for the unusual circumstance that the only genes thus far located in this region—*ah, nv, pum, Tm*$_2$, and *wd*—are all tightly linked. The low crossover rate generally characteristic of heterochromatin is probably responsible.

Our study constitutes another example of the unique advantages offered by the tomato for relating chromosome structure with genetic activity.

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Irreparable Mutations and Ethionine Resistance in Neurospora

Abstract. *Some ethionine-resistant mutants of* Neurospora crassa *are temperature-sensitive, in that they fail to grow in the upper temperature range at which wild type* Neurospora *grow best. Two of these mutants have lost an indispensable function since at elevated temperatures they are unable to grow on a variety of complex media.*

Horowitz and Leupold (*1*) examined the frequency of microbial mutants which have lost an indispensable function—one that cannot be circumvented by furnishing the organism in question with any given nutrient or combination of nutrients. Since such mutations are by definition lethal, it might be thought that such strains would defy isolation and investigation. Horowitz first pointed out that conditional lethals, which apparently grow normally at low temperatures, but which fail to grow on either simple or complex media at higher temperatures, provide an opportunity to estimate the frequency of such mutants. Since that time, a number of ingenious studies of irreparable mutants have been conducted (*2*). For obvious reasons, however, little is known of the biochemical lesion associated with any given temperature-conditional lethal mutant.

In the present study, wild type, ethionine-sensitive conidia of *Neurospora crassa* (mating type *a* derived from Em 5297*a*) were suspended in water and irradiated with a germicidal lamp until about 95 percent were killed; then portions were plated on agar containing Fries' minimal salts (*3*), sucrose (1.5 percent), and L-ethionine (50 μg/ml). The plates were incubated at 24°C until centers of ethionine-resistant growth appeared. To assure the independent origin of the various strains, we picked only one growth center from each plate. To assure homokaryosis of the isolates, we transferred them to the crossing medium of Westergaard and Mitchell (*4*) supplemented with ethionine as already described. After 6 days' incubation at 24°C, the resulting protoperithecia were fertilized with wild type conidia of strain STA-4*A*. We then plated, at low density, random ascospores from each cross onto Fries-sucrose-ethionine agar and induced the spores to germinate by the usual heat-shock treatment. After 12 hours' incubation at 24°C, we isolated ethionine-resistant sporelings. Of 18 original isolates, 16 passed through such a cross successfully and were deemed to be true mutants.

Linear growth rates were measured by the method of Ryan *et al.* (*5*), with comparisons being made between growth on solid Fries' minimal medium containing 1.5 percent sucrose and 1.5 percent agar, with or without L-ethionine (50 μg/ml). All strains were tested on both media at 25° and 38°C. The parental wild type strain grew 20 to 30 percent more rapidly at 38° than at 25°C. Strain STA-4*A* showed similar behavior. In the 16 mutants, the degree of resistance to ethionine at 24°C, expressed as the ratio of growth rate with ethionine to the growth rate without ethionine, ranged from 0.12 to 0.99, with an average value of 0.68. The parental strains had a value of less than 0.02 at both temperatures.

Of the 16 mutants, eight resembled wild type in growing more rapidly at 38° than at 25°C on minimal medium; one grew at an identical rate at both temperatures, and seven grew more slowly or not at all at 38°C. One of the latter, henceforth referred to as *r-eth-1*, was chosen for further study on the basis of its high temperature sensitivity, relatively high ethionine resistance (0.82), good fertility in crosses, and abundant conidiation. The steady-state growth rates of wild type and *r-eth-1* on Fries-sucrose medium at several temperatures are shown in Table 1. It will be noted that the growth rate of the mutant drops sharply between 35.0° and 36.5°C, suggesting that a macromolecule with a large Arrhenius energy of inactivation is being denatured. The failure of *r-eth-1* to grow at elevated temperature was not relieved to any measurable degree by supplementation of Fries' minimal medium with methionine or ethionine at a variety of concentrations, nor by supplementation with the following complex nutrients at a concentration of 2 percent: Difco Bacto-peptone, Bacto-tryptone, yeast extract, malt extract, nutrient broth, and acid-hydrolyzed casein. None of the complex media inhibited growth of the mutant at 24°C or of wild type at either temperature.

Table 1. Growth rates of wild type and *r-eth-1* on Fries' minimal medium at various temperatures.

Strain	Growth rate (mm/hr)			
	24.0°C	35.0°C	36.5°C	38.0°C
Wild type	3.4	5.0	5.1	4.5
r-eth-1	3.0	2.4	0.0	0.0

In order to test the possibility that *r-eth-1* contained separate mutations for ethionine resistance and temperature sensitivity, which were only fortuitously related, we outcrossed the strain to wild type (*r-eth-1*a × STA-4-*A*). Of a total of 248 cultures from random spores, 112 grew at 38°C when tested on spot plates, and 136 failed to grow. This ratio is not significantly different from the expected one-to-one segregation. Of these cultures, we tested 180 for resistance to ethionine. All the temperature-sensitive cultures were ethionine-resistant, and vice versa. As a more sensitive test for segregation, larger numbers of random spores were germinated on ethionine plates, which were then incubated at 38°C. Among 1681 germinated spores examined, we found no ethionine-resistant, temperature-permissive segregant. This, coupled with the independent isolation of a

number of such strains, establishes a causal connection between the ethionine resistance and temperature sensitivity of *r-eth-1*.

Of 97 cultures from the aforementioned cross which we tested for mating type, we found four *r-eth-1*-A and two wild type-a recombinants, the remainder being of the parental mating types. Thus the gene in question is on chromosome 1, roughly six crossover units from the mating type locus. Having established the linkage group to which *r-eth-1* belongs, we obtained all the known irreparable mutants which are located on chromosome 1. Of these strains, STL-6-A, b-39-a, *44409-t*-A, *46006-t*-a, and *55701-t*-A, the latter was ethionine-resistant in the usual test system, and we found it to be irreparable on the same complex media that we used to demonstrate this point with *r-eth-1*. Strain STL-6-A grew slowly and with an abnormal morphology on ethionine. The remaining three strains were fully sensitive to ethionine.

The *55701-t* gene is known to be extremely closely linked to mating type, whereas *46006-t* is located about six crossover units to the right of the latter, at or close to the centromere (*6, 7*). Strain b-39 is far distal to mating type on the left arm of chromosome 1 (*8*); *44409-t* and STL-6 are on the right arm, not closely linked to the centromere (*7*).

We crossed *55701-t* to *r-eth-1* and plated random spores on minimal agar (200 spores to a petri dish), subjected them to heat shock, allowed them to germinate for 6 to 8 hours at 24°C, and then incubated the plates for 12 hours at 38° to 40°C. Of a total of 2450 spores examined, 72 temperature-permissive recombinants were observed. The germination rate was 91 percent. If it is assumed that all the inviable spores were mutant, and that the double mutant was temperature-sensitive, these two loci must be about six crossover units apart. We transferred 12 of the presumed wild-type recombinants onto minimal agar. All were similar to wild type in their growth rate at 38° to 40°C and were fully ethionine-sensitive. We found all of them to be of mating type *a*, consistent with the notion that *55701-t* is much more closely linked to mating type than is *r-eth-1*.

Strain *46006-t*-a was similarly crossed to *r-eth-1*-A. Germination of the random ascospores was virtually 100 percent. Of 2000 sporelings, 19 wild type growth centers appeared, corresponding to a map distance of about 1.9 crossover units. Thus, *r-eth-1* lies to the right of mating type and *55701-t*, near the centromere of chromosome 1. All of the wild type recombinants were mating type a, showing that *r-eth-1* is to the left of *46006-t*.

As expected, crosses of *r-eth-1* to STL-6 and to *44409-t* showed relatively weak linkage, with many wild type growth centers. It is rather surprising to find the two ethionine-resistant strains *r-eth-1* and *55701-t* reasonably closely linked, but in positions sufficiently remote that they are not part of the same cistron or probably even of the same operon.

The *r-eth-1* mutant synthesizes excessive quantities of methionine (*9*), and appears to have lost repression control over this pathway. Thus the basis of resistance does not seem to be an activating enzyme mutation, as found by Fangman and Neidhardt (*10*) in the case of a fluorophenylalanine-resistant mutant of *Escherichia coli*, nor is our mutant similar to the ethionine-resistant strains of *Coprinus* described by Lewis (*11*). Numerous cases of drug resistance have been successfully explained on the basis of overproduction of the normal congener of the drug (*12*), and Ames has recently made a useful summary of additional mechanisms by which a microorganism may acquire resistance to an inhibitory analog (*13*). However, it remains to be explained why the loss of control over methionine synthesis should result in an irreparable failure to grow at elevated temperatures. One attractive possibility is that an indispensible macromolecule, perhaps the methionyl transfer RNA, normally functions as the repressor substance for the methionine pathway; in the mutant under study, this may have been so altered that it is temperature-labile and concomitantly fails to be recognized as a repressor.

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Intranuclear Site of Histone Synthesis

Abstract. *The nucleolus is implicated as a site of synthesis of nuclear basic proteins. Some of these newly synthesized proteins derived from the nucleolus have been characterized chemically as histones.*

In higher organisms histones are of considerable importance in chromosome structure and regulation of DNA transcription (*1*) and replication (*2*). Although predominantly associated with the cell nucleus, histones have also been found in cytoplasmic ribosomes (*3*). The extreme heterogeneity of the histones, as revealed by column chromatography (*4*) and electrophoresis (*5*) may reflect a similarly large diversity of function.

We showed previously that the normally concurrent production of histone and DNA (*6*) may be uncoupled by the action of 5-fluorodeoxyuridine which inhibits DNA synthesis while permitting histone synthesis to continue (*7*). We concluded that histone synthesis is not under the direct control of new synthesis of DNA and that the production of the histone molecule may well precede its association with DNA.

We have now investigated the role of subnuclear components in the synthesis of both basic and residual proteins. For the purpose of this report histones are defined according to standard extraction procedures as proteins soluble in acid, precipitated by base and lacking-SH groups as indicated by their solubility in the Mirsky-Pollister reagent (*8*).

Nuclei were prepared from 36-hour-old pea seedlings (*9*) or 5-day-old exponentially dividing cells of tobacco