s1.m7.p3 **Yeast copper thionein: solving a small but long-standing puzzle.** V. Calderone[#], B. Dolderer°, H.-J. Hartmann°, H. Echner°, C. Luchinat[§], C. Del Bianco[§], S. Mangani[#], U. Weser°. [#] *Department of Chemistry, University of Siena, via Aldo Moro, 53100 Siena, Italy.* [§] *CERM, University of Florence, Italy.* ° *Anorganische Biochemie, University of Tubingen, Germany. E-mail: vito.calderone@unisi.it*

Keywords: Thionein; Copper; Cellular Metal Trafficking

In these postgenomic days the Protein Data Bank is featuring protein structures of increasingly large size, thanks to the progress in the power of the X-ray sources and in the computer programs to solve X-ray structures.

Yet, there are small proteins whose structures remain elusive. One of the most striking examples is a stable and well-folded protein whose existence has been known for nearly thirty years, whose central importance in eukaryotes metabolism is undisputed, whose size is ridiculously small (M_r 5655), but *whose detailed structure is still unknown*. Such protein is yeast copper thionein (Cu-MT) (Figure 1).

Strange enough, solving this particular structure turned out to be a puzzle. Molecular replacement techniques using the protein solution structure as a model failed, probably due to its lack of eight strong anomalous scatterers (the Cu atoms) and direct methods could not be applied for phasing because the resolution, although good, was not at the true atomic level. On the other hand, the enormous anomalous signal present in the data (about 20% of the diffracted intensities) could not be straightforwardly used for the structure solution due to the high number of anomalous scatterers and to the high symmetry of the crystal cell (cubic, $P4_332$).

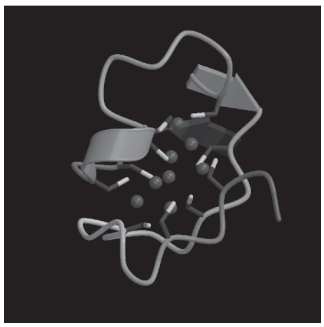

Figure 1

s1.m7.p4 **Human Phosphate Binding Protein.** Carlos Contreras-Martel,[a] Renaud Morales,[a] Murielle Nicodeme,[b] Frederique Renaud,[c] Marie-Laure Chesne,[d] Phillipe Carpentier,[a] Juan-Carlos Fontecilla-Camps,[a] Daniel Rochu,[c] Patrick Masson[c] and Eric Chabriere[e], [a]*Laboratoire de Cristallogénese et Cristallographie des Protéines, Institut de Biologie Structurale J.-P. EBEL, CEA-CNRS, 41 rue Jules Horowitz, 38027 Grenoble, France,* [b]*Laboratoire des BioSciences de l'Aliment, Université Henri Poincaré, Nancy 1, BP239, 54506 Vandoeuvre les Nancy, France,* [c]*Département de Toxicologie, Unité d'Enzymologie, Centre de Recherches du Service de Santé des Armées, 38702 La Tronche, France,* [d]*Laboratoire de Cristallographie Macromoleculaire, Institut de Biologie Structurale J.-P. EBEL, CEA-CNRS, 41 rue Jules Horowitz, 38027 Grenoble, France, and* [e]*Laboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques, CNRS-Université Henri Poincaré, Faculté des Sciences, BP 239, 54506 Vandoeuvre-les-Nancy, France. E-mail: eric.chabriere@lcm3b.uhp-nancy.fr*

Keywords: ABC transporter; Phosphate; Atherosclerosis

We isolated and solved the structure of a plasma phosphate carrier (human). Surprisingly, despite the high importance of phosphate, none plasma phosphate carrier has been described so far. Due to the difficulty to obtain heavy atom derivative, we solved the structure at very-low resolution (25 Å) using a new direct method requiring neither heavy atom derivative nor model [1]. The atomic structure was finally solved at 1.8 Å resolution by classical SIRAS structure determination with one heavy atom derivative, which was obtained using a new family of molecule specially designed to produce better heavy atom derivatives (Kahn et al., in preparation). The structure revealed two globular domains with a fold very similar to the phosphate binding proteins of *E. coli* and *M. tuberculosis* [2]-[3], primary ABC phosphate carriers. ABC carriers have been found in eukaryote kingdom but no primary ABC carrier has been described in this kingdom.

We showed that this protein is associated to paraoxonase in human plasma. Paraoxonase is a lipoprotein associated to HDL ("good cholesterol"). This phosphotriestrase is involved in protection against atherosclerosis development. Knowing that hyperphosphatemia is a risk factor in atherosclerosis, the human phosphate binding protein could be a preclinic marker of cardiovascular pathologies.

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