Int. J. Dev. Biol. 53: 411-424 (2009)
doi: 10.1387/ijdb.082680jm

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

The developing female genital tract: from genetics to epigenetics

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ABSTRACT The mammalian female reproductive tract develops from the Müllerian ducts which differentiate, in a cranial to caudal direction, into oviducts, uterine horns, cervix and the anterior vagina. The developmental processes taking place during this organogenesis are notably under the control of steroid hormones, such as members of the *Wnt* and *Hox* families, which regulate key developmental genes. At later stages, steroid hormones also participate in the development of the female genital tract. Chemical compounds homologous to steroids can thus act as agonists or antagonists in fetuses exposed to them. These so-called endocrine disruptors are nowadays found in increasing amounts in the environment and may therefore have a particular impact on such developing organs. Epidemiological studies have revealed that endocrine disruptors have had drastic effects on female health and fertility during the last decades. Furthermore, these adverse effects might be transmitted to subsequent generations through epigenetic modifications. Given the potential hazard of inherited epigenetic marks altering reproduction and/or human health, such molecular mechanisms must be urgently investigated. This review aims to summarize the cellular and molecular mechanisms involved in female genital tract development, to highlight key genes involved in this process and to present epigenetic mechanisms triggered by endocrine disruptors and their consequences in regard to female reproductive tract development.

KEY WORDS: *genital tract, Hox, Wnt, genetics, epigenetics*

Development of the urogenital system

Relation between urinary and genital tracts

Mammalian genital tract development is closely related to the urinary system embryogenesis. This relation is ancient in evolution and phylogenetically well conserved. In simple organisms like annelids, they both consist in metamerized nephritic tubules composed of a ciliated funnel oriented towards the coelomic cavity and connected to a vascularised duct that opens to the exterior. This system is involved in metabolites and mature genital cells excretion. In higher vertebrates, the urogenital apparatus comprises the kidneys, gonads, urinary and reproductive ducts. The urinary tract embryonic formation is the result of a three steps process that gives rise to three successive structures emerging rostrocaudally in the intermediate mesoderm. Nephrotomes first develop in the cervical part of the embryo; they consist in a ciliated funnel emerging in the coelomic cavity and in a nephritic tubule connected to a common excretory duct (the Wolffian duct, WD). At this stage, they form a primitive kidney called pronephros which is not functional. This transitory organ can only be observed in vertebrate embryos and agnathe larvae (Saxen and Sariola 1987; Bouchard *et al.* 2002). Then, while the pronephros is regressing, the mesonephros develops posteriorly, allowing the WD to grow in the same direction. This intermediary kidney, comprising a Bowman capsule and an internal glomerule, is functional and constitutes the major excretory organ of certain species of fishes and anamniotes (Saxen and Sariola 1987; Sainio *et al.* 1997; Sainio and Raatikainen-Ahokas 1999). In higher vertebrates, an ultimate kidney structure develops from the fusion of the most posterior nephrotomes. This so called metanephric kidney or

Abbreviations used in this paper: BPA, bisphenol A; DES, diethylstilbestrol; En, n days post-coitum; FRT, female reproductive tract; MD, Müllerian duct; Pn, n days post partum; WD, Wolffian duct.

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Published online: 24 April 2009.

ISSN: Online 1696-3547, Print 0214-6282

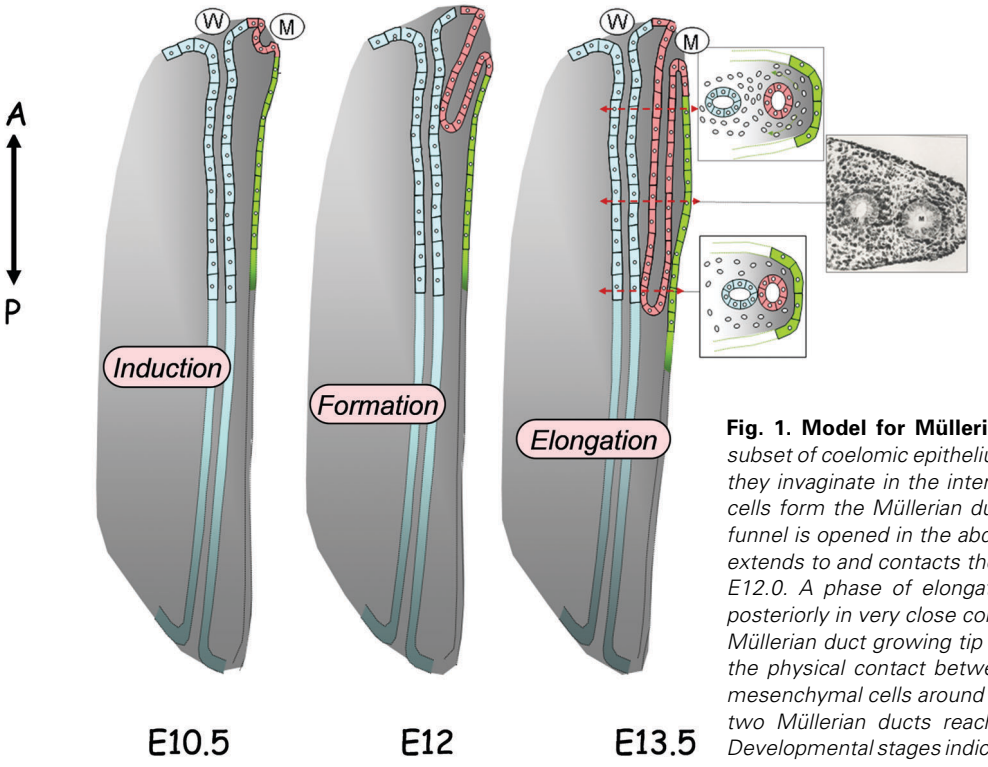

Fig. 1. Model for Müllerian duct development. *At E11.75, after a subset of coelomic epithelium cells (represented in green) are specified, they invaginate in the intermediate mesoderm. Then, the invaginating cells form the Müllerian duct (M, represented in pink). Anteriorly, the funnel is opened in the abdominal cavity, and caudally, the growing tip extends to and contacts the Wolffian ducts (W, represented in blue) at E12.0. A phase of elongation allows the Müllerian duct to elongate posteriorly in very close contact with the Wolffian duct. As soon as the Müllerian duct growing tip has deposited cells and elongated caudally, the physical contact between the ducts is lost by the appearance of mesenchymal cells around the Müllerian duct epithelium. At E13.5, the two Müllerian ducts reach the urogenital sinus and fuse together. Developmental stages indicated in this figure correspond to mice stages.*

metanephros is first connected to the WD, and then to the bladder, through the ureter. The individualization of the metanephros from the mesonephros leads to a separation between urinary and genital tracts; the reproductive tract will later develop from the mesonephros. While in males, the caudal end of the urethra forms a common excretory duct with the spermiduct, a total separation of the female urinary and reproductive tracts occurs, allowing the internal fertilization and subsequently the development of the embryo in the uterus (Oppelt *et al.* 2005a).

As the urinary system develops, another tubular structure called Müllerian duct (MD) appears (Aamar and Frank 2004). The MDs grow rostro-caudally, adjacent to the WD, until they join at the urogenital sinus (Kobayashi and Behringer 2003). At embryonic stage 13.5 days *post-coitum* (E13.5), the WDs and MDs which are respectively the anlagen of the male and female genital tracts, are both present in males and females (Jacob *et al.* 1999; Carroll *et al.* 2005). After sexual differentiation, the MDs regress in males and the WDs differentiate into male genital tract. In females, the mesonephric ducts regress and the MDs give rise to the oviducts, uterus, cervix and vagina.

Müllerian duct formation

The embryonic origin of the MDs has been a controversial issue for several years. Although they develop after pronephros resorption, it has nevertheless been suggested that the MDs were pronephretic derivatives. Indeed, some authors initially supported the idea that the anterior part of the oviducts was derived from fused nephrotomes remaining in the coelomic epithelium as small secondary ducts (Wrobel *et al.* 2002). However, it is now well established that MD formation is initiated in the cervical part of the intermediate mesoderm of the mammalian embryo (E11.75 in mice) by the invagination of the coelomic epithelium (Kobayashi *et al.* 2004; Orvis and Behringer 2007), as illustrated in Fig. 1. The mechanism by which invaginating cells acquire an MD fate remains unclear, although *Wnt4* expression in the coelomic epithelium or the mesonephros seems to be necessary for MD initiation (Vainio *et al.* 1999). After initiation, the MDs elongate posteriorly, in close contact with the WDs, until they join at the urogenital sinus (Basile and De Michele 2001b). More precisely, a recent study from Orvis and Behringer, clearly showed that the Müllerian epithelium, at its most posterior end, is in physical contact with the Wolffian duct epithelium and is separated from the coelomic epithelium only by a basement membrane (Orvis and Behringer 2007). Based on genetic markers, the same study revealed that, in spite of its epithelial morphology, MD epithelium expresses mesenchymal markers and could thus be considered as a meso-epithelium tissue (like the coelomic epithelium which also expresses epithelial and mesenchymal markers) (Orvis and Behringer 2007). Furthermore, it has been demonstrated that WD experimental interruption leads to MD incomplete formation, highlighting the link between Wolffian and Müllerian ducts (Gruenwald 1941). More recent data have allowed to precise this information. Indeed, it seems that the first phase of MD development is WD-independent, whereas the close contact with the Wolffian duct is essential for Müllerian duct elongation (Vainio *et al.* 1999; Kobayashi *et al.* 2005; Orvis and Behringer 2007). The origin of Müllerian cells along the duct was still not clear until recently. Indeed, two hypotheses were proposed. The first one was in favour of Wolffian duct cells contribution to Müllerian duct formation (Balfour 1879), whereas the second supported that the WD just acted as a guide (Dohr *et al.* 1987b). Very recent data ruled out the hypothesis of the involvement of Wolffian duct cells as source of Müllerian duct epithelium (Dohr *et al.* 1987a; Guioli *et al.* 2007; Orvis and Behringer 2007). By lineage-tracing of

coelomic epithelium, in chick and mice explants, the authors showed that a discrete population of coelomic epithelium cells, probably located in the transition area between pronephros and mesonephros, segregates and gives rise to the entire anlagen of the Müllerian duct epithelium (Guioli *et al.* 2007). In addition, the origin of Müllerian ducts was corroborated by another approach using recombinant transplant cultures (Orvis and Behringer 2007). While no cell contribution from the Wolffian ducts is required for Müllerian duct formation, these cells play nevertheless a role in this process by sending paracrine signals (Carroll *et al.* 2005).

In addition to the demonstration of the origin of Müllerian duct cells, both groups of authors have described the cellular processes leading to MD elongation and mesenchyme formation (Guioli *et al.* 2007; Orvis and Behringer 2007): they showed that, in both chick and mouse, the full length Müllerian duct epithelium possesses BrdU and histone H3 positive cells, suggesting that the MDs can extend dependently of widespread cell proliferation along the developing duct. In addition, by removing the rostral part of the Müllerian duct on mesonephros explants, it has been demonstrated that the posterior tip cells of the duct are sufficient for laying the foundation of the forming Müllerian duct (Orvis and Behringer 2007). Concomitantly to Müllerian epithelium elongation, a spatial organization of mesenchyme surrounding cells takes place and physically separates the Wolffian and Müllerian ducts. The mechanism by which these surrounding mesenchymal cells get a Müllerian fate is still not clear. In fact, the Müllerian duct mesenchyme seems to derive from *in situ* mesonephros mesenchyme as well as local delamination of the coelomic epithelium cells situated along the length of the mesonephros (Guioli *et al.* 2007).

Differentiation of the Müllerian duct

The Wolffian and Müllerian ducts are discrete primordia which temporarily coexist in undifferentiated embryo until genetic sex triggers differentiation of either ovaries or testes. In males, MDs regress due to the sexual dimorphic expression of the anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting substance (MIS), a member of the transforming growth factor-β (TGF-β) family, secreted by the Sertoli cells of the foetal testis (Josso *et al.* 1993). In addition, testosterone production by Leydig cells, allows androgen-dependant growth and differentiation of Wolffian ducts into epididymides, vas deferens and seminal vesicles (Hannema and Hughes 2007). In females, in absence of testicular hormones, Müllerian ducts differentiate into Fallopian tubes, uterus, cervix and upper part of the vagina while the Wolffian ducts degenerate (Jost 1953).

At the end of the elongation step, the growing tips of the MDs converge and join at the urogenital sinus (Orvis and Behringer 2007). The Müllerian tubules then fuse and form a one-luminar tube, the utero-vaginal duct, which will give rise to the upper vagina, cervix and uterus. The anterior non-fused region of the MD differentiates into oviducts and infundibulum. The morphology of mammalian uterus markedly varies between species, depending on the width of the ducts fusion. For instance, uterus is duplex in most rodents, generally bicornuate in big quadrupeds and simplex in most primates, including humans (Kobayashi and Behringer 2003). Although still controversial, it is generally admitted that the lower vagina is a derivative of the urogenital sinus. However its differentiation requires an induction mechanism by the MD (Drews *et al.* 2002; Kobayashi *et al.* 2005). Data obtained from studies on human malformative syndromes clearly argue in that direction. Indeed, the Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a genetic disease that affects notably the development of inner genital tract (Basile and De Michele 2001a) (Morcel *et al.* 2007). These women lack Müllerian derivatives (uterus, upper vagina and optionally oviducts) but possess the lower part of the vagina, showing that upper and lower parts have different embryologic origins (Oppelt *et al.* 2005b). At birth, the female genital tract has undergone a regional morphologic specialisation that differentiates oviducts, uterus, cervix and upper vagina but it is still composed of simple structures that will acquire a full maturation aspect after birth and until puberty.

Maturation of Müllerian ducts

The MD maturation proceeds in three steps: 1) region specific Müllerian duct epithelium differentiation, 2) formation and organization of endometrium and myometrium, 3) uterine adenogenesis.

Firstly, the single-layered epithelium acquires different morphologies and cyto-architectures along its anterior-posterior axis. While the uterine epithelium is composed of simple columnar epithelial cells, the posterior cervix and the vaginal epithelia are made up with of stratified squamous cells and the oviducts epithelium possesses of ciliary and secretory cells. This process seems to occur perinatally since, at E16-18, the oviductal, uterine, cervical and Müllerian vaginal epithelial cells are morphologically undistinguishable (Komatsu and Fujita 1978; Kurita *et al.* 2001).

Distinct markers then appeared in mice at around 1-3 days *post-partum* (P1-3), and as regional patterning of epithelial differentiation takes place in the uterus, cervix and Müllerian vagina (Kurita *et al.* 2001). Oviductal epithelium ciliary cells differentiate at P5-10 and active secretory cells are observed from P23 (Komatsu and Fujita 1978). At the utero-vaginal level, the Müllerian duct epithelium undergoes these region-specific morphogenetic changes under mesenchymal cells paracrine influence (Cunha 1975;1976b;a). Indeed, heterotypic recombinant assays have demonstrated that uterine and Müllerian vaginal epithelia are both able to undergo either uterine or vaginal differentiation when induced by uterine or Müllerian vaginal mesenchyme, respectively. This characteristic is however stage-dependent: epithelial cells are the most responsive to mesenchyme signals at around P2-P5 but lose this competence by P9 (Cunha 1976b). At P10, only a subset of epithelial cells has kept some plasticity (Kurita *et al.* 2001). Uterine mesenchymal inductive activity has been shown in P2 up to P7 neonates whereas vaginal stroma keeps its inductive activity from 2 up to 150 days after birth (Cunha 1976b). Subsequently, in the developing uterus, like in many other organs (intestine, bladder, etc.), mesenchymal cells in close proximity to the epithelium differentiate into fibroblasts, to form the endometrium, whereas the most distant ones differentiate into smooth muscle, so forming the myometrium (Brody and Cunha 1989). It has been further demonstrated that myometrium differentiation and spatial organisation require the epithelium which can therefore be considered as a key mesenchymal inducer for endometrial/myometrial segregation and subsequent formationn (Cunha *et al.* 1989; Cunha *et al.* 1992).

The female genital tract development ends with adenogenesis, *i.e.* the formation of uterine glands, which is the paroxysm of its functional differentiation. These glands are essential to later

survival and development of the conceptus by secreting histotrophic substances. Uterine adenogenesis involves differentiation and budding of glandular epithelium from luminal epithelium, penetration of uterine stroma by tubes of glandular epithelium and extensive branching and coiling of glandular epithelium (Gray *et al.* 2001). The timing of uterine adenogenesis is highly variable among mammalian species. In mice, it begins around P5 by epithelial invagination of glandular epithelium into the luminal epithelium (Brody and Cunha 1989); and completion is observed by P7 (Branham *et al.* 1985). This process seems to be governed by site-specific alterations in cell proliferation and movement, as well as by paracrine, cell-cell and cell-extracellular matrix interactions, and specific endocrine-, paracrine- and juxtacrin-acting factors and receptors (Gray *et al.* 2001).

Molecular genetics of Müllerian duct development

Most of the genes known to be essential for MD embryonic development have been characterized either by description of Human syndromes affecting the female reproductive tract (FRT) formation, regression and differentiation or by studies of knocked-out mice (Table 1 and Fig. 2). Analysis of the molecular and cellular mechanisms of MD development depicts an emergent genetic cascade for this process (Kobayashi and Behringer 2003).

Early onset of Müllerian ducts

As aforementioned, the initial steps of MD formation in both sexes briefly consist in invagination of coelomic epithelium and caudal elongation towards the urogenital sinus. These two mechanisms depend on correct expression of various transcription factors and signalling molecules. As previously described, the elongation phase, as well as further maintenance of MD, seems not only to depend on the intrinsic nature of the MD, but also to require the presence of WD (Roberts *et al.* 2002). As a consequence, genes involved in WD development are of critical importance for the subsequent onset of MD. Amongst factors required for FRT development, *Lim1* (a.k.a. *Lhx1*), *Pax2*, *Emx2*, *Wnt4*, *Wnt9b*, *Tcf2*, *Dach1* and *Dach2* seem to be essential for the initial biphasic process of MD formation and are therefore detailed below.

Lim1, a homeodomain-containing transcription factor, shows a dynamic expression pattern in the epithelium of the developing MD, beginning at E11.5 in the mouse. This suggests a role for Lim1 in the very early steps of MD formation. Indeed, *Lim1*-null mutant mice display complete absence of mesonephric- and paramesonephric-derived structures, a phenotype consistent with the expression of the gene in the epithelium of both sexual ducts (Kobayashi *et al.* 2004). Furthermore, specific inactivation of *Lim1* in the WD epithelium, causing its degeneration, leads to impaired development of MD but does not affect their initial formation, highlighting the WD-dependent processes of MD elongation and maintenance but not initiation (Kobayashi *et al.* 2005). *Pax2*, a member of the *paired-box* gene family encoding transcription factors, is also expressed in the epithelium of the Wolffian and Müllerian ducts. This gene, involved in multiple developmental processes, appears to be essential for the development of the epithelial components derived from intermediate mesoderm. Indeed, *Pax2*-null mutant mice, which die perinatally due to absent kidneys, have neither Wolffian nor Müllerian derivatives (Torres *et al.* 1995). Nevertheless, unlike the phenotype observed in *Lim1*-null mutant mice, both sexual ducts initially form in *Pax2*-null

TABLE 1

GENES INVOLVED IN THE DEVELOPMENT OF FEMALE GENITAL TRACT

	Spatio-temporal expression of genes during FRT development				
Gene	**Early[1]**	**Late[2]**	**Adult (sexual cycle)**	**Phenotype in KO female mice**	**References**
Lim 1	CE, WE, ME	ME	NA	No FRT	Kobayashi, 2004
Pax2	CE, WE, ME	ME	Oviducts and proliferative epithelium	No FRT	Torres, 1995 Tong, 2007
Emx2	WE, ME	MMET	Endometrial development	No FRT	Myamoto, 1997
Wnt4	CE, MM	Restricted to uterine mesenchyme	Dynamic expression	No FRT	Vainio, 1999 Miller, 1998b
Wnt5a	MM, ME	MM	Dynamic expression	Defective posterior growth of MD. No uterine glands	Miller, 1998b Mericskay, 2004
Wnt7a	ME	ME	Dynamic expression	Posteriorization of genital tract. No uterine glands	Miller & Sassoon, 1998 Parr, 1998; Carta & Sassoon, 2004
Wnt9b	WE (*), NE in MD	WE (until E14.5), NE in MD	NE	Absent uterus and upper vagina	Carroll, 2005
RAR α,β,γ	NA			Combined mutants: various degrees of affection	Mendelsohn, 1994
Dlgh1	WE (*)	NE	NA	Cervix and vagina aplasia	Iizuka-Kogo, 2007
β-catenin	All compartiments			Hypotrophic uterine horns + defective oviduct coiling. Myogenesis to adipogenesis switch (**)	Arango, 2005 Deutscher, 2007
Hoxa10	Colinear expression along the A/P axis		Steroid-dependent expression	Homeotic transformation of the anterior part of the uterus to an oviductal morphology	Benson, 1996
Hoxa11				Partial anteriorization: hypoplastic uterus,decreased endometrial glands.	Gendron, 1997
Hoxa13 (***)				Agenesis of the caudal portion of MD.	Warot, 1997

MD: Müllerian duct; ME: Müllerian epithelium; WD: Wolffian duct; WE: Wolffian epithelium; CE: coelomic epithelium; MMET: Müllerian Mesenchyme-Epithelium Transition; FRT: Female Reproductive Tract; NA: not available; NE: not expressed.

[1] Early refers to determination, invagination and elongation steps of MD. [2] Late corresponds to E13.5 (time of MD regression in the male) to puberty. * Specific WD genes are indicated since MD growth initially depends on the presence of WD. ** Targeted knock-out, efficient from E15.5. *** Compound *Hoxa13*$^{+/-}$;*Hoxd13*$^{-/-}$-mutant females display severe urogenital and rectal anomalies (Warot, 1997) whereas *Hoxd13*$^{-/-}$-mutant female are still fertile (Dollé *et al.* 1993).

embryos but degenerate soon after. In addition, the homeobox-containing gene *Emx2*, is expressed in the epithelial components of the urogenital system and plays a major role in the development of this organ. Similarly to *Pax2* mutants, both sexes of homozygous null mice for *Emx2* display complete absence of urogenital tract and die soon after birth (Miyamoto *et al.* 1997). Nevertheless, WD develops normally at E10.5 in these mice but degenerates the day after and, as expected, no MD is observed later. Finally, the POU domain-containing *TCF2* gene (formerly *v-HNF1* or *HNF-1beta*) has been shown to be expressed in many organs during development (Coffinier *et al.* 1999) and seems to play a general role in epithelial differentiation (Coffinier *et al.* 1999; Kolatsi-Joannou *et al.* 2001). Expression of this gene is particularly noticeable from the earliest steps of urogenital tract formation up to adult stage in the mouse model (Coffinier *et al.* 1999; Reber and Cereghini 2001). It was originally found associated with MODY-type diabetes (Horikawa *et al.* 1997) and with diabetes mellitus, renal cysts and other renal developmental disorders (Bingham *et al.* 2001; Kolatsi-Joannou *et al.* 2001). Interestingly, genital malformations such as bicornuate uterus (Bingham *et al.* 2002), uterus didelphys (Bingham *et al.* 2002) and Müllerian aplasia (Lindner *et al.* 1999) were also found together with renal anomalies in patients showing a defective gene, tending thus to show a major role of the *TCF2* gene during uro-genital development. Transcriptional co-factors such as Dach1 and -2 seem to also take place in the molecular cascade of MD formation. Whereas inactivation of each corresponding gene does not appear to affect this pathway, combined knock-out results in drastic defect of Müllerian derivatives, tending to show a functional redundancy of these factors (Davis *et al.* 2008).

In addition to the key role of the above homeodomain transcription factors in the early steps of MD formation, some signalling molecules have been shown to be involved in this process. Amongst them, *Wnt9b* and *Wnt4*, two members of the *Wnt* gene family encoding secreted glycoproteins, homologues to the *Drosophila* segment polarity gene *wingless,* are crucial paracrine factors. In mice, *Wnt9b* is expressed in the WD epithelium from E9.5 to E14.5 in both sexes and seems to be necessary for MD extension (Carroll *et al.* 2005). Interestingly, the phenotype of *Wnt9b* mutants can be rescued by activation of *Wnt1* within the WD, identifying the canonical Wnt pathway as a determinant signalling process in Müllerian ducts elongation. Another member of the family, *Wnt4*, is expressed in mesenchymal cells surrounding the newly formed MD on E12.5 in the mouse. *Wnt4*-null female mice exhibit a complete absence of FRT and, strikingly, are masculinised, probably due to ectopic activation of testosterone biosynthesis as initially hypothesized (Vainio *et al.* 1999; Heikkila *et al.* 2005). In fact, no Müllerian structures are observed both in male and female mutant mice on E11.5, before normal regression of MD takes place in the male. Thus, this reversal of sexual development in mutant female indicates a requirement for *Wnt4* in the initial steps of MD formation in both sexes but also for the suppression of male differentiation pathway in female gonad. Incidentally, a mutation in the *Wnt4* gene was discovered in a 46, XX woman presenting a somewhat similar phenotype: complete absence of MD-derived structures and clinical signs of androgens excess (Biason-Lauber *et al.* 2004). Interestingly, Wnt4 and Fgf9 seem to constitute mutual antagonistic signals in the bipotential gonad, regulating gonadal differentiation into female or male pathway by imbalance between them (Kim *et al.* 2006).

Another well-studied signalling molecule in the context of embryonic development is retinoic acid (RA), a morphogen derived from vitamin A. RA seems to be particularly involved in antero-posterior patterning both along the body axis and in developing limb bud (Dreyer and Ellinger-Ziegelbauer 1996; Robert and Lallemand 2006). Compound null mutations of its cognate receptors (RARs and RXRs) lead to a broad range of developmental abnormalities, among which severe defects of the urogenital system (Mendelsohn *et al.* 1994). More precisely, RARαβ2 double mutants lack identifiable MD in E12.5, a phenotype not imputable to a defect in WD formation, making RA signalling a specific pathway required to ensure the correct formation of MD. Similarly, abnormalities of the urinary and genital tracts have been recently described in *Dlgh1*-null mutant mice in both sexes (Iizuka-Kogo *et al.* 2007). The most interesting defect here is the aplasia of the uterine cervix and vagina due to defective lateral fusion and impaired caudal elongation of MD (Iizuka-Kogo *et al.* 2007). At the present time, no relation between retinoic acid and *Dlgh1* gene has been established. Finally, a

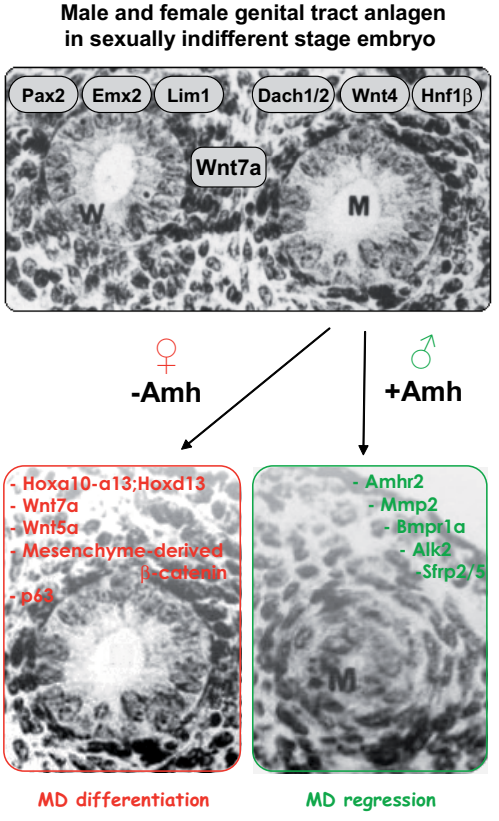

Fig. 2. Genes involved in the regression or development of the Müllerian ducts. *At the so-called sexually indifferent stage (bipotential gonad), both male and female reproductive duct primordials co-exist. Sexual differentiation takes place according to the genetic sex of the embryo, determining its gonadal status and consequently allowing the differentiation into male or female reproductive organs. In female embryos, in the absence of specific male hormones, the WDs degenerate while the MDs ducts develop. In male embryos, the MDs degenerate and the WDs give rise to the male genital tract. Key genes involved in such processes are indicated on the figure.*

requirement of PI3K/AKT signalling pathway for MD tip elongation has been uncovered (Fujino *et al.*, 2008), highlighting the intricacy of multiple signalling pathways for MD development.

Regression of Müllerian ducts in the male

As mentioned earlier, both male and female reproductive duct primordials coexist at the so-called sexually indifferent stage. Subsequently, sexual differentiation takes place according to the genetic sex of the embryo, determining its gonadal status and consequently allowing the differentiation into male or female reproductive organs. In female embryos, the absence of androgens seems to be sufficient to cause the degeneration of the WD, whereas regression of MD in male embryos is an active process mediated by AMH solely secreted by the foetal testis.

Molecular and cellular studies, mainly in the mouse, have shown that AMH exerts its effect on MD through a paracrine mechanism, by binding to its type II receptor (AMHRII). This latter is initially expressed in the coelomic epithelium of the mesonephros, prior to regression (Zhan *et al.* 2006). Binding of AMH to AMHRII induces the AMHRII-expressing cells to migrate into the area adjacent to the MD and eventually around the MD at ~E15.5, corresponding thus to an epithelial-to-mesenchymal transition. In the female, in absence of AMH, AMHRII expression remains located in the coelomic epithelium at least until stage E15.5 (Zhan *et al.* 2006). *Amh*- or *AmhrII*-mutant male mice show an identical phenotype: they exhibit a morphologically normal male reproductive system but are infertile because of the persistence of a complete FRT blocking sperm passage (Behringer *et al.* 1994; Mishina *et al.* 1996). Mutations in *AMH* or *AMHRII* genes have been reported in patients affected by Persistent Müllerian Duct Syndrome type I and type II respectively (Di Clemente and Belville 2006). These evidences tend to show that AMHRII is probably the unique receptor of type II mediating AMH signalling. In the mouse, Bmpr1a (a.k.a. Alk3) has been identified as a type I receptor, necessarily associated to AmhrII to mediate Amh-induced regression of Müllerian derivatives (Jamin *et al.* 2002). It is noteworthy that other Tgf-β family type I receptors can transduce Amh signal in absence of Bmpr1a (Clarke *et al.* 2001; Jamin *et al.* 2003). Interestingly, Alk2 (a.k.a. Acvr1) seems to mediate Amh signalling by inducing migration of AmhrII-expressing epithelial cells from the coelomic epithelium into the MD mesenchyme, accounting for the sexually dimorphic pattern of AmhrII expression during the regression (Zhan *et al.* 2006). According to their spatiotemporal expression pattern, Alk2 and Bmpr1a type I receptors are though to act sequentially in MD regression. Moreover, an epithelial-derived *Wnt7a* signal is required to induce or to maintain *AmhrII* expression in the Müllerian mesenchyme of both sexes, and thus responsiveness to Amh signalling. Indeed, *Wnt7a*-deficient mice do not express *AmhrII* in the ductal mesenchyme and are infertile due to persistent MD in the male and abnormal morphogenesis of the oviduct and uterus in the female (Parr *et al.* 1998; Parr and Mcmahon 1998). The direct effect of *Wnt7a* inactivation was corroborated by the description of β-catenin/Tcf4 complex binding onto AmhrII promoter to activate the gene (Hossain and Saunders 2003). These data highlight the multiple roles of *Wnt* signalling in the molecular cascades triggering sexual development. One of the earliest events following Amh signalling, but prior to MD regression seems to be *Sfrp2* and *-5* gene upregulation. However single or combined inactivation of either gene does not lead to any overt anomaly of the reproductive tract development, suggesting that other members of the *Sfrp* gene family may redundantly be involved in this process (Cox *et al.* 2006).

Regression of MD is a cranial-to-caudal process achieved by both apoptosis of epithelial cells and epithelio-mesenchymal transition. A positive correlation has been found between the expression pattern of AmhrII and β-catenin cytoplasmic accumulation in the peri-Müllerian mesenchymal cells and seems to follow the wave of apoptosis spreading chronologically along the cranio-caudal axis (Allard *et al.* 2000). Furthermore, β-catenin co-localizes with Lef1 (another member of the nuclear TCF/LEF family) in the nucleus of mesenchymal cells treated with Amh, and therefore might alter gene expression and cell fate, making β-catenin/Lef1 complex a possible mediator of Amh action. Indeed, Allard and colleagues have observed that apoptosis of epithelial cells occurs only after disruption of the MD basement membrane and is followed by an entry of healthy epithelial cells into the mesenchymal compartment where they are subject to epithelio-mesenchymal transformation (Allard *et al.* 2000). One of the putative targets of Amh signalling is the Matrix metalloproteinase 2 gene (*Mmp2*). This gene is expressed in a sexually dimorphic pattern with an upregulation in the male Müllerian mesenchyme during regression; this male-specific expression pattern is lost in *Amh*-null mutant mice (Roberts *et al.* 2002). Additionally, inhibition of Mmp2 protein activity blocks MD regression *in vitro* and is correlated with a decrease of apoptotic cells in the MD epithelium. Although *Mmp2*-null mutant mice develop a normal urogenital tract, probably due to redundancy with other gene (s) (Oppelt *et al.* 2005b), Mmp2 remains a putative actor of Amh-induced regression (Itoh *et al.* 1997). More recently, Wilms' tumour suppressor gene (*Wt1)* has been described as another regulator of *AmhrII* expression (Klattig *et al.* 2007), providing a novel function for *Wt1* in the process of sexual development.

Differentiation of Müllerian ducts

In the female foetus, the differentiation of MD along the antero-posterior (A-P) axis depends on local inductive interactions between the MD epithelium and the surrounding mesenchyme. The correct regionalization of FRT, *i.e.* acquisition of tissue identity in cervix, uterus and vagina, relies on spatiotemporally regulated interactions between transcription factors and signalling molecules. Most of the genes acting to determine Müllerian cell fate and thus the proper MD identity are still expressed throughout differentiation. Amongst these genes, homeogenes such as *Hox* genes play a crucial role in specifying cell characteristics along the A-P axis.

The homeobox (*Hox*) genes belong to a large family of 39 genes organized in four clusters, *Hoxa to Hoxd* (*HOXA-D* in Human), each on a different chromosome. These major developmental genes are involved in patterning the animal body axis by providing positional identity through the molecular "*Hox* code", emerging from highly complex spatiotemporal combinations of Hox proteins (Hombria and Lovegrove 2003). Some members of the *Abdominal-B* (*Abd-B*) like *Hox* gene family, *Abd-B Hoxa9* to *13* and *Hoxd9* to *13*, are expressed in partially nested patterns in the mesenchyme of the developing MD, defining a specific *Hox* code along the A-P axis (Dollé *et al.* 1991; Taylor *et al.* 1997). Evidence of their roles was provided, particularly by *in vivo* inactivation of *Hoxa10*, *Hoxa11*, *Hoxa13* and *Hoxd13* genes in the mouse, that

led to region-specific alterations of the reproductive tract. *Hoxa10*-null mutant mice exhibit abnormal utero-tubular junction and uterine epithelium and an anterior homeotic transformation of the upper part of the uterus into oviduct-like structure (Benson *et al.* 1996). In *Hoxa11*-deficient mice, the uterus is thinner and shorter than normal and endometrial glands have not developed, suggesting an anteriorized phenotype (Gendron *et al.* 1997). Moreover, replacement of the homeodomain of *Hoxa11* by that of *Hoxa13* results in a posterior homeotic transformation of the uterus into cervix- and vagina-like structures (Zhao and Potter 2001). Besides, *Hoxa13*-null mice show agenesis of the distal portion of the MD, indicating a role for *Hoxa13* not only in the differentiation but also in the formation of MD (Warot *et al.* 1997). Subsequently, mutations in the *HOXA13* coding region have been shown to cause Hand-foot-genital syndrome (HFGS) in Human, characterized by hands and feet defects, hypospadias in males, Müllerian duct fusion defect in females and urinary tract malformations in both sexes (Mortlock and Innis 1997; Goodman *et al.* 2000).

Despite its involvement during MD regression in the male, *Wnt7a* is also required for correct patterning of the murine FRT. Initially expressed throughout the MD epithelium, *Wnt7a* expression becomes postnatally restricted to the oviductal and uterine epithelium, declining in the vaginal epithelium (Miller *et al.* 1998b). Loss of *Wnt7a* expression in the differentiating MD results in partial homeotic transformation of the oviduct to uterus and of the uterus to vagina (Miller and Sassoon 1998). Since *Wnt7a* is not required to induce *Hoxa* gene maintenance, *Wnt7a* and *Hoxa* genes are likely acting in parallel pathways during FRT development. Indeed, a combinatorial regulation by *Hox* and *Wnt* genes probably occurs in most organogenesis and therefore during FRT development (for a review, see Bondos 2006). Moreover, *Wnt7a* mutant uterus in adult presents an atrophic aspect due to postnatal uterine growth failure, a phenotype partly related to the role of *Wnt7a* as a suppressor of cell death in the FRT (Carta and Sassoon 2004). In addition, the absence of oviduct coiling in the *Wnt7a*-null mice has been reproduced by specific inactivation of β-catenin in the MD mesenchyme using an *Amhr2-cre* mouse line, without any observed alteration in *Wnt5a* and *Wnt7a* expression patterns (Deutscher and Hung-Chang Yao 2007). This defect on oviduct differentiation is accompanied by an overall hypotrophic aspect of uterine horns, a phenotype correlated with a decreasing cell proliferation in both the epithelium and the mesenchyme of the Müllerian derivatives. In the end, deletion of β-catenin directed by the *Amhr2-cre* transgene leads to adipose tissue deposition in the uterine wall in adult, probably due to cell fate switch from myogenesis to adipogenesis (Arango *et al.* 2005; Deutscher and Hung-Chang Yao 2007), without any ovarian phenotype. Nevertheless, recent *in vitro* studies have uncovered the requirement of β-catenin for normal ovarian steroidogenesis (Hernandez-Gifford *et al.* 2009).

Given the specific expression of *Wnt7a* in the luminal epithelium of the uterus (Miller *et al.* 1998b), the phenotype of *Wnt7a*-null mutant mice implies crucial paracrine interactions between uterine epithelium and stromal cells. *Wnt5a*, a component of the non-canonical Wnt signalling pathway, that is expressed in mesenchyme of the uterus, cervix and vagina (Miller *et al.* 1998b), is an actor of such interactions. Indeed, *Wnt5a* is required for proper development of the posterior region of FRT and for uterine adenogenesis (Mericskay *et al.* 2004). Therefore, involvement of *Wnt7a* and *Wnt5a* for glandular genesis in the uterus highlights the necessary cooperation between epithelium- and stroma-derived signals during the essential process of cyto-differentiation. In this context of reciprocal interactions, *p63*, a homologue of *p53* tumour suppressor gene, is considered as an identity switch for MD epithelial differentiation into uterine or cervicovaginal cell fate (Ince *et al.* 2002; Kurita *et al.* 2004). Besides, the homeodomain transcription factor Msx2, promotes normal vaginal epithelial differentiation and, interestingly, seems to be involved in regression of the caudal region of WD in females by promoting apoptosis (Yin *et al.* 2006).

In conclusion, differentiation of MD into a functional reproductive tract relies on a complex genetic cascade wherein cooperation between *Wnt* and *Hox* genes is fundamental for correct patterning and maturation of the FRT. It is noteworthy that many of the developmental genes presented above, especially *Hox* genes, are also regulated by steroid hormones during both embryogenesis and adulthood (Daftary and Taylor 2006). The role of this endocrine regulation in the reproductive tract is now well established during embryonic development but appears to be also essential in adult developmental-like processes such as menstrual cycle, embryo implantation and pregnancy.

Studying the consequences of prenatal or perinatal exposure to endocrine disruptors such as diethylstilbestrol (DES) gives new insights on hormonal regulation of these genes during critical periods of FRT development and may be useful to address the question of epigenetic regulations of *Hox* genes, an extensively studied research field in recent years.

What about epigenetics?

Epigenetics and environment

The term epigenetics refers to the overall molecular phenomenon which is heritable from parents and that regulates gene expression without any alteration of the genomic DNA sequence. These heritable epigenetic changes include DNA methylation, post-translational modifications of histones tails (acetylation, methylation, phosphorylation,..) and chromatin remodelling. The epigenome is the set of epigenetic prints present in a given genome, at a given time and in a given cell type.

Most of the studies on epigenetic regulation have shown that environment can play an important role in this respect. Indeed, both environmental chemicals and toxins have recently been shown to alter DNA methylation patterns, resulting in epigenetic phenotypes (Cisneros 2004; Anway *et al.* 2005).

Several studies have shown that, during critical periods of differentiation, exposure to low environmentally relevant doses of some chemicals, may alter developmental programming. In particular, two studies demonstrated that xenobiotic chemicals such as tributyltin present in PVC plastics as well as some fungicides, alter normal development and homeostatic control over adipogenesis and energy balance, leading to obesity (Grun and Blumberg 2006; Tabb and Blumberg 2006). Moreover, recent works on mice by Newbold and collaborators, support the idea that a brief exposure to environmental chemicals, especially those with estrogenic activity, can increase body weight in correlation with age (Newbold *et al.* 2007b; Newbold *et al.* 2007c). Precisely, treatment of females with diethylstilbestrol does not

affect the body weight during the time of exposure, but provokes, later on, an increase of the body weight associated with an augmentation of body fat. In addition, exposure of adult animals to metals such as cadmium or nickel, has been associated with tumorigenesis. In fact, these metals seem to interact with the epigenome and induce carcinogenic effects through abnormal DNA methylation (Salnikow and Costa 2000; Poirier and Vlasova 2002).

Among harmful chemicals, the so-called endocrine disruptors have been described to present strong effects on the development of organisms. Indeed, various natural or synthetic molecules can interfere with the endocrine system through the binding to members of the intracellular steroid receptors family. This interference ultimately disturbs the normal function of tissues and organs, in particular those of the reproductive tract. Exposure to endocrine-disrupting chemicals present in the environment has been reported to cause many disorders in mammals like abnormal thyroid and immune function, alteration of the developing reproductive tract such as de-masculinization and feminization of males, associated with decreasing fertility. These adverse effects were reported for multiple chemicals such as industrial waste and pesticides (reviewed in Colborn *et al.* 1993).

Recently, several lines of evidence have tended to show that epigenetic adaptations, in response to *in utero* nutritional and environmental factors, play an important role in developmental plasticity and diseases susceptibility. Waterland and collaborators have demonstrated that the epigenetic gene regulation of the imprinted gene *Igf2* may be influenced by early post-natal nutritional diet in the mouse model. Indeed, in normal environmental conditions, the *Igf2* gene is expressed from the paternal allele whereas the maternal gene is repressed by imprinted silencing. When a methyl-donor deficient diet is administered to animals for 60 days post-weaning, a paternal hypomethylation of the gene is observed, in correlation with a significant loss of imprinted regulation of the *Igf2* gene (Waterland *et al.* 2006). Therefore, nutrition may alter the epigenome of these young animals (Waterland *et al.* 2006).

Besides experimentally diet-mediated imprinting, general environment conditions may also lead to epigenetic variations in humans. As another evidence of environment modification of the epigenome, is the following study of Fraga and collaborators (Fraga *et al.* 2005). From a set of monozygotic twins of various ages and living in different environments, an epigenetic profile of each person was performed by measuring the global CpG methylation and the acetylation on H3 and H4 histones. This showed that, although twins are epigenetically indistinguishable during the early years of life, older twins exhibit remarkable differences in their overall content and genomic distribution of DNA methylation and histone acetylation, which can thus bring variations in each subject's gene expression pattern. Furthermore, these data clearly showed a more important difference of the epigenome between older twins and between twins who have lived separately more than 50% of their lifetime (Fraga *et al.* 2005).

Epigenetic modifications can therefore be induced by natural and synthetic chemicals present in the environment. These epigenetic changes seem to lead to some genetic programs variations and consequently may have drastic effects on human development and health. Amongst organs that can be affected by these compounds, the reproductive tract is a very sensitive target. The following paragraphs will address the developmental alterations of the reproductive tract, which can be triggered by natural and synthetic epigenetic effectors.

Effects of estrogens on female reproductive tract development

As mentioned earlier, the development of the female reproductive tract (FRT) is regulated by steroid hormones and in particular by oestrogens acting through two different receptors, designated ERα and ERβ. ERα is predominantly expressed in the uterus, vagina, mammary gland, and thecal cells of the ovary whereas ERβ is mainly expressed in the granulosa cells of the ovary (Couse and Korach 1999; Muramatsu and Inoue 2000). The particular role of ERα in FRT development has been revealed by disrupting the corresponding gene in the mouse, driving to hypoplastic uterine and vaginal tissues (Couse and Korach 1999; Hewitt and Korach 2002).

The molecular mechanism triggered by steroid hormones is relatively well known. Indeed, the steroid hormones linked to specific nuclear receptors which are able to bind to the promoter of target genes and then regulate proliferation and/or differentiation processes (Groothuis *et al.* 2007). Considering the importance of steroid hormones in FRT development, exposure to endocrine disruptors may thus have a strong impact on this organ system. As a matter of fact, aberrant temporal or over stimulation of oestrogens signalling pathway during development is now known to lead to various long-term or irreversible abnormalities (Newbold *et al.* 1990). During the last decades, large quantities of various chemicals with oestrogenic activity have been released into the environment. Many of them can potentially influence the endocrine system and therefore modify organs responsiveness to endocrine signals during pre- and/or post-natal life, by disrupting the proper expression of oestrogen-regulated genes.

Amongst these compounds, genistein, a nonsteroidal phytoestrogen, is present in food, particularly in soy products. Human foetuses may thus be exposed to this molecule during *in utero* development as well as in infancy through lactation. Newbold and colleagues have demonstrated that neonatal exposure of mice to genistein leads to an increase of uterine adenocarcinoma later in life (Newbold *et al.* 2001). Moreover, this phytoestrogen can exert dose-dependent adverse effects on the ovary: neonatal exposure to genistein at environmentally relevant doses alters ovarian differentiation and development, and leads to multioocyte follicles still visible later in life; at a higher dosage, females become infertile (Jefferson *et al.* 2002). In addition, at lower doses, females show minor rates of pregnancy coupled with smaller and fewer implantation sites (Jefferson *et al.* 2002).

Bisphenol A (BPA) is a synthetic compound used in the manufacture of many plastics including food containers and dental composites. This molecule presents an oestrogenic activity and as such, is able to induce malformations of the FRT. Indeed, recent experimental studies on mice have highlighted the association of BPA with deformity of the reproductive tract and with gynaecologic cancers. In particular, it has been reported that exposure to BPA leads to earlier vaginal opening (Honma *et al.* 2002), to altered ovarian morphology (Markey *et al.* 2003; Kim *et al.,* 2009), to proliferative lesions of the uterus (Newbold *et al.* 2007a) and to a strong incidence of cystic ovaries (Newbold *et al.* 2007a).

The most dramatic report on FRT malformations induced by a

synthetic nonsteroidal estrogen-mimetic molecule, is the abnormalities observed in women exposed *in utero* to DES. Although, this chemical had been demonstrated to be ineffective as soon as 1953 (Dieckmann *et al.* 1953), it was prescribed for miscarriage and other pregnancy complications between 1938 and 1971. Several studies have demonstrated that prenatal exposure of female to DES was associated with subsequent development of reproductive tract abnormalities. First, women prenatally exposed to DES presented with a "T shaped" uterus and an increased incidence of clear cell adenocarcimas of the vagina and cervix. Second, these women also presented an enhanced risk of spontaneous abortion, ectopic pregnancy and preterm delivery (Kaufman *et al.* 2000). These epidemiologic studies were corroborated by experimental studies performed on mice exposed to DES during development. Indeed, these mice showed many malformations of the uterus, characterized by squamous metaplasia of the luminar and glandular epithelium, and by endometrial hyperplasia and increased risk of leiomyomas (Kitajewski and Sassoon 2000). In addition, these mice showed persistent epithelium cornification of the vagina associated with adenocarcinoma, and oviduct proliferative lesions (Mclachlan *et al.* 1980; Couse *et al.* 2001; Couse and Korach 2004).

The overall data cited above demonstrate clearly that natural or synthetic compounds, with oestrogenic activity, can have adverse effects on the FRT and consequently disrupt reproductive functions. Moreover, the detrimental effects of steroid disruptors are generally not visible before the offspring reaches maturity or even middle age. Such molecules have been shown to have long time effects on the female mouse or woman reproduction. Indeed epidemiologic and experimental studies have pointed out that developmental alterations of the reproductive tract could be inherited to the next generation, suggesting that DES exposure could affect durably gene regulation. However, the molecular mechanisms involved in such inheritance of altered genetic traits are far to be clearly understood.

Effects of DES on developmental genes

As a first step to understand the molecular mechanisms involved in the alteration of the female reproductive tract exposed to DES, target genes for such molecules need to be clearly identified. Amongst these genes, the *Hoxa* genes are under control of both oestrogen and progesterone (Ma *et al.* 1998). Moreover, the phenotype associated with developmentally experimental exposure to DES is similar to those observed in *Hoxa* genes knocked out mice. In fact, DES-induced abnormalities include loss of the boundary between the oviduct and the uterus, associated with a loss of uterotubal junction, a phenotype also present in *Hoxa-10* mutant mice. Ma and collaborators have demonstrated that expression of *Hoxa9*, *Hoxa10* and *Hoxa11* is repressed by DES in the mouse developing reproductive ducts following foetal or neonatal exposure to DES (Ma *et al.* 1998). This repression of *Hoxa* genes provides a putative explanation to the teratogenic effects of DES on the developing FRT. Other features of DES exposure, *i.e.* vaginal adenosis, abnormal urethral openings in the vagina and failure of distal MD to form a common cervical canal, correlate with those of the *Hoxa-13* mutant mice. In order to further correlate DES exposure and *Hoxa* genes expression, Block and collaborators, have treated mice with DES from days 9 to 16 of gestation (Block *et al.* 2000). Using *in situ* hybridization experiments, they revealed a posterior shift of *Hoxa9* and *Hoxa10* expression in the reproductive tract of female offspring. A similar decrease was observed in the *Hoxa11* anterior expression domain. In this study, the authors suggested that the DES-induced homeotic transformations of the reproductive tract could correspond to the uterine morphological changes seen in up to 70% of the women exposed *in utero* to DES ("T-shaped" uterus). In fact, the decrease of *Hoxa10* and *Hoxa11* expression and the increase of *Hoxa9* expression may cause the uterus to develop in the tissue normally fated by *Hoxa9 i.e.* the oviduct. The T-shape of the uterus in DES-exposed women, may then stand for a transformation into an oviduct-like structure (Block *et al.* 2000).

In addition to these studies on *Hox* genes, other investigations have shown that DES is acting through multiple gene pathways to cause structural changes in the FRT. For instance, DES *in utero* exposure leads to a down-regulation of the *Wnt7a* gene in the foetal uterus up to birth; normal regulation of the gene is only restored 5 days after birth (Miller *et al.* 1998a). Interestingly, prenatally DES-exposed mice and *Wnt7a*$^{-/-}$ mutants show similar deformity of the FRT. One hypothesis to explain this, would be that the DES-induced transient down-regulation of *Wnt7a* during a critical window of time, is sufficient to account for the DES syndrome. During the down-regulation of the *Wnt7a* gene, the female reproductive tract would lose the competency to respond appropriately to surrounding signals. This hypothesis is consistent with grafting experiments showing that vaginal and uterine epithelia are responsive to stromal induction when grafting is performed between P3 to P5. It is noteworthy that epithelia are no more responsive when the experiment is performed at later stages (Cunha 1976b).

The expression of the developmental gene *Msx2* has also been described to be altered in the female reproductive tract following foetal exposure to DES. Indeed, *Msx2* is involved in vaginal epithelial differentiation and is required for Tgf-β2 and -β3 expression in the reproductive tract (Yin *et al.* 2006). In mice, after exposure to DES, the level of *Msx2* transcripts is significantly lower than physiological level in the developing uterus (Huang *et al.* 2005) and in vaginal epithelium (Yin *et al.* 2006). Moreover, a much more severe DES-induced vaginal phenotype is observed in *Msx2-/-* mutant mice exposed to DES, suggesting an important role for this gene during the estrogen-dependent growth of Müllerian derivatives and therefore in the protection of adverse effects of DES (Yin *et al.* 2006). However this protective mechanism remains unknown.

Many developmental genes involved in organogenesis of the FRT have been shown to be deregulated following *in utero* or neonatal exposure to the pharmacological endocrine disruptor DES. In spite of this, the mechanism by which these deregulations would be transmitted through generations is completely unclear. Indeed, no alteration of genomic DNA methylation pattern has been described yet under this condition. In particular, no variation in the methylation pattern of *Hoxa10* and *Hoxa11* promoters was observed in the mouse uterus after neonatal DES exposure (Li *et al.* 2001a). This transmission of phenotypic traits without alteration of methylation marks on DES-regulated genes is thus extremely puzzling and needs to be clarified.

DES and epigenetics

During more than 30 years, DES was heavily prescribed to pregnant women to prevent miscarriages and other pregnancy

problems. Much later, correlation between this chemical compound and reproductive dysfunctions was uncovered. Epidemiologic studies revealed that structural uterine, cervical or vaginal abnormalities may be as high as 33% in women with *in utero* exposure and that overall pregnancy outcomes in DES-exposed women were worse than those in unexposed women (Kaufman *et al.* 2000). Moreover, a small cell ovarian carcinoma was found in a 15 year old girl whose maternal grand mother was treated by DES during her pregnancy (Blatt *et al.* 2003).

This observation was later established in the mouse, in experiments aiming at understanding genetic and epigenetic mechanisms involved in both adverse and transgenerational effects of DES exposure. Overall outcomes showed that foetal exposure to DES led to poor reproductive outcome and to gynaecologic tumours later on. Furthermore, it appeared that the adverse effects of DES, such as tumour susceptibility, could be transmitted to subsequent generation (s) of both males and females. More precisely, experiments dealt with mice exposed to DES during various stages of gestation and with F1 and F2 females mated to unexposed males. It first appeared that F2 females' fertility was not affected unlike F1's. However F2 mice showed a high frequency of tumours, about similar to that assessed in F1 (Walker and Haven 1997). This study was further corroborated although F2 females exhibited a tumour incidence (including uterine adenocarcinoma) higher than controls but lower than F1 mice (Newbold *et al.* 1998).

The transmission of specific lesions of the female reproductive tract, such as uterine adenocarcinoma, to subsequent generation(s) seems to be difficult to explain without evoking epigenetic mechanisms. While no epigenetic modification was detected on key developmental genes such as *Hoxa* genes (Li *et al.* 2001b), modifications of DNA methylation have been reported for other genes following exposure to DES (Newbold *et al.,* 1997; Tang *et al.,* 2008). For example the estrogen-responsive lactoferrin gene seems to be up-regulated in the mouse uterus, after neonatal exposure to DES; this abnormal expression persists later in life (Nelson *et al.* 1994) and may be attributed to an abnormal demethylation in the lactoferrin gene promoter, which seems to occurr specifically in response to neonatal DES exposure (Li *et al.* 1997). Moreover, this demethylation state is continuously maintained in uterine tumours of DES-exposed mice, suggesting that neonatal DES treatment may not only induce tumour formation but also gene-specific demethylation, through a common cellular process, such as alterations of the expression of DNA methyltransferases and methylation of genomic DNA (Li *et al.* 1997; Sato *et al.* 2009). Subsequent work demonstrated that other developmental genes, up-regulated after exposure to DES, also exhibit modified methylation patterns. As such, the proto-oncogene *c-fos* is one of the early and persistently induced genes in uterine epithelium of mice exposed to oestrogen stimulation (Loose-Mitchell *et al.* 1988). Moreover this gene is known to play an important role in uterine epithelial proliferation and in uterine tumorigenesis. As a matter of fact, the yield of unmethylated CpGs in the exon-4 of this gene, is higher in neonatally DES-exposed mice than in untreated controls (Li *et al.* 2003). Although, the consequences of the hypomethylation level of these two genes remain unclear, it has been proposed that methylation pattern of genomic DNA can be transmitted to subsequent generation (s) (Holliday 1990). The fact that F2 mice, which were not directly exposed to DES, develop uterine adenocarcinoma would thus be explained by the transmission of modified methylation pattern of genes involved in cell homeosis such as the proto-oncogene *c-fos*. In addition, we previously mentioned, that genistein was a compound presenting an oestrogenic activity, and able to induce formation of multioocyte follicles on DES-treated neonates (Jefferson *et al.* 2002). Interestingly, a complementary study showed that F2 female mice also present multioocyte follicles although they were not formerly exposed to genistein (Jefferson *et al.* 2007).

The transmission of epigenetic pattern has been difficult to accept for many scientists until recently. Indeed, for several years, it was thought that methylation prints were removed from DNA as it was packaged into germ cells, wiping the epigenetic state clean for next generation. The whole literature on DES, as well as other studies performed by Cropley and collaborators, strengthen the relevance of a transmission of the epigenome through following generations (Cropley *et al.* 2006). Indeed, these authors showed that feeding pregnant A^{vy}/a mice with methyl donor in their diet not only shifted the coat colours of their offspring towards the brown end of the spectrum (Wolff *et al.* 1998), but also affected the next generation in the same way, showing that the grandmother's diet can affects the epigenetic state of her grandchildren (Cropley *et al.* 2006) through a stable modification of the germline epigenetic state (Cropley *et al.* 2007).

Conclusion

The venue of epigenetic research has allowed scientists to interpret some inexplicable malformative pathologies or environmental induced diseases such as cancers or allergies. Indeed, epigenetics could explain not only the discordances observed between monozygous twins but also phenomena such as incomplete penetrance, variable expression, sporadic cases and provide a novel viewpoint for understanding normal and aberrant development. The study of epigenetic mechanisms involved in such normal and pathologic processes constitutes thus a new exciting approach of investigation. In addition, considering that most epigenetic alterations are reversible both *in vitro* and *in vivo*, it suggests that a new therapy targeting complexes that catalyse epigenetic modifications could be found in the future.

Acknowledgments

IP wishes to sincerely thank Dr. F. Omilli for helpful comments on the manuscript; Agnes Burel for technical assistance. JM was supported by a grant from the "Conseil Régional de Bretagne" and TW was supported by a French Ministry fellowship. This work was supported by the CNRS and by grants from "Rennes Métropole" and the "Conseil Régional de Bretagne".

References

AAMAR, E. and FRANK D. (2004). *Xenopus* Meis3 protein forms a hindbrain-inducing center by activating FGF/MAP kinase and PCP pathways. *Development* 131: 153-163.

ALLARD, S., ADIN P., GOUEDARD L., DI CLEMENTE N., JOSSO N., ORGEBIN-CRIST M.C., PICARD J.Y. and XAVIER F. (2000). Molecular mechanisms of hormone-mediated Mullerian duct regression: involvement of beta-catenin. *Development* 127: 3349-3360.

ANWAY, M.D., CUPP A.S., UZUMCU M. and SKINNER M.K. (2005). Epigenetic

transgenerational actions of endocrine disruptors and male fertility. *Science* 308: 1466-1469.

ARANGO, N.A., SZOTEK P.P., MANGANARO T.F., OLIVA E., DONAHOE P.K. and TEIXEIRA J. (2005). Conditional deletion of beta-catenin in the mesenchyme of the developing mouse uterus results in a switch to adipogenesis in the myometrium. *Dev Biol* 288: 276-283.

BALFOUR, F.M., SEDGEWICK,A. (1879). On the existence of a head-kidney in the embryo chick, and on certain points in the development of Müllerian duct. *K.J.Microsc. Sci.* 19: 1-19.

BASILE, C. and DE MICHELE V. (2001a). Renal abnormalities in Mayer-Rokitanski-Kuster-Hauser syndrome. *J Nephrol* 14: 316-318.

BASILE, C. and DE MICHELE V. (2001b). Renal abnormalities in Mayer-Rokitanski-Kuster-Hauser syndrome. *J Nephrol*. 14: 316-318.

BEHRINGER, R.R., FINEGOLD M.J. and CATE R.L. (1994). Mullerian-inhibiting substance function during mammalian sexual development. *Cell* 79: 415-425.

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.

BIASON-LAUBER, A., KONRAD D., NAVRATIL F. and SCHOENLE E.J. (2004). A WNT4 mutation associated with Mullerian-duct regression and virilization in a 46,XX woman. *N Engl J Med* 351: 792-798.

BINGHAM, C., BULMAN M.P., ELLARD S., ALLEN L.I., LIPKIN G.W., HOFF W.G., WOOLF A.S., RIZZONI G., NOVELLI G., NICHOLLS A.J. and HATTERSLEY A.T. (2001). Mutations in the hepatocyte nuclear factor-1beta gene are associated with familial hypoplastic glomerulocystic kidney disease. *Am J Hum Genet* 68: 219-224.

BINGHAM, C., ELLARD S., COLE T.R., JONES K.E., ALLEN L.I., GOODSHIP J.A., GOODSHIP T.H., BAKALINOVA-PUGH D., RUSSELL G.I., WOOLF A.S., NICHOLLS A.J. and HATTERSLEY A.T. (2002). Solitary functioning kidney and diverse genital tract malformations associated with hepatocyte nuclear factor-1beta mutations. *Kidney Int* 61: 1243-1251.

BLATT, J., VAN LE L., WEINER T. and SAILER S. (2003). Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. *J Pediatr Hematol Oncol* 25: 635-636.

BLOCK, K., KARDANA A., IGARASHI P. and TAYLOR H.S. (2000). In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing mullerian system. *FASEB J* 14: 1101-1108.

BONDOS, S. (2006). Variations on a theme: Hox and Wnt combinatorial regulation during animal development. *Sci STKE* 2006: pe38.

BOUCHARD, M., SOUABNI A., MANDLER M., NEUBUSER A. and BUSSLINGER M. (2002). Nephric lineage specification by Pax2 and Pax8. *Genes Dev* 16: 2958-7290.

BRANHAM, W.S., SHEEHAN D.M., ZEHR D.R., RIDLON E. and NELSON C.J. (1985). The postnatal ontogeny of rat uterine glands and age-related effects of 17 beta-estradiol. *Endocrinology* 117: 2229-2237.

BRODY, J.R. and CUNHA G.R. (1989). Histologic, morphometric, and immunocytochemical analysis of myometrial development in rats and mice: I. Normal development. *Am J Anat* 186: 1-20.

CARROLL, T.J., PARK J.S., HAYASHI S., MAJUMDAR A. and MCMAHON A.P. (2005). Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Dev Cell* 9: 283-292.

CARTA, L. and SASSOON D. (2004). Wnt7a is a suppressor of cell death in the female reproductive tract and is required for postnatal and estrogen-mediated growth. *Biol Reprod* 71: 444-454.

CISNEROS, F.J. (2004). DNA methylation and male infertility. *Front Biosci* 9: 1189-1200.

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.

COFFINIER, C., BARRA J., BABINET C. and YANIV M. (1999). Expression of the vHNF1/HNF1beta homeoprotein gene during mouse organogenesis. *Mech Dev* 89: 211-213.

COLBORN, T., VOM SAAL F.S. and SOTO A.M. (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101: 378-384.

COUSE, J.F., DIXON D., YATES M., MOORE A.B., MA L., MAAS R. and KORACH K.S. (2001). Estrogen receptor-alpha knockout mice exhibit resistance to the developmental effects of neonatal diethylstilbestrol exposure on the female reproductive tract. *Dev Biol* 238: 224-238.

COUSE, J.F. and KORACH K.S. (1999). Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 20: 358-417.

COUSE, J.F. and KORACH K.S. (2004). Estrogen receptor-alpha mediates the detrimental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract. *Toxicology* 205: 55-63.

COX, S., SMITH L., BOGANI D., CHEESEMAN M., SIGGERS P. and GREENFIELD A. (2006). Sexually dimorphic expression of secreted frizzled-related (SFRP) genes in the developing mouse Mullerian duct. *Mol Reprod Dev* 73: 1008-1016.

CROPLEY, J.E., SUTER C.M., BECKMAN K.B. and MARTIN D.I. (2006). Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. *Proc Natl Acad Sci USA* 103: 17308-17312.

CROPLEY, J.E., SUTER C.M. and MARTIN D.I. (2007). Methyl donors change the germline epigenetic state of the A (vy) allele. *FASEB J* 21: 3021; author reply 3021-302.

CUNHA, G.R. (1975). The dual origin of vaginal epithelium. *Am J Anat* 143: 387-392.

CUNHA, G.R. (1976a). Epithelial-stromal interactions in development of the urogenital tract. *Int Rev Cytol* 47: 137-194.

CUNHA, G.R. (1976b). 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.

CUNHA, G.R., BATTLE E., YOUNG P., BRODY J., DONJACOUR A., HAYASHI N. and KINBARA H. (1992). Role of epithelial-mesenchymal interactions in the differentiation and spatial organization of visceral smooth muscle. *Epithelial Cell Biol* 1: 76-83.

CUNHA, G.R., YOUNG P. and BRODY J.R. (1989). Role of uterine epithelium in the development of myometrial smooth muscle cells. *Biol Reprod* 40: 861-871.

DAFTARY, G.S. and TAYLOR H.S. (2006). Endocrine regulation of HOX genes. *Endocr Rev* 27: 331-355.

DAVIS, R.J., HARDING M., MOAYEDI Y. and MARDON G. (2008). Mouse Dach1 and Dach2 are redundantly required for Mullerian duct development. *Genesis* 46: 205-213.

DEUTSCHER, E. and HUNG-CHANG YAO H. (2007). Essential roles of mesenchyme-derived beta-catenin in mouse Mullerian duct morphogenesis. *Dev Biol* 307: 227-236.

DI CLEMENTE, N. and BELVILLE C. (2006). Anti-Mullerian hormone receptor defect. *Best Pract Res Clin Endocrinol Metab* 20: 599-610.

DIECKMANN, W.J., DAVIS M.E., RYNKIEWICZ L.M. and POTTINGER R.E. (1953). Does the administration of diethylstilbestrol during pregnancy have therapeutic value? *Am J Obstet Gynecol* 66: 1062-1081.

DOHR, G., TARMANN T. and SCHIECHL H. (1987a). Different antigen expression on Wolffian and Mullerian cells in rat embryos as detected by monoclonal antibodies. *Anat Embryol (Berl)* 176: 239-242.

DOHR, G., TARMANN T. and SCHIECHL H. (1987b). Different antigen expression on Wolffian and Mullerian cells in rat embryos as detected by monoclonal antibodies. *Anat Embryol (Berl).* 176: 239-242.

DOLLE, P., DIERICH A., LEMEUR M., SCHIMMANG T., SCHUHBAUR B., CHAMBON P. and DUBOULE D. (1993). Disruption of the Hoxd-13 gene induces localized heterochrony leading to mice with neotenic limbs. *Cell* 75: 431-441.

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: 1767-7.

DREWS, U., SULAK O. and SCHENCK P.A. (2002). Androgens and the development of the vagina. *Biol Reprod* 67: 1353-1359.

DREYER, C. and ELLINGER-ZIEGELBAUER H. (1996). Retinoic acid receptors and nuclear orphan receptors in the development of *Xenopus laevis*. *Int J Dev Biol.* 40: 255-262.

FRAGA, M.F., BALLESTAR E., PAZ M.F., ROPERO S., SETIEN F., BALLESTAR M.L., HEINE-SUNER D., CIGUDOSA J.C., URIOSTE M., BENITEZ J., BOIX-CHORNET M., SANCHEZ-AGUILERA A., LING C., CARLSSON E., POULSEN P., VAAG A., STEPHAN Z., SPECTOR T.D., WU Y.Z., PLASS C. and ESTELLER M. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 102: 10604-10609.

FUJINO, A., ARANGO N.A., ZHAN Y., MANGANARO T.F., LI X., MAC LAUGHLIN D.T. and DONAHOE P.K. (2009). Cell migration and activated PI3K/AKT-directed elongation in the developing rat Müllerian duct. *Dev Biol* 325: 351-362.

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.

GOODMAN, F.R., BACCHELLI C., BRADY A.F., BRUETON L.A., FRYNS J.P., MORTLOCK D.P., INNIS J.W., HOLMES L.B., DONNENFELD A.E., FEINGOLD M., BEEMER F.A., HENNEKAM R.C. and SCAMBLER P.J. (2000). Novel HOXA13 mutations and the phenotypic spectrum of hand-foot-genital syndrome. *Am J Hum Genet* 67: 197-202.

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.

GROOTHUIS, P.G., DASSEN H.H., ROMANO A. and PUNYADEERA C. (2007). Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. *Hum Reprod Update* 13: 405-417.

GRUENWALD, P. (1941). The relation of the growing Mullerian duct to the Wolffian duct and its importance for the genesis of malformations. *Anat. Rec.* 81: 1-19.

GRUN, F. and BLUMBERG B. (2006). Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology* 147: S50-S55.

GUIOLI, S., SEKIDO R. and LOVELL-BADGE R. (2007). The origin of the Mullerian duct in chick and mouse. *Dev Biol* 302: 389-398.

HANNEMA, S.E. and HUGHES I.A. (2007). Regulation of Wolffian duct development. *Horm Res* 67: 142-151.

HEIKKILA, M., PRUNSKAITE R., NAILLAT F., ITARANTA P., VUORISTO J., LEPPALUOTO J., PELTOKETO H. and VAINIO S. (2005). The partial female to male sex reversal in Wnt-4-deficient females involves induced expression of testosterone biosynthetic genes and testosterone production, and depends on androgen action. *Endocrinology* 146: 4016-4023.

HERNANDEZ GIFFORD, J.A., HUNZICKER-DUNN M.E. and NILSON J.H. (2009). Conditional deletion of b-catenin mediated by Amhr2cre in mice causes female infertility. *Biol Reprod* DOI:10.1095/biolreprod.108.072280.

HEWITT, S.C. and KORACH K.S. (2002). Estrogen receptors: structure, mechanisms and function. *Rev Endocr Metab Disord* 3: 193-200.

HOLLIDAY, R. (1990). DNA methylation and epigenetic inheritance. *Philos Trans R Soc Lond B Biol Sci* 326: 329-338.

HOMBRIA, J.C. and LOVEGROVE B. (2003). Beyond homeosis—HOX function in morphogenesis and organogenesis. *Differentiation* 71: 461-476.

HONMA, S., SUZUKI A., BUCHANAN D.L., KATSU Y., WATANABE H. and IGUCHI T. (2002). Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod Toxicol* 16: 117-122.

HORIKAWA, Y., IWASAKI N., HARA M., FURUTA H., HINOKIO Y., COCKBURN B.N., LINDNER T., YAMAGATA K., OGATA M., TOMONAGA O., KUROKI H., KASAHARA T., IWAMOTO Y. and BELL G.I. (1997). Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17: 384-385.

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.

HUANG, W.W., YIN Y., BI Q., CHIANG T.C., GARNER N., VUORISTO J., MCLACHLAN J.A. and MA L. (2005). Developmental diethylstilbestrol exposure alters genetic pathways of uterine cytodifferentiation. *Mol Endocrinol* 19: 669-682.

IIZUKA-KOGO, A., ISHIDAO T., AKIYAMA T. and SENDA T. (2007). Abnormal development of urogenital organs in Dlgh1-deficient mice. *Development* 134: 1799-1807.

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. *Am J Pathol* 161: 1111-1117.

ITOH, T., IKEDA T., GOMI H., NAKAO S., SUZUKI T. and ITOHARA S. (1997). Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. *J Biol Chem.* 272: 22389-22392.

JACOB, M., KONRAD K. and JACOB H.J. (1999). Early development of the mullerian duct in avian embryos with reference to the human. An ultrastructural and immunohistochemical study. *Cells Tissues Organs* 164: 63-81.

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.

JAMIN, S.P., ARANGO N.A., MISHINA Y., HANKS M.C. and BEHRINGER R.R. (2003). Genetic studies of the AMH/MIS signaling pathway for Mullerian duct regression. *Mol Cell Endocrinol* 211: 15-19.

JEFFERSON, W.N., COUSE J.F., PADILLA-BANKS E., KORACH K.S. and NEWBOLD R.R. (2002). Neonatal exposure to genistein induces estrogen receptor (ER)alpha expression and multioocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions. *Biol Reprod* 67: 1285-1296.

JEFFERSON, W.N., PADILLA-BANKS E. and NEWBOLD R.R. (2007). Disruption of the female reproductive system by the phytoestrogen genistein. *Reprod Toxicol* 23: 308-316.

JOSSO, N., CATE R.L., PICARD J.Y., VIGIER B., DI CLEMENTE N., WILSON C., IMBEAUD S., PEPINSKY R.B., GUERRIER D., BOUSSIN L. and *et al.* (1993). Anti-mullerian hormone: the Jost factor. *Recent Prog Horm Res* 48: 1-59.

JOST, A. (1953). Problems of fetal endocrinology: the gonadal and hypophyseal hormones. *Recent Prog Horm Res* 8: 379-418.

KAUFMAN, R.H., ADAM E., HATCH E.E., NOLLER K., HERBST A.L., PALMER J.R. and HOOVER R.N. (2000). Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. *Obstet Gynecol* 96: 483-489.

KIM, Y., KOBAYASHI A., SEKIDO R., DINAPOLI L., BRENNAN J., CHABOISSIER M.C., POULAT F., BEHRINGER R.R., LOVELL-BADGE R. and CAPEL B. (2006). Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biol* 4: e187.

KIM, H., HAYAHI S., CHAMBON P., WATANABE H., IGUCHI T. and SATO, T. (2009). Effects of diethystilbestrol on ovarian follicle development in neaonatal mice. *Reprod. Toxicol.* 27: 55-62.

KITAJEWSKI, J. and SASSOON D. (2000). The emergence of molecular gynecology: homeobox and Wnt genes in the female reproductive tract. *Bioessays.* 22: 902-910.

KLATTIG, J., SIERIG R., KRUSPE D., BESENBECK B. and ENGLERT C. (2007). Wilms' tumor protein Wt1 is an activator of the anti-Mullerian hormone receptor gene Amhr2. *Mol Cell Biol* 27: 4355-4364.

KOBAYASHI, A. and BEHRINGER R.R. (2003). Developmental genetics of the female reproductive tract in mammals. *Nat Rev Genet* 4: 969-980.

KOBAYASHI, A., KWAN K.M., CARROLL T.J., MCMAHON A.P., MENDELSOHN C.L. and BEHRINGER R.R. (2005). Distinct and sequential tissue-specific activities of the LIM-class homeobox gene Lim1 for tubular morphogenesis during kidney development. *Development* 132: 2809-2823.

KOBAYASHI, A., SHAWLOT W., KANIA A. and BEHRINGER R.R. (2004). Requirement of Lim1 for female reproductive tract development. *Development* 131: 539-549.

KOLATSI-JOANNOU, M., BINGHAM C., ELLARD S., BULMAN M.P., ALLEN L.I., HATTERSLEY A.T. and WOOLF A.S. (2001). Hepatocyte nuclear factor-1beta: a new kindred with renal cysts and diabetes and gene expression in normal human development. *J Am Soc Nephrol* 12: 2175-2180.

KOMATSU, M. and FUJITA H. (1978). Electron-microscopic studies on the development and aging of the oviduct epithelium of mice. *Anat Embryol (Berl)* 152: 243-259.

KURITA, T., COOKE P.S. and CUNHA G.R. (2001). Epithelial-stromal tissue interaction in paramesonephric (Mullerian) epithelial differentiation. *Dev Biol* 240: 194-211.

KURITA, T., MILLS A.A. and CUNHA G.R. (2004). Roles of p63 in the diethylstilbestrol-induced cervicovaginal adenosis. *Development* 131: 1639-1649.

LI, S., HANSMAN R., NEWBOLD R., DAVIS B., MCLACHLAN J.A. and BARRETT J.C. (2003). Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol Carcinog* 38: 78-84.

LI, S., MA L., CHIANG T., BUROW M., NEWBOLD R.R., NEGISHI M., BARRETT J.C. and MCLACHLAN J.A. (2001a). Promoter CpG methylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Mol Carcinog* 32: 213-219.

LI, S., MA L., CHIANG T., BUROW M., NEWBOLD R.R., NEGISHI M., BARRETT J.C. and MCLACHLAN J.A. (2001b). Promoter CpG methylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Mol Carcinog* 32: 213-219.

LI, S., WASHBURN K.A., MOORE R., UNO T., TENG C., NEWBOLD R.R., MCLACHLAN J.A. and NEGISHI M. (1997). Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res* 57: 4356-4359.

LINDNER, T.H., NJOLSTAD P.R., HORIKAWA Y., BOSTAD L., BELL G.I. and SOVIK O. (1999). A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1beta. *Hum Mol Genet* 8: 2001-2008.

LOOSE-MITCHELL, D.S., CHIAPPETTA C. and STANCEL G.M. (1988). Estrogen regulation of c-fos messenger ribonucleic acid. *Mol Endocrinol* 2: 946-951.

MA, L., BENSON G.V., LIM H., DEY S.K. and MAAS R.L. (1998). Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in mullerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol* 197: 141-154.

MARKEY, C.M., COOMBS M.A., SONNENSCHEIN C. and SOTO A.M. (2003). Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs. *Evol Dev* 5: 67-75.

MCLACHLAN, J.A., NEWBOLD R.R. and BULLOCK B.C. (1980). Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Res* 40: 3988-3999.

MENDELSOHN, C., LOHNES D., DECIMO D., LUFKIN T., LEMEUR M., CHAMBON P. and MARK M. (1994). Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development* 120: 2749-2771.

MERICSKAY, M., KITAJEWSKI J. and SASSOON D. (2004). Wnt5a is required for proper epithelial-mesenchymal interactions in the uterus. *Development* 131: 2061-2072.

MILLER, C., DEGENHARDT K. and SASSOON D.A. (1998a). Fetal exposure to DES results in de-regulation of Wnt7a during uterine morphogenesis. *Nat Genet* 20: 228-230.

MILLER, C., PAVLOVA A. and SASSOON D.A. (1998b). Differential expression patterns of Wnt genes in the murine female reproductive tract during development and the estrous cycle. *Mech Dev* 76: 91-99.

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.

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.

MIYAMOTO, N., YOSHIDA M., KURATANI S., MATSUO I. and AIZAWA S. (1997). Defects of urogenital development in mice lacking Emx2. *Development* 124: 1653-1664.

MORCEL, K., CAMBORIEUX L. and GUERRIER D. (2007). Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome. *Orphanet J Rare Dis* 2: 13.

MORTLOCK, D.P. and INNIS J.W. (1997). Mutation of HOXA13 in hand-foot-genital syndrome. *Nat Genet* 15: 179-80.

MURAMATSU, M. and INOUE S. (2000). Estrogen receptors: how do they control reproductive and nonreproductive functions? *Biochem Biophys Res Commun.* 270: 1-10.

NELSON, K.G., SAKAI Y., EITZMAN B., STEED T. and MCLACHLAN J. (1994). Exposure to diethylstilbestrol during a critical developmental period of the mouse reproductive tract leads to persistent induction of two estrogen-regulated genes. *Cell Growth Differ* 5: 595-606.

NEWBOLD, R.R., BANKS E.P., BULLOCK B. and JEFFERSON W.N. (2001). Uterine adenocarcinoma in mice treated neonatally with genistein. *Cancer Res* 61: 4325-4328.

NEWBOLD, R.R., BULLOCK B.C. and MCLACHLAN J.A. (1990). Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res* 50: 7677-7681.

NEWBOLD, R.R., HANSON R.B. and JEFFERSON W.N. (1997). Ontogeny of lactoferrin in the developing mouse uterus: a marker of early hormone response. *Biol Reprod* 56: 1147-1157.

NEWBOLD, R.R., HANSON R.B., JEFFERSON W.N., BULLOCK B.C., HASEMAN J. and MCLACHLAN J.A. (1998). Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis* 19: 1655-1663.

NEWBOLD, R.R., JEFFERSON W.N. and PADILLA-BANKS E. (2007a). Long-term adverse effects of neonatal exposure to bisphenol A on the murine female reproductive tract. *Reprod Toxicol* 24: 253-258.

NEWBOLD, R.R., PADILLA-BANKS E., SNYDER R.J. and JEFFERSON W.N. (2007b). Perinatal exposure to environmental estrogens and the development of obesity. *Mol Nutr Food Res* 51: 912-917.

NEWBOLD, R.R., PADILLA-BANKS E., SNYDER R.J., PHILLIPS T.M. and JEFFERSON W.N. (2007c). Developmental exposure to endocrine disruptors and the obesity epidemic. *Reprod Toxicol* 23: 290-296.

OPPELT, P., S P.R., KELLERMANN A., BRUCKER S., G A.H., K S.L., P L.S., STRICK R., WALLWIENER D. and M W.B. (2005a). Clinical aspects of Mayer-Rokitansky-Kuester-Hauser syndrome: recommendations for clinical diagnosis and staging. *Hum Reprod.* 21: 792-797.

OPPELT, P., STRISSEL P.L., KELLERMANN A., SEEBER S., HUMENY A., BECKMANN M.W. and STRICK R. (2005b). DNA sequence variations of the entire anti-Mullerian hormone (AMH) gene promoter and AMH protein expression in patients with the Mayer-Rokitanski-Kuster-Hauser syndrome. *Hum Reprod* 20: 149-157.

ORVIS, G.D. and BEHRINGER R.R. (2007). Cellular mechanisms of Mullerian duct formation in the mouse. *Dev Biol* 306: 493-504.

PARR, B.A., AVERY E.J., CYGAN J.A. and MCMAHON A.P. (1998). The classical mouse mutant postaxial hemimelia results from a mutation in the Wnt 7a gene. *Dev Biol* 202: 228-234.

PARR, B.A. and MCMAHON A.P. (1998). Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a. *Nature* 395: 707-710.

POIRIER, L.A. and VLASOVA T.I. (2002). The prospective role of abnormal methyl metabolism in cadmium toxicity. *Environ Health Perspect* 110 Suppl 5: 793-795.

REBER, M. and CEREGHINI S. (2001). Variant hepatocyte nuclear factor 1 expression in the mouse genital tract. *Mech Dev* 100: 75-78.

ROBERT, B. and LALLEMAND Y. (2006). Anteroposterior patterning in the limb and digit specification: contribution of mouse genetics. *Dev Dyn.* 235: 2337-2352.

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.

SAINIO, K., HELLSTEDT P., KREIDBERG J.A., SAXEN L. and SARIOLA H. (1997). Differential regulation of two sets of mesonephric tubules by WT-1. *Development* 124: 1293-1299.

SAINIO, K. and RAATIKAINEN-AHOKAS A. (1999). Mesonephric kidney—a stem cell factory? *Int J Dev Biol* 43: 435-439.

SALNIKOW, K. and COSTA M. (2000). Epigenetic mechanisms of nickel carcinogenesis. *J Environ Pathol Toxicol Oncol* 19: 307-318.

SATO, K., FUKATA H., KOGO Y., OHGANE J., SHIOTA K. and MORI, C. (2009). Neonatal exposure to diethylstilbestrol alters expression of DNA methyltransferases and methylation of genomic DNA in mouse uterus. *Endocrinol. J.* 56: 131-139.

SAXEN, L. and SARIOLA H. (1987). Early organogenesis of the kidney. *Pediatr Nephrol* 1: 385-392.

TABB, M.M. and BLUMBERG B. (2006). New modes of action for endocrine-disrupting chemicals. *Mol Endocrinol* 20: 475-482.

TANG, W.Y., NEWBOLD R., MARDILOVICH K., JEFFERSON W., CHENG R.Y.S., MEDVEDOVIC M. and HO, S.M. (2008). Persistent hypomethylation in the

promoter of nucleosomal binding protein 1 (Nsbp1) correlates with overexpression of Nsbp1 in mouse uteri neonatally exposed to diethylstilbestrol or genistein. *Endocrinology* 149: 5922-5931.

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: 1338-1345.

TONG, G.X., CHIRIBOGA L., HAMELE-BENA D. and BORCZUK A.C. (2007) Expression of PAX2 in papillary serous carcinoma of the ovary: immunohistochemical evidence of fallopian tube or secondary Müllerian system origin? *Mod Pathol* 20: 856-863.

TORRES, M., GOMEZ-PARDO E., DRESSLER G.R. and GRUSS P. (1995). Pax-2 controls multiple steps of urogenital development. *Development* 121: 4057-4065.

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.

WALKER, B.E. and HAVEN M.I. (1997). Intensity of multigenerational carcinogenesis from diethylstilbestrol in mice. *Carcinogenesis* 18: 791-793.

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.

WATERLAND, R.A., LIN J.R., SMITH C.A. and JIRTLE R.L. (2006). Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (Igf2) locus. *Hum Mol Genet* 15: 705-716.

WOLFF, G.L., KODELL R.L., MOORE S.R. and COONEY C.A. (1998). Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. *FASEB J* 12: 949-957.

WROBEL, K.H., HEES I., SCHIMMEL M. and STAUBER E. (2002). The genus Acipenser as a model system for vertebrate urogenital development: nephrostomial tubules and their significance for the origin of the gonad. *Anat Embryol (Berl)* 205: 67-80.

YIN, Y., LIN C. and MA L. (2006). MSX2 promotes vaginal epithelial differentiation and wolffian duct regression and dampens the vaginal response to diethylstilbestrol. *Mol Endocrinol* 20: 1535-1546.

ZHAN, Y., FUJINO A., MACLAUGHLIN D.T., MANGANARO T.F., SZOTEK P.P., ARANGO N.A., TEIXEIRA J. and DONAHOE P.K. (2006). Mullerian inhibiting substance regulates its receptor/SMAD signaling and causes mesenchymal transition of the coelomic epithelial cells early in Mullerian duct regression. *Development* 133: 2359-2369.

ZHAO, Y. and POTTER S.S. (2001). Functional specificity of the Hoxa13 homeobox. *Development* 128: 3197-3207.

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