INTRODUCTION

When a cell encounters a problem such as damaged DNA or a block to replication, it can call upon a variety of mechanisms to fix the problem. But these mechanisms can take time, and it is often crucial that the cell does not continue through the cell cycle until the problem is fixed. That is where cell cycle checkpoints come in. The checkpoints recognize the problem and delay cell cycle progression by inhibiting the basic cell cycle machinery until the problem is fixed (Hartwell and Weinert, 1989; Elledge, 1996; Rhind and Russell, 1998a).

These checkpoints can also regulate transcription and may directly regulate repair machinery, but these functions are beyond the scope of this Commentary. Many, if not all, of the major cell cycle transitions are regulated by one or another checkpoint. However, we will concern ourselves here with the DNA damage and replication checkpoints, which have served as the prototypic checkpoint pathways. These checkpoints are triggered by various forms of DNA damage and various treatments that block replication, respectively.

It is useful to think of checkpoints as divided into three parts: a sensor, a transduction pathway and a target. The transduction pathways for the DNA damage and replication checkpoints are composed of a shared group of conserved proteins that may also serve as the sensors. The pathways have been recently reviewed and will only be briefly addressed here (Elledge, 1996; Longhese et al., 1998; Rhind and Russell, 1998a; Dasika et al., 1999). Of the proteins known to be involved, the most interesting is a large protein kinase of the DNA-PK family, known as Rad3 in the fission yeast Schizosaccharomyces pombe, Mec1 in budding yeast Saccharomyces cerevisiae, MEI-41 in the fruit fly Drosophila melanogaster, and X-ATM in the frog Xenopus laevis (Zakian, 1995). Two homologs, ATM and ATR, have been identified in humans and mice (Westphal, 1997). For convenience, we will refer to these homologs generically as ATMs but use the specific name when referring to a specific organism. By analogy with DNA-PK, a kinase that is activated by binding to DNA ends, it is proposed that ATM acts to recognize the DNA damage or stalled replication forks and initiate the checkpoint signal (Hartley et al., 1995; Bentley et al., 1996). The other members of this pathway could serve as regulatory subunits of a complex that has ATM as its core (Longhese et al., 1998). Although it is appealing, there is little direct evidence for this model.

However, the fact that the checkpoints share many upstream proteins suggests that they may recognize a similar or overlapping set of DNA structures. What is known is that downstream of ATM in each species are homologs of the Chk1 and Cds1 protein kinases (Fig. 1; Weinert et al., 1994; Murakami and Okayama, 1995; Walworth and Bernards, 1996; Sanchez et al., 1997; Kumagai et al., 1998; Blasina et al., 1999; Brown et al., 1999; Chaturvedi et al., 1999; Sanchez et al., 1999; Sibon et al., 1999; Tominaga et al., 1999; Guo and Dunphy, 2000; Liu et al., 2000b).

Chk1 was originally identified in fission yeast as a kinase required for the DNA damage checkpoint but not for the replication checkpoint (Walworth et al., 1993). Genetic experiments place fission yeast Chk1 as the most downstream
Fig. 1. A schematic representation of the DNA damage and replication checkpoints in various organisms. The human/mouse pathway is based on evidence from both organisms. Bold arrows indicate some evidence for direct biochemical regulation, although this evidence may only be in vitro phosphorylation. Dashed arrows indicate that the regulation has been shown in vivo not to be sufficient for establishment of cell cycle arrest. In both cases the regulation seems to be more important for maintenance of the arrest. A question mark after a protein indicates that there is only circumstantial evidence for its involvement. A question mark without a protein indicates that there is evidence for some protein at that point in the pathway, but no evidence as to its identity.

THE VARIOUS TARGETS OF CHK1 AND CDS1

Targets in yeast

Fission yeast cells spend most of their time in G2, and the DNA damage and replication checkpoints prevent cell cycle progression by blocking the G2-M transition (Rhind and Russell, 1998a). They do this by inhibiting Cdc2, the kinase that drives mitosis, through Tyr15 phosphorylation (Rhind et al., 1997; Rhind and Russell, 1998b). The tyrosine phosphorylation of Cdc2 is regulated by the Wee1 and Mik1 tyrosine kinases and the Cdc25 tyrosine phosphatase (Coleman and Dunphy, 1994). Thus, the checkpoints could act to upregulate Wee1 and/or Mik1, or inhibit Cdc25. In fact, the checkpoints seem to both upregulate Mik1 and inhibit Cdc25.

In fission yeast, Cdk1 is the effector of the DNA damage checkpoint pathway and specifically downstream of Rad3 (Walworth and Bernards, 1996). Chk1 homologs have been identified in all other eukaryotes examined, and, where the experiments have been done, the function of Chk1 as a downstream checkpoint effector seems to be conserved (Fogarty et al., 1997; Sanchez et al., 1997; Kumagai et al., 1998; Sanchez et al., 1999; Liu et al., 2000b). The Chk1 homologs share a similar N-terminal kinase domain as well as scattered similarity throughout their C-termini (Sanchez et al., 1997).

The founding member of the Cds1 family is budding yeast Rad53 (Allen et al., 1994). Like Chk1, Rad53 is widely conserved (Murakami and Okayama, 1995; Matsuoka et al., 1998; Blasina et al., 1999; Brown et al., 1999; Chaturvedi et al., 1999; Tominaga et al., 1999; Guo and Dunphy, 2000). Since these homologs are generally called Cds1, we will refer to them generically as Cds1 but use the specific name when referring to a specific organism. The Cds1 homologs are recognizable by a similar kinase domain and an N-terminal forhead associated (FHA) domain (Blasina et al., 1999). FHA domains, originally recognized in the forhead transcription factor, are believed to act as protein-protein interaction domains, and in some instances bind specifically to phosphorylated partners (Hofmann and Bucher, 1995; Sun et al., 1998; Durocher et al., 1999). Rad53 is unique among the Cds1 homologs in possessing a second C-terminal FHA domain.

The wide conservation of Chk1, Cds1 and the upstream effectors seems to both upregulate Mik1 and inhibit Cdc25. In fact, the checkpoints seem to both upregulate Mik1 and inhibit Cdc25. Although this idea holds in general, it is becoming increasingly clear that there are significant differences in the details. One area in which differences were inevitable is the targets of the checkpoint effectors, Chk1 and Cds1. Although the basic cell cycle machinery is well conserved, there are important differences in the points of the cell cycle at which organisms impose regulation (Murray and Hunt, 1993). Thus the checkpoints need to target different points in the cell cycle, and it could have been predicted that the targets of the effector kinases would vary. More surprising is the fact that the checkpoints in which each effector kinase acts seem not to be conserved – for example, Chk1 being required for the DNA damage checkpoint in fission yeast and the replication checkpoint in frogs and flies (Walworth et al., 1993; Kumagai et al., 1998; Sibon et al., 1999). Here, we focus on the various targets of Chk1 and Cds1 and on which checkpoints activate them to regulate these targets.
checkpoint pathway (Walworth et al., 1993). Chk1 is phosphorylated in a Rad3-dependent manner in response to activation of the DNA damage checkpoint, but not the replication checkpoint, and this phosphorylation correlates with its ability to arrest cells in G_{2} (Walworth and Bernards, 1996). Of its targets, Cdc25 is the best understood. In vivo experiments show that Cdc25 is strongly inhibited in response to activation of the DNA damage checkpoint, and this inhibition requires Chk1 (Furnari et al., 1997; Rhind et al., 1997). This inhibition is presumed to be due to direct regulation by Chk1, which binds to Cdc25 in vivo and phosphorylates it in vitro (Furnari et al., 1997; Zeng et al., 1998). The phosphorylation of Cdc25 by Chk1 has two effects. First, in vitro phosphorylation by Chk1 inhibits its phosphatase activity (Blasina et al., 1999; Furnari et al., 1999). Second, activation of the DNA damage checkpoint results in reduced nuclear localization of Cdc25 (Lopez-Girona et al., 1999). This relocalization of Cdc25 is blocked by mutation of Chk1 phosphorylation sites in Cdc25, which suggests that it is due to Chk1 phosphorylation (Zeng and Piwnica-Worms, 1999).

The phosphorylation of Cdc25 on these sites promotes the binding of Rad24, a 14-3-3 protein, and the binding of Rad24 is believed to sequester Cdc25 in the cytoplasm, away from its substrate, Cdc2 (Zeng et al., 1998; Lopez-Girona et al., 1999). It is not clear to what extent each of these two modes of regulation contributes to the inhibition of Cdc25 in vivo. Part of the problem is that Cdc25 is phosphorylated in vitro on at least 12 sites (Zeng and Piwnica-Worms, 1999). Mutation of nine of these sites impairs the 14-3-3 binding of Cdc25, its relocalization after damage, and the DNA damage checkpoint (Zeng and Piwnica-Worms, 1999). However, the mutations may also impair the regulation of Cdc25 phosphatase activity, and thus do not distinguish between the two possible mechanisms of regulation.

The other cell cycle target of the DNA damage checkpoint in fission yeast is Mik1 (Baber-Furnari et al., 2000; Christensen et al., 2000; Rhind and Russell, unpublished data). Although Cdc25 regulation is alone sufficient to arrest cells in response to activation of the DNA damage checkpoint, regulation of Mik1 alone suffices only to produce an attenuated DNA damage checkpoint delay (Rhind and Russell, unpublished data). It appears that Mik1 is regulated at two levels by the DNA damage checkpoint. Mik1 is able to contribute to the establishment of a DNA damage checkpoint delay, in a manner that does not require increased Mik1 accumulation (Rhind and Russell, unpublished data). In addition, after a prolonged activation of the DNA damage checkpoint, Mik1 accumulates through a post-transcriptional mechanism (Baber-Furnari et al., 2000; Christensen et al., 2000). This accumulation correlates with its requirement for maintenance of an extended checkpoint arrest (Baber-Furnari et al., 2000). It is not known whether Chk1 directly phosphorylates Mik1 to effect its regulation or whether other substrates are involved.

The role of Cds1 in fission yeast is as the effector of the replication checkpoint pathway (Murakami and Okayama, 1995; Boddy and Russell, 1999). Cds1 is activated by the checkpoint and is required for cells to survive treatments that block replication (Boddy et al., 1998; Lindsay et al., 1998). However, the role of Cds1 as effector of the replication checkpoint is complicated by the fact that, in the absence of Cds1, Chk1 can act to impose a checkpoint delay (Boddy et al., 1998; Zeng et al., 1998; Brondello et al., 1999). Although Chk1 may be involved in the replication checkpoint in wild-type cells, it does not appear to play an important role (Walworth et al., 1993; Boddy and Russell, 1999; Brondello et al., 1999). In vivo, Cdc25 is inhibited by the replication checkpoint (Rhind and Russell, 1998b). Cds1 seems to regulate Cdc25 in a similar manner to Chk1. Cds1 phosphorylates Cdc25 in vitro on sites similar to Chk1, and inhibits Cdc25 in vitro (Zeng et al., 1998; Furnari et al., 1999). The effect of the replication checkpoint on Cdc25 localization has yet to be described.

In addition to Cdc25, Mik1 is also an important target of Cds1 (Boddy et al., 1998; Christensen et al., 2000; Rhind and Russell, unpublished data). Like Cdc25, regulation of Mik1 alone is sufficient to arrest cells in response to activation of the replication checkpoint (Rhind and Russell, unpublished data). In a Cds1-dependent manner, Mik1 accumulates in replication checkpoint arrested cells (Boddy et al., 1998; Christensen et al., 2000). The accumulation of Mik1 correlates with the accumulation of its mRNA. The upregulation of several other S-phase-specific transcripts in response to activation of the replication checkpoint requires the Cdc10-dependent S-phase transcription factor (Baum et al., 1997). So it seems plausible that Cds1 acts through Cdc10 machinery to maintain the S-phase transcription program during a replication checkpoint arrest, although its direct targets are unknown.

Although both Cdc25 and Mik1 are in vivo targets of the checkpoints, Wee1 does not seem to be. This conclusion is drawn from genetic experiments showing that Wee1 is neither necessary nor sufficient for checkpoint function in vivo (Barbet and Carr, 1993; Rhind and Russell, unpublished data, but see also Raleigh and O’Connell, 2000). This conclusion was somewhat surprising given previous in vitro results implicating Wee1 as a target in both checkpoints. In response to activation of the replication checkpoint, Cds1 binds to and phosphorylates exogenous Wee1 in cell lysates (Boddy et al., 1998). Furthermore, Chk1 can phosphorylate Wee1 in vitro (O’Connell et al., 1997). These phosphorylations do not appear to be important for the establishment of either checkpoint. However, it is possible that they play a role in maintenance of, or adaptation to, the checkpoints.

The targets of the checkpoints in budding yeast are dictated by the unusual organization of the cell cycle in this yeast. Specifically, budding yeast has no well-defined G_{2} phase (Lew et al., 1997). Rather, events traditionally defined as mitotic and requiring the tyrosine dephosphorylation of Cdc28 (the budding yeast Cdc2 homolog), such as spindle formation, occur before S phase is complete (Lim et al., 1996; Lew et al., 1997). Thus, regulating the tyrosine phosphorylation of Cdc28 is not practical for the budding yeast checkpoints* (Elledge, 1996). Instead, they prevent cell cycle progression by blocking the metaphase to anaphase (M-A) transition (Yamamoto et al., 1996).

In response to DNA damage, both Rad53 and Chk1 are required to arrest fully cells in metaphase. One, and possibly the major, target of Rad53 in the DNA damage pathway is thought to be Dun1, another homolog in the Cds1 family.

*However, the bud morphogenesis checkpoint, which acts before spindle formation, does use tyrosine phosphorylation of Cdc28 (Lew and Reed, 1995).
and Cln2 does not override the checkpoint. There must be other targets, since ectopic expression of Cln1 would be predicted following inhibition of Swi6. However, regulate the G1-S transition, is reduced during the G1 delay, as transcription of Rad53 (Sidorova and Breeden, 1997). Furthermore, the checkpoint appears to be the transcription factor Swi6. Swi6 is Mec1- and Rad53-dependent manner (Siede et al., 1994; yeast DNA damage checkpoint also delays cells in G1 in a dependent manner, and it is directly phosphorylated in vitro by phosphorylated in vivo in a DNA-damage-induced and Rad53-induced metaphase arrest, whereas cells lacking both Chk1 and Rad53, Pds1 and Rad53 or Pds1 and Dun1 show no metaphase arrest, suggests that there is another Rad53-dependent target regulating establishment of the arrest (Gardner et al., 1999; Sanchez et al., 1999; Liu et al., 2000b). Alternatively, in the absence of Chk1, inhibition of Cdc5 may be sufficient to delay anaphase, although this would not be predicted by the known phenotypes of cdc5 mutants.

The replication checkpoint in budding yeast seems to be separated into two parts. When cells are blocked in early replication, they arrest in a Rad53-dependent manner (Allen et al., 1994; Weinert et al., 1994). Although Rad53 regulates Dun1 in the transcriptional response to blocked replication, it is not known whether Dun1 is required for the metaphase arrest, or what other targets Rad53 might have. In the absence of Rad53, cells blocked in replication proceed with anaphase, but arrest in telophase (Allen et al., 1994). This telophase arrest indicates that there are other targets in the replication checkpoint. One of those other targets appears to be Pds1 (Clarke et al., 1999). There is no evidence that Chk1 plays a role in the budding yeast replication checkpoint, and it is unknown how Pds1 might be regulated by the replication checkpoint.

In addition to regulating the M-A transition, the budding yeast DNA damage checkpoint also delays cells in G1 in a Mec1- and Rad53-dependent manner (Siede et al., 1994; Sidorova and Breeden, 1997). One target of Rad53 in this checkpoint appears to be the transcription factor Swi6. Swi6 is phosphorylated in vivo in a DNA-damage-induced and Rad53-dependent manner, and it is directly phosphorylated in vitro by Rad53 (Sidorova and Breeden, 1997). Furthermore, the transcription of CLN1 and CLN2, two G1 cyclin genes that regulate the G1-S transition, is reduced during the G1 delay, as would be predicted following inhibition of Swi6. However, there must be other targets, since ectopic expression of Cln1 and Cln2 does not override the checkpoint.

Targets in metazoans

The cloning of ATM from humans and its identification as a homolog of Rad3 and Mec1 was the first clue that the yeast DNA damage and replication checkpoints are conserved in humans (Savitsky et al., 1995). Since then, homologs of most of the fission yeast checkpoint genes have been found in humans, including hCHK1 and hCDS1, also known as CHK2 (Matsuoka et al., 1998; Rhind and Russell, 1998a). Furthermore, the human checkpoints seem to regulate the G2-M transition in the same way as fission yeast ones, through ATM to the tyrosine phosphorylation of CDC2 (Blasina et al., 1997; Westphal, 1997). Although the checkpoint regulation of the G2-M transition appears to be similar between fission yeast and humans, the predominant DNA damage checkpoint in humans is the p53-dependent G1 arrest pathway (Wahl et al., 1997). ATM is required for p53 activation in response to gamma-ray-induced DNA damage, but not other types of damage, such as that induced by UV radiation, which is speculated to act through ATR (Kastan et al., 1992; Wright et al., 1998). Neither ATM nor ATR is thought to be involved with the DNA-damage-independent activation of p53 of the kind induced by growth factor withdrawal. These results indicate that the ATM-dependent DNA damage checkpoint pathway regulates both the G1-S and G2-M transitions in humans in response to ionizing radiation, and suggest that ATR may serve a similar role in response to UV radiation. It is proposed that hCHK1 and hCDS1 act downstream of ATM and ATR in these pathways.

In vitro, hCHK1 phosphorylates the three isoforms of human CDC25: CDC25A, CDC25B, and CDC25C (Sanchez et al., 1997). It phosphorylates CDC25C on Ser216 and other sites and inhibits its in vitro phosphatase activity (Sanchez et al., 1997; Blasina et al., 1999). Ser216 is a major site of in vivo phosphorylation of CDC25C. It is phosphorylated to high stoichiometry during a normal cell cycle by processes other than the DNA damage checkpoint (Ogg et al., 1994). Phosphorylation of Ser216 leads to 14-3-3 binding (Peng et al., 1998). It is proposed that the binding of 14-3-3 to this site leads to the inhibition of CDC25, either directly or through nuclear exclusion, although inactivation of CDC25 by in vitro phosphorylation does not correlate with 14-3-3 binding (Peng et al., 1997; Weinert, 1997; Blasina et al., 1999). Furthermore, expression of CDC25C-S216A, which cannot be phosphorylated on Ser216, causes only a minor disruption of either the DNA damage or replication checkpoint in human cells (Peng et al., 1997). Thus it is unlikely that Ser216 is the only site of DNA-damaged-induced phosphorylation on CDC25C. hCHK1 has also been implicated in the regulation of CDC25A stability in response to DNA damage (Mailand et al., 2000). In addition to phosphorylating CDC25, hCHK1 phosphorylates p53 in vitro on Ser20, a site of damage-inducible phosphorylation in vivo (Shieh et al., 2000).

An in vivo role for CHK1 in the mammalian G2 DNA damage checkpoint is supported by analysis of Chkl-mutant mouse cells. These analyses are complicated by the fact that CHK1 appears to be required for cellular viability, at least in embryonic cells. However, two strategies have been employed in which Chkl-mutant cells can be tested for checkpoint defects before they die (Liu et al., 2000a; Takai et al., 2000). Chkl-/- blastocysts fail to proliferate, and die between 3.5 and 6.5 days post-fertilization. If wild-type blastocysts are
irradiated at day 3.5, the percentage of cells in mitosis drops substantially, which is consistent with activation of a G₂ checkpoint. In contrast, Chkl⁻/⁻ blastocysts contain many mitotic cells after irradiation, which suggests that CHK1 is required to prevent mitosis in response to DNA damage (Takai et al., 2000). Another approach to study the role of CHK1 has been to delete Chkl by induced recombination in embryonic stem (ES) cell cultures. ES cells lacking Chkl die after about 48 hours. During those 48 hours, the cells continue to enter mitosis in spite of radiation-induced DNA damage (Liu et al., 2000a). An important caveat for both sets of experiments is that the Chkl⁻/⁻ cells are destined to die during or shortly after the time course of the experiments, and it is not clear whether the accumulation of mitotic cells is due to a specific DNA damage checkpoint defect or a general mitotic-catastrophe phenotype.

Additional evidence that hCHK1 plays a role in the human DNA damage checkpoint is that the CHK1 inhibitors UCN01 and SB-218078 abrogate the DNA-damage-induced G₂ arrest in human cell lines (Busby et al., 2000; Graves et al., 2000; Jackson et al., 2000). It should be noted, however, that both inhibitors are structurally related to staurosporine, a competitive inhibitor of ATP binding, and may well inhibit other kinases (Jackson et al., 2000). Specifically, UCN01 also inhibits C-TAK1, a kinase that can phosphorylate CDC25C on Ser216 (Busby et al., 2000). Since C-TAK1 has been proposed to be a major CDC25C-inhibitory kinase, it is conceivable that a C-TAK1 inhibitor could activate CDC25C and override the checkpoint (Ogg et al., 1994; Peng et al., 1998). Curiously, neither UCN01 nor SB-218078 causes cell lethality, despite the fact that they are both strong inhibitors of CHK1. These observations raise the possibility that CHK1 is essential for embryonic cell growth but not for somatic cell viability.

The role of Chkl in the replication checkpoint in metazoans is supported by studies in frogs and flies. In a Xenopus cell-free egg extract system, XChkl is phosphorylated in response to a replication block but not DNA damage (Kumagai et al., 1998). Furthermore, depletion of XChkl reduces the mitotic delay in response to unreplicated DNA but not DNA damage. Cdc25 is thought to be at least one of the targets of XChkl. In response to unreplicated DNA, XChkl phosphorylates Cdc25 on several sites, including Ser287, a 14-3-3 binding site thought to be analogous to Ser216 of human CDC25C (Kumagai et al., 1998). But, again, there are other Ser287 kinases in Xenopus. And, whereas Cdc25-S287A does impair the DNA damage and replication checkpoints in the egg extract system, it also advances mitosis in untreated extracts and thereby may override other modes of checkpoint control (Kumagai et al., 1998).

Studies in flies tell a similar story. MEI-41 and Grapes (GRP), the ATM/ATR and Chkl homologs in flies, are required for the replication-block-induced delay of mitosis in embryonic cells (Hari et al., 1995; Fogarty et al., 1997; Sibon et al., 1997; Sibon et al., 1999). In the absence of either protein, embryos fail to maintain String, a Cdc25 homolog, or tyrosine phosphorylation of Cdc2, although it has yet to be determined whether these changes are the cause or the effect of the failure of the checkpoint (Sibon et al., 1999). As in mice, both GRP and MEI-41 are required for viability (Hari et al., 1995; Fogarty et al., 1997). However, the requirement is limited to early embryogenesis and does not appear to affect the function of somatic cells, providing a precedent for the suggestion that CHK1 may be required for embryonic, but not somatic, cell growth in mammals. Nevertheless, the fly studies provide strong in vivo evidence that, in metazoans, Chkl can function in the replication checkpoint and suggest that it may target Cdc25. GRP is also required for the regulation of the abundance of cyclin A (Su et al., 1999). However, flies lacking the homolog of Weel appear to have phenotypes similar to those lacking GRP, which suggests that a major role of GRP is to regulate the tyrosine phosphorylation of CDC2 (Price et al., 2000). The requirement for GRP in the DNA damage checkpoint has not been directly addressed.

A strong case can be made for the involvement of hCDS1 in the human DNA damage checkpoint. Like hCHK1, hCDS1 can phosphorylate CDC25C on Ser216 and other sites, and this phosphorylation leads to the inhibition of CDC25C in vitro (Matsuoka et al., 1998; Blasina et al., 1999; Brown et al., 1999; Chaturvedi et al., 1999). Likewise hCDS1 can phosphorylate p53 on Ser20 (Chehab et al., 2000; Hirao et al., 2000; Shieh et al., 2000). hCDS1 is also phosphorylated and activated in response to DNA damage and replication arrest (Matsuoka et al., 1998; Brown et al., 1999; Chaturvedi et al., 1999; Tominga et al., 1999). This regulation requires ATM in the case of gamma-radiation-induced DNA damage but not in the case of UV-radiation-induced DNA damage or replication arrest. Mouse thymocytes lacking CHK2, the mouse homolog of CDS1, fail to upregulate p53 in response to gamma radiation but not UV radiation (Hirao et al., 2000). Moreover, mutations in hCDS1 have been associated with Li-Fraumeni syndrome, a syndrome usually caused by mutations in the gene encoding p53 (Bell et al., 1999). Taken together, these results show that in mammals, CDS1 is required for the p53-dependent G1 DNA damage response and suggest that ATM acts through CDS1, instead of directly regulating p53.

Although CDS1 seems to play an important role in the DNA damage checkpoint pathway in humans, two results suggest that hCDS1 is not required for the regulation of G₂-M transition. First, UCN01, which strongly inhibits the G₂ DNA damage checkpoint response, does not inhibit hCDS1 (Busby et al., 2000; Graves et al., 2000). Second, mouse embryonic stem cells lacking the mouse homolog of CDS1 arrest properly in response to G₂ to DNA damage, although they leak through the arrest at later timepoints (Hirao et al., 2000). Similarly, although Xcds1 is activated by DNA ends in a cell-free Xenopus egg extract, Xcds1 is not required for the delay of mitosis induced by DNA ends in that system (Guo and Dunphy, 2000).

Other targets

In addition to the targets discussed above, there are certainly other targets of Chkl and Cds1. For instance, in fission yeast, Cds1 is required to maintain cell viability during a replication arrest, which is independent of its role in preventing mitosis (Murakami and Okayama, 1995). Rad53 is also required for budding yeast to survive replicative stress (Desany et al.,
1998). It probably has several targets, which may be indirectly regulated through Dun1, including induction of gene expression through regulation of the transcriptional repressor Rfx1/Crt1 (Zhou and Elledge, 1993; Huang et al., 1998). These functions may be related to a checkpoint that we have not discussed, the intra-S checkpoint, which slows DNA replication in response to DNA damage. This checkpoint has been studied in humans, budding yeast and fission yeast, and requires ATM and Cds1, although the role of hCDS1 has yet to be determined (Dasika et al., 1999). In budding yeast this checkpoint inhibits origin firing and regulates primase phosphorylation in a Rad53-dependent manner, implicating Rad53, and possibly other Cds1s, in the regulation of origin function (Santocanale and Diffley, 1998; Shirahige et al., 1998; Pelliccioli et al., 1999). In addition, hCDS1 binds to and phosphorylates BRCA1, a protein implicated in DNA damage repair and recombination that may function in the intra-S and other DNA damage checkpoint pathways (Dasika et al., 1999; Lee et al., 2000).

**CONCLUDING REMARKS**

Studies in a variety of organisms have led to the discovery of a largely conserved pathway for the DNA damage and replication checkpoint pathways. These pathways are best understood in fission and budding yeast. It is thought that most of the players have been identified, and the biochemical mechanisms of many of the steps are beginning to be understood.

Although the studies in fission and budding yeast provide a useful framework for understanding the human checkpoints, it is clear that much work remains to be done in humans. In particular it is still unclear what proteins act downstream of ATM in the G2 checkpoints. Since most human cancer cells lack the p53-dependent G1 DNA damage checkpoint, the G2 DNA damage checkpoint is particularly important for cancer cell survival. As such, it is a promising target for chemotherapy, because drugs that specifically inhibit the G2 checkpoints should sensitize cancer cells to DNA damage.

Understanding the molecular details of this pathway is crucial for both design and assay of new checkpoint inhibiting drugs. Structural studies, such as the recent crystal structure of CHK1, may well help (Chen et al., 2000). It is also an open question as to why CHK1 should be required for cell viability in mice. The development of more in vivo techniques will provide clearer understanding of the roles of the yeast homologs in mammalian cells. In this respect, establishment and analysis of further mouse mutant strains will certainly play a key role.

At a more theoretical level, it is interesting to speculate as to why Chk1 and Cds1 seem to play different roles in different organisms. One interesting observation is that all of the known or suspected targets of Chk1 are cell cycle regulatory proteins. Cds1, by contrast, seems to be also involved in regulating DNA repair, DNA recombination and transcription. It may be easiest to posit an ancestral situation in which, in response to both DNA damage and replication blocks, Chk1-regulated cell cycle progression and Cds1 regulated repair. From here, it is possible to imagine how Cds1 could acquire cell cycle roles and come to be associated with checkpoints that most require DNA repair, such as those before and during replication. However these systems evolved, it appears that much of the plasticity centers on the functions of Chk1 and Cds1. Thus, these kinases act as linchpins that link the checkpoint signal transduction pathways with the basic cell cycle and DNA repair machinery.

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