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Science. Author manuscript; available in PMC 2013 June 27.

Published in final edited form as:
Science. 2012 November 9; 338(6108): 798–802. doi:10.1126/science.1219969.

Germ Cell Migration across Sertoli Cell Tight Junctions

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Abstract

The blood-testis barrier includes strands of tight junctions between somatic Sertoli cells that restricts solutes from crossing the paracellular space, creating a microenvironment within seminiferous tubules and providing immune privilege to meiotic and postmeiotic cells. Large cysts of germ cells transit the Sertoli cell tight junctions (SCTJs) without compromising their integrity. We used confocal microscopy to visualize SCTJ components during germ cell cyst migration across the SCTJs. Cysts become enclosed within a network of transient compartments fully bounded by old and new tight junctions. Dissolution of the old tight junctions releases the germ cells into the adluminal compartment, thus completing transit across the blood-testis barrier. Claudin 3, a tight junction protein, is transiently incorporated into new tight junctions and then replaced by claudin 11.

Tight junctions are specialized anchoring connections between cells and are composed of several integral and peripheral membrane proteins (1, 2). The elaborate fibrils formed by the tight junction components play a critical role in multi-cellular organisms by sealing off the space between neighboring epithelial cells, thereby partitioning compartments within an organism (3).

Spermatogenesis takes place within seminiferous tubules of the testis. Extensive tight junctions between somatic Sertoli cells, referred to ultrastructurally as basal ectoplasmic specializations, create one of the body's tightest epithelial barriers and in doing so create separate functional compartments for diploid spermatogonia in the basal compartment, and differentiating spermatocytes and haploid spermatids in the adluminal compartment (fig. 1A). To prevent autoimmunity and maintain a conducive microenvironment for differentiation, differentiating germ cells must cross this barrier without disrupting its functional integrity (4). To compound the challenge, germ cells are contained within cysts, which can be composed of hundreds of cells connected by intercellular bridges formed as a result of incomplete cytokinesis during transit amplification of differentiating spermatogonia (5). The mechanism by which large syncytial chains of preleptotene spermatocytes move from the basal to the adluminal compartment without causing damage to the SCTJs is unknown.

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Supplementary Materials
Figs. S1 and S2
Movie S1
Materials and Methods

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To visualize the 3-dimensional (3D) organization of the SCTJs, we stained whole seminiferous tubules dissected from adult mice for claudin 11 (CLDN11) and F-actin, both of which are known constituents of the SCTJs (6, 7). The tubules were then imaged by confocal microscopy from the basal surface into the tubule in ~200 optical sections, which were rendered into a single image with a depth of approximately 15μm (fig. 1A).

First, we imaged regions of the seminiferous tubules where no germ cells were migrating across the SCTJs. CLDN11 and bundles of filamentous-actin, labeled with phalloidin, both marked the SCTJs. In these regions, CLDN11 circumscribed each Sertoli cell near the basement membrane (fig. 1B and C), and delineated the basal and adluminal compartments by passing between the spermatogonia and pachytene spermatocytes (fig. S1, A and B).

We then imaged regions of the seminiferous tubules where preleptotene spermatocytes were migrating across the SCTJs, using spermiation (release of spermatids into the lumen) as a marker for this transit (8). During preleptotene spermatocyte translocation across the SCTJs, CLDN11 tight junctions were observed on both the basal and apical sides of the migrating spermatocytes (fig. 2A and B). Additionally, we observed vertical cross-linked connections between the basal and apical tight junction fibrils, enclosing the spermatocytes in small compartments. These vertical tight junctions were found only at the tricellular junctions of three Sertoli cells (fig. 2A arrowhead), possibly suggesting a role of these specialized tight junction structures in the formation of the compartments (9). These tight junction-demarcated compartments were observed only enclosing leptotene spermatocytes. Therefore, as initially suggested more than three decades ago (10), leptotene spermatocytes are temporarily enclosed in an intermediate compartment (fig. 2B).

Because leptotene spermatocytes are syncytial, forming long chains of cells joined by cytoplasmic bridges (5), and the compartments we observed generally contained only two or three leptotene spermatocytes, we hypothesized that the cytoplasmic bridges must pass between the enclosed compartments at the regions of the tricellular junctions. To image cysts of leptotene spermatocytes in the context of the SCTJ compartments, seminiferous tubules were stained with both CLDN11 and testis expressed gene 14 (TEX14), which localizes to a portion of each cytoplasmic bridge (11). As expected, TEX14 was observed between cells within individual compartments, and as hypothesized, we observed intercellular bridges, marked by TEX14, within tricellular junctions (fig. 2A arrowhead), suggesting that cells within a common syncytia span compartments (fig. 2B). In no cases did we observe co-staining of TEX14 with either the apical (old) or basal (new) tight junction fibrils, suggesting that intercellular bridges connecting germ cells within a cyst pass between the smaller compartments, linking them to ultimately comprise a single large network of compartments enclosing the entire cyst.

Our observation that migrating spermatocytes are bounded both apically and basally by SCTJs, suggests that the basal surface of the Sertoli cells form new Sertoli-Sertoli cell contacts to accommodate the formation of the new tight junctions. To visualize the 3D structure of the Sertoli cells, we generated mice that express EGFP only within the cytoplasm of Sertoli cells. The seminiferous tubules from the resulting mice were then stained for EGFP to visualize the Sertoli cell body, and CLDN11 and F-actin, to visualize the SCTJs. Prior to leptotene migration across the SCTJs, we observed the basal surfaces of Sertoli cells adhered to the basement membrane, except where spermatogonia were present (fig. S2A). To accommodate spermatogonia that were also bound to the basement membrane, the basal surfaces of neighboring Sertoli cells were displaced away from the basement membrane, cradling the spermatogonia in trough-like structures (fig. S2A). The SCTJs were present at the Sertoli-Sertoli cell contacts just below the spermatogonia (movie S1). As spermatogonia continue to proliferate and differentiate, the chains of cells expand

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between the Sertoli cells, such that by Stage VII of spermatogenesis, just before the leptonema migration, the basal surfaces of the Sertoli cells no longer contact one another (fig. S2B). As the preleptotene spermatocytes lose contact with the basement membrane, the basal surface of the Sertoli cells intercalate between the preleptotene spermatocytes (now leptotene spermatocytes) and the basement membrane (fig. S2C), enclosing the migrating spermatocytes in a tunnel-like structure (fig. S2D). This change in Sertoli cell shape also allows for the formation of new Sertoli-Sertoli cell contacts and new tight junctions on the basal side of the leptotene spermatocytes, while still maintaining the old SCTJs.

We have previously shown that CLDN3 is transiently expressed at the time of SCTJ remodeling and translocation of leptotene spermatocytes from the basal to the adluminal compartment (12). To determine whether CLDN3 localizes to new or old tight junctions, we stained adult seminiferous tubules for CLDN3 and with phalloidin, which stains bundles of F-actin anchored at CLDN11-delineated TJ fibrils (fig. 1B). We first observed CLDN3 staining in Stage VII, just prior to the leptonema migration. Rather than localizing to the SCTJs as expected, CLDN3 was distributed across the basal surface of the Sertoli cells (fig. 3A), consistent with the observation that there are no Sertoli-Sertoli cell contacts on the basal surface of Sertoli cells at this stage to allow for tight junction formation.

At Stages VIII-IX of spermatogenesis, when the leptotene spermatocytes are crossing the SCTJs, CLDN3 was integrated into SCTJs and no longer distributed on the basal surface (fig. 3B). Specifically, CLDN3 was localized to the newly forming tight junction at the new Sertoli-Sertoli cell contacts near the basement membrane, bridging gaps in the SCTJs where migrating preleptotene spermatocytes were being enclosed in the SCTJs. By Stage XI, when the leptotene spermatocytes emerge into the adluminal compartment, CLDN3 was almost completely absent from the seminiferous tubule (fig. 3C).

Based on these findings, we propose a model for spermatocyte translocation across the SCTJs. In this model, as the preleptotene spermatocytes lose contact with the basement membrane, the basal membrane of the Sertoli cells is loaded with CLDN3 (fig. 4A). The Sertoli cells then intercalate between the basement membrane and the preleptotene spermatocytes, and new CLDN3-containing tight junctions form at regions of new Sertoli-Sertoli cell contact (fig. 4B). CLDN11 then replaces CLDN3, enclosing the chains of leptotene spermatocytes within the SCTJs. Once the chain is fully enclosed within the SCTJs, the “old” tight junctions are removed, releasing zygotene spermatocytes into the adluminal compartment (fig. 4C).

We have shown that portions of syncytial chains of preleptotene spermatocytes become enclosed within a network of transient compartments, bounded by apical and basal TJ fibrils and sealed by vertical tight junction strands that form at tricellular junctions. By visualizing a portion of the cytoplasmic bridges using TEX14, we were able to conclude that the intercellular bridges that connect germ cells within a syncytium span neighboring compartments by crossing the tricellular junctions, thereby allowing a complete syncytium to remain intact while also being enclosed within the SCTJs. Our studies resolve the long-standing question of movement of an entire syncytium across the blood-testis barrier and provide insight into the molecular and cellular mechanism of new tight junction formation.

Supplementary Material

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Acknowledgments

We thank M. Lessard and J. Denegre for assistance with confocal imaging and S. Leighton for artistic rendering of the images. This research was supported by a grant to REB from NICHD/NIH (HD12629) as part of the Specialized Cooperative Centers Program in Reproduction Research and from NCI (CA34196) in support of The Jackson Laboratories shared services.

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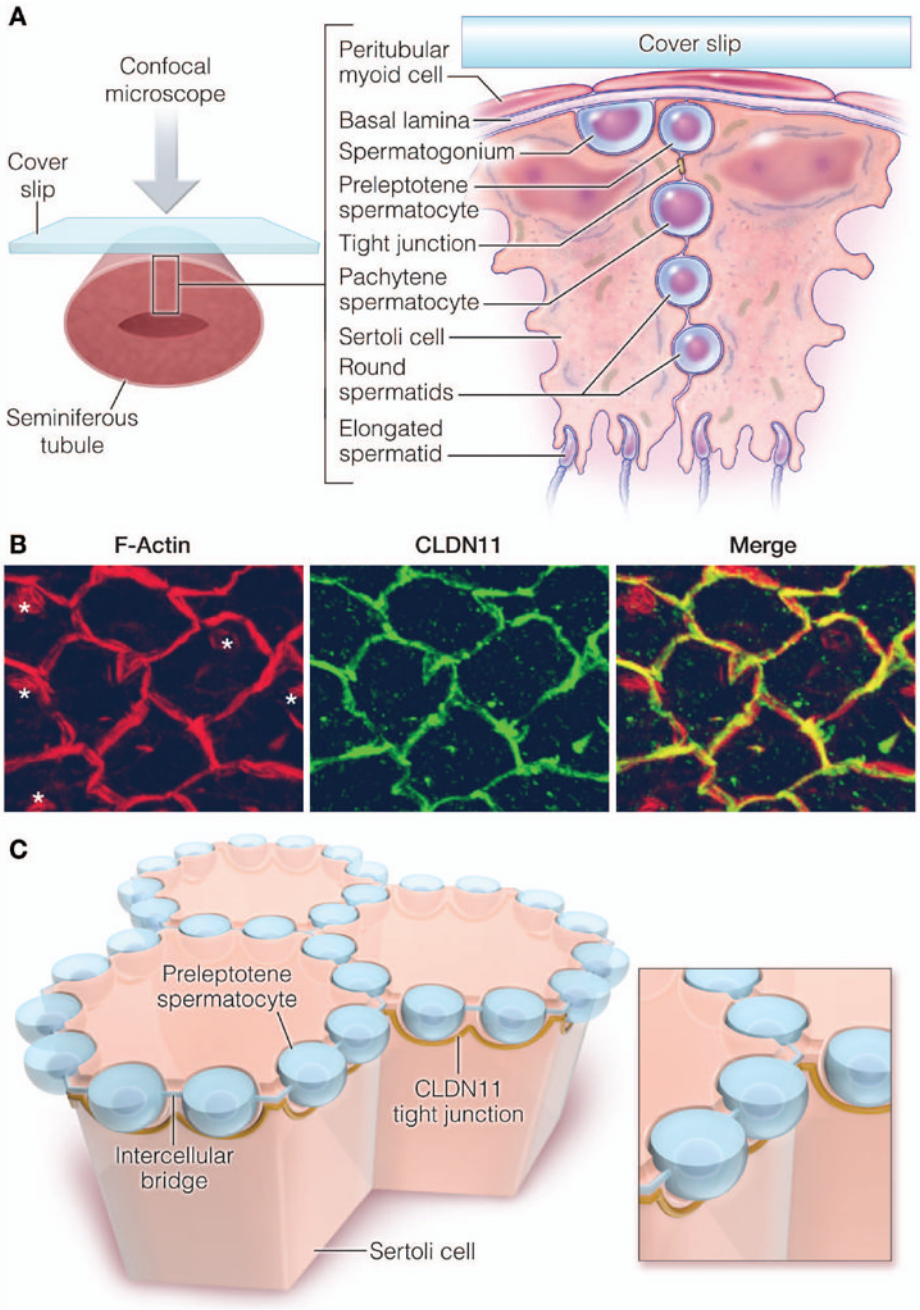

Fig. 1. Imaging tight junction fibrils between Sertoli cells

A) The schematic on the left illustrates the orientation of the confocal microscope with respect to the seminiferous tubule. The area imaged is demarcated with a rectangle. The schematic on the right depicts a region of the epithelium prior to preleptotene migration showing germ cells at different steps of differentiation along with supporting Sertoli and peritubular myoid cells. B) Confocal image of SCTJs visualized during Stage IV, a stage in which germ cells are not transiting the SCTJs, using two different markers, F-actin and CLDN11. Bundles of F-actin, labeled with phalloidin, were detected at both the SCTJs and the apical ectoplasmic specializations surrounding the heads of elongating spermatids (*). CLDN11 localized to just the SCTJs. The merged image shows that both markers label the SCTJs. C) An artist's rendition of the confocal images shown in (B), fig. S2 and movie S1,

illustrating three Sertoli cells, the CLDN11-containing tight junctions circumscribing each Sertoli cell and chains of preleptotene spermatocytes connected by intercellular bridges.

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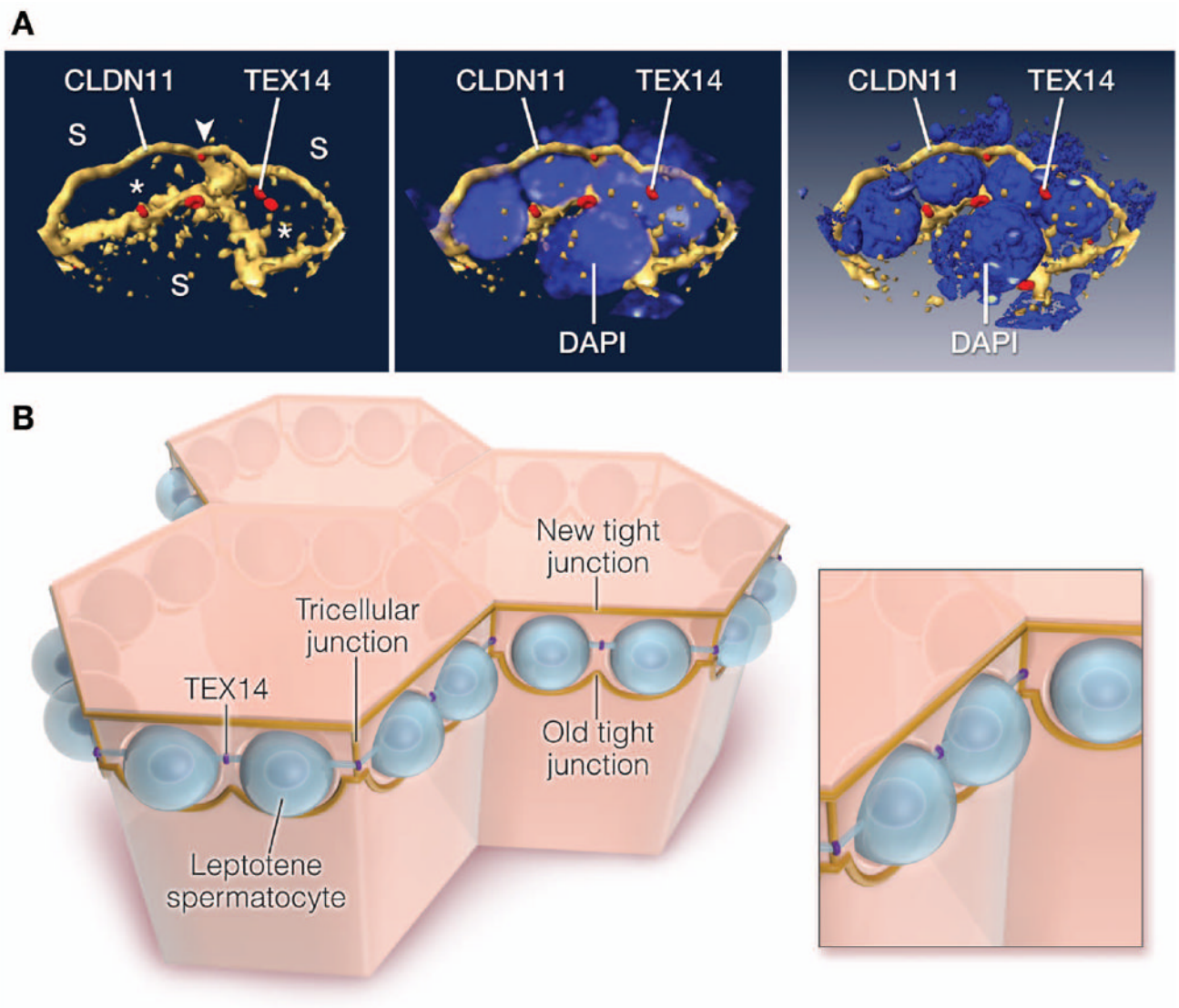

Fig. 2. Formation of transient compartments bounded by tight junctions during migration of preleptotene spermatocytes across Sertoli cell tight junctions

A) During germ cell migration across the SCTJs, Stage IX of the seminiferous epithelium cycle, the leptotene spermatocytes are enclosed within compartments (*) bounded by SCTJs. A double-layer tight junction that sandwiches the leptotene spermatocytes forms the compartments. The arrowhead shows connections between tight junction layers formed at the tricellular junction of three Sertoli cells (S). TEX14 (red) marks the cytoplasmic bridges between the leptotene spermatocytes. TEX14 localization to the tricellular junctions (arrowhead) reveals that the cytoplasmic bridges between spermatocytes pass through the tricellular junctions. B) A schematic of (A) illustrating germ cell migration across the SCTJs showing that the leptotene spermatocytes are transiently enclosed within a network of compartments bounded by tight junctions both apically and basally.

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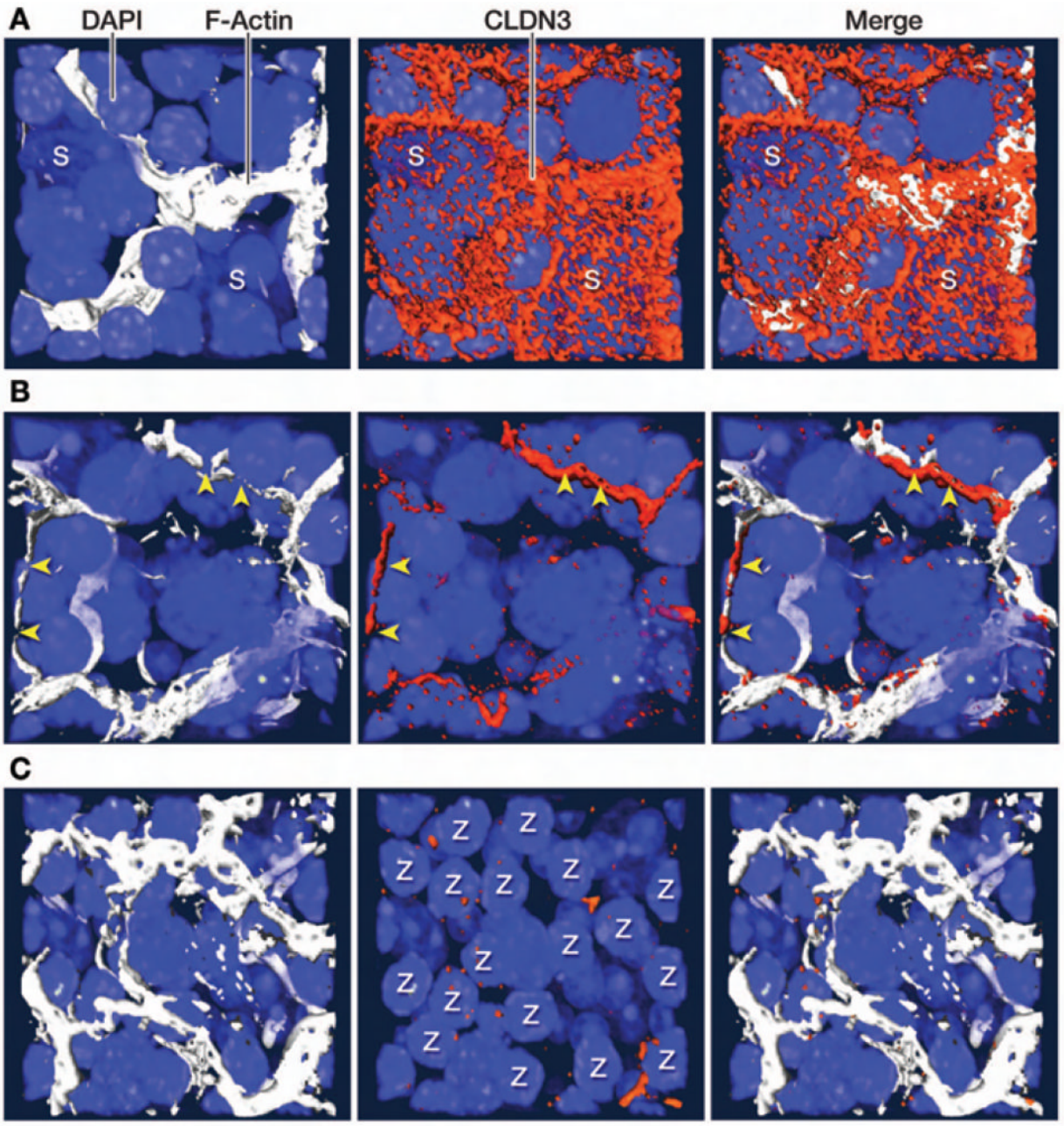

Fig 3. CLDN3 localizes to the newly forming SCTJs

A) Just prior to the migration of preleptotene spermatocytes across the SCTJs, in stage VII of the seminiferous epithelium cycle, CLDN3 (orange) is initially localized to the basal surface of the Sertoli cells (S) rather than the SCTJs (white), labeled with phalloidin. B) In Stage IX of the seminiferous epithelium cycle, when the leptotene spermatocytes migrate across the SCTJs, the spermatocytes are enclosed within the SCTJs (white). However, in this image the basal side of these compartments is incomplete as visualized as breaks in the tight junctions (yellow arrowheads). CLDN3 (orange) localizes to these breaks in the incomplete tight junction fibrils. C) During stage XI of the seminiferous epithelium cycle, after the zygotene spermatocytes (Z) have emerged into the adluminal compartment, CLDN3

(orange) has been almost completely removed from the SCTJs (white). By stage XI, much of the old (apical) tight junctions have been removed.

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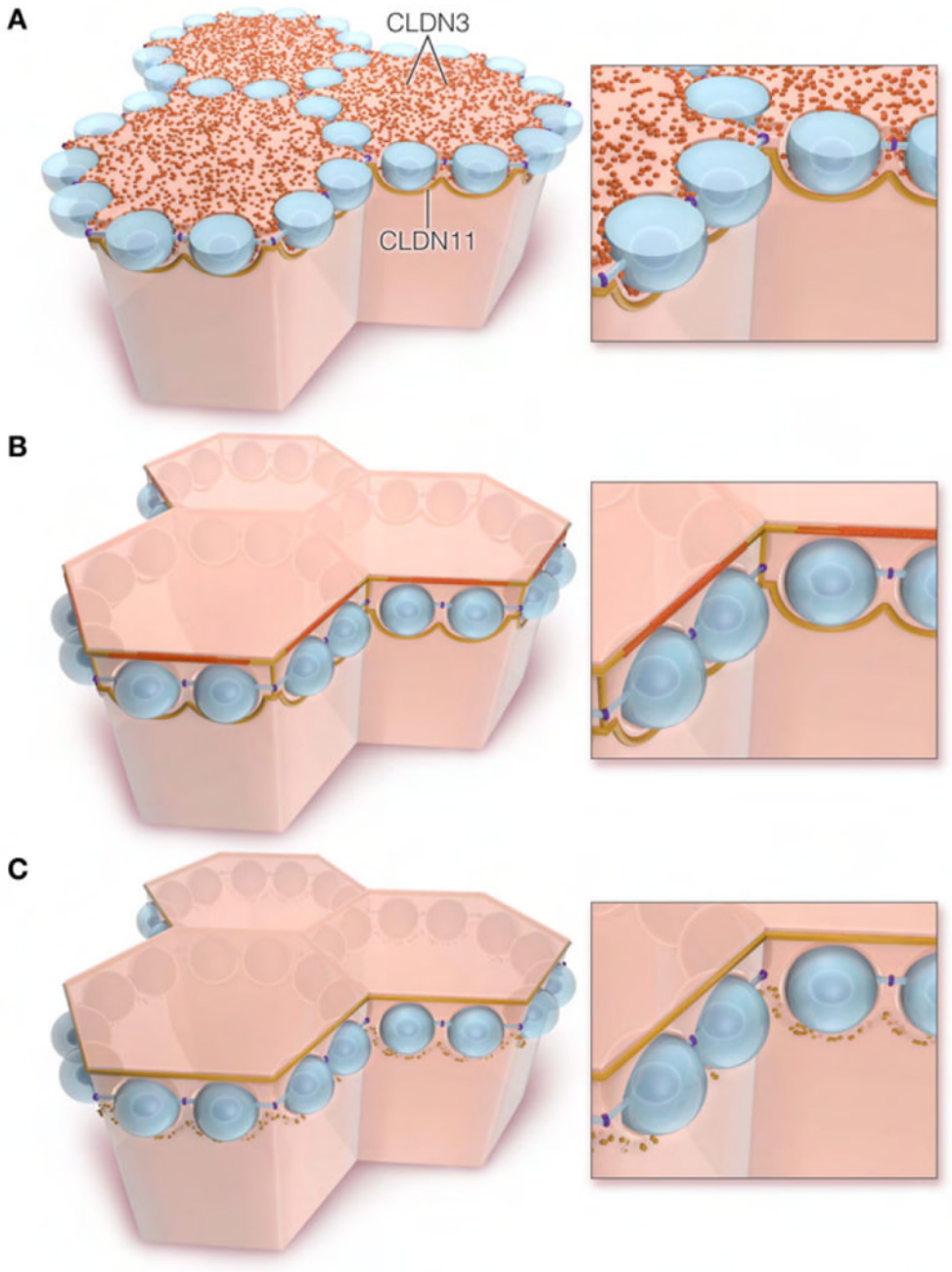

Fig 4. Model for tight junction remodeling during germ cell cyst transit across the blood-testis barrier

A) Schematic illustration of the confocal image in fig. 3A. B) Drawing representing the confocal image in fig. 3B showing segmental localization of CLDN3 (red) and CLDN11 (brown) in areas of new tight junction formation. CLDN11 is usually found in and near tricellular junctions. Once the preleptotene spermatocyte leaves the basement membrane, Stage VIII, the basal surface of the Sertoli cells intercalates between the basement membrane and the preleptotene spermatocyte, forming new Sertoli-Sertoli cell contacts. CLDN3 (red) then localizes to this new contact and forms a new tight junction that encloses the preleptotene spermatocytes. Inset on right shows passage of an intercellular bridge, marked by TEX14 (purple) through a tricellular junction connecting two compartments. C)

Illustration of confocal image in fig. 3C showing replacement of CLDN3 with CLDN11 in the new tight junction and removal of the old tight junction.

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