Fungal Genetics Reports

Volume 37 Article 23

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Recommended Citation
SACHS, M. S., and D. EBBOLE (1990) "The use of lacZ gene fusions in Neurospora crassa," *Fungal Genetics Reports*: Vol. 37, Article 23. https://doi.org/10.4148/1941-4765.1488

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Abstract

Systematic analyses of gene expression in diverse organisms have relied on genetic fusions in which the regulated expression of the product of the *Escherichia coli lacZ* gene, ß-galactosidase, is used to assay gene activity. Because there are low but readily detectable levels of ß-galactosidase in *Neurospora crassa* (e.g. Landman, Arch. Biochem. Biophys. 52:93-109, 1954), the use of *E. coli lacZ* as a reporter gene in this organism has not been extensively investigated. Here we report that *lacZ* fusion proteins can be used to analyze the regulation of two *N. crassa genes, arg-2* and *con-10*. The levels of ß-galactosidase produced by strains carrying the fused genes indicate that they are developmentally regulated in a manner similar to the intact genes (Davis, Microbiol. Rev. 50: 280-313, 1986; Orbach, Sachs, and Yanofsky, J. Biol. Chem. in press 1990; Roberts, Berlin, Hager and Yanofsky, Mol. Cell. Biol. 8:2411-2418, 1988; Sachs and Yanofsky, in preparation).

The use of *lacZ* gene fusions in *Neurospora crassa*

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Systematic analyses of gene expression in diverse organisms have relied on genetic fusions in which the regulated expression of the product of the *Escherichia coli lacZ* gene, ß-galactosidase, is used to assay gene activity. Because there are low but readily detectable levels of ß-galactosidase in *Neurospora crassa* (e.g. Landman, Arch. Biochem. Biophys. 52:93-109, 1954), the use of *E. coli lacZ* as a reporter gene in this organism has not been extensively investigated. Here we report that *lacZ* fusion proteins can be used to analyze the regulation of two *N. crassa genes, arg-2* and *con-10*. The levels of ß-galactosidase produced by strains carrying the fused genes indicate that they are developmentally regulated in a manner similar to the intact genes (Davis, Microbiol. Rev. 50: 280-313, 1986; Orbach, Sachs, and Yanofsky, J. Biol. Chem. in press 1990; Roberts, Berlin, Hager and Yanofsky, Mol. Cell. Biol. 8:2411-2418, 1988; Sachs and Yanofsky, in preparation). Expression of the *arg-2*/*lacZ* fusion gene is high in germinating conidia and under conditions of amino acid starvation; expression is reduced by growth in arginine-containing media. Expression of the *con-10*/*lacZ* fusion is high in conidia and in conidiating cultures. We anticipate that the use of ß-galactosidase fusions will help extend our knowledge of how these and other Neurospora genes are regulated.

Experimental. Plasmid pUD284, derived from pAE1 (Orbach et al.,1990), contains *lacZ* fused in-frame to codon 10 of *arg-2*. Nucleotides 1479-2705 of *arg-2* were removed (by *Sty*I-digestion of pAE1) and replaced with the 3.4 kb *Sma*I-*Xba*I fragment of pC4Bgal (Thummel, Boulet and Lipshitz, Gene 74:445-456,1988) using appropriate DNA manipulations. The DNA regions containing the *arg-2* mRNA 5' and 3' ends were left intact. Plasmid pUD234 contains (i) the 1.9 kb *Kpn*I-*Bam*HI fragment of pCon10a, which includes the DNA upstream of the *con-10* mRNA start through codon 40 of the *con-10* coding sequence (Roberts et al., 1988), followed by (ii) the *Bam*HI *lacZ* cartridge from pMC1871 (Shapira, Chou, Richaud and Casadaban Gene 25:71-82, 1983) and (iii) the *Bam*HI-*Xba*I fragment containing the RNA termination region from the *Aspergillus nidulans trpC* gene (Cullen, Leong, Wilson and Henner, Gene 57:21-26, 1987). Both pUD plasmids also contain truncated *N. crassa his-3* genes (DNA downstream of the *Hin*dIII site of pNH60, Legerton and Yanofsky, Gene 39:129-140, 1985) to select for site-specific integration in Neurospora, as well as additional sequences to maintain the plasmids in bacteria.

The fused genes were placed into the Neurospora genome by transformation of *his-3* spheroplasts (FGSC#462) and selection for his+ prototrophs on minimal medium. Prototrophic homokaryons were purified by microconidiation (see accompanying article). Analyses of genomic DNA from transformants by Southern blotting showed that the *lacZ* fusions were present as single copy genes integrated at the *his-3* locus.

The hydrolysis of o-nitrophenyl-ß-d-galactopyranoside (ONPG) was used to assay ß-galactosidase enzyme levels in clarified whole cell extracts (J. H. Miller, Experiments in Molecular Genetics, 1972, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). Germinating and mycelial cultures were grown in Vogels minimal medium/2% sucrose ± 0.4 mg/ml arginine, or in medium to induce microcyclic macroconidiation (Guignard et al., 1984),

and harvested on Whatman 541 filter paper by vacuum filtration. After rinsing with water, the cells were resuspended (0.5-1 g wet weight cells/10 ml) in either ice cold HK (1 x HK buffer is 20 mM HEPES pH 7.9, 100 mM KCl, 2 mM DTT) or Z buffers containing 1 mM phenylmethylsulfonyl fluoride (added immediately prior to use from a 0.1 M stock prepared in isopropanol) by vortex mixing. Cells were broken by passage through a French press twice at 16000 psi. Clarified supernatants were obtained from the extracts following centrifugation at 4°C for 10 min at 17000 xg in a Sorvall SS34 rotor. For long term storage, glycerol was added to 20% (v/v); aliquots were quick-frozen on dry ice and stored at -70°C.

Neurospora cultures containing *arg-2*/*lacZ* fusions were also broken by sonication. Cells (0.1 g wet weight) were resuspended in one ml of cold Z buffer in a 1.5 ml eppendorf tube and were sonicated with two 10-second bursts (Heat Systems - Ultrasonics micro tip 415 attached to a model W225R sonicator; power level 4-5) with cooling between bursts. Clarified supernatants were obtained from extracts following centrifugation for 5 min at 4°C in an eppendorf microcentrifuge.

Protein levels were determined using the Bradford assay with bovine serum albumin as the reference standard. ß-galactosidase activity was measured as described (Miller, 1972). Endogenous ß-galactosidase in 74-OR23-IVA and FGSC #462 ranged from 0.5-2 units/mg protein in extracts prepared with the French press, and from 0.1-0.5 units/mg protein in extracts prepared by sonication.

Results. We constructed translational fusions of *lacZ* to *arg-2* and *con-10* and introduced the fusions into the *N. crassa* genome by transformation. Strains were analyzed for ß-galactosidase levels at different developmental stages. Strains containing the *con-10*/*lacZ* and the *arg-2*/*lacZ* fusion genes produced higher levels of ß-galactosidase than untransformed strains under all growth conditions examined. Compared to ß-galactosidase levels during mycelial growth in Vogel's medium (20 units), ß-galactosidase levels in the *con-10*/*lacZ* strain were 70-fold greater in conidia and were increased 12-fold when conidiation was induced in liquid culture by nitrogen limitation (Guignard et al., 1984). Levels of ß-galactosidase in the *arg-2*/*lacZ* strains were higher in germinating conidia than in mycelia. Levels of ß-galactosidase were reduced at least 3.5-fold in mycelia by growth in arginine-containing medium. In other experiments in which we integrated *arg-2*/*lacZ* fusion genes into *arg-12s* and wild type *N. crassa* strains without targeting integration to a specific site, ß-galactosidase expression in minimal medium was greater for the *arg-12s* transformants, which are starved for arginine in minimal medium, than for wild type transformants. Expression of the *arg-2*/*lacZ* fusion was reduced more than 10-fold by growth of *arg-12s* transformants in arginine-supplemented medium.

Conclusion. *lacZ* is a reliable reporter gene in *N. crassa*. For the most rigorous quantification of *lacZ* activity, it may be necessary to separate *lacZ* from the endogenous ß-galactosidases, e.g., by using immunochemical or biochemical methods.

Acknowledgements. We gratefully acknowledge the support and encouragement of Charles Yanofsky. We thank Michael Plamann for suggesting the sonication protocol, and both Michael Plamann and Karl Hager for establishing the utility of *his-3* targeting in *N. crassa*. M. Sachs and D. Ebbole contributed equally to this work.