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A Gene Modifying the Thiamine-requirement of Strains of *Neurospora crassa*

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SUMMARY: A new gene, *thi-1o*, is described in *Neurospora crassa*. This gene is shown to be a modifier of the nutritional response of strains containing the *thi-1* gene, and is not nutritionally detectable in the absence of the *thi-1* mutant locus. The gene *thi-1o* is additionally characterized by a decreased ability to synthesize thiamine from thiazole and pyrimidine; *thi-1o* appears to modify only *thi-1* strains because of their unusually high thiamine requirements. The nutritional responses of strains containing either *thi-2* or *thi-3* loci are not affected by the presence of *thi-1o*. Both *thi-1o* and *thi-4* are anomalous in the condensation of pyrimidine and thiazole. The probability that *thi-1o* may be an isoallele of *thi-4* is based upon a similarity of metabolic pattern and upon location on chromosome III. The failure to obtain recombination in crosses between strains containing *thi-1o* and *thi-4* constitutes additional support for their allelism.

The metabolic pathway of thiamine synthesis in *Neurospora* is not entirely agreed upon. Tatum & Bell (1946) concluded that thiamine is derived by a condensation of substances equivalent to vitamin pyrimidine (2-methyl-4-amino-5-ethoxymethyl pyrimidine) and vitamin thiazole (4-methyl-5- β -hydroxyethyl thiazole). In subsequent work, Harris (1953) stated that such a scheme does not account for the requirements and the accumulation products of the *Neurospora* thiamine-requiring mutants. Harris (1955) also proposed an alternative scheme in which a pyrimidine-incomplete-thiazole intermediate is formed prior to the formation of intact thiamine. The quantitative nutritional response of the thiamineless strains is critical in the formulation of either of these alternative proposals. According to Barratt, Newmeyer, Perkins & Garnjobst (1954), the various thiamine-requiring strains of *Neurospora* have these qualities:

Symbol	Isolation no.	Linkage group	Growth responses			
			Thiamine	Thiazole	Pyrimidine	Pyrimidine & thiazole
<i>thi-1</i>	56501/17084	I	+	-	-	+
<i>thi-2</i>	9185	III	+	-	-	-
<i>thi-3</i>	18558	VII	+	+	-	+
<i>thi-4</i>	85902	III	+	-	-	-

As part of the continued investigation of this problem (Eberhart, 1956), several thiamineless strains have been examined for the presence of modifying

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genes which might affect quantitative or qualitative requirements of these thiamineless mutants. Although no modifiers were found which contribute to a re-evaluation of the thiamine metabolism in *Neurospora*, a gene modifying the *thi-1* locus was found in certain wild-type strains. The purpose of this paper is to describe this new gene, *thi-1o*, in terms of its genetic behaviour and metabolic expression.

METHODS

The basal medium for growing *Neurospora* was that of Beadle & Tatum (1945). Crosses were made using the chemically defined medium of Westergaard & Mitchell (1947).

Coccarboxylase determinations essentially followed the procedure of Westenbrink & Steyn-Parvé (1950) as follows: 1 g. dried yeast* was suspended in 20 ml. of tris (2-amino-2-hydroxymethyl propane-1:3-diol; Sigma Chemical Co., St Louis, Mo.) buffer at pH 9.0 (0.1 M). The preparation was centrifuged, and three similar washings with distilled water followed quickly. For optimal results this procedure took no more than 30 min. The yeast was then suspended in phosphate buffer (pH 6.2, 0.1 M) with a final volume of 12 ml. One ml. of a 0.1 M-magnesium chloride solution was added, followed by 1 ml. of a thiamine solution (equiv. 100 μ g. thiamine). The yeast preparation was stored at 5° until used within 24 hr.). Storage by freezing destroyed only half of the carboxylase activity. One-half ml. of this preparation was added to each Warburg flask (total volume 3 ml.). The substrate consisted of 0.2 ml. of a 2% (w/v) pyruvate solution.

Thiamine and thiamine pyrophosphate were determined by the thiochrome technique of Stiller (1950). Thiochrome was assayed in a Nefluorophotometer (Fisher Scientific Co., New York, N.Y.). In experiments in which the thiamine concentrations were high, direct water-phase thiochrome determinations were successful without the usual isobutanol extraction.

Thiamine synthesis and thiamine phosphorylation were measured by the incorporation of exogenous thiazole and pyrimidine or of thiamine into 'bound' thiamine (i.e. thiochrome not soluble in isobutanol) in mycelia of optimally grown strains of *Neurospora*. Typically, 5 μ g. thiamine/100 ml. were included in the growth media of all strains tested. The strains were grown with agitation for 40 hr. at 30° (exceptions are noted), and filter-sterilized thiazole and pyrimidine or thiamine were then added. After further incubation, the extracts were examined for 'bound' thiamine and for total thiamine (thiochrome soluble after takadiastase treatment).

Air-dried mycelia with carboxylase activity were prepared from *Neurospora* cultures which were filtered on paper in a Buchner funnel, and washed with three volumes of distilled water. During the final washing the mycelia were suspended so that the resulting pad was spread evenly on the filter-paper. The damp mycelial pad was removed from the funnel and placed on a

* The authors had no success with dried 'Active' Bakers' yeast preparations, but found air dried Brewers' yeast (Falstaff of San Jose, California) to be satisfactory for cocarboxylase determination.

glass surface. The pad was gently pressed so that it would stick to the glass, and an air stream from an electric fan was used to dry the pad as quickly as possible. When thoroughly dry (coolness of the pad indicates evaporation is still in progress) the mycelial pad was removed from the glass, weighed, and stored in a desiccator in the dark. Mycelial preparations of this type retain carboxylase activity for as long as a year.

The carboxylase activity of dry *Neurospora* mycelia was measured in a Warburg-Barcroft manometer. Weighed samples of mycelium (about 20 mg.) were placed in the manometer flask, and 2.8 ml. of 0.1 M-phosphate buffer (pH 5.6) were added. Pyruvate substrate solution (0.2 ml. of a 2%, w/v, solution) was added to the side arm. When the closed manometric system reached equilibrium in a 33° water bath, the substrate was added and readings were taken at 5 min. intervals. The first three readings were critical because of the inhibitory effects of the acetaldehyde produced from the pyruvate.

The strains of *Neurospora crassa* used in this study were: (wild types) STA, STa, SY7A, SY4a, (thiamine mutants) 56501a (*thi-1*), 17084-44602 (*thi-1*, pyridoxine), 9185A (*thi-2*), 18558a (*thi-3*), 85902A (*thi-4*). All other strains are derived from crosses of the above strains (Table 1). These strains were obtained from the lyophilized collection at Stanford University, California.

Table 1. Crosses described in the test

Cross no.	Strains in cross	Type of auxotrophy*				
		Proto-trophs	Auxo-trophs	<i>B</i> ₁ or <i>Tz</i> + <i>Py</i>	<i>B</i> ₁	<i>B</i> ₁ or <i>Tz</i>
56501-3	56501a × SY7A (<i>thi-1</i> × <i>thi-1o</i>)	59	59	37	22	—
56501-6	56501-3-69A × 9185a (<i>thi-1</i> ; <i>thi-1o</i> × <i>thi-2</i>)	47	46	46	5	41
56501-7	56501-5(11-5) × SY7A (<i>thi-1</i> ; <i>thi-3</i> × <i>thi-1o</i>)	(See text)				
56501-17	56501-3-90A × 56501-3-88a (<i>thi-1</i> × <i>thi-1</i> ; <i>thi-1o</i>)	—	—	—	—	—
18558-1	18558A × 56501-7-54a (<i>thi-3</i> × <i>thi-1o</i>)	35	34	—	—	34
85902-2	85902A × 56501-3-88a (<i>thi-4</i> × <i>thi-1</i> ; <i>thi-1o</i>)	15	55	—	55†	—
85902-3	85902A × 56501-3-93a (<i>thi-4</i> × <i>thi-1</i>)	26	64	28	36	—
9185-1	STA × 9185a (wild × <i>thi-2</i>)	0	69	—	69	—

* Will grow on: *B*₁ = thiamine, *Tz* = thiazole, *Py* = pyrimidine.

† 15 *thi-1*, *thi-1o* and 40 *thi-4* strains as determined by 5 day growth response.

RESULTS

Since the possibility of the existence of modifier genes in *thi-1* stocks had never been adequately investigated, a cross of *thi-1* and wild type SY7A was made (cross 56501-3). In the random isolates of this cross a number of novel thiamine-requiring strains were found, which were characterized by their inability to grow on thiazole and pyrimidine (either together or separately) in a

standard 3-day assay at 25°. The requirements of these new strains were therefore qualitatively similar to those of *thi-2* or *thi-4* strains, while their quantitative responses to intact thiamine remained quite characteristic of normal *thi-1* strains (Table 2). Subsequent investigation showed that a new gene, *thi-1o*, is responsible for this modification of the *thi-1* phenotype in these unusual strains. This gene apparently came from SY7A stocks.

Table 2. *The growth response of isolates from cross 56501-3*

Test cultures grown three days at 25° in 20 ml. minimal with supplements noted above.

Additions (μ g./20 ml.)	Isolate number				
	8	40	69	71	77
	(<i>thi-1</i>)	(<i>thi-1</i>)	(<i>thi-1</i> ; <i>thi-1o</i>)	(<i>thi-1</i> ; <i>thi-1o</i>)	(<i>thi-1</i>)
	dry wt. mycelium (mg.)				
Thiamine					
0	6	4	4	2	5
0.05	14	12	14	13	14
0.1	16	15	16	18	20
1.0	40	43	49	45	42
5.0	63	66	70	56	75
20.0	65	72	80	64	70
Thiazole					
0.05	4	3	2	4	5
0.1	4	5	3	3	4
1.0	6	5	3	4	6
5.0	7	7	3	3	8
20.0	13	13	5	5	15
Pyrimidine					
5.0	4	4	3	3	4
20.0	4	3	3	2	2
Thiazole + pyrimidine					
0.1 0.05	6	+0	3	2	6
0.5 0.05	8	7	3	2	6
1.0 0.05	9	8	3	2	6
10.0 0.05	12	12	4	6	14

Mechanism of thi-1o action

Additional insight into the metabolic basis of the *thi-1o* modifier was sought by studying the thiamine synthesizing abilities of various strains containing the *thi-1o* modifier. The steps involved in the biological condensation of thiazole with pyrimidine, and the subsequent phosphorylation of thiamine, were investigated directly. In designing a test system for the *in vivo* thiamine metabolism of these strains, there were certain general considerations involved. The *in vivo* thiamine metabolism has at least three steps: the condensation of thiazole with pyrimidine, the phosphorylation of thiamine, and the binding of thiamine diphosphate within the cell. Since it is not yet possible to block any of these steps successfully without interfering with metabolism in general, an examination of the over-all system is required.

For example, the thiazole-pyrimidine condensing system must be measured in terms of 'bound' thiamine, because the ability of *Neurospora* strains to

phosphorylate and maintain thiamine becomes a potentially limiting factor when bound thiamine is used as an index of pyrimidine and thiazole condensation. The fate of exogenous thiamine was, therefore, considered to be the first logical step in the study of these systems. Figure 1 illustrates the ability of several *Neurospora* strains to take up and maintain thiamine. Each culture was grown for 40 hr. at 30° on minimal medium + 0.05 µg. thiamine/ml. and then incubated with 50 µg. thiamine. The uptake and maintenance of thiamine by these strains are quite similar despite their extremely different thiamine requirements.

Fig. 1. Thiamine synthesis by *Neurospora*. Cultures were grown 40 hr. at 30° with 2.5 µg. thiamine/50 ml. minimal medium. At 40 hr. 50 µg. thiamine (a) or 50 µg. thiazole + 50 µg. pyrimidine (b) were added. In control flasks there were no additions (c). Strains used were: □, *thi-1* (56501a); ●, *thi-1*; *thi-1o* (17084-1-3); ○, *thi-3* (18558A); ○, *thi-1o* + (STA).

A test for the presence of the *thi-1* gene in *Neurospora* strains was devised by incubating optimally-grown mycelia cultures in the presence of 50 µg., each of thiazole and pyrimidine and measuring the thiamine synthesized by thiochrome fluorescence. The results are also shown in Fig. 1. With the exception of the *thi-1o* containing strain, these cultures produced similar amounts of thiamine from exogenous pyrimidine and thiazole. The *thi-1o* containing strain was unable to produce a significant amount of thiamine from its precursors. These results and the results in Table 3 indicate that this test is a valid means of detecting the presence of *thi-1o* in strains independently of nutritional tests.

Other strains were tested for their ability to condense pyrimidine and thiazole. The experiment illustrated in Fig. 2 shows the relative synthetic rates of a few of these strains. The substrates of 100 µg. each of pyrimidine and thiazole were introduced into 48 hr.-old mycelial cultures grown in the standard optimal media. Both *thi-1* and *thi-3* strains show good rates of thiamine synthesis,

while the *thi-2* and *thi-10* strains show very little increase in thiamine. Interestingly, even the SY7A (*thi-10*) strain had relatively low activity in this test system, despite the fact that the growth of this wild-type strain was unaffected by thiamine.

Table 3. *The pyrimidine and thiazole condensing abilities of Neurospora mycelia*

Cultures were grown in 300 ml. Erlenmeyer flasks with 150 ml. minimal medium + 5 μ g. thiamine/100 ml. Cultures were shaken at 30° for 41 hr., then 100 μ g. of pyrimidine and thiazole were added aseptically and the cultures were shaken for another 12 hr.

Strain		No addition		Pyrimidine 100 μ g., thiazole 100 μ g.	
		Final dry wt. mycelium (mg.)	Relative thiochrome fluor- escence	Final dry wt. mycelium (mg.)	Relative thiochrome fluor- escence*
56501 a	<i>thi-1</i>	0.386	8	0.372	35
56501-3-70 a	<i>thi-1</i>	0.449	9	0.478	50
56501-3-71 A	<i>thi-1</i> ;	0.350	6	0.400	8
	<i>thi-10</i>				
56501-3-75 a	<i>thi-1</i>	0.384	6	0.360	34
56501-3-77 a	<i>thi-1</i>	0.378	9	0.455	45
56501-3-86 A	<i>thi-1</i>	0.370	9	0.400	33
56501-3-87 A	<i>thi-1</i>	0.380	7	0.413	32
56501-3-88 a	<i>thi-1</i> ;	0.450	8	0.420	9
	<i>thi-10</i>				
56501-5-(15-8)	<i>thi-3</i>	0.434	4	0.440	38
56501-5-(15-5)	<i>thi-1</i>	0.430	8	0.480	40
56501-5-(15-3)	<i>thi-1</i>	0.448	8	0.460	40

* Relative fluorescence here is directly proportional to thiamine (thiochrome) concentration in the mycelium.

Since the nutritional response of a 'wild-type' strain was unmodified by the presence of *thi-10*, it was of interest to study the effect of *thi-10* on the *thi-3* nutritional phenotype. This was done by making crosses which would result in *thi-3*; *thi-10* double mutants and testing the nutritional and thiamine synthesizing capacities of various random isolates. Results of two experiments are reported.

In the first experiment a *thi-1*; *thi-3*; *thi-10* + double mutant was crossed to *thi-10* (SY7A), (cross 56501-7). Strains were randomly isolated. The following nutritional types of strains were found: 'wild', *thi-1*, *thi-3*, and strains which responded like *thi-1*; *thi-10*. In thiamine-synthesizing tests, both the 'wild' and *thi-3* types showed either high or low activity (+ or - *thi-10*), while the strains which were like *thi-1* nutritionally showed only high thiamine synthesizing abilities. All strains which responded nutritionally like *thi-1*; *thi-10* doubles had low thiamine-synthesizing capacities. Typical thiamine-synthesizing abilities of some of these strains are shown in Table 4. These results are consistent with the idea that *thi-10* drastically lowers the ability of *thi-3* strains to condense pyrimidine and thiazole, without modifying

their nutritional requirements. A similar decrease in synthetic capacity by *thi-1o* in *thi-1* strains results in a change in nutritional requirements.

A second experiment was designed to show the interactions of *thi-1o* and *thi-3* genes. Strain 18558A (*thi-3*), which did not contain *thi-1o*, was crossed (cross 18558-1) to a thiamine-independent strain (56501-7-54a) which contained *thi-1o* as shown by a lowered ability to condense pyrimidine and thiazole. Of the seventy random spores dissected from this cross, thirty-six were

Fig. 2. Thiamine synthesis from pyrimidine and thiazole by *Neurospora*. Cultures were grown on 50 ml. minimal medium + 2.5 μ g. thiamine. At 48 hr. 100 μ g. thiazole + 100 μ g. pyrimidine were added. Strains used were: □, *thi-1* (56501a); ●, *thi-1*; ○, *thi-1o* (17084-1-3); ○, *thi-2* (9185a); ○, *thi-3* (18558A); ●, *thi-1o* (Sy7A).

wild and thirty-four were like *thi-3* nutritionally. Several isolates were examined for their synthetic abilities and bound thiamine (thiamine pyrophosphate) was measured in terms of the relatively specific yeast cocarboxylase assay. The data given in Table 5 show the cocarboxylase-synthesizing abilities of *thi-1o* +, *thi-3*; *thi-1o* and *thi-3* strains. In the longer growth and incubation of these cultures, *thi-1o* can be shown not to be a complete block. Nevertheless, under these conditions there is still at least a twofold difference in the synthetic performance of modified and unmodified strains. The *thi-3*; *thi-1o* strains are nutritionally similar to *thi-3* in their ability to respond to exogenous thiazole as a source of thiamine.

It was thought that carboxylase activities in these strains might be a useful index of the presence of *thi-1o* in its role as modifier of cocarboxylase synthesis from thiazole and pyrimidine. Yet, this test system was not applicable to optimally grown mycelia since maximal carboxylase activity is found in

thio-1o strains. Table 5 includes the carboxylase activities of *thi-1o* +, *thi-3*; *thi-1o* and *thi-3*. These results indicate that *thi-1o* is not distinguishable in terms of the carboxylase activities of optimally grown strains. Carboxylase activity apparently does not reflect differences of internal cocarboxylase concentrations at these high cocarboxylase levels. The apocarboxylase in these mycelia is probably saturated with coenzyme at the high cocarboxylase

Table 4. *Thiamine-synthesizing abilities* of Neurospora isolates from cross 56501-7*

Cultures were grown in shake culture in 50 ml. of minimal medium + 2.5 μ g. thiamine. At 40 hr. cultures were supplemented with 50 μ g. each of pyrimidine and thiazole, shaken an additional 12 hr., then harvested.

Strain	Nutritional phenotype†	Additions at 40 hr.		
		None	Thiamine	Thiazole + pyrimidine
		Relative thiochrome concentrations*		
56501-7-20	<i>thi-1</i>	5	24	48
23	<i>thi-1</i>	9	58	48
34	<i>thi-3</i>	5	49	51
64	<i>thi-3</i> ; <i>thi-1o</i>	4	39	8
72	<i>thi-1</i> ; <i>thi-1o</i>	3	55	12
75	<i>thi-1</i> ; <i>thi-1o</i>	4	60	13
76	<i>thi-1</i> ; <i>thi-1o</i>	6	51	11
77	<i>thi-3</i> ; <i>thi-1o</i>	7	44	10
56501-3-69	<i>thi-1</i> ; <i>thi-1o</i>	8	39	14
71	<i>thi-1</i> ; <i>thi-1o</i>	5	42	12
73	<i>thi-1</i> ; <i>thi-1o</i>	7	60	18
87	<i>thi-1</i>	4	26	35
SY7A	<i>thi-1o</i>	10	54	13
9185a	<i>thi-2</i>	2	30	4

*Values in table are in relative thiochrome concentrations (see methods).

† *thi-3*; *thi-1o* doubles are nutritionally identical to *thi-3* strains; *thi-1*; *thi-1o* strains are quantitatively like *thi-1* (high thiamine requirement), but they will eventually show growth on thiazole- and pyrimidine-containing media at times when the minimal cultures are still blank.

Table 5. *Cocarboxylase synthesis and carboxylase activity in modified and unmodified Neurospora strains*

Cultures were grown with shaking in 50 ml. of minimal medium + 2.5 μ g. thiamine in 125 ml. Erlenmeyer flasks at 30°. After 46 hr. of growth, solutions containing 50 μ g. thiamine, or 50 μ g. each of pyrimidine and thiazole, sterilized by filtration were added and the cultures were shaken an additional 14 hr. and harvested.

Values in the table are: *a* = μ g. cocarboxylase/g. dry wt. mycelium; *b* = carboxylase activity as μ l. CO₂/25 mg. dry wt. mycelium/5 min. (average of three readings).

Strain	Genetic composition	Additions at 46 hr.					
		None		Thiazole 50 μ g. + pyrimidine 50 μ g.		Thiamine 50 μ g.	
		<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
STA	<i>thi-1o</i> +	37	158	105	172	105	168
18558-1-9	<i>thi-3</i> ; <i>thi-1o</i> +	8	78	86	140	92	143
18558-1-8	<i>thi-3</i> ; <i>thi-1o</i>	16	105	54	147	96	150
18558-1-10	<i>thi-3</i> ; <i>thi-1o</i>	19	110	41	110	101	112

concentrations resulting from optimal growth. From these experiments and others it was seen that the presence of *thi-1o* suppresses the condensation of pyrimidine and thiazole in every strain tested. The nutrition of *thi-1* strains is modified by *thi-1o*, while the nutritional responses of wild-type (Table 6) and *thi-3* strains are not impaired by *thi-1o*. Consequently the only means of detecting *thi-1o* in *thi-3* and wild-type stocks is a test of the synthetic abilities of their mycelia.

Table 6. Growth response of *Neurospora* strains *thi-1o* and *thi-1o* + to thiamine-active substances

The strains were grown 45 hr. at 30° in 125 ml. Erlenmeyer flasks containing 20 ml. minimal medium + supplements indicated. Figures represent dry weight of mycelium in mg.

Strain Number Genotype		Supplements added (2 µg./20 ml.)			
		None	Thiamine	Thiazole	Pyrimidine
			Dry wt. mycelium (mg.)		
56501-7-54a	<i>thi-1o</i>	34, 34	33, 34	32, 31	31, 29
56501-7-40A	<i>thi-1o</i>	19, 16	17, 17	18, 15	14, 13
SY7A	<i>thi-1o</i>	39, 38	38, 42	41, 38	38, 38
STa	<i>thi-1o</i> +	57, 48	51, 51	50, 46	46, 51
STA	<i>thi-1o</i> +	56, 53	54, 55	55, 60	60, 49

Genetic relationships of *thi-1o*

Since *thi-1o* expresses itself as a nutritional anomaly only in the presence of *thi-1*, a cross was made (cross 56501-17) between two *thi-1* strains, one of which contained *thi-1o*. In fourteen complete asci tested, *thi-1o* showed only first division segregation, which indicated a map location close to the centromere. The 2:2 segregation data found indicated that the *thi-1o* character was associated with a single gene. Additionally, the data from the original cross (cross 56501-3) in which *thi-1o* segregated from SY7A are also consistent with the hypothesis that *thi-1o* was a single gene in this 'wild-type' stock.

When *thi-1o* was detected in *thi-1* stocks, it became important to know whether it was present in the various other thiamine-requiring stocks. The presence of *thi-1o* in *thi-2* or *thi-4* stocks would make obtaining and studying of double mutants with *thi-1* extremely difficult.

A cross of a *thi-1*; *thi-1o* strain by a *thi-2* strain (cross 56501-6) was made, and a number of recombinant *thi-1* strains were recovered which did not contain *thi-1o*, thus showing that *thi-1o* is not present in the *thi-2* parent (9185a), and that *thi-2* is not allelic with *thi-1o*. In a cross of *thi-4* by *thi-1* (without *thi-1o*) (cross 85902-3), the randomly selected unmodified *thi-1* types were approximately equal to the wild types suggesting there are no modifiers in *thi-4* not linked to *thi-4*.

The possibility that *thi-1o* might be linked to or allelic with *thi-4* was considered because of the similarity of action of these loci. From the linkage data summarized by Barratt *et al.* (1954), *thi-1* is in linkage group I, *thi-2* and *thi-4* are in group III, while *thi-3* is in group VII. A cross of a *thi-4* strain by *thi-1*; *thi-1o* strain (cross 85902-2) was made, and of fifty-five thiamine-requiring random isolates, not one was a recombinant *thi-1*; *thi-1o* + nutritional

type. Of these fifty-five isolates forty were *thi-4*-containing strains, and fifteen were *thi-1*; *thi-10* parental types. The parental *thi-1*; *thi-10* types could be differentiated from isolates which contained *thi-4* because the *thi-1*; *thi-10* eventually grew on test media containing pyrimidine and thiazole in 5-6 days at 30°. Strains containing the *thi-4* gene did not grow under comparable conditions. These results were in agreement with the proposed parental genotypes. These results are also consistent with the location of *thi-10* in linkage group III. Since neither *thi-4* or *thi-10* have shown recombination with their centromere nor with each other, and since their function is at the same metabolic step, their allelism is strongly suggested.

DISCUSSION

Considerable attention has been paid to the mechanism of gene suppressor action in *Neurospora* since the initial work of Houlahan & Mitchell (1947). This is mainly due to the relative ease of obtaining this type of modifier. Relatively little attention has been directed to other types of modifiers in *Neurospora*. There is no reason at present to believe that the basis of suppressor action is fundamentally different from that of other modifiers. The difference may only be a definitional one concerning the direction (towards or away from wild-type phenotype) of the modification rather than a fundamental difference in mechanism.

Modifier action can be considered as operating at two levels: (a) at the enzyme-forming level where apoenzyme specificity is determined; (b) at the enzyme activity level, where co-factor, pH, substrate and other factors are involved. While some of these inter-relationships have been shown in suppressor systems (Yanofsky 1956) the examples from other modifier systems are limited. Haskins & Mitchell (1952) and Newmeyer & Tatum (1953) studied modifiers in *Neurospora* which apparently have their effect at the substrate level. These investigators independently found that the phenotype of a gene (*nt*), which is involved in nicotinic acid and tryptophan biosynthesis, is affected both qualitatively and quantitatively by the presence of modifying genes. Strauss (1956) studied the gene *at* which acts as a modifier of succinate (*suc*) requiring strains. Strains containing *at*; *suc* are unable to grow immediately on acetate, while *suc* strains can use this substrate.

The gene *thi-10* appears to illustrate gene modifier action at the substrate level. The *thi-10* mechanism of modification in the strains where it has been studied is clearly that of modulating the ability to condense pyrimidine with thiazole. Still *thi-10* is a modifier in the strictest sense, because its primary phenotypic expression is not discernible in terms of the wild-type nutritional phenotype, while it specifically modifies the nutritional phenotype of the *thi-1* strains tested. The fact that thiamine mutants derived from only 17084 and 56501 strains are modified by *thi-10* is in agreement with their probable allelism. The *thi-10*; *thi-1* mutant strains are similar to *at*; *suc* strains in that they eventually grow on thiazole + pyrimidine just as *at*; *suc* strains will eventually grow on acetate. As with *thi-10* strains, the *at* strains show a wild-type growth rate.

The gene *thi-10* is also of interest as a probable isoallele of *thi-4*. In this role it represents a class of subthreshold alleles in a series which would range from mutants possessing a complete block through 'leaky-mutants' to mutants with no auxotrophic requirements at all. Members of this last class are most poorly represented in the catalogue of mutant types because simple nutritional techniques are inadequate to detect them. Subthreshold alleles (or wild-type isoalleles) can be picked up by specific physiological techniques as with the sub-threshold tryptophan mutants studied by Giles (1955). Alternatively, as suggested by the work of Goldschmidt & Piterich (1956) with the effects of subthreshold alleles on phenocopies in *Drosophila*, and as indicated by the work with *thi-10*, these isoalleles may be detected by their interaction with other genes like *thi-1* which can lower the nutritional threshold to a point where the subthreshold gene can express itself.

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