5
Chromatin Remodeling and Nucleosome Positioning

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5.1
Introduction

The human body consists of roughly 10^{12} cells, constituting more than 200 different cell types that are characterized by their own gene expression program. Moreover, the cellular gene expression programs change in a defined manner during development from a fertilized egg cell to a fully developed organism. The basic information stored in the DNA genome sequence is essentially identical in all cells of a given organism. Regulatory proteins control the cell type-specific gene expression programs. Their activity is targeted to certain genomic loci by recognizing specific DNA and RNA sequences as well as epigenetic signals associated with histone and DNA modifications (Chapters 1–4). Notably, the eukaryotic DNA is highly compacted by their association with histone proteins to form nucleosomes containing 147 bp of DNA wrapped in almost two turns around a disc-shaped protein core of histones (Chapter 3). Human DNA (2 m in total length) is partitioned into linear fragments, the chromosomes, which comprise 1.7–8.5 cm DNA each when fully extended. Three-quarters of the DNA genome is associated with core histones in about 30 million nucleosomes, with only one-quarter of the DNA being present in the more accessible linker DNA region that connects the nucleosomes [1]. This chain of nucleosomes and $\sim$50 bp linker DNA folds into higher order structures on different scales as discussed in Chapters 6, 9, 17 and 20 [2–4]. Thus, the assembly of nucleosomes and the folding of the nucleosome chain tightly packages the DNA sequence elements. At the same time the DNA needs to be made accessible in a defined manner for DNA-dependent processes like transcription, DNA replication, recombination, and repair. In particular, changes of the gene expression program require switching and establishment of accessible and repressed regulatory regions to allow the binding of regulatory proteins [5]. Recent studies from yeast to human have revealed that a surprisingly large number of nucleosomes have well defined positions that restrict DNA access for sequence-specific binding proteins and basal transcription factors. Chromatin remodeling complexes are able to translocate nucleosomes along the DNA upon hydrolysis of ATP. In this manner they can facilitate protein binding to

Genome Organization And Function In The Cell Nucleus, First Edition. Edited by Karsten Rippe.

Published 2012 by WILEY-VCH Verlag GmbH & Co. KGaA

previously occluded regions. Thus, nucleosome dynamics and positioning have a strong impact on chromatin structure and on the binding of regulatory protein factors associated with the regulation of gene expression as well as all other processes that involve protein–DNA interactions.

5.2 Chromatin Remodeling Complexes

5.2.1 ATPase Families

Chromatin remodeling enzymes are abundant cellular proteins, present at a ratio of about 1 per 10 nucleosomes in yeast [6, 7]. They belong to the helicase superfamily 2 (SF2) and contain a common core of two RecA domains that is also found in DNA helicases [8]. ATP hydrolysis is linked to a change of the relative orientation of the RecA domains [9]. Proteins with a helicase-like region similar to the Snf2p protein in the *Saccharomyces cerevisiae* form the Snf2 family and many, but not all of the Snf2 family members, have been identified as part of enzyme complexes able to remodel chromatin [10, 11]. The first detailed sequence alignment by Eisen and colleagues subdivided the Snf2 family into various subfamilies named according to the archetypical members Snf2 (*S. cerevisiae*), Snf2L (ISWI, *Drosophila melanogaster*), Chd1 (mouse), as well as Rad54, ETL1, MOT1, ERCC6, and Rad16 (all *S. cerevisiae*) [12]. A more recent study classified the Snf2 family members according to their helicase region and their three-dimensional structure [13]. The authors identified 24 distinct Snf2 subfamilies with 11 being ubiquitously represented in eukaryotic genomes. Many of these subfamilies correlate with specific functions [10, 13]. Besides the helicase domain, additional domains present in the proteins are more generally used to divide the enzymes into four groups [14]. The Snf2 subfamily members contain a C-terminal bromodomain, the ISWI members contain a SANT and SLIDE domain, Chd1, Mi-2, and CHD7 members contain a chromodomain and the Ino80 members are characterized by a "split" helicase, separating the DExx and HELICc (Helicase_C subdomain) domain by a long insertion [13].

The remodeling enzymes and associated subunits contain several protein domains that direct remodeler–nucleosome interactions, recognize histone tails and their modification states (bromodomain, BAH, CHD, PHD, SANT), the globular domain of histones (PHD), and nucleosomal DNA (SLIDE), and/or are directly involved in the mechanism of nucleosome remodeling (CHD, SLIDE). Although the diversity of protein domains points to the recognition of specialized nucleosomal substrates, the conserved helicase domain argues for a rather similar remodeling mechanism within the different subfamilies of remodeling ATPases. Unlike other helicases, the remodeling ATPases are not generally associated with the separation of DNA strands, but they exert dynamic transitions in chromatin

structure with different outcomes. ATP-dependent remodeling enzymes have been shown to generate accessible DNA in chromatin, to affect DNA and chromatin topology, to disrupt or evict nucleosomes, to exchange histones within the particle, and to catalyze translocations of the intact histone octamer along the DNA [15].

5.2.2 Accessory Subunits of Chromatin Remodeling Complexes

The remodeling ATPases are catalytically active as isolated subunits *in vitro*. However, the enzymes are usually found in large multiprotein complexes that modulate the activity of the ATPase motor protein. SWI/SNF complexes purified from *S. cerevisiae* are composed of 8–14 subunits. ISWI remodelers contain 2–4 subunits, the chromodomain containing remodeling enzymes have 1–10 subunits and the Ino80 enzymes harbor more than 10 subunits (reviewed in ref. [10]). In addition, the same enzyme has been detected in different multiprotein complexes. For example, the Snf2H and Snf2L ATPases were found in biochemically purified mammalian remodeling complexes like ACF, CHRAC, NoRC, NURF, CERF, WICH, B-WICH, RSF, and associate with NURD, cohesins and DNA methyltransferases [16–25]. Many more variants of these ISWI family remodelers may exist in different cell types or developmental stages. In addition, the diversity of mammalian complexes is further increased by the exchange of the molecular motors. The human ISWI subfamily contains two different isotypic ATPase subunits Snf2H and Snf2L. Furthermore, Snf2L is present in several splice variants that include Snf2L1, Snf2L2, Snf2LΔNLS and Snf2L + 13 [16]. On the sequence level, Snf2H and Snf2L are 80% identical and even 87% of the amino acids are functionally conserved. The catalytic RecA-like ATP-dependent domains DEXDc and HELICc, like the substrate- and protein-interacting HAND, SANT, and SLIDE domains are almost identical, whereas C- and N-termini are highly divergent. The enzymes are differentially expressed and the Snf2L protein is predominantly found in terminally differentiated neurons of mouse, whereas Snf2H is ubiquitously expressed [26]. Currently, it is not known whether these isotypic complexes exert the same or distinct functions [17]. In addition, many of the accessory subunits of the remodeling complexes exist as multiple-splice variants, such as the large subunits CECR2 [17], BPTF [27, 28], Tip5, and Baz2B [29] further increasing the number of remodelers with potentially distinct functions. Another example for the combinatorial assembly of the complexes is the BAF complex that exists either as a Brg1- or a Brm-containing form [30]. In addition to the BAF complex the related PBAF complex was described that differs in the subunits BAF250 and BAF180 present in the one but not in the other complex [30]. The variations in ATPase and associated subunits are likely to result in the presence of several hundreds of distinct and abundant remodeling complexes in the cell (Figure 5.1). This suggests that those complexes fulfill specific functions in the organization and regulation of chromatin structure and DNA-dependent processes.

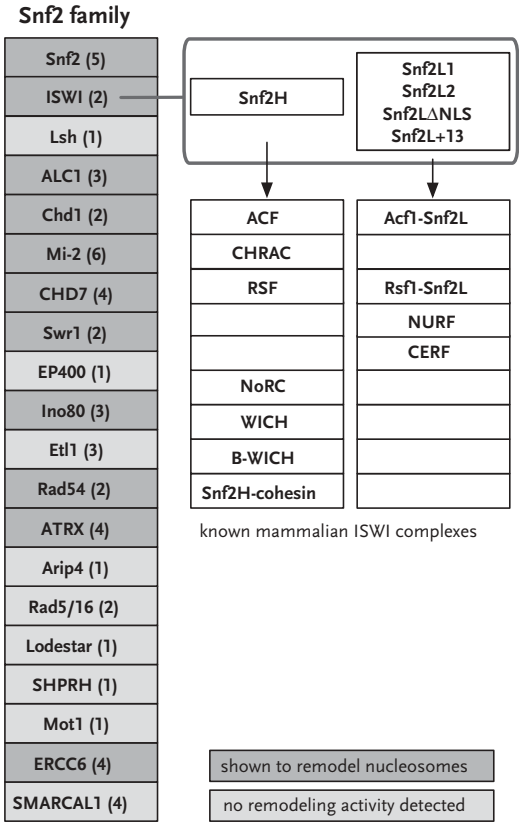

Figure 5.1 Mammalian chromatin remodeling complexes are highly diverse. The left panel lists the Snf2 family members present in humans with the number of individual proteins within a subfamily in brackets. The 11 subfamilies in dark gray were shown to possess ATP-dependent chromatin remodeling activities. Each of these subfamilies comprises many different members. As an example the multiple ISWI ATPase complexes known to date are shown on the right side of the table.

5.2.3 Activities of Chromatin Remodeling Factors

Besides the presence of a conserved ATPase motif in many different remodeling complexes, additional subunits specify the chromosomal target of the complexes. This occurs via the interaction with DNA-binding factors or the presence of DNA-binding domains in the complex itself, or the participation in defined nuclear processes. Specific chromatin remodeling complexes have been shown to play a role in gene activation (Swi/Snf complexes, Nurf) [31, 32], gene repression (yISW1, NuRD, NoRC) [33–35], DNA replication (WSTF, NoRC) [36, 37], chromatin assembly (ACF, CHRAC, Chd1) [20, 38–40], and DNA repair (Ino80-, Swr1-containing complexes) [41, 42]. This list is by no means comprehensive, but

illustrates the specialization of these molecular machines. A more detailed description of remodeler-specific activities is given by Clapier and Cairns [10].

5.3
Mechanisms of Nucleosome Translocations

5.3.1
The Loop–Recapture Mechanism for Nucleosome Translocation

Analysis of the structure of SWI/SNF and RSC remodelers by electron microscopy revealed a multilobed conformation with a central trough that is likely to represent the nucleosome binding site [43–46]. In this complex, the energy of ATP hydrolysis is used to transiently disrupt histone–DNA interactions. Subsequently, these perturbations are translated into nucleosomal movement along the DNA, nucleosome disruption, or even complete eviction of the histone octamer from the DNA. Two main models were suggested for envisioning nucleosome movements [47]: the DNA twisting model involves DNA rotation relative to the histone octamer, while the loop–recapture model has a DNA loop within the nucleosome as its characteristic intermediate (Figure 5.2). Most of the

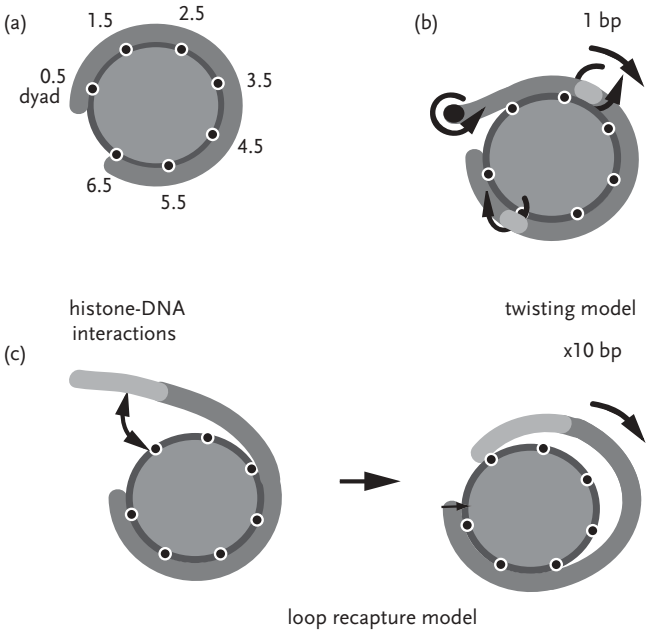

Figure 5.2 Nucleosome architecture and possible remodeling mechanisms. (a) Schematic drawing showing the location of major histone–DNA contact sites. (b) Transmission of a DNA twisting. (c) A loop is formed at the DNA entry–exit site and then propagated around the histone octamer core.

experiments propose variations of the loop–recapture model as the underlying mechanism of nucleosome translocation. The remodeler stably interacts with the nucleosome, and during the ATP cycle linker DNA is pushed into the nucleosome, resulting in the detachment of a segment of DNA at the entry/exit site of the nucleosome. According to this model, the segment of detached DNA interacts with a different position on the histone octamer, creating a DNA loop on the nucleosomal surface. The directional propagation of the DNA loop around the histone octamer changes the translational position of the nucleosome, according to the loop size. Loop propagation over the histone octamer surface requires only little energy, since for each histone–DNA contact broken ahead, similar contacts are reformed behind the DNA bulge. Complexes shown to move nucleosomes according to the loop recapture mechanism were ACF [48] and subsequently ISW2, Swi/Snf and RSC [49–51].

Differences in the remodeling reaction have been reported, like translocation of the nucleosome by ISWI complexes without transient destabilization of the histone octamer [52] versus translocation in conjunction with nucleosome destabilization for Swi/Snf complexes [53]. These may be related to the DNA loop size and the kinetics of the remodeling reaction and may not necessarily reflect distinct remodeling mechanisms. DNA loop sizes are probably small for ISWI-like complexes (about 10–20 bp [49, 54]) and rather large for SWI/SNF complexes (up to 100 bp [50, 55]), correlating with the relative instability of SWI/SNF-remodeled nucleosomes. From *in vitro* experiments the velocity of chromatin remodeling can be estimated to be on the scale of ~ 10 bp s^{-1}. A value of 13 bp s^{-1} was measured for SWI/SNF remodelers [50], while ensemble FRET studies with purified mononucleosomes and Snf2H resulted in a rate of 17 bp within a few seconds [56].

5.3.2
Mechanisms for Targeting Nucleosomes to Certain Site

In a number of *in vitro* studies using mononucleosome substrates it has been shown that the end position(s) of the remodeling reaction depend both on the type of chromatin remodeler and on the DNA sequence [57–59]. An example for this is given in Figure 5.3.

To mechanistically explain how a remodeling machine is able to direct the nucleosome to a specific position it is instructive to consider the nucleosome translocation as an enzymatic reaction that follows a Michaelis–Menten-like model. "Good" substrates for the chromatin remodeling complex are characterized by a high affinity of enzyme and its nucleosome substrate (low value of Michaelis–Menten constant, K_M) and a high catalytic conversion rate k_{cat} of the enzyme–substrate complex to nucleosome at the end position of the translocation reaction. Thus, the k_{cat}/K_M ratio is high as expected for an efficient catalytic process. The opposite would be true for "bad" nucleosome remodeling substrates, that is, having a low k_{cat}/K_M ratio. This view leads to a mechanism in which the nucleosome translocation reaction proceeds by moving nucleosomes from sites where the DNA sequence environment or other signals makes them "good" substrates to sites where they are "bad" substrates according to a "release" or an "arrest" mechanism (Figure 5.4).

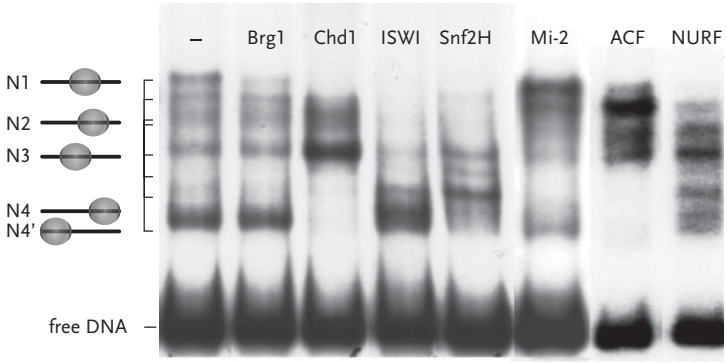

Figure 5.3 Chromatin remodeling complexes position nucleosomes in a DNA sequence-specific manner. The nucleosome substrate was reconstituted by salt dialysis on a radioactively labeled 350-bp fragment carrying the hsp70 promoter. A mixture of a single nucleosome at five different major positions (indicated as N1, N2, N3, N4, and N4') was obtained [60]. This mixed nucleosome population was used as the same substrate for all seven remodelers shown [57]. The end point of the nucleosome translocation reaction obtained after incubation for 90 min at 26 °C in the presence of ATP is shown for recombinant Brg1, Chd1, dISWI, Snf2H, Mi-2, ACF, and NURF as indicated.

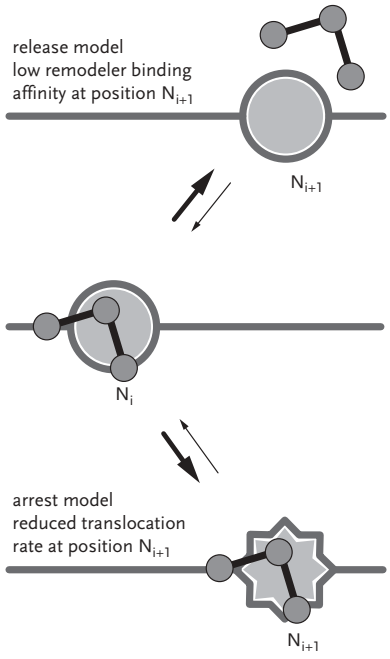

Figure 5.4 Mechanisms of nucleosome positioning by chromatin remodeling complexes. The remodeler *R* can translocate a nucleosome *N* from position *i* to $i + 1$ according to two mechanisms: In the *release* model the binding affinity at position $i + 1$ is reduced as compared to the initial nucleosome position so that the remodeler dissociates. For the *arrest* model the catalytic rate constant k_{cat} for translocation from position $i + 1$ is reduced as compared to the other position.

Indeed, experimental evidence for a change in the remodeler-nucleosome binding affinity (i.e., the value of K_M) with DNA sequence position has been detected. Binding assays showed that for the Chd1 and ACF remodeling complexes the binding affinity was reduced for nucleosomes at positions that reflected the end points of the remodeling reaction [57]. This behavior was referred to as a "release" mechanism. Its mode of operation is similar to transcription termination by specific DNA terminator sequences that form a hairpin structure in the RNA, which then disrupt the binding of RNA polymerase to the template so that the elongation reaction stops at this site [61, 62].

5.4 Positioning Nucleosomes in the Genome

5.4.1 DNA Sequence-Dependent Binding Affinities of the Histone Octamer

The DNA sequence encodes a number of signals that modulate its interactions with chromosomal proteins and regulatory factors (Chapter 1). Although the nucleosome is a prototypic example for a non-specific protein–DNA complex, it shows some sequence preferences (Chapter 3). The idea that nucleosome positioning is directed by the DNA sequence was first proposed theoretically in the 1980s after the discovery that genomic DNA carries a periodic pattern of dinucleotides repeated almost each 10 bp [63, 64]. This phasing of DNA sequence motifs at a distance coinciding with the pitch of the double helix was subsequently studied experimentally in the context of intrinsic DNA curvature and bendability to facilitate wrapping the DNA around the histone octamer core [65]. Indeed, it seems that the strongest protein–DNA contacts in the nucleosome are separated by $\sim$10 bp along each DNA strand [66], and the optimal nucleosomal sequences are characterized by the 10-bp dinucleotide periodicity [67]. Corresponding dinucleotide periodicities have been found in most tested organisms but with different sequence preferences: In *S. cerevisiae*, 14 dinucleotides (all dinucleotides except AC, GT) are repeated with a periodicity of 10.4 bp; *D. melanogaster* has four repeated dinucleotides (AA, TT, CG, GC); and in *Homo sapiens* only CG showed a repeat pattern that correlated with the helical rise of the DNA double helix [68]. The latter finding suggests that the role of dinucleotide periodicities probably decreases with the increasing complexity of the organism and points to reduced direct DNA sequence effects in higher eukaryotes. Interestingly, a $\sim$10 bp periodicity (or a multiple thereof) seems to be also present as the step size with which remodelers translocate nucleosomes. For example, the remodeling complex NURF or ISW2 repositions nucleosomes in increments of $\sim$10 bp, while for SWI/SNF a step length of around 50 bp has been reported [49, 69]. In *in vitro* experiments, a number of nucleosome-excluding and nucleosome-favoring DNA motifs were identified [70, 71]. It was found that the energy difference for histone octamer–DNA binding between natural DNA sequences varies from zero to -2.4 kcal mol^{-1},

and up to -4.1 kcal mol^{-1} for some artificial sequences, which would correspond to 1000-fold differences in histone-DNA affinities [72]. A particularly high-affinity nucleosome "601" binding sequence was identified by *in vitro* selection [73, 74]. However, this type of high-affinity binding sites appears not to be present in the genome, pointing to an evolutionary selection against DNA sequences that bind the histone octamer with highest affinity. In addition, some sequences seem to have a general nucleosome-excluding effect. This has been reported for example for poly(dA : dT) sequences, so-called A-tracts, *in vitro* and *in vivo* in *S. cerevisiae* (Chapter 3) [74]. However, it is noted that poly(dA : dT) elements are not enriched in nucleosome-depleted regions of *Schizosaccharomyces pombe* [75]. It is assumed that the physical mechanism of nucleosome exclusion by A-tracts is a unique 3-D structure, which is both bent and stiff and thus counteracts wrapping around the histone octamer protein core. Interestingly poly(dA : dT) also have a specific role in prokaryotes, where they facilitate transcription initiation by wrapping around RNA polymerase [76]. Thus, both for pro- and eukaryotes A-tracts upstream of the promoter appear to facilitate transcription initiation, albeit due to different mechanisms. Eukaryotic A-tracts are frequently flanking a gene on both sides, thus providing some nucleosome ordering within coding regions. A number of other DNA sequence motifs not related to A-tracks, such as $(CCGNN)_n$ have also been identified as nucleosome-excluding sequences [77].

5.4.2
Genome-Wide Analysis of Nucleosome Positions

Genome-wide analysis of nucleosome positioning has become possible due to the recent advances in high-throughput DNA sequencing [78–81]. In these experiments the linker DNA is removed from isolated chromatin by digestion with micrococcal nuclease (MNase). The DNA fragments associated with the histone octamer are then analyzed either on microarrays or by DNA sequencing as recently reviewed in Refs. [82–84]. A list of experimental data sets can be found in Ref. [85]. The genome-wide nucleosome-positioning experiments have led to the development of a number of models that predict the propensity of a given DNA sequence to be associated with a nucleosome [86–90]. They confirmed the finding from early studies that the average nucleosome repeat length (NRL) varies between organisms as well as different cells of the same organism [1]. For example, the most frequently found NRL is around 154 bp ($\sim$7 bp linker) in *S. pombe*, 165 bp ($\sim$18 bp linker) in *S. cerevisiae*, 175 bp ($\sim$28 bp linker) in *D. melanogaster* and *Caenorhabditis elegans*, and 185 bp ($\sim$38 bp linker) in *H. sapiens* [75, 82]. The average nucleosome repeat length can also vary in different cell types of the same organism, for example, 173 bp for human cortical neurons and 207 bp for human cortical glial cells [1]. Furthermore, cells can change their NRL during development: Rat cerebral neuronal chromatin has a repeat length of 164 bp at 12 months and 199 bp at 30 months [91]. During erythropoiesis in chicken, the nucleosome repeat length increases from 190 to 212 bp [92]. Recent genome-wide studies revealed significant nucleosome rearrangements upon activation of human $CD4^+$

T-cells [93], which could also affect the nucleosome repeat length at least locally. Finally, when chromosome segments from *S. pombe* were inserted into mouse chromosomes their nucleosome repeat length increased by about 30 bp to that of mouse chromatin [94].

5.4.3 Nucleosome Positions at Promoters and Enhancers

The nucleosome patterns at regulatory regions such as enhancers and promoters are distinct, but some common features can be derived from averaging data for certain genomic elements. For example, exon regions have more nucleosomes than introns [95]. Yeast promoters and enhancers are nucleosome-depleted, while human regulatory regions show the opposite trend [96]. It is noted that the genome-wide statistical averaging could artificially amplify some features and ignore others. This might be relevant for the nucleosome distribution at the promoter regions, which was initially thought to follow a general pattern for all promoters. However, a subsequent more detailed analysis concluded that promoters that are actively transcribed or contain a paused RNA polymerase are distinct by a region with low nucleosome occupancy immediately upstream of the transcription start site (TSS) [97].

Recent studies have raised the question why the regular spacing of nucleosomes at the promoters is sometimes different from the nucleosome maps found on the same DNA sequences *in vitro* [98]. To some extend this can be explained by nucleosome depletion upstream of the TSS being dependent on the transcription activity of a given promoter and the presence of RNA polymerase [99]. In the open promoter state the first nucleosome downstream the nucleosome-depleted region, the so-called "+1 nucleosome", is well positioned (Figure 5.5). This is probably due to statistical positioning by the boundary created by the nucleosome-depleted region

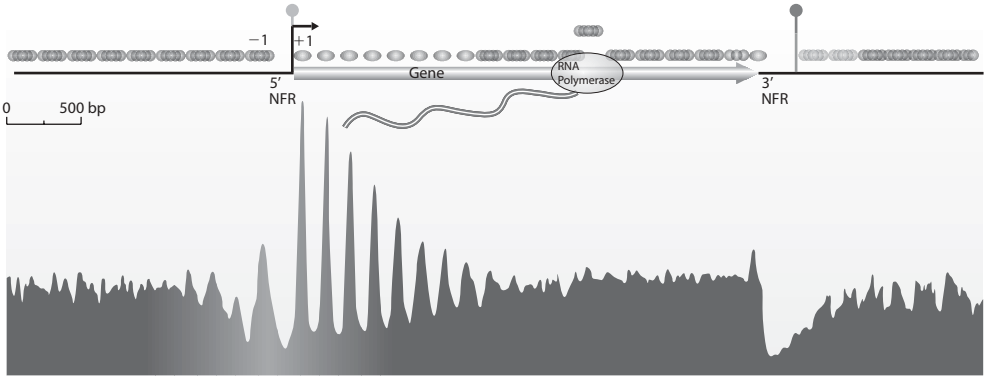

Figure 5.5 Nucleosome positions relative to transcription start sites averaged over all yeast genes. The 3′ and 5′ regions of transcribed genes depleted of nucleosomes are denoted as NFR ("nucleosome-free regions"). The Figure is reprinted from [82].

[100]. Such statistical positioning effects are not specific to nucleosomes and apply to any DNA-binding protein where the probability of protein occupancy oscillates close to the DNA boundary [101]. Experimental studies [102–105] and theoretical considerations [106, 107] confirm that the boundary effect is indeed important in nucleosome positioning. An even stronger barrier is imposed by the CTCF insulator binding protein that covers 50–60 bp of DNA [108]. Although it excludes just one nucleosome or might even bind in the linker region between two nucleosomes, it can statistically position up to 20 nucleosomes in its vicinity [104]. This positioning is symmetric on both sides of the bound protein. However, Figure 5.5 shows that the nucleosome-depleted promoter region positions nucleosomes in a different way upstream and downstream of TSS. The oscillations of nucleosome occupancies are more pronounced downstream than upstream of TSS. However, it is conceivable that this is an artifact of the computational averaging over a large number of structurally unrelated genes. Upstream of TSS both the length and the number of TF binding sites of the regulatory region could be very different, while a similar pattern is observed downstream of the TSS in the absence of strong nucleosome positioning effects. In such a scenario averaging over many upstream regions characterized by their own distinct nucleosome ordering could lead to the observed absence of oscillations of nucleosome positions.

5.4.4 Prediction of Nucleosome Positions from the DNA Sequence

The multiple *in vitro* studies mentioned above suggest a strong role of the DNA sequence in nucleosome positioning. Accordingly, several approaches have been developed to predict nucleosome positions from the DNA sequence in the absence of *trans*-acting factors such as competitive protein binding and remodeler action [66, 80, 81, 105, 109–116]. The most straightforward way to do this would be to construct the weight matrix for the nucleosome in analogy to the weight matrices composed for transcription factors. Such a matrix would contain the weights corresponding to the probabilities to find one of four nucleotides at a given position in the nucleosome consensus sequence. However, unlike typical proteins, which cover $\sim$10 bp, the nucleosome covers 147 bp, and therefore accurately constructing such a 147×4 weight matrix would require testing $\sim 4^{147}$ different sequences. Thus, predicting nucleosome positioning from the sequence is a challenging problem even in the absence of *trans*-acting factors.

Several possible ways to predict sequence-dependent nucleosome positioning have been proposed. Biophysical approaches usually attempt to predict the flexibility of different sequence motifs and the corresponding free energies involved in nucleosome formation [66, 85, 105, 114–117]. In contrast, bioinformatical approaches try to collect as many experimentally determined nucleosome positions as possible. These are used to train computer algorithms to predict the probabilities to find a nucleosome on new positions not included in the initial experimental dataset [80, 81, 109–113, 118–120]. For example, the algorithm of Segal and coworkers assigns specific weights for the two main features of

nucleosome positioning: the repetitions of dinucleotides and the 5-nucleotide motifs [81, 112]. The justification to score 5-mer motifs is just technical, since higher lengths would be more difficult to take into account. Algorithms based on 4-mer motifs also exist [121]. In the algorithm of Trifonov and coworkers, the elementary motif length is chosen as a 10-mer [122], motivated by the assumption that all DNA positions along the nucleosome are equivalent, provided they are in phase. In this case, the optimal nucleosomal sequence has been identified as $(GGAAATTTCC)_n$, and all genomic sequences are considered as deviations from this sequence with respect to their nucleosome formation strength [123]. As mentioned above, the number of possible 147 bp nucleosomal sequences is much higher than the length of any genome. Therefore, although nucleosome-positioning rules could be the same in all genomes, an algorithm based solely on the knowledge of nucleosome positioning in one genome (e.g., yeast) might not be suited to predict sequence preferences of histone–DNA binding in another genome. Furthermore, the fraction of nucleosome positions that can be predicted from the DNA sequence in yeast is controversially discussed [97, 98, 124–126]. While the resolution of this issue has to await further experimental studies, the emerging view is that the DNA sequence is the dominant positioning element for only a subset of 15–25% of the nucleosomes in yeast [88].

The propensity of a nucleosome positioning sequence identified *in vitro* to direct nucleosome positioning *in vivo* can be evaluated by integrating it into different sites of the genome. The limited data available from this type of experiments suggest that strong nucleosome positioning sequences alone are not sufficient to position nucleosomes *in vivo*. A 40 bp $(A/T)_3NN(G/C)_3NN$ sequence identified by Satchwell and co-workers exhibited strong nucleosome positioning capability *in vitro*, comparable to the strongest native sequences [127]. However, when this sequence was introduced into different locations of the yeast genome and on plasmids, it failed to position nucleosomes [128, 129]. In *Drosophila*, a similar sequence was more frequently found in the linker regions than incorporated into nucleosomes [130]. The *in vivo* nucleosome positioning capability of the 601 sequence was tested after stable integration into the mouse genome [131]. While the 601 sequence did transiently impose a specific chromatin structure, the effect was lost when the transgene vector became inactive. Finally, sequences that excluded nucleosomes *in vitro* like poly(dA dT) tracts did not display the same behavior *in vivo* [132, 133]. These experiments together with the changes in the nucleosome repeat length within the same organism (Section 5.4.2) clearly show that *in vivo* additional factors exist that can override the nucleosome positioning due to an increased or decreased affinity of the DNA sequence for the histone octamer core.

5.4.5
Effects of Chromatin Remodelers on Nucleosome Positioning

The nucleosome positions derived from high-throughput experiments reflect a complex interplay of numerous factors that include the activity of chromatin remodeling factors as well as the competitive binding of TFs and the histone

octamer to the DNA. In contrast, the positioning of a single nucleosome can be investigated *in vitro* under well defined conditions, when a DNA fragment that comprises several hundreds of base pairs of known sequence is studied in the absence and/or in the presence of certain chromatin remodeling complexes [57, 59, 60, 105, 134, 135]. Nucleosome assembly in such experiments is usually conducted via salt dialysis in a multistage process that is determined by the initial recruitment of histones H3 · H4 to the DNA followed by the addition of H2A · H2 as reviewed previously [136] and in Chapter 3. The resulting distributions nucleosomes at different positions on the DNA are then quantified, for example, with the help of gel electrophoresis, microscopy, or spectroscopy measurements (Figure 5.3).

In addition, *in vitro* experiments provide detailed mechanistic insights for a remodeler acting on a single nucleosome at a DNA segment of known sequence [57–59, 137]. These studies have revealed that the remodeler activities depend not only on the remodeler type but also on the DNA sequence. Furthermore, remodelers might be affected by the covalent histone modifications of a specific nucleosome [138–141]. The enrichment/removal of nucleosomes at/from a certain DNA site can be explained either by lowering the remodeler binding affinity to the nucleosomes at the target DNA sequence (the "release" model) or by a reduced

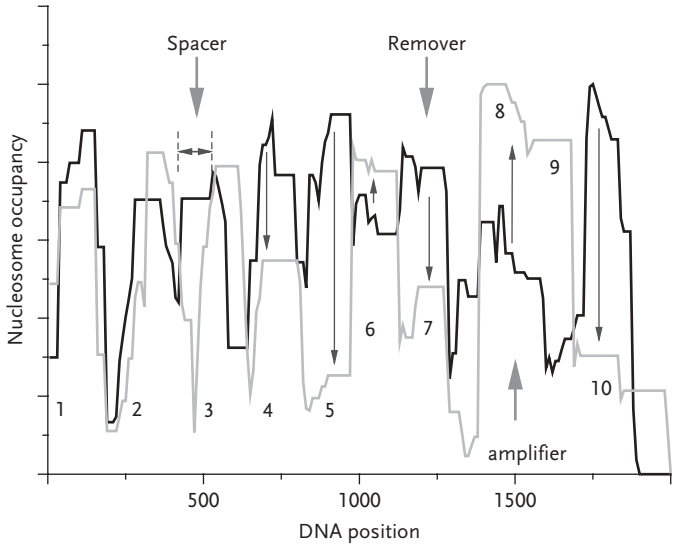

Figure 5.6 Genome-wide changes of nucleosome positions. Experimental nucleosome occupancy for resting (black line) and activated human $CD4^+$ T-cells (gray line) at a region on chromosome 5 (from 132 026 342 to 132 1028 342) [93]. The numbers indicate 10 nucleosome positions identified in this locus. Three main types of predicted remodeler activities are indicated by arrows [107]: (i) a "spacer" introduces a certain separation distance between two positions as indicated here between position 2 and 3, (ii) a "remover" mediates translocations of nucleosomes (e.g., 4, 5, 7, 10), and (iii) an "amplifier" enriches nucleosomes as indicated in the example for positions 8 and 6.

translocation rate away from this site (the "arrest" model) as discussed in Section 3.2. In the genome-wide context also the spacing of nucleosomes with respect to each other is affected by chromatin remodeling. Genome-wide nucleosome position maps can be evaluated to delineate three main types of chromatin remodeler activity [107]: (i) the establishment of regular nucleosome spacing in the vicinity of a strong positioning signal acting as a boundary, (ii) the enrichment of nucleosomes through amplification of intrinsic chromatin-encoded signals, and (iii) the removal of nucleosomes from certain sites. These effects can be quantitatively described in calculations of single- and multiple-nucleosome distributions and have been assigned to changes in nucleosome positioning in T-cells undergoing activation as depicted in Figure 5.6 [93, 107]. All three theoretically predicted classes of remodeler activity have been found in recent experimental studies [178, 192].

5.5
Gene Regulation via Nucleosome Positioning

5.5.1
Competitive Binding of Transcription Factors and Nucleosomes

The dynamic nucleosome structure modulates DNA accessibility and plays a significant role in gene regulation. In many instances nucleosome positions coincide at least partially with transcription factor binding site and inhibit their sequence-specific DNA binding [71, 87, 88, 142–145]. As discussed above transcription factors (TFs) can gain access to the nucleosomal DNA via nucleosome translocation along the DNA double helix [83, 87, 88, 107]. Interestingly, the observed reduction in binding affinity of a TF to nucleosomal DNA was found to be highly variable between twofold and >1000-fold relative to the free DNA. Thus, a number of protein factors exist that show only a relatively small reduction in binding affinity if a nucleosome is present. According to their ability to recognize a DNA target site occluded in a nucleosome two categories of chromatin binding factors are distinguished. *Pioneering* factors are able to bind to a DNA target site within the nucleosome. This process might be facilitated by a specific rotational phasing of the nucleosomal DNA to exposes the binding DNA sequence outwards from the histone octamer. A list of potential pioneering factors is shown in Table 5.1. These frequently function to initiate downstream events like chromatin remodeling or recruitment of other *effector* proteins. The effector factors do not recognize a nucleosomal DNA binding site on their own. As factors that can promote their binding two other aspects of the interaction of TFs with nucleosomal DNA need to be considered. These are the partial disassembly of the nucleosome and the unwrapping of DNA from the histone octamer core [71, 142–145, 164–168]. To describe this process quantitatively, it is not sufficient to consider the histone octamer as a single entity that interacts with 147 bp but more detailed theoretical considerations are required (Figure 5.7) [90, 170].

Table 5.1 Pioneering protein factors that can bind their recognition site in the presence of a nucleosome.

Factor	Description
Amt1	The copper responsive transcription factor Amt1 binds with only a threefold reduced affinity to its nucleosomal binding site [146, 147].
Adf1	Adf1 binds to developmentally regulated promoters in *Drosophila* and is capable of recognizing its binding site reconstituted with nucleosomes. Adf1 binding induces a distorted DNA path on the nucleosome, generating a DNase I hypersensitive site [148].
HSF	HSF displaces stably positioned nucleosomes in *Saccharomyces cerevisiae* at the core promoter of the HSP82 gene leading to activation of transcription [149].
HNF3/FoxA	The forkhead box transcription factor FoxA has a "winged helix" DNA binding domain that has a folding motif similar to that found in histones. The presence of the specific binding site results in increased affinity and the formation of a defined footprint on the nucleosome [150, 151].
GAGA	GAGA is a ubiquitous transcription factor binding to several GA-rich promoters in drosophila. GAGA and Adf1 bind non-cooperatively but with high affinity to their nucleosomal recognition sites [148].
Gal4	Multiple Gal4 sites occur at some native promoters. It was shown with a reconstituted nucleosome carrying multiple Gal4 binding sites that the protein did bind to the individual sites and induced the disassembly of the nucleosome [152, 153].
GATA-1	Binding of GATA-1 to the nucleosome partially dissociates the DNA from the nucleosome without displacing histones. Disruption of the nucleosome is entirely reversible [154].
GR	The glucocorticoid receptor GR binds sequence specific to its nucleosomal target site located in the mammary tumor virus long terminal repeat. Upon GR binding local alterations of DNA structure are observed without unfolding the nucleosome [155, 156].
PR	Sequence specific binding of the progesterone receptor (PR) is strongly affected by the translational and rotational settings of the nucleosome [157].
SP1	The ubiquitous transcription factor SP1 binds to numerous viral and cellular genes including the constitutive housekeeping genes. Sp1 recognizes its GC box reconstituted into nucleosomes with an affinity reduced by about 20- to 40-fold compared to free DNA [158, 159].
TFIIIA	The TFIIIA binding site within the 5S gene overlaps by 35 bp with a positioned nucleosome in the gene. TFIIIA binds with high affinity to the nucleosomal DNA and displaces the DNA from the histone octamer [160].

(*Continued*)

Table 5.1 (Continued)

Factor	Description
TR	The binding of the thyroid hormone receptor (TR) to the TR response element reconstituted into nucleosomes is not influenced by histone H1 but by the rotational position of the DNA relative to the histone octamer [161].
TTF-I	TTF-I is a RNA polymerase I specific termination factor that also binds to the rRNA gene promoter. Binding of the factor to its nucleosomal target site results in the reorganization of the rRNA gene chromatin structure [162].
USF	USF can bind in a sequence specific manner to the nucleosomal DNA. However, this interaction is significantly reduced by the linker histone H1 [153, 159, 163].

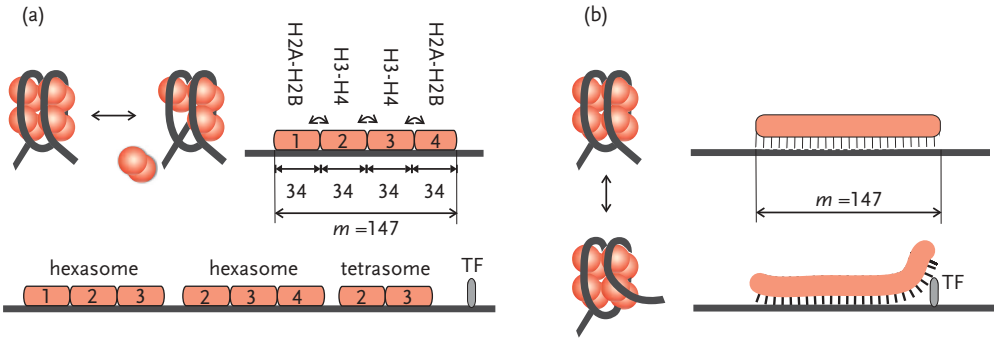

Figure 5.7 Transcription factor access to nucleosomal DNA and corresponding DNA lattice models. (a) The nucleosome can lose one or more histone dimers to form subnucleosomal particles [90, 136, 168, 169]. (b) Unwrapping DNA from the nucleosome. The histone octamer core is considered as an extended ligand, which covers $m = 147$ bp upon interactions of the DNA with the protein. If unwrapping takes place a fraction of interactions at both ends of the protein-covered DNA region can be disrupted. A transcription factor can bind the DNA partially unwrapped from the histone octamer [170].

The partial disassembly of the nucleosome could, for example, involve the dissociation of one H2A · H2B dimer as reviewed in refs. [90, 136, 168, 169]. In the other scenario, the transcription factor binds the nucleosomal DNA when it is partially unwrapped from the histone core, either due to a thermal fluctuation or induced by other protein factors [71, 142–145, 164–167, 170]. Partial nucleosome unwrapping implies that transcription factor binding at the promoter is inherently cooperative, even in the absence of direct protein–protein interactions [145, 166, 193]. This is due to the fact that the pioneering factor frees the nucleosomal DNA for the second protein binding. In addition, nucleosome arrangements on the DNA depend on whether the nucleosomes can protect a fixed or varied DNA length, and whether they can invade each other's binding sites [90, 170].

5.5.2
Remodeler and Nucleosomes as Molecular Switches

Specifically positioned nucleosomes play an essential role in the organization of regulatory regions of eukaryotic genes [82, 87, 88, 171–176]. From a number of recent studies the view is emerging that the DNA sequence is the dominating factor of only a limited subset of nucleosome positions *in vivo*, particularly in higher eukaryotes as discussed above. Thus, the changes of nucleosome positions that occur due to the activity of chromatin remodeling complexes on the same DNA sequence are likely to present an important regulatory system in the cell. Significant deviations of the genome-wide nucleosome maps were identified in yeast in dependence of the RSC [177] and ISW1 [178] remodelers. In addition, nucleosome position pattern change globally during reprogramming of cellular function within the same genome. As mentioned above, striking differences in nucleosome repeat length exist between different human tissues with values ranging from 173 ± 6 (cortical neurons) to 207 ± 8 (cortical glial cells) [1] and genome-wide change of nucleosome positions have also been observed also for activation of human T-cells [93]. These are likely to involve chromatin remodeling activity. The switching of repressive and activating nucleosome positions mediated by chromatin remodeling complexes appears to be a general mechanism for transcription activation [179–181]. DNA binding factors like the α2-MCM1 complex actively position nucleosomes at repressed genes in yeast α-cells. This process requires the intact histone H4 tail [182, 183], a target of the ISWI-containing remodeling machines [139]. Similarly, the Ssn6-Tup1 complex is a global co-repressor responsible for nucleosome positioning at a number of genes and the recombination enhancer of the silent mating-type loci in budding yeast, and it requires the ISW2 chromatin remodeler at the RNR3 gene [184–189]. Another example for repression/activation via nucleosome positioning is found at the rRNA genes [21, 35]. In this system the NoRC remodeling complex moves the promoter bound nucleosome about 25 bp downstream of the position found at inactive genes [140]. Histone modifications could represent signals that direct remodeling activity as demonstrated in several reports [138–141, 190]. This implies that remodeler activity depends on the covalent modifications of histone tails, which might modify binding affinities for remodelers or other proteins recruiting remodelers to the nucleosome. Furthermore, it appears that the reverse dependence also applies: the action of histone-modifying enzymes depends on the history of remodeling of a given nucleosome by a given remodeler type. For example, a recent study has revealed that SET domains of histone methyltransferases recognize ISWI-remodeled nucleosomal species [191].

5.6
Conclusions

The factors that govern the positioning of nucleosome at promoter and enhancer regions (and thus access to the associated DNA) are emerging as important

regulators for the control of gene expression. In the eukaryotic cell nucleus, a complex chromatin remodeling machinery operates that comprises numerous different types of ATPase motors. These molecular machines can associate with different subunits to form remodeling complexes with distinct biological functions. Their high combinatorial complexity leads to the estimate of several hundreds of different chromatin remodeling complexes in humans. They consume ATP to control nucleosome positions that, in a simple equilibrium system, would be determined by the DNA sequence-dependent competitive binding of the histone octamer, transcription factors, and other chromosomal proteins. Thus, chromatin remodeling complexes may be viewed as molecular machines that transform this equilibrium into a different steady state. Either due to sequence specificity of remodeler–DNA interactions or via chromatin signals at certain nucleosomes (e.g., histone modifications, incorporation of histone variants, or the presence of interacting proteins) these nucleosomes would become high-affinity substrates that are repositioned efficiently. In this manner, chromatin remodeling complexes can establish specific nucleosome positioning patterns that define the accessibility of DNA and with it the "on" or "off" states for DNA-dependent processes.

Acknowledgments

We thank Fabian Erdel for discussion. We gratefully acknowledge funding by the DFG in projects FOR1068 (G.L.) and Ri 1283/8-1 (K.R.), support from the Bavarian State Ministry of Sciences, Research and the Arts (Baygene program; G.L.), a fellowship of the German CellNetworks Cluster of Excellence (EXC81), and grant B10M-060 of the Belarus Foundation of Fundamental Research (V.T.).

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