Two different Swi5-containing protein complexes are involved in mating-type switching and recombination repair in fission yeast

Yufuko Akamatsu*†, Dorota Dziadkowiec†‡, Mitsunori Ikeguchi*, Hideo Shinagawa†, and Hiroshi Iwasaki*§

*Graduate School of Integrated Science, Yokohama City University, 1-7-29, Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan; †Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan; and ‡Institute of Microbiology, Wroclaw University Przybyszewskiego 63/77, Wroclaw 51-148, Poland

Edited by Kiyoshi Mizuuchi, National Institutes of Health, Bethesda, MD, and approved October 24, 2003 (received for review May 14, 2003)

Homologous recombination is an important biological process that occurs in all organisms and facilitates genome rearrangements and repair of DNA double-strand breaks. Eukaryotic Rad51 proteins (Rad51^{sp} or Rhp51 in fission yeast) are functional and structural homologs of bacterial RecA protein, an evolutionarily conserved protein that plays a key role in homologous pairing and strand exchange between homologous DNA molecules *in vitro*. Here we show that the fission yeast *swi5*$^{+}$ gene, which was originally identified as a gene required for normal mating-type switching, encodes a protein conserved among eukaryotes and is involved in a previously uncharacterized Rhp51 (Rad51^{sp})-dependent recombination repair pathway that does not require the Rhp55/57 (Rad55/57^{sp}) function. Protein interactions with both Swi5 and Rhp51 were found to be mediated by a domain common to Swi2 and Sfr1 (*S*wi *f*ive-dependent *r*ecombination repair protein *1*, a previously uncharacterized protein with sequence similarity to the C-terminal part of Swi2). Genetic epistasis analyses suggest that the Swi5–Sfr1–Rhp51 interactions function specifically in DNA recombination repair, whereas the Swi5–Swi2–Rhp51 interactions may function, together with chromodomain protein Swi6 (HP1 homolog), in mating-type switching.

DNA double-strand breaks (DSBs) are caused by DNA-damaging agents, such as ionizing irradiation, and also arise during normal DNA replication. These accidentally generated DSBs are critical genotoxic lesions in all organisms. However, the DSBs can be repaired efficiently by several cellular mechanisms that maintain genome stability. Homologous recombination is one of the most important pathways for DSB repair.

Eukaryotic Rad51 protein is regarded as the structural and functional counterpart of the *Escherichia coli* RecA protein and plays a central role in homologous recombination in eukaryotic cells (1). Purified Rad51 forms nucleoprotein filaments, like RecA (2), and promotes homologous pairing and strand exchange between homologous DNA strands *in vitro* (3, 4). However, eukaryotes possess additional RecA-like proteins such as Rad55^{sc}/Rhp55 (Rad55^{sp}) and Rad57^{sc}/Rhp57 (Rad57^{sp}) in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and Rad51B, Rad51C, Rad51D, Xrcc2, and Xrcc3 in vertebrates (1).

Biochemical studies have demonstrated that these RecA paralogs stimulate Rad51-mediated strand exchange (1, 5, 6). Rad55 (Rhp55) and Rad57 (Rhp57) form heterodimers and bind to Rad51 (Rhp51) (7, 8). Null mutants of the *S. pombe rhp51* gene are hypersensitive to DNA-damaging agents, and *rhp51* mutations are epistatic to *rhp55* or *rhp57* mutations. In addition, paralog mutants are cold-sensitive, and overexpression of Rad51 (Rhp51) partially suppresses their DNA repair defects (9–12). Furthermore, the formation of Rhp51 foci in irradiated *S. pombe* cells depends on the *rhp55* function (13). The dependence of Rad51 foci on paralogs has also been demonstrated in *S. cerevisiae* (14) and in chicken DT40 cells (12, 15). These observations are consistent with the idea that these paralogs function as accessory proteins for Rad51 (Rhp51)-mediated homologous strand exchange by facilitating the formation of Rad51 (Rhp51) nucleoprotein filaments. However, the fact that the damage-sensitivity of *rhp55* and *rhp57* mutants is much more moderate than that of *rhp51* mutants (11, 16) in *S. pombe* also suggests the possibility that there is some factor other than the paralogs that facilitates formation of the Rhp51 filaments.

Whereas accidental DSBs are serious threats to all organisms, spatiotemporally regulated DSBs also play very important biological roles in the initiation of genetic rearrangements such as meiotic recombination (17), V(D)J recombination (18), and mating-type switching of budding yeast (19). Highly regulated specific endonucleases, Spo11, Rag1/Rag2, and HO nuclease, respectively, produce the DSBs that initiate DNA rearrangement in these cases.

S. pombe mating-type switching is also assumed to be initiated by a transient DSB associated with DNA replication, although an HO-type endonuclease has not been identified. *S. pombe* normally has haploid cells of two mating types called *P* (plus) and *M* (minus), which differ at the *mat1* locus. After two consecutive asymmetric divisions, only one of four granddaughter cells undergoes a mating-type switch (20), in which genetic information is transferred to *mat1* from either one of two silent cassettes (*mat2-P* and *mat3-M*). This switching pattern probably results from an imprinting event at *mat1* that marks one of the two sister chromatids in a strand-specific manner. The imprint locus allows the formation of a DSB during replication, and this DSB plays an important role in initiating mating-type switching *in vivo* (refs. 21 and 22; and for a review, see ref. 23).

Previous genetic studies resulted in the isolation of 10 *swi* mutants and one recombination repair mutant, *rad22*, with a reduced rate of mating-type switching (*swi* mutants) and showed that they fall into three classes (Ia, Ib, and II) based on their phenotype (24, 25). Class Ia mutants (*swi1*, *swi3*, and *swi7*) have a reduced frequency of the switch-specific imprint (24, 26). Class Ib mutants (*swi2*, *swi5*, and *swi6*) have normal imprinting but switch with reduced frequency, probably because of inefficient and/or improper utilization of the DSB for gene conversion (21, 24, 27). Class Ia mutations are epistatic to class Ib mutations. Interestingly, *swi6*$^{+}$ encodes a chromodomain protein homologous to *Drosophila* HP1 and is involved in silencing at the *mat* locus (28). Like class Ib mutants, class II (*swi4*, *swi8*, *swi9*, *swi10*, and *rad22*) mutants also have normal imprinting but produce a high proportion of heterothallic progeny containing extensive DNA rearrangements at the mating-type locus. The genes in this class may be involved in the processing of intermediates formed during mating-type switching (24).

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: DO, dropout; DSB, double-strand break.

Data deposition: The sequences reported in this paper have been deposited in the DDBJ/EMBL/GenBank databases [accession nos. AB089498 (*swi5* genomic DNA), AB089499 (*swi5* cDNA), and AB089500 (*sfr1* genomic DNA and cDNA sequences)].

§To whom correspondence should be addressed. E-mail: iwasaki@tsurumi.yokohama-cu.ac.jp.

S. pombe Rad22 is a homolog of Rad52 that plays an important role in homologous recombination and DSB repair in *S. cerevisiae* (29), and *swi5* is the only *swi* mutant that is deficient in meiotic homologous recombination and DSB repair (30). Thus, we assumed that *swi5* might yield clues about the mechanistic connection between the two different but related DNA rearrangement reactions. Here, we show that *swi5*$^+$ is involved in a previously uncharacterized Rhp51-dependent recombination repair pathway that does not require the Rhp55/57 dimer. We found that protein interactions with both Swi5 and Rhp51 were mediated by a domain common to Swi2 and Sfr1 (a previously uncharacterized protein with sequence similarity to the C-terminal part of Swi2). The Sfr1-dependent interaction functions specifically in DNA recombination repair, whereas the Swi2-dependent interaction functions together with Swi6 in mating-type switching.

Materials and Methods

S. pombe Strains, Culture Conditions, and Genetic Methods. *S. pombe* strains used in this study were; SP157 (*h*^{90} *leu1-32 ura4*-D18), SP168 (*h*^{90} *swi5-39 leu1-32 ura4-D*18), YA119 (*Msmt-0 leu1-32 ura4-D18 his3-D1 arg3-D1*), T3 (as YA119 but *rhp51*Δ::*his3*$^+$), T5 (as YA119 but *rhp57*Δ::*his3*$^+$), YA177 (as YA119 but *swi5*Δ::*his3*$^+$), YA244 (as YA119 but *swi5*Δ::*ura4*$^+$ *rhp51*Δ::*his3*$^+$), YA250 (as YA119 but *swi5*Δ::*ura4*$^+$ *rhp57*Δ::*his3*$^+$), YA431 (as YA119 but *sfr1*Δ::*ura4*$^+$), YA452 (as YA119 but *sfr1*Δ::*ura4*$^+$ *swi5*Δ::*his3*$^+$), YA424 (as YA119 but *swi5*Δ::*ura4*$^+$ *rhp51*Δ::*his3*$^+$ *rhp57*Δ::*his3*$^+$), YA455 (as YA254 but *sfr1*Δ::*ura4*$^+$), YA474 (as YA119 but *sfr1*Δ::*ura4*$^+$ *rhp51*Δ::*his3*$^+$), YA478 (as YA119 but *rhp57*Δ::*his3*$^+$ *sfr1*Δ::*ura4*$^+$), YA254 (*h*^{90} *leu1-32 ura4-D18 his3-D1 arg3*-D1), YA175 (as YA254 but *swi5*Δ::*his3*$^+$), YA252 (as YA254 but *swi5*Δ::*ura4*$^+$), and YA492 (as YA254 but *h*^{90} *swi2*Δ::*ura4*$^+$). Standard procedures were used for cultivation and genetic manipulations (31).

Cloning of the *swi5*$^+$ Gene. SP168 (*swi5-39*) cells were transformed with a *S. pombe* genomic library (a generous gift from H. Masukata, Osaka University) and spread on EMM plates containing uracil (225 mg/liter). Transformants were examined for plasmid-dependent UV resistance. *swi5*$^+$ cDNA was constructed with RNA from wild-type strain SP157 by using PolyATtract System 1000 (Promega) and amplified by using RNA LA PCR kit Version 1.1 (Takara Shuzo, Kyoto). The primers had the sequences 5′-GTTTAAATCGTATGGAAAAG-3′ and 5′-TTATTCTGACCCATTAACTC-3′. 5′-RACE was carried out by using a 5′-Full RACE Core kit (Takara Shuzo). The RT-PCR primer 5′-TTATTCTGACCCATTAACTC-3′ and two sets of primers (5′-ATACGGGATATTGCTTTGGG-3′ and 5′-TATAGGTATGGAGTAGATCG-3′; 5′-GATTGGCAAAGTGGCAGAGC-3′ and 5′-ACAGTTTGCTTAGCATCTCG-3′) were used in this experiment. The 3′-terminal region of *swi5*$^+$ cDNA was amplified from a pcD2-based cDNA library (a generous gift from H. Nojima, Osaka University) with the set of primers 5′-GTTTAAATCGTATGGAAAAG-3′ from *swi5* and 5′-ACTGCCTTCTAGTGTAGCC-3′ from the vector plasmid pcD2.

Yeast Two-Hybrid Assay. Gal4-based Matchmaker Two-Hybrid System 3 (Clontech) was used for the yeast two-hybrid assay and screening according to the manufacturer's instructions. The indicated proteins were fused to the GAL4 activation domain (AD) in pGADT7 vector and the GAL4 DNA-binding domain (DBD) in pGBKT7, and expressed in *S. cerevisiae* tester strain AH109. For screening, an *S. pombe* cDNA library constructed with pGADGH vector was purchased from Clontech.

Immunoprecipitation. *S. pombe* YA254 cells harboring pREP42HAN (empty vector) or pREP42HAN-*swi2*$^+$ were grown in EMM containing 10 μM thiamin. YA119 cells were used as the host strain for pREP42HAN-*sfr1*$^+$. Protein expression was induced by incubation in thiamin-free EMM for 17 h, and 3×10^8 exponentially growing cells were collected and resuspended in IP buffer (50 mM Tris·HCl, pH 8.0/120 mM NaCl/0.5% Nonidet P-40) containing a protease inhibitor mixture (Complete, Roche) according to the manufacturer's instructions. The cells were disrupted by vortexing with glass beads. One milliliter of the supernatant of an HA-Swi2 overproducer, which was obtained by centrifugation of the crude extract at 18,000 × *g* for 20 min, was incubated with 50 μl of anti-HA matrix (Roche Applied Science) for 2 h at 4°C. When anti-Swi5 and Rhp51 antibodies were used, the supernatant was preincubated with Protein G Sepharose 4B (Amersham Biosciences) for 10 min at 4 °C, before incubation with each polyclonal antiserum for 1 h. Fifty microliters of Protein G Sepharose 4B was added to the reaction mixtures, and the incubation was continued for 1 h. The immunoprecipitate was washed three times with immunoprecipitation buffer, and the proteins were subjected to immunoblotting. Mouse anti-HA monoclonal antibody (12CA5, Roche Molecular Biochemicals), rabbit anti-Rhp51, or rat anti-Swi5 antiserum was used as the first antibody, and horseradish peroxidase-conjugated anti-rat or -mouse IgG antibody was used as the second antibody. For analysis of the HA-Sfr1-mediated interaction, Dynabeads Protein G (Dynal, Great Neck, NY) was used instead of Protein G Sepharose. The ECL Plus system (Amersham Biosciences) was used for detection of immunocomplexes.

GST Pull-Down Assay. An expression plasmid for GST-fused Sfr1 protein was constructed by using pGEX6P-1 (Amersham Biosciences). Expression plasmids for recombinant Swi5 and Rhp51 proteins were constructed by using the plasmid pET11a (Novagen). GST and GST-Sfr1 fusion protein were expressed in *E. coli* (UT481). The induced cells were suspended in buffer G (50 mM Tris·HCl, pH 7.6/100 mM NaCl/1 mM EDTA/0.1% Nonidet P-40 and protein inhibitors) and disrupted by sonication. The cleared lysates, which were obtained by centrifugation at 15,000 × *g* for 30 min, were incubated with 250 μl of Glutathione Sepharose 4B (50% slurry) (Amersham Biosciences) for 5 h. The beads were washed three times with 5 ml of buffer G. The recombinant Swi5 and Rhp51 proteins were expressed in *E. coli* BL21-CodonPlus (DE3) (Stratagene). The induced cells suspended in buffer G were disrupted by sonication, and the cleared lysate was obtained by centrifugation at 15,000 × *g* for 30 min. The lysates (25 μg of proteins) were incubated with 10 μl of the bead-immobilized GST or GST-Sfr1 proteins for 3 h. The beads were washed five times with buffer G and boiled in SDS/PAGE sample buffer to elute bound proteins. The proteins were subjected to immunoblotting.

Results and Discussion

Cloning of the *swi5*$^+$ Gene. To clone the *S. pombe swi5* gene, we isolated an *S. pombe* genomic clone that complements the UV sensitivity of *swi5-39*. The clone encodes a single ORF, SPBC409.03, that maps to the *swi5* locus (25). DNA sequencing revealed a single G:C to A:T transition codon in *swi5-39* (Fig. 1*A*). We then constructed strains in which the *swi5* ORF was disrupted with either a *ura4*$^+$ or *his3*$^+$ marker cassette; these strains were defective in mating-type switching and sensitive to UV irradiation, confirming that SPBC409.03 is the *swi5* gene. cDNA analysis revealed that *swi5* has two introns and encodes a small protein of 85 aa, and that the *swi5-39* mutation changes Gln-38 to an ochre nonsense. Database searches indicate that putative Swi5 homologs exist at least in humans, rats, mice, chickens, and frogs, implying that Swi5 is a conserved protein that probably plays a common important role in these organisms (Fig. 1*B*). Alignment of the amino acid sequences reveals particularly high conservation in the C-terminal half: this region may contain a previously uncharacterized protein motif or domain. *S. cerevisiae SAE3*, which has been shown to be involved in meiotic recombination, is proposed to encode a 55-aa protein (32). However, the genomic region giving one of the best hits using TBLASTX shows a promising canonical intron that would extend *SAE3*, producing a 102-aa protein. The

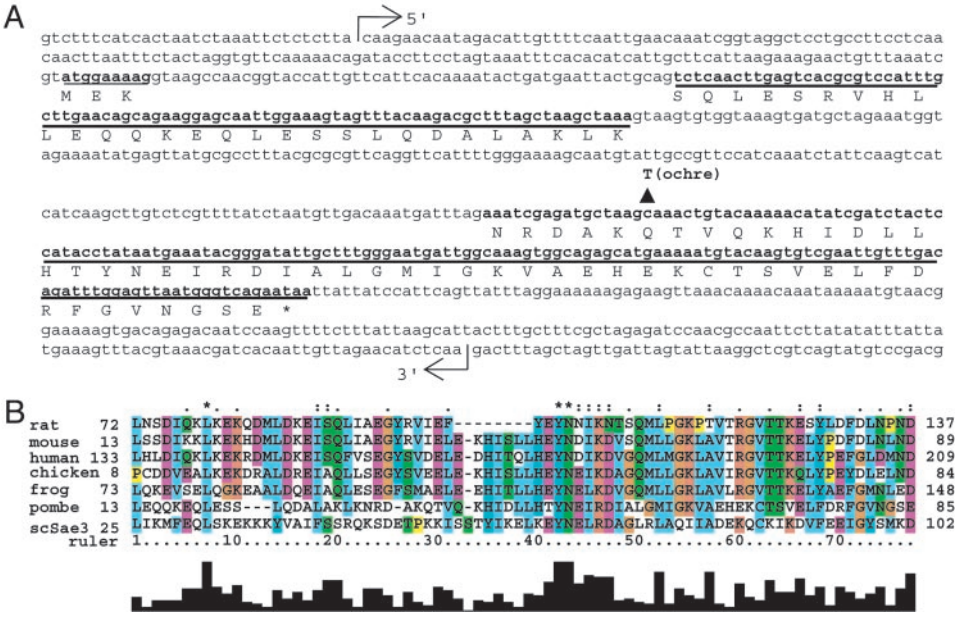


Fig. 1. (*A*) The nucleotide sequence of the *swi5* region and the deduced sequence of Swi5 protein. The 5′-end sequence of the mRNA was determined by 5′-RACE, and the 3′-end sequence was determined from the cDNA. There are two introns in the *swi5* gene. The *swi5-39* mutation is a single G:C to A:T transition that changes Gln-38 to an ochre nonsense codon. (*B*) Sequence alignment of Swi5 proteins from various organisms. Multiple sequence alignment was performed by using the CLUSTALX program (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX). Sequence data were obtained from databases. The accession numbers are XP_232740 for rat, AAH21748.1 for mouse, AAH29911.1 for human, BU480721 for chicken, and AL657116 for frog. A 102-aa form of *S. cerevisiae* Sae3 was predicted by V. Wood (personal communication)

C-terminal half of the extended Sae3 protein contains a C-terminal sequence conserved among putative Swi5 homologs (V. Wood, personal communication) (Fig. 1*B*). However, *S. cerevisiae sae3* mutants, unlike *S. pombe swi5* mutants, did not show any detectable defects in DNA repair during mitosis (unpublished data) (32).

Swi5 Works in a Previously Uncharacterized Rhp51 (Rad51^{sp})-Dependent Recombination Repair Pathway That Is Independent of Rhp57 (Rad57^{sp}) Function. To determine whether *swi5*$^+$ is involved in Rhp51-dependent recombination repair in *S. pombe*, the DNA damage sensitivities of *rhp51*Δ and *swi5*Δ *rhp51*Δ were compared. The *rhp51*Δ single mutant was as sensitive as the *swi5*Δ *rhp51*Δ double mutant to γ-rays, UV irradiation, and methyl methanesulfonate (MMS) (Fig. 2*A* and data not shown), indicating that *swi5* is epistatic with *rhp51* and that *swi5*$^+$ functions in Rhp51-dependent recombination repair.

The effect of *rhp51*$^+$ overexpression on *swi5* mutants was also examined. When a multicopy plasmid expressing *rhp51*$^+$ was introduced into *swi5*Δ, the MMS, γ-ray, and UV sensitivities of *swi5*Δ were partially suppressed (Fig. 2*B* and data not shown). This result suggests functional interaction(s) between the Rhp51 and Swi5 proteins.

Because we have shown that overexpression of Rhp51 partially suppresses the repair defect phenotype of *rhp57*Δ mutants (11), genetic interactions between *swi5* and *rhp57* were also examined. The *swi5*Δ *rhp57*Δ double mutant was more sensitive to γ-rays and UV irradiation than the *swi5*Δ or the *rhp57*Δ single mutant, suggesting that *swi5*$^+$ functions independently of *rhp57*$^+$ (Fig. 2*A*). Moreover, the sensitivities of the *swi5*Δ *rhp57*Δ double mutant to γ-rays and UV irradiation were almost the same as those of the *rhp51*Δ single mutant, indicating that the double mutant is completely defective in Rhp51-dependent DNA repair. The *swi5*Δ *rhp57*Δ *rhp51*Δ triple mutant also exhibited the same UV sensitivity as the *rhp51*Δ single mutant (Fig. 2*A*). These results suggest that there are two subpathways in Rhp51-dependent repair: one subpathway involves *rhp57*$^+$ and the other subpathway involves *swi5*$^+$.

Fen-1 flap endonuclease is involved in the processing of Okazaki fragments during lagging-strand DNA synthesis. The products of the fission yeast *rad2*$^+$ gene and the budding yeast *RAD27* gene are homologs of Fen-1, and yeast with mutations in these genes in combination with mutations that inactivate homologous recombination are often nonviable (33, 34). This suggests that Fen-1 mutants accumulate DSBs during replication fork arrest and that these DSBs are lethal in the absence of homologous recombination (11). Surprisingly, a *rad2*Δ *swi5*Δ double mutant was found to be viable and had no detectable growth defect (data not shown), although *rad2*Δ *rhp57*Δ is a nonviable combination (11). This suggests that DSBs formed during S phase in *rad2* mutant cells may be repaired exclusively through a Rhp57- and Rhp51-dependent pathway that is independent of Swi5.

The *rhp51* epistasis group includes *rhp51*, *rhp55*, *rhp57*, and *rad32*, and mutants in these genes have a checkpoint-dependent delay in

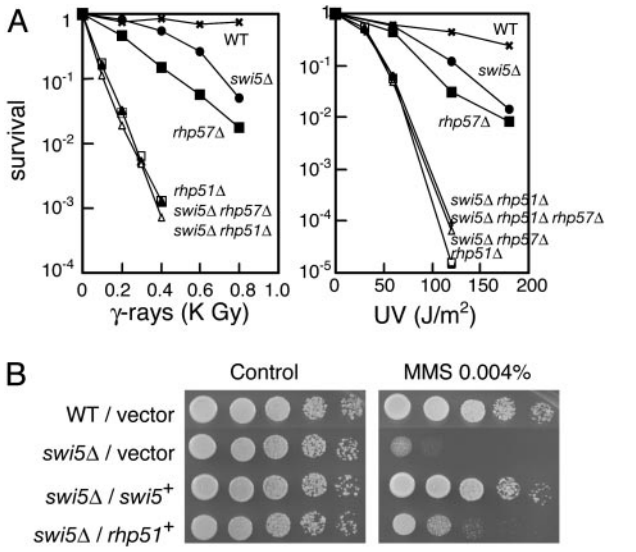
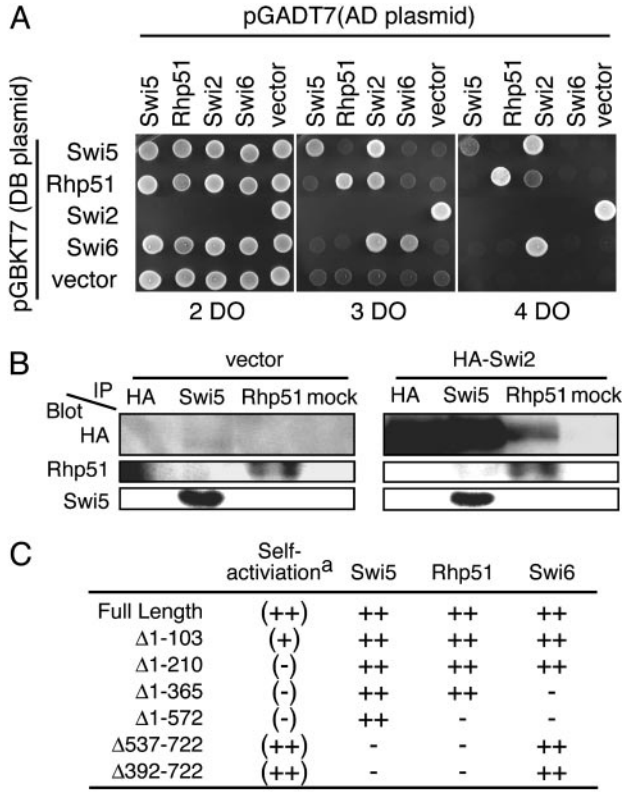


Fig. 2. Swi5 is involved in Rhp51-dependent and Rhp57-independent recombination repair. (*A*) Epistasis of DNA repair activity among *swi5*Δ, *rhp51*Δ, and *rhp57*Δ. Exponentially growing cells were irradiated with the indicated doses of γ-rays (*Left*) or UV light (*Right*), and colonies were counted. WT, wild-type YA119 (Xs); *swi5*Δ, YA177 (filled circles); *rhp51*Δ, T3 (filled triangles); *rhp57*Δ, T5 (filled squares); *swi5*Δ *rhp51*Δ, YA244 (open triangles); *swi5*Δ *rhp57*Δ, YA250 (open squares); *swi5*Δ *rhp51*Δ *rhp57*Δ, YA424 (crosses). (*B*) Multicopy plasmid expressing *rhp51*$^+$ suppresses the DNA repair defect of *swi5*Δ. MMS sensitivity of the strains *swi5*Δ (YA177) and WT (YA119) carrying the *rhp51*$^+$ gene on multicopy plasmid vector pSP102 was judged by performing spot tests as described (11).



	Self-activiation[a]	Swi5	Rhp51	Swi6
Full Length	(++)	++	++	++
Δ1-103	(+)	++	++	++
Δ1-210	(-)	++	++	++
Δ1-365	(-)	++	++	-
Δ1-572	(-)	++	-	-
Δ537-722	(++)	-	-	++
Δ392-722	(++)	-	-	++

Fig. 3. Physical interactions of Swi5 with recombination repair and mating-type switching proteins. Swi5 interacts with Rhp51 and Swi6 via Swi2. (*A*) Two-hybrid interactions were judged by spot tests on three types of dropout (DO) plates: 4DO (SD-adenine, -histidine, -leucine, and -tryptophan; high-stringency condition), 3DO (SD-histidine, -leucine, and -tryptophan; medium-stringency condition), and the control 2DO (SD-leucine and -tryptophan). Reciprocal combinations of fusions with the GAL4-activation domain (AD) and the GAL4 DNA-binding domain (DBD) were examined, except for combinations with the DBD fusion of Swi2, because it showed strong self-activation even in the presence of empty vector pGADT7. (*B*) Coimmunoprecipitation among Swi2, Swi5, and Rhp51. Proteins in extracts from cells transformed with HA-Swi2 plasmid (*Right*) or empty vector (*Left*) were immunoprecipitated with antibody against HA, Rhp51, or Swi5. The immune complexes were separated by SDS/PAGE and immunoblotted with antibody against HA, Rhp51, or Swi5. (*C*) The two-hybrid interactions of Swi2-truncated alleles with Swi5, Rhp51, and Swi6. Full-length *swi2* (encoding a 722-aa protein) or *swi2*-truncated alleles were subcloned in the AD plasmid pGADT7, and Swi5, Rhp51, and Swi6 were subcloned in the DBD plasmid pGBTK7. Two-hybrid interactions were expressed as follows: ++, very strong interaction (grown on 4DO plates); +, strong interaction (grown on 3 DO plates); −, no detectable interaction. a, Swi2-induced self-activation that does not depend on the AD plasmid was assayed by judging the growth of the reporter strain with the *swi2* derivative on pGBKT7 and the pGADT7 empty vector on 4DO or 3DO plates and expressed in parentheses by using the same criteria as above.

the cell cycle that results in a cell-elongation phenotype (16, 35). However, *swi5*Δ single mutants do not exhibit this kind of abnormal cell morphology. In addition, *swi5* mutants are much more resistant to hydroxyurea than *rhp57* mutants (data not shown). Taken together, the results presented above indicate that there are *swi5* and *rhp57* subpathways of Rhp51-dependent repair and that these perform distinct functions during recombination repair.

Swi5 Interactors and Mating-Type Switching. The genetic interaction between *swi5*$^+$ and *rhp51*$^+$ suggests that Swi5 might interact physically with Rhp51, for example, by forming a complex analogous to that formed by the Rhp55–Rhp57 dimer with Rhp51 (8). However, we detected no interaction between Swi5 and Rhp51 by the two-hybrid assay (Fig. 3*A*). We therefore searched for additional protein interactors by using Swi5 as bait in a two-hybrid screening of approximately one million transformants with an *S. pombe* cDNA expression library, and thereby identified two candidate plasmids: both clones expressed the C-terminal 151 aa of Swi2. Full-length Swi2 was then tested and found to interact with Swi5 and Rhp51 (Fig. 3*A*).

To analyze the physical interaction of Swi2 with Swi5 and Rhp51, we attempted coimmunoprecipitation experiments with HA-tagged Swi2. HA-Swi2 was fully active as judged by a complementation test for the mating-type switching defect of a *swi2* mutant. However, we could not detect the genomically expressed HA-tagged Swi2 protein from its own promoter in a whole-cell extract by immunoblotting or immunoprecipitation (data not shown). We then overexpressed the HA-Swi2 from a plasmid by using the *nmt1* promoter. Antibody against Swi5 or Rhp51 coimmunoprecipitated HA-Swi2 from the extract of cells overproducing HA-Swi2 but not from the extract of cells containing the empty vector (Fig. 3*B*). However, anti-HA antibody did not coimmunoprecipitate Rhp51 or Swi5. A very small amount of Rhp51 was coimmunoprecipitated by the anti-Swi5 antibody, and Swi5 was hardly detected in the coprecipitate by anti-Rhp51 antibody. These results suggest that Swi2 interacts with Swi5 and Rhp51 very weakly and/or transiently *in vivo*.

The reciprocal two-hybrid analysis also indicated that Swi5 could form a homooligomer (Fig. 3*A*). Indeed, purified Swi5 that had been expressed in *E. coli* eluted as a protein of ≈60 kDa from gel filtration column (Superdex 75, Amersham Biosciences) chromatography (data not shown), implying that Swi5 forms an oligomer, probably a hexamer, in solution, because the calculated monomer size is 9,745.

Interestingly, a fusion construct combining full-length Swi2, but not Swi2 with an N-terminal truncation (the same insert as the originally isolated clone), with the Gal4 DNA-binding domain in pGBKT7 activated the reporter genes by itself even under conditions of high stringency (4DO SD plate). Truncation analysis revealed that the N-terminal 103 aa were important for the transcriptional activator activity of the protein itself (self-activation) (Fig. 3*C*).

Next, we performed another two-hybrid screening to search for additional interactors by using Swi2 with truncation of the N-terminal 103 aa as bait and thereby found Swi6; the results of the reciprocal two-hybrid interaction are shown in Fig. 3*A*. Truncation analysis of Swi2 revealed that amino acid residues 211–391, 366–722, and 573–722 are important for interaction with Swi6, Rhp51, and Swi5, respectively (Fig. 3*C*).

swi5, *swi2*, and *swi6* belong to the same class Ib epistasis group for mating-type switching, but *swi2* and *swi6* are not defective in DNA repair (data not shown and ref. 30). Mutants with *swi2 swi5* or *swi5 swi6* double mutation show defects in both mating-type switching and DNA repair, but no cumulative effects on either mating-type switching or repair are observed (25). Thus, the Rhp51-Swi5 protein interaction, which is mediated by Swi2, is likely to play a specific role in mating-type switching.

Sfr1, a Previously Uncharacterized Swi2-Like Protein, Interacts with both Swi5 and Rhp51. If Swi5 and Rhp51 form a protein complex that is mating-type switching specific, it seems possible that Swi5 and Rhp51 may form a second complex that is recombination repair specific, and that formation of such a recombination repair complex might require a specific mediator analogous to Swi2. This idea was supported by the results of a BLAST search for proteins with homology to Swi2 that identified an ORF, SPBC28F2.07, exhibiting similarity to the C-terminal region of Swi2 (Fig. 4*A*). However, the Swi2 sequence exhibited no similarity to other proteins or motifs, as indicated by searches of current databases. cDNA analysis indicated that SPBC28F2.07 has no introns (data not shown). Fusion plasmids were accordingly constructed for the two-hybrid assay. The results indicated that the gene product encoded by

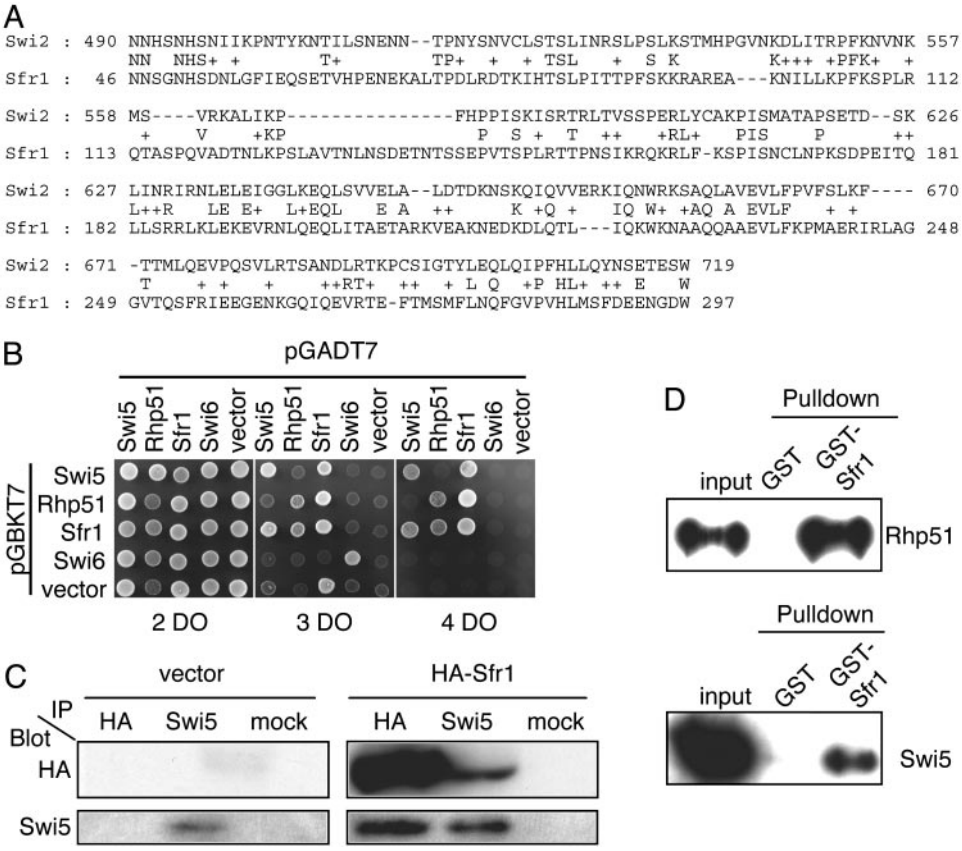


Fig. 4. Sfr1 binds to both Swi5 and Rhp51. (*A*) Sequence alignment of Swi2 and Sfr1. The two proteins show 23% identity and 41% similarity. (*B*) Sfr1 two-hybrid interaction with Swi5 and Rhp51. The interactions of the indicated proteins were examined by using the two-hybrid assay as described in Fig. 2. (*C*) Sfr1 coimmunoprecipitates with Swi5. Proteins in extracts from cells transformed with HA-Sfr1 plasmid (*Right*) or empty vector (*Left*) were immunoprecipitated with polyclonal antibody against HA or Swi5. The immunocomplexes were separated by SDS/PAGE and immunoblotted with anti-HA or -Swi5 antibodies. Note that HA-Sfr1 was fully active as judged by a complementation test of the DNA repair defect of an *sfr1* mutant, YA431 (data not shown; see Fig. 5). (*D*) Coimmunoprecipitation of HA-Sfr1 with Swi5. Proteins in extracts from cells transformed with HA-Sfr1 plasmid (*Right*) or empty vector (*Left*) were immunoprecipitated with anti-HA monoclonal antibody (12CA5, Roche Applied Science). The immune complexes were separated by SDS/PAGE and immunoblotted with rat anti-Swi5 antibody.

SPBC28F2.07 interacts with both Swi5 and Rhp51, as shown in Fig. 4*B*. We named SPBC28F2.07 *sfr1* on the basis of the experiments reported below. The data from the two-hybrid assay also suggest that Sfr1 does not interact with Swi6 and that Sfr1 forms a homooligomer.

The physical interactions were analyzed by coimmunoprecipitation experiments. However, nonspecific interactions between HA-Sfr1 and Protein G Sepharose were observed under conditions of physiological ionic strength, whereas under high stringency conditions, no interactions were observed among these proteins (data not shown). We therefore used magnet-conjugated protein G beads to conduct the experiment under physiological conditions (Fig. 4*C*). HA-Sfr1 was coimmunoprecipitated from cells overproducing HA-Sfr1, but not from cells containing the empty vector, by the antibody against Swi5. Swi5 was also coimmunoprecipitated by the antibody against HA, suggesting that Sfr1 interacts with Swi5 *in vivo*. However, antibody against Rhp51 did not immunoprecipitate Rhp51 itself under the same conditions (data not shown). Rhp51 protein was not detected in the immunocomplexes that were pulled down by antibody against HA or against Swi5. Therefore, we next analyzed the Sfr1-mediated interactions by GST pull-down assays (Fig. 4*D*). Extracts from *E. coli* cells expressing Rhp51 or Swi5 were incubated with GST-Sfr1 or GST proteins that were immobilized on Glutathione Sepharose 4B beads. After the beads were washed thoroughly, the protein complexes were separated by SDS/PAGE and then subjected to immunoblotting. Rhp51 and Swi5 were pulled down by GST-Sfr1 but not by GST, suggesting that Sfr1 interacts with Rhp51 and Swi5. The fact that Rhp51 is not detected in immunoprecipitation experiments may indicate that the physical interactions are very weak and/or transient *in vivo*. A similar pull-down assay for Swi2-mediated interaction was not conducted because the GST-Swi2 fusion protein could not be recovered in the soluble fraction from an *E. coli* overproducer. Whether the Swi2 and Sfr1 interaction proteins simultaneously form a ternary complex should be clarified in future studies.

Sfr1 Is Involved in Swi5-Mediated Recombination Repair, but Not in Mating-Type Switching. We constructed an *sfr1* deletion strain and characterized it. Although the deletion mutant was fully viable, it exhibited similar sensitivity to UV and γ-ray irradiation as the *swi5*Δ mutant (Fig. 5*A*). Moreover, an *sfr1*Δ *swi5*Δ double deletion mutant was no more sensitive to UV and γ-ray irradiation than the respective single mutants, and the *sfr1*Δ *rhp51*Δ double mutant was as sensitive to these reagents as *rhp51*Δ (Fig. 5*A*). Finally, an *sfr1*Δ *rhp57*Δ double mutant was as sensitive to UV irradiation as *rhp51*Δ and *rhp57*Δ *swi5*Δ (Fig. 5*A*).

The *sfr1*Δ mutation did not affect mating-type switching, as judged by iodine staining (Fig. 5*B*). No interaction between Sfr1 and Swi6 could be detected by the two-hybrid assay (Fig. 4*B*), consistent with the fact that Srf1 does not possess a region corresponding to the Swi6 interaction region in Swi2. In addition, *sfr1*Δ, like *swi5*Δ, was not lethal when combined with *rad2*Δ, and a *rad2*Δ *sfr1*Δ double mutant had no detectable growth defect (data not shown). Taken together, these findings suggest that Sfr1 plays a specific role in DNA repair and that it functions together with Swi5. Sfr1 stands for "*S*wi *f*ive-dependent *r*ecombination repair protein *1*" to reflect the experimental findings described above.

Conclusions and Perspectives. Our studies provide genetic evidence that two different Swi5-containing protein complexes are involved in mating-type switching and a previously uncharacterized recombination repair in fission yeast (Fig. 6). This subpathway of recombination repair is Rhp51-dependent but Rhp55/57-independent. In vertebrates, *RAD51* is essential for cell viability, whereas paralog mutants exhibit much milder defects in cell proliferation (12, 36), implying the existence of an independent and partially redundant pathway. Lambert and Lopez (37) also suggested that there are

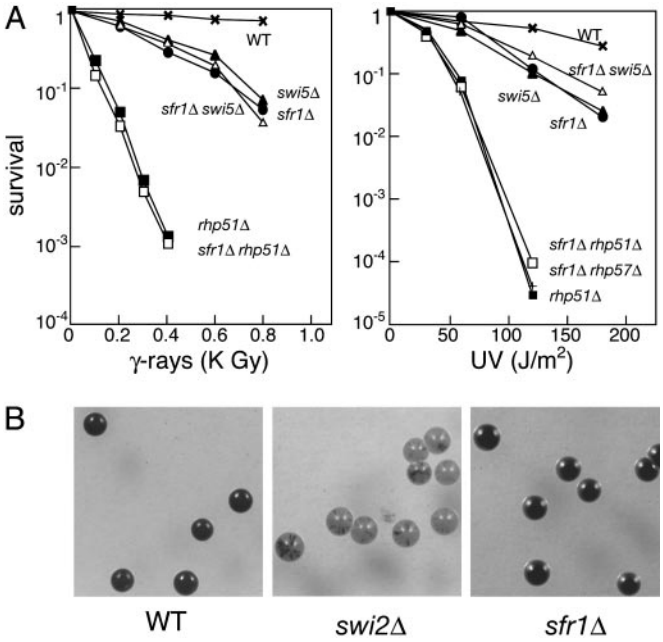


Fig. 5. Sfr1 protein is involved in the same repair pathway as Swi5 but not in mating-type switching. (*A*) The *sfr1*$^+$ gene functions in the same repair pathway for γ-ray (*Left*) and UV (*Right*) irradiation as the *swi5*$^+$ gene. WT, wild-type YA119 (Xs); *sfr1*Δ, YA431 (filled circles); *swi5*Δ, YA177 (filled triangles); *rhp51*Δ, T3 (filled squares); *sfr1*Δ *swi5*Δ, YA452 (open triangles); *sfr1*Δ *rhp51*Δ, YA474 (open squares); *sfr1*Δ *rhp57*Δ, YA478 (crosses). (*B*) *sfr1*Δ is proficient in mating-type switching, as judged by the iodine staining assay. WT, wild-type, YA254; *swi2*Δ, YA492; and *sfr1*Δ, YA455.

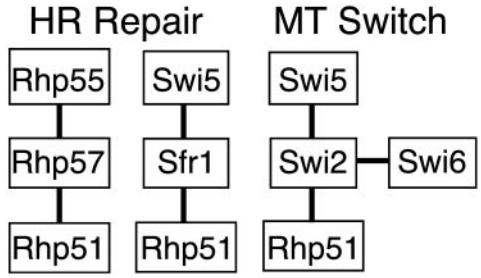


Fig. 6. A summary model. Three distinct pathways are involved in mitotic DNA recombination-related events, one for mating-type switching and the other two for DNA repair. Swi5 is involved in both DNA repair and mating-type switching. The Swi5/Sfr1 repair pathway is completely parallel to the Rhp55/57 pathway. Sfr1 functions specifically in repair, whereas Swi2 functions specifically in mating-type switching. Note that *swi2*, *swi5*, and *swi6* belong to the same class 1b epistasis group for mating-type switching (24, 25).

different recombination pathways that depend on Rad51 in the mouse. The two proposed *S. pombe* Rhp51-dependent subpathways are distinct, as shown by the synthetic lethality with *rad2* and the checkpoint-dependent delay in the cell cycle, implying that the two subpathways use different substrates for initiation of recombination repair. Although *S. cerevisiae* Rad52 plays a role in various processes, mouse and chicken cells deficient for *RAD52* are not hypersensitive to DNA damage (38, 39). Instead, Rad52 apparently acts redundantly to the Rad51 paralogs (40). The genetic relationship between the redundancy and Swi5/Sfr1 pathway in *S. pombe* is very interesting. Additional studies will be needed to clarify how different recombination pathways discriminate between different recombination substrates. Such studies will be important for elucidating the precise mechanisms of homologous recombination and recombination repair in eukaryotes.

We thank B. Arcangioli, A. M. Carr, T. Morishita, Y. Tsutsui, T. Hishida, and T. Kokubo for helpful discussions and yeast strains, and V. Wood for sharing the information on the Sae3 homology. Y.A. and D.D. were supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists and Foreign Researchers, respectively. This work was supported, in part, by Grants-in-Aid for Scientific Research of Priority Areas B (to H.S.) and C (to H.I.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. H.I. was supported by the Human Frontier Science Program and Core Research for Evolutional Science and Technology.

1. Baumann, P. & West, S. C. (1998) *Trends Biochem. Sci.* **23,** 247–251.
2. Ogawa, T., Yu, X., Shinohara, A. & Egelman, E. H. (1993) *Science* **259,** 1896–1899.
3. Sung, P. (1994) *Science* **265,** 1241–1243.
4. Baumann, P., Benson, F. E. & West, S. C. (1996) *Cell* **87,** 757–766.
5. Paques, F. & Haber, J. E. (1999) *Microbiol. Mol. Biol. Rev.* **63,** 349–404.
6. Morrison, C. & Takeda, S. (2000) *Int. J. Biochem. Cell Biol.* **32,** 817–831.
7. Sung, P. (1997) *Genes Dev.* **11,** 1111–1121.
8. Tsutsui, Y., Khasanov, F. K., Shinagawa, H., Iwasaki, H. & Bashkirov, V. I. (2001) *Genetics* **159,** 91–105.
9. Lovett, S. T. & Mortimer, R. K. (1987) *Genetics* **116,** 547–553.
10. Johnson, R. D. & Symington, L. S. (1995) *Mol. Cell. Biol.* **15,** 4843–4850.
11. Tsutsui, Y., Morishita, T., Iwasaki, H., Toh, H. & Shinagawa, H. (2000) *Genetics* **154,** 1451–1461.
12. Takata, M., Sasaki, M. S., Tachiiri, S., Fukushima, T., Sonoda, E., Schild, D., Thompson, L. H. & Takeda, S. (2001) *Mol. Cell. Biol.* **21,** 2858–2866.
13. Caspari, T., Murray, J. M. & Carr, A. M. (2002) *Genes Dev.* **16,** 1195–1208.
14. Gasior, S. L., Olivares, H., Ear, U., Hari, D. M., Weichselbaum, R. & Bishop, D. K. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 8411–8418.
15. Takata, M., Sasaki, M. S., Sonoda, E., Fukushima, T., Morrison, C., Albala, J. S., Swagemakers, S. M., Kanaar, R., Thompson, L. H. & Takeda, S. (2000) *Mol. Cell. Biol.* **20,** 6476–6482.
16. Khasanov, F. K., Savchenko, G. V., Bashkirova, E. V., Korolev, V. G., Heyer, W. D. & Bashkirov, V. I. (1999) *Genetics* **152,** 1557–1572.
17. Cromie, G. A., Connelly, J. C. & Leach, D. R. (2001) *Mol. Cell* **8,** 1163–1174.
18. Sadofsky, M. J. (2001) *Nucleic Acids Res.* **29,** 1399–1409.
19. Haber, J. E. (1998) *Annu. Rev. Genet.* **32,** 561–599.
20. Miyata, H. & Miyata, M. (1981) *J. Gen. Appl. Microbiol.* **27,** 365–369.
21. Arcangioli, B. & de Lahondes, R. (2000) *EMBO J.* **19,** 1389–1396.
22. Dalgaard, J. Z. & Klar, A. J. (1999) *Nature* **400,** 181–184.
23. Dalgaard, J. Z. & Klar, A. J. (2001) *Trends Genet.* **17,** 153–157.
24. Egel, R., Beach, D. H. & Klar, A. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3481–3485.
25. Gutz, H. & Schmidt, H. (1985) *Curr. Genet.* **9,** 325–331.
26. Dalgaard, J. Z. & Klar, A. J. (2000) *Cell* **102,** 745–751.
27. Thon, G. & Klar, A. J. (1993) *Genetics* **134,** 1045–1054.
28. Lorentz, A., Ostermann, K., Fleck, O. & Schmidt, H. (1994) *Gene* **143,** 139–143.
29. Ostermann, K., Lorentz, A. & Schmidt, H. (1993) *Nucleic Acids Res.* **21,** 5940–5944.
30. Schmidt, H., Kapitza-Fecke, P., Stephen, E. R. & Gutz, H. (1989) *Curr. Genet.* **16,** 89–94.
31. Moreno, S., Klar, A. & Nurse, P. (1991) *Methods Enzymol.* **194,** 795–823.
32. McKee, A. H. & Kleckner, N. (1997) *Genetics* **146,** 817–834.
33. Murray, J. M., Tavassoli, M., al-Harithy, R., Sheldrick, K. S., Lehmann, A. R., Carr, A. M. & Watts, F. Z. (1994) *Mol. Cell. Biol.* **14,** 4878–4888.
34. Symington, L. S. (1998) *Nucleic Acids Res.* **26,** 5589–5595.
35. Jang, Y. K., Jin, Y. H., Shim, Y. S., Kim, M. J., Yoo, E. J., Seong, R. H., Hong, S. H. & Park, S. D. (1995) *Biochem. Mol. Biol. Int.* **37,** 329–337.
36. Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., Matsushiro, A., Yoshimura, Y. & Morita, T. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 6236–6240.
37. Lambert, S. & Lopez, B. S. (2001) *Oncogene* **20,** 6627–6631.
38. Yamaguchi-Iwai, Y., Sonoda, E., Buerstedde, J. M., Bezzubova, O., Morrison, C., Takata, M., Shinohara, A. & Takeda, S. (1998) *Mol. Cell. Biol.* **18,** 6430–6435.
39. Rijkers, T., Van Den Ouweland, J., Morolli, B., Rolink, A. G., Baarends, W. M., Van Sloun, P. P., Lohman, P. H. & Pastink, A. (1998) *Mol. Cell. Biol.* **18,** 6423–6429.
40. Fujimori, A., Tachiiri, S., Sonoda, E., Thompson, L. H., Dhar, P. K., Hiraoka, M., Takeda, S., Zhang, Y., Reth, M. & Takata, M. (2001) *EMBO J.* **20,** 5513–5520.