CHROMATIN ASSEMBLY BY DNA-TRANSLOCATING MOTORS

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Chromatin assembly is required for the duplication of eukaryotic chromosomes and functions at the interface between cell-cycle progression and gene expression. The central machinery that mediates chromatin assembly consists of histone chaperones, which deliver histones to the DNA, and ATP-utilizing motor proteins, which are DNA-translocating factors that act in conjunction with the histone chaperones to mediate the deposition of histones into periodic nucleosome arrays. Here, we describe these factors and propose possible mechanisms by which DNA-translocating motors might catalyse chromatin assembly.

HIGH MOBILITY GROUP PROTEINS (HMG proteins). Abundant, non-histone chromosomal proteins. There are three families of HMG proteins: HMGB (HMG1/2), HMGN (HMG14/17) and HMGA (HMG-I/-Y).

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In the eukaryotic nucleus, DNA is packaged into a periodic nucleoprotein complex, known as chromatin (BOX 1). The assembly of the genome into chromatin enables the DNA — which amounts to approximately two metres of DNA per cell in humans — to be compacted within the nucleus, which has an average diameter of 10 μm in a human cell. Importantly, the DNA is packaged in a way that is compatible with essential DNA processes, such as transcription, replication, repair and recombination. So, the organization and utilization of the eukaryotic genome is dependent on the assembly of DNA into chromatin (for recent reviews, see REFS 1–5).

Chromatin assembly functions at the interface of cell-cycle progression and gene expression. The replication of the genome requires not only the duplication of the DNA, but also the assembly of the newly synthesized DNA into chromatin. In addition, the control of gene activity is strongly influenced by chromatin structure, and the establishment of the correct nucleoprotein structures of genes requires chromatin assembly. Therefore, it is important to consider the potential effects of chromatin assembly factors on processes that are related to cell growth and gene expression.

Chromatin assembly does not generate a uniform and static product, but rather it forms a structure with a diverse and dynamic chemical composition. The properties of chromatin can be modulated by covalent modification of histones[6], the incorporation of histone variants such as H2A.Z (REF. 7) and H3.3 (REF. 8), the association of non-histone chromosomal proteins such as heterochromatin protein 1 (HP1) and HIGH MOBILITY GROUP (HMG) PROTEINS[9], and DNA methylation[10]. These specific and dynamic variations in chromatin structure are important for many nuclear processes. For example, acetylation of histones generally correlates with gene activation; phosphorylation of serine 10 of histone H3 occurs during mitosis; methylation of lysine (Lys) 9 of histone H3 creates a binding site for HP1; and methylation at position 5 of cytosine in CpG dinucleotides correlates with transcriptional repression.

The simplest definition of chromatin assembly is the process by which DNA is packaged into nucleosomes. In this sense, chromatin assembly is equivalent to nucleosome assembly. The basic chromatin assembly process is mediated by histone chaperones and ATP-utilizing factors that catalyse the deposition of the histones onto DNA to yield periodic arrays of nucleosomes (FIG. 1). As well as the basic process, there are factors that are involved in the coupling of chromatin assembly to processes such as DNA replication and repair. In addition, as noted above, it is important to consider that native chromatin comprises not only nucleosomes, but also linker histones, such as histone H1, and non-histone chromosomal proteins, such as HP1 and the HMG proteins. So, from a broader perspective, the term 'chromatin assembly' includes the assembly of higher-order forms of chromatin.

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Box 1 | **Composition and structure of chromatin**

The basic repeating unit of chromatin is the nucleosome. Each nucleosome core consists of 146 base pairs of DNA wrapped with ~1.7 superhelical turns around a molecular spool that consists of an octamer of core histone proteins. The histone octamer consists of two copies each of the histones H2A, H2B, H3 and H4. In the absence of DNA at physiological ionic strength, the prevalent forms of the histones are $(H3–H4)_2$ tetramers and H2A–H2B dimers. To form a complete octamer, two H2A–H2B dimers assemble onto each side of an $(H3–H4)_2$ tetramer. Nucleosome cores are separated by linker DNA to give an overall nucleosome repeat length that is approximately 180–200 bp. At physiological ionic strength, nucleosome arrays fold into a more highly compacted form, such as the 30-nm chromatin filament, in a process that is facilitated by linker histones, for example histone H1 (REF. 107). High-resolution structural information is available for the nucleosome core particle[108], whereas little is known about the three-dimensional structures of the higher-order states of chromatin[109,110].

In this review, we will provide an overview of the factors that mediate nucleosome assembly. Recent studies have led to the purification and cloning of the histone chaperones and molecular motors that mediate the basic assembly process. In addition, we will discuss possible models by which the energy of ATP hydrolysis might be used to assemble periodic arrays of nucleosomes.

Chromatin assembly *in vivo*

In cycling cells, chromatin assembly occurs immediately after DNA replication. At the replication fork, histones from the parental chromosome are randomly distributed to the two daughter DNA strands, and the remaining complement of nucleosomes is assembled from newly synthesized histones (for a review, see REF. 4). The newly synthesized histones are acetylated at conserved positions on H3 and H4 before their incorporation into nucleosomes, and the acetyl groups are removed shortly after assembly[1,11]. The specific function of the conserved acetylation of newly synthesized histones is still unclear. For example, the acetylation of the amino termini of histones H3 and H4 does not seem to be required for chromatin assembly *in vitro*[12,13].

Chromatin assembly occurs independently of DNA replication — for example, during DNA repair, recombination, transcription and histone exchange/turnover[14]. Replication-independent chromatin assembly involves histone variants, such as H2A.Z and H3.3, which can be referred to as 'replacement histones'. These replication-independent histones differ from the S-phase-specific histones in certain ways. Most notably, the replication-independent histones are synthesized throughout the cell cycle rather than specifically in S phase. For example, the histone variant H3.3, which differs from the S-phase-specific H3 by only four amino-acid residues, is deposited into chromatin throughout the cell cycle[15]. It is also notable that transcripts that encode S-phase-specific histones lack a polyadenylate (polyA) tail, whereas the transcripts for replication-independent histones are polyadenylated. So, their genes are transcribed by fundamentally different mechanisms. Although histones are relatively stable proteins, they are still slowly degraded. So, in long-lived cells, such as neurons, the replication-independent histones are needed to replace histones in chromatin as they are turned over (hence the name 'replacement' histones).

The factors that are involved in the assembly of replication-independent histones might be distinct from those used in the assembly of S-phase-specific histones[15,16]. For example, S-phase-specific H3 that is ectopically expressed throughout the cell cycle does not exhibit replication-independent deposition[15]. This observation indicates that there might be chromatin assembly factors that function with the replication-independent histones, but not with the S-phase-specific histones.

Finally, it is important to consider that the cell-cycle-independent exchange of nucleosomal histones is a potential mechanism for switching epigenetic states. For example, methylation of histone H3 on Lys9 is associated with gene silencing and HETEROCHROMATIN formation, and one way that methylated histones could be converted to the unmethylated state is through histone exchange[17].

Chromatin assembly factors

Chromatin assembly is an active process that involves histone chaperones and ATP-dependent motor proteins (FIG. 1). When mixed together at physiological ionic strength, purified core histones and DNA aggregate to form an insoluble precipitate. This aggregation is driven by the ionic interactions between the negatively charged DNA backbone and the positively charged basic residues in the histones. This non-specific precipitation can be prevented by the addition of histone-transfer vehicles — negatively charged polymers that shield the positive charge of the histones[1–3]. A variety of molecules, including RNA, polyglutamic acid and several histone-binding proteins, have been found to function as histone-transfer vehicles *in vitro*.

Here, we will focus on the chaperones that have been found to associate with histones *in vivo*[1–3,18]. These histone chaperones not only prevent aggregation between histones and DNA, but also function as shuttle proteins that deliver histones to the appropriate sites of chromatin

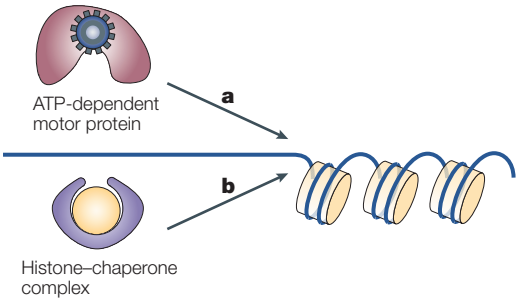

Figure 1 | **A simple view of chromatin assembly. a** | Core histones are delivered to the site of chromatin assembly by histone chaperones, such as chromatin assembly factor 1 (CAF1), nucleosome assembly protein 1 (NAP1) or the anti-silencing function 1 (Asf1) subunit of replication-coupling-assembly factor (RCAF). **b** | The histones are then deposited onto the DNA in conjunction with an ATP-dependent motor protein, such as ATP-utilizing chromatin assembly and remodelling factor (ACF) or remodelling and spacing factor (RSF). The product of this reaction is a periodic array of nucleosomes.

HETEROCHROMATIN
Chromatin that remains in a condensed state throughout the cell cycle; for example, centromeres and telomeres are heterochromatic regions. Few protein-coding genes are located in heterochromatin and most protein-coding genes are located in euchromatin, which decondenses during interphase.

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Table 1 | **Chromatin assembly factors**

Assembly factor	Functional roles	Binding interactions	References
CAF1	Histone chaperone Replication-coupled chromatin assembly DNA repair Silencing Cell-cycle progression	H3–H4 PCNA Asf1	24–39,48,49
Asf1	Histone chaperone Replication-coupled chromatin assembly DNA repair Silencing Cell-cycle progression	H3–H4* CAF1 Brahma $TAF_{II}250$/CCG1 RAD53 SAS-I Hir1, Hir2	40–55
NAP1	Histone chaperone Nuclear import of histones	H2A–H2B‡ H3–H4‡ Kap114 p300	60–68
HIR	Histone gene regulation Histone chaperone Replication-independent chromatin assembly Silencing	H2A–H2B H3–H4 Asf1	16,38,49, 50,56–59, 69
Nucleoplasmin	Maternal storage of histones in oocytes Histone chaperone during rapid rounds of replication in early embryo	H2A–H2B‡ H3–H4‡	18
N1/N2	Maternal storage of histones in oocytes Histone chaperone during rapid rounds of replication in early embryo	H3–H4	18
Spt6	Histone-transfer vehicle Transcription-elongation factor	H3–H4§ H2A–H2B§	70
DF31	Histone-transfer vehicle Chromatin structural protein		71,72
Nucleophosmin/ B23	Histone transfer vehicle Ribosome biogenesis Replication stimulatory factor	H3 RNA	73,74
ACF	DNA-translocating motor Chromatin assembly factor (requires chaperone) Chromatin remodelling factor		19,21–23, 78–80,93, 98,99
RSF	Stimulates transcription *in vitro* Chromatin assembly factor (does not require chaperone) Chromatin remodelling factor		81,100

*Replication-coupling assembly factor (RCAF) is the complex of anti-silencing factor 1 (Asf1) and specifically acetylated histones H3 and H4. The H3 and H4 in the RCAF complex are acetylated with the same pattern as newly synthesized H3 and H4. ‡Nucleosome assembly protein 1 (NAP1) and nucleoplasmin bind with higher affinity to histones H2A–H2B than to histones H3–H4. §Spt6 protein binds with higher affinity to histones H3–H4 than to histones H2A–H2B. ACF, ATP-utilizing chromatin assembly and remodelling factor; CAF1, chromatin assembly factor 1; PCNA, proliferating cell nuclear antigen; RSF, remodelling and spacing factor.

assembly in the cell. *In vitro*, histone-transfer vehicles can mediate the deposition of histones onto negatively supercoiled DNA. Negative supercoiling facilitates histone deposition because the negative superhelical tension is relieved when the DNA is wrapped around the histones[19,20]. However, this process does not yield regularly spaced nucleosome arrays[19], as seen in bulk native chromatin.

The ATP-dependent assembly of periodic nucleosome arrays requires an ATP-driven motor protein as well as the histone chaperones. With relaxed DNA templates, ATP-dependent motor proteins catalyse the deposition of histones onto DNA, as well as the generation of periodic arrays of nucleosomes[19,21–23]. The bulk of the eukaryotic genome seems to possess little superhelical tension. Therefore, the use of relaxed DNA templates for chromatin assembly is preferable to the use of negatively supercoiled DNA. In the following section, we will discuss the histone chaperones and ATP-driven motors that are thought to be involved in chromatin assembly. These factors are also summarized in TABLE 1.

Histone chaperones. A variety of proteins interact with core histones and function as histone-transfer vehicles. Most of these factors have a preference for either H3–H4 or H2A–H2B. Although these proteins all bind to histones, they do not seem to be related at the level of their primary amino-acid sequences. So, these chaperones, which escort histones to the sites of chromatin assembly, might have evolved independently into histone-binding proteins, several of which are described below.

Chromatin assembly factor 1 (CAF1) is a heterotrimeric protein that comprises the p150, p60 and p48 subunits[1,24–28]. It can also be isolated in a complex, known as CAC, that contains histones H3 and H4, where the H4 has an acetylation pattern that is similar to that of newly synthesized H4 (REF. 25). CAF1 was discovered through a DNA-replication-coupled chromatin-assembly assay[28]. In this assay, CAF1 is required for the preferential assembly of nucleosomes onto newly synthesized DNA, relative to bulk unreplicated DNA[27]. CAF1 is recruited to replication foci[29,30] by a mechanism that involves an interaction with the DNA polymerase through the PCNA sliding-clamp protein[31–33]. In yeast, cells that lack Caf1 are viable but defective in transcriptional silencing[34–39]. Many lines of evidence also indicate a role for CAF1 in DNA repair[24].

Replication-coupling-assembly factor (RCAF) is a complex of anti-silencing function 1 (Asf1) protein and specifically acetylated histones H3 and H4 (REF. 40). Like CAF1, RCAF participates in DNA-replication-coupled chromatin assembly. Notably, the histones H3 and H4 in the RCAF complex show the same pattern of acetylation as newly acetylated histones. In addition to its function in replication-coupled chromatin assembly as a subunit of RCAF, Asf1 protein has been shown to have histone chaperone activity in the absence of DNA replication and might also be involved in DNA-replication-independent chromatin assembly[41,42]. *asf1* was originally identified in yeast as a gene that derepresses transcriptional silencing when overexpressed[43,44]. In yeast, deletion of the *asf1* gene causes silencing defects, slow growth and sensitivity to DNA-damaging agents[40,43,44]. Like CAF1, Asf1 seems to be important for DNA repair[40,42,45,46]. Furthermore, Asf1 (RCAF) has been found to function synergistically with CAF1 for chromatin assembly *in vitro* and to interact directly with CAF1 (REFS 40,47–50) In addition, Asf1 interacts with the chromatin remodelling protein Brahma[51], the $TAF_{II}250$/CCG1 subunit of basal transcription factor

PCNA
(Proliferating cell nuclear antigen). PCNA is a sliding-clamp protein that forms a doughnut-shaped structure around the DNA, and functions to increase the processivity of DNA polymerases.

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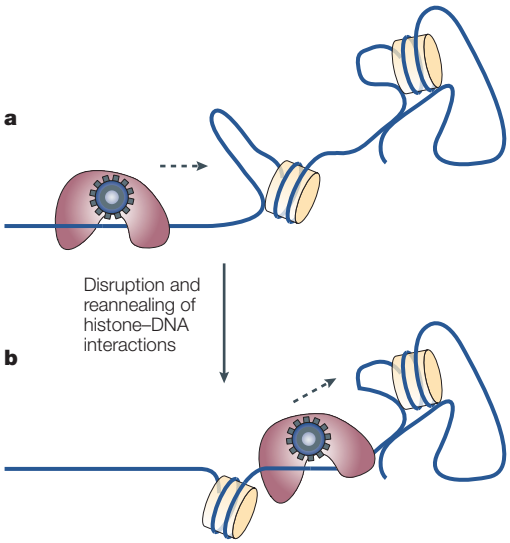

Figure 2 | **Iterative-annealing model of chromatin assembly.** In this model, **a** | non-nucleosomal histone–DNA complexes are initially formed upon deposition of the histones onto the DNA by chaperones, such as chromatin assembly factor 1 (CAF1) or nucleosome assembly protein 1 (NAP1). **b** | Next, a DNA-translocating motor, such as ATP-utilizing chromatin assembly and remodelling factor (ACF) or remodelling and spacing factor (RSF), disrupts the histone–DNA contacts to allow reannealing of the histones and DNA into nucleosomes. Note, however, that this process yields randomly distributed nucleosomes. The generation of periodic arrays of nucleosomes, as seen *in vivo*, would require the subsequent rearrangement of the nucleosomes. Dashed arrows indicate the direction of translocation by the motor protein.

TFIID[52], the RAD53 checkpoint protein kinase[42], the SAS-I histone acetyltransferase complex[53,54], and Hir1 and Hir2 proteins[50,55]. The HIR proteins were originally found to be involved in the regulation of histone gene expression[56–58], and they have since been shown to interact with histones *in vitro*[16,59]. The collective biochemical and genetic data support a model in which Asf1 (RCAF) and HIR proteins function in a chromatin assembly pathway that partially overlaps with an assembly pathway that involves CAF1 and PCNA. Furthermore, the interactions of Asf1 with Brahma, RAD53, $TAF_{II}250$/CCG1 and SAS-I indicate that Asf1 (RCAF) functions in a complex network of biological processes that regulate gene expression and cell growth.

Nucleosome assembly protein 1 (NAP1) was identified as a histone-binding protein that facilitates the random deposition of histones into nucleosomes[60,61]. NAP1 is a homomultimer of a single polypeptide with an acidic carboxyl terminus[62,63], and it binds preferentially to H2A–H2B relative to H3–H4 (REFS 62,64,65). *Drosophila* NAP1 and the related human NAP2 have been shown to move from the cytoplasm into the nucleus specifically during S phase[62,66]. NAP1 has been found to interact directly with the Kap114 KARYOPHERIN and participate in the nuclear import of histones H2A and H2B[67,68].

Several other histone-binding proteins have been identified. As noted above, the HIR proteins bind to histones and participate in the chromatin assembly process, particularly in conjunction with Asf1 (RCAF)[16,38,49,50,59,69]. In *Xenopus*, nucleoplasmin and N1/N2 are abundant histone-binding proteins in the early embryo (for a review and additional references therein, see REF. 18). Nucleoplasmin binds preferentially to H2A–H2B, whereas N1/N2 binds to histones H3–H4. These factors, which are present at high concentrations in the egg cytoplasm, seem to be important for the storage and deposition of histones during the rapid cycles of DNA replication that occur before the midblastula transition[18]. There are no apparent homologues of nucleoplasmin in yeast or humans. Other histone-binding factors that have been shown to have histone-transfer-vehicle activity include Spt6 (REF. 70), DF31 (REFS 71,72) and nucleophosmin/B23 (REFS 73,74). These proteins are likely to have important roles in chromosome assembly and maintenance.

ATP-dependent chromatin-assembly factors. The assembly of periodic arrays of nucleosomes, which is characteristic of native chromatin, is an ATP-dependent process[75]. The fractionation and purification of an ATP-dependent chromatin-assembly activity led to the identification of ATP-utilizing chromatin assembly and remodelling factor (ACF)[22]. ACF consists of two subunits, ACF1 and the ISWI (imitation switch) ATPase, which function cooperatively in the assembly of chromatin[21]. In addition to its role in chromatin assembly, ACF can function as a chromatin remodelling factor[22,76].

Periodic nucleosome arrays can be assembled by using purified ACF, purified core histones, DNA, ATP and a purified histone chaperone such as NAP1 (REFS 21,22). With relaxed DNA, ACF is required not only for the periodic spacing of nucleosomes, but also for the efficient deposition of histones onto DNA[19,21–23]. With negatively supercoiled DNA, NAP1 alone can deposit histones onto DNA because the wrapping of DNA around the histones relieves negative superhelical tension, but the resulting nucleoprotein complexes do not resemble canonical nucleosomes, as assessed by atomic-force microscopy[19].

ACF functions as a processive, ATP-driven DNA-translocating enzyme[23]. The molecular engine of ACF is ISWI, an ATPase that is a common subunit of the ACF, CHRAC, NURF and TRF2 complexes in *Drosophila*. ISWI-containing complexes have also been found in yeast[77], *Xenopus*[78] and humans[79–83]. ISWI, which is an essential protein in *Drosophila*[84], belongs to the SNF2-like family of ATPases[85,86]. Other members of the SNF2-like ATPase family have also been shown to function as catalytic subunits in complexes that have chromatin remodelling activity (for reviews, see REFS 87–91).

When discussing ACF, it is important to note its similarity to CHRAC. CHRAC was identified by its ability to increase the accessibility of nucleosomal DNA to digestion by restriction enzymes[92]. Although CHRAC was originally thought to contain TOPOISOMERASE II, it is now thought to consist of ACF1, ISWI and two small subunits, CHRAC14 and CHRAC16 (REFS 79,93,94). CHRAC14 and CHRAC16 are expressed specifically in the early *Drosophila* embryo[94] and are not required for chromatin remodelling activity[93]. So, CHRAC is closely

KARYOPHERIN
Nuclear import receptor, also known as importin.

CHRAC
(Chromatin accessibility complex). CHRAC was originally identified as a factor that increases the accessibility of restriction enzymes to DNA that is packaged into chromatin.

NURF
(Nucleosome-remodelling factor). NURF was isolated on the basis of its ability to modify the chromatin structure at the *hsp70* promoter in cooperation with transcription factors.

TRF2
(TATA-box-binding protein (TBP)-related factor 2). TRF2-containing complexes are involved in transcriptional regulation.

TOPOISOMERASE II
An abundant, ATP-dependent topoisomerase that functions by creating a double-stranded break in the DNA, passing another DNA molecule through this break, and then resealing the double-stranded break. The strand passage reaction relaxes supercoiled substrates and requires ATP.

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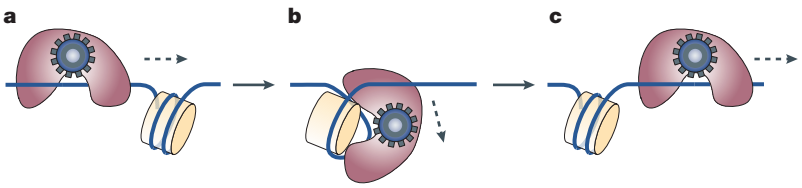

Figure 3 | **Nucleosome mobilization by a DNA-translocating enzyme.** A model for the movement of nucleosomal histones relative to DNA as a consequence of the passage of an ATP-driven DNA translocating enzyme, such as ATP-utilizing chromatin assembly and remodelling factor (ACF) or remodel the structure of chromatin (RSC). In this postulated mechanism, **a** | the DNA-translocating protein tracks along DNA and **b** | dissociates a segment of DNA from one end of the nucleosome. **c** | As the motor continues to track along the DNA, the DNA 'in front' of the motor dissociates from the nucleosomal histones, whereas the DNA 'behind' the motor reassociates with the histones. In this manner, a bulge or loop of DNA is propagated across the surface of the nucleosome. After the motor has traversed the nucleosome, the nucleosome has 'moved' because there is a net change in the position of the nucleosomal histones relative to the DNA. Dashed arrows indicate the direction of translocation by the motor protein.

related to ACF. By contrast, the NURF[95,96] and TRF2 (REF. 97) complexes are not related to ACF or CHRAC, except for the presence of its ISWI subunit.

ACF1, the largest subunit of ACF and CHRAC, enhances the function of the ISWI motor protein. ACF (ACF1 and ISWI) is ~30 times more active in chromatin assembly than ISWI alone[21]. So, ISWI is the motor of ACF, and ACF1 'programmes' the motor to function in chromatin assembly. In addition, ACF1 significantly changes the ability of the ISWI motor to function in chromatin remodelling. In a NUCLEOSOME SLIDING assay, ISWI catalyses the movement of nucleosomes from the centres to the ends of short (< 300 base pairs) DNA fragments, whereas ACF catalyses the opposite movement of nucleosomes from the ends to the centres of DNA fragments[93]. This effect might be due to the binding of ACF1 to the ends of the DNA fragments. ACF1 contains a WAC MOTIF, a WAKZ MOTIF, a DDT DOMAIN, two PHD FINGERS and a BROMODOMAIN[21,98]. The WAC motif is involved in the interaction of ACF1 with DNA, and the DDT domain is in the region of ACF1 that interacts with ISWI[98]. Depletion of ACF1 from HeLa cells by RNA interference caused an alteration in the temporal pattern of bromodeoxyuridine (BrdU) incorporation relative to wild-type cells[99]. These results are consistent with the possibility that ACF1 is involved in the late stages of DNA replication — for example, the replication of heterochromatic DNA. An alternative interpretation is that, on depletion of ACF1, DNA replication progresses faster and the time required to complete S phase is thereby shortened.

In addition to ACF and related complexes, the RSF (remodelling and spacing factor) chromatin-remodelling complex has been shown to mediate the ATP-dependent assembly of nucleosome arrays[100]. RSF was originally identified as a factor that facilitates transcription from chromatin templates[81]. It was purified from cultured human cells and was found to consist of a 325-kDa polypeptide and hSNF2H, which is related to the *Drosophila* ISWI protein[81]. The mechanism of chromatin assembly by RSF might be distinct from that by ACF. For example, chromatin assembly by purified RSF occurs in the absence of a core-histone chaperone such as NAP1 (REF. 100), whereas ACF requires the presence of a histone chaperone for chromatin assembly. Also, the number of nucleosomes assembled per molecule of ACF is several-fold higher than the number of nucleosomes assembled per molecule of RSF. These findings indicate that RSF might possess histone chaperone activity as well as ATP-dependent assembly activity.

Models of ATP-dependent chromatin assembly

ACF seems to function processively as a DNA-translocating enzyme during chromatin assembly[23]. After initiation of the assembly reaction *in vitro*, ACF becomes engaged to the DNA template, as assessed by template commitment analysis[23]. In addition, the initial products of ACF-mediated assembly were found to be periodic nucleosome arrays, rather than randomly distributed nucleosomes. These data are consistent with a model in which ACF binds to the DNA template and assembles periodic arrays of nucleosomes as it translocates along the DNA.

Studies of the functions of other chromatin remodelling proteins provide evidence in support of a DNA-translocation model. For example, the RSC (remodel the structure of chromatin) complex, whose catalytic subunit Sth1 is a member of the SWI2/SNF2-like subfamily of ATPases, has triplex DNA displacement activity, as shown in a TRIPLEX DNA DISPLACEMENT ASSAY[101]. In addition, the ISWI polypeptide, which is the ATPase subunit of ACF, CHRAC and NURF, also displaces triplex DNA[102]. It should be noted, however, that RSC complex does not seem to be involved in chromatin assembly and ISWI polypeptide is a subunit of several protein complexes, at least one of which (NURF) does not seem to catalyse chromatin assembly. Moreover, the ISWI experiments were performed in the absence of NAP1–histone complexes, which are required to observe template commitment by ACF. So, the results of the experiments with the RSC complex and ISWI polypeptide are useful in relation to DNA translocation by ACF, but might not be directly applicable to the assembly of chromatin by ACF.

How can a DNA-translocating motor, such as ACF, assemble chromatin? We describe below two possible models by which a DNA-translocating protein could mediate chromatin assembly. In the first mechanism ('iterative annealing'), the DNA-translocating motor disrupts undesired histone–DNA interactions to allow the proper annealing of histones and DNA into periodic arrays of nucleosomes. In the second mechanism ('directed deposition'), the DNA-translocating motor directly deposits histones onto DNA in periodic nucleosome arrays.

It is also important to note that the deposition of histones H3–H4 *in vivo* does not seem to occur concurrently with the deposition of H2A–H2B (for example, see REFS 103,104). For simplicity, however, we depict the deposition of the four core histones in a single step. Higher-resolution versions of these models could be envisioned, in which H3–H4 tetramers are deposited before the incorporation of H2A–H2B dimers.

NUCLEOSOME SLIDING
The translational movement of nucleosomal histones relative to the DNA. Because of the asymmetry of the histone–DNA contacts in the nucleosome, it is unlikely that the histones actually 'slide' along the DNA.

WAC MOTIF
A protein sequence motif that was initially found in WSTF (Williams syndrome transcription factor), ACF1 and cbp147. The WAC motif in ACF1 was found to be required for binding of ACF to DNA.

WAKZ MOTIF
A protein sequence motif that was initially found in WSTF (Williams syndrome transcription factor), ACF1, KIAA0314 and ZK783.4.

DDT DOMAIN
A protein sequence motif found in transcription and chromatin-modifying factors. The sub-region of ACF1 that interacts with ISWI contains a DDT domain.

PHD FINGER
A protein sequence motif that was termed plant homeodomain finger. The PHD finger is found in many proteins that function with chromatin.

BROMODOMAIN
A protein sequence motif that is present in many chromatin-modifying proteins. Bromodomains have been found to bind to acetylated lysine residues.

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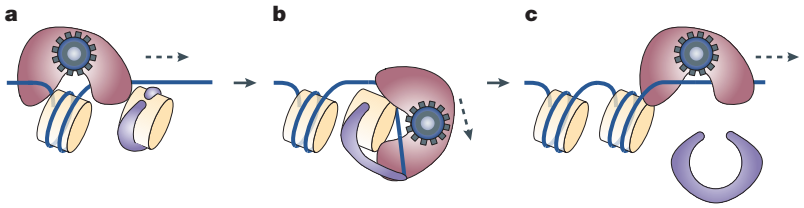

Figure 4 | **Directed-deposition model of chromatin assembly.** In this model, a DNA-translocating motor, such as ATP-utilizing chromatin assembly and remodelling factor (ACF), uses the energy of ATP hydrolysis to mediate histone deposition in a processive fashion. One possible version of a directed-deposition mechanism is depicted in this diagram. **a** | First, the motor translocates along the DNA and associates with a histone–chaperone complex (such as nucleosome assembly protein 1; NAP1). **b** | Then, in a processive fashion, the motor protein dissociates histone–chaperone interactions while it establishes histone–DNA contacts. **c** | The motor continues to translocate along the DNA until the histones are completely dissociated from the chaperone and a nucleosome is formed. Dashed arrows indicate the direction of assembly (direction of translocation).

Iterative-annealing model. The iterative-annealing model for chromatin assembly is similar to the mechanism of protein folding that is used by the bacterial CHAPERONIN GroEL. Proteins that fold with difficulty *in vivo* are thought to become trapped in undesired conformations, known as kinetic traps. These kinetic traps represent aggregates or misfolded states that are occupied during the process of protein folding. GroEL is a large barrel-shaped machine that uses the energy of ATP hydrolysis to disrupt improperly folded proteins in a manner that allows repartitioning of the protein into folded and misfolded states[105]. Through several iterations of unfolding and folding by GroEL, the protein is ultimately able to achieve its native conformation.

In an analogous fashion, it is possible that a DNA-translocating motor, such as ACF, could mediate chromatin assembly through an iterative mechanism. First, the chaperones deliver histones to the DNA to form non-nucleosomal histone–DNA complexes. The chaperones could then remain associated with the histone–DNA complexes at this point. Then, these non-nucleosomal complexes are resolved into nucleosomes by the iterative disruption and re-establishment of histone–DNA contacts by an ATP-driven DNA-translocating enzyme (FIG. 2). The chaperones could also facilitate the disruption and reformation of histone–DNA contacts by binding to the histones.

In addition, by a related mechanism, a DNA-translocating motor could mediate NUCLEOSOME MOBILIZATION or REMODELLING. ATP-dependent chromatin remodelling factors, such as ACF, can catalyse the movement of nucleosomes, which are intrinsically immobile under physiological conditions[106]. A DNA-translocating motor could catalyse nucleosome movement by disruption of histone–DNA contacts, followed by reassociation of the histones with an adjacent stretch of DNA[86,106] (FIG. 3).

The iterative-annealing model provides a mechanism for the formation of randomly distributed nucleosomes, but it is important to remember that the product of ATP-dependent chromatin assembly is a periodic array of nucleosomes. In this regard, it is possible that the folding or packing of nucleosomes into a higher-order structure, such as the 30-nm chromatin fibre, is the basis of the nucleosome periodicity. The packing of nucleosomes (which are mobile due to the action of chromatin remodelling factors such as ACF) into a higher-order structure is one possible mechanism by which a periodic nucleosomal array could be attained.

So, in this two-step process, a periodic nucleosome array could be formed with an ATP-driven DNA-translocating enzyme through the iterative-annealing mechanism. First, non-specific histone–DNA contacts are reorganized into nucleosomes (FIG. 2). Then, by catalysis of nucleosome mobilization (FIG. 3), a periodic array of nucleosomes is generated by packing the chromatin into a higher-order structure to give an evenly packed, regular array of nucleosomes.

Directed-deposition model. In the directed-deposition model, which is more straightforward than the iterative-annealing mechanism, the DNA-translocating motor functions in conjunction with the histone–chaperone complex to mediate the processive formation of nucleosome arrays. Variations of the directed-deposition mechanisms are possible. For example, the translocating motor could simultaneously dissociate histone–chaperone interactions and form histone–DNA contacts, as shown in FIG. 4. Alternatively, DNA translocation by the assembly factor could generate a specialized DNA structure to which histones are rapidly transferred.

The nucleosomes that are assembled by a direct deposition mechanism could be evenly spaced. Alternatively, if the initial reaction product is irregularly distributed nucleosomes, then the chromatin could be rearranged into periodic arrays through a nucleosome-mobilization and chromatin-folding process, similar to that described above in the iterative-annealing section. Finally, it is important to note that ACF, RSF and related complexes could potentially function in either or both of the iterative-annealing and directed-deposition reactions. For example, ACF might assemble chromatin through a directed-deposition mechanism (see FIG. 4) and mobilize nucleosomes by an iterative-annealing process (see FIG. 3).

Summary and perspectives

Chromatin assembly is crucially important for the replication, maintenance and activity of chromosomes. At present, many of the factors that are involved in chromatin assembly have been identified, but it is likely that there are additional chromatin assembly factors that remain to be discovered. Moreover, as indicated by many recent studies, chromatin assembly factors might function in a specialized, rather than general, fashion. So, it will be important to elucidate the specific biological activities of these factors. Such endeavours would include the analysis of the linkage of chromatin assembly to other processes, such as DNA replication, DNA repair and progression through the cell cycle. In a separate line of experiments, it will be interesting to investigate the mechanisms that are involved in the assembly of nucleosomes. From a biochemical perspective, the *in vitro* assembly of periodic arrays of nucleosomes

TRIPLEX DNA DISPLACEMENT ASSAY
A test for DNA translocation in which a short oligonucleotide that binds in the major groove of a pyrimidine-rich target sequence is displaced by motor proteins that translocate through the sequence.

CHAPERONIN
ATP-dependent protein complex that mediates protein folding.

NUCLEOSOME MOBILITY
The ability of nucleosomal histones to move along the DNA. Under physiological conditions, nucleosomes are essentially immobile, but some chromatin-remodelling factors are able to catalyse the movement of nucleosomes.

NUCLEOSOME REMODELLING
Also known as chromatin remodelling. Any detectable change in histone–DNA interactions in a nucleosome. Chromatin-remodelling factors alter the structure of nucleosomes in an ATP-dependent manner.

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from individual purified components is a remarkable process. It will be fascinating to learn how ATP-driven molecular motors, such as ACF and RSF, mediate this process.

Finally, the assembly of periodic nucleosome arrays with core histones and DNA is only the first step in the assembly of 'native' chromatin, as it exists in the nucleus. It will be essential to study subsequent steps in the chromatin assembly process, the next one being the assembly of chromatin that contains linker histones as well as non-histone chromosomal proteins, such as the HMG proteins. In this way, the study of 'nucleosome assembly' becomes the study of 'chromatin assembly' and, eventually, the study of 'chromosome assembly'.

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Flybase: http://flybase.bio.indiana.edu/
Brahma | CHRAC14 | CHRAC16 | DF31 | NAP1 | ISWI

***Saccharomyces* Genome Database:** http://genome-www.stanford.edu/saccharomyces/
Asf1 | Hir1 | Hir2 | Kap114 | Spt6

Swiss-Prot: http://www.expasy.ch/
ACF1 | CAF1 | HP1 | N1/N2 | NAP2 | nucleophosmin/B23 | nucleoplasmin | RAD53 | hSNF2H | TFIID

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