Arguably, sequence-specific transcription factors constitute the most important and diverse gene-regulatory mechanism. The combinatorial diversity afforded by transcription-factor binding is an effective means of coordinating the regulation of complex sets of genes. Moreover, signalling cascades regulate gene expression predominantly by modulating transcription-factor activity.

With transcription factors, the 'central dogma' had come full circle: protein regulates gene, hence message and protein.

Bernd Pulverer, Editor, Nature Cell Biology

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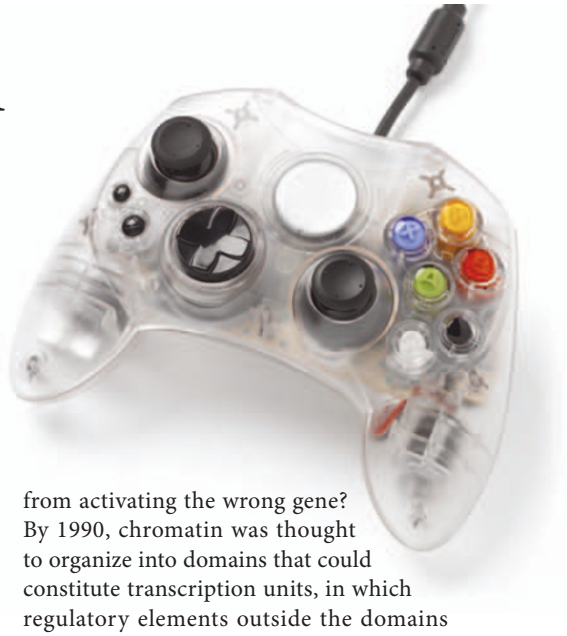
MILESTONE 13

Remote control



Although studies in prokaryotes have identified promoter (see Milestone 5) and operator DNA elements involved in transcription regulation, eukaryotes have markedly different mechanisms for this process. In 1980, it was known that an AT-rich region (the TATA box) ~30 base pairs (bp) upstream of a transcription-initiation site could function as a promoter *in vitro*. However, removal of the TATA box in the simian virus 40 (SV40) early genes did not abolish protein expression *in vivo*. Instead, transcription of these genes required ~200 bp of DNA upstream of the TATA box, including three tandem GC-rich 21-bp repeats and two 72-bp repeats. Benoist and Chambon showed that the presence of at least one of the two 72-bp repeats is necessary for expression of SV40 early genes, indicating that gene expression in eukaryotes can be influenced by remote DNA elements.

Is regulation by remote elements unique to the SV40 early genes? Studies during the early 1980s showed that other sequences upstream of the TATA box of certain genes were essential for expression. In 1981, Schaffner and colleagues provided further evidence that regulation by remote elements might be a general phenomenon. They showed that the SV40 72-bp repeats, which they called 'enhancers', could drive the expression of the heterologous rabbit haemoglobin β1 gene in HeLa cells. In addition, these enhancers could exert their effect even when placed thousands of base pairs upstream or downstream of the transcription-initiation site, independent of the orientation of the enhancer.

Using the native SV40 system, Fromm and Berg confirmed the long-range effect of the enhancers and showed that DNase I hypersensitivity, which occurs within the SV40 enhancer and is an indicator of open chromatin structure, was introduced into the sites where the enhancers were moved. These studies solidified the idea of long-range transcription regulation in eukaryotes, extended the reach of the remote regulatory elements and implicated open chromatin structures in the activity of the enhancers.

The surprising observation that remote enhancers could affect transcription on either side of a gene, irrespective of their orientation, was not readily accepted in the early 1980s. It took a decade of further analysis for this idea to take hold, which then raised another question: what prevents an enhancer from activating the wrong gene? By 1990, chromatin was thought to organize into domains that could constitute transcription units, in which regulatory elements outside the domains have no effect on the gene activity within them. To test this idea, Kellum and Schedl developed an assay using the *Drosophila melanogaster* heat-shock-gene boundary elements, scs and scs′. They showed that heterologous gene constructs enclosed within scs and scs′ are insulated from positive and negative regulatory effects of surrounding elements. Notably, scs and scs′ themselves do not have either positive or negative regulatory activity. This study established a functional definition for 'insulators', and provided a link between chromatin domains and transcription regulation.

The composite proximal and distal *cis*-regulatory elements are essential for combinatorial transcriptional regulation. This type of regulation is particularly important for complex organisms, in which diverse gene-expression patterns that define the many different cell types are driven by the mixing and matching of transcription factors. Much is now known about the transcriptional complexes that bind to some of these regulatory elements. Nevertheless, we still do not have a complete picture of how these elements communicate over long distances to affect transcription.

Hwa-ping Feng, Senior Editor,
Nature Structural & Molecular Biology

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isolated and cloned by the Sharp and Guarente groups, and the Chambon group, in 1988 — could substitute for TFIID in certain circumstances.

Defining the minimal set of transcription factors required for pol-II-mediated transcription paved the way for subsequent studies of basal and activated transcription — experiments that would not have been possible without the right tools.

Arianne Heinrichs, Chief Editor,
Nature Reviews Molecular Cell Biology

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