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CELLULAR RESPONSES TO DNA DAMAGE

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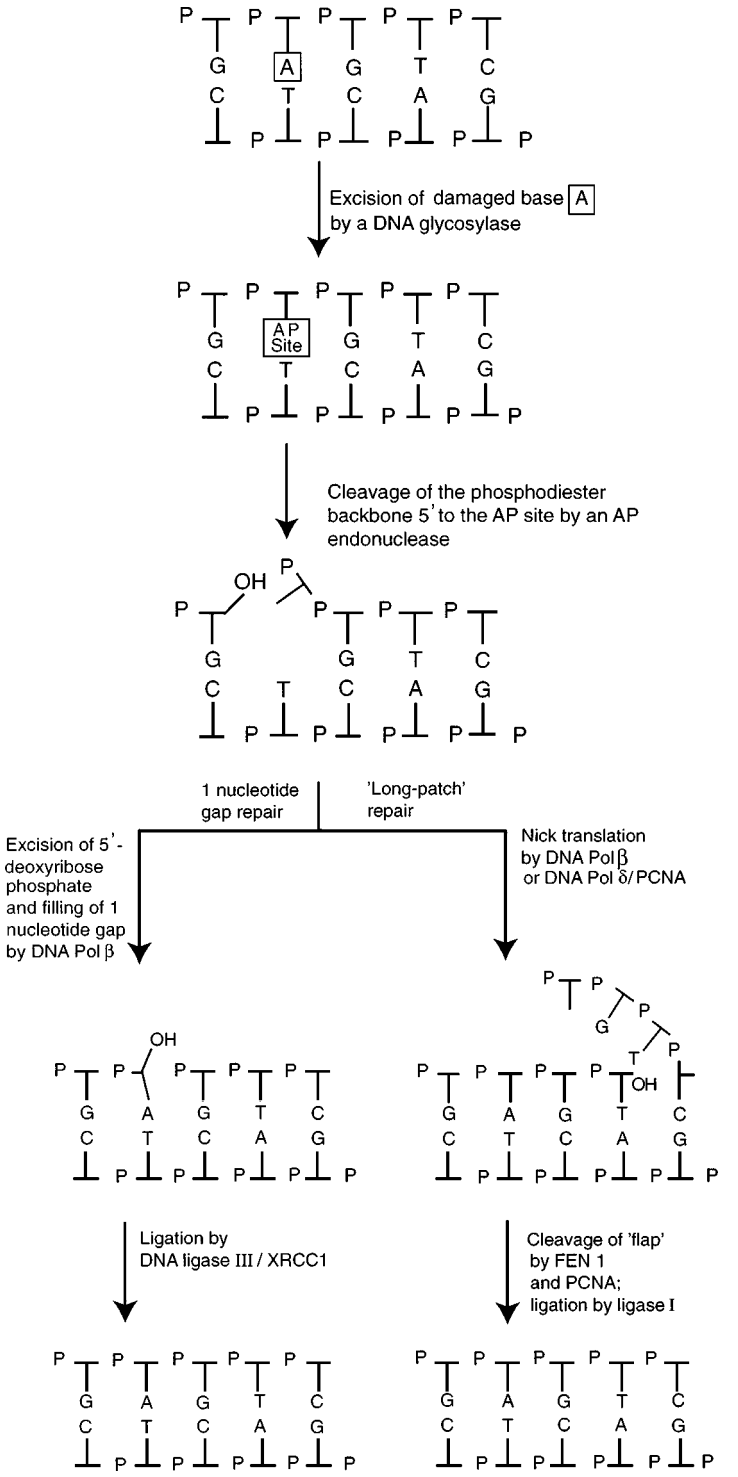
■ **Abstract** Cells are constantly under threat from the cytotoxic and mutagenic effects of DNA damaging agents. These agents can either be exogenous or formed within cells. Environmental DNA-damaging agents include UV light and ionizing radiation, as well as a variety of chemicals encountered in foodstuffs, or as air- and water-borne agents. Endogenous damaging agents include methylating species and the reactive oxygen species that arise during respiration. Although diverse responses are elicited in cells following DNA damage, this review focuses on three aspects: DNA repair mechanisms, cell cycle checkpoints, and apoptosis. Because the areas of nucleotide excision repair and mismatch repair have been covered extensively in recent reviews (1–6), we restrict our coverage of the DNA repair field to base excision repair and DNA double-strand break repair.

BASE EXCISION REPAIR

The major forms of DNA damage arising from the actions of endogenous agents are (*a*) hydrolytic depurination, (*b*) hydrolytic deamination of cytosine and 5-methylcytosine bases, (*c*) formation of covalent adducts with DNA, and (*d*) oxidative damage to bases and to the phosphodiester backbone of DNA. The vast majority of these "small" lesions are repaired by the base excision repair (BER) pathway.

The BER pathway has been reviewed in detail elsewhere (7–16) and is depicted in Figure 1. DNA glycosylases, of which there are several classes (see below), recognize abnormal DNA bases and catalyze hydrolytic cleavage of the N-glycosyl bond linking the base to the sugar. Following the generation of an apurinic/apyrimidinic (AP) site, in most cases the BER pathway can proceed utilizing a common set of proteins. The objective of the AP endonucleases and phosphodiesterases is to generate a single nucleotide gap containing 3′ hydroxyl and 5′ phosphate termini that permits a DNA polymerase to fill the gap. Finally, a DNA ligase can seal the remaining nick. Although this represents the primary pathway for BER, there are variations on the theme. First, instead of an AP endonuclease cleaving the phosphodiester backbone 5′ to the AP site, some glycosylases remove


Excision of damaged base A by a DNA glycosylase
AP Site
Cleavage of the phosphodiester backbone 5' to the AP site by an AP endonuclease
1 nucleotide gap repair
'Long-patch' repair
Excision of 5'-deoxyribose phosphate and filling of 1 nucleotide gap by DNA Pol β
Nick translation by DNA Pol β or DNA Pol δ/PCNA
Ligation by DNA ligase III / XRCC1
Cleavage of 'flap' by FEN 1 and PCNA; ligation by ligase I

the damaged base and cleave the phosphodiester backbone 3′ to the resulting AP site via β-elimination (so-called AP lyases). In this case, the unsaturated sugar fragment left on the 3′ site of the nick is removed by a phosphodiesterase to generate the necessary single nucleotide gap. One source of this phosphodiesterase action is a second activity exhibited by the AP endonuclease (17–21). A second variation in BER is for the polymerization step of BER to involve a gap larger than one nucleotide (Figure 1). In this so-called "long-patch" pathway, approximately 2–10 nucleotides are excised and replaced by the combined actions of a DNA polymerase (usually Polδ or ε but possibly Polβ) proliferating cell nuclear antigen (PCNA), replication factor C (RF-C), and an endonuclease that cleaves "flap" structures (FEN-1) (22–25). The long-patch pathway seems to predominate when repair is initiated at oxidized or reduced AP sites generated by X-rays or chemical agents, whereas the single nucleotide gap repair pathway occurs when "regular" AP sites are generated. In the single nucleotide gap pathway the XRCC1 protein directs specific interactions with both Polβ and DNA ligase III (12, 26–29), and hence recruits this ligase to sites of ongoing repair. In long-patch BER the nick is probably sealed by DNA ligase I. Both branches of BER have been reconstituted in vitro (30–33).

The protein components of the BER pathway (see below) have been conserved both structurally and functionally during evolution, underscoring the vital role that BER plays in defending genome integrity. This role is further emphasized by the finding that disruption of BER function in mammalian cells is generally not compatible with viability. Thus, for example, mice deficient in HAP1 (34) and XRCC1 (35) die during embryonic development.

DNA Glycosylases for Repair of "Inappropriate" DNA Bases

Inappropriate bases include those not normally found in DNA, such as uracil, as well as normal bases found in the wrong context, such as thymine arising from deamination of 5-methyl cytosine. Uracil arises in DNA through deamination of cytosine residues, or through incorporation of dUTP during DNA replication. Uracil DNA glycosylase (UDG) excises uracil residues from DNA but not from RNA (7, 36). Probably because of a requirement to efficiently distinguish between uracil and thymine in DNA, which are very similar in structure, UDG has little flexibility in its range of substrates, being limited to uracil and its derivatives such as 5′ fluorouracil (7, 36). Recent studies have provided a structural basis for this substrate specificity (37, 38). Owing to the shape of the active site pocket, the target uracil nucleotide must adopt an extrahelical conformation. This "nucleotide

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Figure 1 Schematic representation of the base excision repair pathway. A damaged base (A) is excised by a DNA glycosylase to generate an apurinic/apyrimidinic site. After apurinic/apyrimidinic endonuclease cleavage the pathway bifurcates into single-nucleotide and long-patch routes. See text for details.

flipping" mechanism is emerging as a conserved feature of the action of several BER enzymes. Human cells contain another UDG activity, designated hSMUG1, which prefers ssDNA containing uracil residues as a substrate (39).

The DNA of many organisms, including man, includes cytosine residues methylated at the C^5 position. Upon deamination of 5-methylcytosine, thymine is generated in the context of a G:T mispair. Any repair system for correction of this mispair has two obvious requirements: The G:T mispair must be repaired to G:C, and thymine residues in A:T pairs must not serve as substrates. The glycosylase responsible for repair of G:T mispairs is thymine DNA glycosylase (40). However, thymine DNA glycosylase is not specific for thymine residues, because it also recognises uracil when mispaired with guanine (41). This "uracil glycosylase" activity has been conserved in organisms that do not methylate cytosine residues.

Glycosylases for Repair of Oxidized DNA Bases

Base oxidation in cellular DNA can arise following exposure to ionizing radiation, radiomimetic chemicals, and intracellular reactive oxygen species (ROS) (7–11). In *Escherichia coli* oxidized purines are excised by the FPG protein (also known as MutM and FAPY glycosylase) (42). FPG removes imidazole ring–opened derivatives, such as FAPY-guanine and FAPY-adenine and 7,8-dihydro-8 oxoguanine (8-OG) (Figure 2) (7). 8-OG can mispair with A and therefore generates GC → TA transversion mutations. *fpg* mutants show an increased frequency of GC → TA mutations, consistent with a defect in 8-OG repair (7, 43, 44).

Yeast and mammals contain proteins that perform roles analogous to those of FPG. The *Saccharomyces cerevisiae* Ogg1 protein has a substrate specificity similar to that of FPG, although their primary sequences are unrelated (45, 46). Instead, Ogg1 shows sequence similarity to the *E. coli* endonuclease (endo) III protein (see below). The human homolog of Ogg1 excises 8-OG paired with cytosine by flipping the 8-OG residue into an extrahelical configuration (47). The ability of Ogg1 to discriminate between 8-OG and guanine appears to be conferred by a single hydrogen bond between an active site glycine residue and the 8-OG moiety.

8-OG residues that have escaped repair prior to DNA replication can mispair with adenine. 8-OG:A mispairs are relatively poor substrates for Ogg1, presumably to exclude the use of the adenine residue as a template during DNA repair synthesis. All cells, therefore, express an enzyme that converts 8-OG:A mispairs to 8-OG:C through acting as an adenine glycosylase (48–50). The gene encoding this activity in *E. coli* is *mutY* (51–54). Because cells counteract 8-OG residues in DNA through the combined actions of FPG/Ogg1 and MutY, *fpg mutY* double mutants show an extremely high rate of GC → TA mutations (48). The MutY protein shows significant sequence similarity with *E. coli* endo III, including a conserved helix-hairpin-helix (HhH) motif and cysteine residues that create a binding site for an iron-sulfur cluster of the $(4Fe\text{-}4S)^{2+}$ type (55). MutY is also conserved in evolution, and the human homolog, MYH, has a very similar substrate specificity to that of its bacterial counterpart (56, 57).

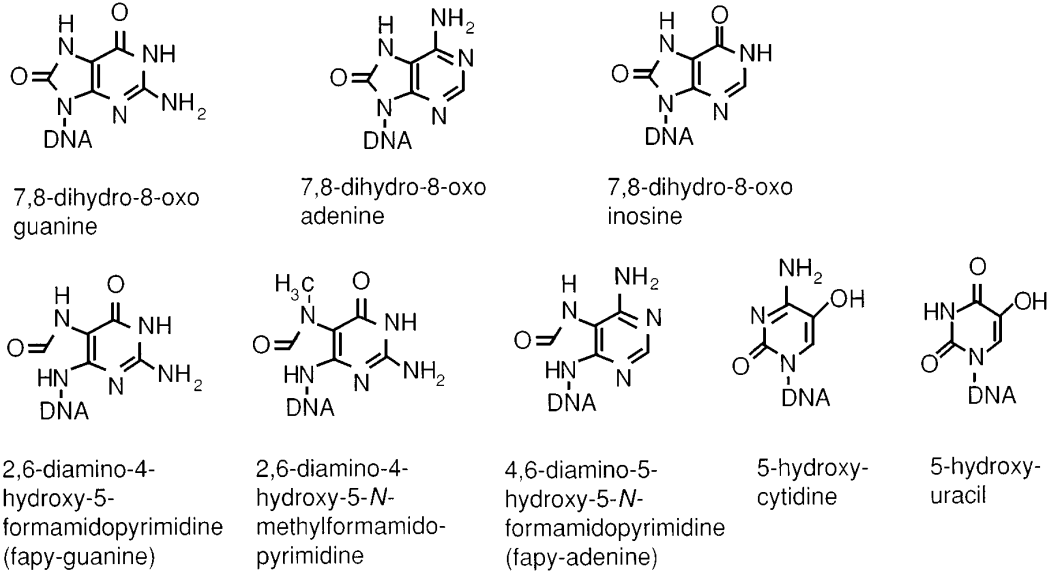


Figure 2 Examples of the structures of oxidized purine substrates for the FPG/Ogg1 class of enzymes.

Endo III excises numerous oxidation products of pyrimidines (7, 58–60). Many substrates result from reaction of ROS at the 5,6-double bond, and include cytotoxic lesions such as thymine and cytosine glycols. However, endo III also recognizes ring-opened, ring-contraction and ring-fragmentation products of cytosine and thymine residues (Figure 3). Budding yeast express two endo III homologs, Ntg1 and Ntg2, the former of which lacks the characteristic iron-sulfur cluster (61, 62). The substrate specificities of these two enzymes are similar but nevertheless different from that of endo III. The only known human endo III homolog, hNTH1, displays the major structural motifs (HhH and Fe-S cluster) found in endo III and has a substrate specificity similar to that of its bacterial counterpart (63, 64).

Glycosylases for Repair of Alkylated Bases

A selection of the wide range of products of base alkylation is shown in Figure 4. These adducts are repaired by a glycosylase designated 3-methyladenine DNA glycosylase (3-MAG) (reviewed in 7, 13). Two such enzymes, AlkA and Tag, exist in *E. coli*, the former being inducible as part of the adaptive response to DNA alkylation. A single major 3-MAG activity with a broad substrate specificity similar to that of AlkA exists in eukaryotes (7, 13). 3-MAG also efficiently excises 1,N^6-ethenoadenine (65). 3-MAG is important for repair of 3-methyladenine and 1,N^6-ethenoadenine residues in vivo, and for the protection of mammalian cells against the cytotoxic effects of certain alkylating agents (66–68). The ability of AlkA to recognize both positively charged and extended aromatic substrates derives from the presence of numerous aromatic side chains lining the catalytic cleft (69, 70). The crystal structure of AlkA indicates a nucleotide flipping mechanism of action.

Alkylation at the O^6 position of guanine is potentially highly mutagenic because of the efficiency with which O^6-alkylguanine mispairs with thymine, leading to GC $\rightarrow$ AT transition mutations. This lesion, which is also cytotoxic, is repaired by a conserved and specialized protein, O^6-alkylguanine DNA alkyltransferase (O^6-AT), reviewed elsewhere (71, 72). The expression of O^6-AT allows cells to resist the cytotoxic and mutagenic effects of a variety of alkylating agents, many of which are used in chemotherapy (71, 72).

AP Endonucleases in BER

AP endonucleases counteract the cytotoxic and mutagenic potential of AP sites. These lesions arise via spontaneous hydrolysis of the N-glycosyl bond linking a base to the phosphodiester backbone, following damage to DNA by alkylating agents or ROS, and through the action of DNA glycosylases (7, 73). There are two major families of AP endonucleases, termed the exonuclease (exo) III and endo IV families, which derive their name from the two AP endonucleases expressed in *E. coli* (7, 9, 11, 73). In addition to AP endonuclease activity, these enzymes possess phosphodiesterase activity for removal of fragmented sugar residues, such as phosphoglycolate, from the 3′ terminus of strand breaks induced by oxidants.

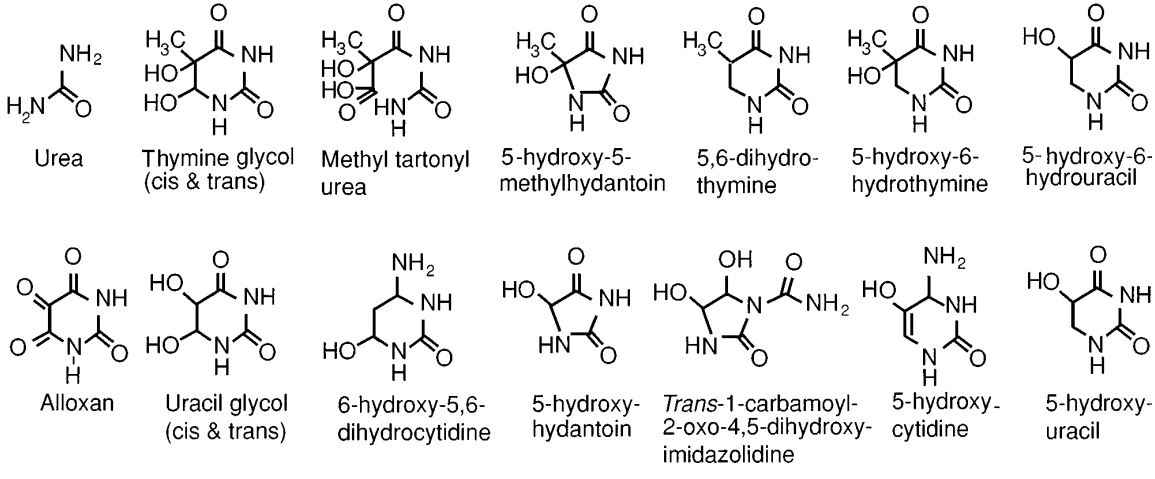


Figure 3 Examples of the structures of oxidized pyrimidine substrates for the endo III class of enzymes.

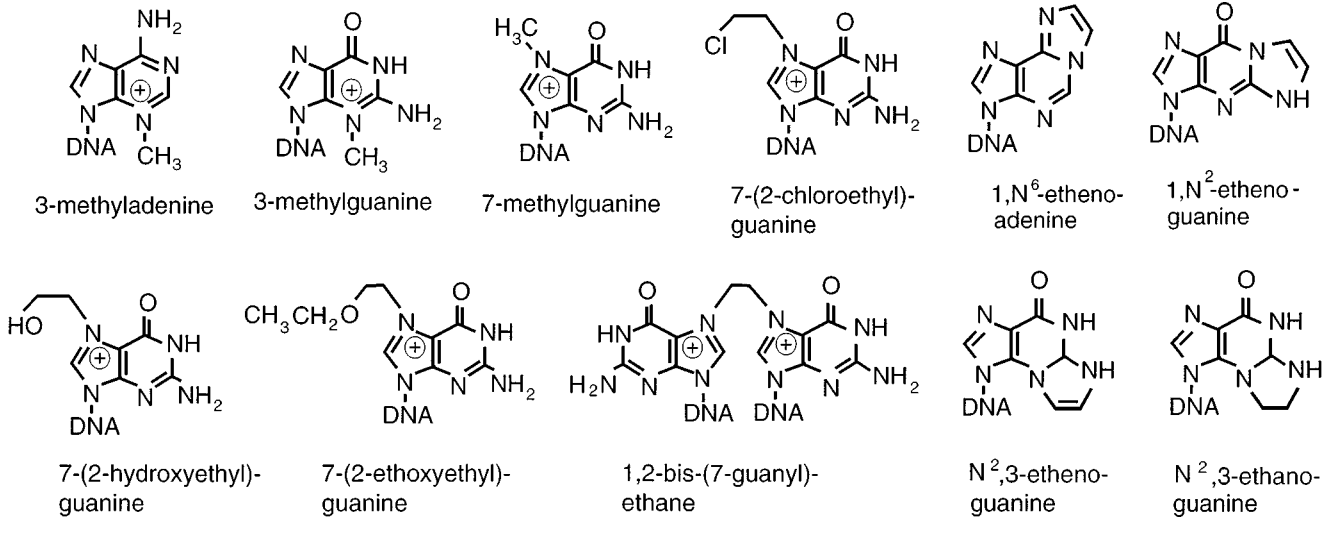


Figure 4 Examples of the structures of substrates for the 3-MAG class of enzymes.

This activity can also remove the 3′ terminal groups left by AP lyases following β-elimination at AP sites (see above). AP lyase activity is a feature of the endo III and FPG families of glycosylases (7, 9, 11).

The catalytic mechanism of action of both families of AP endonucleases has been revealed by X-ray crystallography. The human homolog of exo III, HAP1 (APE/Ref-1), shows structural similarity to DNase I (74). The abasic deoxyribose lies in an extrahelical conformation and is stabilized in the HAP1 active site by interactions with specific residues (75). Several active site residues required for the hydrolysis reaction have been identified by site-directed mutagenesis (17, 73, 76–80). Structural studies of *E. coli* endo IV have revealed that both the abasic deoxyribose and its partner nucleotide are flipped out of the duplex (81).

Structural and site-directed mutagenesis studies have suggested that HAP1 might coordinate different steps of the BER process through engaging in protein:protein interactions (12). HAP1 interacts with and displaces glycosylases that are bound to the AP sites generated by their action. Moreover, following cleavage of the phosphodiester backbone, HAP1 remains bound to the nicked DNA until direct interactions with Polβ initiate the subsequent phosphodiesterase/ polymerization steps, helping to explain how BER can be such an efficient and rapid process in vivo.

Late Stages in BER

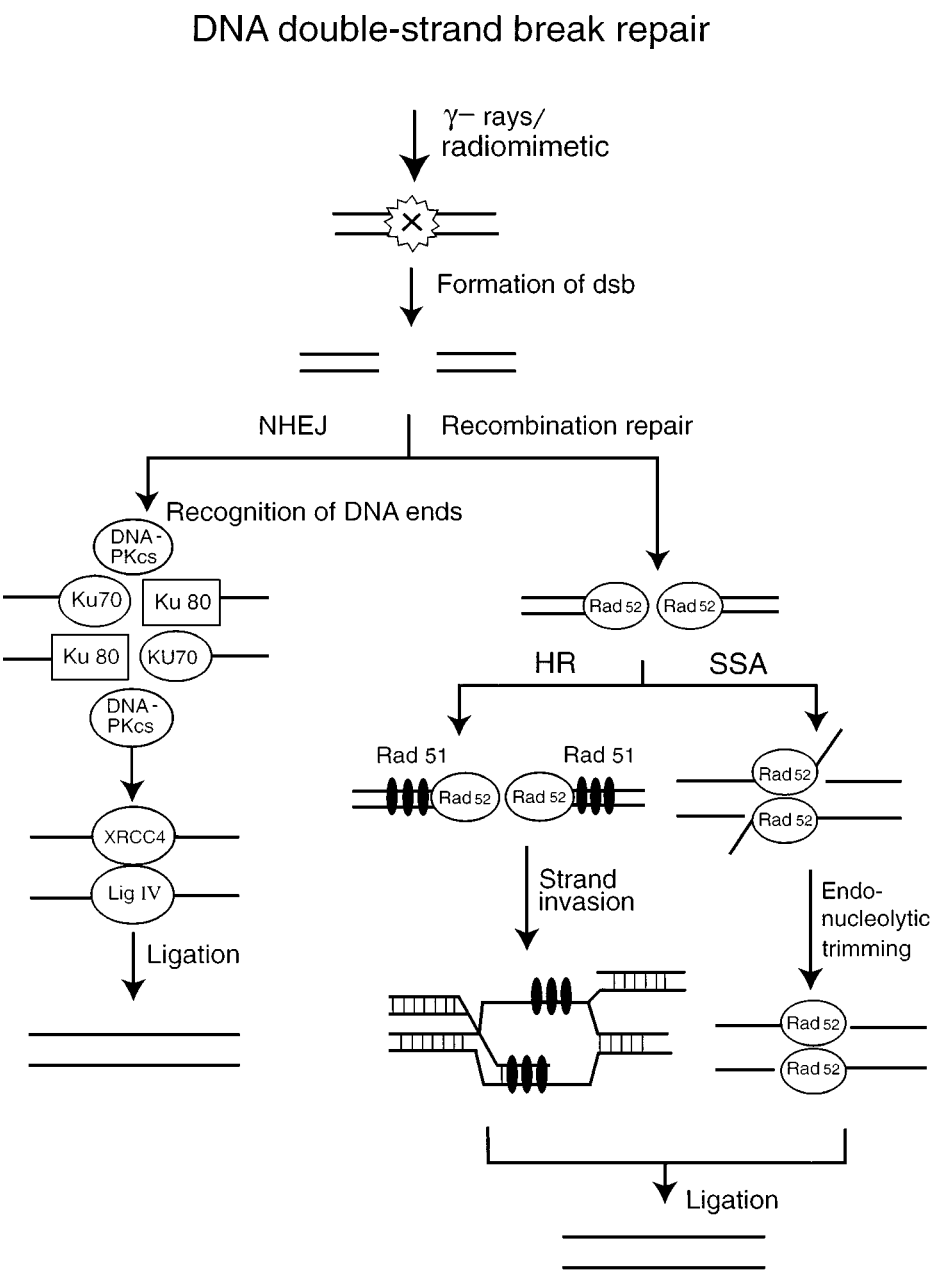
Polβ can catalyze both the 5′ phosphodiesterase and polymerization steps in BER (Figure 1). Perhaps surprisingly, recent data using Polβ-deficient mouse cells indicate that only the phosphodiesterase action of Polβ is essential for protection against methyl methane sulfonate (82). As well as binding to a HAP1:DNA complex, Polβ makes interactions with the XRCC1 protein, which appears to play a scaffolding role in BER rather than any specific enzymatic function. XRCC1 then recruits DNA ligase III to sites of ongoing repair (83).

DNA DOUBLE-STRAND BREAK REPAIR

DNA double-strand breaks (DSBs) arise in cellular DNA via a number of different routes, including exposure to ionizing radiation and radiomimetic chemicals, through the interaction of a replication fork with a single-stranded break in the template, and in a programmed manner during meiosis and immunoglobulin gene rearrangement (reviewed in 84, 85). The three primary pathways for repair of DNA DSBs (Figure 5) are discussed below.

Homologous Recombination

Knowledge of the mechanisms of homologous recombination (HR) and the protein components of the HR pathway has come primarily from genetic analyses in yeasts and subsequent biochemical studies. In *S. cerevisiae* HR is dependent upon


DNA double-strand break repair
γ– rays/
radiomimetic
Formation of dsb
NHEJ
Recombination repair
Recognition of DNA ends
DNA-
PKcs
Ku70
Ku 80
Ku 80
KU70
DNA-
PKcs
XRCC4
Lig IV
Ligation
Rad 52
Rad 52
HR
SSA
Rad 51
Rad 51
Rad 52
Rad 52
Rad 52
Rad 52
Strand
invasion
Endo-
nucleolytic
trimming
Rad 52
Rad 52
Ligation

the *RAD52* epistasis group of genes, which includes *RAD50, RAD51, RAD52, RAD54, RAD55, RAD57, RAD59, MRE11*, and *XRS2* (reviewed in 84, 86). Rad51 is the eukaryotic homolog of *E. coli* RecA, which initiates HR through catalyzing homologous pairing and DNA strand exchange. Rad51 has a substantially reduced catalytic rate compared with RecA. To compensate for this apparent catalytic deficiency, eukaryotic Rad51 acts in concert with other proteins. Replication Protein A (RPA) enhances the efficiency of DNA strand-exchange reactions mediated by Rad51, but only if added to reactions after Rad51 has created the characteristic nucleoprotein filaments on ssDNA in which strand exchange occurs (87–91). Rad52 and the Rad55/57 heterodimer both counteract the inhibitory effect of RPA, presumably by mediating exchange of Rad51 for RPA on ssDNA (87–91). Moreover, Rad54 can stimulate Rad51-catalyzed pairing of homologous DNA molecules in vitro (92, 93). Rad54 is a DNA-dependent ATPase of the Swi/Snf family whose precise role is unclear (94). Several mammalian Rad55/57-related proteins have been identified, but whether any of these act as functional homologs remains unclear. Two of these, XRCC2 and XRCC3, were identified by functional complementation of X-ray-sensitive hamster cell mutants defective in DSB-induced HR (95).

HR functions are vital for repair of DSBs (reviewed in 84–86). In a recent model proposed by Van Dyck et al (96) Rad52 acts to initiate DSB repair through binding DNA ends. This binding protects DNA ends from exonucleolytic attack and facilitates end-to-end joining. Rad52 then loads Rad51 onto the DNA through direct protein:protein interaction. This end-binding function probably explains why Rad52 also acts during the single-strand annealing (SSA) pathway (see below). It is possible that Rad52 acts in partial competition with the Ku DNA end-joining complex to direct DSB repair down the HR pathway instead of the NHEJ pathway (Figure 5).

In *S. cerevisiae RAD52* is essential for DSB repair and HR (86). However, *RAD52*$^{-/-}$ mice are viable and fertile, and their cells do not display deficiency in DSB repair (97). *RAD54*$^{-/-}$ mice are also viable and fertile, despite having a cellular defect in the repair of DNA cross-links. These mice are hypersensitive to γ-rays at the embryonic, but not adult, stages (98, 99). In contrast, *RAD51*$^{-/-}$ mice die during early embryonic development.

The roles played by other DSB repair proteins are not fully elucidated. In yeast the Rad50/Mre11/Xrs2 complex provides nuclease activity and is implicated in

Figure 5 Schematic representation of the three pathways for DNA double-strand break repair. DNA double-strand breaks generated by γ-rays or radiomimetic drugs are processed during either the nonhomologous end-joining (NHEJ) or the recombination-dependent pathways (right), possibly by competition between the Ku70/Ku80 complex and Rad52 for binding the ends. In NHEJ (left pathway) the Ku complex recruits DNA-PK$_{cs}$ and the XRCC4/ligase IV heterodimer to effect rejoining. In homologous recombination Rad51 is recruited by Rad52 to promote DNA strand invasion of an intact sister-chromatid or homologous chromosome. In single-strand annealing, resection and annealing of short complementary sequences precedes trimming of the noncomplementary ssDNA tails and ligation. Figure adapted from Karran (85).

resection of DSBs to create the 3′ ssDNA tail necessary for Rad51 to initiate strand exchange (84–86). Although the Rad50 and Mre11 proteins are highly conserved, Xrs2 is not. However, a protein with limited similarity to Xrs2, called NBS1 (or nibrin), appears to be the functional analog of Xrs2 (100–103). NBS1 has recently been shown to be the protein defective in the ataxia telangiectasia-like disorder, Nijmegen breakage syndrome.

HR is probably more complex in human cells than in yeasts. Two proteins that appear to play important roles in DSB repair are BRCA1 and BRCA2, both of which are tumor suppressor genes (104). Mouse fibroblasts deficient in BRCA1 or BRCA2 show sensitivity to DNA-damaging agents and excessive chromosomal aberrations (105, 106). BRCA1 and BRCA2 interact directly or indirectly with each other and with RAD51, providing a connection with HR (107). A suggested role for BRCA1/2 is in transducing signals from factors that recognize DNA damage to the DNA repair machinery (see below).

Single-Strand Annealing (SSA)

SSA, like HR, is a process for rejoining DSBs using homology between the ends of the joined sequences (Figure 5). However, SSA differs from HR in the manner in which this is achieved. The DNAs to be joined are first resected to generate ssDNA tails. When this resection has proceeded sufficiently to reveal complementary sequences, the two DNAs can than be annealed prior to DNA ligation. Inevitably, SSA has the potential to create deletions between repetitive sequences. SSA requires the Rad52 protein in *S. cerevisiae* but not Rad51, consistent with a lack of a requirement for DNA-strand invasion. The role for Rad52 is probably to bind the DNA ends and effect DNA annealing (84–86).

SSA requires the Rad50, Mre11, Xrs2 protein complex in *S. cerevisiae* and the functionally analogous proteins in humans: RAD50, MRE11, and NBS1 (84–86). It is likely that this complex is involved in the resection of DNA ends. After resection and annealing of complementary DNA sequences, the ssDNA tails must be trimmed (Figure 5). This trimming is probably performed by the structure-specific XPF-ERCC1 endonuclease (Rad1/Rad10 in yeast) in human cells.

Nonhomologous End-Joining (NHEJ)

NHEJ is a process whereby dsDNA ends can be rejoined even where there is little or no base-pairing at the site of the junction. This pathway for DSB repair is apparently unique to eukaryotes. A conserved set of proteins, designated Ku70, Ku80, DNA ligase IV and XRCC4 in humans, is required for NHEJ (Figure 5) (84–86). In vertebrates the catalytic subunit of DNA-dependent protein kinase (DNA PK_{CS}) is also required (108). NHEJ is not only relevant to repair of dsDNA breaks induced by DNA damaging agents, but also to the processing of breaks arising during immunoglobulin gene rearrangement. Indeed, severe combined immune-deficiency in mice is associated with inactivation of DNA PK_{CS} (108). Mice with a targeted

disruption of the Ku80 gene show γ-ray sensitivity, a severe combined immune-deficiency phenotype, and an increase in chromosomal aberrations (109–112). In contrast, disruption of the XRCC4 or DNA ligase IV genes in mice leads to embryonic lethality (113–115). Hence, deletion of the genes encoding DNA PK_{CS}, Ku subunits or ligase IV reveals significant differences in phenotypes, suggesting that these proteins may play independent roles outside the NHEJ pathway. The XRCC4/ligase IV heterodimeric complex is recruited to DNA ends by the Ku complex (116). NHEJ of linearized plasmid DNA has been achieved using cell free extracts (117).

CELL CYCLE CHECKPOINTS ACTIVATED IN RESPONSE TO DNA DAMAGE

DNA repair is an important aspect of genomic maintenance whether or not cells are actively proliferating, but proliferation brings with it added complicating factors. Failure to complete repair before chromosomal replication or segregation can lead to fixation of mutations in the genome, or to irretrievable chromosome breakage. Genome integrity is therefore preserved in part by mechanisms that ensure that cell cycle progression is delayed until DNA damage is removed. These so-called checkpoint mechanisms can lead to cell cycle delay in the G1, G2, or S phase. Loosely analogous mechanisms may act to delay DNA replication in bacteria (118), but this discussion is restricted to checkpoint controls in eukaryotes. Many checkpoint components are not required for cell cycle progression per se, but are brought into play specifically when DNA is damaged or replication blocked. In addition to their inhibitory cell cycle roles, several checkpoint components have functions related to activation of DNA repair, nucleotide metabolism, telomere maintenance, or induction of cell death.

Checkpoints were first defined through the identification of radiation-sensitive (*rad*) yeast mutants that fail to delay progression into mitosis after DNA damage (119). Artificial imposition of cell cycle arrest, for example by the use of spindle poisons, can be sufficient to correct the DNA-damage sensitivity of checkpoint mutants, underlining the importance of this delay for cell survival. Genetic screens in the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* have defined the pathways linking checkpoint gene products, and these interactions are increasingly being clarified at the biochemical level (120, 121). In general, the radiation sensitivity of checkpoint mutants parallels sensitivity to other agents inducing DNA double-strand breaks or forming DNA adducts, though the defects in some mutant strains are lesion specific.

Mec1/Rad3/ATM/ATR Activation: A Fundamental Eukaryotic Checkpoint Response to DNA Damage

Checkpoint genes functionally related to those identified in yeast model systems have been identified in multicellular organisms through analysis of mutants

TABLE 1 Orthologous checkpoint proteins in yeast and mammalian cells[a]

S. pombe	*S. cerevisiae*	Mammals	Protein family	Interacting proteins
Rad1	Rad17Sc	hRAD1	PCNA-like; 3′–5′ exonuclease	Rad9Sp/Ddc1/hRAD9, Hus1/Mec3/hHUS1
Rad3	Mec1	ATM, ATR	PI3-K-like protein kinase	Rad26, Chk1, Cds1, NBS1/nibrin, BRCA1, BLM, c-Abl
Rad9Sp	Ddc1	hRAD9	PCNA-like	Rad1/Rad17Sc/hRAD1, Hus1/Mec3/hHUS1, BCL-2, BCL-xL
Rad17Sp	Rad24	hRAD17	RF-C-like	RF-C
Rad26	?	?	?	Rad3
Hus1	Mec3	hHUS1	PCNA-like	Rad1/Rad17Sc/hRAD1, Rad9Sp/Ddc1/hRAD9
Rhp9/Crb2	Rad9Sc	BRCA1?	BRCT repeat-containing	Chk1 (*S. pombe*), Rad4/Cut5, Rad53
Rad4/Cut5	Dbp11	?	BRCT repeat-containing	Rhp9/Crb2
Chk1	Chk1	CHK1	Ser/Thr protein kinase	Rad3, Rhp9/Crb2, Cut5, Rad24, Rad25 (*S. pombe*)
Cds1	Rad53	hCDS1/CHK2	Ser/Thr protein kinase	Rad3

[a]Abbreviations: PCNA, proliferating cell nuclear antigen; ATM, ataxia-telangiectasia mutated; ATR, ATM-related; RF, replication factor.

sensitive to radiation and by sequence database searches. Although the corresponding checkpoint pathways in various eukaryotic species differ in their detail, their overall organization is conserved (Table 1).

Many of the checkpoint determinants defined genetically have been found to be phosphoproteins and/or protein kinases. Prominent among these are members of a family of large kinases including *S. cerevisiae* Mec1, *S. pombe* Rad3, and the human proteins ATM and ATR. These proteins belong to the phosphoinositide 3-kinase superfamily, but phosphorylate protein substrates rather than lipids. Each is activated in response to DNA damage, and a number of phosphorylation events have been identified that are dependent on activation of these kinases. On this basis, the Mec1/Rad3/ATM/ATR family is thought to act early in the checkpoint pathway, either as DNA-damage detectors or in close association with such detectors. In mammalian cells, these kinases are activated in a somewhat lesion-specific fashion, with ATM being specific for agents that induce DNA, DSB, and ATR, probably responding to UV-induced damage (122). In contrast, the yeast members of the family are activated in response to a wide variety of DNA structures, including stalled replication forks.

Additional conserved checkpoint components that function in concert with the Mec1/Rad3/ATM/ATR kinases have been identified. In *S. pombe* these include the "checkpoint Rad" proteins Rad1, Rad9Sp, Rad17Sp, Rad 26, and Hus1, loss of any one of which confers radiation sensitivity and checkpoint defects very similar to those seen in cells lacking Rad3. Double mutants combining defects in any two of the checkpoint *rad* genes have phenotypes indistinguishable from the corresponding single mutants, suggesting that all of these gene products act in a common pathway.

Many of the checkpoint Rad proteins display low level, but significant, similarity to proteins involved in DNA replication. Specifically, Rad1, Rad9Sp, and Hus1 are related to the DNA polymerase accessory protein PCNA, whereas Rad17Sp is related to subunits of RF-C. These sequence similarities are reflected in physical associations, as Rad1, Rad9Sp, and Hus1 are found together in a complex (123, 124), as are their equivalents in *S. cerevisiae* (125) and human cells (126), whereas Rad17Sp (like Rad24, its equivalent in *S. cerevisiae*) is associated with authentic RF-C subunits on chromatin (127, 128). During DNA replication RF-C functions to load PCNA onto chromatin. By analogy, it might be expected that Rad17Sp, in association with conventional RF-C subunits, might load the Rad1/Rad9Sp/Hus1 complex onto DNA in response to DNA damage. Just as PCNA acts by tethering replicative polymerases to their template, Rad1/Rad9Sp/Hus1 could form a sliding clamp, which has the dual purpose of checkpoint signaling and recruiting DNA repair enzymes (129). Additional evidence supporting this model has come from studies of temperature-sensitive mutants with defects in RF-C subunits. Such mutants not only fail to suppress mitosis during DNA replication, but also display sensitivity to DNA-damaging agents, associated with loss of checkpoint control (127, 130). In human cells hRAD17 is localized to the nucleolus and redistributed to the nucleoplasm following UV irradiation (131), but the significance of this potential mode of regulation has yet to be established.

A straightforward checkpoint pathway model would comprise damage detectors, signal transducers and cell cycle inhibitory effector mechanisms, but this type of linear representation may not be adequate to explain all of the experimental observations relating to Mec1/Rad3/ATM/ATR and the other checkpoint Rad proteins. For example, studies in *S. cerevisiae* using damage in the form of single-stranded DNA near telomeres, induced by a defect in the Cdc13 protein, place Mec1 downstream from Rad17Sc, Rad24, Mec3, and the checkpoint determinant Rad9Sc (132). These studies have given rise to an alternative view of the DNA-damage checkpoint pathway, in which Rad17Sc, Rad24, and Mec3 serve to activate an exonuclease (perhaps Rad17Sc itself). This nuclease processes primary lesions in a manner that leads to the generation of a checkpoint signal. Conversely, protein phosphorylation studies suggest that Rad9Sc acts downstream from Mec1 (133, 134). Similarly, Rhp9/Crb2, the Rad9Sc analogue in *S. pombe*, appears to act downstream from Rad3 (135, 136), and DNA damage–induced, Rad3-dependent phosphorylation of Rad26 is independent of all other known checkpoint proteins

(136). It has been suggested that some of these phosphorylation events could reflect the activation of feedback controls, rather than the transmission of the checkpoint signal itself (137); hence, an all-embracing model describing the order in which these checkpoint components act is still elusive.

Effectors of the Checkpoint Rad Pathway

Checkpoint signaling downstream from Mec1/Rad3/ATM/ATR and the other checkpoint Rad proteins is focused on serine/threonine protein kinases typified by the yeast proteins Chk1 and Rad53/Cds1, and their equivalents in vertebrates, CHK1 and CDS1/CHK2. The positioning of these kinases downstream from the checkpoint Rad proteins reflects the fact that Chk1 overexpression can partially suppress the DNA-damage sensitivity of checkpoint *rad* mutants (138), and that Chk1, Cds1, CHK2, or Rad53 activation after DNA damage is checkpoint Rad dependent (139–142). The detailed requirements for activation of these kinases, and the consequences of their inactivation, differ within and between species. Chk1 mutants are radiation sensitive in *S. pombe*, but not in *S. cerevisiae* (143), where the Cds1-like kinase, Rad53, assumes a major checkpoint signaling role after DNA damage. Conversely, in fission yeast Cds1 is activated specifically during S phase, and is required for S-phase retardation in response to DNA damage (141), but *cds1* mutants are not appreciably radiation sensitive.

A variety of mechanisms have been identified downstream from these Ser/Thr kinases that can transduce checkpoint signals to cell cycle effector proteins. In fission yeast and human cells the key mitotic cyclin-dependent kinase, Cdc2, is a principal target of the checkpoint pathway activated in response to DNA damage or inhibition of replication. Cdc2–cyclin B heterodimers act to trigger all the major events of mitosis (144, 145), and inhibition of this protein kinase is sufficient to prevent mitotic entry. Inhibition of Cdc2–cyclin B in response to checkpoint activation is associated with phosphorylation of the Cdc2 subunit at inhibitory residues Thr14 and/or Tyr15 (reviewed in 121). Phosphorylation at these sites is carried out principally by the Wee1 protein kinase, with secondary activities attributable to the kinases Mik1 and Myt1 in *S. pombe* and human cells, respectively. These cell cycle inhibitory kinases are counterbalanced by the Cdc25 protein phosphatase, which activates Cdc2 by removing phosphate from Tyr15. An abrupt switch between interphase and mitosis is ensured by positive feedback mechanisms, through which active Cdc2 serves both to inhibit Wee1 and to activate Cdc25. During the checkpoint response to DNA damage, activation of Chk1 or Cds1 has the opposite effect. These kinases phosphorylate Cdc25 on Ser216, generating a recognition signal for 14-3-3 proteins, which sequester Cdc25 in the cytoplasm (Figure 6). The biological activity of Wee1 may also be increased by Chk1-mediated phosphorylation, and both Wee1 and Mik1 are stabilized in a Chk1-dependent manner (146, 147). The importance of these mechanisms that converge on Thr14/Tyr15 phosphorylation of Cdc2 is underlined by the checkpoint-defective phenotypes induced in

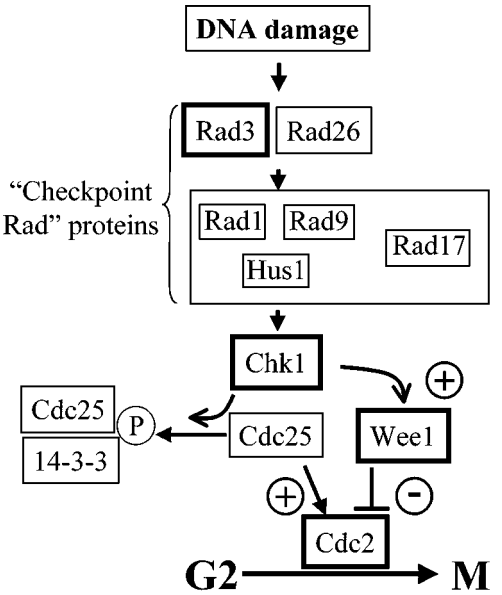


Figure 6 Components of the G2 DNA damage checkpoint pathway. The DNA damage signal is transduced via the checkpoint Rad proteins and the Chk1 protein kinase. Inhibition of the mitotic inducer Cdc2 is brought about by inactivation of Cdc25 and activation of Wee1. Protein kinases are indicated by bold boxes. For simplicity, only the fission yeast nomenclature is shown; for further details see text.

S. pombe or human cells by mutant Cdc2 proteins lacking these phosphorylation sites (148, 149).

In *S. cerevisiae* inhibition of Cdc28, the Cdc2 homolog, is not a significant feature of the DNA-damage checkpoint, which instead operates to block onset of mitotic anaphase as well as exit from mitosis (143). These effects are respectively brought about through the Chk1-dependent stabilization of the Pds1 sister chromatid cohesion protein and the Rad53-dependent inhibition of the Cdc5 protein kinase. Analogous mechanisms may operate in other organisms, but are perhaps masked by the Cdc2 pathway, which leads to arrest at an earlier stage in the cell cycle.

The BRCA1 tumor suppressor protein is a substrate of both ATM and CHK2 (150, 151), and *BRCA1* mutant cells are defective in DNA damage–induced G2 arrest (152), possibly because they fail to downregulate cyclin B transcription (153). Mutated forms of BRCA1 lacking sites of ATM/CHK2-dependent phosphorylation are unable to reverse the radiation hypersensitivity of BRCA1-deficient cell lines, suggesting that these phosphorylation events are indeed involved in transduction of the checkpoint signal.

A number of observations suggest a significant link between the DNA damage checkpoint and telomere maintenance. Telomere shortening has been observed in $ATM^{-/-}$ (154) and checkpoint *rad* mutants (155–157). Furthermore, the Tel1 protein, which is principally concerned with telomere maintenance, can partially compensate for loss of Mec1 (144, 158). However, there is a functional

distinction between DNA damage checkpoint and telomere maintenance functions of checkpoint Rad proteins in *S. pombe*, as the latter function does not require Chk1 or Cds1 (155). The telomere maintenance function may, therefore, be the evolutionary precursor from which the DNA-damage checkpoint mechanism was derived.

Slowing of DNA replication in response to DNA DSB is checkpoint Rad and Rad53/Cds1 dependent in yeasts (159, 160) and ATM dependent in human cells (161), but the effector mechanism(s) in this case are not clearly understood. NBS1 is phosphorylated by ATM and may act downstream in this pathway (162–164). The involvement of NBS1 in the S-phase damage checkpoint may be secondary to its principal role in DNA repair, as discussed above. NBS1, like Xrs2, its functional counterpart in budding yeast, is associated with MRE11 and RAD50, which are required both for DSB repair and for telomere maintenance (165, 166). The overlap between checkpoint controls, DNA repair, and telomere maintenance is therefore extensive.

Damage Tolerance and Cell Cycle Resumption

In yeasts protracted failure to repair DNA damage can be followed by cell cycle re-entry in the presence of persistent DNA strand breaks (167, 168). Cell cycle resumption under these circumstances requires Ku70, in the absence of which residual breaks are subject to 5′ to 3′ degradation. Tolerance of DNA strand breaks necessarily involves the suppression of cell cycle arrest; ssDNA generated in the absence of Ku70 presumably triggers a checkpoint signal that cannot be suppressed in this way. In line with this interpretation, mutations in Mre11 or Rad50, which reduce the extent of ssDNA generation, also suppress the permanent arrest seen after damage in Ku70 mutants. In *S. pombe*, cell cycle restart after arrest at the DNA-damage checkpoint requires phosphorylation of Crb2 by Cdc2 (169), but it is not yet clear whether analogous mechanisms operate in more complex organisms.

Cell Cycle Checkpoint Roles of p53

DNA-damage checkpoints in higher eukaryotes have acquired additional levels of complexity not found in yeast cells. The tetrameric transcription factor and tumor suppressor p53 is central to these higher eukaryotic checkpoint controls. The requirement for p53 in DNA damage–induced G1 arrest is largely, though not completely, explicable in terms of transcriptional induction of the cyclin-dependent kinase inhibitor $p21^{WAF1}$, which binds to and inhibits G1 cyclin-dependent kinases. This mode of cell cycle arrest can range from transient to very protracted depending on the cell type concerned (170). Activation of p53 in response to DNA damage and other stresses involves complex posttranslational modification of p53 and its negative modulator MDM-2, which is itself induced by p53 at the transcriptional level. MDM-2 targets p53 for proteolysis by the ubiquitin/proteasome pathway

(171), and downregulation of this proteolytic pathway following DNA damage allows p53 accumulation.

Activation of p53 also contributes to DNA damage–induced G2 arrest, through the increased expression of both 14-3-3σ, which sequesters Cdc2–cyclin B heterodimers in the cytoplasm, and $p21^{WAF1}$, which can inhibit Cdc2 (172–175). Transcription of the genes encoding CDC2 and cyclin B can also be repressed in a p53-dependent manner (176–178). These p53-dependent mechanisms act in parallel with the checkpoint Rad pathway. Cells lacking functional p53 are therefore wholly reliant on the checkpoint Rad proteins and exhibit a less robust damage-induced G2 arrest than do cells with functional p53. Because p53 function is lost by one means or another in a large proportion of many tumor types, it may be possible to exploit this generality by using DNA damaging agents in combination with drugs that inactivate the already weakened checkpoint responses of p53-compromised cells. Checkpoint-inhibitory drugs with these properties include staurosporines, which can inhibit Chk1 (179), and caffeine, which probably targets Mec1/Rad3/ATM/ATR (180, 181).

Regulation of p53 Following DNA Damage

The p53 pathway has arisen relatively recently during eukaryotic evolution and appears to have taken advantage of the pre-existing checkpoint Rad DNA damage signaling pathway (Figure 7). Human p53 is phosphorylated at serine residues 15, 20, 33, and 37 in response to DNA damage (reviewed in 182), potentially modulating its interaction with MDM-2. Stabilization of p53 and its phosphorylation at Ser15 following DNA DSB are ATM dependent, whereas following UV irradiation, Ser15 phosphorylation of p53 is ATM independent and can be blocked by expression of a dominant negative form of ATR. Phosphorylation at Ser15 and Ser37 may promote the interaction of p53 with the TFIID transcription factor. However, recent evidence suggests that phosphorylation at Ser20 is more significant for p53 stabilization (183). CHK1 and CHK2 phosphorylate p53 at Ser20, and the ATM/ATR-dependent activation of these kinases partly explains the requirement for ATM/ATR in p53 stabilization (183, 184). In line with this interpretation, cells lacking CHK2 are defective for p53 stabilization (185), and germ-line mutations in *CHK2* can give rise to Li-Fraumeni syndrome, a cancer-prone disorder that can also be caused by germ-line mutations in the *TP53* gene encoding p53 (186). ATM-dependent phosphorylation of MDM-2 also modulates the p53-MDM-2 interaction and hence contributes to DNA damage–induced p53 stabilization (187).

Additional DNA damage–induced posttranslational modifications of p53 occur in the C-terminal region, which modulates the sequence-specific DNA-binding activity of the protein. Phosphorylation of Ser392 stimulates this activity and is induced by UV but not by ionizing radiation (188). Ser376 and Ser378 are phosphorylated in unirradiated cells, but IR induces rapid and ATM-dependent

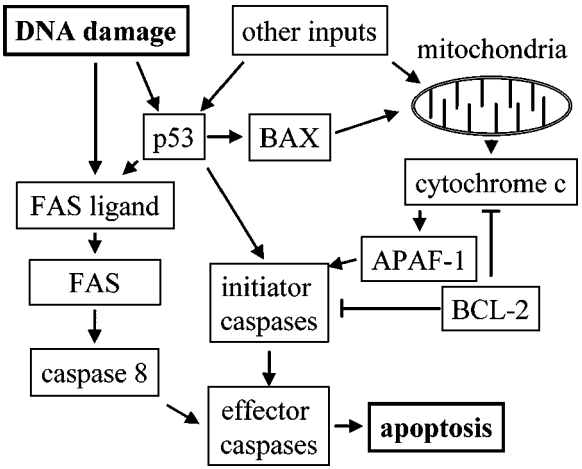


Figure 7 Activation of p53 following DNA damage. The DNA damage-activated protein kinases ATM/ATR and CHK1/CHK2 serve to inhibit the p53 antagonist MDM-2 and activate the transcriptional activity of p53. A parallel activating pathway involves the DNA damage-induced acetylation of p53 by PCAF and p300/CBP. Activation of p53 can lead to apoptotic cell death or cell cycle arrest, according to the cellular context.

dephosphorylation of Ser376. This unmasks a site to which 14-3-3 proteins bind, leading to elevated sequence-specific DNA binding by p53. ATM is presumed to activate the phosphatase that acts on Ser376.

p53 is modified by acetylation on Lys320 by the PCAF acetyl transferase after exposure of cells to UV, and on Lys373 and Lys382 by p300/CBP in response to both IR and UV (189, 190). Acetylation of these residues activates sequence-specific DNA-binding activity of p53 in vitro. Significantly, p53 that is phosphorylated either on Ser15 or on both Ser33 and Ser37 is a preferred substrate for p300/CBP, whereas phosphorylation at Ser378 strongly inhibits acetylation by PCAF (190). Hence, DNA damage–induced modifications of p53 are carefully coordinated and interdependent.

Checkpoint Controls and DNA Repair: Further Connections

Activation of the DNA-damage checkpoint in *S. cerevisiae* leads to the transcriptional induction of a number of genes encoding DNA repair proteins. This pathway requires the Dun1 protein kinase in addition to Mec1 and Rad53 (120). Related mechanisms are likely to operate in other eukaryotes and may help explain experimental observations that suggest a role for ATM in DNA repair by NHEJ (191).

ATM was recently found to be a component of a very large complex that also contains BRCA1, MRE11/RAD50/NBS1, the BLM helicase, MSH2, MSH6, MLH1, and RF-C (192). This complex is potentially involved in the recognition of

diverse abnormal DNA structures, in the transmission of checkpoint signals, and in various aspects of DNA repair itself. When a more comprehensive overview of these processes becomes available, the boundaries between them are likely to be rather indistinct.

CELL DEATH

As an alternative to DNA repair or damage tolerance, multicellular eukaryotes have at their disposal a dramatic strategy for dealing with DNA damage, namely the engagement of the apoptotic cell death pathway. Although this is often viewed as a mechanism for eliminating irreparably damaged cells that might otherwise threaten the health of the organism, the threshold level of damage required to trigger apoptosis is highly cell type–dependent. Levels of damage sufficient to induce rapid apoptosis in thymocytes, for example, induce cell cycle arrest followed by apparently complete repair in other cell lineages. Apoptosis induced by DNA damage is indistinguishable at the cellular level from genetically programmed cell death that occurs during the development of a variety of tissues. Characteristic alterations seen in both cases include cell rounding, plasma membrane blebbing, peripheral condensation of the nuclear DNA without disassembly of the nuclear envelope, and internucleosomal DNA cleavage. These changes distinguish apoptosis from necrotic cell death, which can also result from DNA damage but is not viewed as an active cellular response and is therefore not discussed here.

The events of apoptosis are brought about by activation of members of a specialized family of cysteine aspartyl proteases, the caspases (reviewed in 193). The importance of these enzymes was first revealed by genetic studies of *C. elegans*, which showed that the caspase product of the *ced-3* gene is essential for developmentally regulated apoptosis. Similarly, specific inhibition of caspases in mammalian cells is sufficient to block the morphological aspects of apoptosis and delay cell death. Active caspases are heterodimeric, consisting of large and small subunits generated by caspase-mediated cleavage of a common procaspase precursor protein; there is therefore the potential for amplification of apoptotic signals through caspase cascades. In human cells caspases 8 and 9 play key regulatory roles in these cascades, whereas other members of the family perform effector roles downstream (Figure 8). In some cases release of cytochrome *c* from mitochondria appears to be an early step in caspase 9 activation. Cytochrome *c* release stimulates the human APAF-1 protein, a structural relative of the pro-apoptotic CED-4 protein in *C. elegans*, to bind and activate procaspase 9.

BCL-2 is the prototypical member of a second family of proteins with important functions in the regulation of apoptosis. A BCL-2 homologue in *C. elegans* is encoded by the *ced*-9 gene and functions as a suppressor of CED-3-mediated cell deaths. Additional BCL-2-related proteins in mammals have roles in either the promotion or the inhibition of apoptosis. Members of the BCL-2 family are capable of forming homo- and heterodimers, and probably function primarily as

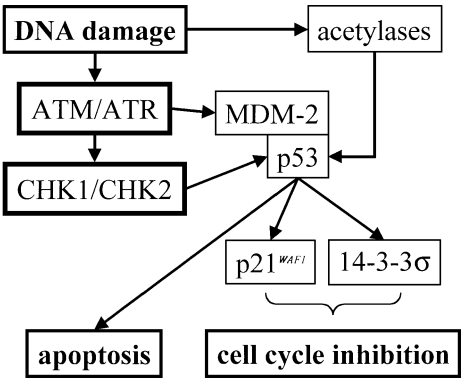


Figure 8 Overview of DNA damage-induced apoptosis. Initiator caspases can be activated by a variety of signals including DNA damage, some of which are transduced by pathways leading to p53 activation. Cytochrome *c* release from mitochondria can also contribute to caspase activation through APAF-1 binding. This process is promoted by BAX, which can be upregulated by p53, and is inhibited by BCL-2 and related anti-apoptotic proteins. Effector caspases can be activated independently via caspase 8 activation following engagement of the FAS receptor. For further details see text.

regulators of caspase activation, in part by governing mitochondrial cytochrome *c* release.

Receptor-Mediated Cell Death

Aside from DNA damage, other physiologically important triggers of apoptosis include growth factor withdrawal, depletion of nucleotide pools, and engagement of transmembrane "death receptors" of the tumor necrosis factor (TNF) receptor superfamily. The best-characterized members of this family are FAS (also known as CD95 or APO1) and the TNF receptor (TNFR1) itself, the intracellular domains of which share a common structure termed the "death domain." The pro-apoptotic ligands for these receptors are homotrimeric peptides that are either soluble or expressed at the surface of adjacent cells. Ligand-induced receptor clustering promotes the binding of a soluble cytosolic adapter protein called FADD, which in turn mediates caspase 8 activation. Perhaps surprisingly, DNA damage–induced apoptosis can be FAS- and FAS ligand–dependent (194–196). Thus, a death signaling pathway presumed to be initiated by DNA damage in the nucleus can include extracellular components. This aspect of apoptotic signaling may help explain "bystander" effects, whereby undamaged cells adjacent to those exposed to DNA-damaging agents are also susceptible to apoptotic stimuli (197).

p53-Dependent and p53-Independent Apoptosis

In addition to its cell cycle regulatory functions, p53 is absolutely required for some forms of apoptosis, such as DNA damage–induced thymocyte death in vivo

(198). The degree to which p53 is activated can be central to the cellular decision of whether to arrest the cell cycle or to initiate cell death after exposure to DNA damage or other stresses (199). Potential mechanisms for this pro-apoptotic effect include p53-mediated transcriptional induction of *FAS* (195), or of *BAX* and *NOXA*, which encode pro-apoptotic members of the BCL-2 family (200–202). Induction by p53 of the transcription factor NF-κB may also be a significant death-promoting pathway (203), and a nontranscriptional apoptotic function for p53 has also been proposed (204).

The extent of DNA damage can influence the extent of subsequent apoptosis without altering p53 levels, and some forms of apoptosis are p53-independent (198, 205, 206). In some such cases, apoptosis results from induction of the FAS ligand through the activation of the transcription factor AP-1, via the c-Jun N-terminal kinase (207, 208). Activation of AP-1 and NF-κB, like activation of p53, is not necessarily pro-apoptotic, however, and in some circumstances each can instead act to delay or block cell death (209, 210).

COORDINATING CELL CYCLE CONTROLS, DNA REPAIR, AND APOPTOSIS

DNA damage–induced apoptosis can be initiated from any point in the cell cycle, as well as from a quiescent [G0] state. There is therefore no obligatory connection between cell cycle regulators and the molecules responsible for the onset of apoptosis. Several experimental observations nonetheless suggest that apoptosis can be triggered by resumption of cycling in cells that have previously been arrested at the G2 checkpoint following DNA damage (see, for example, 211). Progression into mitosis with DNA strand breaks could generate secondary damage capable of triggering a powerful apoptotic stimulus. In normal epithelial cells DNA-damage–induced, p53-independent apoptosis has delayed kinetics in comparison with p53-dependent death (212). This delay could reflect a requirement for cell cycle progression and/or lesion processing in p53-independent apoptotic induction.

As discussed above, the checkpoint Rad pathway is a primordial eukaryotic DNA-damage response to which additional effector mechanisms have been added during the course of evolution. This applies not only to p53, but also to other apoptotic regulators. For example, in human cells hRAD9 interacts with BCL-2 in a manner that appears to promote apoptosis (213). Enforced expression of BRCA1, which may recapitulate some aspects of the DNA-damage response routed through ATM, leads to c-Jun N-terminal kinase–dependent apoptosis (214). Furthermore MRT-2, the Rad1 homolog in *C. elegans*, is required for DNA damage–induced apoptosis as well as cell cycle arrest (215). The extent to which activation of the checkpoint Rad pathway is involved in the human p53-dependent and p53-independent apoptotic pathways outlined above awaits further clarification.

LITERATURE CITED

1. Wood RD. 1996. DNA repair in eukaryotes. *Annu. Rev. Biochem.* 65:135–67
2. de Laat WL, Jaspers NG, Hoeijmakers JHJ. 1999. Molecular mechanism of nucleotide excision repair. *Genes Dev.* 13:768–85
3. Batty DP, Wood RD. 2000. Damage recognition in nucleotide excision repair of DNA. *Gene* 241:193–204
4. Fishel R. 1999. Signaling mismatch repair in cancer. *Nat. Med.* 5:1239–41
5. Buermeyer AB, Deschenes SM, Baker SM, Liskay RM. 1999. Mammalian DNA mismatch repair. *Annu. Rev. Genet.* 33:533–64
6. Jiricny J. 2000. Mediating mismatch repair. *Nat. Genet.* 24:6–8
7. David SS, Williams SD. 1998. Chemistry of glycosylases and endonucleases involved in base-excision repair. *Chem. Rev.* 98:1221–61
8. Lindahl T. 1993. Instability and decay of the primary structure of DNA. *Nature* 362:709–15
9. Demple B, Harrison L. 1994. Repair of oxidative damage to DNA: enzymology and biology. *Annu. Rev. Biochem.* 63:915–48
10. Loeb L, Preston B. 1986. Mutagenesis by AP sites. *Annu. Rev. Genet.* 20:201–30
11. Wallace S. 1988. AP endonucleases and DNA glycosylases that recognise oxidative DNA damage. *Environ. Mol. Mutagen.* 12:431–77
12. Wilson SH, Kunkel TA. 2000. Passing the baton in base excision repair. *Nat. Struct. Biol.* 7:176–78
13. Seeberg E, Berdal KG. 1997. Repair of alkylation damage to DNA. See Ref. 216, pp. 151–68
14. Wilson SH. 1998. Mammalian base excision repair and DNA polymerase beta. *Mutat. Res.* 407:203–15
15. Sancar A. 1995. DNA repair in humans. *Annu. Rev. Genet.* 29:69–105
16. Parikh SS, Mol CD, Tainer JA. 1997. Base excision repair enzyme family portrait: integrating the structure and chemistry of an entire DNA repair pathway. *Structure* 5:1543–50
17. Barzilay G, Mol CD, Robson CN, Walker LJ, Cunningham RP, et al. 1995. Identification of critical active site residues in the multifunctional human DNA repair enzyme HAP1. *Nat. Struct. Biol.* 2:561–67
18. Chen DS, Herman T, Demple B. 1991. Two distinct human DNA diesterases that hydrolyze 3′-blocking deoxyribose fragments from oxidized DNA. *Nucleic Acids Res.* 19:5907–14
19. Wilson DM III, Takeshita M, Grollman AP, Demple B. 1995. Incision activity of human apurinic endonuclease (Ape) at abasic site analogs in DNA. *J. Biol. Chem.* 270:16002–7
20. Suh D, Wilson DM 3rd, Povirk LF. 1997. 3′-phosphodiesterase activity of human apurinic/apyrimidinic endonuclease at DNA double-strand break ends. *Nucleic Acids Res.* 25:2495–500
21. Winters TA, Weinfeld M, Jorgensen TJ. 1992. Human HeLa cell enzymes that remove phosphoglycolate 3′-end groups from DNA. *Nucleic Acids Res.* 20:2573–80
22. Matsumoto Y, Kim K, Bogenhagen DF. 1994. Proliferating cell nuclear antigen-dependent abasic site repair in Xenopus laevis oocytes: an alternative pathway of base excision DNA repair. *Mol. Cell. Biol.* 14:6187–97
23. Frosina G, Fortini P, Rossi O, Carrozzino F, Raspaglio G, et al. 1996. Two pathways for base excision repair in mammalian cells. *J. Biol. Chem.* 271:9573–78
24. Stucki M, Pascucci B, Parlanti E, Fortini P, Wilson SH, et al. 1998. Mammalian base excision repair by DNA polymerases delta and epsilon. *Oncogene* 17:835–43

25. Fortini P, Pascucci B, Parlanti E, Sobol RW, Wilson SH, et al. 1998. Different DNA polymerases are involved in the short- and long-patch base excision repair in mammalian cells. *Biochemistry* 37:3575–80
26. Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE, et al. 1996. Reconstitution of DNA base excision-repair with purified human proteins: interaction between DNA polymerase beta and the XRCC1 protein. *EMBO J.* 15:6662–70
27. Marintchev A, Mullen MA, Maciejewski MW, Pan B, Gryk MR, et al. 1999. Solution structure of the single-strand break repair protein XRCC1 N-terminal domain. *Nat. Struct. Biol.* 6:884–93
28. Caldecott KW, Aoufouchi S, Johnson P, Shall S. 1996. XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick- sensor' in vitro. *Nucleic Acids. Res.* 24:4387–94
29. Cappelli E, Taylor R, Cevasco M, Abbondandolo A, Caldecott K, et al. 1997. Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. *J. Biol. Chem.* 272:23970–75
30. Pascucci B, Stucki M, Jonsson ZO, Dogliotti E, Hubscher U. 1999. Long patch base excision repair with purified human proteins. DNA ligase I as patch size mediator for DNA polymerases delta and epsilon. *J. Biol. Chem.* 274:33696–702
31. Dianov G, Price A, Lindahl T. 1992. Generation of single-nucleotide repair patches following excision of uracil residues from DNA. *Mol. Cell. Biol.* 12:1605–12
32. Klungland A, Lindahl T. 1997. Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J.* 16:3341–48
33. Matsumoto Y, Kim K, Hurwitz J, Gary R, Levin DS, et al. 1999. Reconstitution of proliferating cell nuclear antigen-dependent repair of apurinic/apyrimidinic sites with purified human proteins. *J. Biol. Chem.* 274:33703–8
34. Xanthoudakis S, Smeyne RJ, Wallace JD, Curran T. 1996. The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice. *Proc. Natl. Acad. Sci. USA* 93:8919–23
35. Tebbs RS, Flannery ML, Meneses JJ, Hartmann A, Tucker JD, et al. 1999. Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. *Dev. Biol.* 208:513–29
36. Krokan HE, Standal R, Bharati S, Otterlei M, Haug T, et al. 1997. Uracil in DNA and the family of conserved uracil DNA glycosylases. See Ref. 216, pp. 7–30
37. Slupphaug G, Mol CD, Kavli B, Arvai AS, Krokan HE, et al. 1996. A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA. *Nature* 384:87–92
38. Savva R, McAuley-Hecht K, Brown T, Pearl L. 1995. The structural basis of specific base-excision repair by uracil-DNA glycosylase. *Nature* 373:487–93
39. Haushalter KA, Stukenberg PT, Kirschner MW, Verdine GL. 1999. Identification of a new uracil-DNA glycosylase family by expression cloning using synthetic inhibitors. *Curr. Biol.* 9:174–85
40. Lettieri T, Jiricny J. 1997. Mammalian mismatch-specific DNA-glycoslyases. See Ref. 216, pp. 45–66
41. Neddermann P, Jiricny J. 1994. Efficient removal of uracil from G.U mispairs by the mismatch-specific thymine DNA glycosylase from HeLa cells. *Proc. Natl. Acad. Sci. USA* 91:1642–46
42. Boiteux S, O'Connor TR, Lederer F, Gouyette A, Laval J. 1990. Homogeneous Escherichia coli FPG protein. A DNA glycosylase which excises imidazole ring-opened purines and nicks DNA at apurinic/apyrimidinic sites. *J. Biol. Chem.* 265:3916–22
43. Michaels ML, Miller JH. 1992. The GO system protects organisms from the

mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *J. Bacteriol.* 174:6321–25
44. Tchou J, Grollman AP. 1993. Repair of DNA containing the oxidatively-damaged base, 8-oxoguanine. *Mutat. Res.* 299:277–87
45. van der Kemp PA, Thomas D, Barbey R, de Oliveira R, Boiteux S. 1996. Cloning and expression in Escherichia coli of the OGG1 gene of Saccharomyces cerevisiae, which codes for a DNA glycosylase that excises 7,8-dihydro-8-oxoguanine and 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine. *Proc. Natl. Acad. Sci. USA* 93:5197–202
46. Nash HM, Bruner SD, Scharer OD, Kawate T, Addona TA, et al. 1996. Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Curr. Biol.* 6:968–80
47. Bruner SD, Norman DPG, Verdine GL. 2000. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature* 403: 859–66
48. Michaels ML, Cruz C, Grollman AP, Miller JH. 1992. Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. *Proc. Natl. Acad. Sci. USA* 89:7022–25
49. Radicella JP, Clark EA, Fox MS. 1988. Some mismatch repair activities in Escherichia coli. *Proc. Natl. Acad. Sci. USA* 85:9674–78
50. Tsai-Wu JJ, Liu HF, Lu AL. 1992. Escherichia coli MutY protein has both N-glycosylase and apurinic/apyrimidinic endonuclease activities on A.C and A.G mispairs. *Proc. Natl. Acad. Sci. USA* 89: 8779–83
51. Nghiem Y, Cabrera M, Cupples CG, Miller JH. 1988. The mutY gene: a mutator locus in Escherichia coli that generates G.C—T.A transversions. *Proc. Natl. Acad. Sci. USA* 85:2709–13
52. Au KG, Cabrera M, Miller JH, Modrich P. 1988. Escherichia coli mutY gene product is required for specific A-G—C.G mismatch correction. *Proc. Natl. Acad. Sci. USA* 85:9163–66
53. Au KG, Clark S, Miller JH, Modrich P. 1989. Escherichia coli mutY gene encodes an adenine glycosylase active on G-A mispairs. *Proc. Natl. Acad. Sci. USA* 86:8877–81
54. Lu AL, Chang DY. 1988. A novel nucleotide excision repair for the conversion of an A/G mismatch to C/G base pair in E. coli. *Cell* 54:805–12
55. Michaels ML, Pham L, Nghiem Y, Cruz C, Miller JH. 1990. MutY, an adenine glycosylase active on G-A mispairs, has homology to endonuclease III. *Nucleic Acids Res.* 18:3841–45
56. McGoldrick JP, Yeh YC, Solomon M, Essigmann JM, Lu AL. 1995. Characterization of a mammalian homolog of the Escherichia coli MutY mismatch repair protein. *Mol. Cell Biol.* 15:989–96
57. Slupska MM, Baikalov C, Luther WM, Chiang JH, Wei YF, et al. 1996. Cloning and sequencing a human homolog (hMYH) of the Escherichia coli mutY gene whose function is required for the repair of oxidative DNA damage. *J. Bacteriol.* 178:3885–92
58. Demple B, Linn S. 1980. DNA glycosylases and UV-repair. *Nature* 287:203–8
59. Breimer LH, Lindahl T. 1984. DNA glycosylase activities for thymine residues damaged by ring saturation, fragmentation, or ring contraction are functions of endonuclease III in Escherichia coli. *J. Biol. Chem.* 259:5543–48
60. Hatahet Z, Kow YW, Purmal AA, Cunningham RP, Wallace SS. 1994. New substrates for old enzymes. 5-hydroxy-2′-deoxycytidine and 5-hydroxy-2′-deoxyuridine are substrates for Escherichia coli endonuclease III and formamidopyrimidine DNA N-glycosylase, while 5-hydroxy-2′-deoxyuridine is a substrate for

uracil DNA N-glycosylase. *J. Biol. Chem.* 269:18814–20
61. Augeri L, Lee YM, Barton AB, Doetsch PW. 1997. Purification, characterization, gene cloning, and expression of Saccharomyces cerevisiae redoxyendonuclease, a homolog of Escherichia coli endonuclease III. *Biochemistry* 36:721–29
62. Eide L, Bjoras M, Pirovano M, Alseth I, Berdal KG, et al. 1996. Base excision of oxidative purine and pyrimidine DNA damage in Saccharomyces cerevisiae by a DNA glycosylase with sequence similarity to endonuclease III from Escherichia coli. *Proc. Natl. Acad. Sci. USA* 93:10735–40
63. Aspinwall R, Rothwell DG, Roldan-Arjona T, Anselmino C, Ward CJ, et al. 1997. Cloning and characterization of a functional human homolog of Escherichia coli endonuclease III. *Proc. Natl. Acad. Sci. USA* 94:109–14
64. Hilbert TP, Chaung W, Boorstein RJ, Cunningham RP, Teebor GW. 1997. Cloning and expression of the cDNA encoding the human homologue of the DNA repair enzyme, Escherichia coli endonuclease III. *J. Biol. Chem.* 272:6733–40
65. Singer B, Antoccia A, Basu AK, Dosanjh MK, Fraenkel-Conrat H, et al. 1992. Both purified human 1,N6-ethenoadenine-binding protein and purified human 3-methyladenine-DNA glycosylase act on 1,N6-ethenoadenine and 3-methyladenine. *Proc. Natl. Acad. Sci. USA* 89:9386–90
66. Hang B, Singer B, Margison GP, Elder RH. 1997. Targeted deletion of alkylpurine-DNA-N-glycosylase in mice eliminates repair of 1,N^6-ethenoadenine and hypoxanthine but not of 3,N^4-ethenocytosine or 8-oxoguanine. *Proc. Natl. Acad. Sci. USA* 94:12869–74
67. Elder RH, Jansen JG, Weeks RJ, Willington MA, Deans B, et al. 1998. Alkylpurine-DNA-N-glycosylase knockout mice show increased susceptibility to induction of mutations by methyl methanesulfonate. *Mol. Cell. Biol.* 18:5828–37
68. Engelward BP, Weeda G, Wyatt MD, Broekhof JL, de Wit J, et al. 1997. Base excision repair deficient mice lacking the Aag alkyladenine DNA glycosylase. *Proc. Natl. Acad. Sci. USA* 94:13087–92
69. Yamagata Y, Kato M, Odawara K, Tokuno Y, Nakashima Y, et al. 1996. Three-dimensional structure of a DNA repair enzyme, 3-methyladenine DNA glycosylase II, from Escherichia coli. *Cell* 86:311–19
70. Labahn J, Scharer OD, Long A, Ezaz-Nikpay K, Verdine GL, et al. 1996. Structural basis for the excision repair of alkylation-damaged DNA. *Cell* 86:321–29
71. Samson L. 1992. The suicidal DNA repair methyltransferases of microbes. *Mol. Microbiol.* 6:825–31
72. Yarosh DB. 1985. The role of O6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. *Mutat. Res.* 145:1–16
73. Barzilay G, Walker LJ, Robson CN, Hickson ID. 1995. Site-directed mutagenesis of the human DNA repair enzyme HAP1: identification of residues important for AP endonuclease and RNase H activity. *Nucleic Acids Res.* 23:1544–50
74. Gorman MA, Morera S, Rothwell DG, de La Fortelle E, Mol CD, et al. 1997. The crystal structure of the human DNA repair endonuclease HAP1 suggests the recognition of extra-helical deoxyribose at DNA abasic sites. *EMBO J.* 16:6548–58
75. Mol CD, Izumi T, Mitra S, Tainer JA. 2000. DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination. *Nature* 403:451–56
76. Rothwell DG, Hickson ID. 1996. Asparagine 212 is essential for abasic site recognition by the human DNA repair endonuclease HAP1. *Nucleic Acids Res.* 24:4217–21
77. Masuda Y, Bennett RA, Demple B. 1998. Rapid dissociation of human apurinic endonuclease (Ape1) from incised DNA induced by magnesium. *J. Biol. Chem.* 273:30360–65

78. Lucas JA, Masuda Y, Bennett RAO, Strauss NS, Strauss PR. 1999. Single-turnover analysis of mutant human apurinic/apyrimidinic endonuclease. *Biochemistry* 38:4958–64
79. Rothwell DG, Hang B, Gorman MA, Freemont PS, Singer B, et al. 2000. Substitution of asp-210 in HAP1 (APE/Ref-1) eliminates endonuclease activity but stabilises substrate binding. *Nucleic Acids Res.* 28:2207–13
80. Erzberger JP, Wilson DM 3rd. 1999. The role of Mg2+ and specific amino acid residues in the catalytic reaction of the major human abasic endonuclease: new insights from EDTA-resistant incision of acyclic abasic site analogs and site-directed mutagenesis. *J. Mol. Biol.* 290:447–57
81. Hosfield DJ, Guan Y, Haas BJ, Cunningham RP, Tainer JA. 1999. Structure of the DNA repair enzyme endonuclease IV and its DNA complex: double-nucleotide flipping at abasic sites and three-metal-ion catalysis. *Cell* 98:397–408
82. Sobol RW, Prasad R, Evenski A, Baker A, Yang XP, et al. 2000. The lyase activity of the DNA repair protein beta-polymerase protects from DNA-damage-induced cytotoxicity. *Nature* 405:807–10
83. Thompson LH, West MG. 2000. XRCC1 keeps DNA from getting stranded. *Mutat. Res.* 459:1–18
84. Haber JE. 2000. Partners and pathways repairing a double-strand break. *Trends Genet.* 16:259–64
85. Karran P. 2000. DNA double strand break repair in mammalian cells. *Curr. Opin. Genet. Dev.* 10:144–50
86. Paques F, Haber JE. 1999. Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. *Microbiol. Mol. Biol. Rev.* 63:349–404
87. New JH, Sugiyama T, Zaitseva E, Kowalczykowski SC. 1998. Rad52 protein stimulates DNA strand exchange by Rad51 and replication protein A. *Nature* 391: 407–10
88. Shinohara A, Ogawa T. 1998. Stimulation by Rad52 of yeast Rad51-mediated recombination. *Nature* 391:404–7
89. Sung P. 1997. Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombinase. *Genes Dev.* 11:1111–21
90. Benson FE, Baumann P, West SC. 1998. Synergistic actions of Rad51 and Rad52 in recombination and DNA repair. *Nature* 391:401–4
91. Song B, Sung P. 2000. Functional interactions among yeast rad51 recombinase, rad52 mediator, and replication protein A in DNA strand exchange. *J. Biol. Chem.* 275:15895–904
92. Clever B, Interthal H, Schmuckli-Maurer J, King J, Sigrist M, et al. 1997. Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. *EMBO J.* 16:2535–44
93. Petukhova G, Stratton S, Sung P. 1998. Catalysis of homologous DNA pairing by yeast Rad51 and Rad54 proteins. *Nature* 393:91–94
94. Swagemakers SM, Essers J, de Wit J, Hoeijmakers JH, Kanaar R. 1998. The human RAD54 recombinational DNA repair protein is a double-stranded DNA- dependent ATPase. *J. Biol. Chem.* 273:28292–97
95. Thacker J. 1999. A surfeit of RAD51-like genes? *Trends Genet.* 15:166–68
96. Van Dyck E, Stasiak AZ, Stasiak A, West SC. 1999. Binding of double-strand breaks in DNA by human Rad52 protein. *Nature* 398:728–31
97. Rijkers T, Van Den Ouweland J, Morolli B, Rolink AG, Baarends WM, et al. 1998. Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. *Mol. Cell Biol.* 18:6423–29
98. Essers J, Hendriks RW, Swagemakers SM, Troelstra C, de Wit J, et al. 1997. Disruption of mouse RAD54 reduces ionizing

radiation resistance and homologous recombination. *Cell* 89:195–204
99. Essers J, van Steeg H, de Wit J, Swagemakers SM, Vermeij M, et al. 2000. Homologous and non-homologous recombination differentially affect DNA damage repair in mice. *EMBO J.* 19:1703–10
100. Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, et al. 1998. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* 93:477–86
101. Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, et al. 1998. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* 93:467–76
102. Matsuura S, Tauchi H, Nakamura A, Kondo N, Sakamoto S, et al. 1998. Positional cloning of the gene for Nijmegen breakage syndrome. *Nat. Genet.* 19:179–81
103. Digweed M, Reis A, Sperling K. 1999. Nijmegen breakage syndrome: consequences of defective DNA double strand break repair. *Bioessays* 21:649–56
104. Venkitaraman AR. 1999. Breast cancer genes and DNA repair. *Science* 286:1100–2
105. Patel KJ, Yu VPCC, Lee H, Corcoran A, Thistlethwaite FC, et al. 1998. Involvement of Brca2 in DNA repair. *Mol. Cell* 1:347–57
106. Cressman VL, Backlund DC, Avrutskaya AV, Leadon SA, Godfrey V, et al. 1999. Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice. *Mol. Cell. Biol.* 19:7061–75
107. Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, et al. 1998. Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol. Cell* 2:317–28
108. Smith GCM, Jackson SP. 1999. The DNA-dependent protein kinase. *Genes Dev.* 13:916–34
109. Vogel H, Lim DS, Karsenty G, Finegold M, Hasty P. 1999. Deletion of Ku86 causes early onset of senescence in mice. *Proc. Natl. Acad. Sci. USA* 96:10770–75
110. Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K, et al. 1996. Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* 382:551–55
111. Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, et al. 2000. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 404:510–14
112. Gu Y, Sekiguchi J, Gao Y, Dikkes P, Frank K, et al. 2000. Defective embryonic neurogenesis in Ku-deficient but not DNA-dependent protein kinase catalytic subunit-deficient mice. *Proc. Natl. Acad. Sci. USA* 97:2668–73
113. Frank KM, Sekiguchi JM, Seidl KJ, Swat W, Rathbun GA, et al. 1998. Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* 396:173–77
114. Barnes DE, Stamp G, Rosewell I, Denzel A, Lindahl T. 1998. Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Curr. Biol.* 8:1395–98
115. Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, et al. 1998. A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* 95:891–902
116. Nick McElhinny SA, Snowden CM, McCarville J, Ramsden DA. 2000. Ku recruits the XRCC4-ligase IV complex to DNA ends. *Mol. Cell Biol.* 20:2996–3003
117. Baumann P, West SC. 1998. DNA end-joining catalyzed by human cell-free extracts. *Proc. Natl. Acad. Sci. USA* 95:14066–70

118. Bridges BA. 1995. Are there DNA damage checkpoints in E. coli? *Bioessays* 17:63–70
119. Weinert T, Hartwell L. 1988. The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* 241:317–22
120. Weinert T. 1998. DNA damage and checkpoint pathways: molecular anatomy and interactions with repair. *Cell* 94:555–58
121. O'Connell MJ, Walworth NC, Carr AM. 2000. The G2-phase DNA-damage checkpoint. *Trends Cell Biol.* 10:296–303
122. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, et al. 1999. A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes Dev.* 13:152–57
123. Kostrub CF, Knudsen K, Subramani S, Enoch T. 1998. Hus1p, a conserved fission yeast checkpoint protein, interacts with Rad1p and is phosphorylated in response to DNA damage. *EMBO J.* 17:2055–66
124. Caspari T, Dahlen M, Kanter-Smoler G, Lindsay HD, Hofmann K, et al. 2000. Characterization of Schizosaccharomyces pombe Hus1: a PCNA-related protein that associates with Rad1 and Rad9. *Mol. Cell. Biol.* 20:1254–62
125. Paciotti V, Lucchini G, Plevani P, Longhese MP. 1998. Mec1p is essential for phosphorylation of the yeast DNA damage checkpoint protein Ddc1p, which physically interacts with Mec3p. *EMBO J.* 17:4199–209
126. St. Onge RP, Udell CM, Casselman R, Davey S. 1999. The human G2 checkpoint control protein hRAD9 is a nuclear phosphoprotein that forms complexes with hRAD1 and hHUS1. *Mol. Biol. Cell* 10:1985–95
127. Shimomura T, Ando S, Matsumoto K, Sugimoto K. 1998. Functional and physical interaction between Rad24 and Rfc5 in the yeast checkpoint pathways. *Mol. Cell. Biol.* 18:5485–91
128. Griffiths D, Uchiyama M, Nurse P, Wang TS. 2000. A novel mutant allele of the chromatin-bound fission yeast checkpoint protein Rad17 separates the DNA structure checkpoints. *J. Cell Sci.* 113:1075–88
129. Thelen MP, Venclovas C, Fidelis K. 1999. A sliding clamp model for the Rad1 family of cell cycle checkpoint proteins. *Cell* 96:769–70
130. Sugimoto K, Ando S, Shimomura T, Matsumoto K. 1997. Rfc5, a replication factor C component, is required for regulation of Rad53 protein kinase in the yeast checkpoint pathway. *Mol. Cell. Biol.* 17:5905–14
131. Chang MS, Sasaki H, Campbell MS, Kraeft SK, Sutherland R, et al. 1999. HRad17 colocalizes with NHP2L1 in the nucleolus and redistributes after UV irradiation. *J. Biol. Chem.* 274:36544–49
132. Lydall D, Weinert T. 1995. Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. *Science* 270:1488–91
133. Vialard JE, Gilbert CS, Green CM, Lowndes NF. 1998. The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage. *EMBO J.* 17:5679–88
134. Emili A. 1998. MEC1-dependent phosphorylation of Rad9p in response to DNA damage. *Mol. Cell* 2:183–89
135. Saka Y, Esashi F, Matsusaka T, Mochida S, Yanagida M. 1997. Damage and replication checkpoint control in fission yeast is ensured by interactions of Crb2, a protein with BRCT motif, with Cut5 and Chk1. *Genes Dev.* 11:3387–400
136. Edwards RJ, Bentley NJ, Carr AM. 1999. A Rad3-Rad26 complex responds to DNA damage independently of other checkpoint proteins. *Nat. Cell Biol.* 1:393–98
137. Gardner R, Putnam CW, Weinert T. 1999. RAD53, DUN1 and PDS1 define two parallel G2/M checkpoint pathways in budding yeast. *EMBO J.* 18:3173–85

138. Walworth N, Davey S, Beach D. 1993. Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. *Nature* 363:368–71
139. Sanchez Y, Desany BA, Jones WJ, Liu Q, Wang B, et al. 1996. Regulation of RAD53 by the ATM-like kinases MEC1 and TEL1 in yeast cell cycle checkpoint pathways. *Science* 271:357–60
140. Walworth NC, Bernards R. 1996. rad-dependent response of the chk1-encoded protein kinase at the DNA damage checkpoint. *Science* 271:353–56
141. Lindsay HD, Griffiths DJ, Edwards RJ, Christensen PU, Murray JM, et al. 1998. S-phase-specific activation of Cds1 kinase defines a subpathway of the checkpoint response in *Schizosaccharomyces pombe*. *Genes Dev.* 12:382–95
142. Matsuoka S, Huang M, Elledge SJ. 1998. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* 282:1893–97
143. Sanchez Y, Bachant J, Wang H, Hu F, Liu D, et al. 1999. Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science* 286:1166–71
144. Naito T, Matsuura A, Ishikawa F. 1998. Circular chromosome formation in a fission yeast mutant defective in two ATM homologues. *Nat. Genet.* 20:203–6
145. Nurse P. 1990. Universal control mechanism regulating onset of M-phase. *Nature* 344:503–8
146. Raleigh JM, O'Connell MJ. 2000. The G2 DNA damage checkpoint targets both wee1 and cdc25. *J. Cell Sci.* 113: 1727–36
147. Christensen PU, Bentley NJ, Martinho RG, Nielsen O, Carr AM. 2000. Mik1 levels accumulate in S phase and may mediate an intrinsic link between S phase and mitosis. *Proc. Natl. Acad. Sci. USA* 97:2579–84
148. Rhind N, Furnari B, Russell P. 1997. Cdc2 tyrosine phosphorylation is required for the DNA damage checkpoint in fission yeast. *Genes Dev.* 11:504–11
149. Jin P, Gu Y, Morgan DO. 1996. Role of inhibitory CDC2 phosphorylation in radiation-induced G2 arrest in human cells. *J. Cell Biol.* 134:963–70
150. Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. 2000. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature* 404: 201–4
151. Cortez D, Wang Y, Qin J, Elledge SJ. 1999. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. *Science* 286:1162–66
152. Xu X, Weaver Z, Linke SP, Li C, Gotay J, et al. 1999. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Mol. Cell* 3:389–95
153. MacLachlan TK, Somasundaram K, Sgagias M, Shifman Y, Muschel RJ, et al. 2000. BRCA1 effects on the cell cycle and the DNA damage response are linked to altered gene expression. *J. Biol. Chem.* 275:2777–85
154. Metcalfe JA, Parkhill J, Campbell L, Stacey M, Biggs P, et al. 1996. Accelerated telomere shortening in ataxia telangiectasia. *Nat. Genet.* 13:350–53
155. Matsuura A, Naito T, Ishikawa F. 1999. Genetic control of telomere integrity in *Schizosaccharomyces pombe: rad3*$^+$ and *tel1*$^+$ are parts of two regulatory networks independent of the downstream protein kinases *chk*1$^+$ and *cds*1$^+$. *Genetics* 152:1501–12
156. Ritchie KB, Mallory JC, Petes TD. 1999. Interactions of TLC1 (which encodes the RNA subunit of telomerase), TEL1, and MEC1 in regulating telomere length in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 19:6065–75
157. Ahmed S, Hodgkin J. 2000. MRT-2 checkpoint protein is required for

germline immortality and telomere replication in *C. elegans*. *Nature* 403:159–64
158. Morrow DM, Tagle DA, Shiloh Y, Collins FS, Hieter P. 1995. *TEL1*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. *Cell* 82:831–40
159. Paulovich AG, Hartwell LH. 1995. A checkpoint regulates the rate of progression through S phase in S. cerevisiae in response to DNA damage. *Cell* 82:841–47
160. Rhind N, Russell P. 1998. The *Schizosaccharomyces pombe* S-phase checkpoint differentiates between different types of DNA damage. *Genetics* 149:1729–37
161. Painter RB, Young BR. 1980. Radiosensitivity in ataxia-telangiectasia: a new explanation. *Proc. Natl. Acad. Sci. USA* 77:7315–17
162. Zhao S, Weng YC, Yuan SS, Lin YT, Hsu HC, et al. 2000. Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products. *Nature* 405:473–77
163. Lim DS, Kim ST, Xu B, Maser RS, Lin J, et al. 2000. ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. *Nature* 404:613–17
164. Gatei M, Young D, Cerosaletti KM, Desai-Mehta A, Spring K, et al. 2000. ATM-dependent phosphorylation of nibrin in response to radiation exposure. *Nat. Genet.* 25:115–19
165. Nugent CI, Bosco G, Ross LO, Evans SK, Salinger AP, et al. 1998. Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr. Biol.* 8:657–60
166. Boulton SJ, Jackson SP. 1998. Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* 17:1819–28
167. Lee SE, Moore JK, Holmes A, Umezu K, Kolodner RD, et al. 1998. *Saccharomyces* Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* 94: 399–409
168. Murray JM, Lindsay HD, Munday CA, Carr AM. 1997. Role of *Schizosaccharomyces pombe* RecQ homolog, recombination, and checkpoint genes in UV damage tolerance. *Mol. Cell. Biol.* 17: 6868–75
169. Esashi F, Yanagida M. 1999. Cdc2 phosphorylation of Crb2 is required for reestablishing cell cycle progression after the damage checkpoint. *Mol. Cell* 4:167–74
170. Di Leonardo A, Linke SP, Clarkin K, Wahl GM. 1994. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev.* 8:2540–51
171. Haupt Y, Maya R, Kazaz A, Oren M. 1997. Mdm2 promotes the rapid degradation of p53. *Nature* 387:296–99
172. Hermeking H, Lengauer C, Polyak K, He TC, Zhang L, et al. 1997. 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol. Cell.* 1:3–11
173. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, et al. 1998. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* 282:1497–501
174. Winters ZE, Ongkeko WM, Harris AL, Norbury CJ. 1998. p53 regulates Cdc2 independently of inhibitory phosphorylation to reinforce radiation-induced G2 arrest in human cells. *Oncogene* 17: 673–84
175. Flatt PM, Tang LJ, Scatena CD, Szak ST, Pietenpol JA. 2000. p53 regulation of G2 checkpoint is retinoblastoma protein dependent. *Mol. Cell. Biol.* 20:4210–23
176. Azzam EI, de Toledo SM, Pykett MJ, Nagasawa H, Little JB. 1997. CDC2 is down-regulated by ionizing radiation in a

p53-dependent manner. *Cell Growth Differ.* 8:1161–69
177. de Toledo SM, Azzam EI, Keng P, Laffrenier S, Little JB. 1998. Regulation by ionizing radiation of CDC2, cyclin A, cyclin B, thymidine kinase, topoisomerase IIalpha, and RAD51 expression in normal human diploid fibroblasts is dependent on p53/p21Waf1. *Cell Growth Differ.* 9:887–96
178. Badie C, Itzhaki JE, Sullivan MJ, Carpenter AJ, Porter AC. 2000. Repression of CDK1 and other genes with CDE and CHR promoter elements during DNA damage-induced G2/M arrest in human cells. *Mol. Cell. Biol.* 20:2358–66
179. Graves PR, Yu L, Schwarz JK, Gales J, Sausville EA, et al. 2000. The Chk1 protein kinase and the Cdc25C regulatory pathways are targets of the anticancer agent UCN-01. *J. Biol. Chem.* 275:5600–5
180. Moser BA, Brondello JM, Baber-Furnari B, Russell P. 2000. Mechanism of caffeine-induced checkpoint override in fission yeast. *Mol. Cell. Biol.* 20:4288–94
181. Zhou BB, Chaturvedi P, Spring K, Scott SP, Johanson RA, et al. 2000. Caffeine abolishes the mammalian G2/M DNA damage checkpoint by inhibiting ataxia-telangiectasia-mutated kinase activity. *J. Biol. Chem.* 275:10342–48
182. Caspari T. 2000. How to activate p53. *Curr. Biol.* 10:R315–17
183. Chehab NH, Malikzay A, Appel M, Halazonetis TD. 2000. Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53. *Genes Dev.* 14: 278–88
184. Shieh SY, Ahn J, Tamai K, Taya Y, Prives C. 2000. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage inducible sites. *Genes Dev.* 14:289–300
185. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, et al. 2000. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science* 287:1824–27
186. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, et al. 1999. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science* 286:2528–31
187. Khosravi R, Maya R, Gottlieb T, Oren M, Shiloh Y, et al. 1999. Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage. *Proc. Natl. Acad. Sci. USA* 96:14973–77
188. Kapoor M, Lozano G. 1998. Functional activation of p53 via phosphorylation following DNA damage by UV but not gamma radiation. *Proc. Natl. Acad. Sci. USA* 95:2834–37
189. Gu W, Roeder RG. 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 90:595–606
190. Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, et al. 1998. DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* 12:2831–41
191. Foray N, Priestley A, Alsbeih G, Badie C, Capulas EP, et al. 1997. Hypersensitivity of ataxia telangiectasia fibroblasts to ionizing radiation is associated with a repair deficiency of DNA double-strand breaks. *Int. J. Radiat. Biol.* 72:271–83
192. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, et al. 2000. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* 14:927–39
193. Thornberry NA, Lazebnik Y. 1998. Caspases: enemies within. *Science* 281: 1312–16
194. Petak I, Tillman DM, Harwood FG, Mihalik R, Houghton JA. 2000. Fas-dependent and -independent mechanisms of cell

death following DNA damage in human colon carcinoma cells. *Cancer Res.* 60:2643–50
195. Muller M, Wilder S, Bannasch D, Israeli D, Lehlbach K, et al. 1998. p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. *J. Exp. Med.* 188:2033–45
196. Hill LL, Ouhtit A, Loughlin SM, Kripke ML, Ananthaswamy HN, et al. 1999. Fas ligand: a sensor for DNA damage critical in skin cancer etiology. *Science* 285:898–900
197. Djordjevic B. 2000. Bystander effects: a concept in need of clarification. *Bioessays* 22:286–90
198. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, et al. 1993. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 362:849–52
199. Sionov RV, Haupt Y. 1999. The cellular response to p53: the decision between life and death. *Oncogene* 18:6145–57
200. Zhan Q, Fan S, Bae I, Guillouf C, Liebermann DA, et al. 1994. Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. *Oncogene* 9:3743–51
201. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, et al. 1994. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene* 9:1799–805
202. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, et al. 2000. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 288:1053–58
203. Ryan KM, Ernst MK, Rice NR, Vousden KH. 2000. Role of NF-kappaB in p53-mediated programmed cell death. *Nature* 404:892–97
204. Caelles C, Helmberg A, Karin M. 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature* 370:220–23
205. Chen X, Ko LJ, Jayaraman L, Prives C. 1996. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev.* 10:2438–51
206. Aladjem MI, Spike BT, Rodewald LW, Hope TJ, Klemm M, et al. 1998. ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Curr. Biol.* 8:145–55
207. Kasibhatla S, Brunner T, Genestier L, Echeverri F, Mahboubi A, et al. 1998. DNA damaging agents induce expression of Fas ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappa B and AP-1. *Mol. Cell* 1: 543–51
208. Kolbus A, Herr I, Schreiber M, Debatin KM, Wagner EF, et al. 2000. c-Jun-dependent CD95-L expression is a rate-limiting step in the induction of apoptosis by alkylating agents. *Mol. Cell. Biol.* 20:575–82
209. Wang C, Mayo MW, Baldwin AS. 1996. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NFκB. *Science* 274:784–87
210. Wisdom R. 1999. AP-1: one switch for many signals. *Exp. Cell Res.* 253:180–85
211. Johnson PA, Clements P, Hudson K, Caldecott KW. 2000. The mitotic spindle and DNA damage-induced apoptosis. *Toxicol. Lett.* 112/113:59–67
212. Uberti D, Schwartz D, Almog N, Goldfinger N, Harmelin A, et al. 1999. Epithelial cells of different organs exhibit distinct patterns of p53-dependent and p53-independent apoptosis following DNA insult. *Exp. Cell Res.* 252:123–33
213. Komatsu K, Miyashita T, Hang H, Hopkins KM, Zheng W, et al. 2000. Human homologue of *S. pombe* Rad9 interacts with BCL-2/BCL-X_L and promotes apoptosis. *Nat. Cell Biol.* 2:1–6
214. Harkin DP, Bean JM, Miklos D, Song YH, Truong VB, et al. 1999. Induction

of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. *Cell* 97:575–86

215. Gartner A, Milstein S, Ahmed S, Hodgkin J, Hengartner MO. 2000. A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C. elegans*. *Mol. Cell* 5:435–43
216. Hickson ID, ed. 1997. *Base Excision Repair of DNA Damage*. Austin, TX: Landes Biosci.