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meeting report

Old dogs and new tricks

Meeting on mechanisms of eukaryotic transcription

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The meeting was held at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, August 29–September 2, 2001. It was organized by Nouria Hernandez, Robert Kingston and Richard Treisman. (Photo courtesy of Margot Bennett, 1995.)

Introduction

The recent Cold Spring Harbor Laboratory meeting 'Mechanisms of Eukaryotic Transcription' was a potent reminder of the astounding depth and complexity of the biological world. Despite years of intensive study and the extraordinary advances that have been made, transcriptional regulation remains a vibrant area of research that retains its ability to inspire and to surprise. Indeed, for the 470 participants who crammed into the Laboratory's Grace auditorium, this meeting served up a unique assortment of talks that not only answered many long-standing questions in the transcription field, but also broke important new ground. In this report, we present just a small sample of the many exciting and provocative stories that were described at this meeting. These stories reveal that new discoveries are still being made in transcription and that some of our oldest and dearest friends still have a few surprises in store.

Tails of RNA polymerase II

RNA polymerase II (pol II) was understandably the focus of much attention at this meeting. Weighing in at well over 500 000 kDa, this highly conserved machine not only transcribes all protein-encoding genes in eukaryotes, but also plays a major role in coordinating transcription with downstream events such as message capping, splicing and polyadenylation.

The first talk of the meeting, given by P. Cramer (Munich, Germany), reported on an extraordinary *tour de force* of X-ray crystallography, carried out in the laboratory of R. Kornberg (Stanford, CA). Cramer presented two X-ray crystal structures of *Saccharomyces cerevisiae* pol II, a refined structure of the free enzyme at 2.8 Å resolution (Cramer *et al.*, 2001), and a structure of a pol II elongation complex at 3.3 Å resolution (Gnatt *et al.*, 2001). Comparison of these structures allowed Cramer to paint a vivid picture of the likely mechanics of RNA synthesis, from the moment duplex DNA enters the polymerase to the point at which the growing RNA strand emerges from the enzyme in the probable vicinity of the pol II C-terminal domain (CTD). Because the CTD is the major site of interaction of pol II with capping and splicing enzymes, this unique topography provides a straight-forward way for the machinery of RNA synthesis to collaborate with the machinery of RNA processing.

The CTD itself continues to be a major focus of research interest, particularly its regulation by phosphorylation. It has been known for many years that phosphorylation of the CTD changes upon transcriptional activation and that these changes play a role in orchestrating interactions of the CTD with RNA processing enzymes. A talk by S. Buratowski (Boston, MA) took our understanding of the dynamics of this process one step further. Using antibodies specific for either phosphorylated serine 2 (S2) or phosphorylated serine 5 (S5) in the CTD repeat, combined with chromatin immunoprecipitation analysis, Buratowski and colleagues elegantly revealed how CTD phosphorylation changes during the transcription process (Komarnitsky *et al.*, 2000). Close to promoters, it is predominantly S5 that is

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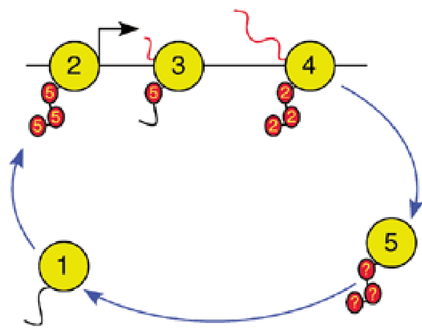

Fig. 1. The yeast transcription cycle. (1) RNA pol II (yellow circles) with an unphosphorylated CTD binds the promoter. (2) TFIIH phosphorylates the CTD repeats at serine 5 (red circles) at the promoter. RNA capping enzymes bind at this stage, and possibly other processing factors (not shown). (3) Serine 5 is rapidly de-phosphorylated after initiation. (4) Serine 2 phosphorylation is regulated during elongation by Ctk1 kinase and Fcp1 phosphatase. (5) The phosphorylation status of the CTD after termination is unknown, but dephosphorylation occurs prior to polymerase recycling. Reproduced with permission from Steve Buratowski.

phosphorylated. As the polymerase elongates away from promoters, however, there is a dramatic shift in the phosphorylation status, and S2 becomes the predominant phosphorylated residue. Buratowski drew an interesting analogy between these changes in CTD phosphorylation and the eukaryotic cell cycle, stating that, as in the cell cycle, specific points in the transcription process are marked by specific phosphorylation events (Figure 1). Although the significance of these phosphorylation changes are not understood, the ability of the CTD to function as an indicator of RNA polymerase status provides a direct way for the cell to appropriately coordinate transcription with multiple RNA processing events.

Just as CTD phosphorylation continues to be a hot topic in the transcription field, so too do the kinases that phosphorylate this domain, particularly pTEF-b, which phosphorylates S2 of the CTD. Although pTEF-b has been studied extensively *in vitro*, comparatively little is known about how pTEF-b regulates pol II *in vivo*. K. Blackwell (Boston, MA) presented a unique genetic analysis of pTEF-b function, using RNA interference (RNAi) to knock out pTEF-b activity in *Caenorhabditis elegans* embryos. Blackwell showed that S2 phosphorylation is eliminated by ablation of pTEF-b and that this correlates with global transcriptional defects within the embryo. Hence, it appears that pTEF-b activity is broadly required for transcription *in vivo*. However, this general requirement does not imply that pTEF-b has the same role at all promoters. Blackwell went on to describe how RNAi-mediated co-inhibition of Spt4 and Spt5, two proteins that functionally interact with pTEF-b, results in promoter-specific effects, suppressing the transcriptional defects of the pTEF-b knockout at two heat-shock promoters, but not at other promoters examined. Thus, although the requirement for pTEF-b activity is general, specific promoters use pTEF-b differently to regulate transcription.

Perhaps the most surprising aspect of the pTEF-b discussion came from two consecutive talks by O. Bensaude (Paris, France) and Q. Zhou (Berkeley, CA), who reported that pTEF-b activity is regulated by its interactions with RNA. Both Bensaude and Zhou isolated the 7SK small nuclear RNA (snRNA) as a pTEF-b-interacting molecule (Nguyen *et al.*, 2001; Yang *et al.*, 2001). The 7SK RNA is an abundant snRNA, but until now its function had remained elusive. Bensaude and Zhou showed that the association of pTEF-b with 7SK RNA antagonizes the ability of pTEF-b to stimulate transcriptional elongation and that this interaction is disrupted by treatment of cells with stress-inducing agents such as UV light and actinomycin D. Thus, these two presentations not only revealed a fascinating mechanism for the rapid induction of gene expression in response to stress, but they also raised the intriguing issue of why an RNA pol III-transcribed snRNA should regulate the elongation activity of RNA pol II.

Relatively speaking...

Establishing complex patterns of gene expression is one of the most daunting challenges facing any multicellular eukaryote. In the past, we have imagined that these patterns are established largely by sequence-specific transcriptional regulators that act in combination to regulate the activity of a conserved, and stoic, basal transcriptional machinery. Several talks at this meeting, however, reinforced the importance of another way in which regulatory complexity is achieved: through the existence of close relatives of the basal machinery that perform specialized functions in cell-type- or promoter-specific gene activation.

M. Fuller (Stanford, CA) shed light on the existence and function of a collection of testis-specific relatives of the TBP-associated factors (TAFs) in *Drosophila*. Fuller presented an elegant analysis of the *Drosophila cannonball* gene, a spermatocyte-specific homolog of the broadly expressed $dTAF_{II}80$ (Hiller *et al.*, 2001). Fuller and co-workers showed that *cannonball* is expressed only in primary *Drosophila* spermatocytes, where it controls transcription of a number of spermatid-differentiation genes; when *cannonball* is knocked out, spermatid differentiation is blocked and *Drosophila* males are sterile. Importantly, there are at least two other testis-specific TAF homologs in *Drosophila* that, all together, play a crucial role in regulating the spermatocyte transcription program. It thus appears that *cannonball* and its colleagues are the tip of the iceberg, and much more remains to be learned about the parallel universe of tissue-specific TAFs.

The importance and function of basal-factor relatives were further reinforced in a talk by R. Tjian (Berkeley, CA), who described a detailed analysis of the TBP-related protein TRF2. Tjian and colleagues isolated a multiprotein (~18 subunit) complex containing TRF-2 from *Drosophila* embryos and showed that this complex contained components of the NURF chromatin remodeling complex, as well as DREF, a promoter-selective DNA-binding protein that controls transcription of DNA-replication-associated genes. The significance of the TRF2–DREF association was reinforced by microarray analysis, which revealed that TRF2 controls the transcription of a number of genes involved in DNA replication, including that encoding the proliferating cell nuclear antigen, PCNA. *PCNA* has a particularly interesting promoter, because it has two characterized transcriptional start sites, one that is dependent on the TRF2–DREF complex and another that depends on good, old-fashioned, TFIID. Thus, Tjian's results demonstrate the extraordinary regulatory potential that basal-factor relatives bring to gene expression, both on their own and in collaboration with their more general counterparts.

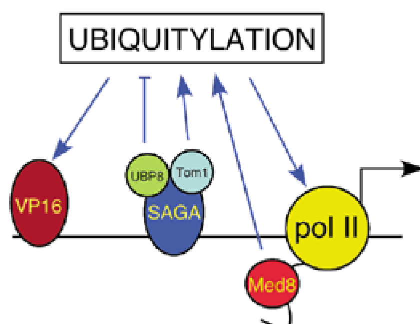

Fig. 2. Ubiquitylation and transcription. A number of talks at the meeting explored the involvement of ubiquitylation in transcription. Blue arrows indicate ubiquitylation of a transcription factor or signaling for ubiquitylation (pointed head) or de-ubiquitylation (blunt head) by a component of the transcription complex. Evidence was presented that (i) the VP16 activation domain signals its own ubiquitylation as part of the activation process, (ii) the UBP8 de-ubiquitylating enzyme associates with SAGA (as does the Ub-ligase Tom1), (iii) the mediator component Med8 recruits a Ub-ligase to the pol II holoenzyme and (iv) ubiquitylation of pol II occurs as a response to the inhibition of polymerase elongation. See text for details.

Transcription and proteolysis: Ub the judge

Transcription is a showcase for the power of the post-translational modification. The roles of phosphorylation, acetylation and methylation in gene control are well documented. It should come as no surprise, therefore, that yet another modification, ubiquitylation, seems to play an important role in transcriptional control. Ubiquitylation generally serves as a signal for protein destruction by the proteasome, although non-proteolytic roles for ubiquitin (Ub) have been described. In recent years, several links between the Ub–proteasome pathway and transcription have emerged. But at this meeting we began to see just how connected these two processes are (Figure 2).

The largest subunit (LSU) of pol II is ubiquitylated and destroyed following exposure of cells to DNA-damaging agents. It has been proposed that ubiquitylation of pol II serves to direct the DNA repair machinery to active sites of transcription, but it has been difficult to test this model because of the lack of a tractable biochemical system. K.-B. Lee (from the laboratory of P. Sharp, Boston, MA) described an *in vitro* system for studying pol II ubiquitylation and showed convincingly that ubiquitylation of pol II is indeed coupled to transcription; not only is LSU ubiquitylation *in vitro* dependent on a DNA template, but it is stimulated when the template is treated with DNA-damaging agents, such as those that induce the formation of *cis*-platin adducts. Lee speculated that this ubiquitylation targets stalled RNA polymerase molecules for destruction, although it is certainly possible that this ubiquitylation serves another purpose—perhaps to recruit the DNA repair machinery. Regardless of the exact mechanism, however, Lee's work has revealed that RNA polymerase ubiquitylation is indeed a 'red flag' for a stalled polymerase.

Perhaps one of the most surprising talks in this area came from J. Conaway (Kansas City, MO), who revealed that pol II associates with an active Ub-ligase, an enzyme responsible for the transfer of Ub to substrate proteins. Conaway showed that mammalian Med8, a component of the RNA polymerase 'mediator' complex, associates with a number of components of the ubiquitylation machinery to form an active Ub-ligase complex. Moreover, Conaway showed that these ubiquitylating components are present in the mammalian mediator complex and that some fraction of the complex prepared by conventional means has associated Ub-ligase activity. Although Conaway has not yet identified a target of this activity, the role of the mediator in connecting activators to the basal transcriptional apparatus suggests that this ubiquitylating function serves some role in transcriptional regulation.

Furthermore, A. Weil (Nashville, TN) reported the identification of a *de*-ubiquitylating enzyme associated with the SAGA chromatin remodeling complex. Weil described a proteomic *tour de force*, performed in collaboration with the laboratory of A. Link (Nashville, TN), aimed at identifying proteins that stably associate with each of the TBP-associated factors in yeast. This analysis led to the identification of a number of previously uncharacterized TAF-interacting proteins, including UBP8, a Ub-specific protease that associates with TAFs in the SAGA complex. Very little is known about UBP8 or its role in SAGA. It is worth noting, however, that UBP8 is the second enzyme from the Ub–proteasome pathway shown to associate with SAGA: the Ub-ligase Tom1 is also a SAGA-associated protein (Saleh *et al.*, 1998). The intriguing link between ubiquitylating and de-ubiquitylating enzymes and the SAGA complex implies that Ub dynamics play an important role in gene expression.

Finally, W. Tansey (Cold Spring Harbor, NY) focused on the role that ubiquitylation of transcriptional activators plays in gene control. Tansey presented evidence that, in order to activate transcription, the prototypical acidic activation domain of VP16 needs to engage the Ub–proteasome pathway (Salghetti *et al.*, 2001). Tansey showed that the ubiquitylating enzyme Met30 interacts with the VP16 activation domain and can signal ubiquitylation and destruction of a synthetic LexA–VP16 activator. Remarkably, in the absence of Met30, LexA–VP16 was not only stable, but also *unable* to activate transcription. This transcriptional defect was rescued by direct fusion of Ub to the LexA–VP16 protein, implying that, in order to activate transcription, the VP16 activator needs to be ubiquitylated. The use of Ub as a dual signal for activation and activator destruction was proposed to be a licensing mechanism, ensuring that transcriptionally active VP16 protein is rapidly destroyed by the proteasome.

Chromatin code

It is fair to say that, in the past, chromatin has not always held the place it deserves at transcription meetings. Chromatin was often viewed as something that had to be dealt with during the process of eukaryotic transcription, but was not directly involved in the control of gene expression. In recent years, however, our understanding of the role of chromatin in the control of gene expression has literally exploded, and chromatin was a hot topic of discussion at this meeting.

Two talks shed important light on the mechanism of action of SWI/SNF chromatin remodeling complexes. T. Owen-Hughes (Dundee, UK) presented evidence that nucleosome mobilization is an important role of the SWI–SNF complex. Owen-Hughes used mutations in histones that circumvent the requirement for SWI–SNF function *in vivo* to probe the function of SWI–SNF

in vitro. He reported that nucleosomes formed from these histones are highly mobile compared with their wild-type counterparts, and thus concluded that a normal function of SWI–SNF is to promote nucleosome mobilization. A slightly different, but not incompatible, take on the function of SWI–SNF was presented by one of the organizers of the meeting, R. Kingston (Boston, MA), who argued that SWI–SNF functions primarily as a protein chaperone, serving to continuously convert nucleosomes to different remodeled states. In an elegant series of biochemical assays using restriction enzymes to probe DNA accessibility within the nucleosome, Kingston showed that the BRG1 subunit of the SWI–SNF complex can generate multiple remodeled states from a single nucleosome. The ability of SWI–SNF to keep nucleosome structure in a constant state of flux provides a simple mechanism to account for the wide-ranging actions of the complex and reveals that specificity in chromatin remodeling must be governed by the action of regulatory transcription factors that stabilize particular nucleosome configurations at their cognate promoters.

Much discussion centered on the role of covalent histone modifications, such as methylation, acetylation and phosphorylation within the histone H3 'tail'. T. Jenuwein (Vienna, Austria) focused on the role of lysine 9 (K9) methylation and its relationship to heterochromatin formation. Jenuwein described how the mammalian Suv39h histone lysine methyltransferases methylates K9 of histone H3, creating a binding site for the heterochromatin-associated protein HP1. Jenuwein also described how Suv39h-deficient mice are specifically defective for K9 methylation at sites of constitutive heterochromatin, but not at other sites in the genome (Peters *et al.*, 2001). Moreover, the Suv39h-deficient mice also display a number of defects in chromosome management, revealing that heterochromatic K9 methylation plays an important role in maintaining genome stability.

S. Grewal (Cold Spring Harbor, NY) presented an elegant combination of genetics and biochemistry that focused on how modifications of the histone tails regulate one another in the establishment of heterochromatin in fission yeast. Grewal showed that deacetylation of lysine 14 (K14) on histone H3 is required for the subsequent methylation of K9, which in turn recruits Swi6, the *Schizosaccharomyces pombe* equivalent of HP1. The elucidation of this epigenetic pathway not only defines the sequence of events that establish heterochromatin, but also establishes a revolutionary 'histone code' (Nakayama *et al.*, 2001), in which modifications of the histone tails carry information for the regulation of gene expression.

The issue of cross-talk between histone modifications was further addressed by S. Berger (Philadelphia, PA), who studied interactions between phosphorylation and acetylation events, also on histone H3. Berger reported that phosphorylation of serine 10 (S10) by the Snf1 histone kinase promotes GCN5-mediated acetylation of K14 (Lo *et al.*, 2001), an event that is linked to gene activation. Thus, full induction of the *INO1* gene, for example, requires targeted recruitment of Snf1, which in turn modifies the chromatin, making it susceptible to the action of histone acetyltransferases. Remarkably, although S10 phosphorylation appears to play a general role in stimulating K14 acetylation, the requirement for Snf1 is promoter specific: the *HO* promoter, for example, is activated perfectly well in the absence of Snf1 function. Berger's work demonstrates how ubiquitous histone modifications can be turned, through the action of promoter-specific modifying enzymes, into gene-specific regulatory events.

A final example of how conserved histone-modifying processes are put to work came from A. Kouzarides (Cambridge, UK), who described how the cellular processes normally involved in heterochromatic gene silencing can also function in repression of gene transcription through the cell cycle. Kouzarides revealed that the retinoblastoma protein Rb, which represses the transcription of E2F target genes during certain phases of the cell cycle, specifically recruits the Suv39h methyltransferase to its target promoters *in vivo*. As with heterochromatic silencing, this targeted recruitment of Suv39h leads to K9 methylation, which in turn recruits HP1, contributing to gene silencing (Nielsen *et al.*, 2001). Thus, although transient gene repression and constitutive heterochromatin apparently have very little in common, a common mechanism appears to be at work for the establishment of these transcriptionally silent states.

Perspectives

What will the 2003 Cold Spring Harbor Transcription meeting look like? The rapid pace of progress in the transcription field makes it almost impossible to predict where we will be in just 2 years. But, based on trends that emerged at the 2001 meeting, a number of things seem certain. First, there will be more participants. In addition, there will be more talks about gene-specific roles for the basal transcriptional machinery and its relatives, more excitement about the role of the Ub–proteasome pathway in transcription, even more emphasis on the role of chromatin and epigenetic gene regulation, and more headaches for whoever has to condense such an extraordinarily rich conference into a meeting report.

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