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Combinatorial function of transcription factors and cofactors

Franziska Reiter*, Sebastian Wienerroither* and Alexander Stark

Differential gene expression gives rise to the many cell types of complex organisms. Enhancers regulate transcription by binding transcription factors (TFs), which in turn recruit cofactors to activate RNA Polymerase II at core promoters. Transcriptional regulation is typically mediated by distinct combinations of TFs, enabling a relatively small number of TFs to generate a large diversity of cell types. However, how TFs achieve combinatorial enhancer control and how enhancers, enhancer-bound TFs, and the cofactors they recruit regulate RNA Polymerase II activity is not entirely clear. Here, we review how TF synergy is mediated at the level of DNA binding and after binding, the role of cofactors and the post-translational modifications they catalyze, and discuss different models of enhancer—core-promoter communication.

Address
Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Campus-Vienna-Biocenter 1, 1030 Vienna, Austria

Corresponding author: Stark, Alexander (stark@starklab.org)
*These authors contributed equally to this work.

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Introduction

Gene regulation is central to development and cellular differentiation [1,2], and erroneous gene expression is linked to many diseases including cancer [3,4]. Gene regulatory information is encoded in the DNA sequences of genomic *cis*-regulatory elements called enhancers [5], which activate or repress transcription from their target genes' core-promoters [2]. Different transcription factors (TFs) bind to short recognition sites within enhancers – thus essentially reading the regulatory information contained in the enhancer sequence – and recruit cofactors (COFs), such as the Mediator complex or the acetyltransferase CBP/p300. Together, these regulatory proteins mediate RNA polymerase II (Pol II) recruitment and activation at core-promoters (Figure 1; reviewed in Refs. [2,6]).

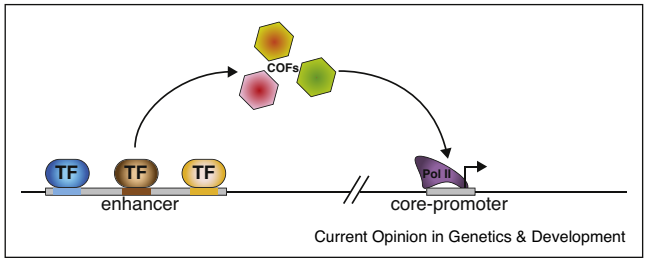
Examples from cellular reprogramming (*e.g.*, [7,8]) or animal development (*e.g.*, [9,10,11•,12]) suggest that gene regulation and cell type determination typically depend on multiple TFs that function in a combinatorial manner. In the early *Drosophila* embryo for example, the *even-skipped* (*eve*) muscle and heart enhancer (MHE) exemplifies how different developmental signals are integrated with cell type-specific gene regulation by TF cooperativity. The MHE is activated by the Wnt, Dpp/TGF-b and RTK pathways, yet its activity relies on additional input from the mesodermal TFs Twist and Tinman [12]. Similarly, in *Ciona intestinalis*, the *Orthodenticle homeobox* (*Otx*)-a enhancer integrates FGF-signaling with GATA-factor activities to achieve specific activation in the anterior neural plate and the dorsal nerve chord [11•]. This dependency of signaling pathway TFs on combinatorial input from partner TFs has been termed 'activator insufficiency' [13] and is a common property of signaling pathways that allows their employment in different cellular contexts in which they typically regulate different target genes via distinct partner TFs.

The combinatorial function of different TFs is typically reflected in the enhancers, which contain short binding site sequences for the respective TFs. Many studies demonstrated the importance of these binding sites for enhancer function, including for example the eve stripe 2 enhancer [9] or sparkling [10] in *Drosophila*, the Otx-a enhancer in *Ciona* [11•], or the human interferon-beta enhancer [14]. Typically, sites for several different TFs are required and none of the individual TFs are sufficient, highlighting the importance of combinatorial regulation [15]. However, how TFs function and how their combined regulatory cues might be integrated at enhancers to synergistically activate transcription has remained a fascinating open question that we discuss in this review.

TF cooperativity at the level of DNA binding

Multiple lines of evidence suggest that cooperativity between TFs can be established at the level of DNA binding. For example, TF binding between different species or between individuals within one species is influenced not only by mutations in the TFs' own recognition sequences (also called *TF motif matches* or *instances*) but also in those of partner TFs [16–19] (recently reviewed in Ref. [20]). Furthermore, experimentally disrupting the recognition sequences of some TFs or

Figure 1



Transcriptional regulation and its main players.
Enhancers contain short sequence motifs that can be recognized by transcription factors (TFs). TFs in turn recruit transcriptional cofactors (COFs), which recruit and activate RNA polymerase II (Pol II) at core-promoters (short sequences surrounding the transcriptional start site) to enable transcription.

depleting the corresponding TF proteins can cause loss of binding of other TFs [21–24,25•,26•].

Given that TFs recognize consensus motifs that are typically only 4–8 bp long, and therefore occur with high frequency in long DNA sequences simply by chance, combinatorial TF binding not only enables higher specificity in binding, but also the cell-type-specific redirection of TFs to different binding sites in different cell types. This becomes possible if the recognition sequences of a given TF at different sites in the genome occur with the recognition sequences of different partner TFs, thus enabling binding only in cell types in which the respective partners are present. Such differential TF binding has indeed been seen for example for Twist, which binds to sites co-bound by Zelda during early *Drosophila* embryogenesis, but to different sites co-bound by different partners at later stages [22,27]. Similarly, in the mammalian hematopoietic lineage, the TF PU.1 for example functions in either B-cell or macrophage development depending on its partner TFs [21,23].

Several mechanisms can explain combinatorial binding of TFs in the context of chromatin. Chromatin at inactive enhancers is typically closed such that the TF binding sites are occupied by nucleosomes, which can function as gatekeepers for TF binding (see *e.g.*, Ref. [23]). While individual TFs might not be able to effectively compete with nucleosomes for DNA binding [28,29], multiple TFs that recognize closely spaced binding sites within enhancers might together evict nucleosomes by 'mass action' thus enabling cooperative binding. Such a passive form of cooperativity, recently called '*collaborative binding*' [20], would not require additional TF or cofactor functions nor direct protein–protein-interactions (PPIs) between the TFs, and could rely solely on the individual TFs' DNA affinities (Figure 2a) [30,31]. This mechanism is consistent with a '*billboard*' model for enhancers [32], which highlights the apparent flexibility of recognition site-arrangements between different enhancers within one species and across orthologous enhancers (reviewed in [33]). Nucleosome competition by the collaborative binding of different TFs is further consistent with the enrichment of cell-type-specific TF binding sequences at sites of accessible DNA (*e.g.*, [34]) and could also explain how accessible sites are re-established after replication [35•] or maintained during mitosis by TFs bound to mitotic chromosomes [36•].

Cooperativity during binding can be enhanced by direct PPIs between TFs. While such interactions rely on defined interaction interfaces and compatible motif spacing, they can confer high specificity and DNA affinity (Figure 2a). Many examples of homodimeric or heterodimeric TF binding are known, including Fos that binds to DNA as a homodimer or a Jun/Fos heterodimer [37]. Interestingly, for some TF pairs, the combined binding preference is composed of the two individual TF motifs, while other pairs have binding preferences different from the ones of the individual TFs [38•] (for a review, see Inukai, Kock and Bulyk, in this issue).

PPIs that favor co-binding or stabilize TFs at active enhancers are not restricted to TF dimers, but can include interactions between TFs and COFs or higher order protein complexes (for a review, see Ref. [6]). An extreme example for cooperative binding is the interferon-beta enhanceosome, which contains binding sites for several TFs (Figure 2a). These TFs, the architectural protein HMG I(Y) and the co-activator p300/CBP create strongly synergistic activating cues that critically rely on the fixed arrangement of the TF binding sites and potentially include the formation of a defined multi-protein complex [14,39].

The discussed mechanisms are not mutually exclusive but can all occur at different enhancers (*e.g.*, billboard-type developmental enhancers and the enhanceosome) or even within a single enhancer (*e.g.*, collaborative binding of individual TFs plus cooperative binding of interacting TF pairs; Figure 2a). The comparison of enhancer sequences between closely related species however suggest that binding site-arrangements in enhancers are typically flexible and that rigid enhanceosome-like enhancer architectures are rare (reviewed in [33]).

A different model for TF binding assumes sequential rather than simultaneous binding of specialized *pioneer TFs* (Figure 2b) such as FoxA or PU.1 in mammals [23] or Zelda in *Drosophila* [24,25•,26•,40]. According to their original definition, pioneer TFs possess distinct biochemical properties that enable binding to nucleosomal DNA within closed chromatin and facilitate the subsequent binding of additional TFs (reviewed in Ref. [41]). In addition to FoxA in liver or PU.1 in the hematopoietic lineage, Sox2 for example enables subsequent Oct4