transduction pathway. Using SCs cultured *in vitro* to allow the assembly of TJs when the TJ-permeability barrier was monitored by the transepithelial electrical resistance (TER) across the SC epithelium, we have examined if the TGF-β3-induced TJ disruption is mediated via one of the four upstream signal transducers, namely MEKKs, Smad2/Smad3, Cdc42/Rac, and Ras. Cellular distribution studies using RT-PCR have shown that both SC and germ cells (GC) express almost similar levels of mRNA encoding for MEKK2, Smad2, and small GTPases, such as Cdc42, Rac and N-Ras. A TGF-β3-induced transient increase in MEKK2 expression, but not Smad2, Cdc42/Rac2, or N-Ras, was detected in SC during the assembly of the TJ- barrier. The TGF-β3-mediated (3 ng/ml) inhibitory effect on the assembly of TJs could be reversed dose-dependently by SB202190 at 0.1 nM-1 mM, a specific p38-MAP kinase inhibitor. We next investigated the protein expression of p-p38-MAP kinase (activated phoshorylated form) *versus* total p38-MAP kinase (nonphosphorylated inactive form) using SC lysates by immunoblottings and specific antibodies against p-p38- and p38-MAP kinase with a chemiluminiscence-based detection system. It was found that the presence of TGF-β3 indeed regulated the production of p-p38 MAP kinase protein during TJ assembly. In summary: the TGF-β3-mediated effects on the inter-Sertoli TJ dynamics and the blood-testis-barrier functionality are regulated via the p38-MAP kinase pathway. [Supported in part by grants from CONRAD (CICCR96-05-A to CYC), and HKRGC (HKU7245/00M to WML/CYC)].

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T3-Regulated Expression of a Novel Attachment Factor, PB-cadherin, may be Critical for Development of Neonatal Testicular Stem Cells

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In the rodent testis, contact-mediated interactions between gonocytes, or neonatal stem cells, and Sertoli cells are critical for development. Previously, we showed that Thyroid Hormone (T3) regulates expression in neonates of at least one Sertoli cell-gonocyte attachment factor, NCAM. We subsequently used a rat cDNA microarray and detected expression of another factor, short-type PB-cadherin (STPB-C) in neonatal Sertoli cell-gonocyte co-cultures. PB-cadherin is a novel cadherin mainly expressed in pituitary gland and brain of adults which is involved in development by regulating Ca2+-dependent cell-cell adhesion. Therefore, our present aims were (1) to explore expression of STPB-C in vivo, (2) to localize STPB-C mRNA in co-cultures with in situ hybridization, and (3) to determine if expression of STPB-C is regulated by T3. RT-PCR was used to generate cDNA for STPB-C from total RNA isolated from cocultures, cDNA was cloned into pPCR-ScriptTM Amp SK(+) cloning vector, and plasmid DNA was isolated and sequenced to confirm the fidelity of the STPB-C cDNA portion of the plasmid. In subsequent Northern analysis of testicular RNA, expression of STPB-C was strong on day 1, then diminished appreciably by day 3, became barely detectable by day 15, and disappeared in testes of adults. When neonatal cocultures were treated with T3 (10 nM, 24 hr) or vehicle, STPB-C mRNA was strongly expressed by neonatal stem cells and weakly by Sertoli cells in situ, while Northern analysis indicated that expression of STPB-C was down-regulated by T3 in vitro. Thus, regulation of PB-cadherin by T3 during the early neonatal period may be critical in development of the stem cell population from which all maturing germ cells subsequently arise. (Supported by NIH HD-15563.)

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Regulation of Catenins in The Rat Epididymis

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Adhering junctions are essential for the formation and regulation of tight junctions. In the epididymis, tight junctions between adjacent principal cells form the blood-epididymal barrier which creates a specific environment within the lumen of the epididymis necessary for sperm maturation. Adhering junctions in the epididymis are composed of a transmembrane protein, cadherin, and catenins (alpha, beta and P120). The objective of this study was to determine the effects of testis and testicular androgens on the immunolocalization of catenins (alpha, beta and P120) in the epididymis. In intact control adult rat epididymis, each of the three catenins were localized along the lateral plasma membranes of adjacent principal cells as well as between principal and both clear and basal cells. Twenty one days following orchidectomy there was a marked increased in the cytosolic staining of both alpha- and beta-catenin, particularly in the corpus and cauda epididymidis, suggesting a loss in the integrity of the adhering junctions. Interestingly, immunostaining for P120 appeared to be unaltered by orchidectomy. In orchidectomized rats that had been given testosterone implants at the time of orchidectomy, the immunolocaiztion of alpha- and beta-catenin was maintained along the lateral plasma membrane of epididymal principal cells. These data suggest that androgens can maintain the integrity of adhering junctions in the epididymis and may represent a mechanism by which androgens can regulate tight junctions and the blood-epididymal barrier in adult rats.

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Regulation of Sertoli(SC)-Germ(GC) Cell Anchoring Junction (AJ) Dynamics by RhoB GTPase

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During spermatogenesis, there are extensive AJ restructuring, however, the mechanistic pathway that regulates AJ dynamics is not known. Rho GTPases have been implicated in the actin organization and cytoskeletal control. For instance, Rho GTPases are known to regulate AJ functionality by redistributing cadherins during AJ assembly. Using RT-PCR, both SC and GC were found to express RhoB. Moreover, the assembly of SC-GC AJs, but not SC TJs, was associated with a transient induction of RhoB expression. These changes were confirmed with a monospecific RhoB antibody for immunoblottings using cell lysates and a chemiluminiscence-based detection system. Disruption of AJs in SC-GC cocultures by hypotonic treatment also induced a surge in RhoB expression, which became visible within 5-min. Moreover, when SC-GC AJs *in vivo* was disrupted by treatment of rats with a single dose of 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide (DCIC, 300 mg/kg b.w. by gavage), a surge in RhoB expression by ~4-fold was also detected within 1-hr. This is long before the depletion of germ cells from epithelium become visible, which required ~10-day. When GCs were added to the SC epithelium (GC:SC, 1:1), which had been cultured for 5-day at $0.5x10^6$ cells/cm^2 on Matrigel-coated dishes, and cultured for 2-day to allow the assembly of AJs; inclusion of DCIC at 250 ng/ml to the cocultures also induced a surge in RhoB expression ~5 min-1 hr. These results thus illustrate that RhoB is activated during the assembly of AJ, and prior to the actual disruption of AJs. These changes are possibly needed because RhoB regulates redistribution of AJ-proteins, such as cadherins. In summary: (i) RhoB is an important signaling molecule that regulates AJ dynamics in the testis; and (ii) the DCIC-induced GC loss from the epithelium is mediated via the Rho GTPase signaling pathway

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The dynamics of Sertoli (SC)-germ cell (GC) anchoring junctions (AJs) are regulated by E-cadherin, N-cadherin and Src

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Earlier studies have shown that the assembly of AJs between SC is associated with a transient induction in N-cadherin/β-catenin expression. Moreover, the junction dynamics in the testis are regulated by the interplay of phosphatases and kinases that regulates the intracellular phosphoprotein content. To expand these earlier studies, we have assessed the role of E-cadherin, N-cadherin and Src (an AJ-associated signaling molecule) in AJ dynamics. When SC were cultured at 0.5 x 10^6 cells/cm^2 or SC-GC were cocultured (SC:GC ratio at 1:1, SC at 0.5 x 10^6 cells/cm^2) *in vitro* on Matrigel-coated dishes to allow the assembly of AJs, it was associated with a transient induction in the expression of E-cadherin, N-cadherin and Src. Similar changes were detected when cell lysates were prepared from these samples for immunoblottings using the corresponding antibodies and a chemiluminiscence-based detection system. Cellular distribution studies by semi-quantitative RT-PCR revealed that while SC expressed almost twice as much N-cadherin when compared to GC, GC expressed almost 3-times as much E-cadherin as SC, suggesting GC play an important role contributing to the AJ-associated protein pool in the testis. Also, the expression of SC E-cadherin and N-cadherin were stimulated by testosterone and DHT by ~ 3-10 fold at 10^{-9}¡V10^{-7} M, suggesting androgens may also regulate AJ functionality via their effects on AJ-associated proteins. Work is now in progress to assess whether there are changes in the phosphorylation status of these proteins during AJ assembly. In summary, these results demonstrate that the dynamics of AJs are regulated, at least in part, by N-cadherin, E-cadherin, Src and androgens. [Supported in part by grants from the CONRAD Program (CICCR96-05-A to CYC) and Hong Kong Research Grant Council (HKU7245/00M to WML/CYC)]

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The role of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) in the dynamics of tight (TJ) and anchoring junctions (AJ)

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NOS catalyzes the enzymatic oxidation of L-arginine to nitric oxide (NO), which plays a critical role in a variety of bioregulatory functions including junction assembly. Also iNOS and eNOS are present in the testis and are implicated in the regulation of spermatogenesis. However, their role(s) in junction dynamics in the testis has not been explored. When SC were cultured at 0.5 x 10^6 cells/cm^2 on Matrigel-coated dishes for up to 7-days, there was a transient induction in eNOS expression, but not iNOS, coinciding with the assembly of inter-Sertoli TJ-permeability barrier. When SCs were cultured at 0.5 x 10^6 cells/cm^2 on Matrigel-coated dishes for 5-days to allow the assembly of both TJs and AJs, freshly isolated GCs were then added onto the SC epithelium at a SC:GC ratio of 1:1 to initiate SC-GC AJ assembly; there was an increase in iNOS expression, but not eNOS, coinciding with the assembly of SC-GC AJs. To further explore the involvement of iNOS in SC-GC AJ dynamics, an in vivo model was used in which 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide (DCIC) was used to induce GC depletion from the epithelium. A significant increase in iNOS, but not eNOS,