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REVIEW ARTICLE

Chromatin dynamics at DNA replication, transcription and repair

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During DNA replication, transcription and DNA repair in eukaryotes, the cellular machineries performing these tasks need to gain access to the DNA that is packaged into chromatin in the nucleus. Chromatin is a dynamic structure that modulates the access of regulatory factors to the genetic material. A precise coordination and organization of events in opening and closing of the chromatin is crucial to ensure that the correct spatial and temporal epigenetic code is maintained within the eukaryotic genome. This review will summarize the current knowledge of how chromatin remodeling and histone modifying complexes cooperate to break and remake chromatin during nuclear processes on the DNA template.

Keywords: chromatin assembly; chromatin remodeling; chromatin; DNA repair; elongation; epigenome; heterochromatin; histone acetylation; histone methylation.

Introduction

The organization of DNA into chromatin and chromosomal structures plays a central role in many aspects of cell biology and development in eukaryotes. Processes ranging from chromosome stability and segregation to gene expression are intimately linked to chromatin configuration. In accessing the genetic material during replication, transcription, recombination and repair, the respective cellular machineries work on chromatin as the native DNA template. Chromatin is generally repressive to extraneous access, and this inhibitory effect must be overcome by regulatory factors. Conversely, the original chromatin condition has to be reinstated after the tasks have been completed. While much is known about how chromatin is accessed, knowledge about chromatin resetting is only starting to accumulate. Many of the mechanistic insights into the dialogue between chromatin and accessory proteins come from the yeast *Saccharomyces cerevisiae*. In the following review, the knowledge from yeast and other organisms will be combined to present models for the dynamic processes on the chromatin template during DNA metabolic processes.

Chromatin is the basic organizational form of DNA in the eukaryotic nucleus. The repeat unit of chromatin is the core nucleosome in which 146 base pairs of DNA are wrapped around the histone octamer that consists of two molecules each of the core histones H2A, H2B, H3 and H4 [1]. Nucleosomal arrays along the DNA are proposed to fold into a 30 nm fiber upon incorporation of the linker histone H1. In addition to the canonical histones, histone variants exist that are structurally related to the 'normal' histones, but are functionally distinct. For example, centromeric nucleosomes carry the CENP-A histone variant (Cse4 in *S. cerevisiae*), which is required for proper kinetochore function (reviewed in [2]). Additional superordinate proteins further modify the higher-order chromatin structure. Genomic domains termed heterochromatin exist that suppress the accumulation of stable transcripts from resident genes [3,4]. In such regions, additional heterochromatin proteins bind nucleosomes and mediate gene silencing. For example, the Sir proteins in *S. cerevisiae* are found at the repressed silent mating-type loci and in subtelomeric regions [5]. Similarly, heterochromatin protein 1 (HP1) in *Drosophila melanogaster* is associated with pericentric heterochromatin and is required for the silencing of genes located in these regions [6]. Another level of chromosome compaction is required at mitosis, where condensin and cohesin proteins bind to chromatin to yield the highly condensed mitotic chromosomes during cell division.

Two main enzymatic activities can be distinguished that regulate chromatin access: chromatin modifying complexes, and chromatin remodeling complexes, which are briefly described below.

Histone modifying complexes

The term 'chromatin modification' describes post-translational modifications on the histones. Potential modifications include histone acetylation, methylation,

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Abbreviations: HAT, histone acetyltransferase; HP1, heterochromatin protein 1; HDAC, histone deacetylase; HMT, histone methyltransferase; ORC, origin recognition complex; NURD, nucleosome remodeling and deacetylation; CAF, chromatin assembly factor; PCNA, proliferating cell nuclear antigen; Hir, histone regulator; PolII, RNA polymerase II; FACT, facilitates chromatin transcription; NER, nucleotide excision repair; DSB, double strand break; NHEJ, non-homologous end-joining.

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phosphorylation, ubiquitylation, sumoylation and ADP-ribosylation. Most modifications were originally observed on the N-terminal tails of histones, with the exception of ubiquitylation, which occurs on the C-terminal tails of H2A and H2B. Recent work has also identified several novel post-translational modifications in the core region of the histones [7].

Histone acetyltransferases

Perhaps best studied among histone modifications is histone acetylation in the amino-terminal tails of the histones, where lysine residues can be post-translationally acetylated at the ε-NH_3^+ position of the side chain. Acetylation is carried out by histone acetyltransferases (HATs). They can be grouped into five families based on homology (for detailed information, see [8]). (a) The Gcn5 family members are most similar to Gcn5 from *S. cerevisiae*. Among them are the Gcn5 homologs from higher eukaryotes as well as PCAF, the cytoplasmic Hat1 and the Elongator component Elp3. (b) The MYST family of HATs is named after the founding members MOZ, Ybf2/Sas3, Sas2 and Tip60 [9]. The MYST HATs have diverse functions in transcription, replication, DNA repair and dosage compensation. (c) The p300 family includes p300 and CREB-binding protein (CBP), two highly related HATs [10] that are invoked in transcription initiation in human cells. (d) The general transcription factor HATs include the TFIID subunit TAF250 [11]; TFIIIC, a general transcription factor in the RNA polymerase III basal machinery [12]; and Nut1 in *S. cerevisiae*, a component of Mediator [13]. (e) The nuclear receptor cofactors ACTR and SRC1 have intrinsic HAT activity. Both stimulate transcriptional activation by nuclear hormone receptors (reviewed in [14]).

Biochemical isolation of HATs has shown that they work in large multiprotein complexes that share some similarities in subunit composition. For example, Gcn5 is the catalytic subunit of the SAGA transcriptional activation complex [15] as well as in the SALSA [16] and SLIK [17] HAT complexes that have a similar composition to SAGA. Sas3 is part of the NuA3 complex [18], and the MYST HAT Esa1, the only essential HAT in yeast, is part of NuA4 [19]. Intriguingly, several HAT complexes contain actin and actin-related proteins [20], which has lead to the hypothesis that they are directed to their site of action by a nuclear scaffold that is yet to be defined.

Histone acetylation is a reversible process and, accordingly, histone deacetylases (HDACs) have been isolated that catalyze this reaction. HDAC families include the HDAC I class that resemble yeast Rpd3, and the HDAC II family that are similar to yeast Hda1 (reviewed in [21]). The yeast HDACs Hos1 and Hos2 are more similar to Rpd3, whereas Hos3 more closely resembles Hda1. A third group of HDACs, the Sirtuin family whose founding member is the Sir2 protein from *S. cerevisiae*, is structurally unrelated to the other two families and has the unusual property of requiring NAD^+ as a cofactor in the deacetylation reaction (reviewed in [22]). Sir2 was characterized early on as a protein that is essential for all forms of silencing in *S. cerevisiae*. However, the NAD^+ requirement and the dissimilarity to known HDACs delayed the discovery of its biochemical activity. Sirtuins are evolutionarily conserved in eukaryotes, prokaryotes and archeae, where they fulfil diverse functions in development, heterochromatin formation and apoptosis.

Histone methyltransferases

In contrast to acetylation, histone methylation seems to be chemically more stable and may be irreversible, as no histone demethylases have yet been isolated. Thus, methylation may only be removed passively by dilution during replication, by exchange on chromatin, or alternatively by proteolytic cleavage of the protein portion carrying the methyl group. Whereas acetylation results in the neutralization of the positive charge on the ε-NH_3 group, methylation leaves the charge intact. Histone methylation is carried out by histone methyltransferases (HMTs). The first HMT identified was Su(var)3–9 [23], and homology comparisons led to the discovery of the SET family of HMTs (Su(var)3–9, Enhancer of zeste and Trithorax). This family also includes six SET homologs from *S. cerevisiae*. An astonishing discovery in the field was the existence of the Dot1 family of HMTs. Dot1 was first characterized in *S. cerevisiae* in that overexpression lead to the disruption of telomeric silencing, and Dot1 was later shown to methylate H3 in the core region at K79. Dot1 shows no sequence similarity to the SET HMTs, but instead methylates histones via a methylase fold [24,25].

A lysine ε-NH_3^+ group can be mono-, di- or trimethylated. It was originally puzzling how an enzyme might be able to catalyze mono-, but not di- or trimethylation. However, insight into this question comes from the crystal structure of HMTs and the study of their catalytic site. In monomethylases such as Set7/9, the ε-NH_3^+ group is bound so tightly in the active site that there is no extra space for a bulky methyl group [26]. Therefore, the enzyme can only catalyze monomethylation. In contrast, di- and trimethylases show more flexibility in their active site. Remarkably, the enzyme specificity can be modified by changing one of the amino acids that controls hydrogen-bonding to the ε-NH_3^+ group [27].

Other histone modifications include lysine ubiquitination, arginine methylation, and phosphorylation on serine or threonine side chains. Histone modifications may affect chromatin structure directly by altering DNA–histone interactions within and between nucleosomes, thus changing higher-order chromatin structure. An alternative, more recent model is that combinations of histone modifications present an interaction surface for other proteins that translate this so-called histone code into a gene expression pattern [28]. This model can explain how the same chemical modification can have different functional consequences depending on the target residue. For example, methylation of H3 K4 is correlated with gene activation [29], whereas H3 K9 methylation results in repression and heterochromatin formation [30,31]. In contrast, histone acetylation is generally correlated with gene activation, although there are exceptions to this rule [32].

Chromatin remodeling complexes

Classically, chromatin remodeling factors have been described as activities that, unlike chromatin modifiers,

leave the biochemical make-up of the nucleosomes unaffected. The remodelers either change the location of the nucleosome along a particular DNA sequence (originally termed 'sliding'), or create a remodeled state of the nucleosome that is characterized by altered histone–DNA interactions (for detailed review, see [33] and references therein). Recent work, however, has significantly broadened the spectrum of their activities by adding the complete removal of histones ('histone eviction') [34,35] as well as histone exchange [36–38] to the palette of reactions catalyzed by chromatin remodeling complexes. In these reactions, remodelers use the energy freed by ATP hydrolysis to loosen DNA–histone contacts and thus to facilitate the movement of the nucleosomes. Depending on the remodeler and the target nucleosome, the new nucleosome location can be more permissive or more inhibitory to other accessory factors.

Common to all chromatin remodeling complexes is an ATPase subunit, the motor of the complex. Hence, remodelers can be categorized based on the sequence features of the ATPase. The first one to be identified was the Swi2/Snf2 ATPase, whose chief activity is to alter histone–DNA contacts within nucleosomes. Mutations in the *SWI2/SNF2* gene cause the inability to undergo mating-type switching (Swi^-) and sucrose nonfermenting (Snf^-) growth defects in *S. cerevisiae* due to defects in gene expression of characteristic sets of genes. Swi2/Snf2 is part of the SWI/SNF multiprotein complex, which possesses ATP-dependent chromatin remodeling activity [39,40]. The SWI/SNF complex is highly related to the in *S. cerevisiae* 'remodels the structure of chromatin' (RSC) complex, with the ATPase Snf two homolog (Sth1) [41]. Interestingly, both complexes contain actin-related proteins (Arps), which are also found in HAT complexes (see above), as well as in the remodeling complex INO80. Another surprising finding in the field was the recognition that the INO80 remodeling activity is modulated by inositol phosphates [42,43]. Again, the initial clue for this observation came from the mutant phenotype of *S. cerevisiae ino80* deletion strains that show inositol auxotrophy.

SWI/SNF-related complexes also exist in higher eukaryotes. Human cells have at least two related complexes, PBAF (or hBRM) and BAF (or BRG complex). The respective ATPases are BRM (related to the *D. melanogaster* ATPase brahma) and BRG (brahma-related group of proteins).

The family of imitation switch (ISWI)-type ATPases were identified based on their similarity to Swi2/Snf2 and belong to the 'sliding' type of remodelers. The three complexes chromatin accessibility (CHRAC), ATP-utilizing chromatin assembly and remodeling factor (ACF) and nucleosome remodeling factor (NURF) were biochemically isolated from *D. melanogaster* and contain ISWI as the ATPase component (reviewed in [33]). Although highly related to each other, these complexes show differences in their subunits as well as subtle biochemical differences *in vitro*. While ISWI is essential in flies, detailed work will be required to dissect the functional roles of the individual ISWI complexes *in vitro*. Two ISWI homologs, Isw1 and Isw2, are found in *S. cerevisiae*. The Isw2 complex is targeted to meiotic genes to repress these during vegetative growth [44] by moving nucleosomes to repressive positions [45]. Isw1 was recently identified in two distinct complexes called Isw1a and Isw1b [46], which have distinct functions in transcription (see below).

A third category of remodeling ATPases are the CHD type of remodelers, which are characterized by the presence of two chromodomains. They are mentioned here for completeness and are more extensively reviewed in [33]. Perhaps best studied among them is Mi-2, which is part of the nucleosome remodeling and deacetylation (NURD) complex. Interestingly, NURD also contains the histone deacetylases HDAC1/2 [47], and remodeling by NURD enhances histone deacetylation on nucleosomes, but not free histones. Thus, NURD provides an example for the combination of chromatin modifying and remodeling activities in one complex.

Replication of chromatin

As cells multiply and give rise to daughter cells, the genome must be accurately duplicated and passed on to the offspring in order to ensure that the genetic information remains constant over generations. One major feat is the duplication of the primary sequence, which is a highly regulated process requiring precise control, for example in the accuracy of duplication and the coordination of events within the cell cycle. However, not only the DNA is replicated, but also the structure of chromatin, which is required to ensure that epigenetic information is also passed on to the daughter cells (Fig. 1). This entails copying the histone code as well as the higher order chromatin structure, by duplicating heterochromatin domains or localizing other chromatin-associated factors to defined genomic regions. In higher organisms, DNA methylation patterns also contribute to the formation of functionally distinct chromatin domains, and accordingly, these patterns also have to be restored after DNA replication. The DNA methylation aspect of epigenetic imprints has been reviewed elsewhere [48,49] and will only be described briefly here.

Opening chromatin before replication

The template for the replication machinery is chromatin. Because chromatin is generally inhibitory to accessory factors, this raises the question of how the machinery obtains access to the DNA. Conceptually, both histone modifications (particularly acetylation) as well as nucleosome remodeling may aid in replication progression, perhaps by loosening chromatin compaction and thus facilitating the partial disassembly of chromatin before passage of the replication fork. While little is known about whether histone modifications affect fork progression, two chromatin remodeling complexes, WSTF-ISWI and ACF1-ISWI, have been implicated in heterochromatin replication. Interestingly, both complexes are targeted to heterochromatic replication foci, and depletion of ACF1 from human cells impairs the replication of pericentric heterochromatin [50,51]. Thus, chromatin remodeling by these complexes may facilitate the movement of the replication fork through heterochromatin domains. An alternative interpretation is that they are required for histone deposition after replication.

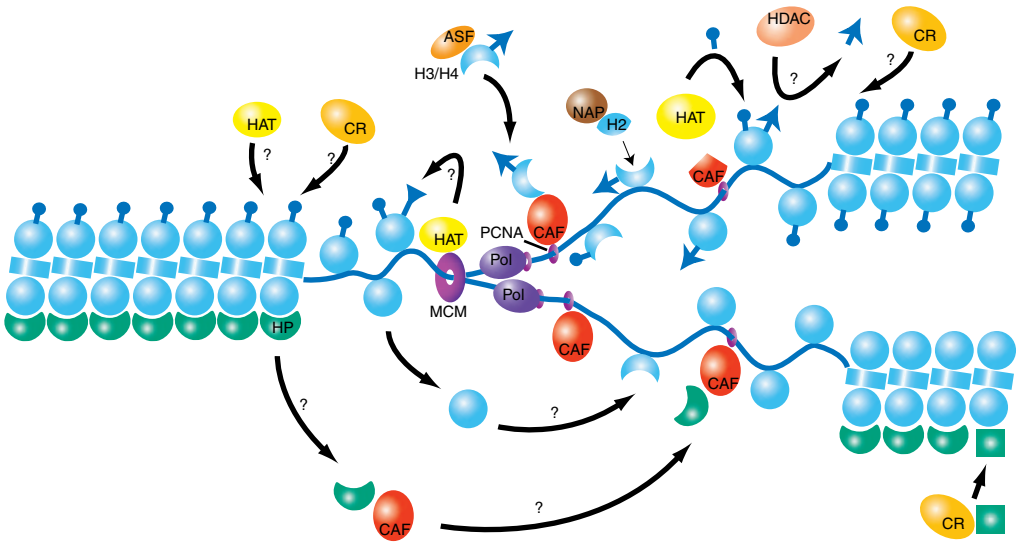

Fig. 1. Model for the events on chromatin surrounding replication. (Top) HATs and chromatin remodeling machineries (CR) loosen the chromatin to allow disassembly of the nucleosomes (blue spheres). The MCM helicase is associated with a HAT that acetylates histones in front of the replication fork. Once the DNA is duplicated by PCNA-aided DNA polymerase (Pol), CAF-I associates with PCNA and builds H3-H4 dimers with a cytoplasmic acetylation pattern (blue arrow) into chromatin. These dimers are delivered to CAF-I by ASF1 (ASF). Nucleosomes are completed by NAP-mediated H2A-H2B delivery. A HAT complex associates with the large subunit of CAF-I and adds a euchromatic acetylation mark (blue ball) to the nucleosomes. HDACs and CRs restore the original chromatin make-up by removing cytoplasmic acetylation patterns and positioning the nucleosomes. (Bottom) How histones and other chromatin components, for instance heterochromatin proteins (HP), are transferred from the parental strand to the daughter strands is unclear (for convenience, the transfer to only one strand is depicted). CAF-I may help transfer HPs. Furthermore, chromatin remodelers install higher order chromatin proteins (e.g. cohesin, green square) on the chromatin. For simplicity, details of the replication machinery are not elaborated on in the model.

An additional function of chromatin remodeling in replication may be to help to strategically position nucleosomes close to origins of replication. Occlusion of the binding site for the replication initiator, the origin recognition complex (ORC), by nucleosomes possibly reduces the binding of ORC as well as other replication factors, and thus is postulated to reduce the efficiency of replication initiation at an origin. Insight into this comes from studies in the yeast *S. cerevisiae*, where nucleosome positioning has been shown to play an important role in the assembly of the prereplication complex (pre-RC) and thus to influence initiation at chromosomal origins of replication [52]. Also, SWI/SNF was shown to be required for replication initiation by compromised yeast plasmid origins, suggesting that it is necessary to maintain a nucleosome structure at the origin that is conducive to initiation [53]. Interestingly, origins of replication in larger eukaryotes are often found close to transcriptional promoter regions [54], and the regulatory elements for transcription and replication overlap [55]. Thus, transcriptional activators at promoters may recruit chromatin remodelers, which fulfil dual functions in that they open chromatin for both transcriptional activation and replication initiation.

Not only the position of a nucleosome but also its acetylation state impinges upon replication initiation. In *S. cerevisiae*, chromatin acetylation regulates the time point during S-phase at which an origin initiates replication. Deletion of the HDAC Rpd3 has been shown to result in higher global levels of acetylation and to promote earlier origin firing. This has been attributed to a direct effect of acetylation, because targeting the Gcn5 HAT to a usually late-firing origin causes its early activation [56]. In this context, it is interesting to note that a putative HAT, human HBO1, associates with ORC1, the large subunit of ORC, as well as with the replication licensing factor MCM2 [57,58]. Perhaps this provides a means to target histone acetylation to chromatin in the vicinity of origins in higher eukaryotes in order to regulate temporal origin firing. Acetylation may enhance nucleosome mobility by recruiting chromatin remodelers, thus improving access of replication factors to the origin.

Replication-coupled chromatin assembly

Once replication initiation has been triggered and as the replication fork plows through chromatin, nucleosomes in front of the fork are disassembled into H3-H4 tetramers and H2A-H2B dimers, and the parental histones are then transferred randomly to the two daughter DNA strands [59]. Chromatin is rapidly reassembled after duplication of the DNA by depositing first H3-H4, then H2A-H2B on the DNA to complete the nucleosomes. Until recently, the general view has been that H3-H4 is deposited on DNA as a tetramer, because an intermediate form of chromatin has properties consistent with it being a tetramer of histones H3 and H4 (reviewed in [60]). This view has now been challenged in that the H3 variant H3.1 is found in an H3-H4 dimer in cellular complexes with chromatin assembly factors before deposition into chromatin [61]. Thus, two

H3.1-carrying assembly complexes may have to deposit their H3-H4 dimers in order to assemble the intermediate H3-H4 tetramer structure.

Chromatin assembly factor-I (CAF-I) was identified as a protein complex that deposits H3 and H4 on DNA in a replication-coupled fashion *in vitro* [62]. CAF-I and other chromatin assembly factors (see below) are also referred to as histone chaperones, because they prevent unspecific aggregation of the positively charged histones with the negatively charged DNA by shielding the histone charge from the DNA. The replication coupling is mediated by the interaction of CAF-I with proliferating nuclear cell antigen (PCNA) [63], which remains associated with the DNA for up to 20 min after replication [64] and thus provides a molecular mark for CAF-I to recognize replicated DNA. CAF-I consists of three subunits, one of which, p48 (Cac3 in *S. cerevisiae*), is similar to proteins found in chromatin-modifying complexes. Deletion of any one of the three subunits of yeast CAF-I, Cac1, Cac2 or Cac3, results in identical phenotypes. These deletions are not lethal, but lead to mild defects in gene silencing at the *HM* loci and the telomeres, a mild sensitivity to UV irradiation [65,66], and defects in kinetochore function, thus reflecting roles in heterochromatin formation, DNA damage repair and centromere assembly, respectively [67]. In contrast, CAF-I in human cells is essential [68], suggesting that here it is important for replication-coupled chromatin assembly, either because it is the major machinery for this purpose, or because it is required for the assembly of critical genomic regions. As cells depleted for CAF-I arrest in mid S-phase, this may indicate a particular role for CAF-I in duplicating the late-replicating heterochromatin, which is interesting in light of the observation that CAF-I interacts with HP1 [69].

The fact that CAF-I is not essential for viability in yeast led to the hypothesis that other histone chaperones exist that can substitute for CAF-I in replication-coupled chromatin assembly. On this basis, *D. melanogaster* replication-coupled assembly factor (RCAF) was isolated as a factor that stimulates CAF-I activity *in vitro* [70]. On its own, it can also assemble chromatin, but does not have a preference for newly replicated DNA. RCAF is homologous to *S. cerevisiae* antisilencing factor 1 (Asf1), which was first characterized as a factor that, when overexpressed, disrupted silencing in yeast [71]. Though Asf1 is a CAF-I accessory factor *in vitro*, Asf1 function *in vivo* only partially overlaps with CAF-I. The deletion of *ASF1* results in silencing defects that are distinct from those of CAF-I deletions, and Asf1 has additional roles in gene regulation and DNA repair not shared by CAF-I. Notably, Asf1 interacts both physically and genetically with the histone regulator (Hir) proteins to regulate the expression of histones and other proteins [72,73]. Again, the Hir proteins and Asf1 have only partially overlapping functions. For example, the Hir proteins have a distinct function in gene silencing in yeast. Combinations of mutations in CAF-I, Asf1 and Hir proteins cause slow growth, but not inviability in yeast. Thus, one might speculate that yeast tolerates the absence of chromatin assembly factors because its genome is relatively gene dense such that chromatin assembly during transcription, with or without uncatalyzed chromatin assembly in intergenic regions, may be sufficient to assemble the genome and allow viability. Alternatively, yeast may harbor additional assembly factors that remain to be identified.

CAF-I and Asf1 deposit H3 and H4 on (replicated) DNA. The addition of H2A and H2B is provided *in vitro* by a histone chaperone specialized for H2A–H2B. This protein, termed NAP-I, purifies with nascent histones from HeLa cells, and its localization within the cell changes during the cell cycle. Accordingly, it is proposed to be an H2A–H2B histone deposition factor that shuttles its histone cargo from the cytoplasm into the nucleus (reviewed in [74]). Thus, NAP-I may cooperate with CAF-I and Asf1 to complete the nucleosomes on replicated DNA.

Epigenetic patterns of histone modification during DNA replication

It is interesting to note that the replication of chromatin is dispersive in that the parental nucleosomes are disassembled into H3-H4 tetramers and H2A-H2B dimers and distributed onto the two daughter strands [75]. To obtain a full complement of histones in the freshly formed chromatin after replication, newly synthesized histones are incorporated along with the parental histones. Thus, the 'old' dimers become mixed with new H3-H4 dimers and H2A-H2B dimers within the individual new nucleosomes [61]. Also, the new histones are acetylated at lysines 5 and 12 of H4, and in *D. melanogaster* at lysine 14 of H3, by cytoplasmic HATs and then transported into the nucleus via transport receptors called karyopherins [76]. Thus, the modification patterns of nucleosomes on the daughter strands are expected to be a mixture of 'old' and 'cytoplasmic' imprints.

Notably, CAF-I is found associated with histones carrying a pattern of histone acetylation characteristic of newly synthesized histones, and thus probably incorporates those into the new chromatin [62]. An open question is how the parental histones are transferred to the daughter strands. However, once deposited, the particular acetyl marks on the histones of cytoplasmic origin are rapidly removed [77]. Thus, equalization of the epigenetic patterns of histone modifications between parental and new histones in the fresh chromatin entails removing some modifications and adding others. In *S. cerevisiae*, H4 K16 acetylation is a global mark for euchromatic regions in that it prevents the binding of the heterochromatic Sir proteins to chromatin outside of their designated genomic areas [78,79]. As cytoplasmic histones are not acetylated on H4 K16, this residue has to become acetylated in the duplicated chromatin.

A mechanistic basis for the re-establishment of acetylation patterns is provided by the observation that the HAT complex SAS-I interacts with Cac1, the large subunit of CAF-I, in *S. cerevisiae* [80]. The SAS-I complex contains as its catalytic subunit the acetyltransferase Sas2, a member of the MYST family of HATs, and the subunits Sas4 as well as Sas5, which is a homolog of the leukemogenic AF-9 protein [81]. One model is that CAF-I is targeted to replicated DNA by interacting with PCNA, where it then deposits newly synthesized H3 and H4. Subsequently, Cac1 recruits SAS-I to the chromatin to acetylate H4 K16. Thus, SAS-I sweeps along the chromatin in the wake of CAF-I and provides global H4 K16 acetylation.

Interestingly, SAS-I also interacts with the histone deposition factor Asf1 [80,82]. Because Asf1 has partially overlapping functions with CAF-I, two interpretations of this interaction are possible that are not mutually exclusive. SAS-I may acetylate chromatin that was assembled by Asf1 acting on its own. Alternatively, the interaction of Asf1 with SAS-I may occur as Asf1 delivers histones to CAF-I at the replication fork.

Resetting epigenetic patterns on chromatin entails more than histone acetylation. While the fate of histone methylation and ubiquitylation patterns at replication is unexplored, it is reasonable to surmise that such modifications also require reinstatement after replication. Thus, perhaps the recruitment of chromatin-modifying activities is a general task of chromatin assembly factors, and it will be interesting to determine whether they interact not only with a HAT complex, but also with other chromatin-modifying complexes. In fact, it is likely that an HDAC is associated with late-replication forks in higher eukaryotes, because the removal of H4 K5 and K12 acetyl groups is sensitive to the HDAC inhibitor trichostatin A [77]. In line with this, PCNA has been found associated with HDAC1 [83], suggesting that this HDAC may perform replication-coupled histone deacetylation. In this context, it is interesting to note that a global (as opposed to promoter-targeted) mode of histone deacetylation has been described in *S. cerevisiae* [84], and that the different HDACs (e.g. Rpd3 and Hda1) are dedicated to individual genomic territories. As an example, global deacetylation by Hda1 is concentrated to contiguous subtelomeric domains [84]. One possibility is that the HDACs are brought to these regions via chromatin assembly during S-phase. Alternatively, they may act constantly, i.e. throughout the cell cycle. Support for this notion comes from the observation that histone acetylation targeted by a transcriptional activator is rapidly reversed upon removal of the activator and independently of the cell cycle [85], suggesting that there is a constant equilibrium between acetylation and deacetylation activities in the yeast genome.

How do chromatin modification patterns propagate themselves during cell division? In copying the histone code onto the new chromatin, one may surmise that some type of recognition of the old code must exist in order to adjust the modification pattern on the daughter chromatin strands, much like a maintenance DNA methyltransferase only methylates hemimethylated DNA. For instance, an enzymatic activity may need to be recruited to the chromatin by interacting with those nucleosomes that already carry its particular modification, for instance like Gcn5 recruitment to promoters is self-enhancing (see below). This then raises the intriguing question of how the machinery recognizes which pattern to propagate, because the new nucleosomes contain the composite patterns of 'old' and 'new' histones.

One solution to this conceptual problem is that, at least in yeast, a histone code-copying mechanism may not be necessary, if one pictures a HAT, an HDAC and an HMT following in the path of chromatin assembly factors at replication, though this simple model may not apply for larger eukaryotes (see below). In this model, the enzymes are specialized for replication-coupled histone modification and ensure that the 'cytoplasmic' modification pattern on the new histones is converted to a 'euchromatic' pattern through their action. This implies that the euchromatic pattern is the default pattern after replication, and that subsequent steps are required to modify it in the different genomic regions. During the replication of active genes, the events at transcriptional activation per se, i.e. chromatin remodeling and modification recruited by transcription factors, may be sufficiently strong to bring about the 'active gene' modification pattern without requiring the aid of a replication-coupled recognition machinery. Similarly, in inactive gene regions such as the *HM* loci and the telomeres, the recruitment of the Sir2 HDAC by the concerted action of DNA binding factors may suffice to remove the transient euchromatic modification pattern and establish silencing after replication.

Propagation of chromatin states during replication

In larger eukaryotes, the restoration of chromatin structures after replication may be more complex than in a unicellular yeast. A striking example of self-propagation that is unlikely to be based on underlying sequence or recruitment factors is the presence of neocentromeres in higher organisms [86]. They form by some unknown cue, but are propagated in a stable fashion throughout many cell divisions, apparently by epigenetic mechanisms. One possibility is that chromatin proteins, for example those that mark the neocentromere, are transferred to the replicated chromatin by transfer factors and thus mark the new chromatin for the purpose it had on the parental strand. In light of this, it is interesting that mouse HP1 interacts with p150, the large subunit of mouse CAF-I [69], suggesting that CAF-I helps to transfer parental (and perhaps newly synthesized) HP1 to replicated heterochromatin. Also, chromatin remodeling regulates the binding of superordinate chromatin proteins, as indicated by the observation that the human cohesin protein hRAD21, which is part of the sister chromatid cohesion machinery, is a component of the human ISWI chromatin remodeling complex [87]. Thus, a picture emerges where chromatin assembly factors and remodelers participate in the rebuilding of higher-order chromatin after DNA replication. Perhaps some chromatin assembly factors are specialized for early replication and recruit a set of chromatin modifying enzymes that install the 'euchromatic' mark, whereas late-replication assembly factors attract a different set that install the 'heterochromatic' mark.

In reconfiguring the chromatin after replication, nucleosomes also need to be installed at the same position on the DNA sequence as in the parental chromatin. This implies a role for chromatin remodelers in this process. Importantly, *D. melanogaster* ASF1 interacts physically and genetically with the Brahma chromatin remodeling complex, a member of the SWI/SNF family of remodelers [88], suggesting that ASF1 may function to target this complex to replicated chromatin in order to restore nucleosome positions. Also, the role of WSTF-ISWI and ACF1-ISWI in heterochromatin replication mentioned above [50,51] may be interpreted as effects on chromatin remodeling after (rather than before) DNA replication and chromatin assembly.

In addition to the canonical histones, chromatin also comprises histone variants, raising the issue of their fate during replication and chromatin assembly. One question is

how CENP-A/Cse4 nucleosomes are assembled at centromeres, as they represent a minority compared to the bulk of H3-containing nucleosomes in the cell. Interestingly, the *D. melanogaster* CENP-A homolog Cid is able to localize to the centromere in the absence of replication or outside of S-phase [89], indicating that a replication-independent pathway for centromere chromatin assembly exists. Also, Cse4 deposition at the centromere in yeast does not require CAF-I, but CAF-I in cooperation with the Hir proteins restricts Cse4 to its centromeric location [67]. Furthermore, the chromatin remodeler RSC is required to restructure centromeric and centromere-flanking nucleosome structure for the accurate transmission of chromosomes [90]. Thus, both CAF-I/Hir and RSC may function in postrecruitment assembly or maintenance of centromeric chromatin.

In contrast to CENP-A/Cse4, other histone variants are more abundant and are present in large numbers of nucleosomes. For example, H3.3 is found in 25% of nucleosomes in *D. melanogaster*, especially on active genes [91], and the H2A variant H2A.Z (Htz1 in *S. cerevisiae*) is estimated to be present in 5–10% of all nucleosomes [92]. How are they incorporated into new chromatin? Interestingly, the chromatin remodeling complex SWR1 was discovered to exchange H2A for H2A.Z in chromatin [36–38]. Thus, one model is that H2A.Z and perhaps other histone variants are not directly incorporated into chromatin at replication, but that specialized remodeling complexes are deployed after replication-coupled chromatin assembly to swap the conventional histones for the histone variants. How the remodelers are targeted to the appropriate genomic regions is still unknown.

DNA methylation during replication

In higher eukaryotes, epigenetic information is also stored in the form of DNA methylation, which generally serves as a marker for transcriptional repression. Accordingly, DNA methylation patterns must be duplicated after replication, as they are semiconservatively passed on to the two daughter strands. Here, DNA methylation occurs primarily through the activity of the DNA methyltransferase DNMT1, a maintenance methyltransferase that is active on hemimethylated DNA and is recruited to replication foci via its interaction with PCNA [93]. Interestingly, DNMT1 interacts with HDAC2 at late replication foci [94]. Perhaps HDAC2 is thus recruited to late-replicating heterochromatin, where it translates the DNA methylation mark into a histone modification (deacetylation) pattern. Another connection between chromatin assembly and DNA methylation is given by the observation that the methyl-CpG binding protein MBD1 interacts with the p150 subunit of CAF-I [95], suggesting a role for these proteins in the inheritance of epigenetic DNA methylation patterns during replication.

Chromatin dynamics during transcription

Gene expression is a highly coordinated and orchestrated process, which ensures that the genes are switched on and off as the respective proteins are needed for their diverse cellular tasks. Transcription takes place on the chromatin template. This raises the question of how the transcriptional machinery gains access to the regulatory sites, how it negotiates the imposing chromatin during transcription elongation, and how the chromatin is restored once the transcriptional machinery has passed and transcription is turned off (Fig. 2).

Chromatin at transcription initiation

In recent years, much attention has focused on the events at transcriptional activation (reviewed in [96]). The current view is that HAT coactivator complexes and chromatin remodeling complexes cooperate with sequence-specific binding factors to help the transcriptional apparatus gain access to the promoter of a given gene. In this scenario a transcriptional activator, such as Gcn4 in *S. cerevisiae*, directs the coactivator complex (e.g. SAGA) to the promoter region [97]. The SAGA recruitment has multiple consequences for transcriptional activation: histone acetylation by Gcn5; recruitment of the transcriptional machinery; and histone deubiquitylation, as detailed below.

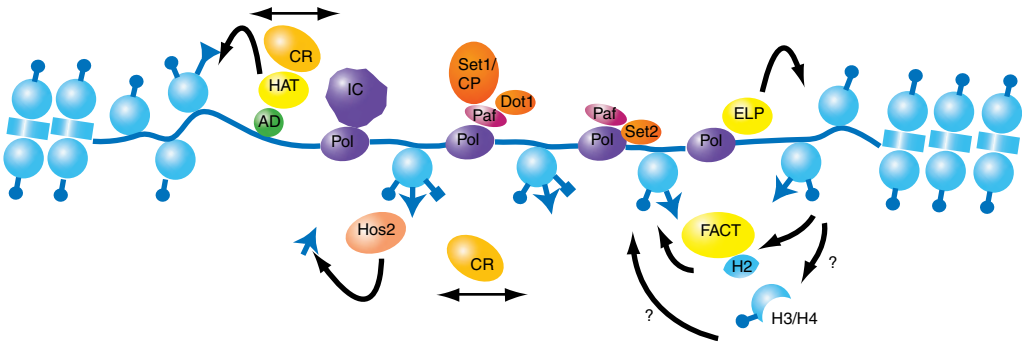

Fig. 2. Model for the processes on chromatin accompanying transcription. A transcriptional activator (AD) recruits HATs and CRs to the promoter. The transcription initiation complex (IC, simplified for clarity) is assembled, and the progressing RNA polymerase (Pol) then associates with the Set1–COMPASS (CP) complex, Dot1, Set2 and Elongator (ELP), which leave acetylation (arrows) and methylation (squares) marks on the chromatin. These marks recruit CRs to remodel chromatin in the body of the gene. FACT facilitates polymerase passage by transferring H2A/H2B (H2) behind the polymerase. The route for H3/H4 transfer is unknown. The HDAC Hos2 travels behind the polymerase to remove transiently added acetyl groups.

The HAT component of SAGA, Gcn5, acetylates nucleosomes in the vicinity of the promoter [98]. This acetylation provides several functions. Firstly, chromatin remodeling factors such as SWI/SNF are thus directed to the promoter due to their bromodomain-containing components. Bromodomains are protein modules that show increased affinity for specifically acetylated histone lysine residues [99]. Once targeted, the remodelers then mobilize nucleosomes in the proximity of the promoter. Interestingly, the action of SWI/SNF remodelers has been shown recently not only to remodel nucleosomes, but also to be able to completely remove them from a target site ('histone eviction') [34,35]. Another consequence of histone acetylation in the promoter vicinity is to stabilize interactions of the transcription apparatus with active chromatin regions, because the transcription factor TAF250 also contains two bromodomains [100]. Furthermore, the acetylation is self-reinforcing in that Gcn5 itself contains a bromodomain and thus is likely to stabilize the association of SAGA with the promoter. Notably, depending on the circumstances, the sequence of chromatin changes at the promoter can also occur in reverse order. In *S. cerevisiae*, genes expressed at mitosis require SWI/SNF remodeling before Gcn5-dependent acetylation occurs [101], suggesting that mitotic chromosomes are less accessible for HAT complexes to perform acetylation.

In addition to the HAT activity of Gcn5, the recruitment of SAGA to the promoter also directly recruits the transcriptional machinery through contacts of Spt3 and Spt8 with TBP [102,103] and thus promotes assembly of the preinitiation complex (Spt indicates 'suppressor of Ty').

Furthermore, the Ubp8 component of SAGA is required for H2B deubiquitylation [104]. H2B ubiquitylation has long been known to correlate with transcription activation [105], and recent work from several labs has shed light on the ubiquitylation events at promoters. In a current model, the E2 ubiquitin-conjugating enzyme Rad6 and the E3 ligase Bre1 cooperate to ubiquitylate H2B in promoter regions [106,107]. The role of SAGA and Ubp8 in this process may be to reset the ubiquitylation state of H2B to allow repeated initiation to occur. Intriguingly, this ubiquitylation affects other modifications like H3 K4 and K79 methylation, which play a role in transcription elongation (see below), in a '*trans*-tail' mode of cross-talk between the different modifications [108,109]. The link between ubiquitylation and histone methylation is given by the proteasomal ATPases Rpt6 and Rpt8, because mutation in the respective genes disrupts H3 K4 and K79 methylation, but not H2B ubiquitylation [110]. Thus, the proteosome components are recruited to the promoter via H2B ubiquitylation and reconfigure chromatin for access by Set1 and Dot1 during trancription elongation.

In summary, there is a highly complex interplay between the different factors, activities and chromatin modifications that all contribute to successful transcription initiation and transition to elongation.

Chromatin changes during transcription elongation

Once the transcriptional apparatus is positioned at the promoter, transcription is initiated by phosphorylation of the C-terminal domain of RNA polymerase II (PolII) on serine 5. As PolII proceeds, serine 5 is dephosphorylated, and serine 2 becomes phosphorylated. While several factors promote elongation by affecting PolII activity directly (reviewed in [111]), other factors exist that modify chromatin to help the elongating polymerase work its way through the chromatin. Elongator is a multiprotein complex containing the HAT Elp3 that acetylates predominantly H3 K14 and H4 K8 [112]. It was isolated biochemically from *S. cerevisiae* as a factor tightly associated with hyperphosphorylated, elongating PolII, and mutations in the genes encoding Elongator subunits in *S. cerevisiae* display phenotypes consistent with a role in elongation. Thus, an attractive model is that Elongator acetylation churns the chromatin in front of PolII and thus facilitates polymerase movement along the body of the gene. Intriguingly, the majority of cellular Elongator is localized in the cytoplasm [113], implicating that Elongator has additional cellular functions that remain to be investigated.

Next to nucleosome acetylation, histone methylation is also associated with elongating polymerase. Interestingly, the H3 K36-specific methyltransferase Set2 is tightly associated with the elongating polymerase, and the deletion of *SET2* in yeast results in phenotypes consistent with Set2 being required for elongation [114–116]. Also, the association of the Paf1 protein complex with PolII recruits other methyltransferase complexes to the elongating polymerase, namely the H3 K4-specific complex COMPASS, which contains the HMT Set1, and Dot1, which methylates K79 within the core region of H3. While the precise function of H3 K79 methylation in elongation is still unclear, H3 K4 methylation, at least at some genes, serves to recruit the nucleosome remodeler Isw1 to chromatin. Yeast Isw1-containing complexes bind *in vitro* preferentially to di- and trimethylated H3 K4, though it is not known which of the two Isw1 complexes do so [117]. H3 K4 di- and trimethylation correlate with high gene activity [29]. The recuitment of Isw1 complexes by Set1-mediated histone methylation serves several purposes. Functional dissection of the two Isw1 complexes revealed that Isw1b influences the elongation step of transcription, whereas Isw1a is required for promoter inactivation by preventing PolII from associating with the promoter, thus placing Isw1 at the crossroads of the transcriptional cycle [118].

The fate of nucleosomes during transcription

An intriguing question for many years has been what happens to the histones as the polymerase passes. How can the chromatin structure allow the movement of the large RNA polymerase along the DNA? Careful biochemical analysis of transcription through nucleosomes *in vitro* suggests that the direct transfer of octamers from in front of the polymerase to behind is an important mechanism allowing the polymerase to work its way through chromatin ([119] and references therein). This transfer mechanism may be a direct consequence of the intrinsic biophysical properties of the histone octamer and the DNA helix, and the process may be aided *in vivo* by chromatin modifications, chromatin remodeling and other activities.

Recent work indicates that factors exist that allow the RNA polymerase to proceed more easily through chromatin. A biochemical approach to identifying such factors

afforded the identification of the 'facilitates chromatin transcription' (FACT) complex, which was shown to displace H2A and H2B during elongation [120]. FACT is also able to assemble chromatin *in vitro*, suggesting a histone chaperone activity for the complex, and thus the ability to reassemble nucleosomes. Therefore, FACT may accelerate the transfer of octamers behind the polymerase. FACT consists of the proteins Pob3 and Spt16 (also known as Cdc68), which was originally characterized in *S. cerevisiae* as a gene that, when mutated, suppressed the repressive effect of a Ty transposon inserted in a promoter on transcription [121].

Another hint at the fate of nucleosomes during transcription and the necessity to reinstate chromatin after polymerase passage comes from the study of the elongation factor Spt6. Mutations in *SPT6* cause aberrant transcription initiation at cryptic promoters and an aberrantly open chromatin structure in the body of a gene [122]. This has led to the view that Spt6 functions to restore chromatin structure in the wake of the polymerase, which is necessary to silence cryptic promoters that would otherwise become inappropriately accessible to the transcriptional machinery. The mechanistic basis for this is not known.

Because the transfer of histones from front to back of an advancing polymerase may be a general characteristic of chromatin processes, it is conceivable that a similar transfer mechanism is at play during DNA replication and the passage of DNA polymerases and helicases. In light of this, it is interesting to note that the FACT components Spt16 and Pob3 are found associated with DNA polymerase α [123]. Interestingly, the *SPT16* gene was independently termed *CDC68*, because mutations cause a cell cycle arrest suggestive of a function for Cdc68 in early S-phase of the cell cycle [124]. Thus, one interpretation is that FACT assists in octamer transfer not only during transcription, but also during DNA replication.

The notion that histones are transferred during transcription, apparently without exchange for free histones, implies that the modifications placed on the histones in front of the polymerase are moved with them to the back, thus leaving the net amount of modifications intact. How then are these histone modification patterns returned to 'normal' after the passage of the transcription machinery? A first glimpse comes from the (at first) counterintuitive observation that the HDAC Hos2 is associated with the coding genes of highly transcribed genes [125] and thus may serve to remove the acetylation that was installed by Elongator. Interestingly, the deletion of *HOS2* leads to slower gene activation. One possibility is that the Hos2 deacetylation is required to return the chromatin to a transcription-competent state for a renewed round of transcription to occur efficiently.

While the removal of temporary acetylation can readily be explained by the action of HDACs, there is no simple explanation for removal of histone methylation, the presumed stable modification, upon transition of a gene to the inactive state. Perhaps the methylated histones are exchanged for unmodified ones by chromatin remodelers in a mode similar to SWR1 exchanging H2A for Htz1. Alternatively, proteolysis of the N-terminus may remove the methylation, though this cannot account for the removal of stable marks in the core region of histones (e.g. H3 K79). It will be interesting to learn how the cellular machinery copes with this issue.

Chromatin and DNA repair

Despite its protection within the chromatin, DNA is subject to widespread damage through exogenous agents such as chemicals or ultraviolet radiation, as well as through endogenous by-products of the cell's own metabolism. DNA lesions can cause physical obstacles to replication and transcription and they, as well as inherently occurring mismatches, must be corrected to prevent mutations. Accordingly, cells have evolved dedicated repair systems to fulfil this task. Among these, nucleotide excision repair (NER) is the best studied. Mutations in the NER repair machinery have long been known to cause genetic diseases in humans, e.g. xeroderma pigmentosum or Cockayne syndrome, where patients show an increased sensitivity to UV light and a predisposition to skin cancer.

Damage repair by NER and other repair systems occurs in several steps (for a detailed overview, see [126]). First, the lesion must be sensed in the cell. One possibility is that a specialized protein scans the genome and binds to the lesion. Alternatively, stalled DNA processing complexes, such as the transcription or replication machineries, can send out signals that activate a cell cycle checkpoint, which causes the cell to arrest in the cell cycle until the break is repaired. It is also possible that alterations in the DNA structure are detected through an altered chromatin topology that serves as a signal for defective DNA and thus transmits a signal through the chromatin fiber. As an example, a single double-strand break (DSB) in the yeast genome can cause checkpoint activation and cell cycle arrest [127].

In a next step, the actual lesion is corrected. Depending on the repair pathway, this may entail removing a larger portion of the DNA. In NER, a short oligonucleotide including the damaged site is excised by nicking one DNA strand on either side of the damage and removing the intervening DNA including the lesion. The resulting gap is then filled in by DNA polymerase, and the nicks are ligated with DNA ligase (reviewed in [126]). In the case of DNA DSBs, the DNA ends are repaired either by homologous recombination or by nonhomologous end-joining (NHEJ). NHEJ involves binding of the Ku70-80 heterodimer to the free DNA ends. In yeast the Mre11–Rad50–Xrs2 complex then processes the break for religation by its endo- and exonucleolytic activities. Finally, the ends are rejoined by ligation [128]. In any case, after repair of the lesion, the original chromatin structure needs to be reinstated at the repair site.

In considering the role of chromatin in DNA repair, similar issues as in replication and transcription come to mind. How does the repair machinery gain access to chromatin? How is chromatin restored after repair? As for replication and transcription, the role of chromatin modifying and remodeling factors will be considered in breaking and remaking the chromatin (Fig. 3).

Histone modifications during repair

Several observations of a role for HAT complexes in DNA repair suggest functional links between histone acetylation

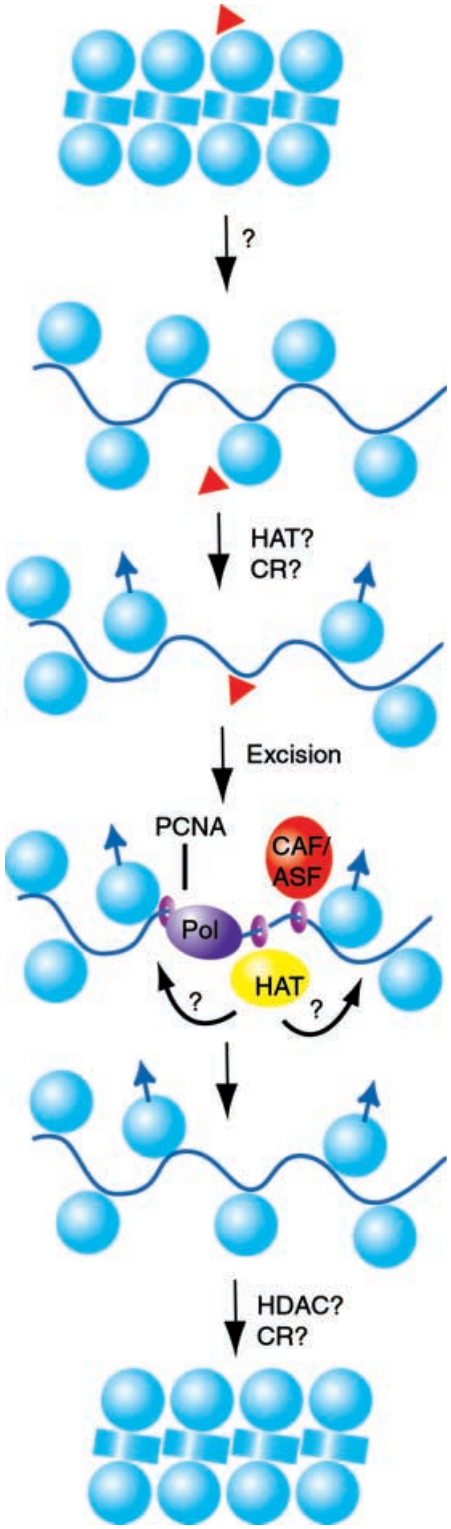

Fig. 3. Model for chromatin alterations during DNA repair. Upon sensing of damage in the DNA (red triangle), chromatin is loosened by the combined action of HATs and chromatin remodeling complexes to allow removal of the damage by the NER machinery. The resulting gap is filled in by DNA polymerase. PCNA recruits a HAT and chromatin assembly factors, which together restore chromatin on the new DNA. HDACs and remodelers then cooperate to install the original chromatin structure at the site.

and repair. Because the effect of histone acetylation on repair *in vitro* is modest [129], the acetylation is postulated to disrupt higher-order chromatin structure *in vivo* and thus to alleviate repair. The *in vivo* function of acetylation in repair comes from observations of mutant phenotypes in yeast and higher eukaryotes, combined with the biochemical analysis of HAT complexes. The human HAT complexes STAGA (a Gcn5-containing complex) and TFTC contain the SAP130 subunit that is able to bind UV-damaged DNA [130,131], suggesting a direct role of the HAT complexes via interaction with the DNA repair machineries. Furthermore, the human MYST HAT Tip60 was biochemically isolated in a complex containing proteins related to the bacterial protein RuvB [132]. In bacteria, RuvB is a Holliday junction helicase and is required for the repair of damaged DNA. Thus, in agreement with a role of Tip60 in DNA repair, the expression of a mutant version of Tip60 causes defects in DNA repair and reduces apoptosis, suggesting that the Tip60 complex is required for signaling DNA damage to the apoptotic aparatus. Similarly, the Tip60-related yeast complex NuA4 with the Esa1 catalytic subunit has been implicated in DSB repair in that mutations in Esa1 cause sensitivity to agents that induce DSBs and defects in NHEJ [133]. Taken together, these observations suggest that histone acetylation participates in the repair of broken chromatin. Beside the disturbance of higher-order chromatin structure, an additional function of the acetylation may be to provide a histone code that helps to recruit repair factors.

Other histone modifications may also help in repair processes, though their precise function is not understood. For instance, poly ADP-ribosylation of histones has long been known to increase upon DNA damage, which has lead to the hypothesis that this modification is involved in DNA repair (reviewed in [134]). Also, phosphorylation of the H2A variant H2AX is implicated in the cellular response to DNA damage in that phosphorylated H2AX in mammalian cells accumulates upon damage in foci and recruits a multitude of other factors implicated in DSB repair to the damaged site in order to mend the damage (reviewed in [126]). Interestingly, H2A in yeast is related to H2AX. Accordingly, phosphorylation of H2A is required for efficient DNA damage repair and NHEJ in yeast [135]. The role of histone methylation in repair process remains to be determined.

Histone acetylation may also be specifically required at the step of reformation of DNA and chromatin, for example in gap filling in NER. This hypothesis is supported by the observation that the HAT p300 binds PCNA and thus is attracted to sites of DNA damage [136]. PCNA serves as a processivity factor for DNA polymerases not only in replication, but also in DNA repair. The p300 recruitment by PCNA may in turn bring in other HAT complexes, all of which may acetylate nucleosomes or other factors in the vicinity of the repair site. Exactly how this affects DNA repair is unknown, but it may include better access of repair proteins on acetylated nucleosomes, higher efficiency of DNA synthesis at the repaired site, or reinstatement of acetylation patterns on the chromatin after repair.

Intriguingly, histone deacetylation has also been implicated in NHEJ. Histone acetylation is decreased in the vicinity

of a chromosomal DSB, and this deacetylation requires the HDAC Sin3–Rpd3. Also, *sin3* or *rpd3* mutants are defective in NHEJ and are susceptible to DSBs [137]. Future investigations of the temporal order of events in chromatin at DSBs will be required to shed light on how both acetylation and deacetylation are necessary for break repair. Perhaps acetylation is required first to relax the chromatin and allow access of repair proteins, and the subsequent deacetylation may serve to locally stabilize the chromatin for rejoining of the DNA ends. The deacetylation may also freeze the nucleosomes in position such that they don't 'slip' off the free end of the DSB.

A number of findings link heterochromatic components to DSB repair in *S. cerevisiae*. The original observation was an interaction between Ku proteins and the Sir4 protein, and *sir* mutants were found to have defects in NHEJ [138]. Though part of the effect was attributed to simultaneous changes in the cell type of *sir* mutant strains [139], the Sir proteins were found to be redistributed from subtelomeric repositories to DSB sites [140,141]. This has been hypothesized to reflect a role of heterochromatic proteins in deacetylating (via Sir2) and stabilizing the nucleosomes at the break point and in restricting access of replication and transcription factors to the site until repair is completed.

Chromatin remodeling during repair

Conceptually, chromatin remodeling complexes can influence DNA repair on several levels. Global remodeling may provide enough chromatin fluidity to increase the exposure of the damaged site for recognition by repair factors. Targeted remodeling may be specifically recruited for the same purpose. Furthermore, remodeling may be necessary to restore nucleosome positions after repair.

In vitro studies have shown that the presence of remodeling activity can stimulate repair on nucleosomal DNA. For example, *S. cerevisiae* SWI/SNF or Isw2 complexes improved the repair of UV-induced cyclobutane pyrimidine dimers in chromatin by photolyase [142], and SWI/SNF stimulates the excision of chemical adducts within the core nucleosome [143]. These findings are supported by the phenotypes observed for remodeler mutations in *S. cerevisiae*, though the precise step at which they act remains to be determined. *ino80* mutants are sensitive to DNA damage by UV, an alkylating agent (MMS), hydroxyurea (HU) and ionizing radiation. Interestingly, the purified Ino80 complex contains RuvB-like proteins [144], as does the Tip60 HAT complex. One hypothesis is that RuvB specifically targets the HATs and remodelers to sites of DNA damage via its recognition of Holliday junctions. Similar to *ino80*, the sensitivity of *rsc* mutants to HU, UV and MMS implies that the RSC remodeler also may directly or indirectly be required to repair defective DNA.

Restoring chromatin after repair

Restoration of chromatin structure is an important final step in the repair of damaged DNA and must ensure that all aspects, including nucleosome position and epigenetic imprints, are re-established. This is performed by CAF-I, and perhaps also by Asf1. *In vitro*, CAF-I specifically deposits histones onto DNA that was subject to DNA repair synthesis, and it is recruited to sites of NER and single-strand break repair, probably through its interaction with PCNA [145]. Accordingly, deletion of CAF-I subunits or of Asf1 in *S. cerevisiae* confers sensitivity to UV radiation. Interestingly, *asf1* mutants are also sensitive to a wide range of other DNA damaging agents, and removal of both CAF-I and Asf1 causes an increased sensitivity [70], which points to independent functions of the two factors in the respective repair processes. One indication of the role of Asf1 comes from the observation of physical and genetic interactions between Asf1 and the DNA damage-checkpoint protein kinase Rad53 [146,147]. In one model, Asf1 is released from a Rad53–Asf1 complex upon DNA damage and thus may facilitate chromatin assembly after repair. Alternatively, Asf1 may help localize Rad53 in order for it to phosphorylate downstream damage response effector proteins.

Concluding remarks

It is evident that maintaining constancy within the packaged eukaryotic genome is a highly complex task that requires accurate coordination and control to prevent errors that infallibly would have dire consequences for the organism. Our knowledge of how chromatin remodeling and histone modifications accompany replication, transcription and repair is only starting to accumulate, and many interesting questions remain to be addressed. For example, how are parental histones and chromatin proteins transferred during replication to establish chromatin domains on the daughter chromatin? What is the fate of histone variants in this process, and what happens to them during transcription and DNA repair? How is histone methylation erased after passage of the transcription machinery and shutdown of the gene? What is the precise order of events on the chromatin at DNA repair? How are chromatin modifying and remodeling complexes recruited to sites of DNA repair? The answers to these questions, and to many others that will undoubtedly arise as new observations become available, will provide important and exciting insights into the dynamic nature of chromatin and its influence on gene expression, growth and development.

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References

1. Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. & Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251–260.
2. Smith, M.M. (2002) Centromeres and variant histones: what, where, when and why*? Curr. Opin. Cell Biol.* **14**, 279–285.
3. Pal-Bhadra, M., Leibovitch, B.A., Gandhi, S.G., Rao, M., Bhadra, U., Birchler, J.A. & Elgin, S.C. (2004) Heterochromatic

silencing and HP1 localization in Drosophila are dependent on the RNAi machinery. *Science* **303**, 669–672.
4. Verdel, A., Jia, S., Gerber, S., Sugiyama, T., Gygi, S., Grewal, S.I. & Moazed, D. (2004) RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* **303**, 672–676.
5. Strahl-Bolsinger, S., Hecht, A., Luo, K. & Grunstein, M. (1997) SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes Dev.* **11**, 83–93.
6. Eissenberg, J.C., Morris, G.D., Reuter, G. & Hartnett, T. (1992) The heterochromatin-associated protein HP-1 is an essential protein in Drosophila with dosage-dependent effects on position-effect variegation. *Genetics* **131**, 345–352.
7. Zhang, L., Eugeni, E.E., Parthun, M.R. & Freitas, M.A. (2003) Identification of novel histone post-translational modifications by peptide mass fingerprinting. *Chromosoma* **112**, 77–86.
8. Roth, S.Y., Denu, J.M. & Allis, C.D. (2001) Histone acetyltransferases. *Annu. Rev. Biochem.* **70**, 81–120.
9. Carrozza, M.J., Utley, R.T., Workman, J.L. & Cote, J. (2003) The diverse functions of histone acetyltransferase complexes. *Trends Genet.* **19**, 321–329.
10. Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H. & Nakatani, Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87**, 953–959.
11. Mizzen, C.A., Yang, X.J., Kokubo, T., Brownell, J.E., Bannister, A.J., Owen-Hughes, T., Workman, J., Wang, L., Berger, S.L., Kouzarides, T., Nakatani, Y. & Allis, C.D. (1996) The TAF (II), 250 subunit of TFIID has histone acetyltransferase activity. *Cell* **87**, 1261–1270.
12. Kundu, T.K., Wang, Z. & Roeder, R.G. (1999) Human TFIIIC relieves chromatin-mediated repression of RNA polymerase III transcription and contains an intrinsic histone acetyltransferase activity. *Mol. Cell. Biol.* **19**, 1605–1615.
13. Lorch, Y., Beve, J., Gustafsson, C.M., Myers, L.C. & Kornberg, R.D. (2000) Mediator–nucleosome interaction. *Mol. Cell.* **6**, 197–201.
14. Sterner, D.E. & Berger, S.L. (2000) Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.* **64**, 435–459.
15. Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L. & Workman, J.L. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.* **11**, 1640–1650.
16. Sterner, D.E., Belotserkovskaya, R. & Berger, S.L. (2002) SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription. *Proc. Natl Acad. Sci. USA* **99**, 11622–11627.
17. Pray-Grant, M.G., Schieltz, D., McMahon, S.J., Wood, J.M., Kennedy, E.L., Cook, R.G., Workman, J.L., Yates, J.R. III & Grant, P.A. (2002) The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. *Mol. Cell. Biol.* **22**, 8774–8786.
18. John, S., Howe, L., Tafrov, S.T., Grant, P.A., Sternglanz, R. & Workman, J.L. (2000) The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF (II) 30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3) -FACT complex. *Genes Dev.* **14**, 1196–1208.
19. Allard, S., Utley, R.T., Savard, J., Clarke, A., Grant, P., Brandl, C.J., Pillus, L. & Workman, J.L. (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *EMBO J.* **18**, 5108–5119.
20. Galarneau, L., Nourani, A., Boudreault, A.A., Zhang, Y., Heliot, L., Allard, S., Savard, J., Lane, W.S., Stillman, D.J. & Cote, J. (2000) Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Mol. Cell* **5**, 927–937.
21. de Ruijter, A.J., van Gennip, A.H., Caron, H.N., Kemp, S. & van Kuilenburg, A.B. (2003) Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem. J.* **370**, 737–749.
22. Buck, S.W., Gallo, C.M. & Smith, J.S. (2004) Diversity in the Sir2 family of protein deacetylases. *J. Leukoc. Biol.* doi: 10.1189/jlb.0903424.
23. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D. & Jenuwein, T. (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593–599.
24. Ng, H.H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y. & Struhl, K. (2002) Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. *Genes Dev.* **16**, 1518–1527.
25. van Leeuwen, F., Gafken, P.R. & Gottschling, D.E. (2002) Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109**, 745–756.
26. Xiao, B., Jing, C., Wilson, J.R., Walker, P.A., Vasisht, N., Kelly, G., Howell, S., Taylor, I.A., Blackburn, G.M. & Gamblin, S.J. (2003) Structure and catalytic mechanism of the human histone methyltransferase SET7/9. *Nature* **421**, 652–656.
27. Zhang, X., Yang, Z., Khan, S.I., Horton, J.R., Tamaru, H., Selker, E.U. & Cheng, X. (2003) Structural basis for the product specificity of histone lysine methyltransferases. *Mol. Cell* **12**, 177–185.
28. Strahl, B.D. & Allis, C.D. (2000) The language of covalent histone modifications. *Nature* **403**, 41–45.
29. Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J. & Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* **419**, 407–411.
30. Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C. & Kouzarides, T. (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124.
31. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120.
32. De Nadal, E., Zapater, M., Alepuz, P.M., Sumoy, L., Mas, G. & Posas, F. (2004) The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. *Nature* **427**, 370–374.
33. Becker, P.B. & Horz, W. (2002) ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **71**, 247–273.
34. Boeger, H., Griesenbeck, J., Strattan, J.S. & Kornberg, R.D. (2003) Nucleosomes unfold completely at a transcriptionally active promoter. *Mol. Cell* **11**, 1587–1598.
35. Reinke, H. & Horz, W. (2003) Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. *Mol. Cell* **11**, 1599–1607.
36. Kobor, M.S., Venkatasubrahmanyam, S., Meneghini, M.D., Gin, J.W., Jennings, J.L., Link, A.J., Madhani, H.D. & Rine, J. (2004) A protein complex containing the conserved Swi2/Snf2-Related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *Plos. Biol.* **2**, E131.
37. Krogan, N.J., Keogh, M.C., Datta, N., Sawa, C., Ryan, O.W., Ding, H., Haw, R.A., Pootoolal, J., Tong, A., Canadien, V., Richards, D.P., Wu, X., Emili, A., Hughes, T.R., Buratowski, S. & Greenblatt, J.F. (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol. Cell.* **12**, 1565–1576.

38. Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S. & Wu, C. (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**, 343–348.
39. Cairns, B.R., Kim, Y.J., Sayre, M.H., Laurent, B.C. & Kornberg, R.D. (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl Acad. Sci. USA* **91**, 1950–1954.
40. Cote, J., Quinn, J., Workman, J.L. & Peterson, C.L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60.
41. Cairns, B.R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Du Tempst, P.J., Laurent, B. & Kornberg, R.D. (1996) RSC, an essential, abundant chromatin-remodeling complex. *Cell* **87**, 1249–1260.
42. Shen, X., Xiao, H., Ranallo, R., Wu, W.H. & Wu, C. (2003) Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science* **299**, 112–114.
43. Steger, D.J., Haswell, E.S., Miller, A.L., Wente, S.R. & O'Shea, E.K. (2003) Regulation of chromatin remodeling by inositol polyphosphates. *Science* **299**, 114–116.
44. Goldmark, J.P., Fazzio, T.G., Estep, P.W., Church, G.M. & Tsukiyama, T. (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell* **103**, 423–433.
45. Fazzio, T.G. & Tsukiyama, T. (2003) Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. *Mol. Cell.* **12**, 1333–1340.
46. Vary, J.C. Jr, Gangaraju, V.K., Qin, J., Landel, C.C., Kooperberg, C., Bartholomew, B. & Tsukiyama, T. (2003) Yeast Isw1p forms two separable complexes in vivo. *Mol. Cell. Biol.* **23**, 80–91.
47. Wade, P.A., Gegonne, A., Jones, P.L., Ballestar, E., Aubry, F. & Wolffe, A.P. (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. *Nat. Genet.* **23**, 62–66.
48. Nakao, M. (2001) Epigenetics: interaction of DNA methylation and chromatin. *Gene* **278**, 25–31.
49. McNairn, A.J. & Gilbert, D.M. (2003) Epigenomic replication: linking epigenetics to DNA replication. *Bioessays* **25**, 647–656.
50. Bozhenok, L., Wade, P.A. & Varga-Weisz, P. (2002) WSTF-ISWI chromatin remodeling complex targets heterochromatic replication foci. *EMBO J.* **21**, 2231–2241.
51. Collins, N., Poot, R.A., Kukimoto, I., Garcia-Jimenez, C., Dellaire, G. & Varga-Weisz, P.D. (2002) An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. *Nat. Genet.* **32**, 627–632.
52. Lipford, J.R. & Bell, S.P. (2001) Nucleosomes positioned by ORC facilitate the initiation of DNA replication. *Mol. Cell* **7**, 21–30.
53. Flanagan, J.F. & Peterson, C.L. (1999) A role for the yeast SWI/SNF complex in DNA replication. *Nucleic Acids Res.* **27**, 2022–2028.
54. DePamphilis, M.L. (1999) Replication origins in metazoan chromosomes: fact or fiction? *Bioessays* **21**, 5–16.
55. Majumder, S. & DePamphilis, M.L. (1994) Requirements for DNA transcription and replication at the beginning of mouse development. *J. Cell. Biochem.* **55**, 59–68.
56. Vogelauer, M., Rubbi, L., Lucas, I., Brewer, B.J. & Grunstein, M. (2002) Histone acetylation regulates the time of replication origin firing. *Mol. Cell* **10**, 1223–1233.
57. Burke, T.W., Cook, J.G., Asano, M. & Nevins, J.R. (2001) Replication factors MCM2 and ORC1 interact with the histone acetyltransferase HBO1. *J. Biol. Chem.* **276**, 15397–15408.
58. Iizuka, M. & Stillman, B. (1999) Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. *J. Biol. Chem.* **274**, 23027–23034.
59. Gruss, C., Wu, J., Koller, T. & Sogo, J.M. (1993) Disruption of the nucleosomes at the replication fork. *EMBO J.* **12**, 4533–4545.
60. Wolffe, A.P. (1998) *Chromatin: Structure and Function*, 3rd edn. Academic Press, London.
61. Tagami, H., Ray-Gallet, D., Almouzni, G. & Nakatani, Y. (2004) Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* **116**, 51–61.
62. Verreault, A., Kaufman, P.D., Kobayashi, R. & Stillman, B. (1996) Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* **87**, 95–104.
63. Shibahara, K. & Stillman, B. (1999) Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* **96**, 575–585.
64. Sporbert, A., Gahl, A., Ankerhold, R., Leonhardt, H. & Cardoso, M.C. (2002) DNA polymerase clamp shows little turnover at established replication sites but sequential de novo assembly at adjacent origin clusters. *Mol. Cell* **10**, 1355–1365.
65. Kaufman, P.D., Kobayashi, R., Kessler, N. & Stillman, B. (1995) The p150 and p60 subunits of chromatin assembly factor I: a molecular link between newly synthesized histones and DNA replication. *Cell* **81**, 1105–1114.
66. Kelley, R.L., Meller, V.H., Gordadze, P.R., Roman, G., Davis, R.L. & Kuroda, M.I. (1999) Epigenetic spreading of the *Drosophila* dosage compensation complex from roX RNA genes into flanking chromatin. *Cell* **98**, 513–522.
67. Sharp, J.A., Franco, A.A., Osley, M.A. & Kaufman, P.D. (2002) Chromatin assembly factor I and Hir proteins contribute to building functional kinetochores in *S. cerevisiae*. *Genes Dev.* **16**, 85–100.
68. Hoek, M. & Stillman, B. (2003) Chromatin assembly factor 1 is essential and couples chromatin assembly to DNA replication *in vivo*. *Proc. Natl Acad. Sci. USA* **100**, 12183–12188.
69. Murzina, N., Verreault, A., Laue, E. & Stillman, B. (1999) Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. *Mol. Cell* **4**, 529–540.
70. Tyler, J.K., Adams, C.R., Chen, S.R., Kobayashi, R., Kamakaka, R.T. & Kadonaga, J.T. (1999) The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* **402**, 555–560.
71. Le, S., Davis, C., Konopka, J.B. & Sternglanz, R. (1997) Two new S-phase-specific genes from *Saccharomyces cerevisiae*. *Yeast* **13**, 1029–1042.
72. Sharp, J.A., Fouts, E.T., Krawitz, D.C. & Kaufman, P.D. (2001) Yeast histone deposition protein Asf1p requires Hir proteins and PCNA for heterochromatic silencing. *Curr. Biol.* **11**, 463–473.
73. Sutton, A., Bucaria, J., Osley, M.A. & Sternglanz, R. (2001) Yeast ASF1 protein is required for cell cycle regulation of histone gene transcription. *Genetics* **158**, 587–596.
74. Adams, C.R. & Kamakaka, R.T. (1999) Chromatin assembly: biochemical identities and genetic redundancy. *Curr. Opin. Genet. Dev.* **9**, 185–190.
75. Sogo, J.M., Stahl, H., Koller, T. & Knippers, R. (1986) Structure of replicating simian virus 40 minichromosomes. The replication fork, core histone segregation and terminal structures. *J. Mol. Biol.* **189**, 189–204.
76. Mosammaparast, N., Ewart, C.S. & Pemberton, L.F. (2002) A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. *EMBO J.* **21**, 6527–6538.
77. Taddei, A., Roche, D., Sibarita, J.B., Turner, B.M. & Almouzni, G. (1999) Duplication and maintenance of heterochromatin domains. *J. Cell. Biol.* **147**, 1153–1166.

78. Kimura, A., Umehara, T. & Horikoshi, M. (2002) Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat. Genet.* **15**, 15.
79. Suka, N., Luo, K. & Grunstein, M. (2002) Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat. Genet.* **32**, 378–383.
80. Meijsing, S.H. & Ehrenhofer-Murray, A.E. (2001) The silencing complex SAS-I links histone acetylation to the assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae*. *Genes Dev.* **15**, 3169–3182.
81. Super, H.G., Strissel, P.L., Sobulo, O.M., Burian, D., Reshmi, S.C., Roe, B., Zeleznik-Le, N.J., Diaz, M.O. & Rowley, J.D. (1997) Identification of complex genomic breakpoint junctions in the t (9; 11) MLL-AF9 fusion gene in acute leukemia. *Genes Chromosomes Cancer* **20**, 185–195.
82. Osada, S., Sutton, A., Muster, N., Brown, C.E., Yates, J.R., 3rd, Sternglanz, R. & Workman, J.L. (2001) The yeast SAS (something about silencing) protein complex contains a MYST-type putative acetyltransferase and functions with chromatin assembly factor ASF1. *Genes Dev.* **15**, 3155–3168.
83. Milutinovic, S., Zhuang, Q. & Szyf, M. (2002) Proliferating cell nuclear antigen associates with histone deacetylase activity, integrating DNA replication and chromatin modification. *J. Biol. Chem.* **277**, 20974–20978.
84. Robyr, D., Suka, Y., Xenarios, I., Kurdistani, S.K., Wang, A., Suka, N. & Grunstein, M. (2002) Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. *Cell* **109**, 437–446.
85. Katan-Khaykovich, Y. & Struhl, K. (2002) Dynamics of global histone acetylation and deacetylation *in vivo*: rapid restoration of normal histone acetylation status upon removal of activators and repressors. *Genes Dev.* **16**, 743–752.
86. Maggert, K.A. & Karpen, G.H. (2000) Acquisition and metastability of centromere identity and function: sequence analysis of a human neocentromere. *Genome Res.* **10**, 725–728.
87. Hakimi, M.A., Bochar, D.A., Schmiesing, J.A., Dong, Y., Barak, O.G., Speicher, D.W., Yokomori, K. & Shiekhattar, R. (2002) A chromatin remodelling complex that loads cohesin onto human chromosomes. *Nature* **418**, 994–998.
88. Moshkin, Y.M., Armstrong, J.A., Maeda, R.K., Tamkun, J.W., Verrijzer, P., Kennison, J.A. & Karch, F. (2002) Histone chaperone ASF1 cooperates with the Brahma chromatin-remodelling machinery. *Genes Dev.* **16**, 2621–2626.
89. Ahmad, K. & Henikoff, S. (2001) Centromeres are specialized replication domains in heterochromatin. *J. Cell. Biol.* **153**, 101–110.
90. Hsu, J.M., Huang, J., Meluh, P.B. & Laurent, B.C. (2003) The yeast RSC chromatin-remodeling complex is required for kinetochore function in chromosome segregation. *Mol. Cell. Biol.* **23**, 3202–3215.
91. McKittrick, E., Gafken, P.R., Ahmad, K. & Henikoff, S. (2004) Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proc. Natl Acad. Sci. USA* **101**, 1525–1530.
92. West, M.H. & Bonner, W.M. (1980) Histone 2A, a heteromorphous family of eight protein species. *Biochemistry* **19**, 3238–3245.
93. Chuang, L.S., Ian, H.I., Koh, T.W., Ng, H.H., Xu, G. & Li, B.F. (1997) Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* **277**, 1996–2000.
94. Rountree, M.R., Bachman, K.E. & Baylin, S.B. (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat. Genet.* **25**, 269–277.
95. Reese, B.E., Bachman, K.E., Baylin, S.B. & Rountree, M.R. (2003) The methyl-CpG binding protein MBD1 interacts with the p150 subunit of chromatin assembly factor 1. *Mol. Cell. Biol.* **23**, 3226–3236.
96. Naar, A.M., Lemon, B.D. & Tjian, R. (2001) Transcriptional coactivator complexes. *Annu. Rev. Biochem.* **70**, 475–501.
97. Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S. & Workman, J.L. (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science* **292**, 2333–2337.
98. Vignali, M., Steger, D.J., Neely, K.E. & Workman, J.L. (2000) Distribution of acetylated histones resulting from Gal4-VP16 recruitment of SAGA and NuA4 complexes. *EMBO J.* **19**, 2629–26240.
99. Syntichaki, P., Topalidou, I. & Thireos, G. (2000) The Gcn5 bromodomain co-ordinates nucleosome remodelling. *Nature* **404**, 414–417.
100. Jacobson, R.H., Ladurner, A.G., King, D.S. & Tjian, R. (2000) Structure and function of a human TAFII250 double bromodomain module. *Science* **288**, 1422–1425.
101. Krebs, J.E., Fry, C.J., Samuels, M.L. & Peterson, C.L. (2000) Global role for chromatin remodeling enzymes in mitotic gene expression. *Cell* **102**, 587–598.
102. Larschan, E. & Winston, F. (2001) The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. *Genes Dev.* **15**, 1946–1956.
103. Bhaumik, S.R. & Green, M.R. (2001) SAGA is an essential in vivo target of the yeast acidic activator Gal4p. *Genes Dev.* **15**, 1935–1945.
104. Henry, K.W., Wyce, A., Lo, W.S., Duggan, L.J., Emre, N.C., Kao, C.F., Pillus, L., Shilatifard, A., Osley, M.A. & Berger, S.L. (2003) Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes Dev.* **17**, 2648–2663.
105. Nickel, B.E., Allis, C.D. & Davie, J.R. (1989) Ubiquitinated histone H2B is preferentially located in transcriptionally active chromatin. *Biochemistry* **28**, 958–963.
106. Hwang, W.W., Venkatasubrahmanyam, S., Ianculescu, A.G., Tong, A., Boone, C. & Madhani, H.D. (2003) A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. *Mol. Cell.* **11**, 261–266.
107. Wood, A., Krogan, N.J., Dover, J., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J.F., Johnston, M. & Shilatifard, A. (2003) Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell.* **11**, 267–274.
108. Sun, Z.W. & Allis, C.D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* **418**, 104–108.
109. Dover, J., Schneider, J., Tawiah-Boateng, M.A., Wood, A., Dean, K., Johnston, M. & Shilatifard, A. (2002) Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *J. Biol. Chem.* **277**, 28368–28371.
110. Ezhkova, E. & Tansey, W.P. (2004) Proteasomal ATPases link ubiquitylation of histone H2B to methylation of histone H3. *Mol. Cell* **13**, 435–442.
111. Shilatifard, A., Conaway, R.C. & Conaway, J.W. (2003) The RNA polymerase II elongation complex. *Annu. Rev. Biochem.* **72**, 693–715.
112. Wittschieben, B.O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C.D., Tempst, P. & Svejstrup, J.Q. (1999) A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Mol. Cell* **4**, 123–128.
113. Pokholok, D.K., Hannett, N.M. & Young, R.A. (2002) Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. *Mol. Cell.* **9**, 799–809.

114. Krogan, N.J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., Richards, D.P., Beattie, B.K., Emili, A., Boone, C., Shilatifard, A., Buratowski, S. & Greenblatt, J. (2003) Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol. Cell. Biol.* **23**, 4207–4218.
115. Xiao, T., Hall, H., Kizer, K.O., Shibata, Y., Hall, M.C., Borchers, C.H. & Strahl, B.D. (2003) Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. *Genes Dev.* **17**, 654–663.
116. Li, B., Howe, L., Anderson, S., Yates, J.R. III & Workman, J.L. (2003) The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J. Biol. Chem.* **278**, 8897–8903.
117. Santos-Rosa, H., Schneider, R., Bernstein, B.E., Karabetsou, N., Morillon, A., Weise, C., Schreiber, S.L., Mellor, J. & Kouzarides, T. (2003) Methylation of histone H3, K4 mediates association of the Isw1p ATPase with chromatin. *Mol. Cell* **12**, 1325–1332.
118. Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N. & Mellor, J. (2003) Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. *Cell* **115**, 425–435.
119. Felsenfeld, G., Clark, D. & Studitsky, V. (2000) Transcription through nucleosomes. *Biophys. Chem.* **86**, 231–237.
120. Belotserkovskaya, R., Oh, S., Bondarenko, V.A., Orphanides, G., Studitsky, V.M. & Reinberg, D. (2003) FACT facilitates transcription-dependent nucleosome alteration. *Science* **301**, 1090–1093.
121. Simchen, G., Winston, F., Styles, C.A. & Fink, G.R. (1984) Ty-mediated gene expression of the *LYS2* and *HIS4* genes of *Saccharomyces cerevisiae* is controlled by the same *SPT* genes. *Proc. Natl Acad. Sci. USA* **81**, 2431–2434.
122. Kaplan, C.D., Laprade, L. & Winston, F. (2003) Transcription elongation factors repress transcription initiation from cryptic sites. *Science* **301**, 1096–1099.
123. Wittmeyer, J. & Formosa, T. (1997) The *Saccharomyces cerevisiae* DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. *Mol. Cell. Biol.* **17**, 4178–4190.
124. Rowley, A., Singer, R.A. & Johnston, G.C. (1991) *CDC68*, a yeast gene that affects regulation of cell proliferation and transcription, encodes a protein with a highly acidic carboxyl terminus. *Mol. Cell. Biol.* **11**, 5718–5726.
125. Wang, A., Kurdistani, S.K. & Grunstein, M. (2002) Requirement of Hos2 histone deacetylase for gene activity in yeast. *Science* **298**, 1412–1414.
126. Gontijo, A.M., Green, C.M. & Almouzni, G. (2003) Repairing DNA damage in chromatin. *Biochimie* **85**, 1133–1147.
127. Bennett, C.B., Lewis, A.L., Baldwin, K.K. & Resnick, M.A. (1993) Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. *Proc. Natl Acad. Sci. USA* **90**, 5613–5617.
128. Lewis, L.K. & Resnick, M.A. (2000) Tying up loose ends: nonhomologous end-joining in *Saccharomyces cerevisiae*. *Mutat. Res.* **451**, 71–89.
129. Anderson, J.D., Lowary, P.T. & Widom, J. (2001) Effects of histone acetylation on the equilibrium accessibility of nucleosomal DNA target sites. *J. Mol. Biol.* **307**, 977–985.
130. Martinez, E., Palhan, V.B., Tjernberg, A., Lymar, E.S., Gamper, A.M., Kundu, T.K., Chait, B.T. & Roeder, R.G. (2001) Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors *in vivo*. *Mol. Cell. Biol.* **21**, 6782–6795.
131. Brand, M., Moggs, J.G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F.J., Stevenin, J., Almouzni, G. & Tora, L. (2001) UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation. *EMBO J.* **20**, 3187–3196.
132. Ikura, T., Ogryzko, V.V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J. & Nakatani, Y. (2000) Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* **102**, 463–473.
133. Bird, A.W., Yu, D.Y., Pray-Grant, M.G., Qiu, Q., Harmon, K.E., Megee, P.C., Grant, P.A., Smith, M.M. & Christman, M.F. (2002) Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature* **419**, 411–415.
134. Althaus, F.R., Hofferer, L., Kleczkowska, H.E., Malanga, M., Naegeli, H., Panzeter, P.L. & Realini, C.A. (1994) Histone shuttling by poly ADP-ribosylation. *Mol. Cell. Biochem.* **138**, 53–59.
135. Downs, J.A., Lowndes, N.F. & Jackson, S.P. (2000) A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* **408**, 1001–1004.
136. Hasan, S., Hassa, P.O., Imhof, R. & Hottiger, M.O. (2001) Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. *Nature* **410**, 387–391.
137. Jazayeri, A., McAinsh, A.D. & Jackson, S.P. (2004) *Saccharomyces cerevisiae* Sin3p facilitates DNA double-strand break repair. *Proc. Natl Acad. Sci. USA* **101**, 1644–1649.
138. Tsukamoto, Y., Kato, J. & Ikeda, H. (1997) Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*. *Nature* **388**, 900–903.
139. Astrom, S.U., Okamura, S.M. & Rine, J. (1999) Yeast cell-type regulation of DNA repair. *Nature* **397**, 310.
140. Martin, S.G., Laroche, T., Suka, N., Grunstein, M. & Gasser, S.M. (1999) Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell* **97**, 621–633.
141. Mills, K.D., Sinclair, D.A. & Guarente, L. (1999) MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell* **97**, 609–620.
142. Gaillard, H., Fitzgerald, D.J., Smith, C.L., Peterson, C.L., Richmond, T.J. & Thoma, F. (2003) Chromatin remodeling activities act on UV-damaged nucleosomes and modulate DNA damage accessibility to photolyase. *J. Biol. Chem.* **278**, 17655–17663.
143. Hara, R. & Sancar, A. (2002) The SWI/SNF chromatin-remodeling factor stimulates repair by human excision nuclease in the mononucleosome core particle. *Mol. Cell. Biol.* **22**, 6779–6787.
144. Shen, X., Mizuguchi, G., Hamiche, A. & Wu, C. (2000) A chromatin remodelling complex involved in transcription and DNA processing. *Nature* **406**, 541–544.
145. Gaillard, P.H., Martini, E.M., Kaufman, P.D., Stillman, B., Moustacchi, E. & Almouzni, G. (1996) Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. *Cell* **86**, 887–896.
146. Emili, A., Schieltz, D.M., Yates, J.R. & 3rd & Hartwell, L.H. (2001) Dynamic interaction of DNA damage checkpoint protein Rad53 with chromatin assembly factor Asf1. *Mol. Cell* **7**, 13–20.
147. Hu, F., Alcasabas, A.A. & Elledge, S.J. (2001) Asf1 links Rad53 to control of chromatin assembly. *Genes Dev.* **15**, 1061–1066.