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Cell cycle, DNA replication, repair, and recombination in the dimorphic human pathogenic fungus *Paracoccidioides brasiliensis*

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ABSTRACT. DNA replication, together with repair mechanisms and cell cycle control, are the most important cellular processes necessary to maintain correct transfer of genetic information to the progeny. These processes are well conserved throughout the Eukarya, and the genes that are involved provide essential information for understanding the life cycle of an organism. We used computational tools for data mining of genes involved in these processes in the pathogenic fungus *Paracoccidiodes brasiliensis*. Data derived from transcriptome analysis revealed that the cell cycle of this fungus, as well as DNA replication and repair, and the recombination machineries, are highly similar to those of the yeast *Saccharomyces cerevisiae*. Among orthologs detected in both species, there are genes related to cytoskeleton structure and assembly, chromosome segregation, and cell cycle control genes. We identified at

least one representative gene from each step of the initiation of DNA replication. Major players in the process of DNA damage and repair were also identified.

Key words: *Paracoccidioides brasiliensis*, Transcriptome, Cell cycle, DNA replication, DNA repair, Recombination

INTRODUCTION

The cell cycle is a sequence of events by which a growing cell duplicates all its components and divides into two daughter cells. Viability of progeny is preserved in each division by temporally and spatially coordinated processes whose most critical function is genome replication and the subsequent segregation of each genome copy into a new daughter cell (Tyson and Novak, 2001).

The eukaryotic cell cycle (Figure 1A) can be subdivided into two main phases: interphase, a long period during which cell contents are duplicated, and mitosis or M phase, a shorter period during which cell contents are segregated. Interphase is further divided into S phase, during which a new copy of each chromosome is synthesized, and the gap phases G1 (between interphase and S phase) and G2 (between S phase and M phase). These gaps allow for cell preparation, protein synthesis and provide the time necessary for cell-cycle input by various intra- and extracellular signals (Nasmyth, 1996).

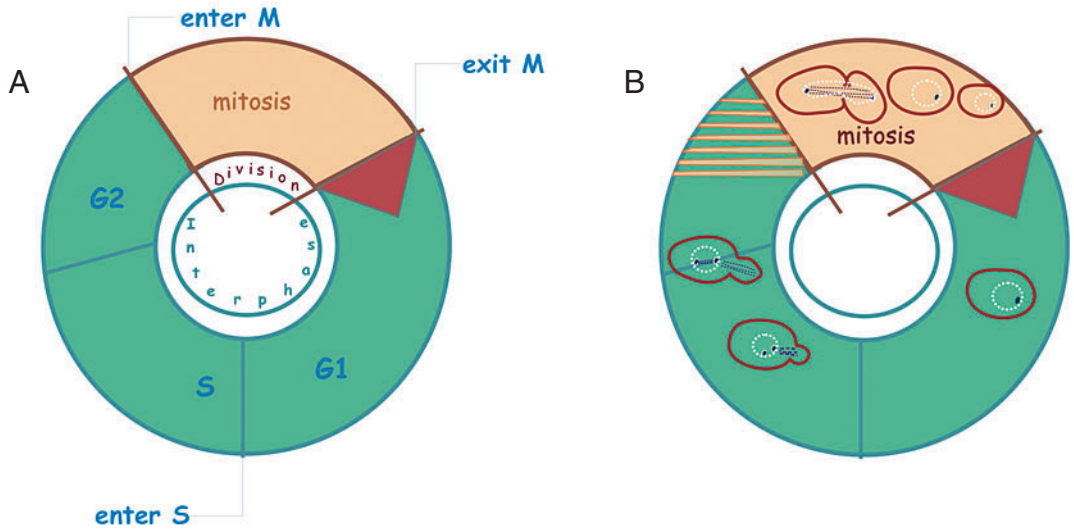

Figure 1. Eukaryotic cell cycle. Arrowheads indicate the clockwise direction of the cycle. **A**, Standard. After continuous growth during interphase, cells divide during the M (mitosis) phase. DNA synthesis is restricted to the S phase. G1 and G2 are the gaps between M and S and S and M phases, respectively. Checkpoints, enter M, exit M, and enter S, are marked. **B**, Budding yeast *S. cerevisiae* performs cell division through normal G1 and S phases. However, G2 does not proceed as in the standard cycle, considering that the microtubule-based spindle begins to form very early in the cycle, during S phase. Unlike fission yeast, the cells of this species divide by budding. As in fission yeast, but in contrast with higher eukaryotic cells, the nuclear envelope remains intact during mitosis.

Phase transitions are well defined. At the G1/S transition (also known as "Start"), the cell commits itself to cell division by checking whether internal and external conditions are favorable for a new round of DNA synthesis and division. When DNA replication is complete and all chromosomes are aligned, the second irreversible transition of the cycle (called "Finish") begins. The sister chromatids are pulled to opposite poles of the mitotic spindle (anaphase). After that, daughter nuclei form around the segregated chromatids (telophase), and daughter cells separate (reviewed by Tyson and Novak, 2001).

Commitment to cell division is made at the first checkpoint, i.e., "Start", during late G1 phase. It is influenced by environmental conditions, such as nutrient supply or the absence of mating pheromones. Once the decision is made and the checkpoint is trespassed, cells typically proceed to complete the cycle even if the conditions are no longer favorable, unless DNA integrity is compromised.

DNA replication initiates at S phase; it is a cumbersome process that demands exceptional accuracy to ensure genetic stability. It is strictly regulated by a set of interrelated factors that operate along the cell cycle. It begins at late G1, when the pre-replicative complex assembles, and it ends at S phase with elongation (Walter and Newport, 2000). Not only must this process occur in a concerted form with other cell processes, such as mitosis and differentiation, but it also must be in harmony with the replication of neighboring cells. Reconstitution of DNA replication by means of purified proteins has provided invaluable insight into the discrete mechanisms that underlie this and other key processes, such as recombination and repair (Challberg and Kelly, 1989; Stillman, 1989; Bambara and Huang, 1995; Bambara et al., 1997).

S-phase checkpoint is essential to the maintenance of genomic stability. Similarly, G2 checkpoint of DNA damage, spindle assembly, as well as tetraploidy, operate subsequently to DNA replication to ensure overall genetic fidelity in cell division (Andreassen et al., 2003). During this phase, the main process of DNA repair and cell viability occurs. Cells have evolved a number of mechanisms to either repair or tolerate DNA damage. In *Saccharomyces cerevisiae*, these pathways include base excision repair (BER), nucleotide excision repair (NER), recombination, and translesion synthesis (Doetsch et al., 2001). BER, which primarily involves the repair of base lesions and abasic sites that cause minimal distortion to the DNA helix, is believed to be the major repair pathway for oxidative DNA damage. In contrast to BER, NER is believed to repair bulky, helix-distorting lesions, such as bipyrimidine UV photoproducts. While BER and NER remove and replace DNA damage, recombination and translesion synthesis are known as bypass/tolerance mechanisms, since neither can actually remove DNA lesions. Translesion synthesis involves DNA polymerases and functions that bypass DNA replication blockage lesions, including oxidative and UV-induced DNA damage (Salmon et al., 2004).

Mitosis, or M phase, consists of four sub-phases: prophase, metaphase, anaphase, and telophase. In prophase, replicated DNA condenses into compact structures known as chromosomes. During metaphase, chromosomes are aligned at cell midplane, and chromosome segregation occurs in anaphase. Centromeric DNA binds a multiprotein complex, the kinetochore, which mediates the binding of chromosomes to the mitotic spindle microtubules. The spindle segregates chromosomes to opposite ends of the cell in anaphase (reviewed by McAinsh et al., 2003). Chromosome binding to the spindle is monitored by the spindle checkpoint that delays sister chromatid separation until all kinetochores attached to the mitotic spindle apparatus are released (Lew and Burke, 2003). Mitosis comes to an end when daughter nuclei are formed and cells begin to divide in telophase.

The dimorphic fungus *Paracoccidioides brasiliensis* is the etiological agent of paracoccidioidomycosis, a systemic disease endemic to Latin America. Infection triggers a differentiation process from the saprophytic mycelium to the pathogenic yeast form, which can be reproduced *in vitro* by switching growth temperature from 23° to 36°C; the transition is equally reversible. *P. brasiliensis* is multinucleate in its pathogenic yeast like form, while a single nucleus is present in both conidia and individual mycelial cells (San-Blas et al., 2002). As is the case with many other fungi, this microorganism has been refractory to classical genetic analysis, and its life cycle is still unclear. An extensive transcriptome analysis, which identified 6,022 expressed genes of *P. brasiliensis,* has recently been carried out in our laboratory, enabling the characterization of ~80% of the genes in this organism (Felipe et al., 2003, 2005). In this work, we examined the genes involved in the cell cycle, DNA replication, DNA repair, and recombination, based on *P. brasiliensis* transcriptome data.

MATERIAL AND METHODS

In order to identify *P. brasiliensis* genes related to the mechanisms of cell cycle control, DNA replication, DNA repair, and recombination, we used *S. cerevisiae* category MIPS table (http://mips.gsf.de/genre/proj/yeast/searchCatalogListAction.do?style=cataloglist.xslt&table = FUNCTIONAL_CATEGORIES&num=10&db=CYGD&order=) as a reference. All genes related to these functions were selected and used to search the bidirectional best hit database. This tool allows the identification of orthologue genes through bidirectional comparative analysis of two or more organism sequences, generating a database. Two bidirectional best hit databases were generated between *P. brasiliensis* and *S. cerevisiae*, and *P. brasilensis* and *Candida albicans*. For DNA repair gene identification, we also used the *Homo sapiens* database (www.cgal.icnet.uk/DNA_Repair_Genes.html).

RESULTS AND DISCUSSION

The *P. brasiliensis* transcriptome was obtained from either stationary yeast or mycelium cultures grown on complex media, resulting in 6,022 identified PbAESTs (*P. brasiliensis* assembled expressed sequence tags). Over the last decades, much information was obtained on cell cycle mechanistic and controlling events in single-cell organisms. One of the best-established eukaryotic models is the yeast *S. cerevisiae*. During analyses of the *P. brasiliensis* functional transcriptome, we mainly used *S. cerevisiae* to find orthologue genes. Although the *S. cerevisiae* cell cycle does not have a canonical G2 phase and S/G2 and G2/M transitions are not well defined (Forsburg and Nurse, 1991; Lew et al, 1997), this fungus was chosen because it has the most complete databank and also because it is a well-accepted model for cell cycle studies in eukaryotic organisms (Figure 1B).

Cell cycle

G1 phase

Transitions in the cell cycle clock are regulated by a family of protein kinases known as cyclin-dependent kinases (Cdks) and by checkpoint controls that hold the cell cycle progression

in check until phase-specific events have been completed. Cdks are composed of a catalytic kinase and a regulatory subunit called cyclin. In addition to being required for kinase activity, cyclins also contribute to substrate specificity. Cdk complexes can be either positively or negatively regulated by phosphorylation and can also be regulated by the binding of Cdk inhibitor proteins (Elledge, 1996). The main kinase activity that controls the cell cycle in *S. cerevisiae* is Cdc28p, which either associates with G1 (*CLN1,2,3*) or G2/M cyclins (*CLB1,2,3,4*), depending on the cell cycle phase (Siegmund and Nasmyth, 1996). Although we have found the orthologue to *CDC28* in the *P. brasiliensis* transcriptome, no cyclin orthologues were found. This was expected, since cyclin genes are specifically expressed during certain phases of the cell cycle in exponential growth cultures.

One way to ensure the unidirectionality of the cell cycle is the rapid and temporal destruction of regulatory proteins by ubiquitin-dependent degradation. In *S. cerevisiae*, this degradation is coordinated by Cdc37p (E2 enzyme), Cdc34p and SCF^{Cdc4} (E3 ligase) complex (DeSalle and Pagano, 2001). We have found the transcripts for *CDC34*, *CDC37* and *CDC4* in *P. brasiliensis.* Other G1 genes, such as *CDC50*, which is apparently involved in polarized growth control (Misu et al., 2003), and *CAK1*, whose product associates with and directs SCF complexes to their substrates (Kaldis et al. 1996), were also identified in the *P. brasiliensis* transcriptome.

S phase and DNA replication

The isolation of a great number of genes encoding proteins that regulate the cell cycle, or that comprise the pre-replicative complex and the replication fork, has greatly contributed to the understanding of the basis of DNA replication (Diffley, 1996; Stillman, 1996; Waga and Stillman, 1998). In eukaryotes, the replicative machinery is evolutionarily conserved (Bell and Dutta, 2002), which also holds true for *P. brasiliensis*, based on computational analyses of transcriptome data. The low expression levels that characterize replication genes may explain our failure to detect many important transcripts. Nonetheless, we were able to identify a number of key genes whose products are involved in DNA replication, which will be described hereafter.

The search for transcripts related to the S phase in *P. brasiliensis* revealed the presence of the key genes involved in the DNA pre-replicative complex, including initiation, elongation and assembly of the mitotic spindle. These include *CDC6*, *CDC45*, *CDC7*, *DBF4*, *MCM3*, *CDC54 (MCM4)*, *CDC46 (MCM5)*, *CDC47 (MCM7)*, *TOP1*, *RFA2*, *PRI2*, *POL12* (Pol α), *CDC2* (Pol δ), *RFC3*, *RFC4*, *SLD3*, *CDC40*, *CDC9*, and *DPB2* (Pol ε).

In *S. cerevisiae*, DNA replication is initiated at well-defined origins of replication, where origin recognition complex (ORC) proteins specifically bind, triggering the process. The pre-replicative complex is formed through the recognition and binding of specific DNA regions by ORC proteins (Figure 2). There are six ORCs in *S. cerevisiae*. Orc1p and Orc5p bind ATP, but only the former hydrolyses it (Takenaka et al., 2004). ATP binding, but not hydrolysis, is a condition to origin recognition by ORCs, and it also allows interaction with Cdc6p, which is another ATP-binding protein that only binds chromatin in association with an ORC (Bell and Dutta, 2002).

Another protein complex involved in initiation, as well as in elongation, is the mini-chromosome maintenance (MCM) complex, which is composed of six proteins (Cdc46p, Cdc47p,

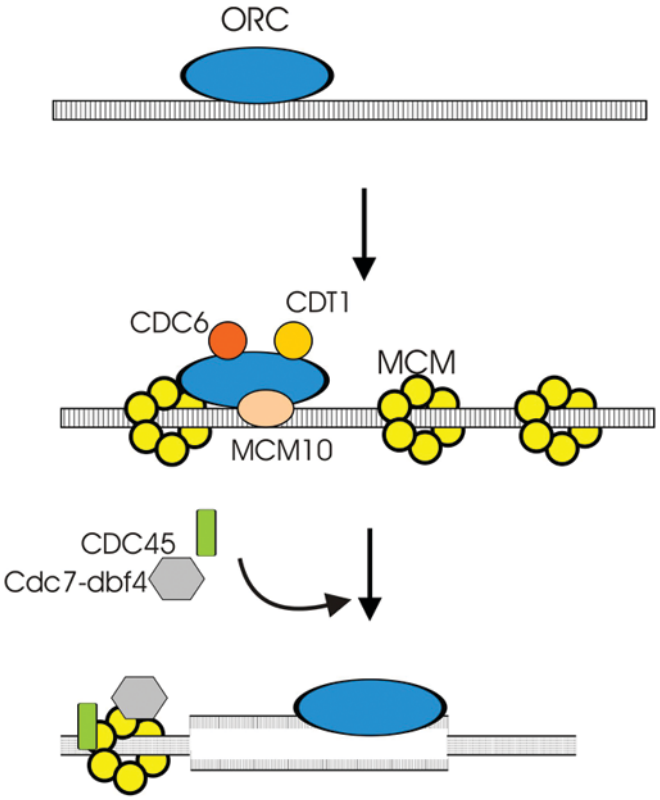

Figure 2. Diagram of the assembly of the pre-replicative complex and of the beginning of replication. ORC = origin recognition complex; MCM = mini-chromosome maintenance.

Cdc54p, Mcm2p, Mcm3p, and Mcm6p), each with a specific function (Aparicio et al., 1997; Kearsey and Labib, 1998; Homesley et al., 2000; Labib et al., 2000). Previous studies have shown that, in mammals (Ishimi, 1997; You et al., 1999), *Schizosaccharomyces pombe* (Lee and Hurwitz, 2000), *S. cerevisiae* (Davey et al., 2003), and *Xenopus*, the MCM complex functions as an ATP-dependent 3'→5' helicase during replication (Pacek and Walter, 2004). Mcm4p (Cdc54p), 6p and 7p (Cdc47p) are responsible for helicase activity during elongation (You et al., 1999; Labib and Diffley, 2001), whereas subunits Mcm2p, 3p and 5p (Cdc46p) exert regulatory functions by inhibiting the others (Ishimi et al., 1998; Lee and Hurwitz, 2000). MCM proteins operate in effector-regulator pairs (3p and 7p, 2p and 6p, and 5p and 4p). They are expressed at higher levels than Orcp, which leads to the hypothesis that they are distributed throughout the chromatin, performing other functions in structural regulation during the passage of the replicative fork (Bell and Dutta, 2002). The fact that no ORC transcripts were found in *P. brasiliensis* could be explained by the fact that these transcripts are generally present in very low quantities throughout cell cycle.

Cdc6p, together with Cdt1p, plays a role in the kinetics of MCM complex assembly, and it is essential because of its association with chromatin (Donovan et al., 1997), although the precise function remains unknown. It is speculated that Cdc6 participates in single-stranded DNA formation through ATP-hydrolysis (Bell and Dutta, 2002).

Another MCM protein, Mcm10p, is a component of pre-replicative complexes. It is necessary for the binding of MCM complex to the origins of replication, providing stability to the association. It seems to play a role in the elongation phase by releasing proteins from the origin and triggering the replication process (Merchant et al., 1997; Homesley et al., 2000).

Cdc45p is also important for initiation (Owens et al., 1997), in that it begins the assembly of the pre-initiation complex (Pacek and Walter, 2004). Experimental evidence shows that this protein is important in elongation as well, although its function is unclear. Studies assessing its interactions with other proteins of the replication machinery suggest that it co-ordinates DNA helicase and DNA polymerase during elongation of both leading and lagging strands (Tercero et al., 2000). Cdc45p is recruited by the MCM complex and probably is involved in activation and/or dissociation of the pre-initiation complex at the beginning of replication (Zou et al., 1997). It may also activate the helicase activity of the MCM complex (Pacek and Walter, 2004), thus releasing it from the origin and further recruiting the replication machinery. The latter process, albeit led by Cdc45p, also seems to depend on another kinase complex called Cdc7p-Dbf4p (Owens et al., 1997), which phosphorylates the Mcm2-7p family of proteins (Burgers, 1998). Cdc54p is one of the four MCM proteins that are substrates for the Cdc7p/Dbf4p protein kinase complex *in vitro* (Lei et al., 1997). Cdc7p and Dbf4p are, respectively, the catalytic and regulatory subunits of a serine/threonine protein kinase required early in the S phase, for the initiation of early-firing origins, as well as in the late S phase, for the initiation of late-firing origins (Bousset and Diffley, 1998). At least two types of aberrant events are monitored during S phase: the failure of the ongoing replication fork to advance and DNA damage. According to Jares et al. (2000), evidence that *cdc7* mutants are hypersensitive to DNA-damaging agents suggests that this protein could play a role in the response to DNA damage.

Elongation phase is characterized by the synthesis of leading and lagging strands. Yeast genetic studies, and the investigation of specific enzymatic reactions, have revealed many proteins that are directly involved in DNA synthesis in the replication fork (Waga and Stillman, 1998). First, 5'→ 3' DNA helicases unwind the DNA duplex downstream to the replication fork, thus creating templates to DNA polymerase. The yeast Dna2p helicase was identified in replication-deficient mutant studies and is a determinant of cell viability (Budd and Campbell, 1995; Budd et al., 1995). Topological stress generated by DNA replication is relieved by the action of topoisomerases, which are enzymes that modify the degree of DNA winding. They participate in many metabolic processes, such as replication, recombination, transcription, and chromosome segregation. Their action is to generate one-stranded (topoisomerase I) or double-stranded (topoisomerase II) breaks, allowing the stressed helix segment to rotate freely and to later rejoin the broken ends (Nitiss, 1998; Wang, 1996). After unwinding, each DNA strand generated by the action of nucleases and topoisomerases is stabilized by the replication protein/factor A (RPA or RFA), which is a single-stranded DNA binding protein that forms a heterotrimer complex and has been found in all nucleated eukaryotic cells that have been investigated so far (Waga and Stillman, 1998). RPA stimulates DNA Pol α/primase activity under certain conditions and is required for replication factor C (RFC)- and proliferating cell nuclear antigen (PCNA)-dependent DNA synthesis by DNA polymerase δ (see below) (Tsurimoto and Stillman, 1991a,b; Braun et al., 1997).

The DNA polymerase α/primase complex is a heterotetramer. In *S. cerevisiae*, one regulatory subunit highly conserved among eukaryotes is probably encoded by the gene *POL12* (Foiani et al., 1994). Besides acting in DNA replication, the DNA polymerase α/primase com-

plex plays an important role in the coordination of TG-repeat synthesis mediated by telomerase, as shown by mutation studies on *POL12* (Grossi et al., 2004). Eukaryotic DNA primase activity derives from two distinct subunits, encoded by the *PRI1* and *PRI2* genes (Santocanale et al., 1992), which present highly conserved homologues in several species, further suggesting an evolutionary conservation of functional domains.

The DNA polymerase α/primase complex acts in DNA replication by synthesizing an RNA primer and then elongating the nascent DNA strand until a short hybrid segment of RNA-DNA about 40 nucleotides long is produced (Bullock et al., 1991; Murakami et al., 1992). Then, this segment is used by DNA polymerase δ as a primer for DNA synthesis in both the leading strand and Okazaki fragments in the lagging strand (Prelich and Stillman, 1988; Weinberg and Kelly, 1989; Tsurimoto et al., 1990; Tsurimoto and Stillman, 1991a, b; Waga and Stillman, 1994). From this step on, synthesis in both strands is radically different; in the leading strand, DNA polymerase δ merely adds desoxyribonucleotides to the primer as unwinding proceeds downstream the replication fork in a continuous process. In the lagging strand, an array of new proteins is needed to synthesize and to mature Okazaki fragments (Turchi et al., 1994; Bambara et al., 1997; Waga and Stillman, 1994, 1998; Hubscher and Seo, 2001; MacNeill, 2001). The RNA-DNA primer made by the DNA polymerase α/primase complex is further elongated by DNA Pol δ, generating Okazaki fragments (Waga and Stillman, 1994). Mossi et al. (2000) have shown that the replacement of DNA Pol α by DNA Pol δ is mediated by RFC; this happens when the primer reaches a critical length of 30 nucleotides. While Okazaki fragments remain hybridized to the template in the lagging strand, the DNA primer can be completely removed by the combined action of RNAseHI and Flap endonuclease 1 (Fen1) (Waga and Stillman, 1998). Recently, it was demonstrated that DNA Pol δ displaces the 5'-end region of downstream Okazaki fragments, during the extension of the upstream ones (Bae and Seo, 2000; Bae et al., 2001). This generates ssDNA flaps that may be efficiently processed by Dna2p and Fen1p (Bae et al., 2001). Rpap plays an essential role in this stage of processing by sequentially regulating Dna2p and Fen1p. Dna2p is responsible for removing most of the ssDNA flaps, but it leaves off a six nucleotide flap region that may be the Fen1p target. Other nucleases, such as Fen2p, end the trimming by leaving nicks that are easily filled by DNA ligase. Although it is generally accepted that Dna2p is an indispensable nuclease for Okazaki fragment processing as a whole, it is still unclear which fraction depends on it (Ryu et al., 2004).

One of the key proteins for recruiting DNA Pol δ to the replication fork is RFC, a five-subunit (p36, p37, p38, p40, and p140) protein complex conserved in all eukaryotes (Waga and Stillman, 1998). Its central role is to load the trimeric structure of the PCNA onto the DNA primer-template junction or onto the DNA duplex nicked site (Tsurimoto and Stillman, 1990, 1991a, b; Lee et al., 1991). The former process is dependent on ATP and is a pre-requisite for the assembly of Pol δ onto template DNA to form the functional holoenzyme (Tsurimoto and Stillman, 1991a, b; Lee et al., 1991; Podust et al., 1992; Fien and Stillman, 1992), which acts during the duplication of both DNA strands in the replication fork.

PCNA, the so-called DNA polymerase clamp, forms a homotrimer and is an accessory factor to DNA polymerase δ. It enhances the efficiency of this enzyme during replication. Its primary amino acid sequence is not phylogenetically conserved; however, the structures of yeast and human forms are tridimensionally identical (Kelman, 1997).

Two DNA polymerases act during the S phase in eukaryotic cells: DNA Pol δ (Hughes et al., 1992; MacNeill et al., 1996; Zuo et al., 1997) and DNA Pol ε, which are distinguished by

proofreading activity and high efficiency, even in the absence of PCNA. PCNA enhances processing capability and RFC might have the further function of disassembly of the complex upon termination. The role of DNA Pol ε in replication is still unclear. Nonetheless, studies on yeasts point to a strong involvement in the process. One possible model for replication would place DNA Pol δ in the leading and DNA Pol ε in the lagging strand. Another suggests that DNA Pol ε is involved in Okazaki fragments maturation (Burgers, 1998).

Since the mechanisms described above are highly conserved in all eukaryotes and at least one representative gene was found for each mechanism, we believe that the genes not identified in this analysis still have orthologues in *P. brasiliensis.*

G2 phase

Only six gene products found in *P. brasiliensis* were reported to play some role in the G2/M transition, some of which also take part in G1/S. Cdc34p forms the SCF complex and regulates cell cycle progression by targeting key substrates for degradation (Giaever et al., 2002). Cdc4p associates with and directs SCF complexes (ubiquitin-protein ligases) to substrates. Cak1, an F-box protein, associates with and directs SCF; *cak1* temperature-sensitive (ts) mutations result in G2 delay, accompanied by low Cdc28p protein kinase activity. Cdc4p mutations arrest meiosis at the mononuclear stage, with duplicated spindle pole bodies (Goh and Surana, 1999). Cdc5p is a Polo-like kinase with multiple functions in mitosis and cytokinesis. Ts *cdc5* mutants form synaptonemal complexes devoid of central elements and arrest division, either at meiosis I, with broken spindles, or at meiosis II, with short spindles. Late shifts to a restrictive temperature result in reduction dyads, each spore thus containing an entire meiosis II short spindle with non-separated chromatids. In some *S. cerevisiae* strains, at a semi-permissive temperature, chromosomes segregate reductively or equally, depending on the centromere (Song et al., 2000). An orthologue was also identified for Cdc20p, a cell-cycle regulated activator of anaphase promoting complex/cyclosome (APC/C) that is required for microtubule function at mitosis (Zachariae and Nasmyth, 1999).

Another function found in G2 phase is the checkpoint of cell size; however, no transcripts related to this mechanism were found in *P. brasiliensis.*

M phase and cytokinesis

In the *P. brasiliensis* transcriptome, we have found the transcripts for *pbsmc2* condensing (Gassmann et al., 2004) and the *pbtrf4* protein involved in cohesion (Wang et al., 2002), both involved in chromosome condensation.

The spindle checkpoint requires APC, a multisubunit ubiquitin ligase that targets proteins for degradation, including cyclins and cohesins, thus triggering chromosome segregation at the metaphase-anaphase transition (Peters, 2002). The orthologs for kinetochore proteins found in *P. brasiliensis* were Spc25p (Ndc80p complex; Le Masson, 2002) and Nnf1p (MIND kinetochore complex; Westermann et al., 2003). Several transcripts involved with the mitotic spindle were also identified: Cdc31p, required for spindle pole body duplication (Paoletti et al., 2003); Cdc42p, a small GTPase of the Rho family involved in regulation of microtubule attachment to kinetochores (Yasuda et al., 2004); Cdc48p, an AAA-ATPase required for spindle disassembly (Cao et al., 2003); Cdc55p, a protein serine/threonine phosphatase 2A subunit involved in spindle

checkpoint and cytokinesis (Koren et al., 2004) and Tub1p, an α-tubulin (microtubule subunit; Abruzzi et al., 2002). We also found ESTs related to the APC, namely *MAD2*, which encodes a protein inhibitor of APC activity (Sugimoto et al., 2004) and Cdc20p, an activator of APC (Irniger, 2002).

Mitosis comes to an end when daughter nuclei are formed and cells begin to divide in telophase. Exit from mitosis, which in *S. cerevisiae* is accomplished through the inactivation of mitotic CDK, requires two signaling networks: the mitotic exit network (MEN) and the Cdc fourteen early anaphase release (FEAR) network. FEAR and MEN control the activity of the protein phosphatase Cdc14p, which initiates mitotic CDK inactivation by reversing CDK-dependent phosphorylation (Geymonat et al., 2002). Cdc14p is activated by FEAR during early anaphase and by MEN during late anaphase and telophase stages (Seshan and Amon, 2004). In these networks, we identified orthologues for Cdc15p, a MAPKKK family member and a MEN subunit (Bardin et al., 2003), Cdc5p, a polo kinase, which belongs to both FEAR and MEN (Visintin et al., 2003), and Fob1p, a negative regulator of the FEAR network (Stegmeier et al., 2004).

Cytokinesis is the process in which a cell divides into two daughter cells during the mitotic cycle (Glotzer, 2003). Septins, a class of proteins functioning in cytokinesis, were first identified in *S. cerevisiae* (reviewed by Longtine and Bi, 2003), whose genome encodes seven of them (Cdc3p, Cdc10p, Cdc1p1, Cdc12p, Spr28p, Spr3p, and Shs1p/Sep7p). In the *P. brasiliensis* transcriptome, homologues were found for Cdc10p, Cdc11p and Cdc12p. In *C. albicans*, *cdc10*Δ and *cdc11*Δ mutants are capable of hyphal growth, both *in vitro* and *in vivo*, although they are defective for invasive growth and have attenuated virulence in mice (Warenda et al., 2003). It would be interesting to determine whether these proteins are determinants of virulence in *P. brasiliensis* as well.

DNA repair

Mismatch repair

Concerning genes involved in this type of DNA repair in *P. brasiliensis*, we were able to identify only three of the 10 specific activity genes: *MSH1*, *PMS1* and *MLH1*. Interestingly, we also found *MBD4* (a *Homo sapiens* homologue), which is a mismatch-specific thymine DNA glycosylase that interacts with the mismatch repair protein Mlh1p. *MBD4* is not normally found in fungi, but it is commonly found in mammals, which could mean that at some time there was an informational exchange between host and pathogen.

Unpaired and mispaired bases in DNA can arise by replication errors, spontaneous or induced base modifications and during meiotic recombination. The main pathway for correction of mismatches arising during replication is the *MutHLS* pathway, described in *E. coli*, and in related pathways in other organisms. *MutS* initiates repair by binding to the mismatch, and together with *MutL*, activates *MutH* endonuclease, which incises at hemimethylated *dam* sites and thereby mediates strand discrimination. Multiple *MutS* and *MutL* homologues have been found in eukaryotes, playing different roles in the mismatch repair pathway or in recombination. However, no *MutH* homologues have been found in eukaryotes, suggesting that strand discrimination is accomplished in a different way from that found in *E. coli* in these organisms (Marti et al., 2002).

Nuclear MMR can be initiated by the eukaryotic heterodimeric *MutS* homologue complexes *MSH2-MSH6* (base-base mismatches and one unpaired nucleotide) and *MSH2-MSH3* (one unpaired nucleotide small insertion/deletion loops with up to eight unpaired nucleotides). *MSH1*, *MSH2*, *MSH3*, and *MSH6* are involved in the interaction with DNA mismatches. *MSH4* and *MSH5* are meiosis-specific and have a role in recombination, but not in mismatch repair. *MSH1* protects mitochondrial DNA from base substitutions (Marti et al., 2002). The main eukaryotic *MutL* activity is a heterodimer of *MLH1* and *PMS1*, which can interact with MSH complexes. It enhances MSH dimer affinity for DNA mismatches and loops. In the presence of ATP, the MSH-MLH-DNA complex is able to move along the DNA. Other factors affecting eukaryotic MMR are: PCNA (*POL30*), which is involved in mismatch recognition and disruption of the MSH-MLH-DNA ternary complex at the end of repair, DNA polymerases (*POL30*, *POL32* - subunit of DNA Pol δ), which are required for excision and re-synthesis steps, and *EXO1* and *RAD27*, which are the exonucleases that participate in the removal of nucleotides during mismatch repair (Marti et al., 2002). In *S. cerevisiae*, there are six *MutS* homologues (*MSH1-6*) and four *MutL* homologues (*PMS1*, *MLH1-3*) (Goldman et al., 2002).

Base excision repair

In *P. brasiliensis*, we have found all the specific activities of base excision repair (BER): the *TDG*, *NTG1*, *MAG1* and *OGG1*glycosylases, *POLB* polymerase, *NTHL1* lyase, and *LIG3* ligase. There are four steps in this repair pathway: 5' end incision, followed by the excision of the damaged base, resynthesis and ligation. The enzymes responsible for base excision are glycosylases (*OGG1*, *NTH1*, *NTG1*, *MAG*, and *UNG*), which break the base and sugar glycosidic bond, leaving an abasic site. A lyase (*NTHL1* or *NTHL2*) then breaks the phosphodiester bond at the 3' side of the lesion. An apurinic/apyrimidinic (AP) endonuclease (*HAP1* or *APE*) removes the abasic sugar and DNA polymerase β (*POLB*) adds a new nucleotide to the site. Finally, a ligase (*LIG1* or *LIG3*) repairs the gap (Lindahl and Barnes, 2000).

Nucleotide excision repair

Another mechanism by which DNA damage is repaired is the nucleotide excision repair (NER), which involves many different genes and activities. In *S. cerevisiae*, once the lesion is recognized by a multiprotein complex (*RAD14*, *RAD4*, *RAD23*), the damaged strand is cleaved several bases away from the 5' side of the lesion by an endonuclease (*RAD1* or *RAD10*). Another endonuclease (*RAD2*) cuts at the 3' side of the lesion. The strand is unwound by a DNA helicase (*RAD25*, *RAD3*, *SSL1*, or *TFB1-4*). DNA Pol η (*RAD30*) or δ (*POLD1*) removes the nucleotides and fills the gap which is subsequently closed by a ligase. *P. brasiliensis* has orthologues for *RAD2*, *RAD23*, *RAD30*, and *POLD1*. We also found a transcript for *RAD16*, which has the same function as *RAD1*, *RAD8* and *DDB1*. *RAD16* is part of a yeast stable heterotrimeric complex (Rad7/Rad16/Abf1) that works in the conserved global genome repair (GGR) pathway. GGR removes lesions from DNA that is not being actively transcribed (Figure 3). Yu et al. (2004) reported that this protein complex generates superhelicity in DNA through the catalytic activity of the Rad16p component. The torsion thus generated is necessary to remove the damage-containing oligonucleotides during excision in NER. Rad8p is a protein that has both a ring finger and helicase domains. The predicted protein is a member of the *SNF2*

subfamily and shows particular homology to *S. cerevisiae* Rad5p. Double mutant analysis demonstrated that the *rad8* mutant is not epistatic to mutants in the excision repair pathway (*RAD13*) or the checkpoint pathway (*RAD9*). Analysis of radiation sensitivity through the cell cycle indicates that, unlike most other *rad* mutants, *rad8* is most sensitive to irradiation during the G1/S period. DNA damage binding protein (DDB) is a heterodimer composed of two subunits, *DDB1* and *DDB2*. This complex is involved in the recognition of UV-induced DNA damage in *Xeroderma pigmentosum* and Cockayne syndrome. DDB has a specialized role in the recognition of DNA damage at the chromatin level, probably helping the NER complex to gain access to the DNA lesions (Wittschieben and Wood, 2003). We also found *RAD26* in *P. brasiliensis*. In addition to the recognition and excision of DNA damage throughout the genome (GGR), there is also a mechanism, the transcription-coupled nucleotide excision repair (TCR), to recognize some types of DNA damage in the transcribed strand in *E. coli*, yeast and mammalian cells (Figure 3). An obstacle to the repair of the transcribed strand of active genes is the RNA polymerase complex stalled at the sites of DNA damage. The stalled RNA polymerase complex may then mediate the recruitment of repair proteins. Proteins that enable TCR are the Cockayne syndrome B protein (CSB) in humans, and its yeast homologue Rad26. Both CSB and *RAD26* belong to the Swi2/Snf2 family of DNA-dependent ATPases that change DNA accessibility to proteins by altering chromatin structure. As TCR takes place in very localized regions of DNA (i.e., within genes) in wild-type cells, Bucheli and Sweder (2004) proposed that the over-expression of recombinant Rad26p increases the accessibility of the damaged DNA in chromatin, favoring the interaction with repair proteins.

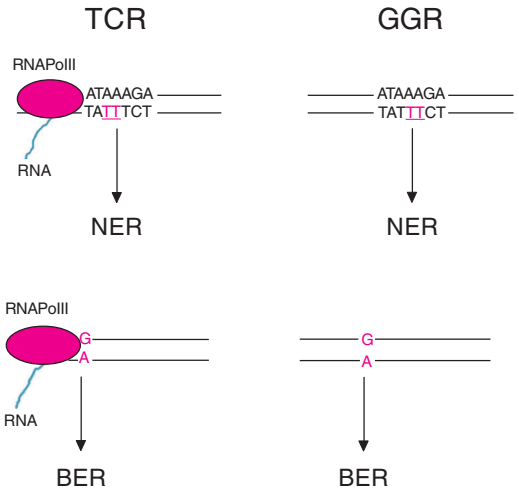

Figure 3. Nucleotide excision repair (NER) and base excision repair (BER) in transcribing (TCR) and non-transcribing (GGR) regions of the genome. TCR = transcription-coupled nucleotide excision repair; GGR = global genome repair.

Recombination repair

Damage in DNA results in base modifications, single- and double-stranded breaks (DSB), and the formation of apurinic/apyrimidinic lesions, many of which are toxic and/or mutagenic (Salmon et al., 2004). DSBs are particularly dangerous because they can cause apoptosis by directly inactivating key genes, or lead to serious chromosomal aberrations. DSBs are repaired

in eukaryotes by the concerted action of two mechanisms. One is based on homologous recombination between sister DNA molecules, and the other on rapid, error-prone non-homologous end-joining (NHEJ - Figure 4A) (Barzilai and Yamamoto, 2004). AP sites, also referred to as abasic sites, are a major type of spontaneous DNA lesion. AP sites are non-coding and highly mutagenic (Zhao et al., 2004).

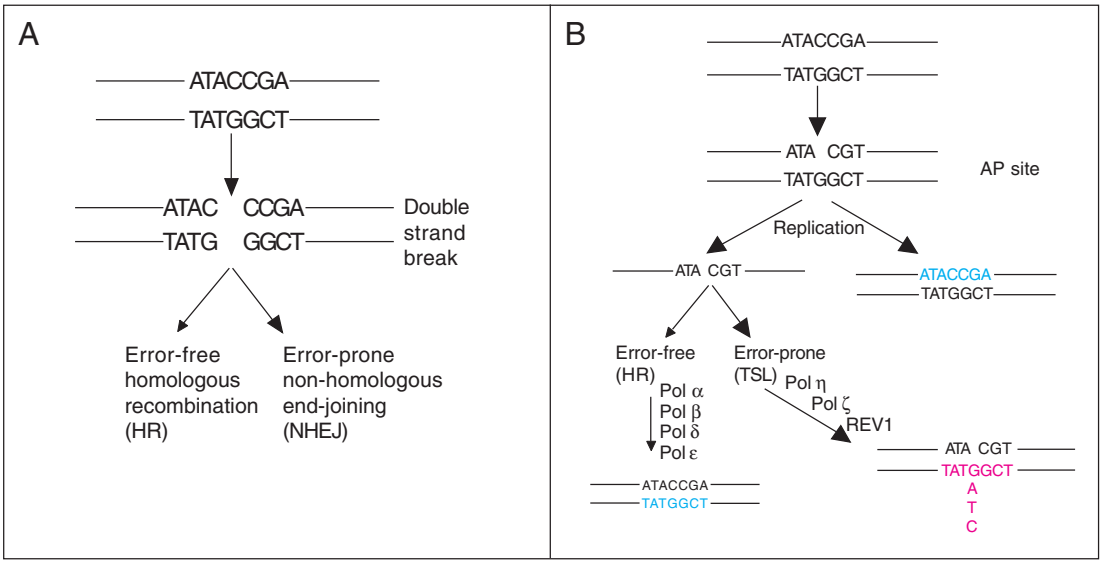

Figure 4. A, Double-strand break repair mechanisms. **B**, Post-replicative apurinic/apyrimidinic (AP) site repair. The undamaged strand is replicated normally, whereas the damaged strand has its replication stalled and the AP site repaired either by homologous recombination with the other replicated double strand or by an error-prone polymerase that inserts any nucleotide opposite the lesion and the damage is repaired either by NER or BER, introducing a mutation as a result.

In *P. brasiliensis*, we found *RAD54*, *RAD52* and *CDC12*, which are necessary for recombination repair during G2 phase. In eukaryotes, two main recombination pathways have been identified. The first, homologous recombination, involves interaction between homologous sequences, whereas the second, NHEJ, involves direct ligation of the strand ends regardless of DNA homology. Studies in *S. cerevisiae* demonstrated that the *RAD52* epistatic group is essential for homologous recombination repair. This group includes several genes, such as *RAD50*, *RAD51*, *RAD52*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *MRE11*, and *XRS2*. *RAD50*, *XRS2* and *MRE11* are believed to form a trimeric protein complex required for the initial processing of DSB by creating 3' single-strand tails that are highly recombinogenic. They attack the homologous intact duplex, searching for strand exchange. This step is mediated by *RAD51*, *RAD52*, *RAD54*, *RAD55*, and *RAD57*. Recombination intermediates produced in this way are then further processed to resolve Holliday junctions and perform DNA synthesis and nick ligation. If the DSB repair takes longer to be processed or no homologous chromosome is available (haploid cells), the damage is repaired by NHEJ, which is error prone since it will ligate two non-related double strands. The NHEJ process is mediated by the DNA-dependent protein kinase catalytic subunit (DNA-PKCs), the Ku70-Ku80 heterodimer that tightly binds DNA ends, and the DNA ligase IV-Xrcc4 complex (Ninomiya et al., 2004). In *P. brasiliensis*, we also found genes involved in NHEJ, such as *BMH1*, *RAD24*, and *DNL4*.

Post-replication repair - translesion synthesis

As for the post-replicative repair mechanism, we found the related genes *RAD6*, *RAD18*, *POLD1* (Pol δ), *REV3* (Pol ζ), *REV1*, and *RAD30* (Pol η) in *P. brasiliensis*. Post-replication repair refers to a network of DNA damage tolerance pathways that allow bacteria and yeast to bypass lesions during replication and correct them later. There are many pathways by which this can be accomplished. The Rad6/Rad18 complex seems to have a central role in organizing post-replication repair in eukaryotes. Translesion synthesis, also referred to as lesion bypass, is the cellular process that directly copies damaged sites of the template during DNA synthesis. It consists of nucleotide insertion opposite to the lesion and extension synthesis from the opposite side of the lesion. According to this definition, a DNA polymerase that performs the insertion step, the extension step, or both, qualifies as a translesion polymerase. Extensive *in vitro* and some *in vivo* studies have indicated that Pol ζ and the Y family polymerases are important translesion polymerases in eukaryotes. Pol ζ belongs to the same B family of DNA polymerases as the replicative Pol α, Pol δ and Pol ε. In *S. cerevisiae*, the Y family consists of Pol η and Rev1. Rev1 possesses a dCMP transferase and is efficient in inserting a C opposite to an AP site *in vitro*, but it cannot catalyze extension from opposite the lesion. However, the combination of Rev1 and Pol ζ results in bypass of the AP site *in vitro* (Figure 4B). Genetic experiments have shown that both Rev1 and Pol ζ are required for AP site-induced mutagenesis in yeast cells. Pol ζ is believed to function in extension synthesis during AP site bypass (Zhao et al., 2004).

CONCLUDING REMARKS

Based on the above findings, we were able to identify the major players in DNA damage repair in *P. brasiliensis*, with the exception of the photo-reactivation repair mechanism. Oxidative DNA damage is mainly repaired by BER, so this kind of repair may play an important role in host defense resistance. Suvarnapunya et al. (2003) suggested that BER-mediated repair might be important for the establishment of *Salmonella enterica serovar Typhimurium* infection. Buchmeier et al. (1995) also suggested that during infection, *Salmonella* cells are subjected to low concentrations of H_2O_2; this situation requires an intact repair system but not a functional catalase. *Paracoccidioides brasiliensis* had shown strong resistance to high concentrations of H_2O_2 (Dantas, A.S., personal communication), which normally would induce oxidative DNA damage. So, BER could be an important mechanism in the response of *P. brasiliensis* against oxidative stress caused by H_2O_2. In a growth-restricting environment, genetic adaptation of a microbial population involves mechanisms (e.g., error-prone polymerases) that lead to an elevated mutation rate. Evidence for the presence of mutagenic pathways in *P. brasiliensis* could explain the high variability observed in the karyotypes of different isolates and the difficulty in determining whether the various *P. brasiliensis* isolates are a single species.

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REFERENCES

Abruzzi, K.C., Magendantz, M. and **Solomon, F.** (2002). An α-tubulin mutant demonstrates distinguishable functions among the spindle assembly checkpoint genes in *Saccharomyces cerevisiae*. *Genetics 161*: 983-994.

Andreassen, P.R., Lohez, O.D. and **Margolis, R.L.** (2003). G2 and spindle assembly checkpoint adaptation, and tetraploidy arrest: implications for intrinsic and chemically induced genomic instability. *Mutat. Res*. *532*: 245-253.

Aparicio, O.M., Weinstein, D.M. and **Bell, S.P.** (1997). Components and dynamics of DNA replication complexes in *S. cerevisiae*: Redistribution of MCM proteins and Cdc45p during S phase. *Cell 91*: 59-69.

Bae, S.H. and **Seo, Y.S.** (2000). Characterization of the enzymatic properties of the yeast dna2 Helicase/endonuclease suggests a new model for Okazaki fragment processing. *J. Biol. Chem*. *275*: 38022-38031.

Bae, S.H., Bae, K.H., Kim, J.A. and **Seo, Y.S.** (2001). RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. *Nature 412*: 456-461.

Bambara, R.A. and **Huang, L.** (1995). Reconstitution of mammalian DNA replication. *Prog. Nucleic Acid Res. Mol. Biol. 51*: 93-122.

Bambara, R.A., Murante, R.S. and **Henricksen, L.A.** (1997). Enzymes and reactions at the eukaryotic DNA replication fork. *J. Biol. Chem. 272*: 4647-4650.

Bardin, A.J., Boselli, M.G. and **Amon, A.** (2003). Mitotic exit regulation through distinct domains within the protein kinase Cdc15. *Mol. Cel. Biol. 23*: 5018-5030.

Barzilai, A. and **Yamamoto, K.** (2004). DNA damage responses to oxidative stress. *DNA Repair 3*: 1109-1115.

Bell, S.P. and **Dutta, A.** (2002). DNA replication in eukaryotic cells. *Annu. Rev. Biochem*. *71*: 333-374.

Bousset, K. and **Diffley, J.F.** (1998). The Cdc7 protein kinase is required for origin firing during S phase. *Genes Dev*. *12*: 480-490.

Braun, K.A., Lao, Y., He, Z., Ingles, C.J. and **Wold, M.S.** (1997). Role of protein-protein interactions in the function of replication protein A (RPA): RPA modulates the activity of DNA polymerase alpha by multiple mechanisms. *Biochemistry 36*: 8443-8454.

Bucheli, M. and **Sweder, K.** (2004). In UV-irradiated *Saccharomyces cerevisiae*, overexpression of Swi2/Snf2 family member Rad26 increases transcription-coupled repair and repair of the non-transcribed strand. *Mol. Microbiol. 52*: 1653-1663.

Buchmeier, N.A., Libby, S.J., Xu, Y., Loewen, P.C., Switala, J., Guiney, D.G. and **Fang, F.C.** (1995) DNA repair is more important than catalase for *Salmonella* virulence in mice. *J. Clin. Invest. 95*: 1047-1053.

Budd, M.E. and **Campbell, J.L.** (1995). A yeast gene required for DNA replication encodes a protein with homology to DNA helicases. *Proc. Nat. Acad. Sci. USA 92*: 7642-7646.

Budd, M.E., Choe, W.C. and **Campbell, J.L.** (1995). DNA2 encodes a DNA helicase essential for replication of eukaryotic chromosomes. *J. Biol. Chem. 270*: 26766-26769.

Bullock, P.A., Seo, Y.S. and **Hurwitz, J.** (1991). Initiation of simian virus 40 DNA synthesis *in vitro*. *Mol. Cell. Biol*. *11*: 2350-2361.

Burgers, P.M.J. (1998). Eukaryotic DNA polymerases in DNA replication and DNA repair. *Chromosoma 107*: 218-227.

Cao, K., Nakajima, R., Meyer, H.H. and **Zheng, Y.** (2003). The AAA-ATPase Cdc48/p97 regulates spindle disassembly at the end of mitosis. *Cell 115*: 355-367.

Challberg, M.D. and **Kelly, T.J.** (1989). Animal virus DNA replication. *Annu. Rev. Biochem*. *58*: 671-717.

Davey, M.J., Indiani, C. and **O'Donnell, M.** (2003). Reconstitution of the Mcm2-7p heterohexamer, subunit arrangement, and ATP site architecture. *J. Biol. Chem*. *278*: 4491-4499.

DeSalle, L.M. and **Pagano, M.** (2001). Regulation of the G1 to S transition by the ubiquitin pathway. *FEBS Lett*. *490*: 179-189.

Diffley, J.F.X. (1996). Once and only once upon a time: specifying and regulating origins of DNA replication in eukaryotic cells. *Genes Dev*. *10*: 2819-2830.

Doetsch, P.W., Morey, N.J., Swanson, R.L. and **Jinks-Robertson, S.** (2001). Yeast base excision repair: interconnections and networks. *Prog. Nucleic Acid Res. Mol. Biol*. *68*: 29-39.

Donovan, S., Harwood, J., Drury, L.S. and **Diffley, J.F.X.** (1997). Cdc6p-dependent loading of Mcm proteins onto pre-replicative chromatin in budding yeast. *Proc. Natl. Acad. Sci. USA 94*: 5611-5616.

Elledge, S.J. (1996). Cell cycle checkpoints: preventing an identity crisis. *Science 274*: 1664-1671.

Felipe, M.S., Andrade, R.V., Petrofeza, S.S., Maranhão, A.Q., Torres, F.A., Albuquerque, P., Arraes,

F.B.M., Arruda, M., Azevedo, M.O., Baptista, A.J., Bataus, L.A., Borges, C.L., Campos, E.G., Cruz, M.R., Daher, B.S., Dantas, A., Ferreira, M.A., Ghil, G.V., Jesuino, R.S., Kyaw, C.M., Leitão, L., Martins, C.R., Moraes, L.M., Neves, E.O., Nicola, A.M., Alves, E.S., Parente, J.A., Pereira, M., Pocas-Fonseca, M.J., Resende, R., Ribeiro, B.M., Saldanha, R.R., Santos, S.C., Silva-Pereira, I., Silva, M.A., Silveira, E., Simões, I.C., Soares, R.B., Souza, D.P., De-Souza, M.T., Andrade, E.V., Xavier, M.A., Veiga, H.P., Venancio, E.J., Carvalho, M.J., Oliveira, A.G., Inoue, M.K., Almeida, N.F., Walter, M.E., Soares, C.M. and **Brigido, M.M.** (2003). Transcriptome characterization of the dimorphic and pathogenic fungus *Paracoccidioides brasiliensis* by EST analysis. *Yeast 20*: 263-271.

Felipe, M.S., Andrade, R.V., Arraes, F.B., Nicola, A.M., Maranhao, A.Q., Torres, F.A., Silva-Pereira, I., Pocas-Fonseca, M.J., Campos, E.G., Moraes, L.M., Andrade, P.A., Tavares, A.H., Silva, S.S., Kyaw, C.M., Souza, D.P., Network P., Pereira, M., Jesuino, R.S., Andrade, E.V., Parente, J.A., Oliveira, G.S., Barbosa, M.S., Martins, N.F., Fachin, A.L., Cardoso, R.S., Passos, G.A., Almeida, N.F., Walter, M.E., Soares, C.M., Carvalho, M.J. and **Brigido, M.M.** (2005). Transcriptional profiles of the human pathogenic fungus *Paracoccidioides brasiliensis* in mycelium and yeast cells. *J. Biol. Chem. 280*: 24706-24714.

Fien, K. and **Stillman, B.** (1992). Identification of replication factor C from *Saccharomyces cerevisiae*: a component of the leading-strand DNA replication complex. *Mol. Cell. Biol. 12*: 155-163.

Foiani, M., Marini, F., Gamba, D., Lucchini, G. and **Plevani, P.** (1994). The B subunit of the DNA polymerase alpha-primase complex in *Saccharomyces cerevisiae* executes an essential function at the initial stage of DNA replication. *Mol. Cell. Biol. 14*: 923-933.

Forsburg, S. and **Nurse, P.** (1991). Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Annu. Rev. Cell. Biol. 7*: 227.

Gassmann, R., Vagnarelli, P., Hudson, D. and **Earnshaw, W.C.** (2004). Mitotic chromosome formation and the condensin paradox. *Exp. Cell Res. 296*: 35-42.

Geymonat, M., Jensen, S. and **Johnston, L.H.** (2002). Mitotic exit: the Cdc14 double cross. *Curr. Biol. 12*: R482-R484.

Giaever, G., Chu, A.M., Ni, L., Connelly, C., Riles , L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., André, B., Arkin, A.P., Astromoff, A., El-Bakkoury, M., Bangham, R., Benito, R., Brachat, S., Campanaro, S., Curtiss, M., Davis, K., Deutschbauer, A., Entian, K.D., Flaherty, P., Foury, F., Garfinkel, D.J., Gerstein, M., Gotte, D., Guldener, U., Hegemann, J.H., Hempel, S., Herman, Z., Jaramillo, D.F., Kelly, D.E., Kelly, S.L., Kotter, P., LaBonte, D., Lamb, D.C., Lan, N., Liang, H., Liao, H., Liu, L., Luo, C., Lussie, M., Mao, R., Menard, P., Ooi, S.L., Revuelta, J.L., Robert, C.J., Rose, M., Ross-MacDonald, P., Scherens, B., Schimmack, G., Shafer, B., Shoemaker, D.D., Sookhai-Mahadeo, S., Storms, R.K., Strathern, J.N., Valle, G., Voet, M., Volckaert, G., Wang, C.Y., Ward, T.R., Wilhelmy, J., Winzeler, E.A., Yang, Y., Yen, G., Youngman, E., Yu, K., Bussey, H., Boeke, J.D., Snyder, M., Philippsen, P., Davis, R.W. and **Johnston, M.** (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature 418*: 387-391.

Glotzer, M. (2003). Cytokinesis: progress on all fronts. *Curr. Opin. Cell Biol. 15*: 684-690.

Goh, P.Y. and **Surana, U.** (1999). Cdc4, a protein required for the onset of S phase, serves an essential function during G(2)/M transition in *Saccharomyces cerevisiae*. *Mol. Cell. Biol. 19*: 5512-5522.

Goldman, G.H., McGuire, S.L. and **Harris, S.D.** (2002). The DNA damage response in filamentous fungi. *Fungal Genet. Biol. 35*: 183-195.

Grossi, S., Puglisi, A., Dmitriev, P.V., Lopes, M. and **Shore, D.** (2004). Pol12, the B subunit of DNA polymerase α, functions in both telomere capping and length regulation. *Genes Dev. 18*: 992-1006.

Homesley, L., Lei, M., Kawasaki, Y., Sawyer, S., Christensen, T. and **Tye, B.K.** (2000). Mcm10 and the MCM2-7 complex interact to initiate DNA synthesis and to release replication factors from origins. *Genes Dev. 14*: 913-926.

Hubscher, U. and **Seo, Y.S.** (2001). Replication of the lagging strand: a concert of at least 23 polypeptides. *Mol. Cell 12*: 149-157.

Hughes, D.A., MacNeill, S.A. and **Fantes, P.A.** (1992). Molecular cloning and sequence analysis of cdc27+ required for the G2-M transition in the fission yeast *Schizosaccharomyces pombe*. *Mol. Gen. Genet. 231*: 401-410.

Irniger, S. (2002). Cyclin destruction in mitosis: a crucial task of Cdc20. *FEBS Lett. 532*: 7-11.

Ishimi, Y. (1997). A DNA helicase activity is associated with an MCM4, -6, and -7 protein complex. *J. Biol. Chem. 272*: 24508-24513.

Ishimi, Y., Komamura, Y., You, Z. and **Kimura, H.** (1998). Biochemical function of mouse minichromosome maintenance 2 protein. *J. Biol. Chem. 273*: 8369-8375.

Jares, P., Donaldson, A. and **Blow, J.J.** (2000). The Cdc7/Dbf4 protein kinase: target of the S phase checkpoint? *EMBO Rep. 41*: 319-322.

Kaldis, P., Sutton, A. and **Solomon, M.J.** (1996). The Cdk-activating kinase (CAK) from budding yeast. *Cell 86*: 553-564.

Kearsey, S.E. and **Labib, K.** (1998). MCM proteins: evolution, properties, and role in DNA replication. *Biochim. Biophys. Acta 1398*: 113-136.

Kelman, Z. (1997). PCNA: structure, functions and interactions. Oncogene *14*: 629-640.

Koren, R., Rainis, L. and **Kleinberger, T.** (2004). The scaffolding A/Tpd3 subunit and high phosphatase activity are dispensable for Cdc55 function in the *Saccharomyces cerevisiae* spindle checkpoint and in cytokinesis. *J. Biol. Chem. 279*: 48598-48606.

Labib, K. and **Diffley, J.F.** (2001). Is the MCM2-7 complex the eukaryotic DNA replication fork helicase? *Curr. Opin. Genet. Dev. 10*: 64-70.

Labib, K., Tercero, J.A. and **Diffley, J.F.** (2000). Uninterrupted MCM2-7 function required for DNA replication fork progression. *Science 288*: 1643-1647.

Le Masson, I., Saveanu, C., Chevalier, A., Namane, A., Gobin, R., Fromont-Racine, M., Jacquier, A. and **Mann, C.** (2002). Spc24 interacts with Mps2 and is required for chromosome segregation, but is not implicated in spindle pole body duplication. *Mol. Microbiol. 43*: 1431-1443.

Lee, J.K. and **Hurwitz, J.** (2000). Isolation and characterization of various complexes of the minichromosome maintenance proteins of *Schizosaccharomyces pombe*. *J. Biol. Chem. 275*: 18871-18878.

Lee, S.H., Kwong, A.D., Pan, Z.Q. and **Hurwitz, J.** (1991). Studies on the activator 1 protein complex, an accessory factor for proliferating cell nuclear antigen-dependent DNA polymerase δ. *J. Biol. Chem. 266*: 594-602.

Lei, M., Kawasaki, Y., Young, M.R., Kihara, M., Sugino, A. and **Tye, B.K.** (1997). Mcm2 is a target of regulation by Cdc7-Dbf4 during the initiation of DNA synthesis. *Genes Dev. 11*: 3365-3374.

Lew, D.J. and **Burke, D.J.** (2003). The spindle assembly and spindle position checkpoints. *Annu. Rev. Genet. 37*: 251-282.

Lew, D.J., Weinert, T. and **Pringle, J.R.** (1997). Cell cycle control in *Saccharomyces cerevisiae*. In: *The Molecular and Cellular Biology of the Yeast Saccharomyces: Cell Cycle and Cell Biology* (Pringle, J.R., Broach, J.R. and Jones, E.W., eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, pp. 607-695.

Lindahl, T. and **Barnes, D.E.** (2000). Repair of endogenous DNA damage. *Cold Spring Harb. Symp. Quant. Biol. 65*: 127-133.

Longtine, M.S. and **Bi, E.** (2003). Regulation of septin organization and function in yeast. *Trends in Cell Biol. 13*: 403-409.

MacNeill, S.A. (2001). DNA replication: partners in the Okazaki two-step. *Curr. Biol. 11*: R842-R844.

MacNeill, S.A., Moreno, S., Reynolds, N., Nurse, P. and **Fantes, P.A.** (1996). The fission yeast Cdc1 protein, a homologue of the small subunit of DNA polymerase delta, binds to Pol3 and Cdc27. *EMBO J. 15*: 4613-4628.

Marti, T.M., Kunz, C. and **Fleck, O.** (2002). DNA mismatch repair and mutation avoidance pathways. *J. Cell Physiol. 191*: 28-41.

McAinsh, A.D., Tytell, J.D. and **Sorger, P.K.** (2003). Structure, function, and regulation of budding yeast kinetochores. *Annu. Rev. Cell Dev. Biol. 19*: 519-539.

Merchant, A.M., Kawasaki, Y., Chen, Y., Lei, M. and **Tye, B.K.** (1997). A lesion in the DNA replication initiation factor Mcm10 induces pausing of elongation forks through chromosomal replication origins in *S. cerevisiae*. *Mol. Cell. Biol. 17*: 3261-3271.

Misu, K., Fujimura-Kamada, K., Ueda, T., Nakano, A., Katoh, H. and **Tanaka, K.** (2003). Cdc50p, a conserved endosomal membrane protein, controls polarized growth in *Saccharomyces cerevisiae*. *Mol. Biol. Cell 14*: 730-747.

Mossi, R., Keller, R.C., Ferrari, E. and **Hubscher, U.** (2000). DNA polymerase switching: II. Replication factor C abrogates primer synthesis by DNA polymerase α at a critical length. *J. Mol. Biol. 295*: 803-814.

Murakami, Y., Eki, T. and **Hurwitz, J.** (1992). Studies on the initiation of simian virus 40 replication *in vitro*: RNA primer synthesis and its elongation. *Proc. Natl. Acad. Sci. USA 89*: 952-956.

Nasmyth, K. (1996). Putting the cell cycle in order. *Science 274*: 1643-1645.

Ninomiya, Y., Suzuki, K., Ishii, C. and **Inoue, H.** (2004). Highly efficient gene replacements in *Neurospora* strains deficient for nonhomologous end-joining. *Proc. Natl. Acad. Sci. USA 101*: 12248-12253.

Nitiss, J.L. (1998). Investigating the biological functions of DNA topoisomerases in eukaryotic cells. *Biochem. Biophys. Acta 1400*: 63-81.

Owens, J.C., Detweiler, C.S. and **Li, J.J.** (1997). CDC45 is required in conjunction with CDC7/DBF4 to trigger the initiation of DNA replication. *Proc. Natl. Acad. Sci. USA 94*: 12521-12526.

Pacek, M. and **Walter, J.C.** (2004). A requirement for MCM7 and Cdc45 in chromosome unwinding during

eukaryotic DNA replication. *EMBO J*. *26*: 1-10.

Paoletti, A., Bordes, N., Haddad, R., Schwartz, C.L., Chang, F. and **Bornens, M.** (2003). Fission yeast cdc31p is a component of the half-bridge and controls SPB duplication. *Mol. Biol. Cell 14*: 2793-2808.

Peters, J.M. (2002). The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol. Cell 9*: 931-943.

Podust, V.N., Georgaki, A., Strack, B. and **Hubscher, U.** (1992). Calf thymus RF-C as an essential component for DNA polymerase δ and ε holoenzymes function. *Nucleic Acids Res. 20*: 4159-4165.

Prelich, G. and **Stillman, B.** (1988). Coordinated leading and lagging strand synthesis during SV40 DNA replication *in vitro* requires PCNA. *Cell 53*: 117-126.

Ryu, G.H., Tanaka, H., Kim, D.H., Kim, J.H., Bae, S.H., Kwon, Y.N., Rhee, J.S., MacNeill, S.A. and **Seo, Y.S.** (2004). Genetic and biochemical analyses of Pfh1 DNA helicase function in fission yeast. *Nucleic Acids Res*. *32*: 4205-4216.

Salmon, T.B., Evert, B.A., Song, B. and **Doetsch, P.W.** (2004). Biological consequences of oxidative stress-induced DNA damage in *Saccharomyces cerevisiae*. *Nucleic Acids Res. 32*: 3712-3723.

San-Blas, G., Niño-Vega, G. and **Iturriaga, T.** (2002). Paracoccidioides brasiliensis and paracoccidioidomycosis: Molecular approaches to morphogenesis, diagnosis, epidemiology, taxonomy and genetics. *Med. Mycol. 40*: 225-242.

Santocanale, C., Locati, F., Falconi, M.M., Piseri, A., Tseng, B.Y., Lucchini, G. and **Plevani, P.** (1992). Overproduction and functional analysis of DNA primase subunits from yeast and mouse. *Gene 113*: 199-205.

Seshan, A. and **Amon, A.** (2004). Linked for life: temporal and spatial coordination of late mitotic events. *Curr. Opin. Cell Biol. 16*: 41-48.

Siegmund, R.F. and **Nasmyth, K.A.** (1996). The *Saccharomyces cerevisiae* start-specific transcription factor Swi4 interacts through the ankyrin repeats with the mitotic Clb2/Cdc28 kinase and through its conserved carboxy terminus with Swi6. *Mol. Cel. Biol 16*: 2647-2655.

Song, S., Grenfell, T.Z., Garfield, S., Erikson, R.L. and **Lee, K.S.** (2000). Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol. Cell. Biol*. *20*: 286-298.

Stegmeier, F., Huang, J., Rahal, R., Zmolik, J., Moazed, D. and **Amon, A.** (2004). The replication fork block protein Fob1 functions as a negative regulator of the FEAR network. *Curr. Biol. 14*: 467-480.

Stillman, B. (1989). Initiation of eukaryotic DNA replication *in vitro*. *Annu. Rev. Cell Biol*. *5*: 197-245.

Stillman, B. (1996). Cell cycle control of DNA replication. *Science 274*: 1659-1664.

Sugimoto, I., Murakami, H., Tonami, Y., Moriyama, A. and **Nakanishi, M.** (2004). DNA replication checkpoint control mediated by the spindle checkpoint protein Mad2p in fission yeast. *J. Biol. Chem. 279*: 47372-47378.

Suvarnapunya, A.E., Lagasse, H.A. and **Stein, M.A.** (2003). The role of DNA base excision repair in the pathogenesis of *Salmonella enterica serovar Typhimurium*. *Mol. Microbiol. 48*: 549-559.

Takenaka, H., Makise, M., Kuwae, W., Takahashi, N., Tsuchiya, T. and **Mizushima, T.** (2004). ADP-binding to origin recognition complex of *Saccharomyces cerevisiae*. *J. Mol. Biol*. *340*: 29-37.

Tercero, J.A., Labib, K. and **Diffley, J.F.** (2000). DNA synthesis at individual replication forks requires the essential initiation factor Cdc45p. *EMBO J. 19*: 2082-2093.

Tsurimoto, T. and **Stillman, B.** (1990). Functions of replication factor C and proliferating-cell nuclear antigen: functional similarity of DNA polymerase accessory proteins from human cells and bacteriophage T4. *Proc. Natl. Acad. Sci. USA 87*: 1023-1027.

Tsurimoto, T. and **Stillman, B.** (1991a). Replication factors required for SV40 DNA replication *in vitro*. I. DNA structure-specific recognition of a primer-template junction by eukaryotic DNA polymerases and their accessory proteins. *J. Biol. Chem*. *266*: 1950-1960.

Tsurimoto, T. and **Stillman, B.** (1991b). Replication factors required for SV40 DNA replication *in vitro*. II. Switching of DNA polymerase α and δ during initiation of leading and lagging strand synthesis. *J. Biol. Chem. 266*: 1961-1968.

Tsurimoto, T., Melendy, T. and **Stillman, B.** (1990). Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. *Nature 346*: 534-539.

Turchi, J.J., Huang, L., Murante, R.S., Kim, Y. and **Bambara, R.A.** (1994). Enzymatic completion of mammalian lagging-strand DNA replication. *Proc. Natl. Acad. Sci. USA 91*: 9803-9807.

Tyson, J.J. and **Novak, B.** (2001). Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions. *J. Theor. Biol*. *210*: 249-263.

Visintin, R., Stegmeier, F. and **Amon, A.** (2003). The role of the polo kinase Cdc5 in controlling Cdc14

localization. *Mol. Biol. Cell 14*: 4486-4498.
Waga, S. and **Stillman, B.** (1994). Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. *Nature 369*: 207-212.
Waga, S. and **Stillman, B.** (1998). The DNA replication fork in eukaryotic cells. *Annu. Rev. Biochem. 67*: 721-751.
Walter, J. and **Newport, J.** (2000). Initiation of eukaryotic DNA replication: origin unwinding and sequential chromatin association of Cdc45, RPA, and DNA polymerase alpha. *Mol. Cell 5*: 617-627.
Wang, J.C. (1996). DNA topoisomerases. *Annu. Rev. Biochem. 65*: 635-692.
Wang, Z., Castaño, I.B., Adams, C., Vu, C., Fitzhugh, D. and **Christman, M.F.** (2002). Structure/function analysis of the *Saccharomyces cerevisiae* Trf4/Pol σ DNA polymerase. *Genetics 160*: 381-391.
Warenda, A.J., Kauffman, S., Sherrill, T.P., Becker, J.M. and **Konopka, J.B.** (2003). *Candida albicans* septin mutants are defective for invasive growth and virulence. *Infect. Immun. 71*: 4045-4051.
Weinberg, D.H. and **Kelly, T.J.** (1989). Requirement for two DNA polymerases in the replication of simian virus 40 DNA *in vitro*. *Proc. Natl. Acad. Sci. USA 86*: 9742-9746.
Westermann, S., Cheeseman, I.M., Anderson, S., Yates III, J.R., Drubin, D.G. and **Barnes, G.** (2003). Architecture of the budding yeast kinetochore reveals a conserved molecular core. *J. Cell Biol. 163*: 215-222.
Wittschieben, B.O. and **Wood, R.D.** (2003). DDB complexities. *DNA Repair 2*: 1065-1069.
Yasuda, S., Oceguera-Yanez, F., Kato, T., Okamoto, M., Yonemura, S., Terada, Y., Ishizaki, T. and **Narumiya, S.** (2004). Cdc42 and mDia3 regulate microtubule attachment to kinetochores. *Nature 428*: 767-771.
You, Z., Komamura, Y. and **Ishimi, Y.** (1999). Biochemical analysis of the intrinsic Mcm4-Mcm6-Mcm7 DNA helicase activity. *Mol. Cell. Biol. 19*: 8003-8015.
Yu, S., Owen-Hughes, T., Friedberg, E.C., Waters, R. and **Reed, S.H.** (2004). The yeast Rad7/Rad16/Abf1 complex generates superhelical torsion in DNA that is required for nucleotide excision repair. *DNA Repair 3*: 277-287.
Zachariae, W. and **Nasmyth, K.** (1999). Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Dev. 13*: 2039-2058.
Zhao, B., Xie, Z., Shen, H. and **Wang, Z.** (2004). Role of DNA polymerase eta in the bypass of abasic sites in yeast cells. *Nucleic Acids Res. 32*: 3984-3994.
Zou, L., Mitchell, J. and **Stillman, B.** (1997). *CDC45*, a novel yeast gene that functions with the origin recognition complex and Mcm proteins initiation of DNA replication. *Mol. Cell. Biol. 17*: 553-563.
Zuo, S., Gibbs, E., Kelman, Z., Wang, T.S., O'Donnell, M., MacNeill, S.A. and **Hurwitz, J.** (1997). DNA polymerase delta isolated from *Schizosaccharomyces pombe* contains five subunits. *Proc. Natl. Acad. Sci. USA 94*: 11244-11249.