Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract

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SUMMARY

The murine female reproductive tract differentiates along the anteroposterior axis during postnatal development. This process is marked by the emergence of distinct cell types in the oviduct, uterus, cervix and vagina and is dependent upon specific mesenchymal-epithelial interactions as demonstrated by earlier heterografting experiments. Members of the Wnt family of signaling molecules have been recently identified in this system and an early functional role in reproductive tract development has been demonstrated. Mice were generated using ES-mediated homologous recombination for the Wnt-7a gene (Parr, B. A. and McMahon, A. P. (1995) Nature 374, 350-353). Since Wnt-7a is expressed in the female reproductive tract, we examined the developmental consequences of lack of Wnt-7a in the female reproductive tract. We observe that the oviduct lacks a clear demarcation from the anterior uterus, and acquires several cellular and molecular characteristics of the uterine horn. The uterus acquires cellular and molecular characteristics that represent an intermediate state between normal uterus and vagina. Normal vaginas have stratified epithelium and normal uteri have simple columnar epithelium, however, mutant uteri have stratified epithelium. Additionally, Wnt-7a mutant uteri do not form glands. The changes observed in the oviduct and uterus are accompanied by a postnatal loss of hoxa-10 and hoxa-11 expression, revealing that Wnt-7a is not required for early hoxa gene expression, but is required for maintenance of expression. These clustered hox genes have been shown to play a role in anteroposterior patterning in the female reproductive tract. In addition to this global posterior shift in the female reproductive tract, we note that the uterine smooth muscle is disorganized, indicating development along the radial axis is affected. Changes in the boundaries and levels of other Wnt genes are detectable at birth, prior to changes in morphologies. These results suggest that a mechanism whereby Wnt-7a signaling from the epithelium maintains the molecular and morphological boundaries of distinct cellular populations along the anteroposterior and radial axes of the female reproductive tract.

Key words: Wnt, Mouse, Female reproductive tract, Anteroposterior patterning, Smooth muscle, Uterine gland

INTRODUCTION

The murine female reproductive tract (FRT) is relatively undifferentiated and rudimentary at birth (Brody and Cunha, 1989). The Müllerian duct consists of simple columnar epithelium which is surrounded by the mesenchyme of the urogenital ridge (Cunha, 1976a). Developmental changes in the uterus occur in response to circulating steroid hormones and are dependent upon specific mesenchymal-epithelial interactions (Cunha, 1976a). Mesenchymal-epithelial interactions are critical for the formation of many organs including lung (Alescio and Cassini, 1962; Wessels, 1970), mammary gland (Sakakura et al., 1976; Daniel and Silberstein, 1987; Kratochwil, 1987) and male and female reproductive tracts (see Cunha, 1976a for review). The contributions of the mesenchymal and epithelial components can be evaluated through epithelial/mesenchymal recombinants prepared from the same or different tissue sources. Tissue recombinant experiments can be performed with the uterus and vagina. The epithelium of the entire Müllerian tract remains plastic and undifferentiated until approximately 5 days after birth (Cunha, 1976a,b). During this period, the epithelium can respond to inductive signals from either uterine or vaginal mesenchyme. When uterine mesenchyme is recombined with vaginal epithelium, the mesenchyme directs the vaginal epithelium along a uterine cytodifferentiation pathway (Cunha, 1976b). The resultant heterograft has a simple columnar epithelium characteristic of the uterus, rather than the stratified squamous morphology normally seen in the adult vagina. These morphogenetic changes are accompanied by changes in gene expression consistent with the uterine developmental program (Pavlova et al., 1994). Similarly, vaginal mesenchyme can induce uterine epithelium to form vaginal-like stratified epithelium (Cunha, 1976b) and express vaginal-specific genes (Pavlova et al., 1994). Uterine epithelium loses the capacity to respond to inductive signals from the vaginal mesenchyme.
between 5 and 7 days after birth (Cunha, 1976a,b). The nature of the inductive signals and the transient capacity to respond to these signals is not understood at the molecular level.

The Drosophila segment polarity gene wingless encodes a secreted molecule (Baker, 1987) that is implicated in patterning and establishment of cell boundaries during embryogenesis (see Moon et al. (1997) for review). Wnt genes are the vertebrate homologs of wingless. The vertebrate Wnt family comprises at least 16 members. Wnt gene expression patterns during embryogenesis and in the adult suggest that they are involved in cell-cell communication and/or regional specification of cell fates (Gavin et al., 1990; Gavin and McMahon, 1992; Parr et al., 1993; Pavlova et al., 1994; Weber-Hall et al., 1994). Targeted deletions of specific members of the Wnt family provide evidence for a key role in patterning and cell-cell communication. Wnt-7a is expressed in the dorsal limb ectoderm and is a dorsalizing molecule since ventralization of the limb occurs in its absence (Parr and McMahon, 1995; Cygan et al., 1997). Wnt-4 mutant mice fail to form kidney tubules due to a failure of cells to undergo mesenchymal-to-epithelial transformation (Stark et al., 1994).

Several members of the Wnt gene family are expressed in the mammary gland (Gavin and McMahon, 1992; Weber-Hall et al., 1994; Bradbury et al., 1995). The morphological changes that occur in the adult mammary gland have been attributed to both hormonal fluctuations and mesenchymal-epithelial interactions (Weber-Hall et al., 1994). The expression of individual Wnt genes is primarily restricted either to mammary stroma or epithelium, and the expression patterns change with pregnancy and lactation (Weber-Hall et al., 1994). Functional data suggest that Wnt genes play a critical role in directing the morphological changes that occur in the adult mammary gland in response to levels of circulating steroid hormones (Bradbury et al., 1995).

Homeobox genes are attractive candidates for the regulation of pattern formation during embryogenesis. Gene disruption and gain-of-function studies have correlated gene expression with developmental defects (Alkema et al., 1995; Horan et al., 1995; Muragaki et al., 1996). Both clustered and non-clustered homeobox-containing genes are expressed in the mouse female reproductive tract (Dollé et al., 1991; Redline et al., 1992; Pavlova et al., 1994; Hsieh-Li et al., 1995; Satokata et al., 1995). The homeobox gene cluster expressed is in spatially restricted domains within the urogenital tract (Dollé et al., 1991). Reduced fertility is seen in females with targeted deletions of hoxa-10 or hoxa-11 (Hsieh-Li et al., 1995; Satokata et al., 1995; Benson et al., 1996). Loss of hoxa-10 or hoxa-11 results in, respectively, a complete or partial anterior transformation of the uterine horn (Benson et al., 1996; Gendron et al., 1997), supporting a role for clustered hox genes in anteroposterior patterning in the female urogenital tract.

We have previously shown that the homeogene Msx1 is a marker of uterine epithelial cytodifferentiation and its expression is dependent upon cell contact with uterine mesenchyme (Pavlova et al., 1994). We observed that uterine mesenchyme expresses high levels of Wnt-5a and that levels of Wnt-5a and Msx1 are coordinately regulated (Pavlova et al., 1994). A number of other interactions between Wnt genes and homeobox-containing genes during patterning events have been described (McMahon et al., 1992; Vogel et al., 1995; Logan et al., 1997). Given the restricted expression patterns of Wnt genes and homeobox genes in the female reproductive tract (Hsieh-Li et al., 1995), it is likely that both Wnt and homeobox-containing genes participate in regulation of anteroposterior patterning in the uterus.

We have identified three members of the Wnt family of signaling molecules expressed in a dynamic pattern in the mouse female reproductive tract (C. Miller et al., unpublished data). We report here that loss of Wnt-7a expression results in a partial posteriorization of the female reproductive tract at gross, cellular and molecular levels. Specifically, the oviduct acquires characteristics of the vagina, although both compartments also retain some characteristics of their own identity. The incomplete shift in the oviduct and the uterus may be due to a postnatal decline in the correct anteroposterior expression of hoxa-10 and hoxa-11. Thus Wnt-7a is required to maintain but not induce hoxa-10 and hoxa-11 expression. We also note that uterine development along the radial (luminal-adluminal) axis is altered. In addition to lacking uterine glands, we note that the smooth muscle layers in the mutant uterus are overgrown and poorly organized. Although the Müllerian ducts are essentially normal in overall morphology at birth in the mutant mice, marked differences in Wnt-5a and Wnt-4 expression can already be observed. We propose a mechanism whereby Wnt-7a acts to regulate the boundaries of expression of Wnt-5a and Wnt-4, which act in concert to establish the correct developmental axes of the uterus.

MATERIALS AND METHODS

Mice

Wnt-7a mutant mice were generated by homologous recombination in ES cells as described previously (Parr and McMahon, 1995). The targeting strategy inserted a neomycin-resistance gene into the second exon of Wnt-7a. 129/Sv sibling or age-matched females were used for control tissues in the described experiments. Neonatal tissues were isolated following timed breedings with the morning of the vaginal plug counted as 0.5 days postcoitum (p.c.). For postnatal tissues, the day of birth is counted as day 0. At least 2 animals were examined for each time point and 10 mutant mice were examined for oviduct morphology.

Histology and in situ hybridization

Tissues were fixed overnight in 4% PBS-buffered paraformaldehyde. Paraffin-embedded tissues were sectioned at 5-6 μm. For histological examination, sections were stained with hematoxylin and eosin. Techniques for in situ hybridization were performed as previously described (Sassoon and Rosenthal, 1993). Antisense riboprobes were generated for Msx1 (Song et al., 1992), Wnt-4 and Wnt-7a (Parr et al., 1993), Wnt-5a (Gavin and McMahon, 1992), smooth muscle myosin heavy chain (SMMHC) (Miano et al., 1994), hoxa-10 (Satokata et al., 1995) and hoxa-11 (Hsieh-Li et al., 1995). Antisense riboprobes were generated under identical reaction conditions and were used at a final concentration of 105,000 dis ints/minute/μl hybridization buffer. Emulsion-coated slides were allowed to expose for 1 or 2 weeks at 4°C. In situ analysis was performed at least twice per tissue sample with each probe and at least two different samples were used per data point.

Intact tissue grafting

Intact uterine and vaginal tissues from newborn Wnt-7a mutant and wild-type mice were surgically inserted underneath the kidney capsule of athymic nude mice (Taconic NCI) (Bigsbey et al., 1986). Control and mutant tissues were grafted to opposite kidneys in the same host.
for 3-4 weeks. Tissues were subsequently isolated and processed for histological examination and in situ hybridization.

**Tissue recombinants**

Recombinants were prepared using techniques described previously (Bigsby et al., 1986). Briefly, intact reproductive tracts were isolated from neonatal (0-2 days postpartum) Wnt-7a mutant and wild-type mice and maintained in calcium/magnesium-free Hank’s buffer (CMF-HBSS, Gibco) at 4°C until use. Uterine horns and vagina were separated, carefully excluding the cervical region. Samples were incubated at 4°C in 1% trypsin (Difco 1:250) in CMF-HBSS for 1-1.5 hours and were rinsed three times with CMF-HBSS supplemented with 10% FCS; the first rinse in the presence of 0.1% deoxyribonuclease 1 (Sigma). The tissues were separated into mesenchymal or epithelial components by gentle teasing with forceps or by drawing into a flame-blunted drawn Pasteur pipette (Bigsby et al., 1986). The mesenchymal and epithelial fragments were made into recombinants on solidified agar medium (Pavlova et al., 1994). Tissues were allowed to re-adhere overnight before grafting under the renal capsule of athymic nude mice.

**RESULTS**

**Wnt-7a is expressed in the developing and adult female reproductive tract**

The expression pattern of Wnt-7a is dynamic during the development of the female reproductive tract (Fig. 1; C. Miller et al., unpublished data). Wnt-7a is expressed throughout the epithelium of the prenatal Müllerian tract (Fig. 1A,B) but becomes restricted to oviduct and uterine luminal epithelium after birth (Fig. 1C,D) and in the adult (Fig. 1E,F). It is not expressed in glandular epithelium in the adult uterus (Fig. 1E,F) or in the epithelium of the adult vagina (Fig. 1G,H).

**(1) Gross and cellular morphology**

Wnt-7a mutant female reproductive tracts are posteriorized

Previous studies revealed that Wnt-7a mutant female mice are sterile, however, the underlying causes were not examined (Parr and McMahon, 1995). Analysis of Wnt-7a mutant and heterozygous female reproductive tracts was undertaken at the gross morphological, cellular and molecular levels. Wnt-7a mutant uteri are smaller and thinner in diameter than wild-type or heterozygote counterparts at the same age (Fig. 2A-C) although the vagina appears unaffected. The oviducts in the mutant mouse are either reduced or absent and there is a variable amount of oviduct coiling (out of 10 mice, 8 had no evident oviducts and 2 had loose oviduct-like coils on one uterine horn). The cell morphology of the wild-type and heterozygote oviducts are indistinguishable (Fig. 2D,E). The malformations in the mutant oviduct are accompanied by alterations in cell morphology. The cytoarchitecture of the mutant oviduct shows a high degree of variation: it can resemble uterine horn (Fig. 2F) or appear similar to normal oviduct and contain raised epithelial folds characteristic of this tissue (Fig. 2G).

We examined whether the differences in the mutant uterus are accompanied by alterations in cell morphology. Uterine horns, oviducts and cervix all derive from the Müllerian tract. The mesenchyme of the Müllerian tract differentiates into peripherally located smooth muscle cells, and an inner layer of stromal cells that is lined by epithelium (Brody and Cunha, 1989). Müllerian epithelium differentiates into both luminal and glandular epithelia postnatally. The epithelia of the oviduct, uterus and vagina are histologically distinct in normal FRTs. Wild-type uterine horn has simple columnar luminal epithelium, stroma containing endometrial glands and two distinct layers of smooth muscle (Fig. 3A; inset). We readily detect perturbations in Wnt-7a heterozygous uterine cytoarchitecture implying a gene dosage effect, although no obvious change in fertility success is noted in these females. We note an excess of uterine glands in heterozygote uteri (Fig. 3B), which increases in severity as the animals age.

There are a number of differences between the wild-type and

![Fig. 1. Wnt-7a is expressed dynamically in the developing and adult female reproductive tract. The panels show the respective bright- and dark-field images of sections hybridized with a probe corresponding to Wnt-7a. (A,B) Longitudinal sections of a 17 d.p.c. female reproductive tract. Wnt-7a is expressed in the simple columnar epithelium of the Müllerian tract in both the future vagina (small arrow) and future uterine horns (ut, arrowhead). (C,D) Sections through a 4 day old anterior uterine horn (ut) and oviduct (ovi). Transcripts are detected in the epithelium of the uterine horn, including the forming glands (g) and in the epithelium of the oviduct. (E,F) A section through an adult uterine horn during metestrus. Indicated are uterine glands (g) and the simple columnar epithelium (double arrows). Wnt-7a is detected in the luminal epithelium of the uterine horns but not in the uterine glands. (G,H) A section through an adult vagina. Wnt-7a is not detected in the vaginal epithelium (double arrows). Scale bar, 200 μm.](image)
the Wnt-7a mutant uterine horns. The appearance of the mutant uterus combines features of the uterus and vagina. Normal vagina has stratified or multilayered epithelium, thin stroma and a layer of disorganized smooth muscle bundles (Fig. 3D). The mutant uterus has a multilayered epithelium, a relatively thin stroma and no glands, but it does have two layers of smooth muscle like the wild-type uterus (Fig. 3C).

The diameter of the mutant uterine horns is generally smaller than that of wild-type or heterozygous uterine horns. Relative to the diameter of the uterus, the longitudinal and circular smooth muscle layers that surround the uterus are much thicker in the mutant uterus than in the wild-type uterus (Fig. 3A,C). In order to verify the identity of the smooth muscle cell bundles and to define more clearly the location of smooth muscle cells,
we performed in situ hybridization using a riboprobe corresponding to smooth muscle myosin heavy chain (SMMHC; Fig. 4). We detect two layers of smooth muscle in the wild-type uterus (Fig. 4A-D); however, there are changes in the distribution of smooth muscle in the mutant uterus. The inner circular layer of smooth muscle, which is normally compact, is composed of irregular and disorganized bundles in the mutant uterus (Fig. 4B,D). These bundles resemble those seen in the wild-type vagina (Fig. 4E,F). The distribution of smooth muscle cells in the heterozygous animals and in the mutant vagina is indistinguishable from wild-type mice (data not shown).

The Wnt-7a mutant FRT phenotype is not due to extrinsic factors

Since Wnt-7a is expressed in many tissues other than the uterus (Parr et al., 1993; Lucas and Salinas, 1997), it could be argued that systemic changes in the mutant mouse, such as the levels of circulating steroid hormones, are responsible for the observed phenotypic differences. To distinguish between local and systemic effects, intact neonatal Wnt-7a mutant uterine horns were grafted under the kidney capsules of female athymic nude mice to assess development in an otherwise normal host environment (Bigsby et al., 1986). The grafts were allowed to grow for 3 weeks in a non-pregnant or pregnant host. Gland formation in the wild-type uterus is only observed in grafts grown in a pregnant host. The phenotype seen in the mutant FRT is recapitulated in the mutant tissue grafts (Fig. 5C,G). We observe multilayered epithelium, little stroma and an increased amount of smooth muscle in the mutant grafts (Fig. 5C,D,G,H). The smooth muscle is located much closer to the epithelium in the mutant grafts than in the control grafts (Fig. 5H versus F). The smooth muscle surrounds the epithelium in the mutant grafts from the same host (Fig. 5G).}

**Wnt-7a regulates uterine smooth muscle development**

The observation that Wnt-7a expression is restricted to the epithelium of the developing and adult uterus suggests that it participates in mesenchymal-epithelial interactions that guide postnatal development. Specifically, the phenotype of the Wnt-7a mutant uterus suggests Wnt-7a is secreted by the epithelium and provides a signal that maintains stromal-smooth muscle boundaries. The excess of smooth muscle in the Wnt-7a mutant mouse suggests Wnt-7a acts in the formation of smooth muscle. These properties were tested using tissue heterografts between Wnt-7a mutant and wild-type tissues. Normal recombinants (UtS + UtE, Fig. 6A) have the same characteristic structure as recombinants prepared with mutant mesenchyme (−/−UtS + UtE, Fig. 6C). They have an outer layer of smooth muscle, a distinct layer of stroma and a simple columnar epithelium. In situ hybridization with the SMMHC probe on nearby sections show the presence of a stromal layer between the epithelium and the smooth muscle (Fig. 6B,D). Recombinants prepared with mutant epithelium mimic the mutant phenotype. They have an excess of smooth muscle, little stroma and stratified epithelium (Fig. 6E). Additionally, the sharp boundary between stromal cells and smooth muscle cells easily noted in the control grafts is not apparent in the grafts containing mutant epithelium (see insets Fig. 6A,E). Smooth muscle cells are not separated from the epithelium by a distinguishable layer of stroma (Fig. 6F). Therefore, loss of Wnt-7a from the uterine epithelium is sufficient to account for changes in the uterine mesenchyme which differentiates into smooth muscle and stroma.

**(2) Analysis of gene expression**

Changes in Wnt gene expression precede the appearance of morphological perturbations

The expression patterns of Wnt-4, and Wnt-5a were examined in developing and adult wild-type, Wnt-7a heterozygous and mutant female reproductive tracts by in situ hybridization. Prior to the emergence of overt phenotypic differences between
mutant and wild-type FRTs (<5 days postnatal), differences already exist in the patterns of gene expression. In the wild-type neonate uterine horn, Wnt-5a is expressed primarily within the uterine mesenchyme (Fig. 7A). In the adult uterus, Wnt-5a expression is regulated by the estrous cycle (C. Miller et al., unpublished data), but its primary site of expression is the stroma (Fig. 7B). In the newborn Wnt-7a heterozygous and mutant uteri, Wnt-5a expression has shifted so that it is detected in both uterine mesenchyme and epithelium (Fig. 7C,E). During postnatal development, Wnt-5a expression is maintained in both the epithelium and stroma of the heterozygote uterine horn (Fig. 7D). Wnt-5a expression declines and becomes undetectable by 12-16 weeks in the mutant stroma (Fig. 7F). In addition, expression of Msx1, a marker of correct uterine cytodifferentiation, is not detectable in the epithelium of the adult Wnt-7a mutant uterus (data not shown).

Wnt-4 is normally expressed solely in the stroma of the neonatal wild-type uterus (Fig. 7G). Wnt-4 undergoes a dynamic pattern of expression in the uterus during the estrous cycle (C. Miller et al., unpublished data), thus we confined our study here to proestrus when expression is detected both within the stroma and epithelium (Fig. 7H). We note abnormal epithelial expression of Wnt-4 in the heterozygote and mutant uteri, both at birth and in the adult. At birth, Wnt-4 is expressed in both uterine mesenchyme and epithelium of the heterozygous and mutant animals (Fig. 7I,K). We observe little or no stromal expression of Wnt-4 in the adult heterozygote or mutant uterus at any stage of the estrous cycle (Fig. 7J,L). However, in contrast to wild-type mice, Wnt-4 is consistently expressed within the uterine epithelium regardless of the stage of the estrous cycle (Fig. 7J,L). Wnt-4 is expressed normally in the vaginal epithelium of both the mutant and heterozygote animals and is expressed in the stroma of the mutant oviduct (data not shown).
**DISCUSSION**

**Wnt-7a guides the development of the anteroposterior axis in the female reproductive tract**

We report here that loss of Wnt-7a activity results in posteriorization of the reproductive tract at gross, cellular and molecular levels. Evidence for posteriorization includes the lack of a discernible oviduct and changes in the uterine horn cytoarchitecture and gene expression patterns. Wnt-7a signaling in the uterus may act through a cascade that includes Wnt-5a (see Fig. 9). The adult mutant uterus exhibits a loss of hoxa-10 and hoxa-11 gene expression, coupled with the appearance of stratified epithelium and disorganized smooth muscle, which are features typical of the vagina. Hoxa-10 and hoxa-11 have been implicated in anteroposterior patterning in the FRT (Benson et al., 1996; Gendron et al., 1997). The loss of hoxa-10, and hoxa-11 expression from the stroma of the mutant uterus precedes the loss of Wnt-5a. The inability to maintain expression of uterine-specific hox genes may account for the intermediate appearance of the Wnt-7a mutant uterine horn. We propose that perinatal expression of hoxa-10 and hoxa-11 help establish segmentation and anteroposterior patterning. Thus the mutant FRT is compartmentalized along the anteroposterior axis to a degree and the uterine horns have some uterine characteristics. The postnatal loss of the uterine-specific gene expression mimics the normal expression pattern in the vagina. This is accompanied by the development of features in the mutant uterus that are similar to the vagina. These results suggest that Wnt-7a directly or indirectly maintains the expression of uterine-specific hox genes, thus identifying a role for Wnt-7a in the anteroposterior patterning of the FRT.

It seems likely that Wnt-7a may be responding to and enforcing positional signals dictated by clustered hox genes in the female reproductive tract. The sharp boundaries between the different regions of the female reproductive tract are likely to be due to the boundaries of hox gene expression. In combination with various members of the hox families, Wnt-7a expression would dictate that a tissue be either uterine or oviduct in nature. When Wnt-7a is not expressed, a default pathway may exist in the oviduct so that it forms uterine-like structures, just as the default pathway in the uterine horns is to take on a vaginal-like cytoarchitecture.

Additionally, we note changes in the boundaries of expression of Wnt-4 and Wnt-5a prior to the appearance of abnormalities in the mutant or heterozygous uterine horns. Levels of Wnt-7a expression may therefore define the limits of expression of other Wnt genes in the uterus. It has been proposed that the Drosophila wingless gene may define its...
pattern of expression by a process termed self-refinement. In this model, Wingless protein mediates the transcriptional repression of *wingless* in neighboring cells (Rulifson et al., 1996). Our results suggest that levels of Wnt-7a protein act to repress transcription of other Wnt genes in the same cells. There may be a threshold of Wnt-7a protein in the uterine epithelium that inhibits Wnt-4 and Wnt-5a expression. Wnt-4 and Wnt-5a expression shift to the epithelium in the homozygous and heterozygous Wnt-7a mouse. Thus, variations in the levels of Wnt-7a may modulate the dynamic expression pattern of Wnt-4 and Wnt-5a during the estrous cycle. Although Wnt-7a may define boundaries of Wnt gene expression by indirectly repressing transcription, in the absence of Wnt-7a expression, Wnt-5a expression in the uterus is not maintained. Thus, Wnt-7a is not required for the early expression of Wnt-5a in the uterus, but is required for its maintenance. However, Wnt-5a is expressed in the stroma of the mutant oviduct even though Wnt-7a expression is missing, indicating that Wnt-7a is not required for the induction and maintenance of Wnt-5a in the oviduct region. The differences between gene expression patterns in the different regions of the FRT may reflect the expression of other regulators that specify positional identity such as the HOM-C genes.

Wnt-7a is expressed throughout the epithelium at birth, but becomes restricted to the uterine luminal epithelium postnatally (Fig. 1; C. Miller et al., unpublished data). The timing of loss of Wnt-7a from the vaginal epithelium corresponds to the onset of epithelial cytodifferentiation (Cunha, 1976b). Vaginal epithelium becomes stratified and unresponsive to inductive signals from uterine mesenchyme. Wnt-7a appears necessary for epithelium to respond to uterine mesenchyme, thus loss of Wnt-7a may lead to vaginal cytodifferentiation. Loss of Wnt-7a expression in the epithelium of the mutant uterine horns results in the tissue mimicking a vaginal-like fate. The normal postnatal decline in vaginal Wnt-7a expression is accompanied by a decline in stromal Wnt-5a levels suggesting that downregulation of both Wnt genes is required for vaginal development.

**Wnt-7a is involved in radial axis patterning in the uterus**

Wnt-7a is required for radial patterning in the uterus as well as for setting up the anteroposterior axis. One major difference between wild-type and mutant uteri is the presence of endometrial glands, which differentiate from luminal epithelium shortly after birth (Cunha, 1976b). We do not observe glands in the mutant uterine horn. The lack of glands in the mutant mice may explain the observed infertility of mutant females (Parr and McMahon, 1995). The importance of uterine glands in fertility is demonstrated by the leukemia inhibitory factor (LIF) mutant mouse. LIF is expressed in uterine glands and is necessary for implantation (Bhatt et al., 1991). Female mice with a targeted deletion of the LIF gene have phenotypically normal uteri but are infertile (Stewart et al., 1992).

Uterine glands are induced in Wnt-7a heterozygous mice; however, higher levels of Wnt-7a expression are required to control glandular hyperplasia. Wnt-7a expression in the uterine epithelium likely stimulates the mesenchyme to induce uterine glands in the Müllerian epithelium (see Fig. 9). Wnt-7a could be signalling to Wnt-5a or a currently unidentified factor in the mesenchyme to promote the formation of glands. Data presented here do not rule out the possibility that glands may form in response to unidentified factors in the mesenchyme that may require stromal expression of Wnt-5a.

**Wnt-7a plays a critical role in uterine smooth muscle patterning**

We observe smooth muscle disorganization in the Wnt-7a mutant uterine horn, which becomes more pronounced during late postnatal development. Newborn uterine mesenchyme differentiates into smooth muscle and stroma. It has been noted previously that smooth muscle formation in uterine mesenchyme is dependent upon the presence of epithelium. Grafts of uterine mesenchyme alone showed little to no smooth muscle (Cunha et al., 1989). We have repeated these experiments and, in contrast, we observe many smooth muscle cells in theserafts using a probe to *SMMHC*, but the cells appear to be scattered throughout the mesenchyme and not organized into layers (C. Miller et al., unpublished data). We suggest that Wnt-7a maintains the organization of the smooth muscle in the uterus and maintains the stroma-smooth muscle boundary. Whether the apparent increase in smooth muscle in the mutant uterus is due to stromal cells becoming smooth muscle cells or due to smooth muscle cell proliferation is unclear. Since Wnt-7a is expressed exclusively within the

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**Fig. 7.** Wnt-7a expression in the uterus sets up boundaries of expression for other Wnt genes and is important for maintenance of uterine genes. Adjacent or neighboring sections of wild-type, heterozygous and mutant uteri were hybridized for Wnt-5a and Wnt-4. The photomicrographs are composites of the phase images and dark-field silver grains (red) to allow for direct comparison of tissue identity and morphology. The luminal epithelium in each section is denoted by double arrows. (A-F) Sections hybridized for Wnt-5a. A neonatal wild-type uterus hybridized for Wnt-5a contains transcripts primarily in the mesenchyme (A). (B) A section of adult uterus with transcripts detectable primarily in the stroma. Little expression is observed in the epithelium or in the smooth muscle layers (m). (C) A section of a neonatal heterozygous uterus with labelling seen both in the mesenchyme and in the epithelium, although little expression is detected in the forming smooth muscle (m). (D) Wnt-5a expression in adult heterozygous uterus. Transcripts are detectable both in the uterine stroma, luminal and glandular epithelium (g). Neonatal Wnt-7a mutant uteri have the same pattern of expression as the heterozygote: transcripts are detectable in both the mesenchyme and the epithelium of the uterine horn (E). In the adult mutant uterus, there is no detectable expression of Wnt-5a in the stroma (s) or the epithelium (F). (G-L) Sections hybridized for Wnt-4. Neonatal wild-type uteri show Wnt-4 transcripts, which are restricted to the mesenchyme of the uterine horn (G). (H) An adult wild-type uterus during proestrus with labelling seen in the epithelium and the stroma adjacent to the epithelium (s). Neonatal heterozygote uteruses shows transcripts primarily in the mesenchyme but also in the epithelium at low levels (I). (J) An adult heterozygous uteruses hybridized for Wnt-4 showing expression primarily in the uterine luminal epithelium. (K) Null neonatal uterine horn shows Wnt-4 transcripts in the mesenchyme and the epithelium. (L) The adult mutant uterus contains Wnt-4 transcripts only within the epithelium of the uterine horn. No detectable labelling is observed in the uterine stroma (s). (M) A schematic representation of the changes in Wnt gene expression in the heterozygote and mutant uteri compared to the wild-type uteruses. The upper row of diagrams indicates the neonatal state while the lower level indicates the situation in adult tissues. Scale bar, 100 μm (A,C,E,F,G,I,K,L); 200 μm (B,D,H,J).
epithelium, its effects on smooth muscle are likely mediated through a molecule in the stroma. Wnt-5a may play a role in this process since its expression in the mutant stroma declines at a time coincident when the smooth muscle phenotype becomes evident. The roles of Wnt-5a in the uterus are currently being addressed utilizing Wnt-5a mutant mice.

Wnt gene expression directs uterine cytodifferentiation

We show that Wnt genes play a key role during postnatal female reproductive tract development, and in the maintenance of adult uterine function. It has been noted that morphogenesis and cytodifferentiation in the FRT occur in response to steroid hormones (Cunha, 1976a). Wnt genes are responsive to changes in the levels of sex steroids both in the mouse mammary gland (Gavin and McMahon, 1992; Weber-Hall et al., 1994) and the female reproductive tract (C. Miller et al., unpublished data; Pavlova et al., 1994). Wnt genes play roles in cell-cell communication, therefore, they may mediate the action of steroid hormones in these tissues. Although the Wnt-7a mutant uterine phenotype is more severely affected than the estrogen receptor knockout (ERKO) mouse uterus, the two have some similarities: both are hypoplastic with reduced amounts of stroma and endometrial glands (Lubahn et al., 1993). It has been noted that prolonged exposure to estrogen results in endometrial glandular hyperplasia (Gusberg, 1947), which we observe in the Wnt-7a heterozygote uterus. It is possible that expression of steroid hormone receptors is altered in the Wnt-7a mutant uterus. Comparison of the effects of sex hormones in the uterus with the phenotypes observed in the Wnt-7a heterozygote and mutant uteri, along with the changes in Wnt-4 and Wnt-5a expression in the estrous cycle, suggests that Wnt gene activity may indeed mediate the effects of sex hormones in the uterus. Therefore, not only do the Wnt genes play a critical role maintaining the correct anteroposterior and radial programs of the female reproductive tract, but likely participate in the hormonally mediated mesenchymal-epithelial signaling events that govern the adult uterine function.

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