Fungal Genetics Reports

Volume 32 Article 2

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Recommended Citation

Feher, Z., M. Schablik, A. Kiss, A. Zsindely, and G. Szabo (1985) "Characterization in inl$^+$ transformants of Neurospora crassa obtained with a recombinant cosmid-pool," *Fungal Genetics Reports*: Vol. 32, Article 2. https://doi.org/10.4148/1941-4765.1567

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Abstract

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The transformability of N. crassa (Case et al. 1979 Proc. Natl. Acad. Sci. USA 76: 5259-5263) makes it possible, in principle, to apply this organism for cloning specific Neurospora genes. Here we describe the attempted cloning of the inl+ gene encoding for myo-inositol-l-P-synthase (MIPS) and our observations regarding the transformants.

An N. crassa gene library was constructed in a BHB 3030 yeast cosmid vector (Feher 1984 Neurospora Newsl. 31: 32). Each E. coli clone of the library contains recombinant DNA molecules i.e. fragments of Neursopora DNA inserted into the BamHI site of the cosmid vector. DNA was prepared from all 5000 clones of the library (a recombinant cosmid-pool) and it was purified further on a CsCl-ethidium bromide gradient (Maniatis et al. 1982 : Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). 20 µg of the cosmid-pool DNA was used to transform protoplasts (Schablik et al. 1983 Neurospora Newsl. 30: 17) of an inl,rg N. crassa strain (R2506), according to the procedure of Case (1982 in: Genetic Engineering of Microorganisms for Chemicals, eds. Hollaender et al. pp. 87-100 Plenum, New York) with slight modifications. Two inl+ transformants (Tl and T3) were obtained. They were back-crossed to an inl (89601) strain and several inl+ ascospores from the F_1 progeny were selected for further genetic and biochemical studies.

The inl+ phenotype proved to be inherited as a chromosomal gene on linkage group V. The map distance from the neighbouring al-3 locus was 0.58% and 2.46% for T1 and T3, respectively. This value in the wild type strain was 1.16%. These results convincingly show that transformants have inl+ genes closely linked to al-3. One of the transformants, T1, exhibited mitotic and meiotic instability.

Cross-immunoelectrophoresis of the purified gene product (MIPS) showed that the transformants synthesize both the "wild type" enzyme and the inactive enzyme-protein. The specific activity (enzymatic activity / µg antigen) of the enzyme purified from the transformants proved to be about 50% lower than that of the wild type enzyme. Physiological regulation of MIPS synthesis -"inositol repression" - was same in the transformants as in the control.

These results suggest that an integration event resulted in a tandem arrangement of *inl* sequences leaving the expression of both *inl*$^+$ and *inl*$^-$ genes intact.

In Southern hybridization experiments, bands characteristic for covalently closed circular plasmids were detected in the DNA isolated from the F1 progeny of the transformants using the vector as a hybridization probe. (The principle of the detection was that the supercoiled plasmid DNA migrates faster than uncleaved chromomsomal DNA in an agarose gel-electrophoresis.) The copy-number of the transforming cosmid sequences was estimated to be 20-25/genome right after transformation and l-2/genome after two months of vegetative propagation on agar slants with periodic transfers. Eleven recombinant plasmids (pNCs) with different restriction patterns were recovered from the transformants by means of *E. coli* transformation. Some of these plasmids proved to be rearranged in *N. crassa*, e . g . deletions within the vector sequences could be detected. None of the pNCs transformed Neurospora to inositol prototrophy. There are a number of explanations for the presence of different plasmids in the F1 progeny of integrative-type transformants: (1) The plasmids were maintained during meiosis by autonomous replication. (2) They are the result of excision from the chromosome. (3) chromosomal DNA fragments carrying vector sequences were taken up and circularized by *E. coli*. - - - Departments of Biology and Biochemistry*, University Medical School, H.-4012 Debrecen, Hungary. $^+$Present Address: Department of Biology, University of Rochester, NY 14627.