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A Phase Separation Model for Transcriptional Control

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Phase-separated multi-molecular assemblies provide a general regulatory mechanism to compartmentalize biochemical reactions within cells. We propose that a phase separation model explains established and recently described features of transcriptional control. These features include the formation of super-enhancers, the sensitivity of super-enhancers to perturbation, the transcriptional bursting patterns of enhancers, and the ability of an enhancer to produce simultaneous activation at multiple genes. This model provides a conceptual framework to further explore principles of gene control in mammals.

Recent studies of transcriptional regulation have revealed several puzzling observations that, as of yet, lack quantitative description, but the further understanding of which would likely afford new and valuable insights into gene control during development and disease. For example, although thousands of enhancer elements control the activity of thousands of genes in any given human cell type, several hundred clusters of enhancers, called super-enhancers (SEs), control genes that have especially prominent roles in cell-type-specific processes (ENCODE Project Consortium, 2012; Hnisz et al., 2013; Lovén et al., 2013; Parker et al., 2013; Kundaje et al., 2015; Whyte et al., 2013). Cancer cells acquire super-enhancers to drive expression of prominent oncogenes, so SEs play key roles in both development and disease (Chapuy et al., 2013; Lovén et al., 2013). Super-enhancers are occupied by an unusually high density of interacting factors, are able to drive higher levels of transcription than typical enhancers, and are exceptionally vulnerable to perturbation of components commonly associated with most enhancers (Chapuy et al., 2013; Hnisz et al., 2013; Lovén et al., 2013; Whyte et al., 2013).

Another puzzling observation that has emerged from recent studies is that a single enhancer is able to simultaneously activate multiple proximal genes (Fukaya et al., 2016). Enhancers physically contact the promoters of the genes they activate, and early studies using chromatin contact mapping techniques (e.g., at the β-globin locus) found that at any given time, enhancers activate only one of the several globin genes within the locus (Palstra et al., 2003; Tolhuis et al., 2002). However, more recent work using quantitative imaging at a high temporal resolution revealed that enhancers typically activate genes in bursts, and that two gene promoters can exhibit synchronous bursting when activated by the same enhancer (Fukaya et al., 2016).

Previous models of transcriptional control have provided important insights into principles of gene regulation. A key feature of most previous transcriptional control models is that the underlying regulatory interactions occur in a stepwise manner dictated by biochemical rules that are probabilistic in nature (Chen and Larson, 2016; Elowitz et al., 2002; Levine et al., 2014; Orphanides and Reinberg, 2002; Raser and O'Shea, 2004; Spitz and Furlong, 2012; Suter et al., 2011; Zoller et al., 2015). Such kinetic models predict that gene activation on a single gene level is a stochastic, noisy process, and also provide insights into how multi-step regulatory processes can suppress intrinsic noise and result in bursting. These models do not shed light on the mechanisms underlying the formation, function, and properties of SEs or explain puzzles such as how two gene promoters exhibit synchronous bursting when activated by the same enhancer.

In this perspective, we propose and explore a model that may explain the puzzles described above. This model is based on principles involving the phase separation of multi-molecular assemblies.

Cooperativity in Transcriptional Control

Since the discovery of enhancers over 30 years ago, studies have attempted to describe functional properties of enhancers

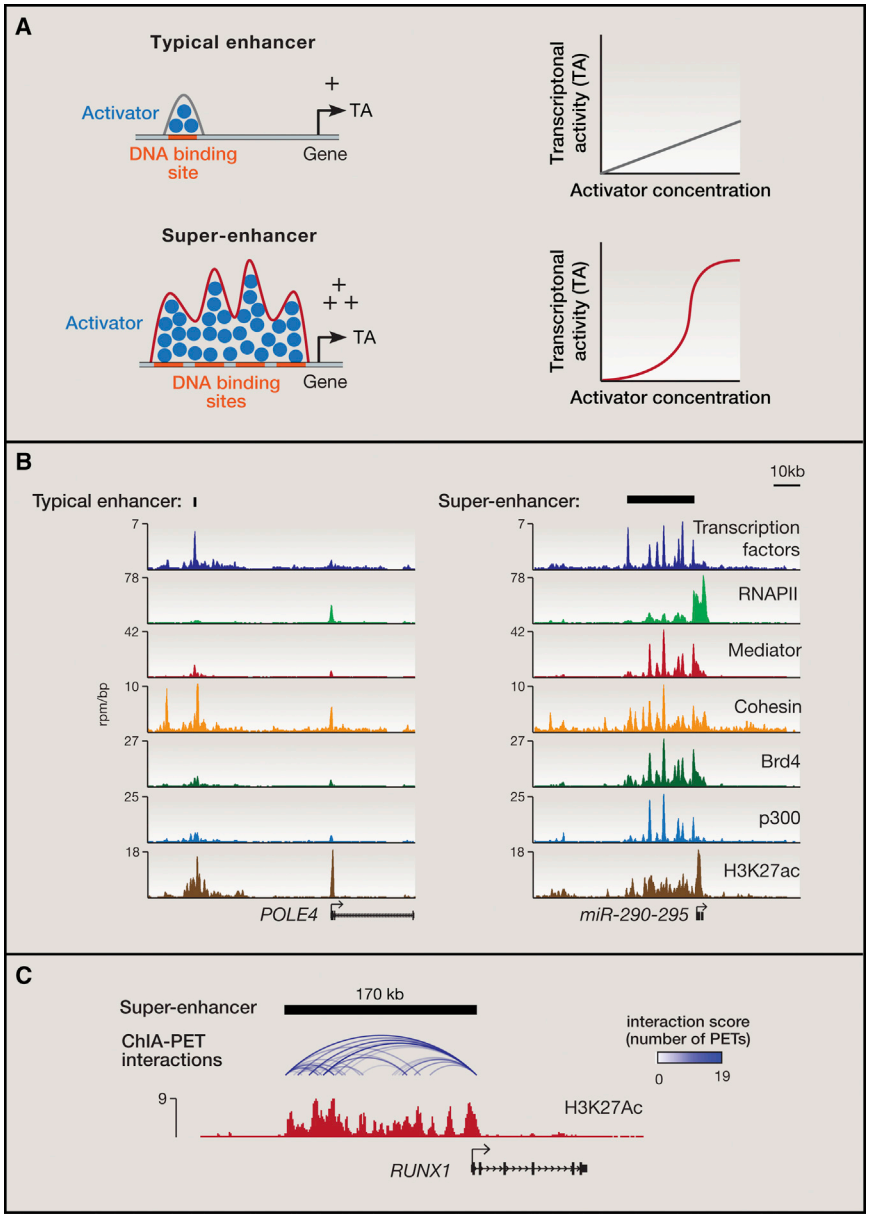

Figure 1. Models and Features of Super-Enhancers and Typical Enhancers

(A) Schematic depiction of the classic model of cooperativity for typical enhancers and super-enhancers. The higher density of transcriptional regulators (referred to as "activators") through cooperative binding to DNA binding sites is thought to contribute to both higher transcriptional output and increased sensitivity to activator concentration at super-enhancers. Image adapted from Lovén et al. (2013).

(B) Chromatin immunoprecipitation sequencing (ChIP-seq) binding profiles for RNA polymerase II (RNA Pol II) and the indicated transcriptional co-factors and chromatin regulators at the *POLE4* and *miR-290-295* loci in murine embryonic stem cells. The transcription factor binding profile is a merged ChIP-seq binding profile of the TFs Oct4, Sox2, and Nanog. rpm/bp, reads per million per base pair. Image adapted from Hnisz et al. (2013).

(C) ChIA-PET interactions at the *RUNX1* locus displayed above the ChIP-seq profiles of H3K27Ac in human T cells. The ChIA-PET interactions indicate frequent physical contact between the H3K27Ac occupied regions within the super-enhancer and the promoter of *RUNX1*.

in a quantitative manner, and these efforts have mostly relied on the concept of cooperative interactions between enhancer components. Classically, enhancers have been defined as elements that can increase transcription from a target gene promoter when inserted in either orientation at various distances upstream or downstream of the promoter (Banerji et al., 1981; Benoist and Chambon, 1981; Gruss et al., 1981). Enhancers typically consist of hundreds of base-pairs of DNA and are bound by multiple transcription factor (TF) molecules in a cooperative manner (Bulger and Groudine, 2011; Levine et al., 2014; Malik and Roeder, 2010; Ong and Corces, 2011; Spitz and Furlong, 2012). Classically, cooperative binding describes the phenomenon that the binding of one TF molecule to DNA impacts the binding of another TF molecule (Figure 1A) (Carey, 1998; Kim and Maniatis, 1997; Thanos and Maniatis, 1995; Tjian and Maniatis, 1994). Cooperative binding of transcription factors at enhancers has been proposed to be due to the effects of TFs on DNA bending (Falvo et al., 1995), interactions between TFs (Johnson et al., 1979), and combinatorial recruitment of large cofactor complexes by TFs (Merika et al., 1998).

Super-Enhancers Exhibit Highly Cooperative Properties

Several hundred clusters of enhancers, called super-enhancers (SEs), control genes that have especially prominent roles in cell-type-specific processes (Hnisz et al., 2013; Whyte et al., 2013). Three key features of SEs indicate that cooperative properties are especially important for their formation and function: (1) SEs are occupied by an unusually high density of interacting factors; (2) SEs can be formed by a single nucleation event; and (3) SEs are exceptionally vulnerable to perturbation of some components commonly associated with most enhancers.

SEs are occupied by an unusually high density of enhancer-associated factors, including transcription factors, co-factors, chromatin regulators, RNA polymerase II, and non-coding RNA (Hnisz et al., 2013). The non-coding RNA (enhancer RNA or eRNA), produced by divergent transcription at transcription factor binding sites within SEs (Hah et al., 2015; Sigova et al., 2013), can contribute to enhancer activity and the expression of the nearby gene in *cis* (Dimitrova et al., 2014; Engreitz et al., 2016; Lai et al., 2013; Pefanis et al., 2015). The density of the protein factors and eRNAs at SEs has been estimated to be

approximately 10-fold the density of the same set of components at typical enhancers in the genome (Figure 1B) (Hnisz et al., 2013; Lovén et al., 2013; Whyte et al., 2013). Chromatin contact mapping methods indicate that the clusters of enhancers within SEs are in close physical contact with one another and with the promoter region of the gene they activate (Figure 1C) (Dowen et al., 2014; Hnisz et al., 2016; Ji et al., 2016; Kieffer-Kwon et al., 2013).

SEs can be formed as a consequence of introducing a single transcription factor binding site into a region of DNA that has the potential to bind additional factors. In T cell leukemias, a small (2–12 bp) mono-allelic insertion nucleates the formation of an entire SE by creating a binding site for the master transcription factor MYB, leading to the recruitment of additional transcriptional regulators to adjacent binding sites and assembly of a host of factors spread over an 8-kb domain whose features are typical of a SE (Mansour et al., 2014). Inflammatory stimulation also leads to rapid formation of SEs in endothelial cells; here again, the formation of a SE is apparently nucleated by a single binding event of a transcription factor responsive to inflammatory stimulation (Brown et al., 2014).

Entire super-enhancers spanning tens of thousands of base pairs can collapse as a unit when their co-factors are perturbed, and genetic deletion of constituent enhancers within an SE can compromise the function of other constituents. For example, the co-activator BRD4 binds acetylated chromatin at SEs, typical enhancers and promoters, but SEs are far more sensitive to drugs blocking the binding of BRD4 to acetylated chromatin (Chapuy et al., 2013; Lovén et al., 2013). A similar hypersensitivity of SEs to inhibition of the cyclin-dependent kinase CDK7 has also been observed in multiple studies (Chipumuro et al., 2014; Kwiatkowski et al., 2014; Wang et al., 2015). This kinase is critical for initiation of transcription by RNA polymerase II (RNA Pol II) and phosphorylates its repetitive C-terminal domain (CTD) (Larochelle et al., 2012). Furthermore, genetic deletion of constituent enhancers within SEs can compromise the activities of other constituents within the super-enhancer (Hnisz et al., 2015; Jiang et al., 2016; Proudhon et al., 2016; Shin et al., 2016), and can lead to the collapse of an entire super-enhancer (Mansour et al., 2014), although this interdependence of constituent enhancers is less apparent for some developmentally regulated super-enhancers (Hay et al., 2016).

In summary, several lines of evidence indicate that the formation and function of SEs involves cooperative processes that bring many constituent enhancers and their bound factors into close spatial proximity. High densities of proteins and nucleic acids—and cooperative interactions among these molecules—have been implicated in the formation of membraneless organelles, called cellular bodies, in eukaryotic cells (Banjade et al., 2015; Bergeron-Sandoval et al., 2016; Brangwynne et al., 2009). Below, we first describe features of the formation of cellular bodies, and then develop a model of super-enhancer formation and function that exploits related concepts.

Formation of Membraneless Organelles by Phase Separation

Eukaryotic cells contain membraneless organelles, called cellular bodies, which play essential roles in compartmentalizing essential biochemical reactions within cells. These bodies are formed by phase separation mediated by cooperative interactions between multivalent molecules (Banani et al., 2017; Banjade et al., 2015; Bergeron-Sandoval et al., 2016; Brangwynne et al., 2009). Examples of such organelles in the nucleus include nucleoli, which are sites of rRNA biogenesis; Cajal bodies, which serve as an assembly site for small nuclear RNPs; and nuclear speckles, which are storage compartments for mRNA splicing factors (Mao et al., 2011; Zhu and Brangwynne, 2015). These organelles exhibit properties of liquid droplets; for example, they can undergo fission and fusion, and hence their formation has been described as mediated by liquid-liquid phase separation. Mixtures of purified RNA and RNA-binding proteins form these types of phase-separated bodies in vitro (Berry et al., 2015; Feric et al., 2016; Kato et al., 2012; Kwon et al., 2013; Li et al., 2012; Wheeler et al., 2016). Consistent with these observations, past theoretical work indicates that the formation of a gel is usually accompanied by phase separation (Semenov and Rubinstein, 1998). Thus, a number of studies show that high densities of proteins and nucleic acids—and cooperative interactions among these molecules—are implicated in the formation of phase separated cellular bodies.

As described above, super-enhancers can be in essence considered to be cooperative assemblies of high densities of transcription factors, transcriptional co-factors, chromatin regulators, non-coding RNA, and RNA Pol II. Furthermore, some transcription factors with low complexity domains have been proposed to create gel-like structures in vitro (Han et al., 2012; Kato et al., 2012; Kwon et al., 2013). We thus hypothesize that formation of a phase separated multi-molecular assembly likely occurs during the formation of SEs and less frequently with typical enhancers (Figure 2A).

We propose a simple model that emphasizes cooperativity in the context of the number and valency of the interacting components, and affinity of interactions between these transcriptional regulators and nucleic acids, to explore the role of a phase separation for SE assembly and function. Computer simulations of this model show that phase separation can explain critical features of SEs, including aspects of their formation, function, and vulnerability. The simulations are also consistent with observed differences between transcriptional bursting patterns driven by weak and strong enhancers, and the simultaneous bursting of genes controlled by a shared single enhancer. We conclude by noting several implications and predictions of the phase separation model that could guide further exploration of this concept of transcriptional control in vertebrates.

A Phase Separation Model of Enhancer Assembly and Function

Many molecules bound at enhancers and SEs, such as transcription factors, transcriptional co-activators (e.g., BRD4), RNA Pol II, and RNA can undergo reversible chemical modifications (e.g., acetylation, phosphorylation) at multiple sites. After such modifications, these multivalent molecules are able to interact with multiple other components, thus forming "cross-links" (Figure 2A). Here, a cross-link can be defined as any reversible feature, including reversible chemical modification, or any other feature involved in dynamic binding and unbinding

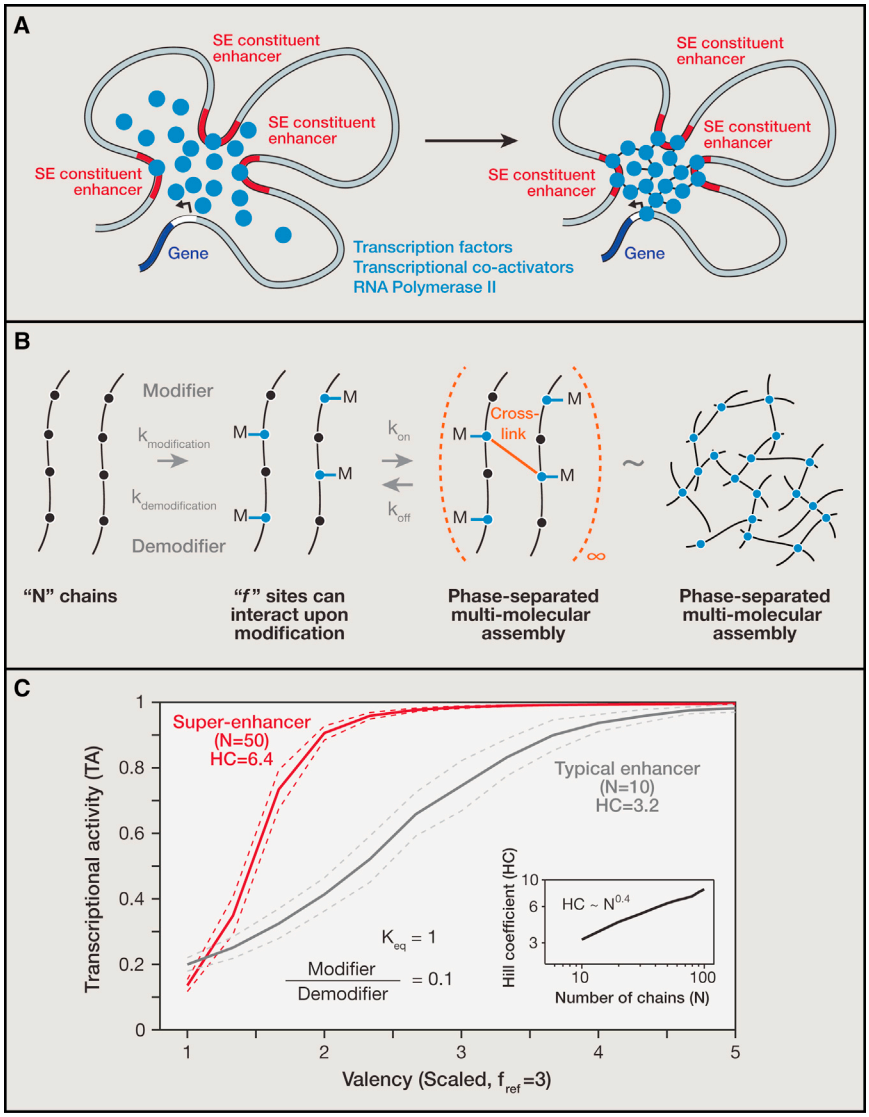

Figure 2. A Simple Phase Separation Model of Transcriptional Control

(A) Schematic representation of the biological system that can form the phase-separated multi-molecular complex of transcriptional regulators at a super-enhancer – gene locus.

(B) Simplified representation of the biological system, and parameters of the model that could lead to phase separation. "M" denotes modification of residues that are able to form cross-links when modified.

(C) Dependence of transcriptional activity (TA) on the valency parameter for super-enhancers (consisting of N = 50 chains), and typical enhancers (consisting of N = 10 chains). The proxy for transcriptional activity (TA) is defined as the size of the largest cluster of cross-linked chains, scaled by the total number of chains. The valency is scaled such that the actual valency is divided by a reference number of three. The solid lines indicate the mean, and the dashed lines indicate twice the standard deviation in 50 simulations. The value of K_{eq} and modifier/demodifier ratio was kept constant. HC, Hill coefficient, which is a classic metric to describe cooperative behavior. The inset shows the dependency of the Hill coefficient on the number of chains, or components, in the system. See also Figures S1–S3.

interactions. In considering whether phase separation may underlie certain observed features of transcriptional control, a simple model is needed to describe the dependence of phase separation on changes in valences and affinities of the interacting molecules, parameters biologists measure. Below, we describe such a model, and explain how the parameters of this model represent characteristics of typical enhancers and super-enhancers.

In the model, the protein and nucleic acid components of enhancers are represented as chain-like molecules, each of which contains a set of residues that can potentially engage in interactions with other chains (Figure 2B). These residues are represented as sites that can undergo reversible chemical modifications, and modification of the residues is associated with their ability to form non-covalent cross-linking interactions between the chains (Figure 2B). Numerous enhancer-components, including transcription factors, co-factors, and the heptapeptide repeats of the C-terminal domain (CTD) of RNA Pol II are subject to phosphorylation and are known to bind other proteins based on their phosphorylation status (Phatnani and Greenleaf, 2006). Our model encompasses such phosphorylation or dephosphorylation that can result in binding interactions, as well as interactions of histones and other proteins found at enhancers and transcriptional regulators modulated by acetylation, methylation, or other types of chemical modifications. For simplicity, we refer to all types of chemical modifications and de-modifications generically as "modification" and "demodification" mediated by "modifiers" and "demodifiers," respectively.

In its simplest form, the model has three parameters: (1) "N" = the number of macromolecules (also referred to as "chains") in the system; this parameter sets the concentration of interacting components—the larger the value of N, the greater the concentration—SEs are considered to have a larger value of N while typical enhancers are modeled as having fewer components. (2) "*f*" = valency, which corresponds to the number of residues in each molecule that can potentially be modified and engage in a cross-link with other chains. Note that in our simplified model, the modification of a residue is required to allow the residue to create a cross-link with another chain. Conceptually, the model works in a similar way if the *demodified* state of a residue is required for cross-link formation, except the enzymatic activities that allow or inhibit cross-link formation are reversed. (3) K_{eq} = (k_{on}/k_{off}) the equilibrium constant, defined by the on and off-rates describing the cross-link reaction or interaction (Figure 2B).

With a few assumptions, such as large chain length and not allowing intramolecular cross-links or multiple bonds between the

same two chains, the equilibrium properties of this model can be obtained analytically (Cohen and Benedek, 1982; Semenov and Rubinstein, 1998). Above a critical concentration of the interacting chains, C*, phase separation occurs creating a multi-molecular assembly. Under these conditions, C* varies as $1/K_{eq}f^2$. Thus, the critical concentration for formation of the assembly depends sensitively on valency and less so on the binding constant.

We carried out computer simulations of the model (relaxing some of the assumptions in the equilibrium theories noted above) to explore its dynamic, rather than equilibrium, properties. In dynamic computer simulations of the model, the valency changes between 0 and "*f*" as the residues are modified and de-modified; the rates of the modification and de-modification reactions are not varied in our studies. The modifier to demodifier ratio (e.g., kinase to phosphatase ratio) in the system determines the number of sites on each component that are modified and can be cross-linked, and is varied in our studies.

The model was simulated with N chains in a fixed volume representing the region where various components of the enhancer or SE are present. We considered various values of N. During the simulation, the chains can undergo modifications and de-modifications with kinetic constants, $k_{mod} = 0.05, k_{demod} = 0.05$. The modifier and demodifier levels (N_{mod}, N_{demod}) are varied. Cross-link formation and disassociation is simulated with kinetic constants, $k_{on} = 0.5$ and $k_{off} = 0.5$ $(K_{eq} = (k_{on}/k_{off}) = 1)$. Only modified residues on different chains were allowed to cross-link; i.e., intra-chain cross-linking reactions are disallowed, but multiple bonds can form between two chains. The simulations were carried out in the limit at which every site on every chain is permitted to cross-link with all other sites on other chains (Cohen and Benedek, 1982; Semenov and Rubinstein, 1998); i.e., while there is an average concentration of interacting sites (determined by N and the number of modified sites), variations in local concentrations within the simulation volume are not considered.

The simulations were carried out using the Gillespie algorithm (Gillespie, 1977), which generates stochastic trajectories of the temporal evolution of the considered dynamic processes (i.e., modifications and cross-linking reactions). Any single trajectory describes the time-evolution of the state of interacting chains, including how they are distributed among connected clusters of varying sizes. All trajectories are initialized with demodified, non-crosslinked chains; i.e., each chain is in a "separate cluster." Simulations are run until steady state is reached, where properties of the system (e.g., average cluster size) are time-invariant. Multiple trajectories (50 replicates) are performed for all calculations to obtain statistically averaged properties when desired.

The proxy for transcriptional activity (TA) in the simulations was defined as the size of the largest cluster of cross-linked chains, scaled by the total number of chains [TA = (size of $Cluster_{max}$) / N]. The approximation of TA with the size of the largest cluster is supported by recent evidence that the concentration of some transcriptional regulators may be rate-limiting for gene activity in mammalian cells (Lin et al., 2012; Nie et al., 2012). When all chains in the system form a single cross-linked cluster ($TA \approx 1$), the phase-separated assembly is the result. This assembly is thought to encompass binding of factors at the enhancer/SE and also at the promoter, which leads to the concentration of components important for enhanced transcription of the gene. We recorded the transcriptional activity generated by the enhancers and SEs as a function of time.

Transcriptional Regulation with Changes in Valency

Modeling transcriptional activity as a function of valency revealed that the formation of SEs involved more pronounced cooperativity than the formation of typical enhancers (Figure 2C). In these simulations, SEs were modeled as a system consisting of N = 50 molecules, and typical enhancers as a system consisting of N = 10 molecules, consistent with an approximately one order of magnitude difference in the density of components at these elements (Hnisz et al., 2013). We then graphed the transcriptional activity (TA) for different valences, while all other parameters remained constant. SEs reached ~90% of the maximum transcriptional activity at a normalized valency value of 2 (i.e., twice the reference value of $f = 3$), while for typical enhancers 90% of the maximum transcriptional activity is attained at a normalized valency value of five. At a normalized valency value of two, typical enhancers reached ~40% of the maximum transcriptional activity (Figure 2C). These results suggest that, under identical conditions, SEs consisting of a larger number of components form larger connected clusters (i.e., undergo phase separation) at a lower level of valency than typical enhancers consisting of a smaller number of components. Furthermore, we observed a sharp increase of transcriptional activity at a normalized valency value of ~1.5 for SEs, while increases in valency lead to a more moderate, smooth increase of transcriptional activity for typical enhancers (Figure 2C), in agreement with previous considerations (Figure 1A) (Lovén et al., 2013).

The sharper change in transcriptional activity of SEs upon changing the valency of the interacting components due to enhanced cooperativity can be quantified by the Hill coefficient. The behavior of SEs is characterized by a larger value of the Hill coefficient, indicating greater cooperativity and ultrasensitivity to valency changes (Figure 2C). Indeed, as the inset in Figure 2C shows, the Hill coefficient increases with the number of components involved in the enhancer as $\sim N^{0.4}$, over a large range of values of N. Also, as expected, the difference between the transcriptional activity of typical enhancers and SEs correlated with the difference in values of "N" that are used to model them; for a sufficiently large difference in N, the behavior reported in Figure 2C is recapitulated (Figure S1).

Super-Enhancer Formation and Vulnerability

These predictions of the phase separation model are qualitatively consistent with previously published experimental data. For example, stimulation of endothelial cells by tumor necrosis factor alpha (TNFα) leads to the formation of SEs at inflammatory genes (Brown et al., 2014). In this study, SE formation was monitored by the genomic occupancy of the transcriptional co-factor BRD4, which is a key component of SEs and typical enhancers. The inflammatory stimulation in these cells resulted in a more pronounced recruitment of BRD4 at the SEs of inflammatory genes as compared to typical enhancers at other genes (Brown et al., 2014). Our phase separation model suggests that this is because stimulation by TNFα led to modifications that change the valency of interacting components, and for

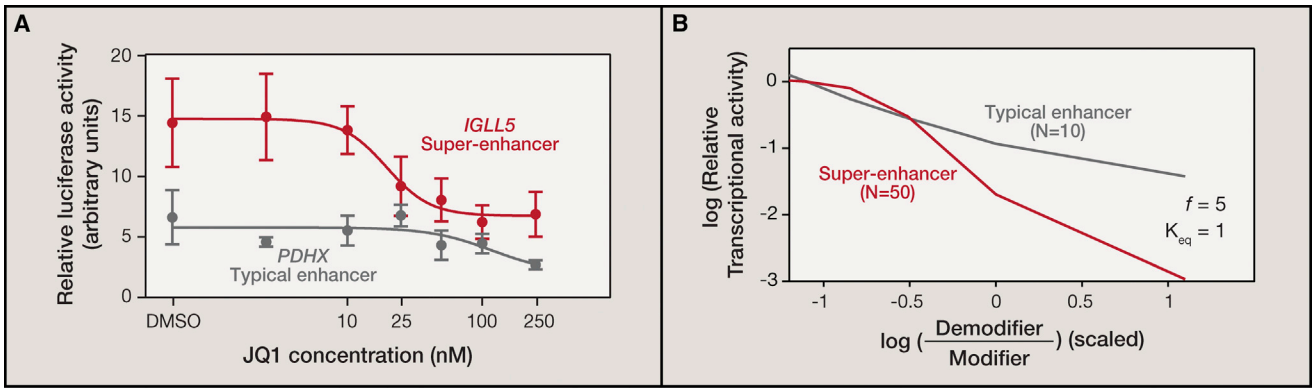

Figure 3. Super-Enhancer Vulnerability

(A) Enhancer activities of the fragments of the *IGLL5* super-enhancer (red) and the *PDHX* typical enhancer (gray) after treatment with the BRD4 inhibitor JQ1 at the indicated concentrations. Enhancer activity was measured in luciferase reporter assays in human multiple myeloma cells. Note that JQ1 inhibits ~50% of luciferase expression driven by the super-enhancer at a 10-fold lower concentration than luciferase expression driven by the typical enhancer (25 nM versus 250 nM). Data and image adapted from Lovén et al. (2013).

(B) Dependence of transcriptional activity (TA) on the demodifier/modifier ratio for super-enhancers (consisting of N = 50 chains), and typical enhancers (consisting of N = 10 chains). The proxy for transcriptional activity (TA) is defined as the size of the largest cluster of cross-linked chains, scaled by the total number of chains. The solid lines indicate the mean and the dashed lines indicate twice the standard deviation of 50 simulations. K_{eq} and f were kept constant. Note that increasing the demodifier levels is equivalent to inhibiting cross-linking (i.e., reducing valency). TA is normalized to the value at log (demodifier/modifier) = −1.5, and the ordinate shows the normalized TA on a log scale.

SEs, phase separation occurs sharply above a lower value of valency compared to typical enhancers, thus resulting in enhanced recruitment of interacting components such as BRD4 (Figure 2C).

Next, we investigated whether the phase separation model explains the unusual vulnerability of SEs to perturbation by inhibitors of common transcriptional co-factors. BRD4 and CDK7 are components of both typical enhancers and SEs, but SEs and their associated genes are much more sensitive to chemical inhibition of BRD4 and CDK7 than typical enhancers (Figure 3A) (Chipumuro et al., 2014; Christensen et al., 2014; Kwiatkowski et al., 2014; Lovén et al., 2013). We modeled the effect of BRD4- and CDK7 inhibitors as reducing valency by changing the ratio of Demodifier/Modifier activity in our system, which shifts the balance of modified sites within the interacting molecules. This is because CDK7 is a kinase which acts as a modifier, and BRD4 has a large valency as it can interact with many components, and so inhibiting BRD4 reduces the average valency of the interacting components disproportionately. As shown in Figure 3B, SEs (N = 50) lose more of their activity sharply at a lower Demodifier/Modifier ratio than typical enhancers (N = 10). These results are consistent with the notion that SE activity is very sensitive to variations in valency because phase separation is a cooperative phenomenon that occurs suddenly when a key variable exceeds a threshold value.

Transcriptional Bursting

Gene expression in eukaryotes is generally episodic, consisting of transcriptional bursts, and we investigated whether the phase-separation model can predict transcriptional bursting. A recent study using quantitative imaging of transcriptional bursting in live cells suggested that the level of gene expression driven by an enhancer correlates with the frequency of transcriptional bursting (Fukaya et al., 2016). Strong enhancers were found to drive higher frequency bursting than weak enhancers, and above a certain level of strength the bursts were not resolved anymore and resulted in a relatively constant high transcriptional activity (Figure 4A). The phase separation model shows that SEs recapitulate the high frequency with low variation (around a relatively constant high transcriptional activity) bursting pattern exhibited by strong enhancers while typical enhancers exhibit more variable bursts with a lower frequency (Figure 4B). Once sustained phase separation occurs (TA saturates), fluctuations are quenched, which results in lower variation in TA for SEs. This difference in bursting patterns can be quantified by translating our results to a power spectrum (data not shown). We expect that strong enhancers, in spite of having fewer components (N) than SEs will form stable phase-separated multi-molecular assemblies more readily than typical enhancers because of higher affinity cross-links. Therefore, a prediction of our model is that SEs, like strong enhancers, should display a different transcriptional bursting pattern compared to weak or typical enhancers.

The phase separation model is also broadly consistent with the intriguing observation that two promoters can exhibit synchronous bursting when activated by the same enhancer (Fukaya et al., 2016); in this case the phase-separated assembly incorporates the enhancer and both promoters (Figure 4C).

Candidate Transcriptional Regulators Forming the Phase-Separated Assembly In Vivo

In our simplified model, phase separation is mediated by changes in the extent to which residues on the interacting components are modified (or valency), with resulting intermolecular-interactions. In reality, however, enhancers are composed of many diverse factors that could account for such interactions, most of which are subject to reversible chemical modifications (Figure 5). These components include transcription factors,

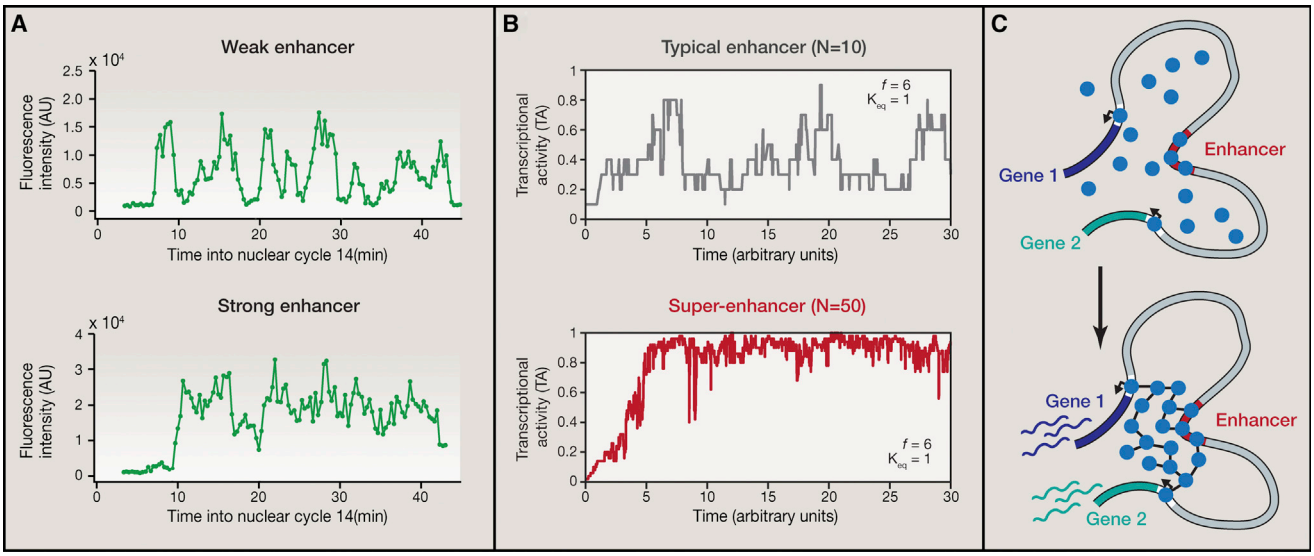

Figure 4. Transcriptional Bursting

(A) Representative traces of transcriptional activity in individual nuclei of *Drosophila* embryos. Transcriptional activity was measured by visualizing nascent RNAs using fluorescent probes. Top panel shows a representative trace produced by a weak enhancer, and the bottom panel shows a representative trace produced by a strong enhancer. Data and image adapted from Fukaya et al. (2016).

(B) Simulation of transcriptional activity (TA) of super-enhancers (N = 50 chains), and typical enhancers (N = 10 chains) over time recapitulates bursting behavior of weak and strong enhancers.

(C) Model of synchronous activation of two gene promoters by a shared enhancer.

transcriptional co-activators such as the Mediator complex and BRD4, chromatin regulators (e.g., readers, writers, and erasers of histone modifications), cyclin-dependent kinases (e.g., CDK7, CDK8, CDK9, and CDK12), non-coding RNAs with RNA-binding proteins, and RNA Pol II (Lai and Shiekhattar, 2014; Lee and Young, 2013; Levine et al., 2014; Malik and Roeder, 2010). Many of these molecules are multivalent, i.e., contain multiple modular domains or interaction motifs, and are thus able to interact with multiple other enhancer components. For example, the large subunit of RNA Pol II contains 52 repeats of a heptapeptide sequence at its C-terminal domain (CTD) in human cells, and several transcription factors contain repeats of low-complexity domains or repeats of the same amino-acid stretch prone to polymerization (Gemayel et al., 2015; Kwon et al., 2013). The DNA portion of enhancers and many promoters contain binding sites for multiple transcription factors, some of which can bind simultaneously to both DNA and RNA (Sigova et al., 2015). Histone proteins at enhancers are enriched for modifications that can be recognized by chromatin readers, and thus adjacent nucleosomes can be considered as a platform able to interact with multiple chromatin readers. RNA itself can be chemically modified and physically interact with multiple RNA-binding molecules and splicing factors. Many of the residues involved in these interactions can create a "cross-link" (Figure 5).

Possible Implications and Predictions of the Phase Separation Model

Our simple phase separation model provides a conceptual framework for further exploration of principles of gene control in development and disease. Below we discuss a few examples of phenomena possibly related to assemblies of phase separated multi-molecular complexes in transcriptional control and some testable predictions of the model.

Visualization of Phase-Separated Multi-molecular Assemblies of Transcriptional Regulators

A critical test of the model is whether phase separation of multi-molecular assemblies of transcriptional regulators can be directly observed in vivo, with the demonstration that phase separation of those complexes is associated with gene activity. Several lines of recent work provide initial insights into these questions. For example, recent studies using high-resolution microscopy indicate that signal stimulation leads to the formation of large clusters of RNA Pol II in living mammalian cells (Cisse et al., 2013) and concordant activation of transcription at a subset of genes (Cho et al., 2016). This, as well as other single molecule technologies (Chen and Larson, 2016; Shin et al., 2017), may thus enable visualization and testing of whether phase separated multi-molecular complexes form in the vicinity of genes regulated by SEs, and whether the simple model we describe here predicts features of transcriptional control. As an example, we hypothesize that the RNA Pol II C-terminal domain, which consists of 52 heptapeptide repeats, is a key contributor to the valency within this assembly, and in cells that express a RNA Pol II with a truncated CTD, the clusters would exhibit significantly lower half-lives.

Signal-Dependent Gene Control

Cells sense and respond to their environment through signal transduction pathways that relay information to genes, but genes responding to a particular signaling pathway may exhibit

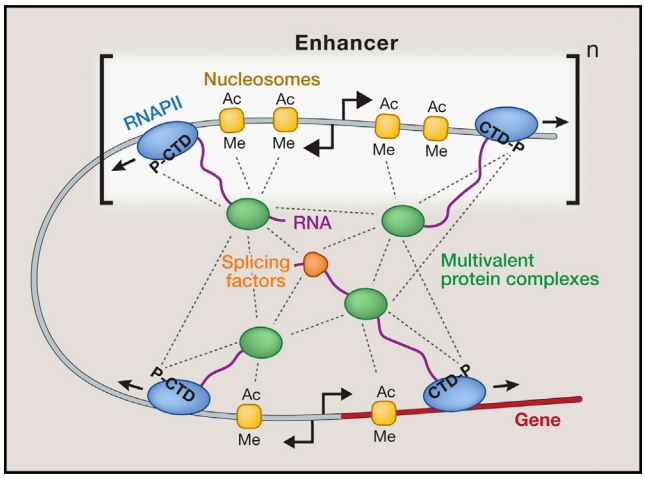

Figure 5. Transcriptional Control Phase Separation In Vivo
Model of a phase-separated complex at gene regulatory elements. Some of the candidate transcriptional regulators forming the complex are highlighted. P-CTD denotes the phosphorylated C-terminal domain of RNA Pol II. Chemical modifications of nucleosomes (acetylation, Ac; methylation, Me) are also highlighted. Divergent transcription at enhancers and promoters produces nascent RNAs that can be bound by RNA splicing factors. Potential interactions between the components are displayed as dashed lines.

different amplitudes of activation to the same signal. We have carried out calculations with the hypothesis that once phase separation occurs, the assembly recruits components that are de-modifiers. Under these conditions, transition to and resolution of phase separation, i.e., transcriptional activity, are more distinct for SEs compared to typical enhancers. Interestingly, such simulations suggest that there is a maximum valency and a maximum number of SE components, which if exceeded, does not allow disassembly in a realistic timescale (Figure S2). This is because the molecules are so heavily cross-linked that it remains in a metastable state for long periods of time. The prediction of the model is that pathological hyperactivation of cellular signaling could underlie disease states through locking cells in an expression program that—at least transiently—becomes unresponsive to signals that would counteract them under normal physiological conditions. We speculate that such states can be artificially induced by increasing the valency or number of interacting components.

Fidelity of Transcriptional Control

Variability in the transcript levels of genes within isogenic population of cells exposed to the same environmental signals—referred to as transcriptional noise—can have a profound impact on cellular phenotypes (Raj and van Oudenaarden, 2008). The phase separation model indicates that because of the high cooperativity involved in the formation of SEs, transcription occurs when the valency (modulated by the modifier/demodifier ratio, which is in fact similar to the developmental signals being transduced through activation cascades) exceeds a sharply defined threshold (Figure 2C). For the smaller number of components in a typical enhancer, the variation of transcription with the environmental signal is more continuous, potentially leading to "noisier" or more error-prone transcription over a wider range of signal strength. In the vicinity of a phase separation point, there are fluctuations between the two phases (low TA and robust TA in our case). Our model shows that these fluctuations (or noise) are confined to a narrow range of environmental signals for SEs compared to the broad range over which this occurs for a typical enhancer (Figure S3). The normalized amplitude of these fluctuations is also smaller for SEs. These results suggest that one reason why SEs have evolved is to enable relatively error free and robust transcription of genes necessary to maintain cell identity. This form of transcriptional fidelity through cooperativity, and not chemical specificity mediated by evolving specific molecules for controlling each gene, may however be co-opted to drive aberrant gene expression in disease states (e.g., SEs in cancer cells).

Resistance to Transcriptional Inhibition

Small molecule inhibitors of super-enhancer components such as BRD4 are currently being tested as anticancer therapeutics in the clinic, where a ubiquitous challenge has been the emergence of tumor cells resistant to the targeted therapeutic agent (Stathis et al., 2016). Interestingly, recent studies revealed that resistance to JQ1, a drug that inhibits BRD4, develops without any genetic changes in various tumor cells (Fong et al., 2015; Rathert et al., 2015; Shu et al., 2016). While JQ1 inhibits the interaction of BRD4 with acetylated histones, BRD4 is still recruited to super-enhancers due to its hyper-phosphorylation in JQ1-resistant cells (Shu et al., 2016). This is consistent with a prediction of our model that BRD4 is a high valency component of SEs, and inhibition of its interaction with acetylated histones (i.e., decrease of its valency) may be compensated for by increasing its valency through the activation of kinase pathways targeting BRD4 itself. In our model, super-enhancers are characterized by a high Hill coefficient, i.e., high cooperativity (Figure 2C), which suggests that inhibition of multiple properly chosen SE components might have a synergistic on effect SE-driven oncogenes in tumor cells. If this prediction is true, resistance to BRD4 inhibitors may be prevented through combined treatment with additional inhibitors of transcriptional regulators.

Concluding Remarks

The essential feature of this phase separation model of transcriptional control is that it considers cooperativity between the interacting components in the context of changes in valency and number of components. This single conceptual framework consistently describes diverse recently observed features of transcriptional control, such as clustering of factors, dynamic changes, hyper-sensitivity of SEs to transcriptional inhibitors, and simultaneous activation of multiple genes by the same enhancer. Cellular signaling pathways could modulate transcription over short time periods by alterations of valency. Selection of cell growth and survival would expand or contract the number of interactions or size of the enhancer over longer times. The model also makes a number of predictions (some noted above) that could be explored in many cellular contexts. Such studies, and others that will be envisaged, will help determine whether a variant of the model we propose underlies transcriptional control in mammals. Also, attractively, this model sets enhancer, and especially super-enhancer -type gene regulation into the broad

family of membraneless organelles, such as the nucleolus, Cajal bodies, and splicing-speckles in the nucleus, and stress granules and P bodies in the cytoplasm, as results of phase-separated multi-molecular assemblies.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one data file and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2017.02.007.

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REFERENCES

Banani, S.F., Lee, H.O., Hyman, A.A., and Rosen, M.K. (2017). Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Bio. Published online February 22, 2017. http://dx.doi.org/10.1038/nrm.2017.7.

Banerji, J., Rusconi, S., and Schaffner, W. (1981). Expression of a beta-globin gene is enhanced by remote SV40 DNA sequences. Cell *27*, 299–308.

Banjade, S., Wu, Q., Mittal, A., Peeples, W.B., Pappu, R.V., and Rosen, M.K. (2015). Conserved interdomain linker promotes phase separation of the multivalent adaptor protein Nck. Proc. Natl. Acad. Sci. USA *112*, E6426–E6435.

Benoist, C., and Chambon, P. (1981). In vivo sequence requirements of the SV40 early promotor region. Nature *290*, 304–310.

Bergeron-Sandoval, L.P., Safaee, N., and Michnick, S.W. (2016). Mechanisms and consequences of macromolecular phase separation. Cell *165*, 1067–1079.

Berry, J., Weber, S.C., Vaidya, N., Haataja, M., and Brangwynne, C.P. (2015). RNA transcription modulates phase transition-driven nuclear body assembly. Proc. Natl. Acad. Sci. USA *112*, E5237–E5245.

Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science *324*, 1729–1732.

Brown, J.D., Lin, C.Y., Duan, Q., Griffin, G., Federation, A.J., Paranal, R.M., Bair, S., Newton, G., Lichtman, A.H., Kung, A.L., et al. (2014). NF-κB directs dynamic super enhancer formation in inflammation and atherogenesis. Mol. Cell *56*, 219–231.

Bulger, M., and Groudine, M. (2011). Functional and mechanistic diversity of distal transcription enhancers. Cell *144*, 327–339.

Carey, M. (1998). The enhanceosome and transcriptional synergy. Cell *92*, 5–8.

Chapuy, B., McKeown, M.R., Lin, C.Y., Monti, S., Roemer, M.G., Qi, J., Rahl, P.B., Sun, H.H., Yeda, K.T., Doench, J.G., et al. (2013). Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. Cancer Cell *24*, 777–790.

Chen, H., and Larson, D.R. (2016). What have single-molecule studies taught us about gene expression? Genes Dev. *30*, 1796–1810.

Chipumuro, E., Marco, E., Christensen, C.L., Kwiatkowski, N., Zhang, T., Hatheway, C.M., Abraham, B.J., Sharma, B., Yeung, C., Altabef, A., et al. (2014). CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. Cell *159*, 1126–1139.

Cho, W.K., Jayanth, N., English, B.P., Inoue, T., Andrews, J.O., Conway, W., Grimm, J.B., Spille, J.H., Lavis, L.D., Lionnet, T., and Cisse, I.I. (2016). RNA Polymerase II cluster dynamics predict mRNA output in living cells. eLife *5*, 5.

Christensen, C.L., Kwiatkowski, N., Abraham, B.J., Carretero, J., Al-Shahrour, F., Zhang, T., Chipumuro, E., Herter-Sprie, G.S., Akbay, E.A., Altabef, A., et al. (2014). Targeting transcriptional addictions in small cell lung cancer with a covalent CDK7 inhibitor. Cancer Cell *26*, 909–922.

Cisse, I.I., Izeddin, I., Causse, S.Z., Boudarene, L., Senecal, A., Muresan, L., Dugast-Darzacq, C., Hajj, B., Dahan, M., and Darzacq, X. (2013). Real-time dynamics of RNA polymerase II clustering in live human cells. Science *341*, 664–667.

Cohen, R.J., and Benedek, G.B. (1982). Equilibrium and kinetic theory of polymerization and the sol-gel transition. J. Phys. Chem. *86*, 3696–3714.

Dimitrova, N., Zamudio, J.R., Jong, R.M., Soukup, D., Resnick, R., Sarma, K., Ward, A.J., Raj, A., Lee, J.T., Sharp, P.A., and Jacks, T. (2014). LincRNA-p21 activates p21 in cis to promote Polycomb target gene expression and to enforce the G1/S checkpoint. Mol. Cell *54*, 777–790.

Dowen, J.M., Fan, Z.P., Hnisz, D., Ren, G., Abraham, B.J., Zhang, L.N., Weintraub, A.S., Schuijers, J., Lee, T.I., Zhao, K., and Young, R.A. (2014). Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. Cell *159*, 374–387.

Elowitz, M.B., Levine, A.J., Siggia, E.D., and Swain, P.S. (2002). Stochastic gene expression in a single cell. Science *297*, 1183–1186.

ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. Nature *489*, 57–74.

Engreitz, J.M., Haines, J.E., Perez, E.M., Munson, G., Chen, J., Kane, M., McDonel, P.E., Guttman, M., and Lander, E.S. (2016). Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature *539*, 452–455.

Falvo, J.V., Thanos, D., and Maniatis, T. (1995). Reversal of intrinsic DNA bends in the IFN beta gene enhancer by transcription factors and the architectural protein HMG I(Y). Cell *83*, 1101–1111.

Feric, M., Vaidya, N., Harmon, T.S., Mitrea, D.M., Zhu, L., Richardson, T.M., Kriwacki, R.W., Pappu, R.V., and Brangwynne, C.P. (2016). Coexisting liquid phases underlie nucleolar subcompartments. Cell *165*, 1686–1697.

Fong, C.Y., Gilan, O., Lam, E.Y., Rubin, A.F., Ftouni, S., Tyler, D., Stanley, K., Sinha, D., Yeh, P., Morison, J., et al. (2015). BET inhibitor resistance emerges from leukaemia stem cells. Nature *525*, 538–542.

Fukaya, T., Lim, B., and Levine, M. (2016). Enhancer control of transcriptional bursting. Cell *166*, 358–368.

Gemayel, R., Chavali, S., Pougach, K., Legendre, M., Zhu, B., Boeynaems, S., van der Zande, E., Gevaert, K., Rousseau, F., Schymkowitz, J., et al. (2015). Variable glutamine-rich repeats modulate transcription factor activity. Mol. Cell *59*, 615–627.

Gillespie, D.T. (1977). Exact stochastic simulation of coupled chemical reactions. J. Phys. Chem. *81*, 2340–2361.

Gruss, P., Dhar, R., and Khoury, G. (1981). Simian virus 40 tandem repeated sequences as an element of the early promoter. Proc. Natl. Acad. Sci. USA *78*, 943–947.

Hah, N., Benner, C., Chong, L.W., Yu, R.T., Downes, M., and Evans, R.M. (2015). Inflammation-sensitive super enhancers form domains of coordinately regulated enhancer RNAs. Proc. Natl. Acad. Sci. USA *112*, E297–E302.

Han, T.W., Kato, M., Xie, S., Wu, L.C., Mirzaei, H., Pei, J., Chen, M., Xie, Y., Allen, J., Xiao, G., and McKnight, S.L. (2012). Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. Cell *149*, 768–779.

Hay, D., Hughes, J.R., Babbs, C., Davies, J.O., Graham, B.J., Hanssen, L.L., Kassouf, M.T., Oudelaar, A.M., Sharpe, J.A., Suciu, M.C., et al. (2016). Genetic dissection of the α-globin super-enhancer in vivo. Nat. Genet. *48*, 895–903.

Hnisz, D., Abraham, B.J., Lee, T.I., Lau, A., Saint-André, V., Sigova, A.A., Hoke, H.A., and Young, R.A. (2013). Super-enhancers in the control of cell identity and disease. Cell *155*, 934–947.

Hnisz, D., Schuijers, J., Lin, C.Y., Weintraub, A.S., Abraham, B.J., Lee, T.I., Bradner, J.E., and Young, R.A. (2015). Convergence of developmental and oncogenic signaling pathways at transcriptional super-enhancers. Mol. Cell *58*, 362–370.

Hnisz, D., Weintraub, A.S., Day, D.S., Valton, A.L., Bak, R.O., Li, C.H., Goldmann, J., Lajoie, B.R., Fan, Z.P., Sigova, A.A., et al. (2016). Activation of proto-oncogenes by disruption of chromosome neighborhoods. Science *351*, 1454–1458.

Ji, X., Dadon, D.B., Powell, B.E., Fan, Z.P., Borges-Rivera, D., Shachar, S., Weintraub, A.S., Hnisz, D., Pegoraro, G., Lee, T.I., et al. (2016). 3D chromosome regulatory landscape of human pluripotent cells. Cell Stem Cell *18*, 262–275.

Jiang, T., Raviram, R., Snetkova, V., Rocha, P.P., Proudhon, C., Badri, S., Bonneau, R., Skok, J.A., and Kluger, Y. (2016). Identification of multi-loci hubs from 4C-seq demonstrates the functional importance of simultaneous interactions. Nucleic Acids Res. *44*, 8714–8725.

Johnson, A.D., Meyer, B.J., and Ptashne, M. (1979). Interactions between DNA-bound repressors govern regulation by the lambda phage repressor. Proc. Natl. Acad. Sci. USA *76*, 5061–5065.

Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J., et al. (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell *149*, 753–767.

Kieffer-Kwon, K.R., Tang, Z., Mathe, E., Qian, J., Sung, M.H., Li, G., Resch, W., Baek, S., Pruett, N., Grøntved, L., et al. (2013). Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. Cell *155*, 1507–1520.

Kim, T.K., and Maniatis, T. (1997). The mechanism of transcriptional synergy of an in vitro assembled interferon-beta enhanceosome. Mol. Cell *1*, 119–129.

Kundaje, A., Meuleman, W., Ernst, J., Bilenky, M., Yen, A., Heravi-Moussavi, A., Kheradpour, P., Zhang, Z., Wang, J., Ziller, M.J., et al.; Roadmap Epigenomics Consortium (2015). Integrative analysis of 111 reference human epigenomes. Nature *518*, 317–330.

Kwiatkowski, N., Zhang, T., Rahl, P.B., Abraham, B.J., Reddy, J., Ficarro, S.B., Dastur, A., Amzallag, A., Ramaswamy, S., Tesar, B., et al. (2014). Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. Nature *511*, 616–620.

Kwon, I., Kato, M., Xiang, S., Wu, L., Theodoropoulos, P., Mirzaei, H., Han, T., Xie, S., Corden, J.L., and McKnight, S.L. (2013). Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains. Cell *155*, 1049–1060.

Lai, F., and Shiekhattar, R. (2014). Enhancer RNAs: the new molecules of transcription. Curr. Opin. Genet. Dev. *25*, 38–42.

Lai, F., Orom, U.A., Cesaroni, M., Beringer, M., Taatjes, D.J., Blobel, G.A., and Shiekhattar, R. (2013). Activating RNAs associate with mediator to enhance chromatin architecture and transcription. Nature *494*, 497–501.

Larochelle, S., Amat, R., Glover-Cutter, K., Sansó, M., Zhang, C., Allen, J.J., Shokat, K.M., Bentley, D.L., and Fisher, R.P. (2012). Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. Nat. Struct. Mol. Biol. *19*, 1108–1115.

Lee, T.I., and Young, R.A. (2013). Transcriptional regulation and its misregulation in disease. Cell *152*, 1237–1251.

Levine, M., Cattoglio, C., and Tjian, R. (2014). Looping back to leap forward: transcription enters a new era. Cell *157*, 13–25.

Li, P., Banjade, S., Cheng, H.C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., et al. (2012). Phase transitions in the assembly of multivalent signalling proteins. Nature *483*, 336–340.

Lin, C.Y., Lovén, J., Rahl, P.B., Paranal, R.M., Burge, C.B., Bradner, J.E., Lee, T.I., and Young, R.A. (2012). Transcriptional amplification in tumor cells with elevated c-Myc. Cell *151*, 56–67.

Lovén, J., Hoke, H.A., Lin, C.Y., Lau, A., Orlando, D.A., Vakoc, C.R., Bradner, J.E., Lee, T.I., and Young, R.A. (2013). Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell *153*, 320–334.

Malik, S., and Roeder, R.G. (2010). The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. Nat. Rev. Genet. *11*, 761–772.

Mansour, M.R., Abraham, B.J., Anders, L., Berezovskaya, A., Gutierrez, A., Durbin, A.D., Etchin, J., Lawton, L., Sallan, S.E., Silverman, L.B., et al. (2014). Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. Science *346*, 1373–1377.

Mao, Y.S., Zhang, B., and Spector, D.L. (2011). Biogenesis and function of nuclear bodies. Trends Genet. *27*, 295–306.

Merika, M., Williams, A.J., Chen, G., Collins, T., and Thanos, D. (1998). Recruitment of CBP/p300 by the IFN beta enhanceosome is required for synergistic activation of transcription. Mol. Cell *1*, 277–287.

Nie, Z., Hu, G., Wei, G., Cui, K., Yamane, A., Resch, W., Wang, R., Green, D.R., Tessarollo, L., Casellas, R., et al. (2012). c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell *151*, 68–79.

Ong, C.T., and Corces, V.G. (2011). Enhancer function: new insights into the regulation of tissue-specific gene expression. Nat. Rev. Genet. *12*, 283–293.

Orphanides, G., and Reinberg, D. (2002). A unified theory of gene expression. Cell *108*, 439–451.

Palstra, R.J., Tolhuis, B., Splinter, E., Nijmeijer, R., Grosveld, F., and de Laat, W. (2003). The beta-globin nuclear compartment in development and erythroid differentiation. Nat. Genet. *35*, 190–194.

Parker, S.C., Stitzel, M.L., Taylor, D.L., Orozco, J.M., Erdos, M.R., Akiyama, J.A., van Bueren, K.L., Chines, P.S., Narisu, N., Black, B.L., et al.; NISC Comparative Sequencing Program; National Institutes of Health Intramural Sequencing Center Comparative Sequencing Program Authors; NISC Comparative Sequencing Program Authors (2013). Chromatin stretch enhancer states drive cell-specific gene regulation and harbor human disease risk variants. Proc. Natl. Acad. Sci. USA *110*, 17921–17926.

Pefanis, E., Wang, J., Rothschild, G., Lim, J., Kazadi, D., Sun, J., Federation, A., Chao, J., Elliott, O., Liu, Z.P., et al. (2015). RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity. Cell *161*, 774–789.

Phatnani, H.P., and Greenleaf, A.L. (2006). Phosphorylation and functions of the RNA polymerase II CTD. Genes Dev. *20*, 2922–2936.

Proudhon, C., Snetkova, V., Raviram, R., Lobry, C., Badri, S., Jiang, T., Hao, B., Trimarchi, T., Kluger, Y., Aifantis, I., et al. (2016). Active and Inactive Enhancers Cooperate to Exert Localized and Long-Range Control of Gene Regulation. Cell Rep. *15*, 2159–2169.

Raj, A., and van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. Cell *135*, 216–226.

Raser, J.M., and O'Shea, E.K. (2004). Control of stochasticity in eukaryotic gene expression. Science *304*, 1811–1814.

Rathert, P., Roth, M., Neumann, T., Muerdter, F., Roe, J.S., Muhar, M., Deswal, S., Cerny-Reiterer, S., Peter, B., Jude, J., et al. (2015). Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. Nature *525*, 543–547.

Semenov, A.N., and Rubinstein, M. (1998). Thermoreversible gelation in solutions of associative polymers. 1. Statics. Macromolecules *31*, 1373–1385.

Shin, H.Y., Willi, M., Yoo, K.H., Zeng, X., Wang, C., Metser, G., and Hennighausen, L. (2016). Hierarchy within the mammary STAT5-driven Wap super-enhancer. Nat. Genet. *48*, 904–911.

Shin, Y., Berry, J., Pannucci, N., Haataja, M.P., Toettcher, J.E., and Brangwynne, C.P. (2017). Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. Cell *168*, 159–171.

Shu, S., Lin, C.Y., He, H.H., Witwicki, R.M., Tabassum, D.P., Roberts, J.M., Janiszewska, M., Huh, S.J., Liang, Y., Ryan, J., et al. (2016). Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer. Nature *529*, 413–417.

Sigova, A.A., Mullen, A.C., Molinie, B., Gupta, S., Orlando, D.A., Guenther, M.G., Almada, A.E., Lin, C., Sharp, P.A., Giallourakis, C.C., and Young, R.A. (2013). Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells. Proc. Natl. Acad. Sci. USA *110*, 2876–2881.

Sigova, A.A., Abraham, B.J., Ji, X., Molinie, B., Hannett, N.M., Guo, Y.E., Jangi, M., Giallourakis, C.C., Sharp, P.A., and Young, R.A. (2015). Transcription factor trapping by RNA in gene regulatory elements. Science *350*, 978–981.

Spitz, F., and Furlong, E.E. (2012). Transcription factors: from enhancer binding to developmental control. Nat. Rev. Genet. *13*, 613–626.

Stathis, A., Zucca, E., Bekradda, M., Gomez-Roca, C., Delord, J.P., de La Motte Rouge, T., Uro-Coste, E., de Braud, F., Pelosi, G., and French, C.A. (2016). Clinical response of carcinomas harboring the BRD4-NUT oncoprotein to the targeted bromodomain inhibitor OTX015/MK-8628. Cancer Discov. *6*, 492–500.

Suter, D.M., Molina, N., Gatfield, D., Schneider, K., Schibler, U., and Naef, F. (2011). Mammalian genes are transcribed with widely different bursting kinetics. Science *332*, 472–474.

Thanos, D., and Maniatis, T. (1995). Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. Cell *83*, 1091–1100.

Tjian, R., and Maniatis, T. (1994). Transcriptional activation: a complex puzzle with few easy pieces. Cell *77*, 5–8.

Tolhuis, B., Palstra, R.J., Splinter, E., Grosveld, F., and de Laat, W. (2002). Looping and interaction between hypersensitive sites in the active beta-globin locus. Mol. Cell *10*, 1453–1465.

Wang, Y., Zhang, T., Kwiatkowski, N., Abraham, B.J., Lee, T.I., Xie, S., Yuzugullu, H., Von, T., Li, H., Lin, Z., et al. (2015). CDK7-dependent transcriptional addiction in triple-negative breast cancer. Cell *163*, 174–186.

Wheeler, J.R., Matheny, T., Jain, S., Abrisch, R., and Parker, R. (2016). Distinct stages in stress granule assembly and disassembly. eLife *5*, 5.

Whyte, W.A., Orlando, D.A., Hnisz, D., Abraham, B.J., Lin, C.Y., Kagey, M.H., Rahl, P.B., Lee, T.I., and Young, R.A. (2013). Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell *153*, 307–319.

Zhu, L., and Brangwynne, C.P. (2015). Nuclear bodies: the emerging biophysics of nucleoplasmic phases. Curr. Opin. Cell Biol. *34*, 23–30.

Zoller, B., Nicolas, D., Molina, N., and Naef, F. (2015). Structure of silent transcription intervals and noise characteristics of mammalian genes. Mol. Syst. Biol. *11*, 823.