THE HISTONE CODE AT DNA BREAKS: A GUIDE TO REPAIR?

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Abstract | Chromatin modifications are important for all cellular processes that involve DNA, including transcription, replication and DNA repair. Chromatin can be modified by the addition of adducts to histone tail residues or by nucleosome remodelling, which requires ATP-dependent chromatin-remodelling complexes. Although the role of these mechanisms in transcription is well studied, their impact on DNA repair has only recently become evident. One crucial chromatin modification, the phosphorylation of histone H2A, links the recruitment of histone modifiers and ATP-dependent chromatin-remodelling complexes to sites of DNA damage.

CHROMATIN
A higher-order structure of DNA folded around histone octamers and stabilized by linker histones and other factors.

NUCLEOSOME
The basic unit of chromatin composed of 147 bp of chromosomal DNA wrapped around an octamer that contains two copies of each histone H2A, H2B, H3 and H4, or appropriate histone variants.

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DNA double-strand breaks (DSBs) are by far the most deleterious type of DNA lesion, and they can be caused either by environmental stress (for example, ionizing radiation) or by the stalling of DNA replication forks. Inefficient or inaccurate repair can cause cell death or genomic instability, which itself can lead to cancer. In order to cope with DSBs, eukaryotic cells have evolved two conserved mechanisms to detect and repair this type of lesion. Homologous recombination (HR) repairs the break using genetic information that is retrieved from an undamaged sister chromatid or chromosomal homologue, whereas non-homologous end joining (NHEJ) involves the direct ligation of DNA ends[1] (BOX 1).

Genomic DNA and histones form a highly condensed structure known as CHROMATIN. Cellular processes that unwind the double helix, such as transcription, replication and DNA repair, have to overcome this natural barrier to DNA accessibility. Genetic and biochemical studies on transcription have identified two classes of enzymes that modify chromatin structure. The first class functions through covalent modifications of histone tails. These include post-translational changes such as serine phosphorylation, lysine ubiquitylation, acetylation and deacetylation, and lysine and arginine methylation[2]. The second class consists of large multi-protein complexes that use the energy from ATP hydrolysis to alter the position or composition of NUCLEOSOMES within chromatin[3] (BOX 2).

In this review we discuss the role of chromatin modifications in the cellular response to DNA damage. We focus on the DNA-damage-induced phosphorylation of histone H2A, which recruits histone-modifying enzymes, ATP-dependent nucleosome remodelling complexes and the sister-chromatid-pairing molecule, cohesin, to sites of DNA damage. Recently, modifications other than histone H2A phosphorylation have also been implicated in the DNA damage response. Indeed, roles for histone H2B phosphorylation and ubiquitylation, histone H3 and H4 acetylation and methylation, and histone H4 phosphorylation have been reported and will also be discussed.

The phosphorylation of histone H2A(X)

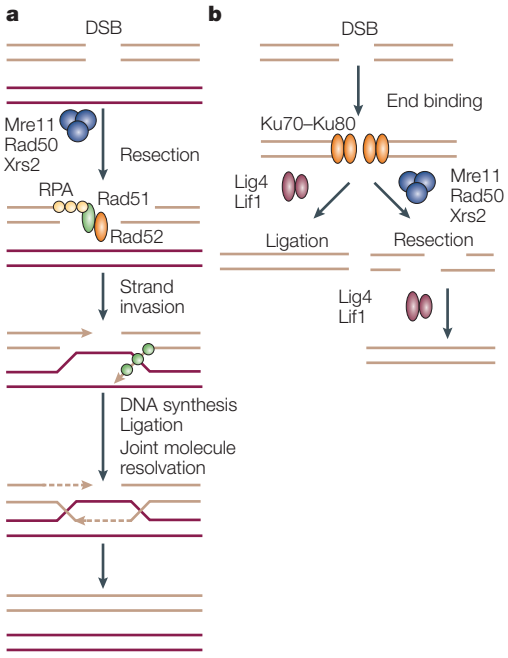
What happens when DNA DSBs are induced in the context of chromatin? One of the first events is the phosphorylation of histone H2A in yeast, or histone H2AX — a variant that constitutes ~10% of nuclear H2A in mammals. Phosphorylation occurs rapidly in response to DNA damage on a serine residue near the C terminus of these proteins (S129 in yeast H2A and S139 in mammalian H2AX). This modification is dependent on the action of members from the phosphatidylinositol 3-kinase (PI3K)-like family of

Box 1 | **Pathways for DNA double-strand break repair**

A DNA double-strand break (DSB) can be repaired by homologous recombination (HR; see figure, part a) or non-homologous end joining (NHEJ; part b).

HR

When a DSB occurs in one of two sister chromatids, the ends of the DSB are recognized by the Mre11–Rad50–Xrs2 (MRX) complex (or Mre11–Rad50–Nbs1 (MRN) complex in mammals). Processing of the ends occurs by the MRX complex and results in the formation of 3′ single stranded (ss) DNA overhangs. The ssDNA-binding protein replication protein A (RPA) binds to the ssDNA overhangs, and Rad51 and Rad52 are recruited to the DSB. Both RPA and Rad52 help to load Rad51 onto ssDNA to form ssDNA–Rad51 nucleoprotein filaments. This nucleoprotein filament searches for the homologous duplex DNA in the undamaged sister chromatid. A successful search results in strand invasion, strand exchange and joint molecule formation. In yeast, these events are facilitated by one or more proteins from the *Rad52* epistasis group, which, as well as Rad51, Rad52 and MRX, includes Rad54, Rad57 and Rad59. In mammals, these events involve the action of BRCA1, BRCA2 and the Rad51-like proteins XRCC2, XRCC3, RAD51B, RAD51C and RAD51D. DNA synthesis by DNA polymerases generates the genetic information that is required to seal the break. Ligation and the resolution of the two double helices joined by strand exchange complete this error-free repair event.



NHEJ

The ends of a DSB are detected and bound by KU, a heterodimer consisting of Ku70 and Ku80 proteins. In mammals, KU forms a complex, known as DNA-PK, with DNA-PK catalytic subunit (DNA-PKcs). It is thought that KU holds the two ends together and facilitates end-to-end ligation by the complex of ligase 4 (Lig4) and ligase-interacting factor 1 (Lif1) in yeast (or XRCC4 in mammals), which usually results in accurate repair of the DSB (left branch of pathway). Alternatively, binding of the ends by KU can be followed by resection of the free ends by the MRX (or MRN) complex. Processing by MRX, followed by Lig4–Lif1-mediated ligation (Lig4–XRCC4 in mammals) has been implicated in the joining of ssDNA overhangs at regions where microhomology exists. This pathway generally leads to error-prone repair of the DSB (right branch of pathway).

kinases[4–6] (FIG. 1), which includes ataxia telangiectasia mutated (ATM), AT-related (ATR) and DNA-dependent protein kinase (DNA-PK). ATM and ATR kinase activities are responsible for the formation of megabase-sized, phospho-H2AX-containing regions around sites of DSBs[7].

In budding yeast, the counterparts of ATM and ATR, Tel1 and Mec1, similarly phosphorylate histone H2A in response to DNA damage[8,9] (FIG. 1). Recent studies mapped H2A phosphorylation to regions flanking a DSB induced by the HO endonuclease, a cleavage event that normally occurs during mating-type switching. CHROMATIN IMMUNOPRECIPITATION (ChIP) experiments using an antibody specific to phosho-H2A showed that H2A becomes rapidly phosphorylated within a ~50-kb region around the site of HO cleavage, with the highest level of phosphorylation at sites ~3–5 kb from the cleavage site[9]. Tel1 and Mec1 could also be detected at the HO-induced DSB, and genetics studies indicated that both kinases were responsible for the formation of the phospho-H2A domain[9] (FIG. 2a–c). Intriguingly, only low levels of phospho-H2A were detected immediately adjacent to the break (within 1–2 kb) despite the presence of both Mec1 and Tel1 in this break-proximal region. Tel1 binds DSBs through its interaction with the Mre11–Rad50–Xrs2 complex (MRX), and Mec1 through its partner Ddc2, also known as Lcd1[10–13]. Because histone H2B, which forms a dimer with H2A (or H2A-P), was not depleted from this region, the low levels of phospho-H2A immediately adjacent to the site of damage are probably not due to nucleosome removal. Instead, they could result from other modifications near S129 or the recruitment of DNA repair proteins, such as Ku80, Rad51 and Mre11, that impair access of the phospho-H2A antibody[9,14–16] (FIG. 2; BOX 1). Alternatively, a rapid turnover of phospho-H2A might occur due to the recruitment of phosphatase activity or histone exchange.

CHROMATIN IMMUNOPRECIPITATION (ChIP). A technique that allows the study of protein–DNA interactions by the amplification of DNA sequences from complexes of crosslinked proteins and DNA, recovered by immunoprecipitation with antibodies against the proteins in question.

The function of H2A(X) phosphorylation

The first evidence for a function of H2A phosphorylation in DNA damage repair came from genetics studies in yeast. Mutation of the C-terminal phospho-acceptor

Box 2 | **ATP-dependent chromatin remodelling**

All ATP-dependent chromatin-remodelling machineries that have been identified so far are multi-protein complexes containing a catalytic subunit that is part of the SWI2/SNF2 superfamily of ATPases. There are four different classes of chromatin-remodelling complexes within this superfamily — SWI/SNF, ISWI, CHD and INO80. The classification of chromatin-remodelling complexes is based on the presence of motifs outside the ATPase region. SWI/SNF members contain a BROMODOMAIN, ISWI members a SANT DOMAIN and CHD members a CHROMODOMAIN and a DNA-binding domain. Members of the INO80 class do not contain any of these domains; instead, their ATPase domain contains an insert that splits it into two segments.

The process of chromatin remodelling generally refers to various changes in chromatin, all of which involve changes in the DNA–histone interaction within nucleosomes. Biochemical studies have shown that chromatin-remodelling complexes use the energy from ATP hydrolysis to induce these changes. They include the mobilization and repositioning of histone octamers in *cis* (along the same DNA template molecule) and in *trans* (from one DNA template molecule to another one), the loss of superhelical torsion of nucleosomal DNA, and the increase in accessibility to nucleosomal DNA for nucleases or proteins involved in transcription. Interestingly, recent studies have shown that ATP-dependent chromatin remodelling also provides a means to change the histone composition of a nucleosome. The yeast SWR1 complex, a member of the INO80 class of remodellers, associates with Htz1, a homologue of the mammalian H2A variant H2AZ. This complex can drive the ATP-dependent replacement of H2A–H2B dimers with Htz1–H2B dimers *in vitro*. *In vivo*, SWR1 catalyses the incorporation of Htz1 into chromatin, which prevents the spreading of heterochromatin regions into regions of EUCHROMATIN.

site of H2A, serine 129, caused a moderate sensitivity to DNA-damaging agents. Recently, such mutations have been shown to influence efficient repair of DSBs during replication[17]. Results from experiments by Downs and co-workers[8] indicated that the efficiency of repair by NHEJ, but not HR, dropped by about twofold in a H2A phospho-acceptor site mutant, but another report detected no changes in NHEJ in a similar mutant[18]. Due to the weak effect of this mutation, it has been difficult to pinpoint the precise function of H2A phosphorylation in DSB repair, although given its conservation, a function is likely to exist.

The impact of H2AX phosphorylation has also been examined in mammalian cells. Mouse embryonic stem (ES) cells deficient for H2AX were shown to be sensitive to the induction of DSBs by ionizing radiation, and exhibit genomic instability and enhanced susceptibility to cancer[19–22]. Several other studies in mammalian cells implicate H2AX in both NHEJ and HR, and a recent report argues that H2AX phosphorylation favours a HR pathway for repair in which sister chromatids are used as a template[23]. Given that the phosphorylation of H2A(X) occurs rapidly after DNA damage induction, it was proposed that phosphorylated H2A(X) might facilitate the recruitment of DNA repair proteins to the site of damage. Indeed, indirect immunofluorescence and live fluorescence microscopy studies in mammalian cells show that the loss of H2AX or the H2A(X) phospho-acceptor site suppresses the formation of DSB-induced foci of DNA repair/checkpoint proteins such as NBS1, BRCA1 and 53BP1/Crb2 (BOXES 1,3)[24,25]. However, microscopy data also indicate that the initial recruitment of these proteins to lesions was not affected. Therefore, it was proposed that H2AX phosphorylation promotes the retention and accumulation of DNA repair proteins at sites of damage, without serving as the primary recognition site.

BROMODOMAIN
An evolutionary conserved protein domain that can bind to acetylated residues of histones.

SANT DOMAIN
An evolutionary conserved protein domain that is important for DNA and histone-tail binding.

CHROMODOMAIN
An evolutionary conserved protein domain that can bind to methylated residues of histones.

EUCHROMATIN
Decondensed regions of chromatin usually associated with active transcription.

HISTONE ACETYLTRANSFERASE (HAT). An enzyme that adds acetyl groups to lysine or arginine residues of a histone.

HISTONE DEACETYLASE (HDAC). An enzyme that removes acetyl groups from lysine or arginine residues of a histone.

Phospho-H2A recruits histone modifiers

Recent work in yeast has addressed the function of histone H2A phosphorylation at sites near DSBs. Downs and co-workers showed that a peptide corresponding to the histone H2A C terminus containing a phosphorylated serine residue interacts with the NuA4 HISTONE ACETYLTRANSFERASE complex *in vitro*. This interaction seems to depend on actin-related protein 4 (Arp4), a subunit of NuA4 and the ATP-dependent chromatin-remodelling complexes INO80 and SWR1 (REFS 26–29; FIG. 3). ChIP analysis showed the binding of the NuA4 subunits Arp4 and Eaf1 and the acetylation of histone H4 (H4 Ac-Lys8) at sites near an HO-induced DSB[30,31] (FIG. 2a,b). However, the *in vivo* importance of the Arp4–phospho-H2A interaction for these events is not known, as it has not yet been shown that *tel1 mec1* or H2A phospho-acceptor mutants that eliminate H2A phosphorylation affect the binding of NuA4 and H4 acetylation activity near the DSB. The NuA4 complex acetylates the first four lysine residues in the N-terminal tail of histone H4 in yeast. Consistently, lysine substitution mutations in the N terminal H4 tail, deletion of the H4 N terminus, or mutations in the NuA4 subunits Esa1 or Yng2, render cells hypersensitive to DSB-inducing agents[30–32]. Taken together, these results strongly indicate a function for NuA4 and histone H4 acetylation in DSB repair, presumably to 'open' or 'loosen' compact nucleosomal structures close to sites of damage.

Jazayeri *et al.*[33] recently showed that deletion of Sin3, a component of the Sin3–Rpd3 HISTONE DEACETYLASE complex, also renders cells defective in the NHEJ repair pathway. Whereas ChIP analyses did not show the binding of Sin3 near an HO-induced DSB, it did show that H4 becomes deacetylated (H4 Ac-K16) near the break in a Sin3-dependent manner[33] (FIG. 2a–c). The occurrence of NuA4- and Sin3-dependent acetylation and deacetylation of H4, respectively, at lesions implies that the acetyl group turnover on the histone H4 N terminus is important for proper repair of DSBs, just as acetylation and deacetylation events cooperate to promote transcription[34].

Phospho-H2A recruits chromatin remodellers

Histone tail modification is one of two key mechanisms that alter chromatin structure. In addition to these covalent modifications, ATP-dependent chromatin remodelling (BOX 2) significantly alters nucleosome positioning, as well as histone composition by selective replacement. Several recent studies have addressed whether ATP-dependent chromatin remodelling has a role in the cellular response to DSBs. Two conserved members of the SWI2/SNF2 superfamily of ATP-dependent chromatin-remodelling complexes, INO80 and SWR1, have been characterized in budding yeast

Figure 1 | **Histone modifications that are implicated in the DNA damage response.** The four core histones H2A, H2B, H3 and H4 and two H2A variants, H2AX (mammals) and H2Av (*Drosophila melanogaster*) possess a histone-fold domain (HFD; dark blue) and have N- and C-terminal tails that contain residues that are important for the DNA damage response. The C-terminal residues of H2AX and H2Av are almost identical to those of yeast histone H2A and contain the conserved SQ motif of which the serine residue is phosphorylated in response to DNA damage (S129 in yeast H2A, S139 in H2AX and S137 in H2Av). H2Av also becomes acetylated on K5 in response to DNA damage, which, in concert with S137 phosphorylation, leads to histone exchange with unmodified H2Av. Although mutations in the yeast H2A residues S122 and T126 render cells sensitive to DNA damage, it has yet to be established whether these residues become phosphorylated in response to DNA damage. Phosphorylation of S14 of mammalian histone H2B occurs in response to DNA damage and has been associated with apoptosis. However, in yeast, phosphorylation of H2B occurs on S10. Although this event has been associated with hydrogen peroxide (H_2O_2)-induced apoptosis, it is unclear whether H2B S10 is phosphorylated in response to DNA damage. Ubiquitylation of H2B K123 and the subsequent methylation of H3 K79 are required for efficient checkpoint activation in the presence of DNA damage in yeast. Similarly, H3K79 methylation in mammalian cells is important for the recruitment of 53BP1 to sites of DNA damage, although it is not clear if this also contributes to checkpoint activation. In fission yeast, H4 K20 methylation is required for the recruitment of the 53BP1 homologue Crb2 to sites of damage, and this was shown to be necessary for checkpoint kinase activation. Furthermore, both the phosphorylation of histone H4 S1 and the acetylation of histone H4 on K5, K8, K12 and K16 are implicated in the repair of DNA DSBs.

and were both shown to contain the RuvB-like proteins Rvb1 and Rvb2 (REFS 26–29; FIG. 3). In bacteria, RuvB is involved in branch migration of Holliday junctions during DNA repair by recombination[35], and the presence of the Rvb subunits in these complexes was proposed to implicate INO80 and SWR1 in DNA repair. Indeed, yeast cells that lack either a functional INO80 or SWR1 complex displayed hypersensitivity to genotoxic agents that induce DSBs[27–29].

Given that both INO80 and SWR1 affect the transcription of hundreds of genes[26,34], it was necessary to determine whether the observed hypersensitivity was a direct or indirect effect of their remodelling function. ChIP studies confirmed the direct recruitment of the Ino80 catalytic subunit, as well as that of several actin-related protein subunits (for example, Arp5 and Arp8), to an HO-induced DSB (FIG. 2a–c). Importantly, the recruitment decreased by about threefold when the phosphorylation of H2A at the DSB was compromised. Furthermore, INO80 recruitment to the break was impaired in strains that lacked both the ATM and ATR homologues, Tel1 and Mec1, and in a H2A phospho-acceptor mutant[30,36,37].

Biochemical analysis has shown a physical interaction between the INO80 complex and phospho-H2A. The INO80 subunit Nhp10 was required for both the INO80–phospho-H2A interaction *in vitro* and the recruitment of INO80 to a DSB *in vivo*[37]. However, one of the INO80 actin-related proteins, Arp4, was shown to physically associate with phospho-H2A[30]. To reconcile the Nhp10-dependent INO80 recruitment to phospho-H2A at DSBs with the reported Arp4–phospho-H2A binding affinity, we propose that both Arp4 and Nhp10 are required for efficient INO80–phospho-H2A interaction. For example, Nhp10 might facilitate Arp4–phospho-H2A binding. Moreover, because INO80 complexes isolated from *nhp10* mutants lack both the Nhp10 and Ies3 (INO eighty subunit 3) subunits (FIG. 3), Ies3 might also facilitate interactions between INO80 and phospho-H2A[37] (FIG. 2c,d).

In addition to the recruitment of Ino80, Arp5 and Arp8 to DSBs, ChIP experiments showed the recruitment of Arp4 and Rvb1 (REFS 30,36,37) — subunits that are shared between the INO80 and SWR1 complexes (FIG. 3). It remains unclear whether this indicates that the Swr1 ATPase is recruited, as neither Swr1 itself nor any other SWR1-specific subunit was monitored. Intriguingly, in contrast to the INO80 complex, the SWR1 complex has a higher affinity for the histone variant Htz1 than for phospho-H2A[26,28,29,37], which suggests that the two complexes are likely to have different roles in DNA repair, even if both are recruited to sites of damage.

If both the SWR1 and INO80 remodellers are recruited to DSBs, what do they do? It was recently shown that yeast strains that lack INO80 chromatin-remodelling activity are less efficient at converting double-stranded DNA into single-stranded DNA at DSB ends[36]. This suggests that chromatin remodelling facilitates the access of end-processing enzymes (FIG. 2d). End-processing is a prerequisite for the initiation of homologous recombination and, indeed, in *Arabidopsis thaliana*, it was shown that INO80 is required for recombinational repair of DSBs[38].

Interest in the SWR1 complex has been stimulated by its similarity to a related mammalian complex known as TIP60. TIP60 has both acetylation and ATPase activities, and contains a number of subunits homologous to subunits of SWR1 and NuA4 (REFS 39,40; FIG. 3). A recent study in *Drosophila melanogaster* showed that TIP60 binds to and acetylates phosphorylated H2Av, an H2AZ-like histone variant that, similar to H2AX, becomes phosphorylated on its C-terminal

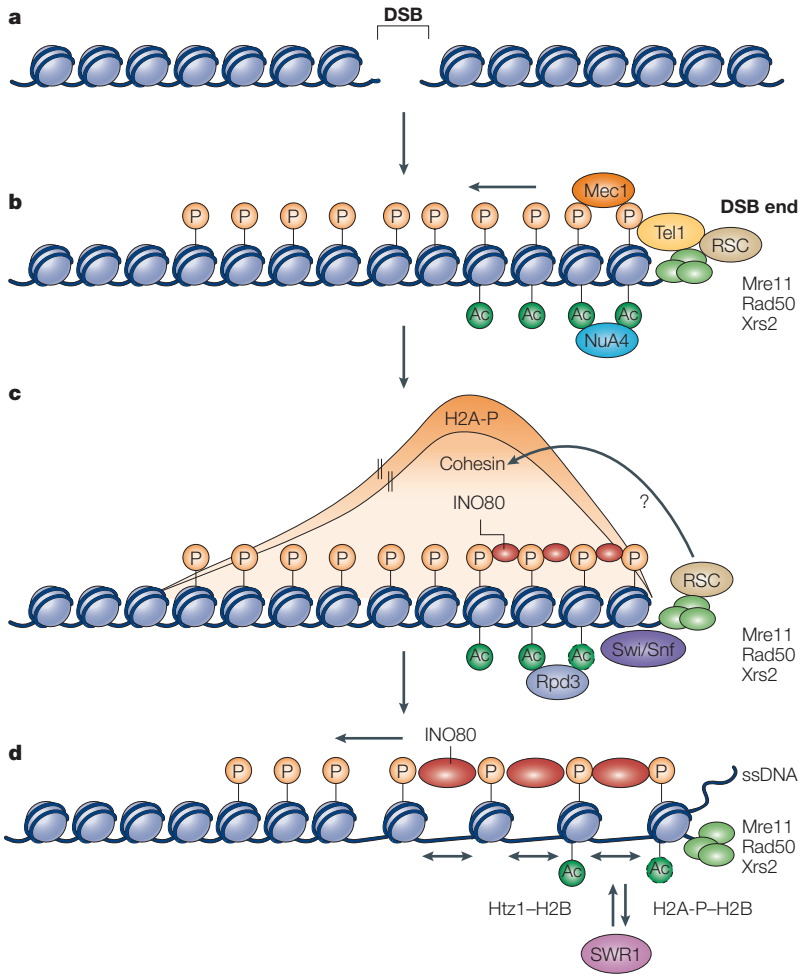


Figure 2 | **A model for the function of phosphorylated H2A at DNA double-strand breaks in budding yeast. a** | Induction of a chromosomal DNA double-strand break (DSB). **b** | The ends of the DSB are rapidly bound by the Mre11–Rad50–Xrs2 (MRX) complex, which might facilitate the recruitment of the Tel1 kinase. Mec1 kinase recruitment requires the presence of its binding partner Ddc2 (not shown). Tel1 and Mec1 phosphorylate H2A over a ~50-kb region, an event that is followed by recruitment of the histone acetyltransferase NuA4 and the subsequent acetylation of histone H4 tails. Mre11 also recruits the ATP-dependent chromatin-remodelling complex RSC. **c** | Presumably, the histone deacetylase Rpd3 is recruited to sites near a break to deacetylate histone H4 tails. MRX and phosphorylated H2A are required for the formation of a ~50-kb cohesin domain that overlaps with the phospho-H2A region. The *de novo* loading of cohesin near the break site, which might itself be facilitated by RSC, facilitates repair of the DSB by sister chromatid recombination (BOX 1). Phosphorylated H2A also leads to the recruitment of the ATP-dependent chromatin-remodelling complexes INO80 and, presumably, SWR1. The ATP-dependent chromatin-remodelling complex SWI/SNF is recruited through an unknown mechanism. Apparently, SWI/SNF helps recruit Rad51 and Rad52 (not shown), thereby facilitating homologous recombination. **d** | INO80 could alter the position of nucleosomes to facilitate the formation of single-stranded DNA by either the MRX complex, the Exo1 nuclease (not shown) or another, as yet unknown, nuclease. SWR1 (or INO80) may also catalyse the exchange of phosphorylated H2A for Htz1 or vice versa.

HETEROCHROMATIN
Condensed regions of chromatin usually associated with the repression of transcription and late replication.

serine 137 residue in response to DNA damage[41] (FIG. 1). Subsequently, TIP60 seems to drive the exchange of phosphorylated and acetylated H2Av with unmodified H2Av in chromatin. In line with this, it was shown that phospho-H2Av accumulates and persists after induction of DSBs in cells that lack a functional TIP60 complex[42]. In yeast, SWR1 was shown to regulate gene expression and the spread of HETEROCHROMATIN by catalysing the exchange of normal H2A with the histone variant Htz1 (H2AZ in mammals), leading to Htz1 incorporation into chromatin[26,28,29]. It is tempting to speculate that SWR1, like TIP60, in concert with NuA4, might exchange phospho-H2A with Htz1 in chromatin near a DSB (FIG. 2d).

In yeast, mutations in the histone acetyltransferase Esa1, which is part of NuA4, not only reduced H4 acetylation, but also the recruitment of the INO80 subunit Rvb1 to a DSB[30]. Given that INO80 also shares subunits with TIP60 (FIG. 3), one could speculate that the yeast INO80, SWR1 and NuA4 complexes work together to facilitate Htz1 or H2A-P exchange at lesions, mimicking the larger TIP60 complex in mammalian cells. Further studies will be required to determine the exact division of labour among these related chromatin remodellers and the end result of their activity.

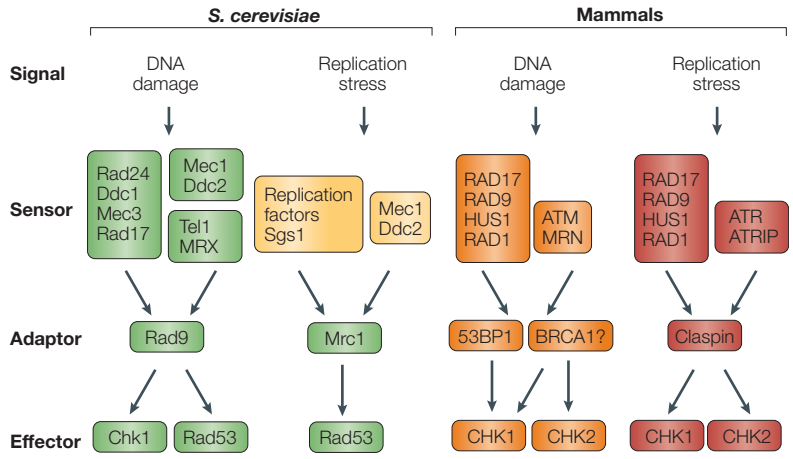
Phospho-H2A-independent recruitment

INO80 and SWR1 are not the only members of the SWI2/SNF2 superfamily of chromatin-remodelling complexes that are implicated in the DNA damage response. Recent reports showed that mutations in subunits of the SWI/SNF (Snf2 and Snf5) and RSC (Rsc1, Rsc2, Sfh1 and Sth1) chromatin-remodelling complexes also render cells hypersensitive to genotoxic agents that induce DSBs[43–46]. Although SWI/SNF is required for the DNA-damage-regulated expression of the ribonucleotide reductase subunit Rnr3, proper expression of which is important for cell survival after DNA damage, mutations in subunits of SWI/SNF or RSC did not lead to misregulation of DNA repair genes[43,47,48]. This would implicate SWI/SNF and RSC directly in DNA repair. Indeed, the SWI/SNF subunit Snf5 and the RSC subunits Rsc8 and Sth1 (the catalytic subunit of RSC) were shown to be recruited to sites near an HO-induced DSB[43,46] (FIG. 2a–c).

The DNA repair proteins Mre11 and Ku70 are recruited with the same kinetics as the RSC subunits Rsc8 and Sth1, and Sth1 recruitment appeared to depend on the presence of Mre11, Ku70 and the RSC subunits Rsc2 and Rsc30 (REFS 9,14,43,46; FIG. 2a–c). Consistently, physical interaction was shown between the RSC subunit Rsc1 and the Mre11 and Ku80 proteins[43]. These results suggest that RSC recruitment, in contrast to that of INO80, does not require H2A phosphorylation or H4 acetylation. The requirements for Snf5 binding are not yet known, and it remains to be examined whether histone tail modifications or DNA repair proteins influence its recruitment.

Mre11 is involved in both HR and NHEJ, whereas the function of Ku80 is unique to NHEJ. The interaction between subunits of RSC and these two repair proteins implicates RSC in both HR and NHEJ, which has been confirmed by two recent reports[43,46]. In addition, Chai *et al.*[46] reported that SWI/SNF, like RSC, has an important role in HR. However, SWI/SNF and RSC seemed to have distinct roles in HR. SWI/SNF is required before or at the strand invasion

Box 3 | **Checkpoints and genome stability**



The aim of dividing cells is to copy and transmit their genetic material in an error-free manner to their offspring. Cells can otherwise accumulate mutations and encounter genetic instability, which in higher eukaryotes can lead to diseases such as cancer. To prevent this, cells possess control mechanisms, known as checkpoints, which delay cell cycle progression in response to DNA damage or replication stress. This delay in cell cycle progression allows cells to repair DNA damage or deal with replication problems and consequently prevents the transmission of mutations, thereby preserving genome stability. The figure summarizes our current knowledge of DNA damage and replication checkpoints in yeast and mammalian cells. Genetic and biochemical analysis of checkpoint pathways has led to the identification of three different classes of checkpoint proteins. Sensor proteins detect different types of DNA damage or replication problems and activate adaptor kinases that transduce a signal to effector kinases. Effector kinases regulate the activity of a number of downstream targets, including those that delay cell-cycle progression and induce the expression of DNA repair genes. *S.cerevisiae, Saccharomyces cerevisiae.*

step, probably to facilitate the recruitment of Rad51 and Rad52, whereas RSC is required once extension of the invading strand has occurred (BOX 1). Additional studies are needed to elucidate which mechanisms regulate the recruitment of these different chromatin-remodelling complexes to sites of damage to orchestrate DNA repair events during HR and NHEJ.

Phospho-H2A recruits cohesin

Sister chromatids become physically linked during S phase by cohesin, a complex consisting of Scc1, Scc3 and two structural maintenance of chromosome (Smc) proteins, Smc1 and Smc3. Not only is the establishment of cohesion crucial for accurate chromosome segregation in mitosis, but loss of cohesion during S phase also leads to defects in post-replicative DNA repair[49]. The human cohesin subunits physically interact with Rad50, a member of the Mre11–Rad50–Nbs1 (MRN, or MRX in yeast) repair complex, and accumulate at sites of laser-induced DNA damage in a Mre11-dependent manner[50].

Two recent studies in yeast have used ChIP to examine the recruitment of cohesin subunits to HO-induced DSBs, and showed that, in G2-phase cells, cohesin becomes enriched throughout a ~50-kb domain around the DSB[51,52]. Remarkably, this cohesin-rich domain showed extensive overlap with the region that contained phospho-H2A, which suggests a link between H2A phosphorylation and cohesin loading at lesions. Indeed, the loading of cohesin around a DSB requires the phosphorylation of histone H2A by Mec1 and Tel1, and the presence of the DNA repair protein Mre11 (REF. 51; FIG. 2a–c). Similar to cohesin loading during a normal S phase, this damage-linked spread of cohesin is dependent on the presence of a functional loading protein, Scc2 (REFS 51,52). Finally, cohesin was shown to facilitate DNA repair, presumably by maintaining sister chromatids in close proximity for post-replicative recombination (BOX 1). We conclude that phospho-H2A has more than one role at DSBs — it may first help recruit remodellers and, second, facilitate the loading and/or spreading of cohesin.

The roles of chromatin remodellers and of cohesin could be linked even further, because cohesin loading along chromosome arms is influenced by the RSC chromatin-remodelling complex[53]. RSC subunits physically interact with Mre11, and recruitment of RSC to sites of damage depends on the presence of Mre11 (REF. 43). It is probable, therefore, that RSC also affects the loading or spreading of cohesin at sites of damage. This would implicate RSC in post-replicative recombination and repair.

Multiple mutations in H2A affect repair functions

Residues other than serine 129 in the C-terminal tail of yeast H2A have recently been shown to be important for the survival of cells in the presence of DNA-damage-inducing agents. Besides S129, the C-terminal 11 residues of H2A contain other potential phosphorylation targets, namely residues S122 and T126 (FIG. 1). Replacement of S122 with an alanine residue renders yeast cells hypersensitive to genotoxic agents that induce DNA breaks. The S122A mutant was also sensitive to the induction of HO-endonuclease-induced DSBs that are repaired by either HR or NHEJ[54]. These results suggest a role for S122 in the repair of DSBs that is different from that of S129, because the S122A S129A double mutant is more sensitive to DNA damage than either single mutant[54]. Although there is evidence that S122 can become phosphorylated *in vivo*, it remains unclear whether S122 phosphorylation occurs in response to DNA damage, nor is it known which kinase mediates the modification[18,54].

The function of T126 in the response to DNA damage has also been investigated. When T126 was replaced with an alanine residue, this mutation rendered cells sensitive to DNA damage by affecting DSB repair by NHEJ, according to one report[18]. On the other hand, Harvey *et al.*[54] showed that this replacement had no effect on cell survival in the presence of break-inducing agents. As there is no evidence for the phosphorylation of T126 in response to DNA damage, it remains unclear what the role of this residue is.

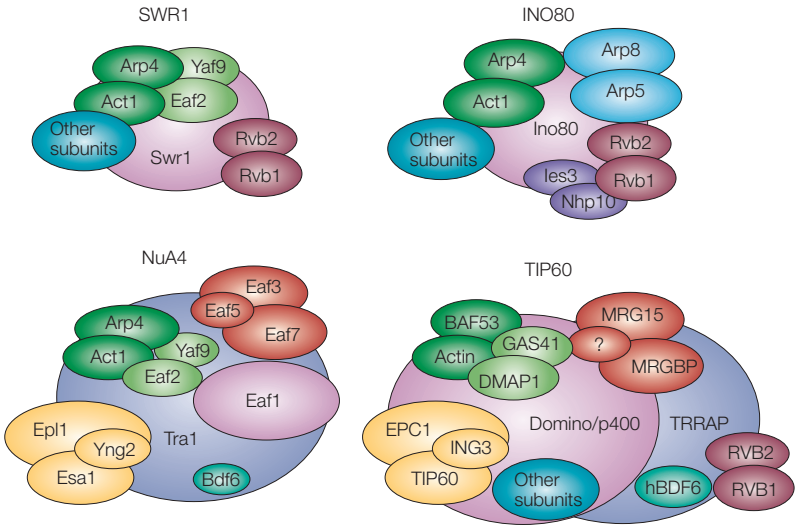


Figure 3 | **Composition of the *Saccharomyces cerevisiae* SWR1, INO80, NuA4 and human TIP60 complexes.** Conserved subunits of the *S. cerevisiae* SWR1, INO80, NuA4 and human TIP60 complexes are colour coded. All the complexes, except NuA4, contain catalytic subunits that are related to the yeast SWI2/SNF2 ATPase (BOX 2). The ATPase subunits in the INO80, SWR1 and human TIP60 complexes are Ino80, Swr1 and Domino/p400, respectively. The catalytic subunit of NuA4 is the histone acetyltransferase Esa1, which has its counterpart in TIP60 that is contained in the human TIP60 complex. As well as the homologues Esa1 and TIP60, the NuA4 and human TIP60 complexes also share Tra1, Epl1, Yng2, Eaf3, Eaf7 and Bdf6 (homologues in TIP60 are TRRAP, EPC1, ING3, MRG15, MRGBP and BDF6, respectively). Eaf5 might be present in both complexes. All four complexes contain actin (Act1) and Arp4 (actin-related protein 4), which is homologous to human BAF53. SWR1, NuA4 and human TIP60 possess the Eaf2 and Yaf9 subunits, which are the homologues of human DMAP1 and GAS41, respectively, in TIP60. SWR1, INO80 and human TIP60 contain Rvb1 and Rvb2, which are related to the bacterial RuvB helicase. Arp5, Arp8, Nhp10 and Ies3 are subunits that are unique to INO80. Eaf1 is a NuA4 subunit that has homology with the Swr1 and Domino/p400 ATPase subunits. However, Eaf1 does not contain an ATPase SWI2/SNF2-related ATPase domain. Because NuA4, SWR1 and INO80 complexes share many subunits with human TIP60, it was proposed that human TIP60 is a hybrid of at least two and possibly all three *S. cerevisiae* complexes.

Modification of histone H2B, H3 and H4

The fact that the phosphorylation of histone H2AX and the acetylation of histone H4 both have important roles in the response to DNA DSBs indicates that DNA-damage-induced histone modifications are not restricted to one type of histone nor to one type of modification.

H2B. Fernandez-Capetillo and co-workers recently reported that in mammalian cells that had been exposed to ionizing radiation or laser treatment, histone H2B becomes phosphorylated on residue serine 14 (REF. 55; FIG. 1). Even though the kinetics of H2B S14 phosphorylation was comparable to that of H2AX S139 phosphorylation, H2B S14-P assembled into foci much later than H2AX S139-P. Although the phosphorylation of H2B S14 was not affected by the loss of H2AX, the formation of H2B S14-P foci was lost in H2AX-deficient cells. Phosphorylation of H2B at serine 14 by the mammalian sterile 20 kinase (MST1) has been closely associated with apoptosis in multicellular eukaryotes[56]. Recently, this finding was extended to unicellular eukaryotes by demonstrating that, in yeast, sterile 20 kinase (Ste20) phosphorylates histone H2B on serine 10 during hydrogen peroxide (H_2O_2)-induced apoptosis[57] (FIG. 1). It will be of interest to know whether Ste20 and MST1 phosphorylate H2B in response to DNA damage, and whether this modification facilitates repair.

Another modification that was found to be important in the cellular response to DNA damage in budding yeast is the ubiquitylation of H2B on lysine 123 by the Rad6–Bre1 complex (comprising the E2 ubiquitin-conjugating enzyme Rad6 and the E3 ubiquitin ligase Bre1)[58]. In the presence of DNA damage, *rad6*, *bre1* and H2B K123A mutants showed impaired activation of the central checkpoint kinase Rad53, most probably due to defects in Rad9 phosphorylation (BOX 3). Studies on gene silencing in *Saccharomyces cerevisiae* have shown that methylation of histone H3 on lysine 79 by the HISTONE METHYLTRANSFERASE Dot1 is dependent on the ubiquitylation of H2B lysine 123 (REF. 59). Interestingly, *dot1* and H3 K79A mutants also displayed Rad9-dependent defects in the activation of Rad53. Because Dot1 influences transcription[60], it is unclear whether these effects are direct or indirect. Nonetheless, it remains possible that ubiquitylation of H2B lysine 123 by Rad6–Bre1 and methylation of H3 lysine 79 might function together to activate the checkpoint response to DNA damage (BOX 3).

H3 and H4. The model discussed above arises in part from a study in mammalian cells by Huyen *et al.*[61] The structure of 53BP1, a p53-binding protein that functions in checkpoint activation, contains two tandem TUDOR DOMAINS comprising residues that are conserved in the budding yeast Rad9 and fission yeast Crb2 orthologues (BOX 3). *In vitro*, the Tudor domains were shown to be essential for binding histone H3 that is methylated on lysine 79. RNA interference experiments showed that reduced levels of the K79 histone methyltransferase Dot1 impaired the formation of ionizing-radiation-induced 53BP1 foci[56]. As the global levels of methylated H3K79 did not change in response to DNA damage induction, a model was proposed in which local changes in chromatin near DNA breaks reveal methylated H3K79 in order to recruit 53BP1 to lesions[56].

A similar mechanism has evolved in fission yeast for the recruitment of Crb2, a checkpoint adaptor protein with homology to 53BP1 (BOX 3). In fission yeast, however, the methylation of histone H4 lysine 20 by a newly identified histone methyltransferase, known as Set9, seems to control the recruitment of Crb2 to sites of damage[62] (FIG. 1). The lack of Set9 or the H4 lysine 20 methyl-acceptor residue renders cells hypersensitive to DNA-damaging agents and impairs activation of the checkpoint protein Chk1 (BOX 3). However, in fission yeast the phosphorylation of H2A on S129, which is mediated by the ATR and ATM homologues Rad3 and Tel1, also influences the recruitment of Crb2 to sites of damage and the activation of the checkpoint kinase Chk1 (REF. 25). In summary, both the methylation of histone H4 on lysine 20 and the phosphorylation of H2A on serine 129 provide

HISTONE METHYLTRANSFERASE
An enzyme that adds methyl groups to lysine or arginine residues of histones.

TUDOR DOMAIN
An evolutionary conserved chromodomain-like protein domain that can bind to methylated residues of histones.

mechanisms for the recruitment of Crb2 to DNA damage and ensure proper checkpoint activation in response to DNA lesions.

Mutational studies of histone H3 residues have implicated residues other than lysine 79 in the response to DNA damage in yeast[63]. Alteration of lysine residues 14 and 23, as well as mutation of the histone acetyltransferase Hat1, renders cells sensitive to DNA-damaging agents (FIG. 1). A more detailed analysis showed that such mutants were defective in HR, but not in NHEJ, which indicates that Hat1 acetylation of lysine residues in the N-terminal part of H3 is important for recombinational repair of DNA DSBs.

In addition to H3 acetylation, a novel modification within the N-terminal histone H4 tail, namely the phosphorylation of serine 1, was reported to occur in response to DSBs[64] (FIG. 1). ChIP analysis showed that H4 S1-P is specifically enriched near HO-induced DSBs. The kinase that mediates this phosphorylation was identified as casein kinase-2 (CK2), and genetic studies suggest that CK2-dependent phosphorylation of H4S1 might be involved in the NHEJ pathway of DSB repair. However, whereas CK2-deficient cells are sensitive to damaging agents, cells that express a form of H4 that lacks the S1 phospho-acceptor site (H4S1A) are not. This could indicate that although H4 S1 phosphorylation occurs, its function can be replaced by other events in the CK2-dependent pathway for DNA repair.

Concluding remarks

Here, we have discussed recent advances that link chromatin modifications with the repair of DSBs. One of the key events in the response to DNA damage seems to be the phosphorylation of histone H2A in yeast or the histone variant H2AX in mammals. This phosphorylation event leads to the subsequent recruitment of histone acetyltransferases, ATP-dependent chromatin remodellers and the cohesin complex to DSBs, and promotes the accumulation of checkpoint and repair proteins at these sites. Future studies will be needed to show whether the chromatin remodelling complexes SWR1 and INO80 catalyse histone exchange at sites of damage, and whether or not these work together with the histone acetyltransferase NuA4. Understanding these events should reveal how remodelling complexes alter chromatin structure near lesions, and if this favours a specific repair pathway. It remains to be clarified whether there is an overlap in the roles of SWR1 and INO80 complexes, and what function Htz1, the H2A variant deposited by SWR1 might have. Finally, the roles of the RuvB-like subunits, Rvb1 and Rvb2, which are present in the INO80 and SWR1 complexes, as well as TIP60, remain to be determined.

We note that, unlike INO80, RSC recruitment to a DSB requires Mre11 and Ku70, but apparently not the phosphorylation of H2A. This could place RSC recruitment upstream of, or parallel to, that of INO80. The function of these remodelling complexes at sites of DNA damage might diverge sharply, as INO80 seems to be involved in DNA end-processing and RSC in cohesin loading. As the recruitment of these remodellers occurs through different mechanisms, an important goal will be to elucidate how these events are coordinated to facilitate proper DSB repair.

Finally, it remains unclear what impact the many other histone modifications have on the cellular response to DNA damage. The complex array of histone modifications and modifying complexes recruited to DNA damage could reflect a range of different signals embedded in chromatin itself, each helping to orchestrate the appropriate repair and checkpoint events depending on chromosomal context and cell-cycle stage. Despite rapid progress in this area, further work is needed to clarify the role of chromatin in DNA-damage signalling and repair.

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Competing interests statement
The authors declare no competing financial interests.

Online links

DATABASES
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http://www.yeastgenome.org
Act1 | Arp4 | Arp5 | Arp8 | Bdf6 | Bre1 | CK2 | Ddc2 | Eaf1 | Eaf2 | Eaf3 | Eaf5 | Eaf7 | Epl1 | Esa1 | Hat1 | Htz1 | HO | Ies3 | INO80 | Ku70 | Ku80 | Lig4 | Lif1 | Mec1 | Mre11 | Nhp10 | Rad6 | Rad9 | Rad50 | Rad51 | Rad52 | Rad53 | Rad54 | Rad57 | Rad59 | Rpd3 | Rvb1 | Rvb2 | Scc1 | Scc2 | Scc3 | Sin3 | Smc1 | Smc3 | Ste20 | SWR1 | Tel1 | Tra1 | Xrs2 | Yaf9 | Yng2
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Online Summary

The repair of chromosomal DNA double-strand breaks, which is essential for the maintenance of genomic stability, occurs within the context of chromatin. Histone modifications correlate with DNA damage and might therefore serve as a code for repair.

Chromatin-remodelling complexes and histone-modifying enzymes are recruited to the sites of DNA damage. Histone tails and core domains also show significant damage-correlated modifications, including acetylation, deacetylation, methylation, phosphorylation and ubiquitylation.

Histone H2A phosphorylation by the ATM/ATR kinase has a key role in the recruitment of the INO80 chromatin remodeller and in the loading of cohesin at sites of double-strand breaks. Cohesin loading near DNA double-strand breaks might be facilitated by both the Mre11 protein and its ligand, the chromatin remodelling complex RSC.

A histone remodeler related to INO80, the SWR1 complex, can exchange histone H2A for a variant known as Htz1. Genetics studies implicate SWR1 in DNA repair, and the analysis of a related complex in *Drosophila,* TIP60, indicates that TIP60 might replace phospho-H2Av with unmodified histone at sites of damage.

Histone acetylation and deacetylation by the NuA4 and Sin3–Rpd3 complexes, respectively, might help open compact nucleosomal fibers at the sites of DNA damage. Roles for other covalent modifications of histones in facilitating repair are suggested but not definitively proven.

Histone H3K79 and H4K20 methylation is important to allow the recruitment of the checkpoint adaptor proteins 53BP1 (mammals) and Crb2 (fission yeast), respectively, to the sites of damage. This links histone modification with checkpoint activation, as the adaptors seem to bind at the sites of damage to stimulate checkpoint effector kinase activation.

Online links

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CK2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=cka1
Ddc2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=ddc2
Eaf1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=eaf1
Eaf2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=eaf2
Eaf3
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=eaf3
Eaf5
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=eaf5
Eaf7
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=eaf7
Epl1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=epl1
Esa1
http://db.yeastgenome.org/cgi-bin/locus.

pl?locus=esa1
Hat1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=hat1
Htz1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=htz1
HO
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=ho
Ies3
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=ies3
INO80
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=ino80
Ku70
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=yku70
Ku80
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=YMR106C
Lig4
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=lig4
Lif1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=lif1
Mec1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=mec1
Mre11
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=mre11
Nhp10
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=nhp10
Rad6
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad6
Rad9
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad9
Rad50
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad50
Rad51
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad51
Rad52
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad52
Rad53
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad53
Rad54
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad54
Rad57
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad57
Rad59
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rad59
Rpd3
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rpd3
Rvb1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rvb1
Rvb2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=rvb2
Scc1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=scc1
Scc2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=scc2
Scc3
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=scc3
Sgs1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=sgs1
Sin3
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=sin3
Smc1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=smc1
Smc3
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=smc3
Ste20
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=ste20
SWR1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=swr1
Tel1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=tel1
Tra1
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=tra1
Xrs2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=xrs2
Yaf9
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=yaf9
Yng2
http://db.yeastgenome.org/cgi-bin/locus.pl?locus=yng2

Swiss-Prot:

http://www.expasy.ch/sprot/
ATM
http://au.expasy.org/cgi-bin/niceprot.pl?Q13315
ATR
http://au.expasy.org/cgi-bin/niceprot.pl?Q13535
53BP1
http://au.expasy.org/cgi-bin/niceprot.pl?Q12888
BAF53
http://au.expasy.org/cgi-bin/niceprot.pl?O96019
BRCA1
http://au.expasy.org/cgi-bin/niceprot.pl?P38398
Chk1
http://au.expasy.org/uniprot/P34208
Crb2
http://au.expasy.org/cgi-bin/niceprot.pl?P87074
DMAP1
http://au.expasy.org/cgi-bin/niceprot.pl?Q9NPF5
DNA-PKcs
http://au.expasy.org/cgi-bin/niceprot.pl?P78527
Domino/p400
http://au.expasy.org/cgi-bin/niceprot.pl?Q96L91
EPC1
http://au.expasy.org/cgi-bin/niceprot.pl?Q9H2F5
GAS41
http://au.expasy.org/cgi-bin/niceprot.pl?O95619
H2AX
http://cn.expasy.org/cgi-bin/niceprot.pl?P16104
H2AZ
http://us.expasy.org/cgi-bin/niceprot.pl?P17317
ING3
http://au.expasy.org/cgi-bin/sprot-search-de?ING3
MRG15
http://au.expasy.org/cgi-bin/niceprot.pl?Q9UBU8
MRGBP
http://au.expasy.org/cgi-bin/niceprot.pl?Q9NV56
MST1
http://au.expasy.org/cgi-bin/niceprot.pl?Q13043
NBS1
http://au.expasy.org/cgi-bin/niceprot.pl?O60934
p53
http://au.expasy.org/cgi-bin/niceprot.pl?P04637
RuvB
http://au.expasy.org/cgi-bin/niceprot.pl?Q8FGR3
Set9

http://au.expasy.org/cgi-bin/niceprot.pl?Q8WTS6
TIP60
http://au.expasy.org/cgi-bin/niceprot.pl?Q92993
TRRAP
http://au.expasy.org/cgi-bin/niceprot.pl?Q9Y4A5
XRCC2
http://au.expasy.org/cgi-bin/niceprot.pl?O43543
XRCC3
http://au.expasy.org/cgi-bin/niceprot.pl?O43542
XRCC4
http://au.expasy.org/cgi-bin/niceprot.pl?Q13426
Susan Gasser's laboratory:
http://www.fmi.ch/html/research/research_groups/epigenetics/susan_gasser/susan_gasser.html