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# Transcription factor binding and activity on chromatin

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**Abstract**

The binding of transcription factors (TFs) via their DNA binding domain at gene promoters or enhancers is part of a multi-step process that leads to transcription activation in eukaryotes. The kinetic on- and off-rates of different TF states are governed by a complex interplay of factors that involve chromatin organization on the level of individual nucleosome positions up to actively transcribed chromatin subcompartments on the mesoscale. Furthermore, not only the TF DNA binding domain but also the activation domain affect TF assembly on chromatin. Here, we summarize recent findings on the interplay between TF binding, chromatin organization, and gene activation to highlight features that need to be considered for constructing quantitative models of eukaryotic gene regulation.

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**Keywords**

Transcription activation, Transcription factor binding, Kinetic proofreading, Chromatin, Nuclear organization.

**Abbreviations**

TF, transcription factor; RNA Pol II, RNA polymerase II; SPT, single particle tracking; CTD, C-terminal domain of RNA polymerase II.

## Transcription activation in eukaryotes versus prokaryotes

The organization of the eukaryotic genome into chromatin has profound implications for transcription activation in comparison to prokaryotes as recognized already more than two decades ago (e.g. Refs. [1,2]). Prokaryotic promoters are in a transcription-competent ground state and RNA polymerase can reach full transcription levels if only the promoter sequence is optimal. Further regulation mostly occurs via the binding of transcription factors (TFs) at or near the promoter, which can block or enhance the binding of RNA polymerase. It is noted that also more complex activation mechanisms exist for some promoters that involve bacterial enhancers and ATP hydrolysis as for example shown recently for the modulation of transcription bursting in *Escherichia coli* [3]. Promoter sequence contribution to activation have been further defined in *E. coli* [4] and the relation between regulatory sequences, TF binding, and gene expression has been systematically mapped [5••]. Thus, increasingly more powerful thermodynamic and kinetic models for bacterial transcription are established. They predict gene expression for a large number of genes [6] with the *lac* promoter as a paradigm for the theoretical description of transcription regulation by equilibrium binding models [7].

In eukaryotes, the binding of general and gene-specific TFs/co-activators at cis-regulatory elements (CREs) that frequently have both promoter and enhancer functions is a key step of gene regulation [8,9]. The coupling of interactions between promoter and enhancers at different genomic loci with subsequent activation steps leads to highly complex non-linear relationships [10••]. Furthermore, multicellular organisms implement cell type-specific gene expression programs and establish patterns of active and silenced regions by organizing the genome into chromatin. This has led to the view that eukaryotic promoters are frequently in a repressed default promoter state [1,2]. Multiple energy-consuming steps are involved to create transcriptionally competent states during transcription activation, which makes it challenging to derive gene regulation functions that quantitatively describe this process [11].

## Transcription factor binding and gene activation in eukaryotes

The additional layers of gene regulation described above are particularly relevant for describing the relation between transcription factor binding and the initiation of gene expression. Simply applying TF equilibrium binding models to rationalize eukaryotic gene regulation from the occupancy of a TF at its target sites is in most cases insufficient to derive meaningful gene regulatory functions. Here we review recent findings on the chromatin-mediated binding of TFs for

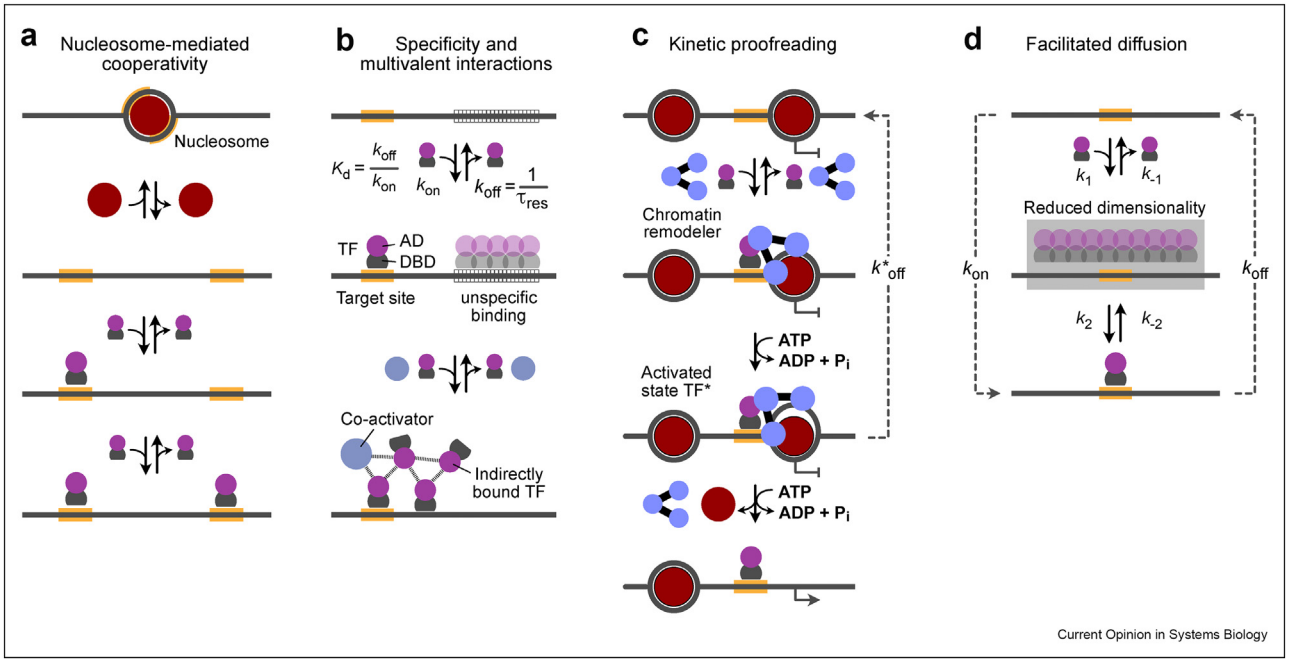
activating transcription by eukaryotic RNA polymerase II (RNA Pol II). A specific focus is on the following aspects: (i) In a multi-step reaction, TFs compete with the histone octamer to gain access to their DNA binding site. This process can lead to the co-binding of TFs to CREs with multiple binding sites (Figure 1a). (ii) TF binding and subsequent gene activation have been considered as two separate consecutive processes attributed to the TF's DNA binding domain (DBD) and the activation domain (AD), respectively. However, it is emerging from recent studies that these two activities need to be considered together, as multivalent interactions by intrinsically disordered regions (IDRs) present in the AD of many TFs [13•] affect DNA binding in a complex manner (Figure 1b). (iii) Several studies conclude that TF binding involves non-equilibrium energy-consuming steps that could increase specificity by a kinetic proofreading mechanism (Figure 1c). (iv) TFs could assemble into a state in which a dimensionality reduction of the search process speeds up target site search (Figure 1d).

## Multi-step promoter activation and energy-dependent steps

The packaging of DNA into a chain of nucleosomes together with other chromosomal proteins and RNAs links transcription initiation to distinct epigenetic signals and chromatin states that regulate TF binding and transcription activation. This multi-step reaction frequently involves energy-consuming steps like the following: (i) Generating active states via the acetylation of histones or the TF itself [14] to regulate CRE activity [15,16]. (ii) TF nucleated formation of silenced chromatin states as for example heterochromatin nanodomains marked by the di- or trimethylation of histone H3 lysine 9, which can repress transcription [17]. (iii) The translocation or eviction of nucleosomes by megadalton-sized chromatin remodeler that can free occluded TF binding sites [18,19].

A certain class of so-called pioneer TFs can bind to nucleosomal DNA [20–22] as characterized in detail for OCT4 and SOX2/11 [23,24]. This suggests a sequential

**Figure 1**




Multi-step TF binding with energy-consuming steps. (**a**) Nucleosome mediated cooperativity. TF binding sites can be inaccessible due to the competitive binding of a histone octamer at this DNA region. TF binding to one site can increase the probability that a second binding site is accessible for TF binding. This mechanism can lead to cooperativity in the absence of direct protein–protein interactions between the bound TFs [12]. (**b**) Specificity and multivalent interactions. The TFs bind with kinetic on- and off-rates ($k_{on}$, $k_{off}$) that define the equilibrium dissociation $K_d$ to their specific target sites as well as unspecifically to other sites in the genome. Additional multivalent interactions of the AD can also affect TF complex formation. (**c**) Multi-step promoter activation with chromatin remodeling as a kinetic proofreading step. An exemplary reaction is depicted where the TF binds a chromatin remodeling complex, which induces an activated state TF* at the nucleosome upon ATP hydrolysis. From this state, the complex can either revert back to the initial state with $k^*_{off}$ or evict/translocate a nucleosome in additional energy-dependent steps and proceed with the transcription activation reaction. (**d**) Facilitated diffusion. After initial binding, TFs form an intermediate state in which the target search process proceeds at reduced dimensionality or in a reduced volume, which speeds up sampling for the presence of the target site.

two-step mechanism in which the binding of a pioneer TF would first remove a nucleosome to facilitate binding by a non-pioneer factor. This view has been recently challenged in a comparison of FOXA1 and HNF4A for which a two-step binding mechanism was not observed [25•]. Single molecule footprinting studies argue in favor of nucleosome-mediated TF binding cooperativity [12,26••,27•,28•]. This type of cooperativity arises as the equilibrium binding of one TF can block histone octamer-DNA interactions so that the binding site of another TF becomes more easily accessible [12] (Figure 1a). As a result, TF binding sites at CREs become preferentially co-occupied although their distance precludes cooperativity via direct interactions between TFs. Furthermore, the relation between TF co-binding and nucleosome occupancy points to an additional non-equilibrium process like chromatin remodeling being involved in the competitive TF-histone octamer binding [26••]. Another study that compared different models for transcription activation of the *hunchback* gene by the Bicoid and Zelda TFs arrives at a similar conclusion [29••]. The two TFs actively induce chromatin accessibility via a series of slow and irreversible steps that could reflect the coupling of Bicoid and Zelda binding to histone acetylation or nucleosome remodeling activity. Thus, an emerging common theme is that TF assembly at CREs in eukaryotes occurs as a non-equilibrium multi-step reaction with energy-consuming steps before the start of RNA Pol II transcription. Furthermore, co-binding of multiple TFs to a CRE frequently appears to arise from independent binding events, i. e. without direct protein−protein interactions between chromatin-bound TFs. Nevertheless, the competitive binding with the histone octamer can lead to cooperative TF binding at CREs.

## Kinetic proofreading and transcription factor residence time

Eukaryotic TF bindings site motifs are typically only 6−12 bp in length. This raises the question how TFs recognize their target sites within Gb large eukaryotic genomes where every residue represents the start of a potential unspecific binding site [30,31]. For energy-consuming multi-step activation mechanisms as described above, a kinetic proofreading mechanism could be one of several mechanisms that largely increase the specificity of the reaction [11,32,33]. In such a mechanism a critical parameter is the TF residence time $\tau_{res}$ in the bound state that is given by the reciprocal value of the dissociation rate constant $k_{off}$ (Figure 1b). The value of $\tau_{res}$ will determine whether a subsequently induced slow energy-consuming reaction to an activated state TF$^*$ will take place efficiently. The reaction can then proceed or fall back to the initial state (Figure 1b). Furthermore, lower affinity off-target sites will become occupied upon increasing the TF concentration, but their activation capacity will remain limited if $\tau_{res}$ is too short to generate the TF$^*$ state efficiently. In a theoretical study, it was shown how this type of kinetic proofreading of activator-DNA recognition via a nucleosome remodeling step increases the specificity of gene activation [34•]. As depicted in Figure 1c, this could involve an energy-consuming transition to an activated state in which the TF interacts with a chromatin remodeling complex that is able to translocate a nucleosome upon ATP hydrolysis [33].

The residence time can be measured by a variety of methods that include single particle tracking (SPT) [35−38] and experiments that suppress rebinding of the dissociated TF [39−41]. The latter approaches rely on competitors or deplete the TF from the nucleus and detect binding by ChIP-seq or expression of a reporter. The resulting $\tau_{res}$ values are typically on the second time scale but large variations have been reported that on the one hand reflect functional differences between TFs [42,43]. For example, chromatin architectural functions are associated with higher $\tau_{res}$ values [42]. On the other hand, technical differences between the methods need to be considered. While SPT provides a direct readout of $\tau_{res}$ at high temporal resolution, it is typically limited to observation periods of up to 20−30 s due to loss of the fluorescence signal over time [42,44•]. Accordingly, SPT cannot detect residence times of minutes or hours. The competitor/depletion methods allow it to observe longer residence times but lack the temporal resolution to resolve processes on the second time scale. Furthermore, TFs like ERα, FOXA1, and CTCF display a power-law distribution of $\tau_{res}$ [44•]. More long-lived binding states reaching the minute time scale exist in the tail of the right-skewed residence time distribution. These binding events might represent a molecular species that is crucial for successful activation events. In line with this view, several studies report a correlation between $\tau_{res}$ and TF activation or repression strength [35−38]. One potential confounding factor is the correlation of longer TF residence times with a higher binding site occupancy. Binding site occupancy is determined by the product of TF concentration and equilibrium binding constant, which is linked to residence time. Experimentally dissecting the individual contributions of binding site occupancy and $\tau_{res}$ in terms of transcription activation strength is difficult. In a carefully designed set of experiments, it was demonstrated that $\tau_{res}$ regulates transcription independently of increased binding site occupancy [45••]. A comparison of synthetic transcription activators showed that changing $\tau_{res}$ had a stronger effect than changing TF concentration. Moreover, both parameters only affected the transcriptional burst frequency and not the burst duration or amplitude. Similar conclusions were made for dCas9-based synthetic activators, whose residence time could be tuned by introducing a single nucleotide mutation into the guide RNA [46••]. It was shown that TF constructs with shorter residence time displayed weaker activation of a reporter array when the

same binding site occupancies were compared. In summary, a number of recent studies show that TF residence time can determine activation strength. However, for most TFs it is not clear how long the residence time needs to be for efficient activation. Accordingly, it will be important to better resolve TF residence times in the 10 sec to minutes time range in relation to productive transcription activation events.

## Accelerating TF target search

In addition to $\tau_{res}$ (or its reciprocal value $k_{off}$) the second fundamental parameter for TF binding is the kinetic on-rate $k_{on}$, which is dependent on the TF target search process. Its value can be increased by confining the space that is searched [30,31,47,48•]. One mechanism, referred to as facilitated diffusion, has been derived from studies of bacterial lac repressor [47]. It is based on reducing the dimensionality of the search process from randomly sampling the complete nuclear space in 3D to lower dimensions, which can largely reduce the target search time. This is accomplished by unspecific binding and sliding along the DNA in 1D to sample for the presence of a specific binding site (Figure 1d). Whether a related diffusion mechanism accelerates the target search of eukaryotic TFs on chromatin has been a long-standing question. Interestingly, IDRs present in the AD of TFs could play a crucial role in this context. According to a "stickers-and-spacers" model, IDRs can be described as flexibly associating polymers that carry interaction-prone motifs separated by more inert segments [49]. Thus, IDRs can establish multiple simultaneous interactions via weak binding patches that can interact in variable configurations to accelerate TF target interaction via different mechanisms [50]. In a recent study, it was reported that IDRs in the AD reduce the TF target search time in a two-step mechanism. A low—specificity association of the IDR with the target region that could be directed by locally enriched chromatin factors is followed by specific DNA binding [51••]. In addition, an SPT study of the HIF-α target search process found its IDR to play an important role in the nuclear search dynamics as well as its targeting [52•].

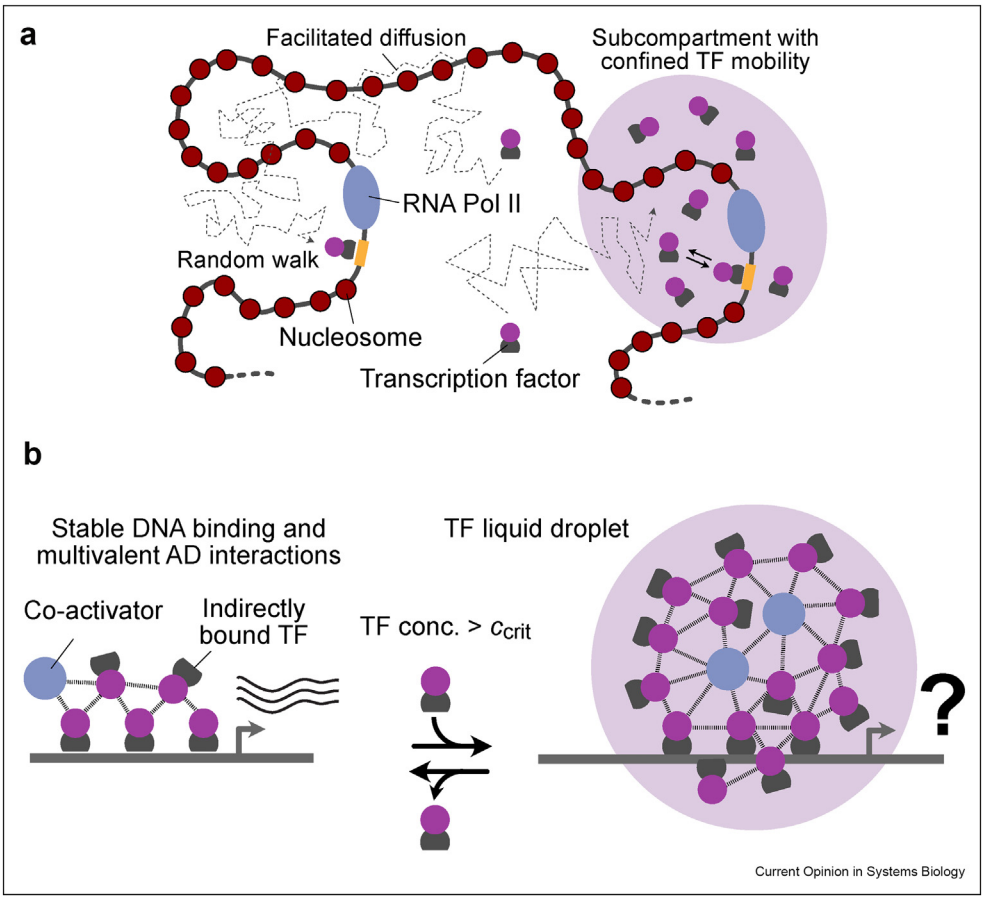
An alternative mechanism to increase $k_{on}$ that involves IDRs is the formation of nuclear subcompartments of high transcriptional activity that enrich RNA Pol II, TFs, and co-activators. These assemblies have been previously characterized as "transcription factories/hubs" while phase-separated "transcriptional condensates" represent a new model for this process as discussed recently [50,53]. The local TF enrichment could arise from various mechanisms like clustering of binding sites, the (cooperative) chromatin binding of protein and RNA factors, bridging interactions between them that fold the nucleosome chain, and the formation of phase-separated condensates via multivalent interactions. Evidence for the clustering of TFs and co-activators at enhancers has been reported [54,55••]. Hallmarks of a phase-separated compartment are sharp transitions of physicochemical properties at the phase boundaries and the existence of a critical concentration above which the compartments form. Recent studies also propose surface condensation of TFs [56,57] or RNA Pol II [58•] as mechanisms to locally nucleate subcompartments rather than inducing a larger scale separation of the nucleoplasm into a dense and a dilute phase. The enrichment of TFs in a confined but yet dynamic chromatin subcompartment via various mechanisms could lead to a reduced target search time [51••,59••,60•] (Figure 1d, Figure 2a).

While binding specificity is frequently assigned to a reduced value of $k_{off}$, the kinetic on rate can also be linked to binding specificity. A recent study of *lac* repressor in vitro found that an increased microscopic on-rate and not the off-rate (corresponding to rates $k_2$ and $k_{-2}$ respectively in Figure 1d) was the main determinant of binding specificity [48•]. Furthermore, the IDR could not only increase $k_{on}$ via the mechanisms discussed above but at the same time also enhance the specificity of binding. This could occur via IDR interactions with pre-existing locally enriched chromatin factors, transcription co-regulators, or other transcription factors during target search/binding [51••,52•,59••,60•].

## TF activity within nuclear subcompartments

In addition to affecting TF binding parameters, the assembly of RNA Pol II nuclear subcompartments could have additional functional implications for the activation reaction. The formation of light-induced phase separated compartments has been related to an enhancement of transcription [61,62]. However, a recent study compared transcription activation below and above the critical concentration for the formation of liquid-like TF droplets and did not find an enhancement of transcription [46••]. The underlying indirect multivalent interaction of the AD were nevertheless crucial for co-activator binding and full transcription activation (Figure 2b). In line with this view, an oncogenic fusion TF showed its highest activity only inside a certain range of multivalent interactions. Enhancing IDR interactions to promote phase separation repressed transcription [63••]. Furthermore, recent studies of promoter architecture demonstrated that the precise location of TF binding sites relative to the core promoter is crucial for gene activation strength [64,65]. These findings suggest that TFs directly bound to DNA at their specific target site govern the activation reaction. Accordingly, it will be important to separate the effect of the directly chromatin-bound fraction of TFs from the effect of those that additionally accumulate non-

**Figure 2**




Potential functional consequences of forming transcriptional nuclear subcompartments. (**a**) Reduction of TF target search time. Formation of a subcompartment by various mechanisms could confine TF diffusion and increase its local concentration. It could reduce the TF target search time by confining the target search to the volume of the subcompartment. In addition, increasing the local TF concentration would increase binding site occupancy. Facilitated diffusion where the dimensionality of the search is reduced from a 3D random walk to a 1D search along the nucleosome chain could also increase $k_{on}$. (**b**) Multivalent AD interactions increase transcription activation capacity by stabilization of binding and in part via interactions with co-activators. Above a critical TF concentration, these multivalent interactions can drive the formation of phase-separated droplets. Conflicting reports exist on if this occurs at physiological expression levels and how the formation of the droplet state affects transcription.

stoichiometrically via phase separation mechanisms. Furthermore, the enrichment of regulatory factors in such subcompartments has been demonstrated as a mechanism that could inhibit Pol I [66] and RNA Pol II transcription [46••,67]. Thus, TF phase separation could play an inhibitory role in regulating RNA Pol II activity in an endogenous cellular environment by establishing refractory promoter states or during overexpression in a disease context. Confining movements of active TFs to a nuclear subcompartment could also enhance a potential non-equilibrium mechanism of enhancer-promoter communication that does not involve direct promoter–enhancer interactions as suggested recently [16]. Thus, enrichment of additional TFs and co-activators via interactions with DNA-bound TFs appears to represent an additional regulatory layer of transcription regulation.

## Conclusions

The dynamic interplay of chromatin organization and TF activity is associated with a variety of additional regulatory mechanisms that go beyond a direct link between the equilibrium binding site occupancy and activation/repression. As discussed above, multi-step activation reactions that include energy-dependent steps for chromatin remodeling can increase the specificity of gene activation. Integrating these chromatin-guided steps of TF binding and transcription activation in the endogenous nuclear environment will be needed to advance eukaryotic gene regulatory functions. The (non-)stoichiometric enrichment of TFs at regulatory elements via multi-valent interactions and their interactions with co-activators represent an additional step that can affect TF activity. Accordingly, it will be important to characterize the fraction and contributions

of directly and indirectly chromatin-bound TFs to transcription activation (or repression) in dependence of TF concentration.

## Conflict of interest statement

Nothing declared.

## Data availability

No data was used for the research described in the article.

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## References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Struhl K: **Fundamentally different logic of gene regulation in eukaryotes and prokaryotes**. *Cell* 1999, **98**:1–4.

2. Kornberg RD: **Eukaryotic transcriptional control**. *Trends Biochem Sci* 1999, **24**:M46–M49.

3. Engl C, Jovanovic G, Brackston RD, Kotta-Loizou I, Buck M: **The route to transcription initiation determines the mode of transcriptional bursting in E. coli**. *Nat Commun* 2020, **11**: 2422.

4. Jensen D, Galburt EA: **The context-dependent influence of promoter sequence motifs on transcription initiation kinetics and regulation**. *J Bacteriol* 2021, **203**:e00512. 00520.

5. •• Ireland WT, Beeler SM, Flores-Bautista E, McCarty NS, Roschinger T, Belliveau NM, Sweredoski MJ, Moradian A, Kinney JB, Phillips R: **Deciphering the regulatory genome of Escherichia coli, one hundred promoters at a time**. *Elife* 2020, **9**, e55308.
The authors introduce an experimental and theoretical framework termed Reg-Seq to systematically identify regulatory sequences, TFs and promoter architectures and apply it to the simultaneous analysis of one hundred promoters in *E. coli*.

6. Morrison M, Razo-Mejia M, Phillips R: **Reconciling kinetic and thermodynamic models of bacterial transcription**. *PLoS Comput Biol* 2021, **17**, e1008572.

7. Landman J, Georgiev RN, Rydenfelt M, Kegel WK: **In vivo and in vitro consistency of thermodynamic models for transcription regulation**. *Phys Rev Res* 2019, **1**, 033094.

8. Grosveld F, van Staalduinen J, Stadhouders R: **Transcriptional regulation by (Super)Enhancers: from discovery to mechanisms**. *Annu Rev Genom Hum Genet* 2021, **22**:127–146.

9. Andersson R, Sandelin A: **Determinants of enhancer and promoter activities of regulatory elements**. *Nat Rev Genet* 2020, **21**:71–87.

10. •• Zuin J, Roth G, Zhan Y, Cramard J, Redolfi J, Piskadlo E, Mach P, Kryzhanovska M, Tihanyi G, Kohler H, *et al.*: **Nonlinear control of transcription through enhancer-promoter interactions**. *Nature* 2022, **604**:571–577.
The relation of transcription activation in dependence of enhancer-promoter contact probability was dissected in mouse embryonic stem cells. The non-linear relationship observed was rationalized by a model in which transient enhancer–promoter interactions are no longer rate-limiting above a certain contact probability as they induce multiple slower activation steps that control promoter bursting dynamics.

11. Wong F, Gunawardena J: **Gene regulation in and out of equilibrium**. *Annu Rev Biophys* 2020, **49**:199–226.

12. Polach KJ, Widom J: **Mechanism of protein access to specific DNA sequences in chromatin: a dynamic equilibrium model for gene regulation**. *J Mol Biol* 1995, **254**:130–149.

13. • Soto LF, Li Z, Santoso CS, Berenson A, Ho I, Shen VX, Yuan S, Fuxman Bass JI: **Compendium of human transcription factor effector domains**. *Mol Cell* 2022, **82**:514–526.
Valuable catalog of 924 effector domains across 594 human TFs that includes their charge, hydrophobicity, disorder and phosphorylation.

14. Shvedunova M, Akhtar A: **Modulation of cellular processes by histone and non-histone protein acetylation**. *Nat Rev Mol Cell Biol* 2022, **23**:329–349.

15. Narita T, Ito S, Higashijima Y, Chu WK, Neumann K, Walter J, Satpathy S, Liebner T, Hamilton WB, Maskey E, *et al.*: **Enhancers are activated by p300/CBP activity-dependent PIC assembly, RNAPII recruitment, and pause release**. *Mol Cell* 2021, **81**:2166–2182 e2166.

16. Karr JP, Ferrie JJ, Tjian R, Darzacq X: **The transcription factor activity gradient (TAG) model: contemplating a contact-independent mechanism for enhancer-promoter communication**. *Genes Dev* 2022, **36**:7–16.

17. Thorn GJ, Clarkson CT, Rademacher A, Mamayusupova H, Schotta G, Rippe K, Teif VB: **DNA sequence-dependent formation of heterochromatin nanodomains**. *Nat Commun* 2022, **13**:1861.

18. Zhu F, Farnung L, Kaasinen E, Sahu B, Yin Y, Wei B, Dodonova SO, Nitta KR, Morgunova E, Taipale M, *et al.*: **The interaction landscape between transcription factors and the nucleosome**. *Nature* 2018, **562**:76–81.

19. Ahmad K, Henikoff S, Ramachandran S: **Managing the steady state chromatin landscape by nucleosome dynamics**. *Annu Rev Biochem* 2022, **91**:183–195.

20. Zaret KS: **Pioneer transcription factors initiating gene network changes**. *Annu Rev Genet* 2020, **54**:367–385.

21. Michael AK, Thoma NH: **Reading the chromatinized genome**. *Cell* 2021, **184**:3599–3611.

22. Morgunova E, Taipale J: **Structural insights into the interaction between transcription factors and the nucleosome**. *Curr Opin Struct Biol* 2021, **71**:171–179.

23. Michael AK, Grand RS, Isbel L, Cavadini S, Kozicka Z, Kempf G, Bunker RD, Schenk AD, Graff-Meyer A, Pathare GR, *et al.*: **Mechanisms of OCT4-SOX2 motif readout on nucleosomes**. *Science* 2020, **368**:1460–1465.

24. Dodonova SO, Zhu F, Dienemann C, Taipale J, Cramer P: **Nucleosome-bound SOX2 and SOX11 structures elucidate pioneer factor function**. *Nature* 2020, **580**:669–672.

25. • Hansen JL, Loell KJ, Cohen BA: **A test of the pioneer factor hypothesis using ectopic liver gene activation**. *Elife* 2022:11.
The comparison of FOXA1 (considered to be a pioneer factor) and the non-pioneer factor HNF4A with respect to DNA binding and target gene activation did not reveal mechanistic difference between the two TFs. No evidence was found for a two-step activation mechanism predicted by the pioneer factor model in which one TF would open up chromatin to potentiate binding of a second TF that would be unable to do so on its own.

26. •• Sonmezer C, Kleinendorst R, Imanci D, Barzaghi G, Villacorta L, Schubeler D, Benes V, Molina N, Krebs AR: **Molecular Co-occupancy identifies transcription factor binding cooperativity in vivo**. *Mol Cell* 2021, **81**:255–267 e256.
Single molecule footprinting shows high CRE co-occupancy with two or more TFs and supports a nucleosome-mediated binding cooperativity. From the correlation of co-binding frequencies and nucleosome occupancy a non-equilibrium energy-consuming mechanism is inferred.

27. • Rao S, Ahmad K, Ramachandran S: **Cooperative binding between distant transcription factors is a hallmark of active enhancers**. *Mol Cell* 2021, **81**:1651–1665 e1654.

This study provides evidence for TF co-occupancy at active enhancers from an integrative analysis of nuclease- and methyltransferase footprinting.

28. • Sahu B, Hartonen T, Pihlajamaa P, Wei B, Dave K, Zhu F, Kaasinen E, Lidschreiber K, Lidschreiber M, Daub CO, *et al.*: **Sequence determinants of human gene regulatory elements**. *Nat Genet* 2022, **54**:283–294.
Unbiased analysis of the DNA sequence space that determines TF activity. TFs were found to activate reporter gene transcription at synthetic CREs in an additive manner without specific combinations of TF motifs beyond the heterodimer level and the requirement for specific TF–TF interactions.

29. •• Eck E, Liu J, Kazemzadeh-Atoufi M, Ghoreishi S, Blythe SA, Garcia HG: **Quantitative dissection of transcription in development yields evidence for transcription-factor-driven chromatin accessibility**. *Elife* 2020, **9**, e56429.
Different models were compared to predict hunchback transcription from the binding of Bicoid and Zelda TFs in the Drosophila embryo. A mechanism where TFs actively induced chromatin accessibility via an energy-consuming step yielded the best description of the experimental data.

30. Mazzocca M, Fillot T, Loffreda A, Gnani D, Mazza D: **The needle and the haystack: single molecule tracking to probe the transcription factor search in eukaryotes**. *Biochem Soc Trans* 2021, **49**:1121–1132.

31. Jana T, Brodsky S, Barkai N: **Speed-specificity trade-offs in the transcription factors search for their genomic binding sites**. *Trends Genet* 2021, **37**:421–432.

32. Hopfield JJ: **Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity**. *Proc Natl Acad Sci U S A* 1974, **71**:4135–4139.

33. Schiessel H, Blossey R: **Pioneer transcription factors in chromatin remodeling: the kinetic proofreading view**. *Phys Rev E* 2020, **101**, 040401.

34. • Shelansky R, Boeger H: **Nucleosomal proofreading of activator-promoter interactions**. *Proc Natl Acad Sci U S A* 2020, **117**:2456–2461.
This theoretical study shows that remodeling promoter nucleosomes can be part of a kinetic proofreading mechanism for gene activation to increase specificity with fast regulatory kinetics.

35. Callegari A, Sieben C, Benke A, Suter DM, Fierz B, Mazza D, Manley S: **Single-molecule dynamics and genome-wide transcriptomics reveal that NF-kB (p65)-DNA binding times can be decoupled from transcriptional activation**. *PLoS Genet* 2019, **15**, e1007891.

36. Loffreda A, Jacchetti E, Antunes S, Rainone P, Daniele T, Morisaki T, Bianchi ME, Tacchetti C, Mazza D: **Live-cell p53 single-molecule binding is modulated by C-terminal acetylation and correlates with transcriptional activity**. *Nat Commun* 2017, **8**:313.

37. Hipp L, Beer J, Kuchler O, Reisser M, Sinske D, Michaelis J, Gebhardt JCM, Knoll B: **Single-molecule imaging of the transcription factor SRF reveals prolonged chromatin-binding kinetics upon cell stimulation**. *Proc Natl Acad Sci U S A* 2019, **116**:880–889.

38. Clauss K, Popp AP, Schulze L, Hettich J, Reisser M, Escoter Torres L, Uhlenhaut NH, Gebhardt JCM: **DNA residence time is a regulatory factor of transcription repression**. *Nucleic Acids Res* 2017, **45**:11121–11130.

39. Hasegawa Y, Struhl K: **Promoter-specific dynamics of TATA-binding protein association with the human genome**. *Genome Res* 2019, **29**:1939–1950.

40. de Jonge WJ, Brok M, Lijnzaad P, Kemmeren P, Holstege FC: **Genome-wide off-rates reveal how DNA binding dynamics shape transcription factor function**. *Mol Syst Biol* 2020, **16**: e9885.

41. Gurdon JB, Javed K, Vodnala M, Garrett N: **Long-term association of a transcription factor with its chromatin binding site can stabilize gene expression and cell fate commitment**. *Proc Natl Acad Sci U S A* 2020, **117**:15075–15084.

42. Lu F, Lionnet T: **Transcription factor dynamics**. *Cold Spring Harbor Perspect Biol* 2021, **13**:a040949.

43. Alvarez JM, Brooks MD, Swift J, Coruzzi GM: **Time-based systems biology approaches to capture and model dynamic gene regulatory networks**. *Annu Rev Plant Biol* 2021, **72**: 105–131.

44. • Garcia DA, Fettweis G, Presman DM, Paakinaho V, Jarzynski C, Upadhyaya A, Hager GL: **Power-law behavior of transcription factor dynamics at the single-molecule level implies a continuum affinity model**. *Nucleic Acids Res* 2021, **49**:6605–6620.
The study challenges the frequently used assumption that TFs display a bimodal binding behavior that reflects specifically versus non-specifically bound complexes. Rather, the residence time distribution follows a power-law with long-lived binding states reaching the minute time scale.

45. •• Popp AP, Hettich J, Gebhardt JCM: **Altering transcription factor binding reveals comprehensive transcriptional kinetics of a basic gene**. *Nucleic Acids Res* 2021, **49**:6249–6266.
Synthetic TFs with different residence times where compared in dependence of their concentration, which was controled by auxin-induced degradation. The residence time was found to be more important for target gene regulation than TF concentration.

46. •• Trojanowski J, Frank L, Rademacher A, Mücke N, Grigaitis P, Rippe K: **Transcription activation is enhanced by multivalent interactions independent of phase separation**. *Mol Cell* 2022, **82**:1878–1893 e1810.
This study shows that TF activation capacity is linked to their propensity to engage in multivalent interactions and to assemble into liquid-like TF droplets. However, the actual formation of liquid-like TF droplets had a neutral or inhibitory effect on transcription activation. It is concluded that multivalent interactions TFs are important for transcription activation as they increase the residence time in the chromatin-bound state and promote interactions with co-activators below the critical concentration needed for TF phase separation.

47. Berg OG, von Hippel PH: **Diffusion-controlled macromolecular interactions**. *Annu Rev Biophys Biophys Chem* 1985, **14**: 131–160.

48. •• Marklund E, Mao G, Yuan J, Zikrin S, Abdurakhmanov E, Deindl S, Elf J: **Sequence specificity in DNA binding is mainly governed by association**. *Science* 2022, **375**:442–445.
The binding kinetics of lac repressor to operator sequences were dissected on a microarray and in single-molecule experiments. It was found that sequence specificity arises to a larger part from an increase of the microscopic kinetic on-rate rather than from a reduced dissociation rate.

49. Choi JM, Holehouse AS, Pappu RV: **Physical principles underlying the complex biology of intracellular phase transitions**. *Annu Rev Biophys* 2020, **49**:107–133.

50. Rippe K, Papantonis A: **Functional organization of RNA polymerase II in nuclear subcompartments**. *Curr Opin Cell Biol* 2022, **74**:88–96.

51. •• Brodsky S, Jana T, Mittelman K, Chapal M, Kumar DK, Carmi M, Barkai N: **Intrinsically disordered regions direct transcription factor in vivo binding specificity**. *Mol Cell* 2020, **79**:459–471. e454.
The authors show that TF IDRs outside the DNA binding domain accelerate TF target search. They suggest a two-step mechanism: low–specificity association of the IDR with the target region followed by DNA binding.

52. • Chen Y, Cattoglio C, Dailey G, Zhu Q, Tjian R, Darzacq X: **Mechanisms governing target search and binding dynamics of hypoxia-inducible factors**. *eLife* 2022, **11**, e75064.
The IDR in the HIF-1alpha activation domain was found to play an important role for nuclear search dynamics and chromatin binding.

53. Rippe K: **Liquid-liquid phase separation in chromatin**. *Cold Spring Harbor Perspect Biol* 2022, **14**:a040683.

54. Nair SJ, Yang L, Meluzzi D, Oh S, Yang F, Friedman MJ, Wang S, Suter T, Alshareedah I, Gamliel A, *et al.*: **Phase separation of ligand-activated enhancers licenses cooperative chromosomal enhancer assembly**. *Nat Struct Mol Biol* 2019, **26**: 193–203.

55. •• Li J, Hsu A, Hua Y, Wang G, Cheng L, Ochiai H, Yamamoto T, Pertsinidis A: **Single-gene imaging links genome topology, promoter-enhancer communication and transcription control**. *Nat Struct Mol Biol* 2020, **27**:1032–1040.
The accumulation of Sox2 and Brd4 at single transcription sites in living mouse embryonic stem cells was analyzed by fluorescence microscopy imaging. Clustering was dependent on the Sox2 DNA-binding motif and the Brd4 bromodomains but not on their IDRs.

56. Quail T, Golfier S, Elsner M, Ishihara K, Murugesan V, Renger R, Jülicher F, Brugués J: **Force generation by protein–DNA co-condensation**. *Nat Phys* 2021, **17**:1007–1012.

57. Morin JA, Wittmann S, Choubey S, Klosin A, Golfier S, Hyman AA, Jülicher F, Grill SW: **Sequence-dependent surface condensation of a pioneer transcription factor on DNA**. *Nat Phys* 2022, **18**:271–276.

58. • Pancholi A, Klingberg T, Zhang W, Prizak R, Mamontova I, Noa A, Sobucki M, Kobitski AY, Nienhaus GU, Zaburdaev V, *et al.*: **RNA polymerase II clusters form in line with surface condensation on regulatory chromatin**. *Mol Syst Biol* 2021, **17**, e10272.
Super-resolution fluorescence microscopy of RNA Pol II cluster in zebrafish embryos revealed subcompartments with actively transcribed chromatin located around a protein rich and DNA depleted core. The authors propose that thes RNA Pol II clusters assemble by a surface condensation process on chromatin.

59. •• Garcia DA, Johnson TA, Presman DM, Fettweis G, Wagh K, Rinaldi L, Stavreva DA, Paakinaho V, Jensen RAM, Mandrup S, *et al.*: **An intrinsically disordered region-mediated confinement state contributes to the dynamics and function of transcription factors**. *Mol Cell* 2021, **81**:1484–1498 e1486.
Single molecule tracking of the glucocorticoid receptor revealed that its IDR promotes confined diffusion of the TF around its target sites.

60. • Kent S, Brown K, Yang CH, Alsaihati N, Tian C, Wang H, Ren X: **Phase-separated transcriptional condensates accelerate target-search process revealed by live-cell single-molecule imaging**. *Cell Rep* 2020, **33**, 108248.
This single particle tracking study of variants of the transcriptional repressor CBX2 found that the target search time was anti-correlated with the propensity to undergo liquid–liquid phase separation in vitro.

61. Schneider N, Wieland FG, Kong D, Fischer AAM, Horner M, Timmer J, Ye H, Weber W: **Liquid-liquid phase separation of light-inducible transcription factors increases transcription activation in mammalian cells and mice**. *Sci Adv* 2021, **7**, eabd3568.

62. Wei MT, Chang YC, Shimobayashi SF, Shin Y, Strom AR, Brangwynne CP: **Nucleated transcriptional condensates amplify gene expression**. *Nat Cell Biol* 2020, **22**:1187–1196.

63. •• Chong S, Graham TGW, Dugast-Darzacq C, Dailey GM, Darzacq X, Tjian R: **Tuning levels of low-complexity domain interactions to modulate endogenous oncogenic transcription**. *Mol Cell* 2022, **82**:2084–2097 e2085.
Increasing IDR–IDR interactions of the oncogenic TF EWS::FLI1 to induce phase separation were found to repress transcription of its endogenous target genes. Furthermore, ectopic IDR mediated multivalent interactions were exploited to sequester this disease-causing TF fusion in the nucleolus to inhibit its activity.

64. de Boer CG, Vaishnav ED, Sadeh R, Abeyta EL, Friedman N, Regev A: **Deciphering eukaryotic gene-regulatory logic with 100 million random promoters**. *Nat Biotechnol* 2020, **38**:56–65.

65. Davis JE, Insigne KD, Jones EM, Hastings QA, Boldridge WC, Kosuri S: **Dissection of c-AMP response element architecture by using genomic and episomal massively parallel reporter assays**. *Cell Syst* 2020, **11**:75–85 e77.

66. Ide S, Imai R, Ochi H, Maeshima K: **Transcriptional suppression of ribosomal DNA with phase separation**. *Sci Adv* 2020, **6**, eabb5953.

67. Rawat P, Boehning M, Hummel B, Aprile-Garcia F, Pandit AS, Eisenhardt N, Khavaran A, Niskanen E, Vos SM, Palvimo JJ, *et al.*: **Stress-induced nuclear condensation of NELF drives transcriptional downregulation**. *Mol Cell* 2021, **81**:1013–1026 e1011.