

Epigenetic Memory Achieved Through Chromatin-Induced Phase Separation

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Chromatin structure is a key determinant of gene expression and epigenetic inheritance. It relies on the post-translational modification of histones, the three-dimensional organization of nucleosomal DNA and interactions between chromatin components. To investigate interplays between these elements, we employed molecular dynamics simulations that integrated polymer modeling with enzymatic reactions on nucleosomes catalyzed by read-write enzymes. We explicitly modeled the diffusible heterochromatic nucleosome reader Swi6 (an HP1 homolog) that engages in homotypic and heterotypic interactions, undergoes and induces conformational changes upon binding to heterochromatic nucleosomes, and facilitates the targeted recruitment of histone deacetylases. The model revealed bistable epigenetic states with a silent state associated to the phase separation of heterochromatic domain condensates. Condensates were supported by Swi6 in a dosage-dependent manner that can be recapitulated in an analytic mean-field approximation. Our model of heterochromatic condensates further showed how a heterochromatic region can induce silencing of another region through spatial proximity in three dimensions. Moreover, extensions of the model suggested a mechanism for how negative feedback from antisilencing factors, here fission yeast Epe1, could confine the spreading of the heterochromatic state by acting on the surface of the condensates, altogether leading to epigenetically stable, spatially restricted chromatin states.

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I. INTRODUCTION

In unicellular and higher eukaryotes alike, the long-term silencing of cell-type specific genes and transposable elements occurs through the deacetylation of nucleosomes, deposition of repressive histone modifications, and formation of a condensed chromatin structure, heterochromatin, that is transmitted from mother to daughter cells [1]. Heterochromatic regions show a propensity to physically interact with each other and to congregate in the same subnuclear compartments in interphase cells [2–5]. The mechanisms and functional significance of these interactions remain mysterious but are likely to contain clues for how domains with particular histone modifications are formed and stably maintained [5]. When they occur, alterations in the heterochromatic landscape can have profound consequences by leading to reversals of cell differentiation, loss of cell identity, and cancerous and aging phenotypes [1,6–9].

The propensity of heterochromatin to cluster in separate subnuclear compartments led to its original cytological identification by Heitz [10]. Dense clusters form at the nuclear periphery, the perinucleolar space, and pericentromeric bodies [2,4]. This localization is favored by the interactions of

heterochromatic regions with structural components such as lamins or nuclear envelope proteins [11–13]. In addition, interactions intrinsic to heterochromatin take place and drive the global 3D compartmentalization of the genome [14–18]. Beyond gene expression, recent work suggests that this organization imparts mechanical stiffness to the nucleus [19].

The modeling of chromatin ranges from polymer physics (chromatin being a polymer of nucleosomes) including the effects of multivalent interactions on polymer folding [20,21] to models of dynamic nucleosomal modifications [22–24]. Alternating periods of chromatin folding and modification has suggested that both might contribute to epigenetic memory: compact heterochromatin would facilitate heterochromatic modifications in interphase cells while during cell division the linear sequence of modified nucleosomes would keep a memory of the compact state to be restored [25,26]. Other models directly couple histone modifications with polymer dynamics [27–36] or with nucleosome contact probabilities [37]. An encountered difficulty is that the simulated polymer dynamics rarely provide the needed physical contacts for read-write enzymes to perform nonlocal nucleosome conversions along the genome. Here we present a model that solves this difficulty through the diffusion of short-lived read-write complexes that effectively allow nucleosome states to spread through condensates supported by these complexes.

A central attribute of heterochromatin is the methylation of histone H3K9 (H3K9me). Research with the fission yeast *Schizosaccharomyces pombe* has provided compelling evidence that histone H3K9me carries epigenetic memory whereby gene expression states are transmitted from mother to daughter cells [38–44]. In this organism, the methyltrans-

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ferase Clr4 (a homolog of the metazoan SUV39 enzymes) both methylates H3K9 [45] and binds to H3K9me with a chromodomain [45–48]. This read-write process induces positive feedback [41,46,48–50]. Additional feedback is provided by the HP1 family proteins Swi6 and Chp2 that bind H3K9me [47,49–52] and function as interaction platforms for multiple chromatin factors [53–56]. Interactions with histone deacetylases (HDACs) facilitate the deacetylation of histones, a necessary step prior to H3K9me [56,57]. However, not all interactors create positive feedback. Thus, the antisilencing factor Epe1 competes with HDACs to associate with Swi6 [56] and counteracts heterochromatin formation through direct demethylation of H3K9 [40,58] or by recruiting euchromatic factors [59].

Swi6 forms stable homodimers in solution [60,61]. Upon binding to H3K9me nucleosomes, these dimers undergo a conformational switch, oligomerize [51,62] and alter the structure of the histone octamer in such a way that Swi6-bound nucleosomes have been proposed to undergo phase separation [63,64]. Consistent with this, Swi6 forms biomolecular condensates in heterochromatic regions *in vivo* [19,65] and the heterochromatic context promotes the interaction of Swi6 and Chp2 with binding partners [52,56,66]. Moreover, ubiquitylated Clr4 undergoes liquid droplet formation *in vitro* with Swi6 [67].

Other HP1 proteins in metazoans also participate in phase separation [21,64,68–71]. In *Drosophila*, liquid-liquid phase separation (LLPS) of HP1 is detected at a very early stage of heterochromatin formation [68]. In later stages and in other systems, it becomes difficult to distinguish the type of condensates formed, arguably due to simultaneous LLPS and polymer-polymer phase separation [70,72]. However, it remains that condensates are now widely expected to be defining features of heterochromatin [73].

Highlighting the relevance of feedback *in vivo*, feedback-defective chromodomain mutants lack the ability to spread and maintain heterochromatin outside nucleation centers [41,46,74]. Conversely, the addition of feedback by fusing a chromodomain to an HDAC enhances the formation of heterochromatin [75]. From a modeling perspective, three-state nucleosome models [22] encapsulate these and other behaviors. In these models, A (Acetylated), U (Unmodified), and M (Methylated at H3K9) nucleosomes are interconverted by read-write enzymes subject to positive feedback. These models can account for the properties of many fission yeast mutants [22,37,76–78]. A recurring finding is that one positive feedback at least has to be nonlocal along the genome. In other words, the models imply that long-range interactions are necessary for the formation and stability of heterochromatin.

Here, we present a model for the interaction between feedback-driven nucleosomal modifications and 3D chromatin folding and compaction in the presence of diffusible HP1 dimers, represented by Swi6. For this, we used the large-scale atomic/molecular massively parallel simulator LAMMPS [79] and a custom version of REACTER, a method for modeling chemical reactions in classical molecular dynamics simulations [80–82]. Current needs in the chromatin modeling field are to consider multiple reactions in 3D models and to smoothly intertwine these reactions with chromatin folding. To this end, positive feedback by diffusible molecules

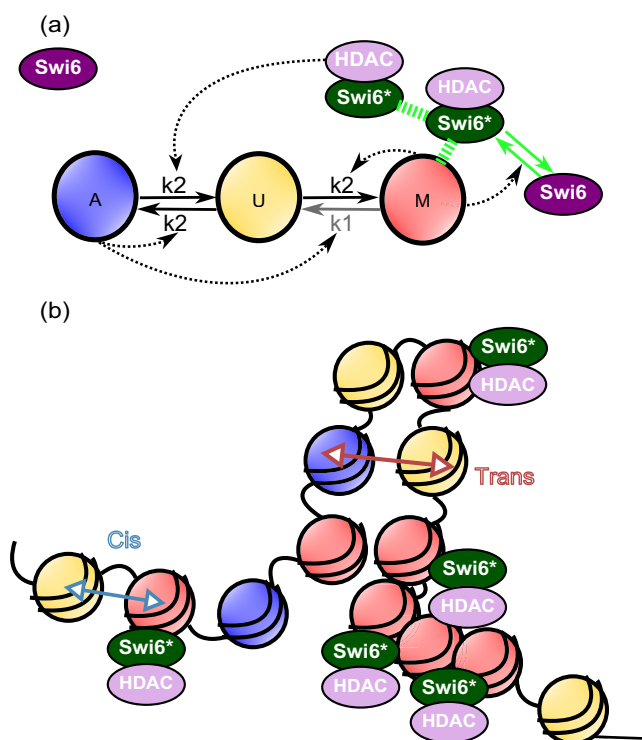


FIG. 1. Coarse-grained model of reactive chromatin. (a) The three-state model with acetylated (A), unmodified (U) and methylated (M) nucleosomes. Diffusible Swi6 dimers (dark purple) are attracted to M nucleosomes; contact causes a reversible conformational change in Swi6 (Swi6*, green) that favors homotypic and heterotypic interactions and accretion of Swi6*HDAC complexes close to M nucleosomes. Swi6* reverts to Swi6 by diffusing away from M nucleosomes. The three nucleosome types interconvert with rate constants k_1 (M to U reaction, gray arrow) or k_2 (other reactions, black arrows) at proximity of a catalyst. A nucleosomes are catalysts for reactions that go towards the A state. M nucleosomes are catalysts for the U to M reaction. Swi6*HDAC complexes are catalysts for the A to U reaction. Solid arrows represent reactions; dotted arrows, the catalysis of a reaction; and dotted green lines, attraction by physical forces. (b) Three-state model in three dimensions illustrating that reactions may be catalyzed in *cis* or *trans*, governed by physical proximity.

capable of multivalent interactions—with nucleosomes and histone-modifying enzymes, as well as self-interactions—was added here to address how these affect chromatin dynamics. Our simulations reveal the *de novo* formation of heterochromatin condensates, the consolidation of unlinked condensates through fusion, and conditions producing bistable and confined chromatin states that are robust to cycles of chromatin replication and mitotic divisions.

II. MODEL

Nucleosomal DNA was represented as a polymer of beads bonded by identical harmonic potentials, free to move in a 3D simulation of Brownian motion. Each bead represents a single nucleosome that could exist in one of three states, A, U, or M.

Enzymatic conversions between the states are depicted in Fig. 1(a). Two types of conversions were considered: recruited conversions representing the direct or indirect recruitment of enzymes by modified nucleosomes and noisy conversions

representing random encounters of nucleosomes with histone-modifying enzymes or nucleosome turnover.

Recruited conversions were governed by the state of nearby nucleosomes or by the presence of nearby Swi6* complexes (see below). They reflect the ability of modified nucleosomes to bind enzymes that catalyze changes towards a particular state. This is generally the state of the recruiting nucleosome, forming positive feedback loops as outlined in Fig. 1. However, negative feedback can also be implemented.

According to its biophysical properties, Swi6 was represented as a diffusible dimer with an affinity for M nucleosomes; it could engage in self-interactions (in addition to dimerization) stimulated by proximity to M nucleosomes; and it could interact with histone-modifying enzymes. For convenience, the simulations distinguished two forms of Swi6: The free diffusing dimers (Swi6, represented in purple) and the form induced by the proximity of M nucleosomes (Swi6*, represented in green). By association with HDACs, Swi6* formed Swi6*HDAC complexes. Except for the Epe1 variant of the model presented at the end, Swi6* and Swi6*HDAC complexes are a single species. In the Epe1 variant, by associating with Epe1, Swi6* could in addition form Swi6*Epe1 complexes. Neither HDAC nor Epe1 were modeled explicitly. Like Swi6, Swi6* complexes diffused. The diffusion was affected by attraction to M nucleosomes and to other Swi6* molecules, as shown in Fig. 1(a) for Swi6*HDAC.

Eligible nucleosomal conversions were checked for on every timestep and determined by the proximity of nucleosomes and Swi6* complexes. Four nucleosome reactions were defined to interconvert U, M, and A nucleosomes, each using a reaction distance cutoff of 2σ . As shown in Fig. 1(a), the four reactions arise from reversible conversions between unmodified and acetylated nucleosomes, and unmodified and methylated nucleosomes. The reaction from M to U was assigned a rate constant of k_1 , while the other reactions were assigned the same rate constant k_2 . Every time an eligible reaction was identified, the probability of that reaction actually occurring was defined as $p_i = k_i dt$, where the time step $dt = 0.001 \tau_{LJ}$. For example, every M nucleosome located close enough to an A nucleosome was converted to U with probability $p_1 = 0.001 k_1$. If multiple A nucleosomes were within the cutoff distance, only the closest nucleosome was considered eligible for a reaction on a given time step. Two additional reactions were defined to convert between Swi6 and Swi6* when in the vicinity of M nucleosomes. Swi6 within 2σ of an M nucleosome is converted to Swi6* and remains Swi6* for a minimum duration of $0.2 \tau_{LJ}$ (see Table I), which is equivalent to a rate constant of $5 \tau_{LJ}^{-1}$. When no longer close to an M nucleosome, Swi6* reverts to Swi6.

The relatively low frequency at which conversions were attempted (in the order of $0.1\text{--}1 \tau_{LJ}^{-1}$) reflects the widely different timescales of polymer dynamics and histone modification. The much faster timescale of polymer dynamics is a challenge in this kind of modeling. Here, for a polymer representing the fission yeast mating-type region with 80 nucleosomes, the polymer was observed to globally reorient itself on a timescale of about $10^3 \tau_{LJ}$. During this time we obtained about 1000 recruitment attempts per nucleosome.

In vivo the number of recruitments would be much smaller, on the order of one per minute. Thus, our simulations are

an approximation, in line with other polymer-based models for epigenetics [27–33,37,83]. A mean-field model presented towards the end bridges this gap for a simplified dynamical version of the model.

Notably, M nucleosomes were not given a particular ability to self-interact in these simulations, in contrast to what is often the case in copolymer models. Swi6, on the other hand, allowed interactions that could potentially lead to a more compact structure for M-dominated states than for other states [Fig. 1(b)]. Furthermore, due to the compaction of the nucleosomal DNA fiber, modifications were expected to be facilitated in both *cis* and *trans* by the nucleosome-mediated recruitment of enzymes [Fig. 1(b)]. Differential compaction and interaction modes will affect the relative reaction rates.

III. RESULTS

A. Swi6-induced chromatin phase transition

We examined the effects of Swi6 in our model (Fig. 1) following the dynamics of an 80-nucleosome polymer surrounded by 400 Swi6 dimers in our $50 \times 50 \times 50 \sigma^3 \sim 0.1 \mu\text{m}^3$ box [Figs. 2(a)–2(d)]. Starting from a polymer predominantly in the A state, due to noisy conversions, some nucleosomes changed to the U or M state. Random encounters led to the capture of Swi6 by M nucleosomes and the accretion of Swi6*HDAC complexes. These, in turn, boosted A-to-U transitions for other nearby nucleosomes opening for transitions toward the M state. Ultimately, Swi6* interactions favored the compaction of a chromatin fiber predominantly composed of M nucleosomes.

Although M nucleosomes were not assigned any direct mutual attraction in our model, their interactions with Swi6* led to a sharp transition to a compact state, quantified as a sharp decrease in the radius of gyration of the polymer [Fig. 2(d)]. Compaction by Swi6 was independently observed when starting with a polymer of nucleosomes in the M state in the absence of enzymatic reactions [Figs. 2(e)–2(g); see Materials and Methods]. As the system evolved from its original decompacted state to a globular state induced by Swi6 to Swi6* conversions, the diffusion properties of Swi6 changed from a very mobile to a less mobile population [Fig. 2(g)].

In general, our model therefore explores Swi6* as a mediator of the positive feedback between the dominance of the M state and the condensation of heterochromatin.

B. Bistability of chromatin states

Having observed that the suggested wiring (Fig. 1) can lead to the formation of a compact heterochromatic domain, we asked whether it can also support bistability. *In vivo*, epigenetic inheritance can lead to bistability where alternate chromatin states are passed on through generations. A prototypic example is the fission yeast ΔK mutant. In this mutant, the mating-type region alternates between two states, a euchromatic, expressed, state and a heterochromatic, repressed state [38,39]. Historically, bistability was simulated in a model with the same three nucleosome states as here [22]. Although the spatial positioning of nucleosomes was not explicitly modeled, the modeling predicted a requirement for polymer-associated long-range feedback to observe bistability.

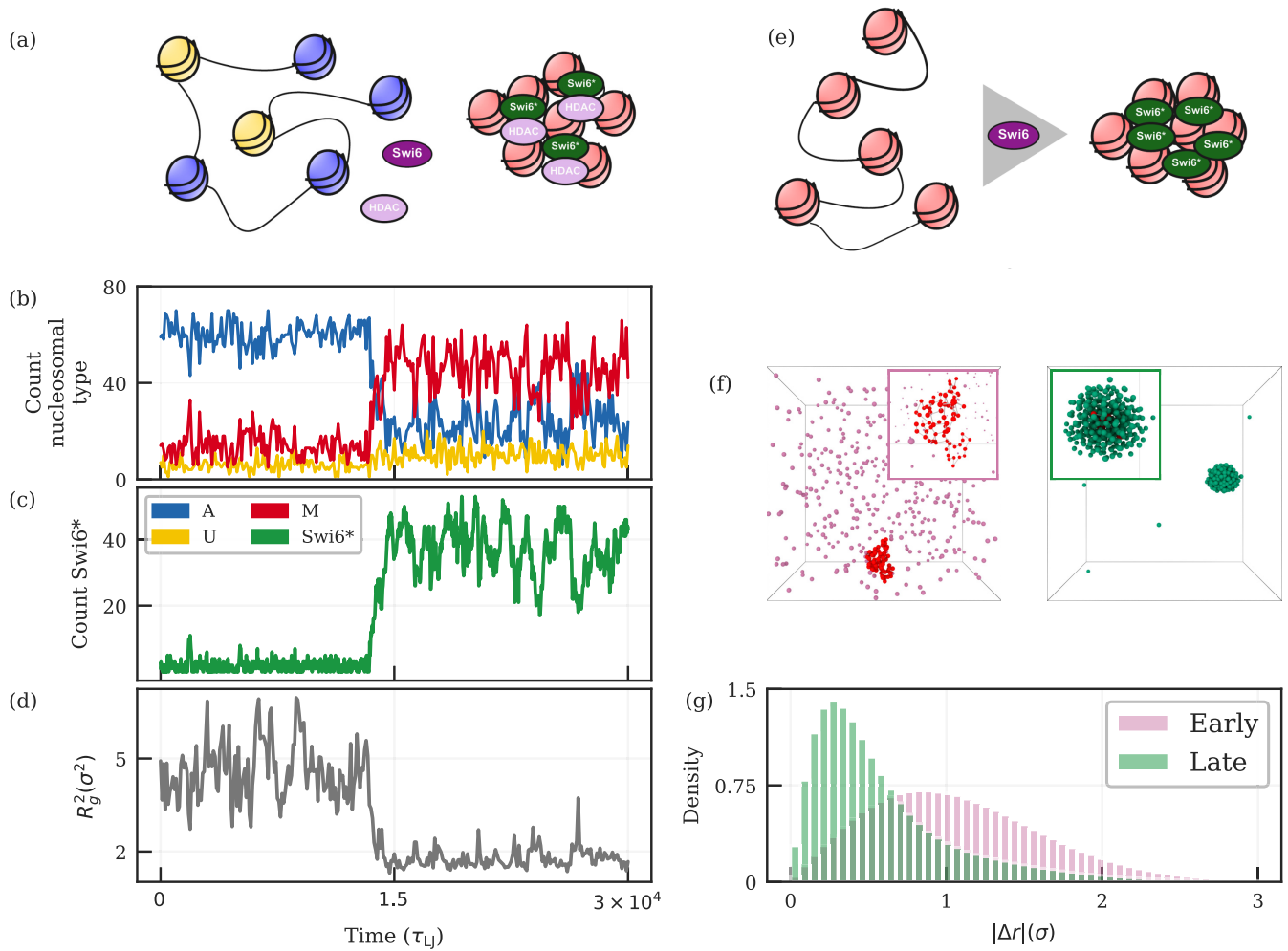


FIG. 2. Swi6-induced chromatin condensation and coil-globule transition. (a)–(d) Chromatin compaction by Swi6 coupled with enzymatic reactions. (a) Schematic representation of the active coil state (left) and repressed globular state (right). The panels below are from a time-course simulation of 80 reactive nucleosomes with 400 diffusive Swi6 dimers, plotting respectively the number of nucleosomes of each type (b), the number of M-associated Swi6* dimers (c), and the radius of gyration of the polymer (d), for $k_1 = 0.1 \tau_{LJ}^{-1}$ and $k_2 = 0.25 \tau_{LJ}^{-1}$. (e)–(g) Chromatin compaction by Swi6 in the absence of reactions. (e) Representation of initially decompacted polymer of 80 M nucleosomes and globular state induced by Swi6 binding. (f) Snapshot of early simulation in the absence of Swi6 interaction with M nucleosomes (400 Swi6 dimers, in purple, on the left) and late simulation following Swi6 to Swi6* conversions (400 Swi6* dimers in green, on the right). Top frames show enlargement on polymer. (g) Displacement histograms corresponding to the populations shown in (f).

Compared to the original model [22], we consider here the actual 3D location of each nucleosome in the system, and moreover the model introduces an asymmetry in positive feedback. Simulations were performed with a system of 80 nucleosomes where we varied the k_1 and k_2 rates of nucleosomal conversions (Fig. 3; Figs. S4 and S5 [84]). In Fig. 3 the simulations were started from the heterochromatic state obtained by assigning the M state to all 80 nucleosomes and by equilibrating the polymer with Swi6*HDAC complexes without allowing conversion reactions. These settings led to the formation of a stable condensate. The reactions were then initiated. During simulations, several values were recorded: the states of nucleosomes as a function of space and time [Figs. 3(b) and 3(c)]; the number of nucleosomes in each state [Fig. 3(d)]; and the number of Swi6* molecules [Fig. 3(e)].

The simulations exhibited spontaneous switching between a condensed M-dominated state and a predominantly A swollen state. These qualitatively recapitulate the observed switches between the heterochromatic and euchromatic state of the ΔK mating-type region [38,39,74]. Within each state, the system was roughly uniform throughout the region [Fig. 3(c)]. Quantitatively, the degree of compaction of the mating-type region is unknown. In our simulation, the radius of gyration of the system was about a factor 4 smaller in the heterochromatic state compared to the euchromatic state. This is consistent with the expectations of random polymers, characterized by the gyration radius scaling with polymer length L as L^ν . For the case of a self-avoiding polymer, Flory scaling predicts $\nu \approx 0.59$ while a polymer with mutual attractive binding has $\nu \approx 1/3$ [85,86]. For our 80-nucleosome region, the ratio between the theoretically expected radii of gyration

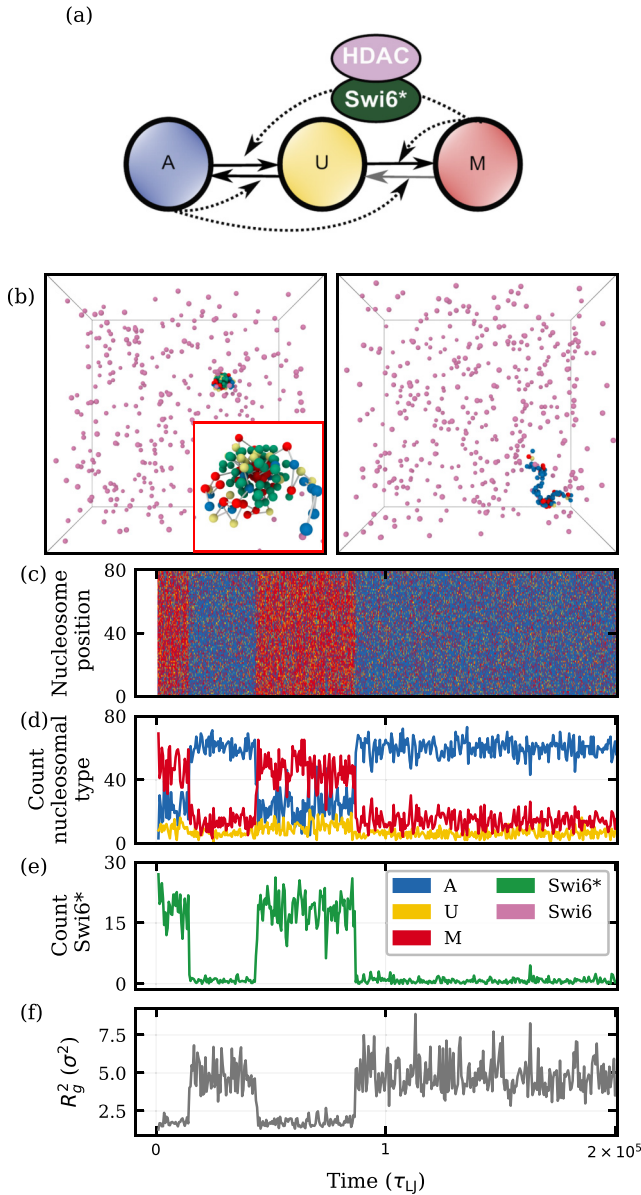


FIG. 3. Bistability of fission yeast ΔK mating-type region. (a) The model shown in Fig. 1 was implemented for a region of 80 nucleosomes and 400 diffusible Swi6 dimers, with rates $k_1 = 0.1 \tau_{LJ}^{-1}$ (gray arrow) and $k_2 = 0.25 \tau_{LJ}^{-1}$ (black arrows). (b) Snapshots of the simulation box for the M-dominated state with Swi6* condensate (left; with zoom on condensate) and the A-dominated state without condensate (right). See Movie S1 [84] for the full simulation and Movie S2 focusing on two transitions. (c) Space-time plot of nucleosome types. (d) Count of nucleosomes of each type as a function of time. (e) Count of Swi6* dimers as a function of time. (f) Radius of gyration of the chromatin polymer. See Fig. S2 for duplicate simulation with different starting conditions.

would be $80^{0.59-\frac{1}{3}} \approx 3.3$ which is consistent with the fourfold obtained in the simulations.

In Supplemental Material [84], we explore k_1 and k_2 reaction rates that support bistability for the 80-nucleosome polymer (Figs. S3–S5). At low values of random conversions, the stability of a starting state might be too large to allow any switch within a reasonable length of simulation. Therefore,

we explored bistability by starting polymers in either the A or the M state (examples are in Fig. S3) and by identifying conditions where both states were sustained for a long time, occasionally for the entire simulation as explained in Materials and Methods.

At low k_2 values ($0.001 - 0.1 \tau_{LJ}^{-1}$ range in Fig. S4a [84], with k_1 fixed at $0.1 \tau_{LJ}^{-1}$, the proportion of M nucleosomes was low, independent of Swi6 concentration. Conversely, for high k_2 values ($2 - 10 \tau_{LJ}^{-1}$ range in Fig. S4a), the M state dominated. Bistability was observed at intermediate k_2 values (see $0.35 \tau_{LJ}^{-1}$ in Fig. S3a and refined scan in Fig. 4), for a large range of Swi6 concentrations (from 100 to 600). On the whole, a mirror image was obtained when varying k_1 and Swi6 at fixed k_2 ($k_2 = 0.25 \tau_{LJ}^{-1}$): the M state dominated for low k_1 values ($0.001 - 0.1 \tau_{LJ}^{-1}$ range in Fig. S3b) and the A state dominated for high k_1 values ($0.1 - 10 \tau_{LJ}^{-1}$ range in Fig. S3b), with bistability in the intermediate range. Finally, a parameter scan where k_1 and k_2 were varied at fixed Swi6 (400 Swi6; Fig. S5) found that the ratio $\frac{k_2}{k_1}$ was an important determinant of bistability as shown by the diagonal defining the different regions of the phase diagram.

The observation that higher Swi6 numbers (Fig. 4; Fig. S4 [84]) cause a persistent heterochromatic state at given conditions are in line with studies of ΔK strains showing that the equilibrium between euchromatic and heterochromatic states can be shifted towards the heterochromatic state by augmenting the nuclear concentration of Swi6 [74,87–89]. To probe whether the system was robust to cell cycle, we first mimicked S phase alone. During DNA replication (S phase), the old histone octamers around which the DNA is wrapped to form nucleosomes are distributed between the two daughter strands and new histones are incorporated to fill in the gaps, diluting the marks. This was implemented by changing half the nucleosomes to U at regular time intervals (Fig. S6 [84]). The operation was conducted at two noise levels, one found to produce very robust bistability in parameter scans and the other more moderate bistability (Fig. S6). The two epigenetic states were maintained at both noise levels upon multiple S phases (Fig. S6).

To mimic a full cell cycle, we added mitosis. Mitosis, where the two daughter chromatids are segregated between daughter cells, constitutes a challenge to epigenetic memory as the 3D folding of chromatin is altered to permit chromosome condensation and segregation. Linked to this process, histone H3 is phosphorylated at S10. H3S10P antagonizes H3K9 methylation and hinders the binding of Swi6 to chromatin [45,90,91]. Binding is not quite abolished though and residual binding has been proposed to constitute an imprint for epigenetic memory [90,92]. Here we mimicked mitosis by allowing polymer dynamics with very reduced reaction rates and very reduced Swi6 association with M nucleosomes and we alternated S phases and mitoses separated by gap phases (G1 and G2) as described in Materials and Methods, for 100 cell cycles. The expected periodic decompaction of the heterochromatin and dissociation of Swi6 can be seen in plots and videos (see increased radius of gyration and reduced Swi6 association in Fig. 5(c) and Movie S3 [84]). Again, each epigenetic state was found to be maintained upon this regime showing bistability can be sustained upon full cell cycles for many generations.

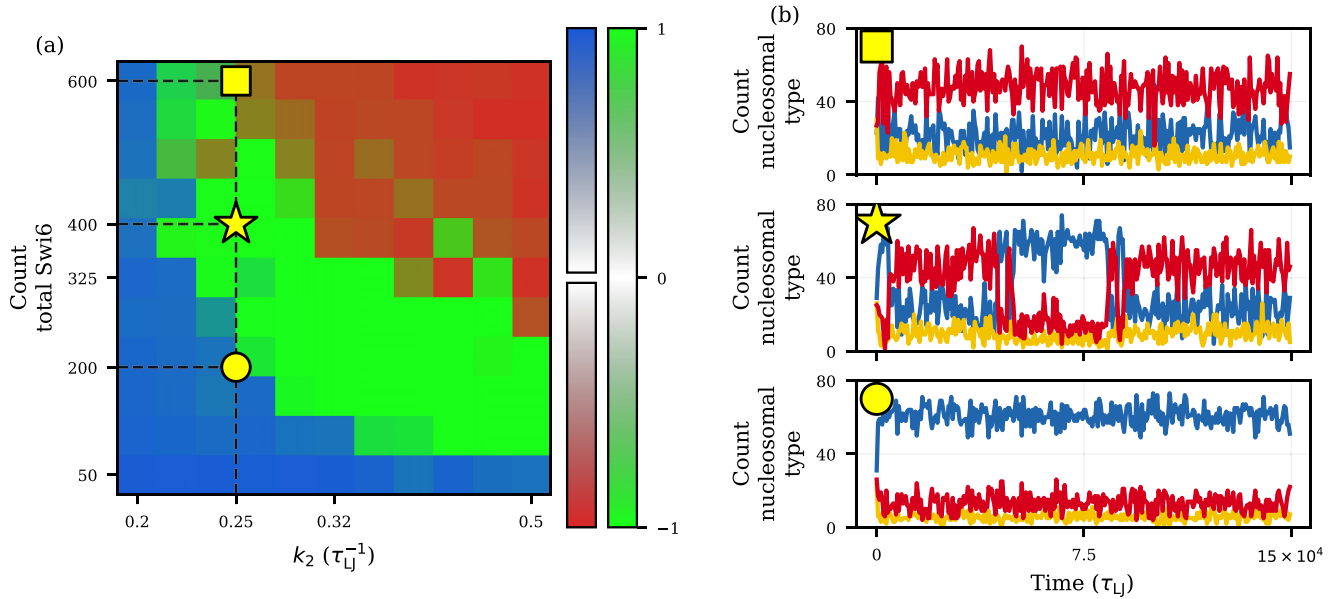


FIG. 4. Bistability is dependent on Swi6 concentration. (a) Parameter scan examining bistability of ΔK mating-type region as a function of k_2 and number of Swi6 dimers in the simulation box, for $k_1 = 0.1 \tau_L^{-1}$. Green denotes bistability; red, M-dominated states; and blue, A-dominated states. Yellow shapes mark the conditions used in (b). (b) Examples of simulations for $k_1 = 0.1 \tau_L^{-1}$ and $k_2 = 0.25 \tau_L^{-1}$ for respectively 600, 400, and 200 Swi6 dimers per box. See also Figs. S3 and S4 [84].

Finally, it has been observed that an epigenetic memory of the ON and OFF states of ΔK regions can be propagated *in cis* when two regions are present at the same time in diploid nuclei, showing the regions adopt independent states [39,74]. Here, by combining two regions in the same simulation box, we observed independent maintenance and switching of the two regions (Fig. S7 and Movies S4 and S5 [84]).

Altogether, the model thus robustly recapitulates characteristic properties of the ΔK mating-type region, bistability, dosage effects, and *cis*-inheritance. Simultaneously keeping track of the modification state and 3D location of each nucleosome in the system reveals concerted changes in histone modification and chromatin compaction.

C. Contact-induced heterochromatin formation

Eukaryotic genomes contain multiple unlinked heterochromatic regions and it is of interest to determine whether physical contacts involving these regions might have functional consequences. In the case of *S. pombe*, in addition to the mating-type region, large heterochromatic regions exist near centromeres and telomeres and at the rDNA clusters. Spatially, the three centromeres coalesce at the spindle-pole body embedded in the nuclear envelope. Telomeres cluster into a few foci, also at the nuclear envelope [12] and they establish contacts with the mating-type region [93]. These heterochromatic domains exhibit liquid-like properties and the clustering of telomeres at least depends on a domain of Swi6 suggested to underlie phase separation [19]. Moreover, the heterochromatic state of a compromised mating-type region is restored by relocalization to the perinucleolar space [94].

To mimic the effect of contact or physical separation on epigenetic states, we added a polymer of 160 nucleosomes to the 80-nucleosome mating-type region (Fig. 6). The 160 nucleosomes were fixed in the M state to represent a region of

stable heterochromatin. The two polymers were either fixed in space or allowed to diffuse freely. When the two polymers were fixed in space, the 80-nucleosome mating-type region did not change state [Fig. 6(b)]. However, when the two polymers could move freely, random encounters induced heterochromatinization of the 80-nucleosome mating-type region and concurrent coalescence of the two regions as seen in Figs. 6(c), 6(d), and 6(f) and Movie S6 [84]. Other examples are shown in Fig. S8 and Movies S7 to S9 with an 80-nucleosome M-state polymer and for varied Swi6 concentrations. Figure S9 shows region-to-region cooperativity with an enhanced local Swi6* concentration when the two regions are in proximity; see Figs. 6 and S8.

D. Confinement of heterochromatin spreading by Epe1

Heterochromatin has a tendency to expand due to multiple positive feedback mechanisms, as illustrated in Fig. 1. This preferential expansion raises the broader question of how heterochromatin is confined *in vivo*. In fission yeast, several factors contribute to establishing heterochromatin boundaries [12,88,89,95,96].

One key factor is Epe1, a protein appertained to the JmjC family of histone demethylases [58,96]. Epe1 mutants exhibit enlarged and stabilized heterochromatic domains whereas Epe1 overexpression leads to reduced heterochromatin [40,41,96–99]. These effects of Epe1 rely on the physical interaction of Epe1 with Swi6 [56,58,61,100] which is stimulated by H3K9me binding [56]. This binding results in a Swi6-dependent accumulation of Epe1 in heterochromatin [101] that is more pronounced at the edges of heterochromatic domains [101,102].

In addition to promoting, perhaps directly, demethylation of H3K9me nucleosomes [40,58], the Swi6-Epe1 interaction antagonizes Swi6*HDAC complex formation [56].

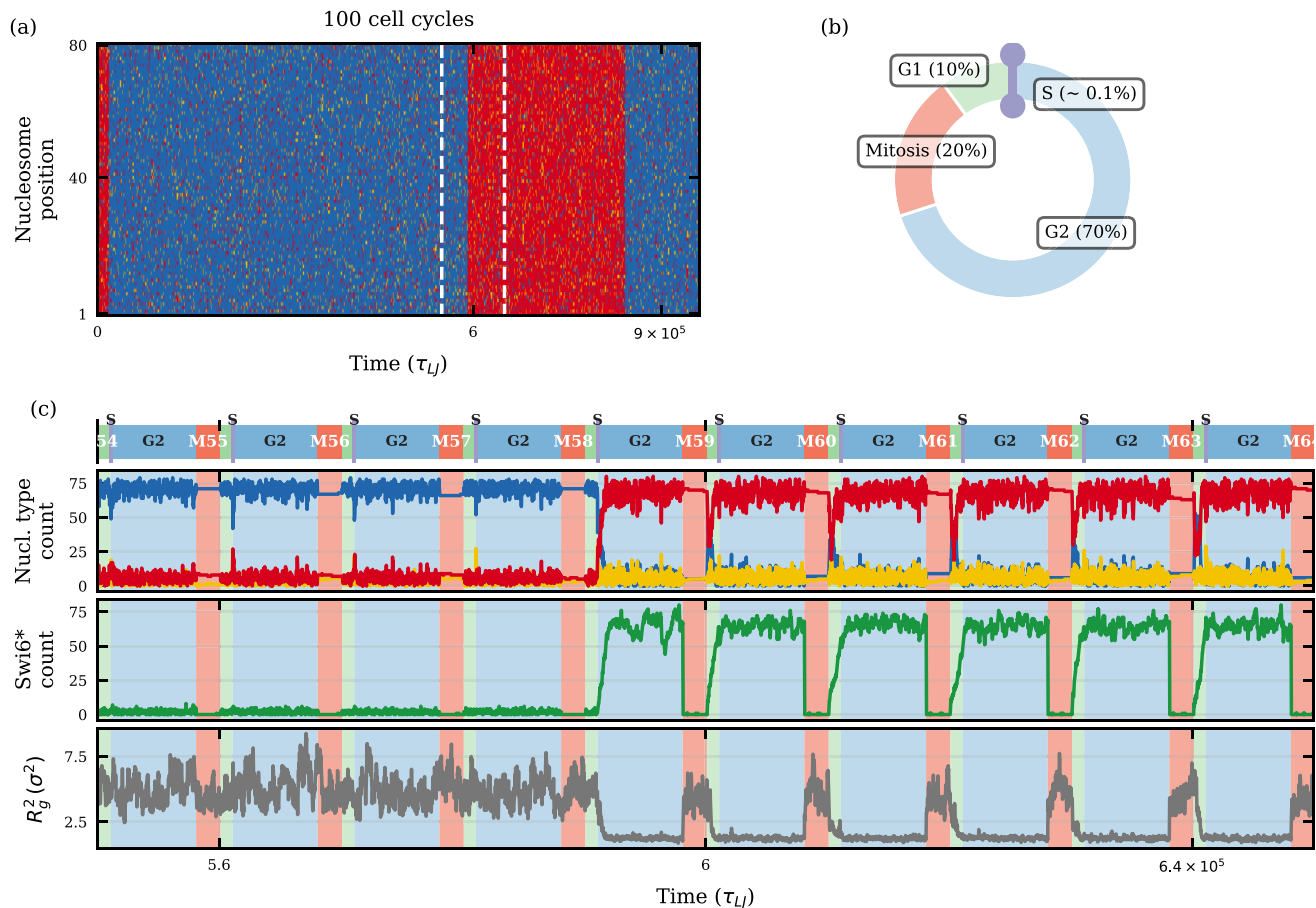


FIG. 5. Robustness to cell cycle. (a) Kymograph of 80-nucleosome region undergoing alternating S phases—where half of the nucleosomes were replaced with U nucleosomes—and mitoses—where the polymer was allowed to decondense at very reduced reaction rates. The period was $10^4 \tau_L$. (b) Simulation scheme for panel (a). G2 was long relative to G1 to mimic the fission yeast cycle. However, the scheme was not strictly meant to represent the phases in real time, in particular mitosis was relatively long in simulations to allow for changes in polymer conformation while this was not necessary for S phase. The parameters were $k_2 = 0.25 \tau_L^{-1}$, $k_2 = 0.1 \tau_L^{-1}$, $\Gamma = 1 \tau_L^{-1}$ and 400 diffusive Swi6 dimers. (c) Nucleosome type and Swi6* counts and gyration index for a window of the simulation. The simulation is shown in Movie S3 [84]. See Fig. S6 for robustness to a simplified cell-cycle with only S phases.

To explore how chromatin boundaries are formed in three dimensions, we introduced Epe1 to our modeling. To reflect the observed Swi6-Epe1 association in heterochromatin, a Swi6*Epe1 interaction was permitted in the vicinity of M nucleosomes while maintaining the Swi6*HDAC interaction. To reflect the more peripheral location of Epe1, the Swi6*Epe1 interaction with M nucleosomes was modeled to act at a greater distance from M nucleosomes than the Swi6*HDAC interaction. The different spatial relationship led to Swi6*HDAC complexes forming an inner shell from which Swi6*Epe1 was largely excluded. This localization is consistent with fluorescence microscopy imaging of Swi6 and Epe1 [52].

We then compared heterochromatin propagation in a long polymer of 500 nucleosomes dependent on the presence of Epe1 (Fig. 7). For this model, heterochromatin was initiated at one, two, or three nucleation regions distributed in the polymer, each region constituted by 25 nucleosomes in the M state. The dynamics within the nucleation regions mimic the *cenH* region in *S. pombe* where RNA interference induces the M state [74]. Sufficient Swi6 was provided for the long

polymer to become entirely heterochromatic. This occurred in the absence of Epe1, as illustrated in Fig. 7(c), left panel, and Movie S10 [84]. In the presence of Epe1 though, heterochromatin propagation was limited to much smaller regions near the nucleation sites [Fig. 7(d), right panels and Movie S11]. These regions fused with each other into a single condensate with the intervening euchromatic domains forming protruding loops [Figs. 7(d) and 7(e) and Movie S11]. The fusion events were reversible during mitosis and the overall organization was robust to cycling conditions (Figs. S10–S13 and Movies S12 and S13).

Thus, like *in vivo*, Epe1 prevented the spread of heterochromatin through its association with Swi6. Precisely positioned boundaries were not obtained [Fig. 7(d), right panels, and Figs. S10–S12 [84]] but this is expected from the literature as Epe1 is only one of several factors establishing the boundaries of the silent mating-type region [88,89,95,96]. Absent other elements, particularly binding sites for the architectural factor TFIIC present in the IR-L and IR-R boundaries, Epe1 prevents the uncontrolled spread of heterochromatin [89] but the heterochromatic domain shrinks or expands around the natural

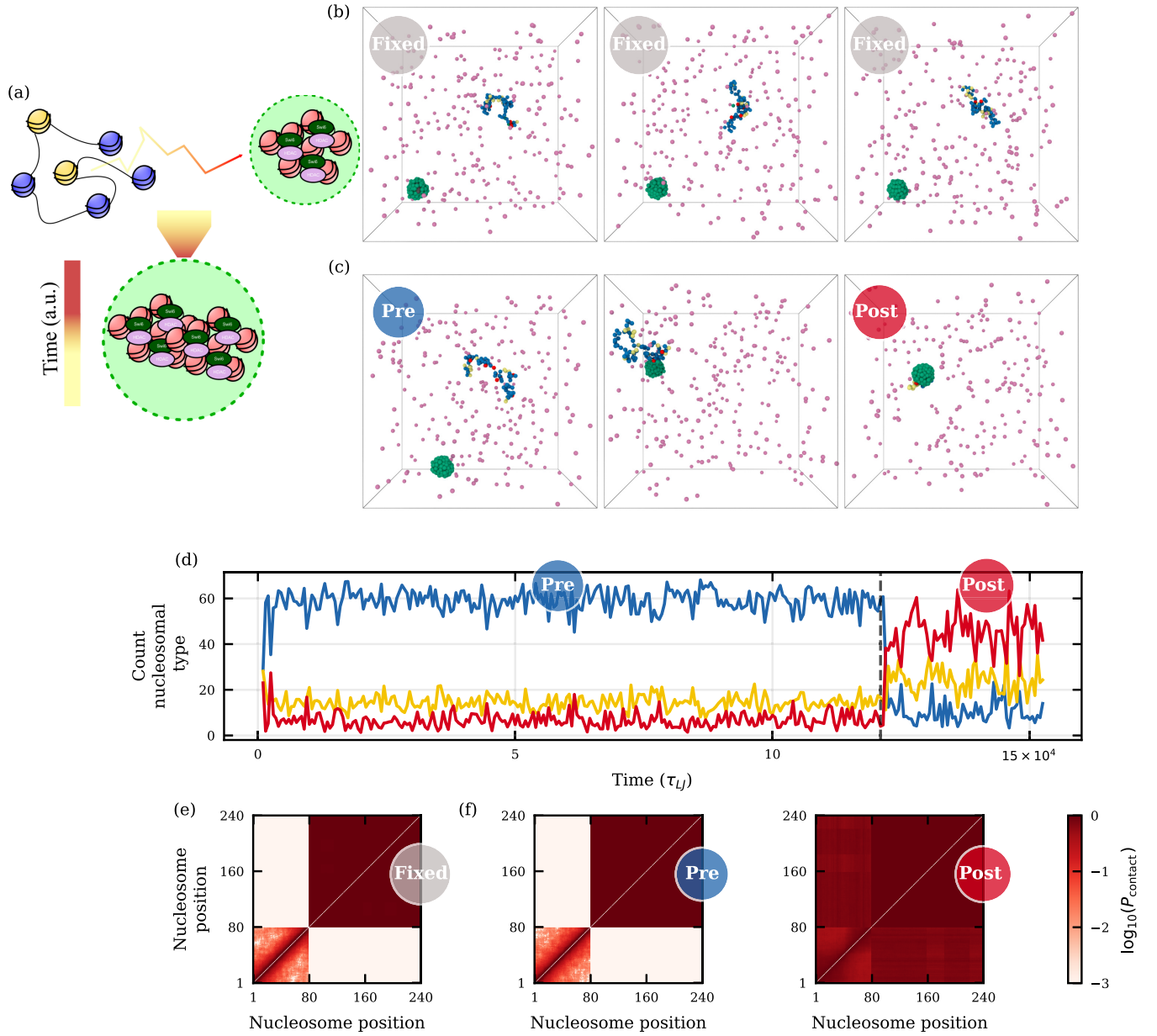


FIG. 6. Contact-induced heterochromatin formation. (a) Representation of the merging of unlinked loci observed in simulations. (b) System with two polymers fixed in space, one of 160 nucleosomes in the M state and the other of 80 reactive nucleosomes, in the presence of 400 Swi6 dimers. Three simulation snapshots are shown. (c) Same as (b) but with diffusing polymers. Full simulation is shown in Movie S6. (d) Time course of nucleosomal composition for diffusing polymer with reactive nucleosomes, showing transition to heterochromatic state upon encounter with polymer in M state (dotted line). (e), (f) Contact maps for fixed (e) and diffusing (f) polymers. Nucleosomes 1–80 are the reactive polymer and 81–240, the M-state polymer. See Fig. S8 for different conditions and corresponding Movies S7–S9 [84].

borders in individual cells [87,95], similar to the outcome of our model. In the future, it will be interesting to add the elements that function in synergy with Epe1 and to explore theoretically a suggested loop formation mechanism [95].

E. Robustness and mean-field description of condensate-mediated epigenetics

The condensate-mediated epigenetics may be explained through a simplified analytical description, formulated in the spirit of the two-state approach to a bistable system of nucle-

osomes by [103]

$$\frac{dm}{dt} = k_2^2 s m (1 - m) - k_1 k_2 m (1 - m)^2 + \Gamma (1 - m) - \Gamma m. \quad (1)$$

Here m denotes the fraction of methylated nucleosomes, while s quantifies the local concentration of Swi6*HDAC complexes, normalized such that its maximum value is unity. The first term represents a gain process, in which de-acetylation, mediated by Swi6*HDAC complexes and therefore proportional to s , is followed by methylation at a rate proportional

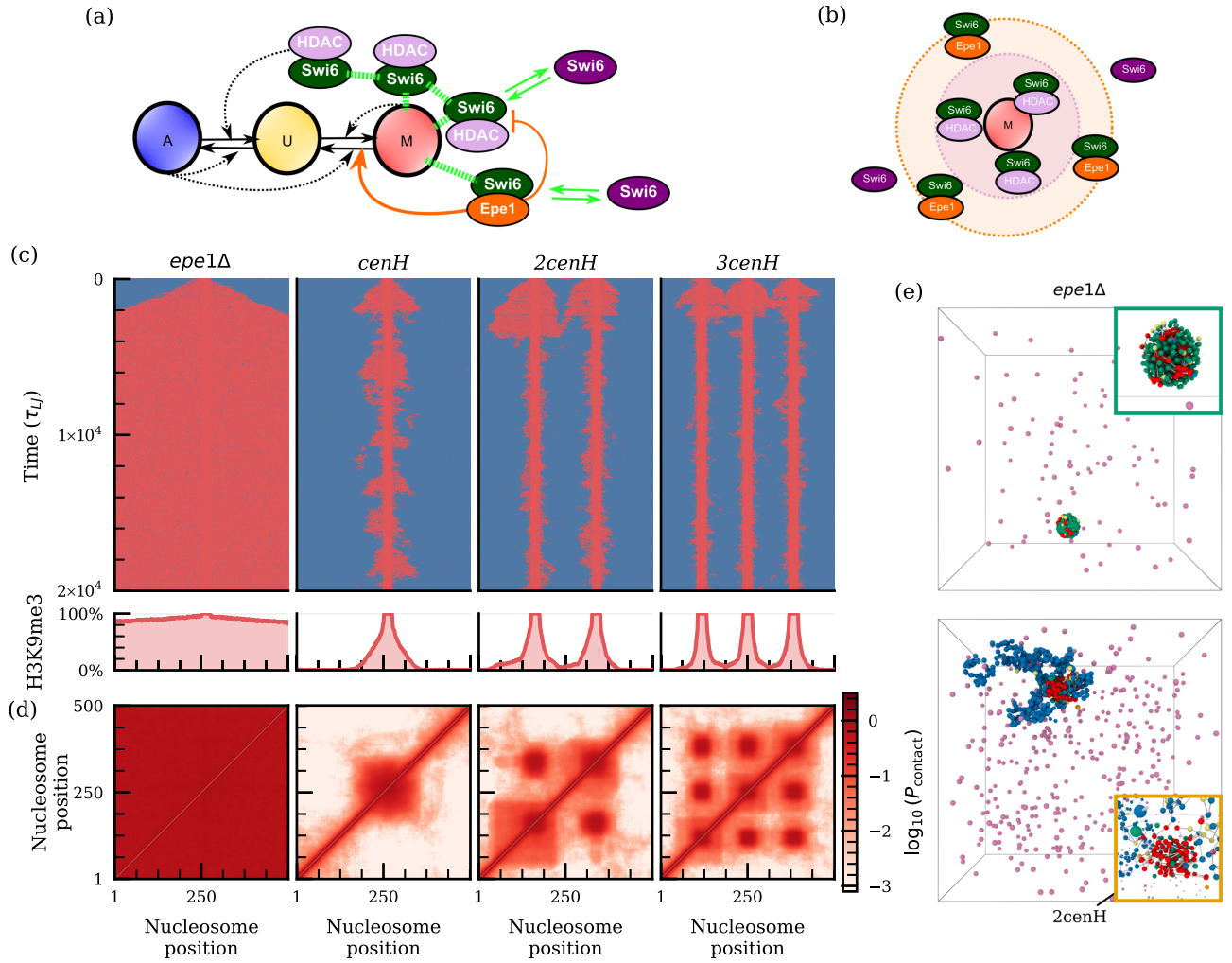


FIG. 7. Limitation of heterochromatin propagation by an Epe1 condensate shell. (a) A negative feedback by the antisilencing factor Epe1 was added to the basic model from Fig. 1. By associating with Swi6*, Epe1 stimulates demethylation of M nucleosomes. Moreover Epe1 competes with HDACs for association with Swi6*. (b) Depiction of the model where Swi6*Epe1 complexes localize in a shell around the Swi6*HDAC condensate. (c) Kymographs of 500-nucleosome polymer with one, two, or three heterochromatin nucleation sites, each of 25 M nucleosomes, during the course of simulations in the absence or presence of Epe1. (d) Contact maps averaged over entire simulations. (e) Snapshots of simulation box and zoom on condensates for indicated conditions. Supplemental movies are available for the simulation in the absence of Epe1 (Movie S10) and in the presence of Epe1 in the case of three heterochromatin nucleation sites (Movie S11 [84]). See Figs. S10–S13 for simulated cell cycles and corresponding Movies S12 and S13.

to the existing methylated fraction m . The loss term describes two consecutive reactions in the opposite direction, promoted by the acetylated fraction $1 - m$. The parameter Γ sets the rate of spontaneous conversion between nucleosome methylation states.

The dynamics of the local Swi6*HDAC concentration is described by

$$\frac{1}{\gamma} \frac{ds}{dt} = \frac{\rho}{V^{1/3}} \frac{mL}{V} \left(1 - \frac{L}{V} - s\right) - s \exp \left[-\beta \left(\frac{mL}{V} + s \right) \right]. \quad (2)$$

Here ρ is proportional to the nuclear density of Swi6, L is the number of nucleosomes in the locus under consideration, and V is the volume occupied by these L nucleosomes. The first term describes diffusion-limited capture of Swi6 molecules within an effective capture radius scaling as $V^{1/3}$, followed by

conversion to Swi6* at a rate proportional to the methylated fraction m . Implicitly, the saturation term assigns the same excluded volume to a nucleosome and a Swi6*. The second term accounts for the escape of Swi6* from a condensate of area $\propto V^{2/3}$ against an attractive potential generated by methylated nucleosomes and locally bound Swi6*HDAC complexes; it is assumed that this escape rate decreases exponentially with the effective potential strength. The parameter β characterizes the strength of effective interactions between Swi6*HDAC complexes and nucleosomes, as well as interactions between Swi6*HDAC complexes themselves. Finally, the prefactor γ absorbs a factor $V^{2/3}$ and sets the time scale of Swi6*HDAC dynamics relative to the nucleosome-state conversion processes.

For simplicity, we assume that the volume of the condensate decreases linearly with both the number of methylated nucleosomes, mL , and the number of locally enriched Swi6*

complexes, sV :

$$V = CL - 3mL - sV \Rightarrow V = \frac{C - 3m}{1 + s} L.$$

Here the linear approximation $V \sim CL$ reflects a polymer-contact scaling consistent with the molten-globule organization reported for nuclear DNA by [3]. The reduction of V with increasing m and s represents the condensate contraction driven by attractive interactions between methylated nucleosomes and Swi6*HDAC complexes.

Noticeably, the model does not invoke a phase transition in the equilibrium properties of the condensate. Instead, condensation emerges as a consequence of nonequilibrium changes in binding interactions between its constituents, as described by Eq. (1) and the recruitment of Swi6*HDAC by m in Eq. (2).

Figure 8 illustrates that the model readily supports the maintenance of epigenetic states across cell divisions. For further exploration of the model dynamics, we refer the reader to the interactive applet provided in [84].

We developed a 3D biophysical model to investigate the mechanisms of heterochromatin formation and its segregation from euchromatin, mediated by the protein Swi6 from the HP1 family. Our model integrates histone modification dynamics with chromatin folding and compaction, both facilitated by Swi6 complexes. Remarkably, we find that several hallmark features of heterochromatin observed *in vivo* emerge from a small set of minimal rules. These include the formation of compact chromatin domains (Fig. 2), bistable epigenetic states (Fig. 3), a strong dependence on Swi6 concentration (Fig. 4), and both *cis*- and *trans*-interactions between heterochromatic loci (Fig. 6). Moreover, an extension of our model incorporating the H3K9me antagonist Epe1 (Fig. 7) reveals how this factor can curtail unchecked heterochromatin growth. The formation of single or multiphasic condensates has previously been considered in analogy to wetting of a spherical substrate, where the condensate itself constitutes the substrate [104–107]. This analogy is grounded in equilibrium thermodynamics associated with differential binding energies, including wetting according to the Young-Dupré law. However, in biological systems, condensate formation may be regulated by active mechanisms that consume energy [108,109]. Our molecular dynamics simulations provide such a framework by combining protein-protein interactions with reactive feedback loops to sustain epigenetic memory. The robustness of alternate epigenetic states is explored numerically in Fig. 4 and through a mean-field approximation in Fig. 8.

Analogous to the Swi6/HP1-mediated feedback that stabilizes heterochromatin, positive feedback from active chromatin states is also supported by diffusible components. For instance, the metazoan bromodomain protein BRD4 undergoes liquid-liquid phase separation (LLPS) [110]. Its fission yeast homolog Bdf2 binds acetylated histones via dual bromodomains and promotes transcriptionally active chromatin by recruiting histone acetyltransferases (HATs), similar to BRD4 [59,111,112]. Future iterations of our model could incorporate such active chromatin regulators to examine the interplay between opposing feedback loops and their capacity to drive or delimit phase separation.

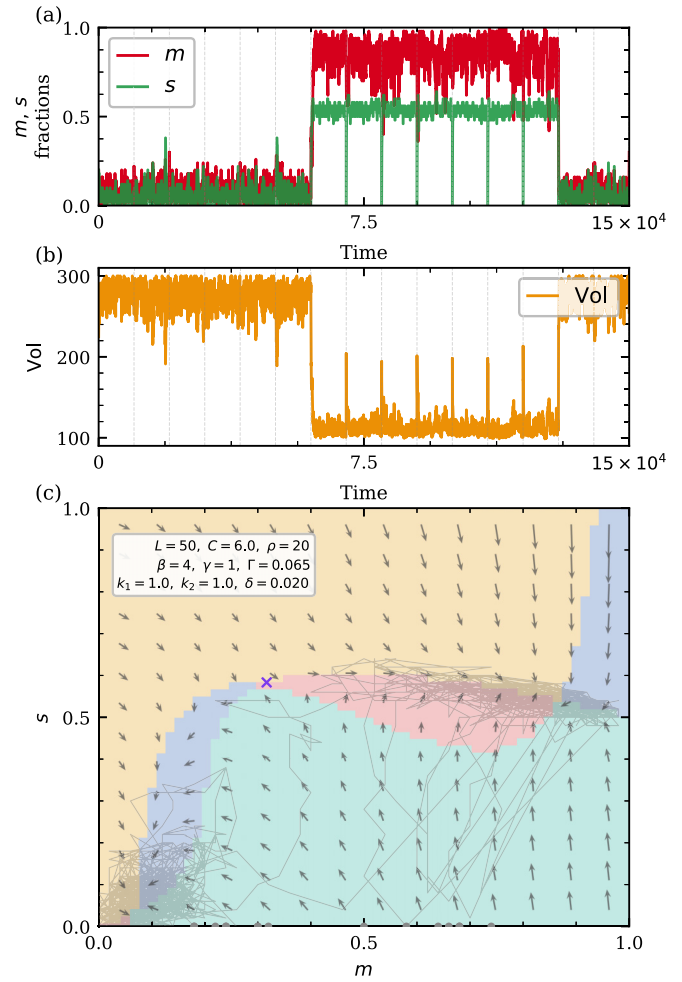


FIG. 8. Mean-field model outlined in Eqs. (1)–(3). (a), (b) The dynamics are simulated using a Gillespie algorithm with independent steps defined in Eqs. (1) and (2), using a step size $\delta = 0.02$. This corresponds to a system size of $L = 50$ nucleosomes. The cell division is treated more brutally than in our spatial simulations, as we at each division set $s = 0$ and select the new $m \in [0, 1]$ selected from a Gaussian with mean $\mu = m/2 + 1/4$ and standard deviation $\sqrt{\mu(1 - \mu)/L}$. The reader is invited to explore this simpler model through the applet in [84]. Panel (c) highlights the two stable fixed points, at low and high m respectively, while the middle fixed point is unstable. The colors in the phase diagram mark the direction of the average (m, s) movement in the corresponding region. The black line indicates the dynamics, with rare transitions between the stable fixed points.

Compared to earlier polymer-based recruitment models, such as that of Skjægstad *et al.* [33], our approach offers key advantages by incorporating diffusible mediators. Specifically, Swi6-mediated feedback in our model permits bistability without assuming an externally compressed euchromatic state. The diffusibility of Swi6 extends the spatial range of nonlocal recruitment, thereby facilitating nucleosome-mediated epigenetic states, including the fact that heterochromatin is more compact than euchromatin.

The inclusion of Bdf2/BRD4-mediated positive feedback introduces further regulatory complexity, particularly through interactions with Epe1 [59]. Notably, Epe1 dissociates more

slowly from Swi6 than Clr3, a histone deacetylase also recruited by Swi6 (or its paralog Chp2) [52]. This kinetic difference allows Swi6*Epe1 complexes to diffuse further from the heterochromatic core than Swi6/Chp2*Clr3, a feature we model by assigning a broader spatial range to Epe1. As a result, Epe1 localizes to a shell at the periphery of the condensate, which can constrain further expansion. This observation suggests that chromatin boundaries may arise at DNA loci that anchor such shells. Proteins or RNAs associated with boundaries could insulate heterochromatin condensates, and amphipathic factors like Epe1, capable of bridging euchromatic and heterochromatic phases, could position the boundaries by anchoring specific DNA elements at the phase interface.

Other models have used enzyme limitation to prevent the spreading of heterochromatin [26,34,37]. However, in fission yeast, overexpressing Swi6 rather than overexpressing enzymes, the HDAC Clr3 or the H3K9 methyltransferase Clr4, increases heterochromatin formation [49]. The effect of Swi6 is seen in our model, and is clear from ρ in the mean-field Eq. (2). Some models also place special nucleosomes at boundaries, for instance Mukherjee *et al.* [36] use nucleosomes whose methylation occurs at a slower time scale than for nucleosomes located within the intervening heterochromatic domain. A limitation in this case [36] is that the efficacy of the boundaries depends on cell-cycle time with marks expanding at reduced growth rates. By contrast, negative feedback limits heterochromatin expansion independent of cell cycle in our model. This is biologically important as cell-cycle length varies greatly depending on the conditions, even for a given cell type. It was recently discovered that negative feedback by HP1 exists also in mammalian systems where ASB7 is recruited to heterochromatin by HP1 to promote SUV39H1 degradation [113]. Thus, it will be interesting to keep exploring negative feedback in future modeling.

At the nuclear scale, our model captures how long-range genomic interactions between loci on different chromosomes or far apart on the same chromosome can nucleate heterochromatin formation. Importantly, this process silences the involved euchromatic locus and stabilizes the initial 3D contact that brought it close to heterochromatin. Thus, chromatin modifications and nuclear organization are mutually reinforcing, offering mechanistic insight into how coupled changes in H3K9me domains and genome topology may emerge during differentiation [14–17] or in disease states [18].

Our spatially explicit model yields several experimentally testable predictions. For instance, the hypothesized Epe1-enriched heterochromatin shell could be examined using super-resolution imaging to map the relative spatial distributions of Epe1, Swi6, and heterochromatin markers, refining previous observations [52]. In addition, the model predicts that proximity between genomic loci can induce heterochromatin formation even across unlinked regions. This prediction could be tested by artificially tethering a euchromatic target locus to a heterochromatic site, such as a centromere, and measuring the capacity of heterochromatin to induce silencing and domain formation *in trans*. Such experiments would further elucidate the role of 3D genome architecture in shaping epigenetic landscapes.

IV. MATERIALS AND METHODS

A. Simulation setup

The molecular dynamics simulations were performed with LAMMPS [79]. One *atom* in LAMMPS terminology corresponds here to one nucleosome or one protein complex. The simulations were conducted in Lennard-Jones (LJ) units with periodic boundary conditions. Atom interactions were modeled using a bond-style interaction to simulate bonded interactions and a pair style *morse/smooth/linear* for non-bonded interactions. The interactions were implemented with a cutoff distance of 3.0 units, representing short-range interactions. The movements of nucleosomes and protein complexes were updated with timestep $\Delta t = 0.001$ LJ time units. The movements of nucleosomes and protein complexes were updated with timestep $\Delta t = 0.001 \tau_{\text{LJ}}$ (simulation time unit) and limited to 1 space unit (σ) per Δt . One time unit is the same as the diffusion time, $\tau_D = \frac{\sigma^2}{D} = 1 = 1 \tau_{\text{LJ}}^{-1}$ (see Table I).

B. Interaction potentials

a. Bond Potentials. To simulate the interactions between DNA-linked nucleosomes we used a harmonic bond potential: $E = K(r - r_0)^2$ with $K = 10k_B T$, representing 10 times the thermal energy, and a preferred distance between neighbor nucleosomes along the polymer $r_0 = 1$ LJ unit.

b. Pair potentials. Every physical interaction except the bonds along the DNA was modeled by a Morse potential [115] (Fig. S1 [84]). The Morse smooth linear version was used to speed up simulations, and to provide continuity in both the energy and the force of the particles:

$$\phi(r) = D_{1/2}[e^{-2\alpha_{1/2}(r-r_{1/2})} - 2e^{-\alpha_{1/2}(r-r_{1/2})}], \quad r < r_c, \quad (3a)$$

$$E(r) = \phi(r) - \phi(r_c) - (r - r_c) \left. \frac{d\phi}{dr} \right|_{r=r_c}, \quad r < r_c, \quad (3b)$$

$$E(r) = 0, \quad r \geq r_c. \quad (3c)$$

Here $D_{1/2}$, $\alpha_{1/2}$, and $r_{1/2}$ depend on the interactions: repulsive and attractive interactions were defined for the heterotypic Swi6* interactions with M nucleosomes and homotypic Swi6* interactions (see Table I). In contrast, only repulsive interactions were used for all other interactions.

C. Groups and atom types

Units (“Atoms”) representing nucleosomes or Swi6 variants were made to interact in a state dependent manner, by separating them into “static groups” in LAMMPS. These groups were defined for specific states and updated dynamically throughout the simulation.

D. Equilibration and thermostat

The system was equilibrated using a combination of velocity perturbations from a normal distribution and Langevin dynamics, maintaining a constant temperature of 1.0 (LJ units). To establish thermal equilibration, the simulation was run for 10^3 time units before any conversion dynamics. The forces on

TABLE I. Comparison of dimensionless Lennard-Jones (LJ) units with real units.

Quantity	Dimensionless LJ Units	Real Units
Energy (ϵ)	1	$k_B T$ (J)
Bond energy (K)	10	$10 k_B T$ (J)
Length ($\sigma = r_0$)	1	Nucleosome bond length
Mass (m)	1	Nucleosome weight (≈ 200 kDa)
Boltzmann constant (k_B)	1	$1.38 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$
Temperature (T^*)	$\frac{k_B T}{\epsilon} = 1$	K (Kelvin)
Time (τ_{LJ})	$\sigma \sqrt{\frac{m}{\epsilon}} = 1$	Characteristic MD time unit
Friction (ζ)	1	kg/s
Diffusion coefficient (D)	$\frac{k_B T}{\zeta} = 1$	m^2/s
Diffusion time (τ_D)	$\frac{\sigma^2}{D} = 1$	s (diffusion over one bead)
Rouse time [114] (τ_R)	$\frac{N^2}{3\pi^2}$	Polymer relaxation time
Reaction time ($1/k_i$)	$1/k_i$	s
Conversion time ($1/\Gamma$)	$1/\Gamma$	s
Repulsive length (r_1)	1.0	Protein-protein repulsive length
Attractive length (r_2)	1.5	Protein-protein attractive length
Repulsive decay (α_1)	3.0	Protein-protein repulsive decay
Attractive decay (α_2)	2.0	Protein-protein attractive decay
Repulsive energy (D_1)	1.0	$k_B T$ (J)
Attractive energy (D_2)	10^{-2}	$0.01 k_B T$ (J)
Cutoff distance (r_c)	3.0	Protein-protein maximum interaction length

each unit were

$$F = F_c + F_f + F_r, \quad (4a)$$

$$F_f = -\zeta m v = -\gamma v, \quad (4b)$$

$$F_r \propto \sqrt{\frac{k_B T m}{\Delta t \zeta}} \quad (4c)$$

as described in the LAMMPS documentation. Here $F_c = -\nabla(E_p)$ is the force of the Morse and Bond potentials and F_f is the friction drag proportional to the velocity of the particle v . $\gamma = \zeta m$ is the friction coefficient, related to a relaxation time τ called *damp* in LAMMPS and set to 1 LJ time unit (τ_{LJ}). The velocity at time t has an exponential decay of τ .

The inertial effect takes 1 τ_{LJ} to take effect. This damping factor has been used in previous studies where $\zeta = 2$ to assure fast computation [116]. Atoms have more inertia than in reality but this will only affect short times. The solvent is modeled implicitly via a random force F_r . The microcanonical ensemble (NVE) allows for the integration of those forces via the Stoermer-Verlet time integration algorithm (velocity-Verlet) via the *fix nve/limit* LAMMPS function. This allows one to constrain the movement of the particle to a maximum of 1 length unit per time step.

E. Reactions and conversions

*a. Nucleosome- and Swi6*HDAC-mediated reactions.* Reactions were simulated using the REACTER protocol, implemented in LAMMPS as *fix bond/react* [80]. This method allows for the dynamic formation and breaking of bonds between specified atom pairs using a probability parameter. This probability was translated to a rate constant for time comparison with $p_i = k_i dt$.

We used a simplified version of REACTER [81,82] optimized for the coarse-grained reactions in this model. REACTER was modified to allow a single reaction template to update atom types, representing the state of nucleosomes or Swi6, regardless of their various chemical environment. This modification allowed “atoms” in proximity to each other to react without affecting their neighboring atoms or bonds. Besides this modification, all conditions described in Fig. 2 of [80] had to be satisfied for a reaction to occur, including the correct reactant types, sufficient proximity, the reaction probability and the selection among possible competing reactions. Reactions involving nucleosomes or Swi6*HDAC complexes were assigned a distance cutoff of 2 σ . All quantities are expressed in reduced Lennard-Jones (LJ) units (σ for space and τ_{LJ} for time), with the equilibrium pairwise interaction distance set to $r_0 = 1 \sigma$ and the cutoff radius for pairwise interactions set to $r_c = 3 \sigma$.

b. Noisy enzyme conversions. A modification loop was implemented to change the type of one randomly picked nucleosome with a rate constant Γ . We explored values from 1 to 4 τ_{LJ}^{-1} but settled mostly on 2 τ_{LJ}^{-1} (value used in simulations unless indicated otherwise). This process simulates the enzyme-catalyzed state changes of nucleosomes in the absence of direct recruitment. The Swi6* to Swi6 conversion was implemented as a deterministic periodic process with characteristic time $\tau_c = 0.2 \tau_{LJ}$.

F. Condensation without reactions

A simulation was performed to visualize the condensation and the coil-globule transition mediated by Swi6* in the absence of reactions [Figs. 2(e)–2(g)]. A more detailed analysis of this transition is presented in [117], where the local concentration of Swi6* in our model can be related to the effective

intermonomer interaction strength ϵ considered in that study. A relaxed polymer of 80 M nucleosomes was placed in a simulation box and 400 freely diffusing Swi6 dimers were added to the box to account for a dilute phase of the protein. The Swi6 dimers were not allowed to interact with M nucleosomes for the first half of the simulation. They were then all converted to Swi6* and allowed to interact accordingly for the second half of the simulation in the absence of Swi6*-to-Swi6 conversions. Histograms were derived for early and late parts of the simulation and analyzed to mimic the two-dimensional ($\Delta r = \sqrt{\Delta x_i^2 + \Delta x_j^2}$, with $i, j \in \{x, y, z\}$) displacement histograms obtained in experimental works by single-particle tracking of chromatin factors [118].

G. Data collection and output

The simulation output included nucleosome-state counts, reaction frequencies, mean square displacements (MSD), and radius of gyration, which was defined through $R_g^2 = \frac{1}{L} \sum_i (r_i - r_{cm})^2$ for a system of L nucleosomes with r_{cm} as their center of mass.

H. Bistability quantification on short time series

To test bistability for multiple variables and values without adding computational cost, we assumed that bistability would be seen not only for long time series (see Fig. 3) but also for pairs of short simulations starting with, respectively, a euchromatic polymer and a heterochromatic polymer bound by Swi6*. To quantitatively assess time series pairs, we computed $\phi(t) = \frac{A(t) - M(t)}{N}$ at every τ_{LJ} to distinguish between an A-dominated state ($\phi > 0$), an M-dominated state ($\phi < 0$) and a purely balanced state ($\phi = 0$). We then computed the relative time spent in each i state starting from a j condition, f_j^i . For example f_M^A is the fraction of time spent in an A-dominant state ($\frac{\tau_{\phi(t)>0}}{\tau_{total}}$), euchromatin, starting from an M-dominant (heterochromatin) condition. The bistability quantification is then $f_A^A f_M^M$, the monostable euchromatin quantification is $f_A^A f_M^A$, and the monostable heterochromatin quantification is $f_A^M f_M^M$. The phase diagrams are the addition of a green, a blue, and a red colormap weighted, respectively, for the bistability, A-monostability and M-monostability.

I. Cell cycle

Up to 100 cell cycles were simulated (see Fig. 5 and Figs. S10 to S13 and Movies S12 and S13 [84]). The total cell cycle length was $10^4 \tau_{LJ}$ with interphase occupying 80% of it (G1+S+G2) and Mitosis 20%. We assumed S phase occurred instantaneously (the *S. pombe* genome is 13 Mb and the total S phase for the whole genome is $\approx 10\%$ of the cell cycle. Thus, S phase in the 15 kb mating-type region would last in the order of 0.01% of the total cell cycle). Chemical reactions and polymer dynamics were performed as usual during the gap phases (G1 and G2). S phase consisted

in changing half of the total nucleosomes, randomly picked, to U nucleosomes. In mitosis, all chemical reactions (including noisy conversions) were slowed down by a factor 10^3 and the Swi6 to Swi6*HDAC conversion was slowed down in total by a factor 10^6 while the Swi6*HDAC to Swi6 conversion was performed at the usual rate. This resulted in extremely low Swi6*HDAC throughout mitosis and decompaction of the chromatin, the radius of gyration adopting a value comparable to an A-dominant state even with methylated histones.

J. Epe1 model

We modeled the formation of the Swi6*Epe1 complex through reactive molecular dynamics. The diffusive Swi6 was converted into Swi6*Epe1 complexes using a distance cutoff of 3 LJ units. However, within a distance of two LJ units, Swi6*Epe1 complexes were converted to Swi6*HDAC complexes to reflect competitions between the two and the more peripheral association of Epe1. This effectively restricts the localization of Swi6*Epe1 to a shell around the condensate of Swi6*HDAC. The Swi6*Epe1 complexes were meant to both compete with Swi6*HDAC and to catalyze the transition of M to U, helping to demethylate our model heterochromatin. This role was explicitly assigned, but according to reaction counts, the most significant outcome was to introduce the competition between Swi6*HDAC and Swi6*Epe1 thereby decreasing long-range catalysis by Swi6*HDAC. The Swi6*Epe1 complexes were assigned a very short life span outside the condensate, modeled by a decay rate to Swi6 of $5 \tau_{LJ}^{-1}$.

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A.B., K.S., and G.T. conceived the modeling; J.R.G. modified the REACTER protocol to permit the simulations in LAMMPS; A.B. performed the simulations; A.B., K.S., and G.T. wrote the manuscript; and all authors reviewed the manuscript.

No competing interest is declared.

DATA AVAILABILITY

The data that support the findings of this article are openly available. Software, source code, and mean-field applet are available at GitHub [119] and Zenodo [120].

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