Letter to the Editor

The Complexity of the Interaction Between *RAD52* and *SRS2*

THE *SRS2* gene of *Saccharomyces cerevisiae* encodes a DNA helicase (RONG and KLEIN 1993). The earliest identification of the gene resulted from a search for suppressors of the ultraviolet sensitivity of *rad6* (LAWRENCE and CHRISTENSEN 1979) and *rad18* mutations (ABOUSSEKHRA *et al.* 1989). The gene was independently identified in a screen for hyperrecombinational mutations (RONG *et al.* 1991). *srs2* mutations, including deletions, confer a dosage-dependent dominance in their suppression of *rad6* and *rad18* mutations (ABOUSSEKHRA *et al.* 1989; SCHIESTL *et al.* 1990; RONG *et al.* 1991). This means that a diploid heterozygous for *srs2* suppresses the *rad6* and *rad18* mutations nearly as well as a strain homozygous for the same *srs2* mutation. *srs2* mutations also exhibit a peculiar dosage dependency in their response to the radiomimetic agent methyl methanesulfonate (MMS) in that diploids homozygous for the *srs2* mutation are more sensitive to the agent than are mutant haploids (ABOUSSEKHRA *et al.* 1989).

Studies on *srs2* mutations have shown that their suppressive effect on *rad6* mutations requires the presence of *RAD52* (SCHIESTL *et al.* 1990), the leader of an epistasis group devoted to double strand break repair and homologous recombination. Although the *RAD52* requirement and the genetic interaction of *SRS2* with three other members of the epistasis group, *RAD50* (ABOUSSEKHRA *et al.* 1989; PALLADINO and KLEIN 1992), *RAD51* (ABOUSSEKHRA *et al.* 1992) and *RAD54* (PALLADINO and KLEIN 1992), intimated its interaction with *RAD52*, no direct involvement has been discovered.

Now, two groups of workers have identified *srs2* mutations as suppressors of *rad52* mutations (MILNE *et al.* 1995; SCHILD 1995). Both groups have found that *srs2* deletions suffice to confer suppression. In this regard *srs2* suppression of *rad52* is much like its suppression of *rad6* and *rad18* mutations. Both groups have also found that suppression does not occur by bypassing the *RAD52*-mediated step because *srs2* mutations cannot suppress a deletion of *RAD52*.

We, too, have identified *SRS2* by its effect on *RAD52* but in a different manner. In our experiment we attempted to clone a suppressor (*rms1*) of a ts *rad52* allele (*rad52–23*). Having characterized the suppressor mutation as recessive, we looked for clones within a *S. cerevisiae* library that would prevent suppression. The clone we pulled out of the library contained *SRS2*. A test for allelism between *SRS2* and our suppressor proved that they are unlinked, *i.e.*, *SRS2* is not our selected suppressor.

We hypothesized that an increased dosage of *SRS2* sensitizes cells to MMS, the agent we use to score *RAD52* competency. This became obvious when we noted that *SRS2* expression from a centromere plasmid sensitizes slightly a *RAD52* strain for growth on MMS, and expression from a high copy plasmid makes the *RAD52* wild-type strain more sensitive to MMS (Figure 1A).

We then conjectured that knocking out *SRS2* should enhance the action of the *rad52* suppressor that we were attempting to identify. Our results clearly showed that a *srs2* deletion in the presence of our suppressor worked to suppress the ts *rad52* mutation better than did the suppressor by itself (Figure 1B).

Obviously, the next question was whether the *srs2* deletion could suppress the ts *rad52* mutation by itself. The answer was negative (Figure 1B). The *srs2* deletion, by itself, does not suppress any of four ts mutations (KAYTOR and LIVINGSTON 1994), a nonsense mutation (BOUNDY-MILLS and LIVINGSTON 1993) or a deletion mutation of *RAD52*. We note that our nonsense mutation, *rad52–327*, results in a truncation equivalent to the allele, *rad52B*, found by MILNE *et al.* (1995) to be suppressed by *srs2*.

We can rationalize our inability to observe the suppressive ability of *srs2* deletions on *rad52* mutations by a number of experimental differences. First, we did not test the *rad52–20* allele used by SCHILD (1995). Furthermore, MILNE *et al.* (1995) expressed all *rad52* alleles from the strong *ADH* promoter, while our *rad52* mutations were chromosomal substitutions using the endogenous *RAD52* promoter. Thus, expression levels, mediated by copy number, could be a contributing factor. In addition, our agar dishes contain approximately tenfold higher MMS concentration than those employed by MILNE *et al.* (1995).

Our intention though is not to quibble about the differences but to point out the complexity of the situation. We all agree that deletion of *SRS2* is beneficial to some *rad52* mutations, and our results suggest that high dosage of *SRS2* is somewhat deleterious to a wild-type strain. The question is, under what circumstances do cells want *SRS2* around? Increased dosage makes cells slightly sick, and feeble *rad52* mutants are better off without *SRS2*. Furthermore, in its relief of *rad6* and *rad18* ultraviolet sensitivity, the conjecture is that its presence turns UV damage into lethal events and that its absence permits degradation of UV single-strand lesions into double-strand breaks for repair by the *RAD52* pathway. Under these circumstances cells seem better off with less or no Srs2p. Cells obviously have a need for *SRS2* because without it they become sensitive to various forms of radiation (ABOUSSEKHRA *et al.* 1989),

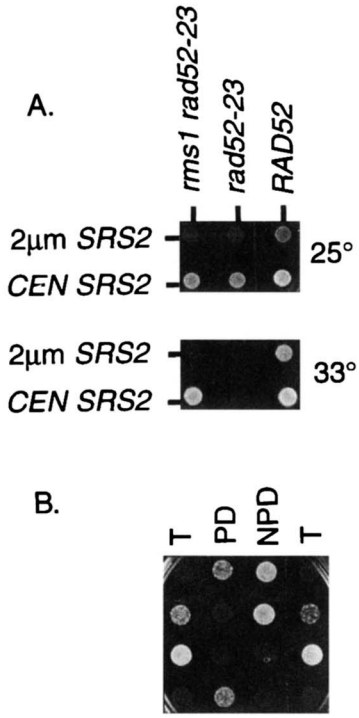

FIGURE 1.—The growth response of yeast strains to the copy number of *SRS2*. (A) *SRS2* inhibits growth on MMS. Three isogenic strains were transformed with *SRS2* on a centromere (*CEN*) or high copy (2 μm) plasmid. Approximately 1×10^4 cells in 5 μl of sterile water were spotted to selective agar containing 0.03% MMS and incubated at 25 or 33°. The three strains are SSL204 (*MATα trp1 his3 leu2 ura3 ade2*), SSL352 same as SSL204 except *rad52–23,* and SSL440 same as SSL204 except *rad52–23 rms1*. *rms1* suppresses *rad52–23*. (B) *srs2*Δ enhances suppression of *rad52–23* by *rms1* but does not suppress by itself. A cross between SSL440 (*rad52–23 rms1*) and SSL416 (*rad52–23 srs2*::*TRP1*) was sporulated, and dissected spore colonies were suspended in water and spotted to MMS agar. The plate was incubated at 33°. Two spore colonies survive on MMS at 33° because the suppressor *rms1* segregates 2:2. In all cases the patches with the more luxuriant growth are Trp^+, *i.e., srs2*Δ. In the case of the tetrad labeled PD, the two spore colonies unable to grow are Trp^+, showing that *srs2*Δ does not suppress *rad52–23*.

hyperrecombinational, balky at sporulation, and liable to synthetic lethality when combined with a *rad54* mutation (PALLADINO and KLEIN 1992). Thus, the gene is not wholly dispensable.

The paradox we see is the following. Dependence of *srs2* suppression of *rad6* UV sensitivity is thought to rely on *RAD52* because the absence of *SRS2* channels damaged DNA into the *RAD52* pathway. Although channeling lesions into the *RAD52* pathway may be palliative in a *RAD52* strain, why should channeling have the effect of suppressing *rad52* mutations? After all, if weakened *rad52* mutant products are having a difficult time keeping up with the demand, why would pushing more lesions their way be helpful? MILNE *et al.* (1995) suggested and tested the possibility that Srs2p physically interacts with Rad52p. Their results were negative. SCHILD (1995) suggested and tested the possibility that the absence of *SRS2* increases expression of *RAD51,* a condition known to suppress certain *rad52* mutants. His results were also negative. We look upon *SRS2* as a negative regulator of a pathway that parallels the *RAD52* pathway. Its removal opens up that pathway, relieving stress on the mutant *rad52* product. In the case of *rad6* suppression, we speculate that the *RAD52* dependence occurs because either the large number of lesions cannot be handled successfully by the pathway turned on by the absence of *SRS2* or there are some lesions that can only be handled by the *RAD52* pathway. This would also explain why *srs2* deletions do not bypass the need for *RAD52*.

The *SRS2* results have certainly piqued the interest of *RAD52* workers. What we might better focus on is the fact that Srs2p is a well characterized DNA helicase, because the importance of this knowledge has not been fully incorporated into our thinking about its interaction with *RAD52*.

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MICHAEL D. KAYTOR, MINH NGUYEN
and DENNIS M. LIVINGSTON
Department of Biochemistry
University of Minnesota
Minneapolis, Minnesota 55455

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