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expression in the testis was detected. It rose by as much as 50-fold 5¡V24 hours after a single dose of DCIC at 40 mg/kg b.w. by gavage, which is ~7 days before any changes in the testis can be detected by histological analysis. These results illustrate iNOS is a sensitive indicator of AJ integrity. Testosterone/dihydrotestosterone/TGF-β1 were also shown to stimulate SC iNOS and eNOS expression dose-dependently in vitro. In summary: iNOS and eNOS are both involved in the regulation of the AJ and TJ dynamics.

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**Extracellular matrix (ECM) regulates the dynamics of tight junctions (TJs) in the testis possibly via its interactions with cytokines and proteases**

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During spermatogenesis, preleptotene/leptotene spermatocytes must translocate across the blood-testis-barrier (BTB) formed by the inter-Sertoli TJ near the basal laminae. Due to this anatomical close proximity between ECM and the BTB, we sought to investigate whether ECM affects TJ dynamics, and the regulatory pathway. When Sertoli cells (SC) were cultured at $1x10^6$ cells/cm$^2$ on Matrigel-coated bicameral units, inter-Sertoli TJs were assembled as manifested by: (i) a rise in transepithelial electrical resistance (TER) across the SC epithelium, (ii) a transient induction in the expression of occludin, ZO-1, and collagen α3(IV), an ECM protein produced by SC/germ cells (GC), and (iii) an increase in collagen protein production. The presence of an anti-collagen IV antibody could perturb the inter-Sertoli TJ-barrier *in vitro* and its removal allowed the resealing of the perturbed TJ-barrier. Since ECM maintains a pool of cytokines, and TGF-β is known to regulate TJ dynamics and collagen expression in kidney mesangial cells, we next examined if TGF-β1 and TNF-α, both are putative SC and/or GC products, affect TJ functionality via their effects on collagen α3(IV). TNF-α and TGF-β1 were found to perturb the inter-Sertoli TJ assembly *in vitro* dose-dependently. However, TNF-α at 10-100 ng/ml, but not TGF-β1, *stimulated SC collagen a3(IV) and gelatinase B (MMP-9) expression*. Moreover, TNF-α also inhibited the timely induction of occludin during the inter-Sertoli TJ assembly. Taking these results collectively, the TNF-α-induced MMP-9 expression apparently is used to cleave the *existing* collagen network in the ECM, thereby perturbing the TJ-barrier, whereas the TNF-α-induced collagen α3(IV) expression is used to replenish the collagen network in the disrupted TJ-barrier so as to maintain the BTB integrity. In summary: the TNF-α-regulated collagen/MMP-9 synthesis plays a critical role in modulating TJ dynamics.

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**Integrin and vinculin regulate the dynamics of anchoring junctions (AJ) in the rat testis**

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Throughout spermatogenesis, developing germ cells (GC) translocate progressively across the seminiferous epithelium. This event of cell movement includes extensive restructuring of cell junctions and cell-matrix. Since ß1-integrin [a signaling focal adhesion complex (FAC) protein] and vinculin (a structural FAC protein) are found in the ectoplasmic specializations (a modified AJ) in the testis, we sought to investigate whether they are involved in the regulation of AJ dynamics. When GCs were cocultured with Sertoli cells (SCs) in vitro, there was a significant induction of ß1-integrin and vinculin expression coinciding with the assembly of AJs. Furthermore, when viable or non-viable GCs were cocultured with SCs, the expression of ß1-integrin and vinculin were also stimulated. These results thus illustrate that ß1-integrin and vinculin play a crucial role in the assembly of AJs, and GCs were an important regulator of SC ß1-integrin and vinculin. We next investigated the effects of a male contraceptive, 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide (DCIC), on ß1-integrin and vinculin expression when GCs were depleted from the epithelium. When rats were treated with DCIC at 40-mg/b.w., i.p., the expression of ß1-integrin and vinculin in the testis were stimulated by ~2-4-fold within ~1-24-hr, long before GCs were depleted from the epithelium, which was not visable histologically until ~10-days post-treatment. Similar observations were obtained when SC-GC cocultures were treated with DCIC *in vitro*. These results thus indicate that the disruption and the reassembly of GC/SC-AJs can stimulate ß1-integrin and vinculin. These results are also consistent with earlier studies that an activation of integrin signaling pathways can affect junction stability. In summary: (i) both SC/GC express ß1-integrin and vinculin, and (ii) the expression of ß1-integrin and vinculin were induced during AJ assembly and disassembly, suggesting these molecules take part in the regulation of AJ dynamics.

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**Mouse Sperm Possess An Intracellular Calcium Store That Is Established Prior To Epididymal Maturation**

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Subsequent to binding to the zona pellucida mammalian sperm undergo a regulated sequence of events that ultimately lead to acrosomal exocytosis. Like most regulated exocytotic processes, a rise in intracellular calcium is sufficient to trigger exocytosis, although the precise mechanism of how this is achieved is still unclear. We are investigating the role of an acrosomal calcium store during the mobilization of intracellular calcium leading to acrosomal exocytosis. Using thapsigargin, a potent inhibitor of the smooth endoplasmic reticular calcium-ATPase (SERCA), we demonstrate that both uncapacitated sperm isolated from the cauda epididymis and sperm isolated from the caput epididymis possess a mobilizable pool of intracellular calcium that is sufficient to induce acrosomal exocytosis. The absence of extracellular calcium in the culture media did not affect the ability of these sperm to undergo thapsigargin-induced acrosomal exocytosis, suggesting that the mobilization of calcium from an intracellular store(s) – most likely the acrosome – is sufficient to initiate exocytosis. Additionally, treatment of uncapacited sperm from the cauda epididymis with thimerosal, an IP3 receptor agonist, also resulted in acrosomal exocytosis in the presence but not absence of extracellular calcium. Taken together these results imply that sperm possess an acrosomal calcium store that is established prior to maturation and transit through the epididymis. Finally, the sensitivity of uncapacitated sperm to thimerosal suggest that sperm mobilize calcium through an IP3 mediated pathway; that this signal transduction pathway requires the presence of extracellular calcium to induce exocytosis and that this pathway is functional prior to capacitation.

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**Different Domains in Rim1 are Responsible for Binding Rab3a and Stimulating Exocytosis**

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Rim1 was identified in brain by its ability to bind Rab3a-GTP and has been postulated to be a Rab3a effector protein. Like Rabphilin3, it modulates secretion and contains a zinc-finger and two C2 domains. We have investigated the structural basis for the ability of Rim1 to bind Rab3a-GTP and to stimulate exocytosis in chromaffin cells. Using the human growth hormone (GH) co-transfection technique for the study of secretion in transfected cells, both full-length and N-terminal Rim1 were found to enhance secretion 40-50% in both intact and permeabilized cells. GST fusion protein pull-down experiments showed that the abilities of Rim1 to enhance secretion and to bind Rab3a-GTP reside on distinct and relatively small domains that act independently. A ~30-amino acid sequence immediately N-terminal of the zinc-finger constitutes the minimal Rab3a-GTP binding domain. This short sequence is not found in Rabphilin3 and is entirely different from the zinc-finger and flanking regions of Rabphilin3 that bind Rab3a-GTP. The zinc-finger domain in Rim1 is unnecessary for Rab3a-GTP binding, but alone enhances secretion. An analysis of the characteristics of the enhancement of secretion in permeabilized chromaffin cells indicates that N-terminal Rim1 does not alter the sensitivity of secretion to Ca2+ but, instead, increases the rate of ATP-dependent priming of secretion. Addition of recombinant zinc finger domain to permeabilized chromaffin cells also enhances secretion. The results indicated that the Rim zinc finger domain stimulates exocytosis at a late step in the secretory pathway through a mechanism that does not require Rab3a.

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**Phosphatidylinositol biosynthesis in regulated exocytosis**

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Mammalian cells control the release of a variety of biologically active peptides and proteins by regulating their secretion through the exocytic pathway. The cell-free system that enable preservation of secretory mechanisms in semi-intact cells has been developed. Using this approach several important regulatory proteins acting at different stages of regulated exocytosis have been identified and characterized. Two of those proteins required for ATP-depended priming reaction were identified as phosphatidylinositol transfer protein (PITP) and phosphatidylinositol 4-monophosphate 5-kinase (PI(4)P-5 kinase), the key enzymes of phosphatidylinositol phosphorylation pathway. PI(4)P-5 kinase is a member of phosphatidylinositol phosphate kinase family and is responsible for phosphate attachment at the 5' position of the inositol ring to generate 5-phosphoinositides, mainly $PI(4,5)P_2$. Inositol phospholipids per se have been identified as important regulators of diverse spectrum of cellular processes, including receptor signaling, vesicle trafficking, and endocytosis and cytoskeletal rearrangement. However, in most cases the specific roles of these lipids in the control of defined pathways remain to be determined. In this work, the roles of PITP and PI(4)P-5 kinase in regulated exocytosis have been investigated. The cell-free system derived from PC12 cells in conjunction with purified PITP and PI(4)P-5 kinase has been used to study regulated exocytosis. In parallel, the production of different inositol phospholipids in prepared membrane fractions has been determined. The results indicate that the maximum secretion effect was seen only upon addition of both proteins. The parallel analysis of phosphatidylinositol production revealed a slight