Roles of KIT and KIT LIGAND in ovarian function

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Evidence from mouse mutants indicates that the Kit gene encoding KIT, a receptor present on the oocyte and theca cells, and the Mgf gene encoding KIT LIGAND, the ligand of KIT, are important regulators of oogenesis and folliculogenesis. Recently, in vitro cultures of fetal gonads, of follicles and of oocytes have identified specific targets for the KIT–KIT LIGAND interaction. In fetal gonads, an anti-apoptotic effect of KIT–KIT LIGAND interactions on primordial germ cells, oogonia and oocytes has been demonstrated. In postnatal ovaries, the initiation of follicular growth from the primordial pool and progression beyond the primary follicle stage appear to involve KIT–KIT LIGAND interactions. During early folliculogenesis, KIT together with KIT LIGAND controls oocyte growth and theca cell differentiation, and protects preantral follicles from apoptosis. Formation of an antral cavity requires a functional KIT–KIT LIGAND system. In large antral follicles, the KIT–KIT LIGAND interaction modulates the ability of the oocyte to undergo cytoplasmic maturation and helps to maximize thecal androgen output. Hence, many steps of oogenesis and folliculogenesis appear to be, at least in part, controlled by paracrine interactions between these two proteins.

Mutations at the dominant white spotting (W) and Steel (Sl) loci in mice affect the proliferation, migration and survival of germ cells, melanocytes and hematopoietic progenitor cells during embryogenesis and adult life. Hence, in homozygous mice, most of these mutations are lethal and mice that survive are generally sterile (Russell, 1979). Different lines of evidence demonstrated that the c-kit proto-oncogene maps to the W locus. First, both the W locus and the Kit gene are located on mouse chromosome 5 and are very tightly linked (Chabot et al., 1988). Second, some specific mutants (W4) are associated with molecular rearrangements in the structure of the Kit gene (Geissler et al., 1988). Third, the Kit gene is expressed in the cells affected by the W mutations (Reith et al., 1990). Similar approaches have also been used to establish the link between KIT LIGAND on chromosome 10 and the Mgf mutations.

The Kit gene encodes a receptor (KIT) belonging to the family of type III transmembrane tyrosine kinase receptors (the family also includes the receptor for colony-stimulating factor 1 and platelet-derived growth factors a and b), and its transcript (5.5 kb) encodes a 975 amino acid protein, which may be glycosylated. The mature protein has a molecular mass ranging from 125 to 160 kDa (Dubreuil et al., 1990; Fleischman, 1993). The Mgf cDNA encodes a protein (KIT LIGAND) of 248 amino acids with a signal peptide, a 189 amino acid extracellular domain, a 23 amino acid acid membrane spanning segment and a 36 amino acid cytoplasmic domain (Williams et al., 1992). There are two membrane-bound forms, KL-1 and KL-2, which can be cleaved, producing a soluble form of KIT LIGAND. The ratio between the two forms appears to vary greatly among tissues.

Expression of the mRNAs coding for the KIT and KIT LIGAND proteins will be described in the first section of this review. Insights into their regulation will be provided in a brief second section. The remainder of the review will outline the relevance of the KIT–KIT LIGAND interaction for ovarian function, mainly in mice and sheep.

The sterility (owing to the absence of germ cells in their ovaries) of most KitW and MgfSl female homozygous mutants indicates a key role for the interaction between KIT and KIT LIGAND in the establishment of the store of primordial follicles during oogenesis. Furthermore, some Sl mutant phenotypes, such as MgfSl–t, in which follicles are present in reduced numbers but fail to initiate follicular growth from the primordial stage, indicate that the interaction between these compounds is relevant for later stages of follicular growth.

Kit/KIT and Mgf/KIT LIGAND expression

The main events occurring throughout oogenesis in mice and in sheep are summarized (Fig. 1).

Kit–KIT and Mgf–KIT LIGAND during oogenesis

As early as embryonic day 7.5, mouse germ cells display small amounts of Kit transcripts (Manova and Bachvarova, 1991). Kit expression is high in proliferating oogonia but stops when oogonia enter meiosis (Manova and Bachvarova, 1991). As a consequence, Kit transcripts are not present between embryonic days 13.5 and 15.5. In the ovaries of late fetuses
Kit transcripts are undetectable by RNA blot analysis (Manova et al., 1990) and a very limited expression (restricted to a few oocytes at the most advanced stages) can be detected using in situ hybridization (Manova et al., 1990) or using a reporter gene (β-galactosidase in transgenic mice in which one copy of Kit has been replaced by a construction including the β-galactosidase gene; K. Reynaud and M.A. Driancourt, unpublished). Hence, in mice, abundant expression of Kit coincides with primordial germ cell migration and mitosis of oogonia.

In the fetal ovaries of mice, Mgf appears to be expressed along the path of migration of primordial germ cells as early as embryonic day 9 (Matsui et al., 1990; Keshet et al., 1991). Expression is also detectable in the genital ridge (Matsui et al., 1990). Once colonization of the genital ridge is complete (at embryonic day 12.5), the expression of Mgf transcripts along the migratory path stops and transcripts are confined in the gonad. This finding indicates that cells expressing KIT LIGAND outline the pathway and guide the KIT-expressing cells towards the genital ridge. Within the gonad, Mgf is not expressed at embryonic day 14.5 (Keshet et al., 1991). Hence, there is tight synchronization of the expression of Kit and Mgf in the developing gonad.

In sheep, Tisdall (1999) reported Kit expression during the early stages of oogenesis and showed that its messenger is expressed as early as embryonic day 24. Expression stays high until embryonic day 55 and stops when meiosis starts. These results are in good agreement with an earlier report that used in situ hybridization to show that meiotic germ cells do not express Kit, while oocytes that have reached the diplotene stage of meiosis and are enclosed in primordial follicles re-initiate Kit expression (Clark et al., 1996). Expression of KIT LIGAND before embryonic day 90 has not yet been investigated. At embryonic day 90, KIT LIGAND is expressed in the mesenchymal cells of the ovarian cortex (Tisdall et al., 1997) and, at embryonic day 100, areas of expression are seen in groups of cells around isolated oocytes and primordial follicles. Therefore, it may be concluded that, in the ovine fetal ovary, as early as embryonic days 90-100, germ cells and somatic cells express Kit and Mgf, respectively.

There are few data available to confirm whether mRNA is translated to produce KIT and KIT LIGAND proteins at any stage of fetal life. In extensive studies, Manova et al. (1990, 1993) did not attempt to localize the KIT and KIT LIGAND proteins in fetal gonads by immunohistochemistry or demonstrate them by western blot analysis. An immunohistochemical study in mice by Horie et al. (1991) did not demonstrate the presence of KIT protein in fetal ovaries at embryonic day 15.5, while Yoshida et al. (1997) reported that Kit was present in mouse ovaries at embryonic day 17.5 and at birth. Tisdall et al. (1999) reported that, in sheep, the KIT protein is present in fetal ovaries even when no message is detected.

Fig. 1. Relationships in mice and sheep between Kit (KIT) and Mgf (KIT LIGAND) expression (right), the main events of oogenesis and folliculogenesis (centre) and associated changes in germ cell numbers (left). Arrows indicate time periods during which specific features of oogenesis–folliculogenesis occur. PGC, primordial germ cells.
Kit–KIT and Mgf–KIT LIGAND during early folliculogenesis

Early folliculogenesis (defined as occurring from birth to day 20 in mice, and from embryonic day 90 to birth in sheep) is characterized by: (1) an increased rate of follicle initiation from the primordial pool (Pedersen, 1969); (2) an accelerated rate of follicular growth (mice: Pedersen 1969); and (3) the absence of follicular atresia (sheep: Sonjaya and Driancourt, 1987).

Expression of Kit at this stage was described in the pioneering study of Manova et al. (1990). Kit expression, measured by RNA blot analysis becomes detectable at birth and is very high at days 8 and 17 of postnatal life (Fig. 2). Abundant expression of mRNA encoding Kit was visualized by in situ hybridization in primordial and growing oocytes in the ovaries of young mice (5, 8 and 10 days of age) (Manova et al., 1990). In addition, ovaries from older mice (17 days of age) contained follicles from a wide range of sizes (primordial to antral), in which all oocytes expressed Kit. This finding was confirmed using Kit<sup>W–lacZ/+</sup> mice, in which one copy of the Kit gene has been replaced by a β-galactosidase reporter gene (for the transgenic model, see Bernex et al., 1996). When this model is used, all oocytes appear to express Kit from birth to about 20 days of age (K. Reynaud and M. A. Driancourt, unpublished; Fig. 3). When quantification of the signal obtained after in situ hybridization was achieved, no difference in the grain density of Kit transcripts could be observed between small (15 μm in diameter), medium (44 μm) and large (58 μm) oocytes (Manova et al., 1990). Finally, starting at days 14–17, significant somatic cell expression (theca–interstitial cells) becomes detectable (Manova et al., 1990), although the Kit protein was not detectable within theca–interstitial cells (Horie et al., 1991). In contrast, the Kit protein is present on oocytes at all stages (Manova et al., 1990; Yoshida et al., 1997). In sheep, there is also evidence (Clark et al., 1996) that all oocytes from primary, secondary and antral follicles present at embryonic days 100, 120 and 135 also express Kit. Hence, there is general agreement that, in these two species, mRNA encoding Kit is expressed in oocytes of all sizes throughout early folliculogenesis.

The main studies on KIT LIGAND have been performed by groups led by Manova and Eppig (Manova et al., 1993; Joyce et al., 1999b). Both studies carefully monitored the amounts of mRNA yielding KL-1 and KL-2. The KL-1 protein occurs principally in a soluble form generated by proteolytic cleavage, while KL-2, which lacks such a cleavage site, is found primarily in the membrane bound form (although it may also occur in a biologically active soluble form). Expression, as measured by RNA blot and RNAse protection assay, was found to be high at birth, low from day 5 to day 8...
and high again after day 12. When in situ hybridization was performed, low expression was detected in primary follicles. Expression of KIT LIGAND mRNA increased in the granulosa cells of three layered follicles (days 8–13), at a time when theca cells differentiate from stroma cells. At this stage, steady state amounts of KL-1 and KL-2 were similar. Expression of KIT LIGAND mRNA remained high in early antral follicles (Manova et al., 1993), but decreased as follicular growth progressed towards the late antral stage, without any significant alteration in the ratio between KL-1 and KL-2 (Joyce et al., 1999b). Essentially similar results have been reported for sheep follicles (Tisdall et al., 1997). In antral follicles of both species, KIT LIGAND expression was restricted to mural granulosa cells and was absent within cumulus cells (Manova et al., 1993; Tisdall et al., 1997). Demonstration of the presence of the KIT LIGAND protein is available only in mice, in which Manova et al. (1993) showed that, as early as day 10 after birth, granulosa cells of preantral follicles were intensively immunostained, mainly along the basal membrane, while the outer layers of granulosa cells from early antral follicles also reacted strongly. A major limitation of all the above studies was that the specific age or follicle size effects could not be assessed since follicle size increases with the age of the animal.

**Kit–KIT and Mgf–KIT LIGAND during adult folliculogenesis**

Adult folliculogenesis features full follicular development to the preovulatory stage (that is, gonadotrophin-sensitive followed by gonadotrophin-dependent follicular growth), major atresia in large follicles and a reduced rate of follicular growth.

There are few studies of Kit–Mgf expression and protein throughout late prepubertal (over 25 days of age) and adult life. There is general agreement that the sites of production of these two compounds are not altered (KIT in oocytes and in some theca–interstitial cells; KIT LIGAND in granulosa cells). However, information is lacking on the effect of follicle size up to the preovulatory stage, of follicle atresia or of the stage of the oestrous cycle on kit expression in oocytes. Data reported by Manova et al. (1990) and Orr-Urtreger et al. (1990) indicate that oocytes contain a Kit message, but it is not clear whether all, or only a fraction of, oocytes do so (see Fig. 3). Kit expression in theca cells does not appear to be affected by follicle size in cattle, as expression is similar in small (< 5 mm), medium (5–10 mm) and large (> 10 mm) follicles (Parrott and Skinner, 1997). Whether Kit expression in theca cells is also affected by follicle atresia–apoptosis is unknown.

There was no link between KIT LIGAND expression and the age of the animal in mice or sheep (Manova et al., 1993; Tisdall et al., 1997). In both species, the KIT LIGAND message and protein are present in preantral follicles and in mural granulosa cells of antral follicles. However, changes in KL-1 or KL-2 associated with preovulatory enlargement in the few days before ovulation in cyclic mice have not yet been described. In contrast, the effects of follicle enlargement throughout gonadotrophin-dependent follicular growth have been characterized in cattle by Parrott and Skinner (1997), who demonstrated higher amounts of mRNA encoding KIT LIGAND (without assessing the respective amounts of KL-1 and KL-2) among granulosa cells of large (> 10 mm) compared with medium (5–10 mm) and small (< 5 mm) follicles. Further studies are required to clarify the effects of atresia on KIT LIGAND expression, to determine whether expression of KIT LIGAND is maintained in atretic follicles, as in sheep (Tisdall et al., 1997), or disappears as it appears to do in mice (Manova et al., 1993), or whether the discrepancy between the two studies is related to the use of follicles at different stages of atresia.

**Insights into the regulation of KIT and KIT LIGAND production**

Kit expression in the oocyte appears to be continuous throughout most of oogenesis and folliculogenesis. The availability...
of natural mutants (Steel Panda: Huang et al., 1993) or of genetically altered mouse models (recombinant growth differentiation factor-9 (GDF-9) knock-out: Elvin et al., 1999) indicates that Kit expression in the oocyte may be independent of GDF-9 expression by the oocyte or Kit LIGAND expression by granulosa cells. The main question that has not yet been clarified is: why does Kit expression cease when germ cells enter meiosis and start again after the diplotene block? A working hypothesis is that cessation of its expression may involve production of proteins of the inhibin–activin family by fetal gonads at this stage, since there is evidence from other cell types (mastocytes, haematopoietic cells) that ligands such as TGFβ or activin can reduce Kit expression (Hino et al., 1995; Sansilvestri et al., 1995). Mechanisms involved in the resumption of Kit expression after the diplotene block have yet to be explored.

Expression of Kit in theca cells appears to be initiated as soon as theca cells, and its regulation may involve LH, since hCG or the ovulatory LH surge have the ability to decrease Kit expression in theca cells (Motro and Bernstein, 1993). Local regulations (by TGFβ–activin) may also be operating.

Regulation of Mgf expression in granulosa cells is complex and needs to be analysed for specific types of follicle. In pre-antral follicles, Joyce et al. (1999b) used cultured intact or oocytecystomized preantral granulosa oocyte complexes to demonstrate that growing oocytes can upregulate expression of both forms of Kit LIGAND. Furthermore, FSH also had the ability to stimulate expression of KL-1 and KL-2 in a dose-dependent manner. In late antral follicles, a paracrine inhibitory effect of fully grown oocytes probably explains why no Kit LIGAND expression is detected in cumulus cells (Joyce et al., 1999b). A possible mediator of this effect is GDF-9, which is present in large oocytes in large amounts which are inversely related to the amount of Kit LIGAND (GDF-9 knock out: Elvin et al., 1999). One explanation for the observation that Kit LIGAND expression is restricted to mural granulosa cells in these follicles may be that testosterone, which stimulates expression of KL-1 and KL-2 (Joyce et al., 1999b), is produced by neighbouring thecal cells. Alternatively, FSH may be involved, since cells close to the basal lamina express more FSH receptors than do the more interior cells (Moniaux and De Reviers, 1989) and FSH can upregulate KL-1 and KL-2 expression. Furthermore, the elegant studies of Parrott and Skinner (1998a) in cattle demonstrated that keratinocyte growth factor (KGF) produced within theca cells can stimulate Mgf expression in granulosa cells and that Kit LIGAND can stimulate KGF expression further. This stimulatory loop may well be involved in the control of Kit LIGAND production at this stage. However, in mice, such a mechanism does not appear to be operative (Joyce et al., 1999b).

KIT–KIT LIGAND interactions: possible roles during oogenesis in mice

Effects on primordial germ cells and oogonia

There is ample evidence that the messengers coding for Kit and Kit LIGAND are present in fetal gonads between embryonic days 8 and 14.5. Hence, it is not surprising that several lines of evidence (Dolci et al., 1991, 1993; Godin et al., 1991; Matsui et al., 1991) show that the Kit–Kit LIGAND interaction is of utmost importance for: (1) germ cell migration to the presumptive gonad, and (2) germ cell proliferation and protection against apoptosis. Evidence supporting the first claim has been obtained using natural W/Sl mutants. For example, in KitW/Wc mice (Buehr et al., 1993), there is no increase in the number of primordial germ cells (PGCs) between embryonic days 8.5 and 10.5, owing to the migration of PGCs to ectopic sites. There is also evidence that the lack of germ cells in the ovaries of MgfSl/SI-Δ mice is produced by an alteration in the migration of primordial germ cells towards the presumptive ovary. Evidence in support of an anti-apoptotic role of the Kit–Kit LIGAND interaction for PGCs or oogonia was obtained after supplementation of in vitro cultured PGCs with Kit LIGAND (30–100 ng ml–1) (Godin et al., 1991; Dolci et al., 1991; Matsui et al., 1991). Whether Kit LIGAND also has a mitogenic effect on PGCs is uncertain.

As other compounds (for example, leukaemia inhibitory factor (LIF): Matsui et al., 1991; Dolci et al., 1993; retinoic acid: Koshimizu et al., 1995; and fibroblast growth factor (FGF): Cheng et al., 1994) also have the potential to increase the number of PGCs in vitro: the possible synergies between Kit LIGAND and these compounds have been evaluated by Matsui et al. (1991) and Dolci et al. (1991). A synergy between LIF (20 ng ml–1) and Kit LIGAND (30–100 ng ml–1) resulting in increased numbers of PGCs was demonstrated. Interactions between Kit LIGAND and bFGF or retinoic acid have not yet been investigated. Potential interactions between Kit LIGAND and thymuline should also be assessed since thymuline appears to be mitogenic for oogonia (Prepin, 1991).

The hypothesis that Kit LIGAND is predominantly a survival factor for PGCs, protecting them from apoptosis, is in good agreement with a number of studies using other cell types (lymphocytes: Carson et al., 1994; haematopoietic cells: Caceres Cortes et al., 1994; type A spermatogonia: Packer et al., 1995).

KIT–KIT LIGAND interactions and the high frequency of apoptotic germ cells observed during late fetal life

Ninety per cent of the germ cells formed earlier during oogenesis undergo apoptosis during late fetal life (defined here as embryonic day 15 to birth; Fig. 1). Since Kit–Kit LIGAND interactions are involved in survival of PGCs, an obvious question is whether they are also relevant to survival of oocytes and primordial follicles.

Few studies have attempted to relate apoptosis in meiotic–postmeiotic germ cells with Kit–Kit LIGAND interactions. In the pioneering study by Morita et al. (1999), addition of Kit LIGAND alone (100 ng ml–1) to cultured fetal mouse ovaries (embryonic day 13.5, cultured for 72 h) did not alter germ cell apoptosis. However, a clear synergy with LIF (100 ng ml–1) was observed, which may be taken as evidence that Kit LIGAND plus LIF can modulate the survival of oocytes at the meiotic stage. Further evidence in support of this claim was obtained by Driancourt et al. (1997) using mice in which one copy of the Kit gene was inactivated by transgenesis. These heterozygous mice, which produce only 50% of the amounts of Kit present in wild-type mice, have a twofold increase (6.8 versus 3.6%) in the frequency of apoptotic oocytes in their ovaries at embryonic day 15.5.
KIT–KIT LIGAND interactions: a stimulatory mechanism for follicular growth from the primordial to the large preantral stage in prepubertal animals?

During the primordial to the large preantral stage, the high expression of KIT and KIT LIGAND, together with the high circulating FSH concentrations may explain the high rate of initiation, the fast growth rate and the absence of atresia observed in juvenile ovaries.

Exit from the primordial pool and transit past the primary follicle stage

There is evidence that the Kit message and KIT protein are present in primordial follicles. However, while the amounts of mRNAs coding for KIT LIGAND are high in ovaries obtained at birth, the KIT LIGAND protein does not appear to be present in primordial–primary follicles. Therefore, it is surprising that clear-cut effects of the blockade of the interaction between KIT and KIT LIGAND on growth initiation were observed. The first group of data showing this effect was generated by the study of natural mutants, particularly those of the Mgf gene. While most of these mutants are sterile, at least two, MgfSl–t (Kuroda et al., 1988) and MgfSl–pan (Huang et al., 1993), have ovaries that contain a limited complement of primordial follicles. In both of these mutants, follicular growth is initiated but follicles rarely grow past the primary stage. The second study, demonstrating the importance of KIT–KIT LIGAND interactions for growth initiation, was based on in vivo injection of an antibody (ACK2) blocking KIT–KIT LIGAND binding. When newborn mice were injected every day from birth to day 12 and killed at day 14, growth initiation was found to have been fully inhibited (Yoshida et al., 1997). When the first injection (at birth) was omitted, initiation proceeded almost normally (Yoshida et al., 1997), indicating that there is a narrow period (from birth to day 2) when the KIT–KIT LIGAND interaction is of importance for initiation. Parrott and Skinner (1999) used an in vitro culture model of postnatal ovaries in the presence of KIT LIGAND or in the presence of an antibody blocking the KIT–KIT LIGAND interaction (ACK2), to demonstrate that KIT LIGAND supplementation produced a marked induction of primordial follicle development. Inclusion of ACK2 in the medium blocked spontaneous and KIT LIGAND-induced growth initiation.

KIT–KIT LIGAND interaction appears to be one of the key regulators of growth initiation from the primordial pool and transition beyond the primary follicle stage. The mechanisms that may be involved in mice are presented (Fig. 4).

Oocyte and granulosa cell function in preantral follicles

At this stage of follicular growth, the KIT and KIT LIGAND messengers and proteins are known to be present, at least in juvenile animals. Their functional importance is demonstrated by two main approaches in vitro.

Using in vitro culture of collagen-enclosed oocytes, Packer et al. (1994) demonstrated a stimulatory role of KIT LIGAND on oocyte growth, reporting that addition of 10–50 ng KIT LIGAND ml–1 to the culture accelerated oocyte growth, with a doubling of growth rate observed at 50 ng KIT LIGAND ml–1.

In addition, oocytes from prepubertal animals appeared to have the ability to increase the amounts of mRNA coding for KIT LIGAND in cultured granulosa cells (Packer et al., 1994). This finding was confirmed by Joyce et al. (1999b). On the basis of these data, a working hypothesis (see Fig. 4) is that a functional loop occurs at this stage, with the oocyte increasing mRNA encoding KIT LIGAND in the surrounding granulosa cells and the increasing amounts of KIT LIGAND produced further stimulating oocyte growth.

In the second approach, injections of ACK2 (a blocking antibody raised against KIT) were given to mice in vivo and effects on cell proliferation of granulosa cells of preantral follicles were assessed after BrdU incorporation (Yoshida et al., 1997). Although no quantitative data are provided, ACK2 injection was reported to reduce proliferation in the granulosa cells of preantral follicles (Yoshida et al., 1997). This result is unexpected since KIT is absent in granulosa cells. An indirect effect (via the oocyte–theca cells sending paracrine messages to the granulosa layers) needs to be postulated but the underlying mechanism remains to be clarified.

A final important effect of the interaction between KIT and KIT LIGAND could be on atresia of preantral follicles. This claim (although not discussed by the authors) is supported by the observation that 2 days of ACK2 injections (day 12 and day 13) resulted in a tenfold reduction in the population of large preantral follicles (Yoshida et al., 1997). Such an effect on survival may be postulated since, at this stage, oocytes are sensitive to apoptosis, and oocyte apoptosis results in preantral follicle atresia (for review, see Morita and Tilly, 1999; Reynaud and Driancourt, 2000). However, such an effect was not demonstrated in preantral follicles cultured in vitro and treated with another blocking antibody, SC1494 (Reynaud et al., 2000).

Theca cell differentiation in preantral follicles

Young preantral follicles are able to stimulate differentiation of stromal–interstitial cells into theca cells by a paracrine mechanism (Magoffin and Magarelli, 1995). This effect is not produced by inhibin, activin, TGFβ or TGFα, but rather by a molecule of 20–25 kDa (Magoffin and Magarelli, 1995). KIT LIGAND may mediate part of this effect since it is able to promote the formation of theca cells around small preantral follicles (Parrott and Skinner, 2000), and to stimulate stromal–theca cell proliferation in a dose-dependent manner (Parrott and Skinner, 1997, 2000) as well as thecal steroid production (Parrott and Skinner, 1997). Once some theca cells are present, they may start to produce KGF and testosterone, which will stimulate KIT LIGAND production by theca cells through a paracrine effect (Parrott and Skinner, 1998a; Joyce et al., 1999b). Such a stimulatory loop may be of utmost importance for follicle morphogenesis.

KIT–KIT LIGAND interaction: a modulatory mechanism for antral follicular growth and maturation?

Assuming that KIT–KIT LIGAND interactions are also operating throughout terminal follicular growth, effects on oocyte maturation and theca cell differentiation may be postulated. There may also be indirect effects of KIT LIGAND on maturation of granulosa cells (Fig. 5).
Effects on the oocyte

In antral follicles, the oocyte is almost fully grown but the nuclear and cytoplasmic compartments still need to undergo maturation, which will allow resumption of meiosis, fertilization and embryo development.

Two lines of evidence support the claim that the KIT–KIT LIGAND interactions are relevant for oocyte maturation. First, when fully grown oocytes are cultured for 24 h in the presence of KIT LIGAND (50 to 500 ng ml\(^{-1}\)), a transient block in resumption of meiosis is observed (Ismail et al., 1996). Second, when Kit antisense oligonucleotides are injected in meiotically arrested rat oocytes, there is an increase in the ability of these oocytes to resume meiosis (Ismail et al., 1997). Hence, in rats, interaction between KIT and KIT LIGAND may be relevant to control the nuclear maturation of the oocyte. The large amounts of KIT LIGAND present around the oocyte in preantral follicles may be one factor preventing resumption of meiosis in such follicles. However, currently available evidence indicates that such a mechanism may not be operative in species other than rats. A possible effect of KIT LIGAND on cytoplasmic maturation is indicated by the positive correlation between the KIT LIGAND concentrations in follicular fluid and pregnancy rate after IVF (Smickle et al., 1998). Confirmation of such an effect has recently been obtained in a study in vitro (Reynaud et al., 2000) in which addition of KIT LIGAND (50 ng ml\(^{-1}\)) to the culture media of mouse follicles cultured for 12 days increased the proportion of oocytes reaching the 2–4-cell stage after IVF. The exact stage of cytoplasmic maturation of the oocyte at which KIT LIGAND needs to be present remains to be identified.

Effects on theca cells

In antral follicles, the KIT LIGAND protein is abundant in granulosa cells along the basal membrane and KIT has appeared in thecal cells. Hence, KIT–KIT LIGAND interactions may also produce effects on somatic cells through paracrine regulations. The elegant studies of Parrott and Skinner (1997, 1998a,b), using serum-free theca cell culture from cows, established that KIT LIGAND can stimulate androstenedione (but not progesterone) output from confluent theca cells. Intact mouse follicles were cultured for 12 days in the presence of 50 ng KIT LIGAND ml\(^{-1}\) to confirm that this effect also occurs in a more physiological system. Before and after induction of ovulation in vitro by hCG plus EGF, follicles treated with KIT LIGAND produced significantly more testosterone than did
The similarity in the findings of both the studies of Parrott and Skinner (1997, 1998a) and Reynaud et al. (2000) indicates that the KIT–KIT LIGAND interaction between granulosa and theca cells contributes to the control of androgen output by theca cells in antral follicles before the dominance phase (Fig. 5a).
Effects on granulosa cells

Antrum formation within granulosa cells appears to be regulated in part by KIT–KIT LIGAND interactions. This claim is supported by the observations showing that ACK2 injection to mice in vivo suppressed antrum formation (Yoshida et al., 1997). Alterations in the pattern of antrum formation in vivo in follicles of KitW–/– compared with Kit+/+ mice (M. A. Driancourt and K. Reynaud, unpublished) support the earlier claim. However, the underlying mechanisms remain to be explored.

Yoshida et al. (1997) reported a blockade of KIT function as a result of injecting ACK2 12–24 h before inducing ovulation, which induced atresia of the prevulatory follicles and ovulation failure. This finding indicates that other effects of KIT LIGAND on granulosa cells are occurring. The observations showing that earlier or later injections had no effect indicate that differentiation of LH receptors occurred on granulosa cells at the time of ACK2 injection and that ACK2 prevented it. Whether this can be confirmed using other approaches (for example, in vitro culture, western blot analysis) remains to be tested. If such an effect was operative, it would have to be indirect.

A working hypothesis relating KIT LIGAND to follicular maturation is presented (Fig 5a: small antral follicle at the beginning of the follicular phase; and Fig 5b: large antral follicle during the late follicular phase). These physiological stages differ in their endocrine environment, which features high versus low circulating FSH concentrations in the early and late follicular phases, respectively. Antral follicles display higher KL-1 and KL-2 expression among the mural granulosa cells during the early follicular phase compared with the late follicular phase (Joyce et al., 1999). In small antral follicles, the high KIT LIGAND concentrations produced by the high FSH and testosterone concentrations (Joyce et al., 1999b) could stimulate KGF production by theca cells (Parrott and Skinner 1998a). High KGF concentration could have two effects. First, it would increase testosterone output by theca cells (Parrott and Skinner, 1997) which would, in turn, increase KIT LIGAND expression further. Second, it would prevent induction of aromatase activity in granulosa cells (Parrott and Skinner, 1998b). In large antral follicles (Fig 5b), owing to decreased FSH support, KIT LIGAND expression may be reduced, lifting the blockade of KIT LIGAND on the induction of aromatase, resulting in increased oestradiol production and differentiation of LH receptors. Hence the dialogue between granulosa and theca cells, involving complex paracrine interactions between KIT LIGAND, FSH, testosterone and KGF, is likely to play a regulatory role in follicular maturation.

Conclusion

From the data summarized in this review, the interactions between KIT and KIT LIGAND appear to be relevant for four main events of oogenesis and folliculogenesis: (1) the formation of the germ cell store; (2) the initiation of follicular growth; (3) antrum and theca formation; and (4) oocyte maturation. Most of these conclusions may be considered as valid, since they are based on a combination of approaches (natural mutants plus demonstration of the presence of the proteins plus in vivo or in vitro approaches). Information obtained from natural mutants (MgfSl–/–, MgfSl–+/–) indicates that oogenesis and initiation of follicular growth are the stages acutely sensitive to the lack of KIT LIGAND or KIT. Several steps of folliculogenesis also appear to have graded requirements for KIT–KIT LIGAND interactions. A limited reduction (by about 50%) in the amounts of KIT LIGAND, such as that observed in heterozygous Wl+/– mice, results in alterations in oocyte growth, granulosa cell proliferation in preantral follicles and in antrum formation (M. A. Driancourt and K Reynaud, unpublished). Any further reduction, through the use of blocking antibodies, would have additional consequences on steroidogenesis and oocyte survival (Reynaud et al., 2000). The complex and intricate communication among the oocyte, the granulosa and theca cells described in this review underlines the need to devise efficient co-culture systems (and not only isolated granulosa or theca cell cultures) to produce, from experiments in vitro, relevant information on the control of follicular growth.

References

Key references are indicated by asterisks.


Parrott JA and Skinner MK (1998a) Thecal cell–granulosa cell interactions involve a positive feedback loop among keratinocyte growth factor, hepatocyte growth factor and kit ligand during ovarian follicular development. *Endocrinology* 139:2240–2245


