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Genetics

Molecular analysis of the *Neurospora qa-1* regulatory region indicates that two interacting genes control *qa* gene expression

(regulatory protein/eukaryotic gene regulation/gene cluster/*Neurospora* transformation)

LAYNE HUIET

Department of Molecular and Population Genetics, University of Georgia, Athens, GA 30602

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ABSTRACT **The *qa-1* regulatory region controls the expression of the three structural genes required for the early reactions in quinic acid catabolism in *Neurospora crassa*. Genetic analysis previously identified two types of noninducible *qa-1* mutants, *qa-1*^{S} and *qa-1*^{F}, which mapped in separate nonoverlapping regions. These mutations were originally interpreted as defining separate domains of a single regulatory protein. This communication describes the further genetic and physical characterization of the *qa-1* regulatory region. Using both *Neurospora* transformation and DNA·RNA hybridization, it has been shown that the *qa-1* region consists of two distinct genes corresponding to the two original mutational types *qa-1*^{S} and *qa-1*^{F}. The analysis of the mRNA species hybridizing to these regions indicates that the *qa-1F* gene encodes a 2.9-kilobase (kb) mRNA, while the *qa-1S* gene encodes related 4.1-kb and 3.4-kb mRNAs. The transcriptional regulation of one of these genes, *qa-1S*, was examined. Evidence is presented that the *qa-1S* gene is induced by quinic acid and is also subject to apparent autogenous regulation as well as to control by the *qa-1F* gene product. Based on these results and earlier genetic analysis, the hypothesis is proposed that one of the two *qa* regulatory genes encodes a repressor protein (qa-1S), and the other encodes an activator protein (qa-1F), both of which control *qa* gene expression.**

The quinate–shikimate catabolic pathway of *Neurospora crassa* constitutes a well-characterized system for studying eukaryotic gene regulation. Initial studies indicated that four genes were required for the organism to catalyze the first three steps in quinic acid degradation and that these four genes were tightly clustered on linkage group VII (1). The three structural genes *qa-2*, *qa-3*, and *qa-4* encode catabolic dehydroquinase, quinate dehydrogenase, and dehydroshikimate dehydratase, respectively. These enzymes are coordinately induced by quinic acid and the regulation of enzyme synthesis is at the transcriptional level (2).

The fourth gene, *qa-1*, was believed to encode a regulatory protein that positively controlled transcription of the structural genes (2, 3). Both pleiotropic negative (noninducible) and constitutive *qa-1* mutants were isolated that affected the synthesis of the three structural gene products. Two types of noninducible *qa-1* mutants were described according to their abilities to complement *qa-2* mutants. Semidominant *qa-1*^{S} mutants show slow (weak) complementation, while recessive *qa-1*^{F} types show fast (strong) complementation (4). Genetically, the *qa-1*^{F} and *qa-1*^{S} mutations map in two distinct nonoverlapping regions at opposite ends of the gene (3). Constitutive *qa-1*^{C} mutants can be obtained directly from wild-type (5) as well as from *qa-1*^{S} mutants, while *qa-1*^{F} mutants do not give rise to constitutives (6). On the basis of these results, it was proposed that the two classes of mutants represented two domains of a regulatory protein, one for inducer binding and the other for DNA binding. The occurrence of pairs of *qa-1*^{S} and *qa-1*^{F} alleles that did not complement suggested that the two mutant types comprised a single cistron (3). However, because the *qa-1*^{S} mutations were partially dominant, the possibility could not be ruled out that *qa-1*^{S} and *qa-1*^{F} mutants defined separate cistrons.

The entire *qa* gene cluster has previously been cloned in *Escherichia coli*, and the individual *qa* genes have been identified by transformation of specific *Neurospora qa* mutants with plasmid subclones (7). The *qa-1* gene was localized to a 5.8-kilobase (kb) region at the centromere–proximal end of the cluster (7).

To precisely localize and characterize the *qa-1* regulatory gene, transformation experiments have been carried out with both *qa-1*^{F} and *qa-1*^{S} mutants using a variety of cloned DNA fragments. These DNA subclones were also hybridized to poly(A)$^+$ RNA to identify mRNAs associated with the *qa-1* gene. These experiments indicate that the *qa-1*^{S} and *qa-1*^{F} regions constitute two different genes that encode distinct mRNA species transcribed in opposite directions. The two genes are now designated *qa-1S* and *qa-1F*. Evidence is also presented that one of these genes, *qa-1S*, is transcribed at a very low uninduced level, but it is induced by quinic acid. This induction of *qa-1S* mRNA requires active gene products of both the *qa-1S* and *qa-1F* genes.

Based on the results presented here and on work of others, it has become necessary to revise the original hypothesis of Case and Giles that the *qa-1* gene encoded a single regulatory protein with separate functional domains (3). A new hypothesis is proposed that the two *qa* regulatory genes encode, respectively, a repressor protein (*qa-1S*) and an activator protein (*qa-1F*) whose interaction controls *qa* gene expression.

MATERIALS AND METHODS

Strains and Plasmids. All the *qa-1* mutants used in these studies except M439 were originally isolated by Howard Rines in an *arom-9* mutant in the wild-type 74A background (4). M439 was isolated from strain M6-11-89601-10A (*arom-9 inos*) after UV mutagenesis. These mutants have been characterized further by Case and Giles (3). All the *qa-1 arom-9* double mutants lack both biosynthetic and catabolic dehydroquinase activities; therefore, they are unable to grow on minimal media without aromatic amino acids. The *qa-1*^{F} mutants used as recipient strains in transformation experiments were M137, M158, and M127. M158 and M127 are stable mutants, while M137 reverts spontaneously at a low frequency. The *qa-1*^{S} mutants used as recipient strains in transformation experiments were M439, M107, M141, and M124, all of which revert spontaneously at a low frequency. The *Neurospora* transformation procedure is that described by Schweizer *et al.* (7). The amount of plasmid DNA used in each experiment was 15 μg. The method of plasmid isolation has been described (7). All plasmids used in transformation

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Abbreviations: kb, kilobase(s); bp, base pair(s).

experiments are derived from wild-type 74A *N. crassa* DNA and are indicated in Fig. 1.

DNA·RNA Hybridization. RNA was isolated from the following strains grown under inducing and noninducing conditions: wild-type 74A, *qa-1*^{F} mutants M158 and M162, a *qa-1*^{C} constitutive mutant M105-R12-1.5, and a *qa-1*^{S} mutant M141. Conditions for growth and subsequent poly(A)$^+$ RNA isolation were according to Patel *et al.* (2). Poly(A)$^+$ RNA (10–20 μg) was fractionated in 1.5% agarose/methylmercury gels and transferred to diazobenzyloxymethyl (DBM)-paper as described (8). Prehybridization and hybridization conditions were according to Tyler *et al.* (9). Filters were exposed to Kodak X-Omat AR film with a Cronex Hi-plus intensification screen at −70°C for 1–7 days.

DNA·DNA Hybridization. *N. crassa* genomic DNA was prepared according to the procedure of Blin and Stafford (10) and subsequently purified using cesium chloride/ethidium bromide gradients. DNA was transferred from agarose gels to nitrocellulose according to Southern (11) and hybridized as described by Geever *et al.* (12). Nick-translations were as described by Rigby *et al.* (13).

Nuclease S1 Mapping. Nuclease S1 mapping procedures were a modification of the method of Berk and Sharp (14, 15). Single ^{32}P-end-labeled DNA fragments were hybridized to 5–10 μg of *Neurospora* poly(A)$^+$ mRNA under conditions favoring DNA·RNA hybridization, but not DNA·DNA hybridization. The temperature used was 52–55°C and depended on the DNA fragment used in the experiment. DNA and RNA were lyophilized and redissolved in 10 μl of 80% formamide/80 mM Pipes, pH 6.8/0.4 M NaCl/10 mM EDTA. The DNA was denatured and hybridized for 6–24 hr in a microfuge tube under paraffin oil. After hybridization, the DNA·RNA hybrids were digested with 50–150 units of endonuclease S1 (Sigma) in 250 mM NaCl/60 mM Na acetate, pH 4.5/1 mM $ZnSO_4$/20 μg of alkali-denatured calf thymus DNA per ml for 30 min at 37°C. The reaction was stopped by adding EDTA to 10 mM, Tris·HCl (pH 9.0) to 50 mM, and 1 μg of tRNA. The digestion products were denatured and then fractionated on an 8% acrylamide/7 M urea sequencing gel. The *qa-1S* mRNA levels in the mutant and wild-type strains were determined from densitometry of several exposures of the autoradiogram shown in Fig. 5 using a Beckman DU-8 spectrophotometer.

RESULTS

Transformation of *Neurospora qa-1* Mutants. Previous experiments of Schweizer *et al.* indicated that a 5.8-kb *Bam*HI fragment (pMSK 375) could transform a *qa-1*^{F} mutant (M158, *qa-1*, *arom-9*) at a high frequency (7). The transformants were detected as colonies able to grow on minimal plates (no aromatic amino acid supplement) because the introduced wild-type *qa-1* gene allows the expression of catabolic dehydroquinase (*qa-2*$^+$ gene product), which can substitute for the missing biosynthetic dehydroquinase (*arom-9*). To define which subregion of the 5.8-kb fragment was responsible for transformation, a restriction map was generated for this region and used to construct subclones and deletions of most of the 5.8-kb *Bam*HI fragment in plasmid pMSK375 (Fig. 1). The results of these transformation experiments are given in Table 1. Only one plasmid, pLH40, which contains the left 3.3 kb of the region, was able to transform the stable *qa-1*^{F} *arom-9* mutant M158. Two plasmids, pLH31 and pLH35, which have part of this region deleted, gave negative results in transformation experiments with M158. These subclones gave identical transformation results with the two other *qa-1*^{F} mutants (M137 and M127) tested (data not shown).

Although all *qa-1*^{S} mutants revert spontaneously, the reversion frequencies were sufficiently low in four mutants (M141, M124, M107, M439) to permit their use in transformation experiments. Surprisingly, none of these *qa-1*^{S} mutants yielded transformants with the plasmids (pMSK375, pLH40) that transformed the *qa-1*^{F} mutants. However, all four of the *qa-1*^{S} mutants were transformed by plasmids pMSK366 and pMSK391, which contain the *Eco*RI and *Bam*HI fragments, respectively, adjacent to the *qa-1*^{F} region (Fig. 1; Table 1). Two subclones, pLH45 and pLH46, were constructed containing either end of the *Bam*HI fragment. Plasmid pLH45, which contains a 1.5-kb *Bam*HI/*Sph* I fragment was the smallest subclone that gave positive transformation results with the *qa-1*^{S} mutant M141. The gene order determined from these experiments agrees with the original genetic order: *qa-3*, *qa-1*^{S}, *qa-1*^{F} (see ref. 3; M. E. Case, personal communication).

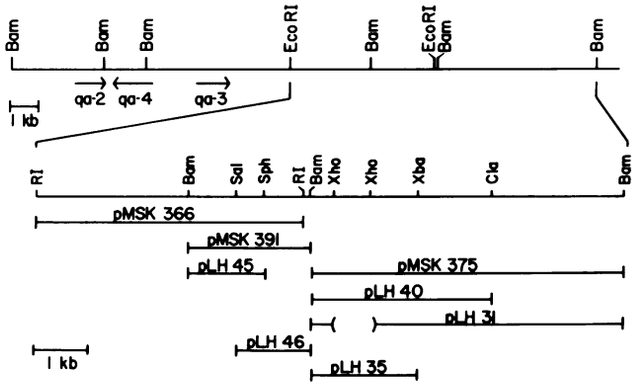

FIG. 1. Cloned DNA fragments used to transform *Neurospora qa-1* mutants. On the restriction endonuclease cleavage map of the *qa* gene cluster, arrows indicate the location and direction of transcription of the structural genes (B. Tyler, personal communication). The *Neurospora* DNA fragments indicated were subcloned into pBR325. ( ) denotes region deleted.

DNA·DNA Hybridization. To try to confirm the locations of the *qa-1*^{S} and *qa-1*^{F} regions, a number of *qa-1*^{S} and *qa-1*^{F} mutants were examined by DNA·DNA hybridization in an attempt to localize specific mutations. Of three *qa-1*^{F} mutants (M162, M158, M127) genetically characterized as possible deletion mutants, M162 had a detectable alteration in the *qa-1*^{F} region, as shown in Fig. 2. This mutation appears to be a large DNA insertion between the right-most *Xho* I site and *Xba* I site (Fig. 1) in the *qa-1*^{F} gene. Five *qa-1*^{S} point mutants were screened using 16 different restriction endonucleases, but none showed a restriction pattern detectably different from that of wild type (unpublished data).

Identification of *qa-1* mRNAs by DNA·RNA Hybridization. To identify mRNA species encoded by the regions that transform *qa-1* mutants and to determine if *qa-1*^{S} and *qa-1*^{F} regions encode different mRNAs (i.e., if they are different genes), an analysis was undertaken using DNA·RNA hybrid-

Table 1. Location of the *qa-1F* and *qa-1S* genes by transformation of *N. crassa qa-1* mutants

Plasmid	Recipient strain	
	qa-1S (M141)*	*qa-1F* (M158)†
pMSK375	0	408
pLH40	0	356
pLH31	0	0
pLH35	0	0
pMSK366	60	0
pMSK391	65	0
pLH45	78	0
pLH46	0	0

*Number of transformants per μg of DNA, above the background reversion frequency.
†Number of transformants per μg of DNA.

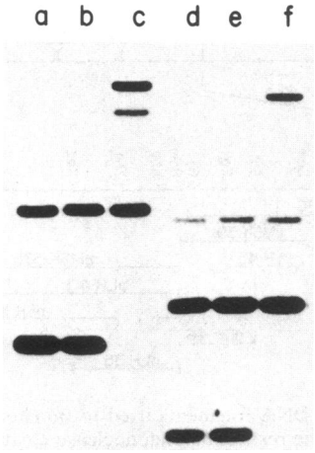

FIG. 2. Hybridization of pLH40 (containing *qa-1*^{F}) to restriction digests of *N. crassa* DNA (5 μg) isolated from three *qa-1*^{F} mutants (3). Lanes a–c, DNA was digested with *Sst* I; lanes d–f, DNA was digested with *Bcl* I. Lanes: a and d, M158; b and e, M127; c and f, M162.

ization (8). *N. crassa* poly(A)$^+$ RNA was fractionated using 1.5% agarose/methylmercury gels, transferred to DBM-paper, and then hybridized. Fig. 3 illustrates the DNA probes used and shows that the left-most probe (probe 1) hybridizes to both a 3.4-kb and a 4.1-kb mRNA. Because this probe is derived from pLH45, which transforms *qa-1*^{S} mutants, these mRNAs presumably are transcribed from the region containing the *qa-1*^{S} mutations. Probes 3 and 4 are derived from pLH40, which transforms *qa-1*^{F} mutants. Fig. 3 shows that these probes hybridized to a different mRNA of 2.9 kb. Presumably, therefore, *qa-1*^{S} mutations and *qa-1*^{F} mutations are located in distinct transcriptional units. Probe 2, which includes the region between probes 1 and 3, hybridized to all three mRNA species (Fig. 3), indicating that both the *qa-1*^{S}-related 3.4- and 4.1-kb mRNAs and the *qa-1*^{F}-related 2.9-kb mRNA are transcribed from this region.

To determine the direction of transcription of these two

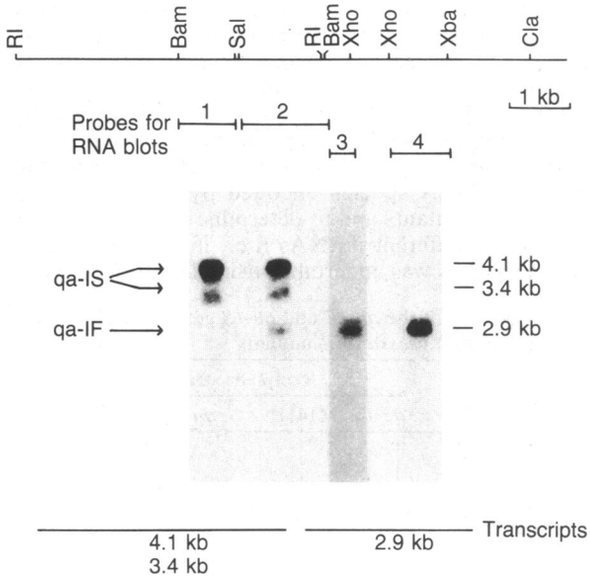

FIG. 3. Localization of *qa-1*-specific mRNAs by RNA·DNA hybridization. *N. crassa* poly(A)$^+$ RNA (10–15 μg) isolated from induced wild type was size fractionated on methylmercury gels and blotted to DBM-paper. Probes were DNA fragments electroeluted from acrylamide gels and nick-translated.

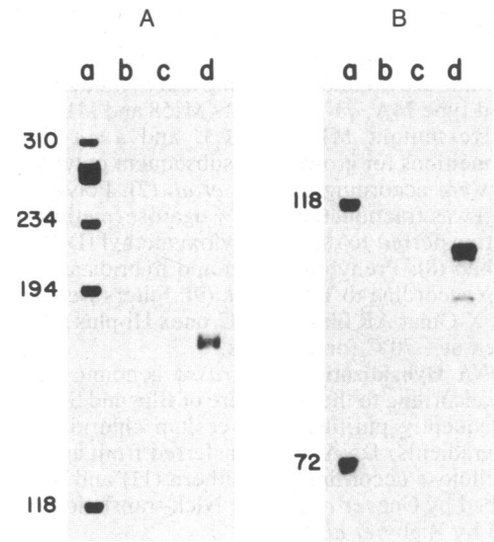

FIG. 4. Direction of transcription of *qa-1* mRNAs determined by nuclease S1 mapping of RNA·DNA hybrids. (*A*) *qa-1S*-related mRNAs (4.1 and 3.4 kb) mapped using probe 2 end-labeled at *Sal* I. (*B*) *qa-1F*-related mRNA (2.9 kb) mapped using probe 2 end-labeled at *Bam*HI. In both *A* and *B*, RNA source was as follows: lanes b, *E. coli* (10 μg); lanes c, uninduced wild-type *N. crassa* poly(A)$^+$ (5 μg); lanes d, induced wild-type *N. crassa* poly(A)$^+$ (5 μg). DNA size markers indicated are the size of φX174 *Hae* III fragments (lanes a) in base pairs (bp).

sets of mRNAs, probe 2 was 5′-end-labeled either at the *Sal* I site or the *Bam*HI site, hybridized to poly(A)$^+$ RNA from induced wild type, and the RNA·DNA hybrids digested with nuclease S1. Fig. 4*A* (lane d) shows that when the probe was labeled at the *Sal* I site, a 175-bp fragment was protected from nuclease digestion, presumably by the 3.4- and 4.1-kb mRNAs. Fig. 4*B* (lane d) shows that when the probe was 5′-end-labeled at the *Bam*HI site, a 105-bp fragment was protected, presumably by the 2.9-kb mRNA. Lanes b and c in Fig. 4 show that no fragments are protected by *E. coli* RNA (lane b) or uninduced *N. crassa* RNA (lane c). When the probe was 3′-end-labeled at either the *Bam*HI or the *Sal* I sites, no fragments were protected, even by RNA from induced wild type (data not shown). These data indicate that the 3.4-kb and 4.1-kb mRNAs are transcribed divergently from the 2.9-kb mRNA and that each mRNA initiates within the *Sal* I/*Bam*HI region defined by probe 2. These results are also consistent with the observation that probe 1 does not hybridize to the 2.9-kb mRNA and probe 3 does not hybridize to the 3.4-kb and 4.1-kb mRNAs. The evidence that the DNA sequences that transform *qa-1*^{S} and *qa-1*^{F} mutants are physically distinct and encode mRNA species of different sizes and orientations indicates that *qa-1*^{S} and *qa-1*^{F} mutations define distinct genes designated as *qa-1S* and *qa-1F*, respectively.

Regulation of *qa-1S* mRNA Synthesis. To begin to understand the interrelationship between the two regulatory genes, *qa-1S* and *qa-1F*, and their involvement in *qa* gene expression, the presence of *qa-1S* mRNA in wild type and in various *qa-1* mutant strains was analyzed. Nuclease digestion of RNA·DNA hybrids was used to provide a more sensitive assay than RNA blots. A 1.4-kb *Sal* I/*Bam*HI restriction fragment labeled at the 5′ end of the *Sal* I cleavage site with [γ-^{32}P]ATP was hybridized to 5 μg of poly(A)$^+$ RNA, digested with nuclease S1, and the resulting 175-bp DNA fragment was resolved on a denaturing acrylamide gel. The RNA was isolated from wild type and various *qa-1* mutant cultures exposed to either inducing or noninducing conditions. The results are shown in Fig. 5. There is a very low level of *qa-1S*

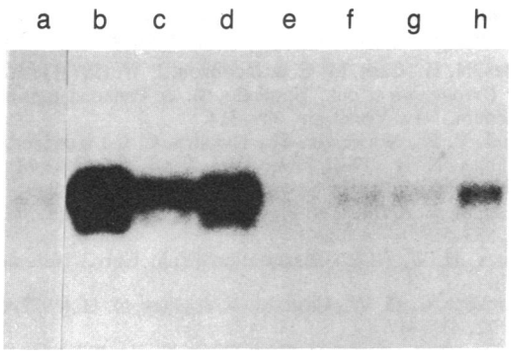

FIG. 5. Regulation of *qa-1S* mRNA transcription determined using nuclease S1 mapping. Probe 2 was end-labeled at the *Sal* I cleavage site and hybridized to 5 μg of poly(A)$^+$ RNA generating a 175-bp protected fragment. Lanes: a, wild-type uninduced; b, wild-type induced; c, 105C uninduced; d, 105C induced; e, M141 (*qa-1S*) uninduced; f, M141 (*qa-1S*) induced; g, M162 (*qa-1F*) uninduced, h, M162 (*qa-1F*) induced.

mRNA in noninduced cultures (lane a), whereas on induction by quinic acid the mRNA level is increased 40-fold (lane b). Lane c shows that the *qa-1S* mRNA level in a *qa-1^C* (constitutive) mutant is much higher than in uninduced wild type (lane a) even when it is grown under noninducing conditions. However, this level is not as high as the induced wild-type level (lane b). Under inducing conditions this constitutive mutant shows an additional increase in *qa-1S* mRNA to ≈75% of the wild-type induced level (lane d). The last four lanes indicate the level of *qa-1S* mRNA in a noninducible *qa-1S* mutant (M141) (lanes e and f) and in a noninducible *qa-1F* mutant (M162) (lanes g and h). Neither mutant showed any appreciable increase as compared to wild type in the level of *qa-1S*-specific mRNA under induced as compared to uninduced conditions. Even under inducing conditions, *qa-1S* mRNA levels in these mutants are all comparable to those found in uninduced wild type. [There does appear to be a slightly higher level (2-fold) of *qa-1S* mRNA in lane h (m162, induced) than in uninduced wild type.] It appears, therefore, that the induction of *qa-1S* mRNA requires active gene products of both the *qa-1S* and *qa-1F* genes.

DISCUSSION

The cloning of a DNA fragment comprising the entire *qa* gene cluster (7) has allowed a physical characterization of the *qa-1* regulatory region. The use of both *Neurospora* transformation and DNA·RNA hybridization experiments has shown here that the *qa-1* region actually consists of two distinct genes corresponding to the two original mutational types *qa-1^S* and *qa-1^F*.

Neurospora transformation results (Table 1; Fig. 1) clearly indicated that the *qa-1^S* and *qa-1^F* mutations were located within distinct DNA sequences, because these mutants were transformed by nonoverlapping DNA fragments. Moreover, the analysis of the mRNA species hybridizing to these two regions showed that the *qa-1F* region encodes a 2.9-kb mRNA, while the *qa-1S* region encodes 4.1-kb and 3.4-kb mRNAs. Subsequent nuclease S1 mapping indicated that these mRNAs associated with the two regions are transcribed in opposite directions and originate within a common 1.4-kb *Sal* I/*Bam*HI fragment (Fig. 3, probe 2). Therefore, these combined results strongly indicate that *qa-1^F* and *qa-1^S* mutations define separate genes, now designated *qa-1F* and *qa-1S*, respectively.

The DNA·RNA hybridization results indicate that two mRNAs of 4.1 kb and 3.4 kb hybridize to the region that transforms *qa-1S* mutations. These two mRNAs appear to be initiated near the same site because they hybridized to probe 2 in approximately the same ratio as to probe 1. Nuclease S1 mapping also indicated that they are transcribed in the same direction. The 4.1-kb mRNA is the predominant species (Fig. 3) and it is possible that the 3.4-kb mRNA is simply a degradation or processing product of the 4.1-kb mRNA. Alternatively, the smaller mRNA could result from termination at a different site to that of the 4.1-kb mRNA.

Both *qa-1S* mRNAs are surprisingly large (3.4 and 4.1 kb) in relation to the smallest *Neurospora* DNA fragment required to transform noninducible *qa-1S* mutants (1.5 kb) (Fig. 1). From the DNA·RNA hybridization data it appears that this 1.5-kb region would correspond to the 5′ end of the 4.1-kb mRNA and would not contain the entire gene. The ability to obtain *qa-1S* transformants with what appears to be only a part of the gene is not fully understood. The region containing the mutation could have been replaced by recombination with the homologous wild-type DNA sequence from the plasmid. However, transformation results with two other *qa* genes (*qa-2* and *qa-1F*) indicate that a complete copy of the gene is required for transformation to occur (ref. 16; unpublished data). Another possibility, in the case of *qa-1S*, is that transformation results from the insertion of the wild-type portion of the gene elsewhere in the genome and that a complementing protein fragment is produced (α-complementation) (17). Because the precise function of the wild-type *qa-1S* gene is not known, it is not yet clear how transformation of *qa-1S$^-$* mutants occurs. For example, if the *qa-1S* gene encodes a repressor (see below), insertion of the transforming plasmid into the *qa-1S$^-$* gene might inactivate the gene, producing a qa-1S^C constitutive phenotype.

The transcriptional regulation of the *qa-1S* gene was examined in wild-type and various *qa-1* mutants under inducing and noninducing conditions. There appears to be a very low level of *qa-1S*-specific mRNA in noninduced cultures. However, after induction by quinic acid, the level of *qa-1S* mRNA increases ≈40-fold. Results obtained with *qa-1F* and *qa-1S* mutants that are noninducible for the structural genes indicate that the induction of *qa-1S* mRNA is dependent on the presence of a wild-type *qa-1F* product and also a wild-type *qa-1S* product. In a *qa-1* constitutive mutant (M105C), *qa-1S* mRNA is produced under noninducing conditions at ≈50% of the wild-type induced level. Thus, the *qa-1S* gene appears to exhibit autogenous regulation. However, it is not clear whether this apparent autogenous regulation is due to direct interaction of the *qa-1S* gene product with its own promoter or to an indirect effect mediated by the *qa-1F* gene product.

The identification of two distinct genes within the *qa-1* regulatory region has made it necessary to revise an earlier model of regulation of the *qa* system that involved only a single regulatory protein (3). It is apparent that two regulatory genes must be involved in the expression of *qa* structural genes and that the different qa-1 mutant phenotypes observed must result from different mutations in these two genes. These phenotypes and the revised interpretation of their genotypes are shown in Table 2. All *qa-1F* mutants exhibit a recessive, noninducible phenotype that is epistatic to *qa-1S^C* mutations in *qa-1S^C qa-1F$^-$* double mutants (3). Based on these genetic data, it therefore seems likely that

Table 2. Genotypes and phenotypes of *qa-1* regulatory mutants (3)

Genotype	Phenotype
qa-1S$^+$ qa-1F$^+$	Wild type, induced by quinic acid
qa-1S$^-$ qa-1F$^+$	Noninducible, semidominant
qa-1S^C qa-1F$^+$	Constitutive, recessive
qa-1S$^+$ qa-1F$^-$	Noninducible, recessive
qa-1S^C qa-1F$^-$	Noninducible, recessive (*qa-1F$^-$* is epistatic to *qa-1S^C*)

the *qa-1F* gene encodes a positive activator protein required for the transcription of all of the *qa* genes, including itself and *qa-1S* (unpublished data).

The existence of semidominant *qa-1S*$^-$ mutants and some recessive *qa-1S*^{C} constitutive mutants (6) suggests that the role of *qa-1S* in *qa* regulation may be a negative one—i.e., it encodes a repressor. Because *qa-1F* is only transcribed at a low level in *qa-1S*$^-$ mutants (unpublished data), the *qa-1S* gene product may regulate the *qa* structural genes by regulating transcription of *qa-1F* in concert with the inducer quinic acid. Constitutive *qa-1S*^{C} mutants would therefore allow transcription of *qa-1F* even in the absence of quinic acid, whereas noninducible *qa-1S*$^-$ mutants would repress *qa-1F* transcription even in the presence of quinic acid. This model for the interaction of the *qa-1S* and *qa-1F* genes does not require that the gene products themselves interact with one another. However, it cannot yet be ruled out that the two gene products interact with one another to form a heteromeric protein, with both negative and positive regulatory functions, similar to the araC protein (which is a single polypeptide). Although the genetic data strongly suggest a classical negative role for the *qa-1S* gene product, such a hypothesis does not predict that *qa-1S* would be subject to induction. This induction of *qa-1S* may have a feedback role in controlling *qa-1F* transcription. However, the possibility cannot be excluded that *qa-1S* may also act positively but that none of the predicted recessive noninducible *qa-1S* mutants have yet been isolated. Further characterization at the molecular level of the *qa-1F* and *qa-1S* genes and their mutations should allow a greater understanding of their roles in *qa* regulation.

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