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Yeast Mutants As a Model System for Identification of Determinants of Chemosensitivity

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***Abstract*——The fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* have become valuable tools for the study of basic cellular functions of eukaryotic cells, including DNA repair mechanisms and cell cycle control. Since the major signaling pathways and cellular processes involved in cellular response to cytotoxic agents are conserved between yeasts and mammalian cells, these simple eukaryotic systems could be excellent models for the identification of molecular/cellular mechanisms of sensitivity to antitumor drugs. We describe relevant biological features of yeast cells and potential applications derived by their genetic manipulation. In particular, we have outlined the role of genes involved in repair processes and in checkpoint control, with specific reference to genes regulating radiation-sensitivity. Specific examples are provided concerning the use of both yeasts in understanding the mechanism of action of platinum compounds and topoisomerase inhibitors. The availability of the genomic sequence of these organisms as well as of new technologies (microarrays, proteomics) is expected to allow the identification of potential drug targets, since the drug discovery process is moving toward a genomic orientation. Among eukaryotic organisms, yeasts are suitable for easy genetic manipulations, and specific genetic alterations are exploitable for assessing the effects of chemotherapeutic agents with different mechanism of action. Although still at an early stage, this fast-moving field shows promise as a novel and potentially useful method for development of target-specific therapeutic approaches.**

I. Introduction

Alterations of genes involved in the cell cycle control and regulation of the cell death process are common genetic changes in human tumor cells. Defects in cell cycle checkpoints that monitor the completion of DNA replication, successful repair of DNA, and the accurate assembly of mitotic spindle contribute to genomic instability, a phenomenon implicated in tumor progression. There is increasing evidence that such alterations can influence the ability of a cell to respond to cytotoxic agents and alter the cellular fate (i.e., decision between cell cycle arrest/DNA repair or cell death). The fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* have become valuable tools for the study of basic cellular functions of eukaryotic cells, including DNA repair mechanisms and cell cycle control. The available evidence supports a high degree of conservation of the major signaling pathways

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and basic cellular processes among simple eukaryotic systems and mammalian cells. Genetic alterations of human tumor cells frequently involve genes that have functional homologs in model systems (Weinert and Hartwell, 1988; Rowley et al., 1992; Carr and Hoekstra, 1995). Thus, yeast could be an excellent model system for the identification of determinants of sensitivity to antitumor drugs, and in this review, we address the rationale for using yeast as a pharmacological tool in the identification of critical determinants of the cellular response to specific cytotoxic injuries. In addition, we summarize the yeast pathways relevant to cellular sensitivity to DNA-damaging agents. Particular emphasis has been given to radiation-sensitive mutants and genes, due to their involvement in pathways regulating the cell cycle or DNA repair. The definition of the molecular context that confers chemosensitivity or the identification of the appropriate target for pharmacological intervention could provide novel approaches to improve the efficacy of antitumor drugs. In addition, integrating basic research through genetic manipulation of model organisms, as well as new technologies designed to facilitate the identification of gene/gene products (e.g., microarray and/or proteomic technology) is expected to provide a more specific and powerful approach to validation of potential drug targets (Dyer et al., 1999). The aim of this review is to outline some of the recent developments in this fast moving field and to anticipate the potential application of future advancements in this area.

II. Integrating Model Systems for Antitumor Pharmacology Studies

Human tumor cells exhibit multiple alterations that have been extensively studied over the last decade. Several lines of evidence indicate that the impairment of any number of possible factors can influence cell sensitivity to antitumor drugs. One of the major difficulties in understanding the specific contribution of each single alteration to the drug-sensitive/-resistant phenotype of a tumor cell is the concomitant presence of multiple alterations. This fact prevents us from defining the biological/molecular background in which a certain drug exerts optimal effects. Since the yeast cell is less complex than a tumor cell, the yeast model system could help in obtaining a more general clarification of the determinants of sensitivity to drugs.

Over the last decade, efforts to develop new drugs effective in the treatment of tumors have been directed toward identifying agents capable of selectively killing tumor cells. This goal has been approached both by trying to improve specific features of the available agents, e.g., improvement of drug distribution to the tumor and/or solubility, and through design of agents targeting alterations thought to be specific for tumor cells, e.g., tyrosine kinase activation. Drug testing in vitro using well characterized human tumor cell lines has provided crucial insights into the mechanism of drug action (O'Connor et al., 1997), but a conclusive elucidation of the molecular background in which drug action is favored has not been reached.

In this context, the yeast system could help in a) clarifying the contribution of a specific gene in regulating sensitivity or resistance to a drug; b) interpreting tumor-specific action of some known antitumor agents; and c) providing novel approaches for identification of new drug targets. In this regard, the use of the genetic technique known as synthetic lethal screening appears promising (see below).

Apoptosis has been recognized as a major mode of cell death after exposure of mammalian cells to antitumor drugs (Hickman, 1996; Zunino et al., 1997). Recent studies indicate that key elements of the apoptotic pathway are present in the yeast cell as overexpression of pro-apoptotic genes including human bax and caspases result in a mode of cell death exhibiting features similar to apoptosis (DNA degradation; Greenhalf et al., 1996; Ryser et al., 1999). The relevance of apoptosis in a unicellular organism like yeast is controversial because the apoptotic process has been mainly implicated in the development and homeostasis of multicellular organisms. However, the possibility of activating the apoptotic pathway in yeast strains through ectopic expression of human genes could provide useful model systems for screening drugs aimed at specific mechanisms (Matsuyama et al., 1999).

III. Relevant Features of Yeast Cells

The fission yeast *S. pombe* and the budding yeast *S. cerevisiae* have been extensively used for studies of cell cycle regulation and DNA repair. Because mutations in cell cycle checkpoint/DNA repair genes are involved in the development of tumors, the cloning of human homologs of yeast genes could be a useful approach for a better understanding of genetic alterations relevant to malignant phenotype as potential targets for novel antitumor strategies. Recent reports suggest that yeast can be exploited for the identification of cellular determinants of chemosensitivity (Hafiz et al., 1995; Perego et al., 1996, 1997, 1998; Perego and Howell, 1997; Rieger et al., 1999; Munder and Hinnen, 1999). Indeed, there are many similarities between relevant physiological processes in yeast and mammalian cells as supported by the cloning of the human homologs of yeast genes (Bentley et al., 1996; Sanchez et al., 1997; Freire et al., 1998). Some physiological processes (e.g., mitosis, cell division) of *S. pombe* are more similar to those of human cells than those of the budding yeast *S. cerevisiae*. Like human cells, *S. pombe* has a distinct G_2 phase so a major checkpoint control is the decision to go from G_2 to M (Russell and Nurse, 1986). On the other hand, the budding yeast has a very short G_2 phase but a long G_1 and

the key transition is G_1/S (Guthrie and Fink, 1991), which is the major damage-responsive checkpoint in human cells. One nice feature of *S. cerevisiae* is the possibility of monitoring progression of cells through cell cycle by cell and nuclear morphology. In G_1 phase, cells are unbudded, and the bud emergence occurs during DNA synthesis; then in G_2 phase, the nucleus is positioned at the neck of the emerging bud, whereas cells progressing through mitosis are elongated and exhibit a bipolar nucleus. Yeast has many advantages as a model system including a small genome (1.4×10^7 bp/cell, about 200 times less than human cells) and a fast doubling time (approximately 2 h). During its life cycle, yeast exists in a haploid or diploid state (Fig. 1). This biological feature allows phenotype analysis of recessive mutations, which are normally masked in a diploid state by the wild-type allele. Assignment of distinct genes to different pathways can be obtained through epistasis analysis in which the phenotype of a double mutant strain is compared to the corresponding single mutant strain. Moreover, the genome of *S. cerevisiae* was sequenced by an international group of laboratories (Zagulski et al., 1998), and the *S. pombe* genome sequencing project is ongoing. The information available can be exploited for pharmacological approaches, as evidenced by the analysis of response of *S. cerevisiae* to an alkylating agent through simultaneous examination of thousands of transcripts by DNA chip technology (Jelin-

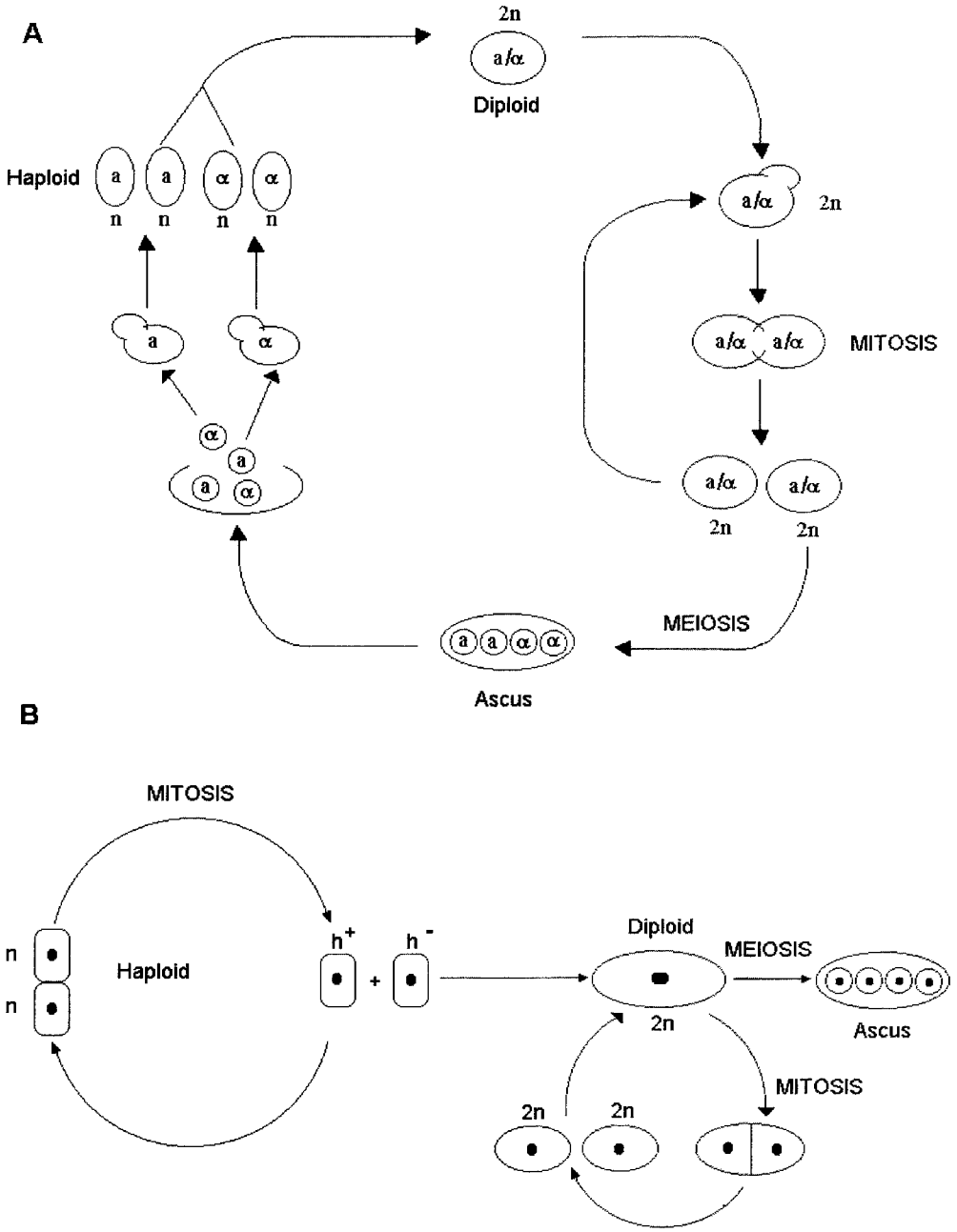

FIG. 1. Advantages of using yeast as a model system: *S. cerevisiae* (A) and *S. pombe* (B) life cycles.

sky and Samson, 1999). In addition, the availability of genome databases describing genes and predicted pathways of simple organisms could help in drug discovery programs (Karp et al., 1999).

Before discussing the individual *rad* mutants, it is worth mentioning that there is confusion with the numbering system used for *S. pombe* and *S. cerevisiae rad* mutants, as none of the mutants with the same number are structural or functional homologs. For the purposes of this review, we will use the standard conventions for which genes are italicized for both organisms (lower case for *S. pombe*, capital letter for *S. cerevisiae*), and proteins are in normal type with only the first letter capitalized. For both yeasts, mutants are italicized and in lowercase. We will refer to genes/proteins for *S. pombe* as *rad#*^{Sp}/Rad#^{Sp} and to *S. cerevisiae* as *RAD#*^{Sc}/Rad#^{Sc}.

IV. Use of Yeast Rad Mutants to Study Drug Mechanisms

Based on the characteristics of yeast cells described above, the use of these organisms may easily permit researchers to dissect out mechanisms that contribute to the multifactorial nature of drug resistance. For example, development of *S. pombe* strains resistant to cisplatin allowed investigation of specific mechanisms of drug resistance (Perego et al., 1996). The analysis of strains with specific mutations in genes affecting the radiation response suggested the utility of using mutants constructed on the same genetic background for studying the cellular response to platinum drugs (Perego et al., 1998). The use of this panel allowed rapid identification of genes relevant to cell ability to withstand the cytotoxicity of cisplatin. Our results indicate that most of *rad* genes influence cisplatin sensitivity. Thus, at least some of the same DNA repair pathways are involved in repair of both cisplatin and radiation damage. Some *rad* genes participate in cellular responses that are quite specific to the type of injury even within a class of drugs that are chemically closely related. These drugs included a) cisplatin analogs containing the diaminecyclohexane carrier ligand and differing in their oxidation state (Pt(IV) for tetraplatin, Pt(II) for oxaliplatin) and b) aminecyclohexylamine Pt(IV) complexes (i.e., JM216). For example, the *rad1*^{Sp}, *rad3*^{Sp} and *rad18*^{Sp} mutations produced very large changes in sensitivity to cisplatin, but had marginal or no effect on sensitivity to other platinum compounds (JM216 or tetraplatin). Moreover, the pattern of sensitivity between tetraplatin and oxaliplatin was markedly different, since most of the screened mutants were hypersensitive to oxaliplatin but not to tetraplatin. The differences in sensitivity between cisplatin and tetraplatin could be related to the different cell capability to recognize specific adducts. This feature might depend on the nature of the carrier ligand and/or time course of DNA lesion formation as expected based on differential lipophilicity. An analysis of the profile of sensitivity of allelic mutants (*rad 5/15-P*^{Sp}, *rad 3–136/19 M*^{Sp}) indicated that the different domains of the same protein may have different effects on the response to cisplatin. However, it is possible that these differences are due to leaky alleles because the studied mutants were obtained by mutagenesis. Although the precise biochemical changes arising from these alleles are not clearly defined, this study suggests that the recognition and/or repair of specific drug-induced DNA lesions are critical determinants of cell response to DNA-damaging agents.

Similarly, the use of *S. cerevisiae* mutants has been proposed as an integrating approach to drug discovery strategies (Hartwell et al., 1997). In particular, the developmental therapeutic program of the NCI/NIH has developed a yeast anticancer drug screening (the Seattle Project) in which the capability of thousands of compounds to inhibit the growth of selected *S. cerevisiae* strains is checked. The strains include single and double mutants carrying mutations in genes involved in DNA repair or cell cycle control including many of the *rad* genes. In particular, the project involves a panel of isogenic strains harboring several DNA repair mutations (including nucleotide excision, base excision, mismatch, postreplication, recombinational repair and reversal of O^6-alkylguanine) and cell cycle checkpoint mutations (including DNA damage and S phase checkpoint, spindle assembly checkpoint). The cytotoxicity profiles emphasize the importance of defects in DNA damage response as a determinant of chemosensitivity. On the basis of the pattern of cellular sensitivity, a variable selectivity of the tested agents was found for damage response defects. The relevance of specific molecular defects is likely dependent on the mechanism of action and suggest that the cellular context (i.e., type of mutations present in individual tumors) could influence the therapeutic outcome. Data obtained from this screening have been recently made available through a web site (Holbeck et al., 2000). The screening program is expected to identify more effective agents or novel molecules (Hartwell et al., 1997; Simon et al., 2000).

V. Rationale for Using Yeast As a Model System

Changes in cellular sensitivity to an antitumor drug can be the result of loss or gain of functions involved in defense mechanisms and/or drug-target interactions, but alterations influencing the ability of the cell to tolerate or recover from the primary lesion may have a critical role in determining the cellular fate. The use of yeast strains in which specific functions have been inactivated through mutation or targeted deletion could help in dissecting out the contribution made to the drug response by alterations described in mammalian cells. Panels of yeast strains carrying specific mutations can be a valuable tool for screening drugs in the attempt to

find an agent more cytotoxic to the mutant than to the wild-type yeast (Hartwell et al., 1997; Perego et al., 1998). Additional genetic methodologies could be used. In particular, synthetic lethal screening is a technique used in yeast to identify mutations that are not lethal per se, but are lethal in combination with another mutation. This technology has been proposed as a tool for new drug targets (Hartwell et al., 1997). Thus, a specific inhibitor of a cellular pathway that contributes to cell survival (e.g., DNA polymerase proofreading activity) could be used in a cell deficient in another pathway concomitantly participating in maintaining cell survival (e.g., mismatch repair). This procedure could be useful in killing cells with a precise defect because it provides other drug targets in addition to a specific mutation whose inactivation may produce an advantage in killing the tumor cell. Another example of this approach of synthetic lethal screening is the possible use of a topoisomerase II inhibitor in a cell carrying a mutation in the human homolog of $rad18^{Sp}$. The $rad18^{Sp}$ mutation is synthetically lethal with topoisomerase II mutant in *S. pombe*, and both gene products are implicated in chromatin organization (Verkade et al., 1999). Thus, the synthetic lethal approach could help in defining the particular combinations of pathways that would be reasonable inactivation targets.

In an effort to identify cellular pathways that are potential targets for drug discovery, genetic selection of peptide inhibitors has been performed in *S. cerevisiae* (Norman et al., 1999). This strategy consists of a) selection of peptides whose binding to unknown targets produces a phenotype, like mutations produce phenotypes by inactivating genes; b) identification of putative targets for the inhibitors (i.e., spindle checkpoint activation) by a combination of two-hybrid system and genetic dissection of the target pathways. This technique allows screening of different peptamers that are presented inside cells on the surface of an inert carrier protein. Genetic selection of inhibitory peptides could identify new targets for drug discovery by finding new elements of a specific pathway. Besides, target proteins inhibited by peptamers could be similarly inhibited with small organic molecules including drugs. Finally, the identification of peptamers with different potency could provide useful information about the correlation between structure and activity of a drug.

Human tumors are often defective in cell cycle checkpoint functions (Hagmann, 1999). Since cell cycle checkpoint pathways have been defined in yeast, this model system should provide further understanding of the critical determinants of cytotoxicity of DNA-damaging agents. In this review, particular emphasis has been given to radiation-sensitive mutants (*rad* mutants), since such mutations involve genes regulating the cell cycle or DNA repair. The original *rad* mutants of *S. pombe* were isolated due to their sensitivity to UV and/or ionizing radiation (Subramani, 1991). Additional *rad* and *rad*-related genes have been identified in further mutant screens in *S. pombe*. A large number of radiation-sensitive mutants have also been characterized in *S. cerevisiae*, based on their altered sensitivity to DNA-damaging agents, increased mutation rates, and defects in recombination and sporulation (Prakash, 1989; Friedberg, 1991; Game, 1993; Ivanov and Haber, 1997; Weinert, 1998). Some *rad* genes encode proteins directly involved in the enzymatic machinery used to recognize and process DNA lesions, incompletely replicated DNA, or recombination substrates. Others, referred to as "checkpoint *rad* " genes, encode proteins that relay signals from repair or replication intermediates to the cell cycle control machinery.

The complexity of cell signaling pathways resulting in cell death might be responsible for the heterogeneous cellular response of human tumors to antitumor agents. Identification of critical molecular defects in tumor cells, which underlie the sensitivity/resistance status of each tumor type, could allow a more rational use of antitumor therapies and identify novel therapeutic strategies. The model organisms described in this review provide a powerful tool for this approach. Therefore, we will begin with discussion of the known or proposed functions of the *rad* genes of *S. pombe* and *S. cerevisiae*.

VI. Molecular Pathways Regulating DNA Damage Responses

A. Checkpoint Control

Progression through the cell cycle can be halted by activation of surveillance mechanisms known as checkpoints that assure that cell cycle events occur in the proper sequence. The first genetic evidence for a checkpoint function was provided in *S. cerevisiae* (Weinert and Hartwell, 1988). Cell cycle checkpoints involve complex pathways that mediate the arrest of the cell cycle in response to alterations, which could result in loss of genomic integrity. Both in *S. pombe* and *S. cerevisiae*, these surveillance systems include the DNA replication and the DNA damage checkpoint, the existence of which is revealed by mutations that abrogate cell cycle arrest normally used for repair following damage. A comparison between *S. pombe* and *S. cerevisiae* indicates that checkpoint pathways are conserved through evolution, although subtle differences exist between different organisms. In higher eukaryotes, activation of cell cycle checkpoint control is accompanied by activation of repair processes or apoptosis (Hetts, 1998). In this regard, *p53* is a crucial gene that is not present in yeast. Defects in cell cycle control lead to genetic instability and neoplastic transformation (Almasan et al., 1995). Although mammalian cell cycle checkpoints may possess a higher complexity than those of yeast cells, the underlying checkpoint mechanisms share similar features. The replication checkpoint (S-M checkpoint) postpones mitosis until DNA replication is completed, and the DNA dam-

age checkpoint postpones mitosis until DNA damage is repaired. Yeast mutants that are unable to delay mitosis following damage are characterized by defects in genes whose products could at least in part be involved in sensing changes in DNA structures (Table 1; Subramani, 1991; Bentley and Carr, 1997; Al-Khodairy and Carr, 1992; Carr, 1997; Weinert, 1998). We have shown that these genes could participate in regulating sensitivity to cisplatin (Perego et al., 1998). Thus, a more complete understanding of their biological role could help in the clarification of the cellular drug response (Perego et al., 1998). In *S. pombe*, mitosis can be prevented in two genetically distinct situations, and a separation of the DNA replication checkpoint from the DNA damage checkpoint can be reached by creating phenotypically distinct mutant alleles in single genes (e.g., $rad1^{Sp}$ or $rad26^{Sp}$; Kanter-Smoler et al., 1995; Uchiyama et al., 1997), or by analyzing distinct mutants defective in one pathway or the other (e.g., $cds1^{Sp}$, $chk1^{Sp}$/$rad27^{Sp}$; Fig. 2; Lindsay et al., 1998; Martinho et al., 1998). A similar distinction exists in *S. cerevisiae*, in which the replication proteins $Pol\epsilon^{Sc}$, $Dpb11^{Sc}$, and $Rfc5^{Sc}$ are involved in sensing replication block and DNA damage during DNA synthesis (replication checkpoint; Araki et al., 1995; Sugimoto et al., 1997), whereas other genes including $RAD9^{Sc}$, $RAD17^{Sc}$, $RAD24^{Sc}$, $MEC3^{Sc}$, $DDC1^{Sc}$, $MEC1^{Sc}$, and $RAD53^{Sc}$ control the DNA damage checkpoint which has been dissected in specific responses (G_1/S, intra S, G_2M) depending on the cell cycle phase at which DNA damage occurs (Fig. 3; Longhese et al., 1998).

In *S. pombe*, the checkpoint *rad* mutants were originally identified as: $rad1^{Sp}$, $rad3^{Sp}$, $rad9^{Sp}$, $rad17^{Sp}$ (Subramani, 1991). These mutants are sensitive to both ionizing and ultraviolet radiation, as well as the DNA synthesis inhibitor hydroxyurea. In *S. cerevisiae* $RAD9^{Sc}$, $RAD17^{Sc}$, $RAD24^{Sc}$, $MEC1^{Sc}$, and $MEC3^{Sc}$ are required for checkpoint control (Carr and Hoekstra, 1995). The $rad1^{Sp}$ gene encodes a protein with limited similarity to *Ustilago maydis* REC1, which is an exonuclease. The $rad1^{Sp}$ mutants are radiosensitive because they fail to delay mitosis until repair of DNA damage has been completed, and, in contrast to the homolog $RAD17^{Sc}$, they are also deficient in the S-M checkpoint control (Rowley et al., 1992). $RAD17^{Sc}$ and $rad1^{Sp}$ are similar to the proliferating cell nuclear antigen (PCNA[2]; Thelen et al., 1999). The role of the $rad1^{Sp}$ gene in checkpoint control can be clarified based on its homology to REC1 and $RAD17^{Sc}$. In particular, an analysis of DNA damage processing in a specific genetic background suggests that DNA damage can occur as a consequence of DNA degradation supporting a role for an exonuclease (Lydall and Weinert, 1995). Processing of damage could lead to cell cycle arrest because checkpoint proteins send a signal for arrest, or damage itself generates a structure that sends a signal (Lydall and Weinert, 1995). The human homolog of $rad1^{Sp}$ maps to 5p14-p13.2, a region that contains tumor suppressor genes (Dean et al., 1998). Two alternative splice variants have been found in humans, one of which has exonuclease activity and has been speculated to be involved in recognition and processing of damage (Parker et al., 1998).

$Rad3^{Sp}$ and $Mec1^{Sc}$ are involved in both DNA damage and replication checkpoint pathways. $Rad3^{Sp}$ and $Mec1^{Sc}$ belong to a family of proteins with homology to "lipid kinases", or phosphatidylinositol 3-kinases, which include $Tel1^{Sc}$, $Tel1^{Sp}$ (Matsuura et al., 1999), DNA-dependent protein kinase (DNA-PK), human ATR (*ataxia telangiectasia* related), and ATM (*ataxia telangiectasia* mutated) (Hunter, 2000). ATM is one of the major upstream regulators of the p53 response to ionizing radiation-induced damage (Hawley and Friend, 1996). ATM-deficient cells have a complex phenotype and may have alterations both in DNA repair and cell cycle checkpoints. ATM has higher homology with $Tel1^{Sc}$ protein than with $Mec1^{Sc}$, and $rad3^{Sp}$ is homologous to the human gene ATR. Therefore, it is likely that in human cells several $rad3^{Sp}$ homologs regulate the DNA damage checkpoint. $Rad3^{Sp}$ acts through its associated protein kinase activity (Bentley et al., 1996). Similarly, the $Mec1^{Sc}$ protein phosphorylates and likely activates specific substrates including $Rad53^{Sc}$, $Rad9^{Sc}$ and $Ddc1^{Sc}$ (Sanchez et al., 1997; Emili et al., 1998; Paciotti et al., 1998). $Rad3^{Sp}$ has been implicated in recognition of specific DNA or protein-DNA structures in which it may be modulated by association with other checkpoint proteins (Bentley et al., 1996). Similarly, another $Rad3^{Sp}$ family member, DNA-PK, is activated in response to DNA damage in association with DNA binding subunits (Hartley et al., 1995; Jeggo et al., 1995). The $rad9^{Sp}$ gene product shows similarity to $DDC1^{Sc}$ (Murray et al., 1991; Longhese et al., 1997), and a human homolog was recently cloned (Lieberman et al., 1996). Since hRAD9 can rescue *S. pombe* cell cycle delay in response to incomplete DNA

TABLE 1
Pathway defects, known biochemical functions, and homologs of relevant rad and rad-related yeast mutants: checkpoint mutants

Mutants		Functions	Human Homologs
S. pombe	*S. cerevisiae*		
rad1	RAD17	Exonuclease	RAD1
rad3	MEC1	Lipid kinase domain	ATR, ATM
rad9	DDC1	G_2 arrest	HRAD9
rad17	RAD24	Nucleotide binding site	RAD17
rad26	none	S phase	None
chk1 (rad27)[a]	CHK1	Kinase	CHK1
rhp9 (crb2)[a]	RAD9	DNA damage checkpoint	None
rad24/rad25	BMH1-2	Phosphoserine binding	14-3-3
hus1	MEC3?	Rad1/rad9 interacting	HUS1
cds1	RAD53	Kinase	CDS1/CHK2

[a] Allelic mutants are indicated in parentheses.

[2] Abbreviations: PCNA, proliferating cell nuclear antigen; DNA-PK, DNA-dependent protein kinase; ATR, *ataxia telangiectasia* related; ATM, *ataxia telangiectasia* mutated; NER, nucleotide excision repair; RPA, replication protein A.

TABLE 2
Pathway defects, known biochemical functions, and homologs of relevant rad and rad-related yeast mutants: nucleotide excision repair mutants

Mutants		Functions	Human Homologs
S. pombe	*S. cerevisiae*		
NER: classical pathway			
rad13	RAD2	Nuclease	ERCC5
rad15 (rad5)	RAD3	Helicase	ERCC2
ERCC3	RAD25	Helicase	ERCC3
rad 16 (rad10, rad20, swi9)[a]	RAD1	Nuclease subunit	ERCC4
Swi10	RAD10	Nuclease subunit	ERCC1
NER: alternative pathway			
rad2	RAD27	Endonuclease	RAD2
UVDE	none	Endonuclease	None
rad18	RHC18	Chromosome structure	None

[a] Allelic mutants are indicated in parentheses.

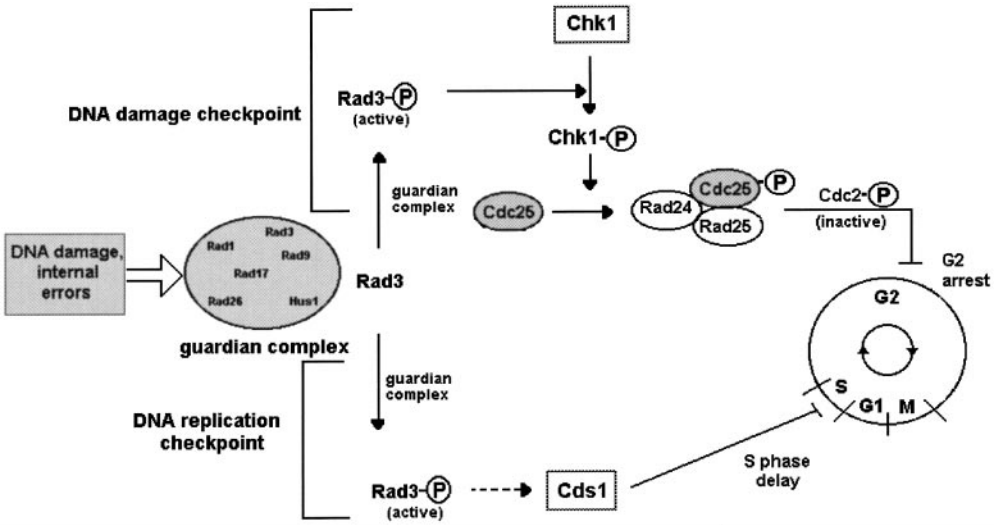

FIG. 2. Model for interplay between *rad* and *rad*-related genes in the fission yeast checkpoint pathway.

replication, but not to DNA damage, it appears that the two pathways may have diverged between yeast and humans (Lieberman et al., 1996). The *rad17*^{Sp} and *RAD24*^{Sc} genes encode nuclear proteins carrying an ATP binding site with homology to DNA replication protein replication factor C/activator 1, which binds DNA at strand breaks and is required to load DNA polymerases onto primed DNA templates during S phase (Griffiths et al., 1995; Waga and Stillman, 1998). This feature suggests the possibility of an association with replication structures and a role in DNA damage recognition. Mapping of the human homolog of *rad17*^{Sp} has shown that the human locus contains tumor suppressor genes (Dean et al., 1998). Rad26^{Sp} is presumed to function in S phase-specific DNA-damage responses, it has been shown to associate with Rad3^{Sp}, and it exhibits Rad3^{Sp}-dependent phosphorylation (Al-Khodairy et al., 1994; Edwards et al., 2000). Hus1^{Sp} acts for all DNA integrity checkpoints with other checkpoint *rad* genes (Dean et al., 1998; Kostrub et al., 1998; Caspari et al., 2000). Recently, *Hus1-B*^{Sp}, likely a homolog of *MEC3*^{Sc}, has been shown to form with Rad9^{Sp} and Rad1^{Sp}, a complex that, based on structural considerations, has been proposed to be a PCNA-like complex (St. Onge et al., 1999; Caspari et al., 2000). *Hus1-B*^{Sp}, a nuclear protein, is under-phosphorylated in its basal state. Phosphorylation is increased following irradiation (Caspari et al., 2000). Interestingly, due to its genomic localization (7p13-p12), the human HUS1 gene has been proposed as a candidate tumor suppressor for ovarian carcinogenesis (Dean et al., 1998; Kostrub et al., 1998).

Additional *S. pombe* genes, *chk1*^{Sp}/*rad27*^{Sp}, *rad24*^{Sp} and *rad25*^{Sp} are involved in checkpoint mechanisms (Walworth et al., 1993; Al-Khodairy et al., 1994; Ford et al., 1994). The *chk1*^{Sp}/*rad27*^{Sp} (checkpoint kinase) gene encodes a serine/threonine protein kinase required for G_2 arrest after DNA damage, but not for S phase arrest. The Chk1^{Sp} response is cell cycle-specific since radiation damage induces Chk1^{Sp} activation/phosphorylation in late S and G_2 (Martinho et al., 1998). Cells lacking Chk1^{Sp} are hypersensitive to a number of DNA-damaging agents including camptothecins (Wan et al., 1999). A human homolog of *chk1*^{Sp}, hChk1, has recently been reported (Sanchez et al., 1997). Human Chk1 activity is expressed at the S to M transition, is independent of ATM function, and is thought to be required for the

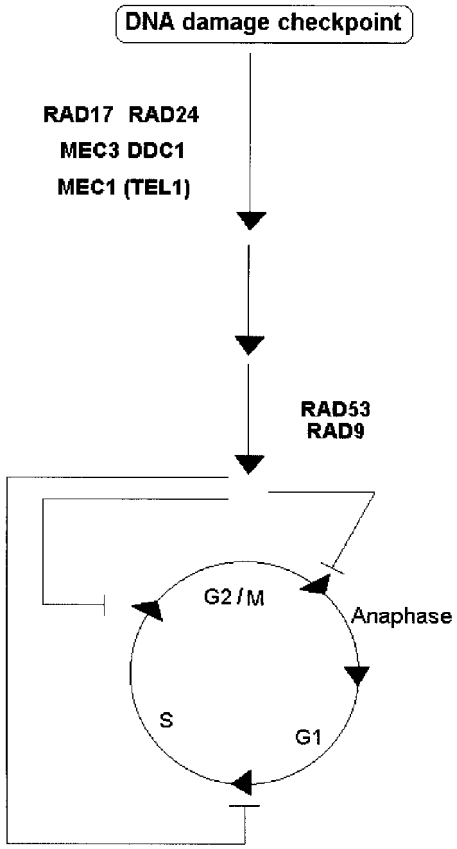

FIG. 3. DNA damage checkpoint pathways in budding yeast.

G_2/M checkpoint in human fibroblasts (Kaneko et al., 1999). hCHK1 may be involved in the DNA damage checkpoint through phosphorylation of hCDC25 (a mitosis-activating phosphatase) on serine 216 (Kaneko et al., 1999). Inhibition of hCHK1 specifically abrogates G_2 checkpoint, thus resulting in sensitization of p53-defective cancer cells to DNA-damaging agents, without cytotoxic effects on normal cells (Suganuma et al., 1999). Recently, a *S. cerevisiae* homolog of *chk1*^{Sp} has been identified (Sanchez et al., 1999).

Also involved only in the damage response is *rhp9*^{Sp} (Wilson et al., 1997) the homolog of *RAD9*^{Sc}. In *S. cerevisiae*, the DNA damage-induced G_2 arrest and its duration are highly dependent on *RAD9*^{Sc} (Weinert and Hartwell, 1988). *RAD9*^{Sc} blocks the entry into M if DNA is broken and acts in maintaining genomic stability. Other checkpoint *rad* mutants include *rad24*^{Sp}, which is defective in the mitotic arrest following damage, and enters mitosis prematurely during normal growth (Ford et al., 1994). Both *rad24*^{Sp} and *rad25*^{Sp} mutants are deficient in genes homologous to the 14-3-3 proteins (Ford et al., 1994).

There are relevant differences between *S. pombe* and *S. cerevisiae* in the checkpoint pathway discussed above (Carr and Hoekstra, 1995). While *rad1*^{Sp} and *rad17*^{Sp} are involved both in the DNA damage and replication checkpoint, the respective *S. cerevisiae* homologs *RAD17*^{Sc} and *RAD24*^{Sc} are involved only in the DNA damage checkpoint. In addition, whereas *RAD53*^{Sc} is required for all checkpoint pathways, the *S. pombe* homolog *cds1*^{Sp} appears to be implicated mainly in the replication checkpoint.

Different mechanisms cause cell cycle arrest in *S. cerevisiae* and *S. pombe* following damage. In *S. cerevisiae* cell cycle arrest involves the nuclear protein *Pds1*^{Sc}, which normally is degraded by a set of proteins that promotes anaphase, but in the presence of damage is phosphorylated in a *MEC1*/*RAD9*-dependent/*RAD53*-independent manner and blocks anaphase (Cohen-Fix and Koshland, 1997). Chk1^{Sc} functions in maintaining the abundance of Pds1^{Sc} through its stabilization (Sanchez et al., 1999). In fission yeast and mammalian cells, mitotic arrest following damage requires inhibitory cyclin-dependent kinase phosphorylation controlled by Chk1^{Sc} (Rhind et al., 1997). In *S. cerevisiae*, inhibition of G_1/S phase transition after damage in G_1 has been proposed to result from inhibition of *CLN1–2*^{Sc} (G_1 cyclins) transcription due to phosphorylation of the transcription regulator Swi6^{Sc} by Rad53^{Sc} (Sidorova and Breeden, 1997).

In *S. pombe*, the complex interaction among the six main checkpoint genes (*rad1*^{Sp}, *rad3*^{Sp}, *rad9*^{Sp}, *rad17*^{Sp}, *rad26*^{Sp}, *hus1*^{Sp}) and the other related genes (*rad24*^{Sp}, *rad25*^{Sp}, *rad27*^{Sp}, *rhp9*^{Sp}) has not been completely elucidated. The products of these genes may function to stabilize replication complexes when DNA is damaged or to stabilize stalled replication forks when DNA synthesis is inhibited. A complex of the six main proteins could operate as a "guardian" that detects changes in DNA structure and generates signals that activate the replication and DNA damage checkpoint (Fig. 2). On the other hand, the Rad3^{Sp}-Rad26^{Sp} complex can respond to DNA damage independently of the other checkpoint proteins (Edwards et al., 1999). Such signals are likely transduced to the mitotic apparatus through proteins including Chk1^{Sp}/Rad27^{Sp}, Rad24^{Sp}, and Rad25^{Sp}. The mechanism linking S phase and mitosis involves Cds1^{Sp}, which has an S phase-specific function, since it is activated by DNA damage only during this phase (Martinho et al., 1998). This activation is dependent on the main six proteins, including Rad26^{Sp}, which physically interacts with Cds1^{Sp} (Lindsay et al., 1998). It is possible that substrates of the Cds1^{Sp} kinase include components of the replication apparatus. Thus, during S phase, the Cds1^{Sp}-mediated response may prevent replication of new replicons and promote collapse of replication fork culminating in irreparable damage (Martinho et al., 1998). There appears to be a direct link between Rad3^{Sp} and the two downstream kinases as suggested by the ability of Rad3^{Sp} to phosphorylate Cds1^{Sp} and Chk1^{Sp} (Walworth and Bernards, 1996; Martinho et al., 1998). Human hCHK1 phosphorylates hCDC25 in vitro and promotes binding to the 14-3-3 proteins, which may prevent hCDC25 spatially from activating hCDC2 (Sanchez et al., 1997). A similar mechanism is likely used in

fission yeast by Rad24^{Sp} and Rad25^{Sp} (Lopez-Girona et al., 1999). Following DNA damage, Cdc2^{Sp} is phosphorylated on inhibitory sites (Y15 regulated through the phosphatase Cdc25) leading to a delay of mitosis (Berry and Gould, 1996). This event is the final effector of the checkpoint control. Because Chk1^{Sp} has been shown to be associated with Cdc25^{Sp} (Furnari et al., 1997), it has been proposed that Chk1^{Sp} stops Cdc2^{Sp} activation by inhibition of Cdc25^{Sp}.

B. Nucleotide Excision Repair

Multiple mechanisms have evolved in eukaryotic cells to repair DNA lesions. Some of these processes play a role in the maintenance of genomic integrity through recombination and DNA rearrangements (Weeda et al., 1993; Sancar, 1996). A subset of the *S. pombe* and *S. cerevisiae rad* mutants are defective in the nucleotide excision repair (NER) pathway (Table 2) (Subramani, 1991; Wang et al., 1997; Prakash and Prakash, 2000). The NER system recognizes DNA lesions including those resulting from UV and cisplatin exposure (Huang et al., 1994). The proteins implicated in processing these lesions include endonucleases, single-stranded binding protein, replication factor C, PCNA, DNA polymerase, and DNA ligase (Fig. 4). Discrete steps in the process have now been well defined and include DNA damage recognition, incision of the DNA backbone on both sides of the lesion, removal of the intervening single-strand containing the damage, filling of the resulting gap and ligation to completely restore the original nucleotide sequence (Boulikas, 1996).

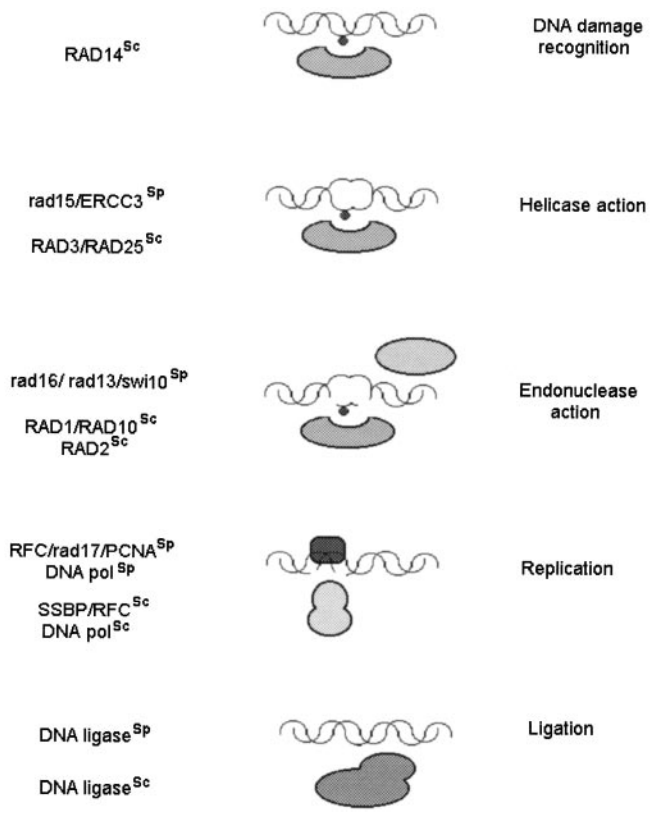

FIG. 4. Classical nucleotide excision repair pathway in *S. pombe* and *S. cerevisiae*.

S. pombe has two NER pathways only one of which is fully conserved in *S. cerevisiae* and humans (Yonemasu et al., 1997). Mutants of the first pathway, originally classified as "*rad5^{Sp}* group" (Subramani, 1991) include those with mutations in genes whose product functions analogously to those of the *RAD3^{Sc}* epistasis group of *S. cerevisiae*. Components of *RAD3^{Sc}* group can complement these *S. pombe rad* mutants (Carr and Hoekstra, 1995). Defects in NER genes of humans (XP-A to XP-G) lead to the cancer-prone syndrome *Xeroderma pigmentosum*.

The NER pathway has been characterized in detail in *S. cerevisiae*, in which several proteins (including Rad1^{Sc}, Rad10^{Sc}, Rad14^{Sc}, and Rad25^{Sc}) are absolutely required. Other proteins including Rad7^{Sc}, Rad16^{Sc} and Rad23^{Sc} are required in specific types of NER (e.g., repair of nontranscribed genes) (Carr and Hoekstra, 1995). Rad14^{Sc} is involved in DNA damage recognition, whereas the two helicases Rad3^{Sc} (homologs to alleles rad15^{Sp} and rad5^{Sp}; Murray et al., 1992) and Rad25^{Sc}/Ssl2^{Sc} (Ercc3^{Sp}) unwind the DNA at the site of damage and generate a junction on the sites of the damage where Rad1^{Sc} (Rad16^{Sp}; Bailis et al., 1992; Carr et al., 1994) and Rad10^{Sc} (Swi10^{Sp}; Schlake et al., 1993) act together to cleave DNA 5′ to the lesion. Incision of DNA 3′ to the lesion is operated by Rad2^{Sc} (Rad13^{Sp}; Carr et al., 1993; Habraken et al., 1993), which is a single-strand DNA endonuclease with different polarity (Bardwell et al., 1994).

In addition to playing a role in repair processes in which unwinding DNA at the site of DNA damage is required, yeast NER proteins including Rad25^{Sc} and Rad3^{Sc} may be components of the RNA polymerase II transcription machinery (Feaver et al., 1993). Their dual role could help in gaining insights into pathways that are not well defined in human cells, in particular transcription-coupled repair, a mechanism that preferentially repairs the transcribed strand of active genes.

The existence of a second NER pathway in *S. pombe* has been detected on the basis that cells deficient in the first NER pathway can still remove photoproducts resulting from UV damage (Yonemasu et al., 1997). The *UVde^{Sp}* gene, encoding a UV dimer endonuclease, is a homolog of the *Neurospora crassa* UV endonuclease that regulates sensitivity to radiation. The *UVde^{Sp}*-mediated pathway is different from the first NER pathway as documented by studies with double mutants. The second NER pathway acts more rapidly than the first pathway and processing of damage involves mechanisms partially dependent on the structure-specific endonuclease Rad2^{Sp}. The human homolog of *rad2^{Sp}*, hRAD2, has been implicated in monitoring chromosome segregation and in the repair of UV-induced damage (Murray et al., 1994). The *rad18^{Sp}* gene also appears to be involved in

the second NER pathway for removing UV damage (Lehmann et al., 1995). *Rad18*^{Sp} is a homolog of *RHC18*^{Sc} and is closely related to genes that regulate chromatin structure. Since epistasis analysis indicates that the recombinational repair gene *rhp51*^{Sp} is required in the *rad2*^{Sp}/*rad18*^{Sp} pathway, recombination processes likely participate in removal of UV damage in the second NER pathway.

C. Recombinational Repair

Recombinational mechanisms play an important role in determining the tolerance of *S. pombe* to DNA damage. Unlike *S. cerevisiae, S. pombe* spends the majority of its time in G_2, when repair of the replicated genome occurs mainly through recombination. In G_2 phase, repair of DNA lesions occurs mainly through recombinational mechanisms. *S. cerevisiae* uses recombination to rejoin double-strand breaks in mitosis (Paques and Haber, 1999). Recombinational repair mutants have a complex phenotype since they are not only defective in response to DNA damage, but also in meiotic recombination (Table 3). The main recombinational repair mutants are defective in double-strand break repair and include *rad32*^{Sp}/*MRE11*^{Sc}, *rad21*^{Sp}/*SSC1*^{Sc}, and *rad22*^{Sp}/*RAD52*^{Sc}. The RAD52-pathway has been widely studied in *S. cerevisiae* (Rattray and Symington, 1994; Sung, 1997; Benson et al., 1998). Recent studies support its importance in maintenance of genomic structure (Liu et al., 1999). The product of *rad32*^{Sp}/*MRE11*^{Sc} has nuclease and double-strand DNA binding activities (Furuse et al., 1998). Rad32^{Sp} acts in a pathway requiring Rhp51^{Sp} and Rad22^{Sp} most likely in a step that processes double-strand breaks early in the sequence of recombinational events (Tavassoli et al., 1995). The human hRAD52 epistasis group of proteins exhibits high expression in testis and functions in complexes similar to their yeast counterparts (Dolganov et al., 1996). In particular, repair of DNA double-strand breaks by radiation appears to be dependent on a complex containing Rad50, Mre11, and the NBS1 gene product. The NBS1 gene, which is altered in the Nijmengen Breakage Syndrome is essential for DNA damage-induced phosphorylation of Mre11 (Dong et al., 1999).

The *rhp54*^{Sp} gene was isolated by homology to *RAD54*^{Sc}, which codes for a putative helicase. The *rhp54*^{Sp} deletion mutant is hypersensitive to radiation and has a high degree of chromosome loss. In addition, cell viability is reduced when the *rhp54*^{Sp} mutant is in a genetic background in which the S phase/mitosis checkpoint is absent. Therefore, *rhp54*^{Sp} has been proposed to play a role in processing replication-specific lesions (Muris et al., 1996).

The *rad21*^{Sp} gene encodes a nuclear cell cycle-regulated phosphoprotein (Birkenbihl and Subramani, 1992). Mutation of *rad21*^{Sp} causes radiation sensitivity, although mutant cells retain the ability to arrest in G_2 after DNA damage (Birkenbihl and Subramani, 1992). The *rad21*^{Sp} gene bears homology to *SCC1*^{Sc}, which is a component of the chromosome cohesion complex (Biggins and Murray, 1999). Like *rad21*^{Sp}, the transcription of the human homolog hHR21 increases in late S phase and peaks in G_2 (McCay et al., 1996). *Rad22*^{Sp} mutants are defective in a terminal step in mating-type switching, which involves the ability to repair double-strand breaks (Ostermann et al., 1993). The human homolog of *rad22*^{Sp} has been isolated. Since the Rad22^{Sp} homolog Rad52^{Sc} interacts with Rad51^{Sc}, an analogous situation may exist for the *rad22*^{Sp} and *rhp51*^{Sp} gene products (Ostermann et al., 1993).

Topoisomerase inhibitors are potent inducers of recombination. Thus, genes involved in repair and recombination might be important in cellular response to these agents, as expected on the basis of the mechanisms of topoisomerase-mediated genotoxic lesions (i.e., formation of double-strand breaks as primary damage). Indeed, sensitization to camptothecin or topoisomerase II inhibitors was found in *S. cerevisiae* as a consequence of *rad52*^{Sc} mutation (Eng et al., 1988; Nitiss and Wang, 1988). The *rad52*^{Sc} mutants are defective in double-strand break repair. In general, postreplication repair is recognized to be a critical function in response to DNA-damaging agents. Accumulation of cells in G_2 phase after DNA damage reflects activation of the G_2 checkpoint to allow postreplication repair. Indeed, mutants defective in multiple functions including postreplication repair (e.g., *rad6*^{Sc}) exhibit hypersensitivity to cisplatin (Montelone et al., 1981; Hartwell et al., 1997).

Several observations suggest that the recombination repair pathway may be an appropriate target for therapeutic intervention. Relevant to this point is the finding that the products of the breast carcinoma susceptibility genes hBRCA1 and hBRCA2 associate with the hRAD51 protein, thus suggesting that breast cancer could arise through defects in recombination (Chen et al., 1999; Hiramoto et al., 1999). In addition, telomeres and telomerase involved in protecting and replicating the ends of chromosomes have a proposed role in the cellular response to antitumor drugs (Park et al., 1998). A link between DNA repair and telomere protection is found with the human Ku autoantigen, which is the DNA binding component of DNA-PK. Yeast homologs have been identified and have been shown to have DNA repair and telomere maintenance functions (Bianchi and

TABLE 3
Pathway defects, known biochemical functions, and homologs of relevant rad and rad-related yeast mutants: recombinational repair mutants

Mutants		Functions	Human Homologs
S. pombe	*S. cerevisiae*		
rad32	MRE11	Double-strand break repair	MRE11
rad21	SSC1	Double-strand break rejoining	HR21
rad22	RAD52	Double-strand break repair	RAD52
rhp51	RAD51	RecA homology	RAD51
rhp54	RAD54	Helicase	None

de Lange, 1999; Featherstone and Jackson, 1999). Recombination repair proteins could play a role in this respect as suggested by the involvement of Rad32^{Sp} and related proteins in telomere length maintenance in *S. pombe* (Wilson et al., 1999). Both in yeast and humans, besides functioning in telomere maintenance Ku70 and Ku80 are involved in the nonhomologous end-joining (also called illegitimate recombination) pathway of double-strand break repair, in which they act in early damage recognition by attracting the catalytic subunit of DNA-PK to DNA (Kanaar et al., 1998). In *S. cerevisiae* DNA damage checkpoint proteins participate in efficient repair of DNA damage by this pathway (De la Torre-Ruiz and Lowndes, 2000).

D. Additional Pathways

Additional cellular pathways function to maintain genome integrity. The DNA mismatch repair has been implicated in recognition of adducts produced by the clinically used platinum-containing drugs (Aebi et al., 1996), and in the generation of signals that trigger apoptosis and activate cell cycle checkpoints (Hawn et al., 1995). *S. pombe* genes involved in such a pathway have been mainly identified on the basis of defects in mating-type switching rather than of altered sensitivity to radiation (Rudolph et al., 1999). In this regard, a protein with a high mobility group domain (Cmb1^{Sp}) has been shown to recognize crosslinks produced by cisplatin (Fleck et al., 1998). In *S. cerevisiae*, another HMG protein, Ixr1^{Sc} binds DNA modified by cisplatin but not by transplatin that is inactive (Brown et al., 1993). These two proteins have been proposed to modulate the DNA repair process with different modes. In fact, Cmb1^{Sp} is supposed to facilitate repair of cisplatin-DNA adducts, whereas Ixr1^{Sc} may shield cisplatin adducts from repair. Indeed, yeasts defective in *cmb1^{Sp}* are more sensitive to cisplatin, while *ixr1^{Sc}* mutants are more resistant than wild-type strains (Brown et al., 1993; Fleck et al., 1998).

Table 4 lists a number of other *rad* mutants that cannot be grouped in the above mentioned classes. Among these, the *rad4^{Sp}* mutant (identical to *cut5^{Sp}*) is deficient in a gene, which is similar to the hXRCC1 gene. However, the sequence similarity seems to represent relevant domains rather than reflect similar functions.

TABLE 4
Pathway defects, known biochemical functions, and homologs of relevant rad and rad-related yeast mutants: miscellaneous mutants

Mutants		Functions	Human Homologs
S. pombe	*S. cerevisiae*		
rad4 (cut5)[a]	DPB11	Replication	XRCC1[b]
rad12 (hus2, rqh1)[a]	SGS1	Helicase	WRN, BLM
rad8	RAD5	Helicase	ERCC6[b]
rad11	RPA	Replication	RPA
rhp6	RAD6	Ubiquitin conjugation	RHH6

[a] Allelic mutants are indicated in parentheses.
[b] Limited homology.

Indeed, a role for *rad4^{Sp}* has been reported in replication/repair and mitosis/cytokinesis. In *rad4^{Sp}* cells, the coordination between cytokinesis and the completion of nuclear division is disrupted and aberrant mitosis occurs even in the absence of irradiation. Although the precise function of Rad4^{Sp} is not known, it has been speculated that it could interact differently with nonduplicated and duplicated chromatid DNA, thus providing a mechanism for distinguishing between post-M (G_1) and post-S (G_2) chromatid DNAs (Saka and Yanagida, 1993). The *rad12^{Sp}* gene is a homolog of the *Escherichia coli* RecQ gene, which is a helicase involved in the RecF recombination pathway. The *rad12^{Sp}* gene, is particularly interesting since alterations in two RecQ-related genes (hWRN, hBLM) are associated with genetic disorders, the Werner's and Bloom's syndromes (German et al., 1979; Ellis et al., 1995; Murray et al., 1997; Stewart et al., 1997). The *S. cerevisiae* homolog of *rad12^{Sp}*, *SGS1^{Sc}*, is required for maintaining genomic stability and in cooperation with other genes it functions in replication and transcription (Watt et al., 1996; Lee et al., 1999). Recently, a possible role for Sgs1^{Sc} in preventing telomere-telomere interactions that can generate chromosome nondisjunction has been proposed based on its capability to unwind G-G paired telomeric sequences (Sun et al., 1999). The *rad8^{Sp}* gene belongs to a family with significant homology to the *SNF2^{Sc}*, a transcriptional activator of genes regulating chromatin structure. *Rad8^{Sp}* also has similarity to *RAD5^{Sc}* and to hERCC6. This homology is relevant since a defective hERCC6 protein is associated with Cockayne's syndrome (Troelstra et al., 1992). The *rad11^{Sp}* mutant is allelic to *rpa1^{Sp}*, which encodes the large subunit of replication protein A (RPA) (Parker et al., 1997). RPA plays a role in the initiation of DNA replication and could be involved in DNA repair since it is part of the enzymatic machinery implicated in this process. Rpa1^{Sp} could be part of a complex required for DNA synthesis that involves Cds1^{Sp} and DNA polymerase α^{Sp} (Parker et al., 1997) and could act to generate the signal that triggers the checkpoint mechanism. Alternatively, RPA may have a direct role in checkpoint control acting as a signal for replication or repair (Parker et al., 1997).

VII. Conclusions

Specific genetic alterations commonly associated with malignant transformation participate in the regulation of cell proliferation, apoptosis, or differentiation. During tumor progression, cells accumulate additional alterations, including changes in DNA repair genes. The realization that the cellular fate in response to genotoxic stimuli depends on "downstream" events, including cell cycle control and regulation of apoptosis, has generated much interest in these processes as determinants of drug action and potential targets for novel therapies (Zunino et al., 1997). Based on the evidence of multiple

alterations in aggressive tumors, it is unlikely that a drug aimed only at a single target would be effective in cancer treatment. A promising approach to improve the antitumor efficacy is the development of a combination therapy including agents that target different cellular pathways and act synergistically. The identification of the exploitable molecular context or the appropriate target in the cell cycle pathway and/or in the DNA repair system and/or in cell death processes could be a relevant goal of this strategy.

The yeast system provides a powerful cellular approach for assessing the effect of specific genetic alterations on the ability of the cell to respond to chemotherapeutic agents. Yeast has the unique advantage of permitting rapid genetic manipulation. Thus, although it cannot completely replace human tumor cells for pharmacological studies, it may be a valuable model system specifically for drug screening and in particular for identifying a) new drugs acting against a specific target; b) eukaryotic genes that control chemosensitivity. Such genes can be unequivocally identified by using strains genetically identical except for mutations in specific genes. By constructing double mutants, it is possible to determine whether two genes work in the same or in different pathways, and whether interactions between pathways are important in controlling drug sensitivity. The molecular mechanisms involved in the detection, processing, and repair of DNA damage and the activation of cell cycle checkpoints and apoptosis appear to play central roles in modulating the sensitivity of tumor cells to antitumor drugs. One advantage of using *S. pombe* or *S. cerevisiae* is that a good deal is already known about the key genes of some of these critical pathways. For many of these, more information is available on their function in *S. pombe* or *S. cerevisiae* than on their homologs in mammalian cells.

Regarding the rationale for using *rad* mutants for drug screening, several examples support the interest of targeting the DNA damage response for therapeutic intervention. Sensitization of p53-deficient tumor cells has been reached through peptide-mediated inhibition of the human homolog of $chk1^{Sp}$ (Suganuma et al., 1999) as well as by radiosensitizing agents targeting the human homolog of $rad3^{Sp}$ or $chk1^{Sp}$ (Sarkaria et al., 1999).

There are limitations to the use of yeast for pharmacological studies primarily related to the relative resistance of yeast cells to antitumor drugs. While physical agents such as UV or ionizing radiation have been useful for defining the DNA damage response in *S. pombe* and *S. cerevisiae*, some DNA-damaging drugs may not affect yeast cells because of the presence of the cell wall or the expression of specific drug transporters (Kolaczkowski and Goffeau, 1997). In other cases, the intrinsic sensitivity of the drug target may be different. Nonetheless, we have provided evidence that *S. pombe* is a suitable model for studying cellular response to platinum compounds (Perego et al., 1998). Methods have been developed to overcome the problem of penetration including the use of yeast permeability mutants defective in cell wall integrity, as already documented for *S. cerevisiae* (Nitiss and Wang, 1988). Several studies provide evidence that both *S. cerevisiae* and *S. pombe* can be successfully used to study the mechanism of action of topoisomerase-targeted drugs and to identify potential inhibitors of different enzymes or isoenzymes (Eng et al., 1988; Nitiss et al., 1996; Keller et al., 1997; Hammonds et al., 1998; Reid et al., 1998; Van Hille et al., 1999). Genetic manipulations of these systems have also been exploited for investigation of specific aspects of cellular response to alkylating agents or bleomycin (Moore et al., 2000).

Among eukaryotic model organisms, yeast cells are easily approachable by genetic and/or biochemical means and their utility in molecular pharmacology of antitumor agents may be quite broad. In addition to the use of *rad* mutants resulting from deletion/inactivation of a specific gene function, yeast cells expressing additional genes (e.g., putative drug targets) could be generated, thus allowing several applications including definition of relevant biomolecular interactions and development of target-oriented bioassay systems (Munder and Hinnen, 1999). In addition, genomic approaches for identification of gene products as specific drug targets can be designed in diploid yeast cells in which the dosage of a single gene has been lowered (haploinsufficient phenotype; Giaever et al., 1999). The availability of the entire yeast genome sequence coupled with advanced array technology should allow the development of transcription profiles that monitor cellular responses to specific drugs. This technology could lead to the identification of functional gene products that are potential drug targets or of novel pathways that could be exploited to improve the efficacy of known agents.

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