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ABSTRACTS / RÉSUMÉS

An in silico approach to analyzing nuclear FRAP protein recovery curves using an extension of the Gillespie algorithm

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Proper recruitment of repair proteins to the foci of DNA double-stranded breaks represent one instance of a fundamental reaction diffusion process in which the geometry and topology within an environment are critically important. Here present a novel computational method to analyze protein diffusion. Our method is based on a previously published extension of the Gillespie algorithm that includes diffusion in 3-D volumes. In particular, our method helps analyze FRAP (fluorescence recovery after photo-bleaching) recovery curves. Simulations are run in idealized nuclei with different geometries (i.e., spheres and cubes). Each nucleus is subdivided into cubic elements (about 64 000 in total) used to model diffusion. The program has 2 adjustable parameters: the initial average concentration of proteins, and the diffusion coefficient. As an illustration, we simulated the bleaching of regions of varying dimensions. Protein molecules of negligible volume were uniformly distributed throughout the simulated nucleus. Proteins had 2 states: fluorescent and bleached. At time 0, only fluorescent molecules were present. At the time of exposure, proteins in the selected region changed states (from fluorescent to bleached) and the recovery of fluorescence owing to diffusion in the bleached region was measured. Our results are in agreement with previous studies and show that varying the initial nuclear protein concentration, the diffusion coefficients, or the geometry of bleached regions changes the FRAP recovery curve. Our study represents an initial step towards better understanding the impact of geometric elements, e.g., chromatin fibers, on proteins as they diffuse into the bleached regions. This research is partially supported by the SCORE National Institutes of Health program grant No. 2S06GM52588-12 and the San Francisco State University Center for Computing in the Life Sciences.

The role of the nuclear cysteine proteinase inhibitor MENT in higher order chromatin condensation and nuclear processes

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A balance between proteolytic activity and protease inhibition is required to maintain the appropriate function of biological systems in which proteases play a role. MENT (myeloid and erythroid nuclear termination protein) is a non-histone heterochromatin-associated protein that is also a functional cysteine protease inhibitor. We have investigated

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the dual functions of this protein, namely chromatin condensation and protease inhibition, to discover whether there is a link between these 2 distinct functions. We found that the interaction between MENT and the papain-like cysteine protease cathepsin V is altered in an environment rich in DNA. We found it accelerated inhibition of cathepsin V up to 60-fold. Importantly, a major part of this effect was found to be mediated through the protease because the interaction between cathepsin V and SCCA-1, a protease inhibitor that is unable to bind DNA, was accelerated 100-fold under the same conditions. Furthermore, cathepsin V was able to bind DNA, and this interaction caused a conformational change in the protease. The presence of nuclear proteases that are capable of interacting with chromatin-associated proteins has broad implications for the regulation and control of chromatin structure.

Roles of the CHD1 chromatin-remodeling factor in *Drosophila* development and chromosome structure

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CHD1 (chromodomain, helicase, DNA binding domain) is a highly conserved chromatin-remodeling factor that is localized to actively transcribed genes. It is unknown whether *Drosophila chd1* is an essential gene, or whether the chromatin-remodeling activity of CHD1 is redundant with that of other SWI2 family members. We have shown through RNA in situ hybridization that *chd1* is expressed ubiquitously in the developing embryo and in the imaginal discs of 3rd instar larvae, suggesting that the CHD1 ATPase may play a broad role in development. To determine whether CHD1 alters the structure of chromosomes in vivo, we used the GAL4 driver system to overexpress CHD1 in the salivary glands of 3rd instar larvae. Overexpression of CHD1 resulted in a range of chromosomal defects, including decondensation of chromatin (chromosome puffing) and overall loss of chromosome structure. To complement our gain of function studies, we generated flies with small deletions in the *chd1* coding sequence by imprecise excision of a P element. The *chd1* mutant flies are viable with wing defects and reduced fertility. The wing phenotypes are similar to phenotypes associated with flies that are mutant for genes in the Delta-Notch pathway, and we observed that our *chd1* mutants genetically enhanced a dominant Delta phenotype. These preliminary studies suggest that CHD1 may decondense chromatin, and may be especially important for the regulation of genes important for fertility and wing development.

Transcriptional regulation of copper homeostasis in *Saccharomyces cerevisiae*

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The *CUP1* gene encodes a copper metallothionein (Cup1p), which functions in maintaining the physiological levels of copper that are essential for activities of numerous proteins. The transcription of *CUP1* is activated rapidly upon exposure of cells to excess copper. Cup1p sequesters excess copper from the cellular environment, thereby minimizing the deleterious effects of the metal ion. However, complete removal of copper by excess metallothionein expression is prevented by rapid shutdown of the gene, the mechanism of which is currently unknown. We are investigating *RUF5*, an antisense RNA generated by the *CUP1* locus, as a candidate RNA involved in downregulation of *CUP1*. Real time RT–PCR assays suggest that *RUF5* transcript levels increase as the *CUP1* is being turned off. This is consistent with the hypothesis that transcription of *RUF5* leads to shut down of *CUP1* through the transcriptional interference mechanism. We obtained further evidence that lower *CUP1* levels correlate with increased *RUF5* levels using mutant strains in which *CUP1* expression is weak and delayed, suggesting a fine-tuning mechanism between *CUP1* and *RUF5* expression to achieve proper levels of Cup1p. We are identifying the key components that are involved in this process, and determining the role of chromatin remodeling–modification in the regulation of *RUF5*.

Role of the histone fold domain in picNuA4-catalyzed acetylation

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Histone acetyltransferases (HATs) are a class of chromatin-remodeling enzymes that catalyze the reversible, post-translational acetylation of histones. Esa1, a member of the MYST family of HATs, is an essential, structurally conserved acetyltransferase found in 2 complexes, NuA4 (nuclesome acetyltranferase of H4) and a smaller trimeric complex, piccolo NuA4. Recent work from our laboratory has shown that Esa1 binds to a core region of H4 (residues 21–52, within the histone fold domain), the loss of which yields a 1000-fold decrease in the rate of H4 acetylation. It is hypothesized that this interaction serves to effectively tether Esa1 to H4 for multiple rounds of histone tail acetylation. Here we characterize the functional properties of the histone fold domain using a chimeric protein consisting of histone H2A (residues 1–14) fused to the globular domain of histone H4 (29–102). We show that the chimeric protein substrate is acetylated with a higher catalytic efficiency than H2A, primarily due to a lower K_m. Additionally, the histone fold domain of H4 leads to a processive-like pattern of acetylation compared with that of authentic H2A. Finally, a substrate competition assay revealed that piccolo NuA4 displays a 5-fold preference for the fusion protein compared with H2A. We conclude that the histone fold domain is uniquely recognized and bound by piccolo NuA4, allowing tail lysine residues to be efficiently acetylated in a processive-like mechanism.

Retinoblastoma binding protein 2 (RBP2) and differentiation

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During differentiation, RBP2 contributes to the role of the pRB tumor suppressor protein as a critical co-ordinator of cell-cycle exit and differentiation; however, how it regulates dependent genes is unknown. RBP2 and 3 homologs in mammals form the JARID1 protein family, and there are single orthologs in fly, worm, and fungi. RBP2 contains several domains, such as JmjC and PHD, which have been recently described in proteins with histone demethylase activity. To gain insight into transcriptional regulation by RBP2, we have identified RBP2 target genes using genome-wide location analysis. Surprisingly, we found that RBP2 target genes frequently encode proteins of mitochondrion and DNA-binding factors. In monocytic cells, many of the RBP2 targets are important in haematopoiesis. Interestingly, in differentiated cells, RBP2 represses genes involved in mitosis, many of which have been shown to be targets of the E2F transcription factor. Genes involved in the regulation of chromatin assembly and disassembly significantly changed in expression in RBP2 depleted cells (both induced and decreased), suggesting that regulation at the level of chromatin is advanced during RBP2-induced differentiation. These results represent an important step toward understanding of how RBP2 regulates gene expression. This work was supported in part by a grant from the American Chemical Society, Illinois Division.

Regulation of DNA damage signaling and repair by Crb2 and histone modifications in fission yeast

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In fission yeast *Schizosaccharomyces pombe*, phosphorylation of histone H2A (γ-H2A) by Rad3 and Tel1 kinases and methylation of histone H4 Lys20 (Me-H4K20) by Set9 histone methyltransferase are both required for rapid large-scale accumulation of the checkpoint mediator Crb2 at DNA double-stranded breaks (DSBs). In a parallel pathway, CDK (cyclin-dependent kinase)-dependent phosphorylation of Crb2 at threonine-T215 also contributes to the recruitment of Crb2 to sites of DSBs by enhancing the interaction between Crb2 and another checkpoint mediator, Cut5. In the absence of Crb2, cells become hypersensitive to IR due to defects in the DNA damage checkpoint and Rqh1-dependent DNA DSB repair. However, this sensitivity can be partially suppressed by eliminating γ-H2A. Eliminating Me-H4K20, on the other hand, cannot suppress IR sensitivity of *crb2*Δ cells. Therefore, γ-H2A appears to have additional roles other than recruitment of Crb2 in response to DSBs. We have found that eliminating a histone chaperon protein complex related to HIRA (Hip1 and Slm9) or eliminating proteins involved in heterochromatin formation (Swi6 and Clr4) can also partially rescue IR sensitivity of *crb2*Δ cells. These suppressions are epistatic to the loss of γ-H2A, suggesting that γ-H2A might influence chromatin remodeling or other histone modifications at DSBs.

Mechanism of chromatin acetylation by a MYST family histone acetyltransferase complex

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The mechanism by which histone acetyltransferases (HATs) modify chromatin is of key interest for understanding the function of these enzymes in DNA damage repair, cell-cycle control, and gene transcription, and for the development of rational therapeutics that target specific HATs. However, many previous studies have used truncated HAT catalytic domains and small peptide substrates, neither of which fully reflects the in vivo environment. Here we determined the mechanism of substrate recognition and catalysis by the yeast HAT complex piccolo NuA4. We find that the histone-fold domain is required for efficient acetylation of histones and nucleosomes, contributing 50 – 10 000-fold to the k_{cat}/K_m. Within histone H4, the site of interaction resides within amino acids 21–52 of the histone-fold domain. Detailed steady-state kinetic analyses revealed a catalytic mechanism involving a ternary complex (i.e., sequential mechanism with direct attack of lysine on the bound acetyl-CoA). This is in direct contrast to the previously proposed report for a ping-pong mechanism, which suggested the obligate formation of an acetyl-cysteine intermediate (Cys304 of Esa1). We find that substitution of the proposed catalytic cysteine (C304A) yields no significant defects in substrate binding or catalysis, suggesting that Cys304 plays no critical role in histone acetylation by Esa1 or piccolo NuA4. This work was performed in collaboration with the laboratories of Song Tan and Jeffrey Hansen.

Histone modifications before and after nucleosome assembly

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Histone post-translational modifications that accompany DNA replication and chromatin assembly were examined in human tissue culture cells. Immunoprecipitations of [^{3}H]thymidine-labeled newly replicated mononucleosomes demonstrated the presence of specific acetylation and methylation patterns of H3 and H4 on new DNA. Microsequencing analysis and chromatin immunoprecipitation further showed that a subset of newly synthesized H3.2/H3.3 is modified by acetylation and methylation at sites that correlate with transcriptional competence. In addition, all nascent H3 variants were deposited in chromatin regions containing K5/K12-acetylated H4. Finally, cytosolic predeposition complexes purified from synchronized cells provided evidence for the preferential acetylation of H3.2 and H3.3 prior to histone deposition. Our experiments provide an in-depth analysis of the histone code associated with chromatin replication and histone deposition in human cells.

Phylogenesis of replication and replacement histone H3

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Nearly 4000 GenBank histone H3 sequences were curated for sequencing errors, reading-frames, and splice sites by comparing all H3 sequences per species and determining consensus loci and protein sequences. Minimal evolution phylogenetic bootstrap analyses (MEGA3) of histone H3 proteins identified duplication events near the root of animal, plant, and fungal phyla. These events could represent distinct outcomes from a single H3 duplication that created the coexistence of 2 functionally distinct H3 variants in all higher eukaryotes. Replication-coupled (RC) H3s form nucleosomes on newly replicated DNA. Replication-independent (RI) H3s cause replacement nucleosomes across DNA sequences to be depleted of nucleosomes by gene transcription. Nonvascular plant H3 proteins clustered with metazoan and fungal species in low-confidence cladograms. The imposition of H3 sequences on tree-of-life phylogenies identified phylum root sequences and excluded recent H3 divergences from minimal evolution analyses. This supports the notion that a single functionally divergent duplication of histone H3 preceded the divergence of animals, plants, and fungi. The absence of distinct RC and RI H3 forms in green algae and ascomycetes suggests that the event may be part of the foundational divergence, possibly as a causative factor, which created the distinct multicellular forms of animals, plants, and fungi. Experimental studies have been started to test this hypothesis by determining H3 multiplicity and RC/RI functionality in the basidiomycete *Ustilago maydis*, in nonvascular plants, in multicellular green algae, and in the unexplored phyla of red and brown algae.

Genetic interaction between fission yeast replication protein Cdc24 and the checkpoint kinase Cds1

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cdc24^{+} is a novel gene in the fission yeast *Schizosaccharomyces pombe*. Cdc24p is suggested to have an important role in DNA replication and is essential for genome stability. Cds1p is a checkpoint protein required for arresting the cell cycle in the presence of DNA replication fork stalling or DNA damage during S phase. Additionally, Cds1p is also responsible for the recovery of S-phase arrested cells. Our preliminary result shows that *cdc24cds1*Δ double mutant exhibits a synthetic growth defect. Specifically, *cdc24* single mutants are viable at 32 °C, but the *cdc24cds1*Δ double mutant cannot form colonies at this semipermissive temperature. This is indicative of a genetic interaction. Published results also show that cdc24 truncation mutants have a novel chromosome breakage phenotype by pulsed-field gel electrophoresis analyses. The truncation mutants with a chromosome breakage phenotype is suggestive of DNA damage; therefore, we hypothesize that Cds1p is activated in the *cdc24* mutant background. Here we expressed Cds1p in wild-type and mutant *cdc24* strains, and we performed transformation and Western blot analyses to test our hypothesis. The detection of the phosphorylated form of Cds1p was observed using a mobility shift assay on Western blot analyses. Our preliminary results show that Cds1p is activated in *cdc24* mutant cells. This suggests that loss of *cdc24* function requires *cds1* function for cells to survive. Funding for this research project was provided by the National Institutes of Health Minority Biomedical Research Support SCORE grant S06 GM 52588 to S.G.P.

Genetic screen to identify novel factors that regulate the dE2F2/RBF repressor in vivo

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E2F and pRB are important regulators of cell proliferation; however, the function of these proteins and their regulation in vivo are not well understood. In *Drosophila*, there are 2 E2F genes, an activator, *de2f1,* and a repressor, *de2f2*. The loss of *de2f1* gives rise to the G_1/S block. The *de2f1* mutant phenotype is rescued by the inactivation of *de2f2*, suggesting that the cell-cycle arrest in *de2f1* mutant cells is due to the repressor activity of dE2F2/RBF. We carried out a screen to identify new regulators of cell proliferation that suppress the *de2f1* mutant phenotype. One isolate was the *B52* gene, encoding a splicing factor SR protein. B52 plays a highly specific role in the regulation of *de2f2* pre-mRNA splicing. In B52-deficient cells, the level of dE2F2 is severely reduced and the dE2F2 specific targets are strongly derepressed. In addition to *B52*, we have isolated mutant alleles of the *Doa* gene, encoding a kinase that has been shown to phosphorylate B52 in vitro. These results uncover a previously unrecognized role of the splicing factors in maintaining the G_1/S block in vivo. Our data demonstrate how the deregulation of splicing machinery observed in some tumor cells may lead to functional inactivation of the pRB pathway.

Evolution and revolutions of nuclear chaperones in chromatin remodeling: the nucleophosmin–nucleoplasmin family

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The proper assembly of basic proteins with nucleic acids is a reaction that must be facilitated to prevent protein aggregation and formation of nonspecific nucleoprotein complexes. The proteins that mediate this orderly protein assembly are generally termed molecular (or nuclear) chaperones. The nucleophosmin–nucleoplasmin (NPM) family of molecular chaperones encompasses members that are ubiquitously expressed in many somatic tissues (NPM1 and NPM3) or specific to oocytes and eggs (NPM2). Although the study of this family of molecular chaperones has experienced a renewed interest in the last few years, information regarding the molecular evolution of these proteins is lacking. The present work shows that this family has been subject to strong purifying selection at the protein level. In contrast to NPM1 and NPM-like proteins in invertebrates, NPM2 and NPM3 have a polyphyletic origin. Furthermore, the presence of selection for high frequencies of acidic residues, as well as the existence of higher levels of codon bias, was detected at the C-terminal ends, which can be ascribed to the critical role played by these residues in constituting the acidic tracts and to the preferred codon usage for phosphorylatable amino acids at these regions.

Estrogen receptor alpha: moving at a Snail's pace

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Estrogen receptor alpha (ERα) plays an important role in breast tumor development and progression. Loss of ERα is correlated with poor prognosis and a higher degree of invasiveness and metastasis. The transcriptional repressor Snail is thought to mediate tumor metastasis by contributing to EMT (epithelial to mesenchymal transitions), which causes an alteration from an organized, epithelial cell structure to a mesenchymal, invasive and migratory phenotype. We previously identified MTA3, a subunit of the Mi-2/NuRD chromatin-remodeling complex, as functioning downstream of ERα to repress Snail. To explore the role of Snail, we used adenovirus overexpression of Snail in the noninvasive MCF-7 breast epithelial cells, which express little to no Snail protein. An important finding was the repression of ERα and the upregulation of TGFβ signaling pathway members by Snail overexpression. While ERα has a stimulatory effect on cell growth and proliferation, TGFβ causes cell-cycle arrest. Here, we show that expression of Snail leads to transcriptional repression of ERα, which appears to be a direct effect of Snail binding at the promoter. Thus, cross-talk among ERα, TGFβ family members, and Snail creates a balance in mammary epithelial cells between a differentiated, epithelial state and EMT. The next step will be to determine the mechanism of repression by Snail.

Critical nonoverlapping functions of WICH and CHRAC complexes in the development of eye and brain in *Xenopus laevis*

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ISWI (imitation switch) is a member of the SWI2/SNF2 superfamily of ATP-dependent chromatin remodelers. Twenty different ISWI complexes have been identified so far in yeast, *Drosophila*, *Xenopus*, and mammals. Three ISWI-containing complexes, WICH, ACF, and CHRAC, have been characterized in *Xenopus*. Loss of ISWI function in *Xenopus* embryos results in severe defects in neural and eye development, including loss of retinal differentiation and the formation of cataracts. We have begun to dissect the contributions of individual ISWI-dependent complexes to development using in situ hybridization and antisense morpholino knockdowns against subunits unique to different ISWI-containing complexes. We show here that inhibition of WSTF (Williams syndrome transcription factor), a component of the WICH complex, results in especially severe defects in eye development and neural defects that may represent a subset of the defects seen when all ISWI complexes are inhibited. In contrast, inhibition of the CHRAC-17 subunit of the CHRAC complex results in loss of facial structures and a different spectrum of eye defects, as well as an overall reduction in body size. We conclude that WICH and CHRAC have critical nonoverlapping functions in the development of the eye and brain in *Xenopus laevis*.

A combinatorial histone tail library to explore the histone code

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A 1-bead, 1-compound combinatorial library based on the N-terminal tail of histone H4 was synthesized in high purity and assayed with an unlabeled antibody directed at phosphoserine 1. The library comprised 800 members and covered all permutations of known histone post-translational modifications within the first 21 amino acids. Modifications include acetylation, methylation (mono-, di-, and tri- for lysine and mono-, di-symmetric, and di-asymmetric for arginine), phosphorylation, and citrullination. A quantum dot detection strategy was developed to identify interacting peptides. Peptides on 90 beads selected from the fluorescence-based screen were sequenced with MALDI–TOF/TOF mass spectroscopy. We found that the antibody bound most tightly to Ser1 phosphorylated peptides with highly positively charged sequences, and bound less tightly to Ser1 phosphorylated with heavily acetylated or citrullinated sequences. None of the nonfluorescent (at 605 nm) beads selected in the screen were found to be phosphorylated. The library should be well suited to the systematic examination of the histone code with proteins that bind chromatin in a modification-dependent manner.

Multiple roles for histone H2A in different DNA damage-response pathways

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Cells are subject to a variety of stressors that result in damage to DNA or other macromolecules. Different classes of DNA damage must be recognized and repaired via specific repair pathways within the context of chromatin. The role of phosphorylation of histone H2A at Ser129 (Ser139 in mammals) in response to double-strand breaks has been studied extensively. We have identified other modifiable residues in both the N- and C-terminal tails of H2A that are important for both the homologous recombination and nonhomologous end-joining pathways of double-strand break repair, as well as for survival of UV irradiation and oxidative damage. We show that the mutation of H2A Ser122 to alanine (S122A) results in a deficient phenotype in each of these assays, while different sets of residues are uniquely required for specific repair pathways. We have identified particular patterns of H2A phosphorylation that occur in response to different types of damage. These data indicate that H2A may be used to present different "damage codes," in which distinct patterns of modifications on both tails of H2A may be used to identify specific types of damage or to promote specific repair pathways.

Characterization of a histone deacetylase in *Tetrahymena thermophila*

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Histone deacetylases (HDACs) have been implicated in several models for the assembly of heterochromatin. To investigate the role of HDACs in heterochromatin formation, we used the biological features of *Tetrahymena* as a model. Vegetative cells possess 2 nuclei: a large somatic, transcriptionally active macronucleus and a smaller germline, transcriptionally silent micronucleus. We have identified a novel putative class II HDAC in *Tetrahymema* (*THD2*) that is alternatively spliced. The transcript for the predominant form of Thd2 (Thd2a) encodes a protein with both an HDAC domain and an inositol polyphosphate kinase domain, which has been implicated in the regulation of ATP-dependent chromatin-remodeling complexes. Our preliminary studies demonstrate that Thd2a is highly enriched in the silent micronucleus during vegetative growth. An expression profile indicated that both forms of *THD2* are not expressed during a brief stage in conjugation in which micronuclei undergo transcription. Together, these results suggest an important role for *THD2* in the maintenance of transcriptionally silent heterochromatin in the micronucleus.

Quantitative analysis of the salt-dependent binding behavior of the H2B tail domain

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The core histone tail domains play an essential role in defining higher order chromatin structures and are the sites of post-translational modifications associated with transcription. However, the structures and interactions of the tails and the effect of post-translational modifications, such as acetylation, on these interactions remains poorly defined. Here we introduce a chemical reactivity based assay to assess the binding state of a core histone tail domain. We found that the rates of reactivity of cysteine residues placed within the H2B tail domain were well described by a pre-equilibrium kinetic model. Thus, we were able to determine conformational equilibrium constants describing the distribution of the tail domain between bound and free states over a wide range of salt conditions. We found that distinct sites within the tail domain exhibited identical salt-dependent behaviors, suggesting a co-operative structure within the bound tail. Analyses of tails containing mutations modeling acetylation indicate that this modification does not cause a global weakening of tail–DNA interactions but rather induces specific and localized structural changes within the tail domain.

The influence of Cenp-A on the structure of the nucleosome and higher order chromatin structure

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The nucleosomes that constitute centromeres in eukaryotes contain a specific histone H3 variant. In humans, the H3 variant Cenp-A specifically locates to active centromeres of human chromosomes and is believed to be a component of the specialized centromeric nucleosomes on which the kinetochores are assembled. We are interested in how Cenp-A influences nucleosome structure and chromatin fiber condensation. To study this we used recombinant histones H2A, H2B, H4, and H3 variant Cenp-A protein complexes to reconstitute nucleosome core particles and biochemically defined nucleosomal arrays. Our in vitro results suggest that Cenp-A containing nucleosomal arrays form higher order chromatin structures under the same conditions as arrays assembled with major type H3. We also monitored nucleosome core particle stability under increasing ionic strength by measuring sedimentation coefficient distributions for Cenp-A containing nucleosomes vs mouse nucleosome core particles using analytical ultracentrifugation. We found that the Cenp-A nucleosome has a salt-dependent stability similar to that of the major type mouse nucleosome. We conclude that the special properties that Cenp-A confers to the centromere are not due to major structural perturbations of either nucleosomes or higher order structure.

SUMO-dependent regulation of thymine DNA glycosylase alters subnuclear localization and CBP/p300 recruitment

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In vertebrates, cytosine methylation at CpG dinucleotides is an important mechanism regulating gene expression and chromatin structure. This epigenetic mechanism also contributes to genomic instability due to spontaneous conversion of methylcytosines to thymines, thereby generating potentially mutagenic guanine–thymine mispairs. Thymine DNA glycosylase (TDG) excises thymine and uracil mispaired with guanine in a CpG context and also functions as a transcriptional cofactor. TDG localizes to euchromatin, and associates with transcription factors, including histone acetyltranferases CBP (CREB-binding protein) and p300. In vitro studies have shown that CBP/p300 and TDG form multi-enzyme complexes that are potentially involved in both DNA repair and transcriptional regulation. Furthermore, acetylation of TDG by CBP/p300 may serve as a regulatory switch between these functions. Interestingly, TDG translocates to discrete nuclear structures, known as promyelocytic leukemia protein oncogenic domains (PODs), which are closely associated with euchromatin. A number of DNA repair and transcription factors, such as CBP, are known to localize to these subnuclear structures. We have shown that the SUMO-1 (small ubiquitin-like modifier-1) protein binding activity of TDG is essential for activation of CBP-dependent transcription and localization to PODs. SUMO-1 binding activity resides in 2 distinct amino- and carboxy-terminal motifs that are negatively regulated by DNA-binding and covalent SUMO-1 conjugation (sumoylation) to Lys341. TDG sumoylation also blocks interaction with CBP, preventing TDG acetylation in vitro. These findings highlight a central role of SUMO-dependent mechanisms in regulating cofactor recruitment and subnuclear localization of TDG.

The histone code of adipogenesis

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Adipogenesis, or the development of adipocytes from undifferentiated precursor cells, is the result of an intertwined network of transcription factors and coregulators with chromatin-modifying activities, which are collectively responsible for the establishment of the gene expression pattern characteristic of the mature adipocyte. We studied the pattern of histone H3 acetylation and methylation throughout adipogenesis using 3T3-L1 cells and found that the promoters of key adipogenic genes display significant levels of dimethylation at the Lys4 of histone H3 in pre-adipocytes, well in advance of the start of transcription of those genes. Moreover, this mark is associated with the recruitment of an as yet inactive RNA polymerase II to the same promoters in pre-adipocytes. The start of transcription, on the other hand, correlates with histone H3 acetylation and trimethylation, as well as recruitment of histone acetylase CBP and the spread of RNA polymerase II to the coding region of the adipogenic genes. Furthermore, we found that the expression levels of several coregulators with histone-modifying activities are regulated throughout adipogenesis. All these data suggest a major role for chromatin and chromatin-modifying enzymes in facilitating the adequate differentiation of adipocytes.

Development of aptamers for recognition imaging

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Atomic force microscopy is a powerful and widely used imaging technique for biological applications, including DNA analysis, chromatin investigation, and molecular crystal and polymer studies. Recognition imaging is a technique developed to give a higher chemical specificity and sensitivity to study molecule interaction. Aptamers as recognition molecules have been proved to show very high specificity and sensitivity in binding interactions. Capillary electrophoresis (CE)-SELEX was used to select DNA aptamers specific for the tails of histone H4 and for acetylated histone H4. A library of ssDNA was incubated with the H4 tail peptide. Sequences bound to H4 tail were isolated using CE, PCR amplified, and purified. They yielded an enriched ssDNA pool suitable for further rounds of selection. Before this study, it was not clear whether smaller and positively charged targets could induce a mobility shift large enough for successful CE-SELEX. H4 tail is a 15-aaa peptide (molecular mass, 1507 g/mol), much smaller than the 84 base ssDNA used in the selection ($\sim$25.5 kDa). Peptide binding aptamers with 1–100 nmol/L dissociation constants were obtained after only 4 rounds of selection. The specificity and affinity of the aptamers were tested using AFM recognition, and included testing the cross-reaction with histones H3, H2A, and H2B.

Analysis of comparative genomic hybridization breast cancer data using algebraic homology groups

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Breast Cancer is one of the most common malignant diseases in American women. During cancer progression, cells accumulate chromosome aberrations. The new technologies of array comparative genomic hybridization (CGH) allow systematic scanning of solid tumors for copy number changes. Array CGH profiles of solid tumors are complex in nature because of the high heterogeneity of chromosome aberrations. This complexity is believed to increase with the stage of the tumor. Here we present a database of array CGH data for breast cancer and we propose new computational methods using techniques from algebraic topology to characterize the data. The recently published dataset we analyzed contained 178 women, with 68 in stage I and 110 in stage II. The database correlates gene density with chromosome idiograms and CGH profiles, creating the opportunity to visually map chromosome aberrations to cytogenetic bands or to gene rich or poor regions and, additionally, to compare CGH profiles among different patients in other cancer stages. Our analysis methods use windowing techniques that associate a "cloud of points" to each CGH profile. Using Plex (A Matlab library for studying simplicial homology), a surface is then interpolated through these points and its topological features are analyzed using homology groups. We propose to use the topological features of this surface to predict stages. For instance, the number of islands (i.e., disconnected pieces) that define the surface (called betti number of order 0) can detect single clone amplifications or deletions. Initial results show our method has a strong potential in staging cancer, as demonstrated by the differences we found in the betti number of order 0 between stage 1 and stage 2 tumor samples. Currently, we are in the process of computing the betti number of order 0 across all chromosomes for every patient. Our results suggest that topological qualities may help us to better predict cancer staging. Stacey Hubbard is the recipient of the Sergio Martens Scholarship. This research receives additional funding from the Center for Computing of Life Science at San Franciso State University and is partially supported by the SCORE National Institutes of Health program grant No. 2S06GM52588–12.

ChromDB: a community chromatin database

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ChromDB is a community database focusing on biological processes among a diverse group of organisms rather than on complete genomes for limited taxa. The database is a repository for homologs of chromatin-associated proteins (CAPs) encoded by diverse genomes (plants, animals, and fungi). CAPs are organized into distinct protein groups that provide a context for comparative analyses across species to convey information to members of the research and teaching communities who are interested in the role played by chromatin proteins in the control of gene expression and genomic organization. The database is funded by the Plant Genome Research Program of the National Science Foundation (DBI-0421679); thus, our first priority is to provide complete sets of plant CAPs. Plant genes are curated individually to ensure the most accurate transcript splice models. Nonplant model organisms are displayed in ChromDB to broaden the relevance and usefulness of the database to the entire chromatin community and to provide a more complete data set for phylogenetic analyses in support of the evolution of the chromatin proteome of important crop species. NCBI RefSeq accessions are used for most of the nonplant sequences and are not subject to the level of curation given to plant sequences.

E2F1: effects of RNAi inhibition

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The dE2F1 gene of *Drosophila* encodes 3 transcripts (PA, PB, and PC), which differ in their 5′ untranslated regions (UTRs). A mutant shows early embryonic lethality of offspring from homozygous mothers. In this mutant the PB transcript is suppressed by the insertion of a transposable element downstream of the 5′-UTR exon controlling the synthesis of PB. Inhibiting the expression of PA and PC has been approached by inserting inverted repeats of the PA- or PC-specific 5′ UTR into the genome. These repeats can be transcribed into an RNA forming a stem loop. They are under the control of an upstream activation sequence that allows tissue-specific expression in combination with different GAL4 strains. The expression of the PC-5′-UTR inverted repeats in early embryos leads to partial male lethality and to mutant phenotypes in the adult, including asymmetrically reduced eye sizes, duplications of an antenna, and wings with defects in their posterior region. Such defects occur in up to 27% of the adults after repeated selection for these phenotypes. The same phenotypes can be obtained by transient transfection with the respective PA-, PB-, or PC-5′-UTR repeats into the embryo, although at lower percentages. We interpreted the defects via quantitative differences in the levels of dE2F1 protein.

When acetylation meets phosphorylation at DNA double-stranded breaks

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Phosphorylation of histone H2AX is the most characterized histone post-translational modification in DNA double-stranded breaks (DSBs). DNA – protein kinase (DNA–PK) is 1 of the 3 phosphatidylinositol-3 kinase-like family of kinase (PIKK) members that is known to phosphorylate histone H2AX during DNA DSB repair. There is a growing body of evidence supporting a role for histone acetylation in DNA DSB repair. It was initially shown that histone acetylation largely enhanced the phosphorylation of H2AX by DNA–PK in vitro, implying that acetylation may precede the phosphorylation of histone H2AX. However, when we repeated the early work, we found that the enhanced histone H2AX phosphorylation by DNA–PK in the presence of acetylated histones is simply the result of differential chromatin solubility under the $MgCl_2$ concentrations used for the phosphorylation reaction. In vitro assembled nucleosomes and HeLa S3 native oligonucleosomes consisting of nonacetylated and acetylated histones are equally phosphorylated by DNA–PK. Using H2AX mutants, we found that DNA–PK can phosphorylate T136 with almost the same efficiency as S139, suggesting a physiological redundancy of these 2 amino acid sites (136TQA138/139SQE141) in the H2AX molecule. Furthermore, the phosphorylation reaction is not inhibited by the presence of H1, which is in itself a substrate of the reaction.

PHD finger proteins as methyl-histone binding modules

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The methylation of Lys4 and Lys36 of histone H3 serve redundant functions in mediating the interaction of the NuA3 histone acetyltransferase complex with chromatin. We have previously identified the PHD finger of the NuA3 subunit, Yng1p (yeast inhibitor of growth 1), as the meH3K4 binding module, but the domain that interacts with meH3K36 was unidentified. Using a combination of genetic and biological techniques, we show that the PHD finger of Nto1p (NuA three orf 1) is required for the interaction of NuA3 with meH3K36. These results identify a novel function for PHD finger proteins in the recognition of methylated H3K36 and shed light on the molecular function of this histone methylation mark. This work was supported in part by grants from the Canadian Institutes of Health Research, the Natural Science and Engineering Research Council, and the Michael Smith Foundation for Health Research.

Sp1 and Sp3: functional roles

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Sp1 and Sp3 are ubiquitously expressed transcription factors in mammalian cells. These transcription factors are involved in regulating the expression of genes involved in a wide range of biological processes, including differentiation, cell-cycle progression, and oncogenesis. Sp3 is structurally similar to Sp1, with similar affinities for Sp binding sites, and both factors are associated with histone deacetylase (HDAC) 1 and phosphorylated HDAC2. However, there are significant differences between these 2 factors. Sp1 typically functions as an activator. In contrast, Sp3 functions as a transcriptional activator or repressor, depending upon promoter context and chromatin structure. We recently demonstrated that Sp1 and Sp3 occupy different subnuclear sites and exhibit different associations with the nuclear matrix. Throughout the mitotic process, Sp1 and Sp3 are displaced from the condensed chromosomes and are dispersed throughout the cell as separate punctate distributions. In late telophase, Sp1 and Sp3 are equally segregated between daughter cells, and their subnuclear organization as distinct foci is restored in a sequential fashion, with Sp3 regrouping into the newly formed nuclei prior to Sp1. Both Sp1 and Sp3 return to the nuclei ahead of RNA polymerase II. Estrogen responsive genes in human breast cancer cells often have an estrogen response element (ERE) positioned next to a Sp1 binding site. In chromatin immunoprecipitation (ChIP) assays, we investigated the binding of estrogen receptor (ER) α , Sp1, and Sp3 to estrogen responsive trefoil factor 1 (TFF1) promoter in MCF-7 breast cancer cells. In the absence of estradiol (E2), Sp1, Sp3, HDAC1, and HDAC2, low levels of acetylated H3 and H4 are associated with the native promoter, with the histones being engaged in dynamic reversible acetylation. Following E2 addition, levels of ER and acetylated H3 and H4 bound to the native promoter increases. There is clearance of Sp1, but not of Sp3, from the promoter, while HDAC1 and HDAC2 remain bound. These data are consistent with a model in which Sp1 or Sp3 aid in recruitment of HDACs and histone acetyltransferases to mediate dynamic acetylation of histones associated with the TFF1 promoter, which is in a state of readiness to respond to events occurring following the addition of estrogen. Cancer cells often exhibit genomic instability. Spectral karyotype analyses of MCF-7 cells revealed that the copies of chromosome 21, which carries the TFF1 gene, ranged from 1 to 5. This observation suggests that cell-to-cell variation in karyotype would complicate ChIP analyses. This work was supported by grants from the National Cancer Institute of Canada with funds from the Canadian Cancer Society, the Canadian Institute of Health Research (CHIR), CancerCare Manitoba Foundation, a Canada Research Chair to J.R.D., a US Army Medical and Material Command Breast Cancer Research Program Foundation Studentship to L.L., and a CIHR Canada Graduate Scholarship to P.S.E.