

## 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.

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## References and links

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## 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, Chrvia *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

