An Altered Invertase in the *cot-2* Mutant of *Neurospora crassa*

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SUMMARY

Because the *cot-2* and *inv* loci of *Neurospora crassa* are closely linked, the invertase from the morphological mutant, *cot-2*, was examined. The *cot-2* strains produce an invertase with altered heat sensitivity, K_m , and ratio of heavy to light forms. The cellular localization of *cot-2* invertase is different from that of the wild type. There were no observable changes in the energy of activation or the pH optimum of *cot-2* invertase, and some of the differences detected were not apparent under culture conditions that promoted wild-type growth. Since recombination (about 5 %) occurred between *cot-2* and *inv* and culture conditions affected the enzyme characteristics, we suggest *cot-2* determines, in part, the carbohydrate composition of the enzyme.

INTRODUCTION

Morphological mutants of *Neurospora crassa* are excellent test systems for studying the effects of gene action on morphology. These strains combine the simplicity of single-gene mutations with drastically altered physical form. Primary enzyme deficiencies are known for 11 such mutants, eight of which cause alterations in the area of carbohydrate metabolism involving glucose-6-phosphate (Brody, 1973).

Brody & Tatum (1966) found that the colonial morphology of the *col-2* strain is the result of a defective glucose-6-phosphate dehydrogenase (G-6-PD) and were the first to use this approach for studying morphogenesis in *Neurospora*. Subsequently, two other mutants, balloon and frost, were shown to possess a defective G-6-PD activity (Scott & Tatum, 1970). A defective 6-phosphogluconic acid dehydrogenase, another enzyme in the hexose monophosphate shunt, has been found in *col-3* and *col-10* (Lechner & Fuscaldo, 1969; Abramsky, Scott & Tatum, 1971; Fuscaldo, Lechner & Bazinet, 1971; Lechner, Fuscaldo & Bazinet, 1971). The ragged-1 and ragged-2 mutants studied by Brody & Tatum (1967) and Mishra & Tatum (1970) possess an altered phosphoglucumutase. As a final example, there is a morphological mutant studied by Murayama (1969) with an altered phosphohexoseisomerase. The three other morphological mutants for which enzyme deficiencies are known possess altered phospholipid metabolism (Crocker & Nyc, 1964; Pina & Tatum, 1967; Scarborough & Nyc, 1967).

cot-2, a morphological mutant of *N. crassa*, is characterized by colonial growth at 34 °C and near wild-type growth at 25 °C (Garnjobst & Tatum, 1967). *cot-2* is located 48 map units distal to the centromere in linkage group V (Morgan, Garnjobst & Tatum, 1967). Interest in the primary biochemical defect responsible for the *cot-2* morphology was stimulated when Sargent & Woodward (1969*b*) discovered and mapped the *inv* gene in the timex strain of *N. crassa*. This gene, a structural gene for invertase (β -D-fructofuranoside

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fructohydrolase, EC. 3.2.1.32), maps at approximately the same location as *cot-2* in the right arm of group V.

The problem, then, was to determine if an altered invertase is responsible for the *cot-2* morphology. The approximate frequency of genetic recombination between *cot-2* and *inv* was determined. The *cot-2* and wild-type invertases were compared biochemically using such parameters as K_m , energy of activation (E_a), pH optimum, thermolability, localization and specific activity. In addition, the physical structures of the two invertases were compared in a preliminary way by disc-gel electrophoresis and column chromatography.

METHODS

Strains and growth conditions. *Neurospora crassa* 74-OR23-1A (FGSC987, Fungal Genetics Stock Center, Humboldt State College, Arcata, California, U.S.A.) was used as the wild-type control. The original *cot-2a*, R1006 (FGSC1512), was backcrossed (Davis & de Serres, 1970) seven times to the wild-type strain to provide an isogenic background. The sets of isolates from each backcross were numbered MLS42-X to MLS48-X, consecutively. Ascospores from each cross were randomly isolated, heat shocked at 60 °C for 30 min and incubated at 34 °C after germination to identify the *cot-2* progeny. Strains used for most biochemical studies on *cot-2* invertase were 45-1, 47-8, and 48-1. Procedures for stock maintenance have been reported (Sargent & Woodward, 1969a).

An invertaseless strain, *inv*, *al*, *thi*, A (MLS49-1) and a strain *cot-2*, *al*, *thi*, a (MLS50-1) were constructed in preparation for determining the *inv* × *cot-2* recombination frequency. After the two strains were crossed, ascospores were collected, heat-shocked and plated on a medium containing 3 % (w/v) sorbose, 0.2 % fructose, 0.02 % thiamine, 1.5 % agar, and 1X Vogel's salts (Vogel, 1964). The *cot*⁺*inv*⁺ recombinant colonies were identified by germination at 35 °C, *in vivo* invertase activity (Sargent & Braymer, 1969) and albino pigmentation.

Mycelia were grown in a minimal-fructose medium (1X Vogel's salts and 1.5 % fructose) inoculated with 10⁵ conidia/ml. All cultures were grown at 25 °C for 72 h unless stated otherwise. Shake cultures (800 ml; linear growth) were incubated on a reciprocal shaker at 100 strokes/min. Bubble cultures (1600 ml; exponential growth) were vigorously aerated and harvested at a density of 1 mg dry wt mycelium/ml medium. In all cases, the mycelia were harvested by filtration and washed with cold (4 °C) water. The mycelia were immediately lyophilized, ground in a Wiley Mill using a 60-mesh screen, and stored at -20 °C.

Assay procedures. Crude extracts were prepared by shaking mycelial powder (50 mg/ml unless otherwise stated) with 50 mM-sodium acetate buffer pH 5.0 at 4 °C for 1 h. The mixture was then centrifuged for 20 min at 20000 g to remove cell debris, and the supernatant fraction (crude extract) collected. Total mycelial activity was determined by assaying the extracts before clarification, while soluble activity was determined from suitable dilutions of the crude extract. Exoenzyme levels were measured by assaying samples of the culture media that had been dialysed for 24 h against three changes of distilled water at 4 °C.

Three different fixed-time assays were used to measure invertase activity. The usual method was that in *Experimental Biochemistry* (1964) modified by increasing the reaction temperature from 25 to 37 °C and decreasing the final assay volume to 15 ml. A more sensitive, but less convenient, method based on Metzenberg (1962) and Somogyi (1952) has been described (Sargent & Woodward, 1969a), and was used when necessary. When excess fructose interfered with the colorimetric reaction, the Glucostat 4X reagent (Worthington Biochemical Corp.) was used specifically to measure glucose formation (Sargent &

Woodward, 1969*b*). A unit of activity is defined as that amount of enzyme that will cleave 1 μ mol sucrose/min under the stated assay conditions. Specific activity refers to units of activity/mg dry wt mycelium unless otherwise stated. Most experiments were repeated several times and enzyme assays were done in duplicate using different enzyme concentrations.

The method of Lowry *et al.* (1951) was employed to determine protein concentration using bovine serum albumin (Sigma) as a standard.

Enzyme kinetics. Heat sensitivity of invertase was determined by placing covered tubes of appropriately diluted crude extract into a water bath at 55 °C. Portions were removed at suitable intervals, placed on ice, and subsequently assayed for invertase activity. The method of Clark (*Experimental Biochemistry*, 1964) was employed to determine the Michaelis-Menton constant, K_m , from a Lineweaver-Burk plot. The energy of activation was determined by assaying crude extracts at several temperatures, and measuring the slope from an Arrhenius plot of the data. Activity as a function of pH was measured by making crude extracts with several different buffers (50 mg/ml) and using these buffers in the assay system (Metzenberg, 1963*a*).

Chromatography and electrophoresis. Separation of invertase forms on the basis of size was accomplished by Sephadex column chromatography (Metzenberg, 1964). All procedures were carried out at 4 °C in a 2 $\times$ 35 cm column using 50 mM-tris-HCl pH 7.5 as the elutant buffer. A 2 ml sample was applied to the top of the column under a 2 cm layer of buffer, and the effluent collected in approximately 2 ml samples for assay of invertase activity.

Polyacrylamide-gel disc electrophoresis (Davis, 1964) was used to examine crude extracts, purified invertase, and exoenzymes present in the growth medium. No spacer or sample gel was used. The running gel was made from 6 % (w/v) acrylamide and 0.2 % *N,N'*-methylenebis-acrylamide. Polymerization was initiated by the addition of 0.14 % ammonium persulphate and 0.06 % *N,N,N',N'*-tetramethylethylenediamine and the gels were allowed to polymerize for 1 h before use. Various buffers, including tris-glycine at pH 8.3 (Davis, 1964) and 50 mM-tris-HCl at pH values 7.2, 7.3 and 7.5, were employed in the running gels, with the former usually giving the best results.

Crude extracts were mixed (1:1, v/v) with 10 % (v/v) glycerol, and 10 μ l samples containing 50 μ g protein were applied to the gels. To study exoenzymes, media samples dialysed as before were concentrated using a flash evaporator (42 °C) until 2 % of the original volume remained. Samples containing 20 μ g protein were applied to the gels as before. The tracking dye was 0.05 % bromophenol blue; one drop was mixed with the sample before application. Gels were run 1 h at 4 °C and 2.5 mA.

Invertase activity in the gels was detected by placing them in 4.0 M-sodium acetate buffer pH 5.0 containing 1 M-sucrose, and incubating for 10 min at 37 °C. After the gels were washed in distilled water, the invertase activity was localized by a colorimetric reaction (Gabriel & Wang, 1969) for liberated fructose. The stained gels were washed and stored in 7.5 % (v/v) acetic acid, and scanned at 360 nm in a Gilford spectrophotometer with a linear transport system. A polar planimeter (Keuffel and Esser Co.) was used for curve integration.

RESULTS

Allelism test between inv and cot-2

To determine whether the *inv* and *cot-2* genes were allelic, a cross was made between an *inv*, *al*, *thi* strain and a *cot-2*, *al*, *thi* strain. Of the 2057 *cot*⁺ colonies tested from random platings, 101 proved to be *cot*⁺, *inv*⁺, thereby yielding a recombination frequency of about 4.9 %. The cross was not fully fertile as the germination rate was about 30 %. Crosses

Table 1. *Level and location of invertase activity*

Strain	Growth temperature (°C)	Invertase specific activity*			
		Total	Soluble	Wall-bound	Extracellular
Shake culture					
Wild type	25	1.68 (100)	0.21 (12.4)	1.46 (86.8)	0.015 (0.8)
<i>cot-2</i> (48-1)	25	1.40 (100)	0.34 (24.5)	1.05 (75.1)	0.005 (0.4)
<i>cot-2</i> (48-1)	34	2.15 (100)	0.74 (34.2)	1.41 (65.2)	0.012 (0.6)
Bubble culture					
Wild type	25	1.79 (100)	1.20 (67.1)	0.54 (29.9)	0.054 (3.0)
<i>cot-2</i> (R1006)	25	3.04 (100)	1.56 (40.8)	0.89 (43.8)	0.592 (15.4)
<i>cot-2</i> (47-8)	25	1.88 (100)	0.59 (31.4)	1.00 (53.0)	0.293 (15.6)
<i>cot-2</i> (48-1)	25	2.56 (100)	0.95 (36.9)	1.07 (42.2)	0.535 (20.9)

* Assays were done on unclarified mycelial suspensions, crude extracts, and the culture medium. Total activity equals the sum of the activities in the unclarified suspension and the culture medium. Soluble activity is that present in the crude extract, while wall-bound activity is obtained by subtracting the soluble activity from that in the unclarified suspension. Figures in parentheses represent percentages of the total activity.

involving *cot-2* characteristically give a low germination rate in this laboratory, and *cot-2* × *cot-2* crosses are known to yield round ascospores (Barry *et al.* 1972). If the low germination rate is accompanied by differential germination of various genotypes, the true recombination frequency may not be 4.9 %. It is clear, however, that the two genes are not allelic.

Growth conditions, morphology and invertase localization

In preliminary experiments it became clear that *cot-2* morphology and invertase characteristics are both affected significantly by mycelial growth conditions. For example, after 72 h growth, wild-type mycelia from shake cultures were well dispersed with an average density of 2 mg dry wt mycelium/ml medium. In contrast, *cot-2* in such cultures at 25 °C grew slowly and in the form of mycelial balls, even after very long growth periods. At higher restrictive temperatures, these mycelial balls were highly compact and adhered tightly to the sides of the flask.

Invertase specific activity and localization in 72 h shake cultures of *cot-2* grown both at a permissive (25 °C) and restrictive (34 °C) temperature were compared with the wild-type control grown at 25 °C. The results (Table 1) indicated that *cot-2* grown at the restrictive temperature had the highest level of total activity, and that *cot-2* had a lower proportion of the enzyme bound to the cell wall, especially at the restrictive temperature. The proportion of wild-type invertase that was wall-bound is comparable to that reported by Sargent & Woodward (1969*b*) and Chang & Trevithick (1972). In general, stationary cultures produced the same ratios of soluble to bound invertase as did the shake cultures.

In contrast to the above, mycelia from bubble cultures of both wild type and *cot-2* were well dispersed and ready for harvesting after 24 h growth. As seen in Table 1, growth in bubble cultures caused a reduction in the amount of wall-bound enzyme. In the wild-type strain the non-bound activity appeared primarily in the soluble fraction, whereas all three *cot-2* strains released much of that activity into the medium. All four strains produced more extracellular invertase when grown in these aerated cultures; 3 % of the total wild-type invertase was extracellular, while *cot-2* strains liberated up to one-fifth of their total invertase activity into the medium.

The level of soluble invertase in crude extracts of *cot-2* was expected to stabilize as the number of backcrosses increased. However, 72 h shake cultures of representatives from

Fig. 1. Heat sensitivity of invertase in crude extracts from shake cultures of (●) wild type and (○) *cot-2* (45-1) grown at 25 °C.

each backcross grown at the permissive temperature had different invertase specific activities (range 0.21 to 0.34 units/mg) with no pattern being evident. It was shown that soluble invertase activity in *cot-2* shake cultures varied with physiological age. However, there was still variation in invertase activity when the *cot-2* backcross representatives were grown in bubble cultures and harvested at the same physiological age.

Invertase heat sensitivity

The thermolability of invertase from the original *cot-2* strain (R1006) in crude extracts from shake cultures grown at 25 °C was slightly different from wild-type invertase at 50 and 60 °C, but radically different at 55 °C (Fig. 1). Representatives from the first four backcrosses, and all fifth-backcross strains had similar heat-sensitive invertases which retained 1 to 8 % of their original activity after 1 h at 55 °C (Table 2). Of the nine random isolates from the sixth backcross, only one (47-8), possessed a typically heat-sensitive invertase. The invertases of the remainder, including the parent strain (47-1) for the seventh backcross, displayed a heat sensitivity intermediate between that of the wild type and *cot-2* (R1006) enzymes. The five isolates of the seventh backcross were further modified, the heat sensitivity of their invertase approaching that of the wild type. The variability between experimental runs was possibly caused by small but critical fluctuations in the water-bath temperature.

When crude extracts of *cot-2* and wild type were diluted to give equal invertase activities, and then combined and assayed, the heat sensitivity of the mixture was intermediate in value. This suggested that the heat sensitivity observed in the *cot-2* enzyme was not due to the absence or inactivation of some stabilizing molecule present in wild-type crude extracts. Metzenberg (1964) reported that light invertase (5.2 S) is considerably more heat sensitive than heavy invertase (10.3 S; a tetramer), but that the *cot-2* strains have, if anything, a higher proportion of the heavy invertase (see below); therefore an altered light/heavy ratio is not responsible for the increased heat sensitivity.

Fig. 2

Fig. 3

Fig. 2. Lineweaver-Burk plot for invertase assayed at 37 °C in crude extracts from shake cultures of (●) wild type and (○) *cot-2* (45-1) grown at 25 °C. [S] is in μmol sucrose/ml.

Fig. 3. Sephadex G 200 elution patterns of soluble invertase from shake cultures of (●) wild type and (○) *cot-2* (45-1) grown at 25 °C.

Table 2. Heat sensitivity of invertase from wild type and *cot-2*

Assays were done on crude extracts from shake cultures grown at 25 °C.

Strain	Invertase specific activity remaining after 1 h at 55 °C (%)		
	Expt 1	Expt 2	Expt 3
Wild type	51.4	70.2	51.8
<i>cot-2</i>			
R1006	8.1	—	—
42-1	5.1	—	—
43-1	3.8	—	—
44-1	3.3	—	—
45-1	6.2	—	—
46-2	3.7	4.3	18.6
47-8	—	—	18.3
47-1	34.2	—	—
47-2	—	30.3	—
47-4	—	35.7	—
47-5	—	36.7	—
47-6	—	39.5	—
47-7	—	—	34.8
47-9	—	27.6	—
48-1	—	—	42.8
48-2	45.8	66.1	47.4
48-3	—	70.3	44.4
48-4	—	73.0	39.7
48-5	—	—	47.7

Invertase K_m and E_a

The K_m for *cot-2* invertase, measured in crude extracts from material grown in shake cultures, was consistently different from that of the wild-type control (Fig. 2; Table 3). This difference, about a 30 % decrease compared with wild type, was apparent for values obtained at three different temperatures, 25, 37 and 45 °C (Table 3). When the strains were grown in bubble cultures, however, the K_m of *cot-2* invertase resembled that of the wild-type control

Table 3. K_m of invertase from wild type and *cot-2*

Assays were done on crude extracts from cultures grown at 25 °C.

Assays on different strains*	K_m (mM)	
	Shake cultures	Bubble cultures
Wild type	7.7	6.4
<i>cot-2</i> (45-1)	5.4	—
<i>cot-2</i> (48-1)	5.0	6.5
<i>cot-2</i> (R1006)	—	6.2
<i>cot-2</i> (47-8)	—	6.5

Assays at different temperatures (°C)†	K_m (mM)	
	Wild type	<i>cot-2</i> (48-1)
25	6.0	4.1
37	7.7	5.0
45	9.3	6.4

* Assays done at 37 °C.

† Crude extracts from shake cultures.

(Table 3). Metzenberg (1963*a*) reported K_m values at 37 °C of 6.1 mM for purified invertase (unspecified wild-type strain) and 6.6 mM for invertase in crude extracts. Our K_m values (6.2 and 6.5) from bubble cultures are similar to Metzenberg's which were obtained on extracts from vigorously aerated cultures.

The E_a for *cot-2* (45-1 and 48-1) invertase in crude extracts was identical to that of wild type and had an experimental value of 9.7 kcal/mol. Likewise, the pH optimum curves for the wild-type and *cot-2* (48-1) invertases were similar, with a broad range of activity between pH 4.0 and 7.0 and an optimum between 4.5 and 5.0 as reported by Metzenberg (1963*a*).

Invertase chromatography and electrophoresis

Elution patterns of wild-type and *cot-2* invertase (crude extracts) from Sephadex G-200 (Fig. 3) revealed two peaks, the first corresponding to the tetramer, heavy invertase, and the second to its monomer, light invertase (Metzenberg, 1964). The data indicate a difference in the relative proportions of heavy and light invertase in *cot-2* extracts from shake cultures as compared with wild type. Integration of the peak areas revealed that 68 % of the *cot-2* invertase was present as the heavy form, while the control strain had a broader peak of heavy invertase containing 57 % of the total activity. In some experiments the overall shape of the curve of wild-type invertase was more like that of the *cot-2* enzyme, but the wild-type invertase consistently had a smaller proportion of the heavy form. There were no reproducible differences in the time of elution between the *cot-2* and wild-type enzymes, and when equal volumes of *cot-2* and wild-type crude extracts were applied to the top of a column, the elution pattern showed no broadening of the activity peaks.

The increased proportion of heavy invertase from *cot-2* grown in shake cultures was also apparent in acrylamide-gel disc electrophoresis. This increase was found with both the soluble and extracellular invertases (Fig. 4*a* to *d*). Approximately 73 % of the *cot-2* soluble invertase was present as the tetramer, compared with 36 % for the wild-type control. The extracellular invertases had 47 and 28 %, respectively, of their activity in the heavy form; they had a smaller fraction of the heavy form than found in the soluble invertases. A

preponderance of the light form in the extracellular invertase is to be expected on the basis of fractionation at the cell wall as described by Trevithick & Metzenberg (1966).

No significant differences were detected between the light/heavy ratios of the wild-type and *cot-2* soluble invertases in crude extracts from bubble cultures (Fig. 4*e, f*). The migration rates (R_m) of the invertase forms of *cot-2* and the wild type differed slightly.

DISCUSSION

After backcrossing *cot-2* for seven generations, and after completing several of the biochemical comparisons, it was discovered that the *cot-2* test strain (48-1) had apparently acquired some modifier gene(s) during the sixth backcross. We have presented the results from strain 48-1 and several strains obtained earlier in the backcrossing protocol, and these show that in all characteristics except heat sensitivity, the invertases from the backcross progeny, including 48-1, are identical.

Most of the comparisons between *cot-2* and wild-type invertase involved mycelium grown at 25 °C, the permissive temperature. Except where otherwise required, the assays were conducted at a restrictive temperature (37 °C), but a permissive growth temperature was usually employed because at higher temperatures the *cot-2* mycelium formed small, sticky balls that prevented the harvest of adequate amounts of mycelium. It is possible that biochemical comparisons between mycelia grown at higher temperatures would have shown larger differences between the respective enzymes. The change in the localization of *cot-2* invertase (Table 1) when *cot-2* was grown at 34 °C rather than 25 °C supports this.

Most of the comparisons between the *cot-2* and wild-type invertases were made using the soluble enzyme (itself a mixture of heavy and light forms). Those experiments (Table 1; Fig. 4) which involved the wall-bound and extracellular invertases showed that the differences were not restricted to the soluble enzyme.

After the sixth backcross, *cot-2* progeny (e.g. 47-1 to 47-7) with an invertase of intermediate heat sensitivity were isolated. These strains probably do not represent segregation of the *cot-2* locus from the gene responsible for the altered invertase because (i) a high proportion of the *cot-2* strains isolated from the sixth backcross have intermediate heat sensitivity, (ii) the heat sensitivity is intermediate and only approaches that of wild-type invertase in the seventh backcross strains, and (iii) the *cot-2* invertases of the sixth and seventh backcrosses still have an altered K_m , heavy to light invertase ratio, and soluble to bound invertase ratio. We suggest that during the sixth and seventh backcrosses, modifier genes that primarily affect the heat sensitivity of invertase were incorporated through recombination into the strains that were isolated.

Recombination between the *inv* and *cot-2* loci indicates that *cot-2* does not play a role in determining the primary structure of the invertase subunit specified by the *inv* gene (Sargent & Woodward, 1969*b*). There is evidence that *Neurospora* invertase may be composed of two non-identical subunits (Meachum, Colvin & Braymer, 1971; H. D. Braymer, personal communication), so it is possible that *cot-2* is the structural gene for the second subunit. A more plausible alternative is that the *cot-2* gene alters the structure of invertase in a more indirect way, perhaps by changing the composition, quantity, or location of the carbohydrate known to be part of this enzyme (Meachum *et al.* 1971). This hypothesis is more compatible with the finding that under conditions (shake culture) that stimulate colonial growth of *cot-2*, the extracted enzyme is quite different from that of the wild-type strain, while under conditions (bubble culture) that stimulate 'wild-type' growth of *cot-2*, some of the differences, i.e. in K_m and light/heavy ratio, are no longer apparent (Table 4). If adequate

Fig. 4. Polyacrylamide gel patterns of wild type and *cot-2* (48-1) invertase using tris-glycine buffer, pH 8.3. (a) Soluble invertase from *cot-2* grown in shake culture; (b) soluble invertase from wild type grown in shake culture; (c) extracellular invertase from *cot-2* grown in shake culture; (d) extracellular invertase from wild type grown in shake culture; (e) soluble invertase from *cot-2* grown in bubble culture; (f) soluble invertase from wild type grown in bubble culture. All cultures were grown at 25 °C.

Table 4. Summarized comparison of invertase from wild type and *cot-2*

Characteristic	Shake culture		Bubble culture	
	<i>cot-2</i>	Wild type	<i>cot-2</i>	Wild type
Fraction wall bound (%)	75	87	42	30
Fraction extracellular (%)	0.4	0.8	21	3.0
Activity after 1 h at 55 °C (%)	4	52	—	—
K_m at 37 °C (mM)	7.7	5.0	6.5	6.4
E_a (kcal/mol)	9.7	9.7	—	—
pH optimum	~5.0	~5.0	—	—
Fraction heavy form (%)	73	36	70	70
Mobility of heavy form (R_m)	0.19	0.21	0.17	0.24

amounts of the *cot-2* invertase could be obtained the hypothesis could be tested directly by comparing the amino acid and carbohydrate content of the *cot-2* and wild-type enzymes.

If the *cot-2* gene somehow affects the structure of invertase, is this the primary function of the *cot-2* gene or a result of other changes in metabolism, e.g. carbohydrate or cell-wall metabolism? Furthermore, if the primary function of the *cot-2* gene is to affect invertase structure, how is the altered invertase responsible for the colonial morphology? Our data provide no direct evidence regarding these questions, but there are several lines of evidence that tie the enzyme invertase to the cell wall, and link the cell wall and its enzymes to fungal morphogenesis (Brody, 1973; Mahadevan & Mahadkar, 1970; Ghosh, Montencourt & Lampen, 1973). It is known that fungal invertases have synthetic transferase activity (the *Neurospora* enzyme may be less active in this regard, however; Trevithick & Metzberg, 1964), that *Neurospora* invertase is physically associated with the cell wall (Metzberg, 1963*b*; Chung & Trevithick, 1970; Chang & Trevithick, 1972; Bigger, White & Braymer, 1972), and that the *inv* mutant is morphologically abnormal (decreased conidiation) even when grown on glucose (Sargent & Woodward, 1969*a*). Hopefully, further study will relate these facts to the specific mechanisms involved.

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