Transcriptional regulation

Meeting on Regulatory Mechanisms in Eukaryotic Transcription

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The Meeting on Regulatory Mechanisms in Eukaryotic Transcription took place between 3 and 8 February 2008, at the Keystone Resort, Colorado, USA, and was organized by K. Struhl & J. Lis.

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transcription factors that promote PIC formation. Once the PIC is formed, Pol II can initiate the synthesis of messenger RNA (mRNA), and this is accompanied by phosphorylation at Ser 5 on the repeats of the carboxy-terminal domain (CTD) of the largest subunit of Pol II (Fig 1). Surprisingly often in higher eukaryotes, Pol II stalls after the synthesis of approximately 20–50 bases of RNA owing to repression by negative elongation factor (NELF) and 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF; Saunders *et al*, 2006). This block is overcome by the cyclin-dependent kinase CDK9 (also known as P-TEFb), which phosphorylates the Pol II CTD at Ser2, as well as NELF and DSIF (Fig 1). These phosphorylations initiate a cascade of events that is required for productive elongation, mRNA metabolism, termination and chromatin modification, by facilitating the recruitment of factors that are essential for these processes to the elongation complex. In the past few years, it has been found that some of the transcription-linked chromatin modifications are crucial for opening up the chromatin to allow the efficient passage of Pol II during transcription, whereas, after the passage of Pol II, other chromatin modifications are essential for re-establishing a more repressive chromatin environment to prevent cryptic transcription initiation.

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Introduction

The broad field of gene regulation encompasses such diverse topics as the biochemical mechanism of transcription factors, chromatin organization and its role in gene regulation, epigenetic control, regulatory networks, and the control of complex biological processes such as development, sex determination and cell identity. This meeting brought together scientists from all of these areas and provided opportunities for interdisciplinary interactions.

Transcription by RNA polymerase II (Pol II) involves the recruitment of general transcription factors, coactivators (such as the Mediator complex) and Pol II to the promoter to form a preinitiation complex (PIC; Hahn, 2004). This step requires both a permissive chromatin environment that allows the transcription machinery to access the promoter DNA and gene-specific

Initiation, elongation and coactivator function

In one of the highlights of the meeting, R. Singer (Bronx, NY, USA) described the methods developed by his laboratory to measure the *in vivo* dynamics of the initiation and elongation process (Darzacq *et al*, 2007). This mammalian cell system consists of many integrated copies of a cassette containing lac operator sites, a regulated promoter, an mRNA that can be detected by the binding of a fluorescent RNA-binding protein and a fluorescent gene product. This allows live-cell imaging of a gene locus, and the measurement of the binding and dissociation kinetics of Pol II and the rate of mRNA production. The data indicate that the Pol II commitment to transcription is highly inefficient, with only 13% of the Pol II molecules that interact with the promoter proceeding to transcription initiation, and less than 10% of these progressing to the elongation step. *In vivo* elongation measurements also indicate that most Pol II elongation is surprisingly fast, but that a small percentage of Pol II elongates much more slowly. Singer also described new methods designed to measure *in vivo* expression in single budding yeast cells.

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Fig 1 | Transcriptional regulation and the pathway of RNA polymerase II transcription. Signalling pathways activate gene-specific transcription factors that bind to gene-regulatory regions. Distant factors can interact with more proximal factors through DNA looping. These gene-specific factors can recruit chromatin-modifying factors, as well as transcriptional coactivator complexes, to the vicinity of the transcription start sites. The coactivators function to recruit, and possibly to stimulate, the activity of the transcription pre-initiation complex (PIC). PIC assembly can be modulated by the repressors Mot1 and negative cofactor 2 (NC2). On initiation of transcription, the RNA polymerase II (Pol II) carboxy-terminal domain (CTD) is phosphorylated at Ser 5 by cyclin-dependent kinase 7 (CDK7). Transcription elongation in higher eukaryotes is often blocked by the action of negative elongation factor (NELF) and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor DSIF (Spt4/5) to generate a paused polymerase. This block is overcome by the action of CDK9, which phosphorylates the Pol II CTD at Ser 2, as well as at NELF and DSIF. The elongating Pol II with the CTD phosphorylated at Ser 2 and Ser 5 recruits factors that are involved in chromatin modification, transcription elongation, messenger RNA (mRNA) processing, mRNA transport and termination. Chromatin-modifying factors that are associated with the transcription–elongation complex function ahead of Pol II to generate a chromatin state that is permissive for transcription, and a more repressive state after the passage of Pol II to prevent cryptic initiation. Long-range chromatin interactions, for example between insulator elements, isolate chromatin domains to prevent the spread of regulatory signals and probably also function in other aspects of gene regulation.

A biochemical analysis that revealed the steps involved in PIC formation and gene activation was also described. M. Meisterernst (Münster, Germany) presented the surprising finding that the general transcription repressor negative cofactor 2 (NC2), which targets the TATA-binding protein (TBP), can rapidly mobilize the sliding of TBP along the DNA (Schluesche *et al*, 2007). This mobilization could either cause gene repression or, alternatively, facilitate promoter location and thereby help to stimulate transcription. NC2 exists in several forms in certain cell types and it will be interesting to investigate whether the other forms also have this activity. In the keynote lecture, R. Roeder (New York, NY, USA) described an alternative form of human POL II containing the factor Gdown1 (Hu *et al*, 2006). As the Mediator coactivator complex is generally required for transcription by POL II *in vivo*, it was surprising that the requirement for Mediator in transcription activation was lost in a system reconstituted with purified general transcription factors and POL II lacking Gdown1. Substitution of purified POL II with the Gdown1 form restored a strong dependence on Mediator for transcription activation in this *in vitro* system. According to the current model, the Gdown form of POL II is repressed for basal and activated transcription, and this repression is alleviated by Mediator.

The pausing of Pol II during early elongation was examined on a global scale by K. Adelman (Research Triangle Park, CA, USA), J. Lis (Ithaca, NY, USA) and R. Young (Cambridge, MA, USA). By using chromatin immunoprecipitation (ChIP)-on-chip technology in *Drosophila* cells and mammalian embryonic stem (ES) cells, respectively, Adelman and Young have found that approximately 20% of genes contain a peak of Pol II positioned near the promoter (Muse *et al*, 2007; Zeitlinger *et al*, 2007). By using permanganate-sensitivity assays, Adelman showed that many of these polymerases had initiated transcription and then paused or stalled 20–30 bases downstream of the start. In both *Drosophila* and ES cells, most genes with stalled Pol II are those required for development. In a complementary approach, Lis created a genome-wide map of engaged Pol II in human primary lung fibroblasts by a nuclear run-on assay followed by sequencing of the resulting products. His observation that about 30% of the genes had paused Pol II agreed well with the above-mentioned findings obtained using different methods. A popular model for the function of paused Pol II is that it allows the rapid induction of target genes when an appropriate environmental or developmental signal is encountered. For example, Adelman proposed that the poised Pol II can exclude

a nucleosome that would otherwise repress initiation from the transcription start site. A counter argument put forward during discussions at the meeting is that the paused Pol II represents a 'dead-end' state that exists simply to repress genes under certain conditions. According to this model, gene activation could occur by blocking pausing and by stimulating initiation through a distinct mechanism. Arguments can be made for both models, and it will be interesting to see whether either one of these extremes or a hybrid model is correct. An *in vivo* measurement of the lifetime of the stalled Pol II will also be informative.

Transcriptional and chromatin regulatory mechanisms

It has been well established that the chromatin state is a crucial factor in gene regulation and in complex biological processes, such as development and cell-fate determination. Although chromatin has been touted as controlling gene expression, it is now relatively well accepted that the chromatin state is regulated by transcription factors. The chromatin state and various histone modifications do not themselves have the specificity required to control gene expression. By contrast, gene-specific transcription factors bound to specific sites in the genome are known to recruit chromatin-remodelling factors and enzymes that covalently modify histones. This leads to the binding of other regulatory factors that act together with chromatin to create a permissive or non-permissive environment for gene expression.

J. Conaway (Kansas City, MO, USA) showed that the deubiquitinating enzyme ubiquitin C-terminal hydrolase 37 (UCH37) is a component of the INO80 chromatin-remodelling complex and also associates with the 19S proteasome subcomplex. By using an active-site probe, Conaway found that UCH37 is largely inactive in the INO80 complex. Furthermore, the catalytic site of UCH37 is subject to auto-inhibition by its C-terminal tail and by other INO80 subunits that can bind to the tail, as well as to activation by the 19S proteasome complex. This indicates that the deubiquitination activity within INO80 is, itself, regulated. By using nucleosomes with either ubiquitinated histone 2A (H2A) or H2B in a non-cleavable form, Conaway showed that nucleosomes containing ubiquitinated H2B are less-efficiently remodelled by INO80 than non-ubiquitinated nucleosomes, and that H2A-Ubiquitin-containing nucleosomes are more efficiently remodelled than non-ubiquitinated chromatin. These results indicate that UCH37 regulates the remodelling activity of INO80.

J. Workman (Kansas City, MO, USA) described the role of the *Drosophila* coactivator Spt-Ada-Gcn5-acetyltransferase (SAGA) complex in accurate axon guidance in the optic lobe (Weake *et al*, 2008). The fruit fly mutant *nonstop* was found to be defective in a ubiquitin-specific protease that is highly related to the *Saccharomyces cerevisiae* ubiquitin protease 8 (Ubp8) SAGA subunit. *nonstop* can complement a yeast *Ubp8* mutant, confirming that *nonstop* is a functional homologue of *Ubp8*. Mutation of the *Drosophila* SAGA subunits Sgf11 or Ada2b also causes defects in axon guidance. Presumably, SAGA is crucial for the regulation of one or more genes that are involved in this axon-guidance decision.

It has long been known that changes in chromatin structure, such as decondensation, accompany gene upregulation. Lis found that at the *Drosophila* heat-shock protein 70 (Hsp70) locus, nucleosome positioning is disrupted shortly after gene induction. Further experiments showed that this loss of nucleosome positioning within the gene is transcription-independent. RNA-interference screens of candidates likely to regulate this process showed that the nucleosome

disruption required polyADP ribose polymerase (Parp), in addition to heat-shock factor and GAGA factor, which are two known Hsp70 regulators. This surprising finding raises the question of how Parp fits into the chromatin remodelling and maintenance pathways.

A. Berk in collaboration with S. Kurdiani, M. Pelligrini and M. Grunstein (Los Angeles, CA, USA) reported a mechanism by which adenovirus infection causes a global change in the acetylation of histone H3K18. Berk and colleagues found that the viral early region 1A (E1A) protein causes relocalization of the histone acetyltransferase p300/CBP to promoter regions of genes that promote cell cycling, resulting in global hypoacetylation of H3K18. This mechanism, combined with E1A-directed localization of the p107 repressor to genes that promote cellular differentiation, can explain how the viral protein contributes to oncogenic transformation and inhibits cellular differentiation.

B. Ren (La Jolla, CA, USA) described genome-wide studies intended to identify transcriptional regulatory elements (Heintzman *et al*, 2007). By profiling chromatin modifications across a large genomic region, it was found that H3K4 methylation marks seemed to predict accurately the location of promoters and enhancers, with trimethylation marking promoters and monomethylation marking enhancers. Ren also examined these marks in different cell lines to determine how they correlated with gene expression and found that the enhancer marks correlated well with actively expressed genes. One point raised during the discussion was that functional studies will be required to confirm that the enhancers identified by these methods are truly functional elements.

Long-range interactions and DNA looping

DNA looping between distant sites on chromosomes was one of the main topics covered at the meeting. Since its discovery as an important bacterial gene-regulatory mechanism more than 20 years ago (Dunn *et al*, 1984), DNA looping has been proposed as a mechanism to explain the action of regulatory sites such as enhancers, locus-control regions and silencers at distant promoters. Methods such as chromosome-conformation capture (3C; Dekker *et al*, 2002) and more sophisticated variants have been used to probe both functional domains within chromatin and the action of gene-regulatory elements.

J. Dekker (Worcester, MA, USA) described results obtained using the 3C carbon copy (5C) technology (Dostie *et al*, 2006) to examine DNA looping over large regions. This method can be performed on a large scale using the Polony sequencing method (Porreca *et al*, 2006). With this technique, Dekker examined a 1-Mb DNA region centred on the β -globin locus to examine whether the locus is physically separated from the surrounding chromatin. In the absence of globin expression, no distal interactions were observed. By contrast, under conditions in which globin was expressed, numerous distal interactions were observed over most of the 1-Mb region. This shows that, in contrast to expectations, the globin locus is not an isolated domain. Closer examination of the data revealed that many of the looping interactions occurred between globin-regulatory sequences and distal CCCTC-binding factor (CTCF)-binding sites that mark insulator elements and other genes. One important question that needs to be addressed is the frequency of these interactions. It is not currently possible to tell whether these distal interactions occur transiently with low frequency and, if so, how they contribute to gene regulation. Also, the mechanism and regulatory consequences of many of these newly discovered interactions remain to be determined. C.-L. Wei (Singapore) described a genome-wide method to examine distal looping interactions termed chromatin-interaction

paired-end ditag (Chia-PET). This method combines ChIP, 3C and paired-end ditagging together with mass sequencing to identify chromosome interactions on a global scale. However, the number of sequencing reads used for this promising new technique does not yet approach the level necessary to identify all interactions—this is one of the goals of future research in this area.

N. Proudfoot (Oxford, UK) described gene looping in *Schizosaccharomyces pombe* (Gullerova & Proudfoot, 2008). By using a locus containing two convergently transcribed genes, he found a DNA loop between the 3' ends of the convergent genes that was independent of their transcription states. Surprisingly, this loop is specific for the G2 phase of the cell cycle and seems to involve cohesin. Another surprise is that the convergent genes correctly terminated only in G2. Under conditions in which termination did not occur, Proudfoot observed heterochromatin marks at the locus that are consistent with read-through transcription into the adjacent gene and double-stranded RNA formation.

M. Hampsey (Piscataway, NJ, USA) showed that all of the *S. cerevisiae* genes examined so far have a loop between the promoter and terminator regions (Singh & Hampsey, 2007). The looping is dependent on both transcription factor IIB (TFIIB) and Ssu72, which is a component of the cleavage and polyadenylation factor. At the galactose (Gal) locus, the looping seems to be stable for up to 4 h after gene shutoff. A specific mutation in TFIIB can eliminate detectable looping, while having only minimal effects on constitutive transcription. By contrast, gene looping at the inducible Gal promoter was found to be important for a second round of induction. Notable goals for future work are the determination of the mechanism by which looping contributes to rapid re-induction and the identification of the protein components that comprise the loop.

Transcriptional silencing in biological regulation

Silencing is a chromatin-dependent biological mechanism that is important for gene regulation and epigenetic control. Heterochromatin formation requires an initiating event followed by the recruitment of silencing proteins, the spread of the silencing factors along the chromosome, and, ultimately, the inheritance of a typically non-acetylated and methylated chromatin state. K. Struhl (Boston, MA, USA) described an inducible yeast system for the study of telomeric silencing (Katan-Khaykovich & Struhl, 2005). In this strain, the silent information-regulator protein 3 (Sir3) is under the control of the Gal promoter. Although Sir3 induction is rapid, complete silencing takes about five cell generations because it requires Sir2-dependent histone deacetylation (around one-cell generation) and demethylation of H3K79 (around five-cell generations). These data led Struhl to propose that the reinforcing of stable 'on' or 'off' states by positive-feedback loops is responsible for stable epigenetic states.

S. Buratowski (Boston, MA, USA) described studies examining the silencing of the ribosomal DNA (rDNA) locus and the role of cryptic non-coding transcripts in this process (Vasiljeva *et al*, 2008). Buratowski found cryptic transcripts originating from the 'non-transcribed' spacer region in yeast rDNA that are terminated by the N-arginine dibasic convertase (Nrd1)—nuclear polyadenylated RNA-binding protein 3 (Nab3) pathway and degraded by the exosome complex. Mutations in Nrd1 that fail to terminate show both decreased silencing and marked changes in histone acetylation and methylation. This change in the heterochromatin state of rDNA also leads to increased recombination. Buratowski postulated that this change in heterochromatin is the result of active

transcription through the rDNA spacer region, and not by the actual RNA product.

In another highlight of the meeting, M. Green (Worcester, MA, USA) described a complex pathway underlying epigenetic silencing by the Ras oncogene (Gazin *et al*, 2007). To probe the mechanism of Ras-mediated silencing, Green screened a short-hairpin RNA library to identify genes that are required for silencing the Fas proapoptotic gene. Surprisingly, his group identified 28 genes, most of which encode nuclear proteins that are involved in repression, transcriptional regulation, chromatin state and genome stability, and at least nine of these factors are recruited to the Fas promoter. In addition, most are ordered in a pathway that ends in DNA methyltransferase 1 (DNMT1). Silencing all of the upstream genes was found to block the Ras-dependent recruitment of DNMT1, resulting in hypomethylation of the Fas promoter. Most factors in the pathway were also required for Ras-dependent silencing of five other oncogenes, showing that this is a general mechanism. One important point is that these studies examined the factors that are required for the maintenance of the silent state; whether or not they are also required for the initiation of the silenced state has yet to be determined.

E. Heard (Paris, France), B. Meyer (Berkeley, CA, USA) and P. Becker (Munich, Germany) all spoke about the distinct dosage-compensation mechanisms used by various organisms to balance the expression of the X chromosome between males and females. Heard described the identification of a new region of the X-inactivation centre (XIC) termed the X-pairing region (XPR) and showed that it is involved in the initial pairing of homologous chromosomes during S phase (Augui *et al*, 2007). She also described an exciting new method for the visualization of a single defined locus in living mouse cells and used this to examine the dynamic interaction of two XICs. Meyer described the latest work on the *Caenorhabditis elegans* dosage-compensation complex (DCC), which binds to highly enriched sequences on the X chromosome. Interestingly, not all X-linked genes were repressed to the same extent, as the most weakly expressed genes were generally the most dosage-compensated, whereas the genes that bound the DCC in the promoter region somehow escaped repression. Subunits of the DCC were also found to be involved in the suppression of double-strand breaks. Normal cells have only one X-chromosome crossover during meiosis (Tsai *et al*, 2008), whereas Meyer discovered that mutants in DCC subunits showed higher levels of both double-strand breaks and crossovers. Becker discussed the fruit fly DCC and its targeting to the X chromosome, which, unlike the DCC of mammals and worms, upregulates the expression of X-linked genes twofold in males. Becker also described evidence that the DCC subunit MOF—a histone acetyltransferase specific for H4K16—can activate transcription from a nucleosome array, and that its activity in cells could be restricted to the required twofold effect in males.

Regulatory networks and master regulatory factors

Another highlight of the meeting included presentations by Young and M. Snyder (New Haven, CT, USA) on regulatory networks. Young described the master transcription regulators in mammalian ES cells octamer-4 (OCT4), SRY sex-determining region Y-box 2 (SOX2) and NANOG, which form an interconnected autoregulatory loop (Boyer *et al*, 2005), and the identification of transcription factor 3 (TCF3) as an additional ES-cell master regulator. These factors are co-associated on approximately 1,000 genes in ES cells, about 50% of which are transcribed and required for cell proliferation. The remaining target genes are not active and most are involved in

specific developmental pathways that must be silenced in ES cells. Many of the silenced genes contain a paused Pol II and are bound by the Polycomb complex (Guenther *et al*, 2007). Young also described the role of Wnt signalling in ES cells and its connection to TCF3. These and other studies show that ES cells are balanced between pluripotency and differentiation, and that this balance is controlled by the action of the master regulatory factors. Young postulated that reprogramming differentiated cells to ES cells requires the transfection of genes that ultimately switch on these master regulatory genes by as yet unknown mechanisms.

Snyder described the results of his work on mapping gene-regulatory networks and identifying master regulatory genes in yeast and human cells. He also addressed the question of what makes species different, asking whether it is a difference in gene sequences and/or the regulation of these genes that differentiate species (Borneman *et al*, 2007). Snyder mapped the binding sites for the transcription factors Ste12 and Tec1 in three closely related budding yeasts that are less than 0.5% different in their gene protein-coding sequences. Importantly, he found surprisingly extensive changes in the transcription factor-binding sites between the three species. For example, the number of binding sites and the particular binding sequence was often not conserved. This analysis indicates that regulatory sequences diverge so quickly that sequence conservation cannot be reliably used to predict binding sites. Snyder postulated that this might be the result of the closely related yeasts existing under different environmental conditions that select for specific modes of gene regulation that are fine-tuned to the environment.

Summary

Overall, the meeting provided an excellent environment for those interested in transcriptional regulation to interact and to hear about a diverse array of topics, which stimulated lively discussions from the interdisciplinary audience. It was a consensus opinion that the meeting was a great success and it is hoped that the keystone symposia will continue to host this meeting in the future.

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