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GOING BEYOND THE LIMIT: IMPACT OF INCREASING GLOBAL TRANSLATION ACTIVITY ON THE PRODUCTIVITY OF RECOMBINANT SECRETED PROTEINS IN *PICHIA PASTORIS*

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Yeasts, especially *Pichia pastoris* (syn. *Komagataella phaffii*), are widely used organisms for commercial, heterologous protein production. *P. pastoris* is known for its high secretory efficiency and biomass yield, however specific productivities are low and tightly coupled to biomass formation. This highly impacts production processes, which are commonly not run at the maximum growth rate, thereby resulting in suboptimal productivities. To tackle this issue, we evaluated transcriptomics datasets of *P. pastoris*. These showed a clear downregulation of protein translation related genes with decreasing growth rates, thus revealing the yeast translation machinery as our cellular engineering target.

By overexpression of selected differentially expressed translation factors, we identified translation initiation as the main rate-limiting step. Specifically, factors associated with the closed-loop conformation, a structure that increases stability and rates of translation initiation before start codon scanning is initiated, showed the strongest effects. Overexpression of these factors alone or in combination increased titers of different heterologous proteins by up to 3-fold in fed-batch processes. Global translational activity, as measured by OPP-labelling assays, correlated nicely to the enhanced secreted recombinant protein levels. Furthermore, selected transcript levels and total protein content were higher in the engineered cells. Translation factor overexpression therefore has a global effect on the cell. Concludingly, our work displays not only the interconnection of different protein synthesis steps but also the capacity *P. pastoris* has for protein production, and indicates that this host organism is not at its limit yet.