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Oocyte–somatic cell communication

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The physical interface between the female germ line and enveloping somatic cells is dynamically modified throughout the course of folliculogenesis. How selective pathways for communication between the oocyte and granulosa cell are established and regulated remains to be determined, but insights into the structural basis for this communication are emerging. This review summarizes the available evidence that supports the notion that the integration of oogenesis with folliculogenesis is achieved by regulated cell interactions between oocytes and granulosa cells.

Introduction

There are multiple forms of cell–cell communication between the oocyte and granulosa cell within the developing ovarian follicle. These forms of communication include both direct cell contact-mediated gap junctions and the exchange of important paracrine signalling factors, such as growth differentiation factor 9 (GDF-9) and Kit ligand. Assessing distinct roles of oocyte–somatic cell communication has been difficult because the ontogeny of female germ cells in animals encompasses phases of prolonged storage, rapid growth and maturation, and mechanisms for releasing oocytes from the ovary (Wallace and Selman, 1990). Ovarian follicles provide the multicellular unit for prolonged storage of oocytes both before and during the reproductive lifespan of females. The follicle, formed by the physical interaction of oocytes and granulosa cells, supports the rapid growth and maturation of oocytes (Canipari, 1994). The somatic granulosa cells surrounding the oocyte undergo periodic changes in structure and function that concurrently attend the needs of the developing oocyte and organismal reproductive cyclicity. The physical linkage between female germ cells and the ovarian soma is finally interrupted during ovulation, even though in many animals the oocyte is released with somatic cells attached to the oolemma or investing extracellular matrices (Suzuki *et al.*, 2000). Thus, from both a phylogenetic and ontogenetic perspective, oogenesis clearly involves a protracted period of contact between the oocyte and surrounding follicle cells. The morphological and physiological relevance of oocyte–granulosa cell contact in the mammalian ovary is considered below, with reference to the form, composition and functions subserved by specialized structures, known as transzonal projections (TZPs).

Structural properties of TZPs

In the mammalian ovary, oocyte–granulosa cell contacts are evident within primordial follicles and appear to include at least gap junction types of specialization (Hertig and Adams, 1967;

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Grazul-Bilska *et al.*, 1997). Once folliculogenesis is initiated, the gradual deposition of the zona pellucida at the oocyte cell surface creates a permanent, but malleable, extracellular matrix barrier between germ cells and ovarian somatic cells for the duration of oogenesis. Specialized properties of granulosa cells have evolved to both breach the zona pellucida and anchor cytoplasmic projections to the oocyte surface to establish and maintain direct physical contact at this heterocellular interface. The devices used by granulosa cells to establish a physical conduit for cell communication have been termed TZPs and their origin from somatic granulosa cells has been confirmed morphologically in live and fixed preparations (Albertini and Rider, 1994). Most of what is known about the structural properties of TZPs derives from earlier electron microscopy studies, but more recently confocal microscopy has been adopted as a valuable tool to explore three-dimensional aspects of cell organization at the oocyte–granulosa interface.

Ultrastructural and confocal analyses

Initial studies by Anderson and Albertini (1976) defined morphological variations in the organization of TZPs at the oocyte surface in several mammals. For example, in the ovarian follicle of the rhesus monkey, TZPs were found to terminate upon broad, non-microvillar plasma membrane domains on the oocyte surface. Focal adhesions at these contacts were flanked by small gap junction plaques. Small gap junctions have also been detected between oocyte microvilli and the tips of small calibre TZPs enriched in actin filaments. Slender actin-laden TZPs appear to be prominent in rodents, whereas larger calibre TZPs with broad terminal contacts and zones of adhesion appear to be more typical of primates and ruminants (Allworth and Albertini, 1993; Albertini and Rider, 1994), including humans (Combelles *et al.*, 2002). Motta *et al.* (1994) have provided further details of the ultrastructure of TZPs in humans with particular reference to changes associated with development of the follicle. These authors report that TZPs of high density are present in preantral follicles where they deeply invaginate the oocyte, sometimes penetrating to close proximity to the germinal vesicle. At later stages of follicle development, TZPs were found to terminate more typically at the oocyte surface and the number per oocyte appears to diminish gradually with advancing stages of follicle development. Clearly, further studies are required to define the spatial organization and composition of these structures during oogenesis, but their prevalence in the mammalian ovary and variable organization indicates some degree of plasticity during follicle development.

The ability to localize specific molecules by immunolabelling procedures, in conjunction with the optical sectioning capabilities of confocal microscopy, has materially extended our understanding of the cytoarchitecture of the oocyte–granulosa interface. Collectively, immunolabelling studies and the use of cytoskeletal inhibitors have defined expression patterns and possible functions for the cytoskeleton within TZPs. Actin filaments are organized in parallel arrays throughout the TZPs in all mammalian species studied (Anderson and Albertini, 1976; Albertini and Rider, 1994). In all TZPs examined, aggregates of F-actin are concentrated at TZP terminals consistent with a role in stabilizing cell contacts between oocytes and granulosa cells (Can *et al.*, 1997). Intermediate filaments of the vimentin type, typically a marker of cells of mesenchymal origin, have been detected in granulosa cells from humans (Czernobilsky *et al.*, 1985), sheep (Gall *et al.*, 1992) and rodents (Albertini and Kravit, 1981) and these appear to be abundant within and at the termini of TZPs of intact cumulus–oocyte complexes (Gall *et al.*, 1992). It has also been shown in humans and sheep that epithelial keratins are co-expressed with vimentin in granulosa cells (Czernobilsky *et al.*, 1985; Gall *et al.*, 1992). The preferential accumulation of these proteins at TZP termini further indicate that more highly differentiated forms of cell adhesion, namely desmosomes, may maintain oocyte–granulosa

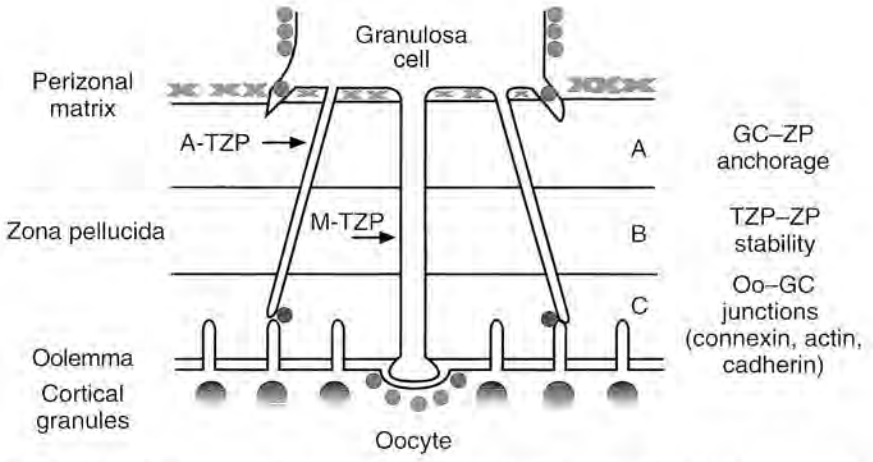

Fig. 1. Model illustrating the coexistence of actin- and microtubule-rich transzonal projections (TZPs) emanating from a single granulosa cell (GC). GC–ZP: granulosa cells–zona pellucida; Oo–GC: oocyte–granulosa cells; TZP–ZP: transzonal projections–zona pellucida; A-TZP: actin-rich TZPs; M-TZP: microtubule-rich TZPs.

cell attachment as physical stresses due to oocyte growth and follicle expansion are increased during folliculogenesis. As the cytoskeletal inhibitors acrylamide (for intermediate filaments, Gall *et al.*, 1992) and cytochalasin D (for actin filaments, De Smedt and Szollosi, 1991) have both been shown to induce meiotic resumption in sheep COCs, it has been suggested that these cytoskeletal components provide a structural framework for the maintenance and function of cell communication pathways between oocyte and granulosa cells.

Microtubule composition and function

It has become clear that TZPs also include microtubules as a major cytoskeletal component (Albertini *et al.*, 2001). Studies on bovine cumulus–oocyte complexes (COCs) illustrated that the oriented elongation of TZPs during hormone-induced oocyte maturation was accompanied by a burst of microtubule assembly within cumulus granulosa cells (Allworth and Albertini, 1993). Subsequent studies have further detailed aspects of microtuble organization and dynamics within TZPs during both follicular development and the periovulatory period. For example, studies on mouse oocytes exposed to the phosphodiesterase inhibitor isobutylmethylxanthine indicate that an increase in granulosa cAMP content results in a marked reduction of microtubles bearing TZPs (Albertini *et al.*, 2001). Furthermore, recent evaluation of tubulin isoforms within human oocytes demonstrates a stable subset of microtubules bearing α-acetylated tubulin epitopes that form hoop-like arrays at the oocyte surface within TZP termini (Combelles *et al.*, 2002). This finding is confirmed by electron microscopy studies illustrating prominent microtubule tracks within human cumulus cells in association with organelles (Tesarik and Dvorak, 1982). Thus, TZPs of two types appear to be present at the oocyte–granulosa interface. Both actin-rich and tubulin-rich TZPs are distinguishable based upon cytoskeletal content and disposition, although whether these distinct structures subserve selective functions remains to be established (Albertini *et al.*, 2001). A model for the coexistence of these two TZP types is shown (Fig. 1).

From a compositional point of view, microtubules consist of traditional heterodimeric subunit isoforms of both α and β epitopes and these are routinely detected by

immunocytochemical studies of TZPs. What remains to be fully elucidated is whether there are post-translational modifications of tubulin that would implicate changes in polymer stability at specific stages of folliculogenesis. α-Acetylated tubulin is seen in TZPs of both COCs and granulosa cells from preantral mouse follicles. A tendency to orient α-acetylated microtubules in preantral follicles (Albertini *et al.*, 2001) indicates that TZPs are preferentially polarized and stabilized at critical times of development, possibly to mediate vectorial exchange of signalling molecules or as a reflection of unique cell cycle control points required for lineage selection of mural or cumulus phenotypes (Herman and Albertini, 1983). Further characterization of microtubule phenotypes in TZPs will be necessary to establish how dynamic these structures are and whether the expression of primary cilia represents modifications that polarize granulosa cells for paracrine signalling.

The recent demonstration that molecules involved in neurotransmission are localized at the oocyte–granulosa interface (Grosse *et al.*, 2000) lends further credence to the idea that TZPs serve as axon-like conduits for macromolecular exchange (Allworth and Albertini, 1993). To date, discriminating markers for a neuronal phenotype have not been systematically tested, with the exception of the above study. However, markers of synaptic transmission machinery, organelle transport and cell-adhesion-based signalling would all be likely candidates for further exploration of the signalling mechanisms operative at the oocyte–granulosa interface (Fagotto and Gumbiner, 1996).

Developmental regulation of oocyte–somatic cell communication

Relatively little is known about how the TZP system is modulated throughout the course of follicular development. It appears that there is significant species variation in the composition, structure and expression of TZPs (Albertini and Rider, 1994; Can *et al.*, 1997). Moreover, there is mounting evidence to indicate that within a given mammalian species, there are significant variations in the organization of TZPs depending upon the stage of follicle development under study. Two broad categories are worth highlighting with reference to TZP remodelling that occurs during the course of folliculogenesis or during the periovulatory period within the COC.

As noted earlier, the studies of Motta *et al.* (1994) revealed variations in TZP organization and density during human ovarian follicle development. Both variations in the extent of TZP penetration of the oolemma, which diminished with advancing folliculogenesis, and TZP density, which followed a similar pattern, were reported. In other species, this has not been well studied from the point of view of TZPs but considerable evidence indicates that modulation of gap junctions is subject to tight spatial and temporal controls (Grazul-Bilska *et al.*, 1997). For example, gap junctions are present even before zona pellucida deposition in primordial follicles at the oocyte–granulosa interface. The number of gap junctions at this interface as well as at the granulosa–granulosa interface are clearly upregulated by oestrogens and FSH (Burghardt and Matheson, 1982). Moreover, a number of studies have extended this work to show transcriptional upregulation of the major granulosa cell connexin, cxn 43 (Grazul-Bilska *et al.*, 1997). Whether cxn 43 participates directly or indirectly in oocyte–granulosa cell communication is not entirely clear. Complicating this prospect is the recent finding that in mice, a unique connexin, the oocyte specific cxn 37, appears to mediate oocyte–granulosa gap junction mediated communication (Carabatsos *et al.*, 2000). Studies on mice in which cxn 37 was disrupted reveal tandem arrest points for oogenesis and folliculogenesis. For oogenesis, growth and meiotic acquisition of competence are incomplete in the absence of functional gap junctions. For folliculogenesis, follicles are arrested at the early antral stage and undergo partial luteinization with advancing age. This phenotype

indicates the need for gap junctions for both compartments to advance developmentally, but clearly defines a degree of development that is independent of gap junctions. Interestingly, microtubule–TZPs are abundant in these animals demonstrating that the formation and stabilization of these structures does not require functional coupling via gap junctions (D. F. Albertini and M. J. Carabatsos, unpublished).

There is substantial evidence to support the notion that somatic cell coupling to the oocyte in terms of both structural integrity and signalling is modified during the periovulatory period. There are two general models that include: (i) the idea that physical uncoupling both removes meiosis-arresting constraints and allows maturation to proceed and (ii) the idea that timely inputs to the oocyte, via the LH surge, stimulate the resumption of meiosis and perhaps modulate the continuation of events that support maturation and the acquisition of developmental competence. Although there is little doubt, especially in rodent models, that physical uncoupling due, in part, to TZP retraction occurs during oocyte maturation and cumulus expansion, the timing of this process and its underlying mechanism remain incompletely understood (Albertini and Rider, 1994). Remodelling of the actin cytoskeleton is likely to be intrinsic to cumulus cell responsiveness to gonadotrophins or other cumulus factors secreted during ovulation. This was shown directly in hamster COCs treated with FSH under conditions that enhance meiotic maturation (Plancha and Albertini, 1994). Both oocyte cortical actin assembly and withdrawal of actin TZPs were necessary remodelling events for maturation to proceed to completion.

In contrast, there is evidence to indicate that withdrawal of actin–TZPs and *de novo* growth of microtubules–TZPs underscores stimulation of COCs in other species, such as cows and primates (Allworth and Albertini, 1993; Albertini and Rider, 1994; Combelles *et al.*, 2002). Although the signalling pathways that mediate these distinct forms of cytoskeletal remodelling await definition, it is likely that extension, anchoring and secretion through hormonally regulated subpopulations of the cumulus underlie critical aspects of oocyte quality determination.

Conclusion

This review discusses the available evidence supporting the idea that physical interactions between the oocyte and granulosa cells coordinate oogenesis with folliculogenesis. The structural basis for this interaction is the TZP, a specialized projection from granulosa cells that terminates on the oolemma after breaching the zona pellucida. TZPs form both gap and adhesion type junctions at the oocyte surface and the density and structure of TZPs is altered during the course of follicle development in mammals. In view of the abundance of microtubules within TZPs, it is proposed that key elements of oocyte–granulosa cell communication are mediated by the effects of paracrine signalling molecules secreted at specific stages of oogenesis and folliculogenesis. On the basis of confocal reconstructions from mouse ovarian follicles, TZPs rich in actin (A-TZP) penetrate the zona pellucida and form gap junction connections with microvilli on the oocyte surface. In contrast, microtubule rich TZPs (M-TZP) directly contact the oocyte surface at non-microvillar sites. Adhesions at the zona pellucida outer surface (zone A) are proposed to anchor granulosa cells to the zona pellucida, whereas after traversing the zona pellucida (zone B), TZP termini would express specific molecules (connexins, actin, cadherins) to form characteristic gap or adhesion based connections at the zona pellucida–oolemmal interface (zone C).

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