Spring 2023 – Epigenetics and Systems Biology
Lecture Outline (Epigenetics)
Michael K. Skinner – Biol 476/576
Week 8 & 9 (February 28 & March 7, 2023)

Epigenetics of Cell and Developmental Biology

- Basic Cell and Developmental Biology
- X Chromosome Inactivation
- Imprinted Genes
- Developmental Epigenetics
- Epigenetics and Stem Cells
- Epigenetics and Developmental Systems

Required Reading

Michael K. Skinner (2011) Environmental Epigenetic Transgenerational Inheritance and Somatic Epigenetic Mitotic Stability. Epigenetics 1;6(7):838-42.

Al-Mousawi J, Boskovic A. Transcriptional and epigenetic control of early life cell fate decisions. Curr Opin Oncol. 2022 Mar 1;34(2):148-154.

Books (Reserve in Library)

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Literature

Zhou JJ, Cho KWY. Epigenomic dynamics of early Xenopus Embryos. Dev Growth Differ. 2022 Sep 27.

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Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability

Michael K. Skinner
Center for Reproductive Biology; School of Biological Sciences; Washington State University; Pullman, WA USA

The majority of environmental factors can not modify DNA sequence, but can influence the epigenome. The mitotic stability of the epigenome and ability of environmental epigenetics to influence phenotypic variation and disease, suggests environmental epigenetics will have a critical role in disease etiology and biological areas such as evolutionary biology. The current review presents the molecular basis of how environment can promote stable epigenomes and modified phenotypes, and distinguishes the difference between epigenetic transgenerational inheritance through the germ line versus somatic cell mitotic stability.

Key words: epigenetic, transgenerational, inheritance, mitotic, environmental, toxicants, evolution, disease etiology

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Correspondence to: Michael K. Skinner;
Email: skinner@wsu.edu

Role of Environmental Epigenetics in Development and Biology

A highly differentiated adult cell type or biological phenotype has been generated through a complex cascade of developmental processes. The stem cell populations of the embryo or selected tissues undergo a cascade of genetic steps through cell fate determinations, development of differentiated cell types, organogenesis, specified physiological states and phenotypes. This genetic process includes classic transitions in transcriptional control to lead to a cascade of specific transcriptomes at each stage of development. This programmed developmental process is hardwired and follows classic genetic processing. The genetic control of developmental biology is stable and integrated into the overall physiology and phenotype of the organism. In contrast to the genetic control of cellular activity, the epigenetic cascade of events is responsive to environmental factors and can directly impact the genetic cascade of events. Just as there is a cascade of genetic steps during development, a cascade of epigenetic steps also exists and impacts the transcriptional stages of cellular differentiation and development (**Fig. 1**). Environmental epigenetics provides a direct molecular mechanism for environmental factors or toxicants to influence the genetic cascade of events involved in development, such that the environment can directly impact biology. An interesting element of these integrated molecular events for developmental biology[1] is the fact that critical windows of susceptibility exist[2] where the environmental factors have a more dramatic ability to modify and impact important stages of development (**Fig. 1**). These critical windows generally are very early in development, such as the fetal or early postnatal periods, when the organ systems are rapidly developing and sensitive to subtle shifts in the epigenome.[3] These critical exposure windows allow an environmental factor or toxicant to permanently modify an epigenome that then continues throughout development to impact genetic programming and result in a modified adult epigenome and genome activity (transcriptome). This promotes a susceptibility to develop disease or creates an increased biological variation in phenotype that will facilitate an adaptation event and influence natural selection (**Fig. 1**).

The stages or cascade of steps in both the genetics and epigenetics are highly integrated and influence each other during the developmental process. Therefore, environmental epigenetics and genetics

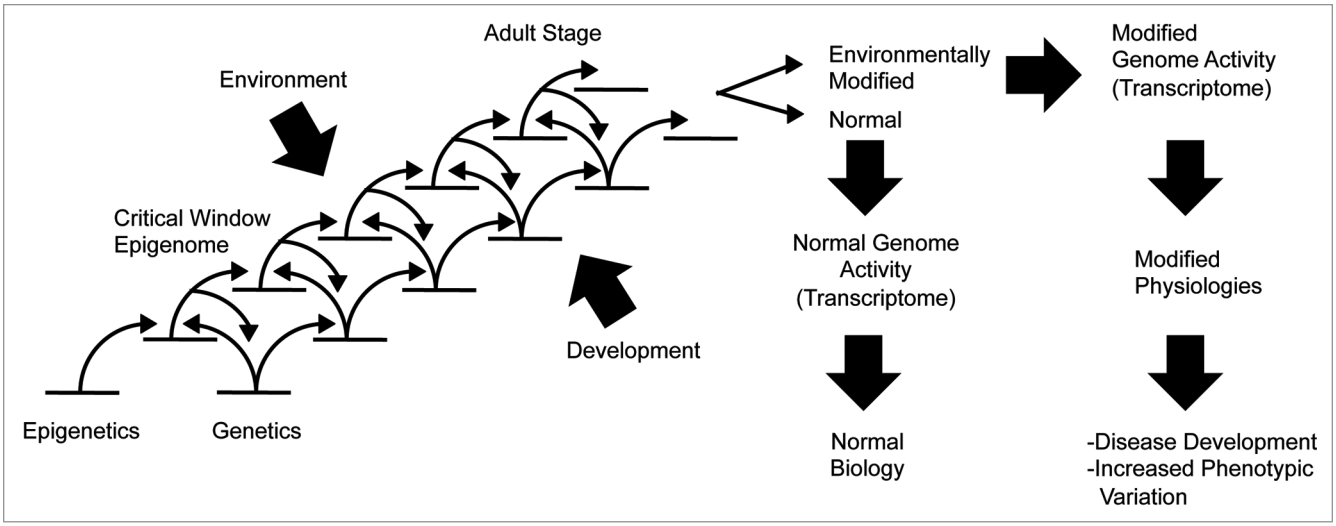

Figure 1. Integration epigenetics and genetics in development.

should not be considered mutually exclusive, but instead highly integrated and dependent on each other. The genome DNA sequence provides the stable nature of an organism that is hardwired and programmed. The epigenome provides a more plastic molecular process[4-6] that is responsive to the environment to impact biology, disease etiology and evolutionary biology. Epigenetics and genetics should be considered cooperative and together provide a more complex and integrated molecular mechanism for the control of development and biology.

Environmental Epigenetic Transgenerational Inheritance

Epigenetic transgenerational inheritance requires germ line transmission of epigenetic information between generations in the absence of direct environmental exposures. During a critical window of germ cell development, embryonic gonadal sex determination in mammals, environmental factors or toxicants have been shown to influence epigenetic programming in the male germ line (sperm), which becomes permanently programmed (imprinted),[7] and then allows the transgenerational transmission of adult onset disease phenotypes.[8,9] The general mechanism for this epigenetic transgenerational inheritance in mammals involves exposure of a gestating female during the period of gonadal sex determination when the primordial germ cell is being reprogrammed at the DNA methylation level.[10] The environmental toxicant alters the DNA methylation to generate new imprinted-like sites that then are transmitted to subsequent generations through the germ line (sperm) (**Fig. 2**). All the somatic cells derived from this germ line will have a baseline shift in their epigenome and, as the cells differentiate, a corresponding shift in genome activity and transcriptomes that in some tissues will promote disease states or phenotypic variation (**Fig. 2**).[11] The transmission of any genetic or epigenetic molecular information between generations requires germ line transmission and permanent alterations in DNA sequence or the epigenome.[11] Due to the reprogramming of the epigenome (DNA methylation) at fertilization,[10,11] the modified epigenetic sites will need to be imprinted-like to escape the demethylation process.[3,8,11,12] The suggestion that an altered epigenome may increase genomic instability and allow genetic mutations to develop in subsequent generations[12] remains a possibility that needs to be investigated further.[7]

A number of environmental factors and toxicants have now been shown to promote epigenetic transgenerational inheritance of disease states or phenotypic variation including the fungicide vinclozolin,[8] plastic compound bisphenol A (BPA),[13] toxicant dioxin,[14] stress responses[15] and nutrition.[16] A critical factor in epigenetic transgenerational inheritance is that the disease states or phenotype be transmitted through the germ line in the absence of direct exposure.[11] If direct exposure of the environmental factor is involved then this would simply be direct exposure toxicology. An example is exposure of a gestating female that has the F0 female, F1 fetus and germ line within the fetus that will generate the F2 generation directly exposed.[17] Therefore, an F3 generation is required to assess a potential transgenerational phenotype from a gestating female exposure.[17] In the event an adult male or female is exposed, the F0 generation adult and the germ line that will generate the F1 generation are directly exposed, such that an F2 generation is required to obtain an epigenetic transgenerational phenotype.[18] Although previous literature has suggested transgenerational phenotypes in F1 or F2 generations, these studies often had direct exposures involved so can not be considered epigenetic transgenerational inheritance phenotypes, but direct exposure toxicology. Epigenetic transgenerational inheritance phenotypes require the lack of direct exposure to be considered transgenerational.

Environmentally induced epigenetic transgenerational inheritance has significant impacts in the areas of disease etiology, inheritance of phenotypic variation and evolutionary biology. This phenomenon provides an alternate to genetic Mendelian inheritance that can provide a molecular mechanism for how the

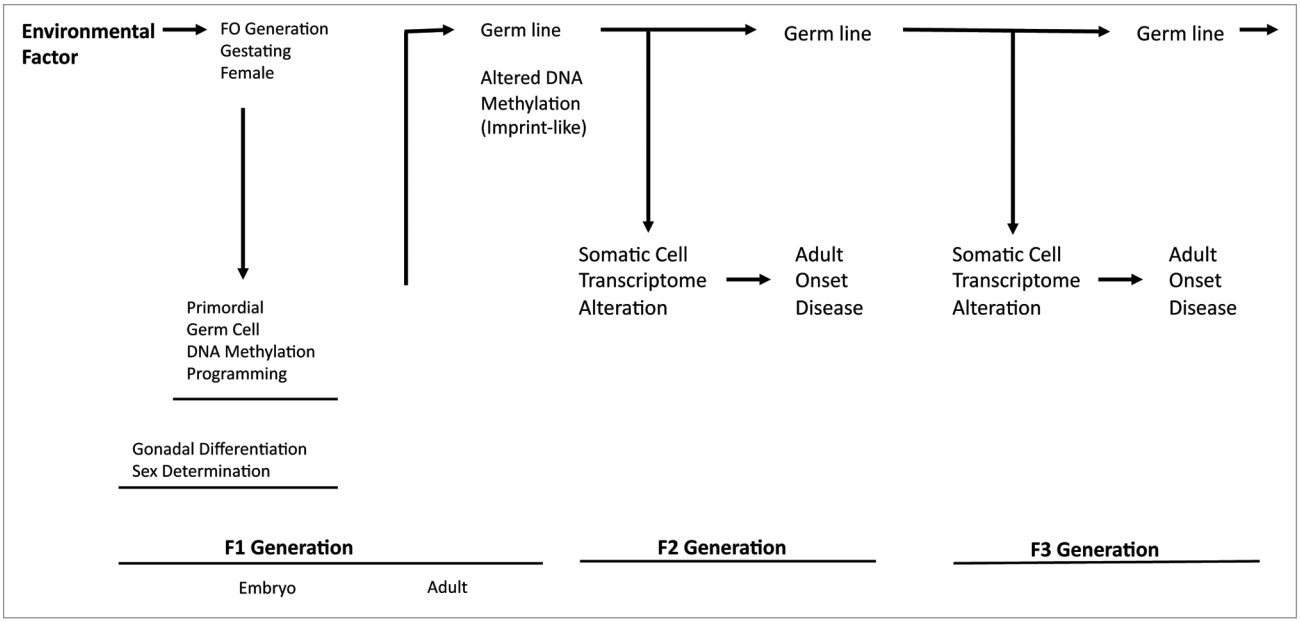

Figure 2. Scheme for epigenetic transgenerational inheritance.

environment can influence disease etiology and general biological phenotypes. In regards to disease etiology, the familial transmission or non-Mendelian characteristics of a variety of disease states can be explained. In regards to evolutionary biology, the ability to acquire an increased biological variation in phenotype following an ancestral environmental exposure will facilitate a potential adaptation event to allow the natural selection process. Environmental epigenetic transgenerational inheritance may provide a molecular process to explain rapid evolutionary events and how environment can influence evolution.

Somatic Epigenetic Mitotic Stability

In the 1940s, when Conrad Waddington described environment-gene interactions as epigenetics, he discussed the stable nature of epigenetics,[4] but had no idea of the molecular aspects of the phenomena. It was not until the 1970's that DNA methylation was described by Robin Holliday[5] and Art Riggs.[6] Riggs discussed the stable nature of the epigenetics as epigenetic inheritance following cell proliferation or mitosis.[19] Unfortunately, this nomenclature of 'inheritance' is not accurate and misleading to suggest generational events. The definition of "inheritance" is transmission of information between generations of an organism, and is accepted by the public and general scientific community as such. The ability of the epigenome to be replicated and transmitted upon cellular proliferation through the mitotic process is distinct and should be considered "mitotic stability" not "inheritance". The use of the term epigenetic inheritance has confused the scientific community and public to consider germline-mediated transgenerational phenomena, rather than simply replication of the epigenome during mitosis. Therefore, the proposal is made to define the replication of the epigenome during mitosis as "Mitotic Stability" and not refer to this as epigenetic inheritance. The definition of epigenetics would be as previously described in reference 11, "molecular factors or processes around DNA that regulate genome activity independent of DNA sequence and that are mitotically stable."

The insight of Art Riggs to suggest the critical need for epigenetic marks to be replicated and stable during mitosis[6,12] was very significant and indeed allows epigenetics to have a profound biological impact. In the event the epigenome was not replicated during mitosis, epigenetics would only impact the immediate cell and not have a long-term impact on the cell population or associated physiology. The ability to maintain a specific epigenome after mitosis is in part how different cell types maintain distinct differentiated states and facilitate a normal developmental process.

The mechanisms involved in the replication of the epigenome during mitosis are understood for DNA methylation and small RNAs, but limited information exists for histone modifications and chromatin structure. The DNA methylation marks are identified on the parental DNA strand during S phase DNA synthesis by DNA methyltransferase (DNMT), which then methylates the newly synthesized strand of DNA to replicate the DNA methylation pattern of the parental cell. Therefore, the DNA methylation marks are replicated during mitosis to maintain the methylome. The non-coding RNAs that act independent of DNA or RNA sequences act as epigenetic components to alter gene expression. The non-coding RNA islands of DNA sequence are replicated through normal DNA synthesis to have mitotic stability of these non-coding RNAs. The histone modifications appear to be replicated following mitosis but the molecular mechanism for replicating the histone code is not known at present.[20] Similarly, replication of the chromatin structure is known to occur, but the

basic replication molecular mechanism remain to be elucidated.[21] Therefore, further research is needed to clarify the basic molecular mechanisms involved in epigenetic mitotic stability.

Although the germ cell is critical for transmission of genetic and epigenetic information between generations, the somatic cells of organism (non-germ cell types) are essential for the basic developmental biology and physiology of an organism. Somatic cells are not capable of transmitting information between generations, but have a critical role in the physiology and disease states of the individual. The reason epigenetic mitotic stability is critical relates to the somatic cell differentiation and function. In the event, as shown in **Figure 1**, an environmental factor modified the epigenome of a somatic cell during a critical window of development, the somatic epigenetic mitotic stability would replicate this epigenome and permanently influence the somatic cell differentiation and function throughout life. Therefore, long after an early life exposure, the modified epigenome will continue to alter gene expression and that cell population. This provides a mechanism for the developmental origins of disease to explain how a transient exposure early in life can promote a susceptibility for disease later in life. The most critical molecular factor involved in this phenomenon is the somatic epigenetic mitotic stability. As previously discussed, the integration of the epigenome to genome activity and the mitotic stability of the epigenome on somatic cells provides a molecular mechanism for environment to influence disease etiology and phenotypic variation associated with evolution.

Summary

Epigenetics provides a molecular mechanism for environmental factors (for example, nutrition) and toxicants to influence biology and disease. The integrated nature of the epigenetics and genetics indicates a highly cooperative interaction to control development and biology (**Fig. 1**). A large number of previous observations have suggested the environment has a major impact on biology, but genetics alone could not explain the phenomena involved. The inclusion of epigenetics in our consideration of basic developmental processes and physiology significantly expands our ability to understand the systems biology of the organism. The ability of the epigenome to be replicated during somatic cell mitosis also can explain how early life exposures can program later life physiology and adult onset disease. This is a new paradigm for disease etiology that needs to be considered. Somatic cell epigenetic mitotic stability provides a somewhat permanent shift in the epigenome following an exposure during a critical window of development, such that later life physiology and disease can be linked (**Fig. 1**).

These somatic cell effects are likely more common and critical for the individual exposed than epigenetic transgenerational inheritance of exposure phenotypes. However, the germ line transmission of a permanent shift in the epigenome will potentially impact all subsequent generations to promote a phenotypic variation and/or disease state (**Fig. 2**). Since all the somatic cells generated from the germ line involved will have a shift in their epigenomes and genome activity, the environmental epigenetic transgenerational inheritance has a profound effect on biology and disease. In the case of disease etiology this can explain non-Mendelian inheritance of disease, environmentally induced increases in disease frequency and regional differences in disease frequencies. Clearly epigenetics will have a critical role in disease etiology and the amount of adult onset disease associated with epigenetic transgenerational inheritance will need to be established.

In regards to environmentally induced epigenetic transgenerational inheritance of biological or phenotypic variation, a significant impact on evolutionary biology needs to be considered.[11] An environmental factor such as nutrition promoting a modification of germ line epigenetic programming that becomes permanently programmed (**Fig. 2**) will have a role in the epigenetic transgenerational inheritance of phenotypic variation. This variation may subsequently impact an adaptation process to facilitate natural selection. An increase in phenotypic variation induced by environmental epigenetics that is heritable will be a molecular mechanism to consider in evolutionary biology. Previously, we have demonstrated an environmental toxicant exposure during fetal gonadal sex determination can promote epigenetic transgenerational inheritance of altered sexual selection phenotypes.[22] Since sexual selection is a major determinant for natural selection, this experiment provides direct evidence that environmental epigenetic transgenerational inheritance may have a role in evolution. This does provide a "neo-Lamarckian influence to facilitate Darwinian evolution" concept for evolutionary biology.

The reviewed environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability will both have significant roles in development, physiology, disease and evolution. These molecular mechanisms and an integration with classic genetics are now required to more fully understand the systems biology of development, physiology and disease, as well as areas of biology such as evolution.

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REVIEW

OPEN

Transcriptional and epigenetic control of early life cell fate decisions

Jasmina Al-Mousawi and Ana Boskovic

Purpose of review
Global epigenetic reprogramming of the parental genomes after fertilization ensures the establishment of genome organization permissive for cell specialization and differentiation during development. In this review, we highlight selected, well-characterized relationships between epigenetic factors and transcriptional cell fate regulators during the initial stages of mouse development.

Recent findings
Blastomeres of the mouse embryo are characterized by atypical and dynamic histone modification arrangements, noncoding RNAs and DNA methylation profiles. Moreover, asymmetries in epigenomic patterning between embryonic cells arise as early as the first cleavage, with potentially instructive roles during the first lineage allocations in the mouse embryo. Although it is widely appreciated that transcription factors and developmental signaling pathways play a crucial role in cell fate specification at the onset of development, it is increasingly clear that their function is tightly connected to the underlying epigenetic status of the embryonic cells in which they act.

Summary
Findings on the interplay between genetic, epigenetic and environmental factors during reprogramming and differentiation in the embryo are crucial for understanding the molecular underpinnings of disease processes, particularly tumorigenesis, which is characterized by global epigenetic rewiring and progressive loss of cellular identity.

Keywords
cell fate, development, embryo, epigenetics, reprogramming, transcription

INTRODUCTION

Development starts at fertilization, when the sperm and egg fuse to create the zygote, which will, through subsequent cleavages and differentiation, give rise to all cells in the new organism. Following fertilization, the specialized and asymmetric epigenomic patterns of the maternal and paternal genomes are largely reset to provide a clean slate supporting the development of the new animal. Embryo-specific organization of the genome is then established with patterning gradually becoming more restricted and specialized, supporting lineage specification during embryogenesis. The first cell differentiation event during mouse development is the distinction of extraembryonic trophectoderm from the pluripotent inner cell mass (ICM) during the morula/blastocyst stage, an event primarily driven by developmental signaling pathways and transcriptional master regulators of the two cell fates.

Generally considered as equipotent, the cells of the early mouse embryo preceding lineage allocation nevertheless harbor some functional differences. In certain cases, these arise as early as two-cell stage of development, when blastomeres are considered totipotent (meaning that they can contribute to both embryonic and extraembryonic tissues). For instance, only a subset of mouse embryos contain two totipotent cells at the two-cell stage, while the majority constitute blastomere pairs in which only one of the blastomeres has the ability to singularly maintain development of a healthy blastocyst [1].

Epigenetics and Neurobiology Unit, European Molecular Biology Laboratory (EMBL), Monterotondo, Italy

Correspondence to Ana Boskovic, Epigenetics and Neurobiology Unit, European Molecular Biology Laboratory (EMBL), Via E. Ramarini 32, Monterotondo 00015 Rome, Italy. Tel: +39 690091218; e-mail: ana.boskovic@embl.it

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KEY POINTS

- In the early mammalian embryo, global epigenetic reprogramming followed by establishment of epigenetic patterns influences the emergence of distinct cell lineages from undifferentiated blastomeres.
- Specification of cell identity during early development is guided by the interaction of transcriptional master regulators with epigenetic factors and chromatin organization.
- Noncanonical distribution of histone and DNA modifications, and asymmetries in epigenetic factor localization are a hallmark of mouse preimplantation blastomeres, with potential to instruct lineage allocation.
- The extent to which parentally inherited epigenomic differences contribute to early embryonic prepatterning and blastomere plasticity, and influence downstream development and differentiation remains to be elucidated.

In this review, we focus on the different gene-regulatory mechanisms influencing chromatin and genome function preceding the first cell differentiation events and discuss how their dynamics and asymmetries influence lineage decisions in the mouse embryo (Fig. 1).

TRANSCRIPTION FACTORS

Transcription factors (TFs) can bind DNA cis-regulatory elements in a sequence-specific manner and modulate transcriptional output of their target genes (reviewed in [2,3]). Recruitment and binding of transcription factors to their cognate sites can be facilitated by cooperative interactions among different transcription factors and by interactions with chromatin components (reviewed in [4]).

The first lineage segregation between the pluripotent ICM and the trophectoderm, which will give rise to the placenta, is guided by lineage-specific transcription factors, resulting from a polarization of the outer cells of the morula and a subsequent activation of the Hippo signaling pathway (reviewed in [5]). Mechanistically, this pathway results in the dephosphorylation of YAP1, allowing for its nuclear translocation where it acts as a co-activator for TEAD4, forming a complex that induces expression of Cdx2 and Gata3, transcriptional master regulators of the trophectoderm lineage [6,7]. The activation of the Hippo pathway leads to downregulation of the pluripotency factor SOX2 in trophectoderm precursors, a mechanism dependent on TEAD4 but not CDX2 [8]. CDX2 itself is dispensable for establishment of the trophectoderm but necessary for the maintenance of its function [9]. CDX2 can be co-expressed with OCT4, a core pluripotency transcription factor, in a cross-antagonistic manner with the transcription factors inhibiting each other's activity [10,11]. Despite Cdx2 expression, morula blastomeres retain a high level of plasticity until the 32-cell stage during which they can interconvert lineages [12]. However, shortly thereafter, cells expressing high CDX2 levels lose their ability to convert to the ICM [13].

After blastocyst formation, the ICM further segregates into the epiblast (Epi), which will give rise to the embryo proper and differentiate into the three germ layers, and the extraembryonic primitive endoderm (PrE), which will contribute to the yolk sac (reviewed in [5]). Initially co-expressed in the early ICM [14,15], the classic Epi specifier NANOG, and PrE-specific transcription factor GATA6 adopt a mutually exclusive 'salt-and-pepper' expression pattern around embryonic day (E) 3.5 [16]. Lack of either factor results in the loss of the cell lineage it specifies [17–20]. During the resolution of the ICM, there is an antagonistic relationship between NANOG and GATA6 [21,23]. Nevertheless, ICM plasticity is retained beyond the bifurcation of NANOG and GATA6 expression patterns, and cells can interconvert between Epi and PrE fates until E4.5 [22,23]. The PrE/Epi divergence is guided by differential Fibroblast Growth Factor (FGF) signaling and activation of the mitogen-activated protein kinase (MAPK) pathway, the action of which causes specification towards PrE [16,24,25]. Phosphorylation of MAPK-effector ERK triggers an initially reversible priming towards PrE through a redistribution of cofactors of the transcriptional machinery, leading to the suppression of pluripotency genes and allowing for the activation of PrE genes [26,27▪]. Expression patterns of MAPK signaling components in the early ICM are heterogeneous with Epi-precursors expressing FGF4 ligand and PrE-precursors expressing FGFR2 receptor [19,28,29]. Modulating the MAPK pathway in embryos shifts the PrE-to-Epi ratio, with Fgf4-null embryos unable to maintain Gata6 expression [25,30,31]. In addition to FGFR2, FGFR1 is expressed throughout the ICM, and its activity is involved in PrE specification, as well as allowing Epi cells to exit the earlier, naive pluripotent state and progress towards a later, primed state [32,33].

CHROMATIN MOBILITY AND LONG NON-CODING RNAS PRECEDING LINEAGE ALLOCATION

As transcription factors function within the chromatin context, it is logical to hypothesize that the

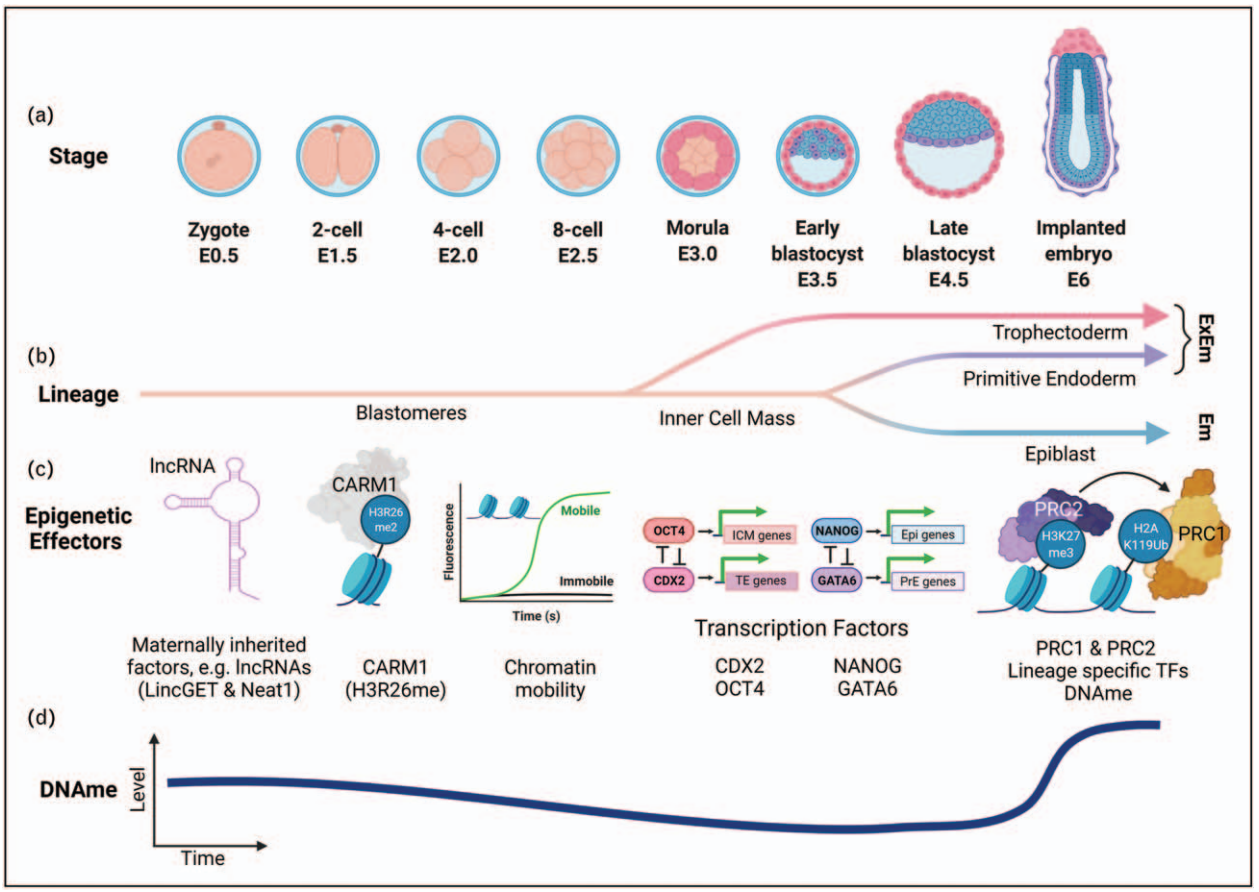

FIGURE 1. Epigenetic and transcription factors regulating cell fate allocation during mouse preimplantation development. (a) Stages of embryonic development from fertilization until implantation and (b) their respective lineage trajectories arising during early differentiation. At the morula stage, the blastomeres adopt either trophectoderm or ICM fate. The ICM subsequently differentiates into the PrE and Epi. ExEm stands for extraembryonic, Em stands for embryonic. (c) Effectors with ascribed instructive roles in the first cell fate decisions depicted below the corresponding developmental stage where they act. Initial heterogeneities are dependent on the distribution of maternally inherited factors, such as lncRNAs (zygote stage), which can impact the tethering of chromatin regulator CARM1 (two-cell stage). CARM1 is in turn associated with an increased level of pluripotency factor expression and chromatin mobility, and higher contribution of cells to the ICM (morula stage). Later, transcription factors ensure proper lineage segregation during the first (trophectoderm/ICM) and second (Epi/PrE) cell fate decisions. Around the time of implantation, DNA methylation (DNAme) and Polycomb Repressive Complexes (PRC) help guide lineage restriction. (d) Loss of DNA methylation levels during reprogramming occurs between the zygote and blastocyst stages, after which the DNA methylation levels are rapidly increased. Figure was made using Biorender.com.

interplay between genome organization and transcription factor action cumulatively contribute to cell plasticity and lineage allocation. In 2011, it was shown that the kinetics of OCT4 on chromatin in four-cell and eight-cell stage embryos differ between individual blastomeres and that differential OCT4 dynamics are predictive of lineage patterning and cell position within the embryo: cells displaying slower OCT4 kinetics are more likely to contribute to inner cells of the morula at compaction [34]. A follow-up study using photo-activatable fluorescence correlation spectroscopy in four-cell embryos found similar results for SOX2: blastomeres with long-lived SOX2 chromatin association contribute more readily towards the pluripotent lineage, in a manner regulated by H3R26 dimethylation [35]. This histone modification, deposited by arginine methyltransferase CARM1, is found to be naturally asymmetrically distributed between cells already at the four-cell stage, depending on the cleavage plane of the two-cell stage blastomeres. Lower levels of H3R26me2 in four-cell stage blastomeres are associated with a subsequent higher propensity of these cells to contribute to trophectoderm compared with ICM [36]. Conversely, increasing H3R26me2 levels through the overexpression of CARM1 in one of the

two-cell stage blastomeres leads to an upregulation of NANOG and SOX2 expression, as well as an increase in histone H3.1 mobility in its progeny [37], and results in higher contribution of these cells to the pluripotent ICM [36]. Presumably, higher accessibility of underlying DNA in ICM-destined cells, caused at least partly by faster histone exchange, facilitates longer and/or more stable association of pluripotency factors with embryonic chromatin.

Additionally, CARM1 has been reported to physically interact with PRDM14 and long non-coding (lnc) RNAs LincGET and Neat1, all of which have been proposed to anchor CARM1 to its cognate sites on chromatin [38,39▪,40▪]. LincGET itself is differentially expressed between the sister blastomeres already at the two-cell stage but only through interaction with CARM1 is it able to induce SOX2 and NANOG expression [40▪]. Similarly, it was found that depletion of Neat1 causes developmental arrest at the morula/early blastocyst stage, possibly due to increased expression of CDX2 [39▪]. Cumulatively, these data point to a dynamic interplay between different epigenetic players, transcription factor levels and underlying genomic context in guiding cell fate allocation during development.

CHROMATIN MODIFICATIONS IN THE EARLY EMBRYO

The first of two genome-wide waves of epigenetic reprogramming in the animal's life cycle takes place immediately after fertilization, with the presumptive aim of 'resetting' the chromatin landscape inherited from the highly specialized gametes. This establishes a clean slate of the embryonic epigenome preceding (and allowing for) cell differentiation. Below, we outline the best characterized chromatin modifications associated with regulation of embryogenesis and differentiation.

DNA methylation

DNA methylation occurs directly on the DNA molecule in a CpG dinucleotide context and is traditionally associated with transcriptional silencing (reviewed in [41]). Although overall stable in somatic tissues, DNA methylation patterns are globally reprogrammed following fertilization and during the specification of the germline.

In the early embryo, progressive loss of DNA methylation takes place, ultimately resulting in a hypomethylated genome at the blastocyst stage (Figure 1d) [42,43]. This occurs as a consequence of the absence of DNA methylation maintenance normally carried out by DNMT1 [42,43], as well as active removal through the action of Ten-eleven Translocation (TET) enzymes. In the zygote, the paternal genome is demethylated more rapidly than the maternal one, through the action of TET3 [44–48]. Maternal chromosomes are protected from this mechanism by STELLA/Dppa3, which recognizes H3K9me2, deposited during oogenesis [49]. This distinction is not clear-cut: TET3 has been reported to demethylate parts of the maternal genome, blurring the segregation of demethylation mechanisms between the parental genomes [50–52]. Although pervasive, it is important to note that DNA demethylation in preimplantation embryos is not absolute, with imprinting control regions and some transposable elements (in particular IAPs) escaping the reprogramming process [53]. From the blastocyst stage, DNA methylation levels increase through the action of de novo DNA methyltransferases DNMT3A and DNMT3B [54]. DNA methylation is dispensable for the formation of extra-embryonic lineages [55], consistent with the reported hypomethylated states in extraembryonic tissues and the higher expression levels of DNMT3A/B in the post-implantation epiblast [56]. Despite the differential requirements and levels of DNA methylation between cell types of the blastocyst, DNA methylation asymmetries in cleavage stage blastomeres have thus far not been implicated as early regulators of the first lineage decision event as they chiefly arise following cell fate allocation.

H3K27me3 and H2AK119Ub1

Polycomb repressive complexes 1 and 2 (PRC1&2) deposit histone modifications H2A monoubiquitylation (H2AUb1) and H3K27 trimethylation (H3K27me3), respectively, which correlate with repression of gene activity and the restriction of cell fate during development in various animal model organisms [57–61]. PRC1 can be recruited to chromatin by its interaction with H3K27me3, suggesting a temporal order of PRC function on chromatin (PRC2 precedes PRC1) [62,63]. However, during preimplantation development, an asymmetric distribution exists between H3K27me3 and H2AK119Ub1 across the genome [64▪▪,65▪▪,66]. After fertilization, global erasure of H3K27me3 and targeted depletion at promoter regions occur at the paternal and maternal genomes, respectively [66–68]. A gradual gain of H3K27me3 follows between the two-cell and morula-to-blastocyst transition and in the postimplantation epiblast [71], concomitantly with the initial cell fate specifications in the embryo. Genetic studies have revealed PRC2 to be dispensable during preimplantation development but essential at the onset of

gastrulation, when cells set a course towards distinct developmental trajectories [69,70]. Interestingly, PRC2 KO has almost no effect on H2AK119Ub1 distribution in the embryo, which is expected in a somatic context [64▪▪,65▪▪,71] after a near-complete loss of H3K27me3. Conversely, in embryos, PRC1 loss-of-function phenotypes are embryonic lethal, causing developmental arrest at the two-cell stage [75]. Recently, variants of PRC1 have been implicated in mediating the noncanonical pattern of H3K27me3. PRC1 variants can mediate the recruitment of PRC2 independently of preexisting H3K27me3. PRC2 can bind H2AK119Ub1, which in turn stimulates its catalytic activity and deposition of H3K27me3 (PRC1 precedes PRC2) [64▪▪,65▪▪,72]. Thus, contrary to the dogma, preimplantation embryos are characterized by a PRC1-mediated regulation of PRC2.

H3K4me3

H3K4me3 is deposited by MLL1 and MLL2 methyltransferases (reviewed in [57]), and generally associated with promoters of actively transcribed genes. In oocytes, H3K4me3 exhibits a noncanonical pattern, which is established gradually during oogenesis through the action of MLL2 [73–75]. These noncanonical domains are broad and abundant (covering promoters, intergenic regions, distal regions and transposable elements), and found on a subset of CpG islands, regardless of their transcriptional status [73,74,76▪]. After fertilization, the pattern of H3K4me3 inherited from the oocyte is reprogrammed through the action of histone demethylases KDM5A and KDM5B [73]. Disruptions of KDM5A/B cause defects in preimplantation development and aberrant resolution of noncanonical H3K4me3 patterning in a transcription-dependent manner [74]. The paternal genome acquires broad, weak regions of H3K4me3, which are replaced by a canonical H3K4me3 pattern at the two-cell stage [74]. Interestingly, H3K4me3 is found over transposable elements at the two-cell stage, which in turn correlates with their transient developmental expression [77]. Both H3K27me3 and H3K4me3 display noncanonical patterning in the oocyte, which is rapidly erased after fertilization. What role could these unique chromatin markings play during oogenesis and are they necessary for proper progression through the earliest developmental stages? The broad distribution of these histone post-translational modifications over large genomic regions argues against their role in fine-tuned regulation of specific genes they decorate and rather points to a more general function prior to transcriptional activation of the genome.

Interestingly, a subset of developmental promoters in the embryonic epiblast harbor both H3K4me3 and the seemingly antagonistic H3K27me3 histone mark. These genomic regions are termed bivalent. Bivalency has been proposed to function as a 'poising' mechanism, pausing genes in an inactive or lowly expressed state, while maintaining the potential for rapid activation upon developmental cues [78–80]. The embryo contains low levels of bivalent chromatin around implantation, which increases in the Epi at peri-implantation. Whether the acquisition and/or resolution of dually marked chromatin domains can play an instructive role in the first cell fate decisions or reflects the transcriptional status of different cell types in the blastocyst remains to be elucidated.

CONCLUSION AND OUTSTANDING QUESTIONS

Despite rapid and pervasive changes in genome organization and function, cell morphology and signaling pathways, early embryogenesis is an incredibly robust and concerted process resulting in the emergence of specialized cell lineages from the same DNA content. Following the principles of regulative development [81], the fate of the cleavage-stage mouse blastomeres is not predetermined by a gradient of maternally provided factors. Nevertheless, differences in chromatin markings, transcription factor dynamics and noncoding RNA species can be detected between cells as early as the two-cell stage. Here, we discussed some of the most-understood gene-regulatory factors influencing early cell fate decisions, and while many more are being continuously uncovered and characterized (such as RNA-binding proteins and metabolites), open questions remain. How are functional asymmetries established and propagated in the near-identical cells of early embryos, and do they play a role in lineage allocation? Are distinct epigenomic patterns between blastomeres a result of differences in local concentrations of epigenetic factors found already in the zygote? How prominent is the role of stochasticity and transcriptional noise in the eventual establishment of regulatory feedback loops and downstream signal amplification? When and how do heterogeneities at the transcription factor level become sufficiently stable to induce lineage allocation, and is chromatin organization instructive during this process? Does the simultaneous expression of different lineage-specifying transcription factors prolong the developmental time window before final lineage commitment? Finally, the extent to which internal and external signals (such as environmental stress or nutrient composition) have the ability to influence the embryonic epigenome and 'nudge' lineage allocation at the onset

of development remains poorly understood. With our increasing ability to molecularly probe early developmental events at unprecedented spatial and temporal resolution, these exciting biological questions will undoubtedly keep developmental biology aficionados busy in the coming years.

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Conflicts of interest

There are no conflicts of interest.

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This article together with reference [65■■] dissects the temporal dynamics and between PRC1 and PRC2 during oocyte growth and after fertilization. Although in oocytes, depletion of PRC1 subunits causes loss of H2AK119Ub1 and leaves H3K27me3 largely unaffected, in the preimplantation embryo there is a dependence of H3K27me3 on the preceding H2AK119Ub1. These results show that PRC1 functions in regulating PRC2 activity after fertilization.

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The authors deplete the catalytic subunit of PRC2, and show that consequent loss of H3K27me3 in the early embryo leaves H2AK119Ub1 mostly unaffected. Additionally, the acute loss of H2AK119Ub1 in the zygote leaves H3K27me3 unaffected until the four-cell stage where the embryos arrest, suggesting together with reference [64■■] that the PRC1-mediated patterning occurs in the oocyte before fertilization.

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The authors profile the establishment of the noncanonical pattern of H3K4me3 in oocytes and find that the patterning occurs independent of transcriptional status. The noncanonical deposition of H3K4me3 is deposited by MLL2, and this chromatin mark can spread to regions marked by DNA methylation in the absence of DNA methyltransferases.

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"Epigenetics and Systems Biology"

Spring 2023 (Odd Years) – Course Syllabus
Biol 476/576 Undergraduate/Graduate Course (3 Credit)
SLN: (476) – 09358, (576) – 09359
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and on Canvas/Panopto and Discussion Sessions 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 - The objective of the course is to learn the concept and critical role of systems to understand molecular, cell, development, physiology and evolutionary aspects of biology with a focus on the role of epigenetics in systems biology.
Schedule/Lecture Outline –

Week 1	January 10 & 12	Systems Biology (History/ Definitions/ Theory)
Week 2	January 17 & 19	Systems Biology (Networks & Emergence)
Week 3	January 24 & 26	Systems Biology (Components: DNA to Phenotype)
Week 4	Jan 31 & Feb 2	Systems Biology (Genomics / Technology)
Week 5	February 7 & 9	Epigenetics (History / Molecular Processes)
Week 6	February 14 & 16	Epigenetics (Molecular Processes & Integration)
Week 7	February 21 & 23	Epigenetics (Genomics and Technology)
Week 8	Feb 28 & March 2	Cell & Developmental Biology
Week 9	March 7 & 9	Epigenetics of Cell & Developmental Biology (& Midterm Exam)
Week 10	**March 13 – 17**	**Spring Break**
Week 11	March 21 & 23	Environmental Impact on Biology
Week 12	March 28 & 30	Environmental Epigenetics
Week 13	April 4 & 6	Disease Etiology
Week 14	April 11 & 13	Epigenetics & Disease Etiology
Week 15	April 18 & 20	Evolutionary Biology & Genetics
Week 16	April 25 & 27	Epigenetics & Evolutionary Biology
Week 17	May 2 & 4	Grant Review/ Study Section Meeting (& Final Exam)

Spring 2023 – Epigenetics and Systems Biology
Lecture Outline (Epigenetics)
Michael K. Skinner – Biol 476/576
Week 8 & 9 (February 28 & March 7, 2023)

Epigenetics of Cell and Developmental Biology

- Basic Cell and Developmental Biology
- X Chromosome Inactivation
- Imprinted Genes
- Developmental Epigenetics
- Epigenetics and Stem Cells
- Epigenetics and Developmental Systems

Required Reading

Michael K. Skinner (2011) Environmental Epigenetic Transgenerational Inheritance and Somatic Epigenetic Mitotic Stability. Epigenetics 1;6(7):838-42.

Al-Mousawi J, Boskovic A. Transcriptional and epigenetic control of early life cell fate decisions. Curr Opin Oncol. 2022 Mar 1;34(2):148-154.

Spring 2023 – Epigenetics and Systems Biology
Discussion Session (Epigenetics and Development)
Michael K. Skinner – Biol 476/576
Week 8 (March 2)

Epigenetics of Cell and Developmental Biology

Primary Papers
1. Hackett, et al., (2013) Science. 339:448. (PMID: 23223451)
2. Bianconi V, Mozzetta C. (2022) Trends Genet. 38(5):501-513. (PMID: 35078651)
3. Wu, et al., (2020) Cell Reports. 33(7):108395. (PMID: 33207205)

Discussion

Student 19 – Ref #1 above
- What stages of development and cells have reduced DNA methylation?
- What technology was used?
- What role does 5hmC have in the process?

Student 20 – Ref #2 above
- What new insights into stem cell development were observed?
- What epigenetic mechanisms are involved?
- What role does chromatin structure have in the process?

Student 21 – Ref #3 above
- What histone modifications and chromatin remodeling proteins involved?
- What is Myeloid and Erythroid progenitors?
- What is the epigenetic observation on the regulation of lineage development?

Spring 2023 – Epigenetics and Systems Biology
Discussion Session (Epigenetics and Development)
Michael K. Skinner – Biol 476/576
Week 9 (March 9)

Epigenetics of Cell and Developmental Biology

Primary Papers
1. Schworer, et al., (2016) Nature 540:428. (PMID: 27919074)
2. Argelaguet, et al. (2019) Nature 576(7787):487-491. (PMID: 31827285)
3. Lyko F, et al., (2010) PLoS Biol. 2;8(11):e1000506. (PMID: 21072239)

Discussion

Student 22 – Ref #1 above
- What is the epigenetic aging effect observed?
- What stem cell effect was observed?
- How do epigenetics and genetics cooperate in this process?

Student 23 – Ref #2 above
- What was the experimental design to investigate gastrulation?
- What technology was used to examine epigenetics?
- What observations regarding gastrulation DNA methylation and transcriptome were made?

Student 24 – Ref #3 above
- What are the cast systems in the bee?
- How does epigenetics influence the development of the bee?
- What is the environmental factor that alters the epigenetic programming?

From Epigenesis to Epigenetics

The Case of C. H. Waddington

epigenesis as "the formation of an organic germ as a new product" with *the theory of epigenesis* defined as "the theory that the germ is brought into existence (by successive accretions), and not merely developed, in the process of reproduction."

"The fact that the word 'epigenetics' is reminiscent of 'epigenesis' is to my mind one of the points in its favour. . . . We all realize that, by the time development begins, the zygote contains certain 'preformed' characters, but that these must interact with one another, in processes of 'epigenesis', before the adult condition is attained. The study of the 'preformed' characters nowadays belongs to the discipline known as 'genetics'; the name 'epigenetics' is suggested as the study of those processes which constitute the epigenesis which is also involved in development" (see also Waddington 1939 [pp. 154–155]).

DEVELOPMENTAL EPIGENETICS: A HOLISTIC ONTOGENY

Seeing Development Epigenetically:
Genotype + Epigenotype = Phenotype

Genetic Developmental Biology Paradigm

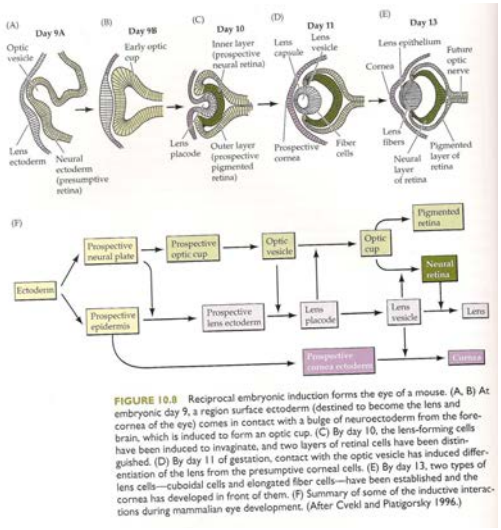


FIGURE 10.8 Reciprocal embryonic induction forms the eye of a mouse. (A, B) At embryonic day 9, a region surface ectoderm (destined to become the lens and cornea of the eye) comes in contact with a bulge of neuroectoderm from the forebrain, which is induced to form an optic cup. (C) By day 10, the lens-forming cells have been induced to invaginate, and two layers of retinal cells have been distinguished. (D) By day 11 of gestation, contact with the optic vesicle has induced differentiation of the lens from the presumptive corneal cells. (E) By day 13, two types of lens cells—cuboidal cells and elongated fiber cells—have been established and the cornea has developed in front of them. (F) Summary of some of the inductive interactions during mammalian eye development. (After Cvekl and Piatigorsky 1996.)

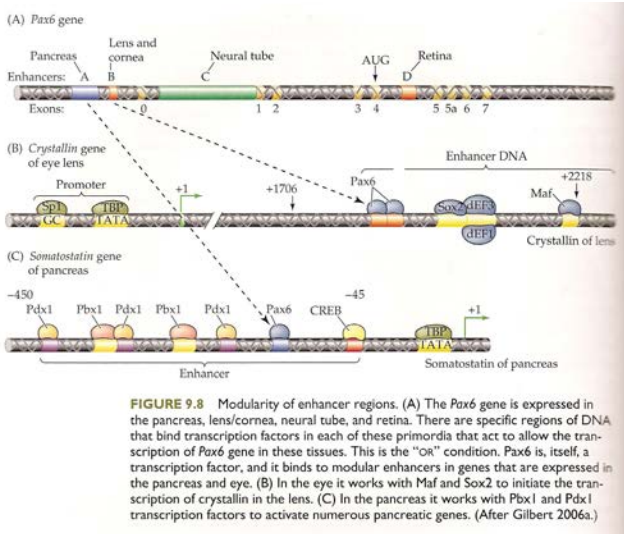
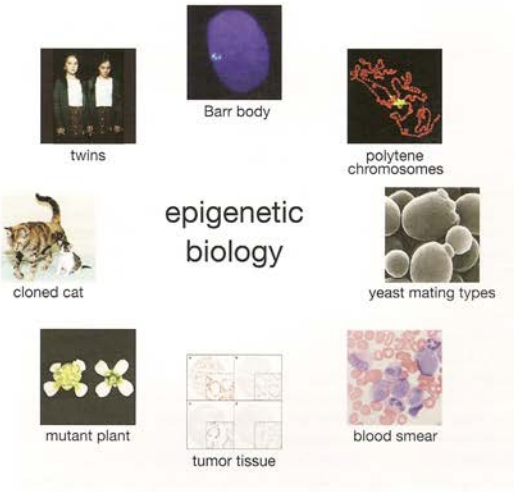
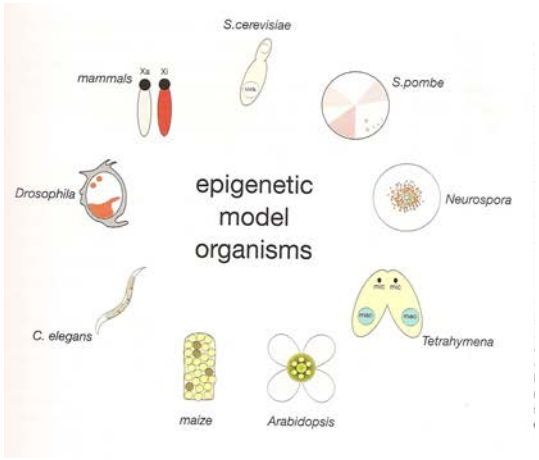
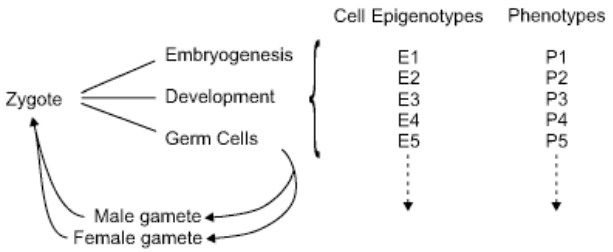


FIGURE 9.8 Modularity of enhancer regions. (A) The *Pax6* gene is expressed in the pancreas, lens/cornea, neural tube, and retina. There are specific regions of DNA that bind transcription factors in each of these primordia that act to allow the transcription of *Pax6* gene in these tissues. This is the "OR" condition. Pax6 is, itself, a transcription factor, and it binds to modular enhancers in genes that are expressed in the pancreas and eye. (B) In the eye it works with Maf and Sox2 to initiate the transcription of crystallin in the lens. (C) In the pancreas it works with Pbx1 and Pdx1 transcription factors to activate numerous pancreatic genes. (After Gilbert 2006a.)

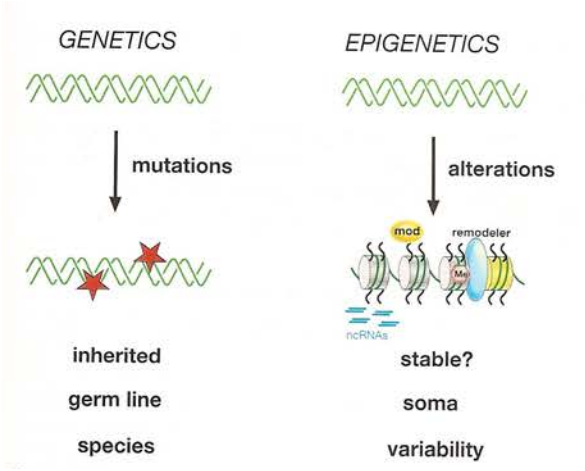
Epigenetics
Developmental Biology Paradigm

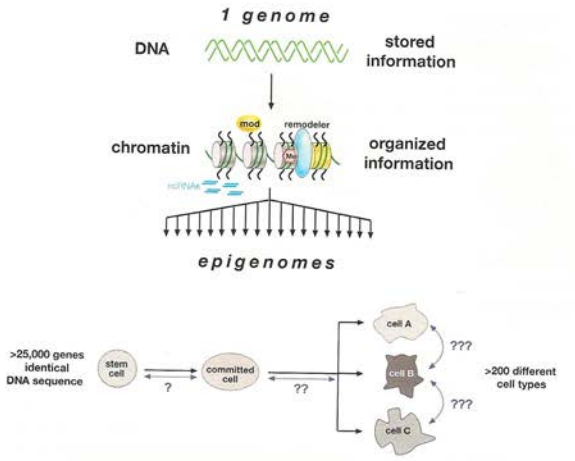






The fertilized egg or zygote divides to form the embryo, which develops to the adult. Within the adult are many cells with different phenotypes, and these phenotypes are determined by the corresponding epigenotypes. The epigenotype determines the spectrum of gene activities in each type of cell. Within the adult are germ line cells that five rise through gametogenesis and meiosis to the sperm and eggs that produce the next generation.





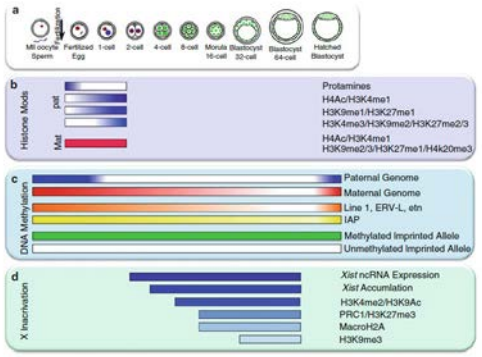
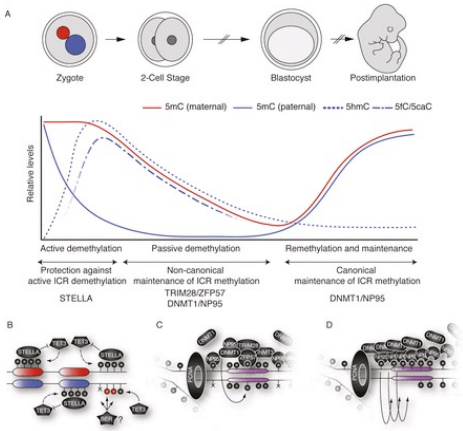
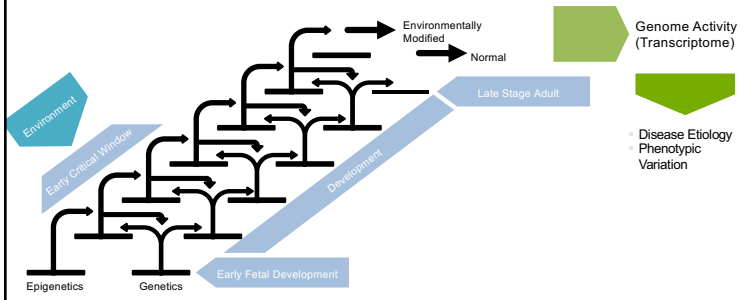


Fig. 1. (a) Epigenetic reprogramming during mammalian preimplantation development. Following fertilization, the maternal and paternal genomes must be epigenetically reprogrammed from a gametic to an embryonic state. Reprogramming is initiated in pronuclear stages and continues throughout preimplantation development. Maternal gametic genome (*red*), paternal gametic genome (*blue*), and embryonic genome (*green*). (b) Histone modifications. Protamines are actively removed from the paternal genome (*shades of blue*) immediately following fertilization. Activating histone modifications are set in the early pronuclear stages (H4Ac/H3K4me1), which are followed by acquisition of repressive histone modifications (H3K4me3/H3K9me2/HeK27me2/3). Both activating (H4Ac/H3K4me1) and repressive (H3K9me2/3/H3K27me1/H4K20me3) histone modifications are abundant throughout the maternal genome (*pink*) during pronuclear stages. (c) DNA Methylation. The paternal genome (*blue*) undergoes active global demethylation immediately following fertilization, while the maternal genome (*red*) is passively demethylated through subsequent cleavage divisions. Line, ERV-L, and etn (*orange*) follow a similar demethylation process, while IAPs (*yellow*) and imprinted loci (*green*) are protected from demethylation events. (d) X-inactivation. A cascade of repressive modifications occurs on the paternal X chromosome in female preimplantation embryos, beginning with paternal-specific *Xist* expression.



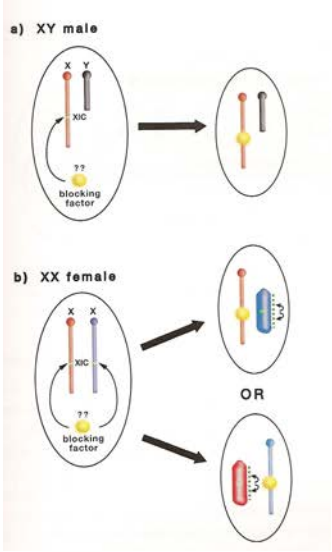


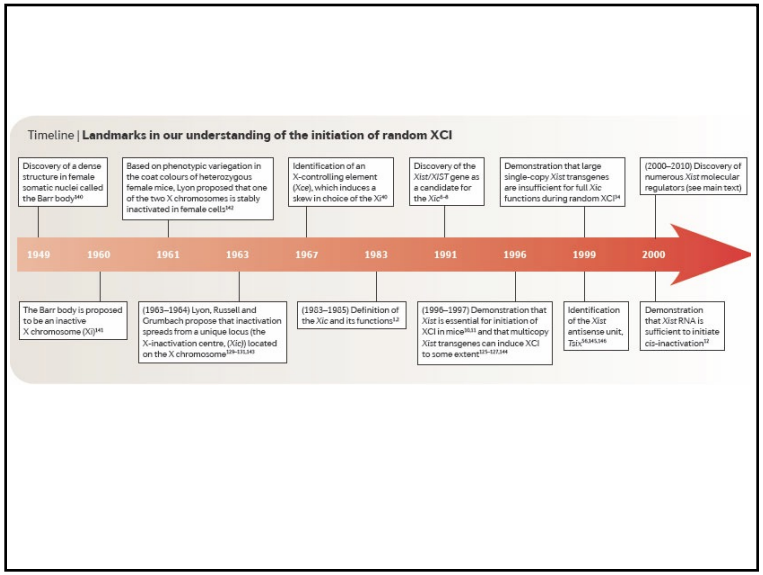
Epigenetics
Biological and Molecular
Processes

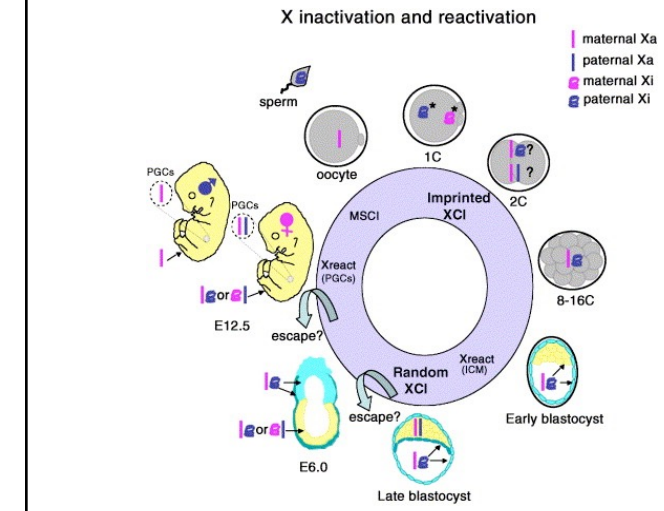
Epigenetic Regulation of Gene Expression

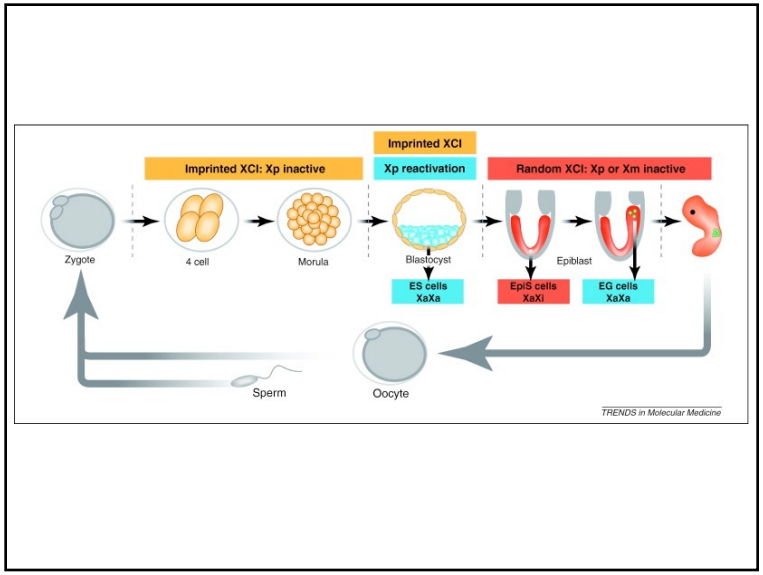
-Genomic Imprinting- Monoallelic Gene Expression
-X-Chromosome Inactivation- Inactivation of one X Chromosome
-Tissue-Specific Gene Expression- Regulation of Subset of Genes
-Developmental Programming- Prepare embryonic and gamete genomes
-Silencing of Repeat (Transposable) Elements

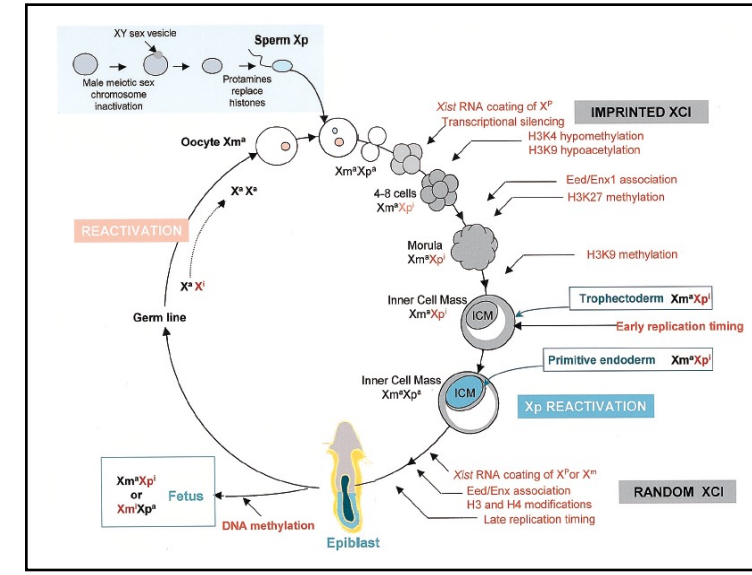
Epigenetics
Biological and Molecular
Processes
(X Chromosome Inactivation)

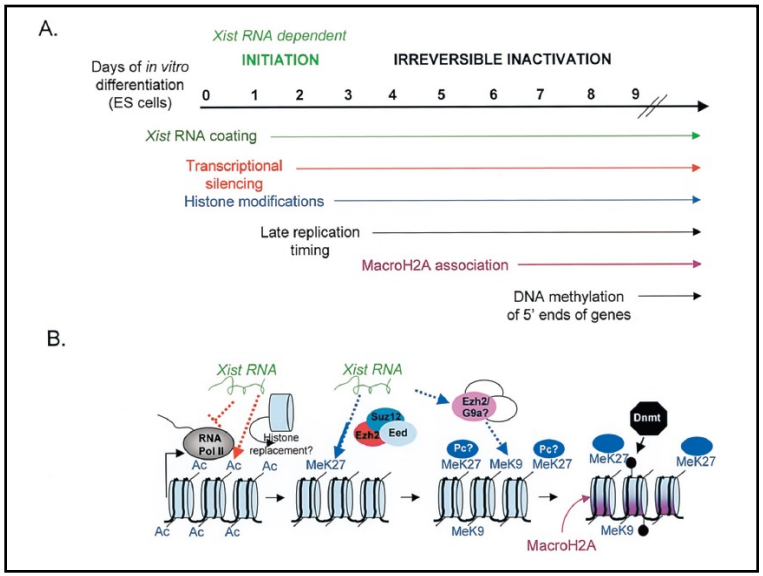



Timeline | Landmarks in our understanding of the initiation of random XCI
Discovery of a dense structure in female somatic nuclei called the Barr body[140]
Based on phenotypic variegation in the coat colours of heterozygous female mice, Lyon proposed that one of the two X chromosomes is stably inactivated in female cells[142]
Identification of an X-controlling element (Xce), which induces a skew in choice of the Xp[143]
Discovery of the Xist/XIST gene as a candidate for the Xic[4–6]
Demonstration that large single-copy Xist transgenes are insufficient for full Xic functions during random XCI[134]
(2000–2010) Discovery of numerous Xist molecular regulators (see main text)
1949
1960
1961
1963
1967
1983
1991
1996
1999
2000
The Barr body is proposed to be an inactive X chromosome (Xi)[141]
(1963–1964) Lyon, Russell and Grumbach propose that inactivation spreads from a unique locus (the X-inactivation centre, (Xic)) located on the X chromosome[126–131,143]
(1983–1985) Definition of the Xic and its functions[1,2]
(1996–1997) Demonstration that Xist is essential for initiation of XCI in mice[10,11] and that multicopy Xist transgenes can induce XCI to some extent[121–127,144]
Identification of the Xist antisense unit, Tsix[14,145,146]
Demonstration that Xist RNA is sufficient to initiate cis-inactivation[12]

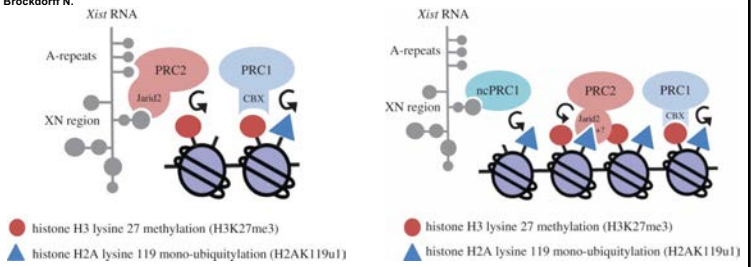

X inactivation and reactivation
maternal Xa
paternal Xa
maternal Xi
paternal Xi
sperm
oocyte
1C
2C
PGCs
PGCs
MSCI
Imprinted XCI
Xreact (PGCs)
8-16C
or
E12.5
escape?
Random XCI
Xreact (ICM)
Early blastocyst
or
escape?
E6.0
Late blastocyst


Imprinted XCI
Imprinted XCI: Xp inactive
Xp reactivation
Random XCI: Xp or Xm inactive
Zygote
4 cell
Morula
Blastocyst
Epiblast
ES cells XaXa
EpiS cells XaXi
EG cells XaXa
Sperm
Oocyte
TRENDS in Molecular Medicine


XY sex vesicle
Sperm Xp
Male meiotic sex chromosome inactivation
Protamines replace histones
Xist RNA coating of Xp
Transcriptional silencing
IMPRINTED XCI
H3K4 hypomethylation
H3K9 hypoacetylation
Oocyte Xm
XmXp
Eed/Enx1 association
H3K27 methylation
4–8 cells
Xm Xp
REACTIVATION
Morula
Xm Xp
H3K9 methylation
Inner Cell Mass
Xm Xp
ICM
Trophectoderm Xm Xp
Early replication timing
Germ line
Primitive endoderm Xm Xp
Inner Cell Mass
Xm Xp
ICM
Xp REACTIVATION
Xist RNA coating of Xp or Xm
Eed/Enx association
H3 and H4 modifications
Late replication timing
RANDOM XCI
Xm Xp or Xm Xp
Fetus
DNA methylation
Epiblast

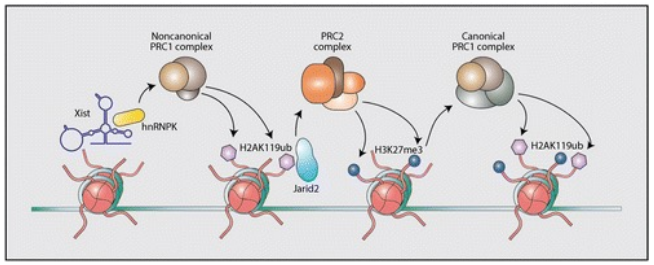


Polycomb complexes in X chromosome inactivation.
Philos Trans R Soc Lond B Biol Sci. 2017 Nov 5;372(1733) Brockdorff N.



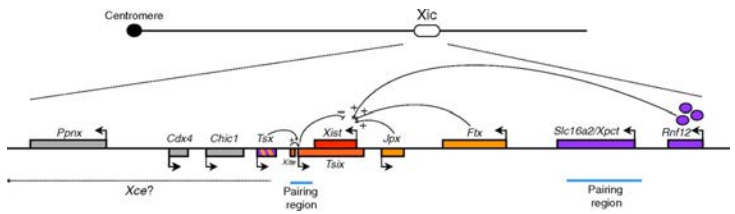
Classical model for Polycomb recruitment by Xist RNA. Early studies proposed direct interaction between core PRC2 subunits and the A-repeat element in Xist RNA. Subsequent studies implicated the Xist XN region and the PRC2 cofactor Jarid2 in initiating PRC2 recruitment. PRC2 functions to catalyse H3K27me3 on underlying nucleosomes. PRC1 recruitment is indicated as occurring downstream through interaction of the PRC1 subunit CBX and PRC2 mediated H3K27me3. Recruitment of PRC1 in turn mediates H2AK119u1 deposition on underlying chromatin.

Revised model for Polycomb recruitment by *Xist* RNA. The Polycomb cascade is initiated by non-canonical (nc) PRC1 complexes that are recruited by the *Xist* XN region. PRC1 mediated H2AK119u1, deposited on underlying nucleosomes, serves to recruit PRC2 through recognition by the cofactor, Jarid2 or through an alternative but currently undefined pathway (+?). PRC2 mediated H3K27me3 then signals recruitment of canonical PRC1 complexes, further reinforcing H2AK119u1 deposition and Polycomb domain formation.

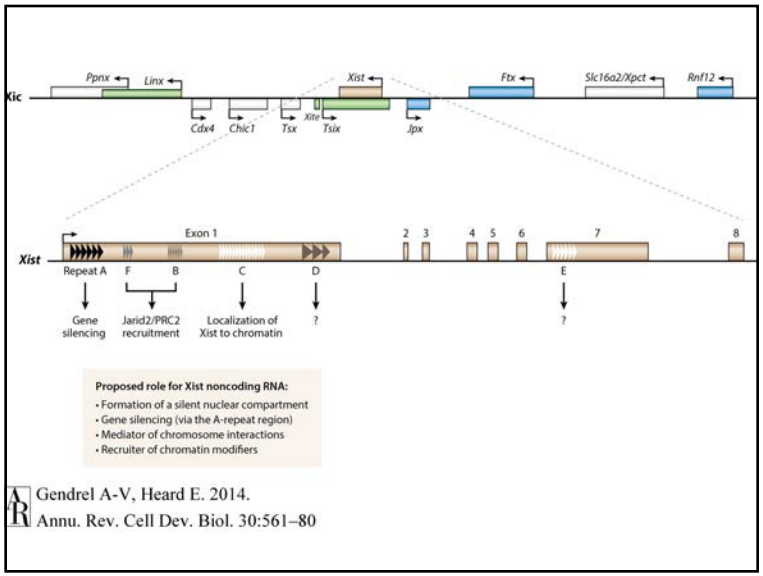


Gallupa R, Heard E. 2018. Annu. Rev. Genet. 52:535–66

Model for PRC1/PRC2 recruitment to the inactive X chromosome. Recruitment of both complexes depends on Xist RNA. Current evidence suggests that noncanonical PRC1 is recruited by Xist RNA via hnRNPK, while canonical PRC1 recruitment is downstream of PRC2 recruitment. PRC2 depends on Jarid2 to be recruited to the Xi, and Jarid2 is able to interact with the PRC1 mark (H2AK119ub). Abbreviations: H2AK119ub, monoubiquitination of histone H2A on lysine 119; H3K27me3, trimethylation of histone H3 on lysine 27; hnRNPK, heterogeneous nuclear ribonucleoprotein K; PRC1, Polycomb repressive complex 1; PRC2, Polycomb repressive complex 2. Figure credit: Bertsy Goic.

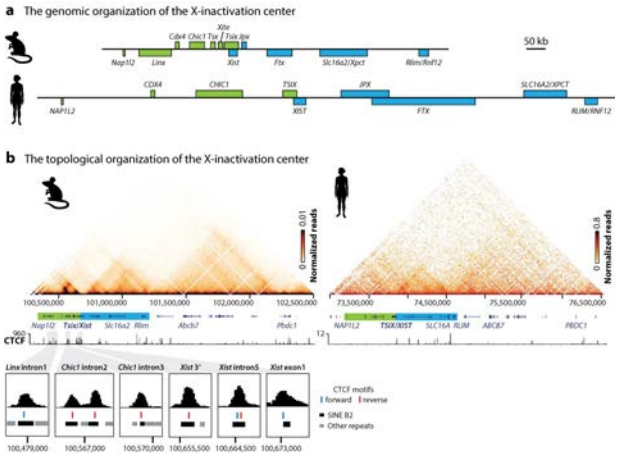


The X-inactivation centre (Xic) in mouse. The Xic on the X chromosome has been defined as the minimum region necessary and sufficient to trigger XCI (Augui et al., 2011). The region around the *Xist* (red) gene harbours a number of non-coding RNA genes (orange), as well as protein-coding genes (purple or grey), and two homologous 'pairing' regions (blue). All genes marked in colour have been shown to have either a positive (+) or a negative (–) effect on *Xist/Tsix* regulation. *Tsx* (striped orange/purple) encodes a protein that is expressed in testes and might also play a role as a ncRNA. *Rnf12* encodes an activator (purple ovals) of *Xist* expression. The potential location of the *Xce* locus, according to genetic mapping, is indicated. *Pprnx (4930519F16Rik); Cdx4*, caudal type homeobox 4; *Chic1*, cysteine-rich hydrophobic domain 1; *Tsx*, testis-specific X-linked gene; Xite, X-inactivation intergenic transcription element; *Tsix*, X (inactive)-specific transcript, antisense; *Xist*, X-inactive-specific transcript; *Jpx (Enox)*, expressed neighbour of *Xist; Ftx (B230206F22Rik)*, five prime to *Xist; Xpct (Slc16a2)*, X-linked PEST-containing transporter; *Rnf12* (*Rlim*), RING finger protein 12.

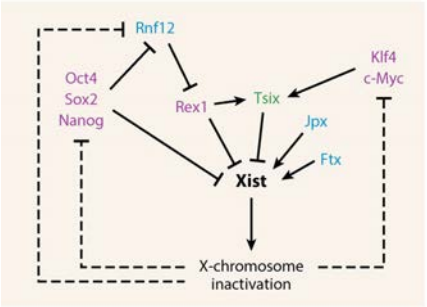


Gendrel A-V, Heard E. 2014.
Annu. Rev. Cell Dev. Biol. 30:561–80

X-Chromosome Inactivation: A Crossroads Between Chromosome Architecture and Gene Regulation
Annu Rev Genet. 2018 Nov 23;52:535-566.
Galupa R, Heard E.



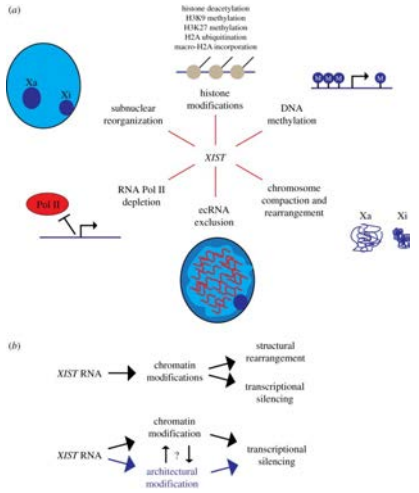
The Xist regulatory network

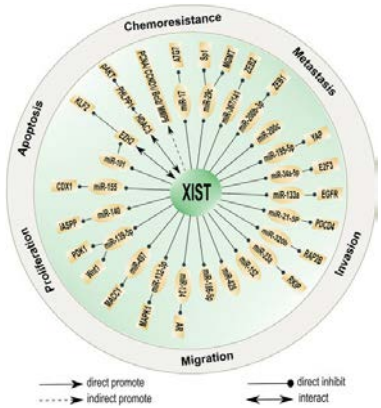


Gendrel A-V, Heard E. 2014.
Annu. Rev. Cell Dev. Biol. 30:561–80

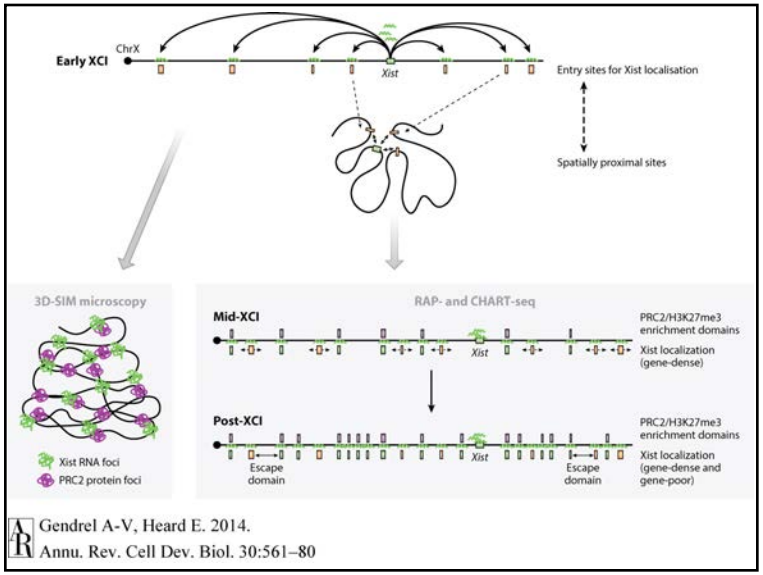
The *Xist* regulatory network. The regulatory network acting on *Xist* involves multiple molecular players acting either in *cis* or in *trans* to regulate *Xist* expression and X-chromosome inactivation (XCI) during development and differentiation. Arrows indicate positive regulation, and inhibition lines indicate negative regulation. Pluripotency factors (purple) can act either positively or negatively on downstream regulators. Putative positive regulators of XCI (blue) and putative negative regulators (green) are shown. Tsix, Linx, Jpx, and Ftx act as noncoding RNAs. In addition, once established, XCI downregulates *Rnf12*, and recently, XCI was shown to feed back on the pluripotency factor network (dashed inhibition lines) (Schulz et al. 2014).

XIST RNA: a window into the broader role of RNA in nuclear chromosome architecture.
Philos Trans R Soc Lond B Biol Sci. 2017 Nov 5;372(1733).
Creamer KM, Lawrence JB.



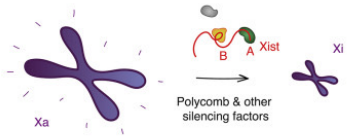


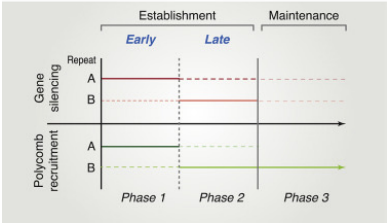
Overview of the regulatory mechanisms of XIST involved in cancer progression. XIST exerts multiple biological effects by interacting with different types of molecules.
Abbreviations: XIST, X-inactive-specific transcript; HDAC3, histone deacetylase 3; PHLPP1, PH domain and leucine rich repeat protein phosphatase 1; KLF2, kruppel-like factor 2; EZH2, enhancer of zeste homolog 2; CDX1, caudal type homeobox 1; iASPP, inhibitor of apoptosis-stimulating protein of p53; PDK1, pyruvate dehydrogenase kinase 1; MACC1, MET transcriptional regulator; MAPK1, mitogen-activated protein kinase 1; AR, androgen receptor; RKIP, raf kinase inhibitor protein; PDCD4, programmed cell death 4; EGFR, epidermal growth factor receptor; E2F3, E2F transcription factor 3; YAP, yes associated protein; ZEB1/2, zinc finger E-box binding homeobox 1/2; SP1, specificity protein 1; MGMT, O6-methylguanine-DNA methytransferase; ATG7, autophagy related 7; PCNA, proliferating cell nuclear antigen; CCND1, cyclin D1; Bcl2, B-cell lymphoma-2; MMP9, matrix metallopeptidase 9.



Gendrel A-V, Heard E. 2014.
Annu. Rev. Cell Dev. Biol. 30:561–80

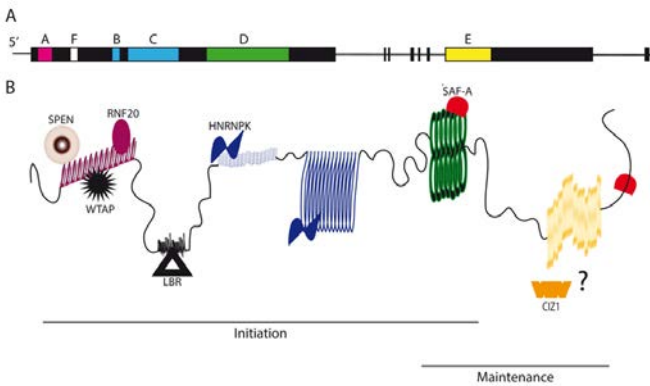
Xist Repeats A and B Account for Two Distinct Phases of X Inactivation Establishment
Colognori D, Sunwoo H, Wang D, et al.
Dev Cell. 2020 Jul 6;54(1):21-32.e5.





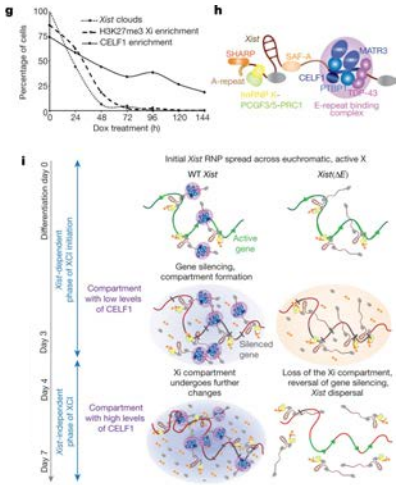
In Brief

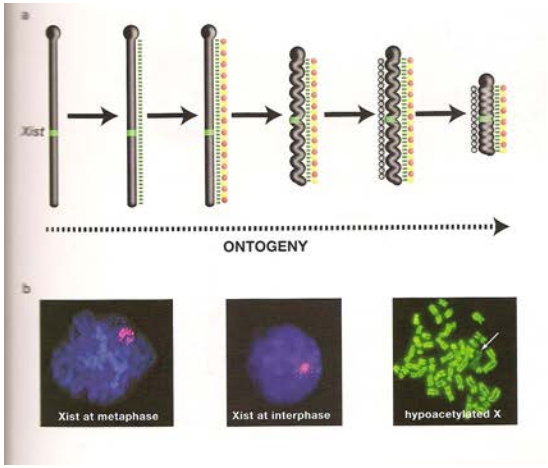
Colognori et al. show that X inactivation establishment is a biphasic process with distinct genetic requirements. Repeat A initiates Polycomb recruitment and gene silencing, whereas repeat B stabilizes them. Surprisingly, X inactivation can initiate without repeat B. Without repeat A, however, differentiating female cells lose one X chromosome to overcome loss of silencing.

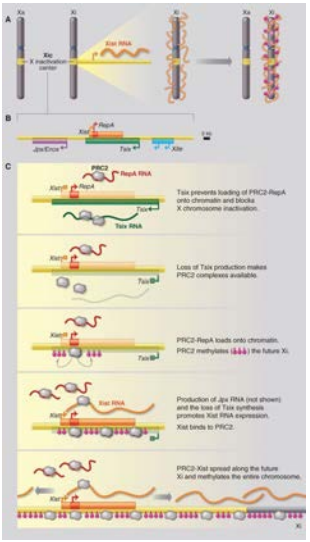


Repeat modules and interactors of Xist RNA.
A) Representation of the mouse Xist gene. Exon1 codes for repeats A–D and F. Exon 7 codes for repeat E. **B)** Mouse Xist RNA with its functional A–F repeats and their corresponding direct interactors. Lines indicate the repeats predicted to contribute to the initiation and maintenance of XCI. The binding of CIZ1 to Xist has not been fully established and is indicated by a question mark. CIZ1, CDKN1A-interacting protein; HNRNPK, heterogeneous nuclear ribonucleoprotein K; LBR, lamin B receptor; SAF-A, scaffold attachment factor A; SPEN, Split Ends; RNF20, ring finger protein 20; WTAP, WT1-associated protein.

A protein assembly mediates Xist localization and gene silencing
Pandya-Jones A, Markaki Y, Serizay J, et al.
Nature. 2020 Nov;587(7832):145-151.

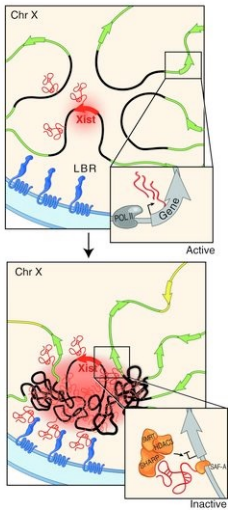




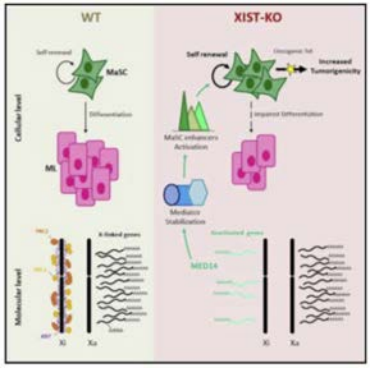


LncRNAs in X-chromosome inactivation. (A) The lncRNA Xist is transcribed from the Xic of the inactive X chromosome (Xi). Xist RNA covers the entire chromosome and silences gene expression through epigenetic modification of histones and DNA. (B) The core region of the Xic and its lncRNAs. (C) LncRNA-protein interactions at the initiation of XCI.

Xist recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing.
Chen CK, Blanco M, Jackson C, et al.
Science. 2016 Oct 28;354(6311):468-472.



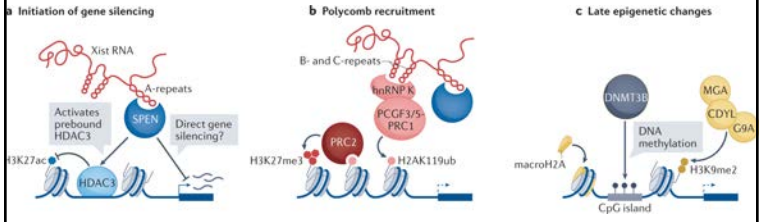
XIST loss impairs mammary stem cell differentiation and increases tumorigenicity through Mediator hyperactivation
Richart L, Picod-Chedotel ML, Wassef M, et al.
Cell. 2022 Jun 9;185(12):2164-2183.e25.



- *XIST*-null cells display reactivation of a few X-linked genes, including *MED14*
- *MED14* overdosage impacts stem cell homeostasis through Mediator stabilization
- Loss of *XIST* enhances the tumorigenic potential of cells upon transformation
- Xi transcriptional reactivation is common among aggressive breast tumors

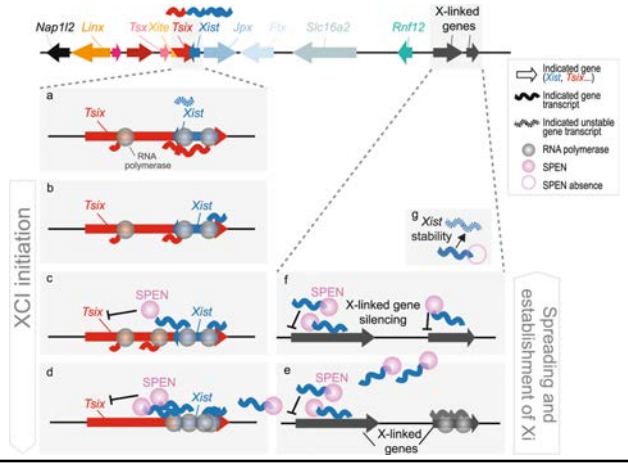
Gene regulation in time and space during X-chromosome inactivation.
Loda A, Collombet S, Heard E.
Nat Rev Mol Cell Biol. 2022 Apr;23(4):231-249.

Molecular mechanisms of X-chromosome inactivation.



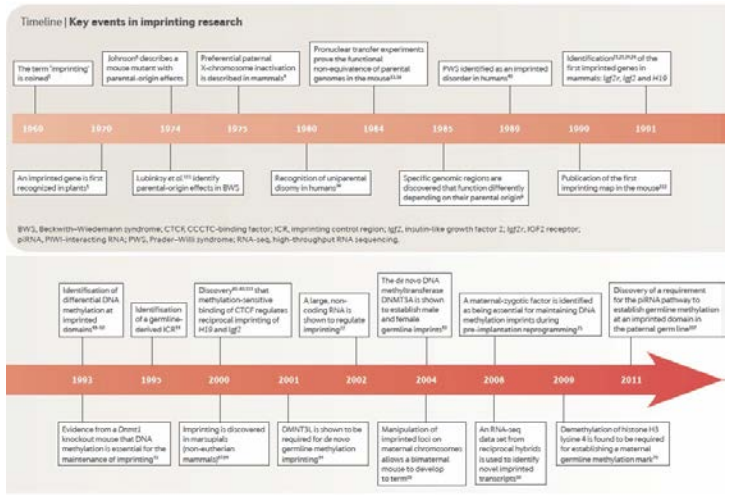
SPEN is required for Xist upregulation during initiation of X chromosome inactivation.
Robert-Finestra T, Tan BF, Mira-Bontenbal H, Timmers E, et al.
Nat Commun. 2021 Dec 1;12(1):7000.

Model of the role of SPEN in XCI



Digging into X chromosome inactivation.
Heard E, Rougeulle C.
Science. 2021 Nov 19;374(6570):942-943.

Epigenetics
Biological and Molecular Processes
(Imprinting)



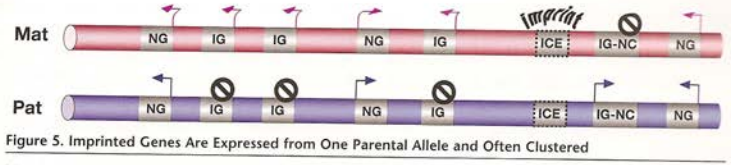
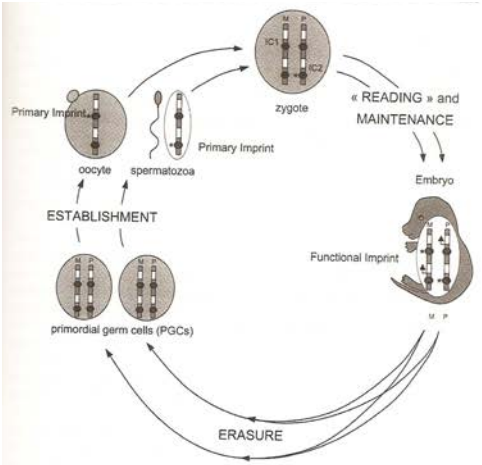
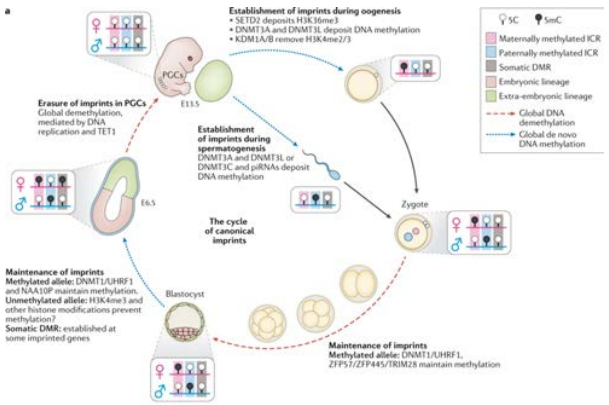


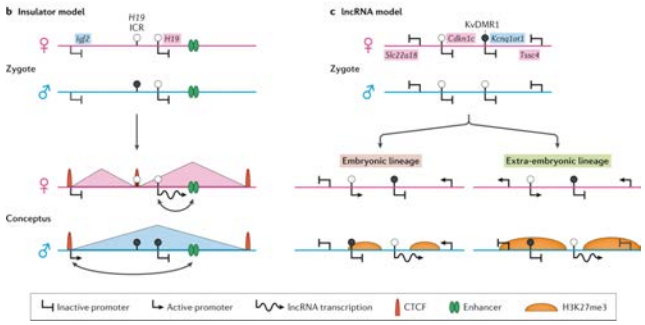
Figure 5. Imprinted Genes Are Expressed from One Parental Allele and Often Clustered

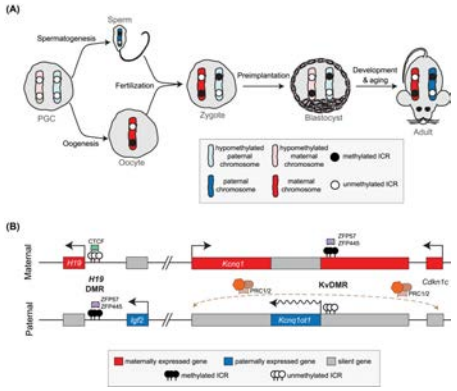
KEY FEATURES OF GENOMIC IMPRINTING IN MAMMALS

- *cis*-Acting mechanism
- A consequence of inheritance, not sex
- Imprints are epigenetic modifications acquired by one parental gamete
- Imprinted genes are mostly clustered together with a noncoding RNA
- Imprints can modify long-range regulatory elements that act on multiple genes
- Imprinted genes play a role in mammalian development









Genomic imprinting regulation by parental allele-specific DNA methylation

Table 1. The function of imprinted genes as determined by gene inactivation

Maternal	Gene function	Paternal
–Igf2r *–Gnas* *–Tssc3/Ipl* *–Mash2* *–Grb10/Meg1* *–/+ Cdkn1c*	growth defects in embryo, placenta, or postnatal stage	*+Igf2* *+Gnasxl* *+Peg1/Mest* *+Peg3/Pw1* *+Rasgrf1* *+Dlk1*
Nesp *Ube3a* *Kcnq1**	behavioral or neurological defects	*+Peg1/Mest* *+Peg3/Pw1* *+Rasgrf1*
Asb1 (spermatogenesis) *Dcn* (Tumor suppressor)	other defects	*Ndn* (strain-specific lethality)
H19 ncRNA *Slc22a2* *Slc22a3*	no obvious defects in embryo or neonate	*Snrpn/Snurf* *Frat3* *Ins2*

(Maternal) Maternally expressed imprinted gene, (Paternal) paternally expressed imprinted genes, (+) growth promoting effect, (–) growth suppressing effect, (–/+) defect in differentiation but growth regulatory status unclear, (*) additional differentiation defect. (Reference to the primary data can be found at: http://www.mgu.har.mrc.ac.uk/research/imprinting/function.html).

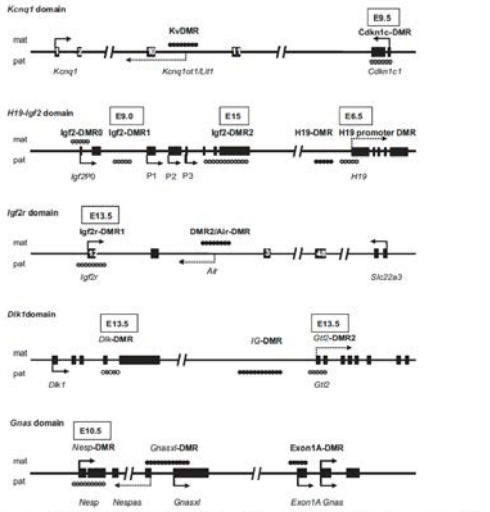
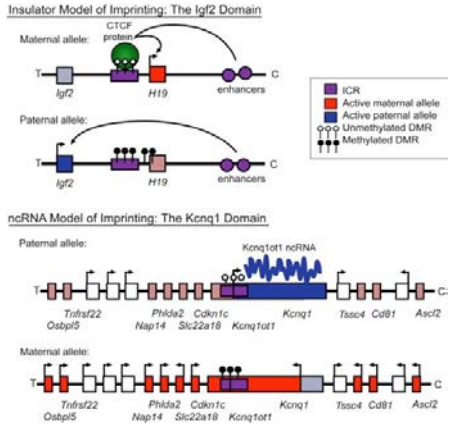


Fig. 2. Schematic representation of five imprinted loci containing somatic DMRs. Exons are represented by filled boxes. Direction of transcription for maternally and paternally expressed genes is denoted by arrows above and below the boxes, respectively. Arrows with dotted lines represent non-coding transcripts. Differentially methylated regions (DMR) are indicated by closed circles (black fill=gametic DMR, gray fill=somatic DMR). The developmental stage at which somatic imprint are known to be present is given above the DMR but some of these imprints may be present at earlier, untested time points.

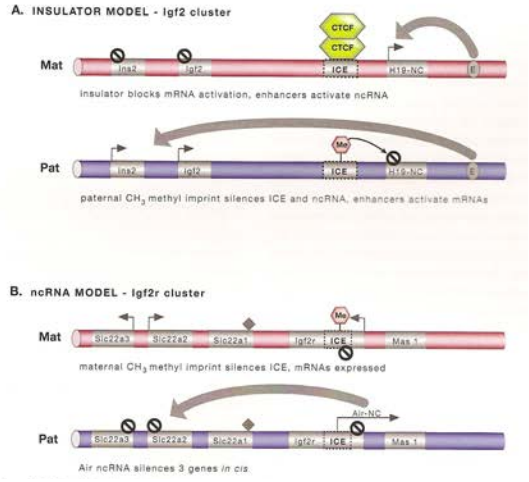
Table 2. Features of imprinted gene clusters in the mouse genome

Cluster type	Cluster name	Chromosome mouse/human	Gametic methylation imprint	Cluster size (kb)	Gene number in cluster	mRNAs and expression	ncRNA and expression	ncRNA orientation
Type I	*Igf2r*	17 / 6	M	400	4	*Igf2r* (M) *Slc22a2* (M) *Slc22a3* (M)	*Air* (P)	antisense to *Igf2r*
	Kcnq1	7 / 11	M	700	10	*Mash2* (M) *Kcnq1* (M) *Cd81* (M) *Cdkn1c* (M) *Msi/t* (M) *Slc22l1* (M) *Ipl* (M) *Tssc4* (M) *Obph1* (M)	*Kcnq1ot1* (P)	antisense to *Kcnq1*
	Pws	7 / 15	M	3000	~7	*Ube3a* (M) Atp10c (M) *Frat3* (P) *Mkrn3* (P) *Ndn* (P) *Magel2* (P) *Snrpn* (P)	**Ube3aas* (P) **IPW* (P) **Mkrn3as* (P) **PEC2* (P) **PEC3* (P) **Pwo1* (P) **may be one long ncRNA*	antisense to *Ube3a* (also overlaps *Snrpn* in sense orientation)
	Gnas	2 / 20	M (x 2)	100	5	*Nesp* (M) *Gnas* (M) *Gnasxl* (P)	[1]*Nespas*(P) [2]*Exon1A* (P)	[1]antisense to *Nesp* [2]sense to *Gnas*
Type II	*Igf2*	7 / 11	P	100	3	*Igf2* (P) *Ins2* (P)	*H19* (M)	sense no overlaps
	Dlk1	9 / 14	P	1000	7	*Dlk1* (P) *Dio3* (P) *Rtl1* (P)	*Gtl2* (M)* *Rian* (M)* *Rtl1as* (M)* *Mirg* (M)* *region may contain longer ncRNAs*	sense to *Dlk1* and also antisense to *Rtl1*

(M) Maternal, (P) paternal, (DMR) differentially methylated region. Details are given in the text. (Modified from Beechey et al. 2005 [http://www.mgu.har.mrc.ac.uk/research/imprinting].)



Mechanism of imprinting. Top. The insulator model is exemplified by the *H19/Igf2* domain. The intergenic ICR is paternally methylated. On the unmethylated maternal allele, CTCF binding prevents enhancers from interacting with the *Igf2* promoter. Instead, the enhancers activate *H19* expression. On the paternal allele, methylation of the ICR spreads to the H19 promoter, silencing its expression, and prevents CTCF from binding the ICR, allowing the enhancers to activate *Igf2* expression. Bottom. The ncRNA model is illustrated by the *Kcnq1* domain. The ICR contains the promoter of the ncRNA *Kcnq1ot1*. On the paternal allele, the ICR is unmethylated, allowing *Kcnq1ot1* expression. *Kcnq1ot1* expression silences the paternal allele of the rest of the imprinted genes in the domain in cis. On the maternal allele, Kcnq1ot1 is not expressed due to methylation of the ICR, and the adjacent imprinted genes are expressed.



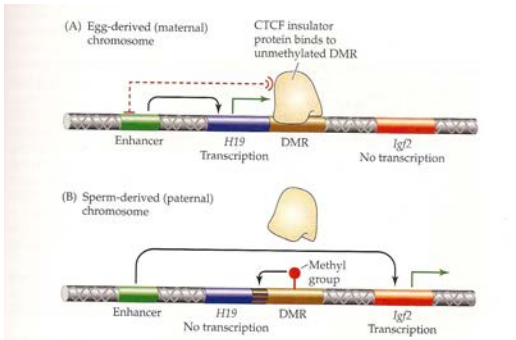
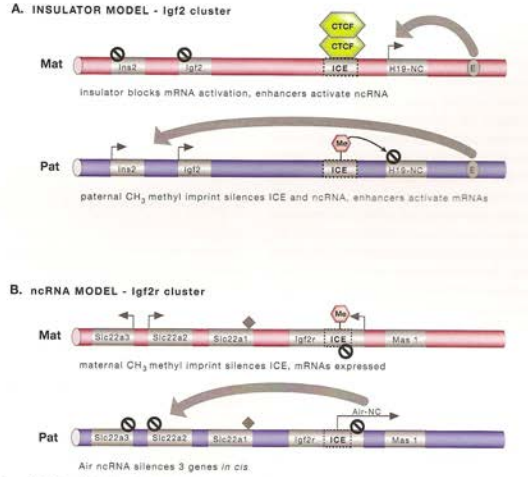
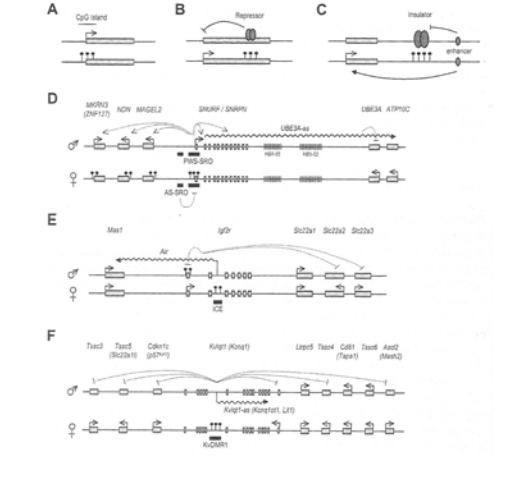
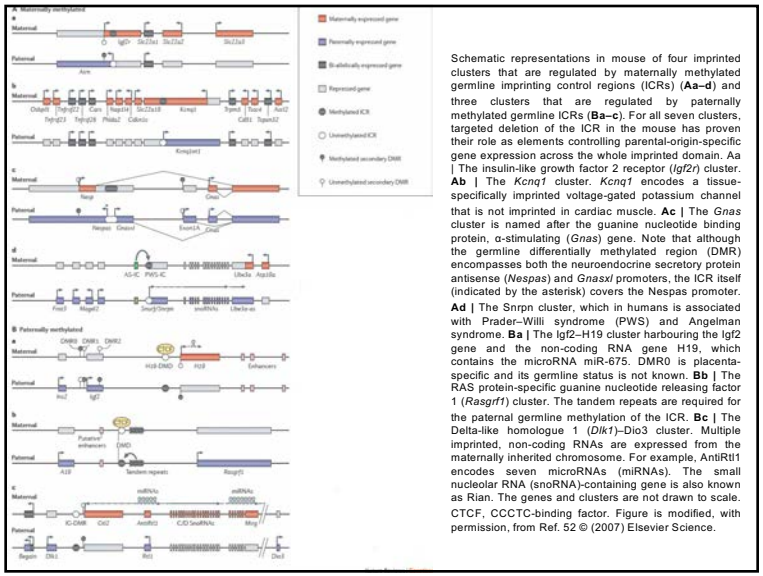


FIGURE 1 Genomic imprinting in the mouse. The *Igf2* gene is activated by an enhancer element it shares with the *H19* gene. A differentially methylated region (DMR) lies between the enhancer and the *Igf2* gene, adjacent to the promoter region of the *H19* gene. (A) In the egg-derived chromosome, the DMR is unmethylated and binds the CTCF insulator protein, thus blocking the enhancer signal and preventing *Igf2* expression. (B) In the sperm-derived chromosome, the DMR is methylated, with two consequences. First, the CTCG insulator protein cannot bind to the DMR and the enhancer can activate *Igf2*. Second, the methylated DMR alters the chromatin of the *H19* promoter, preventing its activation by the enhancer. Therefore, the maternal chromosome expresses *H19* but not *Igf2*, whereas the paternally derived chromosome expresses *Igf2* but not *H19*.







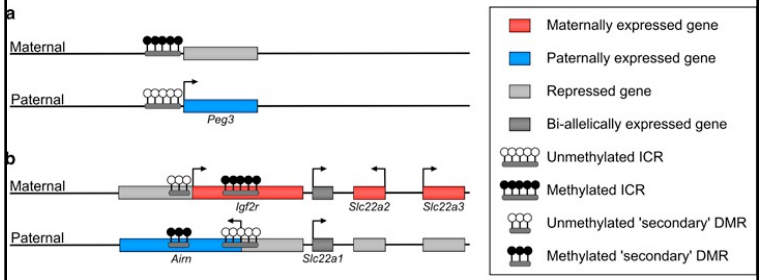
Schematic representations in mouse of four imprinted clusters that are regulated by maternally methylated germline imprinting control regions (ICRs) (**Aa–d**) and three clusters that are regulated by paternally methylated germline ICRs (**Ba–c**). For all seven clusters, targeted deletion of the ICR in the mouse has proven their role as elements controlling parental-origin-specific gene expression across the whole imprinted domain. Aa | The insulin-like growth factor 2 receptor (*Igf2r*) cluster. **Ab** | The *Kcnq1* cluster. *Kcnq1* encodes a tissue-specifically imprinted voltage-gated potassium channel that is not imprinted in cardiac muscle. **Ac** | The *Gnas* cluster is named after the guanine nucleotide binding protein, α-stimulating (*Gnas*) gene. Note that although the germline differentially methylated region (DMR) encompasses both the neuroendocrine secretory protein antisense (*Nespas*) and *Gnasxl* promoters, the ICR itself (indicated by the asterisk) covers the Nespas promoter. **Ad** | The Snrpn cluster, which in humans is associated with Prader–Willi syndrome (PWS) and Angelman syndrome. **Ba** | The Igf2–H19 cluster harbouring the Igf2 gene and the non-coding RNA gene H19, which contains the microRNA miR-675. DMR0 is placenta-specific and its germline status is not known. **Bb** | The RAS protein-specific guanine nucleotide releasing factor 1 (*Rasgrf1*) cluster. The tandem repeats are required for the paternal germline methylation of the ICR. **Bc** | The Delta-like homologue 1 (*Dlk1*)–Dio3 cluster. Multiple imprinted, non-coding RNAs are expressed from the maternally inherited chromosome. For example, AntiRtl1 encodes seven microRNAs (miRNAs). The small nucleolar RNA (snoRNA)-containing gene is also known as Rian. The genes and clusters are not drawn to scale. CTCF, CCCTC-binding factor. Figure is modified, with permission, from Ref. 52 © (2007) Elsevier Science.

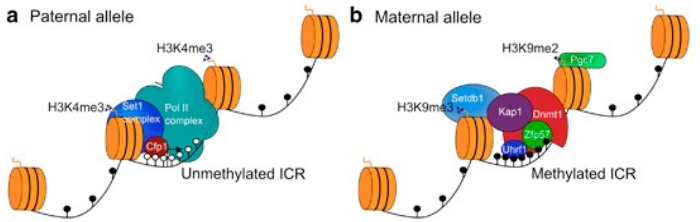
Table 1 Dynamics of H3K4me3 and H3K27me3 profiles at imprinted genes between cell types.

	ESCs		More differentiated cells		
Gene	*Expression*	*Histone modification*	*Expression*	*Histone modification*	*Cell type*
Gatm	Absent	H3K4me3 H3K27me3	Present	H3K4me3	NPC
			Present	H3K4me3 H3K27me3	MEF
Peg12	Absent	H3K4me3 H3K27me3	Present	H3K4me3	NPC+MEF
Tfpi2	Absent	H3K4me3 H3K27me3	Present	None	MEF
Asb2	Absent	H3K4me3 H3K27me3	Absent	H3K4me3 H3K27me3	NPC+MEF
Calcr	Absent	H3K4me3 H3K27me3	Absent	H3K27me3	NPC+MEF
Kcnq1	Absent	H3K4me3 H3K27me3	Absent	H3K27me3	NPC+MEF
Rasgrf	Absent	H3K4me3 H3K27me3	Absent	H3K4me3 H3K27me3	NPC+MEF
Slc22a3	Absent	H3K4me3 H3K27me3	Absent	H3K27me3	NPC+MEF
Tfpi2	Absent	H3K4me3 H3K27me3	Absent	None	NPC

Expression and histone modification profiles at developmentally repressed imprinted gene transcription start sites (TSSs) enriched with both H3K4me3 and H3K27me3 in mouse embryonic stem cells (ESCs) are detailed for neural progenitor cells (NPCs) and mouse embryonic fibroblasts (MEFs). The presence of these two marks in ESCs sometimes resolves to H3K4me3 in the more differentiated cell types at genes that become expressed (upper panel). Resolution (to H3K27me3) also occurs at some genes that do not change expression status and remain repressed (lower panel). The expression status and histone enrichment profile at the TSS of imprinted genes were identified using source data from Mikkelsen *et al.* [50].

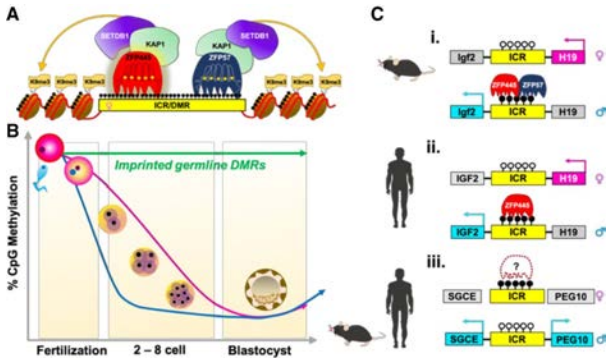
The specification of imprints in mammals.
Hanna CW, Kelsey G.
Heredity (Edinb). 2014 Aug;113(2):176-83.





Factors involved in the maintenance of DNA methylation at imprinting control regions (ICRs) during early embryonic development. In the preimplantation embryo, the parental alleles of ICRs are bound by specific complexes to maintain their differential methylation status during the genome-wide epigenetic reprogramming. (a) The unmethylated ICR has the properties typical of a CpG island. It coincides with an expressed promoter and is, therefore, associated with the RNA polymerase II (pol II) complex. Unmethylated CpG sites are bound by the Cfp1 protein, which recruits the Set1 complex to trimethylate H3K4 which, in turn, reinforces the DNA hypomethylated state, as methylated H3K4 is antagonistic to binding of Dnmt3a/Dnmt3L. Protection against *de novo* DNA methylation of the unmethylated ICR allele could, therefore, be accomplished by generic factors acting at CpG islands, rather than imprint-specific factors. (b) The methylated ICR is bound by the Zfp57:Kap1 complex and Uhrf1 to direct the action of Dnmt1; the complex also interacts with the Setdb1 methyltransferase, which deposits the repressive histone modification H3K9me3. In addition, recognition of H3K9me2 by the PGC7/Stella protein may protect against demethylation in the zygote. Maintenance of DNA methylation at an ICR, therefore, involves the interplay between the imprint-specific factor Zfp57 and generic factors, such as Dnmt1.

Evolving imprinting control regions: KRAB zinc fingers hold the key.
Genes Dev. 2019 Jan 1;33(1-2):1-3.
Juan AM, Bartolomei MS.



Model for ZFP57 and ZFP445-KAP1-mediated regulation of genomic imprints in the post-fertilization embryo. (*A*) An example of a maternally silenced imprinted gene. ZFP445 and ZFP57 bind to the methylated ICR (dark lollipops) and recruit scaffold protein KAP1 and histone methyltransferase SETDB1 to deposit H3K9me3 on the silenced allele (other components of the complex are not depicted for simplicity). (*B*) Shortly after fertilization, the highly specialized genomes of the gametes are demethylated to produce a totipotent zygote or blastocyst. (Pink line) Maternal genome; (blue line) paternal genome. Imprinted ICRs/germline differentially methylated regions (DMRs) are protected from this wave of DNA demethylation (green line) to maintain parental allele-specific methylation. (**C**) ZFP57 and ZFP445 protect ICRs from post-fertilization DNA demethylation in a species- and ICR-specific manner. In rodents, ZFP57 and ZFP445 may cooperate to preserve *H19* imprints (panel *i*), while, in humans, ZFP445 alone appears sufficient (panel *ii*). (Panel *iii*) In both species, *PEG10*/*Peg10* was not regulated by either ZFP57 or ZFP445, suggesting that a different KZFP is involved.

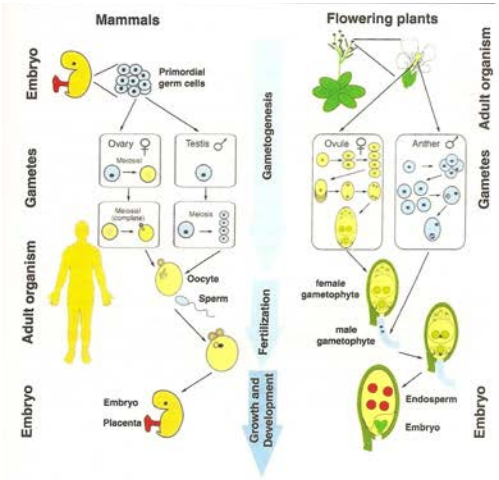
Table 1. Selected disorders of genomic imprinting

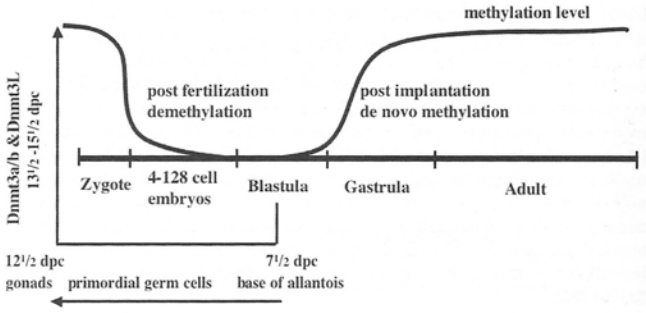
Disorder	Gene	Comments	Gene(s) involved
Prader-Willi syndrome	deletion, UPD, imprint defect	15q11-q13	snoRNAs and other (?)
Angelman syndrome	deletion, UPD, imprint defect, point mutation, duplication[a]	15q11-q13	*UBE3A*
Beckwith-Wiedemann syndrome	imprint defect, UPD, 11p15.5 duplication, translocation point mutation	11p15.5	*IGF2, CDKN1C*
Silver-Russell syndrome	UPD, duplication translocation, inversion	7p11.2	several candidates in the region
	epimutation	11p15.5	biallelic expression of H19 and decrease of IGF2
Pseudohypoparathyroidism	point mutation, imprint defect, UPD	20q13.2	*GNAS1*

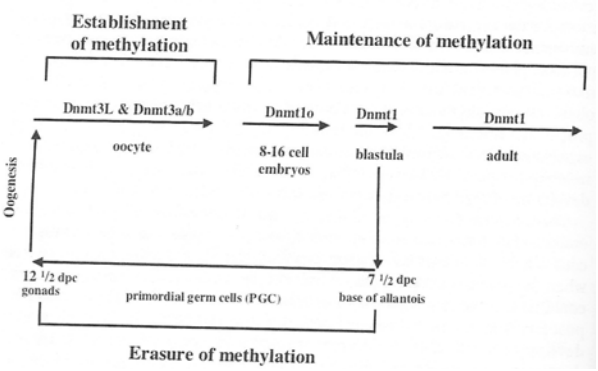
[a]Maternal duplications, trisomy, and tetrasomy for this region cause autism and other developmental abnormalities.

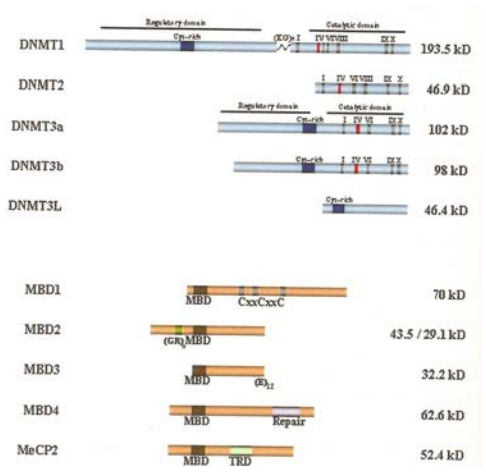
Developmental Epigenetics

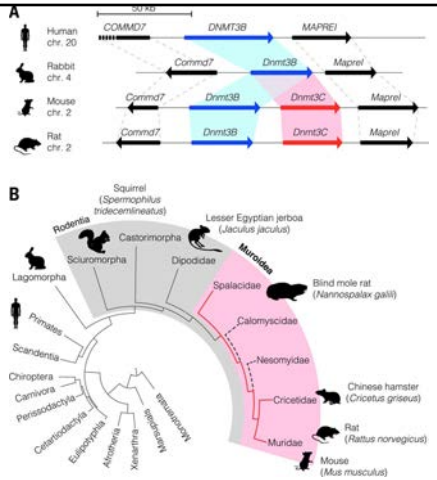
Developmental Epigenetics
(Germ line and early embryo)


Mammals
Flowering plants
Embryo
Gametes
Adult organism
Primordial germ cells
Ovary
Testis
Meiosis
Oocyte
Sperm
Gametogenesis
Fertilization
Growth and Development
Ovule
Anther
female gametophyte
male gametophyte
Endosperm
Embryo
Placenta
Adult organism


methylation level
Dnmt3a/b &Dnmt3L
13½-15½ dpc
post fertilization
demethylation
post implantation
de novo methylation
Zygote
4-128 cell
embryos
Blastula
Gastrula
Adult
12½ dpc
gonads
primordial germ cells
7½ dpc
base of allantois


Establishment
of methylation
Maintenance of methylation
Dnmt3L & Dnmt3a/b
oocyte
Dnmt1o
8-16 cell
embryos
Dnmt1
blastula
Dnmt1
adult
Oogenesis
12 ½ dpc
gonads
primordial germ cells (PGC)
7 ½ dpc
base of allantois
Erasure of methylation


DNMT1
193.5 kD
DNMT2
46.9 kD
DNMT3a
102 kD
DNMT3b
98 kD
DNMT3L
46.4 kD
MBD1
70 kD
MBD2
43.5 / 29.1 kD
MBD3
32.2 kD
MBD4
62.6 kD
MeCP2
52.4 kD
MBD
CxxCxxC
Repair
TRD

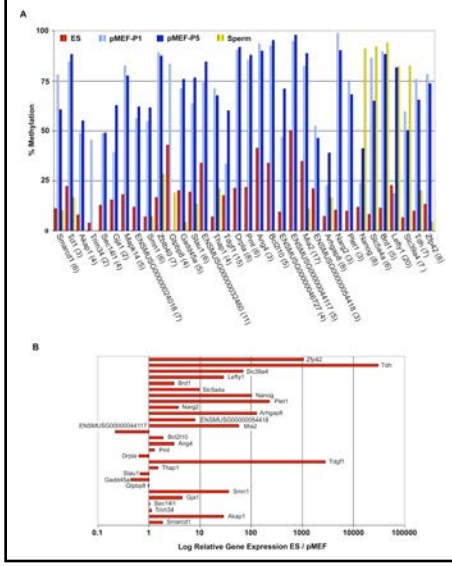


Phylogenetic distribution of Dnmt3C (A) Locus organization and synteny around the Dnmt3B (blue) and Dnmt3C (red) genes in human, rabbit, mouse, and rat genomes. (B) Dnmt3C distribution in the mammalian phylogenetic tree with a focus on the Rodentia order. Species in detail have available sequenced genomes. Red branches: Muroidea families in which Dnmt3C was identified; dashed branches: Muroidea families without available genome sequence.

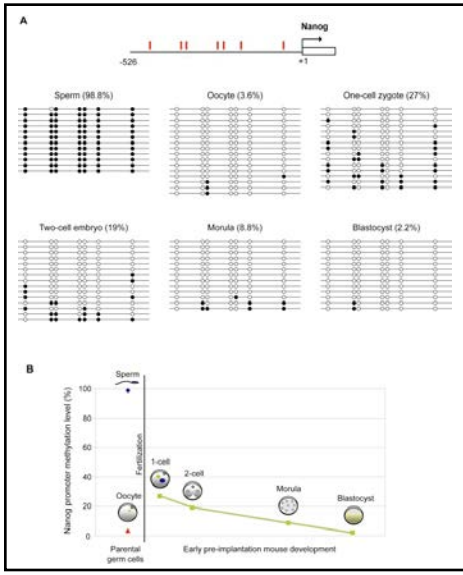
Global mapping of DNA methylation in mouse promoters reveals epigenetic reprogramming of pluripotency genes.

Farthing CR, Ficz G, Ng RK, Chan CF, Andrews S, Dean W, Hemberger M, Reik W.

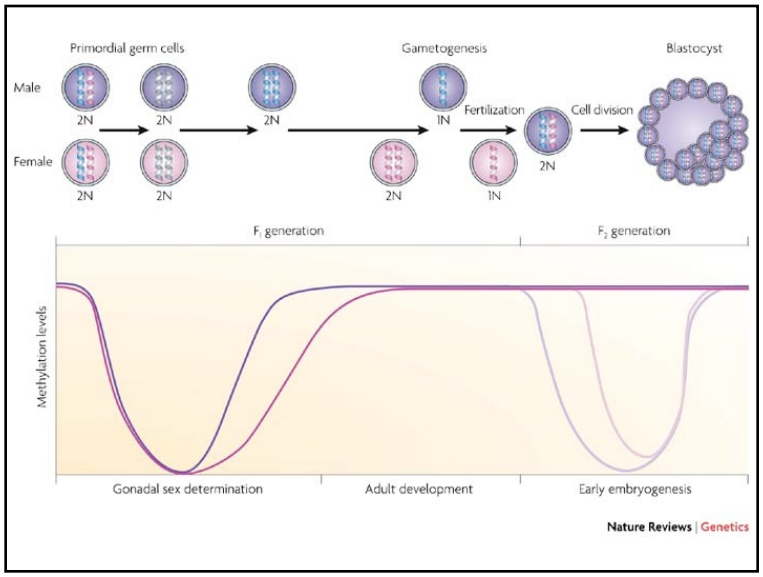
PLoS Genet. 2008 Jun 27;4(6):e1000116.

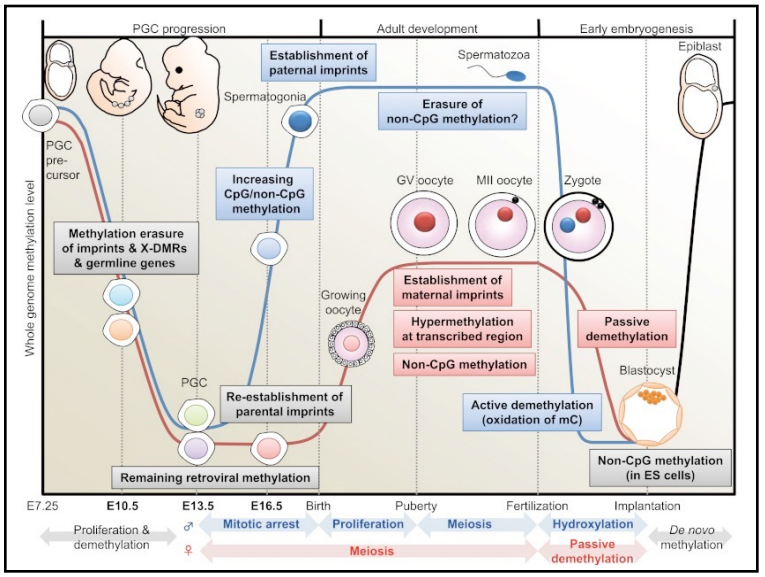


Promoter methylation and gene expression compared between ES cells and pMEFs.
(A) Promoter methylation patterns in ES cells (red bars), early passage pMEFs (pMEFs-P1, light blue bars), late passage pMEFs (pMEFs-P5, dark blue bars) and sperm (yellow bars). Candidate promoter regions were identified by the meDIP screen and validated by Sequenom analysis. The number of differentially methylated CpGs analysed for each gene are given in brackets. (B) Gene expression differences between ES cells and pMEFs (P1) as determined by quantitative RT-PCR analysis. The x-axis gives the log-fold expression difference between the cell types (i.e., log [*ES/pMEF*]). Three reference genes (*Dynein, Rsp23* and *Hdac10-11*) were used for normalization between cell types.



Epigenetic reprogramming of the *Nanog* promoter during preimplantation development.
(A) Methylation patterns of the *Nanog* promoter in gametes and in early fertilised embryos were determined by bisulphite sequencing analysis. The Nanog promoter is highly methylated in sperm but hypomethylated in fertilised embryos. CpG dinucleotides are represented as open circles (unmethylated) or closed circles (methylated). The percentage of CpG methylation is indicated in brackets. (B) Summary of *Nanog* promoter methylation during preimplantation mouse development. The level of methylation at the *Nanog* promoter is given as a percentage. Methylation levels are given for the gametes and at the preimplantation stages indicating that the Nanog promoter undergoes both active and passive demethylation after fertilisation.

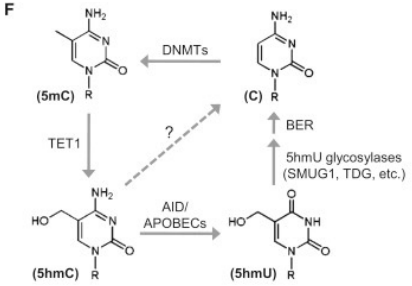




Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain.
Guo JU, Su Y, Zhong C, Ming GL, Song H.
Cell. 2011 Apr 29;145(3):423-34.

Abstract

Cytosine methylation is the major covalent modification of mammalian genomic DNA and plays important roles in transcriptional regulation. The molecular mechanism underlying the enzymatic removal of this epigenetic mark, however, remains elusive. Here, we show that 5-methylcytosine (5mC) hydroxylase TET1, by converting 5mCs to 5-hydroxymethylcytosines (5hmCs), promotes DNA demethylation in mammalian cells through a process that requires the base excision repair pathway. Though expression of the 12 known human DNA glycosylases individually did not enhance removal of 5hmCs in mammalian cells, demethylation of both exogenously introduced and endogenous 5hmCs is promoted by the AID (activation-induced deaminase)/APOBEC (apolipoprotein B mRNA-editing enzyme complex) family of cytidine deaminases. Furthermore, Tet1 and Apobec1 are involved in neuronal activity-induced, region-specific, active DNA demethylation and subsequent gene expression in the dentate gyrus of the adult mouse brain in vivo. Our study suggests a TET1-induced oxidation-deamination mechanism for active DNA demethylation in mammals.

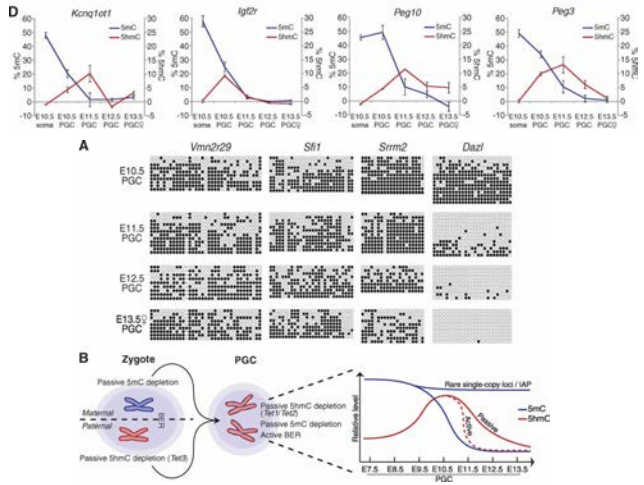


Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation.
Ficz G, et al.
Nature. 2011 May 19;473(7347):398-402.

Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells.
Pastor WA, et al.
Nature. 2011 May 19;473(7347):394-7.

TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity.
Williams K, et al.
Nature. 2011 May 19;473(7347):343-8.

Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine.
Hackett JA, et al. (2013)
Science. 2013 Jan 25;339(6118):448-52.



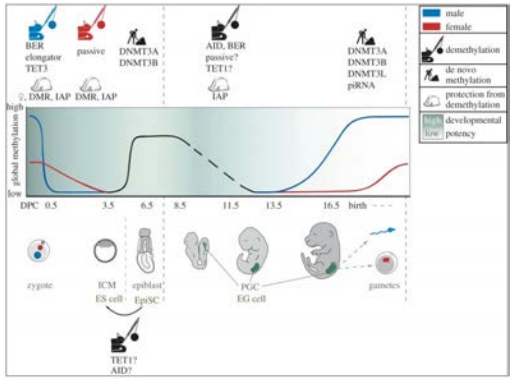
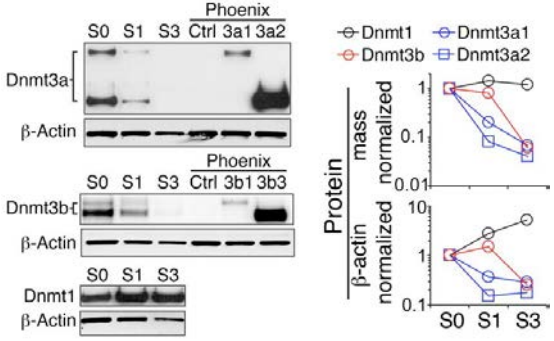


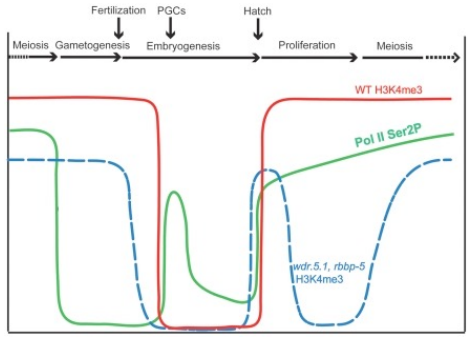
Figure 1. DNA methylation reprogramming in the mammalian life cycle. DNA methylation marks represent an epigenetic barrier in mammalian development that is demolished when developmental potency has to be restored and subsequently re-built with the commitment to a particular cell fate. This first occurs following fertilization, when the DNA methylation marks of the parental gametes are erased in two waves of demethylation. In the first wave, the paternal pronucleus (shown in blue) undergoes rapid demethylation in the zygote, which is followed by a passive loss of DNA methylation marks in the maternal genome (shown in red) over the subsequent cell divisions. Re-establishment of DNA methylation marks commences in the ICM of the developing embryo, which forms an epigenetic barrier (dashed line) in the developmentally more restricted epiblast. PGCs (shown in green) inherit the epigenetic signature from the epiblast, and DNA methylation is again erased on a global scale concomitant with the restoration of developmental potency. Note that DNA methylation at DMRs of imprinted genes become reset in PGCs but are protected from reprogramming in the early embryo. With further development into fully specialized gametes, DNA methylation marks are re-established and developmental potency is restricted. This epigenetic barrier (dashed line) will be demolished once more in the zygote of the next generation as part of the continuous cycle of DNA methylation reprogramming.

Global DNA demethylation during mouse erythropoiesis in vivo.
Shearstone JR, et al.
Science. 2011 Nov 11;334(6057):799-802.

Abstract
In the mammalian genome, 5'-CpG-3' dinucleotides are frequently methylated, correlating with transcriptional silencing. Genome-wide demethylation is thought to occur only twice during development, in primordial germ cells and in the pre-implantation embryo. These demethylation events are followed by de novo methylation, setting up a pattern inherited throughout development and modified only at tissue-specific loci. We studied DNA methylation in differentiating mouse erythroblasts in vivo by using genomic-scale reduced representation bisulfite sequencing (RRBS). Demethylation at the erythroid-specific β-globin locus was coincident with global DNA demethylation at most genomic elements. Global demethylation was continuous throughout differentiation and required rapid DNA replication. Hence, DNA demethylation can occur globally during somatic cell differentiation, providing an experimental model for its study in development and disease.

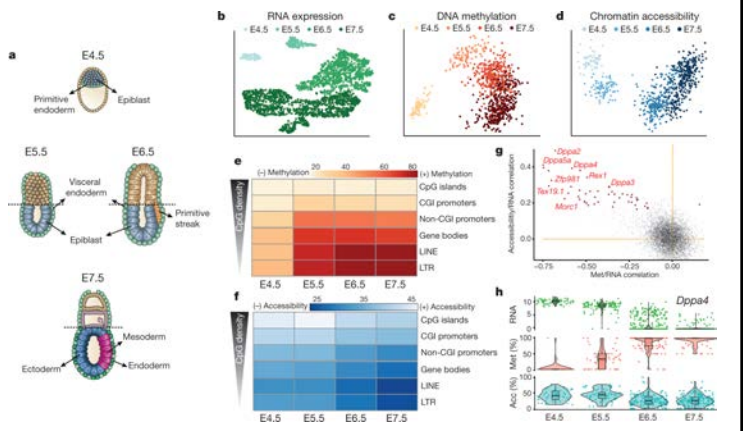


A role for Set1/MLL-related components in epigenetic regulation of the Caenorhabditis elegans germ line.
Li T, Kelly WG.
PLoS Genet. (2011) 7(3):e1001349.

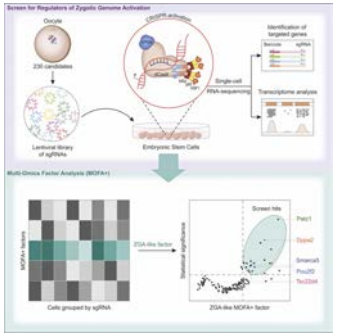


Summary of H3K4me3 and RNA polymerase C-terminal domain phosphorylation dynamics during the C. elegans germline cycle.
Relative abundance of H3K4me3 in germ cell chromatin at different stages of germ cell development (indicated across the top of the graph) is plotted for wild type (WT; red solid line) and both *wdr-5.1* and *rbbp-5* mutants (blue dotted line). Superimposed on this are the dynamics observed (in WT) for the phosphorylation of Serine 2 of the C-terminal domain repeat of RNA Pol II (pSer2; green line; data from [68]). Notice that pSer2 is absent in the P-cells, in which H3K4me3 is maintained, and that loss of H3K4me3 occurs in the P-cell/PGC stage despite the appearance of pSer2.

Multi-omics profiling of mouse gastrulation at single-cell resolution
Argelaguet R, Clark SJ, Mohammed H, et al.
Nature. 2019 Dec;576(7787):487-491.



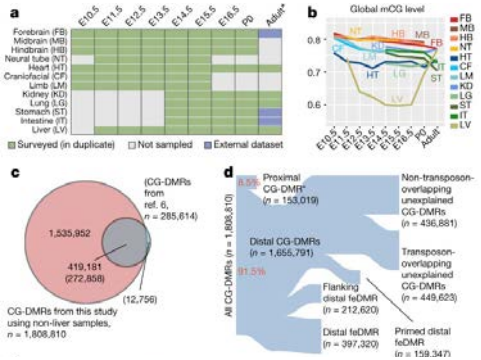
A Single-Cell Transcriptomics CRISPR-Activation Screen Identifies Epigenetic Regulators of the Zygotic Genome Activation Program
Celia Alda-Catalinas C, Bredikhin D, Hernando-Herraez I, et al.
Cell Syst. 2020 Jul 22;11(1):25-41.e9.



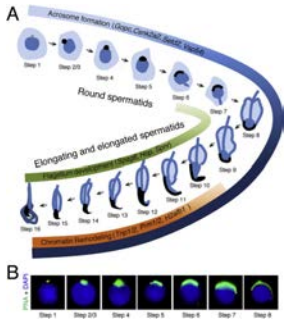
In Brief

Alda-Catalinas et al. developed a pooled CRISPR-activation screen combined with single-cell transcriptomics to identify regulators of zygotic genome activation (ZGA) using an *in vitro* proxy of early mouse embryos, embryonic stem cells. After interrogation of 230 candidate regulators, 24 hits were identified using multi-omics factor analysis (MOFA+), including the transcription factor ***Patz1***, the DNA-binding protein ***Dppa2***, and the chromatin remodeler ***Smarca5***. Follow-up functional experiments demonstrated that ***Smarca5***'s regulation of ZGA-like transcription is dependent on ***Dppa2***.

Spatiotemporal DNA methylome dynamics of the developing mouse fetus.
He Y, Hariharan M, Gorkin DU, Dickel DE, et al.
Nature. 2020 Jul;583(7818):752-759.

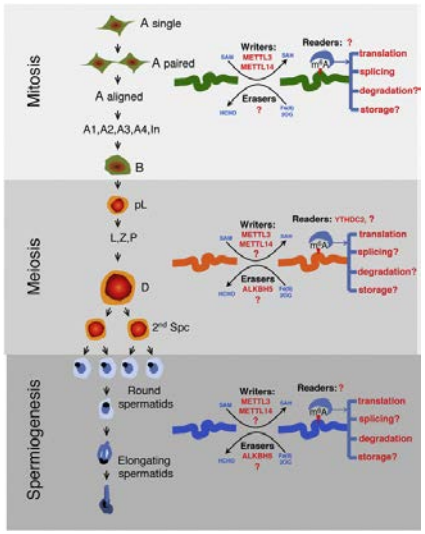


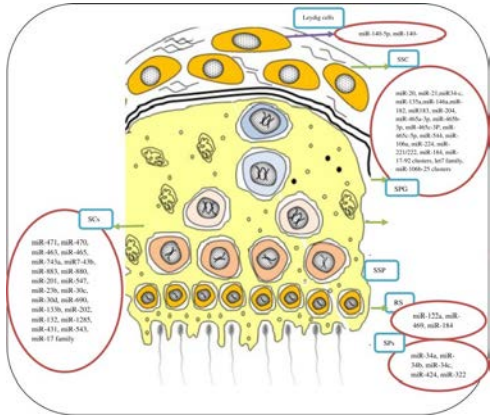
m ^{6}A mRNA modification regulates mammalian spermatogenesis
Lin Z, Tong M-H.
Biochim Biophys Acta Gene Regul Mech. 2019Mar;1862(3):403-411.



List of representative spermatogenesis-associated genes with m^{6}A peaks on their transcripts.

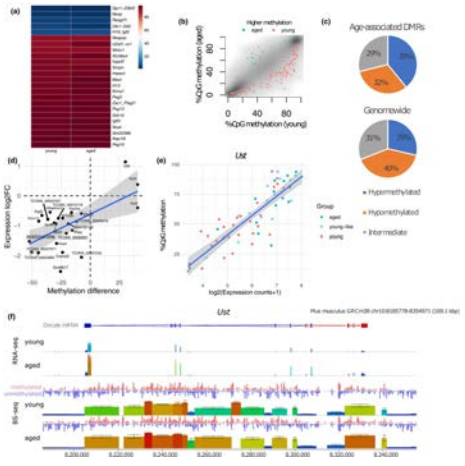
Stage-specific events	Name of genes
Spermatogonial self-renewal and proliferation	*Etv5, Bcl6b, Ubx1, Pou3f1, Id4, Gfra1, Ret, Pou5f1, Foxo1, Lin28a, Ptpf, Sox3, Tqf4b, Ssx3*
Spermatogonial differentiation	*Sra8, Kit, Sohlh2, Dmc3b, Cosd2*
Meiotic recombination	*Prdm9, Spo11, Gm960, Rec114, Mei4, Hormad1, Rpa1, Brca1, Brca2, Dmc1, Rad51, Rec8, Asm, Mlh4/5, Mlh1/3*
Acrosome biogenesis	*Gopc, Pick1, Csnk2a2, Spaca1, Sesd2, Gba2, Agfg1, Spaca1*
Chromatin remodeling	*Prm1, Csnk4, Pygo2*





The expression of relevant miRNAs in testicular cells. A large number of miRNAs are responsible for the normal function of reproductive organ and spermatogenesis. They control all levels of spermatogenesis including 1-Leydig and Sertoli cells development, 2-spermatogonial stem cells formation and their differentiation to 3-spermatogonia, 4-primary spermatocytes, 5-secondary spermatocytes, 6-round spermatids, and 7-mature sperm cells. Data from Harchegani et al.53
Abbreviations: SSCs indicates spermatogonial stem cells; SPG, spermatogonia; PSPC, primary spermatocytes; SSPC, secondary spermatocytes; RS, round spermatids; SPs, sperm cells; SCs, sertoli cells; miRNAs, microRNAs.

Increased transcriptome variation and localised DNA methylation changes in oocytes from aged mice revealed by parallel single-cell analysis
Castillo-Fernandez J, Herrera-Puerta E, Demond H, et al.
Aging Cell. 2020 Nov 17;e13278.



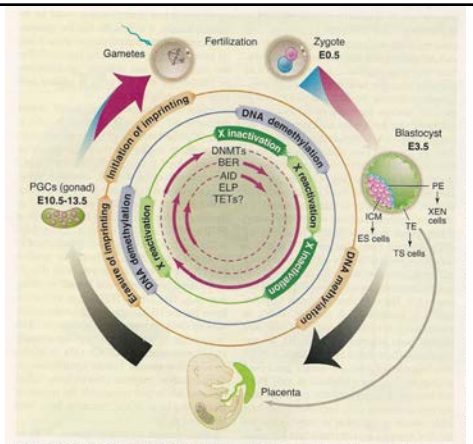
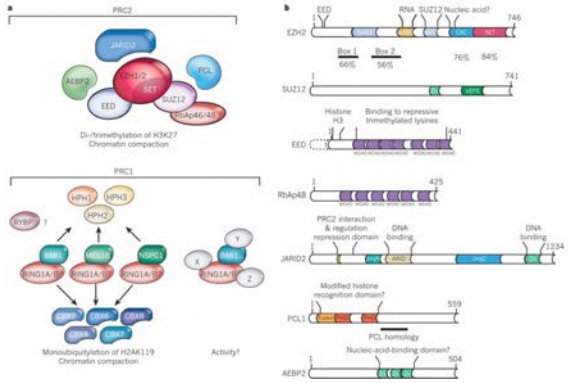
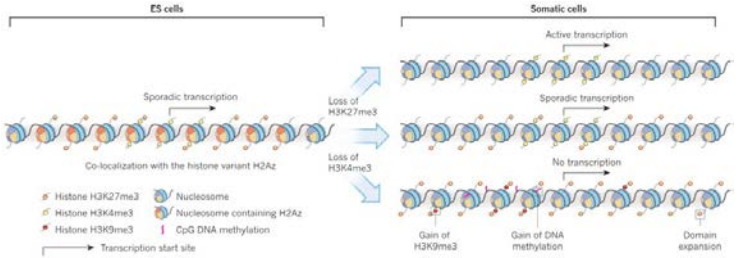


Fig. 2. The two major phases of genome-wide erasure of DNA methylation in the early embryo and in primordial germ cells (PGCs) of the mouse. Thickness of the outer arrows indicates levels of DNA methylation. Red, maternal genome; blue, paternal genome. After fertilization, the paternal genome is more rapidly demethylated than the maternal one. During gametogenesis, de novo methylation in spermatogenesis occurs earlier than in oogenesis. The inner circle shows factors or candidate factors that are implicated in de novo methylation, the maintenance of methylation, and demethylation, respectively. Solid arrows in the inner circle show at what developmental time these epigenetic regulators are thought to act. ES cells, TS cells, and XEN cells are stem cell lines that are derived from the inner cell mass (ICM), trophectoderm (TE), and primitive endoderm (PE) of the blastocyst, respectively.

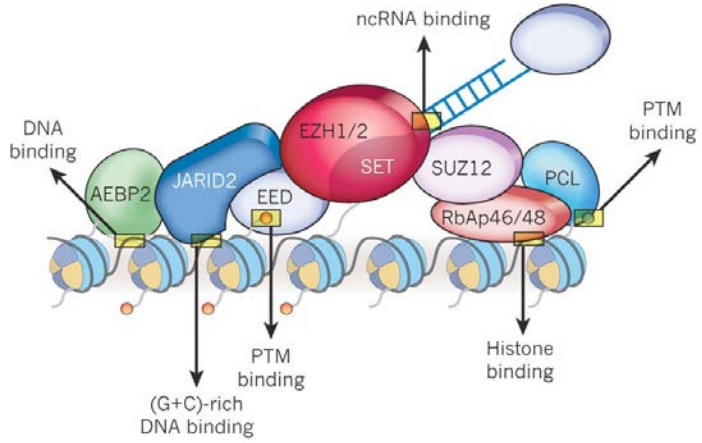
Developmental Epigenetics (Chromatin Remodeling and Development)



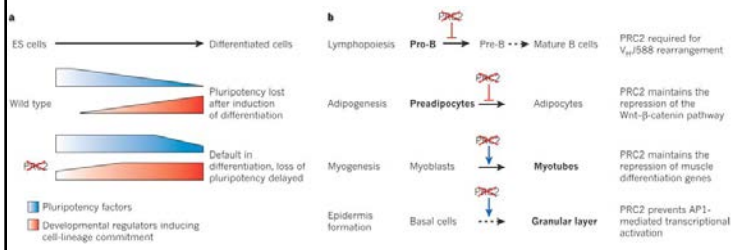
a, Diagrams representing the composition of PRC2 and PRC1 are shown. In PRC1, the diagrams shown on the left correspond to the classical PRC1 complexes, whereas those on the right correspond to the so-called PRC1-like complexes. Owing to their homology with the *Drosophila* PSC protein, we assumed that the BMI1-, MEL18- and NSPC1-containing PRC1 complexes could compact chromatin. The 'pocket' shape of the CBX proteins represents the chromodomain that specifically recognized H3K9/27me3. HPH1, 2 and 3 denote human polyhomeotic homologue 1, 2 and 3. X, Y and Z denote various proteins such as SCMH1/2, FBXL10, E2F6 and JARID1D that could contribute to the formation of PRC1-like complexes, whose exact composition is still enigmatic. **b**, Characterized domains with potential functions are indicated for each PRC2 component. In EZH2, box 1 and 2 refer to domains based on sequence homology, and the numbers below the scheme indicate the percentage similarity between mouse and Drosophila homologues for the corresponding domain. CXC, cysteine-rich domain; ncRBD, non-coding-RNA-binding domain; SANT, SWI3, ADA2, N-CoR and TFIIIB DNA-binding domain; SET, Su(var)3-9, enhancer of zeste, trithorax domain; VEFS, conserved among VRN2–EMF2–FIS2–SU(Z)12; WD40, short ~40 amino acid motifs.



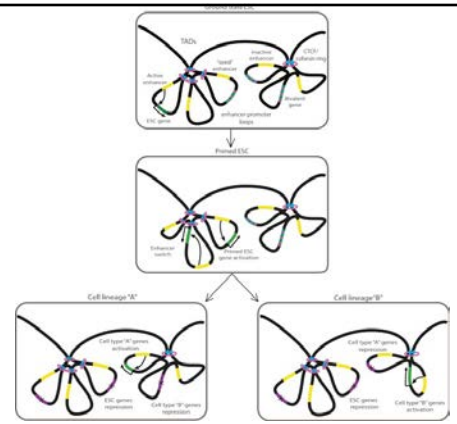
Schematic representation of chromatin at PcG target genes as a function of ES-cell differentiation. In ES cells, most PcG targets are methylated on both H3K4 and H3K27 and co-localize with the histone variant H2Az. During differentiation, H2Az is removed, and some bivalent domains are resolved. For example, genes that are actively transcribed lose H3K27me3. A substantial proportion of PcG targets that retain H3K27me3 but lose H3K4me3 are targeted by other silencing pathways such as DNA methylation or H3K9 trimethylation.



Schematic representation of the PRC2 holoenzyme at chromatin. Putative interactions with either DNA or histones that could explain PRC2 recruitment are highlighted.

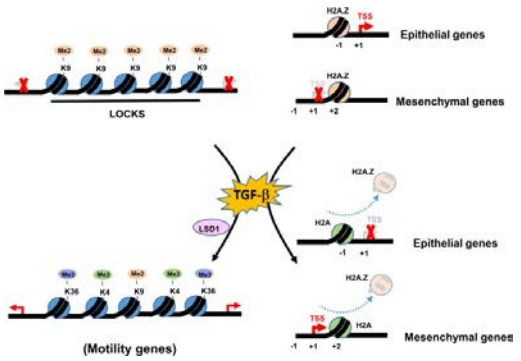


a, Comparison of expression levels of pluripotency factors and factors that induce cell commitment during ES-cell differentiation in wild-type and PRC2-impaired ES cells. **b**, The consequences of PRC2 inactivation for cell differentiation. For simplicity, intermediate differentiation steps are not shown in some cases (dashed arrows).



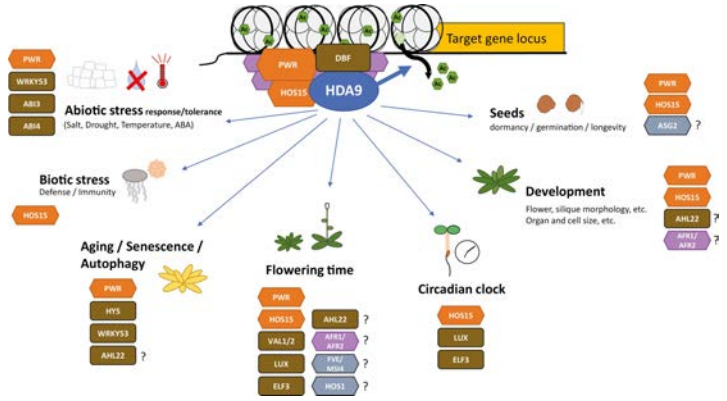
Role of Chromatin Conformation in ESC in Lineage Commitment. Enhancer usage during ESC differentiation. "Ground-state" ESCs achieve expression (green) of pluripotency genes through specific enhancer clusters or super-enhancers (yellow), while differentiation genes are repressed (purple gene with black cross) or in a bivalent state (green and purple). The blue dots and purple ring represent CTCF and Cohesin holding the chromatin loop together. In primed ESC genes, expression of pluripotency-associated genes is often achieved via a switch in enhancer usage, which also may involve local loop rearrangement. Additional primed ESC genes may also start to be expressed. Upon cell-fate determination, lineage-specific genes are activated while pluripotency-associated and alternative-fate genes lose active marks and acquire a repressive chromatin environment.

Epigenetic regulation of epithelial-mesenchymal transition: focusing on hypoxia and TGF-β signaling
Lin YT, Wu KJ. J
Biomed Sci. 2020 Mar 2;27(1):39.

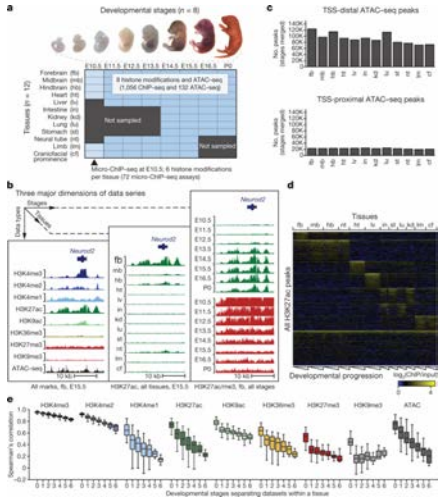


The chromatin changes induced by TGF-β during the EMT process. Overall changes in different histone marks (e.g. decrease in H3K9me2 and increase in H3K4me3/HeK36me3) and removal of a nucleosome variant (H2A.Z) in different positions are shown.

The diverse and unanticipated roles of histone deacetylase 9 in coordinating plant development and environmental acclimation
de Rooij PGH, Perrella G, Kaiserli E, van Zanten M.
J Exp Bot. 2020 Oct 22;71(20):6211-6225.



An atlas of dynamic chromatin landscapes in mouse fetal development
Gorkin DU, Barozzi I, Zhao Y, et al.
Nature. 2020 Jul;583(7818):744-751.



Developmental Epigenetics (HOX Gene Cluster)

Fig. 1. *Hox* clusters in mammals and *Drosophila*. (A) The 4 *Hox* clusters (*HoxA–D*) and their constituent *Hox* genes are shown. *Hox* clusters have arisen from genome duplication, and individual *Hox* genes within clusters have been lost during evolution. (B) The arrangement of the approximately 300 kb bithorax complex (BX-C) in *Drosophila*. The line represents genomic DNA. The boxes below the lines indicate the extent of regulatory regions that control expression of the coding genes in the numbered parasegment (PS). Regulatory regions with equivalent stippling regulate the same coding gene (PS5 and 6 control *Ubx*; PS7–9 control *abd-A*; and PS10–14 control *Abd-B*). The names of each PS-specific regulatory region are given below each box. The position and genomic structure of coding gene transcripts are shown at the bottom of the figure.

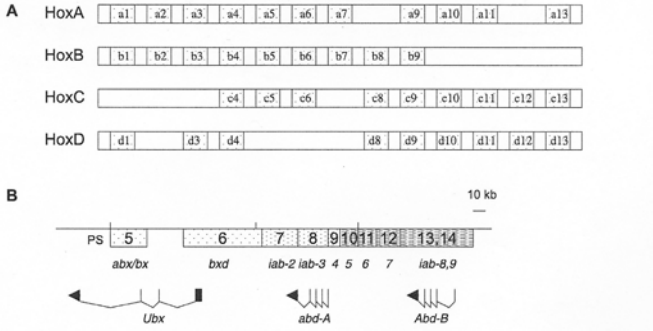


Fig. 2. Noncoding (nc)RNAs of the BX-C. (A) The central line indicates genomic DNA, with the conventional coordinates, in kb, indicated above. Below are indicated the positions of the *abd-A* and *Abd-B* transcripts, plus the *iab-4* and *iab-8* micro (mi)RNAs, with the direction of transcription indicated by the arrowheads. Above is shown expansions of the *iab-4* region, to illustrate the genomic structure of the *iab-4* miRNA and to show the locations of the hairpins that identify *iab-4-5p* and *iab-4-3p*, respectively. (B) A map of the *Ubx* and *bxd* regulatory region, with the scale, in kb, indicated above the line, indicating genomic DNA. The genomic structure of *bxd* ncRNAs and of *Ubx* is indicated below. The black box below the genomic DNA indicates the position of the maintenance element.

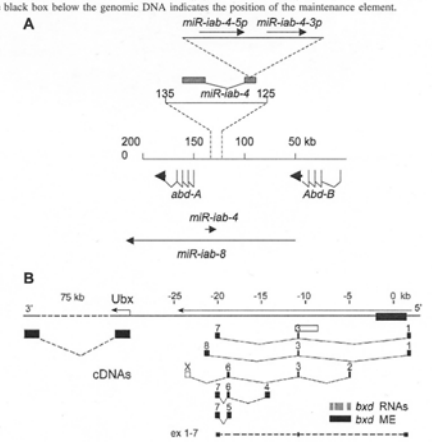
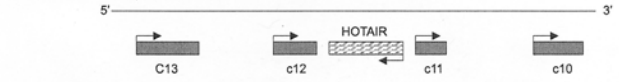
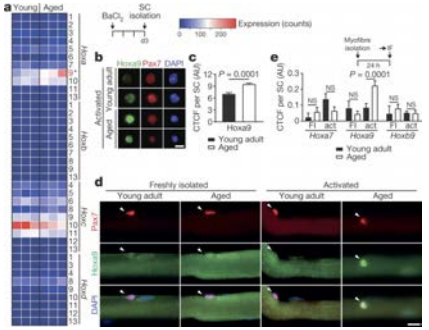


Fig. 3. A map of the 5′ end of the *HOXC* cluster, showing the relative locations of the *Hox* genes and of the HOTAIR ncRNA. Direction of transcription is indicated by the arrowheads.



Epigenetic stress responses induce muscle stem-cell ageing by Hoxa9 developmental signals.
Schwörer S, Becker F, Feller C, et al.,
Nature. 2016 Dec 15;540(7633):428-432.



a–c, Analysis of freshly isolated, in vivo activated SCs (3 days after muscle injury with BaCl2) from young adult and aged mice. a, Heatmap showing the mRNA expression of all Hox genes as determined by RNA-sequencing analysis. b, Representative immunofluorescence staining for Hoxa9 and Pax7. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). c, Corrected total cell fluorescence (CTCF) for Hoxa9 per SC as shown in b. AU, arbitrary units. d, e, Immunofluorescence (IF) staining for Hoxa9 and Pax7 in myofibre-associated SCs that were quiescent (freshly isolated (FI) myofibres) or activated (act; 24 h culture of myofibres). d, Representative images with arrowheads denoting Pax7+ cells. e, CTCF for indicated Hox genes. Note the specific induction of Hoxa9 in activated SCs isolated from aged mice. Scale bars, 5 μm (b) and 20 μm (d). P values were calculated by two-sided Mann–Whitney U-test (c) or two-way analysis of variance (ANOVA) (e). NS, not significant. n = 3 mice in a; n = 134 nuclei (young), n = 181 nuclei (aged) from 3 mice in c; n = 12/13/17/56 nuclei (Hoxa7), n = 9/42/102/62 nuclei (Hoxa9), n = 7/35/34/25 nuclei (Hoxb9) from 2 young and 4 aged mice in e.

"Epigenetics and Systems Biology"

Spring 2023 (Odd Years)
Biol 476/576
Schedule/Lecture Outline –

Week 1	January 10 & 12	Systems Biology (History/ Definitions/ Theory)
Week 2	January 17 & 19	Systems Biology (Networks & Emergence)
Week 3	January 24 & 26	Systems Biology (Components: DNA to Phenotype)
Week 4	Jan 31 & Feb 2	Systems Biology (Genomics / Technology)
Week 5	February 7 & 9	Epigenetics (History / Molecular Processes)
Week 6	February 14 & 16	Epigenetics (Molecular Processes & Integration)
Week 7	February 21 & 23	Epigenetics (Genomics and Technology)
Week 8	Feb 28 & March 2	Cell & Developmental Biology
Week 9	March 7 & 9	Epigenetics of Cell & Developmental Biology (& Midterm Exam)
Week 10	**March 13 – 17**	**Spring Break**
Week 11	March 21 & 23	Environmental Impact on Biology
Week 12	March 28 & 30	Environmental Epigenetics
Week 13	April 4 & 6	Disease Etiology
Week 14	April 11 & 13	Epigenetics & Disease Etiology
Week 15	April 18 & 20	Evolutionary Biology & Genetics
Week 16	April 25 & 27	Epigenetics & Evolutionary Biology
Week 17	May 2 & 4	Grant Review/ Study Section Meeting (& Final Exam)

Spring 2023 – Epigenetics and Systems Biology
Lecture Outline (Epigenetics)
Michael K. Skinner – Biol 476/576
Week 8 & 9 (February 28 & March 7, 2023)

Epigenetics of Cell and Developmental Biology

- Basic Cell and Developmental Biology
- X Chromosome Inactivation
- Imprinted Genes
- Developmental Epigenetics
- Epigenetics and Stem Cells
- Epigenetics and Developmental Systems

Required Reading

Michael K. Skinner (2011) Environmental Epigenetic Transgenerational Inheritance and Somatic Epigenetic Mitotic Stability. Epigenetics 1;6(7):838-42.

Al-Mousawi J, Boskovic A. Transcriptional and epigenetic control of early life cell fate decisions. Curr Opin Oncol. 2022 Mar 1;34(2):148-154.

Spring 2023 – Epigenetics and Systems Biology
Discussion Session (Epigenetics and Development)
Michael K. Skinner – Biol 476/576
Week 9 (March 9)

Epigenetics of Cell and Developmental Biology

Primary Papers

1. Schworer, et al., (2016) Nature 540:428. (PMID: 27919074)
2. Argelaguet, et al. (2019) Nature 576(7787):487-491. (PMID: 31827285)
3. Lyko F, et al., (2010) PLoS Biol. 2;8(11):e1000506. (PMID: 21072239)

Discussion

Student 22 – Ref #1 above

- What is the epigenetic aging effect observed?
- What stem cell effect was observed?
- How do epigenetics and genetics cooperate in this process?

Student 23 – Ref #2 above

- What was the experimental design to investigate gastrulation?
- What technology was used to examine epigenetics?
- What observations regarding gastrulation DNA methylation and transcriptome were made?

Student 24 – Ref #3 above

- What are the cast systems in the bee?
- How does epigenetics influence the development of the bee?
- What is the environmental factor that alters the epigenetic programming?

Epigenetics and Stem Cells

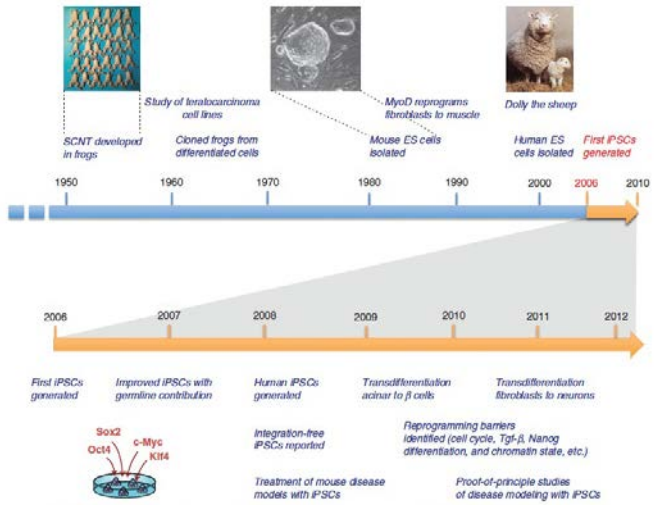


Figure 1. Historic time line of reprogramming research. Shown are seminal discoveries leading to the first generation of iPSCs in 2006, as well as progress in the generation and subsequent application of iPSCs.

Table 1. Definition of some terms

Potency	Sum of developmental options accessible to the cell
Totipotent	Ability to form all lineages of the organism; in mammals, only the zygote and first cleavage blastomeres are totipotent.
Pluripotent	Ability to form all lineages of the body (e.g., embryonic stem cells).
Multipotent	Ability of adult stem cells to form multiple cell types of one lineage (e.g., hematopoietic stem cells).
Unipotent	Cells form one cell type (e.g., spermatogonial stem cells, which can only generate sperm).
Reprogramming	Increase in potency and dedifferentiation; can be induced by nuclear transfer, cell fusion, genetic manipulation.
Transdifferentiation, plasticity	Notion that somatic stem cells have broadened potency and can generate cells of other lineages, a concept that is controversial in mammals. More recently, transdifferentiation also refers to transcription factor–induced lineage conversions among differentiated cell types.

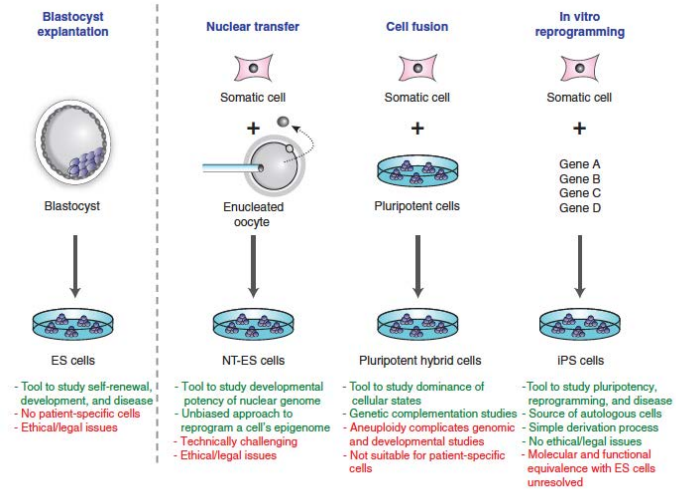


Figure 2. Sources of pluripotent stem cells. Comparison of different strategies used to derive pluripotent stem cell lines; their advantages (in green) or disadvantages (in red) are summarized at the *bottom* of each column. ES cells, embryonic stem cells; NT-ES cells, nuclear transfer-ES cells.

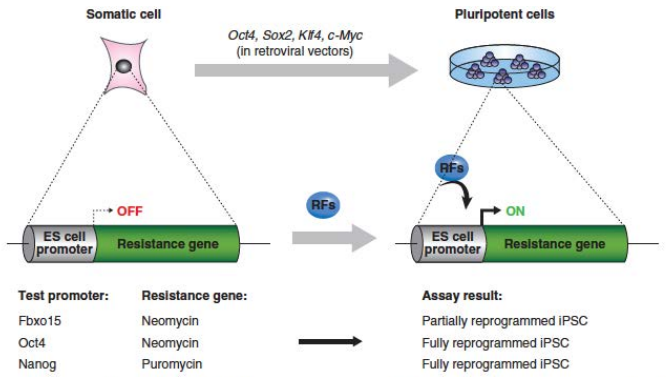


Figure 3. Strategy to derive iPSCs. (*Top*) Schematic representation of the first successful attempt to produce iPSCs by Takahashi and Yamanaka. (*Bottom*) The genetic assay system used to screen for factors that could reprogram to pluripotency (reprogramming factors [RFs]). Partial reprogramming to iPSCs was achieved by viral infection of cells with Oct4, Sox2, Klf4, and c-Myc, followed by drug selection for *Fbxo15*-expressing cells. In contrast, subsequent modifications to the assay selecting for *Oct4*- or *Nanog*-expressing cells gave rise to fully reprogrammed iPSCs. Note that drug selection is not essential for producing high-quality iPSCs, but was used as part of the assay to identify factors that induced embryonic gene expression (see text).

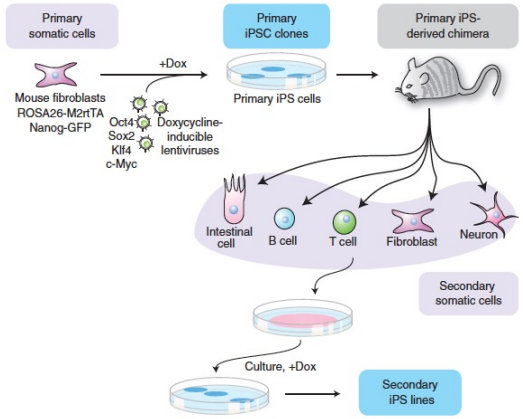
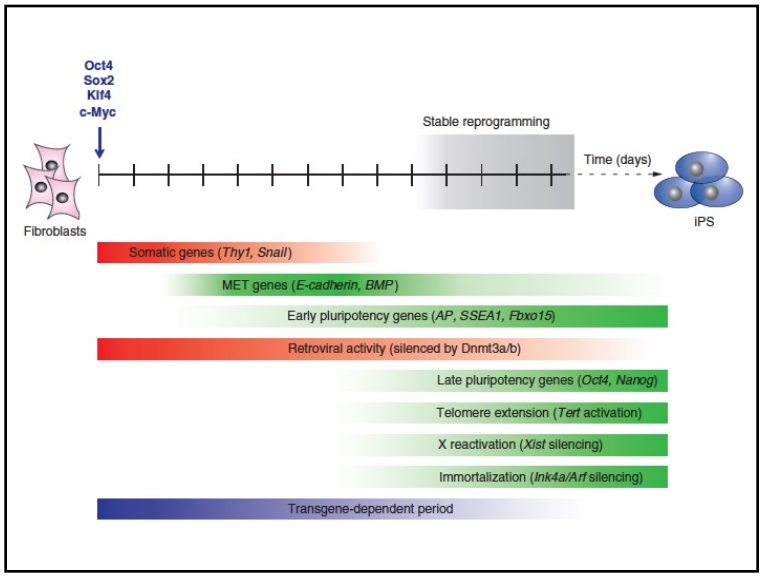
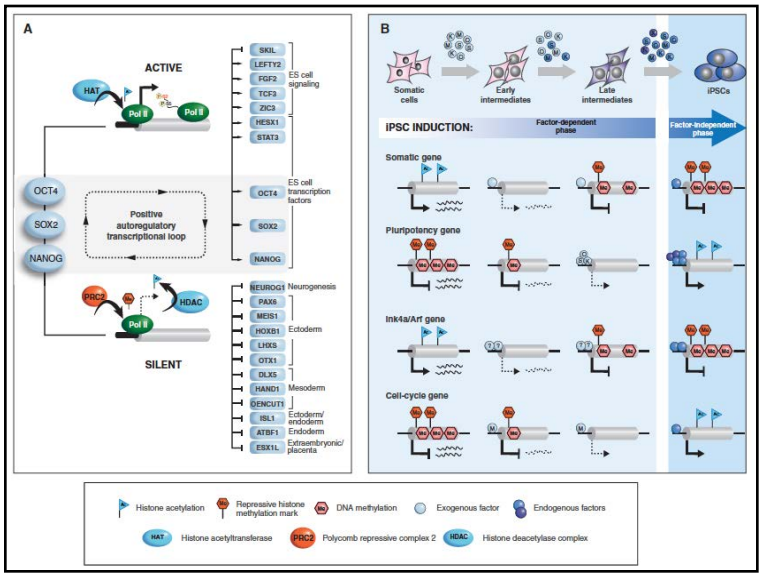
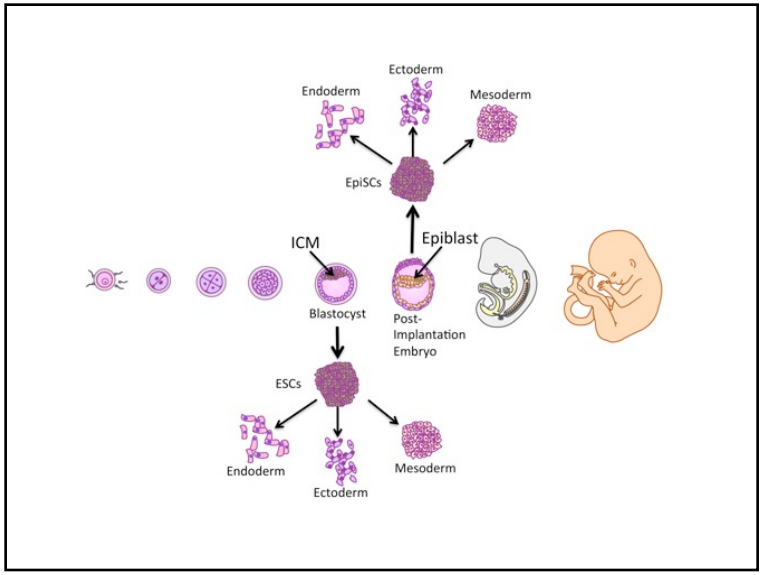
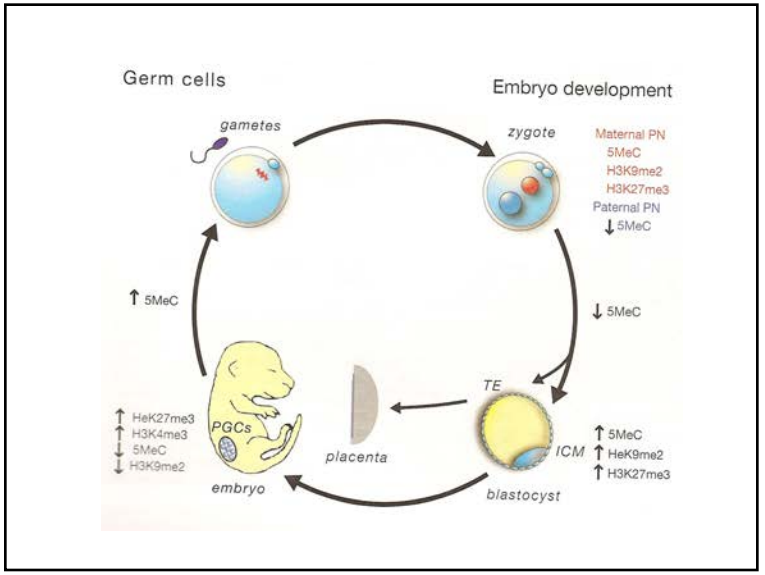


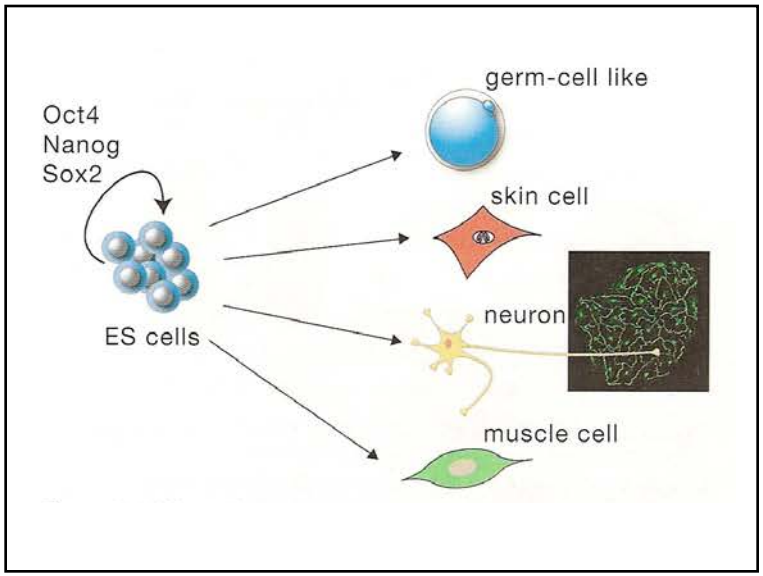
Figure 4. Generation of genetically homogeneous cell cultures for epigenetic reprogramming. Scheme for obtaining genetically homogeneous somatic cells that are more efficient at pluripotency induction. Primary somatic cells with stable integrations of a Nanog-GFP (green fluorescent protein) marker and reverse tetracycline transactivator (M2rtTA) are infected with DOX-inducible lentiviruses encoding the four reprogramming factors. Primary iPSCs are generated by culturing the cells in DOX to activate the factors. After DOX withdrawal, the primary iPSCs are injected into mouse blastocysts and "secondary" somatic cells carrying the DOX-inducible vectors are cultured in the presence of DOX to produce secondary iPSCs. The key advantage of this system is that reprogramming can be induced without new virus infection at a much higher efficiency. (Modified, with permission, from Hanna et al. 2008a.)


Oct4
Sox2
Klf4
c-Myc
Stable reprogramming
Time (days)
Fibroblasts
iPS
Somatic genes (Thy1, Snail)
MET genes (E-cadherin, BMP)
Early pluripotency genes (AP, SSEA1, Fbxo15)
Retroviral activity (silenced by Dnmt3a/b)
Late pluripotency genes (Oct4, Nanog)
Telomere extension (Tert activation)
X reactivation (Xist silencing)
Immortalization (Ink4a/Arf silencing)
Transgene-dependent period

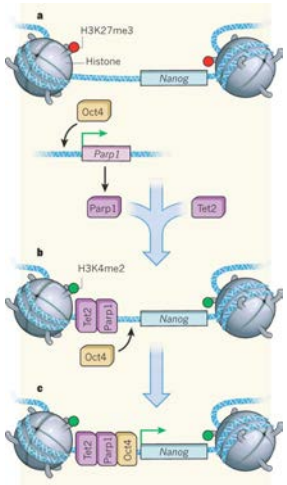

A
ACTIVE
HAT
Pol II
SKIL
LEFTY2
FGF2
TCF3
ZIC3
HESX1
STAT3
ES cell signaling
OCT4
SOX2
NANOG
Positive autoregulatory transcriptional loop
ES cell transcription factors
PRC2
HDAC
SILENT
NEUROG1
PAX6
MEIS1
HOXB1
LHX5
OTX1
DLX5
HAND1
CENCUT1
ISL1
ATBF1
ESX1L
Neurogenesis
Ectoderm
Mesoderm
Ectoderm/ endoderm
Endoderm
Extraembryonic/ placenta
B
Somatic cells
Early intermediates
Late intermediates
iPSCs
IPSC INDUCTION:
Factor-dependent phase
Factor-independent phase
Somatic gene
Pluripotency gene
Ink4a/Arf gene
Cell-cycle gene
Histone acetylation
Repressive histone methylation mark
DNA methylation
Exogenous factor
Endogenous factors
Histone acetyltransferase
Polycomb repressive complex 2
Histone deacetylase complex


Ectoderm
Endoderm
Mesoderm
EpiSCs
ICM
Epiblast
Blastocyst
Post-Implantation Embryo
ESCs
Endoderm
Ectoderm
Mesoderm

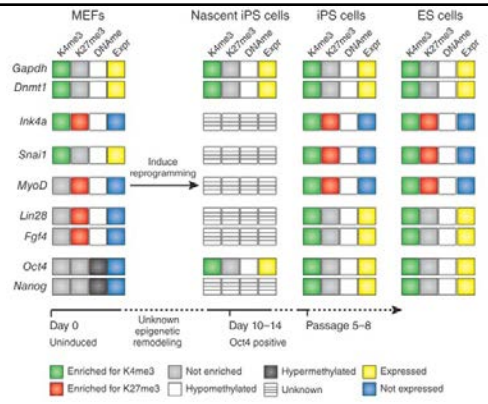

Germ cells
Embryo development
gametes
zygote
Maternal PN
5MeC
H3K9me2
H3K27me3
Paternal PN
↓5MeC
↑5MeC
↓5MeC
TE
ICM
↑5MeC
↑HeK9me2
↑H3K27me3
blastocyst
placenta
PGCs
↑HeK27me3
↑H3K4me3
↓5MeC
↓H3K9me2
embryo



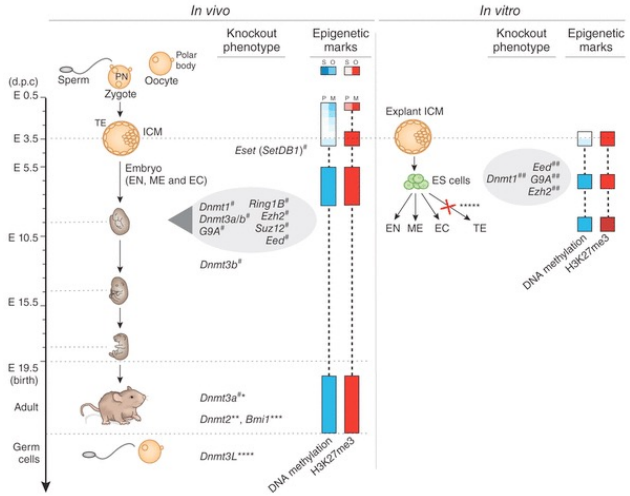
Epigenetics: Actors in the cell reprogramming drama
Kyle M. Loh & Bing Lim (2012)
Nature 488, 599–600

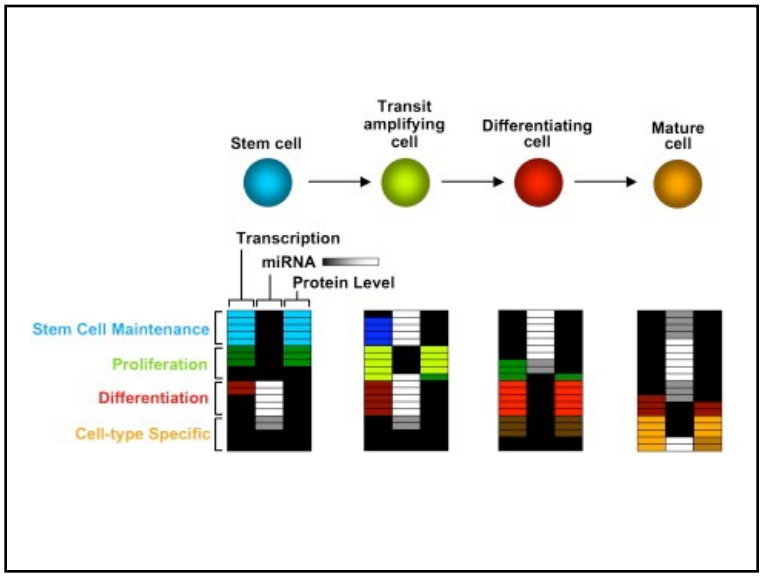


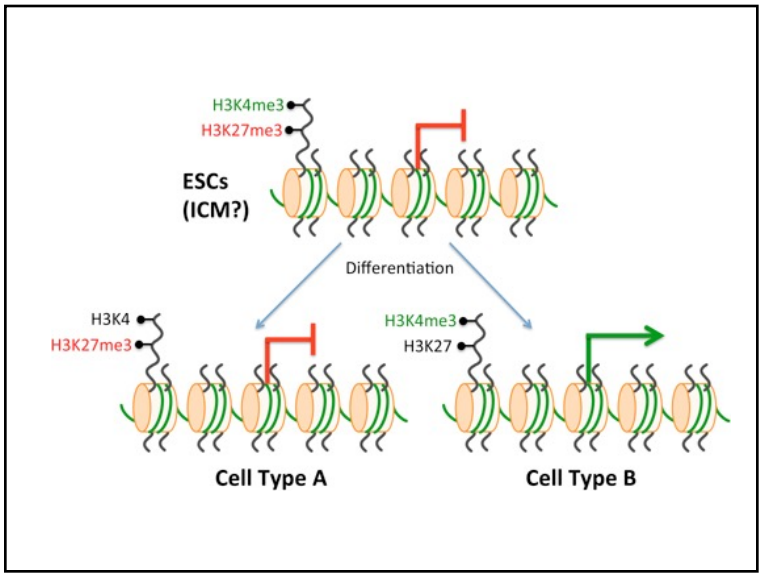
The transformation of skin cells into stem cells is a fascinating but poorly understood process. At last, the molecular characters underlying the initial steps have been revealed.

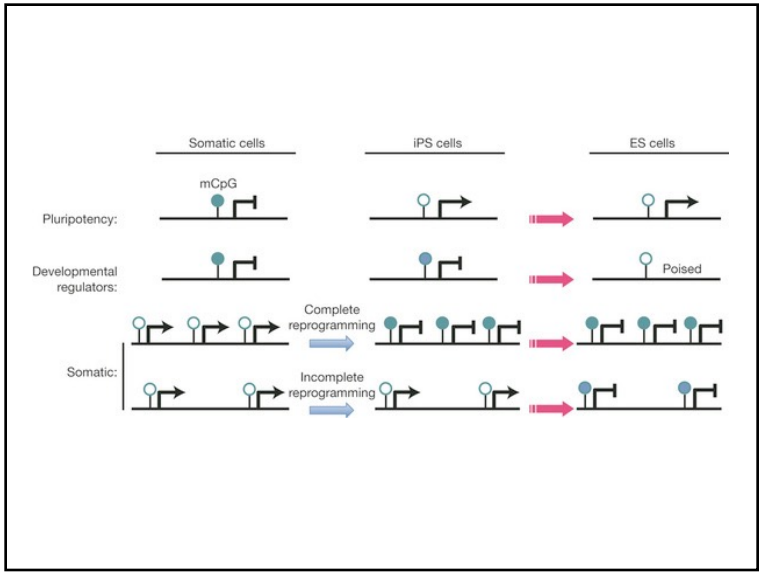


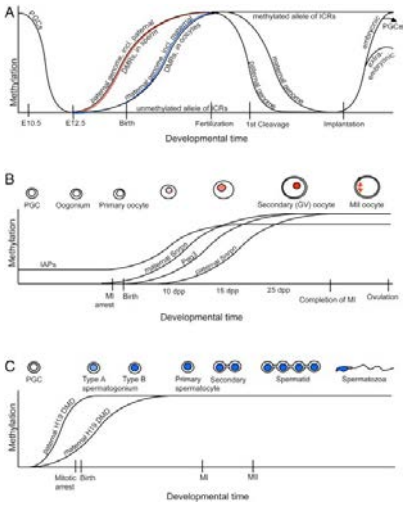
Shown are selected genes with their chromatin and expression state across distinct cell types color-coded as shown on the bottom (data taken from ref. 81). Data are for uninduced mouse embryonic fibroblasts (MEFs), a hypothetical primary iPS cell colony (nascently reprogrammed) as well as an established iPS (MCV8.1) and ES (V6.5) cell line. Upon induction of Oct4, Sox2, Klf4 and c-Myc (OSKM), the MEF epigenome begins remodeling. The initial events and required factors have not been described yet. After ~10–14 days, iPS cell colonies appear that express markers such as Oct4-GFP. The expression of housekeeping genes is not affected, and they remain active throughout the reprogramming process (Gapdh and Dnmt1 are representative examples). With the exception of a few marker genes, the global extent of remodeling is unknown for this stage. Primary colonies are then picked and expanded as clonal iPS cell lines. Usually several more passages are needed before extensive marker stains can be performed. Typically, at least 5 -8 passages are required to obtain sufficient material for genome-wide studies. The chromatin and expression states for the selected marker genes are identical in the iPS cell line MCV8.1 and in a wild-type ES cell line (V6.5) used to construct this schematic81. Ink4a (Cdkn2a) remains bivalent and sensitive to rapid induction in normal (not transformed or immortalized) cells. Overexpression of OSKM or extended cell culture can induce expression of Ink4a, but ES and iPS cells show bivalent marks and lack of DNA methylation. Snai1 is an expressed somatic gene that becomes repressed and regains bivalency upon reprogramming. MyoD is silent in both MEF and iPS cells, but switches from H3K27 only to a bivalent state upon reprogramming. Lin28 and Fgf4 are repressed by H3K27 methylation, whereas Oct4 and Nanog are repressed by DNA methylation, and all become transcriptionally reactivated only upon reprogramming.





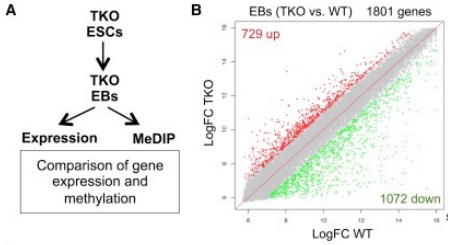


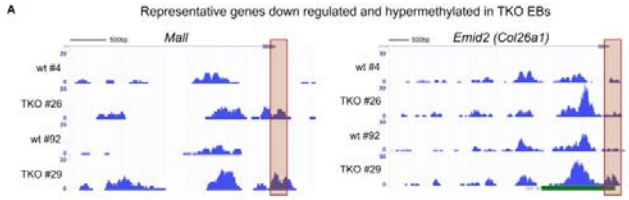


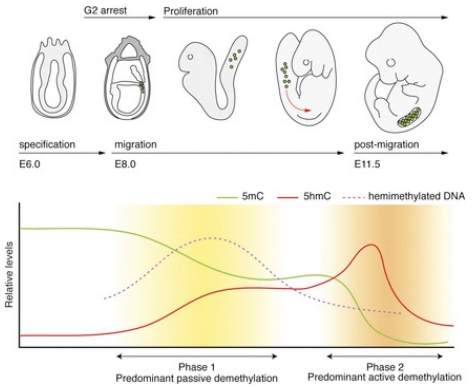


Methylation dynamics in the germline and the embryo. (A) Methylation of imprinted genes versus the genome as a whole during gametogenesis and after fertilization. (B) Imprint establishment in the female germline. (C) Imprint establishment in the male germline.

Loss of Tet enzymes compromises proper differentiation of embryonic stem cells.
Dawlaty MM, et al.
Dev Cell. 2014 Apr 14;29(1):102-11.

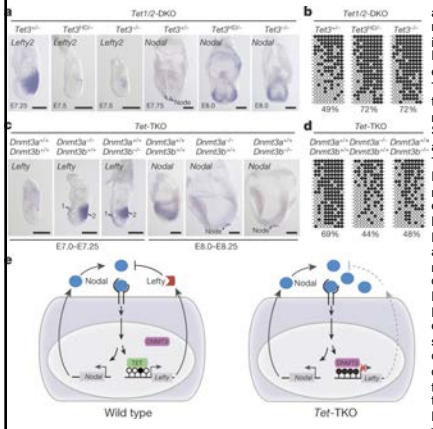






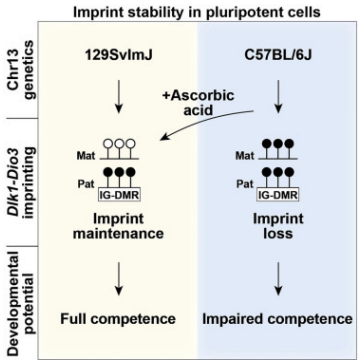
Biphasic demethylation dynamics in mouse PGCs. PGCs are derived from the embryonic ectoderm of the E6.5 embryo and display high (somatic) 5mC levels (green lines) and low 5hmC levels (red lines). Upon migration, PGCs proliferate, and 5mC levels are passively diluted. Coincidently, hemimethylated DNA strands accumulate transiently and are subsequently lost (purple dashed line). Post-migratory PGCs enter a phase of active DNA demethylation, resulting in an almost complete loss of 5mC and a transient enrichment of 5hmC. At E13.5, both 5mC and 5hmC levels are low.

TET-mediated DNA demethylation controls gastrulation by regulating Lefty-Nodal signalling.
Dai HQ, Wang BA, Yang L, et al.
Nature. 2016 Oct 27;538(7626):528-532.



a, TET catalytic activity is required for Lefty2 and Nodal regulation. Tet1/2-DKO embryos carrying a catalytically inactive allele of Tet3 (mutated H and D residues; denoted HD/−) show diminished Lefty2 and unrestricted Nodal expression compared to the embryos retaining a wild-type Tet3 allele (+/−). Images for Lefty2 are lateral views and the others are anterior views. b, TET catalytic activity is required for hypomethylation at the Lefty2 enhancer. Shown are the methylation profiles for Lefty2-expressing areas dissected from the posterior domains of E7.25 Tet1/2-DKO embryos expressing a Tet3 wild-type or an HD mutant allele at approximately E7.5. c, Deletion of methyltransferase genes in Tet-TKO embryos restores the expression patterns of Lefty and Nodal. 1, 2 indicate Lefty1 and Lefty2 expression. Lateral views are shown. d, Dependence of Lefty2 enhancer methylation on Dnmt3a and Dnmt3b. Bisulfite sequencing shows much decreased methylation in dissected Lefty2-expressing compartments of E7.0–E7.25 TKO embryos deficient in Dnmt3a or Dnmt3b. e, A model illustrating the roles of TET and DNMT3 enzymes in the Lefty–Nodal feedback loops in embryonic development. In wild-type embryos, Nodal signalling is balanced by a positive feedback loop and the connected Lefty-based negative feedback loop. In Tet-deficient embryos, the Lefty1 and Lefty2 genes are subject to hypermethylation (filled circles) that presumably impairs their response to the transcriptional activation driven by Nodal signalling, resulting in reduction in Lefty signalling, which in turn causes Nodal overexpression. Scale bars, 200 μm.

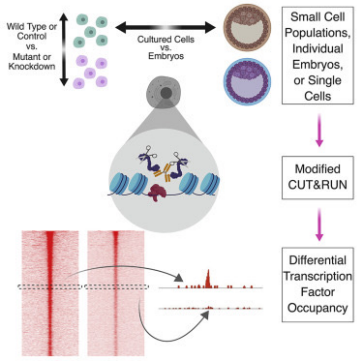
A Susceptibility Locus on Chromosome 13 Profoundly Impacts the Stability of Genomic Imprinting in Mouse Pluripotent Stem Cells
Swanzey E, McNamara TF, Apostolou E, Tahiliani M, Stadtfeld M.
Cell Rep. 2020 Mar 17;30(11):3597-3604.e3.



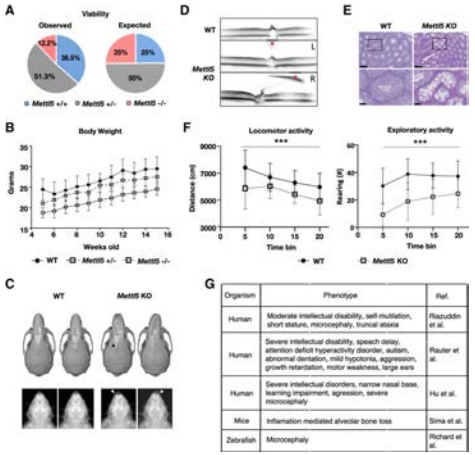
Highlights

- Imprint instability is a cell-line-intrinsic property of mouse pluripotent stem cells
- Susceptibility to imprint instability greatly varies among inbred mouse strains
- A strong genetic determinant of imprint instability maps to chromosome 13

Profiling of Pluripotency Factors in Single Cells and Early Embryos
Hainer SJ, Bošković A, McCannell KN, et al.
Cell. 2019 May 16;177(5):1319-1329.e11.

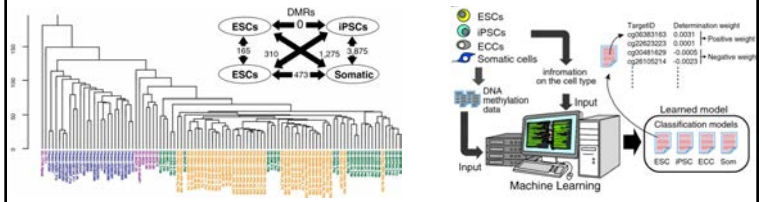


The rRNA m6A methyltransferase METTL5 is involved in pluripotency and developmental programs
Ignatova VV, Stolz P, Kaiser S, et al.
Genes Dev. 2020 May 1;34(9-10):715-729.

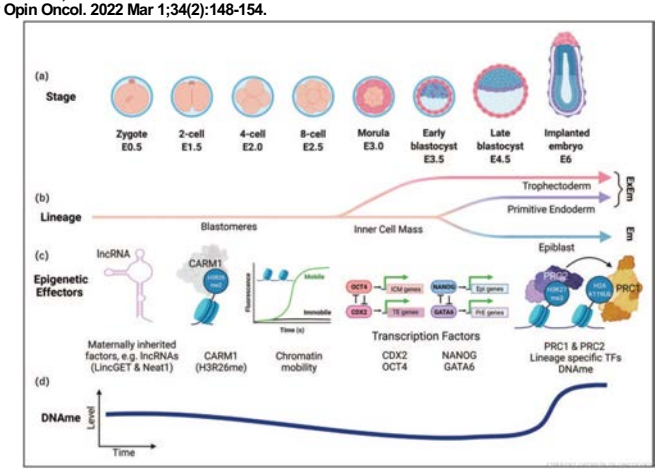


Mettl5 KO mice are subviable and display multiple phenotypic aberrations, including behavioral defects.

Identification of an epigenetic signature in human induced pluripotent stem cells using a linear machine learning model.
Nishino K, Takasawa K, Okamura K, Arai Y, Sekiya A, Akutsu H, Umezawa A.
Hum Cell. 2020 Oct 12. doi: 10.1007/s13577-020-00446-3.

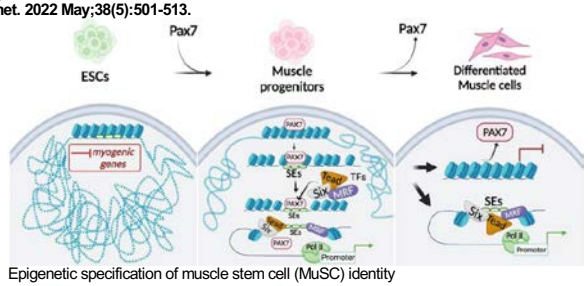


Transcriptional and epigenetic control of early life cell fate decisions.
Al-Mousawi J, Boskovic A.
Curr Opin Oncol. 2022 Mar 1;34(2):148-154.

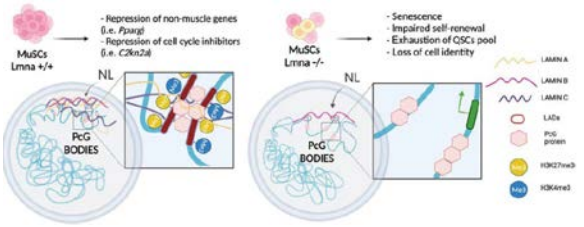


Epigenetic and transcription factors regulating cell fate allocation during mouse preimplantation development

Epigenetic control of muscle stem cells: time for a new dimension
Bianconi V, Mozzetta C.
Trends Genet. 2022 May;38(5):501-513.



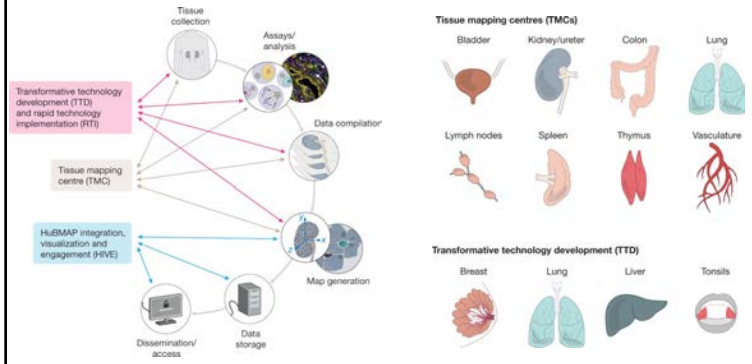
Epigenetic specification of muscle stem cell (MuSC) identity

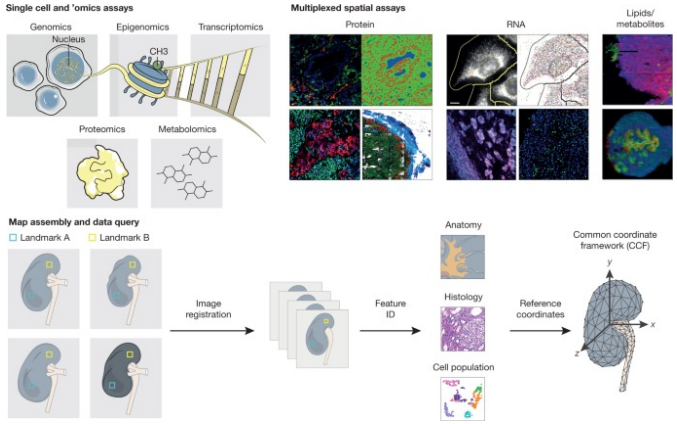


Polycomb group (PcG)-lamin A/C functional interactions maintain muscle stem cell (MuSC) identity

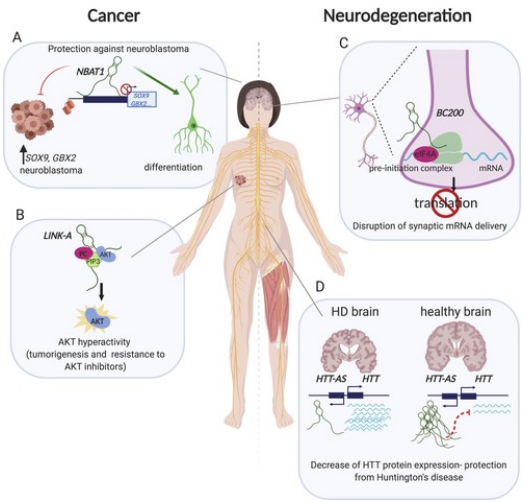
Epigenetics and Developmental Systems

The human body at cellular resolution: the NIH Human Biomolecular Atlas Program
HuBMAP Consortium
Nature. 2019 Oct;574(7777):187-192.

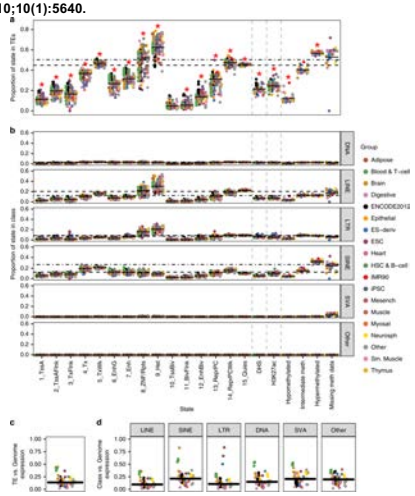




Map generation and assembly across cellular and spatial scales.

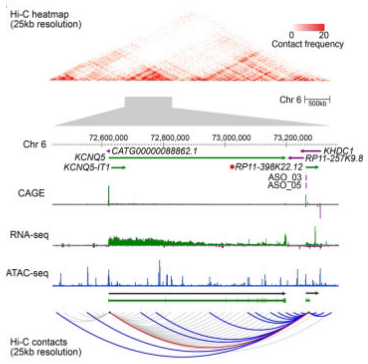


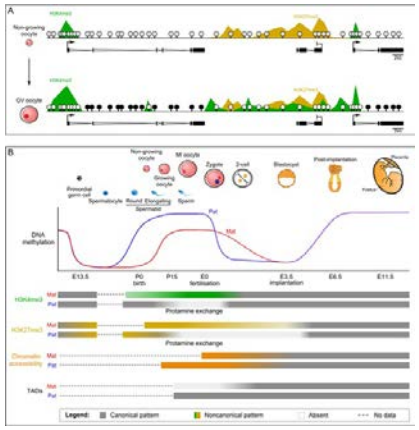
The epigenomic landscape of transposable elements across normal human development and anatomy
Pehrsson EC, Choudhary MNK, Sundaram V, Wang T.
Nat Commun. 2019 Dec 10;10(1):5640.



Variation in TE contribution to epigenetic states by epigenome classification.

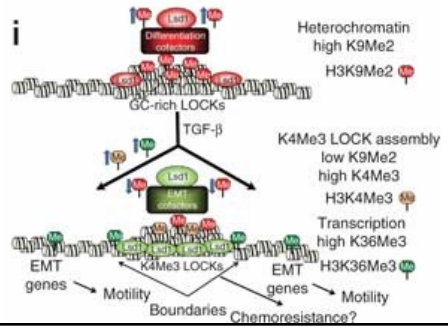
Functional annotation of human long noncoding RNAs via molecular phenotyping
Ramilowski JA, Yip CW, Agrawal S, et al.
Genome Res. 2020 Jul;30(7):1060-1072.





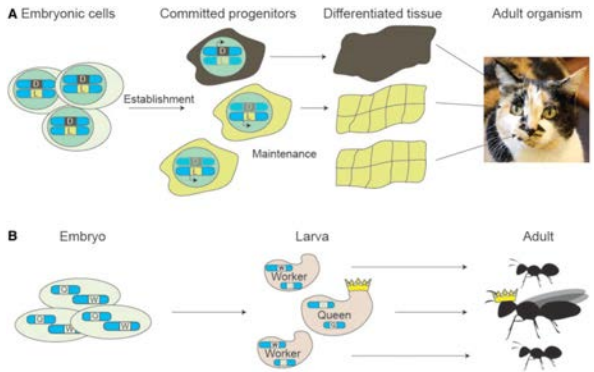
Genome-scale epigenetic reprogramming during epithelial-to-mesenchymal transition.
McDonald OG, Wu H, Timp W, Doi A, Feinberg AP.
Nat Struct Mol Biol. 2011 Jul 3;18(8):867-74.

Abstract
Epithelial-to-mesenchymal transition (EMT) is an extreme example of cell plasticity that is important for normal development, injury repair and malignant progression. Widespread epigenetic reprogramming occurs during stem cell differentiation and malignant transformation, but EMT-related epigenetic reprogramming is poorly understood. Here we investigated epigenetic modifications during EMT mediated by transforming growth factor beta. Although DNA methylation was unchanged during EMT, we found a global reduction in the heterochromatin mark H3 Lys9 dimethylation (H3K9Me2), an increase in the euchromatin mark H3 Lys4 trimethylation (H3K4Me3) and an increase in the transcriptional mark H3 Lys36 trimethylation (H3K36Me3). These changes depended largely on lysine-specific demethylase-1 (Lsd1), and loss of Lsd1 function had marked effects on EMT-driven cell migration and chemoresistance. Genome-scale mapping showed that chromatin changes were mainly specific to large organized heterochromatin K9 modifications (LOCKs), which suggests that EMT is characterized by reprogramming of specific chromatin domains across the genome.

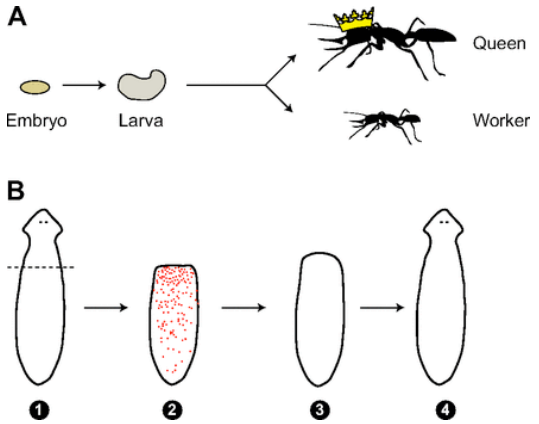


Various Organisms

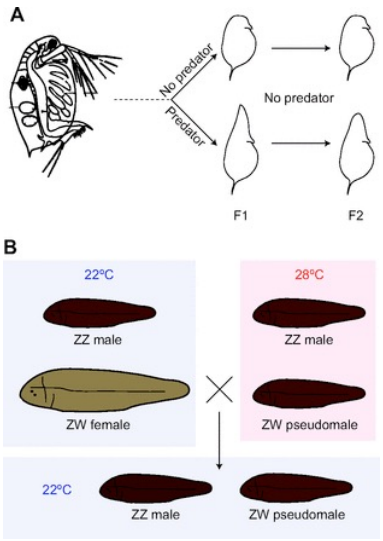
The role of chromatin and epigenetics in the polyphenisms of ant castes.
Bonasio R.
Brief Funct Genomics. 2014 May;13(3):235-45.



Epigenetics and polyphenism in ants. (A) Cellular epigenetics in mammals. The embryo of a calico cat contains genomic information encoding both dark pigmentation ('D') and light pigmentation ('L'). Through the process of random X inactivation, only one of the two phenotypes remains active and its state is epigenetically transmitted to the developing tissues, giving rise to clonal patches of dark or light skin in the adult individual. Photo courtesy photos-public-domain.com. (B) The ant genome encodes both queen ('Q') and worker ('W') traits (left), here for simplicity, depicted as single gene loci, although in all likelihood, hundreds of genes are involved. During development one or the other phenotype is activated (middle), possibly in response to environmental cues, and this results in the observable polyphenisms in the adult individuals that belong to different castes (right).



Extreme epigenetics. (A) Eusocial insects, such as ants, encode different developmental destinies in the same genome. In most species embryos (left) and larvae (middle) are genetically indistinguishable, but they give rise to entirely different adults (right); specifically, reproductives (queens) and non-reproductives (workers). These differ not only in size and morphology, but also in physiology and behavior. (B) Adult planarians can regenerate all body tissues and structures after amputation (1). Pluripotent adult stem cells known as neoblasts (red dots) migrate to the wound site (2), create a regenerating structure called blastema (3), which eventually restores all organs of the adult animal, including the nervous system (4).

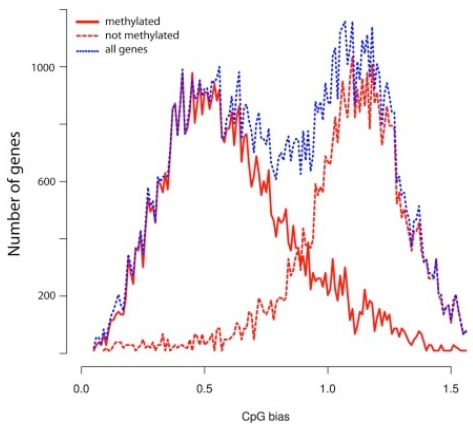


Examples of epigenetic plasticity in development. (A) In *Daphnia*, alternative phenotypes can emerge as a consequence of exposure to predator-derived chemicals (kairomones). In this case, genetically identical F1 (products of parthenogenesis) present differences in the form of a defensive structure called a 'helmet'. The presence of the helmet can persist in later generations despite the absence of the originating stimulus. (B) In the half-smooth tongue sole *Cynoglossus semilaevis*, sex is genetically determined by the presence of two Z chromosomes (male) or one Z and one W chromosome (female). However, higher temperatures during the juvenile phase induce conversion of ZW females to 'pseudomales'. Interestingly, the ZW progeny of these pseudomales will develop into more pseudomales even if the temperature remains low, suggesting an epigenetic transmission of the sex conversion.

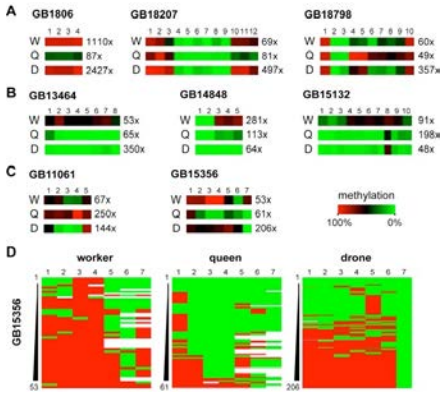
The honey bee epigenomes: differential methylation of brain DNA in queens and workers.

Lyko F, Foret S, Kucharski R, Wolf S, Falckenhayn C, Maleszka R.

PLoS Biol. 2010 Nov 2;8(11):e1000506.



CpG (o/e) bias of protein-coding regions in the honey bee genome. Since the profiles for both queens and workers are virtually identical, only the queen profile is shown.

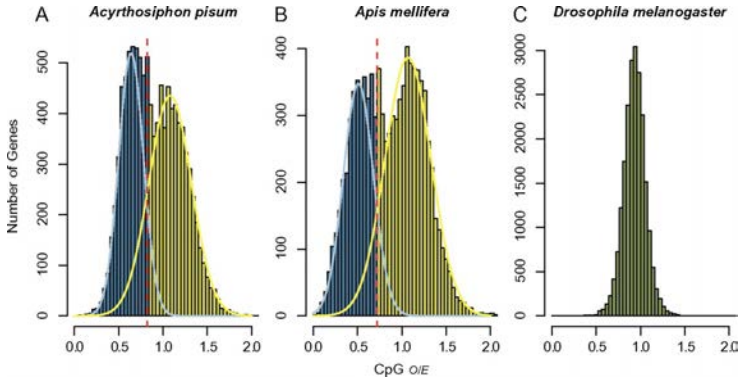


Detailed analysis of deep sequencing of selected genes.
The bisulfite converted amplicons of selected genes were sequenced using 454 technology. The selection was based on differential methylation in brains of queens and workers, but DNA from male brains (drones) was also used in this experiment. The panels illustrate the uniqueness of brain methylation patterns in bees. 3A: Genes showing similar methylation patterns in workers and drones, but a distinct methylation pattern in queens. 3B: Genes with similar methylation patterns in queens and drones, but a distinct pattern in workers. 3C: Gene with distinct methylation patterns in all three castes. Panel 3D shows the full methylation heatmaps of GB15356. This result is discussed in the chapter "Detailed Analysis of Methylation Patterns in Selected Amplicons by Deep Bisulfite Sequencing." Gene annotations: GB18798 - ubiquitin conjugation factor; GB13464 - RhoGAP93B. For other genes, see Figure 2.

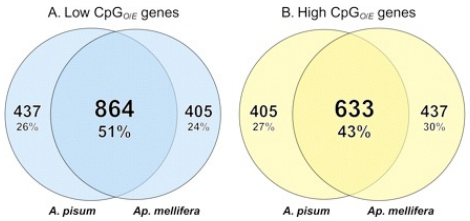
Functional conservation of DNA methylation in the pea aphid and the honeybee.

Hunt BG, Brisson JA, Yi SV, Goodisman MA.

Genome Biol Evol. 2010;2:719-28. Epub 2010 Sep 20.



Distributions of normalized CpG dinucleotide content ($CpG_{O/E}$). (A) Acyrthosiphon pisum and (B) *Apis mellifera* exhibit bimodal distributions of $CpG_{O/E}$ among genes, signifying variation in germline DNA methylation levels. Dashed red lines indicate cutoffs used to divide low $CpG_{O/E}$ genes (blue) from high $CpG_{O/E}$ genes (yellow). In contrast to A. *pisum* and Ap. *mellifera*, (C) Drosophila melanogaster has a unimodal distribution of $CpG_{O/E}$ and does not exhibit substantial levels of CpG methylation.

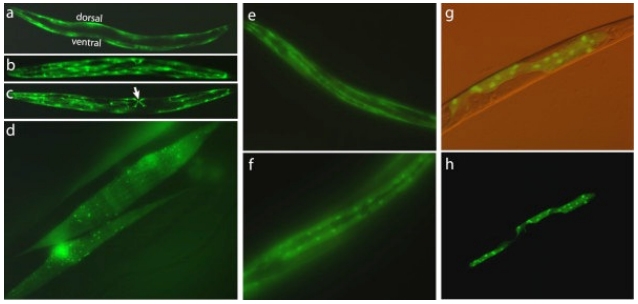


Pan-genomic high $CpG_{O/E}$ status is less conserved than low $CpG_{O/E}$ status. Analysis of orthologs in *Acyrthosiphon pisum* and *Apis mellifera* show that a higher proportion of (A) low $CpG_{O/E}$ genes are conserved with respect to normalized CpG content than (B) high $CpG_{O/E}$ genes. Each circle represents the number of genes from one species belonging to the designated $CpG_{O/E}$ class; overlap designates the number of orthologs with agreement in $CpG_{O/E}$ classification in both species.

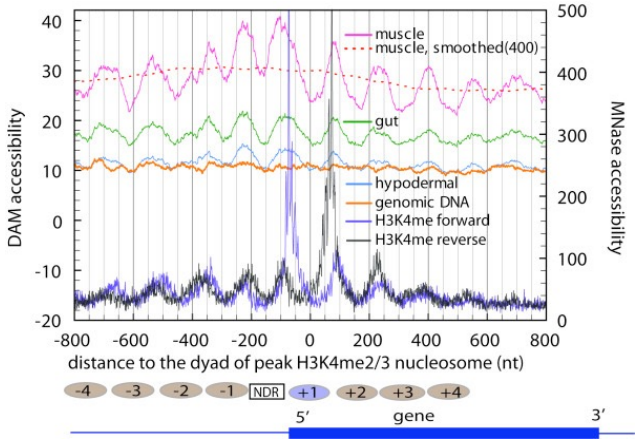
Distributed probing of chromatin structure in vivo reveals pervasive chromatin accessibility for expressed and non-expressed genes during tissue differentiation in C. elegans.

Sha K, Gu SG, Pantalena-Filho LC, Goh A, Fleenor J, Blanchard D, Krishna C, Fire A.

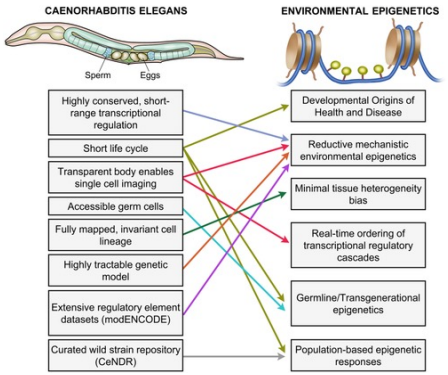
BMC Genomics. 2010 Aug 6;11:465.



Promoter specific expression of DAM methyltransferase in transgenic animals. (a-d) PD5122 animals expressing DAM-GFP fusion driven by the myo-3 (body wall muscle) promoter. (a = L4,10X; b = adult,10X; c = adult,10X; d = adult,100X). (e-f) PD3995 animals expressing a DAM-GFP fusion construct driven by the rol-6 (hypodermal) promoter. (e = 200X; f = 400X). (g-h) PD3997 animals expressing a DAM-GFP construct driven by the vit-2 (gut) promoter. (g = 200X; h = 200X)



Caenorhabditis elegans as an emerging model system in environmental epigenetics.
Environ Mol Mutagen. 2018 Aug;59(7):560-575.
Weinhouse C, Truong L, Meyer JN, Allard P.



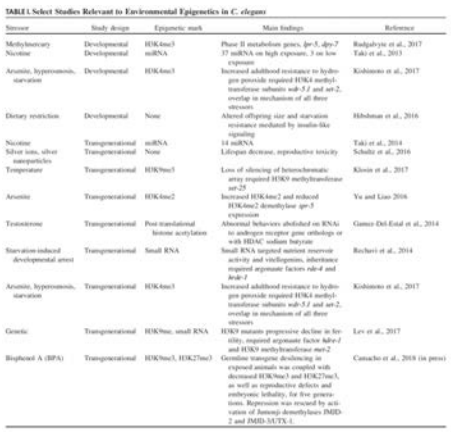
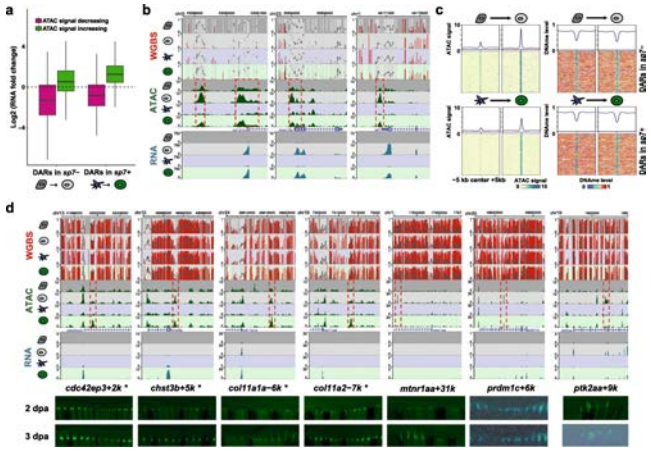


TABLE 1. Select Studies Relevant to Environmental Epigenetics in *C. elegans*

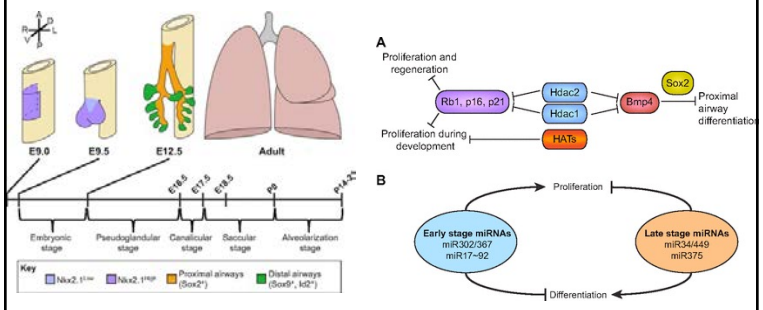
Stressor	Study design	Epigenetic mark	Main findings	Reference
Methylmercury	Developmental	H3K4me3	Phase II metabolism genes, *lpr-5*, *dpy-7*	Rudgalvyte et al., 2017
Nicotine	Developmental	miRNA	37 miRNA on high exposure, 3 on low exposure	Taki et al., 2013
Arsenite, hyperosmosis, starvation	Developmental	H3K4me3	Increased adulthood resistance to hydrogen peroxide required H3K4 methyltransferase subunits *wdr-5.1* and *ash-2*, overlap in mechanism of all three stressors	Kishimoto et al., 2017
Dietary restriction	Developmental	None	Altered offspring size and starvation resistance mediated by insulin-like signaling	Hibshman et al., 2016
Nicotine	Transgenerational	miRNA	14 miRNA	Taki et al., 2014
Silver ions, silver nanoparticles	Transgenerational	None	Lifespan decrease, reproductive toxicity	Schultz et al., 2016
Temperature	Transgenerational	H3K9me3	Loss of silencing of heterochromatic array required H3K9 methyltransferase *set-25*	Klosin et al., 2017
Arsenite	Transgenerational	H3K4me2	Increased H3K4me2 and reduced H3K4me2 demethylase *spr-5* expression	Yu and Liao 2016
Testosterone	Transgenerational	Post-translational histone acetylation	Abnormal behaviors abolished on RNAi to androgen receptor gene orthologs or with HDAC sodium butyrate	Gamez-Del-Estal et al., 2014
Starvation-induced developmental arrest	Transgenerational	Small RNA	Small RNA targeted nutrient reservoir activity and vitellogenins, inheritance required argonaute factors *rde-4* and *krsp-1*	Rechavi et al., 2014
Arsenite, hyperosmosis, starvation	Transgenerational	H3K4me3	Increased adulthood resistance to hydrogen peroxide required H3K4 methyltransferase subunits *wdr-5.1* and *ash-2*, overlap in mechanism of all three stressors	Kishimoto et al., 2017
Genetic	Transgenerational	H3K9me, small RNA	H3K9 mutants progressive decline in fertility, required argonaute factor *hrde-1* and H3K9 methyltransferase *met-2*	Levi et al., 2017
Bisphenol A (BPA)	Transgenerational	H3K9me3, H3K27me3	Germline transgene desilencing in exposed animals was coupled with decreased H3K9me3 and H3K27me3, as well as reproductive defects and embryonic lethality, for five generations. Repression was rescued by activation of Jumonji demethylases JMJD-2 and JMJD-3/UTX-1.	Camacho et al., 2018 (in press)

Regenerating zebrafish fin epigenome is characterized by stable lineage-specific DNA methylation and dynamic chromatin accessibility.
Lee HJ, Hou Y, Chen Y, Dailey ZZ, Riddihough A, Jang HS, Wang T, Johnson SL.
Genome Biol. 2020 Feb 27;21(1):52.

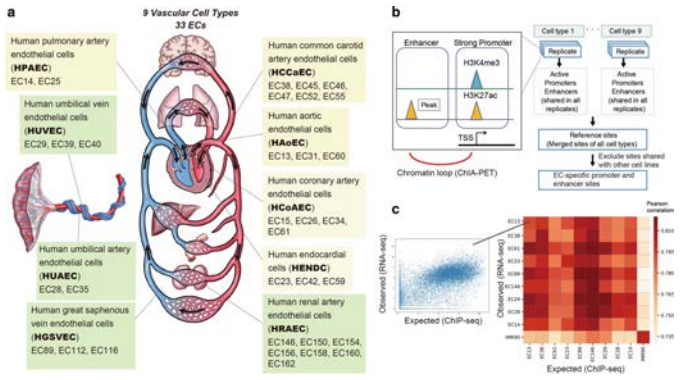


Regeneration-specific gene activation with gain of chromatin accessibility.

Lung development: orchestrating the generation and regeneration of a complex organ.
Herriges M, Morrisey EE.
Development. 2014 Feb;141(3):502-13.



Comprehensive epigenome characterization reveals diverse transcriptional regulation across human vascular endothelial cells
Nakato R, Wada Y, Nakaki, et al.
Epigenetics Chromatin. 2019 Dec 19;12(1):77.

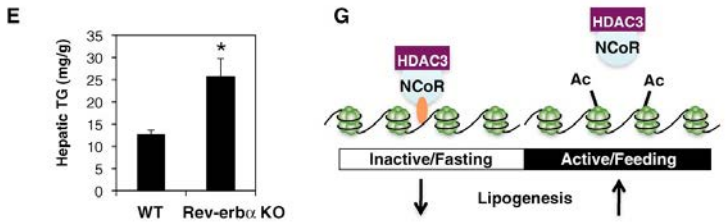


Summary of the cell types and histone modifications analyzed in this project. **a** Schematic illustration of the cardiovascular system, nine EC types and 33 individual samples (indicated by the prefix "EC") used in this paper. The yellow and green boxes indicate EC types from the upper body and lower body, respectively. **b** Workflow to identify the reference sites for ECs. The active promoter and enhancer sites of each sample were identified. For each cell type, the shared sites across all samples were extracted as the reference sites. These were integrated into a single set of reference sites for ECs, which was used for the downstream analyses. ChIA-PET data were utilized to identify the corresponding gene for the reference enhancer sites. **c** Correlation between observed and expected (from ChIP-seq analysis using linear regression model) gene expression data. Left: example scatterplot of observed and expected gene expression level for genes (data from EC13). Right: Pearson correlation heatmap for representative samples of nine cell types and IMR90 cells (as a negative control)

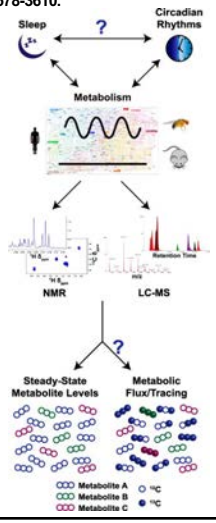
Circadian Clock

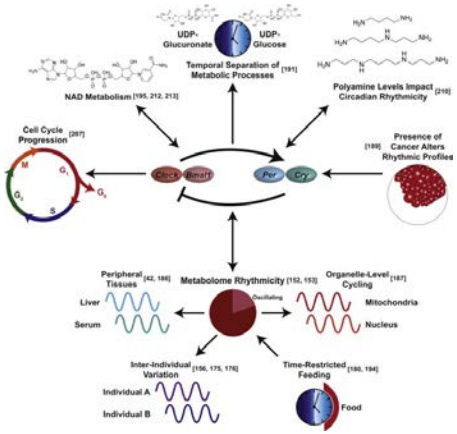
A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism.
Feng D, et al. (2012)
Science. 11;331(6022):1315-9.

Abstract
Disruption of the circadian clock exacerbates metabolic diseases, including obesity and diabetes. We show that histone deacetylase 3 (HDAC3) recruitment to the genome displays a circadian rhythm in mouse liver. Histone acetylation is inversely related to HDAC3 binding, and this rhythm is lost when HDAC3 is absent. Although amounts of HDAC3 are constant, its genomic recruitment in liver corresponds to the expression pattern of the circadian nuclear receptor Rev-erbα. Rev-erbα colocalizes with HDAC3 near genes regulating lipid metabolism, and deletion of HDAC3 or Rev-erbα in mouse liver causes hepatic steatosis. Thus, genomic recruitment of HDAC3 by Rev-erbα directs a circadian rhythm of histone acetylation and gene expression required for normal hepatic lipid homeostasis.



Circadian and Sleep Metabolomics Across Species
Malik DM, Paschos GK, Sehgal A, Weljie AM.
J Mol Biol. 2020 May 29;432(12):3578-3610.

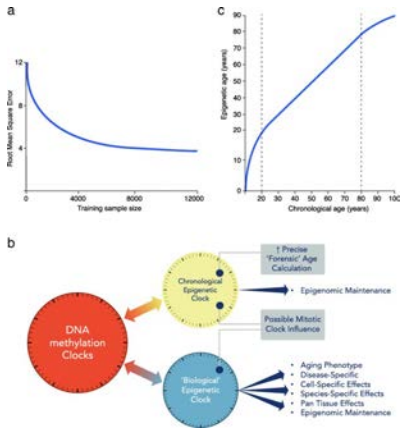




A wide range of metabolic processes are influenced by the circadian clock and some in return also impact the clock. Representative studies are indicated in brackets, and additional detail for the studies can be found in Table 1.

Aging

DNA methylation aging clocks: challenges and recommendations
Bell CG, Lowe R, Adams PD, et al.
Genome Biol. 2019 Nov 25;20(1):249.



Chronological age estimation error. With increasing training sample size, improved measurement of chronological age is expected, even using current array data (adapted from Zhang et al. [46]). y-axis: root mean square error (RMSE) of the predicted age. b DNA methylation clocks contain both chronological and biological information. The relative proportions of each will depend on the CpG probes employed in the construction of the clock. Therefore, there are multiple clocks that can be deconvoluted from aging-related epigenetic changes. Moving forward, more precise chronological (forensic age clock) and biological clocks, specific for particular diseases, informative of health or disease state need to be defined and separated. c Epigenetic age trajectory. Epigenetic age is not linear over the life course. Chronological age in years (x-axis) and epigenetic age in years (y-axis)

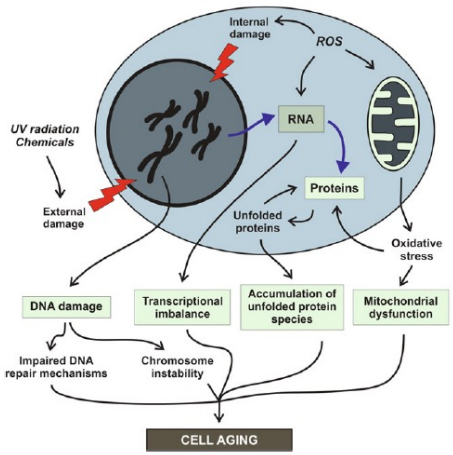


Fig. 1 Molecular damage induced by internal or external factors contribute to the cellular aging process, founded in four pillars: DNA damage, transcriptional imbalance, accumulation of unfolded proteins and mitochondrial dysfunction. The molecular imbalance observed in aged cells is mainly triggered by chemical or physical stress, produced from either internal or external sources. Chemical stress can be originated by external chemicals or by the reactive oxygen species (*ROS*) formed as a consequence of the cellular oxidative metabolism

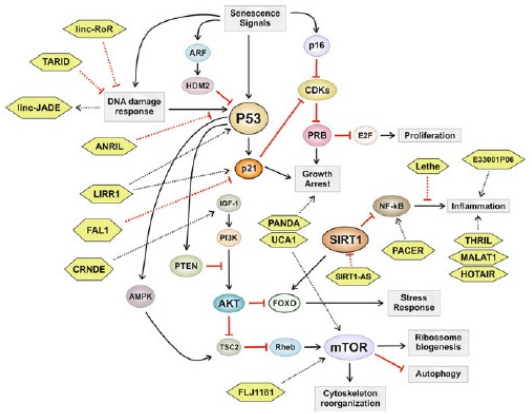


Fig. 3 Potential regulatory role of selected lncRNAs within the aging pathways. Aging-related pathways are schematically represented using only the main key players, connected with lines to pinpoint their regulatory relationships. The lncRNAs are depicted in *solid hexagons*, connected with their regulated targets by *dotted lines*

Table 1 Long noncoding RNAs with demonstrated regulatory activity over processes and pathways involved in cellular and organism aging

lncRNA	Process	Comments	Reference
ANRIL	DNA damage	ANRIL is transcriptionally up-regulated by the transcription factor E2F1 in an ATM-dependent manner following DNA damage	Wan et al. (2013b)
CCAT2	Wnt signaling	CCAT2 interacts with TCF7L2 resulting in an enhancement of WNT signaling activity	Ling et al. (2013)
CRNDE	Insulin pathway	lncRNA regulated by insulin/IGFs and related to nuclear transcripts involved in the modulation of cellular metabolism	Ellis et al. (2014)
E330013P06	Insulin pathway	Its overexpression in macrophages from type 2 diabetic mice induced inflammatory genes	Reddy et al. (2014)
ERIC	DNA damage	Inhibition of ERIC expression increased E2F1-mediated apoptosis in a negative feedback loop that modulates E2F1 activity	Feldstein et al. (2013)
FAL1	Senescence	Association by the epigenetic repressor BMI1 and modulation of expression of CDKN1A	Hu et al. (2014)
FLJ11812	mTOR pathway	Derived from the 3′ untranslated region (3′UTR) of TGFB2, it could bind with miR-4459 targeting ATG13 (autophagy-related 13)	Ge et al. (2014)
GAS5	Apoptosis	Promoted apoptosis by PI3 K/mTOR inhibition	Pickard and Williams (2014)
HOTAIR	Inflammation	IL-6 up-regulates HOTAIR in an autocrine manner, contributing to the EMT and defining a link between inflammation and EMT in malignant cell transformation	Liu et al. (2015)
	Senescence	Up-regulated in senescent cells as a mechanism to prevent premature senescent	Yoon et al. (2013)
	Wnt signaling	Repressed by Wnt/β-catenin signaling	Carrion et al. (2014)
JADE	DNA damage	Transcriptionally activates Jade1, a key component in the HBO1 histone acetylation complex	Wan et al. (2013a)
Lethe	Inflammation	Selectively induced by proinflammatory cytokines via NF-κB or glucocorticoid receptor agonist, and functions in negative feedback signaling to NF-κB	Rapicavoli et al. (2013)
LIRR1	DNA damage	Regulation of DNA damage response in a p53-dependent manner	Jiao et al. (2015)
IL7R	Inflammation	Regulation of inflammatory mediators by epigenetic control of promoters	Cui et al. (2014)
MALAT1	Inflammation	The cross talk between MALAT1 and p38 MAPK signaling pathways is involved in the regulation of endothelial cell function and inflammation	Liu et al. (2014)

(continued)

The Mechanism of Stem Cell Aging.
Mi L, Hu J, Li N, Gao J, Huo R, Peng X, Zhang N, Liu Y, Zhao H, Liu R, Zhang L, Xu K.
Stem Cell Rev Rep. 2022 Apr;18(4):1281-1293.

The Hallmarks of stem cell senescence. The scheme enumerates the seven hallmarks described in this Review: cellular senescence, DNA damag and muations, telomere and Telomerase, epigenetic alterations, microenvironment, deregulated nutrient sensing and cell polarity and proteostasis

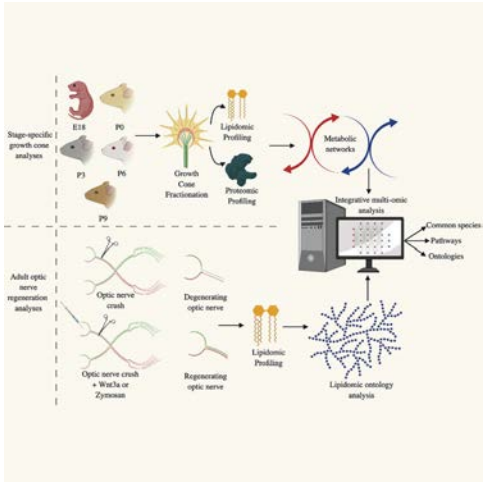
Regulation of Developmental Cell Death in the Animal Kingdom: A Critical Analysis of Epigenetic versus Genetic Factors.
Montero JA, Lorda-Diez CI, Hurle JM.
Int J Mol Sci. 2022 Jan 21;23(3):1154.

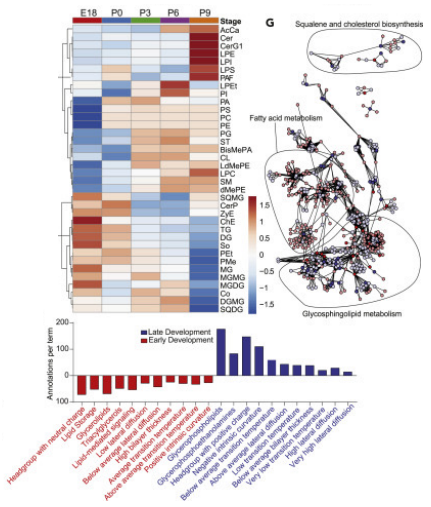
Abstract

The present paper proposes a new level of regulation of programmed cell death (PCD) in developing systems based on epigenetics. We argue against the traditional view of PCD as an altruistic "cell suicide" activated by specific gene-encoded signals with the function of favoring the development of their neighboring progenitors to properly form embryonic organs. In contrast, we propose that signals and local tissue interactions responsible for growth and differentiation of the embryonic tissues generate domains where cells retain an epigenetic profile sensitive to DNA damage that results in its subsequent elimination in a fashion reminiscent of what happens with scaffolding at the end of the construction of a building. Canonical death genes, including Bcl-2 family members, caspases, and lysosomal proteases, would reflect the downstream molecular machinery that executes the dying process rather than being master cell death regulatory signals.

Neuronal Development

Multi-Omic Analyses of Growth Cones at Different Developmental Stages Provides Insight into Pathways in Adult Neuroregeneration
Chauhan MZ, Arcuri J, Park KK, Zafar MK, et al.
iScience. 2020 Feb 21;23(2):100836.





Lipidomic Profiling in Growth Cones Across Developmental Stages

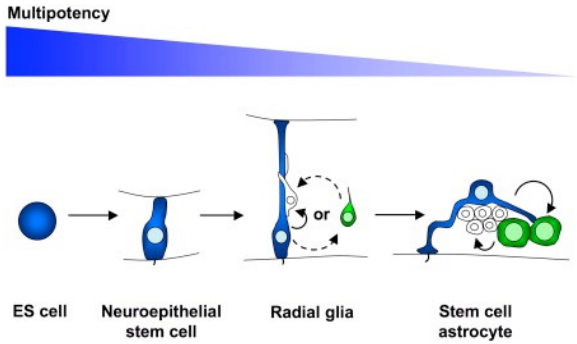
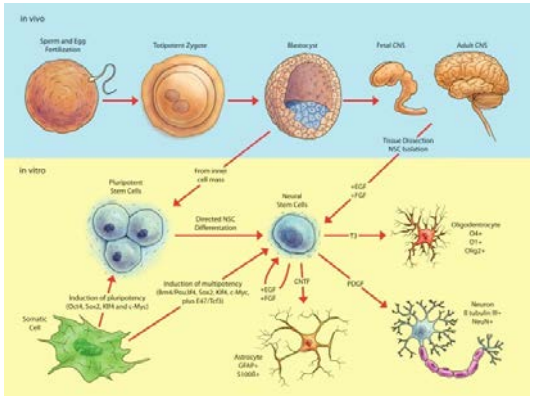


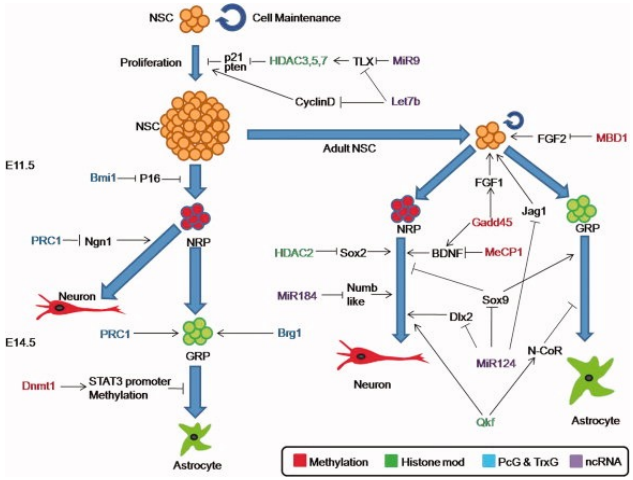
Figure 1. Stem Cells Become Progressively More Restricted over Time

Pluripotent ES cells derived from the inner cell mass of the blastocyst can differentiate into all cell types. During development, neural stem cells are contained in the neuroepithelial→radial glia→astrocyte lineage. However, stem cell potential is retained by the transit amplifying progeny (green cells) of adult stem cell astrocytes when exposed to appropriate growth factors (reviewed in Doetsch, 2003). The multipotency of stem cells is reduced over time due to progressive gene silencing. ES cell, embryonic stem cell.

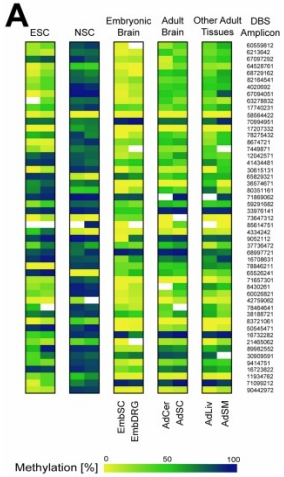


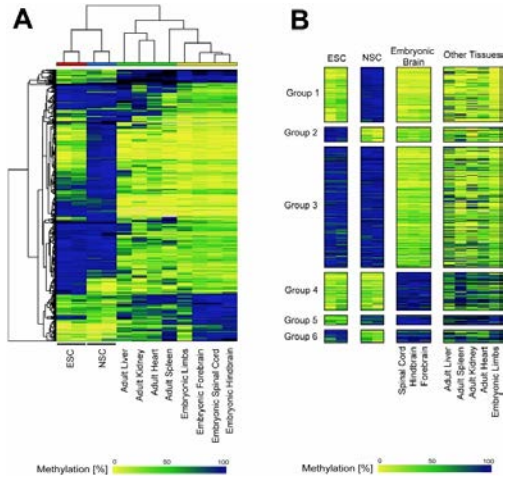
Pathway of pluripotent stem cells to neural cell populations. Fertilization and subsequent cellular divisions create the embryonic blastocyst, where pluripotent ESC are derived (from the inner cell mass; ICM). Additionally, pluripotent and multipotent-like cells can be created via transduction of various factors into differentiated tissue, such as fibroblasts. In vitro analyses of pluripotent and multipotent neural stem cells are integral for understanding aspects of neural differentiation. The in vivo niche of stem cells contains a considerable diversity of biomolecules whose roles still need be deciphered. Exposure of ESC in vitro to various growth factors in serum free media such as fibroblast growth factor 2 (FGF2) and epithelial growth factor (EGF) allows selection of cell lines possessing a neural fate. Neural stem cells can also be acquired from adult tissue and expanded in vitro.

Epigenetic regulation of self-renewal and fate determination in neural stem cells.
Mohamed Ariff I, Mitra A, Basu A.
J Neurosci Res. 2012 Mar;90(3):529-39.



Genome-Wide Screen for Differential DNA Methylation Associated with Neural Cell Differentiation in Mouse
Rene Cortese, et al.
PLoS One. (2011) 6(10): e26002.





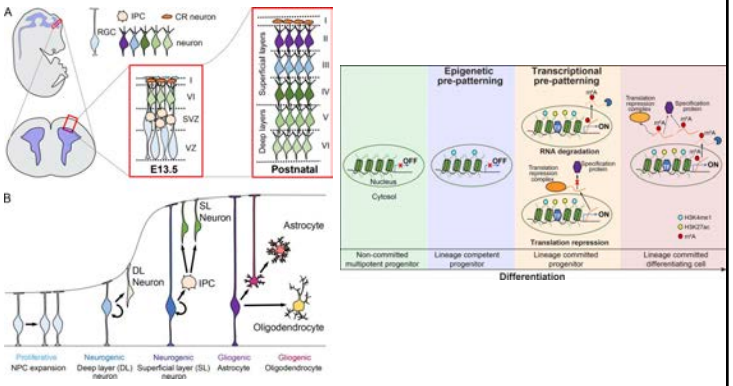
TSS-associated tDMRs define distinct groups in NSCs, ESCs and embryonic brain.
A) Unsupervised clustering of top-ranked candidates. B) ANOVA of methylation percentage in ESCs, NSCs and embryonic brain defined 6 tDMR groups. 382 candidate tDMRs were selected and ranked. Color code as detailed in Figure 2.

Histone deacetylation during brain development is essential for permanent masculinization of sexual behavior.
Matsuda KI, et al.
Endocrinology. 2011 Jul;152(7):2760-7.

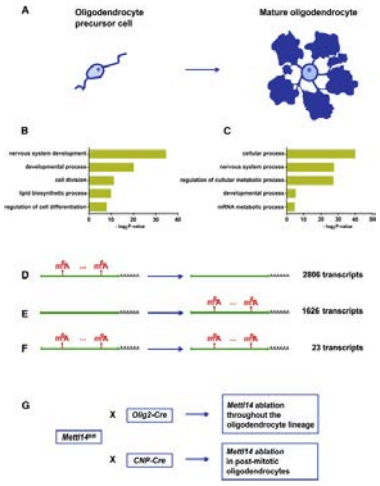
Neuronal activity modifies the DNA methylation landscape in the adult brain.
Guo JU, et al.
Nat Neurosci. 2011 Aug 28;14(10):1345-51.

Cell cycle restriction by histone H2AX limits proliferation of adult neural stem cells.
Fernando RN, et al.
Proc Natl Acad Sci U S A. 2011 Apr 5;108(14):5837-42.

Epigenetics and epitranscriptomics in temporal patterning of cortical neural progenitor competence.
J Cell Biol. 2018 Jun 4;217(6):1901-1914.
Yoon KJ, Vissers C, Ming GL, Song H

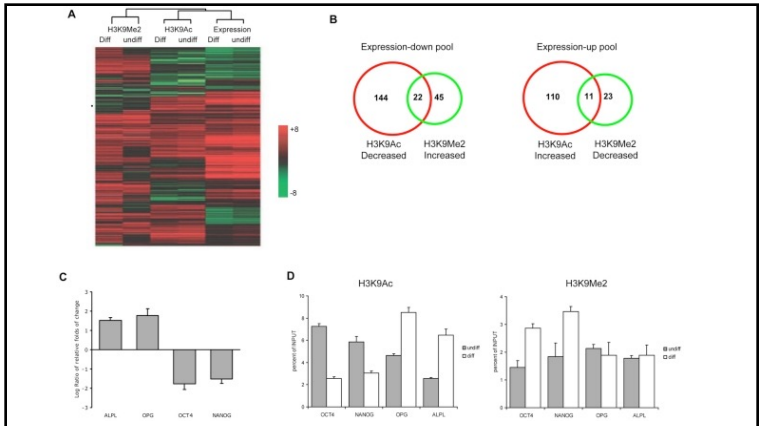


m ^{6} A mRNA Methylation Is Essential for Oligodendrocyte Maturation and CNS Myelination
Xu H, Yulia Dzhashiashvili Y, Shah A, et al.
Neuron. 2020 Jan 22;105(2):293-309.e5.

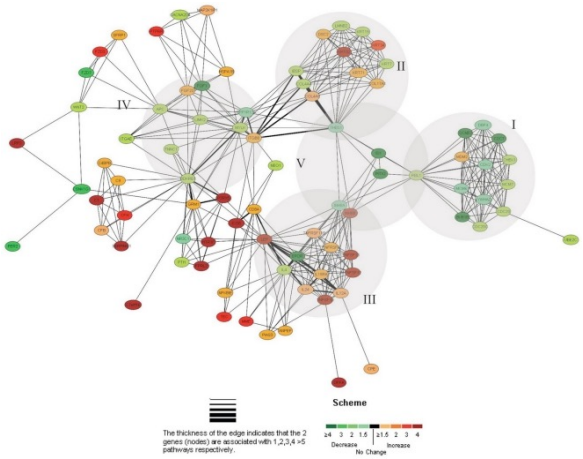


Oligodendrocyte Lineage Progression Is Accompanied by Changes in m^{6}A Modification on Numerous Transcripts

Osteogenic Development

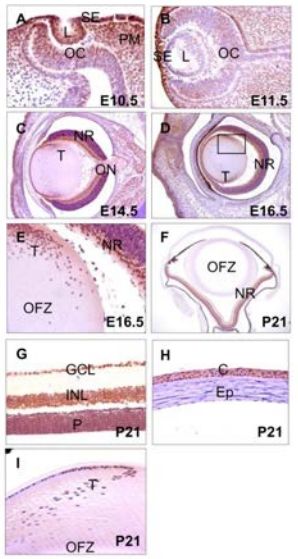


Comparative analyses of the data from gene expression patterns and from promoter H3K9 modification profiles. (A) Hierarchical cluster analysis of the enrichments of H3K9Ac/H3K9Me2, and the data of gene expression upon MSC osteogenic differentiation. (B) Venn diagrams of H3K9Ac-decreased genes versus H3K9Me2-increased genes in expression-down pool, and H3K9Ac- increased genes versus H3K9Me2-decreased genes in expression-up pool. (C) Real-time RT-PCR verification of gene expression of OCT4, NANOG, OPG and ALPL. The relative expression values were normalized against GAPDH. (D) Real-time ChIP-PCR verification of H3K9Ac and H3K9Me2 of promoter regions of OCT4, NANOG, OPG and ALPL upon MSC osteogenic differentiation. Standard error bars of three individual experiments are indicated.

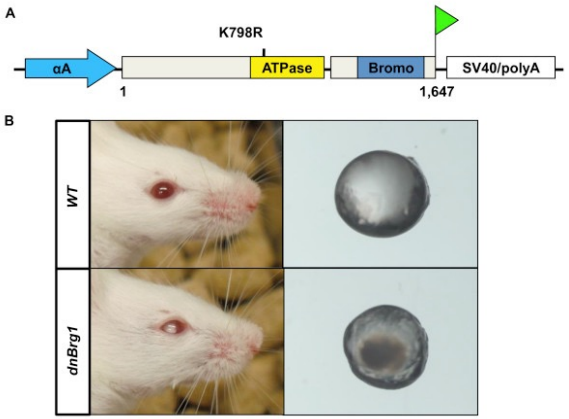


An informational network of the genes regulated by H3K9Ac and H3K9Me2 upon MSC osteogenic differentiation. The informational pathway networks were generated by using BioRag (http//:www.biorag.org). The most affected pathways are the cell cycle pathway (cluster I), the cell communication (cluster II), the cytokine-cytokine receptor interaction (cluster III), the regulation of actin cytoskeleton (cluster IV), and the TGF-beta signaling pathway (cluster V)

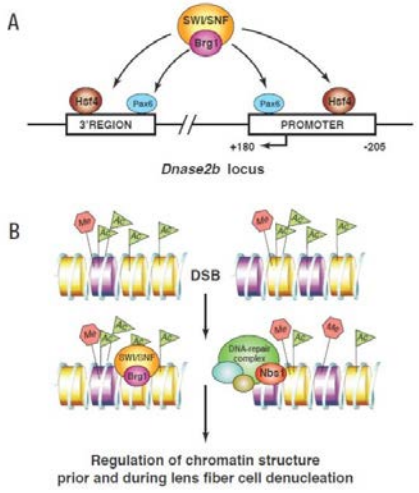
Ocular Development



Brahma-related gene 1 (Brg1) expression profile during mouse ocular development. (A-I) Sagittal sections were immunostained with antibody recognizing Brg1 (brown) and counterstained lightly with hematoxylin (purple) at embryonic days E10.5 (A), E11.5 (B), E14.5 (C) and E16.5 (D and E), as well as postnatal day P21 (F-I). Higher-magnification areas stained with the Brg1 antibody indicated in (D) are shown in (E). (G-I) Brg1 at different ocular regions of (F). C: cornea, Ep: cornea epithelium, GCL: ganglion cell layer, INL: inner nuclear layer, L: lens, NR: neural retina, OC: optic cup, ON: optic nerve, P: photoreceptors, PM: periocular mesenchyme, SE: surface ectoderm, T: transition zone. Magnification: (A), ×460; (B and C), ×320; (D), ×250; (E), ×400; (F), ×60; and (G-I), ×320.

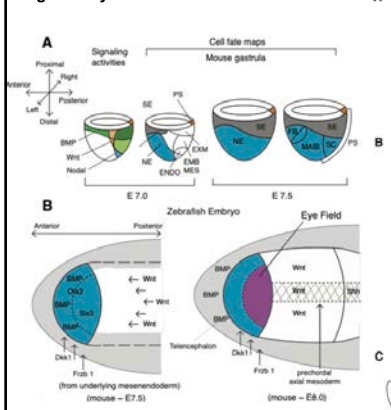


Generation and initial evaluation of the dnBrg1 transgenic mice. (A) Schematic of the transgenic construct. αA-crystallin promoter fragment (-366 to + 46, Cryaa; blue arrow) was used to express mutated Brg1 (K798R) with a C-terminal FLAG tag (green triangle). ATPase domain (yellow) and bromodomain (blue) are shown. (B) Two-month-old wild-type (WT) and transgenic mice. Note the cataract formation in the transgenic mouse and in isolated lens.

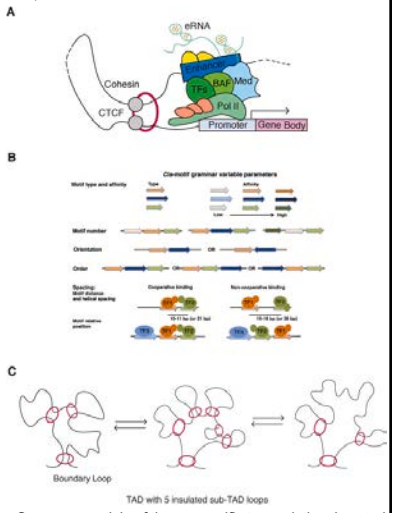


Summary of two complementary models illustrating Brg1's role during lens fiber cell differentiation. (A) A schematic of the *Dnase2b* locus including its evolutionarily conserved promoter region (-205 to + 180). Multiple Pax6- and Hsf4-binding sites were identified in the *DNase2b* promoter and 3'-downstream evolutionary conserved region. Hsf4 and Pax6 recruit (switch/sucrose nonfermentable) (SWI/SNF) complexes as described elsewhere [29,30]. (B) A schematic of a DNA double-strand break (DSB) accompanied by insertions of H2A histone family, member X (H2AX) histone variant (nucleosomes shown in purple). Both SWI/SNF (including Brg1) and DNA repair (including Nbs1) complexes are then recruited to the chromatin. Both complexes are thought to regulate chromatin structure prior to and during lens fiber cell denucleation. In mouse, Nbs1-deficient lenses show incomplete denucleation of lens fiber cells [71].

Cell fate decisions, transcription factors and signaling during early retinal development
Diacou R, Nandigrami P, Fiser A, Liu W, Ashery-Padan R, Cvekl A.
Prog Retin Eye Res. 2022 Jul 8:101093.



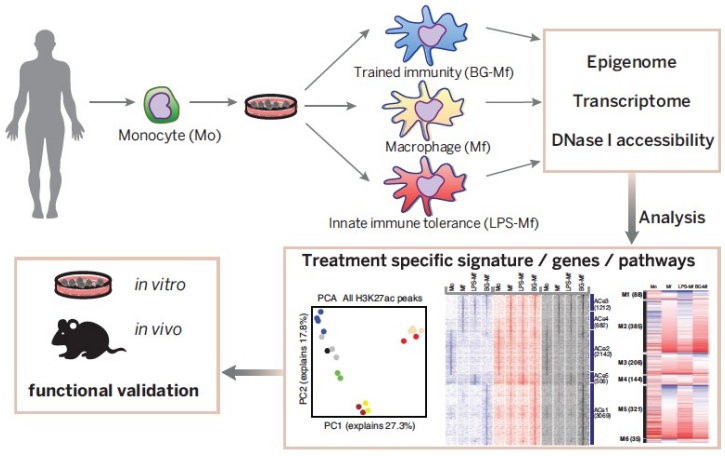
The earliest stages of neuroectoderm formation and eye field formation.



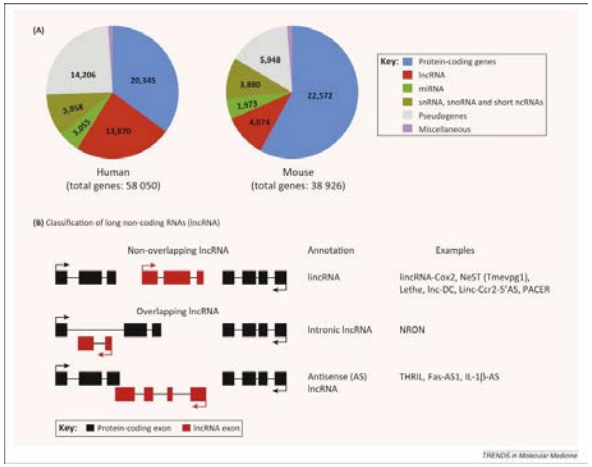
Summary models of tissue-specific transcriptional control.

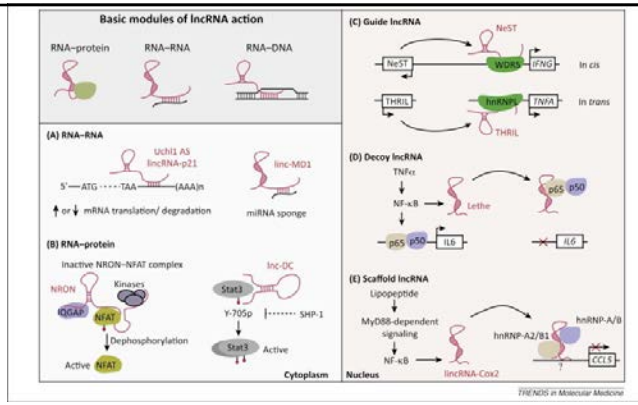
Immune Cell Development

Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity.
Saeed S, et al.
Science. 2014 Sep 26;345(6204):1251086.



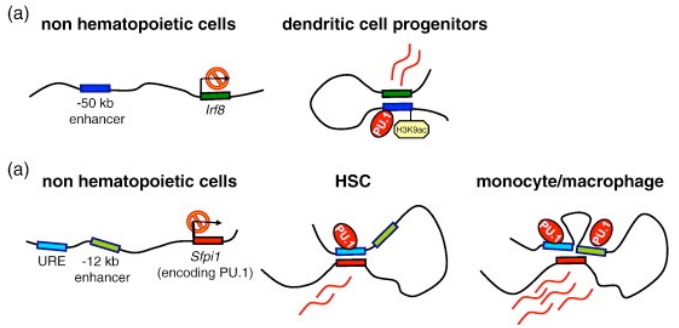
Long non-coding RNAs and control of gene expression in the immune system.
Atianand MK, Fitzgerald KA.
Trends Mol Med. 2014 Nov;20(11):623-31

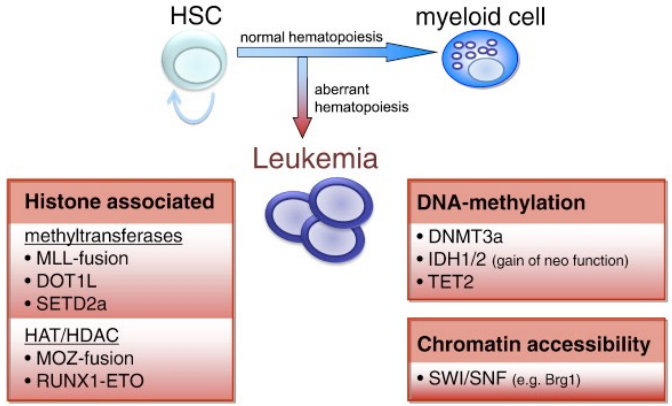




Long non-coding RNAs (lncRNAs) are versatile regulators of gene expression. lncRNAs employ three basic modules, RNA–RNA, RNA–protein and RNA–DNA interactions, to exert their functions either in the cytosol (A,B) or the nucleus (C–E). (A) In the cytosol lncRNAs interact with target mRNAs through base-pairing to either enhance translation (e.g., Uchl1 AS), or repress translation (e.g., lincRNA-p21). In addition, cytosolic lncRNAs are also known to regulate mRNA stability. For example, the lncRNA termed ½-Staufen binding site (sbs) RNA forms an RNA duplex with mRNAs containing partial complementarity in the 3'-untranslated region (UTR) to promote Staufen 1 binding, which drives Staufen-mediated mRNA degradation. (B) Cytosolic lncRNAs are also known to regulate immune signaling pathways through RNA–protein interactions. NRON and lnc-DC act as a scaffold for the transcription factors NFAT and Stat3, respectively, regulating phosphorylation status and thereby the expression of target genes in immune cells. (C–E) In the nucleus, lncRNAs function by acting as a guide (C, NeST; THRIL), decoy (D, Lethe), or scaffold (E, lincRNA-Cox2) to interact with specific protein(s) to silence gene expression. Abbreviations: hnRNP, heterogeneous ribonucleoprotein; IQGAP, IQ motif-containing GTPase activating protein; MD1, muscle differentiation 1; NFAT, nuclear factor of activated T cells; NRON, non-coding repressor of NFAT; THRIL, TNFα and hnRNPL related immunoregulatory lincRNA; Uchl1, ubiquitin carboxy-terminal hydrolase L1; WDR5, WD repeat-containing protein 5.

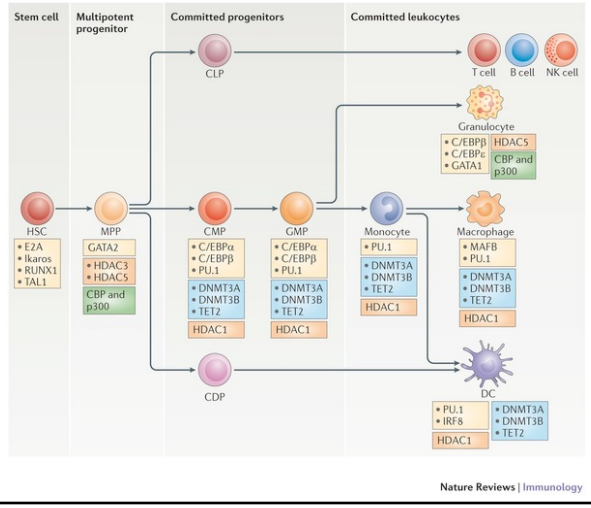
Chromatin Dynamics during Differentiation of Myeloid Cells.
Schönheit J, Leutz A, Rosenbauer F.
J Mol Biol. (2015) 427(3) Pages 670–687



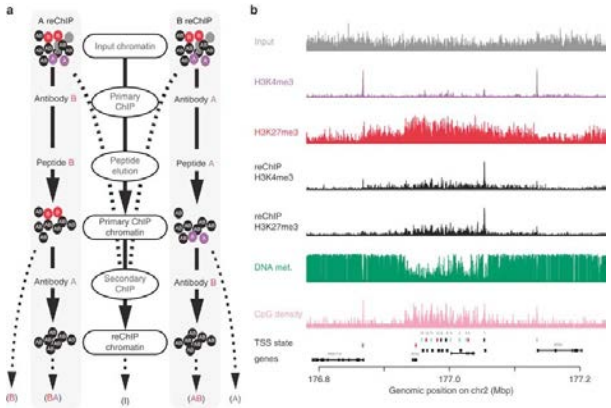


Aberrant epigenetic modifications involved in myeloid leukemia. Epigenetic mechanisms are involved in myeloid cell commitment and three major functions are associated with the onset of leukemia, aberrant histone modification, aberrant DNA methylation and altered chromatin accessibility (red boxes).

Epigenetic control of myeloid cell differentiation, identity and function.
Álvarez-Errico D, et al.
Nat Rev Immunol. 2014 Dec 23;15(1):7-17.



reChIP-seq reveals widespread bivalency of H3K4me3 and H3K27me3 in CD4(+) memory T cells
Kinkley S, Helmuth J, Polansky JK, et al.
Nat Commun. 2016 Aug 17;7:12514.



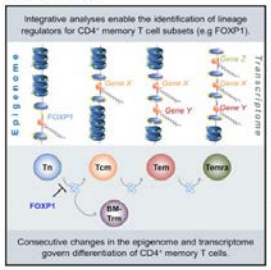
The reChIP-seq method.(a) Experimental design. Black, purple, red and grey circles denote chromatin containing A and B antigens, only A antigens, only B antigens, or neither A nor B antigens, respectively. (b) ChIP- and reChIP-seq at the human HOXD locus. The colours of the boxes in the TSS state track indicate the co-occupancy patterns as described in Fig. 2.

Resource

Immunity

Epigenomic Profiling of Human CD4⁺ T Cells Supports a Linear Differentiation Model and Highlights Molecular Regulators of Memory Development

Graphical Abstract



Authors

Pawel Durek, Karl Nordström, Gilles Gasparoni, ..., Jörn Walter, Alf Hamann, Julia K. Polansky

Correspondence

julia.polansky@drfz.de

In Brief

As part of the IHEC consortium, Durek et al. (2016) generated deep epigenomes and transcriptomes of CD4⁺ memory T cell subsets to infer their lineage relationships and to demonstrate the impact of epigenetic regulation on known and novel molecular regulators involved in memory generation. Explore the Cell Press IHEC webportal at www.cell.com/consortium/IHEC.

Highlights

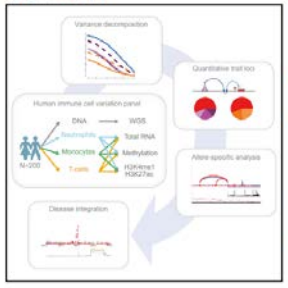
- Comprehensive epigenomes for human CD4⁺ T memory subsets generated and analyzed
- Integrative analyses support a linear model of memory T cell differentiation
- Epigenetic control of transcriptional regulators of memory differentiation revealed
- Chromatin changes highlight novel regulators for T memory cell differentiation

Resource

Cell

Genetic Drivers of Epigenetic and Transcriptional Variation in Human Immune Cells

Graphical Abstract



Authors

Lu Chen, Bing Ge, Francesco Paolo Casale, ..., Kate Downes, Tomi Pastinen, Nicole Soranzo

Correspondence

tomi.pastinen@mcgill.ca (T.P.), ns6@sanger.ac.uk (N.S.)

In Brief

As part of the IHEC consortium, this study integrates genetic, epigenetic, and transcriptomic profiling in three immune cell types from nearly 200 people to characterize the distinct and cooperative contributions of diverse genomic inputs to transcriptional variation. Explore the Cell Press IHEC web portal at http://www.cell.com/consortium/IHEC.

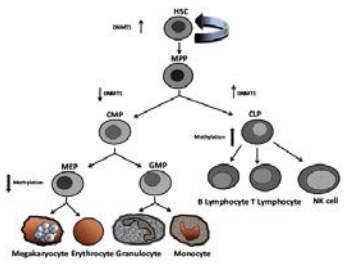
Highlights

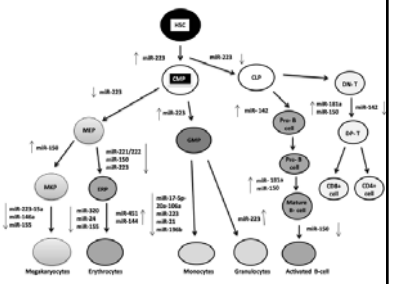
- Genome, transcriptome, and epigenome reference panel in three human immune cell types
- Identified 4,418 genes associated with epigenetic changes independent of genetics
- Described genome-epigenome coordination defining cell-type-specific regulatory events
- Functionally mapped disease mechanisms at 345 unique autoimmune disease loci

Data Resources

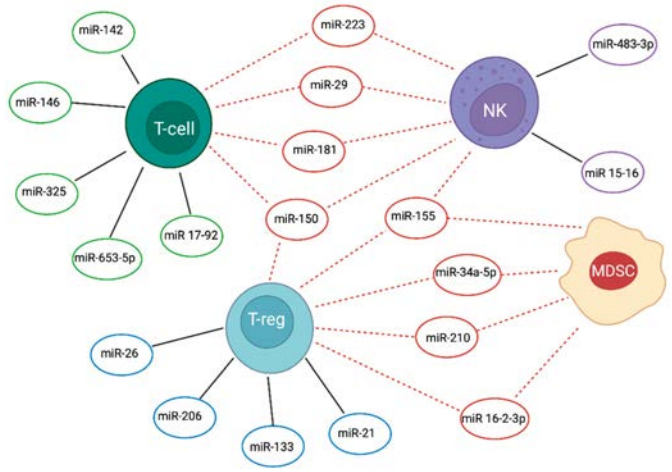
EGAD00001002663
EGAD00001002671
EGAD00001002674
EGAD00001002675
EGAD00001002670
EGAD00001002672
EGAD00001002673
EGAS00001001456

Epigenetic Mechanisms: Role in Hematopoietic Stem Cell Lineage Commitment and Differentiation
Raghuwanshi S, Dahariya S, Kandi R, et al.
Curr Drug Targets. 2018;19(14):1683-1695.





MicroRNAs as Modulators of the Immune Response in T-Cell Acute Lymphoblastic Leukemia
Del Gaizo M, Sergio I, Lazzari S, Cialfi S, Pelullo M, Screpanti I, Felli MP.
Int J Mol Sci. 2022 Jan 13;23(2):829.



Common and cell-specific miRNAs in immune cell responders against ALL.

Orofacial Development

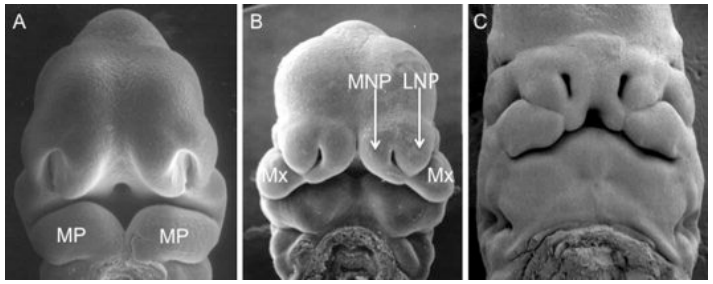
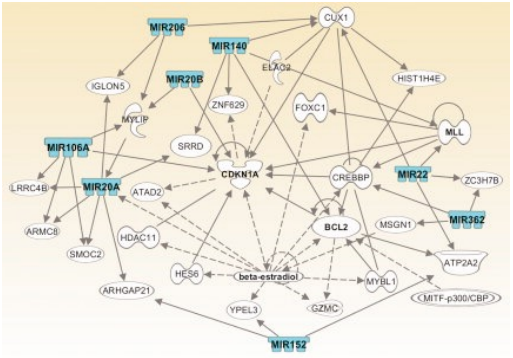
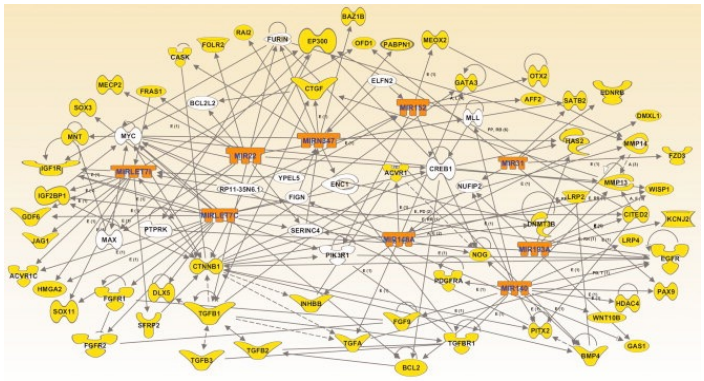


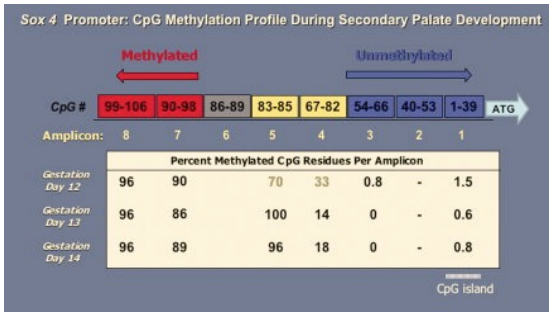
Figure 1. Development of the midfacial primordia. Scanning electron micrographs of the developing orofacial region showing the prominences that give rise to the main structures of the face. (A) mouse gestational day (GD) 10, equivalent to human 5th week of development, (B) mouse GD 11, equivalent to human 6th week of development, and (C) human 6th week of development. The mandible is formed by merging of the homologous mandibular processes (MP) of the first branchial arch. The upper lip is formed by merging of the bilateral maxillary processes (MX) of the first branchial arch with the medial nasal processes (MNP), which merge with each other. The lateral nasal processes (LNP) give rise to the alae, or sides, of the nose. Reprinted with the permission of Dr. Kathleen Sulik, University of North Carolina, Chapel Hill, N.C.



Computational gene interaction predictions from a selected microRNA gene network in murine orofacial development. Several differentially regulated microRNA genes in developing murine orofacial tissue (shown in aqua) were used to construct a gene association map using Ingenuity Systems Pathway Analysis (IPA) software. Solid lines specify direct relationships between genes whereas dotted lines indicate indirect interactions.



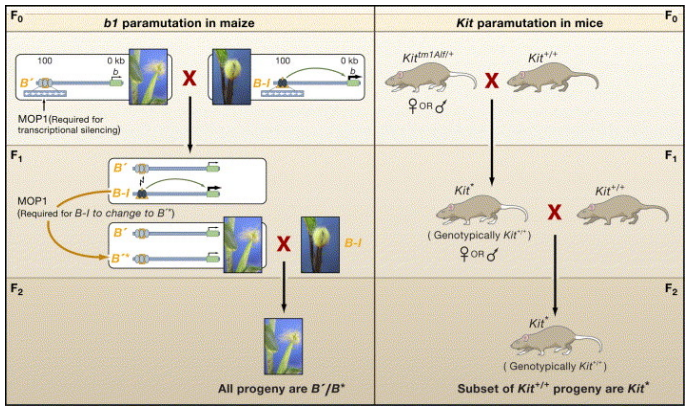
Computational gene interaction predictions: gene network with microRNAs demonstrating enhanced expression in developing orofacial tissue (GD-13 vs. GD-12) and their target genes. A network with selected genes encoding microRNAs (orange) that demonstrate increased expression between GD-12 and GD-13 of orofacial development, and their known or predicted target genes (yellow) critical for orofacial ontogenesis was constructed with Ingenuity Systems Pathway Analysis (IPA) software and the miRDB (http://mirdb.org/miRDB/) database. Solid lines specify direct relationships between genes whereas dotted lines indicate indirect interactions.

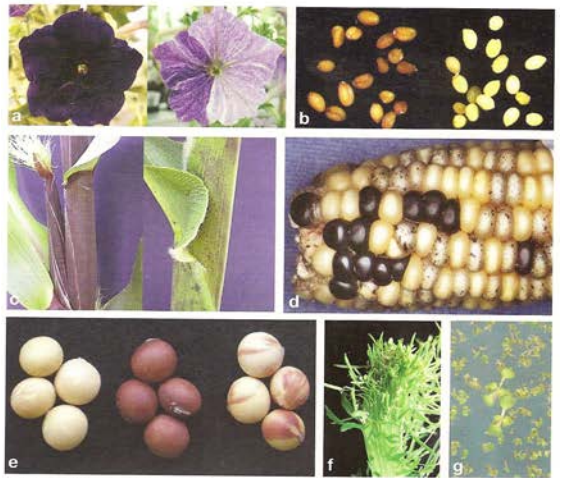


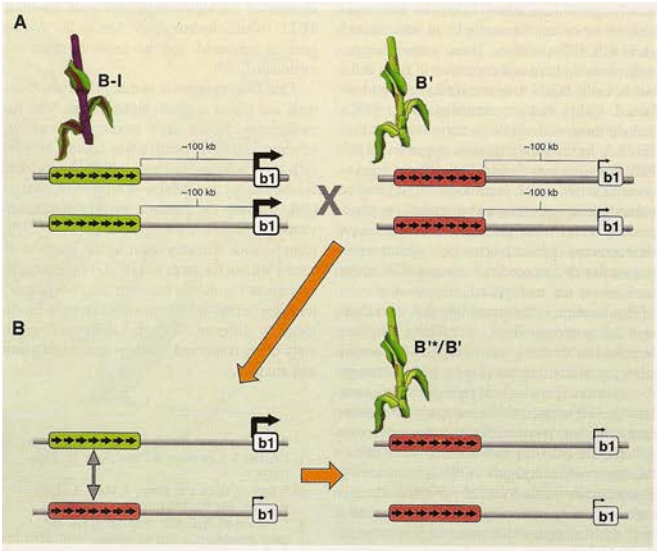
CpG methylation profile of the Sox4 gene upstream regulatory region during murine secondary palate development. An 1.7 kb upstream region of the Sox 4 gene, beginning from the ATG start site (shown on the right), is defined by eight amplicons (boxed). Individual CpG residues are numbered within the boxes. Percentage methylation—the average of all methylated CpG residues within an amplicon—is shown below each amplicon for murine gestation day (GD) 12, GD 13, and GD 14 secondary palate. Yellow boxes represent differentially methylated regions (DMRs); blue boxes represent unmethylated regions; and red boxes represent highly methylated regions. Amplicon 2 was not analyzed as it was presumed to be unmethylated based on analysis of amplicons 1 and 3. Amplicon 6 could not be amplified. The CpG island, an area of high CpG density is located in the area of amplicon #1.

Paramutation

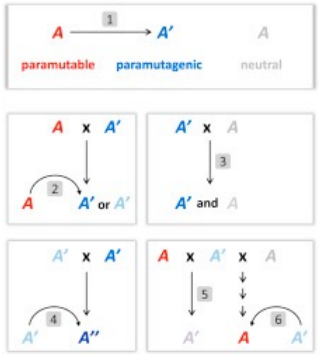
PARAMUTATION



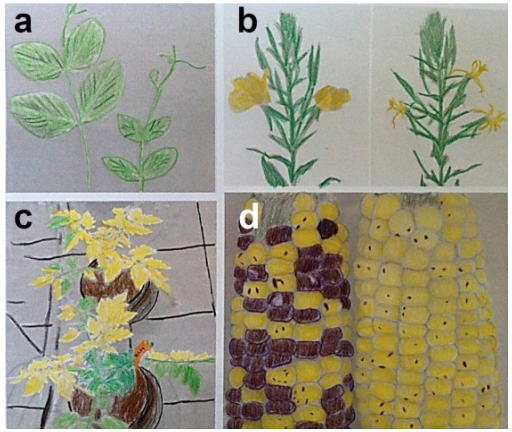




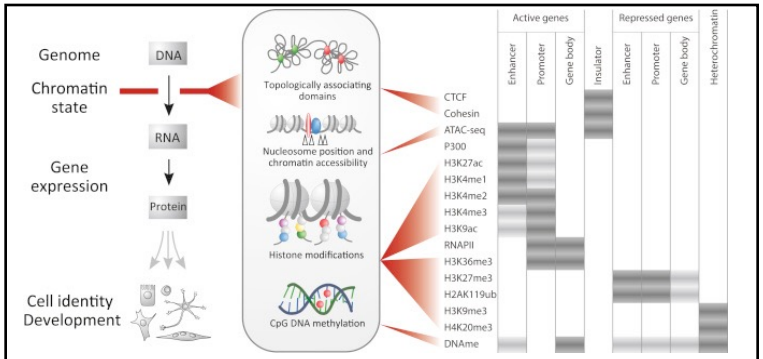
Paramutation phenomena in plants.
Pilu R.
Semin Cell Dev Biol. 2015 Aug;44:2-10.



General outline of the paramutation phenomenon. (1) A paramutable allele (red) can spontaneously change into a paramutagenic (dark blue) allele; neutral alleles (grey) are stable. (2) Crossing an individual carrying the paramutagenic A' allele with another carrying the paramutable A allele causes A' to paramutate A; the new A' allele can be as inactive as the original A' (dark blue) or less silenced than the original one (light blue). (3) No trans-interaction takes place between a paramutagenic A' allele and a neutral A allele. (4) Crossing the paramutated A' allele with the original strong A' allele, reinforcing of the silencing can occur as A'' haplotype. (5) Secondary paramutation (violet A') can occur when an individual carrying the paramutated A' allele is crossed with an individual carrying a new paramutable A allele. Silencing can be weaker than after the primary paramutation. (6) If a paramutated A' allele is no longer exposed to the original A' allele, e.g. after a few few backcrosses to a neutral allele, it can revert to the original A paramutable allele. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



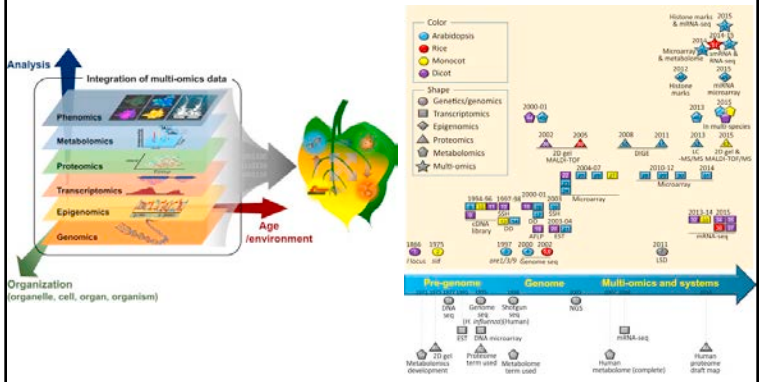
The first cases of paramutation described in plants. (a) 'Rogue' character (on the right) in Pisum sativum conferring an "inferior" phenotype compared to wild type [after 23]. (b) Crociata character (on the right) in Oenothera species conferring an aberrant development of the petals compared to wild type [after 26]. (c) Sulfurea locus in Lycopersicon esculentum conferring yellow chlorophyll-deficient sectors [after 24]. (d) Red colour 1 locus (R-r paramutable allele) in maize conferring dark purple seeds (on the left) that when combined with the R-stippled paramutagenic allele (on the right) is heritably silenced, acquiring a very light pigmentation (designated R-r') [after 27]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



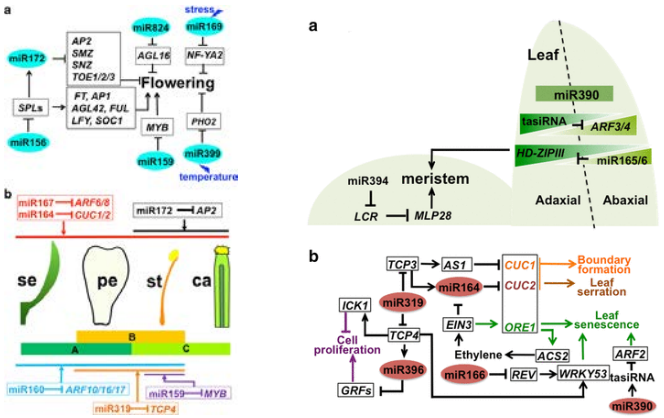
Overview of Epigenetic Modifications and Its Role in Development. The role of chromatin as a filter for genomic information in gene expression and the determination of cell identity (left panel). Chromatin acts at various scales of size (middle panel), from topological organization of chromatin (middle panel, top; different colors represent A and B compartments), chromatin accessibility (middle panel, second from top; triangles represent chromatin accessibility, gray spheres represent nucleosomes), and histone modifications (middle panel, third from top; colored spheres represent modified histone H3 residues) to DNA methylation (middle panel, bottom; methyl-cytosine indicated in red). Chromatin is decorated by modifications and binding of different factors that are specific for the function of the sequence and its transcriptional state (right panel). H3 and H4 refer to histone H3 and H4, respectively, K to the lysine residue involved, me1, me2, and me3 to mono-, di-, and trimethylation, and ac and ub to acetylation and ubiquitination, respectively. CTCF and Cohesin are involved in the organization of chromosomal loops. ATAC sequencing (indicated) and DNase I sequencing (not shown) can be used to assess chromatin accessibility. The p300 coactivator protein acetylates H3K27 and is mainly found at enhancers. RNAPII causes deposition of H3K36me3 in actively transcribed genes. Lighter gray shades indicate a lower or variable degree of factor binding or histone-tail modification. For DNA methylation (DNAme), light gray indicates that the sequence could be either methylated or unmethylated depending on the example considered. H3K27me3-repressed genes tend to be unmethylated. Active promoters tend to be unmethylated unless they have a low CpG density.

Plant Development

Toward Systems Understanding of Leaf Senescence: An Integrated Multi-Omics Perspective on Leaf Senescence Research.
Mol Plant. 2016 Jun 6;9(6):813-25.
Kim J, Woo HR, Nam HG.



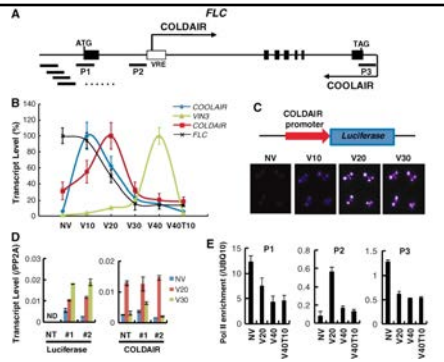
Small but powerful: function of microRNAs in plant development.
Plant Cell Rep. 2018 Mar;37(3):515-528.
Liu H, Yu H, Tang G2,, Huang T.



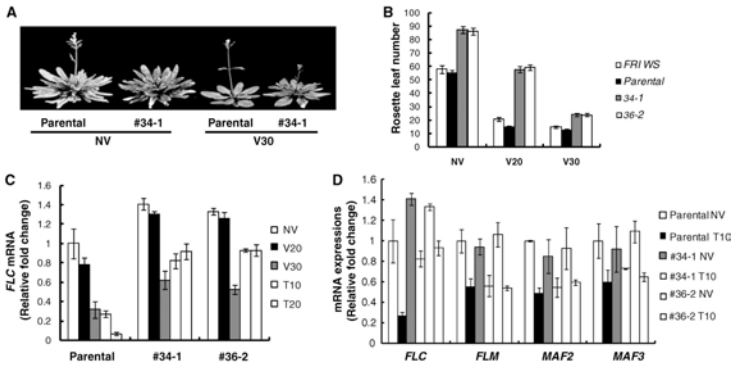
Vernalization-Mediated Epigenetic Silencing by a Long Intronic Noncoding RNA

Jae Bok Heo and Sibum Sung

Science 7 January 2011: Vol. 331 no. 6013 pp. 76-79



COLDAIR, an intronic long ncRNA of the *FLC*. (A) Schematic representation of transcription start sites for COLDAIR and COOLAIR and the location of VRE at the *FLC* genomic region. (B) Transcript expression patterns of COOLAIR, COLDAIR, VIN3, and FLC during the course of vernalization. Relative levels of mRNA expressions were calculated compared with those of the control, PP2A. Maximum expression for each gene is set as 100%, and relative levels are shown. Mean ± SD of quantitative RT-PCR data are shown (n = 3). NV, nonvernalized. V10, 10 days of vernalization treatment. V20, 20 days of vernalization treatment. V30, 30 days of vernalization treatment. (C) Luciferase expression in COLDAIR-promoter::*Luciferase* transgenic lines during the course of vernalization. (D) Expression patterns of luciferase (left) and COLDAIR (right) transcripts in two stable representative COLDAIR-promoter::*Luciferase* transgenic lines (1 and 2) and nontransgenic line (NT). Mean ± SD of quantitative RT-PCR data compared with the control, PP2A, are shown (*n* = 3). ND, not detectable. (E) Transient increase in RNAPII occupancy at the COLDAIR promoter region. Chromatin immunoprecipitation (ChIP) using RNAPII antibody (8WG16). Relative occupancies of RNAPII at FLC regions were calculated by comparison to occupancy of RNAPII at the *UBQ10* promoter region. Relative locations of P1 to P3 are shown in (A). Mean ± SD of quantitative ChIP-PCR data are shown (*n* = 3). V40, 40 days of vernalization treatment; V40T10, 40 days of vernalization treatment followed by 10 days of normal growth temperature.



COLDAIR is required for proper repression of *FLC* during the course of vernalization. (A) Flowering behaviors of the parental line and a representative COLDAIR knockdown line. (B) Flowering times are determined by the rosette leaf number at the timing of flowerings of *FRI* in Ws, the parental line, and two representative COLDAIR knockdown lines (34-1, 36-2). (C) Changes in *FLC* mRNA during the course of vernalization in the parental line and two representative COLDAIR knockdown lines. Data (fold change; mean ± SD of quantitative RT-PCR; n = 3) are relative to the *FLC* mRNA level in the parental line before vernalization. T20, 30 days of vernalization treatment followed by 20 days of normal growth temperature. (D) Changes in *FLC, FLM, MAF2*, and *MAF3* mRNA during the course of vernalization in the parental line and two representative COLDAIR knockdown lines. Data (fold change; mean ± SD of quantitative RT-PCR; n = 3) are relative to the *FLC, FLM, MAF2,* and *MAF3* mRNA levels in the parental line before vernalization.

Epigenetic regulation in the shoot apical meristem.
Nguyen V, Gutzat R.
Curr Opin Plant Biol. 2022 Oct;69:102267.

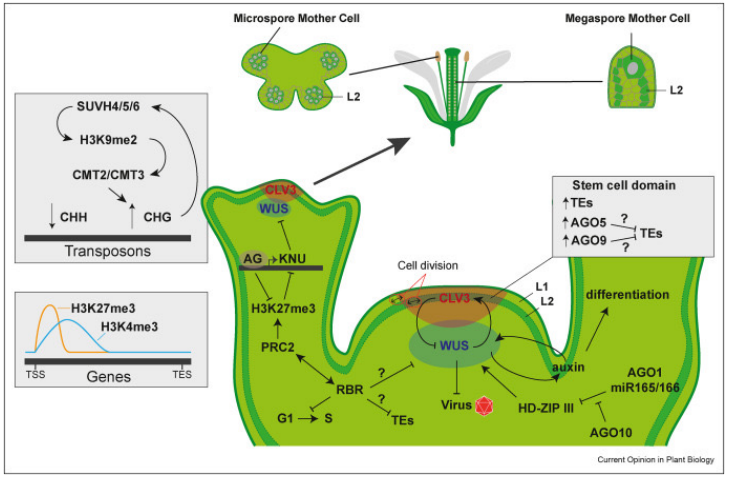
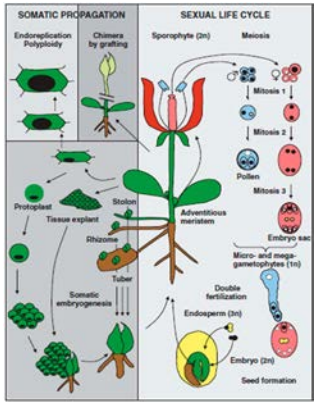
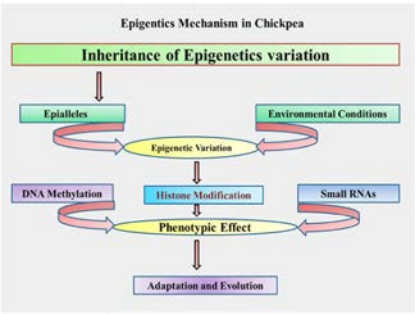


Illustration of epigenetic mechanisms in the Arabidopsis SAM.

Epigenomics as Potential Tools for Enhancing Magnitude of Breeding Approaches for Developing Climate Resilient Chickpea.
Chandana BS, Mahto RK, Singh RK, Ford R, Vaghefi N, Gupta SK, Yadav HK, Manohar M, Kumar R. Front Genet. 2022 Jul 22;13:900253.

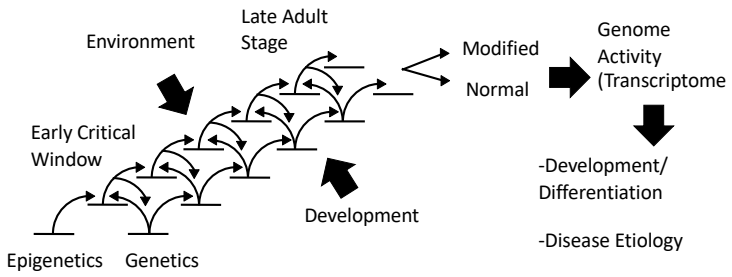


Unique aspects of the plant life cycle



Proposed schematic mechanism/process of epigenetics in chickpea.

Developmental Epigenetics Summary



"Epigenetics and Systems Biology"

Spring 2023 (Odd Years)
Biol 476/576
Schedule/Lecture Outline –

Week 1	January 10 & 12	Systems Biology (History/ Definitions/ Theory)
Week 2	January 17 & 19	Systems Biology (Networks & Emergence)
Week 3	January 24 & 26	Systems Biology (Components: DNA to Phenotype)
Week 4	Jan 31 & Feb 2	Systems Biology (Genomics / Technology)
Week 5	February 7 & 9	Epigenetics (History / Molecular Processes)
Week 6	February 14 & 16	Epigenetics (Molecular Processes & Integration)
Week 7	February 21 & 23	Epigenetics (Genomics and Technology)
Week 8	Feb 28 & March 2	Cell & Developmental Biology
Week 9	March 7 & 9	Epigenetics of Cell & Developmental Biology (& Midterm Exam)
Week 10	**March 13 – 17**	**Spring Break**
Week 11	March 21 & 23	Environmental Impact on Biology
Week 12	March 28 & 30	Environmental Epigenetics
Week 13	April 4 & 6	Disease Etiology
Week 14	April 11 & 13	Epigenetics & Disease Etiology
Week 15	April 18 & 20	Evolutionary Biology & Genetics
Week 16	April 25 & 27	Epigenetics & Evolutionary Biology
Week 17	May 2 & 4	Grant Review/ Study Section Meeting (& Final Exam)