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Chromatin Structure and Dynamics: Meeting Review State-of-the-Art

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A meeting entitled "Chromatin Structure and Dynamics: State-of-the-Art" organized by Jordanka Zlatanova and Sanford Leuba was held at the NIH from May 8–10, 2002. It was a timely meeting and addressed our current understanding of chromatin structure, dynamics, and function.

It is now almost 30 years since chromatin was shown by Hewish and Burgoyne to be a repeating subunit structure (Hewish and Burgoyne, 1973), and their now famous DNA ladder continues to be of major use. The identification of histone complexes by Thomas and Kornberg led Kornberg to propose a chromatin subunit, subsequently called the nucleosome, containing about 200 bp DNA, two H2A/H2B dimers, an $[H3/H4]_2$ tetramer, and one linker histone. Two well-defined subnucleosome particles were described: the chromatosome containing the full complement of histones and 168 bp DNA, and the nucleosome core particle (NCP) containing the histone octamer and 146 bp DNA. Neutron scatter solution studies of the NCP showed that the DNA was coiled around the histone octamer. The crystal structures of the histone octamer (Arents et al., 1991) and the NCP have been determined at increasingly higher resolution (Richmond et al., 1984; Luger et al., 1997; Harp et al., 2000; White et al., 2001). These structures have revealed how the core histone molecules adopt the "histone fold" and interact with each other and with DNA. Unfortunately, electron density was not observed for the histone tails, because they are disordered in the crystal. The lack of knowledge of their physiological binding sites and interactions is a major obstacle to our understanding of histone function, particularly since all reversible post-synthetic modifications of histones that are associated with major chromosome functions (phosphorylation, acetylation, methylation, and ubiquitination) occur in the histone tails.

Electron and atomic force microscopy have revealed the familiar "beads-on-a-string" structure of chromatin at low ionic strength and of linker histone-depleted chromatin. On addition of linker histones and a small increase in ionic strength, chromatin looks like a zig-zag of nucleosomes. At physiological ionic strength, chromatin contracts into the "30 nm fiber," of which we have very limited understanding. The current model is that the hydrated 30 nm fiber is a supercoil of nucleosomes with a diameter of 33 nm, a pitch of 11 nm, and mass per unit length corresponding to 5 to 6 nucleosomes per turn. This supercoil has an 11 nm central hole along its axis, and neutron scattering results indicate that the bulk of the linker histone mass lies within this hole (Graziano et al., 1994).

A major long-standing question concerns the location of the linker histones on the nucleosome. The crystal structure of the globular domain of linker histone by Ramakrishnan's group contains two possible binding sites for DNA that suggest a location of this domain on or close to the pseudodyad axis of the nucleosome (Ramakrishnan et al., 1993). Nevertheless, it seems that all possible sites and some improbable sites have been proposed for the binding of the globular domain of the linker histone to the nucleosome. Even less is known of the physiological binding sites and interactions of the basic flexible N- and C-terminal tails of the linker histones that are subjected to major reversible phosphorylations through G2 to metaphase, at the time of chromosome condensation. Much of the above is discussed at length in van Holde's book on chromatin (van Holde, 1988).

The meeting addressed the continuing themes arising from earlier studies as outlined above and identified emerging themes, particularly: (1) the structures and functions of histone variants; (2) the dynamics of nucleosomes and the intranuclear dynamics of chromatin proteins in vivo; and (3) the development of model systems for higher order chromosome organization and gene regulation. The meeting also revealed the power of physics-based methodologies (such as FRAP; optical tweezers, magnetic tweezers, and other single-molecule techniques; and new imaging techniques, such as AFM and cryo-EM) that allow us to seek answers to new questions.

Nucleosome Core Particle Structure

Considerable progress has been made in the high-resolution structure determination of the NCP. Gerry Bunick (Oak Ridge National Laboratory, USA) described the 2.5 Å resolution structure of a NCP assembled from chicken histone octamer and a nucleosome positioning palindromic human α-satellite DNA sequence constructed in his group (PDB access code 1EQZ) (Harp et al., 2000). This structure differs from previous published models in having stronger experimental support for the atomic positions of the DNA and the placement of 153 more amino acid residues in the histone tails. This structure and the structures by Tim Richmond (Luger et al., 1997) and Karolin Luger (White et al., 2001) provide a wealth of data about the NCPs from yeast and higher eukaryotes. Although most histone tails are largely disordered, some tails are observed to contact adjacent NCPs in the crystal. Whether such contacts have physiological relevance is unclear, especially in view of prior crosslinking results showing that some histone tails (H2A C terminus) bind to different locations on isolated core particles and on particles within the context of chromatin (Usachenko et al., 1994). Two other features

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of the NCP still attract considerable attention: the presence or absence of 2-fold symmetry, and the exact structure of the DNA at the dyad. Andrew Travers (MRC, UK) used hydroxyl radical mapping of sequences around the dyad axis to show different helical periodicities for the proximal sequences and those in the flanking DNA. For some sequences, there was no perfect symmetry at the dyad axis, whereas with Jonathan Widom's (Northwestern University, USA) synthetic sequences (see below), the symmetry was perfect. A lack of symmetry at the dyad axis of native nucleosomes implies an asymmetric binding of the globular domain of the linker histones to the nucleosomes.

Nucleosome Positioning

Nucleosomes are often found positioned upstream of genes, presumably for functional reasons. Much interest, therefore, continues to be generated in identifying DNA sequences and their structural parameters required for nucleosome positioning. Wilma Olson (Rutgers University, USA) summarized recent simulations on the deformability of the DNA double helix as a major factor in its interactions with proteins, and more specifically in the translational and rotational positioning of nucleosomal DNA (Kosikov et al., 2002). She reported that the ability of a sequence to be incorporated into nucleosomes depends on both the intrinsic structure and the deformability of dimer steps, and that charge neutralization on one face of the DNA induces significant bending toward the neutralized face. Widom discussed new DNA sequence rules for high-affinity binding of the histone octamer to DNA and sequence-directed nucleosome positioning (Widom, 2001). Using the SELEX methodology, he identified high-affinity sequences out of a large pool of chemically synthesized random DNA sequences. Of relevance to the function of nucleosomes, these sequences had stronger nucleosome positioning ability than any naturally occurring DNA sequence. An important new finding was the significance of the TA dinucleotide which was periodically spaced at $\sim$10 bp along both the synthetic and genomic DNA positioning sequences. Travers studied the effect of DNA flexibility on the binding affinity of the histone octamer for DNA by band shift competition analysis using DNA fragments with base analog substitutions. An inverse correlation was found between the persistence length of DNA and histone octamer affinity, providing the substitutions did not result in simultaneous changes in intrinsic curvature.

Conformational Dynamics of Nucleosomal Structure

Another outstanding theme was a newly acquired comprehension of the dynamic features of nucleosome conformation. Ariel Prunell (Institut Jacques Monod, France) has developed a system of DNA minicircles ranging from 350 bp to 370 bp in length that incorporate a nucleosome positioning sequence (Sivolob et al., 2000). These minicircles were reconstituted with histone octamers and were further relaxed by topoisomerase I. Three distinct conformational states were identified: a conformation with a negative crossing of the entering and exiting DNAs (reflecting the left-handed coiling of DNA around the histone octamer), an open conformation with no crossing, and a third state showing half-positive crossing. Prunell suggested that fluctuations of individual nucleosomes between these conformations within the fiber explain the so-called linking number paradox and its dependence on histone acetylation. For the tetrasome (the subnucleosomal particle made of DNA wrapped by less than a turn around the H3-H4 tetramer), two conformational states exist in which the DNAs cross either negatively or positively. The two different wrapping polarities of the tetrasome may be explained by an unhinging of the two H3/H4 dimers and a rotation around each other; such a rotation would switch the proteinaceous superhelical ramp around which DNA wraps from a left-handed to a right-handed conformation. Such a tetrasome chiral transition may play a role in nucleosome dynamics during transcription elongation. Widom also discussed the conformational dynamics of nucleosomes in which transient progressive release of DNA from the octamer surface starting from one end of the nucleosomal DNA leads to exposure of internally localized DNA sites.

Jeff Hayes (University of Rochester, USA) reported using engineered H2A to include cysteines as sites for cleavage reagents. The H2A tails make localized contacts with the DNA and probably adopt defined conformations on DNA binding. The interactions of the H2A C-terminal tail, which exits the NCP near the pseudodyad axis, depend on the length of DNA in the particle, in agreement with earlier data from Bradbury's lab (Usachenko et al., 1994). In a separate project, Hayes has observed that human DNA ligase 1 and Fen1 endonuclease can access their target sites within the context of the nucleosome. Ligase activity is not affected by the binding of linker histones but is sensitive to the disposition of the histone octamer tails (Chafin et al., 2000). Another important observation on the structural role of N-tails of histones came from AFM studies. It was shown that the tails of H3 and the linker histone were structurally redundant: chromatin fiber morphology required the linker histone globular domain and either the linker or H3 histone tails (Leuba et al., 1998).

Histone Variants and Postsynthetic Modifications

Much excitement was generated by reports describing the emerging biological importance of replacement histone variants that modulate chromatin structure for specialized functions. Unlike major histones that are synthesized only when DNA replicates, replacement histones are synthesized throughout the cell cycle and in terminally differentiated cells. One replacement histone variant, H2A.X, has a C-terminal extension containing a conserved serine residue. Considerable interest attaches to the findings by William Bonner (NCI, NIH, USA) that the induction of DNA double-strand breaks (DSBs), but not other types of DNA damage, is correlated with an immediate phosphorylation of this serine in a highly amplified response involving about 1000 H2A.X molecules distributed over several Mb of DNA flanking each DSB. Heretofore, the first known cellular response to DNA DSBs was the assembly of repair complexes at damage sites hours later. Thus, the initial cellular response to DNA DSBs is in a chromatin context and involves a region the size of a large chromatin domain. Physiological DSBs (e.g., those that occur during V(D)J recombina-

tion) also induce the phosphorylation of H2A.X. Bonner reported that mice lacking H2A.X are small, are sensitive to ionizing radiation, and have molecular defects in class switch recombination, spermatogenesis, and maintenance of chromosome integrity (Celeste et al., 2002).

Another H2A variant, H2A.Z, plays an essential role during early metazoan development. How H2A.Z modifies chromatin structure to carry out its unique functions is not known. Luger's group reported only minor changes in the crystal structure of a NCP containing H2A.Z, including alterations to the octamer surface (Suto et al., 2000). David Tremethick (John Curtin School of Medical Research, Australia) described how subtle changes to the nucleosome structure could have large effects on higher order chromatin structures. In vitro assembled nucleosome arrays containing H2A.Z showed a significantly altered fiber folding pathway (Fan et al., 2002). Tremethick proposed that H2A.Z may establish a "poised chromatin template" that could be activated or remain repressed, depending on the factors that bind. An important question is whether H2A.Z exists in chromatin in contiguous arrays. Tremethick found that H2A.Z is enriched in specific regions of heterochromatin in early mouse embryo cells but is absent from the inactive X chromosome. Interestingly, macroH2A was not found in regions containing H2A.Z. Mitchell Smith (University of Virginia, USA) reported that H2A.Z in budding yeast plays a positive role in gene expression, acting downstream of the reorganization of the promoter nucleosomes that occurs following induction. This function is partially redundant with that of the Swi/Snf complex. Genetic screens for synthetic lethal mutations revealed interactions with the components of transcriptional elongation machinery, including some subunits of RNA polymerase II.

John Pehrson (University of Pennsylvania, USA) addressed how macroH2A fits into the theme of histone variants adapting nucleosome structure to specialized functions (Changolkar and Pehrson, 2002). MacroH2A is preferentially located in the inactive X chromosome, suggesting a role in transcriptional silencing. Xist RNA plays a critical role in targeting macroH2A to the inactive X chromosome. However, macroH2A functions are not limited to X inactivation, as evidenced by its wide distribution in chromatin of male and female cells and its high evolutionary conservation in nonmammalian vertebrates that do not have X inactivation. Unlike the core histone variants H2A.X, H2A.Z, and CENP-A (an H3 analog), macroH2A is not present in nonvertebrate organisms such as *C. elegans*, *Drosophila*, and yeast. MacroH2A is also distinguished from the other variants by the existence of multiple subtypes and genes that are expressed in cell type specific patterns during development. Steven Henikoff (Fred Hutchinson Cancer Research Center, USA) reported on the functions of the *Drosophila* histone variant H3.3, which differs from the major H3 subtype in only four amino acids (Ahmad and Henikoff, 2002). Whereas the major H3 is assembled into chromatin strictly during DNA replication, the incorporation of H3.3 into chromatin is replication independent. In contrast to the deposition of H3, H3.3 deposition does not require its N-terminal tail. Of considerable interest is the finding that the variant H3.3 is deposited at particular loci, including rDNA arrays. The replication-independent deposition of H3.3 may provide a mechanism for activation of genes, silenced by modifications on the major H3 by simply replacing it. It is also highly significant that H3.3 is the only H3 variant in *S. cerevisiae*.

Juan Ausió (University of Victoria, Canada) also addressed the role of histone variability (variants and modifications) in chromatin folding and dynamics (Ausió et al., 2001). It was reported that histone H2A.Z and histone acetylation have significant destabilizing effects at the level of both the nucleosome and the chromatin fiber. In contrast, histone H2A ubiquitination did not have any major effects, suggesting that this modification has either an informational role (signaling) contributing to the "histone code," or that any structural effects on chromatin folding are exerted synergistically with other histone variations yet to be identified. Concerning current ideas on a histone code, Anthony Annunziato (Boston College, USA) presented interesting findings on the specificity of histone acetyltransferase Hat1, the catalytic subunit of the HAT-B complex thought to acetylate newly synthesized H4 at the conserved K5/K12 sites (Makowski et al., 2001). In agreement with structure-based predictions, it was found that Hat1 cannot acetylate the H4 tail when it is already acetylated on K8 and K16. Interestingly, the different variants of H3 can be differentially modified before or during their assembly onto newly synthesized DNA. Continuing the histone modification theme, Jim Davie (Manitoba Institute of Cell Biology, Canada) reported on the phosphorylation of histone H3 and linker histone $H1^{s}$-3 (formerly H1b) caused by Ras-mitogen activated protein kinase pathways (Davie and Spencer, 2001). They identified MSK1 as the H3 kinase and Cdk2 as the kinase for H1. The persistent activation of the Ras-MAPK pathway in oncogene-transformed cells results in the elevated phosphorylation level of these two histones and, hence, the aberrant gene expression in these cells.

Non-Core Chromatin Proteins

Andrzej Jerzmanowski (Warsaw University, Poland) discussed the advantages of using plant systems to study linker histone contributions to gene regulation. Jerzmanowski's lab developed mutant tobacco lines with reversed proportions of major to minor H1 variants. Interestingly, this reversal affected the phenotype of flower development, specifically the pairing and separation of homologous chromosomes in meiosis (Prymakowska-Bosak et al., 1999).

David Brown (University of Mississippi Medical Center, USA) presented in vivo data on the dynamics of H1 binding to chromatin (Misteli et al., 2000). In collaboration with T. Misteli, Brown has applied FRAP to cells expressing either H1c-GFP or $H1^{\circ}$-GFP fusion proteins. After photobleaching an area in the nucleus, recovery curves (fluorescence intensity versus time) can be interpreted in terms of the dynamics of binding to the underlying nuclear structures. Their significant results show that the entire population of H1 subtypes is bound to chromatin at any one time; despite this, H1 is continuously exchanged among chromatin regions, with residence times between exchange events of several minutes. Histone hyperacetylation induced by TSA treatment of the cells reduced the residency time. In a continuation of this work, the Brown/Misteli team has

constructed a series of H1-GFP mutants with substitutions of individual amino acids suggested to be located in the two DNA binding domains of the globular portion of H1; FRAP with these mutants confirmed the participation of these amino acids in DNA binding.

Two talks addressed the functions of the major nonhistone chromatin proteins, the HMGs. Michael Bustin (NCI, NIH, USA) reported on the role of HMGN (formerly HMG-14/-17) in modulating linker histone binding to chromatin (Bustin, 2001). Footprinting revealed an overlap of HMGN and H1 binding sites. In vitro studies indicated that HMGN could alleviate histone H1-mediated transcriptional repression and chromatin condensation. He has also used FRAP to examine possible interactions between H1 and HMGN in vivo. Apparently, HMGN proteins can compete with H1, on the basis of increased mobility of the bound H1 fraction. Ray Reeves (Washington State University, USA) presented an excellent overview of the world of HMGA (formerly HMGI/Y) (Reeves, 2001). These proteins exhibit an inherent degree of intrinsic flexibility and the ability to undergo complex and extensive patterns of postsynthetic modifications that modulate their biological functions. With functions too numerous to list here, HMGAs are being referred to as "hubs" of nuclear function. HMGAs are among the very few known transcription factors that can bind DNA on the surface of the core particle at specific regions. The binding is mediated through the "AT-hook" and causes moderate remodeling effects. Interestingly, AT-hooks are also found in bona fide chromatin remodeling factors. The HMG-related theme was further elaborated by David Landsman (NCBI, NLM, NIH, USA), who presented a bioinformatics study on the distribution, structure, and expression of HMG pseudogenes in the human genome. He has identified close to 250 insertions in the genome, the majority of which were classified as processed pseudogenes and the rest as potential intronless paralogs. Interestingly, no HMG insertions were found on the Y chromosome, and an abundance was reported on chromosome 15.

Higher Order Structure

The organization of the higher-order packing of the chromatin fiber remains a major problem resistant to present experimental approaches. The abiding interest in chromatin higher order structure, though, was demonstrated in two modeling talks. Joan-Ramon Daban (Universitat Autonoma de Barcelona, Spain) discussed the importance of using local concentrations of DNA in metaphase chromosomes instead of linear DNA packing ratios to validate models for chromosome organization and structure (Daban, 2000). To explain the high DNA concentrations in chromosomes, more compact structures than the "30 nm fiber" must be considered, such as interdigitated solenoid models with stacking of nucleosomes in secondary helices. High concentrations of the chromatin-packing ions, magnesium and calcium, have been recently reported in metaphase chromosomes, further supporting the view of extremely high DNA compaction in these structures. Jörg Langowski (German Cancer Research Center, Germany) presented a new Monte Carlo model for the 30 nm fiber in which the DNA is modeled as a flexible polymer chain (Wedemann and Langowski, 2002). NCPs are represented by oblate ellipsoids whose interaction potential is calibrated with data from nucleosome liquid crystals. For a chain of 100 nucleosomes of a 200 bp repeat length, the simulated fiber diameter, linear mass density, and inclination of the nucleosomal discs to the fiber axis agree well with experimental data. The persistence length of the fiber is 265 nm for uniform twist between adjacent nucleosomes, but decreases for nonuniform twist. Stretching the fiber gives force-extension curves similar to those obtained from single molecule stretching experiments (see Zlatanova and Leuba, below). For stretching forces up to 20 pN, the elastic energy is distributed equally between DNA bending and twisting, while at higher forces the bending contribution dominates.

Single-Molecule Imaging and Manipulation

Single-molecule approaches have emerged over recent years as powerful techniques for digital imaging of individual biological macromolecules (AFM) and their mechanical micromanipulation (AFM, optical and magnetic tweezers) (Leuba and Zlatanova, 2001). They allow observations of individual chromatin fibers and assessment of the variability among individuals in a population. The two organizers presented their joint research. Jordanka Zlatanova (Brooklyn Polytechnic University, USA) discussed the application of AFM imaging to native, linker histone-depleted, and reconstituted chromatin fibers. These results, together with the cryo-EM imaging studies of Chris Woodcock's group, support the straight-linker model for the extended, low ionic strength chromatin fibers. Images of linker histone-depleted chromatin fibers show the "beads-on-a-string" morphology, whereas native fibers are irregularly folded in three dimensions. AFM studies of the effects of DNA methylation on chromatin compaction showed that this compaction requires the presence of linker histones. Of considerable interest is Zlatanova's finding that core histone analogs from *Archaea* can organize DNA templates into fibers with chromatin morphology. Other sophisticated imaging techniques, albeit at a lower spatial resolution, were used successfully by David Bazett-Jones in his studies of PML bodies (see below).

Two presentations concentrated on the use of single-molecule manipulation techniques to assess the forces that govern chromatin and chromosome structure and to reveal structural transitions caused by force application to fibers and chromosomes. Sanford Leuba (NCI, NIH, USA) used optical tweezers to determine the forces required to unravel nucleosomes on a chromatin construct (Bennink et al., 2001). The unraveling forces were in 20 to 40 pN range, comparable to the forces exerted by transcribing polymerases on DNA templates. Magnetic tweezers were used to follow chaperone-mediated chromatin assembly of single chromatin fibers in real time. The rate of assembly was strongly dependent on the exerted force, and assembly was strongly inhibited at forces exceeding 10 pN. During assembly, abrupt increases of fiber length were occasionally observed, giving a clear demonstration of the dynamic equilibrium between assembly and disassembly at the level of a single chromatin fiber.

John Marko (University of Illinois, Chicago, USA) studied the reversible hypercondensation and decondensation of mitotic chromosomes caused by changing ionic

concentrations (Poirier et al., 2002). Chromosomes were extracted from living cells and held between two glass pipettes positioned by computer-controlled micromanipulators; the deflections of one of the pipettes were used to monitor the tension in the suspended chromosome. High salt concentrations led to unfolding, which was reversible upon lowering the salt concentration to physiological levels. Lower NaCl concentrations also caused unfolding, interpreted as inconsistent with the existence of a central proteinaceous chromosome "scaffold." The ion-induced structural transitions indicate a large degree of conformational freedom of the chromosome-contained chromatin fiber.

Transcriptional Activation and Remodeling

Chromatin remodeling has been a major focus of research effort recently. Tom Owen-Hughes (University of Dundee, UK) has used a triplex-forming oligonucleotide displacement assay to study the activity of the chromatin remodeling ISWI factor on a mononucleosomal particle. The results led to the hypothesis that ISWI peels away a bulge of DNA from the surface of the nucleosome; the transit of such a bulge around the octamer surface would result in the alteration of nucleosome positioning by discrete steps of around 40 bp. Such a mechanism of nucleosome repositioning may be shared by both ISWI-driven and thermally induced nucleosome sliding.

Another outstanding issue is the fate of the nucleosome during transcription. Vaughn Jackson (Medical College of Wisconsin, USA) described in vitro transcription experiments through nucleosomes using T7 RNA polymerase at physiological ionic strengths (Peng and Jackson, 2000). Transcription-induced stresses are temporarily maintained even when only three nucleosomes are present on a 6 kb plasmid. Jackson reconstituted his nucleosomal templates on either positively or negatively supercoiled DNA. He showed that H2A/H2B is readily released to a histone chaperone when on positively coiled DNA, even in the absence of transcription. Only when transcription was present, however, would such transfer occur on negatively coiled DNA. During transcription, topologically constrained DNA does not release H3/H4, whereas linear DNA templates do. The presence of transcription-induced stresses may explain the in vivo observations that H2A/H2B dimers tend to release during transcription. Vasily Studitsky (Wayne State University, USA) discussed transcription elongation through NCP by RNA polymerase II in vitro (Kireeva et al., 2002). At physiological and lower ionic strengths, NCPs presented a strong block to transcription elongation that could be relieved by increasing the ionic strength. The passage of Pol II through the NCP resulted in the quantitative loss of one of the two H2A/H2B dimers without changing the position of the NCP. This finding agrees with earlier reports of partial depletion of H2A/H2B dimers from actively transcribed genes (Baer and Rhodes, 1983) and with the model proposed by van Holde a decade ago (van Holde et al., 1992). The mechanisms of transcription through NCPs by Pol II and SP6 RNAP are clearly different, since SP6 polymerase passage is accompanied by histone octamer transfer behind the enzyme.

Several presentations addressed chromatin structure alterations upon gene activation in yeast. David Clark (NIDDK, NIH, USA) studied the induction of the yeast *HIS3* gene in vivo. Using a small episome containing *HIS3*, he compared chromatin structure from induced and uninduced cells. The induced chromatin exhibited a dramatic loss of nucleosomal negative supercoils only after chromatin isolation, and this change required Gcn4p and SWI/SNF. Clark concluded that a chromatin domain containing the entire *HIS3* gene is remodeled to allow transcription. Randall Morse (Wadsworth Center, USA) presented studies on RAP1-dependent opening of the chromatin structure at the promoter of the *HIS4* gene in living budding yeast (Yu et al., 2001). RAP1 binding is needed continuously for GCN4-mediated *HIS4* activation, again indicating a dynamic quality to chromatin structure. In addition, the importance of the H3 amino terminus (now H3.3) in regulation of genes was shown by genome-wide microarray studies.

Uli Laemmli (University of Geneva, Switzerland) presented a fascinating story on the function of chromatin boundaries in budding yeast (Ishii et al., 2002). Laemmli and collaborators constructed a "boundary-trap" strain, with two selectable genes inserted into the silenced mating-type locus *HML*. Synthetic DNA binding sites for proteins flanked one of the inserted genes. The expression status depended on the presence of bound boundary proteins that blocked spreading of heterochromatin through binding to the Nup2p receptor of the nuclear pore complex. Thus, physical tethering of genes to pore complexes can affect transcriptional activity. Vadim Karpov (Engelhardt Institute of Molecular Biology, Russia) has identified a new upstream activating sequence (5P-GGTGGCAAA-3P) in the promoters of 27 out of the 32 genes so far characterized in the *S. cerevisiae* ubiquitin-proteomosomal degradation pathway. This "proteosome-associated control element" (PACE) binds Rpn4p, a protein containing a C_2H_2-type finger motif and two acidic domains (Kapranov et al., 2001). The role of Rpn4p as a transregulator in yeast was supported by its ability to stimulate PACE-driven expression of reporter genes. These findings could indicate a novel regulatory network involved in the control of the ubiquitin-proteosome pathway in yeast. Robert Simpson (Pennsylvania State University, USA) has studied genes that change expression levels according to the yeast mating type by cloning them into circular yeast plasmids, purifying the plasmids with associated proteins from the cells, and further analyzing them by ChIP and EM. The strategy allows the three-dimensional structures of specific gene chromatin with cognate regulatory proteins to be analyzed. Inactive minichromosomes containing the *STE6* gene contained the corepressor Tup1p and had a compact segment that appeared as a hairpin or loop in the region presumed to contain the ten *STE6* nucleosomes. In contrast, the same construct, when active, did not contain Tup1p and appeared as an open circle. Unique chromatin structures were also observed in minichromosomes containing the *MFA1* gene and *HMR* locus.

Chromatin and Nuclear Architecture

Finally, exciting results have been reported on the gross organization of chromatin in the cell nucleus and the participation of nuclear territories in nuclear function and gene expression. David Bazett-Jones (Hospital for

Sick Children, Canada) reported on the composition, structure, and mobility of the so-called PML (*prom*yelocytic *l*eukemia) bodies (Boisvert et al., 2000). PML bodies are subnuclear domains known to be important in cell differentiation, growth, and cancer. These bodies disappear in acute leukemia cells but reappear upon reversal of the disease phenotype. Analytical electron microscopy revealed that the core of a PML body is a protein-only structure surrounded by blocks of chromatin. Nascent RNA transcripts are visualized between the chromatin and the protein core. The bodies are dynamic structures that break down when cells are subjected to stress, such as heat shock, and reappear as the cells recover from the shock. Bazett-Jones hypothesized that the domains where PML bodies are localized are determined by underlying chromatin or nuclear organization.

Mark Groudine (Fred Hutchinson Cancer Research Center, USA) discussed the β-globin gene locus control region (LCR), located upstream of the β-globin gene cluster and known to be essential for transcriptional activation of genes in the cluster (Bulger et al., 2002). The LCR contains multiple binding sites for transactivators, including the erythroid specific factor NF-E2, a heterodimer of p18MafK (the DNA binding partner) and p45 (the transactivator partner). Prior to the induction of differentiation, MafK colocalizes with the β-globin locus in heterochromatin. In contrast, p45 does not colocalize with MafK until the induction of terminal differentiation. FISH analysis showed that the differentiation-linked transcriptional activation of the locus is accompanied by relocation of the locus from heterochromatin to euchromatin and relocation of NF-E2 away from heterochromatin, suggesting that these processes are linked.

The tight focus of the meeting, the extensive questions, and the extended coffee breaks among the posters led many participants to suggest having the meeting again, and in particular, having Zlatanova and Leuba reprise their role as the organizers. Preliminary plans are that it would be in three years' time.

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