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# 4D Epigenomics: deciphering the coupling between genome folding and epigenomic regulation with biophysical modeling

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## Abstract

Recent experimental observations suggest a strong coupling between the 3D nuclear chromosome organization and epigenomics. However, the mechanistic and functional bases of such interplay remain elusive. In this review, we describe how biophysical modeling has been instrumental in characterizing how genome folding may impact the formation of epigenomic domains and, conversely, how epigenomic marks may affect chromosome conformation. Finally, we discuss how this mutual feedback loop between chromatin organization and epigenome regulation, via the formation of physico-chemical nano-reactors, may represent a key functional role of 3D compartmentalization in the assembly and maintenance of stable - but yet plastic - epigenomic landscapes.

# Introduction

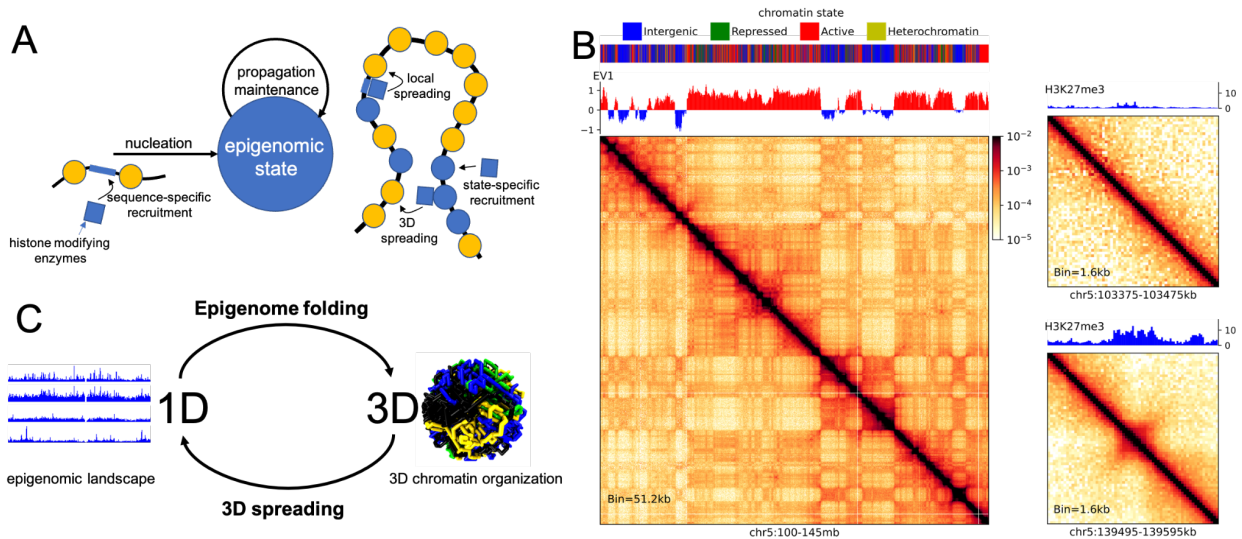


Figure 1: **Epigenome-3D Genome coupling.** (A) General rules of epigenome assembly and regulation: initiation by sequence-specific factors and propagation and maintenance by reader/writer histone modifying enzymes that may spread a mark in 3D. Inspired by [1,2]. (B) Hi-C map of chromosome 5 in mouse ES cells (data from [3] visualized with cooler [4]). On the top, we plot the A/B compartments (EV1) and also the chromatin states inferred from many epigenomic Chip-Seq profiles [5], that for simplicity we clustered into 4 meta-classes: intergenic, (Polycomb)-repressed, active and (constitutive) heterochromatin. A-compartment is enriched in active and repressed states while B-compartment in intergenic and heterochromatin states. On the right, we selected two regions around Polycomb-target genes: one embedded in a highly compact environment (bottom) exhibiting extended strong H3K27me3 profiles, one in a less compact environment (top) having a weaker profile. (C) Correlation between the 1D epigenome and the 3D chromatin organization may arise from the impact of genome folding on epigenomic regulation via the 3D spreading of histone marks coupled to the impact of the epigenomic landscape on genome folding via epigenetic-dependent interactions mediated by architectural proteins.

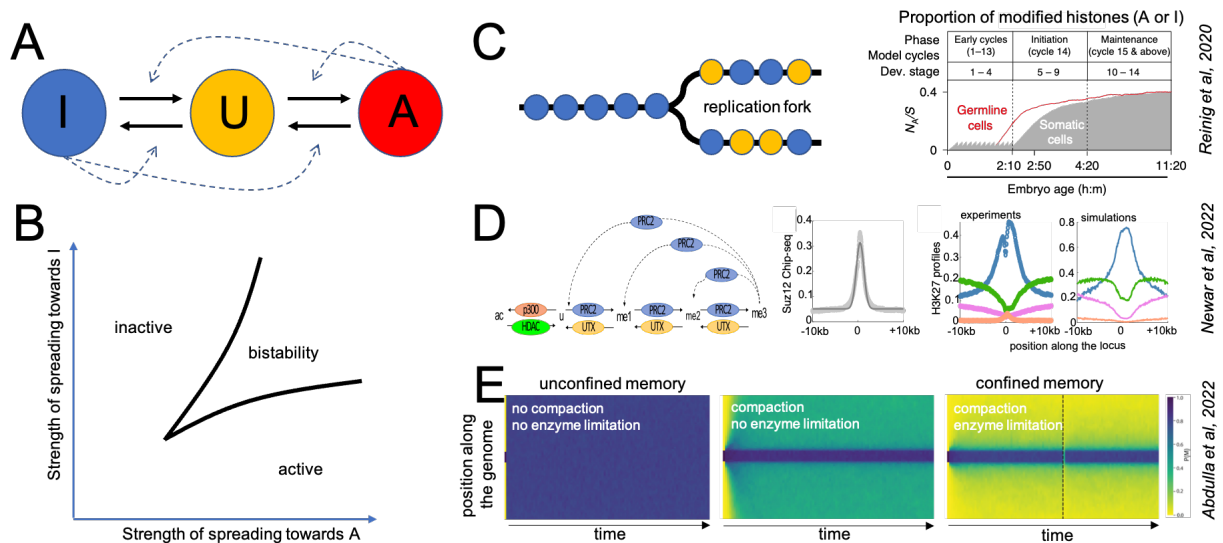
Genome activity (e.g., transcription, replication) is in part regulated at the chromatin level via the controlled deposition of biochemical modifications of DNA or histone tails, the so-called epigenomic or epigenetic marks. These modifications may accumulate along the genome to form contiguous epigenomic domains of various size and composition. The assembly of these regions, either active/early-replicating or inactive/late-replicating, are believed to be conducted by generic principles involving the combined action of various chromatin regulators [6–8] (Fig.1A). Indeed, the *de novo* establishment of epigenomic states proceeds by sequence-specific recruitment of histone modifying enzymes (HMEs). Upon initiation, modifications may then propagate locally around the initiation sites. The “reader-writer” capacity of some HMEs to be also recruited (“reader”) by the epigenomic mark they catalyze (“writer”) results in a cooperative positive feedback loop that enables the further spreading of the mark, thus leading to the formation of chromatin domains that can be stably retained across multiple cell divisions — even in the absence of any external stimuli. However, the detailed spreading mechanisms underlying the long-range propagation of epigenetic marks far from the HME recruitment sites, along with the molecular processes driving the maintenance of a stable epigenomic memory, remain unclear.

In the last 15 years, several experimental evidences suggested that the 3D chromosome organization may be a central player in the regulation of the epigenome. Indeed, the spatial compartmentalization of the genome into 3D structures, like the so-called A/B compartments and topologically-associated domains (TADs) observed in Hi-C experiments (Fig.1B), or heterochromatic chromocenters, Polycomb foci and transcription factories visible by microscopy, are strongly associated with epigenomics [9–12]. In particular, the formation of these 3D patterns is often thought to be driven by architectural proteins (e.g., HP1, PRC1) that specifically bind to given epigenetic marks [13–17]. Loci carrying the same epigenetic marks often

tend to be colocalized (more contacts than expected) and spatially segregated (less contacts than expected) from regions having a different epigenomic content (Fig.1B) [9]. Interestingly, genomic regions localized inside compact 3D domains (e.g., a TAD with a high number of self-contacts) exhibit epigenetic profiles that are more extended than loci inside weakly compacted regions (Fig.1B right) [18]. All this suggests that there exists a coupling between the 3D chromosome organization and the epigenome regulation (Fig.1C): (i) genome folding may impact the formation of epigenomic domains along the genome as it can bring in close spatial proximity two distant loci and favor the 3D, long-range spreading of an epigenomic mark; and (ii) a given epigenomic mark may impact genome folding via the recruitment of architectural proteins. This potential feedback loop between epigenomics and 3D genome strengthens the hypothesis that 3D genome folding is key to chromatin states assembly and maintenance.

Testing this hypothesis experimentally remains very challenging as it would involve the combined perturbations of many factors that often have several functions. Therefore, over the years, biophysical modeling has played an important role to infer the generic mechanistic rules of epigenome regulation, in particular its relation to chromosome organization. In the following, we review the various theoretical approaches that have been developed to study such an interplay between epigenomics and the 3D genome.

## Regulation of the epigenome



**Figure 2: Epigenome regulation.** (A) Example of chromatin state transitions between inactive (I), unmodified (U) and active (A) states. A modified nucleosome (A or I) may influence locally or at long-range the transition rates of other nucleosomes (dashed arrows). (B) Stability diagram of the model shown in (A). Depending on the strength of positive feedbacks towards A or I, the epigenomic state can be monostable (active, inactive or mixed) or bistable. Inspired by [19]. (C) (Left) Random repartition of the ‘old’ maternal nucleosomes among the two sister chromatids, empty positions being filled with ‘new’ unmodified nucleosomes. (Right) Progressive increase of the proportion of modified histones as cell cycle duration increases during development. Extracted from [20]. (D) (Left) Scheme of the regulation of H3K27 modifications around Polycomb-target genes in mouse ES cells. (Center) Average sequence-specific PRC2 binding profile around TSS. (Left) Predicted profiles of H3K27 marks driven by the long-range spreading from PRC2 binding sites and comparison with experimental ones. Adapted from [18]. (E) Enzyme limitation and 3D compaction. Evolution of the probability to be in a modified state as a function of time: (Left) Without enzyme limitation and compaction, strong positive feedback leads to unconfined epigenetic memory. (Center) With only 3D compaction, memory is better confined but exhibits significant ‘flooding’ outside the compacted region. (Right) With enzyme limitation and compaction, an epigenetic signal can be maintained and confined in the compacted region over many cell generations. Adapted from [2].

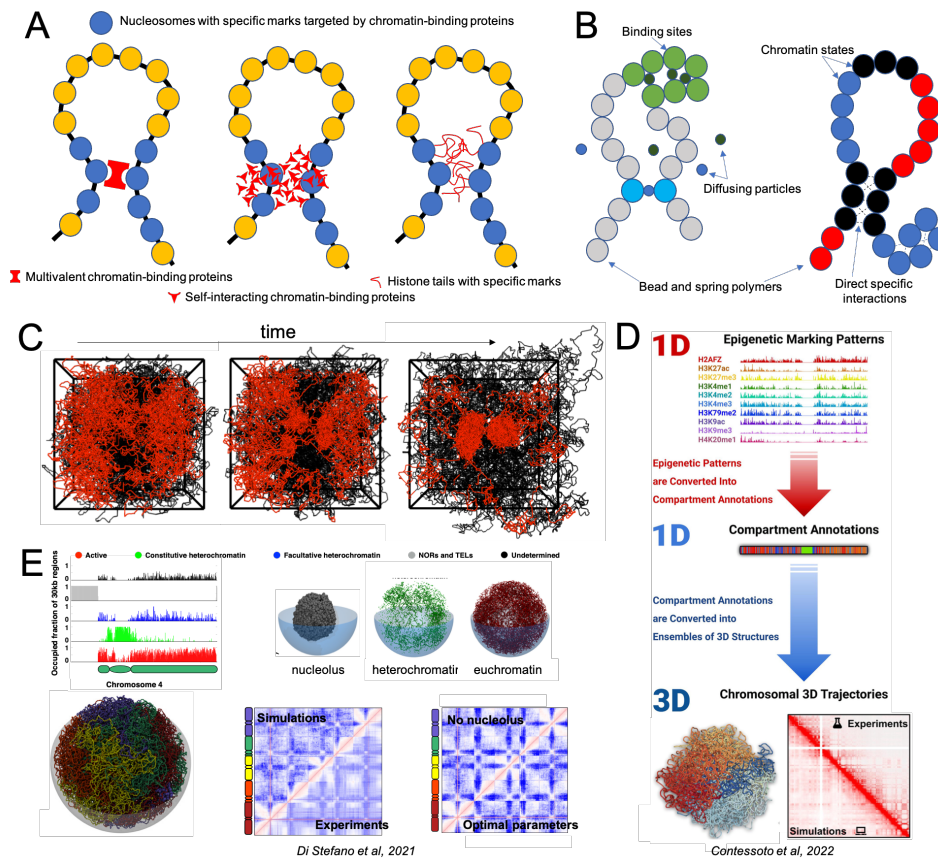
Epigenomic marks, like histone modifications, are only partially transmitted through replication. The maintenance of a stable cellular identity thus necessitates regulatory mechanisms that sustain a memory of the chromatin landscape. The first theoretical models that investigate the maintenance of a stable epigenetic memory were developed 15 years ago in the context of gene silencing in yeasts [21,22]. These seminal works as well as their following theoretical improvements [2,19,23–30] and applications to other systems [18,20,31–34] rely on very similar generic rules that integrate the key molecular processes involved in the regulation of histone modifications (Fig.2A): (1) Chromatin is modeled as an array of nucleosomes whose local chromatin state can fluctuate between different flavors (e.g., histone modifications like H3K27ac/me1/me2/me3). (2) The stochastic switching between these states is controlled by several contributions: (i) “random” conversions accounting for leaky activities of HMEs as well as histone turnover; (ii) “recruited” conversions accounting for the “reader-writer/eraser” ability of some HMEs (e.g., PRC2) to be recruited by specific histone marks (e.g., H3K27me3) and to catalyze the addition of the same mark or the removal of an antagonistic mark (e.g., H3K27ac) on other proximal nucleosomes.

In this framework, epigenetic memory and the maintenance of stable (e.g., active or repressed), extended chromatin domains, arises from the reader-writer capacity of HMEs to spread a mark at “long-range” along the genome, i.e. distant (from few kbps to Mbps away) from the locus where they have been recruited to [21]. Indeed, after a local perturbation of the epigenetic landscape (e.g. during transcription where histone turnover is increased [26,31] or replication where histone modifications are diluted among the two sister chromatids [20,35], Fig.2C left), the large reservoir of local and distant - still modified - nucleosomes [33] may serve as a template to allow a full recovery of the initial state at efficient-enough cooperative recruitment of long-range-spreading HMEs. For example, such a model suggests that epigenetic memory may be strongly challenged by cell cycle duration (Fig.2C right), fast cycles (i.e., faster than the typical spreading rates) not allowing robust maintenance and thus the emergence of stable states [35]. For example, the gradual establishment of Polycomb repression via the deposition of H3K27me3 marks by PRC2 (at a typical rate  $\sim 1h^{-1}$ , [17]) in early fly embryogenesis is concomitant with the increase in cell cycle duration from a few minutes to hours at the end of the maternal-to-zygotic transition [20]. In fact, the long-range spreading capacity emerges naturally from the polymeric nature of the chromatin fiber (Fig.1A): a HME bound at a given position may be in spatial proximity with another nucleosome which is distant along the genome, thanks to 3D chromatin looping [36]. This key contribution is introduced via spreading terms in the recruited conversion rate that depends on the contact probability between distant loci [2,21,25], as measured by Hi-C. In this case, the more compact the region, the more efficient the long-range-spreading and the easier the maintenance of stable epigenetic memory [2]. Interestingly, as suggested by several quantitative studies, the long-range recruited conversions might be only a feature of repressive states, active marks rather contributing only locally with weak or no reader-writer processes [26,29,33,34,37]. More generally, any processes that favor the “communication” between distant loci may contribute to epigenomic stability [2]. For example, SMC-mediated loop extrusion, a major mechanism orchestrating genome folding and TAD formation [38–40], was proposed to participate in the spreading of H2AX phosphorylation around ( $\sim 2$ Mbp) double strand breaks via the formation of transient loops between the ATM kinase bound at the break site and distant loci [41].

Historically, theoretical models of epigenetic regulation focused on characterizing how a stable epigenetic state can sustain itself solely via the reader-writer mechanism. However, the *de novo* assembly and very long-term maintenance of chromatin state were suggested to actually rely on the sequence-dependent recruitment of HMEs at specific genomic sites, thanks to their co-association with DNA-binding proteins or via RNAi-based pathways [6,42–44]. Recently, the formalization of such an interplay between sequence-specific and state-specific (reader-writer) recruited conversions provided quantitative frameworks that can successfully capture the experimental profiles of histone marks around nucleation sites [2,18,25] (Fig.2D).

These models suggest that, *in vivo*, the positive feedback may not be sufficient to maintain a stable epigenome but may require a constant sequence-dependent signaling [2,18,42]. Importantly, chromatin states are often confined within domains of finite sizes delineated by boundary elements (often termed “barriers” or “insulators”) [45][24]. However, such stable confinement is hardly compatible with standard models with long-range positive feedback that predict only stable, unconfined states [2,24,25]. Unless, the spreading of the state outside the region is limited by non-homogeneous recruited conversions [25] via, for example, a strong 3D compaction of the region coupled to a titrated number of HMEs [2] (Fig.2E).

## Compartmentalization of the epigenome



**Figure 3: Epigenome folding.** (A) Three possible mechanisms of epigenomic-driven interactions mediated by chromatin-binding, architectural proteins (left, center) or histone tails (right). Inspired by [46]. (B) Two different kinds of polymer modeling: string-and-binders-switch-like (left) and copolymer-like (right) models. (C) Formation kinetics of a micro-phased configuration for heterochromatin (red monomers) simulated using a copolymer model starting from a random initial configuration. (D) Typical workflow used to model a given system: from the different epigenomic profiles (input) to a predicted Hi-C map (output). Adapted from [47]. (E) Illustration of such a workflow on the genome-wide modeling of the 3D chromosome organization in *Arabidopsis thaliana* accounting for nucleolus formation. Adapted from [48].

Many 3D structural elements of the genome like A/B compartments or TADs are correlated with the 1D epigenomic information along the sequence (Fig.1B). There exists a growing body of evidence showing that some chromatin-binding architectural proteins or complexes, specific to particular epigenomic marks, have the capacity (Fig.3A) to bind to several chromatin regions and/or to oligomerize or self-interact. Thus, such molecules may drive direct or effective physical interactions (or loops) between loci sharing the same chromatin state, possibly distant along the sequence. This has been observed for both euchromatin-associated factors [15,49], like BRD4 that bind to acetylated histones at active super-enhancers [50] and



form small liquid droplets ( $\varnothing \sim 10^{1-2}$  nm) [15], and heterochromatin-associated factors [12–14,16,17], like HP1 that bind to H3K9me2/3 histones at pericentromeric regions and may dimerize and assemble into large condensates ( $\varnothing \sim 10^{2-3}$  nm) [12–14], chemically excluded from BRD4 bodies [15]. There are also recent experiments showing that histone tails themselves may directly promote interactions between nucleosomes depending on the biochemical modifications they carry on [17,51,52].

All this suggests the existence of physico-chemical mechanisms, regulated by the epigenome, that significantly participate in structuring the 3D genome either by stabilizing short-range interactions between genomic loci or by hindering them. To describe how such epigenomic-driven interactions may shape the 3D genome, several mechanistic biophysical models have been developed along the years [53]. They usually model chromatin as a coarse-grain, bead-on-spring, polymer (Fig.3B) where each monomer represents a given portion of chromatin (typically between 1 to 50kbp, depending on the model resolution). The spatio-temporal dynamics of the polymer is then driven by direct or effective interactions between monomers.

In the first class of models, sometimes termed string-and-binders-switch-like models (Fig.3B, left), the polymeric chain (one chromosome or a piece of chromosome) is simulated along with diffusing particles of several kinds. They represent architectural proteins that can bind to specific monomers whose corresponding genomic regions are enriched in some epigenomic marks or TF binding sites [54–59]. In these frameworks, folding of the chain is driven by the capacity of the diffusing particles to bind several chromatin monomers at the same time, but possible self-interactions between diffusing particles were mostly not considered so far, except in very recent theoretical works that highlight their importance in driving genome folding [60,61]. In the second class of models, sometimes termed copolymer-like models (Fig.3B, right), chromatin monomers directly interact via short-range, pairwise potentials whose strengths of interaction depend on the local epigenomic content of each monomer [62–65]. In its simplest form, each monomer is defined by a single chromatin state (e.g., active, Polycomb/H3K27me3-associated, HP1/H3K9me2/3-associated), and only monomers of the same state can interact.

These two classes of models (and their variations) lead qualitatively to the same physical behavior. Since loci sharing the same epigenomic state are usually arranged in long blocks along the genome [9,66] (contiguous 10-100kbp-long regions with homogeneous epigenomic content, Fig.1B top), formation of TADs may emerge from the - full or partial - collapse of each block [54,67] and large-scale A/B compartmentalization from a microphase separation of the different chromatin states [62,65,68,69] (Fig.3C). In the 3D micro-phased organization [70], blocks of the same state colocalize forming more or less extended spatial domains depending on the strength of interaction and of the linear arrangement of the 1D blocks [71]. This generic principle of microphase separation, characteristics of block copolymer [70], has been contextualized to various biological systems. Taking specific 1D epigenomic landscapes as inputs, it generates predictions in very good agreement with Hi-C experiments (Fig.3D,E) and explain quantitatively the 3D compartmentalization observed in many species from *Drosophila* [68,72] to mammals [47,55,59,69,73] and plants [48]. In many cases, it is found that the interaction strengths that best fit the data are weak (fraction of  $k_B T$ ), consistent with a plastic, stochastic and dynamical chromatin organization [64,68,69,74–76].

As expected, epigenomic-driven interactions alone cannot explain all the main features of chromosome organization. Recently, to improve our quantitative understanding of the nuclear chromosome organization, copolymer models were decorated with other key mechanisms. For example, many modeling works investigated the interactions with key nuclear landmarks such as the lamina [65,77–80] or the nucleolus [48,69], highlighting their strong impact on the large-scale nuclear positioning of TADs and A/B compartments, even if having a minor contribution to the patterns observed in Hi-C maps (Fig.3E). Another

example is the work of Nuebler et al [81] that theoretically studied the interplay between epigenomic-driven (micro)phase separation and cohesin-mediated loop extrusion. Loop extrusion was shown to interfere with microphase separation via active mixing, in good agreement with experiments [82]: extruders' activity leading to TAD formation reduce A/B compartmentalization while epigenomic-driven interactions have little influence on the compaction of cohesin-mediated TADs [81].

## Interplay between epigenome and 4D genome

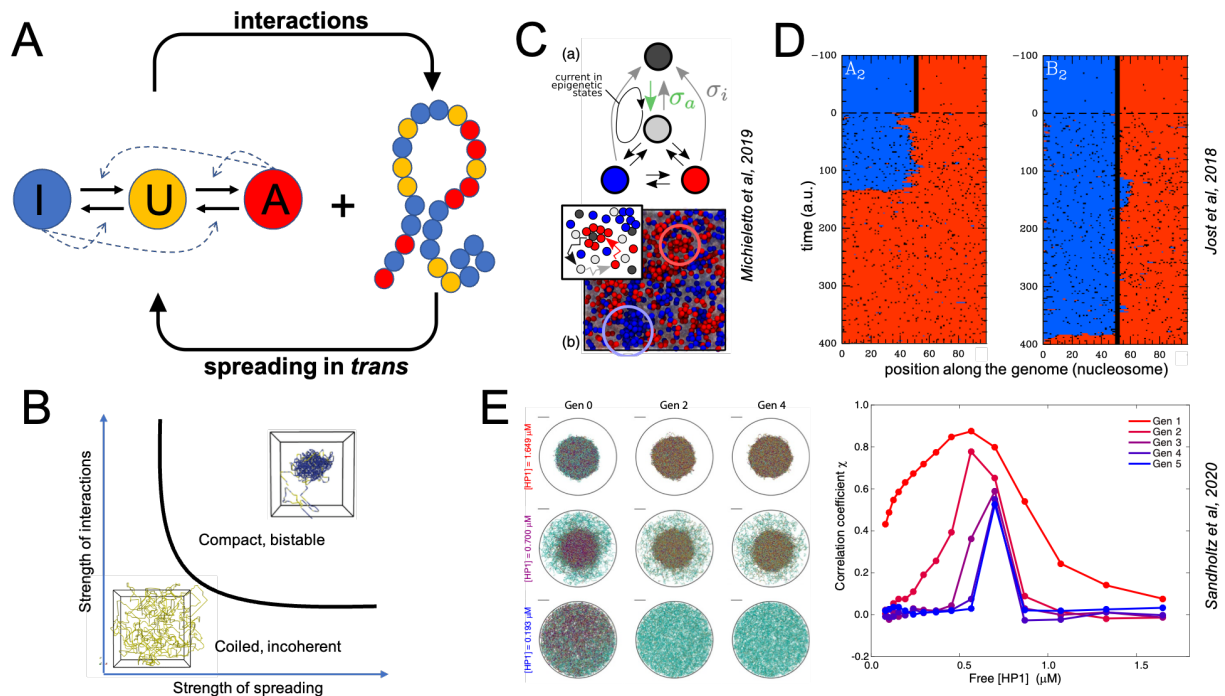


Figure 4. **4D Epigenomics.** (A) Scheme of the regulation of epigenome in space and time coupling a description of the chromatin state dynamics (Fig.2A) with the copolymer framework (Fig.3B). (B) Stability diagram of a single chromatin domain for the model shown in (A). Depending on the strengths of spreading (towards A or I) and of 3D interaction, the system reaches a compact, bistable (active/inactive) configuration or a coiled structure with poorly defined chromatin state. Inspired by [83]. (C) Non-equilibrium model of chromatin state conversion that may lead to a stable micro-phase separation. Extracted from [84]. (D) Kymographs showing the time evolution of the epigenomic state. The stability of an epigenomic landscape with two neighboring antagonistic (active vs inactive) domains is greatly enhanced by the presence of a 1D barrier (right, black line) that prevents *cis* spreading (which is present on the left panel). Adapted from [83]. (E) (Left) Example of configurations for different HME (HP1) concentration as a function of cell generation. (Right) The correlation coefficient between the current epigenomic profile and the initial one is maximal at an intermediate HME (HP1) concentration. Adapted from [85].

In the previous sections, we reviewed how an established 3D chromosome organization may impact epigenome regulation (3D  $\rightarrow$  1D) and how a fixed epigenomic landscape may drive genome folding (1D  $\rightarrow$  3D). To investigate the full dynamical coupling between the 1D epigenome and the 3D genome (1D  $\leftrightarrow$  3D), several polymer models were recently developed [83,85–88] integrating both 3D-mediated chromatin state transitions and epigenomic-driven interactions.

Usually, these approaches combine a copolymer framework to describe the 3D polymer dynamics with reader-writer conversion rules to account for the local chromatin state dynamics (Fig.1C,4A): (1) the spreading process is directly linked to the current 3D configuration by assuming that the transition rate of a monomer towards an epigenetically-modified state is proportional to the current number of other modified monomers that are in spatial proximity; (2) the pairwise interactions driving polymer dynamics are directly



dependent on the current epigenomic state of each monomer. Such positive feedback between chromatin regulation and folding leads to a phase transition between coiled configurations with a poorly-defined epigenetic state and compact globular structures with a stable, coherent epigenetic state (Fig.4B) [83–85,88–91]. Indeed, at strong enough spreading rates and interaction strengths, the 3D colocalization of monomers sharing the same chromatin state increases the local concentration of HMEs bound to them, thus reinforcing their spreading capacity in the 3D neighborhood. In particular, these studies underlined the higher efficiency of *trans* (i.e., in 3D) spreading activity of HMEs compared to on-site - *cis* - action in maintaining a robust epigenomic memory [83,86]. More generally, any biophysical processes that may favor *trans* effects, like increasing compaction or polymer mobility [83,86], would stabilize chromatin state.

In their simplest form, these frameworks may maintain, for a limited period of time, a 1D segmentation of the genome into consecutive active and inactive chromatin states, thanks to the formation of globular, TAD-like domains or of 3D compartments [83,84,88,90]. However, in the absence of constant nucleation signaling or bookmarking, they cannot lead to long-term epigenomic memory, as also observed for standard models of epigenome regulation (see above). Thus, many theoretical developments have been done to investigate various possible biologically-relevant mechanisms to add to the models to better stabilize a confined and partitioned memory of the epigenome. For example, stable micro-phase separation between active and inactive compartments may arise from a non-equilibrium model of chromatin state conversions where the local epigenomic state may switch to a long-lived, inert, refractory state [84,89]. The presence of insulator proteins like CTCF at the boundaries between two antagonistic epigenomic domains (active and inactive) may also participate in domain stability by preventing *cis* spreading between nearest-neighbor regions and thus limiting the contamination of one domain by the epigenomic state of the other [83]. Such insulation, coupled to enough genomic bookmarking (sequence-dependent recruitment of HMEs) [86,92] may significantly enhance stability over many cell generations [83]. Limitation of the number of HMEs coupled to 3D spreading and 3D compaction/segregation, as observed for the Polycomb regulation during fly embryogenesis [93], is also compatible with stable confined memory [85,87]. In this case, an optimal HME concentration may be a prerequisite for robust maintenance of an epigenomic landscape: at too low concentrations ( $\ll 1\mu M$ ), the spreading strength is not strong enough to overcome the perturbations occurring at replication while, at too high concentrations ( $\gg 1\mu M$ ), it may lead to the formation of spurious epigenomic domains and to unconfined memory [85].

## Discussion

In this review, we discussed how the observed correlations between the epigenome regulation and 3D chromatin organization can be consistently accounted for by biophysical models and how such models can be instructive to test mechanistic hypotheses and develop new concepts that can be challenged experimentally. In particular, it illustrates how the reader-writer capacity of HMEs, the positive feedback (1D $\leftrightarrow$ 3D) and long-range spreading via 3D contacts are cornerstones of epigenetic maintenance and inheritance.

By considering chromatin as a copolymer with state-specific interactions mediated by architectural proteins (APs) and with local state-switching dynamics depending on 3D and mediated by chromatin regulators (CRs) like HMEs, such models essentially formalize the concept of bio-chemical “nano-reactors” that we introduced few years ago [91]: the AP-mediated spatial compartmentalization of (epi)genomic loci able to recruit CRs increases locally the concentration of CRs and buffers their fluctuations; it thus enhances their chemical activity within the compartments, leads to less variable epigenomic profiles and avoids spurious propagation outside the targeted regions. This nano-reactor concept is somehow a eukaryotic extension of the bacterial lac-operon system where the gene repression level is controlled by the spatial colocalization

of the auxiliary and main operator sites [94] via generic site-site DNA loops mediated by dimeric repressors. Similarly, in eukaryotes, epigenome regulation also relies on site-specific CRs recruitment (both for *de novo* assembly and for maintenance) and compartmentalization of these sites may result from the binding of multimerizing APs primarily targeted at these recruitment sites. However, in contrast to the simple lac system, the “spreading” of the epigenomic states to the neighboring sequences (in *cis* and in *trans*) introduces additional secondary recruitment sites for both CRs and APs, further enhancing compartmentalization and spreading. Such state-specific - 3D-based - global control of the local CRs concentration might be an evolutionary add-on, increasing the epigenetic stability of such nano-reactors. An interesting perspective would be that 3D compartmentalization might not only buffer epigenomic states against internal and external cellular variability but also against genetic variability, i.e., mutations at the level of the CR coding sequences or of their genomic recruitment sites. Whether compartmentalization may compensate for the weakening of site(s) affinity in the course of evolution remains to be explored.

In this respect, it is pertinent to build a quantitative bottom-up framework to predict and understand the functional output associated with a given epigenomic state. Fine investigation of the interplay between the components of epigenome regulation (strength and distribution of recruitment sites, the CRs concentrations, recruitment modes (sequence- or state-specific) and activities, 3D compartmentalization) would need a refinement of the current models, in particular by explicitly modeling the CRs and APs spatial dynamics in parallel of the polymer and chromatin state dynamics [61]. Of particular interest, would be to better characterize theoretically and experimentally the multiple - and potentially conflicting roles - of SMC-mediated loop extrusion in epigenomic regulation [95]. Indeed, on one side, loop extrusion might interfere with epigenomic-driven compartmentalization [81,82] but, on the other, it may promote efficient spreading by mediating DNA loops between CRs recruitment sites and distant loci [41,96]. All this will allow a finer description of the crosstalk between the structural and functional epigenomic regulatory pathways in normal and pathological conditions.

Specific engineered/synthetic systems of CRs [1,97] may provide a template to study the aforementioned components of epigenome regulation, while development of single cell experimental techniques might facilitate deciphering the CR recruitment mode (thereby the mechanism of spreading) *in vivo* [93]. Importantly, there is also a need to improve our current description of the epigenetic inheritance during and after replication (distribution of the maternal marks among the sister chromatids, dynamics of re-establishment during G2, etc.) and mitosis (retention levels of APs/CRs in condensed chromosomes, re-activation of the epigenomic regulation in early G1, etc.) by accounting for the role of the spatial redistribution of CRs, APs and epigenomic marks and of the structural changes of chromatin, during both cell cycle phases; thus exploring, in a sense, if 3D chromosome organization can be considered as an epigenetic factor *per se*, i.e., directly participating in epigenetic memory via its inheritance during replication and cell division.

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