The role of the DNA damage checkpoint in regulation of translesion DNA synthesis

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The DNA damage checkpoint is a signal transduction pathway that integrates DNA repair with cell cycle arrest and other cellular responses. The checkpoint response is also directly associated with mutagenic translesion DNA synthesis (TLS). For example, checkpoint activation requires complexes with roles in TLS regulation, and leads to elevated mutation levels. A role in TLS regulation implies that the checkpoint contributes to the generation of mutations, rather than their prevention. It can also explain several currently obscure aspects of this response.

Introduction

Genomic DNA is constantly subjected to damage from both exogenous and endogenous sources. DNA damage interferes with gene activity and can result in sequence alterations. Cells accordingly possess multiple mechanisms for the tolerance of DNA damage. In addition to a multitude of DNA lesion reversal pathways, eukaryotic cells possess a conserved series of pathways that coordinate cell cycle progression and additional global responses with the tolerance of DNA damage and DNA replication blocks. These so-called ‘checkpoint’ pathways are commonly divided according to the precise DNA structure that serves to initiate the pathway, i.e. DNA damage or blocked replication forks, and the cell cycle stage in which the response is elicited, i.e. G1, S or G2/M. These different pathways nonetheless share most of their attributes. This includes a protein kinase cascade that transduces the signal and is composed of PI3K-like kinases (PIKKs) and downstream effector kinases. PIKKs include Mec1 and Tel1 in the budding yeast *Saccharomyces cerevisiae*, Rad3 and Tel1 in the fission yeast *Schizosaccharomyces Pombe* and ATM-Rad3-related (ATR) and ataxia telangiectasia mutated (ATM) in mammals. The different proteins are generally specialized for dealing with specific forms of DNA structures. The effector kinases are the Chk1 protein in all the above species, and Rad53, Cds1 and Chk2 in budding yeast, fission yeast and mammals, respectively. Another central group of proteins responsible for activation of the checkpoint responses is the budding yeast Rad24 epistasis group, which includes the Rad17–Mec3–Ddc1 complex. The fission yeast and mammalian counterparts of these proteins are Rad17 and Rad9–Rad1–Hus1 (the 9-1-1 complex). The checkpoint responses mediate cell cycle arrest, transcriptional reprogramming, changes in chromatin structure and activation of several DNA repair and damage bypass pathways. Comprehensive reviews of the DNA damage and replication checkpoints have been published (1–4). The following text refers to the checkpoint responses as whole (as the ‘DNA damage checkpoint’), with important distinctions made when appropriate.

As a response to DNA damage that is also required for its tolerance, the DNA damage checkpoint is generally viewed as required for the maintenance of genome stability and the prevention of cell death and genetic alterations, and its attributes are usually regarded in the context of DNA repair. Thus, cell cycle arrest is considered to provide time to repair the damage to the DNA before cell division (5); the transcriptional response and chromatin modifications associated with checkpoint activation are thought to support DNA repair and the involvement of the checkpoint in DNA repair pathways is considered to be centred on their activation as mechanisms for lesion removal.

The role of the DNA damage checkpoint in translesion DNA synthesis

Interestingly, recent studies have begun to establish a link between the DNA damage checkpoint and the error-prone pathway of translesion DNA synthesis (TLS). TLS is mediated by specialized DNA polymerases that have a poor discrimination ability for correct Watson–Crick base pairs, owing to the relative open structure of their polymerase active cleft, and also lack a proofreading exonuclease domain. This, on the one hand, enables them to incorporate nucleotides across non-informative templates such as DNA lesions, thus bypassing lesion sites and preventing replication fork stalling. On the other hand, TLS polymerases carry a nucleotide misincorporation rate several orders of magnitude higher than that of replicative polymerases. They are accordingly responsible for most damage-induced mutations, as well as >50% of spontaneous mutations, as shown in budding yeast [reviewed in (6,7)].

An early association between the DNA damage checkpoint and mutagenesis was the finding that budding yeast cells carrying mutations in either *RAD24*, the 9-1-1 genes, or *RAD9* exhibit reduced levels of DNA-damage-induced mutations (8). A similar effect was described earlier in several fission yeast mutants that were later annotated as checkpoint mutants (9). This suggests a causative role for the checkpoint in the formation of mutations, rather than their prevention. Another unanticipated finding relates to the biochemical analogy between several of the most upstream checkpoint factors, traditionally considered sensors of DNA damage, and the machinery for loading DNA polymerases onto DNA. Replicative DNA polymerases are tethered to the DNA by the ring-shaped PCNA sliding clamp, itself loaded onto DNA by the replication factor C (RFC) complex, composed of subunits Rfc1–Rfc5. Intriguingly, Rad24/Rad17 can replace the large Rfc1 subunit to form an RFC-like complex. This complex is responsible for the loading onto DNA of the 9-1-1 complex, which forms a PCNA-like clamp complex [reviewed in (10)]. Similarly to PCNA (11), 9-1-1 interacts with various partners, for instance...
several base excision repair proteins (12). However, perhaps most significant in light of the analogy to PCNA is the interaction of 9-1-1 with the TLS polymerases $\zeta$ in *S. cerevisiae* (13) and $\kappa$ in *S. Pombe* (14). It was further shown that the interaction between 9-1-1 and Polz in *S. cerevisiae* is required for recruitment of the polymerase to sites of DNA damage, and that Polz-mediated spontaneous mutagenesis is partially dependent on the 9-1-1 complex (13). Similarly to Rad24, the *S. cerevisiae* Elg1 and Ctf18 proteins also form RFC-like complexes [reviewed in (10)] and are required for full checkpoint activation (15,16). Both complexes probably interact with PCNA rather than 9-1-1. A role for these proteins in TLS has not been demonstrated, but could be expected given their similarity to RFC and the significant genetic interactions the corresponding genes exhibit with RAD24 (15–17). Another yeast protein that has been implicated as both an upstream checkpoint factor and a TLS regulatory protein is Srs2 (18), which is thought to channel lesions into the TLS pathway (19–21). Knockdown of the human gene encoding the TLS Pol1 itself has also been reported to impair checkpoint signalling by ATM (22). Additional links between the checkpoint and TLS activity are the requirement for budding yeast Mec1, albeit not Rad24 nor Ddc1, for association of the Polz partner Rev1 with sites near a double-strand break (DSB) (23), and the relocation of human Polz to sites of active DNA synthesis concomitantly with checkpoint activation (24). Furthermore, TLS polymerase activity is regulated by monoubiquitination of PCNA (25–27), and it was recently shown that down-regulation of ATR or Chk1 reduces PCNA monoubiquitination and prevents interactions between PCNA and Polz (28).

Finally, the involvement of checkpoint proteins in controlling cellular dNTP levels may also be part of their role in TLS regulation. Checkpoint activation in response to DNA damage leads to a 6- to 8-fold increase in dNTP levels in budding yeast (29). Intriguingly, elevation of dNTP levels significantly increases resistance to DNA damage, but at the same time leads to higher mutation rates (29), concordant with the activation of a mutagenic pathway. This pathway is most likely TLS, especially if considering that TLS polymerases often require $>10$ times higher concentrations of dNTPs for their activity compared to replicative DNA polymerases (29,30).

Taken together, a role for the checkpoint in regulation of TLS and associated mutagenesis is strongly supported. Furthermore, TLS factors, especially the RFC-like complexes and the 9-1-1 clamp, appear to be not only controlled by the checkpoint, but also required for its actual activation; this could indicate that the checkpoint has a specific role in modulation of the progression of TLS rather than its mere activation, since at least a certain degree of activity and localization to target sites of TLS factors occurs independently of the checkpoint.

A role for the DNA damage checkpoint in modulation of lesion bypass as opposed to activation of DNA repair

Many of the attributes of the DNA damage checkpoint are interpreted as required for DNA repair, and the DNA damage sensitivity of checkpoint mutants is considered to be the result of failure to repair DNA. However, results from many studies seem inconsistent with this straightforward association. In fact, the main determinant of the sensitivity of checkpoint mutants to DNA damage is the irreversible breakdown of replication forks stalled at sites of DNA damage (31). Thus, budding yeast mecl and rad53 mutant cells held in G1, or after completion of DNA replication but prior to mitotic entry, are only slightly sensitive to methyl methane sulfonate, indicating that they are proficient for DNA repair. In contrast, entry into $S$ phase in the presence of DNA damage causes replication fork breakdown at damage sites and consequent cell death (32). A similar effect occurs in rad53 cells treated with the replication inhibitor hydroxyurea (33). It is well recognized that TLS and recombinational pathways enable the bypass of fork stalling obstacles [reviewed in (6,34)]. In agreement, disruption of TLS is strongly associated with the inability to recover from $S$-phase checkpoint arrest in human cells, probably due to persistence of stalled forks (24,28). This supports the possibility that an inability to properly execute TLS and/or recombinational pathways significantly contributes to the sensitivity of checkpoint mutants to DNA damage and replication blocks. This is also supported by the importance of the checkpoint-mediated control of dNTP production for DNA damage resistance [see above; (29,35)].

In contrast, other central checkpoint features traditionally associated with DNA repair seem to be less important for the tolerance of DNA damage. For example, it is frequently argued that cell cycle arrest provides time for DNA repair and by doing so supports DNA damage resistance. However, imposing an artificial cell cycle arrest on arrest-deficient cells seldom rescues their sensitivities to DNA damage. This has been demonstrated with several yeast and human checkpoint mutants and for several forms of DNA damage (32,35–39). Also inconsistent with the centrality of cell cycle arrest for the tolerance of DNA damage is the frequent lack of correlation between the ability to arrest the cell cycle and the sensitivity to DNA damage. Yeast and human checkpoint mutant cells have been described which are checkpoint defective without a corresponding sensitivity to various forms of DNA damage (39–45). Conversely, several checkpoint gene mutants exhibit an intact checkpoint response but are nonetheless sensitive to DNA damage (39,46). These evidences suggest that cell cycle arrest is not central for conferring resistance to DNA damage. The same arguments also apply to the DNA replication checkpoint (37,45,47,48). Interestingly, in the bacterial SOS response, which is responsible for activation of TLS in response to DNA damage (6), cell cycle arrest is correlated to activation of TLS, but not DNA repair, with respect to the strength of the activating signal required for inducing each pathway (49–51). This suggests a link between cell cycle arrest and TLS, which may also exist in eukaryotes.

Similar arguments apply to the checkpoint-mediated transcriptional response to DNA damage. Originally postulated to be centred on DNA repair genes, genome-wide studies failed to find an over-representation of this functional class among the induced genes in neither budding nor fission yeasts, and this response is in fact very similar to the environmental stress response (52,53). Furthermore, the induced genes are not required for survival of DNA damage (54,55).

Even more perplexing is the occasional lack of correlation between DNA damage sensitivity of checkpoint mutants and actual DNA repair ability. For instance, both ATM- and Mre11-deficient human cells generally do not exhibit a gross defect in repair ability, but are nonetheless highly sensitive to gamma irradiation. This contrasts with the clear DSB repair deficiency of cells carrying mutations in the non-homologous end-joining DSB repair pathway, which exhibit a lower sensitivity to DNA damage (39,56–59). Instead of a gross defect in rejoining DSBs, ATM-deficient (A-T) cells exhibit abnormal DSB repair kinetics and low repair fidelity, associated with
mutations and sequence rearrangements (39,56,57,60). Similarly, budding yeast rad24 mutant cells do not show a general inability to repair DSBs, but rather a defect in repair kinetics, associated with high sensitivity to this form of damage (38). These examples suggest that the checkpoint is more likely to function in controlling the kinetics and fidelity of DNA damage repair or bypass pathways than in their activation per se.

Regulation of TLS activity independently of DNA damage and in specific genomic sites

Several attributes of the DNA damage checkpoint and TLS are interesting to consider in light of a possible regulatory link between these pathways. For instance, TLS can also take place independently of DNA damage. Thus, TLS polymerases are error prone even when replicating across non-damaged DNA (7), and their actual recruitment to intact sites in the genome has been demonstrated by their artificial expression in bacteria, which leads to elevated mutagenesis in the absence of DNA damage induction (49,61–63). This so-called ‘untargeted mutagenesis’ is not confined to bacteria, as exposure of haploid S. cerevisiae cells to DNA damage followed by mating with an unexposed partner strongly induces DNA Polε-dependent mutagenesis in the undamaged strain cells (49,64). It is not known how cells normally restrict the activity of TLS polymerases towards intact sites in the genome, but a role for the DNA damage checkpoint in such regulation is an interesting possibility. This may also be related to the poorly understood significance of several checkpoint activities that are independent of DNA damage, such as the regulation of dNTP levels (35,65,66) and replication origin-activation timing (67–69), and the maintenance of replication fork stability (70) even in cells not exposed to DNA damage.

A related subject is whether there is any site preference for the activity of the DNA damage checkpoint with regard to TLS regulation. For example, the budding yeast RFC-like complex components and checkpoint factors Rad24, Elg1 and Ctf18 have been shown to suppress spontaneous ectopic recombination specifically between repetitive Ty retrotransposon elements (17). Such ectopic recombination can result in chromosomal rearrangements (71) and must therefore be restricted. Given the putative role of RFC-like complexes as TLS polymerase-loading factors (see above), it is possible that suppression of recombination is achieved by channelling lesions to the alternative pathway of TLS; the DNA damage checkpoint may thus have a role in mediating this choice of pathways at specific sites in the genome.

Perhaps more significant is the involvement of the DNA damage checkpoint in maintaining the stability of fragile sites, specific genomic sites which tend to break and undergo rearrangements under conditions of partial replication inhibition. Fragile sites were originally defined in mammalian cells; however, recent evidence also indicates a similar behaviour of budding yeast Ty sequences and tRNA-rich sites (72,73). The budding yeast checkpoint proteins Mec1, Rad9, Rad17 and Mrc1 are required for preventing breaks and genomic rearrangements in Ty and tRNA sites (70,73). Similarly, in human cells, deficiency of the ATR (74), BRCA1 (75) or TopBP1 (76) checkpoint factors leads to a significant increase in DNA breakage at fragile sites. For BRCA1, it was shown that increased fragility is correlated with a defect in cell cycle arrest after replication stalling induced by aphidicolin (75). The role of the checkpoint response in preventing DNA breakage at fragile sites points to an activity that is site specific. An interesting possibility is that this activity is the regulation of TLS. Indeed, fragile sites are characterized by highly flexible AT-rich sequences which can form secondary structures (77–79), and by perturbed or slowed DNA replication which frequently causes the stalling of replication (70,79–83), two traits reminiscent of a need for bypass activity that can be provided by TLS, although also by homologous recombination. Thus, the ability of the checkpoint response to prevent DNA breakage at these specific sites could be explained by its involvement in the regulation of TLS and/or homologous recombination. This possibility, however, requires further proof.

Summary

Genetic changes are not an inevitable result of chemical alterations uncontrolled by the cell. Rather, specialized pathways, mainly TLS and homologous recombination, are responsible for fixation of mutations and genomic rearrangements in the genome. As such, TLS and recombination are expected to be controlled by elaborate regulatory systems. For instance, one such system that is currently under intensive investigation involves the ubiquitination and sumoylation of the PCNA polymerase clamp, which affects both pathways (20,21,25–27,84).

The DNA damage checkpoint may comprise another system for the control of these pathways. Several of the most upstream checkpoint activators seem to be in fact TLS factors, in addition to emerging roles of the checkpoint in the actual regulation of TLS (Figure 1). The most plausible explanation for these
interactions is that the DNA damage checkpoint is involved in the modulation of specific aspects of TLS progression, rather than its mere activation. Thus, initiation of TLS is followed by checkpoint activation, which in turn feeds back to regulate the outcome of TLS activity. The checkpoint may regulate, for instance, the choice between several optional TLS polymerases for the bypass of a particular lesion, or the choice between TLS and homologous recombination. Additional evidence suggests that the checkpoint also affects the rates, partner choice, resolution pathway and fidelity of homologous recombination (38,39,56,57,60,85). An intriguing possibility is that this checkpoint-mediated control of TLS (and homologous recombination) is active even independently of DNA damage, and may also affect synthesis of intact sites in the genome, perhaps in a site-specific manner (Figure 1). These possibilities represent a certain deviation from the checkpoint paradigm, particularly with regard to its mode of activation (by specific DNA structures or the initiation of synthetic pathways), mode of action (pathway activation as opposed to modulation) and genetic consequences (preservation or change of genomic sequences). Most significantly, a role for the DNA damage checkpoint in regulation of TLS implies that it may not simply act to prevent genetic alterations and maintain genomic stability, but can in certain situations possibly alter the probabilities of the generation of particular genetic changes. Such a role would add to the already established roles of the DNA damage checkpoint in DNA repair, and could explain some of the currently obscure aspects of the checkpoint response, such as the DNA damage sensitivity of checkpoint mutants.

References

References:


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