CellPress

Just Took a DNA Test, Turns Out 100% Not That Phase

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https://doi.org/10.1016/j.molcel.2020.03.029

Heterochromatin protein 1 (HP1) has been proposed to drive heterochromatin formation by liquid-liquid phase separation. In this issue of *Molecular Cell,* however, Erdel et al. establish that heterochromatin can adopt digital compaction states that are independent of HP1 phase separation.

A resurgent concept in biology is the way cells organize themselves into discrete membraneless compartments by a process called liquid-liquid phase separation (LLPS). LLPS occurs in nearly all aspects of cell biology and provides a facile way to spatiotemporally organize subcellular components and their biochemistry (Boeynaems et al., 2018).

In the nucleus, the genome is packaged into chromatin by being wrapped around histone proteins. However, chromatin is not completely uniform—rather, it is compartmentalized into distinct domains including actively transcribing genes that are loosely arranged (termed euchromatin) and repressed and silenced regions that are tightly packaged (termed heterochromatin). This compartmentalization is an important mechanism that enables selective gene expression for specialized cells. Heterochromatin can be readily visualized in the nuclei of DAPI-stained cells as intense fluorescent foci termed chromocenters.

Heterochromatin formation involves elevated DNA methylation and repressive histone modifications, especially trimethylation on the ninth lysine residue on histone 3 (H3K9me3), which recruits a set of proteins including heterochromatin protein 1 (HP1). This process elicits a series of recruitment events that spread, thereby further compacting chromatin and establishing the repressed state. HP1 phosphorylation seems to further enhance its affinity and specificity during heterochromatin formation (Hiragami-Hamada et al., 2011). However, heterochromatin cannot be an impervious barrier, since it has to allow access to some proteins, such as the DNA-repair machinery in case of DNA damage. To balance these two seemingly competing needs, heterochromatin is organized in membraneless domains that enable proteins to dynamically come and go. Two recent studies have proposed HP1 LLPS as the driving force underlying heterochromatin formation.

The first team (Larson et al., 2017) made an unexpected discovery while working with purified HP1α, one of the three human forms of HP1. Phosphorylation, addition of DNA, or removal of salt promoted HP1α condensation (Figure 1A). Fusion of multiple HP1 droplets assembling on stretches of DNA were proposed to condense to form heterochromatin domains and spread locally to establish the chromocenter. The other team (Strom et al., 2017) also demonstrated LLPS of purified HP1 and analyzed heterochromatin formation and fusion of GFP-HP1 condensates in *Drosophila* embryos. They also showed dispersal of HP1α droplets upon treatment with 1,6-hexanediol, which disrupts weak hydrophobic interactions, providing further evidence for liquid-like properties of HP1 condensates.

Subsequently, a recent study focused on fission yeast HP1 protein, Swi6, pointing to a role of liquid-like HP1 in heterochromatin compaction (Sanulli et al., 2019), adding to the list of LLPS events in genome organization (Hnisz et al., 2017). However, does the *in vitro* behavior of highly concentrated (> 40 μM) and phosphorylated HP1 accurately recapitulate the *in vivo* biology with physiological HP1 levels (~1μM)? In this issue of *Molecular Cell*, Erdel et al. (2020) systematically investigate the mechanism underlying heterochromatin formation and the biophysical properties of chromocenters. Intriguingly, they propose an alternative way for heterochromatin compaction that does not rely on HP1 phase separation.

First, they confirmed the previous results that purified HP1α or a GFP-tagged HP1α could form liquid droplets when phosphorylated or mixed with DNA. To test if HP1α is concentrated locally in chromocenters, they used high-resolution microscopy to visualize chromocenters in mouse fibroblasts and found robust enrichment of DAPI, HP1α and H3K9me3. Surprisingly, however, when they removed H3K9me3 and HP1α, the chromocenter was still retained, suggesting that HP1α is not required for chromocenter compaction. In fact, they found that HP1α formed sporadic clusters instead of being homogenously distributed as expected from LLPS. In addition, the local HP1α concentration in heterochromatin was ~3 μM, far below the concentration required for *in vitro* LLPS. Thus, the authors suggest that HP1α does not phase separate at physiological concentrations in cells and that HP1α is not essential for establishing the compaction of chromocenters.

Next, the authors harnessed the optodroplet system (Shin et al., 2017) to tune the extent of HP1α nucleation by shining blue light on human osteosarcoma (U2OS) cells. The short lifetime of the HP1α droplets compared to other proteins known to phase separate reflected a transient nature of the induced HP1 droplets. To directly test if HP1α LLPS is

required to repress transcription, they tethered HP1α to an array of DNA-binding sites, introduced it into U2OS cells, and measured its ability to recruit additional untethered HP1 molecules. The tethered HP1α recruited ~20 times fewer molecules than the positive control, PML. Despite the low level of recruitment, the tethered HP1α was still able to repress transcription even without droplet formation. Thus, HP1α LLPS is divorced from transcriptional repression.

The authors performed several further experiments to test if HP1α displays liquid droplet-like behaviors. They used a clever half-bleaching FRAP of chromocenters to reveal that HP1α did not exhibit the internal mixing expected for a liquid droplet. Additionally, the rotational diffusion of HP1α molecules inside and outside of chromocenters was comparable. The exclusion of inert molecules (e.g., GFP) expected from a chromocenter was observed regardless of the presence or absence of HP1α. Importantly, they tested the concentration buffering function and dissolution pattern of HP1α by segmenting cells with varying HP1α expression level. Their results clash with a simple LLPS mechanism but are consistent with a collapsed chromatin-globule model, which posits that HP1 and related proteins induce chromatin compaction in a digital, switch-like manner by bridging two chromatin segments (Figure 1B). Local accumulation of such digital bridging would result in heterochromatin formation.

The work of Erdel et al. serves as a cautionary tale in the difficulty in understanding complex membraneless compartments like heterochromatin based on simple phase-separated condensates formed from minimal components. Another example is found with 53BP1, a DNA-repair factor, which is proposed to phase separate (Kilic et al., 2019) but forms complex hollow structures in cells (Ochs et al., 2019). It is possible that as we obtain higher-resolution biophysical insight, what appear to be simple phase-separated condensates may be shown to be well-defined, complex structures that assemble and disassemble through highly specific molecular interactions. If so, this study by Erdel et al. will be one of the first to advance us toward a more enlightened view of the complexity of membraneless compartments that is underpinned by atomistic structural understanding. Indeed, a major reverse-engineering challenge lies in using purely synthetic components to reconstitute entire, *functional* membraneless organelles that phenocopy their cellular counterparts.

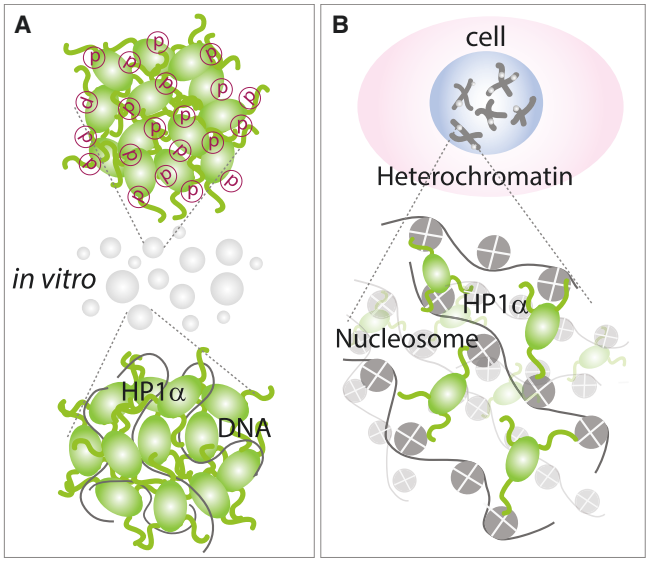

Figure 1. Two Models Proposed for the Role of HP1α in Heterochromatin Formation

(A) *In vitro*, HP1α phase separation is promoted by phosphorylation and DNA. (B) *In vivo*, HP1α undergoes digital compaction to compartmentalize heterochromatin.

DECLARATION OF INTERESTS

J.S., T.H., and S.M., declare no competing interests.

A.D.G. has served as a consultant for Aquinnah Pharmaceuticals, Prevail Therapeutics, and Third Rock Ventures and is a scientific founder of Maze Therapeutics.

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