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Comparative studies of the quinic acid (*qa*) cluster in several *Neurospora* species with special emphasis on the *qa-x-qa-2* intergenic region

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Summary. The organization of the quinic acid (*qa*) genes in *Neurospora crassa* has been compared to that in several other *Neurospora* species. This gene cluster was found to be highly conserved in all species examined. However, there are numberous restriction fragment length polymorphisms that distinguish the heterothallic and homothallic species. Catabolic dehydroquinase assays indicated that *qa-2* gene expression in the homothallic species is subject to induction by quinic acid, as is the case in *N. crassa*. The *qa-x-qa-2* intergenic region of the homothallic species *N. africana* was cloned and sequenced. Conserved *qa* activator (*qa-1F*) binding sites have been identified in this region. When the *qa-x-qa-2* intergenic region of *N. crassa* was replaced with its *N. africana* counterpart, *qa-2* gene expression was reduced; however repression by glucose appeared normal. Furthermore, the *N. africana* start site for *qa-2* transcription (which differs from the *N. crassa* start site) was utilized in the transformant. The overall evidence suggests that a weakening of the −120 activator binding site in the *qa-x-qa-2* intergenic region may be responsible for these differences.

Key words: *Neurospora crassa* – *Neurospora africana* – Quinic acid (*qa*) cluster

Introduction

The quinic acid (*qa*) gene cluster of *Neurospora crassa* provides a well-characterized system for studying gene regulation in a multicellular, yet relatively simple, eukaryotic organism. The *qa* gene cluster consists of five structural (*qa-x, qa-2, qa-4, qa-3* and *qa-y*) and two regulatory (*qa-1F* and *qa-1S*) genes, all of which are grouped together on approximately 17 kb of DNA on *Neurospora* linkage group VII (Giles et al. 1985). Three of the structural genes, *qa-2, qa-3* and *qa-4*, code for enzymes that enable *Neurospora* to utilize quinic acid as a carbon source, another gene, *qa-y*, has been shown to code for a permease that transports quinic acid into the cell (Geever et al. 1989; M. Case et al. unpublished), and the function of the other structural gene, *qa-x*, is unknown. The DNA sequence for the entire *qa* cluster has been determined and the direction of transcription of each gene as well as the location of the start sites for transcription established (Geever et al. 1989). Three pairs of genes are divergently transcribed from common 5′ regions. These gene pairs are *qa-1F, qa-1S; qa-3, qa-4;* and *qa-2, qa-x*. Only *qa-y* does not share a 5′ upstream region with another gene of the cluster.

Expression of all the genes in the *qa* cluster is primarily controlled by the two regulatory genes, *qa-1F* and *qa-1S*. The *qa-1F* gene encodes an activator protein which controls the expression of the various *qa* genes, including itself, in the presence of the inducer, quinic acid, by binding to specific sites upstream of the various *qa* genes. These targets of the activator have been determined by DNase I protection experiments to be 16 bp sequences with the consensus sequence GGRTAARYR-YTTTATCC (Geever et al. 1989; Baum et al. 1987). There are 14 of these sites in the *qa* gene cluster and the relative affinity of each binding site for the activator protein has been determined in vitro. Two of the gene pairs contain several activator binding sites in their 5′ regions, while one gene pair has only one binding site between them (Geever et al. 1989; Baum et al. 1987). The other regulatory gene, *qa-1S*, codes for a repressor protein. However, there is no evidence that the *qa-1S* protein is a DNA-binding protein; indirect evidence suggests that the target of the *qa-1S* repressor is in fact the activator protein itself (Giles et al. 1987; Avalos et al. unpublished).

The interaction of the *qa-1F* and *qa-1S* proteins, in response to the level of quinic acid present in the cell,

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constitutes the first regulatory circuit controlling transcription of the *qa* cluster. A second control circuit, which is superimposed on the first, acts to repress expression of the *qa* cluster in the presence of a preferred carbon source such as glucose. The mechanisms by which this circuit operates are unknown, although evidence from the galactose system in *Saccharomyces cerevisiae* (Flick and Johnston 1990) suggests that one possible regulatory mechanism could be the interaction of carbon repressors with sequences 5′ to the various *qa* genes to block transcription in the presence of a preferred carbon source. However, the presence of such sequences has yet to be demonstrated in the *qa* system.

R. Geever et al. (unpublished data) found that the enzymes coded for by the *qa* gene cluster were present in several closely related *Neurospora* species. In order to understand better the molecular mechanisms involved in the control of the *qa* cluster in *N. crassa*, we sought to examine the genes involved in quinic acid utilization in those species of *Neurospora*. A high degree of conservation of the *qa* gene cluster was observed between the homothallic and heterothallic species of *Neurospora*, as determined by Southern analysis. The heterothallic species were very similar to *N. crassa* even in restriction fragment sizes, while the homothallic species exhibited greater differences. *N. africana* was chosen as a representative of the homothallic species for further study by cloning and sequencing of the *qa-x-qa-2* intergenic region. This region was chosen for investigation for the following reasons: (i) the presence in *N. crassa* of four binding sites possessing widely varying affinities for the activator protein; (ii) our ability to use assays for catabolic dehydroquinase to measure *qa-2* gene expression; (iii) the fact that the *qa-2* gene is an excellent selectable marker for transformation experiments in which *N. crassa* sequences could be replaced by *N. africana* sequences using targeted transformation. Our overall studies indicate that there is a high degree of conservation in the control mechanisms for the *qa* gene cluster between *N. crassa* and the other *Neurospora* species.

Materials and methods

Strains and media. Neurospora strains used in this study are listed in Table 1. *N. crassa* and the heterothallic *Neurospora* strains were grown on Fries minimal media supplemented where required with aromatic amino acids, *p*-amino-benzoic acid, inositol, and methionine. Homothallic *Neurospora* species were grown on a complete medium (Davis and de Serres 1970). *Neurospora* crosses were made on Westergaard's crossing media (Westergaard and Mitchell 1947). Lambda phages were propagated on *Escherichia coli* ER1458 (New England Biolabs). M13 phages and all plasmids used were grown on JM101.

Library construction. N. africana, N. dodgei, N. terricola and *N. lineolata* genomic DNA was digested to completion with *Bam*HI and ligated to *Bam*HI-cleaved arms of the lambda vector EMBL-4 (Frischauf et al. 1983). After ligation the mixture was packaged using the in vitro packaging procedure of Grosveld et al. (1981) and the diluted packaging mix was plated on strain ER1458. Clones were identified by the method of Benton and Davis (1977) using nitrocellulose membranes (Schleicher and Schuell) according to the recommendations of the manufacturer. Probes were labeled with ^{32}P by the method of Feinberg and Vogelstein (1984).

DNA and RNA isolation. Neurospora DNA was prepared by the method of Yelton et al. (1984). Plasmid DNA was prepared by the method of Ish-Horowitz and Burke (1981). *N. crassa* and *N. africana* RNA were prepared by the method of Patel et al. (1981).

Enzymology. The growth of *Neurospora* strains, induction procedures and assay conditions for catabolic dehydroquinase were described previously (Case et al. 1977). Specific conditions are noted in Table 2.

Sequencing. The intergenic region between *qa-x* and *qa-2* was sequenced by the method of Sanger and Coulson

Table 1. *Neurospora* strains used in this study

Strains	Genotype	Source
Neurospora crassa 74A (heterothallic)	wild-type	FGSC No. 2489[a]
N. tetasperma (heterothallic)	wild-type	FGSC No. 1270[a]
N. sitophila (heterothallic)	wild-type	FGSC No. 2216[a]
N. intermedia (heterothallic)	wild-type	FGSC No. 2316[a]
N. galapagosensis (heterothallic)	wild-type	FGSC. No. 2290[a]
N. lineolata (homothallic)	wild-type	FGSC No. 1910[a]
N. africana (homothallic)	wild-type	FGSC No. 1740[a]
N. dodgei (homothallic)	wild-type	FGSC No. 1690[a]
N. terricola (homothallic)	wild-type	FGSC No. 1889[a]
N. crassa	*qa-2*$^-$ *aro-9*$^-$ *inl A*	Authors stock collection[b]
N. crassa	*met-7*$^-$ *a*	Authors stock collection[c]
N. crassa (275-5-1)	*qa-2*$^+$ *aro9*$^+$	This work[d]

[a] Obtained from the Fungal Genetics Stock Center, Department of Microbiology, Immunology, and Molecular Genetics, University of Kansas Medical Center, Kansas City, KA 66103, USA
[b] FGSC No. 3952
[c] FGSC No. 4088
[d] *qa-2*$^+$ transformant containing the *N. africana qa-x-qa-2* intragenic region

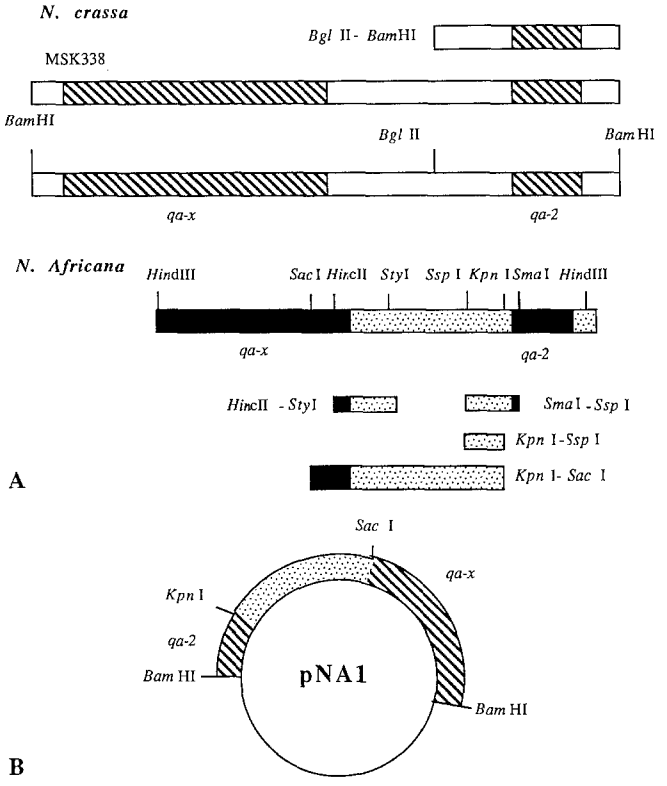

Fig. 1. **A** Diagrammatic representation of the *qa-x-qa-2* region. *Filled lines* represent the *Neurospora africana qa-2* and *qa-x* genes. *Dotted regions* represent the *N. africana* intergenic region between *qa-x* and *qa-2*. The *N. crassa qa-x* and *qa-2* genes are represented by the *striped* areas while the *open boxes* represent the intergenic region between *qa-x* and *qa-2* of *N. crassa*. Probes used for Southern hybridizations (MSK338, *Bgl*II-*Bam*HI and *Kpn*I-*Sac*I) and for S1 analysis (*Sma*I-*Ssp*I, *Hinc*II-*Sty*I, and *Kpn*I-*Ssp*I) are indicated. **B** Diagrammatic representation of the plasmid pNA1

(1975) using M13 cloning vectors (Messing 1983). Sequence analysis was performed using the University of Wisconsin computer programs (Devereux et al. 1984).

Construction of plasmid pNA1. Plasmid pNA1 (Fig. 1 B) was constructed by ligating a 1.1 kb *Kpn*I-*Sac*I fragment from the plasmid p1740 (M. Orejas, unpublished), which contains the *N. africana qa-2* gene and part of the *qa-x* gene on a 2.4 kb *Hin*dIII fragment (Fig. 1 A), into *Kpn*I-*Sac*I cleaved pRD9-95. The resulting plasmid contained the inactive *qa-2* gene from *N. crassa,* the intergenic region from *N. africana,* and an inactivated *qa-x* gene from *N. crassa*. The *qa-x* gene is inactivated in plasmid pRD9-95 (J. Baum, unpublished) by the destruction of a *Hin*dIII site in the coding region, while the *qa-2* gene is inactive due to the destruction of an *Sph*I site in the *qa-2* coding region.

Neuropsora transformation. Neurospora transformation was performed as described earlier by Case et al. (1979) with modifications for use of Novozyme 234 (Vollmer and Yanofsky 1986). Transformants were isolated on minimal medium, stable isolates were crossed to a *met-7*$^-$ *aro-9*$^+$ strain, and *aro-9*$^+$ *met-7*$^+$ progeny were selected. Progeny strains were then tested for their ability to grow on 0.3% quinic acid as sole carbon source. *aro-9*$^+$ *qa-2*$^+$ *met-7*$^+$ progeny were selected for further analysis.

DNA hybridization. Digested genomic DNA (2 µg) was fractionated on 0.8% agarose gels for 12–20 h, then transferred to nylon membranes (Nytran™, Schleicher and Schuell), hybridized, and rinsed as recommended by the manufacturer. Probes were labeled with ^{32}P by the method of Feinberg and Vogelstein (1984).

S1 nuclease analysis. S1 nuclease analysis was performed by the procedure of Berk and Sharp (1977). To map the transcriptional start site of the *qa-2* gene a ^{32}P end-labeled *Sma*I-*Ssp*I probe from the *N. africana qa-2* region (Fig. 1 A) was hybridized to poly(A)$^+$ RNA from either *N. africana* or from the *N. crassa* strain 275-5-1 at 52° C for 16 h. 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). The *qa-2* start site was confirmed using a *Kpn*I-*Ssp*I probe (Fig. 1 A) under similar conditions. The start site of the *qa-x* gene was mapped using a *Hinc*II-*Sty*I fragment as a probe and hybridizing at 52° C.

Results

Characteristics of the qa-x-qa-2 *gene region in other* Neurospora *species*

To determine the degree of conservation between *N. crassa* and other *Neurospora* species in the *qa* cluster particularly in the *qa-x-qa-2* intergenic region, genomic DNAs from the heterothallic species *N. tetrasperma, N. intermedia, N. galapagosensis,* and *N. sitophila,* as well as from the homothallic species *N. dodgei, N. africana, N. lineolata,* and *N. terricola* were digested with *Bgl*II, *Hin*dIII and *Bam*HI. These DNAs were fractionated by electrophoresis, blotted, and probed with ^{32}P-labeled probes from the *qa* gene cluster of *N. crassa*. An example of the data obtained with *Hin*dIII-digested DNA is shown in Fig. 2. All species tested show enough conservation at the DNA sequence level to hybridize to *N. crassa* probes from the *qa* cluster. The heterothallic species *N. tetrasperma, N. intermedia, N. galapagosensis,* and *N. sitophila* (lanes B–E) show a striking conservation of restriction fragment size with *N. crassa* using *Bam*HI, *Bgl*II, and *Hin*dIII as restriction enzymes. However, a high degree of polymorphism was observed between the homothallic species tested and *N. crassa*. None of the restriction fragments in *N. crassa* containing the *qa-x* and *qa-2* genes were conserved in the homothallic species (lanes F–I). The homothallic species tested did, however, show restriction fragment similarities with each other, with the exception of *N. terricola* (lane I), which showed no restriction fragment conservation with the other three species tested. Further analysis revealed that the *qa-x-qa-2* intergenic region was located on a 2.4 kb *Hin*dIII fragment in *N. africana, N. dodgei,* and *N. lineolata*. An

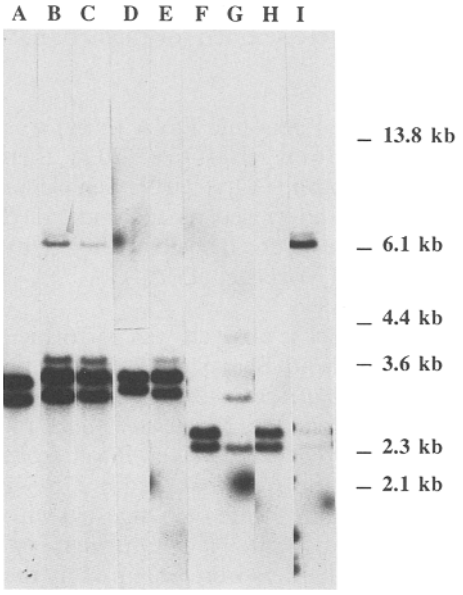

Fig. 2. Genomic Southern blot of various species of *Neurospora*. DNAs were digested with *Hin*dIII and probes with ^{32}P-labeled pMSK338 (Fig. 1A). Lane A, *N. crassa;* lane B, *N. galapagosensis;* lane C, *N. intermedia;* lane D, *N. tetrasperma;* lane E, *N. sitophila;* lane F, *N. lineolata;* lane G, *N. africana;* lane H, *N. dodgei;* lane I, *N. terricola*

analysis of these genomic Southern blots and Southern analysis of the subsequent lambda clones revealed that despite the restriction fragment length polymorphisms (RFLPs) between the homothallics and *N. crassa,* the *N. crassa qa* gene order (*qa-x, qa-2, qa-4, qa-3, qa-y, qa-1S, qa-1F*) is conserved (R. Geever et al. unpublished).

To determine if the same control circuits are operating in the homothallic species and in *N. crassa,* the level of *qa-2* gene expression was assayed by measuring the activity of the *qa-2* gene product (catabolic dehydroquinase) in homothallic strains induced on no carbon source (non-inducing conditions), 0.1% quinic acid as a sole carbon source (inducing conditions) and 0.1% quinic acid and 2% dextrose (catabolite repression conditions) *N. africana* was chosen as a representative of the homothallic species for further studies. However assays done on the other species showed levels of *qa-2* gene expression that were very similar to those in *N. africana. N. africana* showed at least a 1500-fold induction over basal levels of expression of the *qa-2* gene product (catabolic dehydroquinase) in the presence of quinic acid (data not shown). This compares to at least a 2000-fold induction of expression of *qa-2* in *N. crassa* under the same conditions. In the presence of quinic acid and a preferred carbon source such as dextrose, *N. africana* showed a 3-fold reduction in expression of the *qa-2* gene compared with an approximately 65-fold reduction in *qa-2* expression in *N. crassa* under the same growth conditions. From these data it was concluded that both control circuits were operating in the homothallic species; however, the carbon repression of the *qa* cluster was not as efficient in the homothallic species as in *N. crassa.*

Cloning of the qa-x-qa-2 *region from homothallic* Neurospora *species*

To examine further the molecular nature of gene control in the homothallic *Neurospora* species, the region between the *qa-2* and *qa-x* genes from *N. africana, N. dodgei* and *N. lineolata* was analyzed at the molecular level. Southern analysis of genomic DNA from *N. africana, N. lineolata,* and *N. dodgei* revealed that the *qa-2* gene region resided on a *Bam*HI fragment of clonable size (data not shown). In order to clone these fragments, libraries of *Bam*HI fragments from each of these species were prepared using the lambda vector EMBL-4. Clones containing the *qa-2* gene were obtained using as probe the *Bgl*II-*Bam*HI fragment containing the *N. crassa qa-2* gene (Fig. 1A). The *qa-2* genes of *N. lineolata* and *N. dodgei* were obtained on a 12.6 kb *Bam*HI fragment while the *N. africana qa-2* gene was cloned on a 9.5 kb *Bam*HI fragment. As stated earlier, the *qa-2* gene had been shown by Southern analysis to reside on a 2.4 kb *Hin*dIII fragment in *N. africana, N. dodgei,* and *N. lineolata.* This 2.4 kb *Hin*dIII fragment was subcloned into plasmid vectors for all three species.

Comparative analysis of the qa-x-qa-2 *gene region between* N. africana *and* N. crassa

Preliminary sequence analysis of the *N. africana* clone revealed that the 2·4 kb *Hin*dIII fragment contains all of the *qa-2* gene, the *qa-x-qa-2* intergenic region, and the first 700 bp of the *qa-x* gene (Fig. 1A). The region between the start codons of *qa-x* and *qa-2* was sequenced completely on both strands (Fig. 3). The intergenic region between *qa-x* and *qa-2* is smaller in *N. africana* (1088 bp versus 1194 bp in *N. crassa*). There is one large area in which sequences present in *N. crassa* are deleted in *N. africana* (*N. crassa* coordinates 2435–2538, Fig. 3). Binding sites for the *qa-1F* activator protein have been characterized by DNase I protection experiments in *N. crassa* (Baum et al. 1987). A consensus sequence for the *qa-1F* binding site had been determined, GGRTAATYTTTATCC (Baum et al. 1987), and comparison of the *qa-x-qa-2* intergenic regions of *N. africana* and *N. crassa* showed that the sequences aligned with three of the four *N. crassa* binding sites had a high degree of conservation, the exception being the −483 binding site which is a very poor binding site in *N. crassa* (Baum et al. 1987; Geever et al. 1989).

Sequence analysis of the DNA-binding domain of the *qa-1F* protein of *N. africana* showed that the DNA-binding domain is identical with that in *N. crassa* (D. Asch and M. Case, unpublished data). These results suggest that *N. africana* probably employs the same binding sites as *N. crassa* for activation of the *qa* cluster. Of the three binding sites which are highly conserved in *N. africana,* only one, the −502 site, would be predicted to have a greater affinity for the *N. crassa* activator protein than its *N. crassa* counterpart based on the degree of symmetry and similarity to the consensus sequence as deter-

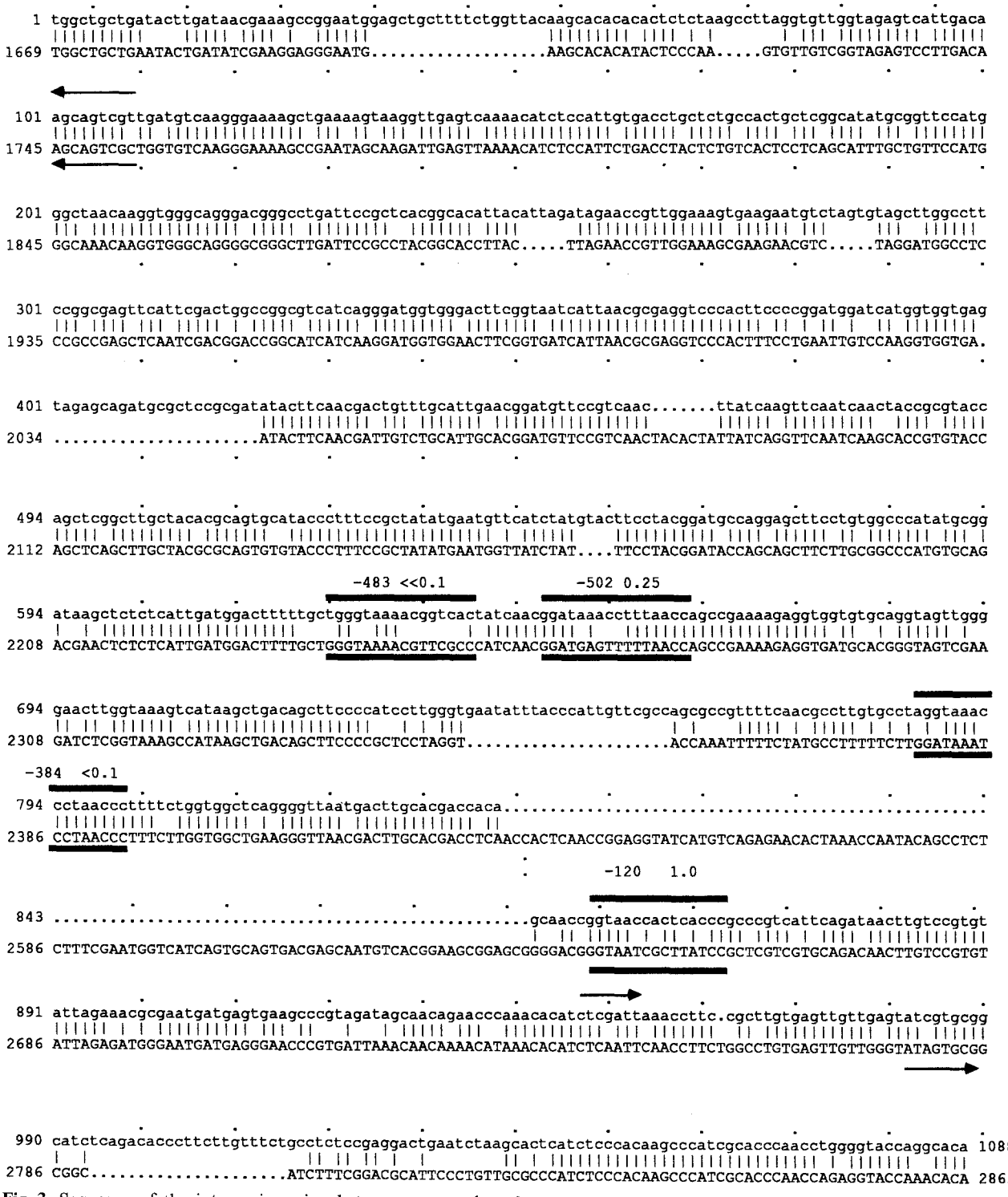

Fig. 3. Sequence of the intergenic region between *qa-x* and *qa-2* in *N. crassa* and *N. africana*. The *N. crassa* sequence is given in *capital letters* while the *N. africana* sequence is in *lower case letters*. Transcriptional start sites are indicatd by *arrows*. *Heavy lines* indicate the binding sites for the *qa-1F* protein. The positions and relative strengths of the binding sites are also indicated

mined by Baum et al. (1987) and Geever et al. (1989). Interestingly the equivalent of the *N. crassa* −120 binding site in *N. africana* would be predicted to be a much poorer binding site in *N. africana* than in *N. crassa*. The −120 binding site between *qa-x* and *qa-2* in *N. crassa* is the strongest binding site of the entire *qa* cluster (Baum et al. 1987). To determine if the *N. crassa qa-1F* protein could bind to the *N. africana* sequences, DNAse I protection experiments were done using *N. crassa qa-1F* protein obtained from the baculovirus expression system (Miller et al. 1986). Protection was only observed with fragments containing the −502 and −384 binding sites. No protection of the −120 binding site of *N. africana* could be detected (M. Orejas, unpublished results).

Placement of the qa-x-qa-2 *gene region from* N. africana *into the gene* qa *cluster of* N. crassa

To determine if *N. africana* does in fact utilize the same mechanisms of *qa* gene control, the intervening region between the *qa-x* and *qa-2* genes of *N. africana* was placed between the *qa-x* and *qa-2* genes in the *N. crassa* gene cluster. To accomplish this replacement a plasmid designated pNA1 was constructed, as described in the Materials and methods (Fig. 1 B). Plasmid pNA1 contains a new *Nhe*I site in the *qa-x* gene which is not present on the *N. crassa* chromosome. This construct was transformed into a *qa-2*$^-$, *aro-9*$^-$ strain of *N. crassa*. Since most transformants in *N. crassa* are the result of non-homologous ectopic integration, the *qa-2* gene in this plasmid was inactivated by the destruction of an *Sph*I site in the *qa-2* coding region (R. Geever, unpublished). Consequently, only integration of the transforming DNA by homologous recombination could produce a *qa-2*$^+$ transformant. This type of approach has been successfully used to target in vitro modifications into the *Neurospora* genome both in the *qa* cluster (M. Case et al. in preparation) and at other loci (Fredrick et al. 1989; Fredrick and Kinsey 1990). Transformants capable of growing on minimal media were selected. Since most *N. crassa* transformants are initially heterokaryotic, transformants were crossed to an *aro-9*$^+$ *qa-2*$^+$ *met-7*$^-$ strain to obtain homokaryotic isolates and the progeny isolates were used for subsequent analysis.

To determine if replacement of the *qa-x-qa-2* intergenic region had occurred, genomic DNA was isolated, digested with *Nhe*I, fractionated by electrophoresis, blotted and probed with a ^{32}P-labeled 1.2 kb *Bgl-Bam* fragment containing the *qa-2* gene of *N. crassa* or a ^{32}P-labeled 1.0 kb *Kpn*I-*Sac*I fragment containing the intergenic region of *N. africana* (Fig. 1 A). In *N. crassa* the wild-type *Nhe*I fragment containing the *qa-2* gene and its 5′ flanking regions is larger than 12 kb. If the replacement has occurred, a 3.2 kb *Nhe*I fragment should be observed, due to the *Nhe*I site created in the *qa-x* gene. Several strains, of which an example is shown in Fig. 4, were examined by Southern analysis and all were found to have the expected modification.

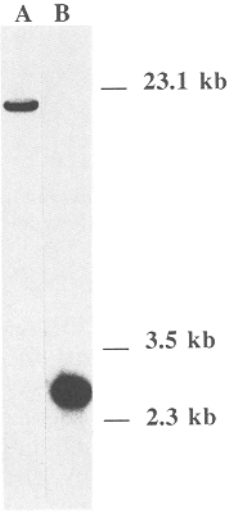

Fig. 4. Genomic Southern blot of wild-type *N. crassa* 74A and transformant 275-1-5 obtained using the plasmid pNA1. DNAs were digested with *Nhe*I. Lane A, 74A probed with the *Bgl*II-*Bam*HI *qa-2* probe (Fig. 1); Lane B, strain 275-1-5 probed with the *Kpn*I-*Sac*I probe containing the intergenic *qa-x-qa-2* region from *N. africana* (Fig. 1)

Table 2. Specific activity for catabolic dehydroquinase in wild-type 74A and transformant 275-1-5

Strains	Induction conditions	Specific activity[a]
Wild-type 74A	Fries	0.0003
	Fries, 0.1% quinic acid	0.6495
	Fries, 0.1% quinic acid, 2% dextrose	0.0155
275-1-5	Fries	0.00003
	Fries, 0.1% quinic acid	0.4588
	Fries, 0.1% quinic acid, 2% dextrose	0.0031

Cultures were grown for 24 h at 25° C with vigorous shaking in a Fries-sucrose medium; cultures were harvested and thoroughly rinsed to remove all sucrose and harvested samples were then incubated for 6 h under the conditions described

[a] Specific activity is expressed as nmol of dehydroshikimate produced per min per milligram of total protein at 37°. Specific activities listed are the mean values from three independent assays. Several independent transformants were isolated and showed similar *qa-2* levels to 275-1-5

One representative transformant, strain 275-1-5, was chosen for further study. To determine if the expression of the *N. crassa qa-2* gene is under the control of the *N. africana qa-x-qa-2* intergenic region, the level of catabolic dehydroquinase (encoded by the *qa-2* gene) was assayed in the transformed strain induced on no carbon source (non-inducing conditions), with 0.1% quinic acid as a sole carbon source (inducing conditions), and 0.1% quinic acid and 2% dextrose (catabolite repression conditions). The transformed strain showed induced levels of *qa-2* enzyme activity which were approximately 70% of those of wild-type *N. crassa* induced on 0.1% quinic acid (Table 2). In the presence of dextrose, *qa-2* enzyme activity in the transformed strain was reduced over 100-fold which is comparable to the degree of repression seen in wild-type *N. crassa*.

S1 mapping studies of N. africana *and the* N. crassa *transformant*

The genes of the *qa* cluster of *N. crassa* use different sites to initiate mRNA transcription (Tyler et al. 1984). One site is used under inducing conditions, i.e. in the presence of quinic acid, while another site is used under non-inducing conditions to produce a low basal level of *qa* gene transcription. To measure the effect of the sequence alterations 5′ to *qa-2* and *qa-x* at the location of the major start sites of transcription, transcriptional start sites were mapped by S1 analysis for the *qa-2* and *qa-x* genes in *N. africana* and in the *N. crassa* transformant containing the *N. africana* intergenic region between *qa-x* and *qa-2*, under inducing conditions (data not given) In *N. africana* the mRNA leader sequence of the *qa-2* gene was 52 bases longer than the leader sequence of *qa-2* in wild-type *N. crassa* (Fig. 3), although

sequences around both start sites were generally conserved. *N. africana* initiated transcription at a different site in the induced culture. The *N. crassa* transformant strain containing the *N. africana* intergenic region initiated transcription at the same site as in *N. africana*. The major start sites of the *qa-x* genes were also mapped. The start sites for *qa-x* transcription are identical in *N. crassa* wild type, *N. africana*, and the transformant containing the *N. africana* intergenic region. However, the *N. africana* mRNA leader sequence for *qa-x* is 24 bp longer than that of *N. crassa* due to two small sequence insertions which are not present in *N. crassa*.

Discussion

The *qa* gene cluster of *N. crassa* is highly conserved in the various species of *Neurospora* which we examined. Very few RFLPs exist in the *qa* gene clusters of the heterothallic species of *Neurospora* examined. However, there are many RFLPs that distinguish *N. crassa* from the homothallic species tested, although the gene organization of the cluster still seemed to be highly conserved. Evidence for organizational conservation came from hybridization studies with heterologous probes (probes from *N. crassa*) as well as from analysis at the DNA sequence level. Interestingly, three homothallic species showed few RFLPs; however, *N. terricola* differed from each of the other species tested.

Given the amount of conservation in the *qa* clusters of the various species examined, any differences in the mechanisms controlling the expression of the *qa* gene cluster might be due to specific sequence differences between the homothallic species and *N. crassa*. To analyze this further we sequenced the intergenic region between *qa-x* and *qa-2* in *N. africana*. The sequencing revealed that while the *N. africana* region between *qa-x* and *qa-2* was smaller than the same region in *N. crassa*, all four binding sites for the activator protein could be aligned with their *N. crassa* counterparts, although the binding sites, with the exception of the −502 site, would be expected to have less affinity for the *qa-1F* protein (Geever et al. 1989). This indicates that the *qa-x* and *qa-2* genes are probably induced by the *qa-1F* protein in *N. africana* using the same mechanism as in *N. crassa*.

Catabolic dehydroquinase assays showed that *qa-2* expression in *N. africana* was induced in the presence of quinic acid. However, *qa-2* expression did not seem to be repressed to the same extent in the presence of a preferred carbon source as is *qa-2* expression in *N. crassa*. To determine if this difference in repression was sequence specific and to examine whether the control circuits controlling *qa* expression in *N. crassa* operate in the presence of *N. africana* sequences located between *qa-x* and *qa-2*, the *qa-x-qa-2* intergenic region of *N. crassa* was replaced with its *N. africana* counterpart. *Qa-2* gene expression in the resulting transformant was inducible in the presence of quinic acid, indicating that the *N. crassa* activator could bind to at least some of the potential binding sites in *N. africana* sequences to activate transcription of the *qa-2* gene. However the level of catabolic dehydroquinase activity was lower in the transformant than in wild-type *N. crassa*, suggesting that this effect may be due to the weakening of the −120 binding site in *N. africana*. Catabolic repression of the *qa-2* gene was essentially as effective in the transformed strain as in wild-type *N. crassa*, indicating that sequence differences between *N. crassa* and *N. africana* in the *qa-x-qa-2* intergenic region had no impact on catabolite repression.

S1 mapping of the transcriptional start sites for both *qa-2* and *qa-x* showed that under inducing conditions the transformed strain utilized the same major inducible start sites of transcription as *N. africana*. *N. africana* uses the same location to initiate transcription of the *qa-x* gene as *N. crassa*; however a different start site for transcription is used for the *qa-2* gene. The location of the transcriptional start site for the *qa-2* gene in *N. africana* seems to be sequence specific. The amount of conservation in the actual *N. crassa* transcriptional start site suggests that the change in location is due to the alteration in the −120 binding site for the activator protein in *N. africana*. However, the possibility cannot be excluded that the changed spatial relationships of the other upstream binding sites in *N. africana* may affect the sites of *qa-2* transcription initiation.

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