Use of Gene Replacement Transformation to Elucidate Gene Function in the qa Gene Cluster of Neurospora crassa

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ABSTRACT

Gene replacement by transformation, employing selective genetic recombination techniques, has been used to delete or disrupt the qa-x, qa-y and qa-IS genes of the qa gene cluster of Neurospora crassa. The growth characteristics of the strain carrying the deletion of the qa-y gene support earlier evidence that this gene encodes a quinic acid permease. The strain containing the deletion of the qa-IS gene (Δqa-IS) was examined with respect to quinic acid induction and carbon catabolite repression. The Δqa-IS strain exhibits constitutive expression of the qa genes supporting earlier evidence that the qa-IS gene codes for a repressor. Several of the qa genes continued to be expressed at high levels even in the presence of glucose in the Δqa-IS strain, which indicates that transcription of these genes is not being affected directly by a repressor molecule in the presence of glucose.

The quinic acid (qa) gene cluster of Neurospora crassa is a well characterized system to study genetic regulation in a multicellular yet relatively simple organism. The qa gene cluster of N. crassa is located on approximately 17.2 kb of DNA on Neurospora linkage group VII (Giles et al. 1985). The DNA sequence of the entire qa cluster is known (Geever et al. 1989). The cluster includes five structural genes and two regulatory genes. Three of the structural genes, qa-2, qa-3 and qa-4 are involved in the first three steps of quinic acid catabolism (Giles et al 1985). A fourth gene, qa-y, is hypothesized to encode a quinic acid permease (Geever et al 1989) based on its similarity to other known permeases and by the phenotype of a strain carrying a deletion of the qa-y gene. The other structural gene, qa-x, is defined by a quinic acid inducible RNA. However, its function is unknown. The other two genes of the qa cluster (qa-IS and qa-1F) code for regulatory proteins on the basis of genetic and molecular analysis (Giles et al. 1985; Geever et al. 1989).

The genes of the qa gene cluster appear to be under two levels of genetic regulation. The first level is mediated by the inducer quinic acid and by the products of the qa regulatory genes. The qa-1F gene encodes a protein of 816 amino acids (Huët 1984). Molecular analysis indicates that the qa-1F protein is an activator protein which plays a positive role in the transcription of all the qa genes by binding to a conserved 16 bp sequence (GGRTAARYRTTATCC) (Baum, Geever and Giles 1987). A total of 14 binding sites for the qa-1F protein have been shown to exist in the qa gene cluster. The DNA binding domain of the activator protein is located in the N-terminal 183 amino acids. Several mutations have been obtained in the qa-1F gene. The resulting mutants are recessive and noninducible which has lead to the hypothesis that they produce defective activators. The qa-IS gene encodes a protein of 918 amino acids (Geever et al. 1987). This protein functions as a repressor molecule, preventing transcription of the qa genes in the absence of quinic acid. Indirect evidence suggests that the qa-IS protein is not a DNA-binding protein but interacts with the qa-1F activator protein to inhibit activator function (Giles et al. 1985, 1991; J. Avalos, R. Geever, M. E. Case and N. H. Giles, unpublished). Two types of mutations have been detected in the qa-IS gene: one produces mutants in which the qa genes are noninducible even in the presence of quinic acid and the second type produces mutants in which the qa genes are constitutively expressed in the absence of inducer. Sequence evidence indicates that the noninducible mutants are the result of nonsense mutations which cause the qa-IS gene product to become a superrepressor that is unable to respond to the presence of the inducer, quinic acid, while the constitutive strains contain nonsense or frame shift mutations which render the qa-IS gene product nonfunctional.

The second control circuit functions to repress transcription of the qa genes in response to the presence of a preferred carbon source such as glucose or sucrose. Wild-type N. crassa strains grown on quinic acid together with a preferred carbon source such as sucrose show a level of induction of the qa enzymes about 1% of the levels of induction observed with

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quinic acid as a sole carbon source. The GAL system in *Saccharomyces cerevisiae* shares many similarities in terms of gene control with the *qa* gene cluster and is also subject to carbon catabolite repression (JOHNSTON 1987). Carbon repression of the GAL genes is thought to operate through several mechanisms such as direct action on the various GAL promoters, inhibition of activator binding and inhibition of galactose transport (JOHNSTON 1987). However, the mechanism(s) of carbon catabolite repression on *qa* gene expression is unknown.

Gene replacement has been widely used in *S. cerevisiae* to study gene function (SCHERER and DAVIS 1979). Since most transforming DNA in *N. crassa* integrates at ectopic sites by nonhomologous recombination (CASE et al. 1979; KINSEY and RAMBOSEK 1984) gene replacement in *N. crassa* has been more difficult. There are two selectable markers in the *qa* gene cluster, *qa-2* and *qa-1F*, which can be used for transformation. Double mutants *qa-2, aro-9* and *qa-1F aro-9* are incapable of growth on minimal media unless supplemented with aromatic amino acids thus permitting *qa-2* and *qa-1F* transformants to be selected on minimal media. Preliminary reports describe the inactivation of the *qa-x* gene (CASE and GEEVER 1987) and the deletion of the *qa-y* gene (GEEVER et al. 1989) by transforming with an inactivated or truncated selectable marker thereby selecting for the rare integration by homologous recombination. A similar approach has been used to select for am*°* (glutamic dehydrogenase) transformants (FREDERICK, ASCH and KINSEY 1989). FREDERICK and KINSEY (1990) also employed this technique to identify those sequences upstream of the *am* gene required for full am gene expression. This paper will describe in more detail the characteristics of the strains carrying an altered *qa*-x gene or a deleted *qa-y* gene. To further our understanding of the role of the repressor protein in the regulation of the *qa* gene cluster, a strain has been constructed by gene replacement transformation in which the *qa-IS* gene has been deleted from the genome. The characteristics of this strain, which makes no repressor protein, have been examined with respect to the roles of both quinic acid induction and carbon catabolite repression in regulating *qa* gene expression. The results have important implications for an understanding of genetic regulation in the *qa* gene cluster of *N. crassa*.

**MATERIALS AND METHODS**

**Strains and media:** *N. crassa* strains used in this study are listed in Table 1. *N. crassa* was grown on Fries minimal medium supplemented with aromatic amino acids, p-aminobenzoic acid, inositol and methionine where required. *N. crassa* crosses were made on WESTERGAARD’s crossing media (WESTERGAARD and MITCHELL 1947). All plasmids used were grown on *Escherichia coli* strain JM101.

**TABLE 1**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
<th>Source</th>
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<tbody>
<tr>
<td><em>N. crassa</em> 74A</td>
<td>Wild type</td>
<td>Authors stock collection</td>
</tr>
<tr>
<td><em>N. crassa</em> qa-2, aro-9, int, A</td>
<td></td>
<td>Authors stock collection</td>
</tr>
<tr>
<td><em>N. crassa</em> qa-1F, aro-9</td>
<td></td>
<td>Authors stock collection</td>
</tr>
<tr>
<td><em>N. crassa</em> met-7-a</td>
<td></td>
<td>Authors stock collection</td>
</tr>
<tr>
<td><em>N. crassa</em> (128-5-3-2)</td>
<td>Δqa-IS</td>
<td>This work</td>
</tr>
<tr>
<td><em>N. crassa</em> (241-9-1-1)</td>
<td>Δqa-y</td>
<td>This work</td>
</tr>
<tr>
<td><em>N. crassa</em> (227-3-5-10)</td>
<td>qa-x°</td>
<td>This work</td>
</tr>
</tbody>
</table>

* FGSC No. 2489.
* FGSC No. 3953.
* FGSC No. 6904.
* FGSC No. 4088.

\[ * Contains deletion of the qa-IS gene.
\]

\[ * Contains deletion of the qa-y gene.
\]

\[ * Contains disruption of the qa-x gene.
\]

**Construction of plasmids:** The plasmid pRD10 (Figure 1A), which contains the disrupted qa-x gene, was constructed by cleaving at a unique HindIII site in the *qa-x* coding region and filling in the staggered ends using the Klenow fragment. This creates a unique NheI site within the *qa-x* coding region. The plasmid pQA-1 was constructed in the following manner. The 5.7-kb BamHI-NheI fragment containing the *qa-3* and *qa-y* genes was ligated to a 323-bp ApII-XbaI fragment from the plasmid pQA-IS. This hybrid fragment was then ligated into the BamHI site of pQA-IS to form a plasmid containing the intact *qa-3, qa-y* and *qa-1F* genes but deleting the entire *qa-IS* gene (Figure 1A). The plasmid pQA-xy was constructed by ligating a 2.6 kb XbaI-NcoI fragment containing the *qa-3* gene to a *BglII*-XbaI fragment containing *qa-IS* and *qa-1F* and the portion of the *qa-IF* gene. This fragment was then ligated into a XbaI cleaved vector. The resulting plasmid contained all of *qa-3* and *qa-IS* and enough of *qa-1F* to complement the *qa-IS* mutation; however the *qa-y* gene had been deleted (Figure 1B).

**Enzymology:** Growth of *N. crassa* strains, induction procedures and assay conditions for catabolic dehydroquinase were described previously (CASE, HAUTALA and GILES 1977). Specific conditions are noted in Table 2.

**Neurospora transformation:** *N. crassa* transformation was performed as described earlier by CASE et al. (1979) modified to use Novozyyme 234 (VOLLMER and YANOFSKY 1986). Transformants were isolated to minimal medium. Stabile isolates were then crossed to a "met-7" *aro-9* strain and *qa-2* *aro-9* "met-7" *qa-1F* *aro-9* "met-7" progeny were selected for further analysis.

**DNA and RNA isolation:** *N. crassa* DNA was prepared by the method of YELTON, HAMER and TIMBERLAKE (1984). Plasmid DNA was prepared by the method of ISH-HOROWICZ and BURKE (1981). *N. crassa* total RNA was prepared by the method of LINDBREN et al. (1990).

**DNA and RNA hybridization:** Digested genomic DNA (2 µg) was fractionated on 0.8% agarose gels for 12–20 hr, then transferred to nylon membranes (Nyttran, Schleicher and Schuell), hybridized and rinsed as recommended by the manufacturer. Total RNA was fractionated by electrophoresis through formaldehyde gels and transferred to Nyttran, hybridized and rinsed as recommended by the manufacturer. Probes were labeled with 32P by the method of FENBERG and VOGELSTEIN (1984).
Figure 1.—Plasmid construction. A. Construction of pRD10 (qa-x) and pΔqa-1s. The crosshatched area represents the fragment used for the construction of pRD10. The HindIII site present in the qa-x gene was filled in to create a unique Nhel site. The squared-in area represents the fragments used in the construction of the plasmid pΔqa-1s. The dotted areas above the line define the probes used in this study. B. Construction of pΔqa-y. The heavy cross-hatched areas show the fragments used to form the plasmid pΔqa-y.

S1 analysis: S1 analysis was performed by the procedure of Berk and Sharp (1977). To map the transcriptional start site of the qa-2 gene a 32P-end-labeled Smal-BglIII probe from the qa-2 region (Figure 1A) was hybridized at 52°C to total RNA from strains containing the Δqa-1S mutation grown under various growth conditions. Hybridizations were then digested with S1 nuclease and fractionated on an 7% denaturing polyacrylamide gel. Sequencing ladders were prepared by the method of Maxam and Gilbert (1980).

RESULTS

Effect of disrupting of the qa-x gene: The qa-x gene was originally identified as a quinic acid-inducible transcript of unknown function (Patel et al. 1981). To examine the function of the qa-x gene, the plasmid pRD10 was constructed in which the qa-x gene was disrupted by filling in a unique HindIII site in the qa-x coding region creating a new Nhel site in the amino-terminal end of the protein as shown in Figure 1A. This plasmid also contained the qa-2 gene, which could be truncated by cleaving at an SphI site (Figure 1A). Strains containing qa-2 and aro-9 mutations were transformed using a fragment containing the truncated qa-2 gene linked to the disrupted qa-x gene. Since the qa-2 mutation in the recipient strain had an A/T deletion in codon 8, the only way qa-2+ transformants could arise would be by homologous recombination in the region between the mutation and the SphI site (Figure 2A).

Transformants were selected for their ability to grow on minimal medium and crossed to a strain...
containing the closely linked met-7 mutation to obtain homokaryotic progeny. The homokaryotic progeny isolates could utilize both quinic acid and chlorogenic acid as carbon sources. The only detected phenotype of strains containing the disrupted qa-x gene is the accumulation of an unidentified brown pigment following growth on quinic acid as a carbon source. Genomic DNA was isolated from these progeny strains, digested with NheI, then probed by Southern blots with a $^{32}$P-labeled BglII-BamHI fragment containing the qa-2 coding region (Figure 1A). In the wild-type strain, this probe would hybridize to a NheI fragment of 3.2 kb in which the qa-x gene had been disrupted the qa-2 gene. The arrowhead indicates the location of the mutation in the qa-1F recipient strain. The filled-box represents sequences upstream of the qa-1F gene.

### Figure 2.—Homologous recombination events necessary to produce qa-2* or qa-1F* transformants. A, Homologous recombination event necessary to produce a transformant of pRD10 which is qa-2* and contains a disruption of the qa-x gene. The arrowhead represents the location of the mutation in the qa-2 recipient strain. B, Homologous recombination events necessary to produce qa-1F* transformants. This strategy was used to delete both the qa-y and qa-1S genes. The stippled box represents the qa-1F gene. The arrowhead indicates the location of the mutation in the qa-1F recipient strain. The filled-box represents sequences upstream of the qa-1F gene.

### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Conditions</th>
<th>Percent wild-type activity</th>
<th>CDHQ</th>
<th>QDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type 74A</td>
<td>Quinic acid</td>
<td>100</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>$\Delta$qa-y</td>
<td>Quinic acid</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>$\Delta$aro-9</td>
<td>Fries</td>
<td>23</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>$\Delta$aro-9</td>
<td>Quinic acid</td>
<td>118</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>$\Delta$qa-y, $\Delta$aro-9</td>
<td>Fries</td>
<td>25</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>$\Delta$qa-y, $\Delta$aro-9</td>
<td>Quinic acid</td>
<td>29</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Cultures were grown for 24 hr at 25°C with vigorous shaking in a Fries, sucrose medium. Cultures were harvested and thoroughly rinsed to remove all sucrose. Samples were then incubated for 6 hr with no carbon source (Fries alone) or with 0.3% quinic acid as a sole carbon source.

### Figure 3.—Genomic Southern blot of selected homokaryotic transformants. DNAs in A were digested with NheI then probed with the BglII-BamHI fragment containing the qa-2 gene (Figure 1A). DNAs in B and C were digested with BamHI and probed with the three BamHI fragments indicated in Figure 1. A, Lane 1, 74A; lane 2, a transformant of pRD10 containing the disruption of the qa-x gene. B, Lane 1, 74A; lane 2, a transformant of the plasmid $\Delta$qa-y containing the deletion of the qa-y gene. C, Lane 1, 74A; lane 2, a transformant of the plasmid $\Delta$qa-1S containing a deletion of the qa-1S gene. The same Southern patterns were observed in nonhomokaryotic transformants.

#### Effect of deleting the qa-y gene: The qa-y gene was originally identified as a quinic acid-inducible transcript of unknown function (PATEL et al. 1981). Subsequent sequence analysis revealed that the qa-y coding region showed a high degree of homology with the qa-x gene in A. HOPPER and E. MATTES (personal communication) have recently shown that the qa-x gene has 31% homology with the GAL12 gene. This gene appears to be involved in the dephosphorylation of the GAL4 activator in the presence of a preferred carbon source. However, we were unable to demonstrate that the disruption of qa-x has any effect on the level of qa-y gene expression in the presence of glucose (data not shown).

Transformants were crossed to a strain containing that specific alterations could be introduced into the qa gene cluster by transforming with a truncated selectable marker. J. HOPPER and E. MATTES (personal communication) have recently shown that the qa-x gene has 31% homology with the GAL12 gene. This gene appears to be involved in the dephosphorylation of the GAL4 activator in the presence of a preferred carbon source. However, we were unable to demonstrate that the disruption of qa-x has any effect on the level of qa-2 gene expression in the presence of glucose (data not shown).
A Wild-type

\[ \Delta qa-y \]

B Wild-type

\[ \Delta qa-1S \]

Figure 4.—The qa gene cluster after transformation with the pΔqa-y and pΔqa-IS plasmids. A, Arrangement of the qa gene cluster before and after transformation with the pΔqa-y plasmid. B, Arrangement of the qa gene cluster after transformation with the plasmid pΔqa-IS.

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>qa-y</td>
<td>10.1 kb</td>
</tr>
<tr>
<td>qa-1S</td>
<td>7.7 kb</td>
</tr>
<tr>
<td>qa-IS</td>
<td>4.3 kb</td>
</tr>
</tbody>
</table>

Effect of deleting the qa-IS gene: The plasmid pΔqa-IS was constructed as shown in Figure 1A. This construct, which retains functional qa-y and qa-1F genes, was used to transform a strain containing the qa-IS, aro-9 mutations to obtain strains in which the qa-IS gene was deleted. Transformants were crossed to a met-7 strain of N. crassa in order to obtain homokaryotic progeny. To determine if the transformants did, in fact, contain deletions of the qa-IS gene, genomic DNA was isolated from several of these progeny strains, digested with BamHI, and probed by Southern blot with the same \(^{32}P\)-labeled fragments from the qa gene cluster as used earlier (Figure 1A). These probes would be expected to hybridize with three genomic BamHI fragments in wild-type DNA at 7.7, 5.5, and 2.3 kb. In strains where deletion of the qa-IS gene had occurred these fragments would be replaced by two fragments, 6.1 and 5.5 kb in size. Several of the transformants had this pattern diagnostic of a deletion of the qa-IS gene. An example is shown in Figure 3C. Representative strains containing the Δqa-IS deletion were selected for further studies. The arrangement of the qa gene cluster after deletion of the qa-IS gene is shown in Figure 4B.

In the GAL system in yeast, strains which are deleted for the GAL80 repressor gene show constitutive levels of GAL gene expression. Expression of the various GAL genes in these strains, however, is still strongly repressed in the presence of glucose (Flick and Johnston). In order to determine if the Δqa-IS strain behaved like the Δgal80 strain in yeast, total RNA was isolated from the Δqa-IS strain as well as wild-type 74A after growth for 6 hr with 2% glucose as a sole carbon source, on 0.1% quinic acid as a carbon source, and in the presence of 0.1% quinic acid and 2% glucose. The RNA was then fractionated by electrophoresis, transferred to a nylon membrane, and probed with the \(^{32}P\)-labeled BglI-BamHI fragment (Figure 1A) which contains the qa-2 coding region or the \(^{32}P\)-labeled H3 (histone) gene. As shown in Figure 5A, the qa-2 message is expressed in the Δqa-IS strain under all three conditions tested, glucose (lane 1), glucose and quinic acid (lane 2), and quinic acid alone (lane 3). In wild-type 74A, the qa-2 message is present only when the strain is grown with quinic acid as the sole carbon source (lane 6) not on glucose (lane 4) or on quinic acid and glucose (lane 5). This indicates that the qa-2 gene is being expressed constitutively in the
Δqa-IS strain and is not subject to the strong catabolite repression as is wild-type 74A. This is in contrast to the situation with the gag genes in yeast where the gag messages are repressed in the presence of a preferred carbon source such as glucose in Δgai80 strains. Northern results similar to those in Figure 5A were observed when the levels of the qa-x and qa-1F messages were determined in the Δqa-IS strain (data not shown). Probing with the H3 gene indicated some variation in the mRNA concentrations (Figure 5B). The variation in the levels of the histone message however cannot account for the repression of the qa-2 message in the presence of glucose in wild-type 74A.

The qa-2 gene of N. crassa utilizes multiple transcriptional start sites which fall into two main groups (TYLER et al. 1984). One group is located about 2% 140 bp from the ATG start codon of qa-2 and is activator independent. These start sites account for the basal level transcripts which are produced under noninducing conditions. The other group of start sites is activator dependent and accounts for the transcripts produced under inducing conditions. These sites are located 70–100 bp upstream of the qa-2 start codon. One model (TYLER et al. 1984) proposes that the binding of the qa-1F activator protein upstream of the qa-2 gene allows transcription to begin at the activator-dependent start sites. To determine if the activator-dependent transcriptional start sites for the qa-2 gene were being used in the presence of glucose, the major transcriptional start sites for the qa-2 gene were determined in the Δqa-IS strain grown with glucose as a carbon source (Figure 6, lane B) and both quinic acid and glucose present (Figure 6, lane C) and compared with the qa-2 start site in wild-type 74A grown with quinic acid as a sole carbon source (Figure 6, lane A). In all cases tested, the major inducible start site for the qa-2 gene was being utilized.

DISCUSSION

While gene replacement and gene disruption have long been useful tools in the study of gene function in S. cerevisiae, the fact that the majority of transformants in N. crassa arise via nonhomologous, ectopic integration has made such studies in N. crassa and other filamentous fungi much more difficult. In this study, three genes of the qa gene cluster (qa-x, qa-IS and qa-y) have been deleted or disrupted by using the “targeted” transformation technique. Southern hybridization of the nonhomokaryotic transformants indicated that only gene replacement had occurred since there was no evidence for ectopic integration. We have shown that this technique can be used to replace or alter specific sequences on the N. crassa genome at a distance of up to 8.0 kb from the selectable marker. Our initial studies resulted in the disruption of the qa-x gene using the qa-2 gene as a selectable marker. Recent data have shown that the qa-x gene has 31% homology with GALI2, a gene controlling carbon-regulated dephosphorylation of the GAL4 activator protein (J. HOPPER and E. MATTHE, personal communication). However, carbon mediated repression of the qa-2 gene seemed unaffected by the disruption in qa-x. Therefore, the role of the qa-x gene in quinic acid catabolism remains unclear.

Subsequently, the qa-y and qa-IS genes were deleted using the qa-1F gene as a selectable marker. This involved the introduction of deletions ending as much as 8.0 kb from the qa-1F gene. The qa-y gene had been shown to have significant homology with the
Gene Replacement in *N. crassa*

quitD gene of *A. nidulans* which is known to be a quinic acid permease (Whittington et al. 1987). Géeven et al. (1989) searched the GenBank data base and found that the qa-γ gene had a probable homology with a glucose transporter of human hepatoma cells. Deletion of the qa-γ gene in *N. crassa* produced a phenotype consistent with the interpretation that the qa-γ gene product is involved in quinic acid transport. Strains containing the Δqa-γ cannot grow on quinic acid as a sole carbon source and have very low inducible levels of qa-2 and qa-3 enzyme activities. In strains containing the aro-9 mutation, the qa genes are partially induced by the action of an internal inducer (DHQ). In strains containing both the Δqa-γ and the aro-9 mutations equivalent partial induction occurs, indicating that the qa genes can be induced if internal inducer is present in the cell. The evidence that Δqa-γ strains have only low levels of inducible qa enzyme activities is thus consistent with the view that the qa-γ gene encodes a quinic acid permease whose absence in the Δqa-γ strain precludes the normal uptake of quinic acid. The low level of induction of the qa-2 and qa-3 genes observed in the Δqa-γ strain in the absence of internal inducer is presumably due to the entry of a low level of quinic acid into the cell by other avenues.

The qa-1S gene was deleted completely by transforming a qa-1F mutant strain with a plasmid construct containing the qa-1F gene as a selectable marker as well as intact qa-3 and qa-γ genes but totally lacking the qa-1S gene and its flanking regions. The resulting transformants had lost the 3.8 kb of sequence from linkage group VII which normally contains the qa-1S gene. However, there was no rearrangement of the surrounding restriction fragments. Transformants containing the Δqa-1S deletion have constitutive levels of expression of the qa-2, qa-1F and qa-γ genes as determined by mRNA analysis, which is consistent with earlier data indicating that the qa-1S gene product is a repressor molecule (Géeven et al. 1989).

The GAL system of *S. cerevisiae* and the qa system of *N. crassa* share many similarities in gene regulation. Both systems are under the control of an activator protein which activates the transcription of the various genes in the presence of substrate and a repressor protein which inhibits activator function in the absence of inducer. Gene expression of both systems is under carbon catabolite repression in that gene expression is severely repressed in the presence of a preferred carbon source even if the inducer is present. It has been shown that carbon repression of the GAL1 gene is mediated by specific sequences upstream of GAL1 as well and by action on the GAL4 binding site (Flick and Johnston 1990). In yeast strains containing a deletion of the GAL80 repressor gene, GAL1 remains strongly repressed in the presence of glucose. However, in *N. crassa* strains containing the Δqa-1S deletion the mRNA levels of the qa-2, qa-γ and qa-1F transcripts are high in the presence of glucose. This suggests that carbon repression of at least some of the genes of the qa gene cluster may be operating by a different mechanism(s), not involving either specific sequences upstream of the qa genes or inhibition of activator binding as in seen with GAL1.

These results may indicate that the qa-1S repressor protein plays a role in catabolite repression of the qa gene cluster. However our present data does not rule out other possible explanations such as control at the level of quinic acid uptake. To determine if the qa-1S protein is involved in carbon repression outside the qa gene cluster we have assayed the mRNA levels of the glucose repressible gene (grg) (McNally and Free 1988) in the Δqa-1S strain grown under conditions in which this gene would be carbon repressed. Under these conditions grg transcription is repressed normally in the Δqa-1S strain (M. E. Case and D. K. Asch, unpublished). Therefore we feel that if the qa-1S gene product plays any role in carbon repression that it may be a target for a general carbon repressor molecule and acts only to repress the qa gene cluster in the presence of a preferred carbon source.

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**LITERATURE CITED**


Frederick, G. D., and J. A. Kinsey, 1990 Distant upstream regulatory sequences control the level of expression of the am


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