

Identification of a gene from *Neurospora crassa* with similarity to a glucoamylase gene from *Schwanniomyces occidentalis*

Daniel J. Ebbole¹ and Lea Madi². Program for the Biology of Filamentous Fungi. ¹Texas A&M University, College Station, TX 77843. ²Current address: Dept. of Plant Pathology and Microbiology, Hebrew University of Jerusalem, Rehovot, Israel.

Glucoamylases are important industrial enzymes used in the conversion of starches to syrups and in other fermentation processes. Previously, the gene encoding the major glucoamylase activity of *N. crassa* was characterized (Stone et al. 1993 Curr. Genet 24:205-211). Here we report the identification of a possible second glucoamylase (*gla-2*) that is similar to a member of a class of glucoamylases represented by the *GAMI* gene of *S. occidentalis* (Dohmen et al. 1990 Gene 95:111-121).

In our analysis of the *rco-3* gene of *N. crassa* we identified *gla-2* through partial sequencing of the DNA adjacent to *rco-3* from two sites in the clone. Homology to *GAMI* was found through a BLAST search using the translated single-stranded DNA sequences. An alignment by the Wisconsin GCG program 'Bestfit' is shown in Fig. 1. *gla-2* and *GAMI* are sufficiently similar in the sequenced regions to suggest that *gla-2* is likely a functional glucoamylase with properties similar to *GAMI*. The 3' end of the *N. crassa gla-2* coding region was sequenced and the predicted protein has a 39 amino acid extension relative to *GAMI*. There is no significant sequence similarity between *gla-2* and *gla-1* of *N. crassa*.

```
Sequence 1
287
So LYFFSGPTPKDAIQQYVKEIGLPAFQPYWSLGYHQCRWGYDTIEKLSEVVENFKKF
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
Nc SISCTLEDLLRYHQVYQQYVGLPAMQQYWTLLGFHQCRWGYSNWTVVKDQVVDNFRKF

                                           392
So NIPLLETIWSIDIDYMSYKDFTYDPRFPLDEYRKFLDELHKNNQHYPVIL
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
Nc GIPLLETIWSIDIDYMKGYRDFENDPD.FSYEEGARFLEELHKNNHMTCLRS

Sequence 2
908                                           958
So VTILGVGHKPKSVKFENANVDFTYKKST.....VFVTGLDKYTKDGAFSKDFITW*
   : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
Nc VTILGVAEAPLGVAINSHLLGSASWSYDSEGEVLSVTELDNFKEGGWASNWTLWS

Nc NSASNSGSSPVQGGGRLEFSSPICSMQLLSASFLAACL*
```

Figure 1. The *S. occidentalis* (So) *GAMI* sequence is numbered with respect to the published initiator Met codon. Two regions of the *N. crassa* (Nc) gene were sequenced and the predicted polypeptide regions were aligned to *GAMI*. Identical residues are indicated by a colon (:). The C-termini of the proteins predicted from DNA sequence data are indicated by an asterisk (*).

The *gla-2* gene and *rco-3* are present in cosmid X10:E5 of the hygromycin cosmid library constructed by M. Orbach and M. Sachs (Orbach, M. J. (1994). *Gene* 150, 159-162; Orbach, M. J., and Sachs, M. S. (1991). *Fungal Genet. Newsl.* 38, 97.). A 10 Kb *Xba*I fragment from this cosmid contains both *rco-3* and *gla-2* with the gene organization shown (Fig 2). The cosmid was mapped by RFLP analysis and confirmed by genetic mapping of *rco-3* with respect to *his-3* (3/65) and the mating type locus (1/65) as being near the centromere of Linkage Group I (Fig. 3). The data suggest that X10:E5 could be placed on either side of *mei-3*.

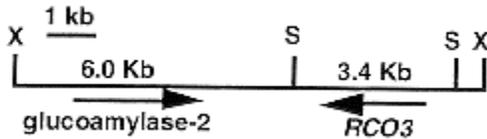


Figure 2. Organization of the *gla-2* and *rco-3* genes of *N. crassa*. The 10 Kb *Xba*I (X) fragment contains a 3.4 Kb *Sma*I (S).

```

AP31a.1      MMMMOOMMMMMOOMMMMMMM-MMOOOOOOOOOO-MOOO--M
AP8v.1      MMMMOOMMMMMOOMMMMMOMMMMMMOOOOOOO-MMOOOO-M
mei-3       MMMMOOMMMMMOOMMMMMMMMMMMMMOOOOOOO-MMOOOO-M
X10:E5      MMM-MOMMMMMOOMM-MMMOM-M--OOOOOOO-MOOOO-M
un-2, his-2 MMMMOOMMMMMOOMMMMMMMMMMMMMOOOOOOO-MMOOOO-M
lys-4       MMMMOOMMMMMOOMMMMMMMMMMMMMOOOOOOO-MMOOOO-M
Fsr-33      MMMMOOMMMMMOOMMMMMMMMMMMMMOOOOOOO-MMOOOO-M

```

Figure 3. RFLP mapping of cosmid X10:E5. The entire cosmid was labeled and used to probe *Bam*HI digested chromosomal DNA from progeny of the Oak Ridge x Mauriceville RFLP mapping cross.

The complete sequence of *gla-2* will be of interest to those interested in the structure and function of glucoamylases. A plasmid (pLMN1) containing the 10 Kb *Xba*I fragment has been deposited in the FGSC. Please contact DJE by e-mail at dje0282@zeus.tamu.edu for DNA sequence files.