Commentary

DNA Replication Control: Liquid-liquid Phase Separation Comes Into Play

Patroula Nathanailidou[1], Stavros Taraviras[2] and Zoi Lygerou[1]

[1]Department of General Biology, School of Medicine, University of Patras, Patras, Greece
[2]Department of Physiology, School of Medicine, University of Patras, Patras, Greece

Received on October 29, 2019; Accepted on December 3, 2019; Published on December 30, 2020

Correspondence should be addressed to Zoi Lygerou; Tel: +30 2610997610; e-mail: lygerou@med.upatras.gr

Abstract

Liquid-liquid phase separation (LLPS) has been recently suggested as a new potential mechanism underpinning various organizational aspects of the cell, from the formation of sub-cellular, biomolecule enrichments to the assembly of organelles. In eukaryotes, DNA replication follows a strict temporal and spatial program that is majorly affected by the chromatin structure, the nuclear organization and the availability of limiting initiation factors; however the regulatory mechanisms driving the process have not been fully elucidated. Original data published lately revealed for the first time that the components of the pre-replicative complex, ORC, Cdc6 and Cdt1, are able to phase separate indicating a possible connection between LLPS and DNA replication. Here, we critically present these preliminary data and propose mechanistic models that could support this regulatory link and lead to new future research directions.

The eukaryotic cell shows remarkable compartmentalization, with specific biological processes taking place within distinct subcellular topologies, which provide appropriate microenvironments. Typically, compartmentalization is achieved in organelles, which are delimited by membranes. There are however numerous cases in which the physical separation from the surrounding milieu is achieved in a membrane independent manner, with the most profound example being the nucleolus. Liquid-Liquid Phase Separation (LLPS) has lately drawn attention as a very tempting theory able to reason several long-standing microscopic observations that remained elusive, including the formation of stress-induced granules (SGs), the formation of specialized transcription foci (transcription factories) and nucleoli organization (Boeynaems *et al.* 2018). The application of LLPS in biological systems is based on the notion that regional biomolecule enrichments are able to generate condensates, that form an alternative material state, ruled by different biophysical properties, which allows them to act as distinct hubs within the cell. These phases present altered density, controlled biomolecule trafficking and different diffusion rates in respect to the environment (Boeynaems *et al.* 2018). Therefore, LLPS has been proposed as a potential mechanism able to drive the formation of membrane-less, in-cell, dynamic compartments that facilitate and regulate specialized functions triggered under different physiological or environmental cues.

It is now well-accepted that DNA replication progresses in a very defined spatial and temporal manner, with specific genomic regions being replicated at specific timepoints during the S phase of the cell cycle (Fragkos *et al.* 2015). Interestingly, several lines of evidence support that genomic regions which replicate concomitantly are organized in higher-order structures which are formed upon mitotic exit and are stable throughout interphase. Recruitment of replication factors to these structures give rise to specialized replication centers, which dynamically assemble and disassemble in an orderly fashion as replication progresses (Fragkos *et al.* 2015; Leonhardt *et al.* 2000). Origins are licensed for replication upon mitotic exit by the formation of the pre-replicative complex (Pre-RC). During pre-RC formation, two licensing factors, Cdt1 and Cdc6, recruit the six-subunit replicative helicase (Mini-chromosome maintenance, MCM2-7) onto the origin-bound

Origin Recognition Complex (ORC). The MCM-helicase is stably loaded but remains in an inactive form. Replication initiation takes place during S phase and is believed to be largely dictated by the recruitment of rate-limiting initiation factors which activate the MCM helicase, leading to origin firing (Rhind & Gilbert 2013). It is however unclear how the 3D organization of replication factories within the eukaryotic nucleus is accomplished and maintained. Phase separation is a highly attractive scenario for replication organization and its control in time and space, which however lacked experimental evidence.

In a recent paper published in Elife, Parker et.al. are the first to provide evidence that phase separation could be taking place during DNA replication, and specifically during DNA replication licensing. The authors showed that the basic components of the pre-replicative complex, ORC, Cdt1 and Cdc6, possess Intrinsically Disordered Regions (IDRs) that enable them to phase separate *in vitro*, in the presence of double stranded DNA, under conditions simulating the cell's environment and protein concentration, in an ATP-independent manner. Interestingly, phase separation of the replicative helicases, MCMs, necessitates the presence of all the other pre-RC components. *In vivo* exploration of these results verified that endogenously expressed Orc1 indeed phase separate and this ability is abolished upon CDK mediated phosphorylation of the protein at the IDR (Parker *et al.* 2019).

Based on these data, the authors propose a two-step, ORC loading mechanism, which involves an initial IDR-mediated ORC binding onto DNA, followed by the ATP-dependent encirclement of DNA, which is necessary for the subsequent loading of MCMs (Parker *et al.* 2019). The ability of MCMs to phase separate only in the presence of the other pre-RC components is consistent with the current model from other studies, supporting the formation of an MCM-Cdt1 complex that is recruited to the DNA bound ORC-Cdc6 complex for licensing to be completed (Bell 2017). Licensing takes place during G1 phase, at a time-window that coincides with the major structural reforming of chromatin, which in turn is considered a critical event for the establishment of the upcoming replication timing program (Dimitrova & Gilbert 1999). Following this notion, it is suggested by the authors that the observed ORC-mediated phase separation could be the mechanism mediating the higher-order organization of origins during G1, through ORC-DNA and ORC-ORC interactions, and therefore could act as a key regulator of the replication program. This scenario is highly attractive and adds DNA replication to the list of cellular processes which could be regulated by LLPS.

The current study could indicate a level of regulation over replication that is exerted at the licensing step. ORC dependent phase separation of spatially co-organized origins could impose different regimes of Cdt1 and Cdc6 recruitment and therefore uneven MCM loading and licensing efficiency at distinct genomic regions. Taking into account a newly proposed replication timing control model, based on which the probability of firing is proportional to the number of MCM complexes loaded on each origin (Das *et al.* 2015; Das & Rhind 2016), the phase separation dependent quantitative biases regarding MCM loading could subsequently affect origin activation during the next S phase.

Finally, the ability of ORC, Cdc6 and Cdt1 to phase separate in a CDK-dependent manner indicates a more profound role concerning the control of the licensing process during the cell cycle, also highlighted by the authors (Parker *et al.* 2019). Phosphorylation of these pre-RC components by CDK during S and G2 seems to prevent their IDR-mediated DNA binding, and along with other established mechanisms, blocks licensing during these phases and therefore prevents genomic instability and cancer emergence (Champeris Tsaniras *et al.* 2018).

The authors suggest that LLPS could be the cause of higher-order origin organization. It is however equally possible that LLPS could be the effect of higher-order origin organization, that could then drive downstream events, such as coordinated origin activation. Considering the already established mechanisms that drive high-order genome organization, it is possible that origins that are brought together by other mechanisms promote regional ORC enrichment and as a result phase-separation of these origins. In this scenario, LLPS would act as a downstream regulator of replication, affecting the diffusion kinetics of rate-limiting factors. Several factors acting at the initiation step has been proposed to be limiting for origin activation, with the DDK kinase being the predominant candidate, and their diffusion rate and residency time onto origins are considered critical for the timing of replication (Rhind & Gilbert 2013). Therefore, a conceivable scenario could be that co-organized licensed origins attract and "trap" DDK within their phase, in that way

promoting efficient and early origin activation at the region. This hypothesis is supported by the fact that indeed the DDK counterpart in Drosophila, along with other initiation and elongation factors, possess IDRs, that could allow their participation in phase separation events (Parker *et al.* 2019). However, this remains to be proven experimentally.

Overall, this is the first study proposing a potential link between LLPS and DNA replication and introduces a new conceptual framework for the regulation of the process. Additional studies and strong experimental support would however be necessary to verify these initial observations and the attractive scenarios resulting from them. Last but not least, it should be stressed that methods for the conclusive evaluation of LLPS formation in cells need to be established: this is a highly novel, emerging field and there is acute need for standardization of experimental procedures in order to avoid misleading interpretations of current and future findings (McSwiggen *et al.* 2019).

Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

PN wrote the manuscript, ST and ZL proofread and finalised the manuscript.

References

Bell SP 2017 Rethinking origin licensing. *eLife* **6** e24052

Boeynaems S, Alberti S, Fawzi NL, Mittag T, Polymenidou M, Rousseau F, Schymkowitz J, Shorter J, Wolozin B, Van Den Bosch L, Tompa P & Fuxreiter M 2018 Protein Phase Separation: A New Phase in Cell Biology. *Trends Cell Biol* **28** 420-435

Champeris Tsaniras S, Villiou M, Giannou AD, Nikou S, Petropoulos M, Pateras IS, Tserou P, Karousi F, Lalioti ME, Gorgoulis VG, Patmanidi AL, Stathopoulos GT, Bravou V, Lygerou Z & Taraviras S 2018 Geminin ablation in vivo enhances tumorigenesis through increased genomic instability. *J Pathol* **246** 134-140

Das SP, Borrman T, Liu VWT, Yang SCH, Bechhoefer J & Rhind N 2015 Replication timing is regulated by the number of MCMs loaded at origins. *Genome Research* **25** 1886-1892

Das SP & Rhind N 2016 How and why multiple MCMs are loaded at origins of DNA replication. *BioEssays* **38** 613-617

Dimitrova DS & Gilbert DM 1999 The spatial position and replication timing of chromosomal domains are both established in early G1 phase. *Mol Cell* **4** 983-993

Fragkos M, Ganier O, Coulombe P & Méchali M 2015 DNA replication origin activation in space and time. *Nat Rev Mol Cell Biol* **16** 360-374

Leonhardt H, Rahn HP, Weinzierl P, Sporbert A, Cremer T, Zink D & Cardoso MC 2000 Dynamics of DNA Replication Factories in Living Cells. *J Cell Biol* **149** 271-280

McSwiggen DT, Mir M, Darzacq X & Tjian R 2019 Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. *Genes & Development* **33** 1619-1634

Parker MW, Bell M, Mir M, Kao JA, Darzacq X, Botchan MR & Berger JM 2019 A new class of disordered elements controls DNA replication through initiator self-assembly. eLife **8** e48562

Rhind N & Gilbert DM 2013 DNA replication timing. *Cold Spring Harb Perspect Biol* **5** a010132