MEETING REVIEW

Exploring the transcription–chromatin interface

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Para lograr lo posible conviene a veces apuntar a lo imposible.

(To achieve the possible, it is sometimes necessary to aim for the impossible.)

Charlas de Café

The control of eukaryotic transcription requires the sequential interaction and precise coordination of a variety of large enzymatic complexes that are recruited by sequence-specific promoter/enhancer-binding proteins (Fig. 1). Regulation is exerted at all levels of the process, which include chromatin recognition and reconfiguration, covalent modification of histones, recruitment of coactivators and basal transcription components, and the assembly of an elongation-competent transcription complex that is capable of moving efficiently through nucleosomal arrays.

Recent years have seen an explosion of new findings concerning how transcriptional regulation is exerted through complex regulatory elements in the context of a chromatin template. The Workshop on “Integration of Transcriptional Regulation and Chromatin Structure,” hosted by the Fundación Juan March in Madrid, Spain, was organized by Juan Ausió, Enrique Palacián, and Jim Kadonaga to address some of the newest emerging themes in chromatin and transcription. Specific sessions focused on the functions of specialized core promoter structures, promoter/enhancer-binding factors and associated coregulators, chromatin remodeling and modification complexes, as well as boundary elements and locus control regions.

ATP-dependent chromatin remodeling complexes

One of the earliest steps of gene activation involves the mobilization of energy-dependent chromatin remodeling complexes. A variety of different complexes capable of assembling and disrupting nucleosome arrays have been identified from yeast, *Drosophila*, and mammalian cells. In general, these complexes are classified according to the identity of the catalytic ATPase and other shared subunits. For example, the *Drosophila* ACF, NURF, and CHRAC complexes use ATPase subunits in the ISWI

family, as do two mammalian remodeling complexes, RSF and hACF/WCRF (Danny Reinberg, UMDNJ). In contrast, SWI/SNF remodeling complexes contain ATPase subunits in the SWI2/SNF2 family. Although the SWI/SNF and ISWI remodeling factors display similar catalytic activities in ATP-dependent nucleosome spacing and mobilization assays in vitro, genetic studies have revealed distinct roles for these complexes in the regulation of specific subsets of genes as well as in other processes such as DNA replication and repair.

At the Workshop, Carl Wu (NIH) described the cloning of the largest subunit of the *Drosophila* NURF complex. This novel protein contains several motifs found in other chromatin remodeling proteins. Analysis of larvae with mutations in this subunit revealed defects in gene expression, consistent with the biochemical role of NURF in transcription on chromatin. The recombinant, four-subunit NURF complex fully reconstitutes nucleosome remodeling functions in vitro. The Wu laboratory has also identified a new yeast remodeling complex, termed the INO80 complex. This multiprotein complex contains the INO80 ATPase, a member of the SNF2/SWI2 superfamily. The INO80 complex appears to be required for transcription of a subset of genes as well as for DNA recombination or repair.

New findings presented by Pierre Chambon (IGBMC, Strasbourg, France) revealed that ligand-dependent activation of the RAR α /RXR α heterodimer in vitro requires the action of both ISWI and SWI/SNF chromatin remodeling complexes as well as histone acetyltransferase complexes (p300 and TIF2). The ISWI remodeling complexes appeared to function before the acetyltransferases and act in part to facilitate tight binding of the nuclear receptor heterodimer to chromatin. Studies with the MMTV promoter demonstrated that chromatin remodeling can also contribute to the synergistic effect of multiple promoter/enhancer factors, as remodeling complexes recruited by hormone receptors allowed subsequent binding of NF1, which in turn stabilizes an open nucleosome complex and facilitates the binding of addi-

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Figure 1. Regulation of transcription by RNA polymerase II. In this model, the initial step in transcription activation involves the recognition of the promoter/enhancer region by sequence-specific DNA-binding factors. These factors recruit ATP-using chromatin remodeling factors to the template. In some cases, sequence-specific DNA-binding factors and remodeling factors may bind to chromatin in a concerted fashion. Chromatin-remodeling factors catalyze the mobilization of nucleosomes, as is needed for the binding of additional transcription factors and coregulators to the DNA template. In addition, the promoter/enhancer-binding factors mediate, directly or indirectly, the association of acetyltransferases (such as CBP/p300, PCAF/Gcn5, SRC/p160) that modify core histones and other proteins essential for transcription initiation. Protein acetylation probably acts through multiple mechanisms to promote unfolding of the chromatin, to modulate the affinity of DNA-binding factors, and to regulate activities of transcription factors and cofactors. Promoter/enhancer-binding factors also interact with a large multisubunit complex, which has related forms known as TRAP, ARC, DRIP, SMCC, NAT, SRB, and Mediator. In turn, this complex interacts with RNA polymerase II to facilitate communication with the basal transcriptional machinery. There are also direct interactions between the TAF subunits (TBP-associated factors) of the TFIID complex and promoter/enhancer-binding factors. Many, and possibly all, of these interactions are required to achieve productive transcription initiation. Transcriptional elongation through chromatin requires the action of the P-TEFb (CycT-Cdk9) and FACT (Spt16-SSRP1) complexes, and is marked by the processive phosphorylation of the carboxy-terminal domain (CTD) of RNA polymerase II. Core promoter elements, such as the TATA box and DPE, can be important for enhancer-to-core promoter communication. Boundary/insulator elements demarcate domains of gene activity. Many of these activation steps can be blocked by specific repressors to prevent inappropriate gene expression or dampen the response to inducers (not shown). The fortuitous placement of heterochromatin near a euchromatic gene can have a repressive effect on transcription (position-effect variegation).

tional hormone receptors (Miguel Beato, Instituto für Molekularbiologie und Tumorforschung, Marburg). Chromatin remodeling at the yeast *PHO5* and *PHO8* promoters was found to require both the activation and DNA-binding domains of Pho4 (Wolfram Hörz, University of Munich, Germany). *PHO8*, in contrast to *PHO5*, requires, in addition, chromatin remodeling and acetyltransferase complexes acting at a step subsequent to binding of factors to the UAS/enhancer. Although activation domains can recruit remodeling complexes, it is the zinc finger domain of the erythroid-specific enhancer factor EKLF that interacts with SWI/SNF (BRG1) complexes to regulate β -globin transcription in vitro (Beverly Emerson, Salk Institute, California). Interestingly, the Emerson laboratory also found that factor-specific tran-

scription activation and remodeling can be achieved in vitro with only two recombinant SWI/SNF subunits.

Chromatin modification complexes and other coregulators

Juan Aurió (University of Victoria, British Columbia, Canada) examined the effects of histone acetylation on chromatin structure. Although hypo- and hyperacetylated nucleosomes contact DNA similarly, the acetylated particle is more asymmetric and the histone tails adopt a higher degree of α -helical structure. In the absence of histone H1 the structural changes result in a less densely packed fiber. Histones H3 and H2B in core particles can also be modified through transglutamina-

tion (Luis Franco, University of Valencia, Spain), which provides a sensitive probe for nucleosome structure and possibly an additional regulatory modification *in vivo*. This decrease in chromatin compaction with histone acetylation is likely to be relevant to the effects of acetyltransferases on transcriptional activity.

The TRAP complex was identified biochemically as a coactivator of thyroid hormone receptor (TR), and has since been found to be required for many other promoter/enhancer-binding factors (Bob Roeder, Rockefeller University, New York). This multisubunit complex appears to be identical to the SRB and MED-containing SMCC complex, and is distantly related to the yeast Mediator complex. Biochemical studies with different transcriptional activators have led to the identification of other coactivators (e.g., DRIP/ARC/NAT) that are closely related to the TRAP complex. These complexes bind with high affinity to several different types of transcription activation domains (Roeder, Robert Tjian, Berkeley, California) and may be a direct conduit between promoter/enhancer-binding factors and RNA polymerase II. For example, TR interacts directly with the TRAP220 subunit of the TRAP complex, and the Roeder laboratory found that fibroblasts from TRAP220 knockout mice, which are subject to severe developmental defects and embryonic lethality, are selectively compromised in their ability to support activation by TR *in vivo*.

Coactivators may also act in an architectural manner to promote assembly of enhancer complexes or to regulate binding of specific promoter/enhancer factors to chromatin. For example, the nonsequence-specific HMG1 protein stimulates the DNA-binding activity of steroid hormone receptors and creates local distortions in the template through its ability to bind to the minor groove and to bend DNA (Marco Bianchi, Milan, Italy). Deletion of the HMG1 gene in mice leads to postnatal hypoglycemia and death, and although fibroblasts from these mice displayed no gross chromatin abnormalities, the Bianchi group found evidence of impaired glucocorticoid receptor activation and increased resistance to dexamethasone-induced apoptosis. Different isoforms of the sequence-specific HMG protein LEF-1 show striking differences in affinity for chromatin that are not seen on DNA, and LEF-1 binding to chromatin is enhanced when it is associated with a non-DNA-binding coactivator (Katherine Jones, Salk Institute, California). Furthermore, promoter/enhancer-binding factors may associate with distinct cofactor complexes in different contexts to switch transcriptional function, as shown by the example of the *Drosophila* GAGA activator, which might also contribute to transcriptional repression upon binding to the SAP18 subunit of the Sin3–HDAC corepressor complex (Fernando Azorín, Institut de Biologia Molecular de Barcelona, Spain).

Gene-specific repression, like activation, can be achieved through a variety of different mechanisms. Epigenetic silencing as a result of DNA methylation involves at least three different methyl-CpG binding proteins that function to target histone deacetylase (HDAC)

complexes for repression (Adrian Bird, Edinburgh, Scotland). HDAC-containing corepressor complexes show sharp differences in their ability to function over short or long distances (Mike Levine, Berkeley, California). In studies of nuclear receptor repression of AP1 and NF- κ B activity, Keith Yamamoto (UCSF, California) demonstrated that whereas repression by TR requires histone deacetylation and the NCoR/SMRT corepressors, glucocorticoid receptor (GR)-mediated repression does not involve histone deacetylation. Instead, GR interaction with the RelA dimerization domain represses transcription at a much later step, subsequent to binding of NF- κ B and recruitment of RNA polymerase II. Interestingly, this repression is accompanied by a selective decrease in RNA polymerase II CTD phosphorylation at the Ser2 position in the heptapeptide repeats (Yamamoto). Thus, repressive complexes can act at many different stages in the transcription process, from the earliest steps of chromatin mobilization to the postinitiation steps that control promoter clearance and elongation.

What's at the core?

Many transcription units contain one of two distinct core promoter structures that comprise a centrally located initiator (Inr) acting in concert with either an upstream TATA box or a downstream promoter element (DPE) to mediate basal transcription (Jim Kadonaga, UC San Diego, California). Both the DPE and the TATA box are binding sites for TFIID, and the DPE appears to be as common as the TATA box in *Drosophila*. Notable differences are observed in the ability of specific core promoters to respond to different enhancers (Kadonaga, Levine). Although most class II promoters respond to TFIID complexes containing TBP, one exception is a core promoter in the *Drosophila* *tudor* gene that is regulated by TRF1 (a tissue-specific TBP-related factor) instead of TBP (Robert Tjian). Moreover, although mammalian TBP is required for transcription by RNA polymerases I, II, and III, it is TRF1 rather than TBP that mediates RNA polymerase III genes in *Drosophila*. Thus, *Drosophila* TRF1 forms a complex with the RNA polymerase III factor BRF to activate tRNA, 5S and U6 RNA genes, and the majority of the TRF and BRF proteins colocalize at polytene chromosome sites containing RNA polymerase III genes (Tjian). Therefore, core promoter components can vary considerably among different tissues and in different species, and the nature of the core promoter (e.g., containing a TATA box or DPE) will determine the responsiveness to different promoters/enhancers.

It is interesting to consider that components of the TFIID complex may also regulate transcription through modification of chromatin or other proteins. Thus, P. Anthony Weil (Vanderbilt University School of Medicine, Nashville, TN) demonstrated that distinct regions of the yeast TAF25p protein function in both the TFIID and SAGA (Spt5/Ada/Gcn5/acetyltransferase) complexes. Moreover, Frank Sauer (ZMBH, Heidelberg, Germany) reported that the TAFII-250 subunit of TFIID is not only both an acetyltransferase and a protein kinase,

but can also catalyze the ATP-dependent ubiquitination of histone H1 in vitro. Hence, TAFII-250 appears to contain both ubiquitin-activating and ubiquitin-conjugating activities, and a point mutation in *Drosophila* TAFII-250 that abolishes its ubiquitination activity in vitro was found to reduce expression of several genes in vivo. These studies suggest a possible role for TAFII-250 and TFIID in the regulation of gene expression by protein ubiquitination, and underscore the fact that regulatory complexes may contain multiple enzymatic activities.

Signals transmitted from the enhancer to the core promoter also affect the transition from initiation to elongation, and enhancer/promoter-specific factors may stimulate either or both of these processes. For example, the HIV-1 Tat protein associates with P-TEFb-CDK9 and strongly enhances CTD phosphorylation at the Ser-5 position (Jones). High-affinity binding of the Tat-P-TEFb complex to nascent TAR RNA was found to require P-TEFb autophosphorylation, and thus to ensure that Tat binds TAR only when complexed with activated P-TEFb and can modulate kinase specificity toward the CTD. Nucleosomal structures present a barrier to elongation through genes, and Danny Reinberg presented an analysis of the factors required for elongation in chromatin. The chromatin-specific transcription elongation factor FACT contains two subunits, Spt16 and SSRP1. The Spt16 subunit interacts directly with the NuA3 HAT complex, whereas SSRP1 binds to the SWI/SNF-related protein CHD1. Thus, FACT may recruit both chromatin-modifying and remodeling activities to the elongating complex. An important function of FACT is its ability to facilitate removal of H2A-H2B from chromatin, and FACT is not able to enhance transcription elongation from chromatin when H2A-H2B dissociation is prevented by cross-linking of the histones. Consistent with these data on FACT from Reinberg, Enrique Palacián (Centro de Biología Molecular "Severo Ochoa," Madrid, Spain) observed that repressive effects of nucleosomes on transcription initiation and elongation were alleviated to a greater extent by replacing histone octamers with H3-H4 tetramers than by the removal of histone tails. In fact, RNA elongation on an H3-H4 tetramers array took place as easily as on free DNA.

The gatekeepers

Within eukaryotic chromosomes, accessible and active genes are interspersed with regions of more densely compacted chromatin, and Joan-Ramon Daban (Barcelona, Spain) discussed different models for these structures and the 30- to 40-nm fiber. Within large regions of the genome, boundary/insulator elements define and separate independent domains of genetic activity. Mike Levine discussed a novel promoter targeting sequence (PTS) element that acts in a dominant fashion to allow an enhancer to overcome the ability of an insulator to block enhancer-to-promoter communication. An intriguing model for long-range enhancer-promoter interactions involving chromatin condensation mediated by a novel class of *Drosophila* proteins that also facilitates

sister chromatid cohesion was proposed by Dale Dorsett (Memorial Sloan-Kettering, New York).

Studies of the β -globin gene locus revealed a dynamic role for the locus control region (LCR) in its association with and dissociation from different genes depending on the LCR-to-promoter distance as well as the presence of key regulatory factors such as EKLF (Frank Grosfeld, Rotterdam, The Netherlands). In addition, removal of one of the hypersensitive domains of the LCR was found to render the locus sensitive to position-effect variegation as well as to cell-cycle timing position effects that arise from nuclear relocalization of the transgenic locus when it is integrated in a (peri)centromeric region of the host genome.

Ulrich Laemmli (Geneva, Switzerland) described an innovative approach to identify important *cis*-acting elements within satellite heterochromatin that control position-effect variegation in *Drosophila*. Minor groove-binding polyamides with different sequence specificities were targeted with high affinity to extended AT-rich tracts and GAGAA repeats present in two different satellite DNAs. Remarkably, the stable and specific association of these compounds, fed to developing flies, resulted in specific gain-of-function and homeotic transformations that could be attributed to the localized disruption of chromatin within each satellite DNA segment. Thus, this new approach provides a powerful complement to genetics and biochemistry to address complex elements that regulate large regions of chromosomes. Studies presented by Susan Gasser (ISREC, Lausanne, Switzerland) included the transcription factor-induced remodeling of nuclear morphology that accompanies zygote formation in budding yeast. The mating pheromone response that activates a MAP kinase cascade and induces transcription factor Ste12p results in a major reorganization of nucleolar and cytoplasmic domains, and induces nuclei to adopt a unique dumbbell shape. Taken together, these presentations highlighted the variety of chromatin remodeling and restructuring activities that affect all levels of chromatin organization, from individual genes and loci to much larger chromosomal domains and nuclear structures.

Summary and perspectives

The many fascinating talks and discussions at the Workshop have brought forth many current concepts. First, it is evident that all of the factors that participate in the transcription process play an active role in the regulation of gene expression. Indeed, even the basal transcription factors and the chromatin template participate in gene-selective transcription, and complexes that were initially identified by their function in general nucleosome assembly have been found to be recruited for gene-specific chromatin remodeling. Second, the mechanisms by which transcription is regulated are of immense complexity. Factors can alternatively act as activators or as repressors, depending on their context or interacting partners, and regulatory complexes of apparently distinct function in transcription may be linked through sharing

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common subunits. Moreover, reversible chemical modifications of chromatin such as methylation (of DNA or histones), acetylation, or ubiquitination can variably affect gene expression. It seems likely that these different covalent modifications of proteins will act in a combinatorial fashion to provide multiple dimensions of transcriptional control. Third, there is the question of how many more regulatory factors remain to be discovered? Have we found most of the relevant factors, or are there many others yet to be identified? Although this remains an open question, there appears to be a pleasing convergence at the moment as newly identified regulatory factors and genes are increasingly found to correlate with previously identified coactivators or corepressors. Finally, we can see the emergence of new approaches and tools for the analysis of chromatin structure and transcriptional regulation. These novel approaches and technologies will help to address the problem of how information is transmitted from nucleosomal arrays and higher order chromatin structures to the core promoter, and will play an important role in future advances and revolutions in our understanding of gene expression.

Acknowledgments

The Fundación Juan March provided the ideal setting for these talks and discussions. We would like to thank Andrés González and Lucia Franco for their superb organization and management, as well as all of our colleagues in Madrid for their participation in this meeting.

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Genes Dev. 2000, **14**:

Access the most recent version at doi:[10.1101/gad.14.16.1992](https://doi.org/10.1101/gad.14.16.1992)

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