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A Gene Controlling the Early Development of Protoperithecium in *Neurospora crassa*

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Summary. A mutation (*ff-1*) which specifically destroys the ability to develop protoperithecia was found in *Neurospora crassa*. This mutation causes no change in vegetative morphology. It is located between *arg-5* and *try-3* on the right arm of linkage group II.

The development of protoperithecium in *Neurospora* and in other Ascomycetes offers a unique system in developmental genetics as reviewed by Esser and Kuenen (1967). Formation of protoperithecium in *Neurospora* proceeds in a definite morphological sequence (Rothschild and Suskind, 1966) and several mutations are known which interfere with protoperithecial development (Dodge, 1946; Westergaard and Hirsch, 1954; Horowitz *et al.*, 1960; Horowitz *et al.*, 1961; Fitzgerald, 1963; McNelly-Ingle and Frost, 1965). However the genetics of these mutants have been poorly studied.

Furthermore, two protoperithecia-less mutants *ty-1* and *ty-2* are also involved in the regulation of tyrosinase synthesis (Horowitz *et al.*, 1960).

Thus combined genetical and biochemical studies on the mutants affecting the development of protoperithecium in *Neurospora crassa* was initiated. This paper reports on the genetic location of our first protoperithecia-less mutant (*ff-1*).

Materials and Methods

Strains. The mutation, *ff-1* was first detected unintentionally (Ho, 1968), while attempting to locate histidinol permeable (*hlp*) mutations, in a *histidine* mutant (K458 *his-3 a*) which itself was derived from Emerson a (Catcheside, 1960).

The following mutants, *his-3* (K458), *arg-5* (K132), *try-3* (A78) *his-7* (K738) *cot-1* (C102), *spray* (B132), *ad-7* (44411), *ylo-1* (Y30539y), *nic-3* (Y31881), *hlp-1* (BS38) and *nt* (65001) were used to locate the *ff-1* mutation.

Methods. The minimal media of Vogel (1964) and Westergaard and Mitchell (1947), with appropriate supplements were used for vegetative growth and crosses, respectively. Vogel's medium plus 0.5% L-sorbose and 0.1% sucrose (SS) with suitable supplements was used for scoring progeny on plates. Vogel's medium plus 0.5% L-sorbose, 0.0125% D-glucose and 0.025% D-fructose (SGF) was used for the germination of ascospores. The quantities of supplements (mg per 100 ml medium) used routinely for crosses, maintenance and scoring of cultures were as follows: 20 L-arginine HCl, L-histidine HCl H_2O, L-tryptophan, adenine; 40 L-histidinol 2HCl; 4 nicotinamide.

Cultures were tested for ability to produce protoperithecia after growing more than 10 days at about 24—26°C in tubes containing appropriately supplemented medium of Westergaard and Mitchell (1947). A piece of filter paper was included in each tube. The cultures carrying the *ff-1* mutation did not produce any protoperithecia (often re-examined

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under low power binocular microscopes) while *ff-1*$^+$ strains produced many. In later work, conidia of two prototrophic strains of opposite mating types carrying the *ff-1* mutation were added together into these cultures to fertilize the protoperithecia. Presence of perithecia was scored about a week after fertilization. The second procedure permitted through melanization the detection of any strain producing few protoperithecia which were often hidden under masses of mycelia.It also demonstrated the complete female sterility of the *ff-1* strains.

The genetics of *ff-1* mutation was carried out by random ascospore analysis.

Results

Cultures carrying the *ff-1* mutation have normal vegetative morphology and as such could not be distinguished from *ff-1*$^+$ cultures. The *ff-1* mutation also did not result in any nutritional requirement and addition of histidine, histidinol, arginine, tryptophan, adenine and nicotinamide into the crossing medium failed to remedy the defect. *ff-1* was completely female sterile but produced normal perithecia and ascospores when used as male parents.

There was good correspondence between the results obtained from the scoring of protoperithecia and that of perithecia. In one preliminary comparison of 386 cultures, only 19 did not correspond in the two tests. Of these, 18 scored as protoperithecia-less produced a few perithecia while 1 culture with protoperithecia did not produce any perithecia. Also, everyone of the 116 progeny tested from a cross between two female fertile strains produced protoperithecia.

The results of testing for linkage of *ff-1* with mutants belonging to each of the seven linkage groups (Table 1) showed that *ff-1* segregated as a single gene mutation. Most of these crosses were originally used to map the *hlp-1* gene (Ho, 1969). *ff-1* was shown to be located on the right arm of linkage group II, first by finding a weak linkage (35%) to *try-3* and then a fairly close linkage (17%) to *arg-5*. There was no significant linkage to mutations in other linkage groups.

Table 1. *Data from crosses of ff-1 with other genes*

Mutant (Linkage group)	Progeny					Recombination fraction	Probability of χ^2 test for 1:1 ratio
	Parental		Recombinant		Total		
	mutant$^+$ *ff-1*	mutant *ff-1*$^+$	mutant$^+$ *ff-1*$^+$	mutant *ff-1*			
try-3 (IIR)	39	25	16	18	98	0.35	0.01—0.001[a]
his-7 (IIIR)	19	17	21	11	68	0.46	0.70—0.50
cot-1 (IVR)	25	39	45	48	157	0.60	0.05—0.02
sp (VR)	7	29	16	7	59	0.43	0.10—0.05
ad-7 (VR)	16	18	12	12	58	0.42	0.20—0.10
yl°-1 (VIL)	14	11	19	14	58	0.57	0.30—0.20
nic-3 (VIIL)	21	19	21	24	85	0.53	0.70—0.50
	mutant$^+$ *ff-1*$^+$	mutant *ff-1*	mutant$^+$ *ff-1*	mutant *ff-1*$^+$			
his-3 (IR)	43	52	61	54	210	0.55	0.20—0.10
arg-5 (IIR)	34	22	8	4	68	0.17	<0.001[a]
hlp-1 (VIIR)	40	24	18	35	117	0.45	0.50—0.30

[a] Significant at the 1% level.

A three point cross (Table 2) showed that the order is *arg-5 ff-1 try-3*, with the distances of 25.5 and 31.4 map units respectively.

It may be noted that in one cross between *his-3 hlp-1 a* with *nt A*, three classes of progeny with respect to protoperithecial development were found. From 64 progeny, 32 were protoperithecia-less, 13 with protoperithecia and 19 without protoperithecia but the medium was covered with black patches of melanin. Presumably, these came from the disintegration of protoperithecia. The same phenomenon was reported previously by Fitzgerald (1963).

Table 2. *Data of a three point cross to place ff-1 with respect to try-3 and arg-5. Scoring of ff-1 mutation by the presence or absence of perithecia*

Parentals	*arg-5*$^+$	*ff-1*	*try-3*	94
	arg-5	*ff-1*$^+$	*try-3*$^+$	81
Single cross overs I	*arg-5*$^+$	*ff-1*$^+$	*try-3*$^+$	35
	arg-5	*ff-1*	*try-3*	32
Single cross overs II	*arg-5*$^+$	*ff-1*	*try-3*$^+$	57
	arg-5	*ff-1*$^+$	*try-3*	33
Double cross overs	*arg-5*$^+$	*ff-1*$^+$	*try-3*	12
	arg-5	*ff-1*	*try-3*$^+$	11
				355

Discussion

The *ff-1* mutation is interesting in several respects. The mutation specifically causes the prevention of the formation of protoperithecium and has no effect on vegetative morphology or nutritional requirement. In other cases, several protoperithecia-less mutants have abnormal vegetative morphology. For instance, *ty-1* has a "velvet" morphology while *ty-2* is morphologically normal (Horowitz *et al.*, 1960). Modifiers (*gul-3, gul-4, gul-6*) of the colonial temperature sensitive (*cot-1*) mutant do not produce any protoperithecia (Terenzi and Reissig, 1967). Several other morphological mutants are also defective in protoperithecial development (personal communications of A. M. Srb and H. G. Kølmark).

Genetically, *ff-1* is not allelic to *ty-1* which is located on the right arm of linkage group III (Walker, 1963) while the location of *ty-2* is not known.

It is probable that the gene products of *ff-1, ty-1, ty-2* and other similar mutants are responsible for the initiation of protoperithecial formation. However, the mechanism of this process is unknown.

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