

The *srs2* suppressor of UV sensitivity acts specifically on the *RAD5*- and *MMS2*-dependent branch of the *RAD6* pathway

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Received June 1, 2001; Revised and Accepted July 10, 2001

ABSTRACT

The *SRS2* gene encodes a helicase that affects recombination, gene conversion and DNA damage repair in the yeast *Saccharomyces cerevisiae*. Loss-of-function mutations in *srs2* suppress the extreme sensitivity towards UV radiation of *rad6* and *rad18* mutants, both of which are impaired in post-replication DNA repair and damage-induced mutagenesis. A sub-branch within the *RAD6* pathway is mediated by *RAD5*, *UBC13* and *MMS2*, and a comprehensive analysis of the *srs2* effect on other known members of the *RAD6* pathway reported here now demonstrates that suppression by *srs2* is specific for mutants within this *RAD5*-dependent sub-system. Further evidence for the concerted action of *RAD5* with *UBC13* and *MMS2* in DNA damage repair is given by examination of the effects of cell cycle stage as well as deletion of other repair systems on the activity of post-replication repair. Finally, it is shown that *MMS2*, like *UBC13* and many other repair genes, is transcriptionally up-regulated in response to DNA damage. The data presented here support the notion that *RAD5*, *UBC13* and *MMS2* encode an ensemble of genetically and physically interacting repair factors within the *RAD6* pathway that is coordinately affected by *SRS2*.

INTRODUCTION

Mechanisms of DNA damage repair are highly conserved from bacteria to higher eukaryotes. The yeast *Saccharomyces cerevisiae* maintains several repair systems that are each responsible for specific types of damage (1,2). The *RAD3* group mediates nucleotide excision repair of pyrimidine dimers and other bulky lesions, the *RAD52* group encodes the factors for double-strand break repair via homologous recombination and the *RAD6* pathway of post-replication repair (PRR) is believed to act upon single-stranded gaps that arise during DNA replication on damaged templates (3). While nucleotide excision repair and homologous recombination are mechanistically quite well understood, little is known about the molecular details of PRR. Extensive genetic analysis,

however, has revealed an intricate system of several independent sub-branches. Notably, two fundamentally different modes of repair can be distinguished within the *RAD6*-dependent repair system: while error-prone repair involves translesion synthesis across a damaged site, error-free repair has been proposed to use information from the undamaged sister chromatid for correct reversal of the damage (3).

RAD6, which is epistatic to all other members of this pathway, encodes a ubiquitin-conjugating enzyme (UBC) whose catalytic activity is essential for function in DNA repair (4,5). Mutations in this gene confer a pleiotropic phenotype, involving a high degree of sensitivity towards DNA-damaging agents, a defect in damage-induced mutagenesis as well as an increased rate of spontaneous mutagenesis, but also defects apparently unrelated to DNA repair, including a reduced growth rate, temperature sensitivity and the inability to sporulate (reviewed in 6). Other members of the *RAD6* pathway exhibit different subsets of the repair phenotypes, with varying degrees of UV sensitivity and variable effects on mutagenesis and recombination. Rad18p, a DNA-binding RING finger protein that forms a stable complex with Rad6p and is believed to target the UBC to sites of DNA damage (7), is apparently involved in all *RAD6*-dependent sub-pathways of DNA repair (8,9). The error-prone system is characterized by a specific damage-tolerant polymerase, Pol ζ , encoded by *REV3* and *REV7*, which is capable of translesion synthesis (10,11). Another *RAD6/RAD18*-dependent translesion polymerase is Pol η , encoded by *RAD30* (12–14). The *RAD5* gene acts independently of both sub-pathways, but again depends on *RAD6* and *RAD18* (15–17). Mutants of *RAD5* exhibit a reduced rate of damage-induced mutagenesis in some assays, but since this effect is limited to specific types of mutations, *RAD5*-dependent repair has also been classified as error free (15–18). *RAD5* encodes a DNA-dependent ATPase (19) with homology to the *SNF2/SWI2* family of helicases and chromatin remodeling factors (20). Recently, it was shown that there appears to be a second error-free pathway in addition to that mediated by *RAD5*: a mutation in the *POL30* gene encoding the polymerase processivity factor PCNA, *pol30-46*, which had previously been assigned to the error-free *RAD6*-dependent sub-system (21), was found to act additively or even synergistically with respect to a *rad5* mutation, suggesting their involvement in alternative pathways (22).

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A second ubiquitin-conjugating activity has been found associated with the *RAD6* pathway: *MMS2*, encoding a UBC-like protein, was cloned by complementation of a mutant sensitive to the alkylating agent methylmethane sulfonate (MMS) and was assigned to an error-free sub-branch (23). The Mms2p protein was later shown to form a heterodimer with Ubc13p, a genuine UBC (24). This complex assembles multi-ubiquitin chains that are linked in a non-standard way, via Lys63 (K63) of ubiquitin instead of the prevalent Lys48 linkage. In fact, mutants of ubiquitin in which K63 is replaced by arginine (*ubiK63R*) display a UV sensitivity identical to that of *mms2* and *ubc13* mutants, indicating that these non-standard multi-ubiquitin chains are relevant to PRR (24,25). A dependence of *UBC13* and *MMS2* on *RAD5* has been established (26), which is supported by the observation that Rad5p protein physically interacts with Ubc13p and recruits the Ubc13p/Mms2p dimer to chromatin, in a manner similar to the recruitment of Rad6p by Rad18p (26).

The *SRS2* gene [independently identified as *HPR5* (27) and *RADH* (28)] represents an unusual member of the *RAD6* pathway: mutation or deletion of this gene was found to partially suppress the UV sensitivities of *rad6* and *rad18* mutants (28–30). Suppression depends on a functional recombination pathway (31). *SRS2* encodes a helicase with 5'→3' polarity (32) and is involved in a variety of processes associated with DNA metabolism, such as recombination and gene conversion, as well as double-strand break repair by single-strand annealing and non-homologous end-joining (33–37). It remains to be elucidated how *SRS2* acts in PRR. In order to define the relation of *SRS2* to the various sub-systems of PRR I have analyzed the effect of an *srs2* deletion on the UV sensitivities of a series of *RAD6* pathway mutants. I present evidence that the suppression effect of *srs2* is specific for the branch mediated by *RAD5*, *UBC13* and *MMS2*. Further support for a concerted action of these factors within the *RAD6* pathway is given by genetic analyses that place *MMS2* and *UBC13* in the *RAD5*-dependent repair system under several different experimental conditions. Finally, I present evidence that *MMS2*, like *UBC13* and most other members of the *RAD6* pathway, is a damage-inducible gene.

MATERIALS AND METHODS

Yeast strains and plasmids

Standard protocols were followed for the preparation of yeast media, transformation, sporulation and tetrad dissection (38,39). With the exception of *UBIK63* and *ubiK63R*, which correspond to SUB280 and SUB413 and were kindly provided by D. Finley (25), all strains are derivatives of the diploid wild-type (*wt*) strain DF5 (40) and its isogenic haploid (*his3-Δ200*, *leu2-3,112*, *lys2-801*, *trp1-1*, *ura3-52*). Construction of *rad18*, *rad5*, *rad30*, *ubc13* and *mms2* deletions has been reported previously (26). The construct used to create a *rev3* deletion strain is based on the *hisG-URA3-hisG* geneblaster and was a gift from W. Kramer, a *rad2::URA3* construct was provided by K. Madura and a *rad52::URA3* plasmid, pSM22, by D. Schild. The *pol30-46* mutation was introduced into the DF5 background by deletion of one *POL30* allele in diploid cells, introduction of pBL230-46, a centromeric plasmid bearing the *pol30-46* allele (21), and sporulation of the transformants.

Plasmid pBL230, encoding the unmutated *POL30* gene, served for construction of an isogenic control strain. pBL230 and pBL230-46 as well as the *pol30::URA3* deletion construct, pBL243, were kindly provided by P. Burgers. The *srs2* strain was constructed by exact replacement of the open reading frame with a HisMX cassette, using a PCR strategy (41). Double and triple mutants with *srs2* were constructed from the corresponding single mutants by mating and tetrad dissection, with the exception of *UBIK63*, *ubiK63R*, *POL30* and *pol30-46*, into which the *srs2* deletion was newly introduced using the same PCR strategy. A KanMX cassette (41) served for replacement of the open reading frame in these cases. Expression vectors for the promoter-*lacZ* fusions are derivatives of YIplac211 (42) carrying the *lacZ* open reading frame as a *Bam*HI-*Hind*III fragment and the transcription terminator from pGBT9 (Clontech). The promoter regions of *RAD6* and *MMS2* were inserted as PCR products (425 and 511 bp upstream of the start codon, respectively).

Determination of UV sensitivities

Unless otherwise noted, cells were grown in liquid YPD medium at 28°C to exponential phase by inoculation from a fresh overnight culture and further incubation with shaking for 4–6 h. For cell cycle experiments, stationary cells were obtained from cultures incubated for 4 days in YPD medium at 28°C. For S phase arrest, exponential cultures were treated with 100 mM hydroxyurea for 4 h, and G₂/M-arrested cells were obtained by incubation with 15 μg/ml nocodazole for 4 h. Arrests were confirmed by microscopic inspection of cell morphology. UV sensitivities were determined according to the two protocols outlined below.

Gradient assay. Suspensions of equal cell densities (~10⁷ cells/ml) were applied to a YPD plate in parallel lines by placing a drop of each culture (12 μl for a round plate of 80 mm diameter, 20 μl for a square plate of 120 × 120 mm) at one end and letting it run across the surface by tilting the Petri dish. The streaks were then irradiated with a gradient of UV dosage by gradually uncovering them from one end to the other during the specified time interval. The UV source was a hand-held lamp (254 nm) fixed at a distance corresponding to a dosage of 0.83 J/(m²·s). Plates were incubated in the dark and examined after 2–3 days.

Survival curves. Survival after UV irradiation was determined by spreading out appropriate dilutions of the respective cultures onto YPD plates and irradiation with specified dosages of UV light. Plates were incubated in the dark and colonies were counted after 3 days. The UV source was a hand-held lamp (254 nm) fixed at a distance corresponding to a dosage of either 0.83 or 1.67 J/(m²·s). All assays were performed two to six times. Standard deviations are shown where they exceed 30% of the respective values.

Northern blots

For UV induction assays *wt* cells grown to logarithmic phase in YPD were washed, resuspended in sterile water at ~2 × 10⁸ cells/ml and irradiated in suspension under constant stirring with a dosage corresponding to ~50% survival (determined by plating a sample before and after irradiation). Cells were harvested, resuspended in YPD prewarmed to 28°C and

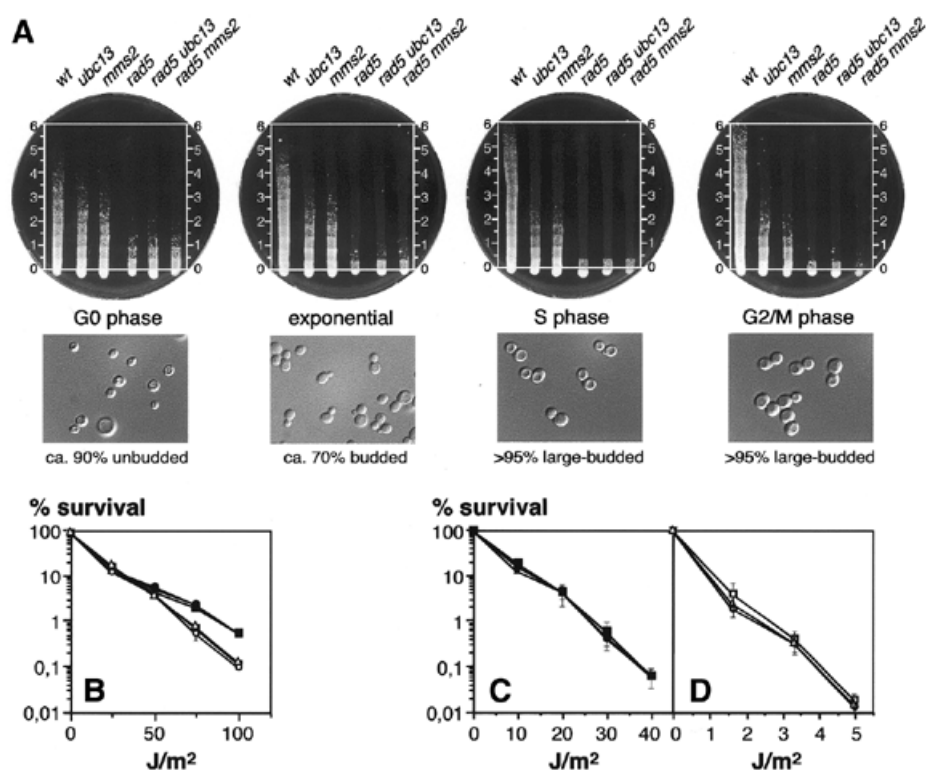


Figure 1. Comparison of the UV sensitivities of *rad5*, *rad5 ubc13* and *rad5 mms2* in different stages of the cell cycle. G₀ cells were derived from stationary cultures, S phase cells by hydroxyurea treatment and G₂/M phase cells by nocodazole treatment. (A) Double mutants were compared to *wt* and single mutants using the qualitative gradient assay as described in Materials and Methods. Cell morphologies were examined microscopically. Irradiation times (min) are indicated on each plate. (B) Survival after irradiation with specified dosages was determined in G₀ (filled symbols) and in S phase (open symbols) for *rad5* (squares), *rad5 ubc13* (circles) and *rad5 mms2* (triangles). (C) Survival in a recombination-deficient background of *rad52 rad5* (squares), *rad52 rad5 ubc13* (circles) and *rad52 rad5 mms2* (triangles). (D) Survival in an excision repair-deficient background of *rad2 rad5* (squares), *rad2 rad5 ubc13* (circles) and *rad2 rad5 mms2* (triangles). Each curve represents the average of two to six independent experiments. Standard deviations are given where they exceed 30% of the respective values.

incubated in the dark. For MMS induction studies MMS was added to a logarithmic culture in YPD to 0.1% (v/v) and incubation was continued for the specified time. Samples of $\sim 5 \times 10^8$ cells were harvested at the indicated time points and total RNA was isolated by hot phenol extraction (43). Samples of 15 μ g RNA each were glyoxylated and separated on a 1% (w/v) agarose gel. Transfer onto GeneScreen Plus membranes (Amersham) and hybridizations were performed as described (38). PCR products of the entire open reading frames excluding the introns were used as probes for the *MMS2* and *UBC13* genes and a 500 bp *ClaI* restriction fragment from a plasmid carrying *ACT1* served as probe for the *ACT1* gene. Bands were visualized using a Fujix BAS1000 Phosphor-imager and quantified with MacBas 2.0 software. Images were edited for better visibility without affecting relative signal strengths.

Measurements of β -galactosidase activities

Yeast cells harboring the promoter-*lacZ* fusion constructs integrated into the *ura3* locus were grown in YPD to logarithmic phase and irradiated with UV light in suspension or treated with 0.1% (v/v) MMS as described above. Samples of 5×10^8 cells were harvested at the indicated times and total protein extracts were prepared by disruption with glass beads in buffer Z (38), followed by low speed centrifugation. β -Galactosidase assays were performed with *o*-nitrophenyl galactoside as

described (38) and specific activities (Miller units) were calculated based on total protein content. All assays were performed in triplicate.

RESULTS

Epistasis of *RAD5* to *MMS2* and *UBC13* is observed throughout the cell cycle

Our previous data indicate that *MMS2* and *UBC13* are both hypostatic to *RAD5* but not to *RAD30* with respect to UV sensitivity (26). In contrast, an independent study found additivity between the UV sensitivities of the *mms2* and *rad5* mutations, while no data were shown for *ubc13* (22). In order to shed light on this issue the relationship between *RAD5* and *MMS2* as well as *UBC13* was re-examined under a number of different experimental conditions. A possible dependence on the cell cycle was examined by irradiation of cells arrested in different stages of the cell cycle and subsequent determination of survival. Two different methods were applied for optimal detection of slight differences in UV sensitivities: overall sensitivities were compared qualitatively by applying a gradient of UV dosage to cells streaked in parallel lines across a Petri dish; in addition, exact survival rates were determined by irradiation with specified dosages and colony counting (see Materials and Methods). Figure 1A shows the survival of *rad5 ubc13* and *rad5 mms2* in comparison to the *wt* and single

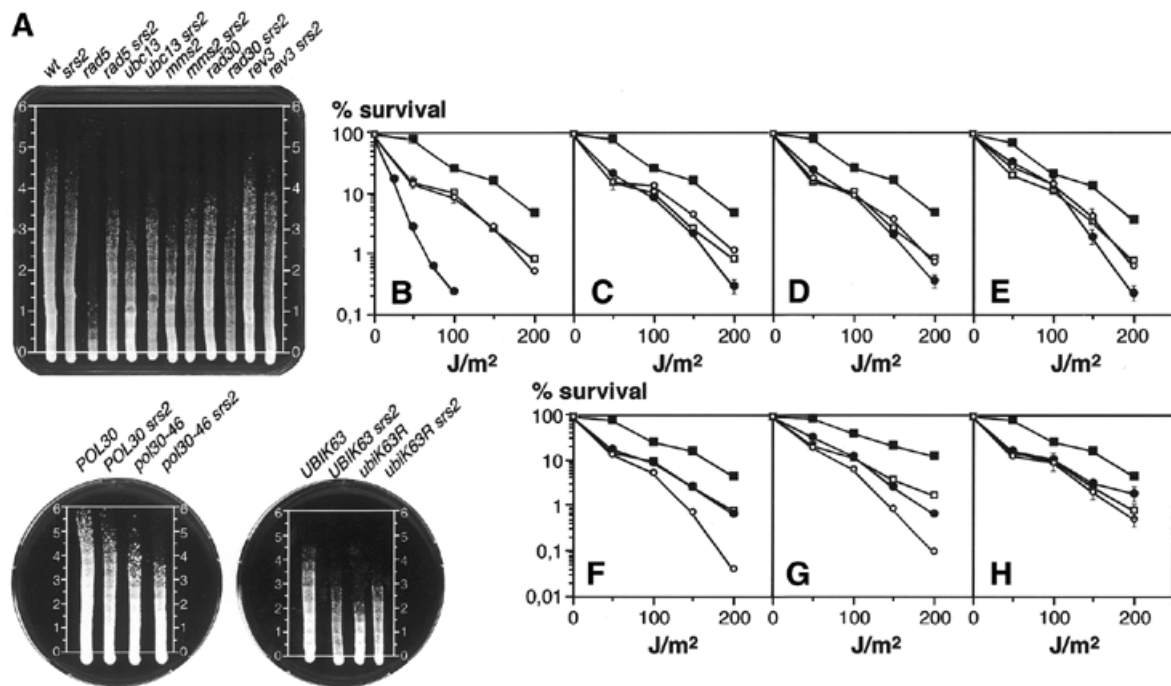


Figure 2. Suppression of UV sensitivity by *srs2* within a sub-set of the *RAD6* pathway. (A) Gradient assays were performed as described in Materials and Methods. Irradiation times (min) are indicated on each plate. (B–H) Survival after UV irradiation was determined as described in Figure 1 for the *wt* (filled squares) and an *srs2* (open squares) deletion mutant and compared to a series of single mutants in other *RAD6* pathway genes (filled circles) as well as the combination of those mutants with *srs2* (open circles): (B) *rad5*; (C) *ubc13*; (D) *mms2*; (E) *ubiK63R*; (F) *rad30*; (G) *pol30-46*; (H) *rev3*. Strains *pol30-46* and *ubiK63R* (SUB413) are plotted in comparison to their respective isogenic *wt* strains, *POL30* and *UBIK63* (SUB280), respectively.

mutants after irradiation of stationary (G_0), exponential, hydroxyurea-arrested (S) and nocodazole-treated (G_2/M) cells. The *wt* cells are significantly more sensitive when irradiated in G_0 than in S or G_2 phase. This situation is reversed in *rad5* mutants, confirming recent observations by Friedl *et al.* (33). Since cells arrested in G_1 with α factor show the same sensitivity as stationary cells (data not shown), this variation must be due to cell cycle stage and not to metabolic differences. Nevertheless, within each stage of the cell cycle the sensitivities of *rad5*, *rad5 ubc13* and *rad5 mms2* cells were indistinguishable (Fig. 1A and B). As it is conceivable that inactivation of other repair pathways might exacerbate any additional defects of the *rad5 ubc13* and *rad5 mms2* double mutants, UV sensitivities were also re-examined in *rad2* and *rad52* backgrounds, defective in nucleotide excision repair and homologous recombination, respectively. Again, identical sensitivities were found for *rad5*, *rad5 ubc13* and *rad5 mms2* cells (Fig. 1C and D). These experiments confirm that in the strain background used for this study the function of *UBC13* and *MMS2* is fully dependent on the presence of *RAD5* and thus support our previous assignment of *UBC13* and *MMS2* to the *RAD5*-dependent branch of the *RAD6* pathway (26).

srs2 acts as a suppressor specific for the *RAD5/UBC13/MMS2*-dependent branch of the *RAD6* pathway

In order to define the position of *SRS2* with respect to the different sub-branches of the PRR system, the effect of an *srs2* deletion on the UV sensitivities of other *RAD6* pathway mutants, *rad5*, *ubc13*, *mms2*, *rad30*, *pol30-46* and *rev3*, was examined. Since many of the single mutants are only slightly more UV-sensitive than *srs2* itself, again the qualitative

gradient assay and the colony assay were both applied for optimal detection of a possible suppression effect. Figure 2A shows that the UV sensitivities of *rad5*, *ubc13* and *mms2* are all suppressed by the *srs2* deletion. Consequently, all three double mutants, *rad5 srs2*, *ubc13 srs2* and *mms2 srs2*, display low UV sensitivities very similar to the *srs2* single mutant. In contrast, the *rad30 srs2* and *pol30-46 srs2* double mutants are significantly more UV-sensitive than each of the corresponding single mutants. In the case of *rev3*, the effect of *srs2* is less clear, since the UV sensitivity of the *rev3* deletion is even weaker than that of *srs2*, precluding the identification of a visible suppression effect.

Quantitative survival curves for the individual strains are shown in Figure 2B–H. Again, the *srs2* suppression effect on *rad5*, *ubc13* and *mms2* is evident (Fig. 2B–D), while additive effects are observed with the *rad30* and *pol30-46* mutants (Fig. 2F and G). Suppression of the *rad5* mutation is in good agreement with recent data (33). Moreover, those double mutants in which suppression is active also display the characteristic curve shape of the *srs2* single mutant itself. This S-shaped curve has been observed previously with exponential *srs2* cells (28) and is due to a sub-population of highly UV-sensitive cells within the culture that likely corresponds to G_1 cells, which are much more sensitive to UV irradiation than G_2 cells. Thus, the *srs2* mutation behaves epistatic to *rad5*, *mms2* and *ubc13* in this respect. Again, the effect of *srs2* on the *rev3* mutant is not easily interpretable (Fig. 2H), but the absence of additivity suggests an epistatic relation, which is also supported by the *srs2*-like shape of the survival curve of the *rev3 srs2* double mutant. In conclusion, the *srs2* suppressor

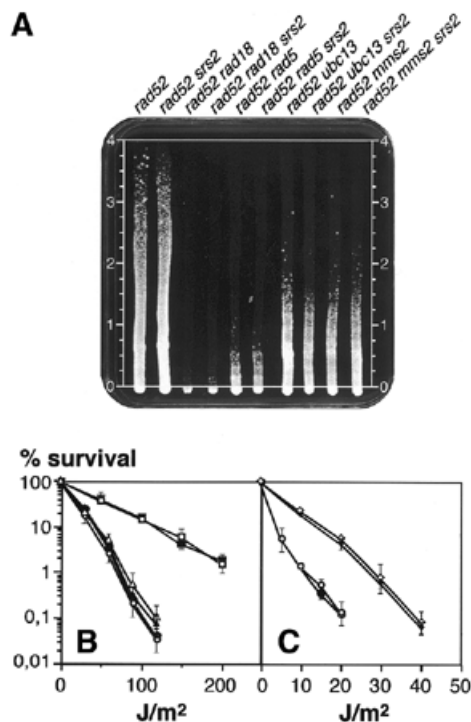


Figure 3. Consequences of defective homologous recombination for suppression of UV sensitivity by *srs2*. (A) Gradient assay. (B) Survival after UV irradiation for *rad52* (filled squares), *rad52 srs2* (open squares), *rad52 ubc13* (filled circles), *rad52 ubc13 srs2* (open circles), *rad52 mms2* (filled triangles) and *rad52 mms2 srs2* (open triangles). (C) Survival after UV irradiation for *rad52 rad18* (filled circles), *rad52 rad18 srs2* (open circles), *rad52 rad5* (filled diamonds) and *rad52 rad5 srs2* (open diamonds).

acts in a manner specific for the *RAD5/UBC13/MMS2*-dependent branch of the *RAD6* pathway.

srs2 acts a suppressor of the *ubiK63R* mutation

Due to the modest UV sensitivities of *ubc13* and *mms2* mutants the extent of suppression by *srs2* is relatively weak compared to the effect on *rad5*. In order to confirm that the observed phenomenon is specific for the error-free repair branch, the consequences of an *srs2* deletion were examined in the *ubiK63R* strain, in which all the repair proteins are present and the only modification is a point mutation in the ubiquitin gene (25). As K63 of ubiquitin serves as a substrate for ubiquitin chain polymerization by the Ubc13p–Mms2p dimer (24), the corresponding mutant should display a phenotype identical to the *mms2* or *ubc13* deletion strains. When compared to the isogenic *wt* strain, suppression of UV sensitivity by *srs2* was indeed observed (Fig. 2A and E).

Suppression by *srs2* depends on a functional recombination system

The suppression effect of *srs2* is dependent on functional homologous recombination, presumably because of a channeling of lesions into recombinational repair in the absence of *SRS2* (31). Thus, if the *srs2* effects on *ubc13*, *mms2* and *rad5* are functionally related, suppression should be abolished in a *rad52* background. Figure 3 shows that this is the case: deletion of *SRS2* in the *rad52 ubc13*, *rad52 mms2* and *rad52 rad5* double mutants did not result in any change in UV

sensitivities. The same situation was observed with the *rad52 rad18* double mutant. Likewise, no suppression of *ubc13* or *mms2* by *srs2* was observed in stationary phase cells, which predominantly exist with an unreplicated genome (in *G*₀) and should thus be incapable of repair by recombination between sister chromatids (data not shown). In conclusion, the relationship between *SRS2* and the members of the *RAD5/UBC13/MMS2*-dependent branch of PRR is comparable to that between *SRS2* and *RAD18* or *RAD6*.

MMS2 is a damage-inducible gene like *UBC13*

Most of the genes involved in post-replication DNA repair have been shown to be inducible by DNA damage, such as *RAD6* itself (44), *RAD18* (45), *RAD30* (12), *RAD5* (46) and *UBC13* (47,48). Considering the close cooperation between Mms2p and the other members of the *RAD6* pathway in DNA repair, the *MMS2* gene should be expected to be regulated in a similar fashion. I therefore investigated the effect of UV irradiation as well as MMS treatment on transcription of the *MMS2* gene by northern analysis. Probes specific for the *ACT1* and *UBC13* transcripts served as controls for a constitutively expressed and a damage-inducible gene, respectively. Figure 4A shows that the *MMS2* mRNA levels increased by a factor of 3 after UV irradiation, while treatment with 0.1% MMS afforded a 6-fold induction. These values are comparable to the roughly 5-fold induction of the *UBC13* transcript (Fig. 4A; 47,48). Interestingly, however, the induction of *MMS2* by MMS followed significantly slower kinetics than that of *UBC13*, with maximal induction after 2 h compared to <1 h for *UBC13*. This observation may explain why *MMS2* had not been identified as MMS-inducible in two previous studies, in which analysis was limited to 60 or 30 min after treatment, respectively (47,48). In order to confirm the damage-inducibility of the *MMS2* gene by an alternative method, its promoter region was fused to the coding sequence of the *lacZ* gene and the resulting construct was integrated into the *ura3* locus. For comparison, the corresponding fusion with the *RAD6* promoter was constructed likewise. Assays for β -galactosidase activity show that both promoters are functional and inducible by UV irradiation as well as MMS treatment in this context, and the relative induction of the *MMS2* promoter as measured by β -galactosidase activity corresponds well to the values obtained by northern analysis (Fig. 4B).

DISCUSSION

The relationship between *RAD5* and *UBC13/MMS2*

Rad5p protein physically interacts with Ubc13p via its RING finger domain and in this manner recruits the Ubc13p–Mms2p dimer to chromatin for DNA repair (26). While at this time it is unknown whether Rad5p acts solely as a recruitment factor for the UBC, as a substrate for ubiquitylation or even as a ubiquitin ligase in cooperation with Ubc13p–Mms2p, the biochemical data are consistent with the observation that the activity of *UBC13* and *MMS2* within the *RAD6* pathway is fully dependent on the presence of the *RAD5* gene (26). Since this relationship has important consequences for the organization of the PRR system, its genetic analysis has been expanded here to account for variations in repair capacities during different stages of the cell cycle. While duplication of the genome

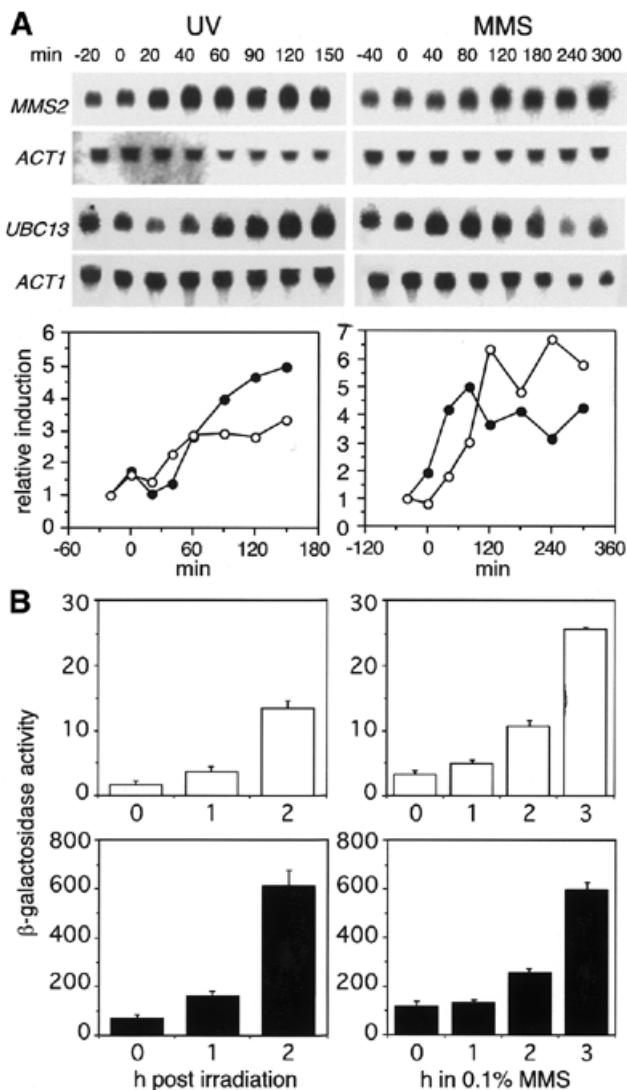


Figure 4. Induction of the *MMS2* and *UBC13* transcripts by DNA damage. (A) Northern blots were analyzed with probes specific for *UBC13* or *MMS2* as indicated, stripped and re-hybridized with a probe specific for the *ACT1* gene. Numbers above each lane denote the time of sampling (min) relative to irradiation (left) or addition of 0.1% MMS to the cultures (right), respectively. Induction levels for *UBC13* (filled circles) and *MMS2* (open circles) were calculated relative to the respective *ACT1* signals. (B) Activities of β -galactosidase (Miller units based on total protein concentration) in cell extracts prepared after UV irradiation or 0.1% MMS treatment, respectively, of strains bearing a reporter construct of the *lacZ* gene fused to the promoter of *MMS2* (open bars) or *RAD6* (solid bars), respectively. Values represent the average of three independent experiments.

confers additional UV resistance to *wt* cells, presumably due to their ability to repair DNA damage by homologous recombination, *rad5* mutants are further sensitized during S and G₂ phase compared to G₁ (Fig. 1; 33). Mutants of *ubc13* and *mms2* exhibit less dependence on the cell cycle, although as with *rad5* the difference from the *wt* is more pronounced in S and G₂ than in G₁ (Fig. 1A). Nevertheless, the presence or absence of *UBC13* and *MMS2* was found to have no influence on UV sensitivity in a *rad5* background in any stage of the cell cycle, thus further demonstrating their dependence on *RAD5* for activity in DNA repair. Similarly, inactivation of other repair

systems that might otherwise obscure the importance of the PRR pathway for survival had no effect on the epistatic relationship of *RAD5* to *UBC13* and *MMS2* either (Fig. 1C and D). In summary, the available data all support an assignment of *UBC13* and *MMS2* to the *RAD5*-dependent sub-branch of the *RAD6* pathway.

The role of *SRS2* in post-replication DNA repair

While deletion of *SRS2* alone leads to relatively mild defects in haploid cells, combination of *srs2* with mutations in the helicase gene *SGS1* or one of several recombination factors results in extremely poor growth or even synthetic lethality, which is, however, suppressible by mutations in other recombination or checkpoint factors (35,49–52). Based on these observations it has been proposed that the absence of *SRS2* and *SGS1* leads to unrestrained recombination and ultimately cell death due to chromosome loss or permanent checkpoint activation, which can only be avoided by preventing the initiation of recombination (53,54).

In PRR *SRS2* has been suggested to act upstream of the *RAD6*-dependent repair machinery. According to this model *SRS2* would exert an anti-recombinogenic effect and suppression by *srs2* would result from a channeling of lesions into recombinational repair in the absence of PRR (30,31,46). Here I have shown that various members of the *RAD6*-dependent repair system are affected differentially by deletion of the *srs2* gene. While the UV sensitivities of *rad5*, *ubc13* and *mms2* mutants are fully suppressed down to the level of the *srs2* single mutant (29,31; unpublished results). Hence, the *RAD30*- and *POL30*-dependent branches of the *RAD6* pathway, which would be crippled by deletion of *RAD6* or *RAD18*, but are not subject to suppression by *srs2*, are likely responsible for this enhanced sensitivity of *rad6 srs2* and *rad18 srs2*. Based upon the biochemical data that suggest a heteromeric complex in which the Ubc13p–Mms2p UBC is recruited to the Rad6p–Rad18p dimer by means of Rad5p, it is attractive to hypothesize that this complex may actually be the target of Srs2p action in error-free PRR.

The interaction of *SRS2* with the *REV3*-dependent, error-prone branch of the *RAD6* pathway is less readily interpretable. Sensitivity of the *rev3* mutant is neither suppressed nor significantly enhanced by deletion of *srs2*. A similar relationship was recently observed between *srs2* and a mutant of *POL32*, which encodes a subunit of polymerase δ and was classified as another member of the error-prone system (55). Likewise, *srs2* does not suppress the deficiency of *rev3* with respect to induced mutagenesis (29). In fact, the *srs2* mutation alone was found to exhibit a significant depression of UV-induced forward mutagenesis, implying that the presence of Srs2p is required for mutagenic translesion synthesis (28). Moreover, a physical interaction between Srs2p and Pol32p

was detected in the two-hybrid system (55). Thus, an influence of *SRS2* on error-prone repair is apparent.

Based on the strong synergistic effect between *mms2* and *rev3* with respect to UV-induced killing it has been proposed that Mms2p and Rev3p compete for a common DNA substrate (56). According to the model of *SRS2* action in PRR outlined above, the Srs2p helicase would thus be involved in creating these substrates, thereby preventing repair by homologous recombination and allowing either mutagenic translesion synthesis or *MMS2*-mediated error-free repair. Srs2p may thus be an important factor contributing to the decision between error-free and error-prone PRR. How this decision is reached, however, remains to be determined. Elucidating the events at the sites of damage on molecular terms, particularly establishing the order in which the individual repair factors are recruited there, will undoubtedly provide valuable insight into the complicated mechanism of the *RAD6*-dependent repair system.

ACKNOWLEDGEMENTS

I would like to thank Regine Kahmann for generous support. Anna Friedl is acknowledged for communicating unpublished data. I also thank Peter Burgers, Dan Finley, Michael Knop, Wilfried Kramer, Kiran Madura and David Schild for plasmids, Sabine Thalmeir for construction of the *srs2* deletion strain and Stefan Jentsch, in whose laboratory part of the work was initiated. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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