Identification of rRNA processing gene homologs of yeast in *Neurospora crassa*

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Abstract
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B. gal-4 and gal-7

C.F. Roberts 1963 (J. Gen. Microbiol. 31:45-58 and Ph.D. thesis, Glasgow 1961) originally found that these two leaky galactose mutants showed only partial complementation and were apparently closely linked: he concluded that the mutations were allelic. He also concluded from a haploidization that gal-4 was probably in linkage group I, but the then switched to mitotic crossing-over experiments with gal-7 and showed that this mutant could not be on either arm of chromosome I, and a further haploidization with gal-7 suggested, on the basis of 8 segregants, that it might be on VIII.

Some time ago, I tried to retest the location of gal-7, and in two haploidizations concluded that it was probably in linkage group IV, but neither experiment was without its difficulties (either of gal classification or of allele ratios) and a third haploidization was totally inconclusive. No meiotic linkage of gal-7 to linkage group markers has been found over the years, although some gal-7 is present on a number of mapping strains. I also failed to find linkage with markers on IV in one cross.

Lacking any hard evidence, in the latest edition of Genetic Maps (Cold Spring Harbor 1986) I have designated gal-7 as belonging to a separate locus: galH, possibly located on IV.

Classification of galactose non-utilizers appears to be a common problem, variable from cross to cross. It seems likely that these mutants are subject to suppression by unsuspected markers in the stocks: it might be worthwhile to test for the effect of ssbA (sorbitol suppressor - E. Käfer 1986, FGN 33:27-28) on galactose mutants. - - - Institute of Genetics, University of Glasgow, Glasgow G11 5JS, Scotland

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Biochem. 55:137-152). Very little is known about the processing of rRNA genes of Neurospora. We were therefore interested, as a first step, in identifying DNA sequences of Neurospora crassa 74A that would hybridize with the rRNA processing genes of yeast using cloned yeast-rRNA-processing genes as probes.

DNA sequences containing rRNA-processing genes of yeast were supplied by Anita Hooper (Pennsylvania State University), Robert L. Last (Carnegie-Mellon University) and Robert J. Crouch (National Institutes of Health). Neurospora crassa wild type 74A DNA was isolated as described by Chambers et al. 1986 (Gene 44:159-164) and neuroblastoma, E. coli and rice DNAs were isolated by following the standard methods described by Verma and Dutta, 1986 (Curr. Genet. 11:309-314). Hybridization conditions were as described by Maniatis et al., 1982 (In: Molecular Cloning - A Lab Manual, Cold Spring Harbor Laboratory).

Plasmid DNAs of RNA1, RNA2, RNA3, RNA4 and RNA5 yeast genes were cloned into E. coli LE392 and named pRP1-pRP5 respectively. Nuclear DNAs from N. crassa wild type 74A and distantly related organisms like animal neuroblastoma cell line NG108, Oryza sativa and E. coli were digested with restriction enzymes PstI, HindIII, EcoRI, BamHI, KpnI and SmaI and run on 0.7% agarose gels. The molecular weight markers were lambda DNA digested with Hind III and pCC103 DNA (Dutta et al., 1986 The Nucleus 29:9-20) digested with either PstI or EcoRI. DNAs from gels were transferred to nitrocellulose filters by Southern technique and hybridized with rRNA processing gene clones (pRP1-pRP5) as probes. Results indicate that clones pRP1, pRP2, pRP3 and pRP4 (containing rRNA processing genes 1, 2, 3 and 4, respectively) hybridized with N. crassa DNA (under highly stringent conditions of hybridization), but not with phage DNA, rice DNA or neuroblastoma DNA. This suggests that analogs of rRNA processing genes exist in the N. crassa strain 74A. Supported in part by a contract with the Department of Energy and partly from an institutional grant from the National Institute of Health to SKD. - - - Depts. of Botany and Genetics & Human Genetics, Howard Univ., Washington DC 20059