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Structural Genomics of Yeast Proteins

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ABSTRACT: Proteinases: Digestion of proteins is required for many biological functions, both intra and extracellular. There are many different families of proteins responsible for this activity, with several different mechanisms (Biochem. J. 290: 205-218, 1993), although some overall similarities in the mechanisms can be detected. Several families of known proteinases still are of unknown fold, and often of unknown mechanism as well, making them ideal targets for a structural genomics approach. For initial testing, members of five of these families were selected: O28616 from serine proteinase family 27, P43764/GCP_HAEIN from metalloproteinase family 22, O25976 from unknown mechanism family 7, P44766/PEPE_HAEIN from unknown mechanism family 28, and P56113/Y169_HELPY from unknown mechanism family 32. To date, O28616, O25976, and P56113 have been successfully cloned. At present all three appear to be of limited solubility; improved expression and lysis conditions, and refolding experiments are underway.

Nuclear Pore Proteins: Nuclear pores are structures found ubiquitously in eukaryotes, permitting and regulating transport across the nuclear double membrane. However, no molecular-level structural data is available for the components of the nuclear pore, which limits the understanding of its gating and other functions. It has been shown that many nuclear pore complex proteins (known as nucleoporins or nups) are broadly conserved (Ann. Rev. Biochem. 64:865-896, 1995)(Ann. Rev. Genet. 31:277-313, 1997). Rout et al. (J.C.B. 148:635-651, 2000) have used subcellular fractionation, mass spectrometry, protein labeling techniques, and yeast genome information to identify the components of the nuclear pore complex. They have identified 39 proteins forming parts of the nuclear pore, as well as 29 other proteins that may associate with it significantly. Inspection of these two lists reveals 24 with properties making them suitable for structural genomics. These include 5 core nups, for which yeast rt-PCR primers are being designed, while Rout et al. are being consulted with regards to the remainder.