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

Jiri Bartek* and Jiri Lukas

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
Department of Cell Cycle and Cancer, Institute of Cancer Biology, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen, Denmark
*e-mail: bartek@biobase.dk
Correspondence: Jiri Bartek

Current Opinion in Cell Biology 2001, **13**:738–747

0955-0674/01/$ – see front matter

Abbreviations

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

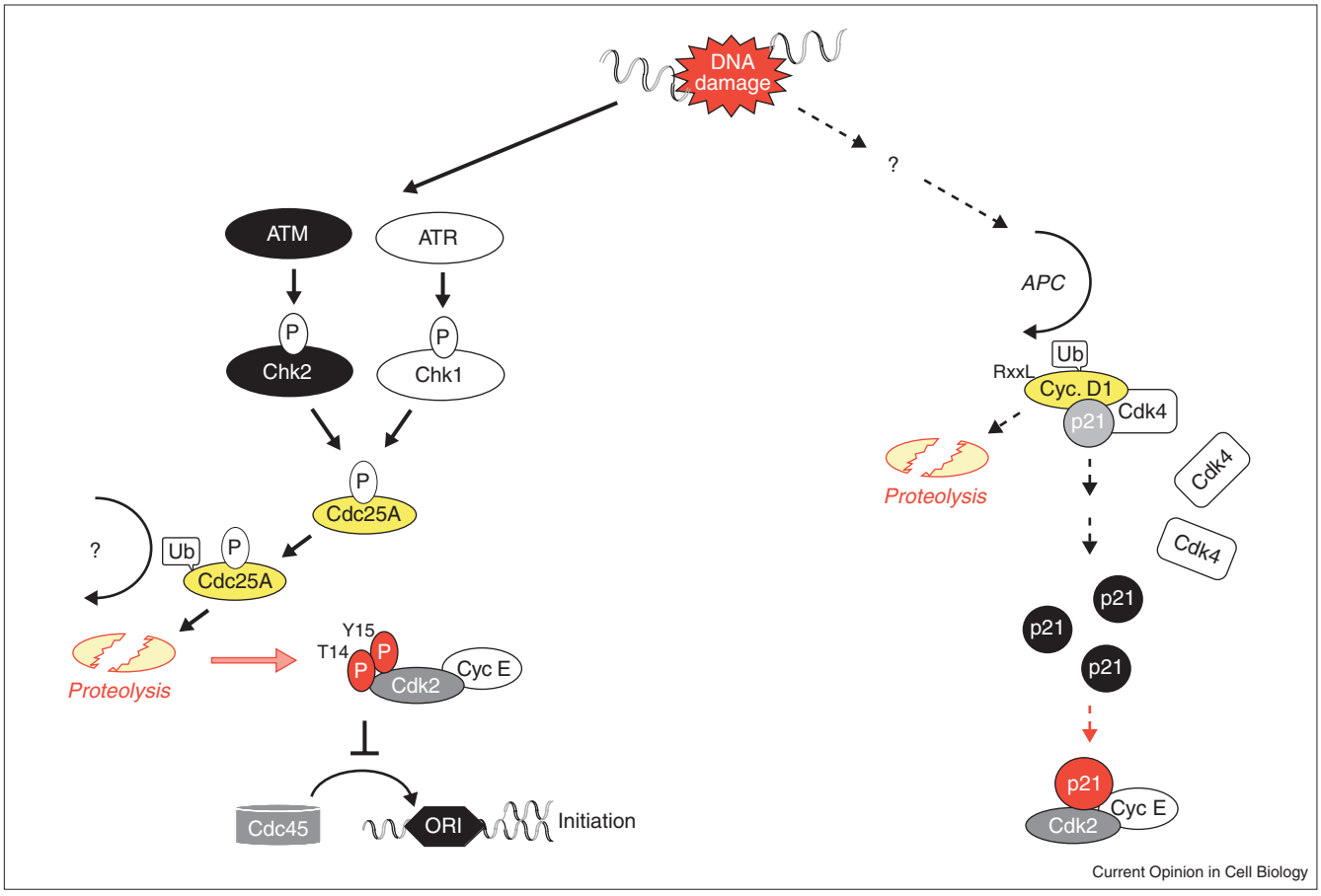
Introduction

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 multi-faceted 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 (phosphatidyl-inositol-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



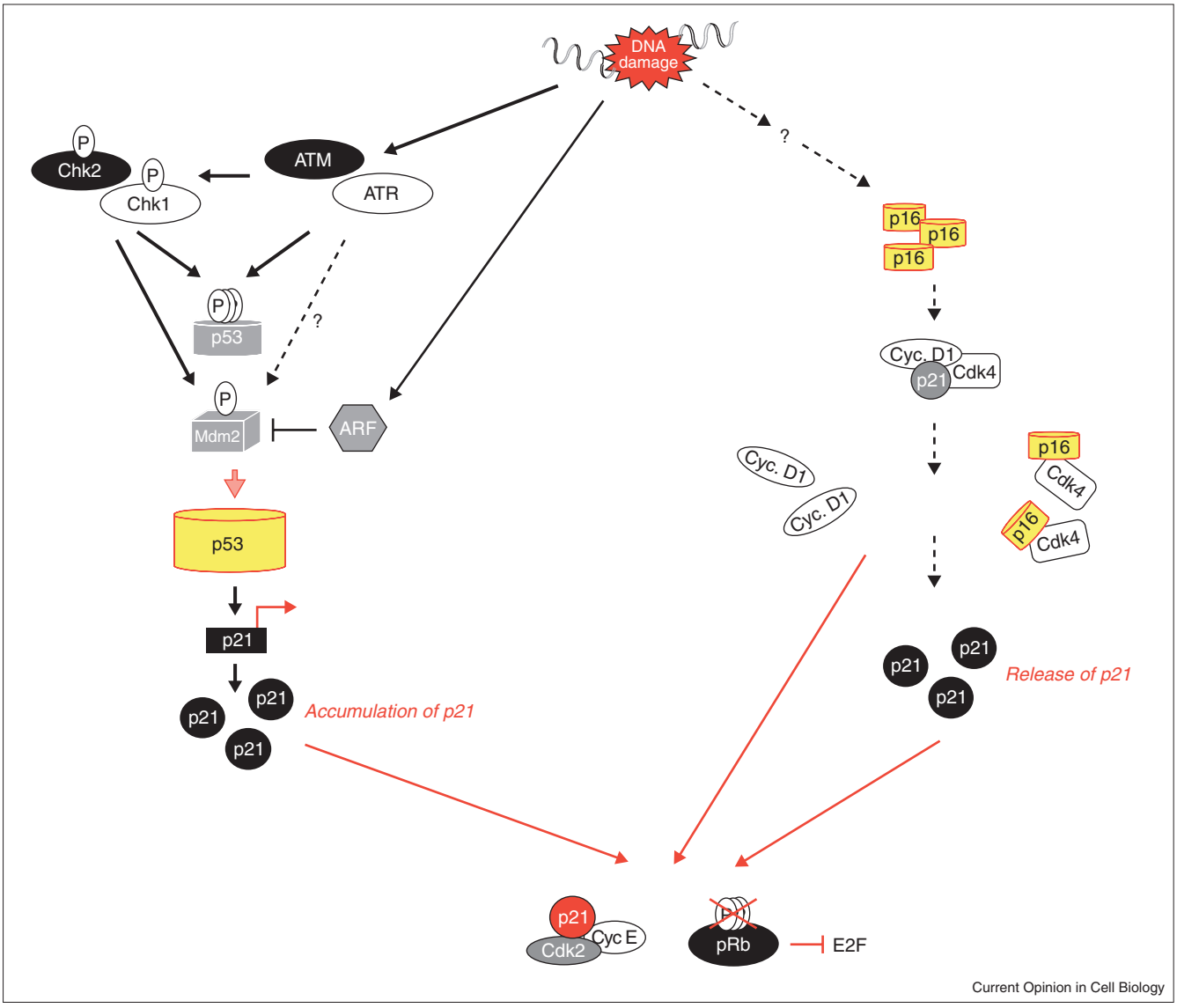
Ubiquitin/proteasome-mediated protein degradation determines rapid G1 arrest in response to DNA damage. DNA damage triggers a rapid cascade of phosphorylation events involving the ATM and Chk2 (upon IR) or ATR and Chk1 (upon UV light) kinases. These cascades culminate at inhibition of the S-phase-promoting 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

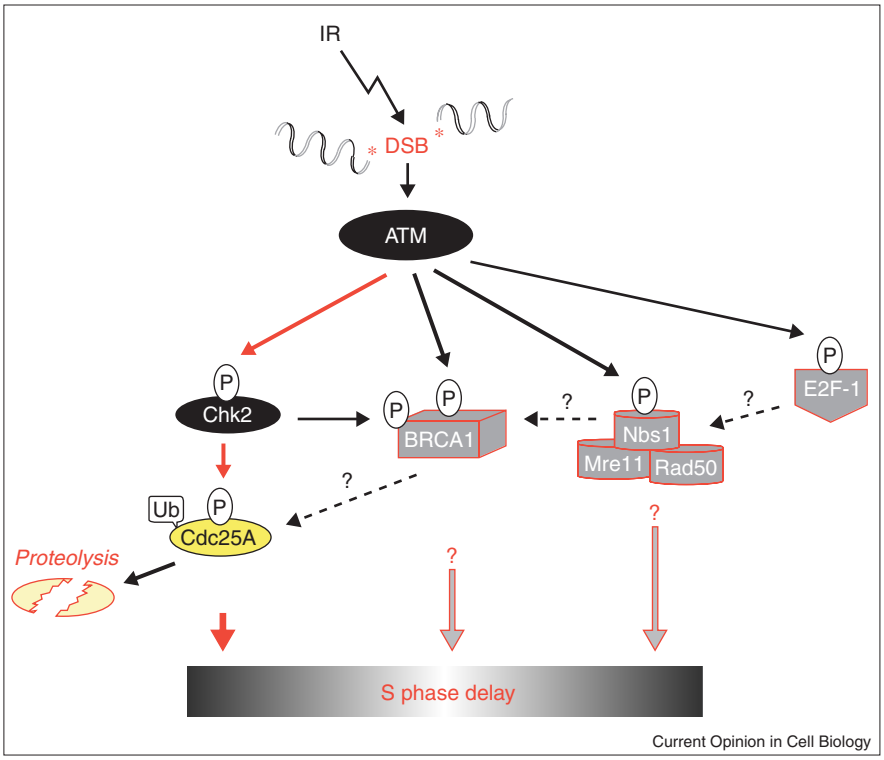
Figure 2



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.

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 $p21^{Waf1/Cip1}$, 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†	Aberrations in human cancer*		
	Molecular basis‡	Sporadic tumours§	Hereditary syndromes/tumours#
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
Cyclin D1 (O)	Gene amplification, translocation, overexpression, etc.	Many types of cancer	NR
Cyclin E (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 $p16^{INK4a}$ 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

Mre-11–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.

Acknowledgements

We are grateful to JHJ Petrini, C Cordon Cardo, J Nevins, and H Nevanlinna for sharing their data before publication, and to the Danish Cancer Society, the Danish Medical Research Council, the John and Birthe Meyer Foundation, and the Nordic Cancer Union for financial support. Our apologies to colleagues whose work could only be cited indirectly in this review.

References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Hoeijmakers JH: **Genome maintenance mechanisms for preventing cancer.** *Nature* 2001, **411**:366-374.

2. Wood RD, Mitchell M, Sgouros J, Lindahl T: **Human DNA repair genes.** *Science* 2001, **291**:1284-1289.

3. Hartwell LH, Kastan MB: **Cell cycle control and cancer.** *Science* 1994, **266**:1821-1828.

4. Zhou BBS, Elledge SJ: **The DNA damage response: putting checkpoints in perspective.** *Nature* 2000, **408**:433-439.

5. Kastan MB, Lim DS. **The many substrates and functions of ATM.** *Nat Rev Mol Cell Biol* 2000, **1**:179-186.

6. Khanna KK, Jackson SP: **DNA double-strand breaks: signaling, repair and the cancer connection.** *Nat Genet* 2001, **27**:247-254.

7. Shiloh Y: **ATM and ATR: networking cellular responses to DNA damage.** *Curr Opin Genet Dev* 2001, **11**:71-77.

8. Durocher D, Jackson SP: **DNA-PK, ATM and ATR as sensors of DNA damage: variations on a theme?** *Curr Opin Cell Biol* 2001, **13**:225-231.

9. Vogelstein B, Lane D, Levine AJ: **Surfing the p53 network.** *Nature* 2000, **408**:307-310.

10. Petrini JH: **The Mre11 complex and ATM: collaborating to navigate S phase.** *Curr Opin Cell Biol* 2000, **12**:293-296.

11. Bartek J, Lukas J: **Pathways governing G1/S transition and their response to DNA damage.** *FEBS Lett* 2001, **490**:117-122.

12. • Lukas C, Bartkova J, Latella L, Falck J, Mailand N, Schroeder T, Sehested M, Lukas J, Bartek J: **DNA damage-activated kinase Chk2 is independent of proliferation or differentiation yet correlates with tissue biology.** *Cancer Res* 2001, **61**:4990-4993.
Evidence that mammalian Chk2 could be activated by ionising radiation throughout the cell cycle, as well as in quiescent and differentiated cells. This study provides also the first insight into tissue-specific expression of the Chk2 kinase.

13. • Bao S, Tibbetts RS, Brumbaugh DK, Fang Y, Richardson DA, Ali A, Chen SM, Abraham RT, Wang XF: **ATR/ATM-mediated phosphorylation of human Rad17 is required for genotoxic stress response.** *Nature* 2001, **411**:969-974.
The authors demonstrate a direct link between the human Rad17 and the checkpoint kinases, ATM and ATR. ATR/ATM-dependent phosphorylation of hRad17 was found to be a critical early event during checkpoint signalling in DNA-damaged cells.

14. •• Mailand N, Falck J, Lukas C, Syljuåsen RG, Welcker M, Bartek J, Lukas J: **Rapid destruction of Cdc25A in response to DNA damage.** *Science* 2000, **288**:1425-1429.
This paper provides the first evidence that the S-phase-promoting Cdc25A phosphatase undergoes rapid ubiquitin/proteasome-mediated degradation in response to DNA damage caused by UV light or ionising radiation. (see also annotation [66••]).

15. •• Costanzo V, Robertson K, Ying CY, Kim E, Avvedoimento E, Gottesman M, Grieco D, Gautier J: **Reconstitution of an ATM-dependent checkpoint that inhibits chromosomal DNA replication following DNA damage.** *Mol Cell* 2000, **6**:649-659.
Using a cell-free system derived from *Xenopus* eggs, this study describes the first biochemical reconstitution of the checkpoint pathway inhibiting initiation of DNA replication after generation of DNA double-stranded breaks. The checkpoint-induced cascade required ATM and culminated in inhibition of cyclin E–Cdk2 activity by phosphorylation of Cdk2 Tyr 15. This resulted in formation of incompletely assembled pre-replicative complexes containing ORC, Cdc6, Cdc7 and MCM proteins but lacking Cdc45. Addition of recombinant Cdc25A but not Cdc25C abrogated the checkpoint and restored DNA replication.

16. •• Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J: **The ATM-Chk2 Cdc25A checkpoint pathway guards against radioresistant DNA synthesis.** *Nature* 2001, **410**:842-847.
This paper reports the discovery of the mechanism regulating the intra-S-phase checkpoint in mammalian cells exposed to ionising radiation (IR). IR-induced formation of DNA double stranded breaks triggered degradation of the Cdc25A phosphatase, which in turn led to inhibition of the S-phase promoting cyclin E–Cdk2 activity and transient blockade of DNA replication. It is shown that Cdc25A destruction required ATM and Chk2-mediated phosphorylation of Cdc25A on Ser 123. Consequently, cells harbouring tumour-associated Chk2 alleles unable to bind and phosphorylate Cdc25A, elevated Cdc25A preventing its efficient degradation, and a Cdk2 mutant unable to undergo inhibitory phosphorylation failed to inhibit S-phase progression when irradiated and underwent radioresistant DNA synthesis.

17. •• Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB: **ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway.** *Nature* 2000, **404**:613-617.
See annotation [20••].

18. •• Wu X, Ranganathan V, Weisman DS, Heine WF, Ciccone DN, O'Neill TB, Crick KE, Pierce KA, Lane WS, Rathbuh G *et al.*: **ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response.** *Nature* 2000, **405**:477-482.
See annotation [20••].

19. •• Zhao S, Weng YC, Yuan SS, Lin YT, Hsu HC, Lin SC, Gerbino E, Song MH, Zdzienicka MZ, Gatti RA *et al.*: **Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products.** *Nature* 2000, **405**:473-477.
See annotation [20••].

20. •• Gatei M, Young D, Cerosaletti KM, Desai-Mekta A, Spring K, Kozlov S, Lavin MF, Gatti RA, Concannon P, Khanna K: **ATM-dependent phosphorylation of nibrin in response to radiation exposure.** *Nat Genet* 2000, **25**:115-119.
This study along with. [17••–19••] link ATM and nibrin (Nbs1) in a common signalling pathway and provide an explanation for phenotypic similarities in the ataxia-telangiectasia and Nijmegen breakage syndrome diseases. Nbs1 was found associated with and phosphorylated by ATM in cells exposed to ionising radiation (IR). Nbs1 alleles mutated at the ATM phosphorylation sites abrogated the IR-induced S-phase checkpoint in normal cells and failed to compensate for this functional deficiency in cells derived from patients with deficient NBS1 gene

21. Bartek J, Bartkova J, Lukas J: **The retinoblastoma protein pathway and the restriction point.** *Curr Opin Cell Biol* 1996, **8**:805-814.

22. • Müller H, Bracken AP, Vernell R, Moroni MC, Christians F, Grassilli E, Prosperini E, Vigo E, Oliner JD, Helin K: **E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis.** *Genes Dev* 2001, **15**:267-285.
Using high-density oligonucleotide arrays, this is so far the most comprehensive and systematic approach to decipher the complex role of E2F-regulated gene expression in various biological processes.

23. • Santoni-Rugiu E, Falck J, Mailand N, Bartek J, Lukas J: **Involvement of Myc activity in a G1/S-promoting mechanism parallel to the pRb/E2F pathway.** *Mol Cell Biol* 2000, **20**:3497-3509.
Evidence for a cooperative role of E2F- and Myc-dependent transcription in stimulating Cdk2 activity and promoting entry into S phase.

24. Sherr CJ, Roberts JM: **CDK inhibitors: positive and negative regulators of G1-phase progression.** *Genes Dev* 1999, **13**:1501-1512.

25. Harbour JW, Dean DC: **The Rb/E2F pathway: expanding roles and emerging paradigms.** *Genes Dev* 2000, **14**:2393-2409.

26. Ekholm SV, Reed SI: **Regulation of G(1) cyclin-dependent kinases in the mammalian cell cycle.** *Curr Opin Cell Biol* 2000, **12**:676-684.

27. Blow JJ: **Control of chromosomal DNA replication in the early *Xenopus* embryo.** *EMBO J* 2001, **20**:3293-3297.

28. • Ekholm SV, Zickert P, Reed SI, Zetterberg A: **Accumulation of cyclin E is not a prerequisite for passage through the restriction point.** *Mol Cell Biol* 2001, **21**:3256-3265.
An important contribution to an ongoing debate about the involvement of distinct cyclin-dependent kinase complexes in passage through the restriction point.

29. Harrington EA, Bruce JI, Harlow E, Dyson N: **pRB plays an essential role in cell cycle arrest induced by DNA damage.** *Proc Natl Acad Sci USA* 1998, **95**:11945-11950.

30. Ryan KM, Phillips AC, Vousden KH: **Regulation and function of the p53 tumor suppressor protein.** *Curr Opin Cell Biol* 2001, **13**:332-337.

31. • Blasina A, Price BD, Turenne GA, McGowan CH: **Caffeine inhibits the checkpoint kinase ATM.** *Curr Biol* 1999, **9**:1135-1138.
The identification of a long-sought molecular target for caffeine, a drug that can override the DNA-damage checkpoint and sensitise cells to killing by genotoxic agents.

32. •• Agami R, Bernards R: **Distinct initiation and maintenance mechanisms cooperate to induce G1 cell cycle arrest in response to DNA damage.** *Cell* 2000, **102**:55-66.
An intriguing concept linking the anaphase-promoting complex-dependent proteolysis of cyclin D1 with rapid, p53-independent G1 arrest in response to DNA damage.

33. Miller SJ, Suthiphongchai T, Zambetti GP, Ewen ME: **p53 binds selectively to 5′ untranslated region of cdk4, an RNA element necessary for transforming growth factor beta- and p53-mediated translational inhibition of cdk4.** *Mol Cell Biol* 2000, **20**:8420-8431.

34. Buschmann T, Fuchs SY, Lee CG, Pan ZQ, Ronai Z: **SUMO-1 modification of Mdm2 prevents its selv-ubiquitination and increases Mdm2 ability to ubiquitinate p53.** *Cell* 2000, **101**:753-762.

35. Ito A, Lai CH, Zhao X, Saito S, Hamilton MH, Appella E, Yao TP: **p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2.** *EMBO J* 2001, **20**:1331-1340.

36. • Maya R, Balass M, Kim ST, Shkedy D, Leal JFM, Shifman O, Moas M, Buschmann T, Ronai Z, Shiloh Y *et al.*: **ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage.** *Genes Dev* 2001, **15**:1067-1077.
This study provides evidence that ATM phosphorylates Mdm2 *in vitro* and *in vivo*. This in turn contributes to nuclear retention, stabilisation and activation of p53 in response to DNA damage. Thus, ATM promotes stabilisation and accumulation of active p53 in the nucleus by phosphorylating p53 itself, as well as its negative regulator Mdm2.

37. Giannakakou P, Sackett DL, Ward Y, Webster KR, Blagosklonny MV, Fojo T: **p53 is associated with cellular microtubules and uses dynein-dependent transport for nuclear accumulation.** *Nat Cell Biol* 2000, **2**:709-717.

38. •• Chehab NH, Malikzay A, Appel M, Halazonetis TD: **Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53.** *Genes Dev* 2000, **14**:278-288.
See annotation [40••].

39. •• Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW: **DNA damage-induced activation of p53 by the checkpoint kinase Chk2.** *Science* 2000, **287**:1824-1827.
See annotation [40••].

40. •• Shieh SY, Ahn J, Tamai K, Taya Y, Prives C: **The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites.** *Genes Dev* 2000, **14**:289-300.
By means of complementary approaches, this paper along with [38••,39••] provide persuasive evidence that stabilisation of p53 and induction of p53-dependent transcripts such as p21 in response to DNA damage requires the activity of the Chk2 or Chk1 kinases. Chk2/Chk1 directly phosphorylate p53 on Ser 20, and thereby interfere with binding of Mdm2, an inhibitor of p53, which masks the p53 transactivation domain, triggers p53 nuclear export and targets p53 for ubiquitin/proteasome degradation. Collectively, these studies provide mechanistic explanation for DNA damage-induced stabilisation of p53 in mammalian cells.

41. • Xhang Y, Xiong Y: **A p53 amino-terminal nuclear export signal inhibited by DNA damage-induced phosphorylation.** *Science* 2001, **292**:1910-1915.
Identification of a previously unknown nuclear export signal (NES) in the amino terminus of p53. The ability of this NES to support export of p53 was specifically inhibited by phosphorylation of p53 on Ser 15 induced by ultraviolet radiation. Ser 15 is a known target of ATM and/or ATR kinases.

42. Sherr CJ, Webster JD: **The ARF/p53 pathway.** *Curr Opin Genet Dev* 2000, **10**:94-99.

43. •• Khan SH, Moritsugu J, Wahl GM: **Differential requirement for p19ARF in the p53-dependent arrest induced by DNA damage, microtubule disruption, and ribonucleotide depletion.** *Proc Natl Acad Sci USA* 2000, **97**:3266-3271.
Prolonged analyses of cellular response to various types of stress revealed alterations in cell-cycle response and kinetics of p53 and p21 induction in the p19ARF-deficient fibroblasts after DNA damage and microtubule disruption. Moreover, the authors provide evidence for a specific increase of p19ARF protein levels in normal cells after exposure to ionising radiation. Thus, in addition to oncogene activation, p19ARF contributes to sustain accumulation of active p53 and generate durable cell-cycle arrest also in cells with damaged DNA or depolymerised microtubules, but not upon other types of stress such as ribonucleotide depletion or RNA synthesis inhibition.

44. Pavey S, Conroy S, Russell T, Gabrielli B: **Ultraviolet radiation induces p16^{CDKN2A} expression in human skin.** *Cancer Res* 1999, **59**:4185-4189.

45. Diffley JF: **DNA replication: building the perfect switch.** *Curr Biol* 2001, **11**:R367-R370.

46. • Gottifredi V, Shieh SY, Taya Y, Prives C: **p53 accumulates but is functionally impaired when DNA synthesis is blocked.** *Proc Natl Acad Sci USA* 2001, **98**:1036-1041.
An interesting study showing that stalled DNA replication impairs transactivation of at least some p53 target genes in cells exposed to ionising radiation, despite the fact that the p53 protein is stabilised and accumulates in cell nuclei.

47. Kastan MB: **Checking two steps.** *Nature* 2001, **410**:766-767.

48. • Larner JM, Lee H, Little RD, Dijkwel PA, Schildkraut CL, Hamlin JL: **Radiation down-regulates replication origin activity throughout the S phase in mammalian cells.** *Nucleic Acids Res* 1999, **27**:803-809.
Evidence that ionising radiation downregulates initiation of both early- and late-firing DNA origins of replication in human cells.

49. • Matsuoka S, Rotman G, Ogawa A, Ahiloh Y, Tamai K, Elledge SJ: **Ataxia telangiectasia-mutated phosphorylates Chk2 *in vivo* and *in vitro*.** *Proc Natl Acad Sci USA* 2000, **97**:10389-10394.
See annotation [50•].

50. • Melchionna R, Chen XB, Blasina A, McGowan CH: **Threonine 68 is required for radiation-induced phosphorylation and activation of Cds1.** *Nat Cell Biol* 2000, **2**:762-765.
Identification of the Thr 68 as a dominant residue of Chk2 targeted by the ATM kinase.

51. Cortez D, Wang Y, Qin J, Elledge SJ: **Requirement of ATM-dependent phosphorylation of BRCA1 in the DNA damage response to double-strand breaks.** *Science* 1999, **286**:1162-1166.

52. Li S, Ting NS, Zheng L, Chen PL, Ziv Y, Shiloh Y, Lee EY, Lee WH: **Functional link of BRCA1 and ataxia telangiectasia gene product in DNA damage response.** *Nature* 2000, **406**:210-215.

53. Desai-Mehta A, Cerosaletti KM, Concannon P: **Distinct functional domains of nibrin mediate Mre11 binding, focus formation, and nuclear localization.** *Mol Cell Biol* 2001, **21**:2184-2191.

54. • Lee JS, Collins KM, Brown AL, Lee CH, Chung JH: **hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response.** *Nature* 2000, **404**:201-204.
Demonstration that human Chk2 interacts and co-localises with BRCA1 in distinct nuclear foci. Upon DNA damage, Chk2 phosphorylated BRCA1, which in turn was required for the release of BRCA1 from Chk2. Phosphorylation of BRCA1 was required for its ability to restore survival after DNA damage in BRCA1-deficient cells.

55. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J: **BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures.** *Genes Dev* 2000, **14**:927-939.

56. Wang JYJ: **New link in a web of human genes.** *Nature* 2000, **405**:404-405.

57. Maser RS, Mirzoeva OK, Wells J, Olivares H, Williams BR, Zinkel RA, Farnham PJ, Petrini JH: **The Mre11 complex and DNA replication: linkage to E2F and sites of DNA synthesis.** *Mol Cell Biol* 2001, **21**:6006-6016.
An intriguing study demonstrating that S-phase checkpoint proficiency relies on a formation of physical complexes between Nbs1/Mre11 and transcription factor E2F-1 near origins of DNA replication.

58. • Xu B, Kim S, Kastan MB: **Involvement of Brca1 in S-phase and G2 phase checkpoints after ionizing irradiation.** *Mol Cell Biol* 2001, **21**:3445-3450.
A thorough study demonstrating that BRCA1 is required for both S-phase and G2 arrests induced by ionising radiation, while Nbs1 is required only for the S-phase arrest. Interestingly, ATM phosphorylation of BRCA1 is required for the G2/M but not the S-phase checkpoint.

59. Scully R, Livingston DM: **In search of the tumour-suppressor functions of BRCA1 and BRCA2.** *Nature* 2000, **408**:429-432.

60. Sherr CJ: **The Pezcoller lecture: cancer cell cycles revisited.** *Cancer Res* 2000, **60**:3689-3695.

61. Carr AM: **Cell cycle. Piecing together the p53 puzzle.** *Science* 2000, **287**:1765-1766.

62. Brown EJ, Baltimore D: **ATR disruption leads to chromosomal fragmentation and early embryonic lethality.** *Genes Dev* 2000 **14**:397-402.

63. de Klein A, Muijtjens M, van Os R, Verhoeven Y, Smit B, Carr AM, Lehmann AR, Hoeijmakers JH: **Targeted disruption of the cell-cycle checkpoint gene ATR leads to early embryonic lethality in mice.** *Curr Biol* 2000 **10**:479-482.

64. • Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Issebacher KJ, Fraumeni JF *et al.*: **Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome.** *Science* 1999, **286**:2528-2531.
The identification of heterozygous germ line mutations in hCHK2 in cells derived from Li-Fraumeni patients and in a colorectal cancer cell line. These observations implicated for the first time hCHK2 as a tumour suppressor whose mutations confer predisposition to diverse types of human malignancies.

65. Stewart GS, Maser RS, Stankivic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM: **The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder.** *Cell* 1999, **99**:577-587.

66. •• Molinari M, Mercurio C, Dominguez J, Goubin F, Draetta GF. **Human Cdc25 A inactivation in response to S phase inhibition and its role in preventing premature mitosis.** *EMBO Rep* 2000 **1**:71-79.
Independent evidence demonstrating rapid ubiquitin/proteasome-mediated degradation of the Cdc25A phosphatase in response to hydroxyurea-induced stalled replication forks. An excess of Cdc25A reduced the cells' ability to arrest cell-cycle progression in the presence of hydroxyurea and lead to initiation of premature chromosome condensation. Thus, proteolysis of Cdc25A appears to play a pivotal role in both replication and DNA damage checkpoints (see also annotation [14••]) and thereby contributes to the maintenance of genetic stability.

67. Bertoni F, Codegoni AM, Furlan D, Tibiletti MG, Capella C, Broggini M: ***CHK1* frameshift mutations in genetically unstable colorectal and endometrial cancers.** *Genes Chromosomes Cancer* 1999, **26**:176-180.

68. Fukuda T, Sumiyoshi T, Takahashi M, Kataoka T, Asahara T, Inui H, Watatani M, Yasutomi M, Kamada N, Miyagawa K: **Alterations of the double-strand break repair gene *MRE11* in cancer.** *Cancer Res* 2001, **61**:23-26.

69. Haruki N, Saito H, Tatematsu Y, Konishi H, Harano T, Masuda A, Osada H, Fujii Y, Takahashi T: **Histological type-selective, tumor-predominant expression of a novel *CHK1* isoform and infrequent *in vivo* somatic *CHK2* mutation in small cell lung cancer.** *Cancer Res* 2001, **61**:4689-4692.

70. Bartkova J, Falck J, Rajpert-De Meyts E, Skakkebæk NE, Lukas J, Bartek J: **Chk2 tumour suppressor protein in human spermatogenesis and testicular germ-cell tumours.** *Oncogene* 2001, **20**:5897-5902

71. • Falck J, Lukas C, Protopopova M, Lukas J, Selivanova G, Bartek J: **Functional impact of concomitant versus alternative defects in the Chk2-p53 tumour suppressor pathway.** *Oncogene* 2001, **20**:5503-5510.
This study demonstrates a concomitant loss of p53 and Chk2 functions in a human colon cancer cell line. Thus, although Chk2 and p53 physically interact and operate partly along a common pathway, concomitant mutations in both of these cell-cycle checkpoint regulators may provide some selective advantage to tumour cells.

72. •• Cangi MG, Cukor B, Soung P, Signoretti S, Moreira G Jr, Ranashinge M, Cady B, Pagano M, Loda M: **Role of the Cdc25A phosphatase in human breast cancer.** *J Clin Invest* 2000, **106**:753-761.
A thorough clinically oriented study demonstrating that overexpression of Cdc25A in a subset of breast cancer was associated with poor survival. The authors suggest that overexpression of Cdc25A contributes to the biological behaviour of primary breast tumours and propose that both Cdc25A and its downstream target CDK2 might represent suitable therapeutic targets in early-stage breast cancer.

73. Angele S, Treilleux I, Taniere P, Martel-Planche G, Vuillaume M, Bailly C, Bremond A, Montesano R, Hall J: **Abnormal expression of the ATM and TP53 genes in sporadic breast carcinomas.** *Clin Cancer Res* 2000 **6**:3536-3544.

74. •• Yu Q, Geng Y, Sicinski P: **Specific protection against breast cancers by cyclin D1 ablation.** *Nature* 2001, **411**:1017-1021.
Elegant *in vivo* study showing that in the breast epithelium, the Neu-Ras oncogenic pathway is connected to the cell cycle machinery via cyclin D1. This explains the absolute dependency on cyclin D1 for the Neu-Ras-mediated oncogenic transformation in the breast.

75. Abraham RT: **Cell cycle checkpoint signalling through the ATM and ATR kinases.** *Genes Dev* 2001, **15**:2177-2196.

Now in press

The work which links the ATM checkpoint kinase with E2F-regulated transcription, referred to in the text as (J Nevins, personal communication), has now been published:

76. • Lin W-C, Lin F-T, Nevins J: **Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation.** *Genes Dev* 2001, **15**:1833-1844.
Together with [57•], this work provides more evidence that cellular responses to DNA damage may operate via the Rb/E2F pathway.