MILESTONE 17

Switched-on chromatin

Studies in the 1980s and early 1990s clearly showed that chromatin structure was different at active and repressed genes. In particular, it was possible to see on gels that nucleosome positioning changed when a gene became transcriptionally active. The events that resulted in this change, and its relationship to transcription, remained a mystery until a series of publications between 1992 and 1995 provided direct evidence for specific chromatin-remodelling complexes that were required for transcriptional activation.

The first evidence of a functional interaction between putative transcriptional activators and chromatin was described by Fred Winston. It was known that *snf5* mutations affected transcription of a set of genes including *SUC2*. In 1992, Winston's laboratory showed that these effects could be reversed by mutations in the genes encoding histones H2A and H2B. In addition, changes in *SUC2* chromatin structure in *swi2* or *snf5* mutants were complemented, regardless of the level of transcription. These results provided genetic evidence that Swi2 and Snf5 were involved in changes in chromatin structure that affected transcription proficiency. But what was happening at a biochemical level?

A breakthrough in the field occurred in 1994, when Carl Wu's laboratory developed an *in vitro* system that recapitulated the changes in chromatin structure occurring at an active promoter. The system used a promoter construct reconstituted with nucleosomes. When GAGA transcription factor was added, the pattern of nuclease sensitivity changed to a more open chromatin conformation. Importantly, the GAGA-dependent pattern of repositioned nucleosomes was similar to that seen *in vivo*. Intriguingly, the reaction was ATP dependent, although GAGA had no ATP-binding motif.

Later that year, a rapid succession of studies from the laboratories of Craig Peterson, Robert Kingston and Michael Green described the purification of the yeast and human Swi/Snf complexes. These 10-subunit complexes were able to stimulate binding of the GAL4 transcription factor to nucleosomal DNA in an ATP-dependent manner — a functional test of what is now known as chromatin remodelling. As anticipated, the Swi/Snf complexes directly interacted with nucleosomal DNA and altered the pattern of nuclease cleavage, and also changed the DNA topology. It was proposed that the Swi/Snf complex would need to interact with a transcriptional activator to localize it to the region targeted for disruption, and would then contact the DNA to disrupt interactions with the histones and increase accessibility for transcription factors.

In 1995, Toshio Tsukiyama and Carl Wu refined the *in vitro* system for GAGA-mediated nucleosome disruption by purifying the ATP-dependent factor of that reaction. This turned out to be a new four-subunit nucleosome-remodelling factor termed NURF. At high concentration, NURF alone could remodel nucleosomes, but it was required at sub-stoichiometric levels in the presence of GAGA. This study established the presence of multiple complexes that are able to remodel nucleosomes. In a related study later that year, Wu and colleagues showed that the 140-kDa subunit of NURF, ISWI, was highly similar in its ATPase domain to SNF2. This provided a link between the two known chromatin-remodelling complexes and indicated that they might share certain unifying principles.

Angela K. Eggleston, Senior Editor, Nature

References and links

ORIGINAL RESEARCH PAPERS Hirschhorn, J. N., Brown, S. A., Clark, C. D. & Winston, F. Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev*. **6**, 2288–2298 (1992) | Tsukiyama, T., Becker, P. B. & Wu, C. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367**, 525–532 (1994) | Côté, J., Quinn, J., Workman, J. L. & Peterson, C. L. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60 (1994) | Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E. & Green, M. R. Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. *Nature* **370**, 477–481 (1994) | Tsukiyama, T. & Wu, C. Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell* **83**, 1011–1020 (1995) | Tsukiyama, T., Daniel, C., Tamkun, J. & Wu, C. *ISW1*, a member of the *SWI2/SNF2* ATPase family, encodes the 140 kD subunit of the nucleosome remodeling factor. *Cell* **83**, 1021–1026 (1995)

MILESTONE 18

Bridging the gap

By the early 1990s, prokaryotic DNA-binding transcription factors were known to directly contact and recruit RNA polymerase subunits to stimulate transcription. However, the situation in eukaryotes was turning out to be more complicated. Initial studies indicated that transcription factors directly recruit components of the basal transcription machinery to activate transcription. But activated transcription could not be reconstituted in the test tube when transcription factors were combined with the known components of the basal machinery. The existence of intermediary proteins was therefore proposed.

In 1991, Dynlacht and colleagues from the Tjian group showed that *Drosophila melanogaster* cells contain a complex of proteins, which they termed 'co-activators', that associate with the TATA-binding protein and are important for activation *in vitro* by specific transcription factors, such as Sp1 and NTF1. Subsequently, co-activators were found to also participate in regulatory signalling pathways — for example, Chrivia *et al*. showed that the transcription factor CREB (cyclic AMP response-element binding protein) recruits the co-activator CBP (CREB-binding protein) during cyclic-AMP-regulated transcription, with phosphorylation of CREB being a prerequisite for co-activator binding.

Biochemical studies by Kelleher *et al*. from the Kornberg group had shown, in 1990, that transcriptional interference caused by overexpressing a transcription factor (which was presumed to sequester a basal transcription factor) could be relieved by a

MILESTONE 19

Gripping tails

For years, biologists have wondered how a relatively small set of genes can generate the many cell types of multicellular organisms. How can such remarkable phenotypic diversity be created by the same genetic template? We now know that the missing piece in this biological conundrum is the chromatin fibre — the histone and non-histone proteins that package DNA into the nucleus. The amino (N)-terminal tails of histones are subject to a range of covalent modifications, which provide binding sites for regulatory proteins that drive specific patterns of gene expression.

In the mid-1990s, it was becoming clear that histone modifications (see Milestone 22) and chromatin remodelling (see Milestone 17) were important regulators of gene expression. Yet, how specific histone modifications translated into altered gene activity remained unclear. Then, in 1995, a landmark study provided the first clue that gene-regulatory proteins directly interacted with chromatin. In *Saccharomyces cerevisiae*, histones are packaged into regions of transcriptionally silent, inaccessible heterochromatin by repressor proteins, such as the silent information regulators SIR3 and SIR4. A series of experiments from Michael Grunstein's laboratory revealed that the N termini of histones H3 and H4 were bound by the SIRs, showing for the first time that histones interact with gene-regulatory proteins. Importantly, further analysis revealed that acetylation of the N terminus of H4 prevented its interaction with SIR3.

Four years later, a team led by Ming-Ming Zhou solved the solution structure of the bromodomain — a motif that is found in many transcriptional co-activators. Zhou identified an acetylated histone lysine as the specific binding site of the bromodomain. It had long been known that active genes were marked by acetylated chromatin, and the Grunstein and Zhou studies provided the first insights into the functional implications of this association — acetylated histone tails prevent the colocalization of repressor proteins and provide specific binding sites for co-activators.

An intensive search for further chromatin-binding modules ensued, which proved fruitful in 2001. Three groups — led by Thomas Jenuwein, Tony Kouzarides and Shiv Grewal — independently verified that the chromodomain of heterochromatin protein-1 (HP1) or its yeast homologue Swi6 interacted with histone H3 when methylated at the Lys9 residue. In contrast to acetyl marks, methylated H3K9 is a signature for heterochromatic domains, and the binding of HP1 at these sites maintains transcriptional silence.

These and other findings lend strong support to the concept of a 'histone code', which predicts that different combinations of histone modifications provide distinct 'readouts' in the form of chromatin-binding proteins. In particular, the binding of proteins that contain bromo- and chromodomains provides illuminating evidence for modification-dependent target specificity on the chromatin template, with contrasting effects on gene expression.

These insights into the selective interactions between histones and effector proteins have transformed our perception of eukaryotic gene regulation. And, although the details of the 'histone code' are still hotly debated, these findings have resolved long-standing mysteries about fundamental processes, such as heterochromatin formation, X-chromosome inactivation and transcriptional memory.

Shannon Amoils, Locum Associate Editor, Nature Reviews Microbiology

References and links

ORIGINAL RESEARCH PAPERS Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. M. & Grunstein, M. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. *Cell* **80**, 583–592 (1995) | Dhalluin, C. *et al*. Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399** 491–496 (1999) | Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001) | Bannister, A. J. *et al*. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124 (2001) | Nakayama, J., Rice, J. C., Strahl, B. D., Allis, C. D. & Grewal, S. I. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292**, 110–113 (2001)

FURTHER READING Hecht, A., Strahl-Bolsinger, S. & Grunstein, M. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature* **383**, 92–96 (1996) | Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001)

partially purified fraction from yeast, and that this fraction did not contain a known basal transcription factor. This observation led the authors to propose the existence of a factor termed 'Mediator'.

In genetic studies reported in 1993, Young and colleagues isolated genes from a screen for mutants that suppressed the defective phenotype caused by truncations in the carboxy-terminal domain of RNA polymerase II (also known as RNA polymerase B). These 'suppressors of RNA polymerase B', or *SRB* genes, were shown by Thompson *et al*. to be required for efficient basal and activated transcription *in vitro*. In subsequent work, Koleske and Young found that a preparation of RNA polymerase II purified from yeast contained the SRB proteins and general transcription factors in a holoenzyme that could mediate the response to activators *in vitro*.

A key breakthrough came in 1994 with the difficult purification of the Mediator complex by Kim *et al*. from the Kornberg group. They were able to reconstitute activated transcription *in vitro* with purified components, finally demonstrating the elusive Mediator activity. There were two particularly surprising findings. First, the Mediator was a huge complex of about 20 subunits that associated with RNA polymerase II. Second, the same SRB proteins identified by Young and colleagues were found to be components of the Mediator complex. Thompson and Young subsequently made the important observation that the Mediator is generally required for transcription in yeast, and we now know that it is also conserved in mammalian cells.

The discoveries of co-activators and the Mediator complexes have led to our present appreciation of the enormous intricacy and diversity of eukaryotic transcription, with large multisubunit complexes integrating the regulated function of transcription factors with the core transcription machinery.

Alex Eccleston, Senior Editor, Nature

References and links

ORIGINAL RESEARCH PAPERS Koleske, A. J. & Young, R. A. An RNA polymerase II holoenzyme responsive to activators. *Nature* **368**, 466–469 (1994) | Kim, Y. J., Bjorklund, S., Li, Y., Sayre, M. H. & Kornberg, R. D. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* **77**, 599–608 (1994)

FURTHER READING Kelleher, R. J., Flanagan, P. M. & Kornberg, R. D. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. *Cell* **61**, 1209–1215 (1990) | Dynlacht, B. D., Hoey, T. & Tjian, R. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **66**, 563–576 (1991) | Chrivia, J. C. *et al*. Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature* **365**, 855–859 (1993) | Thompson, C. M., Koleske, A. J., Chao, D. M. & Young, R. A. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. *Cell* **73**, 1361–1375 (1993) | Thompson, C. M. & Young, R. A. General requirement for RNA polymerase II holoenzymes *in vivo*. *Proc. Natl Acad. Sci. USA* **92**, 4587–4590 (1995)