Transformation of *Neurospora crassa* with the Cloned *am* (Glutamate Dehydrogenase) Gene

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We used DNA containing the *am* gene of *Neurospora crassa*, cloned in the λ replacement vector λ L-47 (this clone is designated λ C-10), and plasmid vector subclones of this DNA to transform *am* deletion and point mutant strains. By means of subcloning, all sequences required for transformation to *am* prototrophy and expression of glutamate dehydrogenase have been shown to reside on a 2.5-kilobase *Bam*HI fragment. We also characterized several *am*⁺ strains that were obtained after transformation with λ C-10. These strains showed Mendelian segregation of the *am*⁺ gene, although less than 50% of the transformed strains showed the normal linkage relationship of *am* with *inl*. In all cases tested, the strains had incorporated λ DNA as well. The λ DNA also showed a Mendelian segregation pattern. In one case, the incorporation of *am* DNA in a novel position was associated with a mutagenic event producing a strain with a very tight colonial morphology. In all cases in which the *am*⁺ gene had become the resident of a new chromosome, glutamate dehydrogenase was produced to only 10 to 20% of the wild-type levels.

Case et al. (1, 2) and Schweizer et al. (13) have developed an efficient transformation procedure for *Neurospora crassa* utilizing the cloned *qa-2* gene and a *qa-2 aro-9* double-mutant strain as recipient. This was a significant step towards facilitating molecular genetics studies with *N. crassa*. Until now, *qa-2* has been the only system available to study *N. crassa* transformation in detail. Recently, however, the *am* gene of *N. crassa*, which codes for NADP-specific glutamate dehydrogenase (GDH), has been cloned on a 9.1-kilobase (kb) *Hind*III fragment (7) in the replacement vector, λ L-47 (9). This λ -chimeric clone (herein referred to as λ C10), which was isolated with a synthetic 17-mer as a probe, was used to transform an *am* deletion mutant strain to prototrophy (7) by the procedure of Schweizer et al. (13).

In this communication, we report the transformation of both deletion and point mutant strains with this clone, as well as with subclones containing the *am* gene, made in the plasmid vector pUC8 (15). By this technique, all sequences required for transformation of *am* auxotrophs to prototrophy and for the expression of GDH were shown to be contained on a 2.5-kb *Bam*HI fragment of the original clone. This fragment has also recently been shown by DNA sequencing to contain all of the coding sequences of the *am* gene (Kinnaird and Fincham, personal communication).

We also characterized several transformants obtained when *am* mutant strains are transformed with λ C10 DNA.

MATERIALS AND METHODS

Strains used. The *am* mutant strains were from our stock collection and have been previously described (8, 12). The *alcoy* strain of Perkins et al. (11) was employed in preliminary mapping of transformed *am* genes. The bacterial strain JM83 and plasmid vector pUC8 (15) were obtained from Bethesda Research Laboratories, Inc.

Construction of plasmid subclones. Figure 1 shows the *N. crassa* DNA insert for each of the constructed subclones as well as a restriction map of the 9.1-kb *Hind*III *N. crassa*

fragment of pJR1 from which they were subcloned. The 2.5-kb *Bam*HI fragment indicated was subcloned into the single *Bam*HI site of pUC8 to form pJR2. The 5.1-kb *Pst*I-*Hind*III fragment was subcloned into appropriately cut pUC8 to form JR3. The 1.9-kb *Bgl*II-*Hind*III fragment was subcloned into *Bam*HI-*Hind*III-cut pUC8 to form pJR4.

Restriction digests. Restriction enzymes were purchased from Bethesda Research Laboratories and were used as directed. Restricted DNA was electrophoresed through 0.65% agarose horizontal slab gels containing 40 mM Tris (pH 8.2), 20 mM sodium acetate, 18 mM sodium chloride, 2mM EDTA, and 1 μ g of ethidium bromide per ml. For analysis of DNA fragments of less than ca. 1 kb, 5% acrylamide gels containing 90 mM Tris (pH 8.0), 90 mM boric acid, and 2 mM EDTA were used. Restriction maps of cloned DNAs were constructed on the basis of the sizes of fragments produced after digestion, either singly or in combination, with restriction enzymes.

DNA isolation. One gram of freeze-dried *N. crassa* mycelia was ground with a mortar and pestle and mixed with 20 ml of 10 mM Tris-1 mM EDTA-4% sodium dodecyl sulfate (SDS)-25% sucrose (pH 8.0). The solution was kept at room temperature for 1 h with occasional swirling, followed by four extractions with phenol-chloroform-isoamyl alcohol (49:49:2). The DNA was precipitated with two volumes of 95% ethanol, resuspended in 5 ml of 10 mM Tris-1 mM EDTA (pH 8.0), and treated with 50 μ g of RNase A for 1 h at 37°C. After extraction with phenol-chloroform-isoamyl alcohol, the DNA solution was overlaid with ethanol and the DNA was spooled out. This method generally yielded 1 to 3 mg of *N. crassa* DNA.

Plasmid DNA was isolated essentially as described by Clewell and Helinski (3). Phage DNA was isolated as previously described (7).

Southern blotting and hybridization. After electrophoresis, DNA was partially hydrolyzed by soaking the gels in 0.25 M HCl for 15 min. DNA was denatured in situ with 0.5 M NaOH-1.5 M NaCl, neutralized with 1.5 M NaCl-0.5 M Tris hydrochloride (pH 7.4), and transferred to nitrocellulose filters in 20 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl plus 0.015 M sodium citrate) as described by Southern (14). After transfer, the filters were washed with 6 $\times$ SSC and baked under

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vacuum for 2 h at 80°C. Before hybridization, the filters were wetted with 6× SSC and prehybridized at 68°C for 2 to 4 h in 6× SSC–0.5% SDS–5× Denhardt solution–100 µg of denatured salmon sperm DNA per ml. Hybridizations were carried out at 68°C for 12 to 16 h in 6× SSC–0.01 M EDTA–5× Denhardt solution–0.5% SDS–100 µg of denatured salmon sperm DNA per ml containing 10⁶ to 10⁷ counts of ³²P-labeled denatured probe. After hybridization, the filters were washed briefly in 2× SSC–0.5% SDS at room temperature. They were then washed at 68°C for 2 h in 0.1% SSC–0.5% SDS, followed by an additional 30-min wash in 0.1% SSC–0.5% SDS.

Transformation of *N. crassa*. The procedure used was based upon a protocol circulated by H. Zalkin (personal communication) which is essentially the same as that of Schweitzer et al. (13). The exact protocol used has been reported in detail previously (7). Transformants were picked to slants of Vogels minimal medium (16) containing 0.02 M glycine, thus maintaining selective pressure for *am*⁺. When vegetative subclones were examined, these were single conidial colony isolates picked from and maintained on selective medium. Crosses of transformants were made on Westergaard and Mitchell salts (17) solidified with 1.5% agar and with a strip of Whatman no. 1 paper as carbon source. Crosses were routinely made in both directions. Crosses were examined and linkage relationships were determined by standard procedures (5). The *am* gene is scored on the basis of resistance to glycine (0.02 M); prototrophs are resistant to this concentration of glycine, and growth of *am* auxotrophs is severely inhibited (essentially no growth after 48 h).

GDH assays. GDH was assayed by the techniques of Coddington et al. (4).

RESULTS

Location of sequences necessary for expression of GDH.

Since the *N. crassa* sequences present on the 9.1-kb *Hind*III fragment cloned in C10 were sufficient to transform the deletion *am*₁₃₂ (7), it seemed likely that all of the coding sequences for the *am* gene were contained within this fragment. To further localize the *am* gene, we constructed restriction site maps and subcloned a number of fragments into the plasmid vector pUC8. These subclones were subsequently used in transformation experiments to identify sequences necessary for expression of GDH. A restriction site map and the fragments subcloned are shown in Fig. 1. pJR1 contained the entire 9.1-kb *Hind*III fragment from λC10. The other subclones shown in Fig. 1 were constructed as described in Materials and Methods.

It was known that the 17-mer used as a probe in cloning the *am* gene hybridized within the 700-base-pair fragment cut from the right end of the 2.5-kb *Bam*HI fragment by *Bgl*III (7). Consequently, we knew that the beginning of the gene was coded for by this region and the rest of the gene lay in the direction of either the *Pst*I or the *Xba*I site. The question of which direction the *am* coding sequences lay was answered by transformation experiments with pJR2, pJR3, and pJR4 as the sources of transforming DNA (Table 1). pJR3 was able to transform *am*₁₃₂, indicating that all of the *am* coding sequences were contained within the 5.1-kb *Pst*I–*Hind*III fragment. pJR2 was also able to transform *am*₁₃₂, whereas pJR4 failed to do so, indicating that all of the coding sequences for the *am* gene, as well as any additional sequences needed for expression, were contained within pJR2, with transcription initiating within the 700-base-pair *Bgl*–*Bam*HI fragment and proceeding toward the *Pst*I site.

Characterization of transformation with λC10. Table 1 shows the results of a typical transformation of *am*₁₃₂ with λC10. This experiment was repeated many times with essentially the same result. The numbers reported are for relatively strong-growing colonies on the original plates. These results represent transformation frequencies considerably higher than those reported for transformation with the *qa-2* gene (1, 2, 13). We have no ready explanation for this difference, nor is it obvious why pJR2 with approximately 10 times as many copies of the *am* gene per microgram of DNA should give transformation frequencies essentially equivalent to those obtained with λC10 DNA. However, as pointed out below (Discussion), the nature of the transformants obtained with pJR2 are very different from those obtained with λC10.

In addition, in every experiment a large number of abortive transformants were observed with λC10 DNA, but no colonies were observed on control plates. Case (1) has previously made similar observations for *N. crassa* transformation with the *qa-2* gene. These abortive transformants initially show substantial growth on selective plates but fail to grow when subcloned to selective medium. We have also repeatedly observed that, even after an initially successful subcloning, some transformant clones fail to grow on the second or third subcloning, suggesting that the λC10 DNA or some part of it containing the *am*⁺ gene is capable of replicating in *N. crassa* in a manner which does not involve stable integration into chromosomes.

A number of transformants that had survived three successive subclonings on selective medium were crossed to *am*₁₃₂ in the opposite mating type. In each case, it was possible to isolate spores that were *am*⁺, although never in Mendelian ratios. This was not an unexpected result, inasmuch as the transformant strains, even after several subclonings, were undoubtedly still heterocaryotic. When *am*⁺ ascospore cultures from these crosses (presumably now homocaryotic) were crossed to *am*₁₃₂, however, we invariably obtained Mendelian ratios of *am*⁺:*am* spores. Shot, unordered tetrads from several of these crosses were analyzed. Sample data for two such crosses involving transformants C10T2 (*am*₁₃₂) × *am*₁₃₂ and C10T3 (*am*₁₃₂) × *am*₁₃₂ are shown in Table 2. In each tetrad the segregation of *inl*, mating type, and colonial morphology (in the case of C10T2 tetrads), as well as *am*, were tested, and all exhibited Mendelian segregation. Figure 2 shows a genomic Southern (14) analysis of *Hind*III-digested DNA from one spore culture of each pair from tetrad 1 of the cross C10T3 (*am*₁₃₂) *inlA* × *am*_{132a}. Each of the two *am*⁺ spores contained an approximately 9-kb *Hind*III fragment which hybridized to the pJR2 probe and an approximately 23-kb fragment that hybridized to the λL47 vector DNA, suggesting that both the *am*-containing *Hind*III fragment and the λ sequence are associated and segregating on a single chromosome.

To confirm this hypothesis, DNA from one of the *am*⁺ tetrad members was digested with *Sst*II and subjected to Southern analysis (14), using either nick-translated pJR3 or λL47 as a probe. *Sst*II makes two adjacent cuts in the *am*⁺ *N. crassa* sequence, one cut in the right arm of λL47 and two adjacent cuts in the left arm of λL47. If the *am*⁺ DNA and the λ sequences are cointegrated, one would expect to see three bands in the above-described Southern analysis when λL47 is used as probe and two bands when pJR3 is used as probe, with one of the bands common to both probes. This expectation was met (data not shown), indicating that *am*⁺ and λ sequences were integrated in adjacent positions.

Figure 3 shows Southern analysis of *Hind*III-digested genomic DNA from ascospore cultures of three other *am*⁺

FIG. 1. Restriction site map of the 9.1-kb *N. crassa* DNA *Hind*III fragment inserted into pUC8 to form pJR1. The fragments subcloned into pUC8 to form pJR2, pJR3, and pJR4 are indicated below the restriction map. h, *Hind*III; b, *Bam*HI; bg, *Bgl*II; e, *Eco*RI; p, *Pst*I; k, *Kpn*I; xb, *Xba*I; t, *Sst*I.

transformants, C10T1, C10T2, and C10T10, each probed with nick-translated pJR2, pJR3, or λ L47. The transformant C10T1 contains two *Hind*III fragments of 14.5 and 3.6 kb; C10T2 contains one fragment of 11.2 kb; and C10T10 contains two fragments of 11.2 and 5.5 kb that hybridize to nick-translated pJR2 and pJR3. In addition, there is a 2.4-kb *Hind*III fragment in all three of the strains that hybridizes to pJR3; this band is also present in the recipient strain *am*₁₃₂ (data not shown). Each of the three transformants also contains one or more *Hind*III fragments that hybridizes to nick-translated λ L47 DNA. The possible association of *am* and λ DNA has not been analyzed in these transformants, nor have we determined whether the multiple bands represent integration at more than one site.

Does incoming λ C10 DNA home to the correct chromosome? The *am* gene is normally a resident of linkage group (LG) V; however, as suggested by the data shown in Table 2, some of the transformed strains have *am* integrated on other chromosomes. We were interested in obtaining some indication of how often the integration occurred on LG V. To examine this question, the *inl* marker present in the parental strain used for transformation was utilized. If the incoming *am*⁺ DNA were integrated into LG V, it should show linkage to *inl*. Only 3 of 10 *am*₁₃₂ transformants examined showed linkage to *inl* (data not shown).

Since λ C10 lacks substantial homology with *am*₁₃₂ DNA, we considered that transformation of a point mutant with λ C10 DNA might result in a substantial increase in the homing of λ C10 DNA to LG V. However, when transformants of *am*₄, a missense mutation (used here because it has a very low reversion frequency) were analyzed in a similar way, only 4 of 10 transformants analyzed were linked to *inl*. Thus, although there is a strong tendency for λ C10 DNA to home to LG V, over half of the transformants tested have

TABLE 1. Transformation of *am*₁₃₂ with cloned *N. crassa* DNA

DNA source	No. of transformants per:	
	μ g of DNA	Viable cell
λ C-10	1.27×10^4	5.5×10^{-3}
λ L47 (vector)	0	0
pJR3	1.23×10^4	3.7×10^{-3}
pJR2	1.2×10^4	1.6×10^{-3}
pJR4	0	0
pUC8 (vector)	0	0

TABLE 2. Unordered tetrads from crosses of transformed strains to *am*₁₃₂

Cross	Tetrad	Spore pair	<i>inl</i>	<i>am</i>	Colonial	MT ^a
C10T-2 (<i>am</i> ₁₃₂) <i>inlA</i> $\times$ <i>am</i> _{132a}	1	1	—	—	+	A
		2	+	—	+	a
		3	—	+	—	A
		4	+	+	—	a
	2	1	+	+	—	A
		2	+	—	+	a
		3	—	+	—	A
		4	—	—	+	a
	3	1	+	—	+	A
		2	—	+	—	a
		3	—	—	+	A
		4	+	+	—	a
	4	1	—	+	—	a
		2	—	—	+	A
		3	+	+	—	a
		4	+	—	+	A
C10T-2 (<i>am</i> ₁₃₂) <i>inlA</i> $\times$ <i>am</i> _{132a}	1	1	—	—		a
		2	+	—		a
		3	+	+		A
		4	—	+		A
	2	1	—	—		A
		2	—	+		a
		3	+	+		a
		4	+	—		A
	3	1	+	—		A
		2	—	—		A
		3	+	+		a
		4	—	+		a
	4	1	—	+		a
		2	+	—		a
		3	—	—		A
		4	+	+		A

^a MT, Mating type.

their *am*⁺ gene integrated on other chromosomes. This appears to be true regardless of whether the recipient strain contains a deletion of the *am* locus or merely a base substitution within the locus.

Using conventional genetic analysis, we have shown that the *am*⁺ sequences of C10T3 are integrated in LG VII linked to *met-7* (9.2%) recombination. C10T1 sequences are integrated in LG V (the normal chromosome for *am*); C10T2 *am*⁺ appears to be on LG II, and C10T10 *am*⁺ sequences on LG I, distantly linked to mating type. More extensive genetic analysis is currently in progress.

Does the transformation event involve major chromosomal rearrangement? D. Perkins (personal communication) had suggested from preliminary analysis of *qa-2* transformants that there might be an association of integration of transforming DNA with translocation. To test this possibility, single ascospore cultures were crossed back to the parental strain and shot asci were analyzed for the frequency of white abortive ascospores (10). A total of 19 transformed cultures were analyzed by this technique, and it was found that all of them were normal in this respect, showing greater than 70% of the asci with eight black ascospores. We therefore found

FIG. 2. Genomic Southern blot analysis of tetrad members from the cross C10T3 (am_{132}) $inl \times am_{132}$. DNA from each strain was digested with *Hind*III, and the mixtures were resolved on a 0.65% agarose gel. After transfer to nitrocellulose, the blots were probed with either nick-translated λ L47 (lanes a through d) or pJR2 (lanes e through h). Lanes a and e, DNA from tetrad 1 (Table 2) member 4; lanes b and f, member 3; lanes c and g, member 2; lanes d and h, member 1.

no support in our limited data for an association of transformation and translocation.

Do the transformants have normal GDH activity? Case et al. observed that *qa-2* transformants had normal dehydrogenase activity both when the *qa-2* gene had been incorporated in its normal site and when it had taken up a new chromosomal location (1). When the GDH activity of am^+ transformants was examined, a different pattern was obtained. Among most transformants in which the am^+ gene is linked to *inl*, GDH activities were normal (C10T1 is the one exception found so far). However, transformants with am^+ unlinked to *inl* had GDH activities considerably lower than normal. A sample of our results with many transformants is shown in Table 3.

Since C-10 DNA is frequently incorporated into the genome in the "wrong" place, can the DNA be mutagenic? One would expect that incoming DNA which is inserted into an incorrect genetic location might occasionally inactivate a resident gene at the site of its insertion. Unless experimental conditions were correctly adjusted, most of these events might either be lethal or escape detection. However, morphological variants require no special conditions for detection and thus make good candidates for use in screening transformants for associated mutagenesis. Of 122 transformants picked from several different transformation experiments, 1 C10T2, exhibited a very tight colonial growth. Random spore analysis from a cross of C10T2 (am_{132}) $inlA \times am_{132}a$ indicated that colonial morphology appeared to segregate with glycine resistance. Table 2 shows unordered tetrad data for this cross indicating cosegregation of am^+ (glycine resistance) and colonial phenotype. To be sure that the am^+ and colonial morphology phenotypes indeed resulted from the same rather than merely two tightly linked independent events, we plated random spores from the above cross on

glycine inositol-containing minimal medium and scored the plates for glycine resistance and colonial morphology. A total of 2,170 glycine-resistant spores were scored; of these, 65 did not have the tight morphological phenotype (an apparent 2.9% recombination). However, when a subset of the glycine-resistant, noncolonial spore cultures (all such spore colonies from one plate) was tested by replating conidia on selective medium, all proved to be heterocaryons (i.e., single conidial colonies that were glycine resistant with tight colonial morphology were observed in every case). This represents an unexpectedly high nondisjunction frequency; however, it does appear that the colonial morphology is directly associated with the am^+ transformation. Thus, it appears that the transformation event can be mutagenic. This opens the possibility of using transformation or mutagenesis to place a known DNA sequence into an intractable *N. crassa* gene, thus providing a convenient handle for the subsequent molecular cloning of that gene.

DISCUSSION

am gene information is on a 2.5-kb *Bam*HI fragment. Using plasmid subclones of λ -C10, we have demonstrated that all sequences required for transformation and expression of the *am* gene are contained on a 2.5-kb *Bam*HI fragment. It should be mentioned at this point that transformants obtained with pJR2 appear to have the *am* gene present in the cell in an unstable, nonintegrated form. A manuscript detailing the nature of pJR2 transformants is in preparation.

λ C10 transformants are integrated. In contrast to the behavior of pJR2 transformants, those obtained with λ C10 appeared at first to be unstable but then became integrated into chromosomes. We found, in our limited sample, a tendency to integrate into the proper LG; however, more than 50% of the transformants are found to be unlinked to the LG V marker *inl*. In a few cases, conventional genetic analysis was used to map the transformed *am* gene to new chromosomal locations. Once integrated, the am^+ genetic information segregates in a conventional, Mendelian fashion, as do λ sequences which appear to be cointegrated. Four transformant strains were examined by means of Southern analysis and all four were found to have incorporated λ sequences.

Nature of the integration event. Perkins has suggested that

FIG. 3. Genomic Southern blot analysis of λ C10 am^+ transformants. The conditions were the same as those described in the legend to Fig. 2. Lanes a through c were probed with nick-translated pJR3, lanes d through f were probed with pJR2, and lanes g through i were probed with λ L47. Lanes a, d, and g, *Hind*III-digested DNA from C10T1; lanes b, e, and h, DNA from C10T2; lanes c, f, and i, DNA from C10T10.

there might be an association of chromosomal rearrangement with transformation in *N. crassa*. Since we were dealing with a linear DNA molecule and had little idea as to how the relevant sequences were becoming associated with chromosomes, we tested this idea for 19 of our primary transformants by examining the pattern of abortive (white) spores in shot, unordered tetrads as described by Perkins and Barry (10). In every cross of a transformant back to the parent strain in opposite mating type, greater than 70% asci with eight black spores were observed. Thus it appears that, at least for *am* transformation, there is no evidence that integration of the transforming DNA sequences is associated with chromosomal rearrangements. The mechanism of integration remains unclear, although it seems relatively certain that, unless the incoming DNA becomes associated with the ends of chromosomes or becomes circularized inside the cell, two recombination events would be required. When the *am*⁺ and λ DNA have integrated in the new location, there is an open question as to whether a piece of resident *N. crassa* DNA lying between two fortuitous bits of homology with the incoming linear DNA is replaced by the incoming DNA, or whether there is some mechanism which makes a complete or partial loop of the linear DNA, allowing one or two adjacent crossovers to integrate the incoming DNA sequences with little or no loss of resident DNA. If segments of the chromosome are routinely deleted during the integration process, one would expect that many, perhaps most, transformation events would be lethal. Again, the contrast in behavior between cells transformed with λ C10 and those transformed with pJR2 should be pointed out. In the latter case, the transformants are unstable and *am*⁺ appears not to be present in a form that is stably integrated into a chromosome. There are several differences between these two vectors which might account for their different behavior, including both size (50 versus 5 kb) and physical form (linear molecule versus covalently closed circle). One obvious possibility is that the presence of free ends favors integration, in which case λ C10 might be an ideal integration vector by virtue of providing both free ends and large buffering stretches of dispensable DNA for the entertainment of rapacious exonucleases.

In at least one case, C10T2, it would appear that the incoming DNA has inactivated a gene involved in morphological development, inasmuch as the transformation event has caused the strain to take on a very compact colonial phenotype. This could have occurred by the integration of the incoming DNA into either the structural gene (or its controlling element), or it could have resulted from the deletion of the gene coding for normal morphology. It should be particularly interesting to analyze the resident DNA sequences where the *am* gene has inserted in this case.

Regardless of the mechanism by which the morphological gene in question was inactivated, this result suggests the possibility of using transformation for mutagenesis, thus placing a known DNA sequence in or near an otherwise intractable gene and giving a simple method for screening clones containing the desired gene.

We are currently recloning the *am* gene from C10T2 and a few other transformant strains to examine in detail the sequence into which it has become integrated as well as the fate of normal sequence at that site.

GDH production by C10 transformants. Case et al. have found that *qa-2* transformants produce essentially wild-type levels of dehydroquinase, whether they are integrated at the correct site or at some non-normal location. This is in direct contrast to the *am* transformants. We find that most of the

TABLE 3. GDH activity of *am*⁺ ascospore isolates from crosses of various λ C10 transformants to *am*₁₃₂

Transformant ^a	Spore no.	Sp act of GDH ^b	Linked to <i>int</i>
T1	1	55	Yes
	2	50	Yes
T2 ^c	1	63	No
	2	51	No
T3	1	82	No
	2	122	No
T4	1	367	Yes
	2	452	Yes
T5	1	407	Yes
	2	462	Yes
T10	1	112	No
	2	77	No
TC3	1	644	Yes
	2	518	Yes
TC5	1	700	Yes
	2	548	Yes
TC8	1	64	No
	2	44	No
Wild-type ORSa	1	556	
	2	468	

^a Transformants with a T prefix were isolated in an *am*₁₃₂ *int* recipient. Those with TC prefix were isolated in an *am*₄ *ure-2 cot-1* recipient.

^b A unit of activity was defined as a change in optical density at 340 nm of 0.001 per min. Specific activity was expressed as units per milligram of protein.

^c T2 spores were grown as heterocaryons with *am*₁₃₂ due to tight colonial growth.

am transformants that are integrated on LGV have approximately wild-type levels of GDH; in contrast, all of the transformants with the *am* gene on a non-normal chromosome produce only 5 to 20% of wild-type levels of GDH. There are two possible explanations for this pattern of GDH production. One hypothesis would suppose that there is some promoter element or enhancer type (6) sequence that has become separated from the *am* gene either in the process of cloning the gene or in the process of integration at non-normal sites. In the absence of this sequence, the promoter would be utilized less efficiently, and thus, GDH production would be reduced. It is interesting to note that, if such an element were separated from the *am* gene by cloning, it must have been more than 1.5 kb upstream from the beginning of the GDH coding sequences. The second, somewhat less appealing hypothesis would be that all of the non-normal integrations have placed the *am* gene in an area of quiet DNA, and there, by virtue of being newly associated with DNA, which is transcriptionally inactive, the *am* promoter has become less accessible to RNA polymerase. One would have to suppose, under this hypothesis, that nonhomologous (or partially homologous) integration in the case of the *am* gene preferentially occurs into transcriptionally inactive DNA, since all of the transformants with *am* not on LGV have less-than-normal levels of GDH activity. There is no obvious reason why this should be so.

With the work presented here, the general applicability of the *N. crassa* transformation system developed by Case and co-workers (1, 2, 13) has been confirmed by using the cloned *am* gene in both λ and plasmid vectors. The capability demonstrated here of transforming *N. crassa* mutants with genes present in a λ vector should be of particular use in screening phage clones selected by using differentially expressed mRNAs as probe. For example, one could clone genes by the use, as a probe, of mRNA expressed specifically during the process of conidiation, as was done by Timberlake's group in *Asperillus* sp. (18). In this manner, one could identify clones that contained genes expressed only during conidiation; however, the function and identity of the gene cloned would remain unknown. Our results indicate that it should be possible to test these clones directly in complementation experiments of aconidial mutants by means of transformation, thus eliminating the time-consuming task of subcloning fragments into plasmids. This should significantly ease the task of identifying the particular gene cloned.

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