The DNA damage response: putting checkpoints in perspective

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The inability to repair DNA damage properly in mammals leads to various disorders and enhanced rates of tumour development. Organisms respond to chromosomal insults by activating a complex damage response pathway. This pathway regulates known responses such as cell-cycle arrest and apoptosis (programmed cell death), and has recently been shown to control additional processes including direct activation of DNA repair networks.

The survival of organisms depends on the accurate transmission of genetic information from one cell to its daughters. Such faithful transmission requires not only extreme accuracy in replication of DNA and precision in chromosome distribution, but also the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations. To achieve this fidelity, cells have evolved surveillance mechanisms that monitor the structure of chromosomes and coordinate repair and cell-cycle progression. Genetic control of cell-cycle transitions in response to DNA damage was first observed in the SOS DNA damage response pathway of *Escherichia coli*[1] and in mammals in ataxia telangiectasia (AT) cells, which are defective for the ataxia telangiectasia mutated (ATM) gene[2]. This control was later observed in yeast and the term checkpoint was applied to the yeast pathway[3].

DNA damage checkpoints were initially defined as non-essential regulatory pathways that control the ability of cells to arrest the cell cycle in response to DNA damage, allowing time for repair. However, recent evidence suggests that the historic definition of the checkpoint pathway is inadequate to explain the function of this pathway completely. In addition to controlling cell-cycle arrest, these pathways have been shown to control the activation of DNA repair pathways[4–8], the composition of telomeric chromatin and the movement of DNA repair proteins to sites of DNA damage[9,10], activation of transcriptional programmes[11], telomere length[12,13] and, in some cell types for reasons not fully understood, induction of cell death by apoptosis[14–17]. Thus, it is now clear that the checkpoint comprises a subroutine integrated into the larger DNA damage response pathway that regulates a multifaceted response (Fig. 1). In addition, several checkpoint genes are essential for cell and organism survival[11,18–21], implying that these pathways are not only surveyors of occasional damage, but are firmly integrated components of cellular physiology.

An example that illustrates the physiological importance of these pathways is the disorder ataxia telangiectasia. Individuals carrying two mutant ATM genes may suffer numerous problems that include loss of motor control owing to Purkinje cell loss, immune deficiencies and high frequencies of cancer. ATM is a central signalling protein in the DNA damage response, and so cells lacking ATM fail to execute many of the cellular responses to DNA damage. However, AT patients have many problems even in the absence of exposure to DNA-damaging agents because numerous chromosome structural defects occur during normal cell duplication. During the process of DNA replication, errors, such as double-strand breaks (DSBs) that arise from stalled replication forks, require attention by the DNA damage response pathway. In addition to these unavoidable errors, cells exist in a highly reactive chemical environment in which reactive species, such as free radicals, are by-products of cellular metabolism or consequences of the cells' existence in an oxygen-rich environment. These reactive species react with DNA, creating more problems that must be addressed. In this regard, ATM has been proposed to be important in the cellular response to oxidative stress[22], which may have particular relevance for long-lived, terminally differentiated cells such as Purkinje cells. Furthermore, DSBs are not solely accidents of nature, but are also regulated components of VDJ recombination and meiosis. ATM is needed to respond to these developmentally programmed DNA alterations as well, which emphasizes its importance to organism physiology.

The recently discovered connections between 'checkpoint' pathways and DNA repair and their physiological effects on the cell prompted us to re-evaluate the role of checkpoint proteins within the context of the overall response to DNA damage. Here we will concentrate primarily on data from mammalian cells, from which a model of the DNA damage response is beginning to take shape. There is much overlap between DNA damage response mechanisms

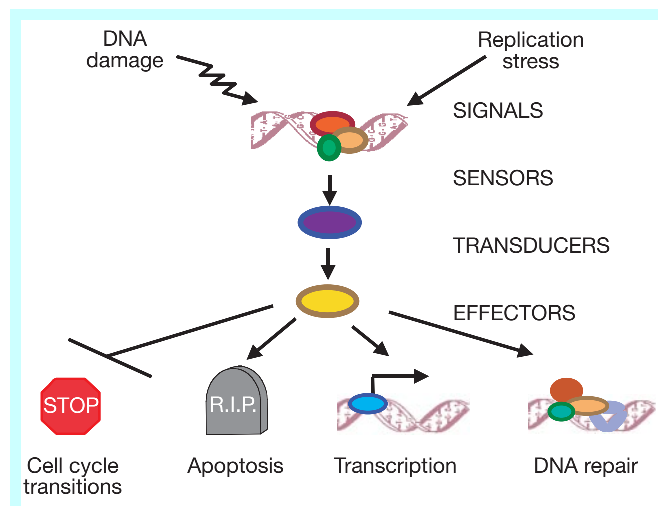

Figure 1 A contemporary view of the general outline of the DNA damage response signal-transduction pathway. Arrowheads represent activating events and perpendicular ends represent inhibitory events. Cell-cycle arrest is depicted with a stop sign, apoptosis with a tombstone. The DNA helix with an arrow represents damage-induced transcription, while the DNA helix with several oval-shaped subunits represents damage-induced repair. For the purpose of simplicity, the network of interacting pathways are depicted as a linear pathway consisting of signals, sensors, transducers and effectors.

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in humans and yeast, and we will attempt to use these similarities to fill the gaps in our knowledge of the mammalian system.

General organization of the DNA damage response pathway

The DNA damage response pathway is a signal transduction pathway consisting of sensors, transducers and effectors (Fig. 1). Although we refer to this as a pathway, it is more accurately described as a network of interacting pathways that together execute the response. The identities of the sensors are not yet known. Much is known about the signal transducers, which are composed of four sets of conserved proteins with recognizable motifs (Table 1). One class is composed of phospho-inositide kinase (PIK)-related proteins which include ATM and ATM-Rad3-related (ATR) in mammals and their homologues in budding and fission yeast. These proteins are central to the entire DNA damage response. Downstream of these proteins are two families of checkpoint kinases (CHK), the Chk1 and Chk2 kinases, and their homologues. These kinases carry out subsets of the DNA damage response in mammals and are targets of regulation by ATM and ATR kinases. The fourth conserved family is the BRCT-repeat containing proteins, which include budding yeast Rad9 and fission yeast Crb2. No clear human orthologues of these proteins exist in mammals, but BRCA1 and 53BP1 are possible candidates. The precise roles of these proteins and where exactly they function with respect to the PIKs are not understood. However, *Saccharomyces cerevisiae* Rad9 is required for phosphorylation of budding yeast Chk1 and Chk2 homologues and interacts physically with both[23–26]. For the Chk2 homologue, the interaction is dependent on the FHA domain, a phospho-amino-acid binding motif[27,28]. Because PIKs are believed to phosphorylate Chk1 and Chk2 directly, it is possible that Rad9 and other BRCT proteins help in recruiting substrates like CHKs to PIKs in response to DNA damage[23].

Below this level of signal transduction are the effectors that execute the functions of the DNA damage response. These include substrates of both PIK and CHK kinases and proteins involved in DNA repair, transcription regulation and cell-cycle control, such as BRCA1, Nbs1, p53 and Cdc25C (Table 2). Depending on the context, certain molecules such as BRCA1 may have multiple functions in this signal transduction pathway.

DNA damage sensors

The proteins that initially sense the aberrant DNA structures, and initiate the signalling response, are currently unknown. Owing to their ability to bind and be activated by DNA strand breaks, poly (ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase (DNA-PK) have long been proposed as DNA damage sensors. However, genetic evidence indicates that these proteins are not activators of the global DNA damage response[29,30]. In place of these molecules, a group of four conserved proteins in yeast have emerged as candidates because they share some of the properties expected for sensors, including an essential genetic role in the activation of the DNA damage response pathway and the potential to interact with nucleic acids. In *Schizosaccaromyces pombe*, three of these proteins, Rad1, Rad9 and Hus1, are related in structure to PCNA (Table 1). Modelling suggests that they may form a doughnut-like heteromer like PCNA, and in principle could be loaded onto damaged DNA just as PCNA is loaded onto primed DNA (reviewed in ref. 31). The human homologues of these proteins form a DNA damage responsive complex[32]. Consistent with yeast studies, inactivation of mouse Hus1 results in impaired responses to genotoxic stress[33].

The fourth conserved checkpoint protein from *S. pombe*, Rad17, shares homology with all five subunits of replication factor C (RFC)[31]. During replication, the RFC1-5 complex binds to DNA primed by primase and DNA pol-α, and recruits PCNA to form a sliding clamp that tethers DNA polymerases to the DNA template. This complex could signal to maintain the replication checkpoint during S phase and, in fact, mutations in RFCs have been found to be defective for the DNA replication checkpoint[34–37]. Analogously, in the DNA damage response, Rad17 homologues, together with the small RFC subunits, can mediate interactions with the Rad1–Rad9–Hus1 complex[38], and are possibly involved in maintaining the damage signal until repair is completed.

The simplest model concerning these putative sensors is that Rad17 complexes bind to damaged DNA directly, and then load the Rad1 complexes onto DNA, which leads to activation of the main damage response kinase Rad3 in *S. pombe*. But in *S. pombe*, although the known biochemical outputs of this damage response, including Rad3-dependent Cds1 and Chk1 phosphorylation, are dependent on Rad1 and Rad17 complexes, Rad3-dependent phosphorylation of Rad26 in response to ionizing radiation (IR) does not require these proteins[39]. Thus, these putative sensors are not always required for Rad3-dependent phosphorylation events. Similar phosphorylation dependency after DNA damage was also observed for a *S. cerevisiae* Rad26 homologue, Ddc2/Lcd1 (refs 40, 41), suggesting that a human homologue could exist and behave in a similar manner (Table 1). But the phosphorylation of Rad26 does not necessarily exclude a sensory role for the Rad1 and Rad17 complexes. It is possible that Rad26 or Rad3 is able to recognize a DSB leading to phosphorylation of the tightly bound Rad26 protein, but full activation of the Rad3 pathway requires the function of the Rad1 and Rad17 complexes to amplify the damage signal. In support of this, Rad26 phosphorylation in response to replication blocks is dependent on the Rad1 complex of proteins[39]. Alternatively, it is possible that there are two separate sensory pathways, each of which must function to fully activate the damage response. This may provide a failsafe mechanism to ensure the pathway is not activated spuriously to arrest the cell cycle inappropriately or activate cell death.

If the above proteins are not the actual sensors, why have the identities of the true sensors not yet emerged from the powerful genetic studies in yeast? One possibility is that we have insufficient biochemical information to recognize them as such. Or there may be many different sensors that have overlapping abilities to sense DNA damage and to signal. This situation is further complicated by the fact that a given DNA damaging agent generates several types of DNA damage, possibly activating several sensors.

Alternative proteins have been suggested as candidate sensors, one of which is the breast cancer protein BRCA1. Mouse cells lacking BRCA1 exon 11 are unable to arrest the cell cycle in G2, suggesting a possible defect in sensing or signalling[42]. BRCA1 is part of a large complex named BASC (BRCA1-associated genome surveillance complex) that contains ATM, the Nbs1–MRE11–RAD50 complex, mismatch proteins (MSH2/6 and MLH2), and the Bloom's helicase (BLM)[43]. This model is speculative, but it is interesting that each of these proteins has the ability to recognize aberrant DNA structures and could thus be involved in transmitting

Table 1 Conserved DNA damage response genes

Functional class	Mammals	*S. cerevisiae*	*S. pombe*
PCNA-like proteins	Rad1 Rad9 Hus1	Rad17 Ddc1 Mec3	Rad1 Rad9 Hus1
RFC-like proteins	Rad17 RFC2-5	Rad24 RFC2-5	Rad17 RFC3
BRCT proteins	BRCA1?, 53BP1?	Rad9 DPB11	Crb2/Rhp9 Cut5
P13K-like proteins	ATR ATM	Mec1 Tel1	Rad3 Tel1
Effector kinases	Chk1	Chk1	Chk1
	Chk2	Rad53	Cds1
Coiled-coil proteins	?	Ddc2/Lcd1	Rad26

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the presence of these structures to ATM and BRCA1. Initial support for this idea is provided by the observation that cells defective for the mismatch repair gene *MLH1* fail to display the ATM-dependent phosphorylation of c-Abl in response to *cis*-platinum treatment[44]. It remains to be seen whether *MLH1* mutants are defective in all responses to *cis*-platinum, for example, in cell-cycle arrest. Interestingly, several of these proteins are also substrates of ATM, suggesting that they may be targets of regulation as opposed to directing signal transduction. At present, it is impossible to distinguish between a sensory role and an effector role for any of these proteins, and it is plausible that the activity of an entire complex must be intact to properly sense and respond to damage.

Proximal kinases ATM and ATR

In contrast to our knowledge of damage sensors, our understanding of signal transducers is more advanced. Two related and conserved proteins, ATM and ATR, are central components of the DNA damage response[11]. Although structurally related to the PI(3)K family members, ATM and ATR are protein kinases. The function of ATM is better understood. Cells from AT patients have mutations in ATM and are defective in several responses to IR including G1 arrest[45], reduction in DNA synthesis[2] and G2 arrest[46]. ATM plays an important part in the response to IR, controlling the initial phosphorylation of several key proteins such as p53, Mdm2, BRCA1, Chk2 and Nbs1 in response to DNA damage (Fig. 2). These proteins are still phosphorylated in γ-irradiated AT cells, but with delayed kinetics, indicating that additional pathways respond to IR. Whereas AT fibroblasts are very sensitive to IR, they show little sensitivity to ultraviolet radiation, alkylating agents or inhibitors of DNA replication.

Less is known about ATR owing to the absence of ATR mutations in human diseases. However, expression of a dominant-negative ATR sensitizes mammalian cells to all forms of DNA damage and diminishes the G2/M checkpoint response induced by γ-radiation[47,48]. Thus, ATR is a good candidate for a pathway that operates in parallel to ATM and responds to different types of damage. Additional support for this model comes from the observation that *S. cerevisiae* ATM and ATR homologues have overlapping but non-redundant roles[11]. Furthermore, ATM and ATR share a number of phosphorylation substrates and often phosphorylate the same residues, although substrate differences have been reported[49]. One substrate that has been examined, p53, is phosphorylated at Ser 15 by both kinases[50–52] *in vitro* and *in vivo*. In addition, ATM and ATR can phosphorylate BRCA1 at Ser 1423, 1524 and other sites[4,53]. BRCA1 phosphorylation in response to IR is largely ATM-dependent but phosphorylation in response to ultraviolet light and hydroxyurea requires ATR[53]. These data fit the emerging model that ATM primarily controls the response to IR, whereas ATR responds to other types of damage. One caveat in the interpretation of the *in vivo* roles of ATR is that most experiments have been performed with a dominant-negative ATR construct that might also interfere with ATM function. Resolution of this will require viable loss-of-function ATR mutants.

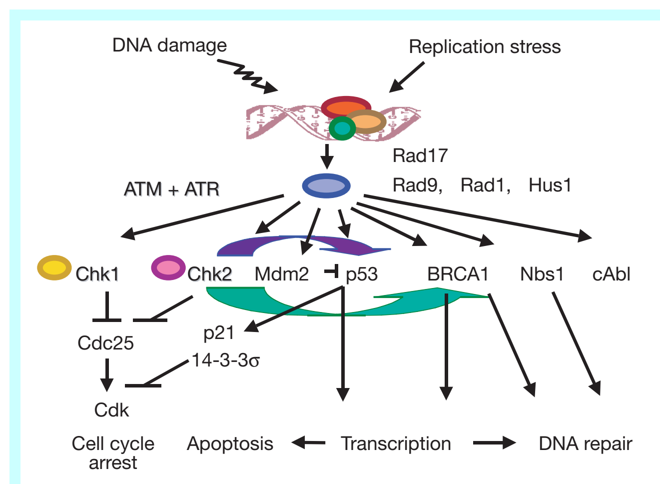

Figure 2 Organization of the mammalian DNA damage response pathway. Arrowheads represent positively acting steps while perpendicular ends represents inhibitory steps. Gene names are shown at the approximate positions where their encoded proteins function in the pathway. Although the general organization of the pathway is correct, some details are omitted, especially concerning the relationship between the ATR/ATM and Hus1/Rad17/Rad9/Rad1 proteins, which may participate in mutual regulation.

The phenotypes of knockout mice have been particularly revealing concerning the function of PIKs. While *ATM* null mice are viable and display growth retardation and infertility[16,54,55], $ATR^{-/-}$ mice die early in embryogenesis[18,19]. $ATR^{-/-}$ blastocyst cells die in culture with a phenotype resembling mitotic catastrophe[18]. The latter result suggests an essential role for ATR, possibly monitoring DNA replication. The *S. cerevisiae* ATR homologue, Mec1, is also essential and has been shown to be required for recovery from DNA replicational stress[56]. Thus, ATR is likely to have a role in each cell cycle, indicating either a constitutive function for ATR or the frequent presence of spontaneous DNA damage or replicational stress during a normal cell cycle.

Precisely how these kinases are controlled in response to various stimuli is unknown. ATM kinase activity can be activated by DNA damage *in vivo*[50,51,57]. Direct activation by DNA is less well established. Some studies report that there is no stimulation by DNA *in vitro*[51,58]; others have found that small amounts of purified ATM bind to and are activated by DNA with DSBs *in vitro*[59]. It is not clear, however, whether ATM alone retains the ability to bind DNA or whether other proteins complexed with it are required. ATM may ultimately resemble DNA-PK, which by itself has a low affinity for DSB-containing DNA, but which acquires a significantly enhanced affinity in the presence of a DNA end-binding factor, the Ku 70/80 complex, which loads DNA-PK onto DNA. Proteins to which ATM is bound are candidate sensors whose identification will be essential for the molecular explanation of ATM activation and DNA damage recognition. Similar activation by damage has not been observed for ATR. However, ATR is likely to be regulated in some fashion because it controls the late phosphorylation of p53 in response to IR[52,60,61] and the ultraviolet-induced phosphorylation of Chk1 (ref. 20). Thus, it is likely that ATR activation is different from that of ATM and may involve a type of substrate accessibility activation that is not readily recapitulated *in vitro*. For example, sensor proteins may become activated and recruit substrates to ATR. This type of mechanism has been suggested to explain the observation that *S. cerevisiae* Rad9, a BRCT-repeat-containing protein, is required for Rad53 phosphorylation in response to DNA damage[23]. Rad9 is phosphorylated in response to DNA damage and phosphorylated Rad9 binds Rad53. A substrate recruitment mechanism has the additional advantage of allowing particular damage sensors to recruit distinct subsets of substrates to tailor the response to each type of damage. Recently, ATR was found to respond to DNA damage and replication blocks by forming foci that overlap with BRCA1 foci[53]. The foci formed in response to replication blocks are at sites of ongoing DNA replication. Thus, it is possible that ATR is regulated and localized to its substrates in response to genotoxic stress. Unravelling ATR regulation promises to be of primary importance in understanding the DNA damage response pathway.

Downstream kinases Chk1 and Chk2

How do ATM and ATR control downstream DNA damage responses? Current evidence suggests it is partially by controlling Chk1 and Chk2 kinases: two serine/threonine kinases that are structurally unrelated but that share some overlapping substrate

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specificity. Chk2 is the homologue of yeast Rad53 and Cds1 (Table 1) that are required for responses to DNA damage and replication blocks, whereas Chk1 kinases in yeast are primarily responsible for cell-cycle arrest in response to DNA damage. Chk2/hCds1 is phosphorylated and activated in response to IR, methyl methane sulphonate and hydroxyurea[62–65]. Its phosphorylation in response to IR is ATM-dependent. It contains a phospho-amino acid binding motif called the FHA domain and a SQ cluster domain (SCD) in its amino terminus. Thr 68 in the SCD is phosphorylated by ATM *in vivo* and *in vitro* in response to damage[66–68]. Chk2 phosphorylation is required for its activation[62,67,68]. ATR also phosphorylates these sites and may regulate the responses to ultraviolet and other agents. *CHK2* null mutant ES cells have been shown to be defective in maintaining but not initiating G2 arrest in response to IR. More importantly, IR-treated *CHK2*$^{-/-}$ thymocytes fail to stabilize and activate p53, to induce p53-dependent inducible genes and to trigger apoptosis[17]. Their inability to activate p53 and p21 indicates that *CHK2* mutants are not able to arrest in G1 in response to IR. Dominant-negative *CHK2* mutants also prevent p53 activation[69]. Chk2 can phosphorylate p53 on Ser 20 *in vitro*[17,69,70], which has been shown to interfere with binding to the ubiquitin ligase Mdm2 (ref. 71). Other studies, however, suggest that Ser 20 phosphorylation is not important for p53 stabilization in certain contexts such as ultraviolet damage[72]. Furthermore, ATM phosphorylates Mdm2 (ref. 73), the significance of which is unknown. Thus, the mechanism of ATM and Chk2 regulation of p53 is still controversial. As ATM and Chk2 both phosphorylate p53, they may synergize to ensure p53 activation only when the pathway is fully active. Genetic evidence for a common pathway for Chk2 and p53 is also provided by the identification of mutations in the *CHK2* gene in a subset of Li–Fraumeni syndrome cancer families that lack p53 mutations[74]. Chk2 can phosphorylate BRCA1 (ref. 75), indicating yet more roles for Chk2.

Unlike Chk2, Chk1 kinase activity does not appear to be increased in response to DNA damage or replication blocks. Nevertheless, Chk1 is phosphorylated in response to IR in mammals[20,76] and in yeast[26,77]. Phospho-specific antibodies recognizing phosphoserine 345 reveal that Chk1 is dramatically phosphorylated on Ser 345 in response to hydroxyurea and ultraviolet light, but only moderately phosphorylated in response to IR. Ser 345 phosphorylation in response to ultraviolet light is ATR-dependent *in vivo*[20] and ATR can phosphorylate this site *in vitro*[49]. The role of Chk1 phosphorylation is currently unknown, but it is likely to facilitate Chk1 function. As the kinase activity of phosphorylated Chk1 is not highly activated toward general substrates *in vitro*, phosphorylation may permit it to assemble with its relevant substrates *in vivo* to execute its function. Mice lacking *CHK1* die in early embryogenesis similarly to *ATR*$^{-/-}$ mice[20,21]. The fact that *ATR* and *CHK1* mutant mice have similar phenotypes and that ATR controls Chk1 phosphorylation suggests that Chk1 is a key effector of the ATR pathway and may be responsible for its essential function.

Studies on Chk1 function have progressed slowly because *CHK1* is an essential gene. Embryonic stem cells expressing a conditional *CHK1* gene die of p53-independent apoptosis after loss of *CHK1*. Before their death, these cells become incapable of preventing mitotic entry in response to IR[20], demonstrating that Chk1 is required for the G2 DNA damage checkpoint in mammals, as previously observed in other organisms[11]. The fact that Chk1 is essential for cell viability suggests that it also has important functions during S phase, possibly facilitating DNA replication and preventing premature mitotic entry.

How do Chk1 and Chk2 prevent mitotic entry? Arrest in G2 is mainly regulated by the maintenance of inhibitory phosphorylation of Cdc2 (ref. 78). Cdc2 dephosphorylation and activation is catalysed by the dual specificity phosphatase Cdc25 (ref. 79). Recent evidence indicates that part of the G2/M DNA checkpoint mechanism involves inactivation and translocation of Cdc25C into the cytoplasm. This is at least partially mediated by phosphorylation on Ser 216 in Cdc25C and its consequent binding with 14-3-3 proteins[80–82]. Chk1 (ref. 76) and Chk2 (refs 62, 64, 65) have been shown to phosphorylate Cdc25C at Ser 216 *in vitro*. This modification is thought to maintain Cdc25C phosphorylation in cells arrested at G2/M in response to DNA damage. Genetic evidence supports this model, but biochemical confirmation using cultured mammalian cells has not yet been achieved, possibly because Cdc25C is normally phosphorylated on Ser 216 during S phase and DNA damage merely prolongs its phosphorylation. Because p53 targets such as p21 and 14-3-3σ have roles in maintaining G2 arrest[83,84], Chk2 regulation of p53 is also expected to be important for the G2 DNA damage checkpoint. It is likely that other components of Cdc2 regulation are controlled in response to DNA damage, such as the inhibitory Wee1 and Myt1 kinases and cyclin B localization, all of which are known to regulate mitotic entry.

DNA damage response and repair are interacting networks

Recent observations have made it clear that the response to DNA damage in mammals is not limited to decisions on cell-cycle arrest and apoptosis, but is intimately involved in controlling repair itself. DNA repair pathways consist of an intricate network of repair systems that each target a specific subset of lesions. Much of DNA repair is constitutive, but a number of regulatory connections between the DNA damage response pathway and DNA repair have emerged (Fig. 3). First, in yeast and mammals, a large number of genes involved in DNA repair are transcriptionally induced in response to DNA damage in a DNA damage response pathway dependent manner, suggesting that many facets of repair are enhanced[11,85]. Second, fibroblasts lacking p53 have been shown to be defective in global excision repair of cyclobutane dimers. The p48 gene, which is mutated in *Xeroderma pigmentosum* group E cells, is induced by DNA damage in a p53-dependent fashion[86], possibly explaining p53's role in excision repair. Recently, a new nuclear localized subunit of ribonucleotide reductase, p53R2, was found to be induced by p53 in response to DNA damage. Blocking p53R2 expression increases cell killing by a variety of DNA-damaging agents[87], supporting a functional role for p53R2 in DNA repair. Regulation of ribonucleotide reductase through the DNA damage response pathway represents a conserved strategy employed by the DNA damage response kinases to facilitate repair[11]. Together these results challenge the long-held notion that p53 functions mainly to induce apoptosis and suggest that p53 also

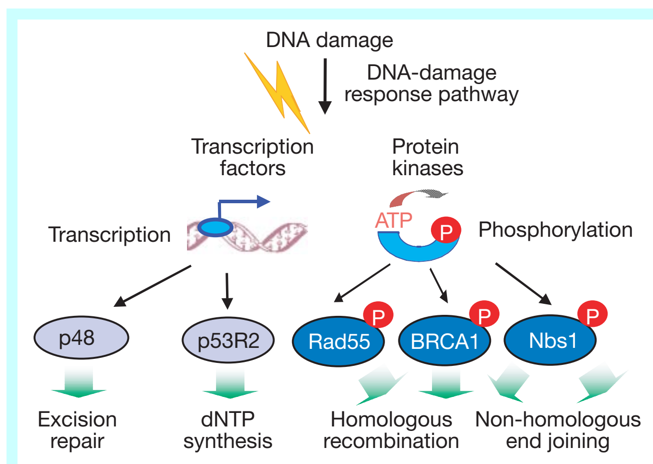

Figure 3 Interactions between the DNA damage response pathway and DNA repair networks. The regulatory connections include transcriptional upregulation of repair proteins such as p48 and p53R2 after DNA damage, and phosphorylation of repair proteins such as BRCA1, Nbs1 and Rad55 after DNA damage. See text for details.

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promotes cell survival in response to DNA damage under certain circumstances.

Another example of regulation of DNA repair comes through studies of Nijmegen breakage syndrome (NBS), which has been classified as a variant of AT on the basis of similarities between the two diseases. The Nbs1 protein resides, together with hMre11 and hRad50 (ref. 88), in a complex that is required for both non-homologous end joining (NHEJ) and homologous recombination repair of DSBs in DNA. Recently, hMre11 was also found to be mutated in individuals with another AT-like disorder[89]. AT cells have a reduced capacity to rejoin breaks[90]. Moreover, both ATM and Nbs1 are required for protection against radio-resistant DNA synthesis. These similarities suggest that ATM and the Nbs1–hMre11–Rad50 complex function in the same pathway. Further evidence has come from recent observations that ATM directly phosphorylates Nbs1 on several sites that are needed for its function *in vivo*[5–8]. This connection helps to explain how ATM regulates recombination and repair in response to DNA damage. In addition, both ATM and the Nbs1–Mre11–Rad50 complex are components of a large complex of proteins, BASC, which contains BRCA1. BRCA1 is also an ATM substrate and is required for DNA damage-induced homologous recombination[91], which suggests that these proteins are all regulated by ATM to execute repair processes (Fig. 2). Finally, the yeast Rad55 recombination protein[92] and the single-strand DNA binding protein RPA[93] are phosphorylated in response to DNA damage, further demonstrating that DNA repair proteins are regulated directly by the DNA damage response pathway. It is likely that identification of other repair proteins as substrates of damage response kinases will soon follow.

Another apparent connection to DNA repair comes from experiments in *S. cerevisiae* that show that *rad9* and *rad17* mutants have opposite effects on the rate of excision of telomeric DNA when the telomeres are no longer protected by telomeric binding proteins[94]. How they regulate these repair activities and why they behave in opposing fashions are not understood. Nevertheless, these proteins clearly play a role in controlling DNA repair functions, giving rise to the possibility that these proteins initially functioned in DNA repair and later evolved into signalling molecules.

Summary and future directions

During the past several years, considerable progress has been made in elucidating the components of the eukaryotic DNA damage response. It has become clear that the checkpoint pathways, originally thought to control primarily cell-cycle decisions, are actually part of a vast DNA damage response pathway, only a branch of which is involved in cell-cycle control (Fig. 1). Thus, for purposes of clarity it is best to refer to the entire pathway as the DNA damage response and to those components specifically involved in controlling cell-cycle progression as the 'checkpoint' branch.

How damaged DNA activates DNA damage response proteins is a central question to be addressed in the next few years. In principle, a sensor protein should have the ability to interact with damaged DNA. There are many ways to damage DNA. Thus, it will be important to determine whether there must be a sensor for each type of damage, or, alternatively, whether each type of damage is converted into one of a few common intermediates (for example, single-strand DNA), that can be detected by a limited number of sensor proteins. In addition, the process of sensing sets up a potential conflict for the cell. If damage itself is sensed through interaction with a sensor protein, this sensor protein may potentially interfere with repair proteins that also need access to damaged DNA. How does the cell resolve this potential conflict? A corollary issue is whether it is the damaged DNA or the process of repair that is sensed. The cell must be aware not only of damage, but also of when the damage is repaired, because completion of repair should be the signal for termination of the response and resumption of the cell cycle. *E. coli* has solved this problem in a parsimonious fashion because its 'sensor' is the single-strand DNA binding protein RecA, the recombinase involved in repairing the damage. Thus, in *E. coli*, it is repair that is monitored. Whether eukaryotes will once again turn out to mimic *E. coli* remains to be seen.

How the damage-signalling kinases are activated and select substrates are critical unresolved issues. Both ATM and Chk2 are activated in response to damage, but ATR and Chk1 regulation remains enigmatic. Furthermore, although many kinase substrates have been identified (Table 2), detailed mechanistic characterization of these substrates and identification of new substrates will be needed to unravel the vast complexities of the DNA damage response.

Why must the cell regulate repair? A plausible explanation is that certain constitutive repair capacities can be deleterious in certain situations. Optimal repair of various types of damage might require the regulated presence of certain repair mechanisms and the absence of others. For example, cells might benefit from a mechanism that favours homologous recombination for DSB repair in sister chromatids as opposed to non-homologous end joining. Alternatively, during DNA replication some structures produced transiently at replication forks may resemble damage, and inappropriate attempts to repair them by constitutive repair functions could disrupt DNA synthesis. This may be in part why cells stop initiation of DNA synthesis globally in the presence of DNA damage while repair is initiated. In addition, repair is unlikely to consist solely of manipulating DNA. If DNA polymerase encounters damage, replication forks may need to be reorganized to facilitate repair, possibly by restructuring chromatin to allow accessibility to repair proteins. Restructuring of chromatin may similarly be required at sites of breaks and would be consistent with the recruitment of Ku and Sirs to sites of DSBs. BRCA1 was also recently shown to be part of a chromatin remodelling complex[95]. A chromatin remodelling capacity of this sort would require tight regulation to avoid interference with ongoing cellular processes.

Another area ripe for future exploration is the relationship between the DNA damage signalling pathway and telomeric DNA structures[96]. It has been proposed that critically shortened telomeres activate the DNA damage response pathway, thereby preventing genomic instability and cancer in ageing cells. Conversely, mutants in *ATM* and *TEL1* result in shortened telomeres and display some aspects of premature ageing. The relationships between ageing, damage response pathways and telomeres need to be better understood and integrated into models for tumorigenesis.

Thus, the pathways through which cells sense and transduce DNA damage signals have begun to come into focus. It is now clear that, although these pathways control cell-cycle transitions and apoptotic decisions, its overall goal is the optimal repair of DNA damage such

Table 2 Targets of damage response kinase-dependent phosphorylation

	Kinase substrate		
Kinase class	Mammals	*S. cerevisiae*	*S. pombe*
PIK homologues	p53*, Mdm2*		
ATM, ATR (Mammals)	Chk1*, Chk2*	Rad53*, Rad9‡	Chk1‡, Cds1‡
Mec1, Tet1 (*S. cerevisiae*)	BRCA1*, CtIP*	Ddc1‡, Ddc2‡	Rad26‡, Hus1‡
Rad3, Tel1 (*S. pombe*)	Nbs1*, c-Abl*	Rpa2‡	Crb2‡
	RPA-p34*		
Chk1 homologues			
Chk1 (Mammals)	Cdc25C†		
Chk1 (*S. cerevisiae*)	Cdc25A†	Pds1‡	Cdc25†
Chk1 (*S. pombe*)	p53*		Wee1†
Chk2 homologues			
Chk2 (Mammals)	Cdc25C†	Crt1‡, Dun1‡	
Rad53 (*S. cerevisiae*)	p53*	Rad55‡, Swi6‡	Cdc25†
Cds1 (*S. pombe*)	BRCA1†	Dbf4‡, Cdc5‡,	Wee1†

* *In vitro* phosphorylation and *in vivo* dependency.
† *In vitro* phosphorylation.
‡ *In vivo* dependency.

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that cells can go on to function and contribute to the long-term survival of the organism and species with minimal levels of genetic variation. The next decade promises to be rich with new insights into this system. □

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