Fungal Genetics Reports

Volume 43 Article 26

Identification of several novel Neurospora genes in the lambda-ZAP I and lambda-ZAP II libraries

Roxanne A. Yamashita
University of Hawaii

W. Dorsey Stuart
University of Hawaii

Recommended Citation

Yamashita, R. A., and W.D. Stuart (1996) "Identification of several novel Neurospora genes in the lambda-ZAP I and lambda-ZAP II libraries," *Fungal Genetics Reports*: Vol. 43, Article 26. https://doi.org/10.4148/1941-4765.1322

Identification of several novel Neurospora genes in the lambda-ZAP I and lambda-ZAP II libraries

Abstract

Polyclonal antibodies to and oligonucleotides derived from N-terminal sequences of media proteins of *Neurospora crassa* were both used to screen the FGSC lambda-ZAP I and lambda-ZAP II cDNA libraries. Primary sequencing of the identified phagemids revealed homologies to a number of known genes. These genes are listed and the cDNA clones are available from our laboratory to interested parties.

Identification of several novel *Neurospora crassa* genes in the lambda- ZAP I and lambda-ZAP II libraries

Roxanne A. Yamashita and W. Dorsey Stuart - Department of Genetics and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96822

Polyclonal antibodies to and oligonucleotides derived from N-terminal sequences of media proteins of *Neurospora crassa* were both used to screen the FGSC lambda-ZAP I and lambda-ZAP II cDNA libraries. Primary sequencing of the identified phagemids revealed homologies to a number of known genes. These genes are listed and the cDNA clones are available from our laboratory to interested parties.

A library screen was initiated in an attempt to identify *Neurospora crassa* genes that code for secreted proteins. The signal sequences and promoters from these genes were to be studied in an effort to increase the levels of secreted heterologous protein in a *N. crassa* production system.

Polyclonal antibodies were generated (HYI-Bio Products) using 2.5 mg of extracellular proteins contained in conditioned media (Vogels with 2% sucrose) from 6 day old *N. crassa* cultures. PAGE-SDS (Novex) analysis of the concentrated media revealed between 17-20 major extracellular proteins of *N. crassa*. Western blot analysis confirmed reactivity to the rabbit antibodies generated. Lambda-ZAP I/II libraries available from the Fungal Genetics Stock Center were screened using this polyclonal antibody. Three phagemids were isolated, purified, and partially sequenced from both ends (Table 1). Additionally, proteins from culture supernatants separated by PAGE-SDS were transferred to a PVDF membrane and five were N-terminal sequenced. A degenerate DNA probe was made to one band from a sucrose grown culture corresponding to a protein of approximately 14.4 kd. This was chosen as a candidate because no extracellular proteins of this weight have been reported to date. The probe was digogxigenin labeled and used to identify clones in the Lambda-ZAP I/II libraries. Eight resulting phagemids were isolated and partially sequenced (Table 1). All sequences were analyzed and tentatively assigned by homology using FastA in the GCG package.

Table 1. Clones from the lambda-ZAP library identified by either the antibody screening method or by oligonucleotide screening method.

Antibody screen		
Clone	Homologous sequence (GCG acession #)	Organism
1c	Fatty acid synthetase (x03977)	S.cerevisiae

4d	Ribosomal protein (S53436)	K. marianus
11c	Ribonucleotide reductase M2 subunit (x68127)	M. auratus (golden hamster)

Oligonucleotide screen

Clone	Homologous sequence (GCG acession #)	Organism
28b	Superoxide dismutase (Z27080)	C. elegans
4a	Sucrose binding protein (L06038)	Glycine max (soybean)
2a	mRNA for mitochondrial ADP/ATP carrier (X00363)	N. crassa
26b	Fructose 1,6-bisphosphate aldolase (D17415)	S. pombe
19b	Photolyase (X58713)	N. crassa
13a	mRNA for protein kinase C inhibitor homologue (X62838)	Oenothera hookeri (evening primrose)
16a	N-myristoyltransfe rase (L25118)	H. capsulatum
1b	mRNA for mitochondrial ADP/ATP carrier (X00363)	N. crassa

Last modified 7/25/96 KMC