Meeting report

An all-round view of eukaryotic transcription
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A report of the Keystone Symposium ‘Regulation of Eukaryotic Transcription: From Chromatin to mRNA’, Taos, USA, 21-26 April 2006.

Transcription of protein-coding genes in eukaryotes involves a complicated yet highly coordinated series of events involving chromatin, chromatin modifiers, the transcriptional machineries and transcriptional regulators. A recent Keystone Symposium on the regulation of eukaryotic transcription covered the topic from a variety of perspectives, both structural and biochemical. This report highlights some of the findings and new approaches reported at the meeting.

Structural views of the transcriptional complex

One key to understanding the mechanism of transcriptional initiation is an atomic-level view of the RNA polymerase pre-initiation complex (PIC). In his keynote address, Roger Kornberg (Stanford University, Palo Alto, USA) described new structural studies of a PIC containing the 12-subunit yeast RNA polymerase II (PolII) and general transcription factors bound to promoter DNA. From this we can see that TATA binding protein (TBP) configures DNA to the PolII surface; transcription factor II B (TFIIB) directs the DNA to the PolII active site and stabilizes the transcription complex; TFIIE recognizes the closed PolII complex and recruits the helicase TFIH, while TFIIF captures the template strand DNA when the DNA duplex melts to form the transcriptional bubble. Finally, TFIH introduces negative supercoiling of the promoter DNA, enabling the polymerase to move away from the promoter.

Patrick Shultz (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France) has used cryo-electron microscopy to view yeast TFIID bound to DNA, revealing that DNA wraps around TFIID and threads through channels formed between three structural modules. Eva Nogales (University of California, Berkeley, USA) has applied a new analytical approach to the cryo-electron microscopic structure of human TFIID - three dimensional variance and conformational flexibility analysis - and has characterized the structure in both closed and open forms.

Transcription factor interactions

The rate-limiting step to transcriptional initiation by PolII is promoter clearance. This is achieved when transcription proceeds independently of TFIH, a short length of hybrid RNA-DNA has formed, and the initial transcription bubble collapses. Donald Luse (Cleveland Clinic Foundation, Cleveland, USA) has found that stability of PolII on the promoter is minimal just before bubble collapse. He observed that the transcription bubble must be 17 nucleotides or longer, and the RNA transcript longer than six nucleotides, for the bubble to collapse, and that TFIIB must be phosphorylated; it is then displaced from the channel on PolII.

Jim Kadonaga (University of California, San Diego, USA) presented a functional analysis of promoter sequence motifs from Drosophila and humans that are required for accurate transcription initiation, which revealed a network of interaction among these elements. MTE (motif 10), for example, can compensate for the loss of the downstream promoter element (DPE) and TATA by increasing the promoter’s affinity for TFIID. Kadonaga has constructed a ‘super’ core promoter containing TATA, the initiator motif (INR), MTE and DPE which exhibits high basal transcriptional activity in vitro and in vivo.

During initiation, RNA polymerase undergoes dramatic conformational changes that could be exploited to inhibit transcription using RNA inhibitors. Jim Goodrich (University
of Colorado, Boulder, USA) has found that both the mouse B2 SINE transcript and the human Alu transcript bind RNA polymerase with high affinity (Kd < 2 nM in the case of B2 SINE RNA) and inhibit transcription. He proposed that these RNA inhibitors might be involved in restricting the transcription of non-heat-shock genes during a heat-shock response. Exactly how the RNAs block transcription is not clear, but Patrick Cramer (University of Munich, Germany) described the structure of an inhibitor RNA binding to the PolII active site; this structure suggests that the inhibitor might act by preventing formation of the open complex.

Although transcriptional initiation and termination are known to be coupled to pre-mRNA processing mechanisms such as capping and polyadenylation, respectively, mechanisms that might coordinate transcriptional elongation, mRNA splicing, and chromatin remodeling remain to be determined. Kathy Jones (Salk Institute, La Jolla, USA) presented an interesting mechanism linking elongation with pre-mRNA processing. She has found that the protein Skip, which is required for basal and Tat-mediated transcription of human immunodeficiency virus (HIV) cDNA, stimulates Tat activation and interacts with both the U5 snRNP (a small nuclear ribonucleoprotein involved in RNA splicing) and pTEFb (positive transcription elongation factor b). Keiko Ozato (National Institutes of Health, Bethesda, USA) showed that the bromodomain protein Brd4 interacts with acetylated histones H3 and H4 and the pTEFb complex, implicating Brd4 in the recruitment of the elongation factor.

Kevin Struhl (Harvard Medical School, Boston, USA) has analyzed mechanisms of nucleosome eviction during transcription elongation in yeast and showed that the protein FACT (facilitates chromatin transcription) is required to restore normal chromatin structure after elongation, which it does by blocking the inappropriate initiation of transcription in the coding sequences. He has also found that the histone chaperone ASF1 travels with PolII and is required for the eviction of H3 but not H2B during elongation.

Real-time analysis of transcription in vivo
John Lis (Cornell University, Ithaca, USA) presented a visualization of the dynamic interactions of transcription factors with the polytene chromatin in Drosophila, obtained by a novel live-cell imaging technique using two-photon microscopy. He showed that in the absence of heat shock there is high turnover of heat-shock factors (HSFs) at their target sites, but on heat shock, HSFs bind extremely stably to the promoters of their target genes. Interestingly, this contrasts with previous real-time imaging observations that suggested a ‘hit and run’ model of transcription factor binding.

By characterizing TBF-associated factor (TAF) complexes with the technique FRAP (fluorescent recovery after photobleaching), Marc Timmers (University Medical Centre Utrecht, Netherlands) has discovered two populations of TBP (the TATA-binding factor) in HeLa cells - a mobile one that corresponds to the TAF1 complex (TBP and one TAF), and an immobile one, suggesting distinct functional pools of TBP in the nucleus. Using the same technique, Marco Bianchi (San Raffaele Scientific Institute, Milan, Italy) has found that the transcription factor NFκB is stripped from DNA by the proteasome but that NFκB with a mutation at Ser26 stays on DNA longer.

Also using in vivo real-time imaging, Mike Marr (University of California, Berkeley, USA) showed that different architectures of the Drosophila metallothionein promoter (indicating an active or inactive promoter) correlated with recruitment of Mediator proteins. An absence of TAF complexes had no effect on the promoter, whereas a lack of Mediators rendered it inactive, but did not affect TFIIID recruitment. In the absence of both Mediators and TFIIID, the promoter could actually be activated.

Nucleosome removal and histone modification
The relationship of chromatin modification to transcription was a strong theme throughout the meeting. Kornberg described a quantitative analysis of chromatin remodeling at the yeast PHO5 locus, which loses two nucleosomes at the promoter and upstream activating sequences upon activation. Disassembly turns out to be the main cause of this nucleosome loss, rather than nucleosome sliding, and Kornberg reported kinetic studies suggesting that nucleosome disassembly is the rate-limiting step for transcription initiation. Also working with yeast, Struhl has found that nucleosome depletion is related not only to promoter activity, but also to transcriptional elongation by RNA polymerase and to the DNA sequence. One of us (B.R.) reported the finding of distinct chromatin signatures associated with active promoters and enhancers in human genes, as detected by chromatin immunoprecipitation followed by DNA microarray (ChIP-chip). This chromatin modification pattern can be used to predict new promoters and enhancers within the genome.

The mechanisms controlling nucleosome removal are not clear, but histone chaperones and other factors are likely to be involved. Karolin Luger (Colorado State University, Fort Collins, USA) presented structural and biochemical analyses of the histone chaperone NAP1 that suggest a role for it as a scavenger of ill-assembled chromatin. Ed Luk (National Institutes of Health, Bethesda, USA) characterized the loss of the yeast histone H2A variant Htz1 from chromatin after deletion of the chromatin-remodeling complex SWR1, and identified Chz1, a novel Htz1 chaperone, as part of the complex. Paul Laybourn (Colorado State University, Fort Collins, USA) has analyzed the effects of the viral transcriptional activator Tax on the local and global chromatin structure of the human T-cell lymphotrophic virus type 1 (HTLV-1) integrated into host-cell chromosomes. He
observed a decrease in acetylated histone H3 along with decreased association of histones at the long terminal repeat (LTR) region when Tax was expressed. These effects were not seen with a Tax Met47 mutant, and Laybourn proposed a model in which Tax activates transcription by displacing histones, SWI/SNF and histone deacetylase from the HTLV long term repeat (LTR).

Jerry Workman (Stowers Institute of Medical Research, Kansas City, USA) described a new function for the SAS (something-about-silencing) complex, which is responsible for the deposition of Htz1 at telomeres. He found that elongating PolII recruits Rpd3S, a component of the SAS complex, to deacetylate histones H3 and H4. Deletion of Rpd3S results in the use of cryptic TATA sites, suggesting that elongating PolII would normally suppress downstream promoters, thus avoiding the production of intergenic transcripts.

New roles for histone modifications are being discovered. Michael Grunstein (University of California, Los Angeles, USA) showed that Htz1 is acetylated on Lys14 at active promoters. It appears, however, that the acetylation is not involved in the histone’s antisilencing function, but is required for its loading onto the promoter. Jane Mellor (University of Oxford, UK) demonstrated a new role for the protein 14-3-3 as a histone-binding protein. Bmh1 and Bmh2, yeast homologs of 14-3-3, enhance H3 acetylation and are required for global acetylation of H3 Lys14 and H3 Lys18 and methylation on H3 Lys4. Judd Rice (University of Southern California, Los Angeles, USA) described the distribution of H4 Lys20 methylation in distinct nuclear compartments. Trimethylated H4 is found at pericentric heterochromatin, while H4 at the nuclear periphery is monomethylated. These modifications are similar to the distribution of di- and trimethylation patterns of histone H3 Lys9, but they occur at distinct loci and do not overlap.

Gordon Hager (National Institutes of Health, Bethesda, USA) presented evidence against the paradigm that deacetylation is associated with repression. He has found that the transcription factor GR is associated with the histone deacetylase HDAC1, which functions as a coactivator in this case. Knockdown of HDAC1 by small interfering RNA reduced the expression of GR target genes. Active chromatin bound by GR was isolated from a transgenic cell line, and the HDAC1 associated with the repressed loci was found to be hyperacetylated. Danny Reinberg (University of Medicine and Dentistry of New Jersey, Piscataway, USA) described a reconstituted chromatin transcription system that his laboratory has developed to investigate the consequences of histone modification. The minimal system includes the retinoic acid receptor (RAR/RXR), Mediator, and the factors SWI/SNF, FACT and p300. He showed that, for transcription elongation to occur, FACT must recruit the RNA polymerase associated factor (PAF) complex, which in turn recruits the ubiquitin ligase RNF40/RNF20, which ubiquitinates H2B Lys120, permitting H3 Lys4 trimethylation. Surprisingly, removal of trimethylation from H3 Lys4 did not appear to affect either the initiation or the rate of transcription. This system holds great promise for understanding the functional consequences of the coordinated chromatin modification that accompanies transcription.

The meeting provided us with much to think about, including novel modes of transcriptional initiation at the promoter, the mechanisms underlying the relationship between chromatin structure and transcription and the unexpected models of gene regulation in living cells revealed by real-time imaging. We look forward to the next meeting on this topic.