Fungal Genetics Reports

Volume 33 Article 8

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Recommended Citation

Koch, J., and I.B. Barthelmess (1986) "Are the cpc-1 and mts-1 mutations of Neurospora allelic?," *Fungal Genetics Reports*: Vol. 33, Article 8. https://doi.org/10.4148/1941-4765.1584

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Abstract

Are the *cpc-1* and *mts-1* mutations of Neurospora allelic?

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Are the cpc-1 and mts-1 mutations of Neurospora crassa allelic?

A characteristic attribute of mutations affecting general or cross-pathway control in yeast as well as in Neurospora is their amino acid analog sensitivity or resistance. This prompted a survey of all the available Neurospora mutant strains from the Fungal Genetics Stock Center reported to be either analog sensitive or resistant (atr-1,bat,eth-1,fpr-1,fpr-3,fpr-4,mtr,mts,mod-5,nap) in an attempt to identify loci concerning cross-pathway regulation. The only locus involved in cross-pathway control so far known in Neurospora, cpc-1 (Davis 1979, Genetics 93:557-575; Barthelmess 1982, Genet. Res. 39:169-185), was identified by several alleles shown to be sensitive to different amino acid analogs (Barthelmess and Meissner, unpubl.), unable to derepress amino acid synthetic enzymes under amino acid limitation and to have reduced basal enzyme levels.

In the various strains, the regulatory properties of amino acid synthetic enzymes were investigated by estimating the specific activity of ornithine carbamoyltransferase (OCT) of arginine biosynthesis or leucine aminotransferase (LAT) of leucine biosynthesis after growth on minimal and growth under condition of amino acid limitation. To impose the latter, the prototrophic strains were grown for 6-8 hrs in the presence of 6 mM 3-amino-1,2,4-triazole (3AT), which was added after germination and initial growth had occurred in minimal medium. 3AT is an inhibitor of a histidine biosynthetic enzyme, thereby causing limitation of endogenous histidine biosynthesis, the signal for enzyme derepression in regulation proficient strains.

Among the mutant strains investigated, only one turned out to be defective in cross pathway control. This carried the mts (MN1) mutation selected by D.E.A. Catcheside (1978, Neurospora Newsl. 25:17-18) via its 5-methyltryptophan sensitivity. It did not only fail to derepress the OCT and LAT enzymes in the presence of 3AT, but showed 50% reduced basal enzyme levels.

To further characterize the failure of the mts mutant to regulate amino acid synthetic enzymes in vivo, it was crossed with arg-12^s (a bradytrophic mutation at the structural locus for OCT with only 2% OCT activity, shown to be dependent on derepressed arginine synthetic enzymes for prototrophic growth). A novel class of slightly leaky arginine auxotrphic segregants was obtained. This was expected if the double mutant mts,arg-12^s were defective in the regulation of at least the arginine synthetic enzymes. (The cpc-1 mutant alleles had indeed been selected via their property to exhibit arginine auxotrophy in the presence of arg-12^s.) When the arginine auxotrophs were grown in liquid shaken culture with limited arginine supplementation, the LAT enzyme failed to derepress.

Catcheside mapped mts to the right of ylo-1 on linkage group VI. This location indicates either identity or close proximity to the cpc-1 locus, which was also located in the vicinity of ylo-1 (Davis 1979). In agreement with tight linkage, 61 segregants out of the cross mts (MNl) x cpc-1 (CD86) were all 3AT sensitive like the two parental strains, i.e. unable to grow on 4 mM 3AT in liquid stagnant culture.

To analyse the possible allelism between mts (MNl) and cpc-1 (CD86, j-5) mutations, complementation tests were performed (Table 1). It was investigated whether in a heterokaryon composed of nuclei that differed in the cpc-1 and mts alelles, the arginine requirement of each individual component in the presence of an arg-12^s mutation (test A), and the analog sensitivity of the individual components against 3AT or para-fluorophenylalanine (pFPA) was complemented (test B). The control heterokaryons no 1, 2, 4 and 5 (Table 1) demonstrated that under each condition, the mutant alleles were recessive to their respective wild-type alleles. Growth of these heterokaryons also implies that a failure to complement cannot be due to the possiblity that the products of the regulatory loci/locus are confined to their nucleus of origin.

The finding that in heterokaryons 3, 6 and 7 neither the arginine requirement (test A) nor the analog sensitivity (test B) was complemented suggests that mts and cpc-1 mutations belong to the same complementation group, and are therefore allelic.

This result, however, may have to be considered with caution, since we are dealing with regulatory loci. A failure to complement might arise if different regulatory genes are interacting with each other in a control cascade (reported for the yeast genes involved in general control of amino acid biosynthesis). In such a situation the dominance of the wild-type alleles - essential for complementation between diferent loci - may no longer be exhibited in mycelia which are heterokaryotic for two regulatory loci simultaneously.

Table 1 Growth tests in liquid stagnant cultures (in replicate). A: test of arginine auxotrophic strains for complementation on minimal medium, B: test of preformed heterokaryons*) or complementation on 3AT (4 mM) or pFPA (0.55 mM) supplemented medium. - no growth, (+) weak growth in the medium, few aerial hyphae, +, ++, or +++ vigorous growth and aerial hyphae formation

A

heterokaryon no.	genotypes	growth on minimal medium (4 days) individual component	heterokaryon
1	mts cpc^+ arg-12^s	(+)	++
	mts^+ cpc^+ arg-12	-	
2	mts^+ cpc(CD86) arg-12^s	(+)	+++
	mts^+ cpc^+ arg-12	-	
3	mts cpc^+ arg-12^s	(+)	(+)
	mts^+ cpc(CD86) arg-12^s	(+)	

B

	genotypes				growth of heterokaryons (7 days) minimal*)	3AT	pFPA
4	mts cpc^+	pan-2	arg-10^+		+++	+	+++
	mts^+ cpc^+	pan-2^+	arg-10				
5	mts^+ cpc(CD86)	arg-12^+	arg-10		+++	+++	+++
	mts^+ cpc^+	arg-12	arg-10^+				
6	mts cpc^+	pan-2	arg-10^+		+++	-	-
	mts^+ cpc(CD86)	pan-2^+	arg-10				
7	mts cpc^+	pan-2	arg-6^+		**+++**	**-**	**-**
	mts^+ cpc(j-5)	pan-2^+	arg-6				

*) Since heterokaryon formation is inhibited in the presence of 3AT (Barthelmess and Kruppa, unpubl.), all heterokaryons were formed on minimal medium via the forcing markers pan-2, arg-10 arg-12 or arg-6, respectively, and then transferred to the test media.

Supported by the Deutsche Forschungsgemeinschaft. - - - Institut fur Angewandte Genetik, Universitat Hannover, 3000 Hannover, FRG.