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Review article

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RNA polymerase II transcription compartments — from factories to condensates

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Abstract

Transcription by RNA polymerase II is a fundamental step in gene regulation that mainly occurs in discrete nuclear foci, or transcription compartments, characterized by a high local concentration of polymerases and nascent RNA. Early studies referred to these foci as transcription factories, proposing that they harbour most transcriptional activity and all relevant protein machinery to produce mature RNAs. However, this model of transcriptional organization has long remained controversial owing to its mechanistic uncertainties. Recently, new insights into how these foci may form are being provided by studies of phase-separated transcriptional condensates that encompass RNA polymerases, transcription factors and RNA. Advances in 3D genomics and chromatin imaging are also deepening our understanding of how transcription compartments might facilitate communication between *cis*-regulatory elements in 3D nuclear space. In this Review, we contrast historical work on transcription factories with recent findings on transcriptional condensates to better understand the architecture and functional relevance of transcription compartments.

Sections

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Introduction

Transcription must be precisely regulated in space and time to ensure cellular homeostasis and the proper response to signalling cues. Transcriptional processes take place in the crowded eukaryotic nucleus, in which rapid, size-dependent diffusion of soluble factors can traverse the chromatin scaffold within seconds[1]. Despite this potential mobility, transcription does not occur diffusely throughout the nucleus but rather at discrete sites with high local concentrations of transcriptional machinery and nascent RNA. For instance, RNA polymerase I (RNAPI) and associated factors form large (up to 500 nm in diameter) proteinaceous cores, known as the fibrillar centres, that draw in and transcribe ribosomal gene arrays to produce nascent ribosomal RNAs. In turn, these ribosomal RNAs extrude into the surrounding outer zone known as the dense fibrillar component[2,3]. Similarly, RNA polymerase II (RNAPII) and scaffold proteins such as NPAT associate in large membraneless formations known as histone locus bodies (HLBs) that transcribe tandem histone genes[4]. These highly organized structures provided early evidence of transcriptional compartmentalization being critical for efficient gene expression and regulation.

Studies in the 1990s demonstrated that similar organizational principles apply to RNAPII when engaged with essentially any gene and its enhancers, with interactions involving active enhancers and promoters on the same or on different chromosomes (Fig. 1). Using electron microscopy and nascent RNA labelling, these studies reported discrete foci of clustered RNAPII, which were termed 'transcription factories', and further described as metastable nuclear substructures consisting of multiple RNAPIIs transcribing different genes[5,6] (Fig. 2a). Other studies subsequently confirmed this observation[7–9]. Nevertheless, the transcription factory model remained controversial given the lack of clear answers to many mechanistic questions: such as what are its constituents beyond active RNAPII, how is assembly controlled, what maintains the high local concentration of its components, is DNA reeled into the factory by RNAPII and is factory formation a cause or a consequence of transcriptional activation[10]?

Technological and conceptual advancements in recent years have created the opportunity to revisit the transcription factory model with newer insights into the mechanisms underlying the formation and function of transcription compartments. In 2017, it was proposed that the assembly of the transcriptional machinery at super-enhancers occurs by phase separation to drive gene expression[11]. Subsequent studies initially focused on liquid–liquid phase separation[12] – a process in which proteins and nucleic acids spontaneously segregate into liquid-like droplets – as the primary mechanism behind the formation of transcriptional condensates that enhance gene expression[13–17] (Fig. 2b and Box 1). In phase separation, diffuse proteins will spontaneously coalesce through multivalent intermolecular interactions once a critical concentration threshold is surpassed. Such interactions often occur between homotypic and heterotypic intrinsically disordered regions (IDRs) of proteins[18,19], as well as between IDRs and RNA or DNA to potentially drive assembly on chromatin[20–22]. Notably, IDRs are particularly prevalent in transcription-related proteins, such as the largest RNAPII subunit, ubiquitous transcriptional co-activators (such as Mediator subunits and BRD4)[14] and most cell-type-specific transcription factors (TFs)[23,24]. This pattern suggests that multivalent interactions involving IDRs are crucial for organizing transcription foci, but their exact roles in this context remain to be fully elucidated[25]. Similarly, the extent to which phase separation of the transcriptional machinery into condensates is functionally relevant in vivo remains unclear. For example, several studies show condensates arising from TF overexpression but do not formally demonstrate that they assemble via a phase transition[21,25–27] (Box 1), whereas others have challenged the assumption that transcription is enhanced by merely driving TF assembly into liquid-like droplets[28–32]. Nevertheless, studies of phase separation provide an intuitive mechanistic framework that could help explain the formation and maintenance of transcription compartments in eukaryotic cells.

In this Review, we examine both historical and recent findings on transcription compartments with the aim of better defining how the observed spatial organization of transcription emerges and how it underpins gene regulation. We first clarify the terminology and then discuss how the descriptions of different types of transcription compartments primarily reflect the fundamental differences of how they were studied. Next, we contrast earlier findings on transcription

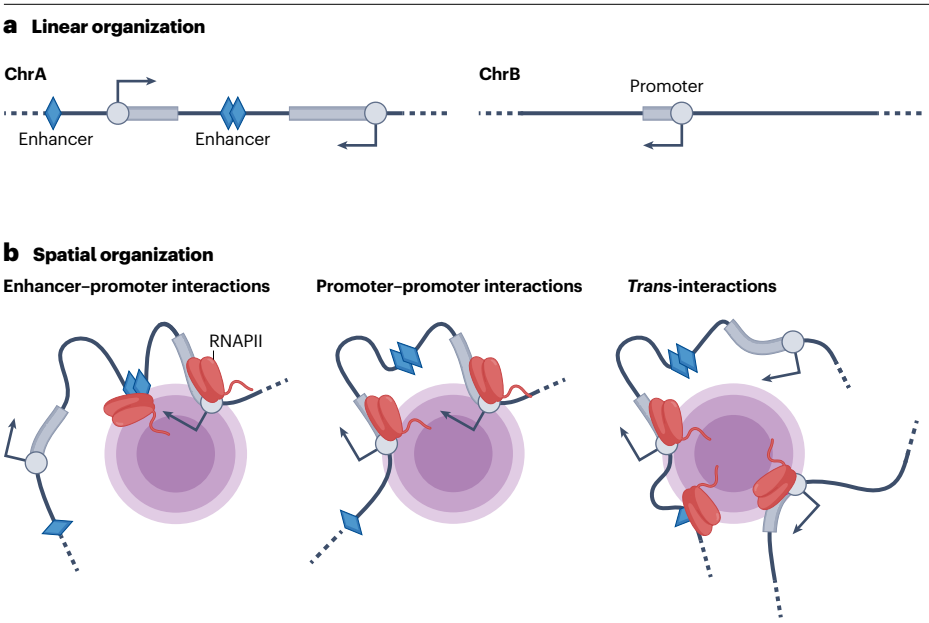

Fig. 1 | Basic organization of mammalian chromatin. Gene promoters (white circles) and enhancers (blue diamonds) are dispersed along linear chromosomes (part **a**), but have been shown to co-associate in 3D space in *cis* (through promoter–enhancer or promoter–promoter interactions) or in *trans* (through interactions between distant elements) when actively transcribed by RNA polymerase II (RNAPII) (red) (part **b**).

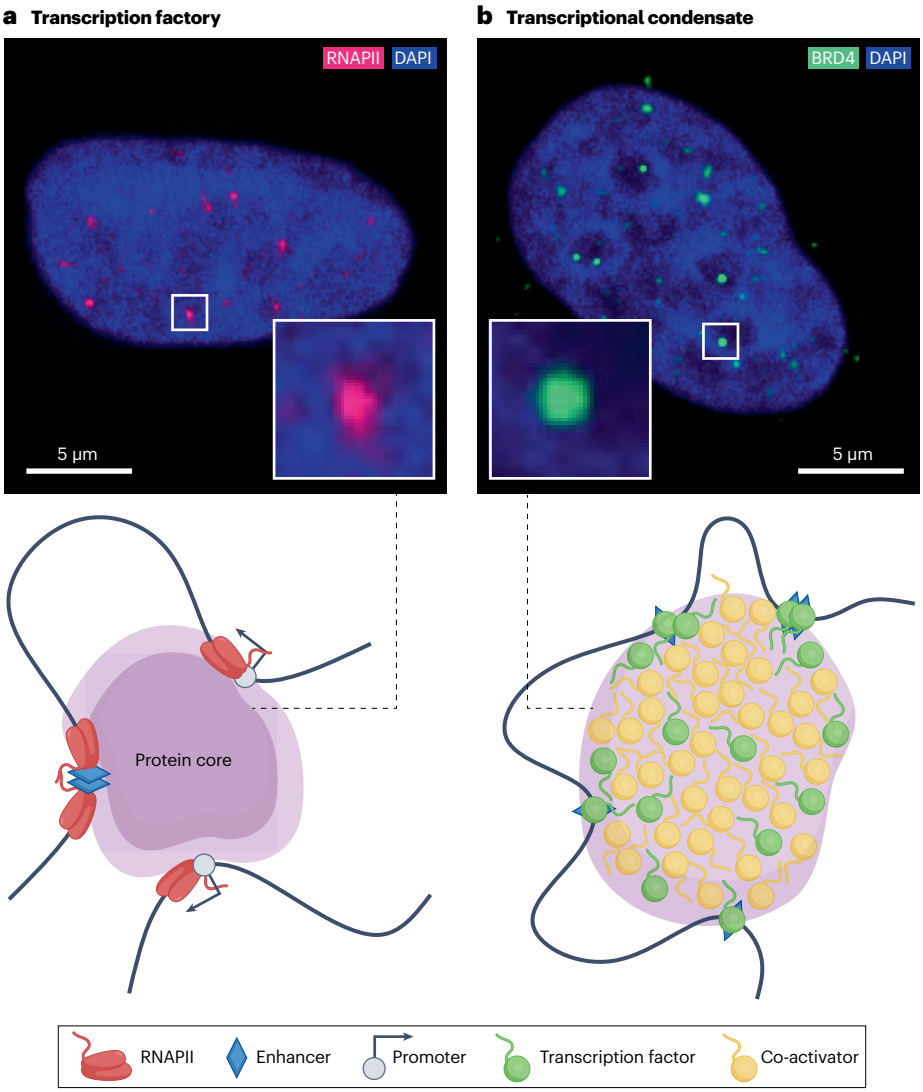

Fig. 2 | Organization of transcription factories and phase-separated transcriptional condensates in mammalian cell nuclei. a, Top: confocal imaging of Ser5-hyperphosphorylated RNA polymerase II (RNAPII) (red) distribution in the nucleus of a human TR-14 cell counterstained by 4′,6-diamidino-2-phenylindole (DAPI) (blue). Inset: magnification of a representative RNAPII cluster. Bottom: model of a transcription factory[3] associated with three transcription units, two promoters (white) and one enhancer cluster (blue) engaged with RNAPII (red). **b**, Top: as in panel **a**, but for BRD4 (green) distribution. Bottom: a transcriptional condensate model as proposed in ref. 14, made up of transcription factors (green) and co-activators (yellow) engaged with a chromatin segment carrying a cluster of enhancers.

factories with more recent evidence on phase-separated condensates to gain insights into transcription compartments in terms of their architecture, mechanisms of formation and dissolution and functional roles. We conclude by integrating the frameworks of factories and condensates and highlighting their common features, as well as those in need of reconciliation, in an effort to better understand gene regulation and its dysregulation in disease.

Definitions and experimental context

Various terms have been coined to describe transcriptional foci, often referring to overlapping phenomena while emphasizing distinct aspects or drawing from specific experimental approaches and concepts (Table 1). In this Review, we use 'transcription compartment' as an overarching term when referring to high local concentrations of transcription-related machinery without implying a specific mechanism of assembly[33–35]. As per its original definition[3], the term transcription factory refers to RNAPII clusters involving at least two transcribed units, that is, two gene promoters or enhancers (Fig. 2a). The term phase-separated transcriptional-condensate (PST-condensate) refers to an assembly of *cis*-regulatory elements and transcription-associated proteins, but not necessarily RNAPII[11], formed by phase separation[13–15] (Fig. 2b). This specification is crucial as it provides a testable biophysical framework for the formation of PST-condensates (Box 2). Of note, the term transcriptional condensate is broader and introduces ambiguity by also potentially referring to condensate formation through other processes[34,36] (Box 1).

To exemplify how the terms transcription factories and PST-condensates often describe similar structures but emphasize different aspects of their organization, we can examine how they are usually identified. Transcription factories are typically visualized in fixed cells as distinct clusters of RNAPII hyperphosphorylated at Ser residues of their C-terminal domains (CTDs), indicating actively transcribing RNAPs[5]. Nevertheless, this experimental context has created the impression that factories represent static structures. By contrast, PST-condensates are primarily studied in living cells using fluorescently tagged proteins, which emphasizes their dynamic properties. However, endogenous protein tagging means that both active and inactive RNAPII

Box 1 | The evolution of transcriptional condensate concepts

The term 'transcriptional condensate' was introduced to describe specialized assemblies forming at super-enhancers through phase separation, which were proposed to enhance transcription by locally concentrating the relevant machinery[11]. It was initially assigned to liquid–liquid phase separation (LLPS) mechanisms forming well-mixed, spherical, liquid-like droplets — often likened to oil droplets in water — with rapid internal dynamics that enhance transcription[13–17].

The LLPS mechanism provides a biophysically grounded, testable model for how transcription factors and co-activators concentrate at enhancers and promoters to enhance gene expression. However, accumulating experimental evidence indicates that transcriptional condensates do not always behave like liquids[27,34,36]. Rather, many transcriptional condensates display heterogeneous composition and reduced dynamics, suggesting a departure from pure liquid behaviour. They can adopt gel-like or dynamically arrested states that resist the fusion and internal mixing expected of liquids[73]. In addition, several studies suggest that transcriptional condensate formation does not necessarily enhance transcription, challenging early assumptions[28–32].

Currently, it often remains unclear whether a transcriptional condensate assembly involves phase separation, hence this mechanism not being used as a defining feature[44]. As such, the biomolecular condensate field is now using the broader term 'phase transition'[139,140], which refers to any shift in the biophysical or organizational state of a system. It includes 'segregative' phase transitions (changes in density) from demixing by LLPS to other forms of phase separation, but also associative transitions such as percolation or gelation, in which connectivity emerges through multivalent interactions giving rise to a network. Thus, instead of describing the assembly of transcriptional condensates with a simple LLPS model, a more complex framework incorporating heterogeneity, time-dependent dynamic arrest, surface-mediated condensation and various classes of phase transitions is considered. It suggests that transcriptional condensates are best described as viscoelastic network fluids with time-dependent properties[139,140] along a continuum from predominantly viscous (liquid-like) to predominantly elastic (solid-like). Positioning on this spectrum is determined by the molecular composition and interaction dynamics of the condensate, as well as of environmental conditions, better reflecting the biophysical diversity observed in vivo. However, these considerations also further complicate the precise testing of the extent and specific type of phase separation involved in the assembly mechanism of a given transcriptional condensate (Box 2).

complexes are studied, as visualizing hyperphosphorylated RNAPIIs in living cells remains challenging[37]. This experimental context results in measurements of PST-condensate clustering dynamics that vary by over two orders of magnitude (from seconds to minutes) and only detect clusters of ≥3 RNAPs by approximately a quarter of all tagged molecules[38,39]. Moreover, such dynamics are often cited as evidence for liquid-like properties but may equally result from transient binding to a scaffold[26,40,41] (Box 2). Equivalent measurements for the clustering of hyperphosphorylated RNAPs in transcription factories are sparse[10]. Therefore, it is likely that such differences between PST-condensates and transcription factories primarily reflect methodological rather than fundamental biological discrepancies and should be cautiously interpreted.

Architecture

Technological advances, such as higher resolution live-cell imaging, have provided insights into the architecture of transcription factories and PST-condensates, specifically elucidating their subnuclear topography (size and density), macromolecular composition (that enables functional specialization), and other architectural features (such as the relative ordering of their components).

Subnuclear topography

Transcription factories are multi-megadalton structures <250 nm in size. Electron and super-resolution imaging measured individual transcription factories as 45–100 nm in diameter in fixed cells[42,43], and subsequent fluorescence microscopy of RNAPII clusters in living cells yielded values of 95–220 nm (ref. 38). The difference in measured sizes between microscopy techniques probably owes to live-cell imaging being unable to separate temporally distinct clustering events originating from neighbouring foci. The size range of PST-condensates is less well defined, but one study reported PST-condensates sizes that range from 100 nm to 500 nm (ref. 44), and others reported sizes of 100 nm to >300 nm for clusters containing three or more RNAPII complexes[38,39]. All are notably consistent with the separation of active enhancer–promoter pairs when bridged by transcription complexes (~340 nm)[45]. However, limited optical resolution of the methods used to measure PST-condensates might under-represent any smaller structures that could be present, as recent work using super-resolution imaging suggested that functional PST-condensates are much smaller than initially thought[46]. Additionally, measurements of PST condensates and transcription factories are not perfectly comparable given that PST-condensates, unlike transcription factories, do not require active RNAPII to be used as a marker and therefore do not necessarily refer to the same entities.

The number of transcription factories per nucleus varies by cell type, ranging from several hundreds to over ten thousand, but remains remarkably constant at 6–8 factories μm^{-3} (refs. 6,25,47). This density persists even when nuclear volume decreases during cell differentiation or increases, for example, in salamander cell nuclei carrying an 11-fold larger genome than human cells[47]. Thus, measurements from fixed cells suggest that the number of RNAPIIs per factory should not substantially fluctuate and — given the known diffusion rates for chromatin loci — any locus can associate with a factory within a few minutes. These two features theoretically ensure efficient and prompt transcription of all active loci in a cell. The densities of PST-condensates remain unclear, probably owing to different definitions of PST-condensates, to technical challenges in counting smaller PST-condensates (resulting from their dynamic nature) and to the variance in condensate formation between cell types and conditions.

Concerning their subnuclear distribution, transcription factories containing RNAPII or RNA polymerase III (RNAPIII) are spatially separated from each other, as well as from the RNAPI machinery of the nucleolus[48]. Additionally, some RNAPII factories might be enriched for specific cell-type-specific or stimulus-specific TFs that could confer transcriptional specialization for gene subsets[49,50]. One such example are HLBs, which are nucleated by Mcx/NPAT bound to histone gene

loci that require specialized factors to be transcribed[4]. Specialization can also be envisioned for PST-condensates through high concentrations of particular TFs bound to cell-type-specific enhancers that are thought to serve as their assembly platform[13]. Finally, transcription factories display a close spatial relationship with nuclear speckles that are enriched with splicing factors[51], and PST-condensates preferentially form around clustered active genes that are highly associated with nuclear speckles[52]. Thus, despite some missing quantitative information, transcription factories and PST-condensates often exhibit similarities in their sizes, density and subnuclear distributions.

Macromolecular composition

The macromolecular composition of transcription factories has been characterized using methods such as imaging and proteomics. Initially, each transcription factory in differentiated mammalian cells was proposed to contain approximately eight active RNAPII molecules on average, which is about 1,000 times the RNAPII concentration in the surrounding nucleoplasm[53]. However, structures resembling transcription factories in other cell types and developmental stages, such as the transcription bodies during early zebrafish development, have been reported to contain several hundred RNAPII complexes[54]. Beyond RNAPs, transcription factories are also thought to harbour >90% of all nascent RNAs in mammalian cells, excluding nucleolar RNAs[42]. Additionally, a key study biochemically isolated very large (>20 MDa) native supramolecular complexes containing RNAPI, RNAPII or RNAPIII and showed through proteomic analysis that each had a distinct macromolecular composition[55]. More specifically, RNAPII factories were found to contain general TFs, Mediator subunits, chromatin remodellers, RNA processing and splicing factors, as well as structural proteins presumably involved in their organization relative to the nucleoskeleton. This same approach, when coupled with RNA sequencing, demonstrated that nascent RNAs are highly enriched in these transcription factory isolates[56].

The compositions of transcription factories and PST-condensates seem to overlap[34,57,58]. Similar to transcription factories, PST-condensates contain TFs and cofactors, including Mediator subunits (MED1 and MED12), BRD3/4, CBP/p300, HDAC1/2, CDK9, SWI/SNF remodellers, RNAPII and general TFs such as TFIID, as well as NPAT and FLASH (key components of HLBs)[58]. Regarding nucleic acids, PST-condensates contain enhancer RNAs, upstream antisense RNAs and nascent transcripts (both protein-coding and long non-coding). Notably, the composition of PST-condensates varies based on the specific locus being studied, cell type or state, transcription cycle stage and signalling conditions, reflecting the diversity of markers used to identify PST-condensates as well as their functional specification.

Architectural features

Both transcription factories and PST-condensates display a distinctive architecture characterized by a protein-rich core surrounded by active RNAPs on the surface (Fig. 2). Electron spectroscopic imaging in human cells revealed that, in transcription factories, this protein-rich core has a diameter of ~90 nm and an estimated mass of >10 MDa (ref. 43). This core is depleted of chromatin and RNA and appears polymorphic rather than spherical. Active RNAPII is located on the core surface, with nascent transcripts extruded at the core–chromatin interface. In mouse erythroblasts, correlative light and electron microscopy identified similarly polymorphic, protein-rich cores decorated with RNAPII, which were ~120 nm in diameter and transcribed cell-type-specific genes[49]. In *Drosophila* embryos during the earlier stages after genome activation, almost all RNAPII organizes in approximately 100 nuclear speckles evenly distributed throughout nuclear space[59]. The two distinctly larger structures are HLBs transcribing histone genes, and the others are associated with activated gene loci. In HLBs that can measure up to 1 µm in diameter, tandem histone gene arrays bind the N terminus of Mxc (the orthologue of mammalian NPAT) to nucleate a protein-rich core with RNAPII, which is in turn surrounded by an outer shell of RNA-processing factors such as FLASH and U7 snRNP that interact with the C terminus of Mxc[4,60,61].

PST-condensates are typically characterized by a round, droplet-like shape. However, those containing RNAPII appeared polymorphic in super-resolution imaging experiments, probably owing to the functional association of the polymerases with enhancers and promoters on chromatin[62]. For instance, RNAPs in zebrafish assemble with chromatin into microphase-separated 'pockets' with a protein-rich core decorated by RNAPII hyperphosphorylated at Ser5 of its CTD[62]. This organization resembles the phase-separated 'RNA nanodomains' seen in human cells and the 'RNAPII compartments' in zebrafish, in which active RNAPII and nascent RNA form exclusionary

Table 1 | Features of transcription compartment terms

Transcription compartment	Marker	DNA content	RNA content	Cellular density	Size	Long-range interactions	Assembly mechanism	Refs.
Transcription factories	Active RNAPII/RNAPIII clusters	*Cis*-regulatory elements, genes	Coding and non-coding RNAs	200–5,000, 6–8 µm^{-3}	45–220 nm	RNAPII-mediated	Chromatin looping, RNAPII depletion–attraction	3,5–10
Active chromatin hubs	Clusters of active genes	*Cis*-regulatory elements, genes	Undefined	Undefined	100–1,000 nm	Promoter–enhancer	Chromatin looping	131
Histone locus bodies	RNAPII clusters, NPAT, FLASH, U7 snRNP	Histone gene clusters	Histone pre-mRNAs	1–2 per nucleus	100–1,000 nm	Histone gene clustering	NPAT/Mxc binding to histone gene clusters, phase separation	4,61,135
TF/co-activator hubs	Accumulations of TFs or co-activators	Undefined	Undefined	Typically 10–100	100–1,000 nm	Undefined	Undefined	132–134
PST-condensates	Accumulated transcription-related proteins	(Super-) enhancers	Undefined	Typically 10–100	100–1,000 nm	Undefined	Phase separation remains often unproven	11,44,76

PST-condensates, phase-separated transcriptional-condensates; RNAPII, RNA polymerase II; RNAPIII, RNA polymerase III; snRNP, small nuclear ribonucleoprotein; TF, transcription factor.

Box 2 | Criteria for assessing phase separation in transcription compartments

Several criteria and experimental approaches can be applied to determine whether a transcription compartment forms through phase separation. In general, these approaches provide insights into the concentration dependence, the stoichiometry of the assembly formed and the dependence on multivalent interactions.

Phase separation occurs above a critical threshold or saturation concentration (C_{sat}), resulting in the coexistence of dense and dilute phases. This concentration dependence can be tested by changing the expression level of a protein of interest and observing threshold-dependent formation of transcriptional condensates. Notably, reaching C_{sat} throughout the entire nuclear volume may not be required. Proteins could first accumulate on chromatin surfaces through multivalent interactions below the bulk phase separation threshold, a process referred to as surface condensation, as is the case for RNA polymerase II clusters[62,85].

Assemblies formed by phase separation are non-stoichiometric, which means that the number or ratio of the interacting components can vary. As the concentration rises beyond C_{sat}, phase-separated compartments may expand, which is in contrast to the stoichiometric binding of a defined number of proteins (for example, during assembly of a virus capsid). However, for heterotypic phase separation arising from interactions between two or more different molecular species (for example, a protein and RNA, or two proteins), droplet size and the total condensed phase volume may remain unchanged.

Phase separation requires multivalent interactions, often mediated by intrinsically disordered regions or repetitive domains. Mutations decreasing valency should inhibit condensate formation if phase separation is indeed the underlying mechanism. Similarly, methods using optogenetics techniques (for example, optodroplets, Corelet and CasDrop) that enhance multivalent interactions can artificially induce phase separation but may not reflect native conditions[141]. Sensitivity to 1,6-hexanediol, although often used as a test for phase separation, is not specific to phase-separated transcriptional-condensates because it targets hydrophobic interactions that can promote various types of macromolecular assemblies[41].

Testing transcriptional condensates specifically for liquid–liquid phase separation-based assembly requires that they are viscous, exhibit deformable spherical shapes and be able to flow or fuse over time, as expected of liquid-like droplets in contrast to solid-like aggregates. However, constraints owing to chromatin binding may produce non-spherical structures, despite their liquid-like properties. To assess their liquid-like material properties, high particle mobility measured by fluorescence recovery after photobleaching (FRAP) is often used as evidence for these properties. However, fast recovery can also result from transient binding to a scaffold[26,40]. Partial bleaching of a transcription compartment in FRAP can help distinguish internal mixing (characteristic of liquids) from its exchange with external molecules[41,142]. In addition, techniques such as microrheology[143] or polarization-dependent fluorescence correlation spectroscopy[142] can directly measure the viscosity in the compartment to assess liquid-like behaviour as well as viscosity differences to the surrounding cellular milieu.

Finally, phase separation-driven formation of transcriptional condensates should be evaluated against alternative mechanisms. The null hypothesis would be stoichiometric binding to clustered sites on chromatin, which could lead to focal local enrichment of transcription-associated proteins without phase separation[21]. A stoichiometric assembly model, in which protein clusters form through bridging interactions, can be combined with a polymer–polymer phase separation mechanism that causes the chromatin chain to segregate into randomly folded coils and denser globule states[40,144,145]. Additionally, phase separation can occur alongside percolation[139], in which multivalent interactions create a system-spanning network once the concentration of factors is sufficiently high (the percolation threshold), trapping molecules in a semi-stable mesh. Notably, pre-percolation clusters of transcription factors can already form below the percolation threshold[138]. Such clusters may have an important functional role distinct from fully formed phase-separated transcriptional-condensates, potentially serving as nucleation sites for the rapid assembly of larger structures in response to cellular cues or once critical concentration thresholds are reached.

structures surrounded by nucleosome clutches marked by active modifications of their histones[63]. Within these structures, active transcription occurs at the RNA–chromatin interface[64]. Analysis of phase-separated (endogenously tagged) MED1 and RNAPII in mouse embryonic stem cells revealed two types of PST-condensates. One type consisted of relatively small PST-condensates (~100 nm), whereas the other types — representing ~10% of all PST-condensates — were larger (>300 nm) and contained approximately 200–400 molecules[46]. However, it remains unclear which of the two types is more implicated in active transcription.

In summary, both transcription factories and PST-condensates contain a protein-rich core decorated with active RNAPs and transcriptional cofactors that interact with chromatin. DNA not engaged with the transcription factory will loop out to form a rosette-like structure, and nascent RNA will be extruded in the area surrounding the core. Therefore, RNAPs could directly mediate 3D chromatin folding, especially between enhancers and promoters (Fig. 3a). Recent high-resolution studies of chromatin conformation now support the notion that RNAPs and associated machinery directly mediate spatial interactions between promoters and enhancers in transcriptionally active domains[65,66]. This mechanism is further supported by observations of engaged RNAPII constraining chromatin movement[67].

Formation and dissolution

The temporal dynamics of PST-condensates have been extensively characterized through live-cell imaging, but our understanding of transcription factory dynamics remains limited owing to their predominant study in fixed cells and the difficulties in visualizing active RNAPII in living cells. This methodological gap has left important questions unanswered about both types of transcription compartments — particularly about how transcription sites initially form, how they are maintained and how they dissolve[10]. The principles underlying PST-condensate formation could provide insights to address these knowledge gaps in transcription factory dynamics.

Temporal dynamics

Live-cell imaging approaches have been used to follow the temporal dynamics of RNAPII and therefore infer dynamic features of transcription factories. Studies of GFP-tagged RNAPII revealed that ~25% of RNAPIIs are engaged in transcription at any given moment, during which they remain relatively immobile for ~20 min on average[68]. Additionally, comparing the dynamics of RNAPII and nascent RNA indicated that transcribed templates are frequently in the vicinity of the transcription factory not associated with active RNAPs. Finally, components of transcription factories other than RNAPII (for example, TFs or co-activators) exchange more rapidly with the nucleoplasm, and the overall size and shape of transcription factories fluctuate on the scale of seconds to minutes[68]. These observations align with live-cell imaging studies of CDK9, a kinase that associates with active RNAPII to phosphorylate Ser5 residues in its CTD. CDK9 forms highly stable foci ($t_{1/2} > 1$ h) that colocalize with hyperphosphorylated RNAPII and exchange rapidly with the diffuse pool[69]. Another study applied Bayesian nanoscopy to live mammalian nuclei and confirmed that RNAPII clusters in transcription factories, which have a diameter of 200 nm (as also observed in fixed cells) and exhibit dynamic size changes on the 10-s scale[70]. This measurement was nearly double the persistence times recorded in an earlier study on clusters <250 nm in size that used time-correlated photoactivation localization microscopy[38].

Notably, altering cellular gene expression through serum stimulation resulted in RNAPII clusters persisting longer (by an order of magnitude to ~50 s) and increasing in size[38], which in turn correlated with elevated mRNA output[71]. It is important to note that this approach cannot distinguish between active (engaged) and inactive (diffusing) RNAPs, a general limitation of studies using fluorescently tagged RNAPII subunits. Approximately 70% of all detected clusters were short-lived, which equals the percentage of RNAPII not engaged with chromatin at any given time[68]. The remaining clusters, ~30%, persisted for up to 10 times longer[38,72]. Taken together, RNAPII clusters associated with transcription factories typically undergo constant

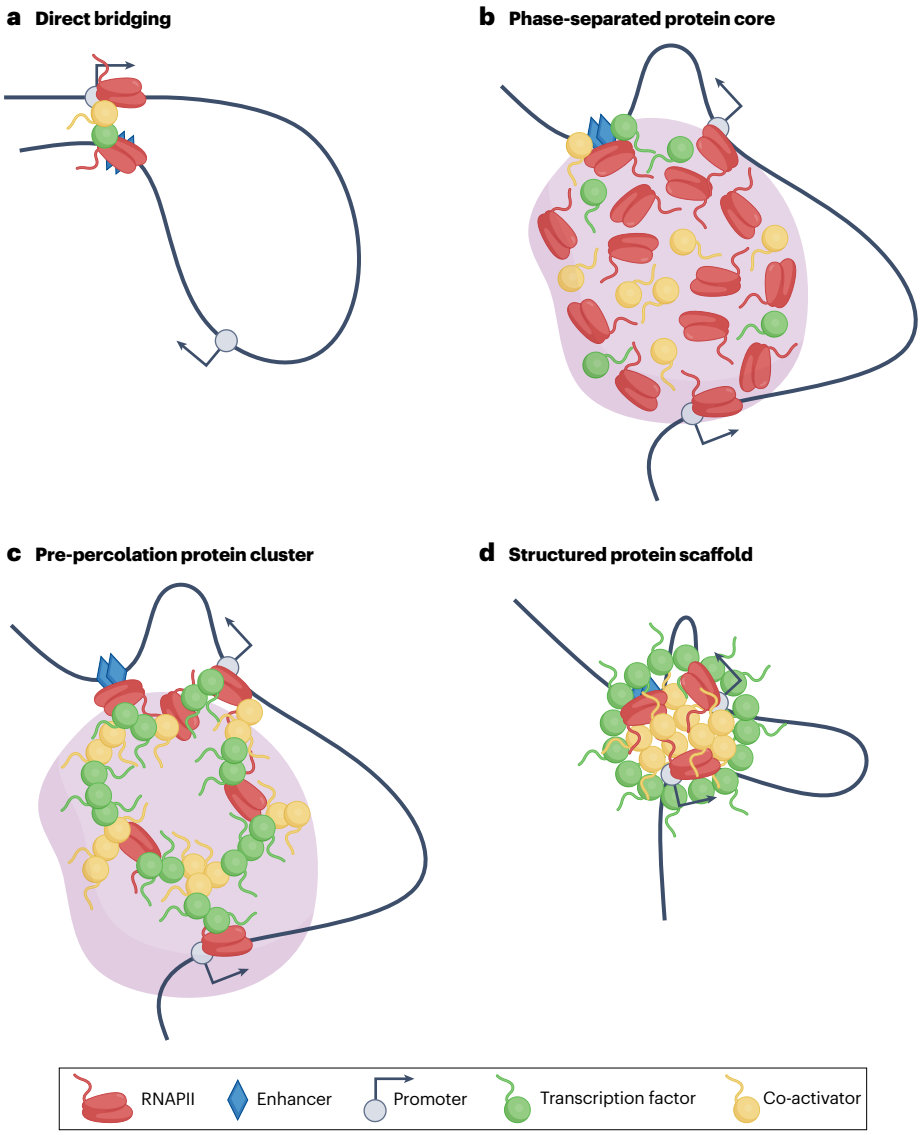

Fig. 3 | Different modes of enhancer–promoter 3D interactions. a, Direct bridging of a gene promoter (white) and enhancers (blue) through RNA polymerase II (RNAPII) (red), transcription factors (TFs) (green) and co-activators such as Mediator (yellow). **b**, Enhancer–promoter interactions mediated by a phase-separated core mixing RNAPII, TF and Mediator molecules. **c**, As in panel **b**, but with RNAPII, TF and Mediator molecules assembling through pre-percolation clustering. **d**, Hierarchical structural scaffold with genes in the interior as described for histone locus bodies.

and asynchronous assembly and disassembly over a duration of 10 s to 1 min.

Similar dynamics have been recorded for PST-condensates. When associated with chromatin, fluorescence recovery after photobleaching (FRAP) analyses revealed rapid dynamics of PST-condensates, with ~90% of RNAPIIs and ~60% of Mediator subunits exchanging within only 10 s (ref. 46). Notably, PST-condensates formed by HOXD13, HOXA13, RUNX2 and TBP TFs displayed rapid recovery rates on the scale of seconds-to-tens-of-seconds under homeostatic conditions. However, disease-associated repeat expansions in the IDRs of these TFs caused the same condensates to transition to a more solid-like state with substantially slower dynamics and altered transcriptional output[73], suggesting a relationship between PST-condensate dynamics and the emergence of pathological states.

Super-resolution imaging of endogenously tagged RNAPII or Mediator subunits in living mouse embryonic stem cells, which detect PST-condensates irrespective of their chromatin association, showed that clusters smaller than 100 nm or larger than 250 nm have markedly different half-lives. Small condensates persisted for only tens of seconds, whereas the larger ones lasted for minutes and sometimes overlapped with active enhancers or gene promoters[14,46]. Curiously, condensates marked by RNAPII compared with those marked by Mediator only sporadically overlapped and were insensitive to transcriptional inhibitors[46], suggesting that RNAPII clustering may not strictly require Mediator assemblies (and vice versa), despite biochemical studies showing PST-condensates to be heterotypic in composition[74,75].

RNA might also influence the assembly and dissolution of transcription compartments, although much remains unknown and debated. In vitro experiments have suggested that nascent RNA accumulation over a certain (yet undefined) threshold can trigger PST-condensate dissolution[76]. Other studies have found that RNA promotes the formation of active chromatin compartments[77], the co-partitioning of Mediator and RNAPII in PST-condensates[78] as well as the formation of HLBs that increase in size with increased histone gene transcription[79]. Importantly, transcription factories were initially discovered as focal accumulations of nascent RNA[5,6]. Moreover, some transcription compartments collapse upon RNAPII inhibition[77,79], whereas others persist[80], further complicating a unified interpretation.

Mechanisms of assembly

As the majority of experiments on transcription factories involves fixed cells, evidence of the precise assembly mechanism of their protein-rich core is lacking[10] despite various theoretical frameworks, such as the depletion-attraction model[81], explaining RNAPII clustering. By contrast, potential phase separation mechanisms have been tested in vitro and in vivo and provide insights into how the local accumulation of various transcription-related factors may be achieved[44]. For example, optogenetic targeting of PST-condensates to specific chromosomal loci by deactivated Cas9 caused chromatin to be excluded from the condensate core, with the loci being maintained in near-constant proximity through interactions of liquid-like nuclear condensates owing to surface tension forces[82]. These forces have also been shown to suffice to actively reposition-specific DNA loci[83]. Such organization is similar to the structures of transcription factories and can be replicated by enforcing ectopic formation of PST-condensates through general (for example, TAF15 (ref. 16)) or cell-type-specific (for example, YAP[84] and TAZ[17]) TFs (Fig. 3b,c). In all cases, TF binding to promoters or enhancers nucleated further protein clustering on chromatin, probably involving surface condensation of proteins on chromatin through multivalent interactions with DNA and nucleosomes[62,85]. This surface-associated condensed protein layer can then nucleate the formation of a PST-condensate through the accumulation of additional factors. This mechanism could explain how PST-condensates can be targeted to specific genes while dynamically exchanging with their surroundings. The process will depend on the number, local density and affinity of available *cis*-regulatory binding sites, as well as on the multivalent interactions among TFs. This mechanism has been shown to occur both in vitro[86] and in living cells[15] once TFs and co-activator concentrations exceed a critical saturation concentration (C_{sat}) threshold. Promoter and enhancer elements associated with the PST-condensate would then enrich hyperphosphorylated RNAPII to decorate its outer surface[16], similar to what is observed for transcription factories[43,49]. This process also applies to the large transcription bodies in zebrafish embryos, in which pluripotency TFs cluster before transcription is activated, followed by RNAPII enrichment[54], whereas in zebrafish cells exiting mitosis, transcription restarts at sites 100–200 nm in diameter with RNAPs at the RNA–chromatin interface[64].

The difficulty in assigning a single mechanism to transcription compartment assembly is exemplified by the case of HLBs, which are hierarchically organized and assemble around histone gene clusters into the 'core–shell' structure described earlier[60,61,87] (Fig. 3d). Unlike PST-condensates, which are thought to predominantly form through multivalent protein–protein interactions, HLB assembly is chromatin-tethered, with a histone gene array at their core being sufficient to initiate formation[87]. Moreover, HLBs persist throughout the cell cycle[61,87], unlike other transcription compartments. However, some evidence suggests that HLBs form through phase transitions regulated by cyclin-dependent kinase (CDK) activity, whereby HLBs exhibit dynamic fusion events indicative of liquid–liquid phase separation[79]. Experiments and simulations have also shown that reducing CDK2 activity or HLB seeding by Mxc results in smaller HLBs and defects in histone mRNA processing, linking HLB size to its functional capacity[79]. Together, these findings support a hybrid model in which HLBs display phase separation properties but remain functionally anchored to histone gene chromatin through the binding of NPAT/Mxc, ensuring precise control of their assembly and activity.

Functional implications

Intuitively, the high local concentration of RNAPII and its associated machinery achieved in PST-condensates and transcription factories should enhance the efficiency of transcriptional activation. At the same time, the association of *cis*-regulatory chromatin within transcription compartments will organize chromatin in 3D space and over time.

Tuning transcriptional output

The high nuclear density of transcription factories (6–8 factories μm^{-3}), which scales with nuclear size[5,6,47], indicates that a promoter or enhancer can associate with a nearby transcription factory within minutes and become activated. Accordingly, RNA fluorescence in situ hybridization (FISH) experiments and simulations have shown that the promoter of a long human gene may associate with more than one RNAPII factory during its transcription cycle through various conformations of its chromatin locus[88].

The apparent high nuclear density of PST-condensates could similarly affect transcriptional activity[46]. Live-cell imaging of the *Sox2* locus suggests that transcriptional bursting is enhanced when RNAPII or Mediator condensates come within <1 μm of a gene and its enhancers[89]. Thus, multiple interactions between a gene and a

transcription factory or PST-condensate could explain the bursting behaviour of eukaryotic transcription, although basal-level bursting still occurs with the gene transcribed being distal to a PST-condensate[89]. However, coordinated transcription of histone genes seems to only occur within HLBs[79].

Overall, the extent to which the multivalent interactions underlying PST-condensates affect transcriptional output remains uncertain. Indeed, studies of ectopic TF condensate formation on target loci have found that, although multivalent interactions can increase the residence time of TFs on chromatin, reaching the C_{sat} threshold for phase separation is not necessary for robust transcriptional activation[28]. Furthermore, there is only a minimal difference between the TF concentrations needed for the transcriptional activation of endogenous human genes (through the formation of IDR-mediated TF interactions) and for transcriptional repression (by further strengthening IDR–IDR interactions in favour of phase separation)[28,29]. Another study demonstrated that <3% of the target genes of the TF N-MYC were transcriptionally activated once phase separation conditions were satisfied (relative to non-phase separation conditions)[31]. Thus, condensate formation by phase separation may not be required for full transcriptional activation.

Tailoring transcription compartments to cellular needs

Regardless of whether transcriptional factory and PST-condensate formation is a prerequisite or a consequence of transcriptional activity, these transcription compartments could still serve several functions. One potential function could be to minimize the search time for TFs and RNAP-associated complexes to find their targets in 3D nuclear space[90,91]. Given the near-constant nuclear density of transcription factories[47], the potential minimization of TF search time would support temporal precision in transcriptional responses. Another possible function could be facilitating the spatiotemporal specialization of transcription, as reflected in the separation of RNAPII and RNAPIII activities in mammalian nuclei and the association of co-regulated genes in specialized transcription factories[48]. For example, genes expressed in mouse erythroid progenitors and co-regulated by KLF1 migrate to shared nuclear sites in a transcription-dependent manner[7,49]. Similarly, primary endothelial cells stimulated by tumour necrosis factor organize coding and non-coding pro-inflammatory genes in a subset of nuclear factor-κB-rich transcription factories[50]. Finally, cells responding to serum stimulation or IFNγ exhibit clustering of RNAPII with stimulus-specific responsive genes and nuclear actin filaments[92] (a documented component of transcription factories[55]). In all cases, responsive genes co-associate preferentially over non-responsive genes[7,50].

Such specialization could occur in PST-condensates through sequence-specific interactions. Biochemical studies suggest that these interactions can be heterotypic and selectively combine specific TFs with RNAPII through an 'IDR grammar', in which the amino acid composition and primary IDR sequence determine how TFs are partitioned into PST-condensates[74,75,93]. For instance, the androgen receptor only mixes in select PST-condensates in response to hormonal signalling, contingent on its IDR length[94]. The transcriptional co-activators BRD4 and SGF29 differentially assemble on chromatin marked by high histone acetylation and lysine 4 trimethylation levels, respectively[95,96], and FIT, a TF controlling plant growth, only co-segregates with other basic helix–loop–helix TFs in PST-condensates that are proximal to nuclear speckles[97]. Furthermore, PST-condensates of Mediator forming on super-enhancers will co-partition with activated nuclear signal transducers to activate specific genes controlling cell identity[98]. Another example is the specialized function of HLBs, in which the DNA-binding scaffold proteins NPAT and FLASH act with RNAPII to nucleate a compartment specifically suited for the co-transcription and co-processing of histone genes, for which even RNAPs themselves require unique modifications[4,99]. Notably, histone gene co-transcription ensures the stoichiometric production of replication-dependent histone mRNAs. Collectively, specialization of both transcription factories and PST-condensates can occur based on the biochemical compatibility of TFs to drive spatial co-regulation of gene subsets.

RNAPs as genome organizers

The 3D organization of the genome facilitates communication between gene promoters and their cognate enhancers and has a fundamental role in regulating gene expression, although the precise nature of this communication remains only partially understood, with different models being considered[66,100–102]. Nevertheless, it is intuitive that 3D genome organization can be influenced by active enhancers and genes dynamically engaging with transcription factories and PST-condensates. However, earlier studies claimed that depleting RNAPI–III[103], Mediator subunits[104] or TAF12 (ref. 105) had no measurable effect on loop or higher-order chromatin domain formation, as measured by high-throughput chromosome conformation capture (Hi-C), suggesting that the 3D organization of the transcription compartments containing these factors was not altered. In addition, higher-resolution studies using transcriptional inhibitors showed only mild effects on enhancer–promoter looping[106], and RNAPII binding to chromatin has even been linked to the emergence of inter-domain insulation[107–109]. Nevertheless, several observations indicated that RNAPs do mediate 3D chromatin interactions. For example, many loops involving active enhancers and promoters in eukaryotic cells remain intact in the absence of functional cohesin (the molecular motor extruding loops[106,110–112]), and techniques orthogonal to Hi-C have uncovered extensive multiway interactions between active genes and enhancers, including those at histone loci[113,114]. These contradictions were reconciled by recent Micro-C experiments that comprehensively mapped enhancer–promoter interactions under-represented in Hi-C data[65,106]. Acute RNAPII depletion from mammalian cells led to the loss of essentially all enhancer–promoter and enhancer–enhancer loops[65], with similar effects also following Mediator depletion[115]. Accordingly, in mature chicken erythrocytes, in which transcription is shut down, promoters bound by 'paused' RNAPII retain 3D interactions to form mini-domains that prevent heterochromatinization[116]. Despite this evidence, it remains uncertain whether such RNAPII-centric genome architecture requires PST-condensate-like structures or is mediated by individual RNAPII–Mediator complexes (Fig. 3a–d).

In orthogonal imaging experiments, sub-diffraction localizations of nascent RNA copied from spatially co-transcribed genes estimated that they are produced in structures that were 50–120 nm in diameter[117]. Enhancer–promoter separations before and after transcriptional activation were found to be 100–200 nm in *Drosophila*[118] and up to 300 nm in mammalian cells[119–121] — measurements consistent with both PST-condensates and transcription factories. Furthermore, enforced TF condensation at enhancers and promoters led to de novo loop formation, selective enrichment of TFs and co-activators and activation of target loci[122]. Finally, for YY1 — a factor contributing to enhancer–promoter loops — phase separation on chromatin was required for nucleating RNAPII-rich PST-condensates at target loci to stimulate transcription[123]. Overall, these data corroborate a

link between PST-condensates and the 3D organization of enhancers and promoters.

Integrating the two frameworks

As described earlier, transcription factories and PST-condensates share several similarities and differences regarding their architecture, underlying mechanisms of formation and dissolution and potential functions. Here, we aim to integrate their respective frameworks to build a consensus model of transcription compartments while highlighting aspects that remain to be elucidated.

Common features

Structurally, both transcription factories and many PST-condensates are organized around a protein-rich core of ~50–200 nm in diameter[43,46], maintaining high local concentrations of TFs and co-activators while dynamically exchanging with the surrounding nucleoplasm. TFs and co-activators in the core can engage in multivalent interactions, often through their IDRs, and scaffolding factors such as nuclear actin may help stabilize the compartment[92]. Another shared feature is the localization of active RNAPII on the outer surface of this protein-rich core[16,43], which enables chromatin loops of enhancers and active genes to emanate and nascent RNA to be extruded at the core–chromatin interface. Multiple enhancer and promoter elements can simultaneously and dynamically engage with RNAPs at this interface[3] (Fig. 4). Interestingly, this organization appears inverted in *Drosophila* HLBs, with a core–shell structure in which the internal core contains the transcribed histone genes associated with the Mxc N terminus[61]. This association nucleates the HLB outer shell assembly enriched with the Mxc C terminus and histone pre-mRNA processing factors (such as FLASH)[61].

The assembly of all these compartments abides by common principles. Initial nucleation occurs through TF binding to *cis*-regulatory sequences of enhancers and promoters[15], or through Mxc/NFAT in HLBs[4]. The compartment then grows through multivalent interactions between IDR-containing proteins, which may occur through phase separation or other mechanisms of molecular clustering (Fig. 3 and Boxes 1 and 2). Continued transcriptional engagement stabilizes the compartment, whereas RNA levels may regulate its properties[76]. Despite maintaining an overall metastable structure, these compartments are highly dynamic, as components rapidly exchange with their surroundings, including attaching and detaching transcription units[46]. Their sizes and shapes are polymorphic and fluctuate on the scale of seconds-to-minutes[68,70,73]. Notably, both transcription factories and PST-condensates can be functionally specialized owing to differential TF enrichment. In this manner, functionally distinct compartments can be created[50] where the physicochemical properties of IDRs determine which TFs will co-segregate[75]. This specialization would enable tissue-specific or stimulus-responsive assemblies potentially capable of coordinated regulation in different transcriptional programmes[98].

These common features suggest that rather than representing fundamentally different structures, transcription factories and PST-condensates probably describe the same organization principles of the transcriptional machinery, viewed through different experimental and conceptual lenses. More specifically, phase separation concepts provide mechanistic insight into how transcription compartments can assemble, whereas the transcription factory model explains how these compartments can interface with chromatin to regulate transcription.

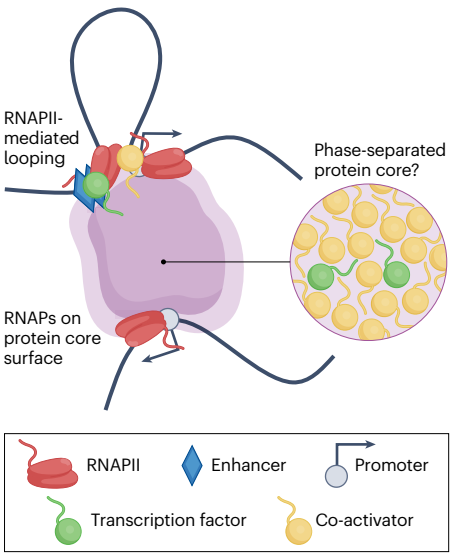

Fig. 4 | Features of an integrated transcription compartment model. Enhancers (blue) and gene promoters (white) organized around a protein-rich, but RNA polymerase II (RNAPII)-depleted, protein core, which presumably forms through phase separation. Enhancer–promoter contacts are mediated by RNAPII (red), transcription factors (green) and co-activators including Mediator subunits (yellow) at the surface of the core of the compartment, enabling gene co-regulation.

Features requiring reconciliation

Many aspects of transcription factories and PST-condensates can be integrated into a consensus model, but several features also need to be reconciled. First, the precise mechanism driving the assembly of the protein-rich core remains to be determined; although phase separation provides an attractive framework[11], other mechanisms of molecular clustering may underlie transcription compartments (Fig. 3b–d). Thus, direct experimental evidence is still needed to distinguish between these mechanistic possibilities (Box 2). A related feature needing reconciliation is how RNAPII interacts with the protein core. This process may occur either by restricting RNAPII binding to *cis*-regulatory elements and genes on the core surface or by directly integrating RNAPII into the core through multivalent interactions involving its CTD. Recent work showing that CTD modifications can affect condensate formation[124] supports the latter, but direct testing is still missing.

The fate of transcription compartments during elongation also requires clarification. The original transcription factory model proposed that the structure persists as DNA is reeled through it[3,117]. However, studies of PST-condensates suggest that escape into elongation and nascent RNA accumulation leads to the 'loosening' of their structure[62] or even dissolution[76]. These contradictory observations need to be reconciled by considering different types of transcription compartments or stages of the transcription cycle. These aspects could be addressed, for example, through new antibody-based methods for live-cell imaging[37,125] combined with rigorous quantification and sub-diffraction colocalization strategies.

The relationship between transcription compartments and architectural proteins such as CTCF and cohesin remains similarly unresolved. RNAPII can stabilize enhancer–promoter loops independently of these factors[65], but architectural proteins may help organize the chromatin scaffold on which compartments assemble[126]. Understanding the relationship between different scales of 3D genome organization represents yet another challenge. Most transcription factories and PST-condensates are 100–200 nm in diameter, whereas

Glossary

Active chromatin hub
A cluster of two or more transcribed genes that are co-regulated.

Genome architecture mapping
A method orthogonal to chromosome conformation capture in which the frequency of co-occurrence of DNA regions in thin cryosections of cell nuclei is used as a proxy of their spatial colocalization. This method does not require ligation of interacting DNA sequences and may, thus, provide information of multiway interactions among regions of interest.

High-throughput chromosome conformation capture
(Hi-C). A method to measure the frequency by which two genomic sequences are found in close spatial proximity. It relies on the ligation and pairwise detection of DNA by high-throughput sequencing.

Histone locus bodies
(HLBs). An assembly of transcription machinery and processing around the histone gene cluster. It drives the highly efficient, coordinated and cell-cycle-associated production of histone mRNAs. HLBs are stable and persist throughout the cell cycle, while uniquely integrating histone-specific processing machinery, such as NPAT, FLASH and U7 small nuclear ribonucleoprotein.

Intrinsically disordered regions
(IDRs). Segments of a protein that lack a defined higher-order structure under physiological conditions.

Liquid–liquid phase separation
The unmixing of proteins and RNA molecules from the surrounding nucleoplasm driven by multivalent interactions that form distinct, liquid-like droplets in the nucleus.

Multivalent
In the case of molecular interactions, multivalent refers to the ability of a molecule or complex to simultaneously interact with two or more other molecules/complexes owing to the presence of multiple interaction sites.

Nuclear speckles
Nuclear condensates, 100–2,000 nm in size, containing polyadenylated RNA and splicing factors that have been associated with the storage, processing and export of mRNAs.

Percolation
A phase transition in which an interconnected network of molecules forms through multivalent interactions. The concentration at which a system transitions from disconnected clusters to a single, system-spanning network is called the percolation limit or threshold.

Phase-separated transcriptional condensates
(PST-condensates). Accumulation of transcription-related factors that occur by a biophysical phase separation mechanism.

Phase separation
Transition in which molecules demix from their bulk environment into a dense condensate and a dilute surrounding phase to form bodies of distinct physicochemical properties.

Phase transition
Change in the physical state or organizational properties of a system. This term includes not only phase separation but also network transitions such as percolation.

RNAPII clusters
Local co-associations of two or more (active) RNA polymerase II (RNAPII) complexes.

Saturation concentration
(C_{sat}). The concentration threshold above which phase separation occurs.

Super-enhancers
Clusters of closely spaced *cis*-regulatory elements typically controlling expression of cell identity genes.

Surface condensation
Initial phase of protein enrichment on the DNA or chromatin surface through multivalent interactions creates a thin, wetting layer, which can nucleate the formation of more defined, phase-separated protein clusters.

Transcription bodies
Large and long-lived focal nucleoplasmic sites of high transcriptional activity harbouring co-expressed genes (for example, the two characteristic bodies emerging upon genome activation during early zebrafish development).

Transcriptional condensates
Assemblies of transcription machinery components and related factors that may form through dynamic multivalent interactions by phase separation or other mechanisms.

some transcription compartments reach the micrometre scale[64]. Whether this variation captures fundamentally different structures or states of the same underlying organization, and how such different organizational scales coexist, remains to be determined.

Finally, the functional connection between transcription compartments dynamics and transcriptional bursting needs elucidation. Recent work suggests that locus proximity to RNAPII or Mediator PST-condensates enhances bursting[89], but the mechanistic basis is not understood. This issue is particularly important given that only ~1% of RNAPII–promoter interactions will produce a complete transcript[127]. However, various specific cases challenge the transcription compartment concept altogether. For example, mammalian genes that span >100 kb and are highly transcribed form micrometre-long rigid structures that occupy substantial nuclear volume, with active RNAPs densely decorating them[128] or exhibiting altered self-interaction patterns in genome architecture mapping data[129]. Nevertheless, during genome activation in *Drosophila*, exemplary activated loci from a 5-Mbp region on chromosome 2 often associate 'individualistically' with any of the ~100 RNAPII speckles that form and remain well separated from one another (by up to 400 nm)[59]. However, a single-cell spatial genomics approach found activated *cis*-regulatory elements and genes coalescing in nucleoplasmic hubs[129]. Although such examples seem less prevalent than the transcription architectures discussed earlier, it will be necessary to understand the extent to which they coexist in a given cell, and how cells accommodate these diverse paradigms and switch between them.

Conclusions and future perspectives

The combined discussion of historical and recent findings on transcription compartments reveals common principles regarding the assembly

of highly dynamic yet metastable structures that harbour high local concentrations of the transcriptional machinery and nascent RNA. Remarkably, this organization appears evolutionarily conserved across species, including the bacterial nucleoid[130].

Moving forward, the variable terms used to describe transcription-associated assemblies need to be reconciled. Relative to the term transcription factory, active chromatin hub is closely related but emphasizes the clustering of two or more active genes[131]. By contrast, 'TF/co-activator hub' designates protein assemblies for which the presence of RNAPs and active genes is not defined[132–134]. As discussed earlier, HLBs represent specialized transcription compartments that form around histone gene clusters[4,61,135]. Collectively, these terms differentiate between transcription-linked assemblies containing chromatin (transcription factories and active chromatin hubs) and potential storage or regulatory bodies (TF/co-activator hubs without active genes or RNAPII). It would also be useful to introduce terminology that defines the precise marker used for each assembly (for example, active RNAPII or TF/co-activator) or explicitly states whether a specific mechanism is involved (for example, PST-condensates). Irrespective of naming, the various types of clusters should be better categorized, and their compositions studied through, for example, proximity labelling[136] or other isolation methods[55,137] coupled with mass spectrometry.

With phase separation providing an attractive mechanistic framework, IDR-mediated multivalent interactions that are essentially ubiquitous among transcription-associated complexes are central to the structural and functional properties of active transcription compartments. Genetic complementation experiments can be valuable for further characterizing their function[57], and it is essential to determine whether their activity depends on an endogenous concentration above the phase separation threshold (that is, the C_{sat}) or whether they already enhance transcription well below this threshold[28,138]. Finally, although PST-condensates could enhance transcription[16,122], they might also represent transcriptionally neutral or repressive states[28–32]. To assess their functional relevance, a persistent challenge is establishing appropriate controls that reflect endogenous conditions in the absence of these structures. In addition, it will be important to clearly define and quantitatively evaluate how higher-order assemblies of the transcriptional machinery affect its various functional stages, from initiation and productive elongation, to co-transcriptional RNA processing and termination.

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Competing interests

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