Molecular analysis of a temperature sensitive allele of the PHO2 gene of *Saccharomyces cerevisiae*

Brian J. McCarthy, Caretha L. Creasy and Lawrence W. Bergman*

Program in Molecular and Cellular Biology, Department of Chemistry, Clippinger Laboratories, Ohio University, Athens, OH 45701, USA

Submitted April 11, 1991

EMBL accession nos X54293, X54294

We have isolated and characterized a temperature sensitive mutation which maps to the yeast PHO2 gene (also termed BAS2 or GRF10), a positive regulatory loci involved in the production of phosphate-repressible acid phosphatase. Various mosaics of the PHO2 gene were constructed containing either temperature sensitive or wild type restriction fragments. These plasmids were then transformed into a strain containing a deletion of the endogenous PHO2 gene and subsequently assayed for acid phosphatase production. This resulted in the localization of the temperature sensitive mutation to an approximately 0.4 kbp BglII–EcoRV restriction fragment. The DNA sequence of this region revealed two separate base pair changes which resulted in amino acid changes. To delineate the temperature sensitive mutation, clones were constructed with the aid of polymerase chain reaction, which contained only a single amino acid change in the encoded PHO2 protein. The temperature sensitive phenotype was localized to the replacement of Asn 164 with a Lys residue.

In sequencing the EcoRV–BglII fragment containing the temperature-sensitive mutation, a large number of sequence differences were observed when comparing the two alleles isolated in this study and the published report of Senstag and Hinnen (1). Therefore the DNA sequence of the region corresponding to nucleotides 400 to 2070 (amino acid 5 to the carboxy-terminus of the protein) was completed for each allele. When comparing the two wild type alleles (the wild type allele isolated from strain GG100-14D in this study vs. the published sequence of Senstag and Hinnen), there were 6 observed nucleotide differences within the sequenced region. This, however, results in only a single amino acid substitution, the

conservative replacement of Asp 157 with a Glu residue. In comparing the sequence of the temperature-sensitive allele isolated from strain YAT475 to the sequence of Senstag and Hinnen (1), 9 nucleotide differences were observed. This results in 4 amino acid replacements in the temperature-sensitive gene product. However, as stated above, only the replacement of Asn 164 with a Lys residue is sufficient to confer the temperature-sensitive phenotype. Finally, the comparison of the two alleles isolated in this study reveals 10 nucleotide differences, resulting in 5 amino acid replacements. Most likely, the large number of differences that were observed are due to the fact that both alleles in this study were isolated from strains (GG100-14D for the wild-type allele and YAT475 for the temperature-sensitive allele) which had been subjected to mutagenesis. Strain GG100-14D contains a *pho5* allele. This mutagenesis would generate nucleotide changes in the PHO2 alleles which would not affect the function of the protein (other than the temperature-sensitive mutation).

ACKNOWLEDGEMENTS

We wish to thank Rose Chambers for preparing the manuscript. This work was supported by grants GM32319 and GM41620 to L.W.B. Accession numbers for the complete sequence information are X54293 (wild type gene) and X54294 (temperature-sensitive gene).

REFERENCE

1. Senstag, C. and Hinnen, A. (1987) *Nucl. Acids Res.* **15**, 233–246.

* To whom correspondence should be addressed