ENGINEERING VACUOLAR SORTING PATHWAYS FOR EFFICIENT SECRETION OF RECOMBINANT PROTEINS

Brigitte Gasser, BOKU University of Natural Resources and Life Sciences ; Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria
brigitte.gasser@boku.ac.at
Lukas Marsalek, BOKU University of Natural Resources and Life Sciences ; Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria
Verena Puxbaum, BOKU University of Natural Resources and Life Sciences ; Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria
Clemens Gruber-Grünwald, BOKU University of Natural Resources and Life Sciences ; Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria
Friedrich Altmann, BOKU University of Natural Resources and Life Sciences ; Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria
Diethard Mattanovich, BOKU University of Natural Resources and Life Sciences ; Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria

Recombinant protein production is an expanding branch of biotechnology with increasing economic importance. Currently, 20% of biopharmaceutical proteins and approximately half of the industrial enzymes are produced in yeasts. Many proteins are efficiently secreted by yeast systems, reaching product titers in the g L^{-1} range. The expression of more complex proteins, however, may overwhelm the folding and secretion capacity of the host cells. This triggers the unfolded protein response (UPR), which aims at restoring endoplasmic reticulum (ER) homeostasis. The UPR, in turn, is thought to activate ER-associated protein degradation (ERAD). Alternatively, trafficking of correctly folded proteins can be hampered on their way to the cell exterior leading e.g. to missorting and subsequent degradation in the vacuole.

The methylotrophic yeast *Pichia pastoris* (*Komagataella* spp.) is a popular microbial host for the production of recombinant proteins. Vacuolar protein sorting has not been investigated in detail so far in *P. pastoris*, although there were a few indications that vacuolar mistargeting of recombinant products might occur also in this yeast. Thus we engineered *the vacuolar sorting pathways in P. pastoris* and investigated their impact on extracellular product titers as well as intracellular localization of the recombinant secretory product. Thereby, differences between *vps* (vacuolar protein sorting) mutant strains disrupted in genes involved either in the CORVET or the HOPS tethering complexes became obvious. Moreover, we were able to show that engineering of the vacuolar sorting pathways has a positive impact on heterologous protein secretion, however, in some cases simultaneous inactivation of specific vacuolar proteases was necessary.

Taken together, these studies allowed us to gain deeper insight into the pathways leading to intracellular degradation of recombinant secretory proteins. Based on these findings, approaches how to efficiently adapt the host cell's secretion capacity will be presented, which confirm that impairment of vacuolar protein sorting is an effective means of enhancing secretion of heterologous proteins.