Unique and Multifunctional Adhesion Junctions in the Testis: Ectoplasmic Specializations

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Summary. In this paper, we review the structure and function of a unique type of actin-related intercellular adhesion junctions in the testis. Based on their ultrastructure, the junctions are divided into five distinct domains. The currently identified molecular components of each domain are summarized. In addition, the architecture of the mammalian system is compared with that of non-mammalian vertebrates. Functionally, the junctions are related to the turnover of adhesion between Sertoli cells, to the attachment of spermatids to the seminiferous epithelium, and to sperm release. They also are part of the mechanism by which spermatids are moved through the epithelium. Evidence consistent with adhesion and motility related functions is discussed. Control, both of junction turnover and of microtubule-based transport, is identified as an important avenue for future research.

The seminiferous epithelium of the testis is composed of two very different populations of cells that have a complex structural and functional interrelationship during the process of spermatogenesis. The architecture of the seminiferous epithelium and provide the physiological environment necessary for the development of sperm cells. Cells of the germ cell lineage, the spermatogenic cells, lie between and are attached to the Sertoli cells. During spermatogenesis, both Sertoli and spermatogenic cells undergo remarkable changes in structure. Terminally differentiated cells of the germ cell line are ultimately released from the epithelium as spermatocytes. Changes in Sertoli cells occur cyclically and correlate with developmental changes occurring in the spermatogenic cells with which they are associated. One of the most interesting structural features in Sertoli cells that changes during spermatogenesis is a unique type of junction that occurs in areas of intercellular attachment. Junctions of this type, known as ectoplasmic specializations, are composed of distinct regions of the plasma membrane, elements of the cytoskeletal system, and components of the endoplasmic reticulum. These junctions are associated with a number of events fundamental to the process of spermatogenesis and to normal fertility in the male.

STRUCTURE

The mammalian system: the archetype

The unique arrangement of elements in the Sertoli cell cytoplasm immediately under the plasma membrane in regions of close association both with spermatids and with neighboring Sertoli cells has been recognized for many years (Brokelmann, 1963; Nicander, 1967). Although initially regarded as some form of junction-related structure, the term "ectoplasmic specialization" later was used to describe the complexes, based on the argument that there was little evidence that the structures actually were junctions (Russell, 1977a). There is now a large body of evidence consistent with the hypothesis that the structures are indeed intercellular junctions and that they are a specialized form of actin-related adhesion junction found generally in epithelia. The plasma membrane has been included in definitions of its structure to reflect this conclusion (Russell et al., 1988; Vogl et al., 1991).

In mammalian Sertoli cells, ectoplasmic specializations consist of the plasma membrane in regions of attachment to adjacent cells, a layer of actin filaments (often arranged in discrete bundles), and a cistern of endoplasmic reticulum. Unlike actin filaments related to junctions in other cell types, those in ectoplasmic specializations of Sertoli cells are hexagonally packed (Franke et al., 1978)—similar to actin filaments in microvilli, and are unipolar (Toyama,
1976). At sites where ectoplasmic specializations occur, the intercellular space narrows to between 70–100 Å (FLICKINGER and FAWCETT, 1967; DYMY and FAWCETT, 1970).

Ectoplasmic specializations occur both at apical and at basal locations in the seminiferous epithelium (Fig. 1). At apical sites, the structures are found at sites of attachment to spermatids. At basal sites, they are present in regions of attachment to neighboring Sertoli cells. Except for minor differences, the general morphology of the structures at the apical (Sertoli/spermatid) and basal (Sertoli/Sertoli) sites is very similar. One problem associated with determining the molecular composition of ectoplasmic specializations is that they are intimately related to other junction types. At basal sites, they form part of a junction complex that includes tight, gap, and desmosome-like junctions. At apical sites, other junction types are not generally apparent; however, even in this location there are indications that, at certain stages of spermatogenesis, intermediate filament-related adhesion junctions may develop within regions occupied by ectoplasmic specializations (AMLANI and VOGL, 1988; GUTTMAN et al., 1999).

Ectoplasmic specializations can be divided into five distinct domains that reflect both the structural and functional attributes of the system (VOGL et al., 1991) (Fig. 2): the extracellular, the plasma membrane, the ectoplasmic, the endoplasmic reticulum, and the cytoplasmic. Based on its ultrastructure, the ectoplasmic domain can be subdivided further into a zone related to the plasma membrane, a zone containing the actin filaments, and a zone related to the ectoplasmic face of the endoplasmic reticulum.

Among elements presumed to be present in the extracellular domain are the parts of integral membrane molecules that project into the intercellular space and are responsible for intercellular attachment. In electron micrographs, fine filamentous linkages have been observed to span the intercellular space between adjacent membranes (RUSSELL et al., 1988).

Surprisingly little is known about the plasma membrane of ectoplasmic specializations. Based on studies using immunofluorescence, it has been established that at least one of the adhesion molecules likely present in the membrane is a β1 integrin (PALOMBII et al., 1992), and is most probably α6β1 (SALANOVA et al., 1995) (Fig. 3). The ligand for this molecule at the junction sites has not been identified, although one potential candidate may be a member of the ADAM family of proteins situated in the plasma membrane of the adjacent cell (ZHOU et al., 1999). The extent to which integrins play a role in establishing and maintaining intercellular adhesion at ectoplasmic specializations has not been determined experimentally.

It has been postulated for many years that a member of the cadherin family of adhesion molecules is present in ectoplasmic specializations; however, available data is inconclusive. What is clear is that E-cadherin, found at sites of adhesion between many epithelial cell types, is not present at ectoplasmic specializations (BEYERS et al., 1994). At least one cadherin, N-cadherin, is expressed in the testis (MACCALMAN et al., 1991; CYR et al., 1992), appears to
participate in Sertoli /spermatogenic cell adhesion (NEWTON et al., 1993), is present at sites of Sertoli/Sertoli contacts during junction formation in the rat (WU et al., 1994), and is reported to label, when signal amplification techniques are employed, apical and basal sites known to contain ectoplasmic specializations (WINE and CHAPMAN, 1999). Antibodies raised against the conserved C-terminus of cadherins react in a punctate fashion with Sertoli/spermatocyte contacts and in a patchy or belt-like fashion at sites interpreted to be Sertoli cell contacts with round and elongate spermatids, but are reported not to react with basal junction complexes between adjacent Sertoli cells (BYERS et al., 1994). Antibodies to α- and β-catenins, cadherin related signaling molecules, react with basal junction complexes (BYERS et al., 1994), but only β-catenin reacts at apical sites interpreted as contacts between Sertoli cells and spermatids. When antigen retrieval and signal amplification techniques are used (WINE and CHAPMAN, 1999), staining with antibodies to β-catenin is reported to stain basal junction complexes and apical Sertoli cell cytoplasm associated with late spermatids and residual bodies. On reviewing all the available data, it is probably premature to definitively assign a cadherin and cadherin associated molecules to ectoplasmic specializations.

Perhaps one of the most interesting regions of the junction plaque is the ectoplasmic domain. Ultrastructurally, this domain consists of three distinct zones: an actin zone separated, by a zone on each side, from the plasma membrane and the endoplasmic reticulum. In addition to actin, a number of molecules tentatively can be assigned to the ectoplasmic domain, although not all have been localized definitively to the site by immunogold labeling. These molecules include vinculin (GROVE and VOGI, 1989) (Fig. 3), α-actinin (FRANKE et al., 1978), espin (BARTLES et al., 1996) (Fig. 4), fimbrin (GROVE and VOGI, 1989), myosin VIIa (HASSON et al., 1997a; WOLFRUM et al., 1998) (Fig. 5), and integrin linked kinase (ILK) (MULHOLLAND and VOGI, 1999). A number of these (espin, fimbrin and α-actinin) are actin binding proteins, likely present in the actin zone, and presumably associated with establishing the pattern of actin filament arrangement in the structure. Unlike in actin networks related to adhesion sites in other epithelia, conventional myosin (myosin II) is not present in the actin zone of ectoplasmic specializations (VOGI and SOUCY, 1985). Vinculin and other molecules, such as ILK, involved with structurally relating the actin filaments to the plasma membrane and/or in signaling cascades are probably located within the plasma membrane related zone. Myosin VIIa also is likely present in this zone, and based on work in other systems (HASSON et al., 1997b), may be involved with anchoring the actin filaments to elements in the plasma membrane. Nothing is known about the endoplasmic reticulum related zone except that it must contain at least some form of structural element that links the cistern of reticulum to the actin filaments.

Little is known about the endoplasmic reticulum of ectoplasmic specializations. Although the endoplasmic reticulum of the junction plaque appears connected to the general intracellular membrane system of Sertoli cells (CLERMONT et al., 1980), the extent to which the junction-related cisternae are specialized has not been defined. In electron micrographs, the intracisternal space of the junction-related edoplasmic reticulum sometimes appears narrower than that of cisternae elsewhere in the cell (personal observa
tion). In addition, the junction-related cisternae remain firmly attached to the junction plaque in mechanically fragmented epithelium. Both of these observations indicate that the junction-related cisternae may have specialized structural features.

Based on the spermatid translocation hypothesis to be discussed below, it is reasonable to predict that the endoplasmic reticulum of the plaque must be reinforced somehow to transmit force from the non-junction-related side of the reticulum to the membrane anchored to the actin filaments. One mechanism for doing this would be with structural linkages that connect adjacent membranes across the cisternal space. Interestingly, linkages such as those predicted here recently have been observed in quick-frozen and deep-etched preparations of endoplasmic reticulum in other systems (Senda and Yoshinaga-Hirabayashi, 1988). The presence of similar linkages in the endoplasmic reticulum of ectoplasmic specializations has not been confirmed.

Because the endoplasmic reticulum generally sequesters calcium in cells, it has long been suspected that the junction related endoplasmic reticulum in Sertoli cells may influence local calcium levels at the junction plaque and thereby play a role in regulating the junction itself (Franchi and Camatini, 1985). This has yet to be investigated.

The cytoplasmic domain of ectoplasmic specializations contains elements that relate the endoplasmic reticulum of the junction plaque to cytoplasmic elements deeper in the cell. In apical regions, there is now evidence that this zone contains microtubule dependent motor proteins (Brach and Vogl, 1999; Miller et al., 1999; Guttman and Vogl, 1999). In apical regions, this zone likely also contains, in addition to motor proteins, structural proteins that relate the plaque both to microtubules and to intermediate filaments.

The non-mammalian system: a basic design

Studies from non-mammalian vertebrate taxa indicate that many of the unusual features of ectoplasmic specializations are restricted to mammals, specifically to placentals mammals (Eutheria). Morphologically similar complexes have not been identified in Sertoli cells of any other vertebrate
group. Nevertheless, structural homologues of the mammalian ectoplasmic specialization do occur in non-mammalian vertebrates. These non-mammalian junctions are identified as homologues of mammalian ectoplasmic specializations based on the following criteria: 1) they occur in the same locations as mammalian ectoplasmic specializations; 2) they are composed of filamentous actin (BACCETTI et al., 1983; STANLEY and LAMBERT, 1985; SPRANDO and RUSSELL, 1987; PFEIFFER and VOGL, 1993, 1994; ARENAS et al. 1995); and 3) they appear to be involved with the process of intercellular adhesion (see “Intercellular adhesion” below).

In non-mammalian vertebrates, the term ectoplasmic specialization has been used mainly in reference to cortical regions of Sertoli cells adjacent to elongating spermatids. Actin-related junctions between adjacent Sertoli cells often resemble typical zonula or puncta adherens (intermediate type junctions) (PFEIFFER and VOGL, 1993, 1994), and in some cases occur at the apex of the epithelium as part of a junction complex (PFEIFFER and VOGL, 1994) (Fig. 6).

Structurally, non-mammalian ectoplasmic specializations differ from their mammalian counterparts in a number of important ways. One of the most notable differences relates to the organization of actin filaments at the junction site. Unlike the highly ordered filament arrays of mammalian ectoplasmic specializations, the actin filaments of non-mammalian Sertoli cell junctions are loosely organized into bundles or networks (SPRANDO and RUSSELL, 1987; PFEIFFER and VOGL, 1993, 1994) (Fig. 6). This loose organization is similar to the filament arrangement seen at actin-related adhesion junctions in other cell types in general. A second difference is that ectoplasmic specializations in non-mammalian vertebrates have contractile properties. Several lines of evidence support this conclusion. The filaments are loosely packed, myosin II is present (ratfish—Chondrichthyes, STANLEY and LAMBERT, 1985; guppy—Osteichthyes, PFEIFFER and VOGL, 1994; turtle—Reptilia, PFEIFFER and VOGL, 1993; rooster—Aves, PFEIFFER and VOGL, 1993), and the structures can be induced to contract in vitro in the presence of ATP (PFEIFFER and VOGL, 1993). A final difference is that the cisternae of endoplasmic reticulum are not a constant feature of ectoplasmic specializations in non-mammalian vertebrates (PFEIFFER and VOGL, 1993, 1994); however, cisternae have been reported in representative species of the classes Chondrichthyes (STANLEY and LAMBERT, 1985), Amphibia (SPRANDO and RUSSELL, 1987) and Reptilia (BACCETTI et al., 1983; SPRANDO and RUSSELL, 1987; PFEIFFER and VOGL, 1993).

Far less is known about the protein composition of non-mammalian than mammalian ectoplasmic specializations. In addition to actin and myosin II, vinculin has been localized at ectoplasmic specializations of one non-mammalian vertebrate species (turtle—PFEIFFER and VOGL, 1993). The presence of this 'junction site marker' in a non-mammalian vertebrate species provides further evidence of the homology between these junctions in non-mammalian vertebrates and mammals. Aside from these three proteins, the remaining elements of these junction sites, including intercellular adhesion proteins, additional actin-binding proteins, and possible signaling elements, remain to be determined in non-mammalian vertebrates. Given the structural differences between mammalian and non-mammalian vertebrate ectoplasmic specializations, one might predict differences at the molecular level as well.
Apical ectoplasmic specializations remain firmly anchored to spermatids when the latter cells are dissociated mechanically from the epithelium in mammals (Romrell and Ross, 1979; Vogl, 1996) and in non-mammalian vertebrates (Stanley and Lambert, 1985; Pfeiffer and Vogl, 1993). Moreover, the pharmacological disruption of actin filaments results in a loss of intercellular attachment between early elongating spermatids and Sertoli cells at sites where ectoplasmic specializations occur (Russell et al., 1988). Vinculin, a protein found at actin-related adhesion junctions in other tissues, is a component of the structures both in mammals (Grove and Vogl, 1989) and in non-mammalian vertebrates (Pfeiffer and Vogl, 1993). In addition, at least one adhesion molecule (α6β1 integrin) has been localized to Sertoli cell regions where ectoplasmic specializations are known to occur (Palombi et al., 1992; Salanova et al., 1995). Also, the appearance and/or disappearance of ectoplasmic specializations is correlated temporally with three major events where changes in intercellular adhesion occur: 1) the positioning of newly elongating spermatids in Sertoli cell crypts, 2) the release of morphologically mature sperm cells from the apices of Sertoli cells, and 3) the movement of spermatocytes from basal to adluminal compartments of the epithelium (Russell, 1977b, 1984; Russell et al., 1979). Finally, comparative studies have demonstrated that the morphology of ectoplasmic specializations in non-mammalian vertebrates closely resembles that of actin-related adhesion junctions generally found in cells (see above). This observation not only supports the argument that ectoplasmic specializations are a form of actin-related adhesion junction, but also indicates that the unique mammalian form of junction probably evolved from one similar to those found in other epithelia.

The selection pressure that led to the dramatic change in junction structure from those present in non-mammalian forms to that present in mammals is not known. It may have been related to improving structural support for junctional areas of the plasma membrane attached to the irregular contours of spermatid heads in mammals. In this case, the selection pressure, applied at apical sites, led to a change in the genetic program for actin-related adhesion junctions generally in the cell. This would account for the same form of junction architecture developing both at apical and basal sites even though the pressure for change was applied only apically. Alternatively, some as yet unidentified selection pressure could have existed both at apical and basal sites.

FUNCTIONS

Numerous functions have been attributed to ectoplasmic specializations. Among the most important of these is a primary role in establishing and maintaining intercellular attachment. Assembly and disassembly of these adhesion junctions is clearly fundamental to maintaining the integrity of the epithelium, to releasing mature spermatids into the duct system, and to moving spermatocytes through basal junction complexes. At apical sites, an additional function may be that of translocating spermatids in the epithelium.

Intercellular adhesion—the primary function

A large body of evidence supports the conclusion that ectoplasmic specializations are involved in intercellular adhesion. Structural linkages are visible between opposing plasma membranes in electron micrographs of ectoplasmic specializations (Russell et al., 1988). These linkages are particularly prominent in some non-mammalian vertebrates (Stanley and Lambert, 1985; Pfeiffer and Vogl, 1994).
Spermatid translocation—a secondary function at apical sites

Mammals

Although the primary function of ectoplasmic specializations is likely intercellular adhesion, the junction plaques also may be involved with positioning and translocating spermatids in the seminiferous epithelium. As spermatids polarize, they become situated in apical invaginations, or crypts, of Sertoli cells. Ectoplasmic specializations develop in regions of the crypt associated with the developing spermatid heads. At certain stages of spermatogenesis, the crypts dramatically deepen, thereby transporting the spermatid heads into basal regions of the epithelium (Fig. 7). In the rat, spermatid heads at stage V often lie adjacent to Sertoli cell nuclei. In subsequent stages, the crypts again become shallow, resulting in the repositioning of spermatids at the apex of the epithelium for their final maturation and eventual release into the lumen of seminiferous tubules. The functional significance of spermatid movement deep into the epithelium is not known, but may be related to providing mechanical support for elongating spermatids or to increasing the surface area of contact between the spermatids and Sertoli cells for exchange.

For many years it has been hypothesized that the mechanism responsible for spermatid translocation is the movement of ectoplasmic specializations, and hence the attached spermatids along microtubule tracts in Sertoli cells (see VOGL, 1988) (Fig. 8). It is proposed that the molecular motors that generate the force for the movement are anchored to the cytoplasmic face of the endoplasmic reticulum component of the junction plaques (REDENBACH and VOGL, 1991; VOGL et al., 1991). Although microtubule-based vesicular transport, involving motor proteins such as the kinesins and cytoplasmic dyneins, is a general phenomenon in cell biology (HIROKAWA, 1998), the potentially significant feature of the Sertoli cell system is that an intracellular transport system is used to move an adjacent cell by coupling the machinery to an intercellular junction.

A number of general observations are consistent with the microtubule-based spermatid translocation hypothesis. First, microtubules are abundant in Sertoli cells. They surround apical crypts in which spermatids are embedded and are arranged in a fashion that is consistent with the proposed direction of movement; that is, microtubules are predominantly arranged parallel to the long axis of the cell and to the direction of translocation. Somewhat surprisingly, Sertoli cell microtubules are nucleated apically (VOGL et al., 1995) and have their plus ends oriented basally (REDENBACH and VOGL, 1991). Second, microtubule-based motor proteins are well represented in the testes. In fact, the concentration of cytoplasmic dynein in the testis is second only to that in the brain (COLLINS and VALLEE, 1989), and the motor appears concentrated in Sertoli cells (NEELEY and BOEKELEHEIDE, 1988). Recently, four isoforms of the dynein heavy chain have been identified in the testis.
Fig. 7. During spermatogenesis, elongate spermatids dramatically change position in the epithelium. This is illustrated in these three images of sequential stages (II-III, V, VII) of rat spermatogenesis. The heads of spermatids are transported deep into the epithelium by stage V of spermatogenesis and are then subsequently returned to the apex of the epithelium in preparation for eventual release into the duct system. The functional significance of this translocation is not known. Bar = 25 μm

(Criswell and Asai, 1998). Numerous members of the kinesin superfamily of motor proteins also are present in the testis, some of which are specific to this organ (Aizawa et al., 1992; Sperry and Zhoa, 1996; Nakagawa et al., 1997). Third, pharmacological disruption (depolymerization) of microtubules in Sertoli cells alters the position of spermatids in the seminiferous epithelium (Vogl et al., 1983; Allard et al., 1993). Fourth, ectoplasmic specializations associated with spermatids are structurally related to Sertoli cell microtubules; in fact, linkages have been described between the endoplasmic reticulum component of ectoplasmic specializations and microtubules in extracted tissue (Russell, 1977a). Fifth, there is support for the translocation of endoplasmic reticulum networks in general by microtubule-based transport mechanisms in other systems (Terasaki et al., 1984; Lee and Chen, 1988; Dabora and Sheetz, 1988; Lee et al., 1989; Vale and Hotani, 1989; Cole and Lippincott-Schwartz, 1995). Moreover, motor proteins (Hollenbeck, 1989; Shmitz et al., 1994; Allan, 1995; Lane and Allan, 1999) and at least one of their "receptors" (Toyoshima et al., 1992) have been localized to this membranous organelle.

If the microtubule-based hypothesis of spermatid translocation in mammals is true, then one can make a number of predictions. In binding assays, more exogenously added microtubules should attach to isolated junction plaques in the absence of nucleotide (ATP) than when nucleotide is present. This is because when ATP is present, microtubules should cycle off of the plaque in the presence of motor proteins. In experiments where exogenous microtubules are mixed with spermatid/junction complexes that have been mechanically dissociated from the seminiferous epithelium, significantly more microtubules do bind to the complexes in the absence of ATP than in the presence of nucleotide (Redenbach et al., 1992; Vogl, 1996). That the microtubules indeed bind to the endoplasmic reticulum component of the junction plaques has been demonstrated using electron microscopy (Vogl, 1996). A second prediction is that in motility assays, isolated junction plaques should transport microtubules. Moreover, this movement should occur both in the plus and the minus directions to account for movement of spermatids in vivo. Spermatid/junction complexes attached to motility chambers do transport fluorescently labeled microtubules in the presence of ATP (Fig. 9; Beach and Vogl, 1999). Significantly, when polarity labeled microtubules are used in the assay system, transport occurs both in the plus and minus directions (Guttman and Vogl, 1999). The latter result is consistent with the prediction that motors capable of transporting the junction plaques along microtubules to the base (plus end direction) and to the apex (minus end direction) of the epithelium are associated with ectoplasmic specializations. Antibodies to cytoplasmic dynein (intermediate chain —IC74), a minus end directed motor, strongly react with sites in the seminiferous epithelium known to contain ectoplasmic specializations associated with spermatid heads (Miller et al., 1999). At the ultrastructural level, these antibodies react with sites associated with the cytoplasmic face of the endoplasmic reticulum of the junction plaques (Kimel and Vogl, unpublished observations). Based on the motility data and the direction of spermatid transport in vivo, a member of the kinesin superfamily of mechanoenzymes (generally plus end directed motors) also is predicted to be present on the junction plaques, but this has yet to be established and the isotype identified. Motor associated elements, such as
dynactin and kinectin, also are predicted to be present, but have not been studied.

Non-mammalian vertebrates

As with mammalian ectoplasmic specializations, the main function of these junctions in non-mammalian vertebrates is likely intercellular adhesion. However, in at least one class of non-mammalian vertebrates, there is evidence to suggest that ectoplasmic specializations may also play a direct role in the positioning of spermatids. In the class Chondrichthyes (the cartilaginous fish), each Sertoli cell encompasses and isolates an isogenic clone of spermatogenic cells. During the process of spermiogenesis, spermatid heads, situated within apical Sertoli cell crypts, become oriented toward the base of the Sertoli cell and packed into tight bundles (STANLEY and LAMBERT, 1985; PUDNEY, 1993, 1995). In the ratfish, Sertoli cells form ectoplasmic specialization-like junctions around the heads of elongating spermatids (STANLEY and LAMBERT, 1985). Significantly, the actin filaments of these
modified junctions extend down to and are presumably linked to the base of the cell, and the filaments are associated with myosin II. It is proposed that contractions of these filament networks position and bundle together the spermatids. Although the mechanisms proposed for positioning spermatids in the ratfish and for positioning and translocating spermatids in mammals both involve ectoplasmic specializations, the two mechanisms fundamentally differ from each other. In the ratfish, spermatid movement is performed by the contraction of actin bundles within ectoplasmic specializations, whereas in mammals, translocation is by a microtubule-based transport mechanism that is coupled to the junction plaque.

Whether or not translocation of spermatids—via an ectoplasmic specialization mediated mechanism—occurs in other non-mammalian classes remains to be determined. In certain reptilian species such as the alligator, elongating spermatids do become situated in deep recesses within Sertoli cells (Fig. 10), similar to those seen in mammals. Also as in mammals, these spermatids shift to an apical location in the epithelium during subsequent stages. The mechanism responsible for this repositioning is unknown. The contractile nature of non-mammalian ectoplasmic specializations certainly indicates to us that contraction of these junctions may participate in the process, at least in the movement of spermatids deep into the epithelium. Ectoplasmic specializations also may be part of the general mechanism by which spermatid orientation is determined in non-mammalian vertebrates. Elongate spermatid heads in these vertebrates are maintained in an orientation perpendicular to the base of the epithelium; that is, the long axis of spermatid heads remains roughly perpendicular to the base of the epithelium. The presence of ectoplasmic specializations in non-mammalian vertebrates and the organization of these junction plaques around elongating spermatid heads are consistent with the hypothesis that ectoplasmic specializations somehow may be involved. The three dimensional organization of microtubules in non-mammalian vertebrate Sertoli cells and the possible function of these cytoskeletal elements in spermatid orientation and translocation in non-mammalian vertebrates is unknown.

Control mechanisms

One of the most interesting and potentially most significant areas yet to be fully explored is the control of ectoplasmic specializations. An understanding of how these adhesion junctions are assembled and disassembled at specific times during sper-

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**Fig. 9.** Ectoplasmic specializations that remain attached to spermatids mechanically dissociated from the seminiferous epithelium transport microtubules in the presence of ATP. In this sequence of images, a microtubule moves (arrow) in an "inch worm-like" fashion along the region of an ectoplasmic specialization associated with the dorsal surface of a spermatid. Actin in the ectoplasmic specialization is labeled with a probe for filamentous actin (red). This series of images is modified from those presented in BEACH and VOGL (1999). Bar=10 μm.
matogenesis is fundamental to our understanding of sperm release, spermatid attachment to the epithelium, and turnover of basal junction complexes between Sertoli cells (see Fig. 8). At apical sites, precise control of filament rearrangements within the plaques and of microtubule-based motor proteins associated with the junctions must also occur, but has not been studied.

Theoretically, control of the assembly and disassembly of these unique adhesion junctions could occur from "outside-in" or from "inside-out". In the "outside-in" scenario, the formation or dissolution of the adhesion bond between adjacent cells would occur by an initial change in adhesion molecules brought about by changes in association with the ligand. These changes would activate a signaling cascade that ultimately leads to the assembly or disassembly of the associated junction structure. Alternatively, in the "inside-out" model, the specific signaling cascade leading to formation or breakdown of intercellular adhesion and the junction plaque would be activated by a stimulus from within the Sertoli cell, possibly in response to some signal received elsewhere at the cell surface. It is possible that the control of assembly and disassembly is activated differently. Complicating matters further is the likely occurrence of multiple levels of signaling cascades from those involving circulating hormones to those at the level of the junction plaque itself. A further complication is that at basal sites, the intercellular junction involves similar cell types (Sertoli cells), whereas at apical sites, the interaction is between cells of entirely different lineages (Sertoli (somatic) cells and spermatogenic (germ) cells).

There is a growing body of evidence that circulating hormones do, at least at some level, influence junctions in the seminiferous epithelium. FSH appears necessary for the appearance of αβ1 integrin heterodimer in basal junction complexes as demonstrated by comparing non-FSH supplemented versus FSH supplemented rat testes in organ cultures (SALANOVA et al., 1998). Testosterone is well known to play a fundamental role in regulating spermatogenesis in general (SAR et al., 1993) and the importance of this hormone to intercellular adhesion in the seminiferous epithelium has been evaluated both in vitro and in vivo (O’DONNELL et al., 1996; PERRYMAN et al., 1996; ORTH et al., 1998). In culture, the binding of round spermatids to Sertoli cells is stimulated by testosterone and the follicle stimulating hormone (PERRYMAN et al., 1996). In vivo, testosterone withdrawal promotes detachment of round spermatids between stages VII and VIII (O’DONNELL et al., 1996), and also results in a failure of round spermatids to progress into elongation (MCLEACHLAN et al., 1994). The relationship of these observations to ectoplasmic specializations is not clear; however, the observations that ectoplasmic specializations form in association with early elongate spermatids during stage VII/VIII (RUSSELL et al., 1979), that sperm release occurs during stage VIII (LEBLOND and CLERMONT, 1952), and that the movement of spermatocytes through basal junction complexes occurs during stages VIII through XI (RUSSELL, 1977b), make this general period of spermatogenesis of particular significance from an ectoplasmic specialization-mediated cell adhesion perspective.

Although there have been many studies on the influence of hormones on development and maintenance of the seminiferous epithelium, there are few studies (WINE and CHAPMAN, 1999) of signaling cascades related directly to the turnover of ectoplasmic specializations. Problems here include a lack of consensus over the actual adhesion components present at the junctions and the lack of a culture system for easily testing proposed models. As mentioned above, the presence of a cadherin at ectoplasmic specializations remains controversial. Although labeling of β1 integrin at the sites not always has been reported as positive (WINE and CHAPMAN, 1999), results from at least two laboratories (PALOMBI et al., 1992; SALANOVA et al., 1995; PFEIFFER et al. 1991)
using different antibodies indicate that an integrin is likely one of the adhesion elements present at the sites; however, this has not been confirmed at the ultrastructural level. The presence of this integrin at the junctions will allow the development of testable models of signaling cascades based on those developed in other systems (DEDDAR AND HANNIGAN, 1996; DEDDAR, 1999; GIANCOTTI and RUOSLAHTI, 1999). ILK (integrin linked kinase), a signaling peptide associated with β1 integrins (HANNIGAN et al., 1996), has been recently localized by immunofluorescence to ectoplasmic specializations (MULHOLLAND and VOGT, 1999). Other control elements have to be determined. The relationship between hormone stimulated signaling cascades, cascades activated by incoming information from adjacent germ cells, and cascades leading to control of adhesion receptors themselves remain to be defined.

How functions secondary to that of intercellular adhesion—such as spermatid translocation—are controlled also remains to be established. Spermatid translocation is particularly interesting because movement of the junction plaque occurs in two directions along microtubule tracts, and movement in each direction occurs at a precise time during spermatogenesis. Control of the process may occur at multiple levels. For example, the motors may be recruited to the sites at specific stages and/or the motors may be activated only at specific times during the spermatogenic cycle.

CONCLUSIONS AND FUTURE DIRECTIONS

Although the basic ultrastructure of ectoplasmic specializations in mammals is now known and the importance of these structures both to intercellular adhesion and to movement of spermatids in the epithelium is appreciated, a number of important issues remain unresolved. Only a few molecular components of the junction have been identified, and not all of these have been localized to specific domains of the structure. The ligand for integrin at the junction has not been identified nor is it known how many non-integrin based adhesion elements are present at the sites. Very little is known about the function of the endoplasmic reticulum at the junction plaque. Structural linkages that bind the reticulum to the actin layer also have not been identified. The biological significance of spermatid translocation still is a mystery, and the isotypes of microtubule-based motor proteins associated with the junction remain to be identified. One of the most exciting avenues of research in the future will be to determine how the adhesion and motility properties of ectoplasmic specializations are controlled. It is possible that defects in the structural or controlling elements of the junctions may be the cause of certain forms of male infertility. Furthermore, if some of these elements are specific to terminally differentiated Sertoli cells, then these elements could be targets for fertility control in men.

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