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Charting the course of ovarian development in vertebrates

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ABSTRACT The decision of the embryonic gonad to differentiate as either a testis or an ovary is a critical step in vertebrate development. The molecular basis of this decision has been the focus of much study, particularly over the past decade. Here we contrast the knowledge of early gonadal development and the switch to testis differentiation with the lack of molecular understanding of ovarian development at early stages. We review current knowledge regarding mechanisms of ovarian morphogenesis and propose a model for the hierarchical control of development of the fetal ovary, incorporating the few genes already known to be important and several signals or factors that are hypothesised to exist in the early ovary.

KEY WORDS: *sex determination, ovary, gonadogenesis, reproduction, germ cells*

Introduction

One of the most striking features of many species is the existence of two distinct sexes, male and female, a distinction that serves a reproductive function. In numerous animal species, including humans, these features have also taken on complex social and psychological significance. The nature of these differences, and how they arise, has exercised the minds of scientists and philosophers for thousands of years. Only recently, with the development of molecular genetics technologies, have the underlying mechanisms of sex determination and early gonadal development started to become clear.

Mammalian sex determination proceeds in three distinct phases (Fig. 1). In the first, genetic sex is determined at fertilisation by the chromosomal complement of the fertilising spermatozoon. Later during embryonic development, this genetic information is translated into gonadal sex - that is, it determines the growth of either a testis or ovary from the bipotential early indifferent gonad. The third stage is phenotypic sex determination, beginning in fetal or early postnatal life and continuing through puberty, a period in which endocrine products of the gonads direct the differentiation of the accessory sex ducts and external genitalia. As developmental biologists, we are interested primarily in the second of these phases, aspects of which form the focus of this review.

Gonadal development is unique in being the only example in the embryo of an entire anlage having two completely different possible organogenetic fates – testis versus ovary – depending ultimately on genetic rather than positional information. Despite this, the cellular and morphogenetic mechanisms – cell migration, proliferation, determination, differentiation, and communication, deposition of matrix components, and apoptosis – that underpin the formation of the gonads are similar to those used in any other developing organ system. It is this blend of unique and typical aspects of gonadal development that continues to fascinate developmental biologists.

Cytogenetic observations around the middle of the last century led to the conclusion that, in mammals at least, development of a testis normally correlates with the presence of a Y chromosome (Ford *et al.*, 1959; Jacobs and Strong, 1959). Classical embryological experiments by Jost (1947) a few years earlier had established that phenotypic sex of a newborn mammal correlates with the presence of a testis in the embryo. Surgical removal of fetal testes in rabbits resulted in development of female internal genitalia and phenotypic sex characteristics, while ovariectomy and implantation of an ectopic testis into a female embryo resulted in development of structures normally seen in males. Together these observations led to the conclusion that male mammalian development is triggered by active signals on the Y chromosome. Female development has therefore been considered for half a century as a default pathway, to be followed by the embryo only in the absence of active "maleness-promoting" signals. Not surprisingly, the latter half of the twentieth century focused on the search for the male determining signal encoded by the mammalian Y chromosome.

Abbreviations used in this paper: AMH, anti-Müllerian hormone; DNA, deoxyribonucleic acid; dpc, days post coitum; FIGα, factor in germline alpha; MIS, Müllerian inhibiting substance; Sf1, Steroidogenic factor 1; Sox9, Sry-like HMG box gene 9; Sry, sex determining region of the Y chromosome; WT1, Wilm's Tumour factor 1.

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Cases of human sex reversal proved especially useful in this search, since XX individuals who developed as males, or conversely XY females, could be mapped for small duplications or deletions. By this means the search for the testis determining gene was narrowed to a small region of the Y chromosome, which in turn was found to contain a gene dubbed *SRY* (Gubbay *et al.*, 1990; Sinclair *et al.*, 1990). *SRY* was found to be the only Y-encoded sequence that is both necessary and sufficient for directing the development of the gonad along the testis pathway, and hence for phenotypic male development (Koopman *et al.*, 1991).

Since the pivotal identification of *SRY* just over a decade ago, work in many labs around the world, including ours, has been largely focussed on testis development, and in particular the the molecular mechanism of action of SRY. Several key regulators of male development have been identified during this time, though the direct target of SRY remains conspicuously elusive. For example, the SRY-related factor Sox9 has been implicated as a key player in testicular differentiation, implicated in the early decision of XY somatic cells of the developing gonad to develop along the male pathway (Kent *et al.*, 1996; Morais da Silva *et al.*, 1996). The *doublesex/mab-3*-related gene *Dmrt1* is implicated in further differentiation of the testis (Raymond *et al.*, 1998; Raymond *et al.*, 2000). Numerous other factors have been identified which direct crucial steps in early gonadal development in both sexes, including WT1, Sf1, Lim1, Lhx9, Emx2, and M33 (Fig. 2). A more detailed discussion of these factors is not warranted here, but can be found in reviews by Swain and Lovell-Badge (1999) and Capel (2000).

In stark contrast to the advances in understanding the development of the testis, ovarian development has remained comparatively mysterious. Many workers have focussed on the postnatal development of the ovary and the function of ovarian follicles in the adult female. From these studies, we know that the functional unit of the mammalian ovary is the follicle, comprising a germ cell or presumptive oocyte, and surrounding layers of somatic cells. We know little about the formation of this unit and the coordination of the several cell types necessary for its early growth. Fetal ovarian structure and histogenesis were studied in some detail in the early part of the last century, but the observations were somewhat subjective, being based on inaccurate theories regarding the origins and fates of various ovarian cell types.

Further, very few cases of sex reversal have yet proved informative regarding active signals that promote ovarian development, and so little is known about the molecular genetics of early ovarian development. Two factors have been identified that are expressed preferentially in the developing ovary, namely the transcriptional regulator Dax1 (Swain *et al.*, 1996) and the signalling molecule

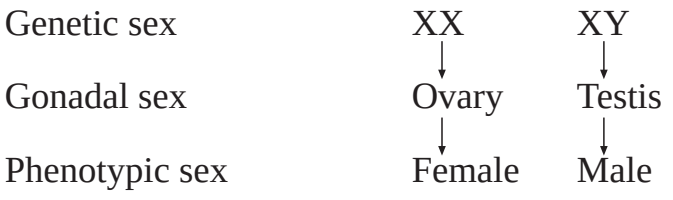

Fig. 1. Three phases of mammalian sex determination. *Mammalian sex determination proceeds in three phases: genetic sex established at fertilisation, gonadal sex during early development and finally phenotypic sex determination beginning in late gestation. Mutations that affect any of these three phases may result in sex reversal or urogenital abnormalities.*

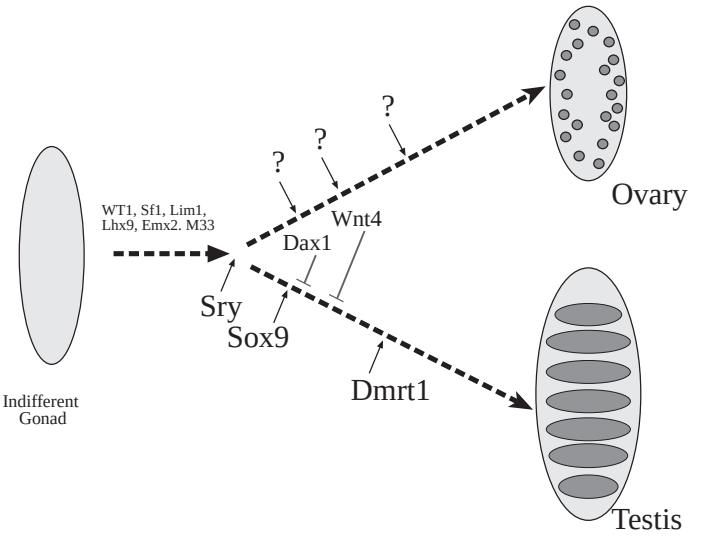

Fig. 2. Molecular interactions in early gonadal development. *Several key regulators of gonadal development and male sex differentiation are known. WT1, Sf1, Lim1, Lhx9, Emx2 and M33 are factors known to be necessary for early urogenital development in both males and females. Sry (sex determining region of the Y chromosome) acts as the initial switch to trigger male development. Sox9 is another early master regulator of male development. Dax1 and Wnt4 are abundantly expressed in early ovarian development but transgenic and knockout analyses indicate a more important role for these factors in repressing the male pattern of development. Active regulators of early ovarian development have not been well characterised.*

Wnt4 (Vainio *et al.*, 1999). However, knockout and transgenic analyses in mice indicate that these function as anti-testis factors to repress male pathways of development, rather than as ovarian determinants *per se* (Swain *et al.*, 1998; Vainio *et al.*, 1999).

In this paper we review current knowledge of sex determination and embryonic gonadal development, but choose the road less travelled, by focusing on ovarian development.

One Way Street: Early Development of the Genital Ridges

The developmental biology and molecular genetics of vertebrate sexual development has been studied most intensively using the mouse as a model organism. This review will therefore refer primarily to data obtained from studies of mice.

Formation of the Intermediate Mesoderm

Early male and female embryos are indistinguishable by morphological criteria. The genetic differences between males and females are not translated into structural differences until midgestation in the mouse (Kaufman and Bard, 1999), or about seven weeks in humans (Moore, 1988). Mesodermal populations arise in the trunk of the embryo after gastrulation between eight and nine days post coitum (dpc), and are referred to as the intermediate mesoderm due to their position between the somitic mesoderm and lateral plate mesoderm. This mesodermal population forms the early precursor of the urogenital system of the adult animal.

Development of the Mesonephros

The urogenital system in most vertebrates forms in three stages. Firstly and most cranially formed is the pronephros, which may have

a temporary excretory function in the embryo but does not remain in the adult. Next to form is the mesonephros, which likewise has a transient excretory function in some species, but has retained other important functions in urogenital development, as described below. Finally and most caudally, the metanephros forms, ultimately differentiating into the adult excretory kidney.

Invaginations of the surface epithelium of the mesonephros form the Wolffian (mesonephric) and Müllerian (paramesonephric) ducts. Both ducts form regardless of the sex of the embryo. In male embryos, the developing testis produces two key endocrine regulators. Testosterone supports differentiation of the Wolffian ducts to form most of the internal genitalia of the male including vasa deferentia and epididymides, while the Müllerian ducts regress under the influence of anti-Müllerian hormone (AMH, also known as Müllerian inhibiting substance, MIS). In the absence of these factors in the female, the Wolffian ducts degenerate and the Müllerian ducts give rise to the oviducts, uterus and upper vagina. Prenatal differentiation of the female internal genitalia appears to be independent of steroid hormonal influences (Couse *et al.*, 2000), but their function in the adult is highly dependent on sex steroids produced by the ovary.

The internal system of tubules in the mesonephros does not have any excretory function in mammals, but are retained in males to give rise to the rete testis, which forms the connecting link between the seminiferous tubules of the testis and the epididymis. The existence of a corresponding region of the ovary, referred to as the rete ovarii, has been discussed by other authors and may have a corresponding embryological origin in the female (for example Byskov, 1978).

Induction of the Urogenital Ridges

An important role of the mesonephros is in inducing the development of the gonad itself. The early gonad of both sexes is first observable morphologically as a thickening of the coelomic epithelium on the ventromedial surface of the mesonephros parallel to the midline, at around 10 dpc in the mouse. This structure is called the gonadal (or genital) ridge; the combination of gonadal ridge and underlying mesonephros is collectively referred to as the urogenital ridge. The urogenital ridge proliferates and differentiates to the point where the extended ovoid gonad can be seen attached to the tubular mesonephros.

Three main somatic cell types make up the genital ridge: the supporting cells, steroidogenic cells and stromal cells. These cell types originate locally in the bipotential indifferent gonad (Merchant, 1975). Each can develop along either a male or female pathway to form a testis or an ovary (Koopman *et al.*, 1991; Bishop *et al.*, 2000) (Fig. 3). Traditionally, this choice has been thought to depend entirely on the presence or absence of male-promoting signals, but the role of positive regulators of ovarian cell differentiation should not be overlooked.

The supporting cell lineage gives rise to the cells that immediately surround and support the development of the germ cells, and are referred to as Sertoli cells in the testis or granulosa cells in the ovary. Cells of the steroidogenic lineage differentiate as either Leydig cells in the male embryo or analogous theca cells in the female ovary. Stromal cells in both testis and ovary are responsible for patterning the structural components of the organ, including production of extracellular matrix and blood vessel formation.

Most of the stromal cells are not identifiably specialised between the two sexes, with one notable exception, the peritubular myoid cells of the testis, which form a layer surrounding the Sertoli cells. These cells interact with Sertoli cells to direct the structural development of the testis cords that give rise to the seminiferous tubules of the mature testis (Skinner *et al.*, 1985). Cord formation is a key developmental difference between early testes and ovaries, and it is therefore significant that peritubular myoid cells originate from the male-specific migration of cells into the gonad from the adjacent mesonephros (Martineau *et al.*, 1997), dependent on the action of *Sry* (Capel *et al.*, 1999).

Colonization of the Genital Ridges by Primordial Germ Cells

While the somatic cells of the developing gonad differentiate from either the mesonephric mesenchyme or the overlying coelomic epithelium, the primordial germ cells have a very different embryological origin. Primordial germ cells are first discernible as a small group of alkaline-phosphatase positive cells clustered in the extraembryonic mesoderm, just after gastrulation begins, at around 7 dpc in the mouse (Ginsburg *et al.*, 1990). This cluster of

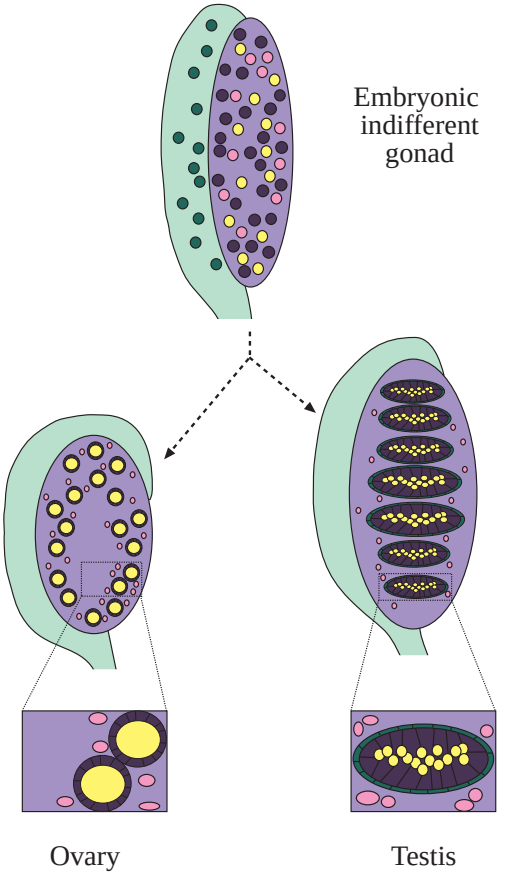

Fig. 3. Sex determination in the major cell types of the gonad. *Indifferent gonads form in both sexes at mid-gestation (top), and can develop as either testes or ovaries depending on genetic information and their hormonal milieu (bottom line represents neonatal ovary or testis). Each of the cell types in the gonad must differentiate accordingly. Germ cells (yellow) give rise to oocytes in the female or sperm in the male. Supporting cells (dark purple), become either Sertoli cells of the testis or granulosa cells in the ovary. Steroidogenic cells (pink) become Leydig cells in the male or theca cells in the female. In the male, Sry induces migration of cells from the mesonephros (green) which include precursors of the peritubular myoid cells.*

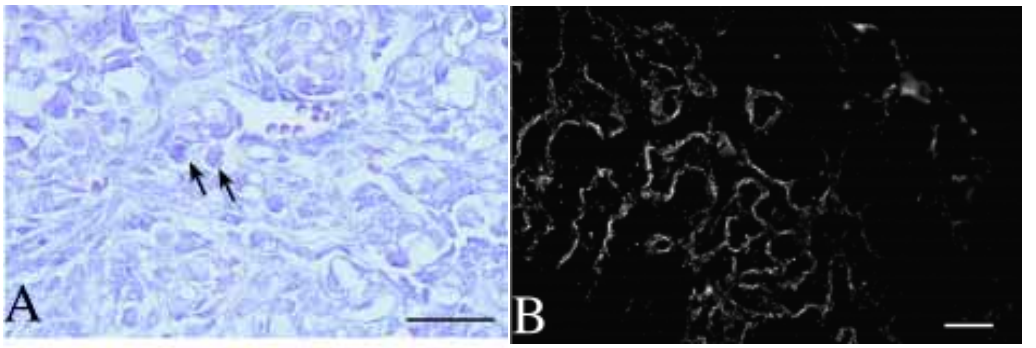

Fig. 4. Development of ovigerous cords. *Sections of 16.5 dpc fetal mouse ovaries demonstrate the presence of clusters of germ cells arranged in loose cords, with surrounding layer of somatic cells, defined by laminin deposition.* **(A)** *8 μm section of formaldehyde fixed, paraffin embedded 16.5 dpc mouse ovary stained with haematoxylin and eosin according to standard methods. Germ cells are large round cells with large nuclei and pale cytoplasm (arrows).* **(B)** *12 μm section of unfixed frozen 16.5 dpc mouse ovary after immunofluorescent detection of laminin. Scale bars indicate 45 μm.*

cells remains shielded from the major morphological rearrangements occurring in the embryo proper, and the germ cells are presumably protected in this position from the differentiating influence of growth factors and morphogens.

Between 8.5 and 12 dpc, primordial germ cells migrate rostrally from the base of the allantois through the hindgut, into the mesoderm of the mesonephros and thence into the gonad itself. While this movement may be partially brought about by morphogenetic rearrangements that shape the whole embryo, evidence suggests that the targeting of these cells to the developing genital ridges is actively controlled by a chemoattractive signal (Godin *et al.*, 1990), and is at least partially dependent on the *c-Kit/Steel* signalling system (Buehr *et al.,* 1993) and on integrins (Anderson *et al.*, 1999). During this journey the population of primordial germ cells proliferates by mitosis, such that the number of cells reaching the indifferent gonad is several thousand (Tam and Snow, 1981).

A Fork in the Road: Sex Determination and Histogenesis of the Ovary

Many cellular and structural changes occur in the developing ovary between the initial appearance of the genital ridge, and formation of the first recognisable follicles, between 1 and 3 days post partum. While these changes are not as rapid and striking as those observed in the developing testis, they are nonetheless important for the development and proper function of the adult ovary.

The Beginnings of Structure: Formation of Ovigerous Cords

The first histologically observable rearrangement seen in the embryonic ovary is the gradual formation, between 12 and 13.5 dpc, of loose cordlike structures classically referred to as ovigerous cords (Odor and Blandau, 1969; Konishi *et al.*, 1986). These cords are not defined by basal laminae as are seen during testicular development, and are not observable at the level of the dissecting microscope, but can be seen by histochemical and immunohistochemical staining of sections (Fig. 4 and Frojdman *et al.*, 1993).

At the core of these cords is a cluster of primordial germ cells, and these are surrounded by somatic cells with a mesenchymal appearance. Close observation of the germ cells has shown them to be physically interconnected via cytoplasmic bridges to form a syncytium or cyst (Pepling and Spradling, 1998). These bridges appear similar to those seen in the germ cell cysts of the Drosophila ovary, termed ring canals, and close apposition is observed between the plasma membranes of the germ cells and surrounding somatic pregranulosa cells at the sites of these canals. More recent observations indicate that the breakdown of these large cysts to form individual primordial follicles is programmed, and occurs rapidly between one and three days post partum (Pepling and Spradling, 2001).

These observations raise the possibility that direct cell-cell communication between developing germ cells may be a means of coordinating their temporal differentiation, and the bridges may be important for the distribution of mitochondria between developing germ cells. Close contact between the germ cells and pregranulosa cells at the sites of ring canals at the time of cyst breakdown is consistent with an active role for the latter in effecting the rapid reorganisation of the cysts to form primordial follicles.

The Central Role of Meiotic Germ Cells

Germ cells are arguably the reason for the existence of the gonads. It is therefore surprising that in the developing male, the presence of germ cells is unnecessary for the appropriate differentiation of the somatic cell complement of the gonad. XY individuals lacking germ cells develop as phenotypically normal, albeit sterile, males (Merchant, 1975). This indicates that cell fate decisions and differentiation of male gonadal somatic cells must be largely independent of signalling from the germ cells.

In the developing ovary, however, the situation is markedly different; the presence of germ cells is essential for the construction and/or maintenance of a normal ovary. Ovarian dysgenesis is observed in germ cell-deficient mouse mutants such as *W* and *Steel* (see Russell, 1979 and references therein). Meiotic germ cells must exert an important regulatory function over the development of the immediately surrounding somatic cells of the ovary. In other mutants in which germ cells populate the genital ridges normally, but subsequently are depleted, the adjacent somatic cells transdifferentiate to take on a Sertoli-like phenotype (Behringer *et al.*, 1990; Hashimoto *et al.*, 1990; Couse *et al.*, 2000). This suggests that the development of several cell types must be exactly coordinated in a precise temporal and spatial fashion for early histogenesis and for the ongoing differentiation of follicles and oocytes in the adult ovary. It is interesting to note that in the normal physiological cycle of the ovary, loss of the oocyte from a follicle triggers differentiation of the surrounding cells to form a corpus luteum, underscoring the importance of interconnections between oocytes and granulosa cells.

Epithelialisation of Pre-Granulosa Cells – Definitive Follicles

Coordinated differentiation of the cells of XX genital ridges brings about the gradual organisation of the ovary into the characteristic

follicular structure observed in the adult organ. Interactions between mesenchymal cells of the genital ridge, cells of the overlying coelomic epithelium, and the immigrating primordial germ cells direct the morphogenesis of the growing ovary. The structural development of the fetal and neonatal mammalian ovary, including morphological classification of early stages of follicle development, is summarized in Fig. 5.

Electron microscopy has shown that somatic cells, presumably pre-granulosa cells, surrounding the germ cells of the early ovary gradually extend fine cytoplasmic processes between the germ cells of the ovigerous cords or germ cell cysts (Odor and Blandau, 1969; Byskov, 1978; Pepling and Spradling, 1998). These somatic cell processes eventually come to form a complete layer separating the clusters into individual oocytes, with each surrounded by a monolayer of squamous, epithelialised granulosa cells (Merchant-Larios and Chimal-Monroy, 1989). Simultaneously, a basal lamina is deposited around the periphery of the follicle, surrounding the granulosa cells and separating them from the stromal cells (Merchant-Larios and Chimal-Monroy, 1989; Rajah *et al.*, 1992). These two features define the primordial follicle.

As has been discussed above, interactions between developing oocytes and pre-granulosa cells appear to be critical for the formation of the follicle cell monolayer. Interactions between the differentiating pre-granulosa cells and the stromal (pre-thecal) cells have been postulated to be responsible in turn for the deposition of the basement membrane which surrounds the definitive follicle (Rajah *et al.*, 1992), in a manner analagous to the cooperation between Sertoli cells and peritubular myoid cells for deposition of basement membrane surrounding the testis cords in the embryonic male (Skinner *et al.*, 1985). Evidence exists in other developmental systems to suggest that the basement membrane, once formed, influences the behaviour and further differentiation of the adjacent cells (Klein *et al.*, 1988). The rapid progression of follicle differentiation after initial formation suggests that this is also likely to occur in the developing ovary.

The close apposition between the plasma membranes of the presumptive oocytes and granulosa cells suggests that this histogenetic process is guided by cues supplied by direct cell-cell contact; perhaps a signalling event triggered by a membrane bound sensor or linked to the integrins involved in cell contact (discussed by Brown *et al.*, 2000), or via a very short range secreted signalling factor. Alternatively, intercellular junctions may be involved in this stage of ovarian morphogenesis. It has long been suspected that presence of gap junctions between the oocyte and its granulosa cells is necessary for maintenance of meiotic arrest as observed in dormant follicles or for coordination of development in activated follicles. Knockout studies confirm the role of connexins, the main component of gap junctions, in these processes (Carabatsos *et al.*, 2000; Ackert *et al.*, 2001). It is possible that as yet uncharacterised junctions are responsible for the developmental coordination observed in the fetal ovary.

Signposts: Molecular Regulation of Fetal Ovarian Development

Many workers over the past century have analysed the development of the ovary at a morphological level. In contrast, understanding of the development and function of the fetal ovary, at both endocrine and molecular levels, is very hazy indeed. Not only are the cell differentiation signals completely mysterious at this stage, but also we suffer from a dearth of molecular markers with which to trace the development, movement and interaction of the various cell types of the developing ovary.

Regulators of Early Cell Fate Restriction

Very little is known about the molecular regulation of early development of the fetal ovary. Prenatal development of the female reproductive system is apparently independent of the action of steroid hormones (Couse *et al.*, 2000), even though the function of the adult organ for reproduction is highly dependent on steroidal regulators. Other factors must therefore control fetal and prepubertal ovarian differentiation.

In the embryonic male, Sry acts cell autonomously as a transcription factor to trigger and regulate the differentiation of the developing testis (Palmer and Burgoyne, 1991; Gubbay *et al.*, 1990; Ferrari *et al.*, 1992). Sox9 is also thought to act as a transcription factor in the developing Sertoli cells (Kent *et al.*, 1996; Morais da Silva *et al.*, 1996). It may therefore be speculated that corresponding factors are likely to exist in the fetal female, and that these postulated factors act in the pre-granulosa cells to regulate their growth and development. However, no such factor has been identified to date.

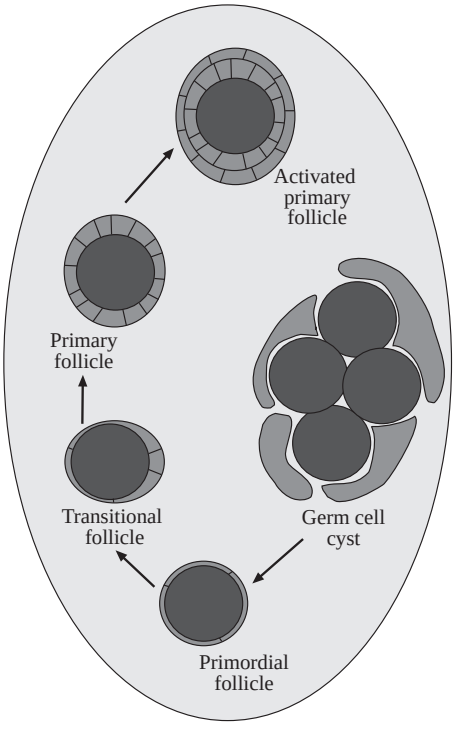

Fig. 5. Development of follicles in the fetal and neonatal mouse ovary. *Clusters of germ cells, or germ cell cysts, are closely surrounded by somatic cells of the developing ovary during fetal development. Around the time of birth, these cysts are rapidly reorganised such that individual oocytes are surrounded by a monolayer of flattened epithelial pre-granulosa cells which deposit a definitive basement membrane, forming primordial follicles. Activation of follicles can occur at any time from a few days after birth onwards, characterised by growth and differentiation of the granulosa cells to form firstly a cuboidal monolayer, then expanding to form multiple layers. Further development of ovarian follicles in preparation for ovulation is not discussed here.*

Cell Interactions for Reciprocal Signalling and Developmental Control

In contrast to the lack of knowledge regarding control of cell differentiation in the developing ovary, at least some candidates exist for effectors of intercellular signalling. Among these, the putative transcription factor FIGα (factor in germline alpha) has been identified in the developing germ cells of early ovary, initially characterised for its role in regulating expression of genes encoding the structural proteins of the zona pellucida (Liang *et al.*, 1997). Gene knockout analysis in mice has indicated that the function of this factor is crucial for formation of primordial follicles (Soyal *et al.*, 2000). This early function of FIGα, prior to activity of its known target genes, indicates that other as yet unidentified targets must exist. Among these targets are likely to be the factors that mediate the granulosa cell survival signal discussed above.

Several members of the transforming growth factor beta (TGFß) superfamily have been shown to be important in regulation of the reproductive function of the adult ovary, and one member of this family, growth and differentiation factor 9 (GDF9) is necessary for early ovarian differentiation. In the absence of this signalling molecule, follicle development is blocked at the primary follicle stage, in which the granulosa cells do not differentiate beyond a cuboidal monolayer and oocyte growth is dysregulated (Elvin *et al.*, 1999a). Further observations suggest that GDF9, produced by the developing oocytes (McGrath *et al.*, 1995), acts on the neighbouring granulosa cells to promote their differentiation (Elvin *et al.*, 1999b). The observation that oocytes are larger than normal and differentiate aberrantly in the absence of this factor indicates that granulosa cells in turn produce an "oocyte growth regulatory factor", via a reciprocal inductive event. The nature of this regulatory signal remains unknown.

A simplified model for the integration of these signals and the cell autonomous factors discussed above is described in Fig. 6.

The Road Ahead

Clearly, the differentiation of the vertebrate ovary is a complex, active process involving co-ordinated differentiation, migration and communication of several cell types. The identification of factors regulating these processes in the developing ovary will be a major challenge in coming years. It is likely that expression screening, positional cloning and candidate gene identification procedures will reveal the genes responsible for differentiation of granulosa and other cell types in the developing ovary.

Recently, a candidate gene for the human syndrome blepharophimosis ptosis epicanthus inversus syndrome (BPES) types I and II has been identified. Mutations in this gene, encoding the putative forkhead transcription factor FOXL2, have been identified in BPES patients (Crisponi *et al.*, 2001; De Baere *et al.*, 2001). *FOXL2* corresponds to a partially characterised murine gene designated *PFrk*, which is known to be expressed in the developing pituitary (Treier *et al.*, 1998). The molecular and cellular role of this factor in pituitary, craniofacial and ovarian development, all of which are affected in BPES, awaits detailed study.

Other syndromes and animal models exist in which ovarian development is abnormal or in which sex reversal or gonadal dysgenesis is observed, and for which no candidate genes have yet been identified. The availability of the sequence of the human genome, and that of various model organisms, will provide valuable tools for the analysis of these cases. Careful investigation of these syndromic cases may provide us with valuable clues regarding the mechanisms of normal sex determination. Additionally, investigation and growing understanding of the molecular causes of premature ovarian failure in women may provide mechanistic insight relating to the developmental plan of this organ. In turn, advances in understanding of early ovarian development may provide clues regarding treatment or prevention of ovarian failure.

Useful insight may also be gained from the investigation of sex determination and ovarian differentiation mechanisms in other species. While ovarian morphology and adult function are very similar amongst vertebrates, molecular mechanisms that underlie this similarity are unknown, and indeed the differences between species with different mechanisms of sex determination are potentially informative. In particular the analysis of sex determination and gonadal development in non-mammalian vertebrates is likely to be a productive means of identifying conserved themes in the evolution of sexual development in vertebrates.

Investigation of the molecular functions and regulatory targets of the few genes implicated in early ovarian development, such as the transcription factor FIGα, and the signalling molecule GDF9, can also be expected to provide insight into the mechanisms by which the complex process of ovarian development is regulated.

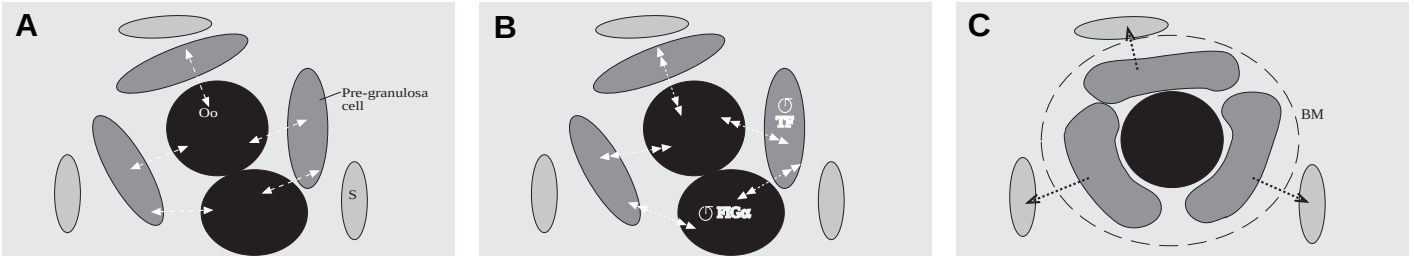

Fig. 6. Molecular control of early ovarian development. *Schematic model of the known and proposed regulatory steps involved in organising primordial follicles shows germ cells (black, Oo), somatic pregranulosa cells and steroidogenic pre-theca cells (S).* **(A)** *Embryonic ovary, in which clusters of germ cells are loosely surrounded by granulosa cells. Close interaction and reciprocal signalling (arrows) between these cells, including the postulated "granulosa cell survival signal" is essential for follicle development.* **(B)** *Representation of a later stage of fetal development, showing expression of the essential germ cell transcription factor FIGα and a postulated granulosa cell specific transcription factor (TF). Arrows represent as yet unidentified signalling events downstream of these transcription factors necessary for coordination of differentiation, such as the "oocyte growth regulatory factor" described in the text.* **(C)** *Neonatal ovary, in which interactions between oocytes and pre-granulosa cells have divided the clusters into individual follicles. A signal of unknown nature from the pre-granulosa cells to the stromal and steroidogenic cells facilitates their interaction and therefore the deposition of a basement membrane (BM, dashed line).*

The means by which the differentiation of several cell types is integrated to generate a functional adult ovary will no doubt be a question which provides workers in the field with challenges and opportunities for many years to come.

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References

ACKERT, C.L., GITTENS, J.E., O'BRIEN, M.J., EPPIG, J.J. and KIDDER, G.M. (2001). Intercellular communication via connexin43 gap junctions is required for ovarian folliculogenesis in the mouse. *Dev. Biol.* 233: 258-270.

ANDERSON, R., FÄSSLER, R., GEORGES-LABOUESSE, E., HYNES, R.O., BADER, B.L., KREIDBERG, J.A., SCHAIBLE, K., HEASMAN, J. and WYLIE, C. (1999). Mouse primordial germ cells lacking ß1 integrins enter the germline but fail to migrate normally to the gonads. *Development* 126: 1655-1644

BEHRINGER, R.B., CATE, R.L., FROELICK, G.J., PALMITER, R.D. and BRINSTER, R.L. (1990). Abnormal sexual development in transgenic mice chronically expressing Müllerian inhibiting substance. *Nature* 345: 167-170.

BISHOP, C.E., WHITWORTH, D.J., QIN, Y., AGOULNIK, A.I., AGOULNIK, I.U., HARRISON, W.R., BEHRINGER, R.R. and OVERBEEK, P.A. (2000). A transgenic insertion upstream of sox9 is associated with dominant XX sex reversal in the mouse. *Nat. Genet.* 26: 490-494.

BROWN, N.H., GREGORY, S.L. and MARTIN-BERMUDO, M.D. (2000). Integrins as mediators of morphogenesis in Drosophila. *Dev. Biol.* 223: 1-16.

BUEHR, M., MCLAREN, A., BARTLEY, A. and DARLING, S. (1993). Proliferation and migration of primordial germ cells in W^e/W^e mouse embryos. *Dev. Dyn.* 198: 182-189.

BYSKOV, A.G. (1978). The anatomy and ultrastructure of the rete system in the fetal mouse ovary. *Biol. Reprod.* 19: 720-735.

CAPEL, B. (2000). The battle of the sexes. *Mech. Dev.* 92: 89-103.

CAPEL, B., ALBRECHT, K.H., WASHBURN, L.L. and EICHER, E.M. (1999). Migration of mesonephric cells into the mammalian gonad depends on *Sry*. *Mech. Dev.* 84: 127-131.

CARABATSOS, M.J., SELLITTO, C., GOODENOUGH, D.A. and ALBERTINI, D.F. (2000). Oocyte-granulosa cell heterologous gap junctions are required for the coordination of nuclear and cytoplasmic meiotic competence. *Dev. Biol.* 226: 167-179.

COUSE, J.F., CURTIS HEWITT, S., BUNCH, D.O., SAR, M., WALKER, V.R., DAVIS, B.J. and KORACH, K.S. (2000). Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science* 286: 2328-2331.

CRISPONI, L., DEIANA, M., LOI, A., CHIAPPE, F., UDA, M., AMATI, P., BISCEGLIA, L., ZELANTE, L., NAGARAJA, R., PORCU, S., RISTALDI, M.S., MARZELLA, R., ROCCHI, M., NICOLINO, M., LIENHARDT-ROUSSIE, A., NIVELON, A., VERLOES, A., SCHLESSINGER, D., GASPARINI, P., BONNEAU, D., CAO, A. and PILIA, G. (2001). The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. *Nat. Genet.* 27: 159-166.

DE BAERE, E., DIXON, M.J., SMALL, K.W., JABS, E.W., LEROY, B.P., DEVRIENDT, K., GILLEROT, Y., MORTIER, G., MEIRE, F., VAN MALDERGEM, L., COURTENS, W., HJALGRIM, H., HUANG, S., LIEBAERS, I., VAN REGEMORTER, N., TOURAINE, P., PRAPHANPHOJ, V., VERLOES, A., UDAR, N., YELLORE, V., CHALUKYA, M., YELCHITS, S., DE PAEPE, A., KUTTENN, F., FELLOUS, M., VEITIA, R. and MESSIAEN, L. (2001). Spectrum of FOXL2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype-phenotype correlation. *Hum. Mol. Genet.* 10: 1591-1600.

ELVIN, J.A., YAN, C., WANG, P., NISHIMORI, K. and MATZUK, M.M. (1999a). Molecular characterization of the follicle defects in the growth differentiation factor 9-deficient ovary. *Mol. Endocrinol.* 13: 1018-1034.

ELVIN, J.A., CLARK, A.T., WANG, P., WOLFMAN, N.M. and MATZUK, M.M. (1999b). Paracrine actions of growth differentiation factor-9 in the mammalian ovary. *Mol. Endocrinol.* 13: 1035-1048.

FERRARI, S., HARLEY, V.R., PONTIGGIA, A., GOODFELLOW, P.N. and LOVELL-BADGE, R. (1992). SRY, like HMG1, recognizes sharp angles in DNA. *EMBO J.* 11: 4497-4506.

FORD, C.E., JONES, K.W., POLANI, P.E., DE ALMEIDA, J.C. and BRIGGS, J.H. (1959). A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's Syndrome). *Lancet* 1: 711-713.

FROJDMAN, K., PARANKO, J., VIRTANEN, I. and PELLINIEMI, L.J. (1993). Intermediate filament proteins and epithelial differentiation in the embryonic ovary of the rat. *Differentiation* 55: 47-55.

GINSBURG, M., SNOW, M. and MCLAREN, A. (1990). Primordial germ cells in the mouse embryo during gastrulation. *Development* 110: 521-528.

GODIN, I., WYLIE, C. and HEASMAN, J. (1990). Genital ridges exert long-range effects on mouse primordial germ cell numbers and direction of migration in culture. *Development* 108: 357-363.

GUBBAY, J., COLLIGNON, J., KOOPMAN, P., CAPEL, B., ECONOMOU, A., MÜNSTERBERG, A., VIVIAN, N., GOODFELLOW, P. and LOVELL-BADGE, R. (1990). A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature* 346: 245-250.

HASHIMOTO, N., KUBOKAWA, R., YAMAZAKI, K., NOGUCHI, M. and KATO, Y. (1990). Germ cell deficiency causes testis cord differentiation in reconstituted mouse fetal ovaries. *J. Expt. Zool.* 253: 61-70.

JACOBS, P.A. and STRONG, J.A. (1959). A case of human intersexuality having a possible XXY sex determining mechanism. *Nature* 183: 302-303.

JOST, A. (1947). Recherches sur la differentiation sexuelle de l'embryon de lapin. *Archs. Anat. Microsc. Morph. Exp.* 36: 271-315.

KAUFMAN, M. and BARD, J. (1999). *The anatomical basis of mouse development*. Academic Press, San Diego.

KENT, J., WHEATLEY, S.C., ANDREWS, J.E., SINCLAIR, A.H. and KOOPMAN, P. (1996). A male-specific role for SOX9 in vertebrate sex determination. *Development* 122: 2813-2822.

KLEIN, G., LANGEGGER, M., TIMPL, R. and EKBLOM, P. (1988). Role of laminin A chain in the development of epithelial cell polarity. *Cell* 55: 331-341.

KONISHI, I., FUGII, S., OKAMURA, H., PARMLEY, T. and MORI, T. (1986). Development of interstitial cells and ovigerous cords in the human fetal ovary: an ultrastructural study. *J. Anat.* 148: 121-135.

KOOPMAN, P., GUBBAY, J., VIVIAN, N., GOODFELLOW, P. and LOVELL-BADGE, R. (1991). Male development of chromosomally female mice transgenic for *Sry*. *Nature* 351: 117-121.

LIANG, L., SOYAL, S.M. and DEAN, J. (1997). *FIGalpha*, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. *Development* 124: 4939-4947.

MARTINEAU, J., NORDQVIST, K., TILMANN, C., LOVELL-BADGE, R. and CAPEL, B. (1997). Male-specific cell migration into the developing gonad. *Curr. Biol.* 7: 958-968.

MCGRATH, S.A., ESQUELA, A.F. and LEE, S.J. (1995). Oocyte-specific expression of growth/differentiation factor-9. *Mol. Endocrinol.* 9: 131-136.

MERCHANT, H. (1975). Rat gonadal and ovarian organogenesis with and without germ cells. An ultrastructural study. *Dev. Biol.* 44: 1-21.

MERCHANT-LARIOS, H. and CHIMAL-MONROY, J. (1989). The ontogeny of primordial follicles in the mouse ovary. *Prog. Clin. Biol. Res.* 296: 55-63.

MOORE, K.L. (1988). *The Developing Human*. W. B. Saunders Company, Philadelphia.

MORAIS DA SILVA, S., HACKER, A., HARLEY, V., GOODFELLOW, P., SWAIN, A. and LOVELL-BADGE, R. (1996). *Sox9* expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. *Nat. Genet.* 14: 62-68.

ODOR, D.L. and BLANDAU, R.J. (1969). Ultrastructural studies on fetal and early postnatal mouse ovaries. 1. Histogenesis and organogenesis. *Am. J. Anat.* 124: 163 - 186.

PALMER, S.J. and BURGOYNE, P.S. (1991). *In situ* analysis of fetal, prepuberal and adult XX×XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. *Development* 112: 265-268.

PEPLING, M.E. and SPRADLING, A.C. (1998). Female mouse germ cells form synchronously dividing cysts. *Development* 125: 3323-3328.

PEPLING, M.E. and SPRADLING, A.C. (2001). Mouse ovarian germ cell cysts undergo programmed breakdown to form primordial follicles. *Dev. Biol.* 234: 339-351.

RAJAH, R., GLASER, E.M. and HIRSHFIELD, A.N. (1992). The changing architecture of the neonatal rat ovary during histogenesis. *Dev. Dyn.* 194: 177-192.

RAYMOND, C.S., MURPHY, M.W., O'SULLIVAN, M.G., BARDWELL, V.J. and ZARKOWER, D. (2000). Dmrt1, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. *Genes Dev.* 14: 2587-2595.

RAYMOND, C.S., SHAMU, C.E., SHEN, M.M., SEIFERT, K.J., HIRSCH, B., HODGKIN, J. and ZARKOWER, D. (1998). Evidence for evolutionary conservation of sex-determining genes. *Nature* 391: 691-695.

RUSSELL, E.S. (1979). Hereditary anemias of the mouse: a review for geneticists. *Adv. Genet.* 20: 357-459.

SINCLAIR, A.H., BERTA, P., PALMER, M.S., HAWKINS, J.R., GRIFFITHS, B.L., SMITH, M.J., FOSTER, J.W., FRISCHAUF, A.-M., LOVELL-BADGE, R. and GOODFELLOW, P.N. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* 346: 240-244.

SKINNER, M.K., TUNG, P.S. and FRITZ, I.B. (1985). Cooperativity between Sertoli cells and testicular peritubular cells in the production and deposition of extracellular matrix components. *J. Cell Biol.* 100: 1941-1947.

SOYAL, S.M., AMLEH, A. and DEAN, J. (2000). FIGalpha, a germ cell-specific transcription factor required for ovarian follicle formation. *Development* 127: 4645-4654.

SWAIN, A. and LOVELL-BADGE, R. (1999). Mammalian sex determination: a molecular drama. *Genes Dev.* 13: 755-767.

SWAIN, A., NARVAEZ, V., BURGOYNE, P.S., CAMERINO, G. and LOVELL-BADGE, R. (1998). *Dax1* antagonizes *Sry* action in mammalian sex determination. *Nature* 391: 761-767.

SWAIN, A., ZANARIA, E., HACKER, A., LOVELL-BADGE, R. and CAMERINO, G. (1996). Mouse *Dax1* expression is consistent with a role in sex determination as well as adrenal and hypothalamus function. *Nat. Genet.* 12: 404-409.

TAM, P.P. and SNOW, M.H. (1981). Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *J. Embryol. Exp. Morphol.* 64: 133-147.

TREIER, M., GLEIBERMAN, A.S., O'CONNELL, S.M., SZETO, D.P., MCMAHON, J.A., MCMAHON, A.P. and ROSENFELD, M.G. (1998). Multistep signaling requirements for pituitary organogenesis in vivo. *Genes Dev.* 12: 1691-1704.

VAINIO, S., HEIKKILA, M., KISPERT, A., CHIN, N. and MCMAHON, A.P. (1999). Female development in mammals is regulated by Wnt-4 signalling. *Nature* 397: 405-409.