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Review

Apoptosis-like yeast cell death in response to DNA damage and replication defects

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Abstract

In budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeast and other unicellular organisms, DNA damage and other stimuli can induce cell death resembling apoptosis in metazoans, including the activation of a recently discovered caspase-like molecule in budding yeast. Induction of apoptotic-like cell death in yeasts requires homologues of cell cycle checkpoint proteins that are often required for apoptosis in metazoan cells. Here, we summarize these findings and our unpublished results which show that an important component of metazoan apoptosis recently detected in budding yeast—reactive oxygen species (ROS)—can also be detected in fission yeast undergoing an apoptotic-like cell death. ROS were detected in fission and budding yeast cells bearing conditional mutations in genes encoding DNA replication initiation proteins and in fission yeast cells with mutations that deregulate cyclin-dependent kinases (CDKs). These mutations may cause DNA damage by permitting entry of cells into S phase with a reduced number of replication forks and/or passage through mitosis with incompletely replicated chromosomes. This may be relevant to the frequent requirement for elevated CDK activity in mammalian apoptosis, and to the recent discovery that the initiation protein Cdc6 is destroyed during apoptosis in mammals and in budding yeast cells exposed to lethal levels of DNA damage. Our data indicate that connections between apoptosis-like cell death and DNA replication or CDK activity are complex. Some apoptosis-like pathways require checkpoint proteins, others are inhibited by them, and others are independent of them. This complexity resembles that of apoptotic pathways in mammalian cells, which are frequently deregulated in cancer. The greater genetic tractability of yeasts should help to delineate these complex pathways and their relationships to cancer and to the effects of apoptosis-inducing drugs that inhibit DNA replication.

Keywords: Apoptosis; Programmed cell death; DNA replication; Checkpoints; Replication forks; DNA damage

1. Introduction

Apoptosis, or programmed cell death (PCD), plays important roles in the development and survival of all metazoans. One role of apoptosis is to eliminate cells with irreparable DNA damage or replication defects that could lead to mutations that cause cancer and other diseases. The importance of this role is indicated by the frequent occurrence in cancer cells of mutations that inhibit the apoptotic machinery (reviewed in [1]). It has also been established that the induction of apoptosis underlies the therapeutic effects of many antitumor drugs, including those that damage DNA or

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inhibit DNA replication, and that defects in apoptosis can lead to drug resistance (reviewed in [2]). Therefore, understanding apoptotic pathways induced by DNA damage or replication inhibition is important to efforts to achieve a more comprehensive understanding of cancer and to more effectively treat this disease.

In recent years, research in this area has uncovered roles for DNA damage checkpoint and other cell cycle regulatory proteins in apoptosis, including apoptosis induced by DNA damage or replication inhibition. The major focus of this research has been on pathways that require the DNA damage checkpoint and tumor suppressor protein p53, which is frequently mutated in tumor cells. Less is known about how DNA damage can lead to apoptosis when p53 is inactivated, despite the fact that elimination by chemotherapeutic drugs of the large fraction of tumor cells with defects in p53 must proceed through p53-independent mechanisms. It is clear, however, that both p53-dependent and -independent apoptotic pathways are regulated by a number of DNA damage response and cell cycle regulatory proteins that, unlike p53, are conserved in yeasts. In fact, genetic experiments in yeasts provided the framework for understanding the checkpoint and cell cycle regulatory functions of these proteins in mammalian cells. Clearly, efforts to understand the apoptotic roles of these and other proteins would similarly benefit from the availability of yeasts and other genetically tractable organisms as experimental systems for studying apoptosis.

Whether apoptosis occurs in unicellular organisms such as yeasts and bacteria has been controversial, due in part to doubts about whether cell suicide programs could contribute to the evolutionary fitness of unicellular organisms. Unlike their growth in laboratory flasks, however, most natural populations of unicellular organisms do not grow in isolation. Instead, they form colonies, or, in the case of bacteria, large networks of cells called biofilms [3–6]. Under these conditions, a damaged or virus-infected cell can compromise the viability of other members of the community by, for example, consuming scarce nutrients or spreading an infection. In the context of the “selfish gene” model for the evolutionary selection of genomes rather than organisms, a cellular suicide program that eliminates these cells would help to ensure the viability and reproductive success of healthier members of the community harboring similar genomes.

A more substantive basis for skepticism concerning the notion of apoptosis in yeasts has been the failure to detect in these organisms several key elements of the apoptotic machinery found in metazoans. This has recently changed, however, with the discovery of a caspase-like molecule encoded by the budding yeast *YCA1* gene that functions very similarly to caspases in mammalian and other metazoan cells [7]. These remarkable similarities include an enzyme activity that cleaves fluorescent caspase substrates typically used to detect caspase activity in mammals, activation of this activity by a self-cleavage event similar to that which occurs during the activation of metazoan caspases, inhibition of caspase activity by the broad-range caspase inhibitor zVAD-fmk, and induction of caspase activity in conjunction with other markers of apoptosis by hydrogen peroxide, which also induces apoptosis in mammalian cells, and by a mutation in the *CDC13* gene [8], which causes the accumulation of DNA damage. Perhaps most importantly, changes in viability or in the ability to detect a standard marker of apoptosis associated with overexpression or deletion of *YCA1* in budding yeast clearly indicate that Yca1p contributes to an apoptotic-like programmed cell death in this organism [7].

In fact, there is now compelling evidence that even bacteria undergo an apoptotic-like cell death in response to DNA damage and other triggers. Indeed, one of the earlier observations of an apoptotic-like phenotype was made in *Escherichia coli* in the beginning days of molecular biology [9], several years before the modern concept of cellular suicide was first articulated [10]. Experiments designed to explore the bacterial DNA replication machinery unexpectedly showed that double-strand DNA (dsDNA) breaks induced in *E. coli* cells harboring a mutation in the recombination protein RecA, which is required for recombinational repair of these breaks, caused disintegration of the *E. coli* chromosome. This apoptosis-like phenomenon—which was not understood as such and has long since been forgotten by most molecular biologists—was only recently described by one of its discoverers, John Cairns, as a cell suicide program induced by irreparable DNA damage [11].

Although the nature of the requirement for mutational inactivation of RecA in this experiment is not clear, it remarkably presaged results of more recent experiments which suggest that the loss of function

of Rad51, a eukaryotic homologue of RecA that has retained RecA's role in recombinational repair, can contribute to DNA damage-induced apoptosis in metazoans [12,13]. Here we summarize recent published and unpublished findings concerning apoptosis or PCD in yeasts and other unicellular organisms and relate these findings to highly conserved DNA damage response pathways that are required for p53-independent apoptosis induced by DNA damage or replication defects in mammalian cells. Some of these findings point to connections between DNA replication, cell cycle regulation and apoptosis that may underlie the antitumor activity of drugs that damage DNA or inhibit DNA replication. These connections may be particularly amenable to investigation in yeasts.

2. Apoptosis in unicellular organisms other than yeasts

One of the first examples of PCD in unicellular organisms that was recognized as such involves the systematic destruction of the mother cell following sporulation of the eubacterium, *Bacillus subtilis*. Sporulation is triggered in *B. subtilis* by a nutrient-poor environment and results in an asymmetric cell division that produces an endospore and a mother cell. The mother cell engulfs the endospore, and, prior to release of the spore, is actively lysed [14,15]. Lysis of the mother cell may provide nutrients to support the final stages of spore formation, an energy-demanding process, or alternatively may simply remove an impediment to spore germination.

Programmed cell lysis has been observed in two additional eubacteria, *Streptococcus pneumoniae* and *E. coli*. In these cases, cell lysis was detected in response to treatment with antibiotics. A screen of transposable element insertion mutants in *S. pneumoniae* identified a number of genes required for cell death in response to penicillin [16], indicating that cell death in this case is an active process.

When *E. coli* is exposed to antibiotics or other growth inhibitors, a small percentage of cells, called persistors, remains viable. Mutants were identified that give rise to a higher percentage of persistors when treated with penicillin [17]. One of the loci was *hipAB*. Mutations in *hipAB* also increase cell survival following thymine starvation [18], or treatment with quinolone antibiotics that target DNA gyrase and topoisomerase [19], and can increase viability of heat shock mutants when exposed to elevated temperature [18]. These results indicate that in *E. Coli*, cell death in response to a variety of lethal agents is an active process.

As indicated previously, cell cycle arrest and cell death can also be induced in *E. coli* by DNA damage. This occurs through a series of steps reminiscent of checkpoint controls in eukaryotic cells [20]. One of the proteins activated by this checkpoint response is SulA, which blocks cell division. Prolonged inhibition of cell division as a result of excessive DNA damage leads to sustained SulA activation and cell lysis. Interestingly, *sulA* mutants are more resistant to DNA-damaging agents than are wild-type cells, implying that the primary role of SulA is not to facilitate DNA repair, but to promote cell death [21]. Therefore, the ability to discard damaged cells via the SulA pathway is clearly more important, in evolutionary terms, than any short-term growth advantage that might result from disruption of the *sulA* gene. This underscores the potential importance of cell death pathways in maintaining the stability and longevity of bacterial cell populations.

Upon starvation, the slime mold, *Dictyostelium discoideum*, aggregates to form a fruiting body that gives rise to two different cell types: dead cells that constitute the stalk and viable spores that reside at the head of the stalk [22]. During development of the stalk, many features of the dying cells mimic those observed during mammalian apoptosis [23,24]. Whether cells live or die can be influenced by genetic factors, providing additional support for the notion that stalk cell death is programmed [25–29].

3. Apoptosis in yeasts

3.1. Induction of yeast cell death by expression of mammalian pro-apoptotic proteins

Yeasts have proved to be powerful model systems for studying complex biological phenomena. Therefore, it is not surprising that they have been employed as model systems to study the functions of mammalian proteins involved in apoptosis

[30–32]. Based on initial failures to detect obvious orthologs of apoptosis-related proteins encoded by yeast genomes, yeasts were considered relatively "clean" systems for examining the effects of pro- and anti-apoptotic mammalian proteins on mitochondrial function, and perhaps for identifying novel apoptotic inhibitors.

Several early studies determined that expression of pro-apoptotic proteins in yeast can cause cell death. These included human Bax and Bak and the related *Caenorhabditis elegans* protein, Ced4 [33–35]. In addition to these proteins, expression of both mammalian caspases 1 and 3 in fission yeast also can lead to cell death [36]. Both caspases are processed normally, are fully active when expressed in fission yeast, and are capable of cleaving co-expressed anti-apoptotic Bcl-2 at predicted sites for caspase cleavage. With the discovery of a caspase-like activity in yeast, it is interesting to speculate that cell death induced by expression of mammalian caspases may occur via an intrinsic pathway activated by these heterologous proteins. This would suggest the feasibility of screening for inhibitors of caspase activity using an in vivo model system.

In fact, genetic screens have identified mitochondrial mutations and novel apoptosis inhibitors that inhibit death in yeast cells overexpressing Bax [37,38]. In one of these studies, genes encoding subunits of the F0F1–ATPase proton pump were identified as critically important for Bax function [38]. Consistent with these results, addition of oligomycin, a pharmacological inhibitor of the proton pump, significantly inhibited cell death in both yeast and mammalian cells [38]. Very recently, a similar approach led to identification of the conserved Ku70 protein—which plays an important role in dsDNA break repair in all eukaryotes—as an additional inhibitor of Bax in mammalian cells [39]. These results underscore the value of applying a genetically tractable model system to the analysis of cell death pathways.

It is also clear that co-expression of the anti-apoptotic proteins Bcl-2 or Bcl-xL in yeasts can rescue cells from the lethality caused by expressing either Bax or Bak. This does not imply the existence of an intrinsic cell death program, because in most cases this suppression is due to direct binding of the mammalian anti-apoptotic proteins to their mammalian apoptosis-promoting counterparts. However, there are at least two examples of mutant Bcl-xL molecules that are able to suppress Bax-mediated lethality without binding to Bax [40]. This clearly suggests that pathways downstream of Bcl-xL are conserved between yeasts and higher eukaryotes. Supporting this notion, expression of Bcl-xL [41] or Bcl-2 [42] can suppress cell death in yeast exposed to oxidative stress.

3.2. Apoptosis can be induced in yeasts independently of mammalian proteins

Some of the features of dying yeast cells expressing pro-apoptotic mammalian proteins are similar to those observed in higher eukaryotic cells undergoing apoptosis, which also suggests that, despite the failure to detect its components, yeasts might contain an intrinsic cell death pathway. Now it is clear that yeasts can undergo PCD independently of the expression of mammalian pro-apoptotic proteins [43]. The first clear evidence was obtained in studies of a budding yeast strain harboring a mutation in the *cdc48* gene, which encodes a protein required for vesicle trafficking [44]. This strain displayed a rapid loss in cell viability following shift to the restrictive temperature, which was accompanied by the appearance of many of the same markers for apoptotic cells detected in mammalian cells (see below for more details) [45].

Soon after the discovery that this *cdc48* mutant can undergo a form of PCD, other researchers detected other stimulants of apoptosis in budding yeast. For example, it was shown that treatment with acetic acid at low concentrations could induce apoptotic cell death [46–48]. Higher concentrations appeared to induce necrosis, a form of passive cell death. Treatment with hydrogen peroxide at low doses also induced an apoptotic-like cell death, similar to its effects in mammals [49]. Apoptosis was also observed in budding yeast exposed to high levels of mating pheromone [50], or that had been deleted of the *ASF1*/*CIA1* gene encoding a histone chaperone [51]. The human homologue of this protein (CIA1) interacts with the largest subunit of the transcription complex TFIID, which, in addition to its role in transcription, has been implicated in apoptosis in mammalian cells [52]. Features of apoptosis have also been observed in budding yeast cells harboring the *orc2-1* mutation in the origin recognition complex (ORC) required for initiation of DNA replication [53].

DNA damage also stimulates apoptosis in budding yeast cells. For example, UV radiation induces some features of apoptosis [54], and apoptosis was recently observed in cells accumulating DNA damage due to a mutation in the *CDC13* gene [8]. Similar to acetic acid, higher levels of UV radiation induce a more necrotic-like cell death. Thus, the apoptosis-like phenotype observed at lower doses of either acetic acid or UV radiation is not a general signature of cell death. Furthermore, exposure of budding yeast cells to lethal levels of a DNA-damaging antitumor agent causes the proteasome-dependent destruction of the DNA replication initiation protein, Cdc6 [55], the mammalian homologue of which is also destroyed by either the proteasome or caspases in mammalian cells undergoing apoptosis [55–57]. Thus, at least one of the proteins destroyed during apoptosis induced by DNA damage and other apoptotic triggers in mammals is similarly attacked in dying yeast cells.

Additional examples of apoptosis-like death in yeasts are provided in Section 4 of this review (below).

3.3. Similar markers of apoptosis in yeasts and mammals

Apoptosis in mammalian cells is distinguished by a number of phenotypic changes that occur during cell death. In addition to caspase activation, these include chromatin condensation and DNA fragmentation, production of reactive oxygen species (ROS), exposure of phosphatidylserine on the cell surface, and production of membrane-enclosed cell fragments called apoptotic bodies. Many of these features are also observed in association with PCD in yeasts. For example, PCD in yeast cells is accompanied by chromatin condensation and nuclear DNA fragmentation following shift to the restrictive temperature of budding yeast strains harboring the apoptosis-inducing *cdc48* [45] or *orc2-1* [53] mutations, or overexpression of mammalian pro-apoptotic proteins, or exposure to oxygen stress. Nuclear condensation was observed following expression of human Bax in both budding [40] and fission yeast [33,34]. Electron microscopic examination of nuclei from cells expressing Bax or Bak [33,34] or following treatment with 3 mM H_2O_2 [49] showed significant chromatin condensation along the nuclear membrane. Interestingly, cells treated with H_2O_2 contain small membrane-associated vesicles that might be analogous to membrane blebbing observed during mammalian apoptosis [49]. DNA of yeast cells undergoing PCD becomes fragmented as revealed by DNA-specific staining or TUNEL staining of nuclei, and this effect is substantially reduced in the presence of the protein synthesis inhibitor cycloheximide [49]. A requirement for protein synthesis to produce the apoptotic phenotype argues that apoptotic-like cell death is an active process. Although DNA chromatin laddering is not observed, pulsed-field gel electrophoresis of chromosomes from fission yeast cells undergoing apoptosis induced by expression of BAK indicates DNA cleavage can be confined to specific regions [33].

The production and accumulation of ROS formed as a by-product of mitochondrial activity occurs in both early and late stages of mammalian apoptosis [58]. In addition to their production in the mitochondria, ROS are also generated anywhere electron transport chains exist, including the endoplasmic reticulum and nuclear membrane. There is considerable evidence that ROS participates in signal transduction pathways that regulate cell death, as well as cell division and senescence. A causal role for ROS in apoptotic pathways is indicated by the fact that, in mammalian cells, production of ROS during apoptosis can occur upstream of other apoptotic events, such as mitochondrial membrane depolarization, cytochrome *c* release, activation of caspases, and nuclear DNA fragmentation.

Similar to mammals, ROS production is also observed in yeast cells undergoing PCD. This is the case, for example, in a temperature-sensitive *cdc48* mutant that also shows many other features of apoptosis when shifted to the restrictive temperature of 37 °C [49]. Induction of apoptosis in yeast with H_2O_2 [49], acetic acid [47], high levels of mating pheromone [50], or by overexpressing mammalian pro-apoptotic genes [49] also results in the production of ROS, which suggests that ROS are a common feature of PCD in yeast. Addition of free radical scavengers to yeast cultures prior to inducing apoptosis led to a significant reduction in lethality, as did incubation in an anaerobic environment, suggesting that ROS contributes to signaling in pathways leading to PCD [49].

Many of the p53-dependent and -independent apoptotic pathways in mammals converge on mitochondria and cause—in addition to ROS production—the release of cytochrome *c* and other proteins into the

cytoplasm. In fact, cytochrome *c* release is another key feature of apoptosis in budding yeast. For example, cytochrome *c* release occurs during apoptosis induced by Bax [59], acetic acid [48], mating pheromone [50], and deletion of the *ASF1*/*CIA1* gene [51]. Furthermore, mutational inactivation of pathways that lead to the production of cytochrome *c* inhibits apoptosis induced by acetic acid [48].

Another marker of apoptosis in metazoans is the appearance of phosphatidylserine residues on the surface of the plasma membrane. These moieties can be detected by annexin V, which binds to phosphatidylserine in the presence of Ca^{++}. Shortly following induction of PCD in budding yeast, spheroplasts incubated with annexin V show strong staining, suggesting that flipping of phosphatidylserine to the outer cytoplasmic membrane occurs, similar to what is observed in metazoans during apoptosis [45,49].

4. Checkpoints, cell cycle regulation and apoptosis in yeasts

4.1. Mammalian paradigms

Although p53 is confined to metazoans, other components of p53-dependent responses to DNA damage are conserved in all eukaryotes. This includes, for example, induction of the activity of members of the conserved phosphoinositide-3-kinase-related family of protein kinases—ATR, ATM, and DNA-dependent protein kinase (DNA-PK)—as well as the conserved Rad9-Rad1-Hus1 (9-1-1) complex (reviewed in [60]). The roles of most of these proteins in either p53-dependent or p53-independent DNA damage responses occur at an early step involving the sensing of DNA damage and/or stalled replication forks and the subsequent transduction of signals to downstream components of checkpoint or DNA repair pathways. The assembly of complexes containing these proteins on chromatin at sites of DNA damage or at stalled replication forks likely promotes the recruitment of additional substrates for ATR- or ATM-dependent phosphorylation and other proteins required for downstream checkpoint signaling or DNA repair. This includes, for example, Chk1 and Chk2, which are conserved serine-threonine kinases that function downstream of ATR and ATM. Ultimately, the p53-dependent and independent checkpoints that require these proteins inhibit the activity of cyclin-dependent kinases (CDKs) that drive the cell cycle forward.

In addition to their roles in DNA damage checkpoints and DNA repair, some of these conserved proteins also play specific roles in apoptosis, particularly in cells suffering irreparable DNA damage. For example, human Rad9 (hRad9) contains a region homologous to the Bcl-2 homology 3 (BH3) domain shared by Bax and other pro-apoptotic members of the Bcl-2 family of apoptosis regulators. hRad9 promotes apoptosis in the absence of p53 by interacting with the anti-apoptotic proteins Bcl-2 and Bcl-xL [61]. Apoptosis induced by the DNA-damaging agent methylmethane sulfonate can be suppressed by antisense knockdown of hRad9, indicating that the pro-apoptotic role of hRad9 can occur in response to DNA damage [61]. hRad9 is a substrate for c-abl phosphorylation in response to DNA damage, and this phosphorylation promotes the association of hRad9 with the anti-apoptotic protein Bcl-xL, thus inhibiting Bcl-xL's anti-apoptotic regulation of pro-apoptotic proteins like Bax [62].

c-abl activation is regulated by its phosphorylation by DNA-PK, as well as by ATM (reviewed in [63]). This places DNA-PK and ATM in pathways that regulate p53-independent apoptosis upstream of hRad9, in addition to their roles in p53-dependent apoptosis. As discussed above, the 70 kDa subunit of the heterodimeric Ku complex, which is a conserved component of DNA-PK, can also inhibit apoptosis by interacting with Bax. This interaction suppresses the translocation of Bax to mitochondria required for its pro-apoptotic function [39]. ATM also has been implicated in p53-dependent and -independent apoptosis through its ability to stabilize E2F1 [64], and it is required for the p53-independent apoptosis induced by the terminal foldback structure of the DNA parvovirus AAV [65]. Chk2 also participates in p53-independent apoptosis downstream of ATM via phosphorylation of the pro-apoptotic proteins PML [66] and E2F1 [67]. Chk2 may also participate in apoptosis independently of ATM via phosphorylation of p53 [68]. The authors of this latter study proposed a role for ATR in regulating Chk2 activation, which, if correct, would place ATR in the category of apoptosis-regulating proteins as well.

4.2. DNA damage checkpoint responses and apoptosis in yeasts

The budding and fission yeast homologues of many of the highly conserved proteins described above play roles in DNA damage checkpoints and DNA repair that are similar, if not identical, to their mammalian counterparts (reviewed in [69]). For example, sensing and responding to DNA damage or stalled replication forks requires the yeast homologues of ATM and/or ATR—Tel1p and Mec1p in budding yeast, and Rad3 in fission yeast. The yeast homologues of the 9-1-1 complex, as well as those of the Chk1 and Chk2 checkpoint kinases and the Ku70 and Ku80 components of DNA-PK are also required for DNA damage responses and have functions similar to their mammalian counterparts. In fact, the genetic framework for understanding DNA damage responses was first determined by studying the functions of these proteins in yeasts. In the context of the now clearly established phenomenon of PCD in yeasts, this raises the important question of whether some of the apoptotic pathways induced by DNA damage in mammals—especially pathways that operate independently of p53—are, like checkpoint responses and pathways leading to DNA repair, conserved in yeasts.

The recently described roles in apoptosis of Rad9 in humans [61] and of the other components of the highly conserved 9-1-1 complex (Hus1 and Rad1) in DNA damage-induced apoptosis in *C. elegans* [13,70] clearly suggest a conserved role for this complex in DNA damage-induced apoptosis. Consistent with this possibility, the BH3 domain of human hRad9 is conserved in fission yeast Rad9, and overexpression of fission yeast Rad9 in mammalian cells causes apoptosis [71]. Furthermore, overexpression of anti-apoptotic Bcl-2 family members in fission yeast can suppress the lethality of DNA-damaging agents [71]. These findings suggest that the function in DNA damage-induced apoptosis of Bcl-2 apoptosis regulators, including Rad9, is conserved in yeasts.

Other DNA damage response genes have also been implicated in PCD in yeasts. For example, in budding yeast, Mec1 (homologue of mammalian ATR and ATM and of fission yeast Rad3) is required for PCD induced by DNA damage generated when *cdc13* cells are grown at a restrictive temperature [8]. In addition, mutations in *MEC1* or deletion of the budding yeast *RAD9* gene —the product of which is required for DNA damage checkpoints downstream of Mec1, but is not equivalent to the products of the fission yeast and human Rad9 genes—can at least partly suppress the lethal effects of the apoptotic *orc2-1* mutation in the origin recognition complex [53,72].

Table 1
Conserved cell cycle and checkpoint proteins and their requirement for apoptotic pathways in yeasts

Mammals	*S. cerevisiae*	*S. pombe*
CDC2	Cdc28	**Cdc2**
ATM	Tel1	Tel1
ATR[a]	**Mec1**	**Rad3**
RAD9	Ddc1	**Rad9**
RAD1	Rad17	Rad1
HUS1	Mec3	Hus1
CHK2	Rad53	**Cds1**
BRCA1	**Rad9**	Rhp9/Crb2
DNA-PK	Ku70, Ku80	Ku70

Bold face indicates that a requirement has been established for apoptosis or an apoptotic phenotype induced by at least some conditions. Absence of bold face does not indicate absence of a role in promoting apoptosis. Instead, it indicates that the possibility of such a role has not yet been tested.

[a] ATR requirement for apoptosis has been inferred (see text).

A summary of mammalian and yeast DNA damage response and cell cycle regulatory proteins that have been implicated in promoting apoptosis is presented in Table 1. This table includes recent observations described in Section 5 below.

5. Apoptosis in yeasts associated with defects in DNA replication

5.1. DNA replication and apoptosis in budding yeast

Many antitumor drugs inhibit DNA replication by blocking the synthesis of precursor molecules (for example, hydroxyurea; HU) or by inhibiting replication fork progression (alkylating agents and other compounds that modify DNA). Although mechanisms by which these drugs induce apoptosis are poorly understood, their interference with DNA replication appears to be a common triggering factor.

In an effort to better understand connections between inhibition of DNA replication and drug mechanisms, a few years ago we screened a number of

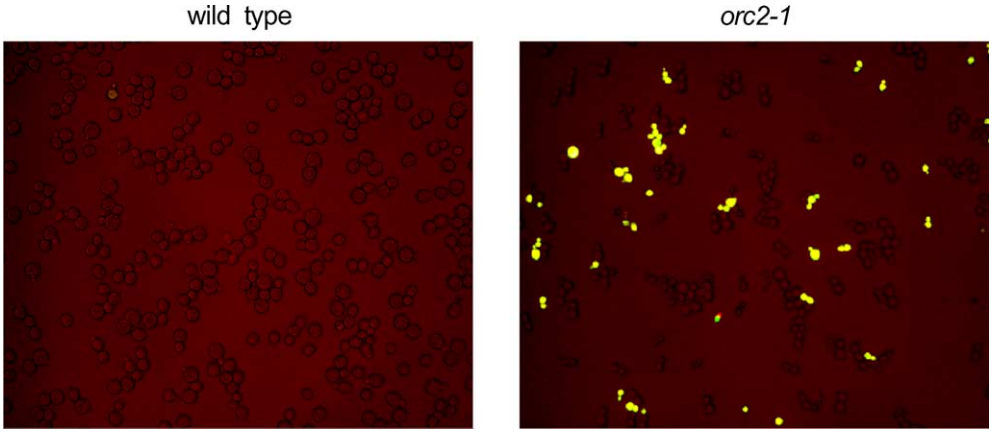

Fig. 1. Detection of ROS in *orc2-1* cells exposed to a DNA-damaging drug. Wild-type and *orc2-1* cells were exposed to the DNA-damaging agent methylmethane sulfonate for 6 h at 30 °C. Cells were then incubated at 30 °C for 80 min with the ROS-specific stain, 2′,7′-dichlorodihydrofluorescein diacetate, which penetrates live cells but does not fluoresce unless oxidized by ROS [90]. Stained cells were harvested and washed with citrate buffer (50 mM sodium citrate, pH 7.0). After resuspension in the same buffer, they were photographed with an epifluorescence microscope equipped with an appropriate filter. Under these conditions, ~0.1% of wild-type cells and 20–30% of *orc2-1* cells produce ROS. Data from Weinberger et al., manuscript in preparation.

budding yeast DNA replication mutants for their sensitivity to an experimental DNA-alkylating antitumor agent [73]. The temperature-sensitive *orc2-1* mutation in the ORC initiation complex was identified as one of several mutations that conferred much greater sensitivity to this drug compared to wild-type cells. The enhanced lethality of this and other DNA-damaging drugs in *orc2-1* cells is accompanied by the production of ROS and other features of apoptosis, which are also induced by shifting *orc2-1* cells to a nonpermissive temperature (Weinberger, Ramachandran, Feng, Marchetti, Huberman and Burhans, manuscript in preparation; Fig. 1). These additional features of apoptosis include chromatin condensation and fragmentation, which were previously detected by others in *orc2-1* cells shifted to a nonpermissive temperature [53]. The partial suppression of *orc2-1* temperature sensitivity by deletion of the *RAD9* gene [53] or by a mutation in *MEC1* [72] indicates that the lethality associated with the *orc2-1* mutation at high temperatures at least partly depends on checkpoints. In fact, our experiments indicate that the same mutation in *MEC1* that partly suppresses temperature sensitivity also suppresses the production of ROS in *orc2-1* cells. These findings establish a role for checkpoints in regulating the apoptotic phenotype of *orc2-1* cells. Interestingly, second site suppressors of the *orc2-1* mutation often arise spontaneously in budding yeast cells harboring this mutation [74], and attempts to introduce the *orc2-1* mutation into budding yeast cells frequently result in *rho*$^{-}$ mutations in the mitochondrial genome (Maki, personal communication). This suggests a tendency to suppress ROS and perhaps other aspects of apoptosis in this strain, similar to the tendency for suppression of apoptotic pathways observed in cancer cells.

An important function of ORC is to assemble on chromatin in G1 cells pre-replicative complexes (pre-RCs) required for establishing DNA replication forks in S phase [75]. ORC and other initiation proteins also participate in a putative G1-M checkpoint that restrains mitosis in G1 in some, but not all, genetic backgrounds [76]. *orc2-1* cells are partly defective in establishing pre-RCs at permissive temperatures, and this defect is even more pronounced at higher temperatures, as measured by DNAse I footprinting of ORC and other protein–DNA interactions that are characteristic of pre-RCs assembled at origins of replication [77]. High levels of DNA damage also alter interactions of initiation proteins with DNA at origins of replication in budding yeast cells (Trabold, Weinberger, Li, and Burhans, manuscript submitted). Furthermore, the sensitivity of *orc2-1* cells to either DNA damage or high temperatures is greatest when they are arrested in G1 [78]. Therefore, at least some

of the temperature- and DNA damage-induced lethality associated with the *orc2-1* mutation is related to loss of ORC function in G1 cells.

However, our experiments also indicate that these lethal effects require subsequent entry into S phase after the temperature shift or exposure to DNA damage in G1 cells. Furthermore, ROS production is greatest under these conditions, but is absent in cells allowed to enter S phase before a shift to high temperatures or exposure to DNA-damaging drugs, after replication forks have been established. This suggests that both lethality and the apoptotic phenotype are caused by entry into S phase with a reduced number of DNA replication forks due to the loss of ORC function in G1 (see [79] in this issue for a discussion of genomic instability associated with entry into S phase with a reduced number of replication forks). The apoptotic phenotype caused by the *orc2-1* mutation may not be related to a catastrophic mitosis produced by loss of the putative G1-M checkpoint that depends on the function of initiation proteins in G1 [76], because divided chromatin, which is a signature of mitotic catastrophe, is not always detected in *orc2-1* cells producing ROS.

How might entry into S phase with a reduced number of replication forks induce apoptosis? Studies by us and others established that intra-S phase DNA damage and DNA replication checkpoints are defective in *orc2-1* cells at permissive temperatures [78,80]. A recent study from the Gasser laboratory demonstrated that the checkpoint defects in *orc2-1* cells are related to the defect in pre-RC assembly caused by this mutation, which reduces the number of DNA replication forks in S phase below a threshold required to fully activate these checkpoints [74]. This reflects a general requirement for DNA replication forks, and thus indirectly initiation proteins, in checkpoints that inhibit CDK-dependent events required for progression through S phase and mitosis ([81,82]).

The absence of intact checkpoints in budding yeast cells with stalled replication forks leads to an irreversible arrest of DNA replication [83–85]. This may occur because in the absence of checkpoint restraint of CDK-dependent events, the cell cycle program regulated by CDKs continues unabated. This could lead to the premature inactivation of replication proteins that would normally be inactivated when DNA replication is complete. Consistent with this model, in mammals, abrogation of checkpoints in cells with stalled replication forks causes the inappropriate phosphorylation of DNA replication proteins (Seiler, Sun, and Burhans; manuscript in preparation) and their departure from recently replicated chromatin [86,87]. The abandonment of replication forks by these proteins may cause them to collapse (to undergo irreversible inactivating structural alterations).

Based on these considerations, we hypothesize that apoptosis in *orc2-1* cells is triggered by collapse of replication forks that occurs when the initiation defect caused by the *orc2-1* mutation causes the number of replication forks to decrease below the threshold required to activate checkpoints and/or rescue forks stalled by protein complexes or DNA structures. Replication fork collapse could be a general feature of apoptosis induced by the unscheduled disassembly of pre-RCs during apoptosis due to the proteolytic destruction of Cdc6 [55–57], which, in mammalian cells, has been shown to contribute to the apoptotic program [56,57]. This model may shed some light on the frequently observed activation during apoptosis of CDKs [88,89], some of which are directly or indirectly (through replication forks) inhibited by Cdc6 and other initiation proteins. It may also help to explain how replication-inhibiting drugs can induce apoptosis, particularly in cancer cells that harbor defective checkpoints.

5.2. ROS production in response to replication and checkpoint defects in fission yeast cells

Possible connections between DNA replication, checkpoints, and apoptosis have not previously been explored in fission yeast. As an initial step toward remedying this deficiency, we carried out a preliminary survey. In the remaining portions of Section 5, we summarize our conclusions based on this survey and on several follow-up experiments in fission yeast (Marchetti, Weinberger, Burhans, and Huberman, manuscript in preparation). As in our budding yeast experiments (above), we employed ROS as an indicator of possible apoptosis-like responses in fission yeast.

ROS production in living fission yeast cells is illustrated in Fig. 2. Wild-type cells and mutant (*dfp1-565*) cells with replication and checkpoint defects were exposed to two different fluorescent stains. The

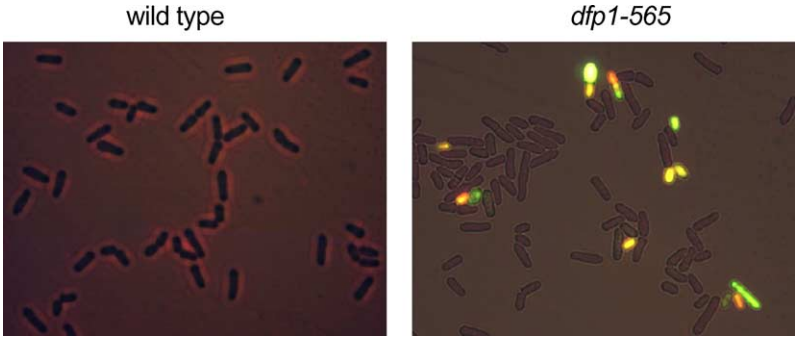

Fig. 2. ROS and propidium iodide staining in a DNA replication mutant strain of fission yeast. Living yeast cells in rich medium were incubated at 25 °C for 80 min with the ROS-specific stain, 2′,7′-dichlorodihydrofluorescein diacetate. The cells were then harvested and washed with citrate buffer as in Fig. 1. The cell pellets were resuspended in the same buffer supplemented with propidium iodide (PI; 10 μg/ml) and then viewed and photographed with an epifluorescence microscope equipped with a filter set capable of detecting both green (ROS) and red (PI) fluorescence. Where ROS and PI signals overlap, the resulting signal is yellow. The picture of wild-type cells is slightly out of focus. The pictures are from Marchetti et al., manuscript in preparation.

ROS-specific stain, 2′,7′-dichlorodihydrofluorescein diacetate—which was also employed in the budding yeast experiment depicted in Fig. 1—can penetrate living cells, but it does not fluoresce unless oxidized by ROS [90]. Its green fluorescence within cells indicates the presence of significant ROS concentrations. The other stain, propidium iodide (PI), cannot enter living cells, but it can enter dead cells in which membrane integrity has been lost. After it enters cells, it binds to nucleic acids within the cells and becomes fluorescent (red).

In Fig. 2, neither green nor red fluorescence is detectable in the wild-type cells. However, a portion of the *dfp1-565* cells, which contain a truncated version of the Dfp1 protein, display green and/or red fluorescence. Notice that some cells show pure green fluorescence, others display a mixture of red and green fluorescence and therefore appear yellowish, and yet others show mostly red fluorescence. These varieties of fluorescence are compatible with a sequence of events in which ROS production within intact cells precedes cell death accompanied by membrane permeabilization. There is a wide range of intensities of green fluorescence per cell; we do not yet know the significance of this variation.

The Dfp1 protein encoded by the *dfp1* gene is the fission yeast homologue of budding yeast Dbf4, which is the regulatory co-factor for the Cdc7 kinase—important for initiation of DNA replication (see [91] elsewhere in this issue of *Mutation Research*). The *dfp1-565* allele is an internal deletion of amino acids 13–240 of Dfp1. This N-terminal-proximal deletion removes a conserved motif (called “N”) from the protein, but does not prevent the protein from participating in the initiation of DNA replication [92,93]. Although the *dfp1-565* gene can complement a *dfp1* gene deletion and thus is largely competent for initiation of replication, versions of Dbf4 lacking domain N are defective in binding to replication origins [92,93]. In addition, versions of Dfp1 lacking domain N are only partially competent at activating the Hsk1 kinase [92]. Note that Hsk1 is the fission yeast version of the budding yeast Cdc7 kinase. Consequently, it is likely that replication origins fire inefficiently in *dfp1-565* cells, and these cells go through S phase with a reduced number of replication forks (similar to budding yeast cells bearing the *orc2-1* mutation; see above). Consistent with this possibility, fission yeast cells with Dfp1 lacking domain N have a reduced growth rate [92]. In addition to their growth rate defect, such cells also appear to exhibit checkpoint defects, as indicated by sensitivity to MMS and, in some cases, sensitivity to HU [92,93]. However, the experiment in Fig. 2 was carried out in the absence of MMS and HU. Consequently, the ROS production evident in Fig. 2 is likely the consequence either of the presumed mild replication defects in the *dfp1-565* mutant or of deficient checkpoint response to problems arising during S phase, such as stalled replication forks.

5.3. ROS generation by a variety of fission yeast replication, repair, recombination and checkpoint mutants

Because fluorescent microscopic results, such as those in Fig. 2, are difficult to quantitate, we employed flow cytometry to measure the number of cells displaying elevated ROS and PI staining. We surveyed strains with mutations in a variety of genes encoding proteins important for aspects of DNA metabolism. The results are summarized in Table 2.

Several interesting trends are suggested by the data in Table 2. First, it is evident that the number of cells positive for PI stain was in all cases roughly proportional to the number of cells positive for ROS stain. This finding is consistent with the observation of doubly-stained cells in Fig. 2. It suggests that ROS production (in living cells) is related to PI staining (in dead cells), and it is consistent with the hypothesis that ROS production precedes cell death.

Second, the most extensive ROS production among the tested strains was by certain mutant alleles affecting proteins required for initiation of replication (Orp2, Orp5, Cdc18 and Dfp1). The effects of *orp2* and *orp5* mutations were highly allele-specific, and in the case of *orp2* the specificity did not correlate with the severity of the replication phenotype. Initiation of replication at the restrictive temperature is more tightly blocked in the *orp2-2* strain than in the *orp2-7* strain [94], yet the *orp2-7* strain generated far more ROS than the *orp2-2* strain (Table 2). Thus ROS production in *orp2* mutants does not appear to be a direct consequence of inhibiting replication. Rather, it may be a consequence of permitting replication

Table 2
ROS production and PI staining in various fission yeast strains

Generic protein name	Relevant genotype	ROS	PI	Strain	Reference
Wild type	Wild type	−	−	501	[99,100]
Dbf4	*dfp1-564* (Δ13-193)	+++	++++	GBY564	[93]
Dbf4	*dfp1-565* (Δ13-240)	+++	+++	GBY565	[93]
Dbf4	*dfp1-566* (Δ183-191)	+++	+++	GBY566	[93]
Dbf4	*dfp1-AF6* (Δ460-545)	++++	+++	AFY6	[93]
Dbf4	*dfp1-AF7* (Δ377-545)	++++	+++	AFY7	[93]
Orc2	*orp2-2* (ts)	+	+	JLP208	[94]
Orc2	*orp2-7* (ts)	++++	+++	JLP216	[94]
Orc5	*orp5-H19* (ts)	+++	+++	H19	Gift from Murakami
Orc5	*orp5-H30* (ts)	+	+	H30	Gift from Murakami
Cdc6	*cdc18-K46*	++++	+++	Cdc18-K46	[101]
DNA pol ε	*cdc20-M10* (ts)	+	−	cdc20-M10	[102]
DNA pol ε	*cdc20-P7* (ts)	++	+	cdc20-P7	[102]
Ku70	*pku70*-Δ	−	ND	1773	[100]
DNA ligase IV	*lig4*-Δ	+	ND	K1	[100]
Damage-specific checkpoint mediator (Rad9 in budding yeast)	*rhp9*-Δ	+	ND	sp.391	[103]
Srs2 helicase	*srs2*-Δ	+	ND	SZ242	[104]
RecQ; Bloom's syndrome helicase	*rqh1*-Δ	++	ND	SZ243	[104]
Mre11	*rad32*-Δ	+	ND	EH62	[105]
Rad50	*rad50*-Δ	++	ND	EH19	[104]
Checkpoint-Rad proteins	*rad1*-Δ, *rad3*-Δ, *rad9*-Δ, *rad17*-Δ, *rad26*-Δ, *hus1*-Δ	−	−	Various	[104]
Chk1	*chk1*-Δ	−	ND	1098	[106]
Chk2/Cds1	*cds1*-Δ	−	ND	1561	[95]

Cells of the indicated strains were incubated at 30 °C (deletion mutants) or at 37 °C (truncation or ts mutants), then stained for ROS and, in some cases, for PI as in Fig. 2. The resulting green or red fluorescence was then quantitated for 10,000 cells by flow cytometry, and the number of cells with fluorescence intensity exceeding a threshold (chosen to exclude most wild-type cells) was determined. For ROS, the % cells with fluorescence exceeding the threshold are: − ≤ 0.2% < + ≤ 0.5% < ++ ≤ 1.2% < +++ ≤ 3.0% < ++++. For PI, the % cells with fluorescence exceeding the threshold are: − ≤ 1.0% < + ≤ 2.0% < ++ ≤ 5.0% < +++ ≤ 12.0% < ++++. Data from Marchetti et al., manuscript in preparation.

to proceed under compromised conditions (a suboptimal number of replication forks, for example, as in the case of the budding yeast *orc2-1* mutant; see above). As indicated above, the results with the Dfp1 N-terminal truncation mutants (*dfp1-564*, *565*, *566*) are also consistent with the possibility that progression through S phase with too few forks can trigger ROS. Table 2 also shows results for Dfp1 C-terminal truncation mutants (*dfp1-AF6*, *AF7*) that eliminate another conserved domain, "C" [92,93]. Like the N-terminal truncation mutants, these mutants are largely competent for DNA replication but are MMS-sensitive and appear to progress through S phase somewhat more slowly than wild-type cells [93]—again consistent with the possibility of a reduced number of replication forks.

DNA polymerase 1 (Table 2; *cdc20* gene) is also required for initiation of replication, but at a later stage. The fact that both tested alleles (*cdc20-M10* and *-P7*) generate relatively little ROS at restrictive temperature suggests either that pol 1 is not as important as the earlier initiation proteins for ROS production, or that we have not yet found and tested *cdc20* alleles that would generate larger amounts of ROS.

The results in Table 2 also suggest that replication initiation mutants, in general, produce more ROS than mutants in genes encoding proteins important for double-strand break repair (Ku70, DNA ligase IV, Rad32 [Mre11] and Rad50) or damage bypass and replication fork stability (Srs2, Rqh1 [RecQ]), at least under the non-stressed conditions employed here (no DNA-damaging agent; no fork-blocking agent). Mutations in additional genes in these pathways need to be tested before one can be confident of this trend. In addition, the fact that one set of data (the replication mutants) was generated with truncation or temperature-sensitive alleles, while the other set of data was generated with complete gene deletions, needs to be taken into account in comparing these results.

Finally, the results in Table 2 suggest that, in the absence of replication fork blockers, DNA-damaging agents, or additional mutations, checkpoint defects by themselves do not cause enhanced ROS production. The next question we asked was: do defective checkpoint pathways prevent, enhance, or have no effect on the ROS generated by replication initiation mutants?

5.4. Requirement in some cases, but not others, for intact checkpoint pathways for ROS production by replication mutants

As a first step toward evaluating the potential role of checkpoint pathways in the ROS production stimulated by fission yeast replication mutants, we created a set of double-mutant cell lines. Deletions of *rad3* or *cds1* were introduced into cells with the *orp5-H19*, *dfp1-564*, or *dfp1-566* mutations (see the description of these genes in Table 2). The Rad3 protein is the fission yeast homologue of mammalian ATR, and Cds1 is the fission yeast homologue of mammalian Chk2/Cds1. Rad3 is the central checkpoint kinase in fission yeast. After being activated by defects in DNA structure or problems in DNA replication (in cooperation with other proteins of the Checkpoint-Rad family; Table 2), it phosphorylates and thus activates one or both of the two downstream kinases, Chk1 and Cds1, depending on cell cycle position. Cds1 is activated in early S phase, and Chk1 is activated in late S phase and G2.

We found that the tendency of cells bearing the *orp5-H19* mutation to produce ROS and PI staining was not significantly affected by additional deletion of *rad3* or *cds1*. Thus ROS generation in the *orp5-H19* strain does not require Rad3 or Cds1.

In contrast, ROS and PI staining in the *dfp1-564* and *-566* strains were abolished by deletion of either *rad3* or *cds1*. In these cases, therefore, the Cds1 branch of the Rad3-dependent checkpoint pathway is required for ROS production. Determining whether the Chk1 branch is also required will require additional experiments.

5.5. Synergism of HU and checkpoint mutations in ROS production

Because the results in Table 2 indicated that mutations in replication proteins frequently lead to ROS production, we asked whether treating fission yeast cells with HU, which inhibits DNA replication, would generate ROS. Since wild-type fission yeast cells can survive for hours in 12 mM HU, we were not surprised to find that adding 12 mM HU to wild-type cells did not lead to ROS production. And since Checkpoint-Rad mutant cells (including *rad3*) are very sensitive to HU, it was not surprising to find that many

cells in each of the six Checkpoint-Rad mutant strains (including *rad3*) displayed ROS staining when treated with HU. What was surprising was the observation that *cds1* mutant cells, which are nearly as sensitive to HU as are Checkpoint-Rad mutants [95], generate no more ROS than do wild-type cells when exposed to HU.

Since Cds1 mediates only a branch of the pathways downstream of the Checkpoint-Rad proteins, we tested whether ROS production might be the responsibility of the other branch, which is mediated by Chk1. We found that *chk1* mutant cells generate no more ROS when incubated in HU than do wild-type or *cds1* mutant cells.

However, *cds1 chk1* double-mutant cells, in which both major checkpoint pathways downstream of the Checkpoint-Rad proteins are abolished, generate as much ROS after exposure to HU as do single mutants in any of the Checkpoint-Rad genes. Thus suppression of ROS in the presence of HU can be accomplished by either the Cds1-mediated or the Chk1-mediated checkpoint pathway. Only when both pathways are eliminated do fission yeast cells produce ROS in the presence of HU.

These results, combined with those described above, indicate that there are at least three different relationships between ROS production and checkpoint pathways in fission yeast. In one case (*orp5-H19*) ROS production is unaffected by checkpoint mutation. In one case (*dfp1* N-terminal truncations), ROS production requires functional checkpoint pathways. And in the situation discussed here (HU-stimulated ROS), ROS production requires combined knockout of both branches of the Checkpoint-Rad pathway. These multiple relationships were not anticipated, and they suggest that further study of apoptosis-like pathways in fission yeast may provide useful clues to understanding the even more complicated apoptotic pathways of animal cells.

5.6. Stimulation of ROS by unregulated CDK activity

The requirement for elimination of both Chk1 and Cds1 in order to generate ROS in the presence of HU reminded us of the requirement for elimination of both Chk1 and Cds1 to efficiently generate catastrophic mitosis in the presence of HU [96]. The reason elimination of both Chk1 and Cds1 is needed for efficient catastrophic mitosis in the presence of HU is that both checkpoint kinases activate pathways that ultimately lead to inhibition of Cdc2, the cyclin-dependent cell cycle kinase (CDK) of fission yeast. Inhibition of Cdc2 is necessary to prevent catastrophic mitosis when S phase is blocked by HU treatment [97]. When only the Chk1 or only the Cds1 pathway is eliminated, the remaining pathway can down-regulate Cdc2 sufficiently to inhibit catastrophic mitosis [96].

To test whether the same pathways might be responsible for ROS generation, we took advantage of the fact that both the Chk1-mediated and the Cds1-mediated pathways ultimately lead to inhibition of Cdc2, and they do this by stimulating the phosphorylation of tyrosine 15 in Cdc2. Phosphorylation of tyrosine 15 inactivates Cdc2. A mutant version of Cdc2 (encoded by *cdc2Y15F*), in which tyosine 15 is replaced by non-phosphorylatable phenylalanine, is constitutively active, displays premature mitosis even in the absence of HU [98], and is insensitive to inhibition by the Checkpoint-Rad checkpoint pathways in the presence of HU [97]. We found that a significant number (corresponding to +++ in Table 2) of *cdc2-Y15F* cells displays ROS staining even in the absence of HU, and this number is enhanced only about two-fold in the presence of HU.

This result indicates that deregulated Cdc2 and premature entry into mitosis can be major sources of ROS production in fission yeast cells. Interestingly, deregulated Cdk1 (the mammalian homologue of Cdc2) and catastrophic mitosis seem to be significant stimuli for apoptosis in mammalian cells as well [88].

Thus, our survey of ROS production in response to replication defects in fission yeast has revealed promising leads for further investigation of the relationships between replication defects and apoptosis. Our results suggest that apoptosis-like pathways in fission yeast mirror those in mammalian cells in several ways, including their complexity. Fortunately, despite the complexity revealed here, fission yeast and budding yeast (in which similar phenomena are evident; see above) are highly amenable to genetic and biochemical analyses and are much simpler than multicellular animals. Consequently, further studies of these phenomena in both yeasts are likely to produce rapid understanding in yeasts and important hints that will assist in understanding the corresponding phenomena in animals.

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References

[1] H.F. Ding, D.E. Fisher, Induction of apoptosis in cancer: new therapeutic opportunities, Ann. Med. 34 (2002) 451–469.
[2] R.W. Johnstone, A.A. Ruefli, S.W. Lowe, Apoptosis: a link between cancer genetics and chemotherapy, Cell 108 (2002) 153–164.
[3] D.G. Davies, M.R. Parsek, J.P. Pearson, B.H. Iglewski, J.W. Costerton, E.P. Greenberg, The involvement of cell-to-cell signals in the development of a bacterial biofilm, Science 280 (1998) 295–298.
[4] P.N. Danese, L.A. Pratt, R. Kolter, Biofilm formation as a developmental process, Methods Enzymol. 336 (2001) 19–26.
[5] L.A. Pratt, R. Kolter, Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili, Mol. Microbiol. 30 (1998) 285–293.
[6] L.A. Pratt, R. Kolter, Genetic analyses of bacterial biofilm formation, Curr. Opin. Microbiol. 2 (1999) 598–603.
[7] F. Madeo, E. Herker, C. Maldener, S. Wissing, S. Lachelt, M. Herlan, M. Fehr, K. Lauber, S.J. Sigrist, S. Wesselborg, K.-U. Frohlich, A caspase-related protease regulates apoptosis in yeast, Mol. Cell 9 (2002) 1–120.
[8] H. Qi, T.K. Li, D. Kuo, E.K.A. Nur, L.F. Liu, Inactivation of Cdc13p triggers MEC1-dependent apoptotic signals in yeast, J. Biol. Chem. 278 (2003) 15136–15141.
[9] J. Cairns, C.I. Davern, Effect of 32P decay upon DNA synthesis by a radiation-sensitive strain of *Escherichia coli*, J. Mol. Biol. 17 (1966) 418–427.
[10] J.F. Kerr, Shrinkage necrosis: a distinct mode of cellular death, J. Pathol. 105 (1971) 13–20.
[11] J. Cairns, A DNA damage checkpoint in *Escherichia coli*, DNA Repair (Amst.) 1 (2002) 699–701.
[12] Y. Huang, S. Nakada, T. Ishiko, T. Utsugisawa, R. Datta, S. Kharbanda, K. Yoshida, R.V. Talanian, R. Weichselbaum, D. Kufe, Z.M. Yuan, Role for caspase-mediated cleavage of Rad51 in induction of apoptosis by DNA damage, Mol. Cell Biol. 19 (1999) 2986–2997.
[13] E.R. Hofmann, S. Milstein, S.J. Boulton, M. Ye, J.J. Hofmann, L. Stergiou, A. Gartner, M. Vidal, M.O. Hengartner, *Caenorhabditis elegans* HUS-1 is a DNA damage checkpoint protein required for genome stability and EGL-1-mediated apoptosis, Curr. Biol. 12 (2002) 1908–1918.
[14] T.J. Smith, S.J. Foster, Characterization of the involvement of two compensatory autolysins in mother cell lysis during sporulation of *Bacillus subtilis* 168, J. Bacteriol. 177 (1995) 3855–3862.
[15] F.A. Nugroho, H. Yamamoto, Y. Kobayashi, J. Sekiguchi, Characterization of a new sigma-K-dependent peptidoglycan hydrolase gene that plays a role in *Bacillus subtilis* mother cell lysis, J. Bacteriol. 181 (1999) 6230–6237.
[16] R. Novak, J.S. Braun, E. Charpentier, E. Tuomanen, Penicillin tolerance genes of *Streptococcus pneumoniae*: the ABC-type manganese permease complex Psa, Mol. Microbiol. 29 (1998) 1285–1296.
[17] H.S. Moyed, K.P. Bertrand, hipA, a newly recognized gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis, J. Bacteriol. 155 (1983) 768–775.
[18] R. Scherrer, H.S. Moyed, Conditional impairment of cell division and altered lethality in hipA mutants of *Escherichia coli* K-12, J. Bacteriol. 170 (1988) 3321–3326.
[19] T.J. Falla, I. Chopra, Joint tolerance to beta-lactam and fluoroquinolone antibiotics in *Escherichia coli* results from overexpression of hipA, Antimicrob. Agents Chemother. 42 (1998) 3282–3284.
[20] G.C. Walker, The SOS response of *Escherichia coli*, ASM Press, Washington, D.C., 1996.
[21] L.J. Piddock, R.N. Walters, Bactericidal activities of five quinolones for *Escherichia coli* strains with mutations in genes encoding the SOS response or cell division, Antimicrob. Agents Chemother. 36 (1992) 819–825.
[22] W.F. Whittingham, K.B. Raper, Non-viability of stalk cells in *Dictyostelium*, Proc. Natl. Acad. Sci. U.S.A. 46 (1960) 642–649.
[23] S. Cornillon, C. Foa, J. Davoust, N. Buonavista, J.D. Gross, P. Golstein, Programmed cell death in *Dictyostelium*, J. Cell Sci. 107 (Pt 10) (1994) 2691–2704.
[24] T. Kawli, B.R. Venkatesh, P.K. Kennady, G. Pande, V. Nanjundiah, Correlates of developmental cell death in *Dictyostelium discoideum*, Differentiation 70 (2002) 272–281.
[25] J.H. Morrissey, P.A. Farnsworth, W.F. Loomis, Pattern formation in *Dictyostelium discoideum*: an analysis of mutants altered in cell proportioning, Dev. Biol. 83 (1981) 1–8.
[26] A. Amagai, S. Ishida, I. Takeuchi, Cell differentiation in a temperature-sensitive stalkless mutant of *Dictyostelium discoideum*, J. Embryol. Exp. Morphol. 74 (1983) 235–243.
[27] Z. Han, R.A. Firtel, The homeobox-containing gene Wariai regulates anterior-posterior patterning and cell-type homeostasis in *Dictyostelium*, Development 125 (1998) 313–325.
[28] H.L. Ennis, D.N. Dao, S.U. Pukatzki, R.H. Kessin, *Dictyostelium amoebae* lacking an F-box protein form spores rather than stalk in chimeras with wild type, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3292–3297.
[29] C.R. Thompson, R.R. Kay, Cell-fate choice in *Dictyostelium*: intrinsic biases modulate sensitivity to DIF signaling, Dev. Biol. 227 (2000) 56–64.
[30] C. Jin, J.C. Reed, Yeast and apoptosis, Nat. Rev. Mol. Cell Biol. 3 (2002) 453–459.

[31] S. Matsuyama, S. Nouraini, J.C. Reed, Yeast as a tool for apoptosis research, Curr. Opin. Microbiol. 2 (1999) 618–623.
[32] H. Zhang, J.C. Reed, Studies of apoptosis proteins in yeast, Methods Cell Biol. 66 (2001) 453–468.
[33] B. Ink, M. Zornig, B. Baum, N. Hajibagheri, C. James, T. Chittenden, G. Evan, Human Bak induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells, Mol. Cell Biol. 17 (1997) 2468–2474.
[34] J.M. Jurgensmeier, S. Krajewski, R.C. Armstrong, G.M. Wilson, T. Oltersdorf, L.C. Fritz, J.C. Reed, S. Ottilie, Bax- and Bak-induced cell death in the fission yeast *Schizosaccharomyces pombe*, Mol. Biol. Cell 8 (1997) 325–339.
[35] C. James, S. Gschmeissner, A. Fraser, G.I. Evan, CED-4 induces chromatin condensation in *Schizosaccharomyces pombe* and is inhibited by direct physical association with CED-9, Curr. Biol. 7 (1997) 246–252.
[36] S. Ryser, E. Vial, E. Magnenat, W. Schlegel, K. Maundrell, Reconstitution of caspase-mediated cell-death signalling in *Schizosaccharomyces pombe*, Curr. Genet. 36 (1999) 21–28.
[37] S. Matsuyama, Q. Xu, J. Velours, J.C. Reed, The Mitochondrial F0F1–ATPase proton pump is required for function of the proapoptotic protein Bax in yeast and mammalian cells, Mol. Cell 1 (1998) 327–336.
[38] Q. Xu, J.C. Reed, Bax inhibitor-1, a mammalian apoptosis suppressor identified by functional screening in yeast, Mol. Cell 1 (1998) 337–346.
[39] M. Sawada, W. Sun, P. Hayes, K. Leskov, D.A. Boothman, S. Matsuyama, Ku70 suppresses the apoptotic translocation of Bax to mitochondria, Nat. Cell Biol. 5 (2003) 320–329.
[40] W. Tao, C. Kurschner, J.I. Morgan, Modulation of cell death in yeast by the Bcl-2 family of proteins, J. Biol. Chem. 272 (1997) 15547–15552.
[41] S.R. Chen, D.D. Dunigan, M.B. Dickman, Bcl-2 family members inhibit oxidative stress-induced programmed cell death in *Saccharomyces cerevisiae*, Free Radic. Biol. Med. 34 (2003) 1315–1325.
[42] V.D. Longo, L.M. Ellerby, D.E. Bredesen, J.S. Valentine, E.B. Gralla, Human Bcl-2 reverses survival defects in yeast lacking superoxide dismutase and delays death of wild-type yeast, J. Cell Biol. 137 (1997) 1581–1588.
[43] K.U. Frohlich, F. Madeo, Apoptosis in yeast—a monocellular organism exhibits altruistic behaviour, FEBS Lett. 473 (2000) 6–9.
[44] M. Latterich, K.U. Frohlich, R. Schekman, Membrane fusion and the cell cycle: Cdc48p participates in the fusion of ER membranes, Cell 82 (1995) 885–893.
[45] F. Madeo, E. Frohlich, K.U. Frohlich, A yeast mutant showing diagnostic markers of early and late apoptosis, J. Cell Biol. 139 (1997) 729–734.
[46] P. Ludovico, F. Rodrigues, A. Almeida, M.T. Silva, A. Barrientos, M. Corte-Real, Cytochrome *c* release and mitochondria involvement in programmed cell death induced by acetic acid in *Saccharomyces cerevisiae*, Mol. Biol. Cell 13 (2002) 2598–2606.
[47] P. Ludovico, F. Sansonetty, M.T. Silva, M. Corte-Real, Acetic acid induces a programmed cell death process in the food spoilage yeast *Zygosaccharomyces bailii*, FEM Yeast Res. 3 (2003) 91–96.
[48] P. Ludovico, M.J. Sousa, M.T. Silva, C. Leao, M. Corte-Real, *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid, Microbiology 147 (2001) 2409–2415.
[49] F. Madeo, E. Frohlich, M. Ligr, M. Grey, S.J. Sigrist, D.H. Wolf, K.U. Frohlich, Oxygen stress: a regulator of apoptosis in yeast, J. Cell Biol. 145 (1999) 757–767.
[50] F.F. Severin, A.A. Hyman, Pheromone induces programmed cell death in *S. cerevisiae*, Curr. Biol. 12 (2002) R233–R235.
[51] M. Yamaki, T. Umehara, T. Chimura, M. Horikoshi, Cell death with predominant apoptotic features in *Saccharomyces cerevisiae* mediated by deletion of the histone chaperone *ASF1/CIA1*, Genes Cells 6 (2001) 1043–1054.
[52] T. Sekiguchi, T. Nakashima, T. Hayashida, A. Kuraoka, S. Hashimoto, N. Tsuchida, Y. Shibata, T. Hunter, T. Nishimoto, Apoptosis is induced in BHK cells by the tsBN462/13 mutation in the CCG1/TAFII250 subunit of the TFIID basal transcription factor, Exp. Cell Res. 218 (1995) 490–498.
[53] K. Watanabe, J. Morishita, K. Umezu, K. Shirahige, H. Maki, Involvement of RAD9-dependent damage checkpoint control in arrest of cell cycle, induction of cell death, and chromosome instability caused by defects in origin recognition complex in *Saccharomyces cerevisiae*, Eukaryot. Cell 1 (2002) 200–212.
[54] R. Del Carratore, C. Della Croce, M. Simili, E. Taccini, M. Scavuzzo, S. Sbrana, Cell cycle and morphological alterations as indicative of apoptosis promoted by UV irradiation in *S. cerevisiae*, Mutat. Res. 513 (2002) 183–191.
[55] F. Blanchard, M.E. Rusiniak, K. Sharma, X. Sun, I. Todorov, M.M. Castellano, C. Gutierrez, H. Baumann, W.C. Burhans, Targeted destruction of DNA replication protein cdc6 by cell death pathways in mammals and yeast, Mol. Biol. Cell 13 (2002) 1536–1549.
[56] C. Pelizon, F. d'Adda di Fagagna, L. Farrace, R.A. Laskey, Human replication protein Cdc6 is selectively cleaved by caspase 3 during apoptosis, EMBO Rep. 3 (2002) 780–784.
[57] H. Yim, Y.H. Jin, B.D. Park, H.J. Choi, S.K. Lee, Caspase-3-mediated cleavage of Cdc6 induces nuclear localization of p49-truncated Cdc6 and apoptosis, Mol. Biol. Cell (2003), published electronically 10.1091/mbc.E03-01-0029.
[58] C. Fleury, B. Mignotte, J.L. Vayssiere, Mitochondrial reactive oxygen species in cell death signaling, Biochimie 84 (2002) 131–141.
[59] S. Manon, B. Chaudhuri, M. Guerin, Release of cytochrome *c* and decrease of cytochrome *c* oxidase in Bax-expressing yeast cells, and prevention of these effects by coexpression of Bcl-xL, FEBS Lett. 415 (1997) 29–32.
[60] A.J. Osborn, S.J. Elledge, L. Zou, Checking on the fork: the DNA-replication stress-response pathway, Trends Cell Biol. 12 (2002) 509–516.
[61] K. Komatsu, T. Miyashita, H. Hang, K.M. Hopkins, W. Zheng, S. Cuddeback, M. Yamada, H.B. Lieberman, H.G. Wang, Human homologue of *S. pombe* Rad9 interacts with

BCL-2/BCL-xL and promotes apoptosis, Nat. Cell Biol. 2 (2000) 1–6.
[62] K. Yoshida, K. Komatsu, H.G. Wang, D. Kufe, c-Abl tyrosine kinase regulates the human Rad9 checkpoint protein in response to DNA damage, Mol. Cell Biol. 22 (2002) 3292–3300.
[63] C. Bernstein, H. Bernstein, C.M. Payne, H. Garewal, DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis, Mutat. Res. 511 (2002) 145–178.
[64] W.C. Lin, F.T. Lin, J.R. Nevins, Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation, Genes Dev. 15 (2001) 1833–1844.
[65] K. Raj, P. Ogston, P. Beard, Virus-mediated killing of cells that lack p53 activity, Nature 412 (2001) 914–917.
[66] S. Yang, C. Kuo, J.E. Bisi, M.K. Kim, PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds1/Chk2, Nat. Cell Biol. 4 (2002) 865–870.
[67] C. Stevens, L. Smith, N.B. La Thangue, Chk2 activates E2F-1 in response to DNA damage, Nat. Cell Biol. 5 (2003) 401–409.
[68] A. Hirao, A. Cheung, G. Duncan, P.M. Girard, A.J. Elia, A. Wakeham, H. Okada, T. Sarkissian, J.A. Wong, T. Sakai, E. De Stanchina, R.G. Bristow, T. Suda, S.W. Lowe, P.A. Jeggo, S.J. Elledge, T.W. Mak, Chk2 is a tumor suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner, Mol. Cell Biol. 22 (2002) 6521–6532.
[69] J. Qin, L. Li, Molecular anatomy of the DNA damage and replication checkpoints, Radiat. Res. 159 (2003) 139–148.
[70] A. Gartner, S. Milstein, S. Ahmed, J. Hodgkin, M.O. Hengartner, A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C. elegans*, Mol. Cell 5 (2000) 435–443.
[71] K. Komatsu, K.M. Hopkins, H.B. Lieberman, H. Wang, *Schizosaccharomyces pombe* Rad9 contains a BH3-like region and interacts with the anti-apoptotic protein Bcl-2, FEBS Lett. 481 (2000) 122–126.
[72] B.A. Desany, A.A. Alcasabas, J.B. Bachant, S.J. Elledge, Recovery from DNA replicational stress is the essential function of the S-phase checkpoint pathway, Genes Dev. 12 (1998) 2956–2970.
[73] M. Weinberger, L. Black, T.A. Beerman, J.A. Huberman, W.C. Burhans, Antitumor drugs and yeast cell cycle checkpoints, in: M.A. Hartwell (Ed.), Genomic Instability and Immortality in Cancer, Plenum Press, New York, 1997, pp. 189–194.
[74] K. Shimada, P. Pasero, S.M. Gasser, ORC and the intra-S-phase checkpoint: a threshold regulates Rad53p activation in S phase, Genes Dev. 16 (2002) 3236–3252.
[75] J.F. Diffley, DNA replication: building the perfect switch, Curr. Biol. 11 (2001) R367–R370.
[76] J.H. Toyn, A.L. Johnson, L.H. Johnston, Segregation of unreplicated chromosomes in *Saccharomyces cerevisiae* reveals a novel G_1/M-phase checkpoint, Mol. Cell. Biol. 15 (1995) 5312–5321.
[77] C. Santocanale, J.F. Diffley, ORC- and Cdc6-dependent complexes at active and inactive chromosomal replication origins in *Saccharomyces cerevisiae*, EMBO J. 15 (1996) 6671–6679.
[78] M. Weinberger, P.A. Trabold, M. Lu, K. Sharma, J.A. Huberman, W.C. Burhans, Induction by adozelesin and hydroxyurea of origin recognition complex- dependent DNA damage and DNA replication checkpoints in *Saccharomyces cerevisiae*, J. Biol. Chem. 274 (1999) 35975–35984.
[79] J. Sidorova, L. Breeden, Precocious G1/S transitions and genomic instability: the origin connection, Mutat. Res. 232 (2003) 5–19.
[80] K. Shirahige, Y. Hori, K. Shiraishi, M. Yamashita, K. Takahashi, C. Obuse, T. Tsurimoto, H. Yoshikawa, Regulation of DNA-replication origins during cell-cycle progression (in process citation), Nature 395 (1998) 618–621.
[81] J.A. Tercero, M.P. Longhese, J.F. Diffley, A central role for DNA replication forks in checkpoint activation and response, Mol. Cell 11 (2003) 1323–1336, and references therein.
[82] K. Labib, S.E. Kearsey, J.F. Diffley, MCM2-7 proteins are essential components of prereplicative complexes that accumulate cooperatively in the nucleus during G1-phase and are required to establish, but not maintain, the S-phase checkpoint, Mol. Biol. Cell 12 (2001) 3658–3667, and references therein.
[83] J.M. Sogo, M. Lopes, M. Foiani, Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects, Science 297 (2002) 599–602.
[84] M. Lopes, C. Cotta-Ramusino, A. Pellicioli, G. Liberi, P. Plevani, M. Muzi-Falconi, C.S. Newlon, M. Foiani, The DNA replication checkpoint response stabilizes stalled replication forks, Nature 412 (2001) 557–561.
[85] J.A. Tercero, J.F. Diffley, Regulation of DNA replication fork progression through damaged DNA by the Mec1/Rad53 checkpoint, Nature 412 (2001) 553–557.
[86] C. Feijoo, C. Hall-Jackson, R. Wu, D. Jenkins, J. Leitch, D.M. Gilbert, C. Smythe, Activation of mammalian Chk1 during DNA replication arrest: a role for Chk1 in the intra-S phase checkpoint monitoring replication origin firing, J. Cell Biol. 154 (2001) 913–923.
[87] D.S. Dimitrova, D.M. Gilbert, Temporally coordinated assembly and disassembly of replication factories in the absence of DNA synthesis, Nat. Cell Biol. 2 (2000) 686–694.
[88] M. Castedo, J.L. Perfettini, T. Roumier, G. Kroemer, Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe, Cell Death Differ. 9 (2002) 1287–1293.
[89] M. Guo, B.A. Hay, Cell proliferation and apoptosis, Curr. Opin. Cell Biol. 11 (1999) 745–752.
[90] C.P. LeBel, H. Ischiropoulos, S.C. Bondy, Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress, Chem. Res. Toxicol. 5 (1992) 227–231.
[91] B. Duncker, G. Brown, Cdc7 kinases (DDKs) and checkpoint responses lessons from two yeasts, Mutat. Res. 232 (2003) 21–27.
[92] K. Ogino, T. Takeda, E. Matsui, H. Iiyama, C. Taniyama, K.-I. Arai, H. Masai, Bipartite binding of a kinase activator

activates Cdc7-related kinase essential for S phase, J. Biol. Chem. 276 (2001) 31376–31387.
[93] A.D. Fung, J. Ou, S. Bueler, G.W. Brown, A conserved domain of *Schizosaccharomyces pombe dfp*1+ is uniquely required for chromosome stability following alkylation damage during S phase, Mol. Cell. Biol. 22 (2002) 4477–4490.
[94] J. Kiely, S.B. Haase, P. Russell, J. Leatherwood, Functions of fission yeast orp2 in DNA replication and checkpoint control, Genetics 154 (2000) 599–607.
[95] H. Murakami, H. Okayama, A kinase from fission yeast responsible for blocking mitosis in S phase, Nature 374 (1995) 817–819.
[96] J.-M. Brondello, M.N. Boddy, B. Furnari, P. Russell, Basis for the checkpoint signal specificity that regulates Chk1 and Cds1 protein kinases, Mol. Cell. Biol. 19 (1999) 4262–4269.
[97] N. Rhind, P. Russell, Tyrosine phosphorylation of Cdc2 is required for the replication checkpoint in *Schizosaccharomyces pombe*, Mol. Cell. Biol. 18 (1998) 3782–3787.
[98] K.L. Gould, P. Nurse, Tyrosine phosphorylation of the fission yeast *cdc*2$^+$ protein kinase regulates entry into mitosis, Nature 342 (1989) 39.
[99] J.M. Murray, C.L. Doe, P. Schenk, A.M. Carr, A.R. Lehmann, F.Z. Watts, Cloning and characterisation of the *S. pombe rad15* gene, a homologue to the *S. cerevisiae* RAD3 and human ERCC2 genes, Nucleic Acids Res. 20 (1992) 2673–2678.
[100] K.G. Manolis, E.R. Nimmo, E. Hartsuiker, A.M. Carr, P.A. Jeggo, R.C. Allshire, Novel functional requirements for non-homologous DNA end joining in *Schizosaccharomyces pombe*, EMBO J. 20 (2001) 210–221.
[101] E. Greenwood, H. Nishitani, P. Nurse, Cdc18p can block mitosis by two independent mechanisms, J. Cell Sci. 111 (1998) 3101–3108.
[102] G. D'Urso, P. Nurse, *Schizosaccharomyces pombe cdc20*$^+$ encodes DNA polymerase epsilon and is required for chromosomal replication but not for the S phase checkpoint, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 12491–12496.
[103] J. Willson, S. Wilson, N. Warr, F.Z. Watts, Isolation and characterization of the *Schizosaccharomyces pombe rhp9* gene: a gene required for the DNA damage checkpoint but not the replication checkpoint, Nucleic Acids Res. 25 (1997) 2138–2146.
[104] M.A. Marchetti, S. Kumar, E. Hartsuiker, M. Maftahi, A.M. Carr, G.A. Freyer, W.C. Burhans, J.A. Huberman, A single unbranched S-phase DNA damage and replication fork blockage checkpoint pathway, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 7472–7477.
[105] M. Tavassoli, M. Shayeghi, A. Nasim, F.Z. Watts, Cloning and characterisation of the *Schizosaccharomyces pombe rad32* gene: a gene required for repair of double strand breaks and recombination, Nucleic Acids Res. 23 (1995) 383–388.
[106] F. al-Khodairy, E. Fotou, K.S. Sheldrick, D.J. Griffiths, A.R. Lehmann, A.M. Carr, Identification and characterization of new elements involved in checkpoint and feedback controls in fission yeast, Mol. Biol. Cell 5 (1994) 147–160.