nuclear structure, with partial clumping of chromatin as well as convolution of nuclear shape. These distortions of nuclear architecture are indicative of disruption of interactions between the nuclear envelope and interphase chromosomes due to BAF loss. We thus conclude that BAF plays essential roles in both cell cycle progression and nuclear organization.

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Barrier-to-Autointegration Factor (BAF), a Protein Involved in Chromatin Decondensation and Nuclear Assembly, Interacts with 'BAF-Like', a Related Protein with Distinct Biochemical Properties

M. Segura-Totten,[1] K. Lee,[1] A. K. Kowalski,[1] R. Craigie,[2] K. L. Wilson[1] ; [1] Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD, [2] National Institutes of Health, Bethesda, MD

Barrier-to-Autointegration Factor (BAF) is a 10 kD DNA-bridging protein, highly conserved in metazoans. BAF binds directly to 'LEM-domain' nuclear membrane proteins, including LAP2 and emerin. We used site-directed mutagenesis and biochemical analysis to map functionally important residues in human BAF, including those required for direct binding to DNA or emerin. We also tested wildtype BAF and 25 point mutants for their effects on nuclear assembly in *Xenopus* egg extracts, which contain ~12 μM endogenous BAF dimers. Exogenous BAF caused two distinct effects: at low added concentrations, wildtype BAF enhanced chromatin decondensation and nuclear growth; at higher added concentrations, wildtype BAF completely blocked chromatin decondensation and nuclear growth. Mutants fell into four classes, including one that defines a novel functional surface on the BAF dimer. Our results suggest that BAF, unregulated, potently compresses chromatin structure, and that BAF interactions with both DNA and LEM proteins are critical for membrane recruitment and chromatin decondensation during nuclear assembly. Adding a new twist to the BAF story, we recently discovered an open reading frame in mammals that is 54% identical to BAF, which we named BAF-like (BAF-L). Similar to BAF, the BAF-L gene encodes a 10 kDa, 90-residue polypeptide. BAF and BAF-L are most divergent in α-helices 4 and 5, and are most conserved in helix 3, which in BAF mediates dimerization. Although BAF and BAF-L are highly similar in sequence and predicted structure, they have distinct biochemical properties. BAF-L can interact with BAF, itself, and LAP2β, but fails to interact with emerin or DNA. The BAF-L and LAP2 genes are both found only in vertebrates. We propose that BAF-L may specifically regulate BAF, LAP2β, or both, in vertebrate cells.

Sperm and Spermatogenesis (2159-2175)

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The adhering junction dynamics in the testis are regulated by an interplay of ß1-integrin, focal adhesion kinase (FAK) and the focal adhesion complex (FAC)-associated proteins

M. Siu,[1,2] D. Mruk,[1] W. Lee,[2] C. Cheng[1] ; [1] Population Council, New York, NY, [2] University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China

During spermatogenesis, the movement of developing germ cells from the basal to the adluminal compartment of the seminiferous epithelium is associated with extensive restructuring of actin-based cell-cell adherens junctions (AJs) between Sertoli and germ cells, such as ectoplasmic specialization (ES). Yet, the mechanism(s) that regulates this event is largely unknown. During Sertoli-germ cell AJ assembly *in vitro*, a transient induction of β1-integrin, vinculin, p-FAK-Tyr^{397} and PI3-kinase, but not the non-phosphorylated FAK, paxillin and p130Cas, was detected. P-FAK-Tyr^{397} was shown to coimmunoprecipitate with β1-integrin, vinculin and c-Src both *in vitro* and *in vivo*. Furthermore, studies by immunohistochemistry and immunofluorescent microscopy have shown that p-FAK-Tyr^{397} and p-FAK-Tyr^{576} were exclusively localized at the site of apical ES, but not basal ES, in the seminiferous epithelium in a stage-specific manner being highest at stages VI-VIII. Also, p-FAK-Tyr^{397} co-localized with vinculin but not N-cadherin as demonstrated by fluorescent microscopy. When rats were treated with 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide (AF-2364) to perturb the Sertoli-germ cell AJs, an induction of β1-integrin, vinculin, p-FAK-Tyr^{397}, PI3-kinase and p130Cas, but not the non-phosphorylated FAK and paxillin, was also detected in the testis. Such induction of gene expression coincided with the time when spermatids began to deplete from the epithelium, indicating their involvement in AJ disassembly. Thereafter, the levels of vinculin, p-FAK-Tyr^{397}, PI 3-kinase and p130Cas in the testis plunged, coinciding with the time when virtually all spermatids were depleted from the epithelium. Taken collectively, these results suggest a bi-functional role of p-FAK, being involved in the events of Sertoli-germ cell AJ assembly and disassembly. In conclusion, the events of AJ dynamics in the testis are regulated by β1-integrin, activated FAK and the focal adhesion complex-associated proteins.

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The role of BARD1 in spermatogenesis: meiotic repair, homeostasis or quality control

A. Feki,[1] C. Jefford,[1] P. Durand,[2] K. Krause,[1] I. T. Irminger-Finger[1] ; [1] Geriatrics, University of Geneva, Geneva/Chene-Bourg, Switzerland, [2] Hopital Debrousse, INSERM, Lyon, France

BARD1 was discovered as a protein interacting with the RING domain of BRCA1. It is structurally homologous to BRCA1 with which it shares the conserved RING finger and BRCT domains. Co-localization of BARD1 with BRCA1 and other repair proteins indicates a function in DNA repair. As reported for BRCA1, BARD1 expression is maximal in testis and spleen. BRCA1 expression is limited to mitotic spermatogonia and early meiotic prophase spermatocytes, where it co-localizes with other repair proteins to recombination nodules on meiotic chromosomes. However, neither the specific localization nor the function of BARD1 have been elucidated during spermatogenesis. Exogenous expression or stress induced upregulation of BARD1 induces apoptosis in vitro and in vivo. Since apoptosis is an important mechanisms for homeostasis of early germ cells, we hypothesized that BARD1 could function in apoptosis induction in spermatogenesis.We have determined that BARD1 is expressed in mitotic spermatogonia and early meiotic prophase spermatocytes, but unlike BRCA1 it is also expressed in secondary spermatocytes of adult rats. Using antibodies directed against different regions of BARD1, we identified two different isoforms of BARD1 by immunohistochemistry and Western blots analysis. RT-PCR on purified cells from rat testes, led to the identification of a new testis specific BARD1 splice variant that retains apoptotic capacity. Our data suggest that only the full length BARD1 acts in concert with BRCA1 in mitotic spermatogonia and early meiotic prophase spermatocytes, and full length BARD1 and the testis specific splice form are associated with apoptosis in spermatogenesis. Survival of male germ cells is determined by a complex network of signals, including paracrine signals as well as endocrine signals. A model, how BARD1 function and the regulation of its expression can be integrated into these known signaling networks, will be proposed.

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Control of Proliferation and Differentiation in *Marsilea*

V. P. Klink, S. M. Wolniak; Cell Biology & Molecular Genetics, University of Maryland, College Park, MD

Spermiogenesis in the water fern, *Marsilea vestita*, is a rapid process that is activated by placing dry microspores into water. The entire developmental process is regulated at a post-transcriptional level; stored mRNAs in the microspore are translated at specific times and in specific cells for gamete development. Successive division cycles produces spermatogenous cells that continue to divide and adjacent sterile jacket cells that lose the capacity to divide. We asked whether the loss of proliferative capacity in jacket cells was the consequence of an absence of proteins involved in DNA replication. We looked at the control of proliferation by focusing on the conserved MCM proteins (MCM-2, -3 -4, and –5), which function in DNA replication as parts of a heximeric complex. We found that anti-MCM antibodies bind specifically to antigens isolated from gametophytes on immunoblots, and binding in sectioned gametophytes is heavily concentrated in spermatogenous cells and virtually absent from sterile jacket cells. Labeling with each of the anti-MCM antibodies is evident in maturing gametes, in association with the elongated nucleus. We then treated cells with trichostatin A, and 5-aza-cytidine, and found that these transcriptional activators arrest gametophyte development at an early stage for 4-8 h. Thereafter, if mitotic divisions occur, they tend to be anomalously symmetric, and the MCM antigens are uniformly distributed in all cells of the gametophyte. Treatment of gametophytes with dsRNA made from *Mv*-mago, the mago nashi homolog in *M. vestita*, results in anomalously symmetric division planes. Here too, the anti-MCM antibody labeling pattern is uniform in all cells of the gametophyte. These results show a link between the presence of conserved MCM antigens and patterns of proliferation and division in the gametophyte of *M. vestita*. (Supported by NSF grant MCB-0090506 to SMW.)

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Sperm of *Zea mays* Express Diverse mRNAs

M. L. Engel,[1] A. Chaboud,[2] C. Dumas,[2] S. McCormick[3,1] ; [1] University of California, Berkeley, Albany, CA, [2] Laboratory of Plant Reproduction and Development, Ecole Normale Supérieure de Lyon, UMR 5667-CNRS-INRA-ENS-Université Lyon1, Lyon, France, [3] Plant Gene Expression Center USDA/ARS, Albany, CA

Plant fertilization is double - each pollen grain carries 2 sperm cells within a larger vegetative cell; one sperm fuses with the egg to give the zygote and the other with the central cell to give the endosperm. Nothing is known about the molecules that mediate gamete fusion. In order to help develop molecular tools for plant gametes, we optimized fluorescence-activated cell sorting to isolate *Zea mays* sperm cells free of the vegetative cell cytoplasm, and constructed a high quality cDNA library. Sequencing of <500 randomly selected cDNAs from the unamplified library already reveals that sperm have a diverse complement of mRNAs. About 7% of the transcripts encode secreted or plasma membrane-localized proteins and are therefore candidates for surface molecules that might mediate gamete interactions. We reasoned that cDNAs that were most similar to proteins annotated as hypothetical in the *Arabidopsis* genome (i.e. not in existing EST databases) might be sperm-specific. RT-PCR analyses indicated that several such transcripts were present only in the sperm cells and not in the larger vegetative cell of mature pollen, but surprisingly some of these were already present at the unicellular stage of pollen development. We used whole mount *in situ* hybridizations to confirm that one of these transcripts, predicted to encode a plasma membrane-localized protein, is sperm cell-specific in mature pollen. In