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Computer simulations of chromatin phase separation

Helmut Schiessel[1]*
[1]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany

ABSTRACT Several simulation studies have recently appeared in *Biophysical Journal* that investigate the formation of biomolecular condensates in the nucleus. These structures explain a large variety of biological phenomena, from epigenetic inheritance, to enhancer-promoter interactions, to the spatial organization of the entire cell nucleus.

Liquid-liquid phase separation into biomolecular condensates, also known as membraneless organelles, has emerged as an important new topic in understanding the organization of cells outside and inside the nucleus (1). As an example from everyday life, consider what happens in a mixture of oil and water. Similarly, one can imagine that the many different ingredients in a cell are not all mixed but separate into different phases. It is not yet understood how this works: why, for example, droplets of certain compositions and sizes occur at different places inside the cell. Computer simulations are crucial to explore the vast parameter space of even highly simplified systems. Here, I highlight five computer simulations, recently published in *Biophysical Journal*, that examine a wide array of phenomena related to condensates in chromatin (2–6). Several of these publications (2–4) have focused on heterochromatin protein 1 (HP1), a moderately self-attracting protein known to spontaneously phase separate in vitro (7, 8). This protein is involved in the formation and maintenance of heterochromatin, the denser, less active variant of chromatin, with the other being euchromatin. There is a direct connection between HP1 and heterochromatin via the nucleosomes, DNA-wrapped protein cylinders that represent the elementary packaging units of chromatin: nucleosomes carrying the epigenetic tag H3K9me3 belong to heterochromatin, and HP1 has a specific binding site for this tag.

Ancona and co-workers (2) study the effect of HP1 on the compaction of heterochromatin. To do this, they model a long stretch of heterochromatin as a polymer where each monomer represents about five nucleosomes. The polymer is in a solution of HP1 molecules, modeled as HP1 dimers, with each represented by seven connected beads. In the molecular dynamics simulation, the attraction between the monomers and HP1 and independently in between HP1 molecules is varied, and the resulting complexes are characterized. When HP1 is highly self-attracting, they find ordinary phase separation regardless of the presence of the polymer. When instead HP1 is strongly attracted to the monomers, the polymer is coated by HP1 but does not collapse. Collapse is only achieved when both interactions are strong and the polymer is adsorbed into the HP1 droplet. Notable is a small range of parameters where the attraction force between HP1 molecules is slightly too small to form droplets themselves for any concentration of HP1 but where a strong interaction with the polymer results in a droplet with a density that depends on the total number of proteins in the simulation box.

Wakim and co-workers (3) use a model where each monomer represents one nucleosome and, remarkably, manage to simulate an entire human chromosome. This requires some tricks, e.g., HP1 molecules are not explicitly modeled, but nucleosomes have two states, HP1 bound or not. The model chromosome methylation profile uses the experimentally determined pattern of H3K9me3 tags. The authors aim to understand how this pattern is inherited by the daughter cells, a major challenge as each cell only obtains half of the nucleosomes from the parent cell, while the missing nucleosomes are replaced with untagged new ones. They suggest that an enzyme, methyltransferase SUV39H1, can reconstruct the missing tags because, after cell division, HP1 helps the chromosome to collapse back into a similar conformation as before the division. This serves as an input for methylation reactions that are performed by solving a system of kinetic equations that depend

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*Correspondence: helmut.schiessel@tu-dresden.de

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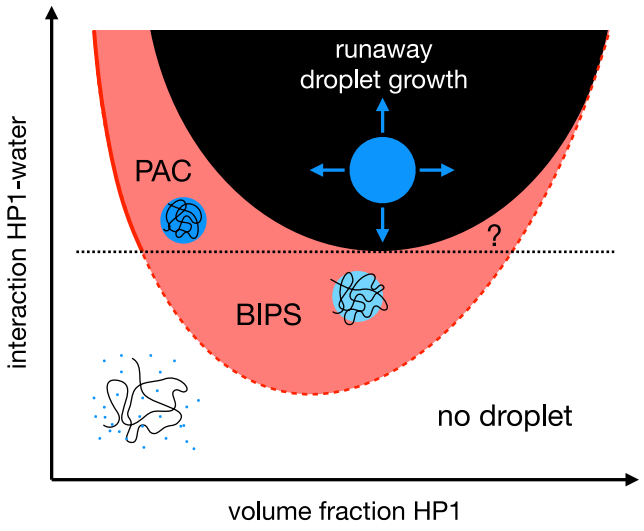

FIGURE 1 Speculative phase diagram of a polymer (e.g., heterochromatin) in a mixture of two types of solvent molecules (e.g., HP1 and water). One axis corresponds to the interaction strength between HP1 and water and the other axis to the volume fraction of HP1. The interaction strength between HP1 and the monomers is attractive and kept constant. There are roughly four phases. Two are unaffected by the presence of the polymer: in the mixed phase (white) there are no droplets, whereas the unmixed phase (black) leads to runaway droplet growth via Ostwald ripening. In the red area outside the runaway regime, finite equilibrium droplets form in the presence of the polymer. Below the critical interaction strength (*dashed black line*), bridging-induced phase separation gives rise to droplets with parameter-dependent densities (2), whereas above that interaction strength, polymer-assisted condensation leads to dense droplets that completely contain the polymer (10). To see this figure in color, go online.

on the local HP1-bound nucleosome density after the system is frozen. After the epigenetic tags have been reconstructed, the polymer is allowed again to relax by a Monte Carlo simulation. Real chromosomes have an additional challenge, as they need to "rediscover" their former configuration with their tags half-diluted and not reconstructed. The authors focus on the question of how a small island of nucleosomes with a specific epigenetic state survives through the cell divisions inside a much longer stretch of the opposite type. Stretches of around 100 nucleosomes and longer are found to be inherited, but shorter stretches are quickly forgotten. The median length of methylated blocks in human chromosomes is shorter, around 50 nucleosomes (9), suggesting that this model captures some, but not all, aspects of how cells copy epigenetic states.

The most microscopic model discussed in these Research Highlights is used in the simulation by Latham and Zhang (4). This system does not include nucleosomes but consists of HP1 molecules, short DNA molecules, and the linker histone H1, a protein that plays an important role in compacting DNA. Each amino acid of the proteins and each nucleotide of DNA is represented by a bead. In their simulation, the authors determine phase diagrams of protein-DNA mixtures. HP1 forms droplets at sufficiently low temperatures, but not H1. In the presence of DNA, droplets form for both molecules, and the stability of the droplets, characterized by the critical temperature, is greatly increased. For tertiary mixtures of HP1, H1, and DNA, it is observed that H1 helps the DNA collapse inside the HP1 droplet as H1 neutralizes charges of the nucleotides. The authors speculate that some of these effects might also persist for strings of nucleosomes, with H1 going to the DNA entry/exit regions of the nucleosomes and with HP1 binding to the H3K9me3 tags.

The other extreme, a whole nucleus with all its chromosomes, is studied by Laghmach and co-workers (5). The simulations are based on a phenomenological model and are formulated in the form of density fields of the different chromatin types: euchromatin and two types of heterochromatin, one of which, constitutive heterochromatin, corresponds to the case discussed in the above-mentioned simulations. Depending on the interactions between the various chromosome types and the interactions with the nuclear periphery, the simulations produced various compartmentalization patterns.

Finally, Chiariello and co-workers (6) study another type of nuclear condensate through a Monte Carlo simulation of a polymer and binders. The binders attract themselves and are also attracted to two sets of binding sites located on the polymer. The two sets represent a gene's promoter and enhancer, and the binders represent transcription factors. It is found that a condensate of transcription factors can attract the two binding regions. Most interesting is a case where the self-attraction between the binders is too weak such that they cannot form droplets by themselves, but where a sufficiently strong attraction to the binding sites creates clusters of binders that are pulled together to form a droplet.

One of the challenges for the cell is to have the right biomolecular condensates of the right size in the right place. The three simulations (2,4,6) that looked at smaller model systems but not at whole chromosomes studied various sets of parameters or combinations of molecules, and all found a possible answer. They show that the polymer can induce droplet formation of the protein under conditions where the attraction between proteins is too weak to form a droplet by themselves at any concentration. However, this scenario requires very strong attractions between the polymer and the proteins, leading to what Ancona and coworkers (2) call bridging-induced phase separation (see Fig. 1). I would like to contrast this to another scenario that is not considered in these simulations: polymer-assisted condensation (PAC) (10) (Fig. 1) In this case, there is enough attraction between the proteins to induce droplet formation, but the protein concentration is so low that the system is in a fully miscible state. The addition of a polymer with a weak attraction for the proteins can put the system into an unstable state such that a protein droplet containing the collapsed polymer forms. The droplet stops growing once all polymer is contained in the droplet, and since the

attraction between all components is weak, the droplet is liquid. The PAC scenario was studied for a simple system containing a flexible polymer, the "chromatin fiber," in a solution of beads, the "HP1 molecules." It will be interesting to see if the PAC scenario also occurs for the models highlighted here. Furthermore, since PAC is extremely robust to parameter changes, it is a promising scenario for the reconstruction of epigenetic tags after cell division, possibly involving various types of condensates as reaction containers, as outlined in ref. (10). PAC could also underlie various other biomolecular condensates where, e.g., RNA molecules might play the role of the condensate-inducing polymers.

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