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

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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.

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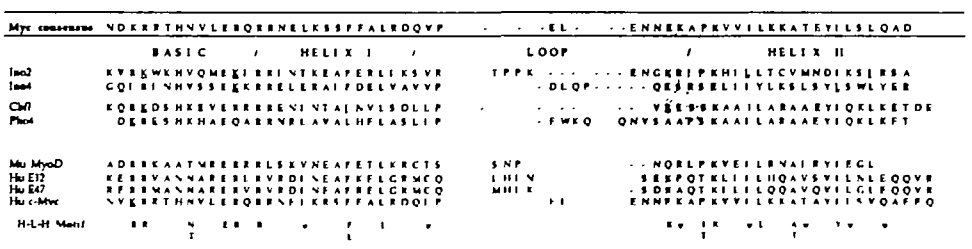

Figure 1. Alignment of several representative helix-loop-helix regulatory proteins. Amino acid similarity between the HLH domain of Ino2p and Ino4p (9), Cbf1 (7), Pho4 (8), MyoD (6), E12 (6), E47 (6) and *c-myc* (6). Identity is indicated in bold face; conservative substitution is denoted by an under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

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