



<b>Title</b>	<b>Transforming Growth Factor- 3-Mediated Regulation of Junctional Adhesion Molecule-B (JAM-B) in Testicular Cells</b>
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to internalize intercellular junctions as part of the sperm release mechanism. In rat, tubulobulbar complexes form in two parallel rows adjacent to each spermatid head. Each tubulobulbar complex consists of a double membrane core formed from the plasma membranes of the two attached cells and is cuffed by a dendritic network of actin filaments. The entire structure is capped by a clathrin-coated pit. Fragments of epithelium, containing mature spermatids and adjacent Sertoli cell regions containing tubulobulbar complexes, from perfusion fixed rat testes, were labeled with probes for actin, spectrin and protein EPB 4.1. The material was imaged using conventional fluorescence or confocal microscopy. The immunological probes for spectrin and EPB 4.1 were co-distributed, but surprisingly, their signals did not overlap with the actin staining; rather their staining appeared to be concentrated between adjacent complexes or to surround the actin cuffs of the complexes. The spectrin antibody reacted with a single band on immunoblots of whole testis and seminiferous epithelium. We suspect that the spectrin network may stabilize the actin cuffs or perhaps function as spacers between adjacent complexes. Our observations add another level of complexity to the cytoskeletal organization of tubulobulbar complexes. Supported by an NSERC Discovery grant to AWW.

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#### **Transforming Growth Factor- $\beta$ 3-Mediated Regulation of Junctional Adhesion Molecule-B (JAM-B) in Testicular Cells.**

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Junctional adhesion molecule-B (JAM-B) is found between Sertoli cells as well as between Sertoli and germ cells in the testis. The expression of JAM-B is highly regulated to facilitate the passage of developing germ cells across the blood-testis barrier as well as the release of mature spermatids. Transforming growth factor beta (TGF- $\beta$ ) family is implicated in the regulation of testicular cell junction dynamics during spermatogenesis. This study aims to investigate the influence of TGF- $\beta$ 3 on the expression of JAM-B as well as the underlying mechanisms. TGF- $\beta$ 3 (5 ng/ml) treatment coupled with RT-PCR and immunoblot analyses have shown that TGF- $\beta$ 3 down-regulates JAM-B expression on mRNA and protein levels in a time-dependent manner in mouse Sertoli cell line, MSC-1 cells. Cycloheximide assay further indicates that the reduction of JAM-B protein by TGF- $\beta$ 3 is mediated via post-translational modification. Moreover, the involvement of ubiquitin-proteasome pathway in TGF- $\beta$ 3-mediated JAM-B protein destabilization was demonstrated by proteasome inhibitor, MG-132, treatment and ubiquitin siRNA knockdown assays. Furthermore, co-immunoprecipitation (Co-IP) assay has further confirmed that JAM-B protein is conjugated by a chain of ubiquitin upon TGF- $\beta$ 3 stimulation in the presence of MG-132. TGF- $\beta$ 3 also speeds up the degradation of JAM-B through Smad-dependent pathway. As knockdown of Smad3 and/or Smad4 effectively abolish TGF- $\beta$ 3-mediated JAM-B degradation. Taken together, the involvement of both ubiquitin-proteasome pathway and Smad-dependent signalling are essential for TGF- $\beta$ 3-mediated JAM-B regulation in mouse Sertoli cells. [This work was supported by Hong Kong Research Grants Council (HKU772009 and HKU773710) and CRCG Seed Funding for Basic Research.]