Loss of cytosolic serine hydroxymethyltransferase in a formate mutant of *Neurospora crassa*

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Summary. In the wild type of *Neurospora crassa* serine hydroxymethyltransferase occurs in the cytosol and mitochondria. The formate mutant (FGSC 9) lacks the cytosolic activity but has increased levels of isocitrate lyase and glyoxylate aminotransferase. Glycine is derived from glyoxylate in the mutant.

Two genetically distinct formate or formaldehyde-requiring mutants of *Neurospora crassa* were originally isolated by Harrold and Fling[1]. Formate could be replaced by adenine and methionine, but not by serine, in their C-24 mutant (FGSC 9). Enzyme studies[2,3] of this mutant subsequently showed a partial deficiency in serine hydroxymethyltransferase (SHMT), a key enzyme for methylenetetrahydrofolate (THFA) biosynthesis. This impairment is apparently offset by increased levels of formylTHFA synthetase and methylene-THFA dehydrogenase[3], but the origins of glycine are not clear. There is now excellent evidence for cytosolic and mitochondrial isoenzymes of SHMT in a variety of tissues[4–9], but there is no comparable data for *Neurospora*. Clearly such information could have a bearing on the lesion resulting in a requirement for a C_1 compound. As part of a study of folate metabolism in *N. crassa*, we have now examined SHMT compartmentation in the wild type and the C-24 for mutant.

Material and methods. *N. crassa* Lindegren A wild type (FGSC 853) and the for mutant (FGSC 9) were cultured for 22 h in liquid media[3], harvested and homogenized in iso-osmotic buffer[10]. Differential centrifugation[10] was followed by isopycnic centrifugation[11] of the mitochondrial pellet. SHMT was assayed by measurement of methyleneTHFA production[12] or by isolation of serine formed in reaction systems containing formaldehyde, THFA and [2-^{14}C] glycine. Isocitrate lyase[13] and alanine:glyoxylate aminotransferase[14] were assayed by standard methods. In [1-^{14}C] glyoxylate feeding experiments mycelia of both strains were fractionated to obtain labelled products as previously described[3].

Results and discussion. Data in table 1 suggest that SHMT activity in *N. crassa* is compartmented as in other eucaryotes. Under our assay conditions, both supernatant and particulate activities favoured serine synthesis, and for both strains this ability was most pronounced in protein sedimented by the 26,000 × g centrifugation. The supernatant fraction of the mutant had essentially no ability to generate methyleneTHFA from serine. In the wild type, this activity was apparently associated with a cytosolic enzyme as higher centrifugation speeds (e.g. 48,000 × g for 40 min) did not reduce specific enzyme activities of the supernatant. The low SHMT activity in the supernatants of the mutant probably resulted from leakage of enzyme from the pellet. This was supported by the observation that partial solubilization occurred when the particulate material was alternately sedimented and resuspended. It is noteworthy that the mitochondrial SHMT of animal tissues is a matrix component and is readily solubilized by mild treatments[9]. Using succinic dehydrogenase as a marker enzyme for *Neurospora* mitochondria[11], we examined the sedimentation of the particulate SHMT in sucrose gradients. A protein band was recovered from both strains which displayed maximal SHMT and marker enzyme activities. After centrifugation, this band was associated with sucrose densities similar to those of characterized *Neurospora* mitochondria[11], and accordingly we conclude that SHMT is associated with this organelle.

Our previous analyses[3] of the mutant showed that the glycine pool was low in comparison with the wild type, and that growth was stimulated some 50% when 2 mM glycine was supplied with the usual formate supplement. The data in table 1 suggests that the mutant has some ability to generate glycine via the mitochondrial SHMT. However the total extractable SHMT activity in the mutant was only 1% of that found in the wild type. Glycine must therefore arise by an alternate pathway during growth of the mutant. Turian's laboratory[15] has presented evidence for an isocitrate → glyoxylate → glycine pathway in *N. crassa*, but its possible importance in mutants deficient in SHMT has not been assessed. Assays of isocitrate lyase and glyoxylate aminotransferase showed that the formate mutant had specific activities that were 2–3 times higher than those of the wild type. Chromatography[16] of the lyase activity revealed that this was due to increases in the amounts of lyase I. In other work we find that the amount of this enzyme in the mutant can be reduced by glycine supplements. Also glycine appears to affect the derepression of this enzyme in sucrose-free media. Thus the pool size of glycine or its metabolic products may have some effect on the amount of isocitrate cleavage in *Neurospora*.

The importance of glyoxylate as a precursor of glycine in the mutant is further indicated by the data in table 2. In both strains, glyoxylate carbon was rapidly incorporated into the free pools of glycine, serine and aspartate. However in the mutant the specific radioactivity of glycine was some 4–5 times higher than the wild type. Serine was also more heavily labelled in the mutant but more ^{14}C entered aspartate in the wild type. The latter strain has approximately 50% more malate synthetase activity than the mutant, so this labelling probably reflects entry of the supplied glyoxylate into the C_4 acid pool.

Table 1. Compartmentation of serine hydroxymethyltransferase

Strain	Cellular fraction	Specific enzyme activity (nmole product/min/mg protein)	
		Serine → glycine	Glycine → serine
Wild type	26,000 × g supernatant	0.416	1.12
	Crude mitochondrial pellet	0.210	2.65
Formate mutant	26,000 × g supernatant	0.003	1.51
	Crude mitochondrial pellet	1.670	5.76

Extracts of 22 h mycelia were prepared in isotonic buffer and fractionated by differential centrifugation. The mitochondrial pellets were washed by resuspension and sedimentation at 26,000 × g. Enzyme activity was assayed in both reaction directions using equal specific radioactivities of [3-^{14}C]-serine and [2-^{14}C]glycine respectively. Data are averages of 2 separate experiments. On a dry wt of mycelium basis the mutant had approximately 1% of the total extractable SHMT of the wild type.

In conclusion, loss of cytosolic SHMT appears to necessitate alternative sources of methyleneTHFA and glycine. The partial reactions of the glyoxylate cycle appear to generate the latter metabolite in the mutant but the localization of this reaction sequence remains to be elucidated. The mitochondrial SHMT may have importance in serine synthesis as is indicated by serine labelling in the mutant (table 2). Further studies of such compartmentation of folate metabolism in *Neurospora* appear warranted. Mutants like those of *Saccharomyces*[4] which lack the mitochondrial enzyme could be useful in this regard.

Table 2. Major products of [1-^{14}C]glyoxylate metabolism

Product	Feeding (min)			
	Wild type		Formate mutant	
	5	15	5	15
Glycine	35.1	223	158	1348
Serine	29.3	84	64	108
Aspartate	87	537	49	216

Data are expressed in cpm $\times 10^{-2}$/μmole of product.

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Natural hybridization in *Drosophila*

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Summary. Although at least 159 cases of interspecific hybridization between closely related species of *Drosophila* have been obtained under laboratory conditions, only 7 cases of natural interspecific hybridization have been recorded. We report yet another case, concerning *D. malerkotliana* and *D. bipectinata.*

The genus *Drosophila* contains well over 1000 valid species, many quite wide-spread[2]. The genus is remarkable in that laboratory-induced interspecific hybridizations between closely related species have been obtained rather commonly but natural hybridization between them seems to be very rare. Only 7 cases of natural interspecific hybridization have been recorded in the genus so far, viz.: *mulleri* and *aldrichi; montana* and *flavomontana; melanogaster* and *simulans; metzii* and *pellewae; pseudoobscura* and *persimilis; setosimentum* and *ochrobasis; heteroneura* and *silvestris*[3]. *Drosophila malerkotliana* Parshad and Paika and *D. bipectinata* Duda are 2 very closely related species which are the commonest, and also coexist in various localities, in the subcontinent of India. Females of these species are practically indistinguishable, while males can be very easily separated on the basis of abdominal tergites pigmentation and of the sex-comb pattern. Male genital structures in these species are apparently identical. However, the male hybrids are easily distinguished from either parental species, being intermediate for tergites pigmentation and sex-comb[4]. Reciprocal crosses between *D. bipectinata* and *D. malerkotliana* in the laboratory produce hybrid individuals of both sexes, males being sterile while females are fertile. Besides this, much is known regarding the karyotypes of the 2 species, and their phylogenetic relationships[5–8].

During the survey of various areas for Drosophilid fauna, it was found that these species are not only sympatric but also dominant in Kushmahi forest, an ecologically undisturbed area. Several collections were undertaken in this region to obtain an adequate number of individuals representing both species. While sorting out the captures, 3 males were found among 5000 specimens belonging to both species and to either sex, which had all the morphological characteristics of F_1 hybrids. These males were then tested for fertility by back-crossing to virgin females of both parental species in separate culture vials, but in no case were offspring produced. It is thus inferred that the female of 1 species had been inseminated by an alien male in nature.

Besides this, 397 wild-caught females representing both species, separated immediately after collection, were tested for the possibility of insemination by alien males in nature. These females were placed singly in separate culture vials. Among them, 78 had no offspring (flies died or no eggs were laid), 318 yielded progeny exclusively belonging either to one or the other species, while 1 culture contained offspring which were intermediate, characteristic of F_1 hybrids. In order to test male hybrids from this vial for fertility, backcross and inbreeding tests were employed which confirmed them as sterile.

Based on the above findings, we infer a certain amount of hybridization and, since hybrid females are fertile, introgression occurs between the 2 species in wild populations. To our knowledge, this is the 8 case of natural interspecific hybridization in the genus reported to-date.

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