NRC Research Press

ABSTRACTS

33rd Annual International Asilomar Chromatin and Chromosomes Conference

Characterization of histone variants in bivalve molluscs and their relevance in the development of chromatin-based tests for evaluating ocadaic acid genotoxicity in the marine environment

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Histone variants are used by the cell to build specialized nucleosomes, replacing canonical histones at different stages of the cell cycle and generating functionally specialized chromatin domains. The exchange of canonical histones for certain histone variants can be related with defined cellular responses to diverse toxic components such as okadaic acid, the most predominant diarrhetic shellfish poisoning biotoxin on the European coasts, with aneugenic and clastogenic effects on the hereditary material (most notably DNA breaks and alterations in DNA repair mechanisms). Thus, the study of the structure and dynamics of these specialized histones may represent the basis underlying the development of novel and powerful chromatin-based tests for evaluating okadaic acid genotoxicity in the marine environment. In the present work we have identified for the first time the presence of histone H2A.X and H2A.Z variants, actively expressed (transcribed and translated) in a bivalve mollusc, the mussel *Mytilus galloprovincialis*, a marine organism widely used in biomonitoring programs. Our results support the functional specialization of these proteins, as suggested by the evolutionary conservation of different molecular features, as well as by their ability to confer specialization to nucleosomes. Given the seminal role of these variants in the maintenance of genomic integrity and regulation of gene expression, their preliminary characterization in a mussel species will be of outstanding relevance for the development of molecular assays allowing the early detection of genotoxic stress in the marine environment.

Following the twist of promoter DNA by learning and not by proving

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Protein/DNA conformations can be described by the helical period of the bound DNA. Since we had previously found that one activator (SyCrp1) in the cyanobacterium *Synechocystis* could have different activator/DNA conformers that were important for measuring their binding affinity, we reasoned that the helical periods suggested by the DNA conformers should somehow fit the ternary promoter complex of activator, RNA polymerase, and promoter DNA. To test this idea, the base pairs were counted between the centers of the activator and Pribnow (the RNA polymerase sigma factor binding site at –10) DNA binding sites. Fourier transforms of these counts found 8.500 and 10.625 bp periods and no other period. The period of the SyCrp1/DNA conformer matched that of the promoter in which it was found in all cases. Promoters bound by other transcription factors also had these same periods, demonstrating that these periods are not unique to SyCrp1. These periods would position the 22 studied activator and –10 binding site pairs on the same face of the DNA, an expected requirement for RNAP and its activator to interact while bound to bacterial promoter DNA. Acknowledgments: Supported in part by grants from the NIH [5 SO6 GM048680, and 1 SC1 GM093998 to M. L. S.].

Chromatin maturation dynamics of histone H3 variants in *Ustilago maydis*

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Newly synthesized replication-coupled histone H3.2 and replication-independent histone H3.1 variants are acetylated at lysine 56, and this results in increased hydrophobicity, the basis for separation from matured H3 polypeptides in reversed-phase hplc fractionation. Tracked by tritiated lysine pulse labeling and subsequent chase reveals loss of H3K56ac acetylation with a half-life of 30 min for H3.2 and 45 min for H3.1 variants in wild-type (wt) *Ustilago maydis*. Loss of H3K56ac acetylation is measured by AUT Coomassie and fluorography gel analysis. This analysis also reveals extensive N-terminal H3 acetylation at up to 5 sites (at lysines 9, 14, 18, 23, and 27) prior to nucleosome assembly. N-terminal acetylation loss rates are constant in new and matured H3 variants at ~10% per hour in wild type and transformed strains alike. Knockout of the H3.1 locus slows down growth rate and H3.2 maturation rate both by ~50%. Knockout of the H3.2 locus limits H3 availability more dramatically, slowing growth more than 2-fold and H3K56ac deacetylation 5-fold. In strains with additional H3 supply by (*i*) constitutive H3.2 expression, (*ii*) addition of an extra, highly expressed H3.1 locus, or (*iii*) replacing the H3.1 variant by an H3.2 sequence, H3.2K56ac deacetylation rates are little affected at wt-like growth rates. In contrast, H3.1K56ac deacetylation half-lives increase 2- to 4-fold. In addition, excess H3 protein production leads to a reduction of transient K56 acetylation from near 100% in wt strains to less than 50%. This suggests a limited capacity of K56 HAT, required chaperones and/or histone H4 synthesis that results in chromatin formation without H3K56 acetylation, a recognized requirement for histone tetramer assembly in yeast. Acknowledgments: Supported by the Missouri Life Sciences Research Board, award 13254, to JHW.

Role of human leukocyte antigen G (HLA-G) in breast cancer pathogenesis

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The HLA-G, a non-classical major histocompatibility complex (MHC) antigen, is associated with various pathological conditions including cancer. The immuno-modulating and tolerogenic properties of HLA-G protect cancer cells from natural killer (NK) cells and various other immune responses. HLA-G has 7 isoforms, including 4 membrane-bound and 3 soluble isoforms, however the differential expression and functions of individual isoforms have not been fully elucidated in breast cancer. In this study, we used 4 cell lines, MCF-10A (normal epithelial cells), T47D (estrogen responsive (ER) positive ductal carcinoma cells), MCF-7 (ER positive adenocarcinoma cells), and MDA-MB-231 (triple negative adenocarcinoma cells) to determine the cell type specific expression of various HLA-G isoforms. We observed differential expression patterns of various HLA-G isoforms between ER positive and negative breast cancer cell lines. Unexpectedly, we found the

soluble HLA-G5 isoform, in the nucleus. Further studies will be needed to explain both the presence and role of HLA-G5 in the nucleus. We are also studying the role of interleukin 10 (IL-10) in HLA-G regulation to determine if an increased IL-10 level in breast cancer is directly up-regulating HLA-G. Our results further suggest that HLA-G has novel oncogenic potential. As such, HLA-G may be used to design new therapeutic strategies to counter breast cancer immune escape. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence).

The effect of Nipbl haploinsufficiency on genome-wide cohesin binding and target gene expression

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Cohesin is a multiprotein complex composed of SMC1 (structural maintenance of chromosome 1), SMC3, Rad21, and SA. The cohesin complex is required for sister chromatid cohesion and chromosome segregation during mitosis, but also plays an important role in gene regulation. However our understanding of cohesin's gene regulatory mechanisms and its target genes remains limited. Cornelia de Lange Syndrome (CdLS) is a severe developmental disorder frequently associated with heterozygous loss-of-function *NIPBL* mutations, the cohesin loading factor. The molecular mechanism underlying the disease phenotype remains poorly understood. A mouse model with heterozygous *Nipbl* mutation exhibits many features observed in CdLS patients, including small size, craniofacial anomalies, microbrachycephaly, heart defects, hearing abnormalities, low body fat, and delayed bone maturation. We use mouse embryonic fibroblasts (MEFs) derived from the *Nipbl* mutant to determine the effect of Nipbl haploinsufficiency on cohesin-mediated gene regulation. We found that only a partial reduction of *Nipbl* level results in decrease of cohesin binding at the majority of its binding sites, including both CTCF and non-CTCF sites and repeat regions. Loss of cohesin binding in the mutant or by Nipbl siRNA depletion leads to a decrease in promoter-distal enhancer interactions. Cohesin binding is highly enriched at gene regions, especially at promoters. Reduced cohesin binding genome-wide due to Nipbl haploinsufficiency correlates well with a decrease of gene expression, suggesting that gene activation is the primary cohesin function sensitive to Nipbl reduction. Over 50% of genes affected in the *Nipbl* mutant MEFs have cohesin binding, indicating that these are the direct cohesin target genes. These targets include developmental regulators and genes involved in adipogenesis. Collectively, the results lead us to conclude that cohesin-dependent activation of key developmental regulators is extraordinarily sensitive to *Nipbl* reduction and is likely to play a major role in CdLS pathogenesis.

SWI/SNF chromatin remodeling enzymes in Sox10-mediated transcriptional activation of glial and melanocyte specific genes

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Melanocytes are derived from bipotent neural crest precursors, which can differentiate into either the melanocyte or the glial lineage. However, the epigenetic mechanisms that regulate the direction of differentiation have not been elucidated. Previous results from our lab have established that SWI/SNF chromatin remodeling enzymes interact with and are required by the master regulator of melanocyte differentiation, namely the microphthalmia-associated transcription factor (MITF).The SRY-related HMG box domain containing factor Sox10 is required for glial gene expression and also synergizes with MITF to activate key melanocyte genes. Utilizing an in vitro model of differentiation, we found that SOX10 requires SWI/SNF chromatin remodeling enzymes to activate both melanocyte and glial specific genes. Moreover, the SWI/SNF-dependent synergistic activity of MITF and Sox10 concomitantly prevents Sox10 from activating glial specific gene expression. Hence we hypothesize that MITF and Sox10 synergy preferentially directs melanocyte gene expression by altering the recruitment or activity of SWI/SNF enzymes at lineage specific promoters. Testing this hypothesis involves elucidating the mechanisms regulating MITF and Sox10 interactions with SWI/SNF components and determining the downstream epigenetic modifications mediated by SWI/SNF enzymes in the regulation of lineage specific gene expression. The precursors of melanocytes, namely the melanoblasts, retain a degree of multipotency that is an important characteristic common to neural crest progenitors. Once the lineage regulation mechanism is deciphered, the downstream modulators could potentially be utilized for therapeutic purposes for individuals suffering from pigmentary disorders and neurocristopathies. Acknowledgements: Funding support from NIAMS grant R01AR059379 to Dr. Ivana l de la Serna.

SWI/SNF chromatin remodeling enzymes in transcriptional regulation of pathological cardiac hypertrophy

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Pathological hypertrophy of adult hearts characterized by an increased cell size and reactivation of the fetal gene program, frequently leads to heart failure and sudden death. Previous studies report that the transcriptional regulator, microphthalmia-associated transcription factor (MITF) regulates cardiac hypertrophy in mice. However, the molecular mechanism(s) that determine MITF activity in the hypertrophic response are unknown. The interaction of transcription factors with chromatin remodeling enzymes such as SWI/SNF is critical for the regulation of cardiac development and function. BRG1, a component of the SWI/SNF complex, is reported to be up-regulated in hypertrophic hearts; however a functional link between BRG1 and transcription factors involved in cardiac hypertrophy has not yet been identified. We previously demonstrated that MITF interacts with BRG1 to activate melanocyte-specific genes in melanoma. Our preliminary data indicate that MITF and BRG1 are up-regulated under hypertrophic conditions in in vitro and in vivo models. Furthermore, transcriptional activation of the hypertrophy marker, B-type natriuretic peptide, is abrogated upon knockdown of MITF and BRG1 under hypertrophic conditions in the in vitro model. Thus we hypothesize that MITF and BRG1 co-operate in response to hypertrophic signaling pathway stimulation to activate expression of hypertrophic genes in cardiomyocytes. A detailed understanding of the mechanism(s) by which MITF regulates cardiac hypertrophy could lead to novel therapeutic options. Acknowledgements: Funding support from *NIAMS* grant R01AR059379 to Dr. Ivana L. de la Serna.