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Abstract

We have been employing the Orbach-Sachs (1991 Fungal Genetics Newsl. 38:97) and Vollmer-Yanofsky (Vollmer and Yanofsky 1986 Proc. Natl. Acad. Sci. USA 83:4869-4873) *N. crassa* cosmid libraries (obtained from the Fungal Genetics Stock Center) in subtractive hybridization experiments designed to identify genes induced under conditions of oxidative stress. Because cosmids containing the 17S and 26S ribosomal RNA genes produce false positives in these experiments, it was necessary for us to identify cosmids in the Orbach-Sachs containing these genes.

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Ribosomal DNA representation in the Orbach-Sachs and Vollmer-Yanofsky *N. crassa* cosmid libraries. Ken Sylvester and Donald O. Natvig - Department of Biology, University of New Mexico, Albuquerque, NM 87131. We have been employing the Orbach-Sachs (1991 Fungal Genetics Newsl. 38:97) and Vollmer-Yanofsky (Vollmer and Yanofsky 1986 Proc. Natl. Acad. Sci. USA 83:4869-4873) *N. crassa* cosmid libraries (obtained from the Fungal Genetics Stock Center) in subtractive hybridization experiments designed to identify genes induced under conditions of oxidative stress. Because cosmids containing the 17S and 26S ribosomal RNA genes produce false positives in these experiments, it was necessary for us to identify cosmids in the Orbach-Sachs containing these genes. Cosmids carrying ribosomal DNA (rDNA) in the Vollmer-Yanofsky library were identified previously (1990 Catalogue of Strains, Fungal Genetics Stock Center, Supplement to Vol. 37:3). We report here an apparent underrepresentation of rDNA cosmids in the Orbach-Sachs library, as determined in a rigorous experimental procedure, together with the locations of three rDNA cosmids in the Vollmer-Yanofsky library that were not previously reported.

In our initial experiments, colony filters from the Orbach-Sachs and Vollmer-Yanofsky libraries were probed with labeled, subtracted cDNAs. Previously identified rDNA cosmids from the Vollmer-Yanofsky library all produced strong signals on the autoradiograms. Only one cosmid from the Orbach-Sachs library, X20:F9, produced a comparable signal.

More definitively, we probed the Orbach-Sachs library for rDNAs using sequences from pRW528, a pBR322-derived plasmid that contains the *N. crassa* 17S and 26S rRNA genes (Russell et al. 1984 Mol. Gen. Genet. 196:275-282; Butler and Metzzenberg 1989 Genetics 122:783-791). An 8.7 kbp *Bam*HI fragment carrying the rRNA genes from this plasmid was gel purified and labeled with ³²P. This fragment was used to probe blotted colonies in the Orbach-Sachs library (with the exception of those on plate X25). This screen again revealed only X20:F9 as a strongly hybridizing cosmid.

In a final experiment, we isolated DNA from cosmid X20:F9 and 88 other Orbach-Sachs cosmids, which included every possible candidate for an above-background hybridization to pRW528 or our labeled cDNAs. These DNAs were bound to nylon membranes (Zetabind) using a Bio-Rad Bio-Dot SF microfiltration apparatus and were probed with the labeled pRW528 rDNA fragment. We also included in these experiments cosmid DNA preparations from 21 strains from the Vollmer-Yanofsky library that had hybridized weakly or strongly to labeled cDNAs, and which did not overlap with the previously identified rDNA cosmids from this library. The 110 cosmid DNAs in question were distributed across three separate membranes. We included on each membrane cosmid X20:F9 and three control DNAs: genomic *N. crassa*, pRW528, and cosmid 13:10G (a known rDNA cosmid from the Vollmer-Yanofsky library).

Restriction digests and gel analyses were used to insure that DNA loading was approximately equal for all cosmids. Hybridization to the three controls was strong on all autoradiograms. Among Orbach-Sachs cosmids, there was detectable hybridization to only X20:F9, which was consistently strong. Three cosmids (in addition to 13:10G) from the Vollmer-Yanofsky library showed strong hybridization: 25:10D, 4:12H and 13:4A. We suggest that these three Vollmer-Yanofsky cosmids contain rDNA. A representative hybridization is shown in Fig. 1.

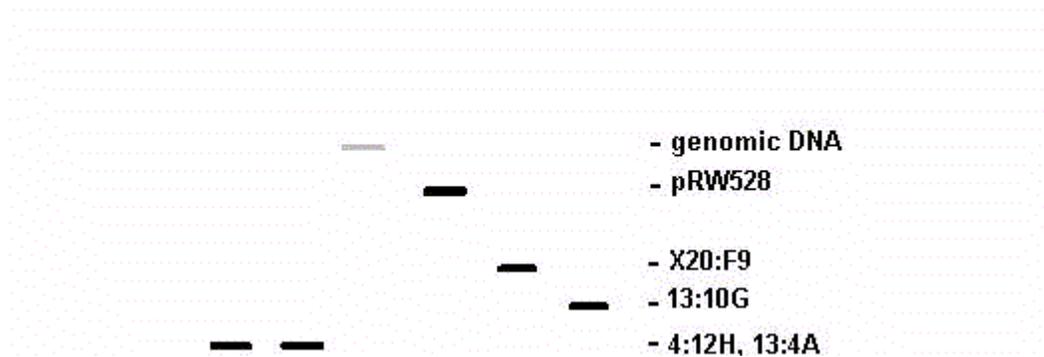


Fig. 1. Hybridization of labeled rDNA from plasmid pRW528 to vacuum-blotted cosmid and genomic DNAs. The membrane shown contained the cosmid DNAs and 35 additional non-hybridizing cosmid DNAs.

We are unable to explain the nearly total lack of rDNA inserts in the Orbach-Sachs cosmid library. In *N. crassa* the rRNA genes are tandemly arranged in approximately 9.2 kbp units (Russell et al. 1984 Mol. Gen. Genet. 196:275-282). The average cosmid therefore would be expected to contain four to five rDNA repeats. One possibility is that promoters in the pMOcosX vector cause significant transcription of *N. crassa* rRNA genes in host cells, resulting in inhibition of bacterial growth. If this is the case, it should perhaps provide a cautionary note to users of the library who are interested in other genes.