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GENE REGULATION

Resolving transcription factor binding

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The binding of transcription factors to DNA *in vivo* is a highly regulated process. Through the refinement of techniques for identifying *in vivo* binding sites or *in vitro* binding affinities, three studies have now improved our understanding of the regulation of transcription factor binding both by DNA sequence and by cofactor interactions.

To increase transcription factor binding site resolution, Rhee and Pugh modified the established chromatin immunoprecipitation followed by sequencing (ChIP–seq) technique. A limitation of ChIP–seq is that some DNA that is not bound by the protein of interest contaminates the sequencing library, resulting in a high rate of false positives. To compensate, high-stringency data filtering is used, but this can result in failure to identify some real binding sites. Rhee and Pugh introduced an exonuclease step after proteins were crosslinked to DNA; this removes DNA flanking the crosslinked site and DNA contaminants. They termed this approach ChIP–exo and used it to identify low-occupancy binding sites at a higher resolution than ChIP–seq. For example, in human cells, they were also able to identify many more binding sites for the transcriptional regulator CTCF than had previously been reported.

STOCKBYTE

Sequence specificity is not the only modulator of protein–DNA binding; cofactor proteins, some with no known DNA binding domain, are able to form complexes with transcription factors and regulate their activity. Slattery *et al.* coupled the systematic evolution of ligands by exponential enrichment (SELEX) technique — which is used to determine the specificity of proteins for a DNA sequence — with high-throughput sequencing to determine how cofactors modulate DNA binding preference. The authors used this SELEX–seq approach to investigate whether the cofactor extradenticle (EXD) modifies the DNA binding specificities of all eight homeobox (HOX) transcription factors from *Drosophila melanogaster*. Despite their different functions, these proteins all bind to highly related sequences *in vitro*. The authors found that EXD binding to the HOX proteins modulated the sequence specificity of these proteins. They identified three classes of binding-site preferences that are collinear with the domains of HOX protein expression along the anterior–posterior axis during *D. melanogaster* development; therefore, these binding preferences might go some way to explain the different functions of the HOX proteins.

In a third study, Siggers *et al.* coupled customized protein-binding microarrays (PBMs) with surface plasmon resonance (SPR) assays to determine how cofactors that do not independently bind to DNA influence the DNA binding specificity of transcription factors. They found that to bind to a particular set of target sites, the yeast transcription factor Cbf1 needs to be in a complex with the Met28 cofactor and the Met4 transcriptional activator protein. The interaction of this protein complex with its target site was enhanced by a specific sequence motif at a fixed distance from the known Cbf1 binding site. Thus, the cofactor that lacks any intrinsic DNA binding specificity itself is involved in the recruitment of the transcription factor complex to its target site.

These three studies highlight the subtlety of DNA–protein interactions, and the methodological improvements presented in these studies will further aid the dissection of these complex regulatory interactions.

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ORIGINAL RESEARCH PAPERS Rhee, A. N. & Pugh, B. F. Comprehensive genome-wide protein–DNA interactions detected at single-nucleotide resolution. *Cell* **147**, 1408–1419 (2011) | Slattery, M. *et al.* Cofactor binding evokes latent differences in DNA binding specificity between Hox proteins. *Cell* **147**, 1270–1282 (2011) | Siggers, T. Non-DNA binding cofactors enhance DNA-binding specificity of a transcriptional regulatory complex. *Mol. Syst. Biol.* 6 Dec 2011 (doi:10.1038/msb.2011.89)

FURTHER READING Stormo, G. D. & Zhao, Y. Determining the specificity of DNA–protein interactions. *Nature Rev. Genet.* **11**, 751–760 (2010) | Park, P. J. ChIP–seq: advantages and challenges of a maturing technology. *Nature Rev. Genet.* **10**, 669–680 (2009)