Chromatin as a three-dimensional memory machine

Jeremy A. Owen[a] and Leonid A. Mirny [b,c]

[a] Department of Chemistry, Princeton University, Princeton, NJ 08540
[b] Institute for Medical Engineering & Science, and
[c] Department of Physics, MIT, 77 Mass Ave, Cambridge, MA 02139

Corresponding authors:
Jeremy A. Owen (jo6038@princeton.edu);
Leonid A. Mirny (leonid@mit.edu)

Abstract

Epigenetic memory—the stable inheritance of a cellular state over cell generations—has long been associated with chromatin modifications. But individual modifications are very dynamic. How can they carry information across cell generations? Recent theoretical work suggests the answer might lie, in part, in the three-dimensional organization of the genome. Cooperation between marks brought together by genome folding can correct epigenetic errors, making stable memory units out of unstable marks. If marks direct the phase separation of chromatin, the resulting bidirectional coupling between marks and structure provides a mechanism for many of these units to operate independently along the genome. Models of bidirectional coupling have helped identify elements, such as formation of a dense compartment, 3D mark spreading, and limited enzyme, which may be key to stable epigenetic memory. An analogy between these 3D models and a classic model of associative memory hints at a way chromatin could perform sophisticated information processing.

Keywords

chromatin, epigenetics, 3D genome organization, cooperativity

Introduction

Classic experiments demonstrated that epigenetic memory of transcriptional state can be held in chromatin [1–3]. Two identical DNA sequences in the nucleus can stably maintain different activities—one copy transcribed, the other silenced—over generations of cell growth and division. However, the fundamental units and precise molecular "seat" of this chromatin-based memory have remained elusive. The bits of genetic information are the individual letters of DNA. What are the bits of epigenetic information?

We have by now a commanding knowledge of chromatin's primary structure and composition—of the histones and their variants, and of the chemical modifications of DNA and histones that form patterns across the genome correlated with transcriptional activity [4–8]. These chemical "marks" decorating chromatin have long been postulated to be the carriers of chromatin-based epigenetic information. However, individual marks are very unstable. They are inevitably diluted at replication, and then remade by noisy molecular processes. This means that a single marked nucleosome, or a single methylated CpG site, cannot serve as a stable bit of memory for more than a few cell divisions. We must look to a larger scale to find the units of epigenetic memory.

Genomic regions bearing marks associated with epigenetic memory commonly exhibit distinctive three-dimensional (3D) organizations which tend to bring marks together—either by the densification or self-association of the regions. Examples range from the inactive X chromosome [9] to Polycomb bodies [10], and from the peripheral localization of heterochromatin to the physical clustering of CENP-A variant nucleosomes within centromeres [11]. Traditionally, these 3D structures have been interpreted as mediating the downstream *effects* of the marks—for example, dense chromatin might physically exclude transcriptional machinery, effecting repression. But recent theoretical work, which we review here, suggests that these structures may be active participants in the memory mechanism itself, serving to correct errors and furnishing the stable bits of epigenetic memory.

Mark spreading as a memory mechanism

The fundamental problem of chromatin-based epigenetic memory is that of *inheritance*—how can information stored in chromatin be stable even as chromatin undergoes the ructions of the cell cycle? In particular, during replication, marks are inevitably diluted as new histones and nucleotides are incorporated. Maintaining marks requires some form of post-replicative restoration [12,13]. If marks themselves are to serve as a seat of memory, the restoration mechanism must be targeted by existing marks.

At first, the most promising substrate for epigenetic memory seems to be DNA methylation, because the semiconservative replication of DNA provides an exact template for its restoration. Replication of a fully methylated CpG site produces a hemimethylated site, which methyltransferase enzymes can recognize and convert back to the fully methylated state. If the enzymes were very fast and absolutely specific for hemimethylation, this might be a perfect memory system. But real enzymes inevitably also have some nonspecific activity—creating errors. Even if the nonspecific activity is 80 times less than the specific one, as reported for a typical maintenance methyltransferase [14], a simple calculation shows that errors are expected at individual sites within 15 cell divisions. [Endnote]

For histone marks, the inheritance mechanism is even less precise. When DNA is replicated, specialized components of the passing replication fork distribute existing histones locally and symmetrically to both

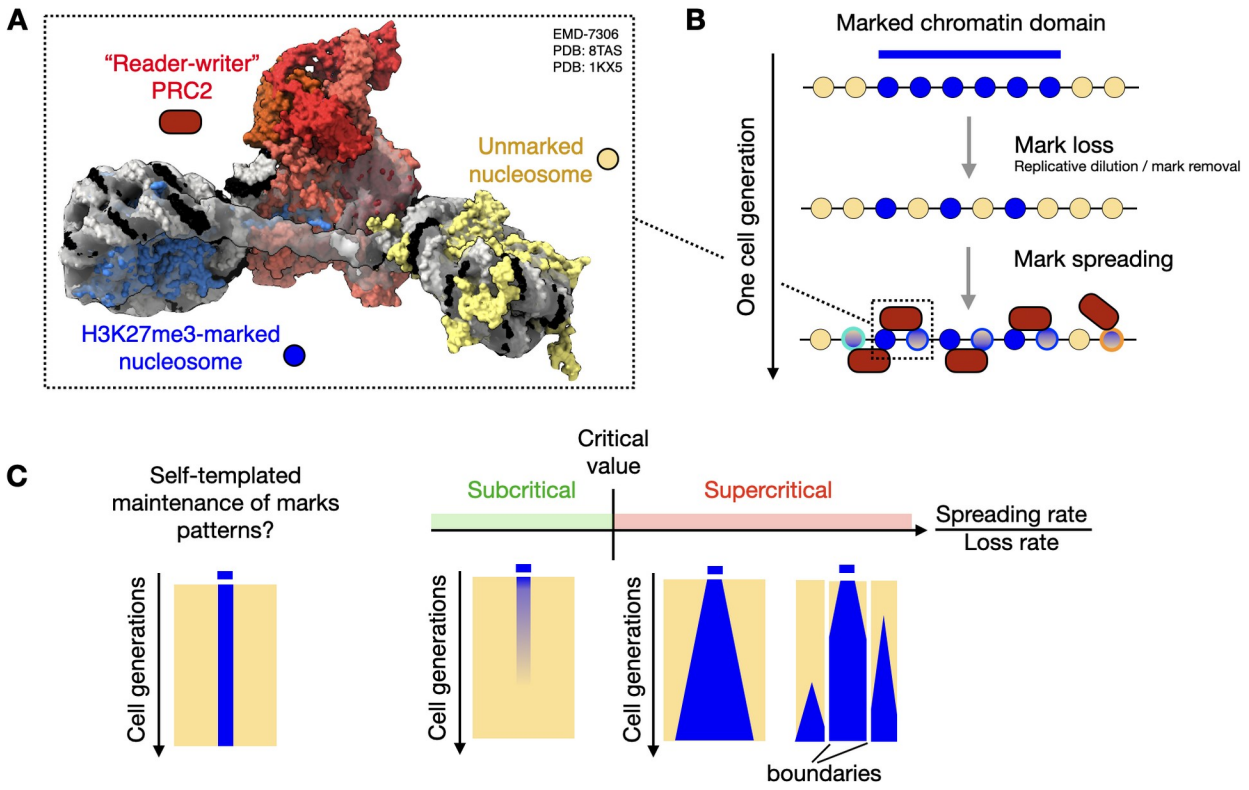

Figure 1. Mark spreading. Many marks "spread" on chromatin due to the action of reader-writer enzymes. (A) This mechanism is beautifully illustrated by the structure [15] of the PRC2 complex—the H3K27me3 reader-writer—engaging a dinucleosome [Created in ChimeraX by fitting PDB:8TAS and PDB:1KX5 into the volume of EMD-7306]. Recognition of H3K27me3 allosterically stimulates the methylation activity of PRC2. (B) Marks (blue) are diluted when DNA is replicated, eroding mark patterns. Activity of reader-writer enzymes (red) stimulated by retained marks could help restore mark patterns, but some errors at the edges of domains (cyan), as well as elsewhere due to unstimulated writer activity (orange), are inevitable. (C) Models of linear mark spreading exhibit a kind of all-or-none behavior that challenges self-templated maintenance of mark patterns—when spreading is fast enough to sustain existing mark domains, marks tend to also spread uncontrollably.

daughter strands, roughly preserving the genomic position of histone marks [16–18]. But unlike DNA methylation, there is no evidence for a *precisely* semiconservative process (such as splitting of the histone octamer) providing an exact template for restoration [12,19].

Many marks [20–22] are made by enzymes that are allosterically stimulated by the presence of that same mark at a nearby site—a mechanism elegantly described as "spreading" marks along chromatin (Figure 1A). Intuitively, mark spreading would seem to solve the inheritance problem by restoring the density of marks in a region i.e., providing inheritance at the level of mark *patterns*, rather than individual marks. Reader-writer enzymes could fill in the gaps in mark patterns left by replicational dilution, recognizing existing marks on the retained parental histones and writing on the adjacent new histones (Figure 1B).

However, mark spreading faces two key challenges. First, there is the problem of uncontrolled spreading, best illustrated by the elegant model of Hodges and Crabtree [23,24] for linear mark spreading. In their model, chromatin is idealized as a linear sequence of sites that can be marked. Marks spread between adjacent sites at a rate k+ and marks are lost at a rate k-, equally at all sites. The fate of epigenetic domains is governed by the ratio of the spreading rate to the loss rate, k+/k-. The model exhibits a sharp phase transition at a critical value of this ratio. When the ratio is above the critical value, marks spread

uncontrollably across the genome. Below the critical value, marks are lost globally. There is no sweet spot in between these behaviors, and there is never faithful, “self-templated” maintenance of mark patterns (Figure 1C).

Second, even if boundaries (such as nucleosome-depleted regions or insulator elements) constrain spreading, linear mark spreading remains highly sensitive to noise [25]. A single mark in the wrong place has a chance to nucleate a new domain, which would then spread until it hits the next boundary. The basal, unstimulated ”writer” activity of reader-writer enzymes, which biochemical studies [21,26] have shown can be significant, provides a constant source of such noise.

Cooperativity and genomic partitioning

A key insight is that the noise problem can be solved by restoration mechanisms that enlist the *cooperation* of many marks [25,27–29]. This leads naturally to a role for 3D genome organization, which can help enable this cooperation via spatial interactions between marks.

In seminal work, Dodd et al. [27] developed a model of histone modification dynamics in a (presumed isolated) chromatin region consisting of 60 nucleosomes. They found that alternative modification states of the region (e.g. largely methylated vs. largely acetylated) can be stably inherited, if the rate of histone modification exhibits cooperativity—that is, if the effective marking rate depends more than linearly on the current number of marks. This can be achieved *directly*, if at least two nucleosomes are involved in the conversion of a third one, but also *indirectly* if there are multiple, successive mark states (e.g. acetylation, unmodified, methylation; or mono- di- and trimethylation at a single site).

Critically, they show it is not sufficient for marks to spread only between linearly-adjacent nucleosomes. Spread between nonadjacent nucleosomes, as could be mediated by higher-order chromatin folding bringing genomically separated nucleosomes into physical proximity, is required. Intuitively, in the absence of this “3D” spread of marks, the marks on one side of the region cannot “know” about marks on the other side, preventing cooperation involving the entire region. 3D spread of marks by reader-writer enzymes has since been demonstrated *in vitro* [21], and is also suggested by *in vivo* observations [30,31], providing support for the plausibility of this mechanism.

Although there is always *some* rate at which a real bistable system can spontaneously flip between states due to noise, Dodd et al. found that, in the presence of cooperativity, this rate can decrease very fast—exponentially—as the number of nucleosomes N in the region increases. This exponential relationship between memory stability and system size is characteristic of bistable chemical systems [32], and is seen in models of a very different sort of epigenetic memory—the genetic toggle switch—where it emerges from cooperative TF binding [33,34]. It seems hard in principle to do any better, because there is always a possibility—about once every 2^N cell cycles—that *all* marks in a cluster of N nucleosomes are lost simultaneously upon replication [35].

Concurrently, Sedighi and Sengupta (2007) [36] developed a coarse-grained model of silencing in which the genome consists of a linear sequence of units (each effectively imagined to consist of many nucleosomes) that are individually bistable due to cooperativity in histone modification. Typically, one state (either silenced or active) spreads from unit to unit at the expense of the other. However, under certain conditions—for example if adjacent elements are sufficiently isolated from one another—propagation from element to element stops, and alternative mark patterns can be stable. Notably, Sedighi and Sengupta also considered titration effects arising from the limitation of enzymes responsible for silencing, an important matter we return to below.

Together, these theoretical works suggest a vision of chromatin-based memory in which the genome is somehow partitioned into discrete memory units, each of which must be internally cooperative through 3D interactions and large enough to suppress noise, while being sufficiently isolated from one another to enable the units to operate independently. The question then turns to understanding how such genome organization could arise.

Self-organized partitioning

Spatial organization of interphase chromosomes can create partitioning of the genome in many different forms [37]. The polymeric nature of chromatin induces a power-law decay of contact probability with genomic distance—this already provides a kind of passive mechanism that could isolate domains without positioned boundaries. Chromatin is also weakly partitioned, by loop extrusion, into the so-called topologically associating domains (TADs), which are defined by explicit boundaries [38]. However, on top of this "mark-independent" structure, the genome also exhibits rich 3D structure *correlated with chromatin marks*—the "compartmentalization" of like-marked regions. The most classic, coarsest example of this phenomenon is the spatial segregation of silenced, heterochromatic regions from active, euchromatic ones. But more detailed, Hi-C-based classification has revealed up to six different types of genomic regions, each with characteristic marks and a tendency for homotypic interaction [39].

Marks probably drive compartmentalization by creating an attraction between regions carrying the same marks [40–43]. Whether direct or mediated by cofactors, affinities between marked regions can induce intramolecular phase separation of the chromatin polymer into spatially segregated, dense and diffuse phases [44,45]. Combined with the possible 3D spread of marks, this suggests a bidirectional coupling: marks direct structure, and structure directs marks (Figure 2). Could this coupling play a role in epigenetic memory?

Models of bidirectional coupling [46–52] share a number of key elements. They idealize chromatin as a dynamic polymer whose monomers represent nucleosomes or short stretches of chromatin, with interactions that depend on their marking status—typically homotypic attraction between similarly marked regions. On top of this, the marks themselves are dynamic, being made or removed by a mechanism that responds to the 3D conformation of the polymer, creating a feedback loop. The details of the mark dynamics vary significantly from model to model, but the resulting behavior is quite universal.

In so-called "magnetic polymer" models [46,50,53–55], a single energy function governs the polymer conformation and the mark dynamics (intuitively, this is like a scenario where the "marks" are particles at some concentration that reversibly stick to the polymer and—when on the polymer—to each other). As the strength of homotypic mark-mark interactions is increased, these physics-inspired models usually exhibit a sudden transition in which the entire polymer collapses and everything gets marked (Figure 2B).

Sandholtz et al. [48] suppose attraction between marks is mediated by a particle (representing HP1), and marks are laid down at a rate dependent on the local HP1 concentration. Fine-tuning of the parameters of this model enables memory of mark patterns over about 5 cell divisions. This was later improved by Mukherjee et al. [56], who posited an exponential dependence between the methylation rate and the HP1 concentration. Other models, such as that of Katava et al. [57], suppose that marks spread at some rate per unit time, between spatially-proximate nucleosomes. This directly models the activity of reader-writer enzymes, and is mathematically very different from a "magnetic polymer". Despite the detailed differences in these models, they all exhibit an "all-or-none" tendency for spreading of marks to the whole polymer, or global loss of marks—reminiscent of the behavior of linear mark spreading [23].

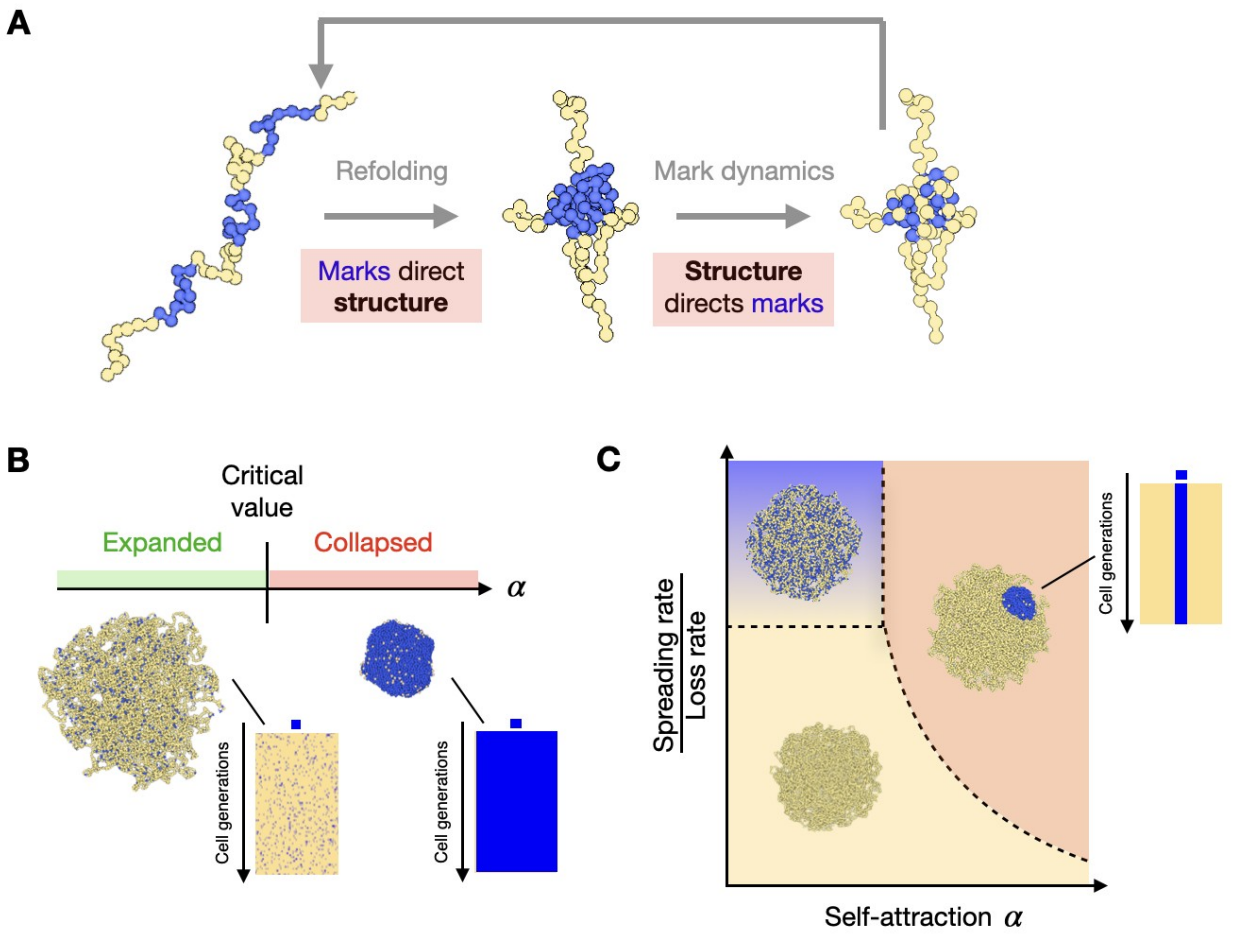

Figure 2. Bidirectional coupling. (A) Chromatin marks (blue) may influence 3D folding of the genome due to direct or mediated self-attraction of marked regions. Conversely, if reader-writer enzymes can "spread" marks in 3D, then 3D structure will influence mark patterns. This bidirectional coupling between marks and structure might help restore mark patterns after replicative dilution, or other perturbations. (B) Many models of bidirectional coupling exhibit an all-or-none tendency where, as a parameter is varied, an expanded state suddenly transitions to a collapsed state where everything is marked the same way. (C) Enzyme limitation eliminates this tendency, yielding a richer "phase diagram" that includes a regime (orange) in which the initial mark pattern can be "remembered" for a long time.

Our own recent model of bidirectional coupling [58] builds on these works by adding a very physiologically plausible element: reader-writer enzymes being limited in abundance relative to their histone substrates. Intuitively, enzyme limitation limits the total rate at which marks can be created in the nucleus. Together with the length of the cell cycle (which controls the rate of loss of marks by replicative dilution), this sets a limit on the total number of marks, which prevents uncontrolled spreading of marks across the whole genome. We find that this then dramatically stabilizes alternative *patterns* of marks, enabling epigenetic memory to remain stable over hundreds of cell divisions (Figure 2C).

Notably, our model exhibits fair resistance to noise, even though there is no direct cooperativity in the mark dynamics. Additionally, there is just one type of mark, so there is no indirect cooperativity of the sort Dodd et al. considered, arising from successive modifications. However, we believe that self-attraction of marks can itself give rise to cooperativity. To see this, note that if marks spread in 3D, the marking rate at a site is proportional to the number of neighbors of that site multiplied by the probability that any neighbor is marked. But if marks self-attract, the number of neighbors (reflecting the local density of chromatin) can

itself depend on the number of marks, creating a nonlinear dependence of the marking rate on the number of marks.

Concurrent work by Murphy et al. [49] found similarly that limited enzyme dramatically stabilizes 3D epigenetic memory. Additionally, they showed that adding successive modification states reduces the need for strong self-attraction—consistent with the idea that these are two different ways to achieve cooperativity which can trade off one another.

Of course, no memory lasts forever. In our model, we find a tendency, akin to that found in other phase-separating systems, for marks to slowly redistribute from small domains to big ones. The physics of this mark redistribution is still under investigation, but it appears that noncontiguous domains coexist for a time proportional to their size. It is notable that this scaling is much less favorable than the exponential scaling of Dodd et al.'s model, suggesting that self-organized partitioning demands larger mark domains to achieve stable memory.

Experiments and implications

We are increasingly able to precisely manipulate chromatin *in vivo*—targeting marks to particular genomic loci [59], or artificially inducing chromatin condensation [60]—and to visualize it at the scale of tens to hundreds of nucleosomes [61]. These technologies allow us to probe chromatin organization at the scales that theoretical models suggest may be most relevant for epigenetic memory.

Superresolution microscopy enables chromatin tracing in single cells, providing direct measurements of cell-to-cell heterogeneity in chromatin compaction at individual loci, which can be directly compared to model predictions [49]. Fujimori et al. [62] applied these methods to show that transient recruitment of an epigenetic "writer" to a locus induces chromatin compaction, and that long-term epigenetic memory formation is associated with this compaction extending over a large region of tens of kilobases—corresponding to 50 to 100 nucleosomes. Reversible silencing occurred without compaction, suggesting a causal role for compaction in memory maintenance.

Striking recent observations of transcriptionally active Y loops in *Drosophila* spermatocytes revealed marks of both activity and repression, forming clusters of about 50 nucleosomes from which the opposing mark was excluded [63]. These structures are remarkably evocative of the picture suggested by theoretical works reviewed earlier, in which epigenetic "bits" are formed by cooperative, 3D interactions between many nucleosomes, providing stability against noise.

At a much broader scale, although the effect of marks on 3D genome organization is well-established, the converse—the role of 3D compartmentalization in the maintenance of histone mark patterns—remains to be examined experimentally. This could be tested by perturbing genome organization and then monitoring changes in marks over cell generations. Global disruption of heterochromatic condensates (e.g. by HP1 depletion [64] or deacetylase inhibition) has not to date been successful in eliminating compartmentalization, but more localized perturbations may be possible. For example, it may be possible to tether a heterochromatic region away from the rest of heterochromatin (e.g. by anchoring it on euchromatic nuclear bodies such as speckles) or by immersing a region without heterochromatic marks into heterochromatin (e.g. by tethering to the nucleolus).

Another aspect of 3D organization whose role in epigenetic memory has not been explored is the anchoring of heterochromatin to the nuclear lamina. In progeria, a mutation in a constituent of the nuclear lamina (lamin A) leads to a dramatic "accelerating aging" phenotype and loss of peripheral

heterochromatin, through a mechanism that remains poorly understood [65]. A number of computational works have investigated how attraction to the lamina affects gross nuclear organization [40,66], but never in the context of a model with labile marks, bidirectionally coupled to structure. While not by itself essential for phase separation [40,67], attraction to the lamina may contribute to memory by reducing the mobility of chromatin during interphase [68]. We found that such mobility, happening simultaneously with mark dynamics, can deteriorate memory [58].

Yet another mystery is the role of loop extrusion in establishing and maintaining epigenetic marks. Loop extrusion by SMC complexes, such as cohesin, creates regions of enhanced 3D contact which are partly isolated from each other—the so-called topologically associating domains (TADs). The TADs are delineated by barriers to extrusion, such as CTCF proteins bound to their DNA sites. If 3D mark spreading were mediated by extrusion, such barriers could serve as potent insulators of spreading. Several distinct molecular mechanisms could give rise to extrusion-mediated spreading of marks. First, a reader-writer enzyme could simply take advantage of local contacts formed by extrusion. Alternatively, the enzyme could colocalize with CTCF, allowing a more directed spreading of marks. Finally, the enzyme might "hitchhike" on the loop extruding complex itself [69]. Recent experiments hint that such mechanisms might be at work for at least some marks, such γH2AX, whose spreading around DNA double-strand breaks is cohesin-mediated [70] and CTCF-constrained [71].

Beyond memory

A striking aspect of chromatin modification systems that models have not yet really addressed is their incredible complexity. Every model we have discussed considers one or two chromatin marks, combined with one or another essentially simple restoration mechanism, to furnish some degree of epigenetic memory. But real chromatin is vastly more intricate. There are dozens of distinct histone modifications, which may be present combinatorially at different sites, and which are made and removed by numerous enzyme complexes, linked together by intricate feedbacks. What is this all for?

This complexity is particularly puzzling when we consider alternative mechanisms for cell memory. Memory can be held and inherited very stably using simple bistable gene circuits [72,73], with the memory encoded in transcription factor concentrations. Chromatin-based systems might provide for more expressive regulation, for example encoding gradations of the transcriptional activity [74] or allowing tunable epigenetic stability [49]. But the key point is that ultimately, cell type specification requires surprisingly little epigenetic information. To see this, note that *just 50 bits* would be enough to uniquely address each one of the ~10^14 cells in a human body (2^50 > 10^14). Why have elaborate chromatin-based systems evolved when simpler mechanisms would seem to suffice for cell memory?

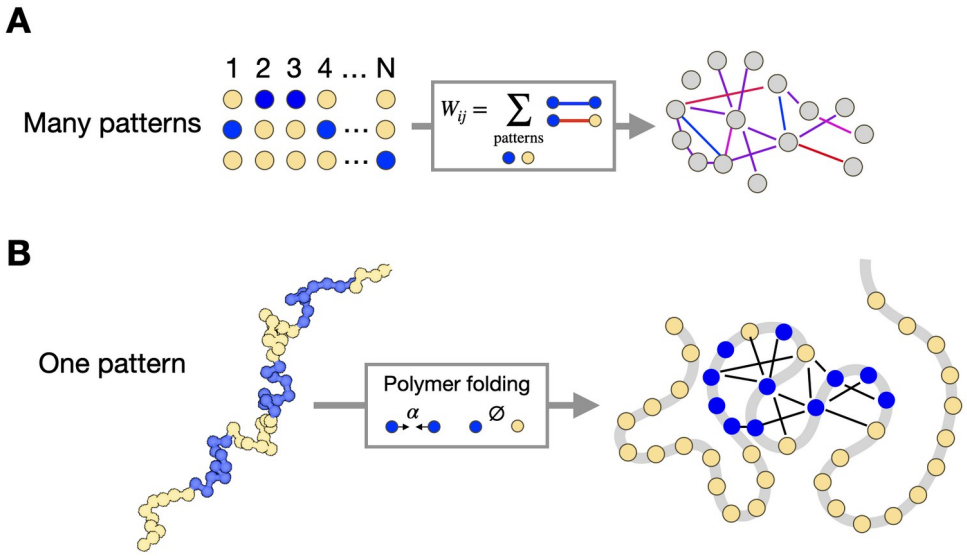

Figure 3. Learning by folding. (A) In a Hopfield network [75], multiple patterns of node activations are encoded simultaneously in the weights of connections, according to a learning rule that strengthens connections between nodes that are correlated across patterns. The network's dynamics then perform a kind of associative recall, reconstructing the stored pattern closest to an imposed initial state. (B) In models of bidirectional coupling, the folding of the chromatin polymer, directed by homotypic attraction of marks, creates 3D contacts that reflect the mark pattern. The dynamics of marks which spread in 3D can then recover the mark pattern even when it is partly erased.

One possibility is that these chromatin systems are in fact performing a much more complex function than memory. Perhaps they are better thought of as *processing* information, rather than merely storing it. For example, might they provide an *associative* memory—a system that can recall a memorized pattern from a noisy or partial presentation of it?

To see how this could work, it is instructive to compare mark-directed chromatin folding with how information is stored in Hopfield's classic model of associative memory [75]. The Hopfield model "learns" patterns by strengthening certain connections between nodes in a network (Figure 3A). These connections then determine the attractor states of the network's dynamics. Analogously, chromatin folding directed by homotypic attraction between marked regions can be viewed as "learning" a mark pattern by encoding it in a 3D conformation in the form of contacts ("connections") between marked elements (Figure 3B). The 3D contacts formed then influence the dynamics of marks spreading in 3D, allowing approximate reconstruction of the learned pattern even when it is partly erased. In this analogy, contacts between marked regions play the role of the strengthened connections in a Hopfield network.

But in a Hopfield network, *many* alternative patterns can be stored simultaneously in the connections, as if in superposition. Starting from an initial state, the network's dynamics can then be viewed as performing *pattern recognition*, reconstructing the closest of the stored patterns—a simple yet powerful form of information processing. Might chromatin do the same? Could differentiating cells "learn" not just a single epigenetic state, but a whole repertoire of cell type-specific possible states, which individual cells choose from dynamically, depending on the external stimuli they receive?

With a single mark, the system is perhaps too degenerate to store multiple patterns. In particular, the polymer's unceasing motion means that the "learning" never stops, and the folding would tend to readjust to match whatever mark pattern is currently present. But a system with many distinct mark types could lift this degeneracy: some marks might direct folding, encoding information in structure, while others mediate dynamic retrieval, or different combinations of marks could represent distinct stored patterns.

Exciting recent work by Murugan, Winfree and colleagues has shown that many-component biomolecular condensates with engineered interactions [76,77] can implement a Hopfield-like associative memory, proving this kind of biochemical computation is possible in principle. Chromatin strikes us as offering at least as rich a palette.

Another key distinction between chromatin memory and a Hopfield network is that chromatin refolds at each cell division, effectively re-training by erasing old and establishing new "connections." The pattern learned in each cycle reflects the mark dynamics ("recall") of the previous one. This process carries the risk of gradual memory deterioration—an aspect discussed above—but also enables adaptation: new stimuli can modify mark patterns, and these changes are immediately memorized. Such capacity to learn in changing environments could be analogous to continual or online machine learning.

Of course, whether any such computational potential is actually realized in chromatin, or whether chromatin's complexity serves entirely different functions (or no function [78]), remains an open question —one that will require marshaling future experimental and theoretical advances to fully resolve.

[Endnote: A restoration process with specific activity a factor S greater than its nonspecific activity, operating on individual marks, gives rise to unrecoverable errors within ~ S / (1 + log(S)) cell divisions. To see this, suppose a methyltransferase effects post-replicative restoration of a hemimethylated CpG site at rate k, with a corresponding off-target *de novo* methylation rate of k / S. Then, in a cell cycle of length T, the probability of "success" – i.e., successful restoration at one site without ectopic methylation at another site is given by (1 - exp(- k T)) * exp(- k T / S). Maximizing this while holding S constant gives the best possible success probability, which is S (1+S)^(-(1+S)/S), from which the claimed result follows approximately when S is large.]

Funding: JAO is supported by a Damon Runyon Quantitative Biology Fellowship (DRQ-19-24). LM is supported by NSF (MCB2044895 and NSF-ANR 2210558) and is a Simons Investigator (Simons Foundation International).

Annotated references

1. Cavalli G, Paro R: **The Drosophila Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis**. *Cell* 1998, **93**:505–518.

2. Grewal SI, Klar AJ: **Chromosomal inheritance of epigenetic states in fission yeast during mitosis and meiosis**. *Cell* 1996, **86**:95–101.

3. Berry S, Hartley M, Olsson TSG, Dean C, Howard M: **Local chromatin environment of a Polycomb target gene instructs its own epigenetic inheritance**. *Elife* 2015, **4**:e07205.

4. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ: **Crystal structure of the nucleosome core particle at 2.8 Å resolution**. *Nature* 1997, **389**:251–260.

5. Allis CD, Jenuwein T: **The molecular hallmarks of epigenetic control**. *Nat Rev Genet* 2016, **17**:487–500.

6. Wong LH, Tremethick DJ: **Multifunctional histone variants in genome function**. *Nat Rev Genet* 2025, **26**:82–104.

7. Allfrey VG, Faulkner R, Mirsky AE: **Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis**. *Proc Natl Acad Sci U S A* 1964, **51**:786–794.

8. Jenuwein T, Allis CD: **Translating the histone code**. *Science* 2001, **293**:1074–1080.

9. Bonora G, Disteche CM: **Structural aspects of the inactive X chromosome**. *Philos Trans R Soc Lond B Biol Sci* 2017, **372**.

10. Boettiger AN, Bintu B, Moffitt JR, Wang S, Beliveau BJ, Fudenberg G, Imakaev M, Mirny LA, Wu C-T, Zhuang X: **Super-resolution imaging reveals distinct chromatin folding for different epigenetic states**. *Nature* 2016, **529**:418–422.

11. Andronov L, Ouararhni K, Stoll I, Klaholz BP, Hamiche A: **CENP-A nucleosome clusters form rosette-like structures around HJURP during G1**. *Nat Commun* 2019, **10**:4436.

12. Zhu B, Reinberg D: **Epigenetic inheritance: uncontested?** *Cell Res* 2011, **21**:435–441.

13. Espinosa-Martínez M, Alcázar-Fabra M, Landeira D: **The molecular basis of cell memory in mammals: The epigenetic cycle**. *Sci Adv* 2024, **10**:eadl3188.

14. Adam S, Klingel V, Radde NE, Bashtrykov P, Jeltsch A: **On the accuracy of the epigenetic copy machine: comprehensive specificity analysis of the DNMT1 DNA methyltransferase**. *Nucleic Acids Res* 2023, **51**:6622–6633.

15. Poepsel S, Kasinath V, Nogales E: **Cryo-EM structures of PRC2 simultaneously engaged with two functionally distinct nucleosomes**. *Nat Struct Mol Biol* 2018, **25**:154–162.

16. Flury V, Groth A: **Safeguarding the epigenome through the cell cycle: a multitasking game**. *Curr Opin Genet Dev* 2024, **85**:102161.

17. Petryk N, Dalby M, Wenger A, Stromme CB, Strandsby A, Andersson R, Groth A: **MCM2 promotes symmetric inheritance of modified histones during DNA replication**. *Science* 2018, **361**:1389–1392.

18. Flury V, Reverón-Gómez N, Alcaraz N, Stewart-Morgan KR, Wenger A, Klose RJ, Groth A: **Recycling of modified H2A-H2B provides short-term memory of chromatin states**. *Cell* 2023, **186**:1050–1065.e19.

19. Xu M, Long C, Chen X, Huang C, Chen S, Zhu B: **Partitioning of histone H3-H4 tetramers during DNA replication-dependent chromatin assembly**. *Science* 2010, **328**:94–98.

20. Margueron R, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ III, Voigt P, Martin SR, Taylor WR, De Marco V, et al.: **Role of the polycomb protein EED in the propagation of repressive histone marks**. *Nature* 2009, **461**:762–767.

21. Müller MM, Fierz B, Bittova L, Liszczak G, Muir TW: **A two-state activation mechanism controls the histone methyltransferase Suv39h1**. *Nat Chem Biol* 2016, **12**:188–193.

22. Ciapponi M, Karlukova E, Schkölziger S, Benda C, Müller J: **Structural basis of the histone ubiquitination read-write mechanism of RYBP-PRC1**. *Nat Struct Mol Biol* 2024, **31**:1023–1027.

23. Hodges C, Crabtree GR: **Dynamics of inherently bounded histone modification domains**. *Proceedings of the National Academy of Sciences* 2012, **109**:13296–13301.

24. Hathaway NA, Bell O, Hodges C, Miller EL, Neel DS, Crabtree GR: **Dynamics and memory of heterochromatin in living cells**. *Cell* 2012, **149**:1447–1460.

25. Nickels JF, Sneppen K: **Confinement mechanisms for epigenetic modifications of nucleosomes**. *PRX Life* 2023, **1**.

* This paper systematically examines ways to achieve bistability in a localized region using histone

modification dynamics. Factors explored include various patterns of positive or negative feedbacks, the role of multiple marks, and the effects of long-range spreading, modeled using a distance-dependent interaction probability scaling.

26. Ge EJ, Jani KS, Diehl KL, Müller MM, Muir TW: **Nucleation and propagation of heterochromatin by the histone methyltransferase PRC2: Geometric constraints and impact of the regulatory subunit JARID2**. *J Am Chem Soc* 2019, **141**:15029–15039.

27. Dodd IB, Micheelsen MA, Sneppen K, Thon G: **Theoretical analysis of epigenetic cell memory by nucleosome modification**. *Cell* 2007, **129**:813–822.

28. Haerter JO, Lövkvist C, Dodd IB, Sneppen K: **Collaboration between CpG sites is needed for stable somatic inheritance of DNA methylation states**. *Nucleic Acids Res* 2014, **42**:2235–2244.

29. Cortini R, Barbi M, Caré BR, Lavelle C, Lesne A, Mozziconacci J, Victor J-M: **The physics of epigenetics**. *Rev Mod Phys* 2016, **88**.

30. Oksuz O, Narendra V, Lee C-H, Descostes N, LeRoy G, Raviram R, Blumenberg L, Karch K, Rocha PP, Garcia BA, et al.: **Capturing the onset of PRC2-mediated repressive domain formation**. *Mol Cell* 2018, **70**:1149–1162.e5.

31. Kraft K, Yost KE, Murphy SE, Magg A, Long Y, Corces MR, Granja JM, Wittler L, Mundlos S, Cech TR, et al.: **Polycomb-mediated genome architecture enables long-range spreading of H3K27 methylation**. *Proc Natl Acad Sci U S A* 2022, **119**:e2201883119.

32. Movilla Miangolarra A, Howard M: **Theory of epigenetic switching due to stochastic histone mark loss during DNA replication**. *Phys Biol* 2024, **22**:016005.

33. Warren PB, ten Wolde PR: **Chemical models of genetic toggle switches**. *J Phys Chem B* 2005, **109**:6812–6823.

34. Biancalani T, Assaf M: **Genetic toggle switch in the absence of cooperative binding: Exact results**. *Phys Rev Lett* 2015, **115**:208101.

35. Yang H, Berry S, Olsson TSG, Hartley M, Howard M, Dean C: **Distinct phases of Polycomb silencing to hold epigenetic memory of cold in Arabidopsis**. *Science* 2017, **357**:1142–1145.

36. Sedighi M, Sengupta AM: **Epigenetic chromatin silencing: bistability and front propagation**. *Phys Biol* 2007, **4**:246–255.

37. Dekker J, Mirny LA: **The chromosome folding problem and how cells solve it**. *Cell* 2024, **187**:6424–6450.

38. Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA: **Formation of chromosomal domains by loop extrusion**. *Cell Rep* 2016, **15**:2038–2049.

39. Spracklin G, Abdennur N, Imakaev M, Chowdhury N, Pradhan S, Mirny LA, Dekker J: **Diverse silent chromatin states modulate genome compartmentalization and loop extrusion barriers**. *Nat Struct Mol Biol* 2023, **30**:38–51.

* By detailed analysis of Hi-C experiments performed on strongly compartmentalized HCT116 colon cancer cells, this paper distinguishes six different kinds of chromatin, each exhibiting distinctive molecular features, and tendency for self-interaction.

40. Falk M, Feodorova Y, Naumova N, Imakaev M, Lajoie BR, Leonhardt H, Joffe B, Dekker J, Fudenberg G, Solovei I, et al.: **Heterochromatin drives compartmentalization of inverted and conventional nuclei**. *Nature* 2019, **570**:395–399.

41. Ghosh SK, Jost D: **How epigenome drives chromatin folding and dynamics, insights from efficient coarse-grained models of chromosomes**. *PLoS Comput Biol* 2018, **14**:e1006159.

42. Jost D, Carrivain P, Cavalli G, Vaillant C: **Modeling epigenome folding: formation and dynamics of topologically associated chromatin domains**. *Nucleic Acids Res* 2014, **42**:9553–9561.

43. Barbieri M, Chotalia M, Fraser J, Lavitas L-M, Dostie J, Pombo A, Nicodemi M: **Complexity of chromatin folding is captured by the strings and binders switch model**. *Proceedings of the National Academy of Sciences* 2012, **109**:16173–16178.

44. Gibson BA, Doolittle LK, Schneider MWG, Jensen LE, Gamarra N, Henry L, Gerlich DW, Redding S, Rosen MK: **Organization of chromatin by intrinsic and regulated phase separation**. *Cell* 2019, **179**:470–484.e21.

45. Strom AR, Emelyanov AV, Mir MR, Fyodorov DV, Darzacq XR, Karpen GH: **Phase separation drives heterochromatin domain formation**. *Biophys J* 2018, **114**:445a.

46. Michieletto D, Orlandini E, Marenduzzo D: **Polymer model with epigenetic recoloring reveals a pathway for the de novo establishment and 3D organization of chromatin domains**. *Physical Review X* 2016, **6**:041047.

47. Jost D, Vaillant C: **Epigenomics in 3D: importance of long-range spreading and specific interactions in epigenomic maintenance**. *Nucleic Acids Res* 2018, **46**:2252–2264.

48. Sandholtz SH, MacPherson Q, Spakowitz AJ: **Physical modeling of the heritability and maintenance of epigenetic modifications**. *Proceedings of the National Academy of Sciences* 2020, **117**:20423–20429.

49. Murphy SE, Boettiger AN: **Polycomb repression of Hox genes involves spatial feedback but not domain compaction or phase transition**. *Nat Genet* 2024, **56**:493–504.

** This paper uses single-cell chromatin tracing to show that a stably Polycomb-silenced locus (HoxA gene cluster) exhibits heterogeneous, frequently decompacted conformations. These observations were found to be consistent with a model of epigenetic memory based on bidirectional coupling, involving multiple marks (successive modification states), weak self-attraction between marks, and limited reader-writer enzymes that spread marks in 3D.

50. Colì D, Orlandini E, Michieletto D, Marenduzzo D: **Magnetic polymer models for epigenetics-driven chromosome folding**. *Phys Rev E* 2019, **100**:052410.

51. Sood A, Schuette G, Zhang B: **Dynamical phase transition in models that couple chromatin folding with histone modifications**. *Phys Rev E* 2024, **109**:054411.

52. Movilla Miangolarra A, Saxton DS, Yan Z, Rine J, Howard M: **Two-way feedback between chromatin compaction and histone modification state explains Saccharomyces cerevisiae heterochromatin bistability**. *Proc Natl Acad Sci U S A* 2024, **121**:e2403316121.

53. Garel T, Orland H, Orlandini E: **Phase diagram of magnetic polymers**. *arXiv [cond-mat.stat-mech]* 1999,

54. Adachi K, Kawaguchi K: **Chromatin state switching in a polymer model with mark-conformation coupling**. *arXiv [physics.bio-ph]* 2019,

55. Dormidontova EE, Grosberg AY, Khokhlov AR: **Intramolecular phase separation of a copolymer chain with mobile primary structure**. *Macromolecular Theory Simulations* 1992, **1**:375–385.

56. Mukherjee S, Skoruppa E, Merlitz H, Sommer J-U, Schiessel H: **A self-organised liquid reaction container for cellular memory**. *arXiv [physics.bio-ph]* 2024,

57. Katava M, Shi G, Thirumalai D: **Chromatin dynamics controls epigenetic domain formation**. *Biophys J* 2022, **121**:2895–2905.

58. Owen JA, Osmanović D, Mirny L: **Design principles of 3D epigenetic memory systems**. *Science* 2023, **382**:eadg3053.

** This paper develops a simple model of bidirectional coupling which reveals that 3D genome organization can stabilize epigenetic memory, as long as (1) there is a large density difference between chromatin compartments, (2) marks spread in 3D, and (3) reader-writer enzymes are limited relative to their substrates.

59. McCutcheon SR, Rohm D, Iglesias N, Gersbach CA: **Epigenome editing technologies for discovery and medicine**. *Nat Biotechnol* 2024, **42**:1199–1217.

60. Eeftens JM, Kapoor M, Michieletto D, Brangwynne CP: **Polycomb condensates can promote epigenetic marks but are not required for sustained chromatin compaction**. *Nat Commun* 2021, **12**:5888.

61. Mateo LJ, Sinnott-Armstrong N, Boettiger AN: **Tracing DNA paths and RNA profiles in cultured cells and tissues with ORCA**. *Nat Protoc* 2021, **16**:1647–1713.

62. Fujimori T, Rios-Martinez C, Thurm AR, Hinks MM, Doughty BR, Sinha J, Le D, Hafner A, Greenleaf WJ, Boettiger AN, et al.: **Single-cell chromatin state transitions during epigenetic memory formation**. *bioRxivorg* 2023, doi:10.1101/2023.10.03.560616.

** This paper combines transient recruitment of repressors to a locus with single-cell chromatin tracing to disentangle the relationship between chromatin compaction and epigenetic memory. The authors find that induced compaction, over tens of kilobases, is predictive of the stability of silencing, even though it is not required for silencing itself. This supports a causal role for 3D structure in memory.

63. Koestler SA, Ball ML, Muresan L, Dinakaran V, White R: **Transcriptionally active chromatin loops contain both "active" and "inactive" histone modifications that exhibit exclusivity at the level of nucleosome clusters**. *Epigenetics Chromatin* 2024, **17**:8.

* This study uses superresolution microscopy to reveal striking epigenetically-correlated spatial organization in individual chromatin fibers in *Drosophila*.

64. Zenk F, Zhan Y, Kos P, Löser E, Atinbayeva N, Schächtle M, Tiana G, Giorgetti L, Iovino N: **HP1 drives de novo 3D genome reorganization in early Drosophila embryos**. *Nature* 2021, **593**:289–293.

65. Dreesen O: **Towards delineating the chain of events that cause premature senescence in the accelerated aging syndrome Hutchinson-Gilford progeria (HGPS)**. *Biochem Soc Trans* 2020, **48**:981–991.

66. Chiang M, Michieletto D, Brackley CA, Rattanavirotkul N, Mohammed H, Marenduzzo D, Chandra T: **Polymer modeling predicts chromosome reorganization in senescence**. *Cell Rep* 2019, **28**:3212–3223.e6.

67. Solovei I, Kreysing M, Lanctôt C, Kösem S, Peichl L, Cremer T, Guck J, Joffe B: **Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution**. *Cell* 2009, **137**:356–368.

68. Bronshtein I, Kepten E, Kanter I, Berezin S, Lindner M, Redwood AB, Mai S, Gonzalo S, Foisner R, Shav-Tal Y, et al.: **Loss of lamin A function increases chromatin dynamics in the nuclear interior**. *Nat Commun* 2015, **6**:8044.

69. Tang W, Costantino L, Stocsits R, Wutz G, Ladurner R, Hudecz O, Mechtler K, Peters J-M: **Cohesin positions the epigenetic reader Phf2 within the genome**. *EMBO J* 2025, **44**:736–766.

70. Teloni F, Takacs Z, Mitter M, Langer CCH, Prlesi I, Steinacker TL, Reuter VP, Mylarshchikov D, Gerlich DW: **Cohesin guides homology search during DNA repair via loops and sister chromatid linkages**. *bioRxiv* 2025, doi:10.1101/2025.02.10.637359.

71. Collins PL, Purman C, Porter SI, Nganga V, Saini A, Hayer KE, Gurewitz GL, Sleckman BP, Bednarski JJ, Bassing CH, et al.: **DNA double-strand breaks induce H2Ax phosphorylation domains in a contact-dependent manner**. *Nat Commun* 2020, **11**:3158.

72. Gardner TS, Cantor CR, Collins JJ: **Construction of a genetic toggle switch in Escherichia coli**. *Nature* 2000, **403**:339–342.

73. Zhu R, del Rio-Salgado JM, Garcia-Ojalvo J, Elowitz MB: **Synthetic multistability in mammalian cells**. *Science* 2021, **375**:eabg9765.

74. Palacios S, Bruno S, Weiss R, Salibi E, Kane A, Ilia K, Del Vecchio D: **Analog epigenetic memory revealed by targeted chromatin editing**. *bioRxiv* 2024, doi:10.1101/2024.02.13.580200.

75. Hopfield JJ: **Neural networks and physical systems with emergent collective computational abilities**. *Proc Natl Acad Sci U S A* 1982, **79**:2554–2558.

76. Chalk C, Buse S, Shrinivas K, Murugan A, Winfree E: **Learning and inference in a lattice model of multicomponent condensates**. 2024, doi:10.4230/LIPICS.DNA.30.5.

    * This computational work shows how multicomponent liquid condensates with appropriately chosen interactions can implement a Hopfield-like associative memory.

77. Evans CG, O'Brien J, Winfree E, Murugan A: **Pattern recognition in the nucleation kinetics of non-equilibrium self-assembly**. *Nature* 2024, **625**:500–507.

    ** This paper shows experimentally that a designed, self-assembling set of DNA tiles can perform a kind of pattern recognition task—selectively assembling one or another higher-order structure, depending on initial concentrations. This frontier demonstration of the computational abilities of biomolecular systems illustrates the broad scope of what complex systems of chromatin modification might, in principle, be capable of.

78. Solovei I, Mirny L: **Spandrels of the cell nucleus**. *Curr Opin Cell Biol* 2024, **90**:102421.