Spotlight

Switching Condensates: The CTD Code Goes Liquid

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Condensates containing RNA polymerase II (Pol II) materialize at sites of active transcription. Young and coworkers now establish that C-terminal domain phosphorylation regulates Pol II partitioning into distinct condensates connected with transcription initiation or splicing. This advance hints that distinct condensates with specialized functional compositions might choreograph distinct stages of transcription.

Liquid–liquid phase separation (LLPS) of RNA polymerase II (Pol II) mediated by its repetitive C-terminal domain (CTD) may explain clustering of Pol II at sites of active transcription [1–4]. Recent work by Young and colleagues now connects CTD-mediated LLPS with the 'CTD code' [5,6]. The CTD code specifies how largely unphosphorylated Pol II is initially recruited to gene promoters and how, as transcription progresses, the CTD becomes dynamically phosphorylated and dephosphorylated in specific patterns [7]. Thus, specific patterns of CTD phosphorylation predominate at 5′ and 3′ ends of genes, with each Pol II phosphoisoform recruiting specific interactomes. In this way, initiation factors give way to elongation and mRNA splicing factors, and eventually termination factors, as Pol II traverses a gene [8]. Guo *et al.* now fuse the concepts of CTD transcriptional condensates with decades of biology delineating how the CTD code might orchestrate transcription and cotranscriptional events [5]. The stage appears to now be set for elucidating nuanced transcriptional condensates of different functional compositions that might drive distinct stages of the transcription cycle.

The Pol II CTD can form liquid droplets and partition into hydrogels comprised of protein low-complexity domains with transcription activation functions [1,9]. In both cases, decreased CTD length or CTD phosphorylation reduced partitioning into these dense phases [1,9]. These observations suggested that Pol II is recruited to promoters via LLPS of the CTD and initiation machinery and subsequently released from promoter-associated condensates as a function of CTD phosphorylation, which accrues after transcription initiation [7]. Now, Guo *et al.* confirm that CTD phosphorylation leads to dissociation of the CTD from mediator condensates and disambiguate that CTD phosphorylation also promotes CTD recruitment to splicing condensates [5]. Thus, the CTD code may govern the CTD interactome as well as the CTD partition coefficient for different functional condensates comprised of distinct components.

Guo *et al.* reconstituted separate condensates *in vitro* containing either the CTD and mediator components, or the CTD and splicing factors, and combined these studies with sophisticated imaging of proteins and nascent RNA in cells [5]. *In vitro*, CTD:mediator condensates exhibited liquid-like behavior [5]. In cells, mediator and Pol II colocalized with nascent RNA from *Nanog* and *Trim28* [5]. Nascent RNA from these genes overlapped with or were immediately adjacent to nuclear speckles (another liquid-like condensate), which contain a host of splicing factors [5]. Notably, unphosphorylated Pol II colocalized more strongly with mediator, while Serine 2 phosphorylated Pol II, a mark for transcription elongation, colocalized more strongly with splicing factors [5]. Proximity of splicing factors with mediator was reduced by drugs that inhibit splicing, whereas colocalization of phosphorylated Pol II with splicing factors was reduced by drugs that inhibit CTD kinases [5].

The differential colocalization of Pol II depending on CTD phosphorylation, coupled with the proximity of actively transcribed loci to clusters of splicing factors and speckle components, suggested that CTD phosphorylation might enable Pol II to migrate from initiation condensates to splicing condensates [5]. In support of this hypothesis, Guo *et al.* reconstituted two distinct CTD condensates. The first contained purified mediator, into which the unphosphorylated CTD partitioned more strongly than CTDs phosphorylated by CDK7 or CDK9 [5]. Conversely, phosphorylated CTD partitioned more favorably than unphosphorylated CTD into condensates containing nuclear-speckle components, SRSF1 and SRSF2, two proteins involved in splicing [5]. The CTD concentration relative to mediator or splicing factors in the two condensates remains a key question, the answer to which may reveal which component scaffolds each condensate versus merely partitions into it. Indeed, is the CTD acting as a scaffold or a client for these condensates [10]? Moreover, does CTD status as scaffold or client shift as a function of phosphorylation? Another open question is whether or not phospho-specific CTD condensates are a general feature of active genes, or are they unique to the super-enhancer regulated genes studied [5]? Regardless, these findings connect CTD condensates to the CTD code, with initiation condensates giving way to splicing condensates as a function of CTD phosphorylation.

What exactly do the separate phospho-specific CTD condensates generated *in vitro* tell us about transcription

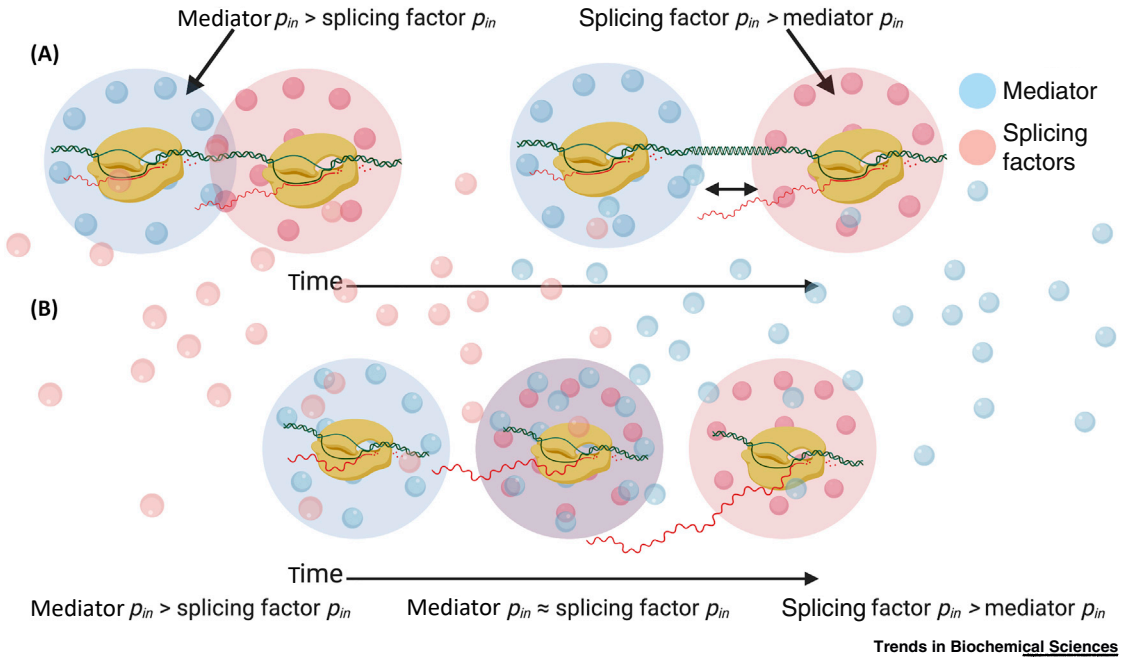

Figure 1. Two Models for Phospho-Specific C-Terminal Domain (CTD) Condensates during Transcription.
(A,B) DNA is depicted in green and Pol II is depicted in yellow. Nascent RNA is depicted in red, which lengthens as a function of time. The partition function of mediator (blue spheres) or splicing factors (red spheres) in the condensate is p_{in}. In one model (A), distinct initiation and splicing condensates exist in close proximity, or transiently contact one another to exchange select components. Here, Pol II would exit one condensate and enter another during elongation. The proximity or contact time between condensates could tune gene output by altering the time initiation gives rise to productive elongation. In a second model (B), one initiation condensate evolves into a splicing condensate as a function of CTD phosphorylation, which changes over time as Pol II traverses a gene. In this model, CTD phosphorylation could tune the partition function of CTD-associated factors, including mediator and splicing factors, for each condensate. Here, gene output would be self-limiting, with initiation ceasing as the partition function for mediator into the droplet decreases and the partition function for splicing factors into the condensate increases. This condensate could conceivably mature into a termination condensate, temporarily shutting down transcription.

in vivo? Does transcription require eviction of Pol II from one condensate and migration into another? Or does one condensate simply evolve into another during the course of transcription in a process driven by CTD phosphorylation? We consider these two models below (Figure 1).

In the first model, there are distinct initiation and splicing condensates, with Pol II leaving the former and entering the latter as it traverses a gene and CTD phosphorylation increases (Figure 1A). Here, these droplets could be distinct and separate immiscible entities, or adjacent drops could touch and transiently exchange components, perhaps akin to kiss-and-run fusion events. Droplet fusion can occur much faster than internal mixing, which may help explain the proximity, but incomplete overlap, of mediator and splicing factors. This model requires elongating Pol II to exit one droplet and enter another, raising questions about how cells would sort which polymerases to extract and which to leave behind? One possibility is that genic DNA radiates outward from mediator droplets in a 5′ to 3′ orientation, with CTD phosphorylation and the energy of NTP hydrolysis driving Pol II across the droplet energy barrier, leaving behind the non-transcribing and hypophosphorylated Pol II to prime re-initiation. Sorting might also arise from phosphorylation by one or more kinases applied processively and primed by an initiation-associated mark, ensuring that elongating Pol II is multiply phosphorylated more rapidly than Pol II that has yet to initiate.

In the second model, there is but a single droplet about a transcribed gene or cluster of genes, with the partition coefficient of specific components evolving over time as a function of phosphorylation of the CTD and other targets of CTD kinases (Figure 1B). In this model, a droplet could evolve through the stages of the transcription cycle, each with its own known phosphorylation patterns and interactome. This model is not mutually exclusive with the existence of separate splicing speckles, which likely also serve as a concentration buffer for

splicing machinery. We note that this model has the advantage of being self-limiting, as an initiation droplet gives way to an elongation droplet, and perhaps so on to a termination droplet. In this model, the CTD and perhaps other components may be expected to have partition coefficients associated with a threshold level of phosphorylation, whereby the composition of a droplet can regulate itself via the net phosphorylation levels of one or more condensate scaffolds or clients.

More complex *in vitro* reconstitution and visualization of catalytically 'active droplets', including regulatory kinases, could help distinguish between these possibilities. Ideally, such experiments would also include specific RNA transcripts, such as *Trim28* and *Nanog*, given the established roles for RNA in regulating condensates. However, this type of sophisticated reconstitution might be challenging. Perhaps more feasible is tracking whether a single Pol II can be captured traversing from initiation to splicing condensates *in vivo* via super-resolution microscopy. Likewise, can a single initiation condensate be observed to evolve into a splicing condensate *in vivo*? Finally, are these condensates a cause or effect of transcription or splicing? Do these condensates truly create specialized microenvironments optimized for the biochemistry of these reactions? The answers to these and other questions will help reveal the mechanisms by which transcriptional and splicing condensates, and potentially transcription and splicing, are regulated.

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https://doi.org/10.1016/j.tibs.2019.10.009

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Spotlight

New Structures Reveal Interaction Dynamics in Respiratory Supercomplexes

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Mitochondrial energy conversion involves a chain of membrane-bound proteins that are wired to conduct an electron current, which drives transmembrane proton translocation. These enzymes associate to form supercomplexes, but the functional relevance of the higher-order structures is unknown. A recent study by Letts *et al.* presents structures of a supercomplex, which suggest how the interaction choreography may control overall functionality.

Cellular respiration is the process by which cells convert energy from exergonic reactions between environmental compounds to produce ATP. Synthesis of ATP from ADP is performed by ATP synthase, a membrane-bound enzyme that uses the free energy stored in a transmembrane difference in both proton concentration and electric potential [1]. The free energy maintaining this electrochemical proton gradient is derived from oxidation of NADH by molecular oxygen. This reduction/oxidation reaction is catalyzed by membrane-bound enzymes wired to transfer electrons from low-potential donors to high-potential acceptors. The electron current drives proton translocation across the membrane from the negative (*n*) to the positive (*p*) side.

Functionally linked respiratory enzymes are collectively referred to as the electron transport chain, which, in eukaryotes, is located in the inner mitochondrial membrane (Figure 1). In mammals, complex I (CI) is the first component of the chain. It catalyzes the oxidation of NADH and reduction of quinone (Q) to quinol (QH_2), and links this electron transfer to proton pumping from the matrix (*n* side) to the intermembrane space (*p* side). The product QH_2 diffuses to reach complex III, which is a homodimer ($CIII_2$) composed of functionally independent monomers. At $CIII_2$, quinol is oxidized to quinone at the Q_p site (also called Q_o), a process linked to proton release at the *p* side. The two-electron oxidation of QH_2 is bifurcated such that one electron is transferred to reduce another quinone bound at the Q_n (also called Q_i) site to form a semiquinone. The other electron from the Q_p site is transferred