Modeling the dynamic epigenome: from histone modifications towards self-organizing chromatin

Epigenetic mechanisms play an important role in regulating and stabilizing functional states of living cells. However, in spite of an increasing amount of experimental data, models of transcriptional regulation by epigenetic processes in particular by histone modifications are rather rare. In this perspective, we focus on epigenetic modes of transcriptional regulation based on histone modifications and their potential dynamical interplay with DNA methylation and higher-order chromatin structure. The main purpose of this article is to review recent formal modeling approaches to the dynamics and propagation of histone modifications and to relate them to available experimental data. We evaluate their assumptions with respect to recruitment of relevant modifiers, establishment and processing of modifications, and compare the emerging stability properties and memory effects. Theoretical predictions that await experimental validation are highlighted and potential extensions of these models towards multiscale models of self-organizing chromatin are discussed.

KEYWORDS: bistability ▪ chromatin remodeling ▪ cooperative binding ▪ epigenetic memory ▪ heritable cell fates ▪ histone modification ▪ multistate conversion ▪ theoretical model ▪ transcriptional regulation

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Epigenetic regulation of heritable cell fates, in other words the regulation and transmission of cellular states by mechanisms not stored in changes of the genome sequence, involves transcriptional repression and activation of genes. It has been implicated in many biological processes including development and stem cell differentiation as well as aging and cancer [1,2]. Epigenetic regulation is based on remodeling of the chromatin structure taking place during different time and length scales. In these processes, DNA methylation, RNA interference and post-translational modifications of histone tails play key roles [3–5].

The fundamental unit of chromatin is the nucleosome, an octamer of histones which consists of two copies of the core histones H2A, H2B, H3 and H4 and approximately 150 bp of DNA wrapped around it [6]. Histones are subject to a large number of covalent post-translational modifications such as methylation, acetylation, phosphorylation, ubiquitination and sumoylation. These modifications can be added and removed by chromatin modifying enzymes in a reversible manner (for a review see [7]). While in yeast many of the processes controlling local recruitment of the modifying enzymes are well studied, in higher eukaryotes and particularly in mammalian cells they are still far from being understood.

As a key part of the epigenetic machinery, histones and their modifications have been implicated in the propagation of gene regulatory states from the mother cell to daughter cells [8]. This epigenetic cell memory is crucial for the functioning of multicellular organisms: while cells share identical genomes, they need to maintain distinct functional identities, often in very similar environments [9]. At the same time, they must be able to adapt in a flexible manner to environmental challenges [10].

The question of how covalent modifications of histones impact the formation of chromatin structures that are capable of generating an epigenetic memory has been the subject of various theoretical investigations. Such models appear as a fundamental step towards general, bottom-up theoretical frameworks of chromatin dynamics that enable to derive quantitative predictions on (epi-)genotype–phenotype maps from general principles. These predictions, in turn, can be tested using experimental epigenetic data already available today. Motivated by these prospects, we review the recent development in this research field.

In the following discussions, we briefly address the historical and conceptual background of model development. Afterward, we review and compare formal models of histone modification dynamics (HMD). Finally, open questions regarding the embedding of these models into a systems biological framework are discussed.

Developing the concept of chromatin computation

In 1929, Emil Heitz discovered the differentiation of chromatin into heterochromatin and euchromatin along the DNA which he described as condensed and extended, respectively. He also proposed that these regions would differ in gene content and considered euchromatin to be genetically more important [11]. Probably inspired by the difference in gene regulation between prokaryotes and eukaryotes, concerning a default 'on' state of gene expression in prokaryotes and a default 'off' state in eukaryotes, Edgar and Ellen Stedman [12], proposed in 1950 that histones could be general repressors. As a consequence, it was assumed that gene activation in eukaryotes requires removal of histones. In 1964, Vincent Allfrey [13] speculated that histone acetylation might be correlated with gene activation. Not before the 1980s, Michael Grunstein [14] demonstrated that enzymatic modifications of histone tails were essential for gene regulation. The discovery by David Allis in 1996 [15] that these gene-regulating histone-modifying enzymes were themselves part of the gene transcription machinery closes the circle. The evidence of a feedback loop in histone modification led to the first models for propagation of chromatin states along the genome and models for transmission of the chromatin state across cell division to be developed.

These days, histone modifications are thought to regulate gene expression by direct modulation of RNA-polymerase recruitment or induction of chromatin conformations that do so. To understand and describe the complexity of the processes leading to this final output is the main subject of conceptual and formal models. The well-known conceptual model of an 'epigenetic code' tries to emphasize the complexity and assumes that there is a code, similar to the genetic code that maps histone modification patters to a biological meaning. The term 'code' is borrowed from semiotics. However, in a semiotic framework, the 'epigenetic code' is not well defined and the question arises as to whether such a code actually exists [16].

Information–theoretical descriptions of chromatin modifications use a set of 'rewrite' rules corresponding to the enzymatic modification reactions and model a sequence of transitions (see FIGURE 1 for an example) [17,18]. Implementation of such a model allows to simulate the impact of different rewriting rules into the dynamics of chromatin modification patterns. Thereby, the combination of different rewrite rules enables qualitatively different modes of information processing, also referred to as 'chromatin computation'. For example, the combination of histone modification readers with histone modification writers allows propagation of histone marks along the chromatin and eventually transmission of marks across cell division [18]. To emphasize the similarity between epigenetic regulation and computer programs, the terminology of computer science is frequently used. The mentioned concept is therefore classified as a finite state automaton.

Quantitative models of the organization, dynamics, stability and inheritance of chromatin, have been developed in the last years [9,19]. They can be regarded as specific applications of the finite state machines mentioned above. In the next section, we focus on such formal modeling approaches.

Formal models of histone modification dynamics

While many different hypotheses have been put forward on how chromatin modification states are established and maintained in living cells, and how combinatorial patterns of these modification states may contribute to transcriptional regulation and cellular memory, very few approaches have so far been developed that rigorously formalize basic dynamical properties of chromatin modification.

In the context of transcription factor (TF) networks, such formal approaches ranging from differential equation systems over Boolean networks to Petri networks (for a review, cf. e.g., [20]) haven proven tremendously successful and constitute a core part of what nowadays is called 'systems biology'. The modeling of chromatin dynamics shares many problems with TF network models, for example the choice of discrete versus continuous dynamics, and stochastic versus deterministic updates; however, additional aspects come into focus. Specifically, spatial effects associated to chromatin structure or DNA looping play a major role and coordinated interactions between different processes (e.g., histone modifications, DNA methylation and transcriptional regulation) impact systems behavior.

Treating all these aspects in a comprehensive, computational model currently appears out of reach and hence, existing models typically focus on subsystems of the epigenetic machinery. In this section, we focus on recently developed formal models of HMD covering different aspects
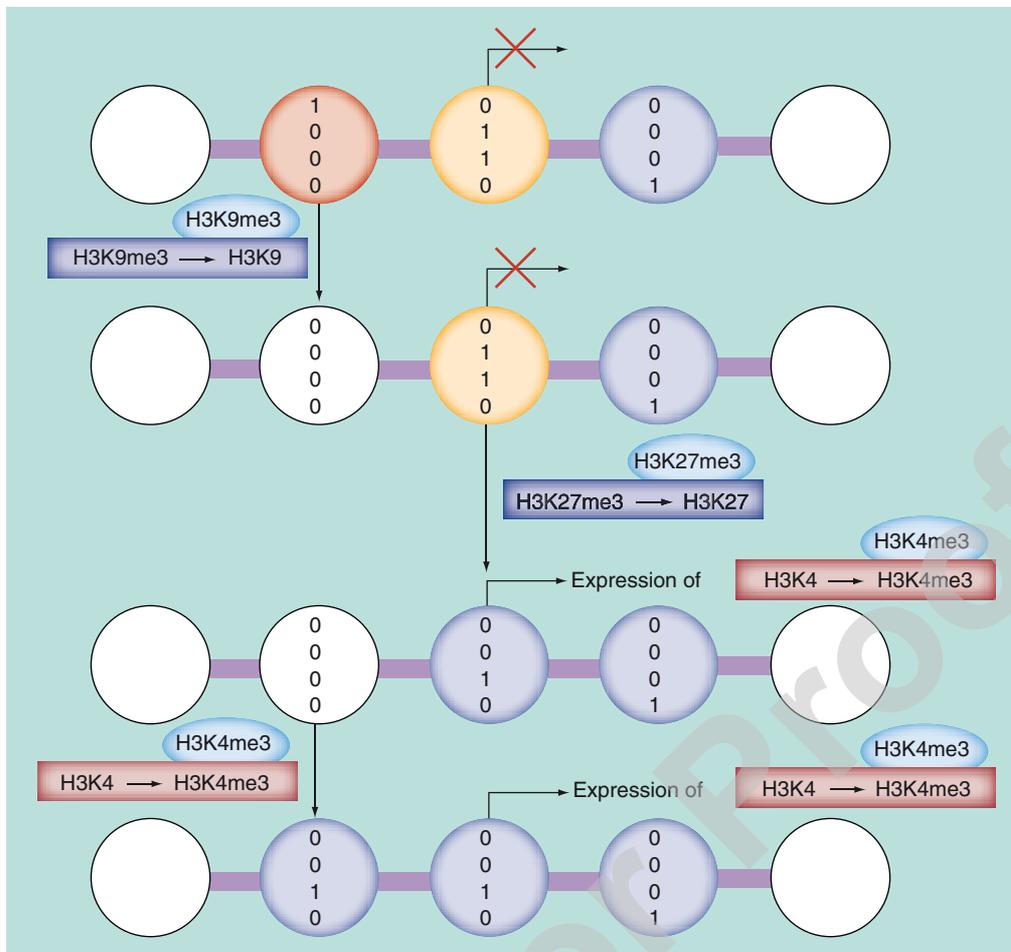
Figure 1. Epigenetic regulatory program. Changes to histone modification states can setup a regulatory program. Here, four different histone marks are considered, H3K9me3, H3K27me3, H3K4me3 and H3K4ac which can either be present or absent, represented by '1' or '0', respectively. The first two are considered to be repressive marks, while activating effects are attributed to both modification of H3K4. Nucleosomes are in a repressive state (red) or activating state (green) if there are more repressive or activating marks, respectively. Otherwise they are bivalent (yellow) or unmodified (white). If a promoter is covered by a nucleosome in an activating or repressing state, the downstream gene is transcribed or repressed, respectively. Here, the gene for a H3K4 methyltransferase is depicted. Changes in the histone modification state are carried out by enzymes composed of a histone modification reader (small white ellipse) and a catalytic domain that carries out a change (colored box). Shown above are examples for H3K9me3 and H3K27me3 demethylation and H3K4 methylation. Action of the enzymes may depend on the modification state. While (0000) becomes H3K4 methylated, (0001) is assumed to be not. Rewriting of histone modifications on the string of nucleosomes can be understood as cellular automaton computing gene-expression patterns.

of this epigenetic subsystem. We review common properties and differences, as well as prospects and limitations of these approaches. Our goal is to understand which types of chromatin-related phenomena can be explained and which predictions can be derived from these models, and how they ultimately could be embedded into a larger systems biology framework.

Different propagation mechanisms considered in HMD models, for example 'jump-like', 'localized' and 'nonlocalized' ones, can enable or exclude bistable behavior. Bistability, in general, is caused by positive feedback loops

between the components of the model which initiate cooperative (i.e., concerted in space and time) and hysteretic (i.e., history dependent) transitions between well distinguished epigenetic states associated with different modes of gene activity. The degree of bistability and the dynamics of switching, in turn, are governed by the relations between the amplitudes of feedback interactions and noise in the systems. The models discussed in the following sections consider different molecular components, mutual interactions and noise contributions.

The first model in this context was developed

by Dodd *et al.* [9]. They studied a stochastic and discrete cellular automata model for HMD based on the silent mating-type region of the yeast *Schizosaccharomyces pombe*. Assuming two mutually exclusive histone modifications and a nonlocal cooperative propagation mechanism of them they observed noise-dependent bistable switching between these states. A different HMD model was introduced by Sedighi and Sengupta [19]. Originally, it was developed to describe epigenetic silencing in budding yeast *Saccharomyces cerevisiae*, where the underlying epigenetic mechanisms are relatively well investigated [21]. In this model, silencing is considered to result from interplay between histone deacetylation and cooperative binding of so-called Sir protein complexes. A HMD-model comparable to that of Sedighi and Sengupta was recently introduced by Binder *et al.* [101] for epigenetic silencing in higher eukaryotes. The model, implemented in terms of time-continuous reaction kinetic equations, is motivated by the structure and function of Polycomb group (PcG) and trithorax group complexes in heterochromatin and euchromatin formation [22]. Binding properties of the complexes with respect to specific DNA sequences, as well as to modified histones are considered, establishing bistable switching behavior and memory effects in a wide parameter range.

Besides these models, we also briefly discuss models that cover additional aspects of HMD dynamics, as transitions between different modification levels [23] and explicit coupling of HMD to transcription and DNA methylation [24].

The following – certainly not exhaustive – list of basic problems regarding mathematical formalization of HMD models and their subsequent analysis will guide our overview: representation of chromatin structure in space and propagation of modifications; mechanisms controlling establishment and maintenance of modifications; cooperativity and bistability in HMD models; inheritance of histone marks; and coupling to transcriptional regulation.

■ Representation of space & propagation of modifications

Nucleosomes are the backbone of chromatin structure. Hence, assumptions about nucleosome positioning and chromatin folding constitute the basic level for theoretical models of HMD. While complex approaches for nucleosome (re-) positioning and association of histones and DNA have been developed [25], current HMD models typically assume a static chromatin structure represented e.g., by 1D linear chains of nucleosomes [9] or neglect space in a mean field-like manner [26]. Nucleosome position variation – at least on a local scale – is known to be limited [27] and hence may be neglected. In contrast, the linear chain approximation represents a strong restriction, since chromosomes undergo 3D folding [28,29] with a large impact on TF binding [30]. Nevertheless, all HDM models discussed in the following rely on the linear chain assumption.

Histone modifications can cover extended genomic regions. Adequate models have therefore been designed to address the question how modifications, initially starting from a small nucleation site, propagate along the nucleosome chain. This question is closely related to the spatial representation of chromatin which may (directly or indirectly) influence the mode of propagation.

Early models for nucleosome modification assume a linear stepwise process, where a modified nucleosome stimulates the modification of its nearest neighbors [4]. Yet, doubts on the effectiveness and stability of such a purely local propagation mechanism soon arose. As a consequence, Dodd *et al.* [9] proposed an alternative propagation scheme: they suggest that, within a confined chromatin region, any other histone potentially affects the modification state of a given histone, thereby allowing a discontinuous, 'jump-like' spreading of modifications. The authors show that this discontinuous propagation significantly improves switching between different (bistable) modification states. Sedighi and Sengupta [19] showed that also a localized propagation mechanism enables robust bistable switching in case that high cooperativity in recruiting (de-)modification complexes is assumed. In a recently developed model, we combined a 'nonlocal' propagation mechanism of modification states with the assumption of cooperative recruitment of modifiers to nucleosomes within a DNA-binding region of definite length. As a consequence, the mean modification state of all nucleosomes within this cooperative region determines the modification state of single histones in a self-consistent manner.

Rhagavan *et al.* [24] show that, in principle, also a coupling between DNA methylation and stochastic histone modification events can lead to propagation of histone modification patterns. These patterns differ depending on the degree of DNA methylation, with histone acetylation (methylation) occurring preferentially at high (low) DNA methylation levels.

Controlling establishment & maintenance of modifications

Chemical modifications of histones are under enzymatic control. The respective 'modifiers' must be recruited to the histones before they can start their 'writing' activity. In *S. cerevisiae* and in *D. melanogaster* the recruitment is known to be governed by sequence-dependent interactions with response elements (RE) (i.e., specific-sequence motifs on the DNA) [22,31]. This fact is often neglected in HMD models [19]. We recently considered sequence-specific recruitment of modifiers by assuming a finite but variable number of binding sites per DNA response element [101]. Unmethylated CpGs have been suggested as candidates for such binding sites in mammalian cells [32].

In general, different modification levels can exist, for example, several lysines can be mono-, di- or tri-methylated. In the abovementioned models, this is neglected. More comprehensive HMD models, however, will have to take into account different modification levels as well since they probably contribute to different regulatory functions [33].

Providing an example how to approach this problem, the coexistence of different histone modification levels in dividing yeast cells has been studied by de Vos *et al.* [23] using rate models. They described the transitions between mono-, di- and tri-methylation of H3 at lysine 79 (H3K79) by the methylase Dot1 using coupled ordinary differential equations. The authors show that the different methylation levels of H3K79 proceed with different kinetics. This result is consistent with a nonprocessive mechanism, where each subsequent reaction is found to be slower than the one before. In consequence, a steady-state modification level is reached for slowly cycling cells only linking different modification levels with cellular growth dynamics. In the more general framework of Rhagavan *et al.* [24] stochastic transitions between different modification levels at single histones are possible too, however, without an explicit link to a biologically motivated reaction kinetics.

Cooperativity & bistability

Development and cell differentiation, as well as cellular response to environmental challenges, require the involvement of bistable elements which switch gene activity between states of high and low expression levels and *vice versa* without changes to DNA sequence. Histone modifications provide one option of epigenetic control of such switching elements. However it remains unclear, how this mechanism can induce stable cell fate decisions in the presence of considerable noise at the single nucleosome level due to effects such as the stochasticity of histone modification reactions, or a high turnover of histones themselves [9,34]. The HMD models discussed here rely on two different mechanisms inducing bistable dynamics: indirect cooperativity induced by a multistep conversion between mutually exclusive histone marks [9]; and direct cooperativity induced by positive feedback between reading and writing of modifications [19].

Bistability as consequence of multistep histone state conversion

In their model, Dodd *et al.* describe HMD in a well-defined ~20 kb chromatin region of the fission yeast *S. pombe* corresponding to approximately n = 60 subsequent nucleosomes. A sketch of the model is shown in FIGURE 2A. Only three relevant kinds of nucleosomes are assumed: unmodified (U), methylated (M) and acetylated (A). In a more general interpretation these three states can be considered as 'unmodified', 'modified' and 'antimodified'. Nucleosomes are actively interconverted by modifying and demodifying enzymes (namely, histone methyl transferases [HMTs], histone acetyl transferases [HATs], histone demethylases [HDMs], and histone deacetylases [HDAs]). Dodd *et al.* assumed that conversions between A and M always proceed via the 'intermediate' U state. The model was implemented as a stochastic cellular automaton. Accordingly, discrete modification states of single nucleosomes are updated in discrete time steps, with either recruited, that is, deterministic, or noisy (random) conversions. The update rules for recruited conversions are given in FIGURE 2B and explained in FIGURE 2's caption.

The cellular automaton defined this way exhibits bistable behavior, which is controlled by the feedback:noise ratio F = α/(1 − α) (see FIGURE 2C). The authors demonstrate that it is the two-step conversion mechanism of the model which inherently implements this cooperative behavior. By limiting recruited modification of nucleosome (n1) to stimulation by adjacent nucleosomes (n2) only, the authors show that stepwise, local propagation of modifications produces poor bistability.

In a subsequent publication, Dodd *et al.* generalize the proposed mechanisms. Now, bistability is considered to be associated with valleys in a so-called 'epigenetic landscape' (see FIGURE 2D, and [35]) in a simplified two-state model (states
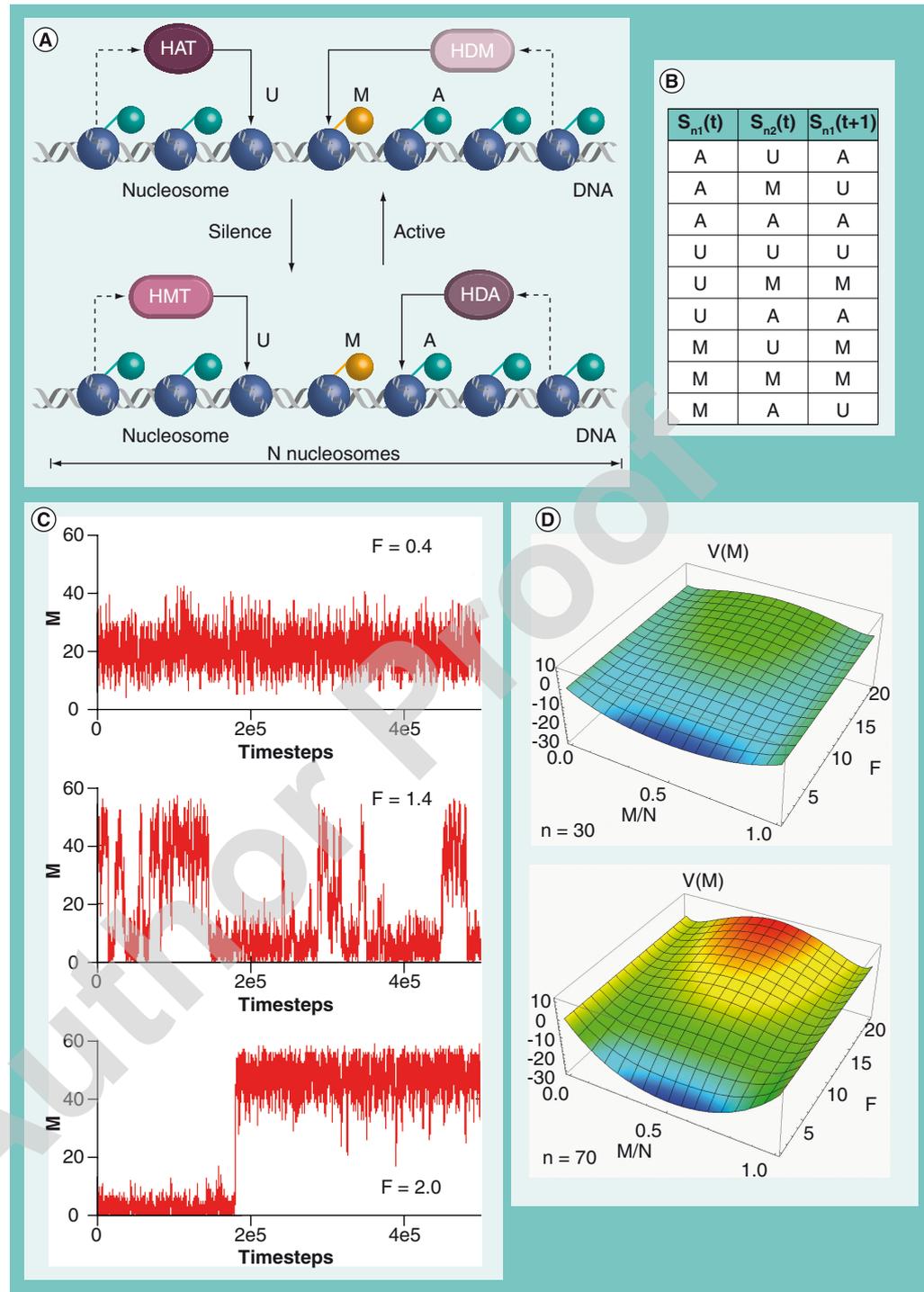
Figure 2. Histone modification dynamic model of acetylation/methylation balance in _Schizosaccharomyces pombe_. Please see facing page for the figure caption.
Reproduced from [9].

M and A). In a formal analysis of this model, changes of histone modification levels are treated as a diffusion process in an effective potential V(m) that describes the shape of the underlying epigenetic landscape. Interestingly, the valley structure of the epigenetic landscape becomes more pronounced with increasing number (n) of cooperatively interacting nucleosomes (**Figure 2D**), leading to an exponentially increasing stability (average lifetime) of epigenetic states with n. It turns out that bistability requires a minimum number of cooperative histones and that the size of the parameter space allowing for bistability increases with n.

Figure 2. Histone modification dynamic model of acetylation/methylation balance in *Schizosaccharomyces pombe*. See facing page. **(A)** The states (M, U and A) of a randomly selected nucleosome triggers the recruitment of specific enzymes (dotted lines) and a subsequent state transition (solid lines) of a second nucleosome. Involved enzymes are: HMTs, HATs, HDMs, HDAs. **(B)** The models' transition rules for recruited (deterministic) conversions. The algorithm selects at each time step (t) randomly a pair of nucleosomes (n1 and n2). Depending on the current modification states ($S_{n1}[t]$ and $S_{n2}[t]$) the new state of n1 at t+1 is determined. For example, the combination A (n1) and M (n2) leads to conversion A → U for n1. Changes of the type A → M or M → A thus, always involve at least two steps. Recruited conversions occur with probability α, additional noisy conversions with probability 1 - α. The latter changes the state of nucleosome (n1) randomly towards the possible neighboring states (e.g., A,M ➜U and *vice versa*). **(C)** Simulation results for the number of nucleosomes in state M over time for different feedback-to-noise ratios F = α/(1 - α), for n = 60 interacting nucleosomes. With increasing F the system starts exhibiting strong bistability. **(D)** Epigenetic landscapes for the simplified two-state model [35] considering different nucleosome numbers (n). Systems dynamics is described in terms of a Fokker-Planck equation (FPE). The effective potential V(M) obtained by solving the FPE is plotted against the fraction of nucleosomes in modification state M and the feedback-to-noise ratio F. The valleys become more pronounced with increasing n.
A: ; HAT: Histone acetyltransferases; HDA: Histone deacetylases; HDM: Histone demethylases; HMT: Histone methyltransferases; M: ; U: .
Reproduced from [9].

Bistability as a consequence of cooperative protein binding to chromatin

Sedighi and Sengupta [19] proposed a different mechanism (and formalism) to study bistable HMD dynamics. Their model is formulated in terms of chemical reaction equations for the local degree of histone acetylation (A) and the local probability of occupation by Sir (S) along a chromatin fiber of undefined length. Independent model parameters are the time-dependent bulk concentration of ambient Sir complexes and the rate of deacetylation. The model can be translated to the more general case of two modification states of chromatin where the production of one state depends nonlinearly on the other. Accordingly, the authors demonstrate that assumption of either cooperative Sir complex binding or of a 'transcriptional' feedback on the histone acetylation are sufficient to obtain bistable behavior in this model. This features are formally introduced by the functions $f(A)=(1-A)^n$ and $g(S)=(1-S)^m$, modulating the rates of Sir binding and of histone acetylation, respectively (n and m are adjustable parameters). The authors discuss the case of finite supply of Sir proteins as an interesting extension of the model. They considered a decrease of the bulk concentration of Sir assuming that deacetylation and Sir binding proceed along extended chromatin regions not subjected to partitioning by boundary elements. Sir binding is assumed to deplete its bulk concentration which in consequence decreases the Sir binding rate and thus slows down the propagation of the modification state. This topic has been investigated in more detail in a subsequent publication [36].

A HMD-model comparable to that of Sedighi and Sengupta was recently introduced by Binder *et al.* for epigenetic silencing in eukaryotes [101]. The model is motivated by the structure and function of PcG and tritho-rax group complexes in heterochromatin and euchromatin formation [22]. The basic framework and formal details of the model are summarized in FIGURE 3A. In this model, cooperative binding of a chromatin modifying enzyme to RE leads to bistable epigenetic states. Reversible binding of a protein complex to these specific genomic loci is described by a classical binding isotherm (FIGURE 3B, EQUATION 1). The bound complex is capable of writing modifications on the histones associated with the RE. Histone modification further facilitates complex binding. This positive feedback loop gives rise to bistability of the transcriptional activity of the genes associated with the RE.

The model shows bistable behavior regarding histone modification depending on the specific binding term ($-n_{BS}\varepsilon_{BS}$), and on the ratio $K_m = k^-/k^+$ characterizing the steady state of the modification reaction which is under enzymatic control. FIGURE 3C shows the respective hyperplane which divides into regions of monostable (blue) and bistable (red) solutions. The monostable region, in turn, is split into two states of high and low modification degree referring to silenced and active gene expression, respectively, in case of a repressive model.

Importantly, the maximum number of histones per RE, N_H, considerably affects systems behavior. It determines the strongest possible attraction that is exerted by the histones on the complex. In consequence, bistability is governed by the length of cooperatively acting chromatin given by N_H. Bistability requires a minimum length of the cooperative unit in agreement with the Dodd model discussed above (see FIGURE 3D).

In summary, the three models discussed above demonstrate that bistability in chromatin states, and thereby regulatory switching and hysteretic behavior, can be generated by different mechanisms that, however, share a common fundamental principle, namely cooperative behavior. In the following, we discuss implications of this principle on inheritance of cell fates.
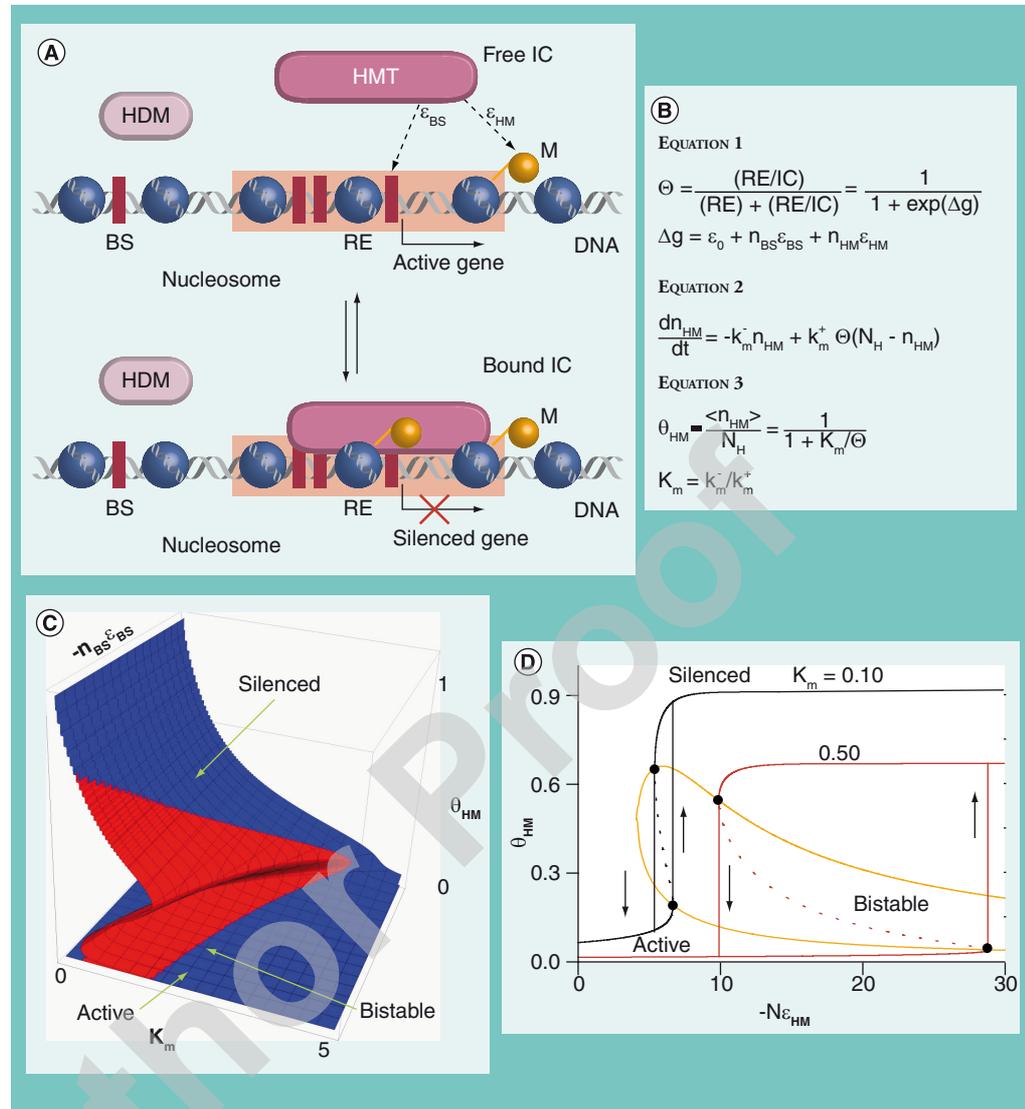
Figure 3. Histone modification dynamic model of histone methylation in eukaryotic cells.
(A) Free ICs interact with BS in the RE as well as with M. Bound ICs trigger additional histone modifications via HMTs that further improve binding. Assuming a repressive model, binding reversibly silences associated genes. Demodification by HDMs occurs permanently. **(B)** Main equations of the model. Equation 1: The free enthalpy Δg of interaction complex binding determines the RE-occupancy Θ depending on (i) a basal repulsive interaction term $\varepsilon_0 > 0$, (ii) an attractive term $\varepsilon_{BS} < 0$ for specific interaction with one of the n_{BS} DNA binding sites, and (iii) an attractive term $\varepsilon_{HM} < 0$ for interaction with one of the n_{HM} modified histones. Equation 2 describes changes of n_{HM} with time t. k^-_m and k^+_m Θ define the rates of demodification and modification, respectively and N_H the number of nucleosomes of the RE. The steady-state-solution of the model for n_{HM} normalized by N_H: $\theta_{HM} = <n_{HM}>/N_H$, is given by Equation 3. **(C)** Solutions of Equation 3 in dependence of the sequence specific binding energy $-n_{BS}\varepsilon_{BS}$ and the demodification constant K_m. The red area indicates bistable solutions while the blue area monostable ones. **(D)** Hysteretic behavior of the system in dependence of N_H in units of $-\varepsilon_{HM}$. The range of bistable solutions (orange line) is defined by the switching points of the trajectories for constant K_m.
BS: Binding site; HDM: Histone demethylases; HMT: Histone methyltransferases; IC: Interaction complex; M: Modified histones; N_H: ; n_{HM}: ; RE: Response element;
Reproduced from [101].

■ Inheritance of histone marks

In multicellular organisms, epigenetic regulation has evolved towards highly complex organization. In these systems, epigenetic cell memory is particularly important, because cells with identical genomes first must achieve distinct phenotypes in course of development and differentiation, but later on also be able to stably maintain their identities under cell divisions. This requires stable inheritance of epigenetic states (cell fates) across

many cell generations. In this context, DNA replication upon cell division poses a major problem: histone modifications are bound to get diluted during DNA replication, requiring a subsequent reconstitution of the parental state for faithful inheritance. A common model of this process, illustrated in FIGURE 4A, assumes that the parental nucleosomes are first randomly partitioned between the two daughter strands and then the diluted histone populations are complemented by *de novo* synthesized and assembled histones [37]. Heritable epigenetic states must be stable against such large-scale perturbations. This problem has been addressed in all three HDM-models discussed above. Consequences of histone dilution are shown in FIGURE 4B. Switches between active and silenced stable transcription states depend from the degree of histone dilution.

In their stochastic model, Dodd *et al.* [9] investigate the ability of high-M and low-M states to be maintained through DNA replication. In simulation series they replaced each nucleosome at the time of replication by a U (unmodified) nucleosome with a probability of a half. They found that high stabilities of modification states can be realized at modest feedback:noise ratios F. Interestingly, the number of switches becomes independent of generation time τ above a minimal value τ_{min}, and for F larger or equal than two. This result demonstrates that transitions between modification states are much more likely to occur immediately after replication than at any other point of the cell cycle. This dichotomy can be interpreted as a gradual development in a well-defined epigenetic landscape (compare FIGURE 2D) between cell divisions, with sudden reshufflings during replication that entirely dominate state transitions [35].

In order to systematically elucidate the conditions for stable inheritance of histone modification states David–Rus *et al.* [26] formulated a general stochastic model of epigenetic inheritance.
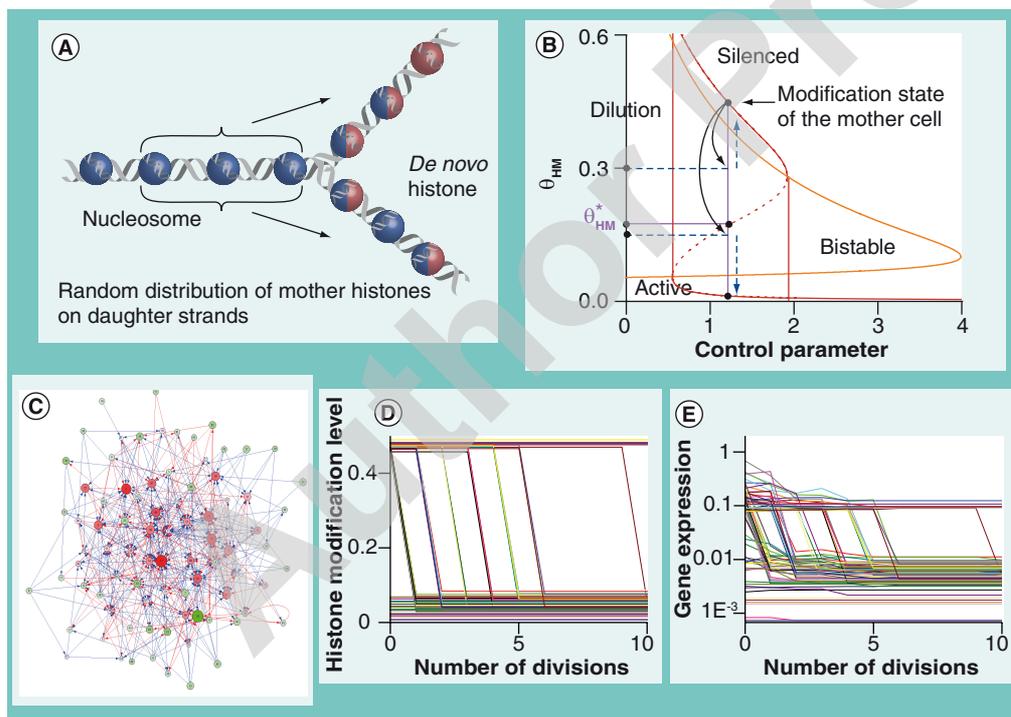

Figure 4. Inheritance of histone marks. (A) Dilution of histone marks. During cell division mother histones (blue) are randomly distributed onto the two daughter strands and complemented with *de novo* synthesized histones (red). **(B)** Consequences of marker dilution in the range of bistable solutions (orange line). Both daughter strands carry initially a lower marker density then the mother strand, which is assumed to be in a high modification state. Depending on whether the diluted states (blue dots) are located in the attractor of the low modification state or not a spontaneous demodification can appear. The attractors separate at the modification level θHM* (pink dot). **(C)** Transcription factor network generated by a random genome (RG) model. Red nodes represent activated genes, green nodes repressed genes. **(D & E)** RG dynamics under the control of an activating histone modification. The gene expression is assumed to be proportional to the histone modification level. Partial demodification of response elements in course of cell division **(D)** leads to decreased expression of genes associated with the response elements **(E)**. Each line represents one response element **(D)**, respective one gene **(E)**.

Subsequently, they developed a mean-field theory of the process, ignoring spatial variation of modifications and replacing them by an average value corresponding to the entire region of chromatin considered. They analyzed two- and three-state models of modification dynamics. The requirement of stability against perturbations at cell division imposes constraints on rate parameters. For two-state models, cooperative conversion of 'unmodified' (U) histones into 'antimodified' (A) ones must occur with a higher rate than vice versa. As a consequence of this asymmetry, fluctuations can flip a low-A state into a high-A states within a few cell cycles.

Three-state models (with the model by Dodd *et al.* [9] as a particular realization) are more stable in this regard. The authors suggest, that "the presence of multiple epigenetic marks is a design criterion for epigenetic stability", where the higher dimensionality of the state space gives rise to the increased stability of the system. We recently discussed an additional option to increase this dimensionality (and thereby stability) [101], namely the superposition of different interaction terms governing complex binding and cooperative modification dynamics. In the parameter range of bistability (compare FIGURE 4B) this can lead to an initial cascade of transitions during the first few generations of cells (reminiscent e.g., of a cell differentiation cascade) that quickly settles onto a stable differentiated stationary state (FIGURE 4C–E).

These studies demonstrate that epigenetic marks can be stable against perturbations only under very specific conditions. Thus, in cycling cells, and to a smaller degree also in quiescent cells, permanent epigenetic remodeling has to be considered, even in stable environments. Notably these changes are not purely random. Instead, changes in a particular chromatin region can be more likely than in other regions.

■ Coupling to transcriptional regulation

Processing of epigenetic states into cellular phenotypes requires transcription, and in the following translation of genes. Effects of chromatin conformations on TF binding are well established [4], however, descriptions of these phenomena often remain qualitative. The HMD models discussed above suggest that this coupling may lead to interesting new phenomena, for example, ultrasensitivity due to highly nonlinear amplification effects.

Sneppen *et al.* [38] showed that noncooperative binding of a TF to a single site can produce a large change in gene expression in response to even a small change in concentration of the TF. This ultrasensitivity is caused by the parallel recruitment of a histone-modifiying enzyme that changes the balance between the assumed feedback loops in histone modification. The resulting asymmetry between modification and antimodification can be interpreted as a TF-induced deformation of the underlying epigenetic landscape [35], pointing at 'dynamic epigenetic landscapes' as a possible general theoretical framework to model developmental and differentiation processes.

Coupling between histone modifications, DNA methylation and transcription is addressed in [24]. Specifically, the authors assume that, in every time step, the probability for transcription increases exponentially with the average number of histone acetylations, and decreases exponentially with average number of histone methylations. Histone modifications, in turn, depend on the degree of DNA methylation. Consequently, the model reproduces a decay of the average transcription rate with increasing DNA methylation.

In addition to TFs, also signal transduction networks can interfere with epigenetic systems [10]. In this context, the model of Dodd *et al.* was applied to a Polycomb-based switch with impact for the epigenetic memory in vernalization of *Arabidopsis* [39] (i.e., the acquisition of a plant's ability to flower or germinate in the spring). Experiments show that Polycomb PRC2-controlled silencing of the floral repressor FLC is involved in this process: H3K27me3 levels continuously increase within a small nucelation region at the *FLC* genomic locus during the cold. At more distal regions the increase of H3K27me3 levels depends additionally on the presence of a subsequent warm period. These results suggest a possible interference of histone modification with environmental signals.

Sedighi and Sengupta [19] and Mukhopadhyay *et al.* [36] show that the recruitment of histone modifying enzymes by TFs (or other molecular complexes) can substantially contribute to the formation of cooperative loops for writing and maintenance of modifications. Hence, current theoretical approaches – although far from realistic levels of complexity – already account for the crosstalk between different epigenetic control systems.

In conclusion, future (more general) models will have to address not only the effect of coupling between different types of histone modifications on (epi-)genotype–phenotype maps, but also

have to integrate the coupled dynamics between TF networks and chromatin-based regulation, as well as higher order effects (e.g., from the 3D structure of chromosomes). Considerations that may guide approaches towards adequate theoretical frameworks will be outlined in the following section.

Towards a systems biology perspective for epigenetics

In order to understand the consequences of a dynamic epigenome on the cell and tissue level, a number extension of the existing models of histone modification have to be considered. We here envision some first steps: adaptation of the models to experimental data; embedding of the models into a multiscale model framework of transcriptional regulation; and explicit representation of the 3D chromatin structure.

Adaptation of the models to experimental data

The genome-wide distribution of various histone modifications can be studied using a combination of ChIP with next generation sequencing (ChIP-seq) technology [40]. Large datasets of different types of modifications in different systems have been published, including data on embryonic stem cells, various lineage committed multipotent progenitors and differentiated cells [41–43]. A comprehensive toolbox is available for processing, analysis and visualization of these 3D datasets [44]. Although the models discussed here are all inspired by experimental results, direct adaptation of them to genome-wide data has been not provided so far. Angel *et al.* [39] for the first time fitted model output data to population averaged modification levels obtained by ChIP experiments. Thus, a major challenge posed in systems biology is the direct quantitative analysis of genome-wide modification data using concepts and hypotheses developed in modeling approaches on the dynamics and inheritance of histone modifications.

A quantitative approach of this kind is not only required for genome-wide modification data, but also for related expression data. However, the models discussed here have not been applied in such a context so far although they link the formation of different chromatin structures with switching of genes between active and silenced transcription in ultra-sensitive regulation circuits [38]. An essential step in that direction would be the integration of histone modification models into multiscale models of transcriptional regulation. Fortunately, the ChIP-seq datasets typically comprise both, modification and expression data, and thus, they can serve as starting point for such joint quantitative approaches (e.g., [14]).

Embedding of the models into a multiscale model framework of transcriptional regulation

Combined simulation of transcriptional regulation by *cis*-regulatory elements and histone modification will support our understanding of the impact of these different layers of regulation, for example, on development and stem cell differentiation. In this context, integration of other epigenetic modes of transcriptional regulation such as DNA methylation may be required. Molecular coupling of DNA methylation and histone methylation has been demonstrated recently [45,46] leading to a coordinated regulation of gene expression involving different time scales [5] and different potentials for stable inheritance of epigenetic information.

Models of the dynamics, stability and inheritance of DNA methylation have been introduced already [47,48]. They highlight the role of cooperative action of maintenance and de novo DNA methylation for stable inheritance of this epigenetic mark. Moreover, genome-wide high-resolution methylation maps are available that complement histone modification data [49]. Rhagavan *et al.* [24] recently demonstrated the possibility to integrate these processes in a common stochastic modeling framework. However, in their (mean-field like) approach DNA sequences, as well as regulatory interactions in TF networks are not explicitly represented. We believe that it is essential to integrate these aspects into realistic multiscale models of transcriptional regulation.

Artificial genomes may help to make progress into this direction. Random genomes (RG) as the simplest type of artificial genomes were developed a decade ago by Reil [50]. Recently, a specially designed RG model has been applied to analyze global gene-expression characteristics [51]. A TF network defined by a particular realization of a RG is shown in **Figure 4C**. We have linked this approach to the histone modification model of Binder *et al.* [101]. We assumed that genomic regions defining the genes in the RG are associated with cooperatively acting chromatin regions (separated from each other by, e.g., insulator elements). Moreover, we assumed direct proportionality between the promoter activity of the genes and the modification level of their regulatory region, as already suggested by Sneppen *et al.* [38]. In an exemplary study, we simulated how an activating modification corresponding, e.g., to H3K4me3 affects

demodification of chromatin regions in the course of proliferation (see FIGURE 4D). FIGURE 4E shows the corresponding progressive decrease of the expression levels of associated genes.

Explicit representation of the 3D chromatin structure

In our RG model each gene is regulated individually. Chromatin modifications, however, can induce coordinated expression changes of groups of neighboring genes. A prominent example of this property of chromatin represents regulation of the *Drosophila Hox* gene cluster. Wit and van Steensel [52] suggested three prototypes of multigene chromatin domains: the spreading of chromatin proteins along the DNA; looping of the chromatin fiber; and compartmentalization of nonadjacent chromatin regions by clustering. The models discussed here refer exclusively to the first option. In this case, a simplified 1D view on chromatin appears to be appropriate. Even these simple models can be made more realistic in straightforward ways, allowing to relate their predictions to available genome-wide data, for example, on the length distributions of modified regions. We were able to reproduce experimentally observed length distributions of modified H3K4me3 and H3K27me3 regions in murine stem cells by assuming insulator elements, which fragment the chromatin fiber into subdomains [53]. The derived values of model parameters such as modification rates or interaction strengths are critically related to bistability of chromatin modifications and associated gene activity [101].

Explicit consideration of chromatin structure reorganization, looping and compartmentalization, however, would require higher dimensional approaches. Note that the models of cooperative binding of histone modifying complexes discussed here already imply spatial effects via nonlocal interactions, however, without providing a physical model to explain them. Histone modifications are known to modify the electrostatic interactions between histones and DNA, as well as between nucleosomes. For example, acetylation of histone tails neutralizes their positive charges and thereby decreases their affinity to DNA, promoting the local formation of euchromatin [54]. Some progress to address these issues in formal models has been made. Arya and Schlick [55], for example, simulated a coarse-grained model of an oligonucleosome incorporating flexible histone tails, reproducing the conformational and dynamical properties of chromatin at various salt milieus. Specifically, they identified the important role of the H3 tails in screening electrostatic

repulsion between entering/exiting linker DNAs and mediating internucleosomal interactions. Including this basic biophysical layer into future HMD models, in principle, could provide a direct link between histone modification patterns and observed chromatin conformations.

Compartmentalization of chromatin into transcription factories providing disjoint local environments has been observed experimentally [56]. Moreover histone modifications and DNA methylation are thought to induce cooperative loopings of the chromatin fiber with implications for the activity of associated genes [56]. For example, looped domains may insulate chromatin from the influence of neighboring domains, and the bases of loops may also act to concentrate proteins locally within the nucleus [57] proposing that higher order folding of the chromatin fiber can serve to maintain active and repressed states of gene expression [58] or regulate the timing of transitions between poised and active gene expression [59]. So far, only a few computational models exist that address the dynamical interplay between transcription factories and chromatin folding [60].

The integration of such spatiotemporal aspects of chromatin organization into multiscale models of transcriptional regulation may represent another future step towards a comprehensive systems level description of the epigenome.

Conclusion

Epigenetic mechanisms of transcriptional regulation pose new problems in mathematical modeling. Particularly, the description of transcriptional regulation by histone modifications is of high relevance for the understanding of many biological processes, including development and differentiation. As the essential model ingredient required for epigenetic memory and inheritance of epigenetic information, the approaches developed so far always identified positive feedback loops based on cooperative nonlocal interactions between the histones and the modifying molecules. Moreover, such regulation circuits imply ultrasensitive responses of gene expression. Despite the success achieved in the description of basic principles of epigenetic regulation the comprehensive integration of whole-genome transcriptional and epigenetic data into modeling is still missing.

Future perspective

Epigenetic memory allows cells with identical DNA to maintain distinct functional identities. Patterns of epigenetic modifications have been demonstrated to diverge in monozygotic twins as

they become older [61]. Such differences have been related early either to environmental factors or to reduced inheritance of the epigenetic information during aging [62]. Regardless of the huge amount of experimental data that has been emerged since then, the mechanisms of epigenetic remodeling are still poorly understood. Reconstructing epigenetic networks ('modification webs') is still a largely unsolved problem [63] that provokes even more detailed and comprehensive measurements. However, there is increasing evidence that generally not all possible combinations of modifications can be observed and that large but specific patterns of modifications can characterize a single functional state [42]. This questions the design of many experimental studies.

Mathematical models of the dynamics, stability and inheritance of epigenetic marks allow to generate well-founded hypotheses regarding the mechanisms on work and to design effective protocols for their experimental validation. Thus, they will support cost-efficient research approaches. However, the prerequisite of a renaissance of such hypotheses-driven research approaches will be an explanatory understanding of measured absolute chromatin modifications levels.

An emerging field in epigenetic research is aging. Epigenetic changes have been linked to a decline in stem cells function [64,65] and epigenetic reprogramming is considered in future therapeutic applications [66,67]. Recent findings demonstrate that age-associated hypermethylation occurs in bivalent modified chromatin domains pointing to a close link between the different layers of regulation also in this process [68]. Aberrant epigenetic changes have also been recognized in cancer development and cancer epigenetics has reached mainstream oncology [69]. Only recently it has been shown that age-dependent DNA methylation at genes that are suppressed in stem cells is a hallmark of cancer explaining age as major risk factor in cancer [70].

In both aging and cancer development, the question arises why clones of cells which carrying a particular modified epigenome become dominate or vanish over time. Thus, in order to understand epigenetic phenomena in aging and cancer clonal competition in stem cell niches has to be considered. This will require simulation on the cell level describing growing populations and regenerating tissues. Individual cell-based models of such systems have been established [71–73]. We envision an integration of complex models of transcriptional regulation with these approaches into a comprehensive model framework.

Executive summary

Conceptual models

- Cooperative interplay between different histone modifications has been suggested to establish a 'histone code'.
- Finite state machines constitute a general, information–theoretical framework to study the impact of different rewriting rules on chromatin modification patterns.

Formal models

- Models of histone modification dynamics use simplified spatial structures (e.g., linear genome chain) and predict that long-range interactions between nucleosomes are essential for effective modification propagation.
- Different types of cooperative interactions can lead to bistability (switching between different modification states), effective parameter range increases with system size.
- Replication is introduced as a global stochastic fluctuation; the models discussed can reproduce stable inheritance of modification marks, as well as differentiation.
- Coupling to transcription is possible and predicts novel effects (e.g., ultra-sensitive gene regulation).

Towards a systems biology perspective for epigenetics

- Quantitative, multiscale approaches are needed that link predictive, formal models of the modification dynamics of different epigenetic marks and transcriptional regulation to genome-wide experimental data.
- Artificial genomes represent a first step towards such a comprehensive modeling framework in this direction.

Future perspective

- Mathematical models of the dynamics, stability and inheritance of epigenetic marks will lead to novel hypotheses guiding future design of experimental protocols.
- Particularly promising application fields are aging and cancer development.

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