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Review

An RNA-centric view of transcription and genome organization

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SUMMARY

Foundational models of transcriptional regulation involve the assembly of protein complexes at DNA elements associated with specific genes. These assemblies, which can include transcription factors, cofactors, RNA polymerase, and various chromatin regulators, form dynamic spatial compartments that contribute to both gene regulation and local genome architecture. This DNA-protein-centric view has been modified with recent evidence that RNA molecules have important roles to play in gene regulation and genome structure. Here, we discuss evidence that gene regulation by RNA occurs at multiple levels that include assembly of transcriptional complexes and genome compartments, feedback regulation of active genes, silencing of genes, and control of protein kinases. We thus provide an RNA-centric view of transcriptional regulation that must reside alongside the more traditional DNA-protein-centric perspectives on gene regulation and genome architecture.

INTRODUCTION

Gene regulation, fundamental to all living processes, has been studied largely from the perspective of DNA-protein and protein-protein interactions.[1–7] As a consequence, the canonical narrative of gene regulation is focused on these interactions. Multiple transcription factor (TF) proteins bind selectively to regulatory DNA elements called enhancers and promoters, facilitated by nucleosome mobilizing enzymes, and recruit components of the transcription apparatus. This DNA-protein assembly is then regulated by various proteins, including kinases and other enzymes, allowing the assembled RNA polymerase II (RNAPII) to undergo multiple steps in transcription initiation, pausing, and processive elongation.

Reports of RNA molecules associated with DNA regulatory elements began to emerge decades ago.[8–12] This stimulated a lively discussion about the possibility that these RNAs were simply a product of promiscuous transcription and had no real regulatory function or whether they actually contribute functionally to gene regulation.[13] Then, it was recognized that enhancers and promoters were often bi-directionally transcribed, which stimulated additional discussion about the underlying mechanisms and potential roles of RNA molecules.[14–16] Evidence that transcription of genes often involves a transcriptional pause,[17,18] where an RNAPII molecule stops ~100 bp downstream of the promoter with a nascent RNA molecule, also led to speculation about the potential role of the nascent RNA molecule in gene regulation.[5,19–23] Thus, investigators reported diverse types of RNA molecules from enhancers, promoters, and pause sites, but the mechanisms by which these might regulate genes were unclear. The lack of evolutionary conservation of enhancer and promoter sequences made it challenging to make a compelling argument for a conserved function of these RNA molecules. By contrast, the mechanisms by which many other RNA molecules contribute to regulatory phenomena, such as microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs), became relatively well established.[24,25]

Here, we discuss recent evidence and insights, aided by recent technological advances (Box 1), which lead to the conclusion that RNA molecules are involved in multiple regulatory mechanisms contributing to gene activation, gene repression, and genome structure. We review evidence that enhancers are transcribed and that enhancer RNAs bind and assemble a plethora of TFs, cofactors, and chromatin regulators. RNA is now known to be a feedback regulator of transcription and may contribute to the discontinuous or burst-like behavior of gene expression. Some RNA molecules attract the gene silencing machinery in heterochromatin and others repress the activity of kinases necessary for gene transcription. We discuss why classical genetic studies provided limited clues to these RNA functions and why more recent genetic and molecular approaches have been more informative. The new concepts that emerge for RNA-mediated regulation create a foundation for future studies that promise to advance our understanding of gene regulation and its dysregulation in disease.

ACTIVE REGULATORY ELEMENTS ARE TRANSCRIBED

The development of nascent RNA sequencing technologies[12,22,26] revealed that transcription of regulatory DNA elements is a ubiquitous feature of the regulatory landscape that is not captured in typical RNA sequencing. Thus, we now know

Box 1. Impact of technological advances on RNA-associated concepts

Recent technological advances have provided new insights into the roles of RNA molecules in transcriptional regulation. Improvements to nascent and single-cell RNA sequencing, as well as high-throughput mass spectrometry, have mapped the genomic landscape of transcribed RNAs and RNA-interacting proteins.[22,26–30] Rapid protein degradation coupled to high-throughput genomics has revealed hidden regulatory features on timescales approaching real-time cellular events.[31–33] Super-resolution and single-molecule microscopy have enabled unprecedented visualization and quantification of dynamic transcriptional processes.[34–38] New RNA aptamers and *in situ* hybridization techniques have revealed RNA distribution and RNA processing in cells.[39–42] High-throughput interrogation of genome structure that integrates multiway contacts between DNA, RNA, and protein has mapped genome organization with increasing spatial resolution.[43–46] Observations derived from these techniques have led investigators to reconsider canonical models of transcriptional regulation and to propose new conceptual models. For example, they have revealed that RNA molecules are not uniformly distributed throughout the nucleus but instead form spatial compartments.[43] Like regulatory DNA sequences, specific RNA sequences have been found to regulate transcriptional output.[47–50] RNA binds to many components of the transcriptional apparatus and regulates the formation and dissolution of large, dynamic assemblies called transcriptional condensates,[51,52] which physically influence genome architecture.[53–56] Some of these observations challenge canonical views of how proteins and DNA regulate transcription,[31,57–59] and they highlight how nascent RNA extruding from active RNA polymerase molecules can function as a feedback regulator to fine-tune transcriptional output.

that virtually all active enhancers and promoters are transcribed[60–62] and that enhancer transcription may be the best indicator of an enhancer's ability to activate genes.[10,60,63–65] The human genome is thought to harbor ~1 million enhancers, promoters for ~20,000 protein-coding genes, and a similar number of long non-coding RNAs (ncRNAs).[66–68] In any one cell type, it is possible that nearly 100,000 enhancers and promoters are transcribed.

Transcription of regulatory elements often occurs bidirectionally; RNA species are typically transcribed divergently from transcription initiation sites[14,15,69] (Figure 1). Some of these RNAs have long half-lives and others short half-lives.[68,70–74] The more stable RNA species that emerge from bidirectional transcription of a locus appear to be stabilized by multiple mechanisms, which include RNA processing and a paucity of the polyadenylation signals that disrupt transcription elongation.[47,48,75–77] The more stable pre-mRNAs or ncRNAs that are destined for the cytoplasm are bundled into ribonuclear protein (RNP) complexes and transported to the cytoplasm, where their half-lives are 20 min to hours.[72] The relatively stable long-noncoding RNAs (lncRNAs), which are often transcribed from enhancer elements,[78] are generally retained at the locus of synthesis and form compartments of RNP complexes.[43] The other, less stable transcripts are often relatively short and, for enhancer RNAs, appear to have a half-life that averages ~7–18 min.[9,76] Although this half-life is short relative to other types of transcripts, these transcripts exist long enough to interact with proteins, where such binding events take place in the order of seconds.[79]

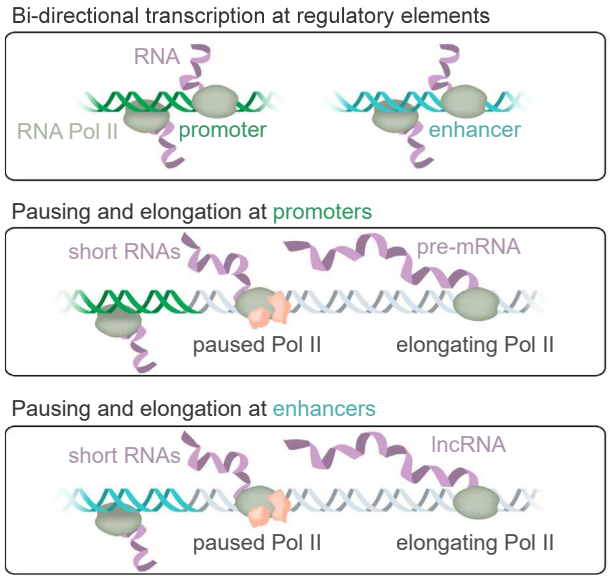
RNA MOLECULES REGULATE TRANSCRIPTION

In prokaryotes and viruses, RNA molecules serve prominent roles in transcriptional regulation. In bacteria, intrinsic terminator sequences are transcribed by elongating RNAP and cause formation of specific RNA secondary structures called terminator hairpins that plug the RNA exit channel of RNAP. Terminator hairpin formation is thought to melt 3–4 base pairs of the upstream RNA-DNA hybrid, which stalls RNAP and destabilizes the elongation complex, promoting its detachment from DNA.[80] Such termination mechanisms are a key component of bacterial riboswitches, a class of RNA molecules that undergo conformational changes upon binding to specific cofactors—including proteins, RNAs, and metabolites—to regulate transcription and post-transcriptional processes.[81] In many cases, the riboswitch and cofactor belong to the same biochemical pathway, thus providing a natural feedback mechanism. RNA molecules are also essential for transcriptional regulation and replication of viruses, including the human immunodeficiency virus 1 (HIV1). The HIV1 transcriptional activator (Tat) binds to a specific hairpin loop and bulge structure transcribed from the 5′ long terminal repeat of the HIV1 genome, called the trans-activation response (TAR) element, to stimulate viral genomic transcription.[82] Tat also binds to similar loop-bulge structures in the human 7SK ncRNA, which normally represses transcription elongation by sequestering the positive transcription elongation factor (P-TEFb) complex.[83] It is thought that Tat stimulates transcription elongation of the HIV1 genome by both binding the nascently transcribed TAR element and by releasing P-TEFb from the 7SK repressive complex.[82–84]

Common themes emerge from these examples in prokaryotes and viruses that highlight the importance of integrating a consideration of RNA into models of transcription regulation. RNA-mediated transcriptional regulation is typically part of autoregulatory feedback networks that enable the cell or virus to quickly respond to changes in the environment.[85] The mechanisms controlling such feedback usually involve specific RNA secondary structures, the most common being hairpin loops, where the terminal loops form complex and specific tertiary structures. Many of these structures are specified in untranslated regions early in genes and operons, and they help control the processivity of RNAP. An open question is the extent to which similar mechanisms are at play in eukaryotic transcription.

In eukaryotes, the pervasive transcription of genomic regulatory elements, as well as the observation that transcription of these elements is the best correlate for function,[60] has prompted many studies into roles for RNA molecules in eukaryotic transcription.[63,86–96] The consensus from perturbation studies is that, across a wide range of cell types, the perturbation of RNA

A



B

Types of RNAs transcribed from active regulatory elements

Type	Acronyms	Estimated number	Half-life	Processing features
pre-messenger RNA	pre-mRNA	19,954	minutes to hours	capped spliced polyA tail
long non-coding RNA	lncRNA lincRNA	30,000	minutes to hours	capped spliced polyA tail
enhancer RNA	eRNA	40,000-65,000	minutes	capped
promoter -associated RNA	uaRNA paRNA PROMPT	Gene and lncRNA promoters	minutes	capped

Figure 1. Active regulatory elements are transcribed

(A) Depiction of the types of gene regulatory elements that exist within gene neighborhoods and are bidirectionally transcribed (RNAPII, RNA polymerase II; pre-mRNA, pre-messenger RNA; lncRNA, long non-coding RNA).

(B) Table of types of RNAs transcribed from gene regulatory elements, including their features and half-lives.[68,70–73] Note that there are many additional types of RNAs, such as short stable miRNAs derived from processed RNA precursors, which are not presented here (uaRNA, upstream antisense RNA; paRNA, promoter-associated RNA; PROMPT, promoter upstream transcript).

molecules near transcribed genes disturbs proper genic transcription. In some cases, these effects seem to target specific stages of transcription, notably the pausing and pause-release of RNAPII that occurs within the first 100 nt of transcription.[48,94] The techniques that have been used to perturb RNA include knockdown by small interfering RNAs and antisense oligos, mutation of transcription start sites, introduction of strong termination signals, and swapping of RNA sequences.[63,86,91,97] The results of these studies can be challenging to interpret because they can disrupt highly dynamic features of transcription that occur on shorter timescales than are typically measured and the results may sometimes reflect downstream and indirect effects.[98] Nonetheless, there are now many examples where there is compelling evidence for RNA molecules that regulate gene transcription in eukaryotes.[47–49,63,86,91,97,99–101]

Common and instructive themes emerge from the study of RNA molecules in transcription. RNAs synthesized at regulatory elements impact the transcription of genes in their spatial proximity. The nature of this regulation is very dynamic: regulatory RNAs are fairly short-lived and the processivity of RNAPII, one of the strongest motors in the cell, is rapid. The timescales of these dynamics are still an order of magnitude longer than typical binding events for proteins and nucleic acids in the cell, which occur within seconds.[79] Thus, there is ample opportunity for transcribed RNAs, even those that are relatively unstable, to interact with proteins. Inspired by the biology of bacteria and viruses, it may be that the bulk of regulatory potential in RNA molecules exists early in their sequence to enable maximal impact on early stages of transcription, RNAPII pausing, and premature termination. In the following sections, we highlight recent work centered on emerging themes that describe sequence-specific and non-sequence-specific mechanisms by which RNA molecules regulate transcription.

RNA MOLECULES ARE BOUND BY TFs AND COFACTORS

In canonical models of transcription, TFs bind enhancer and promoter-proximal DNA sequences and recruit the transcriptional apparatus. Early reports noted that some eukaryotic TFs can also bind RNA[102,103] and, in some cases, linked RNA binding to TF function.[104–111] High-throughput mass spectrometry of proteins capable of binding RNA suggested that some DNA-binding proteins lacking canonical structured RNA-binding domains might be moonlighting as RNA-binding proteins.[27,112] More recently, a systematic effort revealed that most TFs bind RNA in cells through a conserved arginine-rich motif (ARM) domain[51] (Figure 2). This ARM domain was originally described in the HIV1 TAT TF, which binds the RNA genome of the virus to activate its transcription. The TF ARM domain is important for normal chromatin occupancy by TFs and, when mutated, causes developmental defects and disease.[51] The TFs tend to bind enhancer- and promoter-derived RNAs produced in proximity to where the TFs bind DNA, indicating that locally produced RNAs contribute to TF occupancy.[51,88,106,113,114]

TFs regulate genes by recruiting transcriptional coactivators and corepressors to specific loci. These cofactors, defined as factors involved in gene regulation but lacking DNA-binding domains, are often multisubunit complexes that regulate transcription through various mechanisms, including mobilizing

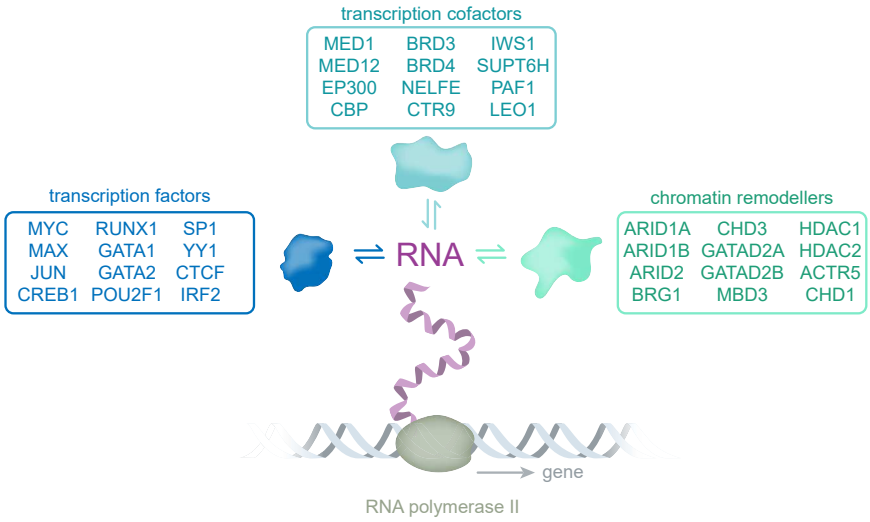


Figure 2. RNA molecules are bound by transcription factors, cofactors, and chromatin remodelers

Many of the protein factors involved in transcription bind RNA at active genes. Boxes include examples of RNA-binding proteins, derived from a study by Oksuz et al.[51] and corroborated by previous studies cited throughout the review.

nucleosomes, modifying chromatin-associated proteins, altering genome structure, recruiting RNAPII, and regulating aspects of transcription initiation and elongation.[6,115,116] Although most studies of cofactor function have focused on protein-protein interactions, many components of cofactor complexes bind RNA in cells.[51,88,94,113,114,117–122] These include components of the Mediator complex, BRD4, CBP, NELF, and members of the SWI/SNF, NuRD, and INO80 chromatin remodeling complexes (Figure 2). Like TFs, most of the RNA-binding cofactors contain ARM domains.[51] Studies have identified RNA-binding regions in the coactivators BRD4[113] and CBP,[114] which appear to overlap the bromodomains of BRD4 or the histone acetyltransferase domain of CBP. These regions are enriched with positively charged basic residues, which share similar properties to ARMs. It is notable that RNA binding can modulate cofactor function. For instance, MED12, part of the kinase module of the Mediator complex, binds to specific ncRNAs, which stimulates histone H3 serine 10 phosphorylation.[88] In a similar manner, RNA binding by CBP stimulates its ability to acetylate histones.[114] RNA can also act as a competitive inhibitor. For instance, nascent RNA binds to the RNAPII pause factor NELF to release it from RNAPII, which helps stimulate transcription elongation.[94,117] These studies demonstrate that many cofactors bind RNA, which modulates their function, suggesting that RNA molecules can act as allosteric regulators of enzymes involved in transcription.

The theme that emerges from these studies is that TFs and their cofactors are frequently able to bind RNA, doing so, at least in part, through a domain that has only been recently recognized.[51] For specific TFs studied so far, this ARM domain makes a positive contribution to the lifetime of the TFs' interaction with regulatory loci that produce RNA.[51]

ACTIVELY TRANSCRIBED LOCI NUCLEATE ASSEMBLY OF PROTEINS AND RNA INTO COMPARTMENTS

Many active genes are regulated by multiple enhancers, and genes with prominent roles in cell identity typically have large clusters of enhancers.[123,124] Several lines of evidence indicate that collections of active enhancers and promoters assemble large amounts of transcription apparatus, forming transcriptional compartments that have been called condensates, hubs, or microcompartments (Figure 3). DNA contact data and imaging studies indicate that active enhancer and promoter DNA sequences come into proximity with one another,[53,125–133] which predicts activity.[134] Other studies have found in specific cases that enhancers and promoters increase their spatial distance upon gene activation.[135,136] Although the observed distances in these studies (250–600 nm) are inconsistent with the view that enhancers and promoters make direct contact, they are consistent with the observed sizes of condensates that are likely forming at these genes and regulatory elements.[137,138] Chromatin immunoprecipitation sequencing (ChIP-seq) data indicate that clusters of enhancer and promoter sequences can be occupied by exceptionally high levels of TFs, cofactors, and RNAPII, and RNA analysis indicates that there are high levels of enhancer RNAs at the enhancer loci.[123,124] Transcription of these elements seems to be important for establishing and maintaining enhancer-promoter contacts.[139,140] Imaging of endogenous tagged cofactors shows that they assemble into dynamic condensates at these loci,[34,137,138,141–143] and quantification reveals large numbers of cofactor and RNAPII molecules clustered into a typical transcriptional condensate in live cells.[35,138] As expected for dynamic assemblies, condensates can be observed to fuse and fission. Recent studies with advanced super-resolution imaging methods suggest that condensates formed at clustered enhancer elements can transiently "kiss" the apparatus assembled at promoters and that the frequency of this interaction may be related to transcriptional output.[34,36]

Most cellular functions involve formation of large protein assemblies that have been termed biomolecular condensates.[144–147] The term condensate, as used here, implies that multiple biomolecules assemble due to favorable interactions that overcome entropy; this may be a consequence of microphase or phase separation but other mechanisms may be involved. Condensate formation is now thought to be involved in the assembly of proteins involved in most cellular functions, including, but not limited to, chromatin state, DNA repair, transcription, RNA processing, ribosome biosynthesis, translation, signaling, and metabolism.[141,148–156] Many condensates harbor RNA components and these can influence the formation and stability of the assembly,[157,158] as discussed below. Because condensate biology is involved in multiple biological processes,

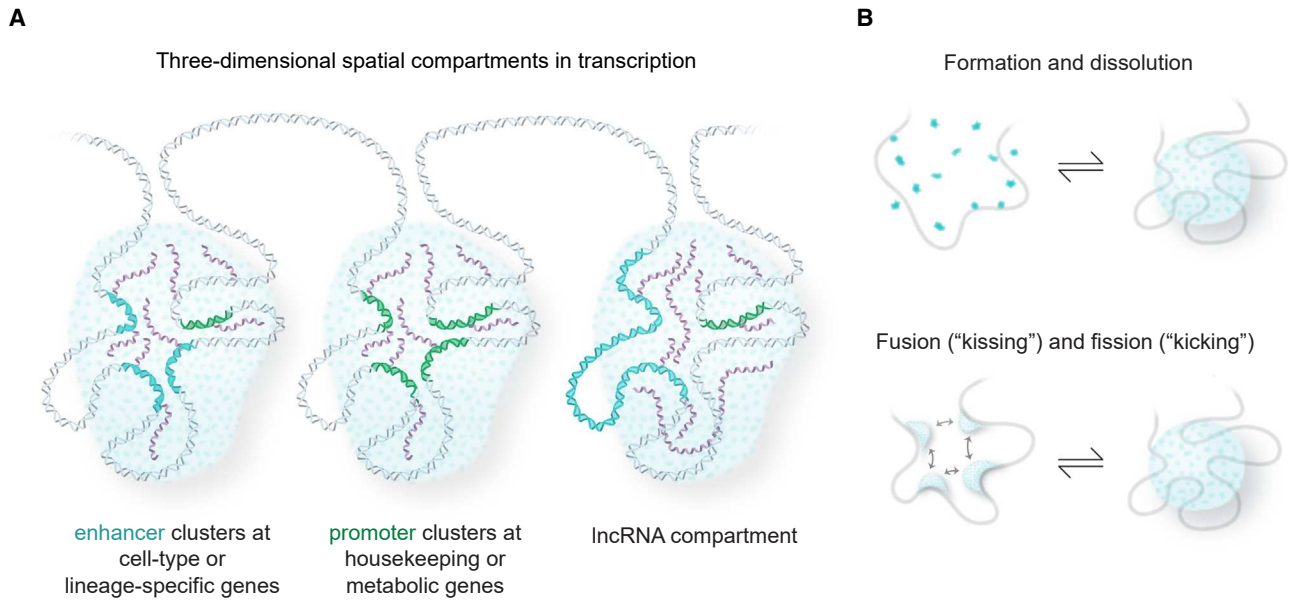


Figure 3. Actively transcribed loci nucleate assembly of proteins into compartments
(A) Types of spatial compartments formed by interactions between protein, DNA, and RNA molecules at active genes.
(B) Principles underlying the formation, dissolution, and dynamics of spatial compartments, mediated by condensates.

specific aspects of condensate biology will be highlighted in relevant parts in the remaining sections.

The genes transcribed by mammalian cells can be roughly divided into two classes: those that have cell-type-specific or lineage-specific roles and those that are active in most cells. The cell-type-specific genes are generally regulated by enhancers and the cell-type-specific TFs that bind them. The genes that are generally active in all cells, called "housekeeping genes," which encode essential metabolic and maintenance functions, often lack cell-specific enhancers. A recent study has shown that the promoters of housekeeping genes, which are clustered in the genome, are bundled into proximity by the TF Ronin.[159] Ronin binds an ultraconserved element in these promoters and acts as a promoter tethering factor. The clustered promoters of housekeeping genes, like the clustered enhancers, form transcriptional condensates.[159] Enhancers and promoter elements share the property of bidirectional transcription initiation and functional features; indeed, the promoters of some genes can function as enhancers for other genes and enhancers contain the transcription initiation sites that have classically been used to define promoters.[160] Thus, it is now understood that clustering of enhancers and clustering of promoters leads to formation of transcriptional condensates[161] (Figure 3).

The human genome also encodes ~20,000 lncRNAs, most of whose functions have long been mysterious.[78,96,162] Two of the most-studied are X-linked X-inactive-specific transcript (*XIST*) and metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*). *XIST* is an ncRNA that spreads across the inactive X chromosome in females and forms a condensate called a Barr body that maintains the chromosome in an inactive state.[163,164] *MALAT1* is an lncRNA that enriches in nuclear speckles, which are condensates enriched in pre-mRNA processing factors that may play a role in coordinating transcriptional and post-transcriptional gene regulation.[165,166] Most lncRNAs do not contain evolutionarily conserved sequences and only occasionally is variation in lncRNAs associated with disease, which has led some to question whether most have any function. Recent studies, however, have revealed an important function for these ncRNA species. Most lncRNAs remain associated with the locus where they are transcribed and assemble spatial compartments in the genome.[43] The transcribed ncRNAs form high-concentration ribonucleoprotein condensates throughout the nucleus, and these apparently shape long-range DNA contacts and can influence local gene expression.[43] It appears likely that tethering by RNAPII and intron retention contribute to retention of these lncRNAs at these loci,[167] and thus the RNA molecules nucleate the formation of a ribonucleoprotein compartment (Figure 3).

The ability of lncRNA molecules to nucleate condensates with important roles in biological regulation is highlighted by evidence that the lncRNA *DIGIT* regulates the formation of transcriptional condensates involved in endoderm differentiation.[168] *DIGIT* is essential for differentiation of definitive endoderm[169] and functions to recruit the bromodomain and extraterminal domain (BET) protein BRD3 to the enhancers of key endoderm TFs, thereby stimulating formation of transcriptional condensates at these sites.[168] With evidence that most lncRNAs remain associated with the locus where they are transcribed, assemble spatial compartments and influence local gene expression,[43] it seems likely that additional examples of lncRNA regulation of developmental processes will be discovered.

The formation of spatial compartments and the physicochemical nature of condensates suggests an interplay between genome organization and condensates. Chromatin is a rich

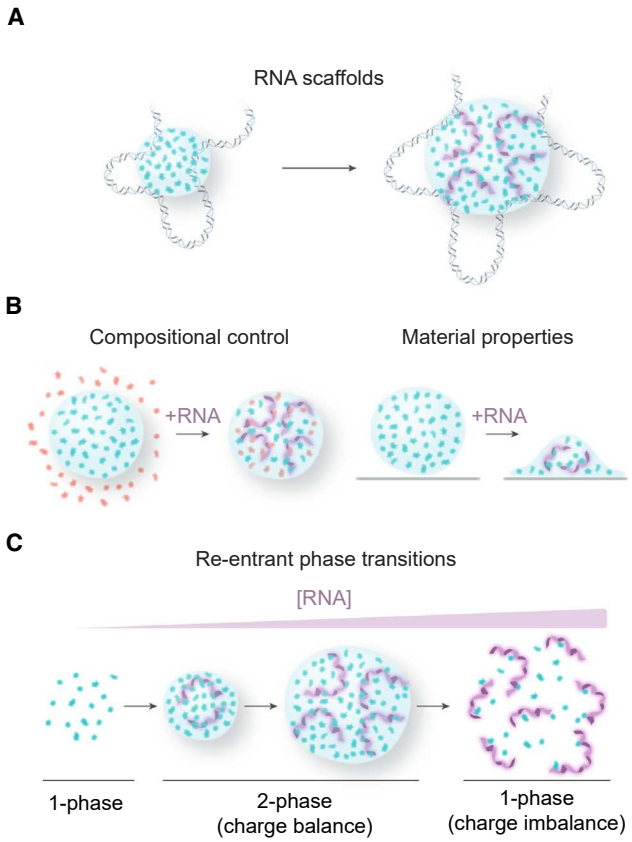


Figure 4. RNA is a general regulator of condensate physicochemistry and dynamics

(A) RNA can act as a scaffolding molecule to stimulate formation of condensates.

(B) RNA modifies condensate composition and material properties, such as surface tension, depicted as wetting against a surface.

(C) Increasing RNA levels at constant protein levels can lead to reentrant phase transitions, where RNA initially stimulates condensate formation near charge balance but then stimulates condensate dissolution through like-like repulsive interactions.

and dense polymeric network that exhibits a range of liquid-like, gel-like, and solid-like material properties.[148,170–172] The nucleosomal building blocks of chromatin can undergo phase separation *in vitro*, which can be altered by histone modifications important in gene control, such as acetylation.[148] As a scaffold for protein-binding and RNA synthesis, chromatin promotes surface condensation of transcriptional proteins, generating a force that pushes against the mechanosensitive chromatin polymer.[54,172,173] Chromatin also acts as an emulsifier that limits coarsening of chromatin-bound condensates.[55] The organization of the genome, reflected in the three-dimensional arrangement of chromatin is, therefore, simultaneously subject to and able to control the formation, diffusion, and dissolution of nuclear condensates. Indeed, high-throughput efforts to map multiway contacts between DNA, proteins, and RNA have discovered that the genome is organized around nuclear condensates, including heterochromatin and nuclear speckles.[43,44] This organization is functional, as proximity to nuclear speckle condensates, for instance, increases the splicing efficiency and expression of proximal genes.[174–176] Thus, the interplay between condensates that form on chromatin and physical limits imposed by the chromatin polymer can tune the behavior of condensates involved in gene expression and affect genome organization.

Evidence suggests that RNA is a structural component of chromatin that modulates its compaction. A recent study revealed a core insoluble RNA and protein network that associates with chromatin and is enriched with repetitive and nascent RNA species.[177–179] This network prevented chromatin compaction, suggesting that nascently transcribed RNA or repetitive RNA plays a key role in chromatin organization.[177,178,180] Consistent with this model, transcriptional activation de-compacts and displaces chromatin in zebrafish embryonic cells.[181] These effects could be due to a variety of factors, including mechanical forces of RNA-mediated condensate formation or competition with histone-DNA interactions via neutralization by RNA.[182] RNA interacts with proteins that modify chromatin structure, such as YY1 and CTCF, which may also contribute to the effect of RNA synthesis on chromatin structure.[106,107,183] A subset of CTCF-mediated chromatin interactions are dependent on the RNA-binding domains of CTCF, and these same domains affect the clustering and diffusion properties of CTCF.[183] Thus, the effect of RNA synthesis on condensate-forming properties could help explain how transcription organizes the three-dimensional genome.

These observations converge on the concept that RNA molecules transcribed from DNA regulatory elements, including enhancers and promoters, can facilitate the assembly of large amounts of transcription apparatus into dynamic condensate compartments. These compartments are an important component of genome architecture.[43,53,62,161,184–186]

RNA IS A GENERAL REGULATOR OF CONDENSATE PHYSICOCHEMISTRY AND DYNAMICS

Condensates are assemblies of biomolecules that form through multivalent interactions of biopolymers and are subject to thermodynamic parameters such as concentration of constituents, the valency of component interactions, temperature, and solvent properties. Condensate formation was initially studied in terms of principles derived from simple, single-component systems undergoing phase transitions at thermodynamic equilibrium. Recent experimental and theoretical studies have extended this understanding to multicomponent and dynamic systems of polymers, where surface condensation, percolated networks, arrested coarsening, and activity-based phase separation are at play.[187–191] A common theme in the regulation of condensates is that nucleic acids like RNA can act as seeds or scaffolds for condensate nucleation[157,192] (Figure 4A). RNA can act as a scaffold due to its ability to form non-stoichiometric and multivalent interactions with proteins. For instance, the ncRNA *XIST* nucleates condensates formed by the RNA-binding protein SHARP to initiate X chromosome inactivation.[163] Global degradation of nuclear RNAs in cells alters many condensates, including Cajal bodies, nuclear speckles, and nucleoli, suggesting that some of these RNAs could act as an integral scaffold for these condensates.[158] Consistent with this observation, specific RNAs have been shown to be crucial structural components for particular

condensates, including ribosomal RNAs in the nucleolus,[193,194] the *NEAT1* transcript in paraspeckles,[195,196] and mRNAs in cytoplasmic stress granules.[197] It has been suggested that even a single, relatively long RNA and its associated proteins could exhibit properties of a condensate.[198] These studies highlight RNA as an important structural component for condensate formation.

RNA also contributes to the regulation of condensates by modulating condensate composition and material properties (Figure 4B). Competition for RNA-binding sites affects the ability of specific RNA-binding proteins to concentrate in the condensed phase, which alters condensate composition and is important for regulation of cytoplasmic condensates such as stress granules and P bodies.[199] The composition and structure of multicomponent condensates can also be tuned by the relative concentration of RNA in the condensed phase. At low RNA concentrations, proteins in the condensed phase tend to be well mixed, but at higher RNA concentrations, new compartments and substructures form that spontaneously organize according to their relative binding affinity for RNA.[200] These structural rearrangements are important for condensates that have unique structures that depend on RNA scaffolds, including the nucleolus and paraspeckles.[201,202] RNA also regulates the material properties of condensates, including viscosity, elasticity, and surface tension.[203,204] Changes in material properties alter condensate dynamics, including the diffusion of molecules within a condensate. Such changes impact binding and reaction rates, and their dysregulation has been linked to condensate pathologies, including those involved in neurodegeneration.[147] Thus, RNA is a powerful regulator of cellular condensates, which inspires a consideration of the role of RNA in the regulation of condensates involved in transcription.

Transcriptional condensates form at active genes through multiple types of molecular interactions between proteins and nucleic acids. Recently, a study showed that proteins with patterns of alternating charge selectively partition into transcriptional condensates, which highlights an important role for electrostatic interactions in these structures.[142] The principles underlying electrostatic interactions of oppositely charged polyions, such as proteins with charged residues and RNA, have been understood for almost a century in a process known as complex coacervation.[205] Complex coacervation refers to the phase separation of mixtures of polymers of opposite charge.[206] In coacervate theory, polycations and polyanions form condensates when their concentrations achieve charge balance. Such mixtures contain two phases, a dense phase that is polymer rich and a supernatant that is polymer poor. A key property of coacervate systems is that addition of excess polycations or polyanions leads to charge imbalance, repulsion of like-like charges, and favors condensate dissolution.[207] This phenomenon of charge-driven demixing (2-phase) followed by remixing (1-phase) upon addition of a polyion is called a reentrant phase transition[208,209] (Figure 4C). Building on this theoretical framework, a recent study revealed that transcriptional condensates behave in a similar manner to complex coacervates in cells. Low RNA levels produced during transcription initiation lead to charge balance and promote condensate formation, while high RNA levels produced during bursts of transcription elongation lead to charge imbalance, causing condensate dissolution.[52] This simple but powerful mechanism of feedback control allows RNA to mediate its own transcription.[210] A subsequent study showed that RNA-binding proteins can modulate the charge balance of transcriptional condensates, which shifts the RNA concentration at which reentrant phase transitions occur.[211] This suggests that the net charge of a transcriptional condensate serves as a regulatory dial that can be tuned to control transcriptional output.

RNA IN GENE REPRESSION

Cells must maintain repetitive elements such as retrotransposons, as well as genes that encode factors that drive other cell identities, in a silent heterochromatic state, and they use diverse types of protein apparatus to accomplish this. TFs can harbor effector domains that recruit corepressors rather than coactivators to silence heterochromatic repeats. For example, KRAB domain TFs recruit the KAP1 corepressor, which in turn recruits histone deacetylases and SETDB1, a histone H3K9 methylase, to facilitate repression.[212] As cells progress along specific lineages during development, they typically deploy polycomb repressive complexes (PRC1 and PRC2) to repress genes that encode factors that drive inappropriate cell identities.[213] And the silencing of the inactive X chromosome in females involves binding to various sites on one X chromosome by the TF SPEN.[163]

Cells also utilize RNA molecules as regulators or intermediates in transcriptional repression. Transcription of heterochromatic repeats was first shown in the fission yeast *Schizosaccharomyces pombe* to be key to silencing of those repeats.[214] Transcribed pericentromeric and centromeric regions of the yeast genome recruit silencing machinery to maintain heterochromatin near centromeres. Recent evidence suggests that transcription of RNA also plays an important role in gene silencing in mammalian cells. The HUSH complex, which is responsible for repressing L1 retrotransposons and lentiviral integrations, does so by interacting with RNA transcribed from these elements.[215] It appears to do this by selectively binding intronless RNAs transcribed from retrotransposons and recruiting repressive chromatin regulators that methylate histone H3K9.

PRC2 is a multiprotein complex that represses specific genes during development and differentiation. PRC2-repressed genes include those encoding TFs whose derepression would disrupt specific developmental processes.[213] Chromatin localization of PRC2 in human pluripotent stem cells is thought to require RNA binding at those loci, although the mechanisms of this requirement are under debate.[216–223] Nevertheless, it has been observed that RNAs, including those transcribed from repetitive regions, contribute to the deposition of repressive marks on chromatin, suggesting that RNA participates in gene silencing.[178,224–226] Such RNAs have also been found to promote the formation of heterochromatin condensates, providing an additional mechanism by which RNAs are involved in repression.[227]

There are also RNA-mediated mechanisms that silence whole chromosomes. To silence one of the two X chromosomes in females, *XIST* RNA initiates a cascade of gene silencing across the

majority of the inactive X chromosome.[164,228] Recent studies have revealed that this assembly of the inactive X into an "*XIST* cloud" occurs through a condensate mechanism, forming what have been called "Barr bodies" in mammalian cells.[228]

Finally, it is notable that an RNA molecule plays a key repressive role at all active genes. During transcription initiation, RNAP typically binds the promoter, initiates transcription of ~100 base pairs, and then pauses due to the action of pause control factors. RNAPII is released from this paused state through the activity of a CDK9/cyclin T complex called P-TEFb. The P-TEFb complex contains an RNA molecule called 7SK that maintains the complex in an inactive state.[229] Removal of this 7SK repressor, likely by TFs or cofactors capable of binding this RNA molecule,[51,230,231] is necessary to derepress CDK9.

RNA-MEDIATED DYSREGULATION IN DISEASE

Disease pathogenesis is best understood in contexts where mutations in the coding regions of the genome alter the structure and function of proteins. Mutations in transcriptional proteins contribute to the pathogenesis of a wide range of diseases, including neurological disorders, developmental syndromes, metabolic diseases, and cancer.[232,233] These mutations can cause loss of function where, for example, TFs are no longer able to activate or repress specific genes, or can lead to a gain of function where, for example, TF fusion proteins activate genes in cellular contexts where they would otherwise be silenced.[234–236] The types of RNA-mediated dysregulation implicated in disease pathogenesis fall into several classes, which include repeat expansions that produce aberrant condensates, RNA overexpression, and variation associated with disease in gene regulatory elements.

There are at least fifty human disorders caused by mutations that expand short tandem repeats in genomic DNA, and transcripts from these repeats are thought in some cases to be pathogenic. Repeat expansion disorders include Huntington's disease, fragile X syndrome, and amyotrophic lateral sclerosis.[237] Although DNA-protein-centric mechanisms explain disease pathogenesis in some cases,[238] it appears likely that RNA is involved in others. Jain and Vale showed that expansions above a critical repeat threshold caused formation of repeat RNA-rich condensates in the nuclei of cells.[239] The formation of these structures is thought to sequester RNA-binding proteins away from their normal sites of action, causing dysregulation and contributing to neurological disease.[239]

Just as overexpression of specific proteins contributes to oncogenesis, altered levels of specific RNA species have been associated with cancers. There are many types of ncRNAs, including lncRNAs, circular RNAs (circRNAs), miRNAs, and piRNAs, which have been implicated as oncogenes or tumor suppressor genes.[240–242] For instance, the lncRNA *MALAT1* is typically over-expressed in patient tumors and related metastases and is often correlated with poorer prognosis.[243] The accumulation of circRNAs, derived from backsplicing during pre-mRNA processing, has been implicated in a variety of disorders.[244,245] Although the mechanisms of their function are still unclear, circRNAs are highly stable due to protection from exonucleases, and this may contribute to a mechanism of pathogenesis. The alteration of specific RNA levels is a common feature in disease, but more work is needed to determine the causality and extent of contribution to pathogenesis for RNAs in each disease.

Genome-wide association studies (GWASs) have identified variants in thousands of non-coding loci that are associated with human diseases and complex traits. Most causal variants are known to occur in non-coding gene regulatory elements such as enhancers.[246–249] In some well-studied cases, these variants have been shown to have small but significant effects on expression of genes regulated by these enhancers.[247,250] These variants alter DNA sequences that can be bound by specific TFs, and thus it is expected that alterations in DNA binding by TFs is the mechanism that accounts for changes in gene expression. However, the understanding that TFs and many other components of the transcription apparatus bind RNA sequences offers an additional potential explanation, where altered RNA sequences change the interaction with TFs at regulatory loci. It seems likely that future studies of GWAS variants will address the potential role of altered RNA sequences in dysregulated gene expression in disease.

CONCLUSIONS AND OUTLOOK

Models of gene regulation and genome organization must incorporate regulatory roles for both protein and RNA. RNA species transcribed from regulatory elements can facilitate recruitment of transcription apparatus and stimulate formation of dynamic condensate assemblies that participate in both gene regulation and genome architecture. RNA produced from intron-lacking repetitive elements can recruit the gene silencing apparatus necessary for heterochromatin formation. Dysregulation of these processes contributes to pathogenic mechanisms in disease.

The study of regulatory roles for RNA is typically more challenging than for proteins, where genetic, biochemical, and AI methodology is more widely used and technically more sophisticated. Productive genetic studies have been more focused on protein than RNA because protein sequences are typically more conserved than RNA sequences and point mutations in protein sequences tend to have more dramatic effects than those in RNA. The diversity of catalytic activities of proteins has provided a far larger universe of *in vitro* assays for biochemical studies than those for RNA molecules. The large databases of protein structures have allowed development of AI methods such as Alphafold3[251] for accurate prediction of protein structures, but ground truth for RNA structures is harder to come by. Nevertheless, there are compelling reasons to study regulatory roles for RNA. For example, human genetic studies showing that most GWAS variation associated with disease occurs in active enhancer elements, coupled with knowledge that the RNA transcribed from these elements is bound by TFs and cofactors, suggests that some disease risk may be associated with changes in the structure and function of RNA molecules transcribed from regulatory DNA.

To realize a more RNA-centric view of transcriptional regulation and genome organization will require an effort equal to the decades of genetics and biochemistry that established the importance of DNA and protein interactions in transcription. The significant structural complexity and flexibility of RNA

compared with DNA naturally makes it more difficult to study. Nevertheless, the field will require more structural insights into specific interactions between RNA and transcriptional proteins. Some of these insights will derive from new computational efforts to predict protein and nucleic acid interactions through Alphafold3[251]; others will require RNA-specific improvements to techniques like nuclear magnetic resonance spectroscopy, cryoelectron microscopy, and *in vivo* determination of RNA structure through methods like SHAPE-MaP.[83,252–254] High throughput and systematic efforts to find specific RNA regulatory motifs will require new experimental designs that attempt to isolate effects to RNA molecules themselves and new fuzzy methods of identifying conservation that do not solely depend on full-sequence similarity.[255,256] Such efforts could help to decode a potential RNA *cis*-regulatory code. The substantial progress made in understanding the physicochemical basis of biomolecular condensate formation in simplified, and sometimes non-physiological, systems must now be extended to complex mixtures of transcriptional proteins and relevant RNAs under physiological conditions. Such efforts could help us understand how RNA contributes to the organization of transcriptional and co-transcriptional processes and, by extension, the organization of the genome. Due to the dynamic and energy-intensive nature of RNA synthesis, both theory and experiment in this space will need to model non-equilibrium effects, production and degradation rates, and energy consumption that do not always lead to intuitive outcomes and extend beyond thermodynamic equilibrium. The consequences of feedback control, which have been deeply characterized in engineering and systems biology, including ultrasensitivity, noise reduction, oscillatory behavior, and coupling, will be important to incorporate into models of transcriptional regulation. Filling these gaps in our understanding of RNA structure and function will advance our understanding of biological regulatory mechanisms in health and disease.

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AUTHOR CONTRIBUTIONS

J.E.H. and R.A.Y. conceptualized the manuscript and wrote and edited subsequent drafts. J.E.H. generated the visualizations.

DECLARATION OF INTERESTS

R.A.Y. is a founder and shareholder of Syros Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics, Dewpoint Therapeutics, and Paratus Sciences; has consulting or advisory roles at Precede Biosciences and Novo Nordisk; and is on the advisory board of *Molecular Cell*.

REFERENCES

1. Furlong, E.E.M., and Levine, M. (2018). Developmental enhancers and chromosome topology. Science *361*, 1341–1345. https://doi.org/10.1126/science.aau0320.

2. Lambert, S.A., Jolma, A., Campitelli, L.F., Das, P.K., Yin, Y., Albu, M., Chen, X., Taipale, J., Hughes, T.R., and Weirauch, M.T. (2018). The human transcription factors. Cell *172*, 650–665. https://doi.org/10.1016/j.cell.2018.01.029.

3. Roeder, R.G. (2019). 50+ years of eukaryotic transcription: an expanding universe of factors and mechanisms. Nat. Struct. Mol. Biol. *26*, 783–791. https://doi.org/10.1038/s41594-019-0287-x.

4. Cramer, P. (2019). Organization and regulation of gene transcription. Nature *573*, 45–54. https://doi.org/10.1038/s41586-019-1517-4.

5. Field, A., and Adelman, K. (2020). Evaluating enhancer function and transcription. Annu. Rev. Biochem. *89*, 213–234. https://doi.org/10.1146/annurev-biochem-011420-095916.

6. Vos, S.M. (2021). Understanding transcription across scales: from base pairs to chromosomes. Mol. Cell *81*, 1601–1616. https://doi.org/10.1016/j.molcel.2021.03.002.

7. Richter, W.F., Nayak, S., Iwasa, J., and Taatjes, D.J. (2022). The Mediator complex as a master regulator of transcription by RNA polymerase II. Nat. Rev. Mol. Cell Biol. *23*, 732–749. https://doi.org/10.1038/s41580-022-00498-3.

8. Tuan, D., Kong, S., and Hu, K. (1992). Transcription of the hypersensitive site HS2 enhancer in erythroid cells. Proc. Natl. Acad. Sci. USA *89*, 11219–11223. https://doi.org/10.1073/pnas.89.23.11219.

9. De Santa, F., Barozzi, I., Mietton, F., Ghisletti, S., Polletti, S., Tusi, B.K., Muller, H., Ragoussis, J., Wei, C.-L., and Natoli, G. (2010). A large fraction of extragenic RNA Pol II transcription sites overlap enhancers. PLoS Biol. *8*, e1000384. https://doi.org/10.1371/journal.pbio.1000384.

10. Kim, T.-K., Hemberg, M., Gray, J.M., Costa, A.M., Bear, D.M., Wu, J., Harmin, D.A., Laptewicz, M., Barbara-Haley, K., Kuersten, S., et al. (2010). Widespread transcription at neuronal activity-regulated enhancers. Nature *465*, 182–187. https://doi.org/10.1038/nature09033.

11. Lam, M.T.Y., Li, W., Rosenfeld, M.G., and Glass, C.K. (2014). Enhancer RNAs and regulated transcriptional programs. Trends Biochem. Sci. *39*, 170–182. https://doi.org/10.1016/j.tibs.2014.02.007.

12. Core, L.J., Martins, A.L., Danko, C.G., Waters, C.T., Siepel, A., and Lis, J.T. (2014). Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. Nat. Genet. *46*, 1311–1320. https://doi.org/10.1038/ng.3142.

13. Struhl, K. (2007). Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat. Struct. Mol. Biol. *14*, 103–105. https://doi.org/10.1038/nsmb0207-103.

14. Seila, A.C., Calabrese, J.M., Levine, S.S., Yeo, G.W., Rahl, P.B., Flynn, R.A., Young, R.A., and Sharp, P.A. (2008). Divergent transcription from active promoters. Science *322*, 1849–1851. https://doi.org/10.1126/science.1162253.

15. Core, L.J., Waterfall, J.J., and Lis, J.T. (2008). Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science *322*, 1845–1848. https://doi.org/10.1126/science.1162228.

16. 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., et al. (2013). Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells. Proc. Natl. Acad. Sci. USA *110*, 2876–2881. https://doi.org/10.1073/pnas.1221904110.

17. Muse, G.W., Gilchrist, D.A., Nechaev, S., Shah, R., Parker, J.S., Grissom, S.F., Zeitlinger, J., and Adelman, K. (2007). RNA polymerase is poised for activation across the genome. Nat. Genet. *39*, 1507–1511. https://doi.org/10.1038/ng.2007.21.

18. Zeitlinger, J., Stark, A., Kellis, M., Hong, J.-W., Nechaev, S., Adelman, K., Levine, M., and Young, R.A. (2007). RNA polymerase stalling at developmental control genes in the Drosophila melanogaster embryo. Nat. Genet. *39*, 1512–1516. https://doi.org/10.1038/ng.2007.26.

19. Henriques, T., Scruggs, B.S., Inouye, M.O., Muse, G.W., Williams, L.H., Burkholder, A.B., Lavender, C.A., Fargo, D.C., and Adelman, K. (2018). Widespread transcriptional pausing and elongation control at enhancers. Genes Dev. *32*, 26–41. https://doi.org/10.1101/gad.309351.117.

20. Mayer, A., Landry, H.M., and Churchman, L.S. (2017). Pause & go: from the discovery of RNA polymerase pausing to its functional implications. Curr. Opin. Cell Biol. *46*, 72–80. https://doi.org/10.1016/j.ceb.2017.03.002.

21. Skalska, L., Beltran-Nebot, M., Ule, J., and Jenner, R.G. (2017). Regulatory feedback from nascent RNA to chromatin and transcription. Nat. Rev. Mol. Cell Biol. *18*, 331–337. https://doi.org/10.1038/nrm.2017.12.

22. Wissink, E.M., Vihervaara, A., Tippens, N.D., and Lis, J.T. (2019). Nascent RNA analyses: tracking transcription and its regulation. Nat. Rev. Genet. *20*, 705–723. https://doi.org/10.1038/s41576-019-0159-6.

23. Schärfen, L., and Neugebauer, K.M. (2021). Transcription regulation through nascent RNA folding. J. Mol. Biol. *433*, 166975. https://doi.org/10.1016/j.jmb.2021.166975.

24. Bartel, D.P. (2018). Metazoan microRNAs. Cell *173*, 20–51. https://doi.org/10.1016/j.cell.2018.03.006.

25. Ozata, D.M., Gainetdinov, I., Zoch, A., O'Carroll, D., and Zamore, P.D. (2019). PIWI-interacting RNAs: small RNAs with big functions. Nat. Rev. Genet. *20*, 89–108. https://doi.org/10.1038/s41576-018-0073-3.

26. Mahat, D.B., Tippens, N.D., Martin-Rufino, J.D., Waterton, S.K., Fu, J., Blatt, S.E., and Sharp, P.A. (2024). Single-cell nascent RNA sequencing unveils coordinated global transcription. Nature *631*, 216–223. https://doi.org/10.1038/s41586-024-07517-7.

27. Gerstberger, S., Hafner, M., and Tuschl, T. (2014). A census of human RNA-binding proteins. Nat. Rev. Genet. *15*, 829–845. https://doi.org/10.1038/nrg3813.

28. Baltz, A.G., Munschauer, M., Schwanhäusser, B., Vasile, A., Murakawa, Y., Schueler, M., Youngs, N., Penfold-Brown, D., Drew, K., Milek, M., et al. (2012). The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. Mol. Cell *46*, 674–690. https://doi.org/10.1016/j.molcel.2012.05.021.

29. Trendel, J., Schwarzl, T., Horos, R., Prakash, A., Bateman, A., Hentze, M.W., and Krijgsveld, J. (2019). The human RNA-binding proteome and its dynamics during translational arrest. Cell *176*, 391–403.e19. https://doi.org/10.1016/j.cell.2018.11.004.

30. He, C., Sidoli, S., Warneford-Thomson, R., Tatomer, D.C., Wilusz, J.E., Garcia, B.A., and Bonasio, R. (2016). High-resolution mapping of RNA-binding regions in the nuclear proteome of embryonic stem cells. Mol. Cell *64*, 416–430. https://doi.org/10.1016/j.molcel.2016.09.034.

31. Mahendrawada, L., Warfield, L., Donczew, R., and Hahn, S. (2023). Surprising connections between DNA binding and function for the near-complete set of yeast transcription factors. Preprint at bioRxiv. https://doi.org/10.1101/2023.07.25.550593.

32. de Wit, E., and Nora, E.P. (2023). New insights into genome folding by loop extrusion from inducible degron technologies. Nat. Rev. Genet. *24*, 73–85. https://doi.org/10.1038/s41576-022-00530-4.

33. Hsieh, T.S., Cattoglio, C., Slobodyanyuk, E., Hansen, A.S., Darzacq, X., and Tjian, R. (2022). Enhancer–promoter interactions and transcription are largely maintained upon acute loss of CTCF, cohesin, WAPL or YY1. Nat. Genet. *54*, 1919–1932. https://doi.org/10.1038/s41588-022-01223-8.

34. Du, M., Stitzinger, S.H., Spille, J.-H., Cho, W.-K., Lee, C., Hijaz, M., Quintana, A., and Cissé, I.I. (2024). Direct observation of a condensate effect on super-enhancer controlled gene bursting. Cell *187*, 331–344.e17. https://doi.org/10.1016/j.cell.2023.12.005.

35. 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. https://doi.org/10.1126/science.1239053.

36. Pownall, M.E., Miao, L., Vejnar, C.E., M'Saad, O., Sherrard, A., Frederick, M.A., Benitez, M.D.J., Boswell, C.W., Zaret, K.S., Bewersdorf, J., et al. (2023). Chromatin expansion microscopy reveals nanoscale organization of transcription and chromatin. Science *381*, 92–100. https://doi.org/10.1126/science.ade5308.

37. Ling, Y.H., Liang, C., Wang, S., and Wu, C. (2024). Live-cell single-molecule dynamics of eukaryotic RNA polymerase machineries. Preprint at bioRxiv. https://doi.org/10.1101/2024.07.08.602478.

38. Baek, I., Friedman, L.J., Gelles, J., and Buratowski, S. (2021). Single molecule studies reveal branched pathways for activator-dependent assembly of RNA polymerase II pre-initiation complexes. Mol. Cell *81*, 3576–3588.e6. https://doi.org/10.1016/j.molcel.2021.07.025.

39. Zhang, M., Eichhorn, S.W., Zingg, B., Yao, Z., Cotter, K., Zeng, H., Dong, H., and Zhuang, X. (2021). Spatially resolved cell atlas of the mouse primary motor cortex by MERFISH. Nature *598*, 137–143. https://doi.org/10.1038/s41586-021-03705-x.

40. Cawte, A.D., Unrau, P.J., and Rueda, D.S. (2020). Live cell imaging of single RNA molecules with fluorogenic Mango II arrays. Nat. Commun. *11*, 1283. https://doi.org/10.1038/s41467-020-14932-7.

41. Cabili, M.N., Dunagin, M.C., McClanahan, P.D., Biaesch, A., Padovan-Merhar, O., Regev, A., Rinn, J.L., and Raj, A. (2015). Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. Genome Biol. *16*, 20. https://doi.org/10.1186/s13059-015-0586-4.

42. Coté, A., O'Farrell, A., Dardani, I., Dunagin, M., Coté, C., Wan, Y., Bayatpour, S., Drexler, H.L., Alexander, K.A., Chen, F., et al. (2024). Post-transcriptional splicing can occur in a slow-moving zone around the gene. eLife *12*, RP91357. https://doi.org/10.7554/eLife.91357.

43. Quinodoz, S.A., Jachowicz, J.W., Bhat, P., Ollikainen, N., Banerjee, A.K., Goronzy, I.N., Blanco, M.R., Chovanec, P., Chow, A., Markaki, Y., et al. (2021). RNA promotes the formation of spatial compartments in the nucleus. Cell *184*, 5775–5790.e30. https://doi.org/10.1016/j.cell.2021.10.014.

44. Quinodoz, S.A., Ollikainen, N., Tabak, B., Palla, A., Schmidt, J.M., Detmar, E., Lai, M.M., Shishkin, A.A., Bhat, P., Takei, Y., et al. (2018). Higher-order inter-chromosomal hubs shape 3D genome organization in the nucleus. Cell *174*, 744–757.e24. https://doi.org/10.1016/j.cell.2018.05.024.

45. Arrastia, M.V., Jachowicz, J.W., Ollikainen, N., Curtis, M.S., Lai, C., Quinodoz, S.A., Selck, D.A., Ismagilov, R.F., and Guttman, M. (2022). Single-cell measurement of higher-order 3D genome organization with scSPRITE. Nat. Biotechnol. *40*, 64–73. https://doi.org/10.1038/s41587-021-00998-1.

46. Cai, Z., Cao, C., Ji, L., Ye, R., Wang, D., Xia, C., Wang, S., Du, Z., Hu, N., Yu, X., et al. (2020). RIC-seq for global in situ profiling of RNA–RNA spatial interactions. Nature *582*, 432–437. https://doi.org/10.1038/s41586-020-2249-1.

47. Almada, A.E., Wu, X., Kriz, A.J., Burge, C.B., and Sharp, P.A. (2013). Promoter directionality is controlled by U1 snRNP and polyadenylation signals. Nature *499*, 360–363. https://doi.org/10.1038/nature12349.

48. Mimoso, C.A., and Adelman, K. (2023). U1 snRNP increases RNA Pol II elongation rate to enable synthesis of long genes. Mol. Cell *83*, 1264–1279.e10. https://doi.org/10.1016/j.molcel.2023.03.002.

49. Chiu, A.C., Suzuki, H.I., Wu, X., Mahat, D.B., Kriz, A.J., and Sharp, P.A. (2018). Transcriptional pause sites delineate stable nucleosome-associated premature polyadenylation suppressed by U1 snRNP. Mol. Cell *69*, 648–663.e7. https://doi.org/10.1016/j.molcel.2018.01.006.

50. Vlaming, H., Mimoso, C.A., Field, A.R., Martin, B.J.E., and Adelman, K. (2022). Screening thousands of transcribed coding and non-coding regions reveals sequence determinants of RNA polymerase II elongation potential. Nat. Struct. Mol. Biol. *29*, 613–620. https://doi.org/10.1038/s41594-022-00785-9.

51. Oksuz, O., Henninger, J.E., Warneford-Thomson, R., Zheng, M.M., Erb, H., Vancura, A., Overholt, K.J., Hawken, S.W., Banani, S.F., Lauman, R., et al. (2023). Transcription factors interact with RNA to regulate genes. Mol. Cell *83*, 2449–2463.e13. https://doi.org/10.1016/j.molcel.2023.06.012.

52. Henninger, J.E., Oksuz, O., Shrinivas, K., Sagi, I., LeRoy, G., Zheng, M.M., Andrews, J.O., Zamudio, A.V., Lazaris, C., Hannett, N.M., et al. (2021). RNA-mediated feedback control of transcriptional condensates. Cell *184*, 207–225.e24. https://doi.org/10.1016/j.cell.2020.11.030.

53. Goel, V.Y., Huseyin, M.K., and Hansen, A.S. (2023). Region Capture Micro-C reveals coalescence of enhancers and promoters into nested microcompartments. Nat. Genet. *55*, 1048–1056. https://doi.org/10.1038/s41588-023-01391-1.

54. Shin, Y., Chang, Y.-C., Lee, D.S.W., Berry, J., Sanders, D.W., Ronceray, P., Wingreen, N.S., Haataja, M., and Brangwynne, C.P. (2018). Liquid nuclear condensates mechanically sense and restructure the genome. Cell *175*, 1481–1491.e13. https://doi.org/10.1016/j.cell.2018.10.057.

55. Lee, D.S.W., Wingreen, N.S., and Brangwynne, C.P. (2021). Chromatin mechanics dictates subdiffusion and coarsening dynamics of embedded condensates. Nat. Phys. *17*, 531–538. https://doi.org/10.1038/s41567-020-01125-8.

56. Wei, M.-T., Chang, Y.-C., Shimobayashi, S.F., Shin, Y., Strom, A.R., and Brangwynne, C.P. (2020). Nucleated transcriptional condensates amplify gene expression. Nat. Cell Biol. *22*, 1187–1196. https://doi.org/10.1038/s41556-020-00578-6.

57. Brodsky, S., Jana, T., Mittelman, K., Chapal, M., Kumar, D.K., Carmi, M., and Barkai, N. (2020). Intrinsically disordered regions direct transcription factor in vivo binding specificity. Mol. Cell *79*, 459–471.e4. https://doi.org/10.1016/j.molcel.2020.05.032.

58. Lang, T.J., Brodsky, S., Manadre, W., Vidavski, M., Valinsky, G., Mindel, V., Ilan, G., Carmi, M., and Barkai, N. (2024). Massively Parallel Binding Assay (MPBA) reveals limited transcription factor binding cooperativity, challenging models of specificity. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.26.600749.

59. Kumar, D.K., Jonas, F., Jana, T., Brodsky, S., Carmi, M., and Barkai, N. (2023). Complementary strategies for directing in vivo transcription factor binding through DNA binding domains and intrinsically disordered regions. Mol. Cell *83*, 1462–1473.e5. https://doi.org/10.1016/j.molcel.2023.04.002.

60. Andersson, R., Gebhard, C., Miguel-Escalada, I., Hoof, I., Bornholdt, J., Boyd, M., Chen, Y., Zhao, X., Schmidl, C., Suzuki, T., et al. (2014). An atlas of active enhancers across human cell types and tissues. Nature *507*, 455–461. https://doi.org/10.1038/nature12787.

61. Lorenzi, L., Chiu, H.-S., Avila Cobos, F., Gross, S., Volders, P.-J., Cannoodt, R., Nuytens, J., Vanderheyden, K., Anckaert, J., Lefever, S., et al. (2021). The RNA Atlas expands the catalog of human non-coding RNAs. Nat. Biotechnol. *39*, 1453–1465. https://doi.org/10.1038/s41587-021-00936-1.

62. Sartorelli, V., and Lauberth, S.M. (2020). Enhancer RNAs are an important regulatory layer of the epigenome. Nat. Struct. Mol. Biol. *27*, 521–528. https://doi.org/10.1038/s41594-020-0446-0.

63. Li, W., Notani, D., Ma, Q., Tanasa, B., Nunez, E., Chen, A.Y., Merkurjev, D., Zhang, J., Ohgi, K., Song, X., et al. (2013). Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. Nature *498*, 516–520. https://doi.org/10.1038/nature12210.

64. Hah, N., Murakami, S., Nagari, A., Danko, C.G., and Kraus, W.L. (2013). Enhancer transcripts mark active estrogen receptor binding sites. Genome Res. *23*, 1210–1223. https://doi.org/10.1101/gr.152306.112.

65. Arner, E., Daub, C.O., Vitting-Seerup, K., Andersson, R., Lilje, B., Drabløs, F., Lennartsson, A., Rönnerblad, M., Hrydziuszko, O., Vitezic, M., et al. (2015). Transcribed enhancers lead waves of coordinated transcription in transitioning mammalian cells. Science *347*, 1010–1014. https://doi.org/10.1126/science.1259418.

66. Heintzman, N.D., Hon, G.C., Hawkins, R.D., Kheradpour, P., Stark, A., Harp, L.F., Ye, Z., Lee, L.K., Stuart, R.K., Ching, C.W., et al. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene expression. Nature *459*, 108–112. https://doi.org/10.1038/nature07829.

67. Dunham, I., Kundaje, A., Aldred, S.F., Collins, P.J., Davis, C.A., Doyle, F., Epstein, C.B., Frietze, S., Harrow, J., and Kaul, R. (2012). An integrated encyclopedia of DNA elements in the human genome. Nature *489*, 57–74. https://doi.org/10.1038/nature11247.

68. Frankish, A., Diekhans, M., Ferreira, A.-M., Johnson, R., Jungreis, I., Loveland, J., Mudge, J.M., Sisu, C., Wright, J., Armstrong, J., et al. (2019). GENCODE reference annotation for the human and mouse genomes. Nucleic Acids Res. *47*, D766–D773. https://doi.org/10.1093/nar/gky955.

69. Preker, P., Almvig, K., Christensen, M.S., Valen, E., Mapendano, C.K., Sandelin, A., and Jensen, T.H. (2011). PROMoter uPstream Transcripts share characteristics with mRNAs and are produced upstream of all three major types of mammalian promoters. Nucleic Acids Res. *39*, 7179–7193. https://doi.org/10.1093/nar/gkr370.

70. McStay, B., and Grummt, I. (2008). The epigenetics of rRNA genes: from molecular to chromosome biology. Annu. Rev. Cell Dev. Biol. *24*, 131–157. https://doi.org/10.1146/annurev.cellbio.24.110707.175259.

71. Li, W., Notani, D., and Rosenfeld, M.G. (2016). Enhancers as non-coding RNA transcription units: recent insights and future perspectives. Nat. Rev. Genet. *17*, 207–223. https://doi.org/10.1038/nrg.2016.4.

72. Schwalb, B., Michel, M., Zacher, B., Frühauf, K., Demel, C., Tresch, A., Gagneur, J., and Cramer, P. (2016). TT-seq maps the human transient transcriptome. Science *352*, 1225–1228. https://doi.org/10.1126/science.aad9841.

73. Hon, C.-C., Ramilowski, J.A., Harshbarger, J., Bertin, N., Rackham, O.J.L., Gough, J., Denisenko, E., Schmeier, S., Poulsen, T.M., Severin, J., et al. (2017). An atlas of human long non-coding RNAs with accurate 5′ ends. Nature *543*, 199–204. https://doi.org/10.1038/nature21374.

74. Ietswaart, R., Smalec, B.M., Xu, A., Choquet, K., McShane, E., Jowhar, Z.M., Guegler, C.K., Baxter-Koenigs, A.R., West, E.R., Fu, B.X.H., et al. (2024). Genome-wide quantification of RNA flow across subcellular compartments reveals determinants of the mammalian transcript life cycle. Mol. cell *84*, 2765–2784. https://doi.org/10.1016/j.molcel.2024.06.008.

75. Preker, P., Nielsen, J., Kammler, S., Lykke-Andersen, S., Christensen, M.S., Mapendano, C.K., Schierup, M.H., and Jensen, T.H. (2008). RNA exosome depletion reveals transcription upstream of active human promoters. Science *322*, 1851–1854. https://doi.org/10.1126/science.1164096.

76. Flynn, R.A., Almada, A.E., Zamudio, J.R., and Sharp, P.A. (2011). Antisense RNA polymerase II divergent transcripts are P-TEFb dependent and substrates for the RNA exosome. Proc. Natl. Acad. Sci. USA *108*, 10460–10465. https://doi.org/10.1073/pnas.1106630108.

77. Lai, F., Gardini, A., Zhang, A., and Shiekhattar, R. (2015). Integrator mediates the biogenesis of enhancer RNAs. Nature *525*, 399–403. https://doi.org/10.1038/nature14906.

78. Mattick, J.S., Amaral, P.P., Carninci, P., Carpenter, S., Chang, H.Y., Chen, L.-L., Chen, R., Dean, C., Dinger, M.E., Fitzgerald, K.A., et al. (2023). Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat. Rev. Mol. Cell Biol. *24*, 430–447. https://doi.org/10.1038/s41580-022-00566-8.

79. Chen, J., Zhang, Z., Li, L., Chen, B.-C., Revyakin, A., Hajj, B., Legant, W., Dahan, M., Lionnet, T., Betzig, E., et al. (2014). Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. Cell *156*, 1274–1285. https://doi.org/10.1016/j.cell.2014.01.062.

80. Ray-Soni, A., Bellecourt, M.J., and Landick, R. (2016). Mechanisms of bacterial transcription termination: all good things must end. Annu. Rev. Biochem. *85*, 319–347. https://doi.org/10.1146/annurev-biochem-060815-014844.

81. Winkler, W.C., and Breaker, R.R. (2005). Regulation of bacterial gene expression by riboswitches. Annu. Rev. Microbiol. *59*, 487–517. https://doi.org/10.1146/annurev.micro.59.030804.121336.

82. Berkhout, B., Silverman, R.H., and Jeang, K.-T. (1989). Tat trans-activates the human immunodeficiency virus through a nascent RNA target. Cell *59*, 273–282. https://doi.org/10.1016/0092-8674(89)90289-4.

83. Pham, V.V., Salguero, C., Khan, S.N., Meagher, J.L., Brown, W.C., Humbert, N., de Rocquigny, H., Smith, J.L., and D'Souza, V.M. (2018). HIV-1 Tat interactions with cellular 7SK and viral TAR RNAs identifies dual structural mimicry. Nat. Commun. *9*, 4266. https://doi.org/10.1038/s41467-018-06591-6.

84. Ken, M.L., Roy, R., Geng, A., Ganser, L.R., Manghrani, A., Cullen, B.R., Schulze-Gahmen, U., Herschlag, D., and Al-Hashimi, H.M. (2023). RNA

conformational propensities determine cellular activity. Nature *617*, 835–841. https://doi.org/10.1038/s41586-023-06080-x.

85. Sherwood, A.V., and Henkin, T.M. (2016). Riboswitch-mediated gene regulation: novel RNA architectures dictate gene expression responses. Annu. Rev. Microbiol. *70*, 361–374. https://doi.org/10.1146/annurev-micro-091014-104306.

86. Lam, M.T.Y., Cho, H., Lesch, H.P., Gosselin, D., Heinz, S., Tanaka-Oishi, Y., Benner, C., Kaikkonen, M.U., Kim, A.S., Kosaka, M., et al. (2013). Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. Nature *498*, 511–515. https://doi.org/10.1038/nature12209.

87. Melo, C.A., Drost, J., Wijchers, P.J., van de Werken, H., de Wit, E., Oude Vrielink, J.A.F.O., Elkon, R., Melo, S.A., Léveillé, N., Kalluri, R., et al. (2013). eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol. Cell *49*, 524–535. https://doi.org/10.1016/j.molcel.2012.11.021.

88. 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. https://doi.org/10.1038/nature11884.

89. Mousavi, K., Zare, H., Dell'orso, S., Grontved, L., Gutierrez-Cruz, G., Derfoul, A., Hager, G.L., and Sartorelli, V. (2013). eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. Mol. Cell *51*, 606–617. https://doi.org/10.1016/j.molcel.2013.07.022.

90. Zhao, Y., Wang, L., Ren, S., Wang, L., Blackburn, P.R., McNulty, M.S., Gao, X., Qiao, M., Vessella, R.L., Kohli, M., et al. (2016). Activation of P-TEFb by androgen receptor-regulated enhancer RNAs in castration-resistant prostate cancer. Cell Rep. *15*, 599–610. https://doi.org/10.1016/j.celrep.2016.03.038.

91. 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. https://doi.org/10.1038/nature20149.

92. Joung, J., Engreitz, J.M., Konermann, S., Abudayyeh, O.O., Verdine, V.K., Aguet, F., Gootenberg, J.S., Sanjana, N.E., Wright, J.B., Fulco, C.P., et al. (2017). Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. Nature *548*, 343–346. https://doi.org/10.1038/nature23451.

93. Yang, F., Tanasa, B., Micheletti, R., Ohgi, K.A., Aggarwal, A.K., and Rosenfeld, M.G. (2021). Shape of promoter antisense RNAs regulates ligand-induced transcription activation. Nature *595*, 444–449. https://doi.org/10.1038/s41586-021-03589-x.

94. Gorbovytska, V., Kim, S.-K., Kuybu, F., Götze, M., Um, D., Kang, K., Pittroff, A., Brennecke, T., Schneider, L.-M., Leitner, A., et al. (2022). Enhancer RNAs stimulate Pol II pause release by harnessing multivalent interactions to NELF. Nat. Commun. *13*, 2429. https://doi.org/10.1038/s41467-022-29934-w.

95. Nair, S.J., Suter, T., Wang, S., Yang, L., Yang, F., and Rosenfeld, M.G. (2022). Transcriptional enhancers at 40: evolution of a viral DNA element to nuclear architectural structures. Trends Genet. *38*, 1019–1047. https://doi.org/10.1016/j.tig.2022.05.015.

96. Ferrer, J., and Dimitrova, N. (2024). Transcription regulation by long noncoding RNAs: mechanisms and disease relevance. Nat. Rev. Mol. Cell Biol. *25*, 396–415. https://doi.org/10.1038/s41580-023-00694-9.

97. Olivero, C.E., Martínez-Terroba, E., Zimmer, J., Liao, C., Tesfaye, E., Hooshdaran, N., Schofield, J.A., Bendor, J., Fang, D., Simon, M.D., et al. (2020). p53 activates the long noncoding RNA Pvt1b to inhibit Myc and suppress tumorigenesis. Mol. Cell *77*, 761–774.e8. https://doi.org/10.1016/j.molcel.2019.12.014.

98. Lee, J.-S., and Mendell, J.T. (2020). Antisense-mediated transcript knockdown triggers premature transcription termination. Mol. Cell *77*, 1044–1054.e3. https://doi.org/10.1016/j.molcel.2019.12.011.

99. Tseng, Y.-Y., Moriarity, B.S., Gong, W., Akiyama, R., Tiwari, A., Kawakami, H., Ronning, P., Reuland, B., Guenther, K., Beadnell, T.C., et al. (2014). PVT1 dependence in cancer with MYC copy-number increase. Nature *512*, 82–86. https://doi.org/10.1038/nature13311.

100. Chen, X., and Huang, C. (2023). Chromatin-interacting RNA-binding proteins regulate transcription. Trends Cell Biol. *33*, 625–629. https://doi.org/10.1016/j.tcb.2023.05.006.

101. Avila-Lopez, P., and Lauberth, S.M. (2024). Exploring new roles for RNA-binding proteins in epigenetic and gene regulation. Curr. Opin. Genet. Dev. *84*, 102136. https://doi.org/10.1016/j.gde.2023.102136.

102. Theunissen, O., Rudt, F., Guddat, U., Mentzel, H., and Pieler, T. (1992). RNA and DNA binding zinc fingers in Xenopus TFIIIA. Cell *71*, 679–690. https://doi.org/10.1016/0092-8674(92)90601-8.

103. Niessing, D., Driever, W., Sprenger, F., Taubert, H., Jäckle, H., and Rivera-Pomar, R. (2000). Homeodomain Position 54 Specifies Transcriptional versus Translational Control by bicoid. Mol. Cell *5*, 395–401. https://doi.org/10.1016/S1097-2765(00)80434-7.

104. Yoshida, Y., Izumi, H., Torigoe, T., Ishiguchi, H., Yoshida, T., Itoh, H., and Kohno, K. (2004). Binding of RNA to p53 regulates its oligomerization and DNA-binding activity. Oncogene *23*, 4371–4379. https://doi.org/10.1038/sj.onc.1207583.

105. Jeon, Y., and Lee, J.T. (2011). YY1 tethers Xist RNA to the inactive X nucleation center. Cell *146*, 119–133. https://doi.org/10.1016/j.cell.2011.06.026.

106. 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. https://doi.org/10.1126/science.aad3346.

107. Saldaña-Meyer, R., Rodriguez-Hernaez, J., Escobar, T., Nishana, M., Jácome-López, K., Nora, E.P., Bruneau, B.G., Tsirigos, A., Furlan-Magaril, M., Skok, J., et al. (2019). RNA interactions are essential for CTCF-mediated genome organization. Mol. Cell *76*, 412–422.e5. https://doi.org/10.1016/j.molcel.2019.08.015.

108. Holmes, Z.E., Hamilton, D.J., Hwang, T., Parsonnet, N.V., Rinn, J.L., Wuttke, D.S., and Batey, R.T. (2020). The Sox2 transcription factor binds RNA. Nat. Commun. *11*, 1805. https://doi.org/10.1038/s41467-020-15571-8.

109. Hou, L., Wei, Y., Lin, Y., Wang, X., Lai, Y., Yin, M., Chen, Y., Guo, X., Wu, S., Zhu, Y., et al. (2020). Concurrent binding to DNA and RNA facilitates the pluripotency reprogramming activity of Sox2. Nucleic Acids Res. *48*, 3869–3887. https://doi.org/10.1093/nar/gkaa067.

110. Xu, Y., Huangyang, P., Wang, Y., Xue, L., Devericks, E., Nguyen, H.G., Yu, X., Oses-Prieto, J.A., Burlingame, A.L., Miglani, S., et al. (2021). ERα is an RNA-binding protein sustaining tumor cell survival and drug resistance. Cell *184*, 5215–5229.e17. https://doi.org/10.1016/j.cell.2021.08.036.

111. Lammer, N.C., Ashraf, H.M., Ugay, D.A., Spencer, S.L., Allen, M.A., Batey, R.T., and Wuttke, D.S. (2023). RNA binding by the glucocorticoid receptor attenuates dexamethasone-induced gene activation. Sci. Rep. *13*, 9385. https://doi.org/10.1038/s41598-023-35549-y.

112. Hudson, W.H., and Ortlund, E.A. (2014). The structure, function and evolution of proteins that bind DNA and RNA. Nat. Rev. Mol. Cell Biol. *15*, 749–760. https://doi.org/10.1038/nrm3884.

113. Rahnamoun, H., Lee, J., Sun, Z., Lu, H., Ramsey, K.M., Komives, E.A., and Lauberth, S.M. (2018). RNAs interact with BRD4 to promote enhanced chromatin engagement and transcription activation. Nat. Struct. Mol. Biol. *25*, 687–697. https://doi.org/10.1038/s41594-018-0102-0.

114. Bose, D.A., Donahue, G., Reinberg, D., Shiekhattar, R., Bonasio, R., and Berger, S.L. (2017). RNA binding to CBP stimulates histone acetylation and transcription. Cell *168*, 135–149.e22. https://doi.org/10.1016/j.cell.2016.12.020.

115. Chen, T., and Dent, S.Y.R. (2014). Chromatin modifiers and remodellers: regulators of cellular differentiation. Nat. Rev. Genet. *15*, 93–106. https://doi.org/10.1038/nrg3607.

116. 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. https://doi.org/10.1038/nrg2901.

117. Schaukowitch, K., Joo, J.-Y., Liu, X., Watts, J.K., Martinez, C., and Kim, T.-K. (2014). Enhancer RNA facilitates NELF release from immediate early genes. Mol. Cell *56*, 29–42. https://doi.org/10.1016/j.molcel.2014.08.023.

118. Skalska, L., Begley, V., Beltran, M., Lukauskas, S., Khandelwal, G., Faull, P., Bhamra, A., Tavares, M., Wellman, R., Tvardovskiy, A., et al. (2021). Nascent RNA antagonizes the interaction of a set of regulatory proteins with chromatin. Mol. Cell *81*, 2944–2959.e10. https://doi.org/10.1016/j.molcel.2021.05.026.

119. Saha, D., Animireddy, S., Lee, J., Thommen, A., Murvin, M.M., Lu, Y., Calabrese, J.M., and Bartholomew, B. (2024). Enhancer switching in cell lineage priming is linked to eRNA, Brg1's AT-hook, and SWI/SNF recruitment. Mol. Cell *84*, 1855–1869.e5. https://doi.org/10.1016/j.molcel.2024.03.013.

120. von Ehr, J., Oberstrass, L., Yazgan, E., Schnaubelt, L.I., Blümel, N., McNicoll, F., Weigand, J.E., Zarnack, K., Müller-McNicoll, M., Korn, S.M., et al. (2024). Arid5a uses disordered extensions of its core ARID domain for distinct DNA- and RNA-recognition and gene regulation. Preprint at bioRxiv. https://doi.org/10.1101/2024.02.29.582703.

121. De Silva, S.M., Dhiman, A., Sood, S., Mercedes, K.F., Simmons, W.J., Henen, M.A., Vögeli, B., Dykhuizen, E.C., and Musselman, C.A. (2023). PBRM1 bromodomains associate with RNA to facilitate chromatin association. Nucleic Acids Res. *51*, 3631–3649. https://doi.org/10.1093/nar/gkad072.

122. Wei, C., Xiao, R., Chen, L., Cui, H., Zhou, Y., Xue, Y., Hu, J., Zhou, B., Tsutsui, T., Qiu, J., et al. (2016). RBFox2 binds nascent RNA to globally regulate polycomb Complex 2 targeting in mammalian genomes. Mol. Cell *62*, 875–889. https://doi.org/10.1016/j.molcel.2016.04.013.

123. 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. https://doi.org/10.1016/j.cell.2013.03.035.

124. 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. https://doi.org/10.1016/j.cell.2013.09.053.

125. Dowen, J.M., Fan, Z.P., Hnisz, D., Ren, G., Abraham, B.J., Zhang, L.N., Weintraub, A.S., Schujiers, J., Lee, T.I., Zhao, K., et al. (2014). Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. Cell *159*, 374–387. https://doi.org/10.1016/j.cell.2014.09.030.

126. Mifsud, B., Tavares-Cadete, F., Young, A.N., Sugar, R., Schoenfelder, S., Ferreira, L., Wingett, S.W., Andrews, S., Grey, W., Ewels, P.A., et al. (2015). Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. Nat. Genet. *47*, 598–606. https://doi.org/10.1038/ng.3286.

127. Schoenfelder, S., Furlan-Magaril, M., Mifsud, B., Tavares-Cadete, F., Sugar, R., Javierre, B.-M., Nagano, T., Katsman, Y., Sakthidevi, M., Wingett, S.W., et al. (2015). The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. Genome Res. *25*, 582–597. https://doi.org/10.1101/gr.185272.114.

128. Pombo, A., and Dillon, N. (2015). Three-dimensional genome architecture: players and mechanisms. Nat. Rev. Mol. Cell Biol. *16*, 245–257. https://doi.org/10.1038/nrm3965.

129. 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. https://doi.org/10.1016/j.stem.2015.11.007.

130. Beagrie, R.A., Scialdone, A., Schueler, M., Kraemer, D.C.A., Chotalia, M., Xie, S.Q., Barbieri, M., de Santiago, I., Lavitas, L.-M., Branco, M.R., et al. (2017). Complex multi-enhancer contacts captured by genome architecture mapping. Nature *543*, 519–524. https://doi.org/10.1038/nature21411.

131. Rubin, A.J., Barajas, B.C., Furlan-Magaril, M., Lopez-Pajares, V., Mumbach, M.R., Howard, I., Kim, D.S., Boxer, L.D., Cairns, J., Spivakov, M., et al. (2017). Lineage-specific dynamic and pre-established enhancer–promoter contacts cooperate in terminal differentiation. Nat. Genet. *49*, 1522–1528. https://doi.org/10.1038/ng.3935.

132. Schoenfelder, S., and Fraser, P. (2019). Long-range enhancer–promoter contacts in gene expression control. Nat. Rev. Genet. *20*, 437–455. https://doi.org/10.1038/s41576-019-0128-0.

133. Long, H.K., Osterwalder, M., Welsh, I.C., Hansen, K., Davies, J.O.J., Liu, Y.E., Koska, M., Adams, A.T., Aho, R., Arora, N., et al. (2020). Loss of extreme long-range enhancers in human neural crest drives a craniofacial disorder. Cell Stem Cell *27*, 765–783.e14. https://doi.org/10.1016/j.stem.2020.09.001.

134. Fulco, C.P., Nasser, J., Jones, T.R., Munson, G., Bergman, D.T., Subramanian, V., Grossman, S.R., Anyoha, R., Doughty, B.R., Patwardhan, T.A., et al. (2019). Activity-by-contact model of enhancer–promoter regulation from thousands of CRISPR perturbations. Nat. Genet. *51*, 1664–1669. https://doi.org/10.1038/s41588-019-0538-0.

135. Benabdallah, N.S., Williamson, I., Illingworth, R.S., Kane, L., Boyle, S., SenGupta, D., Grimes, G.R., Therizols, P., and Bickmore, W.A. (2019). Decreased enhancer-promoter proximity accompanying enhancer activation. Mol. Cell *76*, 473–484.e7. https://doi.org/10.1016/j.molcel.2019.07.038.

136. Alexander, J.M., Guan, J., Li, B., Maliskova, L., Song, M., Shen, Y., Huang, B., Lomvardas, S., and Weiner, O.D. (2019). Live-cell imaging reveals enhancer-dependent Sox2 transcription in the absence of enhancer proximity. eLife *8*, e41769. https://doi.org/10.7554/eLife.41769.

137. Sabari, B.R., Dall'Agnese, A., Boija, A., Klein, I.A., Coffey, E.L., Shrinivas, K., Abraham, B.J., Hannett, N.M., Zamudio, A.V., Manteiga, J.C., et al. (2018). Coactivator condensation at super-enhancers links phase separation and gene control. Science *361*, eaar3958. https://doi.org/10.1126/science.aar3958.

138. Cho, W.-K., Spille, J.-H., Hecht, M., Lee, C., Li, C., Grube, V., and Cisse, I.I. (2018). Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. Science *361*, 412–415. https://doi.org/10.1126/science.aar4199.

139. Barshad, G., Lewis, J.J., Chivu, A.G., Abuhashem, A., Krietenstein, N., Rice, E.J., Ma, Y., Wang, Z., Rando, O.J., Hadjantonakis, A.-K., et al. (2023). RNA polymerase II dynamics shape enhancer–promoter interactions. Nat. Genet. *55*, 1370–1380. https://doi.org/10.1038/s41588-023-01442-7.

140. Isoda, T., Moore, A.J., He, Z., Chandra, V., Aida, M., Denholtz, M., Piet van Hamburg, J., Fisch, K.M., Chang, A.N., Fahl, S.P., et al. (2017). Non-coding transcription instructs chromatin folding and compartmentalization to dictate enhancer-promoter communication and T cell fate. Cell *171*, 103–119.e18. https://doi.org/10.1016/j.cell.2017.09.001.

141. Boija, A., Klein, I.A., Sabari, B.R., Dall'Agnese, A., Coffey, E.L., Zamudio, A.V., Li, C.H., Shrinivas, K., Manteiga, J.C., Hannett, N.M., et al. (2018). Transcription factors activate genes through the phase-separation capacity of their activation domains. Cell *175*, 1842–1855.e16. https://doi.org/10.1016/j.cell.2018.10.042.

142. Lyons, H., Veettil, R.T., Pradhan, P., Fornero, C., De La Cruz, N., Ito, K., Eppert, M., Roeder, R.G., and Sabari, B.R. (2023). Functional partitioning of transcriptional regulators by patterned charge blocks. Cell *186*, 327–345.e28. https://doi.org/10.1016/j.cell.2022.12.013.

143. Patil, A., Strom, A.R., Paulo, J.A., Collings, C.K., Ruff, K.M., Shinn, M.K., Sankar, A., Cervantes, K.S., Wauer, T., St Laurent, J.D., et al. (2023). A disordered region controls cBAF activity via condensation and partner recruitment. Cell *186*, 4936–4955.e26. https://doi.org/10.1016/j.cell.2023.08.032.

144. Shin, Y., and Brangwynne, C.P. (2017). Liquid phase condensation in cell physiology and disease. Science *357*, eaaf4382. https://doi.org/10.1126/science.aaf4382.

145. Lyon, A.S., Peeples, W.B., and Rosen, M.K. (2020). A framework for understanding the functions of biomolecular condensates across scales. Nat. Rev. Mol. Cell Biol. *22*, 215–235. https://doi.org/10.1038/s41580-020-00303-z.

146. Sabari, B.R. (2020). Biomolecular condensates and gene activation in development and disease. Dev. Cell *55*, 84–96. https://doi.org/10.1016/j.devcel.2020.09.005.

147. Alberti, S., and Hyman, A.A. (2021). Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. Nat. Rev. Mol. Cell Biol. *22*, 196–213. https://doi.org/10.1038/s41580-020-00326-6.

148. Gibson, B.A., Doolittle, L.K., Schneider, M.W.G., Jensen, L.E., Gamarra, N., Henry, L., Gerlich, D.W., Redding, S., and Rosen, M.K. (2019). Organization of chromatin by intrinsic and regulated phase separation. Cell *179*, 470–484.e21. https://doi.org/10.1016/j.cell.2019.08.037.

149. Pessina, F., Giavazzi, F., Yin, Y., Gioia, U., Vitelli, V., Galbiati, A., Barozzi, S., Garre, M., Oldani, A., Flaus, A., et al. (2019). Functional transcription promoters at DNA double-strand breaks mediate RNA-driven phase separation of damage-response factors. Nat. Cell Biol. *21*, 1286–1299. https://doi.org/10.1038/s41556-019-0392-4.

150. Guo, Y.E., Manteiga, J.C., Henninger, J.E., Sabari, B.R., Dall'Agnese, A., Hannett, N.M., Spille, J.-H., Afeyan, L.K., Zamudio, A.V., Shrinivas, K., et al. (2019). Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. Nature *572*, 543–548. https://doi.org/10.1038/s41586-019-1464-0.

151. Greig, J.A., Nguyen, T.A., Lee, M., Holehouse, A.S., Posey, A.E., Pappu, R.V., and Jedd, G. (2020). Arginine-enriched mixed-charge domains provide cohesion for nuclear speckle condensation. Mol. Cell *77*, 1237–1250.e4. https://doi.org/10.1016/j.molcel.2020.01.025.

152. Riback, J.A., Zhu, L., Ferrolino, M.C., Tolbert, M., Mitrea, D.M., Sanders, D.W., Wei, M.-T., Kriwacki, R.W., and Brangwynne, C.P. (2020). Composition-dependent thermodynamics of intracellular phase separation. Nature *581*, 209–214. https://doi.org/10.1038/s41586-020-2256-2.

153. Klein, I.A., Boija, A., Afeyan, L.K., Hawken, S.W., Fan, M., Dall'Agnese, A., Oksuz, O., Henninger, J.E., Shrinivas, K., Sabari, B.R., et al. (2020). Partitioning of cancer therapeutics in nuclear condensates. Science *368*, 1386–1392. https://doi.org/10.1126/science.aaz4427.

154. Roden, C.A., and Gladfelter, A.S. (2022). Design considerations for analyzing protein translation regulation by condensates. RNA *28*, 88–96. https://doi.org/10.1261/rna.079002.121.

155. Zamudio, A.V., Dall'Agnese, A., Henninger, J.E., Manteiga, J.C., Afeyan, L.K., Hannett, N.M., Coffey, E.L., Li, C.H., Oksuz, O., Sabari, B.R., et al. (2019). Mediator condensates localize signaling factors to key cell identity genes. Mol. Cell *76*, 753–766.e6. https://doi.org/10.1016/j.molcel.2019.08.016.

156. Dall'Agnese, A., Platt, J.M., Zheng, M.M., Friesen, M., Dall'Agnese, G., Blaise, A.M., Spinelli, J.B., Henninger, J.E., Tevonian, E.N., Hannett, N.M., et al. (2022). The dynamic clustering of insulin receptor underlies its signaling and is disrupted in insulin resistance. Nat. Commun. *13*, 7522. https://doi.org/10.1038/s41467-022-35176-7.

157. Roden, C., and Gladfelter, A.S. (2020). RNA contributions to the form and function of biomolecular condensates. Nat. Rev. Mol. Cell Biol. *22*, 183–195. https://doi.org/10.1038/s41580-020-0264-6.

158. Decker, C.J., Burke, J.M., Mulvaney, P.K., and Parker, R. (2021). RNA is required for the maintenance of multiple cytoplasmic and nuclear membrane-less organelles. Preprint at bioRxiv. https://doi.org/10.1101/2021.08.16.456519.

159. Dejosez, M., Dall'Agnese, A., Ramamoorthy, M., Platt, J., Yin, X., Hogan, M., Brosh, R., Weintraub, A.S., Hnisz, D., Abraham, B.J., et al. (2023). Regulatory architecture of housekeeping genes is driven by promoter assemblies. Cell Rep. *42*, 112505. https://doi.org/10.1016/j.celrep.2023.112505.

160. Kim, T.-K., and Shiekhattar, R. (2015). Architectural and functional commonalities between enhancers and promoters. Cell *162*, 948–959. https://doi.org/10.1016/j.cell.2015.08.008.

161. Dall'Agnese, A., and Young, R. (2023). Regulatory architecture of cell identity genes and housekeeping genes. Trends Cell Biol. *33*, 1010–1013. https://doi.org/10.1016/j.tcb.2023.08.007.

162. Gil, N., and Ulitsky, I. (2019). Regulation of gene expression by cis -acting long non-coding RNAs. Nat. Rev. Genet. *21*, 102–117. https://doi.org/10.1038/s41576-019-0184-5.

163. Jachowicz, J.W., Strehle, M., Banerjee, A.K., Blanco, M.R., Thai, J., and Guttman, M. (2022). Xist spatially amplifies SHARP/SPEN recruitment to balance chromosome-wide silencing and specificity to the X chromosome. Nat. Struct. Mol. Biol. *29*, 239–249. https://doi.org/10.1038/s41594-022-00739-1.

164. Markaki, Y., Chong, J.G., Wang, Y., Jacobson, E.C., Luong, C., Tan, S.Y.X., Maestrini, D., Banerjee, A.K., Mistry, B.A., Dror, I., et al. (2021). Xist nucleates local protein gradients to propagate silencing across the X chromosome. Cell *184*, 6174–6192.e32. https://doi.org/10.1016/j.cell.2021.10.022.

165. Miyagawa, R., Tano, K., Mizuno, R., Nakamura, Y., Ijiri, K., Rakwal, R., Shibato, J., Masuo, Y., Mayeda, A., Hirose, T., et al. (2012). Identification of cis- and trans-acting factors involved in the localization of MALAT-1 noncoding RNA to nuclear speckles. RNA *18*, 738–751. https://doi.org/10.1261/rna.028639.111.

166. Arun, G., Aggarwal, D., and Spector, D.L. (2020). MALAT1 long non-coding RNA: functional implications. Noncoding. RNA *6*, 22. https://doi.org/10.3390/ncrna6020022.

167. Yeom, K.-H., Pan, Z., Lin, C.-H., Lim, H.Y., Xiao, W., Xing, Y., and Black, D.L. (2021). Tracking pre-mRNA maturation across subcellular compartments identifies developmental gene regulation through intron retention and nuclear anchoring. Genome Res. *31*, 1106–1119. https://doi.org/10.1101/gr.273904.120.

168. Daneshvar, K., Ardehali, M.B., Klein, I.A., Hsieh, F.-K., Kratkiewicz, A.J., Mahpour, A., Cancelliere, S.O.L., Zhou, C., Cook, B.M., Li, W., et al. (2020). lncRNA DIGIT and BRD3 protein form phase-separated condensates to regulate endoderm differentiation. Nat. Cell Biol. *22*, 1211–1222. https://doi.org/10.1038/s41556-020-0572-2.

169. Daneshvar, K., Pondick, J.V., Kim, B.-M., Zhou, C., York, S.R., Macklin, J.A., Abualteen, A., Tan, B., Sigova, A.A., Marcho, C., et al. (2016). DIGIT is a conserved long noncoding RNA that regulates GSC expression to control definitive endoderm differentiation of embryonic stem cells. Cell Rep. *17*, 353–365. https://doi.org/10.1016/j.celrep.2016.09.017.

170. Wang, L., Gao, Y., Zheng, X., Liu, C., Dong, S., Li, R., Zhang, G., Wei, Y., Qu, H., Li, Y., et al. (2019). Histone modifications regulate chromatin compartmentalization by contributing to a phase separation mechanism. Mol. Cell *76*, 646–659.e6. https://doi.org/10.1016/j.molcel.2019.08.019.

171. Strickfaden, H., Tolsma, T.O., Sharma, A., Underhill, D.A., Hansen, J.C., and Hendzel, M.J. (2020). Condensed chromatin behaves like a solid on the mesoscale in vitro and in living cells. Cell *183*, 1772–1784.e13. https://doi.org/10.1016/j.cell.2020.11.027.

172. Strom, A.R., Kim, Y., Zhao, H., Orlovsky, N., Chang, Y.-C., Košmrlj, A., Storm, C., and Brangwynne, C.P. (2023). Condensate-driven interfacial forces reposition DNA loci and measure chromatin viscoelasticity. Preprint at bioRxiv. https://doi.org/10.1101/2023.02.27.530281.

173. Quail, T., Golfier, S., Elsner, M., Ishihara, K., Murugesan, V., Renger, R., Jülicher, F., and Brugués, J. (2021). Force generation by protein–DNA co-condensation. Nat. Phys. *17*, 1007–1012. https://doi.org/10.1038/s41567-021-01285-1.

174. Bhat, P., Chow, A., Emert, B., Ettlin, O., Quinodoz, S.A., Strehle, M., Takei, Y., Burr, A., Goronzy, I.N., Chen, A.W., et al. (2024). Genome organization around nuclear speckles drives mRNA splicing efficiency. Nature *629*, 1165–1173. https://doi.org/10.1038/s41586-024-07429-6.

175. Alexander, K.A., Coté, A., Nguyen, S.C., Zhang, L., Gholamalamdari, O., Agudelo-Garcia, P., Lin-Shiao, E., Tanim, K.M.A., Lim, J., Biddle, N., et al. (2021). p53 mediates target gene association with nuclear speckles for amplified RNA expression. Mol. Cell *81*, 1666–1681.e6. https://doi.org/10.1016/j.molcel.2021.03.006.

176. Chen, Y., and Belmont, A.S. (2019). Genome organization around nuclear speckles. Curr. Opin. Genet. Dev. *55*, 91–99. https://doi.org/10.1016/j.gde.2019.06.008.

177. Creamer, K.M., Kolpa, H.J., and Lawrence, J.B. (2021). Nascent RNA scaffolds contribute to chromosome territory architecture and counter

chromatin compaction. Mol. Cell *81*, 3509–3525.e5. https://doi.org/10.1016/j.molcel.2021.07.004.

178. Frank, L., and Rippe, K. (2020). Repetitive RNAs as regulators of chromatin-associated subcompartment formation by phase separation. J. Mol. Biol. *432*, 4270–4286. https://doi.org/10.1016/j.jmb.2020.04.015.

179. Hall, L.L., Carone, D.M., Gomez, A.V., Kolpa, H.J., Byron, M., Mehta, N., Fackelmayer, F.O., and Lawrence, J.B. (2014). Stable C0T-1 repeat RNA is abundant and is associated with euchromatic interphase chromosomes. Cell *156*, 907–919. https://doi.org/10.1016/j.cell.2014.01.042.

180. Marenda, M., Michieletto, D., Czapiewski, R., Stocks, J., Winterbourne, S.M., Miles, J., Fleming, O.C., Lazarova, E., Chiang, M., Aitken, S., et al. (2024). Nuclear RNA forms an interconnected network of transcription-dependent and tunable microgels. Preprint at bioRxiv. https://doi.org/10.1101/2024.06.16.599208.

181. Hilbert, L., Sato, Y., Kuznetsova, K., Bianucci, T., Kimura, H., Jülicher, F., Honigmann, A., Zaburdaev, V., and Vastenhouw, N.L. (2021). Transcription organizes euchromatin via microphase separation. Nat. Commun. *12*, 1360. https://doi.org/10.1038/s41467-021-21589-3.

182. Dueva, Akopyan, R., K., Pederiva, C., Trevisan, D., Dhanjal, S., Lindqvist, A., and Farnebo, M. (2019). Neutralization of the positive charges on histone tails by RNA promotes an open chromatin structure. Cell Chem. Biol. *26*, 1436–1449.e5. https://doi.org/10.1016/j.chembiol.2019.08.002.

183. Hansen, A.S., Hsieh, T.-H.S., Cattoglio, C., Pustova, I., Saldaña-Meyer, R., Reinberg, D., Darzacq, X., and Tjian, R. (2019). Distinct classes of chromatin loops revealed by deletion of an RNA-binding region in CTCF. Mol. Cell *76*, 395–411.e13. https://doi.org/10.1016/j.molcel.2019.07.039.

184. Misteli, T. (2020). The self-organizing genome: principles of genome architecture and function. Cell *183*, 28–45. https://doi.org/10.1016/j.cell.2020.09.014.

185. Feric, M., and Misteli, T. (2021). Phase separation in genome organization across evolution. Trends Cell Biol. *31*, 671–685. https://doi.org/10.1016/j.tcb.2021.03.001.

186. Kawasaki, K., and Fukaya, T. (2024). Regulatory landscape of enhancer-mediated transcriptional activation. Trends Cell Biol. https://doi.org/10.1016/j.tcb.2024.01.008.

187. Snead, W.T. (2019). The control centers of biomolecular phase separation: how membrane surfaces, PTMs, and active processes regulate condensation. Mol. Cell *76*, 11.

188. Shrinivas, K., and Brenner, M.P. (2021). Phase separation in fluids with many interacting components. Proc. Natl. Acad. Sci. USA *118*, e2108551118. https://doi.org/10.1073/pnas.2108551118.

189. Morin, J.A., Wittmann, S., Choubey, S., Klosin, A., Golfier, S., Hyman, A.A., Jülicher, F., and Grill, S.W. (2022). Sequence-dependent surface condensation of a pioneer transcription factor on DNA. Nat. Phys. *18*, 271–276. https://doi.org/10.1038/s41567-021-01462-2.

190. Mittag, T., and Pappu, R.V. (2022). A conceptual framework for understanding phase separation and addressing open questions and challenges. Mol. Cell *82*, 2201–2214. https://doi.org/10.1016/j.molcel.2022.05.018.

191. Goychuk, A., Kannan, D., Chakraborty, A.K., and Kardar, M. (2023). Polymer folding through active processes recreates features of genome organization. Proc. Natl. Acad. Sci. USA *120*, e2221726120. https://doi.org/10.1073/pnas.2221726120.

192. Garcia-Jove Navarro, M.G.-J., Kashida, S., Chouaib, R., Souquere, S., Pierron, G., Weil, D., and Gueroui, Z. (2019). RNA is a critical element for the sizing and the composition of phase-separated RNA–protein condensates. Nat. Commun. *10*, 1–13. https://doi.org/10.1038/s41467-019-11241-6.

193. Shav-Tal, Y., Blechman, J., Darzacq, X., Montagna, C., Dye, B.T., Patton, J.G., Singer, R.H., and Zipori, D. (2005). Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. Mbo C *16*, 2395–2413. https://doi.org/10.1091/mbc.e04-11-0992.

194. Dash, S., Lamb, M.C., Lange, J.J., McKinney, M.C., Tsuchiya, D., Guo, F., Zhao, X., Corbin, T.J., Kirkman, M., Delventhal, K., et al. (2022). rRNA transcription is integral to liquid-liquid phase separation and maintenance of nucleolar structure. Preprint at bioRxiv. https://doi.org/10.1101/2022.11.14.516489.

195. Fox, A.H., Nakagawa, S., Hirose, T., and Bond, C.S. (2018). Paraspeckles: where long noncoding RNA meets phase separation. Trends Biochem. Sci. *43*, 124–135. https://doi.org/10.1016/j.tibs.2017.12.001.

196. Yamazaki, T., Souquere, S., Chujo, T., Kobelke, S., Chong, Y.S., Fox, A.H., Bond, C.S., Nakagawa, S., Pierron, G., and Hirose, T. (2018). Functional domains of NEAT1 architectural lncRNA induce Paraspeckle assembly through phase separation. Mol. Cell *70*, 1038–1053.e7. https://doi.org/10.1016/j.molcel.2018.05.019.

197. Buchan, J.R., and Parker, R. (2009). Eukaryotic stress granules: the ins and outs of translation. Mol. Cell *36*, 932–941. https://doi.org/10.1016/j.molcel.2009.11.020.

198. Forman-Kay, J.D., Ditlev, J.A., Nosella, M.L., and Lee, H.O. (2022). What are the distinguishing features and size requirements of biomolecular condensates and their implications for RNA-containing condensates? RNA *28*, 36–47. https://doi.org/10.1261/rna.079026.121.

199. Sanders, D.W., Kedersha, N., Lee, D.S.W., Strom, A.R., Drake, V., Riback, J.A., Bracha, D., Eeftens, J.M., Iwanicki, A., Wang, A., et al. (2020). Competing protein-RNA interaction networks control multiphase intracellular organization. Cell *181*, 306–324.e28. https://doi.org/10.1016/j.cell.2020.03.050.

200. Kaur, T., Raju, M., Alshareedah, I., Davis, R.B., Potoyan, D.A., and Banerjee, P.R. (2021). Sequence-encoded and composition-dependent protein-RNA interactions control multiphasic condensate morphologies. Nat. Commun. *12*, 872. https://doi.org/10.1038/s41467-021-21089-4.

201. Yao, R.-W., Xu, G., Wang, Y., Shan, L., Luan, P.-F., Wang, Y., Wu, M., Yang, L.-Z., Xing, Y.-H., Yang, L., et al. (2019). Nascent Pre-rRNA sorting via phase separation drives the assembly of dense fibrillar components in the human nucleolus. Mol. Cell *76*, 767–783.e11. https://doi.org/10.1016/j.molcel.2019.08.014.

202. Yamazaki, T., Yamamoto, T., Yoshino, H., Souquere, S., Nakagawa, S., Pierron, G., and Hirose, T. (2021). Paraspeckles are constructed as block copolymer micelles. EMBO J. *40*, e107270. https://doi.org/10.15252/embj.2020107270.

203. Elbaum-Garfinkle, S., Kim, Y., Szczepaniak, K., Chen, C.C.-H., Eckmann, C.R., Myong, S., and Brangwynne, C.P. (2015). The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics. Proc. Natl. Acad. Sci. USA *112*, 7189–7194. https://doi.org/10.1073/pnas.1504822112.

204. Zhang, H., Elbaum-Garfinkle, S., Langdon, E.M., Taylor, N., Occhipinti, P., Bridges, A.A., Brangwynne, C.P., and Gladfelter, A.S. (2015). RNA controls PolyQ protein phase transitions. Mol. Cell *60*, 220–230. https://doi.org/10.1016/j.molcel.2015.09.017.

205. Overbeek, J.T.G., and Voorn, M.J. (1957). Phase separation in polyelectrolyte solutions. Theory of complex coacervation. J. Cell. Physiol. Suppl. *49*, 7–26. https://doi.org/10.1002/jcp.1030490404.

206. Sing, C.E., and Perry, S.L. (2020). Recent progress in the science of complex coacervation. Soft Matter *16*, 2885–2914. https://doi.org/10.1039/D0SM00001A.

207. Aumiller, W.M., and Keating, C.D. (2016). Phosphorylation-mediated RNA/peptide complex coacervation as a model for intracellular liquid organelles. Nat. Chem. *8*, 129–137. https://doi.org/10.1038/nchem.2414.

208. Banerjee, P.R., Milin, A.N., Moosa, M.M., Onuchic, P.L., and Deniz, A.A. (2017). Reentrant phase transition drives dynamic substructure formation in ribonucleoprotein droplets. Angew. Chem. Int. Ed. Engl. *56*, 11354–11359. https://doi.org/10.1002/anie.201703191.

209. Milin, A.N., and Deniz, A.A. (2018). Reentrant phase transitions and non-equilibrium dynamics in membraneless organelles. Biochemistry *57*, 2470–2477. https://doi.org/10.1021/acs.biochem.8b00001.

210. Sharp, P.A., Chakraborty, A.K., Henninger, J.E., and Young, R.A. (2022). RNA in formation and regulation of transcriptional condensates. RNA *28*, 52–57. https://doi.org/10.1261/rna.078997.121.

211. Shao, W., Bi, X., Pan, Y., Gao, B., Wu, J., Yin, Y., Liu, Z., Peng, M., Zhang, W., Jiang, X., et al. (2022). Phase separation of RNA-binding protein promotes polymerase binding and transcription. Nat. Chem. Biol. *18*, 70–80. https://doi.org/10.1038/s41589-021-00904-5.

212. Urrutia, R. (2003). KRAB-containing zinc-finger repressor proteins. Genome Biol. *4*, 231. https://doi.org/10.1186/gb-2003-4-10-231.

213. Comet, I., Riising, E.M., Leblanc, B., and Helin, K. (2016). Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. Nat. Rev. Cancer *16*, 803–810. https://doi.org/10.1038/nrc.2016.83.

214. Holoch, D., and Moazed, D. (2015). RNA-mediated epigenetic regulation of gene expression. Nat. Rev. Genet. *16*, 71–84. https://doi.org/10.1038/nrg3863.

215. Seczynska, M., Bloor, S., Cuesta, S.M., and Lehner, P.J. (2022). Genome surveillance by HUSH-mediated silencing of intronless mobile elements. Nature *601*, 440–445. https://doi.org/10.1038/s41586-021-04228-1.

216. Hall Hickman, A.H., and Jenner, R.G. (2024). Apparent RNA bridging between PRC2 and chromatin is an artifact of non-specific chromatin precipitation upon RNA degradation. Cell Rep. *43*, 113856. https://doi.org/10.1016/j.celrep.2024.113856.

217. Healy, E., Zhang, Q., Gail, E.H., Agius, S.C., Sun, G., Bullen, M., Pandey, V., Das, P.P., Polo, J.M., and Davidovich, C. (2024). The apparent loss of PRC2 chromatin occupancy as an artifact of RNA depletion. Cell Rep. *43*, 113858. https://doi.org/10.1016/j.celrep.2024.113858.

218. Guo, J.K., Blanco, M.R., Walkup, W.G., Bonesteele, G., Urbinati, C.R., Banerjee, A.K., Chow, A., Ettlin, O., Strehle, M., Peyda, P., et al. (2024). Denaturing purifications demonstrate that PRC2 and other widely reported chromatin proteins do not appear to bind directly to RNA *in vivo*. Mol. Cell *84*, 1271–1289.e12. https://doi.org/10.1016/j.molcel.2024.01.026.

219. Long, Y., Hwang, T., Gooding, A.R., Goodrich, K.J., Hanson, S.D., Vallery, T.K., Rinn, J.L., and Cech, T.R. (2024). Evaluation of the RNA-dependence of PRC2 binding to chromatin in human pluripotent stem cells. Preprint at bioRxiv. https://doi.org/10.1101/2023.08.17.553776.

220. Long, Y., Bolanos, B., Gong, L., Liu, W., Goodrich, K.J., Yang, X., Chen, S., Gooding, A.R., Maegley, K.A., Gajiwala, K.S., et al. (2017). Conserved RNA-binding specificity of polycomb repressive complex 2 is achieved by dispersed amino acid patches in EZH2. eLife *6*, e31558. https://doi.org/10.7554/eLife.31558.

221. Song, J., Gooding, A.R., Hemphill, W.O., Love, B.D., Robertson, A., Yao, L., Zon, L.I., North, T.E., Kasinath, V., and Cech, T.R. (2023). Structural basis for inactivation of PRC2 by G-quadruplex RNA. Science *381*, 1331–1337. https://doi.org/10.1126/science.adh0059.

222. Zhang, Q., McKenzie, N.J., Warneford-Thomson, R., Gail, E.H., Flanigan, S.F., Owen, B.M., Lauman, R., Levina, V., Garcia, B.A., Schittenhelm, R.B., et al. (2019). RNA exploits an exposed regulatory site to inhibit the enzymatic activity of PRC2. Nat. Struct. Mol. Biol. *26*, 237–247. https://doi.org/10.1038/s41594-019-0197-y.

223. Gail, E.H., Healy, E., Flanigan, S.F., Jones, N., Ng, X.H., Uckelmann, M., Levina, V., Zhang, Q., and Davidovich, C. (2024). Inseparable RNA binding and chromatin modification activities of a nucleosome-interacting surface in EZH2. Nat. Genet. *56*, 1193–1202. https://doi.org/10.1038/s41588-024-01740-8.

224. Velazquez Camacho, O., Galan, C., Swist-Rosowska, K., Ching, R., Gamalinda, M., Karabiber, F., De La Rosa-Velazquez, I., Engist, B., Koschorz, B., Shukeir, N., et al. (2017). Major satellite repeat RNA stabilize heterochromatin retention of Suv39h enzymes by RNA-nucleosome association and RNA:DNA hybrid formation. eLife *6*, e25293. https://doi.org/10.7554/eLife.25293.

225. Johnson, W.L., Yewdell, W.T., Bell, J.C., McNulty, S.M., Duda, Z., O'Neill, R.J., Sullivan, B.A., and Straight, A.F. (2017). RNA-dependent stabilization of SUV39H1 at constitutive heterochromatin. eLife *6*, e25299. https://doi.org/10.7554/eLife.25299.

226. Chow, J.C., Ciaudo, C., Fazzari, M.J., Mise, N., Servant, N., Glass, J.L., Attreed, M., Avner, P., Wutz, A., Barillot, E., et al. (2010). LINE-1 activity in facultative heterochromatin formation during X chromosome inactivation. Cell *141*, 956–969. https://doi.org/10.1016/j.cell.2010.04.042.

227. Novo, C.L., Wong, E.V., Hockings, C., Poudel, C., Sheekey, E., Wiese, M., Okkenhaug, H., Boulton, S.J., Basu, S., Walker, S., et al. (2022). Satellite repeat transcripts modulate heterochromatin condensates and safeguard chromosome stability in mouse embryonic stem cells. Nat. Commun. *13*, 3525. https://doi.org/10.1038/s41467-022-31198-3.

228. Pandya-Jones, A., Markaki, Y., Serizay, J., Chitiashvili, T., Mancia Leon, W.R., Damianov, A., Chronis, C., Papp, B., Chen, C.-K., McKee, R., et al. (2020). A protein assembly mediates Xist localization and gene silencing. Nature *587*, 145–151. https://doi.org/10.1038/s41586-020-2703-0.

229. Peterlin, B.M., and Price, D.H. (2006). Controlling the elongation phase of transcription with P-TEFb. Mol. Cell *23*, 297–305. https://doi.org/10.1016/j.molcel.2006.06.014.

230. Yang, Z., Yik, J.H.N., Chen, R., He, N., Jang, M.K., Ozato, K., and Zhou, Q. (2005). Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Mol. Cell *19*, 535–545. https://doi.org/10.1016/j.molcel.2005.06.029.

231. Jang, M.K., Mochizuki, K., Zhou, M., Jeong, H.-S., Brady, J.N., and Ozato, K. (2005). The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Mol. Cell *19*, 523–534. https://doi.org/10.1016/j.molcel.2005.06.027.

232. Lee, T.I., and Young, R.A. (2013). Transcriptional regulation and its misregulation in disease. Cell *152*, 1237–1251. https://doi.org/10.1016/j.cell.2013.02.014.

233. Bradner, J.E., Hnisz, D., and Young, R.A. (2017). Transcriptional addiction in cancer. Cell *168*, 629–643. https://doi.org/10.1016/j.cell.2016.12.013.

234. Ahn, J.H., Davis, E.S., Daugird, T.A., Zhao, S., Quiroga, I.Y., Uryu, H., Li, J., Storey, A.J., Tsai, Y.-H., Keeley, D.P., et al. (2021). Phase separation drives aberrant chromatin looping and cancer development. Nature *595*, 591–595. https://doi.org/10.1038/s41586-021-03662-5.

235. Terlecki-Zaniewicz, S., Humer, T., Eder, T., Schmoellerl, J., Heyes, E., Manhart, G., Kuchynka, N., Parapatics, K., Liberante, F.G., Müller, A.C., et al. (2021). Biomolecular condensation of NUP98 fusion proteins drives leukemogenic gene expression. Nat. Struct. Mol. Biol. *28*, 190–201. https://doi.org/10.1038/s41594-020-00550-w.

236. Chandra, B., Michmerhuizen, N.L., Shirnekhi, H.K., Tripathi, S., Pioso, B.J., Baggett, D.W., Mitrea, D.M., Iacobucci, I., White, M.R., Chen, J., et al. (2022). Phase separation mediates NUP98 fusion oncoprotein leukemic transformation. Cancer Discov. *12*, 1152–1169. https://doi.org/10.1158/2159-8290.CD-21-0674.

237. Malik, I., Kelley, C.P., Wang, E.T., and Todd, P.K. (2021). Molecular mechanisms underlying nucleotide repeat expansion disorders. Nat. Rev. Mol. Cell Biol. *22*, 589–607. https://doi.org/10.1038/s41580-021-00382-6.

238. Basu, S., Mackowiak, S.D., Niskanen, H., Knezevic, D., Asimi, V., Grosswendt, S., Geertsema, H., Ali, S., Jerković, I., Ewers, H., et al. (2020). Unblending of transcriptional condensates in human repeat expansion disease. Cell *181*, 1062–1079.e30. https://doi.org/10.1016/j.cell.2020.04.018.

239. Jain, A., and Vale, R.D. (2017). RNA phase transitions in repeat expansion disorders. Nature *546*, 243–247. https://doi.org/10.1038/nature22386.

240. Anastasiadou, E., Jacob, L.S., and Slack, F.J. (2018). Non-coding RNA networks in cancer. Nat. Rev. Cancer *18*, 5–18. https://doi.org/10.1038/nrc.2017.99.

241. Slack, F.J., and Chinnaiyan, A.M. (2019). The role of non-coding RNAs in oncology. Cell *179*, 1033–1055. https://doi.org/10.1016/j.cell.2019.10.017.

242. Goodall, G.J., and Wickramasinghe, V.O. (2021). RNA in cancer. Nat. Rev. Cancer *21*, 22–36. https://doi.org/10.1038/s41568-020-00306-0.

243. Arun, G., and Spector, D.L. (2019). MALAT1 long non-coding RNA and breast cancer. RNA Biol. *16*, 860–863. https://doi.org/10.1080/15476286.2019.1592072.

244. Kristensen, L.S., Andersen, M.S., Stagsted, L.V.W., Ebbesen, K.K., Hansen, T.B., and Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. Nat. Rev. Genet. *20*, 675–691. https://doi.org/10.1038/s41576-019-0158-7.

245. Liu, C.-X., and Chen, L.-L. (2022). Circular RNAs: characterization, cellular roles, and applications. Cell *185*, 2016–2034. https://doi.org/10.1016/j.cell.2022.04.021.

246. Freedman, M.L., Monteiro, A.N.A., Gayther, S.A., Coetzee, G.A., Risch, A., Plass, C., Casey, G., De Biasi, M., Carlson, C., Duggan, D., et al. (2011). Principles for the post-GWAS functional characterization of cancer risk loci. Nat. Genet. *43*, 513–518. https://doi.org/10.1038/ng.840.

247. Nasser, J., Bergman, D.T., Fulco, C.P., Guckelberger, P., Doughty, B.R., Patwardhan, T.A., Jones, T.R., Nguyen, T.H., Ulirsch, J.C., Lekschas, F., et al. (2021). Genome-wide enhancer maps link risk variants to disease genes. Nature *593*, 238–243. https://doi.org/10.1038/s41586-021-03446-x.

248. Maurano, M.T., Humbert, R., Rynes, E., Thurman, R.E., Haugen, E., Wang, H., Reynolds, A.P., Sandstrom, R., Qu, H., Brody, J., et al. (2012). Systematic localization of common disease-associated variation in regulatory DNA. Science *337*, 1190–1195. https://doi.org/10.1126/science.1222794.

249. Farh, K.K.-H., Marson, A., Zhu, J., Kleinewietfeld, M., Housley, W.J., Beik, S., Shoresh, N., Whitton, H., Ryan, R.J.H., Shishkin, A.A., et al. (2015). Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature *518*, 337–343. https://doi.org/10.1038/nature13835.

250. Choudhuri, A., Trompouki, E., Abraham, B.J., Colli, L.M., Kock, K.H., Mallard, W., Yang, M.-L., Vinjamur, D.S., Ghamari, A., Sporrij, A., et al. (2020). Common variants in signaling transcription-factor-binding sites drive phenotypic variability in red blood cell traits. Nat. Genet. *52*, 1333–1345. https://doi.org/10.1038/s41588-020-00738-2.

251. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A.J., Bambrick, J., et al. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature *630*, 493–500. https://doi.org/10.1038/s41586-024-07487-w.

252. Kappel, K., Zhang, K., Su, Z., Watkins, A.M., Kladwang, W., Li, S., Pintilie, G., Topkar, V.V., Rangan, R., Zheludev, I.N., et al. (2020). Accelerated cryo-EM-guided determination of three-dimensional RNA-only structures. Nat. Methods *17*, 699–707. https://doi.org/10.1038/s41592-020-0878-9.

253. Corley, M., Flynn, R.A., Lee, B., Blue, S.M., Chang, H.Y., and Yeo, G.W. (2020). Footprinting SHAPE-eCLIP reveals transcriptome-wide hydrogen bonds at RNA-protein interfaces. Mol. Cell *80*, 903–914.e8. https://doi.org/10.1016/j.molcel.2020.11.014.

254. Siegfried, N.A., Busan, S., Rice, G.M., Nelson, J.A.E., and Weeks, K.M. (2014). RNA motif discovery by SHAPE and mutational profiling (SHAPE-MaP). Nat. Methods *11*, 959–965. https://doi.org/10.1038/nmeth.3029.

255. Ross, C.J., Rom, A., Spinrad, A., Gelbard-Solodkin, D., Degani, N., and Ulitsky, I. (2021). Uncovering deeply conserved motif combinations in rapidly evolving noncoding sequences. Genome Biol. *22*, 29. https://doi.org/10.1186/s13059-020-02247-1.

256. Kirk, J.M., Kim, S.O., Inoue, K., Smola, M.J., Lee, D.M., Schertzer, M.D., Wooten, J.S., Baker, A.R., Sprague, D., Collins, D.W., et al. (2018). Functional classification of long non-coding RNAs by k-mer content. Nat. Genet. *50*, 1474–1482. https://doi.org/10.1038/s41588-018-0207-8.