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Identification of rRNA processing

gene homologs of yeast in

<u>Neurospora</u> crassa

Ribosomal RNA genes play a vital role in the development of an organism. In eucaryotes, the precursor ribosomal RNA genes are transcribed and then processed into mature rRNAs viz. 5.8s. 17S and 26S. This processing of pre-rRNA is believed to be regulated by protein products of specific genes. In yeast (<u>Saccharomyces</u> <u>cerevisiae</u>) some of the rRNA processing genes have been identified, cloned and sequenced (Sollner-Webb, B. and J. Tower 1986 Ann. Rev.

Biochem. <u>55</u>: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 <u>Neurospora</u> 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 <u>E. coli</u> LE392 and named pRP1-pRP5 respectively. Nuclear DNAs from <u>N. crassa</u> wild type 74A and distantly related organisms like animal neuroblastoma cell line NG108, <u>Oryza sativa</u> and <u>E. coli</u> 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 <u>N. crassa</u> 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