

Neurospora Spore Killers *Sk-2* and *Sk-3* Suppress Meiotic Silencing by Unpaired DNA

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Manuscript received December 3, 2006
Accepted for publication February 16, 2007

ABSTRACT

In *Neurospora crassa*, pairing of homologous DNA segments is monitored during meiotic prophase I. Any genes not paired with a homolog, as well as any paired homologs of that gene, are silenced during the sexual phase by a mechanism known as meiotic silencing by unpaired DNA (MSUD). Two genes required for MSUD have been described previously: *sad-1* (suppressor of ascus dominance), encoding an RNA-directed RNA polymerase, and *sad-2*, encoding a protein that controls the perinuclear localization of SAD-1. Inactivation of either *sad-1* or *sad-2* suppresses MSUD. We have now shown that MSUD is also suppressed by either of two Spore killer strains, *Sk-2* and *Sk-3*. These were both known to contain a haplotype segment that behaves as a meiotic drive element in heterozygous crosses of *killer* × *sensitive*. Progeny ascospores not carrying the killer element fail to mature and are inviable. Crosses homozygous for either of the killer haplotypes suppress MSUD even though ascospores are not killed. The killer activity maps to the same 30-unit-long region within which recombination is suppressed in *killer* × *sensitive* crosses. We suggest that the region contains a suppressor of MSUD.

SEXUAL reproduction in *Neurospora crassa* is mediated by the fusion of two haploid nuclei that carry compatible mating-type genes (*mat A* and *mat a*). The haploid nuclei proliferate in specialized premeiotic ascogenous tissue (precroziers and three-celled croziers) within the fertilized fruiting body (perithecium). Two nuclei of opposite mating type that are sequestered in the subapical cell of the crozier then migrate into a tubular sac (ascus) and fuse to create the zygote, which immediately undergoes two meiotic divisions and a postmeiotic mitosis. The resulting eight haploid nuclei reside in the same cytoplasm until they are sequestered into eight linearly ordered ascospores. The mating-type locus and other segregating markers show 1:1 segregation (RAJU 1992; DAVIS 2000).

Meiotic drive is a phenomenon in which certain chromosomal loci show a progeny ratio different from 1:1 and one allele is favored over the other. Examples of meiotic drive in animals include the segregation *distorter* (*SD*) system of *Drosophila melanogaster* and the *t* haplotype in the mouse (LYTTLE 1991a,b; SCHIMENTI 2000; KUSANO *et al.* 2003). In heterozygous males, the meiotic products that do not carry the drive element (*SD* chromosome or *t* haplotype) fail to differentiate into functional sperm. Although meiotic drive is widespread in insects, mammals, plants, and fungi, very few cases

have been dissected at the molecular level (PENNISI 2003; BURT and TRIVERS 2006).

In *Neurospora*, Spore killers are chromosomal elements that cause the death of ascospores that do not contain the killer element (TURNER and PERKINS 1979, 1991). For example, in a cross of Spore killer × wild type (*Sk* sensitive), every mature ascus contains four normal-sized, black, viable ascospores and four tiny, undeveloped ascospores that are inviable (see Figure 2A). All survivors carry the killer element. When both killer and sensitive nuclei are enclosed in the same ascospore (as occurs in the giant-spore *N. crassa* mutant *Banana* and in the four-spored species *N. tetrasperma*), the sensitive nuclei are sheltered (RAJU 1979, 1994, 2002; RAJU and PERKINS 1991; VAN DER GAAG *et al.* 2000). There is little or no ascospore death in homozygous *killer* × *killer* or *sensitive* × *sensitive* crosses. It was inferred that the killer has a resistance factor that protects not only its own nucleus from getting killed, but also other nonkiller nuclei in the same ascospore.

Three Spore killers have been studied extensively. *Spore killer-1* (*Sk-1*) was found in *N. sitophila*, while *Spore killer-2* (*Sk-2*) and *Spore killer-3* (*Sk-3*) were found in *N. intermedia* (TURNER and PERKINS 1979). *Sk-2* and *Sk-3* have been introgressed into *N. crassa* for detailed genetic analysis. Both have been mapped to the same general region on linkage group (LG) III. When either one is heterozygous, crossing over is blocked in a 30-MU region that spans the centromere (Figure 1) (CAMPBELL and TURNER 1987; TURNER 2003).

We dedicate this article to the memory of David Perkins for his enthusiastic support of our work on Spore killers and meiotic silencing.

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Loci conferring resistance to Spore killers have been found in wild populations (TURNER and PERKINS 1979, 1993; CAMPBELL and TURNER 1987). For example, the Spore-killer-resistant gene *r(Sk-2)* prevents killing of ascospores that do not contain the *Sk-2* haplotype. The recombination block observed in a heterozygous Spore killer cross is not suppressed by the resistant allele, however. An allele conferring partial resistance to *Sk-2* has also been reported (TURNER 2003). *pr(Sk-2)* gives only partial resistance to *Sk-2* killing when acting alone. However, when paired with a modifier allele, *mod(pr)*, *pr(Sk-2)* confers a high level of resistance. In a wild-type cross (*i.e.*, in the absence of a Spore killer), the resistant alleles do not affect crossover frequencies and can therefore be mapped precisely. Both *r(Sk-2)* and *pr(Sk-2)* are located within the recombination block region, while *mod(pr)* is located just outside of it (Figure 1).

We are interested in another seemingly unrelated ascus-dominant phenomenon called meiotic silencing by unpaired DNA (MSUD). If a copy of a gene is not properly paired with its homolog during prophase I, the MSUD mechanism creates a sequence-specific signal, which transiently silences all copies of that gene during meiosis and the subsequent mitosis (SHIU *et al.* 2001). For example, if an ectopic copy of the β -*tubulin* gene is inserted into one mating partner but not the other, all three copies (including those paired canonically) will be silenced. The resulting ascus is devoid of microtubules and will be arrested before metaphase I. Evidently, the presence of an unpaired copy of a gene is a red flag for mischief, which will alert the silencing system to destroy all transcripts similar to it (SHIU *et al.* 2001).

The MSUD mechanism suggests an explanation for the nature of many long-known ascus-dominant mutants, such as *Round spore*, *Peak*, and *Banana*. For example, the *Round spore* gene is formally categorized as ascus dominant because, in a heterozygous cross, all nuclei of the tetrad give rise to round ascospores; even those nuclei that contain a wild-type allele are round. The cause of dominance is probably rearrangements and/or deletions that prevent the wild-type allele from pairing with a homolog. MSUD is implicated in these ascus-dominant phenotypes of these mutants by virtue of the fact that a suppressor of meiotic silencing can eliminate the dominance. Although any gene can be subject to the action of MSUD, only those that encode a meiotic function will give an abnormal sexual phenotype. Genes that are responsible for nonmeiosis-specific products (such as amino acids) can be silenced but may not show a mutant sexual phenotype.

MSUD is likely to involve the production of double-stranded (ds)RNA and homologous mRNA degradation, since an RNA-directed RNA polymerase (RdRP), as encoded by *sad-1* ("suppressor of ascus dominance"), is involved (SHIU and METZENBERG 2002). RdRP is known to be important in other RNA interference (RNAi) and post-transcriptional silencing systems (AHLQUIST 2002).

For example, quelling, an RNA-silencing mechanism that targets hyperhaploid transgenes during the vegetative phase of *N. crassa*, requires an RdRP (QDE-1) (COGONI and MACINO 1999). Other genes involved in the meiotic silencing process include *sad-2*, which encodes a novel protein that controls the perinuclear localization of SAD-1 (SHIU *et al.* 2006), and *sms-2*, which encodes an argonaute-like protein (LEE *et al.* 2003). We report here that the Spore killer strains *Sk-2* and *Sk-3* contain a previously unidentified element that suppresses MSUD. Both *Sk-2* and *Sk-3*, like *Sad-1^A* and *Sad-2^{RIP}* mutants, allow the meiotic gene expression of many unpaired loci that otherwise would result in silencing by MSUD. The mechanism of suppression by the Spore killers, however, is different from those of *Sad-1* and *Sad-2*.

MATERIALS AND METHODS

Strains and crosses: Genotypes of the *Neurospora* strains used in this study are listed in Table 1. Individual genes are described in PERKINS *et al.* (2001) or RADFORD'S e-Compendium (http://www.bioinf.leeds.ac.uk/~gen6ar/newgenelist/genes/gene_list.htm). Various crosses between Spore killer strains and strains containing ectopically inserted genes (*e.g.*, $::act^+$, $::asm-1^+$) and two dominant mutants (*Dip-1*, *Round spore*) are shown in Table 2. Although multiple strains in both mating types were often used for various crosses, only a single representative cross of each mutant with wild type, with *Sad*, and with *Sk* is shown in Table 2. Culturing, crossing media, and genetic techniques were utilized as described in DAVIS and DE SERRES (1970), and DAVIS (2000).

Generally each of these duplication strains was crossed with a wild-type strain and with *Sk-2* and *Sk-3*. Many of the same duplication strains had previously been examined in homozygous and heterozygous crosses and in crosses with *Sad-1^A* and *Sad-2^{RIP}* (SHIU *et al.* 2001, 2006; SHIU and METZENBERG 2002).

For scoring fertility, crosses were made by simultaneous inoculation of the two mating types at opposite sides of petri plates containing crossing medium. Ejected ascospores were examined on the petri plate lid 3 weeks after simultaneous inoculation of the parents.

Cytological methods: Crosses for examining ascus development were made in petri plates. The protoperithecial parent was grown for 5 days (25°) and then fertilized by spreading conidia of the male parent. The scoring of fertility of each cross was initially based on ejected ascospores. Perithecia were opened for detailed observations on developing asci and for photographing rosettes of asci. Four to 6-day-old perithecia containing asci at all stages of meiosis and ascospore delimitation were stained using iron-hematoxylin or acriflavin (RAJU and NEWMAYER 1977; RAJU 1986). Rosettes of maturing asci from 7- to 10-day-old perithecia were lightly stained using 10- to 20-fold dilutions of ferric acetate and hematoxylin solutions. Developing asci from crosses of GFP-tagged strains were examined using an epifluorescence microscope (FREITAG *et al.* 2004). Each perithecium typically contains 200–400 asci. Over a thousand asci were scored for each cross.

RESULTS

Characteristics of *Sk-2* and *Sk-3*: The map locations of *Sk-2* and *Sk-3* and several linked genes in linkage group III are shown in Figure 1. The behavior of *Sk-2* and *Sk-3*

TABLE 1
Strains used in this study and their genotypes

Strain name or symbol ^a	Genotype	Strain nos. ^b
ORS <i>a</i>	Oak Ridge wild-type <i>mat a</i>	FGSC 2490
OR23-IV A	Oak Ridge wild-type <i>mat A</i>	FGSC 2489
Houma-1 <i>a</i>	Wild-type <i>mat a</i>	FGSC 2221
<i>fluffy</i> (OR) A	<i>fl mat A</i>	FGSC 4317
<i>fluffy</i> (RL) <i>a</i>	<i>fl mat a</i>	FGSC 6683
:: <i>act</i> ⁺ A	<i>mep his-3</i> ⁺ :: <i>act</i> ⁺ ; <i>pan-2 mat A</i>	95-20
:: <i>act</i> ⁺ <i>a</i>	<i>mep his-3</i> ⁺ :: <i>act</i> ⁺ ; <i>pan-2 mat a</i>	95-21
:: <i>asm-1</i> ⁺ A	<i>mep his-3</i> ⁺ :: <i>asm-1</i> ⁺ ; <i>pan-2 mat A</i>	01-39, 80-21, 80-22
:: <i>asm-1</i> ⁺ <i>a</i>	<i>mep his-3</i> ⁺ :: <i>asm-1</i> ⁺ ; <i>pan-2 mat a</i>	95-07
:: <i>Bml</i> ^R A (β -tubulin gene)	<i>mep his-3</i> ⁺ :: <i>Bml</i> ^R ; <i>pan-2 mat A</i>	95-24
:: <i>Bml</i> ^R <i>a</i> (β -tubulin gene)	<i>mep his-3</i> ⁺ :: <i>Bml</i> ^R ; <i>pan-2 mat a</i>	95-25
:: <i>Bml</i> ⁺ -GFP A	<i>rid his-3</i> ⁺ :: <i>Bml</i> ⁺ -GFP <i>mat A</i>	FGSC 9520, N2527
:: <i>Bml</i> ⁺ -GFP <i>a</i>	<i>rid his-3</i> ⁺ :: <i>Bml</i> ⁺ -GFP <i>mat a</i>	N2505
Diploid ascospores-1 A	<i>Dip-1 mat A</i>	24-40, 84-02
Diploid ascospores-1 <i>a</i>	<i>Dip-1 mat a</i>	20-39, 25-39
:: <i>hH1</i> -GFP A	<i>his-3</i> ⁺ :: <i>hH1</i> -GFP <i>mat A</i>	FGSC 9518
:: <i>hH1</i> -GFP <i>a</i>	<i>rid his-3</i> ⁺ :: <i>hH1</i> -GFP <i>mat a</i>	FGSC 9517
:: <i>hH3hH4</i> -1 A	<i>mep his-3</i> ⁺ :: <i>hH3hH4</i> -1 ⁺ ; <i>pan-2 mat A</i>	95-22
Δ <i>mat</i> (mating-type deletion)	<i>fl; nic-3; \Delta mat</i>	92-40
<i>mat A</i> (IL \rightarrow VR)	Δmat ; <i>am::mat A</i>	89-27
<i>Dp</i> (VIIR \rightarrow IL)5936 A	<i>fl; Dp</i> (VIIR \rightarrow IL)5936 <i>mat A</i>	18-13
:: <i>mei-3</i> ⁺ A	<i>mep his-3</i> ⁺ :: <i>mei-3</i> ⁺ ; <i>pan-2 mat A</i>	15-39
:: <i>mei-3</i> ⁺ <i>a</i>	<i>mep his-3</i> ⁺ :: <i>mei-3</i> ⁺ ; <i>pan-2 mat a</i>	15-30
Round spore A	<i>R; fl; inl mat A</i>	92-38
Round spore <i>a</i>	<i>R; fl; inl mat a</i>	92-39
<i>Sad-1</i> Δ A	<i>Sad-1</i> Δ <i>mat A</i>	96-02, 48-25
<i>Sad-1</i> Δ <i>a</i>	<i>Sad-1</i> Δ <i>mat a</i>	96-01
<i>Sad-2</i> ^{RIP} A	<i>Sad-2</i> ^{RIP} <i>mat A</i>	30-03
<i>Sad-2</i> ^{RIP} <i>a</i>	<i>Sad-2</i> ^{RIP} <i>mat a</i>	30-04, 33-12
<i>Sk-2</i> A	<i>Sk-2 mat A</i>	30-38, 60-38, FGSC 6648
<i>Sk-2 a</i>	<i>Sk-2 mat a</i>	FGSC 6647, DDP 3120
<i>Sk-2</i> :: <i>hH1</i> -GFP <i>a</i>	<i>Sk-2; his-3</i> ⁺ :: <i>hH1</i> -GFP <i>mat a</i>	24-37
Resistant to <i>Sk-2 a</i>	<i>r</i> (<i>Sk-2</i>) <i>acr-7 mat a</i>	88-01
Partially resistant to <i>Sk-2 a</i>	<i>pr</i> (<i>Sk-2</i>) <i>mod</i> (<i>pr</i>) <i>mat a</i>	97-40
<i>Sk-3</i> A	<i>Sk-3 mat A</i>	24-16, FGSC 7076
<i>Sk-3 a</i>	<i>Sk-3 acr-2 mat a</i>	FGSC 7077
<i>Sk-3</i> :: <i>hH1</i> -GFP <i>a</i>	<i>Sk-3; his-3</i> ⁺ :: <i>hH1</i> -GFP <i>mat a</i>	46-02

^a “::” indicates that an extra copy of a wild-type gene is inserted ectopically at the *his-3* locus on LG IR or at the *am* locus on LG VR.

^b The four-digit strain numbers (e.g., 95-20, 95-21) are from R. L. Metzberg’s collection. N2505 and N2527 are from M. Freitag, Oregon State University, Corvallis. The remaining strains are from either the Fungal Genetics Stock Center (FGSC) or the Perkins collection (DDP 3120) at Stanford University.

in heterozygous crosses with wild type and in homozygous crosses (*Sk* \times *Sk*) is shown in Figure 2A and described in the Introduction.

***Sk-2* suppresses the ascus-dominant mutant Diploid ascospores:** In crosses of *Diploid ascospores* (*Dip-1*) \times wild type, asci frequently contain two to four large ascospores instead of the normal eight (Figure 2B). *Dip-1* is thus ascus dominant. The large ascospores are expected to be either heterozygous diploids or heterokaryons, and upon germination they produce one or more heterokaryons. A cross between *Sad-1* Δ and *Dip-1* yields mostly normal eight-spored asci (Figure 2C). Expression of *Dip-1*, which presumably contains a deletion or a chromosome rearrangement, is suppressed by *Sad-1* Δ .

When a cross is heterozygous for Spore killer, the *Sk*-sensitive nuclei are sheltered from killing in a giant ascospore when the ascospore also contains one or more killer nuclei (RAJU 1979, 2002; RAJU and PERKINS 1991). While we were gathering evidence that *Dip-1* indeed causes diploidy or heterozygosity, we crossed *Dip-1* with *Sk-2* to determine whether the spore killer death is expressed in the large-spored asci. We predicted that the sensitive chromosome would be protected because it would be in the same large ascospore as the killer chromosome; *i.e.*, we expected to observe four black ascospores in most asci. Surprisingly, the cross produced almost entirely eight-spored asci, and these showed 4:4 ascospore patterns because of spore killing

TABLE 2
Suppression of meiotic silencing of unpaired loci by *Sad-1/Sad-2*, *Sk-2*, and *Sk-3*

Parent 1 ^a	Parent 2					
	× Wild type	× <i>Sad-1</i> ^Δ or <i>Sad-2</i> ^{sup}	× Spore killer-2	× Spore killer-3	× <i>r(Sk-2)</i>	× <i>pr(Sk-2)</i> <i>mod(pr)</i>
:: <i>act</i> [†]	Silenced, lollipop asci arrested in meiosis I	Not silenced, normal asci	Not silenced, 4:4 asci	Partially silenced, 4:4 asci	Silenced (no suppression)	Silenced (no suppression)
:: <i>asm-1</i> ⁺	Silenced, mostly white ascospores	Not silenced, 8-spored asci	Partially silenced, 50% 4:4 asci	Partially silenced, 25% 4:4 asci	Silenced	Silenced
:: <i>Bml</i> ^{RS} (mutant β -tubulin)	Silenced, all asci blocked in meiosis I	Not silenced, 8-spored asci	Not silenced, 4:4 asci	Not silenced, 4:4 asci	Silenced	Silenced
:: <i>Bml</i> ⁺ <i>-GFP</i>	Silenced, asci blocked in meiosis I	Not silenced, 8-spored asci	Not silenced, 4:4 asci for fluorescence and Sk	Not silenced, 4:4 asci for fluorescence and Sk	ND	ND
<i>Dip-1</i> (<i>Diploid ascospores</i>)	Silenced, asci mostly 2-4 spored	Not silenced, 8-spored asci	Not silenced, 4:4 asci	Silenced, asci mostly 2-4 spored	Silenced	Silenced
Dp(VIIR → IL)5936	Silenced, barren perithecia	Not silenced	Not silenced, 4:4 asci	Not silenced, 4:4 asci	Silenced	Silenced
:: <i>hH1-GFP</i>	Silenced, no fluorescent nuclei in meiosis	Not silenced, all nuclei fluoresce	Not silenced, nuclei fluoresce, 4:4 asci	Not silenced, nuclei fluoresce, 4:4 asci	ND	ND
:: <i>hH3hH4-1</i> ⁺	Silenced, asci showed meiotic defects	Not silenced	Not silenced, 4:4 asci	Not silenced, 4:4 asci	Silenced	Silenced
<i>mat A(IL → VR)</i>	Silenced, reduced fertility	Not silenced	Not silenced, 4:4 asci	Not silenced, 4:4 asci	Silenced	Silenced
:: <i>mei-3</i> ⁺	Silenced, meiotic defects, aborted asci	Not silenced, 8-spored asci	Not silenced, 4:4 asci	Partially silenced, meiotic defects	Silenced	Silenced
<i>Round spore</i>	Silenced, round ascospores	Not silenced, normal ascospores	Silenced, round ascospores, 4:4 asci	Silenced, round ascospores, 4:4 asci	Silenced	Silenced
<i>Sk-2</i> (<i>Spore killer-2</i>)	4K:4S ascospores in all asci	4K:4S ascospores in all asci	No killing, all 8 ascospores viable	All ascospores killed (mutual killing)	Partial killing, mostly 8:0, some 4:4 asci	Partial resistance, mostly 4:4 asci
<i>Sk-3</i> (<i>Spore killer-3</i>)	4K:4S ascospores in all asci	4K:4S ascospores in all asci	All ascospores killed (mutual killing)	No killing, all 8 ascospores viable	ND	ND

[†]“Silenced” indicates that the mutation or the inserted gene failed to function during ascus development because of meiotic silencing; “Not silenced” indicates that the mutation or the inserted gene functioned normally because of suppression of silencing of unpaired loci by *Sad-1/Sad-2*^{sup}, *Sk-2*, or *Sk-3*; “ND” (no data) indicates that the cross was not examined.

^a“::” indicates that an extra copy of a wild-type gene is inserted ectopically at the *his-3* locus on LG IR or at the *am* locus on LG VR.

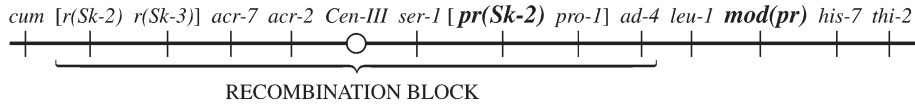


FIGURE 1.—Partial map of linkage group III showing the recombination block (RB) in crosses heterozygous for *Sk-2* or *Sk-3*. The RB contains the

locus or loci responsible for spore killing, but more precise mapping of *Sk* is impossible because the Spore killer element does not recombine with loci in the RB. Intervals in the diagram are not proportional to genetic map distances. (The figure is from TURNER 2003 with permission of the author.)

(Figure 2D). The eight-spored condition resembles that in a cross of *Dip-1* × *Sad-1^Δ*. This suggests that *Sk-2* might contain a dominant suppressor of meiotic silencing, and it prompted us to test the behavior of *Sk-2* and *Sk-3* in crosses with several loci previously shown to experience meiotic silencing (SHIU *et al.* 2001). *Sk-2* suppressed the *Dip-1* phenotype but *Sk-3* did not; the asci from *Dip-1* × *Sk-3* resembled those in *Dip-1* × wild type (Figure 2E).

***Sk-2* and *Sk-3* suppress the meiotic silencing of several unpaired loci:** Strains having a gene-sized duplication due to the insertion of an extra copy at an ectopic location (:: *act⁺*, :: *asm-1⁺*, :: *mei-3⁺*, :: *Bml^R*, and :: *hH3/hH4-1*), a gene relocated to an ectopic site [*mat A*(*IL* → *VR*)], or a deletion allele, as is believed to be the case for *Round spore* and *Dip-1*, will each have unpaired DNA that will be silenced throughout meiosis when crossed to a wild-type strain. The result is a dramatic reduction of ascospore production and/or a mutant ascus phenotype. The mutant phenotypes, for example, include ascospore maturation defects (:: *asm-1⁺*), swollen lollipop asci (:: *act⁺*), and round ascospores (*Round spore*). To determine whether *Sk-2* and *Sk-3* carry a dominant suppressor of meiotic silencing, we made

crosses heterozygous for unpaired MSUD-sensitive loci containing *Sk-2* and *Sk-3*, respectively.

Cytological observations on the suppression of meiotic silencing by *Sk-2* and *Sk-3* are given here for several partial duplication strains that contain an ectopically inserted gene at the *his-3* locus on linkage group I.

***actin*:** Partial duplication strains for the wild-type *actin* (*act⁺*) gene grew normally in the vegetative phase, but they showed dramatic deleterious effects in the sexual phase. In crosses of wild type × :: *act⁺*, >90% of young asci became abnormally swollen like lollipops (Figure 3A). These asci, which usually contained a single nucleus, were arrested in meiotic prophase and subsequently degenerated. Apparently, both the unpaired :: *act⁺* and its native paired homologs were silenced during meiosis. The remaining 5–10% of asci that were less severely swollen progressed through meiosis and a postmeiotic mitosis. These often delimited one or two large ascospores and less frequently up to eight normal-sized ascospores (SHIU *et al.* 2001). In contrast, crosses of :: *act⁺* × *Sad-1^Δ* and :: *act⁺* × *Sk-2* showed completely normal ascus development through the stages of ascospore delimitation and formation of linearly ordered ascospores (Figure 3, B and C). The maturing asci of

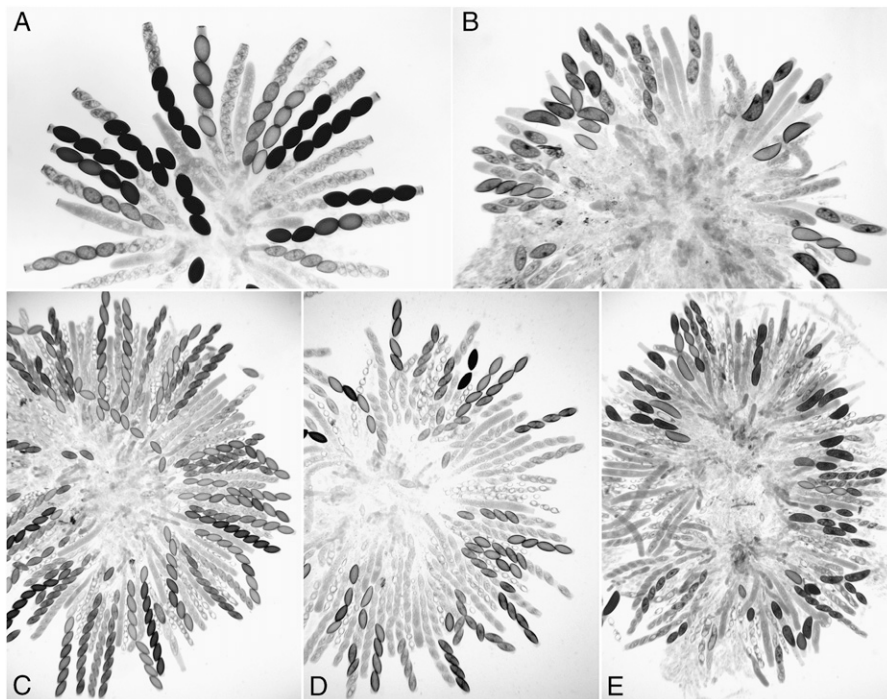


FIGURE 2.—Rosettes of maturing asci showing the phenotype of *Dip-1* and its suppression by *Sk-2* and *Sad-1^Δ* 8 days after fertilization. (A) *Sk-2* × wild type. Maturing asci show four normal (larger) spores and four small aborted spores. All the mature ascospores carry the *Sk-2* killer. The first-division segregation (4K:4S) in all asci indicates that there is no crossing over between *Sk-2* and the centromere (from RAJU 1979). (B) *Dip-1* × wild type. Asci showing two to four abnormally large ascospores because of MSUD. (C) *Dip-1* × *Sad-1^Δ*. A rosette of asci, all showing eight normal-size ascospores. The *Dip-1* phenotype is completely suppressed by *Sad-1^Δ*. (D) *Dip-1* × *Sk-2*. A rosette of eight-spored asci, where the *Dip-1* phenotype is completely suppressed by *Sk-2*. The asci show the characteristic 4:4 *Sk* phenotype. (E) *Dip-1* × *Sk-3*. Asci showing mostly large ascospores. The infrequent eight-spored asci show a 4:4 *Sk* phenotype. The *Dip-1* phenotype is not suppressed by *Sk-3*.

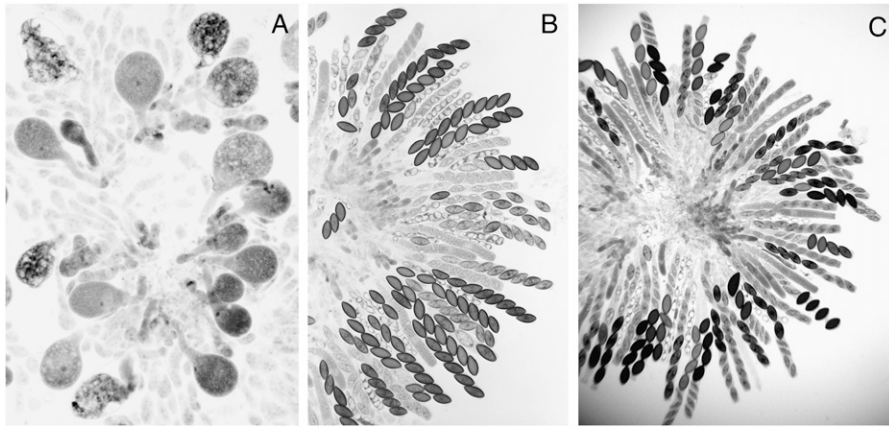


FIGURE 3.—Rosettes of asci showing the consequences of the meiotic silencing of actin and the suppression of silencing by *Sad-1 Δ* and *Sk-2*. (A) $::act^+$ \times wild type 6 days after fertilization. Abnormally swollen asci resulting from silencing of actin genes. Karyogamy is normal in the swollen asci but almost all asci abort and degenerate without completing meiosis I. (B) $::act^+$ \times *Sad-1 Δ* 8 days after fertilization. Meiosis and postmeiotic mitoses are completely normal; the silencing of actin is suppressed by *Sad-1 Δ* . (C) $::act^+$ \times *Sk-2* 8 days after fertilization. As in the case of *Sad-1 Δ* , the meiotic silencing of actin is suppressed by *Sk-2*.

The 4:4 ascospore patterns result from the death of the *Sk*-sensitive ascospores. The consequence of the suppression of MSUD by *Sk-2* is identical to that of *Sad-1 Δ* , except that there is no ascospore death in the *Sad-1 Δ* cross. *Sk-3* also suppresses the MSUD of actin but to a lesser degree than with *Sk-2*.

$::act^+$ \times *Sk-2* contained four viable black ascospores and four inviable unpigmented ascospores (4K:4S) because of the Spore killer. Thus *Sad-1 Δ* and *Sk-2* completely suppressed the meiotic silencing of the unpaired $::act^+$ gene.

Sk-3 also suppressed the meiotic silencing of $::act^+$, but to a lesser degree. Eight-spored asci were produced, but many young asci were slightly swollen, suggesting that actin levels were less than normal. Nevertheless, the asci progressed through meiosis, postmeiotic mitoses, and ascospore development.

β -tubulin: As with $::act^+$, the mycelia of duplication strains grew normally if they carried an ectopic $::Bml^R$ gene [a mutant β -tubulin (*Bml*) gene, which confers resistance to benomyl]. However, in crosses of $::Bml^R$ \times wild type, asci became elongated but failed to undergo meiosis or delimit ascospores. The asci were arrested and contained a single, condensed nucleus, and no division spindle was formed (Figure 4A). Apparently, the β -tubulin genes were meiotically silenced when an

unpaired copy was present in the cross (SHIU *et al.* 2001). In *Sk-2* \times $::Bml^R$, meiotic silencing of *Bml* was completely suppressed by *Sk-2*: asci developed normally and produced 4:4 ascospore patterns for *Sk* (Figure 4B). *Sk-3* also suppressed the meiotic silencing of *Bml*. However, nuclear divisions were delayed in up to 25% of asci. The tardy asci produced fewer than eight ascospores and also showed defects in ascospore maturation.

***meiotic-3*, an ortholog of *recA* and *RAD51*:** Heterozygous crosses of $::mei-3^+$ with wild type resulted in the meiotic silencing of the *meiotic-3* (*mei-3*) genes, as expected. Asci showed perturbations during meiosis and almost all asci aborted at or shortly after spore delimitation; <5% of asci contained any maturing ascospores (Figure 4C). In *Sk-2* \times $::mei-3^+$, ascus development was fully restored, and all maturing asci showed normal 4:4 ascospore patterns for *Sk* (Figure 4D). Thus, the suppression of meiotic silencing of *mei-3* genes by *Sk-2* is complete. *Sk-3* also suppressed the silencing of *mei-3*, but only partially. In *Sk-3* \times $::mei-3^+$, asci underwent meiosis

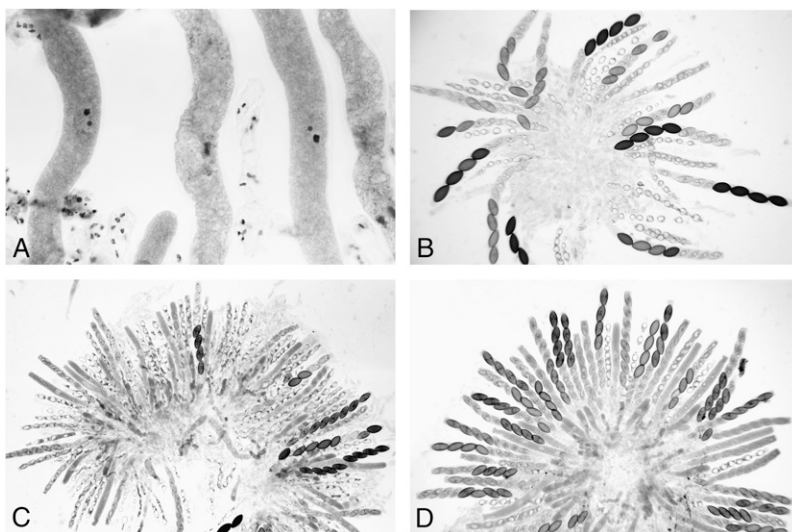


FIGURE 4.—Abnormal ascus development resulting from the meiotic silencing of *Bml* and *mei-3* genes and the suppression of silencing by *Sk-2*. (A) $::Bml^R$ \times wild type 5 days after fertilization. The β -tubulin genes are silenced, and ascus development is arrested prior to metaphase I. (B) $::Bml^R$ \times *Sk-2* 8 days after fertilization. Meiotic silencing of β -tubulin is suppressed by *Sk-2*, and asci continue to develop through ascospore maturation. The maturing asci show *Sk*-caused 4:4 ascospore abortion patterns. (C) $::mei-3^+$ \times wild type 8 days after fertilization. A rosette of mostly aborted asci; <5–10% of asci contain mature ascospores. Nuclear divisions do occur in the aborted asci, albeit abnormally, and most of the asci delimit eight ascospores, which become vacuolated and degenerate. (D) $::mei-3^+$ \times *Sk-2* 7 days after fertilization. Meiotic silencing is completely suppressed by *Sk-2*, and the ascus development through spore delimitation is normal. The maturing asci show 4:4 patterns for *Sk*.

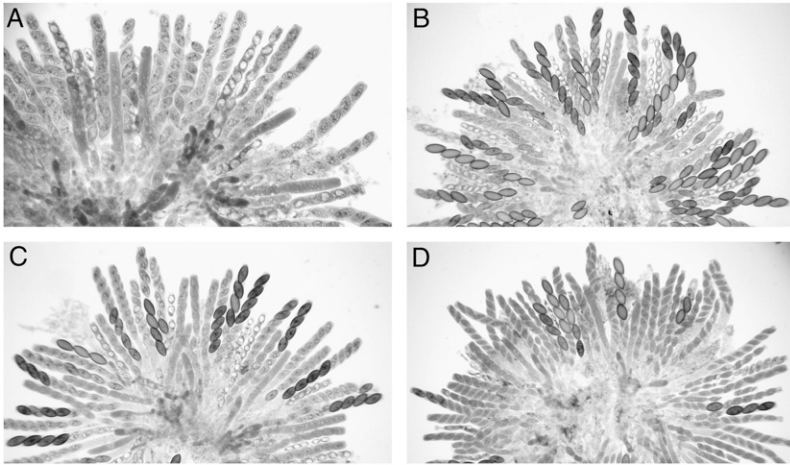


FIGURE 5.—Rosettes of asci showing the consequences of the meiotic silencing of *asm-1*, and the suppression of meiotic silencing by *Sad-1 Δ* , *Sk-2*, and *Sk-3* 8 days after fertilization. (A) $::asm-1^+ \times$ wild type. Early ascus development is normal through spore delimitation and the first mitosis in young ascospores. All eight ascospores are subsequently arrested and fail to mature (in $>95\%$ of asci) because of the meiotic silencing of *asm-1* genes. (B) $::asm-1^+ \times Sad-1\Delta$. The meiotic silencing of *asm-1* is suppressed by *Sad-1 Δ* and the ascospores develop and mature in the majority of asci. (C) $::asm-1^+ \times Sk-2$. A rosette of maturing asci at 8 days. Meiotic silencing is suppressed by *Sk-2* in $\sim 50\%$ of asci. These asci show normal ascospore development (4:4 ascospore abortion patterns). (D) $::asm-1^+ \times Sk-3$. A rosette of maturing asci at 8 days. Suppression of *asm-1* by *Sk-3* occurs in $<25\%$ of asci. These

minority asci show 4:4 ascospore abortion patterns, and there is no suppression in the majority of asci, where all eight ascospores are aborted, just as in $::asm-1^+ \times$ wild type.

and a postmeiotic mitosis, and they delimited ascospores. However, ascospore development was abnormal and all eight ascospores were aborted in $\sim 50\%$ of the asci. The remaining asci contained only one to four maturing ascospores.

***asm-1* (APSES transcription factor):** A mutant of the *ascospore maturation-1* gene (*Asm-1 Δ*) showed dominant effects in crosses with a wild type, where all eight ascospores failed to mature in $>95\%$ of asci (Figure 5A; ARAMAYO and METZENBERG 1996). This result was shown to be due to MSUD, and the abnormality could be suppressed by *Sad-1 Δ* , restoring near-normal fertility (Figure 5B; SHIU *et al.* 2001). We have tested $::asm-1^+$ in crosses with *Sk-2* and *Sk-3*. In *Sk-2* $\times ::asm-1^+$, ascus development was completely normal through meiosis and a postmeiotic mitosis. Ascospore delimitation and the first mitosis in young ascospores were also normal. In $\sim 50\%$ of asci, further development of all eight ascospores was arrested soon after the first mitosis, just as in wild type $\times ::asm-1^+$ (or *Asm-1 Δ*). The two nuclei in the arrested ascospores were much condensed. In the remaining 50% of asci, up to four ascospores developed to full size and maturity (Figure 5C). In *Sk-3* $\times ::asm-1^+$, early ascus development was the same as that in *Sk-2* $\times ::asm-1^+$, except that $<20\%$ of asci showed any maturing ascospores (Figure 5D). Thus *Sk-2* suppresses MSUD of $::asm-1^+$ more efficiently than does *Sk-3*.

***hH3hH4-1 $^+$* , *mat A*, and *Dp(VIIR \rightarrow IL)5936*:** Three additional strains, $::hH3hH4-1^+$ (ectopic insert of histone H3/H4-1), *mat A* (*IL \rightarrow VR*) (relocation of mating-type A), and *Dp(VIIR \rightarrow IL)5936* (a long segmental duplication of VIIR inserted into IL), were tested. Crosses of these three strains to wild type, *Sad-1 Δ* , *Sad-2^{RIIP}*, *Sk-2*, and *Sk-3* were scored for fertility and for the numbers of black ascospores ejected; they were not examined cytologically, however. In crosses with wild types, all three strains produced very few ascospores (silenced). *Sad-1 Δ* , *Sad-2^{RIIP}*, *Sk-2*, and *Sk-3* restored the

fertility of all three strains to near-normal levels (*i.e.*, silencing was suppressed) (Table 2).

Use of GFP-tagged histone H1 and β -tubulin gene inserts for visualizing the suppression of MSUD by *Sk-2* and *Sk-3*: In wild type $\times ::hH1-GFP$, an unpaired copy of an ectopically integrated *histone H1-GFP* gene and its homologs were silenced throughout meiosis and until after ascospore delimitation (Figure 6A); the silenced *hH1-GFP* was expressed in the developing ascospores ~ 20 – 24 hr after spore delimitation (FREITAG *et al.* 2004). In contrast, in a cross of *Sad-2^{RIIP}* (or *Sad-1 Δ*) $\times ::hH1-GFP$, the *hH1-GFP* is expressed throughout meiosis owing to the suppression of MSUD. As a result, the nuclei fluoresced throughout meiosis, a postmeiotic mitosis, and the early development of the young ascospores (Figure 6B). To determine whether *Sk-2* and *Sk-3* gave the same chronological suppression pattern as *Sad-1 Δ* and *Sad-2^{RIIP}*, we crossed $::hH1-GFP$ with *Sk-2* and *Sk-3*. In both crosses, nuclei fluoresced throughout meiosis, a postmeiotic mitosis, and ascospore delimitation, just as in *Sad-1 Δ* (or *Sad-2^{RIIP}*) $\times ::hH1-GFP$ (Figure 6, C and D). Initially, all eight young ascospores (both *hH1-GFP* and non-*hH1-GFP*) showed the nuclear fluorescence. As the ascospores developed and matured, the nuclear fluorescence gradually faded away in the four non-*hH1-GFP* ascospores. Subsequently, four of the eight ascospores that carried the *Sk*-sensitive nuclei stopped developing, and only the four ascospores that contained the killer nuclei matured. Because $::hH1-GFP$ and *Sk* are unlinked, the four maturing ascospores showed fluorescence in only 50% of the asci. Just as with $::hH1-GFP \times Sk$, crosses of $::Bml-GFP \times Sk-2$ or *Sk-3* (or *Sad-1 Δ*) showed suppression of meiotic silencing, and asci showed normal development through meiosis and spore delimitation (Figure 6, E–H). β -Tubulin–GFP fluorescence could be seen in the developing asci and in four of the eight ascospores (Figure 6F). Thus, the suppression of MSUD by *Sk-2* and *Sk-3* is qualitatively

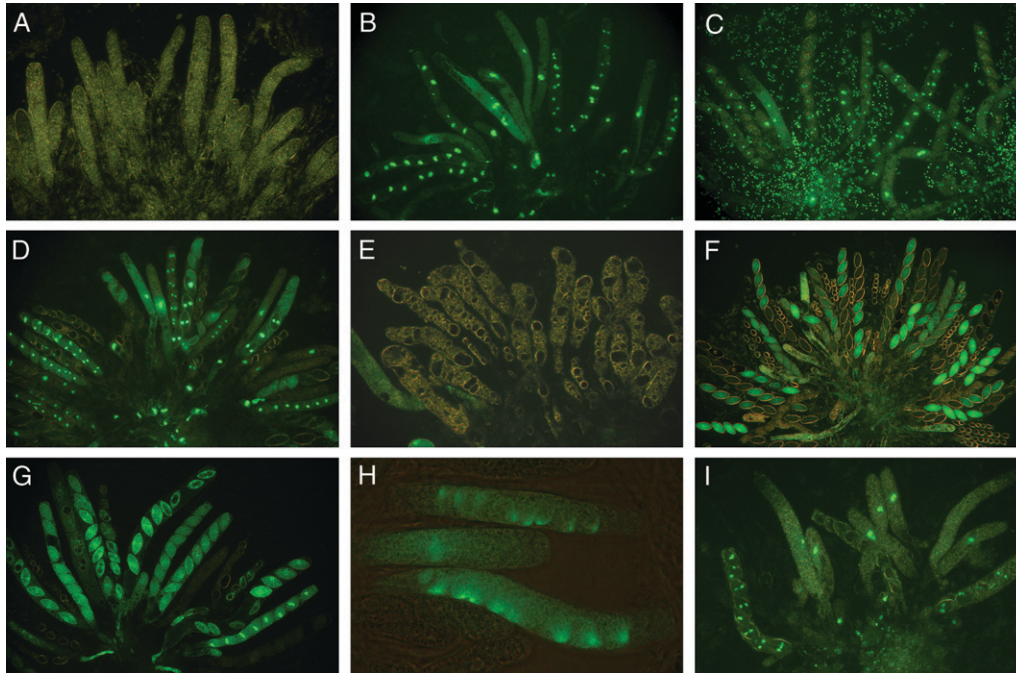


FIGURE 6.—The use of GFP-tagged *hH1* and β -tubulin genes for visualizing meiotic silencing and its suppression by *Sad-2^{RIP}*, *Sk-2*, and *Sk-3*. (A) Wild type \times $::hH1-GFP$ 4 days after fertilization. In the heterozygous asci, the unpaired *hH1-GFP* is completely silenced in young asci throughout meiosis, a postmeiotic mitosis, and spore delimitation. (B) *Sad-2^{RIP}* \times $::hH1-GFP$ 5–6 days after fertilization. Meiotic silencing of *hH1-GFP* is suppressed by *Sad-2^{RIP}*, and the nuclei fluoresce throughout meiosis, a postmeiotic mitosis, and in the young ascospores. As the ascospores develop, the nuclear *hH1-GFP* fluorescence gradually fades away in four of the eight non-*hH1-GFP* ascospores. (C) $::hH1-GFP$

(female) \times *Sk-2* (male) 5–6 days after fertilization. Meiotic silencing of *hH1-GFP* is suppressed by *Sk-2*, and the nuclei fluoresce throughout meiosis and in the young ascospores. The background fluorescent nuclei are in the nonascogenous paraphyseal cells of the female parent. (D) *Sk-3* \times $::hH1-GFP$ 6 days after fertilization. Meiotic silencing of *hH1-GFP* is suppressed by *Sk-3*, just as in *Sad-2^{RIP}* \times $::hH1-GFP$ (6B). (E–H) Meiotic silencing of β -tubulin and its suppression by *Sad-1 Δ* , *Sk-2*, and *Sk-3*. (E) Wild type \times $::Bml-GFP$ 6 days after fertilization. The expression of β -tubulin is silenced, and the asci are arrested early in meiosis. (F) *Sad-1 Δ* \times $::Bml-GFP$ 8 days after fertilization. Meiotic silencing of β -tubulin is suppressed by *Sad-1 Δ* . Developing asci and four of the eight ascospores of each ascus show β -tubulin–GFP. (G) *Sk-2* \times $::Bml-GFP$ 6 days after fertilization. Meiotic silencing of β -tubulin is suppressed by *Sk-2*, resulting in normal ascus and ascospore delimitation. The far right ascus shows a bright spindle in each ascospore at the first mitosis. *Sk*-caused death of four of the eight ascospores is just visible in two asci. (H) *Sk-3* \times $::Bml-GFP$ 5 days after fertilization. Meiotic silencing of β -tubulin is suppressed by *Sk-3*. Two asci at spore delimitation (top and bottom) show fluorescent spindle pole bodies at one end of each young ascospore. (I) *Sk-2* \times *Sk-2*; $::hH1-GFP$ 5 days after fertilization. Meiotic silencing of *hH1-GFP* is suppressed by *Sk-2* even when *Sk-2* is homozygous, and there is no ascospore killing. Thus suppression of MSUD is independent of ascospore killing. (A and B are from SHIU *et al.* 2006.)

and chronologically similar to that of conventional *Sad* mutants.

Heterozygosity for *Sk-2* or *Sk-3* is not required for the suppression of meiotic silencing: We tested whether meiotic silencing is defective in crosses that are homozygous (and hence completely paired) for *Sk-2* or for *Sk-3* and heterozygous for either $::hH1-GFP$ or $::Bml-GFP$. Our results demonstrate that MSUD was suppressed in these crosses. For example, in *Sk-2* \times *Sk-2*; $::hH1-GFP$, nuclei fluoresced throughout meiosis and ascospore development, indicating that meiotic silencing of $::hH1-GFP$ was suppressed (Figure 6I). Similar suppression of MSUD was observed in *Sk-3* \times *Sk-3*; $::hH1-GFP$, *Sk-2* \times *Sk-2*; $::Bml-GFP$, and *Sk-3* \times *Sk-3*; $::Bml-GFP$. These observations confirm that MSUD is inoperative (*i.e.*, suppressed) when *Sk-2* or *Sk-3* is homozygous, as well as when it is heterozygous, and that the unpaired $::hH1-GFP$ and $::Bml-GFP$ genes are expressed normally. Thus, unlike spore killing, where *Sk-2* or *Sk-3* must be heterozygous, heterozygosity for the *Sk* complex is not required for the suppression of meiotic silencing by either of the *Sk* haplotypes.

Loci that confer resistance to *Sk-2* killing do not suppress MSUD: To determine whether the resistant alleles can suppress MSUD, we crossed strains containing *r(Sk-2)* and *pr(Sk-2) mod(pr)*, respectively, to strains containing different unpaired loci. Results show that these Spore-killer-resistant alleles do not suppress meiotic silencing in any of the unpaired loci tested (Table 2). These data suggest that, unlike the *Sk-2* and *Sk-3* complexes, the *Sk-2*-resistant strains do not contain a dominant suppressor of MSUD.

Interactions of *Sk-2* and *Sk-3* with *Sad-1 Δ* and *Sad-2^{RIP}*: We have seen that the two Spore killers suppress the meiotic silencing of several gene inserts nearly as efficiently as do the two previously described *Sad* mutants. Clearly, the *Sk* and *sad* genes are not allelic: *Sk-2* and *Sk-3* are on linkage group III (tight centromere linkage), *sad-1* is on linkage group IL, and *sad-2* is on linkage group VR. We have examined the interactions of *Sad-1 Δ* and *Sad-2^{RIP}* with *Sk-2* and *Sk-3* in various intercrosses. In *Sk-2* \times *Sad-2^{RIP}*, for example, ascus development was completely normal through ascospore delimitation, and four of the eight ascospores failed to mature, just as in *Sk-2* \times wild

type. Similarly, $Sk-3 \times Sad-1^A$ also produced 4:4 ascospore patterns, indicating that *Sad* mutants do not prevent the death of *Sk*-sensitive ascospores.

DISCUSSION

Meiotic silencing is a phenomenon in which a gene that is unpaired in meiosis generates a signal that silences all copies of that gene. Since presence of a sequence found in one mating partner but not in the other could result from a virus on the move, targeting unpaired DNA for silencing could represent a defense mechanism that protects the organism during times of vulnerability. In other words, the MSUD mechanism could prevent the invasion of a partner genome by an insertion element during a period when other pre-meiotic silencing mechanisms such as quelling and repeat-induced point (RIP) mutation are dormant (GALAGAN and SELKER 2004). Furthermore, SHIU *et al.* (2001) have shown that a dominant suppressor of meiotic silencing can confer fertility (albeit marginal) on otherwise barren interspecific crosses in the genus *Neurospora*. This suggests that MSUD not only could provide surveillance against invading sequences but also might play a role in reproductive isolation.

Meiotic silencing works by examining the pairing between homologous sequences during prophase I. One possible model is that the MSUD scanning machinery can sense the presence of an unpaired DNA sequence and subsequently generate a single-stranded aberrant (a)RNA from it. The aRNA is then duplicated into a dsRNA species by the action of SAD-1 (an RNA-directed RNA polymerase). SAD-2 is required to recruit SAD-1 to the perinuclear region, which could be the central location for RNAi activity. The dsRNA, through the action of dicers, is then processed into short-interfering RNAs, which subsequently direct the specific destruction of mRNA similar in sequence to the unpaired DNA.

Previously, we identified two dominant suppressors of MSUD ($Sad-1^A$ and $Sad-2^{RIP}$) via the selection of mutants that can bypass the pairing requirement of genes coding for wild-type spore production (SHIU and METZENBERG 2002; SHIU *et al.* 2006). Initially, we expected the dominant $Sad-1$ mutant to contain a hypermorphic allele that was able to interfere with the normal function of a wild-type SAD-1 protein. As it turned out, the original $Sad-1$ mutant is dominant over wild type because it has a truncation at its 3'-end (SHIU *et al.* 2001; SHIU and METZENBERG 2002). This deletion deprives the wild-type allele of a pairing partner in a $Sad-1^A \times$ wild-type cross and therefore remains under the radar of the MSUD mechanism. Consequently, the silencer will be silencing itself to some low level via a negative feedback mechanism, thus creating a dominant phenotype for the $Sad-1^A$ allele. Similarly, the original $Sad-2$ mutant contains numerous RIP mutations that leave the wild-

type allele unpaired in a heterozygous $Sad-2$ cross and therefore subject to self-silencing.

This article describes the finding that two meiotic drive elements in *Neurospora* ($Sk-2$ and $Sk-3$) act as dominant suppressors of meiotic silencing in a cross. One may wonder what could be the nature of their sequences that make them dominant over wild type. The $Sk-2$ and $Sk-3$ loci, which originated in *N. intermedia*, are likely to have a gene order in linkage group III different from that of wild-type *N. intermedia* and *N. crassa*. One possible explanation for our findings is that an *Sk* chromosome contains a deletion allele of a *sad* gene, which, unlike the two known *sad* genes, is not needed for the completion of meiosis. More plausibly, the suppression of MSUD could be due to a suppressor protein that interferes with the gene-silencing pathway. The gene coding for the suppressor protein, however, is unlikely to be represented by the $r(Sk-2)$ - and $pr(Sk-2)$ -resistant loci, since they themselves do not suppress MSUD.

Results presented here show that $Sk-2$ and $Sk-3$ indeed suppress most of the unpaired loci tested (Table 2), suggesting that $Sk-2$ and $Sk-3$ contain a dominant suppressor of MSUD and superficially resemble $Sad-1^A$ and $Sad-2^{RIP}$ in suppressing meiotic silencing. $Sk-2$ suppresses the meiotic silencing of $::act^+$, $::asm-1^+$, $::Bml^R$, and $::mei-3^+$ more efficiently than does $Sk-3$. However, any differences between the suppression by $Sk-2$ and $Sk-3$ are quantitative, not qualitative, except in the case of $Dip-1$. Spore killers differ from the two *Sad* mutants in several respects: (1) spore killers are meiotic drive elements that distort genetic ratios of *Sk*-linked genes; (2) spore killers are not simple mutant alleles, but rather putative complex rearrangements that show a recombination block in a 30-MU area (and are thus haplotypes); (3) both $Sk-2$ and $Sk-3$ are homozygous fertile, whereas $Sad-1^A$ and $Sad-2^{RIP}$ are barren when homozygous in a cross (RAJU 2002; SHIU *et al.* 2006); and (4) neither $Sk-2$ nor $Sk-3$ suppresses the MSUD associated with the unpairing of the *Round spore* locus.

It remains unclear whether there is an evolutionary benefit of having a dominant suppressor of meiotic silencing in the *Sk* region. At first glance, it could increase the mating potential of fungi carrying an *Sk* haplotype, since they can breed with strains that have a different genome arrangement. Normally, a cross lacking perfect DNA pairing may have some of its meiotic genes silenced, thus affecting overall fertility. The wider selection of potential mating partners could help the *Sk* elements to transmit themselves into a population. It will be of interest to determine whether elimination of the putative MSUD suppressor would affect the mating potential of strains carrying an *Sk* haplotype.

The studies of *SD*, *het-s*, *Sk*, and other examples described by LYTTLE (1991a,b), DALSTRA *et al.* (2003, 2005), and BURT and TRIVERS (2006) show that there are several diverse cellular mechanisms that can trigger meiotic drive. However, the *Podospora* [*Het-s*] prion,

the *Drosophila* *SD* locus, and the mouse *t* locus are the only three meiotic drive elements that have been characterized at the molecular level (SCHIMENTI 2000; KUSANO *et al.* 2001, 2003; MADDELEIN *et al.* 2002; DALSTRA *et al.* 2003, 2005). For example, *SD* in *Drosophila* causes preferential transmission of *SD* chromosomes because *SD*⁺-bearing spermatids fail to mature in *SD/SD*⁺ males. *Sd* encodes a mutant form of RanGAP protein, which is an important player in the Ran-signaling pathway required for nuclear transport, spindle assembly, and other nuclear functions that ultimately affect spermatid elongation and maturation (MERRILL *et al.* 1999; KUSANO *et al.* 2001).

Neurospora Spore killers provide yet another example of the diverse themes of segregation distortion. *Sk-2* and *Sk-3* behave like the two previously characterized dominant suppressors of meiotic silencing (*Sad-1* and *Sad-2*), yet little is known about their mode of action in killing spores or in overcoming meiotic silencing. Future studies concerning the Spore killer loci and molecular identification of the killing and resistance factors, as well as their suppressor function, will be fascinating for those who are interested in meiotic silencing, sexual selection, and how evolutionary accidents can lead to the creation of selfish DNA elements.

N.B.R., R.L.M., and P.K.T.S. were supported by National Science Foundation grants MCB0417282 (to D. Perkins), MCB0234421 (to R.L.M.), and MCB0544237 (to P.K.T.S.).

LITERATURE CITED

- AHLQUIST, P., 2002 RNA-dependent RNA polymerases, viruses, and RNA silencing. *Science* **296**: 1270–1273.
- ARAMAYO, R., and R. L. METZENBERG, 1996 Meiotic transvection in fungi. *Cell* **86**: 103–113.
- BURT, A., and R. TRIVERS, 2006 *Genes in Conflict: The Biology of Selfish Genetic Elements*. The Belknap Press of Harvard University Press, Cambridge, MA.
- CAMPBELL, J. L., and B. C. TURNER, 1987 Recombination block in the Spore killer region of *Neurospora*. *Genome* **29**: 129–135.
- COGONI, C., and G. MACINO, 1999 Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase. *Nature* **399**: 166–169.
- DALSTRA, H. J., K. SWART, A. J. DEBETS, S. J. SAUPE and R. F. HOEKSTRA, 2003 Sexual transmission of the [Het-S] prion leads to meiotic drive in *Podospora anserina*. *Proc. Natl. Acad. Sci. USA* **100**: 6616–6621.
- DALSTRA, H. J., R. VAN DER ZEE, L. SWART, R. F. HOEKSTRA, S. J. SAUPE *et al.*, 2005 Non-Mendelian inheritance of the HET-s prion or HET-s prion domains determines the *het-S* spore killing system in *Podospora anserina*. *Fungal Genet. Biol.* **42**: 836–847.
- DAVIS, R. H., 2000 *Neurospora: Contributions of a Model Organism*. Oxford University Press, New York.
- DAVIS, R. H., and F. J. DE SERRES, 1970 Genetic and microbiological research techniques for *Neurospora crassa*. *Methods Enzymol.* **17A**: 79–143.
- FREITAG, M., P. C. HICKEY, N. B. RAJU, E. U. SELKER and N. D. READ, 2004 GFP as a tool to analyze the organization, dynamics and function of nuclei and microtubules in *Neurospora crassa*. *Fungal Genet. Biol.* **41**: 897–910.
- GALAGAN, J. E., and E. U. SELKER, 2004 RIP: the evolutionary cost of genome defense. *Trends Genet.* **20**: 417–423.
- KUSANO, A., C. STABER and B. GANETZKY, 2001 Nuclear mislocalization of enzymatically active RanGAP causes segregation distortion in *Drosophila*. *Dev. Cell* **1**: 351–361.
- KUSANO, A., C. STABER, H. Y. CHAN and B. GANETZKY, 2003 Closing the (Ran)GAP on segregation distortion in *Drosophila*. *BioEssays* **25**: 108–115.
- LEE, D. W., R. J. PRATT, M. MCLAUGHLIN and R. ARAMAYO, 2003 An argonaute-like protein is required for meiotic silencing. *Genetics* **164**: 821–828.
- LYTTLE, T. W., 1991a Segregation distorters. *Annu. Rev. Genet.* **25**: 511–557.
- LYTTLE, T. W. (Editor), 1991b The genetics and evolutionary biology of meiotic drive. *Am. Nat.* **137**: 281–456.
- MADDELEIN, M. L., S. DOS REIS, S. DUVEZIN-CAUBET, B. COULARY-SALIN and S. J. SAUPE, 2002 Amyloid aggregates of the HET-s prion protein are infectious. *Proc. Natl. Acad. Sci. USA* **99**: 7402–7407.
- MERRILL, C., L. BAYRAKTAROGU, A. KUSANO and B. GANETZKY, 1999 Truncated RanGAP encoded by the Segregation Distorter mutation in *Drosophila*. *Science* **283**: 1742–1745.
- PENNISI, E., 2003 Meiotic drive: bickering genes shape evolution. *Science* **301**: 1837–1839.
- PERKINS, D. D., A. RADFORD and M. S. SACHS, 2001 *The Neurospora Compendium*. Academic Press, San Diego/London.
- RAJU, N. B., 1979 Cytogenetic behavior of Spore killer genes in *Neurospora*. *Genetics* **93**: 607–623.
- RAJU, N. B., 1986 A simple fluorescent staining method for meiotic chromosomes of *Neurospora*. *Mycologia* **78**: 901–906.
- RAJU, N. B., 1992 Genetic control of the sexual cycle in *Neurospora*. *Mycol. Res.* **96**: 241–262.
- RAJU, N. B., 1994 Ascomycete spore killers: chromosomal elements that distort genetic ratios among the products of meiosis. *Mycologia* **86**: 461–473.
- RAJU, N. B., 2002 Spore killers: meiotic drive elements that distort genetic ratios, pp. 275–296 in *Molecular Biology of Fungal Development*, edited by H. D. OSIEWACZ. Marcel Dekker, New York.
- RAJU, N. B., and D. NEUMEYER, 1977 Giant ascospores and abnormal croziers in a mutant of *Neurospora crassa*. *Exp. Mycol.* **1**: 152–165.
- RAJU, N. B., and D. D. PERKINS, 1991 Expression of meiotic drive elements *Spore killer-2* and *Spore killer-3* in asci of *Neurospora tetrasperma*. *Genetics* **129**: 25–37.
- SCHIMENTI, J., 2000 Segregation distortion of mouse *t* haplotypes: the molecular basis emerges. *Trends Genet.* **16**: 240–243.
- SHIU, P. K. T., and R. L. METZENBERG, 2002 Meiotic silencing by unpaired DNA: Properties, regulation, and suppression. *Genetics* **161**: 1483–1495.
- SHIU, P. K. T., N. B. RAJU, D. ZICKLER and R. L. METZENBERG, 2001 Meiotic silencing by unpaired DNA. *Cell* **107**: 905–916.
- SHIU, P. K. T., D. ZICKLER, N. B. RAJU, G. RUPRICH-ROBERT and R. L. METZENBERG, 2006 SAD-2 is required for meiotic silencing by unpaired DNA and perinuclear localization of SAD-1 RNA-directed RNA polymerase. *Proc. Natl. Acad. Sci. USA* **103**: 2243–2248.
- TURNER, B. C., 2003 Analysis of two additional loci in *Neurospora crassa* related to Spore killer-2. *Fungal Genet. Biol.* **39**: 142–150.
- TURNER, B. C., and D. D. PERKINS, 1979 Spore killer, a chromosomal factor in *Neurospora* that kills meiotic products not containing it. *Genetics* **93**: 587–606.
- TURNER, B. C., and D. D. PERKINS, 1991 Meiotic drive in *Neurospora* and other fungi. *Am. Nat.* **137**: 416–429.
- TURNER, B. C., and D. D. PERKINS, 1993 Strains for studying Spore killer elements in four *Neurospora* species. *Fungal Genet. Newsl.* **40**: 76–78.
- VAN DER GAAG, M., A. J. M. DEBETS, J. OOSTERHOF, M. SLAKHORST, J. A. G. M. THIJSSSEN *et al.*, 2000 Spore-killing meiotic drive factors in a natural population of the fungus *Podospora anserina*. *Genetics* **156**: 593–605.

Communicating editor: M. S. SACHS