

# Chromatin topology, condensates and gene regulation



© 2019. Published by The Company of Biologists Ltd | Development (2019) 146, dev182766. doi:10.1242/dev.182766

SPOTLIGHT

# Chromatin topology, condensates and gene regulation: shifting paradigms or just a phase?

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## ABSTRACT

In the past decade, two major advances in our understanding of nuclear organization have taken the field of gene regulation by storm. First, technologies that can analyze the three-dimensional conformation of chromatin have revealed how the genome is organized and have provided novel insights into how regulatory regions in the genome interact. Second, the recognition that many proteins can form membraneless compartments through liquid-liquid phase separation (LLPS) has challenged long-standing notions of how proteins within the nucleus are organized and has offered a tantalizing general mechanism by which many aspects of nuclear function may be regulated. However, the functional roles of chromatin topology and LLPS in regulating gene expression remain poorly understood. These topics were discussed with great fervor during an open discussion held at a recent workshop titled 'Chromatin-based regulation of development' organized by The Company of Biologists. Here, we summarize the major points covered during this debate and discuss how they tie into current thinking in the field of gene regulation.

**KEY WORDS: Chromatin topology, Condensates, Gene regulation, Genome organization, Phase separation, Transcription**

## Introduction

The cell nucleus is a crowded environment. This is easily appreciated by considering that the almost 2 m long DNA polymer that comprises the human genome is packaged into a ∼6-10 µm diameter cell nucleus along with all the biological macromolecules required to replicate, maintain and interpret genetic information. The challenges posed by this crowding are appreciated when considering that distal regulatory elements that modulate transcription, such as enhancers, can be located hundreds of kilobases away from the genes that they act on, and that regulatory proteins, such as transcription factors, have to find their specific binding sites among billions of non-target sites. In the face of these challenges, elegant mechanisms have evolved that take advantage of this crowded environment and organize the nucleus, both in terms of the three-dimensional architecture of DNA packaged into chromatin, and of the spatial and temporal distributions of the macromolecular complexes that act on chromatin to regulate the genome. Understanding how such organization is achieved, its functional implications, and how it changes during development and disease progression has become a major focus of inquiry in the life sciences.

Driven by advances in technology, two major paradigms have emerged that dominate emerging models of nuclear organization. First, that the genome is compartmentalized at multiple spatial scales and, second, that some biological macromolecules can form membraneless compartments through the creation of two distinct phases, most commonly through liquid-liquid phase separation (LLPS) as occurs in the familiar case of oil drops in water. However, despite a tremendous amount of research on both genome topology and LLPS, the underlying mechanisms and functional implications of both remain poorly understood. This obscurity is due to a lack of experimental evidence and appropriate tools, as well as contradictory findings in the literature. These issues were discussed at great length in a recent workshop, organized by The Company of Biologists, which took place in April 2019 and was titled 'Chromatin-based regulation of development'. A facilitated open discussion was held at the workshop, motivated by two timely questions: (1) does LLPS enable gene regulation within a crowded nucleus? (moderated by Mustafa Mir and Geeta Narlikar); and (2) is chromatin topology important for gene expression? (moderated by Wendy Bickmore and Eileen Furlong). Here, we summarize the major points raised during this discussion, which we think reflect ongoing debates in the field, and provide some perspective on how they relate to current thinking on the regulation of gene expression.

## What is LLPS and why are people so excited by it?

The notion that the interior of cells resembles and behaves as an emulsion, composed of both membrane-bound and membraneless organelles, has existed in the literature since the 19th century (Wilson, 1899; Montgomery, 1898). More recently, renewed interest in the mesoscopic organization of proteins within the cell has led to the discovery that such membraneless compartments can form through the well-understood mechanism of LLPS (Brangwynne et al., 2009, 2011; Li et al., 2012; Kato et al., 2012). This has led to an explosion of research into which proteins can undergo LLPS, the physico-chemical properties of the resulting compartments, and the mechanistic principles that govern these properties.

In general, LLPS offers a dynamic and versatile mechanism by which the nucleoplasm may be spontaneously organized (i.e. without requiring additional energy) into distinct compartments across a broad range of spatial and temporal scales. The potential functional implications of such compartmentalization are far reaching, from creating distinct local high-concentration environments that increase the probability of specific biochemical reactions, to buffering the levels of free protein in the nucleoplasm, and to rapidly mediating chromatin organization into active and inactive regions (Holehouse and Pappu, 2018). These possibilities have captured the imagination of many biologists, with LLPS often being used brazenly to explain many currently mysterious phenomenon despite the fact that the functional roles of LLPS compartments remain largely theoretical.

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In our discussion at the workshop, we reviewed the current evidence for LLPS occurring in nuclei and its implications for the regulation of gene expression.

## Identifying liquid-liquid phase separated compartments *in vivo*: seeing is not believing

To reconcile the general excitement around LLPS with current experimental evidence, our discussion was initiated by surveying the room for 'validated' examples of phase separation in nuclei. The responses comprised classical and commonly cited examples, such as nucleoli (Brangwynne et al., 2011), and more recent examples, such as heterochromatin (Strom et al., 2017; Larson et al., 2017) and Polycomb bodies (Tatavosian et al., 2019; Plys et al., 2019). Objections were quickly raised in all cases, necessitating a conversation about how we define phase separation and what counts as sufficient evidence to claim that a specific protein is incorporated into a membraneless compartment through LLPS *in vivo*.

LLPS occurs when the concentration of a molecule reaches a critical point beyond which it is supersaturated. Under such conditions, it may be energetically favorable for the molecule in question to partition into low- and high-concentration phases; these distinct phases are viewed by biologists as membraneless compartments. The critical concentration at which LLPS occurs depends on a number of factors, including temperature, the properties of the solvent (such as ionic strength mediated by salt concentration), and the properties of the molecule being considered, as is best summarized by a phase diagram (Fig. 1). For example, proteins that tend to undergo LLPS typically have large, low-complexity and/or intrinsically disordered regions (IDRs) that enable hetero- and homotypic multivalent interactions (Chong et al., 2018), or have other tendencies that lead to oligomeric states. The existence of such domains in a protein are often motivation to suggest that a protein may be capable of LLPS (Banani et al., 2017).

However, the rigorous definitions of LLPS that have emerged from classical physics and chemistry research are at odds with the way LLPS and its associated terminology are used in the life sciences. Moreover, the inability to modulate important parameters, such as concentration or temperature, *in vivo* has led to an overwhelming reliance on less-rigorous, qualitative descriptors of LLPS. This reliance on qualitative descriptors was revealed when the question was posed of what experimental evidence exists for the case of nucleoli being formed by LLPS. The overwhelming response was that they are round in shape (indicating surface tension-driven morphology) and that they undergo fusion and fission events indicative of liquid-like properties. Similar criteria were described for P granules from *Caenorhabditis elegans* in which fusion, dripping and wetting behaviors have contributed substantially to conceptualizations of membraneless organelles (Brangwynne et al., 2009). At the same time, it was also discussed how such qualitative descriptors do not suffice as evidence for LLPS in the absence of measuring concentration or temperature dependence to determine the phase diagram for a given system.

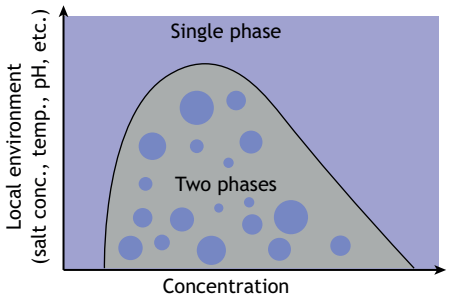


**Fig. 1. A phase diagram.** An illustration of a phase diagram showing how a system may spontaneously transition from a single homogenous phase (a miscible solution) into two distinct phases (two immiscible solutions, with one forming droplets) through a variety of paths. This may occur, for example, through an increase in the concentration of the molecule in question (*x*-axis) or through a change in the local environment (e.g. in temperature, pH or salt concentration; *y*-axis).

A frequently used, but commonly misunderstood, quantitative assay, fluorescence recovery after photobleaching (FRAP), was also brought up as a more rigorous test beyond qualitative descriptors. This assay is based on the idea that the rapid recovery rate of fluorescence within a droplet, caused by rapid internal rearrangement, is indicative of its liquid-like nature (Alberti et al., 2019). It was quickly pointed out that the idea of 'rapid liquid-like recovery' is also qualitative, as recovery times spanning from sub-seconds to minutes have been reported in the literature as being diagnostic of LLPS. Furthermore, rapid recovery in FRAP can result from a variety of mechanisms (Mueller et al., 2010; Sprague et al., 2006), and unless proper controls are conducted and multiple models are tested, the use of recovery time as a marker of LLPS is insufficient.

We agreed (mostly) upon a set of criteria for defining LLPS *in vivo*, including: evidence for a membraneless compartment, a different set of chemical rules inside and outside the compartment, and concentration- and temperature-dependent properties. For practical reasons, measurements such as concentration and temperature dependence are often tested *in vitro* and these results are used to claim *in vivo* LLPS. However, there was general agreement that, although *in vitro* experiments are informative, concluding that a molecule can undergo LLPS *in vivo* requires quantitative measurements in the endogenous cellular context. Thus, although qualitatively 'seeing' evidence for *in vivo* LLPS using microscopy assays is an essential starting step, it is not sufficient in the absence of quantitative measurements. The conclusion for this part of the discussion can thus be best summarized as 'just seeing is not believing'.

## Does LLPS enable gene regulation within a crowded nucleus?

Part of the appeal of LLPS is that it can enable distinctive regulatory mechanisms. One often-used example is the potential to regulate local concentrations of transcriptional activators and repressors in a locus-dependent manner. We thus considered a thought experiment in which multiple enhancers are in spatial proximity to a single gene, all working synergistically with multiple DNA-binding proteins in a single phase-separated body, without requiring actual physical contact between individual enhancers and promoters. Such a mechanism would confer robustness, as the disruption of a single enhancer would not lead to loss of activation. At the same time, the plasticity and reversibility of the multivalent interactions that lead to LLPS could provide a way to tune the components and thus reactions 'permitted' in the compartment and also provide a mechanism to shut-off activation or repression rapidly, for example through post-translational modifications that make LLPS energetically unfavorable. Also discussed was another unique advantage that LLPS potentially holds: to pull together and organize big swaths of the genome without expending energy by relying on the thermodynamically favorable fusion of smaller droplets. This mechanism may explain how small droplets of heterochromatin regions undergo coalescence to create larger domains of repression, as has been observed in *Drosophila* embryos (Strom et al., 2017). In the process of discussing the dynamic

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nature of phase-separated states, we came up with an analogy to 'pop-up' restaurants. In this analogy, phase-separated states get assembled at different times and locations to serve specific functions and then get disassembled when their purpose has been served. Thus, the limited nuclear space can be used multiple times to carry out distinct functions.

The types of experiments needed to determine whether such mechanisms exist were also briefly discussed. A common approach is to show that a protein requires a certain disordered domain to phase separate *in vitro*, and that deletion or mutation of that domain leads to loss of nuclear puncta and a measurable effect on transcription *in vivo*. However, it is difficult to separate direct and indirect effects in such experiments, as the disordered regions of proteins have likely evolved to play multiple roles (such as in the activation domains of transcription factors) independent of their ability to enable LLPS *in vitro*. It was suggested that trying to understand the evolutionary pressures that have led to the ability of some proteins to phase separate may provide some insight into the functional roles of LLPS.

In summary, it was clear that there is a lot of interest in LLPS but very limited understanding of how it is defined and what it can do functionally. Although there are some clear-cut examples of LLPS occurring *in vivo*, there are many more for which the evidence is weak. Furthermore, recent studies have described regulatory hubs within nuclei that qualitatively resemble LLPS compartments but appear to form via independent mechanisms (McSwiggen et al., 2019; Chong et al., 2018; Mir et al., 2018). For example, viral replication compartments exhibit many of the usual qualitative descriptors of LLPS (such as roundness, the ability to coalesce and high local concentrations) but form through transient non-specific DNA binding rather than LLPS and allow for unrestricted diffusion across boundaries (McSwiggen et al., 2019). Such alternative mechanisms that lead to high local concentrations of proteins may also enable many of the possible functions associated with LLPS and thus must also be considered when LLPS is suspected. Therefore, to address the question of how ubiquitous LLPS is and its functional roles with necessary rigor, we must incorporate what we can from physical sciences and form interdisciplinary teams to design new experimental approaches and tools.

### What do we mean by chromatin topology?

Metazoan genomes are organized hierarchically, at multiple size scales. The smallest structural feature is the nucleosome, around which ∼140 bp of DNA are wrapped. At the ∼100 kb to ∼1 Mbp scale, chromatin is organized into topological associating domains (TADs) (Nora et al., 2012; Dixon et al., 2012; Sexton et al., 2012). These domains exhibit a higher than expected interaction frequency between the genomic regions within them, as measured by chromosome conformation capture (3C)-based methods such as Hi-C. In a large number of cases, architectural proteins and promoters are present at TAD boundaries (Dixon et al., 2012; Rao et al., 2014). At these scales and higher, chromatin is also organized into 'active and inactive' compartments that are thought to form independently of TADs (Nora et al., 2017; Schwarzer et al., 2017; Haarhuis et al., 2017). At an even larger scale, chromosomes fall into their own distinct territories (Cremer et al., 2014).

In our discussion of the functional role of chromatin topology, we agreed to focus on TADs and compartments in interphase nuclei, as these are the features of chromatin topology that have been unveiled most recently. We also placed emphasis on recent evidence from single cell studies using 3C-based methods (Stevens et al., 2017; Flyamer et al., 2017; Nagano et al., 2013) and imaging approaches (Bintu et al., 2018; Finn et al., 2019; Cattoni et al., 2017), both of which have suggested that chromatin topology exhibits significant cell-cell variability and is likely far more dynamic than previously imagined (Hansen et al., 2018; Finn et al., 2019). Keeping this hierarchical and dynamic topology in mind, we discussed the role of chromatin topology in gene repression and activation.

### Is chromatin topology and packing important for gene repression?

The relationship between chromatin accessibility or compaction in heterochromatic regions of the genome has been long established. We discussed if the way constitutive heterochromatin is folded is important, or whether compaction alone is sufficient for repression. The point was raised that heterochromatic regions are known to have sub-structure (Maison et al., 2010), but it is unclear whether any sort of deterministic folding is necessary, or even if the substructure is the cause or consequence of large-scale compaction. These considerations become more important when considering facultative heterochromatin formation, which is dynamic during early development. In this context, the discussion turned to the role of large membraneless compartments formed by Polycomb-group (PcG) proteins in gene repression. These compartments, which are termed 'PcG bodies', are known to contain many repressed genes. This results in an increased spatial proximity of co-repressed genes and suggests that higher order chromatin structure could play an important role in repression (for a recent review, see Schuettengruber et al., 2017).

We discussed whether the presence of a Polycomb response element (PRE) is sufficient for repression, or whether large domains and topology are necessary. Occupancy of a single PRE by a PcG protein close to a promoter or enhancer can be sufficient for repression (Papp and Muller, 2006), suggesting that topology is not always necessary. However, at other loci, looping is required for PcG-mediated repression (Ogiyama et al., 2018; Cheutin and Cavalli, 2018), suggesting that some level of higher order structure is needed. Likewise, if PcG body formation is inhibited, only subtle phenotypes are observed in the absence of other perturbations (Bantignies et al., 2011). These and related observations led to the tentative conclusion that although the formation of large PcG bodies might not be necessary for repression, they have likely evolved as a mechanism to provide additional robustness to the system. Furthermore, there was no consensus on how to design experiments that distinguish the role of local binding of PcG proteins to PREs from a role in the formation of large PcG bodies.

### What is the role of chromatin topology in gene activation by distal enhancers?

Enhancers provide a powerful lens through which to consider the role of chromatin topology in gene regulation because of their many interesting properties: they can be located at great genomic distances from their target genes, they do not always regulate the gene that is most proximal to them, they function independently of their orientation or location, and they are classically thought to function by making physical contact with their target genes' promoter through protein-protein interactions, although chromosome topologies inconsistent with this idea have been reported (Benabdallah et al., 2019). These observations implicate the regulation of chromatin topology in enhancer function but require further investigation.

Our discussion of distal regulation by enhancers was initiated by the question of what happens if you systematically increase the distance between an enhancer and a promoter. Evidence in *Drosophila* embryos suggests that although spatial patterns of expression do not change as a function of enhancer-promoter

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distance, transcription levels (i.e. the probability of enhancer function) are decreased (Mikhaylichenko et al., 2018; Fukaya et al., 2016). This is in contrast to observations in the mouse genome where distance dependence for enhancers appears less evident (Anderson et al., 2014). However, we were reminded repeatedly that in the context of the nucleus, linear genomic distance becomes less relevant than 3D spatial proximity (although they are generally correlated), which may be regulated by a variety of mechanisms, including currently known topological features such as TADs and loops. Thus, to understand better the role of topology in enhancer function it was prudent to first discuss the evidence for the functional roles of TADs.

## What are the functional roles of TADs?

Measurements of genome-wide interaction frequencies indicate that the spatial distance between genomic loci is generally reduced within TADs compared with loci at equal linear distance between TADs. Enhancers and their target genes are generally contained within the same TAD (Symmons et al., 2014) and thus are more likely to interact with higher frequency than if they are contained in different domains. However, TAD boundaries do not represent strict physical barriers – contact probabilities within a TAD are only 2- to 3-fold greater than contact frequencies across a TAD boundary. It is also important to bear in mind that, although the presence of TADs has been confirmed by microscopy, they are essentially data features that emerge from Hi-C analyses, and how TADs are defined largely depends on the resolution of the sequencing performed. With higher-resolution maps, more nested structures emerge with large TADs containing weaker sub-TADs. However, a large number of TAD boundaries are well conserved over evolutionary time scales and are marked by genomic features such as promoters and the binding of architectural proteins such as CTCF in mammals (Dixon et al., 2012).

Importantly, it was pointed out that there is contradictory evidence on the phenotypic consequences of disrupting TADs on gene expression, or of moving genes and enhancers into separate TADs at a given locus (Paliou et al., 2019; Kane et al., 2019 preprint; Despang et al., 2019; Laugsch et al., 2019; Ghavi-Helm et al., 2019). If TAD formation is widely disrupted, for example through the removal or depletion of cohesin (Sofueva et al., 2013; Wutz et al., 2017; Schwarzer et al., 2017; Rao et al., 2017; Haarhuis et al., 2017) or CTCF (Nora et al., 2017), the activity of only a subset of genes is disrupted, whereas the expression of many genes appears unperturbed. Similarly, extensive genetic rearrangements or deletions that fuse and shuffle TADs have only modest effects on gene expression, as seen for many TADs in *Drosophila* (Ghavi-Helm et al., 2019) and for the TFAP2A (Laugsch et al., 2019), HoxD (Rodríguez-Carballo et al., 2019), Sox9-Kcnj2 (Despang et al., 2019) and Shh (Williamson et al., 2019) containing TADs in vertebrates.

These results converge with emerging data showing cell-to-cell variability in TAD structure: evidence from imaging experiments and single cell 3C-based studies indicate that, far from being composed of strict stable structures, as suggested for long-range high-frequency loops, chromatin topology is highly heterogeneous from cell to cell and likely very dynamic (Cattoni et al., 2017; Finn et al., 2019; Flyamer et al., 2017; Nagano et al., 2013; Stevens et al., 2017). Given that transcription factor residence times on DNA and cohesin-mediated loop extrusion (thought to bring enhancers and promoters together) are in the order of tens of seconds and are thought to occur frequently and repetitively (Hansen et al., 2018), it is likely that transient but very frequent interactions may be sufficient to initiate and maintain transcription at a given locus. In such a model, the probability of interactions between some enhancer-promoter pairs may be high enough without requiring a constrained topology. For some pairs, however, a well-regulated topology might be crucial to force a higher frequency of interaction. Such a model may explain the mild effects of disrupting processes, such as extrusion (e.g., by deleting CTCF or cohesin), on transcription levels at the genome-wide scale, as well as the large effects observed at specific loci. Although such models still imply that regulating chromatin topology is important, they call into question current textbook models of stable and hierarchical chromatin organization.

Results indicating that the presence of TADs is not necessary, or permissive, for the expression of many genes (at least as measured by standard *in situ* hybridization and RNA-seq) question why TADs are so well conserved over evolution. Perhaps TADs provide an additional layer of robustness or precision to gene expression, with some genes being more dependent on this layer compared with others. The genomic regions spanning TADs are highly correlated with blocks of conserved synteny (Engstrom et al., 2007; Harmston et al., 2017), suggesting that they serve as conserved units during recombination to avoid separating enhancers and promoters. TADs may also reflect gene transcription, being stabilized or reinforced during gene expression, which could explain their conserved synteny. Recent data have also linked TADs to DNA replication (Pope et al., 2014; Sima et al., 2019) and to DNA damage (Canela et al., 2017), implying alternative and more general roles for TADs in genome functions.

In summary, it is clear that we have only just begun to appreciate the role of chromatin topology. Although some principles, such as extrusion and insulation, may apply broadly, it is likely that more nuanced and locus-specific considerations will be necessary to elucidate the functional role of genome topology.

## Outlook

Although extraordinary progress is being made in understanding 3D genome architecture and nuclear organization, it was clear from our discussions that further studies are required to convince scientists of the functional role of LLPS and TADs in the regulation of gene expression. At this point, refraining from creating generalized models is prudent, as they are easily challenged by contradictory data. Although LLPS- and chromatin topology-based models may or may not provide the unifying principles to explain gene regulation that we are desperately searching for, both phenomena are highly interconnected and likely play important roles by virtue of their existence in multiple organisms spanning large evolutionary time scales. Understanding these roles will continue to require interdisciplinary studies and the development of new technologies that allow us to both visualize and dissect the molecular mechanisms that dictate nuclear organization and function.

#### Acknowledgements

We thank all participants of the 2019 The Company of Biologists Workshop on 'Chromatin-based regulation of development' for the lively discussions that provided the material and motivation for this article; Joanna Wysocka (Stanford) and Benoit Bruneau (UCSF) for organizing the workshop; and The Company of Biologists for sponsoring it. We also thank Anders S. Hansen and David T. McSwiggen for useful comments on the manuscript.

#### Competing interests

The authors declare no competing or financial interests.

#### Funding

W.B. acknowledges support from the UK Medical Research Council (MC_UU_00007/2). G.N. acknowledges support from the National Institutes of Health (R35 GM127010). E.E.M.F. acknowledges support from the European

Research Council (ERC) Advanced Grant DeCRyPT (787611). Deposited in PMC for release after 12 months.

## References


**Alberti, S., Gladfelter, A. and Mittag, T.** (2019). Considerations and challenges in studying liquid-liquid phase separation and biomolecular condensates. *Cell* **176**, 419-434. doi:10.1016/j.cell.2018.12.035

**Anderson, E., Devenney, P. S., Hill, R. E. and Lettice, L. A.** (2014). Mapping the Shh long-range regulatory domain. *Development* **141**, 3934-3943. doi:10.1242/dev.108480

**Banani, S. F., Lee, H. O., Hyman, A. A. and Rosen, M. K.** (2017). Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* **18**, 285-298. doi:10.1038/nrm.2017.7

**Bantignies, F., Roure, V., Comet, I., Leblanc, B., Schuettengruber, B., Bonnet, J., Tixier, V., Mas, A. and Cavalli, G.** (2011). Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. *Cell* **144**, 214-226. doi:10.1016/j.cell.2010.12.026

**Benabdallah, S., Williamson, I., Illingworth, R. S., Kane, L., Boyle, S., Sengupta, D., Grimes, G. R., Therizols, P. and Bickmore, W. A.** (2019). Decreased enhancer-promoter proximity accompanying enhancer activation. *Mol. Cell.* S1097-2765(19)30593-3. doi:10.1016/j.molcel.2019.07.038

**Bintu, B., Mateo, L. J., Su, J.-H., Sinnott-Armstrong, N. A., Parker, M., Kinrot, S., Yamaya, K., Boettiger, A. N. and Zhuang, X.** (2018). Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science* **362**, eaau1783. doi:10.1126/science.aau1783

**Brangwynne, C. P., Eckmann, C. R., Courson, D. S., Rybarska, A., Hoege, C., Gharakhani, J., Julicher, F. and Hyman, A. A.** (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* **324**, 1729-1732. doi:10.1126/science.1172046

**Brangwynne, C. P., Mitchison, T. J. and Hyman, A. A.** (2011). Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 4334-4339. doi:10.1073/pnas.1017150108

**Canela, A., Maman, Y., Jung, S., Wong, N., Callen, E., Day, A., Kieffer-Kwon, K.-R., Pekowska, A., Zhang, H., Rao, S. S. P. et al.** (2017). Genome organization drives chromosome fragility. *Cell* **170**, 507-521.e18. doi:10.1016/j.cell.2017.06.034

**Cattoni, D. I., Cardozo Gizzi, A. M., Georgieva, M., Di Stefano, M., Valeri, A., Chamousset, D., Houbron, C., Déjardin, S., Fiche, J.-B., González, I. et al.** (2017). Single-cell absolute contact probability detection reveals chromosomes are organized by multiple low-frequency yet specific interactions. *Nat. Commun.* **8**, 1753. doi:10.1038/s41467-017-01962-x

**Cheutin, T. and Cavalli, G.** (2018). Loss of PRC1 induces higher-order opening of Hox loci independently of transcription during Drosophila embryogenesis. *Nat. Commun.* **9**, 3898. doi:10.1038/s41467-018-05945-4

**Chong, S. S., Dugast-Darzacq, C., Liu, Z., Dong, P., Dailey, G. M., Cattoglio, C., Heckert, A., Banala, S., Lavis, L., Darzacq, X. et al.** (2018). Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science* **361**, 378. doi:10.1126/science.aar2555

**Cremer, T., Cremer, C. and Lichter, P.** (2014). Recollections of a scientific journey published in human genetics: from chromosome territories to interphase cytogenetics and comparative genome hybridization. *Hum. Genet.* **133**, 403-416. doi:10.1007/s00439-014-1425-5

**Despang, A., Schöpflin, R., Franke, M., Ali, S., Jerković, I., Paliou, C., Chan, W.-L., Timmermann, B., Wittler, L., Vingron, M. et al.** (2019). Functional dissection of the Sox9-Kcnj2 locus identifies nonessential and instructive roles of TAD architecture. *Nat. Genet.* **51**, 1263-1271. doi:10.1038/s41588-019-0466-z

**Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J. S. and Ren, B.** (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485**, 376-380. doi:10.1038/nature11082

**Engstrom, P. G., Ho Sui, S. J., Drivenes, O., Becker, T. S. and Lenhard, B.** (2007). Genomic regulatory blocks underlie extensive microsynteny conservation in insects. *Genome Res.* **17**, 1898-1908. doi:10.1101/gr.6669607

**Finn, E. H., Pegoraro, G., Brandao, H. B., Valton, A.-L., Oomen, M. E., Dekker, J., Mirny, L. and Misteli, T.** (2019). Extensive heterogeneity and intrinsic variation in spatial genome organization. *Cell* **176**, 1502-1515.e10. doi:10.1016/j.cell.2019.01.020

**Flyamer, I. M., Gassler, J., Imakaev, M., Brandão, H. B., Ulianov, S. V., Abdennur, N., Razin, S. V., Mirny, L. A. and Tachibana-Konwalski, K.** (2017). Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. *Nature* **544**, 110-114. doi:10.1038/nature21711

**Fukaya, T., Lim, B. and Levine, M.** (2016). Enhancer control of transcriptional bursting. *Cell* **166**, 358-368. doi:10.1016/j.cell.2016.05.025

**Ghavi-Helm, Y., Jankowski, A., Meiers, S., Viales, R. R., Korbel, J. O. and Furlong, E. E. M.** (2019). Highly rearranged chromosomes reveal uncoupling between genome topology and gene expression. *Nat. Genet.* **51**, 1272-1282. doi:10.1038/s41588-019-0462-3

**Haarhuis, J. H. I., Van Der Weide, R. H., Blomen, V. A., Yanez-Cuna, J. O., Amendola, M., Van Ruiten, M. S., Krijger, P. H. L., Teunissen, H., Medema, R. H., Van Steensel, B. et al.** (2017). The Cohesin release factor WAPL restricts chromatin loop extension. *Cell* **169**, 693-707.e14. doi:10.1016/j.cell.2017.04.013

**Hansen, A. S., Cattoglio, C., Darzacq, X. and Tjian, R.** (2018). Recent evidence that TADs and chromatin loops are dynamic structures. *Nucleus* **9**, 20-32. doi:10.1080/19491034.2017.1389365

**Harmston, N., Ing-Simmons, E., Tan, G., Perry, M., Merkenschlager, M. and Lenhard, B.** (2017). Topologically associating domains are ancient features that coincide with Metazoan clusters of extreme noncoding conservation. *Nat. Commun.* **8**, 441. doi:10.1038/s41467-017-00524-5

**Holehouse, A. S. and Pappu, R. V.** (2018). Functional implications of intracellular phase transitions. *Biochemistry* **57**, 2415-2423. doi:10.1021/acs.biochem.7b01136

**Kato, M., Han, T. W., Xie, S., Shi, K., Du, X., Wu, L. C., Mirzaei, H., Goldsmith, E. J., Longgood, J., Pei, J. et al.** (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell* **149**, 753-767. doi:10.1016/j.cell.2012.04.017

**Larson, A. G., Elnatan, D., Keenen, M. M., Trnka, M. J., Johnston, J. B., Burlingame, A. L., Agard, D. A., Redding, S. and Narlikar, G. J.** (2017). Liquid droplet formation by HP1$\alpha$ suggests a role for phase separation in heterochromatin. *Nature* **547**, 236-240. doi:10.1038/nature22822

**Laugsch, M., Bartusel, M., Rehimi, R., Alirzayeva, H., Karaolidou, A., Crispatzu, G., Zentis, P., Nikolic, M., Bleckwehl, T., Kolovos, P. et al.** (2019). Modeling the pathological long-range regulatory effects of human structural variation with patient-specific hiPSCs. *Cell Stem Cell* **24**, 736-752.e12. doi:10.1016/j.stem.2019.03.004

**Li, P., Banjade, S., Cheng, H.-C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J. V., King, D. S., Banani, S. F. et al.** (2012). Phase transitions in the assembly of multivalent signalling proteins. *Nature* **483**, 336-340. doi:10.1038/nature10879

**Maison, C., Quivy, J.-P., Probst, A. V. and Almouzni, G.** (2010). Heterochromatin at mouse pericentromeres: a model for de novo heterochromatin formation and duplication during replication. *Cold Spring Harbor Symp. Quant. Biol.* **75**, 155-165. doi:10.1101/sqb.2010.75.013

**Mcswiggen, D. T., Hansen, A. S., Teves, S. S., Marie-Nelly, H., Hao, Y., Heckert, A. B., Umemoto, K. K., Dugast-Darzacq, C., Tjian, R. and Darzacq, X.** (2019). Evidence for DNA-mediated nuclear compartmentalization distinct from phase separation. *eLife* **8**, e47098. doi:10.7554/eLife.47098

**Mikhaylichenko, O., Bondarenko, V., Harnett, D., Schor, I. E., Males, M., Viales, R. R. and Furlong, E. E. M.** (2018). The degree of enhancer or promoter activity is reflected by the levels and directionality of eRNA transcription. *Genes Dev.* **32**, 42-57. doi:10.1101/gad.308619.117

**Mir, M., Stadler, M. R., Ortiz, S. A., Hannon, C. E., Harrison, M. M., Darzacq, X. and Eisen, M. B.** (2018). Dynamic multifactor hubs interact transiently with sites of active transcription in Drosophila embryos. *eLife* **7**, e40497. doi:10.7554/eLife.40497

**Montgomery, T. S. H.** (1898). Comparative cytological studies, with especial regard to the morphology of the nucleolus. *J. Morphol.* **15**, 265-582. doi:10.1002/jmor.1050150204

**Mueller, F., Mazza, D., Stasevich, T. J. and Mcnally, J. G.** (2010). FRAP and kinetic modeling in the analysis of nuclear protein dynamics: what do we really know? *Curr. Opin. Cell Biol.* **22**, 403-411. doi:10.1016/j.ceb.2010.03.002

**Nagano, T., Lubling, Y., Stevens, T. J., Schoenfelder, S., Yaffe, E., Dean, W., Laue, E. D., Tanay, A. and Fraser, P.** (2013). Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature* **502**, 59-64. doi:10.1038/nature12593

**Nora, E. P., Lajoie, B. R., Schulz, E. G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., Van Berkum, N. L., Meisig, J., Sedat, J. et al.** (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* **485**, 381-385. doi:10.1038/nature11049

**Nora, E. P., Goloborodko, A., Valton, A.-L., Gibcus, J. H., Uebersohn, A., Abdennur, N., Dekker, J., Mirny, L. A. and Bruneau, B. G.** (2017). Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. *Cell* **169**, 930-944.e22. doi:10.1016/j.cell.2017.05.004

**Ogiyama, Y., Schuettengruber, B., Papadopoulos, G. L., Chang, J.-M. and Cavalli, G.** (2018). Polycomb-dependent chromatin looping contributes to gene silencing during Drosophila development. *Mol. Cell* **71**, 73-88.e5. doi:10.1016/j.molcel.2018.05.032

**Paliou, C., Guckelberger, P., Schöpflin, R., Heinrich, V., Esposito, A., Chiariello, A. M., Bianco, S., Annunziatella, C., Helmuth, J., Haas, S. et al.** (2019). Preformed chromatin topology assists transcriptional robustness of Shh during limb development. *Proc. Natl. Acad. Sci. USA* **116**, 12390-12399. doi:10.1073/pnas.1900672116

**Papp, B. and Muller, J.** (2006). Histone trimethylation and the maintenance of transcriptional ON and OFF states by trxG and PcG proteins. *Genes Dev.* **20**, 2041-2054. doi:10.1101/gad.388706

**Plys, A. J., Davis, C. P., Kim, J., Rizki, G., Keenen, M. M., Marr, S. K. and Kingston, R. E.** (2019). Phase separation of Polycomb-repressive complex 1 is governed by a charged disordered region of CBX2. *Genes Dev.* **33**, 799-813. doi:10.1101/gad.326488.119

**Pope, B. D., Ryba, T., Dileep, V., Yue, F., Wu, W., Denas, O., Vera, D. L., Wang, Y., Hansen, R. S., Canfield, T. K. et al.** (2014). Topologically associating domains are stable units of replication-timing regulation. *Nature* **515**, 402-405. doi:10.1038/nature13986

**Rao, S. S. P., Huntley, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., Robinson, J. T., Sanborn, A. L., Machol, I., Omer, A. D., Lander, E. S. et al.** (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665-1680. doi:10.1016/j.cell.2014.11.021

**Rao, S. S. P., Huang, S.-C., St Hilaire, B. G., Engreitz, J. M., Perez, E. M., Kieffer-Kwon, K.-R., Sanborn, A. L., Johnstone, S. E., Bascom, G. D., Bochkov, I. D. et al.** (2017). Cohesin loss eliminates all loop domains. *Cell* **171**, 305-320.e24. doi:10.1016/j.cell.2017.09.026

**Rodríguez-Carballo, E., Lopez-Delisle, L., Yakushiji-Kaminatsui, N., Ullate-Agote, A. and Duboule, D.** (2019). Impact of genome architecture on the functional activation and repression of Hox regulatory landscapes. *BMC Biol.* **17**, 55. doi:10.1186/s12915-019-0677-x

**Schuettengruber, B., Bourbon, H.-M., Di Croce, L. and Cavalli, G.** (2017). Genome regulation by polycomb and trithorax: 70 years and counting. *Cell* **171**, 34-57. doi:10.1016/j.cell.2017.08.002

**Schwarzer, W., Abdennur, N., Goloborodko, A., Pekowska, A., Fudenberg, G., Loe-Mie, Y., Fonseca, N. A., Huber, W., Haering, C. H., Mirny, L. et al.** (2017). Two independent modes of chromatin organization revealed by cohesin removal. *Nature* **551**, 51-56. doi:10.1038/nature24281

**Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A. and Cavalli, G.** (2012). Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell* **148**, 458-472. doi:10.1016/j.cell.2012.01.010

**Sima, J., Chakraborty, A., Dileep, V., Michalski, M., Klein, K. N., Holcomb, N. P., Turner, J. L., Paulsen, M. T., Rivera-Mulia, J. C., Trevilla-Garcia, C. et al.** (2019). Identifying cis elements for spatiotemporal control of mammalian DNA replication. *Cell* **176**, 816-830.e18. doi:10.1016/j.cell.2018.11.036

**Sofueva, S., Yaffe, E., Chan, W.-C., Georgopoulou, D., Rudan, M. V., Mira-Bontenbal, H., Pollard, S. M., Schroth, G. P., Tanay, A. and Hadjur, S.** (2013). Cohesin-mediated interactions organize chromosomal domain architecture. *EMBO J.* **32**, 3119-3129. doi:10.1038/emboj.2013.237

**Sprague, B. L., Müller, F., Pego, R. L., Bungay, P. M., Stavreva, D. A. and Mcnally, J. G.** (2006). Analysis of binding at a single spatially localized cluster of binding sites by fluorescence recovery after photobleaching. *Biophys. J.* **91**, 1169-1191. doi:10.1529/biophysj.105.073676

**Stevens, T. J., Lando, D., Basu, S., Atkinson, L. P., Cao, Y., Lee, S. F., Leeb, M., Wohlfahrt, K. J., Boucher, W., O'shaughnessy-Kirwan, A. et al.** (2017). 3D structures of individual mammalian genomes studied by single-cell Hi-C. *Nature* **544**, 59-64. doi:10.1038/nature21429

**Strom, A. R., Emelyanov, A. V., Mir, M., Fyodorov, D. V., Darzacq, X. and Karpen, G. H.** (2017). Phase separation drives heterochromatin domain formation. *Nature* **547**, 241-245. doi:10.1038/nature22989

**Symmons, O., Uslu, V. V., Tsujimura, T., Ruf, S., Nassari, S., Schwarzer, W., Ettwiller, L. and Spitz, F.** (2014). Functional and topological characteristics of mammalian regulatory domains. *Genome Res.* **24**, 390-400. doi:10.1101/gr.163519.113

**Tatavosian, R., Kent, S., Brown, K., Yao, T., Duc, H. N., Huynh, T. N., Zhen, C. Y., Ma, B., Wang, H. and Ren, X.** (2019). Nuclear condensates of the Polycomb protein chromobox 2 (CBX2) assemble through phase separation. *J. Biol. Chem.* **294**, 1451-1463. doi:10.1074/jbc.RA118.006620

**Williamson, I., Kane, L., Devenney, P. S., Flyamer, I. M., Anderson, E., Kilanowski, F., Hill, R. E., Bickmore, W. A. and Lettice, L. A.** (2019). Developmentally regulated *Shh* expression is robust to TAD perturbations. *Development* **146**, dev.179523. doi:10.1242/dev.179523

**Wilson, E. B.** (1899). The structure of protoplasm. *Science* **10**, 33-45. doi:10.1126/science.10.237.33

**Wutz, G., Várnai, C., Nagasaka, K., Cisneros, D. A., Stocsits, R. R., Tang, W., Schoenfelder, S., Jessberger, G., Muhar, M., Hossain, M. J. et al.** (2017). Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. *EMBO J.* **36**, 3573-3599. doi:10.15252/embj.201798004