Vol. 179, No. 1, 1991
August 30, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 423-427

OVEREXPRESSION OF THE ACTIN GENE IS ASSOCIATED WITH THE MORPHOGENESIS OF *CANDIDA ALBICANS*

Vijay Paranjape and Asis Datta

Molecular Biology Laboratory, School of Life Sciences,
Jawaharlal Nehru University, New Delhi 110 067,
India

Received July 17, 1991

A progressive increase in the synthesis of actin mRNA was observed by Northern blot analysis, when cells were induced to form germ tubes at 37°C by N-acetyl-D-glucosamine. Presence of trifluoperazine, a calmodulin inhibitor, or incubation of cells at 25°C, or by replacing N-acetyl-D-glucosamine with glucose which inhibited germ tube formation lowered this synthesis. Furthermore, in vitro translation of total RNA revealed an increase in the synthesis of actin (45 kDa) during germ tube formation. These results suggest for the first time that the expression of actin gene is regulated during morphogenesis of *C. albicans*.

Candida albicans, a pathogenic dimorphic yeast, can grow in yeast or hypha form depending on environmental conditions (Odds, 1988; Datta etal., 1989). Germ tube, an intermediate stage in yeast to hypha transition, can be induced by a number of factors including N-acetyl-D-glucosamine (GlcNAc) (Shepherd etal., 1980; Natarajan etal., 1984). There are several reports on the identification of morphology specific gene products in *C. albicans* by separation of yeast and hyphal cytoplasmic proteins using one- and two- dimensional polyacrylamide gel electrophoresis (Odds, 1988). Unfortunately these proteins have not been characterised and their role in morphogenesis is not known. Hence a direct analysis of gene transcription by investigation of RNA in yeast and germ tube would help in the identification of morphology specific genes in *C. albicans*.

We had earlier reported that calcium and calmodulin regulate morphogenesis in *C.albicans* (Paranjape etal., 1990) and other fungi (Paranjape and Datta, 1990). Calcium and calmodulin are also known to regulate cytoskeletal organization (Manalan and Klee, 1982) and actin polymerization (Greer and Schekman, 1982; Sobue etal., 1982). Actin granules are associated with the growth zones of yeast and hypha of *C. albicans* (Anderson and Soll, 1986). It was therefore of interest to investigate whether calmodulin

0006-291X/91 $1.50

is involved in regulation of actin gene expression and thereby controlling morphogenesis. In this paper, by Northern blot hybridization and in vitro translation of total RNA we have examined the expression of actin gene during GlcNAc-induced germ tube formation in *C. albicans*.

EXPERIMENTAL PROCEDURES

C. albicans SC 5314 was maintained on a medium containing 2% peptone, 1% yeast extract, 2% glucose and 2% agar (all w/v). The cells were grown for 17 h in a liquid medium containing 1% glucose, 0.5% peptone and 0.3% KH_2PO_4 and then transferred to a new medium (with half the concentration of glucose) and grown for 11 h into stationary phase. Germ tubes were induced as described (Shepherd etal., 1980; Natarajan etal., 1984). Approximately $5x10^7$ cells/ml were incubated at 37°C in a 20 mM imidazole/HCl buffer (pH 6.6) containing 0.2 mM $MnCl_2$ and 5 mM GlcNAc as inducer. Total RNA was isolated at various times during germ tube formation by a published procedure (Chomczynski and Sacchi, 1987). RNA (8-10 *fg*) was denatured with glyoxal, and electrophoresed on 1.2% agarose gel and transferred to GeneScreen Plus (Dupont, USA) membrane. Prehybridization, hybridization and washing conditions were as described by the supplier of the membrane. The DNA used to prepare probe (Feinberg and Vogelstein, 1984) was a 1.49 kb Cla I-EcoR I fragment of *C. albicans* actin clone which was obtained from Dr. Joachim F. Ernst, FRG. It codes for a single mRNA species of about 1.5 kb (Losberger and Ernst, 1989). The blots were exposed to preflashed Kodak XAR film for 1-5 days with a single intensifying screen. For hybrid selected translation of actin mRNA, the procedure of Ricciardi etal (1979) was followed. Hybridized RNA was eluted and translated in vitro in rabbit reticulocyte lysate. [^{35}S]-labeled translated product was separated on 12% SDS-PAGE.

RESULTS AND DISCUSSION

Northern blot analysis of total RNA isolated at various stages of germ tube formation indicates that there is a progressive increase in the level of actin mRNA as cells form germ tubes (Fig. 1). There was a 2-3 fold increase in the level of actin mRNA in 3 h after addition of inducer (GlcNAc) (Fig. 1). About 90-95% of the cells form germ tubes in 3 h (data not shown). The Nothern blot was quantitated by densitometry with a densitometer (Hirschmann). Ethidium bromide staining of a parallel agarose gel revealed comparable amount of RNA in each lane (data not shown).

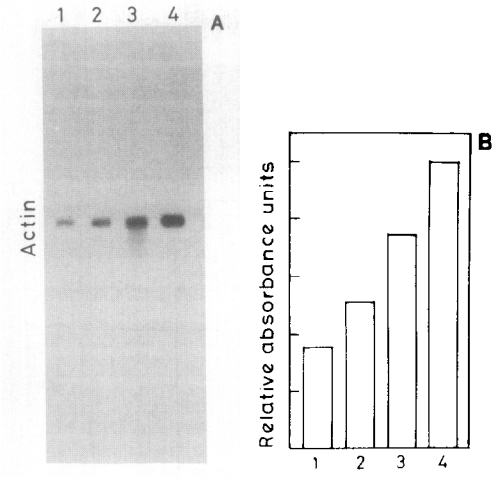

Fig.1. Time course of actin mRNA synthesis during germ tube formation in *Candida albicans* induced by GlcNAc. Total RNA isolated at indicated times was electrophoresed on 1.2 % agarose gel. Resolved RNA was transferred to GeneScreen Plus membrane and hybridized to [^{32}P]- labeled actin probe. **A.** Lane 1 to 4, total RNA isolated at 0, 60, 120, 180 min. respectively, after addition of GlcNAc. **B.** Densitometric scan of the Northern blot.

Germ tubes induced by GlcNAc can be blocked either by trifluoperazine (TFP), a calmodulin inhibitor, or by incubating cells at 25°C (Paranjape etal, 1990). Furthermore, replacing GlcNAc (inducer of germ tube formation) in the medium by glucose (5 mM) also prevented cells from forming germ tubes. In all these three conditions cells remain as yeast form and viable. Total RNA isolated from all these yeast and germ tube forming cells were analyzed by Northern blot using actin gene as a probe (Fig.2). The intensity of rRNA bands, observed by ethidium bromide staining (Fig.2B) was unchanged whereas a 2-3 fold increase in the level of actin mRNA in germ tube forming cells as compared to yeast cells was observed (Fig.2A,C). This suggests that expression of actin gene is associated with a change in morphology. Furthermore, the effect of calmodulin inhibitor on germ tube formation (Paranjape etal, 1990) and actin gene expression (Fig.2) indicates that calmodulin is involved in this morphogenetic pathway, as we proposed earlier (Paranjape and Datta 1990).

In order to study the synthesis of actin during germ tube formation, total RNA was translated <u>in vitro</u> using rabbit reticulocyte lysate (Boehringer, Mannheim). SDS-PAGE analysis of <u>in vitro</u> translated products of total RNA isolated from germ tubes and yeast cells revealed differences in the protein synthesis pattern (Fig. 3). An increase in the synthesis of three proteins (50 kDa, 45 kDa and 28 kDa) encoded by the RNA, indicated the accumulation of these mRNA species during germ tube formation. The

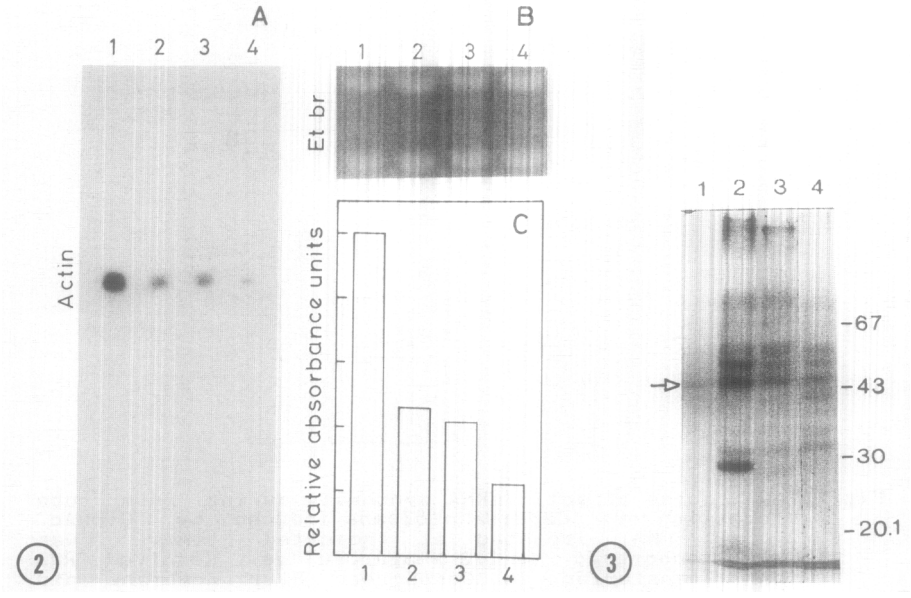

Fig.2. Northern blot analysis of actin mRNA of yeast and germ tube forms of *C. albicans*. Germ tube formation was induced by GlcNAc at 37°C and inhibited either by adding trifluoperazine (20 μM) or by incubating cells at 25°C or by replacing GlcNAc in the medium with Glucose. Total RNA was isolated from both morphological forms and subjected to Northern blot analysis as described in the legend to Fig.1. **A.** Lane 1, cells incubated at 37°C (Germ tube); Lane 2, cells incubated at 37°C in presence of 20 μM trifluoperazine (yeast); Lane 3, cells incubated at 25°C (yeast); Lane 4, cells incubated at 37°C in presence of glucose instead of GlcNAc (Yeast). **B.** Ethidium bromide staining of the total RNA samples run on a parallel agarose gel. **C.** Densitometric scan of the Northern blot.

Fig. 3. SDS-PAGE analysis of in vitro translation products ([^{35}S] methionine labeled) of total RNA and of mRNA species isolated by hybrid selection with immobilized DNA of actin clone. Germ tube formation was induced by GlcNAc and inhibited as described in the legend to Fig.2. Total RNA was isolated and translated in vitro in rabbit reticulocyte lysate. Translated products were analysed on 12% polyacrylamide gels and fluorographed. Dried gels were exposed to preflashed Kodak X-ray film at -20°C for 3-4 days. Lane 1, RNA hybrid selected with DNA of actin clone; Lane 2, total RNA from cells forming germ tubes; Lane 3, total RNA from trifluoperazine treated cells; Lane 4, total RNA from cells incubated at 25°C. Position of molecular weight standards are indicated on the right. Arrow indicates the position of actin.

identity of 50 kDa and 28 kDa protein is not known at present. A protein of Mol.wt.45 kDa is identified as actin since it is comigrated with the translated product of total RNA hybrid selected by the actin clone. This suggests that the level of actin mRNA is more in germ tube forming cells as compared to yeast cells. These observations, supported by the results of the Northern blot anlaysis, further confirm that the expression of actin gene is regulated during morphogenesis of *C. albicans*. As germ tube

formation is accompanied by an increase in the cell volume, the increase in the level of actin in germ tubes is most probably required to accomodate the increasing size of the cells (Mc Cairns et al., 1984). Increased level of mRNA could be the result of either increased rate of transcription or enhanced stability of actin mRNA accompanying change in morphology. It will be of interest to study the stability of actin mRNA and also determine whether the same or different upstream sequences control the transcription of actin gene during morphogenesis. To our knowledge this is the first report of a differentially expressed gene during mophogenesis of *C. albicans*.

ACKNOWLEDGMENTS

We acknowledge Dr. Joachim F. Ernst, FRG for the gift of actin clone from *C. albicans* and Dr. K. Natarajan for doing the hybrid selection translation experiment and providing the translated product. This work has been supported by a grant from Council of Scientific and Industrial Research, India.

REFERENCES

1. Anderson, J.M., and Soll, D.R. (1986) J. Gen. Microbiol. **132**, 2035-2047.
2. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. **162**, 156-159.
3. Datta, A., Ganesan, K and Natarajan, K. (1989) Adv. Microbial Physiol. **30**, pp.53-88. Academic Press, London.
4. Feinberg, A.P., and Vogelstein, B. (1984) Anal. Biochem. **137**, 266-267.
5. Greer, C., and Schekman, R. (1982) Mol. Cell. Biol. **2**, 1279-1286
6. Losberger, C., and Ernst, J.F. (1989) Nucleic Acid Res. **17**, 22.
7. Manalan, A. S., and Klee, C.B. (1982) Adv. Cyclic Nucleotide Protein Phosphorylation Res. **18**, 227-278.
8. McCairns, E., Fahey, D., Muscat, G.E.O., Murray, M., and Rowe, P.E. (1984) Mol. Cell. Biol. **4**, 1754-1760.
9. Natarajan, K., Rai, Y.P., and Datta. A. (1984) Biochem. Int. **9**, 735-744.
10. Odds, F.C. (1988) In Candida and Candidiasis, pp.42-59, Bail liere Tindal, London.
11. Paranjape, V.D., Gupta Roy, B., and Datta, A. (1990) J. Gen. Microbiol. **136**, 2149-2154.
12. Paranjape, V.D., and Datta, A. (1990) In Calcium as an Intracellular Messenger in Eucaryotic Microbes, (D.H.O' Day Ed.), pp. 362-374. ASM Publication. USA.
13. Ricciardi, R.P., Millers, J.S., and Roberts, B.E. (1979) Proc. Natl. Acad. Sci.(USA) 76, 4927-4931.
14. Shepherd, M.G., Chiew, Y.Y., Ram, S.P., and Sullivan, P.A. (1980) Can. J. Microbiol. **26**, 21-26.
15. Sobue, K., Morimoto, K., Kanda, K., Maruyama, K., and Kakiuchi, S. (1982) FEBS Lett. **138**, 289-292.