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ChIPping away at protein–DNA interactions

We now know that chromatin is not simply an inert packaging structure for DNA, but provides a dynamic environment with an important role in regulating processes such as gene transcription, DNA repair and replication. However, over much of the twentieth century the study of chromatin progressed more slowly than that of DNA, often because appropriate tools were not available.

The chromatin immunoprecipitation (ChIP) assay has become an indispensable tool, but the required techniques took many years to evolve. The assay allows transcription factors, chromatin proteins (such as histones) or post-translational modifications to these proteins to be mapped to specific regions of genomic DNA. A crucial aspect of the ChIP assay is to preserve physiologically relevant interactions between DNA and chromatin proteins, which was made possible by crosslinking methods.

Formaldehyde crosslinking became popular as it works well with histones and is easily reversible. In an early report in 1978, Jackson used formaldehyde crosslinking and electrophoresis to demonstrate histone–DNA and histone–histone interactions in isolated nuclei. Some years later, Varshavsky, Lis and their colleagues published influential papers in which an immunoprecipitation step was introduced with specific histone antibodies, which was performed after protein–DNA complexes had been formaldehyde or ultraviolet crosslinked and sheared. This approach allowed investigators to show, for example, that chromatin at the heat-shock protein 70 promoter of *Drosophila* was perturbed upon heat shock.

A further key advance was the development of selective antibodies, such as those to modified histones, for immunoprecipitating specific protein–DNA complexes. In the late 1980s, ChIP with these antibodies provided a functional link between histone acetylation and transcription.

Later, PCR was used to amplify the DNA purified from the antibody complexes; however, with the advent of DNA microarrays, the ChIP assay was dramatically extended to locate transcription factor binding sites on a genome-wide scale, a technique that acquired the moniker 'ChIP-chip'. In two pioneering papers, Ren, Iyer and their colleagues combined ChIP of transcription factors in yeast with a PCR step to amplify and label the immunoprecipitated DNA before hybridization to microarrays of gene promoters. A genome-wide view of the sites bound by transcription factors under various cellular conditions could now be combined with microarray gene expression analysis to determine the direct function of these factors. Since then, ChIP-chip has also allowed mapping of chromatin proteins and histone modifications across the genome, and so has proved a powerful tool to investigate the influence of chromatin on gene transcription and other DNA processes.

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