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Genetics

Expression in *Escherichia coli* K-12 of the structural gene for catabolic dehydroquinase of *Neurospora crassa*

(recombinant DNA/pBR322 plasmid/eukaryotic gene regulation/*qa* cluster)

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ABSTRACT **The inducible quinic acid catabolic pathway of *Neurospora crassa* is controlled by four genes, the *qa* cluster which includes structural genes *qa-2, qa-3, qa-4* for three enzymes and a regulatory gene, *qa-1*. In this paper we report the molecular cloning of at least the *qa-2* gene which encodes the catabolic dehydroquinase (5-dehydroquinate hydro-lyase, EC 4.2.1.10). Endo·R·*Hin*dIII restriction endonuclease fragments of *N. crassa* DNA from a *qa-1*^{C} (constitutive) mutant and of *Escherichia coli* plasmid pBR322 DNA were ligated *in vitro* and used to transform an *aroD6* (5-dehydroquinate hydrolyase deficient) strain of *E. coli* K12. The recombinant plasmid (pVK55) isolated from one AroD^{+} transformant (SK1518) contained, in addition to pBR322, two *N. crassa Hin*dIII fragments with molecular weights of 2.3×10^{6} and 1.9×10^{6}. Derivatives of SK1518 cured of plasmid DNA were phenotypically Amp^{S} and AroD^{-}. These cured strains, retransformed with pVK55, were phenotypically Amp^{R} and AroD^{+}. Strains transformed with pVK55 possessed 5-dehydroquinate hydrolyase activity but no activity was present in any AroD^{-} strain. The enzyme extracted from strains containing the recombinant plasmid was identical to *N. crassa* catabolic dehydroquinase by the criteria of heat stability, ammonium sulfate fractionation, immunological crossreactivity, molecular weight, and purification characteristics. This identity demonstrates that the *N. crassa qa-2*$^{+}$ gene is carried by the recombinant plasmid and is apparently transcribed and translated with complete fidelity. Furthermore, subunit assembly of the *N. crassa* polypeptides also occurs in *E. coli*, because the catabolic dehydroquinase is a multimer composed of approximately 20 identical subunits.**

Recent progress in the elucidation of molecular mechanisms involved in genetic regulation in prokaryotes has depended on the isolation of DNA sequences carrying both structural and regulatory genes. Purification of these DNA sequences has led to the establishment of *in vitro* systems in which the mode of action of regulatory proteins could be directly tested (1) and, in addition, has permitted the sequencing of promotor and operator regions (2–4). By using molecular cloning techniques, it should now be possible to isolate similar DNA sequences from eukaryotes. Of particular significance is the recent demonstration by Struhl *et al.* (5) and Ratzkin and Carbon (6) that either λ or plasmid hybrids containing specific yeast genes can be selected by their ability to complement auxotrophic mutants of *Escherichia coli*. Cloned DNA sequences obtained by this method will be useful for the study of eukaryotic gene regulation only if they contain both structural and regulatory genes.

One such DNA sequence is the *qa* gene cluster of *Neurospora crassa* which controls the first three reactions in the inducible quinic acid catabolic pathway (Fig. 1A) (7, 8). Three of the loci are the structural genes for the individual *qa* enzymes: *qa-2*, catabolic dehydroquinase (5-dehydroquinate hydro-lyase, EC 4.2.1.10); *qa-3*, quinate dehydrogenase (quinate:NAD^{+} oxidoreductase, EC 1.1.1.24); and *qa-4*, dehydroshikimate dehydrase. The fourth gene, *qa-1*, encodes a regulatory protein which in conjunction with the inducer, quinic acid, controls the expression of the three structural genes. The catabolic dehydroquinase enzyme of *N. crassa* has been well characterized (9) and is capable of complementing aromatic amino acid auxotrophs deficient in the biosynthetic dehydroquinase isozyme (10). This combination of structural genes under the control of a tightly linked regulatory locus and a well-defined enzymatic activity (catabolic dehydroquinase) which should complement an *aroD*$^{-}$ mutant of *E. coli* (see Fig. 1) makes the *qa* gene cluster an ideal DNA sequence for cloning on a recombinant plasmid.

In this communication we describe the isolation and preliminary characterization of a pBR322–*N. crassa* DNA recombinant plasmid that complements an *aroD6* (5-dehydroquinate hydrolyase deficient) strain of *E. coli*. The transformed strains contain a 5-dehydroquinate hydrolyase activity which has been shown to be biochemically and immunologically identical to the *N. crassa* catabolic dehydroquinase.

MATERIALS AND METHODS

Materials. Reagents were obtained from the following sources: CsCl (high purity), Penn Rare Metals; Sarkosyl NL-97, Geigy Chemical Co.; lysozyme, Worthington Biochemical Corp.; "ultra pure" $(NH_4)_2SO_4$ and enzyme grade sucrose, Schwarz/Mann; agarose, Seakem Laboratories; ethidium bromide, Calbiochem; bovine serum albumin, Miles Research Laboratories; goat anti-rabbit antiserum, Grand Island Biological Co.; chloramphenicol, tetracycline, streptomycin sulfate, and RNase IIA, Sigma Chemical Co.; ampicillin (Omnipen), Wyeth Pharmaceutical Co. Spectinomycin sulfate was the generous gift of the Upjohn Co. T4 DNA ligase was purified by the method of Weiss *et al.* (11). Endo·R·*Hin*dIII was obtained from New England Biolabs, Inc. Endo·R·*Eco*RI was purified by the method of Green *et al.* (12). One unit of either enzyme is defined as that amount required to completely digest 1 μg of λ DNA in 1 hr at 37°. All other chemicals were reagent grade. Bacteriophage λ DNA was graciously supplied by R. Mural.

Bacterial Strains and Media. The relevant genotypes and origins of the bacterial strains are listed in Table 1. Bacterial nomenclature conforms to the suggestions of Demerec *et al.* (13) and Bachmann *et al.* (14).

The procedure and media for conjugational crosses were those of Willetts *et al.* (15). Specific genotypic characteristics were determined by using the replica plating techniques of Clark and Margulies (16). The presence or absence of the *hsdR4*

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Table 1. Bacterial strains

Strain	Sex	*proA*	*argE*	*his*	*aroD*	*rpsE*	*rpsL*	*hsdR*	*hsdM*	Other	Source*
AB1360	F⁻	2	3	4	6	+	+	+	+		B. Bachmann
C600	F⁻	+	+	+	+	+	+	+	+	*thr, leu,* pBR322	D. Vapnek
HB94	Hfr	+	+	+	+	+	+	4	+		B. Bachmann
KL208	Hfr	+	+	+	+	+	+	+	+	Origin, 30 min	K. B. Low
KL226	Hfr	+	+	+	+	+	+	+	+	Origin, 15 min	K. B. Low
SK274	F⁻	2	3	4	6	−	+	+	+		A
SK1478	F⁻	+	3	4	6	−	+	4	+		B
SK1485†	F⁻	+	3	4	6	−	−	4	+		C
SK1516†	F⁻	+	3	4	6	−	−	4	+	pVK53	This paper
SK1518	F⁻	+	3	4	6	−	−	4	+	pVK55	This paper
SK1520	F⁻	+	3	4	6	−	−	4	+		This paper
SK1524†	F⁻	+	3	4	6	−	−	4	+	pVK55	This paper
SK1529	F⁻	+	3	4	6	−	−	4	+	pVK55	This paper

* A = Spc^R mutant of AB1360 obtained with ethyl methanesulfonate; B = SK274 × HB94 Pro$^+$ conjugant; C = Str^R mutant of SK1478 obtained with ethyl methanesulfonate.

† These strains contain an unknown mutation that prevents high levels of complementation of the *aroD6* allele in the presence of pVK55. This mutation has reverted or been suppressed in SK1518 and its derivatives.

allele was tested for by the ability to plate P*vir*·K or P*vir*·B. Cells were grown in Luria (L) broth (17) or K medium (18). Minimal medium consisted of M56/2 buffer (15) supplemented with glucose (0.5%), appropriate amino acids (50 μg/ml), thiamin (0.001%), and ampicillin (20 μg/ml). AroD$^+$ transformants were selected by the ability of colonies to grow on minimal agar plates lacking aromatic amino acids.

Purification of Plasmid and *N. crassa* DNA. Plasmid pBR322 DNA was obtained from *E. coli* C600 (pBR322) grown in L broth (plus ampicillin, 20 μg/ml) by the method of Vapnek *et al.* (19). *N. crassa* DNA was isolated from a *qa-1^C* (constitutive) mutant, strain M105-R12-1.5 (20), by the method of Hautala *et al.* (21). The DNA was further purified by digestion with preheated RNase followed by Sepharose 4B chromatography in 10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.2% Sarkosyl.

Construction of Recombinant pBR322 Plasmids. *N. crassa* DNA and pBR322 DNA were simultaneously digested to completion with Endo·R·*Hin*dIII. The reaction mixture (340 μl) containing 32 μg of *N. crassa* DNA, 10 μg of pBR322 DNA, 6 mM Tris·HCl, (pH 7.5), 50 mM NaCl, 6 mM $MgCl_2$, 40 μg of bovine serum albumin, and 100 units of Endo·R·*Hin*dIII was incubated for 75 min at 37°. After heating at 65° for 10 min to inactivate the enzyme, the mixture was further incubated for 5 min at 37° prior to addition of phage T4 ligase. Ligation was performed in a final volume of 400 μl by the method of Tanaka and Weisblum (22), followed by dialysis for 4 hr against 1 liter of 10 mM Tris·HCl, pH 7.0/1 mM EDTA.

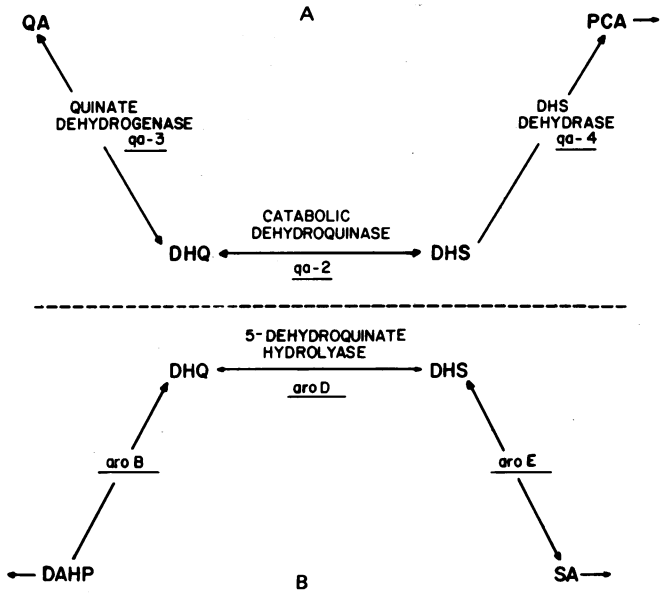

FIG. 1. Relationship of the initial reactions of the inducible quinic acid catabolic pathway in *N. crassa* (*A*) with the initial reactions of the aromatic amino acid biosynthetic pathway in *E. coli* (*B*). Abbreviations: QA, quinic acid; PCA, protocatechuic acid; DHQ, dehydroquinic acid; DHS, dehydroshikimic acid; DAHP, 3-deoxy-D-arabinoheptolusonic acid 7-phosphate; SA, shikimic acid.

Bacterial Transformation. Recipient strains were transformed as described (19) except that DNA uptake was allowed to take place during a 1-min incubation at 43.5°. After growth at 37° in L broth for 18 hr, the cells were concentrated by centrifugation such that approximately 2 × 10^8 cells were plated on each minimal agar selective plate (plus ampicillin, 20 μg/ml). Some cells were plated on L agar plates containing ampicillin (20 μg/ml). The transformation proficiency of the initial experiment was approximately 1 × 10^4 transformants per μg of DNA.

Transformation of recipient strains with purified recombinant plasmid DNAs was carried out by the same technique except that 0.5 μg of DNA was used per 10^8 cells and overnight expression was omitted. In addition, the cells were washed twice with M56/2 buffer prior to plating on minimal selective agar plates.

Purification and Analysis of Recombinant Plasmid DNAs. Recombinant plasmid DNAs were purified as described by Vapnek *et al.* (19). Plasmid DNAs were digested with Endo·R·*Hin*dIII and electrophoresed on 0.8% agarose gels as outlined (19).

Biochemical Procedures. The assay procedure for 5-dehydroquinate hydrolyase has been reported (23). One unit of activity represents 1 nmol of dehydroshikimate produced per min at 37°.

For enzyme assays, 500-ml cultures were grown in L broth (plus ampicillin, 20 μg/ml) to 2.5 × 10^8 cells per ml, washed with 50 mM Tris·HCl, pH 7.5/10% sucrose, and stored at −70°. The cells were lysed according to the procedure of Wickner *et al.* (24) with the following modifications. The frozen cell pellet was resuspended in 50 mM Tris·HCl, pH 7.5/10% sucrose (0.75 g wet weight of cells per 10 ml of buffer), frozen in a −50° dry ice/ethanol bath, and thawed at 20°. To each 10 ml of cell suspension were added 1.25 ml of 0.1 M EDTA, 0.25 ml of 4 M

NaCl, and 1.0 ml of lysozyme (2.5 mg/ml in 0.25 M Tris·HCl, pH 7.5). After 30 min at 0° and 20 min at 37°, the suspension was centrifuged at 100,000 × *g* for 60 min, and the clear supernatant was made 0.4 mM in dithiotreitol and 0.1 mM in phenylmethylsulfonyl fluoride.

Thermal stability studies were performed by heating the crude supernatants at 71° for 10 min. The 0–50% ammonium sulfate fractions were obtained from the crude, unheated supernatants. The precipitates were dissolved in buffer A (10 mM potassium phosphate, pH 7.5/0.4 mM dithiothreitol/0.1 mM EDTA/0.1mM phenylmethylsulfonyl fluoride and dialyzed overnight against the same buffer prior to assay. Double-immunoprecipitation experiments were performed using the dialyzed ammonium sulfate fractions or, in the case of SK1313, the crude supernatant was dialyzed against buffer A. The samples were treated with the gamma globulin fraction of rabbit antiserum prepared against pure catabolic dehydroquinase from *N. crassa* (25) followed by treatment with goat anti-rabbit antiserum (26).

The 5-dehydroquinate hydrolyase activity from the transformed strain SK1518 was purified according to the procedure reported (9) for the isolation of catabolic dehydroquinase from *N. crassa* (9) except that the RNase/DNase treatment was omitted and the activity was precipitated with 50% ammonium sulfate.

Protein concentrations were determined by the microbiuret (27) and Lowry (28) techniques. Analytical polyacrylamide disc gel electrophoresis was performed according to the method of Davis (29). Sucrose density gradient centrifugations were performed according to the method of Martin and Ames (30) at 37,000 rpm and 4° for 18 hr in an SW 65 rotor.

Containment. The experiments were carried out under P2/EK1 conditions as specified by the "National Institutes of Health Guidelines for Recombinant DNA Research."

RESULTS

Construction of Hybrid Plasmids and Bacterial Transformation. pBR322 is a ColE1 related plasmid (molecular weight 2.6×10^6) that carries resistance to ampicillin (Amp^R) and tetracycline (Tc^R) and contains a single Endo·R·*Hin*dIII cleavage site (31). Because Endo·R·*Hin*dIII cleaves within the promotor for the Tc^R gene, introduction of foreign DNA into this site results in sensitivity to tetracycline and facilitates the identification of hybrid plasmids (31).

The mixture of *N. crassa* nuclear DNA and plasmid pBR322 DNA prepared as described in *Materials and Methods* was used to transform an *aroD6* (5-dehydroquinate hydrolyase deficient), *hsdR4* (restriction deficient) strain of *E. coli* K12 (SK1485). Apparent complementation of the *aroD6* allele was detected by plating transformed cells on minimal agar plates lacking aromatic amino acids. Presumptive transformants that appeared were tested for the other chromosomal markers carried by SK1485 as well as for the phenotypic properties expected of strains carrying a pBR322 recombinant plasmid (Amp^R, Tc^S).

Approximately 2×10^4 colonies appeared, and 2000 were tested. Of these, one had the properties expected of a strain carrying a pBR322 recombinant plasmid that could complement the chromosomal *aroD6* mutation. Although this isolate initially grew slowly on minimal agar medium, upon purification one faster growing colony appeared. This colony (SK1518) was further purified for analysis.

Electrophoretic Analysis of Recombinant Plasmid DNA. Plasmid DNA was prepared from SK1518 and from a strain carrying a putative hybrid plasmid that did not complement the *aroD6* mutation (SK1516). The purified DNAs, along with pBR322 DNA, were analyzed by agarose slab gel electrophoresis after Endo·R·*Hin*dIII digestion. λ DNA treated with Endo·R·*Eco*RI was included as a molecular weight standard (Fig. 2, lane A). Treatment of pBR322 with Endo·R·*Hin*dIII yielded a single DNA band of 2.6×10^6 daltons (Fig. 2, lane B). When the plasmid obtained from SK1518 (pVK55) was digested with Endo·R·*Hin*dIII, three distinct fragments appeared (Fig. 2, lane D). One of these corresponded to pBR322, while the other two were calculated to be 1.9×10^6 and 2.3×10^6 daltons, based on the λ molecular weight standard. Cleavage of the plasmid from SK1516 (pVK53) with Endo·R·*Hin*dIII produced two DNA bands, one corresponding to pBR322 and a small DNA fragment of about 3×10^5 daltons (Fig. 2, lane E).

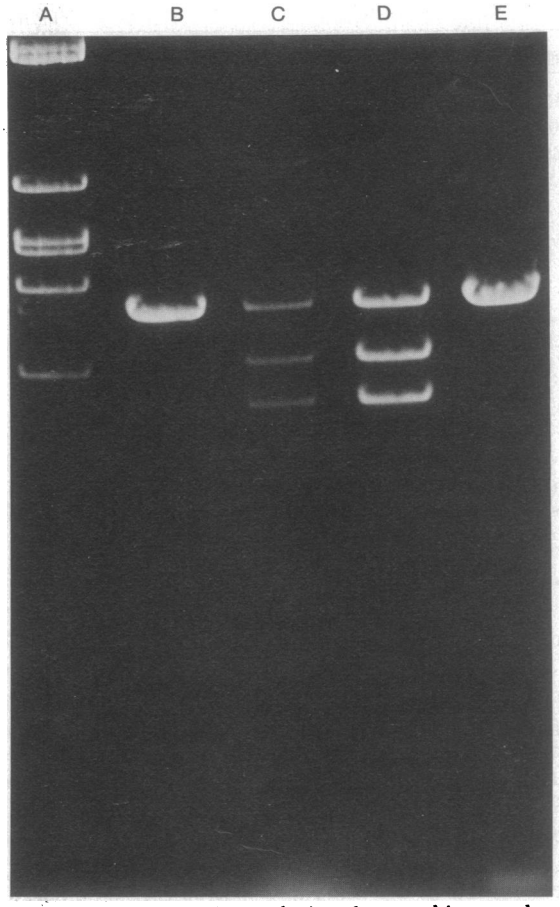

FIG. 2. Electrophoretic analysis of recombinant plasmids. Plasmid DNAs were isolated and digested with Endo·R·*Hin*dIII (B-E) as described in *Materials and Methods*. Lanes: A, λ DNA digested with Endo·R·*Eco*R1; B, pBR322; C, pVK55 from SK1524; D, pVK55 from SK1518; E, pVK53.

Properties of pVK55. To confirm that pVK55 carried the gene that resulted in complementation of the *E. coli aroD6* allele, SK1518 was cured of the plasmid by overnight growth at 44° in the presence of acridine orange (50 μg/ml). The cells were subsequently plated on L agar plates and tested for the appropriate phenotypic properties. Of 81 surviving colonies tested, 4 were sensitive to ampicillin and unable to grow in the absence of aromatic amino acids.

One of these cured derivatives (SK1520) and the original *aroD6* strain (SK1485) were used as recipients for transformation with pVK55 plasmid DNA. All Amp^R transformants of SK1485 analyzed ($n = 200$) grew extremely poorly in the absence of added aromatic amino acids (750-min doubling time), whereas the Amp^R transformants of SK1520 grew as well

Table 2. Levels of 5-dehydroquinate hydrolyase activity from *N. crassa* and various *E. coli* derivatives

Strain	Genotype	Plasmid	Total activity,* units	Total protein, mg	Specific activity, units/mg
SK1313	*aroD*+	—	1.86	44.2	0.042
SK1516	*aroD6*	pVK53	<0.03	44.7	<0.0007
SK1518	*aroD6*	pVK55	0.44	35.0	0.013
SK1520	*aroD6*	—	<0.03	52.0	<0.0006
SK1524	*aroD6*	pVK55	0.42	53.1	0.008
SK1529	*aroD6*	pVK55	0.48	47.9	0.010
M16†	*qa-2*+	—	2.4	76.5	0.031

* Determined as described in *Materials and Methods.*
† Strain M16 of *N. crassa* (32), induced with quinic acid.

Table 3. Comparison of 5-dehydroquinate hydrolyase activity from *N. crassa* and various *E. coli* strains

Strain	Plasmid	% recovery of activity: Heat shock	Ammonium sulfate* Pellet	Ammonium sulfate* Supernatant	Immunoprecipitate
SK1313	—	1.5	1.6	86	0
SK1518	pVK55	80	46	4	96
SK1524	pVK55	87	60	9	96
SK1529	pVK55	100	58	0	94
M16†	—	99	78	3	94

* 50% saturation; see *Materials and Methods.*
† Strain M16 of *N. crassa* (32), induced with quinic acid.

on minimal medium as SK1518 (136-min doubling time). In contrast, an *aroD*+ strain (SK1313) had a doubling time of 66 min, whereas the *aroD6* mutant (SK1485) showed no detectable growth without the addition of aromatic amino acids.

When the plasmids isolated from retransformants of SK1485 (SK1524) and SK1520 (SK1529) were treated with Endo·R·*Hin*dIII and examined by gel electrophoresis, they were found to be identical to pVK55. The electrophoretic pattern of one of these, the plasmid from SK1524, is shown in Fig. 2, lane C.

These results suggested that a plasmid-carried activity was responsible for the complementation of the *aroD6* allele but that the extent of complementation depended on the presence or absence of an additional chromosomal mutation. In order to confirm this hypothesis, both SK1518 and SK1524 were mated with either KL208 or KL226 donor Hfr strains. Among the Arg^+, Str^R recombinants obtained in a cross of SK1524 and KL208, greater than 90% showed high levels of complementation, whereas all the conjugants obtained with KL226 retained their poor growth characteristics in the absence of aromatic amino acids. No alteration in the level of complementation was observed when SK1518 was used as a recipient in similar crosses. These results suggested that SK1485 carried a second mutation that prevented a high level of complementation of the *aroD6* mutation by the pVK55 recombinant plasmid.

Characterization of Dehydroquinate Hydrolyase Activity. Several *E. coli* strains transformed with either pVK53 or pVK55 were lysed and assayed for 5-dehydroquinate hydrolyase activity. The *AroD*− strains tested [SK1516 (pVK53) and SK1520] showed no detectable 5-dehydroquinate hydrolyase activity (Table 2). In contrast, three derivatives carrying pVK55 (SK1518, SK1524, and SK1529) all contained significant levels of enzyme although the activities were lower than those from an *aroD*+ *E. coli* control strain (SD1313) or a quinic acid induced strain (M16) of *N. crassa.*

The characteristics of the 5-dehydroquinate hydrolyase activity obtained from the *AroD*+ transformants (SK1518, SK1524, and SK1529) were compared with those of the wild-type enzyme from *E. coli* and the catabolic enzyme from *N. crassa* (Table 3). By the criteria of heat stability, ammonium sulfate fractionation, and immunoprecipitation, the enzyme activity isolated from pVK55 transformed strains of *E. coli* appeared identical to *N. crassa* catabolic dehydroquinase. The biosynthetic 5-dehydroquinate hydrolyase obtained from SK1313 (*aroD*+) was significantly different by each of these criteria. An additional distinction between the two enzymes is the native molecular weight. The *E. coli* biosynthetic enzyme has a reported molecular weight of approximately 40,000 (33), whereas the catabolic enzyme from *N. crassa* is considerably larger, 220,000 (9). Sucrose density gradient centrifugation demonstrated that the enzyme obtained from pVK55 transformed strains had a molecular weight identical to the *N. crassa* catabolic dehydroquinase (Fig. 3).

5-Dehydroquinate hydrolyase was further purified from SK1518 by the procedure described for the *N. crassa* catabolic enzyme (9). The activity obtained behaved identically to the catabolic *N. crassa* enzyme with respect to both Sephadex G-200 gel filtration and DEAE-cellulose chromatography. The active fractions obtained from the DEAE-cellulose column were pooled, concentrated, and analyzed by polyacrylamide gel electrophoresis. A single protein species, which comigrated with *N. crassa* catabolic dehydroquinase, was observed.

DISCUSSION

The results presented in this communication demonstrate unequivocally that eukaryotic DNA can be faithfully transcribed and translated in a prokaryotic host. The presence of functional *N. crassa* catabolic dehydroquinase in *E. coli* also demonstrates that assembly of subunits takes place, because the *N. crassa* enzyme is a multimer composed of approximately 20 10,000-dalton subunits (9).

The *N. crassa* DNA cloned in pVK55 was isolated from a strain carrying a *qa-1*^{C} mutant (constitutive for synthesis of catabolic dehydroquinase). Because the *qa* cluster is under positive control exerted by the *qa-1* gene, transcription and translation of the *qa-2*+ gene in this mutant occurs only if the *qa-1*^{C} gene is expressed. However, when carried by the recombinant plasmid, the *qa-2*+ gene could be expressed by one of three alternative mechanisms: (*i*) transcription initiation from a pBR322 promoter; (*ii*) transcription initiation from a normal *N. crassa* promoter by a mechanism that does not require the presence of a regulatory protein: (*iii*) transcription initiation by the normal *N. crassa* mechanism which requires the presence of an expressed *qa-1*^{C} gene. If the third mechanism should prove to be the one operating, pVK55 must contain the entire *qa* cluster because *qa-1* and *qa-2* are the proximal and distal genes of the cluster (34). Although there may be enough *N. crassa* DNA contained in pVK55 to encode for the entire *qa* cluster, initial experiments have not demonstrated the presence of the *qa-4* gene (dehydroshikimate dehydrase) which is adjacent to *qa-2* (34). Furthermore, it is not clear if the two Endo·R·*Hin*dIII fragments carried by pVK55 are in fact contiguous on the *N. crassa* chromosome. Preliminary experiments suggest that a plasmid containing only the smaller (1.9×10^6 dalton) fragment complements *aroD6* mutant strains of *E. coli.*

The different levels of complementation obtained with pVK55 in various *aroD6* strains of *E. coli* point out a potential difficulty in attempting to clone eukaryotic functions in *E. coli.* Strain SK1485 apparently contained a mutation in addition to

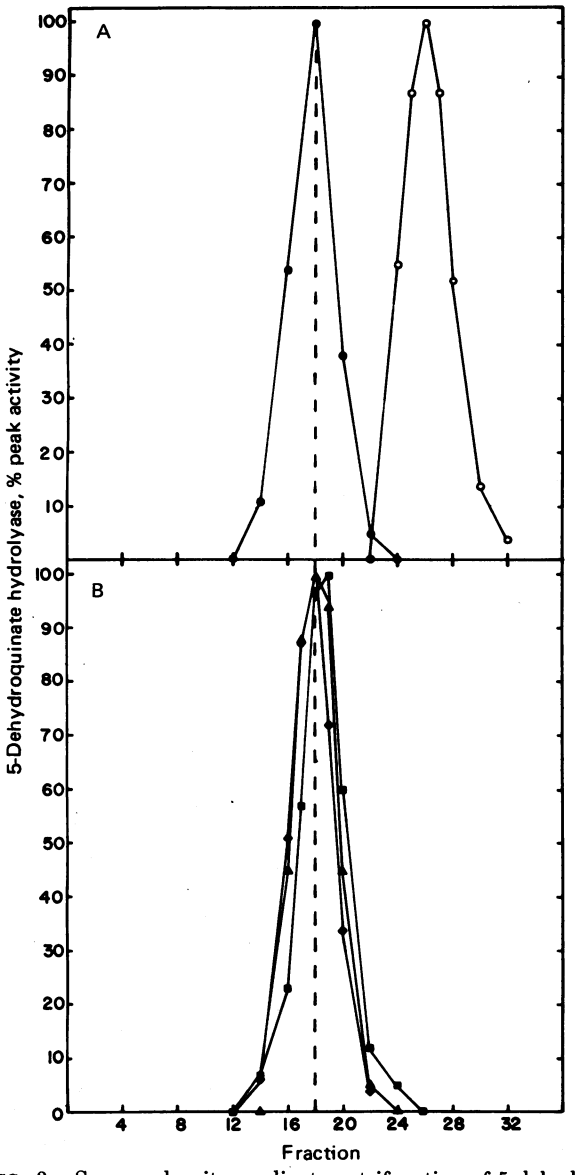

FIG. 3. Sucrose density gradient centrifugation of 5-dehydroquinate hydrolyase activity. (*A*) *N. crassa* strain M16 (●) and wild-type *E. coli* strain SK1313 (○). (*B*) pVK55 transformed strains SK1518 (■), SK1524 (♦), and SK1529 (▲).

aroD6 that prevented high levels of complementation and was only detected in the presence of the recombinant plasmid. This additional mutation was not present in AB1360, SK274, or SK1478 and was apparently introduced when SK1478 was made Str^R by ethyl methanesulfonate mutagenesis. In the absence of the spontaneous reversion or suppression of this allele in SK1518, pVK55 would not have been isolated. Therefore, care must be exercised in determining the genotype of strains to be used.

Another significant factor involved in the successful functional expression of the *N. crassa qa-2*$^+$ gene may be the unusual stability of catabolic dehydroquinase (9). In a prokaryotic background, eukaryotic enzymes with less inherent stability may be subject to proteolytic degradation or other conditions that prevent their detection.

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