A TEMPERATURE INDEPENDENT MUTATION AT THE rib-1t LOCUS IN NEUROSPORA CRASSA

LAURA GARNJOBST

The Rockefeller Institute, New York 21, New York

Received December 13, 1961

ONLY two riboflavin mutants have been described so far in *Neurospora crassa*, 51602t(*rib-1*) (MITCHELL and HOULAHAN 1946) in linkage group VI, and Y30539 (*rib-2*) (GARNJOBST and TATUM 1956) in group IV. MCNUTT and FORREST (1958) list two other riboflavin mutants which were used in testing their pteridine derivative from Eremothecium as a growth substance. One of these is 76R5 (C168, METZENBERG, personal communication, 1961). The other, C107, a riboflavin-requiring poky mutant, was sent to us from the California Institute of Technology collection by W. S. MCNUTT, who obtained negative results in cross-feeding experiments between this strain and the two known riboflavin strains (personal communication, 1954). Since no report of the genetics of C107 (*rib-3*) has appeared in the literature, it has seemed worthwhile to describe the brief study made in this laboratory.

To free the new strain from the poky character, C107a was first crossed with a St.L wild type *A*, and several fast-growing (i.e., at rate of wild type) riboflavin-requiring reisolates selected for further study. One of these, C107–1(5–5)a, was crossed with SY7A wild type. All of the germinants of the two crosses which failed to grow in minimal medium at 31°C required riboflavin for growth also at 25°C. Table 1 shows the growth response of one riboflavin reisolate from the cross with SY7A in a series of increasing amounts of riboflavin in minimal medium at three temperatures, 25°C, 30°C and 34°C.

TABLE 1

Growth response of C107-2(2-3)a to increasing amounts of riboflavin at three different temperatures

Riboflavin added* µg/20 ml	Dry wt of mycelium† mg/20 ml 25°C	30°C	34°C
1. 0 (Control)	...	...	...
2. 0.1	...	...	...
3. 0.3	...	...	...
4. 0.6	...	±	6.0
5. 1.0	±	6.0	21.5
6. 3.0	27.0	53.2	46.8
7. 6.0	40.0	56.5	47.0
8. 10.0	43.6	55.4	43.8

* Fries two percent sucrose minimal medium.
† Three days growth from conidia.

Heterocaryon tests of the *rib-3* strain were made with reisolates of Y30539 (*rib-2*), 51602t (*rib-1*), and 37401 (*inos*) which were previously found to form heterocaryons with one another and with many other strains derived from the Lindegren wild types 1A and 25a, including SY7A (GARNJOBST 1955), but not with strains derived from the St. Lawrence wild types (GARNJOBST, unpublished). Therefore, six riboflavin reisolates from the cross C107-1(5-5)a × SY7A were tested for heterocaryon formation with Y30539 (*rib-2*). Of these, two were negative and four positive. The difference in response was no doubt due to difference in heterocaryon genotype since the same two isolates which were negative with Y30539 also gave negative heterocaryon responses with the inositol mutant 37401. The reisolates of C107 (*rib-3*) which formed heterocaryons with 37401 did not do so (at 31°C) with 51602t-5145-2A, a reisolate found to be incompatible with 37401, *nor* with a 51602t reisolate capable of forming heterocaryons with 37401 and Y30539. According to these tests *rib-3* and *rib-2* are nutritionally complementary but *rib-2* and *rib-1* are not.

The genetic data were obtained from four crosses. Slow growth from ascospores (or slowness in ripening of ascospores) is characteristic of this new riboflavin strain as well as of the previously described strains. Consequently, some of the riboflavinless germinants were missing from the asci isolated in order from two crosses of C107–1(5–5)a with two different *rib-2* isolates, Y30539–3–47A and Y30539–215(5–3)A. One or two pairs of wild types in many of the asci (20 isolated from each cross), however, show that *rib-3* is not an allele of *rib-2* in linkage group IV.

In contrast, no wild types appeared in any of 20 asci isolated in order from the cross of C107–1(5–5)a with 51602t–5145–2A (*rib-1*). In 12 of these asci at least one member of each pair of ascospores grew and often both members of a pair were present; both members of three pairs germinated in seven asci; and in one ascus two different contiguous pairs were represented. The progeny obtained were tested in minimal medium and in minimal plus riboflavin at 31°C and at 25°C. The temperature independent isolates (*rib-3*) were clearly distinguishable from the temperature sensitive (*rib-1*) within three days. Two asci showing second division segregation were obtained in 19 asci; thus a distance of 5.2 units from the centromere is indicated for *rib-3* (and *rib-1*). STADLER (1956) in his intensive study of mutant genes in linkage group VI found the centromere distances in different crosses to vary, the highest obtained for the *rib-1* locus being 4.2 (see also FROST 1961).

An additional cross was made with a marker strain in linkage group VI, Y30539y (*ylo*) which produces yellow conidia. According to STADLER, *ylo* is located in the left arm and in one of his maps (p. 542) is placed at 7.4 units from the centromere. The 20 asci isolated in order from the cross C107–3(2–3)a (*rib-3*) with Y30539–6(2–2)A (*ylo*) gave good germination. They show a recombination percentage of 7.5 (i.e., three tetratype and 17 parental ditype asci). In a previous study (GARNJOBST and TATUM 1956) a recombination percentage of 1.5 in 33 asci was obtained from a cross of Y30539–6(2–2)A with 51602t–1–1a. (The latter reisolate was obtained from a cross of 51602t–5145–2A

with SY4a wild type.) In the 20 asci mentioned, the centromere distance for yellow is 10.0 and *rib-1* segregated in the first division.

SUMMARY

It seems clear from the data presented that *rib-3* is an allele of *rib-1* but differs from it in that the riboflavin requirement of the C107 strain is temperature independent. In accordance with the nomenclature proposed in BARRATT, NEWMEYER, PERKINS and GARNJOBST (1954) it is suggested that hereafter the locus be referred to as *rib-1*; the allele in strain 51602 as *rib-1t*; and the allele in strain C107, as *rib-1*. A culture of C107 has been sent to the Fungal Genetics Stock Center, Dartmouth College.

NOTE ADDED IN PROOF

In preliminary genetic tests still in progress, two additional riboflavinless strains, C168, recently obtained from DR. METZENBERG, and K28t from the Dartmouth culture collection, appear to show strong linkage to *ylo*, and hence probably also represent additional mutations at the *rib-1* locus.

LITERATURE CITED

BARRATT, R. W., D. NEWMEYER, D. D. PERKINS, and L. GARNJOBST, 1954 Map construction in *Neurospora crassa*. Advances in Genet. **6**: 1–93.

FROST, L. C., 1961 Heterogeneity in recombination frequencies in *Neurospora crassa*. Genet. Research **2**: 43–62.

GARNJOBST, L., 1955 Further analysis of genetic control of heterocaryosis in *Neurospora crassa*. Am. J. Botany **42**: 444–448.

GARNJOBST, L., and E. L. TATUM, 1956 A temperature-independent riboflavin-requiring mutant of *Neurospora crassa*. Am. J. Botany **43**: 149–157.

MCNUTT, W. S., and H. S. FORREST, 1958 The incorporation of the C^{14} of adenine into a pteridine derivative by *Eremothecium ashbyii*. J. Am. Chem. Soc. **80**: 951–952.

MITCHELL, H. K., and M. B. HOULAHAN, 1946 Neurospora. II. A temperature sensitive riboflavin mutant. Am. J. Botany **33**: 31–35.

STADLER, D. B., 1956 A map of linkage group VI of *Neurospora crassa*. Genetics **41**: 528–543.