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Chromatin modifiers in transcription and DNA repair

A. Verger* and M. Crossley

School of Molecular and Microbial Biosciences, University of Sydney, New South Wales, 2006 (Australia),
Fax: +61 2 9351 4726, e-mail: a.verger@mmb.usyd.edu.au

Abstract. In eukaryotes, the packaging of DNA into nucleosomes and the organization of chromatin fibres generate constraints for all nuclear processes involving DNA, including replication, repair, recombination and transcription. The three major processes that regulate chromatin structure and counterbalance its repressive effects are ATP-dependent chromatin remodeling, post-translational modification of histones and histone replacement. While many of these processes have been intensively studied with respect to their effects on transcription, there is also evidence that they affect other nuclear processes involving DNA. This review explores the functions of chromatin-remodeling factors and histone-modifying enzymes in gene regulation and summarizes recent findings which suggest a role for these chromatin modifiers in DNA repair.

Key words. DNA damage; repair; chromatin; transcription.

Introduction

DNA damage resulting from exposure to intracellular or external mutagens presents a major threat for a living cell. In higher eukaryotes, the accumulation of genomic changes is one of the leading causes of oncogenesis and is thought to contribute to cellular aging and senescence [1]. For example, one mutagenic agent, ultraviolet light (UV), can induce dimerization of adjacent pyrimidines in DNA, which may act as obstacles to DNA transcription and replication. Accordingly, cells have developed diverse mechanisms to recognize defects in DNA structure and then respond by either repairing the lesion or undergoing apoptosis [2–4]. Failure of these mechanisms can lead to serious disease consequences, as well illustrated in the human hereditary diseases xeroderma pigmentosum (XP), hereditary non-polyposis colon cancer (HNPCC) and some forms of breast cancer [5].

Although the repair of different types of DNA lesions relies on different sets of proteins (fig. 1), the various forms of DNA damage nevertheless trigger common signal transduction pathways, which collectively bring about what is known as the DNA damage response [6]. One well-established feature of the DNA damage response is the slowing or arrest of cell cycle progression, with delays to key cell cycle transitions occurring until repair is effected. Other aspects of the DNA damage response include changes in chromatin structure at the site of damage and the transcriptional induction and post-translational modification of various proteins involved in DNA repair [7–13]. This review is aimed at discussing the problem of how DNA repair enzymes can gain access to DNA lesions within chromatin, repair the damaged DNA strand and restore the chromatin structure present prior to the formation of DNA damage. In order to understand how this genomic maintenance is achieved, we will first explore some of the findings related to the role of chromatin modifiers in regulating transcription. We will then discuss selected examples that relate transcription and chromatin structure to DNA repair.

Chromatin packaging

Mammalian DNA must be tightly packaged in order to fit within the nucleus. The first level of packaging involves the winding of about 150 bp of DNA around an octamer of core histone proteins H2A, H2B, H3 and H4. The histone core and its DNA is referred to as a nucleosome. Strings of nucleosomes can exist as an extended 10-nm

* Corresponding author.

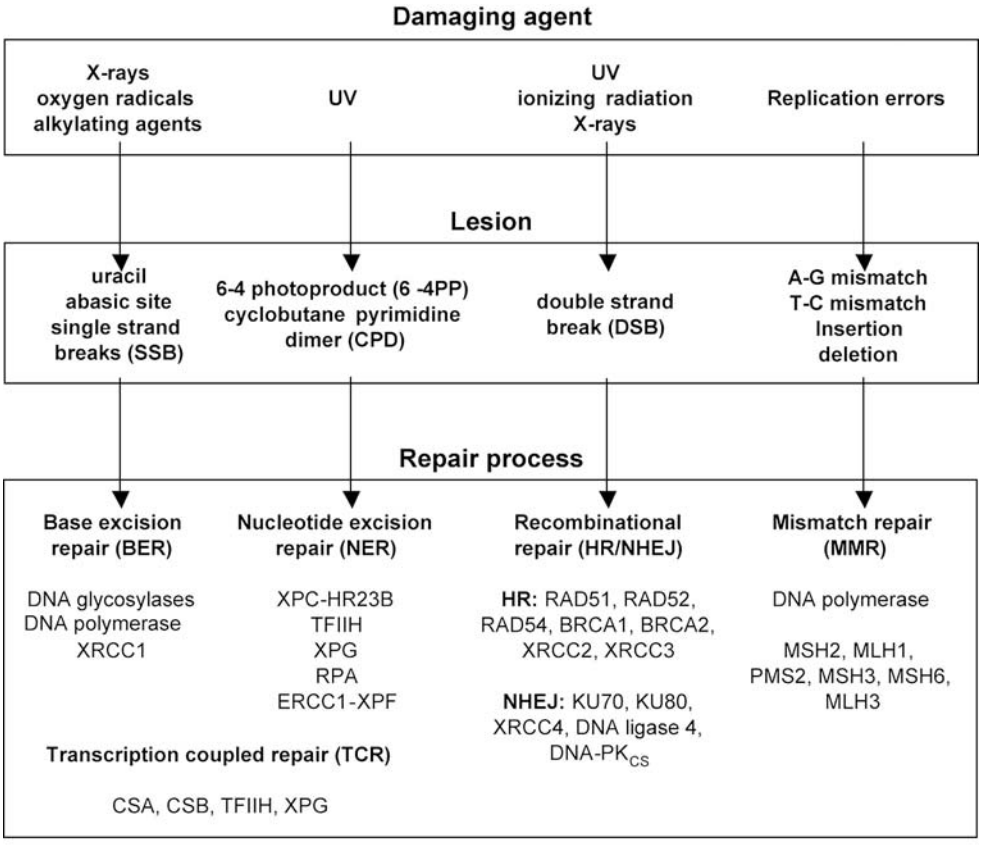

Figure 1. The repair pathway. Adapted from [2]. DNA-damaging agents, examples of DNA lesions and the relevant repair mechanisms are shown.

fibre, reminiscent of beads on a string, or can be further condensed to become a coiled 30-nm fibre. This can then be further wound into a large solenoid structure which is thought to be looped together to form the chromosome proper [14].

The chromatin template undergoes dynamic changes during many genetic processes. These include necessary structural reorganizations that occur during DNA replication and cell cycle progression, spatially and temporally coordinated gene expression, as well as DNA repair and recombination events. Recent studies have revealed that there are different types of protein complexes capable of altering the chromatin, and these act in a physiological context to modulate DNA accessibility. One type includes multi-protein complexes that utilize the energy derived from ATP hydrolysis to mobilize or alter the structure of nucleosomes (table 1) [15]. Another class includes protein complexes that modify the histone tails covalently (table 2) [16]. The central involvement of these two classes of protein complexes in transcriptional regulation has provided new directions for the exploration of DNA repair in chromatin [7–13].

Chromatin-remodeling complexes in transcription

Remodeling involves the breaking and reforming of histone-DNA contacts and results in the mobilization of nucleosomes on the chromatin template. Several different remodeling complexes have been identified [15, 17]. These complexes can be divided into three main classes based on the identity of their catalytic ATPase subunits (table 1). They also contain additional subunits that may affect regulation, efficiency and specificity.

Chromatin-remodeling factors act by catalysing fluidity in the position and conformation of nucleosomes in an ATP-dependent manner. They are thought to do this by catalysing the interconversion between various chromatin states via an activated intermediate consisting of the remodeling factor and a nucleosome with weakened histone-DNA contacts [15]. As this model posits only that remodeling complexes increase the rate of interconversion between chromatin states, an important implication is that the action of remodeling complexes does not in itself specify whether the resulting chromatin state is positive or negative for transcription. Indeed, genome-wide analysis of the effects of loss of the Swi2-remodeling factor in yeast indicates that this factor has positive roles in

Table 1. ATP-dependent chromatin-remodeling factors. Adapted from [16]. The ATPase subunit is indicated in bold. ISWI, imitation switch; ACF, ATP-utilizing chromatin assembly and remodeling factor; WICH, WSTF (Williams syndrome transcription factor)-ISWI chromatin-remodeling complex; CHRAC, chromatin accessibility complex; NURF, nucleosome-remodeling factor; RSF, remodeling and spacing factor; NcoR-C, nuclear receptor corepressor complex; SWI/SNF, switch/sucrose-non fermentation; RSC, remodels the structure of chromatin; INO80, Inositol80; CHD1, chromodomain-helicase-DNA binding protein 1; NuRD, nucleosome-remodeling and deacetylase repressor complex.

ATPase family	Complex	Species	Subunits
ISWI	ISWI1	yeast	**ISWI1**, p110, p105, p74
	ISWI2	yeast	**ISWI2**, p140
	ACF	*Drosophila*, *Xenopus*, human	Acf-1, **ISWI (hSNF2H)**
	WICH	*Xenopus*, mouse	WSTF, **hSNF2H**
	CHRAC	*Drosophila*, human	Acf-1, **ISWI (hSNF2H)**, CHRAC16, CHRAC14
	NURF	*Drosophila*	NURF301, **ISWI**, NURF55, NURF38
	RSF	human	Rsf-1 (p325), **hSNF2H**
	NCoR-C	human	Tip5, **hSNF2H**
	ISWI-D	*Xenopus*	p195, **ISWI**
SWI2/SNF2	SWI/SNF	yeast, *Drosophila*, mouse, human	**Brg1/Brm**, ~10 subunits depending the species
	RSC	yeast	**Sth1**, ~15 polypeptides complex
INO	INO80	yeast	**Ino80**, Rvb1, Rvb2, Arp
CHD	CHD1	yeast, human	**CHD1**
	NuRD	*Drosophila*, *Xenopus*, human	**CHD4 (Mi-2)**, MTA2, MBD3, HDAC1/2, RbAp48/46

Table 2. Examples of histone acetyl transferase factors. Adapted from [16]. GNAT, GCN5-related N-acetyltransferase; MYST, MOZ, Ybf2/Sas3, Sas2, Tip60; NR, nuclear receptor.

HAT family	HAT enzyme	Species	Complex	Function
GNAT	Gcn5	yeast to human	STAGA, ADA, ADA2	coactivator
	PCAF	human, mouse	PCAF	coactivator
	Hat1	yeast	HatB	histone deposition, silencing
MYST	Sas2	yeast		silencing
	Sas3	yeast	NuA3	silencing
	TIP60	human	TIP60	coactivator, DNA repair, apoptosis
	Esa1	yeast	NuA4	Cell cycle progression
	MOF	*Drosophila*	MSL	X-chromosome dosage compensation
	HBO1	human	HBO1	DNA replication
	MOZ	human	AML1	transcriptional activation
p300/CBP	p300	human, mouse		coactivator
	CBP	human, mouse		coactivator
NR	ACTR	human, mouse		NR coactivator
	SRC-1	human, mouse		NR coactivator
	TIF2	human, mouse		NR coactivator
TAFII250	TAFII250	yeast to human	TFIID	RNA polymerase II complex

transcription at some genes and negative roles at others [18].

Chromatin-modifying enzymes in transcription

When the structure of the nucleosome was solved by X-ray crystallography [19, 20], it became apparent that the termini of the histone subunits, the so-called histone tails, protruded outside the nucleosome, and it was hypothesized that these might either influence the general level of packaging by mediating nucleosome-nucleosome contacts and/or be instrumental in forming the binding sites for additional proteins. There is now considerable evidence that this is essentially correct and that the histone tails are of vital importance to the regulation of gene expression. It has become apparent that a 'histone code' exists [21, 22], whereby different modifications to the tails of the different subunits do indeed alter packaging and form the docking sites for enzymes that ultimately lead to either activation or repression of particular genes.

In general, the acetylation of histones is linked to transcriptional activation. It is thought that histone acetylation decreases internucleosome interaction and the interaction of nucleosome tails with linker DNA, thereby allowing greater accessibility, but acetylation also has additional effects. Acetylated lysine can be recognized and bound by activating proteins that contain bromodomains. Such proteins include SWI/SNF components and one of the TBP-associated factors, TAF1 (formerly TAF_{II}-250 [23]), and the coactivator CREB-binding pro-

tein (CBP) (table 2) [24]. Thus, acetylation of lysine can theoretically lead to further recruitment of activators and sustained gene activation. For example, the recruitment of CBP might lead to the acetylation of lysines within the promoter; this may facilitate the recruitment or retention of SWI/SNF through binding of the acetyl lysine to its bromodomain-containing subunits. SWI/SNF may then loosen the chromatin packaging, and the acetyl lysines may contribute to the recruitment of TAF1 and further CBP to ensure that the gene remains stably on as the cells terminally differentiate. It is a hallmark of mammalian gene expression that some genes turned on during differentiation must be kept on thereafter for the life of the cell, and epigenetic marks such as the acetylation of histones could facilitate the establishment of an active chromatin state so that the gene remains on.

Histone modifications have also been associated with repression of gene expression. The first histone-modifying enzymes associated with repression were the histone deacetylase or HDAC enzymes [25]. The deacetylation of lysine will restore the positive charge and possibly thereby allow strong binding of the histone tails to DNA, including nucleosome-nucleosome contacts, and this may ultimately lead to a tightening of chromatin structure. Recently, another effect of deacetylation has become apparent. Deacetylated lysine can be targeted by methyltransferase enzymes, and it appears that histone deacetylases and certain methyltransferases can cooperate in a pathway to repression [26]. Moreover, just as acetylated lysine can recruit activating proteins that contain bromodomains, such as certain SWI/SNF subunits, methylated lysine residues can be bound by repressive proteins that contain so-called chromodomains, such as polycomb and heterochromatin protein 1 (HP1) [26].

While acetylation is readily reversible by deacetylase enzymes, there is as yet no evidence for demethylases that remove the methyl groups from lysine and arginine residues. Thus, methylation of histones can presumably only be reversed by rounds of DNA replication and repackaging with new, unmodified histones [27, 28]. Thus methylation appears to be a relatively permanent mark and is likely to be ideal for maintaining chromatin states in terminally differentiated non-dividing cells, where patterns of gene expression remain relatively static.

In summary, transcriptional activation involves the opening of chromatin around specific genes and the recruitment of RNA polymerase and its associated proteins to the beginning of these genes, that is to the gene promoter. This process is orchestrated initially by the binding of sequence-specific DNA-binding proteins or transcription factors to specific regulatory sequences, promoters or enhancers, and the subsequent recruitment of chromatin-remodeling enzymes and chromatin modifiers to open the chromatin structure, followed by the recruitment of RNA polymerase to transcribe the gene. As we shall see, there are several parallels with pathways of repair, as specific lesions in DNA also recruit chromatin-remodeling and -modifying enzymes, which then recruit and allow access to DNA repair machinery.

Repair in chromatin

An early indication that chromatin structure played a significant role in regulating DNA repair came from the observation that nucleosomes had an inhibitory effect on repair in vivo. It was found that 48 h after ultraviolet (UV) irradiation of human fibroblasts, there were more cyclobutane pyrimidine dimers (CPDs) in the nuclease-resistant fraction of chromatin (i.e. the highly packaged DNA) than in the nuclease-accessible fraction [29]. Analysis of damage distribution at the nucleosomal level revealed that Pyr-Pyr dimers were produced with a 10.3-bp periodicity in the core nucleosome and that this periodicity was maintained during the repair period, indicating that there was no preferential repair along the nucleosome [30]. More detailed studies of the effect of chromatin structure on repair have been carried out in yeast using a mini-chromosome with well-defined nucleosome phasing and transcriptionally active and inactive regions. These studies [31, 32] conclusively showed that both Pyr-Pyr and (6-4) photoproducts (6-4PPs) were repaired at faster rates in nucleosome-free regions and in the linker DNA than were photoproducts in the nucleosome core.

Further evidence on the role of nucleosomes came from in vitro experiments. Crude cell extracts were used to perform nucleotide excision repair (NER), and it was shown that repair synthesis is strongly reduced on UV-irradiated DNA preassembled in nucleosome [33] or UV-irradiated simian virus 40 mini-chromosomes [34], compared to naked DNA. Furthermore, NER repair studies that used UV-irradiated reconstituted nucleosomes as templates with bacterial repair enzymes or *Xenopus* oocyte repair extracts demonstrated that nucleosome assembly reduces efficiencies of DNA repair [35, 36].

Recently, defined nucleosomal templates containing synthetic 6-4PPs at unique sites were used for NER reactions reconstituted with purified human NER cofactors [37–39]. As expected for a negative role of chromatin structure on DNA repair, the efficiency of damage excision is lower on reconstituted nucleosomes than on naked DNA. Interestingly, it seems that this inhibitory state could be extended to other repair pathways, as the DNA base excision repair (BER) of uracil residues is reduced three- to ninefold in nucleosomes compared with naked DNA [40].

One interpretation of these experiments is that nucleosomes inhibit the access of the repair machinery. If an active access mechanism is required before DNA repair can

take place, what processes are involved? In the same way that chromatin remodeling allows some transcriptional complexes access to their binding sites, accumulating evidence suggests that DNA repair may also involve recruitment of chromatin-remodeling complexes or histone-modifying enzymes as well as architectural factors [7–13]. Indeed, in vitro studies and yeast genetics have begun to identify factors that connect chromatin dynamics with repair (table 3).

Repair and chromatin remodeling

The recent purification of a yeast SNF2-related ATPase complex that may function in DNA repair underscores a connection between repair and remodeling activities [41]. Interestingly, this complex called INO80 (tables 2 and 3) contains two proteins related to the bacterial RuvB DNA helicase that catalyses branch migration of Holliday junctions [42]. Consistent with the activity of bacterial RuvB, the INO80 complex contains DNA helicase activity, and mutations in INO80 cause sensitivity to hydroxyurea, methyl methane sulfonate (MMS), UV and ionizing radiation, suggesting a function of INO80 in repair [41]. Another example concerns the chromatin assembly and remodeling complex ACF (tables 2 and 3), which was reported to facilitate NER of a specific lesion in linker DNA of a dinucleosome in vitro, but not of a lesion positioned in the centre of one of the nucleosomes [38]. In this study, the dinucleosome templates have 50–80 bp of linker DNA, thus shorter than the ~100-bp stretch apparently required for excision of a lesion from naked DNA by the human NER complex [43]. These results suggest that ACF may promote NER by assisting histone octamer sliding, which then may extend the nucleosome-free DNA region around the lesion and thereby permit the access of NER factors to the damaged sites [38].

Another member of the ATP-dependent remodeling enzymes, SWI/SNF (tables 2 and 3), has been shown to stimulate the excision of acetylaminofluorene-guanine (AAF-G) adduct from the nucleosome core particle [44, 45]. Interestingly, the three NER factors implicated in damage recognition, RPA, XPA and XPC, stimulate the remodeling activity of SWI/SNF, which in turn stimulates the removal of the AAF-G adduct. This result raises the intriguing possibility that these three proteins may be the functional analogs of transcription factors which recruit remodeling factors to target genes to initiate gene expression [44, 45]. A further link between DNA repair and chromatin-remodeling activity has been shown recently, as SWI/SNF and ISWI can positively modulate the DNA damage accessibility to photolyase [46].

Some repair-related proteins may directly remodel chromatin during repair. The Cockayne syndrome B protein (CSB) that is involved in transcription-coupled repair (TCR) and its yeast homologue Rad26 both belong to the SNF2 family [47]. Recombinant CSB was shown to remodel undamaged nucleosomes and nucleosome arrays in vitro, thus being the first repair enzyme with remodeling activity [48]. Rad7-Rad16 is a complex of the NER pathway in *S. cerevisiae* that recognizes UV lesions in an ATP-dependent way in vitro [49, 50]. Rad16, like CSB, has homology to SNF2 and might play a role in nucleosome remodeling to generate space for the other NER proteins [11]. Finally, two recent reports indicate that the

Table 3. Chromatin modifiers implicated in repair. Adapted from [7, 12]. See text for relevant discussion and references.

Proteins	Chromatin connection	Repair connection
ACF	ATP-dependent chromatin remodeler	promotes NER on dinucleosome template in vitro
CAF-1	histone chaperone	required for nucleosome reassembly afer NER
CSB	belongs to the SWI2/SNF2 family	required for TCR
Rad26	yeast homologue of CSB	required for TCR
H2AX	histone H2A variant	specifically phosphorylated after DSB
TIP60	HAT	involved in DSB
Esa1	HAT	acetylates histone H4 after DSB
INO80	ATP-remodeling complex	mutation of INO80 causes sensitivity to MMS, UV
Rad54	catalyzes bidirectional nucleosome redistribution	required for homologous recombination
TFTC	acetylates histone H3	binds sites of UV damage
CBP/p300	HAT	associates with Fen-1, DDB, PCNA, TDG
SWI/SNF	ATP remodeling complex	stimulates excision of AAF-G adduct in nucleosome
Hat1	HAT	mutation causes sensitivity to MMS and defect in homologous recombination
Sir	histone deacetylase NAD dependent	inactivation renders cells more sensitive to DNA damage
PARP-1	ADP ribosylation relaxes chromatin structure	ADP ribosylation of histones associated with DNA damage
HMGN1	destabilizes higher-order chromatin structure	knockout in mice suggests a role in facilitating DNA damage access in chromatin context

recombinational repair of double-strand DNA break is facilitated by the chromatin-remodeling activity of the SWI2/SNF2 member Rad54 [51, 52], again consistent with the view that DNA repair efficiency and chromatin remodeling are interconnected.

Remodeling of chromatin subsequent to DNA repair is also important as the restoration of chromatin to its original state is likely to be required for the maintenance of patterns of gene expression. In the case of UV damage, the chromatin assembly factor-1 (CAF-1) is the best-studied factor involved in the restoration of chromatin structure after repair [53]. CAF-1 is able to perform assembly of chromatin specifically onto plasmids that have been repaired by NER [54]. Furthermore, in vitro and in vivo, CAF-1 can be recruited to UV-damaged DNA in a PCNA-dependent manner [55, 56]. Interestingly, the recruitment of CAF-1 is restricted to damage sites and depends on NER, suggesting that the function of CAF-1 is directly linked to local chromatin rearrangements instead of global chromatin relaxation [55]. However, a non-mutually exclusive mechanism of global chromatin remodeling is also possible in response to damage in order to expose the individual damages sites for recognition [57]. The precise response, however, may vary in each instance, depending on the specific repair mechanism and on the exact location of the damage in the nucleosome (i.e. some sites may be accessible and thus may not need chromatin relaxation, whereas other may be not accessible and repaired only after significant remodeling).

Repair and chromatin-modifying enzymes

The covalent modification of histones may also influence damage site accessibility. In vitro, histone H3 acetyltransferase activity can be directed to sites of UV damage via the TBP-free TAFII complex (TFTC), which contains both a DNA damage binding subunit (SAP130 that shares homology with UV-damaged DNA-binding protein UV-DDB p127), and a HAT subunit (GCN5) [58]. H3 acetylation is also implicated in repair of DSBs in yeast. Mutants of specific H3 lysines or HAT1, a component of a nuclear HAT complex (tables 2 and 3), are sensitive to the mutagen MMS and also defective in homologous recombination [59]. Another HAT protein implicated in DNA repair is TIP60 (tables 2 and 3) [60]. Ectopically expressed TIP60 lacking HAT activity results in cells with defective DSB repair and impaired apoptotic competence. Remarkably, like the yeast INO80 complex, two TIP60-interacting proteins turned out to be the human homologues of the bacterial helicase RuvB [8, 60]. Moreover, the yeast counterpart of the human TIP60, namely Esa1, acetylates histone H4, a modification that appears to be essential for DSB repair [61].

Additional evidence for an association between HAT proteins and DNA repair has come from studies that identified new partners of the well-known p300/CBP transcriptional coactivator and other HAT complexes. Thus, the damage-specific DNA-binding proteins XP-E and DDB, which are involved in the initial steps of NER, have been found to interact with the p300 and STAGA HAT complexes, respectively [62, 63]. Moreover, thymine DNA glycosylase, an enzyme involved in the initiation step of BER, can also recruit CBP/p300 [64]. Interestingly, p300 also interacts with PCNA [65]. All these interactions very likely allow the recruitment of HAT proteins to damaged sites to induce acetylation of histones at or near the lesion, thus increasing DNA repair machinery accessibility by locally altering chromatin structure.

Just as transcription factors are regulated via post-translational modifications, cells may regulate DNA repair by modifying repair proteins and thereby regulate their activity. For example, acetylation of the human Flap endonuclease 1 (Fen1) by p300 is significantly enhanced after UV irradiation [66]. The consequence of the acetylation of this structure-specific nuclease involved in BER is a downregulation of its activity due to a decrease in its DNA binding activity [66]. While this is a useful example of how repair proteins can be regulated, further work may be necessary to understand why the activity of this repair protein seems to be diminished, rather than enhanced as a result of exposure to UV light.

Remarkably, deacetylase enzymes that catalyze the opposite reaction to that of HATs have also been linked to DNA repair. Mutations of the NAD-dependent histone deacetylase Sir2 result in an increased sensitivity to DSB in yeast [67]. Similarly, inactivation of the human Sir2 homologue makes cells more sensitive to DNA damage [68, 69]. However, in this case, the effect might be attributable to a increase in levels of p53 acetylation directing the damaged cells towards apoptosis [68, 69].

Recent intriguing evidence suggests a correlation between the ADP-ribosyl-transferase and deacetylase activities of a *Trypanosoma brucei* Sir2 homologue and effective repair responses [70]. The authors proposed that the catalytic activity of Sir2 might facilitate chromatin remodeling by ADP ribosylation of histones and, consequently, favour access to the damaged region [70]. Interestingly, it appears that ADP ribosylation of histones is a hallmark of damaged DNA, and this post-translational modification is also induced by PARP-1, the founding member of the poly ADP-ribose polymerase family [71]. Indeed, repair of DNA damage is associated with increased ADP ribosylation of core histones as well as other nuclear proteins, and ADP ribosylation of histones by PARP-1 is known to facilitate chromatin remodeling [72] and to facilitate access to the lesion [73]. All these findings support the view that ADP ribosylation is associated with DNA repair.

Finally, architectural chromosomal factors, that is proteins like the nucleosome-binding protein HMGN1, which bind to and stabilize or generate bends in DNA, may also play an active role during DNA repair. HMGN proteins are known to destabilize the higher-order chromatin structure by targeting histone H1 and the N-terminal tail of histone H3 [74]. Interestingly, *Hmgn1–/–* mouse embryonic fibroblasts are hypersensitive to UV irradiation, and the removal rate of photoproducts from the chromatin is decreased as compared with the chromatin of wild-type cells, suggesting that HMGN1 may enhance the rate of DNA repair by facilitating access to UV-damaged DNA sites [75].

Transcription and DNA repair

Another connection between repair and chromatin remodeling arose from the important discovery that repair occurs more rapidly in actively transcribing genes than in the genome overall [76]. This process, referred to as transcription-coupled repair (TCR), is a sub-pathway of NER. Although the current hypothesis for TCR revolves around the targeted recruitment of the repair machinery to an RNA polymerase II stalled at a damage site on the transcribed strand, transcription might also affect DNA repair through the nucleosome remodeling that accompanies RNA polymerase II progression. Nevertheless, there is also evidence that transcriptional activators can induce NER in a TCR-independent pathway [77]. It appears that the binding of activators to their cognate sequences can induce a local chromatin remodeling mediated by ATP-dependent chromatin remodelers and acetyl-transferase activities, and this can also facilitate DNA repair [77]. In some instances, transcriptional activators may act by directly recruiting DNA repair factors [78].

A 'histone code' for DNA repair?

The 'histone code' hypothesis [21, 22] predicts that modification marks on the histone tails provide binding sites for effector proteins. Given that repair of DNA damage is associated with increased ADP ribosylation of core histones [70, 71], one can imagine that this post-translational modification may act as a marker for damaged chromatin and thus may help in recruiting specific repair proteins. Other markers may also be important. Interestingly, phosphorylation of serine 139 in the unique carboxy-terminal tail of histone H2AX (a histone variant of H2A [79]) is observed to be one of the first cellular responses to DSB [80]. In accordance with a role in repair factor recruitment rather than simply structural chromatin remodeling, this modification is required for the concentration of repair proteins into foci but not their initial relocation to break sites [81]. Furthermore, the actin-related protein Arp4 that interacts specifically with acetylated histone H4 tail is recruited to the site of DSB in vivo [61]. This association could help to target the Esa1 HAT-containing complex (NuA4) to acetylate chromatin in the vicinity of a break.

Conclusion

It is now apparent that the use of histone modifications and chromatin remodeling as regulatory mechanisms is not restricted to transcription, but may be a hallmark of all chromatin-templated processes, including DNA repair, recombination and replication. The observation discussed here that chromatin-remodeling complexes and -modifying enzymes can act on damaged nucleosomes and alter the accessibility, at least in vitro, to DNA repair factors, suggests that similar activities can indeed play a key role in vivo. However, because DNA lesions are generated all over the genome and need to be removed everywhere, whereas transcription is relatively localised, the absolute number of chromatin modifier complexes required for efficient DNA repair may be greater than those required for transcription [13]. This implies either that specialized complexes capable of both damage recognition and nucleosome remodeling are involved, or that DNA damage needs to be recognized first, before nucleosome-remodeling activities can be recruited. The identification of new genes that are involved in biological responses to DNA damage is a major challenge for the future. One recent study using RNA interference identified 61 genes in *Caenorhabditis elegans* that affect genomic stability in somatic cells and spontaneous mutagenesis in the germ line [82]. Strikingly, many of the genes uncovered are involved in chromatin organization and remodeling.

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