Regulation of Protein Overexpression by Ribosomal Proteins in Pichia pastoris: Postprint

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Abstract

In regulatory studies of protein overexpression, research on the molecular mechanisms of gene transcription, protein modification, folding, and secretion pathways, as well as their application in protein synthesis regulation, has been relatively extensive. Due to the structural complexity of ribosomes, reports on research and regulation of protein overexpression at the ribosomal translational level remain limited.

Pichia pastoris, as an important industrial strain for protein expression and production, has become a key model for investigating the regulation of high-efficiency protein expression. Previous studies have achieved the goal of enhancing protein synthesis and expression yields through selecting strong promoters to elevate protein gene transcription levels, overexpressing endogenous UPR-related factors to improve protein folding capacity, and optimizing secretion signal peptides. Our earlier research observed significant downregulation of numerous ribosomal proteins and decreased cell growth in P. pastoris cells overexpressing proteins. In-depth analysis of ribosomal proteins and ribosomal structural function holds promise for investigating the molecular regulatory mechanisms of ribosomal proteins on cellular protein overexpression at the translational level.

This study employed iTRAQ LC-MS/MS technology to analyze the differential expression of recombinant Pichia pastoris ribosomal proteins and energy metabolism pathways, revealing that 30 ribosomal proteins were downregulated during xylanase overexpression. For these downregulated ribosomal proteins, gene deletion was performed to construct P. pastoris mutant strains. Using a Cre/loxP selectable marker-recyclable gene knockout system, screening and functional characterization of ribosomal protein mutant strains were conducted in the P. pastoris GS115 host, successfully obtaining 28 ribosomal protein mutant strains including RPL22Δ, RPL24Δ, and RPL26Δ. Using enhanced green

fluorescent protein (eGFP) and phytase (phy) as reporter proteins, we preliminarily analyzed the cell growth characteristics and protein synthesis efficiency of the mutant strains, and determined their polysome profiles. The results demonstrated that ribosomal protein deletion exerted varying degrees of impact on cell growth and protein overexpression, and could substantially reduce cellular translational activity. Combined with translatomics and analysis of nascent polypeptide chain folding stability, the findings indicate that deletion of non-essential ribosomal proteins affects ribosomal translation initiation and elongation rates, subsequently influencing protein co-translational folding efficiency and thereby regulating protein yield through synthesis quality. This provides a foundation for further analyzing the effects of ribosomal proteins on translation, ER stress tolerance, and protein synthesis/secretion functions, and for deeply exploring the molecular regulatory mechanisms of ribosomal proteins on recombinant P. pastoris cell growth and protein overexpression.

Full Text

Preamble

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Regulation of Ribosomal Proteins on Protein Overexpression in *Pichia pastoris*

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Research on protein overexpression regulation has extensively investigated molecular mechanisms governing gene transcription, protein modification, folding, and secretion pathways, along with their applications in controlling protein synthesis. However, due to the structural complexity of ribosomes, studies on protein overexpression at the translational level remain relatively scarce.

Pichia pastoris serves as a crucial industrial strain for protein expression and production, making it a key model organism for investigating high-efficiency expression regulation. Previous studies have successfully enhanced protein synthesis and yield through various strategies, including selecting strong promoters to increase transcriptional levels, overexpressing endogenous UPR-related factors to improve protein folding, and optimizing secretory signal peptides. Preliminary studies in our group have observed significant downregulation of numerous ribosomal proteins alongside reduced cell growth in *P. pastoris* cells overexpressing target proteins. Comprehensive analysis of ribosomal proteins and their structural functions promises to elucidate the molecular regulatory mechanisms by which ribosomal proteins control cellular protein overexpression at the translational level.

In this study, we employed iTRAQ LC-MS/MS technology to analyze differential expression of recombinant *P. pastoris* ribosomal proteins and energy metabolism pathways, revealing that 30 ribosomal proteins were downregulated

during xylanase overexpression. We subsequently constructed *P. pastoris* mutant strains through gene deletion of these downregulated ribosomal proteins. Utilizing a Cre/loxP marker recycling system for gene knockout in the *P. pastoris* GS115 host, we successfully generated and functionally characterized ribosomal protein mutants, including RPL22Δ, RPL24Δ, RPL26Δ, among others.

Analysis of mutant cell growth characteristics and protein synthesis efficiency, coupled with polysome profiling, demonstrated that ribosomal protein deletions exert varying effects on cell growth and protein overexpression while substantially reducing translational activity. Integrating translatomic analysis with assessments of nascent polypeptide chain folding stability, our findings reveal that deletion of non-essential ribosomal proteins impairs ribosomal translation initiation and elongation rates, consequently affecting co-translational folding efficiency. This regulation of protein yield through synthesis quality provides a foundation for further investigating the impacts of ribosomal proteins on translation, ER stress tolerance, and protein secretion, as well as for elucidating the molecular mechanisms by which ribosomal proteins govern recombinant *P. pastoris* cell growth and protein overexpression.

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Note: Figure translations are in progress. See original paper for figures.