REVIEW

Interfaces Between the Detection, Signaling, and Repair of DNA Damage

John Rouse and Stephen P. Jackson

Left unrepaired, the myriad types of damage that can occur in genomic DNA pose a serious threat to the faithful transmission of the correct complement of genetic material. Defects in DNA damage signaling and repair result in genomic instability, a hallmark of cancer, and often cause lethality, underlining the importance of these processes in the cell and whole organism. The past decade has seen huge advances in our understanding of how the signal transduction pathways triggered by DNA damage radically alter cell behavior. In contrast, it is still unclear how primary DNA damage is detected and how this interfaces with signal transduction and DNA repair proteins.

The rapid detection of DNA lesions poses a potentially major problem for the cell. Endogenous DNA damage occurs at high frequency—for example, it has been estimated that base loss owing to spontaneous hydrolysis of DNA glycosyl bonds is in the order of 10^4 events per day for a mammalian cell (*1*). Furthermore, a bewildering array of physically dissimilar DNA lesions must be efficiently recognized. For example, ultraviolet (UV) light can induce dimerization of adjacent pyrimidines in DNA, resulting in potentially deleterious obstacles to DNA transcription and replication. The nucleotide excision repair (NER) proteins ultimately repair these photoproducts and other bulky DNA adducts (*2*). In contrast, ionizing radiation (IR) or the reactive oxygen intermediates produced as a consequence of oxidative metabolism can cause DNA double-strand breaks (DSBs), which are ultimately repaired by homologous recombination (HR) or nonhomologous end-joining (NHEJ) (*3*). DSBs can also arise when replication forks stall at sites of DNA damage (*4*). Efficient detection of DNA damage is particularly important for dividing cells where replication or segregation of chromosomes bearing unrepaired lesions could seriously compromise genome integrity.

Although the repair of different types of DNA lesion relies on different sets of proteins, the various forms of DNA damage nevertheless trigger common signal transduction pathways, which collectively bring about what is known as the DNA damage response. One well-established feature of the DNA damage response is the slowing or arrest of cell-cycle progression, as a result of what are termed DNA damage "checkpoints" (*5*, *6*), which delay key cell-cycle transitions until repair has occurred. Other aspects of the DNA damage response include changes in chromatin structure at the site of DNA damage and the transcriptional induction and posttranslational modification of various proteins involved in DNA repair (*5*–*8*). All of these outputs combine to enhance the ability of the organism to survive DNA damage.

How is DNA damage initially detected? Is the cell armed with a battery of "sensors," each capable of recognizing a different DNA lesion? Pathways of DNA repair are generally equipped with proteins that bind preferentially to certain classes of DNA lesion. For example, the MutS proteins bind to mismatched bases (*9*), the Ku heterodimer binds to DSBs (*10*), and the *Xeroderma pigmentosum* (XP) group C protein (XPC) involved in NER is one of several proteins that selectively recognize UV-induced DNA photoproducts (*2*). Over the past few years, evidence has accumulated for the dynamic physical localization of many DNA repair and DNA damage signaling proteins to sites of DNA lesions within the cell. As discussed below, these developments provide a framework for understanding how cells initially sense DNA damage and how the various aspects of the DNA damage response may be coordinated with ongoing DNA repair.

In addition to detecting different types of DNA lesions, the cell must also be able to recognize very low levels of DNA damage anywhere in the genome. Elegant studies in budding yeast have indicated that even a single persistent DSB can be detected and, under some circumstances, can trigger a global DNA damage response (*11*). It is remarkable that a single break, in a genome of 15 million base-pairs (in yeast) of DNA tightly packed into chromatin, can be quickly detected. The rapidity and potency of the DNA damage response indicates that the signaling proteins involved are very sensitive and have the capacity to greatly amplify the initial stimulus. These are key features of the Mec1p/Tel1p (ATR/ATM) signal transduction network, a highly conserved protein kinase cascade that is critical for cellular responses to many types of DNA damage (Fig. 1, Table 1).

The Mec1p/Tel1p (ATR/ATM) signaling network

Most components of the Mec1p/Tel1p signaling network were originally discovered in yeast, where defects in this pathway cause hypersensitivity to genotoxins, loss of DNA damage checkpoints, and genomic instability (*5*, *6*). The central regulator of the pathway in budding yeast, Mec1p, belongs to a family of protein kinases termed PIKKs (phosphatidylinositol 3-kinase–like protein kinases), and Mec1p functions in a partially redundant manner with Tel1p, another PIKK family member (*5*, *6*). Orthologs of Mec1p and Tel1p have been identified in many species, including humans (ATR and ATM, respectively; Table 1) (*6*). ATM seems to be involved predominantly in responding to DSBs, but ATR responds to a wider variety of lesions and has a particularly important role in genome surveillance during DNA replication. Disruption of ATR or Mec1p causes cell lethality (*6*), and *mec1* null cells can be rescued from lethality by increases in intracellular deoxyribonucleotide levels, although the molecular basis for this observation is still unclear (*6*). ATM and Tel1p are not essential proteins, although ATM defects give rise to ataxia telangiectasia (A-T), a debilitating human neurodegenerative and cancer predisposition disease whose symptoms include hypersensitivity to agents that cause DSBs (*6*).

Several cellular proteins become rapidly phosphorylated in a Mec1p/Tel1p–dependent manner in response to DNA damage (*5*, *6*). Some of these, such as Rad53p and Chk1p (hCHK2 and hCHK1, respectively, in humans) (Table 1), are themselves protein kinases that are activated by Mec1p/Tel1p (ATR/ATM)–dependent phosphorylation. When activated these proteins phosphorylate and modulate the activities of key effectors of the DNA damage response (*5*, *6*). Like *MEC1* and ATR, *RAD53* and *hCHK1* are essential genes. Mutations in *hCHK2* have been linked to increased breast cancer susceptibility (*12*) and to a variant of the Li-Fraumeni cancer predisposition syndrome (*13*).

Full Mec1p-dependent activation of downstream targets, such as Rad53p, requires several

The Wellcome Trust/Cancer Research UK Institute (of Cancer and Developmental Biology) and Department of Zoology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, UK. E-mail: spj13@mole.bio.cam.ac.uk; jwr24@mole.bio.cam.ac.uk

additional factors, some of which form two discrete protein complexes. The first of these resembles the pentameric replication factor C (RFC), except that the Rad24p checkpoint protein (hRAD17 in humans) (see Table 1) replaces the Rfc1p large subunit, forming a complex with the four small RFC subunits, Rfc2-5p (*14*). The proteins of the second complex—Rad17p, Ddc1p and Mec3p (Fig. 1, Table 1)—show sequence similarity to the proliferating cell nuclear antigen (PCNA) "sliding clamp" (*15*). The classical RFC complex physically loads the homotrimeric PCNA clamp onto primer-template junctions and is thus referred to as a "clamp loader." As discussed below, the similarity of the Rad17p-Ddc1p-Mec3p and the Rad24p/Rfc2-5p checkpoint complexes to PCNA and RFC, respectively, has prompted speculation on their mode of action. Mec1p also exists in complex with the Lcd1p/Ddc2p/Pie1p protein (referred to hereafter by its curated name, Lcd1p) that is required for all known functions of Mec1p (*16*–*18*). ATRIP, which interacts with ATR, is likely to be an ortholog of Lcd1p (*19, 20*).

Mec1p/Tel1p (ATR/ATM) network proteins associate with sites of DNA damage

Researchers studying budding yeast have developed tools that enable them to generate discrete numbers and types of DNA lesions at precise genomic sites. Several groups have combined these advances with chromatin immunoprecipitation (ChIP) or fluorescence analyses with green fluorescent protein (GFP)–tagged proteins to show that both the Rad24p/Rfc2-5p and the Rad17p-Ddc1p-Mec3p complexes translocate to sites of DNA damage (*21, 22*). Moreover, this work has shown that the recruitment of the Rad17p/Ddc1p-Mec3p complex to sites of DNA damage requires Rad24p but not Mec1p-Lcd1p (*21, 22*). Similarly, it was shown that hRAD17 is required for DNA damage–induced association of hRAD1-hRAD9-hHUS1 with bulk chromatin (*23*) (Table 1). Taken together, these data support a model in which Rad24p–Rfc2-5p loads Rad17p-Ddc1p-Mec3p (referred to hereafter as the RFC-like and PCNA-like complexes, respectively) onto sites of DNA damage, in a manner analogous to the RFC-mediated loading of PCNA onto sites of DNA replication.

Through similar approaches, it has been shown that the Mec1p-Lcd1p complex is also recruited to sites of DNA damage, independently of the PCNA-like and RFC-like complexes (*21, 22, 24*). Moreover, although loss of Mec1p does not prevent the recruitment of Lcd1p to sites of DNA damage, Mec1p is not targeted to such sites when Lcd1p is absent (*24*). In addition, Mec1p and Lcd1p can be retrieved from cell extracts by immobilized linear single-stranded (ss) or double-stranded (ds) DNA bearing free ends, and the binding of Mec1p to DNA in such assays requires Lcd1p (*24*). Mutations in a basic motif in Lcd1p, which do not affect the Mec1p-Lcd1p interaction or the subcellular localization of the complex, result in a phenotype similar to that of *lcd1*Δ null cells (*21, 24*). These mutations also abrogate the ability of the Mec1p-Lcd1p complex to bind DNA in vitro and to localize to sites of DNA damage in vivo (*24*). These data therefore suggest that direct DNA binding by Lcd1p may contribute to recruitment of Mec1p-Lcd1p to sites of DNA damage, and indicate that this is likely to be essential for the DNA damage response. Notably, it has been shown that ATR (*25, 26*) and ATM (*27*) in cell extracts can also be retrieved with different types of DNA. It is not yet clear, however, whether this requires ATRIP in the case of ATR, or some other associated factor in the case of ATM.

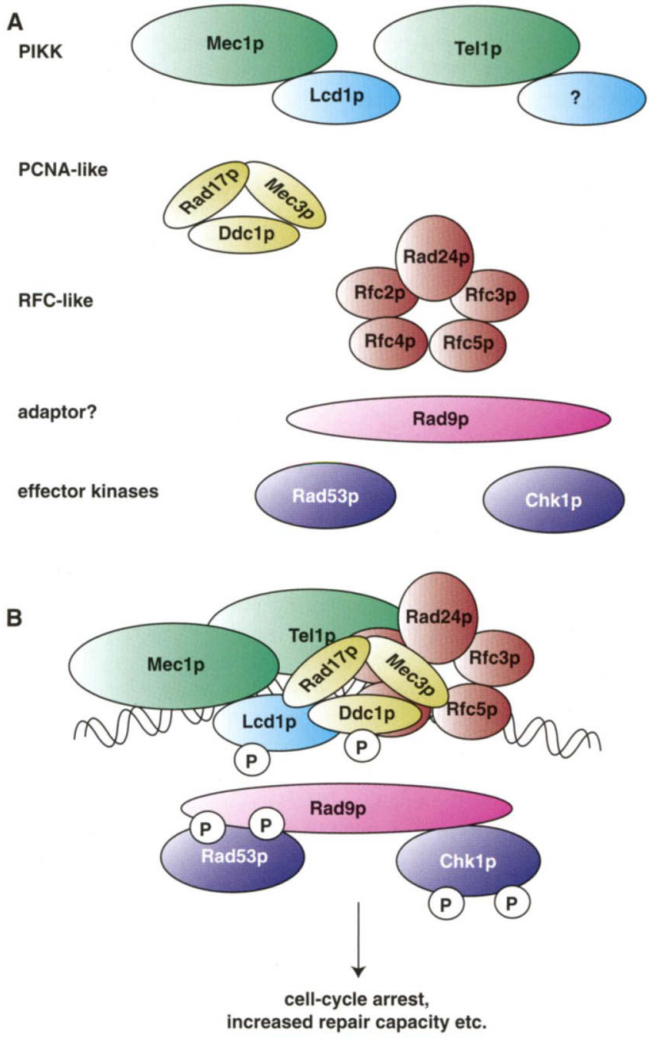

Fig. 1. The Mec1p/Tel1p signal transduction network. (**A**) The various categories of proteins involved in propagating the DNA damage signal are indicated on the left. It is not yet clear whether Tel1p has an associated regulatory subunit (or subunits). (**B**) After recognition of DNA damage, probably by lesion-specific repair factors, Mec1p-Lcd1p translocates to sites of damage independently of the RFC-like (Rad24p/Rfc2-5p) and PCNA-like (Rad17p-Ddc1p-Mec3p) complexes. Similarly, the RFC-like and PCNA-like complexes load onto these sites independently of Mec1p-Lcd1p. Exactly how these complexes are directed to DNA lesions and the precise nature of the structures that they recognize are still unclear. Activation of Rad53p and the resulting cell-cycle arrest occur after loading of these complexes. For simplicity, Tel1p is shown at sites of DNA damage, although this has not yet been demonstrated experimentally.

Potential roles for the RFC- and PCNA-like checkpoint complexes.

Mec1p-dependent activation of downstream targets, such as Rad53p, in response to DNA damage is severely reduced in the absence of the RFC-like and PCNA-like complexes (*28*). Because of this, it has been frequently concluded that loading of these complexes onto sites of DNA damage is required for the initial triggering of Mec1p signaling. However, data from both budding and fission yeasts indicate that this is unlikely. Specifically, DNA damage–induced phosphorylation of proximal Mec1p targets such as core histone H2A (*29*) and Lcd1p (*17*) requires Mec1p but not the RFC-like or PCNA-like complexes. Similarly, Rad3-dependent phosphorylation of Rad26 (see Table 1) in fission yeast does not require Rad1-Rad9-Hus1(*30*).

Thus, although the RFC-like and PCNA-like complexes translocate to sites of DNA damage, they are clearly not needed for the initial translocation of Mec1p there or for the phosphorylation of at least a subset of proximal Mec1p targets. Why, then, are they required for Mec1p to phosphorylate other targets such as Rad53p? One possibility is that these complexes are required to recruit the downstream targets of Mec1p to DNA lesions so that they can be efficiently phosphorylated by Mec1p. Consistent with this idea, Mec1p-dependent activation of Rad53p requires interaction of the latter with the Rad9p adaptor protein, after Rad9p has itself been phosphorylated by

Mec1p (*31, 32*). This inducible interaction is mediated by the FHA domains of Rad53p, which recognize phosphorylated threonine residues in particular sequence contexts (*31*). It will be interesting to investigate, through a sensitive approach such as ChIP, whether Rad9p translocates to DNA lesions and whether this translocation requires the RFC-like and PCNA-like complexes. Another possibility is that these complexes are required to maintain Mec1p in an active form, possibly by stabilizing the interaction of Mec1p-Lcd1p with DNA damage. This could be brought about by the physical interaction of the RFC-like and PCNA-like complexes with Mec1p-Lcd1p at these sites, or by their facilitating the processing of DNA lesions to form a structure that somehow sustains elevated Mec1p activity. Indeed, Rad17p has limited sequence homology to the Rec1 nuclease of the fungus *Ustilago maydis*, although several groups have failed to detect nuclease activity associated with Rad17p orthologs [see, for example, (*33*)]. As discussed below, it may be that the RFC-like and PCNA-like complexes are only recruited to DNA lesions that are more difficult to repair than others because recent data suggest that, for several different types of DNA damage, it is only persistent lesions that trigger activation of Rad53p.

Is processing of DNA lesions required for the DNA damage response?

The observations that many different types of DNA lesion can trigger the DNA damage response and that checkpoint complexes translocate to these lesions raise the questions of whether components of the Mec1p/Tel1p signaling network can recognize all of these lesions directly, whether they need a series of "adaptor" DNA damage recognition factors for their recruitment, or whether primary lesions need to be modified to allow Mec1p/Tel1p recruitment. It seems most unlikely that Mec1p-Lcd1p and the RFC-like and PCNA-like complexes could directly recognize all of the various primary lesions that arise in the cell. In this light, interesting links between lesion-specific DNA repair factors and activation of Mec1p/Tel1p signaling have been seen. For example, treatment of budding yeast cells with UV light when arrested in the G_1 phase of the cell cycle causes a delay in exit from G_1 when the cells are released from arrest. This G_1 checkpoint depends on Mec1p-Lcd1p, and components of the RFC-like and PCNA-like complexes (*34, 35*). However, in cells lacking the *RAD1* or *RAD14* NER genes, Mec1p-dependent G_1 arrest does not occur (*34, 35*). Instead, cells progress into S phase, where they arrest in a Mec1p-dependent manner, probably as a result of conversion of UV-induced photoproducts to other structures such as DSBs that can be recognized independently of NER proteins (*34*). Similarly, UV-induced accumulation of p53 in G_1, which requires ATR, is defective in mammalian cells lacking XPA (XP group A protein) (*36*).

During NER, the XPA (Rad14p in budding yeast), XPC (Rad4p), or XPE proteins bind directly to DNA photoproducts (*2*) and then recruit the transcription factor complex TFIIH, which, through the action of the XPB and XPD helicases, locally unwinds DNA to form an open protein–ssDNA structure before excision of the DNA patch containing the photoproduct (carried out by Rad1p and Rad2p nucleases) (*2*). Taken together, the available evidence therefore suggests that the NER proteins physically recruit Mec1p (or ATR) to sites of UV-induced DNA damage or that the enzymatic activity of NER components creates some intermediate DNA structure that is recognized by Mec1p-Lcd1p and the RFC-like and PCNA-like complexes. At present, it is not clear what the nature of such an intermediate might be, although one candidate is ssDNA (see below). Genetic manipulation of the successive steps in NER, coupled with the monitoring of the recruitment of checkpoint factors (as discussed below) and analysis of Rad53p activation, may help to resolve this important issue. Whatever the mechanism, these key studies suggest that the actions of lesion-specific repair proteins provide an important link between primary DNA lesions and initiation of Mec1p/Tel1p signaling. Significantly, ATR has been found to interact physically with MSH2 and MSH6 (*37*), components of the mismatch repair machinery that directly recognize mismatched bases, raising the possibility that such proteins may function to recruit ATR (or Mec1p) to this type of lesion.

DNA DSBs are potent activators of the DNA damage response, and accumulating evidence suggests that these lesions become rapidly processed. A single DSB can be introduced into the budding yeast genome by induction of the HO endonuclease, which is normally involved in mating-type switching (*11*). Studies from several laboratories have shown that HO-induced DSBs, in cells where these DSBs are made to persist by inactivation of HR, are quickly resected by a 5′–3′ exonuclease activity, resulting in the formation of ssDNA (*11*). Naked ssDNA is highly recombinogenic and is normally coated with the abundant ssDNA binding protein, replication protein A (RPA) (*38*). Because RPA-coated ssDNA is generated during many different repair processes such as NER (see above), base excision repair (BER), and HR, this structure is a good candidate for recognition by components of the Mec1p (or ATR) signaling apparatus. Consistent with this idea, Mec1p and Lcd1p, together with RPA, can be retrieved from cell extracts with ssDNA (*24*), and specific mutations in RPA show defects in DNA damage checkpoints (*38*).

Many proteins translocate to DNA damage–induced "foci."

In mammalian cells, indirect immunofluorescence analyses have shown that many proteins that play key roles in the DNA damage response become physically localized to sites of DNA damage. In many cases, these are observed as brightly staining spots or "foci" within the cell nucleus. Such proteins include ATR, ATRIP (*23*), the MRE11-RAD50-NBS1 (MRN) complex (*39*), activated forms of hRAD17 (*23*) and hCHK2 (*40*), the BRCA1 breast cancer susceptibility gene product (*41*), 53BP1 (*42*), NER factors (*43*), and proteins such as RAD51 and RAD52 that are involved in HR (*44, 45*). Although these foci have attracted much attention and have often been used as a readout of the DNA damage response in a range of contexts, the exact composition of these foci and their biological importance remain unclear.

Nuclear focus formation by the human MRN complex in response to IR has been the subject of particularly intense investigation. This complex, which is involved in a variety of responses to DSBs including checkpoint control and DNA repair, forms several differ-

Table 1. Components of the Mec1p/Tel1p (ATR/ATM) signaling network in different organisms.

Protein	Organism		
	Budding yeast	Fission yeast	Humans
PIKK	Mec1p	Rad3	ATR
	Tel1p	Tel1	ATM
PIKK subunit	Lcd1p/Ddc2p/Pie1p	Rad26	ATRIP/hRAD26
RFC-like	Rad24p	Rad17	hRAD17
PCNA-like	Rad17p	Rad1	hRAD1
	Ddc1p	Rad9	hRAD9
	Mec3p	Hus1	hHUS1
Adaptor?	Rad9p	Crb2	?
Transducer kinase	Rad53p	Cds1	hCHK2/hCDS1
	Chk1p	Chk1	hCHK1

ent types of focus in a temporally regulated manner (*39*). Most attention has been focused on Type III foci, which occur relatively late—usually several hours after exposure to DSB-inducing agents (*39*). By this time, most DSB repair is complete and type III foci may thus correspond to lesions that are difficult to repair. Formation of these late foci is defective in cells lacking ATM (*46*), linking checkpoint pathways to repair of difficult lesions.

Several lines of evidence indicate that nuclear foci correspond to sites of DNA damage. For example, when human cell nuclei are irradiated with ultra soft x-rays in defined subnuclear volumes, strong staining for the MRN complex is observed only in those parts of the nucleus that have been exposed to radiation (*47*). Furthermore, in budding yeast, an HO-induced DSB results in a single Ddc1p or Lcd1p focus (*22*), whereas when telomeric DNA damage is induced, the number of Ddc1p or Lcd1p foci corresponds roughly to the number of telomere clusters (*22*). In addition, through experiments that used porous polycarbonate filters during UV irradiation to allow DNA damage to be generated only in certain parts of the nucleus, it was shown that NER factors accumulate, in a temporally regulated manner, only in parts of the nucleus corresponding to bona fide sites of DNA damage (*43*). This technique should be useful in examining the recruitment of checkpoint factors, e.g., ATR, to DNA photolesions, and the availability of cell lines defective in different steps in the NER process could allow analysis of the genetic dependences of such events.

Protein targeting to sites of DNA damage is a dynamic process.

Many different proteins become localized to sites of DNA damage, and in several cases some of these have roles in both DNA repair and DNA damage signaling. How, then, is DNA repair coordinated with DNA damage signaling? For example, once bound, does a particular complex remain tethered at the site of damage, thus preventing binding by other complexes? These issues can be illuminated by considering studies in which the mobility of GFP-tagged proteins in living cells has been analyzed by the use of FRAP (fluorescence recovery after photobleaching). In such experiments, a small strip of the cell nucleus is bleached (which quenches GFP fluorescence) with a short laser pulse, then recovery of fluorescence is monitored at regular intervals. By ascertaining the diffusion coefficients of various proteins in such studies, it has been shown that IR-induced DNA damage results in transient immobilization of the HR proteins RAD51, RAD52, and RAD54 in mammalian cells (*48*), consistent with their translocation to sites of DNA damage (*48, 49*). Remarkably, after bleaching of a single IR-induced RAD52-containing focus, the fluorescence of the focus was found to recover over time, indicating that unbleached RAD52 molecules from the nucleoplasm can exchange with bleached RAD52 molecules in the DNA damage–induced structures. Similar results were obtained for the NER repair endonuclease complex, ERCC1-XPF (*49*). These results indicate that, although these factors are less mobile at sites of DNA damage than they are elsewhere in the nucleus, they are not irreversibly tethered there, but instead, exist in a state of equilibrium. The dynamic nature of these protein-DNA interactions could, in principle, allow several DNA damage–binding complexes to interact with the same DNA lesion within the same time frame. As discussed further below, this might allow DNA damage signaling to be coordinated with DNA repair and might permit a dynamic monitoring process that dictates how far the cellular response to DNA damage proceeds (Fig. 2).

An integrated model for DNA damage sensing, signaling, and repair.

In light of the studies discussed above (and below), we suggest a revised model of the DNA damage response (Fig. 2). In this model, DNA damage is initially detected by specific repair factor(s) that have an affinity for specific types of primary DNA lesion. In some cases, the lesion may be relatively easy to repair so that the DNA damage becomes rapidly reversed after initial detection. Under these circumstances, repair would occur sufficiently quickly to prevent recognition by components of the Mec1p/Tel1p signaling network and initiation of the DNA damage response. Consistent with this, it has been reported that HO-induced DSBs only trigger Rad53p activation when repair of these lesions is prevented by inactivation of HR (*50*). Similarly, even though hydrogen peroxide–induced DNA base damage does not trigger Rad53p activation during G_1 or G_2 in wild-type yeast cells, decreasing the efficiency of BER allows Rad53p activation in response to this type of DNA damage (*51*). Furthermore, when recognition of endogenous abasic DNA lesions is prevented, *RAD9*-dependent G_2-M checkpoint and cell death are triggered, but neither of these responses are seen in wild-type cells (*52*). Thus, lesions that are quickly repaired do not trigger the global DNA damage response.

Sometimes a DNA lesion might persist because it cannot be rapidly repaired. This could be because of the nature of the lesion—for example, some DSBs induced by IR also have damaged bases—and/or its genomic location. We propose that in such instances, the Mec1p-Lcd1p com-

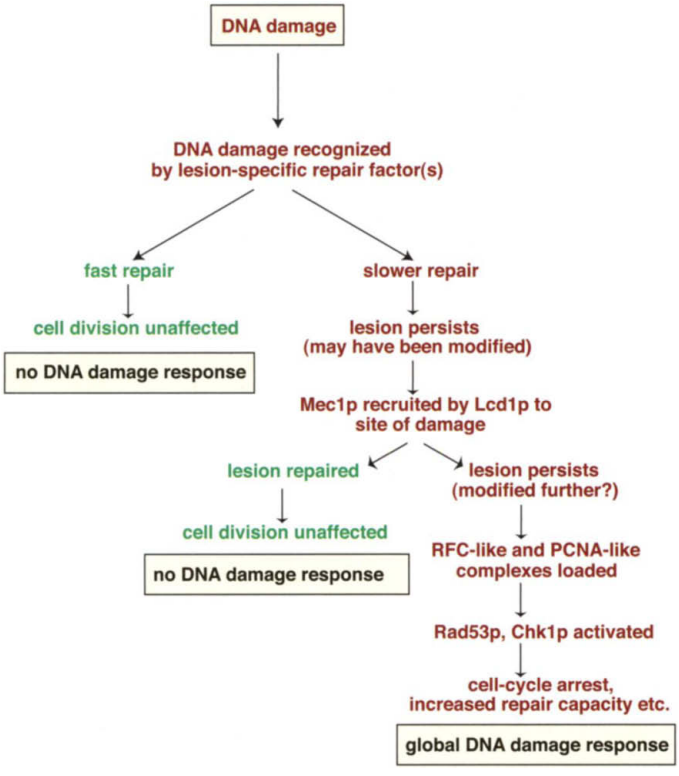

Fig. 2. An integrated model for DNA damage sensing, signaling, and repair. After detection by lesion-specific repair factors, DNA damage is either quickly repaired or it persists, depending on the nature of the lesion and/or the genomic context. If the lesion is not repaired sufficiently quickly, then Mec1p-Lcd1p is recruited to sites of DNA damage, which have probably been modified by the action of the lesion-specific repair factors. Mec1p now phosphorylates targets that are in the vicinity of the lesion, such as H2A and Lcd1p (which may be considered a "local" response), and if full repair occurs, the global DNA damage response is averted. However, if DNA repair still cannot be completed, the RFC-like (Rad24p/Rfc2-5p) and PCNA-like (Rad17p-Ddc1p-Mec3p) complexes are recruited to sites of damage that have probably been modified further, which allows Mec1p-dependent activation of Rad53p and Chk1p. This triggers a global DNA damage response including cell-cycle arrest, further chromatin modulation, and up-regulation of the repair capacity of the cell, all of which combine to facilitate repair of recalcitrant lesions and to prevent key cell-cycle transitions.

plex will translocate to sites of DNA damage, most likely in a manner that depends on the activity of lesion-specific repair factors. Mec1p will then catalyze phosphorylation of proximal targets located in the vicinity of the DNA lesion. If DNA repair now occurs [perhaps facilitated by Mec1p-dependent histone H2A phosphorylation and alteration of local chromatin structure (*29*)], then a global DNA damage response is averted (Fig. 2). By contrast, if DNA repair still cannot be completed, the lesion may then undergo more extensive modification and the RFC-like and PCNA-like complexes translocate to these sites. Only then would Mec1p-dependent activation of Rad53p and Chk1p occur (Fig. 2). This series of events would trigger the full DNA damage response, including cell-cycle arrest, possibly further chromatin modulation, and the up-regulation of the repair capacity of the cell, all of which would combine to facilitate repair of recalcitrant lesions. Because several separate complexes must independently assemble at the appropriate location as a prerequisite for the global DNA damage response, this elaborate control mechanism may provide this system with the necessary specificity and may minimize the risk of launching this response inappropriately.

In the case of UV-induced damage, it is clear that the action of the proteins that function early in NER is required for triggering Mec1p and ATR signaling, and it will be interesting to explore the molecular mechanisms involved. As discussed earlier, only when base damage persists (in BER-defective cells) is Mec1p signaling triggered by this type of damage. If it becomes possible to induce base damage at precise regions of the genome, this would facilitate analysis of the potential involvement of BER factors in recruitment of checkpoint factors to these sites. For DSBs, removal of the predominant repair pathways (HR or NHEJ) has no apparent effect on the Mec1p-dependent metaphase checkpoint induced by HO expression in budding yeast (*11*). It is possible that, because of the highly deleterious nature of DSBs, checkpoint complexes have evolved the ability to recognize this type of DNA lesion directly. In this light, the Mec1p/Tel1p network plays an important role in responding to persistent DSBs during meiosis [reviewed in (*53*)], and there is genetic evidence to suggest that recognition of these DSBs by Tel1p in meiosis does not require processing (*54*).

This model of the DNA damage response predicts that, for many types of lesions, translocation of Mec1p-Lcd1p (ATR-ATRIP) to sites of DNA damage might precede the arrival of the RFC-like and PCNA-like complexes. This hypothesis could be tested by live imaging experiments with GFP-tagged forms of these proteins. Of course, it could be that all of these complexes translocate to sites of damage at the same time, but that for some reason, the RFC-like and PCNA-like complexes can only allow Mec1p-dependent activation of Rad53p after a delay, perhaps after further lesion modification and/or processing. Genetic screens to identify factors required for the ability of the RFC-like and PCNA-like complexes to trigger Rad53p activation, combined with biochemical analyses of these checkpoint complexes, might resolve these issues. In vitro reconstitution experiments with purified proteins should also shed further light on the precise modes of action of the RFC-like and PCNA-like complexes. For example, elucidation of the types of DNA structures that are recognized by these factors and the identification of factors that interact with these complexes, on and off DNA, will be of great interest.

Conclusion

As our knowledge of the proteins involved in DNA damage detection, repair, and signaling improves, continued studies on mechanisms that integrate these processes will provide exciting new directions for research. Determining the precise structures with which DNA repair and signaling proteins interact at sites of DNA damage and analyzing how these structures are created represent critical issues and it is likely that a combination of experimental approaches—biochemical, structural, and genetic—will be required to address these problems. The question of how lesion-specific repair factors are initially directed to DNA lesions also represents a major challenge for future research.

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