THE ORGANIZATION OF THE *QA* GENE CLUSTER IN *NEUROSPORA CRASSA* AND ITS EXPRESSION IN *ESCHERICHIA COLI*

(eucaryotic gene regulation, quinic acid catabolic enzymes, recombinant DNA, pBR322 plasmid)

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SUMMARY

The qa gene cluster of Neurospora crassa comprises four genes on the right arm of linkage group VII. Three of these genes encode inducible enzymes which catalyze the first three reactions in the catabolism of quinic acid. All three enzymes have been purified and partial amino-terminal sequences determined for each one. The fourth gene (qa-1) encodes a regulatory protein. Our current working hypothesis (based on both biochemical and genetic evidence) states that genetic regulation in the system occurs at the level of transcription. The model proposes that the product of the qa-1 gene, a multimeric activator protein, contains two functionally distinct regions, one of which interacts with the inducer (quinic acid) and the other with initiator sites within the qa gene cluster. The genetically determined order of the four genes is qa-1, qa-3, qa-4, qa-2. Combined genetic and amino acid sequence data have established the direction of transcription of the qa-3 gene.

By the use of recombinant DNA techniques, expression of one of the qa genes has been achieved in E. coli. In these molecular cloning experiments, restriction endonuclease fragments of N. crassa DNA from a qa-1^{c} (constitutive) mutant and of plasmid pBR322 DNA were ligated in vitro and used to transform an aroD^{-} E. coli mutant which lacks biosynthetic dehydroquinase (5-dehydroquinate hydrolyase). Aro^{+} transformants have been shown to contain hybrid plasmids, to become Aro^{-} when cured of their plasmids, and to regain their Aro^{+} phenotype when retransformed. The dehydroquinase activity extracted from E. coli strains carrying the complementing recombinant plasmids has been shown to be identical to N. crassa catabolic dehydroquinase and completely different from the E. coli biosynthetic enzyme by a variety of criteria: heat stability, ammonium sulfate fractionation, immunological cross-reactivity, molecular weight, and chromatographic properties. Experiments employing RNA-DNA hybridization have demonstrated the presence of specific regions of transcription within

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the cloned DNA. Evidence has also been obtained that the orientation of insertion of the qa-2$^+$ gene into pBR322 has no effect on the level of expression. This result indicates that the E. coli RNA polymerase is able to recognize a promoter site on the inserted Neurospora DNA adjacent to the qa-2$^+$ gene. Furthermore, immunoprecipitation tests indicate that minicells carrying hybrid plasmids produce catabolic dehydroquinase. Taken together, these experiments demonstrate that transcriptional and translational starts and stops exist within the cloned Neusorpora DNA. Therefore, this DNA fragment should be ideally suited for a determination of the nucleotide. sequence of the controlling regions presumably recognized by the qa-1 activator protein and by the RNA polymerase in this eucaryote.

INTRODUCTION

Great progress has been made during the past decase in the elucidation of molecular mechanisms involved in genetic regulation in procaryotes. Comparable investigations with eucaryotes have only recently become feasible. Of particular importance are eucaryotic systems in which detailed genetic studies can be combined with detailed biochemical studies of both the DNA sequences corresponding to structural genes and their associated regulatory elements as well as the amino acid sequences of the final gene products.

The *qa* gene cluster of *Neurospora crassa* appears to provide an exceptionally favorable system for such combined studies. This cluster contains a regulatory gene which controls the expression of three tightly linked structural genes whose protein products (enzymes) have been purified and partially sequenced. In addition, at least part of the *qa* cluster has been cloned on a recombinant plasmid as demonstrated by the production in transformed *E. coli* strains of an enzyme which is biochemically and immunologically identical to one of the Neurospora *qa* enzymes. This cloned Neurospora DNA should provide information on the DNA sequences of controlling sites involved in regulating the synthesis of the three *qa* enzymes. The *qa* system should also be well-suited for the establishment of an *in vitro* system in which the mode of action of the regulatory protein can be directly tested.

This paper will summarize the current status of our studies on genetic regulatory mechanisms operating in the *qa* gene cluster. The first part of the discussion will deal with our interpretations of the organization and regulation of the *qa* cluster in *Neurospora* and the second part will discuss the present status of studies on cloning the *qa* genes in *E. coli*.

THE ORGANIZATION OF THE *QA* GENE CLUSTER IN *NEUROSPORA CRASSA*

The *qa* gene cluster of *Neurospora crassa* comprises four genes, three of which encode inducible enzymes catalyzing the first three reactions in the catabolism of quinic acid (FIGURE 1) (RINES, 1968; CHALEFF, 1974a). Mutants in each of these three genes are noninducible for only the specific enzyme encoded in that gene. These three enzymes are quinate (shikimate) dehydrogenase (quinate: NAD$^+$ oxidoreductase, EC 1.1.1.24) encoded in the *qa-3* gene, catabolic dehydroquinase (5-dehydroquinate hydro-

lyase, EC 4.2.1.10) encoded in the *qa* gene, and 3-dehydroshikimate dehydratase (dehydrase) encoded in the *qa-4* gene. The fourth gene encodes a regulatory protein which when combined with the inducer quinic acid becomes an activator and exerts positive control over the synthesis of the three *qa* enzymes (CASE and GILES, 1975). Genetic evidence for positive control comes from the fact that mutants in the *qa-1* gene are pleiotropic types, non-inducible for all three *qa* enzymes, and that constitutive mutations in the *qa-1* gene *(qa-1^{C})* (which do not require the inducer, quinic acid for synthesis of the *qa* enzymes) are partially dominant over wild type *(qa-1^{+})* in heterocaryons (VALONE, CASE, and GILES, 1971).

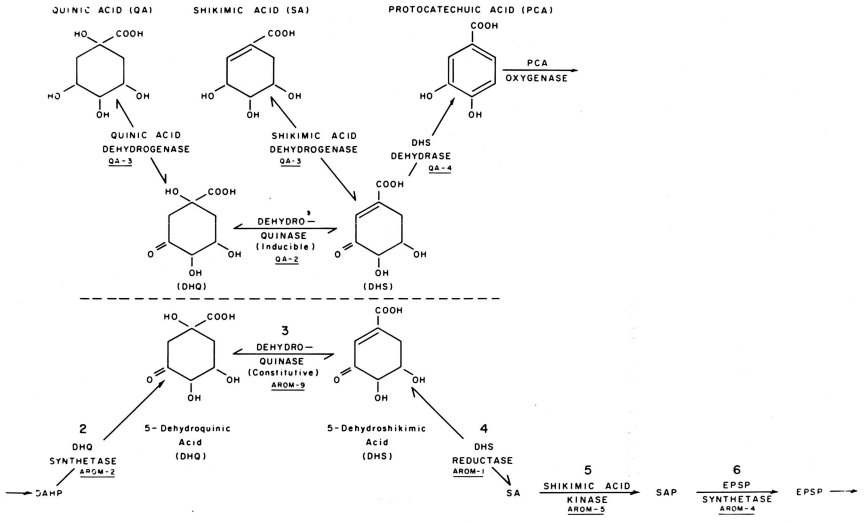

Figure 1. Diagram of gene-enzyme relationships in the inducible quinate-shikimate catabolic pathway (shown above the dotted line) in *Neurospora crassa*. Also indicated (below the dotted line) are the reactions in the constitutive common aromatic synthetic pathway catalyzed by enzymes encoded in the *arom* cluster-gene.

The *qa* gene cluster is located close to the centromere in linkage group VII and is apparently proximal to the closely linked *me-7* locus in the right arm. The order of the four genes has been determined by special genetic crosses to be *qa-1 qa-3 qa-4 qa-2* (CASE and GILES, 1976). The four genes are contiguous, apparently occupying a continuous segment of DNA which does not include other unrelated genes.

Recent biochemical evidence indicates that the coordinate induction of the *qa* enzymes, which is mediated by the activator protein in the presence of the inducer quinic acid (CHALEFF,

1974b), involves *de novo* protein synthesis. This evidence eliminates the alternative hypothesis that the *qa-1* protein simply activates preformed polypeptides derived from the three structural genes. This conclusion is based on data from experiments in which the enzymes catabolic dehydroquinase and quinic dehydrogenase are labeled with deuterium by inducing the cultures in deuterium oxide instead of water. These experiments demonstrated that, when induced in deuterium oxide, both enzymes band in cesium chloride at a higher density than when induced in water. The band position is independent of whether the cultures are grown in water or in deuterium oxide prior to induction. These results provide conclusive evidence that these two *qa* enzymes are synthesized *de novo* during induction (REINERT and GILES, 1977).

Preliminary biochemical evidence supports the view that expression of the *qa* cluster is regulated at the level of transcription rather than translation. This conclusion is based on the detection of mRNA for at least one of the *qa* enzymes, quinate dehydrogenase, in induced but not uninduced cultures (JACOBSON, *et al.*, unpublished). Whether some type of mRNA processing occurs involving, for example, the excision of one or more RNA segments complementary to DNA sequences not encoding amino acids in the final *qa* enzymes (GILBERT, 1978) cannot yet be determined. However, evidence that the gene for catabolic dehydroquinase is expressed with apparent complete fidelity when present on an *E. coli* recombinant plasmid (see below) would appear to make such processing unlikely, at least in the case of that particular mRNA.

Our current working hypothesis states that genetic regulation in this system functions at the level of transcription and that the product on the *qa-1* gene, a multimeric regulatory protein, contains two functionally distinct regions, detected initially by the kinds of complementation exhibited in heterocaryons by mutants mapping in these two non-overlapping regions (CASE and GILES, 1975). One group of recessive mutants (fast-complementing--$qa\text{-}1^F$) is presumed to alter the amino acid sequence of that portion of the regulatory protein which interacts with the inducer (quinic acid), while the other group of semi-dominant mutants (slow-complementing--$qa\text{-}1^S$) is presumed to alter the amino acid sequence of that portion of the regulatory protein which interacts with an initiator site (or sites) within the *qa* cluster. (In this discussion, initiator will be used as a general term to include DNA controlling sites involved in activator recognition and/or polymerase binding). The binding of the inducer to the regulatory protein presumably causes an allosteric change producing an activator protein capable of binding to the initiator site(s) and facilitating the initiation of transcription by a DNA-dependent RNA polymerase. In support of this model is the genetic evidence that $qa\text{-}1^C$ mutants, which do not require an inducer to synthesize high levels of all three *qa* enzymes, map within the $qa\text{-}1^S$ region which presumably encodes that portion of the amino acid sequence of the regulatory protein which binds to the initiator region (FIGURE 2).

Preliminary genetic evidence suggests that at least one

initiator site lies within the *qa* cluster, apparently located in the region of the *qa-4* gene. Certain mutants which map in this region are pleiotropic types which not only abolish activity for the *qa-4* enzyme, but also affect the activities of both the *qa-3* and *qa-2* enzymes (CASE and GILES, unpubl.). One possible model to explain the organization of the *qa* cluster on the basis of the evidence just discussed is shown in FIGURE 2. In this model, an internal initiator site(s) exists within the *qa* cluster from which transcription presumably proceeds in both directions. Combined genetic and biochemical data which support the view that transcription, at least for the *qa-3* gene, occurs from right to left in FIGURE 2 as orientated will now be discussed.

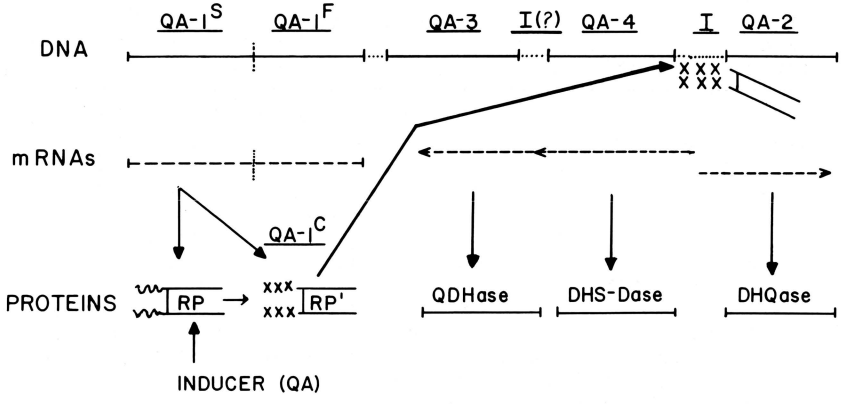

Figure 2. Possible model for the organization of the *qa* gene cluster in *N. crassa*. For further details see text.

Genetic analyses have localized 26 *qa-3* mutants at a minimum of 11 different sites within the *qa-3* gene. Certain of these mutants (e.g., M16 and M45) map at opposite ends of the *qa-3* gene. Data from crosses of homoalleles of these two mutants with one mutant (M124) in the adjacent *qa-1* gene indicate that *qa-3* mutant M45 is closer to *qa-1* mutant M124 than is *qa-3* mutant M16. These results indicate the following orientation of the *qa-3* gene within the *qa* cluster: *qa-1*, *qa-3* mutant M45 ("left" end), *qa-3* mutant M16 ("right" end), *qa-4*, *qa-2* (CASE and GILES, 1978). These genetic data, in conjunction with biochemical data on the amino-terminal amino acid sequences of quinate dehydrogenase from wild type and from revertants M16 and M45, have made it possible to determine the direction of transcription of the *qa-3* gene.

All three of the *qa* enzymes have now been purified and characterized. Catabolic dehydroquinase is a 220,000 molecular weight aggregate composed of 20 to 22 identical subunits of ap-

proximately 10,000 daltons each. (HAUTALA, JACOBSON, CASE, and GILES, 1975). Quinate dehydrogenase is a monomer of approximately 41,000 daltons (BAREA and GILES, 1978). Dehydroshikimate dehydratase is also a monomer with a molecular weight of approximately 37,000 daltons, (STRØMAN, REINERT, and GILES, 1978). The availability of purified material has made it possible to obtain a partial sequence, beginning at the amino-terminal end, for the wild type form of each of the three enzymes, (REINERT, STRØMAN, and GILES, unpublished). These sequences are shown in FIGURE 3.

In the case of quinate dehydrogenase, additional partial amino-terminal sequences have been obtained for two revertants, one induced in M45 and one induced in M16. As indicated previously, these two mutant sites are located at opposite ends of the *qa-3* genetic map (see FIGURE 3). The sequence for the first ten amino acids for R45 (from M45) is identical to that of wild type. However, in the sequence for R1 (from M16), an isoleucine is substituted for the proline of wild type at amino acid position three (FIGURE 3). The genetic code indicates that a change from a codon for proline to one for isoleucine requires two base pair changes. Thus, the evidence strongly favors the conclusion that the original M16 mutant resulted from a single base pair change in wild type, giving a mutant phenotype, and that this mutant codon (as yet undefined) mutated by a second base pair change such that the revertant (R1) produces a partially active quinate dehydrogenase not equivalent to wild type. The requirement for two base pair changes to explain the origin of M16-R1 appears to make highly unlikely an alternative possibility that this revertant represents a "second site" (suppressor) revertant not at the original M16 mutant site which results in partially restored enzyme activity. Thus these results permit the conclusion that M16 is near the amino-terminal end of the *qa-3* gene, whereas M45 is presumably near the carboxyl-terminal end. Consequently, it can be concluded that the direction of transcription of the *qa-3* gene is from right to left on the basis of the indicated orientation of its mutant sites within the *qa* cluster (FIGURE 3). As yet, no clear evidence has been obtained concerning the number of mRNAs produced by the three *qa* structural genes. Since polycistronic mRNAs probably do not occur in eucaryotes (BIGELIS, KEESEY, and FINK, 1977), it seems probable that there is an initiator site between the *qa-3* and *qa-4* gene as well.

THE EXPRESSION OF THE *QA* GENE CLUSTER IN *Escherichia coli*

The use of recombinant DNA techniques for cloning eucaryotic DNA into a procaryotic host appears to hold great promise in helping elucidate at the molecular level the organization of the eucaryotic genome and its mode of genetic regulation. Of particular importance are eucaryotic systems such as the *qa* gene cluster in which a single segment of DNA contains both structural and regulatory genes. Consequently, the successful cloning of at least one of the *qa* genes coupled with its functional expression in *E. coli* (VAPNEK, HAUTALA, JACOBSON, GILES, and KUSHNER, 1977) has important implications for a further understanding of genetic regulatory mechanisms in eucaryotes.

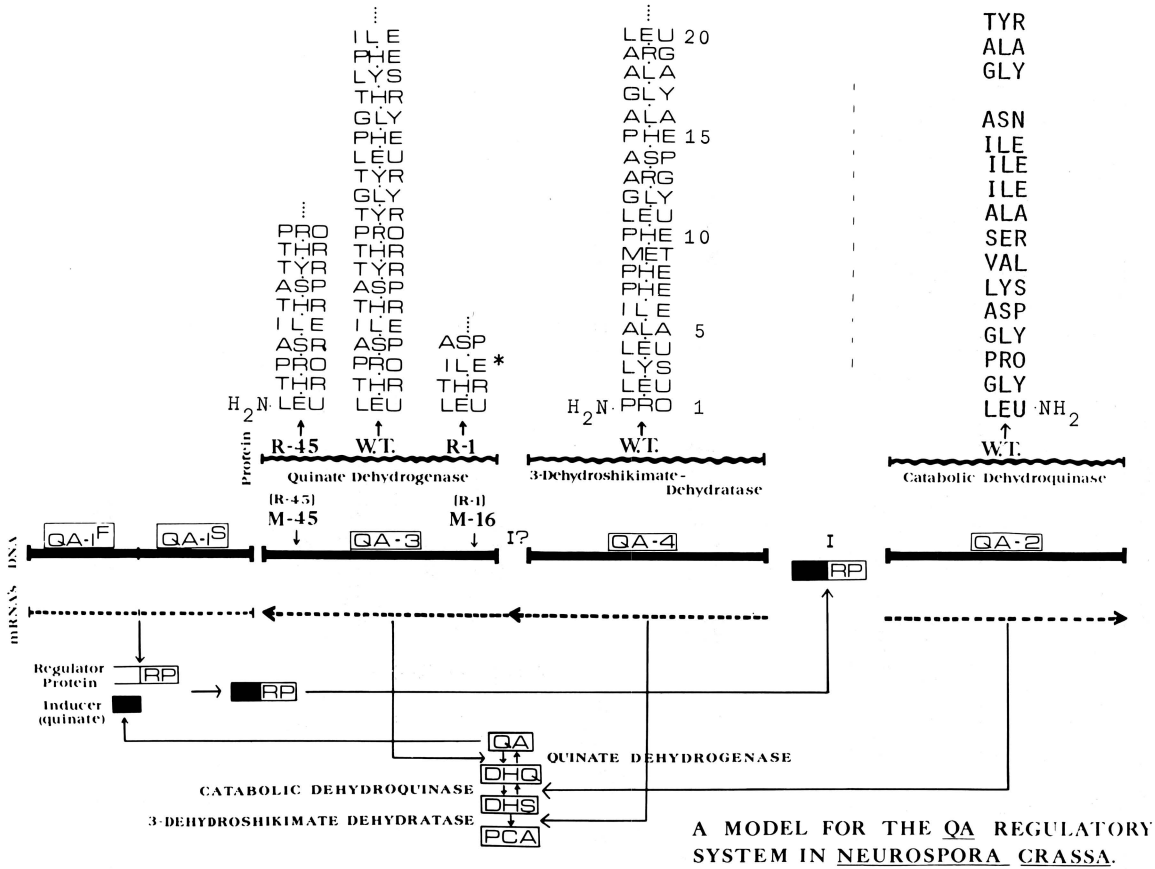

Figure 3. Wild type amino-terminal amino acid sequences for the three *qa* enzymes. Also shown are the amino-terminal sequences for revertants of two *qa*-3 mutants. For further details see text.

In Neurospora it has long been known that mutants *(arom-9)* defective for biosynthetic dehydroquinase can utilize catabolic dehydroquinase (the product of the *qa-2*$^{+}$ gene) to convert dehydroshikimic acid to dehydroquinic acid and thereby supply their aromatic amino acid requirements (FIGURE 1) (RINES, CASE, and GILES, 1968). The initial experiments which succeeded in isolating at least part of the *qa* cluster on an *E. coli* recombinant plasmid relied on the expectation that this same relationship would hold for *E. coli*, i.e., that Neurospora DNA carrying a *qa-2*$^{+}$ gene capable of producing active catabolic dehydroquinase when inserted into an *E. coli* plasmid should complement an *aroD*$^{-}$ *E. coli* mutant which lacks biosynthetic dehydroquinase (5-dehydroquinate hydrolyase) (FIGURE 4). (*E. coli* lacks all three of the *qa* enzymes and is unable to utilize quinic acid as a carbon source)

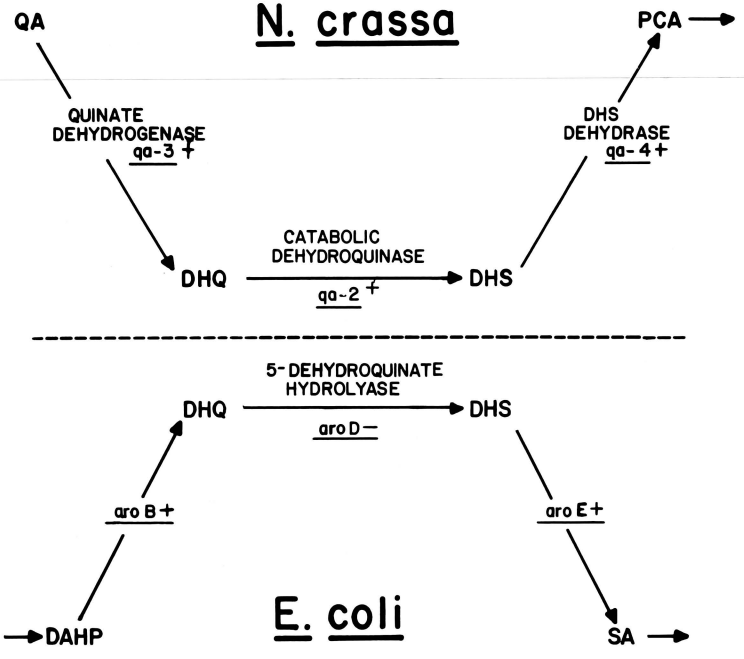

Figure 4. Relationship of the initial reactions of the inducible quinic acid catabolic pathway in *N. crassa* with early reactions in the constitutive common aromatic synthetic pathway in *E. coli*. Abbreviations as in Figure 1.

In the initial molecular cloning experiments (VAPNEK, *et al.*, 1977) restriction endonuclease fragments (Endo·R·*Hin*d III digestion) of *N. crassa* DNA from a *qa-1*^{C} (constitutive) mutant and of plasmid pBR322 DNA were ligated *in vitro* and used to transform an *aroD*$^{-}$ strain of *E. coli*. The first Aro^{+} transformant obtained was found to contain a hybrid plasmid (pVK55) which yielded three distinct DNA fragments when digested with Endo·R·*Hin*d III. One corresponded to pBR322 (2.6 x 10^{6} daltons) while the other two were calculated to be 1.9 x 10^{6} and 2.3 x 10^{6} daltons. To confirm that pVK55 carried the gene that resulted in complementation of the *E. coli aroD*$^{-}$ allele, the strain was cured of the plasmid and such cured isolates were shown to be AroD^{-}. When retransformed with plasmid pVK55, these isolates became AroD^{+} again.

Assays for dehydroquinase activity were performed on these (and related control) strains and in all cases the complementing AroD^{+} strains were found to have dehydroquinase activity, while those which retained the AroD^{-} phenotype lacked activity (Table 1). Additional experiments (VAPNEK *et al.*, unpubl.) have demonstrated that only plasmids carrying the 1.9 x 10^{6} dalton

Table 1. Levels of dehydroquinase (5-dehydroquinate hydrolyase) activities from various *E. coli* strains with hybrid plasmids.

Strain	Plasmid	Characteristics	Activity Units/G Cells
SK1516	pVK53	Does not Complement *aroD*$^{-}$	< 0.03
SK1518	pVK55	Complements *aroD*$^{-}$	0.44
SK1520	---	Cured *E. coli*	< 0.03
SK1529	pVK55	Retransformed (Complements)	0.48
SK1530	pVK56	1.9 x 10^{6}D. DNA Orientation A (Complements)	0.42
SK1555	pVK78	1.9 x 10^{6}D. DNA Orientation B (Complements)	0.36
SK1534	pVK60	2.3 x 10^{6}D. DNA (Does Not Complement)	< 0.03

fragment (i.e., pVK56) complement and yield activity in transformed strains. The 2.3 x 10^{6} dalton fragment (in pVK60) does not result in complementation nor activity (Table 1). Appropriate hybridization experiments (VAPNEK, *et al.*, unpubl.) have demonstrated that all the inserted DNA in the plasmid is from Neurospora. The 2.3 x 10^{6} dalton fragment has been shown not to be contiguous on Neurospora DNA to the 1.9 x 10^{6} dalton fragment. The original plasmid pVK55 apparently arose from the simultaneous insertion into pBR322 of two unrelated pieces of Neurospora DNA, only one of which represented a portion of the

qa cluster.

The dehydroquinase activity extracted from transformed AroD$^+$ *E. coli* strains has been shown to be identical to *N. crassa* catabolic dehydroquinase and completely different from the *E. coli* biosynthetic activity by a variety of criteria: heat stability, ammonium sulfate fractionation, immunological cross-reactivity, molecular weight, and chromatographic properties (VPANEK, *et al.*, 1977) (Table 2). This identity demonstrates

Table 2. Comparative characteristics of dehydroquinase (5-dehydroquinate hydrolyase) activities from *N. crassa* and various *E. coli* strains.

Strain	Plasmid	Percent Recovery of Activity			
		Heat[1] Shock	Ammonium Sulfate[2] Pellet	Ammonium Sulfate[2] Supernatant	Immuno-[3] Precipitation
E. coli SK1313	---	1.5	1.6	86	0
SK1518	pVK55	80	46	4	96
SK1529	pVK55	100	58	0	94
SK1530	pVK56	90	62	14	86
SK1555	pVK78	82	64	7	94
N. crassa M16[4]	---	99	78	3	94

[1]71°, 10 min; [2]50% saturation; [3]antibody prepared against *N. crassa* catabolic dehydroquinase; [4]induced on quinic acid.

that the Neurospora *qa-2$^+$* gene is carried by the recombinant plasmids and is transcribed and translated with apparent fidelity. Furthermore, assembly of the 20 subunit multimeric structure of native Neurospora catabolic dehydroquinase must also occur in *E. coli*. By appropriate genetic experiments, the recombinant plasmid pVK56 has been introduced into an *E. coli* strain carrying an *aroD$^+$* gene on its chromosome (KUSHNER, *et al.*, unpubl.). Tests of this strain demonstrate that it is producing both the Neurospora catabolic and the *E. coli* biosynthetic dehydroquinases since the two different dehydroquinases differ markedly in their molecular weights and in their heat stabilities (HAUTALA, *et al.*, unpubl.) (FIGURE 5).

Additional cloning experiments (VAPNEK, *et al.*, unpubl.) employing Endo·R·*Pst* 1 digestion have produced a much larger fragment (4.5 x 10^6 daltons) carrying the *qa-2$^+$* gene (pVK88). Restriction mapping has demonstrated that the 1.9 x 10^6 dalton fragment is totally contained within the *Pst* 1 fragment (FIGURE 6). When the orientation of the 1.9 x 10^6 dalton fragment within pBR322 was reversed by cleaving plasmid pVK57 (a resiolate of pVK56) with Endo·R·*Hind* III followed by religation and transfor-

formation of *E. coli*, no effect was observed on either the ability of the hybrid to complement an *aroD*⁻ auxotroph or on the level of dehydroquinase activity in transformed strains (Table 1). The same result has also recently been shown for pVK88.

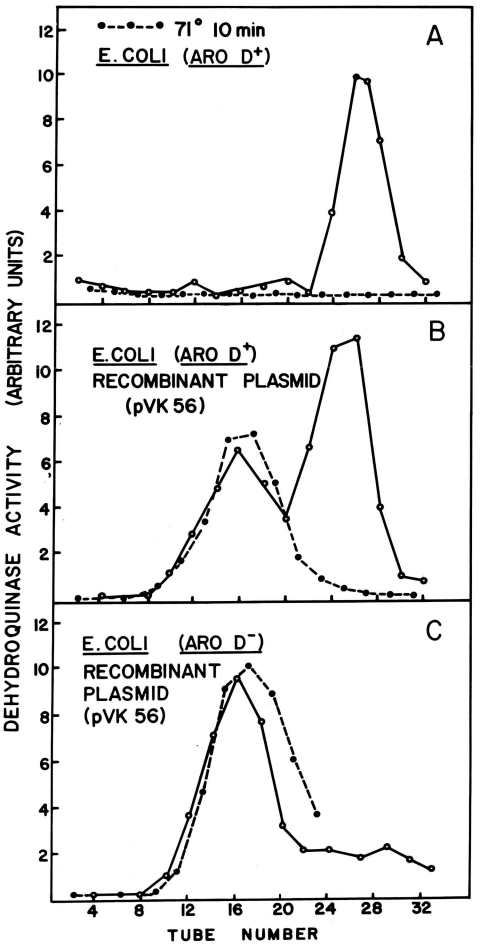

Figure 5. Sucrose density gradient centrifugation of dehydroquinase (5-dehydroquinate hydrolyase) activity. Panels A, B, and C indicate results with three different *E. coli* strains of the designated genotypes. The thermolability of the activity is indicated.

The regions of the cloned Neurospora DNA in pVK88 which are transcribed were determined by the techniques developed by SOUTHERN (1975). The results demonstrate the presence of specific regions of transcription within the cloned DNA. Employing RNA-DNA filter hybridization, it has been shown that about 60% of the transcription from the 4.5 x 10^6 daltons of Neurosport DNA is localized in the 1.9 x 10^6 *Hin*d III fragment carrying the dehydroquinase gene. (VAPNEK, *et al.*, unpubl.).

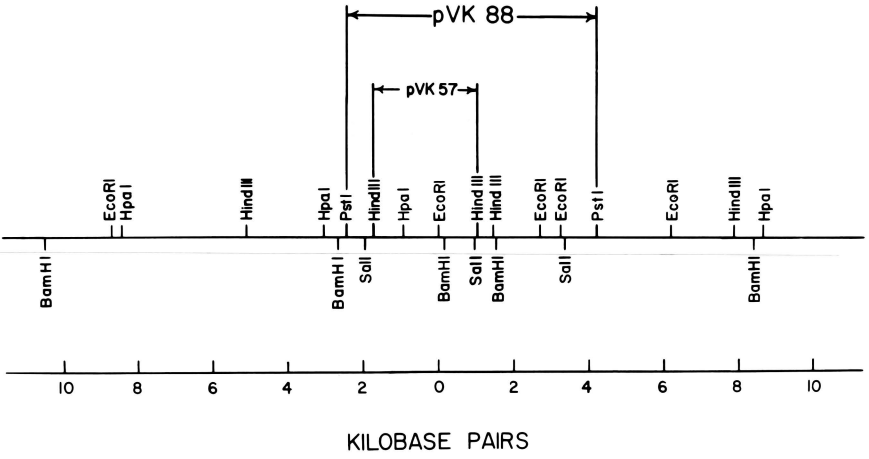

Figure 6. Restriction endonuclease map of *N. crassa* DNA in the *qa* gene cluster region. Neurospora catabolic dehydroquinase is expressed in *E. coli* strains carrying plasmids pVK57 (*Hin*d III) and pVK88 *(Pst)*. For further details see text.

In additional experiments, (VAPNEK, *et al.*, unpubl.), the hybrid plasmids carrying Neurospora DNA in both orientations were segregated into minicells. After labeling these minicells with ^{35}S-methionine, a limited number of specific polypeptides was identified. Immunoprecipitation tests indicate that one of the products in the minicells is Neurospora catabolic dehydroquinase. Taken together, these experiments demonstrate that transcriptional and translational starts and stops exist within the cloned Neurospora DNA. Apparently *E. coli* RNA polymerase is able to recognize a promoter site on the inserted Neurospora DNA adjacent to the *qa-2*$^+$ gene.

To date, all tests have failed to detect activity for either of the other two *qa* enzymes in strains of *E. coli* transformed with plasmids carrying an active *qa-2*$^+$ gene. Experiments in

which the two Neurospora enzymes were added to *E. coli* extracts suggest that the activities are not destroyed by *E. coli* proteases, at least *in vitro* (HAUTALA, *et al.*, unpubl.). At present there is no evidence to indicate whether either the *qa-3* or the *qa-4* gene is actually contained in any of the recombinant plasmids. The known molecular weights for the monomers of the three *qa* enzymes make it possible to calculate that these three genes could be present in a DNA fragment of ca. 1.8×10^6 daltons, assuming no spacer DNA for control regions. Thus the 1.9×10^6 dalton fragment is large enough to include these three *qa* genes plus some additional DNA for controlling regions such as initiators. However, it should be recalled that the *qa-2* gene is at one end of the *qa* cluster and its position within the 1.9×10^6 dalton fragment has not been precisely defined. It appears much more probable that one or both of the two other *qa* genes which encode enzymes (*qa-3* and *qa-4*) may be present on the 4.5×10^6 dalton fragment (pVK88). Indeed, this fragment should be more than large enough to carry the entire *qa* cluster, including the *qa-1* gene, on the assumption that the cluster comprises a continuous segment of DNA without exceptionally long spacer regions and that the activator protein has a reasonable molecular weight (e.g., 35,000 daltons). Again, however, the $qa\text{-}2^+$ gene could be quite near one end of pVK88 (see FIGURE 6) and therefore this plasmid might also contain only one complete *qa* gene.

It is, of course, possible that the other *qa* genes, even if present, may not be able to produce active protein products in *E. coli*. Recombinant DNA experiments with yeast suggest that perhaps no more than 25% of yeast genes encoding enzymes also known to be present in *E. coli* can be detected as expressed on the basis of transformation-complementation tests (RATZKIN & CARBON, 1977). Lack of expression in *E. coli* could involve problems at any one of a number of levels and additional experiments will be required to clarify the situation.

Another intriguing question is whether the synthesis of Neurospora catabolic dehydroquinase is regulated in *E. coli*. The Neurospora DNA employed in experiments to date was extracted from a $qa\text{-}1^C$ (constitutive) mutant which does not require the inducer quinic acid for the synthesis of high levels of the three *qa* enzymes in Neurospora. However, as already indicated, it appears unlikely that the *qa-1* gene can be contained within the original 1.9×10^6 dalton fragment (pVK56). Furthermore, assay data indicate that *E. coli* isolates carrying this plasmid in either orientation produce equivalent amounts of catabolic dehydroquinase (Table 1). Taken together, these results suggest that the *E. coli* RNA polymerase recognizes an initiator site on the inserted Neurospora DNA and that the Neurospora activator protein is probably not required for significant synthesis of catabolic dehydroquinase in *E. coli*. Additional transformation experiments currently in progress utilizing both wild type ($qa\text{-}1^+$) and *qa-1* mutant DNAs should help clarify this matter.

The ultimate aim of these studies is to elucidate, at the molecular level, the manner in which the synthesis of the three *qa* enzymes is regulated. Of great importance in this regard

would be a knowledge of the nucleotide sequences of the controlling regions presumably recognized by the activator protein and by the Neurospora RNA polymerase. Ultimately, the determination of the nucleotide sequence of Neurospora *qa* DNA inserted into the *E. coli* plasmid, combined with amino acid sequence data for the *qa* enzymes (FIGURE 3), should make it possible to determine the limits of the structural genes and to identify the presence and organization of controlling regions in the *qa* gene cluster.

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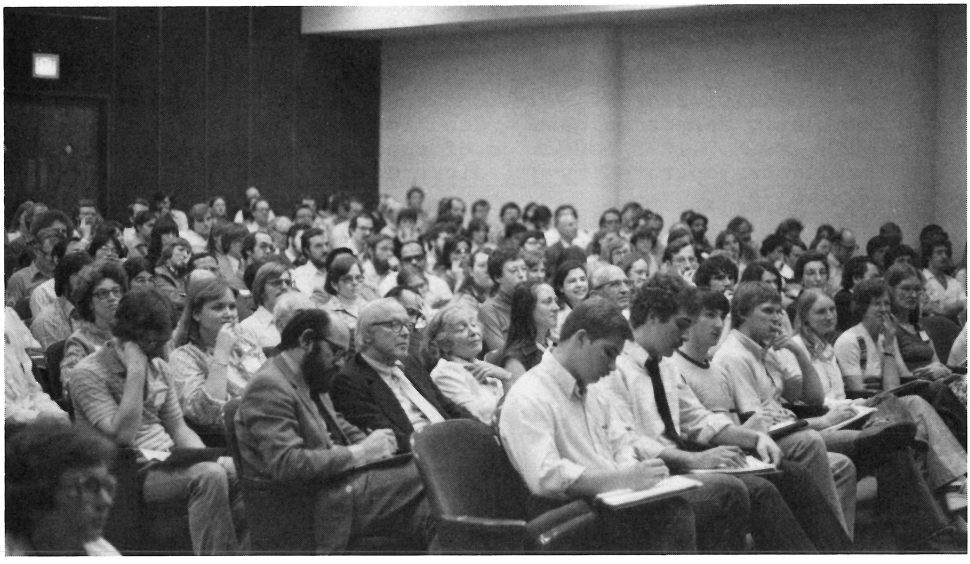

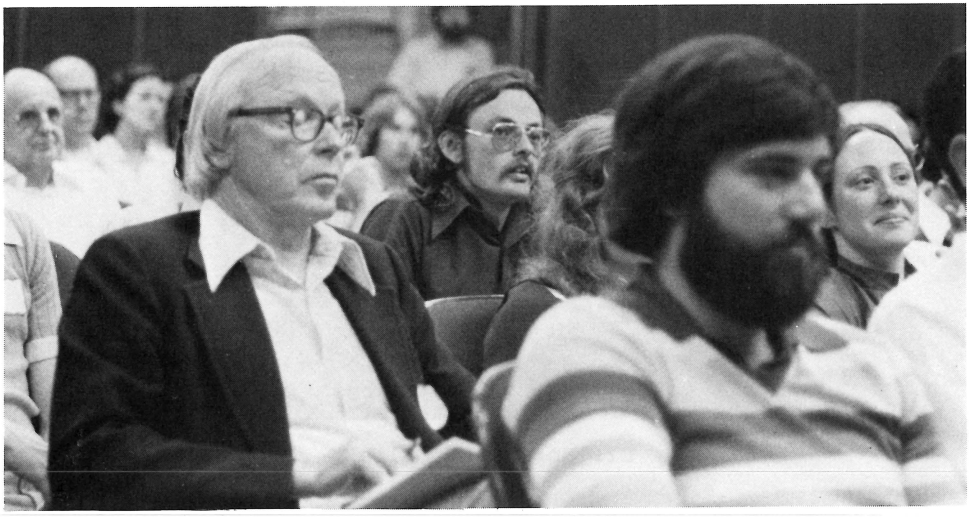

Dr. Norman H. Giles at the Symposium