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Three-Dimensional Structure of the Developing Mouse Genital Ridge [and Discussion]

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Three-dimensional structure of the developing mouse genital ridge

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SUMMARY

We are interested in understanding how the field of cells which forms the gonad arises, and how the testis-determining gene, *Sry*, controls morphogenesis of a testis within this field of cells. To appreciate changes in the three-dimensional structure of the mouse genital ridge at this time in development, whole-mount genital ridges taken from male and female embryos over the developmental period when the initiation of testis cord morphogenesis takes place, were stained with an antibody against laminin. Samples were visualized using confocal microscopy. Anti-laminin illuminates the elaborate array of mesonephric duct and tubules which occupy the cranial two-thirds of the mesonephros at the earliest timepoint. This complex structure gradually regresses as testis cords form in male gonads. No structural organisation is recognized by this antibody in the female gonadal region during this period. Confocal sections in the Z-plane reveal continuous cellular connections between 3–6 mesonephric tubules and the gonadal primordium. These cellular bridges are present in male and female gonads, so they do not depend on the expression of *Sry*. We consider the possibility that these bridges constitute the pathways of the founder cells of the gonadal primordium.

1. INTRODUCTION

In eutherian mammals, sex determination is defined as the initiation of testis development because all secondary sexual differentiation is dependent upon and subsequent to this initial event controlled by a gene on the Y chromosome, *Sry* (Gubbay *et al*. 1990; Sinclair *et al*. 1990; Lovell-Badge 1992). It has been shown, in transgenic mice, that the presence of this single gene on an otherwise XX background leads to the initiation of testis-cord formation and complete sex reversal (Koopman *et al*. 1991). On the other hand, deletion of this gene in an XY mouse leads to development of a fertile female (Lovell-Badge & Robertson 1990; Gubbay *et al*. 1992). Expression of *Sry* occurs in a narrow window of time immediately before the divergence of development between male and female gonadal primordia is apparent at the light microscope level (Hacker *et al*. 1995) (see figure 1). Expression is limited to the gonadal portion of the urogenital ridge at 11.5 days *post coitum* (DPC) as shown by *in situ* hybridization (Koopman *et al*. 1990). Expression of *Sry* is thought to be required only in the pre-Sertoli cell based on genetic analysis of XX↔XY chimeras (Burgoyne & Palmer 1993).

It is believed that the testis-determining gene exerts its dominant effect by acting as a genetic switch to divert development of the indifferent gonad from the female pathway to the male pathway of development (McLaren 1988). *Sry* is a member of a family of DNA-binding proteins, and is thought to act as a transcription factor, regulating a cascade of gene expression required to trigger testis development (Sinclair *et al*. 1990; Harley *et al*. 1992; Pontiggia *et al*. 1994).

At 11.5 DPC, male and female gonads are identical, however, a cursory comparison of male and female gonads at 12.5 DPC reveals a larger gonad in males than in females, as well as a cellular organisation into testis-cord structures specific to male gonads. Reorganisation of the cells of the indifferent gonad into testis cords can be visualized in male gonads within ~ 36 h of the initiation of *Sry* expression. Expression of *Sry* must trigger multiple new pathways of gene expression which lead to these changes in cell-cell signalling, extracellular matrix deposition, cell associations, cell movements and cell proliferation. This primary event in sex determination does not occur in females or in XY mice in which the *Sry* gene has been deleted (Lovell-Badge & Robertson 1990; Gubbay *et al*. 1992). In the absence of *Sry* expression, development proceeds along the ovarian pathway: the earliest (follicular) structures appear approximately 4 days later (16.5 DPC).

The origin of the cells which contribute to the gonadal tissue, where *Sry* is expressed at 11.5 DPC, has long been a controversial issue. The urogenital system arises from the intermediate mesoderm which lies between the somites and the lateral plate mesoderm. In mouse, at about 9 DPC this mesoderm develops as a pair of mounds on the coelomic surface of the dorsal side of the embryo, lying on either side of the neural tube and dorsal aorta, and running the full length of the body cavity. The most anterior region of this mesodermal field, the pronephros, disappears soon after it arises in vertebrates, and has no known function. The gonad develops within the mid-section of this field, the mesonephros. The most posterior section, the metanephros, gives rise to the definitive kidney.

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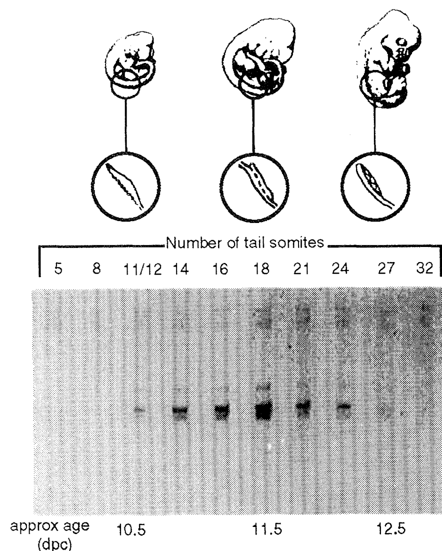

Figure 1. Diagram indicating *Sry* expression relative to the stage of the developing gonad. RNase protection experiments (Hacker *et al.* 1995) identify the *Sry* urogenital ridge transcript specifically in the male gonad between the 11–27 tail-somite stages, during the narrow window of development in which testis cords begin to organise.

The mesonephric duct first appears within the mesonephros in segments at the anterior end, eventually extending as a continuous duct, and ending in the cloaca at the posterior end of the embryo. The differentiation of the kidney is initiated by the growth of the ureteric bud near the caudal end of the mesonephric duct where it has joined the cloaca, back into the metanephric region. Growth of this epithelial duct induces the organisation of kidney tubules within the metanephric region (Grobstein 1956; Wartiovaara *et al.* 1974).

The gonad begins to condense as a distinct tissue at about 10.5 DPC in the mouse, and is first visible in scanning electron micrographs as a region which can be distinguished from the mesenchymal tissue on the inside of the mesonephros (Capel & Lovell-Badge 1993). In the mesonephric region, a complex set of S-shaped tubules form, connecting with the mesonephric duct, and extending toward the gonad. It has been argued on the basis of data from electron microscopy, that these tubules contribute cells directly to the gonad. Common staining characteristics in electron micrographs have been observed between cells of the mesonephric tubules and the colonizing cells of the early gonad, suggesting that the pre-Sertoli cell is contributed from the mesonephric tubule cells (Merchant-Larios 1979; Upadhyay *et al.* 1979; Zamboni and Upadhyay 1982; Satoh 1985; Kanai *et al.* 1989; Wartenberg *et al.* 1991). Recent support for this mechanism is suggested by analogy to the case in kidney where cell-marking studies have demonstrated that epithelialized cells from the ureteric bud actually migrate away from the bud, passing through a mesenchymal phase before re-epithelializing in condensing kidney tubules (Herzlinger *et al.* 1993).

Organ culture experiments have also indicated that cells from the mesonephros move into the gonad and participate in cord formation, but in this case it is the peritubular myoid, endothelial, and perhaps Leydig cell types which are contributed. If the mesonephros and gonad are separated by a permeable membrane, cord formation does not occur (Buehr *et al.* 1992; Merchant-Larios *et al.* 1993, also see results detailed in this paper).

We are interested in characterizing population cell movements in the male gonad during testis organogenesis. We would like to understand how these cell movements are related to the expression of *Sry*, and how they are important for organogenesis of a testis. For example, the expression of *Sry* may initiate the signal for this cell movement to occur or, alternatively, *Sry* expression may render the cells already present in the gonad responsive to the influx of cells from other regions. Distinguishing between these possibilities is important for understanding how *Sry* acts to initiate the process of cord formation in males. As a step toward achieving a better understanding of the 3-D relation of the cells of the mesonephros and gonad during this period of development, gonads between 11.5 DPC and 13.5 DPC were labelled as whole-mounts with a polyclonal antibody to laminin. The samples were then visualized in a confocal microscope, using a secondary antibody conjugated to fluorescein isothiocyanate. This antibody illuminates the basement membrane of cells forming the mesonephric duct and tubules, as well as the basal lamina of testis cords, and of the coelomic epithelium as these structures appear in the male gonad. This system has facilitated visualization of cellular connections between the mesonephros and gonad during the early phases of gonadal morphogenesis.

2. MATERIALS AND METHODS

(*a*) *Tissues*

Gonads used for the whole-mount immunohistochemistry were collected from timed matings of CD1 mice. The developmental stage was defined by counting noon of the day the plug was found as 0.5 DPC. 11.5 DPC gonads were dissected between 11h00 and 14h00, when tail somite number ranged between 18–20. 12.0 DPC gonads were dissected between 22h00 and 24h00 (tail somite = 22–24). 12.5 DPC (tail somite = 27–28) and 13.5 DPC were dissected at approximately 12h00. For more accurate staging between 11.5–12.5 DPC, somites were counted posterior to the hind limb bud according to the method of Hacker *et al.* (1995). Amnions were prepared as previously described (Palmer & Burgoyne 1991) for stages where sex could not be determined by inspection in the light microscope (before 12.5 DPC), and examined for the presence or absence of a chromatin body marking the inactive X in female embryos.

(*b*) *Whole-mount immunohistochemistry*

The dissected gonads were washed several times in PBS, fixed overnight at 4 °C in 4 % paraformaldehyde, washed in PBS and treated with 0.1 M glycine/PBS for

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30–60 mins at room temperature (RT) to quench the reaction. Samples were blocked for 4–10 h in blocking buffer (5% BSA/0.1% TX-100/PBS), and incubated on a rocker at 4 °C overnight with polyclonal anti-laminin antibody raised in rabbit against purified laminin from the EHS tumour, kindly provided by H. P. Erickson (used at 1:200 dilution in the blocking buffer). The samples were then washed 4–5 h at 4 °C in washing buffer (1% BSA/0.1% TX-100/PBS), changing the washing buffer once, followed by two 1 h washes at RT. The secondary antibody goat anti-rabbit IgG, conjugated to fluorescein isothiocyanate (Gibco-BRL), was incubated O/N at 4 °C (1:100 dilution in blocking buffer). The gonads were then washed as before. Samples were mounted in DABCO (Kodak) and sealed in chambers made by suspending a coverslip above the slide with ~ 100–200 μ florist clay 'posts'. Images were collected on a Zeiss LSM410 confocal microscope.

3. RESULTS

(*a*) *Comparison of male and female genital ridges at stages 11.5–13.5 DPC*

Genital ridges were dissected from staged embryos between 11.5–13.5 DPC. Samples were stained with a polyclonal antibody α-laminin and labelled with a secondary antibody conjugated to fluorescein isothiocyanate. Male and female samples were then compared as whole-mounts in a confocal microscope.

(*b*) *11.5-12.0 DPC (18–24 tail somites)*

At 11.5 DPC the mesonephric duct is well developed, with a prominent basal lamina illuminated by α-laminin antibody (see figure 2*a*, *b*). Numerous highly convoluted tubules (MT) occupy the cranial two-thirds of the mesonephros, and are directly connected to the mesonephric duct (MD). These tubules are branched and intermingled, and coil extensively in the region between the mesonephric duct and the gonad. In both male and female ridges, tubules can be seen to extend into the gonadal primordia at the most cranial end of the gonad (G) in this sectional plane (near the ventral surface of the gonad).

The gonadal primordium at 11.5 DPC consists of a layer of cells which are closely apposed and 4–8 cells deep. This layer increases to > 12 cell thickness over this period of growth. There is a fine meshwork of laminin deposited on the cell surface of cells throughout this population. A discontinuous layer of laminin is localized at the base of cells forming the coelomic epithelial layer covering the surface of the gonad.

No consistent differences in the structure, location, or organisation of the tubules, or of the cells of the gonadal primordium were observed between male and female samples at these stages.

(*c*) *12.5 DPC (26–28 tail somites)*

The female gonad (see figure 2*c*) shows no clear organisational changes from earlier periods although it is approximately twofold larger than at 11.5 DPC. Mesonephric tubules still occupy nearly half the length of the mesonephros. Both degeneration of these tubules and extensive vascularization are less advanced in the female gonad than in the male (see figure 2*d*).

At the 28-tail somite stage, male gonads were clearly distinguishable by the organisation of testis cords in the gonadal region and by a more than twofold size increase from 11.5–12.5 DPC (see figure 2*d*). Laminin is deposited on the basal surface of pre-Sertoli cells which have epithelialized to form testis cords (TC). The mesonephric tubules (MT) have pulled away from the gonadal primordia at this stage, but persist at the cranial end of the mesonephros, although the space which they occupy is diminished relative to the gonad. Vasculature within the mesonephros (V), which is lightly staining with α-laminin antibody, is increasing in complexity.

(*d*) *13.5 DPC*

The female gonadal region (G) at this stage reveals no clear structural organisation with the α-laminin antibody (see figure 2*e*). Tubules in the mesonephros (MT) appear to be degenerating and fragmented. This feature is apparent in male mesonephros as well. The mesonephric duct is less clearly defined with α-laminin at this stage in females. The Müllerian duct (MU) is clearly apparent.

At 13.5 DPC, partitioning of the testis cords (TC) in the male gonad is complete: their coiled structure is well defined by a continuous basal lamina in regions both proximal and distal to the mesonephros. The entire structure has the appearance of a continuous looped tube (see figure 2*f*). The confocal plane of section is optimized to reveal the structure of the testis cords rather than the mesonephric tubules (MT) in this image. The structure of the mesonephric tubules, more visible in other sectional planes, is similar to the view in female mesonephros. The mesonephric duct (MD) remains strongly positive whereas the Müllerian duct is difficult to visualize (not shown). Vascularization is extensive in both the mesonephros and the gonadal regions.

(*e*) *Z-section series through an 11.5 DPC male genital ridge*

The use of whole-mount immunohistochemistry coupled with confocal microscopy visualizes the overall structure of the organ and additionally allows the organ to be seen in a series of Z-section planes. Male and female genital ridges stained with α-laminin have been sectioned in the confocal from their dorsal to ventral surfaces, in ~ 10 μ sections (see figure 3*a*–*h*). Mesonephric tubules (MT) can be seen to connect in 4–6 places with the mesonephric duct (MD) (see figure 3*e*, *f*), and extend in a winding path toward the gonad. Cells of the tubules are strongly positive with α-laminin, whereas other cells of the mesonephros are not reactive with this antibody. In deeper sectional planes, at least 3–4 tubules can be seen to connect directly to the gonadal primordium (arrows) (see figure 3*g*, *h*). When these connections are viewed at × 45, cells of the mesonephric tubules (MT) can be seen to be continuous with the cell population of the gonad (G) (see

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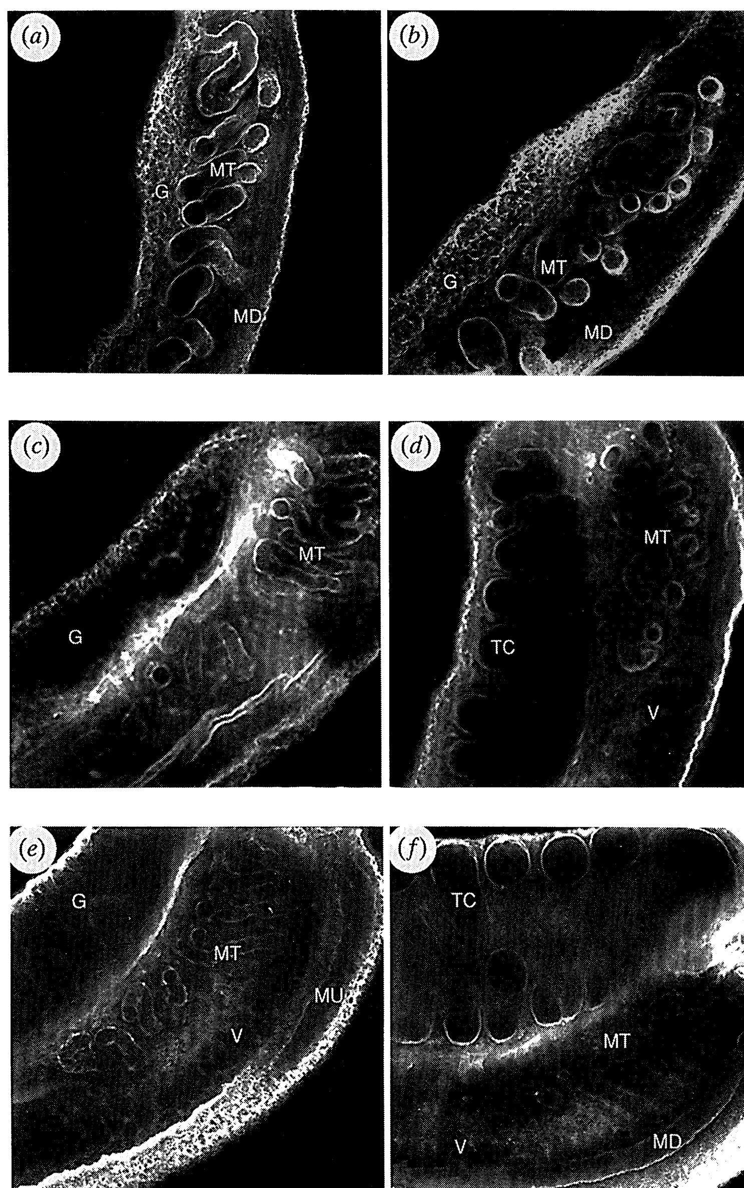

Figure 2. Laminin staining of 11.5, 12.5, and 13.5 DPC male and female genital ridge, magnified ×20. (*a*) 11.5 DPC female genital ridge. (*b*) 11.5 DPC male genital ridge. In both samples the mesonephric tubules (MT) take up much of the space in the cranial two-thirds of the mesonephros, and can be seen to join the mesonephric duct (MD). At the most cranial end of the gonad (G) a tubule connects directly to the gonadal primordium. (*c*) 12.5 DPC female. Cells of the gonad (G) stain lightly around their peripheries with α-laminin. Laminin is deposited in a discontinuous layer at the base of cells of the coelomic epithelium. No other structural organisation is apparent in the female gonad at this stage. Mesonephric tubules (MT) are limited to the cranial half of the genital ridge. Both Müllerian and mesonephric ducts are visible at this stage (although not in this plane of section) (*d*) 12.5 DPC male gonad. Cells in the region of the gonad have organised into testis cords (TC) and deposited a basal lamina . The size of the male gonad has increased more rapidly than the female gonad, while the size of the mesonephros is similar between males and females. Mesonephric tubules (MT) have regressed to a more cranial position in male genital ridge; both mesonephric and Müllerian ducts are visible (out of this sectional plane). (*e*) 13.5 DPC female gonad. The proportion of the tubules in the mesonephros is now much smaller and the gonad has grown to a bigger size, although still only 1/2 the size of the male gonad. The Müllerian duct (MU) is visible in this sectional plane. (*f*) 13.5 DPC male gonad. The male gonad is now nearly twice as large as the female. The basal lamina is complete around the testis cords (TC). The mesonephric tubules (MT) show a similar configuration as in the female. Extensive vasculature has developed in both sexes (V). In figures *a-f* the cranial end of the gonad is toward the right.

figure 4). Although the genital ridge illustrated in this Z-section series is male, similar connections are routinely observed in the female gonad at this stage. By 12.0 DPC, only the 1–2 most anterior connections remain between mesonephric tubules and the gonad in the genital ridge of either sex. By 12.5 DPC, no connections remain in the male gonad: the tubules have pulled away from the gonad (see figure 2*d*). This process may be delayed in the female (see figure 2*c*).

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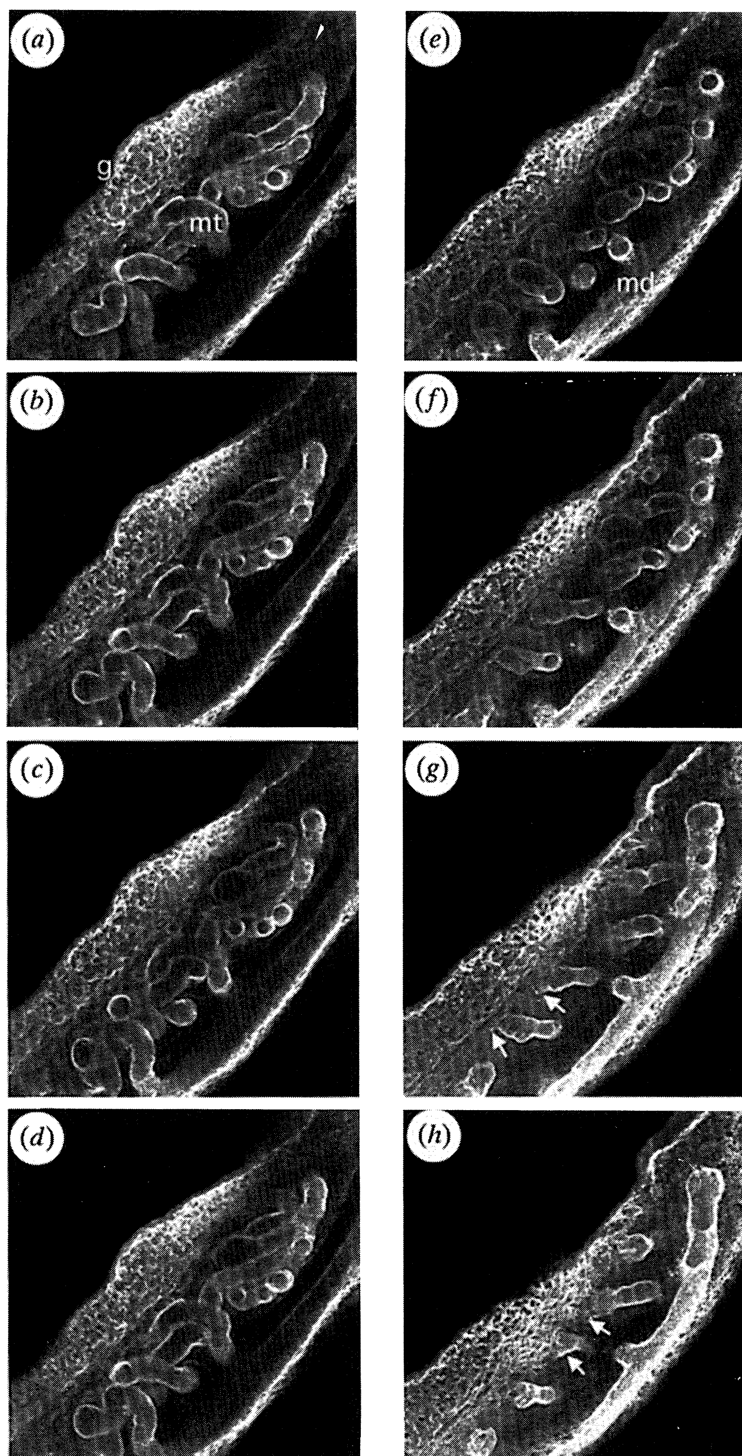

Figure 3. Confocal Z section through the 11.5 DPC male genital ridge, magnified ×20. Section thickness is 8.5 μm. The convoluting tubules are shown in sequential sections from the ventral to the dorsal surface of the genital ridge. There are multiple connections in different planes of section between the tubules and the mesonephric duct which can be seen throughout the series. The tubule to gonad connections can be seen in (*g*) and (*h*), (arrows). The cranial end of the genital ridge is to the right.

4. DISCUSSION

Mouse male and female genital ridges dissected at stages from 11.5 DPC to 13.5 DPC, the period of time over which testis cords organise in the male gonad, have been stained with an antibody against laminin. The purpose of this study was to illuminate the 3-D structure of the gonad over this period of development, using confocal microscopy. Laminin is expressed at high levels in the cells which form the mesonephric tubules and the mesonephric duct at the earliest stages tested. Laminin is expressed at low levels in all cells of the gonadal blastema at 11.5 DPC, and is deposited in the basement membrane which partitions the testis cords from the interstitial space as cords form by 12.5 DPC in the male gonad. Extensive electron and light microscopy has suggested that cells from the mesonephric tubules are continuous with the gonadal

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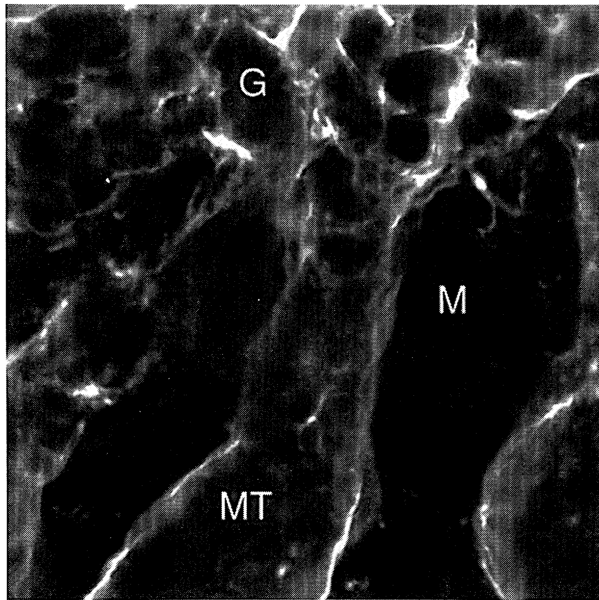

Figure 4. High magnification of mesonephric tubule connections to the gonad. 11.5 DPC male genital ridge sectioned by confocal microscopy at ×45 showing a higher magnification view of cells at the ends of tubules which are continuous with the gonadal primordium. The figure is oriented craniocaudal from right to left. Similar connections are routinely observed in female gonads at 11.5 DPC.

primordium in some sections (Merchant-Larios 1979; Upadhyay *et al.* 1979; Zamboni & Upadhyay 1982; Satoh 1985; Kanai *et al.* 1989; Wartenberg *et al.* 1991). This method has facilitated the 3-D visualization of cellular connections between the distal ends of the mesonephric tubules and the gonadal primordia at 11.5 DPC. At least 3–6 such connections exist at 11.5 DPC in both male and female gonads. These cellular bridges have disappeared by 12.0 DPC except for occasional residual connections at the most cranial end of the genital ridge. The earliest time of appearance of these connections to the gonad has not been determined, but there is a decline in the elaboration of the mesonephric tubules after 11.5 DPC which suggests that these structures reach their zenith of development before or at 11.5 DPC. We suspect this means that we are observing the end of this phase of gonadogenesis at the 11.5–12.0 DPC timepoint.

These images suggest that at least some of the cells which form the gonad are contributed by mesonephric tubules at the earliest stages of gonad formation. At present there is no direct evidence that cells from these tubules actually move into the gonadal primordia. There is no significant difference between male and female genital ridges in the structure, timing, or position of these mesonephric tubule-gonad connections. The difficulty comes in reconciling this data, which we and many other investigators have documented, with data from cell labelling experiments in organ culture (Buehr *et al.* 1992; Merchant-Larios *et al.* 1993). In organ culture experiments, which are initiated at 11.5 DPC using marked cells from the mesonephric donor, we and others have observed that mesonephric cells move into the male gonad, and contribute to the myoid, endothelial and perhaps Leydig cell populations. These movements appear to be largely exclusive to the male genital ridge (Nordquist *et al.*, unpublished data), whereas the tubule connections observed at 11.5 DPC show no sexual dimorphism.

We suggest two possible explanations for this discrepancy. The mesonephric tubule to gonad connections may be present in both the male and female but not be functionally equivalent. The migration of cells from the tubules into the gonad may depend on *Sry* expression at this stage of development. If this were true, specific migration into the male gonad in organ culture experiments could be explained. Alternatively, there may be no difference between male and female genital ridges with respect to this phase of cell movement. The influx of cells from the mesonephric tubules may be a universal step in formation of the 'indifferent' gonadal primordium. If this is the case, it is an event of gonad formation which occurs upstream of *Sry* expression, and may be essential to the assembly of the cell population common to male and female genital ridge. It is this population in which *Sry* is expressed in male gonads, and must act to initiate differentiation of testis cords. There may be later phases of cell migration into the gonad which are assayed in organ culture experiments, are male specific and depend on a signal from the testis-determining gene, *Sry*. It should be possible to distinguish between these two alternatives using dye-injection techniques to label individual cells in the mesonephric ducts and to follow the movement of these cells in male and female genital ridges placed in an organ culture system.

The earliest cell type(s) present in the gonadal primordia are still unknown, but several lines of investigation point to the presence of the pre-Sertoli cell in this population. The Sertoli cell is thought to be required to initiate the signaling pathways which lead to testis organogenesis. It is the only cell type of the testis which requires the presence of a Y chromosome in an XX↔XY chimera. (Burgoyne & Palmer 1993). These data, in conjunction with *in situ* expression data which localize *Sry* expression to the gonadal primordia at 11.5 DPC (Koopman *et al.* 1990), have led to the hypothesis that *Sry* expression is required only in the pre-Sertoli cell, which is present at early times in the gonadal primordium. Although expression of *Sry* is detected by RNase protection in late 10.5 DPC embryos (Hacker *et al* 1995, and figure 1), cellular localization of the transcript by *in situ* hybridization at this stage has not been successful so far. It would be interesting to determine the cellular localization at this earlier period of gonad formation.

Other evidence supports the idea that both pre-Sertoli and pre-Leydig cells are contributed early to the cell population of the gonadal primordia. When the male gonad is separated from the mesonephros at 11.5 DPC, and cultured separately *in vitro*, cords do not form; however, both AMH, known to be expressed in Sertoli cells, and 3β-HSD, an enzyme in the steroidogenic pathway which characterizes Leydig cells, are reportedly expressed (Merchant-Larios *et al.* 1993). These data suggest that both these cell types are already present in the gonadal region at 11.5 DPC.

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The possibility that steroid-producing cells are contributed to the gonad in this early phase of gonadogenesis suggests an interesting link to the origin of the cells of the adrenal gland. In the pig, a giant mesonephric duct is thought to contribute cells to both the adrenal gland and the gonad (Upadhyay & Zamboni 1982). In the mouse, the adrenal gland arises from a group of cells at the most cranial end of the gonad, visible by *in situ* hybridization with a probe for steroidogenic factor 1 (SF1) at 10.5 DPC (Ikeda *et al.* 1994), and as a discrete organ by scanning electron microscopy at 11.5 DPC (Capel & Lovell-Badge 1993). SF1 is also expressed in the gonadal primordium. In a mouse functionally depleted of the SF1 gene, neither adrenals nor gonads form, although the mesonephros appears normal: at 11.5 DPC the region of the gonadal primordia has not expanded and is undergoing apoptosis (Luo *et al.* 1994). It will be interesting to determine whether the complex of mesonephric tubules is normal in the SF1 knock-out animals by α-laminin staining and whole-mount immunohistochemistry.

Finally, these experiments do not address the contribution of the coelomic epithelium to the gonadal primordium. It has been suggested that cells from the coelomic epithelium may invaginate into the interior of the condensing gonad (Pelliniemi 1976; Smith & MacKay 1991), forming early 'cords' of cells in male and female gonads, and contributing to the expanding cell population in this region. At least in chick, where dye labeling has been used to mark cells of the coelomic epithelium, this seems to occur in association with migrating germ cells which enter the region at this time (Rodemer-Lenz 1989). Scanning electron micrographs from 10.5 DPC show pores from the surface epithelium into the interior of the gonad, which could reveal a similar phenomenon in mouse (Capel & Lovell-Badge 1993). Experiments using dye labelling of individual cells can address the contribution of the coelomic epithelium to the establishment of the principal somatic cell types of the gonad.

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Discussion

M. A. Ferguson-Smith (*Department of Pathology, Cambridge University, U.K.*). Have similar studies on the relation of the mesonephros to gonadal development been possible on the ovotestis found in XX/XY mouse chimaeras? Can the invariable finding of a cranial ovarian and a caudal testicular arrangement be explained by differential contribution of the mesonephros?

B. Capel. We have not yet done studies on XX↔XY chimaeras, or on any ovotestes yet. It will be interesting to look at mutants where ovotestes form, like Y^{POS}, where the orchestration of this event could be out of synchrony. It is my understanding that, at least in Y^{POS}, the testicular region is commonly central, whereas the ovarian regions occur at the ends. This effect might be similar to the case in a number of white-spotting mutants, where a defect in the migration of neural crest leads to a failure of melanoblasts to reach distal regions before 'closure'. This experiment might tell us more about the importance of timing in this migration event.

A. McLaren (*Wellcome/CRC Institute, Cambridge, U.K.*). From *in situ* hybridization, is *Sry* expressed throughout the urogenital ridge at 10.5 DPC? At 11.5 DPC is *Sry* expressed only in the genital ridge, or also in the mesonephros?

B. Capel. Our original *in situ* hybridization results were convincing only for 11.5 DPC where expression of *Sry* was shown to be restricted to the genital ridge itself. This experiment was done several years ago. Now that we have a better understanding of the time course of *Sry* expression in the ridge we know the best antisense probes to use, from the RNase protecion experiments which Adam Hacker has done, it makes sense to repeat the *in situ*'s using digoxigenin to try to localize expression to specific cells during this 10.5–12.5 DPC time period.

S. L. Ullmann (*Division of Environmental & Life Sciences, Institute of Biomedical & Life Sciences, University of Glasgow, G12 8QQ, FCI, U.K.*). Dr Capel described two distinct cell migrations from the mesonephros into the gonad primordium: one at the anterior end, from the Wolffian duct, and a later one from more posterior regions of the organ. Do either of these cell migrations contribute to the formation of the gonadal blastema, or do these events occur subsequent to the formation of the blastema?

B. Capel. Our current hypothesis is that the cellular bridges from the mesonephric tubules to the gonad, which we have observed at 11.5 DPC are involved in the formation of the gonadal blastema. Based on the fact that these bridges are localized in the anterior third of the genital ride at 11.5 DPC and sequentially disappear toward the anterior by 12 DPC, we suspect we are witnessing the end of this phase at 11.5 DPC. We need to look earlier at 10.5 and 11.0 day ridges to see if these connections are already present at that time and could be involved in the earliest formation of the gonadal blastema. The second phase of cell migration ocurs subsequent to the formtion of the gonadal blastema. We have observed this movement between 11.5 DPC and the formation of testis cords, but we do not yet know how time-restricted it is. We have seen no evidence that it is limited to any particular region: it appears that cells can migrate from the anterior, posterior, or middle of the mesonephros into the gonad.

P. Burgoyne (*NIMR, The Ridgeway, Mill Hill, London, NW7 1AA, U.K.*) I have two questions: first, with regard to the first migration from the mesonephros into the developing gonad, specifically the cells which appear to derive form the mesonephric tubules, you suggest that these might be precursors of Leydig cells. I have previously been attracted to Dr Zamboni's suggestion that these cells give rise to the supporting cell lineage, because this would represent one epithelial cell type giving rise to another. Secondly, do your observations shed any light on Dr Merchant-Larios' recent finding tht XY gonad/XY mesonephros combinations subsequently produce more testosterone that XY gonad/XX mesonephros combinations?

B. Capel. In answer to your first question: it is Dr Upadhyay and Dr Zamboni's report that the mesonephric ducts contribute to the adrenal cortex as well as to the gonad, that leads us to think that there may be some common steroidogenic precursor. The regional localization at the anterior end of the gonad makes sense, and there is also the information tht SF1 knock-out mice form no gonads or adrenals. The supporting cells may also come from these tubules. However, the coelomic epithelium is an alternative source of epithelialized cells, which seems to contribute some portion of the gonadal blastema in chick, where dye-labelling experiments have been done. In answer to your second question: we have some evidence that there may be slightly more (second phase) migration in an XY gonad/XY mesonephos combination that in an XY gonad/XY mesonephros conbimation. However, there is a lot of variability and we need more data to address this point. I don't know what to make of this finding yet. There seems to be some question about whether this effect is also seen whent he XY gonad is abutted to a piece of XX or XY limb bud.

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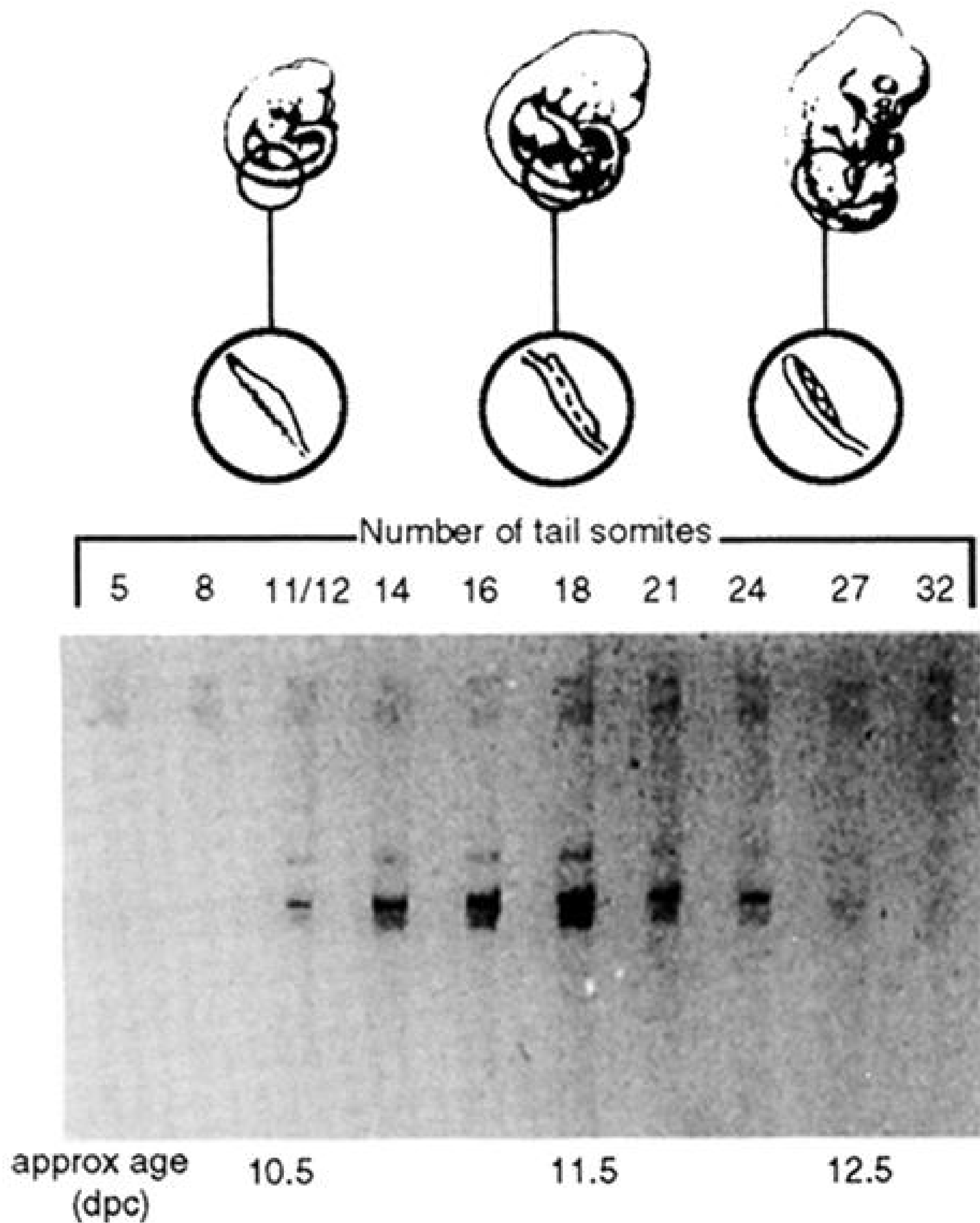

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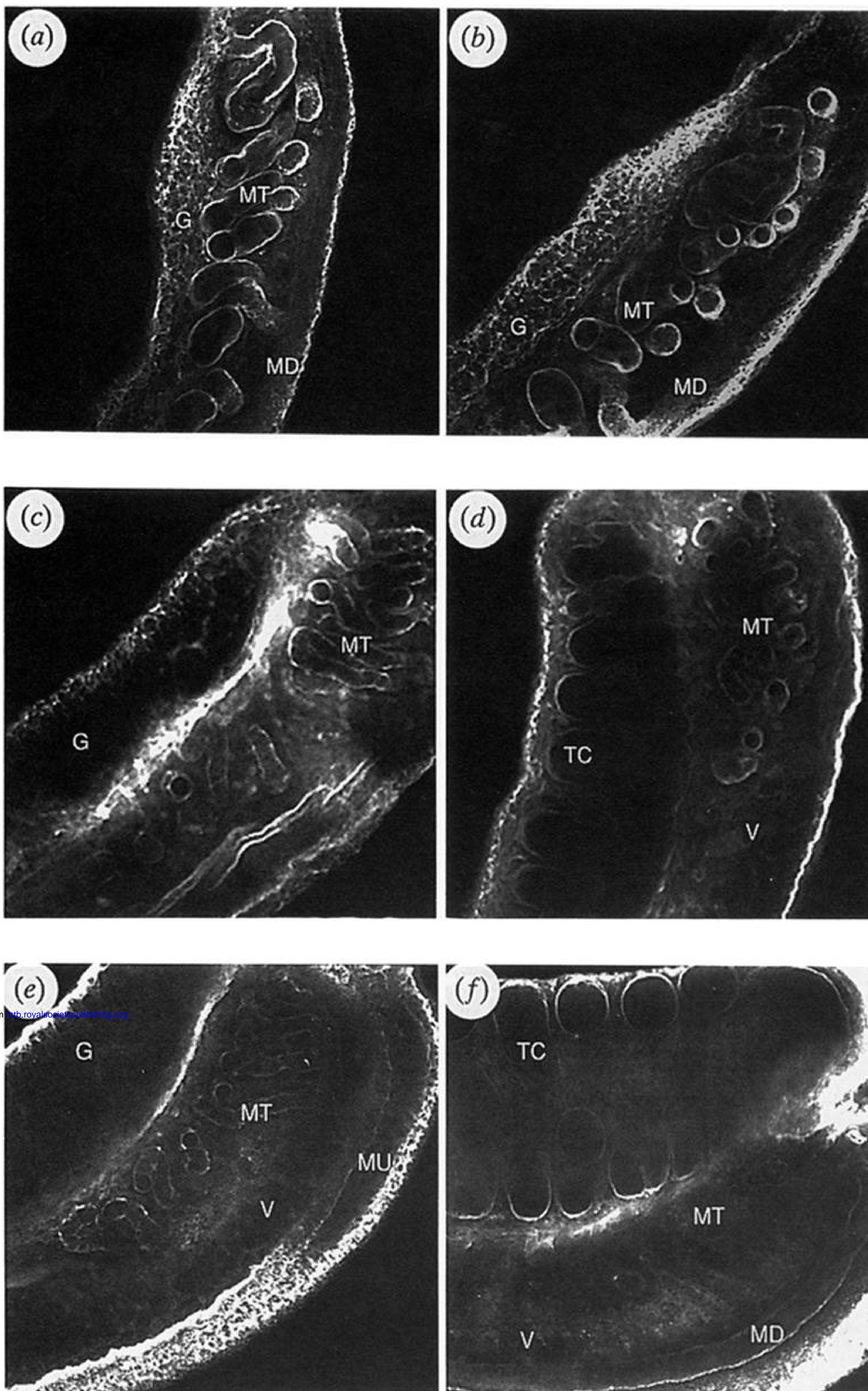
gure 1. Diagram indicating *Sry* expression relative to the ıge of the developing gonad. RNase protection experiments lacker *et al.* 1995) identify the *Sry* urogenital ridge anscript specifically in the male gonad between the 11–27 il-somite stages, during the narrow window of development which testis cords begin to organise.



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igure 2. Laminin staining of 11.5, 12.5, and 13.5 DPC male and female genital ridge, magnified ×20. (*a*) 11.5 DPC male genital ridge. (*b*) 11.5 DPC male genital ridge. In both samples the mesonephric tubules (MT) take up much the space in the cranial two-thirds of the mesonephros, and can be seen to join the mesonephric duct (MD). At e most cranial end of the gonad (G) a tubule connects directly to the gonadal primordium. (*c*) 12.5 DPC female. Cells the gonad (G) stain lightly around their peripheries with α-laminin. Laminin is deposited in a discontinuous layer the base of cells of the coelomic epithelium. No other structural organisation is apparent in the female gonad at is stage. Mesonephric tubules (MT) are limited to the cranial half of the genital ridge. Both Müllerian and esonephric ducts are visible at this stage (although not in this plane of section) (*d*) 12.5 DPC male gonad. Cells in e region of the gonad have organised into testis cords (TC) and deposited a basal lamina . The size of the male onad has increased more rapidly than the female gonad, while the size of the mesonephros is similar between males nd females. Mesonephric tubules (MT) have regressed to a more cranial position in male genital ridge; both esonephric and Müllerian ducts are visible (out of this sectional plane). (*e*) 13.5 DPC female gonad. The proportion the tubules in the mesonephros is now much smaller and the gonad has grown to a bigger size, although still only /2 the size of the male gonad. The Müllerian duct (MU) is visible in this sectional plane. (*f*) 13.5 DPC male gonad. he male gonad is now nearly twice as large as the female. The basal lamina is complete around the testis cords (TC). he mesonephric tubules (MT) show a similar configuration as in the female. Extensive vasculature has developed both sexes (V). In figures *a-f* the cranial end of the gonad is toward the right.

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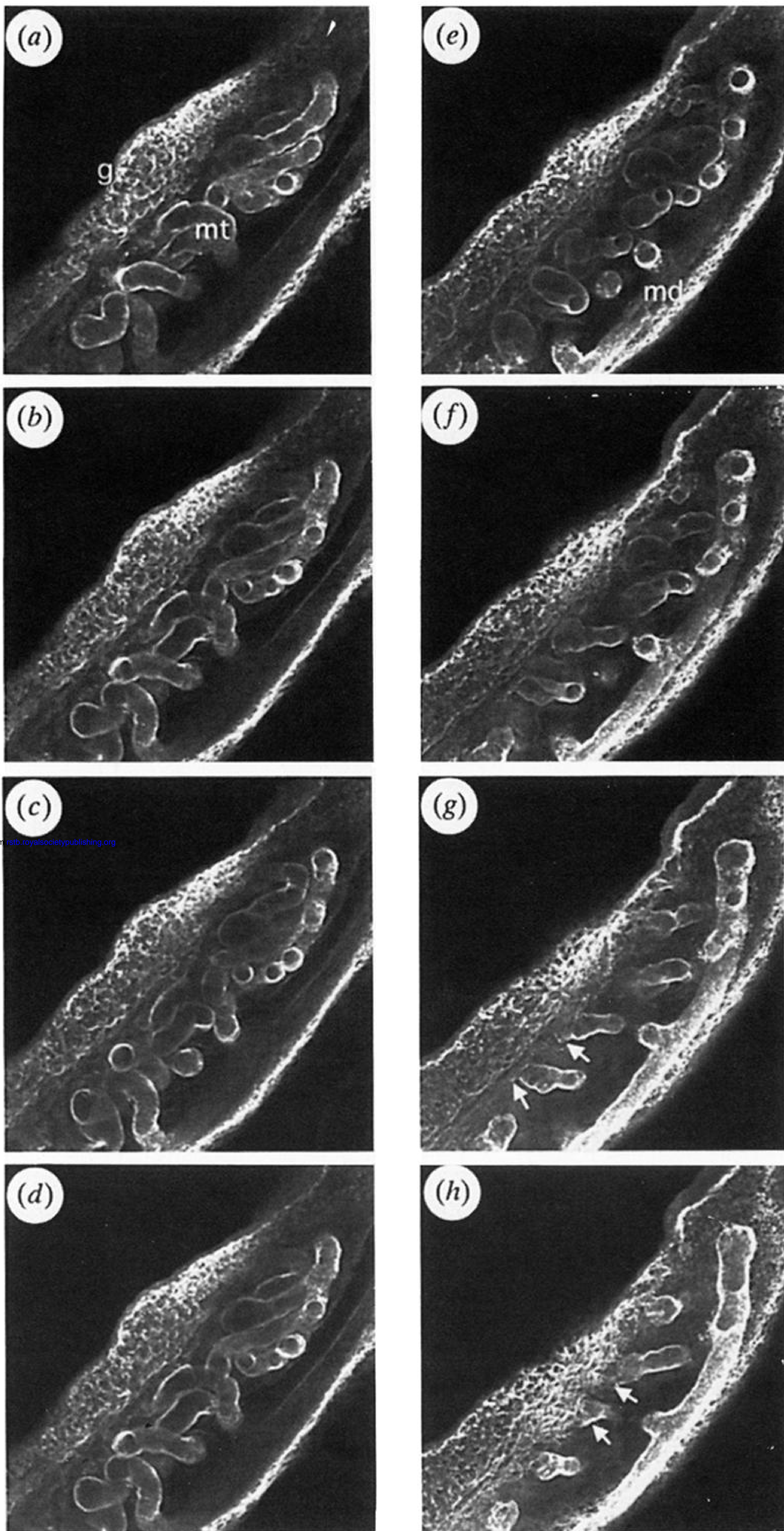

igure 3. Confocal Z section through the 11.5 DPC male genital ridge, magnified ×20. Section thickness is 8.5 μm. he convoluting tubules are shown in sequential sections from the ventral to the dorsal surface of the genital ridge. here are multiple connections in different planes of section between the tubules and the mesonephric duct which ın be seen throughout the series. The tubule to gonad connections can be seen in (*g*) and (*h*), (arrows). The cranial ıd of the genital ridge is to the right.

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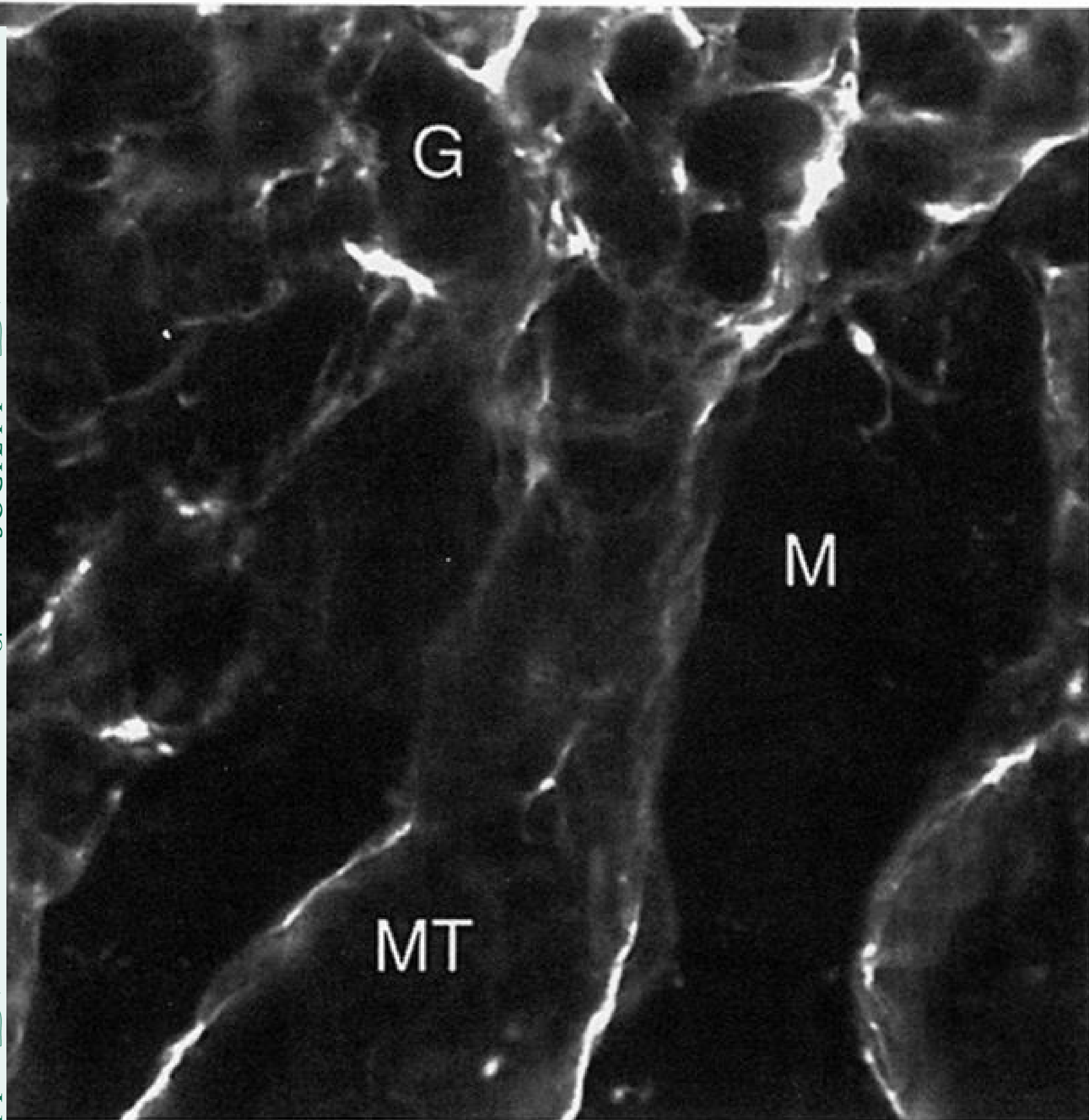

gure 4. High magnification of mesonephric tubule connections to the gonad. 11.5 DPC male genital ridge sectioned confocal microscopy at ×45 showing a higher magnification view of cells at the ends of tubules which are ntinuous with the gonadal primordium. The figure is iented craniocaudal from right to left. Similar connections e routinely observed in female gonads at 11.5 DPC.

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