Spring 2018 – Systems Biology of Reproduction
Lecture Outline – Male Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
January 30, 2018
Week 4

Male Reproductive Tract Development & Function

Embryonic Development and Reproductive Tract Organogenesis
- Overview
- Development of Mullerian Duct vs. Duct Wolffian Duct Derivatives
- Mullerian Inhibiting Substance (MIS)

Male Urogenital Tract Organogenesis
- Prevention of Programmed Cell Death in the Wolffian Duct
- UGS/Prostate/Seminal Vesicle
    1. Prostate Morphogenesis (ductal branching)
    2. Cell-Cell Interactions and Paracrine Factors
    3. Prostate Cancer
- Epididymis/Ductus Deferens
- Role of Androgens (T versus DHT)
    1. Androgen Metabolism
    2. 5 α Reductase Inhibitors
    3. Organ Culture
- Endocrine Disruption

Required Reading

Mullen RD and Behringer RR (2014) Sex Dev. 8(5):281-296.

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Sexual Development

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Molecular Genetics of Müllerian Duct Formation, Regression and Differentiation

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Key Words

Anti-Müllerian hormone · Human reproductive tract disorders · Müllerian duct · Regression · Reproductive tract organogenesis · Sex differentiation · Signal transduction

Abstract

The Müllerian duct (MD) forms the female reproductive tract (FRT) consisting of the oviducts, uterus, cervix, and upper vagina. FRT function is vital to fertility, providing the site of fertilization, embryo implantation and fetal development. Developmental defects in the formation and diseases of the FRT, including cancer and endometriosis, are prevalent in humans and can result in infertility and death. Furthermore, because the MDs are initially formed regardless of genotypic sex, mesenchymal to epithelial signaling is required in males to mediate MD regression and prevents the development of MD-derived organs. In males, defects in MD regression result in the retention of FRT organs and have been described in several human syndromes. Although to date not reported in humans, ectopic activation of MD regression signaling components in females can result in aplasia of the FRT. Clearly, MD development is important to human health; however, the molecular mechanisms remain largely undetermined. Molecular genetics studies of human diseases and mouse models have provided new insights into molecular signaling during MD development, regression and differentiation. This review will provide an overview of MD development and important genes and signaling mechanisms involved.

The Müllerian duct (MD) is the embryonic structure that develops into the female reproductive tract (FRT), including the oviduct, uterus, cervix, and upper vagina. The FRT has essential functions in mammals, providing the site of fertilization, embryo implantation and fetal development. Defects in human FRT formation, thought to arise from abnormal embryonic development, are estimated to occur in up to 3% of births and often result in fertility problems. Diseases of the FRT are also prevalent in adult women and include uterine and cervical cancers as well as endometriosis. Furthermore, the reproductive tracts of males and females initially contain identical pairs of fully formed Wolffian ducts (WDs) and MDs. During male sex differentiation, signaling between the MD mesenchyme and epithelium mediates MD regression and

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prevents its development into a FRT. In males, defects in MD regression result in the retention of MD-derived organs and have been described in human persistent Müllerian duct, Urioste and Denys-Drash syndromes. Although to date not reported in humans, activation of the signaling pathways responsible for MD regression in females results in aplasia of the FRT [Kobayashi and Behringer, 2003]. While an understanding of MD development is clearly important to human health, the cellular and molecular mechanisms of these processes remain largely unknown. Recent molecular genetics studies of human diseases and mouse models have identified multiple genes important for MD development (table 1). This review will provide an overview of MD formation, regression and differentiation and important genes and signaling mechanisms involved.

Embryology of the Urogenital System

In vertebrates, the urogenital system originates from the intermediate mesoderm and consists of the kidneys, the gonads as well as the urinary and reproductive tracts. Differentiation of the intermediate mesoderm into the urogenital tract begins shortly after gastrulation.

First, signaling from the somite and surface ectoderm transduces mesenchymal to epithelial conversions in the intermediate mesoderm and the anterior to posterior formation of the nephric ducts, a pair of epithelial tubes joined at the cloaca [Obara-Ishihara et al., 1999; Mauch et al., 2000]. Next, the primary kidney or pronephros transiently forms in the posterior region of the nephric ducts and subsequently degenerates [Saxen and Sariola, 1987; Bouchard et al., 2002]. Then, posterior to the degenerating pronephros, the mesonephric duct (WD) develops and extends in an anteroposterior direction. The metanephros arises from inductive interactions between the ureteric bud that branches from the caudal WD and mesenchyme [reviewed in Little et al., 2010]. Soon after formation of the WD, the paramesonephric duct (MD) appears and grows rostral to caudal adjacent to the WD until the duct joins at the urogenital sinus. Initially, the reproductive tracts of males and females are identical, containing 2 pairs of fully formed WDs and MDs. After sex determination, hormones produced in the fetal testis, anti-Müllerian hormone (AMH), testosterone and insulin-like 3 (INSL3), trigger regression of the MD, differentiation of the WD into the male genital tract, consisting of the vasa deferentia, epididymides and seminal vesicles, and testicular descent, respectively. In females, lack of AMH, testosterone and INSL3 in this developmental window permits differentiation of the MD into the FRT, consisting of the oviducts, uterus and upper vagina, passive degeneration of the WD and maintenance of the ovaries in an abdominal position, respectively (fig. 1) [reviewed in Kobayashi and Behringer, 2003].

The elongating MDs reach and fuse with the urogenital sinus to form the utero-vaginal duct that will give rise to the caudal uterus, cervix and upper vagina [Orvis and Behringer, 2007]. The rostral region of the MD develops into the oviducts and rostral uterus. Uterine morphology between different mammalian species is highly diverse and varies in part because of differences in the extent of rostral MD fusion. For example, fusion in rodents is minimal, resulting in a duplex uterus (consisting of 2 individual uterine horns connected at the cervix), while in primates fusion extends more rostrally, resulting in a simplex uterus (consisting of a single uterine cavity) [Kobayashi and Behringer, 2003].

MD Formation

The MD forms in 3 distinct phases: initiation, invagination and elongation (fig. 2A). The first phase, initiation, begins with the formation of a placode-like thickening and expression of the LIM *(lin-11*, *isl1* and *mec-3)* class homeodomain transcription factor, LHX1, in the rostral mesonephric epithelial cells fated to become MD epithelial cells [Orvis and Behringer, 2007]. *Lhx1* has important functions in reproductive tract development of both sexes. One *Lhx1* null male neonate had normal testes but lacked WD-derived organs [Kobayashi et al., 2004]. *Lhx1* null female mice form normal gonads but lack all MD-derived reproductive tract structures, including the oviducts, uterus and upper vagina [Kobayashi et al., 2004]. Transcriptional co-factors, DACH1 and DACH2, function redundantly in and are required for the formation of the MD. The WD forms normally; however, double *Dach1/2* mutant mice have severe defects in MD formation and differentiation and reduced MD expression of *Lhx1* and *Wnt7a.* This suggests that DACH proteins act upstream of *Lhx1* and *Wnt7a* and regulate expression, either directly or indirectly, of these and possibly other factors important for MD formation [Davis et al., 2008].

In the second phase of MD formation, invagination, MD-specified cells from the mesonephric epithelium extend caudally towards the WD. Expression of *Wnt4* in the mesonephric mesenchyme is necessary to signal the MD progenitor cells to begin invagination [Vainio et al., 1999;

Table 1. Mouse and human genes involved in MD development

Gene	Molecule encoded	Expression (mouse)	Reproductive tract phenotype [References]	
			mouse	human
Amh	TGF-β superfamily-secreted protein	Sertoli cells	ectopic FRT in males (N) [Behringer et al., 1994]	PMDS type I, ectopic FRT in males (AR) [Belville et al., 1999, 2009; di Clemente and Belville, 2006; Salehi et al., 2012]
Amhr2	TGF-β superfamily type II Ser/Thr trans-membrane receptor	MM	ectopic FRT in males (N) [Mishina et al., 1996]	PMDS type I, ectopic FRT in males (AR) [Belville et al., 1999, 2009; di Clemente and Belville, 2006; Salehi et al., 2012]
β-catenin (Ctnnb1)	signaling protein adhesion	MM, ME, WE, WM	males: ectopic FRT (C) [Kobayashi et al., 2011] females: hypotrophic uterine horns and defective oviduct coiling; myogenesis to adipogenesis switch (C) [Arango et al., 2005; Deutscher and Hung-Chang Yao, 2007; Jeong et al., 2009]	NA
Dlgh1	scaffolding protein	WE	cervix and vagina aplasia (N) [Iizuka-Kogo et al., 2007]	NA
Emx2	HTF	ME, WE	no FRT (N) [Miyamoto et al., 1997]	NA
Hoxa10	HTF	MM, WM	homeotic transformation of anterior uterus to oviduct (N) [Benson et al., 1996]	defects in MD fusion (AD) [Cheng et al., 2011; Ekici et al., 2013]
Hoxa11	HTF	MM, WM	partial homeotic transformation of uterus to oviduct (N) [Gendron et al., 1997]	NA
Hoxa13	HTF	MM, WM	agenesis of the caudal MD (N) [Warot et al., 1997] homeotic transformation of cervix to uterus *(Hd-* dominant negative allele of *Hoxa13)* [Post et al., 2000] homeotic transformation of anterior cervix to uterus (compound *Hoxa13*$^{+/-}$*; Hoxd13*$^{-/-}$) [Warot et al., 1997]	HFG syndrome (AD) [Mortlock and Innis, 1997; Goodman et al., 2000]
Lhx1	HTF	ME, WE	no FRT (N) [Kobayashi et al., 2004]	MRKH syndrome (AD) [Ledig et al., 2012]
Pax2	HTF	ME, WE	no FRT (N) [Torres et al., 1995]	NA
Rara, Rarb, Rarg	retinoic acid receptors	NA	compound mutants; varying degrees of MD defect (malformation to absence) [Mendelsohn et al., 1994]	NA
Tcf2	HTF	NA	NA	MODY5 with vaginal aplasia and rudimentary uterus (AD) [Lindner et al., 1999; Bingham et al., 2002]
Wnt4	Wnt-secreted protein	MM	no FRT (N) [Vainio et al., 1999] stratified luminal epithelial layer and reduced uterine gland numbers (C) [Franco et al., 2011]	MRKH syndrome (AD) [Biason-Lauber et al., 2007]
Wnt5a	Wnt-secreted protein	MM, ME (<E13.5) MM (>E13.5)	posterior MD growth defects; absence of uterine glands (N) [Mericskay et al., 2004]	NA
Wnt7a	Wnt-secreted protein	ME	males: ectopic FRT (N) [Parr and McMahon, 1998] female: homeotic transformation of oviduct to uterus and uterus to vagina; no uterine glands, abnormal mesenchyme differentiation (N) [Miller and Sassoon, 1998; Parr and McMahon, 1998]	NA
Wnt9b	Wnt-secreted protein	WE	absence of uterus and upper vagina (N) [Carroll et al., 2005]	NA
Wt1	zinc finger transcription factor	MM	NA	DDS, ectopic FRT in males in some cases [Denys et al., 1967; Barakat et al., 1974; Manivel et al., 1987]

This table lists known genes involved in the development and differentiation of the MD as demonstrated by mouse models and disease-causing gene mutations in humans. AD = Autosomal dominant; AR = autosomal recessive; C = conditional; DDS = Denys-Drash syndrome; FRT = female reproductive tract; HFG = hand-foot-genital; HTF = homeodomain transcription factor; ME = Müllerian epithelium; MM = Müllerian mesenchyme; MODY5 = maturity-onset diabetes of the young type 5; MRKH = Mayer-Rokitansky-Küster-Hauser; N = null; NA = not available; PMDS = persistent Müllerian duct syndrome; WE = Wolffian epithelium; WM = Wolffian mesenchyme.

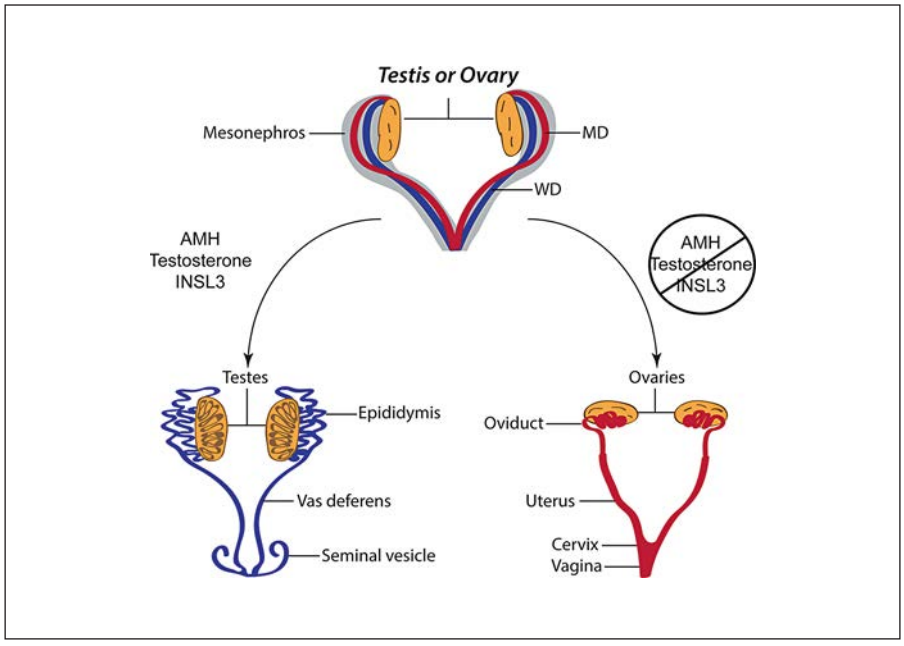

Fig. 1. Sexual differentiation of the reproductive tracts. The reproductive tracts prior to sexual differentiation are equivalent and contain a fully formed WD (blue) and MD (red). Hormones produced in the fetal testis, AMH, testosterone and INSL3, enable regression of the MD, differentiation of the WD into the male genital tract and testicular descent, respectively. In females, lack of AMH, testosterone and INSL3 at this developmental time permits differentiation of the MD into the FRT, passive degeneration of the WD and maintenance of the ovaries in an abdominal position, respectively. The WD differentiates into the male reproductive tract consisting of the vasa deferentia, epididymides and seminal vesicles. The MD develops into the FRT consisting of the oviducts, uterus and upper vagina. Adapted from Kobayashi and Behringer [2003].

Kobayashi et al., 2004]. The MD is absent in male and female *Wnt4* null mice at E11.5 and E12.5 [Vainio et al., 1999]. Loss of *Wnt4* does not alter *Lhx1* expression in the MD precursor cells; however, these cells fail to invaginate, indicating that *Wnt4* is necessary for invagination but not specification [Kobayashi et al., 2004].

The final phase of MD formation, elongation, begins when the invaginating tip of the MD contacts the WD. MD elongation continues in close proximity to the WD until the MD fuses at the urogenital sinus [Orvis and Behringer, 2007; Masse et al., 2009]. By E12.5 in the mouse, the MD has reached approximately the halfway point of its elongation path and crosses over the WD to be located medially. Elongation is complete by ~E13.5 with the MD reaching the urogenital sinus (fig. 2B) [Gruenwald, 1941; Orvis and Behringer, 2007]. While controversial in the past, it is now believed that the origin of the MD epithelium cells is a cell population of the mesonephric epithelium likely found in the transition area between the pronephros and mesonephros [Guioli et al., 2007; Orvis and Behringer, 2007]. There are cellular markers that are distinct between the WD, MD and mesonephric epithelium during MD formation. In mouse at E12.5, the WD expresses the epithelial markers cytokeratin 8 (CK8), pan cytokeratins and E-cadherin (CDH1) and lacks expression of the mesenchymal marker vimentin (Vim). Initially, the newly formed caudal portion of the MD is mesenchymal in nature and expresses Vim but is CDH1-negative. Later, the MD differentiates and expresses the standard epithelial cell markers with expression of CDH1 first evident in the most rostral region of the MD. At mouse E12.5 and E13.5, the mesonephric epithelium expresses both epithelial and mesenchymal cell markers [Orvis and Behringer, 2007].

Development of the MD is independent of sex genotype and occurs rostral to caudal. Only MD cells at the most caudal tip are in physical contact with WD cells during elongation (fig. 2B) [Orvis and Behringer, 2007]. Mesenchymal cells are present between the MD and WD and mesonephric epithelium in regions rostral to the caudal tip of the MD [Gruenwald, 1941; Orvis and Behringer, 2007]. Specification of MD precursor cells and the initial invagination of the mesonephric epithelium occur independently of the WD. However, elongation requires signaling and structure from the WD.

Organ culture studies demonstrated that physical disruption of the WD causes MD truncation, highlighting the link between WD and MD formation [Gruenwald, 1941]. The dependence of MD elongation on the WD has also been shown in several mutant mouse models in which the WD either fails to form (*Lhx1* and *Pax2* mutants), degenerates shortly after formation (*Emx2* mutants) or lacks key signaling molecules (*Wnt9b* mutants). In *Lhx1* null mice, the WD is completely absent and the MD fails to form [Kobayashi et al., 2004]. Paired-box gene 2 *(Pax2)* is expressed in the WD and MD and is nec-

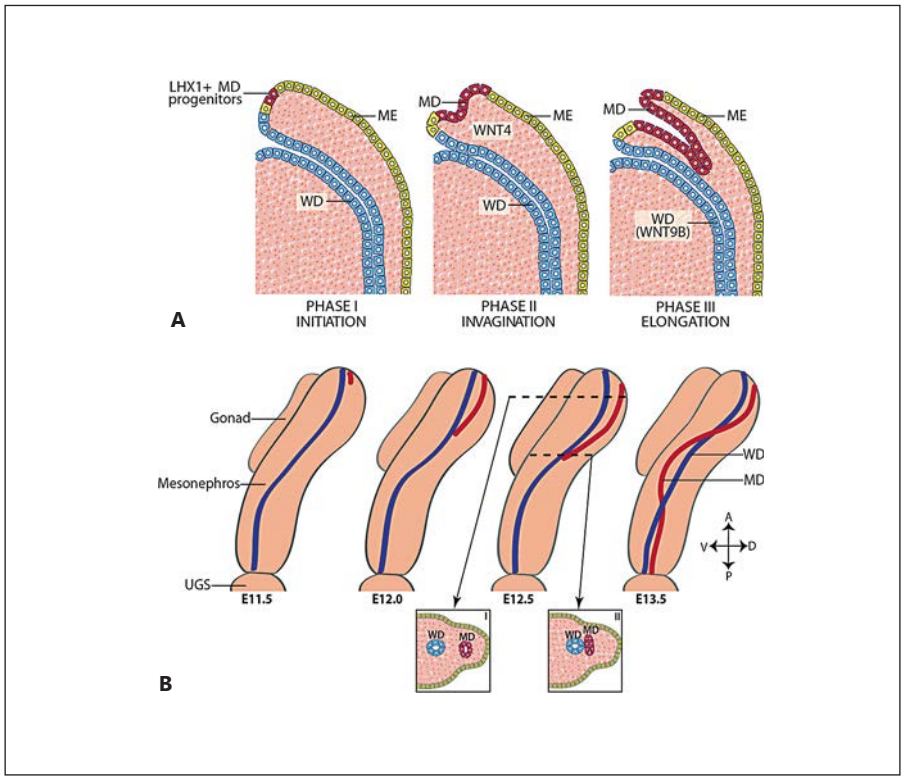

Fig. 2. MD formation. **A** MD (red) formation occurs in 3 phases: initiation, invagination and elongation. Phase I (initiation): MD progenitor cells in the mesonephric epithelium (ME) (yellow) are specified and begin to express LHX1. Phase II (invagination): in response to WNT4 signaling from the mesenchyme, LHX1+ MD progenitor cells invaginate caudally into the mesonephros towards the WD (blue). Phase III (elongation): the tip of the MD contacts the WD and elongates caudally in close proximity to the WD requiring structure and WNT9B signaling from the WD. **B** Beginning at ~E11.5 in mice, the MD invaginates and extends posteriorly guided by the WD. During elongation, mesenchymal cells separate the WD and MD anterior to the growing tip (**inset I**). However at the MD tip, the MD and WD are in contact (**inset II**). At ~E12.5, the MD crosses over the WD to be located medially. Elongation is complete by ~E13.5 with the MD reaching the urogenital sinus (UGS). Adapted from Kobayashi and Behringer [2003]. A = anterior (dorsal); D = dorsal; P = posterior (caudal); V = ventral.

essary for MD formation. *Pax2* null mice die shortly after birth, failing to form kidneys and reproductive tracts. In *Pax2* homozygous null mice, the rostral portion of the WD forms at E9.5 but does not elongate. By E12.5 the truncated WD has begun to degenerate. MD initiation and invagination occur normally but MD elongation occurs only along the truncated WD. By E16.5, the truncated WD and MD are absent [Torres et al., 1995]. Homeobox gene *Emx2* knockout mice lack kidneys, reproductive tracts and gonads in males and females and die shortly after birth due to renal dysfunction. The WD forms normally at E10.5, but by E11.5 the WD is degenerating. The MD fails to form in mutants and is absent at E13.5 [Miyamoto et al., 1997]. Although the structure of the WD is unaffected in *Wnt9b* mutant mice, MD elongation is blocked. This suggests that the WD guides elongation through the secreted WNT9B signal. As in studies with a disruption of WD structure, loss of WNT9B signaling did not affect MD specification and initial invagination, only caudal elongation [Carroll et al., 2005].

While the primary cause of MD loss in *Lhx1, Pax2* and *Emx2* null mice is likely a result of WD defects, these homeodomain transcription factors are suggested to have later functions in MD development. Chimera studies suggest that *Lhx1* is required cell-autonomously for the formation of the MD epithelium [Kobayashi et al., 2004]. It is also probable that *Pax2* functions cell-autonomously during MD formation and/or maintenance. PAX2 protein is expressed in both the MD and WD epithelium at E13.5. Further, *Pax2* is thought to be required for the mesenchyme to epithelium transitions in the intermediate mesoderm necessary for both WD and MD formation [Torres et al., 1995]. Similarly, *Emx2* is expressed in both the WD and MD epithelium at E13.5 suggesting additional roles in MD development [Miyamoto et al., 1997].

Caudal growth of the MD is thought to occur primarily as a result of proliferation of the MD epithelium. Cells from the WD or from the mesonephric epithelium following MD specification do not contribute significantly to the growing MD [Guioli et al., 2007; Orvis and Behringer, 2007]. During elongation, studies in mouse and chick have shown that proliferation is occurring along the length of the MD [Jacob et al., 1999; Guioli et al., 2007; Orvis and Behringer, 2007]. Organ culture of mouse urogenital ridges in which the rostral MD has been removed leaving the caudal MD tip region shows completion of MD elongation. Therefore, cells contained

in the caudal MD tip are capable of completing MD elongation in the absence of the rostral MD [Orvis and Behringer, 2007]. In organ culture studies of rat urogenital ridges, migration of MD epithelial cells has been shown to occur in the rostral to caudal direction during MD elongation. Furthermore, following extended culture with BrdU, both dividing and non-dividing cells are found in the MD tip suggesting migration may contribute to MD elongation [Fujino et al., 2009].

The PI3K/AKT pathway also has a role in MD elongation. Treatment with PI3K inhibitors in rat urogenital organ culture blocks MD elongation. PI3K inhibition also deterred lateral migration of the mesenchymal cells that separate the WD and MD. However, PI3K inhibition did not affect rostral to caudal migration of the MD epithelial cells in the already formed portions of the duct. Slight increases in apoptosis were observed in the MD after PI3K inhibition, but likely do not explain the MD elongation defect. The authors hypothesize that PI3K may be required to activate enzymes that break down the extracellular matrix that would otherwise block invasion by the caudal tip [Fujino et al., 2009].

Retinoic acid (RA) signaling is also required for the formation and/or maintenance of the MD. RA, a morphogen derived from vitamin A, has important functions in antero-posterior patterning of the body axis and during limb development [Dreyer and Ellinger-Ziegelbauer, 1996; Robert and Lallemand, 2006]. RA receptor *(Rar)* genes have redundant function in MD formation. In mouse, single gene mutants of *Rara1, Rara2, Rarb2*, or *Rarg* have no defects in FRT development. However, the MD is completely absent at E12.5 in *Rara1*/*Rarb2* compound mutants not attributable to any defects in WD formation. Additionally, other combinations of *Rar* mutations resulted in partial MD loss caudally, thus suggesting RA signaling has important functions in MD but not WD formation [Mendelsohn et al., 1994; Kastner et al., 1997]. Caudal defects in MD elongation are also observed in discs large homologue 1 *(Dlgh1)* null mice that cause MD fusion failure and obstruction which results in aplasia of the cervix and vagina [Iizuka-Kogo et al., 2007].

Sex Differentiation: MD Regression in Males

AMH Signaling Pathway

During male development, mesenchyme-epithelia interactions mediate MD regression to prevent its development into a uterus and oviduct (fig. 1). MD regression requires binding and signal transduction from the transforming growth factor-β (TGF-β) family member AMH secreted from the Sertoli cells of the fetal testis and its type 1 and 2 receptors expressed in MD mesenchyme [reviewed in Josso et al., 1993; Kobayashi and Behringer, 2003]. The transcription of the *Amh* gene is directly regulated by multiple factors in the testis-determining pathway including SRY-box containing gene 9 *(Sox9)*, steroidogenic factor 1 *(Sf1* alias *Nr5a1)*, Wilms tumor homologue *(Wt1)*, and DSS-AHC critical region on the X-chromosome gene 1 *(Dax1* alias *Nr0b1)* [Shen et al., 1994; De Santa Barbara et al., 1998; Nachtigal et al., 1998; Arango et al., 1999]. Females do not express AMH during fetal development thus allowing differentiation of the MD.

The first observable histological change during regression in males is the appearance of the 'sworl' pattern of the mesenchymal cells surrounding the MD in the most rostral region [Dyche, 1979; Orvis and Behringer, 2007]. At the onset of regression, differences in the MD also appear at the cellular level between the sexes. Initially the forming MD is mesoepithelial in nature with cell markers consistent with a mesenchymal cell tube; however, the morphology is consistent with a true epithelial cell tube [Dyche, 1979; Orvis and Behringer, 2007]. Beginning at E13.5, the female MD begins to express the epithelial cell marker CDH1 apically and to show evidence of apicobasal polarity while the MD in males remains unchanged [Orvis and Behringer, 2007]. This has also been observed in several other species including rat [Paranko and Virtanen, 1986; Dohr et al., 1987], human [Magro and Grasso, 1995], chick [Jacob et al., 1999], but not in golden hamster [Viebahn et al., 1987]. AMH-induced MD regression occurs in a specific window in time during development and after this time the MD is no longer sensitive to AMH-induced regression [Josso et al., 1976]. This window corresponds with the time frame in which the MD is not yet expressing epithelial-specific markers [Orvis and Behringer, 2007]. One hypothesis is that the mesoepithelial nature of the MD may facilitate regression in males.

Genetic experiments in mouse and naturally occurring mutations in humans have demonstrated that AMH is necessary and sufficient for MD regression. *Amh* null male mice have normal development of the testis and male reproductive tract; however, MD-derived tissues develop, causing infertility by physically blocking sperm release [Behringer et al., 1994]. Furthermore, female transgenic mice ectopically expressing human AMH lack MD-derived tissues [Behringer et al., 1990]. Additionally, mutations in the human *AMH* gene are causative of ~45%

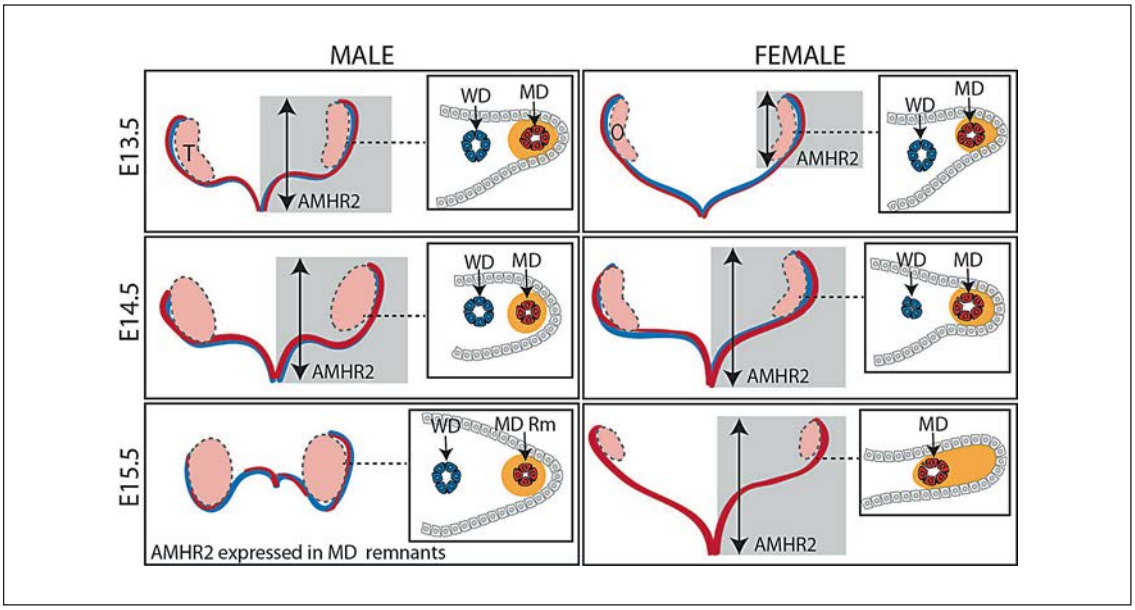

Fig. 3. MD regression. At E13.5 in mice, male and female WD (blue) and MD (red) are fully formed. In males, AMHR2 is expressed throughout the length (gray box and arrow) and in the MD mesenchyme (orange) in a tight ring around the MD epithelium (cross-section inset). In females, AMHR2 is caudally expressed and found in the MD mesenchyme on the antimesometrial side of the MD epithelium. By E14.5, expression of AMHR2 is found along the length of the MD in both sexes. At E14.5 in females, the WD is beginning to degenerate and is absent by E15.5. Portions of the MD have regressed in males by E15.5 in an apparently random pattern, and AMHR2 expression is limited to the remaining MD remnants. O = Ovary; Rm = remnant; T = testis.

of persistent Müllerian duct syndrome (PMDS) cases, a rare autosomal recessive disorder. Like *Amh* null mice, male patients with PMDS are normally virilized, but have female reproductive organs including a uterus and fallopian tubes. PMDS is most often diagnosed because of cryptorchidism, a failure of the testis to descend, and/or inguinal hernia [Belville et al., 1999, 2009; di Clemente and Belville, 2006; Salehi et al., 2012].

Amhr2-positive cells in the MD mesenchyme transduce the AMH hormone signal secreted from the fetal testis [Mishina et al., 1999]. AMH signaling occurs in a paracrine manner and begins with AMH binding to its type 2 receptor (AMHR2). AMHR2 then forms a heteromeric complex with and after that phosphorylates and activates a type 1 receptor. This activation results in the phosphorylation of an R-SMAD and supposedly formation of an R-SMAD/SMAD-4 complex that translocates into the nucleus to transcriptionally activate AMH signaling pathway target genes. AMH type 1 receptors ALK2 (AVCR2) and ALK3 (BMPR1A) as well as AMH R-SMAD effectors (SMAD1, SMAD5 and SMAD8) function redundantly in MD regression and are shared with the bone morphogenetic protein (BMP) pathway. ALK3 is considered the primary type 1 receptor required for regression; however, ALK2 is capable of transducing the AMH signal in the absence of ALK3. Conditional knockout of *Alk2* in the MD mesenchyme does not block MD regression. However, approximately half of all *Alk3* conditional mutant males and 100% of mutants with conditional knockout of both *Alk2* and *Alk3* failed to regress the MD [Jamin et al., 2002; Orvis et al., 2008].

ALK2 and ALK3 are believed to function in a distinct temporal and spatial manner during regression. Initially, *Amhr2* is expressed in the mesonephric epithelium. In the mesonephric epithelium, ALK2 appears to mediate AMH signaling which directs *Amhr2*-positive cells to undergo an epithelial to mesenchymal transition and migrate to surround the MD epithelium forming the distinct 'sworl' pattern observed at E15.5 [Zhan et al., 2006]. This is followed by the breakdown of the basement membrane and subsequent loss of the epithelium [Dyche, 1979; Trelstad et al., 1982; Orvis and Behringer, 2007]. In females, *Amhr2* expressing cells are found in the mesonephric epithelium on the antimesometrial side of the MD, but without AMH signaling, these cells do not undergo migration to surround the MD epithelium or cellular changes up to at least E15.5 (fig. 3) [Zhan et al., 2006; Arango et al., 2008; Orvis et al., 2008].

Mechanisms known to be involved in regression include epithelial cell migration, epithelial to mesenchymal transformations and apoptosis [Hutson et al., 1984; Austin, 1995; Allard et al., 2000]. The pattern of regression of the MD is hypothesized to occur in a rostral to caudal wave corresponding to *Amhr2* expression. A statically imaged time course of ex vivo cultured rat urogenital ridges (males and AMH-treated females) during regression showed rostrally a reduction in MD diameter [Picon, 1969; Tsuji et al., 1992]. Furthermore, Allard et al. [2000] showed a positive correlation between the rostral-caudal wave of *Amhr2* expression and the pattern of increased apoptosis of the MD epithelium during regression in rat. In contrast, studies from the *Amhr2-lacZ* knock-in mouse model indicate that, although *Amhr2* expression initiates in a rostral to caudal wave, it is expressed along the entire length of the MD at E13.5 well before any overt changes in the male MD (fig. 3) [Arango et al., 2008]. Further studies will be needed to clarify the spatiotemporal patterning and cell behaviors, including migration and apoptosis, during MD regression.

Genetic studies in mouse and human indicate that AMHR2 is the sole type 2 receptor required for AMH signaling and is likely dedicated to the AMH signaling pathway. Male *Amhr2* and *Amh* mutant mice have identical phenotypes: normally virilized with persistent MD-derived organs [Behringer et al., 1994; Mishina et al., 1996, 1999]. Additionally, the phenotype of hAMH expressing transgenic female mice is rescued by *Amhr2* mutation [Behringer et al., 1990; Mishina et al., 1999]. Furthermore, mutation of the *AMHR2* gene accounts for about half of the cases of PMDS with known molecular etiology [di Clemente and Belville, 2006; Belville et al., 2009; Salehi et al., 2012].

The *Wt1* gene is a direct activator of *Amhr2* transcription. *Amhr2* transcript levels are reduced in *Wt1* null mice and both transcripts are co-expressed in the developing MD. Additionally, *Wt1* expression mirrors the sexually dimorphic pattern observed for *Amhr2* expression during MD regression in the urogenital ridge. Furthermore, in vitro assays show that WT1 activates transcription of *Amhr2* and binds to elements in the *Amhr2* proximal promoter [Klattig et al., 2007]. Two alternate splice variants of *Amhr2*, *Amhr2*-Δ exon 2 and *Amhr2*-Δ exon 9 and 10, are found in adult rat and mouse. The *Amhr2* splice variants act in a dominant negative manner when co-transfected with full-length AMHR2 and AMH in in vitro luciferase reporter assays. The dominant negative effect of the splice variants was reduced at higher levels of AMH concentration corresponding to local levels of AMH in the gonads. The authors hypothesize that splice variants may be expressed at high levels only in a particular subset of gonadal cells and regulate AMH signaling in these cells. Alternatively, splice variants may have site-specific effects independent of the presence of AMH ligands or may have a role in the transport of ligands into the cell and might be important in trafficking across the blood brain barrier [Imhoff et al., 2013]. Expression of these splice variants during MD regression has not been determined, and it is currently unknown what, if any, role they have during reproductive tract development.

Wnt signaling plays multiple roles in MD development and is needed for formation, regression and differentiation. Prior to the onset of MD regression in males, WNT7A signaling from the epithelium to mesenchyme of the MD activates *Amhr2* expression in both sexes and is also required for appropriate differentiation of the MD. *Wnt7a* mutant males retain MD-derived organs because *Amhr2* expression is lost in the MD mesenchyme, thus blocking the AMH signaling pathway. Consistent with the differentiation defects observed in the *Wnt7a* mutant FRT, in mutant males, the ectopic FRT shows no evidence of oviduct coiling and is a simple epithelial tube [Parr and McMahon, 1998]. This is in contrast to *Amh* and *Amhr2* null mouse models where the differentiation of the mutant female and male MD-derived organs is relatively normal [Behringer, 1994; Behringer et al., 1994; Mishina et al., 1996]. The frizzled *(Fzd)* genes encode the 7 transmembrane protein receptors for the WNT ligand which are required for both canonical and non-canonical WNT signaling pathways. A dedicated FZD receptor has not been identified for WNT7A in the MD. Previous studies showed that interactions between WNT7A and FZD10 activated the WNT pathway [Kawakami et al., 2000]. Additionally, in mouse, *Wnt7a* and *Fzd10* have overlapping expression patterns in the MD [Nunnally and Parr, 2004]. Although this identified FZD10 as a potential candidate receptor for WNT7A, *Fzd10* knockout mice have no reproductive tract phenotype. Similar to *Fzd10, Fzd1* expression is found in the MD mesenchyme and epithelium at E14.5, but *Fzd1* knockout males have no MD regression defects [Deutscher and Hung-Chang Yao, 2007; Lapointe et al., 2012]. This suggests that multiple FZD receptors are capable of interaction with WNT7A and function redundantly during MD regression.

AMH Signaling: Downstream Molecular Mechanisms

Several studies suggest that WNT signaling is also important to the downstream molecular signaling cascade required for MD regression during male reproductive tract differentiation. However, the exact role of WNT sig-

naling following activation of the AMH signaling pathway remains unclear. Either inactivation or constitutive activation of β-catenin (CTNNB1) in the MD mesenchyme causes retention of MD-derived tissues in mutant males independent of AMH expression, suggesting tight control of CTNNB1 activation is necessary for MD regression [Tanwar et al., 2010; Kobayashi et al., 2011]. In the canonical WNT signaling pathway, nuclear-localized CTNNB1 in a complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors regulates expression of target genes. CTNNB1 is also known to have roles in cell tight junction formation and adhesion and may therefore be functioning independently of WNT signaling during regression [Brembeck et al., 2006]. CTNNB1 activates *Lef1* transcription and upregulates *Lef1* promoter activity in vitro [Filali et al., 2002; Vadlamudi et al., 2005]. Additionally, *Ctnnb1* inactivation results in the loss of LEF1 upregulation normally observed in the MD mesenchyme of males during regression [Kobayashi et al., 2011]. This suggested LEF1 may be required downstream of WNT/β-catenin signaling to induce MD regression during male reproductive tract differentiation. However, *Lef1* null male mice have normal MD regression [van Genderen et al., 1994; Mullen and Behringer, unpubl. observations]. Several additional WNT pathway factors have also been identified that are expressed in a sex-specific pattern in the mesenchyme during AMH-induced MD regression including *Wnt4, Wnt5a* and frizzled-related WNT pathway genes *Sfrp1, Sfrp2* and *Sfrp5*. Knockout of *Wnt4* in the MD mesenchyme does not interfere with MD regression [Kobayashi et al., 2011]. No defects in MD regression have been reported in $Wnt5a^{-/-}$ mice [Mericskay et al., 2004]. Likewise, loss of function of *Sfrp2* and *Sfrp5* caused no defects in MD regression [Cox et al., 2006]. Double knockout $Sfrp1^{-/-}/Sfrp2^{-/-}$ mice appear to have a slight delay, but MD regression is complete at later embryonic stages [Warr et al., 2009]. These results suggest that WNT pathway factors have redundant function during MD regression or alternatively are not required for regression. Further studies will be needed to clarify the roles of WNT signaling during later stages of MD regression.

Sexually dimorphic expression patterns during regression have also been identified for matrix metalloproteinase 2 *(Mmp2)*. *Mmp2* is upregulated in the male MD mesenchyme during regression, and this upregulation is lost in *Amh* null males. Morpholino knockdown of *Mmp2* in organ culture blocks regression and decreases MD epithelium apoptosis [Roberts et al., 2002]. *Mmp2* null mice, however, have no defects in MD regression. This may suggest redundant function with other genes [Itoh et al., 1997; Roberts et al., 2002]. The PI3K/AKT pathway may also have a role in AMH signal transduction during regression. Activated phospho-AKT (p-AKT) is present in equal amounts in the WD and MD in both sexes prior to regression. In rats, synchronous with the initiation of MD regression, p-AKT is decreased in males at E15.5 and is undetectable at E16.5. Females maintain p-AKT expression. This pattern is also observed in mice [Fujino et al., 2009]. Although PI3K signaling has been shown to prevent apoptosis and the epithelial to mesenchymal transitions that take place during MD regression, it is not clear if this reduction in p-AKT is a cause or effect of regression [Dyche, 1979; Trelstad et al., 1982; Allard et al., 2000; Zhan et al., 2006; Fujino et al., 2009]. AMH signaling has been shown to inactivate the PI3K pathway by blocking autophosphorylation of the EGF receptor in the MD epithelium by inhibiting tyrosine kinase [Hutson et al., 1984; Hurst et al., 2002]. Although multiple genes have been identified using candidate approaches, the role of many of these signaling pathways and molecules remains unclear due to the possibility of functional redundancy.

MD Differentiation in Females

Once the MD is formed, it differentiates into a functional oviduct, uterus, cervix, and upper vagina. Correct patterning and differentiation of the MD is dependent on a complex network of *Hox* and *Wnt* genes. Furthermore, it is known that steroid hormones also regulate many of the genes necessary for proper MD differentiation during organogenesis and adulthood [Masse et al., 2009]. Abdominal B *(AbdB)* homeobox genes *(Hoxa9*, *Hoxa10*, *Hoxa11*, and *Hoxa13)* of the mammalian *Hoxa* cluster are required for differentiation and segmental patterning of the MD. *AbdB* genes are expressed along the anterior-posterior axis of the MD according to their 3′ to 5′ order in the *Hoxa* cluster. *Hoxa9* is expressed in the oviduct, *Hoxa10* in the mesenchyme of the uterus, *Hoxa11* in the posterior uterus and cervix, and *Hoxa13* in the cervix and upper vagina [Taylor et al., 1997; Warot et al., 1997]. *Hoxa10* expression is necessary for correct specification of tissue boundaries in the male and female reproductive tract. In *Hoxa10* null mice, male and female reproductive tracts display posterior to anterior homeotic transformation. At E17.5, *Hoxa10* is present only in the portion of the MD that will differentiate into the uterus. Mutations in *Hoxa10* result in homeotic transformation of

25% of the proximal uterus into oviduct [Benson et al., 1996]. Three heterozygous mutations in the *HOXA10* gene with predicted loss of function have been associated with uterine malformations. Patients with *HOXA10* mutations had uterine defects ranging from septate uterus and vagina with a duplex cervix to a didelphic uterus indicative of MD fusion defects [Cheng et al., 2011; Ekici et al., 2013]. In the adult uterus, *Hoxa10* represses *Emx2* and is found in an inverse expression pattern suggesting a further role for *Emx2* in MD patterning and differentiation [Troy et al., 2003]. Overlapping expression patterns of *Hoxa10* and *Hoxa11* suggest they have partially redundant function during MD differentiation. Furthermore, exposure to the non-steroidal estrogen diethylstilbestrol (DES) in mice caused a posterior shift in *Hoxa9* expression, likely a result of down-regulation of *Hoxa10* and *Hoxa11* [Block et al., 2000]. Sex steroids mediate *Hoxa10* and *Hoxa11* expression levels. Women exposed to DES during development have malformations of the reproductive tract consistent with anterior transformation [Taylor et al., 1999; Cermik et al., 2003]. *Hoxa11* null mice have a thinner and shorter uterus lacking glands consistent with a partial homeotic transformation [Gendron et al., 1997]. Mutant mice in which the *Hoxa11* homeodomain was replaced with the *Hoxa13* homeodomain displayed posterior homeotic transformation of the FRT with the posterior uterus becoming cervix/vagina. This demonstrates that *Hoxa11* and *Hoxa13* have unique functions in MD differentiation and that *Hoxa13* is upstream of factors required for differentiation of the MD into cervix and vagina [Zhao and Potter, 2001]. Although *Hoxa13* null mutants die between E13.5 and E14.5, mutant female embryos are missing the caudal portion of the MD, suggesting *Hoxa13* has a function during MD formation in addition to MD differentiation. Defects in caudal MD formation were also observed in *Hoxa13* paralogue, *Hoxd13,* mutant females at birth [Warot et al., 1997].

The WNT pathway is required for MD patterning and differentiation. *Wnt7a* has important functions in both MD regression in males and MD differentiation in females. *Wnt7a* is expressed throughout the MD epithelium prior to birth. After birth, expression is maintained in the oviductal and uterine epithelium but is down-regulated in the vaginal epithelium [Miller et al., 1998]. In adult and neonate *Wnt7a* mutant females, the uterus is smaller in length and diameter, the uterine wall is thinner with less smooth muscle, and uterine glands are not present. Oviduct differentiation occurs but coiling and elongation are absent. Posteriorly, a homeotic transformation occurs of oviduct to uterus and uterus to vagina in the *Wnt7a* null females [Miller and Sassoon, 1998; Parr and McMahon, 1998]. *Wnt7a* also appears to be necessary for the maintenance of *Hoxa10* and *Hoxa11* expression with the *Wnt7a* null females showing reduced expression of the 2 genes. Additionally, the similarity between the *Hoxa11* null and *Wnt7a* null mutant phenotype (thin, small uterus lacking glands) is consistent with upstream regulation of *Hoxa11* by *Wnt7a* [Miller and Sassoon, 1998; Parr and McMahon, 1998].

Recent studies using conditional knockouts and tissues explanted under the kidney capsule have also clarified the role for WNT signaling at later stages of MD differentiation. These WNT signaling molecules include *Wnt4, Wnt5a* and *Ctnnb1,* which were not previously described because of early embryonic lethality *(Wnt5a* and *Ctnnb1)* or early roles in MD formation *(Wnt4)*. The role of *Wnt4* in MD formation is well established. A recent conditional knockout study also demonstrates that *Wnt4* is important for MD differentiation. Conditional inactivation of *Wnt4* in the uterine luminal and glandular epithelium, stroma and myometrium using progesterone receptor *(PR)-Cre* resulted in reduced uterine gland numbers and a stratified luminal epithelial layer instead of a simple columnar epithelial cell layer [Franco et al., 2011]. The receptor for *Wnt4* during MD development has not been identified. However, *Fzd1* expression has been found in the developing mesonephros in both the MD mesenchyme and epithelium [Deutscher and Hung-Chang Yao, 2007]. Furthermore, 3 of 17 $Fzd1^{-/-}$ females had a uterine phenotype similar to the *Wnt4* conditional mutant females suggesting FZD1 may be acting as the WNT4 receptor [Lapointe et al., 2012]. However, because of the low penetrance of uterine defects in the $Fzd1^{-/-}$ mutant females, it is likely that other FZD receptors are also able to transduce the WNT4 signal.

The *Wnt5a* gene is required for the development of the posterior (caudal) region of the MD and glandular genesis. *Wnt5a* null mice have short, coiled uterine horns, but lack a cervix and vagina. In kidney capsule explant studies of mutant uterine horns, both *Wnt7a* in the luminal epithelium and *Wnt5a* in the uterine stroma were required for gland formation independently of canonical pathway member *Lef1* [Mericskay et al., 2004]. Conditional deletion of *Ctnnb1* in the MD mesenchyme using *Amhr2-Cre* causes a hypoplastic uterus with uterine hypotrophy, reduced uterine glands and uncoiled oviducts. In *Ctnnb1* conditional mutants, reduced proliferation but not apoptosis

contributes to the hypoplasia of the uterus. No differences were found in the expression patterns of *Wnt4* and *Wnt5a* in the mesenchyme or *Wnt7a* in the epithelium [Deutscher and Hung-Chang Yao, 2007]. The mutant phenotype including lack of coiling mimics the *Wnt7a* null phenotype [Parr and McMahon, 1998]. Distinct from the *Wnt7a* null mice, deletion of *Ctnnb1* in the MD mesenchyme resulted in the differentiation of smooth muscle cells into adipose tissue postnatally causing the uterus to become fat-filled. This suggests other WNTs may be needed for uterine differentiation or, alternatively, the role of *Ctnnb1* in the mesenchyme is independent of WNTs and is functioning instead to control cell adhesion or the formation of cell tight junctions. These studies suggest that the initial differentiation of the myometrium does not require *Ctnnb1*, but in its absence there is a progressive shift from smooth muscle tissue to adipose tissue [Arango et al., 2005; Deutscher and Hung-Chang Yao, 2007]. *Ctnnb1* conditional ablation using *PR-Cre* resulted in a thinner uterus of normal length with reduced gland numbers at sexual maturity. Constitutive activation of CTNNB1 using *PR-Cre* reduced uterus length and caused hyperplasia of uterine glands. Reductions in the size of the uterus as a result of ablation or activation of *Ctnnb1* suggest tight control of WNT signaling is required for proper MD differentiation and development [Jeong et al., 2009].

The *Wnt4* and *Ctnnb1* conditional mutant females and *Wnt7a*$^{-/-}$ and *Wnt5a*$^{-/-}$ null females have similar defects in MD differentiation. These are also similar to the uterine phenotype caused by DES exposure suggesting a link between WNT signaling and estrogen signaling. WNT signaling ligands in the luminal epithelium may be required to prevent the formation of a stratified epithelial layer in the uterus in response to estrogen signaling [Franco et al., 2011].

Human MD Formation and Differentiation Defects

Aberrant development of the MD is a relatively frequent cause of human birth defects. Defects include MD aplasia, MD persistence as well as MD fusion and patterning defects. Multiple medical syndromes are associated with FRT abnormalities. Molecular genetic studies of these patients have identified candidate factors involved in some cases. However, the molecular genetic cause of these syndromes remains unknown in the majority of cases [Kobayashi and Behringer, 2003]. Several well-characterized syndromes are described: Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome (OMIM 277000), maturity-onset diabetes of the young type 5 (MODY5; OMIM 604284), PMDS (OMIM 261550), Urioste syndrome (OMIM 235255), Denys-Drash syndrome (DDS; OMIM 194080) and Hand-foot-genital (HFG) syndrome (OMIM 140000).

MD Aplasia Syndromes

MRKH syndrome occurs in about 1 in 4,500 female births and is characterized by the absence of the uterus and upper vagina in genetic females (46,XX) [Folch et al., 2000]. In the majority of affected patients, ovarian development is normal, and the lower third of the vagina is present. The molecular etiology of most cases of MRKH is unknown; MRKH syndrome occurs most often due to sporadic mutations but familial cases have been described with autosomal dominant inheritance with incomplete penetrance and variable expressivity. MRKH syndrome is further classified as type I (typical; restricted to the reproductive tract) or type II (atypical; associated with additional developmental defects) [Ledig et al., 2011]. MD aplasia, unilateral renal agenesis and cervicothoracic somite anomalies (MURCS) association is a severe form of MRKH type II. Patients with MURCS association have renal and skeletal defects in addition to uterine and vagina aplasia or agenesis [Oppelt et al., 2006]. Plausible causes of MRKH syndrome are mutations increasing either AMH or AMHR2 activity and/or expression that could result in MD regression in females. However, no defects in either the *AMH* or *AMHR2* genes have been discovered to date [Oppelt et al., 2005].

Multiple genes have been associated with MRKH syndrome, including *WNT4, TCF2* (also known as *HNF1β* or *v-HNF1*), *LHX1*, and short stature homeobox *(SHOX)*. Heterozygous loss-of-function mutations in the human *WNT4* gene cause a complete absence of the uterus and upper vagina. In addition, female patients with *WNT4* mutations also have excess androgens and symptoms of virilization [Biason-Lauber et al., 2004, 2007]. This is consistent with the phenotype of *Wnt4* null mice in which the MD does not develop and testosterone biosynthesis is ectopically activated in the ovary [Vainio et al., 1999]. The most common mutations associated with MRKH syndrome are deletions of chromosomal region 17q12 which contains both *TCF2* and *LHX1* genes with ~6% of examined cases of MRKH carrying this deletion. TCF2 is a POU domain containing transcription factors widely expressed during development with function in epithelial differentiation [Coffinier et al., 1999a; Kolatsi-Joannou et al., 2001]. Heterozygous mutations of *TCF2* were first associated with MODY5 with additional malformations in renal development and function. A subset of female patients with heterozygous muta-

tions in *TCF2* have malformations of the reproductive tract including bicornuate uterus, uterus didelphys as well as MD aplasia and renal defects in the absence of diabetes [Lindner et al., 1999; Bingham et al., 2002]. This suggests an important role for *TCF2* in urogenital tract formation and maintenance. In mouse, *Tcf2* is expressed in the reproductive tract epithelium during development and persists in the adult [Coffinier et al., 1999a; Reber and Cereghini, 2001]. However, the function of *Tcf2* in mouse urogenital development remains undetermined due to the early embryonic lethality of *Tcf2* null mutant mice and the normal phenotype of the heterozygous mutants [Barbacci et al., 1999; Coffinier et al., 1999b]. To date, MRKH syndrome has also been associated with 5 heterozygous mutations in the human *LHX1* gene; 4 missense mutations and a frame shift mutation leading to a stop codon [Ledig et al., 2011, 2012; Sandbacka et al., 2013]. Additionally, *Lhx1* has been shown in mice to be essential for MD formation [Kobayashi et al., 2004]. Partial duplication of the *SHOX* gene was found in 2 daughters with MRKH type I and their unaffected father [Gervasini et al., 2010].

MD Persistence Syndromes

The development of a uterus and oviduct in human males has been noted in 3 syndromes: PMDS, Urioste syndrome and DDS. PMDS patients have normal testis development and the presence of MD-derived female reproductive organs. The syndrome is usually diagnosed while correcting undescended testes in pediatric patients. Reduced fertility is common in PMDS patients, and potential causes include structural abnormalities caused by MD remnants, cryptorchidism past the age of 2 years and damage to the vas deferens during orchidopexy. There is also an increased risk of malignancy in the ectopic MD-derived organs if not surgically removed and in the testes due to cryptorchidism. Eleven cases of malignancy in the retained MD organs have been reported and laparoscopic removal of the MD structures in PMDS patients is recommended [Farikullah et al., 2012]. PMDS is further classified with type I males having undetectable levels of AMH and type II males with normal AMH levels. The majority of PMDS cases are caused by mutations in the *AMH* (type I) or *AMHR2* (type II) genes with each representing about half of the cases with known molecular etiology [di Clemente and Belville, 2006; Belville et al., 2009; Salehi et al., 2012].

Urioste syndrome is an autosomal recessive disorder associated with the retention of MD-derived tissues in males. In addition to a persistent MD phenotype, patients also have lymphangiectasia and postaxial polydactyly. The molecular basis of this syndrome is currently unknown [Urioste et al., 1993].

DDS is characterized by partial gonad dysgenesis, congenital or infantile nephropathy and Wilms' tumor. The molecular cause of DDS in almost all cases is dominant loss-of-function mutations in the zinc finger DNA binding domain of WT1. In multiple cases of DDS, patients have MD-derived uterus and vagina remnants in addition to developed vas deferens and epididymis [Denys et al., 1967; Barakat et al., 1974; Manivel et al., 1987]. *Amh* and *Amhr2* are regulated by WT1; therefore, reductions in AMH ligand and its receptor are postulated to cause the defects in MD regression seen in DDS patients [Nachtigal et al., 1998; Hossain and Saunders, 2003; Klattig et al., 2007]. The most common *WT1* gene mutation in DDS is a missense mutation in exon 9, 1180C>T (R394W). The presence of retained MD structures is found in some but not all patients including those from the same family [Coppes et al., 1992; Zhu et al., 2013]. Mouse models heterozygous either for the *Wt1* null allele or the R394W mutation have no evidence of MD regression defects [Gao et al., 2004]. Together this suggests that genetic background and/or environmental factors may play an important role in determining the penetrance of MD regression defects in DDS patients.

MD Fusion and Patterning Defect Syndrome

HFG is an autosomal dominant syndrome that results in shortened thumbs and big toes and genital defects including hypospadias in males and a range of FRT defects from a longitudinal vagina or double vagina to double uterus and cervix. Incomplete MD fusion during embryogenesis gives rise to these defects in females with HFG [Goodman and Scambler, 2001]. The similarity of the hypodactyly *(Hd)* mutant mouse phenotype with a spontaneous dominant negative mutation in the first exon of the *Hoxa13* gene to the limb and genital defects in humans first identified *Hoxa13* as a potential candidate gene [Post et al., 2000]. Although MD fusion defects are not present in *Hd* and *Hoxa13*$^{-/-}$ mice, mild hypospadias of the vagina is observed in a portion of the mutant females [Warot et al., 1997; Post et al., 2000]. Additionally, 1 in 6 female compound mutants of *Hoxa13* and its paralogue, *Hoxd13*, had MD fusion defects [Warot et al., 1997]. Furthermore, 6 heterozygous mutations in the human *HOXA13* gene have been reported in families with HFG to date [Mortlock and Innis, 1997; Goodman et al., 2000].

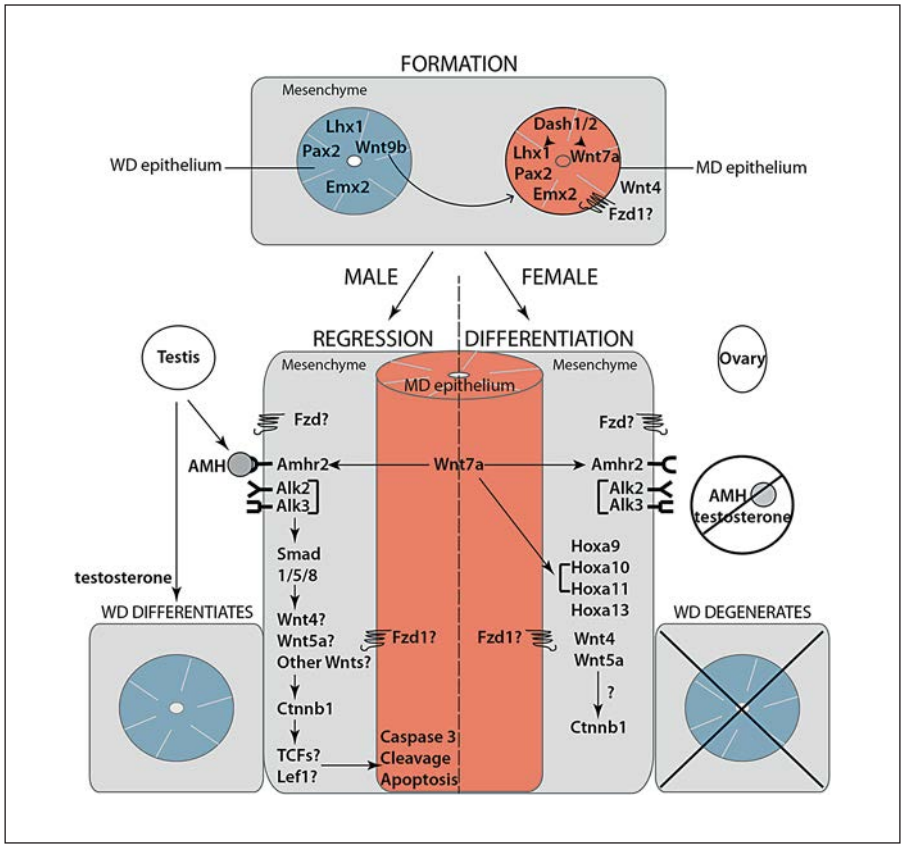

Fig. 4. Genes involved in MD development. Multiple factors and signaling pathways in the mesenchyme (gray) and epithelium of both ducts (WD epithelium, blue; MD epithelium, red) act in concert to direct MD development. Hormones and key genes required for MD formation, regression in males and differentiation in females are indicated in this figure.

Conclusions

Much progress has been made in understanding the molecular genetics of MD development, and several important signaling pathways have been identified (fig. 4). Study of knockout and conditional knockout mouse model phenotypes and molecular genetic studies of human diseases of FRT development have provided key insights into the complex signaling cascade involved. However, potential functional redundancy of many of these factors, including WNT signaling pathway members, MMPS and HOX genes, has made it difficult to assess their in vivo function. For example, MD regression requires tight regulation of CTNNB1 activation, but thus far a single WNT required for MD regression has not been identified. Furthermore, it is likely that other factors yet to be identified are involved in MD development. Expression profiling using next-generation sequencing technologies should identify genes that are differentially expressed during MD formation and differentiation. By using *Cre* recombinase lines expressing in the MD epithelium *(Wnt7a-Cre)* and mesenchyme *(Amhr2-Cre),* it will be possible to globally uncover the transcriptome of these juxtaposed tissue types. Mesenchyme-epithelia interactions are important regulators of development, and many defects in FRT development are the result of aberrant cell-cell communication and signaling. Understanding the molecular and cellular mechanisms of MD formation and differentiation will give key insights into FRT development and disease.

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Zhan Y, Fujino A, MacLaughlin DT, Manganaro TF, Szotek PP, et al: Müllerian inhibiting substance regulates its receptor/SMAD signaling and causes mesenchymal transition of the coelomic epithelial cells early in Müllerian duct regression. Development 133:2359–2369 (2006).

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Spring 2018 – Systems Biology of Reproduction
Lecture Outline – Male Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
January 30, 2018
Week 4

Male Reproductive Tract Development & Function

Embryonic Development and Reproductive Tract Organogenesis
- Overview
- Development of Mullerian Duct vs. Duct Wolffian Duct Derivatives
- Mullerian Inhibiting Substance (MIS)

Male Urogenital Tract Organogenesis
- Prevention of Programmed Cell Death in the Wolffian Duct
- UGS/Prostate/Seminal Vesicle
  1. Prostate Morphogenesis (ductal branching)
  2. Cell-Cell Interactions and Paracrine Factors
  3. Prostate Cancer
- Epididymis/Ductus Deferens
- Role of Androgens (T versus DHT)
  1. Androgen Metabolism
  2. 5 α Reductase Inhibitors
  3. Organ Culture
- Endocrine Disruption

Required Reading

Mullen RD and Behringer RR (2014) Sex Dev. 8(5):281-296.

Spring 2018 – Systems Biology of Reproduction
Discussion Outline – Male Reproductive Tract Development & Function
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
February 1, 2018
Week 4

Reproduction Tract Development & Function

Primary Papers:

1. Murashima, et al. (2015) Asian J Andrology 17:749-755
2. Okazawa, et al. (2015) Developmental Biology 400(1):139-47
3. Liu, et al. (2017) Sexual Development 11:190-202

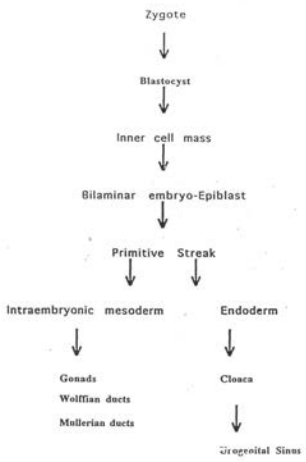
Discussion

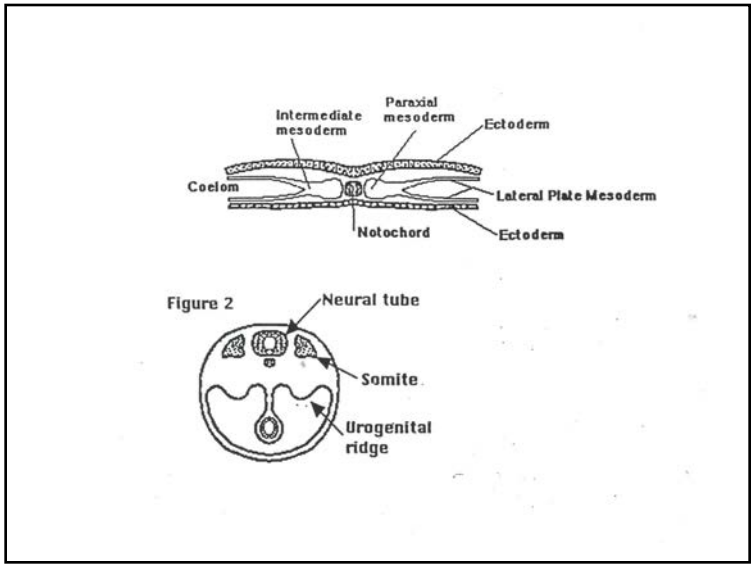
Student 7: Classic Reference 1 above
- What are the developmental steps of the Wolffian/epididymal duct?
- What are the Phenotypes of knockouts that explain the development?
- What technology was used

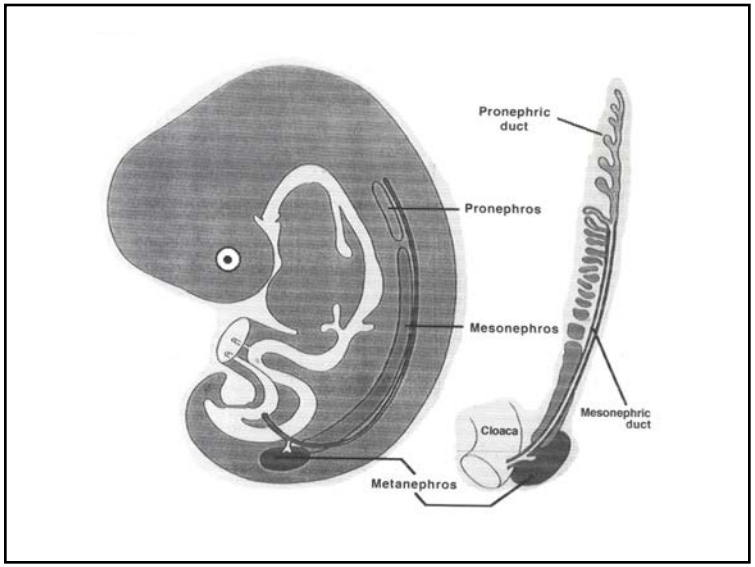
Student 8: Reference 2 above
- What is the technology used?
- Where is the expression pattern of the FGF receptor?
- What does the knockout phenotypes show on regional actions of FGF receptor?

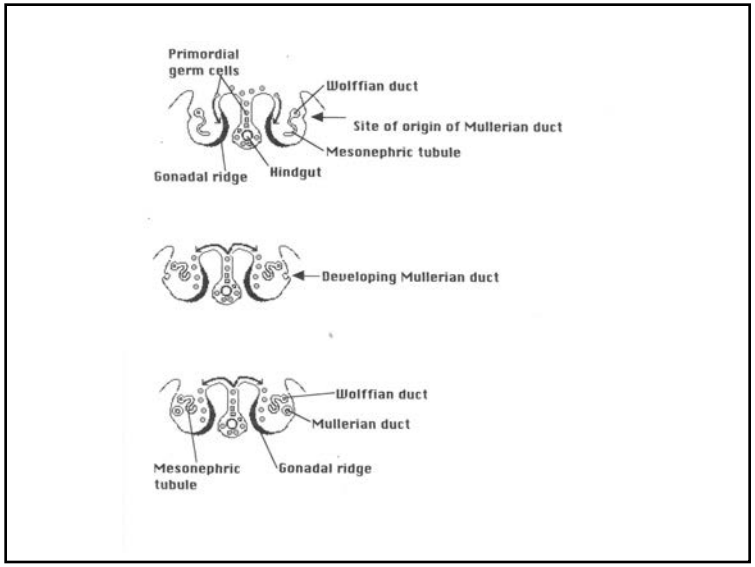
Student 9: Reference 3 above
- What is the technology used?
- What androgen alterations in actin localization were observed?
- What basic information on male reproductive tract development was obtained?

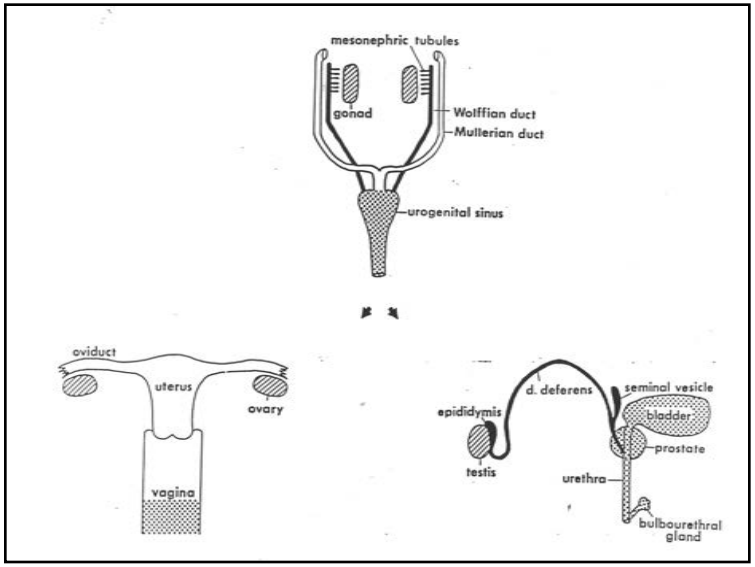
Development




Intermediate mesoderm
Paraxial mesoderm
Ectoderm
Coelom
Lateral Plate Mesoderm
Notochord
Ectoderm
Figure 2
Neural tube
Somite
Urogenital ridge


Pronephric duct
Pronephros
Mesonephros
Mesonephric duct
Cloaca
Metanephros


Primordial germ cells
Wolffian duct
Site of origin of Mullerian duct
Mesonephric tubule
Gonadal ridge
Hindgut
Developing Mullerian duct
Wolffian duct
Mullerian duct
Mesonephric tubule
Gonadal ridge


mesonephric tubules
gonad
Wolffian duct
Mullerian duct
urogenital sinus
oviduct
uterus
ovary
vagina
epididymis
d. deferens
seminal vesicle
bladder
prostate
testis
urethra
bulbourethral gland

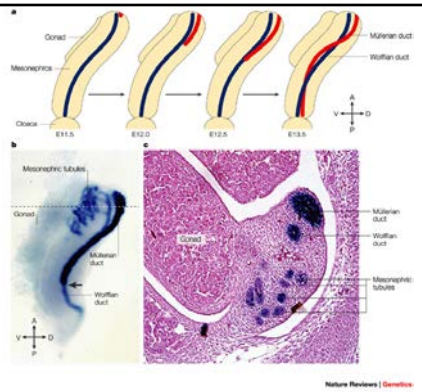
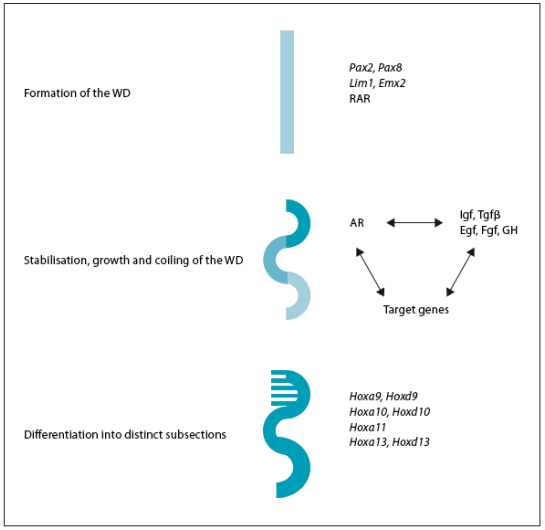
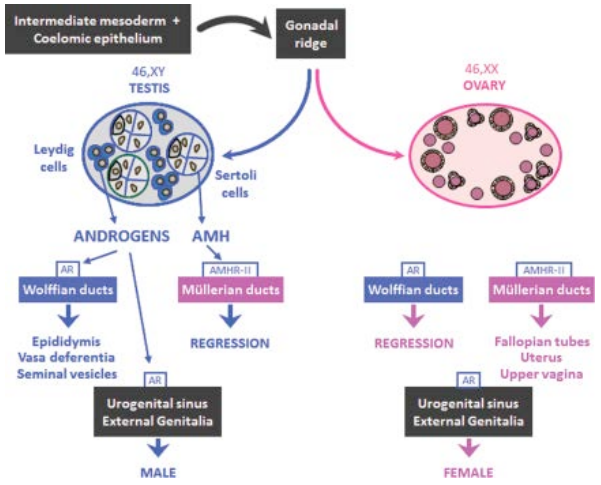
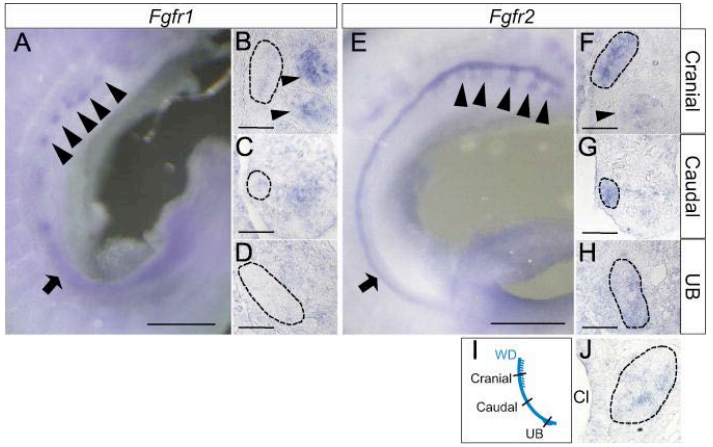


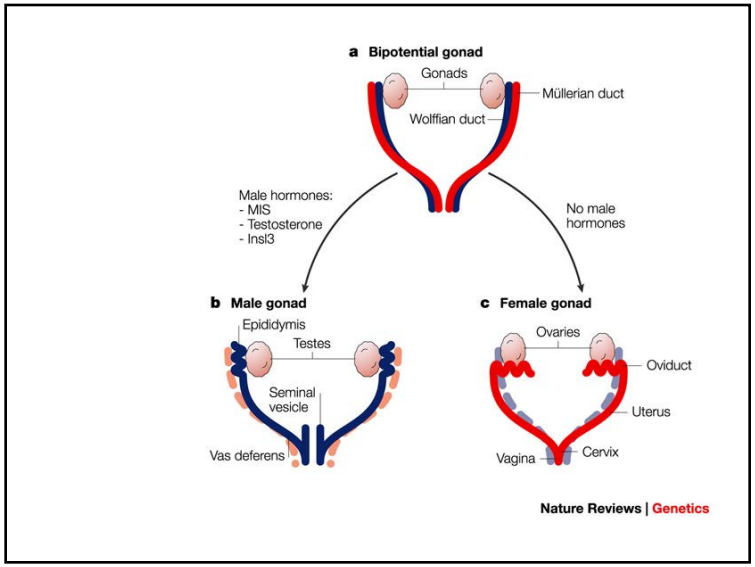
Figure 2 | **Formation of the Müllerian ducts.** **a** | Schematic diagram of Müllerian duct formation in mammals. The Müllerian duct forms as an invagination of the surface epithelium of the MESONEPHROS at around embryonic day (E) 11.5 in mice and this epithelial invagination extends posteriorly until it reaches the CLOACA at E13.5. **b** | The extending epithelium of the Müllerian duct is visualized at E12.5 by *Lim1 (Lhx1)-lacZ* expression11. Note that the Wolffian duct (blue) has reached the cloaca posteriorly, but the Müllerian duct is still in the process of extending posteriorly. The grey arrow points to the posterior tip of the extending Müllerian duct. **c** | Cross section of the gonadal/mesonephric region (dashed line in **b**). Blue staining by *Lim1–lacZ* expression is observed in the epithelium of the Wolffian and Müllerian ducts and the mesonephric tubules. A, anterior (cranial); D, dorsal; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; P, posterior (caudal); V, ventral. Panel **c** adapted from Ref. 11 © (2003) The Company of Biologists Ltd.





Region-specific regulation of cell proliferation by FGF receptor signaling during the Wolffian duct development.
Okazawa M, et al.
Dev Biol. 2015 Apr 1;400(1):139-47.





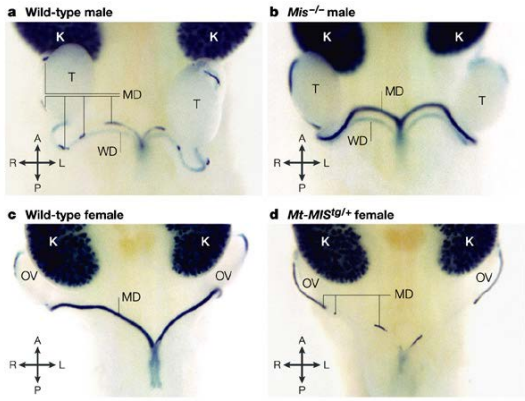
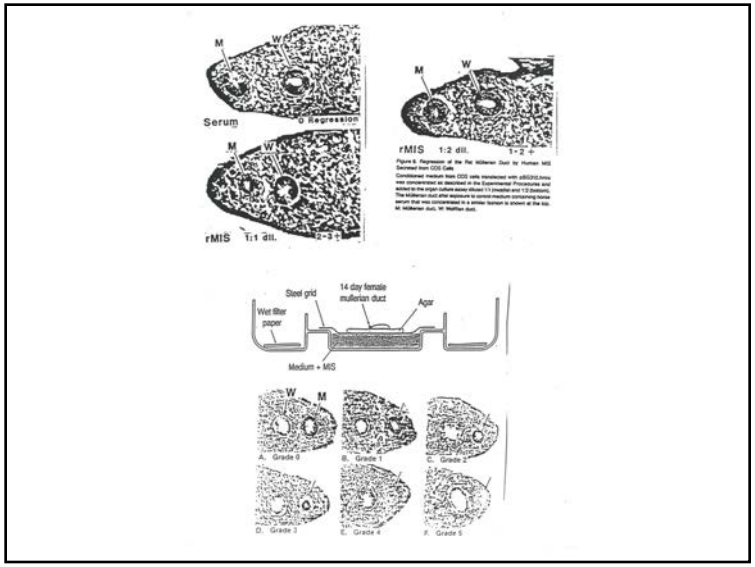


Figure 3 | **Müllerian duct regression.** The developing Müllerian ducts are visualized by *Lim1–lacZ* expression11 in the mouse embryo at embryonic day (E) 15. **a** | In XY male mice, Müllerian-inhibiting substance (MIS) is produced by the testes and eliminates the Müllerian ducts. The regressing Müllerian ducts (MD) have a fragmented pattern at this stage. **b** | When *Mis* is mutated by gene targeting in XY mice, there is no Müllerian duct regression. **c** | There is no Müllerian duct regression in the absence of MIS in XX female mice. **d** | When human *MIS* (*AMH*) is overexpressed using a metallothionein (*Mt*) promoter in XX mice, ectopic regression of the Müllerian duct is observed. A, anterior (cranial); K, kidney; L, left; *Lim*, *lin-11*, *Isl1* and *mec-3* transcription-factor homologue; OV, ovary; P, posterior (caudal); R, right; T, testis; WD, Wolffian duct. Panels **c** and **d** adapted from Ref. 11 © (2003) The Company of Biologists Ltd.



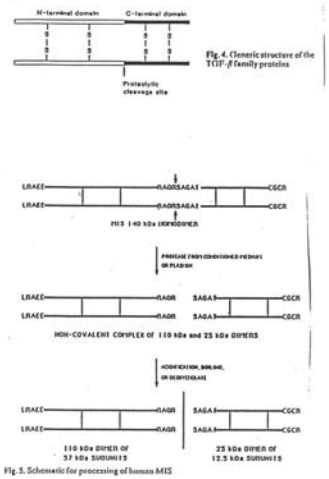


Fig. 4. Generic structure of the TGF-β family proteins

Fig. 5. Schematic for processing of human MIS

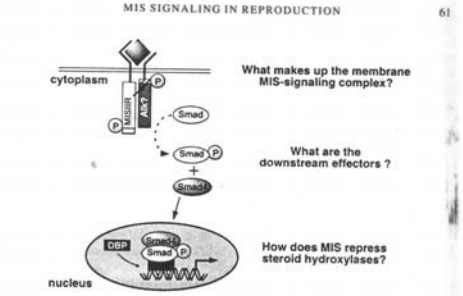


FIG. 5. MIS signaling cascade (hypothetical). Binding of MIS to the MISIIR triggers subsequent binding to a type I receptor. Cross-phosphorylation of the type I receptor upon ligand binding results in phosphorylation of SMAD cytoplasmic proteins that, in turn, are shuttled into the nuclei, where they heterodimerize with other DNA-binding proteins. This heterodimeric complex binds cis-elements to affect gene expression. Identification of MIS target genes in the embryo and adult awaits further work.

Table 1. Effect of protease inhibitors on Müllerian duct regression

Protease inhibitor	Target protease	% Regressed*	n†
Control analog	None	95	19
GM6001	Metalloproteinases	5	22
Phosphoramidon	Metalloproteinases	50	8
Ecotin	Serine proteases	45	11
Aprotinin	Serine proteases	86	7
Leupeptin	Serine proteases	100	7
Boc-D-FMK	Caspases	0	9

*Genital ridges with bo Müllerian duct.
†Total number of ridges tested. Experiments were repeated on 3-11 separate litters. Control analog and GM6001 were tested on male and female ‡MIS cultures; all other inhibitors were tested on males.

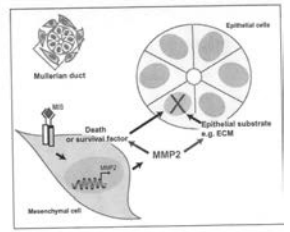


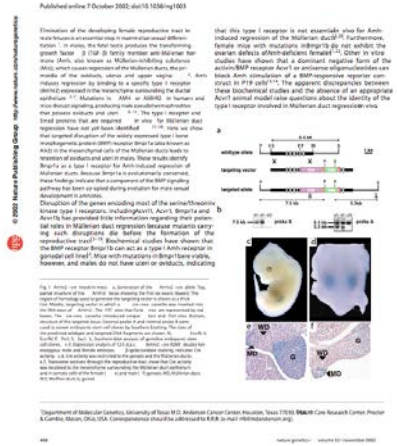
Fig. 6. Potential roles of MMP2 in mediating Müllerian duct regression. Activation of the MIS signaling cascade induces expression of MMP2 in the Müllerian duct mesenchyme. Latent or pro-MMP2 is secreted into the extracellular space, where it is activated to its mature form by membrane bound metalloproteinases such as MMMP14. Cell death of the Müllerian duct epithelium may occur by MMP2 cleavage of a factor secreted by the mesenchyme. MMP2 activity may result in the degradation of a survival factor or activation of a death factor. Alternatively, MMP2 could cause apoptosis by cleaving substrates on the epithelial cell, such as the epithelial cell basement membrane.

letter

Requirement of Bmpr1a for Müllerian duct regression during male sexual development

Soazik P. Jamin[1], Nelson A. Arango[1], Yuji Mishina[1], Mark C. Hanks[2] & Richard R. Behringer[1]

Published online 7 October 2002; doi:10.1038/ng1003



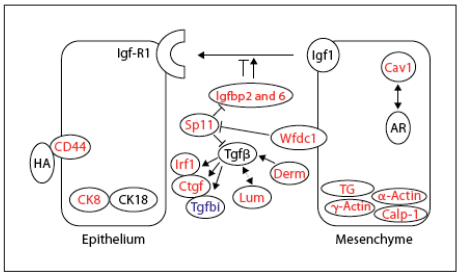


Fig. 4. Model of the interaction of several genes in the developing WD, previously shown to be upregulated (red) and downregulated (blue) between E17.5 and E20.5 [39]. Calp1 = Calponin-1; Cav1 = caveolin-1; CK8 = cytokeratin-8; Ctgf = connective tissue growth factor; Derm = dermatopontin; HA = hyaluronan; Irf1 = interferon regulatory factor-1; Lum = lumican; Sp11 = serine protease 11; TG = transgelin; Tgfbi = Tgfβ-induced; Wfdc1 = WAP four disulfide core domain-1.

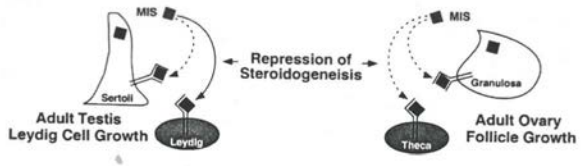
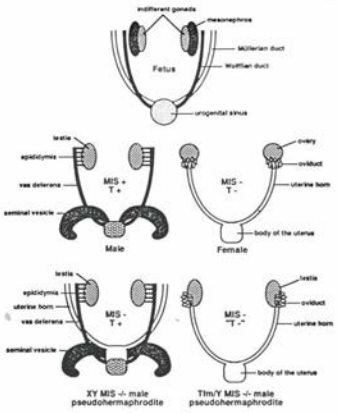


FIG. 2. MIS: a cellular signal in the adult reproductive system. In the adult testis and ovary, MIS is expressed in Sertoli and granulosa cells, respectively. The MISIIR is co-expressed with the MIS ligand in granulosa cells as well as the internal theca layer and in Leydig cells. Proposed functions for the MIS in the ovary have been implied from loss-of-function MIS or MISIIR mouse mutants (Durlinger *et al.*, 1999). In the testis, MIS represses Leydig cell growth. For both gonads, MIS is implicated in repression of steroid production.



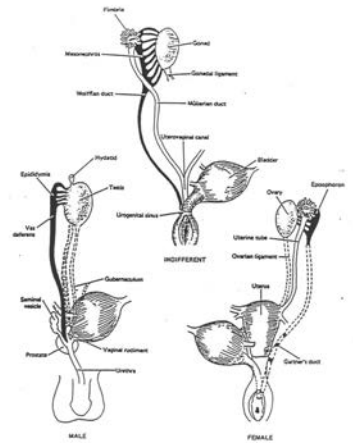
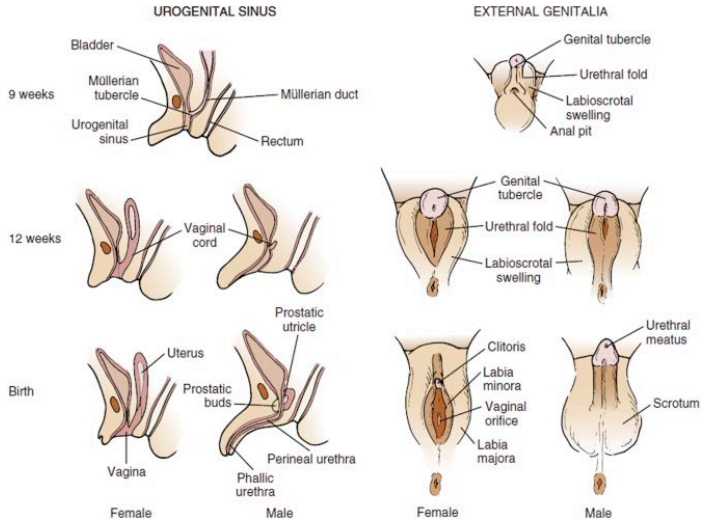
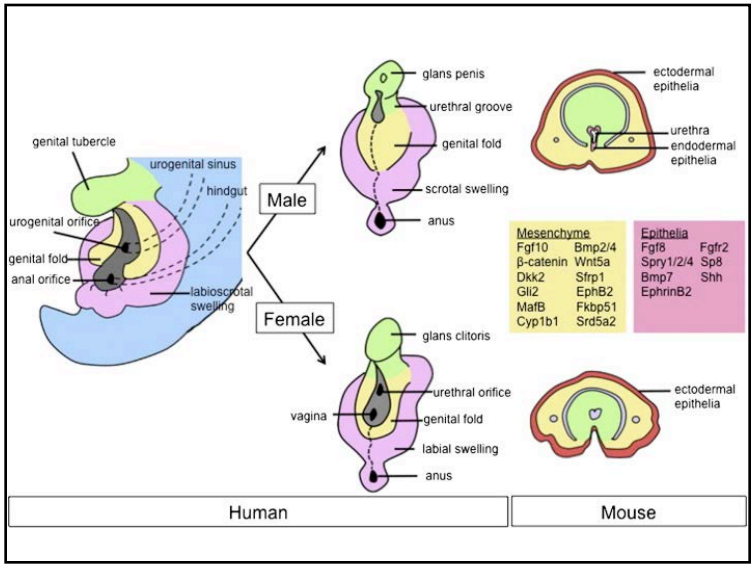
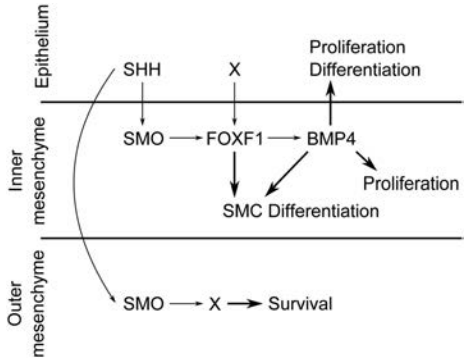


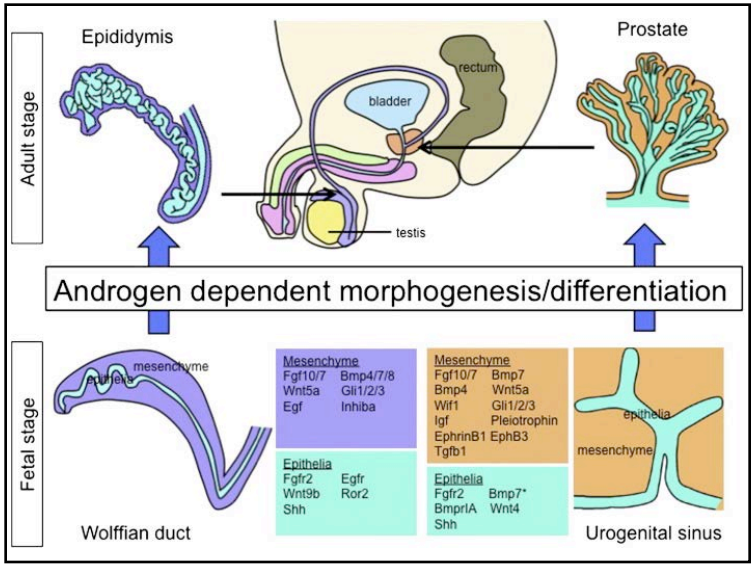
Figure 22–6. Embryonic differentiation of male and female internal genitalia (genital ducts) from wolffian (male) and müllerian (female) primordia. (Alter Corning HK, Wilkins L, Redrawn and reproduced, with permission, from Van Wye J, Grumbach M in: *Textbook of Endocrinology*. 5th ed. Williams RH [editor]. Saunders, 1974.)



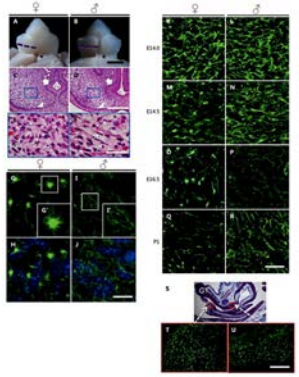


A SHH-FOXF1-BMP4 signaling axis regulating growth and differentiation of epithelial and mesenchymal tissues in ureter development.
Bohnenpoll T, Wittern AB, Mamo TM, Weiss AC, Rudat C, Kleppa MJ, Schuster-Gossler K, Wojahn I, Lüdtke TH, Trowe MO, Kispert A.
PLoS Genet. 2017 Aug 10;13(8):e1006951.

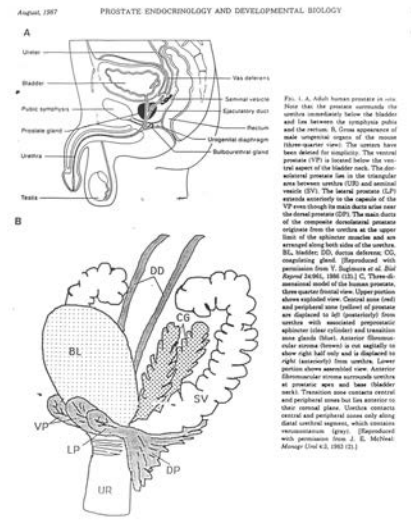


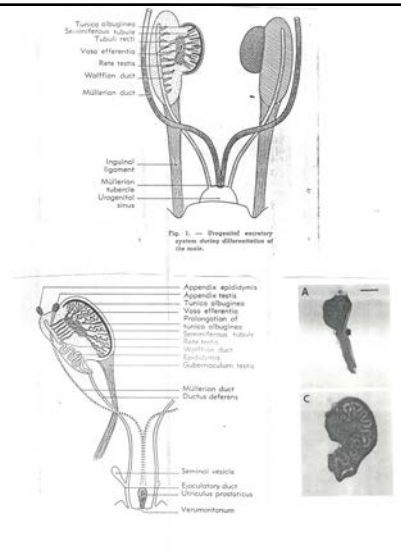


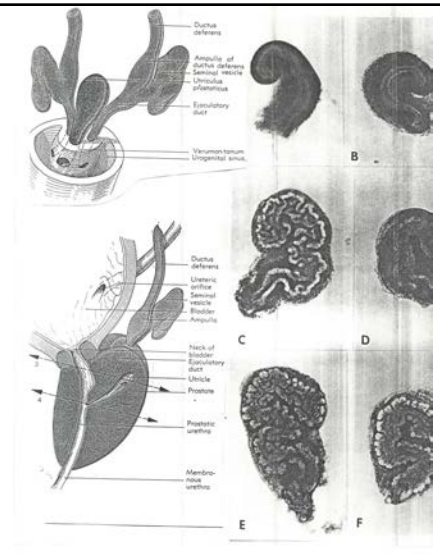
Androgen Regulates Dimorphic F-Actin Assemblies in the Genital Organogenesis.
Liu L, Suzuki K, Chun E, Murashima A, Sato Y, Nakagata N, Fujimori T, Yonemura S, He W, Yamada G.
Sex Dev. 2017;11(4):190-202.

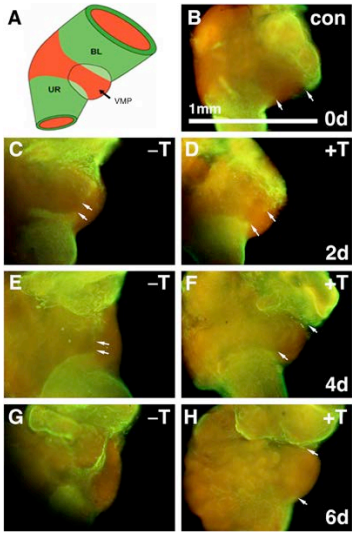


Prostate and Seminal Vesicle








A
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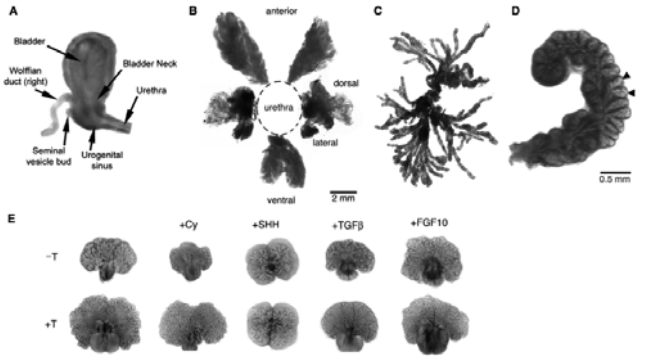
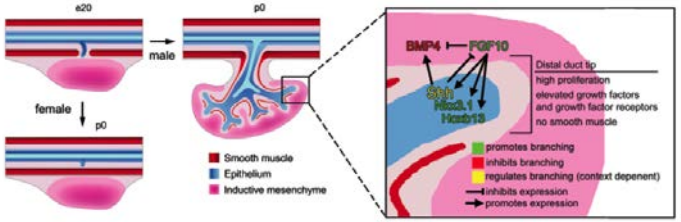
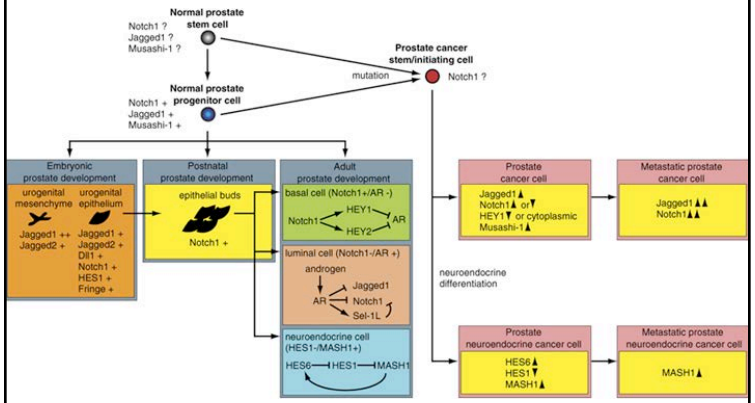

A
Bladder
Wolffian duct (right)
Bladder Neck
Urethra
Seminal vesicle bud
Urogenital sinus
B
anterior
dorsal
urethra
lateral
ventral
2 mm
C
D
0.5 mm
E
+Cy
+SHH
+TGFβ
+FGF10
–T
+T


e20
male
p0
female
p0
Smooth muscle
Epithelium
Inductive mesenchyme
BMP4
FGF10
Shh
Hoxb13
Distal duct tip
high proliferation
elevated growth factors
and growth factor receptors
no smooth muscle
promotes branching
inhibits branching
regulates branching (context depenent)
inhibits expression
promotes expression


Normal prostate stem cell
Notch1 ?
Jagged1 ?
Musashi-1 ?
Prostate cancer stem/initiating cell
mutation
Notch1 ?
Normal prostate progenitor cell
Notch1 +
Jagged1 +
Musashi-1 +
Embryonic prostate development
urogenital mesenchyme
urogenital epithelium
Jagged1 ++
Jagged2 +
Jagged1 +
Jagged2 +
Dll1 +
Notch1 +
HES1 +
Fringe +
Postnatal prostate development
epithelial buds
Notch1 +
Adult prostate development
basal cell (Notch1+/AR -)
Notch1
HEY1
HEY2
AR
luminal cell (Notch1-/AR +)
androgen
AR
Jagged1
Notch1
Sel-1L
neuroendocrine cell (HES1-/MASH1+)
HES6
HES1
MASH1
neuroendocrine differentiation
Prostate cancer cell
Jagged1▲
Notch1▲ or▼
HEY1▼ or cytoplasmic
Musashi-1▲
Metastatic prostate cancer cell
Jagged1▲▲
Notch1▲▲
Prostate neuroendocrine cancer cell
HES6▲
HES1▼
MASH1▲
Metastatic prostate neuroendocrine cancer cell
MASH1▲

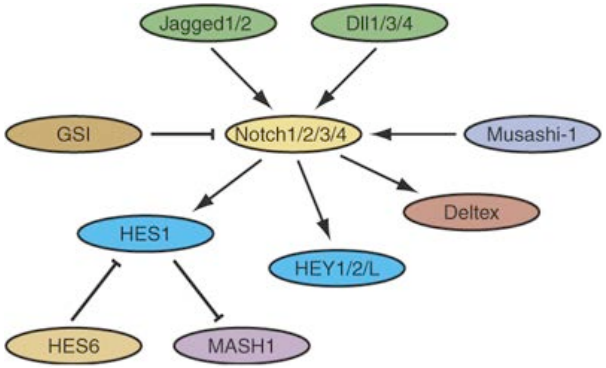


Table 1 Proteins affecting branching morphogenesis in the prostate or seminal vesicles

Protein	Role in branching?	Supporting data for		Supporting evidence		References
		Prostate	Seminal vesicles	*In vitro* studies	Genetic studies	
AR	Promote	X	X	X	X	Takeda et al. (1986), Brown et al. (1988), Lubahn et al. (1989), Charest et al. (1991), Gaspar et al. (1991), He et al. (1991)
BMP4	Inhibit	X		X	X	Lamm et al. (2001)
BMP7	Inhibit	X		X	X	Grishina et al. (2005)
FGF7	Promote	X	X	X		Alarid et al. (1994), Sugimura et al. (1996)
FGF10	Promote	X	X	X	X	Thomson and Cunha (1999), Donjacour et al. (2003)
FST	Promote	X		X		Cancilla et al. (2001)
GDF7	Promote		X		X	Settle et al. (2001)
GHR	Promote	X			X	Ruan et al. (1999)
GLI2	Promote	X		X	X	Doles et al. (2006)
HOXA10	Promote	X	X		X	Podlasek et al. (1999c)
HOXA13	Promote	X	X		X	Podlasek et al. (1999b)
HOXB13	Promote	X			X	Economides and Capecchi (2003)
HOXD13	Promote	X	X		X	Podlasek et al. (1997), Economides and Capecchi (2003)
IGF1	Promote	X			X	Ruan et al. (1999)
INHBA	Inhibit	X		X		Cancilla et al. (2001)
NKX3.1	Promote	X			X	Bhatia-Gaur et al. (1999), Schneider et al. (2000), Tanaka et al. (2000)
p63	Promote	X			X	Signoretti et al. (2000)
SFRP1	Promote	X		X		Joesting et al. (2005)
TGFβ	Inhibit	X		X		Itoh et al. (1998), Tomlinson et al. (2004)
SHH	Context dependent regulator	X		X	X	Podlasek et al. (1999a), Freestone et al. (2003), Wang et al., (2003), Lamm et al., (2002), Berman et al. (2004), Doles et al. (2006)
SMO	Context dependent regulator	X		X	X	(Podlasek et al. (1999a), Freestone et al. (2003), Wang et al. (2003), Lamm et al. (2002), Berman et al. (2004), Doles et al. (2006)
SRD5A2	Promote	X	X		X	Andersson et al. (1991), Mahendroo et al. (2001)

BMP, bone morphogenetic protein; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; SHH, sonic hedgehog.

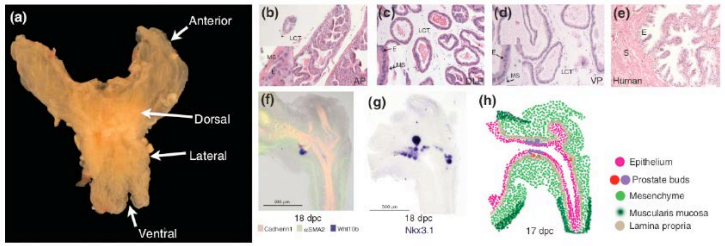
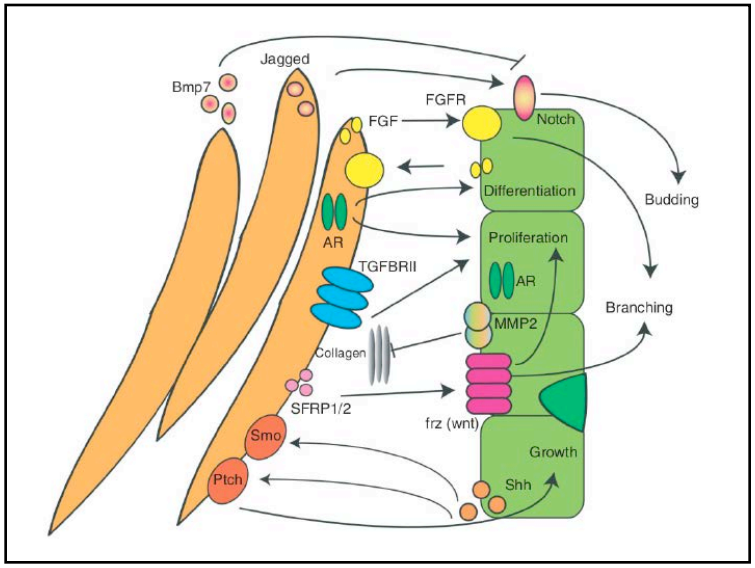
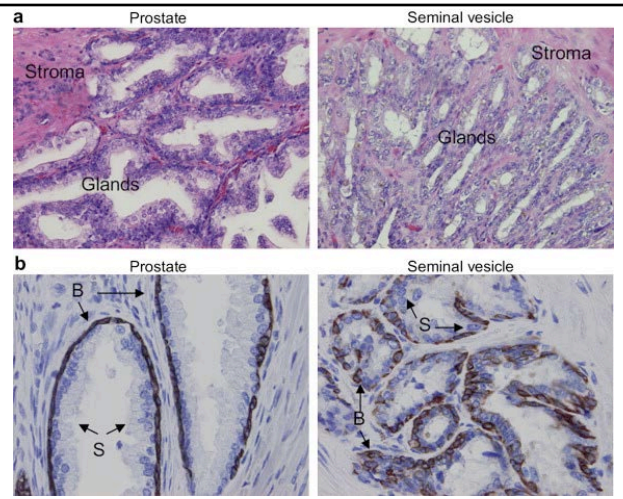


TABLE 1 | Recent Advances in Paracrine Regulation of Prostate Development

Name	Process	Reference
Axin2	Expressed in budding and branching epithelium	18
Bmp2	Marker of the ventral prostate	16
Bmp7	Mesenchymal expression inhibits Notch and restricts budding	19
Lef1	Expressed in budding and branching epithelium	18
FGF10	Stromal expression promotes branching	20, 21
FGFR2	Epithelial expression is required for proper branching and optimal androgen responsiveness	20, 22
MMP2	Epithelial expression required for branching and reducing collagen deposition of stroma	23
Notch	Required for terminal differentiation of epithelium	24
SFRP1	Prostate initiation gene signature and branching	12, 13
Shh	Required for epithelial growth	25
SOX9	Promotes prostate budding (particularly VP and AP) and deletion reduces FGFR2 expression	26
Sulf1	Inhibits ductal branching and FGFR signaling	27
Wnt4	Prostate epithelium marker	18
Wnt7a	Prostate epithelium marker	18
Wnt9b	Prostate epithelium marker	18
Wnt10b	Marker for prostate buds and epithelium	16, 18





Histology of SVs and the prostate. The glandular structure of SVs and the prostate is visualized in standard paraffin-embedded tissue sections. The sections were stained with hematoxylin-eosin (HE) (A) and for basal cytokeratins 5/14 (B) as described in Jäämaa et al. (2010). The epithelium of both tissues consists of two major cell types, basal (B) and secretory (S) cells. Note the discontinuous layer of basal cells in SVs.

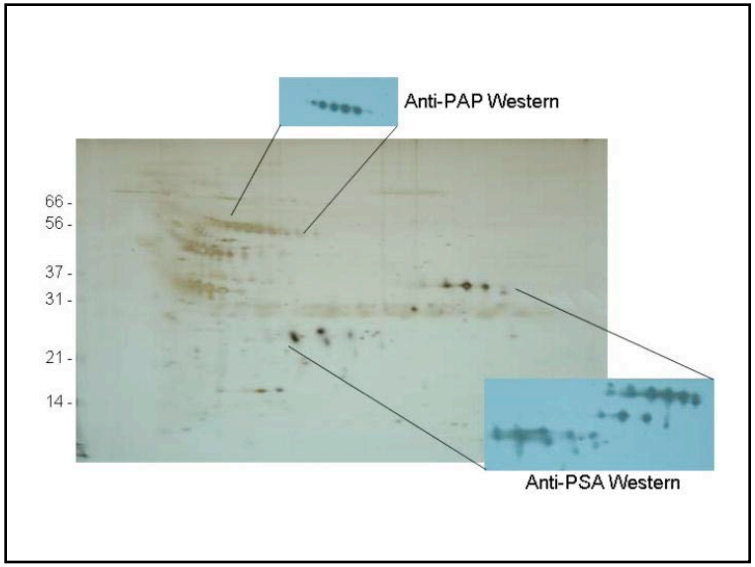
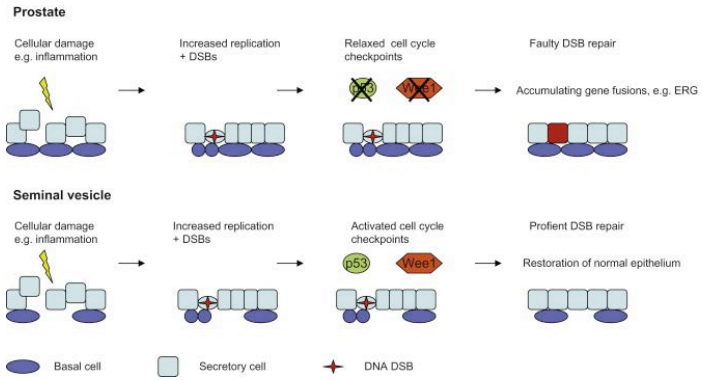


Table 1 – Abundant EPS urine proteins identified following 2D-gel separation.

Protein name*	Score	Peptide matches
AMBP/Alpha-1-microglobulin	1160	254
Gelsolin	1097	97
Serotransferrin	1001	32
Basement membrane-specific HS proteoglycan	981	60
Serum albumin	890	66
Ig kappa chain C regions	673	102
Inter-alpha-trypsin inhibitor heavy chain H4	657	45
Alpha-1B-glycoprotein	518	28
Prostate-specific antigen	478	31
Vesicular integral-membrane protein, VIP36	477	12
Ig lambda chain C regions	455	41
Complement C3	432	9
Actin, cytoplasmic 1	391	18
Epithelial cadherin	352	7
Prostatic acid phosphatase	349	17
Protein S100-A9	312	11
Cell adhesion molecule 4	234	5
Apolipoprotein D	230	9
Prostaglandin-H2 D-isomerase	227	5
Carboxypeptidase E	217	6
Zinc-alpha-2-glycoprotein	208	7
Triosephosphate isomerase	202	4
Cathepsin H	185	7
Alpha-1-antitrypsin	151	2
Histidine-rich glycoprotein	134	2
Annexin A5	133	5
Uromodulin precursor	124	5
14-3-3 protein epsilon	119	3
Proactivator polypeptide/Saposin-A	118	10
Monocyte differentiation antigen CD14	85	2
Endothelial protein C receptor	77	3
Metalloproteinase inhibitor 1	76	2
14-3-3 protein beta/delta	70	2
Neural cell adhesion molecule 1	68	2

*Gel spots were excised from 2D gels, then reduced, alkylated and digested with trypsin to release peptides. Mass spectrometric analysis was performed on an LTQ™ Linear Ion Trap (ThermoFinnigan, San Jose, CA) mass spectrometer in the data-dependent acquisition mode. Survey full scan MS spectra (from m/z 300 to 1800) were acquired and the four most intense ions in a scan were sequentially isolated and fragmented in the linear ion trap (MS/MS). The peptide sequences were identified from their tandem mass spectra using Mascot a probability based search engine (www.matrixscience.com) using the SwissProt database. The following search criteria were used: variable modifications; carbamido-methylation of cysteine and oxidation of methionine residues, 1 missed enzyme cleavage site and an error tolerance of 1.5 Da for MS and 0.8 Da for MS/MS.

Prostate Cancer



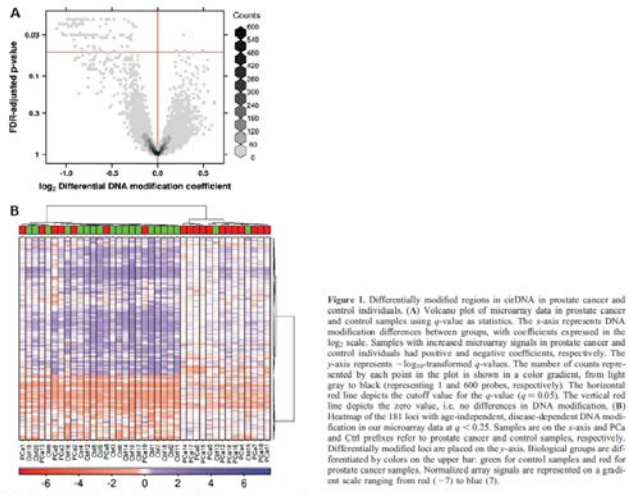
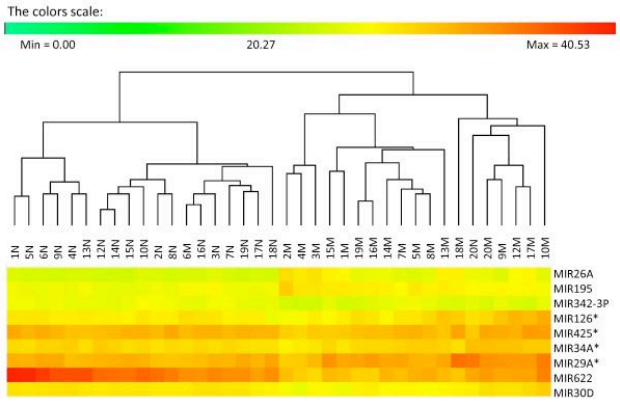
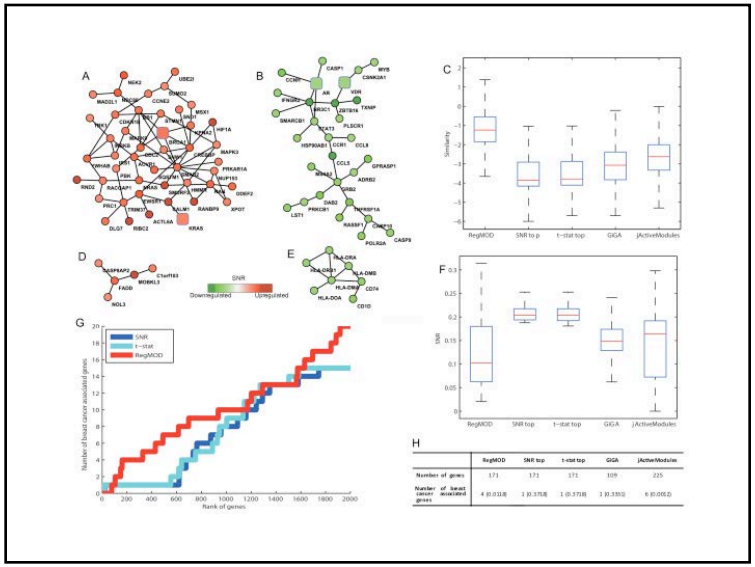


Figure 1. Differentially modified regions in cirDNA in prostate cancer and control individuals. **(A)** Volcano plot of microarray data in prostate cancer and control samples using q-value as statistics. The x-axis represents DNA modification differences between groups, with coefficients expressed in the $\log_2$ scale. Samples with increased microarray signals in prostate cancer and control individuals had positive and negative coefficients, respectively. The y-axis represents $-\log_{10}$-transformed q-values. The number of counts represented by each point in the plot is shown in a color gradient, from light gray to black (representing 1 and 600 probes, respectively). The horizontal red line depicts the cutoff value for the q-value ($q = 0.05$). The vertical red line depicts the zero value, i.e. no differences in DNA modification. **(B)** Heatmap of the 181 loci with age-independent, disease-dependent DNA modification in our microarray data at $q < 0.25$. Samples are on the x-axis and PCa and Ctrl prefixes refer to prostate cancer and control samples, respectively. Differentially modified loci are placed on the y-axis. Biological groups are differentiated by colors on the upper bar: green for control samples and red for prostate cancer samples. Normalized array signals are represented on a gradient scale ranging from red (-7) to blue (7).

A miRNA expression signature that separates between normal and malignant prostate tissues.

Carlsson J, Davidsson S, et al. Cancer Cell Int. 2011 May 27;11(1):14.





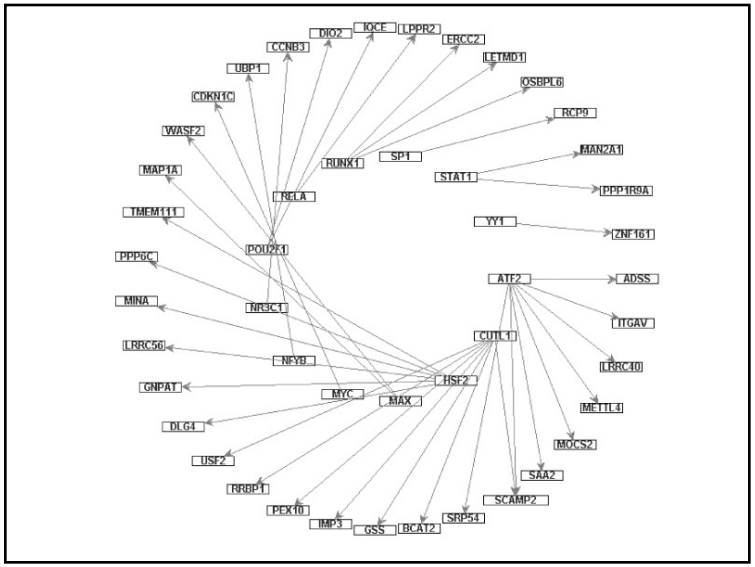


TABLE II. Genes Identified in the Genetic Prognostic Signature and the Hybrid Genetic and Clinical (Marked by ¶) Predictive Model

Gene symbol	Gene title	Mean expression in recurrent tumors	*P*-value	Occurrence frequencies
PAK3¶	P21 (CDKN1A)-activated kinase 3	Under-expressed	<9.0e – 6	78 (79)
RPL23¶	Ribosomal protein L23	Over-expressed	<5.0e – 5	79 (79)
E124¶	Etoposide-induced 2.4 mRNA	Over-expressed	<3.0e – 7	79 (79)
TGFB3¶	Transforming growth factor, beta 3	Under-expressed	<1.0e – 5	79 (3)
RBM34¶	RNA-binding motif protein 34	Over-expressed	<3.0e – 4	62 (8)
PCOLN3	Procollagen (type III) N-endopeptidase	Under-expressed	<3.0e – 5	78
FUT7	Fucosyl transferase 7 (alpha (1,3) fucosyl transferase)	Under-expressed	<3.0e – 3	30
RICS Rho	GTPase-activating protein	Over-expressed	<3.0e – 6	8
MAP4K4	Mitogen-activated protein kinase 4	Over-expressed	<3.0e – 5	5
CUTL1	Cut-like 1, CCAAT displacement protein (*Drosophila*)	Over-expressed	<3.0e – 5	2
ZNF324B	Zinc finger protein 324B	Under-expressed	<5.0e – 4	1

The *P*-values, computed using a *t*-test, quantify the up- or down-regulation of a gene between patients with, and without recurrence. The value inside and outside of the brackets in the last column is the number of iterative models in which a gene was selected in the hybrid and genetic models, respectively.

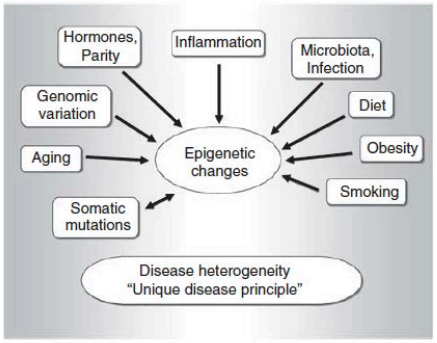


Figure 1 A variety of endogenous and exogenous etiological factors contribute to epigenetic changes leading to heterogeneity of disease processes, which is implicated by the 'unique disease principle'. To simplify, only selected examples of those etiological factors are demonstrated. There are numerous interactions between the factors, which are not depicted for simplicity.

Cortese R, Kwan A, et al. (2012) Epigenetic markers of prostate cancer in plasma circulating DNA. Hum Mol Genet. 15;21(16):3619-31.

Table 1. Summary of pyrosequencing results

Loci	Sample set 1 % Mod. Pca[a]	% Mod. Ctrl[a]	Disease p_LME[b]	Age p_LME[b]	Sample set 2 % Mod. Pca[a]	% Mod. Ctrl[a]	Disease p_LME[b]	Age p_LME[b]
DLG2	48.7 ± 22.0	45.6 ± 23.6	**0.02**	**0.02**	42.9 ± 14.7	41.9 ± 18.3	0.85	0.07
GNG7	38.4 ± 3.0	38.2 ± 2.4	0.74	0.40	37.9 ± 11.1	36.8 ± 7.8	0.93	0.21
HPSE2	22.7 ± 13.7	16.1 ± 8.3	0.13	**0.02**	40.6 ± 30.0	29.6 ± 27.1	0.32	0.82
KIAA1539	38.8 ± 5.8	37.1 ± 10.9	$\mathbf{1 \times 10^{-3}}$	$\mathbf{8 \times 10^{-4}}$	30.8 ± 10.0	36.2 ± 6.4	$\mathbf{1 \times 10^{-4}}$	0.05
NUDCD3	59.4 ± 6.1	63.6 ± 9.0	0.09	0.19	67.6 ± 11.6	69.7 ± 11.1	0.47	0.76
PCDHB1	66.6 ± 23.3	50.9 ± 36.2	0.51	0.69	58.4 ± 27.6	58.0 ± 25.0	0.97	0.77
RNF219	35.0 ± 18.1	55.0 ± 17.6	$\mathbf{3 \times 10^{-41}}$	$\mathbf{3 \times 10^{-9}}$	35.3 ± 31.8	46.2 ± 23.7	$\mathbf{1 \times 10^{-4}}$	0.14

Statistically significant differences ($p < 0.05$) are in bold.
[a]Mean cirDNA modification per amplicon.
[b]LME model p-value.

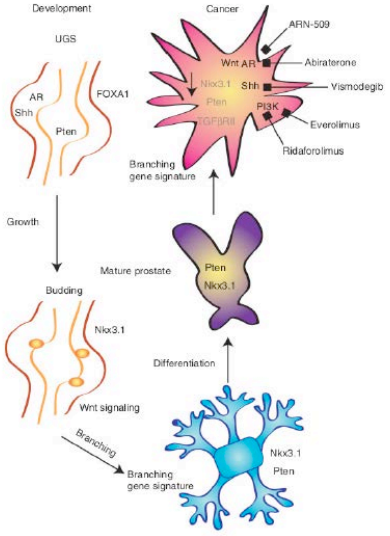


TABLE 2 | Drugs Targeting Prostate Development Pathways

Drug Name	Target	Phase of Development	Reference
Abiraterone	CYP17A1 inhibitor	Approved for castration-resistant prostate cancer, current trials with various drug combinations	58
ARN-509	AR antagonist	Phase I (NCT01171898) and Phase II (NCT01709734) trials in castration-resistant prostate cancer	57
Bicalutamide	AR antagonist	Approved for prostate cancer, current trials in combination with other drugs	62
Dutasteride	5α reductase type II inhibitor	Approved BPH, current trials for combination therapy for BPH and prostate cancer	63
Enzalutamide	AR antagonist	Approved for castration-resistant prostate cancer, current trials in combination with abiraterone, leuprolide, and bicalutamide	64
Everolimus	PI3 kinase/mTOR inhibitor	Phase I (NCT01642732) and II (NCT01313559) clinical trials for advanced prostate cancer	59
Finasteride	5α reductase type II inhibitor	Approved for BPH	63
Flutamide	AR antagonist	Approved for prostate cancer, current trials in combination therapies	62
Leuprolide	GnRH antagonist	Approved for prostate cancer, current trials in various combination therapies	62
Ridaforolimus	mTor inhibitor	Phase II prostate cancer trial (NCT00777959 and NCT00110188)	60
Vismodegib	Shh inhibitor	Phase I/II prostate cancer trial (NCT01163084) FDA approved for metastatic basal cell carcinoma	61

Epididymis

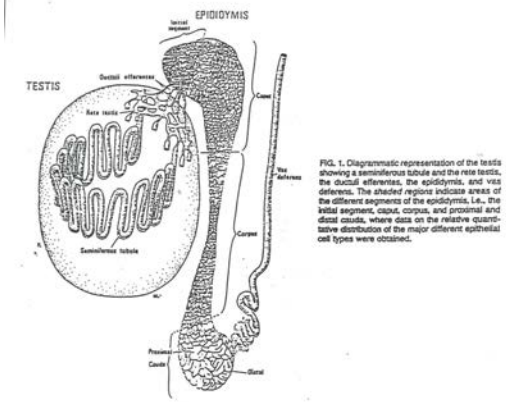
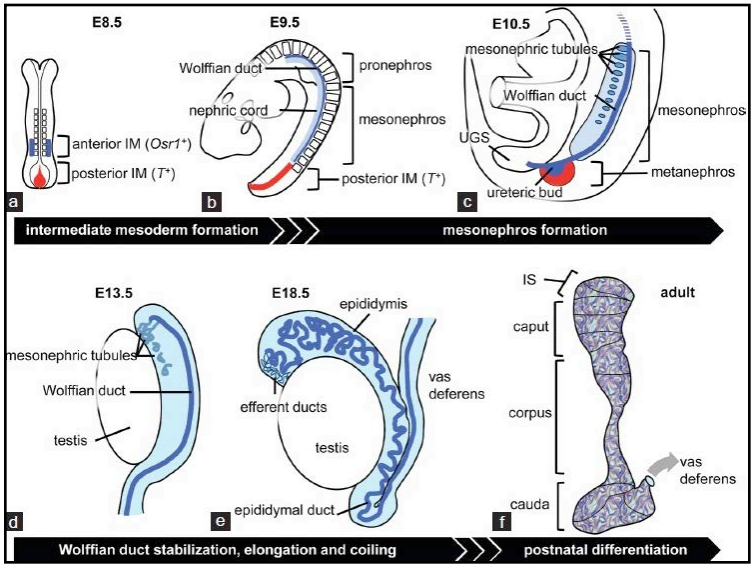
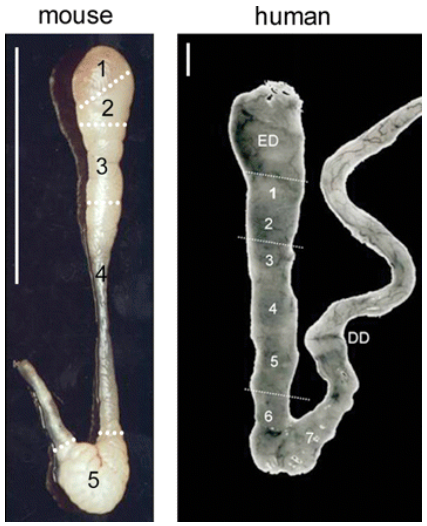


FIG. 1. Diagrammatic representation of the testis showing a seminiferous tubule and the rete testis, the ductuli efferentes, the epididymis, and vas deferens. The shaded regions indicate areas of the different segments of the epididymis, i.e., the initial segment, caput, corpus, and proximal and distal cauda, where data on the relative quantitative distribution of the major different epithelial cell types were obtained.



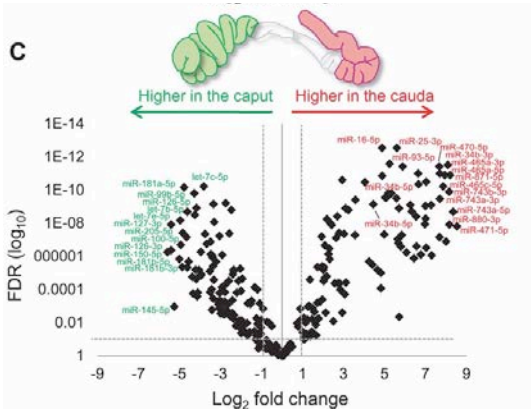


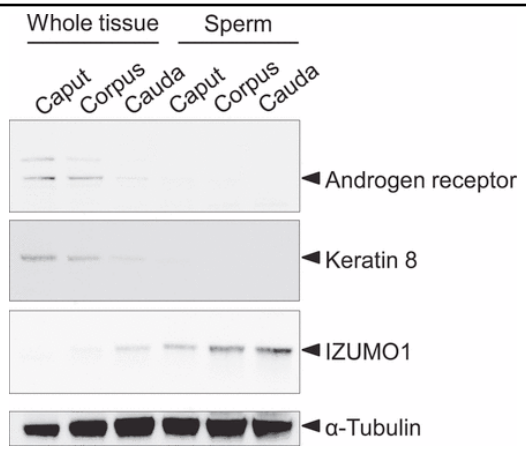
Mouse and human epididymides.

Mouse: 1, initial segment, proximal caput; 2, midcaput; 3, distal caput; 4, corpus; 5, cauda. Adapted (regional divisions by the author) from Trevor Cooper, University of Muenster (Yeung and Cooper, 2002), with permission of author and publisher, Springer. Human: ED, efferent ducts; 1, anterior caput; 2, posterior caput; 3, anterior corpus; 4, midcorpus; 5, posterior corpus; 6, anterior cauda; 7, posterior cauda; DD, ductus deferens. Reprinted by permission of the author (Dacheux et al., 2006), UMR INRA-CNRS and publisher, Elsevier Limited. Scale bar, 1 cm.

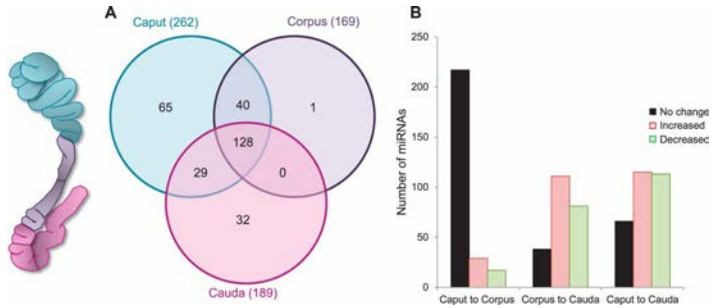
The microRNA signature of mouse spermatozoa is substantially modified during epididymal maturation.

Nixon B, Stanger SJ, Mihalas BP, Reilly JN, Anderson AL, Tyagi S, Holt JE, McLaughlin EA. Biol Reprod. 2015 Oct;93(4):91.

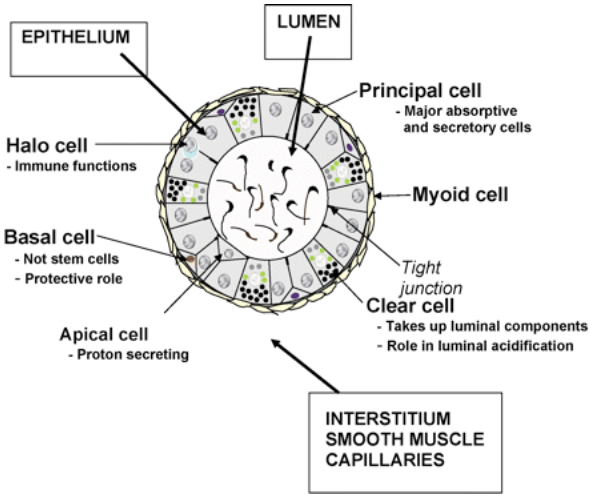


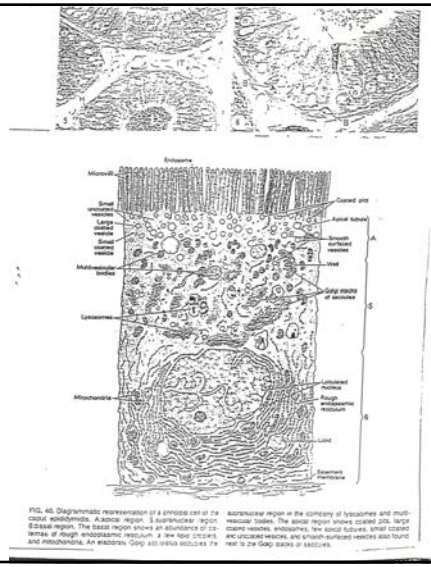


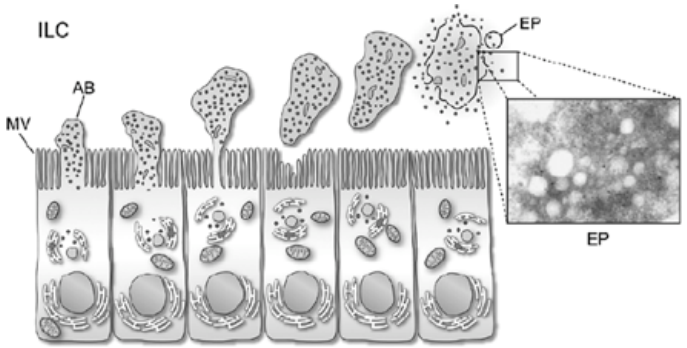
Isolation and assessment of epididymal spermatozoa. The purity of sperm cell suspensions isolated from the caput, corpus, and cauda epididymis was assessed by immunoblotting with antibodies against tissue- (androgen receptor, 110 kDa; keratin 8, 54 kDa) and sperm- (IZUMO1, 60 kDa) specific markers. Anti-α-tubulin was included as a loading control. This analysis was repeated for each isolation and representative immunoblots are shown.



Determination of the miRNA signature present in mouse epididymal spermatozoa. A) Venn diagram illustrating the number of miRNAs that were identified by next-generation sequencing and their disruption within spermatozoa sampled from the caput, corpus, and cauda regions of the adult mouse epididymis. B) Graphical representation of miRNA distribution highlighting the number of significantly up- and down-regulated (threshold = ± ≥2-fold change and FDR of <0.05) miRNAs positively identified in spermatozoa between each epididymal region. For the purpose of these analyses, an average count value of >10 across two biological replicates (with each replicate comprising pooled miRNA from a minimum of nine animals) was used as the threshold for positive identification of all miRNAs.





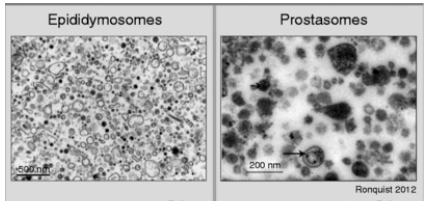


Sullivan R, Saez F. (2013) Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology. Reproduction. 2013 Jun 14;146(1):R21-35.

Table 1 Proteins associated with epididymosomes[a]. Proteins from epididymosomes with known or proposed functions once transferred to spermatozoa during maturation.

Name	Abbreviation	Functions	Particularities	References
Macrophage migration inhibitory factor	MIF	Associated with sperm dense fibers; involved in motility	Chelation of Zn; disulfide-bound formation	Eickhoff *et al.* (2004, 2006) and Frenette *et al.* (2002, 2003, 2004, 2005, 2006, 2010)
Liprin α3	Ppfia3	Acrosome reaction	Estrogen-responsive element in the 5′UTR	Joshi *et al.* (2012)
Kinases cSrc	cSrc	Signaling cascade of capacitation	Essential in cauda epididymal development	Krapf *et al.* (2012)
Glutathione peroxidase 5	GPX5	Protection against oxidative stress (DNA integrity)	Seleno-independent GPX	Chabory *et al.* (2009)
Ubiquitin	UBC	Elimination of defective spermatozoa	Involved in proteasome activity	Fraile *et al.* (1996) and Sutovsky *et al.* (2001)
Epididymal sperm binding protein 5	CD52 (HE5)	Protection against immune response	Highly glycosylated GPI anchored to sperm surface	Kirchhoff & Hale (1996), for review
Epididymal sperm binding protein 1	ELSPBP1	Elimination of defective spermatozoa	Zn-dependent transfer from epididymosomes to spermatozoa	D'Amours *et al.* (2012*a*, 2012*b*)
P26h (hamsters), P25b (bovine)	P26h/P25b	Sperm–zona pellucida interaction	GPI-anchored to sperm surface	Legare *et al.* (1999) and Frenette & Sullivan (2001)
Sperm adhesion molecule 1	SPAM1 (PH-20)	Different roles in fertilization	GPI-anchored to sperm surface	Martin-DeLeon (2006) and Griffiths *et al.* (2008)
Glioma pathogenesis-related protein 1	GLIPR1L1	Roles in fertilization	Belongs to the CAP family, GPI-anchored to sperm surface	Caballero *et al.* (2012) and Gibbs *et al.* (2010)
A desintegrin metalloproteases	ADAM2, ADAM3, ADAM7	Involved in fertilization	Behave as integral membrane proteins once transferred to sperm	Oh *et al.* (2005)
Methylmalonate-semialdehyde dehydrogenase	MMSDH	Unknown	Behave as peripheral and integral membrane protein once transferred to sperm	Suryawanshi *et al.* (2012)

[a]Proteomes of ram epididymosomes (exosomes-like), bovine caput, and cauda epididymosomes and of human 'epididymosomes' collected from the proximal vas deferens during vasectomy procedures have been published by Gatti *et al.* (2005), Thimon *et al.* (2008*a*, 2008*b*) and Girouard *et al.* (2011) respectively.



Ronquist 2012

	Reference		Reference
Size: 25–300 nm	(1)	Size: 30–500 nm	(2)
Cellular origin: epididymal principal cells	(3)	Cellular origin: prostate acinar cells	(2)
Extracellular release: Apocrine secretion	(3, 4)	Extracellular release: Exocytosis of storage vesicles	(5)
Function: Sperm maturation, transter of proteins to the sperm surface	(6)	Function: Sperm motility, immunosuppressive, and antioxydant factor, and sperm capacitation	(7)
Lipids: cholesterol: phospholipid ratio = 0.5	(8, 9)	Lipids: cholesterol: phospholipid ratio = 2. High amount of sphingomyelin.	(10)

Table I Epididymal sperm proteins

Sperm proteins modified or relocalized during epididymal transit	Epididymal proteins that interact with spermatozoa
Spam1[1]	CRISP1[11]
ADAM2[2], ADAM3[3], ADAM15[4], ADAM24[5]	P26h[12]
α-mannosidase[6]	Clusterin[13]
CE9[7]	HE1[14], HE2[15], HE4[16], HE5[17], HE12[18]
β-galactosidase[8]	HEL75[19]
Basigin[9]	SPAG11[20]
α-enolase[10]	Eppin[21]
Grp78/Hsp70[10]	Cystatin 11[22]
Endoplasmin[10]	SED1[23]
Phosphatidylethanolamine binding protein[10]	
Lactate dehydrogenase 3[10]	
Testis lipid-binding protein[10]	
Cytokeratin[10]	
β-subunit F1-ATPase[10]	

[1](Phelps *et al.*, 1990); [2](Lum and Blobel, 1997); [3](Frayne *et al.*, 1998); [4](Pasten-Hidalgo *et al.*, 2008); [5](Zhu *et al.*, 2001); [6](Tulsiani *et al.*, 1995); [7](Nehme *et al.*, 1993); [8](Scully *et al.*, 1987); [9](Saxena *et al.*, 2002); [10](Baker *et al.*, 2005); [11](Cohen *et al.*, 2000); [12](Legare *et al.*, 1999); [13](Sylvester *et al.*, 1991); [14](Kirchhoff *et al.*, 1996); [15](Osterhoff *et al.*, 1994); [16](Kirchhoff *et al.*, 1991); [17](Kirchhoff and Hale, 1996); [18](Saalmann *et al.*, 2001); [19](Lin *et al.*, 2008); [20](Yenugu *et al.*, 2006); [21](Richardson *et al.*, 2001); [22](Hamil *et al.*, 2002); [23](Ensslin and Shur, 2003).

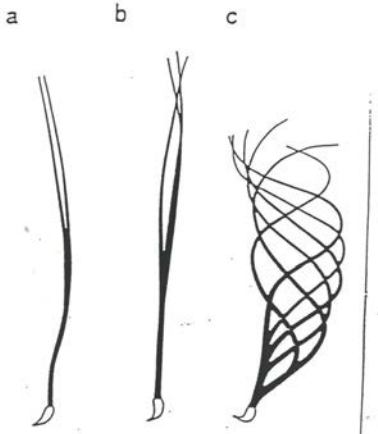


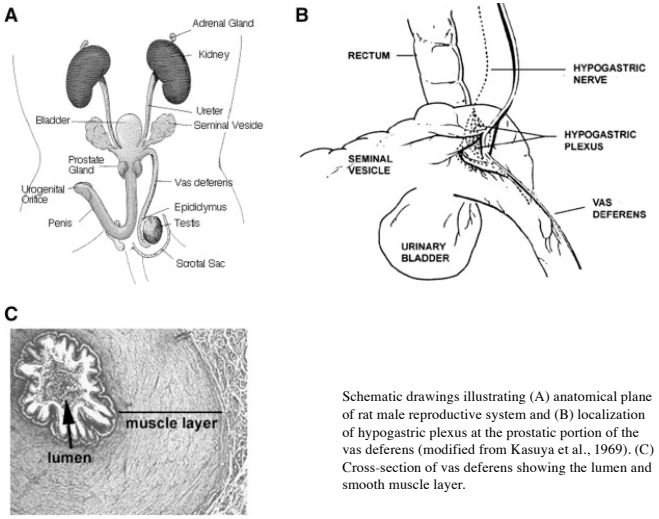
FIGURE 3. Changes in the beating pattern of golden hamster spermatozoa during the development of sperm motility: a, testicular; b, caput epididymal; and c, cauda epididymal spermatozoa. The time interval between successive images is 1.5 sec in a, 0.5 sec in b, and 1/60 sec in c. The scale bar = 30 μm.

Vas Deferens

Mechanisms of adaptive supersensitivity in vas deferens.

Quintas LE, Noël F.
Auton Neurosci. 2009 Mar 12;146(1-2):38-46.

Adaptive supersensitivity is a phenomenon characteristic of excitable tissues and discloses as a compensatory adjustment of tissue's response to unrelated stimulatory endogenous and exogenous substances after chronic interruption of excitatory neurotransmission. The mechanisms underlying such higher postjunctional sensitivity have been postulated for a variety of cell types. In smooth muscles, especially the vas deferens with its rich sympathetic innervation, the mechanisms responsible for supersensitivity are partly understood and appear to be different from one species to another. The present review provides a general understanding of adaptive supersensitivity and emphasizes early and recent information about the putative mechanisms involved in this phenomenon in rodent vas deferens.



Schematic drawings illustrating (A) anatomical plane of rat male reproductive system and (B) localization of hypogastric plexus at the prostatic portion of the vas deferens (modified from Kasuya et al., 1969). (C) Cross-section of vas deferens showing the lumen and smooth muscle layer.

Table 1
Alteration of signaling proteins in supersensitive vas deferens

Protein	Species	Model	Technique	Change	Reference
α1-adrenoceptor	Rat	Res	[^{3}H]WB4101 binding	=	Watanabe et al., 1982
		Den	[^{125}I]BE-2254 binding	=	Abel et al., 1985
		Res	[^{125}I]BE-2254 binding	=	Nasseri et al., 1985
		Den	[^{3}H]WB4101 binding	↓	Hata et al., 1981
	Guinea pig	Den	[^{3}H]WB4101 binding	=	Hata et al., 1980
		Den, Res	[^{3}H]WB4101 binding	=	Cowan et al., 1985
α2-adrenoceptor	Rat	Den, Res, OHDA	[^{3}H]Clonidine binding	↑	Watanabe et al., 1982
		Den	[^{3}H]Rauwolscine binding	ND	Abel et al., 1985
		Res	[^{3}H]Rauwolscine binding	ND	Nasseri et al., 1985
Muscarinic receptor	Rat	Den	[^{3}H]QNB binding	=	Hata et al., 1981
		Guan	[^{3}H]QNB binding	↑	Higuchi et al., 1985a
	Guinea pig	Den	[^{3}H]QNB binding	↑	Hata et al., 1980
Na^+/K^+-ATPase α1 (or α1T) isoform	Rat	Den	Immunoblot	=	Quintas et al., 2000
	Guinea pig	Res	Immunoblot	=	Hershman et al., 1993
Na^+/K^+-ATPase α2 isoform	Rat	Den	Immunoblot, [^{3}H] ouabain binding	↓	Quintas et al., 2000
	Guinea pig	Den, Dec, Res, OHDA	[^{3}H]Ouabain binding	↓	Wong et al., 1981
		Res	Immunoblot	↓	Hershman et al., 1993
		Res	Immunoblot	↓	Hershman et al., 1995
SERCA 2	Rat	Den	Immunoblot	↓	Quintas et al., 2005
PMCA	Rat	Den	Immunoblot	=	Quintas et al., 2005
Ryanodine receptor	Rat	Den	[^{3}H]Ryanodine binding	↓	Quintas et al., 2005
L-Type Ca^{2+}-channel	Rat	Den	[3H]Isradipine binding	↓	Jurkiewicz et al., 1994

Den — denervation; Dec — decentralization; Res — reserpine; Guan — guanetidine; OHDA — 6-hydroxydopamine; ND — not detected; WB4101 — 2-[(2′,6′-dimethoxy)-phenoxyethylamino]methylbenzodioxane; QNB — quinuclidonyl-benzilate; BE — [2-[(1-[4-hydroxyphenyl]ethylaminomethyl]-tetralone]; SERCA — sarco(endo)plasmic reticulum Ca^{2+}-ATPase; PMCA — plasma membrane Ca^{2+}-ATPase.

Endocrine

Role Testosterone -

1) Wolffian Duct Development
2) Male ReproductionGenetalia
3) External Genetalia

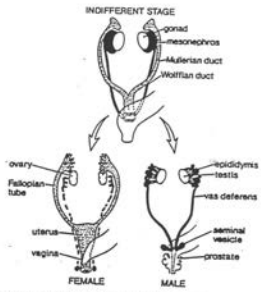


FIG. 7. Differentiation of the internal genitalia. (From ref. 256.)

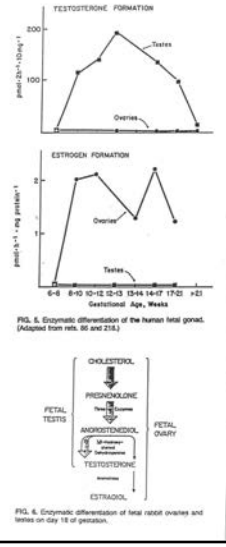
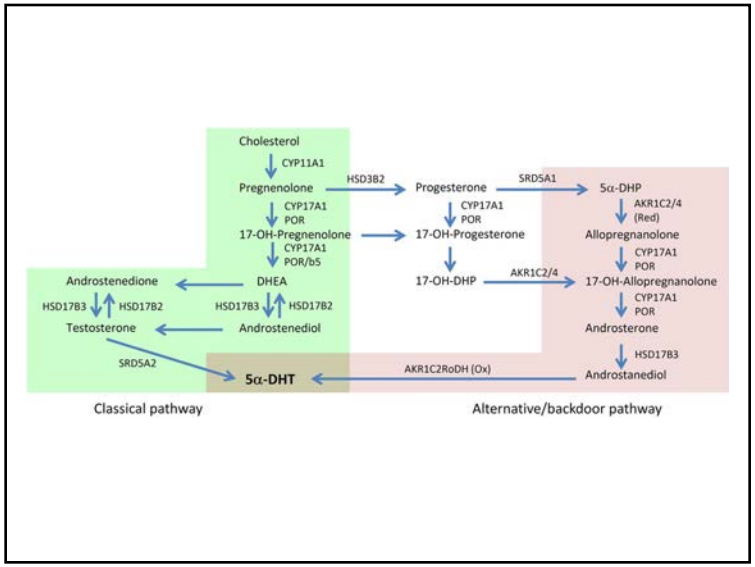


FIG. 5. Enzymatic differentiation of the human fetal gonad. (Adapted from refs. 86 and 218.)

FIG. 6. Enzymatic differentiation of fetal rabbit ovaries and testes on day 18 of gestation.



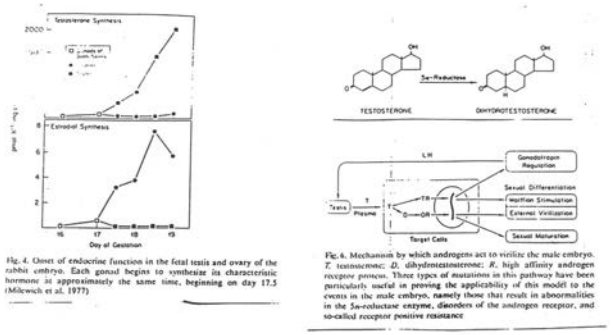
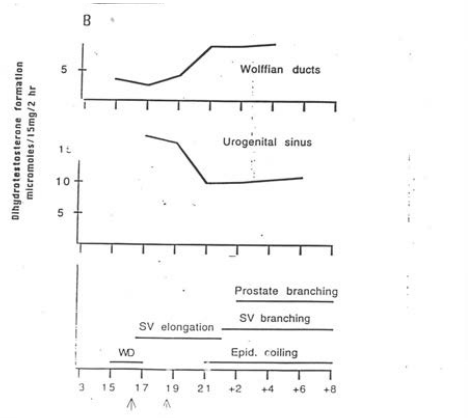
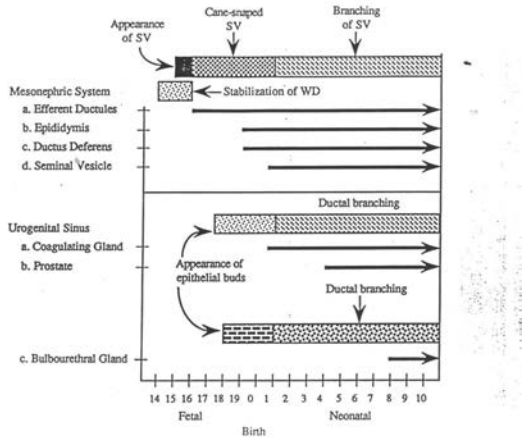


Fig. 4. Onset of endocrine function in the fetal testis and ovary of the rabbit embryo. Each gonad begins to synthesize its characteristic hormone at approximately the same time, beginning on day 17.5 (Milewich et al. 1977)

Fig. 6. Mechanism by which androgens act to virilize the male embryo. *T*, testosterone; *D*, dihydrotestosterone; *R*, high affinity androgen receptor protein. Three types of mutations in this pathway have been particularly useful in proving the applicability of this model to the events in the male embryo, namely those that result in abnormalities in the 5α-reductase enzyme, disorders of the androgen receptor, and so-called receptor positive resistance





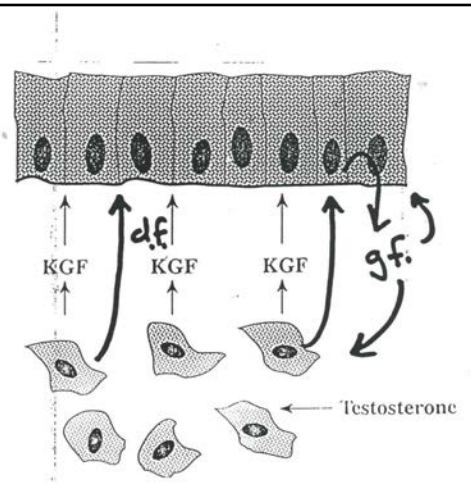
Prostatic growth and development are regulated by FGF10

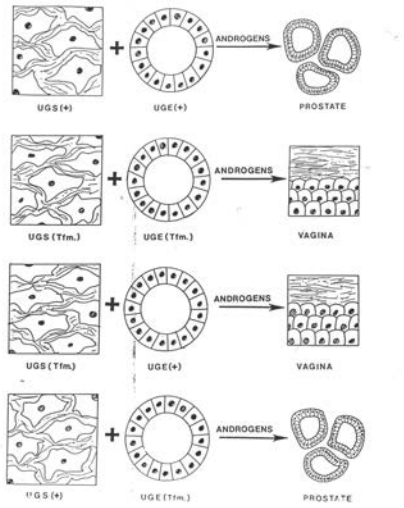
A. A. Thomson*,† and G. R. Cunha

Anatomy Department, Box 0452, University of California, San Francisco, 513 Parnassus Avenue, San Francisco CA 94143-0452, USA

SUMMARY

We have examined the role of Fibroblast Growth Factor 10 (FGF10) during the growth and development of the rat ventral prostate (VP) and seminal vesicle (SV). FGF10 transcripts were abundant at the earliest stages of organ formation and during neonatal organ growth, but were low or absent in growth-quiescent adult organs. In both the VP and SV, FGF10 transcripts were expressed only in a subset of mesenchymal cells and in a pattern consistent with a role as a paracrine epithelial regulator. In the neonatal VP, FGF10 mRNA was expressed initially in mesenchymal cells peripheral to the peri-urethral mesenchyme and distal to the elongating prostatic epithelial buds. At later stages, mesenchymal cells surrounding the epithelial buds also expressed FGF10 transcripts. During induction of the SV, FGF10 mRNA was present in mesenchyme surrounding the lower Wolffian ducts and, at later stages, FGF10 transcripts became restricted to mesenchymal cells subadjacent to the serosa. We investigated whether the FGF10 gene might be regulated by androgens by analysing the levels of FGF10 transcripts in SV and VP organs grown in serum-free organ culture. While FGF10 transcript levels increased after treatment with testosterone in the SV (but not VP), these changes were not sensitive to anti-androgen treatment, and thus it is likely that FGF10 mRNA was not directly regulated by testosterone. Also, FGF10 mRNA was observed in the embryonic female reproductive tract in a position analogous to that of the ventral prostate in males suggesting that FGF10 is not regulated by androgens in vivo. Recombinant FGF10 protein specifically stimulated growth of Dunning epithelial and BPH1 prostatic epithelial cell lines, but had no effect on growth of Dunning stromal cells or primary SV mesenchyme. Furthermore, FGF10 protein stimulated the development of ventral prostate and seminal vesicle organ rudiments in serum-free organ culture. When both FGF10 and testosterone were added to organs in vitro, there was no synergistic induction of development. Additionally, development induced by FGF10 was not inhibited by the addition of the anti-androgen Cyproterone Acetate demonstrating that the effects of FGF10 were not mediated by the androgen receptor. Taken together, our experiments suggest that FGF10 functions as a mesenchymal paracrine regulator of epithelial growth in the prostate and seminal vesicle and that the FGF10 gene is not regulated by androgens





Endocrine Disruption and Disruptors

Compounds that alter with hormone receptor and/or signal transduction to alter hormone actions.

Anti-androgenic chemicals that impact the androgen signaling pathway can affect male reproductive development via several different mechanisms of action resulting in slightly differing profiles of effects

		↓ Testosterone production			
Compound	Binds AR	↓ Insl3	↓mRNA	↓activity w/no ↓mRNA	'Low dose' prominent malformations
Vinclozolin	X	0	0	0	Retained nipples; Hypospadias; Agenesis of ventral prostate
Procymidone	X	0	0	0	Similar to Vinclozolin
Linuron	X	0	0	X	Epididymal and testis abnormalities; No gubernacular agenesis
Prochloraz	X	0	0	X	Similar to Vinclozolin
Dibutyl phthalate	0	X	X	-	All three phthalates produce epididymal and testis abnormalities' Gubernacular agenesis
Benzybutyl phthalate	0	X	X	-	
Diethylhexyl phthalate	0	X	X	-	

↓: decrease, X: a known mechanism, 0: does not act through this mechanism, ↓activity w/no ↓mRNA: an x here indicates enzyme activity is decreased but expression levels of mRNA for the enzyme are not affected.

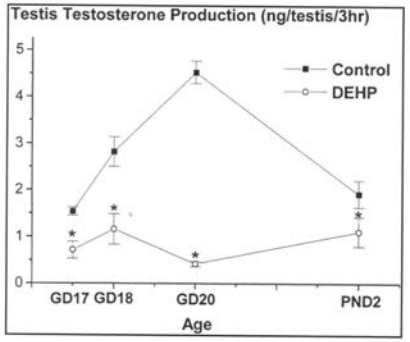


FIG. 2. Basal levels of testosterone production (ng/testis/3 h) during incubation of one testis (GD 17–20) or paired testes (PND 3) for control and DEHP (750 mg/kg/day from GD 14–PND 3) treatment groups from gestational day 17 to postnatal day 2. Graph values represent litter means with standard error bars, and asterisks indicate statistical significance at the $p \leq 0.05$ level, based on litter means analyses ($n = 4$ litters for GDs 17, 18, and 20, and $n = 5$ litters for PND 2).

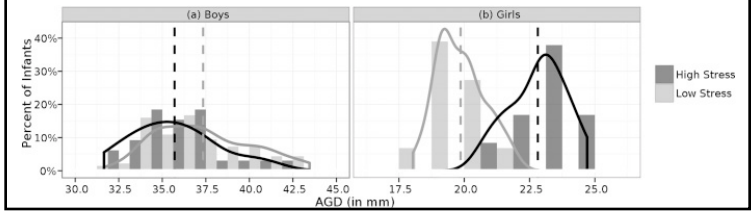
Stress and Androgen Activity During Fetal Development

Endocrinology. 2015 Oct;156(10):3435-41

Barrett ES, Swan SH

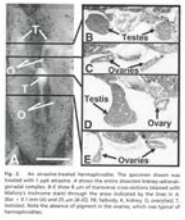
Abstract

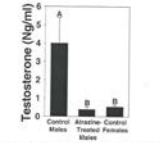
Prenatal stress is known to alter hypothalamic-pituitary-adrenal axis activity, and more recent evidence suggests that it may also affect androgen activity. In animal models, prenatal stress disrupts the normal surge of testosterone in the developing male, whereas in females, associations differ by species. In humans, studies show that (1) associations between prenatal stress and child outcomes are often sex-dependent, (2) prenatal stress predicts several disorders with notable sex differences in prevalence, and (3) prenatal exposure to stressful life events may be associated with masculinized reproductive tract development and play behavior in girls. In this minireview, we examine the existing literature on prenatal stress and androgenic activity and present new, preliminary data indicating that prenatal stress may also modify associations between prenatal exposure to diethylhexyl phthalate, (a synthetic, antiandrogenic chemical) and reproductive development in infant boys. Taken together, these data support the hypothesis that prenatal exposure to both chemical and nonchemical stressors may alter sex steroid pathways in the maternal-placental-fetal unit and ultimately alter hormone-dependent developmental endpoints.



Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses

Tyrone B. Hayes*, Atif Collins, Melissa Lee, Magdalena Mendoza, Nigel Noriega, A. Ali Stuart, and Aaron Vonk





Reproductive Biology and Endocrinology

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Review

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Estrogen in the adult male reproductive tract: A review

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Abstract

Testosterone and estrogen are no longer considered male only and female only hormones. Both hormones are important in both sexes. It was known as early as the 1930's that developmental exposure to a high dose of estrogen causes malformation of the male reproductive tract, but the early formative years of reproductive biology as a discipline did not recognize the importance of estrogen in regulating the normal function of the adult male reproductive tract. In the adult testis, estrogen is synthesized by Leydig cells and the germ cells, producing a relatively high concentration in rete testis fluid. Estrogen receptors are present in the testis, efferent ductules and epididymis of most species. However, estrogen receptor-? is reported absent in the testis of a few species, including man. Estrogen receptors are abundant in the efferent ductule epithelium, where their primary function is to regulate the expression of proteins involved in fluid reabsorption. Disruption of the ? -receptor, either in the knockout (? ERKO) or by treatment with a pure antiestrogen, results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increased secretion of Cl^-, and eventual decreased fertility. In addition to this primary regulation of luminal fluid and ion transport, estrogen is also responsible for maintaining a differentiated epithelial morphology. Thus, we conclude that estrogen or its ? -receptor is an absolute necessity for fertility in the male.

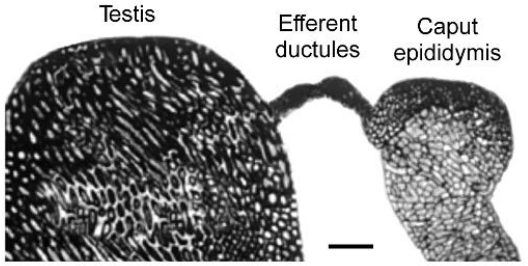


Figure 4
Testis, efferent ductules and epididymis. The surrounding fat pad was dissected away to show the efferent ductules that lie between the testis and caput epididymis. Bar = 2 mm.

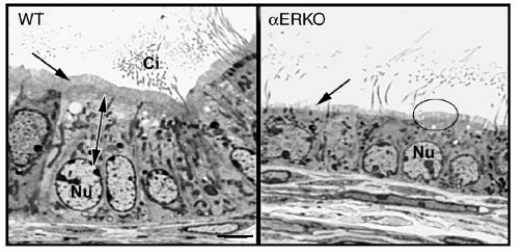


Figure 6
Histology of the efferent ductule epithelium in ? ERKO mouse. The wild-type (WT) ductule epithelium is columnar in shape with nonciliated cells that contain large spherical to oblong shaped nuclei (Nu) and extensive apical cytoplasm (double arrow). The nonciliated cell has a tall microvillus brush border (arrow) and extensive endocytotic apparatus. The ciliated cells have motile cilia (Ci) that extend into the lumen. The ? ERKO efferent ductule epithelium has a low cuboidal shape, with the apical cytoplasm reduced in size and the nucleus (Nu) also smaller. Microvilli are sparse on some cells (arrow) and reduced in height in other cells (circle). Bar = 10 μm.

Estrogen and Its Inhibition in the Male Reproductive Tract

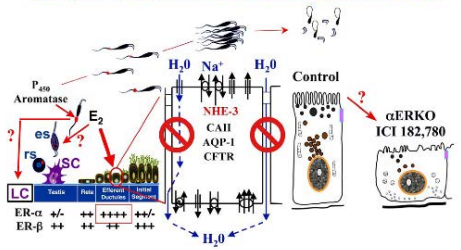
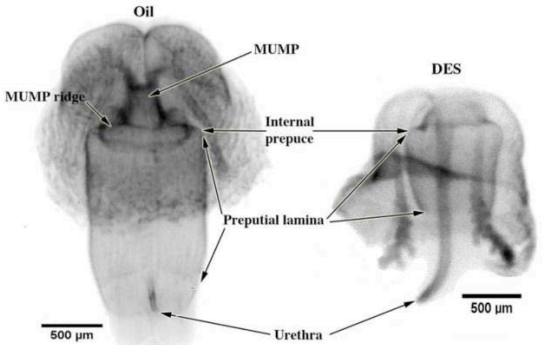


Figure 7
Estrogen and its inhibition in the male reproductive tract: a summary. In adult males, germ cells, as well as Leydig cells (LC) contain P450 aromatase and actively synthesize estrogen (E2), which produces a relatively high concentration in rete testis fluid. This luminal estrogen targets estrogen receptors that are abundant throughout the male reproductive tract, but particularly ER? that is localized in the efferent ductule epithelium, where its expression is more abundant than even the female reproductive tract. In the testis, E2 may also feedback to influence the function of LC and spermatids, either round spermatids (rs) or elongated spermatids (es). Estrogen's primary function in the male tract is the regulation of fluid reabsorption in the efferent ductules via ER? , which increases the concentration of sperm prior to entering the epididymis. Disruption of ER? , either in the knockout (? ERKO) or by treatment with a pure antiestrogen ICI 182,780, results in a decrease in Na^+ transport from lumen to interstitium and thus a decrease in water (H_2O) and fluid reabsorption. This inhibition is mediated by a decrease in the expression of NHE3 mRNA and protein and also decreases in carbonic anhydrase II (CAII) and aquaporin I (AQP-1) proteins. There is also an increase in cystic fibrosis transmembrane conductance regulator protein and mRNA, which adds to the NHE3 effect by secreting Cl^- into the lumen by the cystic fibrosis transmembrane conductance regulator (CFTR) [64]. This inhibition of fluid reabsorption results in the dilution of cauda epididymal sperm, disruption of sperm morphology, and eventual decreased fertility. In addition to this primary regulation of luminal fluids and ions, estrogen is also responsible for maintaining a differentiated epithelial morphology through an unknown mechanism.

Prenatal diethylstilbestrol induces malformation of the external genitalia of male and female mice and persistent second-generation developmental abnormalities of the external genitalia in two mouse strains.
Mahawong P, et al.
Differentiation. 2014 Sep-Oct;88(2-3):51-69.



Optical projection tomography images stained with anti-E-cadherin of day 5 postnatal penises derived from mice treated prenatally with oil or DES as indicated. Note overall reduction in size of all structures, specifically reduction in overall length of the preputial lamina and truncation of distal structures destined to form the penile urethral meatus.

TABLE 1. *CLASSIFICATION OF DISORDERS OF SEX DEVELOPMENT*

A. Non-endocrine DSD (malformative DSD)	B. Endocrine DSD
A.I. Defective morphogenesis of the gonadal ducts	*B.I. Dysgenetic DSD (abnormal gonadal differentiation)*
Müllerian duct aplasia	Complete or partial gonadal dysgenesis
Other uterine malformations	Asymmetric gonadal differentiation
Absence of the vasa deferentia	Ovotesticular DSD
A.II. Defective morphogenesis of the cloaca and urogenital sinus	*B.II. Non-dysgenetic DSD*
Cloacal malformations	Defective male hormone production or action in 46,XY
Exstrophy of the bladder	Defects of androgen production
Prostate defects	Androgen insensitivity (androgen receptor mutations)
A.III. Defective morphogenesis of the primordia of the external genitalia	Persistent Müllerian duct syndrome: defects of AMH production or
Aphallia and micropenis	AMH receptor mutations
Diphallia and bifid phallus	Excess of male hormones in 46,XX
A.IV. Associated defects in the morphogenesis of the urogenital sinus and	Congenital adrenal hyperplasias
the primordia of the external genitalia	Placental aromatase defects
Isolated hypospadias	Adrenal or ovarian tumors
Epispadias	Iatrogenic
Penoscrotal transposition	

TABLE 2. *MALFORMATIVE (NONENDOCRINE) DISORDERS OF SEX DEVELOPMENT IN WHICH A GENETIC ETIOLOGY HAS BEEN IDENTIFIED*

Syndrome	Gene mutation	Defect	OMIM[a]
Mayer–Rokitansky–Küster–Hauser	*WNT4, LHX1, TBX6*	Müllerian derivative malformation or agenesis	277000
MURCS association		Müllerian derivative malformation or agenesis, unilateral renal agenesis, and cervico-thoracic somite dysplasia	601076
RCAD (renal cysts and diabetes)	*HNF1B*	Müllerian aplasia and renal dysfunction	137920
McKusick–Kaufman	*MKKS*	Vaginal atresia	236700
Hand-foot-genital	*HOXA13*	Hypospadias; vagina, uterus, and bladder malformations	140000
Velocardiofacial	Del 22q11.2, *TBX1*	Hypospadias; Müllerian aplasia and renal dysfunction	192430
Cystic fibrosis	*CFTR*	Congenital absence of vasa deferentia	277180
Robinow	*WNT5A*	Aphallia or micropenis	180700
Opitz G/BBB	*MID1*	Hypospadias	300000
Bladder exstrophy and epispadias complex	*PERP*	Epispadias associated to bladder exstrophy	600057

[a]OMIM: Online Mendelian Inheritance of Man (available at: http://www.ncbi.nlm.nih.gov/omim).

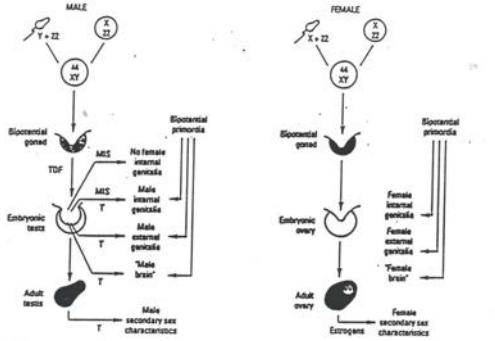
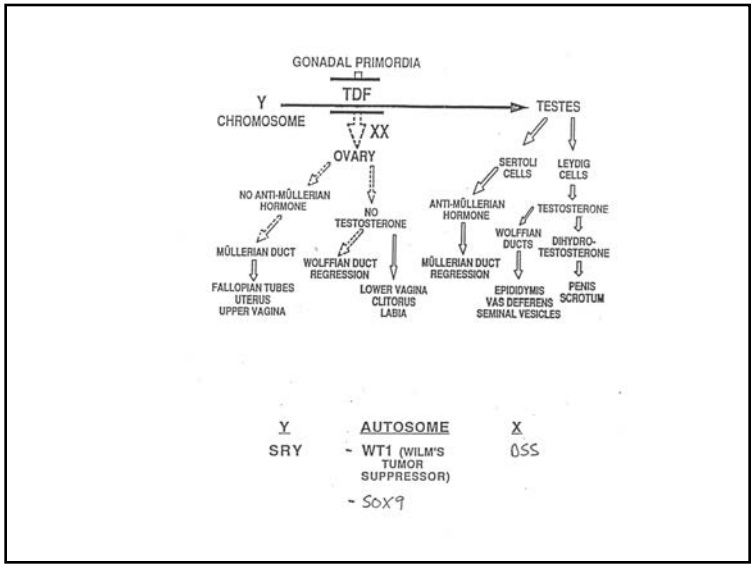


Figure 23–5. Diagrammatic summary of normal sex determination, differentiation, and development in humans. MIS, müllerian inhibiting substance; T, testosterone or other androgen.



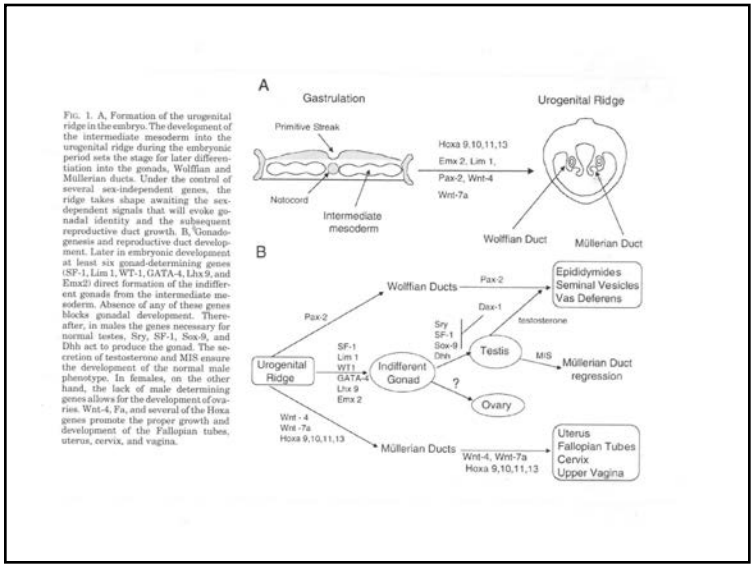


FIG. 1. A, Formation of the urogenital ridge in the embryo. The development of the intermediate mesoderm into the urogenital ridge during the embryonic period sets the stage for later differentiation into the gonads, Wolffian and Müllerian ducts. Under the control of several sex-independent genes, the ridge takes shape awaiting the sex-dependent signals that will evoke gonadal identity and the subsequent reproductive duct growth. B, Gonadogenesis and reproductive duct development. Later in embryonic development at least six gonad-determining genes (SF-1, Lim 1, WT-1, GATA-4, Lhx9, and Emx2) direct formation of the indifferent gonads from the intermediate mesoderm. Absence of any of these genes blocks gonadal development. Thereafter, in males the genes necessary for normal testes, Sry, SF-1, Sox-9, and Dhh act to produce the gonad. The secretion of testosterone and MIS ensure the development of the normal male phenotype. In females, on the other hand, the lack of male determining genes allows for the development of ovaries. Wnt-4, Fa, and several of the Hoxa genes promote the proper growth and development of the Fallopian tubes, uterus, cervix, and vagina.