JOURNAL OF BACTERIOLOGY, Mar. 1969, p. 1114-1117
Vol. 97, No. 3

Interaction Between Analogue Resistance and Amino Acid Auxotrophy in *Neurospora*[1]

JOHN A. KINSEY[2] AND DAVID R. STADLER
Department of Genetics, University of Washington, Seattle, Washington 98105

Received for publication 16 December 1968

A new *p*-fluorophenylalanine resistant mutant of *Neurospora* (*fpr-1*) was isolated. It is unaffected by the suppressor of a previously described resistance mutant, *mtr*. The *fpr-1* locus is on linkage group V, tightly linked to *act-2*. The expression of resistance to *p*-fluorophenylalanine by *fpr-1* can be suppressed by genes controlling a requirement for lysine or arginine. The suppression seems to involve an increased sensitivity of the lysine and arginine auxotrophs to *p*-fluorophenylalanine.

The growth of wild-type *Neurospora* is inhibited by the addition of either of the amino acid analogues 4-methyltryptophan (4MT) or *p*-fluorophenylalanine (FPA) to minimal medium. Mutants resistant to both inhibitors have been isolated on such media after treatment of conidia with ultraviolet light (UV). The resistance has been shown to result from a mutation at the *mtr* locus which stops or drastically slows the normal uptake of exogenous tryptophan, phenylalanine, and certain other amino acids (1, 2).

Revertants of these mutants were selected by Stadler (3), employing an *mtr tryp* double mutant. This strain required exogenous tryptophan but could not grow on medium supplemented with very low levels of tryptophan. This served as a selective medium for the mutational recovery of uptake. Revertants selected in this way were analyzed and were found to result not from backmutation at the *mtr* locus but from a suppressor mutation (su^{mtr}) at an unlinked locus on linkage group I (*mtr* is on linkage group IV). Nine such suppressors were studied, and all appeared to be identical genetically and phenotypically. They were not allele-specific; each suppressor was effective in combination with any *mtr* allele with which it was crossed. Studies of the uptake properties of these suppressed mutant strains indicated that the su^{mtr} mutation had modified a second uptake system, increasing its substrate range to include tryptophan.

The present study was originated to find out whether mutation at *mtr* or any other locus could bring about resistance to 4MT and FPA in the presence of an *mtr* suppressor.

[1] A preliminary report of part of this paper was presented at the 36th annual meeting of the Genetics Society of America, Stanford, Calif., 1967.

[2] Present address: Department of Microbiology, University of Kansas Medical Center, Kansas City, Kan. 66103.

MATERIALS AND METHODS

The strains employed and the origin of *mtr* and su^{mtr} strains have been described previously (2, 3). Auxotrophic stains were obtained either from the authors' stock collections or from the Fungal Genetics Stock Center. Selective media used for isolation and maintenance of resistance stocks have been described previously (2, 3).

RESULTS AND DISCUSSION

A heavy suspension of conidia of a suppressor strain (su^{mtr}) was plated on minimal medium plus FPA. Several resistant colonies were isolated, and these were retested on the same medium. The one showing the strongest growth on retest was designated *fpr-1*. (One portion of the conidial suspension had been treated with UV before the plating in FPA medium, but the *fpr-1* isolate came from one of the plates containing untreated conidia; *fpr-1* was previously called R-2.) The *fpr-1* strain is also resistant to 4MT.

To insure that the *fpr-1* strain was genetically pure, it was reisolated from single ascospores from a cross to wild type. We wished to confirm that resistance would be expressed in a pure strain carrying the suppressor, so we sought an ascospore culture that was *fpr-1* su^{mtr}. The suppressor could not be recognized by any phenotypic effects in the progeny of this cross (short of uptake studies), but we were helped by the knowledge that the suppressor is linked to mating type. A single resistant ascospore of the same mating type as the resistant parent was isolated.

The presence of su^{mtr} in this strain was confirmed by a cross to *col-4 mtr*. This colonial mutant is tightly linked to *mtr*. Analysis of *col* ascospores from this cross revealed that about one-fourth of them were sensitive to FPA. This is the result which would be expected if the suppressor of *mtr* were present in one parent and

if the resistance of *fpr-1* were controlled by a single gene unlinked to *mtr* (as will be confirmed below).

Complete tetrads were analyzed from the cross *fpr-1* su^{mtr} × *pdx-1*. This gene for pyridoxine requirement is closely linked to *mtr* and *col-4* on linkage group IV. The analysis of 27 complete asci from this cross showed that the resistance of *fpr-1* was determined by a single gene; it segregated 2:2 in every ascus. The same asci showed that the *fpr-1* locus is unlinked to *pdx-1*. The ratio PD:NPD:T (parental ditype to nonparental ditype to tetratype) for these two traits was 7:5:15. Resistance segregated in the first division in 21 of the 27 asci, and *pdx* showed first-division segregation in 18 asci. These are the results expected in a cross segregating for allelic pairs at two loci linked to the centromeres of different chromosomes. Resistance also segregated independently of mating type in this cross. The mating-type locus is linked to the centromere of linkage group I. This showed that *fpr-1* is not linked to su^{mtr}, which is very near that centromere.

Crosses of *fpr-1* to other centromere markers showed no linkage except in the case of *lys-1* on group V. However, the analysis of this cross (as well as crosses of *fpr-1* to other lysine and arginine auxotrophs) was complicated by phenotypic interactions.

Nineteen complete asci were analyzed from the cross *fpr-1* × *lys-1*. All showed 2:2 segregation for lysine requirement. However, only 11 of the asci had 2:2 segregation for resistance to FPA. The other eight asci each had three sensitive spore pairs and only one resistant spore pair. Moreover, one of the expected phenotypic classes (FPA-resistant, lysine-requiring) did not occur in these 19 asci, nor among viable random spores from this cross. This led to the hypothesis that *lys-1* or something closely linked to it was capable of suppressing *fpr-1*.

All of the FPA-sensitive members of each of the eight asci showing 3:1 segregation were tested by crossing to sensitive wild-type strains. The progeny from these crosses were checked for FPA resistance. The occurrence of resistant spores in the progeny of such a cross signals the presence of an unexpressed *fpr-1* gene in the strain under test. In each of the eight asci, one of the lysine-requiring spore pairs contained an unexpressed *fpr-1*. Thus, all of the asci from the cross *fpr-1* × *lys-1* segregated 2:2 for *fpr-1* as well as for *lys*. The asci which showed 3:1 segregation for sensitivity were actually tetratypes. We will demonstrate below that the factor which suppressed *fpr-1* expression in this cross was the *lys-1* gene itself. Other lysine and arginine auxotrophs are also capable of this suppression.

The analysis of the asci discussed above revealed that *fpr-1* was linked to *lys-1*; the ratio PD:NPD:T was 11:0:8. The position of *fpr-1* was determined more precisely by crossing it to other markers on linkage group V. A stock containing a group 2 isoleucine-valine mutant (*iv-T318*) and the morphological marker spray (*sp*) was crossed to *fpr-1*. The *iv-T318* locus is distal to *lys-1* and *sp* is distal to *iv-T318* on the right arm of linkage group V. Analysis of eight complete asci from this cross gave consistent 2:2 segregation for each of the three traits, showing that the scoring was not obscured by phenotypic interactions. The results of the analysis of 96 unselected random spores from this cross are given in Table 1. The predominance of parental combinations confirms the linkage of all three of these loci. It appears that the *fpr-1* locus is between the other two, because the two classes which are absent from the progeny are those which would require a double crossover in that situation.

A strain carrying a gene for cycloheximide resistance (*act-2*), which is known to be in this region of linkage group V, was also crossed to *fpr-1*. Seventy-six unselected random spores were tested for resistance to FPA and to cycloheximide; there were no recombinants among them (wild type or double resistant). Subsequently, a large number of random spores from this cross were germinated on medium supplemented with both FPA and cycloheximide. Among 2,730 germinated spores, there were only 10 which continued to grow. These were retested, and all appeared to be authentic double mutants. Thus, *fpr-1* appears to be very closely linked to *act-2*.

The locus of *lys-2* (lysine requirer) is also in this region of linkage group V. Ten complete asci from the cross *lys-2* × *fpr-1* were analyzed. Nine were parental ditypes, and one showed 3:1 segregation for sensitivity to FPA. The three sensitive members of this ascus were crossed to wild type, and one of the lysine requirers was found to contain an unexpressed resistance gene. Again, as in the cross to *lys-1*, a factor seemed to enter the cross with the lysine-requiring parent, which was closely linked to the lysine marker in question, and which had the capability of suppressing the expression of *fpr-1*.

To study the linkage of the putative suppressor (su^{fpr-1}), a cross was made in which its segregation could be followed directly: *lys-1* su^{fpr-1} *fpr-1* × *fpr-1*. Among 157 unselected random spores, there were no recombinants between *lys-1* and su^{fpr-1}; all were either lysine-requiring and sensitive or lysine-independent and resistant.

TABLE 1. *Analysis of 96 random progeny from the cross iv-T318 × fpr-1*

Genotype			No. observed
fpr-1$^+$	*iv*	*sp*	36
fpr-1	*iv*$^+$	*sp*$^+$	37
fpr-1$^+$	*iv*$^+$	*sp*$^+$	8
fpr-1	*iv*	*sp*	3
fpr-1$^+$	*iv*	*sp*$^+$	4
fpr-1	*iv*$^+$	*sp*	8
fpr-1$^+$	*iv*$^+$	*sp*$^+$	0
fpr-1	*iv*	*sp*	0

This tight linkage of the suppressor to *lys-1* and its presence in the *lys-2* stock led us to postulate that the lysine mutant genes were themselves the suppressing factors. Two kinds of experiments have been performed to test this idea: (i) reversion studies of both *lys-1* and the suppressor of *fpr-1*; (ii) tests of the capability of other auxotrophs to suppress *fpr-1*.

Suspensions of conidia of the genotype *lys-1* *su*$^{fpr-1}$ *fpr-1* were irradiated with UV and plated in minimal medium supplemented with lysine and FPA. Since the conidia were sensitive to FPA before the irradiation, only those which had gained resistance would survive. The presence of lysine in the medium assured that no demand would be placed on the *lys-1* gene. The expectation was that if the *lys-1* gene were itself the suppressor of *fpr-1*, resistant mutants from this experiment would fall into two classes. One class would represent the production of new resistance factors and would still require lysine. The second class would represent back-mutation at the *lys-1* locus (relieving suppression) and would no longer require lysine. Fifteen resistant mutants were tested in this experiment. Twelve were found to require lysine and were tested no further. Three, however, had simultaneously lost their lysine requirement and gained FPA resistance. Subsequent tests of these three strains showed in each case that the resistance factor present was *fpr-1* and that the reversion event had apparently taken place at the *lys-1* locus (that is, there was no indication of unlinked suppressors of *lys*).

Irradiated conidia of the same strain were also plated on minimal medium. This medium selects for lysine independence but makes no demand for resistance to FPA. Five mutants to prototrophy were isolated in this experiment. All five were found to have simultaneously gained lysine independence and FPA resistance.

In the second type of experiment, *fpr-1* was crossed to a variety of other lysine auxotrophs to test their ability to suppress the resistance gene (Table 2). All of the lysine mutants tested gave at least partial suppression. The *lys-3* and *lys-6* auxotrophs, like *lys-1* and *lys-2*, rendered *fpr-1* strains completely sensitive to FPA. A similar suppression was shown by *lys-5* at the standard 48-hr scoring time; however, longer incubation revealed some growth. Three of four arginine mutants tested showed complete or partial suppression. A variety of other auxotrophs gave no suppression (Table 2).

Physiological investigations of the basis of resistance to amino acid analogues in *fpr-1* are in progress. Preliminary results indicate that *fpr-1* is impaired in its ability to concentrate FPA, as well as the normal amino acids phenylalanine, tyrosine, and tryptophan. In short-term (10 min) measurements of phenylalanine uptake into germinating conidia of *fpr-1*, the rate was approximately 55% of that into wild-type conidia under similar conditions. Whether a difference in uptake of this magnitude is sufficient to explain the resistance of the *fpr-1* strain is not clear. It is also of interest to note that FPA incorporation into protein in *fpr-1* seems to be essentially identical to that in the wild type. This raises some questions as to what is the actual basis of FPA inhibition in *Neurospora*.

The manifestation of what we have called suppression is the inability of the *fpr-1 lys-1* strain to grow on medium supplemented with lysine and FPA. It has been assumed that this demonstrated the sensitivity of this strain to FPA. The failure to grow could also result from an inability to assimilate lysine from this medium, but the following observations argue against this explanation. The *fpr-1 lys-1* strain grows well on lysine medium without FPA, so *fpr-1* cannot be affecting the normal utilization of lysine. FPA could be interfering with the utilization of lysine by this strain, but it would not in the prototroph, since *fpr-1* cells grown in the

TABLE 2. *Suppression of fpr-1 by certain auxotrophs*

Auxotrophs showing			
Complete suppression	Partial suppression	No suppression	
lys-1	*lys-5*	*arg-8*	*prol-1*
lys-2	*arg-4*	*hist-2*	*inos*
lys-3	*arg-10*	*hist-4*	*nic-1*
lys-6		*hist-7*	*pab-1*
arg-2		*cys-5*	*tryp-4*
		iv-T318	*pan-1*
		me-7	*tryp-2*
		pdx-1	

presence of FPA must carry out functions required for the utilization of endogenous lysine. The rate of lysine uptake was measured in 48-hr pads of *fpr-1* and *fpr-1 lys-1* and was found to be 80 and 85%, respectively, of the wild-type rate of lysine uptake. In the presence of FPA, the rate of uptake of lysine by pads of *fpr-1 lys-1* was 92% of the rate in the absence of FPA. In all of the experiments, the lysine concentration was 10^{-3} M. When FPA was added, its concentration was 10^{-4} M. The *fpr-1 lys-1* strains will not grow on media containing 10^{-3} M lysine and 10^{-4} M FPA. Moreover, after as much as 5 hr of preincubation with FPA, the uptake rate of lysine was still 80% of that seen in the absence of FPA. Thus, there appears to be no competition between lysine and FPA for uptake, nor does there seem to be a labile lysine-uptake system which is inactivated by FPA. In some experiments, the lysine precursor α-aminoadipic acid (AAA), which will substitute for lysine as a growth factor for the *lys-1* strain, was used. Although AAA is presumably concentrated by a different uptake system than lysine, *fpr-1 lys-1* strains will not grow on AAA-FPA media.

The above observations make it seem doubtful that lysine starvation is involved. Thus, it appears that the suppression is, in fact, due to increased sensitivity to FPA. Genes for lysine and arginine requirement may cause increased sensitivity to FPA even in the absence of *fpr-1*. Growth tests indicate that these auxotrophs are more sensitive than the wild type (although the rate of uptake of FPA by the auxotrophs is no greater than that of the wild type).

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Fellowship 5-F2-GM-30,034-02 and by a grant from the National Science Foundation.

LITERATURE CITED

1. Lester, G. 1966. Genetic control of amino acid permeability in *Neurospora crassa*. J. Bacteriol. 91:677–684.
2. Stadler, D. R. 1966. Genetic control of the uptake of amino acids in *Neurospora*. Genetics 54:677–685.
3. Stadler, D. R. 1967. Suppressors of amino acid uptake mutants of *Neurospora*. Genetics 57:935–942.