Building an Ovary: Insights into Establishment of Somatic Cell Lineages in the Mouse

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Development · FOXL2 · Granulosa cells · Ovary · Sex determination · Theca cells · WNT pathway

Abstract

The molecular pathways that drive the differentiation of somatic cell populations in the testis and ovary have been the subjects of intensive research over the past decade. It is now clear that ovarian differentiation is a coordinate event driven by secreted factors including R-spondin1, WNT4, and follistatin and transcriptional regulators such as β -catenin and FOXL2. These factors direct bipotential somatic cell lineages toward an ovarian fate and simultaneously suppress the emergence of testis-determining processes. This review summarizes the molecular pathways responsible for establishment of the ovary and discusses the current hypotheses on the origin(s) of somatic cell lineages and how these somatic cells acquire the characteristics necessary for their function during ovarian development and maintenance.

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During organogenesis, cells acquire tissue-specific identities that shape their developmental fate and future functions in a given organ. This process of cell fate specification is facilitated by coordinate interactions among numerous signals which originate from either the cell itself, or the surrounding environment, or both. Sex determination of the gonad is an ideal model for the study of cell lineage specification as 2 distinctively different organs (testis and ovary) arise from a common bipotential organ called the genital ridge or gonadal primordium. In mammals, expression of the testis-determining gene *SRY* (sex-determining region of the Y chromosome) tips the balance towards testis differentiation [Sinclair et al., 1990; Koopman et al., 1991; Albrecht and Eicher, 2001]. In a normal XX individual (or in the absence of the *SRY* gene), the bipotential gonad undergoes ovarian differentiation which eventually leads to the formation of follicles consisting of a single oocyte surrounded by somatic granulosa cells and theca cells. Over the past decade, significant progress has been made toward understanding ovarian differentiation at both the molecular and cellular level. This review focuses on the differentiation of the somatic compartment of the mammalian ovary, using the mouse as the model organism, with emphases on granulosa and theca cells populations, their potential fetal origins, and the molecular pathways that drive their differentiation and maintenance.

Morphological Changes from the Bipotential Gonad to an Ovary

Around embryonic day 10 (E10) in the mouse, the somatic compartment of the bipotential gonad arises from the thickening of coelomic epithelium on the surface of the mesonephros, a transient nephric organ that later contributes to reproductive tract formation [Swain and Lovell-Badge, 1999; Kusaka et al., 2010]. Germ cells, in contrast to somatic cells, originate from the epiblast outside of the bipotential gonad [Ginsburg et al., 1990; Lawson and Hage, 1994; Lawson, 1999] and migrate into the bipotential gonad between E10.5 and E11.5. Gonadal sex determination in the mouse embryo starts at E10.5 with a transient expression of *Sry* in a particular somatic cell population that becomes the Sertoli cells, the supporting cell lineage in the testis [Sinclair et al., 1990; Koopman et al., 1991; Albrecht and Eicher, 2001]. As a consequence of SRY action, dramatic morphological changes (increased cell proliferation and formation of testis cords and the coelomic vessel) occur in the fetal testis. At this stage, the ovary is smaller than the testis, and ovarian morphology remains similar to the bipotential gonad. The first distinguishable sign of ovarian differentiation appears around E13.5 when germ cells enter meiosis in an anterior to posterior fashion [McLaren and Southee, 1997; Menke et al., 2003; Yao et al., 2003]. Female germ cells then arrest at prophase of meiosis I and initiate folliculogenesis close to the time of birth when they form primordial follicles [for a review see Edson et al., 2009]. Each primordial follicle is composed of a single oocyte surrounded by a layer of flattened supporting cells called granulosa cells. A basal lamina separates the oocyte and granulosa cells from the ovarian interstitium or mesenchyme. As follicles grow, the granulosa cells become cuboidal in the primary follicle and proliferate into multilayers in the secondary follicle and antral follicles. Starting at the secondary follicle stage, the steroidogenic cell population called theca cells appears and surrounds the granulosa cell layers outside of the basal lamina. The major role of these theca cells is to produce androgens that are converted to estrogens by the granulosa cells and to support vascularization of the follicle [Magoffin, 2005].

Molecular Pathways That Drive Ovarian Differentiation and Maintenance

Ovarian differentiation was once considered to be a default process that occurs in the absence of SRY. This perception is based on the fact that no histological trans-

formation appears in the ovary during the time of sex determination, in contrast to the dramatic morphological changes in the testis at this time. Indeed, spontaneous human mutations and mouse genetic studies have uncovered numerous genes in the testis-determining pathway downstream of SRY [Vigier et al., 1989; Sinclair et al., 1990; Kent et al., 1996; Schafer et al., 1996; Colvin et al., 2001], further supporting the conclusion that testis formation is actively driven. In parallel to the role of SRY in testis determination, an ovarian determining (*Od*) gene(s) was proposed to trigger ovarian differentiation, and that this gene is negatively regulated by the testis-determining gene in the XY gonads [Eicher and Washburn, 1983; Washburn and Eicher, 1983]. However, the identification of XX individuals that develop testes in the absence of the SRY gene offers a different view on how ovarian differentiation is initiated [McElreavey et al., 1993; Vaiman et al., 1996]. Such cases of ovary-to-testis sex reversal in the absence of SRY have led to the hypothesis that an ovary-specific *Z* gene inhibits the testis-determining pathway while promoting ovarian differentiation. In the testis, the *Z* gene is suppressed by SRY, therefore allowing the progression of testis development [McElreavey et al., 1993]. Genetic analyses of ovary-to-testis sex reversal cases in human and animals have identified potential candidates for the *Z* gene, including *WNT4* [Vainio et al., 1999; Biason-Lauber et al., 2004, 2007], *RSPO1* [Parma et al., 2006; Chassot et al., 2008; Tomizuka et al., 2008], and *FOXL2* [Crisponi et al., 2001; Pailhoux et al., 2001, 2002]. In the mouse gonad, all of these cited genes are expressed in an ovary-specific pattern before any morphological changes are observed in the ovary [Nef et al., 2005].

Both *Wnt4* and *Rspo1* are already expressed in the somatic cells of the bipotential gonad, and their expression becomes ovary specific at E11.5 [Vainio et al., 1999; Parma et al., 2006]. Knockout mouse models reveal that *WNT4* and *RSPO1* are parts of a common signaling pathway that acts through β -catenin (fig. 1A). Global knockouts of *Wnt4* and *Rspo1* and somatic cell specific ablation of β -catenin all result in similar phenotypes in the ovary, characterized by the formation of testis-specific vasculature, appearance of ectopic adrenal cells, and a loss of germ cells [Vainio et al., 1999; Chassot et al., 2008; Tomizuka et al., 2008; Liu et al., 2009]. The involvement of β -catenin as a downstream regulator of *WNT4* and *RSPO1* is further supported by the observation that ectopic activation of β -catenin in the somatic cells of *Wnt4* or *Rspo1* knockout ovaries rescues the phenotypes of these mice [Chassot et al., 2008; Liu et al., 2010]. The expression of *Wnt4*, but not *Rspo1*, appears to be regulated in an au-

Fig. 1. Molecular pathways involved in granulosa cell specification and maintenance. **A** Fetal granulosa cell fate is driven primarily by 2 secreted proteins, RSPO1 and WNT4, which presumably act on the pregranulosa cells in an autocrine/paracrine manner. Based on the evidence from other model systems, WNT4/RSPO1 synergistically activate β -catenin that induces transcription of ovarian factors such as *Fst*, *Bmp2*, and *Wnt4* itself. FST inhibits activin B which causes formation of testis-specific coelomic vessel and loss of female germ cells. In addition, the WNT4/RSPO1/ β -catenin

pathway antagonizes the emergence of Sertoli cell fate by suppressing *Sox9*. Transcription factor FOXL2 alone does not have a determining role in ovarian specification in mice; however, it participates to some degree in the suppression of *Sox9* and activation of other ovarian genes such as *Fst*. **B** In the adult ovary, granulosa cell identity is maintained by FOXL2 and estrogen receptor (ER) which suppress the transcription of *Sox9*. ER α is activated by the presence of estrogens (E2) as a result of the conversion of androgens by the steroidogenic enzyme aromatase CYP19.

ocrine or paracrine manner as the expression of *Wnt4* is downregulated in the absence of *Rspo1* or β -catenin [Chassot et al., 2008; Liu et al., 2009]. Additional downstream targets of the WNT4/RSPO1/ β -catenin pathway have been identified, including follistatin (*Fst*) and *Bmp2* [Yao et al., 2004; Manuylov et al., 2008; Liu et al., 2009]. *Fst* expression is detected in the fetal mouse ovary as early as E11.5 [Menke and Page, 2002; Yao et al., 2004]. In addition to the WNT4/RSPO1/ β -catenin pathway, *Fst* is also cooperatively regulated by FOXL2 and BMP2 [Kashimada et al., 2011]. FST prevents the formation of testis-specific vasculature and germ cell death through inhibition of activin B [Yao et al., 2004, 2006; Liu et al., 2010]. At the molecular level, the WNT4/RSPO1/ β -catenin pathway exhibits similar anti-testis activity, a characteristic of the Z factor. Without *Wnt4* or *Rspo1*, fetal mouse ovaries show signs of sex reversal with the expression of testis markers *Sox9* and *Amh*. This antagonism of *Sox9* expression by the ovarian pathway could be due to the inhibitory capacity of β -catenin at the *Sox9* enhancer [Maatouk et al., 2008; Manuylov et al., 2008; Bernard et al., 2012]. There are also mutual antagonisms between *Rspo1* and *Sox9* and between *Wnt4* and *Fgf9* [Kim et al.,

2006; Jameson et al., 2012a; Lavery et al., 2012]. Removal of *Rspo1* in the *Sox9* knockout testis restores testis development, indicating that *Rspo1* is responsible for the testis-to-ovary sex reversal in the *Sox9* knockout male [Lavery et al., 2012]. Similarly, loss of *Wnt4* rescues the testis-to-ovary sex reversal in *Fgf9* or *Fgfr2* knockout males [Jameson et al., 2012a]. Therefore, testis differentiation requires repression of *Rspo1* and *Wnt4*, as expected for a potential Z gene. However, similar to the *Rspo1/Sox9* double knockout, absence of *Fgf9* does not rescue the sex reversal phenotype of *Wnt4* knockout ovaries, suggesting that ovarian development requires repression of other masculinizing factors [Jameson et al., 2012a; Lavery et al., 2012].

The anti-testis nature of these ovarian genes is also demonstrated in the context of fetal testes. Overexpression of *Wnt4* in fetal mouse testes affects testis vasculature and steroid production but is not sufficient to induce testis-to-ovary sex reversal [Jeays-Ward et al., 2003; Jordan et al., 2003]. However in humans, duplication of a portion of chromosome 1p that contains both *WNT4* and *RSPO1* is sufficient to disrupt testis differentiation and induce testis-to-ovary sex reversal [Jordan et al., 2001; Tevosian and Manuylov, 2008]. A similar testis-to-ovary sex

reversal phenotype is observed in XY mice when β -catenin is ectopically expressed in the somatic cell lineage [Maa-touk et al., 2008]. FOXL2 also has anti-testis properties, as its overexpression in the fetal testis results in disorganized seminiferous tubules and reduced expression of *Sox9*, *Amh*, and other testis-specific genes [Ottolenghi et al., 2007].

The involvement of the WNT4/RSPO1/ β -catenin pathway in early ovarian differentiation is beyond any doubt; however, it is worth noting that the phenotypes in *Wnt4*, *Rspo1*, and *Ctnnb1* (coding for β -catenin) knock-out ovaries are not identical. Although a weak and transient expression of *Sox9* is detected in both *Rspo1* and *Wnt4* knockout ovaries at E11.5, a strong upregulation of *Sox9* is only detected in *Rspo1* knockout ovaries at the time of birth [Vainio et al., 1999; Kim et al., 2006; Ottolenghi et al., 2007; Chassot et al., 2008]. In addition, no upregulation of *Sox9* expression is found in somatic cell-specific *Ctnnb1* knockout ovaries [Manuylov et al., 2008; Liu et al., 2009]. These observations imply that the WNT4/RSPO1/ β -catenin pathway is not linear and could involve other unknown factors in a synergistic or redundant manner.

While the initial establishment of ovarian somatic cell fate is controlled by the WNT4/RSPO1/ β -catenin pathway, differentiation and maintenance of ovarian somatic cell identity require the transcription factor FOXL2 [Schmidt et al., 2004; Ottolenghi et al., 2005]. Unlike *Rspo1* or *Wnt4* knockout mice, inactivation of *Foxl2* does not disturb early ovarian differentiation [Ottolenghi et al., 2005]. In the fetal mouse ovary, FOXL2 protein appears in the nuclei of some somatic cells at E12.5, and this expression is maintained through adult life in granulosa cells [Loffler et al., 2003]. The ovary-specific expression of *Foxl2* is highly conserved among vertebrates [Baron et al., 2005]. In humans, mutations of the *FOXL2* gene cause the blepharophimosis epicanthus inversus syndrome, characterized by premature ovarian failure among other symptoms [Crisponi et al., 2001]. The same ovarian phenotype is also observed in *Foxl2* knockout mice in which follicles are arrested at the primordial stage as a result of the failure of granulosa cells to differentiate from a squamous to a cuboidal form [Schmidt et al., 2004; Uda et al., 2004]. In the adult ovary, granulosa cell identity requires FOXL2 as conditional ablation of *Foxl2* leads to transdifferentiation of granulosa cells into Sertoli cells, characterized by the expression of Sertoli-cell specific markers such as *Sox9* and *Dmrt1* [Uhlenhaut et al., 2009]. A similar transdifferentiation phenotype is observed in the adult mouse ovary when estrogen signaling is inhibited. In the

absence of the estrogen-producing enzyme aromatase (or *Cyp19*) or receptors for estrogen (estrogen receptor α and β), granulosa cells transdifferentiate into Sertoli cells in the adult ovary, yet early ovarian differentiation appears normal [Couse and Korach, 1999; Britt et al., 2001; Britt and Findlay, 2003]. In marsupials and some non-mammalian species, estrogen signaling prevents translocation of SOX9 into the nucleus, therefore blocking the initiation of testis differentiation [for a review see Pask, 2012]. In the adult mouse ovary, FOXL2 acts in conjunction with ER α as a direct transcriptional repressor of the testis pathway by binding to the regulatory sequence of *Sox9* [Uhlenhaut et al., 2009]. These genetic evidences demonstrate that, at least in mice, the differentiated state of adult granulosa cells is reversible, and FOXL2 and estrogen signaling serve as guardians of granulosa cell identity (fig. 1B).

The involvement of FOXL2 in ovarian differentiation seems to be a species-specific event. In female goats with polled intersex syndrome (PIS), a 11.7-kb DNA fragment that contains *FOXL2* and 2 long non-coding RNAs is deleted [Pailhoux et al., 2001]. As a result of this deletion, the PIS females develop a complete ovary-to-testis sex reversal during fetal life, in contrast to the normal ovarian development observed in *Foxl2* knockout mouse embryos [Pailhoux et al., 2002]. The difference in response to the loss of *FOXL2* in these 2 species may be due to either an involvement of the 2 non-coding RNAs in the PIS goats or a difference in steroidogenic activity in the fetal ovaries between mouse and goat. Fetal mouse ovaries are steroidogenically inactive; however, the fetal goat ovary is capable of producing and responding to estrogens based on the expression of *Cyp19* and ER β in pregranulosa cells [Pannetier et al., 2006]. The presence of both FOXL2 and estrogen signaling in the fetal goat ovary could result in an environment similar to that in the adult mouse ovary, where granulosa cells require both FOXL2 and the estrogen pathway to maintain their identities.

Although loss of *Foxl2* does not appear to affect fetal ovarian differentiation in the mouse, subtle molecular changes are observed in the *Foxl2* knockout fetal ovary. It shows an upregulation of testis-specific genes such as *Sox9* and *Inhbb* and downregulation of ovarian genes such as *Fst* [Ottolenghi et al., 2007; Garcia-Ortiz et al., 2009]. Interestingly, double knockout of *Wnt4* and *Foxl2* or *Rspo1* and *Foxl2* leads to an earlier and more pronounced ovary-to-testis sex reversal phenotype than a single knockout of *Wnt4* or *Rspo1* [Ottolenghi et al., 2007; Auguste et al., 2011]. This observation suggests a complementary action between the WNT4/RSPO1 pathway and

FOXL2 in ovarian differentiation. It is worth noting that no complete ovary-to-testis sex reversal is observed in these double knockout models, indicating the involvement of other unidentified factors. All these data accumulated over the years suggest that instead of a single Z gene, multiple ovarian genes may act independently or synergistically to promote ovarian differentiation while suppressing the testis pathway.

Granulosa Cells: Where Do They Come From?

Different sources of granulosa cells in mammalian ovaries have been proposed, including the gonadal mesenchyme [Pinkerton et al., 1961], the mesonephros [Byskov and Lintern-Moore, 1973], or the surface epithelium [Gondos, 1975; Juengel et al., 2002; Sawyer et al., 2002]. In the bovine ovary, a particular cell type from the surface epithelium of the mesonephros has been shown to give rise to both granulosa cells and the surface epithelium of the ovary [Hummitzsch et al., 2013]. Evidence from a series of experiments utilizing chimeric mouse embryos led Burgoyne and colleagues to propose that Sertoli cells in the testis and granulosa cells in the ovary are derived from a common supporting cell lineage. This hypothesis is based on the findings that some XX somatic cells in the chimeric XX/XY testis become Sertoli cells, whereas some XY somatic cells in the chimeric XX/XY or XO/XY ovary differentiate into granulosa cells [Palmer and Burgoyne, 1991a, b; Patek et al., 1991]. Later studies involving a transgenic mouse model that contains an EGFP reporter gene under the control of the *Sry* promoter (termed *Sry-EGFP*) further confirmed this hypothesis [Albrecht and Eicher, 2001]. *Sry-EGFP*-positive cells are detected in the bipotential gonads of both sexes immediately below the coelomic epithelium and extending to the center of gonads near the mesonephros. In the fetal ovary, *Sry-EGFP*-positive cells become granulosa cells in the primordial follicle and express FOXL2 [Mork et al., 2012]. These findings together demonstrate that the supporting cell types in the ovary share a common origin with their male counterparts and that both cell populations possess the molecular machinery necessary for the induction of *Sry* expression.

Although granulosa cells likely derive from a common supporting cell lineage in the bipotential gonad, emerging evidence suggests that the granulosa cell population is heterogeneous. Examining the same *Sry-EGFP* model described above, Mork et al. [2012] discovered that cells of the ovarian surface epithelium (or coelomic epithelium)

move into the ovarian proper and then become positive for *Sry-EGFP* and FOXL2. However, some FOXL2-positive cells are *Sry-EGFP* negative, implying that these FOXL2-positive/*Sry-EGFP* negative cells come from different sources. This discovery leads to a new model of granulosa cell specification: in the bipotential gonad, some cells from the coelomic epithelium migrate into the mesenchyme at the center of the gonad and give rise to the first set of granulosa cells. These pregranulosa cells express FOXL2 and participate in the formation of primordial follicles in the medulla immediately after birth. During later fetal development and shortly after birth (E14.5 to postnatal day 4), some cells from the ovarian surface epithelium continue to move into the ovary and give rise to a second wave of granulosa cells. These cells will form the follicles in the cortex that are activated during adult life [for a schematic view of this model see Mork et al., 2012]. However, these findings do not exclude the possibility that somatic cells present in the bipotential gonad are also a source of granulosa cells. The concept of heterogeneous populations of granulosa cells is also corroborated by the findings by Auguste et al. [2011]. In the absence of *Rspo1*, FOXL2 expression is missing from the medial region of the ovary under the surface epithelium but is maintained in the other half of the ovary adjacent to the mesonephros. These data suggest that at least 2 different FOXL2-positive populations exist within the fetal ovary: one expressing *Foxl2* in an *Rspo1*-dependent manner under the surface epithelium, and the other population expressing *Foxl2* independent of *Rspo1* close to the mesonephros. Heterogeneity within granulosa cells population was further brought to light by Harikae et al. [2013]. They identified a subpopulation of pre-granulosa cells that continuously retains an 'SRY-dependent SOX9-inducibility' (SDSI) throughout fetal and early postnatal stages whereas this ability should be lost by E12.5. Interestingly, these SDSI-positive pre-granulosa cells are located near the mesonephros and participate in the formation of primordial follicles in the medulla at birth, similarly to the first set of FOXL2-positive granulosa cells observed by Mork et al. [2012]. A study of the developmental dynamics of primordial follicles described the presence of 2 populations of follicles with one population in the medulla that is simultaneously activated right after birth and the other population in the cortex that is progressively activated throughout adulthood [Zheng et al., 2013]. This study seems to support the notion that the first wave of granulosa cells contributes to folliculogenesis in the medulla, whereas the second wave of granulosa cells participates in follicle formation in the cortex.

Examination of the expression patterns of various ovary-specific genes reveals an even more complicated picture of the composition of the somatic cell populations in the fetal ovary. For example, *Rspo1* mRNA is present throughout the fetal ovary including the ovarian surface epithelium, whereas *Wnt4*, *Foxl2*, and *Fst* are expressed in the ovarian proper but not in the surface epithelium domain [Chen et al., 2012]. *Bmp2*, another ovary-specific gene, is produced by a population of cells located right underneath the surface epithelium [Yao et al., 2004]. Cell-type specific microarray analysis showed that *Bmp2* is expressed in a variety of somatic cell lineages in the fetal ovary, including the *Sry*-EGFP population, suggesting that some *Bmp2*-positive cells belong to the pregranulosa cell population [Jameson et al., 2012b]. One explanation for the diverse expression pattern of ovarian genes is that it simply reflects the differentiation progress of one somatic cell population. However, the simultaneous expression of these genes in different domains of the ovary argues against this hypothesis. Instead, these data seem to favor the notion that granulosa cells are composed of different sub-populations expressing slightly different sets of genes. Whether these different sub-population of cells have unique functions remains to be determined.

Theca Cells, the Other Major Somatic Cell Type of the Follicle

Theca cells are fibroblast-like flattened cells that constitute the vascularized and steroidogenic layers of the mature follicle. Mature theca cells are characterized by expression of luteinizing hormone receptor (LHR) and genes involved in steroidogenesis (steroidogenesis acute regulatory protein (*STAR*), *CYP17A1*, *CYP11A1*, and *HS-D3B*) [Magoffin and Weitsman, 1993]. Theca cells synthesize androgens which are then converted into estrogens by granulosa cells [Erickson et al., 1985]. These steroidogenic enzymes are used as markers for theca cells in the adult ovary and are first detected adjacent to early secondary follicles, suggesting that theca cells are only activated postnatally [Magoffin and Magarelli, 1995]. In vitro studies using the bovine or human ovarian stromal tissue show that recruitment and activation of theca cells from the stroma requires pituitary-derived LH and local factors including insulin-like growth factor (IGF), KIT ligand, and growth differentiation factor 9 (GDF9) [for a review see Young and McNeilly, 2010]. In particular, the oocyte-derived GDF9 appears to be involved in theca cell differentiation, as the folliculogenesis in *Gdf9* knockout

mouse and *GDF9* mutant sheep arrests at the primary stage, and theca cell recruitment is blocked [Dong et al., 1996; Elvin et al., 1999; Nicol et al., 2009]. GDF9 may act directly on theca cells or indirectly through the stimulation of specific molecular pathways by the granulosa cells [Elvin et al., 1999]. Indeed, granulosa cells secrete various factors, such as KIT ligand or IGF, which are involved in theca cell recruitment and activation [for a review see Young and McNeilly, 2010]. When co-cultured with granulosa cells, stromal cells from the mature bovine ovary differentiate into theca cells, suggesting that precursors of the theca cells are present in the mesenchyme of the ovary [Orisaka et al., 2006]. Honda et al. [2007] isolated and cultured putative theca cell precursors from neonatal mouse ovaries. When exposed to granulosa-cell conditioned medium or a cocktail of IGF, KIT ligand, and GDF9, these cells expressed markers of theca cells. Moreover, when transplanted into donor ovaries, these differentiated cells integrated into the theca cell layers of follicles. Thus, the theca cell lineage appears to be already determined at the onset of folliculogenesis.

Similar to the hypothesis that a common precursor exists for the somatic supporting cells in the testis and ovary (Sertoli cells vs. granulosa cells), a common precursor for steroidogenic cells (Leydig cells in the testis and theca cells in the ovary) is suspected. In the testis, some Leydig cell precursors appear to be present already at the bipotential stage, as these gonads are able to produce testosterone in culture [Merchant-Larios et al., 1993]. In search for the progenitor population of the interstitial cells in the gonads, DeFalco et al. [2011] uncovered that 2 MAF transcription factors (MAFB and C-MAF) mark the interstitial cell population in both fetal testes and ovaries. Cell lineage tracing of these markers showed that distinct progenitor lineages, the surface epithelium and an unknown cell type along the vascularized gonad-mesonephros border, give rise to the interstitial/stromal compartment of the gonad, and that some of these cells can differentiate into fetal Leydig cells in the testis. In the fetal ovary, the MAFB/C-MAF-positive cells are negative for the granulosa cell lineage marker FOXL2, suggesting that they could be the progenitors for the theca cells.

Based on their similar steroidogenic activity and regulation, knowledge on how the Leydig cell population is established could be applied to understand how theca cell differentiation is initiated. The signaling pathway induced by the ligand Desert hedgehog (DHH) is responsible for the specification of fetal Leydig cells in mice and rats [Pierucci-Alves et al., 2001; Kawai et al., 2011]. Mutations or loss of *Dhh* lead to decreased Leydig cell numbers

and consequent androgen deficiency [Pierucci-Alves et al., 2001; Yao et al., 2002]. Moreover, chemical inhibition of the hedgehog pathway abolishes the fetal Leydig cell population, suggesting that other hedgehog ligands are required for Leydig cell development [Yao and Capel, 2002; Barsoum and Yao, 2011]. The hedgehog pathway acts through its receptor PTCH1 and intracellular regulator GLI1. In the postnatal and adult mouse ovary, theca cells are positive for *Ptch1* and *Gli1*, while the ligands *Dhh* and Indian hedgehog (*Ihh*) are produced by the granulosa cells [Wijgerde et al., 2005]. Moreover, in vitro culture of bovine theca cells in the presence of Sonic hedgehog induces cell proliferation, androgen synthesis, and *Gli1* expression [Spicer et al., 2009]. These evidences implicate a potential role for the hedgehog pathway in theca cell differentiation in the ovary.

Conclusions and Perspectives

With the molecular mechanisms underlying ovarian differentiation gradually coming to light, new questions emerge regarding where these factors are produced and how they act to control the establishment of various somatic cell lineages. The unique expression patterns of *Wnt4*, *Rspo1*, *Foxl2*, and other ovary-specific genes imply the presence of subpopulations of granulosa cells. Identification of lineage markers for the interstitial cells reveals that potential progenitors of theca cells reside in the fetal

ovary long before their differentiated progeny appear in the adult ovary. The origins of somatic cells in the ovary are more complex than expected, with probably multiple sources for both granulosa and theca cells. The surface epithelium that encloses the gonad, mesenchymal cells that are already present in the gonads, or cells that migrate from the mesonephros are potential contributors to the somatic cell lineages. Cell tracing of early markers of somatic cells is a powerful tool to uncover the origins of theca or granulosa cell populations, and recent studies using this strategy provide promising results [DeFalco et al., 2011; Liu et al., 2012; Mork et al., 2012]. Cell-specific microarrays lead to the identification of many novel genes specifically expressed in the somatic compartment of the ovary at the time of sex determination [Bouma et al., 2004; Jorgensen and Gao, 2005; Nef et al., 2005; Beverdam and Koopman, 2006; Chen et al., 2012; Jameson et al., 2012b]. Challenges await us to identify the functional roles of these novel candidates and to connect the molecular pathways with cell fate specification processes that ultimately determine the identities of ovarian cells.

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