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Review

"Structure"-function relationships in eukaryotic transcription factors: The role of intrinsically disordered regions in gene regulation

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SUMMARY

Many principles of bacterial gene regulation have been foundational to understanding mechanisms of eukaryotic transcription. However, stark structural and functional differences exist between eukaryotic and bacterial transcription factors that complicate inferring properties of the eukaryotic system from that of bacteria. Here, we review those differences, focusing on the impact of intrinsically disordered regions on the thermodynamic and kinetic parameters governing eukaryotic transcription factor interactions—both with other proteins and with chromatin. The prevalence of unstructured domains in eukaryotic transcription factors as well as their known impact on function call for more sophisticated knowledge of what mechanisms they support. Using the evidence available to date, we posit that intrinsically disordered regions are necessary for the complex and integrative functions of eukaryotic transcription factors and that only by understanding their rich biochemistry can we develop a deep molecular understanding of their regulatory mechanisms.

INTRODUCTION

Across all domains of life, DNA sequences in *cis*-regulatory elements (CREs) are interpreted by DNA-binding proteins to produce functional effects on transcription. However, it is now evident that the chemical rules governing transcription factor (TF) function vary widely between bacteria and eukaryotes. A conspicuous difference is how DNA is packaged in eukaryotes versus bacteria, necessitating that TFs engage in distinct ways with the chromosome. These differences in genome organization and modes of TF engagement are reflected in wide differences in TF structure. Bacterial TFs (bTFs) are generally well-folded proteins that support structured and stoichiometric interactions with DNA and other proteins. Eukaryotic TFs (eTFs), on the other hand, engage less stably with a lower specificity for target DNA sites. Additionally, eTFs typically comprise several functional domains, which influence both their chromatin interactions and regulatory activities. The least understood eTF domains—which are nonetheless the most functionally critical in many cases—are intrinsically disordered and thus make eTFs less amenable to conventional structural and biochemical studies. Despite such profound differences between bacterial and eTFs, the field has historically imposed on both essentially the same regulatory logic, leading to much success in the case of the former and causing much confusion in that of the latter. Here, we take an initial step toward a fundamentally different path to understanding eTF mechanisms by considering the root chemical disparities resulting from their distinctive molecular compositions. Specifically, we suggest that the intrinsically disordered regions (IDRs) present in eTFs enable complex regulation by facilitating non-structured binding interactions and by integrating diverse chemical processes. Note: we use general language throughout to describe bTFs and eTFs for the sake of clarity and concision, but we remind ourselves and the readers that when we do so, we overlook many interesting exceptions.

SALIENT DIFFERENCES BETWEEN BACTERIAL AND EUKARYOTIC TRANSCRIPTION FACTORS (BOX 1)

Eukaryotic transcription factors defy expectations from studies of their bacterial counterparts

We can generically describe TFs, regardless of the species, as being composed of a DNA-binding domain (DBD) and some other domain(s) that imparts activity or regulation (e.g., activation domain, ligand-binding domain, dimerization domain, etc.). DBDs function by recognizing and binding to their cognate motifs—DNA sequences for which they have an increased binding affinity—in target CREs, thereby directing their associated activity to those sites (Stewart et al., 2012). The elucidation of DBD structures, the molecular intricacies underlying their behavior, and the sequences they bind were crucial steps in our molecular understanding of gene regulation, as were early experiments showing the necessity of specific *cis* sequences for the proper *trans* function of TFs. Discovering DBDs seemed to close the regulatory loop starting with DNA sequence (in a TF-encoding

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Box 1. Key points

(1) bTFs are folded proteins that bind stably to information-rich DNA sequences (typically >20 bp), which generally match binding motifs found in vitro and regulate gene expression by forming stoichiometric protein:DNA complexes.
(2) eTFs contain both folded DNA-binding domains and intrinsically disordered regions, bind only transiently to short and degenerate DNA motifs (5–15 bp) that often differ from those observed as binding sites *in vitro* and indirectly regulate gene expression in a non-stoichiometric and non-equilibrium regime.
(3) PTFs are folded proteins that bind stably to specific DNA sequences (typically >20 bp) of the prokaryotic genome which generally match motifs found *in vitro* and directly regulate gene expression by forming stoichiometric protein:DNA complexes.
(4) eTFs contain both folded DNA-binding domains and intrinsically disordered regions, bind remarkably transiently to shorter DNA motifs (5–15 bp) that often differ from those observed in vitro and indirectly regulate gene expression in a non-stoichiometric and non-equilibrium regime.

gene) going to protein structure (of the TF) and returning to DNA sequence (in a CRE), allowing for genetic encoding of transcriptional circuitry.

In bacteria, a simple flow of information does indeed seem to describe *cis/trans* relationships in gene regulation: CREs harbor DNA motifs that stably and specifically bind TFs for as long as they are present and binding-competent, and bound TFs act directly upon the DNA or other bound proteins to immediately affect transcription (Ptashne and Gann, 1997) (Figure 1). This logic has proven powerful in predicting promoter activity from sequence, as has been done since 1987 in a seminal paper by Berg and von Hippel (Berg and von Hippel, 1987; Chen et al., 2018), and in mathematically modeling transcriptional networks, as is being done with increasing sophistication (Kim et al., 2009). However, against our early expectations, this elegant picture does not apply even to the simplest eukaryotic systems (Ball et al., 2016; Donovan et al., 2019; Hahn and Young, 2011; Nguyen et al., 2021). eTFs typically recognize much shorter and more information-sparse motifs, providing less specificity in their DNA recognition than bTFs while navigating a much greater genome size (10^6 versus 10^9 bp). eTFs are therefore subject to many more off-target, non-functional binding events, and even on-target eTF binding events are poorly predictive of gene expression, indicating an uncoupling of binding and regulation (Wunderlich and Mirny, 2009). Although target binding is not only necessary but also sufficient for bTFs to function, it is far from sufficient for many eTFs, and the mode of binding is quite distinct, as discussed below. Hence, responses to eTF concentrations are generally more difficult to model mathematically in a thermodynamics/statistical-mechanics framework (Eck et al., 2020), and eukaryotic promoter activity is far more difficult to predict based on sequence (Irie et al., 2011). It is therefore evident that eTFs are not mere elaborations of bTFs, following the same rules tweaked for a new context; rather, they seem to have evolved to operate by fundamentally different principles owing to their distinct molecular properties, which we will now explore.

bTFs and eTFs interact with the genome in remarkably different ways

In bacterial systems, it is often the case that the direct action of a bTF binding to a particular gene is conferred either directly by the DBD or through the conjugated functional domain, allowing binding of a bTF to directly elicit a transcriptional consequence. Because of this 1:1 relationship between a particular bTF binding a promoter and a direct functional consequence, the equilibrium dissociation constant (K_D), and more specifically the rate of complex dissociation (k_d), are key parameters of bTFs, making their affinity for DNA a principal, indeed elegant, focus of regulation. bTF concentrations are often very low—between 10 and 30

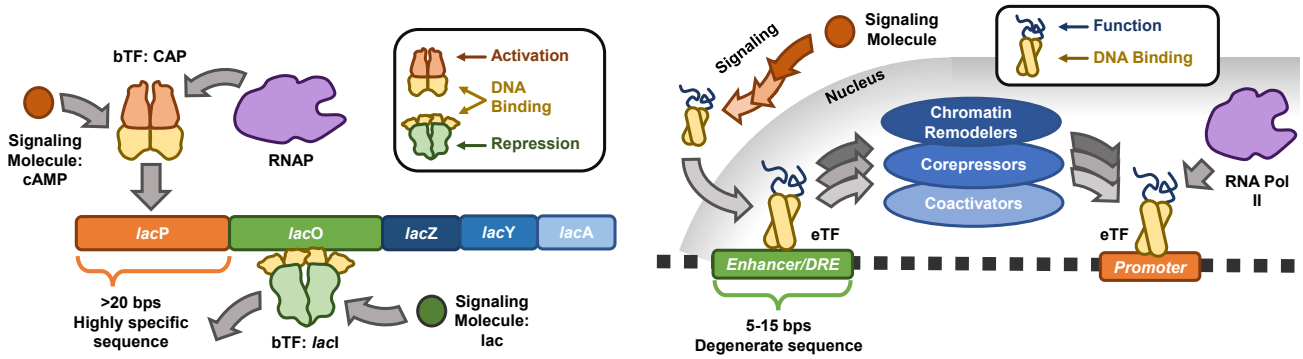

Figure 1. Overview of bacterial and eukaryotic regulation

(Left) The catabolite activator protein binds to the lac promoter (lacP) following binding of cyclic adenosine monophosphate (cAMP). Binding of lactose (lac) to the lac repressor (lacI) releases lacI from the lac operon (lacO), allowing for transcription of the lac genes (lacZ, lacY, lacA) by the recruitment of RNAP via bound catabolite activator protein (CAP). Right. Cell signaling processes in the nucleus result in the translocation of eTFs which can bind at distal regulator elements (DREs) such as enhancers or at promoters. The binding of these eTFs results in the recruitment and licensing of general transcription machinery, eventually culminating in the recruitment and licensing of RNA polymerase II (RNA Pol II) and gene activation.

molecules per cell (Taniguchi et al., 2010). Nevertheless, due to the stable, high-affinity interactions with their targets, even a small number of bTFs can sustain an enduring occupancy (residency time ~10 min) of their target sites (Hammar et al., 2014). Increases in bTF concentration should therefore be able to activate promoters with weaker binding sites, which is in fact a strategy used in conjunction with positive feedback loops to turn on targets successively (Gao et al., 2015; Kalir et al., 2001). Moreover, since functional binding requires the formation of specific molecular interactions between the bTF and DNA as well as other DNA-bound proteins, the stoichiometry of functionally relevant binding events is strictly defined.

Like their bacterial counterparts, eTFs contain ordered DBDs, although eTF DBDs are quite distinct from those in bTFs. Although bTFs stably associate with target sequences through extensive structured interactions with DNA, eTFs recognize considerably shorter and more-degenerate sequences (5–15 bp) (Stewart et al., 2012) (Figure 1). Compared with typical bTF binding to target sequences, which can last for many minutes (Marklund et al., 2022), live-cell imaging techniques such as single-particle tracking have revealed that binding interactions between eTFs and chromatin in living cells are often on the order of milliseconds to seconds (Liu and Tjian, 2018). Although single-particle tracking experiments measure global eTF behavior and may be blind to potentially functional rare events, fluorescence recovery after photobleaching (FRAP) experiments of glucocorticoid receptor at a target gene array also demonstrated that rapid dynamics can maintain a functional response (McNally et al., 2000; Stavreva et al., 2004). It is important to note, however, that although a given eTF remains bound for only a short period of time, the binding frequency or association rate can compensate such that the relative occupancy—time-averaged ratio of a site being occupied by at least one TF over total time the site is available to be bound—for eTF target sites could theoretically approach unity (Chen et al., 2014).

Such kinetics reveal remarkable divergences between the bacterial and eukaryotic systems with important ramifications for understanding regulation (discussed in Thermodynamics of IDRs and eTF-chromatin interactions). The low-turnover, bacterial system requires high on and off rates, which appear to be coupled, for bTFs binding to DNA motifs in strong promoters (Marklund et al., 2022). Conversely, the high-turnover eukaryotic system accommodates relatively low-affinity eTF:DNA interactions, which is reflected by observations that unlike bTFs, the relative affinities of eTFs for specific DNA sequences cataloged *in vitro* do not correspond well with binding preferences observed *in vivo* (Carr and Biggin, 1999; Kribelbauer et al., 2019). Although both can achieve high time-averaged occupancy, the eukaryotic system is dizzyingly dynamic and seems to rely on a much higher ratio of eTFs to accessible DNA sites. Consider that copy numbers of bTFs tend to be ~1,000-fold lower than those of eTFs (Wunderlich and Mirny, 2009), whereas eTF genomes are ~1,000-fold greater in size. Therefore, without chromatinization, the TF:DNA ratios would be approximately equal. However, given that the average cell in a human has only ~100,000 DNase hypersensitivity sites (Meuleman et al., 2020), there is clearly a huge reduction in DNA accessibility due to chromatinization, driving the TF:DNA ratio much higher in eukaryotes. Finally, unlike bTFs, many eTFs do not appear to be bound to chromatin with defined stoichiometry; rather, it appears that self-association generates transient regions of local high concentration (discussed in Controlling eTF binding through chromatin remodeling) that are important for eTF function. Together, these observations suggest that the processes through which bTFs and eTFs find their binding sites and the mechanisms by which they perform their functions may be fundamentally different.

bTFs and eTFs function in differentiated contexts

Another significant difference between bTFs and eTFs lies in their mechanisms of activation. bTFs are typically (in)activated in one of two ways with direct consequences on DNA binding: their effector domain either directly senses a small-molecule signal or is phosphorylated by a protein partner (Ali and Seshasayee, 2020). bTFs either do not depend on enzymatic regulation or participate in two- to three-step cascades with minimal integration, and the regulatory consequence is often a conformational change that alters protein-DNA or protein-protein interactions. eTFs, on the other hand, are invariably subject to enzymatic modifications as part of various and intricate signaling cascades, even when they directly sense a signaling molecule—e.g., ligand-gated nuclear receptors (Latchman, 2008). Crucially, these post-translational modifications (PTMs) do not merely toggle the DNA-binding capacity of eTFs but can alter protein-protein interactions, nuclear import/export, protein stability, and even the *trans*-activating/repressing ability of the eTFs. eTF PTMs are deposited both in the cytoplasm (primarily phosphorylation) and in the nucleus (primarily acetylation) (Filtz et al., 2014). Since single eTF molecules acquire various PTMs by different signaling-regulated enzymes in multiple subcellular compartments, complex signal integration can occur within a single polypeptide of an eTF as well as through its recruitment and activation of yet other enzymes (see A new lens on eTF regulation, Box 5).

bTFs and eTFs differ further in the way they induce transcriptional responses. Of course, they are generally operating in opposing regulatory contexts: the default state of many prokaryotic promoters is active, whereas eukaryotic promoters are often inert until activated (Struhl, 1999). bTFs directly alter the biochemical properties of a promoter—either by deforming the DNA, cooperatively stabilizing another TF or RNA polymerase (RNAP) or by sterically hindering access to underlying sequences. To what extent such mechanisms are utilized in the eukaryotic system remains unclear, but many other mechanisms have been assigned to eTFs, mostly involving the recruitment or activation of an array of protein cofactors: chromatin remodelers, chromatin writers, transcription coactivators, and components of the general transcription initiation complex. Precise molecular understanding of many of these mechanisms remains elusive but that vast subject lies beyond the scope of this review. What should be appreciated here is that there are many layers of regulation both upstream and downstream of eTFs hinging on signal integration mediated through the action of enzymes (revisited in A new lens on eTF regulation).

From a mechanistic perspective, it is highly significant that the remarkably dynamic behavior as well as the integrative functions

Box 2. Key points

(1) IDRs populate structural ensembles dictated by patterning of charged and hydrophobic residues and can occupy larger volumes than a folded polypeptide of similar length.
(2) Such patterning of amino acid sequences supports specific IDR-IDR interactions by integrating several multivalent, weak interactions distributed across the sequence.
(3) IDRs can also fold in the context of a partner; different IDRs can bind to distinct interaction surfaces of the same folded protein partner.

of eTFs coincide with the evolutionary emergence of large and conformationally dynamic IDRs exemplified by the first such low-complexity domain discovered in the human eTF Sp1 (Kadonaga et al., 1988). Although only 5% of bTFs contain an IDR, 80% or more of eTFs possess at least one IDR (Liu et al., 2006; Xhani et al., 2020). This is not to say that primordial proteins preceding those observed in bacteria were not disordered, as has been speculated based on the proposed temporal additional of amino acids and the fact that initial polypeptides were not likely folded, but that organismal complexity appears to require disordered systems (Uversky, 2013). Although there is an overall enrichment in disordered regions in eukaryotes compared with bacteria, the enrichment is dramatically outsized for TFs compared with other types of proteins (Liu et al., 2006). Critically, eTF activation domains themselves are often unstructured (Wright and Dyson, 1999; Liu et al., 2006), underscoring that IDRs are not mere structural linkers or non-functional sequence expansions but are rather the very domains that confer eTF function (Courey et al., 1989; Kadonaga et al., 1988). By dissecting the distinct properties of IDR-containing DNA-binding proteins, we hope to provide molecular intuition about the distinct principles at work, respectively, in bacterial and eukaryotic gene regulation.

BUILDING AN INTUITION FOR IDRs (BOX 2)

IDRs are more conformational labile and less spatially constrained than structured domains

An IDR is any protein stretch that does not adopt a stable secondary structure in isolation. Instead of populating a restricted set of compacted states, IDRs dynamically interconvert between many conformational states, termed a structural ensemble. Unrestrained by backbone interactions characteristic of secondary structure, IDR conformations are limited by dihedral-angle energetics and little else. Many labs have used *in vitro* methods, including single-molecule FRET, NMR, and more, to develop a more rigorous understanding of the behavior and the interactions that give rise to these structural ensembles (Camacho-Zarco et al., 2022; Schuler et al., 2016; van der Lee et al., 2014). The average behavior of these ensembles is largely determined by the sequence composition. Hydrophobic content and hydrophobic/charge-patterning within the primary sequence play significant roles in determining both local and global behaviors, as the interactions of hydrophobic and charged amino acids directly give rise to self-attraction or self-repulsion (Das and Pappu, 2013; Zheng et al., 2020). In addition to assuming collapsed structures or compact globules (Holehouse and Pappu, 2018), IDRs can occupy volumes dramatically different than ordered proteins (for example, a 100 residue ordered protein has an $R_g \approx$ 14 Å, whereas an extended disordered protein of equal length has an $R_g \approx$ 38 Å) (Hofmann et al., 2012). IDRs present in eTFs are often very long (>250 residues) compared with other eukaryotic IDRs, leading to remarkable differences in effective size between bTFs and eTFs that undoubtedly impact their diffusion properties and protein-protein interaction networks (Lobley et al., 2007).

IDRs support a diversity of protein-protein interactions

IDRs—even so-called low-complexity domains, composed of amino acid repeats or a very limited subset of amino acids—can support a broad range of protein-protein interactions. These include both homotypic interactions between two IDRs of identical sequence as well as heterotypic interactions with IDRs of different sequences, more-ordered proteins, or other biomolecules (van der Lee et al., 2014). Unlike ordered proteins which present relatively stable and confined interactions surfaces, the broad and dynamic interaction surfaces presented by disordered proteins allows for highly exchangeable, multivalent interactions that can be integrated across the protein surface to allow favorable and selective interactions. As with ordered proteins, hydrophobic packing and hydrogen bonding can stabilize interactions between disordered monomers, as has been observed for phase separation of FUS *in vitro* (Murthy et al., 2019). It is worth noting that unlike ordered proteins, maintenance of dynamics is important for function, as mutations of proline residues that maintain dynamic, hydrogen bond supported, cross-β sheet interactions between copies of TDP-43, NFL, tau, or hnRNPA2 cause disease and aggregate formation (Zhou et al., 2022). Moreover, the Pappu group among others have demonstrated that typically weak and highly geometrically constrained interactions less frequently utilized by ordered proteins, such as cation-pi and pi-pi interactions, can also support heterotypic and homotypic phase separation (Wang et al., 2018). Indeed, the role of interspersed aromatic residues in supporting IDR:IDR interactions was recognized prior to the development of a more formal understanding of the molecular mechanism. Investigation of poly-Q Huntingtin aggregates' ability to divert TF target search uncovered that Phe and Tyr residues sparsely distributed within Q-rich regions of Sp1 were necessary for Sp1 to interact with Huntingtin aggregates and that aromatic residues could be added to non-functional Q-rich regions of Sp1 to gain activation function (Li et al., 2016; Staller et al., 2022). Integration of these interactions can even support high-affinity interactions, illustrated by the picomolar affinity interaction between IDRs of linker histone H1 and the nuclear chaperone

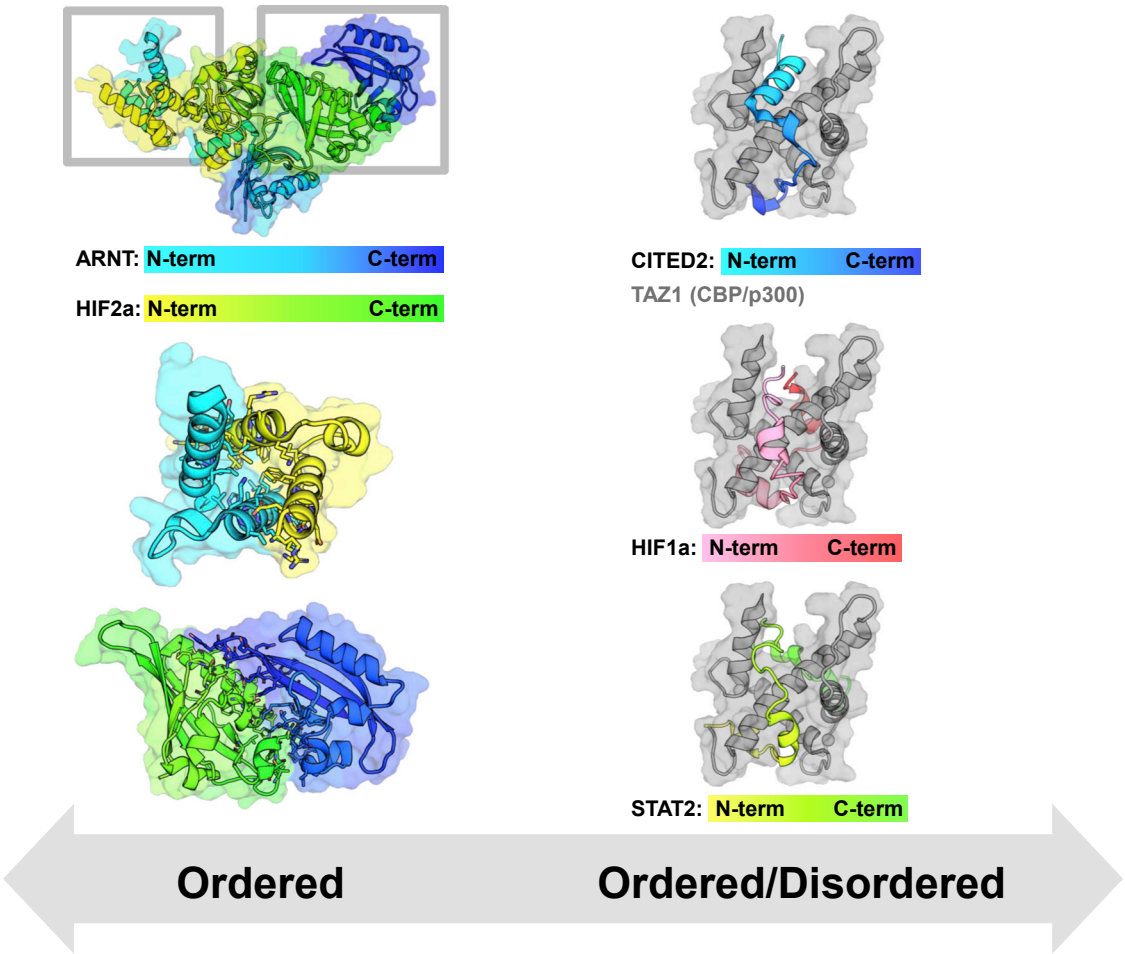

Figure 2. Differences in interactions between ordered, mixed, and disordered proteins

(Left, top) ARNT and HIF2a (PDB: 4ZP4) form multiple highly organized interfaces to support their dimerization. The N-terminal regions feature well-packed helices supporting inter-protein hydrogen bonds and salt bridges (middle) while in the C-terminal region similar interactions occur across a more limited protein-protein interface (bottom). Right. The CBP/p300 TAZ1 domain interacts with IDRs from several different transcription factors, including CITED2 (top: PDB: 1R8U), HIF1a (middle: PDB: 1L8C), and STAT2 (bottom: PDB: 2KA4), which bind with differing directionalities and to different faces of the TAZ1 surface. Images show single states from multi-state, fuzzy binding interactions measured by solution-phase NMR.

prothyomsin-α (Borgia et al., 2018). Here, histone H1 and prothymosin-α form a fuzzy complex, in which both members remain disordered but are stably associated with one another. Moreover, the electrostatic interactions that support this interaction are still highly sensitive to the surrounding environment, allowing the binding affinity of the complex to be modulated by six orders of magnitude by changing pH and salt concentration (Borgia et al., 2018). Similarly, changes in protein solvation associated with events such as phase separation can result in significant increase in the local dielectric constant which in turn strengthens polar and charge-based interactions (Brady et al., 2017). This suggests that the behavior of eTFs and their interactions may be highly subject to their local environment and behave differently on the chromatin surface versus in the nucleoplasm. Therefore, it appears that size, content, and pattern complementarity of IDRs can be added to the shape complementarity typical of ordered domains in determining interaction affinities and specificities. However, more detailed molecular rules that govern specificity have yet to be established as little work has been done to decouple interactions required for binding from those that dictate specificity. To establish clear specificity rules for disordered systems, assays need to be developed that directly identify and report on specificity *in vivo* (Chong et al., 2018) combined with *in vitro* characterization (Camacho-Zarco et al., 2022). Continued development of our understanding of such interactions is crucial as it has been shown that fuzzy binding is sufficient to confer specificity on eTF:eTF interactions, which are essential for eukaryotic gene regulation (Chong et al., 2018; Sanborn et al., 2021; Staller et al., 2022).

IDRs can also interact with folded proteins through multiple mechanisms. Fuzzy complexes can form not only between two IDRs but also between an ordered protein and an IDR. Such interactions have been observed via solution-phase NMR for a variety of eTF IDRs binding to the folded TAZ1 domain of the key transcriptional coactivators CBP/p300 (De Guzman et al., 2004). Unlike ordered interactions, where secondary and tertiary

structures position amino acids to pack together or engage in specific hydrogen bonds, fuzzy binding interactions feature an IDR stretching across a broad surface of the folded partner, mimicking the interactions observed in IDR-IDR interactions (Figure 2). Interactions between a folded domain and different IDRs can vary in the directionality of the IDR, interacting face(s) of the folded partner, specific amino acids, and secondary structure of the IDR (Berlow et al., 2017; Dames et al., 2002; De Guzman et al., 2004; Wojciak et al., 2009). This not only allows for many and divergent IDRs to interact with a single folded protein, which may be of critical importance for sequence-specific eTFs to license the general transcription machinery, but also allows IDRs to compete for ordered protein binding, allowing fuzzy complex formation or disruption to serve as a regulatory process. Indeed, it has been observed that although HIF1a, which induces the expression of hypoxia-induced genes, and CITED2, which counteracts HIF1a activity, both bind with almost equal affinity to the TAZ1 domain of CBP, CITED2 is 50-fold more effective at competing HIF1a off of the TAZ1 domain than HIF1a is at competing off CITED2 (Berlow et al., 2017). Although the competition and binding affinities appear to be in direct contradiction, the unique binding topology of the two factors enables this unidirectional, switch-like behavior (Berlow et al., 2022).

Finally, IDRs can undergo disordered-to-ordered transitions in the presence of a binding partner. Both short stretches (3–11 contiguous amino acids called short linear motifs or SLiMs) that undergo such transitions and larger protein segments (termed molecular recognition features or MoRFs) have been observed in transcription-related proteins (van der Lee et al., 2014). These SliMs and MoRFs can engage in interactions typical of structured domains, including hydrogen bonds, salt bridges, and hydrophobic packing. Such transitions can also occur between multiple copies of the same IDR, as is observed in the formation of previously mentioned cross-β sheet structures (Zhou et al., 2022). However, it is worth stating that the formation of ordered complexes may or may not be required for an IDR's activity, although the activity may rely on the same subset of interactions.

There exist several examples of activation domains that utilize fuzzy binding modes or undergo disordered-to-ordered transitions in interactions with the general transcriptional machinery. eTFs contain activation domains—IDRs that interact with other coregulators to activate a particular gene—which are enriched in sequences that have an increased propensity for forming α-helices (Alerasool et al., 2022). For example, the activation domain of the herpes simplex virus VP16 has been shown to adopt a helical conformation in complex with a subunit of TFIID (Uesugi et al., 1997). GCN4's activation domain has been shown to interact with activator binding domain of Med15 through a well characterized fuzzy binding interaction (Brzovic et al., 2011; Tuttle et al., 2018). Moreover, an artificial TF screen using yeast TF activation domains demonstrated that 73% bound to the MED15 domain of Mediator (Sanborn et al., 2021). The p53 tumor suppressor activation domain has been shown to undergo binding partner-induced helix formation when interacting with Mdmx (Popowicz et al., 2008), HMGB1 (Rowell et al., 2012), and TFIIH (Okuda and Nishimura, 2014) while showing fuzzy binding interactions with the TAZ2 domain of p300 (Krois et al., 2016). Therefore, it appears that eTFs make use of all the interaction modes available to IDRs and that a further understanding of eTF behavior requires an in-depth characterization of their manifestations.

THERMODYNAMICS OF IDRs AND eTF-CHROMATIN INTERACTIONS (BOX 3)

IDRs have different interaction energetics than structured domains

It has long been recognized that a TF's ability to bind to a specific DNA motif and a select set of interaction partners underpins its role in regulating transcription. The favorability of a binding event (Equation 1—Gibbs free energy) and the probability that such an event will occur (Equation 2—Boltzmann distribution) are dictated by the associated change in free energy:

$$\Delta G = \Delta H - T\Delta S \quad \text{(Equation 1)}$$

$$p \propto e^{-\Delta G/k_B T} \quad \text{(Equation 2)}$$

Negative values of ΔH and positive values of ΔS favor the forward reaction and thereby increase the probability (p) of the event occurring. The molecular interactions described in IDRs support a diversity of protein-protein interactions, and those seen through structural biology, contribute to decreasing the enthalpy of the system. However, experimental difficulties in tallying entropy often leads to its contribution to the free energy being ignored, despite it being the driving force of important biochemical processes. The most obvious biochemical example to highlight the role of entropy is protein folding, a process largely driven by the desolvation of hydrophobic amino acids. In a somewhat simplistic view, the compaction of the protein reduces the protein's entropy while reducing the protein's enthalpy by allowing hydrophobic residues no longer interacting with water to form favorable van der Waals interactions (Privalov, 1990). However, the loss of entropy in the protein is offset by the gain in entropy by surrounding water molecules, which are no longer required to form ordered solvation shells around hydrophobic residues. Conversely, IDRs remain unfolded partly because their relative deficiency in hydrophobic amino acids reduces the enthalpic favorability of folding the polypeptide and reduces the entropic favorability of desolvation associated with compaction. This is not at all to say that hydrophobic residues are not present or required for IDR function; rather because they are limited in number and patterned in ways that prevent hydrophobic collapse (Staller et al., 2022; Zheng et al., 2020), IDRs can expose their limited hydrophobic surface area to enable entropically driven intermolecular interactions that generally bury these hydrophobic residues (Mészáros et al., 2007).

What remains little appreciated is that enthalpy and entropy are often coupled. Bimolecular binding interactions, which restrict the number of different orientations possible for a given system, almost always reduce the entropy of a system. For folded proteins, this increase in the free energy is offset by a larger reduction in enthalpy, resulting in a favorable net ΔG.

Box 3. Key points

(1) Exchange of enthalpy for entropy makes predicting the impact of amino acid substitutions on IDR interactions challenging.
(2) The multivalency of IDRs results in higher entropic contributions to protein-protein interactions compared with stably folded proteins, enabling similar IDRs to engage in distinct interactions with a shared partner through enthalpy-entropy compensation.
(3) The broad range of interactions supported by IDRs along with their size can facilitate eTF target search by reducing the target search space.

Although the same general rules apply for disordered systems, the absence of rigid scaffolds affording the integration of many enthalpically favorable interactions over a small surface area can be offset by greater entropic contributions. For example, mutations that might remove a hydrogen bond, perturb a salt bridge, or introduce a steric clash—which can greatly perturb the interactions between ordered systems—can have a negligible impact on fuzzy binding (Hobor et al., 2022). Although such mutations do reduce the enthalpic contribution to the free energy, by the same token, they also increase the number of states that possess a similar enthalpy, thus increasing the entropy of the bound state of the mutant protein relative to the wild type and producing a minimal change in ΔG (Hobor et al., 2022). This concept known as enthalpy-entropy compensation has been shown for a variety of systems and makes structure-based prediction of the behavior of such systems highly challenging (Fox et al., 2018; Sherry et al., 2017; Theisen et al., 2021).

This considerably complicates evolutionary inferences of IDR function based on degree of conservation: because of entropy-enthalpy compensation, an IDR interaction could be conserved even while its sequence diverges. Typically, sequences of IDRs are less conserved than their ordered counterparts (Uversky, 2013; van der Lee et al., 2014). However, specific properties of IDRs such as residues that support interactions, sequence length, conformational propensities, or net charge have been shown to be conserved (González-Foutel et al., 2022; van der Lee et al., 2014). IDRs are often referred to as weak, multivalent interactors, meaning that the enthalpic contribution supported by any one residue is minimal and the interaction network is spread across a relatively large protein surface. The strength of IDR binding arises not from the stability of any single bound configuration but from the great multiplicity of ways in which it can bind. A given bound state of an IDR is typically weaker than the bound state of a structured domain (enthalpy); however, the IDR can occupy many more possible bound states (entropy). The ability of these weak enthalpic interactions to be easily exchanged, combined with the intrinsic flexibility of IDRs, makes entropy a significant determinant of IDR binding interactions. Hence, mutational studies targeted at IDRs often require a significant number of mutations to be made before a functional impact is realized (Wang et al., 2018). A mutation that weakens one bound state may nonetheless lead to greater binding overall by making available a greater diversity of binding modes. This point can be further illustrated by recognizing that some IDRs serve as entropic tethers or flexible linkers that allow attached domains to explore specific conformation ensembles (González-Foutel et al., 2022; Sherry et al., 2017). These ensembles, rooted in aspects of sequence length and charge, can have a significant impact on the interaction strengths of adjacent domains with their binding partners (González-Foutel et al., 2022; Sherry et al., 2017). Therefore, to understand the mechanisms behind IDR functions in regulating transcription unavoidably requires contending with both their enthalpic and entropic landscapes. (For the qualitative contributions of enthalpy and entropy to different eTF interactions, see Figure 4.)

IDR effects on eTF-chromatin binding kinetics

IDRs will influence both an eTF's ability to find its target sites—a process called target search, abstracted in the parameter k_{on}—and its binding interactions once there, which will determine its dissociation rate k_{off}. As seen in the modified Smoluchowski equation below, which gives the diffusion-limited rate for binding of a single TF to a particular binding site (k_s), the dimensionality of the search process and the size of the target largely determine the search efficiency and mean first passage time (Mirny et al., 2009; Wunderlich and Mirny, 2009).

$$k_s \approx 4\pi D_{3D}\left(\frac{\tau_{3D}}{\tau_{1D}+\tau_{3D}}\right)na \qquad \text{(Equation 3)}$$

Here, D_{3D} is the diffusion coefficient of the protein, τ_{3D} is the mean diffusion-limited time experienced by the protein prior to interacting with DNA, τ_{1D} is the mean diffusion-limited time experienced by the protein search for its target site in 1D while bound to DNA, n is the number of base-pairs constituting the target site, and a is the fraction of the protein surface that constitutes the binding interface. It is worth noting that the Smoluchowski equation was originally formalized for coagulation, describing the time evolution and number density of aggregating particles (Smoluchowski, 1916). Therefore, this equation can be validly applied to bTFs, which remain stably bound once reaching their binding site, but its utility in describing eTF binding is unclear, as the binding process is not unidirectional. It has been long known that bTFs reduce the dimensionality of their target search by sliding along DNA through non-specific interactions between the DNA and DBDs, reducing the dimension of their diffusion while allowing them to sample conformational space at many positions along the chromosome to find their target (Elf et al., 2007). eTFs, however, must navigate a much more complicated DNA-protein complex, and evidence is emerging that they do so via their IDRs as well as their DBDs (Brodsky et al., 2020; Chen et al., 2021; Gera et al., 2022; Hansen et al., 2020). We can imagine a process analogous to bTFs' DNA sliding mediated by the weak, multivalent interactions supported by eTF IDRs. Since chromatin is carpeted with histone tails as well as IDRs of eTFs and cofactors, IDR-IDR interactions may bring eTFs to the chromatin, reducing the dimensionality of their target search while minimizing entropic loss by the exchangeability of these

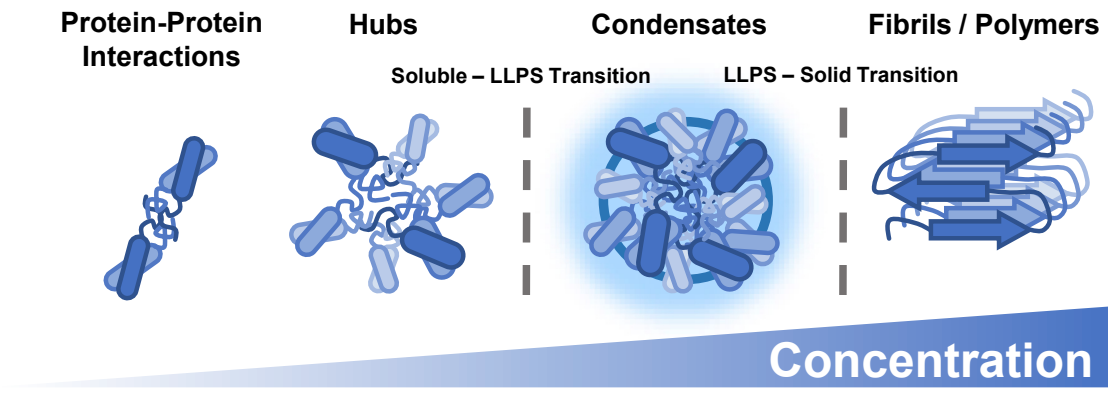

Figure 3. Structures supported by IDR-IDR interactions
As the concentration of an eTF is increased, IDR-IDRs can mediate the formation of various structures. Structures such as condensates and fibrils that occur at higher concentrations form through phase transitions including liquid-liquid phase separation (LLPS) and present a variety of emergent properties (solubility, diffusion, dielectric constant, etc.).

interactions (Hansen et al., 2006)—decreasing τ_{3D} in Equation 3. IDRs could also impact target search by modulating the target size—increasing the product *na* in Equation 3. If other eTFs or coactivators whose IDRs can interact with the eTF even weakly reside at the target site, the effective size of the target is now much larger than the DNA motif alone. By displaying a protein surface with affinity for the eTF much larger than the small DNA recognition sequence of typical eukaryotic CREs, IDRs could effectively funnel eTFs to their target sites with potentially enormous increases in k_{on} and the associated ΔG. We speculate that such a mechanism was part of what allowed eukaryotic genomes to multiply their size without compromising eTF target search. Indeed, it has been observed that even at vanishingly low concentrations of an eTF with a typically high k_{off}, small transient hubs of locally high concentration can be achieved through a protein partner with an extensive IDR (Mir et al., 2017). Finally, it is worth noting that the conformational dynamics of IDRs can directly impact the folding and dynamics of associated folded domains, such that IDRs can modulate the dimerization and DNA-binding affinity of eTFs—providing yet another avenue for modulating target search (Liu et al., 2008; Xhani et al., 2020).

IDRs AND SELF-ENRICHMENT (BOX 4)

IDRs and phase separation

Condensates, phase separation, and protein hubs have become popular topics in transcription over the past decade, with their role in transcription a subject of significant debate. At the root of each of these phenomena is the process of local enrichment, whereby a particular protein, usually an intrinsically disordered protein (IDP), is concentrated locally relative to the surrounding milieu. Many biological features that display local enrichment have been termed condensates—a word that remains loosely defined but originated from descriptions of membraneless organelles such as P-bodies, stress granules, and nucleoli that are observable by light microscopy (Feric et al., 2016; Luo et al., 2018; Molliex et al., 2015). The mechanism governing these formations has been linked to the physical processes of phase separation and reversible polymerization, as proteins enriched within these condensates have been shown to undergo liquid-liquid phase separation and fibril formation *in vitro* (Dignon et al., 2020; Kato and McKnight, 2018). These *in vitro* studies have provided useful insights, revealing the previously mentioned (see IDRs support a diversity of protein-protein interactions) importance of local hydrophobic collapse, pi-pi stacking, and cation-pi interactions. However, in many experiments where eTF condensates have been reported, there was either gross overexpression of the eTF or the addition of some fixative, both of which are highly perturbative and unphysiological (Erdel et al., 2020; McSwiggen et al., 2019a; Teves et al., 2016). Indeed, local enrichment processes are highly concentration dependent (Figure 3), requiring non-physiological micromolar eTF concentrations to form condensates *in vitro*. Importantly, in several cases where eTF phase-separated condensates have been induced in living cells, the result was loss or misregulation of transcription (Chong et al., 2022). The high concentrations required to form condensates and fibrils, and the association of these processes with phase separation, have led us to propose the term "hubs" to describe these much smaller and transient clusters which are not phase separated (McSwiggen et al., 2019b). Interestingly, recent observations show that proteins that undergo phase separation produce heterogeneous distributions of clusters, similar to hubs, at concentrations below the phase separation saturation concentration (Kar et al., 2022). A framework for understanding such behavior has recently been articulated which helpfully distinguishes between percolation and liquid-liquid phase separation (Mittag and Pappu, 2022). Crucially, in a system of multivalent interactions that can reversibly form networks, beneath the percolation threshold, there will be clusters of constituents exhibiting a large distribution of sizes. Such sub-percolation clusters are uncannily similar to what have historically been called hubs. Because these clusters or hubs can form at low (i.e., physiological) concentrations and do not depend on discontinuous phase transitions, they seem to better correspond with observed eTF self-association behavior (Mir et al., 2017). We anticipate that bona fide liquid-liquid phase separation (LLPS) and condensates may also play a role under

Box 4. Key points

(1) IDRs facilitate phase separation *in vitro*, although the evidence for eTF phase separation and function *in vivo* remains elusive.
(2) The same weak, multivalent interactions that support phase separation also serve as the basis for small, dynamic IDR interaction hubs distinct from condensates observed in cells.
(3) Local enrichment can be facilitated through a mechanism distinct from liquid-liquid phase separation by favorable interactions between eTF IDRs.
(4) IDRs allow eTF DBDs to rapidly cycle between specific and non-specific interactions with DNA to form an ensemble eTF:DNA interaction in which the enthalpic gain is distributed between eTFs, whereas entropy is increased by having multiple eTF:DNA configurations. Such interactions would be maximal at clustered eTF binding motifs.

physiologically relevant concentrations for some processes such as those involving RNAPs and other well characterized cellular puncta (P-bodies, Cajal bodies, stress granules etc.), but their role in mediating transcription activation seems remote except perhaps in the context of repression under certain conditions (Chong et al., 2022). Overall, we believe that further investigation of eTFs in live-cell imaging experiments at physiological concentrations is likely required to determine the relevance of these phenomena (McSwiggen et al., 2019b; Mir et al., 2017).

Molecular mechanisms of IDR self-enrichment distinct from phase separation

IDR-driven local enrichment has deep implications for eTF-chromatin interactions that should not be subsumed or overshadowed by phase separation processes. The multiplicity of interactions experienced by eTFs unveils a distinct mechanism by which IDRs can support local enrichment, without requiring that the system phase separate or form polymers and fibrils (Figure 4). eTF domains that support dimerization and DNA binding are often ordered, allowing enthalpically favorable interactions across a limited interface to offset the loss of entropy associated with complex formation (Figure 4, classical dimer formation/classical DNA binding). Conversely, IDR interactions may or may not be sufficient to support the formation of such complexes in solution (Figure 4, transient multimer formation) as binding requires the enthalpically weak interactions to offset the loss of entropy incurred by dimerization. However, at high concentrations, enthalpic or entropic contributions from IDRs can be sufficient to trigger phase transitions, leading to the formation of phase-separated species (Figure 4, phase separation) (Martin and Mittag, 2018). Although IDRs might only interact transiently in solution, chromatin provides a scaffold for cooperativity in which entropic costs can be offset. Most often, we think about TF DBDs binding to DNA at specific motifs through enthalpically favorable, specific interactions that offset the reduction in entropy associated with binding of a single site (Figure 4, classic DNA binding). Recall that DBDs can also bind to chromatin through relatively enthalpically weak, non-specific interactions with DNA that allow for sliding (Figure 4, DBD sliding) (see IDR effects on eTF-chromatin binding kinetics), which increases the entropy of the interactions relative to the DBD remaining bound to a single site (Figure 4, classic DNA binding). A similar process could occur for IDRs, replacing the non-specific DBD:DNA interactions with relatively weak and highly exchangeable IDR:IDR interactions, which could allow non-DNA-bound factors to slide on a carpet of bound IDR-containing factors or histone tails (Figure 4, IDR sliding).

Crucially, eTF IDR:IDR interactions allow a mode of cooperative binding, once a eTF is anchored to a CRE by its DBD, distinct from classical cooperativity. Compared with a single factor which does not interact with adjacent factors on the DNA (Figure 4, classical binding), the entropy of an eTF which maintains IDR interactions while bound to DNA is increased (Figure 4, self-enriched binding). Moreover, compared with eTFs interacting through their IDRs in solution (Figure 4, transient multimer formation), eTFs interacting through their IDRs when bound to DNA possess a significantly more favorable enthalpy through the DBD:DNA interactions to combat the entropy reduction associated with complex formation (Figure 4, self-enriched binding). These interacting factors can also rapidly exchange with one another, through processes like facilitated displacement—the binding of a factor to a site occupied by another factor resulting in eviction of the initially bound protein from the DNA (Graham et al., 2011; Kamar et al., 2017), further increasing the entropy of the system. Additional copies can take part in this dynamic complex formation resulting in a self-enriching binding process, where a single eTF grounded at a specific DNA site (or a limited number of eTFs at clustered sites) can participate in a rapidly exchangeable and dynamic mode of DNA binding in which multiple copies of an eTF interacting through their IDRs can cycle in and out. Importantly, this enrichment does not require as many molecules as phase separation nor does it dramatically reduce the ability of factors to exchange with the surrounding environment. Rather, if many copies are present, microscopic, facilitated displacement would enable rapid macroscopic exchange. This mechanism of self-enriched binding may represent what we have termed "small transient hubs" and could appear similar to prewetting transitions observed for Klf4 binding to DNA *in vitro* (Morin et al., 2022), both of which are distinct from phase-separated condensates.

Although the discussion so far has considered homotypic interactions between like eTFs, such interactions could also occur heterotopically and facilitate the enrichment of multiple distinct eTFs that specifically engage adjacent DNA sites (Staller, 2022). Indeed, this could partly rationalize why eukaryotic CREs often have multiple copies of eTF binding sites or multiple different eTF binding sites. In the traditional model of cooperativity, in which eTFs interact with each other in structured and stoichiometric complexes, building *cis*-regulatory elements that integrate multiple inputs from different eTFs would be evolutionarily difficult. If, on the other hand, eTF IDRs allow

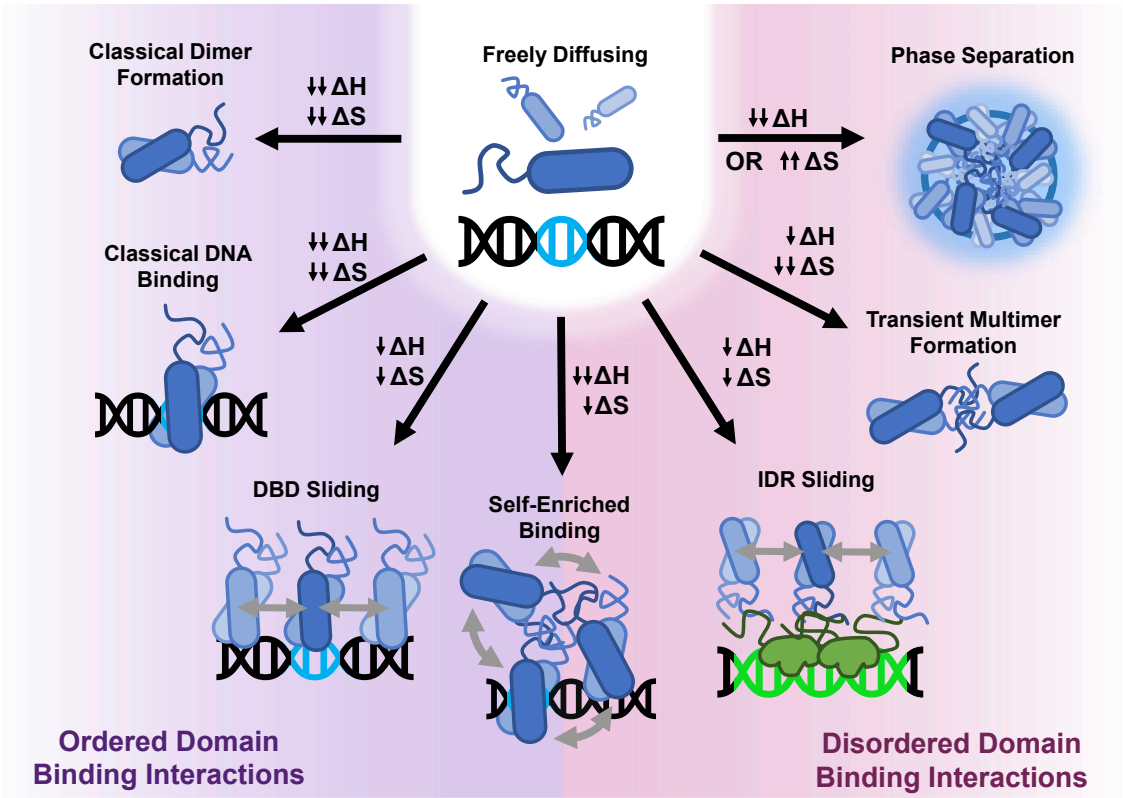

Figure 4. Thermodynamics of eTF interactions
Illustrations of the diverse interactions supported by the DBD and IDRs of eTFs along with the expected changes in enthalpy and entropy associated with each interaction.

local enrichment around the DNA element via interactions unconstrained by rigid structure or defined stoichiometry, many more combinations of DNA sequence with functional outcomes become available. Even the requirement that an eTF's motif be present in the CRE would be relaxed in that its mere ability to associate with DNA non-specifically might contribute enough enthalpy for it to engage in a hub of eTFs with compatible IDRs.

This mechanism of self-enrichment also affects the kinetics of eTF-chromatin interactions (discussed in IDR effects on eTF-chromatin binding kinetics) because it hones the target search process by making the binding of each successive eTF increase the effective size of the target. The site specificity of such self-enriched binding still depends on differences in the collective ΔG of the factors bound to the set of sequences at one locus versus all other sets of DNA sequences at other loci. Importantly, at some threshold, the ΔG associated with adding another eTF copy to enrich a locus will not be as favorable as having the same eTF bind at some other locus, as the addition of many factors will restrict the likelihood that any given eTF can maintain both favorable IDR and DNA interactions. This free energy competition likely prevents self-enriched binding from serving as a nucleation event for uncontrolled aggregation or phase separation at physiological concentrations of eTFs. It is worth reemphasizing that the intrinsic difficulty associated with tracking entropy and the multiplicity of potential interactions supported by eTF, as shown in Figure 4, makes it quite challenging to directly assign mechanisms to the currently observable behaviors of eTFs. Indeed, it may be the case that particular eTFs exhibit particular interactions and not others, or utilize multiple interactions to varying degrees.

A NEW LENS ON eTF REGULATION (BOX 5)

How are eTF-chromatin interactions regulated and how do they regulate?

Although mechanisms mediating eTF target search and CRE engagement are widely divergent from their bTF counterparts, how the eTF system is dynamically regulated remains largely unaddressed by the foregoing sections. The observed rapid dynamics of eTF-chromatin interactions make it likely that passive binding via mass action quickly reaches equilibrium in the nucleus. Indeed, data from single-particle tracking of fluorescently labeled eTFs in live cells suggest that energy expenditure is taking place, keeping the system out of equilibrium, whereas transcription burst assays using synthetic TF systems suggest that parameters like residence time, not fractional occupancy, correlate with function (Biddle et al., 2019; Popp et al., 2021). If binding of a particular factor is indeed at equilibrium, then regulation, turning on and off particular genes by that factor,

Box 5. Key points

(1) Since eTF dynamics likely allow them to rapidly reach binding equilibrium in the nucleus, regulation must occur either by modulating binding equilibria, through processes like chromatin remodeling, or through non-equilibrium, enzymatic processes.
(2) Post-translational modification of eTF IDRs could mediate the necessary integration of information to support transcription regulation.

must therefore occur either by shifting equilibria or through some non-equilibrium process. An example of the former mechanism is the influx of eTFs to the nucleus via ligand-induced dimerization. However, as was mentioned, bTFs and eTFs interact with the genome in remarkably different ways, active—i.e., non-equilibrium—enzymatic processes are at play in regulating eTFs even when they are also regulated by passive mechanisms. Hence, understanding eTF regulation requires answering the following questions: What active processes govern the binding of eTFs to target sites? And what active processes translate the binding of eTFs to their target sites into transcriptional output?

Controlling eTF binding through chromatin remodeling

The chromatinization of eukaryotic genomes presents a physical barrier to every biochemical transaction with the DNA, including eTF binding. Without histone mediated passivation of naked DNA, eTFs would likely bind to so many off-target sites that target search would become impossible (McSwiggen et al., 2019a). It was also shown that for *Drosophila* TFs, DNA accessibility tightly and quantitatively correlates with TF binding, whereas TF sequence specificity is a subordinate determinant—almost a tuning parameter for where TFs bind (Li et al., 2011). Because nucleosome positioning can be modulated by chromatin remodelers, there exists a layer of enzymatic regulation upstream of TF binding. (A discussion of "pioneering" factors and how chromatin remodelers are directed to specific loci is beyond the scope of this review.)

PTMs in signaling and transcription

Although chromatin opening is an important regulatory step, it is not sufficient to induce transcription (Wang et al., 2022), and it is far from the only enzymatically regulated step in the process. For example, release of Pol II from promoter-proximal pausing requires phosphorylation of its C-terminal domain (an extended IDR) and of the Pol II-associated factors DSIF and NELF by the kinase P-TEFb, which is itself highly regulated through PTMs (Cho et al., 2010). P-TEFb may be recruited to promoters by binding BRD4, which recognizes acetylated lysines via its two bromodomains (Yang et al., 2005). Finally, the lysine acetyltransferase p300 is often activated by phosphorylated eTFs, such as CREB and STAT proteins, whose kinases are members of larger enzymatic cascades beginning in the cytoplasm or at the plasma membrane (Ortega et al., 2018). This one example pathway makes clear that there is a multiplicity of regulated steps and suggests that transcription regulation is largely an extension of cellular signaling, which operates through enzymatic relays (Karr et al., 2022). It is therefore no coincidence that the two categories of eukaryotic proteins most enriched for IDRs are TFs and cellular signaling proteins (Bondos et al., 2021; Liu et al., 2006). The solvent accessibility, conformational flexibility, and interaction surface size of IDRs make them prime candidates for both receiving and relaying chemical signals. Their compositional plasticity and length variability also allow them to evolve multiple PTM sites, and the breadth of PTMs deposited on IDPs is impressive (van der Lee et al., 2014)—possibly providing a platform for combinatorial signals. These marks not only can elicit intra-domain changes in conformation but can also allosterically modulate adjacent functional domains. PTMs also create new binding interactions, particularly with protein partners bearing corresponding reader domains.

The intrinsic multivalency of IDRs uniquely positions them for the task of signal integration, allowing for the deposition of several distinct PTMs and the binding of several distinct partners at once. Moreover, because virtually all the eTF properties conferred by IDRs explored in this review are regulable by PTM deposition, disordered domains are likely the regulatory nexus not just for particular eTFs but for the entire system of eukaryotic gene regulation. Finally, this regulatory mechanism complements the observed binding kinetics of eTFs—although eTFs' rapid binding kinetics prevent the formation of stable complexes which integrate signals only through structured binding interactions, the short residence times of eTFs could allow them to be turned over rapidly as substrates of transcriptional coactivators and thereby propagate PTM-eTF signals to adjacent CREs (Karr et al., 2022).

Conclusion

Most textbook discussions of eTF target search, target binding, and mechanisms of transcription regulation have adopted principles gleaned from bTFs. However, closer inspection of eTF properties strongly suggests that because the differences between the two systems are so profound and numerous, we have likely been misapplying many bTF paradigms. In general, bTFs engage in stable, structured, stoichiometric interactions with a DNA substrate, whereas eTFs bind a chromatin substrate only transiently and are largely aided through disordered interactions which are likely not stoichiometric. bTFs recognize longer, content-rich, and highly specific CRE sequences which they find through sliding along DNA, whereas eTFs bind short, highly degenerate sequences likely influenced by IDR-mediated interactions with other eTFs. bTFs modulate transcription in response to direct or closely linked signals by binding promoters and promoting or disfavoring binding of RNAP. eTFs modulate transcription in response to highly integrated and multifaceted signaling pathways eventually leading to specific binding sites being opened by chromatin remodelers (whose own activity is also modulated by PTMs), which is read out through a downstream chain of enzyme activities that eventually ends in Pol II initiation and elongation. All these properties

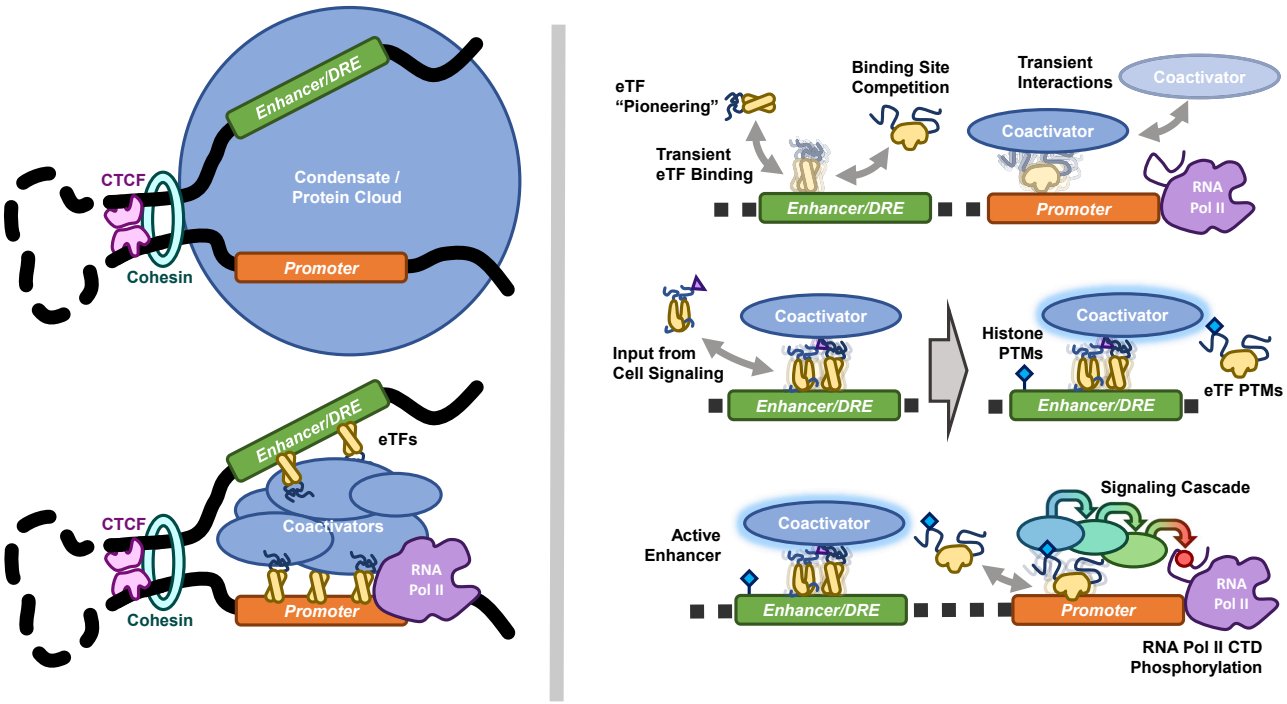

Figure 5. Comparing classical illustrations of eukaryotic regulation with an updated model

(Left) The formation of stable enhanceasome structures between two CREs brought into proximity by CTCF/cohesin mediated DNA looping, potentially allowing the formation of condensates (top) or the stable binding of several transcription factors and coactivators (bottom) to facilitate recruitment and licensing of Pol II. Right. Top. eTFs interact transiently and compete for accessible regions of DNA or facilitate the opening of specific regions, while also mediating transient interactions with coactivator and the general transcription machinery. (Middle) Arrival of a PTM-eTF following cell signaling allows for coactivator activation and the deposition of new PTMs on eTFs and histones. (Bottom) This now active enhancer can communicate with distal promoters through the point source originated diffusion of PTM eTFs and through subsequent signaling cascades facilitated by other coactivators, terminating in RNA Poll II C-terminal domain (CTD) phosphorylation and transcription.

of eTFs are biochemically supported by their long IDRs, which are almost absent in bTFs.

Here, we propose a model in which out-of-equilibrium processes, like nucleosome remodeling, change the available binding sites on chromatin, whereas subsequent passive processes, including the rapid binding/unbinding of eTFs or recruitment of coactivators, are approximately at equilibrium. Since the order of events is only well defined for out-of-equilibrium processes which do not obey detailed balance, the equilibrium occupation of an eTF at a particular site is largely determined by ΔG and may not be highly influenced by the target search time. Additional active processes, including cytosolic signaling leading to eTF nuclear translocation or PTM deposition on eTFs by coactivators, act alongside binding at accessible loci to influence subsequent protein interactions and gene activation (Figure 5). Compared with traditional models, which utilize the same component factors to form stable complexes and, in many ways, mimic prokaryotic systems, this updated model attempts to re-envision transcriptional regulation through the lens of dynamic interactions and non-equilibrium regulatory steps. We hope that an appreciation of the chemical, structural, and functional divide between bTFs and eTFs will lead to new hypotheses and models about eTF mechanisms that do not depend on stable structures regulated by binding but on highly dynamic processes regulated by ordered enzymatic steps.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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