Mutations blocking development of perithecium

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Abstract
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It is in efforts to discover those genes in known that several female-sterile types. Combinaton of incubation times have of the development of protoperithecio have be con be in specific mutation also mops on linkage group II between of linkage vegetative hyphae.

The development of the protoperithecium or the female sexual organ in Neurospora, though essential for sexual development, is nevertheless dispensable for completion of the life cycle, due to alternative vegetative reproduction by conidia and vegetative hyphae. Mutants defective in the formation of protoperithecium are therefore valuable non-lethal developmental mutants in efforts to discover those genes that are responsible for the initiation of a developmental pathway. For genetical studies, these female-sterile mutants can be used or male parents to fertilize the protoperithecia from strains of opposite mating type.

Three classes of mutations blocking different stages of the development of protoperithecio have now been obtained. All of them are spontaneous mutations. In most cases (except ty-1 and ty-2) only mutants with normal vegetative morphology and good growth rate were chosen, so that it can be certain that the mutation specifically affects the development of protoperithecio. It is already known that several morphological mutants, such as the modifiers of the colonial temperature-sensitive mutant (Terenz and Reissig 1967 Genetics 56:321), have defective protoperithecio and are female sterile.

The mutations of the first group (ff-1, ff-2 ond others) specifically prevent the formation of protoperithecio and have no effect on vegetative morphology or nutritional requirements. The ff-1 mutation was mopped on the right arm of linkage group II between org-5 and try-3 (Tan and Ho 1970 Molec. Gen. Genet. 107:158). Another protoperithecio-less mutant also mops on linkage group II, but its precise location is not known. The location of ff-2 is uncertain.

The second class of mutants (ty-1, ty-2) was first discovered by Westergaard, and the regulation of their tyrosinase synthesis was studied intensively (Horowitz et al. 1960 J. Mol. Biol.2:96). They form a few small protoperithecio, which are generally defective in function. Rarely, a few of their protoperithecio can be mated to form perithecia. The mutant ty-1 has on abnormal vegetative morphology called "velvet", in that the aerial hyphae are short and bear few conidio. Velvet is inseparable from female sterility. Most ascoscospores of ty-1 are also probably lethal, as indicated by a large deficiency of ty-1 in the progeny of all crosses as determined by random ascospore analysis. The aerial hyphae of ty-2 are also shorter than those of the wild type. The mutant ty-1 was tentatively mopped by Walker (1963 Neurospora NewsL.3:15) near tyrosine-1 on the for right on of linkage group III. The present work confirms his result. The genesis located to the right of albino-2 on the right arm of linkage group 1. It is not allelic to the T locus (Horowitz and Fling 1956 Proc. Natl Acad. Sci. U.S.42:498), the structural gene of tyrosinase, which is proximal to al-2.
The last class of mutants (ff6) produces many large and black protoperithecia which cannot be mated to form perithecia. They also excrete large amounts of black pigments, presumably melanin, into the medium. This excretion of pigments may not be the cause of the functional defect, for there are similar excretor mutants which are female fertile. The gene ff-6 is located close to ty-1.

The protoperithecia-less mutants (ff-1, ff-2) are strong candidates for the regulatory gene or genes that switch on the development of protoperithecia. If this is true, it is expected that these mutants may show a deficiency of the various enzymes and proteins involved in the development of this organelle. The nature of the ff-6 mutation is unknown. * * = Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.

Courtright, J. B. Inhibition of Neurospora protein synthesis by erythromycin. Erythromycin has been reported to be an inhibitor of mitochondrial specific protein synthesis in yeast and the mutations conferring resistance to this antibiotic are cytoplasmically inherited. Such mutants possess mitochondria which are insensitive to the effects of erythromycin in a cell-free protein synthesizing system. We have attempted to extend these observations to N. crassa, but have found that the effects of this antibiotic in N. crassa are significantly different from the effects found in yeast.

The inhibition of Neurospora growth at high concentrations of erythromycin (greater than 5 mg/ml) has been examined with regard to the effect of this antibiotic on in vivo protein synthesis. Conidia from wild type strain LSIT A (Harding et al. 1970 Arch. Biochem. Biophys. 138:653) were grown in Vogel's minimal medium with shaking at 30°C for four hours. The cells were collected by centrifugation, adjusted to a concentration of about 10^6/ml in minimal sucrose medium, and incubated with varying concentrations of erythromycin for fifteen minutes. At the end of the incubation period, ^14C-L-lysine (20 mC/mM) was added to a final concentration of 0.1 mC/ml and the cells were incubated another fifteen minutes at 30°C. The cells were collected on filter paper, washed five times with 5 ml each time 5% TCA made 0.1 M with respect to unlabeled leucine, and finally counted in Bray's solution. Under these conditions, 7100 cpm/ml were incorporated by cells in the absence of erythromycin, while duplicate samples containing 5 mg/ml erythromycin only incorporated 320 cpm/ml, which represents a 95% inhibition of leucine incorporation.

In addition, cells which had been incubated with ^14C-L-leucine for 30 minutes, with and without erythromycin, were digested by treatment with Glusulase and disrupted by osmotic lysis. One ml aliquots were layered on a continuous 5-20% sucrose gradient and centrifuged for one hour at 25,000 rpm in a Beckman SW 50 rotor. Fractions of 0.5 ml were collected and assayed for incorporation of ^14C leucine into hot TCA precipitable, NaOH soluble material. Under these conditions, it was found that the mitochondrial fractions from the erythromycin treated culture had incorporated only 37% as much leucine as that found in the mitochondrial fractions from the untreated culture. On the other hand, the cytosol fractions from the erythromycin treated culture contained only 3.4% as much labeled leucine as that found in the fractions from the untreated culture.

Taken together, these data suggest that erythromycin inhibits growth of Neurospora through inhibition of cytoplasmic protein synthesis, although it is still possible that mitochondrial protein synthesis is also inhibited. The fact that no mutants resistant to erythromycin have yet been obtained may indicate that more than one system is inhibited by this antibiotic. This work was supported by NIH grant # 12323 to R. P. Wagner. * * = Department of Zoology, University of Texas, Austin, Texas 78712.

Perkins, D. D. Response of thi-5 and thi-1 to vitamin pyrimidine. The mutant 50005 was listed as a thiamine auxotroph by Houlahan, Beadle and Calvin (1949 Genetics 34: 493), who showed that it was not allelic with thi-a designated thi-5 when mapped near pan-1 in linkage group IV (Perkins et al. 1962 Can. J. Genet. Cytol. 4: 187). Specific growth responses were not reported, and thi-5 was not included by Tatum and Bell (1946 Am. J. Botany 33:15) or by Eberhart and Tatum (1959 J. Gen. Microbiol. 20: 43; 1961 Am. J. Botany 48: 702; 1963 Arch. Biochem. Biophys. 101: 378) in their studies of thiamine biosynthesis in Neurospora.

When thi-5 is tested auxanographically, a clear response is obtained to vitamin pyrimidine (2-methyl-4-amino-5-aminomethyl pyrimidine, Nutritional Biochemicals). Of the other thiamine mutants, thi-1, -3 and -4 (9185, 18555, and 85902) do not respond when tested in the same way. But thi-1 resembles thi-5 in showing a strong response to 2-methyl-4-amino-5-aminomethyl pyrimidine, and this is true of both thi-1 strains 56501 and 17084. Our auxanographic tests were made at 34°C in minimal medium containing the antagonist pyritiamine (0.01 μg/ml; Calbiochem) to reduce background growth. Visibly turbid suspensions of conidio from fresh cultures were plated in molten agar, and the test substance was added at a marked position on each plate as soon as the agar had solidified.

Eberhart and Tatum (1959) reported no response of thi-1 (56501) to vitamin pyrimidine in flask assays where vitamin pyrimidine was added to liquid medium at concentrations of 0.25 and 1 μg/ml. It may be significant that the vitamin pyrimidine used by Tatum and Bell and by Eberhart and Tatum was 5-ethoxyethyl rather than 5-aminomethyl. Another possible explanation for the difference in response, suggested by B. M. Eberhart, is that pyrimidine may be antagonizing the antagonist and restoring background growth in the auxanographic tests.

Neither our laboratory nor those of Eberhart or Tatum expect to pursue this problem further. * * = Department of Biological Sciences, Stanford University, Stanford, California 94305.