© 1992 Oxford University Press Nucleic Acids Research, Vol. 20, No. 12 3253

The *INO2* gene of *Saccharomyces cerevisiae* encodes a helix-loop-helix protein that is required for activation of phospholipid synthesis

D.Michele Nikoloff, Patricia McGraw+ and Susan A.Henry*

Department of Biological Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA

Submitted May 1, 1992 EMBL accession no. X66066

In the yeast *Saccharomyces cerevisiae*, biochemical and genetic evidence have established that a number of phospholipid biosynthetic enzymes are coordinately regulated in response to the soluble precursors inositol and choline and a common set of regulatory factors (1). The *ino2* and *ino4* mutants show pleiotropic defects in phospholipid metabolism. Recessive mutations at the *INO2* locus lead to reduced phosphatidylcholine synthesis and inositol auxotrophy due to an inability to derepress expression of the *INO1* structural gene (which encodes inositol-1-phosphate synthase) (2), (3). *Ino2* mutant extracts also lack a specific DNA-protein complex that is present in wildtype extracts (4). Thus, the *INO2* locus encodes a positive regulatory factor required for derepression of the coregulated phospholipid biosynthetic enzymes.

The wildtype *INO2* gene was isolated by functional complementation of the inositol auxotrophy in an *ino2* mutant. Upon transformation with a partial *Sau*3A genomic library, one plasmid harboring a 1.8 kilobase *Sma*I–*Xba*I insert restored inositol prototrophy. Integrative transformation established linkage to the *ino2-21* mutation. In a cross between an integrant and wildtype strain 48 tetrads showed 4:0 segregation for the Ino+ phenotype, confirming that the cloned DNA represents the authentic *INO2* locus. The nucleotide sequence of the *INO2* gene was determined on both strands by the Sanger dideoxy chain termination method (5). Computer-assisted sequence analysis revealed 912 base pair open reading frame, capable of encoding a 304 amino acid protein with a predicted molecular mass of 34,234 daltons. The Ino2 protein (Ino2p) is largely hydrophilic and acidic (pI = 5.76). Proline residues comprise 8.5% of the protein. A potential structural similarity between the carboxy-terminus of Ino2p and the helix-loop-helix (HLH) domain of the proto-oncogene *c-myc* mapped to residues 253–291 of Ino2p (see Figure 1) (6). Basic residues precede the HLH domain of Ino2p. Interestingly, the Ino4 protein, which encodes a known transcriptional activator of phospholipid synthesis shares sequence similarity to Ino2p in a region that is restricted the the 68 amino acid HLH structural domain (9). Extracts prepared from *ino4* mutants lack the same DNA-protein complex that is missing in extracts prepared from *ino2* mutants (4). Thus, it is tempting to speculate that Ino2p and Ino4p may be dimerization partners that associate in a DNA-protein complex to regulate the expression of phospholipid structural genes.

ACKNOWLEDGEMENTS

We thank Dr John E.Hill for expertise in the computer sequence analysis. Supported by PHS grant GM-19629 to S.A.H. from the NIH and a predoctoral fellowship to D.M.N. from the AHAPA.

REFERENCES

1. Nikoloff,D.M. and Henry,S.A. (1991) *Annu. Rev. Genet.* **25**, 559–583.
2. Hirsch,J.P. and Henry,S.A. (1986) *Mol. Cell. Biol.* **6**, 3320–3328.
3. Loewy,B.S. and Henry,S.A. (1984) *Mol. Cell. Biol.* **4**, 2479–2485.
4. Lopes,J.M. and Henry,S.A. (1991) *Nucleic Acids Res.* **19**, 3987–3994.
5. Sanger,F.*et al.*, (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
6. Murre,C., McCaw,P.S. and Baltimore,D. (1989) *Cell* **56**, 777–783.
7. Cai,M. and Davis,R. (1990) *Cell* **61**, 437–446.
8. Berben,G., Legrain,M., Gilliquet,V. and Hilger,F. (1990) *Yeast* **6**, 451–454.
9. Hoshizaki,D.K., *et al.*, (1990) *J. Biol. Chem.* **265**, 4736–4745.

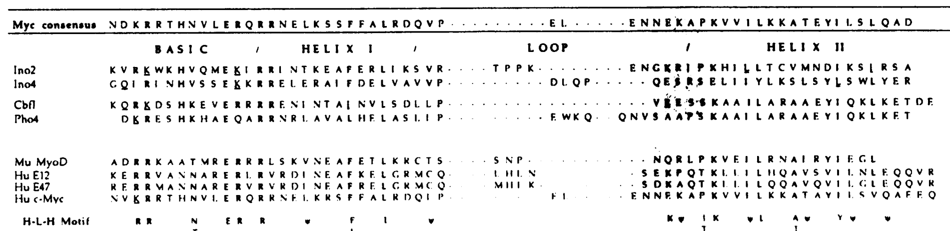

Figure 1. Alignment of several representative helix-loop-helix regulatory proteins. Amino acid similarity between the HLH domain of Ino2p and Ino4p (9), Cbf1 (7), Pho4 (8), MyoD (6), E12 (6), E47 (6) and *c-myc* (6). Identity is indicated in bold face; conservative substitution is denoted by an under line; Y represents hydrophobic amino acid residues.

* To whom correspondence should be addressed

+Present address: Department of Biological Sciences University of Maryland, Catonsville, MD 21228, USA