IOP Publishing Phys. Biol. 13 (2016) 035006 doi:10.1088/1478-3975/13/3/035006

Physical Biology

RECEIVED
1 December 2015

REVISED
11 March 2016

ACCEPTED FOR PUBLICATION
8 April 2016

PUBLISHED
24 June 2016

PAPER

Computational strategies to address chromatin structure problems

Ognjen Perišić[1] **and Tamar Schlick**[2,3]

[1] Big Blue Genomics, 11000 Belgrade, Serbia
[2] Department of Chemistry, New York University, NY 10003, USA
[3] Courant Institute of Mathematical Sciences, New York University, NY 10012, USA

E-mail: schlick@nyu.edu

Keywords: chromatin structure, molecular modeling, molecular dynamics, coarse grained modeling

Abstract

While the genetic information is contained in double helical DNA, gene expression is a complex multilevel process that involves various functional units, from nucleosomes to fully formed chromatin fibers accompanied by a host of various chromatin binding enzymes. The chromatin fiber is a polymer composed of histone protein complexes upon which DNA wraps, like yarn upon many spools. The nature of chromatin structure has been an open question since the beginning of modern molecular biology. Many experiments have shown that the chromatin fiber is a highly dynamic entity with pronounced structural diversity that includes properties of idealized zig-zag and solenoid models, as well as other motifs. This diversity can produce a high packing ratio and thus inhibit access to a majority of the wound DNA. Despite much research, chromatin's dynamic structure has not yet been fully described. Long stretches of chromatin fibers exhibit puzzling dynamic behavior that requires interpretation in the light of gene expression patterns in various tissue and organisms. The properties of chromatin fiber can be investigated with experimental techniques, like *in vitro* biochemistry, *in vivo* imagining, and high-throughput chromosome capture technology. Those techniques provide useful insights into the fiber's structure and dynamics, but they are limited in resolution and scope, especially regarding compact fibers and chromosomes in the cellular milieu. Complementary but specialized modeling techniques are needed to handle large floppy polymers such as the chromatin fiber. In this review, we discuss current approaches in the chromatin structure field with an emphasis on modeling, such as molecular dynamics and coarse-grained computational approaches. Combinations of these computational techniques complement experiments and address many relevant biological problems, as we will illustrate with special focus on epigenetic modulation of chromatin structure.

Introduction

Celebrated as one of the highest achievements in science, the first mapping of the human genome in the dawn of the 21st century [1, 2], raised fundamental questions that may take a whole century to answer satisfactorily. The human genome, and genomes of all other higher organisms, contain much less genes than expected, but the amount of genes expressed in majority of tissues is higher than anticipated [3]. Moreover, simpler organisms can have comparable or even higher number of genes than human. These findings suggest that the diversity of living species stems not only from the multitude of genes, but also from many ways of their expression [3]. Although short segments of RNA can have enzymatic roles, they are not sufficient to account for the variety of cell types and cell signaling processes. Chromatin, the fiber that stores the genomic material in eukaryotes, holds one of the keys to the connection between the limited number of genes and the complex nature of higher organisms. Chromatin's primary role, to compress 2 m of total genomic DNA in humans into a micrometer sized cell nucleus, is accompanied by a more active role, namely the control of gene expression.

The chromatin fiber is made of double helical DNA wrapped around protein-octamer globules. Nucleosomes, chromatin's building blocks, are built of about 147 base pairs of DNA wrapped around highly conserved, four histone proteins (H2A, H2B, H3 and H4) [4], with the addition of the dynamically bound linker histone (LH) H1/H5. The interplay

between positively charged tails protruding from the histone cores with DNA and neighboring nucleosome particles and various chromatin binding factors controls the level of chromatin compaction [5, 6], from accessible double stranded DNA to fully formed chromosomes. That level may determine the type of cell, its status, and its future. Modern day genomics coupled with the next generation sequencing [7–9] reveals that the perturbation of the gene expression patterns can disrupt cell-signaling processes and affect human health [10, 11]. High throughput genome studies aimed at deciphering the spatial connectivity of chromosomal genomic regions [12] are suggesting that the structural diversity of chromosomes is related to gene expression patterns [13] and that genes and corresponding regulatory elements can be, sequentially and spatially, far apart in the genome [13, 14]. Therefore, deciphering chromatin structure and dynamics is of the crucial importance for understanding gene expression control in various organisms and tissues.

In this paper, we describe challenges associated with chromatin structure and the histone code, and then outline modeling approaches to study chromatin structure, dynamics, and the effect of epigenetic modifications on chromatin structure. We end by advocating code/resource sharing approaches to accelerate both experimental and modeling research in this area and to help make the necessary links between them.

Chromatin structure and the histone code

The internal organization of chromosomes and the corresponding structure of the chromatin fiber are still open questions [15, 16]. The internal organization of the 30 nm fiber, often observed *in vitro* via various optical techniques, was for a long time a holy grail of structural biology [17]. Two theoretical models were developed to address the 30 nm organization, one-start solenoid with bent DNA linkers [18, 19] and two-start zig-zag with straight linkers [20–22]. Zig-zag fibers are often observed under idealized experimental conditions [23–27], with mild ionic environments that discourage linker bending. Multiple experiments and simulations showed that the chromatin fiber is a highly dynamic entity with a very pronounced structural diversity sharing properties of both theoretical models [15, 16, 28, 29]. For example, cryo-electron microscopy and synchrotron x-ray scattering experiments observed a fractal-like organization of chromosome in human mitotic HeLa cells, without prominent 30 nm fiber like structures [30]. The absence of 30 nm motif was also demonstrated in recent cross-linking experiments of HeLa cells in combination with modeling that revealed zigzag motifs associated with hierarchically looped chromatin fiber in interphase and metaphase chromatin [31]. Real-world fibers interact with various enzymatic particles that disrupt the simple two-start nucleosome ordering. They do not fold into identically ordered structures in every cell because the entropic cost of such a folding process would be enormous [32]. Increased ion concentrations screen electrostatic repulsion between positively charged DNA fibers and allow their bending [28]. The naturally occurring variability of DNA linkers connecting successive nucleosome cores introduces a diversity in the fiber structure [28, 33, 34]. The H1/H5 LH dynamically binds to the nucleosome between two DNA strands (entering and exiting), draws them together, and establishes the nucleosome stem.

Prominent sources of fiber diversity are post-translational modifications of histones and histone tails [35–39]. Histone tails are typical targets for modification because they have prominent roles in inter- and intra-nucleosomal interactions and interactions with DNA linkers [28, 35, 39–41]. Modifications of histone core and tails also influence the stability and dynamics of nucleosomes [36, 38, 42–46], although early experiments did not observe a prominent role of tails in nucleosome stabilization [47, 48]. Therefore, histone modifications either induce partial unfolding of the chromatin fiber (euchromatin) or stabilize a tightly locked fiber (heterochromatin).

Enzymes introducing histone modifications are usually very selective [49]. They act upon a specific residue or a small group of selected residues. The three major covalent histone tail modifications are acetylation, phosphorylation, and methylation, see figure 1. These chemical changes reduce the tails charges and modify tails dynamics and thus influence their interactions with neighboring nucleosomes and DNA linkers. Other notable modifications with similar influences are ubiquitylation, sumoylation, ADP ribosylation, histone tail clipping, histone proline isomerization and β-N-acetylglucosamine modification [5].

The histone modifications have been a research topic for many years, but their importance came to prominence with the realization that the DNA contains much less genes than expected, and that their expression strongly depends on those heritable [49], albeit reversible modifications. Seemingly simple, these chemical changes produce perplexing effects, alone or in combination with other factors [5]. Acetylation neutralizes the positive charge of lysine and in this manner weakens interactions between positively charged histones and negatively charged DNA and exposes DNA to transcription mechanisms [49–55]. It is also involved in gene silencing [56], and DNA damage response [57–59]. Acetylation affects numerous lysines on all tails (see table 1), and the high number of possible sites where it may occur is an indication of the existence of hyper-acetylated regions that are devoid of tail charges and thus transcriptionally active [60, 61]. Phosphorylation adds a negative charge to serines, threonines and tyrosines, mostly in the histone *N*-terminal tails, but its mechanism of action is not based on electrostatic screening only. It is a highly dynamic, site-specific modification active in mitosis,

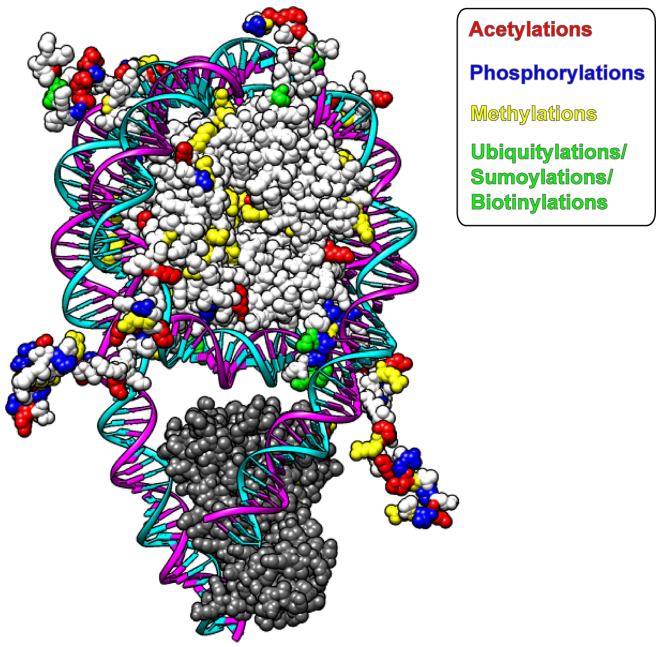

Figure 1. Positions of targets for major covalent histone modifications (pdb ID 1KX5). The histone octamer is white and DNA is pink and cyan. Acetylations are colored red, phosphorylation blue, ubiquitylations, sumoylations and biotinylations green, and methylations yellow. The linker histone (PDB IDs 1HST and 1C7Y) is gray.

meiosis, transcription activation, cell death, DNA repair, DNA replication and recombination [62–67] (see table 1). Phosphorylation is a strong signaling factor dependent on the cell cycle, and its dysregulation is highly correlated with cancer [66, 68]. Methylation is a physically small modification that does not alter the charge of a histone protein but can have a profound effect as a signaling factor for various cell processes. It modifies all histone molecules, including LHs. Methylation is associated with transcriptional activation, repression, silencing, euchromatin formation, and antagonistic or supportive cross-talks with other modifications as well as with DNA methylations [69–73] (see table 1). Ubiquitylation produces the largest physical modification of all reactions mentioned here because it attaches ubiquitin, a 76-amino acid polypeptide, to the lysine's Ɛ-amino group. Ubiquitin is a cell-signaling factor mostly involved in the degradation of proteins [74]. Histone ubiquitylation induces a change of the overall nucleosome conformation and in turn disrupts intra and inter nucleosomal interactions, as well as interactions with DNA or with other chromatin-binding factors. It has a role in gene silencing, meiosis, transcriptional activation and in euchromatin formation [75–77]. The same enzymes that attach ubiquitin are involved in sumoylation [78], a reaction that attaches small, an ubiquitin-like modifier molecules to histone lysines. This chemical change antagonizes acetylations and ubiquitylations that address the same lysine side chains [79, 80], and it is primarily associated with the transcriptional repression, though its direct physical effects are not yet known [5, 77, 79, 81]. Biotinylation is a modification that covalently attaches the vitamin biotin to a protein. Biotin is a small molecule, when compared to ubiquitin, but its binding to histone proteins influences gene expression, cell proliferation [82], transcription repression and histone dimerization [83], as well as the cell's response to DNA damage [84, 85]. Biotinylation may affect other histone modifications via crosstalk [82]. Gene expression can be also regulated though histone tail clipping, a procedure that removes residues from a histone tail. The procedure is present in many systems, from yeast to mammals [86–88].

The effects of histone modifications are sequence and context dependent. Besides directly affecting chromatin structure, they often attract factors that induce or remove modifications on neighboring residues, or they impair access to gene promoting or suppressing factors [72, 89]. Biochemical and physiological effects caused by histone modifications cannot be interpreted solely through their linear combination and could be perceived as a type of code that controls gene expression [90]. This histone code is, therefore, complementary to the four-letter genetic code that holds information for protein synthesis [90–92].

Chromatin modeling

The roles of histone components and histone modifications in chromatin structure formation can be addressed using various biophysical, biochemical, genetic or epigenomic experimental techniques. These techniques offer insights into the structure, and more importantly dynamics of chromatin fibers

Table 1. List of major histone covalent modification (A—acetylation, P—phosphorylation, U—ubiquitylation, S—sumoylation, M—methylation) listed by the histone and sequence position. The number of histone modifying factors (9th column) represents the relative importance a residue has within a histone code.

Tail	Residue	A	P	U	S	B	M	# modifying factors	Description
H1	Lys26						x	1	Transcription silencing [72, 89]
	Ser27		x					1	Transcription silencing, chromatin decondensation [72, 89]
	Lys48		**x**					1	Oxidative DNA damage [38, 93, 94]
	Arg54						x	1	Chromatin compaction, transcription [95]
	Lys65	x					x	2	Oxidative DNA damage [94, 96]
	Lys66						x	1	Oxidative DNA damage [94, 96]
	Tyr73		x					1	Unknown [96]
	Lys92	x					x	2	Oxidative DNA damage [93, 96]
	Lys99						x	1	Unknown [93]
H2A	Ser1		x					1+	Mitosis, chromatin assembly, transcription repression [97, 98]
	Arg3						x	4	Transcription activation, transcription repression [73]
	Lys4 (*S. cerevisiae*)	x						1	Transcription activation [99]
	Lys5 (mammals, *S. cerevisiae*)	x						3	Transcription activation, unknown [50, 100, 101]
	Lys7 (*S. cerevisiae*)	x						1	Transcription activation [99, 102]
	Lys9					x		1	Transcription repression [83]
	Lys13					x		1	Transcription repression [83]
	Lys36	x						1	Unknown [96, 103]
	Arg42					x		1	Unknown [38, 96]
	Lys74					x		1	Unknown [104]
	Lys75					x		1	Unknown [104]
	Arg77					x		1	Unknown [104]
	Arg88					x		1	Unknown [96]
	Lys95					x		1	Unknown [104]
	Gln105					x		1	Transcription [105]
	Lys119 (mammals)			x				1	Gene silencing [75]
	Thr119 (*D. melanogaster*)		x					1	Mitosis [106]
	Thr120 (mammals)		x					2	Mitosis, transcription repression [107, 108]
	Ser122 (*S. cerevisiae*)		x					1	DNA repair [64]
	Lys125					x		1	Histone dimerization [83]
	Lys126 (*S. cerevisiae*)				x			1	Transcription repression [81]
	Lys127					x		1	Histone dimerization [83]
	Lys129					x		1	Histone dimerization [83]

Table 1. (Continued.)

Tail	Residue	A	P	U	S	B	M	# modifying factors	Description
	Ser129 (*S. cerevisiae*)		x					2	DNA repair [109, 110]
	Ser139 (mammalian H2A.X)		x					3	DNA repair [111–113]
	Thr142 (mammalian H2A.X)		x					1	Apoptosis, DNA repair [114]
H2B	Lys5	x						2	Transcription activation [50, 115]
	Lys6 (*S. cerevisiae*)				x			1	Transcription repression [81]
	Lys7 (*S. cerevisiae*)				x			1	Transcription repression [81]
	Ser10 (*S. cerevisiae*)		x					1	Apoptosis [116]
	Lys11 (*S. cerevisiae*)	x						1	Transcription activation [102]
	Lys12 (mammals)	x						2	Transcription activation [50, 115]
	Ser14 (vertebrates)		x					1+	Apoptosis, DNA repair [63, 117]
	Lys15 (mammals)	x						2	Transcription activation [50, 115]
	Lys16 (mammals)	x						2	Transcription activation [102]
	Lys20	x					x	2	Transcription activation [50]
	Lys23						x		Unknown [96]
	Ser33 (*D. melanogaster*)		x					1	Transcription activation
	Ser36		x					1	Transcription activation [118]
	Lys43						x	1	Unknown [103]
	Lys57						x	1	Unknown [96]
	Arg79						x	1	Unknown [96]
	Lys85						x	1	Unknown [96]
	Arg99						x	1	Unknown [96]
	Lys120 (mammals)			x				1	Meiosis, development [76]
	Lys123 (*S. cerevisiae*)			x				1	Transcription activation, elongation, euchromatin [77]
	Arg2						x	2	Transcription repression [73]
	Thr3		x					2	Mitosis [119]
	Lys4 (*S. cerevisiae*)	x				x	x	8	Transcription activation (tri-me), gene expression, cell proliferation, permissive euchromatin (di-me) [82, 99, 120–124]
	Thr6		x					1	Unknown [125]
	Arg8						x	3	Transcription activation, transcription repression [126, 127]
	Lys9	x				x	x	10	Transcription activation, transcription repression (tri-me), genomic imprinting, histone deposition, gene expression, cell proliferation [51, 70, 71, 82, 128–131]
	Ser10		x					5	Transcription activation, mitosis, meiosis, immediate-early gene activation, DNA methylation [62, 132–135]

Table 1. (Continued.)

Tail	Residue	A	P	U	S	B	M	# modifying factors	Description
H3	Thr11 (mammals)		x					2	Mitosis [136]
	Lys14(12)	x						12	Transcription activation (elongation), DNA repair, transcription control, histone deposition, RNA polymerase II transcription, cell growth [50, 51, 56–58, 99, 100, 120, 128, 129, 137–139]
	Arg17						x	1	Transcription activation [140]
	Lys18	x				x		3	Transcription activation, histone deposition, gene expression, cell proliferation [50, 51, 82, 140]
	Lys23	x						3	Transcription activation, histone deposition, DNA repair [50–52, 128, 140]
	Arg26						x	1	Transcription activation [73]
	Lys27	x					x	3	Transcription silencing, X inactivation [130, 141]
	Ser28 (mammals)		x					3	Mitosis, immediate-early gene activation [133, 142, 143]
	Lys36	x					x	1	Transcription activation (elongation) [144, 145]
	Tyr41		x					1	Transcription activation [67]
	Arg42						x	1	Transcription activation [146]
	Thr45		x					1	Apoptosis [147]
	Lys56	x	x					1	Transcription activation, DNA repair, oxidative DNA damage [53, 59, 96, 148, 149]
	Lys57		x					1	Unknown [150]
	Arg63						x	1	Unknown [96]
	Lys64	x					x	2	Transcription [96, 104, 151]
	Lys79						x	1	Euchromatin, transcription activation (elongation), checkpoint response [152–154]
	Thr80		**x**					1	Mitosis [150]
	Thr118		**x**					1	Transcription, DNA repair [103, 155, 156]
	Lys122	**x**					**x**	2	Transcription, DNA repair [94, 96, 157]
	Arg128						**x**	1	Unknown [96]
	Ser1		x					2+	Mitosis, chromatin assembly, DNA repair [65, 97]
	Arg3						x	5	Transcription activation, transcription repression [73, 126, 158]
	Lys5	x						7	Transcription activation, histone deposition, DNA repair [50, 57, 58, 99, 100, 115, 120, 159]
	Lys8	x				x		9	Transcription activation (elongation), DNA repair [50, 57, 58, 84, 85, 99, 100, 115, 137, 160]

Table 1. (Continued.)

Tail	Residue	A	P	U	S	B	M	# modifying factors	Description
H4	Lys12	x				x		7	Transcription activation (elongation), DNA repair, histone deposition, telomeric silencing, euchromatin [50, 57, 58, 84, 85, 99, 100, 120, 159, 161]
	Lys16	x						7	Transcription activation, transcription silencing, DNA repair [39, 56–58, 99, 100, 102, 115, 160, 162]
	Lys20						x	5	Transcription activation, transcription silencing (mono-me), heterochromatin (tri-me), checkpoint response [163–165]
	Arg35						x		Unknown [96]
	Ser47		x						Nucleosome assembly [166]
	Arg55						x		Unknown [96]
	Lys59						x	1+	Transcription silencing, oxidative DNA damage [96, 167]
	Arg67						x	1	Unknown [96]
	Lys77	x					x	2	Unknown [96]
	Lys79	x					x	2	Oxidative DNA damage [94, 96]
	Tyr88		x					1	Unknown [96]
	Lys91 (*S. cerevisiae*)	x			.			2	Chromatin assembly [96, 168]
	Arg92						x	1	Unknown [96, 104]

[22–27, 168, 169], but they cannot fully describe the chromatin structural patterns. The difficulties stem from the tightly packed and still not fully accessible chromatin fiber interior and from the small size of histone modifications. Furthermore, laboratory preparations often put analyzed specimens in conditions not present *in vivo*. For example, *in vitro* fibers have equally spaced nucleosomes and usually lack histone modifications [168, 169]. In principle, systematic modeling can help address the influence of histone modifications on chromatin structure. This is a challenging task because the effects of histone modifications cover various spatial and temporal scales, which means that different levels of chromatin fiber organization have to be described using different theoretical and computational methods [6]. The first, basic level modeling has to cover is the influence of histone modifications on histone tails themselves. The second level is the modeling of the structure and behavior of nucleosome cores with and without modifications. Next, the behavior and structure of oligonucleosomes, with and without histone modifications, has to be accurately determined. The final level is the modeling of longer stretches of chromatin fiber and the modeling of whole chromosomes. All those four levels of modeling have to apply different tools and strategies in a selective manner, but they have to be consistent in the interpretation of fiber behavior.

Nucleosome and oligonucleosome modeling

Molecular dynamics uses the atom level interpretation of molecules to model their behavior in natural environments (for a detailed review see [170]). Its numerical complexity makes MD simulations of biomolecules applicable to systems with a limited number of atoms, usually less than ten million [171], including solvent and salt atoms [170]. The maximum time span MD can cover is in the range of microseconds [171–173], or milliseconds on special architectures (Anton [174]). The full atom interpretation of a dinucleosome in explicit solvent requires 800 000 atoms [39], which means that besides simulations of individual tails only a limited number of nucleosome related simulations have been conducted so far (see [175] for details). Although limited in scope, those simulations provide important insights into the behavior of the nucleosome and its components.

Many methods have been developed in attempt to improve the efficiency of molecular dynamics (MDs)

simulations [176–184]. The most commonly method used to reduce the numerical cost of MD is implicit solvent. This approach reduces the number of simulated atoms by representing solvent as a continuous medium, following the assumption that polar or charged molecules stay in water [185, 186]. This method is used to explore the protein folding and dynamic behavior of biological polymers, DNA, RNA and proteins. Its applicability can be limited in a tightly packed environment of the chromatin fiber interior, in which individual water molecules may affect the behavior of small peptides such as histone tails.

Many other methods have been developed, but their application to complex systems like the chromatin fiber remains to be shown as advantageous.

Coarse-graining interprets biological molecules less accurately than MD, but allows simulations of larger systems and makes possible more efficient sampling of the configurational space. Early coarse-grained models, although simple, were able to interpret basic structural patterns of the chromatin fiber and approach experimental findings [187–191]. Those models usually represented DNA as a worm-like-chain and nucleosomes as simple, rigid particles that interact via simple potentials. Advanced models use more detailed representations of nucleosomes and apply explicit tails.

Woodcock *et al* applied a model of the oligonucleosome fiber, based on a simple representation of rotatable nucleosome core particle (NCP) and variable-length DNA linkers with linker entry-exit angles, to interpret EM chromatin images [187]. A similar early coarse-grained model by Katritch, Bustamante and Olson was able to reproduce force-extension curves of real chromatin pulling experiments [192]. Later on, Wedemann and Langowski modeled NCPs as oblate ellipsoids in an elastic fiber [189]. Their Monte Carlo simulations of 100-NCP chains with elastic and electrostatic interactions reproduced experimental properties of 30 nm fibers with medium DNA linker lengths (they achieved a mass density of 6.1 NCP per 11 nm with 32 nm wide fiber). Later on, they added various chromatin structural elements to their model, such as the nucleosome stem [190] or irregularly spaced nucleosomes [193]. Many other groups also developed coarse-grained models to address the stability of nucleosome, its response to stretching, and to analyze interactions between DNA and cylindrical particles (nucleosomes) under physiological conditions [194–198]. See [6] for a recent overview of coarse-grained and multiscale modeling of chromatin.

Our group was among the first to address the role of histone tails in chromatin compaction using a detailed coarse-grained model of the nucleosome. The model uses different coarse-graining techniques to model basic chromatin building blocks. Nucleosomes, histone tails, DNA linkers and LHs are modeled as separate entities using appropriate theoretical models [15, 28, 33–35, 37, 199–205]. The nucleosome core (eight histones plus wrapped DNA), without histone tails, is modeled as a rigid object with irregular surface interpreted via approximately 300 charged beads [201]. The charge of each bead is assigned using the discrete surface charge optimization algorithm developed by Beard and Schlick [206] based on the Debye–Hückel approximation. The nucleosome core model robustly reproduces the electrostatic field of the nucleosome at physiological monovalent salt concentrations. DNA linkers between consecutive nucleosomes are interpreted as worm-like-chains. Their negative charges are modeled using Stigter's procedure [202]. The core histone tails are separately modeled, using the Warshel–Levitt united-atom protein model, with one bead per five amino-acids [35]. The LH was initially modeled as a rigid three-bead structure [15]. A recent refinement accounts for the inherently dynamic and disordered nature of the LH [199, 203]. The refined model includes flexible *C*-terminal domains and a globular head attached to the nucleosome [200]. Chromatin fiber configurations are sampled using an efficient set of Monte Carlo moves [35, 37].

Comparison with real-world experimental data has been used to validate the model. For instance, the model reproduces values of sedimentation coefficients, radios of gyration and packing ratios encountered in *in vitro* experiments [15, 28, 33, 34, 37, 200, 204, 205]. Various applications have detailed the influence of dynamic tails [35], variable linker lengths [207] and divalent Mg^{2+} ions [15], and the LH binding affinity [205, 208] on chromatin's structure [28]. Furthermore, fiber models share properties of both theoretical models (zig-zag and solenoid) [28]. The model with improved LH was able to explain the binding asymmetry and relate the LH condensation and nucleosome stem formation with the global condensation of chromatin [200]. A linear relationship between the LH concentration and the DNA linker length observed experimentally could also be related to the formation of a compact zig-zag fiber [209]. Forced induced unfoldings showed that LH increases fiber's resistance to unfolding, and that dynamic binding/unbinding of LH reduces it [204, 205]. Heterogeneous elements promote super beads-on-a-string configurations during stretching. Those configurations are biologically advantageous because they selectively expose DNA as 10 nm linkers between super-beads. The fibers with non-uniform linker lengths also exhibit smoother transitions because of a more continuous range of similar stable configurations [210]. The absence of 30 nm fibers was also shown in recent modeling work in collaboration with cross-linking experiments. Persistence of zig-zag motifs within a new model of hierarchical looping for metaphase chromosomes helps reconcile current models of polymer melts on one hand and other models that define clear chromosomal boundaries [31].

The histone tails have been a major topic of modeling studies due to their intrinsic disorder and their roles in fiber compaction. The tails were addressed using full-atom MD [45, 211] as well as coarse-graining [35, 37, 39, 41]. The researchers explored the roles of individual tails and their interactions with nucleosomes and DNA before shifting their focus to tail modifications. Our early studies, although less detailed than full-atom MD simulations, revealed that tails have multiple roles and are crucial for fiber compaction. They delineated roles of each tail, especially the role of the H4 tail in mediating internucleosomal interactions in highly compacted fibers with LHs, followed by roles of 'H3, H2A, *and* H2B *tails in decreasing order of importance*' [35, 37], a result concordant with experimental finding on the role of H4 tail in fiber condensation [54]. The nanosecond timescale MD simulations of the nucleosome core by Roccatano and coworkers [211] showed that at physiological salt concentrations histone tails adopt conformations in which tail segments preferentially interact with major and minor grooves of DNA and produce a slightly more compact and solvent-protected nucleosome, a result that contradicts experimental finding that tails adopt a largely solvent-exposed structure at salt concentrations above 50 mM NaCl [212]. H3 in their simulations exhibited the most noticeable conformational changes, correlated with the increased number of close tail-DNA contacts, while H2A domains showed affinity toward the DNA minor groove and higher mobility, likely caused by the presence of the H2A's long *C*-terminal tail. Roccatano and coworkers also showed that the canonical nucleosome particle is mostly rigid under physiological conditions during the nanosecond simulation runs. Over longer time scales, however, nucleosomes exhibit conformational heterogeneity and spontaneous unwrapping/rewrapping of nucleosomal DNA, as shown by the Langowski and Widom groups [213, 214]. MD simulations by Biswas and coworkers [45] showed that nucleosome simulations with truncated H2A and H3 tails produce less compact nucleosomes. The removal of these tails disrupts the electrostatic potential around the H2A histone, which in turn destabilizes the docking between the H2A–H2B dimer and the H3–H4 tetramer. The multiscale study of nucleosome unwrapping by the Langowski group [41], based on coarse-grained modeling and all-atom MD simulations, suggested that histone tails could have an opposite effect on the mononucleosome. While the attraction between the H3 tail and the 'acidic patch', formed on the nucleosome surface by seven acidic residues from H2A and H2B, can trigger partial unwrapping of DNA, and prevent the DNA rewrapping, the attraction between the H4 tail and the patch promotes full wrapping of the nucleosome. That indicates that the H3 tails actively participate in the initiation of the nucleosome remodeling. A recent study from the same group [40], based on replica-exchange and MDs simulations showed that the H2B and H4 tails have a single dominant binding configuration with DNA, while the H2B and H3 tails have multiple DNA binding configurations. However, large portions of tails were found not to be bound to DNA, which is an indirect conformation of their complex roles. The Rippe group studied the nucleosome's resistance to forced unwrapping using the steered MD (SMD) [215]. The analysis of SMD trajectories proposed a multistep process in which histone tails have prominent roles in the resistance to unwrapping. The authors also suggested that there are two main base-pair related barriers to unwrapping. However, results obtained with SMD simulations have to be taken with precaution because SMD perturbations are five to six orders of magnitude faster than experimental techniques. The Langowski group applied Brownian dynamics to a coarse-grained model with a uniform, distance-dependent potential to examine the mechanical unfolding of DNA from the torus-like nucleosome core [216]. Their simulations suggested a gradual unwrapping of DNA from nucleosome and reproduced force-extension curves for the low-force loading rate. Dobrovolskaia and Arya used coarse-grained models of nucleosome and DNA with experimentally derived position-dependent free energy profile to address the influence of non-uniform interactions between histone octamer and wrapped DNA on the nucleosome's resistance to external force [217].

An effective modeling of histone modifications requires both MD and coarse-grained approaches to describe the direct influence of modifications on the nucleosome and their influence on longer stretches of the chromatin fiber. Yang and Arya [218] confirmed the experimental finding [219] that the interactions between the H3 and H4 histone tails and the nucleosome's acid patch play a crucial role in the regulation of the nucleosome structure and may have a significant role in the overall fiber architecture. They suggested that the acetylation of the lysine 16 from the H4 tail reduces the alpha-helix forming propensity of H4 and destabilizes its binding to the acidic patch. In a similar study, Potoyan and Papopian [220] found that the acetylation of H4K16 induces partial ordering of the H4 histone and enhances its propensity toward alpha-helical organization. The ordering of the intrinsically disorder protein reduces the tail's binding affinity toward neighboring nucleosomes and weakens internuclosomal contacts, a result in concordance with experimental findings [54]. The acetylation of the H4 tail, despite reducing the positive tail charge produces a stronger attraction between the tail and DNA by inducing a partial collapse of the tail that enables it to make more hydrophobic contacts with the surface of DNA [220]. Our recent multiscale study follows the path established by our earlier studies and addresses seven major histone acetylations [39]. In that study Collepardo-Guevara and coworkers show through combined MD and

coarse-grained Monte Carlo simulations of 24 oligonucleotides that acetylations change the overall histone-tail flexibility and decrease the disorder of tails. The more folded tails have reduced interaction surface areas that limit inter-nucleosomal interactions in oligonucleosomes and trigger chromatin fiber opening. For instance, a H4 tail with acetylated residues has a limited ability to extend and reach neighboring nucleosomes but has a higher affinity toward parental DNA. The results clearly underscore many factors that affect the chromatin compaction in cooperative manner, and confirm that the hyperacetylation is not a prerequisite for a significant structural change. The acetylation of a single H4 lysine (H4K16) prohibits the formation of compact fiber and affects the formation of both higher order chromatin structures and functional interactions between chromatin fiber and non-histone proteins [54, 220].

Chromatin-protein interactions are an attractive research topic also. An MD study by Papamokos and coworkers [221] suggested that the binding of a protein important for the formation of the hypercondensed mitotic chromosomes (Heterochromatin Protein 1—HP1, [6]) to the H3 tail is strongly affected by the H3S10 phosphorylation. That study discovered that the phosphorylated H3S10 residue forms a salt bridge with H3R8 and thus impacts the HP1 binding to the methylated H3K9me2/3. A similar MD based study depicted the non-covalent binding of lysine specific demethylase-1 enzyme (LSD1) with its corepressor protein (CoREST) to chromatin [222]. This study is notable because LSD1 is one of the most promising epigenetic targets for drug discovery against cancer and neurodegenerative diseases [222]. The authors showed that the LSD1/CoREST complex binds to H3 via an induced-fit mechanism, an information potentially useful in designing LSD1 inhibitors. The combined docking, Brownian dynamics, and normal mode analysis by the Wade group [199] showed that the LH H1/H5 can adopt various docking positions near the nucleosome's dyad axis, a results in concordance with the latest experimental studies that suggests that asymmetric, on- and off-dyad, binding of the LH globular domain may lead toward distinct higher order chromatin structures [223]. The variable LH positioning is important because it allows the LH to influence the higher order structure of chromatin fiber in adaptable fashion through the modulation of entering/exiting angles of DNA linkers [224].

Polymer and continuum models

Polymer models are less refined than mesoscale models, but cover much larger spatial scales. They interpret chromatin as a chain of spherical beads that interacts through simple harmonic and Lennard–Jones systems, where each bead represents more than one nucleosome core. The polymer models have been developed as a response to large-scale experimental observations by various chromatin conformation capture techniques based on cross-linking of sequentially distant DNA segments. Those techniques produce kilo-base (3C), mega-base (4C and 5C) and genome-wide (Hi-C) interaction frequencies between genome loci in the nucleus as statistical averages over cell populations [225]. Interaction frequencies in single cells can be analyzed via 3C [226] and fluorescence *in situ* hybridization techniques [227]. Continuum models, on the other hand, were developed to interpret experimental data related to *in vivo* cell dynamics. They primarily use analytical approaches, based on fluid dynamics, to explain the behavior of large segments of chromatin in the cell nucleus.

Both experimental observations and simulations established the fractal model of the structural organization of chromosomes (first introduced by Grosberg and coworkers [228, 229]). The model assumes globular organization of chromosomes at almost all scales. In that case the contact probability of genomic loci as a function of the genome distance (or sub-chain length) follows the same scaling law. That law is the outcome of the self-similar polymer (chromatin) domains organized into non-equilibrium hierarchical structures with open-state and untangled topologies that rarely interfere with each other [230]. The Mirny lab introduced the fractal framework using a 20 nm bead polymer chain in which each bead covers six nucleosomes [231]. They were able to generate a fractal-like organization of their chain with a power law contact probability with the -1 exponent. However, the equilibration of that chain produced a uniform distribution, incompatible with the interphase chromosomes. Later on, they decreased the bead size to 10 nm and applied attractive and repulsive Lennard–Jones potentials, softened at short distances to allow chain crossing [232]. This refined model produced contact probabilities consistent with experimental data and proposed local loop formations, without reproducible radial positions, with the rest of their model chromosome being in disordered state. The model suggested a power law dependency of contact probability on genomic distance with the exponential factor -0.5 for short distances, that sharply decays with the increase in distance [232]. Barbieri and coworkers showed that fractal organization also depends on the concentration of protein cofactors able to bridge different genomic regions [233]. Simulations based on a polymer model composed of two set of beads by the Langowski lab [234] showed that yeast chromosomes have preferential positions in cell nucleus with dynamical clustering of functional elements of genomes. Jost and coworkers addressed epigenetic modifications through block organized chromatin model [235]. In their interpretation, the chromatin chain is organized into various blocks of identical monomers that preferentially interact with other monomers of the same

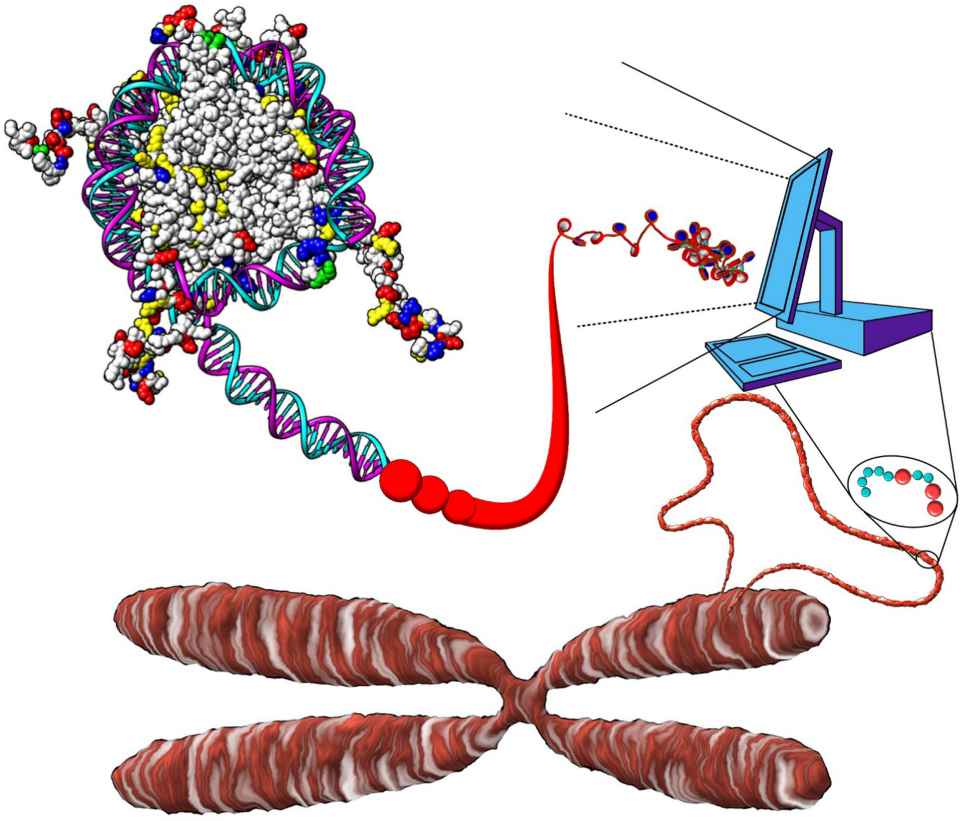

Figure 2. Multiscale chromatin modeling covers all levels of modeling, from atomic models of nucleosomes and histone modifications, via oligomers and polymer models to continuous models that interpret global chromatin motion in cell nucleus.

chromatin type over interactions with monomers of other types. They applied Langevin dynamics and Gaussian like potentials to sample fiber configurations. Their results are consistent with Hi-C data obtained from 10 Mbp drosophila regions. The topological domains they generated are related epigenetically and form a multistable fiber with coiled and collapsed microphase regions that separate different epigenetic domains. Bruinsma and coworkers [236] produced a two-fluid analytical model (nucleoplasm as a solvent and chromatin as a solute) to interpret experimental observation of coherent movements beyond single chromosomes [237]. They showed that the nucleus in ATP-depleted cells is characterized with passive longitudinal thermal fluctuations, while ATP-active cells have intense transverse long wavelength velocity fluctuations driven by force dipoles dependent on ATP. Isaacson and coworkers examined the time required to find specific DNA binding site as a function of the chromatin density (expressed as the volume exclusivity, a potential term that excludes a diffusing protein from a given volume filled with chromatin) [238, 239]. Their results based on a continuum model indicate that the time sharply decreases as the exclusivity increases and reaches a minimum value and then slowly increases with the increase in volume exclusivity.

Discussion

The chromatin structure problem has remained a challenge despite many recent advances in both experimentation and modeling. Its appeal comes not only from the puzzling nature of chromatin architecture and still unknown relationship between local fiber properties and behavior of the chromatin fiber at large, but also from the chromatin's key role in gene expression. And the deciphering that role has very practical implications because the disruption of DNA packaging has serious implications on health. Indeed, chromatin structure can be easily disrupted by histone modifications. That disruption can affect overall chromosome integrity and alter gene expression, including the aberrant regulation of oncogenes and/or tumor suppressors. However, new treatment avenues arise because the majority of histone modifications is reversible.

The detailed modeling of histone modifications is required for a proper interpretation of chromatin fiber structure and dynamics because experimentation is still limited in scope and resolution. The current modeling efforts addressed only general properties of chromatin fiber and described a very small number of histone modifications. To understand the chromatin fiber structure and its behavior, all modifications and their interplay have to be addressed in a systematic fashion.

That enormous task will require MD simulations of all tail modifications, within mononucleosome and without it. The puzzling role and behavior of LH has to be further examined, using docking, MD and coarse-graind simulations. Furthermore, MD simulations have to be performed on structures larger than dinucleosomes, with and without histone modifications. Simulations with four, or even more, nucleosomes are necessary if we want to fully grasp the role of tails in internucleosomal interactions. Those simulations have to based on different crystal structures because majority of current simulations has been based on the same high resolution structure, 1KX5 [175]. The role of DNA sequence in nucleosome positioning and fiber dynamics also has to be addressed in a systematic fashion. That will require all atom representations of DNA linkers and, probably, combination of classical and quantum MD. The nucleosome's mechanical resistance to unfolding has been addressed using only fast SMD [215], orders of magnitude faster that experimental stretching. New simulations have to be performed using much slower pulling on single nucleosomes with and without modifications, and, if possible, on equilibrated systems with more nucleosome cores.

All-atom simulations are, despite recent advances in hardware, numerically still very expensive. That means that longer stretches of fiber have to be simulated using coarse grained models. Current models are very detailed, but have to be improved to include all histone modifications, as well as LH and nucleosome core dynamics, especially during mechanical stretching. Moreover, they have to accurately interpret the influences of various binding factors and ion environments.

To facilitate research and collaboration, the modeling approaches should be able to record chromatin simulation trajectories, corresponding starting configurations, and force filed parameters in a standardized, human readable format(s) based on attribute-value pairs. Similarly, software tools should be open sourced and deposited in easily accessible online repositories. This can enable an approach to modeling where an appropriate algorithm can be called if necessary in semi-autonomous fashion. For instance, a coarse-grained model can invoke an atom-based model to interpret an unusual configuration. This could, in principle, allow zooming-in and zooming-out of chromatin regions, see figure 2, because information between modeling levels can be easily exchanged using a common data format. In that case, the long chromatin fiber is already in energetically favorable configuration which means that only sampling on a limited scale with spatial constraints, using local neighborhoods would have to be performed. Such a gradual, object-oriented modeling enables inheritance of general properties from one modeling level to another without the burden of increased numerical complexity. However, it has to be applied with caution because an apparently small modification with a limited influence on a local fiber neighborhood can have a crucial role in the behavior of longer stretches of the chromatin fiber. Those longer stretches often exhibit puzzling dynamic behavior that requires a detailed interpretation in the light of gene expression patterns in various tissue and organs.

Chromatin structure and its relation to the histone code remains a central question in structural/molecular biophysics and genetics. Addressing it fully requires a joint effort of experimentalist and modelers. To facilitate these efforts, a more facile information flow and data/program sharing between different teams could be valuable.

References

[1] International Human Genome Sequencing Consortium 2001 Initial sequencing and analysis of the human genome *Nature* **409** 860–921

[2] Venter J C *et al* 2001 The sequence of the human genome *Science* **291** 1304–51

[3] Uhlén M *et al* 2015 Tissue-based map of the human proteome *Science* **347** 1260419

[4] Luger K, Mäder A W, Richmond R K, Sargent D F and Richmond T J 1997 Crystal structure of the nucleosome core particle at 2.8 Å resolution *Nature* **389** 251–60

[5] Bannister A J and Kouzarides T 2011 Regulation of chromatin by histone modifications *Cell Res.* **21** 381–95

[6] Ozer G, Luque A and Schlick T 2015 The chromatin fiber: multiscale problems and approaches *Curr. Opin. Struct. Biol.* **31** 124–39

[7] Mardis E R 2008 The impact of next-generation sequencing technology on genetics *Trends Genet.* **24** 133–41

[8] Quail M A, Smith M, Coupland P, Otto T D, Harris S R, Connor T R, Bertoni A, Swerdlow H P and Gu Y 2012 A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers *BMC Genomics* **13**

[9] Pabinger S, Dander A, Fischer M, Snajder R, Sperk M, Efremova M, Krabichler B, Speicher M R, Zschocke J and Trajanoski Z 2013 A survey of tools for variant analysis of next-generation genome sequencing data *Briefings Bioinformatics* **15** 256–78

[10] The Cancer Genome Atlas Research Network 2008 Comprehensive genomic characterization defines human glioblastoma genes and core pathways *Nature* **455** 1061–8

[11] Brennan C W *et al* 2013 The somatic genomic landscape of glioblastoma *Cell* **155** 462–77

[12] Dekker J, Rippe K, Dekker M and Kleckner N 2002 Capturing chromosome conformation *Science* **295** 1306–11

[13] Dekker J 2008 Gene regulation in the third dimension *Science* **319** 1793–4

[14] Naumova N and Dekker J 2010 Integrating one-dimensional and three-dimensional maps of genomes *J. Cell Sci.* **123** 1979–88

[15] Grigoryev S, Arya G, Correll S, Woodcock C and Schlick T 2009 Evidence for heteromorphic chromatin fibers from analysis of nucleosome interactions *Proc. Natl Acad. Sci* **106** 13317–22

[16] Schlick T, Hayes T and Grigoryev S 2012 Toward convergence of experimental studies and theoretical modeling of the chromatin fiber *J. Biol. Chem.* **287** 5183–91

[17] Grigoryev S and Woodcock C L 2012 Chromatin organization—the 30 nm fiber *Exp. Cell Res.* **318** 1448–55

[18] Thoma F, Koller T and Klug A 1979 Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin *J. Cell Biol.* **83** 403–27

[19] Finch J T and Klug A 1979 Solenoidal model for superstructure in chromatin *Proc. Natl Acad. Sci.* **73** 1897–901

[20] Staynov D Z 1983 Possible nucleosome arrangements in the higher-order structure of chromatin *Int. J. Biol. Macromol.* **5** 3–9
[21] Williams S P, Athey B D, Muglia L J, Schappe R S, Gough A H and Langmore J P 1986 Chromatin fibers are left-handed double helices with diameter and mass per unit length that depend on linker length *Biophys. J.* **49** 233–48
[22] Athey B D, Smith M F, Rankert D A, Williams S P and Langmore J P 1990 The diameters of frozen-hydrated chromatin fibers increase with DNA linker length: evidence in support of variable diameter models for chromatin *J. Cell Biol.* **111** 795–806
[23] Horowitz R A, Agard D A, Sedat J W and Woodcock C L 1994 The three-dimensional architecture of chromatin in situ: electron tomography reveals fibers composed of a continuously variable zig-zag nucleosomal ribbon *J. Cell Biol.* **125** 1–10
[24] Dorigo B, Schalch T, Kulangara A, Duda S, Schroeder R R and Richmond T J 2004 Nucleosome arrays reveal the two-start organization of the chromatin fiber *Science* **306** 1571–3
[25] Schalch T, Duda S, Sargent D F and Richmond T J 2005 X-ray structure of a tetranucleosome and its implications for the chromatin fibre *Nature* **436** 138–41
[26] Scheffer M P, Eltsov M and Frangakis A S 2011 Evidence for short-range helical order in the 30 nm chromatin fibers of erythrocyte nuclei *Proc. Natl Acad. Sci.* **108** 16992–7
[27] Song F, Chen P, Sun D, Wang M, Dong L, Liang D, Xu R M, Zhu P and Li G 2014 Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units *Science* **344** 376–80
[28] Perišić O, Collepardo-Guevara R and Schlick T 2010 Modeling studies of chromatin fiber structure as a function of DNA linker length *J. Mol. Biol.* **403** 777–802
[29] Eltsov M, MacLellan K M, Maeshima K, Frangakis A S and Dubochet J 2008 Analysis of cryo-electron microscopy images does not support the existence of 30 nm chromatin fibers in mitotic chromosomes *in situ Proc. Natl Acad. Sci.* **105** 19732–7
[30] Nishino Y *et al* 2012 Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30 nm chromatin structure *EMBO J.* **31** 1644–53
[31] Grigoryev S A, Bascom G, Buckwalter J M, Schubert M B, Woodcock C L and Schlick T 2016 Hierarchical looping of zigzag nucleosome chains in metaphase chromosomes *Proc. Natl Acad. Sci.* **113** 1238–43
[32] Mirny L A 2011 The fractal globule as a model of chromatin architecture in the cell *Chromosome Res.* **19** 37–51
[33] Schlick T and Perišić O 2009 Mesoscale simulations of two nucleosome-repeat length oligonucleosomes *Phys. Chem. Chem. Phys.* **11** 10729–37
[34] Collepardo-Guevara R and Schlick T 2014 Chromatin fiber polymorphism triggered by variations of DNA linker lengths *Proc. Natl Acad. Sci.* **111** 8061–6
[35] Arya G and Schlick T 2006 Role of histone tails in chromatin folding revealed by a new mesoscopic oligonucleosome model *Proc. Natl Acad. Sci.* **103** 16236–41
[36] Mersfelder E L and Parthun M R 2006 The tale beyond the tail: histone core domain modifications and the regulation of chromatin structure *Nucleic Acids Res.* **34** 2653–62
[37] Arya G and Schlick T 2009 A tale of tails: how histone tails mediate chromatin compaction in different salt and linker histone environments *J. Phys. Chem.* A **113** 4045–59
[38] Tessarz P and Kouzarides T 2014 Histone core modifications regulating nucleosome structure and dynamics *Nat. Rev. Mol. Cell Biol.* **15** 703–8
[39] Collepardo-Guevara R, Portella G, Frenkel M D, Schlick T and Orozco M 2015 Chromatin unfolding by epigenetic modifications explained by dramatic impairment of internucleosome interactions: a multiscale computational study *J. Am. Chem. Soc.* **137** 10205–15
[40] Erler J, Zhang R, Petridis L, Cheng X, Smith J C and Langowski J 2014 The role of histone tails in the nucleosome: a computational study *Biophys. J.* **107** 2911–22
[41] Voltz K, Trylska J, Calimet N, Smith J C and Langowski J 2012 Unwrapping of nucleosomal DNA ends: a multiscale molecular dynamics study *Biophys. J.* **102** 849–58
[42] Brower-Toland B, Wacker D A, Fulbright R M, Lis J T, Kraus W L and Wang M D 2005 Specific contributions of histone tails and their acetylation to the mechanical stability of nucleosomes *J. Mol. Biol.* **346** 135–46
[43] Ferreira H, Somers J, Webster R, Flaus A and Owen-Hughes T 2007 Histone tails and the H3 alpha-N helix regulate nucleosome mobility and stability *Mol. Cell. Biol.* **27** 4037–48
[44] Henikoff S 2008 Nucleosome destabilization in the epigenetic regulation of gene expression *Nat. Rev. Genet.* **9** 15–26
[45] Biswas M, Voltz K, Smith J C and Langowski J 2011 Role of histone tails in structural stability of the nucleosome *PLoS Comput. Biol.* **7** e1002279
[46] Iwasaki W, Miya Y, Horikoshi N, Osakabe A, Taguchi H, Tachiwana H, Shibata T, Kagawa W and Kurumizaka H 2013 Contribution of histone *N*-terminal tails to the structure and stability of nucleosomes *FEBS Open Biol.* **3** 363–9
[47] Widlund H R, Vitolo J M, Thiriet C and Hayes J J 2000 DNA sequence- dependent contributions of core histone tails to nucleosome stability: differential effects of acetylation and proteolytic tail removal *Biochemistry* **39** 3835–41
[48] Gottesfeld J M and Luger K 2001 Energetics and affinity of the histone octamer for defined DNA sequences *Biochemistry* **40** 10927–33
[49] Marmorstein R and Trievel R C 2009 Histone modifying enzymes: structures, mechanisms, and specificities *Biochim. Biophys. Acta* **1789** 58–68
[50] Schiltz R L, Mizzen C A, Vassilev A, Cook R G, Allis C D and Nakatani Y 1999 Overlapping but distinct patterns of histone acetylation by the human coactivators p300 and PCAF within nucleosomal substrates *J. Biol. Chem.* **274** 1189–92
[51] Grant P A, Eberharter A, John S, Cook R G, Turner B M and Workman J L 1999 Expanded lysine acetylation specificity of Gcn5 in native complexes *J. Biol. Chem.* **274** 5895–900
[52] Howe L, Auston D, Grant P, John S, Cook R G, Workman J L and Pillus L 2001 Histone H3 specific acetyltransferases are essential for cell cycle progression *Genes Dev.* **15** 3144–54
[53] Xu F, Zhang K and Grunstein M 2005 Acetylation in histone H3 globular domain regulates gene expression in yeast *Cell* **121** 375–85
[54] Shogren-Knaak M, Ishii H, Sun J M, Pazin M J, Davie J R and Peterson C L 2006 Histone H4-K16 acetylation controls chromatin structure and protein interactions *Science* **311** 844–7
[55] Yang X J and Seto E 2007 HATs and HDACs: From structure, function and regulation to novel strategies for therapy and prevention *Oncogene* **26** 5310–8
[56] Sutton A, Shia W J, Band D, Kaufman P D, Osada S, Workman J L and Sternglanz R 2003 Sas4 and Sas5 are required for the histone acetyltransferase activity of Sas2 in the SAS complex *J. Biol. Chem.* **278** 16887–92
[57] Bird A W, Yu D Y, Pray-Grant M G, Qiu Q, Harmon K E, Megee P C, Grant P A, Smith M M and Christman M F 2002 Acetylation of histone H4 by esa1 is required for DNA double-strand break repair *Nature* **419** 411–5
[58] Ikura T, Ogryzko V V, Grigoriev M, Groisman R, Wang J, Horikoshi M, Scully R, Qin J and Nakatani Y 2000 Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis *Cell* **102** 463–73
[59] Masumoto H, Hawke D, Kobayashi R and Verreault A 2005 A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response *Nature* **436** 294–8
[60] Kiefer C M, Hou C, Little J A and Dean A 2008 Epigenetics of beta-globin gene regulation *Mutat. Res.* **647** 68–76
[61] Wang Z *et al* 2008 Combinatorial patterns of histone acetylations and methylations in the human genome *Nat. Genet.* **40** 897–903
[62] Hendzel M J, Wei Y, Mancini M A, Van Hooser A, Ranalli T, Brinkley B R, Bazett-Jones D P and Allis C D 1997 Mitosis-specific phosphorylation of histone H3 initiates primarily

within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation *Chromosoma* **106** 348–60
[63] Fernandez-Capetillo O, Allis C D and Nussenzweig A 2004 Phosphorylation of histone H2B at DNA double-strand breaks *J. Exp. Med.* **199** 1671–7
[64] Harvey A C, Jackson S P and Downs J A 2005 *Saccharomyces cerevisiae* histone H2A Ser122 facilitates DNA repair *Genetics* **170** 543–53
[65] Cheung W L, Turner F B, Krishnamoorthy T, Wolner B, Ahn S H, Foley M, Dorsey J A, Peterson C L, Berger S L and Allis C D 2005 Phosphorylation of histone H4 serine 1 during DNA damage requires casein kinase II in *S. cerevisiae Curr. Biol.* **15** 656–60
[66] Oki M, Aihara H and Ito T 2007 Role of histone phosphorylation in chromatin dynamics and its implications in diseases *Subcell Biochem.* **41** 319–36
[67] Dawson M A, Bannister A J, Göttgens B, Foster S D, Bartke T, Green A R and Kouzarides T 2009 JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin *Nature* **461** 819–22
[68] Yamada Y, Warren A J, Dobson C, Forster A, Pannell R and Rabbitts T H 1998 The T cell leukemia LIM protein Lmo2 is necessary for adult mouse hematopoiesis *Proc. Natl Acad. Sci.* **95** 3890–5
[69] Rea S *et al* 2000 Regulation of chromatin structure by site-specific histone H3 methyltransferases *Nature* **406** 593–9
[70] Tamaru H and Selker E U 2001 A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa Nature* **414** 277–83
[71] Johnson L, Cao X and Jacobsen S 2002 Interplay between two epigenetic marks DNA methylation and histone H3 lysine 9 methylation *Curr. Biol.* **12** 1360–7
[72] Daujat S, Zeissler U, Waldmann T, Happel N and Schneider R 2005 HP1 binds specifically to Lys26-methylated histone H1. 4, whereas simultaneous Ser27 phosphorylation blocks HP1 binding *J. Biol. Chem.* **280** 38090–5
[73] Di Lorenzo A and Bedford M T 2011 Histone arginine methylation *FEBS Lett.* **585** 2024–31
[74] Glickman M H and Ciechanover A 2002 The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction *Physiol. Rev.* **82** 373–428
[75] Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones R S and Zhang Y 2004 Role of histone H2A ubiquitination in polycomb silencing *Nature* **431** 873–8
[76] Zhu B, Zheng Y, Pham A D, Mandal S S, Erdjument-Bromage H, Tempst P and Reinberg D 2005 Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation *Mol. Cell* **20** 601–11
[77] Robzyk K, Recht J and Osley M A 2000 Rad6-dependent ubiquitination of histone H2B in yeast *Science* **287** 501–4
[78] Seeler J S and Dejean A 2003 Nuclear and unclear functions of SUMO *Nat. Rev. Mol. Cell Biol.* **4** 690–9
[79] Shiio Y and Eisenman R N 2003 Histone sumoylation is associated with transcriptional repression *Proc. Natl Acad. Sci.* **100** 13225–30
[80] Nathan D *et al* 2006 Histone sumoylation is a negative regulator in saccharomyces cerevisiae and shows dynamic interplay with positive-acting histone modifications *Genes Dev.* **20** 966–76
[81] Nathan D *et al* 2006 Histone sumoylation is a negative regulator in saccharomyces cerevisiae and shows dynamic interplay with positive-acting histone modifications *Genes Dev.* **20** 966–76
[82] Kobza K, Camporeale G, Rueckert B, Kueh A, Griffin J B, Sarath G and Zempleni J 2005 K4, K9 and K18 in human histone H3 are targets for biotinylation by biotinidase *FEBS J.* **272** 4249–59
[83] Chew Y C, Camporeale G, Kothapalli N, Sarath G and Zempleni J 2006 Lysine residues in *N*-terminal and *C*-terminal regions of human histone H2A are targets for biotinylation by biotinidase *J. Nutritional Biochem.* **17** 225–33
[84] Camporeale G, Shubert E E, Sarath G, Cerny R and Zempleni J 2004 K8 and K12 are biotinylated in human histone H4 *Eur. J. Biochem.* **271** 2257–63
[85] Kothapalli N, Sarath G and Zempleni J 2005 Biotinylation of K12 in histone H4 decreases in response to DNA double-strand breaks in human JAr choriocarcinoma cells *J. Nutrition* **135** 2337–42
[86] Santos-Rosa H *et al* 2009 Histone H3 tail clipping regulates gene expression *Nat. Struct. Mol. Biol.* **16** 17–22
[87] Duncan E M *et al* 2008 Cathepsin L proteolytically processes histone H3 during mouse embryonic stem cell differentiation *Cell* **135** 284–94
[88] Allis C D, Bowen J K, Abraham G N, Glover C V and Gorovsky M A 1980 Proteolytic processing of histone H3 in chromatin: a physiologically regulated event in *Tetrahymena micronuclei Cell* **20** 55–64
[89] Kuzmichev A, Jenuwein T, Tempst P and Reinberg D 2004 Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3 *Mol. Cell* **14** 189–93
[90] Jenuwein T and Allis C D 2001 Translating the histone code *Science* **293** 1074–80
[91] Strahl B D and Allis C D 2000 The language of covalent histone modifications *Nature* **403** 41–5
[92] Rando O J 2012 Combinatorial complexity in chromatin structure and function: revisiting the histone code *Curr. Opin. Genet. Dev.* **222** 148–55
[93] Wisniewski J R, Zougman A, Krüger S and Mann M 2006 Mass spectrometric mapping of linker histone H1 variants reveals multiple acetylations, methylations, and phosphorylation as well as differences between cell culture and tissue *Mol. Cell. Proteomics* **6** 72–87
[94] Jiang T, Zhou X, Taghizadeh K, Dong M and Dedon P C 2007 *N*-formylation of lysine in histone proteins as a secondary modification arising from oxidative DNA damage *Proc. Natl. Acad. Sci.* **104** 60–5
[95] Christophorou M A *et al* 2014 Citrullination regulates pluripotency and histone H1 binding to chromatin *Nature* **507** 104–8
[96] Tan M *et al* 2011 Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification *Cell* **146** 1016–28
[97] Barber C M *et al* 2004 The enhancement of histone H4 and H2A serine 1 phosphorylation during mitosis and *S*-phase is evolutionarily conserved *Chromosoma* **112** 360–71
[98] Zhang Y, Griffin K, Mondal N and Parvin J D 2004 Phosphorylation of histone H2A inhibits transcription on chromatin templates *J. Biol. Chem.* **279** 21866–72
[99] Clarke A S, Lowell J E, Jacobson S J and Pillus L 1999 Esa1p is an essential histone acetyltransferase required for cell cycle progression *Mol. Cell. Biol.* **19** 2515–26
[100] Kimura A and Horikoshi M 1998 Tip60 acetylates six lysines of a specific class in core histones in vitro *Genes Cells* **3** 789–800
[101] Verreault A, Kaufman P D, Kobayashi R and Stillman B 1997 Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase *Curr. Biol.* **8** 96–108
[102] Suka N, Suka Y, Carmen A A, Wu J and Grunstein M 2001 Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin *Mol. Cell* **8** 473–9
[103] Zhang L, Eugeni E E, Parthun M R and Freitas M A 2003 Identification of novel histone post-translational modifications by peptide mass fingerprinting *Chromosoma* **112** 77–86
[104] Freitas M A, Sklenar A R and Parthun M R 2004 Application of mass spectrometry to the identification and quantification of histone post-translational modifications *J. Cell Biochem.* **92** 691–700
[105] Tessarz P, Santos-Rosa H, Robson S C, Sylvestersen K B, Nelson C J, Nielsen M L and Kouzarides T 2014 Glutamine

methylation in histone H2A is an RNA-polymerase-I-dedicated modification *Nature* **505** 564–8
[106] Aihara H *et al* 2004 Nucleosomal histone kinase-1 phosphorylates H2A Thr 119 during mitosis in the early Drosophila embryo *Genes Dev.* **18** 877–88
[107] Yamagishi Y, Honda T, Tanno Y and Watanabe Y 2010 Two histone marks establish the inner centromere and chromosome bi-orientation *Science* **330** 239–43
[108] Kim K, Kim J M, Kim J S, Choi J, Lee Y S, Neamati N, Song J S, Heo K and An W 2013 VprBP Has intrinsic kinase activity targeting histone H2A and represses gene transcription *Mol. Cell* **52** 459–67
[109] Downs J A, Lowndes N F and Jackson S P 2000 A role for saccharomyces cerevisiae histone H2A in DNA repair *Nature* **408** 1001–4
[110] Shroff R, Arbel-Eden A, Pilch D, Ira G, Bonner W M, Petrini J H, Haber J E and Lichten M 2004 Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break *Curr. Biol.* **14** 1703–11
[111] Ward I M and Chen J 2001 Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress *J. Biol. Chem.* **276** 47759–62
[112] Burma S, Chen B P, Murphy M, Kurimasa A and Chen D J 2001 ATM phosphorylates histone H2AX in response to DNA double-strand breaks *J. Biol. Chem.* **276** 42462–7
[113] Park E J, Chan D W, Park J H, Oettinger M A and Kwon J 2003 DNA-PK is activated by nucleosomes and phosphorylates H2AX within the nucleosomes in an acetylation-dependent manner *Nucleic Acids Res.* **31** 6819–27
[114] Xiao A *et al* 2008 WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity *Nature* **457** 57–62
[115] Kawasaki H, Schiltz L, Chiu R, Itakura K, Taira K, Nakatani Y and Yokoyama K K 2000 ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation *Nature* **405** 195–200
[116] Ahn S H, Cheung W L, Hsu J Y, Diaz R L, Smith M M and Allis C D 2005 Sterile 20 kinase phosphorylates histone H2B at serine 10 during hydrogen peroxide-induced apoptosis in *S. cerevisiae Cell* **120** 25–36
[117] Cheung W L *et al* 2003 Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase *Cell* **113** 507–17
[118] Bungard D, Fuerth B J, Zeng P Y, Faubert B, Maas N L, Viollet B, Carling D, Thompson C B, Jones R G and Berger S L 2010 Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation *Science* **329** 1201–5
[119] Dai J, Sultan S, Taylor S S and Higgins J M 2005 The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment *Genes Dev.* **19**
[120] Angus-Hill M L, Dutnall R N, Tafrov S T, Sternglanz R and Ramakrishnan V 1999 Crystal structure of the histone acetyltransferase Hpa2: a tetrameric member of the Gcn5-related N-acetyltransferase superfamily *J. Mol. Biol.* **294** 1311–25
[121] Briggs S D, Bryk M, Strahl B D, Cheung W L, Davie J K, Dent S Y, Winston F and Allis C D 2001 Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae *Genes Dev.* **15** 3286–95
[122] Wang H, Cao R, Xia L, Erdjument-Bromage H, Borchers C, Tempst P and Zhang Y 2001 Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase *Mol. Cell* **8** 1207–17
[123] Nakamura T, Mori T, Tada S, Krajewski W, Rozovskaia T, Wassell R, Dubois G, Mazo A, Croce C M and Canaani E 2002 ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation *Mol. Cell* **10** 1119–28
[124] Sedkov Y, Cho E, Petruk S, Cherbas L, Smith S T, Jones R S, Cherbas P, Canaani E, Jaynes J B and Mazo A 2003 Methylation at lysine 4 of histone H3 in ecdysone-dependent development of drosophila *Nature* **426** 78–83
[125] Metzger E *et al* 2010 Phosphorylation of histone H3T6 by PKCbeta(I) controls demethylation at histone H3K4 *Nature* **464** 792–6
[126] Pal S, Vishwanath S N, Erdjument-Bromage H, Tempst P and Sif S 2004 Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes *Mol. Cell Biol.* **24** 9630–45
[127] Su X, Zhu G, Ding X, Lee S Y, Dou Y, Zhu B, Wu W and Li H 2014 Molecular basis underlying histone H3 lysine-arginine methylation pattern readout by spin/Ssty repeats of Spindlin1 *Genes Dev.* **28** 622–36
[128] Sobel R E, Cook R G, Perry C A, Annunziato A T and Allis C D 1995 Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4 *Proc. Natl Acad. Sci.* **92** 1237–41
[129] Spencer T E *et al* 1997 Steroid receptor coactivator-1 is a histone acetyltransferase *Nature* **389** 194–8
[130] Tachibana M, Sugimoto K, Fukushima T and Shinkai Y 2001 Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3 *J. Biol. Chem.* **276** 25309–17
[131] Schultz D C, Ayyanathan K, Negorev D, Maul G G and Rauscher F J 3rd 2002 SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins *Genes Dev.* **16** 919–32
[132] Hsu J Y *et al* 2000 Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and Glc7/PP1 phosphatase in budding yeast and nematodes *Cell* **102** 279–91
[133] Soloaga A, Thomson S, Wiggin G R, Rampersaud N, Dyson M H, Hazzalin C A, Mahadevan L C and Arthur J S 2003 MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14 *EMBO J.* **22** 2788–97
[134] Anest V, Hanson J L, Cogswell P C, Steinbrecher K A, Strahl B D and Baldwin A S 2003 A nucleosomal function for IkappaB kinase-alpha in NF-kappaB-dependent gene expression *Nature* **423** 659–63
[135] Lo W S, Duggan L, Emre N C, Belotserkovskya R, Lane W S, Shiekhattar R and Berger S L 2001 Snf1—a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription *Science* **293** 1142–6
[136] Preuss U, Landsberg G and Scheidtmann K H 2003 Novel mitosis-specific phosphorylation of histone H3 at Thr11 mediated by Dlk/ZIP kinase *Nucleic Acid Res.* **31** 878–85
[137] Winkler G S, Kristjuhan A, Erdjument-Bromage H, Tempst P and Svejstrup J Q 2002 Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo *Proc. Natl Acad. Sci.* **99** 3517–22
[138] Hsieh Y J, Kundu T K, Wang Z, Kovelman R and Roeder R G 1999 The TFIIIC90 Subunit of TFIIIC Interacts with multiple components of the RNA polymerase III machinery and contains a histone-specific acetyltransferase activity *Mol. Cell. Biol.* **19** 7697–704
[139] Mizzen C A *et al* 1996 The TAF(II)250 subunit of TFIID has histone acetyltransferase activity *Cell* **87** 1261–70
[140] Daujat S, Bauer U M, Shah V, Turner B, Berger S and Kouzarides T 2002 Crosstalk between CARM1 methylation and CBP acetylation on histone H3 *Curr. Biol.* **12** 2090–7
[141] Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones R S and Zhang Y 2002 Role of histone H3 lysine 27 methylation in Polycomb-group silencing *Science* **298** 1039–43
[142] Goto H, Yasui Y, Nigg E A and Inagaki M 2002 Aurora-B phosphorylates Histone H3 at serine28 with regard to the mitotic chromosome condensation *Genes to Cells* **7** 11–7
[143] Zhong S, Jansen C, She Q B, Goto H, Inagaki M, Bode A M, Ma W Y and Dong Z 2001 Ultraviolet b-induced phosphorylation of histone h3 at serine 28 is mediated by msk1 *J. Biol. Chem.* **276** 33213–9

[144] Morris S A, Rao B, Garcia B A, Hake S B, Diaz R L, Shabanowitz J, Hunt D F, Allis C D, Lieb J D and Strahl B D 2007 Identification of histone H3 lysine 36 acetylation as a highly conserved histone modification *J. Biol. Chem.* **282** 7632–40
[145] Krogan N J *et al* 2003 Methylation of histone H3 by Set2 in saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II *Mol. Cell. Biol.* **23** 4207–18
[146] Casadio F, Lu X, Pollock S B, LeRoy G, Garcia B A, Muir T W, Roeder R G and Allis C D 2013 H3R42me2a is a histone modification with positive transcriptional effects *Proc. Natl Acad. Sci.* **110** 14894–9
[147] Hurd P J *et al* 2009 Phosphorylation of histone H3 Thr-45 is linked to apoptosis *J. Biol. Chem.* **284** 16575–83
[148] Ozdemir A, Spicuglia S, Lasonder E, Vermeulen M, Campsteijn C, Stunnenberg H G and Logie C 2005 Characterization of lysine 56 of histone H3 as an acetylation site in *Saccharomyces cerevisiae J. Biol. Chem.* **280** 25949–52
[149] Yu Y, Song C, Zhang Q, DiMaggio P A, Garcia B A, York A, Carey M F and Grunstein M 2012 Histone H3 lysine 56 methylation regulates DNA replication through its interaction with PCNA *Mol. Cell* **46** 7–17
[150] Vermeulen M *et al* 2010 Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers *Cell* **142** 967–80
[151] Di Cerbo V *et al* 2014 Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription *eLife* **3** 1–23
[152] Feng Q, Wang H, Ng H H, Erdjument-Bromage H, Tempst P, Struhl K and Zhang Y 2002 Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain *Curr. Biol.* **25** 1052–8
[153] Krogan N J *et al* 2003 The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation *Mol. Cell* **11** 721–9
[154] Huyen Y, Zgheib O, Ditullio R A Jr, Gorgoulis V G, Zacharatos P, Petty T J, Sheston E A, Mellert H S, Stavridi E S and Halazonetis T D 2004 Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks *Nature* **432** 406–11
[155] North J A *et al* 2011 Phosphorylation of histone H3(T118) alters nucleosome dynamics and remodeling *Nucleic Acids Res.* **39** 6465–74
[156] Kruger W, Peterson C L, Sil A, Coburn C, Arents G, Moudrianakis E N and Herskowitz I 1995 Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription *Genes Dev.* **9** 2770–9
[157] Tropberger P *et al* 2013 Regulation of transcription through acetylation of H3K122 on the lateral surface of the histone octamer *Cell* **152** 859–72
[158] Strahl B D *et al* 2001 Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1 *Curr. Biol.* **11** 996–1000
[159] Parthun M R, Widom J and Gottschling D E 1996 The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism *Cell* **87** 85–94
[160] Kuo M H, Brownell J E, Sobel R E, Ranalli T A, Cook R G, Edmondson D G, Roth S Y and Allis C D 1996 Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines *Nature* **383** 269–72
[161] Kelly T J, Qin S, Gottschling D E and Parthun M R 2000 Type B histone acetyltransferase Hat1p participates in telomeric silencing *Mol. Cell Biol.* **20** 7051–8
[162] Hilfiker A, Hilfiker-Kleiner D, Pannuti A and Lucchesi J C 1997 mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in drosophila *EMBO J.* **16** 2054–60
[163] Nishioka K *et al* 2002 PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin *Mol. Cell* **9** 1201–13
[164] Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D and Jenuwein T 2004 A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin *Genes Dev.* **18** 1251–62
[165] Sanders S L, Portoso M, Mata J, Bähler J, Allshire R C and Kouzarides T 2004 Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage *Cell* **119** 603–14
[166] Kang B, Pu M, Hu G, Wen W, Dong Z, Zhao K, Stillman B and Zhang Z 2011 Phosphorylation of H4 Ser 47 promotes HIRA-mediated nucleosome assembly *Genes Dev.* **25** 1359–64
[167] Ye J, Ai X, Eugeni E E, Zhang L, Carpenter L R, Jelinek M A, Freitas M A and Parthun M R 2005 Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly *Mol. Cell* **18** 123–30
[168] Simpson R T, Thoma F and Brubaker J M 1985 Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure *Cell* **42** 799–808
[169] Lowary P T and Widom J 1998 New DNA sequence rules for highaffinity binding to histone octamer and sequence-directed nucleosome positioning *J. Mol. Biol.* **276** 19–42
[170] Schlick T, Collepardo-Guevara R, Halvorsen L A, Jung S and Xiao X 2011 Biomolecularmodeling and simulation: a field coming of age *Q. Rev. Biophys.* **44** 191–228
[171] Sun Y, Zheng G, Mei C, Bohm E J, Phillips J C and Kalé L V 2012 Optimizing fine-grained communication in a biomolecular simulation application on cray XK6 *Proc. SC '12 Proc. Int. Conf. on High Performance Computing, Networking, Storage and Analysis* **55** 1–11
[172] Phillips J C, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel R D, Kalé L and Schulten K 2005 Scalable molecular dynamics with NAMD *J. Comput. Chem.* **26** 1781–802
[173] Kumar S, Huang C, Zheng G, Bohm E, Bhatele A, Phillips J C, Yu H and Kalé L V 2008 Scalable molecular dynamics with NAMD on the IBM blue gene/L system *IBM J. Res. Dev.* **52** 177–88
[174] Grossman J P, Towles B, Greskamp B and Shaw D E 2015 Filtering, reductions and synchronization in the Anton 2 network *IEEE 29th Int. Parallel and Distributed Proc. Symp.* 860–70
[175] Biswas M, Langowski J and Bishop T C 2013 Atomistic simulations of nucleosomes *WIReS Comput. Mol. Sci.* **3** 378–92
[176] Schlick T 2009 Monte Carlo, harmonic approximation, and coarse-graining approaches for enhanced sampling of biomolecular structure *F1000 Biol. Rep.* **1**
[177] Schlick T 2009 Molecular-dynamics based approaches for enhanced sampling of long-time, large-scale conformational changes in biomolecules *F1000 Biol. Rep.* **1**
[179] Wabik J, Kmiecik S, Gront D, Kouza M and Kolinski A 2013 Combining coarse-grained protein models with replica-exchange all-atom molecular dynamics *Int. J. Mol. Sci.* **14** 9893–905
[179] Perišić O and Lu H 2014 On the improvement of free-energy calculation from steered molecular dynamics simulations using adaptive stochastic perturbation protocols *PLoS One* **9** e101810
[180] Abrams C and Bussi G 2014 Enhanced sampling in molecular dynamics using metadynamics, replica-exchange, and temperature-acceleration *Entropy* **16** 163–99
[181] Akimov A V and Prezhdo O V 2015 Large-scale computations in chemistry: a bird's eye view of a vibrant field *Chem. Rev.* **115** 5797–890
[182] Bernardi R C, Melo M C R and Schulten K 2015 Enhanced sampling techniques in molecular dynamics simulations of biological systems *Biochim. Biophys. Acta* **1850** 872–7
[183] Fujisaki H, Moritsugu K, Matsunaga Y, Morishita T and Maragliano L 2015 Extended phase-space methods for enhanced sampling in molecular simulations: a review *Frontiers Bioeng. Biotechnol.* **3**

[184] Pan A C, Weinreich T M, Piana S and Shaw D E 2016 Demonstrating an Order-of-Magnitude Sampling Enhancement *Molecular Dynamics Simulations of Complex Protein Systems Journal of Chemical Theory and Computation* **12** 1360–7
[185] Richards F M 1977 Areas, volumes, packing, and protein structure *Annu. Rev. Biophys. Bioeng.* **6** 151–76
[186] Roux B and Simonson T 1999 Implicit solvent models *Biophys. Chem.* **78** 1–20
[187] Woodcock C, Grigoryev S, Horowitz R and Whitaker N 1993 A chromatin folding model that incorporates linker variability generates fibers resembling the native structures *Proc. Natl Acad. Sci.* **90** 9021–5
[188] Schiessel H, Gelbart W M and Bruinsma R 2001 DNA folding: structural and mechanical properties of the two-angle model for chromatin *Biophys. J.* **80** 1940–56
[189] Wedemann G and Langowski J 2002 Computer simulation of the 30 nm chromatin fiber *Biophys. J.* **82** 2847–59
[190] Stehr R, Kepper N, Rippe K and Wedemann G 2008 The effect of internucleosomal interaction on folding of the chromatin fiber *Biophys. J.* **95** 3677–91
[191] Stehr R, Schöpflin R, Ettig R, Kepper N, Rippe K and Wedemann G 2010 Exploring the conformational space of chromatin fibers and their stability by numerical dynamic phase diagrams *Biophys. J.* **98** 1028–37
[192] Katritch V, Bustamante C and Olson W 2000 Pulling chromatin fibers: computer simulations of direct physical micromanipulations *J. Mol. Biol.* **295** 29–40
[193] Müller O, Kepper N, Schöpflin R, Ettig R, Rippe K and Wedemann G 2014 Changing chromatin fiber conformation by nucleosome repositioning *Biophys. J.* **107** 2141–50
[194] Kunze K-K and Netz R R 2000 Salt-induced DNA-Histone complexation *Phys. Rev. Lett.* **85** 4389–92
[195] Schiessel H 2003 The physics of chromatin *J. Phys. Condens. Matter* **15** 699–774
[196] Cherstvy A G and Winkler R G 2004 Complexation of semiflexible chains with oppositely charged cylinder *J. Chem. Phys.* **120** 9394–400
[197] Korolev N, Lyubartsev A P and Laaksonen A 2004 Electrostatic background of chromatin fiber stretching *J. Biomol. Struct. Dyn.* **22** 215–26
[198] Fenley A T, Adams D A and Onufriev A V 2010 Charge state of the globular histone core controls stability of the nucleosome *Biophys. J.* **99** 1577–85
[199] Pachov G V, Gabdoulline R R and Wade R C 2011 On the structure and dynamics of the complex of the nucleosome and the linker histone *Nucleic Acids Res.* **39** 5255–63
[201] Luque A, Collepardo-Guevara R, Grigoryev S and Schlick T 2014 Dynamic condensation of linker histone *C*-terminal domain regulates chromatin structure *Nucleic Acids Res.* **42** 7553–60
[201] Zhang Q, Beard D and Schlick T 2003 Constructing irregular surfaces to enclose macromolecular complexes for mesoscale modeling using the discrete surface charge optimization (DISCO) algorithm *J. Comput. Chem.* **24** 2063–74
[202] Schlick T, Li B and Olson W K 1994 The influence of salt on the structure and energetics of supercoiled DNA *Biophys. J.* **67** 2146–66
[203] Caterino T L and Hayes J J 2011 Structure of the H1 *C*-terminal domain and function in chromatin condensation *Biochem. Cell Biol.* **89** 35–44
[204] Collepardo-Guevara R and Schlick T 2011 The effect of linker histone's nucleosome binding affinity on chromatin unfolding mechanisms *Biophys. J.* **101** 1670–80
[205] Collepardo-Guevara R and Schlick T 2012 Crucial role of dynamic linker histone binding and divalent ions for DNA accessibility and gene regulation revealed by mesoscale modeling of oligonucleosomes *Nucleic Acids Res.* **40** 8803–17
[206] Beard D and Schlick T 2001 Modeling salt-mediated electrostatics of macromolecules: the discrete surface charge optimization algorithm and its application to the nucleosome *Biopolymers* **58** 106–15
[207] Collepardo-Guevara R and Schlick T 2014 Chromatin fiber polymorphism triggered by variations of DNA linker lengths *Proc. Natl Acad. Sci.* **111** 8061–6
[208] Collepardo-Guevara R and Schlick T 2011 The effect of linker histone's nucleosome binding affinity on chromatin unfolding mechanisms *Biophys. J.* **111** 1670–80
[209] Luque. A and Schlick T 2016 A sensitive interplay between nucleosome repeat length, linker histone density, and histone tail interactions *Biophys. J.* in revision
[210] Ozer G, Collepard-Guevara R and Schlick T 2015 Forced unraveling of chromatin fibers with nonuniform linker DNA lengths *J. Phys.: Condens. Matter.* **27** 064113
[211] Roccatano D, Barthel A and Zacharias M 2007 Structural flexibility of the nucleosome core particle at atomic resolution studied by molecular dynamics simulation *Biopolymers* **85** 407–21
[212] Mangenot S, Leforestier A, Vachette P, Durand D and Livolant F 2002 Salt-induced conformation and interaction changes of nucleosome core particles *Biophys. J.* **82** 345–56
[213] Gansen A, Valeri A, Hauger F, Felekyan S, Kalinin S, Toth K, Langowski J and Seidel C A M 2009 Nucleosome disassembly intermediates characterized by single-molecule FRET *Proc. Natl Acad. Sci.* **106** 15308–13
[214] Li G, Levitus M, Bustamante C and Widom J 2004 Rapid spontaneous accessibility of nucleosomal DNA *Nat. Struct. Mol. Biol.* **12** 46–53
[215] Ettig R, Kepper N, Stehr R, Wedemann G and Rippe K 2011 Dissecting DNA-histone interactions in the nucleosome by molecular dynamics simulations of DNA unwrapping *Biophys. J.* **101** 1999–2008
[216] Wocjan T, Klenin K and Langowski J 2009 Brownian dynamics simulation of DNA unrolling from the nucleosome *J. Phys. Chem.* B **113** 2639–46
[217] Dobrovolskaia I V and Arya G 2012 Dynamics of forced nucleosome unraveling and role of nonuniform histone-DNA interactions *Biophys. J.* **103** 989–98
[218] Yang D and Arya G 2011 Structure and binding of the H4 histone tail and the effects of lysine 16 acetylation *Phys. Chem. Chem. Phys.* **13** 2911–21
[219] Zhou J, Fan J Y, Rangasamy D and Tremethick D J 2007 The nucleosome surface regulates chromatin compaction and couples it with transcriptional repression *Nat. Struct. Mol. Biol.* **14** 1070–6
[220] Potoyan D A and Papoian G A 2012 Regulation of the H4 tail binding and folding landscapes via Lys-16 acetylation *Proc. Natl Acad. Sci.* **109**
[221] Papamokos G V, Tziatzos G, Papageorgiou D G, Georgatos S D, Politou A S and Kaxiras E 2012 Structural role of RKS motifs in chromatin interactions: a molecular dynamics study of HP1 bound to a variably modified histone tail *Biophys. J.* **102** 1926–33
[223] Vellore N A and Baron R 2013 Molecular dynamics simulations indicate an induced-fit mechanism for LSD1/CoREST-H3-histone molecular recognition *BMC Biophys.* **6** 15
[223] Zhou B R, Jiang J, Feng H, Ghirlando R, Xiao T S and Bai Y 2015 Structural mechanisms of nucleosome recognition by linker histones *Mol. Cell* **59** 1–11
[224] Zhou B R, Feng H, Kato H, Dai L, Yang Y, Zhou Y and Bai Y 2013 Structural insights into the histone H1-nucleosome complex *Proc. Natl Acad. Sci.* **110** 19390–5
[225] Marti-Renom M A and Mirny L A 2011 Bridging the resolution gap in structural modeling of 3D genome organization *PLoS Comput. Biol.* **7** e1002125
[227] Nagano T, Lubling Y, Stevens T J, Schoenfelder S, Yaffe E, Dean W, Laue E D, Tanay A and Fraser P 2013 Single-cell Hi-C reveals cell-to-cell variability in chromosome structure *Nature* **502** 59–64
[227] Rouquette J, Cremer C, Cremer T and Fakan S 2010 Functional nuclear architecture studied by microscopy: present and future *Int. Rev. Cell Mol. Biol.* **282** 1–90

[228] Grosberg A Yu, Nechaev S K and Shakhnovich E I 1988 The role of topological constraints in the kinetics of collapse of macromolecules *J. Phys.* **49** 2095–100
[229] Grosberg A, Rabin Y, Havlin S and Neer A 1993 Crumpled globule model of the three-dimensional structure of DNA *Europhys. Lett.* **23** 373–8
[230] Mirny L A 2011 The fractal globule as a model of chromatin architecture in the cell *Chromosome Res.* **19** 37–51
[231] Lieberman-Aiden E *et al* 2009 Comprehensive mapping of long-range interactions reveals folding principles of the human genome *Science* **326** 289–93
[232] Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie B R, Mirny L A and Dekker J 2013 Organization of the mitotic chromosome *Science* **342** 948–53
[233] Barbieri M, Chotalia M, Fraser J, Lavitas L M, Dostie J, Pombo A and Nicodemi M 2012 Complexity of chromatin folding is captured by the strings and binders switch model *Proc. Natl Acad. Sci.* **109** 16173–8
[234] Gehlen L R, Gruenert G, Jones M B, Rodley C D, Langowski J and O'Sullivan J 2012 Chromosome positioning and the clustering of functionally related loci in yeast is driven by chromosomal interactions *Nucleus* **3** 370–83
[235] Jost D, Carrivain P, Cavalli G and Vaillant C 2014 Modeling epigenome folding: formation and dynamics of topologically associated chromatin domains *Nucleic Acids Res.* **42** 9553–61
[236] Bruinsma R, Grosberg A Y, Rabin Y and Zidovska A 2014 Chromatin hydrodynamics *Biophys. J.* **106** 1871–81
[237] Zidovska A, Weitz D A and Mitchison T J 2013 Micron-scale coherence in interphase chromatin dynamics *Proc. Natl Acad. Sci.* **110** 15555–60
[238] Isaacson S A, McQueen D M and Peskin C S 2011 The influence of volume exclusion by chromatin on the time required to find specific DNA binding sites by diffusion *Proc. Natl Acad. Sci.* **108** 3815–20
[239] Isaacson S A, Larabell C A, Le Gros M A, McQueen D M and Peskin C S 2013 The influence of spatial variation in chromatin density determined by x-ray tomograms on the time to find DNA binding sites *Bull. Math. Biol.* **75** 2093–117