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

PAOLA PEREGO, GRETCHEN S. JIMENEZ, LAURA GATTI, STEPHEN B. HOWELL, AND FRANCO ZUNINO

Istituto Nazionale Tumori, Milan, Italy (P.P., L.G., F.Z.); Vical Inc., San Diego, California (G.S.J.); and Department of Medicine and the Cancer Center, University of California, San Diego, La Jolla, California (S.B.H.)

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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.
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 (Matuyama 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 similar to those of human cells than those of the budding yeast S. cerevisiae. Like human cells, S. pombe has a distinct G₂ phase so a major checkpoint control is the decision to go from G₂ to M (Russell and Nurse, 1986). On the other hand, the budding yeast has a very short G₂ phase but a long G₁ and
the key transition is G₁/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 G₁ phase, cells are unbudded, and the bud emergence occurs during DNA synthesis; then in G₂ 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 × 10⁷ 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-

![Diagram of yeast life cycles](image)
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 of this review, we will use the standard conventions for 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 rad1Sp, rad3Sp and rad18Sp 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-PSp, rad 3–136/19 MSp) 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 O6-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
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., rad1Sp or rad26Sp; Kanter-Smoler et al., 1995; Uehi yama et al., 1997), or by analyzing distinct mutants defective in one pathway or the other (e.g., cdc1Sc, chk1Sp, rad2Sp; Fig. 2; Lindsay et al., 1998; Martinho et al., 1998). A similar distinction exists in S. cerevisiae, in which the replication proteins PoleSc, Dpb11Sc, and Rfc5Sc are involved in sensing replication block and DNA damage during DNA synthesis (replication checkpoint; Arak i et al., 1995; Sugimoto et al., 1997), whereas other genes including RAD9Sc, RAD17Sc, RAD24Sc, MEC3Sc, DDC1Sc, MEC1Sc, and RAD53Sc control the DNA damage checkpoint which has been dissected in specific responses (G1/S, intra S, G2/M) 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: rad1Sp, rad3Sp, rad9Sp, rad17Sp (Subramani, 1991). These mutations are sensitive to both ionizing and ultraviolet radiation, as well as the DNA synthesis inhibitor hydroxyurea. In S. cerevisiae RAD9Sc, RAD17Sc, RAD24Sc, MEC1Sc, and MEC3Sc are required for checkpoint control (Carr and Hoekstra, 1995). The rad1Sp gene encodes a protein with limited similarity to Ustilago maydis REC1, which is an exonuclease. The rad1Sp mutants are radiosensitive because they fail to delay mitosis until repair of DNA damage has been completed, and, in contrast to the homolog RAD17Sc, they are also deficient in the S-M checkpoint control (Rowley et al., 1992). RAD17Sc and rad1Sp are similar to the proliferating cell nuclear antigen (PCNASc; Thelen et al., 1999). The role of the rad1Sp gene in checkpoint control can be clarified based on its homology to RAD1 and RAD17Sc. 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 rad1Sp 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).

Rad3Sp and Mec1Sc are involved in both DNA damage and replication checkpoint pathways. Rad3Sp and Mec1Sc belong to a family of proteins with homology to “lipid kinases”, or phosphatidylinositol 3-kinases, which include Tel1Sc, Tel1Sp (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 Tel1Sc protein than with Mec1Sc, and rad3Sp is homologous to the human gene ATR. Therefore, it is likely that in human cells several rad3Sp homologs regulate the DNA damage checkpoint. Rad3Sp acts through its associated protein kinase activity (Bentley et al., 1996). Similarly, the Mec1Sc protein phosphorylates and likely activates specific substrates including Rad53Sc, Rad9Sc and Ddc1Sc (Sanchez et al., 1997; Emili et al., 1998; Pacciotti et al., 1998). Rad3Sp 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 Rad3Sp 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 rad9Sp gene product shows similarity to DDC1Sc (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

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<th>Mutants</th>
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<td>S. pombe</td>
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<td>rad1</td>
<td>RAD17</td>
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<td>Rad1/rad9 interacting</td>
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* 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.
replication, but not to DNA damage, it appears that the two pathways may have diverged between yeast and humans (Lieberman et al., 1996). The rad17Sp and RAD24Sc 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 rad17Sp has shown that the human locus contains tumor suppressor genes (Dean et al., 1998). Rad26Sp is presumed to function in S phase-specific DNA-damage responses, it has been shown to associate with Rad3Sp, and it exhibits Rad3Sp-dependent phosphorylation (Al-Khodairy et al., 1994; Edwards et al., 2000). Hus1Sp 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-BSp, likely a homolog of MEC3Sc, has been shown to form with Rad9Sp and Rad1Sp, 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-BSp, 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, chk1Sp/rad27Sp, rad24Sp and rad25Sp are involved in checkpoint mechanisms (Walworth et al., 1993; Al-Khodairy et al., 1994; Ford et al., 1994). The chk1Sp/rad27Sp (checkpoint kinase) gene encodes a serine/threonine protein kinase required for G2 arrest after DNA damage, but not for S phase arrest. The Chk1Sp response is cell cycle-specific since radiation damage induces Chk1Sp activation/phosphorylation in late S and G2 (Martinho et al., 1998). Cells lacking Chk1Sp are hypersensitive to a number of DNA-damaging agents including camptothecins (Wan et al., 1999). A human homolog of chk1Sp, 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
G2/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 G2 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 G2 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). Chk1Sc 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 Chk1Sc (Rhind et al., 1997). In *S. cerevisiae*, inhibition of G1/S phase transition after damage in G1 has been proposed to result from inhibition of *CLN1-2*Sc (G1 cyclins) transcription due to phosphorylation of the transcription regulator Swi6Sc by Rad53Sc (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 Rad3Sp-Rad26Sp 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 Chk1Sp/Rad27Sp, Rad24Sp, and Rad25Sp. The mechanism linking S phase and mitosis involves Cds1Sp, 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 Rad26Sp, which physically interacts with Cds1Sp (Lindsay et al., 1998). It is possible that substrates of the Cds1Sp kinase include components of the replication apparatus. Thus, during S phase, the Cds1Sp-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 Rad3Sp and the two downstream kinases as suggested by the ability of Rad3Sp to phosphorylate Cds1Sp and Chk1Sp (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

**Fig. 3.** DNA damage checkpoint pathways in budding yeast.
fission yeast by Rad24\textsuperscript{Sp} and Rad25\textsuperscript{Sp} (Lopez-Girona et al., 1999). Following DNA damage, Cdc2\textsuperscript{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\textsuperscript{Sp} has been shown to be associated with Cdc25\textsuperscript{Sp} (Furnari et al., 1997), it has been proposed that Chk1\textsuperscript{Sp} stops Cdc2\textsuperscript{Sp} activation by inhibition of Cdc25\textsuperscript{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 \textit{S. pombe} and \textit{S. cerevisiae} \textit{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).

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

The NER pathway has been characterized in detail in \textit{S. cerevisiae}, in which several proteins (including Rad1\textsuperscript{Sc}, Rad10\textsuperscript{Sc}, Rad14\textsuperscript{Sc}, and Rad25\textsuperscript{Sc}) are absolutely required. Other proteins including Rad7\textsuperscript{Sc}, Rad16\textsuperscript{Sc} and Rad23\textsuperscript{Sc} are required in specific types of NER (e.g., repair of nontranscribed genes) (Carr and Hoekstra, 1995). Rad14\textsuperscript{Sc} is involved in DNA damage recognition, whereas the two helicases Rad3\textsuperscript{Sc} (homologs to alleles rad15\textsuperscript{Sp} and rad5\textsuperscript{Sp}; Murray et al., 1992) and Rad25\textsuperscript{Sc}/Ssl2\textsuperscript{Sc} (Ercc3\textsuperscript{Sp}) unwind the DNA at the site of damage and generate a junction on the sites of the damage where Rad1\textsuperscript{Sc} (Rad16\textsuperscript{Sp}; Bailia et al., 1992; Carr et al., 1994) and Rad10\textsuperscript{Sc} (Swi10\textsuperscript{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\textsuperscript{Sc} (Rad13\textsuperscript{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\textsuperscript{Sc} and Rad3\textsuperscript{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 \textit{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\textsuperscript{Sp} gene, encoding a UV dimer endonuclease, is a homolog of the \textit{Neurospora crassa} UV endonuclease that regulates sensitivity to radiation. The UVde\textsuperscript{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\textsuperscript{Sp}. The human homolog of \textit{rad2}\textsuperscript{Sp}, hRAD2, has been implicated in monitoring chromosome segregation and in the repair of UV-induced damage (Murray et al., 1994). The \textit{rad18}\textsuperscript{Sp} gene also appears to be involved in

![Fig. 4. Classical nucleotide excision repair pathway in \textit{S. pombe} and \textit{S. cerevisiae}.](image-url)
the second NER pathway for removing UV damage (Lehmann et al., 1995). *Rad18*<sup>Sp</sup> is a homolog of *RHC18<sub>Sc</sub>* and is closely related to genes that regulate chromatin structure. Since epistasis analysis indicates that the recombinational repair gene *rhp51<sup>Sp</sup>* is required in the *rad2<sup>Sp</sup>/rad18<sup>Sp</sup>* 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<sub>2</sub>, when repair of the replicated genome occurs mainly through recombination. In G<sub>2</sub> 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 postreplication repair, which involves the ability to repair double-strand breaks by recombination (Table 3). The main recombinational repair mutants are defective in double-strand break repair and include *rad32<sup>Sp</sup>/MRE11<sub>Sc</sub>*, *rad21<sup>Sp</sup>/SSC1<sub>Sc</sub>*, and *rad22<sup>Sp</sup>/RAD50<sub>Sc</sub>*. 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<sup>Sp</sup>/MRE11<sub>Sc</sub>* has nuclease and double-strand DNA binding activities (Furuse et al., 1998). Rad32<sup>Sc</sup> acts in a pathway requiring Rhp51<sup>Sp</sup> and Rad22<sup>Sp</sup> 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 Nijmegen Breakage Syndrome is essential for DNA damage-induced phosphorylation of Mre11 (Dong et al., 1999).

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

The *rad21<sup>Sp</sup>* gene encodes a nuclear cell cycle-regulated phosphoprotein (Birkenbihl and Subramani, 1992). Mutation of *rad21<sup>Sp</sup>* causes radiation sensitivity, although mutant cells retain the ability to arrest in G<sub>2</sub> after DNA damage (Birkenbihl and Subramani, 1992). The *rad21<sup>Sp</sup>* gene bears homology to *SSC1<sub>Sc</sub>*,** which is a component of the chromosome cohesion complex (Biggins and Murray, 1999). Like *rad21<sup>Sp</sup>*,** the transcription of the human homolog hHR21 increases in late S phase and peaks in G<sub>2</sub> (McCay et al., 1996). *Rad22<sup>Sp</sup>* 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<sup>Sp</sup>* has been isolated. Since the Rad22<sup>Sp</sup> homolog Rad52<sub>Sc</sub> interacts with Rad51<sup>Sc</sup>, a homologous situation may exist for the *rad22<sup>Sp</sup>* and *rhp51<sup>Sp</sup>* 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<sup>Sc</sup>* mutation (Eng et al., 1988; Nitiss and Wang, 1988). The *rad52<sup>Sc</sup>* 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<sub>2</sub> phase after DNA damage reflects activation of the G<sub>2</sub> checkpoint to allow postreplication repair. Indeed, mutants defective in multiple functions including postreplication repair (e.g., *rad6<sup>Sc</sup>*<sub>Sc</sub>) 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

<table>
<thead>
<tr>
<th>Mutants</th>
<th><em>S. pombe</em></th>
<th><em>S. cerevisiae</em></th>
<th>Functions</th>
<th>Human Homologs</th>
</tr>
</thead>
<tbody>
<tr>
<td>rad32</td>
<td>MRE11</td>
<td>Double-strand break repair</td>
<td>MRE11</td>
<td></td>
</tr>
<tr>
<td>rad21</td>
<td>SSC1</td>
<td>Double-strand break rejoicing</td>
<td>HR21</td>
<td></td>
</tr>
<tr>
<td>rad22</td>
<td>RAD52</td>
<td>Double-strand break repair</td>
<td>RAD52</td>
<td></td>
</tr>
<tr>
<td>rhp51</td>
<td>RAD51</td>
<td>RecA homology</td>
<td>RAD51</td>
<td></td>
</tr>
<tr>
<td>rhp54</td>
<td>RAD54</td>
<td>Helicase</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>
Indeed, a role for rad4<sup>sp</sup> has been reported in replication/repair and mitosis/cytokinesis. In rad4<sup>sp</sup> 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<sup>sp</sup> is not known, it has been speculated that it could interact differently with nonduplicated and duplicated chromatin DNA, thus providing a mechanism for distinguishing between post-M (G<sub>1</sub>) and post-S (G<sub>2</sub>) chromatin DNAs (Saka and Yanagida, 1993). The rad12<sup>sp</sup> gene is a homolog of the Escherichia coli RecF gene, which is a helicase involved in the RecF recombination pathway. The rad12<sup>sp</sup> 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<sup>sp</sup>, SGS1<sup>Sc</sup>, 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<sup>Sc</sup> 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<sup>sp</sup> gene belongs to a family with significant homology to the SNF2<sup>Sc</sup>, a transcriptional activator of genes regulating chromatin structure. Rad8<sup>sp</sup> also has similarity to RAD5<sup>Sc</sup> and to hERCC6. This homology is relevant since a defective hERCC6 protein is associated with Cockayne’s syndrome (Troelstra et al., 1992). The rad11<sup>sp</sup> mutant is allelic to rpa1<sup>sp</sup>, 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<sup>sp</sup> could be part of a complex required for DNA synthesis that involves Cds1<sup>sp</sup> and DNA polymerase ε<sup>sp</sup> (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 (Suganuma et al., 1999) as well as by radiosensitizing agents targeting the human homolog of rad3 or chk1 (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; Hammond 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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