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QIP, a Protein That Converts Duplex siRNA Into Single Strands, Is Required for Meiotic Silencing by Unpaired DNA

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ABSTRACT

RNA interference (RNAi) depends on the production of small RNA to regulate gene expression in eukaryotes. Two RNAi systems exist to control repetitive selfish elements in *Neurospora crassa*. Quelling targets transgenes during vegetative growth, whereas meiotic silencing by unpaired DNA (MSUD) silences unpaired genes during meiosis. The two mechanisms require common RNAi proteins, such as RNA-directed RNA polymerases, Dicers, and Argonaute slicers. We have previously demonstrated that, while Quelling depends on the redundant dicer activity of DCL-1 and DCL-2, only DCL-1 is required for MSUD. Here, we show that **Q**DE-2-**i**nteracting **p**rotein (QIP), an exonuclease that is important for the production of single-stranded siRNA during Quelling, is also required for MSUD. QIP is crucial for sexual development and is shown to colocalize with other MSUD proteins in the perinuclear region.

NEUROSPORA crassa is a filamentous fungus that grows by hyphal tip extension and branching (Glass *et al.* 2000). Since septa (cross walls) between individual cells are normally incomplete, deleterious elements such as viruses or selfish DNA can easily infiltrate the entire network of hyphae (known as the mycelium). To combat these repetitive elements, several genome surveillance systems have evolved and are maintained in *N. crassa*. For example, repeat-induced point mutation, a premeiotic process operating during the haploid dikaryotic stage, introduces extensive GC-to-AT mutations to duplicated sequences (Cambareri *et al.* 1989). Quelling and meiotic silencing by unpaired DNA (MSUD), on the other hand, target transcripts generated from potential intruders during vegetative growth and meiosis, respectively (Romano and Macino 1992; Shiu *et al.* 2001). These surveillance mechanisms presumably allow *N. crassa* to be virtually free of active transposons and viruses (Catalanotto *et al.* 2006).

In the Quelling model, large tandem arrays of a transgene often alert the host defense mechanism, presumably during DNA replication (Nolan *et al.* 2008). QDE-3, a DNA helicase (Cogoni and Macino 1999b), may play a role in resolving secondary structures of tandem transgenes, enabling the transcription of single-stranded aberrant RNA and their conversion to double strands by QDE-1, a DNA- and RNA-directed RNA polymerase (DdRp and RdRP, respectively; Cogoni and Macino 1999a; Lee *et al.* 2009). The double-stranded RNA (dsRNA) molecules are further processed into 21- to 25-nucleotide small interfering RNA (siRNA) by the redundant dicer activity of DCL-1 and DCL-2 (Catalanotto *et al.* 2004). The siRNA duplexes are then loaded into the QDE-2 Argonaute, a component of the RNA-induced silencing complex (RISC) (Catalanotto *et al.* 2002). One of the siRNA strands (the passenger strand) is nicked by the slicer activity of QDE-2 and later degraded by the **Q**DE-2-**i**nteracting **p**rotein (QIP) (Maiti *et al.* 2007). The remaining single-stranded siRNA (the guide strand) can subsequently recognize homologous mRNA by base complementarity and target them for QDE-2-dependent cleavage.

In addition to propagation through mycelial growth and dispersal of conidia (asexual spores), *N. crassa* can also enter a sexual cycle. After fertilization and nuclear proliferation, opposite mating-type nuclei, *A* and *a*, pair and migrate into a dikaryotic hypha (for review, see Shiu and Glass 2000). Karyogamy occurs between *A* and *a* nuclei in the ascus (spore sac) mother cell, with meiosis and ascospore development following immediately afterward. Meiosis represents a window of opportunity for the expansion of selfish elements, as the two genomes are aligned intimately during the homologous pairing stage. In *N. crassa*, the mechanism known as MSUD exists to silence unpaired and potentially harm-

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ful genes. In MSUD, a gene not paired with a homologous partner generates a signal that silences all copies of that gene during sexual development. The current MSUD model suggests that an unpaired gene is detected and transcribed into aberrant RNA. The aberrant RNA are converted into dsRNA by the SAD-1 RdRP (Shiu and Metzenberg 2002), whose localization is controlled by the SAD-2 protein (Shiu *et al.* 2006). The dsRNA in turn are diced into siRNA by DCL-1 (Alexander *et al.* 2008). SMS-2, an Argonaute protein, is responsible for the siRNA-guided destruction of mRNA (Lee *et al.* 2003). The involvement of DCL-1 in both Quelling and MSUD suggests that a crosstalk exists between the two RNA-silencing mechanisms. In this work, we have set out to determine whether QIP, a part of the Quelling machinery, is also required for MSUD.

MATERIALS AND METHODS

Strains, media, and growth: The Neurospora strains used in this study are described in Table 1. Auxotrophic and other mutant strains were acquired from the Fungal Genetics Stock Center (FGSC; McCluskey 2003). The description of individual genes, including their mapping information, can be obtained from the Neurospora Compendium (Perkins *et al.* 2001) and the e-Compendium (http://bmbpcu36.leeds.ac.uk/~gen6ar/newgenelist/genes/gene_list.htm). The *qip*$^{\Delta}$ deletion strain contains the replacement of a sequence encompassing the *qip* open reading frame (positions −206 to 2070; supporting information, Figure S1) with a hygromycin-resistant gene (*hph*) (Colot *et al.* 2006). The *qip*^{fs} (frameshift) allele was constructed from the wild-type gene by cleavage at the *Nsi*I site (positions 149–154) followed by a Klenow fill-in reaction. Preparation of culturing and crossing media was as previously described (Westergaard and Mitchell 1947; Vogel 1964). Homokaryons were isolated using the method of Ebbole and Sachs (1990). Standard procedures for growth, crosses, and other Neurospora manipulations were followed throughout (Davis and de Serres 1970).

Nucleic acid methods and transformation: Standard molecular techniques were used according to Sambrook and Russell (2001). Fungal DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). Custom oligonucleotide primers, as listed in Table S1, were obtained from Integrated DNA Technologies (Coralville, IA). DNA amplification by polymerase chain reaction (PCR) was conducted in a PTC-100 Peltier Thermal Cycler (MJ Research, Waltham, MA), using either the AccuPrime Pfx system (Invitrogen, Carlsbad, CA) or the Expand Long Range dNTPack (Roche Applied Science, Indianapolis). PCR products, when necessary, were cloned into the pCRII-TOPO vector (Invitrogen). Bacterial plasmid DNA was purified with the HiSpeed Plasmid Midi Kit (Qiagen). DNA sequencing was performed by the University of Missouri DNA core (Columbia, MO). For integration at the *his-3* locus, the *qip*^{fs} allele and various green and red fluorescent protein (GFP and RFP, respectively) constructs were built using pBM61 (Margolin *et al.* 1997), pMF272 (Freitag *et al.* 2004), and pMF334 (Freitag and Selker 2005), respectively. For GFP integration at native loci, fusion PCR products from genomic DNA and a *gfp-hph*-containing plasmid (pTH1067.9) were obtained by double-joint PCR (Yang *et al.* 2004; Yu *et al.* 2004) and were used as the transforming DNA. DNA-mediated gene placement in Neurospora was performed according to Margolin *et al.* (1997).

TABLE 1

Neurospora strains used in this study

Strain	Genotype
F1-05	*fl a*
F2-01	*fl A*
F2-06	*fl; qip*$^{\Delta}$*::hph A*
F2-29	*rid R*$^{\Delta}$*::hph; fl A*
F2-35	*his-3*$^{+}$*::act*$^{+}$*; fl A*
F2-36	*his-3*$^{+}$*::Bml*^{R}*; fl A*
F3-23	*rid his-3*$^{+}$*::asm-1*$^{+}$*; fl; Asm-1*$^{\Delta}$*::hph A*
P3-07	Oak Ridge wild-type *A* (FGSC 2489)
P3-08	Oak Ridge wild-type *a* (FGSC 2490)
P3-25	*mep Sad-1*$^{\Delta}$*::hph a*
P5-52	*Sad-1*$^{\Delta}$*::hph rid his-3 a*
P6-07	*rid A*
P6-08	*rid a*
P6-62	*rid his-3*$^{+}$*::sad-2-rfp; inv Sad-2*^{RIP} *a*
P9-39	*qip*$^{\Delta}$*::hph a* (FGSC 12130)
P10-16	*rid his-3*$^{+}$*::hH1-gfp a*
P10-18	*rid his-3*$^{+}$*::dcl-1-gfp; dcl-1*$^{\Delta}$*::hph mus-52*$^{\Delta}$*::bar A*
P11-21	*rid his-3; qip*$^{\Delta}$*::hph; mus-51*$^{\Delta}$*::bar a*
P11-36	*rid his-3*$^{+}$*::r*$^{+}$*; mus-52*$^{\Delta}$*::bar A*
P11-38	*rid his-3*$^{+}$*::qip*^{fs}*; mus-52*$^{\Delta}$*::bar A*
P11-41	*rid his-3*$^{+}$*::r*$^{+}$*; qip*$^{\Delta}$*::hph; mus-51*$^{\Delta}$*::bar a*
P12-13	*rid his-3*$^{+}$*::hH1-gfp; mus-51*$^{\Delta}$*::bar; qip*$^{\Delta}$*::hph a*
P12-28	*rid his-3; qip*$^{\Delta}$*::hph; mus-51*$^{\Delta}$*::bar A*
P13-15	*sad-1-gfp::hph A*
P14-05	*rid his-3*$^{+}$*::qip-gfp; qip*$^{\Delta}$*::hph; mus-51*$^{\Delta}$*::bar A*
P15-02	*rid his-3*$^{+}$*::sms-2-rfp; mus-52*$^{\Delta}$*::bar; qip-gfp::hph A*
P15-03	*rid his-3*$^{+}$*::sms-2-rfp; mus-52*$^{\Delta}$*::bar; qip-gfp::hph a*

FGSC, Fungal Genetics Stock Center.

Reverse-transcriptase PCR: Total RNA extraction was performed as previously described (Shiu and Glass 1999). Poly(A^{+}) mRNA was enriched using the Oligotex mRNA kit (Qiagen). Reverse transcription, using a first-strand cDNA synthesis kit (Amersham Biosciences, Piscataway, NJ), was conducted according to the manufacturer's specifications. Primers used in the PCR amplification of a region spanning four *qip* introns are listed in Table S1. The PCR product of the *qip* cDNA is 1414 bp in length, as compared to the 1666 bp of that of genomic DNA. The identities of reverse transcriptase PCR (RT–PCR) products, from vegetative (P3-07) and perithecial (F1-05 × P3-07) mRNA, were confirmed by DNA sequencing. Our intron 2 sequence (Figure S1), which is based on cDNA sequencing, is 96 nucleotides shorter than the sequence depicted in version 3 of the *qip* predicted open reading frame and is in agreement with the sequence found in version 4 (http://www.broadinstitute.org/annotation/genome/neurospora/MultiHome.html).

Sample preparation and cytological methods: Perithecia fixation, mounting, and viewing using the Zeiss LSM510 were as described (Alexander *et al.* 2008). Some samples were imaged using a Zeiss LSM710 confocal laser scanning microscope, equipped with a PlanNeofluar ×40 (NA1.3) oil immersion objective and standard Zeiss software (ZEN). Multi-fluorophore images were scanned sequentially. Visualization of the GFP was achieved by use of a 488-nm Argon laser line for excitation with the detector set to collect emission bandwidth at 494–536 nm; RFP visualization was achieved by use of a 560-nm diode laser line for excitation with the detector set to collect emission bandwidth at 565–620 nm;

and DAPI visualization was achieved by use of a 405-nm diode laser excitation with the detector set to collect emission bandwidth at 410–470 nm.

RESULTS

***qip* is expressed during both vegetative and sexual stages:** *qip* encodes a 600-amino-acid polypeptide and is located between *mus-52* and *tim14*, on the right arm of linkage group III (Figure S1). To determine the expression pattern of *qip*, we obtained total RNA from mycelia (vegetative cells) and two perithecial (fruiting body) preparations (4 and 6 days after fertilization). cDNA products of *qip*, whose identities were confirmed by sequencing, could be detected from all conditions tested (Figure 1). The expression of *qip* in the perithecial tissue suggests that *qip* may play a role during sexual development.

A cross homozygous for *qip*$^{\Delta}$ is barren: Maiti *et al.* (2007) did not report any vegetative defect other than a Quelling deficiency in a *qip* strain. To determine if *qip* is important for sexual development, we performed crosses heterozygous and homozygous for *qip*$^{\Delta}$. While ascospores are produced in a *qip*$^{+}$ × *qip*$^{\Delta}$ cross, a cross homozygous for *qip*$^{\Delta}$ is completely barren. Although pigmented perithecia are produced in such a cross, they have just a hint of a beak (Figure 2C). Perithecial contents showed no asci, not even their rudiments (Figure 2D). DAPI staining showed only fluorescent nuclei in the background paraphysal tissue (Figure 2E).

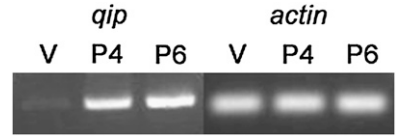

Figure 1.—*qip* is expressed in both asexual and sexual tissues. RT–PCR products for *qip* and *actin* (control) are shown (1414 and 227 bp, respectively). RNA from vegetative (V) and perithecial (P4 and P6: perithecial at 4 and 6 days) preparations were used for the amplification reactions.

Perithecial development was apparently arrested very early. Crosses homozygous for *dcl-1*$^{\Delta}$ have a similar phenotype (Alexander *et al.* 2008), suggesting that both of these genes are important for early sexual development.

***qip*$^{\Delta}$ does not act as a dominant suppressor of meiotic silencing:** QIP is important for the degradation of the passenger siRNA strand in Quelling (Maiti *et al.* 2007). While there are two paralogs for both RdRP and Argonaute in the *N. crassa* genome (one set for Quelling and another for MSUD), only one *qip* gene is present. These observations suggest either that MSUD utilizes a different method of passenger strand removal or that QIP is important for both vegetative and meiotic silencing, as is the case for DCL-1 (Alexander *et al.* 2008). Many deletion mutants of genes encoding components of the MSUD machinery, such as *Sad-1*$^{\Delta}$ and *Sad-2*$^{\Delta}$ (Shiu *et al.* 2001, 2006), act as a dominant

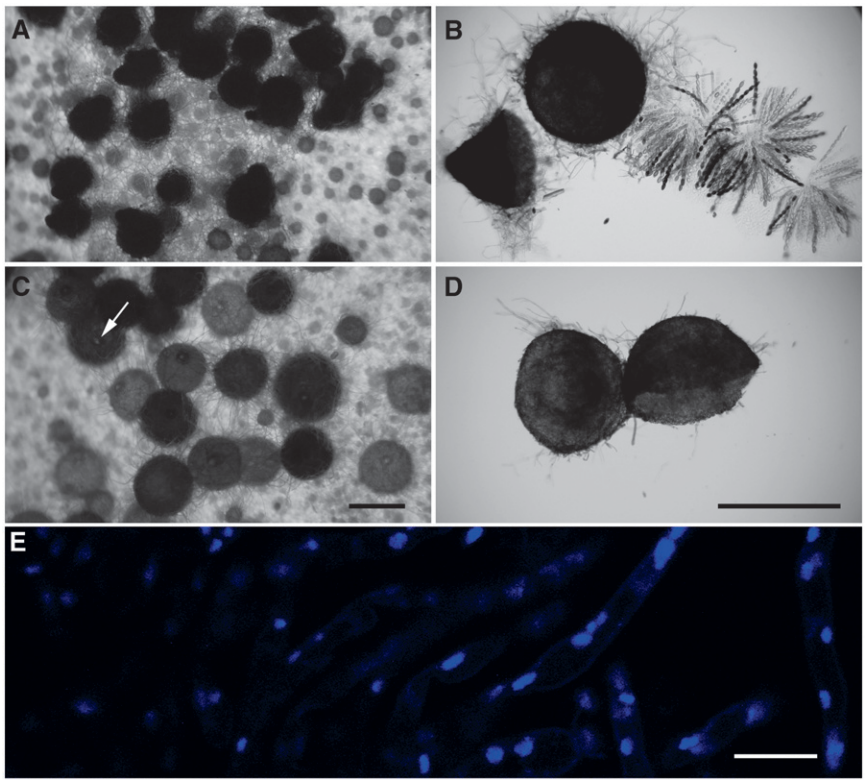

Figure 2.—Perithecial examination of various crosses demonstrates the requirement of *qip* in early sexual development. (A and B) *qip*$^{+}$ × *qip*$^{+}$ (F2-01 × P3-08). Normal perithecia and rosettes of eight-spored asci can be seen from the control cross. (C and D) *qip*$^{\Delta}$ × *qip*$^{\Delta}$ (F2-06 × P9-39). Undersized beaks (arrow in C) and the absence of asci (perithecial cross section in D) in a cross homozygous for *qip*$^{\Delta}$. Bars, 500 μm. (E) Only paraphysal tissue is found in perithecia from a *qip*$^{\Delta}$ × *qip*$^{\Delta}$ (F2-06 × P9-39) cross, suggesting a severe defect in perithecial development in a *qip*-null background. DAPI stain was used. Bar, 10 μm.

suppressor of meiotic silencing in a cross. For example, the unpaired r^+ (*Round spore*) gene is silenced in a wild type × R^Δ cross (which gives round spores) while it is expressed in a *Sad-1*$^\Delta$ × R^Δ cross (which gives wild-type spindle-shaped spores resembling an American football) (Shiu *et al.* 2001). The logic behind the dominant suppression in a *Sad-1*$^\Delta$ × R^Δ cross is that the *sad-1*$^+$ gene itself is unpaired, allowing the silencer to silence itself and thereby defeating the silencing mechanism (Shiu and Metzenberg 2002). To determine whether *qip* suppresses MSUD in a dominant fashion, we introduced *qip*$^\Delta$ to crosses containing various unpaired genes, including *actin*, *ascospore maturation-1*, β-*tubulin*, and *Round spore*. These unpaired genes, in an MSUD-proficient background, lead to various aberrant ascus/ascospore phenotypes (lollipop asci, white ascospores, elongated asci, and round ascospores, respectively). Our data indicate that the presence of a single *qip*$^\Delta$ allele does not suppress the meiotic silencing of any unpaired gene tested (Table 2). We reached the same conclusion with a visual *gfp* expression assay using a histone *hH1-gfp* reporter gene (Raju *et al.* 2007; Alexander *et al.* 2008). In a *qip*$^\Delta$ × *::hH1-gfp* cross, the unpaired *hH1-gfp* gene is silenced as usual and does not give rise to fluorescent nuclei in developing asci (Figure 3, A and B). Taken together, these results indicate that *qip*$^\Delta$, unlike *Sad-1*$^\Delta$ or *Sad-2*$^\Delta$, does not act as a dominant MSUD suppressor in a cross.

The *qip* gene product is required for meiotic silencing: The fact that a *qip*$^\Delta$ mutant does not dominantly suppress MSUD suggests either that *qip* is not involved in the meiotic silencing pathway or that one unpaired copy of the *qip* gene is not sufficient to silence the silencer. Since *qip* is required for early sexual development, we cannot examine the expression of unpaired genes in a *qip*$^\Delta$ × *qip*$^\Delta$ cross (which is completely barren) and unequivocally determine the role of *qip* in meiotic silencing. To circumvent this technical problem, we utilized a "two unpaired copy knockdown" scheme that was proven successful previously (Alexander *et al.* 2008). Basically, we constructed a cross heterozygous for *qip*$^\Delta$, heterozygous for an insertion of *qip*^{fs} (a frameshift null allele), and heterozygous for an insertion of *hH1-gfp* (an unpaired reporter gene), *i.e.*, *his-3*$^+$ *::qip*^{fs} × *his-3*$^+$ *::hH1-gfp qip*$^\Delta$. In this cross, the presence of a single *qip*$^+$ gene allows the perithecia to go through early ascus development. However, the single wild-type *qip* gene is inactivated by meiotic silencing at later stages due to two unpairing events (*qip*$^+$ unpaired with *qip*$^\Delta$*::hph* at the native *qip* locus and *qip*^{fs} unpaired with *hH1-gfp* at the *his-3* locus). Results from our cytological examination indicate that the unpaired *hH1-gfp* reporter gene is expressed throughout meiosis, suggesting that meiotic silencing is indeed deficient in a low QIP background (Figure 3C). We repeated the experiment using r^+ as the reporter gene (in a *his-3*$^+$ *::qip*^{fs} × *his-3*$^+$ *::r*$^+$ *qip*$^\Delta$ cross).

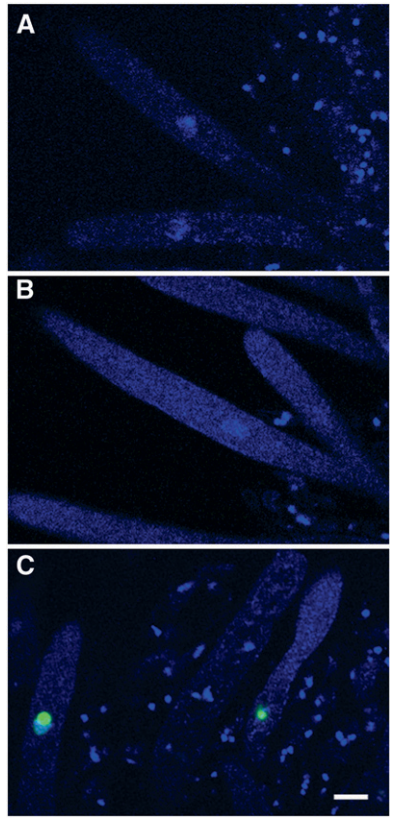

Figure 3.—Meiotic silencing of the unpaired *histone H1-gfp* (*his-3*$^+$ *::hH1-gfp*) gene is suppressed in a QIP-deficient cross. (A) *qip*$^+$ × *::hH1-gfp* (P6-07 × P10-16). Since the unpaired *hH1-gfp* gene is silenced during meiosis in a developing ascus, no fluorescence is observed. (B) *qip*$^\Delta$ × *::hH1-gfp* (P12-28 × P10-16). *qip*$^\Delta$ does not dominantly suppress the meiotic silencing of unpaired *hH1-gfp*. The nuclei remain nonfluorescent. (C) *::qip*^{fs} × *::hH1-gfp qip*$^\Delta$ (P11-38 × P12-13). Silencing of the unpaired *hH1-gfp* gene is suppressed in a *qip* knockdown cross, resulting in the presence of nuclear fluorescence. Bar, 10 μm.

Our results indicate that the unpaired r^+ gene is expressed and that the progeny are of wild-type spindle-shape in the *qip* knockdown cross (Figure 4). These results indicate that QIP is a necessary component of the MSUD machinery.

***qip* is localized in the perinuclear region:** QIP interacts with the QDE-2 Argonaute during the Quelling process (Maiti *et al.* 2007). If Quelling and MSUD function in a similar manner, at least for the RNA-degradation portion of the pathway, one would expect QIP also to associate with the MSUD Argonaute protein (SMS-2). Previously, we have shown that components of the MSUD machinery, including SAD-1, SAD-2, DCL-1, and SMS-2, are localized in the perinuclear region (Alexander *et al.* 2008). To determine the subcellular localization of QIP as well as its possible association with other MSUD proteins, we have constructed vectors expressing various green and red fluorescent fusion proteins. Our results indicate that QIP colocalizes with the other MSUD proteins, including the SMS-2 Argo-

TABLE 2

***qip*$^{\Delta}$, unlike *Sad-1*$^{\Delta}$, does not act as a dominant suppressor of MSUD**

Unpaired gene	Parent 1 (*mat A*): ectopic insertion at *his-3* (*::*) and/or deletion (Δ)	Parent 2 (*mat a*): mutation at *sad-1* or *qip*	Ascospore count/phenotype	Predominant ascus phenotype
actin	*::act*$^{+}$	Wild type	208×10^3	Lollipop asci
	::act^{+}	*qip*$^{\Delta}$	320×10^3	Lollipop asci
	::act^{+}	*Sad-1*$^{\Delta}$	2408×10^3	Normal
Ascospore maturation-1	*::asm-1*$^{+}$*; Asm-1*$^{\Delta}$	Wild type	1.6% black	White ascospores
	::asm-1^{+}*; Asm-1*$^{\Delta}$	*qip*$^{\Delta}$	1.4% black	White ascospores
	::asm-1^{+}*; Asm-1*$^{\Delta}$	*Sad-1*$^{\Delta}$	86% black	Black ascospores
β-*tubulin*	*::Bml*^{R}	Wild type	2.9×10^3	Arrests before metaphase
	::Bml^{R}	*qip*$^{\Delta}$	2.4×10^3	Arrests before metaphase
	::Bml^{R}	*Sad-1*$^{\Delta}$	4008×10^3	Normal
Round spore	*R*$^{\Delta}$	Wild type	0% football	Round ascospores
	R$^{\Delta}$	*qip*$^{\Delta}$	0% football	Round ascospores
	R$^{\Delta}$	*Sad-1*$^{\Delta}$	100% football	Football-shaped ascospores

Meiotic silencing of meiotically important genes, such as *act*$^{+}$, *asm-1*$^{+}$, *Bml*^{R}, and *r*$^{+}$, leads to the reduced production of black (mature), American football (spindle)-shaped ascospores. Crosses carrying *Sad-1*$^{\Delta}$, not *qip*$^{\Delta}$, can improve the production of normal ascospores. Strains used in this experiment include F2-29, F2-35, F2-36, F3-23, P3-08, P3-25, and P9-39.

naute, in the perinuclear region (Figure 5). The colocalization pattern is in agreement with the notion that these MSUD proteins are related functionally and spatially and that they may form an RNA-processing complex.

DISCUSSION

The vegetative silencing machinery in *N. crassa* is important for the preservation of genome integrity (Chicas *et al.* 2004; Nolan *et al.* 2005), the maintenance of ribosomal DNA copies (Cecere and Cogoni 2009), and DNA damage response (Lee *et al.* 2009). The QIP exonuclease, first identified as a QDE-2-interacting protein, functions to degrade the passenger strand of an siRNA duplex and to activate the RISC during Quelling (Maiti *et al.* 2007). QIP is of special importance to the delineation of the RNA interference (RNAi) pathway, as the mechanism for passenger strand removal was not obvious before its identification. In this work, we have demonstrated that QIP is also required for MSUD. Thus far, we have shown that at least two proteins, DCL-1 and QIP, have dual functions in the genome surveillance of *N. crassa*. These results suggest that the crosstalk between the vegetative and meiotic silencing mechanisms is more prevalent than once thought. All the MSUD proteins reported previously, including SAD-1, SAD-2, SMS-2, and DCL-1, are important for sexual development (Shiu *et al.* 2001, 2006; Lee *et al.* 2003; Alexander *et al.* 2008). Homozygous crosses for *sad-1* or *sad-2* are arrested in prophase, suggesting that some degree of meiotic silencing may be a required checkpoint for cell cycle progression (Shiu *et al.* 2001, 2006). Unlike *sad-1* and *sad-2*, a cross homozygous for *qip*$^{\Delta}$ does not produce any asci. This observation suggests that *qip* is important for early ascus develop-

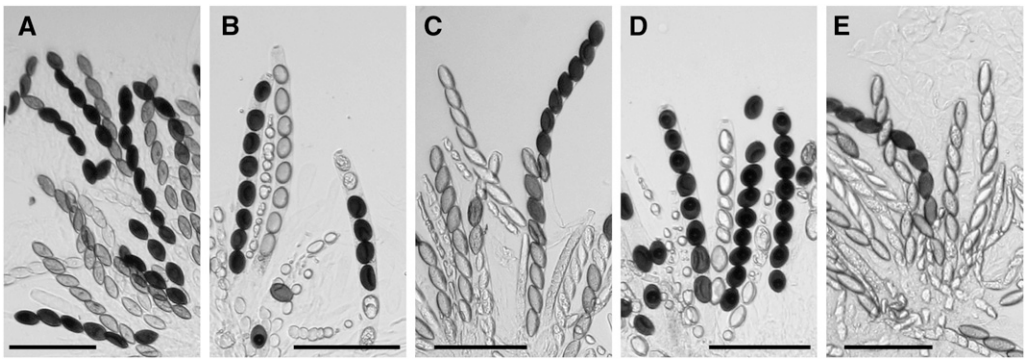

Figure 4.—Meiotic silencing of the unpaired *Round spore* (*r*$^{+}$) gene is suppressed in a QIP-deficient cross. (A) *r*$^{+}$ × *r*$^{+}$ (P3-07 × P3-08). Normal spindle-shaped (American football-like) ascospores are observed when the *r*$^{+}$ gene is expressed. (B) *r*$^{+}$ × *::r*$^{+}$ (P6-08 × P11-36). Meiotic silencing of the *r*$^{+}$ gene leads to the production of round ascospores. (C) *Sad-1*$^{\Delta}$ × *::r*$^{+}$ (P5-52 × P11-36). *Sad-1*$^{\Delta}$ suppresses the meiotic silencing of *r*$^{+}$, resulting in the presence of spindle-shaped ascospores. (D) *qip*$^{\Delta}$ × *::r*$^{+}$ (P11-21 × P11-36). *qip*$^{\Delta}$ does not act as a dominant suppressor of MSUD, resulting in predominantly round ascospores. (E) *::qip*^{fs} × *::r*$^{+}$ *qip*$^{\Delta}$ (P11-38 × P11-41). The silencing of the unpaired *r*$^{+}$ gene is suppressed in a *qip* knockdown cross, resulting in the presence of spindle-shaped ascospores. Bars, 100 μm.

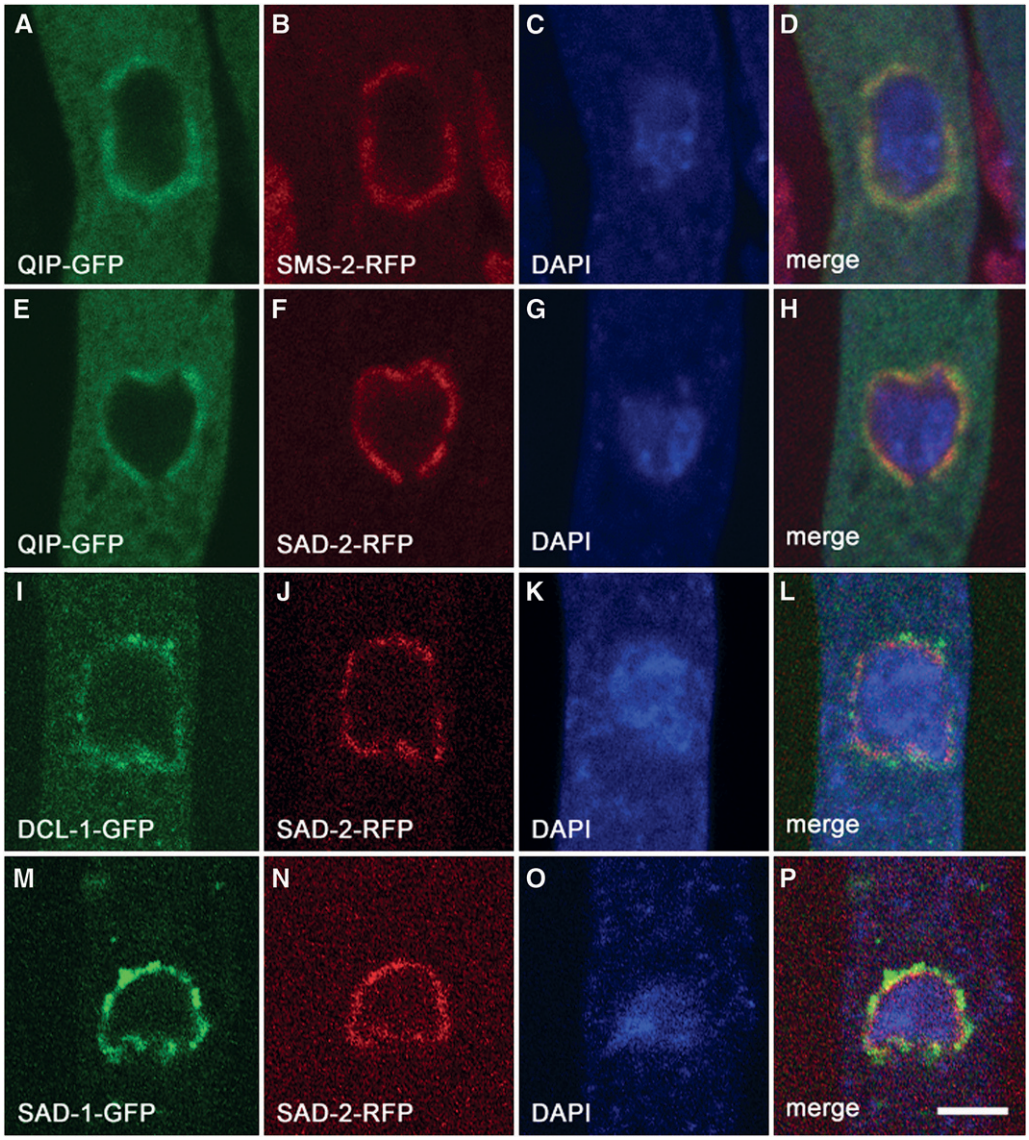

FIGURE 5.—Colocalization of MSUD proteins in the perinuclear region. Micrographs illustrate prophase asci expressing (A–D) *qip-gfp* and *sms-2-rfp* (P15-02 × P15-03). (E–H) *qip-gfp* and *sad-2-rfp* (P14-05 × P6-62). (I–L) *dcl-1-gfp* and *sad-2-rfp* (P10-18 × P6-62). (M–P) *sad-1-gfp* and *sad-2-rfp* (P13-15 × P6-62). The chromatin was stained with DAPI. Bar, 5 μm.

ment, much like *dcl-1* (ALEXANDER *et al.* 2008). Since QIP and Dicers are essential to the biogenesis of certain microRNA-like RNA (LEE *et al.* 2010), the lack of these proteins may affect the expression of genes that regulate sexual development. Alternatively, they could regulate endogenous genes that are naturally transcribed in both sense and antisense orientations (FULCI and MACINO 2007).

All known components of the MSUD machinery (SAD-1, SAD-2, SMS-2, DCL-1, and QIP), with the exception of *Sk-2* and *Sk-3* (which have not been molecularly characterized; RAJU *et al.* 2007), are localized in the perinuclear region (SHIU *et al.* 2001; ALEXANDER *et al.* 2008). This observation is in contrast with the one made in the Quelling mechanism, in which QDE-1 (and hypothetically, QDE-3) has affinity for repetitive transgenic loci (NOLAN *et al.* 2008). In mammalian cells, siRNA have been shown to accumulate in the perinuclear region, and their proper localization is correlated with the efficiency of RNAi (GRÜNWELLER *et al.* 2003; CHIU *et al.* 2004). Furthermore, some RNAi proteins have been shown to localize in this region in Drosophila and mouse germ cells (KOTAJA and SASSONE-CORSI 2007; LIM and KAI 2007; PANE *et al.* 2007). These observations suggest that the perinuclear region may be an RNAi center for meiotic silencing. It is possible the MSUD machinery examines each RNA molecule as it exits the nucleus, processing any aberrant RNA before it has a chance to reach the exonucleases or the translational machinery. Colocalization of related MSUD proteins may allow the coupling of consecutive reactions and therefore increase the efficiency of the silencing process.

Meiotic silencing can be a useful tool in determining gene functions during meiosis and sexual development. A wide variety of genes, including those encoding actin and β-tubulin, have been silenced using MSUD (SHIU *et al.* 2001). Although some MSUD mutants, such as *Sad-1*$^{\Delta}$, *Sad-2*$^{\Delta}$, *Sk-2*, and *Sk-3*, behave as strong dominant MSUD suppressors via the "silencing the silencer"

negative feedback system (Shiu *et al.* 2001, 2006; Raju *et al.* 2007), others may have difficulties in achieving similar effectiveness. Our use of two unpaired copies in a cross may prove to be useful in silencing genes that are especially hard to silence via the standard "wild type × Δ" scheme, such as those that are highly expressed or those that need few transcripts for normal operation.

The silencing of unpaired chromosomal regions during meiosis is not restricted to fungi. Some form of meiotic silencing is also found in worms, mice, and humans (Bean *et al.* 2004; Turner *et al.* 2005; Ferguson *et al.* 2008). In mammals, the phenomenon is known as meiotic silencing of unsynapsed chromatin, and it is responsible for meiotic sex chromosome inactivation (Burgoyne *et al.* 2009). Further identification of the genetic factors controlling these phenomena should shed light on the mechanisms involved in targeting unpaired DNA and on whatever similarity or difference there might be among various eukaryotes.

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Note added in proof: See Lee *et al.* in this issue (pp. 127–133) for a related work.

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QIP, a Protein That Converts Duplex siRNA Into Single Strands, Is Required for Meiotic Silencing by Unpaired DNA

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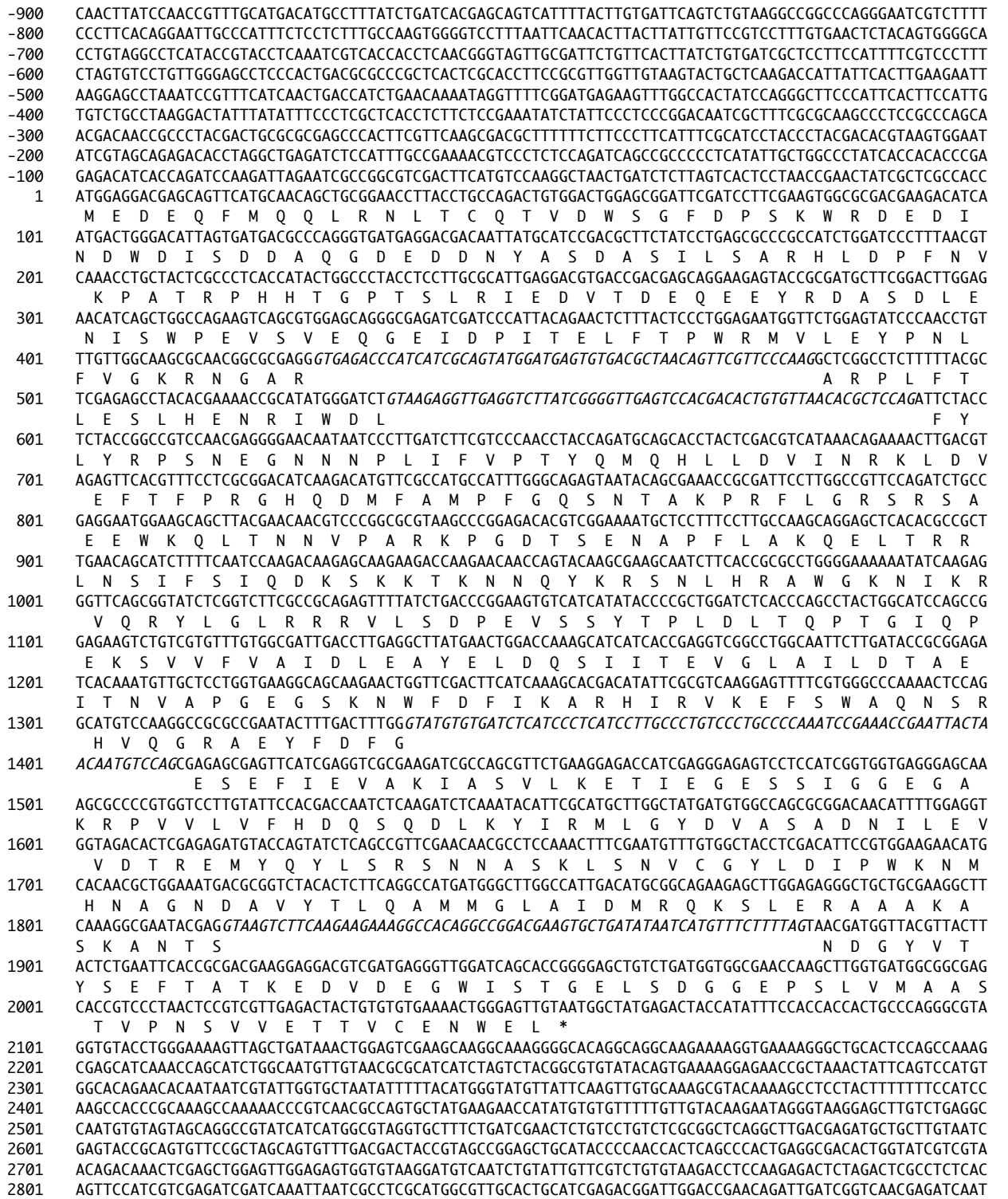

FIGURE S1.—The nucleotide and amino-acid sequence of *qip* (NCU00076). The intron sequences are italicized. The DNA sequence listed here corresponds to the reverse complement of nucleotides 2528149-2531948 of *N. crassa* supercontig 3 (http://www.broadinstitute.org/annotation/genome/neurospora/MultiHome.html).

TABLE S1

Primers used in this study

Primer	Sequence (5' to 3')	Amplification of
Act-507F	507 CGTTGGTCGTCCCCGTTATCATG 529	cDNA spanning *actin* intron 4
Act-811R	811 TGGGAGCCTCGGTAAGAAGGACG 789	cDNA spanning *actin* intron 4
Gfp-t-hph-F1	GGGGCA-3060 GGAGCTGGTGCAGGCGCTGGAGCCAT 3035	*gfp-hph*
Gfp-t-hph-R1	331 GCCCTTGTTAACTGATATTGAAGGAGCAT 359	*gfp-hph*
Qip-298792R	313 CCAGAAGTCAGCGTGGAGCAG 333	cDNA spanning *qip* introns 1-4
Qip-297127F	1978 GTTCGCCACCATCAGACAGCTC 1957	cDNA spanning *qip* introns 1-4
Qip-299824R	-720 GTGAACTC-TCTAGA-GGGGCACCTGTAGG -693	*qip* for frameshift construction
Qip-296819F	2286 GTTTA-GCGGCCGC-CCTTTTCACTGTATACAC 2256	*qip* for frameshift construction
Qip-299128R	-24 CTA-ACTAGT-CTATCGCTCGCCACCAT 2	*qip* for *gfp* fusion[1]
Qip-297035F	2070 GTAGTCTCAT-TTAATTAA-CAACTCCCAGTTTTC 2038	*qip* for *gfp* fusion[1]
Qip-A	793 GATCTGCCGAGGAATGGAAGCAG 815	*qip* left flank for *gfp* fusion[2]
Qip-gfp1	CAGCGCCTGCACCAGCTCCTGCCCC-	*qip* left flank for *gfp* fusion[2]
	2052 CAACTCCCAGTTTTCACACACAGTAGTC 2025	
Qip-gfp2	CTCCTTCAATATCAGTTAACAAGGGC	*qip* right flank for *gfp* fusion[2]
	- 2219 CTGGCAATGTTGTAACGCGCATC 2241	
Qip-B	3322 TGTTCAGTCCGCCTTGTCGTTGT 3300	*qip* right flank for *gfp* fusion[2]
Sad1-A	5420 ATACACGCACAAGCGATGGCAAG 5441	*sad-1* left flank for *gfp* fusion[2]
Sad1-gfp-1	CAGCGCCTGCACCAGCTCCTGCCCC-	*sad-1* left flank for *gfp* fusion[2]
	6372 AAGCGCCGCCATCTGTGCATAAC 6350	
Sad1-gfp-2	CTCCTTCAATATCAGTTAACAAGGGC-	*sad-1* right flank for *gfp* fusion[2]
	6630 CCCCATCCTCATCTTCCATCACC 6652	
Sad1-B	7923 CACTACCTCGAATCCCCCACCAA 7901	*sad-1* right flank for *gfp* fusion[2]
Sms2-107713F	107713 CTCTTGCCTCAACCAGTACC-ACTAGT-ATGTCTGCTCCTGG	*sms-2* for *rfp* fusion[1]
	107752	
Sms2-110724R	110724 GCCAAAGCGACCAAG-TCTAGA-CCCACCACATGGTGTTGTG 110685	*sms-2* for *rfp* fusion[1]

Restriction sites, including *Not*I (GCGGCCGC), *Spe*I (ACTAGT), *Pac*I (TTAATTAA), and *Xba*I (TCTAGA) are underlined. Nucleotide positions for *qip* are numbered according to Figure S1. [1]*his-3* integration. [2]Native integration.