Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Reproduction Overview and Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesdays & Thursdays
January 9, 11, 16, 2024
Weeks 1 and 2

Reproductive Biology Overview

Importance of Reproduction
- Define animal (mineral/vegetable/animal)
- Define area in biology

History Reproduction/Thought
- Testis/Ovary
- Aristotle
- Harvey
- 1930's Steroids/Inhibin/Cycle

Reproductive Biology Areas

Reproduction Problems/Questions
- Contraception/Society problems

Required Reading

Ganong W., Medical Physiology, Development and Function of Reproductive System, Chapter 23

Skinner MK, Jegou B (2018) Historic Considerations in Male Reproduction in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 1: 3-9.

Reproduction Background References

Encyclopedia of Reproduction, Second Edition (2018) Volumes 1-6, Editor-in-Chief Michael K. Skinner.
Open Access through WSU library:
https://www.sciencedirect.com/referencework/9780128151457/encyclopedia-of-reproduction

Systems Biology

- History and Definitions
- Revolutionary and Evolutionary Systems Biology
- Reductionism/ Genetic Determination
- Holism/ Emergentism/ Homeostasis or Robustness
- Networks and Computational Biology
- Basic Molecular and Cellular Components
- Omics and Technology

Required Reading

Knepper, et al., (2014) Systems biology versus reductionism in cell physiology. Am J Physiol Cell Physiol 307:C308-309

Systems Biology Background Book References

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Literature

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a LANGE medical book

Review of

Medical Physiology

twenty-second edition

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Lange Medical Books/McGraw-Hill
Medical Publishing Division

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The Gonads: Development & Function of the Reproductive System

23

INTRODUCTION

Modern genetics and experimental embryology make it clear that, in most species of mammals, the multiple differences between the male and the female depend primarily on a single chromosome (the Y chromosome) and a single pair of endocrine structures, the testes in the male and the ovaries in the female. The differentiation of the primitive gonads into testes or ovaries in utero is genetically determined in humans, but the formation of male genitalia depends on the presence of a functional, secreting testis; in the absence of testicular tissue, development is female. Evidence indicates that male sexual behavior and, in some species, the male pattern of gonadotropin secretion are due to the action of male hormones on the brain in early development. After birth, the gonads remain quiescent until adolescence, when they are activated by gonadotropins from the anterior pituitary. Hormones secreted by the gonads at this time cause the appearance of features typical of the adult male or female and the onset of the sexual cycle in the female. In human females, ovarian function regresses after a number of years and sexual cycles cease (the menopause). In males, gonadal function slowly declines with advancing age, but the ability to father children persists.

In both sexes, the gonads have a dual function: the production of germ cells (**gametogenesis**) and the secretion of **sex hormones.** The **androgens** are the steroid sex hormones that are masculinizing in their action; the **estrogens** are those that are feminizing. Both types of hormones are normally secreted in both sexes. The testes secrete large amounts of androgens, principally **testosterone,** but they also secrete small amounts of estrogens. The ovaries secrete large amounts of estrogens and small amounts of androgens. Androgens are secreted from the adrenal cortex in both sexes, and some of the androgens are converted to estrogens in fat and other extragonadal and extraadrenal tissues. The ovaries also secrete **progesterone,** a steroid that has special functions in preparing the uterus for pregnancy. Particularly during pregnancy, the ovaries secrete the polypeptide hormone **relaxin,** which loosens the ligaments of the pubic symphysis and softens the cervix, facilitating delivery of the fetus. In both sexes, the gonads secrete other polypeptides, including **inhibin B,** a polypeptide that inhibits FSH secretion.

The secretory and gametogenic functions of the gonads are both dependent on the secretion of the anterior pituitary gonadotropins, FSH, and LH. The sex hormones and inhibin B feed back to inhibit gonadotropin secretion. In males, gonadotropin secretion is noncyclic; but in postpubertal females an orderly, sequential secretion of gonadotropins is necessary for the occurrence of menstruation, pregnancy, and lactation.

SEX DIFFERENTIATION & DEVELOPMENT

CHROMOSOMAL SEX

The Sex Chromosomes

Sex is determined genetically by two chromosomes, called the **sex chromosomes** to distinguish them from the **somatic chromosomes (autosomes).** In humans and many other mammals, the sex chromosomes are called X and Y chromosomes. The Y chromosome is necessary and sufficient for the production of testes, and the testis-determining gene product is called SRY (for sex-determining region of the Y chromosome). SRY is a DNA-binding regulatory protein. It bends the DNA and acts as a transcription factor that initiates transcription of a cascade of genes necessary for testicular differentiation, including the gene for MIS (see below). The gene for SRY is located near the tip of the short arm of the human Y chromosome. Male cells with the diploid number of chromosomes contain an X and a Y chromosome (XY pattern), whereas female cells contain two X chromosomes (XX pattern). As a consequence of meiosis during gametogenesis, each normal

ovum contains a single X chromosome, but half the normal sperms contain an X chromosome and half contain a Y chromosome (Figure 23–1). When a sperm containing a Y chromosome fertilizes an ovum, an XY pattern results and the zygote develops into a **genetic male.** When fertilization occurs with an X-containing sperm, an XX pattern and a **genetic female** result. Cell division and the chemical nature of chromosomes are discussed in Chapter 1.

Human Chromosomes

Human chromosomes can be studied in detail. Human cells are grown in tissue culture; treated with the drug colchicine, which arrests mitosis at the metaphase; exposed to a hypotonic solution that makes the chromosomes swell and disperse; and then "squashed" onto slides. Fluorescent and other staining techniques make it possible to identify the individual chromosomes and study them in detail (Figure 23–2). There are 46 chromosomes: in males, 22 pairs of autosomes plus an X chromosome and a Y chromosome; in females, 22 pairs of autosomes plus two X chromosomes. The individual chromosomes are usually arranged in an arbitrary pattern (**karyotype**). The individual autosome pairs are identified by the numbers 1–22 on the basis of their morphologic characteristics. The human Y chromosome is smaller than the X chromosome, and it has been hypothesized that sperm containing the Y chromosome are lighter and able to "swim" faster up the female genital tract, thus reaching the ovum more rapidly. This supposedly accounts for the fact that the number of males born is slightly greater than the number of females.

Sex Chromatin

Soon after cell division has started during embryonic development, one or the other of the two X chromosomes of the somatic cells in normal females becomes functionally inactive. In abnormal individuals with more than two X chromosomes, only one remains active. The process that is normally responsible for inactivation is initiated in an X-inactivation center in the chromosome, probably via the transactivating factor CTCF, which is also induced in gene imprinting. However, the details of the inactivation process are still incompletely understood. The choice of which X chro-

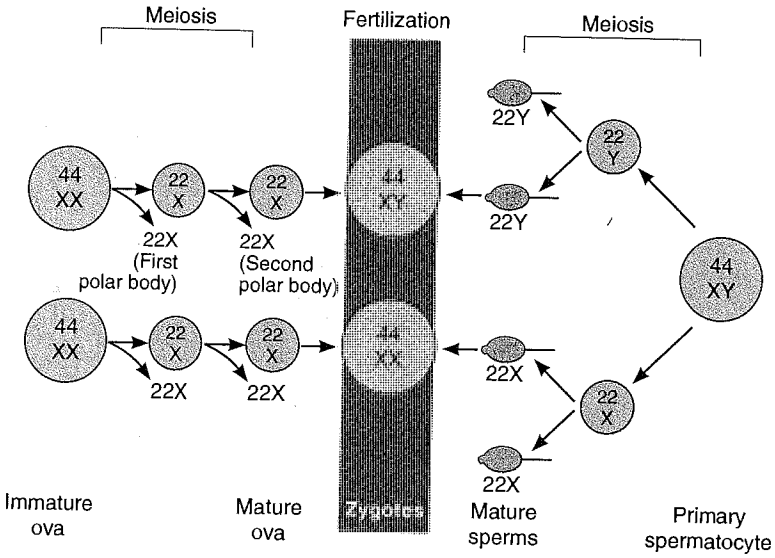


Figure 23–1. Basis of genetic sex determination. In the two-stage meiotic division in the female, only one cell survives as the mature ovum. In the male, the meiotic division results in the formation of four sperms, two containing the X and two the Y chromosome. Fertilization thus produces a male zygote with 22 pairs of autosomes plus an X and a Y or a female zygote with 22 pairs of autosomes and two X chromosomes. Note that for clarity, this figure and Figures 23–6 and 23–7 differ from the current international nomenclature for karyotypes, which lists the total number of chromosomes followed by the sex chromosome pattern. Thus, XO is 45, X, XY is 46, XY, XXY is 47, XXY, etc.

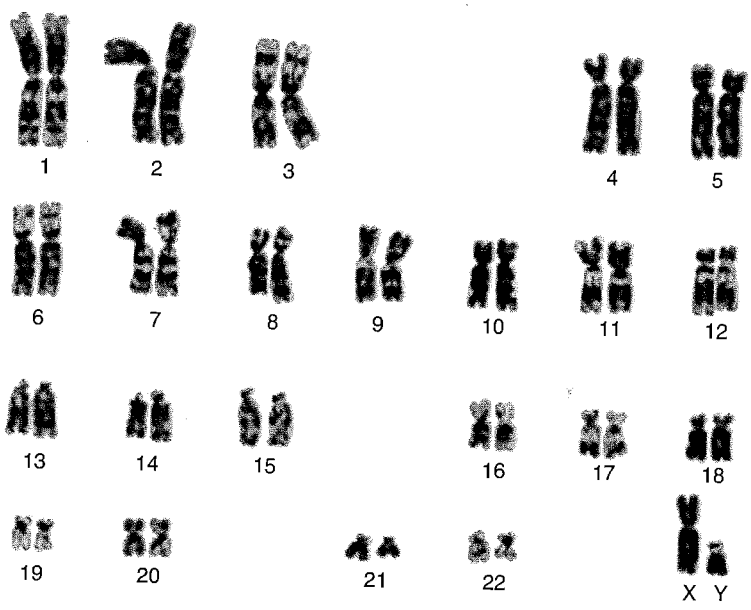


Figure 23–2. Karyotype of chromosomes from a normal male. The chromosomes have been stained with Giemsa's stain, which produces a characteristic banding pattern. (Reproduced, with permission, from Lingappa VJ, Farey K: *Physiological Medicine.* McGraw-Hill, 2000.)

mosome remains active is random, so normally one X chromosome remains active in approximately half of the cells and the other X chromosome is active in the other half. The selection persists through subsequent divisions of these cells, and consequently some of the somatic cells in adult females contain an active X chromosome of paternal origin and some contain an active X chromosome of maternal origin.

In normal cells, the inactive X chromosome condenses and can be seen in various types of cells, usually near the nuclear membrane, as the **Barr body,** also called sex chromatin (Figure 23–3). Thus, there is a Barr body for each X chromosome in excess of one in the cell. The inactive X chromosome is also visible as a small "drumstick" of chromatin projecting from the nuclei of 1–15% of the polymorphonuclear leukocytes in females but not in males (Figure 23–3).

EMBRYOLOGY OF THE HUMAN REPRODUCTIVE SYSTEM

Development of the Gonads

On each side of the embryo, a primitive gonad arises from the genital ridge, a condensation of tissue near the adrenal gland. The gonad develops a **cortex** and a

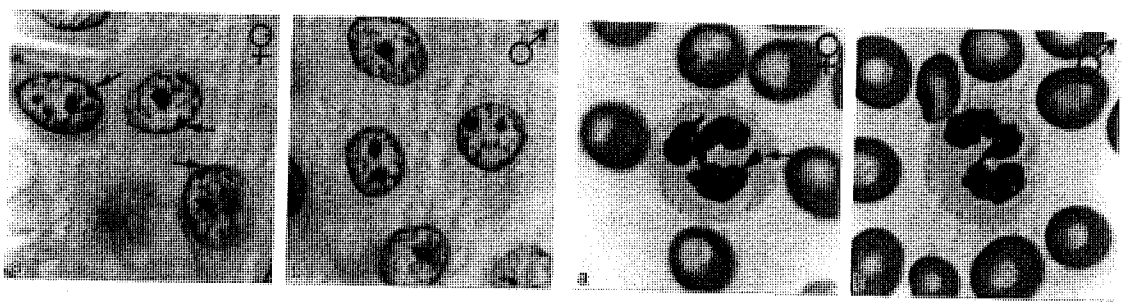


Figure 23–3. **Left:** Barr body (arrows) in the epidermal spinous cell layer. **Right:** Nuclear appendage ("drumstick") identified by arrow in white blood cells. (Reproduced, with permission, from Grumbach MM, Barr ML: Cytologic tests of chromosomal sex in relation to sex anomalies in man. Recent Prog Horm Res 1958;14:255.)

medulla. Until the sixth week of development, these structures are identical in both sexes. In genetic males, the medulla develops during the seventh and eighth weeks into a testis, and the cortex regresses. Leydig and Sertoli cells appear, and testosterone and müllerian inhibiting substance are secreted. In genetic females, the cortex develops into an ovary and the medulla regresses. The embryonic ovary does not secrete hormones. Hormonal treatment of the mother has no effect on gonadal (as opposed to ductal and genital) differentiation in humans, although it does in some experimental animals.

Embryology of the Genitalia

The embryology of the gonads is summarized in Figures 23–4 and 23–5. In the seventh week of gestation, the embryo has both male and female primordial genital ducts (Figure 23–4). In a normal female fetus, the müllerian duct system then develops into uterine tubes (oviducts) and a uterus. In the normal male fetus, the wolffian duct system on each side develops into the epididymis and vas deferens. The external genitalia are similarly bipotential until the eighth week (Figure 23–5). Thereafter, the urogenital slit disappears and male genitalia form, or, alternatively, it remains open and female genitalia form.

When the embryo has functional testes, male internal and external genitalia develop. The Leydig cells of the fetal testis secrete testosterone, and the Sertoli cells secrete **müllerian inhibiting substance (MIS;** also called müllerian regression factor, or MRF). MIS is a 536-amino-acid homodimer that is a member of the TGFβ superfamily of growth factors, which includes inhibins and activins (see below). In their effects on the internal as opposed to the external genitalia, MIS and testosterone act unilaterally. MIS causes regression of the müllerian ducts by apoptosis on the side on which it is secreted, and testosterone fosters the development of the vas deferens and related structutes from the wolffian ducts. The testosterone metabolite dihydrotestosterone (see below) induces the formation of male external genitalia and male secondary sex characteristics (Figure 23–6).

MIS continues to be secreted by the Sertoli cells, and it reaches mean values of 48 ng/mL in plasma in 1- to 2-year-old boys. Thereafter, it declines to low levels by the time of puberty and persists at low but detectable levels throughout life. In girls, MIS is produced by granulosa cells in small follicles in the ovaries, but plasma levels are very low or undetectable until puberty. Thereafter, plasma MIS is about the same as in adult men, ie, about 2 ng/mL. The functions of MIS after early embryonic life are unsettled, but it is probably involved in germ cell maturation in both sexes and in control of testicular descent in boys (see below).

Development of the Brain

At least in some species, the development of the brain as well as the external genitalia is affected by androgens early in life. In rats, a brief exposure to androgens during the first few days of life causes the male pattern of sexual behavior and the male pattern of hypothalamic control of gonadotropin secretion to develop after puberty. In the absence of androgens, female patterns develop (see Chapter 15). In monkeys, similar effects on sexual behavior are produced by exposure to androgens in utero, but the pattern of gonadotropin secretion remains cyclic. Early exposure of female human fetuses to androgens also appears to cause subtle but significant masculinizing effects on behavior. However, women with adrenogenital syndrome due to congenital adrenocortical enzyme deficiency (see Chapter 20) develop normal menstrual cycles when treated with cortisol. Thus, the human, like the monkey, appears to retain the cyclic pattern of gonadotropin secretion despite the exposure to androgens in utero.

ABERRANT SEXUAL DIFFERENTIATION

Chromosomal Abnormalities

From the preceding discussion, it might be expected that abnormalities of sexual development could be caused by genetic or hormonal abnormalities as well as by other nonspecific teratogenic influences, and this is indeed the case. The major classes of abnormalities are listed in Table 23–1.

An established defect in gametogenesis is **nondisjunction,** a phenomenon in which a pair of chromosomes fail to separate, so that both go to one of the daughter cells during meiosis. Four of the abnormal zygotes that can form as a result of nondisjunction of one of the X chromosomes during oogenesis are shown in Figure 23–7. In individuals with the XO chromosomal pattern, the gonads are rudimentary or absent, so that female external genitalia develop. Stature is short, other congenital abnormalities are often present, and no sexual maturation occurs at puberty. This syndrome is called **gonadal dysgenesis** or, alternatively, **ovarian agenesis** or **Turner's syndrome.** Individuals with the XXY pattern, the most common sex chromosome disorder, have the genitalia of a normal male. Testosterone secretion at puberty is often great enough for the development of male characteristics. However, the seminiferous tubules are abnormal, and the incidence of mental retardation is higher than normal. This syndrome is known as **seminiferous tubule dysgenesis** or **Klinefel-**

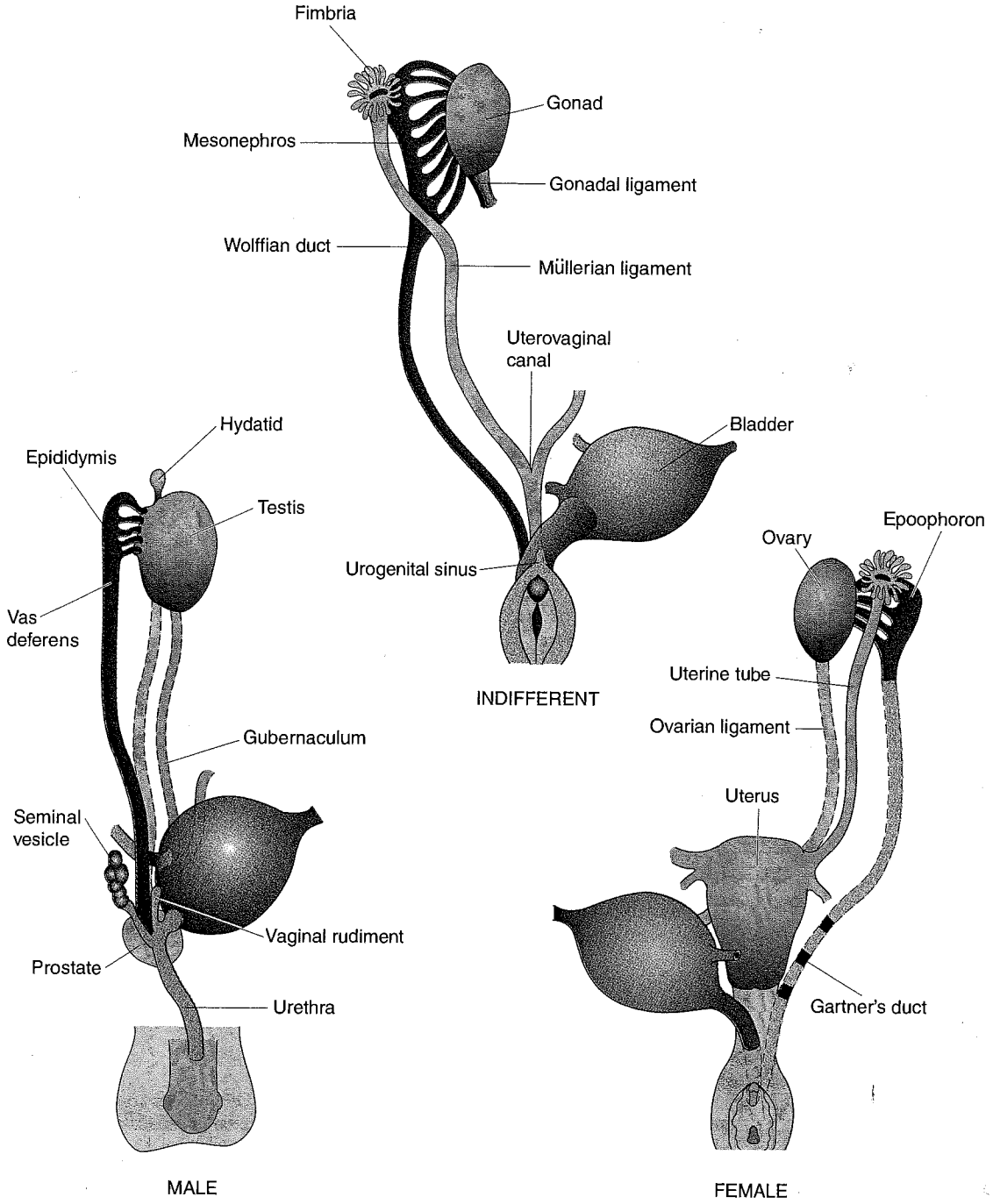


Figure 23–4. **Embryonic differentiation of male and female internal genitalia (genital ducts) from wolffian (male) and müllerian (female) primordia.** (After Corning HK, Wilkins L. Redrawn and reproduced, with permission, from *Williams Textbook of Endocrinology,* 7th ed. Wilson JD, Foster DW [editors]. Saunders, 1985.)

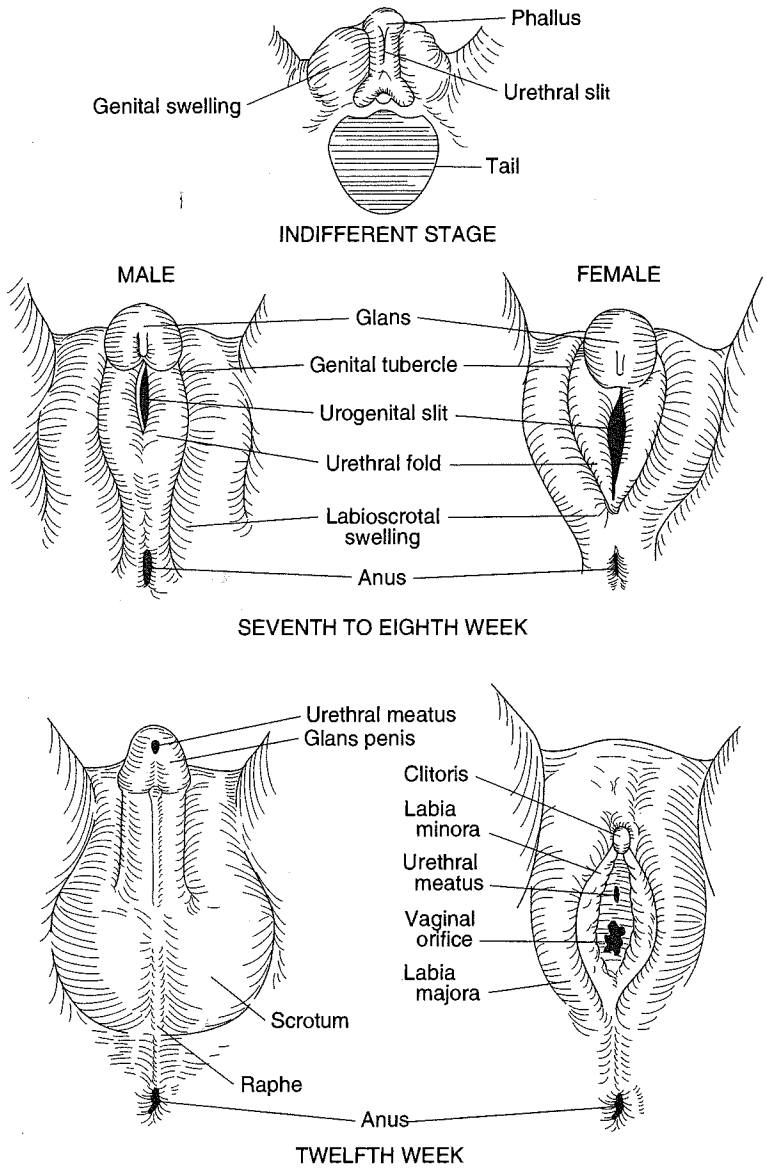


Figure 23–5. Differentiation of male and female external genitalia from indifferent primordial structures in the embryo.

ter's syndrome. The XXX ("superfemale") pattern is second in frequency only to the XXY pattern and may be even more common in the general population, since it does not seem to be associated with any characteristic abnormalities. The YO combination is probably lethal.

Meiosis is a two-stage process, and although nondisjunction usually occurs during the first meiotic division, it can occur in the second, producing more complex chromosomal abnormalities. In addition, nondisjunction or simple loss of a sex chromosome can occur during the early mitotic divisions after fertilization. The result of faulty mitoses in the early zygote is the production of **mosaicism**, in which two or more populations of cells have different chromosome complements. **True hermaphroditism,** the condition in which the individual has both ovaries and testes, is probably due to XX/XY mosaicism and related mosaic patterns, although other genetic aberrations are possible.

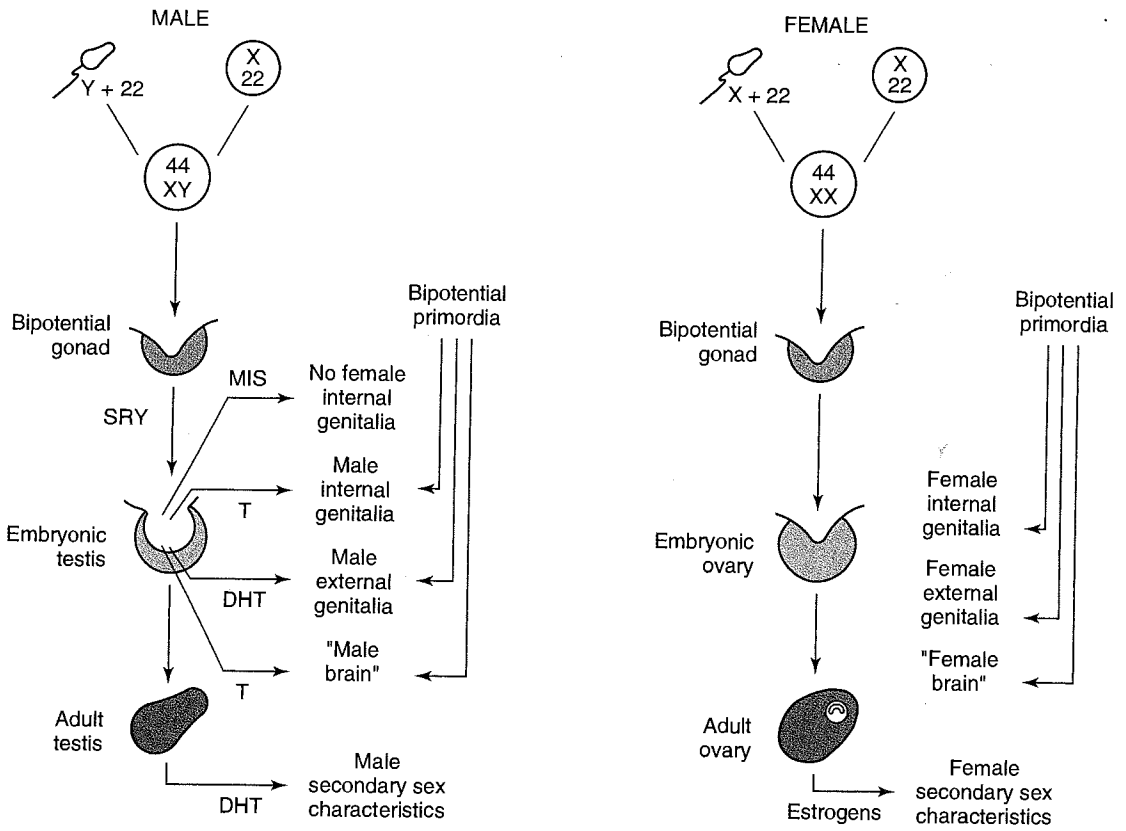


Figure 23–6. Diagrammatic summary of normal sex determination, differentiation, and development in humans. MIS, müllerian inhibiting substance; T, testosterone; DHT, dihydrotestosterone.

Chromosomal abnormalities also include transposition of parts of chromosomes to other chromosomes. Rarely, genetic males are found to have the XX karyotype because the short arm of their father's Y chromosome was transposed to their father's X chromosome during meiosis and they received that X chromosome along with their mother's. Similarly, deletion of the small portion of the Y chromosome containing SRY produces females with the XY karyotype.

Nondisjunction of chromosome 21 produces **trisomy 21,** the chromosomal abnormality associated with **Down's syndrome** (mongolism). The additional chromosome 21 is normal, so Down's syndrome is a pure case of gene excess causing abnormalities.

Many other chromosomal abnormalities occur as well as numerous diseases due to defects in single genes. These conditions are generally diagnosed in utero by analysis of fetal cells in a sample of amniotic fluid collected by inserting a needle through the abdominal wall **(amniocentesis)** or, earlier in pregnancy, by examining fetal cells obtained by a needle biopsy of chorionic villi **(chorionic villus sampling).**

Hormonal Abnormalities

Development of the male external genitalia occurs normally in genetic males in response to androgen secreted by the embryonic testes, but male genital development may also occur in genetic females exposed to androgens from some other source during the eighth to the thirteenth weeks of gestation. The syndrome that results is **female pseudohermaphroditism.** A pseudohermaphrodite is an individual with the genetic constitution and gonads of one sex and the genitalia of the other. After the thirteenth week, the genitalia are fully formed, but exposure to androgens can cause hypertrophy of the clitoris. Female pseudohermaphroditism may be due to congenital virilizing adrenal hyperplasia (see Chapter 20), or it may be caused by androgens administered to the mother. Conversely, one cause of the development

Table 23–1. Classification of the major disorders of sex differentiation in humans. Many of these syndromes can have great variation in degree and, consequently, in manifestations.

Chromosomal disorders
Gonadal dysgenesis (XO and variants)
"Superfemales" (XXX)
Seminiferous tubule dysgenesis (XXY and variants)
True hermaphroditism
Developmental disorders
Female pseudohermaphroditism
Congenital virilizing adrenal hyperplasia of fetus
Maternal androgen excess
Virilizing ovarian tumor
Iatrogenic: Treatment with androgens or certain synthetic progestational drugs
Male pseudohermaphroditism
Androgen resistance
Defective testicular development
Congenital 17α-hydroxylase deficiency
Congenital adrenal hyperplasia due to blockade of pregnenolone formation
Various nonhormonal anomalies

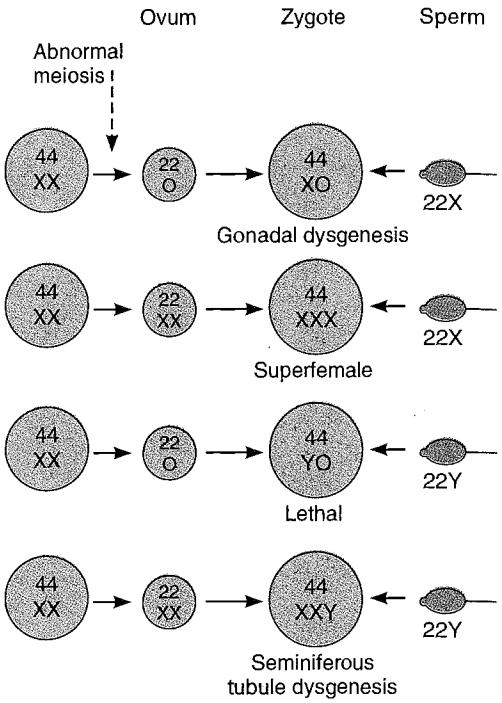


Figure 23–7. Summary of four possible defects produced by maternal nondisjunction of the sex chromosomes at the time of meiosis. The YO combination is believed to be lethal, and the fetus dies in utero.

of female external genitalia in genetic males **(male pseudohermaphroditism)** is defective testicular development. Because the testes also secrete MIS, genetic males with defective testes have female internal genitalia.

Another cause of male pseudohermaphroditism is **androgen resistance,** in which, as a result of various congenital abnormalities, male hormones cannot exert their full effects on the tissues. One form of androgen resistance is a **5α-reductase deficiency,** in which the enzyme responsible for the formation of dihydrotestosterone, the active form of testosterone, is decreased. The consequences of this deficiency are discussed in the section on the male reproductive system. Other forms of androgen resistance are due to various mutations in the androgen receptor gene, and the resulting defects in receptor function range from minor to severe. Mild defects cause infertility with or without gynecomastia (see below). When the loss of receptor function is complete, the **testicular feminizing syndrome,** now known as **complete androgen resistance syndrome,** results. In this condition, MIS is present and testosterone is secreted at normal or even elevated rates. The external genitalia are female, but the vagina ends blindly because there are no female internal genitalia. Individuals with this syndrome develop enlarged breasts at puberty and usually are considered to be normal women until they are diagnosed when they seek medical advice because of lack of menstruation.

It is worth noting that genetic males with congenital blockage of the formation of pregnenolone are pseudohermaphrodites because testicular as well as adrenal androgens are normally formed from pregnenolone. Male pseudohermaphroditism also occurs when there is a congenital deficiency of 17α-hydroxylase (see Chapter 20).

PUBERTY

As noted above, a burst of testosterone secretion occurs in male fetuses before birth (Figure 23–8). In the neonatal period there is another burst, whose function is unknown, but thereafter the Leydig cells become quiescent. There follows in all mammals a period in which the gonads of both sexes are quiescent until they are activated by gonadotropins from the pituitary to bring about the final maturation of the reproductive system. This period of final maturation is known as **adolescence.** It is often also called **puberty,** although puberty, strictly defined, is the period when the endocrine and gametogenic functions of the gonads have first developed to the point where reproduction is possible. In

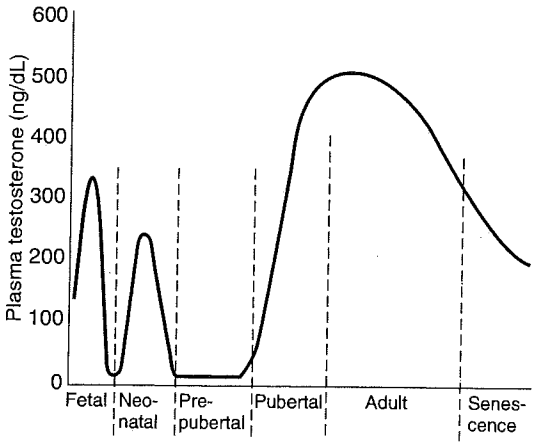


Figure 23–8. Plasma testosterone levels at various ages in human males.

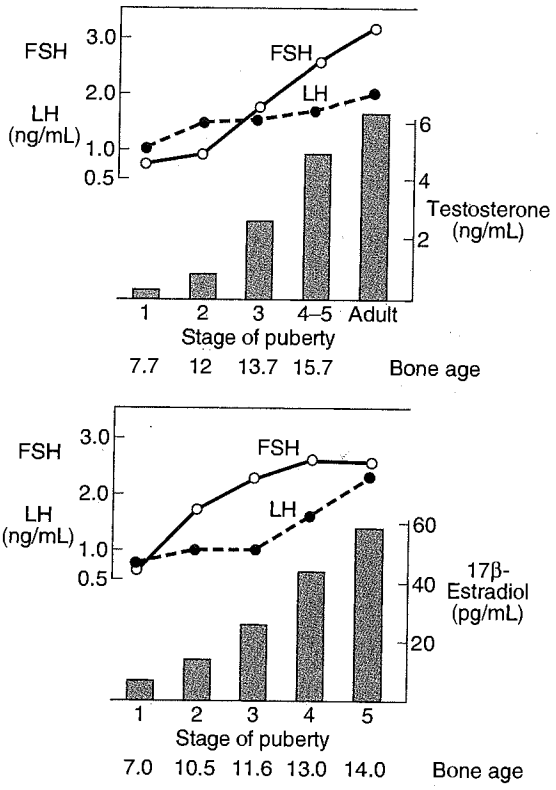


Figure 23–9. Changes in plasma hormone concentrations during puberty in boys **(top)** and girls **(bottom).** Stage 1 of puberty is preadolescence in both sexes. In boys, stage 2 is characterized by beginning enlargement of the testes, stage 3 by penile enlargement, stage 4 by growth of the glans penis, and stage 5 by adult genitalia. In girls, stage 2 is characterized by breast buds, stage 3 by elevation and enlargement of the breasts, stage 4 by projection of the areolas, and stage 5 by adult breasts. (Modified and reproduced, with permission, from *Puberty: Biologic and Psychosocial Components.* Berenberg SR [editor]. HE Stenfoert Kroese BV, 1975.)

girls, the first event is **thelarche,** the development of breasts, followed by **pubarche,** the development of axillary and pubic hair, and then by **menarche,** the first menstrual period. The initial periods are generally anovulatory, and regular ovulation appears about a year later. In contrast to the situation in adulthood, removal of the gonads during the period from soon after birth to puberty causes only a small increase in gonadotropin secretion, so gonadotropin secretion is not being held in check by the gonadal hormones. In children between the ages of 7 and 10, a slow increase in estrogen and androgen secretion precedes the more rapid rise in the early teens (Figure 23–9).

The age at the time of puberty is variable. In Europe and the United States, it has been declining at the rate of 1–3 months per decade for more than 175 years. In the United States in recent years, puberty generally occurs between the ages of 8 and 13 in girls and 9 and 14 in boys.

Another event that occurs in humans at the time of puberty is an increase in the secretion of adrenal androgens (see Figure 20–14). The onset of this increase is called **adrenarche.** It occurs at age 8–10 years in girls and age 10–12 years in boys. DHEA values peak at about 25 years of age in females and slightly later than that in males. They then decline slowly to low values in old age. The rise appears to be due to an increase in the lyase activity of 17α-hydroxylase.

Control of the Onset of Puberty

The gonads of children can be stimulated by gonadotropins; their pituitaries contain gondanotropins; and their hypothalami contain GnRH (see Chapter 14). However, their gonadotropins are not secreted. In immature monkeys, normal menstrual cycles can be brought on by pulsatile injection of GnRH, and they persist as long as the pulsatile injection is continued. Thus, it seems clear that pulsatile secretion of GnRH brings on puberty. During the period from birth to puberty, a neural mechanism is operating to prevent the normal pulsatile release of GnRH. The nature of the mechanism inhibiting the GnRH pulse generator is unknown. However, one or more genes produce products that stimulate secretion of GnRH, and inhibition of these genes before puberty is an interesting possibility.

Relation to Leptin

It has been argued for some time that a critical body weight must normally be reached for puberty to occur. Thus, for example, young women who engage in strenuous athletics lose weight and stop menstruating. So do girls with anorexia nervosa. If these girls start to eat and gain weight, they menstruate again, ie, they "go back through puberty." It now appears that leptin, the satiety-producing hormone secreted by fat cells (see Chapter 14), may be the link between body weight and puberty. Obese ob/ob mice that cannot make leptin are infertile, and their fertility is restored by injections of leptin. Leptin treatment also induces precocious puberty in immature female mice. However, the way that leptin fits into the overall control of puberty remains to be determined.

PRECOCIOUS & DELAYED PUBERTY

Sexual Precocity

The major causes of precocious sexual development in humans are listed in Table 23–2. Early development of secondary sexual characteristics without gametogenesis is caused by abnormal exposure of immature males to androgen or females to estrogen. This syndrome should be called **precocious pseudopuberty** to distinguish it from **true precocious puberty** due to an early but otherwise normal pubertal pattern of gonadotropin secretion from the pituitary (Figure 23–10).

Constitutional precocious puberty—ie, precocious puberty in which no cause can be determined—is more common in girls than in boys. In both sexes, tumors or infections involving the hypothalamus cause precocious puberty. Indeed, in one large series of cases, precocious puberty was the most common endocrine symptom of hypothalamic disease. In experimental animals, precocious puberty can be produced by hypothalamic lesions. Apparently the lesions interrupt a pathway that normally holds pulsatile GnRH secretion in check. Pineal tumors are sometimes associated with precocious puberty, but evidence indicates that these tumors are associated with precocity only when there is secondary damage to the hypothalamus.

Precocious gametogenesis and steroidogenesis can occur without the pubertal pattern of gonadotropin secretion (gonadotropin-independent precocity). At least in some cases of this condition, the sensitivity of LH receptors to gonadotropins is increased because of an activating mutation in the G protein that couples the receptors to adenylyl cyclase.

Figure 23–10. Constitutional precocious puberty in a 3-year-old girl. The patient developed pubic hair and started to menstruate at the age of 17 months. (Reproduced, with permission, from Jolly H: *Sexual Precocity.* Thomas, 1955.)

Table 23–2. Classification of the causes of precocious sexual development in humans.

True precocious puberty
Constitutional
Cerebral: Disorders involving posterior hypothalamus
Tumors
Infections
Developmental abnormalities
Gonadotropin-independent precocity
Precocious pseudopuberty (no spermatogenesis or ovarian development)
Adrenal
Congenital virilizing adrenal hyperplasia
Androgen-secreting tumors (in males)
Estrogen-secreting tumors (in females)
Gonadal
Leydig cell tumors of testis
Granulosa cell tumors of ovary
Miscellaneous

Delayed or Absent Puberty

The normal variation in the age at which adolescent changes occur is so wide that puberty cannot be considered to be pathologically delayed until the menarche has failed to occur by the age of 17 or testicular development by the age of 20. Failure of maturation due to panhypopituitarism is associated with dwarfing and evidence of other endocrine abnormalities. Patients with the XO chromosomal pattern and gonadal dysgenesis are also dwarfed. In some individuals, puberty is delayed even though the gonads are present and other endocrine functions are normal. In males, this clinical picture is called **eunuchoidism.** In females, it is called **primary amenorrhea** (see below).

MENOPAUSE

The human ovaries become unresponsive to gonadotropins with advancing age, and their function declines, so that sexual cycles disappear **(menopause).** This unresponsiveness is associated with and probably caused by a decline in the number of primordial follicles, which becomes precipitous at the time of menopause (Figure 23–11). The ovaries no longer secrete progesterone and 17β-estradiol in appreciable quantities, and estrogen is formed only in small amounts by aromatization of androstenedione in peripheral tissues (see Chapter 20). The uterus and the vagina gradually become atrophic. As the negative feedback effect of estrogens and progesterone is reduced, secretion of FSH and LH is increased, and plasma FSH and LH increase to high levels. Old female mice and rats have long periods of diestrus and increased levels of gonadotropin secretion, but a clear-cut "menopause" has only been described in women.

In women, the menses usually become irregular and cease between the ages of 45 and 55. The average age at onset of the menopause has been increasing since the end of the 19th century and is currently 52 years.

Sensations of warmth spreading from the trunk to the face (hot flushes; also called hot flashes), night sweats, and various psychologic symptoms are common after ovarian function has ceased. Hot flushes are said to occur in 75% of menopausal women and may continue intermittently for as long as 40 years. They also occur when early menopause is produced by bilateral ovariectomy, and they are prevented by estrogen treatment. In addition, they occur after castration in men. Their cause is unknown. However, they coincide with surges of LH secretion. LH is secreted in episodic bursts at intervals of 30–60 minutes or more **(circhoral secretion),** and in the absence of gonadal hormones these bursts are large. Each hot flush begins with the start of a burst. However, LH itself is not responsible for the symptoms, because they can continue after removal of the pituitary. Instead, it appears that some estrogen-sensitive event in the hypothalamus initiates both the release of LH and the episode of flushing.

Although the function of the testes tends to decline slowly with advancing age, the evidence is clear that there is no "male menopause" **(andropause)** similar to that occurring in women.

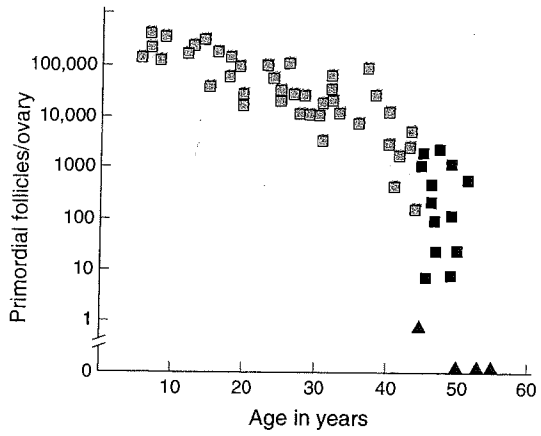


Figure 23–11. Number of primordial follicles per ovary in women at various ages. Colored squares, premenopausal women (regular menses); black squares, perimenopausal women (irregular menses for at least 1 year); black triangles, postmenopausal women (no menses for at least 1 year). Note that the vertical scale is a log scale and that the values are from one rather than two ovaries. (Redrawn by PM Wise and reproduced, with permission, from Richardson SJ, Senikas V, Nelson JF: Follicular depletion during the menopausal transition: Evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 1987;65:1231.)

■ PITUITARY GONADOTROPINS & PROLACTIN

Chemistry

FSH and LH are each made up of an α and a β subunit whose nature is discussed in Chapter 22. They are glycoproteins that contain the hexoses mannose and galactose, the hexosamines *N*-acetylgalactosamine and *N*-acetylglycosamine, and the methylpentose fucose. They also contain sialic acid. The carbohydrate in the gonadotropin molecules increases their potency by markedly slowing their metabolism. The half-life of

human FSH is about 170 minutes; the half-life of LH is about 60 minutes. Loss-of-function mutations in the FSH receptor cause hypogonadism. Gain-of-function mutations cause a spontaneous form of **ovarian hyperstimulation syndrome,** a condition in which many follicles are stimulated and cytokines are released from the ovary, causing increased vascular permeability and shock.

Human pituitary prolactin contains 199 amino acid residues and three disulfide bridges (Figure 23–12) and has considerable structural similarity to human growth hormone and hCS. The half-life of prolactin, like that of growth hormone, is about 20 minutes. Structurally similar prolactins are secreted by the endometrium and by the placenta (see below).

Receptors

The receptors for FSH and LH are serpentine receptors coupled to adenylyl cyclase through Gs (see Chapter 1). In addition, each has an extended, glycosylated extracellular domain.

The human prolactin receptor resembles the growth hormone receptor and is one of the superfamily of receptors that includes the growth hormone receptor and receptors for many cytokines and hematopoietic growth factors (see Chapters 1, 22, 24, and 27). It dimerizes and activates the JAK–STAT and other intracellular enzyme cascades.

Actions

The testes and ovaries become atrophic when the pituitary is removed or destroyed. The actions of prolactin and the gonadotropins FSH and LH, as well as those of the gonadotropin secreted by the placenta, are described in detail in succeeding sections of this chapter. In brief, FSH helps maintain the spermatogenic epithelium by stimulating Sertoli cells in the male and is responsible for the early growth of ovarian follicles in the female. LH is tropic to the Leydig cells and, in females, is responsible for the final maturation of the ovarian follicles and estrogen secretion from them. It is also responsible for ovulation, the initial formation of the corpus luteum, and secretion of progesterone.

Prolactin causes milk secretion from the breast after estrogen and progesterone priming. Its effect on the breast involves increased action of mRNA and increased production of casein and lactalbumin. However, the action of the hormone is not exerted on the

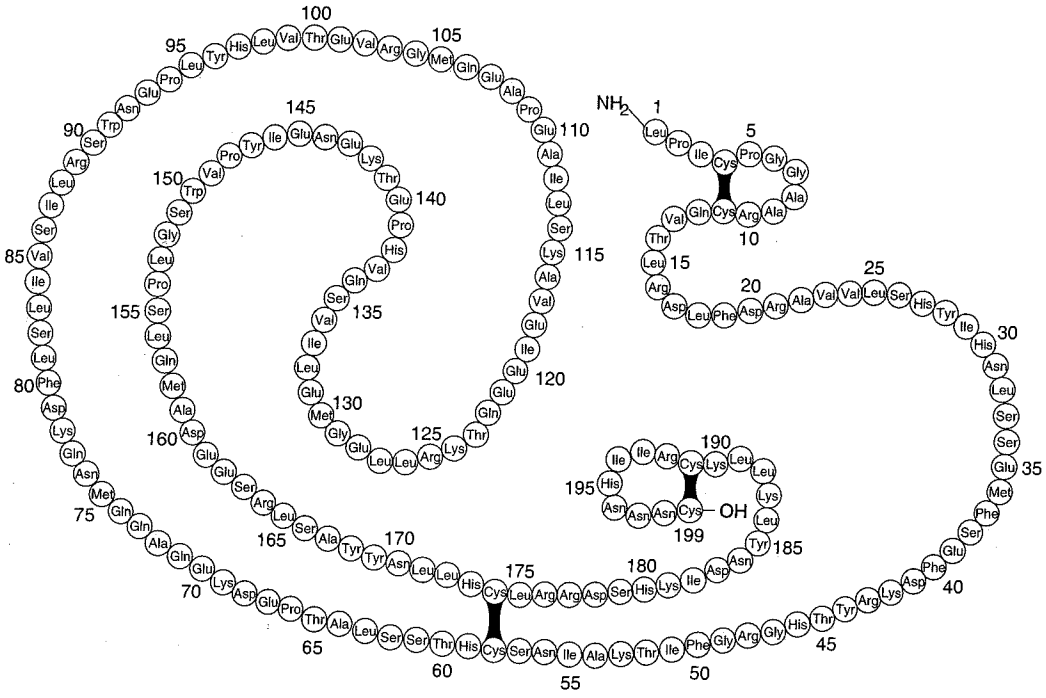


Figure 23–12. Structure of human prolactin.

cell nucleus and is prevented by inhibitors of microtubules. Prolactin also inhibits the effects of gonadotropins, possibly by an action at the level of the ovary. Its role in preventing ovulation in lactating women is discussed below. The function of prolactin in normal males is unsettled, but excess prolactin secreted by tumors causes impotence. An action of prolactin that has been used as the basis for bioassay of this hormone is stimulation of the growth and "secretion" of the crop sacs in pigeons and other birds. The paired crop sacs are outpouchings of the esophagus which form, by desquamation of their inner cell layers, a nutritious material ("milk") that the birds feed to their young. However, prolactin, FSH, and LH are now regularly measured by radioimmunoassay.

Regulation of Prolactin Secretion

The normal plasma prolactin concentration is approximately 5 ng/mL in men and 8 ng/mL in women. Secretion is tonically inhibited by the hypothalamus, and section of the pituitary stalk leads to an increase in circulating prolactin. Thus, the effect of the hypothalamic prolactin-inhibiting hormone (PIH) dopamine is normally greater than the effects of the various hypothalamic peptides with prolactin-releasing activity. In humans, prolactin secretion is increased by exercise, surgical and psychologic stresses, and stimulation of the nipple (Table 23–3). The plasma prolactin level rises during sleep, the rise starting after the onset of sleep and persisting throughout the sleep period. Secretion is increased during pregnancy, reaching a peak at the time of parturition. After delivery, the plasma concentration falls to nonpregnant levels in about 8 days. Suckling produces a prompt increase in secretion, but the magnitude of this rise gradually declines after a woman has been nursing for more than 3 months. With prolonged lactation, milk secretion occurs with prolactin levels that are in the normal range.

L-Dopa decreases prolactin secretion by increasing the formation of dopamine, and bromocriptine and other dopamine agonists inhibit secretion because they stimulate dopamine receptors. Chlorpromazine and related drugs that block dopamine receptors increase prolactin secretion. TRH stimulates the secretion of prolactin in addition to TSH, and additional polypeptides with prolactin-releasing activity are present in hypothalamic tissue. Estrogens produce a slowly developing increase in prolactin secretion as a result of a direct action on the lactotropes.

It has now been established that prolactin facilitates the secretion of dopamine in the median eminence. Thus, prolactin acts in the hypothalamus in a negative feedback fashion to inhibit its own secretion.

Table 23–3. Factors affecting the secretion of human prolactin and growth hormone.

Factor	Prolactin[a]	Growth Hormone[a]
Sleep	I+	I+
Nursing	I++	N
Breast stimulation in nonlactating women	I	N
Stress	I+	I+
Hypoglycemia	I	I+
Strenuous exercise	I	I
Sexual intercourse in women	I	N
Pregnancy	I++	N
Estrogens	I	I
Hypothyroidism	I	N
TRH	I+	N
Phenothiazines, butyrophenones	I+	N
Opiods	I	I
Glucose	N	D
Somatostatin	N	D+
L-Dopa	D+	I+
Apomorphine	D+	I+
Bromocriptine and related ergot derivatives	D+	I

[a]I, moderate increase; I+, marked increase; I++, very marked increase; N, no change; D, moderate decrease; D+, marked decrease.

Hyperprolactinemia

Up to 70% of the patients with chromophobe adenomas of the anterior pituitary have elevated plasma prolactin levels. In some instances, the elevation may be due to damage to the pituitary stalk, but in most cases, the tumor cells are actually secreting the hormone. The hyperprolactinemia may cause galactorrhea, but in many individuals no demonstrable endocrine abnormalities are present. Conversely, most women with galactorrhea have normal prolactin levels; definite elevations are found in less than a third of patients with this condition.

Another interesting observation is that 15–20% of women with secondary amenorrhea have elevated prolactin levels, and when prolactin secretion is reduced, normal menstrual cycles and fertility return. It appears that the prolactin may produce amenorrhea by blocking the action of gonadotropins on the ovaries, but definitive proof of this hypothesis must await further research. The hypogonadism produced by prolactinomas

is associated with osteoporosis due to estrogen deficiency.

As noted above, hyperprolactinemia in men is associated with impotence and hypogonadism that disappear when prolactin secretion is reduced.

THE MALE REPRODUCTIVE SYSTEM

STRUCTURE

The testes are made up of loops of convoluted **seminiferous tubules,** in the walls of which the spermatozoa are formed from the primitive germ cells **(spermatogenesis).** Both ends of each loop drain into a network of ducts in the head of the **epididymis.** From there, spermatozoa pass through the tail of the epididymis into the **vas deferens.** They enter through the **ejaculatory ducts** into the urethra in the body of the **prostate** at the time of ejaculation (Figure 23–13). Between the tubules in the testes are nests of cells containing lipid granules, the **interstitial cells of Leydig** (Figures 23–14 and 23–15), which secrete testosterone into the bloodstream. The spermatic arteries to the testes are tortuous, and blood in them runs parallel but in the opposite direction to blood in the pampiniform plexus of spermatic veins. This anatomic arrangement may permit countercurrent exchange of heat and testosterone. The principles of countercurrent exchange are considered in detail in relation to the kidney in Chapter 38.

GAMETOGENESIS & EJACULATION

Blood–Testis Barrier

The walls of the seminiferous tubules are lined by primitive germ cells and **Sertoli cells,** large, complex glycogen-containing cells that stretch from the basal lamina of the tubule to the lumen (Figure 23–15). Germ cells must stay in contact with Sertoli cells to survive, and this contact is maintained by cytoplasmic bridges. Tight junctions between adjacent Sertoli cells near the basal lamina form a **blood–testis barrier** that prevents many large molecules from passing from the interstitial tissue and the part of the tubule near the basal lamina (basal compartment) to the region near the tubular lumen (adluminal compartment) and the lumen. However, steroids penetrate this barrier with ease, and evidence suggests that some proteins pass from the Sertoli cells to the Leydig cells and vice versa in a paracrine fashion. In addition, maturing germ cells must pass through the barrier as they move to the lumen. This appears to occur without disruption of the barrier by progressive breakdown of the tight junctions above the germ cells, with concomitant formation of new tight junctions below them.

The fluid in the lumen of the seminiferous tubules is quite different from plasma; it contains very little protein and glucose but is rich in androgens, estrogens, K^+, inositol, and glutamic and aspartic acids. Maintenance of its composition presumably depends on the blood–testis barrier. The barrier also protects the germ cells from blood-borne noxious agents, prevents antigenic products of germ cell division and maturation from entering the circulation and generating an autoimmune

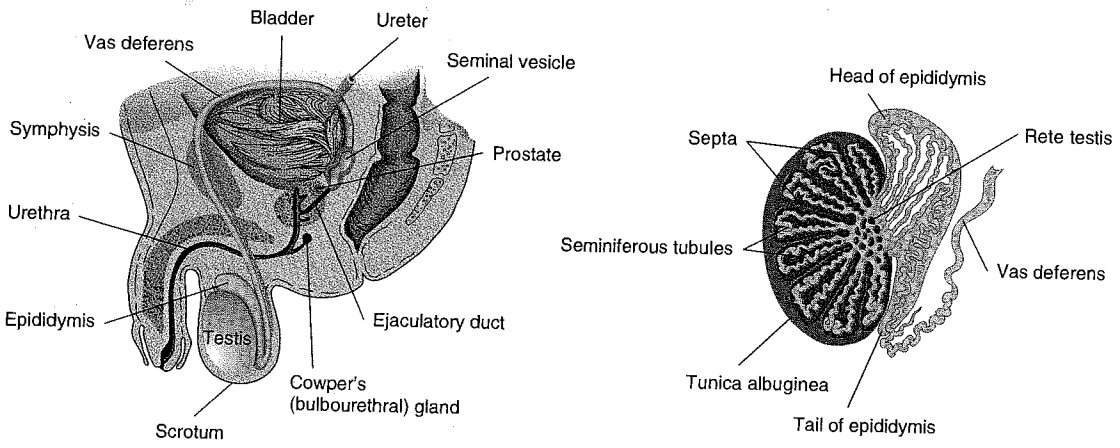


Figure 23–13. **Left:** Male reproductive system. **Right:** Duct system of the testis.

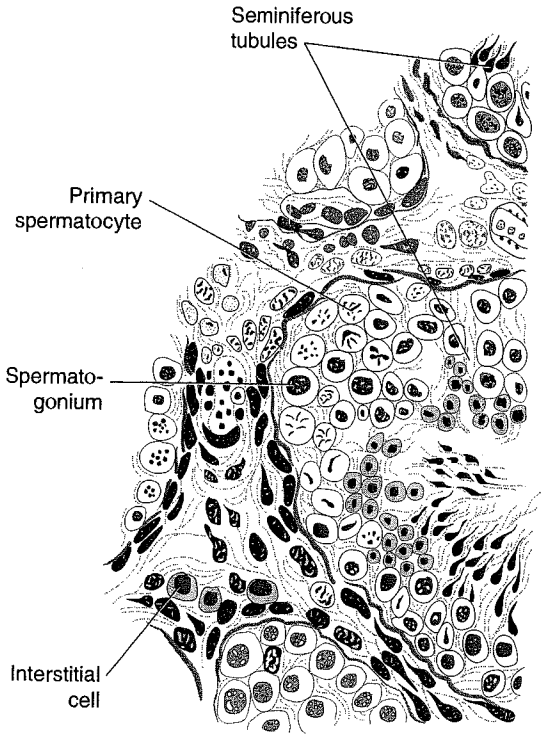


Figure 23–14. Section of human testis.

response, and may help establish an osmotic gradient that facilitates movement of fluid into the tubular lumen.

Spermatogenesis

The **spermatogonia,** the primitive germ cells next to the basal lamina of the seminiferous tubules, mature into **primary spermatocytes** (Figure 23–15). This process begins during adolescence. The primary spermatocytes undergo meiotic division, reducing the number of chromosomes. In this two-stage process, they divide into **secondary spermatocytes** and then into **spermatids,** which contain the haploid number of 23 chromosomes. The spermatids mature into **spermatozoa (sperms).** As a single spermatogonium divides and matures, its descendants remain tied together by cytoplasmic bridges until the late spermatid stage. This apparently ensures synchrony of the differentiation of each clone of germ cells. The estimated number of spermatids formed from a single spermatogonium is 512. In humans, it takes an average of 74 days to form a mature sperm from a primitive germ cell by this orderly process of spermatogenesis.

Each sperm is an intricate motile cell, rich in DNA, with a head that is made up mostly of chromosomal material (Figure 23–16). Covering the head like a cap is the **acrosome,** a lysosome-like organelle rich in enzymes involved in sperm penetration of the ovum and other events involved in fertilization. The motile tail of the sperm is wrapped in its proximal portion by a sheath holding numerous mitochondria. The membranes of late spermatids and spermatozoa contain a special small form of angiotensin-converting enzyme called **germinal angiotensin-converting enzyme** (see Chapter 24). The function of this enzyme in the sperms is unknown, although male mice in which the function of the angiotensin-converting enzyme gene has been disrupted have reduced fertility.

The spermatids mature into spermatozoa in deep folds of the cytoplasm of the Sertoli cells (Figure 23–15). Mature spermatozoa are released from the Sertoli cells and become free in the lumens of the tubules. The Sertoli cells secrete **androgen-binding protein (ABP), inhibin,** and **MIS.** They do not synthesize androgens, but they contain **aromatase (CYP19),** the enzyme responsible for conversion of androgens to estrogens, and they can produce estrogens. ABP probably functions to maintain a high, stable supply of androgen in the tubular fluid. Inhibin inhibits FSH secretion (see below). MIS causes regression of the müllerian ducts in males during fetal life (see above).

FSH and androgens maintain the gametogenic function of the testis. After hypophysectomy, injection of LH produces a high local concentration of androgen in the testes, and this maintains spermatogenesis. The stages from spermatogonia to spermatids appear to be androgen-independent. However, the maturation from spermatids to spermatozoa depends on androgen acting on the Sertoli cells in which the developing spermatozoa are embedded. FSH acts on the Sertoli cells to facilitate the last stages of spermatid maturation. In addition, it promotes the production of ABP.

An interesting observation is that the estrogen content of the fluid in the rete testis (Figure 23–13) is high, and the walls of the rete contain numerous ERα estrogen receptors. In this region, fluid is reabsorbed and the spermatozoa are concentrated. If this does not occur, the sperm entering the epididymis are diluted in a large volume of fluid, and infertility results.

Further Development of Spermatozoa

Spermatozoa leaving the testes are not fully mobile. They continue their maturation and acquire motility during their passage through the epididymis. Motility is obviously important in vivo, but fertilization occurs in vitro if an immotile spermatozoon from the head of the

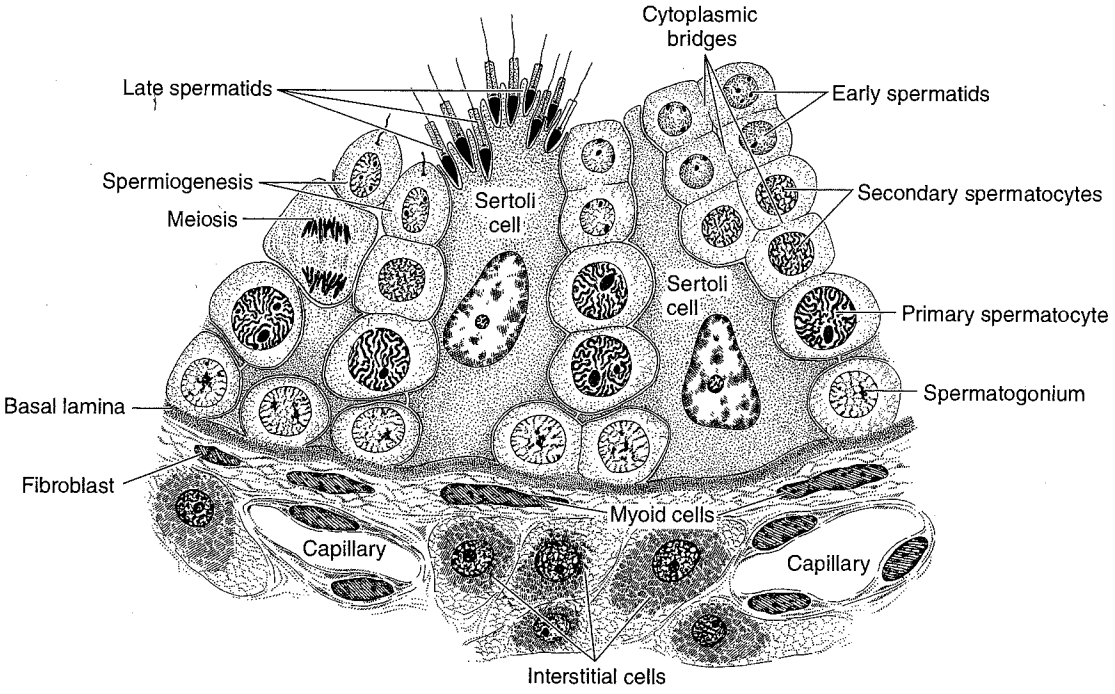


Figure 23–15. Seminiferous epithelium. Note that maturing germ cells remain connected by cytoplasmic bridges through the early spermatid stage and that these cells are closely invested by Sertoli cell cytoplasm as they move from the basal lamina to the lumen. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas,* 10th ed. McGraw-Hill, 2003.)

epididymis is microinjected directly into an ovum. The ability to move forward **(progressive motility),** which is acquired in the epididymis, involves activation of a unique protein called **CatSper,** which is localized to the principal piece of the sperm tail. This protein appears to be a Ca^{2+} ion channel that permits cAMP-generalized Ca^{2+} influx. In addition, spermatozoa express olfactory receptors, and ovaries produce odorant-like molecules. Recent evidence indicates that these molecules and their receptors interact, fostering movement of the spermatozoa toward the ovary (chemotaxis; see below).

Ejaculation of the spermatozoon (see below) involves contractions of the vas deferens mediated in part by P2X receptors for ATP (see Chapter 4), and fertility

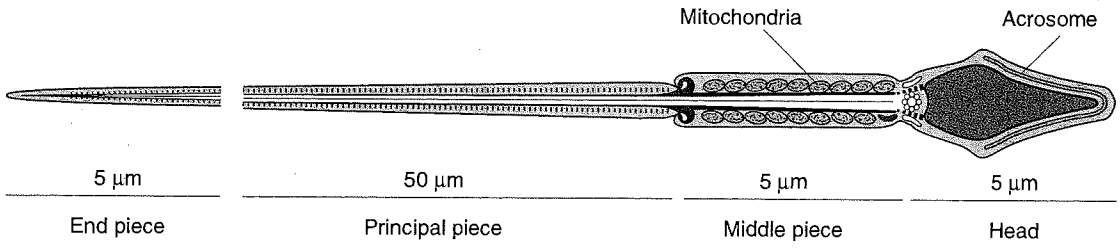


Figure 23–16. Human spermatozoon, profile view. Note the acrosome, an organelle that covers half the sperm head inside the plasma membrane of the sperm. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas,* 10th ed. McGraw-Hill, 2003.)

is reduced in mice in which these receptors are knocked out.

Once ejaculated into the female, the spermatozoa move up the uterus to the isthmus of the uterine tubes, where they slow down and undergo **capacitation.** This further maturation process involves two components: increasing the motility of the spermatozoa and facilitating their preparation for the acrosome reaction. However, the role of capacitation appears to be facilitatory rather than obligatory, because fertilization is readily produced in vitro. From the isthmuses the capacitated spermatozoa move rapidly to the tubal ampullas, where fertilization takes place (see below).

Effect of Temperature

Spermatogenesis requires a temperature considerably lower than that of the interior of the body. The testes are normally maintained at a temperature of about 32 °C. They are kept cool by air circulating around the scrotum and probably by heat exchange in a countercurrent fashion between the spermatic arteries and veins. When the testes are retained in the abdomen or when, in experimental animals, they are held close to the body by tight cloth binders, degeneration of the tubular walls and sterility result. Hot baths (43–45 °C for 30 minutes per day) and insulated athletic supporters reduce the sperm count in humans, in some cases by 90%. However, the reductions produced in this manner are not consistent enough to make the procedures reliable forms of male contraception. In addition, evidence suggests a seasonal effect in men, with sperm counts being greater in the winter regardless of the temperature to which the scrotum is exposed.

Semen

The fluid that is ejaculated at the time of orgasm, the **semen,** contains sperms and the secretions of the seminal vesicles, prostate, Cowper's glands, and, probably, the urethral glands (Table 23–4). An average volume per ejaculate is 2.5–3.5 mL after several days of continence. The volume of semen and the sperm count decrease rapidly with repeated ejaculation. Even though it takes only one sperm to fertilize the ovum, each milliliter of semen normally contains about 100 million sperms. Fifty percent of men with counts of 20–40 million/mL and essentially all of those with counts under 20 million/mL are sterile. The presence of many morphologically abnormal or immotile spermatozoa also correlates with infertility. The **prostaglandins** in semen, which actually come from the seminal vesicles, are in high concentration, but the function of these fatty acid derivatives in semen is unknown. Their structure and their multiple actions in other parts of the body are discussed in Chapter 17.

Table 23–4. Composition of human semen.

Color: White, opalescent	
Specific gravity: 1.028	
pH: 7.35–7.50	
Sperm count: Average about 100 million/mL, with fewer than 20% abnormal forms	
Other components:	
Fructose (1.5–6.5 mg/mL)	From seminal vesicles (contributes 60% of total volume)
Phosphorylcholine	
Ergothioneine	
Ascorbic acid	
Flavins	
Prostaglandins	
Spermine	From prostate (contributes 20% of total volume)
Citric acid	
Cholesterol, phospholipids	
Fibrinolysin, fibrinogenase	
Zinc	
Acid phosphatase	
Phosphate	Buffers
Bicarbonate	
Hyaluronidase	

Human sperms move at a speed of about 3 mm/min through the female genital tract. Sperms reach the uterine tubes 30–60 minutes after copulation. In some species, contractions of the female organs facilitate the transport of the sperms to the uterine tubes, but it is unknown if such contractions are important in humans.

Erection

Erection is initiated by dilation of the arterioles of the penis. As the erectile tissue of the penis fills with blood, the veins are compressed, blocking outflow and adding to the turgor of the organ. The integrating centers in the lumbar segments of the spinal cord are activated by impulses in afferents from the genitalia and descending tracts that mediate erection in response to erotic psychologic stimuli. The efferent parasympathetic fibers are in the pelvic splanchnic nerves **(nervi erigentes).** The fibers presumably release acetylcholine and the vasodilator VIP as cotransmitters (see Chapter 4).

Nonadrenergic noncholinergic fibers are also present in the nervi erigentes, and these contain large amounts of **NO synthase,** the enzyme that catalyzes the forma-

tion of nitric oxide (NO; see Chapter 31). NO activates guanylyl cyclase, resulting in increased production of cGMP, and cGMP is a potent vasodilator. Injection of inhibitors of NO synthase prevents the erection normally produced by stimulation of the pelvic nerve in experimental animals. Thus, it seems clear that NO plays a prominent role in the production of erection. Sildenafil (Viagra) inhibits the breakdown of cGMP by phosphodiesterases and has gained worldwide fame for the treatment of impotence. The multiple phosphodiesterases (PDEs) in the body have been divided into seven isoenzyme families, and sildenafil is most active against PDE5, the type of phosphodiesterase found in the corpora cavernosa. It is worth noting, however, that sildenafil also produces significant inhibition of PDE6. This is the type of phosphodiesterase found in the retina, and one of the side effects of sildenafil is transient loss of the ability to discriminate between blue and green (see Chapter 8).

Normally, erection is terminated by sympathetic vasoconstrictor impulses to the penile arterioles.

Ejaculation

Ejaculation is a two-part spinal reflex that involves **emission,** the movement of the semen into the urethra; and **ejaculation** proper, the propulsion of the semen out of the urethra at the time of orgasm. The afferent pathways are mostly fibers from touch receptors in the glans penis that reach the spinal cord through the internal pudendal nerves. Emission is a sympathetic response, integrated in the upper lumbar segments of the spinal cord and effected by contraction of the smooth muscle of the vasa deferentia and seminal vesicles in response to stimuli in the hypogastric nerves. The semen is propelled out of the urethra by contraction of the bulbocavernosus muscle, a skeletal muscle. The spinal reflex centers for this part of the reflex are in the upper sacral and lowest lumbar segments of the spinal cord, and the motor pathways traverse the first to third sacral roots and the internal pudendal nerves. Carbon monoxide may be involved in the control of ejaculation, since HO2, the enzyme that catalyzes its production in the nervous system (see Chapter 4), is abundant in the pathways concerned with ejaculation, and ejaculatory performance is diminished when the gene for HO2 is knocked out.

PSA

The prostate produces and secretes into the semen and the bloodstream a 30-kDa serine protease generally called **prostate-specific antigen (PSA).** The gene for PSA has two androgen response elements. PSA hydrolyzes the sperm motility inhibitor semenogelin in semen, and it has several substrates in plasma, but its precise function in the circulation is unknown. An elevated plasma PSA occurs in prostate cancer and is widely used as a screening test for this disease, though PSA is also elevated in benign prostatic hyperplasia and prostatitis.

Vasectomy

Bilateral ligation of the vas deferens (vasectomy) has proved to be a relatively safe and convenient contraceptive procedure. However, it has proven difficult to restore the patency of the vas in those wishing to restore fertility, and the current success rate for such operations, as measured by the subsequent production of pregnancy, is about 50%. Half of the men who have been vasectomized develop antibodies against spermatozoa, and in monkeys, the presence of such antibodies is associated with a higher incidence of infertility after restoration of the patency of the vas. However, the antisperm antibodies do not appear to have any other adverse effects.

ENDOCRINE FUNCTION OF THE TESTES

Chemistry & Biosynthesis of Testosterone

Testosterone, the principal hormone of the testes, is a C_{19} steroid (see Chapter 20) with an —OH group in the 17 position (Figure 23–17). It is synthesized from cholesterol in the Leydig cells and is also formed from androstenedione secreted by the adrenal cortex. The biosynthetic pathways in all endocrine organs that form steroid hormones are similar, the organs differing only in the enzyme systems they contain. In the Leydig cells, the 11- and 21-hydroxylases found in the adrenal cortex (see Figure 20–8) are absent, but 17α-hydroxylase is present. Pregnenolone is therefore hydroxylated in the 17 position and then subjected to side chain cleavage to form dehydroepiandrosterone. Androstenedione is also formed via progesterone and 17-hydroxyprogesterone, but this pathway is less prominent in humans. Dehydroepiandrosterone and androstenedione are then converted to testosterone.

The secretion of testosterone is under the control of LH, and the mechanism by which LH stimulates the Leydig cells involves increased formation of cAMP via the serpentine LH receptor and G_s. Cyclic AMP increases the formation of cholesterol from cholesteryl esters and the conversion of cholesterol to pregnenolone via the activation of protein kinase A.

Secretion

The testosterone secretion rate is 4–9 mg/d (13.9–31.33 μmol/d) in normal adult males. Small amounts of testosterone are also secreted in females, probably from the ovary but possibly from the adrenal as well.

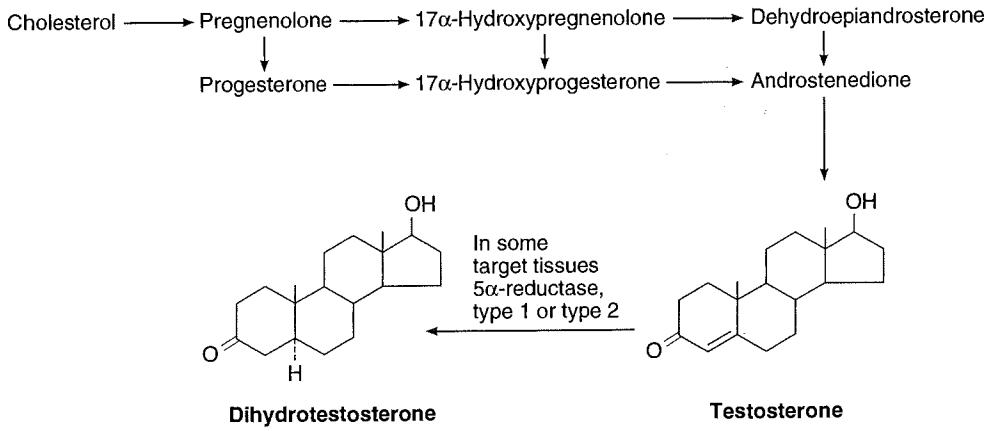


Figure 23–17. Biosynthesis of testosterone. The formulas of the precursor steroids are shown in Figure 20–8. Although the main secretory product of the Leydig cells is testosterone, some of the precursors also enter the circulation.

Transport & Metabolism

Ninety-eight percent of the testosterone in plasma is bound to protein: 65% is bound to a β-globulin called **gonadal steroid-binding globulin (GBG)** or **sex steroid-binding globulin,** and 33% to albumin (Table 23–5). GBG also binds estradiol. The plasma testosterone level (free and bound) is 300–1000 ng/dL (10.4–34.7 nmol/L) in adult men (Figure 23–8) and 30–70 ng/dL (1.04–2.43 nmol/L) in adult women. It declines somewhat with age in males.

A small amount of circulating testosterone is converted to estradiol (see below), but most of the testosterone is converted to 17-ketosteroids, principally androsterone and its isomer etiocholanolone (Figure 23–18), and excreted in the urine. About two thirds of the urinary 17-ketosteroids are of adrenal origin, and one third are of testicular origin. Although most of the 17-ketosteroids are weak androgens (they have 20% or less the potency of testosterone), it is worth emphasizing that not all 17-ketosteroids are androgens and not all androgens are 17-ketosteroids. Etiocholanolone, for example, has no androgenic activity, and testosterone itself is not a 17-ketosteroid.

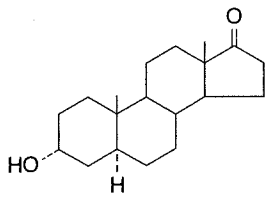
Table 23–5. Distribution of gonadal steroids and cortisol in plasma.[a]

Steroid	% Free	% Bound to CBG	% Bound to GBG	% Bound to Albumin
Testosterone	2	0	65	33
Androstenedione	7	0	8	85
Estradiol	2	0	38	60
Progesterone	2	18	0	80
Cortisol	4	90	0	6

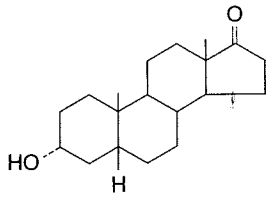
[a]CBG, corticosteroid-binding globulin; GBG, gonadal steroid-binding globulin. (Courtesy of S Munroe.)

Actions

In addition to their actions during development, testosterone and other androgens exert an inhibitory feedback effect on pituitary LH secretion; develop and



Androsterone



Etiocholanolone

Figure 23–18. Two 17-ketosteroid metabolites of testosterone.

maintain the male secondary sex characteristics; exert an important protein-anabolic, growth-promoting effect; and, along with FSH, maintain spermatogenesis (see above).

Secondary Sex Characteristics

The widespread changes in hair distribution, body configuration, and genital size that develop in boys at puberty—the male **secondary sex characteristics**—are summarized in Table 23–6. The prostate and seminal vesicles enlarge, and the seminal vesicles begin to secrete fructose. This sugar appears to function as the main nutritional supply for the spermatozoa. The psychic effects of testosterone are difficult to define in humans, but in experimental animals, androgens provoke boisterous and aggressive play. The effects of androgens and estrogens on sexual behavior are considered in detail in Chapter 15. Although body hair is increased by androgens, scalp hair is decreased (Figure 23–19). Hereditary baldness often fails to develop unless dihydrotestosterone is present.

Anabolic Effects

Androgens increase the synthesis and decrease the breakdown of protein, leading to an increase in the rate of growth. It used to be argued that they cause the epiphyses to fuse to the long bones, thus eventually stopping growth, but it now appears that epiphysial closure is due to estrogens (see below and Chapter 22). Secondary to their anabolic effects, androgens cause moderate sodium, potassium, water, calcium, sulfate, and phosphate retention; and they also increase the size of the kidneys. Doses of exogenous testosterone that exert significant anabolic effects are also masculinizing and increase libido, which limits the usefulness of the hormone as an anabolic agent in patients with wasting diseases. Attempts to develop synthetic steroids in which the anabolic action is divorced from the androgenic action have not been successful.

Table 23–6. Changes at puberty in boys (male secondary sex characteristics).

External genitalia: Penis increases in length and width. Scrotum becomes pigmented and rugose.
Internal genitalia: Seminal vesicles enlarge and secrete and begin to form fructose. Prostate and bulbourethral glands enlarge and secrete.
Voice: Larynx enlarges, vocal cords increase in length and thickness, and voice becomes deeper.
Hair growth: Beard appears. Hairline on scalp recedes anterolaterally. Pubic hair grows with male (triangle with apex up) pattern. Hair appears in axillas, on chest, and around anus; general body hair increases.
Mental: More aggressive, active attitude. Interest in opposite sex develops.
Body conformation: Shoulders broaden, muscles enlarge.
Skin: Sebaceous gland secretion thickens and increases (predisposing to acne).

Mechanism of Action

Like other steroids (see Chapter 1), testosterone binds to an intracellular receptor, and the receptor–steroid complex then binds to DNA in the nucleus, facilitating transcription of various genes. In addition, testosterone is converted to **dihydrotestosterone (DHT)** by 5α-reductase in some target cells (Figures 23–17 and 23–20), and DHT binds to the same intracellular receptor as testosterone. DHT also circulates, with a plasma level that is about 10% of the testosterone level. Testosterone–receptor complexes are less stable than DHT–receptor complexes in target cells, and they conform less well to the DNA-binding state. Thus, DHT formation is a way of amplifying the action of testosterone in target tissues. Humans have two 5α-reductases, encoded by different genes. Type 1 5α-reductase is present in skin throughout the body and is the dominant enzyme in the scalp. Type 2 5α-reductase is present in genital skin, the prostate, and other genital tissues.

Testosterone–receptor complexes are responsible for the maturation of wolffian duct structures and consequently for the formation of male internal genitalia during development, but DHT-receptor complexes are needed to form male external genitalia (Figure 23–20). DHT–receptor complexes are also primarily responsible for enlargement of the prostate and probably of the penis at the time of puberty, as well as for the facial hair, the acne, and the temporal recession of the hairline. On the other hand, the increase in muscle mass and the development of male sex drive and libido depend primarily on testosterone rather than DHT.

Congenital 5α-reductase deficiency, in which the gene for type 2 5α-reductase is mutated, is common in certain parts of the Dominican Republic. It produces an interesting form of male pseudohermaphroditism. Individuals with this syndrome are born with male internal genitalia including testes, but they have female external genitalia and are usually raised as girls. However, when they reach puberty, LH secretion and circulating testosterone levels are increased. Consequently, they develop male body contours and male libido. At this point, they usually change their gender identities and "become boys." Their clitorises enlarge ("penis-at-12 syndrome") to the point that some of them can have intercourse with women. This enlargement probably

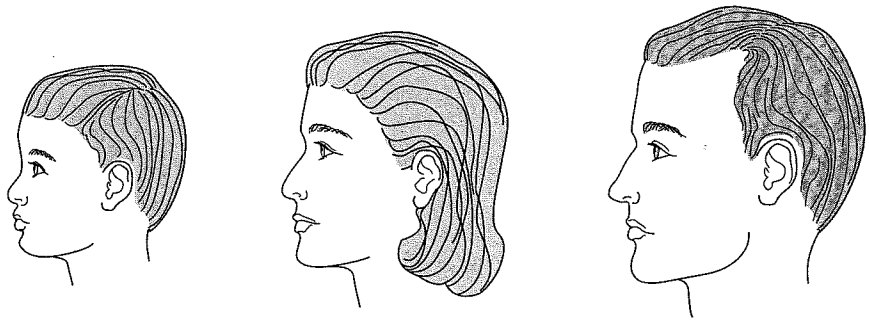


Figure 23–19. Hairline in children and adults. The hairline of the woman is like that of the child, whereas that of the man is indented in the lateral frontal region.

occurs because with the high LH, enough testosterone is produced to overcome the need for DHT amplification in the genitalia.

5α-Reductase-inhibiting drugs are now being used clinically to treat benign prostatic hyperplasia, and finasteride, the most extensively used drug, has its greatest effect on type 2 5α-reductase.

Testicular Production of Estrogens

Over 80% of the estradiol and 95% of the estrone in the plasma of adult men is formed by extragonadal and extraadrenal aromatization of circulating testosterone and androstenedione. The remainder comes from the testes. Some of the estradiol in testicular venous blood comes from the Leydig cells, but some is also produced by aromatization of androgens in Sertoli cells. In men, the plasma estradiol level is 20–50 pg/mL (73–184 pmol/L) and the total production rate is approximately 50 μg/d (184 nmol/d). In contrast to the situation in women, estrogen production moderately increases with advancing age in men.

CONTROL OF TESTICULAR FUNCTION

FSH is tropic to the Sertoli cells, and FSH and androgens maintain the gametogenic function of the testes. FSH also stimulates the secretion of ABP and inhibin. Inhibin feeds back to inhibit FSH secretion. LH is tropic to the Leydig cells and stimulates the secretion of testosterone, which in turn feeds back to inhibit LH secretion. Hypothalamic lesions in animals and hypothal-

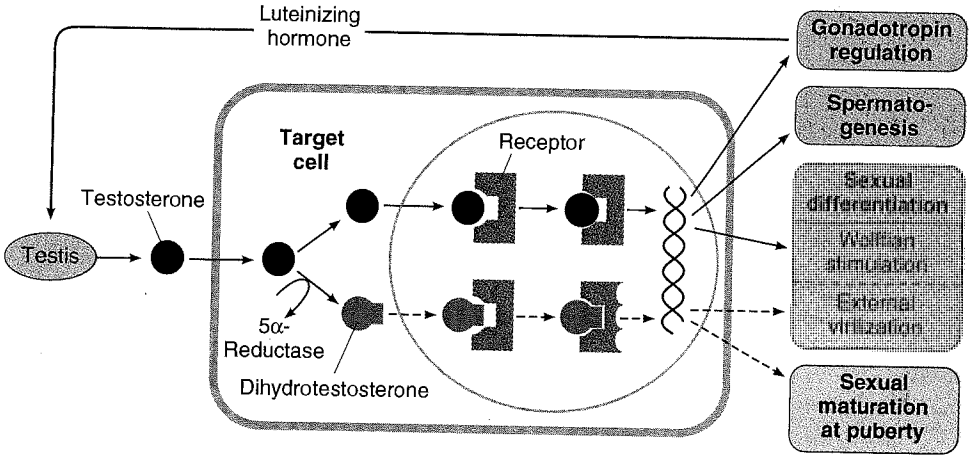


Figure 23–20. Schematic diagram of the actions of testosterone (solid arrows) and dihydrotestosterone (dashed arrows). Note that they both bind to the same receptor, but DHT binds more effectively. (Reproduced, with permission, from Wilson JD, Griffin JE, Russell W: Steroid 5a-reductase 2 deficiency. Endocr Rev 1993;14:577. Copyright © 1993 by The Endocrine Society.)

amic disease in humans lead to atrophy of the testes and loss of their function.

Inhibins

Testosterone reduces plasma LH, but except in large doses, it has no effect on plasma FSH. Plasma FSH is elevated in patients who have atrophy of the seminiferous tubules but normal levels of testosterone and LH secretion. These observations led to the search for **inhibin,** a factor of testicular origin that inhibits FSH secretion. There are two inhibins in extracts of testes in men and in antral fluid from ovarian follicles in women. They are formed from three polypeptide subunits: a glycosylated α subunit with a molecular weight of 18,000, and two nonglycosylated β subunits, β_A and β_B, each with a molecular weight of 14,000. The subunits are formed from precursor proteins (Figure 23–21). The α subunit combines with β_A to form a heterodimer and with β_B to form another heterodimer, with the subunits linked by disulfide bonds. Both $\alpha\beta_A$ (inhibin A) and $\alpha\beta_B$ (inhibin B) inhibit FSH secretion by a direct action on the pituitary, though it now appears that it is inhibin B that is the FSH-regulating inhibin in adult men and women. Inhibins are produced by Sertoli cells in males and granulosa cells in females.

The heterodimer $\beta_A\beta_B$ and the homodimers $\beta_A\beta_A$ and $\beta_B\beta_B$ are also formed. They stimulate rather than inhibit FSH secretion and consequently are called **activins.** Their function in reproduction is unsettled. However, the inhibins and activins are members of the TGFβ superfamily of dimeric growth factors that also includes MIS (see above). Two **activin receptors** have been cloned, and both appear to be serine kinases. Inhibins and activins are found not only in the gonads but also in the brain and many other tissues. In the bone marrow, activins are involved in the development of white blood cells. In embryonic life, activins are involved in the formation of mesoderm. All mice with a targeted deletion of the α-inhibin subunit gene initially grow in a normal fashion but then develop gonadal stromal tumors, so the gene is a tumor suppressor gene.

In plasma, α_2-macroglobulin binds activins and inhibins. In tissues, activins bind to a family of four glycoproteins called **follistatins.** Binding of the activins inactivates their biologic activity, but the relation of follistatins to inhibin and their physiologic function remain unsettled.

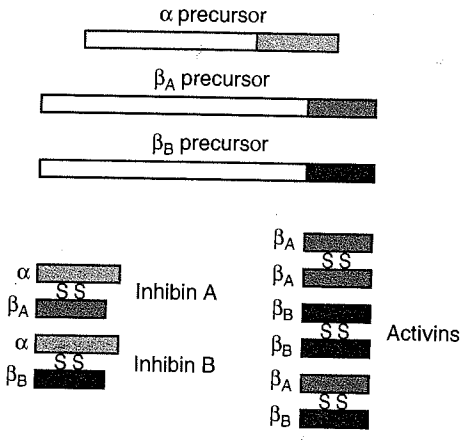


Figure 23–21. Inhibin precursor proteins and the various inhibins and activins that are formed from the carboxyl terminal regions of these precursors. SS, disulfide bonds.

Steroid Feedback

A current "working hypothesis" of the way the functions of the testes are regulated is shown in Figure 23–22. Castration is followed by a rise in the pituitary content and secretion of FSH and LH, and hypothala-

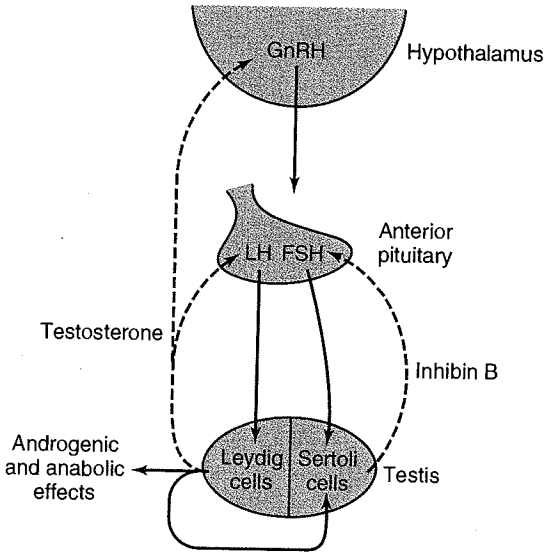


Figure 23–22. Postulated interrelationships between the hypothalamus, anterior pituitary, and testes. Solid arrows indicate excitatory effects; dashed arrows indicate inhibitory effects. Compare with Figures 18–12, 20–21, 22–10, and 23–35.

mic lesions prevent this rise. Testosterone inhibits LH secretion by acting directly on the anterior pituitary and by inhibiting the secretion of GnRH from the hypothalamus. Inhibin acts directly on the anterior pituitary to inhibit FSH secretion.

In response to LH, some of the testosterone secreted from the Leydig cells bathes the seminiferous epithelium and provides the high local concentration of androgen to the Sertoli cells that is necessary for normal spermatogenesis. Systemically administered testosterone does not raise the androgen level in the testes to as great a degree, and it inhibits LH secretion. Consequently, the net effect of systemically administered testosterone is generally a decrease in sperm count. Testosterone therapy has been suggested as a means of male contraception. However, the dose of testosterone needed to suppress spermatogenesis causes sodium and water retention. The possible use of inhibins as male contraceptives is now being explored.

ABNORMALITIES OF TESTICULAR FUNCTION

Cryptorchidism

The testes develop in the abdominal cavity and normally migrate to the scrotum during fetal development. **Testicular descent** to the inguinal region depends on MIS, and descent from the inguinal region to the scrotum depends on other factors. Descent is incomplete on one or, less commonly, both sides in 10% of newborn males, the testes remaining in the abdominal cavity or inguinal canal. Gonadotropic hormone treatment speeds descent in some cases, or the defect can be corrected surgically. Spontaneous descent of the testes is the rule, and the proportion of boys with undescended testes **(cryptorchidism)** falls to 2% at age 1 year and 0.3% after puberty. However, early treatment is now recommended despite these figures because the incidence of malignant tumors in is higher in undescended than in scrotal testes and because after puberty the higher temperature in the abdomen eventually causes irreversible damage to the spermatogenic epithelium.

Male Hypogonadism

The clinical picture of male hypogonadism depends on whether testicular deficiency develops before or after puberty. In adults, if it is due to testicular disease, circulating gonadotropin levels are elevated **(hypergonadotropic hypogonadism);** if it is secondary to disorders of the pituitary or the hypothalamus (eg, Kallmann's syndrome; see Chapter 14), circulating gonadotropin levels are depressed **(hypogonadotropic hypogonadism).** If the endocrine function of the testes is lost in adulthood, the secondary sex characteristics regress slowly because it takes very little androgen to maintain them once they are established. The growth of the larynx during adolescence is permanent, and the voice remains deep. Men castrated in adulthood suffer some loss of libido, although the ability to copulate persists for some time. They occasionally have hot flushes and are generally more irritable, passive, and depressed than men with intact testes. When the Leydig cell deficiency dates from childhood, the clinical picture is that of **eunuchoidism.** Eunuchoid individuals over the age of 20 are characteristically tall, although not as tall as hyperpituitary giants, because their epiphyses remain open and some growth continues past the normal age of puberty. They have narrow shoulders and small muscles, a body configuration resembling that of the adult female. The genitalia are small and the voice high-pitched. Pubic hair and axillary hair are present because of adrenocortical androgen secretion. However, the hair is sparse, and the pubic hair has the female "triangle with the base up" distribution rather than the "triangle with the base down" pattern (male escutcheon) seen in normal males.

Androgen-Secreting Tumors

"Hyperfunction" of the testes in the absence of tumor formation is not a recognized entity. Androgen-secreting Leydig cell tumors are rare and cause detectable endocrine symptoms only in prepubertal boys, who develop precocious pseudopuberty (Table 23–2).

THE FEMALE REPRODUCTIVE SYSTEM

THE MENSTRUAL CYCLE

The reproductive system of women (Figure 23–23), unlike that of men, shows regular cyclic changes that teleologically may be regarded as periodic preparations for fertilization and pregnancy. In humans and other primates, the cycle is a **menstrual** cycle, and its most conspicuous feature is the periodic vaginal bleeding that occurs with the shedding of the uterine mucosa **(menstruation).** The length of the cycle is notoriously variable in women, but an average figure is 28 days from the start of one menstrual period to the start of the

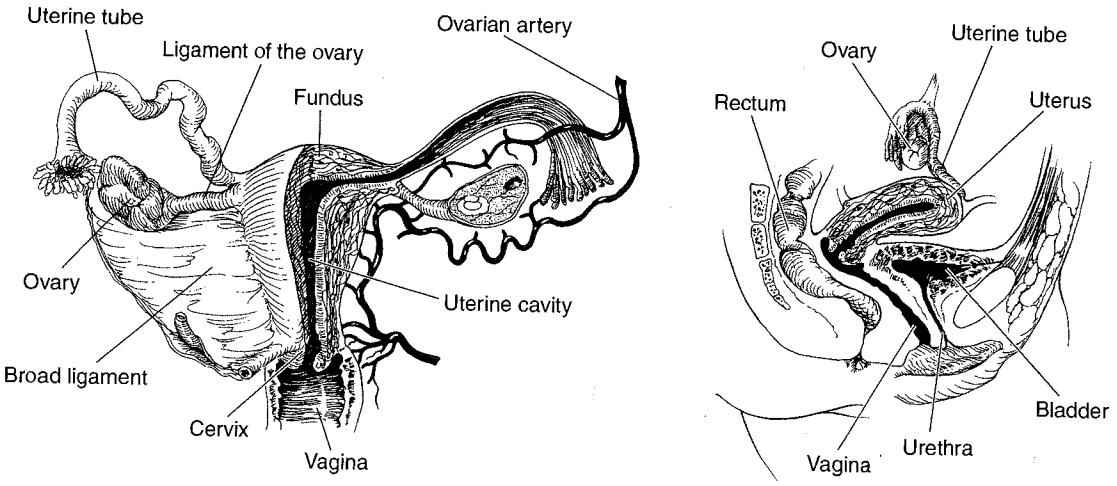


Figure 23–23. The female reproductive system.

next. By common usage, the days of the cycle are identified by number, starting with the first day of menstruation.

Ovarian Cycle

From the time of birth, there are many **primordial follicles** under the ovarian capsule. Each contains an immature ovum (Figure 23–24). At the start of each cycle, several of these follicles enlarge, and a cavity forms around the ovum **(antrum formation).** This cavity is filled with follicular fluid. In humans, usually one of the follicles in one ovary starts to grow rapidly on about the sixth day and becomes the **dominant follicle,** while the others regress, forming **atretic follicles.** The atretic process involves apoptosis. It is uncertain how one follicle is selected to be the dominant follicle in this **follicular phase** of the menstrual cycle, but it seems to be related to the ability of the follicle to secrete the estrogen inside it that is needed for final maturation. When women are given highly purified human pituitary gonadotropin preparations by injection, many follicles develop simultaneously.

The structure of a maturing ovarian **(graafian)** follicle is shown in Figure 23–24. The cells of the **theca interna** of the follicle are the primary source of circulating estrogens. However, the follicular fluid has a high estrogen content, and much of this estrogen comes from the granulosa cells (see below).

At about the 14th day of the cycle, the distended follicle ruptures, and the ovum is extruded into the abdominal cavity. This is the process of **ovulation.** The ovum is picked up by the fimbriated ends of the uterine tubes (oviducts). It is transported to the uterus and, unless fertilization occurs, on out through the vagina.

The follicle that ruptures at the time of ovulation promptly fills with blood, forming what is sometimes called a **corpus hemorrhagicum.** Minor bleeding from the follicle into the abdominal cavity may cause peritoneal irritation and fleeting lower abdominal pain ("mittelschmerz"). The granulosa and theca cells of the follicle lining promptly begin to proliferate, and the clotted blood is rapidly replaced with yellowish, lipid-rich **luteal cells,** forming the **corpus luteum.** This initiates the **luteal phase** of the menstrual cycle, during which the luteal cells secrete estrogens and progesterone. Growth of the corpus luteum depends on its developing an adequate blood supply, and there is evidence that VEGF (see Chapter 30) is essential for this process.

If pregnancy occurs, the corpus luteum persists and usually there are no more periods until after delivery. If pregnancy does not occur, the corpus luteum begins to degenerate about 4 days before the next menses (24th day of the cycle) and is eventually replaced by scar tissue, forming a **corpus albicans.**

The ovarian cycle in other mammals is similar, except that in many species more than one follicle ovulates and multiple births are the rule. Corpora lutea form in some submammalian species but not in others.

In humans, no new ova are formed after birth. During fetal development, the ovaries contain over 7 million primordial follicles. However, many undergo atresia (involution) before birth and others are lost after birth. At the time of birth, there are 2 million ova, but 50% of these are atretic. The million that are normal

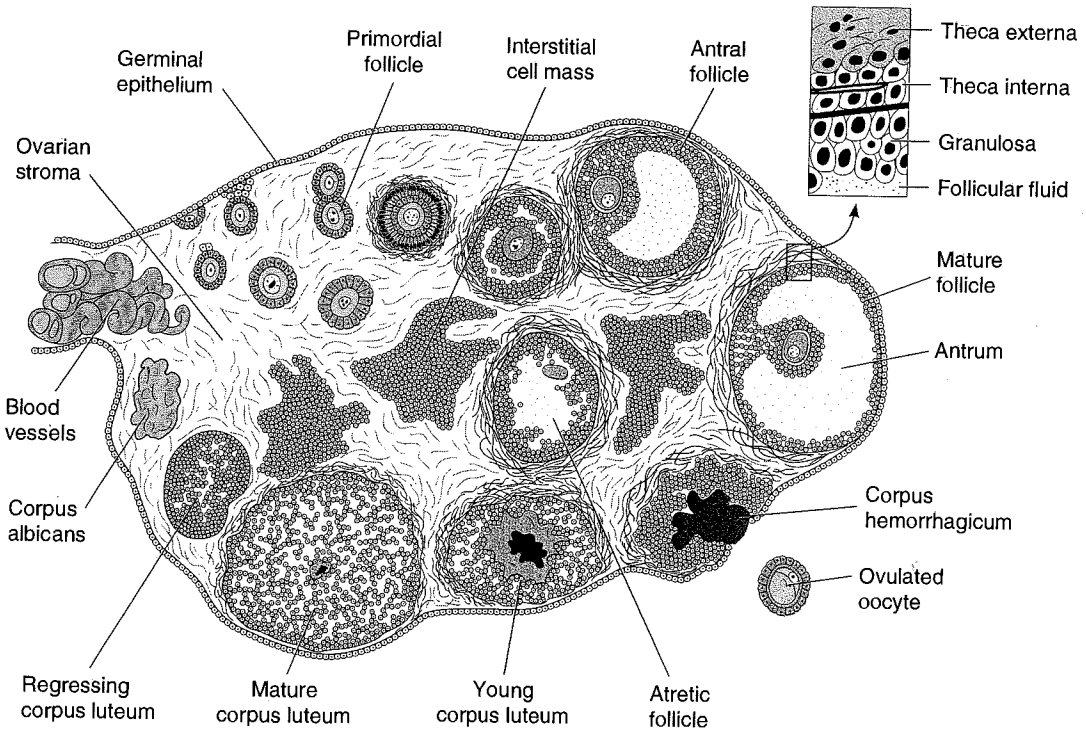


Figure 23–24. Diagram of a mammalian ovary, showing the sequential development of a follicle, formation of a corpus luteum, and, in the center, follicular atresia. A section of the wall of a mature follicle is enlarged at the upper right. The interstitial cell mass is not prominent in primates. (Reproduced, with permission, from Gorbman A, Bern H: *Textbook of Comparative Endocrinology*. Wiley, 1962.)

undergo the first part of the first meiotic division at about this time and enter a stage of arrest in prophase in which those that survive persist until adulthood. Atresia continues during development, and the number of ova in both of the ovaries at the time of puberty is less than 300,000 (Figure 23–11). Only one of these ova per cycle (or about 500 in the course of a normal reproductive life) normally reaches maturity; the remainder degenerate. Just before ovulation, the first meiotic division is completed. One of the daughter cells, the **secondary oocyte,** receives most of the cytoplasm, while the other, the **first polar body,** fragments and disappears. The secondary oocyte immediately begins the second meiotic division, but this division stops at metaphase and is completed only when a sperm penetrates the oocyte. At that time, the **second polar body** is cast off and the fertilized ovum proceeds to form a new individual. The arrest in metaphase is due, at least in some species, to formation in the ovum of the protein **pp39**^{mos}, which is encoded by the ***c-mos*** protooncogene. When fertilization occurs, the pp39^{mos} is destroyed within 30 minutes by **calpain,** a calcium-dependent cysteine protease.

Uterine Cycle

At the end of menstruation, all but the deep layers of the endometrium have sloughed. A new endometrium then regrows under the influence of estrogens from the developing follicle. The endometrium increases rapidly in thickness from the fifth to the fourteenth days of the menstrual cycle. As the thickness increases, the uterine glands are drawn out so that they lengthen (Figure 23–25), but they do not become convoluted or secrete to any degree. These endometrial changes are called proliferative, and this part of the menstrual cycle is sometimes called the **proliferative phase.** It is also called the preovulatory or follicular phase of the cycle. After ovulation, the endometrium becomes more highly vascularized and slightly edematous under the influence of estrogen and progesterone from the corpus luteum. The glands become coiled and tortuous (Figure

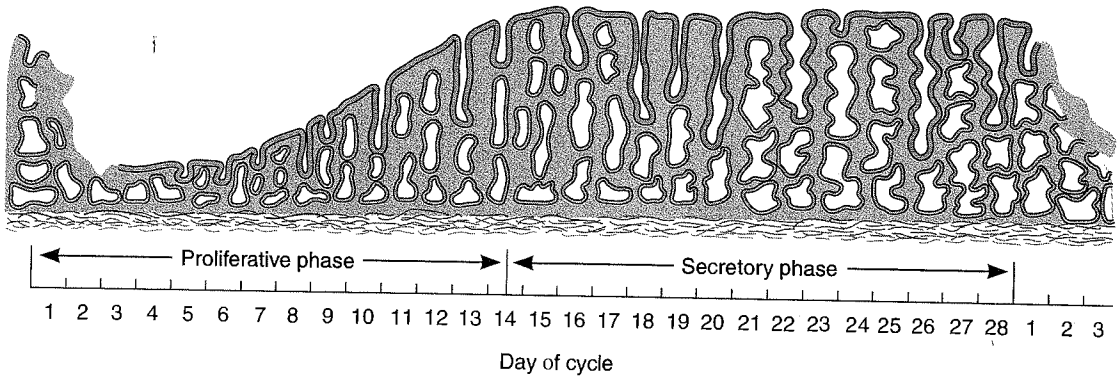


Figure 23–25. Changes in the endometrium during the menstrual cycle.

23–25), and they begin to secrete a clear fluid. Consequently, this phase of the cycle is called the **secretory** or **luteal phase.** Late in the luteal phase, the endometrium, like the anterior pituitary, produces prolactin, but the function of this endometrial prolactin is unknown.

The endometrium is supplied by two types of arteries. The superficial two thirds of the endometrium that is shed during menstruation, the **stratum functionale,** is supplied by long, coiled **spiral arteries** (Figure 23–26), whereas the deep layer that is not shed, the **stratum basale,** is supplied by short, straight **basilar arteries.**

When the corpus luteum regresses, hormonal support for the endometrium is withdrawn. The endometrium becomes thinner, which adds to the coiling of the spiral arteries. Foci of necrosis appear in the endometrium, and these coalesce. In addition spasm and degeneration of the walls of the spiral arteries take place, leading to spotty hemorrhages that become confluent and produce the menstrual flow.

The vasospasm is probably produced by locally released prostaglandins. Large quantities of prostaglandins are present in the secretory endometrium and in menstrual blood, and infusions of $PGF_{2\alpha}$ produce endometrial necrosis and bleeding.

From the point of view of endometrial function, the proliferative phase of the menstrual cycle represents restoration of the epithelium from the preceding menstruation, and the secretory phase represents preparation of the uterus for implantation of the fertilized ovum. The length of the secretory phase is remarkably constant at about 14 days, and the variations seen in the length of the menstrual cycle are due for the most part to variations in the length of the proliferative phase. When fertilization fails to occur during the secretory phase, the endometrium is shed and a new cycle starts.

Normal Menstruation

Menstrual blood is predominantly arterial, with only 25% of the blood being of venous origin. It contains tissue debris, prostaglandins, and relatively large amounts of fibrinolysin from endometrial tissue. The fibrinolysin lyses clot, so that menstrual blood does not normally contain clots unless the flow is excessive.

The usual duration of the menstrual flow is 3–5 days, but flows as short as 1 day and as long as 8 days can occur in normal women. The amount of blood lost may range normally from slight spotting to 80 mL; the average amount lost is 30 mL. Loss of more than 80 mL is abnormal. Obviously, the amount of flow can be affected by various factors, including the thickness of the endometrium, medication, and diseases that affect the clotting mechanism.

Anovulatory Cycles

In some instances, ovulation fails to occur during the menstrual cycle. Such anovulatory cycles are common for the first 12–18 months after menarche and again before the onset of the menopause. When ovulation does not occur, no corpus luteum is formed and the effects of progesterone on the endometrium are absent. Estrogens continue to cause growth, however, and the proliferative endometrium becomes thick enough to break down and begins to slough. The time it takes for bleeding to occur is variable, but it usually occurs in less than 28 days from the last menstrual period. The flow is also variable and ranges from scanty to relatively profuse.

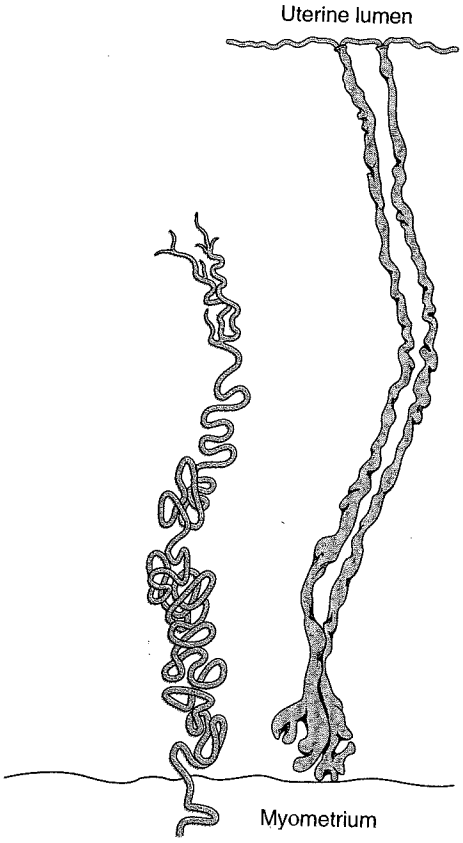


Figure 23–26. Spiral artery of endometrium. Drawing of a spiral artery **(left)** and two uterine glands **(right)** from the endometrium of a rhesus monkey; early secretory phase. (Reproduced, with permission, from Daron GH: The arterial pattern of the tunica mucosa of the uterus in the *Macacus rhesus*. Am J Anat 1936;58:349.)

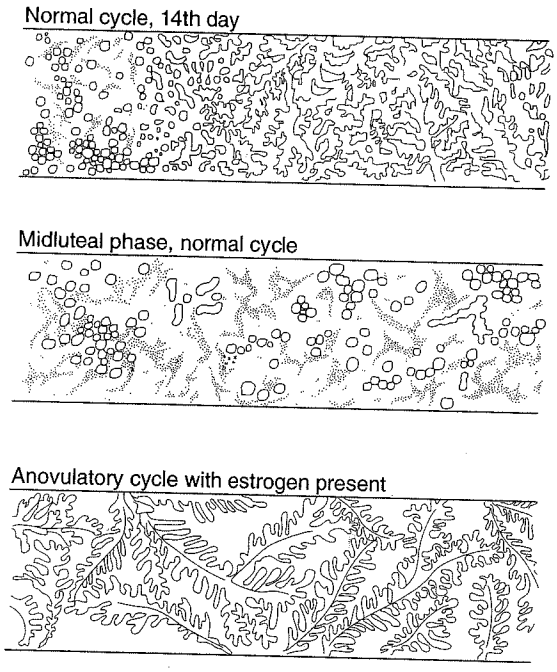


Figure 23–27. Patterns formed when cervical mucus is smeared on a slide, permitted to dry, and examined under the microscope. Progesterone makes the mucus thick and cellular. In the smear from a patient who failed to ovulate **(bottom),** no progesterone is present to inhibit the estrogen-induced fern pattern.

Cyclic Changes in the Uterine Cervix

Although it is continuous with the body of the uterus, the cervix of the uterus is different in a number of ways. The mucosa of the uterine cervix does not undergo cyclic desquamation, but there are regular changes in the cervical mucus. Estrogen makes the mucus thinner and more alkaline, changes that promote the survival and transport of sperms. Progesterone makes it thick, tenacious, and cellular. The mucus is thinnest at the time of ovulation, and its elasticity, or **spinnbarkeit,** increases so that by midcycle, a drop can be stretched into a long, thin thread that may be 8–12 cm or more in length. In addition, it dries in an arborizing, fern-like pattern (Figure 23–27) when a thin layer is spread on a slide. After ovulation and during pregnancy, it becomes thick and fails to form the fern pattern.

Vaginal Cycle

Under the influence of estrogens, the vaginal epithelium becomes cornified, and cornified epithelial cells can be identified in the vaginal smear. Under the influence of progesterone, a thick mucus is secreted, and the epithelium proliferates and becomes infiltrated with leukocytes. The cyclic changes in the vaginal smear in rats are relatively marked. The changes in humans and other species are similar but not so clear-cut.

Cyclic Changes in the Breasts

Although lactation normally does not occur until the end of pregnancy, cyclic changes take place in the breasts during the menstrual cycle. Estrogens cause proliferation of mammary ducts, whereas progesterone causes growth of lobules and alveoli. The breast swelling, tenderness, and pain experienced by many

women during the 10 days preceding menstruation are probably due to distention of the ducts, hyperemia, and edema of the interstitial tissue of the breast. All these changes regress, along with the symptoms, during menstruation.

Changes During Intercourse

During sexual excitement in women, fluid is secreted onto the vaginal walls, probably because of release of VIP from vaginal nerves. A lubricating mucus is also secreted by the vestibular glands. The upper part of the vagina is sensitive to stretch, while tactile stimulation from the labia minora and clitoris adds to the sexual excitement. These stimuli are reinforced by tactile stimuli from the breasts and, as in men, by visual, auditory, and olfactory stimuli, which may build to the crescendo known as orgasm. During orgasm, autonomically mediated rhythmic contractions occur in the vaginal walls. Impulses also travel via the pudendal nerves and produce rhythmic contraction of the bulbocavernosus and ischiocavernosus muscles. The vaginal contractions may aid sperm transport but are not essential for it, since fertilization of the ovum is not dependent on orgasm.

Indicators of Ovulation

Knowing when during the menstrual cycle ovulation occurs is important in increasing fertility or, conversely, in family planning. A convenient and reasonably reliable indicator of the time of ovulation is a change—usually a rise—in the basal body temperature (Figure 23–28). The rise starts 1–2 days after ovulation. Women interested in obtaining an accurate temperature chart should use a thermometer with wide gradations and take their temperatures (oral or rectal) in the morning before getting out of bed. The cause of the temperature change at the time of ovulation is probably the increase in progesterone secretion, since progesterone is thermogenic (see Chapter 14).

A surge in LH secretion triggers ovulation (see below), and ovulation normally occurs about 9 hours after the peak of the LH surge at midcycle (Figure 23–28). The ovum lives for approximately 72 hours after it is extruded from the follicle, but it is fertilizable for a much shorter time than this. In a study of the relation of isolated intercourse to pregnancy, 36% of women had a detected pregnancy following intercourse on the day of ovulation, but with intercourse on days after ovulation, the percentage was zero. Isolated intercourse on the first and second day before ovulation also led to pregnancy in about 36% of women. A few pregnancies resulted from isolated intercourse on day 3, 4, or 5 before ovulation, although the percentage was much lower, eg, 8% on day 5 before ovulation. Thus, some sperms can survive in the female genital tract and fertilize the ovum for up to 120 hours before ovulation, but the most fertile period is clearly the 48 hours before ovulation. However, for those interested in the "rhythm method" of contraception, it should be noted that there are rare but documented cases in the literature of pregnancy resulting from isolated coitus on every day of the cycle.

The Estrous Cycle

Mammals other than primates do not menstruate, and their sexual cycle is called an **estrous cycle.** It is named for the conspicuous period of "heat" (**estrus**) at the time of ovulation, normally the only time during which the sexual interest of the female is aroused (see Chapter 15). In spontaneously ovulating species with estrous cycles, such as the rat, no episodic vaginal bleeding occurs but the underlying endocrine events are essentially the same as those in the menstrual cycle. In other species, ovulation is induced by copulation (reflex ovulation).

OVARIAN HORMONES

Chemistry, Biosynthesis, & Metabolism of Estrogens

The naturally occurring estrogens are **17β-estradiol, estrone,** and **estriol** (Figure 23–29). They are C18 steroids (see Figure 20–7) which do not have an angular methyl group attached to the 10 position or a Δ^4-3-keto configuration in the A ring. They are secreted primarily by the granulosa cells of the ovarian follicles, the corpus luteum, and the placenta. Their biosynthesis depends on the enzyme **aromatase** (CYP19), which converts testosterone to estradiol and androstenedione to estrone (Figure 23–29). The latter reaction also occurs in fat, liver, muscle, and the brain.

Theca interna cells have many LH receptors, and LH acts via cAMP to increase conversion of cholesterol to androstenedione. Some of the androstenedione is converted to estradiol, which enters the circulation. The theca interna cells also supply androstenedione to the granulosa cells. The granulosa cells make estradiol when provided with androgens (Figure 23–30), and it appears that the estradiol they form in primates is secreted into the follicular fluid. Granulosa cells have many FSH receptors, and FSH facilitates their secretion of estradiol by acting via cAMP to increase their aromatase activity. Mature granulosa cells also acquire LH receptors, and LH also stimulates estradiol production.

Two percent of the circulating estradiol is free, and the remainder is bound to protein: 60% to albumin

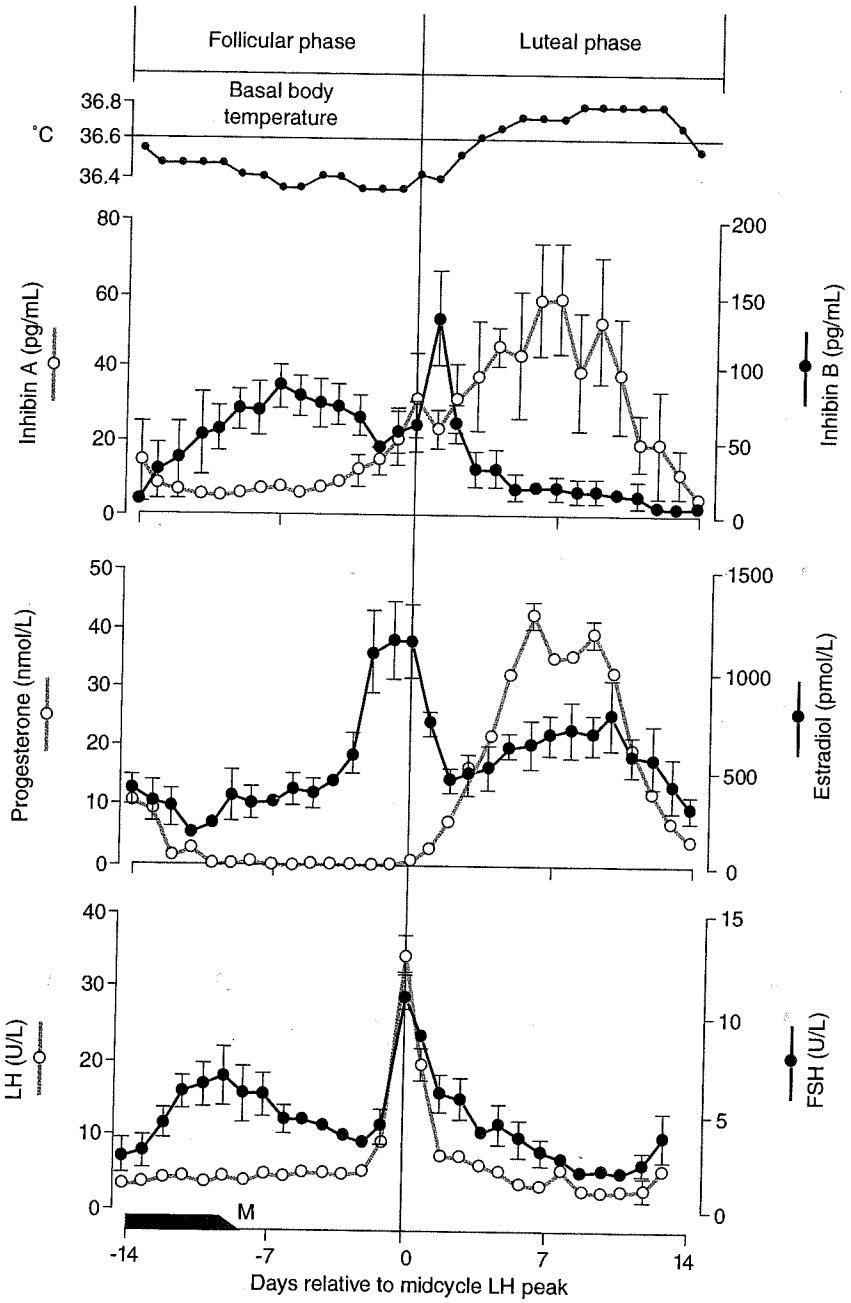


Figure 23–28. Basal body temperature and plasma hormone concentrations (mean ± standard error) during the normal human menstrual cycle. Values are aligned with respect to the day of the midcycle LH peak.

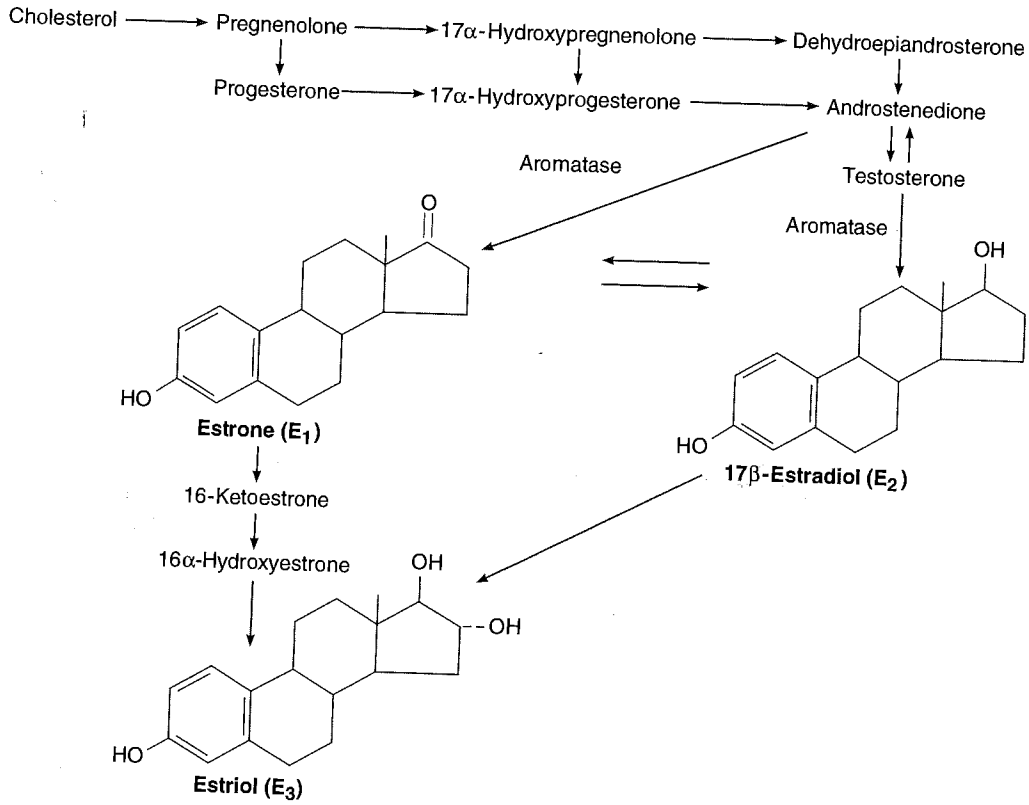


Figure 23–29. Biosynthesis and metabolism of estrogens. The formulas of the precursor steroids are shown in Figure 20–8.

and 38% to the same gonadal steroid-binding globulin (GBG) that binds testosterone (Table 23–5).

In the liver, estradiol, estrone, and estriol are converted to glucuronide and sulfate conjugates. All these compounds, along with other metabolites, are excreted in the urine. Appreciable amounts are secreted in the bile and reabsorbed into the bloodstream (enterohepatic circulation).

Secretion

The concentration of estradiol in the plasma during the menstrual cycle is shown in Figure 23–28. Almost all of this estrogen comes from the ovary, and two peaks of secretion occur: one just before ovulation and one during the midluteal phase. The estradiol secretion rate is 36 μg/d (133 nmol/d) in the early follicular phase,

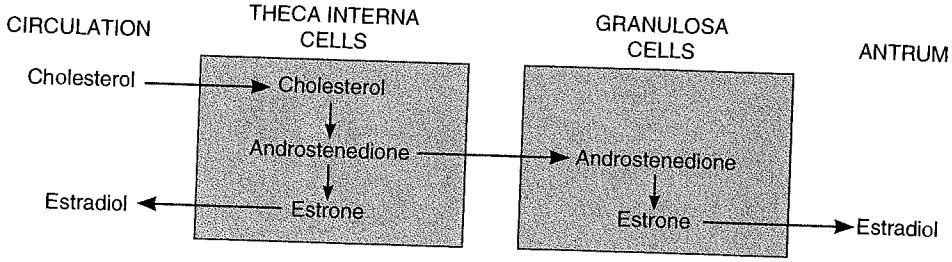


Figure 23–30. Interactions between theca and granulosa cells in estradiol synthesis and secretion.

380 µg/d just before ovulation, and 250 µg/d during the midluteal phase (Table 23–7). After menopause, estrogen secretion declines to low levels.

As noted above, the estradiol production rate in men is about 50 µg/d (184 nmol/d).

Effects on the Female Genitalia

Estrogens facilitate the growth of the ovarian follicles and increase the motility of the uterine tubes. Their role in the cyclic changes in the endometrium, cervix, and vagina is discussed above. They increase uterine blood flow and have important effects on the smooth muscle of the uterus. In immature and castrate females, the uterus is small and the myometrium atrophic and inactive. Estrogens increase the amount of uterine muscle and its content of contractile proteins. Under the influence of estrogens, the muscle becomes more active and excitable, and action potentials in the individual fibers become more frequent (see Chapter 3). The "estrogen-dominated" uterus is also more sensitive to oxytocin.

Chronic treatment with estrogens causes the endometrium to hypertrophy. When estrogen therapy is discontinued, sloughing takes place with **withdrawal bleeding.** Some "breakthrough" bleeding may occur during treatment when estrogens are given for long periods.

Effects on Endocrine Organs

Estrogens decrease FSH secretion. Under some circumstances, they inhibit LH secretion (negative feedback); in other circumstances, they increase LH secretion (positive feedback; see below). Women are sometimes given large doses of estrogens for 4–6 days to prevent conception after coitus during the fertile period (postcoital or "morning-after" contraception). However, in this instance, pregnancy is probably prevented by interference with implantation of the fertilized ovum rather than changes in gonadotropin secretion.

Estrogens cause increased secretion of angiotensinogen (see Chapter 24) and rhyroid-binding globulin (see Chapter 18). They exert an important protein anabolic effect in chickens and cattle, possibly by stimulating the secretion of androgens from the adrenal, and estrogen treatment has been used commercially to increase the weight of domestic animals. They cause epiphysial closure in humans.

Effects on the CNS

The estrogens are responsible for estrous behavior in animals, and they increase libido in humans. They apparently exert this action by a direct effect on certain neurons in the hypothalamus (Figure 23–31). The relation of estrogens, progesterone, and androgens to sexual behavior is discussed in Chapter 15. Estrogens increase the proliferation of dendrites on neurons and the number of synaptic knobs in rats.

Effects on the Breasts

Estrogens produce duct growth in the breasts and are largely responsible for breast enlargement at puberty in girls; they have been called the growth hormones of the breast. They are responsible for the pigmentation of the areolas, although pigmentation usually becomes more intense during the first pregnancy than it does at puberty. The role of the estrogens in the overall control of breast growth and lactation is discussed below.

Female Secondary Sex Characteristics

The body changes that develop in girls at puberty—in addition to enlargement of breasts, uterus, and vagina—are due in part to estrogens, which are the "feminizing hormones," and in part simply to the absence of testicular androgens. Women have narrow shoulders and

Table 23–7. Twenty-four-hour production rates of sex steroids in women at different stages of the menstrual cycle.

Sex Steroids	Early Follicular	Preovulatory	Midluteal
Progesterone (mg)	1.0	4.0	25.0
17-Hydroxyprogesterone (mg)	0.5	4.0	4.0
Dehydroepiandrosterone (mg)	7.0	7.0	7.0
Androstenedione (mg)	2.6	4.7	3.4
Testosterone (µg)	144.0	171.0	126.0
Estrone (µg)	50.0	350.0	250.0
Estradiol (µg)	36.0	380.0	250.0

Modified and reproduced, with permission, from Yen SSC, Jaffe RB, Barbieri RL: *Reproductive Endocrinology,* 4th ed. Saunders, 1999.

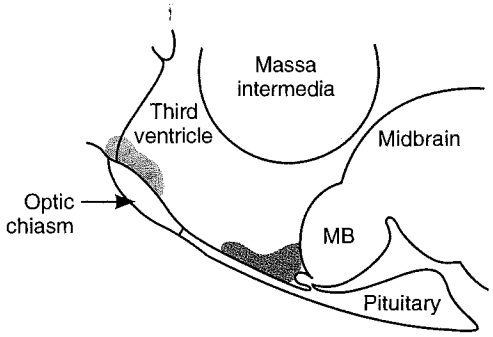


Figure 23–31. Loci where implantations of estrogen in the hypothalamus affect ovarian weight and sexual behavior in rats, projected on a sagittal section of the hypothalamus. The implants that stimulate sex behavior are located in the suprachiasmatic area above the optic chiasm (gray area), whereas ovarian atrophy is produced by implants in the arcuate nucleus and surrounding ventral hypothalamus (colored area). MB, mamillary body.

broad hips, thighs that converge, and arms that diverge (wide **carrying angle**). This body configuration, plus the female distribution of fat in the breasts and buttocks, is seen also in castrate males. In women, the larynx retains its prepubertal proportions and the voice stays high-pitched. Women have less body hair and more scalp hair, and the pubic hair generally has a characteristic flat-topped pattern (female escutcheon). However, growth of pubic and axillary hair in both sexes is due primarily to androgens rather than estrogens.

Other Actions

Normal women retain salt and water and gain weight just before menstruation. Estrogens cause some degree of salt and water retention. However, aldosterone secretion is slightly elevated in the luteal phase, and this also contributes to the premenstrual fluid retention.

Estrogens are said to make sebaceous gland secretions more fluid and thus to counter the effect of testosterone and inhibit formation of **comedones** ("blackheads") and acne. The liver palms, spider angiomas, and slight breast enlargement seen in advanced liver disease are due to increased circulating estrogens. The increase appears to be due to decreased hepatic metabolism of androstenedione, making more of this androgen available for conversion to estrogens.

Estrogens have a significant plasma cholesterol-lowering action (see Chapter 17), and they rapidly produce vasodilation by increasing the local production of NO. However, it is uncertain whether they prevent heart attacks and strokes. Their action on bone is discussed in Chapter 21.

Mechanism of Action

There are two principal types of nuclear estrogen receptors: estrogen receptor α (ERα) encoded by a gene on chromosome 6; and estrogen receptor β (ERβ), encoded by a gene on chromosome 14. Both are members of the nuclear receptor superfamily (see Chapter 1). After binding estrogen, they form homodimers and bind to DNA, altering its transcription. Some tissues contain one type or the other, but overlap also occurs, with some tissues containing both ERα and ERβ. ERα is found primarily in the uterus, kidneys, liver, and heart, whereas ERβ is found primarily in the ovaries, prostate, lungs, gastrointestinal tract, hemopoietic system, and CNS. They also form heterodimers with ERα binding to ERβ. Male and female mice in which the gene for ERα has been knocked out are sterile, develop osteoporosis, and continue to grow because their epiphyses do not close. ERβ female knockouts are infertile, but ERβ male knockouts are fertile even though they have hyperplastic prostates and loss of fat. Both receptors exist in isoforms, and like thyroid receptors, can bind to various activating and stimulating factors. In some situations ERβ can inhibit ERα transcription. Thus, their actions are complex, multiple, and varied.

Most of the effects of estrogens are genomic, ie, due to actions on the nucleus, but some are so rapid that it is difficult to believe they are mediated via production of mRNAs. These include effects on neuronal discharge in the brain and, possibly, feedback effects on gonadotropin secretion. Evidence is accumulating that these effects are mediated by cell membrane receptors that appear to be structurally related to the nuclear receptors and produce their effects by intracellular mitogen-activated protein kinase pathways. Similar rapid effects of progesterone, testosterone, glucocorticoids, aldosterone, and 1,25-dihydroxycholecalciferol may also be produced by membrane receptors.

Synthetic and Environmental Estrogens

The ethinyl derivative of estradiol is a potent estrogen and—unlike the naturally occurring estrogens—is relatively active when given by mouth, because it is resistant to hepatic metabolism. The activity of the naturally occurring hormones is low when they are administered by mouth because the portal venous drainage of the intestine carries them to the liver, where they are inactivated before they can reach the general circulation. Some nonsteroidal substances and a few compounds found in plants have estrogenic activity. The plant estrogens are rarely a problem in human nu-

trition, but they may cause undesirable effects in farm animals. **Dioxins,** which are found in the environment and are produced by a variety of industrial processes, can activate estrogen response elements on genes. However, they have been reported to have antiestrogenic as well as estrogenic effects, and their role, if any, in the production of human disease remains a matter of disagreement and debate.

Because natural estrogens have undesirable as well as desirable effects (eg, they preserve bone in osteoporosis but can cause uterine and breast cancer), there has been an active search for "tailor-made" estrogens that have selective effects in humans. Two compounds, **tamoxifen** and **raloxifene**, show promise in this regard. Neither combats the symptoms of menopause, but both have the bone-preserving effects of estradiol. In addition, tamoxifen does not stimulate the breast, and raloxifene does not stimulate the breast or uterus. The way the effects of these selective estrogen receptor modulators (**SERMs**) are brought about is related to the complexity of the estrogen receptors (see above) and hence to differences in the way receptor–ligand complexes they form bind to DNA.

Chemistry, Biosynthesis, & Metabolism of Progesterone

Progesterone is a C_{21} steroid (Figure 23–32) secreted by the corpus luteum, the placenta, and (in small amounts) the follicle. It is an important intermediate in steroid biosynthesis in all tissues that secrete steroid hormones, and small amounts apparently enter the circulation from the testes and adrenal cortex. About 2% of the circulating progesterone is free (Table 23–5), whereas 80% is bound to albumin and 18% is bound to corticosteroid-binding globulin (see Chapter 20). Progesterone has a short half-life and is converted in the liver to pregnanediol, which is conjugated to glucuronic acid and excreted in the urine.

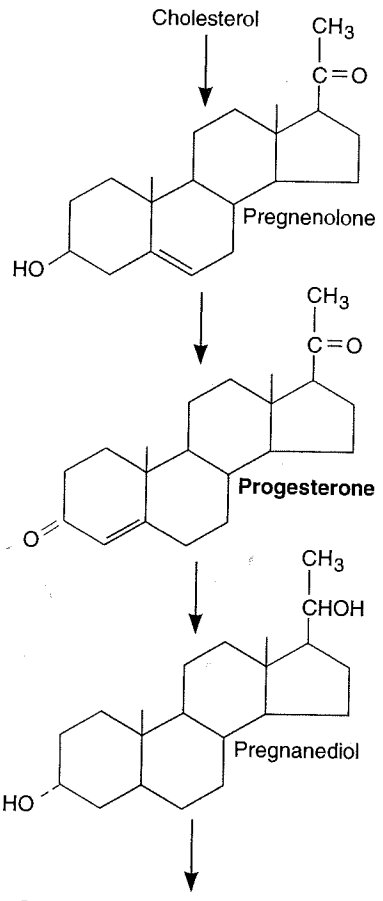


Figure 23–32. Biosynthesis of progesterone and major pathway for its metabolism. Other metabolites are also formed.

Secretion

In men, the plasma progesterone level is approximately 0.3 ng/mL (1 nmol/L). In women, the level is approximately 0.9 ng/mL (3 nmol/L) during the follicular phase of the menstrual cycle (Figure 23–28). The difference is due to secretion of small amounts of progesterone by cells in the ovarian follicles; theca cells provide pregnenolone to the granulosa cells, which convert it to progesterone. Late in the follicular phase, progesterone secretion begins to increase. During the luteal phase, the corpus luteum produces large quantities of progesterone (Table 23–7) and plasma progesterone is markedly increased to a peak value of approximately 18 ng/mL (60 nmol/L).

The stimulating effect of LH on progesterone secretion by the corpus luteum is due to activation of adenylyl cyclase and involves a subsequent step that is dependent on protein synthesis.

Actions

The principal target organs of progesterone are the uterus, the breasts, and the brain. Progesterone is responsible for the progestational changes in the endometrium and the cyclic changes in the cervix and vagina described above. It has an antiestrogenic effect on the myometrial cells, decreasing their excitability, their sensitivity to oxytocin, and their spontaneous electrical activity while increasing their membrane potential. It also decreases the number of estrogen receptors

in the endometrium and increases the rate of conversion of 17β-estradiol to less active estrogens.

In the breast, progesterone stimulates the development of lobules and alveoli. It induces differentiation of estrogen-prepared ductal tissue and supports the secretory function of the breast during lactation.

The feedback effects of progesterone are complex and are exerted at both the hypothalamic and pituitary levels. Large doses of progesterone inhibit LH secretion and potentiate the inhibitory effect of estrogens, preventing ovulation.

Progesterone is thermogenic and is probably responsible for the rise in basal body temperature at the time of ovulation. It stimulates respiration, and the alveolar P_{CO_2} (see Chapter 34) in women during the luteal phase of the menstrual cycle is lower than that in men. In pregnancy, the P_{CO_2} falls as progesterone secretion rises. However, the physiologic significance of this respiratory response is unknown.

Large doses of progesterone produce natriuresis, probably by blocking the action of aldosterone on the kidney. The hormone does not have a significant anabolic effect.

Mechanism of Action

The effects of progesterone, like those of other steroids, are brought about by an action on DNA to initiate synthesis of new mRNA. As noted in Chapter 1, the progesterone receptor is bound to a heat shock protein in the absence of the steroid, and progesterone binding releases the heat shock protein, exposing the DNA-binding domain of the receptor. The synthetic steroid **mifepristone (RU 486)** binds to the receptor but does not release the heat shock protein, and it blocks the binding of progesterone. Since the maintenance of early pregnancy depends on the stimulatory effect of progesterone on endometrial growth and its inhibition of uterine contractility, mifepristone causes abortion. In some countries, mifepristone combined with a prostaglandin is used to produce elective abortions.

There are two isoforms of the progesterone receptor—PR_A and PR_B—produced by differential processing from a single gene. PR_A is a truncated form, but it is likely that both isoforms mediate unique subsets of progesterone action.

Substances that mimic the action of progesterone are sometimes called **progestational agents, gestagens,** or **progestins.** They are used along with synthetic estrogens as oral contraceptive agents (see below).

Relaxin

Relaxin is a polypeptide hormone that is produced in the corpus luteum, uterus, placenta, and mammary glands in women and in the prostate gland in men. During pregnancy, it relaxes the pubic symphysis and other pelvic joints and softens and dilates the uterine cervix. Thus, it facilitates delivery. It also inhibits uterine contractions and may play a role in the development of the mammary glands. In nonpregnant women, relaxin is found in the corpus luteum and the endometrium during the secretory but not the proliferative phase of the menstrual cycle. Its function in nonpregnant women is unknown. In men, it is found in semen, where it may help maintain sperm motility and aid in sperm penetration of the ovum.

In most species there is only one relaxin gene, but in humans there are two genes on chromosome 9 that code for two structurally different polypeptides which both have relaxin activity. However, only one of these genes is active in the ovary and the prostate. The structure of the polypeptide produced in these two tissues is shown in Figure 23–33.

CONTROL OF OVARIAN FUNCTION

FSH from the pituitary is responsible for the early maturation of the ovarian follicles, and FSH and LH together are responsible for their final maturation. A burst of LH secretion (Figure 23–28) is responsible for ovulation and the initial formation of the corpus luteum. A smaller midcycle burst of FSH secretion also occurs, the significance of which is uncertain. LH stimulates the secretion of estrogen and progesterone from the corpus luteum.

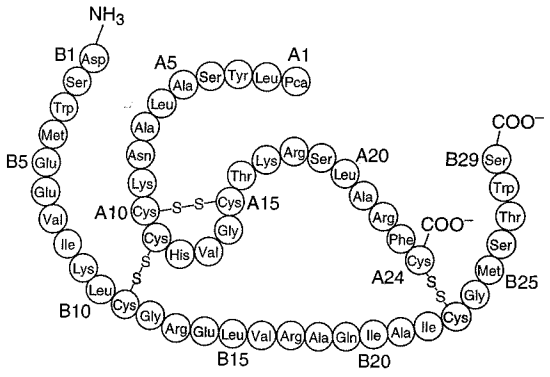


Figure 23–33. Structure of human luteal and seminal relaxin. Note the similarity to the structure of insulin, IGF-I, and IGF-II (see Figure 22–7). Pca, pyroglutamic acid. (Modified and reproduced, with permission, from Winslow JW et al: Human seminal relaxin is a product of the same gene as human luteal relaxin. Endocrinology 1992;130:2660. Copyright © 1992 by The Endocrine Society.)

Hypothalamic Components

The hypothalamus occupies a key position in the control of gonadotropin secretion. Hypothalamic control is exerted by GnRH secreted into the portal hypophysial vessels (see Chapter 14). GnRH stimulates the secretion of FSH as well as LH, and it is unlikely that there is an additional separate FRH.

GnRH is normally secreted in episodic bursts, and these bursts produce the circhoral peaks of LH secretion. They are essential for normal secretion of gonadotropins. If GnRH is administered by constant infusion, the GnRH receptors in the anterior pituitary down-regulate (see Chapter 1) and LH secretion declines to zero. However, if GnRH is administered episodically at a rate of one pulse per hour, LH secretion is stimulated. This is true even when endogenous GnRH secretion has been prevented by a lesion of the ventral hypothalamus.

It is now clear not only that episodic secretion of GnRH is a general phenomenon but also that fluctuations in the frequency and amplitude of the GnRH bursts are important in generating the other hormonal changes that are responsible for the menstrual cycle. Frequency is increased by estrogens and decreased by progesterone and testosterone. The frequency increases late in the follicular phase of the cycle, culminating in the LH surge. During the secretory phase, the frequency decreases as a result of the action of progesterone (Figure 23–34), but when estrogen and progesterone secretion decrease at the end of the cycle, the frequency once again increases.

At the time of the midcycle LH surge, the sensitivity of the gonadotropes to GnRH is greatly increased because of their exposure to GnRH pulses of the frequency that exist at this time. This self-priming effect of GnRH is important in producing a maximum LH response.

The nature and the exact location of the GnRH pulse generator in the hypothalamus are still unsettled. However, it is known in a general way that norepinephrine and possibly epinephrine in the hypothalamus increase GnRH pulse frequencies. Conversely, opioid peptides such as the enkephalins and β-endorphin reduce the frequency of GnRH pulses.

The down-regulation of pituitary receptors and the consequent decrease in LH secretion produced by constantly elevated levels of GnRH has led to the use of long-acting GnRH analogs to inhibit LH secretion in precocious puberty and in cancer of the prostate (see below).

Feedback Effects

Changes in plasma LH, FSH, sex steroids, and inhibin during the menstrual cycle are shown in Figure 23–28, and their feedback relations are diagrammed in Figure 23–35. During the early part of the follicular phase, inhibin B is low and FSH is modestly elevated, fostering follicular growth. LH secretion is held in check by the negative feedback effect of the rising plasma estrogen level. At 36–48 hours before ovulation, the estrogen feedback effect becomes positive, and this initiates the burst of LH secretion (LH surge) that produces ovulation. Ovulation occurs about 9 hours after the LH peak. FSH secretion also peaks, despite a small rise in inhibin, probably because of the strong stimulation of gonadotropes by GnRH. During the luteal phase, the secretion of LH and FSH is low because of the elevated levels of estrogen, progesterone, and inhibin.

It should be emphasized that a moderate, constant level of circulating estrogen exerts a negative feedback effect on LH secretion, whereas during the cycle, an elevated estrogen level exerts a positive feedback effect and

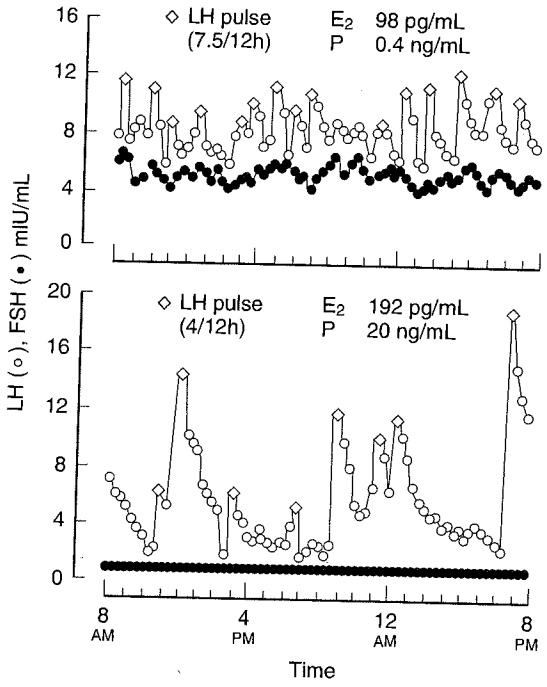


Figure 23–34. Episodic secretion of LH (s) and FSH (d) during the follicular stage **(top)** and the luteal stage **(bottom)** of the menstrual cycle. The numbers above each graph indicate the numbers of LH pulses per 12 hours and the plasma estradiol (E_2) and progesterone (P) concentrations at these two times of the cycle. (Reproduced, with permission, from Marshall JC, Kelch RO: Gonadotropin-releasing hormone: Role of pulsatile secretion in the regulation of reproduction. N Engl J Med 1986;315:1459.)

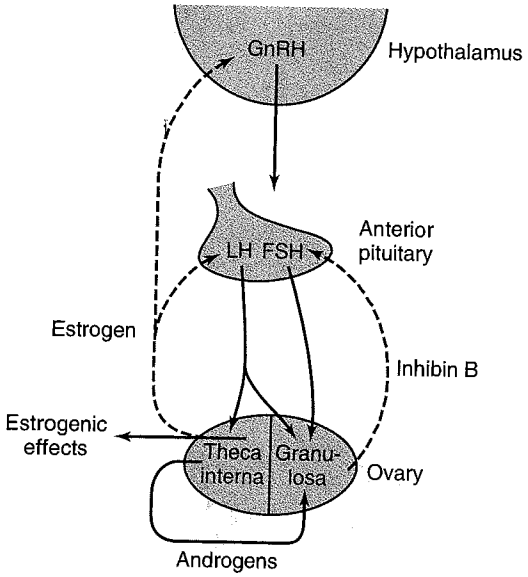


Figure 23–35. Feedback regulation of ovarian function. The cells of the theca interna provide androgens to the granulosa cells, and theca cells also produce the circulating estrogens that inhibit the secretion of GnRH, LH, and FSH. Inhibin from the granulosa cells inhibits FSH secretion. LH regulates the thecal cells, whereas the granulosa cells are regulated by both LH and FSH. The dashed arrows indicate inhibitory effects and the solid arrows stimulatory effects. Compare with Figures 18–12, 20–21, 22–10, and 23–22.

stimulates LH secretion. It has been demonstrated that in monkeys estrogens must also be elevated for a minimum time to produce positive feedback. When circulating estrogen was increased about 300% for 24 hours, only negative feedback was seen; but when it was increased about 300% for 36 hours or more, a brief decline in secretion was followed by a burst of LH secretion that resembled the midcycle surge. When circulating levels of progesterone were high, the positive feedback effect of estrogen was inhibited. There is evidence that in primates, both the negative and the positive feedback effects of estrogen are exerted in the mediobasal hypothalamus, but exactly how negative feedback is switched to positive feedback and then back to negative feedback in the luteal phase remains unknown.

Control of the Cycle

In an important sense, regression of the corpus luteum **(luteolysis)** starting 3–4 days before menses is the key to the menstrual cycle. $PGF_{2\alpha}$ appears to be a physiologic luteolysin, but this prostaglandin is only active when endothelial cells producing ET-1 (see Chapter 31) are present. Therefore it appears that at least in some species luteolysis is produced by the combined action of $PGF_{2\alpha}$ and ET-1. In some domestic animals, oxytocin secreted by the corpus luteum appears to exert a local luteolytic effect, possibly by causing the release of prostaglandins. Once luteolysis begins, the estrogen and progesterone levels fall and the secretion of FSH and LH increases. A new crop of follicles develops, and then a single dominant follicle matures as a result of the action of FSH and LH. Near midcycle, estrogen secretion from the follicle rises. This rise augments the responsiveness of the pituitary to GnRH and triggers a burst of LH secretion. The resulting ovulation is followed by formation of a corpus luteum. Estrogen secretion drops, but progesterone and estrogen levels then rise together, along with inhibin B. The elevated levels inhibit FSH and LH secretion for a while, but luteolysis again occurs and a new cycle starts.

Reflex Ovulation

Female cats, rabbits, mink, and some other animals have long periods of estrus, during which they ovulate only after copulation. Such **reflex ovulation** is brought about by afferent impulses from the genitalia and the eyes, ears, and nose that converge on the ventral hypothalamus and provoke an ovulation-inducing release of LH from the pituitary. In species such as rats, monkeys, and humans, ovulation is a spontaneous periodic phenomenon, but neural mechanisms are also involved. Ovulation can be delayed 24 hours in rats by administering pentobarbital or various other neurally active drugs 12 hours before the expected time of follicle rupture. In women, menstrual cycles may be markedly influenced by emotional stimuli.

Contraception

Methods commonly used to prevent conception are listed in Table 23–8, along with their failure rates. Once conception has occurred, abortion can be produced by progesterone antagonists such as mifepristone.

Implantation of foreign bodies in the uterus causes changes in the duration of the sexual cycle in a number of mammalian species. In humans, such foreign bodies do not alter the menstrual cycle, but they act as effective contraceptive devices. Intrauterine implantation of pieces of metal or plastic **(intrauterine devices, IUDs)** has been used in programs aimed at controlling population growth. Although the mechanism of action of IUDs is still unsettled, they seem in general to prevent sperms from fertilizing ova. Those containing copper appear to exert a spermatocidal effect. IUDs that slowly

Table 23–8. Relative effectiveness of frequently used contraceptive methods.

Method	Failures per 100 Woman-Years
Vasectomy	0.02
Tubal ligation and similar procedures	0.13
Oral contraceptive	
> 50 mg estrogen and progestin	0.32
< 50 mg estrogen and progestin	0.27
Progestin only	1.2
IUD	
Copper 7	1.5
Loop D	1.3
Diaphragm	1.9
Condom	3.6
Withdrawal	6.7
Spermicide	11.9
Rhythm	15.5

Data from Vessey M, Lawless M, Yeates D: Efficacy of different contraceptive methods. Lancet 1982;1:841. Reproduced with permission.

release progesterone or synthetic progestins have the additional effect of thickening cervical mucus so that entry of sperms into the uterus is impeded. IUDs can cause intrauterine infections, but these usually occur in the first month after insertion and in women exposed to sexually transmitted diseases.

Women undergoing long-term treatment with relatively large doses of estrogen do not ovulate, probably because they have depressed FSH levels and multiple irregular bursts of LH secretion rather than a single midcycle peak. Women treated with similar doses of estrogen plus a progestational agent do not ovulate because the secretion of both gonadotropins is suppressed. In addition, the progestin makes the cervical mucus thick and unfavorable to sperm migration, and it may also interfere with implantation. For contraception, an orally active estrogen such as ethinyl estradiol is often combined with a synthetic progestin such as norethindrone. The pills are administered for 21 days, then withdrawn for 5–7 days to permit menstrual flow, and started again. Like ethinyl estradiol, norethindrone has an ethinyl group on position 17 of the steroid nucleus, so it is resistant to hepatic metabolism and consequently is effective by mouth. In addition to being a progestin, it is partly metabolized to ethinyl estradiol, and for this reason it also has estrogenic activity. Small as well as large doses of estrogen are effective (Table 23–8).

Implants made up primarily of progestins such as levonorgestrel are now seeing increased use in some parts of the world. These are inserted under the skin and can prevent pregnancy for up to 5 years. They often produce amenorrhea, but otherwise they appear to be effective and well tolerated.

ABNORMALITIES OF OVARIAN FUNCTION

Menstrual Abnormalities

Some women who are infertile have **anovulatory cycles;** they fail to ovulate but have menstrual periods at fairly regular intervals. As noted above, anovulatory cycles are the rule for the first 1–2 years after menarche and again before the menopause. **Amenorrhea** is the absence of menstrual periods. If menstrual bleeding has never occurred, the condition is called **primary amenorrhea.** Some women with primary amenorrhea have small breasts and other signs of failure to mature sexually. Cessation of cycles in a woman with previously normal periods is called **secondary amenorrhea.** The commonest cause of secondary amenorrhea is pregnancy, and the old clinical maxim that "secondary amenorrhea should be considered to be due to pregnancy until proved otherwise" has considerable merit. Other causes of amenorrhea include emotional stimuli and changes in the environment, hypothalamic diseases, pituitary disorders, primary ovarian disorders, and various systemic diseases. Evidence suggests that in some women with hypothalamic amenorrhea, the frequency of GnRH pulses is slowed as a result of excess opioid activity in the hypothalamus. In encouraging preliminary studies, the frequency of GnRH pulses has been increased by administration of the orally active opioid blocker naltrexone.

The terms **hypomenorrhea** and **menorrhagia** refer to scanty and abnormally profuse flow, respectively, during regular periods. **Metrorrhagia** is bleeding from the uterus between periods, and **oligomenorrhea** is reduced frequency of periods. **Dysmenorrhea** is painful menstruation. The severe menstrual cramps that are common in young women quite often disappear after the first pregnancy. Most of the symptoms of dysmenorrhea are due to accumulation of prostaglandins in the uterus, and symptomatic relief has been obtained by treatment with inhibitors of prostaglandin synthesis (see Chapter 17).

Some women develop symptoms such as irritability, bloating, edema, emotional lability, decreased ability to concentrate, depression, headache, and constipation during the last 7–10 days of their menstrual cycles. These symptoms of the **premenstrual syndrome (PMS)** have been attributed to salt and water retention.

However, it seems unlikely that this or any of the other hormonal alterations that occur in the late luteal phase are responsible because the time course and severity of the symptoms are not modified if the luteal phase is terminated early and menstruation produced by administration of mifepristone. The antidepressant fluoxetine (Prozac), which is a serotonin reuptake inhibitor, and the benzodiazepine alprazolam (Xanax) produce symptomatic relief, and so do GnRH-releasing agonists in doses that suppress the pituitary–ovarian axis. How these diverse clinical observations fit together to produce a picture of the pathophysiology of PMS is still unknown.

Genetic Defects

A number of single-gene mutations cause reproductive abnormalities when they occur in women. Examples include (1) Kallmann's syndrome, which causes hypogonadotropic hypogonadism (see above); (2) GnRH resistance, FSH resistance, and LH resistance, which are due to defects in the GnRH, FSH, or LH receptors, respectively; and (3) aromatase deficiency, which prevents the formation of estrogens. These are all caused by loss-of-function mutations. An interesting gain-of-function mutation causes the **McCune–Albright syndrome,** in which Gsα becomes constitutively active in certain cells but not others (mosaicism) because a somatic mutation after initial cell division has occurred in the embryo (see Chapter 1). It is associated with multiple endocrine abnormalities, including precocious puberty and amenorrhea with galactorrhea.

PREGNANCY

Fertilization & Implantation

In humans, **fertilization** of the ovum by the sperm usually occurs in the ampulla of the uterine tube. Fertilization involves (1) chemoattraction of the sperm to the ovum by substances produced by the ovum; (2) adherence to the **zona pellucida,** the membranous structure surrounding the ovum; (3) penetration of the zona pellucida and the acrosome reaction; and (4) adherence of the sperm head to the cell membrane of the ovum, with breakdown of the area of fusion and release of the sperm nucleus into the cytoplasm of the ovum (Figure 23–36). Millions of sperms are deposited in the vagina during intercourse. Eventually, 50–100 sperms reach the ovum, and many of them contact the zona pellucida. Sperms bind to a sperm receptor in the zona, and this is followed by the **acrosomal reaction,** ie, the breakdown of the acrosome, the lysosome-like organelle on the head of the sperm (Figure 23–18). Various enzymes are released, including the trypsin-like protease **acrosin.** Acrosin facilitates but is not required for the penetration of the sperm through the zona pellucida. When one sperm reaches the membrane of the ovum, fusion to the ovum membrane is mediated by **fertilin,** a protein on the surface of the sperm head that resembles the viral fusion proteins which permit viruses to attack cells. The fusion provides the signal that initiates development. In addition, the fusion sets off a reduction in the membrane potential of the ovum that prevents polyspermy, the fertilization of the ovum by more than one sperm. This transient potential change is followed by a structural change in the zona pellucida that provides protection against polyspermy on a more long-term basis.

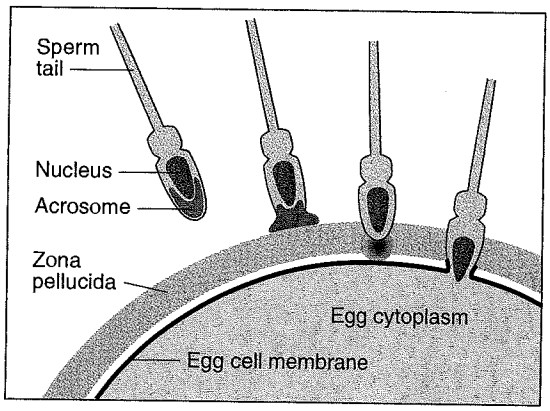


Figure 23–36. Sequential events in fertilization in mammals. Sperm are attracted to the ovum, bind to the zona pellucida, release acrosomal enzymes, penetrate the zona pellucida, and fuse with the membrane of the ovum, releasing the sperm nucleus into its cytoplasm. Current evidence indicates that the side—rather than the tip—of the sperm head fuses with the egg cell membrane. (Modified from Vacquier VD: Evolution of gamete recognition proteins. Science 1999;281:1995.)

The developing embryo, now called a **blastocyst,** moves down the tube into the uterus. This journey takes about 3 days, during which the blastocyst reaches the 8- or 16-cell stage. Once in contact with the endometrium, the blastocyst becomes surrounded by an outer layer of **syncytiotrophoblast,** a multinucleate mass with no discernible cell boundaries, and an inner layer of **cytotrophoblast** made up of individual cells. The syncytiotrophoblast erodes the endometrium, and the blastocyst burrows into it (**implantation).** The implantation site is usually on the dorsal wall of the

uterus. A placenta then develops, and the trophoblast remains associated with it.

Failure to Reject the "Fetal Graft"

It should be noted that the fetus and the mother are two genetically distinct individuals, and the fetus is in effect a transplant of foreign tissue in the mother. However, the transplant is tolerated, and the rejection reaction that is characteristically produced when other foreign tissues are transplanted (see Chapter 27) fails to occur. The way the "fetal graft" is protected is unknown. However, one explanation may be that the placental trophoblast, which separates maternal and fetal tissues, does not express the polymorphic class I and class II MHC genes and instead expresses ***HLA-G,*** a nonpolymorphic gene. Therefore, antibodies against the fetal proteins do not develop. In addition, there is a Fas ligand on the surface of the placenta, and this bonds to T cells, causing them to undergo apoptosis (see Chapter 1).

Infertility

The vexing clinical problem of infertility often requires extensive investigation before a cause is found. In 30% of cases the problem is in the man; in 45%, the problem is in the woman; in 20%, both partners have a problem; and in 5% no cause can be found. **In vitro fertilization,** ie, removing mature ova, fertilizing them with sperm, and implanting one or more of them in the uterus at the four-cell stage is of some value in these cases. It has a 5–10% chance of producing a live birth.

Endocrine Changes

In all mammals, the corpus luteum in the ovary at the time of fertilization fails to regress and instead enlarges in response to stimulation by gonadotropic hormones secreted by the placenta. The placental gonadotropin in humans is called **human chorionic gonadotropin (hCG).** The enlarged **corpus luteum of pregnancy** secretes estrogens, progesterone, and relaxin. The relaxin helps maintain pregnancy by inhibiting myometrial contractions. In most species, removal of the ovaries at any time during pregnancy precipitates abortion. In humans, however, the placenta produces sufficient estrogen and progesterone from maternal and fetal precursors to take over the function of the corpus luteum after the sixth week of pregnancy. Ovariectomy before the sixth week leads to abortion, but ovariectomy thereafter has no effect on the pregnancy. The function of the corpus luteum begins to decline after 8 weeks of pregnancy, but it persists throughout pregnancy. hCG secretion decreases after an initial marked rise, but estrogen and progesterone secretion increase until just before parturition (Table 23–9).

hCG

hCG is a glycoprotein that contains galactose and hexosamine. It is produced by the syncytiotrophoblast. Like the pituitary glycoprotein hormones, it is made up of α and β subunits. hCG-α is identical to the α subunit of LH, FSH, and TSH. The molecular weight of hCG-α is 18,000, and that of hCG-β is 28,000. hCG is primarily luteinizing and luteotropic and has little FSH activity. It can be measured by radioimmunoassay and detected in the blood as early as 6 days after conception. Its presence in the urine in early pregnancy is the basis of the various laboratory tests for pregnancy, and it can sometimes be detected in the urine as early as 14 days after conception. It appears to act on the same receptor as LH. hCG is not absolutely specific for pregnancy. Small amounts are secreted by a variety of gastrointestinal and other tumors in both sexes, and hCG has been measured in individuals with suspected tumors as a "tumor marker." It also appears that the fetal liver and kidney normally produce small amounts of hCG.

hCS

The syncytiotrophoblast also secretes large amounts of a protein hormone that is lactogenic and has a small amount of growth-stimulating activity. This hormone has been called **chorionic growth hormone-prolactin (CGP)** and **human placental lactogen (hPL),** but it is now generally called **human chorionic somatomammotropin (hCS).** The structure of hCS is very similar to that of human growth hormone (see Figure 22–3), and it appears that these two hormones and prolactin evolved from a common progenitor hormone. Large quantities of hCS are found in maternal blood, but very little reaches the fetus. Secretion of growth hormone

Table 23–9. Hormone levels in human maternal blood during normal pregnancy.

Hormone	Approximate Peak Value	Time of Peak Secretion
hCG	5 mg/mL	First trimester
Relaxin	1 ng/mL	First trimester
hCS	15 mg/mL	Term
Estradiol	16 ng/mL	Term
Estriol	14 ng/mL	Term
Progesterone	190 ng/mL	Term
Prolactin	200 ng/mL	Term

from the maternal pituitary is not increased during pregnancy and may actually be decreased by hCS. However, hCS has most of the actions of growth hormone and apparently functions as a "maternal growth hormone of pregnancy" to bring about the nitrogen, potassium, and calcium retention, lipolysis, and decreased glucose utilization seen in this state. These latter two actions divert glucose to the fetus. The amount of hCS secreted is proportionate to the size of the placenta, which normally weighs about one sixth as much as the fetus, and low hCS levels are a sign of placental insufficiency.

Other Placental Hormones

In addition to hCG, hCS, progesterone, and estrogens, the placenta secretes other hormones. Human placental fragments probably produce POMC. In culture, they release CRH, β-endorphin, α-MSH, and dynorphin A, all of which appear to be identical to their hypothalamic counterparts. They also secrete GnRH and inhibin, and since GnRH stimulates and inhibin inhibits hCG secretion, locally produced GnRH and inhibin may act in a paracrine fashion to regulate hCG secretion. The trophoblast cells and amnion cells also secrete leptin (see Chapter 14), and moderate amounts of this satiety hormone enter the maternal circulation. Some also enters the amniotic fluid. Its function in pregnancy is unknown. The placenta also secretes prolactin in a number of forms.

Finally, the placenta secretes the α subunits of hCG, and the plasma concentration of free α subunits rises throughout pregnancy. These α subunits acquire a carbohydrate composition that makes them unable to combine with β subunits, and their prominence suggests that they have a function of their own. It is interesting in this regard that the secretion of the prolactin produced by the endometrium also appears to increase throughout pregnancy, and it may be that the circulating α subunits stimulate endometrial prolactin secretion.

The cytotrophoblast of the human chorion contains prorenin (see Chapter 24). A large amounts of prorenin is also present in amniotic fluid, but its function in this location is unknown.

Fetoplacental Unit

The fetus and the placenta interact in the formation of steroid hormones. The placenta synthesizes pregnenolone and progesterone from cholesterol. Some of the progesterone enters the fetal circulation and provides the substrate for the formation of cortisol and corticosterone in the fetal adrenal glands (Figure 23–37). Some of the pregnenolone enters the fetus and, along

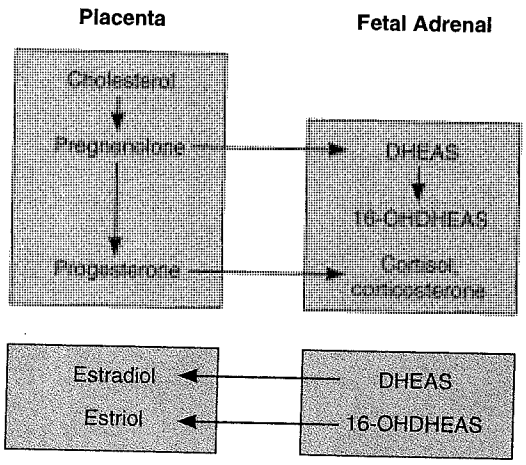


Figure 23–37. Interactions between the placenta and the fetal adrenal cortex in the production of steroids.

with pregnenolone synthesized in the fetal liver, is the substrate for the formation of dehydroepiandrosterone sulfate (DHEAS) and 16-hydroxydehydroepiandrosterone sulfate (16-OHDHEAS) in the fetal adrenal. Some 16-hydroxylation also occurs in the fetal liver. DHEAS and 16-OHDHEAS are transported back to the placenta, where DHEAS forms estradiol and 16-OHDHEAS forms estriol. The principal estrogen formed is estriol, and since fetal 16-OHDHEAS is the principal substrate for the estrogens, the urinary estriol excretion of the mother can be monitored as an index of the state of the fetus.

Parturition

The duration of pregnancy in humans averages 270 days from fertilization (284 days from the first day of the menstrual period preceding conception). Irregular uterine contractions increase in frequency in the last month of pregnancy.

The difference between the body of the uterus and the cervix becomes evident at the time of delivery. The cervix, which is firm in the nonpregnant state and throughout pregnancy until near the time of delivery, softens and dilates, while the body of the uterus contracts and expels the fetus.

There is still considerable uncertainty about the mechanisms responsible for the onset of labor. One factor is the increase in circulating estrogens produced by increased circulating DHEAS. This makes the uterus more excitable, increases the number of gap junctions

between myometrial cells, and causes production of more prostaglandins, which in turn cause uterine contractions. In humans, CRH secretion by the fetal hypothalamus increases and is supplemented by increased placental production of CRH. This increases circulating ACTH in the fetus, and the resulting increase in cortisol hastens the maturation of the respiratory system. Thus, in a sense, the fetus picks the time to be born by increasing CRH secretion.

The number of oxytocin receptors in the myometrium and the decidua (the endometrium of pregnancy) increases more than 100-fold during pregnancy and reaches a peak during early labor. Estrogens increase the number of oxytocin receptors, and uterine distention late in pregnancy may also increase their formation. In early labor, the oxytocin concentration in maternal plasma is not elevated from the prelabor value of about 25 pg/mL. It is possible that the marked increase in oxytocin receptors causes the uterus to respond to normal plasma oxytocin concentrations. However, at least in rats, the amount of oxytocin mRNA in the uterus increases, reaching a peak at term; this suggests that locally produced oxytocin also participates in the process.

Premature onset of labor is a problem because premature infants have a high mortality rate and often require intensive, expensive care. Intramuscular 17α-hydroxyprogesterone (see Figure 20–8) causes a significant decrease in the incidence of premature labor. The mechanism by which it exerts its effect is uncertain, but it may be that the steroid provides a stable level of circulating progesterone. Progesterone relaxes uterine smooth muscle, inhibits the action of oxytocin on the muscle, and reduces the formation of gap junctions between the muscle fibers. All these actions would be expected to inhibit the onset of labor.

Once labor is started, the uterine contractions dilate the cervix, and this dilation in turn sets up signals in afferent nerves that increase oxytocin secretion (Figure 23–38). The plasma oxytocin level rises, and more oxytocin becomes available to act on the uterus. Thus, a positive feedback loop is established that aids delivery and terminates on expulsion of the products of conception. Oxytocin increases uterine contractions in two ways: (1) It acts directly on uterine smooth muscle cells to make them contract; and (2) it stimulates the formation of prostaglandins in the decidua. The prostaglandins enhance the oxytocin-induced contractions.

During labor, spinal reflexes and voluntary contractions of the abdominal muscles ("bearing down") also aid in delivery. However, delivery can occur without bearing down and without a reflex increase in secretion of oxytocin from the posterior pituitary gland, since paraplegic women can go into labor and deliver.

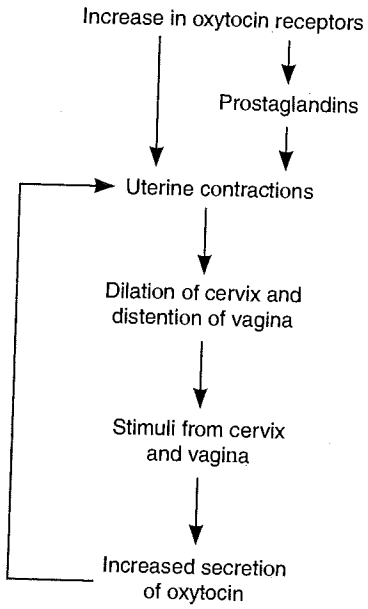


Figure 23–38. Role of oxytocin in parturition.

■ LACTATION

Development of the Breasts

Many hormones are necessary for full mammary development. In general, estrogens are primarily responsible for proliferation of the mammary ducts and progesterone for the development of the lobules. In rats, some prolactin is also needed for development of the glands at puberty, but it is not known if prolactin is necessary in humans. During pregnancy, prolactin levels increase steadily until term, and levels of estrogens and progesterone are elevated as well, producing full lobuloalveolar development.

Secretion & Ejection of Milk

The composition of human and cows' milk is shown in Table 23–10. In estrogen- and progesterone-primed rodents, injections of prolactin cause the formation of milk droplets and their secretion into the ducts. Oxytocin causes contraction of the myoepithelial cells lining the duct walls, with consequent ejection of the milk through the nipple. The reflex release of oxytocin initiated by touching the nipples and areolas (milk ejection reflex) is discussed in Chapter 14.

The transfer of antibodies to the infant by colostrum is discussed in Chapter 26.

Table 23–10. Composition of colostrum and milk. (Units are weight per deciliter.)

Component	Human Colostrum	Human Milk	Cows' Milk
Water, g	...	88	88
Lactose, g	5.3	6.8	5.0
Protein, g	2.7	1.2	3.3
Casein:lactalbumin ratio	...	1:2	3:1
Fat, g	2.9	3.8	3.7
Linoleic acid	...	8.3% of fat	1.6% of fat
Sodium, mg	92	15	58
Potassium, mg	55	55	138
Chloride, mg	117	43	103
Calcium, mg	31	33	125
Magnesium, mg	4	4	12
Phosphorus, mg	14	15	100
Iron, mg	0.09[2]	0.15[a]	0.10[a]
Vit A, µg	89	53	34
Vit D, µg	...	0.03[a]	0.06[a]
Thiamine, µg	15	16	42
Riboflavin, µg	30	43	157
Nicotinic acid, µg	75	172	85
Ascorbic acid, mg	4.4[a]	4.3[a]	1.6[a]

Reproduced, with permission, from Findlay ALR: Lactation. Res Reprod (Nov) 1974;6(6).

[a]Poor source.

Initiation of Lactation After Delivery

The breasts enlarge during pregnancy in response to high circulating levels of estrogens, progesterone, prolactin, and possibly hCG. Some milk is secreted into the ducts as early as the fifth month, but the amounts are small compared with the surge of milk secretion that follows delivery. In most animals, milk is secreted within an hour after delivery, but in women it takes 1–3 days for the milk to "come in."

After expulsion of the placenta at parturition, the levels of circulating estrogens and progesterone abruptly decline. The drop in circulating estrogen initiates lactation. Prolactin and estrogen synergize in producing breast growth, but estrogen antagonizes the milk-producing effect of prolactin on the breast. Indeed, in women who do not wish to nurse their babies, estrogens may be administered to stop lactation.

Suckling not only evokes reflex oxytocin release and milk ejection; it also maintains and augments the secretion of milk because of the stimulation of prolactin secretion produced by suckling (see above).

Effect of Lactation on Menstrual Cycles

Women who do not nurse their infants usually have their first menstrual period 6 weeks after delivery. However, women who nurse regularly have amenorrhea for 25–30 weeks. Nursing stimulates prolactin secretion, and evidence suggests that prolactin inhibits GnRH secretion, inhibits the action of GnRH on the pituitary, and antagonizes the action of gonadotropins on the ovaries. Ovulation is inhibited, and the ovaries are inactive, so estrogen and progesterone output falls to low levels. Consequently, only 5–10% of women become pregnant again during the suckling period, and nursing has long been known to be an important if only partly effective method of birth control. Furthermore, almost 50% of the cycles in the first 6 months after resumption of menses are anovulatory.

Chiari–Frommel Syndrome

An interesting although rare condition is persistence of lactation (**galactorrhea**) and amenorrhea in women who do not nurse after delivery. This condition, called the **Chiari–Frommel syndrome,** may be associated with some genital atrophy and is due to persistent prolactin secretion without the secretion of the FSH and LH necessary to produce maturation of new follicles and ovulation. A similar pattern of galactorrhea and amenorrhea with high circulating prolactin levels is seen in nonpregnant women with chromophobe pituitary tumors and in women in whom the pituitary stalk has been sectioned in treatment of cancer.

Gynecomastia

Breast development in the male is called **gynecomastia.** It may be unilateral but is more commonly bilateral. It is common, occurring in about 75% of newborns because of transplacental passage of maternal estrogens. It also occurs in mild, transient form in 70% of normal boys at the time of puberty and in many men over the age of 50. It occurs in androgen resistance. It is a complication of estrogen therapy and is seen in patients with estrogen-secreting tumors. It is found in a wide variety of seemingly unrelated conditions, including eunuchoidism, hyperthyroidism, and cirrhosis of the liver. Digitalis can produce it, apparently because cardiac glycosides are weakly estrogenic. It can also be caused by many other drugs. It has been seen in malnourished prisoners of war, but only after they were liberated and eating an adequate diet. A feature common to many and perhaps all cases of gynecomastia is an increase in the plasma estrogen:androgen ratio due to either increased circulating estrogens or decreased circulating androgens.

Hormones & Cancer

About 35% of carcinomas of the breast in women of childbearing age are **estrogen-dependent;** their continued growth depends on the presence of estrogens in the circulation. The tumors are not cured by decreasing estrogen secretion, but symptoms are dramatically relieved, and the tumor regresses for months or years before recurring. Women with estrogen-dependent tumors often have a remission when their ovaries are removed. Inhibition of the action of estrogens with **tamoxifen** also produces remissions, and inhibition of estrogen formation with drugs that inhibit **aromatase** (Figure 23–29) is even more effective.

Some carcinomas of the prostate are **androgen-dependent** and regress temporarily after the removal of the testes or treatment with GnRH agonists in doses that are sufficient to produce down-regulation of the GnRH receptors on gonadotropes and decrease LH secretion.

The formation of pituitary tumors after removal of the target endocrine glands controlled by pituitary tropic hormones is discussed in Chapter 22.

TESTIS

Historic Considerations in Male Reproduction

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Introduction

The objective of this article is to provide a number of early historic observations and theories that influenced the development of the field of Male Reproductive Biology. The use of castration by ancient man to domesticate animals indicate the testis was the first organ studied in regards to its impact on male reproductive physiology. The differences between males and females also lead to the first biological theories on why these differences exist. One of the first uses of the microscope was the examination of sperm and the initial theories developed that impeded the initial theory of fertilization. The basis for sex determination was also one of the first biological considerations of Aristotle which impacted the biological sciences for over 2000 years. This article will present a number of historic considerations (**Table 1**) that were for the most part inaccurate and misleading, but have significantly impacted the progression of the field of reproductive biology.

Historic Castration (Testis) Considerations

Ancient civilizations used the removal of testis (i.e., castration) to domesticate animals including pigs, cattle, and sheep. The origins of castration, most likely developed at the time of the emergence of faming (i.e., neolitic period ~10,000 BC), either accidentally or intentionally are unknown but had a significant impact on ancient civilizations (Diamandopoulos et al., 2005). Therefore, this biological manipulation is the first documented physiological observation on the impacts of a specific organ on the biology of an organism. The first writings on castration was around 1500 BC by Assyrians in a tablet relating castration as punishment for sexual crimes. Then, the Greek medical writer Hippocrates in the 5th—4th century BC elaborated on the concept requiring the two parents. Physical traits on voice and feminization in eunuchs (i.e., human castrations) were documented by Aristotle in the 4th—3rd centuries BC (Hippocrates, 1915). He commented on the effects of castration in the rooster and man and gave a detailed description

Table 1 Historic considerations impacting male reproduction

Reproductive topic	*Observation*	*Period*
Castration	Testis first organ interest Domestication animals	Prehistoric/neolitic
	Eunuch characteristics	Assyrian/egyptian/greek societies
Reproduction theory	Theory male provide seed (testis involved) *active* role and female provide soil *passive* role (prevailing theory 2000 years)	Aristotle (384–322 BC)
Environmental sex determination	Theory environmental factors such as heat (fire) and cold (water) promotes sex determination for male or female	Aristotle (384–322 BC)
Spermist theory/animalculism	One of the two theory of preformationism for which sperm head contained preformed offspring (homunculus) and female provide passive environment for development	Nicolaas Hartsoeker 1600s
Ovism	The other theory of preformation for which "the embryo is preformed in the ovum (egg) while the sperm only provides an aura seminalis," that is a vital essence	1600s–1700s
Female ovary and egg role reproduction	Egg produced in ovary and has active role reproduction with sperm	William Harvey and Bishop Niels Stenson 1600s
Male reproductive tract organs anatomy	Detailed anatomy of male reproductive tract organs and specialized roles	Vesale 1543s and De Graaf 1600s
Genetic sex determination theory	The role of genetics followed by gonadal followed by phenotypic sex in mammals	Alfred Jost (1940s and 1950s)
Male reproduction endocrine theory	The role of endocrine hormones in male reproduction integrating the testis, brain and other reproductive organs	Berthold 1879; Alfered Jost (1940s and 1950s); Mc Cullagh (1930s and 1940s)

of the genital tract in mammals. Aristotle also developed early theories or speculations on the role of the testis in male biology (Hippocrates, 1962). Over the next 1000 years debates and theories on the impacts of the testis on male biology developed ranging from the impact of temperature on the testis to the effects of the testis on the heart, brain, and muscle development (Diamandopoulos et al., 2005). One of the more interesting speculations was the role of a testis derived liquid on the males biology (Diamandopoulos et al., 2005; Galen, 1964). Although the majority of these theories and speculations were neither accurate or based on specific observations, Aristotle's concepts had significant impacts for centuries on our approach and progress of scientific advances (**Table 1**).

The use of castrations in ancient societies in humans to create eunuchs was reported in Assyrian, Egyptian, and Greek cultures. Observations of the physiological impacts of castration were documented in all these societies. This included infertility, brain behavioral effects, voice changes, weight, and metabolism alterations and more. These observations led to a variety of early theories and speculations. A prominent link between the testis and the brain was discussed by Aristotle and others throughout history, which was the first speculations of the brain and gonadal axis today known to be endocrine based. Although these early speculations and theories were not completely accurate, they laid the foundation for much of the early male reproduction research in the last century.

Reproduction and Sperm Theory

One of the first recorded reproduction theories or speculations was proposed by Aristotle (**Table 1**). Observations with castration and role of the testis to create semen Aristotle proposed the male provided the seed having an active role in reproduction and the female provided the soil having a passive role in reproduction. Although this reproduction theory was based on observations at the time, this Aristotle concept persisted for nearly 2000 years and impacted the majority of reproductive biology research and society. A classic example involving one of the first observations following the development of the single lens microscope in the 1600s by Leewenhock (1678) was made on his own sperm which lead him to describe the presence and vigor of "animalcules," the name that he gave to spermatozoa. Under the name of "homunculus" the speculation was made by Hartsoeker (1694) that the spermatozoon contained the entire preformed human (homunculus)/animal (animalcules) with a number of supporting microscopic observations (Hartsoeker, 1696). The concept named "animalculism" was that the sperm provided the seed that contained the preformed animal (**Fig. 1**) and that the female provided the soil to allow its growth. Therefore, Aristotle's reproduction theory was supported.

This preformation sperm theory was debated for the next 100 years. The work of the Reverent Lazzaro Spallanzani (1717, 1780) lead to the description of the mobility of spermatozoa (the "small worms" as he wrote) and he also provided the first artificial insemination using a dog (Inza, 1964). Although all of Spallanzani's work supported the role of the sperm in reproduction, he believed in ovism and the role of a preformed person in the egg. The very long standing controversy about the role of spermatozoa in reproduction really ended with Prevost and Dumas in 1824 who rediscovered after Spallanzani the relationship between the male gamete and reproductive abilities. The named "spermatozoa" was first coined by Van Baer soon after (1827), and it was van Kolliker in 1841 who introduced the fact that spermatozoa derive from germ cells in the testis.

During the same period, it was William Harvey (1578–1657), who discovered circulation of blood, but he also studied the ovary anatomy making major observations on the histology of the ovary and anatomy of the female reproductive system. Observations led Harvey to the proposal that ovulation and the ovary had a critical role in reproduction (Harvey, 1651). Harvey did not discover the egg as during the same period Bishop Niels Stensen (1638–86) using fish ovaries to discover the egg and later using the mule, horse and donkey described the role of the egg in reproduction (Volker, 1989). The debate that the female and egg played an active role in reproduction was contested during the 1600s and 1700s period due in part to the predominant influence of the early Aristotle concepts. The active role of the female in reproduction slowly became accepted, however, this provides an example of how early scientific theories can develop into dogma and paradigms that can persist and sometimes impair the progression of science.

Male Reproductive Tract Organ and Anatomy

Anatomy was one of the first biological fields to develop and was documented by artists and scientists from early Egyptian, Greek, and Roman periods through the modern era. All those societies had documentation of testis and ovary which suggested their

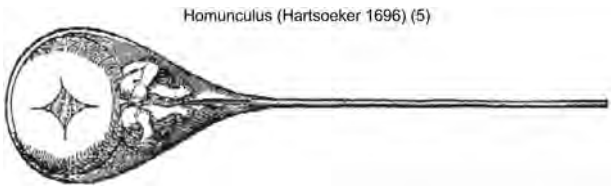

Fig. 1 Homunculus involving preformed human/animal in the head of the sperm to then allow growth in the female From Hartsoeker, N. Principes de Physique. Paris, 1696.

understanding of the link with reproduction. Later artists such as Leonardo Di Vinci (1452–1519) created drawings of reproductive systems and organs such as the male reproductive tract. This included an anatomical representation of coitum of a hemisected man and women, and is best known for his analysis of fetal developmental anatomy (O'Malley, 2003). Many artists and scientists documented the anatomy of many reproductive organs, such as the work of Vesale in 1513, but one of the most influential and informative scientists was Regneri de Graaf in the 1600s (de Graaf, 1677). The most complete and insightful male and female reproductive tract anatomy was provided by de Graaf and detailed the specific organs, connections and hypothetical functions. An example of his detailed drawings of male reproductive tract anatomy is presented in **Figs. 2 and 3**. In addition to the testis, rete testis and epididymis, seminal vesical, vas deferens and penis are presented in detail (de Graaf, 1677). The work of early anatomists such as de Graaf set the ground work for our modern understanding of male reproduction. The development of the microscope in the late 1600s allowed more detailed anatomy and histology to be performed of male reproductive tract tissues. This analysis advanced the concepts of the "Cell Theory" which suggested tissues and organs had specific cells that allowed them to function. One of the first tissues studied was the testis due to its importance for reproduction and having more understanding than most tissues. Following the understanding sperm was produced within the seminiferous tubules, the first

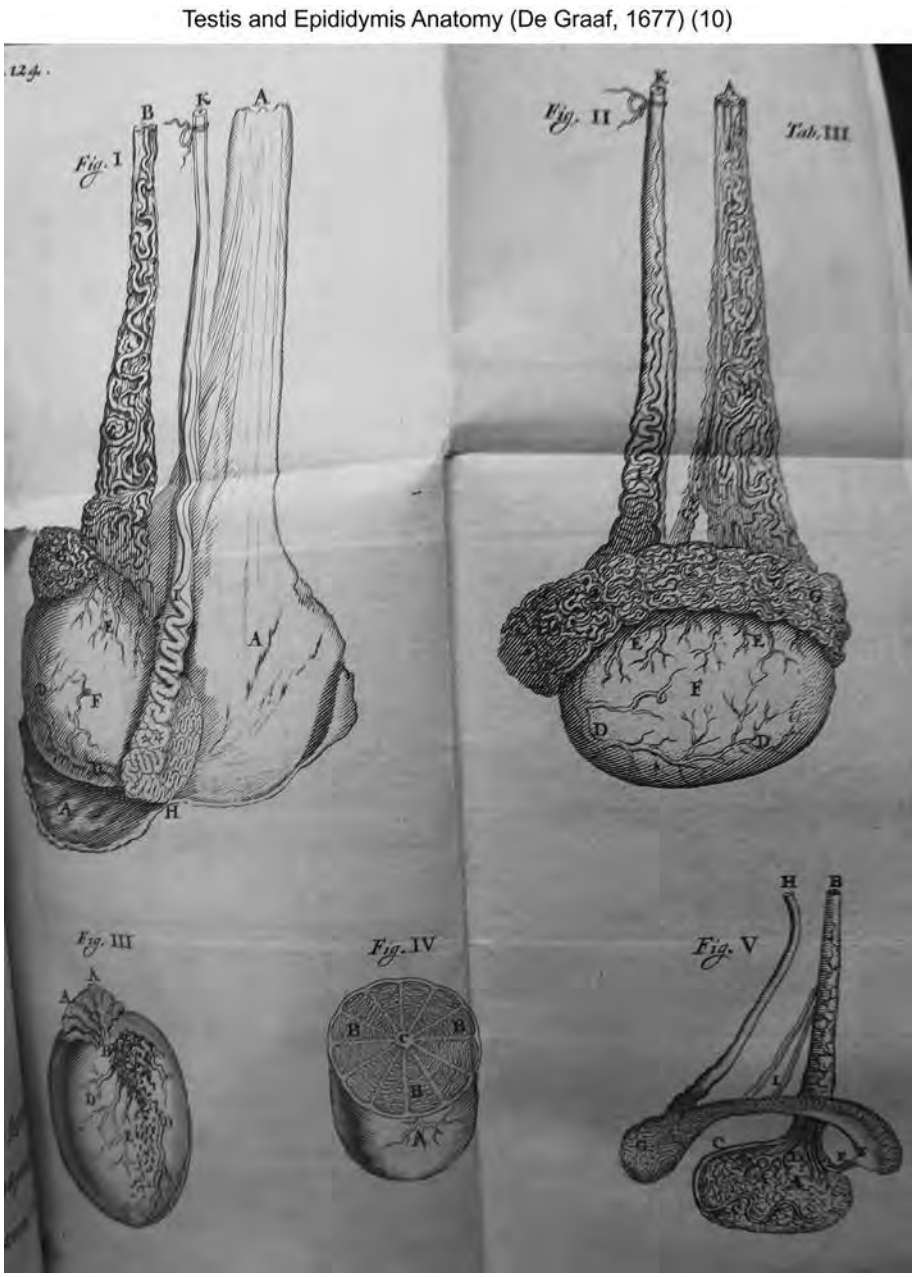

Fig. 2 Testis and epididymis male reproductive tract anatomy drawing From de Graaf, R. Opera Omnia: Lugd. Batav. Ex Officina Hackiana, 1677.

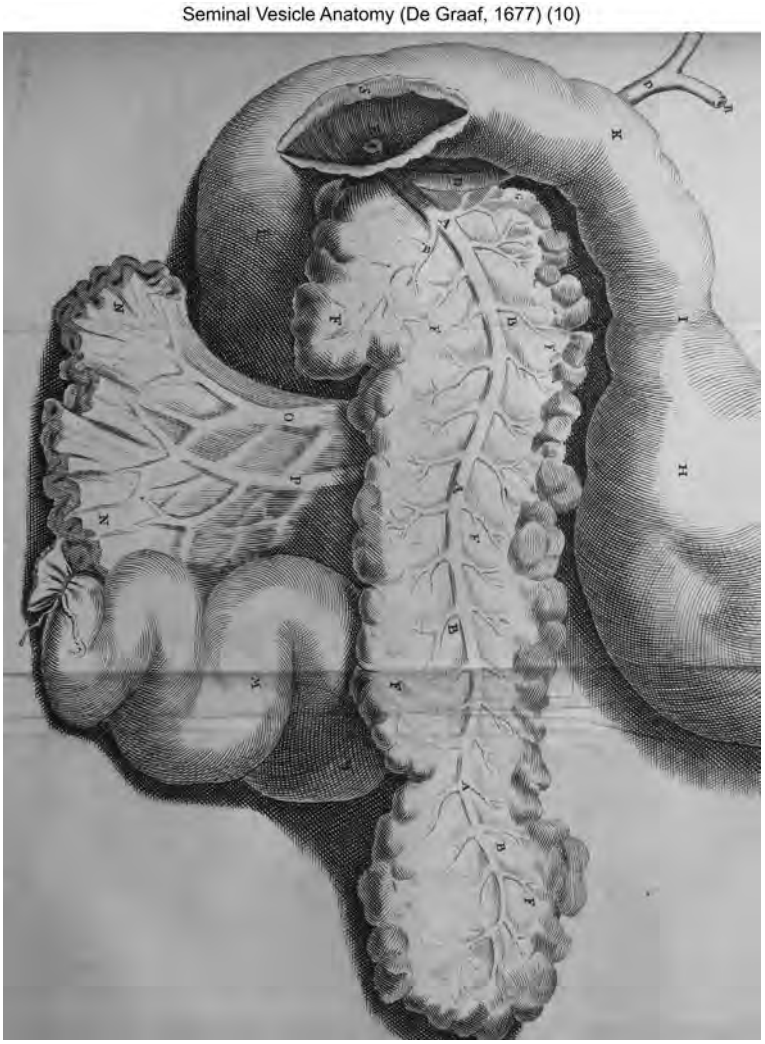

Fig. 3 Seminal vesicle and male reproductive tract anatomy drawing From de Graaf, R. Opera Omnia: Lugd. Batav. Ex Officina Hackiana, 1677.

somatic cell identified at this level was the Sertoli cell. Enrico Sertoli was an Italian physician that used the early microscope to describe the structure and histology of this critical cell type. Sertoli elaborated the crucial concept that Sertoli cells are the "nurse cells" of germ cells (Anon, 1987). His early diagrams of these cells that now have his name are shown in **Fig. 4** (Sertoli, 1865). The various structures of the Sertoli cell and its role in the development of the seminiferous tubule are described from this 1865 observation (Sertoli, 1865). This is simply the first of many different observations by many scientists to describe specific cell types in various male reproduction tract organs. Clearly the organ anatomy and cellular histology of the 1600s–1800s help establish the initial building blocks for current reproduction research.

Early Sex Determination Theory

The origins of males versus females through the process of sex determination was also considered in early societies, but the first documented theory or speculation was provided by Aristotle (**Table 1**). The concept was that environment promoted sex determination and Aristotle suggested heat initiated male development and cold initiated female development (**Fig. 4**). Although this does exist in some organisms such as turtles, this is now known not to be a factor in mammals and most organisms. This theory has persisted even into the modern era. An example is in the 1980s when the prince of England was about to have a child the fact he had been a helicopter pilot was determined by the popular press to indicate he would have a male child. Therefore, even today the concept environment can impact sex determination persists from these early Aristotle concepts.

This environmental impact on sex determination persisted until the mid-1900s. The first modern theory of sex determination was provided by Alfred Jost, University of Paris in the 1940s and 1950s (Jost, 1945, 1970). The development of genetics in the early 1900s led to the identification of sex chromosomes that then through experiments by Jost on early fetal sex determination led to his

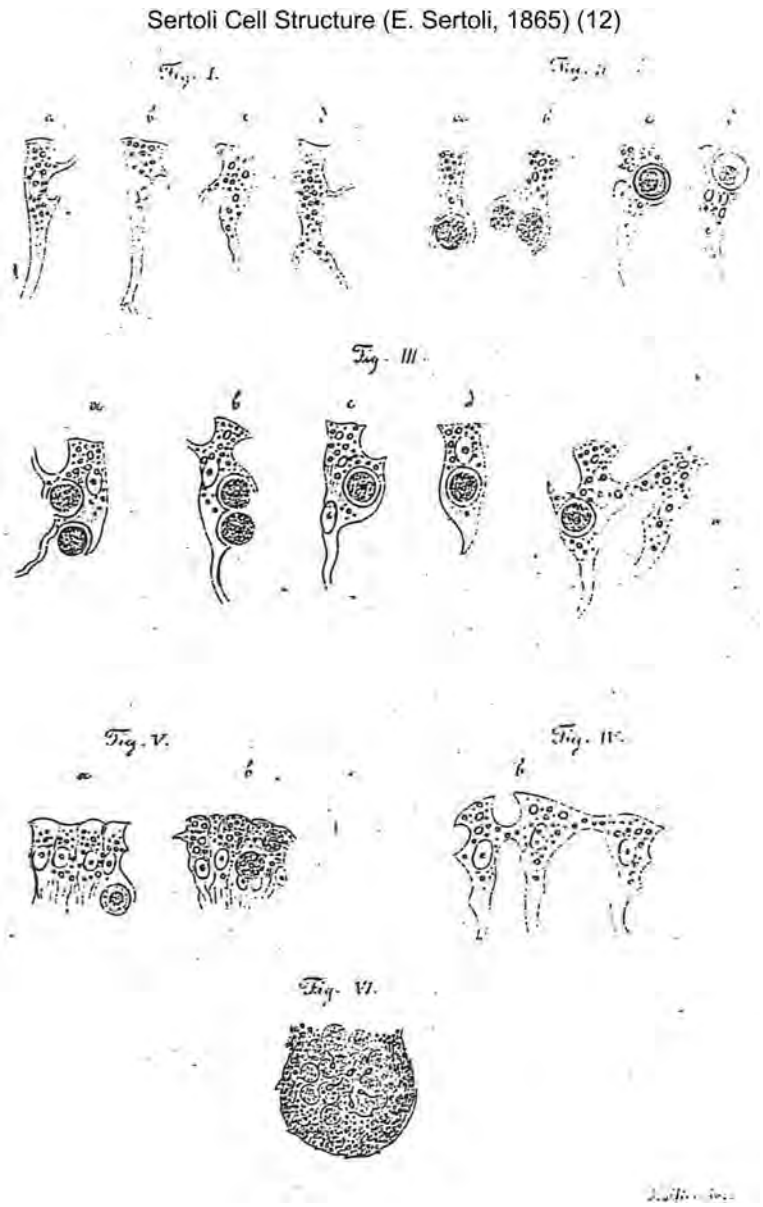

Fig. 4 Aristotle sex determination theory. The relative preponderances of the four elements and their qualities in males and females. The greater innate heat of the male allows for a greater degree of concoction of the nourishment and hence a more concentrated white semen is formed. From Sertoli E. (1865). Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano. Morgagni 7, 31–40.

theory for sex determination (**Table 1**). This involved the theory that genetic sex (sex chromosomes) promote gonadal sex (testis or ovary) that then promotes phenotypic sex (male or female) (Jost, 1945, 1970), **Fig. 5**. This sex determination theory displaced the early Aristotle concept of environmental sex determination, but surprisingly Aristotle's theory was predominant for nearly 2000 years. Another example of how early historic concepts can impede the progression of science by development of strong dogma or paradigms.

Early Endocrine Observations

The development of early anatomy and cell histology in the 1600s and 1700s established an understanding of the complexities of male reproduction in a number of different species. The first major advance to the next level was made by Claude Bernard in the 1850s with the advent of the field of physiology. He is considered the father of the field through his insight and experiments that different organ systems interact to influence their functions. He described the concept of cybernetic systems between organs and tissues through the internal milieu as the basis of how the organism functions (De La, 1872). Although he did not focus on

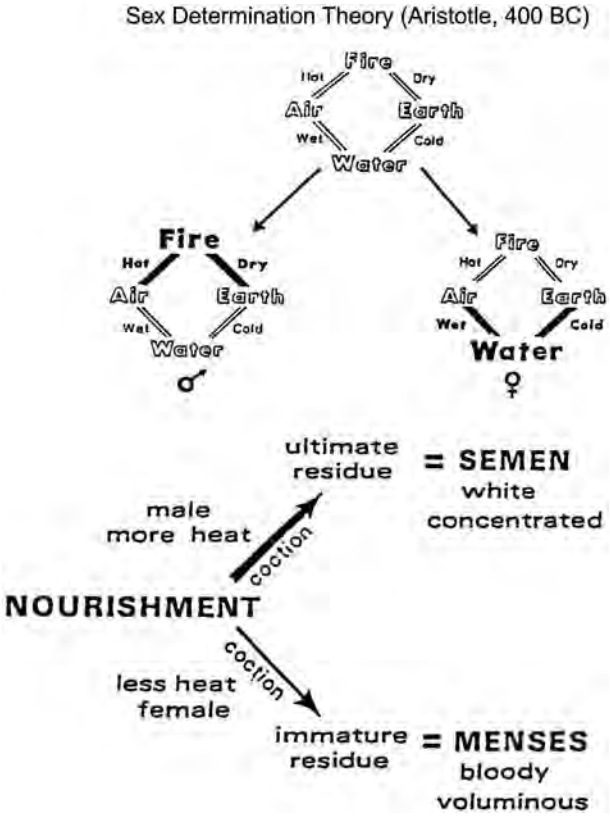

Fig. 5 Jost sex determination theory. Role of genetic sex (sex chromosomes) in promoting gonadal sex (testis or ovary) that promote male or female phenotypic sex characteristics (Jost, 1945).

reproduction, the concepts and theory initiated the fields of physiology, endocrinology and cell biology. One of the systems he did consider was the brain-gonadal interaction. Therefore, Bernard set the ground work for reproduction physiology and indirectly led to the field of endocrinology.

With this in mind and the summary of the main milestones concerning the testis itself, the seminiferous tubules and spermatozoa (see above) it is interesting to note that the first demonstration of the role of a "gland," was provided by Berthold in 1849 when he showed that while castration was followed by regression of the cockscomb, transplantation of the testes to the castrated cocks restored its size. Very soon afterward Leydig (1850) provided the first microscopic description of the interstitial cells of the testis which were named the Leydig cells and are now known to produce the hormones named androgens, and Insulin-like factor 3 the latter being responsible for testicular descent in mammals.

The early 1900s involved a large number of physiology experiments to examine how organ systems influence each other and identified substances that mediate those interactions. Some of the first hormones identified were shown to be produced by the gonads and impact a wide variety of tissues (**Table 1**). This involved the identification of testosterone being produced by the testis (McCullagh, 1948). In addition, one of the first protein hormones inhibin being produced by the testis and impacting the brain hypothalamus (McCullagh, 1948). One of the prominent scientists involved was (McCullagh, 1948). This initiated in the following decades an increasing number of endocrine focused experiments to help understand male reproduction. Another example is related to the work of Alfred Jost, **Fig. 6**, who during his work on sex determination identified a testis determining factor that we now know is a specific gene (SRY), as well as a hormone anti-Müllerian hormone (AMH) which is critical in both male and female fetal sex determination and in the adult (Jost, 1945, 1970). Over the past 100 years the advances have been far more rapid than in previous centuries.

Summary

The review of ancient and early history has revealed that early history concepts for science are often not completely accurate, but they do have an important role in the progression of science. The advantage is that a theory is developed that can be tested through experiment and during that process new observations can be made to progress science. The disadvantage is that the theory developed can turn into dogma and a strong paradigm that becomes so ingrained that it can impede the progression of science. In considering the history and concepts of male reproduction, there have been good examples of both. Although the insight and

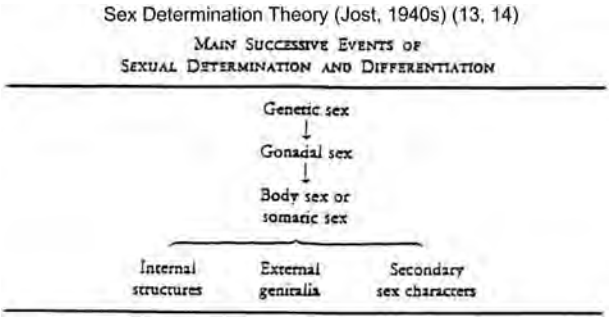

Fig. 6 Sex determination theory. From Jost, A. (1970). Hormonal factors in the sex differentiation of the mammalian foetus. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* **259**(828), 119–130; Jost, A. (1945). Sur l'action de divers androgenes dans la differenciation embryonnaire du sexe. *Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales* **139**, 670–672.

theories of Aristotle were surprising, the development of dogma around these concepts clearly impeded the progression of science in subsequent centuries. We have displaced many of these concepts, but even today we have our paradigms. The future will need to assess these paradigms and not resist the paradigm shifts required to advance science.

Considering the male reproduction historic concepts can assist in our understanding of the origins of current science and theories. Perhaps the biggest value of this historic understanding is to put the current activities in perspective, which will only help the advances of male reproductive sciences in the future.

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Sertoli, E. (1865). Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano. *Morgagni, 7*, 31–40.

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WASHINGTON STATE UNIVERSITY

World Class. Face to Face.

COURSE ANNOUNCEMENT

SPRING 2024

Biol 475/575
Systems Biology of Reproduction

3 Credits

Tues, Thurs 10:35-11:50 A.M. Room CUE 418
Lectures – Live and Recorded Online
Discussions Live and on Zoom, Thurs. 10:35-11:50 A.M.

Course Director: Dr. Michael Skinner
Line Number:
SLN (475) – 06763
SLN (575) – 06764

Lectures offered live and through Canvas / Panopto and Discussion Sessions live and on Zoom for other WSU campuses

Course Contents:
Current literature based course on the Systems Biology of Mammalian Reproduction. Systems approaches to the biology of reproduction from a molecular level to physiological level of understanding will be considered.

First Lecture January 9, 2024

Online version of course offered on Global Campus
SLN: (475) – 13946, (575) – 13947

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review

Instruction Format –
- One 1.5 hour overview/lecture per week (In person and Panopto Recording)
- One 1.5 hour literature review presentations/discussion session per week (In person and Live and Zoom session Thursdays 10:30-Noon)

Course Requirements –

1. Attendance
2. Participation in literature and discussion sessions

Graduate Students:

3. Grant Proposal (12 page limit) due week 16
4. Student Grant Review session week 17

Undergraduate Students:

3. Two take home exams (weeks 9 & 17)

Late Work is discouraged but can be arranged if notified ahead of time for uncontrolled circumstances, such as health issues.

Grading Policy –
- Both in class attendance (10%) and discussion participation & answers (25%) and (graduate students) the proposal (65%) or (students) exams (65%) will be factors considered.
- Grading scale A(90%), B(80%), C(70%), D(60%), F(<60%)

References and Textbooks –
- Reading literature and references provided one week prior to session
- No required textbook (suggested reading provided from selected literature and review articles)

Graduate Students

Grant Proposal

Outline:
- Title
- Abstract
- Specific Aims Page
- Background
- Preliminary Results
- Experimental Design and Methods (Approach)
- References (not part 12 pages)

(12 page maximum single spaced typed limit)

Key Points:
- Specific aims should be focused, concise and investigate hypothesis
- Be as concise and direct as possible
- Work significance of proposal into grant when appropriate
- Use only critical preliminary results (literature derived results fine)

Additional Information:
- Propose short-range studies to address long-range goals
- Write grant for 3 to 4 year period to complete studies
- Feasibility of success is critical, ask right type of question
- Experimental design needs to address hypothesis

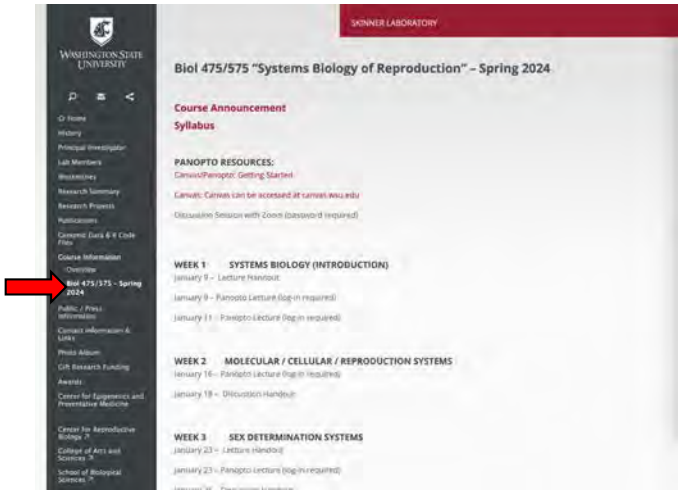
Score/Rating:
Factors involved: Type question addressed, organization of thoughts, preliminary results, feasibility, reasonable completion expectations, focus of aims and proposed studies.

Score		
1.0 - 1.5	**Outstanding**	Funded
1.5 - 2.0	**Excellent**	Probably Funded
2.0 - 2.5	**Good**	Accepted, but not Funded
2.5 - 3.0	**Satisfactory**	
3.0 - 3.5	**Adequate**	
3.5 - 4.0	**Fair**	
4.0 - 5.0	**Acceptable**	

Review:
NIH Study Section style review with all students/fellows participating in the review. Primary and secondary reviewers will be selected and all grants will be critiqued.

Note:
Welcome to use opportunity to prepare grants for student orals or fellowship applications.

www.skinner.wsu.edu



PANOPTO GUIDE FOR STUDENTS

1) Downloading Recorder

- Log in to wsu.hosted.panopto.com
- Download link is in the upper right-hand corner

2) Getting Help

- Call Panopto at 1 (855.726.6786) between 5 a.m. 5 p.m. M-F
- Email support@panopto.com
- Visit http://support.panopto.com/documentation
- Visit CougTech in CUE 302

3) Recording Spaces

- Reserve a study space at calendars.libraries.wsu.edu
- AMS in Holland 150. Phone: (509) 335-4535
- CougTech in CUE 302 also has a limited amount of spaces

4) Laptop Checkout

- AMS has a small number of laptops available for checkout
  - Up to 90-minute checkouts
- The WSU Libraries also have laptops available
  - Must stay within library buildings
- CougTech rents laptops to students, rates below:
  - https://cougtech.wsu.edu/Services/RentalRates.aspx

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Reproduction Overview and Systems Biology
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesdays & Thursdays
January 9, 11, 16, 2024
Weeks 1 and 2

Reproductive Biology Overview

Importance of Reproduction
- Define animal (mineral/vegetable/animal)
- Define area in biology

History Reproduction/Thought
- Testis/Ovary
- Aristotle
- Harvey
- 1930's Steroids/Inhibin/Cycle

Reproductive Biology Areas
Reproduction Problems/Questions
- Contraception/Society problems

Required Reading

Ganong W., Medical Physiology, (2012) Chapter 23

Skinner MK, Jegou B (2018) Historic Considerations in Male Reproduction in: Encyclopedia of Reproduction 2nd Edition, Ed: MK Skinner. Elsevier. Vol 1: 3-9.

Reproduction Background References

Encyclopedia of Reproduction, Second Edition (2018) Volumes 1-6, Editor-in-Chief Michael K. Skinner.
Open Access through WSU library:
https://www.sciencedirect.com/referencework/9780128151457/encyclopedia-of-reproduction

Systems Biology

- History and Definitions
- Revolutionary and Evolutionary Systems Biology
- Reductionism/ Genetic Determination
- Holism/ Emergentism/ Homeostasis or Robustness
- Networks and Computational Biology
- Basic Molecular and Cellular Components
- Omics and Technology

Required Reading

Knepper, et al., (2014) Systems biology versus reductionism in cell physiology. Am J Physiol Cell Physiol 307:C308-309

Systems Biology Background Book References

Eberhard Voit (2012) A First Course in Systems Biology, Garland Science

Capra and Luisi (2014) The Systems View of Life, Cambridge University Press.

Spring 2024 – Systems Biology of Reproduction
Discussion Outline (Systems Biology)
Michael K. Skinner – Biol 475/575
Weeks 1 and 2 (January 18, 2024)

Systems Biology

Primary Papers

1. Westerhoff & Palsson (2004) Nat Biotech 22:1249-1252
2. Joyner (2011) J Appl Physiol 111:335-342
3. Stanoev, et al. (2021) Development 148:dev197608
4. Zhang, et al. (2023) Human Reproduction Update 00(0), 1-17

Discussion

Student 1 - Ref #1 above
-How does this support evolutionary systems biology?
-Give an example that supports this perspective.

Ref #2 above
-What is the problem with reductionism?
-What is the void?
-What is the solution?

Student 2 - Ref #3 above
-What is robustness?
-What is the role of inhomogeneous steady state?
-What insights in the systems biology of cellular differentiation are described?

Student 3- Ref #4 above
-What are spatial transcriptomes?
-What is the experimental design?
-What reproductive tissues used and insights obtained?

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Reproductive Biology Areas

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Skinner MK, Encyclopedia of Reproduction (2018) Volume 1, Chapter 2

REPRODUCTIVE BIOLOGY

- Define animal

- Central to any biological system

• Reproduction ⟷ Propagation of Species

Required
- Survival Species
- Genetic Diversity
- Propagate Numbers

REPRODUCTIVE BIOLOGY

• Oldest medical discipline (obstetrics)/Scientific Area

• Many Species only Reproduce

• Theology- Atheism – Reprod Purpose Life

Historic Considerations Impacting Male Reproduction

Reproductive Topic	Observation	Period
Castration	Testis first organ interest Domestication animals Eunuch characteristics	Prehistoric / neolitic Assyrian / Egyptian / Greek societies
Reproduction Theory	Theory male provide seed (testis involved) active role and female provide soil passive role (prevailing theory 2000 years)	Aristotle (384 – 322 BC)
Environmental Sex Determination	Theory environmental factors such as heat (fire) and cold (water) promotes sex determination for male or female	Aristotle (384 – 322 BC)
Spermist Theory / Animalculism	One of the two theory of preformationism for which sperm head contained preformed offspring (homunculus) and female provide passive environment for development	Nicolaas Hartsoeker 1600s
Ovism	The other theory of preformation for which the embryo is preformed in the ovum (egg) while the sperm only provides an aura seminalis", that is a vital essence	1600s-1700s
Female Ovary & Egg Role Reproduction	Egg produced in ovary and has active role reproduction with sperm	William Harvey and Bishop Niels Stenson 1600s
Male Reproductive Tract Organs Anatomy	Detailed anatomy of male reproductive tract organs and specialized roles	Vesale 1543s and De Graaf 1600s
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HISTORY REPRODUCTION:

TESTIS

First organ interest (prehistoric periods)

Castration
- Domestication of animals
- Greek Mythology
- Society (punishment, eunuch, biblical) – *every known society*
- Assyrian Societies (noted)

Link to reproduction noted/importance?

OVARIES

- Egyptians (3000 BC) hicroglyph ♈ (womb)

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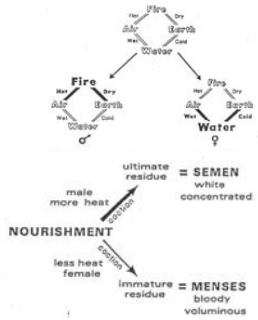
ARISTOTLE – (384 – 322 BC)

Castration (testis/ovary) → no reproduction
(first recorded ovorectomy procedure)

Reproductive Theory
- Man provide seed (testis involved) active
- Woman provided soil to allow growth (ovary involved) passive

(Man propagate active) 2000 years prevailing theory

Sex Determination Theory (Aristotle, 400 BC)



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Harvey Introduced –
LEEWENHOCK (1678) with crude microscope, 1696 Hartsoeker see in sperm (animal)

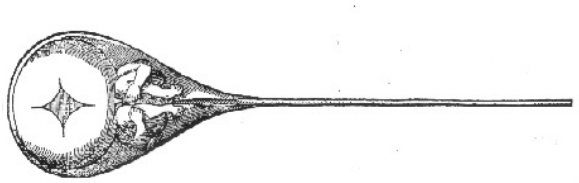
Sperm origin

- Aristotle → Brain → Testis
- Testis process from blood
- With microscope → testis origin

Spallanzani (1780)

- Spermatozoa are fertilizing agent and successfully artificially inseminated a dog

Homunculus (Hartsoeker 1696)



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WILLIAM HARVEY (1578 – 1657)

- Discovered circulation blood
- Observed follicle development in ovary
- Theory ovary play important role in reproduction so woman active role not just soil – (major debate of time). Counter Aristotle idea
- Did not discover egg

During same time as Harvey –
BISHOP NIELS STENSEN – (1638 – 1686)

- First examined fish ovaries → egg
- Later examine mule/horse/donkey due to lack eggs in donkey – no reproduction so proposed egg import reproduction

Fig. 3.9. Detail from the frontispiece of William Harvey's book *De Generatione Animalium*, published in 1651. The hands are those of Jove.

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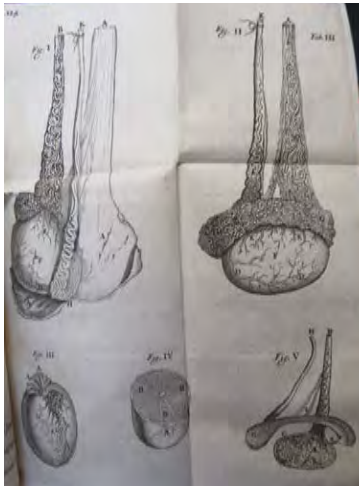
DI VINCHI (1452 – 1519 AD)

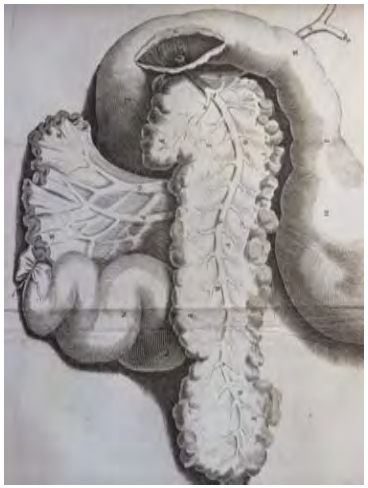
- Example anatomist
- Drawings – ovary recorded

(Anatomy prevalent science)

Testis and Epididymis Anatomy (De Graaf, 1677)



Seminal Vesicle Anatomy (De Graaf, 1677)



Sertoli Cell Structure (E. Sertoli, 1865)

Historic Considerations Impacting Male Reproduction

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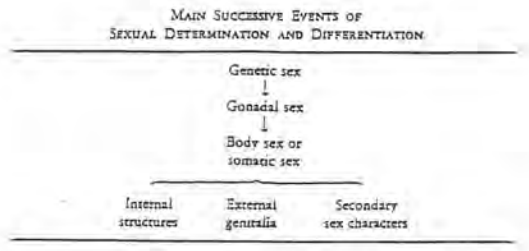
Dumas (1825)

- Proved sperm were the fertility agent

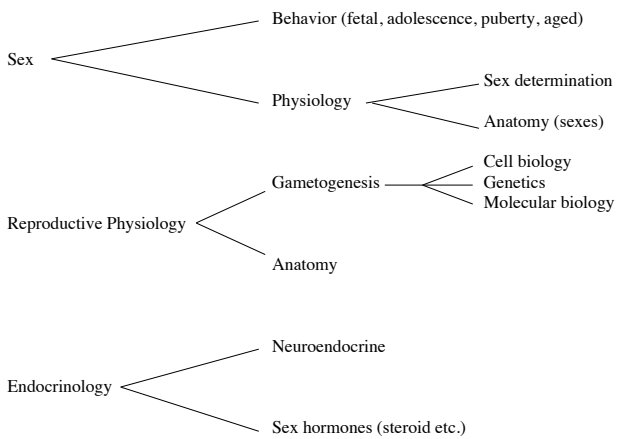
1930's

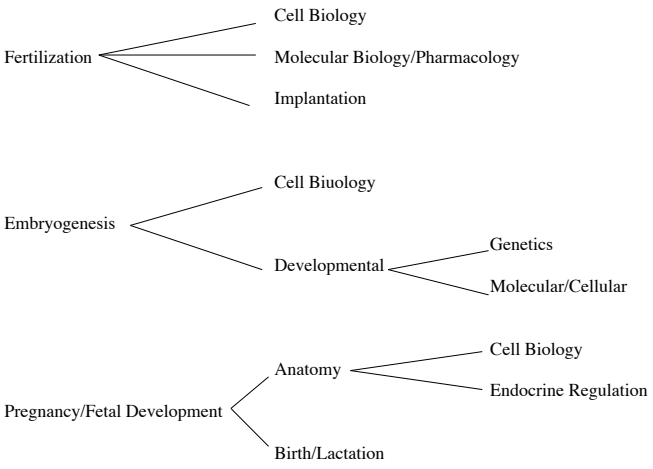
- Steriods discovered - Estrogen/Androgen
- Pituitary/Gonadal axis
- Inhibin discovered

Sex Determination Theory (Jost, 1940s)



GENERAL AREAS OF REPRODUCTIVE BIOLOGY



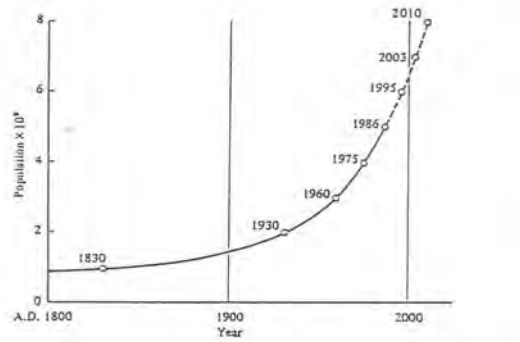


GENERAL BIOLOGICAL PROBLEMS/QUESTIONS WHICH CAN UTILIZE REPRODUCTIVE BIOLOGY

- Fertility/Contraception
- Disease
  - Reproductive Medicine
  - Immunology
- Cancer
  - Breast Cancer
  - Prostate Cancer
- Cell Biology
  - Growth Regulation
  - Cellular Communication
  - Cell Differentiation and Development
- Molecular Biology
  - Gene Structure
  - Transcriptional Regulation

GENERAL WORLD PROBLEMS WHICH CAN BENEFIT FROM REPRDOCUTIVE BIOLOGY RESEARCH

- Sociological & Economic Problems in Underdeveloped Countries
- World Famine
- Green House Effect
- Conservation Biology



CURRENT AND FUTURE

ASSISTED REPRODUCTIVE TECHNOLOGY

IVF
Fertility
Cloning
Transgenes/Genetic Modify/Oocyte Modify
Stem Cells

INDUSTRY

- Cattle
- Dairy
- Aquaculture
- Agriculture

COURSE GOALS UTILIZE SYSTEMS BIOLOGY STUDY REPRODUCTIVE TISSUE OR PROCESSES

To address general problems/questions

- Cellular & molecular biology
- Reproductive Biology/ Physiology
- Classic/current/advanced research reviewed
- Molecular ⟷ Physiological

Systems Biology

- History and Definitions
- Revolutionary and Evolutionary Systems Biology
- Reductionism/ Genetic Determination
- Holism/ Emergentism/ Homeostasis or Robustness
- Networks and Computational Biology
- Basic Molecular and Cellular Components
- Omics and Technology

Required Reading

Knepper, et al., (2014) Systems biology versus reductionism in cell physiology. Am J Physiol Cell Physiol 307:C308-309

Antony, et al., (2012) From systems biology to systems biomedicine. Curr Opin Biotechnol. 23(4):604-8

Systems Biology

Definition
History
Theory
Paradigm Shift
Parameters

Systems biology is a comprehensive quantitative analysis of the manner in which all the components of a biological system interact functionally over time. Such an analysis is executed by an interdisciplinary team of investigators that is also capable of developing required technologies and computational tools. In this model, biology dictates what new technology and computational tools should be developed, and, once developed, these tools open new frontiers in biology for exploration. Thus, biology drives technology and computation, and, in turn, technology and computation revolutionize biology.

"systems biology is the study of *an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions which give rise to life"(Hood* 2005).

Systems Biology Theory

Evolutionary Systems Biology- Extension of classical biology paradigm with new technology

Revolutionary Systems Biology- New paradigm shift in biology with altered perspective on causal relationships and systems

Evolutionary Systems Biology History

Systems biology extension current paradigm and history of biology with new technology

300BC Aristotle, System has 4 properties or causes: Material, Formal, Efficient,Teleological
200AD Galen (Roman Physician), Teleological important role in organism function
1500s Fernel, Systematic approach Anatomy
1600s Harvey, Physiology, Cell Biology, Circulation
1700s Newton, Physics leads to mechanistic determinism to explain systems
La Mettrie, Define Biological Machine (eg Clock)
1800s Bernard, Father physiology and integration biological systems (milieu interieur)
1900s Cannon, Biological equalibrium and homeostatsis
-Discovery DNA/Structure/Genes (Molecular Biology)
-Computational Biology (non-equalibrium thermodynamics and kinetics metabolism)
2000s -Genome Sequence
-Omics Technology

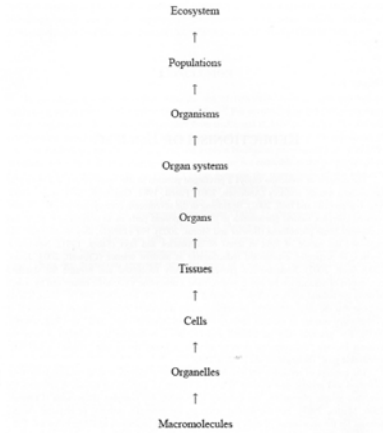


Figure 1. Hierarchical relationships involved in reductionism. The figure is not complete or exhaustive since non-represented phenomena exist at both ends; rather, it is simply illustrative of hierarchical, causal relationships.

Evolutionary System Biology Definitions

Extension of traditional biological paradigm

Marc Kirchner 2005

"Systems biology is the study of the behavior of complex biological organization and processes in terms of the molecular constituents"

Westerhoff and Alberghina 2005

Systems biology is "nothing but good old physiology" or that is "molecular biology claiming additional money"

Sorger 2005

"System biology aim is to build numerical models of biological processes and test the models experimentally"

Revolutionary Systems Biology History

Jan Smuts (1870-1950), South Africa, Defined-**Holism** (Tendency in nature to form wholes that are greater than the sum of the parts through creative evolution)
Alfred Whitehead (1861-1947), USA, Defined- **Organisms** (Philosophy of organism to explain the complexity of natural processes- including biological organisms)
Ludwig von Bertalanffy (1901-1972), Austria, Defined- **Disequalibrium** (Biological organisms are open systems, which respond to changes in environment, such that dis equalibrium is state of living organism and equalibrium is death)
Norbert Wiener (1894-1964), USA, Defined- **Cybernetics** (Application mathematics to explain biological mechanisms)
Joseph Woodger (1894-1981), UK, Defined- **Bauplan** (Bauplan as the essential structural plan or morphology of an organism body plan, eg vertebrates)
Conrad Waddington (1905-1975), Scotland, Defined- **Epigenetics** (Discuss later)
Walter Elsasser (1904-1991), Hungarian, Defined- **Biotonic** (Laws not reducible to physical or chemical laws)

1980s Theoretical Biology Holism (Elsasser and Laszlo) (Butterfly Effect)
Chaos Theory (Mathematical approach complex systems)
1990s High throughput sequencing and expansion epigenetic area
2000s Sequence genome and transcriptome (Omics technologies)

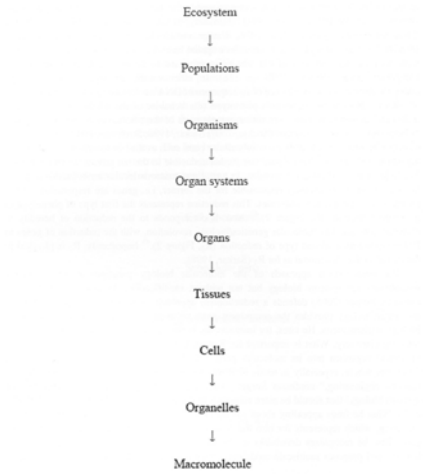


Figure 3. Hierarchical relationships involved in holism. The figure again is not complete or exhaustive but rather illustrative of hierarchical, causal relationships.

Definitions for Systems Biology

Leroy Hood (2005)

"The inter-relationships of all the elements in a system rather than studying them one at a time"

Methodological Approach-

1) Develop simple descriptive, graphical, or mathematical model of how system functions
2) Identify and define the various components of the system and their state (eg omics)
3) Disturb the system with external perturbation and document changes in the components
4) Integration of the two data sets from step 3 and comparison to model in step 1
5) Adjust model until harmony or conjunction exists between data and model

Hiroaki Kitano (2002)

Four factors for comprehensive systems biology definition

1) System Structure, organization of components (macromolecules, genes, cells, tissues etc
2) System Dynamics, interactions between or relationships of the various hierarchical levels over time
3) Systems Control Method, regulatory mechanisms involved in the maintenance of the organizational hierarchy
4) Systems Design Method, hierarchical organization with specific properties and manipulate

Table 1. Comparison of features for revolutionary and evolutionary systems biology

Revolutionary systems biology	Evolutionary systems biology
1. Holism	Reductionism
2. Top-down causation	Bottom-up causation
3. Epigenetics	Genetic determinism
4. Emergentism	Mechanism
5. Synergism	Synthesis
6. Robustness	Homeostasis
7. Nonlinear dynamics	Linear stasis

Reductionism

The view that the ultimate scientific understanding of a range of phenomena is to be gained exclusively from looking at the constituents of these phenomena and their properties

Ontological Reductionism

That complex phenomena are reducible to or determinable by simpler entities and forces that compose them (eg genetic determinism) and (bottom-up or upward causation)

Methodological Reductionism

Reducing wholes to parts and explaining the higher levels in terms of lower ones as the ultimate direction for all scientific research (eg physics)

Epistemological Reductionism

Reduction of scientific knowledge, whether in terms of theories, laws, or explanations, from a higher level of organization to that of a lower or more basic one

The fall and rise of pharmacology--(re-)defining the discipline?
Winquist RJ, Mullane K, Williams M.
Biochem Pharmacol. 2014 Jan 1;87(1):4-24.

Abstract

Pharmacology is an integrative discipline that originated from activities, now nearly 7000 years old, to identify therapeutics from natural product sources. Research in the 19th Century that focused on the Law of Mass Action (LMA) demonstrated that compound effects were dose-/concentration-dependent eventually leading to the receptor concept, now a century old, that remains the key to understanding disease causality and drug action. As pharmacology evolved in the 20th Century through successive biochemical, molecular and genomic eras, the precision in understanding receptor function at the molecular level increased and while providing important insights, led to an overtly reductionistic emphasis. This resulted in the generation of data lacking physiological context that ignored the LMA and was not integrated at the tissue/whole organism level. ***As reductionism became a primary focus in biomedical research, it led to the fall of pharmacology.*** However, concerns regarding the disconnect between basic research efforts and the approval of new drugs to treat 21st Century disease tsunamis, e.g., neurodegeneration, metabolic syndrome, etc. has led to the reemergence of pharmacology, its rise, often in the semantic guise of systems biology. Against a background of limited training in pharmacology, this has resulted in issues in experimental replication with a bioinformatics emphasis that often has a limited relationship to reality. The integration of newer technologies within a pharmacological context where research is driven by testable hypotheses rather than technology, together with renewed efforts in teaching pharmacology, is anticipated to improve the focus and relevance of biomedical research and lead to novel therapeutics that will contain health care costs.

Neuropharmacology beyond reductionism - A likely prospect.
Margineanu DG.
Biosystems. 2016 Mar;141:1-9.

Abstract

Neuropharmacology had several major past successes, but the last few decades did not witness any leap forward in the drug treatment of brain disorders. Moreover, current drugs used in neurology and psychiatry alleviate the symptoms, while hardly curing any cause of disease, basically because the etiology of most neuro-psychic syndromes is but poorly known. This review argues that this largely derives from the unbalanced prevalence in neuroscience of the analytic reductionist approach, focused on the cellular and molecular level, while the understanding of integrated brain activities remains flimsier. The decline of drug discovery output in the last decades, quite obvious in neuropharmacology, coincided with the advent of the single target-focused search of potent ligands selective for a well-defined protein, deemed critical in a given pathology. However, all the widespread neuro-psychic troubles are multi-mechanistic and polygenic, their complex etiology making unsuited the single-target drug discovery. An evolving approach, based on systems biology considers that a disease expresses a disturbance of the network of interactions underlying organismic functions, rather than alteration of single molecular components. Accordingly, systems pharmacology seeks to restore a disturbed network via multi-targeted drugs. This review notices that neuropharmacology in fact relies on drugs which are multi-target, this feature having occurred just because those drugs were selected by phenotypic screening in vivo, or emerged from serendipitous clinical observations. The novel systems pharmacology aims, however, to devise ab initio multi-target drugs that will appropriately act on multiple molecular entities. Though this is a task much more complex than the single-target strategy, major informatics resources and computational tools for the systemic approach of drug discovery are already set forth and their rapid progress forecasts promising outcomes for neuropharmacology.

Holism (Revolutionary Systems Biology)

The living world consists in a reality that can be understood only in its global and inseparable unity. The whole is fundamental, not any one level. The whole is greater than the sum of its parts or of its levels.

Ontological Holism

Putting together the parts will not produce the wholes (such as living systems) or account for their properties and behaviors. Downward causation claims that higher order entities determine causally the properties or behavior of lower-level entities.

Methodological Holism

That life can only be understood by studying it as a whole. The world is disordered and it recognized that each hierarchical level requires its own research strategy not reducible to the methodological strategy below it.

Epistemological Holism

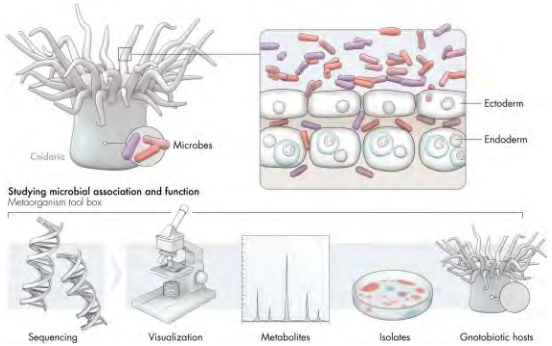
Complex wholes are considered not to be understandable from the mere knowledge of the behavior of the parts in isolation; only properties of the system as a whole may offer understanding.

The new holism: P4 systems medicine and the medicalization of health and life itself.
Vogt H, Hofmann B, Getz L.
Med Health Care Philos. 2016 Jun;19(2):307-23.

Abstract

The emerging concept of systems medicine (or 'P4 medicine'-predictive, preventive, personalized and participatory) is at the vanguard of the post-genomic movement towards 'precision medicine'. It is the medical application of systems biology, the biological study of wholes. Of particular interest, P4 systems medicine is currently promised as a revolutionary new biomedical approach that is holistic rather than reductionist. This article analyzes its concept of holism, both with regard to methods and conceptualization of health and disease. Rather than representing a medical holism associated with basic humanistic ideas, we find a technoscientific holism resulting from altered technological and theoretical circumstances in biology. We argue that this holism, which is aimed at disease prevention and health optimization, points towards an expanded form of medicalization, which we call 'holistic medicalization': Each person's whole life process is defined in biomedical, technoscientific terms as quantifiable and controllable and underlain a regime of medical control that is holistic in that it is all-encompassing. It is directed at all levels of functioning, from the molecular to the social, continual throughout life and aimed at managing the whole continuum from cure of disease to optimization of health. We argue that this medicalization is a very concrete materialization of a broader trend in medicine and society, which we call 'the medicalization of health and life itself'. We explicate this holistic medicalization, discuss potential harms and conclude by calling for preventive measures aimed at avoiding eventual harmful effects of overmedicalization in systems medicine (quaternary prevention).

Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches.
Jaspers C, Fraune S, Arnold AE, Miller DJ, Bosch TCG, Voolstra CR; Consortium of Australian Academy of Science Boden Research Conference Participants.
Zoology (2019) 133:81-87.



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7. Nonlinear dynamics	Linear stasis

Genetic Determinism

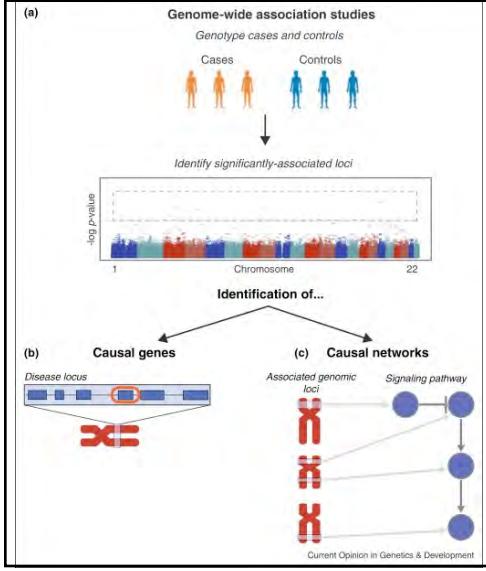
The view that genes (genotype) cause traits (phenotype)

Genetic determinism also referred to as- Geneticism, Genetic Essentialism and Genetic Fatalism

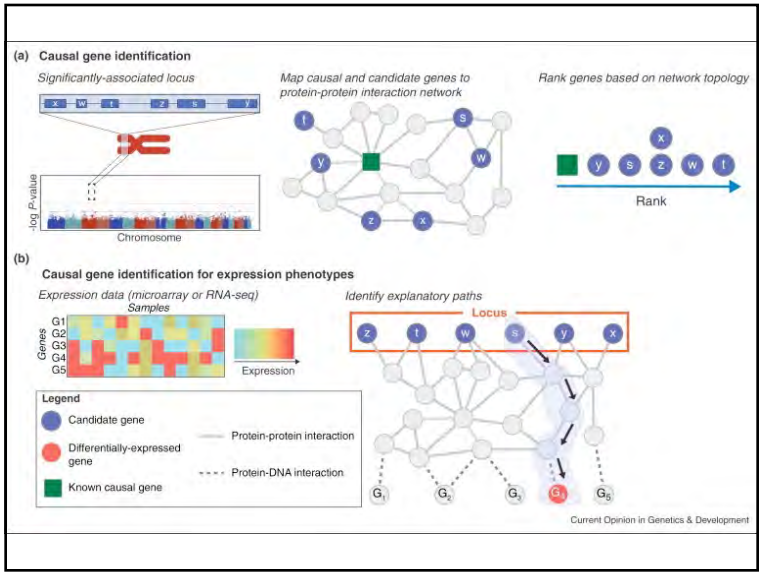
Strong Genetic Determinism- genotype "always" dictates phenotype

Weak Genetic Determinism- genotype "sometimes" dictates phenotype, also potentials or predispositions

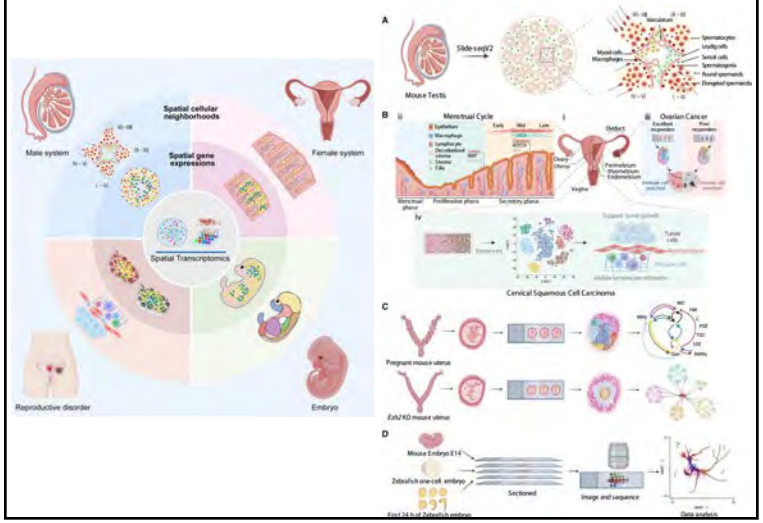
Classical Genetics (Mendel) to Molecular Genetics (DNA) to Molecular Biology



Two applications of network-based analyses of GWAS. (a) GWAS analysis computes the association between a SNP and case/control, reporting a P-value for each SNP. (b) Causal gene identification is the problem of identifying a single causal gene (circled in red) for the phenotype from a larger locus of candidate genes that is significantly associated with the phenotype. (c) Causal network identification is the problem of finding a group of interacting genes (e.g. a signaling pathway or protein complex) containing SNPs that distinguish cases and controls.



Dissecting mammalian reproduction with spatial transcriptomics
Zhang X, Cao Q, Rajachandran S, Grow EJ, Evans M, Chen H.
Hum Reprod Update. 2023 Jun 23:dmad017. doi: 10.1093/humupd/dmad017. Online ahead of print.



After geneticization.

Arribas-Ayllon M.
Soc Sci Med. 2016 Jun;159:132-9.

Abstract

The concept of geneticization belongs to a style of thinking within the social sciences that refers to wide-ranging processes and consequences of genetic knowledge. Lippman's original use of the term was political, anticipating the onerous consequences of genetic reductionism and determinism, while more recent engagements emphasise the productivity and heterogeneity of genetic concepts, practices and technologies. This paper reconstructs the geneticization concept, tracing it back to early political critiques of medicine. The argument is made that geneticization belongs to a style of constructionist thinking that obscures and exaggerates the essentializing effects of genetic knowledge. Following Hacking's advice, we need a more literal sense of construction in terms of 'assembly' to give a clearer account of the relationship between processes and products. Using the 'assemblage' concept to explore the social ontology of genetics, the paper reviews three areas of the empirical literature on geneticization - disease classification, clinical practice and biosociality - to show that a new style of thinking has appeared within the social sciences. In the final assessment, the conditions that gave rise to geneticization are now obsolete. While it may serve as a useful ritual of debate, conceptually geneticization offers a limited account of the heterogeneity of socio-technical change.

Epigenetics

Waddington (1940s) coined term to describe environment-gene interactions that promote phenotype.

Non-genetic factors in the control of developmental processes and phenotype (? anti-genetic determinism)

Art Riggs (1996), defined as "mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence"

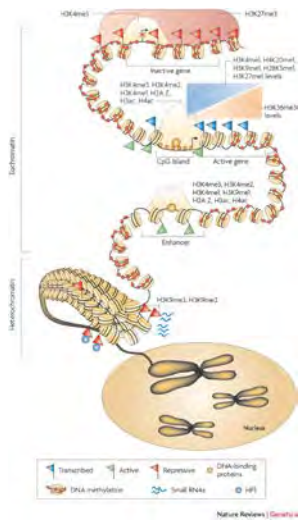
Epigenetics represents for many systems biologists a promise for control of biological phenomena unfulfilled by genetic determinism (Silverman 2004)

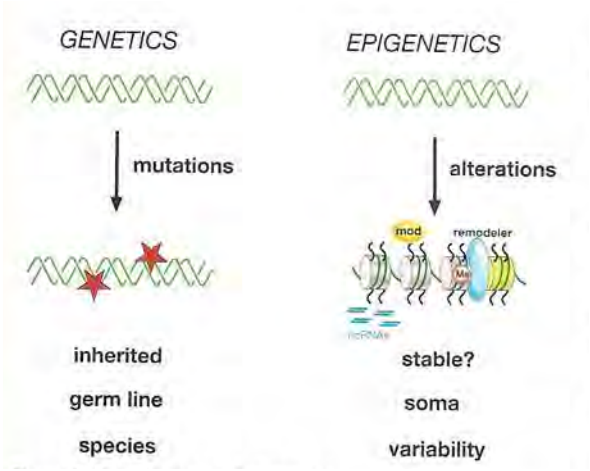
Epigenetics

Molecular factors/processes around the DNA that regulate genome activity, independent of DNA sequence, and are mitotically stable

Epigenetic Mechanisms of Gene Regulation

-DNA Methylation
-Histone Modification
-Chromatin Structure
-DNA Organization into Domains (eg Loops)
-Nuclear Compartmentalization (eg nuclear matrix)
-Noncoding functional RNAs





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7. Nonlinear dynamics	Linear stasis

Mechanism and Emergence

Mechanism-

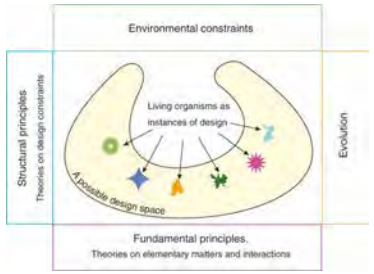
Glennan 2002- "is a complex system that produces that behavior by the interaction of a number of parts, where the interactions between parts can be characterized by direct, invariant, change relating generalizations"

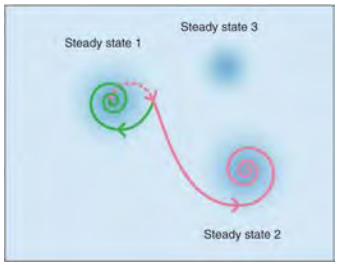
Machamer, Darden, Craver 2000- "are intities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions" (A to B to C)

Mechanisms are especially open to investigation particularly through experimentation

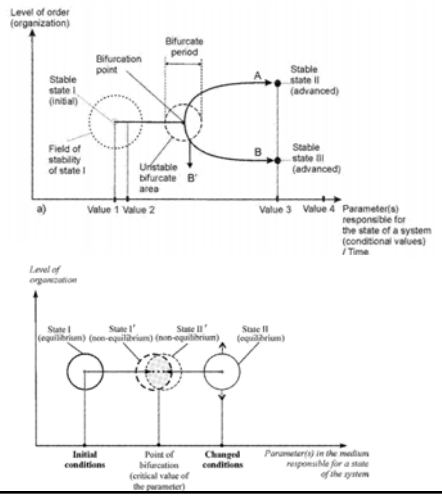
Emergence. Complex systems display properties, often called "emergent properties," that are not demonstrated by their individual parts and cannot be predicted even with full understanding of the parts alone. For example, understanding the properties of hydrogen and oxygen does not allow us to predict the properties of water. Life is an example of an emergent property. It is not inherent in DNA, RNA, proteins, carbohydrates, or lipids but is a consequence of their actions and interactions. A comprehensive understanding of such emergent properties requires systems-level perspectives and cannot be gleaned from simple reductionist approaches.

"What is the difference between a live cat and a dead one? One scientific answer is systems biology. A live cat is the emergent behavior of the system incorporating those parts."





Emergence of biological organization through thermodynamic inversion.
Kompanichenko V.
Front Biosci (Elite Ed). 2014 Jan 1;6:208-24.



Homeostasis vs Robustness

Homeostasis-

Claude Bernard (1800s)- "internal milieu's constancy"

Cannon (1939)- "steady states in the body…..a condition that may vary, but is relatively constant"

Miglani (2006)- "a mechanism for promoting the stability of phenotypic expression of a genotype when grown over a wide range of environments"

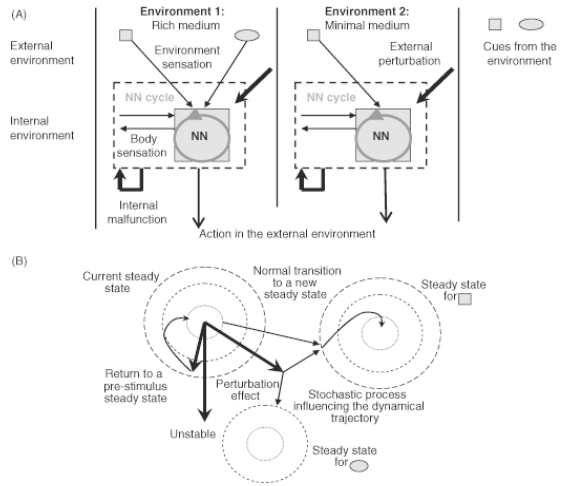
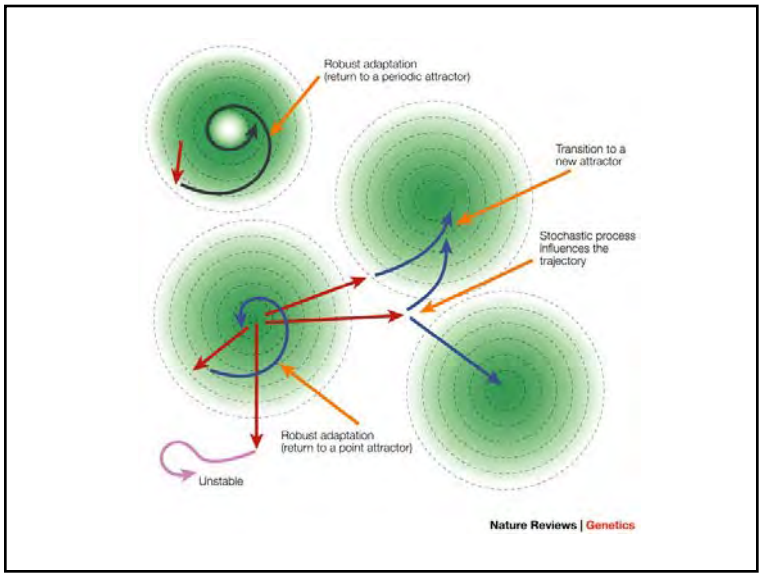


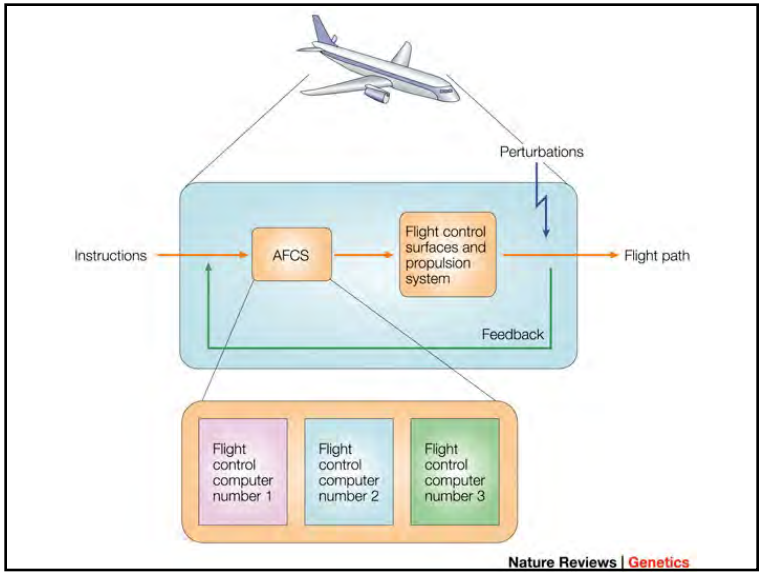
Illustration of environmental influences and the effect of perturbations on inner dynamics. In (A), two environments are shown (rich and minimal media). Plots adapted from (Freilich et al., 2010). In (B), a current state of an internal control can be modified by small or large perturbations (thick black arrows) pushing the agent–internal dynamics within the current boundary of attraction or far from it. NN, neural network. See main text for further details.

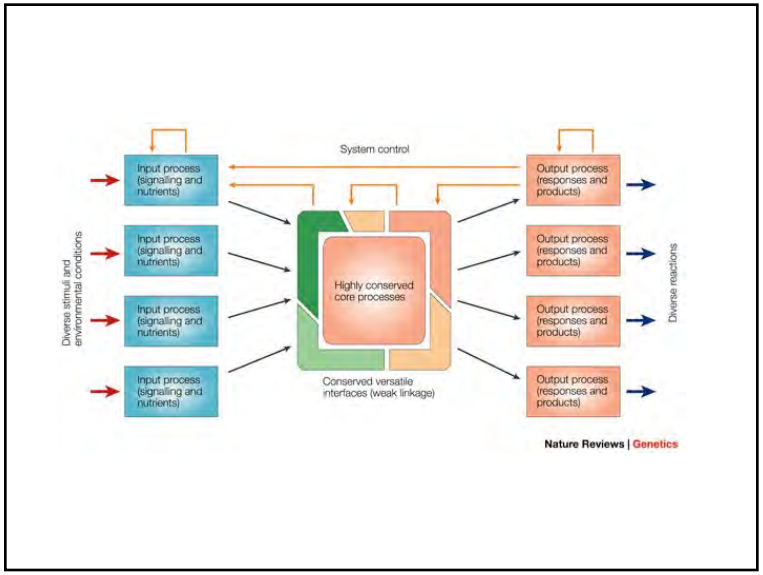
Robustness. Biological systems maintain phenotypic stability in the face of diverse perturbations imposed by the environment, stochastic events, and genetic variation. Robustness often arises through positive and negative feedback loops and other forms of control that constrain a gene's output. This feedback insulates the system from fluctuations imposed on it by the environment. Positive feedback, in general, enhances sensitivity, whereas negative feedback can dampen noise and reject perturbations. Robustness is an inherent property of all biological systems and is strongly favored by evolution.

Robustness as an organizational principle

Robustness enables the system to maintain its functionalities against external and internal perturbations. This property has been widely observed across many species, from the level of gene transcription to the level of systemic homeostasis.







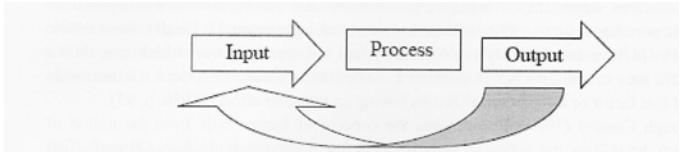


Figure 4. Feedback mechanism or loop.

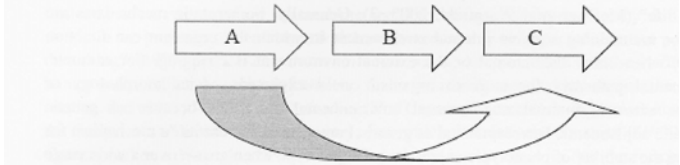
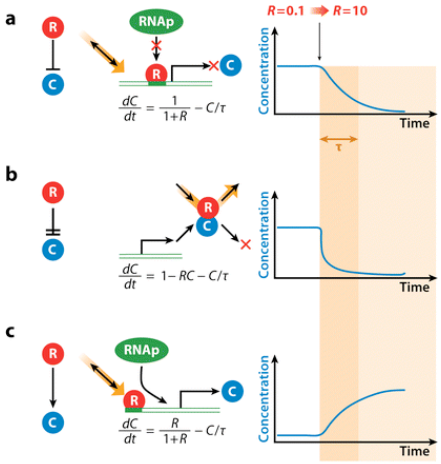
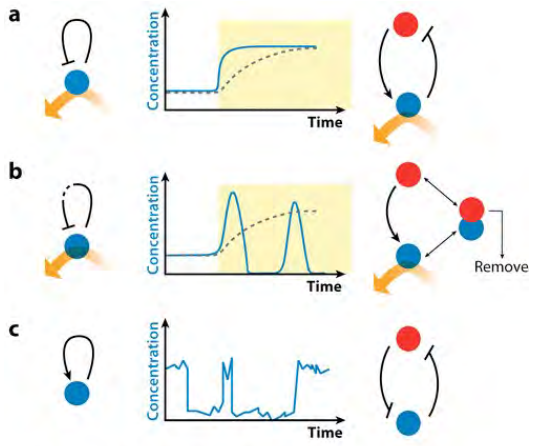


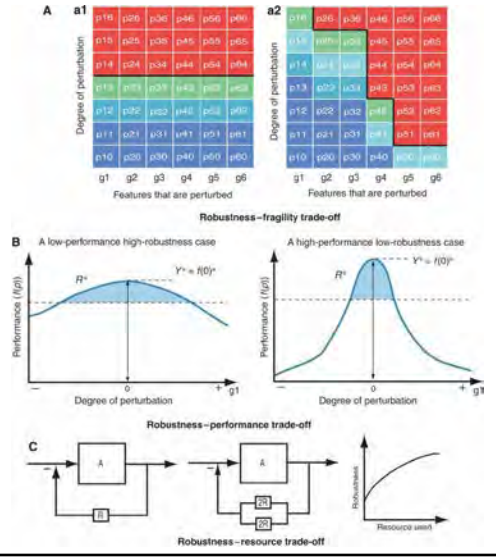
Figure 5. Feedforward mechanism or loop.

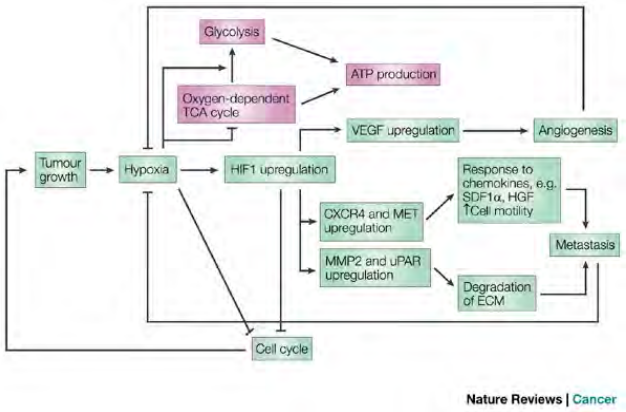


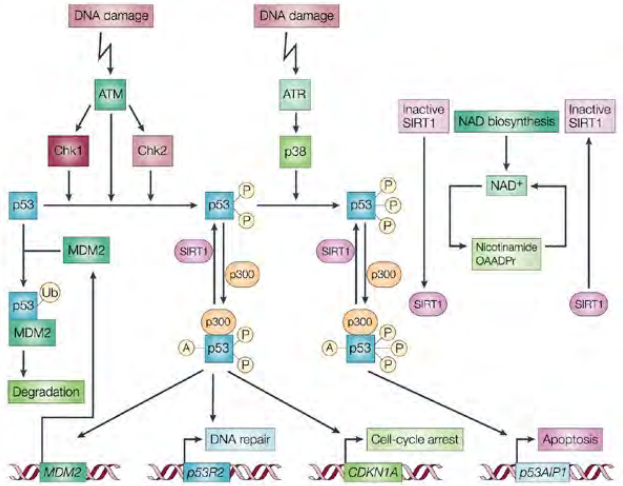
Sneppen K, et al. 2010.
Annu. Rev. Biophys. 39:43–59



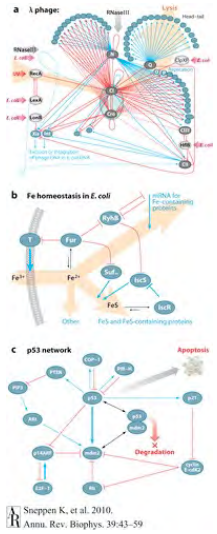
Sneppen K, et al. 2010.
Annu. Rev. Biophys. 39:43–59







Modularity. A further characteristic of complex systems is their modularity. Multiple useful definitions of a module exist. To an engineer, a module is a functional unit, a collection of parts that interact together to perform a distinct function. Such a module would have distinct inputs, things it is sensitive to, and outputs, things it controls. To a biologist, a module in a network is a set of nodes that have strong interactions and a common function. Modularity can contribute to both robustness of the entire system, by confining damage to separable parts, and to evolution, by simply rewiring modules. Furthermore, modularity decreases the risk of failure of the system by preventing the spread of damage in one part of the network throughout the entire network.



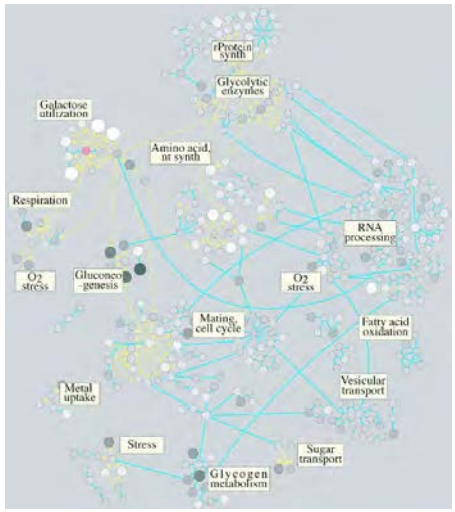


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Table 1. Categorisations of systems biology

	Type One	Type Two
Haubelt et al., 2000[37]		
Label	*Biological systems biology*	*Systems-oriented biology*
Precursors	Reductionist molecular biology	Cybernetics; network theory in electronics; biochemical systems theory (BST) and metabolic control analysis (MCA); cell biology
Focus	Integration of data from different levels & sources	System functions and properties
Huang, 2003[11]		
Label	*Localists*	*Globalists*
Precursors	Classical molecular biology	General networks (physics perspective); Kauffman[38]
Focus	Large datasets of constituent parts; 'pathway-centric'	Deeper principles of complex systems; wholes
Levesque & Benfey, 2004[39]		
Label	*Panomicists*	*Dynamicists*
Precursors	Reductionist molecular biology; genomics	Systems theory
Focus	Components; reconstruction of networks from high-throughput data	Modelling networks as complex systems; applying principles of systems theory
Westerhoff & Palsson, 2004[36]		
Label	*Biology-rooted systems biology*	*Systems-rooted biology*
Precursors	Mainstream molecular biology; genomics	Non-equilibrium thermodynamics; self-organisation; BST & MCA
Focus	Pattern recognition and phenomenological modelling of macromolecular interactions	New functional states arising from simultaneous interactions of multiple molecules; fundamental principles and laws

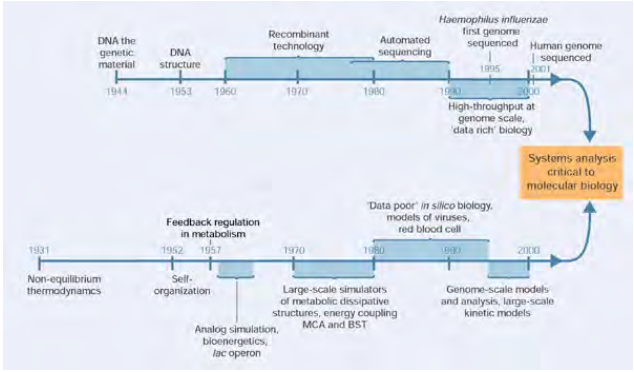
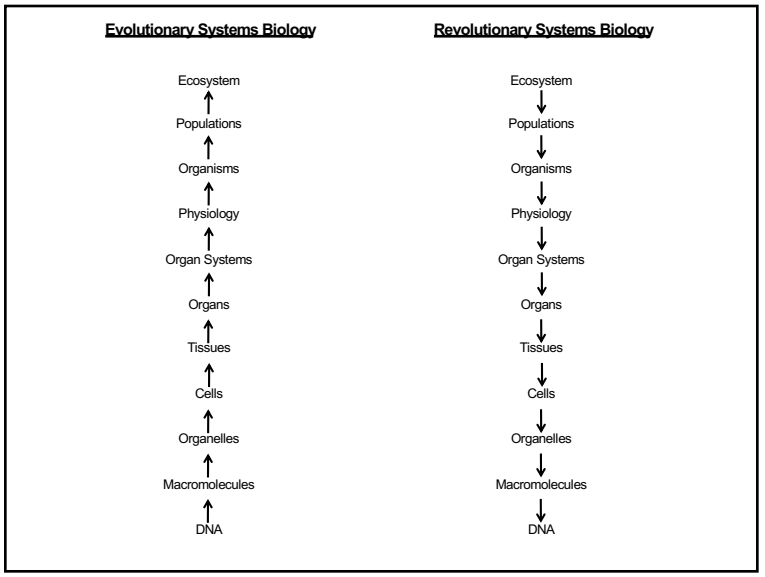
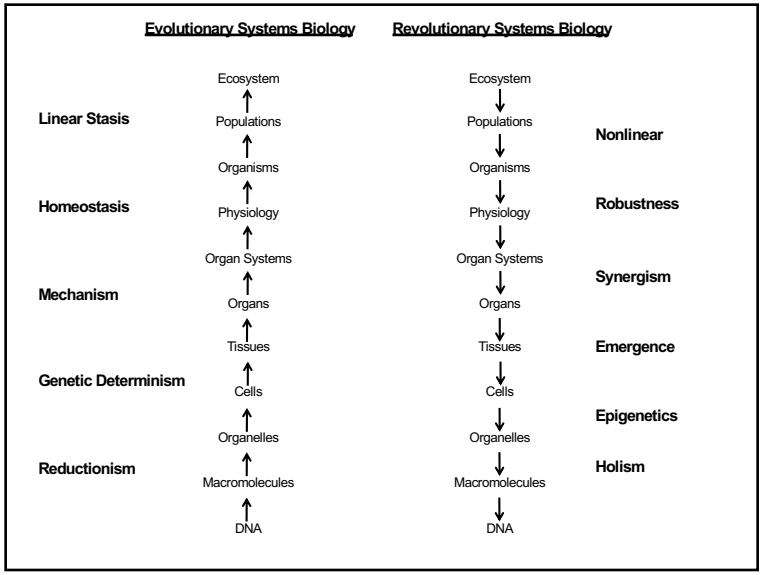
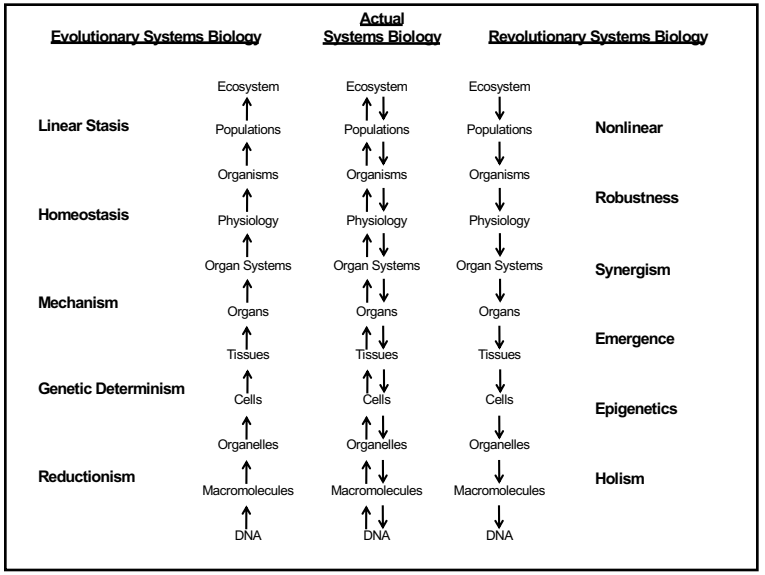


Table 1. Comparison of features for revolutionary and evolutionary systems biology

Revolutionary systems biology	Evolutionary systems biology
1. Holism	Reductionism
2. Top-down causation	Bottom-up causation
3. Epigenetics	Genetic determinism
4. Emergentism	Mechanism
5. Synergism	Synthesis
6. Robustness	Homeostasis
7. Nonlinear dynamics	Linear stasis







Top-down models in biology: explanation and control of complex living systems above the molecular level.

Pezzulo G, Levin M.

J R Soc Interface. 2016 Nov;13(124).

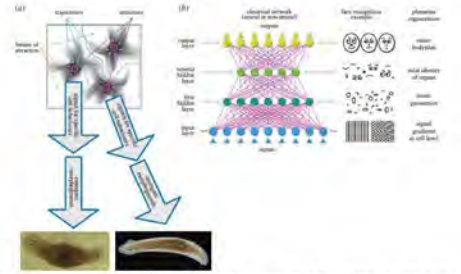


Figure 5. Representation of multiple levels within bioelectric (neural-like) networks. (a) Neural networks of different kinds—e.g. deep (set), attractor nets—are able to learn internal representations of external entities directly from (unannotated) input data, at multiple levels, as they become tuned to the statistics of their inputs during learning [47]. For example, generative neural networks trained to reproduce in output the same figures (e.g. faces, objects) or numbers) they receive in input often learn simpler-to-more-complex object or face features at increasingly higher hierarchical layers of the network, in ways that are (sometimes) biologically realistic [119,159]. Learning proceeds differently depending on the specific neural network architecture: For example, basins of attraction in the state space of attractor neural networks can represent specific memories [65]. If somatic tissues undergoing remodelling can be represented by similar frameworks, then it is possible that stable states in the network can represent specific patterning outcomes (like the one- or two-headed flatworms that result from editing of bioelectric networks in vivo [120–122]). (b) One advantage of neural network architectures is that they show a proof of principle of how collections of cells can represent higher-level topological information; here are shown analogies between information representation in an ANN during a face recognition tasks and in flatworm regeneration. Neural network layers represent progressively increasingly abstract features of the input, in well-understood ways. The functioning of specific neural network architectures such as attractor, generative or deep nets (or other [123–125]) may give clues as to how collections of somatic cells can learn and store memories about tissues, organs, and entire body plan layouts. The current state of the art in the field of developmental bioelectricity is that it is known, at the cellular level, how resting potentials are translated into downstream gene cascades, as well as which transcriptional and epigenetic targets are sensitive to change in developmental bioelectrical signals [126–128]. What is largely missing however (and may be provided by a network approach or other possible conceptualizations) is a quantitative understanding of how the global dynamics of bioelectric circuits make decisions that orchestrate large numbers of individual cells, spread out over considerable anatomical distances, towards specific pattern outcomes. The mechanisms by which bioelectrics and chromatin state interact at a single cell level will be increasingly clarified by straightforward reduction analysis. The more difficult, major advances in prediction and control will require a systems-level model of pattern memory and encoding implemented in somatic bioelectrical networks whose output is signals that control growth and form. Images in panels (a) and (b) drawn by Jeremy Guay of Peregrine Creative.

Systems Biology

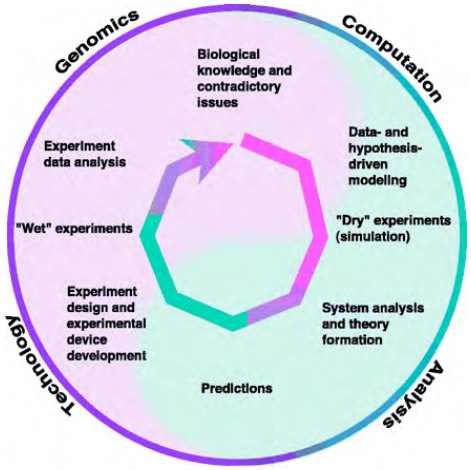
- History and Definitions
- Revolutionary and Evolutionary Systems Biology
- Reductionism/ Genetic Determination
- Holism/ Emergentism/ Homeostasis or Robustness
- Networks and Computational Biology
- Basic Molecular and Cellular Components
- Omics and Technology

Required Reading

Knepper, et al., (2014) Systems biology versus reductionism in cell physiology. Am J Physiol Cell Physiol 307:C308-309

Antony, et al., (2012) From systems biology to systems biomedicine. Curr Opin Biotechnol. 23(4):604-8

Evolutionary Systems Biology		Actual Systems Biology		Revolutionary Systems Biology	
Linear Stasis	Ecosystem ↑ Populations ↑ Organisms	Ecosystem ↑↓ Populations ↑↓ Organisms		Ecosystem ↓ Populations ↓ Organisms	**Nonlinear**
Homeostasis	↑ Physiology	↑↓ Physiology		↓ Physiology	**Robustness**
Mechanism	↑ Organ Systems ↑ Organs	↑↓ Organ Systems ↑↓ Organs		↓ Organ Systems ↓ Organs	**Synergism**
Genetic Determinism	↑ Tissues ↑ Cells	↑↓ Tissues ↑↓ Cells		↓ Tissues ↓ Cells	**Emergence** **Epigenetics**
Reductionism	↑ Organelles ↑ Macromolecules ↑ DNA	↑↓ Organelles ↑↓ Macromolecules ↑↓ DNA		↓ Organelles ↓ Macromolecules ↓ DNA	**Holism**



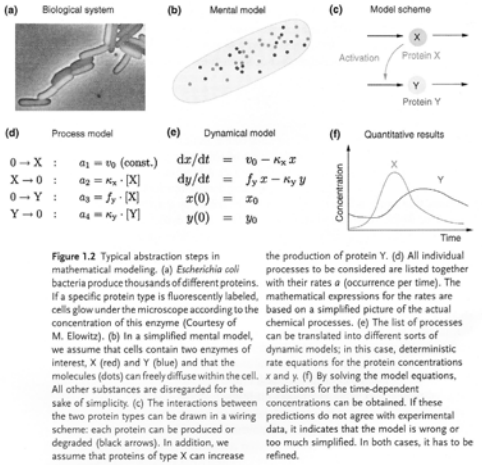


Figure 1.2 Typical abstraction steps in mathematical modeling. (a) *Escherichia coli* bacteria produce thousands of different proteins. If a specific protein type is fluorescently labeled, cells glow under the microscope according to the concentration of this enzyme (Courtesy of M. Elowitz). (b) In a simplified mental model, we assume that cells contain two enzymes of interest, X (red) and Y (blue) and that the molecules (dots) can freely diffuse within the cell. All other substances are disregarded for the sake of simplicity. (c) The interactions between the two protein types can be drawn in a wiring scheme: each protein can be produced or degraded (black arrows). In addition, we assume that proteins of type X can increase the production of protein Y. (d) All individual processes to be considered are listed together with their rates a (occurrence per time). The mathematical expressions for the rates are based on a simplified picture of the actual chemical processes. (e) The list of processes can be translated into different sorts of dynamic models; in this case, deterministic rate equations for the protein concentrations x and y. (f) By solving the model equations, predictions for the time-dependent concentrations can be obtained. If these predictions do not agree with experimental data, it indicates that the model is wrong or too much simplified. In both cases, it has to be refined.

Computational Biology

- Mathematical modeling
- Data set analysis to develop models

Computational Models

- Model Scope (mathematical elements)
- Model Statement (equations)
- System State (dynamic, snapshot)
- Variables, Parameters and Constants
- Model Behavior (environmental and internal processes)
- Model Assignment (biology described mathematical)
- Data Integration (omics data)

1.3.6
Model Classification

For modeling, processes are classified with respect to a set of criteria.

- A structural or *qualitative* model (e.g., a network graph) specifies the interactions among model elements. A *quantitative* model assigns values to the elements and to their interactions, which may or may not change.
- In a *deterministic* model, the system evolution through all following states can be predicted from the knowledge of the current state. *Stochastic* descriptions give instead a probability distribution for the successive states.
- The nature of values that time, state, or space may assume distinguishes a *discrete* model (where values are taken from a discrete set) from a *continuous* model (where values belong to a continuum).
- *Reversible* processes can proceed in a forward and backward direction. Irreversibility means that only one direction is possible.
- *Periodicity* indicates that the system assumes a series of states in the time interval $\{t, t + \Delta t\}$ and again in the time interval $\{t + i\Delta t, t + (i + 1)\Delta t\}$ for $i = 1,2,\ldots$.

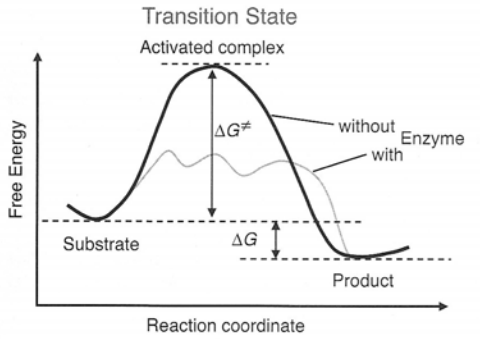


Figure 2.1 Change of free energy along the course of a reaction. The substrate and the product are situated in local minima of the free energy; the active complex is assigned to the local maximum. The enzyme may change the reaction path and thereby lower the barrier of free energy.

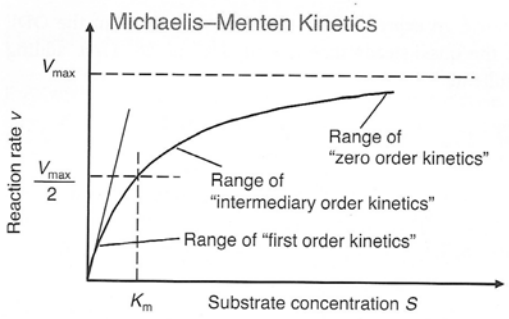
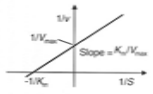
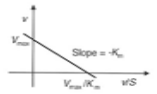
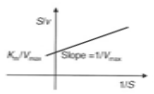


Figure 2.2 Dependence of reaction rate v on substrate concentration S in Michaelis–Menten kinetics. V_{max} denotes the maximal reaction rate that can be reached for large substrate concentration. K_m is the substrate concentration that leads to half-maximal reaction rate. For low substrate concentration, v increases almost linearly with S, while for high substrate concentrations v is almost independent of S.

Table 2.2 Different approaches for the linearization of Michaelis–Menten enzyme kinetics.

	Lineweaver–Burk	Eadie–Hofstee	Hanes–Woolf
Transformed equation	$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{S} + \frac{1}{V_{max}}$	$v = V_{max} - K_m \frac{v}{S}$	$\frac{S}{v} = \frac{S}{V_{max}} + \frac{K_m}{V_{max}}$
New variables	$\frac{1}{v}; \frac{1}{S}$	$v; \frac{v}{S}$	$\frac{S}{v}; S$
Graphical representation			

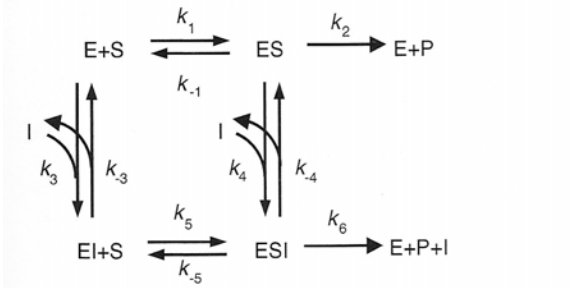


Figure 2.3 General scheme of inhibition in Michaelis–Menten kinetics. Reactions 1 and 2 belong to the standard scheme of Michaelis–Menten kinetics. Competitive inhibition is given, if in addition reaction 3 (and not reactions 4, 5, or 6) occurs. Uncompetitive inhibition involves reactions 1, 2, and 4, and noncompetitive inhibition comprises reactions 1, 2, 3, 4, and 5. Occurrence of reaction 6 indicates partial inhibition.

Table 2.3 Types of inhibition for irreversible and reversible Michaelis–Menten kinetics[a].

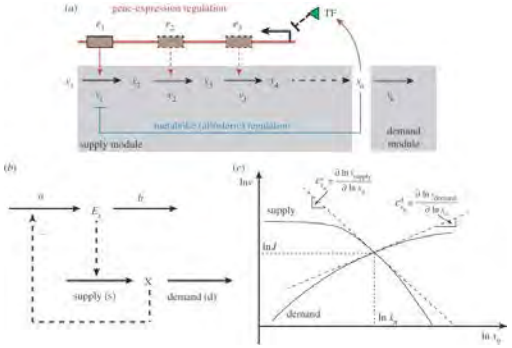
Name	Implementation	Equation – irreversible case	Equation – reversible case	Characteristics
Competitive inhibition	I binds only to free E; P-release only from ES complex $k_{2,4}=k_{-1,5}=k_6=0$	$v=\frac{V_{max}S}{K_m\cdot i_I+S}$	$v=\frac{V^f_{max}(S/K_{mS})-V^r_{max}(P/K_{mP})}{(S/K_{mS})+(P/K_{mP})+i_I}$	K_m changes, V_{max} remains same. S and I compete for the binding place; high S may out compete I.
Uncompetitive inhibition	I binds only to the ES complex; P-release only from ES complex $k_{1,3,5}=k_{2,3,5}=k_6=0$	$v=\frac{V_{max}S}{K_m+S\cdot i_{II}}$	$v=\frac{V^f_{max}(S/K_{mS})-V^r_{max}(P/K_{mP})}{1+[(S/K_{mS})+(P/K_{mP})]i_{II}}$	K_m and V_{max} change, but their ratio remains same. S may not out compete I
Noncompetitive inhibition	I binds to E and ES; P-release only from ES $K_{I,3}=K_{I,4}$, $k_6=0$	$v=\frac{V_{max}S}{(K_m+S)i_{II}}$	$v=\frac{V^f_{max}(S/K_{mS})-V^r_{max}(P/K_{mP})}{(1+(S/K_{mS})+(P/K_{mP}))i_{II}}$	K_m remains, V_{max} changes. S may not out compete I
Mixed inhibition	I binds to E and ES; P-release only from ES $K_{I,3}\neq K_{I,4}$, $k_6=0$	$v=\frac{V_{max}S}{K_m\cdot i_I+S\cdot i_{II}}$		K_m and V_{max} change. $K_{I,3}>K_{I,4}$: competitive–noncompetitive inhibition $K_{I,3}<K_{I,4}$: noncompetitive–uncompetitive inhibition
Partial Inhibition	I may bind to E and ES; P-release from ES and ESI $K_{I,3}\neq K_{I,4}$, $K_6\neq 0$	$v=\frac{V_{max}S[1+\{(k_6/I)/(k_2K_{I,3})\}]}{K_m i_I+Si_{II}}$		K_m and V_{max} change. if $k_6>k_2$: activation instead of inhibition.

[a]These abbreviations are used: $K_{I,3}=\frac{k_{-3}}{k_3}$, $K_{I,4}=\frac{k_{-4}}{k_4}$, $i_I=1+\frac{I}{K_{I,3}}$, $i_{II}=1+\frac{I}{K_{I,4}}$.

Synthetic biology and regulatory networks: where metabolic systems biology meets control engineering.

He F, Murabito E, Westerhoff HV.

J R Soc Interface. 2016 Apr;13(117).



An unbranched metabolic pathway under hierarchical regulation. (a) The first enzyme is regulated through both transcriptional repression and allosteric activity inhibition by the end product. Enzymes in other steps might also be regulated through gene expression (in dashed arrows), but this is not explicitly considered here. TF denotes transcription factor. (b) The hierarchical supply–demand representation of the pathway (a). The lower part represents the classical metabolic supply–demand system, in which only the metabolic regulation (in this case allosteric inhibition) is considered. The letter 'X' denotes the penultimate product xn, still in pathway. The supply is catalysed by enzyme Es (i.e. e1 here or enzymes stemming from an entire operon). (c) Illustration of the steady-state properties of a supply–demand system in terms of changes in the flux, intermediate concentration and elasticity coefficients.

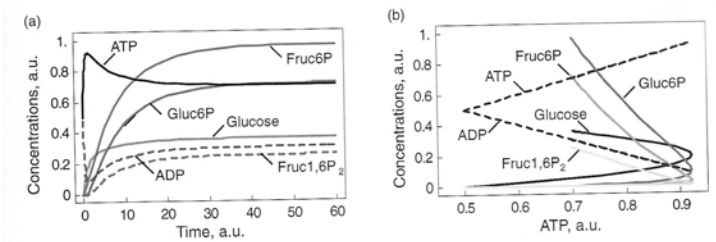


Figure 3.3 Dynamic behavior of the upper glycolysis model (Figure 3.2 and Eq. (3.1)). Initial conditions at $t=0$: *Glucose*$(0)=$ *Gluc*-6P$(0)=$ *Fruc*-6P$(0)=$ *Fruc*-1,6P$_2(0)=0$ and *ATP*$(0)=$ *ADP*$(0)=0.5$ (arbitrary units). Parameters: $k_1=0.25, k_2=1, k_3=1, k_{-3}=1, k_4=1, k_5=1, k_6=1$, and $k_7=2.5$. The steady-state concentrations are *Glucose*$^{ss}=0.357$, *Gluc*-6P$^{ss}=0.714$, *Fruc*-6P$^{ss}=0.964$, *Fruc*-1,6P$_2{}^{ss}=0.2$, and *ATP*$^{ss}=0.7$, and *ADP*$^{ss}=0.25$. The steady-state fluxes are $J_1=J_2=J_3=J_5=J_6=0.25, J_4=0.5$, and $J_7=0.75$. (a) Time-course plots (concentration versus time), (b) phase-plane plot (concentration versus concentration of ATP with varying time); all curves start at *ATP* $=0.5$ for $t=0$.

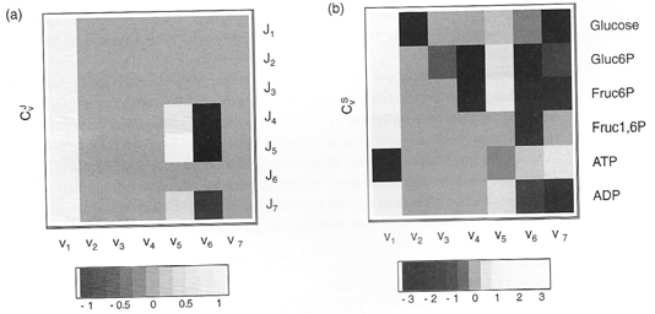


Figure 3.4 Flux and concentration control coefficients for the glycolysis model in Figure 3.2 with the parameters given in the legend of Figure 3.3. Values of the coefficients are indicated in gray-scale: gray means zero control, white or light gray indicates positive control, dark gray or black negative control, respectively.

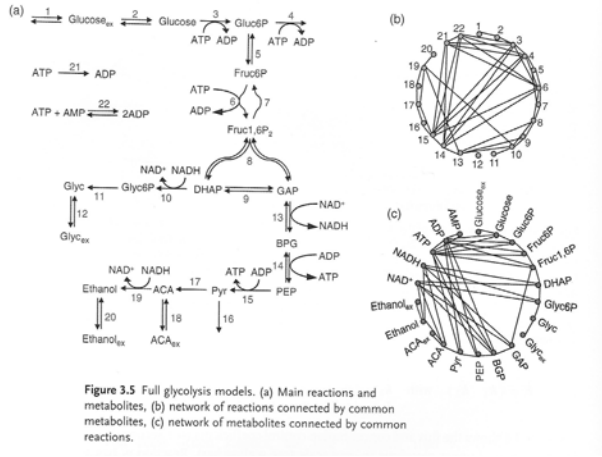


Figure 3.5 Full glycolysis models. (a) Main reactions and metabolites, (b) network of reactions connected by common metabolites, (c) network of metabolites connected by common reactions.

4.1
Data for Small Metabolic and Signaling Systems

Summary

The mathematical equations that are used to develop kinetic models of biochemical systems are so complex that, except for the most simple cases, it is impossible to solve them analytically. Therefore, numerical simulations are required to predict how concentrations develop over time and when and if the system will reach a steady state. But numerical simulations need numerical data to assign specific values to a large number of molecule properties. Among these properties are Michaelis–Menten constants, K_m, and maximal velocities, V_{max}, (for enzymes), but also biological half-lives, binding constants, molecule concentrations, and diffusion rates. In the early days of mathematical modeling, it was very difficult to obtain enough data of sufficient quality to make reliable model predictions. In such a situation, only qualitative models can be constructed that investigate the question if a certain behavior is at all possible or not. Although such a model provides valuable information about a system of biochemical reactions, most models today aim to be quantitative. This means that the model should agree well with measured concentrations and also predictions regarding changes of molecule concentrations are given as specific numbers instead of a qualitative up or down statement. To develop quantitative models, it is therefore essential to obtain a large number of reliable data for the model parameters. One source are specialized databases, which will be discussed in this section. But the process of filling these databases is currently very time-consuming, since most kinetic data have to be extracted by hand from the existing literature. Recently developed experimental techniques aim to improve the situation by enabling researchers to measure large numbers of kinetic data with high accuracy. Some of these techniques will be described at the end of chapter 4.1.

4.2
Parameter Estimation

Summary

Parameters in a model can be determined by fitting the model to experimental data. In the method of least squares, a common approach in parameter estimation, the sum of squared residuals between model predictions and data is minimized. For data with additive standard Gaussian errors, this method is equivalent to maximum likelihood estimation. The variability of parameter estimates due to noisy and insufficient data can be assessed by repeating the estimation with resampled data ("bootstrapping") and the quality of model predictions can be tested by cross-validation. In Bayesian parameter estimation, parameter sets are scored by how well they agree with both available data and with certain prior assumptions, which are expressed by probability distributions of the parameters. The parameter estimation often leads to minimization problems, which can be solved with a variety of local or

global optimization algorithms. Local optimizers are relatively fast, but they may get stuck in suboptimal local optima. Global optimizers like simulated annealing or genetic algorithms can evade local minima, but they may be numerically demanding.

Parameter Estimations

- Regression (minimum of the function)
- Estimators (distance measure)
- Maximum likelihood estimation (Gaussian noise)
- Identifiability (landscape in parameter space)
- Bootstrapping (sampling and noisy data)
- Cross Validation (model fitting and prediction)
- Baysian Parameter Estimation (parameter not fixed, random variables)
- Local and Global Optimization
- Genetic Algorithms (simulations)

(Mathematica / Matlab / Systems Biology Markup Language, SBML)

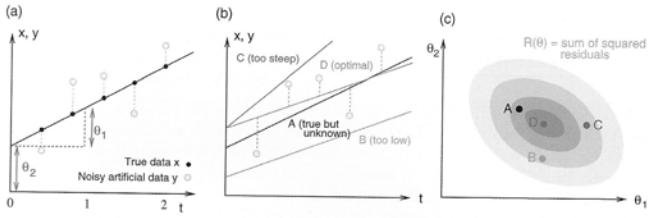
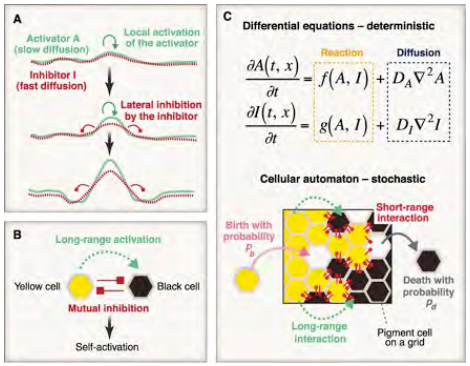
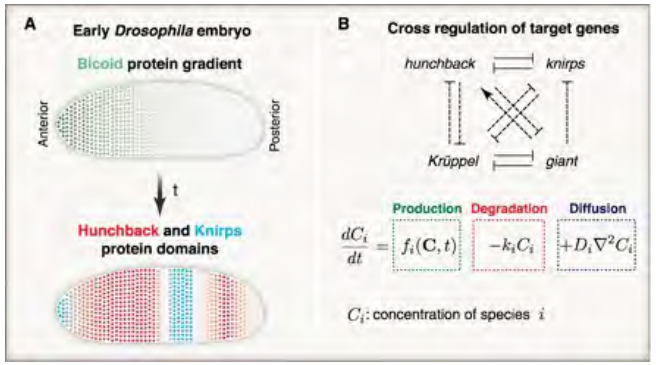


Figure 4.2 Linear regression leads to an optimization problem. (a) Artificial data points (t_m, y_m) (grey) are created by adding Gaussian noise to data points (black) from a model $x(t) = \theta_1(t) + \theta_2$ (straight line). Each possible line is characterized by two parameters, the slope θ_1 and the offset θ_2. The aim in linear regression is to reconstruct the unknown true parameters from noisy data (in this case, the artificial data set **y**). (b) The distance between a possible line (four lines A, B, C, and D are shown) and the data points can be measured by the sum of squared residuals (SSR). The residuals are shown for line D (red dashed lines). (c) Each of the lines A, B, C, and D corresponds to a point (θ_1, θ_2) in parameter space. The SSR as a function $R(\theta)$ forms a landscape in parameter space (schematically shown by shades of pink, dark pink indicates small SSR). Line D minimizes the SSR value.



Patterning with activator-inhibitor systems. (A) Local activation and lateral inhibition generates spatially heterogeneous patterns. (B) Interactions between black and yellow pigment cells produce Turing patterns in zebrafish skin. Mutual inhibition between them functions as self-activation for the yellow cells. Each yellow cell activates distant black cells. Therefore, inhibition of the yellow cell by the black cell works as a lateral inhibition. (C) Different modeling approaches to spontaneous pattern formation.



Patterning with signaling gradients. (A) Schematic of early fruit fly embryo showing the maternal gradient of Bicoid protein at cycle 13 that directs the formation of precise target gene domains such as hunchback and knirps. (B) Proposed gene regulatory network showing cross-regulation of target genes (9). The four genes are also under control of Bicoid and other players. t, time.

Parameter Estimations

- Regression (minimum of the function)
- Estimators (distance measure)
- Maximum likelihood estimation (Gaussian noise)
- Identifiability (landscape in parameter space)
- Bootstrapping (sampling and noisy data)
- Cross Validation (model fitting and prediction)
- Baysian Parameter Estimation (parameter not fixed, random variables)
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- Genetic Algorithms (simulations)

(Mathematica / Matlab / Systems Biology Markup Language, SBML)

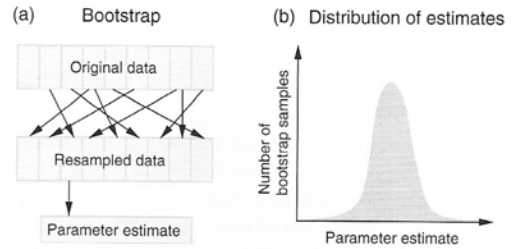

Figure 4.4 The bootstrapping method. (a) Hypothetical data sets are created by resampling data values from the original data set. Each resampled data set yields a parameter estimate $\hat{\theta}$. (b) The distribution of the parameter estimates, obtained from the bootstrap samples, approximates the true distribution of the estimator $\hat{\theta}$. A good approximation requires a large original data set.

Parameter Estimations

- Regression (minimum of the function)
- Estimators (distance measure)
- Maximum likelihood estimation (Gaussian noise)
- Identifiability (landscape in parameter space)
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- Genetic Algorithms (simulations)

(Mathematica / Matlab / Systems Biology Markup Language, SBML)

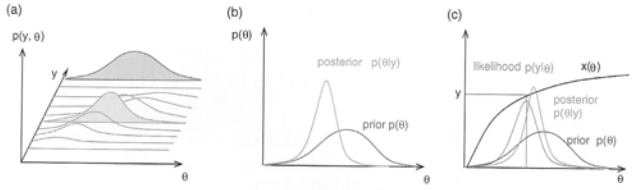


Figure 4.6 Bayesian parameter estimation. (a) In Bayesian estimation, the parameters θ and the data $\mathbf{y}$ follow a joint probability distribution with density $p(\mathbf{y}, \theta)$. The marginal probability density $p(\theta)$ of the parameters is called the prior (blue), while the conditional density $p(\theta|\mathbf{y})$ given a certain data set is called the posterior (magenta). (b) The posterior (magenta) is more narrow than the prior (blue), which reflects the information gained by considering the data. (c) Prior, likelihood and posterior. In a model, the data $\mathbf{y}$ are given by a mean prediction $\mathbf{x}(\theta)$ (black line) plus Gaussian noise. An observed value $\mathbf{y}$ gives rise to a likelihood function $L(\theta|\mathbf{y}) = p(\mathbf{y}|\theta)$ in parameter space. The posterior is proportional to the product of prior and likelihood function.

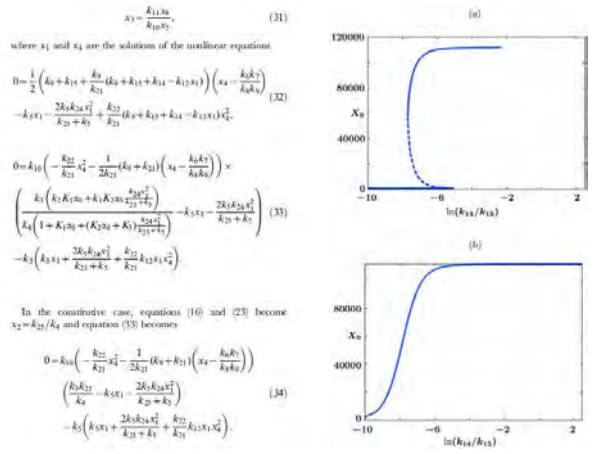
Parameter Estimations

- Regression (minimum of the function)
- Estimators (distance measure)
- Maximum likelihood estimation (Gaussian noise)
- Identifiability (landscape in parameter space)
- Bootstrapping (sampling and noisy data)
- Cross Validation (model fitting and prediction)
- Baysian Parameter Estimation (parameter not fixed, random variables)
- Local and Global Optimization
- Genetic Algorithms (simulations)

(Mathematica / Matlab / Systems Biology Markup Language, SBML)

Machine Learning Modeling

- Large data set with manipulations
- Test data set with known outcomes parameters (learning data set)
- Mathematical Algorithm development from training set
- Refine Algorithm development with large data set
- Final Algorithm should be correct with training set and reveal new biology insight



Bifurcation diagrams for the deterministic reaction rate equations.
The diagrams are constructed using XPPAUT for equations (1)–(13) and the parameter values given in Results. Numbers of reporter protein molecules produced are plotted against the natural logarithm of the external signal ln(k_{14}/k_{15}), in the a) autoregulated and b) constitutive cases, showing a bistable and graded response respectively. Bold lines denote stable solutions and dashed lines denote unstable solutions. doi:10.1371/journal.pcbi.1002396.g003

Methods of information theory and algorithmic complexity for network biology.
Zenil H, Kiani NA, Tegnér J.
Semin Cell Dev Biol. 2016 Mar;51:32-43.

Abstract

We survey and introduce concepts and tools located at the intersection of information theory and network biology. We show that Shannon's information entropy, compressibility and algorithmic complexity quantify different local and global aspects of synthetic and biological data. We show examples such as the emergence of giant components in Erdös-Rényi random graphs, and the recovery of topological properties from numerical kinetic properties simulating gene expression data. We provide exact theoretical calculations, numerical approximations and error estimations of entropy, algorithmic probability and Kolmogorov complexity for different types of graphs, characterizing their variant and invariant properties. We introduce formal definitions of complexity for both labeled and unlabeled graphs and prove that the Kolmogorov complexity of a labeled graph is a good approximation of its unlabeled Kolmogorov complexity and thus a robust definition of graph complexity.

4.3
Reduction and Coupling of Models

Summary

The aim in model reduction is to simplify complex models, i.e., to capture their key dynamical properties with fewer equations and parameters. This facilitates understanding, numerical and analytical calculations, and model fitting. A reduced model has to emulate the behavior of relevant variables under relevant conditions and on the relevant time scale. To reduce a model, elements can be omitted, lumped, or replaced by effective descriptions, and global model behavior can be approximated by global modes or simplified black-box models. Important simplifying concepts like quasi-equilibrium or quasi-steady state can be justified by a distinction between fast and slow processes. Once models for parts of the cell have been established, they may be combined to form move complex models, which may show new emergent behavior.

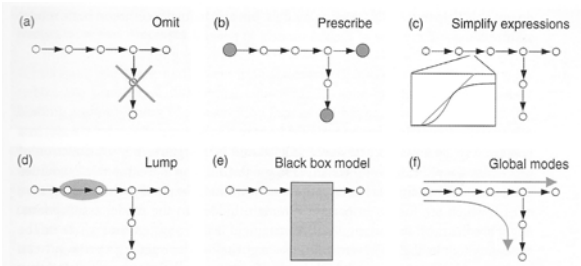


Figure 4.8 Simplifications in biochemical models. The scheme shows a branched pathway of metabolites (circles) and reactions (arrows). (a) Omitting substances or reactions. (b) Predefining the values of concentrations or fluxes or relations between them. (c) Simplifying the mathematical expressions (e.g., omitting terms in a kinetic law, using simplified kinetic laws [21], neglecting insensitive parameters [22]). (d) Lumping the substances, for instance, similar metabolites, protonation states of a metabolite, or metabolite concentrations in different compartments. Likewise, subsequent reactions in a pathway or elementary steps in a reaction can be replaced by a single reaction of the same velocity; for parallel reactions, like the action of isoenzymes, the velocities are summed up; for the two directions of a reaction, the velocities are subtracted. (e) Replacing the model parts by a dynamic black-box model that mimics the input–output behavior [23]. (f) Describing the dynamic behavior by global modes (e.g., elementary flux modes or eigenmodes of the Jacobian).

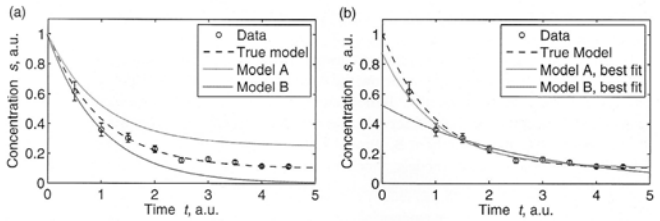


Figure 4.15 Fit of the example models. (a) Artificial data (a concentration time series, black dots) were generated by adding Gaussian noise to results of the true model (dashed line). Solid curves show simulations from model A (red) and B (blue) with fixed parameters. (b) After estimating the parameters of models A and B, a better fit is obtained.

Table 4.3 Calculation of selection criteria for the running example.[a]

	σ large		σ small	
	Model A	Model B	Model A	Model B
n	3	2	–	
k	9	9		
$2k$	6	4	–	
$2k + \frac{2k(k+1)}{n-k-1}$	4.67	2.33		
$k \log n$	6.59	4.39		
Weighted SSR	4.98	6.13	4.99	19.81
AIC	10.98	10.13	10.99	23.81
AICc	9.64	8.46	9.66	22.14
BIC	11.57	10.52	11.58	24.20

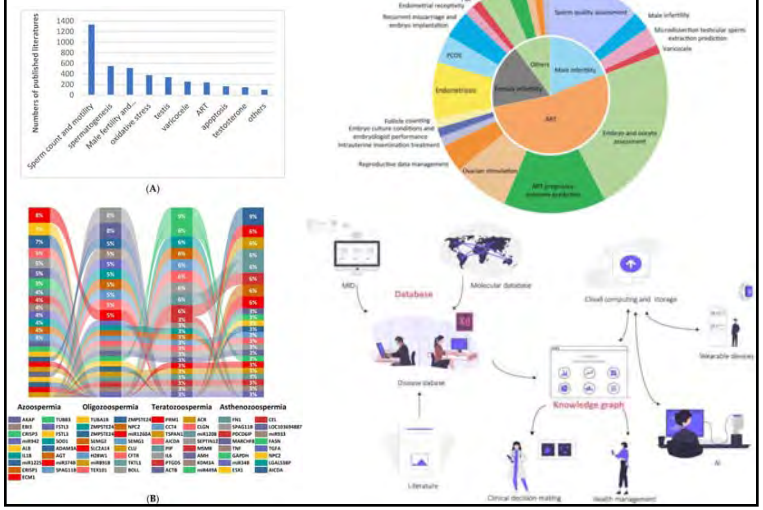
[a] For each of the criteria (weighted sum of squared residuals (SSR), Akaike information criteria (AIC and AICc), and Schwarz criterion (BIC), the more favorable values are shown in red.

Parameter Estimations

- Regression (minimum of the function)
- Estimators (distance measure)
- Maximum likelihood estimation (Gaussian noise)
- Identifiability (landscape in parameter space)
- Bootstrapping (sampling and noisy data)
- Cross Validation (model fitting and prediction)
- Baysian Parameter Estimation (parameter not fixed, random variables)
- Local and Global Optimization
- Genetic Algorithms (simulations)

(Mathematica / Matlab / Systems Biology Markup Language, SBML)

Translational Bioinformatics for Human Reproductive Biology Research: Examples, Opportunities and Challenges for a Future Reproductive Medicine.
Liu K, Zhang Y, Martin C, Ma X, Shen B.
Int J Mol Sci. 2022 Dec 20;24(1):4.



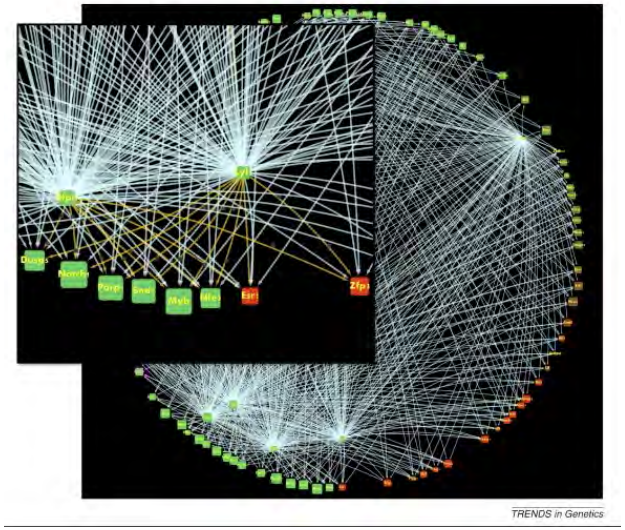
Networks

- Modules
- Nodes
- Clusters
- Interactomes

8.1
Structure of Biochemical Networks

Summary

The structure of complex biochemical systems – e.g., metabolism or transcriptional regulation – can be represented by networks. Nodes typically correspond to molecule types or genes, while edges represent, for instance, molecular interactions, causal influences, or correlations in high-throughput data. To detect significant structures that deserve further explanation, networks can be compared to random graphs with defined statistical properties. Various characteristic structures have been found in biological networks, including scale-free degree distributions, small average path lengths, modules and clustering, as well as network motifs.



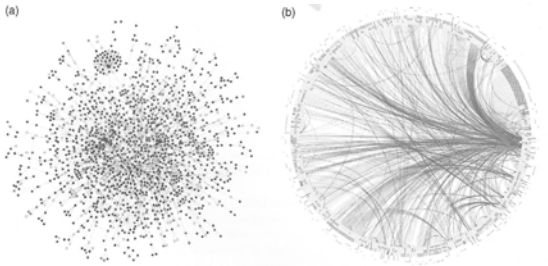
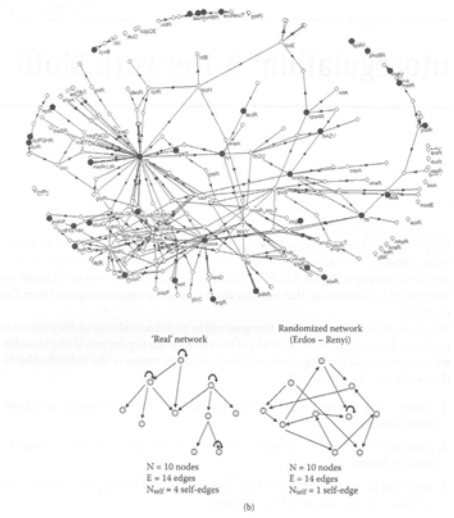


Figure 8.1 Biological networks. (a) Network of protein–protein interactions in yeast. From Jeong et al. [4]. (b) Regulatory interactions between *E. coli* genes. Genes shown as colored segments associated with the structural description of the gene's main function. Curve colors express the nature of relation (red: inhibition, blue: activation, green: dual regulation), and the traces around the circle indicate autoregulation. Courtesy of S. Ortiz, L. Rico, and A. Valencia.



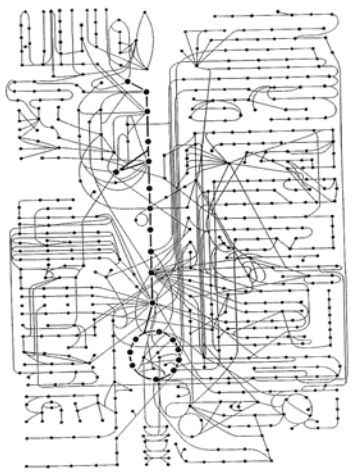
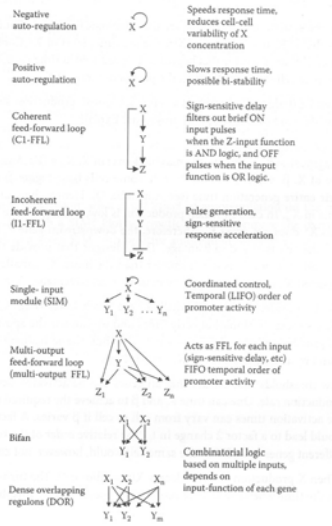
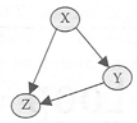
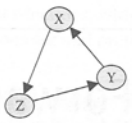


Figure 7.1 A section of the metabolic network of a "simple" bacterium. Note that each point (each chemical compound) is linked to any other point via the complexity of the network.



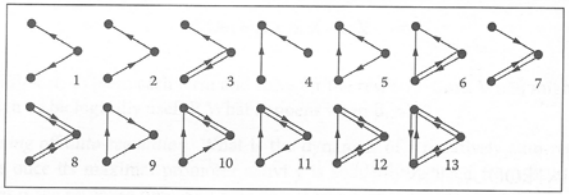


Feed-forward loop



3-node feedback loop (cycle)

(a)



(b)

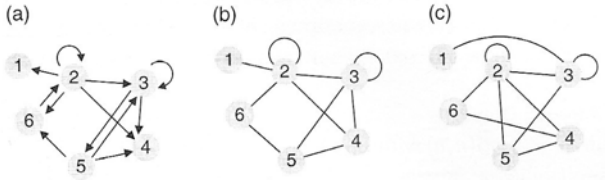
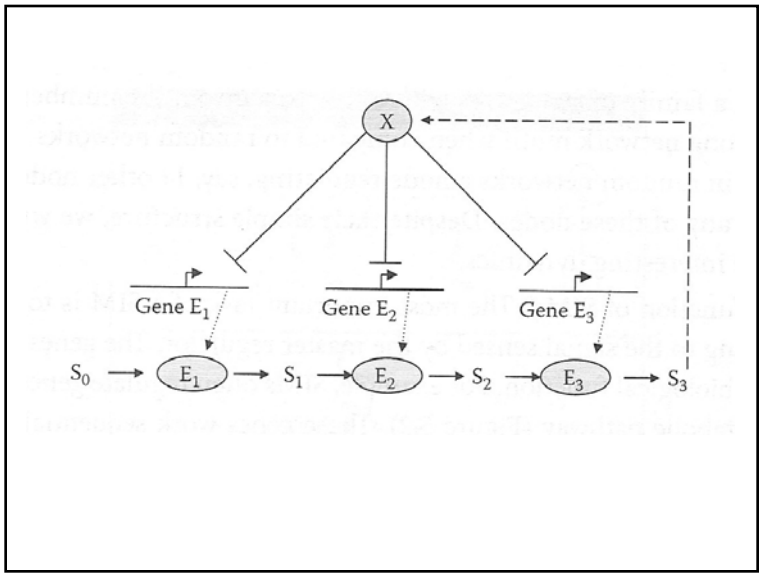
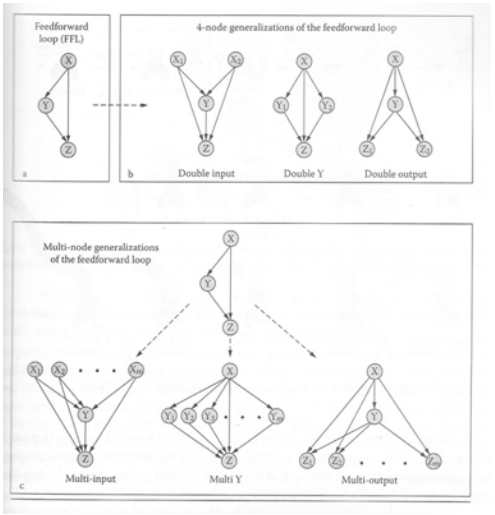
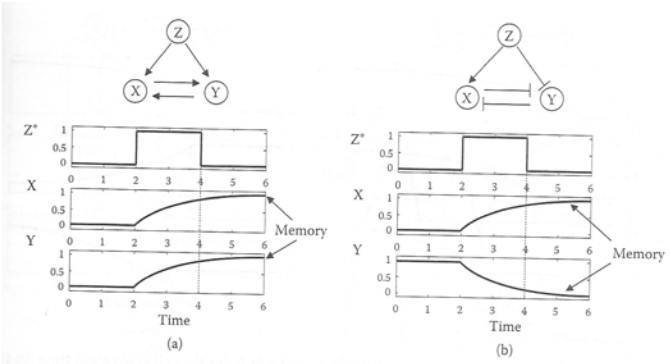
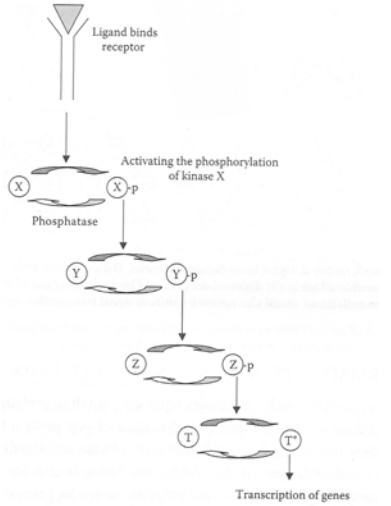


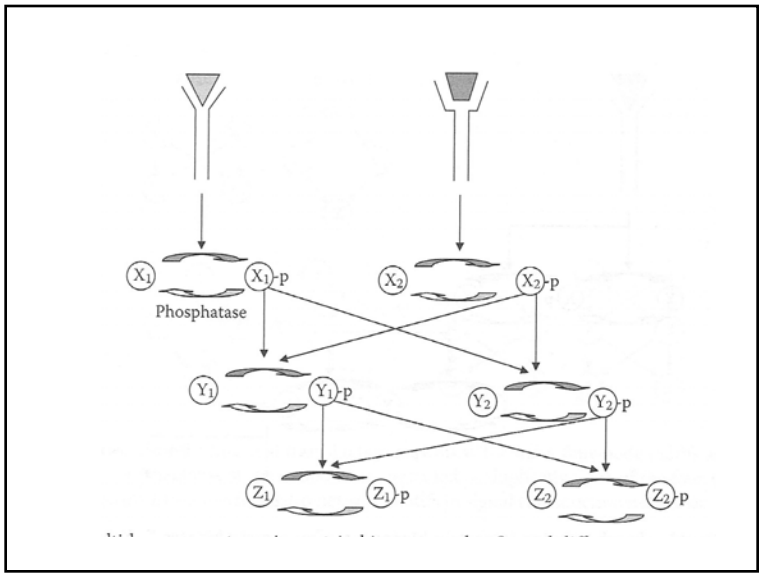
Figure 8.2 Small graphs. (a) Directed graph with 6 nodes and 9 edges. (b) An undirected graph with similar topology. (c) By rewiring, we can obtain a new graph without changing the degrees k_i.

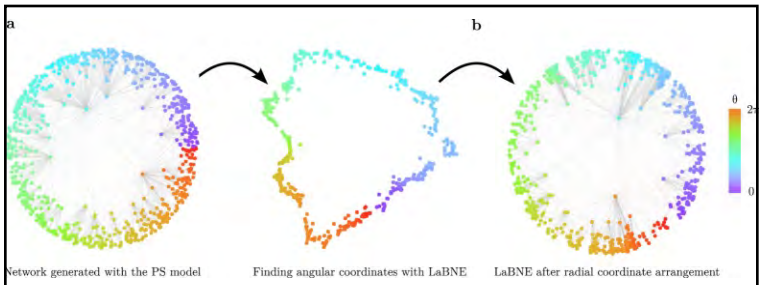

X
Gene E_1
Gene E_2
Gene E_3
S_0
E_1
S_1
E_2
S_2
E_3
S_3


Feedforward loop (FFL)
4-node generalizations of the feedforward loop
Double input
Double Y
Double output
Multi-node generalizations of the feedforward loop
Multi-input
Multi Y
Multi-output


Z*
X
Y
Memory
Time
(a)
(b)


Ligand binds receptor
Activating the phosphorylation of kinase X
Phosphatase
Transcription of genes





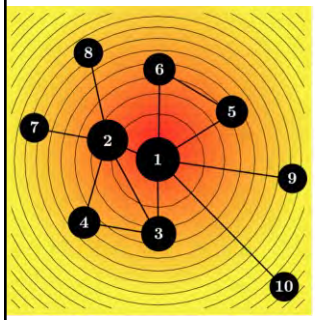
(a) A network of 750 nodes was generated by means of the PS model, with target average node degree 2m = 10, scaling exponent γ = 2.75 and network temperature T = 0. The network is embedded to the hyperbolic plane H^2 An external file that holds a picture, illustration, etc. Object name is srep30108-m31.jpg with LaBNE to reveal the angular position of the nodes in the hyperbolic circle containing the network. (b) Finally, the radial coordinates of the nodes are assigned, so that they resemble the rank of each node according to its degree. By the colour of the nodes, which highlights their angular coordinates, one can note that the embedding by LaBNE is rotated by some degrees with respect to the actual node angular coordinates obtained with the PS model. This does not impact the hyperbolic, distance-dependent connection probabilities, because distances are invariant under rotations. Edges in the raw embedding by LaBNE are not shown for clarity.

Input: A, the $N \times N$ adjacency matrix representing network $G = (V, E)$
Output: $Y_{\mathbb{H}^2}$, the hyperbolic coordinates for the set of nodes V

Compute the average node degree of the network $2m$
Determine the network's scaling exponent γ
$\beta \leftarrow 1/(\gamma - 1)$
$R \leftarrow 2 \ln(N) - 2 \ln\left[\frac{2(1 - e^{-\ln(N)(1-\beta)})}{\pi m(1-\beta)}\right]$
Compute the degree matrix D
$L \leftarrow D - A$
Embed G to $\mathbb{H}^2$ via $L\mathbf{v}_{k+1} \approx \lambda_{k+1} D\mathbf{v}_{k+1}$ with $k = 2$
Since the smallest eigenvalue is 0, $Y_{emb} = [\mathbf{y}_1 = \mathbf{v}_2, \mathbf{y}_2 = \mathbf{v}_3]$
Sort nodes decreasingly by degree and label them $i = \{1, 2, \ldots, N\}$
Assign each node with radial coordinates $\mathbf{r}(i) = 2\beta \ln(i) + 2(1 - \beta) \ln(N)$
$\boldsymbol{\theta} \leftarrow \arctan(\mathbf{y}_2/\mathbf{y}_1)$
Finally, $Y_{\mathbb{H}^2} \leftarrow [\mathbf{r}, \boldsymbol{\theta}]$

Note that to embed a network G to H^2, the truncated spectral decomposition of L is used. This gives the closest approximation to the eigen-decomposition by a matrix λk+1 of rank λ_{k+1} and ensures that the computational complexity of LaBNE is $O(N^2)$.

Efficient embedding of complex networks to hyperbolic space via their Laplacian.
Alanis-Lobato G, Mier P, Andrade-Navarro MA.
Sci Rep. 2016 Jul 22;6:30108.



$f(i)$ = Degree of node i

$$L = \begin{array}{c} \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \\ 10 \end{array} \begin{array}{c} \begin{array}{cccccccccc} 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \end{array} \\ \begin{bmatrix} 6 & -1 & -1 & 0 & -1 & -1 & 0 & 0 & -1 & -1 \\ -1 & 5 & -1 & -1 & 0 & 0 & -1 & -1 & 0 & 0 \\ -1 & -1 & 3 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & -1 & 2 & 0 & 0 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & 2 & -1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 & -1 & 2 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \end{array} \quad f(i) = \begin{bmatrix} 6 \\ 5 \\ 3 \\ 2 \\ 2 \\ 2 \\ 1 \\ 1 \\ 1 \\ 1 \end{bmatrix} = \begin{bmatrix} 22 \\ 12 \\ -4 \\ -4 \\ -4 \\ -4 \\ -4 \\ -4 \\ -5 \\ -5 \end{bmatrix}$$

$f(i) = \Sigma_j A_{i,j}(f(i) - f(j))$

Summary Points

1. Feedback is an essential part of molecular networks. It allows the cell to adjust the repertoire of functional proteins to current needs.

2. A FL is primarily characterized by its sign: negative feedback for maintaining homeostasis, positive feedback for obtaining ultrasensitivity or multiple stable states of the cellular composition.

3. Negative feedback can cause oscillations if signal propagation around the FL is sufficiently slow. High Hill coefficients, additional positive FLs, or saturated degradation facilitates oscillations in a negative FL.

4. Positive feedback can come from strong self-activation of a gene, from mutual repression between proteins, or by autocatalytic processes. In all cases one can obtain bistability if reactions involve some sort of cooperativity.

5. Metabolism of small molecules is characterized by a separation of scales. Typically, the intracellular pool of available small molecules is much smaller than the total amount of small molecules consumed during one cell generation.

6. Combinations of FLs in small-molecule uptake and metabolism can result in new behavioral features that are significantly different from a simple sum of the behaviors of single loops.

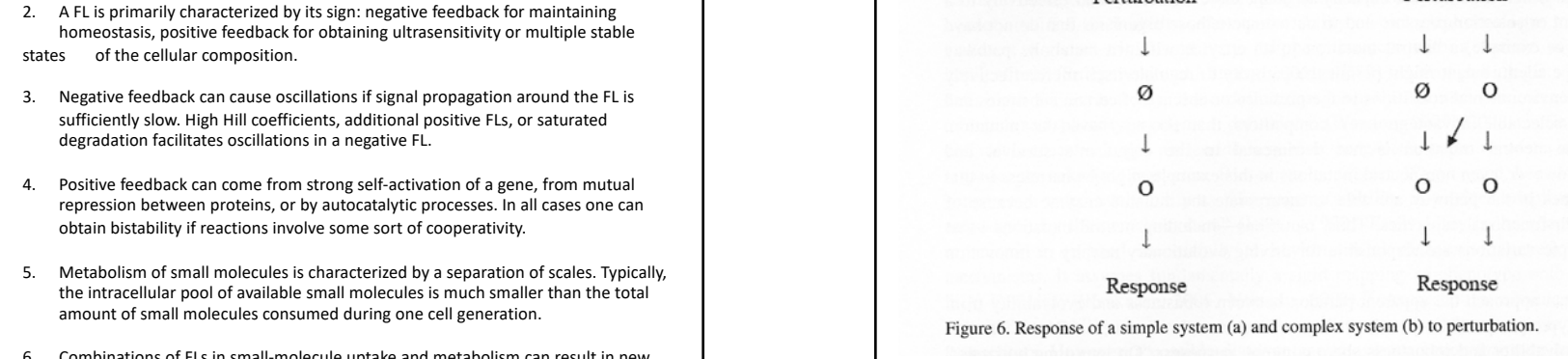
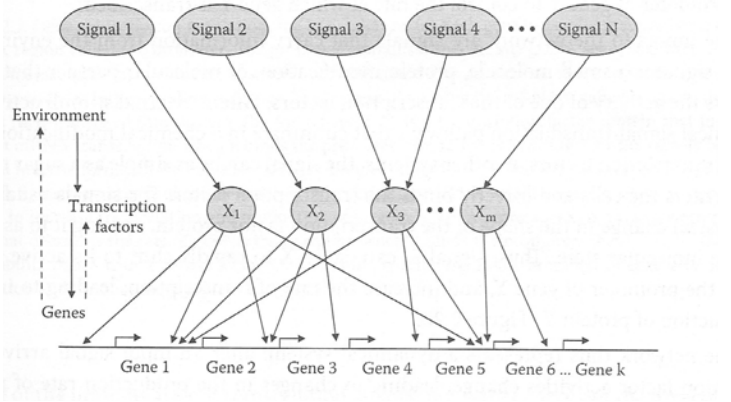


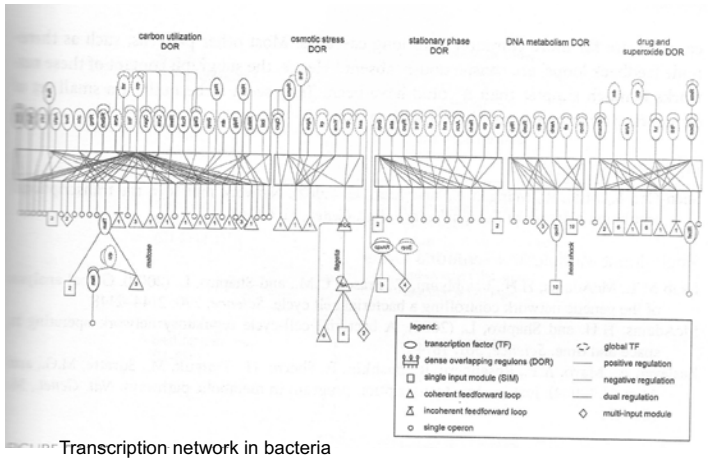
Figure 6. Response of a simple system (a) and complex system (b) to perturbation.



8.2
Network Motifs

Summary

Signal transduction pathways and transcription networks process biochemical signals, which are coded in the concentrations, modifications, and localization of molecules. Regulatory networks contain characteristic motifs, which may reveal small subsystems with typical dynamic behavior and specific regulatory functions. The adapation motif, for instance, translates jumps of its input signal into a transient response, but in steady-state situations, its response is completely independent of the magnitude of the input. Other typical motifs comprise negative feedback loops, which speed up response times and contribute to stability, but also to oscillations, and the feed-forward loops, which can act as filters, sign-sensitive delays, or pulse generators.



Transcription network in bacteria

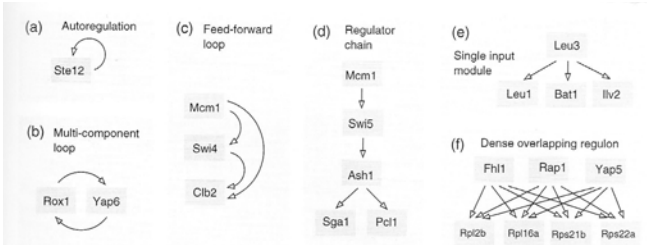


Figure 8.7 Network motifs in the transcription network of the yeast *S. cerevisiae*. Gene names refer to specific examples in the network. Redrawn from Lee *et al.* [19].

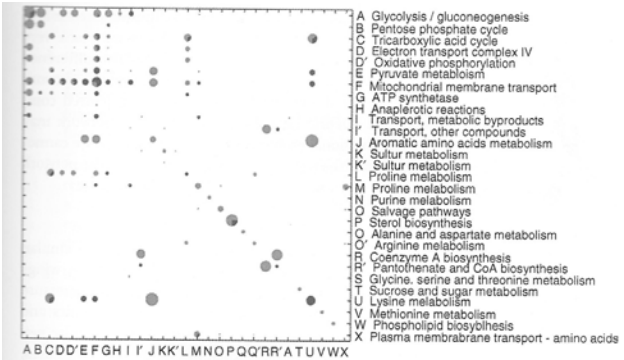
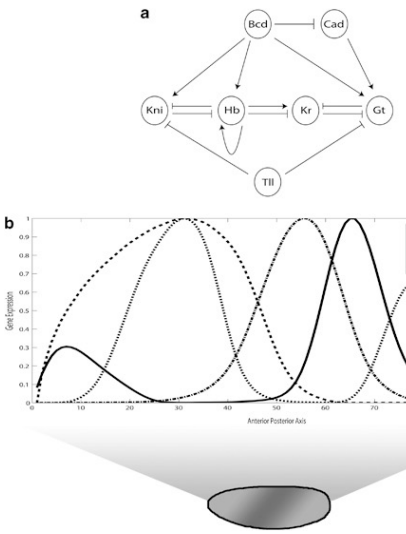


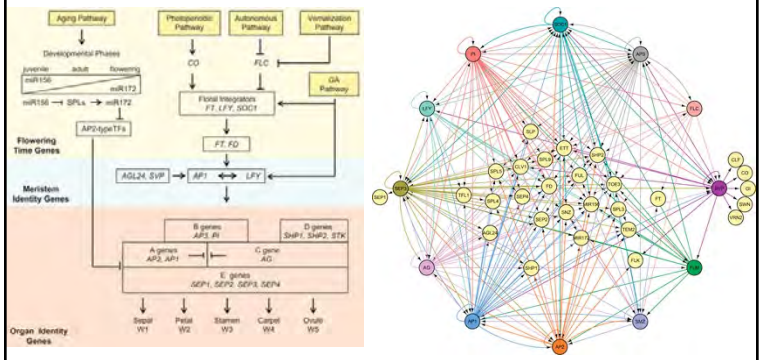
Figure 8.15 Epistatic effects reflect the shared functions of genes. Circles show the abundance of epistatic interactions between genes belonging to functional groups (rows and columns). Circle radii represent numbers of epistatic interactions. Aggravating and buffering interactions are shown as red and green pie slices, respectively. From Segrè *et al.* [66].

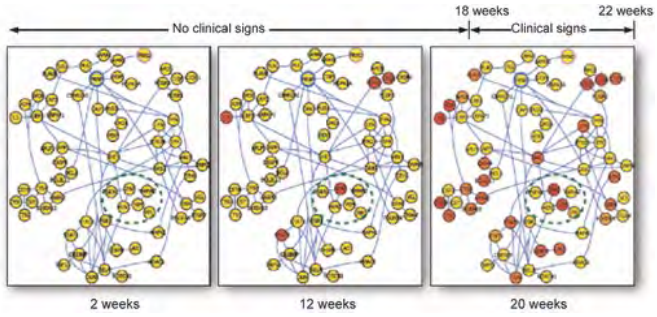
Network Application Examples



(a) Gene regulatory network for Drosophila gap genes, showing relationship between input genes (Bcd, Cad, Hb, Tll) and output genes (Kni,Hb,Kr,Gt). (After figure 1 of Papatsenko and Levine (2011)). (b) Concentration of Gap genes along the anterior posterior axis of the embryo. Model was fitted to this data. Hb, hunchback; Gt, giant; Kr, Kruppel; Kni, Knirps.

The (r)evolution of gene regulatory networks controlling Arabidopsis plant reproduction: a two-decade history.



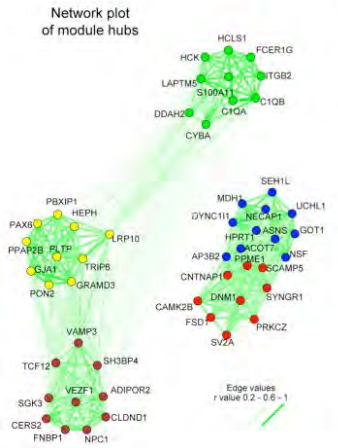


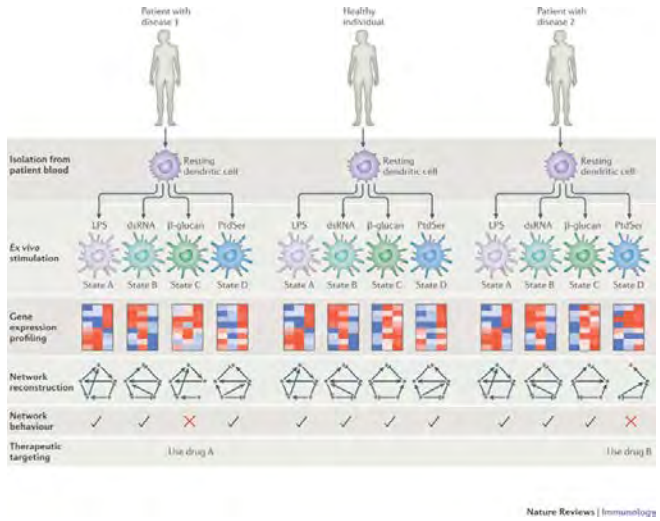
A schematic of the network perturbations of one neural degenerative network over the 20 weeks of the progression of this disease in a mouse model. The red nodes indicate mRNAs that have become disease perturbed as compared with the brain transcripts of normal mice. The spreading of the disease-perturbed networks at the three different times points is striking – indicating the progressive disease perturbation of this neurodegenerative network.

Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders.

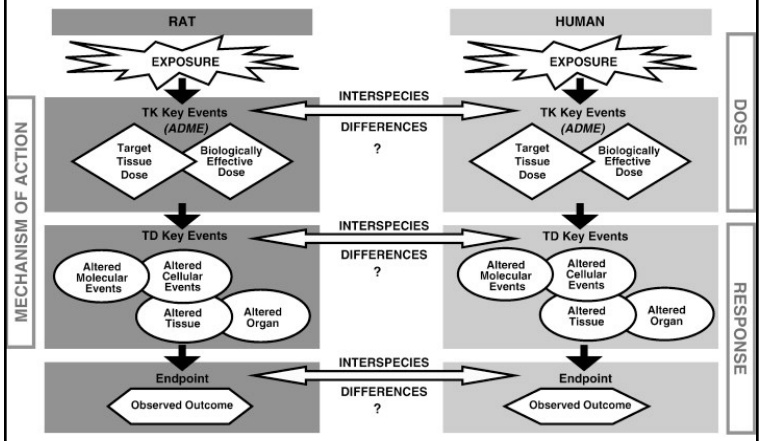
Parikshak NN, Gandal MJ, Geschwind DH.

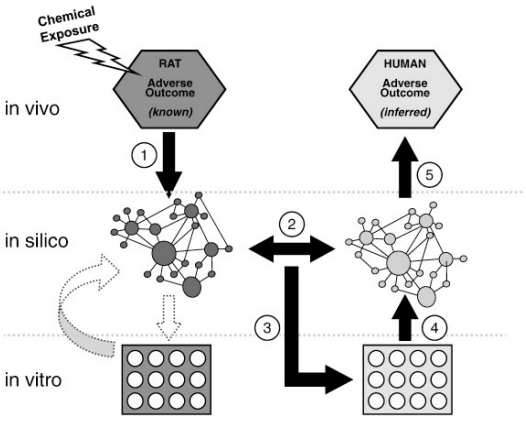
Nat Rev Genet. 2015 Aug;16(8):441-58.





Use of comparative genomics approaches to characterize interspecies differences in response to environmental chemicals: challenges, opportunities, and research needs.





Interspecies comparisons and human relevance — a modified parallelogram approach for integrating in vivo, in vitro, and computational approaches in interspecies extrapolation of toxicity perturbation. The parallelogram approach proposed by Nesnow (2004) and referred to by the National Research Council (National Research Council, 2006) is modified here by the incorporation of computational comparative genomics approaches. Using rat and human as examples: 1) a rat network perturbation model is developed based on in vivo data; 2) the rat and human networks are computationally compared; 3) differences and similarities found by the interspecies network comparison are tested via human in vitro assays (e.g., primary human cell lines); 4) quantified in vitro perturbations are mapped back to the compared networks; and, 5) human in vivo outcomes are inferred. In addition, rat in vivo assays, driven by network-based hypotheses or otherwise (as represented by the white arrows), can inform the rat network model and the compared network model.

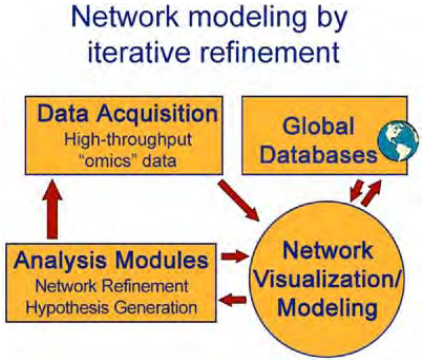
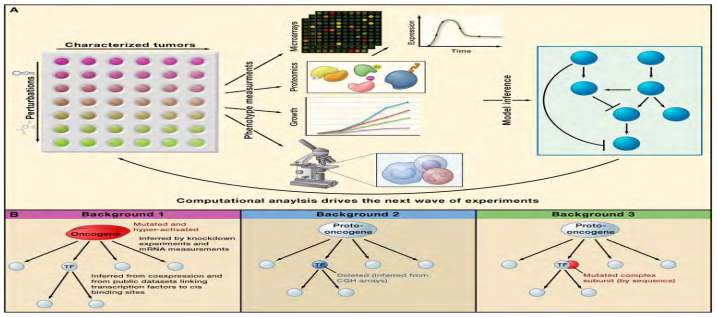


Table 1. **Summary of the datasets used in systems biology based drug design paradigm and the nature of the hypothesis that can be inferred from these analyses.**

Data type	Parts list	Hypothesis from a retrospective analysis of the interactions
Chemoinformatics	Nodes: Chemicals Node Attributes: *Proteins, Domains, Substructures, Enriched fragments[3], Pharmacophores, Toxicophores, Physicochemical properties, Structural descriptors*, etc	(a) Chemical similarity network analysis that can complement chemoproteomic and chemogenomic analysis.
Proteomics	Nodes: Proteins Node Attributes: *Domain definitions,Sequence motifs(linear and non-linear), Superfamily definitions, Sequence descriptors, Cognate ligands, Pathways, other protein interacting partners*	(a) Protein similarity and (b) protein interaction networks:
Genomics	Nodes: Genes/ transcripts Node Attributes: *Phenotypes/indications, Perturbagens[7] (small molecule or si/shRNA), motifs, regulators (TFs, Epigenetic factors, Master regulators), Pathways, Literature gene sets.*	(a) Finding and interpreting genes/ transcripts associated with phenotypic changes or perturbations.
Phenomics	Nodes: Diseases/ Indications/ phenotypes Node Attributes: *in vivo*[1] Biochemical data, Hematology, Organ Weight, Pathology Data, Histology, Pathways, Genes, Proteins (drug targets), Chemicals, Chromatin regulators.	(a) Studying the genotype–phenotype map, (b) Identifying the genetic basis of complex traits.
Chemoproteomics (bipartite networks – edges between chemicals and proteins only)	Nodes: Chemicals, Proteins Edge Attributes: Activation, inhibition, degradation.	(a) Analyzing the pharmacological map of the druggable proteome and discovering ligands for undruggable proteome, (b) drug target discovery.
Chemogenomics (bipartite networks – edges between chemicals and genes only)	Nodes: Chemicals, Genes Node Attributes: : *in vivo* Biochemical data, Hematology, Organ Weight, Pathology Data, Histology, Pathways, Genes, Proteins (drug targets), Chemicals, Chromatin regulators. Edge attributes: *activation, repression.*	(a) Determining mode of action, (b) drug repurposing and drug target identification
Qualitative and quantitative network models	Nodes: Chemicals, Genes, Proteins, protein complexes, phenotypes Node Attributes: Activity levels inferred from mRNA or protein expression activity data. Edge Attributes: Regulatory interactions, PTMs,	(a) Represent existing knowledge of biological systems, (b) predict the effect of perturbations on other components of the pathway, (c) identify missing components in a pathway, (d) determine the most critical components of the pathway,



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Cell Volume 144, Issue 6 2011 839 - 841
http://dx.doi.org/10.1016/j.cell.2011.02.044

Systems biology: perspectives on multiscale modeling in research on endocrine-related cancers.
Clarke R, Tyson JJ, Tan M, Baumann WT, Jin L, Xuan J, Wang Y.
Endocr Relat Cancer (2019) 26(6):R345–R368

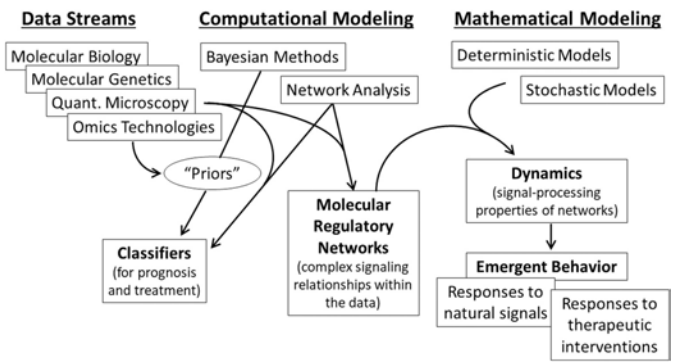

Table 2 Methods of mathematical modeling.

Method	Dynamic variables	Time	Example
Boolean networks	$X(t) = 0$ or 1 $Y(t) = 0$ or 1	t = integer (0, 1, 2, ...)	X inhibits synthesis of Y and Y inhibits synthesis of X $X(t+1) = \neg Y(t)$ $Y(t+1) = \neg X(t)$
Ordinary differential equations	$X(t)$ = positive real number $Y(t)$ = positive real number	t = real number ($t \geq 0$)	X inhibits synthesis of Y and Y inhibits synthesis of X $\frac{dX}{dt} = \frac{k_{sx}}{1+Y^p} - k_{dx}X$ $\frac{dY}{dt} = \frac{k_{sy}}{1+X^q} - k_{dy}Y$
Stochastic models	$M(t)$ = positive integer	t = real number ($t \geq 0$)	Propensity of mRNA synthesis = k_{sm} Propensity of mRNA degradation = $k_{dm}M$ Probability density function for number of mRNA molecules in the cell is $P(M) = e^{-\lambda}\frac{\lambda^M}{M!}$, where $\lambda = \frac{k_{sm}}{k_{dm}}$
Hybrid deterministic-stochastic models	$M(t)$ = positive integer $P(t)$ = positive real number	t = real number ($t \geq 0$)	Genetic regulatory network: Simulate mRNA fluctuations, $M(t)$, with a stochastic model and protein dynamics, $P(t)$, with ordinary differential equations

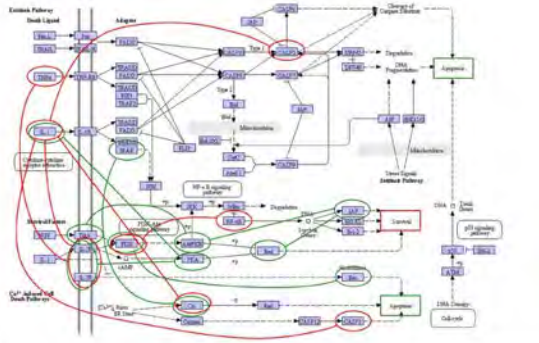

Figure 2
Differential dependency network focused on the KEGG apoptosis pathway (Kanehisa & Goto 2000). Recurrent breast cancers (uniquely featured by red edges) showed the imbalance between apoptosis and survival with only one route into the cell through IL1B-induced inhibition of proapoptotic CASP3. Non-recurrent breast cancer (uniquely featured by green edges) had a cascade of signaling pathways inside the cell that provides the balance between apoptosis and survival. Copyright Kanehisa Laboratories. Reproduced with permission from KEGG.

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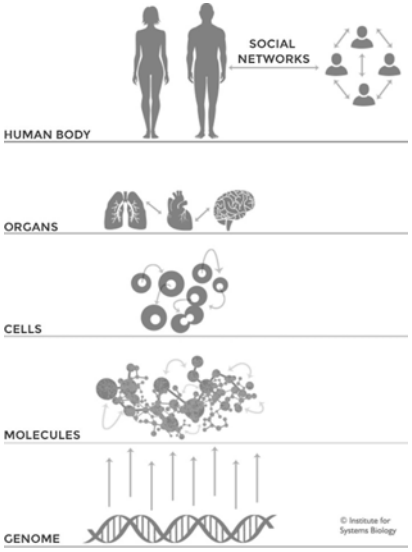
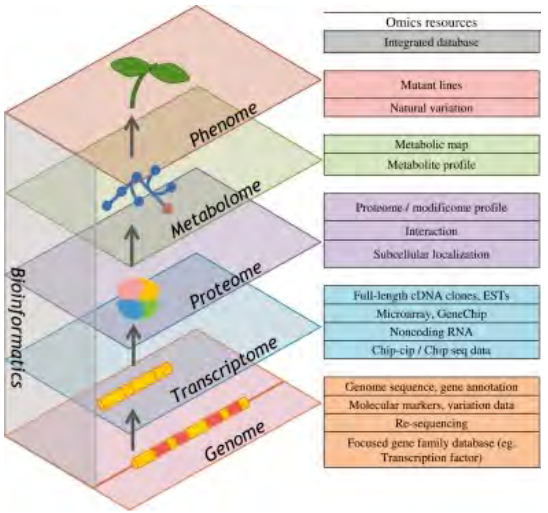
http://dx.doi.org/10.1016/j.cell.2011.02.044

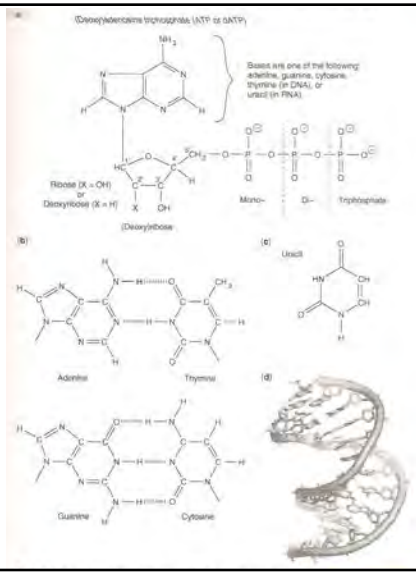
Systems Biology Components and Technology

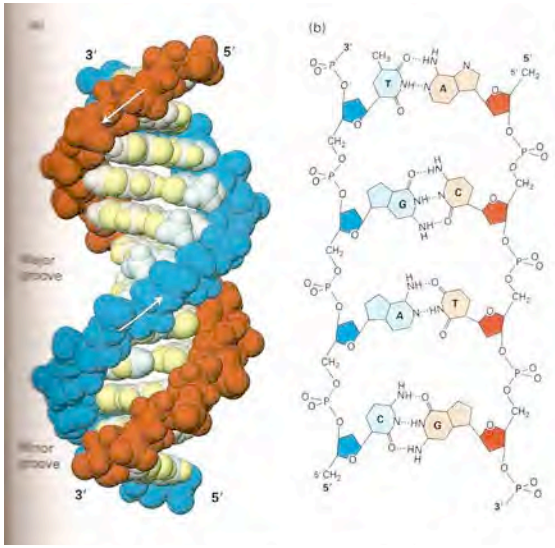
- Components
  - DNA , Expression (Transcriptome), Protein, Cellular, Organ, Physiology, Organism
  - Differentiation, Development, Phenotype, Evolution
- Technology
  - Genomics, Transcriptomics, Proteomics
  - Interactome, Signaling, Metabolism
- Omics
  - Computational Biology, Data Processing
  - Data Resources

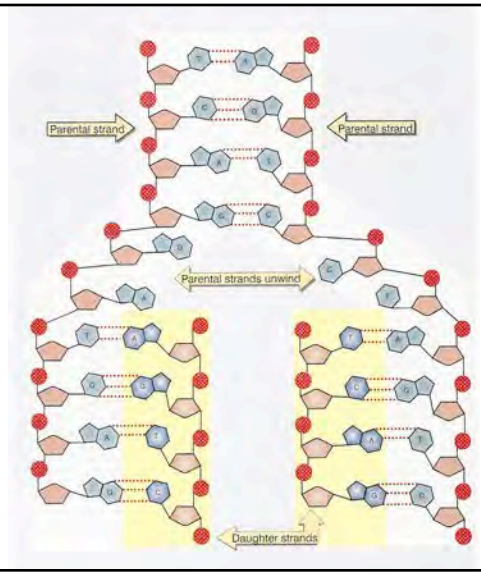


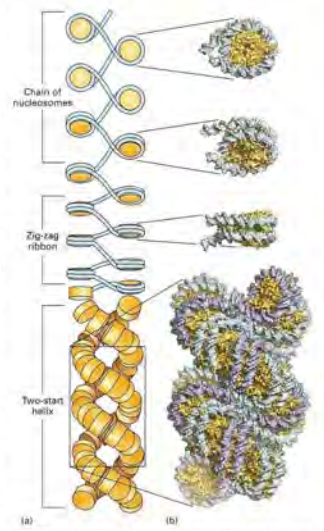
The human being as a dynamic network of networks. In systems medicine the human organism is envisioned as a system of systems or network of networks. At every scale of biological organization (molecular, cellular, organ, individual and social/environmental) systems are portrayed as giving rise to and embedding each other. At all levels the network of networks is seen as a dynamic or four-dimensional process (as opposed to a static thing) (Copyright: The Institute for Systems Biology, used with permission)











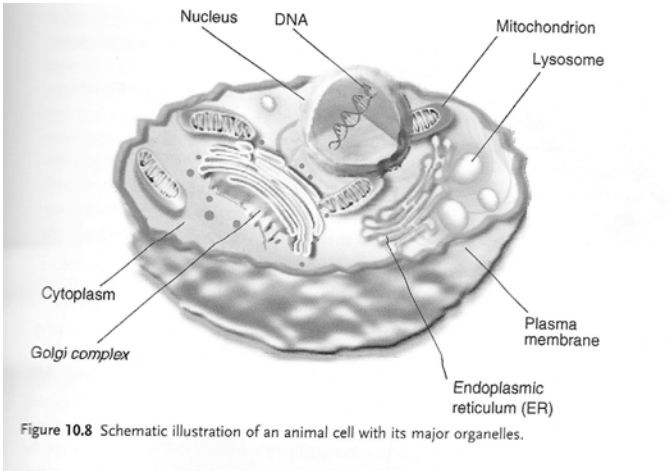
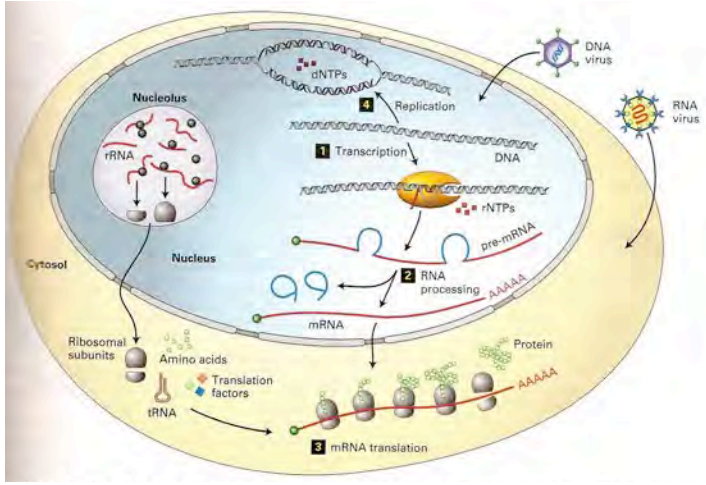
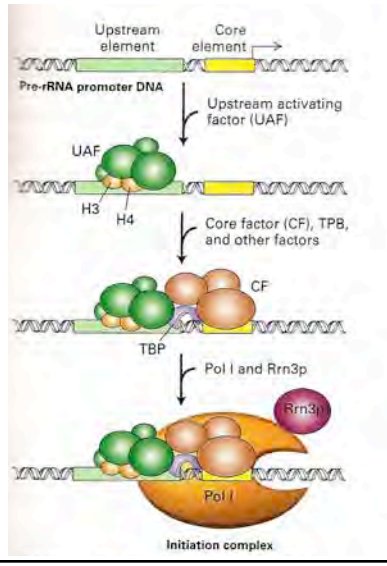
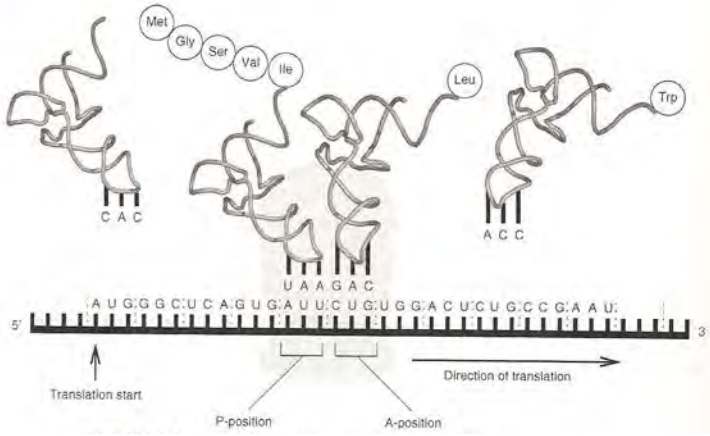


Figure 10.8 Schematic illustration of an animal cell with its major organelles.







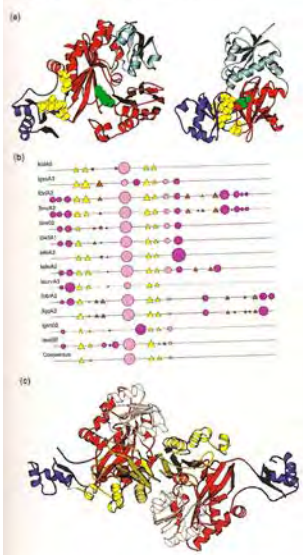
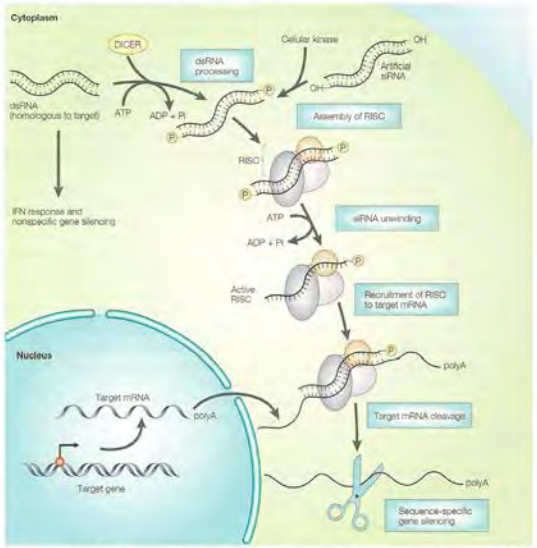
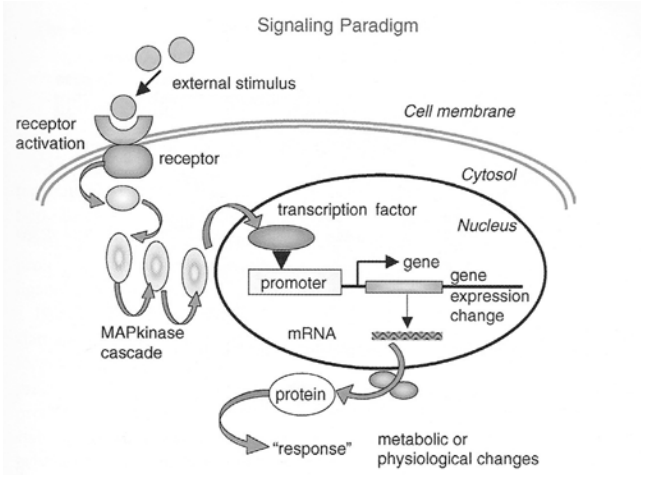
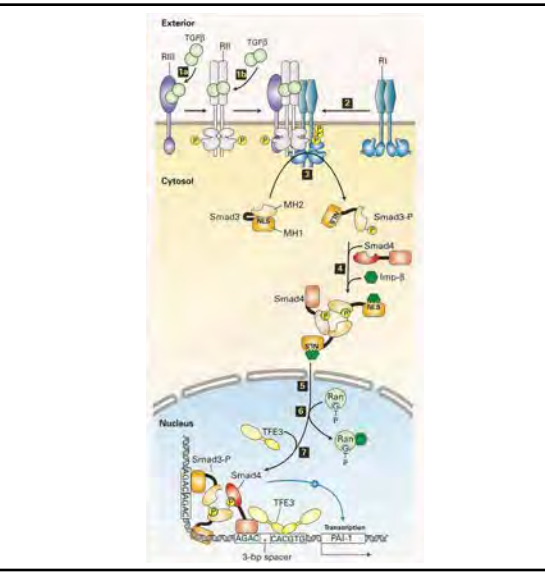


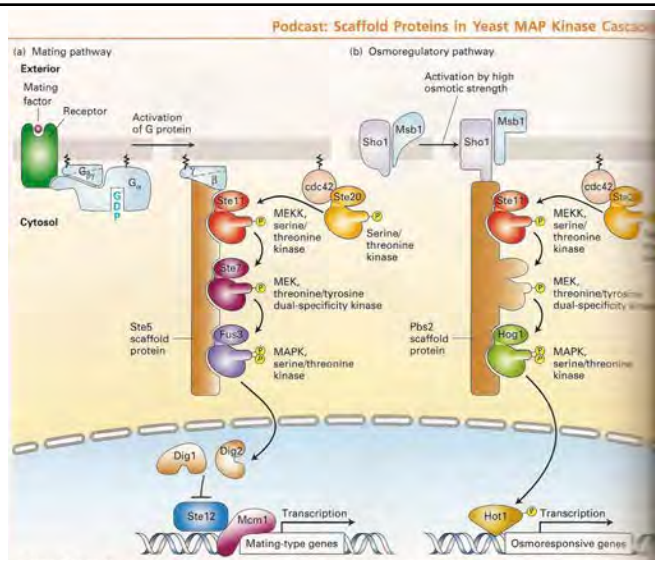
TABLE 2.2 Timescales for the Reactions in the Transcription Network of the Bacterium *E. coli* (Order of Magnitude)

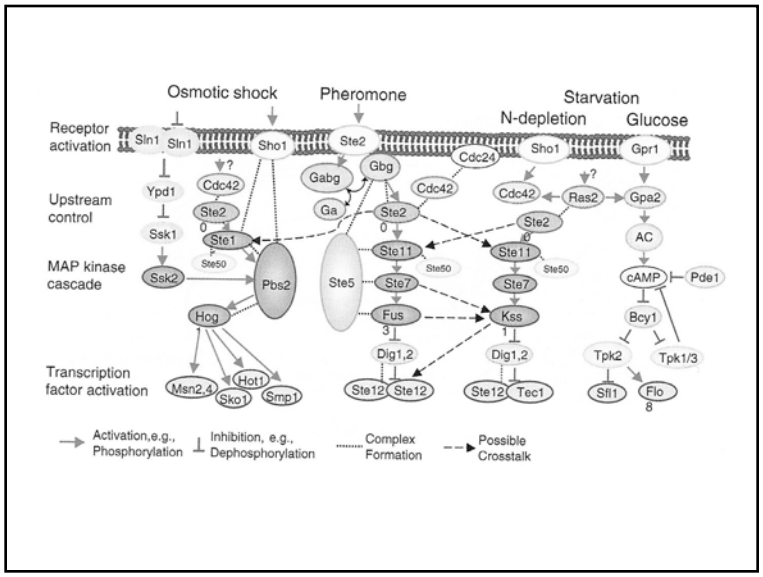
Binding of a small molecule (a signal) to a transcription factor, causing a change in transcription factor activity	~1 msec
Binding of active transcription factor to its DNA site	~1 sec
Transcription + translation of the gene	~5 min
Timescale for 50% change in concentration of the translated protein (stable proteins)	~1 h (one cell generation)

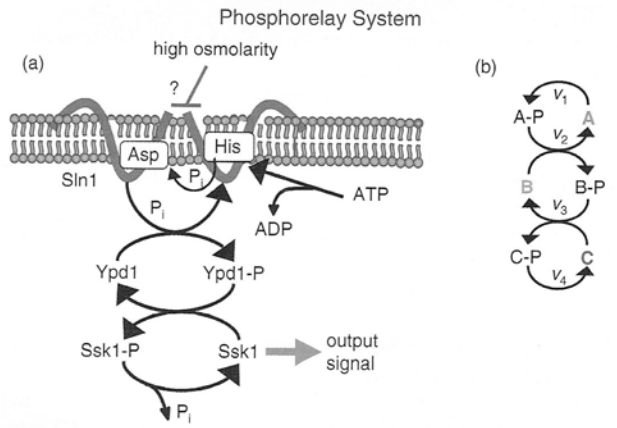

Cytoplasm
DICER
dsRNA processing
Cellular kinase
Artificial siRNA
dsRNA (homologous to target)
ATP
ADP + Pi
Assembly of RISC
RISC
IFN response and nonspecific gene silencing
ATP
ADP + Pi
siRNA unwinding
Active RISC
Recruitment of RISC to target mRNA
Nucleus
polyA
Target mRNA
polyA
Target mRNA cleavage
Target gene
polyA
Sequence-specific gene silencing

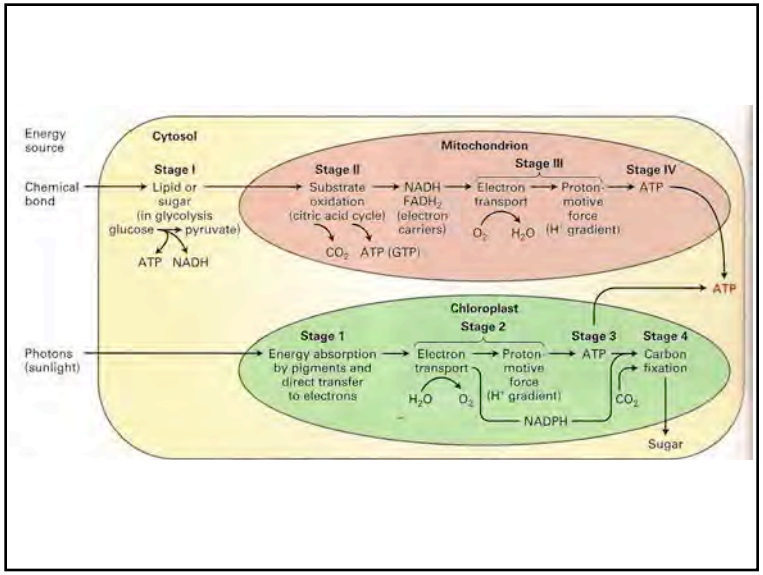

Signaling Paradigm
external stimulus
receptor activation
Cell membrane
receptor
Cytosol
transcription factor
Nucleus
promoter
gene
gene expression change
MAPkinase cascade
mRNA
protein
"response"
metabolic or physiological changes

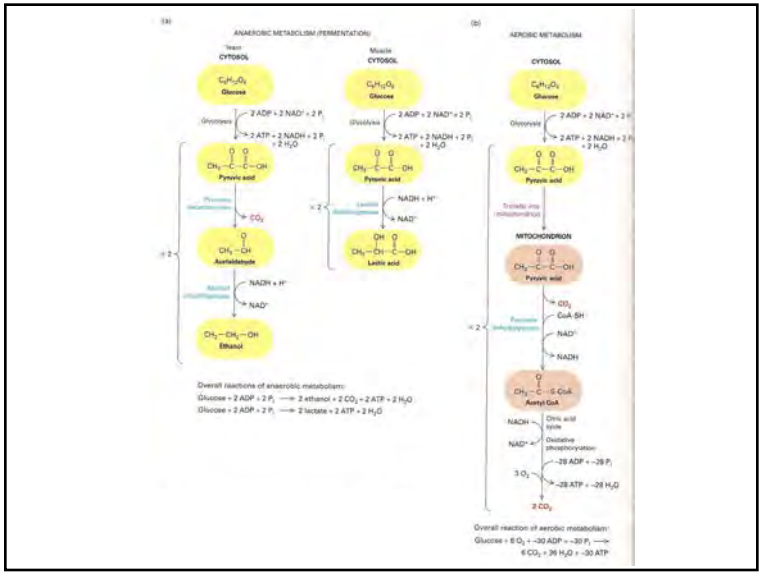

Exterior
TGFβ
RIII
RII
TGFβ
RI
Cytosol
MH2
Smad3
MH1
Smad3-P
Smad4
Imp-β
Smad4
NPC
Nucleus
Ran
TFE3
Ran
Smad3-P
Smad4
TFE3
Transcription
AGAC
CACTTGTG
PAI-1
3-bp spacer

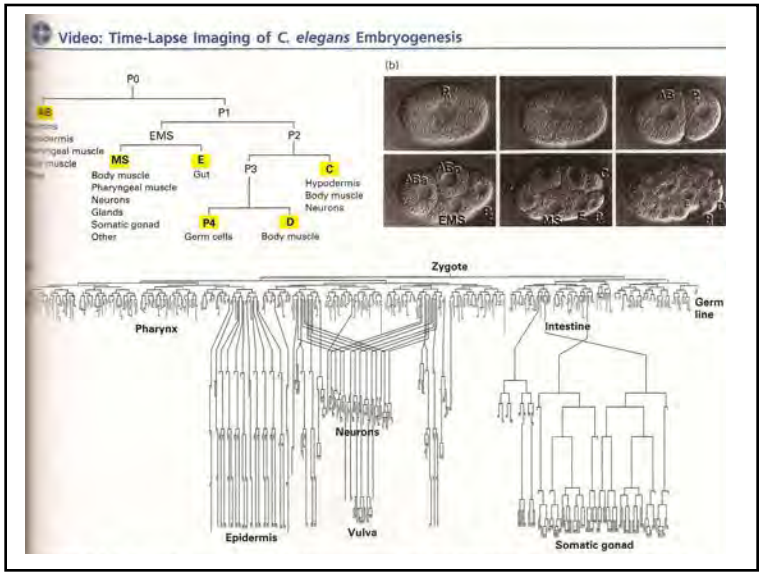

Podcast: Scaffold Proteins in Yeast MAP Kinase Cascade
(a) Mating pathway
(b) Osmoregulatory pathway
Exterior
Mating factor
Receptor
Activation of G protein
Activation by high osmotic strength
Sho1
Msb1
Cytosol
cdc42
Ste20
MEKK, serine/threonine kinase
Serine/threonine kinase
Ste11
Ste7
MEK, threonine/tyrosine dual-specificity kinase
Ste5 scaffold protein
Fus3
MAPK, serine/threonine kinase
Pbs2 scaffold protein
Hog1
Dig1
Dig2
Ste12
Mcm1
Transcription
Mating-type genes
Hot1
Transcription
Osmoresponsive genes

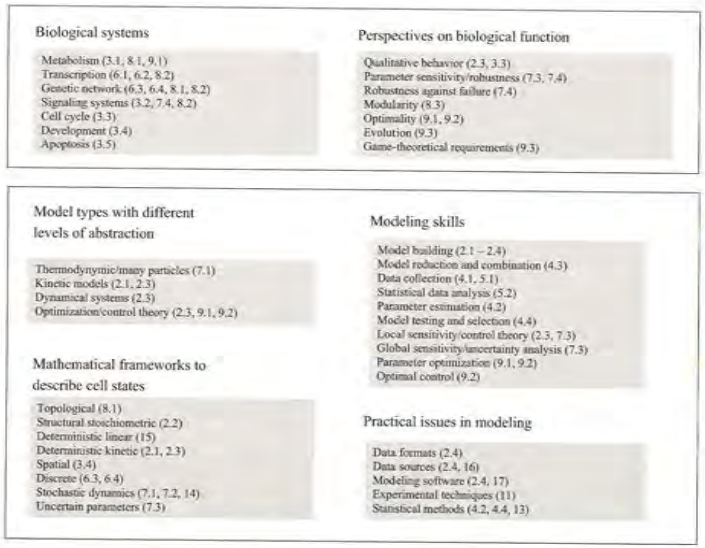

Osmotic shock
Pheromone
Starvation
N-depletion
Glucose
Receptor activation
Upstream control
MAP kinase cascade
Transcription factor activation
Sln1
Sho1
Ste2
Gbg
Gabg
Ga
Cdc24
Gpr1
Ras2
Gpa2
AC
cAMP
Pde1
Bcy1
Tpk2
Tpk1/3
Ypd1
Cdc42
Ste1
Ste50
Ssk1
Ssk2
Pbs2
Hog
Ste5
Ste11
Ste7
Fus
Dig1,2
Ste12
Kss
Tec1
Sfl1
Flo
8
Msn2,4
Hot1
Sko1
Smp1
Activation,e.g., Phosphorylation
Inhibition, e.g., Dephosphorylation
Complex Formation
Possible Crosstalk


Phosphorelay System
high osmolarity
(a)
(b)
Sln1
Asp
His
ATP
ADP
Ypd1
Ypd1-P
Ssk1-P
Ssk1
output signal
A-P
B-P
C-P

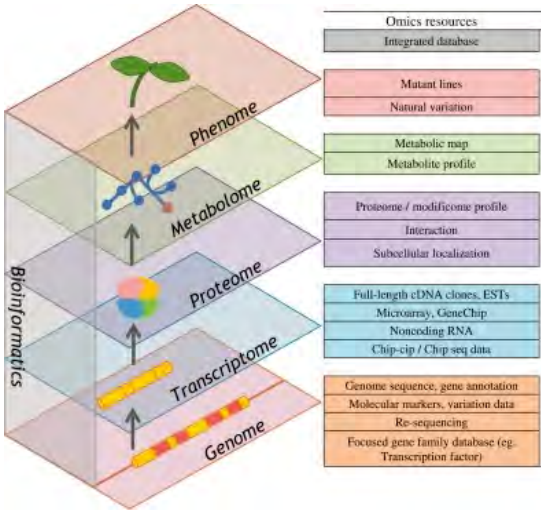

Energy source
Chemical bond
Photons (sunlight)
Cytosol
Mitochondrion
Chloroplast
Stage I
Stage II
Stage III
Stage IV
Stage 1
Stage 2
Stage 3
Stage 4
Lipid or sugar (in glycolysis glucose → pyruvate)
ATP NADH
Substrate oxidation (citric acid cycle)
NADH FADH2 (electron carriers)
Electron transport
Proton-motive force (H+ gradient)
ATP
CO2 ATP (GTP)
O2 H2O
Energy absorption by pigments and direct transfer to electrons
Electron transport
Proton-motive force (H+ gradient)
ATP
Carbon fixation
H2O O2
NADPH
CO2
Sugar

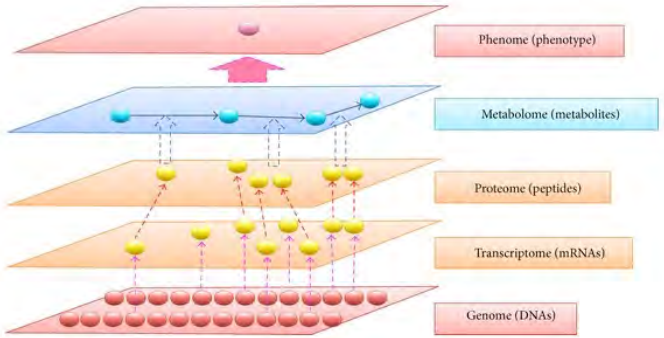

ANAEROBIC METABOLISM (FERMENTATION)
AEROBIC METABOLISM
CYTOSOL
MITOCHONDRION
Glucose
Pyruvic acid
Acetaldehyde
Ethanol
Lactic acid
Acetyl CoA
NADH
NAD+
CO2
CoA-SH

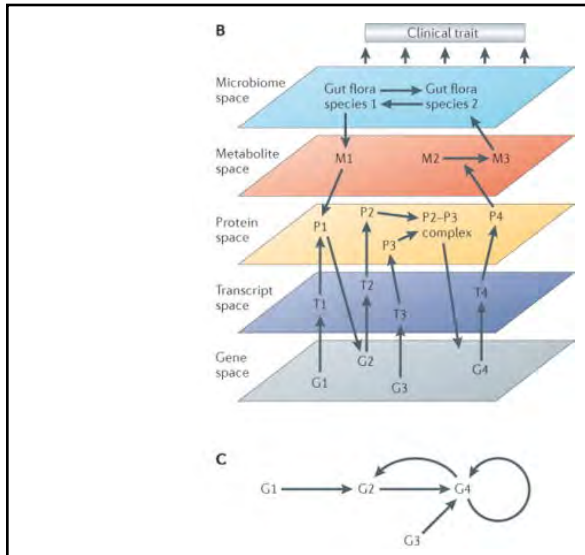


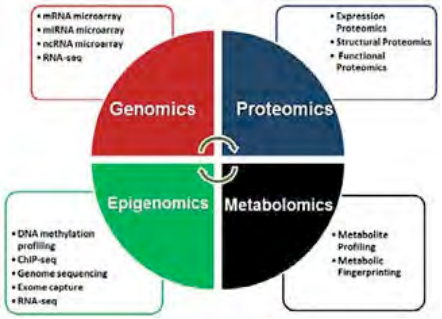


Omics Technology

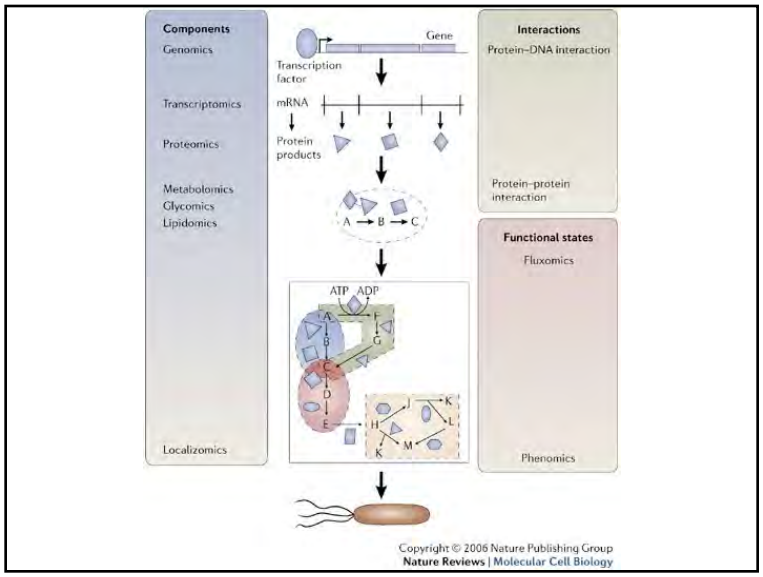


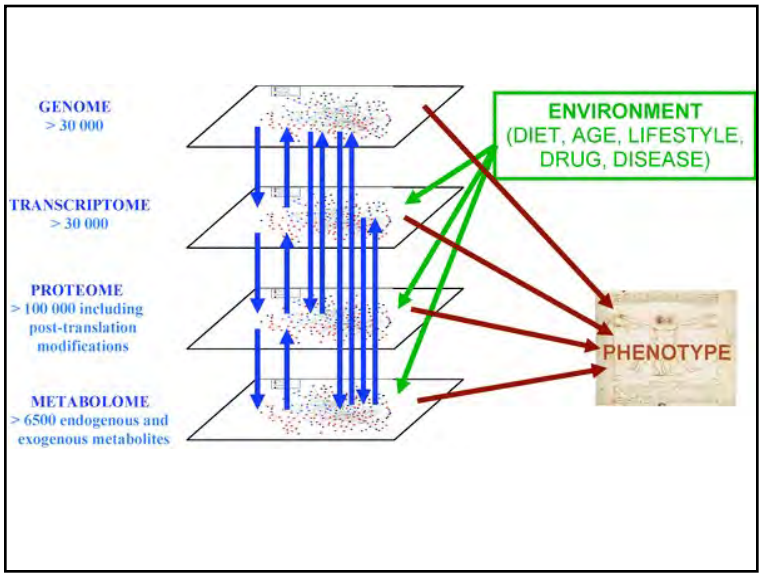






Genomics	Transcriptomics	Proteomics	Metabolomics	Protein–DNA interactions	Protein–protein interactions	Fluxomics	Phenomics
Genomics (sequence annotation)	• ORF validation • Regulatory element identification[4]	• SNP effect on protein activity or abundance	• Enzyme annotation	• Binding-site identification[70]	• Functional annotation[71]	• Functional annotation	• Functional annotation[43,161] • Biomarkers[81]
	Transcriptomics (microarray, SAGE)	• Protein–transcript correlation[80]	• Enzyme annotation[160]	• Gene-regulatory networks[74]	• Functional annotation[68] • Protein complex identification[67]		• Functional annotation[162]
		Proteomics (abundance, post-translational modification)	• Enzyme annotation[81]	• Regulatory complex identification	• Differential complex formation	• Enzyme capacity	• Functional annotation
			Metabolomics (metabolite abundance)	• Metabolic–transcriptional response		• Metabolic pathway bottlenecks	• Metabolic flexibility • Metabolic engineering[163]
				Protein–DNA interactions (ChIP–chip)	• Signalling cascades[69,164]		• Dynamic network responses[68]
					Protein–protein interactions (yeast 2H, coAP–MS)		• Pathway identification activity[69]
						Fluxomics (isotopic tracing)	• Metabolic engineering
							Phenomics (phenotype arrays, RNAi screens, synthetic lethals)

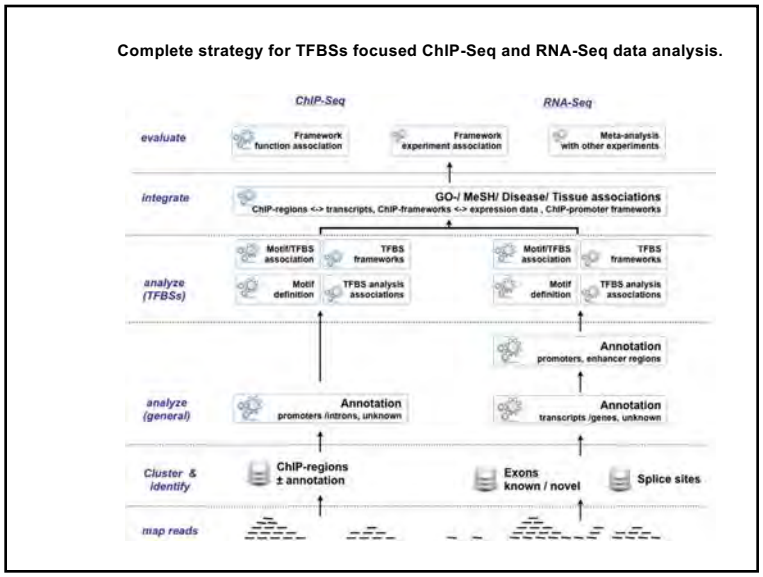


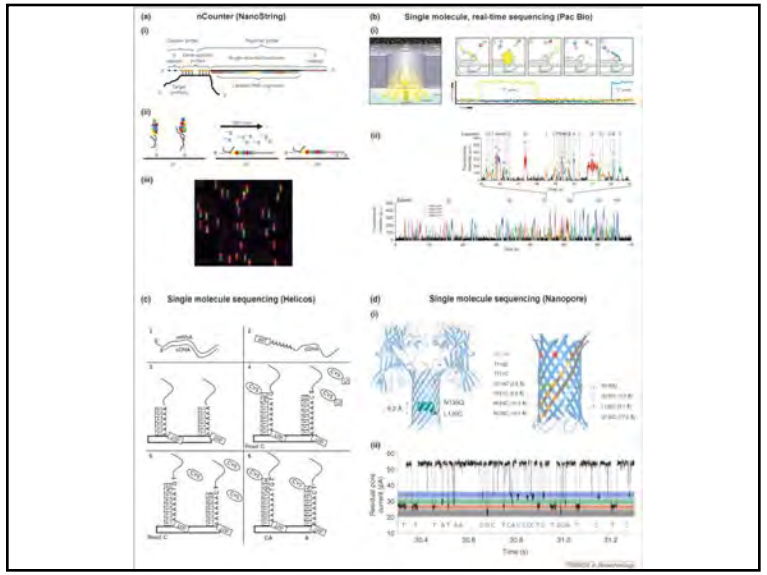


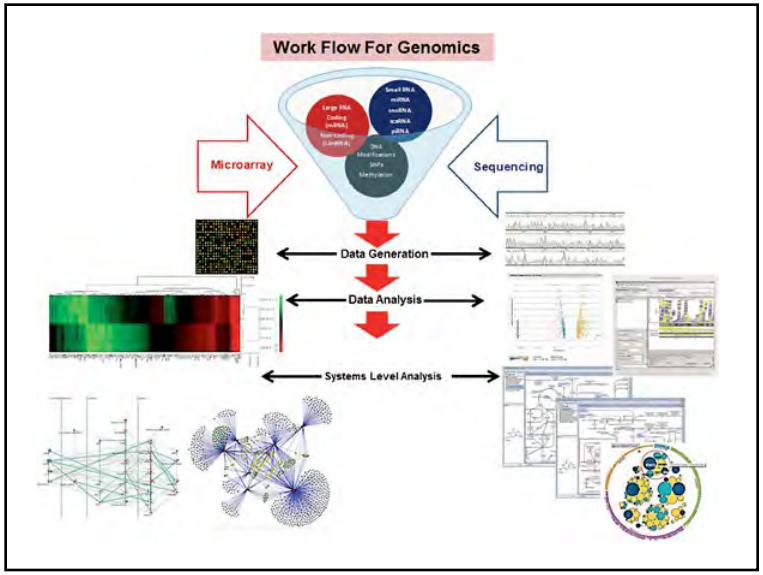
Genomics

Tiling array

A high-density microarray that contains evenly spaced, or ‘tiled’, sets of probes that span the genome or chromosome, and can be used in many experimental applications such as transcriptome characterization, gene discovery, alternative-splicing analysis, ChIP-chip, DNA-methylation analysis, DNA-polymorphism analysis, comparative genome analysis and genome resequencing.







Transcriptome

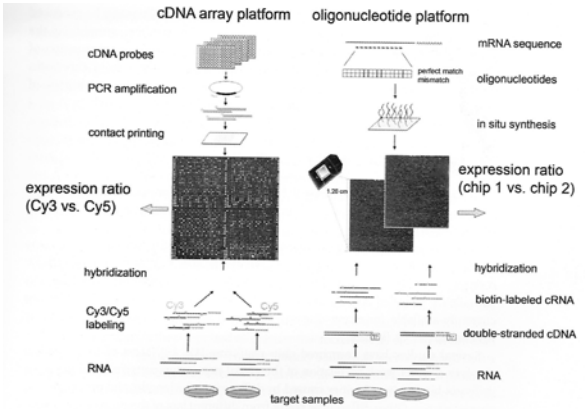
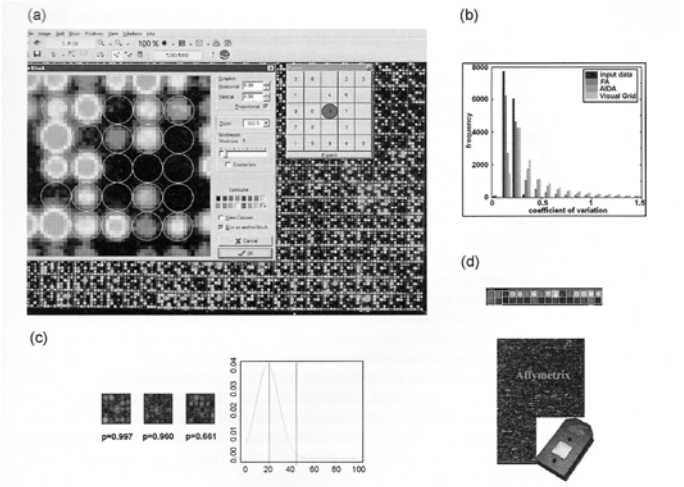


Figure 5.1 Scheme of DNA array analysis using cDNAs (left) and oligonucleotides (right, here Affymetrix technology) as probes. Probe design and construction of the array is shown in the upper part of the figure and the different preparation steps of the target samples are shown in the lower part. In the center of the figure, the strategy for case-control studies is shown using a two-color approach (left panel) and a single-color approach (right panel). Array images were taken from [69] and from http://www.affymetrix.com.



5.2
Analysis of Gene Expression Data

Summary

The analysis of genome-wide gene expression data involves basic concepts from multivariate statistics. Most applications belong to two groups: the first group consists of case-control studies comparing a certain transcriptome state of the biological system (e.g., disease state, perturbed state) to the control situation; the second group of applications consist of multiple case studies involving different states (e.g., drug response time series, groups of patients, etc.). The analysis of case-control studies involves testing of statistical hypotheses. Here, expression changes are observed that deviate from a predefined hypothesis and this deviation is judged for significance. The basic methods for multicase studies are clustering and classification. Here, groups of coexpressed genes serve to identify functionally related groups of genes or experiments. These types of analysis result in the identification of marker genes and their related interactions, which are the basis for further network studies.

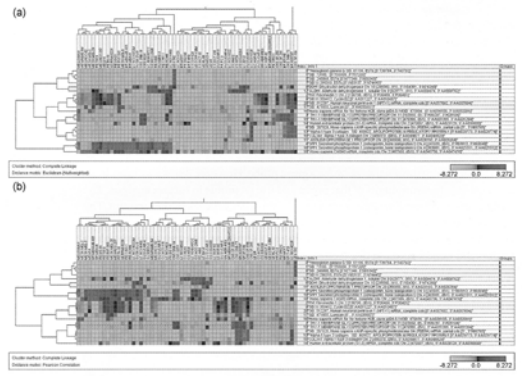
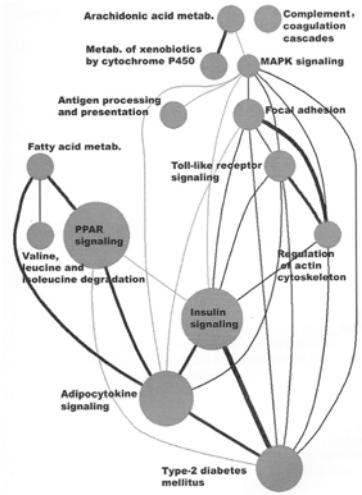
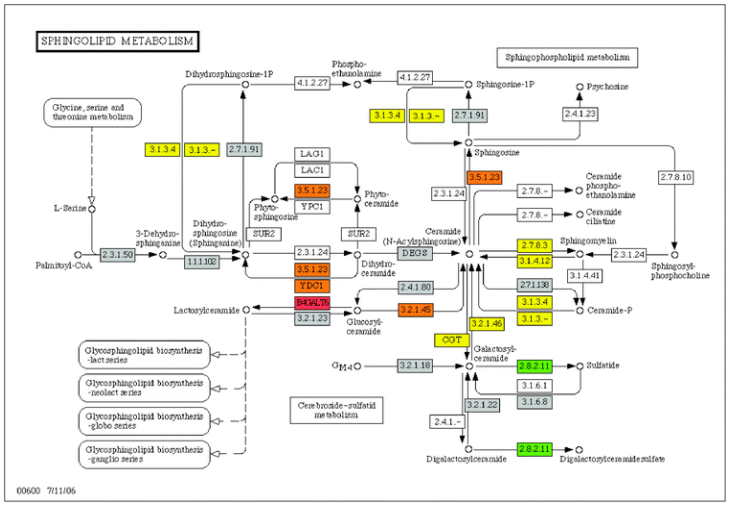


Figure 5.5 Influence of similarity measure on clustering. Two dendrograms of a subgroup of genes using the microarray expression data of Ross *et al.* [70] were generated using hierarchical clustering with Euclidean distance (a) and Pearson correlation (b) as pairwise similarity measure. Although all other parameters are kept constant, results show differences in both gene and cancer cell line groupings. Clustering was performed with the *J-Express Pro* software package (Molmine, Bergen Norway).





6.3
Dynamic Models of Gene Regulation

Summary

In order to comprehend the functioning of organisms at the molecular level, we wish to know which genes are expressed, to what level, where, and when. A network of interactions between DNA, mRNA, proteins, and other molecules realizes the regulation of gene expression. This network comprises many components. According to the central dogma of molecular biology formulated by Francis Crick [71], there is a forward flow of information from gene to mRNA to protein. Moreover, positive and negative feedback loops and information exchange with signaling pathways and energy metabolism ensure the appropriate regulation of expression according to the current state of the cell and the environment.

Modeling of gene expression is used here as an example to apply different modeling techniques. The dynamics and the regulatory patterns of gene expression will be mathematically described with various graphs, Boolean networks, Bayesian networks, ordinary and partial differential equation systems, stochastic processes, and with rule-based formalisms.

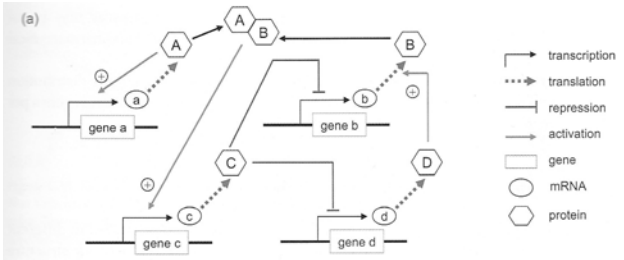
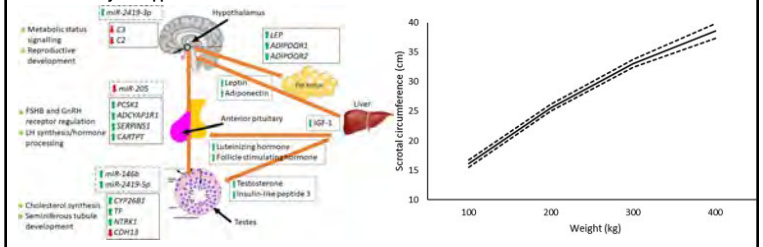
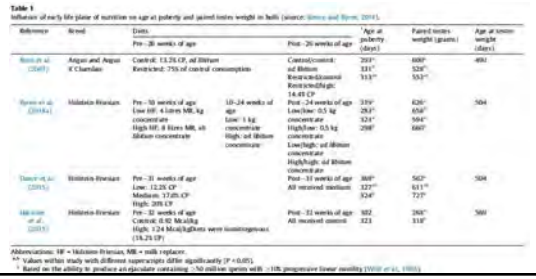


Figure 6.9 Gene regulatory network with four genes and mutual regulation.

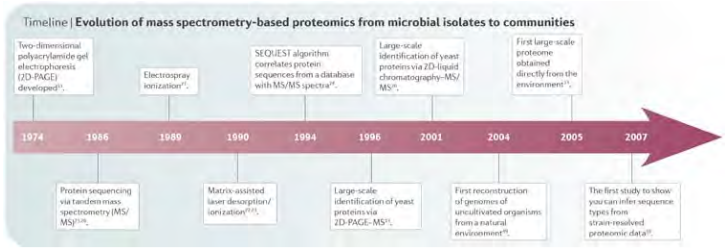
Role of early life nutrition in regulating sexual development in bulls
C J Byrne, K Keogh, D A Kenny
Animal. 2023 May:17 Suppl 1:100802.





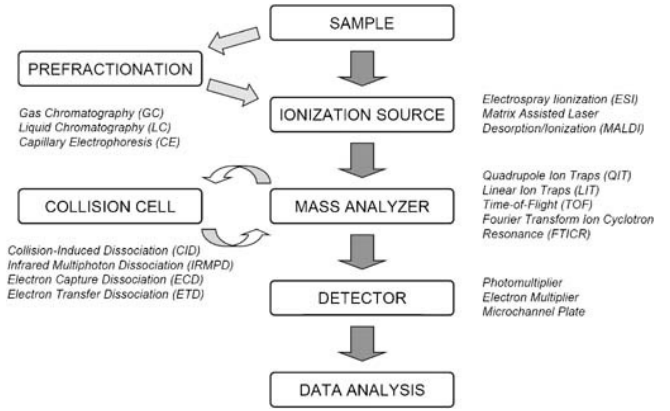
Proteome

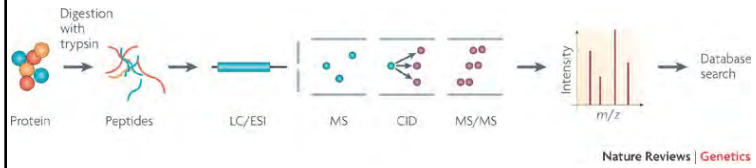
Mass spectrometry
An analysis technique that identifies biochemical molecules (such as proteins, metabolites or fatty acids) on the basis of their mass and charge.



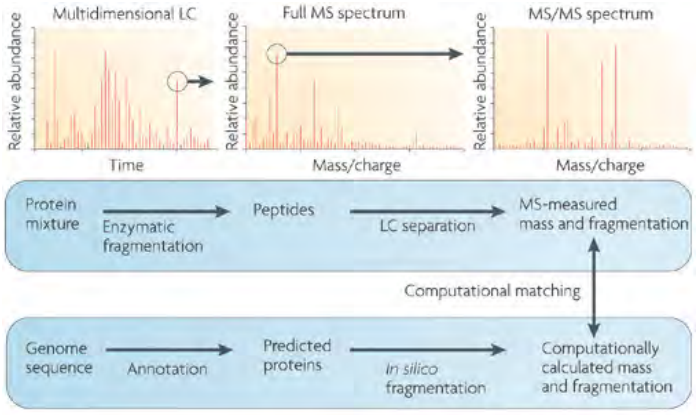
Tandem mass spectrometry

This combines two mass spectrometers: one (MS1) for the detection and selection of precursor ions, which is followed by a second (MS2) for the analysis of fragment ion spectra generated from selected precursor ions after collision-induced fragmentation. The information from the fragment ion spectra is used for peptide identification.

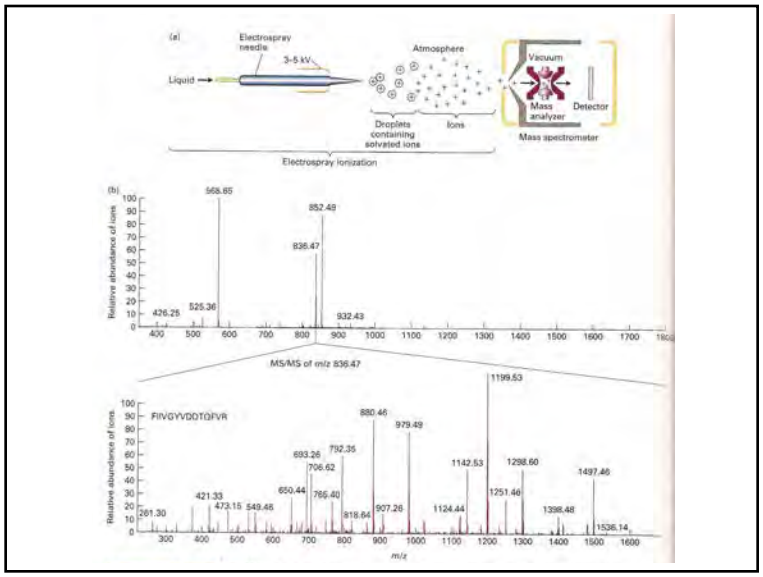




Nature Reviews | Genetics



Nature Reviews | Microbiology



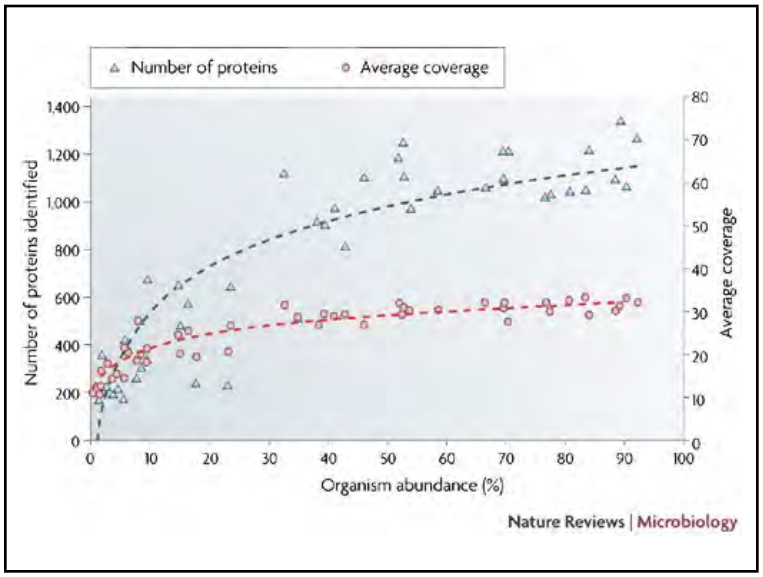
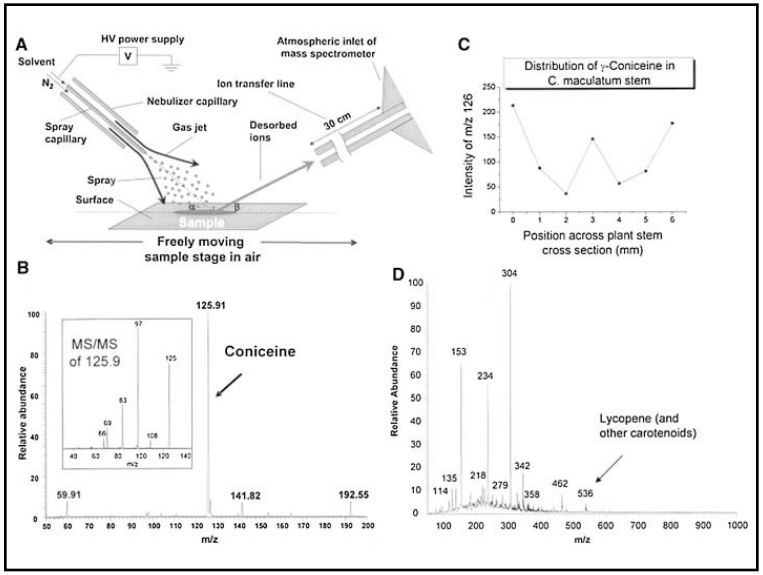
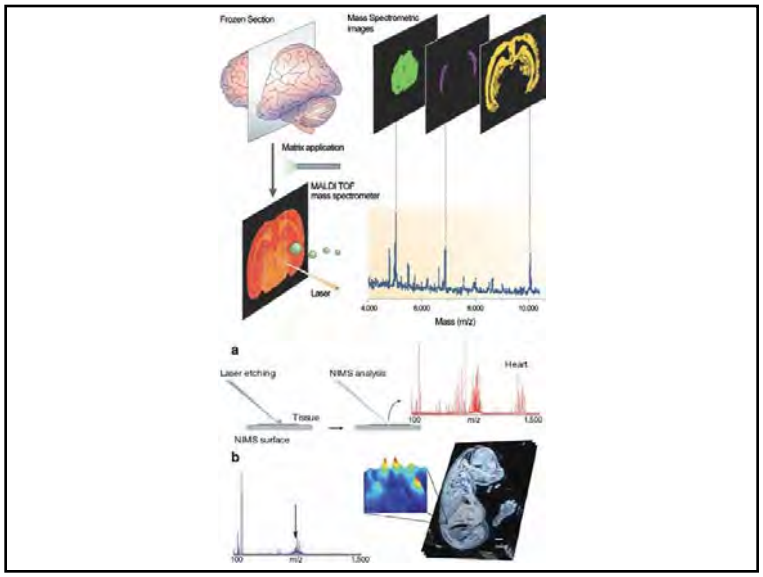
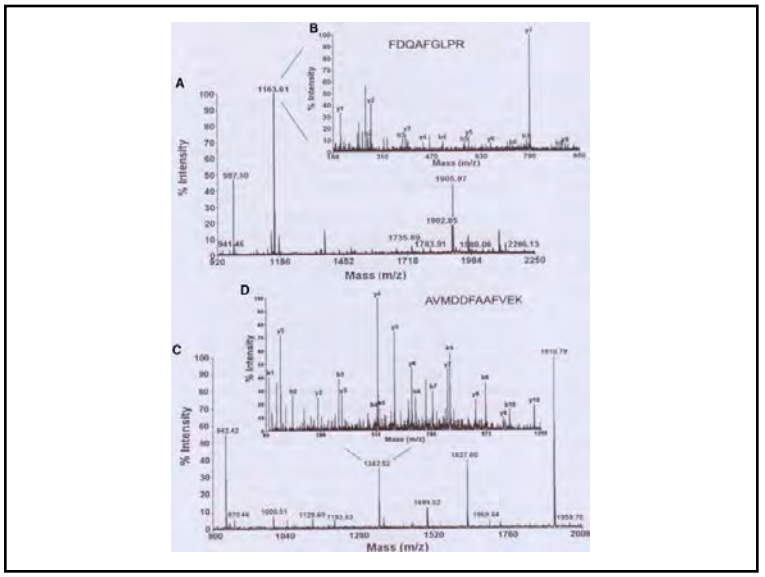


TABLE 1. Potential Cancer Biomarkers Identified by Mass Spectrometry-Based 'Omics' Technologies

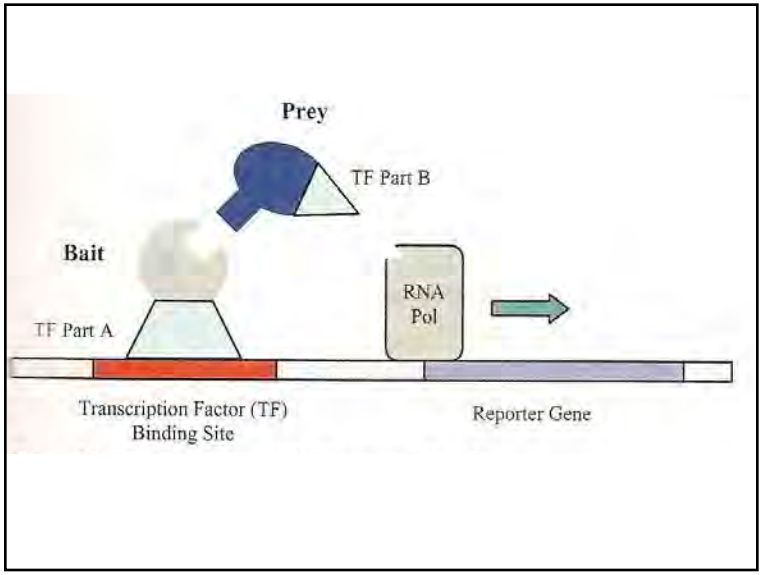
Biomarkers	"omics" platforms	MS methods	Sample source	Cancer type	References
Apolipoprotein A1, Inter-α-trypsin inhibitor Haptoglobin-a-subunit Transthyretin	Proteomics	SELDI-TOF	Serum	Ovarian	Ye et al., 2003; Zhang et al., 2004
Vitamin D-binding protein	Proteomics	SELDI-TOF	Serum	Prostate	Hlavaty et al., 2003
Stathmin (Op18), GRP 78	Proteomics	ESI-MS	Tissue	Lung	Chen et al., 2003
14-3-3 isoforms, Transthyretin Protein disulfide Isomerase					
Peroxiredoxin, Enolase Protein disulfide Isomerase	Proteomics	MALDI-TOF, LC-MS	Tissue	Breast	Somiari et al., 2003
HSP 70, α-1-antitrypsin HSP 27	Proteomics	MALDI-TOF	Serum	Liver	Feng et al., 2005
Annexin I, Cofilin, GST Superoxide dismutase Peroxiredoxin, Enolase Protein disulfide Isomerase	Proteomics	MALDI-TOF, ESI-MS, Q-TOF	Tissue	Colon	Seike et al., 2003; Stierum et al., 2003
Neutrophil peptides 1-3	Proteomics	SELDI-TOF	Nipple aspirate fluid	Breast	Li et al., 2005b
PCa-24	Proteomics	MALDI-TOF	Tissue	Prostate	Zheng et al., 2003
Alkanes, Benzenes	Metabonomics	GC-MS	Breath	Lung	Phillips et al., 1999
Decanes, Heptanes	Metabonomics	GC-MS	Breath	Breast	Phillips et al., 2003
Hexanal, Heptanal	Metabonomics	LC-MS	Serum	Lung	Deng et al., 2004
Pseu, m1A, m1I	Metabonomics	HPLC, LC-MS	Urine	Liver	Yang et al., 2005b

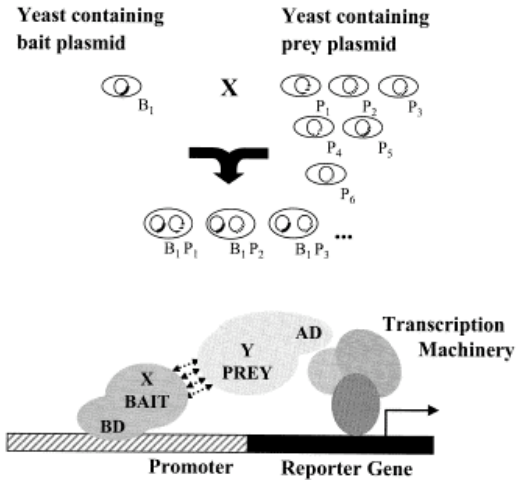


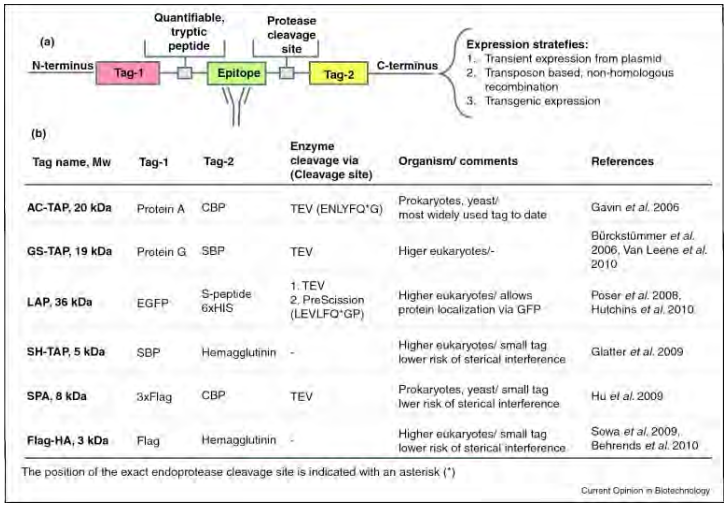




Protein Interactome







Tag name, Mw	Tag-1	Tag-2	Enzyme cleavage via (Cleavage site)	Organism/ comments	References
AC-TAP, 20 kDa	Protein A	CBP	TEV (ENLYFQ*G)	Prokaryotes, yeast/ most widely used tag to date	Gavin *et al.* 2006
GS-TAP, 19 kDa	Protein G	SBP	TEV	Higer eukaryotes/-	Bürckstümmer *et al.* 2006, Van Leene *et al.* 2010
LAP, 36 kDa	EGFP	S-peptide 6xHIS	1. TEV 2. PreScission (LEVLFQ*GP)	Higher eukaryotes/ allows protein localization via GFP	Poser *et al.* 2008, Hutchins *et al.* 2010
SH-TAP, 5 kDa	SBP	Hemagglutinin	-	Higher eukaryotes/ small tag lower risk of sterical interference	Glatter *et al.* 2009
SPA, 8 kDa	3xFlag	CBP	TEV	Prokaryotes, yeast/ small tag lwer risk of sterical interference	Hu *et al.* 2009
Flag-HA, 3 kDa	Flag	Hemagglutinin	-	Higher eukaryotes/ small tag lower risk of sterical interference	Sowa *et al.* 2009, Behrends *et al.* 2010

The position of the exact endoprotease cleavage site is indicated with an asterisk (*)

Current Opinion in Biotechnology

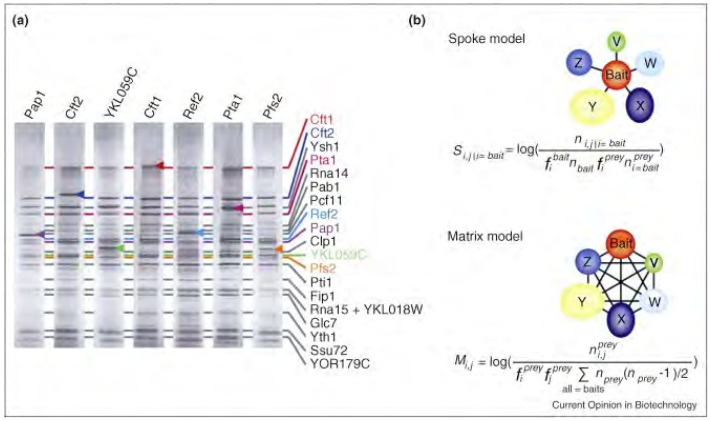
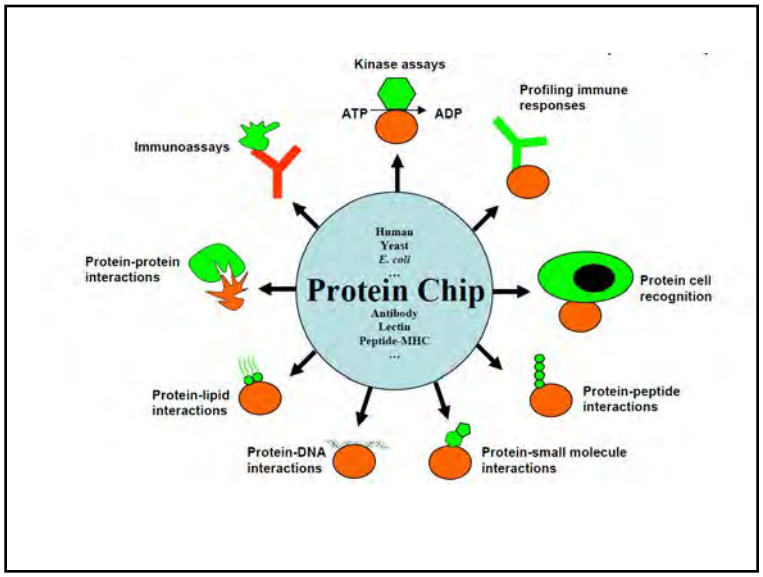
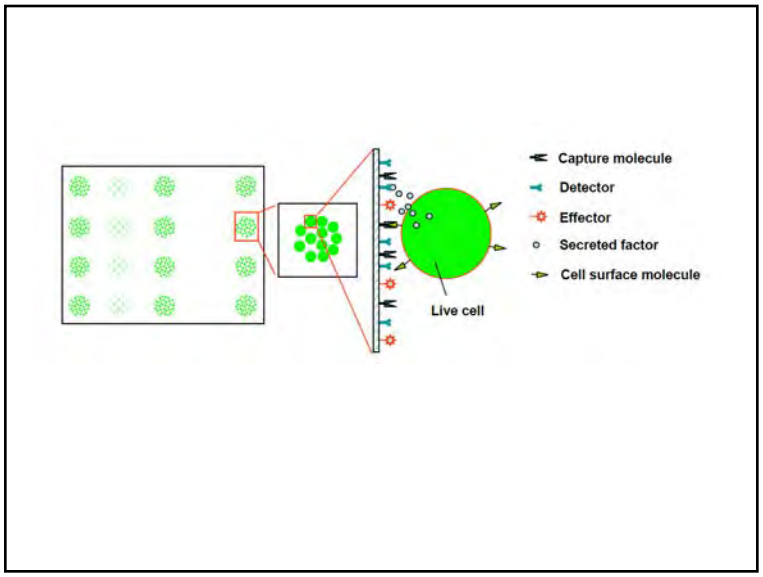
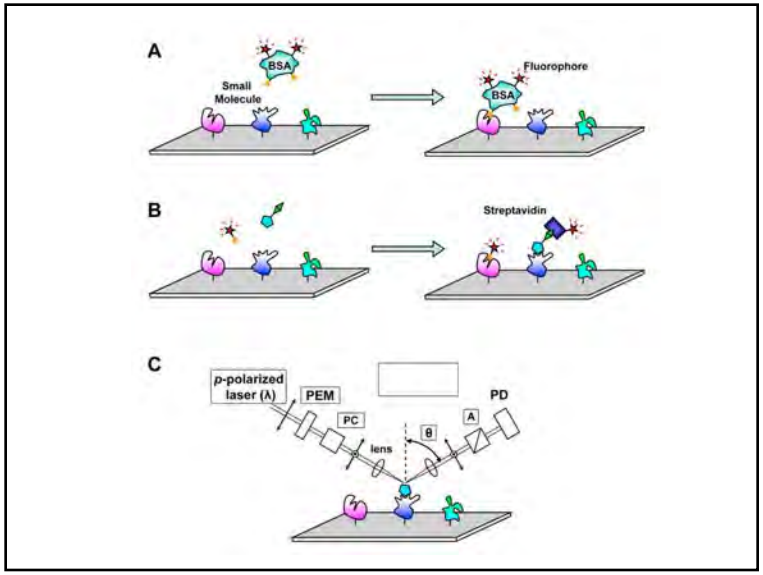


Fig. (1). Image of a typical protein microarray. A yeast protein microarray has been probed with an anti-GST antibody, followed by a Cy3-conjugated secondary antibody. An enlarged image of one of the 48 blocks is shown below the protein chip.

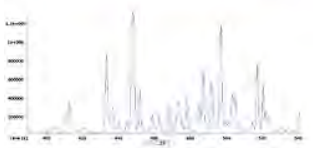

Kinase assays
ATP
ADP
Profiling immune responses
Immunoassays
Protein-protein interactions
Human
Yeast
E. coli
...
Protein Chip
Antibody
Lectin
Peptide-MHC
...
Protein cell recognition
Protein-lipid interactions
Protein-peptide interactions
Protein-DNA interactions
Protein-small molecule interactions


Capture molecule
Detector
Effector
Secreted factor
Cell surface molecule
Live cell


A
BSA
Small Molecule
Fluorophore
BSA
B
Streptavidin
C
p-polarized laser (λ)
PEM
PC
lens
θ
A
PD

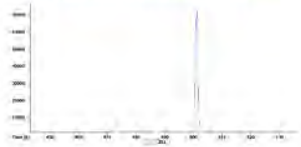
Metabolome

METABOLIC PROFILING

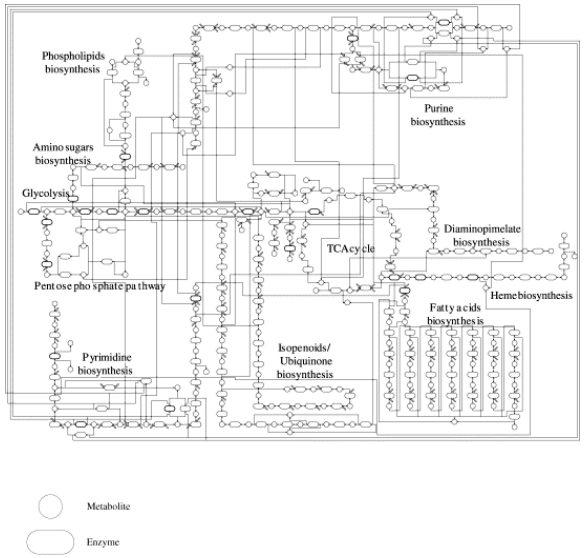


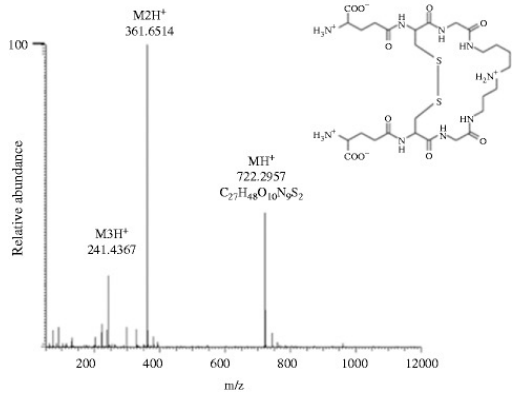
- (semi)-quantitative (global) detection of a wide range of metabolites
- data acquisition without *a priori* knowledge of biologically interesting metabolites
- search for the proverbial 'needles in the metabolic haystack'
- **inductive/hypothesis generating** experiment
- appropriate experimental design and data analysis is essential
- **MIDDLE-IN strategy**

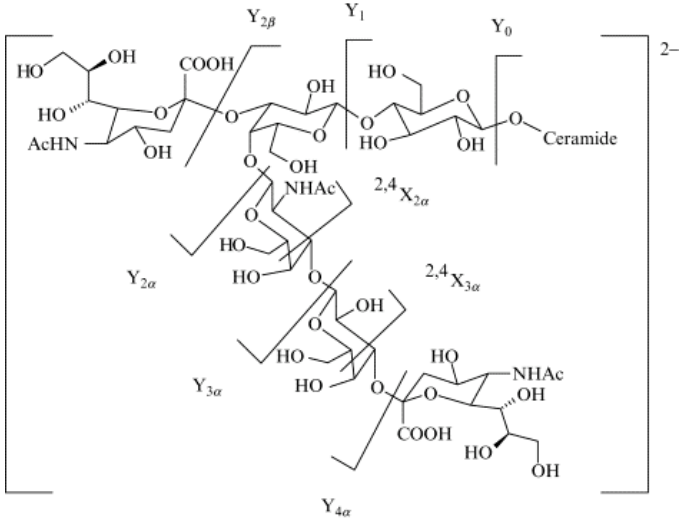
TARGETED ANALYSIS

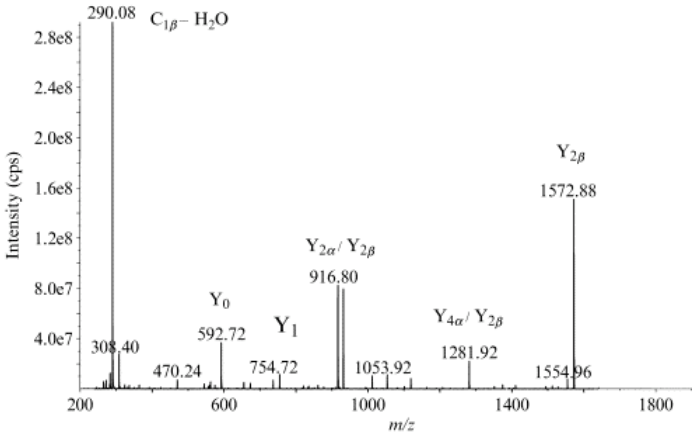


- quantification of a small number of related metabolites for **hypothesis testing** or systems biology modelling
- specificity provided by extensive sample preparation and MS/MS
- absolute quantification using isotopic internal standards or standards addition
- **BOTTOM-UP strategy**









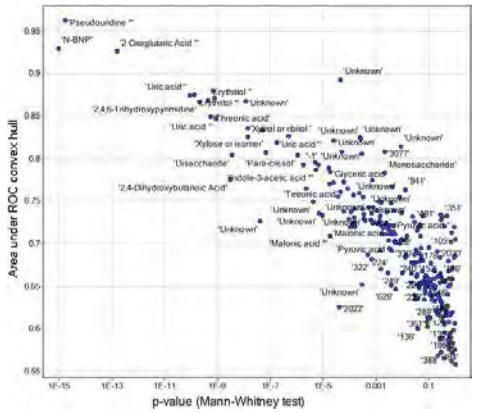


Fig. 13 An example of plots describing the relationship between area under ROC curve and p-values for various metabolites. These plots are applicable when comparing univariate biomarkers or multiple model predictions. The more effective biomarkers approach the top left hand corner of the plot (i.e., low p-value and high AuROC). Kindly reprinted from a study related to heart failure203 with permission from Springer.

Databases

16

Databases

Summary

With the rapid increase of biological data, it has become even more important to organize and structure the data in a way so that information can easily be retrieved. As a result, the number of databases has also increased rapidly over the past few years. Most of these databases have a web interface and can be accessed from everywhere in the world, which is an enormously important service for the scientific community. In the following, various databases are presented that might be relevant for systems biology.

Moreover, the journal *Nucleic Acids Research* offers a database issue each year in January dedicated to factual biological databases and in addition to this a web server issue each year in July presenting web-based services.

Databases Sources

- National Center for Bioinformatics NCBI
- European Bioinformatics Institute
- EMBL
- Ensembl
- Interpro
- Protein databank
- Bionumbers
- Gene Ontology
- Pathway- KEGG
- Consensus Path DB

Table 1 Examples of the most commonly used endocrine-related breast cancer public omic datasets.

Database	URL	Data spaces
CPTAC	https://proteomics.cancer.gov/data-portal	Proteome
EGA	https://ega-archive.org/datasets	Genome, Transcriptome
EMBL-EBI	https://www.ebi.ac.uk/services/all	Genome, Transcriptome, Proteome, Metabolome
GNPS/Massive	https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp	Metabolome
ICGC	https://dcc.icgc.org/	Genome, Transcriptome
MassIVE	https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp	Proteome
Metabolomics Workbench	https://www.metabolomicsworkbench.org/	Metabolome
NCBI-GEO	https://www.ncbi.nlm.nih.gov/gds	Genome, Transcriptome
ONCOMINE	https://www.oncomine.org/resource/login.html	Genome, Transcriptome
ProteomeXchange (PX) Consortium	http://www.proteomexchange.org/	Proteome
ProteomicsDB	https://www.proteomicsdb.org/	Proteome
TCGA	https://portal.gdc.cancer.gov/	Genome, Transcriptome

Primary data and metadata quality vary across and within these sites. For example, clinical metadata for human subjects are often limited. The platform used for data collection in each omics space also can vary across and within these sites. While most provide access to the raw (unprocessed) data, ONCOMINE primarily exposes only processed data; the method of data processing can vary across individual studies.

Data mining

An analytical discipline that is focused on finding unsuspected relationships and summarizing often large observational data sets in new ways that are both understandable and useful to the data owner.

***In silico* prediction**

A general term that refers to a computational prediction that usually results from the analysis of a mathematical or computational model.

Unsupervised analysis: Unsupervised analysis includes methods used for grouping of features (sample, metabolites and spectral features) according to the molecular data measured. These methods are used for the analysis of features when no prior information is available about the system. Depending on the method, the analysis might or might not require the user to define the number of clusters. In terms of cell culture metabolomics, this method is ideal for discovery of novel classes.

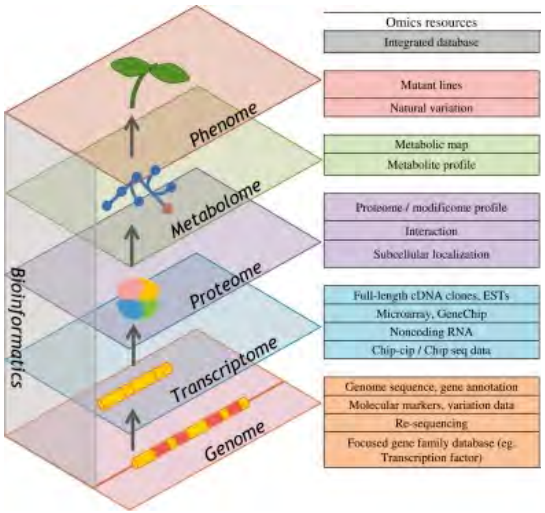
Supervised analysis: Supervised analysis defines methods for sample grouping or classification and for selection of major sample defining features. In supervised analysis, a set of features is pre-assigned to a class and it is used as a training set for the method of choice to define a classifier that will be used for classification of an unknown sample. Supervised analysis creates a model from the training set and, thus, can only be accurately used for classification of a different dataset (i.e. supervised analysis requires application of cross-validation for the determination of accuracy of the classifier).

Table 1 | **'Omics' data repositories***

Data types	Online resource	Description	URL
Components			
Genomics	Genomes OnLine Database (GOLD)	Repository of completed and ongoing genome projects	http://www.genomesonline.org
Transcriptomics	Gene Expression Omnibus (GEO)	Microarray and SAGE-based genome-wide expression profiles	http://www.ncbi.nlm.nih.gov/geo
	Stanford Microarray Database (SMD)	Microarray-based genome-wide expression data	http://genome-www.stanford.edu/microarray
Proteomics	World-2DPAGE	Links to 2D-PAGE data	http://us.expasy.org/ch2d/2d-index.html
	Open Proteomics Database (OPD)	Mass-spectrometry-based proteomics data	http://bioinformatics.icmb.utexas.edu/OPD
Lipidomics	Lipid Metabolites and Pathways Strategy (LIPID MAPS)	Genome-scale lipids database	http://www.lipidmaps.org
Localizomics	Yeast GFP Fusion Localization Database	Yeast genome-scale protein-localization data	http://yeastgfp.ucsf.edu
Interactions			
Protein–DNA	Biomolecular Network Database (BIND)	Published protein–DNA interactions	http://www.bind.ca/Action/
	Encyclopedia of DNA Elements (ENCODE)	Database of functional elements in human DNA	http://genome.ucsc.edu/ENCODE/index.html
Protein–protein	Munich Information Center for Protein Sequences (MIPS)	Links to protein–protein interaction data and resources	http://mips.gsf.de/proj/ppi
	Database of Interacting Proteins (DIP)	Published protein–protein interactions	http://dip.doe-mbi.ucla.edu
Functional states			
Phenomics	RNAi database	*C. elegans* RNAi screen data	http://rnai.org
	General Repository for Interaction Datasets (GRID)	Synthetic-lethal interactions in yeast	http://biodata.mshri.on.ca/grid
	A Systematic Annotation Package For Community Analysis of Genomes (ASAP)	Single-gene-deletion microarray data for *E. coli* phenotypes	http://www.genome.wisc.edu/tools/asap.htm

*This table details some of the databases that store and distribute genome-scale omics data sets through publicly accessible Web sites. Some omics technologies do not yet have associated data dissemination resources — notably metabolomics, glycomics and fluxomics — and are therefore not included in this table. It should also be noted that this table does not represent all publicly available omics data resources, but, rather, provides a reasonably broad sample of the data that are readily accessible to researchers today. *C. elegans*, *Caenorhabditis elegans*; 2D-PAGE, two-dimensional polyacrylamide-gel electrophoresis; *E. coli*, *Escherichia coli*; GFP, green fluorescent protein; RNAi, RNA interference; SAGE, serial analysis of gene expression.

Omics Data Integration



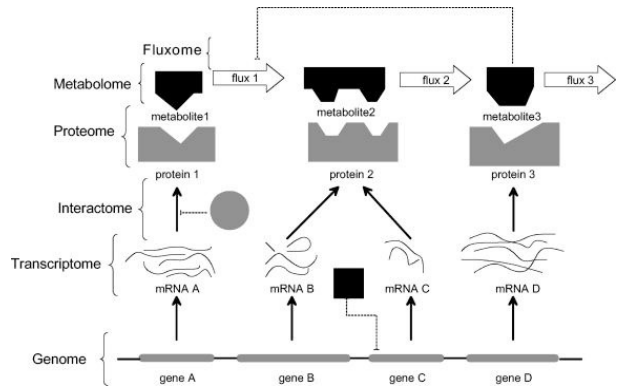
Omics data integration

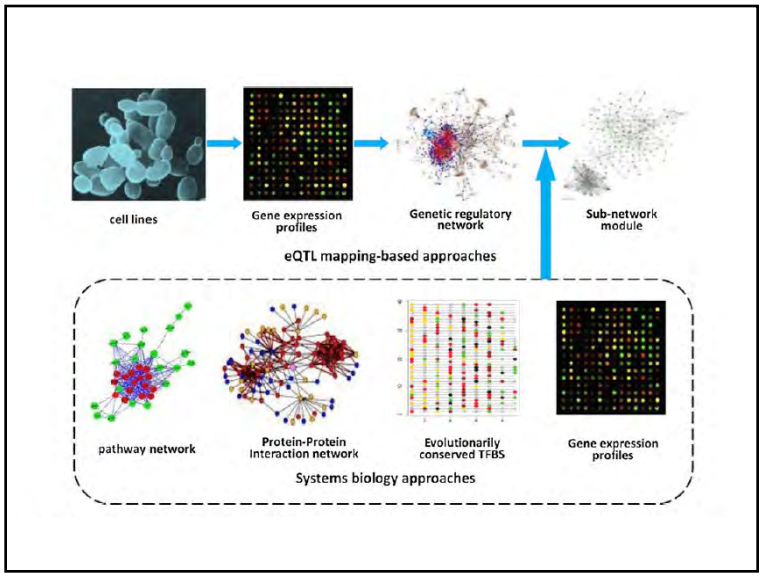
The simultaneous analysis of high-throughput genome-scale data that is aimed at developing models of biological systems to assess their properties and behavior.

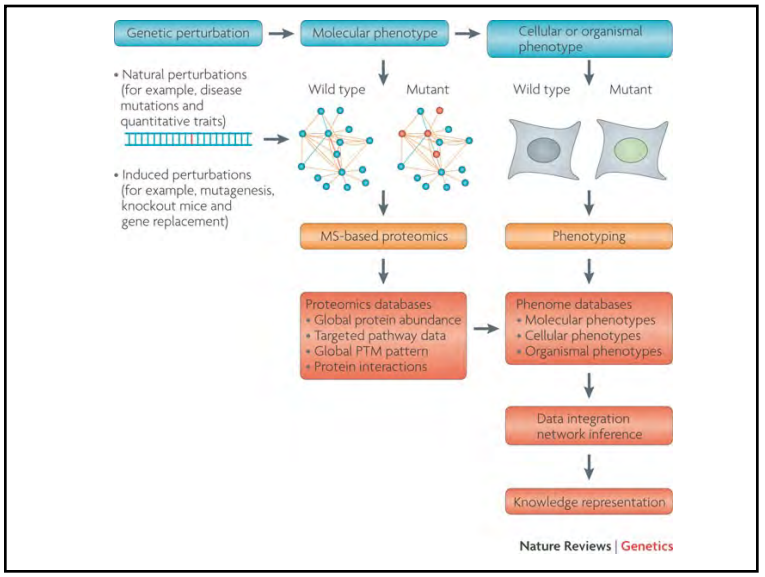
Table 3 | **Software for 'omics' integration***

Package	Accessibility	Functionality	URL
Biotapestry	Open source	• Build developmental gene-regulatory network models • Visualize network • Simulate and analyse network behaviour	http://www.biotapestry.org
CellDesigner	Open source	• Build interaction maps • Visualize process diagrams	http://www.celldesigner.org
Cytoscape	Open source	• Build interaction maps • Visualize high-throughput data • Conduct graph theoretical analysis	http://www.cytoscape.org
Pajek	Free for non-commercial use	• Build interaction maps • Visualize network structure • Conduct graph theoretical analyses	http://vlado.fmf.uni-lj.si/pub/networks/pajek/default.htm
SimPheny	Commercial	• Build COBRA models • Integrate high-throughput data • Visualize high-throughput data • Simulate and analyse network behaviour	http://www.genomatica.com
ToPNet	Open source	• Represent interaction maps as PetriNets • Visualize high-throughput data	http://www.biosolveit.de/ToPNet
YeastHub	Open source	• Integrate high-throughput data	http://yeasthub.gersteinlab.org

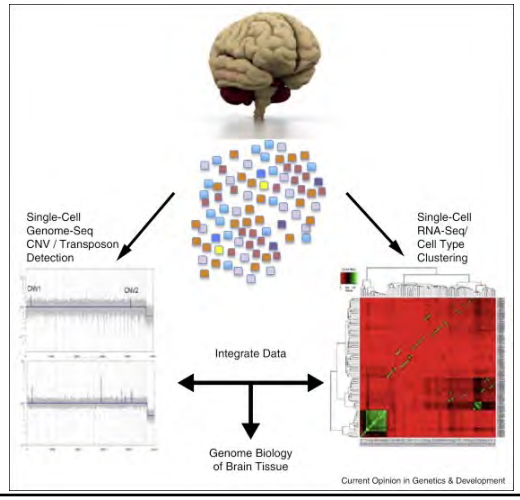
*As omics data sets become increasingly available, the need has grown for software tools to effectively integrate, structure, analyse and visualize omics data. This table compiles several of the useful tools that are currently available to researchers, briefly describes their utility, and includes links to their associated online resources. The Systems Biology Markup Language (SBML) Web page provides links to many other software tools for network visualization and analysis (http://www.sbml.org). COBRA, constraint-based reconstruction and analysis.







Single cell sequencing approaches for complex biological systems.
Baslan T, Hicks J.
Curr Opin Genet Dev. 2014 Jun;26:59-65.

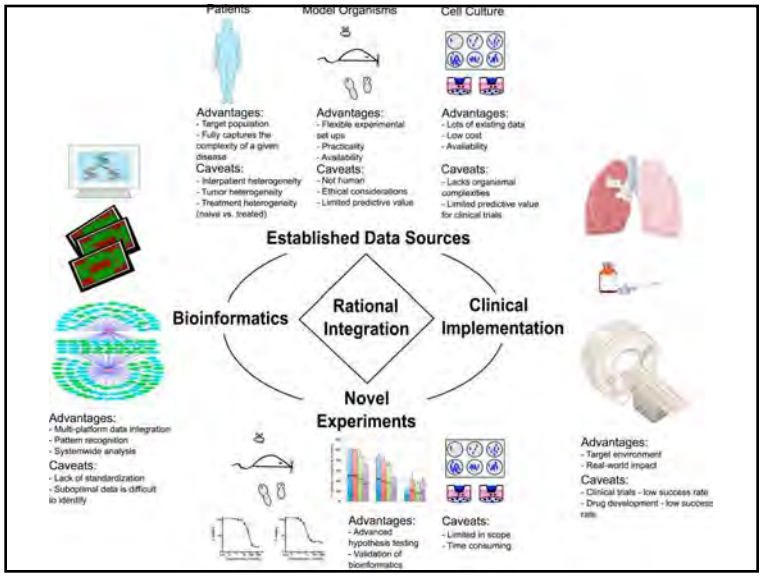


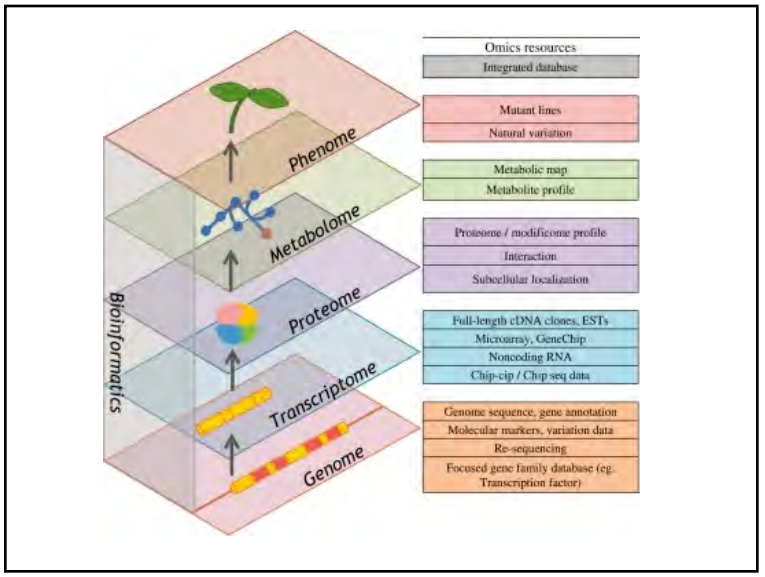
Single-cell RNA-sequencing of retrieved human oocytes and eggs in clinical practice and for human ovarian cell atlasing.
Machlin JH, Shikanov A.
Mol Reprod Dev. 2022 Dec;89(12):597-607.

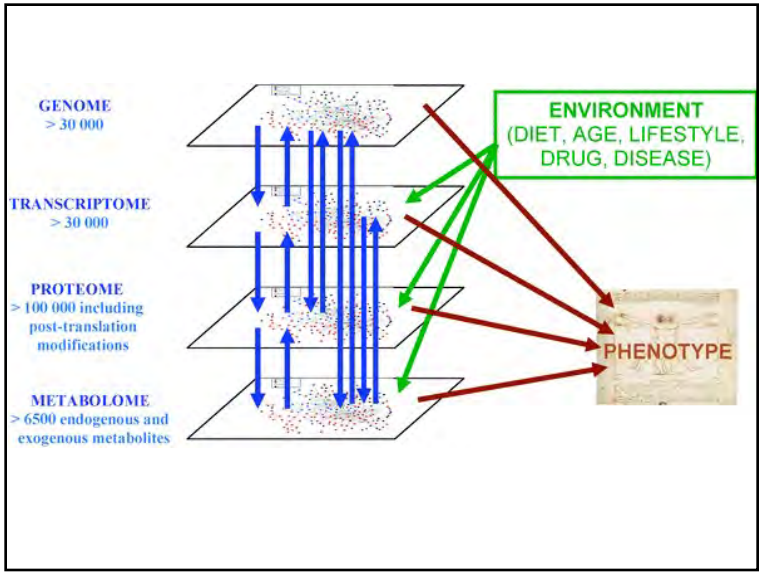
TABLE 1 Single-cell isolation techniques used in the human ovary

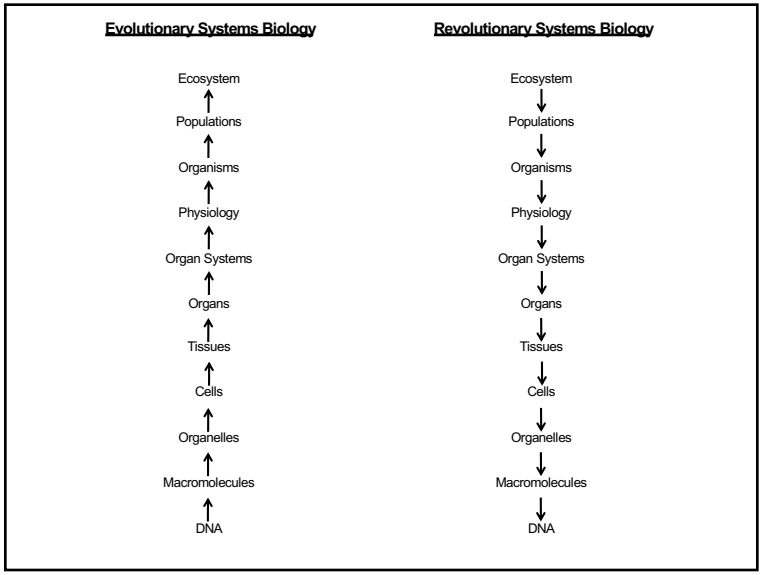
Technique	Description	Infographic	Targeted or untargeted	Fixed or fresh	Limitations	scRNA-seq paper using the technique
Direct cell lysis (DCL)	• Researcher mechanically isolates single cells of interest and places them directly into a lysis or storage buffer.		Untargeted	Fresh	• Technically the most challenging and time-consuming • Not ideal for "whole tissue" atlasing	• Li et al. (2017) • Y. Zhang et al. (2018) • Fan et al. (2019) • Fan et al. (2021) • [a]
Fluorescence-activated cell sorting (FACS)	• Specialized flow cytometry. Sorts heterogeneous cell mixture one cell at a time based on light scattering and fluorescent characteristics of the cell. • Used in conjunction with DCL		Targeted	Fresh	• Requires a cell surface marker for separation so excludes rare cell types • Bulk groups of cells are isolated so an additional method of individual isolation is needed	• Li et al. (2017) • Fan et al. (2019) • Man et al. (2020) • Wagner et al. (2020)
Magnetic-activated cell sorting (MACS)	• Target cells are tagged with magnetic particles bound to antibodies and passed through a magnetic field. Isolates cells into bulk groups. • Using in conjunction with DCL		Targeted	Fresh	• Requires a cell surface marker for separation so excludes rare cell types • Bulk groups of cells are isolated so an additional method of individual isolation is needed	• Li et al. (2017)
Laser-capture microdissection (LCM)	• Tissue is paraffin-embedded and sectioned. Outline of cells of interest is marked and cut using a UV laser. Cells are lifted onto a sterile cap for RNA collection.		Untargeted	Fixed	• Tissue fixation could alter/damage RNA quality • Imprecise and subjective method of cutting out cells of interest	• Ernst et al. (2017)

[a]All improvement of IVF outcomes papers used the DCL method. Infographics created in BioRender.com.


Patients
Model Organisms
Cell Culture
Advantages:
- Target population
- Fully captures the complexity of a given disease
Caveats:
- Interpatient heterogeneity
- Tumor heterogeneity
- Treatment heterogeneity (naive vs. treated)
Advantages:
- Flexible experimental set ups
- Practicality
- Availability
Caveats:
- Not human
- Ethical considerations
- Limited predictive value
Advantages:
- Lots of existing data
- Low cost
- Availability
Caveats:
- Lacks organismal complexities
- Limited predictive value for clinical trials
Established Data Sources
Bioinformatics
Rational Integration
Clinical Implementation
Novel Experiments
Advantages:
- Multi-platform data integration
- Pattern recognition
- Systemwide analysis
Caveats:
- Lack of standardization
- Suboptimal data is difficult to identify
Advantages:
- Advanced hypothesis testing
- Validation of bioinformatics
Caveats:
- Limited in scope
- Time consuming
Advantages:
- Target environment
- Real-world impact
Caveats:
- Clinical trials - low success rate
- Drug development - low success rate


Omics resources
Integrated database
Phenome
Mutant lines
Natural variation
Metabolome
Metabolic map
Metabolite profile
Proteome
Proteome / modificome profile
Interaction
Subcellular localization
Transcriptome
Full-length cDNA clones, ESTs
Microarray, GeneChip
Noncoding RNA
Chip-cip / Chip seq data
Genome
Genome sequence, gene annotation
Molecular markers, variation data
Re-sequencing
Focused gene family database (eg. Transcription factor)
Bioinformatics


GENOME
> 30 000
TRANSCRIPTOME
> 30 000
PROTEOME
> 100 000 including post-translation modifications
METABOLOME
> 6500 endogenous and exogenous metabolites
ENVIRONMENT
(DIET, AGE, LIFESTYLE, DRUG, DISEASE)
PHENOTYPE


Evolutionary Systems Biology
Ecosystem
Populations
Organisms
Physiology
Organ Systems
Organs
Tissues
Cells
Organelles
Macromolecules
DNA
Revolutionary Systems Biology
Ecosystem
Populations
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Organs
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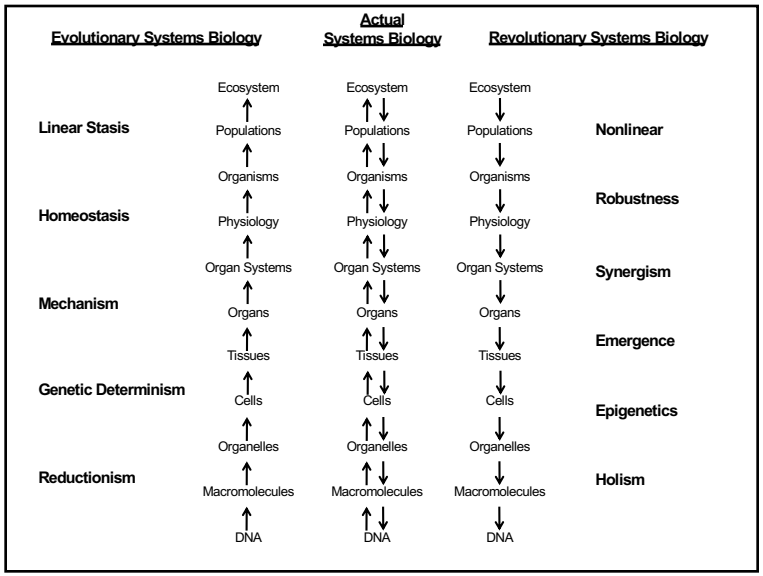

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January	9 & 11	Week 1	Systems Biology Introduction
	16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
	23 & 25	Week 3	Sex Determination Systems
Jan /Feb	30 & 1	Week 4	Male Reproductive Tract Development & Function
February	6 & 8	Week 5	Female Reproductive Tract Development & Function
	13 & 15	Week 6	Gonadal Developmental Systems Biology
	20 & 22	Week 7	Testis Systems Biology
	27 & 29	Week 8	Ovary Systems Biology
March	5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
	11 – 15	**Week 10**	**Spring Break**
	19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
	26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April	2 & 4	Week 13	Reproductive Endocrinology Systems
	9 & 11	Week 14	Fertilization & Implantation Systems
	16 & 18	Week 15	Fetal Development & Birth Systems
	23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May	30 & 2	Week 17	Exam or Grant Review