

## Structural Studies of the Yeast Phosphorelay Protein YPD1

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Abstract No. Xu0678

Beamline(s): X12B, X12C

**Introduction:** In *Saccharomyces cerevisiae*, YPD1 functions as a phospho-protein intermediate required for phosphoryl group transfer from a membrane-bound sensor histidine kinase (SLN1) to two distinct response regulator proteins (SSK1 and SKN7). This branched multi-step phosphorelay signaling pathway is primarily involved in adaptive responses to hyperosmotic and oxidative environmental stress in yeast.

In 1999, we reported the X-ray structure of the histidine-containing phosphotransfer (HPT) protein YPD1, which was solved at a resolution of 2.7 Å by conventional multiple isomorphous replacement methods. The tertiary structure of YPD1 consists of six  $\alpha$ -helices (designated A-E, and G) and a short  $3_{10}$ -helix (helix F). A four-helix bundle, composed of helices B, C, D, and G, forms the "core" of the molecule in which the histidine that is phosphorylated (His64) is located in the middle of helix C and is completely exposed to solvent.

**Methods and Materials:** Based on our analysis of the YPD1 structure, we identified several highly conserved amino acid residues surrounding the site of phosphorylation (His64) that we postulated might be critical for phosphoryl transfer activity. Site-directed mutagenesis was carried out and the YPD1 mutants were subjected to *in vitro* biochemical assays in order to assess the effect of each mutation on phosphoryl transfer activity between YPD1 and response regulator domains associated with SLN1 and SSK1. Substitution of a highly conserved glycine residue (G68), which is located one turn of the helix away from His64, with a bulkier glutamine side chain resulted in the most severe defect with respect to phosphoryl transfer activity. During the last year, we have been able to collect X-ray data from both native crystals and YPD1 mutant crystals at the BNL/NSLS synchrotron beamlines X12B and X12C.

**Results:** We have obtained a higher resolution (1.75 Å) structure of YPD1 using data collected from native tetragonal crystals (space group  $P4_32_11$ ). The G68Q mutant crystallized in a different space group ( $P3_121$ ) and we have now solved its structure to 2.2 Å resolution.

**Conclusions:** It appears that steric hindrance imposed by the bulkier side chain in the G68Q mutant reduces the accessibility of His64 and affects the ability of YPD1 to interact with phosphodonor or phosphoreceiver domains.

**Acknowledgments:** The authors gratefully acknowledge the expert assistance of beamline personnel at the NSLS and financial support of this project from the National Institutes of Health (GM58311), the Oklahoma Center for the Advancement of Science and Technology (OCAST Grant #HR99-080), and Research Corporation (Cottrell Scholar Award #CS0607).

**References:** Q. Xu and A. H. West, "Conservation of Structure and Function Among Histidine-Containing Phosphotransfer (HPT) Domains as Revealed by the Crystal Structure of YPD1," *J. Mol. Biol.*, **292**, 1039 (1999); Q. Xu, F. Janiak-Spens, and A. H. West, "Importance of Glycine 68 in the Histidine-Containing Phosphotransfer (HPT) Protein YPD1," *Manuscript in preparation*.