Cell cycle checkpoint control

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Abbreviations: ORC, origin-recognition complex; RF-C, replication factor-C; HU, hydroxyurea; ATM, ataxia telangiectasia mutated gene product; CAK, CDC2-activating kinase

Abstract

Genetic instability is considered to be a major driving force of malignancy of cancer cells, and at least some of cancer-associated genetic instability is known to be caused by defects in the cell cycle checkpoint control. Patients of the cancer-prone genetic disorder ataxia telangiectasia frequently develop malignant lymphoma and their cells are defective in γ -irradiation responsive checkpoint control, whereas cells inactivated for the p53 recessive oncoprotein are defective in DNA damage-induced checkpoint control and develop genetic instability. Cells contain two major cell cycle checkpoint control systems: DNA-replication checkpoint, DNA-damage checkpoint. These checkpoint systems are thought to consist of three functionally distinct components: sensors, checkpoint signal transducers and cell cycle effectors. Recent rapid progress in the identification of these components is beginning to prove this conceptual model and the generality of the checkpoint system among eukaryotes. The full understanding of the cell cycle checkpoint control system will provide deeper insights into the highly complex mechanisms of carcinogenesis and highlight possible targets for cancer therapy.

Keywords: cell cycle, cancer, ataxia telangiectasia, DNA damage, DNA replication

Introduction

One of the key properties of malignant transformed cells is genetic instability. Genetic instability itself, however, has no contribution to malignant phenotypes but is considered to act as a powerful engine driving

activation and inactivation of a variety of oncogenes and recessive oncogenes, which ultimately lead to the expression of malignant phenotypes. Recent advances in cell cycle research indicate that a defect in a checkpoint control may be responsible for the genetic instability of at least some cancer cells (Hartwell and Kastan, 1994). The p53 recessive oncogene, which is inactivated in a variety of cancer cells, is actually required for genetic stability and γ -irradiation induced G1 checkpoint arrest (Ko and Prives, 1996; Sherr, 1996), whereas ATM, the causative gene for cancer-prone ataxia telangiectasia, structurally resembles a checkpoint gene in yeast and is essential for γ -irradiation induced checkpoint arrest (Savitsky *et al.*, 1995; Elledge, 1996; Sherr, 1996).

The current understanding of the mechanisms of cell cycle checkpoint control is still fragmentary and far from complete, yet in the past few years a remarkable progress has been made regarding its key elements. In this review, we outline the current understanding of general cell cycle checkpoint control and discuss its implication to cancer research.

Checkpoint controls in the cell cycle

Duplication of the genome and its faithful transmittance to the daughter cells are vital to the propagation of all organisms. The cell cycle-associated checkpoint controls have been evolved as a surveillance mechanism to ensure the proper occurrence of these fundamental cellular events (Hartwell and Weinert, 1989). It monitors whether or not the genetic information is completely duplicated or thoroughly repaired if it is damaged and controls cell cycle progression, much like a quality control in a factory, they have to be produced in an orderly fashion and checked for quality before shipment. Cells contain several distinct checkpoint controls (Nurse, 1994; Diffley, 1996; Edgar and Lehner, 1996; Elledge, 1996; King *et al.*, 1996; Nasmyth, 1996; Okayama *et al.*, 1996; Sherr, 1996; Stillman, 1996). One called DNA replication checkpoint control is present to prevent entry into mitosis during S phase. One called DNA damage checkpoint control is to block cell cycle progression until damage is completely repaired. There are other types of checkpoint controls, which ensure duplication of the genome only once per cycle and faithful progression of mitosis. Moreover, all the cell cycle controls by various extracellular and intracellular signals, such as those for nutrient availability, cell size and cell-cell interactions, could also be considered to be one type of checkpoint control. Because of their high relevance to

carcinogenesis, we like to focus on DNA replication-, DNA damage- and endoreplication-checkpoint controls in this review.

Conceptually, these checkpoint control systems are thought to be made up of three functionally distinct components: a sensor(s), a checkpoint signal transducer(s) and a cell cycle effector(s). The sensor would detect, via a specific object(s), the events and, if the events are in progress, generate checkpoint signal, which is mediated by the signal transducer(s) and controls the effector to block cell cycle progression. The effector is actually a key component of the cell cycle progression machinery. In this concept, the sensor

would generally have no function other than monitoring the event and the checkpoint signal generated by the sensor always acts to block cell cycle progression. If the object(s) were absent or the sensor(s) were inactivated, the checkpoint signal would not be generated. Consequently, cells in which the sensor or the object is missing would display checkpoint defects. Recent identification of key checkpoint factors began to prove this conceptual model.

The components of the checkpoint control system have been identified in the fission yeast *Schizosaccharomyces pombe*, the budding yeast *Saccharomyces cerevisiae*, *Aspergillus nidulans*, mammal and other

Table 1. Checkpoint related genes in yeast

<i>S. pombe</i>	DNA replication	DNA damage	Activity	Putative <i>S. cerevisiae</i> counterpart	DNA replication	DNA damage
<i>rad1</i>	+	+	Nuclease	RAD17	-	+
<i>rad3</i>	+	+	PI-kinase	MEC1	+	+
<i>rad9</i>	+	+	?	?	?	?
<i>rad17</i>	+	+	RFC-related	RAD24	-	+
<i>rad26</i>	+	+	?	?	?	?
<i>hus1</i>	+	+	?	?	?	?
<i>rad24, rad25</i>	-	+	14-3-3	BMH1, BMH2	?	?
<i>chk1</i>	-	+	protein kinase	?	?	?
<i>cds1</i>	+	-	protein kinase	RAD53	+	+
?	?	?	?	RAD9	-	+
?	?	?	?	MEC3	-	+

Figure 1. Checkpoint signal transduction.

organisms (Table 1; Figure 1). The former three organisms are genetically tractable and have many features common to mammals. The important feature of the fission yeast as well as most of other eukaryotes is that mitosis and other mitotic events never start until the completion of S phase. On the contrary, in budding yeast, some mitotic events, such as spindle pole body duplication, initiate even before the onset of S phase. Since such difference in cell cycle progression would profoundly affect the way of checkpoint regulation, the objects to be sensed and the cell cycle targets for the checkpoint control might entirely differ between budding yeast and other organisms. We, therefore, will describe the fission yeast system as the primary example and discuss those of budding yeast, *A. nidulans* and mammals.

DNA replication-monitoring checkpoint control

How the DNA replication checkpoint system monitors S phase has begun to be understood. The recent identification of a major sensor monitoring S phase has revealed that S phase is monitored in three separated sub-phases: pre-replication, mid-replication and post-replication (Murakami and Okayama, 1995).

Pre-replication S phase

The primary cellular event in this phase is activation of origins of replication which is achieved by their association with the pre-replicative complex that is composed of origin-recognition complex (ORC), MCM proteins, Cdc18 (Cdc6 homologue) and probably a Cdc7-related protein kinase (Diffley, 1996; Stillman, 1996). Interestingly, fission yeast cells deleted for a component of ORC (Muzi-Falconi and Kelly, 1995; Grallert and Nurse, 1996; Leatherwood *et al.*, 1996), Cdc7-related kinase (Masai *et al.*, 1995), a MCM homologue (Maiorano *et al.*, 1996), or Cdc18 (Kelly *et al.*, 1993) enter mitosis without DNA synthesis, indicating that the pre-replication complex is likely to be a major object detected by a checkpoint sensor. However, the nature of the sensor is currently unknown. A *cut5⁻* mutant, which arrests before DNA replication upon shift to the nonpermissive temperature, is also defective in checkpoint control (Saka and Yanagida, 1993). But, the function of Cut5 and its possible relation to the pre-replication complex is not known at present.

Mid-replication S phase

In this phase, DNA is synthesized by several functionally distinct polymerases. DNA polymerase α initiates DNA replication as a primer complex and synthesizes the lagging strand whereas DNA polymerase δ synthesizes the leading strand as well as

elongates the lagging strand (Sugino, 1995). Fission yeast cells deleted for DNA polymerase α enter mitosis without DNA synthesis (D'Urso *et al.*, 1995). In contrast, cells deleted for DNA polymerase δ or ϵ arrest in S phase and never proceed into mitosis (D'Urso *et al.*, 1995; D'Urso and Nurse, 1996). In addition, cells deleted for Cdc1, a subunit of DNA polymerase δ , have intact checkpoint function (MacNeill *et al.*, 1996). Furthermore, Cdc27, a factor associated with Cdc1, is not involved in checkpoint control (MacNeill *et al.*, 1996). Thus, DNA polymerase α is likely to be an object detected by a sensor. Recently, a factor that fits the criteria of a checkpoint sensor in this sub-phase was identified. It is Cds1 kinase (Murakami and Okayama, 1995). The *cds1⁺* gene was isolated as a multicopy suppressor of a temperature-sensitive DNA polymerase α mutant. It encodes a serine/threonine protein kinase homologous with Rad53 in budding yeast. Cells deleted for *cds1⁺* are viable and have no detectable defect in DNA synthesis, but display a checkpoint defect to DNA synthesis inhibitors but not to DNA damage. When treated with hydroxyurea (HU, an inhibitor of ribonucleotide reductase, the key enzyme for the synthesis of deoxyribonucleotides), cells lacking Cds1 initially arrest in early S. However, they prematurely enter mitosis as soon as DNA polymerase α starts synthesizing DNA as an enough amount of deoxyribonucleotides accumulates due to leaky inhibition of ribonucleotide reductase. Thus, the checkpoint defect of cells lacking Cds1 is closely associated with the start of DNA synthesis, well matching with the expected properties of a checkpoint sensor. The object monitored by Cds1 is likely to be only DNA polymerase α because Cds1 is not required for checkpoint control when cell cycle progression is inhibited by inactivation of other factors essential for S phase progression (Murakami and Okayama, 1995; Murakami and Okayama, unpublished results).

It is not known whether the checkpoint signal generated by Cds1 is directly transmitted to the cell cycle target or mediated by some factor(s) although several checkpoint *rad* genes have been known. The cell mutants of the checkpoint *rad* genes were initially isolated as those highly sensitive to irradiation, but subsequently found to be defective in damage-responsive checkpoint control (Al-Khodairy and Carr, 1992; Rowley *et al.*, 1992a). *rad1⁺*, *rad3⁺*, *rad9⁺*, *rad17⁺*, *rad26⁺* and *hus1⁺* are among them (Enoch *et al.*, 1992; Rowley *et al.*, 1992a; Al-Khodairy and Carr, 1992; Al-Khodairy *et al.*, 1994). Interestingly, these genes are also required for DNA replication checkpoint controls, and their mutants show very similar checkpoint defects and viability loss to both ultraviolet (UV) irradiation and hydroxyurea treatment (Al-Khodairy and Carr, 1992; Enoch *et al.*, 1992; Rowley *et al.*, 1992a; Al-Khodairy *et al.*, 1994). There is no evidence to indicate that any one of them acts upstream or downstream of

the others. High level expression of *rad1*⁺ and *rad9*⁺ is unable to rescue the other checkpoint *rad* mutants (Murakami and Okayama, unpublished data). Since the mutants of these *rad* genes have similar defects in checkpoint function and none of them shows any clear upstream-downstream relationship, their products might function as a large protein complex. But, there is no biochemical evidence to support this possibility.

There is, however, a clear interaction between *cds1*⁺ and these checkpoint *rad* genes. High level expression of *cds1*⁺ efficiently rescues the DNA replication checkpoint defect of at least *rad1*⁺, *rad3*⁺ and *rad9*⁺ mutants (Murakami and Okayama, 1995). However, this rescue is specific to DNA replication checkpoint defect, and the similar high expression of *cds1*⁺ is unable to rescue the DNA damage checkpoint defect of these mutants. Such relationship holds true for *chk1*⁺ (*rad27*⁺) but for their DNA damage checkpoint defects (see below for *chk1*⁺). Overexpression of *chk1*⁺ rescues the DNA damage checkpoint defect (Walworth *et al.*, 1993) but not the DNA replication checkpoint defect of these mutants (Murakami and Okayama, unpublished observations). Regarding the functions of the checkpoint sensor and these checkpoint *rad* genes, these observations suggest that simple overproduction of a sensor would not generate checkpoint signals unless the proper object is present and also in action.

Some of the checkpoint Rad proteins, however, have interesting structural features or similarity to some known proteins. Rad3, a putative phosphatidylinositol 3-kinase, has a significant functional and structural similarity with, and therefore is considered to be a homologue of, the ataxia telangiectasia mutated gene product (ATM) in mammals (Savitsky *et al.*, 1995). Rad17 protein shows a limited homology to replication factor-C (RF-C), an accessory factor for DNA polymerases δ and ϵ (Griffiths *et al.*, 1995). It is not clear whether its similarity to RF-C is meaningful because the mutant of at least DNA polymerases δ are intact in checkpoint control as already described. In addition, Rad1 protein has a homology to a nuclease (Lydall and Weinert, 1995; Siede *et al.*, 1996a) whereas Rad9 (Lieberman *et al.*, 1992) and Rad26 (Al-Khodairy *et al.*, 1994) have no significant homology with any known proteins.

In budding yeast, the objects monitored by checkpoint sensors seem radically different from those in fission yeast. POL2 (DNA polymerase ϵ) (Navas *et al.*, 1995), DPB11 (Araki *et al.*, 1995) and RFC5 (Sugimoto *et al.*, 1996) are known to be required for replication checkpoint, and cells with a point mutation of POL2 fail to arrest in response to HU but not to DNA damage. In addition, DPB11, essential for DNA replication, is required for checkpoint arrest to HU. This protein is homologous with Cut5 in fission yeast that seems to be involved in some checkpoint pathway and has a genetic

interaction with POL2. On the other hand, Rfc5 protein is a subunit of multiprotein complex called RF-C. These observations suggest that, unlike in fission yeast, DNA polymerase ϵ is a potential candidate for an object for a DNA replication checkpoint sensor in this organism. This radical difference in the checkpoint sensing objects between the two organisms may reflect the difference in the control of the onset of mitosis.

The budding yeast mutant of RAD53 (SPK1, MEC2, SAD1), a kinase structurally similar to Cds1, is defective in both DNA replication and damage checkpoint control, but unlike Cds1, it is essential for viability (Stern *et al.*, 1991; Allen *et al.*, 1994; Weinert *et al.*, 1994). Cells deleted for RAD53 die with large buds, suggesting that RAD53 is required for S phase progression (Zheng *et al.*, 1993). The reason for RAD53 requirement for S phase progression is unknown. MEC1 (ESR1, SAD3), a homologue of ATM in humans and Rad3 in fission yeast, is involved in both DNA replication and DNA damage checkpoint controls (Kato and Ogawa, 1994; Weinert *et al.*, 1994; Siede *et al.*, 1996a). MEC1 is also essential for viability, but why it is required for viability remains unknown. The lethality of *mec1* mutation is rescued by overexpression of RAD53 and phosphorylation of Rad53 protein is dependent on MEC1, suggesting that RAD53 acts downstream of MEC1 (Sanchez *et al.*, 1996; Sun *et al.*, 1996). In addition, overproduction of Rad53 protein rescues a *rfc5* mutation, similarly suggesting that Rad53 kinase acts downstream of RFC5 (Sugimoto *et al.*, 1996).

Post-replication S phase

It is not known what determines the completion of S phase and what factors are required for this. At the end of DNA replication, gaps and nicks in the DNA strand have to be sealed by DNA polymerase δ and DNA ligase. Therefore, at least such steps would be a potential candidate for the objects for checkpoint monitoring. However, such enzymes are unlikely to be the objects. As already described, these mutants have no detectable checkpoint defects. Unlike the pre-replication and mid-replication S phase, this stage of S phase seem to be monitored via a DNA structure rather than some specific enzymes and protein factors involved.

The *chk1*⁺ gene was initially isolated by phenotypic suppression of a cold sensitive *cdc2* mutant and found to play an important role in DNA damage checkpoint control and checkpoint control for nicked DNA induced by inactivation of DNA ligase (Walworth *et al.*, 1993). Subsequently, this factor was found to be also essential for checkpoint control during S arrest by inactivation of DNA polymerase δ (Francesconi *et al.*, 1995). Furthermore, this gene is required for checkpoint control at the arrest points of the *cdc1* (a subunit for Pol ϵ), *cdc19* (a mcm homologue), *cdc21* (a mcm homologue), *cdc24*

(essential for S phase progression) and *cdc27* (binds Cdc1) mutants (Carr *et al.*, 1995). Perhaps, at these arrest points, unrepaired nicks and gaps are left on the DNA strands. Whether Chk1 protein is a sensor of these aberrant structures of DNA or simply a signal transducer remains to be answered. Regarding its unique function, however, it is noteworthy that Chk1 contains a kinase domain as well as a leucine zipper motif which is essential for activity (Murakami and Okayama, unpublished observation).

Cell cycle target for checkpoint control

A key cell cycle target for the checkpoint signal is Cdc2 (Cdk1) kinase, which plays a central role in the onset and progression of mitosis in virtually all eukaryotic cells (Figure 2, 3) (Nurse, 1990; Okayama *et al.*, 1996). For its mitotic activity, Cdc2 requires association with cyclin B (Moreno *et al.*, 1989) and phosphorylation on the Thr 167 residue performed by CDC2-activating kinase

(CAK) (Gould *et al.*, 1991; Buck *et al.*, 1995; Damagnez *et al.*, 1995). The timing of Thr 167 phosphorylation is unclear (Gould *et al.*, 1991) and its regulatory role in the cell cycle control is somewhat controversial partly because CAK is also involved in general transcription control (Roy *et al.*, 1994; Feaver *et al.*, 1994). Until mitosis is ready to start, the Cdc2-cyclinB complex is held inactive by phosphorylation on the Tyr 15 residue (and also Thr 14) of the Cdc2 molecule. Tyr 15 phosphorylation is catalyzed predominantly by Wee1 kinase (Featherstone and Russell, 1991; Parker *et al.*, 1992). This phosphorylation is also catalyzed by a minor Mik1 kinase (Lundgren *et al.*, 1991). The inhibitory effect of Thr 14 phosphorylation of Cdc2 by Myt1 kinase is also reported in vertebrates (Mueller *et al.*, 1995), while the significance of Thr 14 phosphorylation is unclear in fission yeast (Den Haese *et al.*, 1995). Just prior to the onset of M phase the phosphorylated Tyr 15 and Thr 14 residues are dephosphorylated by

Figure 2. Regulation of Cdc2 activity.

Figure 3. Target of checkpoint regulation.

the Cdc25 dual tyrosine and threonine phosphatase and a minor Pyp3 phosphatase (Millar *et al.*, 1991, 1992), resulting in rapid activation of the Cdc2-cyclin B complex. The major regulatory event for the final activation of the Cdc2-cyclinB complex seems to be the abrupt dephosphorylation at Tyr 15. During S and G2 phases, the Tyr 15-phosphorylated form of the Cdc2-cyclin B complex accumulates (Hayles and Nurse, 1995). Cells in which wild type Cdc2^{Y15} is replaced by unphosphorylatable Cdc2^{F15} greatly advances into mitosis with some premature mitotic events (Gould and Nurse, 1989). This phenotype is somewhat similar to that seen when *mik1*⁺ and *wee1*⁺ are both inactivated (Lundgren *et al.*, 1991). Both these mutant cells are defective in DNA replication checkpoint control (Enoch *et al.*, 1991; Lundgren *et al.*, 1991). Thus, Tyr 15 phosphorylation of Cdc2 is a critical target for DNA replication checkpoint control in fission yeast.

However, it is unclear at present how Tyr 15 dephosphorylation is blocked by the checkpoint control system. The blocking may be achieved by either activating Wee1 kinase or inhibiting Cdc25 phosphatase. In fact, overexpression of Cdc25 phosphatase (Enoch and Nurse, 1990) or inactivation of both Wee1 and Mik1 (Lundgren *et al.*, 1991) overrides the arrest imposed by HU. However, cells lacking Wee1 kinase is intact in DNA-replication checkpoint and display no HU sensitivity (Enoch and Nurse, 1990). Similarly, cells lacking Mik1 kinase have little HU sensitivity (Rowley *et al.*, 1992b). Furthermore, fission yeast cells in which both Wee1 and Cdc25 are deleted can still be arrested by HU, demonstrating that Wee1 and Cdc25 are not the sole target for the checkpoint control (Enoch *et al.*, 1992). In this situation, Tyr 15 of Cdc2 is phosphorylated by minor Mik1 kinase and dephosphorylated by minor Pyp3 phosphatase.

In *A. nidulans*, NIMX kinase, a homologue of Cdc2, and NIMA kinase are essential for the initiation of mitosis (Osmani *et al.*, 1991; Osmani *et al.*, 1994; Osmani and Ye, 1996). The regulatory role of Tyr phosphorylation of NIMX is also conserved in this organism (O'Connell *et al.*, 1992). The NIMA kinase is only found in *A. nidulans*, although it is thought that a NIMA-like mitotic pathway may also exist in other organisms (O'Connell *et al.*, 1994; Lu and Hunter, 1995). The activity of NIMA kinase is regulated independently of NIMX kinase but coordinate activation of NIMA and NIMX are required for proper M phase initiation (Osmani *et al.*, 1991). Like in fission yeast, in *A. nidulans*, Tyr 15 phosphorylation of NIMX is required for slowing down the initiation of mitosis in response to partial inhibition of DNA replication whereas Tyr 15 phosphorylation and BIME are required for G2 arrest in response to complete inhibition of DNA replication (Ye *et al.*, 1996). Recently, BIME protein homologue was identified as an anaphase-promoting complex in fission

yeast (Yamashita *et al.*, 1996), *Xenopus* (Peters, *et al.*, 1996) and budding yeast (Zachariae *et al.*, 1996), which promotes ubiquitination of target proteins for proteolytic degradation by the 26S proteasome (King *et al.*, 1996).

In mammalian cells, the regulation of Cdc2 at G2/M transition is conserved and the regulatory factors are also identified (Okayama *et al.*, 1996). Expression of the mutant form of Cdc2 unphosphorylatable on both Thr 14 and Tyr 15 does not induce mitosis when DNA replication is completely inhibited, but induces low levels of premature mitosis during progression through S and G2 phase (Jin *et al.*, 1996). This phenotypic difference between the complete and partial inhibitions might be explained by whether the cells were blocked in early S before induction of cyclin B or not. Or like in *A. nidulans* cells, cooperative activation of Cdc2 and inactivation of BIME-like protein might be required for inducing complete checkpoint defects regardless the state of DNA replication.

Unlike most eukaryotes, DNA replication checkpoint is fully active in budding yeast cells containing unphosphorylatable Cdc28^{F15}, a homologue of Cdc2, clearly showing that they utilize other mechanisms (Amon *et al.*, 1992; Sorger and Murray, 1992). This difference might be caused by the peculiar feature of budding yeast cells, in which spindle pole body duplication initiates just before S phase begins as pointed already. Unlike in fission yeast, NIMA-like kinase might be the target for checkpoint control in budding yeast. As already noted, the role of Tyr 15 phosphorylation of Cdc2 in checkpoint regulation is highly conserved among many organisms. However, it is also clear that this regulation is not the sole mechanism for DNA replication checkpoint control.

DNA damage-responsive checkpoint control

The checkpoint sensors and the objects sensed for the detection of DNA damage are not well understood. Damaged DNA is repaired by specific enzymes. For example, when pyrimidine dimers are formed in DNA by UV irradiation, they are detected and excised by the excision repair complex, and the gaps left by excision are filled with DNA polymerase δ . At least the system involving Chk1 kinase, which we have postulated detects aberrant DNA structures rather than factors involved in repair in the previous section, plays a major role in DNA damage-responsive checkpoint control. Cells deleted for *chk1*⁺ are defective in DNA damage-induced cell cycle arrest (Walworth *et al.*, 1993). In addition, as described in the previous section, the *rad1*⁺, *rad3*⁺, *rad9*⁺, *rad17*⁺, *rad26*⁺ and *hus1*⁺ genes are essential for damage checkpoint control. Very similar to the relationship between *cds1*⁺ and these *rad*

genes in replication checkpoint control, overproduction of Chk1 partially suppresses the UV sensitivity of at least *rad1*, *rad3* and *rad9* mutants, but any of the checkpoint *rad* genes can not suppress the checkpoint defect of Chk1-minus cells (Murakami and Okayama, unpublished results). In addition, Chk1 autophosphorylates and its autophosphorylation is dependent on the checkpoint *rad* genes (Walworth and Bernards, 1996). These observations imply that the checkpoint *rad* genes may be required at least for a full function of Chk1 kinase which is partly compensated by its overproduction. Several observations suggest that Chk1 kinase functions in vicinity of Cdc2 kinase. *chk1*⁺ was initially isolated as a multicopy suppressor of a cold-sensitive *cdc2*⁻ mutant (Walworth *et al.*, 1993). Chk1 kinase phosphorylates Wee1 kinase *in vitro* though it does not affect Wee1 kinase activity (O'Connell *et al.*, 1997). Despite its great significance in checkpoint control, structural homologues of Chk1 have not been found in other organisms.

Rad24 protein, a member of the 14-3-3 protein family that has diverse biochemical properties (Aitken *et al.*, 1995) and associates with polyomavirus middle tumor antigen (Pallas *et al.*, 1994), is partly involved in damage but not replication checkpoint (Ford *et al.*, 1994). Cells lacking this gene show γ -ray sensitivity and partial checkpoint deficiency. On the other hand, Rad25, another member of the 14-3-3 family, seems to have no obvious checkpoint function. Cells lacking both *rad24*⁺ and *rad25*⁺ are lethal. But, it is not known whether this lethal phenotype is caused by checkpoint loss or not. Because of their low level involvement in checkpoint control, their relation to Chk1 and the checkpoint Rad proteins is unknown.

In budding yeast, RAD9, RAD17, RAD24 and MEC3 have been identified as those specifically involved in damage checkpoint control (Weinert *et al.*, 1994). RAD17, RAD24 and MEC3 are required for removal of damaged nucleotide (Lydall and Weinert, 1995). It has been postulated that the resulting single strand DNA region may be detected by a damage checkpoint sensor, such as the system involving Chk1 in fission yeast. Actually, RAD17, a fission yeast homologue of Rad1, encodes a 3'-5' exonuclease (Lydall and Weinert, 1995; Siede *et al.*, 1996a). RAD24, a fission yeast homologue of Rad17 (Griffiths *et al.*, 1995), have a limited homology with RF-C that binds gapped DNA and promotes repair DNA synthesis by DNA polymerase δ . Rad24 protein would be a candidate for a DNA damage recognition protein although its biochemical properties are not known. In contrast, RAD9 is not directly involved in the above process and thought to be activated by a single strand DNA, but its role is unclear (Lydall and Weinert, 1995).

Much like for replication checkpoint control, a key cell cycle target for damage checkpoint control is Cdc2

kinase. However, the involvement of the Y15 phosphorylation regulation in damage checkpoint control is less clear at present. Cells deficient in Wee1 are sensitive to UV and γ -ray (Rowley *et al.*, 1992b), but this sensitivity is not caused by the checkpoint deficiency (Barbet and Carr, 1993). Cells having the dominant *cdc2* mutation, *cdc2-3w*, are defective in replication checkpoint control (Enoch and Nurse, 1990) but intact in damage checkpoint control (Al-Khodairy and Carr, 1992). This fact also indicates that the replication and damage checkpoint control systems are mutually independent.

In mammals, several factors are known to be involved in damage checkpoint control. Cells of ataxia telangiectasia are defective in damage checkpoint control in both G1 and G2 phases and show a reduced induction of p53 after γ -irradiation, suggesting that p53 induction is governed by ATM (Kastan *et al.*, 1992; Lu and Lane, 1993), the causative gene of this hereditary disease, which was recently cloned and shown to have a limited homology with the budding yeast and fission yeast checkpoint *rad* genes, *MEC1* and *rad3*⁺ (Savitsky *et al.*, 1995). p53 is a recessive oncoprotein and its mutation is associated with malignancy of many human tumor cells (Greenblatt *et al.*, 1994). Cells mutated for p53 are defective in γ -irradiation induced G1 arrest. p21 was identified as a potent inhibitor of Cdk2 (Xiong *et al.*, 1993), and subsequently found to be transcriptionally regulated by p53 (El-Deiry *et al.*, 1993; Dulic *et al.*, 1993). Consequently, this cascade was initially thought to be the mechanism for damage-induced G1 arrest. But, cells lacking p21 are only partially lost the ability to arrest in G1 (Deng *et al.*, 1995; Brugarolas *et al.*, 1995). In addition to the ATM-p53-p21 cascade (Macleod *et al.*, 1995), the regulation of Cdk4 by tyrosine phosphorylation plays a major role in DNA-damage induced G1 arrest (Terada *et al.*, 1995). The importance of this regulation in damage-induced G1 arrest was shown by loss of checkpoint function by the expression of unphosphorylatable Cdk4^{F17}. The cells expressing Cdk4^{F17} suffer severe chromosomal aberration after UV irradiation as a consequence of the inability to arrest in G1, demonstrating that a damage-responsive checkpoint defect is directly linked to a chromosome aberration in mammals. But, in contrast to the ATM-p53-p21 system, this checkpoint system is operative only during cell cycle entry from the stationary phase induced by contact inhibition and serum starvation (Jinno and Okayama, unpublished observation). Thus, at least two independent mechanisms are operated for damage checkpoint control in the G1 phase of mammalian cells.

In the G2 phase of mammalian cells, tyrosine phosphorylation of Cdc2 is required for DNA damage-dependent checkpoint arrest (Kharbanda *et al.*, 1994; Jin *et al.*, 1996). Cells expressing unphosphorylatable Cdc2^{A14F15} are partially defective in G2 arrest when

irradiated with X-ray, indicating that additional mechanisms are likely to be present, just like in G1.

In *A. nidulans*, like in other eukaryotes, the cell cycle target for damage checkpoint control is NIMX (Ye *et al.*, 1997). DNA damage induces rapid phosphorylation of Tyr 15 of NIMX with its inactivation but has little effect on NIMA kinase activity. Cells expressing unphosphorylatable mutant NIMX are deficient in damage checkpoint control.

Endoreplication checkpoint

Virtually all eukaryotic cells duplicate their genome only once per cell cycle. This rigid control is called endoreplication checkpoint and ensured by at least two restrictions in the cell cycle progression. The first, replication origins are activated only once per S phase. The second, S phase always follows the M phase of the previous cell cycle. Thus, endoreplication checkpoint differs from the former two checkpoints in a sense that these rigid controls are essential parts of the cell cycle progression system.

In fission yeast, the Cdc2-CyclinB complex plays a central role in endoreplication checkpoint control (Nurse, 1994). Inactivation of the Cdc2-Cyclin B kinase activity either by inactivation of Cdc2 itself or cyclin B, or by overexpression of Rum1, a Cdc2-Cyclin B inhibitor, causes the cell's entry into multiple rounds of S phase (Broek *et al.*, 1991; Moreno and Nurse, 1994; Hayles *et al.*, 1994; Correa-Bordes and Nurse, 1995). In these cases, an endoreplication checkpoint that restricts activation of DNA replication origins once a S phase seems to remain intact. Strangely enough, inactivation of the all known CyclinB in fission yeast cells renders the cells unable to initiate DNA replication (Fisher and Nurse, 1996; Martin-Castellanos *et al.*, 1996). It is postulated that a low activity of Cdc2-CyclinB complex is required for the initiation of S phase whereas high activity is necessary for the start of M phase. On the other hand, overexpression of Cdc18 induces multiple rounds of DNA replication without nuclear division (Nishitani and Nurse, 1995; Muzi-Falconi *et al.*, 1996), indicating that the regulation of Cdc18 activity might be required for the restriction of origin activation once a S phase. Regarding endoreplication checkpoint control, an intricate regulatory cascade among Cdc2-Cyclin B, Rum1 and Cdc18 has been postulated (Jallepalli and Kelly, 1996). In this cascade, Rum1 negatively regulates Cdc2-Cyclin B, which negatively regulates Cdc18, thereby ensuring origin activation once a S phase.

Concluding remarks

In conclusion, the activity of Cdc2-CyclinB complex is a key to all the checkpoint regulations stated above. The

ratio of Tyr 15 phosphorylation of Cdc2 may vary depending on a checkpoint regulation and on organisms. In mammals, the situation is more complex since there are many CDKs and cyclins. However, further studies in yeast will provide invaluable insights to us.

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