RNA polymerase II transcription compartments: from multivalent chromatin binding to liquid droplet formation?

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Recent studies invoke phase separation as a mechanism underlying the formation of 'transcriptional condensates'. However, similarities between transcriptional condensates and the previously introduced 'transcription factories' model raise the question of what distinguishes the two models. One crucial aspect to consider is the contribution of intrinsically disordered regions in transcription-relevant factors.

In cell nuclei, concentration gradients equilibrate within seconds owing to fast diffusion of macromolecules. Thus, one would expect proteins and RNA involved in transcription to become homogeneously distributed as soon as they are released from chromatin. However, transcription by RNA polymerase II (Pol II) was shown already in the early 1990s to preferentially occur in distinct 50–180 nm nuclear foci of active Pol II holoenzymes named 'transcription factories'[1]. Approximately 1,000–2,500 such factories can be detected per nucleus, depending on cell type. Active Pol II in its preinitiation state at these loci displays residence times of ~10 seconds, whereas elongating Pol II is stably bound to the DNA until a transcript is synthesized[1,2]. The mechanisms guiding the assembly of Pol II transcription compartments, however, remained enigmatic. A recent suggestion is that RNA polymerases, promoter and enhancer sequences and associated factors undergo phase separation through multivalent interactions, to create an exclusionary local protein–RNA environment with characteristic physicochemical properties[3–6]. A frequently used, yet simplistic analogy for this process is the demixing of oil in water.

Inside cells, phase-separated foci can be described as macromolecules of flexibly associating polymers that are patterned with interaction-prone motifs separated by more-inert segments[3,6]. In particular, protein intrinsically disordered regions (IDRs) and RNA fit well in such a 'stickers-and-spacers' model[6]. Above a critical concentration threshold, multivalent IDR and RNA interactions can induce separation into a dense polymer phase, which coexists with a diluted, polymer-depleted phase. Molecules in the dense phase have liquid-like properties if interactions are weak and transient, but they can adopt gel-like or solid-like states with reduced mobility as interaction strength increases[6]. Can this mechanism explain how Pol II transcription is spatially confined to certain parts of the nucleus and the genome?

Are Pol II transcription factories liquid droplets?

The characteristics of chromatin affect the structure of active Pol II clusters. At the μm scale, chromatin has mostly solid-like properties as its polymeric nature restricts nucleosome to 50–100 nm movements[2]. A parsimonious transcription factory assembly would thus involve (cooperative) binding of proteins and RNA onto a mostly immobile chromatin scaffold. The resulting complex would nevertheless be highly dynamic as the residence time of most factors on chromatin is limited to just a few seconds[2]. Protein–protein interactions involving these chromatin-bound factors may occur at a distance to establish bivalent bridging interactions and loop out parts of the nucleosome chain[1,2]. Thus, the folded chromatin template itself (likely influenced by transcription-linked forces) defines a transcription compartment comprising directly and indirectly bound factors that bring enhancers and promoters into spatial proximity[1,2]. A feature suggested from the physical persistence of such a compartment during transcript elongation is it that the DNA template is reeled through the factory (as opposed to Pol II translocating along a mostly static chromatin), and co-transcription of the different Pol II units locally accumulates RNA.

Alternatively, Pol II, transcription factors and *cis*-regulatory sequences could assemble through phase separation into transcriptional condensates around chromatin-bound factors serving as nucleation sites[3,4,7], similar to water droplets on a spider web. Such a phase separation mechanism provides a novel and intriguing explanation for the previously enigmatic role of the IDRs found in a large fraction of transcription regulators

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(for example, the Pol II C-terminal domain (CTD), general or cell-type-specific transcription factors, and transcription co-activators such as Mediator and BRD4). By driving phase separation, IDRs can maintain a high local concentration of all necessary factors with a sharp boundary with the nucleoplasm. IDR-mediated multivalent interactions may also keep promoter and enhancers in (transient) proximity through the assembly of liquid droplet to amplify transcription activation[3–7]. Hence, invoking phase separation does not oppose the core of the transcription factory model, but rather provides a mechanism for the maintenance of high local concentrations of the transcription machinery in transcriptionally active foci.

Another feature of transcription factories, which was uncovered using electron spectroscopic imaging, is that active polymerases decorate the periphery of a protein-rich core[1]. Using optogenetic tools, the transcription factor TAF15 was shown to prime particular loci for activation, and light-induced formation through phase separation of ectopic TAF15–Pol II droplets on chromatin amplified the expression of particular loci[7]. Notably, actively transcribing polymerases therein adopt a topology similar to that described for transcription factories: they concentrate at the periphery of these phase-separated clusters, the core of which being rich in transcription factors and (presumably) in co-activators[7]. Here too, the two models share a common feature.

The contribution of IDRs to Pol II clusters

IDR-mediated assembly of transcription compartments through phase separation is a novel feature not included in the original Pol II factory model. By varying the affinity and the number and density of IDR–IDR and DNA–transcription factor interactions, sharp thresholds for the occurrence of phase separation can be defined[4]. Whether these conditions are indeed fulfilled at endogenous transcribed loci in living cells is a critical question. Most experimental assays affirming the formation of liquid droplets are performed in vitro or in artificial contexts. Moreover, even live-cell imaging studies have predominantly focused on µm-size condensates, which constitute a clear minority inside nuclei compared with the ~100-nm clusters of active Pol II (marked by hyper-phosphorylation of Ser5 in their CTD)[1]. Is then the main function of IDRs to drive phase separation, or do they promote transcription initiation even below the critical concentration regime required for the formation of phase-separated transcriptional condensates? Work from a carefully conducted recent study in yeast is particularly instructive for discussing possible IDR functions[8]. The study showed that shortening the Pol II CTD reduced gene expression and transcription bursting. Remarkably, this phenotype could be reverted by simply fusing the phase-separation-prone FUS or TAF15 IDRs to the end of shortened CTDs, which also rescued the ability of Pol II to cluster.

One interpretation of these findings is that it is sufficient to simply restore the formation of Pol II droplets through these fusions to allow for efficient and accurate transcription. However, considering the FUS and TAF15 IDRs as standalone, functionally independent modules that drive phase separation might be a misrepresentation of their function in vivo. It is also difficult to imagine how their substitution of the CTD would mimic the Pol II CTD phosphorylation code associated with the transition of Pol II into an active state. Given that both FUS and TAF15 can interact with the Pol II CTD[9], they could simply directly replace an activating function that is otherwise mediated by the CTD. Furthermore, as previously proposed, unstructured domains can reduce the occurrence of unproductive encounters between two diffusing macromolecules in a 'wrong' steric orientation[10]. In this scenario, IDRs would be interchangeable as they simply serve to stabilize an intermediate state that increases the kinetic rate for forming specific interactions during the assembly of multi-subunit complexes such as the Pol II preinitiation complex or the Mediator complex. Such an IDR function would be unrelated to phase separation.

In-depth analyses are needed to examine whether IDR-mediated interactions are exploited inside cells to drive phase separation, or whether the interactions they mediate are sufficient to drive transcription below the critical factor concentration thresholds required for phase separation. Furthermore, it would be important to elucidate how phase separation might accommodate the dynamic functional transitions that enable efficient elongation, co-transcriptional splicing and transcription termination within Pol II clusters. Addressing such questions and revisiting the founding model of transcription factories and its associated experimental data could help to attain a more holistic view of how transcription is organized in nuclear space and time.

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Competing interests

The authors declare no competing interests.