DNA damage checkpoints: from initiation to recovery or adaptation
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In response to diverse genotoxic stresses, cells activate DNA damage checkpoint pathways to protect genomic integrity and promote survival of the organism. Depending on DNA lesions and context, damaged cells with alarmed checkpoints can be eliminated by apoptosis or silenced by cellular senescence, or can survive and resume cell cycle progression upon checkpoint termination. Over the past two years a plethora of mechanistic studies have provided exciting insights into the biology and pathology of checkpoint initiation and signal propagation, and have revealed the various ways in which the response can be terminated: through recovery, adaptation or cancer-prone subversion. Such studies highlight the dynamic nature of these processes and help us to better understand the molecular basis, spatiotemporal orchestration and biological significance of the DNA damage response in normal and cancerous cells.

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Introduction: DNA damage checkpoints and genome integrity
Hand in hand with the fundamental ability of all organisms to reproduce comes the challenge to faithfully copy their genomes and maintain them in a pristine state through successive cell division cycles. To preserve genomic integrity, eukaryotes employ complex surveillance mechanisms, called checkpoints, which counteract the omnipresent DNA-damaging effects of endogenous (metabolic) and environmental genotoxic insults. The DNA damage checkpoint network is composed of DNA damage sensors, signal transducers and various effector pathways, and its central components are the phosphoinositide 3-kinase related kinases (PIKKs) ATM, ATR and DNA-PK, whose many substrates mediate cell cycle arrest in G1, S or G2 phases, DNA repair and cell death [1–4]. Reflecting the biological significance of the DNA damage response (DDR) and the causative links between genetic defects in checkpoint components and major life-threatening pathologies such as cancer, immune deficiency and neurodegenerative disorders, recent years have witnessed rapid progress in this fruitful area of research [4,5]. Focusing mainly on mammalian cells, here we highlight the exciting advances in our understanding of the molecular mechanisms and spatiotemporal orchestration of checkpoint initiation and physiological termination (checkpoint recovery), and discuss how cells can escape from checkpoints through adaptation or disease-promoting malfunction.

Initiation of DNA damage checkpoints
To illustrate the initial phases of checkpoint activation, we chose the example of the response to DNA double strand breaks (DSBs), possibly the deadliest type of DNA lesion, and the one to which all three PIKKs respond in concert. While the immediate and dominant role of the ATM kinase in this process, the temporally delayed action of ATR, and the participation of DNA-PK are well established [1,4], recent studies have identified candidate DSB sensor(s), revealed the basis of cooperation between ATM and ATR, and provided insights into mechanisms of PIKK activation, the key events in checkpoint initiation.

The DSB lesions are recognized by the multifunctional Mre11–Rad50–Nbs1 (MRN) complex [6]. Five lines of evidence support MRN as the best candidate for a DSB sensor: first, the direct recognition of DNA by the Rad50 subunit [7]; second, the fact that Nbs1 is required for recruitment of ATM to DSB lesions [8–9]; third, the hypermorphic nature of the Rad50S mutant that constitutively upregulates ATM signalling [10]; fourth, the extremely rapid assembly of Nbs1 at the DSB sites, as monitored in real time directly in live human cells [11]; and fifth, the fact that the initial recruitment of the MRN complex at DSBs is independent of any other DNA damage response protein examined so far (Figure 1).

The initial interaction of MRN with the DSB lesion is transient [12], yet competent to recruit the ATM kinase. This recruitment is mediated through a protein–protein interaction between the C-terminal motif of Nbs1 and the so-called HEAT repeats of ATM [8–9]. The issue of whether this step also activates ATM or whether ATM might be recruited as an already pre-activated kinase is still a matter of debate, since experiments with different models have yielded partly conflicting results [8–9, 13,14]. In any case, the activation process involves
Co-operation between ATM and ATR kinases in response to DNA double strand breaks (DSBs). DSBs are recognized by the Mre11–Rad50–Nbs1 (MRN) complex, which recruits ATM. The activated ATM then triggers two pathways to rearrange the chromatin and process the DNA in the vicinity of the DSB lesion, events essential for checkpoint signalling and DSB repair. First, ATM phosphorylates histone H2AX, which then provides a docking platform for Mdc1 and thereby facilitates a build-up of protein complexes that also involves MRN, 53BP1, BRCA1 and ATM itself. The locally accumulated, active ATM then targets many substrates, including the effector kinase Chk2, to further spread the damage signal throughout the nucleus. Whereas this chromatin modification pathway is very rapid and operates throughout the cell cycle in a CDK-independent
autophosphorylation of human ATM on serine residues S1981 [13], S367 and S1893 [15]. Interestingly, while each of these phosphorylation events is required for proper function of human ATM [13,15], and phosphorylated S1981 serves as a marker of activation for human ATM in vitro [13,15] and in vivo [16,17], this regulatory mode appears to be less important for the activity of mouse ATM [18].

Following its recruitment to DSBs, the MRN–ATM complex triggers two main pathways culminating in local rearrangements of DNA and the neighbouring chromatin (Figure 1). These events are central to support DNA repair as well as to initiate the checkpoint signalling. First, ATM phosphorylates the C-terminal tail of histone H2AX, which in turn serves as a docking platform for the Mdc1 adaptor/mediator protein [19]. Mdc1 combines two important features: it binds directly to γ-H2AX through its tandem BRCT domains [19], and it also physically interacts with the Nbs1 subunit of the MRN complex [20]. This dual function allows a gradual spreading of H2AX phosphorylation that provides the basis for a productive assembly of most (if not all) other components of the DNA damage-modified chromatin [11,20–22]. Apart from Mdc1, proteins currently known to interact specifically with the DSB-flanking chromatin include 53BP1, BRCA1, the MRN complex and indeed ATM itself [21]. The increased local concentration of ATM is important to boost phosphorylation of ATM targets, including pan-nuclear signal messengers such as the Chk2 kinase and the Kab1 protein, the latter of which is implicated in the regulation of global chromatin compaction [4,23]. Importantly, the above-described chromatin response is very fast (the first cytological manifestations of protein assembly at the DSB-flanking chromatin are detectable less than a minute after DSB generation), operates with similar pace throughout the entire interphase, and could be facilitated by the local chromatin expansion that, surprisingly, does not seem to be γ-H2AX-dependent [11,21,22,24].

Second, MRN and ATM are also essential to initiate DSB resection and formation of ssDNA, the critical structural intermediate for DNA repair by homologous recombination and for ATR-dependent signalling (Figure 1) [25,26,27,28]. Importantly, and in contrast to the chromatin response, DSB resection also requires the activity of cyclin-dependent kinases (CDK) [27], and it is restricted to S and G2 phases of the cell cycle [21,27]. After DSB resection, the ssDNA is coated and stabilized by RPA, which in turn facilitates recruitment of the ATR–ATRIP complex [29]. Full activation of the ATR kinase requires TopBP1, a protein that is recruited to the ssDNA independently of the ATR–ATRIP complex [30]. At this point, ATR is competent to phosphorylate most of its substrates, with the notable exception of Chk1 [31], whose productive phosphorylation (followed by its release as the active kinase to the nucleus) requires the Claspin mediator protein [32]. Interestingly, the assembly of Claspin on the ssDNA generated by replication stress requires ATR-dependent phosphorylation of Rad17, another important protein that specifically interacts with the ssDNA micro-compartments [33]. Whether the same mechanism also operates on ssDNA generated after DSB resection has yet to be tested.

Finally, although we only discuss in depth the initiation of checkpoint signalling from DSB, checkpoints induced upon recognition of other types of DNA lesions operate through similar principles and share some of the key elements, including the ATR kinase. In general, ATM and DNA-PK respond mainly to DSBs, whereas ATR is activated by single-stranded DNA and stalled DNA replication forks [4]. For all these kinases, activation involves their recruitment to DNA lesions through conserved C-terminal motifs in Nbs1, ATRIP and Ku80 proteins, the ‘specificity factors’, which are required for their direct protein–protein interaction with ATM, ATR and DNA-PKS, respectively [8,9]. These motifs are critical for recruitment of PIKKs to sites of damage, as well as for the signalling events that govern the DDR machinery. These recent discoveries reveal a unifying feature shared by the upstream DDR kinases, and indicate that their mechanisms of engagement in the early phases of checkpoint signalling are more conserved than was previously thought.

Checkpoint recovery

Arguably, the major biological mission of DNA damage checkpoints is to allow time to repair the damage so that checkpoint-arrested cells can eventually resume cell cycle progression and continue their physiological programme. Until recently, however, very little was known about the molecular basis of such checkpoint termination and recovery. Here we highlight the progress in our understanding of the recovery from the G2 checkpoint, an important component of DDR that reflects the ability of cells to delay mitotic entry in the presence of unrepaired DNA lesions.

After completion of DNA repair, cells regain the ability to exit the G2 block and enter mitosis (Figure 2). Recent
SCFβ-TrCP-mediated proteolysis: a molecular switch involved in both initiation and recovery from the DNA-damage-induced G₂ cell-cycle checkpoint. Upon recognition of a DNA lesion and checkpoint initiation, the Chk1 kinase is activated in an ATR/Claspin-dependent manner and phosphorylates Cdc25A, thereby ‘earmarking’ this Cdk-stimulatory phosphatase for degradation through recognition by the SCFβ-TrCP ubiquitin ligase. As a result, the Cdk1- and Cdk2-containing kinase complexes remain in their tyros-phosphorylated, inactive form and the mitotic onset is blocked. During checkpoint recovery, the same SCFβ-TrCP enzyme targets Claspin and Wee1 (the kinase that carries out the inhibitory tyrosine phosphorylations on Cdk1/2), which are phosphorylated by the Plk1 kinase (see section in the text on ‘Checkpoint recovery’ for more mechanistic details). This cancels Chk1 activation and allows Cdc25A accumulation and activation of the mitosis-promoting Cdk1/2 complexes. Thus, all components of this functional switch are present in proliferating cells, and depending on cellular context (DNA damage and repair), SCFβ-TrCP chooses its substrates and thereby helps to dictate the phenotypic outcome in terms of G₂ arrest versus resumed mitotic entry.

Although attractive, this model (Figure 2) still awaits clarification of a crucial point, namely the mechanism behind the reactivation of Plk1 at the time of checkpoint recovery. In addition, it will be important to further explore the mechanisms that maintain Claspin stability during the S and G₂ phases, when it is required to support Chk1 activity. In this regard, two recent studies provide intriguing insights. First, Claspin’s turnover could be stabilized by a feedback loop including its phosphorylation by Chk1 [46]. In addition, Claspin protein can be ‘shielded’ from the proteasome via its recently discovered association with the USP28 de-ubiquitylating enzyme [47]. Finally, it should be kept in mind that other factors must contribute to the G₂ checkpoint recovery, as the Claspin/Wee1 degradation step, despite being essential, is insufficient to account for the entire process. For instance, there is emerging evidence that some protein phosphatases could counteract the phosphorylations carried out by the checkpoint kinases ATM, ATR and DNA-PK, thereby shifting the balance back to the steady state before the DNA-damaging insult. For example, the Wip1 and PP2A phosphatases, through de-phosphorylation of γ-H2AX, Chk1, Chk2, p53 and possibly other checkpoint components, are likely to represent additional rate-limiting factors involved in recovery from the DNA-damage-induced cell cycle arrest [48–51].

**Checkpoint adaptation**

Related to, but conceptually distinct from, checkpoint recovery is checkpoint adaptation, a phenomenon known from work with the yeast *Schizosaccharomyces cerevisiae* to confer on cells the ability to divide following a sustained checkpoint-imposed cell cycle arrest despite the presence of persistent DNA damage [52]. While it is easier to argue why an escape from long-term arrest would be beneficial for survival of unicellular organisms such as yeast, it
seemed unlikely that the cells of higher eukaryotes, including humans, could also undergo this process [53]. However, recent studies showed that adaptation to persistent genotoxic stress also occurs in frog cells exposed to chronic DNA replication block [54] and in human cells exposed to DSBs induced by persistent ionizing radiation in G2 [55]. Mechanistically, the mitotic re-entry of such adapted cells facing incomplete DNA replication [54] or unrepaired DNA breakage [55] is facilitated by resumed activity of Plx1/Plk1 and inhibition of Chk1 kinases, through an incompletely understood mechanism that involves the adaptor/mediator protein Claspin [54] and possibly other factors [55]. Whether the only mission of adaptation in vertebrates is to eliminate such defective cells with unrepairable damage through ‘mitotic catastrophe’ or related ‘mitosis-linked cell death’ from the body remains to be seen, especially given that a subset of such cells can undergo several cell cycles and some might perhaps survive.

**DNA damage checkpoint function and malfunction in cancer**

Apart from restarting the cell cycle in checkpoint recovery or adaptation, as discussed above, cells can also escape proliferation arrest or cell death as a result of checkpoint malfunction. For example, defects in DDR components such as the p53, ATM, Chk2, BRCA1 and BRCA2 tumour suppressors probably contribute to the pathogenesis of all types of human cancer [5], through allowing cell proliferation at the expense of enhanced genomic instability. But although maintenance of genomic integrity seems universally targeted in tumorigenesis [5], the biological basis for selection of cells with aberrant DDR in cancer has remained poorly understood. Recent studies gave a major insight into this issue by providing evidence that the DDR machinery is constitutively activated in early, pre-malignant lesions of major types of human solid tumours, and in cell culture models transformed by various oncogenes (Figure 3), thereby providing an inducible barrier against cancer progression [17\*,56\*,]. This activation involved both the ATR–Chk1 and the ATM–Chk2 DDR signalling modules and was attributable to oncogene-induced ‘replication stress’, including replication fork stalling, collapse and DNA breakage [57\*,58\*,]. The resulting DDR activation led to cell death or cell cycle arrest [17\*,56\*,], the latter sometimes permanent, manifested as oncogene-induced senescence [57\*,58\*,].

Most but not all tested oncogenes elicited the DDR barrier alarm [59], indicating how some early lesions can expand without experiencing suprathreshold DNA damage. Whether the underlying oncogenes or some other factors are responsible for the apparent involvement of DDR in some [60], but not all, mouse models of tumorigenesis [61,62] remains to be elucidated. This emerging concept of DDR as an endogenous anti-cancer barrier is attractive not only as a potential explanation of the selective pressure that allows outgrowth of malignant cells with acquired defects in the DDR network (Figure 3), but also because it suggests potential therapeutic implications [17\*,56\*,63].

**Conclusions and open questions**

The examples of recent progress discussed in this review illustrate that the key components of the DDR machinery, and their interplay during the dynamic checkpoint responses from initiation to termination, are emerging. The pace of discovery has accelerated over the past few years, owing to novel technologies and the availability of complete sequences of human and other genomes, yet many questions remain unanswered and new issues, both conceptual and mechanistic, pop up as we go. For example, we need to know more about the molecular mechanisms of cell fate decisions, such as temporary cell cycle arrest, senescence and cell death, for various cell types. Additional challenges for future research include the basis for maintenance and recovery of the checkpoints.
in G₁, S and G₂: the roles of posttranslational protein modifications such as acetylation, ubiquitylation or sumoylation in checkpoint signalling; and the spectrum and impact on genome integrity of various disease-predisposing or -promoting checkpoint defects. Both the precise mechanism and biological significance of checkpoint adaptation in higher organisms remain elusive, as does the role of DDR as an anti-cancer barrier relative to other processes that may prevent or slow down tumorigenesis. On the other hand, many DDR components have stimulated drug discovery, and some small molecules that target checkpoint signalling or effector pathways are under evaluation in clinical trials. Given this appreciation of the intimate involvement of DDR in human diseases and the therapeutic potential of checkpoint targeting [5], we believe that the basic discoveries will soon be translated into clinical practice and, through individualized therapy, will save the lives of patients.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

20. Using a combination of structural approaches and functional assays, this paper demonstrates a direct protein–protein interaction between MDC1 and the phosphorylated histone H2AX, and illustrates the functional impact of this interplay on chromatin modification surrounding DSBs and on regulation of downstream events within the DDR machinery.


See annotation to [27].


See annotation to [27].


In this paper and in [25, 26, 28], the authors show a surprising dependency of ATR activation upon upstream functions of ATM and the MRN complex, in response to DSBs. In addition, the dependency of ATR recruitment and activity on CDK activity in S and G2 phases of the cell cycle and the link to homologous recombination repair are demonstrated, in contrast to ATM, which functions throughout interphase.


See annotation to [27].


By demonstrating that interaction of TopBP1 with the ATRIP/ATR complex greatly enhances the ATR kinase activity, this study changed the prevailing model of ATR ‘activation by recruitment’, which postulated that the ATRIP/ATR complex is constitutively active and only targeted to substrates, but not directly activated, upon replication stress or DNA damage.


By providing evidence for the role of the SCFbetaTrCP ubiquitin ligase in the degradation of Claspin during both normal G2/M transition and recovery from the G2 DNA damage checkpoint, this study and [35, 36] help to elucidate the molecular basis of checkpoint recovery and propose an elegant model for SCFbetaTrCP as a switch between cell fate decisions in G2.


See annotation to [34].


See annotation to [34].


This study, based on analysis of the G2 checkpoint and mitotic entry in human cells after ionizing radiation, is the first to show that the phenomenon of DNA damage checkpoint adaptation also occurs in human cells.


See annotation to [17*].

This paper and [58*] provide evidence, based on cell culture and mouse models, as well as data obtained with clinical specimens, that oncogene-induced cellular senescence is one of the possible outcomes of the DNA damage checkpoints activated in response to oncogene-induced DNA replication stress.

See annotation to [57*].


