Meeting report

Transcription regulation: no holy grail, but many treasures
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How does a transcription factor bring about gene regulation? This key question has been tackled for decades, but there is no unifying answer. Some researchers emphasize recruitment mechanisms by which activators target the basal machinery, in effect RNA polymerase, to the promoter; and some put the emphasis on regulation through alterations in chromatin structure and histone modifications. A recent meeting on transcription in London provided an opportunity to learn about progress in this field.

Focus on RNA polymerase

In a meeting on transcription, a surprising statement by Peter Cook (University of Oxford, UK) was that transcription does not happen very often. But this is what his analysis of transcript abundance in *Escherichia coli* and yeast suggests. Even when RNA polymerase has been recruited to a promoter, only a small minority of the transcripts initiated will make it to full-length RNA, as the processes of initiation, elongation and termination are subject to multiple levels of regulation, as described in several other talks at the meeting.

The take-home message given by Jesper Svejstrup (Cancer Research UK, London, UK) may be just as paradoxical as Cook's, namely that RNA polymerase II (pol II) degradation can be good for transcription. This is because stalled pol II could be fatal. A DNA strand break can lead to pol II arrest, and as RNA polymerase cannot bypass the lesion, the stalled pol II will lead to a pile-up of polymerases with subsequent gene shutdown that could be lethal in a haploid organism such as yeast. Svejstrup's lab has identified the targeted ubiquitin-mediated degradation of stalled polymerases by

the Rad26-Def1 complex as a mechanism for dealing with this problem and has reconstituted the reaction *in vitro*.

After RNA polymerase has successfully transcribed through a gene, termination of transcription becomes an important regulatory step that may, in turn, impact on subsequent transcriptional reinitiation. Analysis of transcriptional termination in the human β-globin gene has revealed the phenomenon of co-transcriptional cleavage (CoTC). Cleavage within the pre-messenger RNA, downstream of the poly(A) addition site, is critical for efficient termination and involves an RNA self-cleaving activity. An important question is whether this is a general phenomenon. Alexandre Akoulitchev (University of Oxford) described his group's use of bioinformatics to identify a novel regulatory element, found both 5′ and 3′ of many genes, which they have termed 'Checkpoint Charlie', and which is believed to play a part in the regulation of CoTC and the definition of transcription borders. Nick Proudfoot (University of Oxford) described his lab's work, which has revealed another CoTC event in the mouse albumin terminator sequence. Given that Proudfoot's work implicated ATP-dependent nucleosome-remodeling factors in termination, it will be interesting to see whether they regulate termination via Checkpoint Charlie, maybe by mediating formation of a loop between the 5′ and 3′ borders of genes.

When people think of transcription most think 'pol II', despite the fact that transcription by RNA polymerase I (pol I), which transcribes the rRNA genes, accounts for 50% of all RNA synthesis within the cell. Brian McStay (University of Dundee, UK) described how pol I transcription is dependent on upstream binding factor (UBF) and selectivity factor 1 (SL1), the latter being essential for targeting pol I to the promoter. He has found that UBF, which contains multiple HMG boxes (motifs that are characteristic of high-mobility group chromatin proteins and that can bend DNA), plays a role in determining chromatin architecture, causing decondensation of the DNA across the ribosomal RNA gene and

also stabilizing the preinitiation complex. Joost Zomerdijk (University of Dundee) presented *in vitro* transcription experiments with a reconstituted system suggesting that UBF also acts after preinitiation complex formation, possibly facilitating escape of the polymerase from the promoter. Robert White (University of Glasgow, UK) has established that human c-Myc, which is only a weak activator of pol II-transcribed genes, directly and markedly enhances pol I transcription of rRNA genes, mediating recruitment of SL1 and increased histone acetylation at these loci. Together, these findings implicate chromatin modification in the regulation of pol I transcription, but also point to the critical roles in transcriptional regulation of downstream events, occurring after pol I or pol II loading.

Regulation of gene expression: switching on the cofactors

The structure of the core promoter of protein-coding genes has an important influence on basal transcription and on the ability to respond to activators. This core comprises the DNA sequences flanking the transcription start site, including the TATA box, the initiator (INR) element and downstream elements, which interact with the general transcription machinery to mediate the formation of the preinitiation complex. Thomas Oelgeschläger (Marie Curie Research Institute, Oxted, UK) presented evidence that the INR element confers resistance to repression by the negative cofactor 2 complex, illustrating that the transcription apparatus does not only have to contend with repression by nucleosomes.

Michael Green (University of Massachusetts Medical School, Worcester, USA) made a case for fluorescence resonance transfer (FRET) for the study of protein-protein interactions in live cells using fluorescence-tagged proteins. The method relies on energy transfer between interacting proteins and the majority of protein functions in yeast do not seem to be impeded by such tags. Green's study of the paradigm locus for gene activation in yeast, the *GAL1* promoter, indicates activation by the transcription factor Gal4 through recruitment of Tra1, a component of the histone acetyltransferase-containing SAGA coactivator complex (Spt/Ada/Gcn5 histone acetyltransferase), with little evidence that the histone-modification enzymes present in SAGA are critical for the activation of this gene. SAGA in turn recruits the Mediator complex, followed by the general transcription factors, to the core promoter. Richard Reece (University of Manchester, UK) also reported the use of FRET to investigate the regulation of the *GAL* genes by Gal4. His work illustrates how the cell 'borrows' and modifies metabolic enzymes through evolution to mold them into transcriptional co-regulators for nutrient-regulated genetic switches, in this case galactokinase. The transcriptional inducer Gal3 shares a high degree of sequence homology with galactokinase, Gal1, but does not itself possess galactokinase activity. The work also provided insights into how transcription factor activity is modulated by co-regulators.

The latter theme was expanded by Andy Sharrocks (University of Manchester), who described how the transcription factor Elk-1 is phosphorylated within its transactivation domain by mitogen-activated protein kinases. This phosphorylation is an activating event, but he has also found that modification of Elk-1 by attachment of the small protein SUMO inhibits gene activation by mediating the recruitment of histone deacetylase 2, leading to decreased histone acetylation at Elk-1 target genes. Surprisingly, Sharrocks has also found that PIASx (protein inhibitor of activated STAT), which belongs to a group of proteins thought to be involved in attaching SUMO, is implicated in counteracting histone deacetylase recruitment and therefore acts to activate transcription by Elk-1.

Neil Perkins (University of Dundee) revealed a switching mechanism from an oncogenic function of the transcription regulator NFκB to that of a tumor suppressor in response to p53, ADP-ribosylation factor (ARF) and cytotoxic stimuli. In the absence of ARF, NFκB activates the transcription of several oncogenes. In the presence of ARF, however, NFκB associates with histone deacetylase 1, resulting in repression of transcription. In summary, the analysis of co-regulators described at the meeting puts the spotlight on chromatin-modifying enzymes as key regulators.

Control through chromatin?

A mammalian ortholog of the SAGA complex of yeast is the TBP-free TAF complex (TFTC). Laszlo Tora (Institute of Genetics and Cell and Molecular Biology (IGBMC), Strasbourg, France) has been investigating the role of the TFTC in the inherited neurodegenerative disease spinocerebellar ataxia type 7. Polyglutamine expansion of ataxin-7 (ATX7), a TFTC subunit, has been linked to this disease, and Tora described a mouse model expressing mutant ATX7 containing a stretch of 90 glutamines in the retina. The TFTC complex also contains a histone acetyltransferase (HAT) and although the mutant ATX7 gets incorporated into the TFTC and does not seem to alter the *in vitro* HAT activity, the mutation leads to a gross alteration of nuclear architecture, massive chromatin decondensation and a downregulation of retinal cell-specific genes associated with an increase in histone H3 acetylation at promoters *in vivo*. Tora suggests a model in which deregulated TFTC function results in general histone hyperacetylation and a diversion of activators away from retina-specific genes.

Tony Kouzarides (University of Cambridge, UK) made a case for the analysis of histone modifiers because many are connected with the cancer process. He revealed proline isomerization as a new type of histone modification involved in the repression of specific genes and has identified cross-talk between histone methylation and proline isomerization. The peptidyl-prolyl isomerase Frp4 of budding yeast binds histone H3 tails and drives proline isomerization at position

38, which is inhibited by methylation of lysine (K) 36. In turn, Frp4 antagonizes H3 K36 methylation.

Smads are a family of intracellular signaling molecules that act downstream of receptors for the transforming growth factor-β family of ligands. Smad complexes translocate to the nucleus, where they are recruited to DNA by site-specific transcription factors and participate in regulating target genes. Caroline Hill (Cancer Research UK, London, UK) described experiments showing that Smad proteins do not affect gene expression *in vitro* unless the DNA is packaged into chromatin, providing further support for chromatin as a key mediator of gene regulation.

Regulation of gene expression via chromatin-modifying enzymes acquires a sinister side when these enzymes are subverted as a result of chromosomal translocations in acute myeloid leukemias. In one translocation, for example, the histone acetyltransferase MOZ (monocytic leukemia zinc finger protein) is fused to the histone acetyltransferase CBP. David Heery (University of Nottingham, UK) revealed that another translocation product, the fusion of a part of the nuclear receptor co-activator TIF2 with MOZ (MOZ-TIF2) results in gene misregulation through its impact on CBP activity. MOZ-TIF2 interacts directly with CBP, leading to aberrant cellular localization and depletion of CBP and inhibition of CBP-dependent transcriptional activation and, thus, ultimately to transformation of hematopoietic progenitor cells.

As well as the histone-modifying enzymes, the ATP-dependent nucleosome-remodeling factors are important in the regulation of gene expression via chromatin and may function by allowing transcriptional regulators access to important DNA elements. Tom Owen-Hughes (University of Dundee) described evidence that histone modifications may regulate the function of nucleosome-remodeling factors. He and his colleagues used a histone-tail peptide ligation approach to create nucleosomes with defined tail modifications, and showed that acetylation of histone H3 leads to an increase in nucleosome sliding mediated by the RSC (remodels structure of chromatin) complex.

One of us (P.V-W.) described work from our group showing that nucleosome-remodeling factors may determine transcriptional states by being involved in chromatin assembly at the site of DNA replication. The Williams syndrome transcription factor (WSTF) interacts with the nucleosome-remodeling ATPase ISWI and both are targeted to sites of DNA replication by a key factor in DNA replication, the sliding clamp (PCNA). Our model suggests that chromatin remodeling by this complex allows the rapid rebinding of transcription regulators that have been evicted from the replication fork back to the new chromatin in the daughter strands, thereby ensuring epigenetic inheritance of chromatin states.

The big picture

An ultimate challenge is to understand the regulation of tissue-specific gene expression during development. The tight connection between gene regulation and chromatin remodeling was illustrated by Wendy Bickmore (MRC Human Genetics Unit, Edinburgh, UK), who described how the sequential activation of Hox genes in cell culture and in embryos leads to a dramatic decondensation of the locus and a looping out of the activated genes from the chromosomal territory. In contrast, her genome-wide analysis indicates that things are not always so clear-cut. She showed that in general, gene-dense regions are found in 'open chromatin' and most heterochromatin is usually condensed, but she has found that open chromatin is not necessarily linked to active gene expression, and some actively expressed genes are embedded within closed chromatin. Nevertheless, Steve Busby (University of Birmingham, UK) described how regulation via 'chromatin' can occur even in prokaryotes. Here, proteins that organize the genome in a general way are involved in the specific regulation of operons, transcription units, and in the response to environmental cues by differential regulation of DNA looping.

Nature takes advantage of every opportunity to regulate transcription, from regulation of chromatin structure through to transcript elongation and termination. Direct recruitment mechanisms that target the preinitiation complex and, ultimately, RNA polymerase to promoters are clearly important, as are subsequent events. Chromatin modification is implicated in transcriptional regulation at almost all steps yet very little is known about the mechanisms by which chromatin modifications regulate transcription. Ultimately, structural analysis may provide the key mechanistic insights. This meeting, the first major meeting with a focus on transcription research performed in the UK, attested the vibrancy of this field here. We hope that it will have a successor in the next few years.