Epigenetic regulation of gene transcription via histone modifications

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Our laboratory is interested in post-translational modifications of histone proteins, with studies ranging from identification of novel modifications to functional characterization of these marks. Ultimately, we seek to provide a greater understanding of how histone modifications work together to form a 'histone code'. This code is thought to regulate the recruitment of effector proteins that regulate the diverse functions associated with DNA, including gene transcription and DNA repair. Our recent studies show that RNA polymerase II recruits a variety of chromatin-modifying enzymes that contribute to the disruption, reassembly and maintenance of chromatin structure during the transcription elongation process. One enzyme we have focused on is Set2, which associates with the transcribing polymerase and methylates nucleosomal H3 on lysine 36. H3K36 methylation results in the recruitment of a histone deacetylase complex which functions to prevent inappropriate transcription initiation from occurring within the transcribed regions of genes. I will discuss our recent progress toward understanding how Set2 contributes to the organization and function of chromatin. In addition, I will highlight our progress on a proteomics project that is providing new insights into how "readers" of the histone code bind their cognate modifications using high-density histone peptide arrays.

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Discovery, profiling and identification of polymorphic mRNA target sites in cattle

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MicroRNAs are small 22 nucleotide-long noncoding RNAs capable of controlling gene expression by inhibiting translation. Sanger and high throughput sequencing of microRNA libraries from *Bos taurus* and *Bos indicus* breeds resulted in the identifications of several bovine microRNAs. This strategy combined with comparative sequence analysis identified 129 sequences that corresponded to mature microRNAs (miR). A total of 107 sequences aligned to known human mir, and 100 of these matched expressed miR. The other seven sequences represented novel miR expressed from the complementary strand of previously characterized human mir. The 22 sequences without matches displayed characteristic mir secondary structures when folded in silico, and 10 of these retained sequence conservation with other vertebrate species. Expression analysis based on sequence identity counts revealed that some miR were preferentially expressed in certain tissues, while bta-miR-26a and bta-miR-103 were prevalent in all tissues examined. Comparative analysis between *Bos taurus* and *Bos indicus* microRNA revealed complete conservation in microRNA sequences. However, comparison of potential microRNA binding sites in the 3´untranslated regions o mRNAs from *Bos indicus* breeds revealed polymorphisms that could be involved in deferential mRNA regulation in these breeds. These results support the premise that differences in regulation of gene expression by miR occur primarily at the level of expression, processing and mRNA targeting.

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Complex alterations in structure and function associated with loss of function of specific genes/proteins in the male reproductive system

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Spermatogenesis is a complex process of cellular and subcellular interactions implicating a plethora of proteins carrying out many diverse functions and involving the production and differentiation of sperm that ultimately mature in the epididymis. Over the past 20 years we have investigated the functions of many proteins with specific roles in male reproduction using knockout mouse models. Cathepsin A (PPCA) is a lysosomal carboxypeptidase highly expressed in Sertoli cells, Leydig cells and macrophages in the testis and in specific cell types and regions of the epididymis. In PPCA-deficient mice, both Sertoli and germ cells appear normal, but there are significant decreases in tubular diameters with age. Major abnormalities in the testis involve macrophages which significantly change in number, size and appearance. Epididymal epithelial cells are vastly altered in morphology, coincident with significant reductions in shape and size of tubules and dependent on specific cell type and regions of the epididymis. Electron microscope analyses reveal grossly enlarged lysosomes which at times engorge the entire cell cytoplasm. Despite a 70% reduction in sperm counts and major increases in slow moving and static sperm, PPCA-/- mice are fertile albeit with reduced litter sizes. β-hexosaminidase (Hex) is a lysosomal enzyme formed from α and β subunits comprising the enzymes Hex A (αβ) and Hex B (ββ). Hex is highly expressed in Sertoli cells and in a cell type-region specific manner in the epididymis. Interestingly, mice deficient in the α or β subunit reveal no apparent abnormalities in the testis, but epididymal epithelial cells have grossly abnormal lysosomes, which dependent on the missing subunit show cell type and region specific differences. Nevertheless, despite the major disruption in the epididymis and reduced sperm counts, young mice are still fertile. Hormone sensitive lipase (HSL), functioning in the release of fatty acids, shows high expression levels in spermatids. Germ cells in HSL-/- mice show structural abnormalities including multinucleation and disrupted intercellular bridges. These mice have reduced sperm counts, and those sperm that can move show almost no progressive motility, with males being infertile. Huntingtin interacting protein 1 (HIP1), an endocytic adaptor protein, is expressed in Sertoli cells and elongating spermatids. Seminiferous tubules in HIP1-/- mice show vacuolation, loss of spermatids and reduced mean profile areas. Spermatids and sperm show deformed heads, flagella and acrosomes. Despite decreased sperm counts and many immobile sperm, and reduced litter sizes, mice are fertile. Cystatin 8 (CRES) is a serine protease inhibitor highly expressed in spermatids of the testis and in epithelial cells of the initial segment of the epididymis. Seminiferous tubules in CRES-/- mice show vacuolation and degeneration and disrupted ectoplasmic specializations. Sperm counts are reduced and the mice are subfertile. Thus, absence of specific proteins generally leads to lower sperm counts and varying alterations to sperm motility characteristics which frequently results in subfertility and in rarer cases complete infertility.

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Sperm chromatin integrity and fertility

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Although the standardized World Health Organization semen analysis protocol is a valuable predictor of fertility when the number, motility or morphology of spermatozoa is extremely low, this analysis has often proven to be an unreliable predictor for both fertility and infertility when this is not true. Over the past twenty years, it has become apparent that we need to understand the quality of the nuclear material being delivered by the spermatozoon to the oocyte. A wide range of techniques have been developed that include determination of the rate of sperm decondensation, of extent of breaks, cross-links and integrity of chromatin, its template function and its chromatin structure, as well as assessment of the epigenetic status. We have used several of these tests to determine the effects of age and cancer chemotherapeutic agents (cyclophosphamide, bleomycin, etoposide and cisplatin) on sperm chromatin structure, sperm production and progeny outcome in a rat model. We also have examined the effects of testicular cancer and chemotherapy on sperm chromatin integrity markers in adult men. In rat studies, we found that each of the assays used (acridine orange, TUNEL, chromomycin A3 and monobromine bimane) provided differing, complementary information about chromatin quality. Furthermore, DNA damage induced by chemotherapeutic alkylating agents was germ cell phase specific, with the most extensive effects occurring during histone hyperacetylation and transition protein deposition, a key point of sperm chromatin remodelling. By investigating the proteome of the sperm nucleus, we found significant alterations in sperm basic proteins as well as the selective induction of numerous nuclear matrix proteins involved in cell defense and detoxification. Spermatozoa with extensive nuclear damage fertilized ova effectively. In our clinical studies, we found that sperm DNA damage and abnormal DNA compaction was greater in men with testis cancer than controls prior to chemotherapy and persisted in cancer patients up 24 months post-chemotherapy. In contrast, the level of free thiols and the extent of protamination in sperm from cancer survivors did not differ from controls by 18 months post-treatment. Indeed, although a consensus has yet to be reached with respect to the optimal methods to assess sperm chromatin integrity, there appears to be little doubt that understanding the integrity of sperm chromatin integrity will not only shed light on the fertility potential of a given male but also provide an indication as to whether his progeny will undergo normal development.

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Epigenetic changes during nuclear reprogramming

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Nuclear reprogramming or nuclear remodeling is the process by which a cell of a specific phenotype changes into a different one under the influence of foreign factors. These factors can be defined in the form of small molecules, DNA, RNA, miRNA or proteins. In the context of our presentation we will discuss nuclear reprogramming of a somatic cell into a pluripotent one. The most striking reprogramming events are those described during somatic cell nuclear transfer (SCNT). With the advent of induced pluripotent stem cells (iPSC) a similar 'end product' - i.e. embryonic stem cell-like cells - can be obtained by using only 3 transcription factors. As a consequence, the use of SCNT as a way of reprogramming differentiated cells has been deemed 'obsolete' however recent evidence has surfaced showing incomplete reprogramming in iPSCs, highlighting the need for more research in both areas. iPSC technology and SCNT rely on massive epigenetic changes. We will discuss their differences and similarities and point to aspects that remain to be addressed before both techniques are implemented as a standard tools in biomedicine. High-resolution analysis of overall genome methylation in iPSC and embryonic stem cells will be discussed as well as genetic and epigenetic changes observed in preimplantation embryos produced by SCNT.

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