Fungal Genetics Reports

Volume 28 Article 5

Unstable dTMP auxotrophs in Neurospora crassa

A. M. DeLange

N. C. Mishra

Recommended Citation

DeLange, A. M., and N.C. Mishra (1981) "Unstable dTMP auxotrophs in Neurospora crassa," *Fungal Genetics Reports*: Vol. 28, Article 5. https://doi.org/10.4148/1941-4765.1650

Unstable dTMP auxotrophs in Neurospora crassa

Abstract

Unstable dTMP auxotrophs in *Neurospora crassa*

DeLange, A.M. and N. C. Mishra

Unstable dTMP auxotrophs in *Neurospora crassa*.

In vivo DNA-specific labelling is not possible in Neurospora because it lacks thymidine kinase (necessary for the conversion of thymidine to dTMP) and is unable to take up exogenous dTMP (Fink, R. M. and Fink, R. 1961 BBRC 6: 7). This problem has been overcome in saccharomyces cerevisiae by the isolation of (tup) mutants capable of dTMP-uptake (Brendel, M. W. et al. 1975 Methods in Cell Biology 11: 287; Wickner, R. B. 1975 Methods in Cell Biology 11: 295). These mutants were selected by their ability to grow on medium containing dTMP when aminopterin (APT) and sulfanilamide (SAA) are added to block endogenous synthesis of dTMP from dUMP. It is also necessary to supplement the above selective growth medium with adenine, glutamic acid, glycine and methionine since APT + SAA also block purine and amino acid synthesis. Two kinds of mutants can grow on such a selective medium, one of which is essentially a class of drug-resistant mutants (called asr for resistance to aminopterin and sulfanilamide) and the other which is capable of dTMP uptake (called tup mutants) and behaves as dTMP auxotrophs in the selective medium.

Here we report the isolation of tup mutants of Neurospora. They were obtained on a selective medium which consisted of minimal plating medium (Davis, R. W., deSerres, F. 1970 Methods in Enzymology 17A: 79) supplemented with casaminoacids (1 g/l), yeast extract (2.5 g/l), adenine (20 mg/l), methionine (80 mg/l), glutamic acid (80 mg/l), glycine (80 mg/l), APT (20 mg/l), SAA (5 mg/l) and dTMP (10 mg/l). Plating of mutagenized(25 mM nitrosoguanidine for 30 min.) conidia of strain uvs-3 (FGSC #1627) produced 19 asr colonies; only three of these mutants were dTMP auxotrophs since these did not grow when dTMP was omitted from the selective medium. In contrast, none of the 177 asr mutants obtained from a similar treatment and plating of microconidia strain pe fl.; cot-1 a were dTMP auxotrophs. The three mutants behaved in an identical manner and exhibited the following characteristics:

1. They had a characteristic colonial morphology similar to that of the frost mutant (Garnjobst and Tatum 1967, Genetics 57: 579) on the selective medium.

2. At 37° C, the mutant had an absolute requirement for dTMP; however, at 25° C, the growth in the absence of dTMP was only about 5-10% of that in the presence of dTMP.

3. On sorbose-free medium, dTMP did not stimulate growth at either 25° C or 37° C (i.e., slow growth at 25° C., no growth at 37° C.).

4. Initial analysis of the crosses ORA (wild type) x asr revealed 5 ascospore isolates (out of 23 tested) with the tup phenotype. In addition, three ascospore isolates showed only asr phenotype. Since the asr mutants were used as the male parent, these observations establish the nuclear nature of these mutants.

5. Ascospore isolates with the tup and asr phenotypes were spot-tested on selective and nonselective (lacking APT and SAA) medium at 37°C and incubated for several weeks. Both types of ascospore isolates, though keeping their ability to grow on nonselective medium, lost the ability to grow on selective medium. This instability of mutant phenotype in ascospore isolates was quantified by determining the percentage of mutant(tup or asr) conidia after growth on nonselective medium and/or selective medium (sorbose was omitted to allow conidiation and growth was at 25° C only). in all cases tested, prior growth on selective medium produced conidia, of which l0-15% formed colonies on selective medium; in contrast, prior growth on nonselective medium produced only O-2% resistant conidia.

In summary, we have obtained mutants that were stimulated by dTMP under conditions not permitting endoqenous dTMP production. The stimulation is expressed in the presence but not in the absence of sorbose and appears correlated with an altered morphology. The mutation is nuclear in origin, and is unstable in all ascospore isolates tested. The instability of the mutant phenotype may have a cytoplasmic determinant since the original mutant isolates, but not ascospore isolates, appeared stable thus this instability may indicate epigenetic and genetic interactions. The mutant phenotype may well be caused by amplification of genetic determinants controlling ars and/or tup phenotypes, in which case the instability would correspond to loss of amplified copies; however, several alternative hypotheses can explain the present data. (Supported by DOE Contract No.-DE AS09-78-EVOl-07). - - - - Department of Biology, University of South Carolina, Columbia, South Carolina 29208.