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

Cary Miller and David A. Sassoon*

Brookdale Center of Developmental and Molecular Biology, One Gustave Levy Place, Box 1126, New York, NY 10029, USA
*Author for correspondence (e-mail: dsassoo@smtplink.mssm.edu)

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 *hoxd* gene cluster is expressed 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 uterus and the uterus 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 disints/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) (Bigsby 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
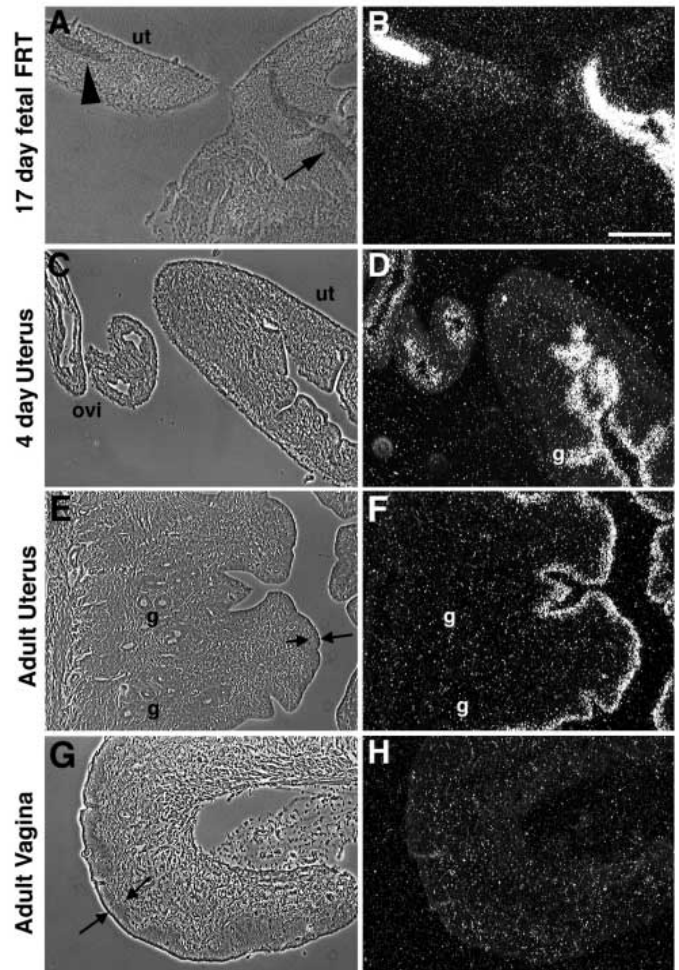
Fig. 1. *Wnt-7a* is expressed dynamically in the developing and adult female reproductive tract. The panels show the respective bright- and dark-field images of sections hybridized with a probe corresponding to *Wnt-7a*. (A,B) Longitudinal sections of a 17 d.p.c. female reproductive tract. *Wnt-7a* is 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.

Fig. 2. The *Wnt-7a* mutant female
reproductive tract shows changes in
morphology. (A-C) Adult wild-type,
Wnt-7a heterozygous and mutant
female reproductive tracts,
respectively, during the proestrus
stage of the estrous cycle. There are
no obvious differences in
morphology between the wild-type
and heterozygous reproductive
tracts. Note that the mutant
reproductive tract is shorter than the
wild-type tract and the mutant
uterine horns (ut) are much thinner
in diameter than the wild-type or
heterozygous uteri. We note some
variability in the degree of oviduct
coiling between samples. The
appearance and size of the ovary
(ov) is normal.
(D-G) Photomicrographs of
hematoxylin- and eosin-stained

sections through the oviduct regions of the respective mice. (D,E) Oviducts of the wild-type and heterozygous mice, respectively. The histology
is indistinguishable. They contain highly convoluted epithelial folds, very little underlying connective tissue, and the loops are surrounded by a
thin layer of smooth muscle. (F) A section through the anterior uterine horn of the reproductive tract from C as marked by the dashed line. The
cytoarchitecture of this region is reminiscent of normal uterus and does not resemble the oviducts seen in D and E. The epithelium is not highly
convoluted, and there is a thick layer of underlying stroma and smooth muscle. Additionally, we observe numerous glands in the mutant oviduct
while no glands are apparent in the wild-type or heterozygous oviducts. We observe significant variability in the degree of oviduct coiling and
cell morphology in the mutant. (G) A mutant oviduct, which has cytoarchitecture similar to that seen in the wild-type oviduct. Scale bar, 4 mm
(A-C), 200 µm (D-G).
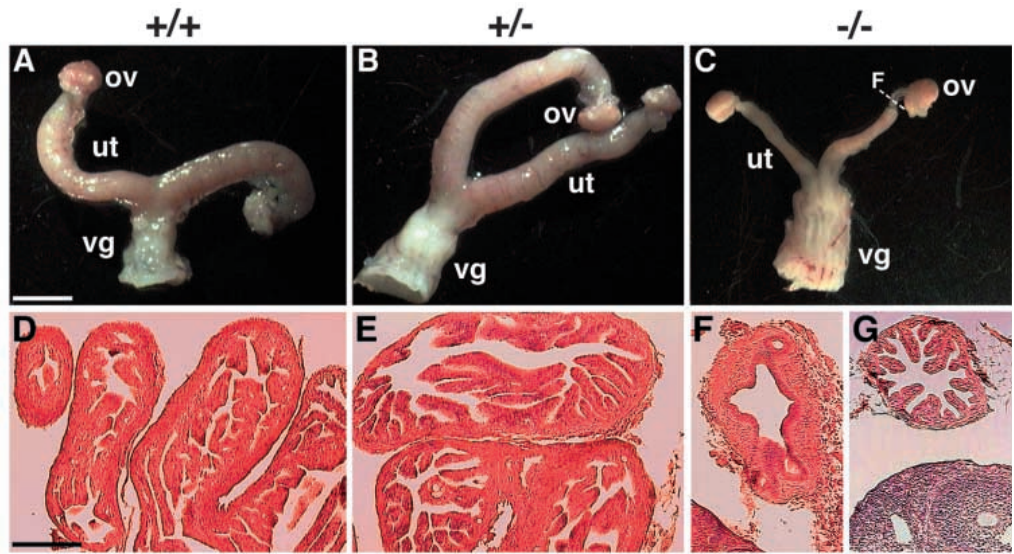
the *Wnt-7a* mutant uterine horns. The appearance of the mutant
uterus combines features of the uterus and vagina. Normal
vagina has stratified or multilayered epithelium, thin stroma
and a layer of disorganized smooth muscle bundles (Fig. 3D).
The mutant uterus has a multilayered epithelium, a relatively
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,
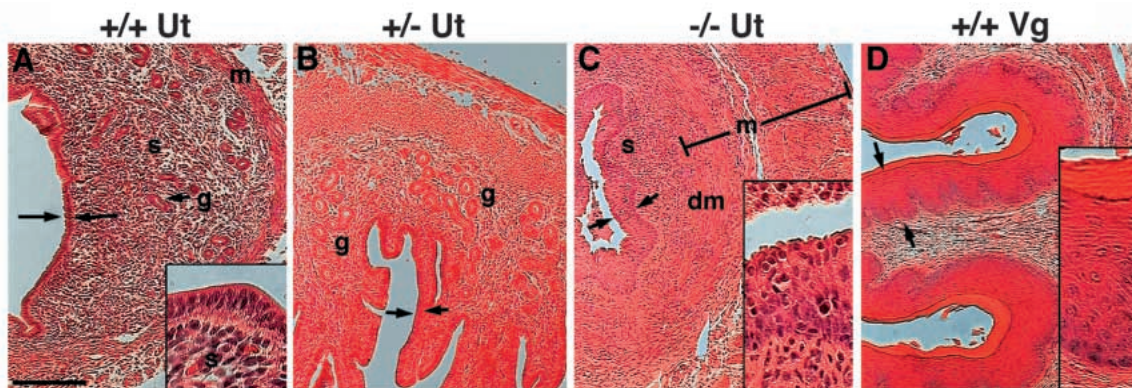
Fig. 3. *Wnt-7a* mutant uterine morphology acquires an intermediate cellular phenotype. (A) A cross-section through adult wild-type uterine
horn showing 2 layers of smooth muscle (m), a thick stroma (s) populated by glands (g), and lined by simple columnar epithelium (double
arrows). Inset is a high magnification of the luminal and glandular epithelium showing that the luminal epithelium is simple columnar. (B) A
section through a heterozygous uterine horn. The tissue cytoarchitecture is identical to that of the wild-type animal except there is an excess of
uterine glands (g). (C) A cross-section through mutant uterine horn. Note that smooth muscle (m) is thicker in the mutant than in the wild-type
uterus. The inner layer of smooth muscle is not only thicker, but has become highly disorganized and irregular (dm). In contrast to the wild-type
uterus, there is no sharp boundary between the stroma and smooth muscle. The thickness of the stroma (s) is greatly reduced in the mutant and
is not populated by any discernible glands. The epithelium of the uterine horn is clearly stratified (double arrows). The inset shows that the
epithelium consists of 6-7 layers of cells versus the 1 layer of cells seen in the wild-type uterus. (D) A transverse section through adult vagina.
The stratified, keratinized epithelium is denoted by double arrows. Scale bar, 200 µm and 50 µm (insets).

Fig. 4. (A,B) Wild-type uterus, (C,D) mutant uterus or (E,F) wild-type vagina, hybridized with a cRNA probe corresponding to *SMMHC*. (A,B) We detect 2 layers of smooth muscle in the wild-type uterine horn. (C,D) Two layers of smooth muscle are also discernible in the mutant uterine horn, but the inner layer of smooth muscle has formed into irregular bundles interspersed by stromal cells. This contrasts with the compact circular layer of smooth muscle observed in the wild-type mouse. (E,F) *SMMHC* transcripts are detected in irregular bundles on the periphery of the vagina. Note the similarity in appearance to the inner layer of smooth muscle bundles in the mutant uterine horn (C). The anteroposterior axis is indicated. Scale bar, 400 μm (A,C,E), and 200 μm (B,D,F).
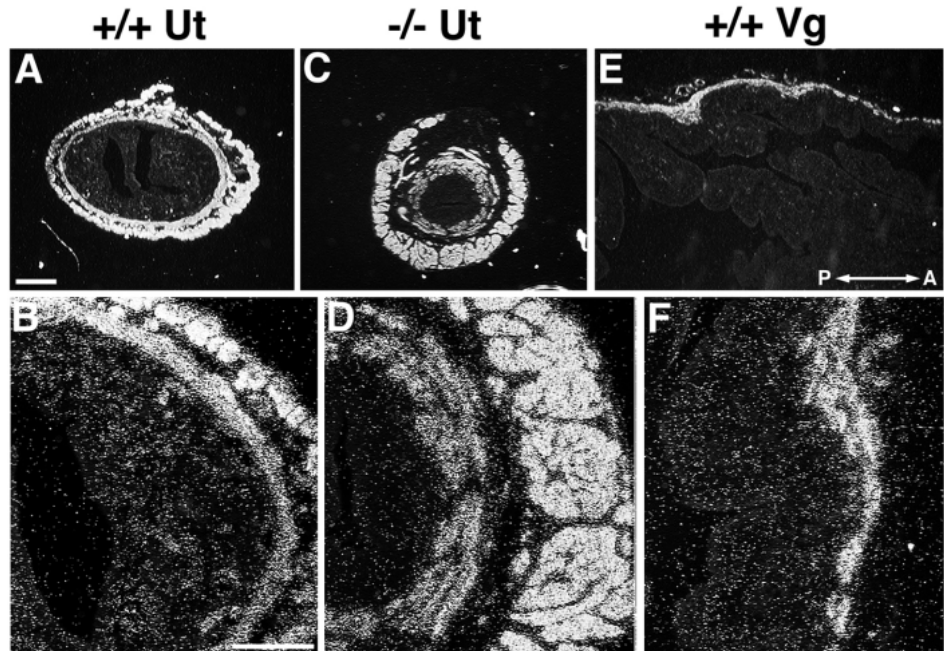
we performed in situ hybridization using a riboprobe corresponding to *smooth muscle myosin heavy chain* (*SMMHC*; Fig. 4). We detect two layers of smooth muscle in both the wild-type and mutant uteri (Fig. 4A-D); however, there are changes in the distribution of smooth muscle in the mutant uterus. The 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 and little or no stroma remains (Fig. 5H). We detect the formation of uterine glands (g) in the control graft from the pregnant host (Fig. 5E), whereas no glands are observed in the mutant tissue 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
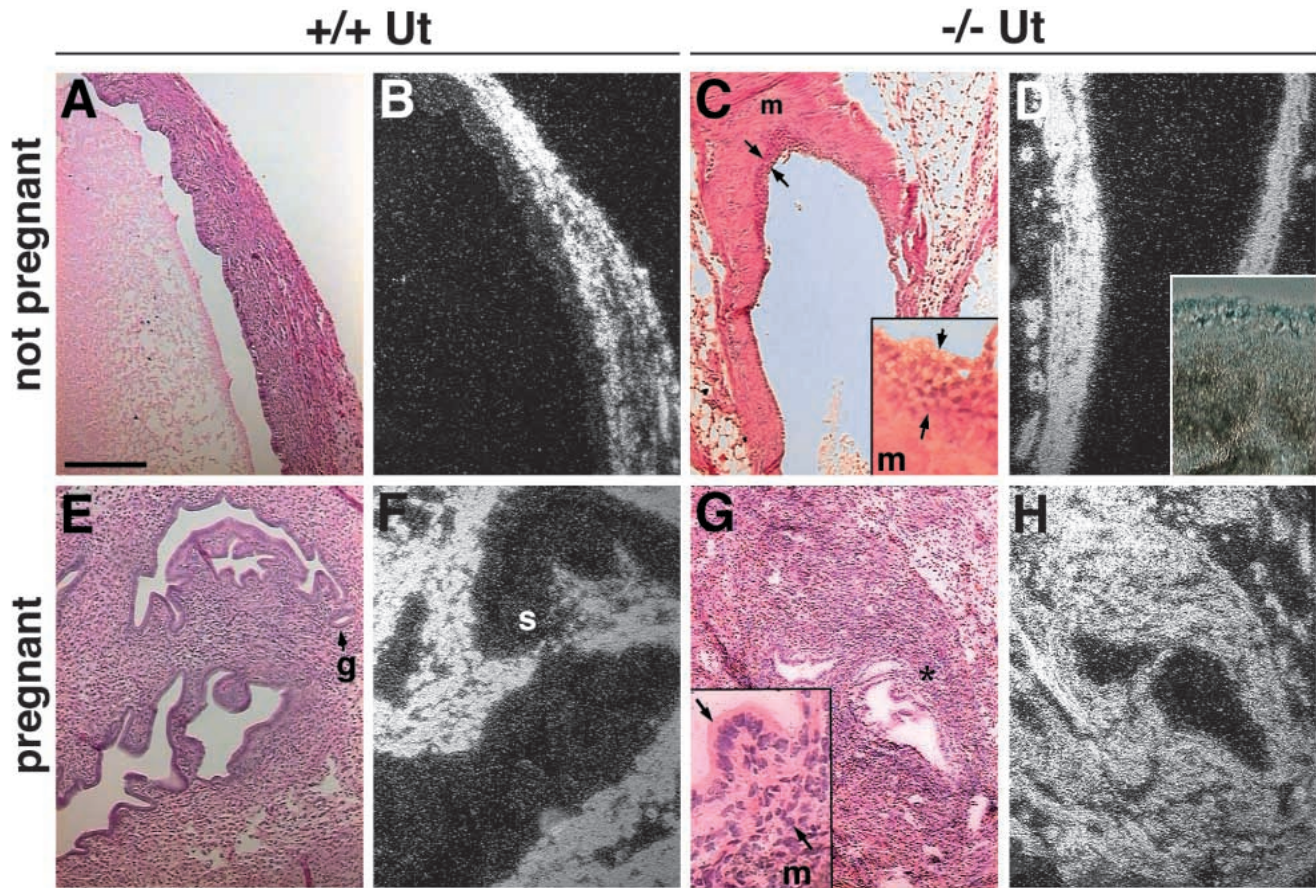
Fig. 5. Loss of *Wnt-7a* in the uterus is responsible for the mutant phenotype. Intact neonatal uterine horns from wild-type and *Wnt-7a* mutant mice were grafted underneath host kidney capsules. The grafts were grown for 3 weeks in a pregnant or non-pregnant host. Gland formation is only observed in the tissues grown in pregnant hosts. Control and mutant grafts, along with the host reproductive tracts, were analyzed. Hematoxylin- and eosin-stained sections or nearby sections hybridized for *SMMHC* are shown. (A) Wild-type tissue from non-pregnant hosts contains simple columnar epithelium, a layer of stroma and a layer of smooth muscle. (B) The smooth muscle is not adjacent to the epithelium. (C) Mutant tissues contain epithelium which varies from simple cuboidal to stratified, an abundance of smooth muscle (m), and little stroma. (C,D) The smooth muscle is immediately adjacent to the epithelium. (E) We observe the formation of glands (g) in the wild-type tissue grown in a pregnant host. (E,F) The tissue has simple columnar epithelium surrounded by a thick layer of stroma (s), and smooth muscle. (G,H) The mutant graft from the pregnant host is comprised almost entirely of smooth muscle. There are only smooth muscle cells detectable adjacent to the epithelium. The epithelium is multilayered, disorganized and clearly abnormal (see inset), and no glands are detected. Scale bar, 200 μm (A-G), and 50 μm (insets).

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).

Wnt-7a maintains expression of *hoxa* genes in the uterine horn

The observation that the changes in the mutant uterine radial axis resemble posterior homeotic transformations in the FRT led us to examine the expression of molecules previously implicated in anteroposterior patterning: *hoxa-10* and *hoxa-11*. *Hoxa-10* and *hoxa-11* have similar patterns of expression in the neonatal uterine stroma (Fig. 8A, and Taylor et al., 1997). Stromal expression is maintained throughout adult life (Fig. 8B). In the *Wnt-7a* mutant uterus, *hoxa-11* is expressed in the neonate stroma (Fig. 8C), but expression becomes undetectable as the animals age and the changes in uterine cytoarchitecture become apparent (Fig. 8D). Finally, the loss of *hoxa-10* and *hoxa-11* expression precedes the loss of other uterine-specific genes (*Wnt-5a* and *Msx1*).

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
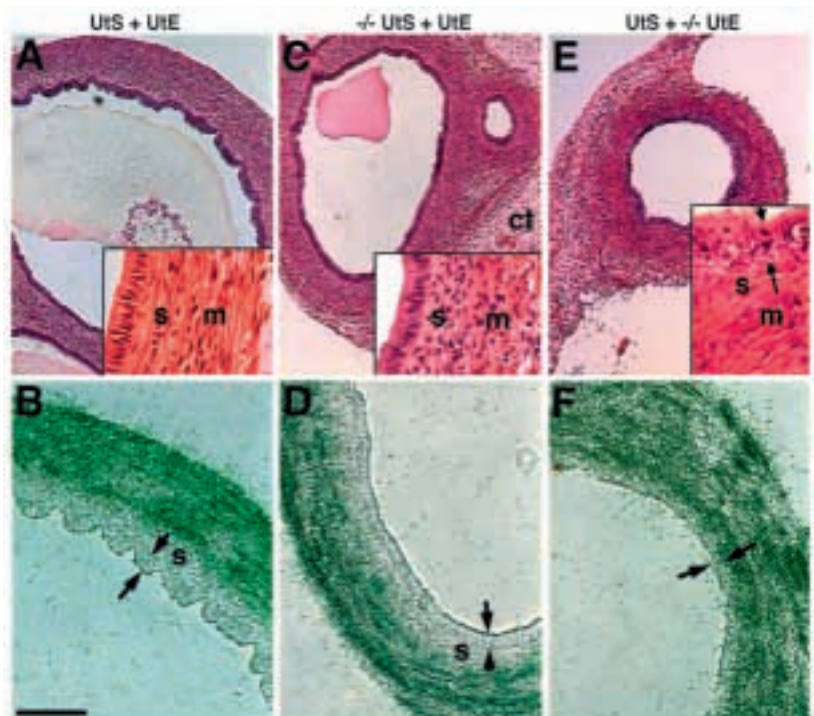
Fig. 6. Loss of *Wnt-7a* expression in the uterine epithelium is directly responsible for the phenotype observed in the *Wnt-7a* mutant mice. Recombinants between wild-type and mutant tissues are shown. The recombinants were grown under the kidney capsules of nude mice for 3 weeks. (B,D,F) Neighboring sections to A,C and E, which were hybridized with a probe corresponding to *SMMHC*. The photomicrographs are composites of the phase images and dark-field silver grains (green) to allow for direct comparison of tissue identity and morphology. The epithelia in B,D and F are denoted by the double arrows. (A) A recombinant composed of wild-type uterine stroma and epithelium. It contains simple columnar epithelium, a distinct layer of stroma (s) and a layer of smooth muscle (m). (B) The smooth muscle in this graft is separated from the epithelium by an intervening layer of stroma (s). (C) The recombination of mutant uterine stroma (−/−UtS) and wild-type uterine epithelium. The high magnification inset shows that it has normal cytoarchitecture. The graft is surrounded by connective tissue (ct), presumably derived from the uterine mesothelium. (D) A layer of stroma separates the epithelium and smooth muscle. (E) A recombinant composed of wild-type uterine stroma and mutant uterine epithelium (−/−UtE). The grafts contain excessive smooth muscle, little stroma (s) and stratified epithelium (double arrows). These features are shown at a higher magnification in the inset. (F) There is no distinct layer of stroma separating the epithelium and smooth muscle; *SMMHC* is expressed in the tissue directly adjacent to the epithelium. Scale bar, 200 μm (A,C,E), 100 μm (B,D,F) and 50 μm (insets).
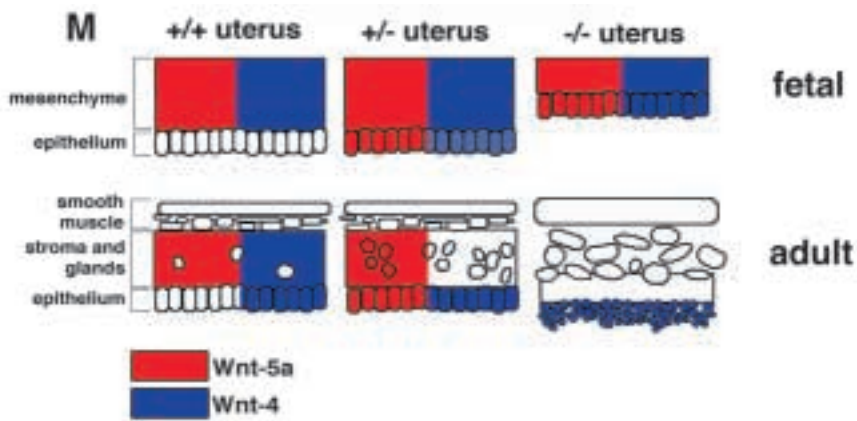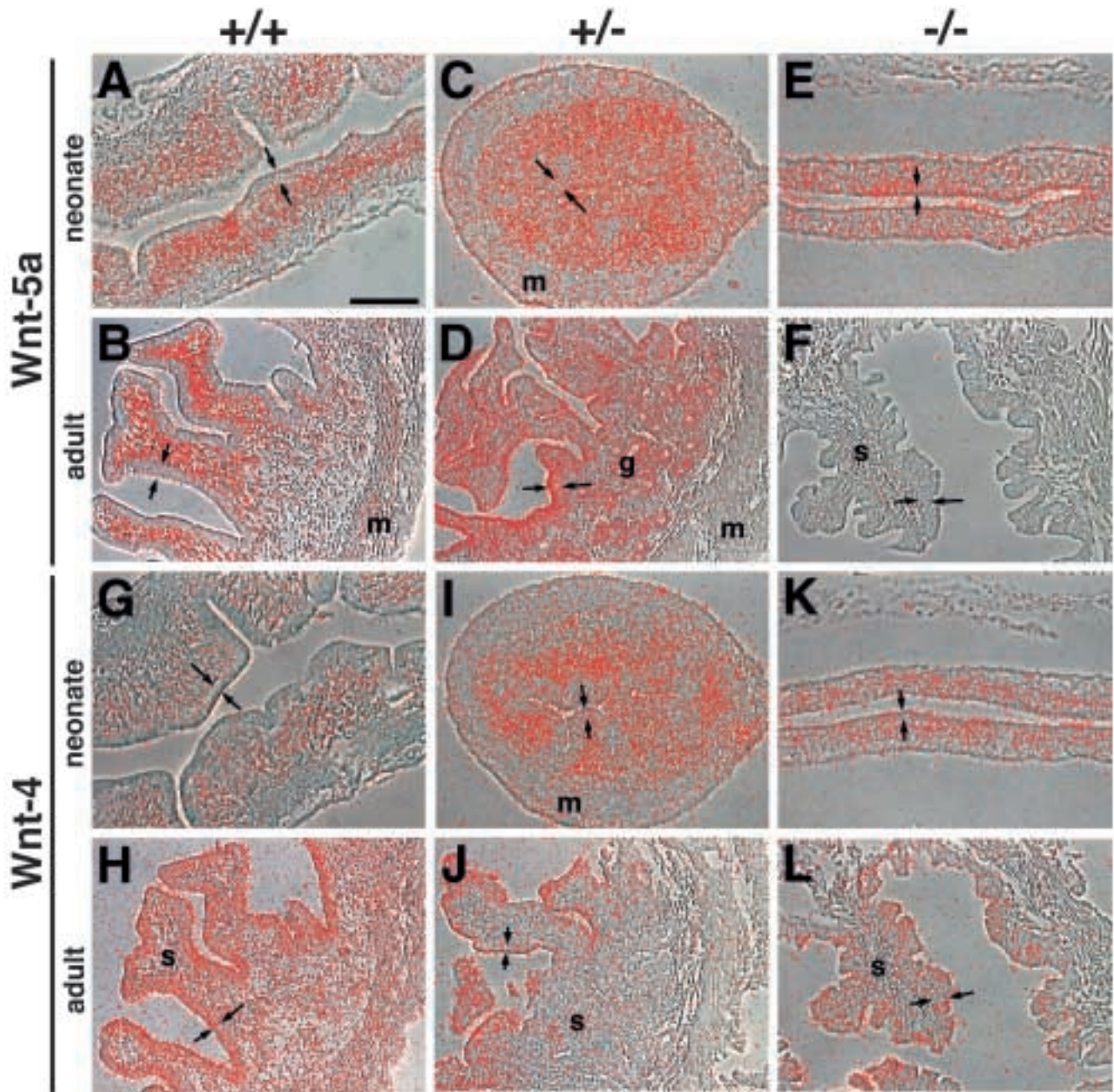
pattern of expression by a process termed self-refinement. In this model, Wingless protein mediates the transcriptional repression of *wingless* in neighboring cells (Rulifson et al., 1996). Our results suggest that levels of Wnt-7a protein act to repress transcription of other Wnt genes in the same 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

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 uterus shows transcripts primarily in the mesenchyme but also in the epithelium at low levels (I). (J) An adult heterozygous uterus 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 uterus. 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).

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 these grafts 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
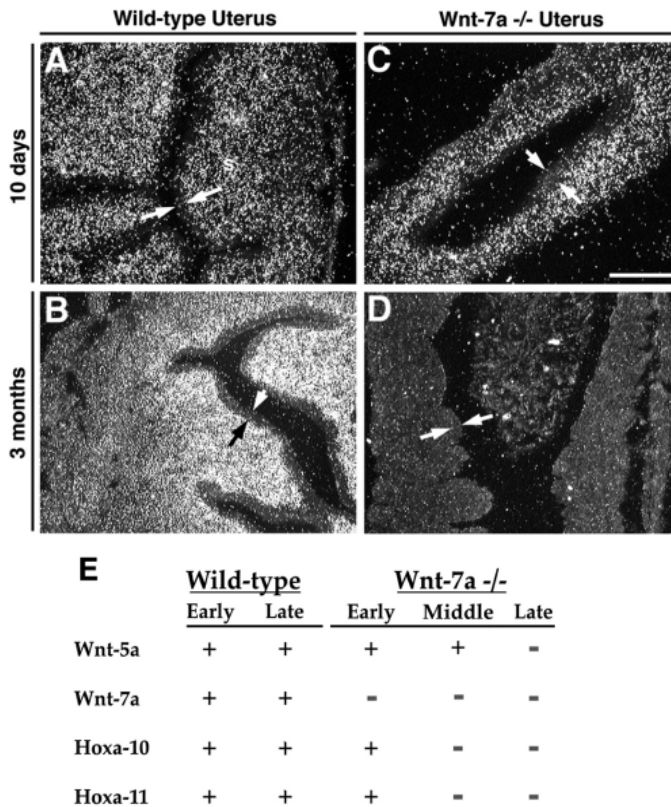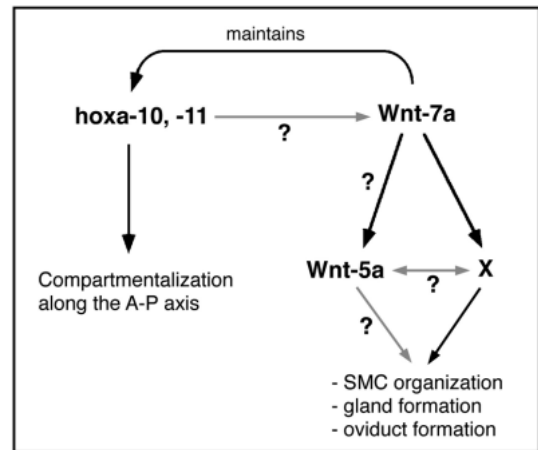
Fig. 8. *Wnt-7a* maintains the expression of uterine-specific *hoxa* genes. Dark-field sections of wild-type and *Wnt-7a* mutant uteri hybridized for *hoxa-11* are shown. The epithelium is denoted by the double arrows. In the presence of Wnt-7a, *hoxa-11* is expressed in the stroma both (A) during neonatal uterine development and (B) during adult life. (C) In the *Wnt-7a* mutant uterus, although we initially observe expression of *hoxa-11* in the stroma, we lose expression in the adult uterus (D). (E) The expression of Wnt and *hoxa* genes in the wild-type and *Wnt-7a* mutant uterus is summarized. Initially in the mutant uterus, we observe expression of *Wnt-5a*, as well as *hoxa-10* and *hoxa-11*. We note the loss of *hoxa-10* and *hoxa-11* from the uterine stroma (5-12 weeks) prior to noting changes in cell morphology. Additionally, we note that loss of *Wnt-5a* expression in the mutant uterus follows the loss of the *hox* genes (12-16 weeks). Scale bar, 100 μm (A,C), 200 μm (B,D).

Fig. 9. Model showing interactions between *Wnt-7a* and *hoxa* genes. *Wnt-7a* expression is required for gland formation, proper oviduct morphology and smooth muscle organization. *Wnt-7a* expression is required to maintain expression of *hoxa-10* and *hoxa-11* (this paper). *Hoxa-10* and *hoxa-11* expression have been implicated in the anteroposterior segmentation of the FRT (Benson et al., 1996; Gendron et al., 1997). Other interactions shown in light grey are tentative (see Discussion).

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.

REFERENCES

Alescio, T. and Cassini, A. (1962). Induction in vitro of tracheal buds by pulmonary mesenchyme grafted on tracheal epithelium. *J. Exp. Zool.* **150**, 83-94.

Alkema, M. J., Van der Lugt, N. M., Bobeldijk, R. C., Berns, A. and Van Lohuizen, M. (1995). Transformation of axial skeleton due to overexpression of *bmi-1* in transgenic mice. *Nature* **374**, 724-727.

Baker, N. E. (1987). Molecular cloning of sequences from *wingless*, a segment polarity gene in Drosophila: the spatial distribution of a transcript in embryos. *EMBO J.* **6**, 1765-1773.

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.

Bhatt, H., Brunet, L. J. and Stewart, C. L. (1991). Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. *Proc. Natn. Acad. Sci. USA* **88**, 11408-11412.

Bigsby, R. M., Cooke, P. S. and Cunha, G. R. (1986). A simple method for separating murine uterine epithelial and mesenchymal cells. *Am. J. Physiol.* **251**, E630-636.

Bradbury, J. M., Edwards, P. A., Niemeyer, C. C. and Dale, T. C. (1995). *Wnt-4* expression induces a pregnancy-like growth pattern in reconstituted mammary glands in virgin mice. *Dev. Biol.* **170**, 553-563.

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

Bui, T. D., Zhang, L., Rees, M. C., Bicknell, R. and Harris, A. L. (1997). Expression and hormone regulation of *Wnt2*, *3*, *4*, *5a*, *7a*, *7b* and *10b* in normal human endometrium and endometrial carcinoma. *Br. J. Cancer* **75**, 1131-1136.

Cunha, G. R. (1976a). Epithelial-Stromal Interactions in Development of the Urogenital Tract. *Int. Rev. Cytology* **47**, 137-94.

Cunha, G. R. (1976b). Stromal induction and specification of morphogenesis and cytodifferentiation of the epithelia of the Müllerian ducts and urogenital sinus during development of the uterus and vagina in mice. *J. Exp. Zool.* **196**, 361-370.

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**, 831-871.

Cygan, J. A., Johnson, R. L. and McMahon, A. P. (1997). Novel Regulatory interactions revealed by studies of murine limb pattern in *Wnt-7a* and *En-1* mutants. *Development* **124**, 5021-5032.

Daniel, C. W. and Silberstein, G. B. (1987). Postnatal development of the rodent mammary gland. In *The Mammary Gland Development, Regulation and Function.* (ed. M. C. Neville and C. W. Daniel). pp. 3-36. New York: Plenum Press.

Dollé, 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**, 1767-1777.

Gavin, B. J. and McMahon, A. P. (1992). Differential regulation of the Wnt gene family during pregnancy and lactation suggests a role in postnatal development of the mammary gland. *Mol. Cell Biol.* **12**, 2418-23.

Gavin, B. J., McMahon, J. A. and McMahon, A. P. (1990). Expression of multiple novel Wnt-1/int-1-related genes during fetal and adult mouse development. *Genes Dev.* **4**, 2319-32.

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.

Gusberg, S. B. (1947). Precursors of Corpus Carcinoma Estrogens and Adenomatous Hyperplasia. *Am. J. Obstetrics Gynecology* **54**, 905-927.

Horan, G. S. B., Ramirez-Solis, R., Featherstone, M. S., Wolgemuth, D. J.,Bradley, A. and Behringer, R. R. (1995). Compound mutants for the paralogous *Hoxa-4*, *Hoxb-4*, and *Hoxd-4* genes show more complete homeotic transformations and a dose-dependent increase in the number of vertebrae transformed. *Genes Dev.* **9**, 1667-1677.

Hsieh-Li, H. M., Witte, D. P., Weinstein, M., Branford, W., Li, H., Small, K. and Potter, S. S. (1995). *Hoxa-11* structure, extensive antisense transcription, and function in male and female fertility. *Development* **121**, 1373-1385.

Kratochwil, K. (1987). Tissue combination and organ culture studies in the development of the embryonic mammary gland. In *Developmental Biology:*

A Comprehensive Synthesis (ed. R.B. L. Gwatkin). pp 315-334; New York: Plenum Press.

Logan, C., Hornbruch, A., Campbell, I. and Lumsden, A. (1997). The role of Engrailed in establishing the dorso ventral axis of the chick limb. *Development* **124**, 2317-2324.

Lubahn, D. B., Moyer, J. S., Golding, T. S., Couse, J. F., Korach, K. S. and Smithies, O. (1993). Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc. Nat. Acad. Sci., USA* **90**, 11162-11166.

Lucas, F. R. and Salinas, P. C. (1997). WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. *Dev. Biol.* **192**, 31-44.

McMahon, A. P., Joyner, A. L., Bradley, A. and McMahon, J. A. (1992). The mid-hindbrain phenotype of *wnt-1⁻/wnt-1⁻* mice results from a stepwise deletion of engrailed-expressing cells by 9.5 days post-coitum. *Cell* **69**, 581-595.

Miano, J. M., Cserjesi, P., Ligon, K. L., Periasamy, M. and Olson, E. N. (1994). Smooth muscle myosin heavy chain exclusively marks the smooth muscle lineage during mouse embryogenesis. *Circ. Res.* **75**, 803-812.

Moon, R. T., Brown, J. B. and Torres, M. T. (1997). WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet.* **13**, 157-162.

Muragaki, Y., Mundlos, S., Upton, J. and Olsen, B. R. (1996). Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. *Science* **272**, 548-51.

Parr, B. A. and McMahon, A. P. (1995). Dorsalizing signal *Wnt-7a* required for normal polarity of D-V and A-P axes of mouse limb. *Nature* **374**, 350-353.

Parr, B. A., Shea, M. J., Vassileva, G. and McMahon, A. P. (1993). Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. *Development* **119**, 247-61.

Pavlova, A., Boutin, E., Cunha, G. and Sassoon, D. (1994). *Msx1* (*Hox-7.1*) in the adult mouse uterus: cellular interactions underlying regulation of expression. *Development* **120**, 335-346.

Redline, R. W., Williams, A. J., Patterson, P. and Collins, T. (1992). Human HOX4E: a gene strongly expressed in the adult male and female urogenital tracts. *Genomics* **13**, 425-430.

Rulifson, E. J., Micchelli, C. A., Axelrod, J. D., Perrimon, N. and Blair, S. S. (1996). Wingless refines its own expression domain on the Drosophila wing margin. *Nature* **384**, 72-74.

Sakakura, T., Nishizuka, Y. and Dawe, C. J. (1976). Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. *Science* **194**, 1439-1441.

Sassoon, D. and Rosenthal, N. (1993). Detection of messenger RNA by in situ hybridization. *Methods Enzymol.,* **225**, 384-404.

Satokata, I., Benson, G. and Maas, R. (1995). Sexually dimorphic sterility phenotypes in *Hoxa10*-deficient mice. *Nature* **374**, 460-463.

Song, K., Wang, Y. and Sassoon, D. A. (1992). Expression of *Hox-7.1* in myoblasts results in inhibition of differentiation and induces cell transformation. *Nature* **360**, 477-481.

Stark, K., Vainio, S., Vassileva, G. and McMahon, A. P. (1994). Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by *Wnt-4*. *Nature* **372**, 679-683.

Stewart, C. L., Kaspar, P., Brunet, L. J., Bhatt, H., Gadi, I., Kontgen, F. and Abbondanzo, S. J. (1992). Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. *Nature* **359**, 76-79.

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.

Vogel, A., Rodriguez, C., Warnken, W. and Izpisua Belmonte, J. C. (1995). Dorsal cell fate specified by chick Lmx1 during vertebrate limb development. *Nature* **378**, 716-720.

Weber-Hall, S. J., Phippard, D. J., Niemeyer, C. C. and Dale, T. C. (1994). Developmental and hormonal regulation of Wnt gene expression in the mouse mammary gland. *Differentiation* **57**, 205-214.

Wessels, N. K. (1970). Mammalian lung development: interaction in formation and morphogenesis of tracheal bud. *J. Exp. Zool.* **175**, 455-466.