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A liquid reservoir for silent chromatin

The protein HP1 mediates compaction of DNA into a repressive structure called heterochromatin. Analysis reveals that HP1 has liquid-like properties, offering a fresh perspective on genome organization.

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In biological phase separation[1], proteins self-organize into liquid-like droplets that grow and fuse, allowing certain molecules to become concentrated while excluding others[2]. This transition occurs abruptly under changing conditions — of protein concentration, solvent conditions or temperature, for instance. Such intracellular phase transitions seem to be a common route to achieving spatial organization of cellular components into dynamic, membrane-less compartments[3]. In two papers online in *Nature*, Strom *et al.*[4] and Larson *et al.*[5] find another role for phase separation in the cell: driving the formation of a repressive DNA domain called heterochromatin.

The human genome consists of a 2-metre-long DNA strand that must be packed into a roughly 10-micrometre-wide nucleus. This is achieved through folding into an organized material called chromatin, which is composed mostly of DNA wrapped around histone proteins. Chromatin is not uniform, but contains several large, compact domains called heterochromatin, in which various repetitive elements and unused genes are kept in a repressed state. This ensures the stability of the genome and helps cells to become specialized by preventing unwanted transcription. Despite the high level of compaction, heterochromatin domains must remain accessible to enzymes so that, for instance, DNA can be efficiently repaired if damaged[6]. A key outstanding question is how geographically distinct regions of chromatin can organize into large, condensed heterochromatin domains while nevertheless remaining accessible.

Heterochromatin protein 1 (HP1) is a key element in heterochromatin formation. It binds to chemical modifications on histones that 'bookmark' transcriptionally silent regions of chromatin, and it recruits other heterochromatin components. It can also bind to other HP1 molecules attached to the chromatin fibre, and as such is thought to keep the domain in a condensed state by creating staple-like connections between HP1-associated chromatin regions.

Strom *et al.* analysed heterochromatin formation in the nuclei of early-stage fruit-fly embryos. Intriguingly, they observed that heterochromatin domains labelled with a form of HP1 called HP1a bear the hallmarks of phase-separated compartments: the domains grow by fusing together; they repel large, inert macromolecules; and they mostly dissolve in the presence of an alcohol that disrupts weak hydrophobic interactions. The group confirmed the liquid-like nature of heterochromatin by showing that purified HP1a phase-separates in solution, forming liquid-like drops.

Larson *et al.* compared the properties of the three human forms of HP1 *in vitro*. They discovered that one, HP1α, can spontaneously phase-separate in solution and form liquid-like droplets. Interestingly, these properties emerged only when the protein was phosphorylated or in the presence of DNA — conditions that evoke conformational changes that allow the protein to bind other HP1 molecules. Using a sophisticated *in vitro* assay, the group showed that liquid-like drops of HP1α can form on a stretched DNA strand and pack it together into a small, dense point. The group also demonstrated that DNA wrapped around histones that carry chemical modifications characteristic of heterochromatin domains becomes locally concentrated through enrichment inside an HP1α drop.

These two studies provide a fresh perspective on how heterochromatic domains perform their function. It seems that the access of molecules to heterochromatin might be determined by selectivity at the interface between the liquid drop and its surroundings, rather than by simple spatial confinement in the dense chromatin meshwork. Selectivity has been seen in other liquid-like cell compartments, such as RNA–protein compartments called nucleoli[7], and could explain why certain large proteins diffuse freely in heterochromatin, whereas some small molecules are excluded.

The dynamic properties of a liquid-like domain might facilitate the repair of DNA breaks within heterochromatin. Liquidity would allow relocation of the damaged DNA segment from the centre to the boundary, where it can be accessed by the large protein complexes required for repair. Complexes that mediate transcription or

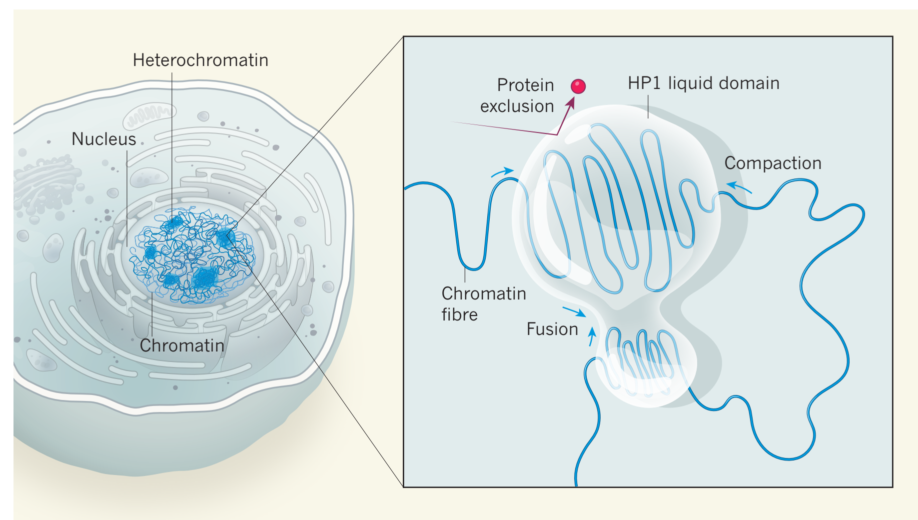

Figure 1 | Chromatin compaction in liquid droplets. In the cell nucleus, DNA is packaged around histone proteins as chromatin. DNA is transcribed from many regions of chromatin, but in places in which transcription must be repressed, chromatin is highly compacted by heterochromatin protein 1 (HP1), which binds to repressive chemical modifications on histones to form heterochromatin. Strom *et al.*[4] and Larson *et al.*[5] report that HP1 mediates heterochromatin formation by phase-separating, forming liquid-like domains in which chromatin becomes enriched and compacted. The fusion of liquid droplets might increase the size of these domains. Some proteins (perhaps those that mediate transcription) are excluded from the droplets.

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replication could also access heterochromatic regions in this way, allowing them to act at the appropriate times without disrupting the domain as a whole.

In both studies, the concentration of HP1 required for phase separation *in vitro* was much larger than that found in nuclei. Together with their other data, this points to an *in vivo* model in which HP1 becomes concentrated locally by binding to chromatin marked with repressive chemical modifications, then phase-separates on the DNA strand and spreads to other regions (Fig. 1). The two studies are focused on constitutive heterochromatin, which is highly repetitive, HP1-enriched and always transcriptionally silenced. It will be interesting to see whether liquid-phase-based organization also governs the formation of facultative heterochromatin, which harbours genes that need to be silenced only in certain tissues and at particular developmental stages.

Although heterochromatin domains are largely silent, RNA transcription from within these domains in fact helps to maintain them, acting as a platform from which to recruit heterochromatin components and as a template to produce small RNAs that reinforce silencing[8]. RNA is a potent trigger for phase separation[9,10], and many phase-separated compartments contain RNA (ref. 3), so it will be interesting to see whether the formation of HP1 liquid-like droplets is triggered by nascent RNA transcripts.

The discovery of HP1-driven phase separation provides a plausible explanation for the fact that heterochromatin is highly sensitive to temperature changes — a key characteristic of phase-separating systems[11]. This sensitivity of heterochromatin was first observed in flies[12], in which high temperatures affect position-dependent heterochromatin silencing. It has also been reported in yeast[13] and certain flowering plants[14]. Temperature-dependent phase separation of heterochromatin could therefore provide a way for organisms to effect large-scale changes in chromatin organization in response to changing environmental conditions.

Phase separation is emerging as a general mechanism for genome organization[15,16]. Combining our understanding of the physical and chemical properties of chromatin with techniques to analyse the 3D structure of the genome and its interactions with other molecules will be an essential step in better understanding the principles that govern the nuclear architecture of heterochromatin. ■

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