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Genetic Evidence on the Organization and Action of the *qa-1* Gene Product: A Protein Regulating the Induction of Three Enzymes in Quinate Catabolism in *Neurospora crassa*

(gene cluster/inducible enzymes/activator molecule)

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ABSTRACT **The first three reactions in the catabolism of quinic acid in *Neurospora crassa* are under the genetic control of the *qa* gene cluster. This cluster consists of three structural genes encoding three inducible enzymes plus a regulatory gene (*qa-1*$^+$) whose diffusible product apparently acts in a positive fashion to initiate coordinate synthesis of the three enzymes when an appropriate inducer is present. Genetic and biochemical evidence for both complementing and temperature-sensitive *qa-1* alleles indicates that the product of the *qa-1*$^+$ gene is an oligomeric (multimeric) protein. On the basis of *cis-trans* tests of appropriate double mutants (plus genetic mapping data for temperature-sensitive mutants), at least certain constitutive mutants (which produce all three *qa* enzymes in the absence of an inducer) are mutants in the regulatory gene and not in controlling elements such as initiators. The detection of stable (non-revertible) *qa-1* intralocus deletion (multisite) mutants provides additional evidence for positive regulation in the *qa* system. Extensive genetic recombination data provide evidence that the two types of *qa-1* mutants—slow-complementing (*qa-1*^{S}) and fast-complementing (*qa-1*^{F})—map in discrete, non-overlapping segments of the *qa-1* locus. These two distinct types of mutants are hypothesized to produce altered regulatory protein molecules that fail to interact either with a DNA initiator site (*qa-1*^{S} types) or with an inducer (*qa-1*^{F} types). The striking similarities between the *qa* system in this lower eukaryote and certain prokaryote operon systems are discussed.**

Much evidence is now available concerning molecular mechanisms involved in the genetic regulation of several enzyme systems in prokaryotes such as *Escherichia coli* (1). By contrast, much less is known about genetic regulatory mechanisms in most eukaryotes. This paper describes recent studies with the lower eukaryote *Neurospora crassa* of one regulatory system that is becoming increasingly well understood, and that appears to possess certain striking similarities to prokaryote operon systems.

In *Neurospora crassa* the degradation of quinic acid (QA) and shikimic acid to protocatechuic acid is catalyzed by three inducible enzymes. The inducer QA acts to effect coordinate induction of these three enzymes, which are encoded in a cluster of three structural genes mapping very close to the methionine-7 (*me-7*) locus in linkage group VII. Mutations in each of these genes—*qa-2* [encoding catabolic dehydroquinase (5-dehydroquinate hydro-lyase, EC 4.2.1.10)], *qa-3* [encoding quinate dehydrogenase (quinate:NAD oxidoreductase, EC 1.1.1.24)], and *qa-4* (encoding 5-dehydroshikimate dehydratase)—result in the partial or complete loss of inducible activity for the enzyme encoded in that gene. Mutants in a fourth gene, *qa-1*, (closely linked to the other *qa* genes) are pleiotropic, i.e., non-inducible for all three activities, and complement mutants in each of the three other *qa* genes (2–5).

Constitutive mutants (designated *qa-1*^{C}) have been obtained as reversions of slow-complementing mutants (6), and as mutants induced directly in *qa-1*$^+$ (wild type—WT) (7). These mutants appear to map within (or very close to) the *qa-1* locus and show various degrees of constitutivity for the three *qa* enzymes in the absence of added inducer. Under non-inducing conditions, some *qa-1*^{C} mutants are dominant or semidominant in heterokaryons with WT (6).

This paper presents evidence that the product of the *qa-1*$^+$ gene is an oligomeric (multimeric) protein. This conclusion is based on allelic complementation between certain *qa-1* mutants, and on genetic and biochemical evidence for various types of temperature-sensitive *qa-1* mutants. The presence of *qa-1* intralocus deletions provides support for a positive, as opposed to a negative, system or regulation. Furthermore, genetic studies indicate that the two categories of *qa-1* mutants—slow- (*qa-1*^{S}) and fast- (*qa-1*^{F}) complementing types (2)—map in discrete, non-overlapping regions of the *qa-1* locus and that some *qa* constitutive mutants map within the *qa-1* locus. The detection of *qa-1*^{C} *qa-1* double mutants, combined with the results of *cis-trans* tests utilizing appropriate double mutants, indicate that certain constitutives are not mutants in controlling elements such as initiators.

These results provide strong additional support for a specific model which describes the organization and function of the *qa-1*$^+$ gene product in *N. crassa* (8). In this model, the *qa-1*$^+$ gene produces an oligomeric regulatory protein that, in the presence of an inducer (effector) such as QA, acts in a positive fashion to initiate the coordinate synthesis of the three quinate catabolic enzymes encoded in the *qa* gene cluster. The *qa-1*$^+$ regulatory protein (activator) is considered to possess two discrete functional regions—one, an amino-acid sequence that interacts with the inducer, and two, a sequence that interacts, presumably directly with a DNA site, to initiate transcription of the adjacent three *qa* structural genes.

MATERIALS AND METHODS

Origin of Genetic Stocks. The *qa-1* and *qa-2* mutants used in these studies (Fig. 1) were originally isolated by Rines (2, 3) in an *arom-9* mutant in WT 74A background. Rines (3) divided

Abbreviations: QA, quinic acid; WT, wild type; UV, ultraviolet.

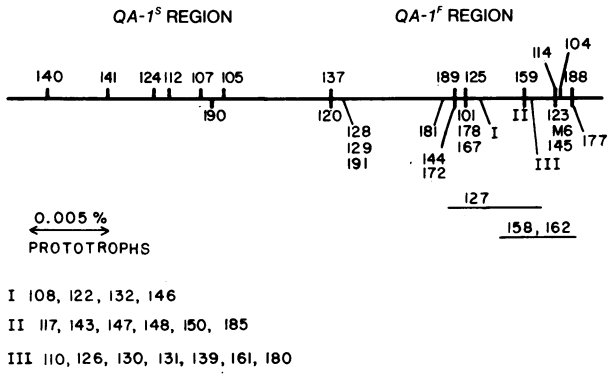

FIG. 1. Fine structure map of the *qa-1* locus based on genetic recombination studies. (For further details, see *text.*)

qa-1 mutants into two groups on the basis of their complementation responses with *qa-2* mutants: slow-complementing types and fast-complementing types. *qa-3* and *qa-4* mutants have been characterized by Chaleff (4). Additional strains used were *me-7* allele 4894 and *pan-2* alleles B23 and B36.

Complementation Tests. Allelic complementation tests were done at both 25° and 35° with *qa-1 arom-9* mutants on liquid Fries minimal medium with either 1.5% sucrose or 0.3% QA as sole carbon source. Temperature-sensitive mutants were omitted from complementation tests. All pairwise combinations were tested. Control tests for heterokaryon compatibility were done with *pan-2* allele B36. To force heterokaryons between two *qa-1* single mutants used for enzyme assays, double *qa-1 pan-2* mutants with complementing *pan-2* alleles were used.

Genetic Analyses. Crosses and ascospore platings were done as previously described (9–11). The plating temperature was usually 35°; however, crosses of temperature-sensitive strains 105 and 190 (which grow at 35°) were plated at 25°. Prototroph frequencies are based on the total number of ascospores plated. A minimum of 10^5 ascospores (often many more, especially in crosses involving presumptive deletion mutants) were plated for each cross.

Growth and Enzyme Assays of Mutants and Heterokaryons. Individual mutants and heterokaryons were grown for enzyme extractions as previously described (12). Specific details are noted for individual experiments. Current assays catabolic dehydroquinase, quinate dehydrogenase, and dehydroshikimate dehydratase have been described (12). Assays were done on 0–50% $(NH_4)_2SO_4$-precipitated and dialyzed extracts. Specific activities are expressed as nmol/min per mg of protein.

Tests for Nonsense Suppressors. To test whether *qa-1* mutants were suppressible, conidia from double mutants of 25 *qa-1* mutants combined with *arom* mutant 54—which has been shown to have a suppressible nonsense mutation (13, 14)—were irradiated with ultraviolet light (UV) and plated on Fries minimal medium alone or supplemented with 80 mg/liter of both phenylalanine and tyrosine. Revertants were isolated and tested for their ability to grow on QA as a carbon source.

Induction of qa Double Mutants. To obtain certain critical types of double mutants and to test the *cis-trans* relationships of constitutive mutants, a second *qa* mutation had to be introduced into these strains. For this purpose, conidia of double *qa-1^C arom-9* mutants were irradiated with UV, subjected to filtration-concentration on either sucrose or QA minimal medium, and all resulting quinate non-utilizing mutants were checked by complementation tests with appropriate *qa* tester stocks.

TABLE 1. *Tests for QA induction of catabolic dehydroquinase in mutants and in heterokaryons between complementing qa-1 mutants 105 (qa-1^S) and 114 (qa-1^F)**

Strains	Specific Activities: Sucrose cultures	Specific Activities: QA cultures
Mutants		
105 (*qa-1^S arom-9*)	0.6	0.5
114 (*qa-1^F arom-9*)	0.1	1.8
Heterokaryon I		
105 (*qa-1^S arom-9*) + 114 (*qa-1^F arom-9*)	67	234
Heterokaryon II		
105 (*qa-1^S pan-2* B23) + 114 (*qa-1^F pan-2* B36)	0	10

* Because of slower growth heterokaryon I was grown as a shake culture for 5 days on minimal sucrose medium and then shifted to 0.3% QA for 4 hr. The mutants and heterokaryon II were grown as shake cultures for 24 hr on minimal sucrose medium, plus a polyaromatic supplement as required, and then shifted to 0.3% QA for 8 hr.

RESULTS

Evidence for Allelic Complementation Between qa-1 Mutants. (A) *Genetic tests:* All tests of allelic complementation on QA medium were negative, presumably because the levels of restored *qa* enzymic activities are not sufficient to support growth on QA as a sole carbon source. However, tests on sucrose minimal medium indicated that many *qa-1 arom-9* mutant pairs complement. Positive complementation responses only require a level of catabolic dehydroquinase activity sufficient to convert 5-dehydroquinate to 5-dehydroshikimate, which is then utilized in the aromatic biosynthetic pathway. Non-complementing mutants were detected in tests at 25° and were more frequent at 35°. All *qa-1* mutants exhibited rapid complementation with the *pan-2* allele, B36. Clear complementation responses were observed only between certain pairs of *qa-1^F* plus *qa-1^S* mutants. One of these complementing pairs (*qa-1^F* 114 + *qa-1^S* 105) was examined to establish that growth was not the result of reverse mutation of either parent, and to determine whether a heterokaryon between two *qa-1* alleles is inducible for dehydroquinase, as would be expected if a functional *qa-1* product is being produced by complementation. To test for reverse mutations, crosses of the heterokaryon between mutants 114 and 105, (as well as of 20 additional presumptive heterokaryons), were made with a *me-7* mutant. No evidence was obtained for reverse mutations in any of the heterokaryons, since all of the *me-7*$^+$ isolates were unable to utilize QA as a carbon source (a characteristic of *qa-1* mutants), while all *me-7* isolates grew on QA.

(B) *Biochemical tests:* If allelic complementation does indeed occur between the *qa-1* mutants 114 and 105, then heterokaryons between these two non-inducible mutants should be inducible. The results of induction tests on two different types

of heterokaryons are presented in Table 1. These data indicate that heterokaryon I, unlike the "parental" mutants, can be induced for dehydroquinase by QA and must be producing an active *qa-1* product as a result of allelic complementation. The enzymic activity observed in heterokaryon I in the absence of QA results from internal induction by dehydroquinate, which accumulates because of the *arom-9* mutant in each member of the heterokaryon (3, 5). Heterokaryon II was "forced" on sucrose minimal by using two complementing *pan-2* alleles. This heterokaryon was also inducible by QA, although to a lesser extent than was heterokaryon I (Table 1).

Temperature-Sensitive qa-1 Mutants. Growth responses on QA at 25° and 35° indicated that several *qa-1 arom-9* mutants are temperature-sensitive. Certain *qa-1*^{F} mutants (e.g., 188, 189, and 191) grow slowly at 25°, and not at all at 35° (Table 2). These mutants may be considered as essentially "conventional" temperature-sensitive types. All three mutants have low levels of catabolic dehydroquinase and are inducible to a limited degree at 25°. At 35° they have essentially no dehydroquinase activity and are incapable of being induced. By contrast, two *qa-1*^{S} mutants (105 and 190) exhibit a reverse temperature-sensitivity, with little or no growth at 25° on QA, but appreciable growth at 35°. At 25°, both mutants have very low levels of dehydroquinase activity and only slight induction occurs after a shift to QA. At 35°, however, both mutants have high levels of dehydroquinase and are not further induced (especially 105) by shifting to QA. Thus these two mutants are particularly unusual in that they are *qa-1* mutants at 25°, but temperature-sensitive constitutives (*qa-1*^{C}) at 35°. Although only dehydroquinase values are reported here, activities for the other two *qa* enzymes follow the same general patterns in the various *qa-1* mutants.

Tests for qa-1 Nonsense Mutants. Suppressible (nonsense) *qa-1* mutants would provide additional evidence that the product of the *qa-1*$^+$ regulatory gene is a protein. Tests for such mutants have involved reversion experiments utilizing a total of 25 *qa-1* mutants (including both *qa-1*^{S} and *qa-1*^{F} types) combined as double mutants with *arom* mutant 54. Revertants were obtained on minimal or minimal supplemented with phenylalanine and tyrosine after UV treatment. These revertants were tested for their ability to grow on QA as a carbon source. Double *arom qa-1* mutants able to grow on QA should be recovered only as a result of a single mutation suppressing nonsense codons in both single mutants. To date, no such revertants have been detected.

Cis and Trans Relationships of Constitutive qa Mutants. Mutants have been obtained that are constitutive for the three *qa* catabolic enzymes when grown in the absence of inducer. Two constitutive mutants derived from wild type, which map within the *qa-1* regulatory gene (as will be discussed later) are temperature-sensitive, suggesting that at least these mutants are making a protein product. Temperature-insensitive constitutive mutants have also been assumed to be producing a diffusible regulatory gene product. However, although these mutants were known to be active in the *cis* configuration in heterokaryons, they had not been tested in *trans* heterokaryons, since most double mutants within the *qa-1* cluster are difficult to obtain.

To test constitutive mutants in the *trans* configuration, mutations in other *qa* genes were induced in two different presumptive *qa-1*^{C} mutants, E6 and 112-R12, both carrying

TABLE 2. *Temperature-sensitivity tests for various qa-1 (arom-9) mutants**

Mutant no.	Growth on QA as carbon source		Specific activities, dehydroquinase			
			Sucrose cultures		QA cultures	
	25°	35°	25°	35°	25°	35°
188 (*qa-1*^{F})	±	0	1.0	0	12	0
189 (*qa-1*^{F})	±	0	2.0	0.3	15	0
191 (*qa-1*^{F})	+	0	6.0	0.6	42	0.5
105 (*qa-1*^{S})	0	±	1.0	335	1.0	342
190 (*qa-1*^{S})	±	+	0.2	173	4.0	230
WT	+	+	0.1	1.3	113	77

* Strains were grown as shake cultures for 24 hr at 25° and 35° on minimal sucrose medium plus a polyaromatic supplement and then shifted to 0.3% QA plus a polyaromatic supplement for 8 hr.

arom-9. Mutant E6 was originally induced in WT 74A (8), while 112-R12 was obtained as a revertant of *qa-1*^{S} mutant 112. Genetic data indicate that the mutation in revertant 112-R12 is within the *qa-1* gene and apparently at the same site as in mutant 112 (Case and Giles, unpublished). These strains differ in that E6 has low constitutive levels of the *qa enzymic* activities, while strain 112-R12 has high levels. The additional *qa* mutations were induced in these strains by UV treatment, and the strains were recovered by filtration-concentration on either sucrose or QA medium.

Complementation tests on QA as a sole carbon source using the four *qa* tester strains indicated that second mutations had been induced in all four of the *qa* genes, including the *qa-1* regulatory gene. All 38 mutants that failed to complement with one *qa* tester strain could be classified into one of the four previously detected *qa* mutant types.

In addition to complementation tests, assays for the three *qa* enzymes were done on heterokaryons of appropriate *qa* strains forced to grow on minimal medium by using the two *pan-2* alleles, B23 and B36. In heterokaryons involving a double mutant of E6 (i.e., heterokaryons of the genotype *qa-1*^{C} *qa-2 qa-4*$^+$ *pan-2* B36 plus *qa-1*$^+$ *qa-2*$^+$ *qa-4 pan-2* B23) no dehydroquinase activity was detected. However, heterokaryons formed between the second constitutive carrying a *qa-3* mutation (112-R12-M72) and a *qa-1*^{F} mutant (i.e. heterokaryons of the genotype *qa-1*^{C} *qa-3 pan-2* B36 plus *qa-1*^{F} *qa-3*$^+$ *pan-2* B23 had quinate dehydrogenase activities markedly above those in the parental strains (Table 3). The nuclear ratios of the parental types in the heterokaryons were obtained by growing up a small sample of the 24-hr shake cultures used for enzyme assays. This ratio is presumed to reflect the nuclear ratios under growth conditions.

Genetic Mapping and Reversion Studies of qa-1 Mutants. The genetic map of the *qa-1* locus is presented in Fig. 1. (Over 95% of the crosses between the strains involved were fertile.) Mutants shown below the line were crossed to all mutants above the line. Additional intragroup crosses will be required to determine whether mutants included in groups I, II, and III are all homoalleles—which seems unlikely. The presence of pseudowild types in crosses of complementing *qa-1* mutants is not a problem, since they appear much later and grow much more slowly than do prototrophs formed by recombination.

Two major conclusions can be drawn from these extensive mapping data: (*1*) The data support the conclusion that *qa-1*^{S}

TABLE 3. *Enzymic activities in forced heterokaryons between strains 112-R12-M72 (qa-1^C qa-3 pan-2 B36) and 125 (qa-1^F qa-3$^+$ pan-2 B23) and in control strains**

	Specific Activities		
	Quinate dehydrogenase	Dehydroquinase	Dehydroshikimate dehydratase
Heterokaryons:			
112-R12-M72 + 125†	4.3	119	27
Control strains:			
112-R12-M72 (*qa-1^C qa-3 pan-2* B36)	0	163	46
125 (*qa-1^F qa-3$^+$ pan-2* B23)	0.01	3.1	0.4
112-R12 (*qa-1^C pan-2* B36)	77	151	61

* Specific activity values have been averaged from three separate experiments. All strains were grown for 24 hr at 25° in 500 ml of minimal medium or minimal medium supplemented with 2 μg/ml of pantothenic acid in 2-liter flasks on a rotary shaker.

† In two different heterokaryons tested, the ratios of the two parental type nuclei (*qa-1^C qa-3*:*qa-1^F*) were 1:1 and 2:1.

and *qa-1^F* mutants map in discrete, non-overlapping regions of the *qa-1* locus; (*2*) Evidence has been obtained for three intralocus deletions of two different types. The conclusion that the "multisite" mutants are deletions is supported by the failure of these three mutants to yield UV-induced revertants. By contrast, about half of the presumptive single site mutants tested did revert. Some of the stable apparent single site mutants may carry small deletions.

DISCUSSION

The detection of allelic complementation between various *qa-1* mutations and of temperature-sensitive *qa-1* alleles provides strong indirect evidence that the product of the *qa-1$^+$* regulatory gene is a protein. There appears to be no evidence that allelic complementation can involve molecules other than proteins (15), and most temperature-sensitive mutants presumably produce thermolabile protein gene products, although instances of temperature-sensitive mutants encoding a tRNA are known in *E. coli* and in yeast (16). The failure to detect nonsense suppressors constitutes only negative evidence, since experiments with non-complementing *arom* mutants indicated that only a few were suppressible (13, 14).

The occurrence of non-complementing *qa-1* mutant types indicates that both *qa-1^S* and *qa-1^F* types are within a single functional gene (cistron) despite the distinct phenotypes exhibited by these two mutant types and their non-overlapping distribution on the *qa-1* map.

The various temperature-sensitive *qa-1* mutants presumably produce regulatory proteins having thermolabilities different from that of WT. Certain of these mutants (*qa-1^F* types) can be classified as "leaky" at the permissive temperature (25°), since they do not grow as well as WT on QA at that temperature. Those mutants presumably produce regulatory proteins that are modified in the inducer-binding region, and are incapable of interacting with the inducer as effectively as does WT regulatory protein. Of particular interest is mutant 105, which is a *qa-1^S* mutant at 25° but a *qa-1^C* mutant at 35°. Presumably this mutant produces an inactive regulatory protein at 25°, while at 35° it produces a protein that has the proper configuration to initiate transcription of the three *qa* structural genes in the absence of an inducer.

In *cis-trans* tests, enzyme assay data (Table 3) indicate that when growth occurs in the absence of inducer quinate dehydrogenase activity is essentially absent in the two parental strains, but present in heterokaryons involving the constitutive mutant carrying a *qa-3* mutation (mutant 112-R12-M72) combined with a *qa-1^F* mutant. Tihs positive *trans* test indicates that this constitutive mutant must be producing a diffusible product and does not have a mutation in a controlling element such as an initiator. However, the quinate dehydrogenase specific activities are much lower than would be expected on the basis of the comparative activities for dehydroquinase and dehydroshikimate dehydratase in the parental strains and heterokaryons. Apparently the *qa-1^C* gene product is much more effective in initiating the synthesis of *qa* enzymes encoded within its own nucleus (*cis*) than in other nuclei (*trans*) in a heterokaryon. This result may mean that the *qa* regulatory protein is synthesized within its own nucleus and acts at the level of transcription. *Trans* activity in a heterokaryon would therefore depend upon the internuclear migration of the *qa-1* gene product. Concentration, activity, rate of diffusion, and stability of the regulatory protein may be important factors in this process. One or more of these factors could account for the failure to detect dehydroquinase activity in the heterokaryons involving constitutive mutant E6. These overall results appear comparable to, but not as extreme as, those observed with the "nucleus limited" product of the *sconc* gene (18).

An alternative interpretation of the negative *trans* test (based on enzyme assay data) with constitutive mutant E6 is that this mutant is only active in *cis* and is a mutation in a controlling element. Complementation tests for growth on QA (even ones utilizing a *qa-1^F* mutant) apparently cannot distinguish between the two categories of constitutive mutants, since in the presence of inducer the *qa-1$^+$* gene present in a *qa* initiator constitutive mutant (*I^C*) could produce a regulatory protein active in *trans* and hence capable of initiating synthesis of the *qa* enzymes encoded in the *qa-1^F* nucleus. Evidence against the interpretation of E6 as an *I^C* mutant comes from the types of double mutants detected in this strain, specifically *qa-1* mutants. The recovery of *qa-1* mutations in this mutant eliminates the possibility that it is an *I^C* mutant, since an *I^C* mutant should be epistatic to a *qa-1* mutant, producing all *qa* enzymes in the absence of either an inducer or a *qa* regulatory protein. Thus *qa-1* mutants should not be recovered as double mutants induced in an *I^C* mutant.

Constitutive mutants in both regulatory genes and controlling elements are known in prokaryotes such as *E. coli* (1), and presumably both might occur in the *qa* system. The possibility has not been excluded that certain other *qa* constitutives may be mutants in a controlling element. However, this cannot be the case for the two temperature-sensitive constitutives, which have also been shown to map within the *qa-1* gene.

The detection of deletion type *qa-1* mutants provides supporting evidence for positive regulatory control in the *qa* system, since they are non-inducible, whereas deletion mutants in a regulatory gene in a negatively controlled system are constitutive (17). More compelling evidence would come from deletions of the entire *qa-1$^+$* gene, but to date none has been detected.

The *qa-1* genetic map indicates a clear segmentation of the *qa-1*$^+$ gene in that *qa-1*^{S} and *qa-1*^{F} mutant sites occur in discrete, non-overlapping regions. *qa-1*^{S} mutants are semidominant in heterokaryons with mutants in the three other *qa* genes. These mutants exhibit negative complementation, probably through formation of hybrid multimeric regulatory proteins that are defective in transcription initiation and therefore would be analogous to the *i*^{d} mutants in the *lac i* gene of *E. coli*. By contrast, *qa-1*^{F} mutants are recessive in heterokaryons with mutants in other *qa* genes. The activator molecules produced by these mutants are presumably defective in their interaction with effector molecules such as QA and thus *qa-1*^{F} mutants appear to be analogous to *i*^{s} mutants in the *lac i* gene. Indeed, there is a striking similarity between the intragenic localization of functionally analogous mutant types in the *qa-1* and *lac* genes (19).

Additional evidence exists for a functional distinction between the two segments of the *qa-1* gene. The only constitutive mutants derived from WT that can be localized on a genetic map (two temperature-sensitive constitutives) occur at sites within the *qa-1*^{S} segment and are *qa-1*^{S} mutants at the non-permissive temperature (25°). Furthermore, only *qa-1*^{S} mutants have yielded constitutive revertants. Presumably *qa-1*^{C} mutants produce regulatory proteins capable of initiating transcription in the absence of an inducer because allosteric transitions produced by inducer binding are not required. These mutants are apparently similar to the *araC*^{C} mutants in *E. coli* (17).

The similarities between the *qa* system in *N. crassa* and certain *E. coli* operon systems such as *lac* and *ara* are striking. In all three systems, three apparently contiguous structural genes encode three different inducible enzymes. An adjacent gene produces a regulatory protein that acts, in either a positive or negative fashion, to control the coordinate synthesis of the three enzymes. In the *qa* and *lac* systems (20) the regulatory gene encoding this protein is divisible into two, presumably analogous, functionally discrete regions. However, present evidence does not permit the conclusion that the *qa* gene cluster is equivalent to a prokaryote operon. Such a conclusion depends upon the detection of mutations in adjacent controlling sites, together with evidence that the three *qa* genes that encode enzymes are transcribed as a single polycistronic mRNA.

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