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Development of the Mammalian Female Reproductive Tract

Yan Yin and Liang Ma*

Division of Dermatology, Department of Medicine, Washington University School of Medicine, St Louis, MO 63110, USA

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The female reproductive tract (FRT), which includes the oviduct, uterus, cervix and vagina, is critical for mammalian reproduction. Recent research using knockout mice has contributed substantially to our understanding of the molecular mechanisms governing FRT development. Aside from satisfying our curiosities about the origin of life, these studies have provided us with a better understanding of FRT disorders and ways to improve female fertility. Here we review genes that are involved in various stages of sexual duct formation and development in mammals. In addition, the effect of exogenous estrogen such as DES on FRT development is also discussed.

Key words: DES, Müllerian duct, uterine adenogenesis, uterus, Wollfian duct.

Müllerian and Wolffian duct formation

The mammalian sex is determined genetically. Individuals with an X and a Y chromosome normally develop as males, whereas those with two X chromosomes develop as females. However, up to a specific stage during embryonic development, embryos of either sex are indistinguishable. In mammals, the urogenital system originates from the intermediate mesoderm. In the mouse, the male reproductive tract develops from the mesonephric duct (a.k.a. the Wolffian duct) by embryonic day 9.5 (E9.5), and can be distinguished from the surrounding mesonephroi as a solid rod. It grows caudally and fuses with the ventrolateral cloacal wall, which gives rise to the future bladder. The paramesonephric duct (a.k.a. the Müllerian duct), giving rise to the majority of FRT, develops in parallel to the the Wolffian duct around E11.5 and reaches the cloaca by E13.5 (*1*). This stage is the aforementioned sexually indifferent stage, as embryos possess both male and female reproductive duct primordials. Subsequently, in male embryos, the Müllerian duct degenerates, whereas the Wolffian duct differentiates into epididymis, vas deferens, seminal vesicles and ejaculatory ducts. In female embryos, however, the Wolffian duct regresses and the Müllerian duct differentiates into oviduct, uterus, cervix and upper one-third of vagina (Fig. 1).

A number of genes have been identified to be essential for FRT development. Among them, *Lim1, Pax2, Emx2* and *Wnt4* are indispensable for the early steps of Müllerian duct development. *Lim1 (Lhx1)*, encoding a LIM-domain containing transcription factor, plays an essential role in mouse head and urogenital system development (*2*, *3*). *Lim1* has a dynamic expression pattern in the Müllerian duct as early as E11.5, suggesting that *Lim1* function is crucial for the initial formation of the Müllerian duct. Although most *Lim1*-null embryos die at mid-gestation due to defects in allantois differentiation, some do survive to birth. Analysis of *Lim1*-null neonates revealed that Müllerian duct derivatives were completely absent. Furthermore, no Wolffian duct derivatives were ever observed in *Lim1*-null neonatal males, demonstrating that *Lim1* is required for the formation of both sexual ducts (*3*). *Pax2*, a member of the *Pax* gene family, possesses a highly conserved paired box which encodes a 128 amino acid DNA binding domain at the N-terminus, termed the Paired domain (*4*). Nine *Pax* genes have been identified in both mouse and human, and knockout of each gene in the mouse resulted in developmental abnormalities (for review, see Ref. *5*). In addition to defects in ear, eye, and brain, *Pax2*-null neonates also lack kidneys, ureters and genital tracts in both males and females (*6*–*8*). Unlike in *Lim1*-null embryos, both the Wolffian and the Müllerian ducts initially form in *Pax2*-null mutants, but later degenerate. This phenotype correlates with *Pax2* expression at this stage in both reproductive tracts on E13.5, indicating a cell-autonomous role for Pax2 in the developing Wolffian and Müllerian ducts (*8*). Another homeobox gene, *Emx2*, a mammalian homologue of the *Drosophila empty spiracles*, is also expressed in the epithelial component of the intermediate mesoderm. *Emx2*-/- mutants completely lack the urogenital system, including kidneys, ureters, gonads and genital tracts (*9*). The Wolffian duct forms in *Emx2* mutant embryos on E10.5, but subsequently degenerates on E11.5. In constrast, the Müllerian duct never forms in these mutants. *Emx2* expression is only detected during early stages of Wolffian and Müllerian duct formation (*9*). These results suggest that this gene is only required in a specific time period during development of the intermediate mesoderm, possibly providing a survival signal.

The mammalian *Wnt* genes are homologues of the *Drosophila* segment polarity gene *Wingless*. They encode secreted signaling glycoproteins that influence multiple processes during development (for review, see Ref. *10*). *Wnt4*-null females exhibit a complete loss of FRTs, whereas male mutants appear normal (*11*). This phenotype is simply due to failure of Müllerian duct formation in both sexes. Up to E11, *Wnt4* expression is detected in the mesenchyme of gonad and mesonephros in both sexes. Subsequently, its expression is downregulated in male gonads but maintained in female gonads. Similarly,

*To whom correspondence should be addressed. Tel: +1-314-454-8771, Fax: +1-314-454-5626, E-mail: lima@im.wustl.edu

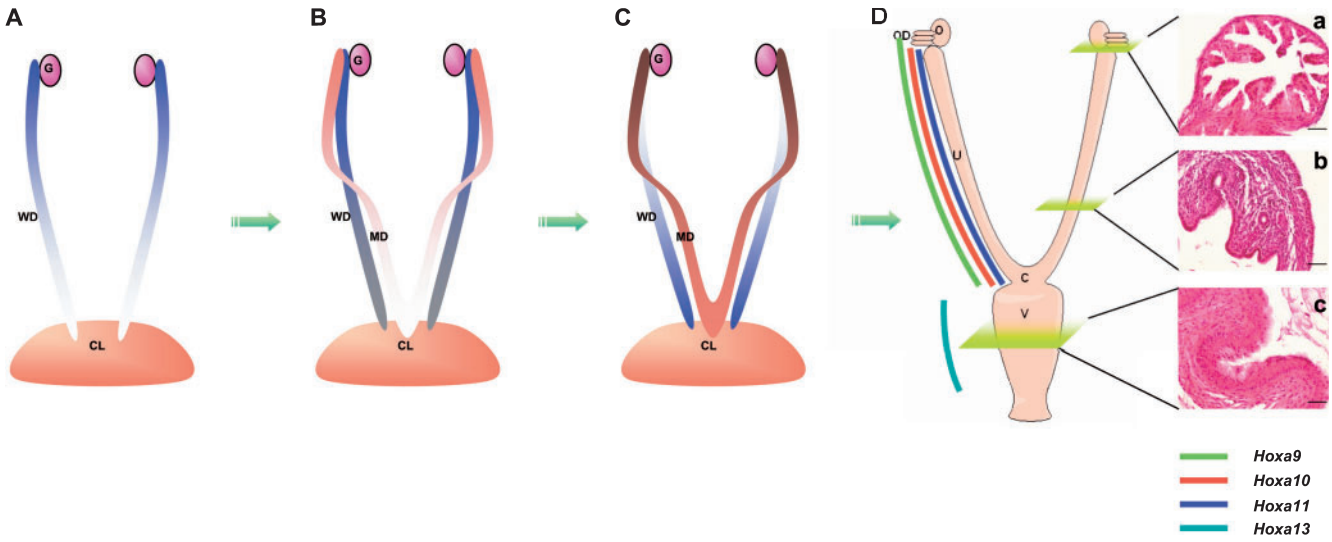

Fig. 1. **A schematic representation of FRT development in the mouse.** A, on E9.5, Wolffian ducts are readily distinguishable from the surrounding mesonephroi and are growing caudally. B, Müllerian ducts grow in parallel to Wolffian ducts at around E11.5 and reach the cloaca by E13.5. At this time, the embryo possesses both the male and female reproductive tract primordials regardless of its genetic sex. C, in females, Wolffian ducts start to degenerate in the absence of androgen, while Müllerian ducts develop in the absence of anti-Müllerian hormone. D, eventually Müllerian ducts differentiate into oviduct, uterus, cervix and upper part of vagina. *AbdB* like *Hox* genes have a nested expression pattern along the A-P axis of the FRT, as illustrated in D. Panels a–c in D, H&E staining of oviductal, uterine and vaginal sections showing morphological differences among the epithelia. G, gonad; WD, Wolffian duct; CL, cloaca; MD, Müllerian duct; OD, oviduct; U, uterus; C, cervix; V, vagina. Scale bars = 50 μm.

its expression was no longer detected in the developing Wolffian duct, but continues to be strongly expressed in the mesenchyme of the newly formed Müllerian duct, indicating a requirement for *Wnt4* during the initial stages of FRT morphogenesis (*11*). Despite these recent exciting discoveries, we still know very little about the nature of the signal(s) that triggers the formation of both sexual ducts from the mesonephric mesenchyme. Future research building on existing mutants may shed light onto this process if upstream regulators and downstream targets of these genes can be identified. Meanwhile, in *Emx2* mutant intermediate mesoderm, *Lim1*, *Pax2* and *Wnt4* are abnormally expressed, whereas *Pax2* expression is abolished in that of *Lim1* mutants, demonstrating the emergence of a genetic pathway in the formation of sexual ducts (*9*, *12*).

Regression of the Müllerian duct in males

As mentioned earlier, mammalian embryos undergo a sexual indifferent stage in which both male and female reproductive tract primordials are present. Since the degeneration of the Müllerian duct requires the presence of male hormones, it is widely accepted that female development is the default pathway. In male embryos, the sex determining region Y gene (*Sry*) is sufficient to induce testis differentiation and subsequent male reproductive tract development (*13*). Sry is an HMG box containing transcription factor (*14*). It is believed that Sry functions by inducing the expression of Müllerian Inhibiting Substance (MIS, a.k.a. anti-Müllerian hormone, AMH), secreted by the sertoli cells in the testis (*15*). *MIS* mutant males exhibit normal testis morphology and function. Nevertheless, ovaries as well as the FRTs persist in these mutants which perturb sperm transfer leading to male infertility (*16*). Molecularly *MIS* expression is regulated by an array of transcription factors, including GATA factors, nuclear receptor subfamily 0 group B member 1 (Nr0b1, a.k.a. Dax1), nuclear receptor subfamily 5 group A member 1 (Nr5a1, a.k.a. Sf1), SRY-box containing protein 9 (Sox9) and Wilms tumour homologue (WT1) (for review, see Ref. *17*). *MIS* encodes a glycoprotein that shows homology to the transforming growth factor beta (TGFβ) superfamily members. Like most other members of the TGFβ superfamily, full activation of MIS requires a proteolytic cleavage near its C-terminus.

TGFβ superfamily ligands transmit their signals by binding to a heterodimeric receptor complex consisted of a type I and a type II TGFβ receptors. Upon ligand binding, the type II receptor, like other serine/ threonine kinase receptors, recruits and phosphorylates the type I receptor which in turn phosphorylates downstream targets (*18*). The MIS-specific type II receptor (*Misr2*) has been identified and cloned in many species (*19–22*). Its expression was detected in the mesenchyme surrounding the Müllerian duct at E13.5 when regression of the Müllerian duct begins. *Misr2*-deficient males exhibit the same phenotype as *MIS* mutant males, indicating that MIS uses Misr2 exclusively for its signal transduction (*22*). Natural mutations in MIS and MISR2 genes have also been reported in patients of Persistent Müllerian Duct Syndrome, a rare form of male pseudohermaphroditism (*23*, *24*). In the case of the type I receptor for MIS, recent studies showed that activin A receptor type 1 (*Acvr1*, a.k.a. *Alk2*) has a spatial and temporal expression pattern overlapping with that of *Misr2* perfectly in the developing Müllerian duct mesenchyme. Furthermore, *in vitro* cell culture experiments confirmed that Acvr1 is required for MIS signaling, suggesting that it could be one of the type I receptors for MIS (*25*). However, this has not been proven in knockout mice due to early

lethality of *Acvr1*-null embryos (*25*). Generation of an *Acvr1* conditional knockout mouse in the Müllerian duct mesenchyme should be able to demonstrate its *in vivo* relevance in MIS signal transduction. Meanwhile, conditional ablation of bone morphogenetic protein receptor type 1A (*Bmpr1a*, a.k.a. *Alk3*) in the developing Müllerian duct mesenchyme using the *cre*/*lox* system revealed that it could also serve as the type I receptor for MIS (*26*). In *Bmpr1a* conditional knockout male embryos, *MIS* and *Misr2* expressions are preserved. However, these mutant male neonates still possess oviduct and uterus, indicating that *Bmpr1a* is required in the MIS signaling pathway (*26*).

Although little is known about the downstream targets of MIS signaling cascades, a few candidates have been proposed. The most promising one is matrix metalloproteinase 2 (Mmp2) (*27*). *Mmp2* has a sexually dimorphic expression pattern during embryogenesis, which is no longer maintained in *MIS*-deficient mice. Inhibition of Mmp2 in organ culture blocked Müllerian duct regression. On the other hand, activation of Mmp2 led to Müllerian duct regression in a ligand-independent manner (*27*). Taken together, Mmp2, and may be other members of the Mmp family, possibly functions downstream of MIS signaling pathway to mediate Müllerian duct degeneration.

One of the upstream signals regulating Misr2 expression is *Wnt-7a*, which is critically required for the development of reproductive tracts in both sexes. *Wnt7a*-null mice of either sex are infertile, due to persisted Müllerian duct in mutant males and abnormal differentiation of the oviduct and uterus in mutant females (*28*). The direct cause of persisted Müllerian duct in *Wnt7a*-deficient males is the absence of *Misr2* expression in these mutants. The regulation of *Misr2* expression by *Wnt7a* is thought to be mediated through the canonical *Wnt* pathway and requires the presence of functional β-catenin (*29*). Transcriptionally, *Misr2* is directly regulated by *Sf1*, suggesting multiple roles played by *Sf1* during male sex differentiation (*30*). In addition, β-catenin and transcription factor 4 (TCF4) are also required for regulation of *Misr2* by Sf1 (*29*).

Patterning and cytodifferentiation of the Müllerian duct

In female mice, the Wolffian duct regresses due to the absence of male hormones which are necessary for its maintenance. The Müllerian duct proliferates and differentiates rostral-caudally, forming the oviduct, uterus, cervix and upper part of vagina, which is completed by two weeks after birth. During this time, the single-layered Müllerian duct epithelium differentiates into distinct morphologies along the anterior-posterior (A-P) axis of the FRT. For example, the uterine epithelium is composed of simple columnar epithelial cells, whereas stratified squamous cells make up epithlia of posterior cervix and vagina. Classical tissue recombination experiments demonstrated that this differential cell fate determination along the A-P axis requires reciprocal interactions between the epithelium and the underlying mesenchyme (*31*). The fate of FRT epithelial cells is determined between postnatal days 5 to 7 by cues from the underlying mesenchyme while some developmental plasticity remains in a subset of epithelial cells for even longer (*32*).

Homeobox genes encode a family of transcription factors sharing a highly conserved 61 amino acid helix-turn-helix domain, termed the homeodomain. In mammals, *Hox* genes are expressed in a temporally and spatially colinear pattern during organogenesis which provides positional information along several body axes (for review, see Ref. *33*). In the developing Müllerian duct, a number of posterior *AbdominalB Hox* genes were found to be expressed in nested patterns along the A-P axis (*34*, *35*). In the case of *AbdB Hoxa* genes, anterior limit of *Hoxa9* expression was detected in the future isthmus region of the oviduct, with that of *Hoxa10* at the uterotubal junction. *Hoxa11* is strongly expressed in the uterus and less intensively in the cervix, whereas *Hoxa13* is expressed only in the cervix and upper vagina (*35*). Targeted mutagenesis of these genes results in region-specific defects along the FRT. *Hoxa10* deficiency causes homeotic transformation of the anterior part of the uterus into oviduct-like structure and reduced fertility in females (*36*). Homeotic transformation of the reproductive tract also exists in male *Hoxa10* mutants (*36*). *Hoxa13* null embryos show agenesis of the posterior portion of the Müllerian duct (*37*). As mentioned earlier, the molecular cues from the mesenchyme induce the differentiation of Müllerian duct epithelium. While the nature of these stromal signals is not clear at present, swapping the homeodomain of Hoxa-11 with that of Hoxa-13 resulted in the expression of a hybrid Hox protein in the uterine stroma and subsequent stratification of the uterine epithelium, suggesting that the stromal signals are likely controlled by Hox proteins (*38*). In response to such stromal signals from the cervix and vagina, epithelial expression of *p63*, a homologue of the *p53* tumor suppressor gene, is induced and is thought to be an identity switch in the differentiation of Müllerian duct epithelium (*39*).

Wnt7a, which has been shown to influence Müllerian duct regression, also plays an important role at later stages of FRT cytodifferentiation (*28*, *40*). *Wnt7a*-deficient females show dramatic posterior transformation of the reproductive tract, in which oviducts are absent and uterus exhibits a cytoarchitecture typical of vagina. Molecular analysis showed that expression of *Hoxa10* and *Hoxa11* was reduced in the mutant uterine stroma, suggesting that *Wnt7a* is required to maintain uterine-specific *Hox* gene expression (*40*). In addition, *Wnt7a* may also be involved in radial axial patterning since *Wnt7a* mutant myometrium is highly disorganized (*40*). *Wnt5a*, another member of this growth factor family, is also critical for FRT development (*41*). *Wnt5a*-deficient females have coiled and shortened uterus and poorly-defined cervix and vagina. Genetic analyses revealed that *Wnt5a* resides in an genetic pathway with *Wnt7a* and *AbdB Hoxa* genes during FRT development (*41*).

Gland formation in the uterus

Endometrial glands are present in the uterus of all mammals. The main biological function of uterine glands is thought to secrete and transport nutrients, termed histotroph, important for embryo implantation and fetal development. In most mammals, uterine gland develop-

ment (also termed uterine adenogenesis) occurs postnatally and the timing is highly species-specific. In rodents, epithelial invagination is first seen on P5, and mature glands can be detected by P15 (*42*), whereas in ungulates, the process begins right after birth (P0) and is completed by P56 in sheep and by P120 in pigs, respectively In humans and other primates, however, this process begins *in utero*, continues after birth and glands reach histological maturity at puberty. Typical uterine adenogenesis includes epithelial invagination, bud formation and tubulogenesis, followed by further branching morphogenesis. Unlike primates and ungulates, rodent uterine glands do not undergo extensive coiling and branching morphogenesis, resulting in a simpler, less coiled and branched structure (*42*, *43*). In rodents, at least two known critical proteins, leukemia inhibitory factor (*Lif*) and calcitonin, are exclusively produced by uterine glands. Perturbation of rat uterine calcitonin expression by antisense oligonucleotides during early pregnancy severely affected implantation, resulting in a reduced number of implanted embryos (*44*). *Lif* is a downstream target of the endogenous estrogen, 17β-estradiol (E2), during pregnancy. *Lif*-null female mice are infertile due to failure of blastocyst invasion and stromal decidulization (*45*). These results indicate that endometrial glands are essential for successful pregnancy in mammals. The cellular and molecular mechanisms governing uterine adenogenesis are not fully understood. However, several lines of evidence implicate that epithelial-mesenchymal interactions, tissue remodeling factors, steroid hormones and their receptors, and prolactin are involved in this process.

Again, Wnt signaling is important in uterine gland formation. *Wnt7a* is expressed exclusively in the uterine luminal epithelium (LE) and in invaginated LE but not in glandular epithelium (GE), suggesting that Wnt7a may play a role in uterine adenogenesis. In contrast, *Wnt5a* is mainly expressed in the uterine stroma during postnatal development. Both *Wnt7a* and *Wnt5a* mutant uteri fail to form uterine glands indicating that Wnt signaling is essential for uterine gland formation. Moreover, recombinant grafts of wild-type stroma and *Wnt5a* mutant epithelium successfully formed glands whereas the reverse recombination failed, indicating that *Wnt5a* is required in the stroma for gland development (*40*, *41*).

Uterine gland development also involves endometrial tissue remodeling. MMPs and their tissue inhibitors (TIMPs) have been shown to be key regulators of branching morphogenesis in various organs (*46–48*). Consistently, three TIMPs (TIMP-1, -2, and -3) are expressed in the uterus and TIMP-1 knockout mouse exhibits accelerated endometrial gland formation (*46*, *49*). In addition, MMP-2, -10, -11, -14, and -23 are all expressed at high levels in the developing uterus with MMP-9 at a lower level. However, other TIMP and MMP knockout mice do not exhibit impaired uterine gland formation, which may be the result of functional redundancy (*50*). Future experiment examining uterine adenogenesis in compound genetic mutants is necessary to reveal the function of these proteins in uterine gland formation.

Insulin-like growth factors (IGFs) are multifunctional regulators involved in various biological processes (*51*). The temporal and spatial expression of IGF1 and several members of IGF-binding proteins have been characterized in the developing rat uterus (*52*). Loss of IGF1 in mice leads to a dramatic hypoplastic uterine phenotype demonstrating its critical role in uterine growth and differentiation (*53*). Studies in ovine uterus revealed that the highest level of IGF1 and IGF2 mRNA is present in the area surrounding the nascent and proliferating glandular epithelium, whereas IGF1R mRNA is most abundant in luminal and glandular epithelium, suggesting that IGF-mediated epithelial-mesenchymal interactions might be critical for uterine gland development (*54*).

Numerous studies in lab rodents and ungulates have shown that postnatal development of uterine glands does not depend on ovary and hormones. Yet abnormal exposure to steroid hormones during critical period of postnatal uterine development can severely affect uterine adenogenesis (*55–57*). In ewe lambs, a distinct uterine gland knockout (UGKO) model was generated by neonatal exposure to a synthetic progestin for as short as eight weeks from birth, which completely abolished uterine adenogenesis (*42*, *58*, *59*). The mechanism for this phenotype may involve suppression of estrogen receptor-α (ER-α) expression, as treatment with an ER-α antagonist also inhibited adenogenesis in neonatal pig uterus (*42*, *59*, *60*). However, ER-α knockout mouse uterus develops glands albeit at a reduced number, suggesting that ER-α may not be required for the initiation of uterine adenogenesis but is probably required for GE differentiation (*42*, *61*, *62*). Prolactin (PRL) is another important player in uterine adenogenesis. In ewes, PRL is present in the serum from P1, peaks on P14, and declines thereafter until the whole process is complete. Expression of PRL receptor (PRL-R) is restricted to nascent glandular epithelium on P7 and to proliferating GE (*63*, *64*). Treatment with PRL from P0 to P56 increased uterine gland number, whereas treatment with a PRL inhibitor bromocriptin decreased gland number in ewe lambs (*64*). These results support the idea that PRL positively regulates uterine gland development. However, the molecular mechanism whereby PRL regulates uterine adenogenesis is still not fully understood. A couple of studies indicated that MAPK signaling cascade and JAK/STAT signaling cascade may be involved in this event (*65*, *66*).

DES-induced reproductive tract phenotypes

Diethylstilbestrol (DES) is the first synthetic estrogenic compound orally administered to pregnant women from 1947 to 1971 in an effort to preserve pregnancy. Despite controlled studies in the 1950s showing no protective effect of DES towards pregnancy, this drug was continually prescribed to pregnant women in the United States and elsewhere in the world for another two decades. During that time at least four million pregnant women and their fetuses were exposed to DES in the United States alone (for review, see Ref. *67*). In the late 1960s, eight young women 15–22 years of age were treated in Boston for clear cell adenocarcinoma of the vagina, a rare tumor usually occurring in women over 50 years old (*68*). Since then, *in utero* exposure to DES has been associated with reproductive tract anomalies such as T-shaped uterus, vaginal adenosis, extensive ectropion, annular cervical rings and clear cell adenocarcinoma in female humans (*69*, *70*). In males who were exposed to DES in utero, cryptorchidism, hypospadias,

Table 1. **Genes involved in FRT development and phenotype of the reproductive tract in their mutants.**

Gene	Knockout phenotype in reproductive tract	Reference
Lim1	Absence of derivatives of Müllerian duct	(*3*)
Pax2	Absence of entire FRT	(*8*)
Emx2	Absence of derivatives of Müllerian duct	(*9*)
Wnt4	Absence of derivatives of Müllerian duct	(*11*)
MIS	Persistence of Müllerian duct derivatives in male mutants	(*16*)
Misr2	Same as MIS-null mice	(*22*)
Alk3	Oviduct and uterus in male mutants	(*26*)
Hoxa10	Homeotic transformation of the anterior part of the uterus into oviduct-like structure	(*36*, *37*)
Hoxa11	Hypoplastic uterus, reduced gland number	(*74*, *75*)
Hoxa13	Agenesis of the posterior portion of the Müllerian ducts	(*37*)
P63	Persistence of columnar epithelium at lower genital tract sites that normally undergo squamous and urothelial differentiation.	(*76*)
Wnt7a	Absence of oviducts and uteri exhibiting a cytoachitecture typical of vagina; failure to form uterine glands	(*28*, *40*)
Wnt5a	Coiled and shortened uterus and poorly-defined cervix and vagina; failure to form uterine glands	(*41*)
Lif1	Failure of blastocyst invasion and stromal decidulization	(*45*)
IGF	Dramatic hypoplasia of the uterus	(*53*)
TIMP1	Accelerated endometrial gland formation	(*46*)
ERα	Hypoplastic uteri, nonovulatory ovaries and reduced gland number	(*61*, *62*)

microphallus, testicular hypoplasia and epididymal cysts were found to be associated with DES exposure. DES has a binding affinity for ER-α that is much higher than that of 17β-estradiol (*71*). Thus it is believed that DES is not readily metabolized in the placenta and therefore can function as a strong estrogen. The establishment of a mouse model for DES-induced reproductive tract defects has made substantial contributions to study the teratogenicity of DES (*72*). DES also induces pleiotropic defects along the developing mouse FRT. Oviductal malformations include loss of the uterotubal junction, paraovarian cycsts, and developmental arrest of the oviduct. Uterine malformations include stratification of the uterine epithelium, delayed adenogenesis, reduced gland formation and stromal layer, and disorganized muscle layers. Defects in cervix and vagina include abnormal presence of glands in the vaginal fornix (vaginal adenosis), abnormal uterthral openings in the vagina (persistent urogenital sinus), and failure of distal Müllerian ducts to form a common cervical canal (*72*). These phenotypes resemble those observed in various *Hoxa* single and compound mutant and *Wnt7a* mutant FRTs. Indeed, *Hoxa-10*, *Hoxa-11* and *Wnt7a* are all repressed by DES in the developing uterus during critical developmental period of FRT patterning offering a possible molecular basis for DES teratogenicity. Our recent studies showed that developmental DES exposure alters uterine epithelial cell fate and induces abnormal epithelial differentiation. A number of stratified epithelial markers were found ectopically expressed in the uterine epithelium (*73*). Moreover, several additional developmental control genes were also identified.

In summary, although investigations into reproductive tract development began centuries ago, it is not until recent decades that more molecular mechanisms underlying this process have been uncovered owing to the rapid development of modern molecular genetics. This article has reviewed some of the most important factors in this process. Table 1 lists a subset of these genes knockout of which produces defects in reproductive tract development. However, we are still not certain how these genes assemble into a genetic pathway to orchestrate a complicated and highly organized reproductive tract developmental process. Future research should be concentrated on identifying additional players and elucidating crosstalks between different genetic pathways.

REFERENCES

1. Bard, J.B.L. and Kaufman, M.H. (1999) *The Anatomical Basis of Mouse Development*, Academic Press, New York
2. Shawlot, W. and Behringer, R.R. (1995) Requirement for Lim1 in head-organizer function. *Nature* **374**, 425–430
3. Kobayashi, A., Shawlot, W., Kania, A., and Behringer, R.R. (2004) Requirement of Lim1 for female reproductive tract development. *Development* **131**, 539–549
4. Treisman, J., Harris, E., and Desplan, C. (1991) The paired box encodes a second DNA-binding domain in the paired homeo domain protein. *Genes Dev.* **5**, 594–604
5. Dahl, E., Koseki, H., and Balling, R. (1997) Pax genes and organogenesis. *Bioessays* **19**, 755–765
6. Torres, M., Gomez-Pardo, E., and Gruss, P. (1996) Pax2 contributes to inner ear patterning and optic nerve trajectory. *Development* **122**, 3381–3391
7. Urbanek, P., Fetka, I., Meisler, M.H., and Busslinger, M. (1997) Cooperation of Pax2 and Pax5 in midbrain and cerebellum development. *Proc. Natl Acad. Sci. USA* **94**, 5703–5708
8. Torres, M., Gomez-Pardo, E., Dressler, G.R., and Gruss, P. (1995) Pax-2 controls multiple steps of urogenital development. *Development* **121**, 4057–4065
9. Miyamoto, N., Yoshida, M., Kuratani, S., Matsuo, I., and Aizawa, S. (1997) Defects of urogenital development in mice lacking Emx2. *Development* **124**, 1653–1664
10. Nelson, W.J. and Nusse, R. (2004) Convergence of Wnt, β-catenin, and cadherin pathways. *Science* **303**, 1483–1487
11. Vainio, S., Heikkila, M., Kispert, A., Chin, N., and McMahon, A.P. (1999) Female development in mammals is regulated by Wnt-4 signalling. *Nature* **397**, 405–409
12. Tsang, T.E., Shawlot, W., Kinder, S.J., Kobayashi, A., Kwan, K.M., Schughart, K., Kania, A., Jessell, T.M., Behringer, R.R., and Tam, P.P. (2000) Lim1 activity is required for intermediate mesoderm differentiation in the mouse embryo. *Dev. Biol.* **223**, 77–90
13. Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P., and Lovell-Badge, R. (1991) Male development of chromosomally female mice transgenic for Sry. *Nature* **351**, 117–121

14. Giese, K., Cox, J., and Grosschedl, R. (1992) The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. *Cell* **69**, 185–195
15. Cate, R.L., Mattaliano, R.J., Hession, C., Tizard, R., Farber, N.M., Cheung, A., Ninfa, E.G., Frey, A.Z., Gash, D.J., Chow, E.P., *et al.* (1986) Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. *Cell* **45**, 685–698
16. Behringer, R.R., Finegold, M.J., and Cate, R.L. (1994) Mullerian-inhibiting substance function during mammalian sexual development. *Cell* **79**, 415–425
17. Rey, R., Lukas-Croisier, C., Lasala, C., and Bedecarras, P. (2003) AMH/MIS, what we know already about the gene, the protein and its regulation. *Mol. Cell. Endocrinol.* **211**, 21–31
18. Wrana, J.L., Attisano, L., Wieser, R., Ventura, F., and Massague, J. (1994) Mechanism of activation of the TGF-beta receptor. *Nature* **370**, 341–347
19. Baarends, W.M., van Helmond, M.J., Post, M., van der, S.choot, P.J., Hoogerbrugge, J.W., de Winter, J.P., Uilenbroek, J.T., Karels, B., Wilming, L.G., Meijers, J.H., *et al.* (1994) A novel member of the transmembrane serine/threonine kinase receptor family is specifically expressed in the gonads and in mesenchymal cells adjacent to the mullerian duct. *Development* **120**, 189–197
20. Teixeira, J., He, W.W., Shah, P.C., Morikawa, N., Lee, M.M., Catlin, E.A., Hudson, P.L., Wing, J., Maclaughlin, D.T., and Donahoe, P.K. (1996) Developmental expression of a candidate mullerian inhibiting substance type II receptor. *Endocrinology* **137**, 160–165
21. di Clemente, N., Wilson, C., Faure, E., Boussin, L., Carmillo, P., Tizard, R., Picard, J.Y., Vigier, B., Josso, N., and Cate, R. (1994) Cloning, expression, and alternative splicing of the receptor for anti-Mullerian hormone. *Mol. Endocrinol.* **8**, 1006–1020
22. Mishina, Y., Rey, R., Finegold, M.J., Matzuk, M.M., Josso, N., Cate, R.L., and Behringer, R.R. (1996) Genetic analysis of the Mullerian-inhibiting substance signal transduction pathway in mammalian sexual differentiation. *Genes Dev.* **10**, 2577–2587
23. Imbeaud, S., Faure, E., Lamarre, I., Mattei, M.G., di Clemente, N., Tizard, R., Carre-Eusebe, D., Belville, C., Tragethon, L., Tonkin, C., *et al.* (1995) Insensitivity to anti-mullerian hormone due to a mutation in the human anti-mullerian hormone receptor. *Nat. Genet.* **11**, 382–388
24. Imbeaud, S., Belville, C., Messika-Zeitoun, L., Rey, R., di Clemente, N., Josso, N., and Picard, J.Y. (1996) A 27 base-pair deletion of the anti-mullerian type II receptor gene is the most common cause of the persistent mullerian duct syndrome. *Hum. Mol. Genet.* **5**, 1269–1277
25. Clarke, T.R., Hoshiya, Y., Yi, S.E., Liu, X., Lyons, K.M., and Donahoe, P.K. (2001) Mullerian inhibiting substance signaling uses a bone morphogenetic protein (BMP)-like pathway mediated by ALK2 and induces SMAD6 expression. *Mol. Endocrinol.* **15**, 946–959
26. Jamin, S.P., Arango, N.A., Mishina, Y., Hanks, M.C., and Behringer, R.R. (2002) Requirement of Bmpr1a for Mullerian duct regression during male sexual development. *Nat. Genet.* **32**, 408–410
27. Roberts, L.M., Visser, J.A., and Ingraham, H.A. (2002) Involvement of a matrix metalloproteinase in MIS-induced cell death during urogenital development. *Development* **129**, 1487–1496
28. Parr, B.A. and McMahon, A.P. (1998) Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. *Nature* **395**, 707–710
29. Hossain, A. and Saunders, G.F. (2003) Synergistic cooperation between the beta-catenin signaling pathway and steroidogenic factor 1 in the activation of the Mullerian inhibiting substance type II receptor. *J. Biol. Chem.* **278**, 26511–26516
30. Shen, W.H., Moore, C.C., Ikeda, Y., Parker, K.L., and Ingraham, H.A. (1994) Nuclear receptor steroidogenic factor 1 regulates the mullerian inhibiting substance gene, a link to the sex determination cascade. *Cell* **77**, 651–661
31. Cunha, G.R (1976) Stromal induction and specification of morphogenesis and cytodifferentiation of the epithelia of the Mullerian ducts and urogenital sinus during development of the uterus and vagina in mice. *J. Exp. Zool.* **196**, 361–370
32. Kurita, T., Cooke, P.S., and Cunha, G.R. (2001) Epithelial-stromal tissue interaction in paramesonephric (Mullerian) epithelial differentiation. *Dev. Biol.* **240**, 194–211
33. Kmita, M. and Duboule, D. (2003) Organizing axes in time and space; 25 years of colinear tinkering. *Science* **301**, 331–333
34. Dolle, P., Izpisua-Belmonte, J.C., Brown, J.M., Tickle, C., and Duboule, D. (1991) HOX-4 genes and the morphogenesis of mammalian genitalia. *Genes Dev.* **5**, 1767–1767
35. Taylor, H.S., Vanden Heuvel, G.B., and Igarashi, P. (1997) A conserved Hox axis in the mouse and human female reproductive system, late establishment and persistent adult expression of the Hoxa cluster genes. *Biol. Reprod.* **57**, 1338–4135
36. Benson, G.V., Lim, H., Paria, B.C., Satokata, I., Dey, S.K., and Maas, R.L. (1996) Mechanisms of reduced fertility in Hoxa-10 mutant mice, uterine homeosis and loss of maternal Hoxa-10 expression. *Development* **122**, 2687–2696
37. Warot, X., Fromental-Ramain, C., Fraulob, V., Chambon, P., and Dolle, P. (1997) Gene dosage-dependent effects of the Hoxa-13 and Hoxd-13 mutations on morphogenesis of the terminal parts of the digestive and urogenital tracts. *Development* **124**, 4781–4791
38. Zhao, Y. and Potter, S.S. (2001) Functional specificity of the Hoxa13 homeobox. *Development* **128**, 3197–3207
39. Kurita, T., Mills, A.A., and Cunha, G.R. (2004) Roles of p63 in the diethylstilbestrol-induced cervicovaginal adenosis. *Development* **131**, 1639–1649
40. Miller, C. and Sassoon, D.A. (1998) Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. *Development* **125**, 3201–3211
41. Mericskay, M., Kitajewski, J., and Sassoon, D. (2004) Wnt5a is required for proper epithelial-mesenchymal interactions in the uterus. *Development* **131**, 2061–2072
42. Gray, C.A., Bartol, F.F., Tarleton, B.J., Wiley, A.A., Johnson, G.A., Bazer, F.W., and Spencer, T.E. (2001) Developmental biology of uterine glands. *Biol. Reprod.* **65**, 1311–1323
43. Bartol, F.F., Wiley, A.A., Floyd, J.G., Ott, T.L., Bazer, F.W., Gray, C.A., and Spencer, T.E. (1999) Uterine differentiation as a foundation for subsequent fertility. *J. Reprod. Fertil. Suppl.* **54**, 287–302
44. Zhu, L.J., Bagchi, M.K., and Bagchi, I.C. (1998) Attenuation of calcitonin gene expression in pregnant rat uterus leads to a block in embryonic implantation. *Endocrinology* **139**, 330–339
45. Chen, J.R., Cheng, J.G., Shatzer, T., Sewell, L., Hernandez, L., and Stewart, C.L. (2000) Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. *Endocrinology* **141**, 4365–4372
46. Zhou, H.E., Zhang, X., and Nothnick, W.B. (2004) Disruption of the TIMP-1 gene product is associated with accelerated endometrial gland formation during early postnatal uterine development. *Biol. Reprod.* **71**, 534–539
47. Kheradmand, F., Rishi, K., and Werb, Z. (2002) Signaling through the EGF receptor controls lung morphogenesis in part by regulating MT1-MMP-mediated activation of gelatinase A/MMP2. *J. Cell Sci.* **115**, 839–848
48. Simian, M., Hirai, Y., Navre, M., Werb, Z., Lochter, A., and Bissell, M.J. (2001) The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. *Development* **128**, 3117–3131
49. Hu, J., Zhang, X., Nothnick, W.B., and Spencer, T.E. (2004) Matrix metalloproteinases and their tissue inhibitors in the developing neonatal mouse uterus. *Biol. Reprod.* **71**, 1598–1604
50. Hulboy, D.L., Rudolph, L.A., and Matrisian, L.M. (1997) Matrix metalloproteinases as mediators of reproductive function. *Mol. Hum. Reprod.* **3**, 27–45

51. Jones, J.I. and Clemmons, D.R. (1995) Insulin-like growth factors and their binding proteins, biological actions. *Endocr. Rev.* **16**, 3–34
52. Gu, Y., Branham, W.S., Sheehan, D.M., Webb, P.J., Moland, C.L., and Streck, R.D. (1999) Tissue-specific expression of messenger ribonucleic acids for insulin-like growth factors and insulin-like growth factor-binding proteins during perinatal development of the rat uterus. *Biol. Reprod.* **60**, 1172–1182
53. Baker, J., Hardy, M.P., Zhou, J., Bondy, C., Lupu, F., Bellve, A.R., and Efstratiadis, A. (1996) Effects of an Igf1 gene null mutation on mouse reproduction. *Mol. Endocrinol.* **10**, 903–918
54. Taylor, K.M., Chen, C., Gray, C.A., Bazer, F.W., and Spencer, T.E. (2001) Expression of messenger ribonucleic acids for fibroblast growth factors 7 and **10**, hepatocyte growth factor, and insulin-like growth factors and their receptors in the neonatal ovine uterus. *Biol. Reprod.* **64**, 1236–1246
55. Tarleton, B.J., Wiley, A.A., Spencer, T.E., Moss, A.G., and Bartol, F.F. (1998) Ovary-independent estrogen receptor expression in neonatal porcine endometrium. *Biol. Reprod.* **58**, 1009–1019
56. Bartol, F.F., Wiley, A.A., Coleman, D.A., Wolfe, D.F., and Riddell, M.G. (1988) Ovine uterine morphogenesis, effects of age and progestin administration and withdrawal on neonatal endometrial development and DNA synthesis. *J. Anim. Sci.* **66**, 3000–3009
57. Branham, W.S. and Sheehan, D.M. (1995) Ovarian and adrenal contributions to postnatal growth and differentiation of the rat uterus. *Biol. Reprod.* **53**, 863–872
58. Allison, G.C., Bartol, F.F., Taylor, K.M., Wiley, A.A., Ramsey, W.S., Ott, T.L., Bazer, F.W., and Spencer, T.E. (2000) Ovine uterine gland knock-out model, effects of gland ablation on the estrous cycle. *Biol. Reprod.* **62**, 448–456
59. Bartol, F.F., Wiley, A.A., Spencer, T.E., Ing, N.H., Ott, T.L., and Bazer, F.W. (1997) Progestin Exposure from birth, Epitenetic induction of a unique adult uterine phenotype in sheep-a glandless endometrium. *Biol. Reprod.* **56**, 133
60. Tarleton, B.J., Wiley, A.A., and Bartol, F.F. (1999) Endometrial development and adenogenesis in the neonatal pig, effects of estradiol valerate and the antiestrogen ICI 182,780. *Biol. Reprod.* **61**, 253–263
61. Curtis, S.W., Clark, J., Myers, P., and Korach, K.S. (1999) Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor alpha knockout mouse uterus. *Proc. Natl Acad. Sci. USA* **96**, 3646–3651
62. Lubahn, D.B., Moyer, J.S., Golding, T.S., Couse, J.F., Korach, K.S., and Smithies, O. (1993) Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc. Natl Acad. Sci. USA* **90**, 11162–11166
63. Taylor, K.M., Gray, C.A., Joyce, M.M., Stewart, M.D., Bazer, F.W., and Spencer, T.E. (2000) Neonatal ovine uterine development involves alterations in expression of receptors for estrogen, progesterone, and prolactin. *Biol. Reprod.* **63**, 1192–1204
64. Carpenter, K.D., Gray, C.A., Noel, S., Gertler, A., Bazer, F.W., and Spencer, T.E. (2003) Prolactin regulation of neonatal ovine uterine gland morphogenesis. *Endocrinology* **144**, 110–120
65. Schwertfeger, K.L., Hunter, S., Heasley, L.E., Levresse, V., Leon, R.P., DeGregori, J., and Anderson, S.M. (2000) Prolactin stimulates activation of c-jun N-terminal kinase (JNK). *Mol. Endocrinol.* **14**, 1592–1602
66. Gubbay, O., Critchley, H.O., Bowen, J.M., King, A., and Jabbour, H.N. (2002) Prolactin induces ERK phosphorylation in epithelial and CD56(+) natural killer cells of the human endometrium. *J. Clin. Endocrinol. Metab.* **87**, 2329–2335
67. Mittendorf, R. (1995) Teratogen update, carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) in utero. *Teratology* **51**, 435–445
68. Herbst, A.L., Ulfelder, H., and Poskanzer, D.C. (1971) Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N. Engl. J. Med.* **284**, 878–881
69. Haney, A.F., Hammond, C.B., Soules, M.R., and Creasman, W.T. (1979) Diethylstilbestrol-induced upper genital tract abnormalities. *Fertil. Steril.* **31**, 142–146
70. Noller, K.L., O'Brien, P.C., Colton, T., Kaufman, R., and Melton, L.J. (1990) Medical and surgical diseases associated with in utero exposure to diethylstilbestrol in *Clinical Practice of Gynecology* (Noller, L.L., ed.) pp. 1–7, Elsevier *Science* Publishing, New York
71. Korach, K.S., Chae, K., Levy, L.A., Duax, W.L., and Sarver, P.J. (1989) Diethylstilbestrol metabolites and analogs. Stereochemical probes for the estrogen receptor binding site. *J. Biol. Chem.* **264**, 5642–5647
72. Newbold, R.R. and McLachlan, J.A. (1982) Vaginal adenosis and adenocarcinoma in mice exposed prenatally or neonatally to diethylstilbestrol. *Cancer Res.* **42**, 2003–2011
73. Huang, W.W., Yin, Y., Bi, Q., Chiang, T.C., Garner, N., Vuoristo, J., McLachlan, J.A., and Ma, L. (2004) Developmental diethylstilbestrol (DES) exposure alters genetic pathways of uterine cytodifferentiation. *Mol. Endocrinol.* In press
74. Gendron, R.L., Paradis, H., Hsieh-Li, H.M., Lee, D.W., Potter, S.S., and Markoff, E. (1997) Abnormal uterine stromal and glandular function associated with maternal reproductive defects in Hoxa-11 null mice. *Biol. Reprod.* **56**, 1097–1105
75. Hsieh-Li, H.M., Witte, D.P., Weinstein, M., Branford, W., Li, H., Small, K., and Potter, S.S. (1995) Hoxa 11 structure, extensive antisense transcription, and function in male and female fertility. *Development* **121**, 1373–1385
76. Ince, T.A., Cviko, A.P., Quade, B.J., Yang, A., McKeon, F.D., Mutter, G.L., and Crum, C.P. (2002) p63 Coordinates anogenital modeling and epithelial cell differentiation in the developing female urogenital tract. *Amer. J. Pathol.* **161**, 1111–1117