Regulation of DNA repair throughout the cell cycle

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Abstract | The repair of DNA lesions that occur endogenously or in response to diverse genotoxic stresses is indispensable for genome integrity. DNA lesions activate checkpoint pathways that regulate specific DNA-repair mechanisms in the different phases of the cell cycle. Checkpoint-arrested cells resume cell-cycle progression once damage has been repaired, whereas cells with unrepairable DNA lesions undergo permanent cell-cycle arrest or apoptosis. Recent studies have provided insights into the mechanisms that contribute to DNA repair in specific cell-cycle phases and have highlighted the mechanisms that ensure cell-cycle progression or arrest in normal and cancerous cells.

Replication fork
The branch-point structure that forms during DNA replication between the two template DNA strands where nascent DNA synthesis is ongoing.

Cyclin-dependent kinases
A group of serine/threonine protein kinases that are activated at specific points during the cell cycle, together with their regulatory cyclin subunits. They regulate cell-cycle transitions by inducing degradation of cell-cycle inhibitory proteins.

Apoptosis
A form of programmed cell death that is well defined in multicellular organisms.

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doi:10.1038/nrm2351
Published online
20 February 2008

The genome is under constant threat of damage from exogenous agents that damage DNA. Endogenous processes can also induce DNA damage; for example, hydrolysis leads to spontaneous DNA depurination; reactive oxygen species (ROS) induce base oxidation and DNA breaks; replication defects can cause mismatches; and replication fork collapse can result in strand breaks[1]. The resulting DNA lesions must be repaired to prevent loss or incorrect transmission of genetic information, as errors can cause developmental abnormalities and tumorigenesis. However, the choice of which repair system to use depends both on the type of lesion and on the cell-cycle phase of the cell (FIG. 1). For example, a DNA double-strand break (DSB) in S and G2 phases is readily repaired by homologous recombination (HR) using the intact sister chromatid (BOX 1). However, as cells progress into G2–M, the chromosomes are condensed in a highly ordered chromatin structure that makes homology search difficult. The coordination of the DNA-repair pathway and the cell cycle is controlled through different cell-cycle activities, such as cyclin-dependent kinases (CDKs). CDKs regulate cell-cycle transitions by inducing degradation of cell-cycle inhibitory proteins (BOX 2) and are periodically activated by their regulatory cyclin subunits, which are differentially expressed during the different cell-cycle phases[2]. Cells integrate DNA-repair processes with transcription and apoptosis in a network that is known as the DNA-damage response (DDR), which is orchestrated by checkpoint proteins. Understanding how DNA repair is modulated according to the cell-cycle phase has important applications for medicine and cancer.

Over the past decade, tremendous progress has been made in the elucidation of the mechanistic intricacies of different repair pathways, and of the spatio–temporal orchestration of DNA repair. Post-translational modifications, such as checkpoint- and CDK-dependent phosphorylation, ubiquitylation and sumoylation, were shown to be crucial for the regulation of the stability and activity of important components of the checkpoint machinery, thereby regulating important cell-cycle events. These post-translational modifications may affect the recruitment of repair proteins to damaged DNA or tune the efficiency or the specificity of the repair machinery towards a certain type of lesion, often to facilitate repair in a specific cell-cycle phase.

In this review, we describe the most important DDR pathways that operate in a eukaryotic cell and discuss their activity through the cell cycle. We then discuss the main regulatory mechanisms that affect the choice of DNA-repair pathways through the cell cycle. Last, we provide examples of how the function of different repair factors are modulated through post-translational modifications and discuss crucial questions that need to be addressed. The connections between DNA repair and other chromosome-metabolism processes, such as replication, transcription, cohesion and condensation, segregation, and DNA topology, have been discussed in other reviews[3–6].

The checkpoint-activation network

Checkpoints are cellular surveillance and signalling pathways that coordinate DNA repair with chromosome metabolism and cell-cycle transitions[7,8]. The checkpoint proteins are often recruited to DNA lesions

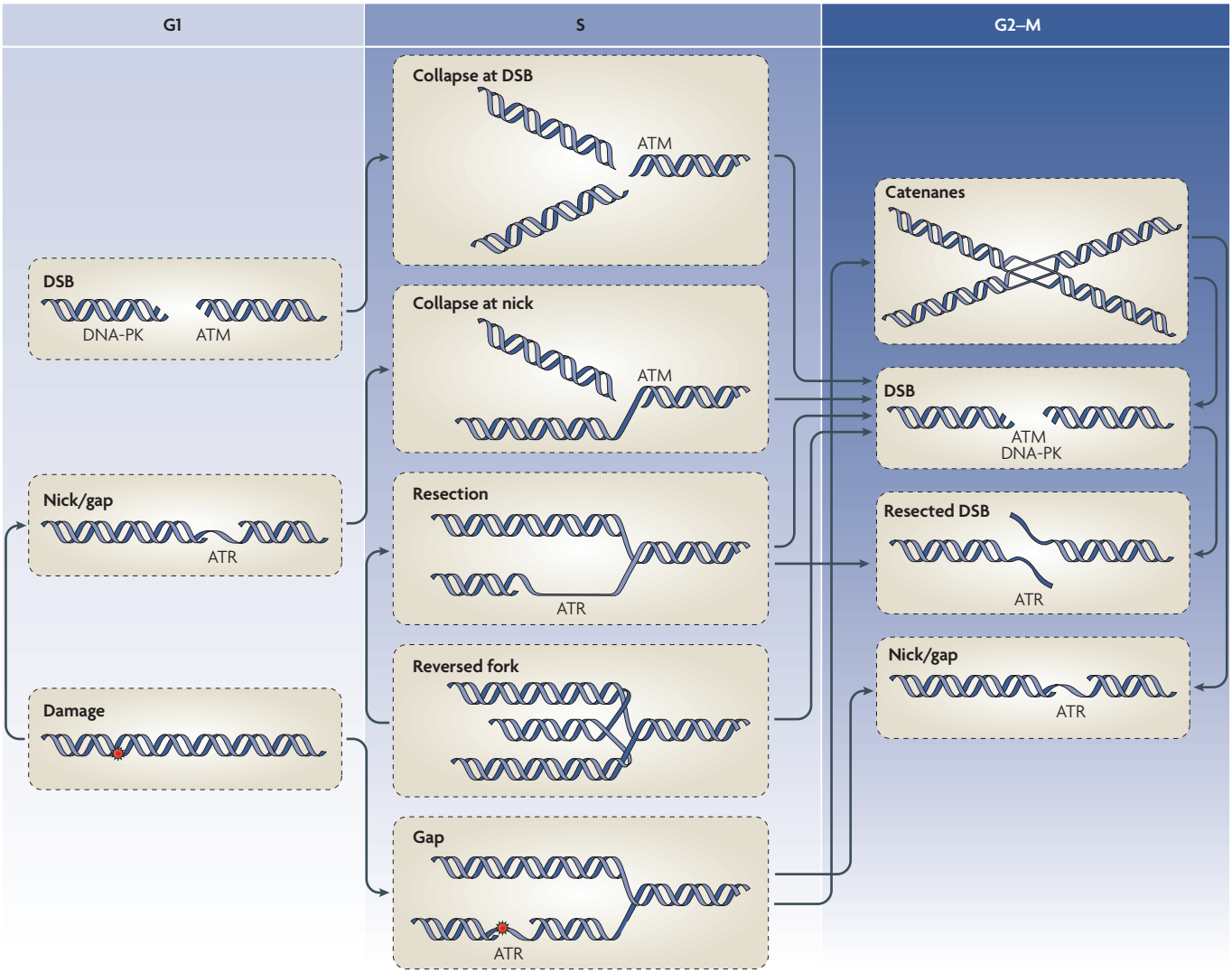


Figure 1 | **Cell-cycle-specific DNA structures and lesions and the checkpoint kinases that respond to them.** During the G1 phase, double-strand breaks (DSBs) lead to activation of the phosphoinositide 3-kinase related kinases DNA-PK and ATM, whereas other types of damage, such as ultraviolet-induced pyrimidine dimers, are processed by nucleotide-excision repair enzymes and lead to ATR activation. DSBs or nicks that are not repaired during G1 result in collapse of replication forks, which activates ATM (following DSBs resection, ATR is also activated). S phase DNA damage, such as stalled forks or gaps that are generated during replication, activate ATR. In pathological conditions — for example, when cells contains mutations in genes of the ATM–ATR pathway — accumulation of reversed forks[155,156] is processed by nucleases[14] that lead to extensive gaps or DSBs. Topological problems during replications can form catenanes, which can result in nicks or, if unresolved, can lead to DSBs during chromosome segregation.

by repair complexes that generate the intermediate DNA structures that function as signals to activate the checkpoint response. For example, nucleotide-excision repair (NER) factors are required to process lesions in budding yeast G1 cells that have been irradiated with ultraviolet (UV). By contrast, DSBs are first processed by the Mre11–Rad50–Xrs2 (MRX) complex in yeast (the MRE11–RAD50–NBS1 (MRN) complex in mammals) to generate long single-stranded (ss)DNA regions that activate the checkpoint response[9–13] (FIGS 1,2). During replication, stalled forks expose ssDNA, and the collapsed forks that are deprived of replisome[4] are processed by the exonuclease Exo1 in budding yeast to expose ssDNA[14]; when coated with replication protein A (RPA), ssDNA becomes an activating signal for the checkpoint[15] (FIGS 1,2). Following activation, the checkpoint transducers transmit and amplify the checkpoint signal to downstream targets such as the DNA-repair apparatus and the cell-cycle machinery[7]. The transmission of the signal or activation of these targets is often achieved by different phosphorylation events that affect the transcription level or activity of repair genes, and modulate cell-cycle transitions by influencing the stability or activity of other proteins that are implicated in checkpoint maintenance or cell-cycle progression (FIG. 2; BOX 2).

Stalled fork
A replication fork at which progress is blocked. Progress may be blocked by the presence of bulky lesions, aberrant DNA structures, protein–DNA complexes or depletion of dNTP pools.

Box 1 | **Cell-cycle-specific DNA-repair events**

	G1	S	G2–M
DSBs or single-strand breaks	NHEJ	HR-mediated fork restart	HR-mediated repair
Mismatches		Mismatch repair	
Bulky lesions	NER	Template-switch-mediated damage bypass	
		TLS-mediated damage bypass	

The main repair pathways that function to repair different types of DNA lesions are shown in the table. Double-strand breaks (DSBs) that occur during G1 phase are mainly repaired through non-homologous end joining (NHEJ), whereas DSBs that are formed during S and G2 phases are predominantly repaired by homologous recombination (HR) mechanisms. Mismatch repair is very important during replication to remove mismatches, or small insertion or deletion loops, that are generated by faulty replication. Nucleotide-excision repair (NER) plays an important role during G1 phase to remove bulky lesions, such as those caused by ultraviolet irradiation. If left unrepaired during G1 phase, bulky DNA lesions can block DNA polymerases. Replication then proceeds by bypassing these lesions using specialized translesion synthesis (TLS) polymerases or template-switch mechanisms that use the newly synthesized sister chromatid as a template. Repair pathways that function throughout the cell cycle or for which cell-cycle regulation is not well understood (such as base-excision repair, NER and transcription-coupled repair) are not shown.

Central components of the checkpoint machinery are the phosphoinositide 3-kinase related kinases ATM, ATR and DNA-PK. ATM and DNA-PK respond mainly to DSBs, whereas ATR is activated by ssDNA and stalled replication forks[8] (FIGS 1,2). Activation and recruitment of these kinases to DNA lesions occurs through direct interactions with the specificity factors NBS1 (for ATM), ATRIP (for ATR) and Ku80 (for DNA-PK)[16,17]. DSB resection, which is less efficient in G1 and is restricted by CDK activity[12,13,18], also leads to ATR activation[13] (FIG. 2). When ATM and ATR are recruited to sites of damage, they target many substrates, including checkpoint kinase-2 (CHK2) and CHK1, respectively (FIG. 2; BOX 2). A comprehensive catalogue of ATM and ATR substrates that might be involved in the damage response has recently been presented[19]. These signalling modules probably contribute to coordinate the checkpoint responses with DNA repair (BOX 2; REFS 8,19).

Cell-cycle specificity of the DDR

Eukaryotic chromosomes experience rounds of DNA replication and segregation, and cycles of condensation and decondensation. Each of these events is coupled with topological transitions that are mediated by topoisomerases[6]. Chromatin structure and compaction is also regulated throughout the cell cycle, and can be influenced by checkpoints and other post-translational modifications[20–22]. Here, we discuss the main types of lesions that occur in different cell-cycle phases and the repair pathways that are likely to operate in these phases (FIG. 1). We also try to address, wherever possible (BOX 1; FIG. 2), how these repair pathways are connected with the checkpoint-activation network and how they are integrated into the overall DDR.

Repair during G1 phase. Cells in G1 phase need to repair accidental damage, such as damage that is generated by endogenous ROS species or by chemical agents, UV or ionizing radiation (IR). This damage has to be repaired preferentially before the onset of replication, when the primary DNA lesions can stall replication or can be converted into other types of DNA damage, with hazardous consequences for the cell (FIG. 1). For example, oxidation of guanine generates oxoG. OxoG is highly mutagenic as it can base-pair with adenine during replication and cause a G:C to T:A transversion[23] — one of the most common mutations in human cancers[24]. OxoG can be removed by DNA glycosylases by the base-excision repair (BER) pathway[25].

The NER pathway is mainly responsible for repairing pyrimidine dimers, which are caused by UV and can block the function of DNA polymerases[1]. Although NER has an important role during G1, its activity is not restricted to this phase of the cell cycle (BOX 1). NER is divided into global repair and transcription-coupled repair (TCR). TCR repairs bulky lesions of transcribed genes, whereas global NER repairs lesions irrespective of genome location and cell-cycle phase[1]. It has also been postulated that NER supports other repair systems and shares components with other repair pathways. NER proteins together with HR proteins promote repair of the DSBs that are generated by crosslinks during replication, and NER proteins can function together with the error-prone polymerases during crosslink repair[1,26].

IR causes DSBs, which are perhaps the most harmful damage to DNA. DSBs can be repaired by different pathways, the most important of which are HR and non-homologous end-joining (NHEJ)[27–29] (BOX 1; FIG. 3). The HR pathway uses the information that is contained in genetically identical, or almost identical, DNA molecules (usually the sister chromatid) to repair damaged DNA. In NHEJ, the Ku heterodimer, which consists of Ku70 and Ku80, binds to the two ends of a DSB and recruits DNA-PK catalytic subunit and Ligase4–XRCC4 to ligate the termini and complete NHEJ (FIG. 3). In NHEJ, ligation occurs regardless of whether the ends come from the same chromosome, and so loss of genetic information and translocation can occur[1].

The high compaction of chromatin and the absence of sister chromatids are important factors that make NHEJ the predominant DSB repair (DSBR) pathway during G1 (BOX 1). The cell-cycle phase is a decisive factor in the control of the DSBR pathway on the basis of the phase-specific sensitivity profile to IR of mutants that are defective in either HR or NHEJ[30,31]. In chicken DT40 cells, a *RAD54*-knockout mutant (HR defective) is sensitive to IR only during the late S and G2 phases, whereas *KU70*-knockout mutants are extremely sensitive to IR during the G1 phase[30]. Sensitivity of NHEJ-defective (severe combined immunodeficiency) mouse cells to IR-induced DSBs is elevated only during G1 and early S phases[31]. In budding yeast, diploid cells downregulate NHEJ in favour of HR, but haploid cells in the G1 phase of the cell cycle rely on NHEJ[32,33]. The choice of the DSBR pathway is also coordinated by the CDK activity, which is low until the S phase and influences HR initiation[12,13,18] (BOX 1) (see below).

Collapsed forks
Disjunction of the two partially replicated sister duplexes at the replication fork that is usually associated with the dissociation of the replisome from the replication fork.

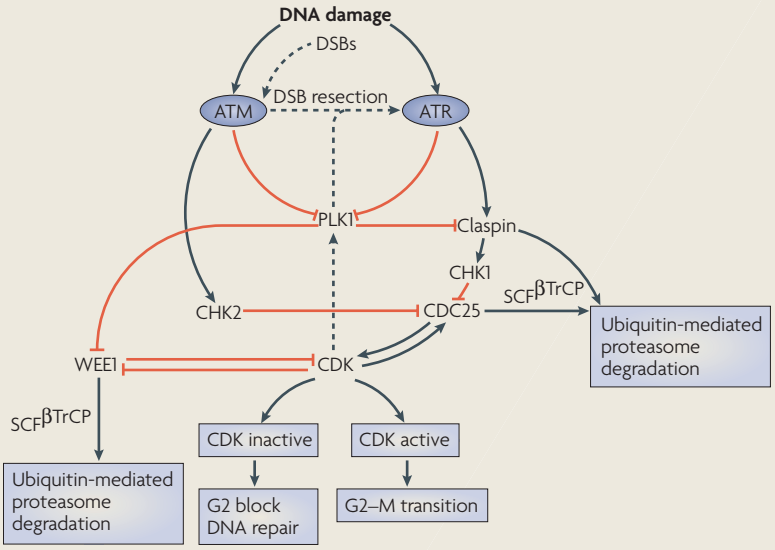
Replisome
Protein machinery that is required to replicate DNA.

Translesion-synthesis polymerases
Low-fidelity and non-processive polymerases that can be used to bypass DNA lesions at the replication fork, often in an error-prone way.

Template switch
(TS). A process that repairs gaps in newly replicated DNA. TS can occur, for example, when a replicative polymerase encounters a lesion on the parental strand. TS uses the information on the newly synthesized sister chromatid as a template to fill in the gaps.

Topoisomerases
Enzymes that remove torsional stress from double-stranded DNA by breaking and rejoining one or two of the DNA strands.

Box 2 | **Checkpoint-mediated cell-cycle arrest and recovery**



An important role of the DNA-damage checkpoints is to sense DNA damage and mediate cell-cycle arrest to allow time to repair DNA lesions. However, once DNA repair is complete, it is also important to terminate the checkpoint activation so that cells can resume cell-cycle progression. The pathways that enable arrest and recovery in mammals are shown in the figure. ATM and ATR kinases respond to different types of DNA damage. ATM mainly responds to double-strand breaks (DSBs), whereas ATR is activated by S phase damage such as single stranded (ss)DNA and stalled forks[141]. DSB resection and activation of ATR requires both ATM and cyclin-dependent kinase (CDK) activity[13]. ATM and ATR are responsible for phosphorylation of different targets, including checkpoint kinase-1 (CHK1) and CHK2 (REFS 8,141). ATR phosphorylation of CHK1 requires the mediator protein claspin[142].

CDK activity is responsible for orchestration of most cell-cycle processes. CDK activation depends on the availability of the cyclins, the levels of which are controlled by the ubiquitin-proteasome system. CDK activation is regulated by inhibitory phosphorylation that is mediated by the WEE1 family of protein kinases at Thr14 and Tyr15, and by CDC25-mediated dephosphorylation of WEE1-mediated phosphorylation[8,103]. Polo-like kinase-1 (PLK1) phosphorylates claspin[143] and WEE1 (REFS 104,144). These post-translational modifications might act as phosphodegrons to promote SKP1–CUL1–F-box ($SCF^{\beta TrCP}$)-mediated ubiquitylation and subsequent degradation of claspin and WEE1 (REFS 143,145,146). Phosphorylation of the phosphatase CDC25 by CHK1 and CHK2 activates $SCF^{\beta TrCP}$ and promotes its degradation. WEE1 degradation eliminates CDK inhibition, whereas CDC25 degradation inhibits CDK activation. In response to DNA damage, checkpoint activation leads to CDC25 degradation, low CDK activity and G2 arrest. However, when DNA repair is complete, PLK1 promotes degradation of both claspin and WEE1 (REFS 143,145,146), which both converge to a build-up of CDK activity that allows G2–M transition.

DNA-repair mechanisms that function in S phase. DNA synthesis is frequently associated with nucleotide misincorporation, accumulation of nicks and gaps, slippage at repetitive sequences, fork collapse at DNA breaks and aberrant transitions at collapsed forks that cause reversed and/or resected forks[4] (FIG. 1). These aberrant replication-fork transitions can endanger the stability of the chromosomes if they are not promptly repaired. Moreover, the torsional stress that is generated when the replication fork advances, when two replicons fuse together at termination or when the forks encounter transcription bubbles causes topological modifications that lead to the accumulation of supercoils and/or precatenanes[6]. The topoisomerase-mediated resolution of these topological constraints allows the completion of S phase, chromosome condensation and segregation during the G2 and M phases (FIG. 1).

Base–base mismatches and small insertion and/or deletion loops that are generated by faulty replication are corrected by the mismatch repair (MMR) pathway, which functions mainly during S phase[34] (BOX 1). This pathway recognizes and removes the flawed stretch of DNA, and then novel DNA synthesis fills in the gap. Chemical alterations of nucleotide bases are often removed by BER, as in G1 phase. BER is also involved in removing misincorporated uracils during S phase[1] (FIG. 1).

Single-strand gaps or nicks often occur during replication (FIG. 1) and seem to be the main source of HR in mitotic cells[35,36]. However, they can also be dealt with by damage tolerance or bypass-replication mechanisms that operate during S phase (BOX 1; FIG. 2). Cells have evolved two mechanisms that promote damage tolerance in S phase. The first mechanism is mediated by translesion synthesis (TLS) polymerases, which replicate across lesion, often in an error-prone manner. Template switch (TS) is an error-free mechanism that fills in gaps in the DNA template by repriming events downstream of the lesion[37,38] (BOX 1). The TS pathway uses the undamaged information of the sister duplex, and the mechanism seems to share similarities with HR. In budding yeast, both pathways depend on the RAD6–RAD18 post-replication repair (PRR) pathway and are largely controlled through covalent modifications of proliferating cell nuclear antigen (PCNA; an essential processivity clamp for DNA polymerases) by ubiquitin[37,39] (BOX 2; FIG. 4).

DSBs can often occur during S phase as a result of replication-fork collapse (FIG. 1). Studies in budding yeast have established that HR is carried out by the products of the conserved *RAD52* epistasis group of genes[28,40]. HR requires 5′–3′ resection of DSBs[12,18], a process that depends largely on the activities of Exo1 and the MRX complex[12,41,42]. The MRX (or MRN in mammals) complex has DNA-binding, endonuclease and 3′–5′ exonuclease activity, and it is thought to function together with a 5′–3′ exonuclease to resect DSBs and create 3′-ended ssDNA that is required to initiate strand invasion (FIGS 2,3). Recently, both the fission yeast protein Ctp1 and its mammalian orthologue CtIP were shown to operate cooperatively with the MRN complex exclusively during the S and G2 phases of the cell cycle to promote DSB resection and HR[43,44]. Studies in budding yeast have shown that the CDK activity facilitates the resection stage of the HR reaction and prevents NHEJ[12,45] (FIGS 2,3). The Ku heterodimer can still bind to DSBs, with even faster kinetics than HR factors[46]; therefore, it is possible that a competition might exist between NHEJ and HR even during the S phase, which suggests that additional factors might suppress the binding of Ku in favour of HR proteins. Recent studies in chicken DT40 cells indicated that both RAD18 and poly(ADP-ribose) polymerase (PARP) function to decrease the affinity of Ku to DSBs and to favour HR[47,48]. The mechanism through which CDK facilitates HR in S phase is just beginning to be elucidated, but it might involve the MRE11-associated

Supercoils
Contortions in DNA that are important for DNA packaging and DNA–RNA synthesis. Topoisomerases sense supercoiling and can either generate or dissipate it by changing DNA topology.

Precatenanes
Cruciform junctions that are formed by the intertwining of the sister duplexes in the replicated portion of a replicone.

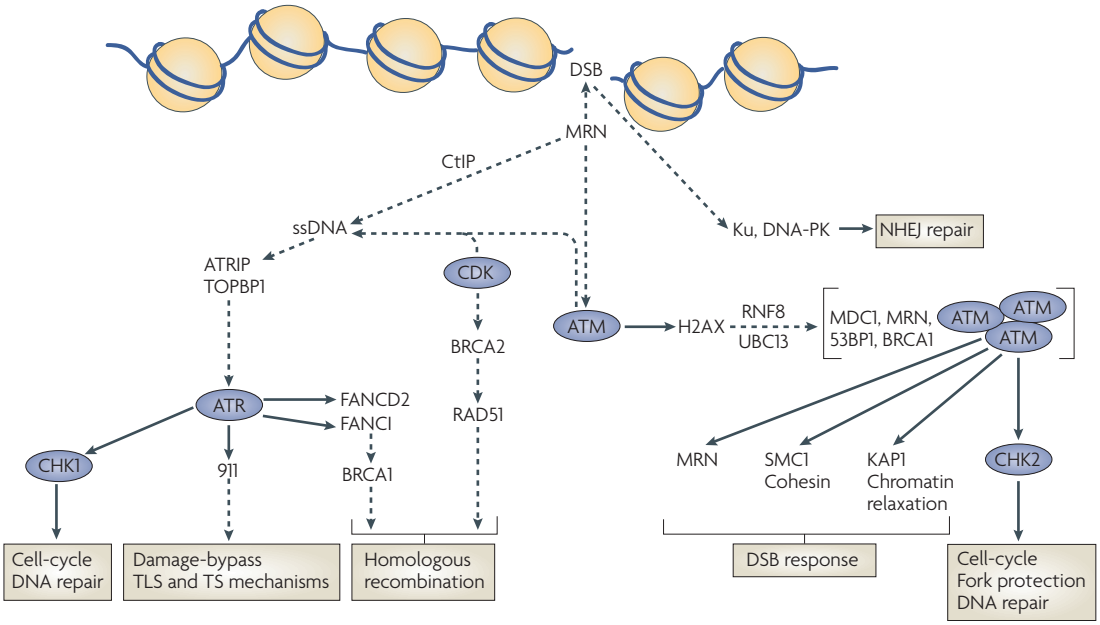


Figure 2 | **Cyclin-dependent-kinase- and checkpoint-mediated regulatory processes influence DNA-repair pathways.** Ku and the MRE11–RAD50–NBS1 (MRN) complex bind to double-strand breaks (DSBs) and activate the kinases DNA-PK and ATM, respectively. Ku and DNA-PK promote non-homologous end joining (NHEJ) repair of DSBs. During S and G2 phases, DSBs are resected to expose single stranded (ss)DNA and activate ATR[12,13,44]. Activated ATM induces chromatin changes around the DSB site through phosphorylation of the histone H2AX, which leads to the recruitment of many checkpoint and repair factors, such as MDC1, MRN and the ubiquitin ligases RNF8 and UBC13. These factors promote the recruitment of 53BP1, BRCA1 (REFS 98–100,102) and ATM itself to facilitate the spreading of the damage signal through the nucleus[8]. ATM-mediated phosphorylation of KAP1 induces chromatin relaxation, whereas other modifications promote repair (such as modification of SMC1 or MRN), or further spreading of the damage signal (such as modification of MRN or checkpoint kinase-2 (CHK2)). ATM- and ATR-mediated phosphorylation of CHK2 and CHK1 promote cell-cycle arrest and DNA repair and reduce cyclin-dependent kinase (CDK) activity. Several targets of ATM and ATR are required for homologous recombination (HR), including SMC1, FANCD2 and FANCI. CDK-mediated phosphorylation of BRCA2 inhibits HR by impairing the interaction of BRCA2 with the HR protein RAD51. In fission yeast, the DNA-damage-checkpoint complex Rad9–Rad1–Hus1 (911) promotes translesion synthesis (TLS) and template switch (TS) damage-bypass mechanisms. Arrows indicate direct phosphorylation events. Dashed arrows indicate indirect events.

factor CtIP[43,44], which is phosphorylated by CDKs[49]. Remarkably, the protein levels of both fission yeast Ctp1 and mammalian CtIP are very low during G1 and high during S and G2 phases of the cell cycle[43,50]; this may also account for S–G2-specific HR (BOX 1; FIG. 2).

DNA repair during G2 and M phases. Gaps and DSBs that occur during replication, if left unrepaired by the end of the S phase, need to be repaired before mitosis. For HR to occur during S and G2 phases using the sister chromatid as a template, it is important that the sister chromatids are in proximity to one another. This is probably established by cohesion, which provides a physical linkage that connects the sister chromatids from S phase until their separation during anaphase. Cohesion depends largely on cohesin, a protein complex that contains two structural maintenance of chromosomes (SMC) proteins, SMC1 and SMC3, held together by sister-chromatid cohesion-1 (SCC1) and SCC3 (REF. 51). Cohesion must be established during S phase[52], and this process requires additional proteins such as Eco1 in budding yeast[53]. However, DSBs can trigger cohesion after DNA replication is complete, and this event is required for sister-chromatid repair in cells in G2 phase[54,55] (FIG. 2). Not surprisingly, mutations that affect the cohesin complex, its loading, or factors that are required to establish cohesion, are severely defective in DSBR[56–58].

The topological problems that arise when two replicons fuse together at termination also need to be resolved during S–G2 in order to prevent chromosome breakage during segregation[6,59]. When the DSBs occur during chromosome segregation — during which time chromosomes are already highly compact and the search for homology is difficult — repair is likely to occur by NHEJ in the subsequent G1 phase if checkpoints or caretaker genes had not caused cell-cycle arrest during G2 and M phases[60–62].

DNA repair also occurs in non-dividing cells. Most DDRs are associated with replication, and it is therefore likely that cells that do not divide (differentiated or senescent cells) may have dedicated repair mechanisms that repair endogenous damage when most DNA-repair pathways that function in dividing cells are attenuated[63]. It has been proposed that accumulating damage in the DNA of the human brain has a crucial role in ageing and in the pathogenesis of many neurological disorders, including Alzheimer's, Parkinson's and amylotrophic lateral sclerosis (ALS)[64,65]. The most predominant type of damage in

Damage tolerance
A post-replicative repair pathway in which the lesions are not repaired, but bypassed (tolerated) during replication. Bypass can be achieved by either using specialized polymerases, or by using the newly synthesized sister chromatid strand as a template.

Epistasis
A group of genes that function in the same biological pathway, usually defined by genetic analysis of double mutants.

Poly(ADP-ribose) polymerase
A polymerase that attaches ADP–ribose moieties to target proteins by means of covalent bonds, which is one of the earliest cellular responses to strand breaks.

Differentiated cells
Cells that are specialized for a particular function (such as neurons and muscle cells) and that cannot proliferate.

Senescent cells
Mitotic cells that cannot divide, but remain metabolically active. Senescence is often caused by stimuli that can cause cancer.

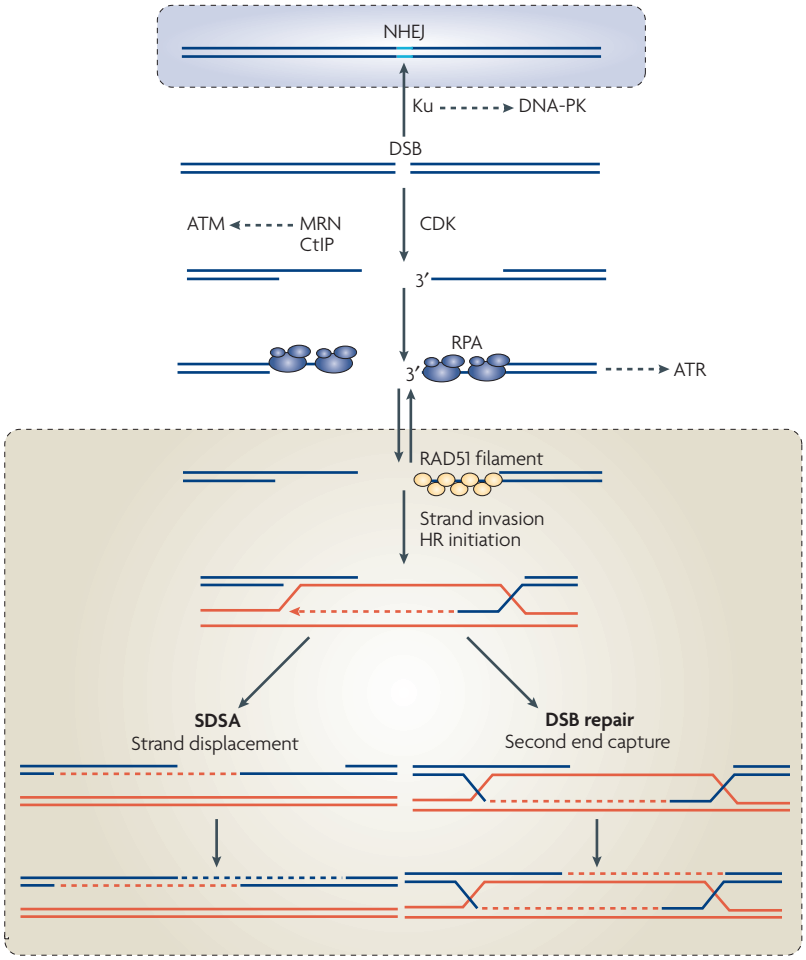


Figure 3 | **Repair of double-strand breaks by non-homologous end joining and homologous recombination.** Double-strand breaks (DSBs) are repaired preferentially by non-homologous end joining (NHEJ) during G1 phase and by homologous recombination (HR) during S and G2 phases of the cell cycle (see text and REF. 27). Binding of the Ku heterodimer to DSBs triggers the recruitment of DNA-PK catalytic subunit and sealing of the DSBs by NHEJ. By contrast, DSBs that occur during S and G2 phases preferentially activate ATM, through the MRE11–RAD50–NBS1 (MRN; Mre11–Rad50–Xrs2 (MRX) in yeast) complex[8]. The higher cyclin-dependent kinase (CDK) activity that is specific for S and G2 phases of the cell cycle promotes DSB resection[12], exposing 3′ overhangs of single stranded (ss)DNA. When the ssDNA of 3′ overhangs is coated with replication protein A (RPA), it activates ATR; RPA can be removed and replaced by RAD51 with the help of mediator proteins such as RAD52. This leads to the formation of RAD51 presynaptic filaments, which initiate HR by invading the homologous region in the duplex to form a DNA joint called a D-loop, which can be further extended by DNA synthesis. Strand displacement of this intermediate by a DNA helicase channels the reaction towards synthesis-dependent strand annealing (SDSA). Alternatively, the second DSB end can be captured, giving rise to a double Holliday junction intermediate, which can be resolved by endonucleases or dissolved by the combined action of a helicase (BLM) and a topoisomerase (TOP3)[40,134].

Double Holliday junction
A central intermediate to homologous recombination.

Ischaemia
A restriction in blood supply, generally due to factors in the blood vessels, that causes tissue damage or dysfunction.

neuronal cells is probably oxidative DNA damage, which arises during normal cellular metabolism[65]. Oxidative base damages are primarily removed by BER[65], whereas DNA adducts are repaired mostly by NER[64]. NER is also important for survival and proper function in neuronal cells and is thought to be essential for repairing endogenous DNA damage, as attested by the severe neurological defects that are observed in patients with Xeroderma pigmentosum (XP) and Cockayne's syndrome (CS). XP and CS are characterized by defects in NER and TCR, respectively[66]. MMR, HR and NHEJ have minor roles in neuronal cells (see REF. 65 and references therein), especially in conditions that occur following ischaemia or apoptotic stimuli. A major clinical effect of cancer treatments is neurocognitive dysfunction and neuropathy, and so much research focuses on understanding the repair pathways that are responsible for the repair of neuronal DNA following chemotherapy and IR[64,65].

Regulation of DNA repair by kinases

Regulation of DNA-repair pathways is important for genome integrity. This can occur by modulating the choice of the repair pathway when a lesion is a potential substrate for two or several repair pathways, by regulating the stability or activity of a repair factor, or by regulating the period of time during which repair can take place. Here we discuss several DNA regulatory pathways that are either activated in a cell-cycle-dependent manner or that crosstalk with the checkpoint machinery to induce cell-cycle arrest and DNA repair.

The checkpoint kinases mediate arrest and allow repair. The checkpoint kinases promote the viability of cells following DNA damage through their ability to mediate cell-cycle arrest, which allows cells to repair DNA damage (BOX 2). Although ATM and ATR respond to different types of lesions (BOX 2), to which they are recruited by different factors (MRN recruits ATM; ATRIP and RPA-coated ssDNA recruit ATR) (FIG. 2), recent evidence suggests that ATR is also activated by IR-induced DSBs in a cell-cycle regulated manner[13]. ATR activation by DSBs requires ATM and MRN–CtIP, it occurs only during S and G2 phases, and it is abolished by CDK inhibition[13,44] (FIG. 2). These results are in agreement with previous findings showing that formation of IR-induced foci that contained the HR protein RAD51 in both yeast and human cells is restricted to S and G2 phases and depends on checkpoint activity[67–69]. The relevant targets of the CDK in this pathway, besides the likely CtIP[44,49], remain to be identified.

In certain occasions, CDK-mediated phosphorylation of checkpoint proteins is required to activate their DNA-repair mediator function. In fission yeast, the checkpoint protein Crb2 is phosphorylated by Cdk1. This modification is important to mediate later steps of HR-mediated DSBR that implicate the RecQ helicase Rqh1 and the topoisomerase Top3 (REF. 70).

Numerous examples have shown that checkpoint-dependent phosphorylation of targets affects their function in repair events that occur in a cell-cycle-specific manner. CHK1 phosphorylation of RAD51 is required for mammalian HR[71]; CHK1 also influences the replacement of RPA on ssDNA with RAD51 and RAD52 in a process that leads to RAD51 presynaptic filament formation and initiation of HR-mediated DSBR[72] (FIG. 3). However, the replication-checkpoint-dependent phosphorylation of fission yeast protein Mus81, a conserved endonuclease that has been proposed to act at stalled replication forks, inhibits recombination[73]. The ATR-dependent phosphorylation of the NBS1 subunit

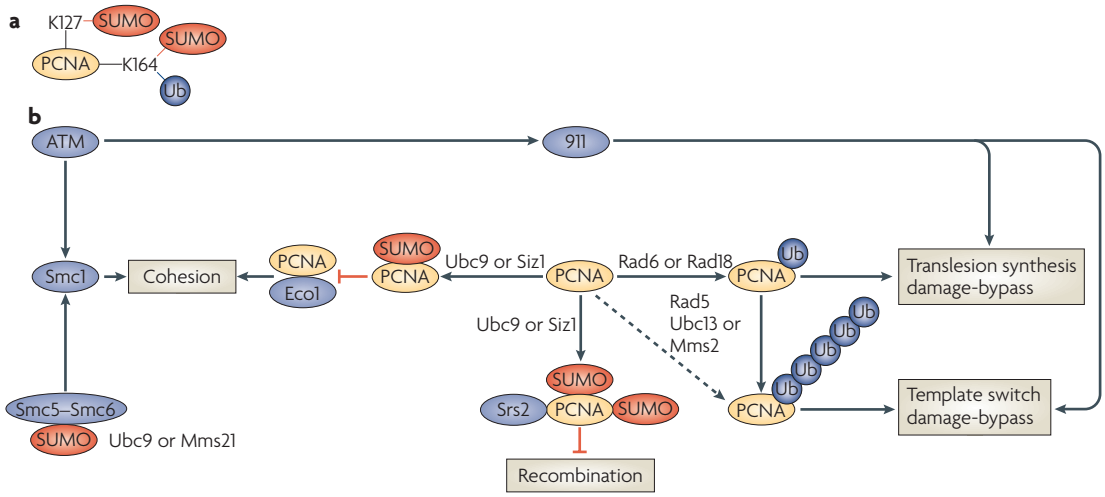


Figure 4 | **Sumoylation and ubiquitylation events that are implicated in modulation of DNA repair.** **a** | Proliferating cell nuclear antigen (PCNA) is modified at the conserved Lys164 by ubiquitylation; it can be monoubiquitylated, Lys63-linked polyubiquitylated or sumoylated[39]. In budding yeast, Lys127 of PCNA can also be modified by small ubiquitin-like modifier (SUMO)[39]. **b** | Monoubiquitylated PCNA promotes translesion synthesis (TLS), probably by promoting the interaction of PCNA with different TLS polymerases[118–120]. PCNA polyubiquitylation is needed for template switch (TS)-mediated gap filling[39]. Sumoylation of PCNA recruits the helicase Srs2 to stalled replication forks[122,123], which inhibits homologous recombination (HR) by disrupting Rad51 filaments[128,129]. In fission yeast, TLS is also promoted by the DNA-damage checkpoint complex Rad9–Rad1–Hus1 (911) (REF. 83), in which Rad3-dependent phosphorylation of the checkpoint clamp protein Rad9 also inhibits recombination and promotes TS via interaction with Mms2 (REF. 86). Both sumoylation and ATM affect double-strand break repair (DSBR) through cohesion. The Mms21–Smc5–Smc6 complex is required for loading of the cohesin subunit Smc1 (REF. 157), which is phosphorylated by ATM[80,81]; both events are important for DSBR. In budding yeast, Eco1 is required to establish cohesion during S phase and for DSBR[56]. Eco1 must interact with PCNA to acquire activity[158]; however, this interaction is counteracted by PCNA sumoylation, and the SUMO-conjugating enzyme Ubc9 competes with Eco1 for PCNA binding[158].

from the MRN complex and of FANCD2 (REF. 74) from the Fanconi anaemia (FA)-crosslink-repair pathway are thought to promote HR repair during S phase[75] (FIG. 2). In budding yeast, Rad53-mediated phosphorylation of Rad55 is required for effective repair of replication-associated damage, perhaps by activating recombination[76], and Mec1–Tel1-dependent phosphorylation of the endonuclease Slx4 is required for Slx4 activity in single-strand annealing (SSA)[77], a sub-pathway of DSBR[28].

As a more general response to DSBs, ATM phosphorylation of KAP1 promotes chromatin relaxation[78]. Furthermore, ATM-dependent phosphorylation of histone H2AX is an early event that is important for efficient DSBR (FIG. 2) and probably contributes to the recruitment of different repair, cohesion and checkpoint factors[8,79]. In mammalian cells, ATM-dependent phosphorylation of the cohesin subunit SMC1 is important for DSBR[80,81] (FIG. 2).

In budding yeast, a role for the replication checkpoint in promoting gap filling has been proposed on the basis of the observation that replication-checkpoint mutants accumulate gaps behind forks after UV treatment[82]. The PCNA-like damage-checkpoint complex (Rad9, Rad1 and Hus1; called the 911 complex) contributes to TLS damage bypass in both budding and fission yeast[83–85]. In fission yeast, Rad3 (the ATR homologue)-dependent phosphorylation of the PCNA-like checkpoint complex was suggested to promote the *RAD6*-mediated error-free PRR while inhibiting recombination[86] (FIG. 2).

CDK activity regulates DNA repair. Recent studies have suggested that CDK activity regulates HR-repair events during the S and G2 phases of the cell cycle (see also above). The role of Cdk1 in activating HR during the S and G2 phases (BOX 1) can be explained by the finding that, in budding yeast, the generation of 3′-overhangs at DSBs is influenced by Cdk1 (REFS 12,18; FIGS 2,3).

In budding yeast, a defect in Cdk1 activity also affects IR-mediated Rad51-foci formation[70]. Likewise, in human cells, RAD51-foci formation is largely restricted to S and G2 phases, and is impaired by the CDK inhibitor roscovitine[13]. Furthermore, the coating of ssDNA with RPA is greatly reduced following CDK inhibition[13]. Collectively, these results suggest that the MRN–CtIP-associated nuclease activity can only efficiently resect DSBs in cells that are in the S and G2 phases of the cell cycle and have high CDK activity[13,44] (BOX 2; FIG. 2).

CDK activity can influence later steps of HR through the phosphorylation of several proteins that are implicated in DSBR. Consistent with this view, CDK targets the budding yeast DNA helicase Srs2 (REF. 87), human BRCA1 (REF. 88) and the fission yeast checkpoint protein Crb2 (REF. 89), which have all been implicated in recombination-mediated repair. Studies using *Brca1*-deficient mouse embryonic stem cells indicated that BRCA1 has a role in promoting HR and in limiting non-homologous repair processes[90]. CDK-dependent phosphorylation of CtIP is essential for physical interactions between CtIP and BRCA1, and the ubiquitin-ligase activity of BRCA1

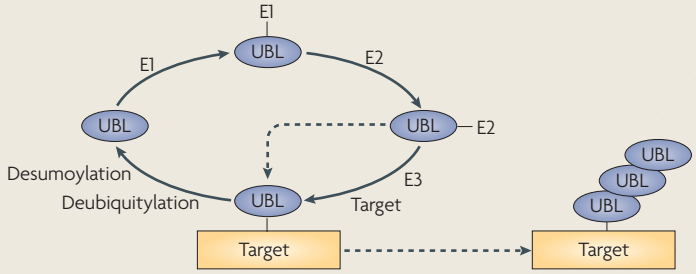
RecQ helicase
A family of helicase enzymes that is important for genome maintenance. They function through unwinding paired DNA and translocate in the 3′→5′ direction.

Fanconi anaemia
A rare genetically inherited disorder that is characterized by congenital abnormalities and increased incidence of cancer.

Srs2
A budding yeast DNA helicase that functions to prevent recombination by disrupting Rad51 filaments.

BRCA1
The product of the first breast cancer susceptibility gene; it is involved in DNA repair, cell-cycle regulation and protein ubiquitylation.

Box 3 | **Ubiquitin and SUMO post-translational modification systems**



Modification of proteins by the ubiquitin or ubiquitin-like proteins (UBL; see figure), such as the small ubiquitin-like modifier (SUMO), controls many signalling networks and is evolutionarily conserved in all eukaryotes[147]. Both ubiquitin and SUMO modifications play important roles in the regulation of the stability and activity of components of the mitotic checkpoint (BOX 2), and in DNA-damage induced G2-checkpoint initiation and recovery[8,103].

The ubiquitin-conjugation machinery involves an E1 activating enzyme, an E2 conjugating enzyme, an E3 ligase (to enhance conjugation) and proteases or isopeptidases (to deconjugate UBLs from target proteins). For both SUMO and ubiquitin pathways, single E1 enzymes function to initiate the activation of individual UBLs. In budding yeast, the ubiquitin E1 is Uba1, whereas the SUMO E1 is a heterodimer of Aos1 and Uba2. However, unlike the ubiquitin system, in which a large number of E2 enzymes and E3 ligases have been found to mediate substrate specificity, the SUMO pathway relies only on Ubc9, a single E2 enzyme that interacts directly with most of the substrates and can link SUMO to specific consensus sequences[147–149]. Ubiquitylation relies on E3 enzymes (of which many have been identified), whereas sumoylation can occur in some cases without the aid of an E3. In contrast to the ubiquitin system, only a few SUMO ligases have been identified. In budding yeast, there are only three characterized bona fide SUMO ligases — Siz1, Siz2 and Mms21 (REF. 149) — although recent evidence suggests that the Slx5–Slx8 complex might also functions as a SUMO ligase[150]. In addition, Slx5–Slx8 has a robust substrate-specific ubiquitin-ligase activity that is stimulated by SUMO attachment to the substrate, which suggests a mechanism through which sumoylation can trigger subsequent ubiquitylation of a target[151,152]. Chain formation through UBL linkage to an internal lysine of the preceding UBL has been well documented for ubiquitin[153], and has also been observed for SUMO[154].

targets H2AX and CtIP[49,50,91,92]. Budding yeast Srs2 has been implicated in channelling DSBR towards synthesis-dependent strand annealing (SDSA), a sub-pathway of HR that leads to non-crossover products[40,93–95] (FIG. 3). In fission yeast, both Cdk1 and Crb2 were found to regulate late steps of HR-dependent DSBR by affecting Top3 and Rqh1 activity[70,95].

CDK-dependent phosphorylation can also inhibit HR. CDK-dependent phosphorylation of BRCA2 was reported to impair the interactions of BRCA2 with RAD51 and to inhibit HR[96]. The CDK phosphorylation site, Ser2391, is a cancer-related mutation site, and levels of Ser3291 phosphorylation decrease following IR[96]. The different roles of CDK on HR could be reconciled by a model in which CDK activity is required for the initial processing of DSBs[12,13,44] to generate 3′-end ssDNA and to activate the checkpoint cascade. The checkpoint cascade then induces G2 arrest and reduces CDK activity (BOX 2; FIG. 2; REF. 8). The temporarily reduced CDK activity (a consequence of IR-induced checkpoint-mediated G2 arrest) might then diminish BRCA2 phosphorylation and allow BRCA2 to interact with RAD51 to promote HR[97].

BRCA2
A tumour suppressor and an integral component of the homologous-recombination machinery.

BRCT repeats
A protein motif with homology to the C-terminal region of BRCA1 that constitutes a phosphopeptide-recognition domain.

Ubiquitylation directs DDR and repair

A number of studies have shown an important role for ubiquitylation (BOX 3) in coordinating cell-cycle-specific DDR and repair processes[8,92,98–102]. Regulators of CDK activities are often targets of ubiquitin-mediated degradation[8,103]. Two ubiquitin ligases, the anaphase-promoting complex/cyclosome (APC/C) and βTrCP, a component of the SKP1–CUL1–F-box (SCF) complex, mediate degradation of many cell-cycle regulators and have been well characterized[8,103] (BOX 3). The $SCF^{\beta TrCP}$ complex has emerged as a key factor in the control of the two important CDK regulators, CDC25a (one of the three mammalian CDC25 phosphatases) and WEE1 (REFS 103–106) (BOX 2). The SCF pathway is under the control of several checkpoint kinases including ATM, ATR, CHK1 and CHK2 (REFS 105,107). Following DNA damage, CDC25a is heavily phosphorylated by CHK1 and CHK2 (REFS 103,107). CHK1-mediated phosphorylation on Thr507 of CDC25a blocks the interaction of CDC25a with CDK and cyclin targets[108]. CDC25a is therefore unable to remove the inhibitory phosphorylation on CDK1 that is mediated by WEE1. By contrast, other phosphorylation events enhance the recognition of CDC25a by the $SCF^{\beta TrCP}$ complex, which mediates CDC25a degradation[103,105,107] (BOX 2).

Ubiquitylation has emerged as an important regulator of cell-cycle-specific repair. FA cells exhibit chromosome instability and increased sensitivity to DNA-crosslinking agents, which is indicative of a defect in DNA repair or recovery from blocked replication forks (see REF. 109 and references therein). The FA core complex is a ubiquitin ligase[110,111], the activity of which is controlled by ATR-dependent phosphorylation[19,112109] (FIG. 2). Ubiquitylation of FANCI and FANCD2, two FA components that interact with each other and the FA core complex, leads to their association with chromatin in nuclear foci that also contain recombination proteins such as RAD51, BRCA1 and BRCA2. BRCA1, which also associates with FANCJ, is a ubiquitin ligase[113,114] that catalyses the formation of unusual polyubiquitin chains that are linked through Lys6 (these chains are often present at sites of DNA damage)[115–117]. BRCA1 was found to form at least three distinct protein complexes, by binding Abraxas and RAP80, BACH1 and BRIP1, or CtIP, in an exclusive manner through its C-terminal BRCT repeats[115–117]. RAP80 binding to Lys63-linked ubiquitin chains[117] is probably mediated by UBC13 and RNF8, which possess ubiquitin-conjugating and -ligase activities, respectively[92,102]. Both UBC13 and RNF8 are required for localization of BRCA1 and Abraxas to sites of damage[92,102,115–117]. Recent evidence suggests that RNF8 binds to MDC1 and ubiquitylates phosphorylated H2AX. These events facilitate the recruitment of factors (such as BRCA1 and 53BP1) that are required for DNA repair and checkpoint signalling, probably through a ubiquitin-ligase cascade[98–100,102] (FIG. 2).

Mono- and polyubiquitylation of PCNA. PCNA, an essential replication factor and an important factor for S phase DNA repair, is also regulated by ubiquitylation. Different covalent modifications of PCNA by ubiquitin are crucial in modulating damage-bypass processes during

replication[39]. PCNA is ubiquitylated at the conserved Lys164 in several species[39]. In budding yeast, PCNA is monoubiquitylated in a manner that is dependent on Rad6-, or is polyubiquitylated via Rad5–Mms2–Ubc13 through non-canonical Lys63 chains that do not promote proteasomal degradation of PCNA[39] (BOX 3; FIG. 4). A similar mechanism also appears to exist in chicken DT40 and human cells.

It is unclear whether PCNA monoubiquitylation always precedes polyubiquitylation. However, although PCNA monoubiquitylation triggers TLS[118], probably by promoting interaction between the TLS polymerases with monoubiquitylated PCNA (as shown for human polymerase η[119,120]), PCNA polyubiquitylation is required for the error-free branch of PRR or TS[37,39] (FIG. 4). The mechanism of TS and the role of polyubiquitylated PCNA in this process are not well understood. The polyubiquitin chains might detach the modified PCNA from the replisome, labelling the site of the lesion for subsequent repair, whereas new PCNA molecules can be loaded downstream of the lesion for normal replication. This model predicts that ssDNA gaps are formed behind the replication forks[85] that could be filled by TLS or recombination (TS) mechanisms. Indeed, such gaps have been observed following UV irradiation[82]. Alternatively, the polyubiquitin chain on PCNA might recruit specialized replication or recombination factors that are required for TS, or it might inhibit TLS by binding and inhibiting TLS polymerases[121].

SUMO modifications modulate DNA repair

Increasing evidence suggests that sumoylation (BOX 3) of specific targets has a role in protecting genome integrity and in modulating DNA repair[39,122–124]. Small ubiquitin-like modifier (SUMO) modification has also been implicated in checkpoint maintenance and in modulating cell-cycle transitions[103]. In budding yeast, sumoylation is required for degradation of Pds1 (also known as securin), a protein that inhibits the protease separase that triggers chromosome segregation at the onset of anaphase by cleaving cohesin[125].

Sumoylation also affects DNA repair through cell-cycle-specific modification of DNA-repair factors or by targeting proteins that function in cell-cycle-regulated DNA-repair pathways. SUMO modification targets budding yeast Rad52 (REF. 124) and Ku70 (REF. 126), which regulate HR and NHEJ, respectively (FIG. 3). Rad52 sumoylation shelters Rad52 from proteasomal degradation[124] and regulates formation of Rad52 foci in the nucleolus and recombination of ribosomal DNA (rDNA)[127].

PCNA is not only ubiquitylated, but it is also sumoylated, at least in budding and fission yeast, *Xenopus* and chicken. In budding yeast, PCNA sumoylation occurs during unperturbed S phase or in response to sublethal doses of DNA damage (methyl methanesulfonate)[39]. Lys164 — the same residue of budding yeast PCNA that is ubiquitylated — is the main target for sumoylation. PCNA is also sumoylated at Lys127 in budding yeast[39] (FIG. 4). Genetic studies have shown that sumoylated PCNA can promote TLS[118], but perhaps its most important function is to inhibit recombination repair. This occurs through recruitment of the helicase Srs2 (REFS 122,123), which disrupts Rad51 filaments[128,129] (FIG. 4). A similar role for Srs2 and PCNA sumoylation has also been reported in fission yeast. Moreover, in fission yeast, the replication and damage checkpoint Rad3-dependent (or Mec1-dependent in budding yeast) phosphorylation of the checkpoint clamp protein Rad9 (or Ddc1 in budding yeast) prevents inappropriate recombination by promoting the error-free branch of PRR through a Pli1-mediated (or Siz1-mediated in budding yeast) sumoylation pathway (BOX 3; FIG. 4; REF. 86). Although Srs2 is not conserved in mammalian cells, its function might be performed by two RecQ helicases: RecQ5 and BLM. Both these helicases interact with PCNA and can disrupt RAD51 filaments and inhibit early steps of HR[130,131].

Gap-filling mechanisms are expected to lead to the transient formation of hemicatenane-like structures, which accumulate in cells in which the RecQ helicase Sgs1 and the topoisomerase activities of Top3 are impaired[132,133]. Consistent with this model, *in vitro* studies have shown that the BLM, the human orthologue of Sgs1, can merge a double Holliday junction (dHJ) (FIG. 3) and create an intermediate that can be subsequently resolved by dissolution through the specific single-strand decatenating activity of Top3 (REFS 95,134). This ability of Sgs1 to resolve the hemicatenane-like molecules formed during damage-bypass processes is regulated by Ubc9- and Mms21-dependent sumoylation, but is independent of PCNA sumoylation[135]. Because Sgs1, like BLM[136], is itself sumoylated, but in an Mms21-independent manner[135], it is possible that other Mms21 targets also exist that cooperate with Sgs1 in this process. Mms21 is part of the Smc5–Smc6 complex, which is implicated in DNA repair, formation of Rad52 recombination foci and rDNA recombination[127,137,138]; furthermore, Smc5 in budding yeast and Smc6 in fission yeast and mammalian cells are sumoylated in an Mms21-dependent fashion[126,139,140]. The Smc5–Smc6 complex also contains a putative ubiquitin E3 ligase (Nse1) activity, which opens the possibility that, like PCNA, this complex might provide another example of functional interplay between ubiquitin and SUMO modifications in S and G2 phase DNA repair.

Concluding remarks and future perspectives

Accurate repair of DNA damage is of paramount importance for genome integrity. In response to DNA damage, intertwined networks of surveillance mechanisms act to temporarily halt cell-cycle progression and to promote DNA repair. The regulation of DNA repair is usually dependent, or takes into account, two different factors: the type of the DNA lesion that needs to be repaired, and the cell-cycle-related substrate characteristics. These characteristics include expression or stability of certain repair proteins, the compaction level of the chromatin and the availability of sister chromatids.

The CDKs stand out as a prime model of cell-cycle regulators that affect DNA repair. We now realize that CDK activity affects and crosstalks with the checkpoint-activation network to mediate cell-cycle arrest and effective DNA repair in an intricate network. So far, the

Hemicatenanes
Cruciform junctions of two double-stranded DNA molecules in which one of the strands of one duplex passes between the two strands of the other duplex (and vice versa).

cellular response to DSBs provides the best understood example of how cell cycle and CDK activity regulate DNA repair. However, many questions remain concerning the targets and the molecular details of this regulation. How does CDK influence MRN–CtIP activity? Is there a lapse between the timing of the 3′-end ssDNA formation and the initiation of the HR reaction? And is this regulated by the oscillation in the level of CDK activity? Although results from different organisms converge into the idea that CDK activity is required to generate the DNA substrate for HR, the roles of CDK activity in late steps of HR are still poorly understood. Clearly, identifying which CDK targets are involved in repair and how their modifications affect repair efficiency will be crucial for our understanding of CDK functions.

Increasing evidence suggests that there is a complex interplay and crosstalk between different regulatory mechanisms; many different players and pathways overlap and interact, competing or collaborating to repair the same lesion. However, many important questions concerning the molecular details of how different post-translational modifications of repair factors influence protein interactions, cellular distribution, turnover and ultimately repair efficiency remain unsolved. For example, the manner in which ubiquitylation and sumoylation modulate different repair events or the choice of the repair pathway is still poorly understood. Sumoylation often affects the cellular localization of its targets, but are these repair events compartmentalized in the cell? Do these modifications affect the affinity of the targets for specific DNA substrates, or the preferences for certain interacting proteins? How does BRCA1 (which is also modified by CDK[88]) enhances HR function, and what are its relevant targets in the recombination-repair process? Similar DNA structures are substrates for different types of DNA-metabolism processes, and there are many repair enzymes with high affinity for binding or processing a certain type of DNA structure. ssDNA is a signal for checkpoint activation when it is coated with RPA, but extensive RAD51 binding leads to presynaptic filament formation and initiation of HR. ssDNA gaps that are left during replication can also be substrates for specialized polymerases with activities that are often enhanced, or that rely upon, different PCNA modifications (such as sumoylation or ubiquitylation). The role of PCNA modifications on DNA repair has been well studied, but the physiological roles of ubiquitylation or sumoylation of many other proteins that are involved in repair remain to be elucidated. The difficulties in these areas of research are numerous. Often only a small fraction of a protein is modified, making the identification of modification sites difficult. Moreover, mutagenesis of the sites that undergo modification might not be associated with a clear phenotype owing to redundancy problems or to the fact that the interacting proteins might also be targeted for the same modification; therefore, the complex might be at least partially functional.

In addition to the mechanisms through which checkpoint activation functions to coordinate repair in a cell-cycle-dependent manner, the molecular mechanisms that regulate the consequences of failed DNA-repair attempts (and induce temporary cell-cycle arrest, senescence and cell death) are also likely to be active areas of research. In the next few years we hope to achieve a better understanding of the mechanisms that control DNA-repair mechanisms and coordinate repair with cell-cycle progression to preserve genome integrity.

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Acknowledgements
The authors apologize for the many interesting articles that could not be cited here owing to space limitations. The work in the authors' laboratories is supported by grants form the Associazione Italiana per la Ricerca sul Cancro, the European Community, Telethon-Italy, the Italian Ministry of Education and the Association for International Cancer Research to M.F. and D.B. D.B. is supported by the Buzzati-Traverso foundation.

DATABASES

UniProtKB: http://ca.expasy.org/sprot
ATM | ATR | ATRIP | CDC25a | CHK1 | CHK2 | CtIP | DNA-PK | FANCD2 | FANCI | Ku80 | Mre11 | NBS1 | Rad50 | RAD51 | RAD52 | Ubc9 | Xrs2

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Marco Foiani's homepage:
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