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Large Scale Purification of Human ABC-Transporters from the Yeast *Pichia pastoris*. Y. Trinh, L. Stalcup, S. Souza-Burzinski, A. Pickert, M. Dumont, M. Dean, I.L. Urbatsch, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

P-glycoprotein (Pgp) confers resistance of cancer cells to therapeutic drugs. It is a member of the ABC transporter family that hydrolyses ATP to drive export of cytotoxic drugs to prevent their intracellular accumulation. Pgp from mouse and human has been expressed in the yeast *P. pastoris*. In this system, the cDNA is stably inserted into the chromosome and expression of the protein is controlled by the methanol-inducible promoter AOXI. Cultures of *P. pastoris* can be grown to high cell density in a fermentor (>300g/L). Pgp is solubilized from microsomal membranes and purified by a two step chromatography procedure. The final material is pure, soluble at low detergent concentrations, and activated to high specific ATPase activity on addition of lipids. From 100 g of cells we routinely obtain 2-5 mg of Pgp with characteristics very similar to protein purified from mammalian sources. We have expressed several other human ABC transporters in *P. pastoris* and tested a variety of detergents for purifications. Co-expression of the putative cholesterol transporters ABCG5/ABCG8 significantly improved yield and purity, opening up avenues for studying the molecular mechanism of this class of transporters.

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