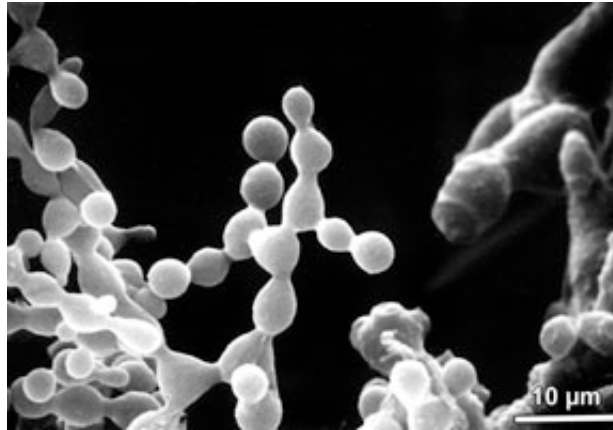


Bread Mold as Fungi Model Receives Grant for \$9 Million

Researchers interested in filamentous fungi will have a new model to derive genetic information from. As part of a five-year, \$9 million grant from the National Institutes of Health, Bethesda, Md., researchers from seven universities will collaborate on projects aimed at elucidating the *Neurospora crassa* (bread mold) genome.

"*Neurospora* is a model for filamentous fungi," says Jay Dunlap, PhD, professor of genetics and biochemistry at Dartmouth Medical School, and principal investigator for the grant, titled "Functional Analysis of a Model Filamentous Fungus."

There are roughly 250,000 different filamentous fungi, which are quite different from yeast in both their biology and molecular biology, says Dunlap. "In *Neurospora*, for example, most of the genes have introns, there are many genes with multiple promoters, and there is alternative splicing. These are characteristics that are unusual in yeast and much more typical of animals."



The genomic characteristics of the filamentous fungi *Neurospora* are unusual in yeast and much more typical of animals. (Source: Matthew Springer, PhD, University of California)

Filamentous fungi are also very important animal and plant pathogens. Although *Neurospora* does not specifically have much significance, it is the most well understood filamentous fungus and offers a good model for these organisms, says Dunlap. "There is a lot to be learned in general about how filamentous fungi work. About 40% of the genes are unknown, so there is an enormous amount of biology that is not described."

The *Neurospora* project will provide a model for finding the functions for genes, to researchers working on other filamentous fungi, says Dunlap, such as *Cochliobolus* or *Magnaportha*, which are two important pathogens for corn and rice, respectively. *Neurospora* is an important model system itself, in specific areas such as DNA methylation and photobiology, he says.

Dartmouth University and the University of California Riverside plan to collaborate on running gene knockouts. Phenotypic analysis of the knockouts, focusing on routine characterization of general biological functions, will be conducted at University of California Los Angeles. Bioinformatics work and the creation of a community-based annotation for the Web will be centered at Massachusetts Institute of Technology with a subcontract to Oregon Health and Science University. Transcriptional profiling will be centered at the University of California Berkeley. Creation of complementary DNA libraries and a single-nucleotide polymorphism (SNP) map will be shared between University of New Mexico and Dartmouth.

The gene knockouts, which are being handled by Dunlap's team at Dartmouth, will be performed using a split-marker strategy by homologous integration. In this process, two fragments are used to knock out a gene. On the left flank is a piece of genomic DNA homologous to that region surrounding that gene and containing two-thirds of a selectable marker. On the right flank is a piece of genomic DNA containing an overlapping two-thirds of the selectable marker. "When the two fragments recombine, you get a completely functional selectable marker flanked on either side by the pieces of DNA," says Dunlap.

The project started in April and is now in the early planning stages. The first milestone for Dunlap's team is to complete the knockout cassettes, the pieces of DNA that will be used to make the knockouts, for all 10,000 genes in *Neurospora*, constructed within a year. "There has been a lot of interest in the fungal genetics community in using these cassettes to knock out genes of particular interest to people," says Dunlap. Although his team plans to

concentrate on genes that are unknown and probably of lesser interest to investigators, the community will be able to use the cassettes to knock out their own sets of genes. He estimates that by the end of five years, they will have knockouts for about one-half of all of *Neurospora's* genes.

"The work should provide a terrific background for analyzing all of the filamentous fungi," says Dunlap, "[thus] opening a door on a whole kingdom of organisms that have been largely unexplored."

—Elizabeth Tolchin