Nature Reviews Genetics | Published online 23 November 2015; doi:10.1038/nrg.2015.11

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GENE REGULATION

Landscape and mechanisms of transcription factor cooperativity

> the cooperative binding of TFs is dependent on the spacing and orientation of their binding motifs

Our understanding of the mechanisms by which individual transcription factors (TFs) interact with enhancers to orchestrate gene expression has greatly advanced in the past decade. However, how multiple TFs and their cofactors cooperate to activate or inhibit gene expression is less well known. Two independent studies in the same issue of *Nature* provide insight into the mechanisms of TF cooperativity during DNA binding and subsequent regulatory functions.

Stampfel *et al.* developed enhancer complementation assays to investigate the regulatory effects of different TFs and cofactors after DNA binding. The researchers constructed fusion proteins in which TFs or transcriptional cofactors were coupled to the GAL4 DNA-binding domain. They also created different 'enhancer contexts' in *Drosophila melanogaster* S2 cells by replacing individual DNA-binding motifs in enhancer sequences with the upstream activating sequence (UAS), which is bound by the GAL4 DNA-binding domain. The transcriptional effects of different TFs and cofactors recruited to these enhancer contexts were then assessed by luciferase assays. In total, the researchers tested 474 TFs and 338 cofactors, each within 24 different enhancer contexts.

Most of the fusion proteins assessed were functional, either repressing or activating gene expression in at least one enhancer context. The researchers categorized the TFs into 15 clusters on the basis of similar functional profiles and saw that the TFs within each cluster seemed to be functionally equivalent across various cell types. The team also noted a similarity between the effects of TFs and cofactors of particular functional classes, for example, between globally activating TFs and globally activating cofactors, and between context-dependent TFs and context-dependent cofactors (although, interestingly, globally repressing TFs form three distinct clusters that appear to function via different cofactors). These similarities in regulatory function enabled Stampfel *et al.* to predict TF–cofactor pairs. The fact that some of these pairs are known to physically interact supports the idea that these correspondences are true functional associations, which the authors validated with additional functional assays.

Jolma *et al.* developed a new technique, consecutive affinity-purification systematic evolution of ligands by exponential enrichment (CAP-SELEX), to systematically identify TF–TF pairs that are cooperatively bound to DNA, and to characterize the sequences recognized by such complexes. The analysis included TFs from a large variety of structural classes and with different binding specificities, which were tagged with either a streptavidin-binding protein (TF1 set) or three Flag tags (TF2 set). Among 9,400 potential interactions, the researchers were able to identify 315 active TF1–TF2 pairs that bind cooperatively to DNA. Many associations involved structurally related TFs, but interactions between TFs from different structural families were also common. Further analysis also indicated that the cooperative binding of TFs is dependent on the spacing and orientation of their binding motifs, and that when TFs bind to sites in close proximity, their specificities are often altered. Structural analysis showed that, for 95% of the interactions, the DNA molecule acts as a scaffold to TFs in such a manner that results in either no contact between the proteins or in only a small number of amino acid contacts.

Taken together, these studies highlight the role of DNA in determining how TFs bind and act cooperatively to regulate gene expression.

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The author declares no competing interests.

ORIGINAL ARTICLES Stampfel, G. *et al.* Transcriptional regulators form diverse groups with context-dependent regulatory functions. *Nature* http://dx.doi.org/10.1038/nature15545 (2015) | Jolma, A. *et al.* DNA-dependent formation of transcriptional factor pairs alters their binding specificity. *Nature* http://dx.doi.org/10.1038/nature15518 (2015)