Abstracts of the 27th Annual West Coast Chromatin and Chromosomes Conference / Résumés de la 27e Conférence sur la Chromatine et les Chromosomes de la Côte Ouest

ABSTRACTS / RÉSUMÉS

Histone H1 function and distribution in chromatin: what does molecular evolution tell us about it?

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The linker histone H1 family shows the greatest number of isoforms among the 5 main histone families (H1, H2A, H2B, H3 and H4). This variation encompasses the somatic, spermatogenesis-specific, oocyte-specific, and replacement subtypes, with each one capable of undergoing a number of post-translational modifications. Since the first fractionation of calf thymus H1 histones almost 40 years ago, the main characteristic defining this family of histones has been the broad range of microheterogeneity presented by its members. Besides establishing a uniform nomenclature for H1, one of the most controversial points arising from this diversity has been the search for the functional meaning of the diversification process. In contrast with the notion that the observed microheterogeneity is the result of genetic drift and that the different H1 subtypes are functionally redundant, it now seems clear that each member of this family has a unique structural and functional identity. Although different reports have already addressed this functional evolution, the evolutionary process involved is still far from being completely understood. Histone H1 genes are often clustered and are supposed to have undergone concerted evolution, a process that would involve homogenization (not diversification) of its family members. After an extensive analysis of the long-term evolutionary pattern of histone H1 in eukaryotes, we did not find any evidence in support of a concerted process acting among members of this histone family. Quite on the contrary, our analysis provides evidence for a mechanism based on the birth-and-death model of evolution under a strong purifying selection. In addition to allowing for a greater degree of H1 functional diversity, this mechanism also explains the origin of the replication-independent H1 variants that occurred before the differentiation of protostomes and deuterostomes.

Histone modifications during H2A/H2B exchange in vivo

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We have investigated histone modifications associated with the deposition and exchange of newly synthesized H2A and H2B in HeLa cells. New histones were radiolabeled with ^{3}H-labeled lysine, and nucleosomes were prepared by micrococcal nuclease digestion and immunoprecipitated using antibodies that recognize acetylated H4 in a site-specific manner. Histones were recovered from immunoprecipitated chromatin and analyzed by polyacrylamide gel electrophoresis and fluorography. It was found that the deposition of nascent core histones, including new H3.3, was targeted to chromatin regions containing H4 acetylated at lysines 5 and 12, in accord with the selective acetylation of new H4 in this pattern. However, a subset of new H2A/H2B was also exchanged into chromatin containing H4 that was acetylated at lysines 8 and 16, independently of new H3/H4 deposition. The assembly of new H2A/H2B into K8/K16-acetylated chromatin continued in the absence of DNA replication, but markedly decreased when Pol II transcription was inhibited. In further experiments, it was found that H2A in the free cellular histone pool can be acetylated at lysine 5. These experiments establish that replication-independent H2A/H2B exchange can be differentiated from replication-coupled nucleosome assembly by the acetylation pattern of H4, and suggest that exchanging H2A can be acetylated at lysine 5. In light of our previous observation that cytosolic H2A/H2B is associated with the nucleosome assembly factor NAP1 (Chang et al. 1997. Biochemistry **36**: 469), our results support a model in which NAP1 facilitates transcription-coupled exchange of H2A/H2B into acetylated chromatin.

 doi:10.1139/O06-901

Characterization of native H2A.Z-containing chromatin structures

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H2A.Z is one of a few histone variants that have garnered considerable research interest in recent years. A heteromorphous variant of canonical H2A forms, some of the interest in H2A.Z may stem from the abundance of seemingly disparate structural and functional roles ascribed to H2A.Z. Earlier structural studies using recombinant forms of H2A.Z yielded contradictory results regarding whether H2A.Z stabilized or destabilized chromatin structures. The objective of the following studies was to resolve this dichotomy by characterizing chromatin structures containing native H2A.Z. Hydroxyapatite dissociation chromatography compared the differential elution of H2A.Z with that of the other major histones. H2A.Z eluted late with H3/H4, indicating a stronger interaction with the tetramer and (or) DNA. H2A.Z elution occurred independently of linker histones and tissue and chromatin type. In mononucleosomes fractionated by sucrose gradients under increasing [NaCl], at 0.9 mol/L NaCl, H2A.Z had a subtle preference for stabilized populations. In pH-dependent gel filtration chromatography of octamers, H2A.Z was present in stable octamers at pH 7.5 and only in destabilized fractions under decreased pH. Induced circular dichroism analysis showed that H2A.Z/H2B folding was highly unstructured compared with H2A/H2B at 100 mmol/L NaCl. While studies of higher chromatin structures suggest a (subtle) stabilizing role for H2A.Z, the unfolded H2A.Z/H2B dimer perpetuates the structural dichotomy of H2A.Z.

Protein scaffold supporting tertiary chromatin structure: implications from biochemical and structural studies of MENT

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During terminal cell differentiation, chromatin is condensed into compact higher-order structures. To assemble such structures, the DNA linkers in zig-zag nucleosome arrays are constrained by architectural chromatin proteins that mediate two discrete activities: longitudinal intrafiber folding (secondary structure) and lateral interfiber bridging (tertiary structure). In myeloid blood cells, chromatin compaction is mediated by chromatin-associated serpins. The first known chromatin architectural serpin MENT contains at least two discrete DNA binding sites. DNase I mapping of MENT location on oligonucleosomes arrays, nucleosome binding experiments, and mutagenesis suggest that this protein interacts with chromatin by simultaneous binding to two DNA linkers at the nucleosome entry/exit site. Upon binding nucleosome arrays, MENT forms oligomers. Biochemical and structural studies of oligomeric MENT reveal loop-sheet linkages that, together with electron microscopy analysis of MENT-condensed chromatin, suggest that a polymeric protein scaffold associated with nucleosome linkers may connect partially intercalated nucleosome arrays to support tertiary chromatin structure.

Changes in H1-chromatin interactions during the differentiation of murine erythroleukemia cells

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In higher eukaryotes, the linker or H1 histones stabilize the folding of chromatin into higher-order structures. Recent studies of the in vivo binding kinetics of H1 using photobleaching recovery techniques demonstrated its ability to exhibit transient interactions with chromatin. We hypothesize that differentiation involves global changes in dynamic H1–chromatin interactions as a means to regulate chromatin structure. To investigate this possibility, we used fluorescence recovery after photobleaching (FRAP) to measure the in vivo kinetics of H1 green fluorescent protein (GFP) variants expressed in differentiating murine erythroleukemia (MEL) cells. Terminally differentiated MEL cells displayed a global increase in residency time of H1 on chromatin, suggesting an overall decrease in chromatin plasticity. Furthermore, this behavior occurred only in cells that were committed to terminal differentiation. Analysis of mutant H1-GFP constructs suggested that dephosphorylation of H1 at concensus cyclin-dependent kinase sites is necessary for differentiation-specific changes in chromatin binding kinetics. Overexpression of a mutant that mimics a hyperphosphorylated form of H1 in MEL cells significantly compromised its ability to commit to differentiation. The results described here suggest that stronger H1–chromatin interactions, caused in part by H1 dephosphorylation, play a crucial role in decreasing chromatin plasticity and the ability of induced MEL cells to become committed to erythroid differentiation. This work was supported by grant No. MCB0235800 from the National Science Foundation (to D.T.B.)

Mapping the interaction surface of the linker histone H1° with the nucleosome of native chromatin in vivo

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The H1 linker histones are major architectural components of metazoan chromatin. H1 binding stabilizes the nucleosome, limits nucleosome mobility, and facilitates the condensation of chromatin. To resolve the structural basis for how H1 mediates these processes, it is essential to determine how H1 interacts with chromatin in vivo and how it is positioned onto the nucleosome. We have developed an approach combining systematic mutagenesis and photobleaching microscopy to measure in vivo binding and structural modeling to determine the binding geometry of the globular domain of the H1° linker histone variant within the nucleosome in unperturbed, native chromatin in living cells. We demonstrate the existence of two distinct DNA-binding sites within the globular domain that are formed by spatial clustering of multiple residues. Molecular modeling of the interaction surface with the nucleosome structure demonstrates that the globular domain is positioned via interaction of one binding site with the major groove near the nucleosome dyad. The second site interacts with linker DNA adjacent to the nucleosome core. The data indicate that multiple residues bind in a cooperative manner to form a highly specific chromatosome structure that provides a mechanism by which individual domains of linker histones interact to facilitate chromatin condensation. This work was supported in part by grant No. MCB0235800 from the National Science Foundation (to D.T.B.)

Multiple roles for histone H2A in different DNA damage response pathways

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There are many types of DNA damage that are repaired by a multiplicity of different repair pathways. All damage and repair occur in the context of chromatin, and histone modifications are clearly involved in many repair processes, though the specific roles of these modifications have not been elucidated in most cases. While many individual histones have been implicated in different repair pathways, most attention has focused on histone H2A (H2AX in mammals), specifically on the role of phosphorylation of the C-terminal tail of H2A in response to double strand breaks. We have extended the analysis of potential modifications of H2A in repair by systematically mutagenizing modifiable residues in both the N-terminal and C-terminal tails of yeast H2A, and testing strains containing these H2A mutations in a number of DNA repair assays. We show that a number of residues in both tails 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 mutation of H2A serine 122 to alanine (S122A) results in a deficient phenotype in each of these assays, and that S122 is phosphorylated in response to double-strand breaks, indicating a role for S122 phosphorylation as a general signal for multiple classes of damage. In addition, we also find that overlapping but nonidentical groups of H2A residues in both tails are involved in different pathways of repair. These data suggest the presence of a set of H2A 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.

Autoregulation of the *CUP1* gene in *Saccharomyces cerevisiae*

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The *CUP1* gene encodes a copper metallothionein (Cup1p), a protein involved in copper homeostasis. The transcription of *CUP1* is activated rapidly upon exposure of cells to copper. Cup1p binds copper ions with a high affinity, and therefore removes excess free copper from the cellular environment, minimizing the deleterious effects of this toxic metal ion. However, if the expression of Cup1p is not turned off rapidly after the excess copper is sequestered, it continues to bind all the copper ions in the cell. Complete removal of copper by the excess metallothionein leads to copper starvation and cessation of growth, since a trace amount of copper is essential for normal functions of numerous proteins. A significant amount of work has been done on mechanisms leading to activation of the *CUP1* gene, and many of the components involved in this process have been identified. However, the mechanism of the shutdown process is still not understood. We are working on elucidating the molecular mechanisms involved in transcriptional shutdown of the *CUP1* gene in Saccharomyces cerevisiae. We are investigating Ruf5, a noncoding antisense RNA generated by the *CUP1* locus, as a candidate RNA involved in down regulation of the *CUP1* gene. Real time RT-PCR assays suggest that Ruf5 transcript levels increase as the *CUP1* gene is being turned off following induction with copper, consistent with the idea that transcription from the *RUF5* gene might lead to shut down of *CUP1* expression through the transcriptional interference mechanism. We are currently trying to identify the key components that are involved in this process, and to determine the role of chromatin remodeling and modification in the regulation of *RUF5*.

Rad54 protein mediates DNA strand exchange within chromatin

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In *Saccharomyces cerevisiae*, the Rad54 protein participates in the recombinational repair of double-strand DNA breaks together with the Rad51, Rad52, Rad55, and Rad57 proteins. Rad54 interacts with the Rad51 nucleoprotein filament and stimulates DNA strand exchange promoted by the Rad51 protein. Rad54 is a SWI2/SNF2-related protein that possesses dsDNA-dependent ATPase activity and catalyzes bidirectional nucleosome redistribution by sliding nucleosomes along DNA. This chromatin remodeling activity is greatly stimulated by interaction with a Rad51 nucleoprotein filament. Here we demonstrate that Rad54 protein efficiently mediates Rad51-promoted DNA strand exchange within mononucleosomes, while displacing histone octamers from the dsDNA. The presence of nucleosome-free dsDNA significantly enhances DNA strand exchange by accepting histone octamers transferred from mononucleosomes during the formation of heteroduplex DNA. The DNA strand exchange promoted by the Rad51 protein in turn stimulates the chromatin remodeling activity of Rad54. These findings indicate that the Rad54 protein facilitates chromatin remodeling at the DNA pairing and DNA strand exchange steps of genetic recombination.

Direct observation of nucleosomes formed on individually trapped dsDNA molecules

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DNA double-strand breaks can lead to genetic instability and malignancy in eukaryotic cells. Such breaks are repaired by homologous recombination with high fidelity. Studies of homologous recombination in vitro are commonly conducted using bare DNA; however, inside a eukaryotic cell, DNA damage recognition and repair occur in the context of DNA packaged as chromatin. We have developed a single-molecule, mini-chromosome system for studying the behavior of DNA repair proteins operating on a chromatin substrate. The system utilizes phage λ DNA modified to contain a tandem repeat of nucleosome positioning sequences. Reconstitution with fluorescently labeled histones and attachment of a polystyrene bead allows capture by a laser trap under buffer flow conditions and simultaneous visualization of single molecules by fluorescence microscopy. In the presence of the DNA stain YOYO-1, both the DNA and chromatin can be observed at separate emission wavelengths. Using this system, rates of DNA translocation can be measured in real time for individual motor proteins on bare DNA and through chromatin. To test the validity of the system, the canonical DNA repair helicase of the *Escherichia coli* RecBCD enzyme was examined. Our results support previous findings that RecBCD enzyme can disrupt nucleosome arrays. The rate of RecBCD translocation through chromatin is greatly reduced (>10-fold) compared with bare DNA, and enzymes often fail to progress through even limited nucleosome arrays.

Temperature sensitivity and thermotolerance in H2A tail mutants

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Our laboratory has previously demonstrated that histone H2A tail modification is required for the induction of stress response pathways due to heavy metal contamination, as well as DNA damage. This project focuses on the highly conserved heat shock proteins (HSPs) and the stress response pathways associated with heat shock. *Saccharomyces cerevisiae* has a normal growth temperature range of 25–30 °C, and growth at elevated temperatures (37–42 °C) is reduced. An increase to 50 °C is fatal for cells unless they have been previously pretreated with a mild heat shock; this acquired viability is termed thermotolerance. We found that H2A tail residues S2, S122, T126, and S129 play a significant role in survival at moderately increased temperatures, while K121 and K127 are important for the acquisition of thermotolerance. A strain bearing a deletion of the entire H2A C-terminus results in severe heat sensitive and thermointolerant phenotypes. Heat shock induced expression of *HSP26*, encoding a small heat shock protein that is rapidly expressed in response to heat shock, and *HSP104*, previously shown to be required for the acquisition of thermotolerance, was analyzed using Northern blot analysis. *HSP26* and *HSP104* mRNA levels are diminished in the C-truncated strain, individual residue mutations are not critical for HSP expression, with the exception of S2, which shows decreased production of *HSP26* mRNA. These results suggest that, while H2A tail residues are crucial for heat stress response and thermotolerance, this is not due to transcriptional defects for the HSP genes tested.

There is more to H2AX phosphorylation than meets the eye

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Although the phosphorylation of histone H2AX, a heteromorphous histone H2A variant, is one of the best-characterized chromatin modification events in DNA double-strand break (DSB) responses, the precise molecular mechanism underlying its role in DSB repair remains to be elucidated. This study was conducted to determine the role of human H2AX in response to DNA DSB damage both structurally and enzymatically. A recombinant human histone H2AX and two of its phosphorylation mimics (E1 and E2, in which relevant serines were replaced by glutamate) were expressed and purified. We show that reconstituted nucleosome core particles consisting of H2AX/E1/E2 and a stoichiometric complement of HeLa S3 histones H2B, H3, and H4 exhibit similar binding affinity to histone H1. DNA dependent protein kinase (DNA-PK), reported to participate in DNA DSB repair, phosphorylates H2AX, as well as E2, within the reconstituted nucleosome core particles. Furthermore, this kinase phosphorylates H2AX in the reconstituted nucleosome core particles even in the presence of H1. Our results suggest the reconstituted nucleosome core particles containing E1 or E2 have the same H1 binding affinity as the nonphosphorylated H2AX nucleosome core particles. Therefore, the phosphorylation of H2AX is not likely to interfere with H1 binding for the accessibility of other DNA repair machineries to chromatin. Preliminary results indicate that, in addition to the conventional phosphorylation at the SQE motif, H2AX is phosphorylated at one or more other positions during DNA DSB repair. Finally, the presence of H1 in nucleosome core particles does not interfere with the phosphorylation of H2AX by DNA-PK.

DNA-mediated Sir3 spreading of silencing

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The current model describing Sir3 silencing mentions its recruitment and repression spreading strictly as a histone tail mediated phenomenon. However, we have recently shown that Sir3 is capable of binding to DNA in a cooperative manner, although with a lower affinity than that observed with chromatin templates. Our initial findings also indicated that Sir3 binding to chromatin templates left the linker DNA in nucleosomal arrays fairly accessible. This would indicate that Sir3 preferentially interacts with DNA that is directly in contact with, or in close proximity to, the core histones. We investigated Sir3 DNA-binding properties in more detail using the well characterized 208–12 DNA fragment and found binding to be cooperative. Several DNA sequences displaying various degrees of inherent bendability were tested by electrophoretic mobility shift assay. Sir3–DNA complexes generated a smear at molar ratios r^{SIR3} (Sir3/208 bp of DNA) between 2 and 5, indicating a serious level of heterogeneity. By monitoring half depletion, we found that our DNA fragment with the lowest curvature was the preferred target for Sir3 deposition. As one would expect internucleosome DNA to adopt a more linear conformation, this result somewhat contradicts our initial assessment of a Sir3 preference for linker DNA. To more precisely test Sir3′s location within a chromatin template, we used a four-way junction to mimic nucleosomal entry–exit DNA. The results indicated that the template is a poor substrate, suggesting that Sir3 recruitment occurs either at the linker or nucleosomal DNA, away from the entry–exit point. Finally, we tested DNA binding as a function of Sir3 molarity at various Sir3/DNA molar ratios. A well defined complex was formed at higher molarity (r^{SIR3}~8). The results indicate that Sir3′s DNA binding affinity is affected by changes in local concentration. We believe that these results, taken together with similar behavior on a 208–7 DNA template, are indicative of a DNA-dependent mechanism for Sir3 spreading and silencing.

Tail-dependent compaction of nucleosomal arrays by MeCP2

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It has been recently demonstrated that MeCP2, the methyl CpG DNA-binding transcriptional repressor binds and tightly compacts in vitro assembled chromatin. To further characterize the nucleosomal determinants for both the binding and compacting activities of MeCP2, we performed an electrophoretic mobility shift assay and electron microscopic imaging on various modified nucleosomal arrays (NAs). Since it has been demonstrated that MeCP2 can form a complex with Sin3 and HDAC1, we first investigated the effect of histone tail acetylation. When complexed with MeCP2, hyperacetylated NAs were unable to achieve the full compaction state observed with underacetylated nucleosomal arrays. Similarly, we demonstrated that MeCP2 binding to tailless NAs was mildly affected, but the ability to form highly compacted complexes was severely diminished. To further characterize the histone tail effect, we reconstituted NAs using various combinations of three full-length overexpressed histones combined with the remaining core tailless histone. The initial results indicate that the strongest component in MeCP2 binding to chromatin is the histone H3 N-terminus. Histones H2A, H2B, and H4 have only a marginal effect on MeCP2 recruitment. The actual MeCP2-mediated compaction effect is still under investigation. In conclusion, it appears that MeCP2 binding and compaction are dependent on the integrity and level of post-translational modification of the core histones.

The evolution of histone H1 antibodies into cancer therapeutics, part IV: vasopermeation enhancement agents as a case study for histone antibody-based therapies

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One of the barriers faced by new therapies targeting solid tumors is the small amount of drug that actually penetrates the cancer. To resolve this problem, we are trying to develop a molecule that causes targeted vascular leakage at the tumor site to improve drug uptake. As part of Peregrine's program to expand the use of antihistone antibodies as drug delivery agents, we have employed our human antihistone H1, known as NHS76, and we have chosen for our first nonradioactive effector molecule a fragment of interleukin-2 (IL-2), which is known to have vascular leakage properties. We have termed these fusion antibody constructs vasopermeation enhancement agents (VEAs). Indeed, any chemotherapeutic or immunotherapeutic drug paired with our VEA compound should, theoretically, have an improved therapeutic index because of the drug's increased uptake at the tumor site. However, an early challenge to the development of these antibodies has been the need for an appropriate screening assay for evaluating potency despite a lack of understanding about the diverse mechanisms responsible for vascular leakage. To surmount this obstacle, we have, in collaboration with Dr. John Lewis at the Scripps Research Institute, begun using the avian embryo chorioallantoic membrane (CAM), a highly vascularized extra-embryonic membrane connected to the embryo through a continuous circulatory system. The CAM system is an in vivo model that combines the versatility of in vitro assays with the higher-order tissue complexity found in vivo. CAMs are easily accessible for experimental manipulation given the external location of the membrane, allowing topical application or intravenous injection of drug candidates and the optical visualization of local responses using fluorescent reagents. Application of VEAs to capillaries in the CAMs has already begun to reveal striking images of vascular leakage. Our next goal is to grow tumors on the surface of these membranes prior to intravenous injection of VEAs. Thus, this system provides for convincing visual data of the VEA fusion antibody's activity, but when combined with localization to a xenografted tumor, it will also demonstrate antibody binding to the accessible regions of chromatin in the tumor core. To provide quantitative measurements of vasoactivity that complement the visual data, we have also developed a method of measuring the extravasation of blue dye or fluorescent reagent into the surrounding tissue upon application of the VEA compound to the CAM. Hence, CAMs provide an attractive alternative to murine models for colocalization studies that evaluate the amount of vasoactivity that can be correlated to VEAs localized on exposed chromatin in the tumor core.

The evolution of histone antibodies into cancer therapeutics, part III: establishing histone antibodies as a carrier platform for therapeutics and diagnostics

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While normally segregated to the nucleus of the cell, histone H1 antigens are exposed to the extracellular environment under a variety of disease conditions. In cancer, these antigens are found in the necrotic lesion present at the core of all solid tumors, exposed by the rupture of dead cells. Thus anti-H1 antibodies target an antigen structure that is universally conserved among solid tumors arising from any tissue, providing a unique opportunity for therapeutic antibody targeting. As a critical cellular component, these antigens are not subject to the usual obstacles of downregulation

or mutation by transformed cells under therapeutic selection pressure. Just as importantly, by localizing to the necrotic core, agents fused with these antibodies have the unique ability to concentrate in the hypoxic zone of the tumor. It is widely accepted that the living tumor cells in this region are particularly resistant to current therapies. Over the last several years these targeting antibodies have evolved into therapeutics to effectively target radiochemical effectors or image enhancement agents to treat or diagnose solid tumors. The first such compound to enter clinical trials, Cotara, a chimeric human/murine antibody carrying ^{131}I, is currently under evaluation for brain tumors. We have further investigated the specificity of a fully human anti-H1 antibody, NHS76. Both antibodies react strongly with the H1 proteins from a diverse range of species, and to a lesser extent, with human core histones. Further epitope mapping revealed that these antibodies recognize the C-terminal tail of H1 proteins, a site providing favorable accessibility for antibody targeting. Indeed, in comparisons with other histone antibodies, we found the chimeric and fully human forms of our antibodies to have greater accessibility to nuclear antigens of neoplastic cells. We have now begun to exploit this characteristic by using these antibodies as the chief delivery platform of effector molecules into the core of a solid tumor.

Analysis of histone modification sites in proteins eluted from fixed and stained high-resolution two-dimensional polyacrylamide gels

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High-resolution two-dimensional polyacrylamide gel electrophoresis in buffers containing acetic acid, urea, and Triton X-100 permits separation of histone subtypes and postsynthetically modified derivatives of these subtypes created by processes such as acetylation and phosphorylation. Recent developments in mass spectrometry permit analysis of the modification sites in the small amounts of protein typically found in individual protein spots in two-dimensional polyacrylamide gels. Efficient methods for protein recovery from fixed and stained polyacrylamide gels are required to combine these powerful technologies so that specific information about the modification sites in the differentially modified histones may be obtained. Here we describe modifications to our two-dimensional polyacrylamide gel system designed to increase resolution and yield of histone subtypes and their modified derivatives. An electroelution system facilitates efficient recovery of individual protein spots from the fixed and stained gels, yielding an acetone precipitate that is suitable for analysis by mass spectrophotometry. Examples and results from these procedures will be presented.

Tissue-specificity of *dE2F1* expression in *Drosophila*

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From the *Drosophila dE2F1* gene, 2 (PB and PC) of 3 transcripts are expressed in a tissue-specific way. Deletion of the transcript dE2F1-PB results in early embryonic lethality due to a maternal effect, which indicates that this gene product is synthesized in the ovary but used in the embryo. Detailed studies of the early embryonic development in the mutant show that it is delayed and that the synchrony of nuclear divisions in the early blastoderm is interrupted. Aneuploid nuclei were found, indicating defects in the segregation of chromosomes during the early cleavages. The transcript dE2F1-PC can be functionally suppressed in by RNAi techniques: GAL4/UAS strains, which express an RNAi specifically directed against the dE2F1-PC transcript, reveal eye and wing defects if the GAL4 expression is in eye or wing disks. Our data reveal different functions associated with the *dE2F1* gene. This relates to the multiple

functions assigned to different *E2F* genes in mammals. The originally single gene appears to have duplicated and diverged to allow different functions to be assigned to separate genes.

The involvement of the high mobility group N1 protein in differentiation, chromatin structure, and SWI/SNF remodeling

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The dynamic modulation of chromatin structure is determined by many factors, including enzymes that modify the core histone proteins, enzymes that remodel the structure of chromatin, and factors that bind to genomic DNA to affect its structure. The nucleosome binding family of high mobility group proteins (HMGN1, formally known as HMG-14) is a chromatin architectural protein that specifically interacts with nucleosomes and has been shown to facilitate the reversal of repressive chromatin structure, thereby making it more conducive for transcription. Expression of key regulatory and tissue-specific proteins necessary for myogenesis and adipogenesis are dependent on functional SWI/SNF enzymes that hydrolyze ATP to remodel chromatin and allow factors access to chromatinized DNA. To determine if HMGN1 functions together with SWI/SNF enzymes in myogenesis or adipogenesis, 2 SWI/SNF-dependent processes, we used RNA interference to create stable cell lines with reduced HMGN1 protein levels and differentiated them along the myogenic and adipogenic pathways. We show that neither differentiation path was affected by reduced HMGN1 protein levels. We further demonstrate that HMGN1 levels naturally decrease as a function of contact-mediated cell cycle arrest, a necessary step to promote differentiation, thereby explaining the lack of requirement for HMGN1 in these cellular differentiation processes. HMGN1 shares several nucleosome structure preserving characteristics with linker histones. We have previously shown that linker histones inhibit SWI/SNF-mediated remodeling on both mononucleosome and nucleosomal arrays. To ascertain how HMGN1 affects SWI/SNF remodeling we reconstituted both nucleosomes and model 208–11 nucleosomal arrays with and without HMGN1and subjected them to SWI/SNF remodeling assays. We demonstrate that HMGN1 does not affect SWI/SNF-dependent chromatin remodeling on either mononucleosomes or nucleosomal arrays, indicating that SWI/SNF functions independently of HMGN1. Structural characterization of the nucleosomal arrays demonstrate that HMGN1 decreases the sedimentation velocity of elongated arrays formed in low ionic strength buffers but has little effect on the structure of more highly folded arrays.