

# Mammalian G1- and S-phase checkpoints in response to DNA damage

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The ability to preserve genomic integrity is a fundamental feature of life. Recent findings regarding the molecular basis of the cell-cycle checkpoint responses of mammalian cells to genotoxic stress have converged into a two-wave concept of the G1 checkpoint, and shed light on the so-far elusive intra-S-phase checkpoint. Rapidly operating cascades that target the Cdc25A phosphatase appear central in both the initiation wave of the G1 checkpoint (preceding the p53-mediated maintenance wave) and the transient intra-S-phase response. Multiple links between defects in the G1/S checkpoints, genomic instability and oncogenesis are emerging, as are new challenges and hopes raised by this knowledge.

### Addresses

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### Abbreviations

<b>ATM</b>	ataxia telangiectasia mutated
<b>ATR</b>	ataxia telangiectasia related
<b>CDK</b>	cyclin-dependent kinase
<b>DSB</b>	double-strand break
<b>IR</b>	ionising radiation
<b>RB</b>	retinoblastoma protein
<b>RDS</b>	radioreistant DNA synthesis
<b>UV</b>	ultraviolet

### Introduction

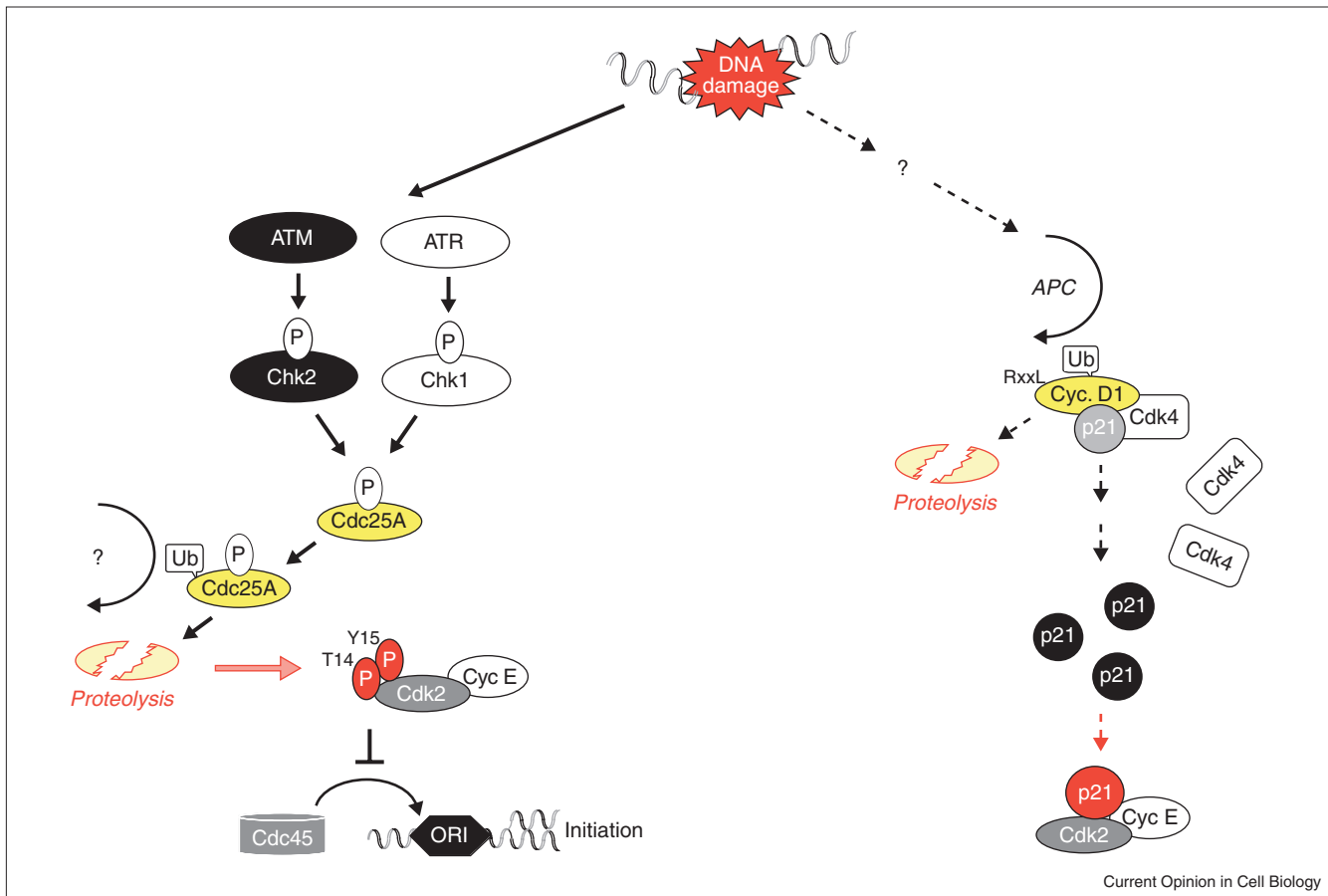
It is arguable that now and then the odd genetic mutation can be a healthy event, particularly in germ cells. Such mutations complement genetic recombination in providing limited genomic plasticity necessary for the process of evolution to select favourable traits for future generations. On the other hand, less is clearly more when it comes to genetic change, and all eukaryotes have evolved a plethora of mechanisms to minimise DNA damage. The threat of excessive genetic change needs constant attention as DNA becomes damaged by inherent errors in processes such as DNA replication, as well as through genotoxic stress from reactive cellular metabolites and exogenous stimuli (e.g. ionising radiation, ultraviolet light, cigarette smoke). Our cells cope with the required monitoring and maintenance of genomic integrity by means of a complex network of DNA repair pathways [1,2] and the so-called cell-cycle checkpoints. The latter are biochemical signalling pathways that sense various types of structural defects in DNA, or in chromosome function, and induce a multifaceted cellular response that activates DNA repair and delays cell-cycle progression [3–7]. When DNA damage is

irreparable, checkpoints eliminate such potentially hazardous cells by permanent cell-cycle arrest or cell death.

Reflecting their distinct positions and functions within the checkpoint cascades, components of the cell-cycle checkpoints have been subclassified into DNA damage sensors, signal transducers, and effectors [4]. To ensure faithful replication and transmission of the genome and to promote survival, checkpoints fulfil at least four tasks: they rapidly induce cell-cycle delay, help activate DNA repair, maintain the cell-cycle arrest until repair is complete, and then actively re-initiate cell-cycle progression. Mechanistic elements of the first three tasks are emerging, yet the molecular basis of the recovery from checkpoint-mediated arrest remains unknown. The biological and (patho)physiological relevance of the checkpoint pathways is supported by their evolutionary conservation [4], and it is evident from the consequences of checkpoint failure. Checkpoint malfunction leads to accumulation of mutations and chromosomal aberrations, which in turn increase the probability of developmental malformations or genetic syndromes and diseases including cancer [3–11].

Despite the response of some checkpoint cascades to DNA damage in quiescent cells [12\*], most checkpoint pathways operate only in cycling cells, which are at higher risk of fixing and propagating deleterious mutations [3–11]. But even among proliferating cells, the choice of checkpoint cascade(s) to be alarmed, and the outcome of such response, depends on many variables. These factors include the type, extent and duration of the DNA-damage stimulus, the type of cell cycle (meiotic versus mitotic; early embryonic versus 'somatic'), the cell type and differentiation stage, and the position of the cell within the cell cycle. Although we are still largely ignorant of the impact of some of these variables on checkpoint control and execution, rapid advances have recently been made in understanding the molecular basis of the checkpoint pathways operating in various phases of the mitotic cycles in mammalian somatic cells. The sensors of DNA damage remain relatively obscure, and may include the Rad1–Rad9–Hus1 complex, Rad17, and possibly the large ATM and ATR kinases of the PI3K family (phosphatidylinositol-3-kinase), which might recognise DNA lesions through so-far elusive subunits analogous to the Ku 70/80 proteins of DNA-PK (DNA-dependent protein kinase) [4,8,13\*]. The choice of transducers of the damage signal (the ATM/ATR and Chk1/Chk2 kinases) reflects the type of DNA damage, though some overlap between the ATM–Chk2 axis and the ATR–Chk1 axis exists [4–7]. These upstream elements of the checkpoint cascades are shared by diverse cell types and cell-cycle phases. In contrast, the downstream checkpoint effectors and their final targets within the cell-cycle machinery may differ in G1, S, or G2/M phases.

Figure 1



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Ubiquitin/proteasome-mediated protein degradation determines rapid G1 arrest in response to DNA damage. DNA damage triggers a rapid cascade of phosphorylation events involving the ATM and Chk2 (upon IR) or ATR and Chk1 (upon UV light) kinases. These cascades culminate at inhibition of the S-phase-promoting cyclin E-CDK2 kinase complex, failure to load Cdc45 on chromatin, and rapid blockade of initiation of the DNA replication origins. The key step in this pathway is the Chk2/Chk1-triggered phosphorylation (P) of the Cdc25A phosphatase, which primes Cdc25A for ubiquitination (Ub) and rapid destruction by the proteasome. The absence of Cdc25A phosphatase activity 'locks' the CDK2 kinase in its inactive form phosphorylated on inhibitory threonine 14 (T14) and tyrosine 15 (Y15). This pathway operates presumably in every cell type and appears to be conserved among vertebrates. Moreover, proteolysis of Cdc25A was also linked with the replication checkpoint, which guards against premature entry into mitosis in the presence of stalled replication

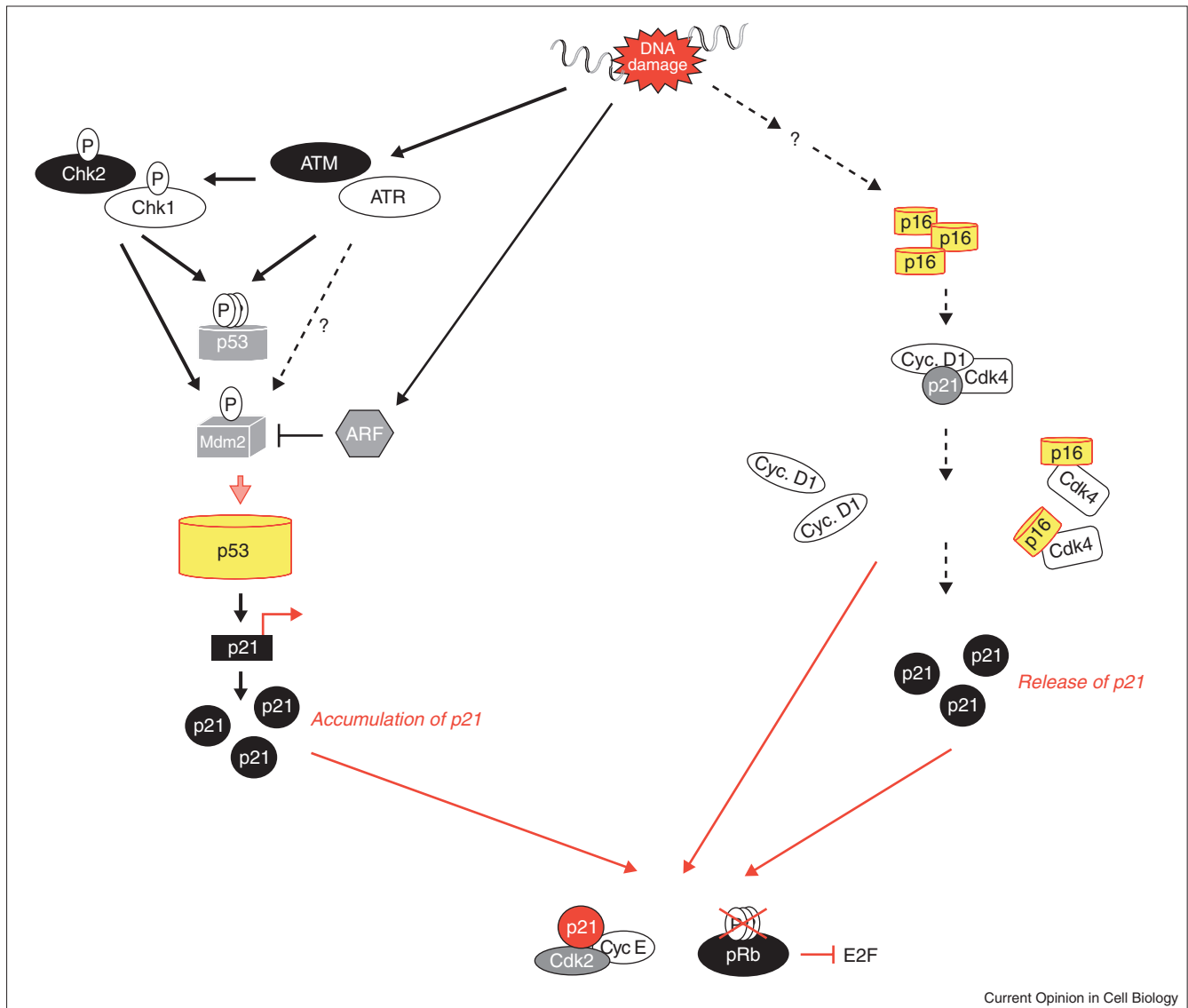
forks [66\*\*]. In some mammalian somatic cells, whose proliferation critically depends on the presence of abundant cyclin D1, another mechanism may contribute to initiate the rapid G1 arrest. Here, DNA damage leads to unmasking of a cryptic 'destruction box' (RxxL) within the cyclin D1 amino-terminus, which leads to its recognition by the anaphase-promoting complex (APC) ubiquitin ligase and priming for rapid destruction by the proteasome. The result is again inactivation of the S-phase-promoting cyclin E-CDK2, in this case by release of the p21 CDK2 inhibitor from the disrupted cyclin D1-Cdk4(6) complexes. Critical steps in both pathways involving proteasome-dependent proteolysis are highlighted by yellow. Question marks indicate the key open questions for future research, namely which ubiquitin ligase primes Cdc25A for degradation, and what is the nature of upstream signalling which couples DNA damage with the proteolysis of cyclin D1 (this pathway has been proposed to be ATM/ATR-independent).

In this review, we discuss the progress in elucidating the mechanisms of the mammalian DNA-damage checkpoints that guard the entry into, and progression through, the S phase. This focus has been motivated by the recent discoveries of the molecular basis for the rapid, p53-independent initiation of the G1 checkpoint [14\*\*,15\*\*], and the intra-S-phase checkpoint [16\*\*–20\*\*]. Furthermore, we provide examples of potential cell-type-restricted checkpoint responses, and the evidence for cancer-promoting aberrations in the G1- and S-phase checkpoints. Finally, we highlight the conceptual significance of these new discoveries and the challenges they raise for future research.

### G1/S control and the two-wave G1 checkpoint response

To appreciate the workings of the G1 DNA damage checkpoint(s), it is helpful to briefly consider the G1/S control. G1 phase is a period when cells make critical decisions about their fate, including the optional commitment to replicate DNA and complete the cell division cycle. Provided mitogens are available and the cellular environment is favourable for proliferation, a decision to enter S phase is made at the so-called 'restriction point' in mid-to-late G1 [21]. In unstressed cells, this commitment to replicate DNA and divide seems irreversible until the

Figure 2



Maintenance of the G1/S arrest after DNA damage is a delayed response that requires transcription, translation and/or protein stabilisation of key checkpoint transducers. Once initiated, the G1 arrest must be maintained and the entry into S phase prevented as long as the cell detects a single unrepaired DNA lesion. As in the rapid response (see Figure 1), the ATM/ATR and Chk2/Chk1 kinases play a pivotal role. Thus, phosphorylation of p53 stabilises the protein by preventing its interaction with Mdm2, which acts as a specific inhibitor of p53 transactivation domain and a p53 ubiquitin ligase. Phosphorylation of both p53 and Mdm2 also inactivates nuclear export of p53. Furthermore, at least some types of DNA damage can upregulate the ARF protein, a specific inhibitor of Mdm2. Collectively (and together with other p53 activating mechanisms such as sumoylation, acetylation,

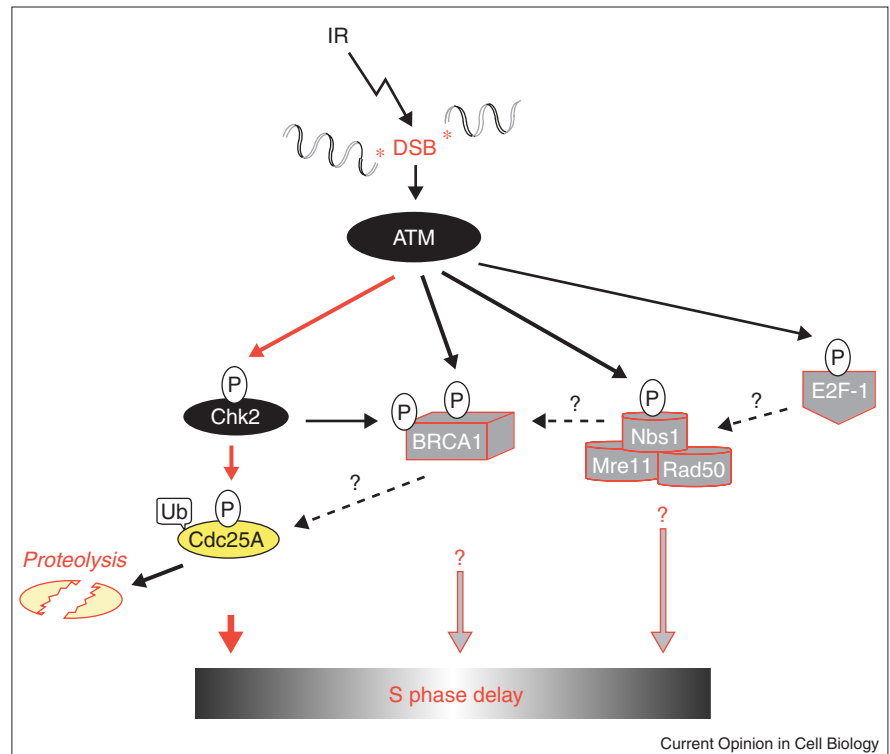
dephosphorylation-dependent interaction with 14-3-3 proteins), this leads to accumulation of a stable and transcriptionally active p53 protein in the cell nucleus. This in turn results in induction of a number of genes including the p21 CDK inhibitor. Exposure of epithelial cells to UV light can lead to yet another mechanism to mobilise the cellular p21. In this case this is a gradual accumulation of p16, a protein that can selectively disrupt cyclin D-CDK4(6) complexes and thereby release already existing pool of p21. When accumulated to a threshold level, p21 can stoichiometrically bind and inhibit all cellular S-phase promoting cyclin E-CDK2, and thereby secure the maintenance of the G1 arrest. Another important consequence of inhibiting both CDK2 and CDK4(6) kinase complexes is dephosphorylation of RB and inhibition of the E2F-dependent transcription of S-phase genes.

next G1 phase. Importantly, the checkpoints alarmed by genotoxic stress can delay cell-cycle progression even when cells have already passed this restriction point. Available data suggest that the restriction point switch, from the growth factor-dependent early G1 to the subsequent mitogen-independent phases, reflects the induction of broad transcriptional programmes regulated

by the parallel retinoblastoma protein (RB) and Myc pathways, which regulate genes critical for G1/S transition and coordination of S-G2-M progression ([11,21,22\*,23\*] and references therein). Within the RB pathway, the molecular switch appears to be the phosphorylation of RB by cyclin D-CDK4(6) kinases [21,24], resulting in the derepression of the RB-regulated E2F transcription

**Figure 3**

Molecular mechanisms of the intra-S-phase checkpoint. In contrast to G1/S and G2/M transitions, S phase can only be delayed, and never permanently blocked in the presence of DNA DSBs generated by IR. ATM- and Chk2-dependent degradation of the Cdc25A phosphatase, and the consequent blockade of *de novo* initiation of replication origins (see Figure 1 for details) represent an essential means to achieve rapid reduction of the rate of DNA synthesis. Other cellular factors directly phosphorylated by ATM and/or Chk2, such as the Nbs1–Mre11–Rad50 complex, BRCA1 and E2F-1 (J Nevins, personal communication) also contribute to the S-phase checkpoint (see also Update). What is the exact hierarchical order among these factors, and whether they converge on downregulation of Cdc25A or operate independently on parallel pathways are among the major challenges for future research.



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factors [21,22\*,25]. E2F and Myc jointly activate the key target gene cyclin E whose product activates the CDK2 kinase necessary for the actual initiation of DNA replication [11,26,27]. Consequently, the cyclin E protein becomes detectable and accumulates only in late G1, a few hours after the passage through the restriction point [28\*]. Both its position at the convergence of the RB and Myc pathways, and its essential and rate-limiting function in G1/S transition, makes cyclin E–CDK2 activity an ideal candidate for a DNA damage checkpoint target [11]. In principle, progression through G1 can be blocked either at the restriction point (by preventing RB phosphorylation), or closer to the G1/S transition by silencing cyclin E–CDK2 activity. Both CDK2 [14\*\*–16\*\*] and RB [29] are indeed targeted by the DNA damage checkpoint(s), yet through temporally distinct mechanisms corresponding to induction and maintenance of the G1 checkpoint, respectively (see below).

For almost a decade, the G1 arrest induced by DNA damage has been ascribed to another transcription factor, the p53 tumour suppressor protein [3,9,11]. Upon diverse stress stimuli, cellular p53 becomes post-translationally modified, stabilised, and competent to induce expression of genes required to halt the cell-cycle progression or trigger programmed cell death [9,30]. Among the genes induced by p53 is the CDK inhibitor p21<sup>Waf1/Cip1</sup>, capable of silencing the CDKs that are essential for entry into S phase [24,26]. However, the transcription-dependent and protein synthesis-dependent role of p53 in the G1 checkpoint is

implemented too slowly to account for the rapid inhibition of CDK2 seen upon genotoxic stress [11]. In addition, the silencing of cyclin E–CDK2 activity in late G1 occurs even in cells lacking p53 or p21 ([14\*\*–16\*\*], and references therein). These facts argue for a two-wave model of the G1 checkpoint response in mammalian cells, in which the initial, rapid, transient and p53-independent response is followed by the delayed yet more sustained G1 arrest imposed by the p53–p21 axis. A strong experimental support for this model comes from the recent identification of a novel pathway that underlies the rapid inhibition of CDK2 upon various types of DNA damage, as discussed in the following section.

### Rapid, p53-independent induction of the G1 checkpoint

To be effective within minutes after DNA damage, induction of the G1 block should exploit a mechanism that is poised to act, independent of transcription and protein synthesis. Recent reports suggest that pathways which fit this definition operate by targeting Cdc25A [14\*\*–16\*\*]. The phosphatase activity of Cdc25A cancels the inhibitory phosphorylation of CDK2 and is essential for G1/S transition [11]. Independent of the p53 status, the abundance and activity of Cdc25A rapidly decreases when mammalian cells are exposed to ultraviolet (UV) light or ionising radiation (IR), reflecting ubiquitination induced by DNA damage and accelerated turnover of the Cdc25A protein by proteasomes [14\*\*,16\*\*]. This novel checkpoint pathway (Figure 1) results in persistent

**Table 1****Aberrations of the G1/S checkpoint components in human tumours**

Gene/protein <sup>†</sup>	Molecular basis <sup>‡</sup>	Aberrations in human cancer*	
		Sporadic tumours <sup>§</sup>	Hereditary syndromes/tumours <sup>#</sup>
ATM (S)	Truncations, missense mutations, deletions, reduced expression	Carcinomas of the breast	Ataxia-telangiectasia
ATR (S/?)	NR	NR	NR
Chk1 (S)	Frameshift mutations	Colorectal and endometrial carcinomas	NR
Chk2 (S)	Missense mutations, truncations, reduced expression	Carcinomas of the breast, lung, colon, urinary bladder, testicular tumours	Li-Fraumeni syndrome
BRCA1 (S)	Diverse types of mutations, deletions, reduced expression	Carcinomas of the breast, ovary	Familial breast and ovarian cancer
Mre11 (S)	Missense and frameshift mutations, truncations.	Carcinoma of the breast, lymphoid tumours	Ataxia-telangiectasia-like disorder
Nbs1 (S)	Truncations	NR	Nijmegen breakage syndrome
p53 (S)	Missense mutations, deletions, etc. HPV-E6-mediated degradation	Many types of cancer	Li-Fraumeni syndrome
Cdc25A (O)	Overexpression	Carcinomas of the breast	NR
p16 (S)	Deletions, promoter silencing, missense mutations	Many types of cancer	Familial melanoma
<i>Cyclin D1</i> (O)	Gene amplification, translocation, overexpression, etc.	Many types of cancer	NR
<i>Cyclin E</i> (O)	Gene amplification, overexpression	Carcinomas of the breast, ovary	NR
RB (S)	Deletions, diverse mutations, promoter silencing, HPV-E7-mediated inactivation	Many types of cancer	Familial retinoblastoma

\*Further reading can be found in references 1–11,30,56,59–63, 67–70,71\*,72\*\* and 73. †(S) = tumour suppressor; (O) = (proto)-oncogene; (S/? ) = candidate suppressor; italics: effectors and targets of checkpoints, as opposed to upstream checkpoint regulators and

transducers (top part, non-italicized). ‡Examples of molecular defects (somatic or germline) found in human tumours. NR = not reported. §The list shows examples of tumour types, it is not exhaustive. NR = Not reported. #All listed syndromes are cancer prone. NR = not reported.

inhibitory phosphorylation of CDK2 on tyrosine 15, and thus inhibition of cyclin E–CDK2 activity leading to the blockage of G1/S transition.

The signal for ubiquitination after UV and IR exposure is created by phosphorylation of Cdc25A mediated by Chk1 [14\*\*] and Chk2 [16\*\*], respectively. The critical residue of Cdc25A targeted by Chk2 is serine 123 [16\*\*]. This pathway is sensitive to caffeine, an inhibitor of the ATM/ATR kinases [14\*\*,16\*\*,31\*], and at least the response to IR depends on ATM [15\*\*,16\*\*] as an activator of Chk2 [4,7] (Figure 1). The end-point target of this cascade is the inhibition of CDK2-dependent loading of Cdc45, an attractant for DNA polymerases, onto DNA pre-replication complexes ([15\*\*]; J Falck, personal communication). Consistent with criteria for a bona fide cell-cycle checkpoint, the extent of DNA damage is enhanced and cell survival decreased after irradiation under conditions when the ubiquitin/proteasome-mediated degradation of Cdc25A is experimentally prevented [14\*\*]. Thus, the ATM/ATR–Chk2/Chk1–Cdc25A–CDK2 pathway(s) seem to account for the p53-independent initiation of the G1 checkpoint, and the need for speedy execution seems

solved by a cascade of protein–protein interactions, phosphorylations, ubiquitination and proteolysis of the key target, the Cdc25A phosphatase (Figure 1).

Interestingly, an analogous concept based on enhanced protein degradation in response to IR has been reported to target cyclin D1, another G1 regulator [32\*\*]. The rapid silencing of CDK2 by this pathway is thought to reflect redistribution of the p21 CDK inhibitor, from cyclin D1–CDK4(6) complexes (for which p21 serves as an assembly factor) to cyclin E–CDK2 complexes, which are inhibited by p21 [24,32\*\*] (Figure 1). If confirmed as a cell-cycle checkpoint, this mechanism would be an example of an ATM-independent, cell-type-restricted response, since cyclins D2 and D3 are not degraded upon DNA damage, and therefore this pathway would have little effect in cell types expressing several D-cyclins, or lacking cyclin D1 (see Update). Nevertheless, such a mechanism may complement the more ubiquitous Cdc25A pathway, which operates in many cell types, upon diverse genotoxic stimuli, and is phylogenetically conserved at least from *Xenopus* [15\*\*] to mammals [14\*\*,16\*\*].

## The p53 pathway and the maintenance of the G1 arrest

Under normal conditions, p53 is a highly unstable protein and its DNA binding capacity is low. After DNA damage, numerous post-translational modifications lead to stabilisation of the p53 protein and activation of its sequence-specific DNA binding [9,30]. Only then can p53 efficiently stimulate transcription of cell-cycle inhibitors such as p21 (Figure 2). Furthermore, the p21 protein has to accumulate to levels sufficiently high to inhibit the CDK-containing complexes, before cell-cycle progression becomes efficiently blocked. Although p53 has recently been described binding to 5' untranslated region of CDK4 mRNA and inhibition of CDK4 translation seemed to be transcription-independent [33], even this process requires time for stabilisation and accumulation of p53, and the subsequent slow decay of the stable CDK4 protein. Thus, depending on the nature of DNA damage, the period from generating the DNA lesion to the effective p53-dependent cell-cycle arrest can last for several hours, consistent with maintenance of the G1 block previously initiated by the Cdc25A pathway.

The events that mobilise p53 after stress, including its protein stabilisation, subcellular trafficking, and transcriptional activation, have been reviewed recently [9,30]. Yet this complex regulatory web is continuously expanding [34,35,36\*,37]. What needs to be emphasised in relation to the two-wave G1 checkpoint concept is that the key upstream regulators, the ATM/ATR and Chk2/Chk1 kinases, are shared by both waves (Figures 1,2) and target Cdc25A and p53 simultaneously within minutes after DNA damage. Phosphorylation on serine 20 of p53 by Chk2/Chk1 helps stabilise p53 by uncoupling it from the Mdm2 ubiquitin ligase [38\*\*–40\*\*], while ATM- (and likely also ATR-) mediated phosphorylations of Mdm2 (Ser 395) [36\*] and p53 (Ser 15 and some other residues) interfere with nuclear export of p53 [41\*], and help activate p53 [5,7,30], respectively. But despite the fact that the initial steps along the G1 checkpoint are common for the Cdc25A and p53 pathways, their impact on CDK2 activity and G1/S blockade are separated in time, due to the dependence of the latter pathway on transcription and protein synthesis (Figures 1,2).

The maintenance of cell-cycle checkpoints may be further prolonged by additional mechanisms. For example, the ARF tumour suppressor (known to sequester Mdm2 in response to oncogenic stimuli [42]) also becomes induced with delayed kinetics after DNA double-strand breaks (DSBs), and may reinforce stabilisation and activation of p53 after DNA damage [43\*\*]. Another example is the delayed increase of the p16<sup>INK4a</sup> CDK inhibitor in human skin keratinocytes and melanocytes following exposure to physiological doses of UV light [44]. The UV-induced elevation of p16 occurred 16 hours after exposure and peaked by 24 hours, being reversible with a decline by 72 hours post-irradiation. Such accessory maintenance

pathways (Figure 2) may act in a stimulus-dependent, cell-type-restricted manner.

## The intra-S-phase checkpoint response

In contrast to the key role of p53 in maintenance of the DNA-induced G1 arrest, no specific roles for p53 or p21 have been implicated in the control of the intra-S-phase checkpoint. This is perhaps not so surprising as the S-phase checkpoint, manifested by a decreased rate of DNA synthesis after generation of DSBs, is by definition a transient phenomenon [5]. The absence of the 'maintenance component' during S phase, contrary to the G1 and G2 checkpoints, might be beneficial for the cells by providing some delay but not permanent arrest with incompletely replicated genome. Long-term intra-S-phase blockade would limit the amount of sister chromatids and therefore reduce available template for efficient repair by homologous recombination. Moreover, work in yeast suggests that complete inhibition of CDKs and prolonged intra-S-phase arrest may cause regaining of replication competence of already fired origins, which would then make the recovery process prone to over-replication of at least parts of the genome [45]. Finally, it is possible that the p53 activation in S phase could be detrimental *per se*, and that there are mechanisms that operate in every S phase to prevent p53 from targeting at least a subset of genes. It has been speculated that induction of a 'full-scale' p53 transcription programme within S phase, when the E2F-1 transcription factor (known to cooperate with p53 to induce apoptosis) is highly active, could promote unwanted cell death [46\*].

Unexpectedly, fresh insights into the intra-S-phase checkpoint mechanisms induced by DSBs have also implicated the above-mentioned Cdc25A-degradation pathway in slowing down ongoing S phase [16\*\*,47]. Thus, the ATM–Chk2–Cdc25A–CDK2–Cdc45 axis emerges as a key mechanism of not only the rapid prevention of S-phase entry in the G1 checkpoint [14\*\*,15\*\*] (Figure 1), but also in the transient intra-S-phase response [16\*\*] (Figure 3), predictably affecting both the early- and late-firing origins of DNA replication [48\*]. Inhibition of CDK2 activity through Cdc25A degradation leads to a several-hour delay of S-phase progression, a timing that correlates well with the transient intra-S-phase checkpoint response [16\*\*]. The physiological relevance of this pathway is documented by the fact that analogous to ATM defects, interference with the Chk2–Cdc25A–CDK2 cascade at any of these steps downstream of ATM results in radioresistant DNA synthesis (RDS) [16\*\*], a phenomenon of persistent DNA synthesis after irradiation, originally described for ataxia telangiectasia patients who harbour mutations in the ATM gene [5,7].

Upon IR-induced activation in S-phase cells, ATM phosphorylates several checkpoint components including Chk2 [49\*,50\*] (which then targets Cdc25A), but also BRCA1 [51,52], and Nbs1 [17\*\*–20\*\*], a component of the

Mre11–Nbs1–Rad50 complex [10,53]. The ATM-mediated phosphorylations of Nbs1 are required for the proper execution of the intra-S-phase checkpoint, since mutating the targeted serine residues (Ser 278, 343 and 397) to alanine resulted in RDS. Reports documenting functional interplay between Chk2, BRCA1, and Nbs1 [54•,55,56], Mre11 complex and E2F-1 [57•], and S-phase checkpoint defects in BRCA1-deficient cells [58•] are tantalising yet so far insufficient to judge whether or not all these regulators feed into the Cdc25A pathway [16•,47], or whether parallel mechanisms co-operate to inhibit DNA replication upon IR (Figure 3).

### G1/S checkpoint defects and cancer

Genetic instability is one of the hallmarks of cancer, and its links to aberrations in DNA repair machinery and the cell-cycle checkpoint pathways is well documented [1–11,30,56,59–61]. Evidence to support this notion continues to accumulate, and here we briefly review the known, and particularly the recently identified, cancer-associated defects of the G1/S checkpoint components (Table 1).

Except for the ATR whose lack causes early embryonic lethality in mice [62,63] and whose somatic defects might result in cell death, all the major G1/S checkpoint transducers and effectors qualify as either tumour suppressors or proto-oncogenes, and their loss-of-function mutations or overexpression have been identified in many types of human malignancies (Table 1). In addition, when mouse models that mimic such defects are available, the resulting phenotypes generally support the putative roles of these checkpoint regulators and effectors in guarding against genomic destabilisation and tumour development. Hereditary mutations in at least ATM [5,7], Chk2 [64•], BRCA1 [59], Mre11 [65], Nbs1 [10], p53 [9,64•], p16 [60], and RB [60] are known to cause familial cancer and/or clinical syndromes that are cancer prone (Table 1). The intimate involvement of cell-cycle checkpoints in molecular pathogenesis of cancer, and their emerging significance for the outcome of chemotherapy and radiotherapy, inspired intensive efforts to explore this new knowledge for diagnostic purposes, and particularly to search for more rational cancer treatment strategies. Global assessment of the checkpoint pathways by functional genomics and proteomics approaches may help predict therapeutic responses of individual cancers or aid in selecting a tailor-made treatment. In terms of new therapies, attempts to develop CDK inhibitors, activators of p53 and particularly its pro-apoptotic effect, as well as attenuators of checkpoint responses that might presensitise tumour cells to radiation and cytotoxic drugs, are well under way [9,11,30] and remain a great promise for the future.

### Conclusions and future directions

The crude molecular anatomy of mammalian cell-cycle checkpoints is taking shape, and we are learning rapidly about their physiology and pathology. The two-wave concept of the G1 checkpoint, the mechanistic insights

into the intra-S-phase checkpoint, and the appreciation of checkpoint aberrations as important determinants of multistep tumorigenesis exemplify the recent advances in this field. One of the unifying features of the G1- and S-phase checkpoints is their joint targeting of the Cdc25A pathway and, more broadly, their rapid effects on protein turnover of the critical checkpoint effectors, namely degradation of Cdc25A, cyclin D1, and protection from degradation of p53. Among the major gaps that remain in our understanding of checkpoint function is the extent and molecular nature of the interdependence between cell cycle effects and DNA repair, along with the signalling, dynamics, and indeed the mechanistic basis of the recovery from activated checkpoints in any cell-cycle phase. Related to this is the somewhat contradictory issue of whether or not cells that activate the p53 response ever resume proliferation.

Research on the specific features of the already known, or possibly still unknown, ‘accessory’ checkpoint pathways restricted to certain cell- and tissue-types and differentiation stages is in its infancy. Clarification of the identity and modus operandi of the ‘true’ sensors of DNA lesions should also be a fruitful area of investigation in the near future. There is also a clear need to find the missing components and connections in the web of the checkpoint signalling cascades, and better understand the significance of the protein–protein interactions within multiprotein complexes, and their dynamic changes in response to distinct types of DNA damage. These studies should greatly benefit from the recently established technologies allowing kinetic and often quantitative analyses of such transient events in living mammalian cells in real time. Yet how to study phosphorylation of particular proteins at key residues, directly in live cells, is still a subject for technological development.

The link between checkpoint failure, genome destabilisation, and cancer, will surely inspire exploration of more rational therapies based on pharmacological intervention with rate-limiting events in checkpoint pathways. Elucidation of the importance of haploinsufficiency in checkpoint genes such as ATM, BRCA1 or Chk2, for cancer predisposition, is required. A closer symbiosis of basic and translational research into how the checkpoint pathways work will lead not only to many more exciting discoveries to satisfy our curiosity about the elementary principles of life, but hopefully also offer a new generation of drugs to treat cancer.

### Update

Experiments with transgenic mice by P Sicinski and colleagues [74••] greatly substantiate the concept that cyclin D1 could indeed represent an important checkpoint target in specific tissues. An excellent review on ATM- and ATR-mediated checkpoint signalling by R Abraham has recently been published [75]. This overview also summarises the current knowledge about the sensors of damaged DNA, including the candidacy of the Rad

protein family members for such function, an issue not discussed in our review.

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