Journal of Molecular Science

Journal of Molecular Science
Volume 32 Issue 1, Year of Publication 2023, Page 9-11
DoI-17.4687/1000-9035.2023.004

Journal of Molecular Science
www.jmolecularsci.com ISSN:1000-9035

Rational Engineering and Synthetic Rewiring of Bacterial Metabolic Pathways for Enhanced Production of Recombinant Therapeutic Proteins

Ewa Kowalska, Mei Li, Antonio Russo, Rosa Pérez, Henrik Sørensen

Article Information
Received: 09-01-2023
Revised: 15-02-2023
Accepted: 03-03-2023
Published: 15-03-2023

Keywords

ABSTRACT

The production of recombinant therapeutic proteins using bacterial hosts has revolutionized modern medicine, providing cost-effective and scalable solutions for treating various diseases. However, native metabolic constraints often limit protein yield and functionality. This study explores the rational engineering and synthetic rewiring of bacterial metabolic pathways to optimize the biosynthesis of recombinant proteins. Strategies such as metabolic flux redirection, promoter engineering, codon optimization, and stress tolerance enhancement are examined. By integrating computational modeling with experimental validation, we demonstrate how synthetic biology approaches can maximize protein yield while maintaining structural fidelity and biological activity.

INTRODUCTION:

Recombinant therapeutic proteins, including monoclonal antibodies, insulin, and growth factors, are essential for treating numerous diseases. Bacterial expression systems, particularly *Escherichia coli*, serve as efficient production platforms. However, bottlenecks such as inclusion body formation, inefficient folding, and metabolic burden limit their full potential. This study focuses on synthetic rewiring strategies to optimize bacterial metabolism for high-yield protein production while preserving post-translational modifications and bioactivity.

Engineering Metabolic Pathways for Enhanced Protein Synthesis

Redirection of Metabolic Flux

By diverting carbon flux from competing pathways toward recombinant protein synthesis, metabolic efficiency can be improved. Strategies include deletion of competing metabolic branches and overexpression of rate-limiting enzymes.

Promoter and Ribosome Binding Site Optimization

Dynamic regulation of transcription and translation enhances protein expression. Promoter engineering and ribosome binding site (RBS) tuning help in balancing protein synthesis rates with host cell viability.

Journal of Molecular Science

Journal of Molecular Science
Volume 32 Issue 1, Year of Publication 2023, Page 9-11
DoI-17.4687/1000-9035.2023.004

Codon Optimization and tRNA Engineering
Codon bias can impact translation efficiency. Engineering host strains with enhanced tRNA pools improves translation rates and reduces ribosomal stalling, resulting in higher protein yields.

Overcoming Stress Responses and Proteostasis Challenges
Enhancing Protein Folding Mechanisms
Proper protein folding is essential for maintaining biological function and preventing the formation of **misfolded aggregates or inclusion bodies**. In recombinant protein production, enhancing folding efficiency is crucial for maximizing yield and functionality. Several strategies can be employed to improve protein solubility and prevent aggregation:

1. Chaperone Co-Expression
Molecular chaperones assist in **protein folding, stabilization, and refolding** of misfolded proteins. Co-expressing chaperones in host cells can enhance the solubility and proper folding of recombinant proteins:

- **Hsp70 and GroEL/GroES (in bacteria)** facilitate ATP-dependent folding and prevent aggregation.
- **PDI (Protein Disulfide Isomerase) and DsbA/DsbC (in E. coli)** promote correct disulfide bond formation in secretory and cytoplasmic proteins.
- **BiP and Calnexin (in eukaryotic systems)** assist in endoplasmic reticulum (ER)-associated folding and quality control.

2. Oxidative Stress Modulation

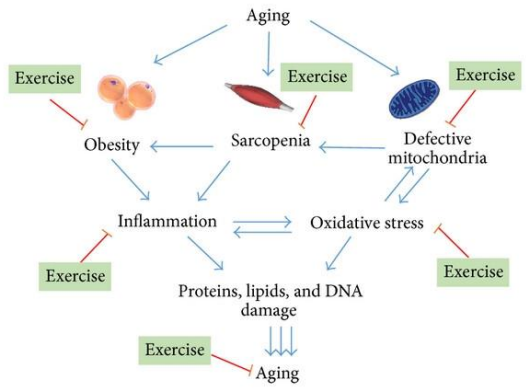

Fig.Oxidative Stress Modulation

The cellular redox environment significantly influences **disulfide bond formation** and protein stability:

- **Glutathione Redox Buffering**: Maintaining an optimal ratio of reduced (GSH) to oxidized glutathione (GSSG) enhances the correct disulfide bond formation.
- **Superoxide Dismutase (SOD) Co-expression**: Reduces oxidative stress, which can denature proteins or cause misfolding.
- **Thioredoxin Overexpression**: Promotes proper disulfide exchange and assists in **correct tertiary structure formation**.
-

3. Folding Optimization Strategies
Fine-tuning environmental and biochemical conditions helps **enhance protein solubility and reduce aggregation**:

- **Lowering Expression Temperature**: Reducing growth temperatures (e.g., **from 37°C to 16-25°C in bacterial cultures**) can slow down folding kinetics, allowing better chaperone-assisted folding.
- **Codon Optimization**: Using host-preferred codons reduces translation errors and misfolded intermediates.
- **Fusion Tags**: Solubility-enhancing tags (e.g., **MBP, SUMO, GST, and Trx** tags) improve proper folding and prevent aggregation.
- **Chemical Additives**: Osmolytes such as **arginine, proline, and trehalose** stabilize folding intermediates.

Controlling Cellular Resource Allocation
The balance between recombinant protein expression and host cell metabolism is crucial for sustainable **high-yield protein production**. Excessive resource utilization can lead to **metabolic stress, reduced growth, and decreased protein yield**. Optimizing cellular resource allocation ensures both **viability and efficient expression**.

1. Regulating Protein Synthesis Rate

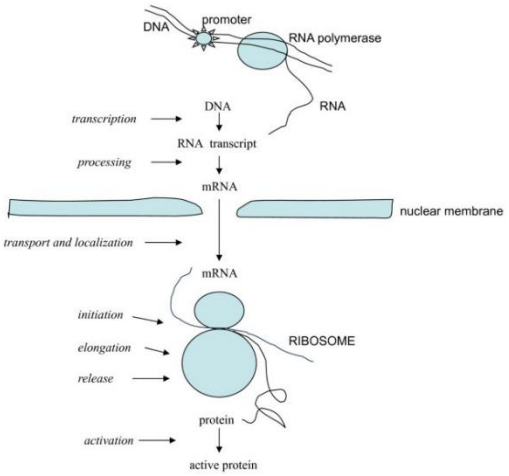

Fig.Regulating Protein Synthesis Rate

- **Inducible Promoters**: Controlled induction (e.g., **IPTG in T7 systems or arabinose in pBAD systems**) prevents early metabolic burden.
- **Ribosome Binding Site (RBS) Engineering**: Adjusting RBS strength regulates translation rates, preventing overloading of translational machinery.
- **mRNA Stability Optimization**: **Codon bias**

Journal of Molecular Science
Volume 32 Issue 1, Year of Publication 2023, Page 9-11
DoI-17.4687/1000-9035.2023.004

adaptation and **5' UTR modifications** ensure efficient ribosomal processing.

2. Balancing Cellular Growth and Expression

- **Fed-Batch Culturing**: Gradual nutrient supplementation avoids excessive metabolic load and sustains high cell density.
- **Energy Redistribution**: Fine-tuning ATP and NADPH availability between **cellular maintenance and protein synthesis** prevents resource depletion.
- **Host Strain Engineering**: Using **protease-deficient strains** (e.g., BL21(DE3) in E. coli) prevents degradation of recombinant proteins.

3. Preventing Excessive Metabolic Burden

- **Tunable Expression Systems**: Using **leaky or tightly regulated promoters** helps prevent unnecessary protein synthesis before induction.
- **Synthetic Circuit Design**: Engineering feedback loops to **dynamically adjust gene expression** based on cellular stress markers.
- **Stress Adaptation Strategies**: Overexpressing **stress response regulators** (e.g., GroEL/GroES, DnaK) improves cellular robustness.

Computational Modeling and Predictive Optimization

In silico metabolic flux analysis and machine learning models provide insights into pathway bottlenecks, enabling rational design of engineered bacterial strains with improved biosynthetic capabilities.

Applications and Industrial Implications

Engineered bacterial systems have broad applications in biopharmaceutical production, including biosimilars, vaccines, and enzyme therapeutics. The scalability and cost-effectiveness of these strategies make them attractive for industrial implementation.

CONCLUSION:

Synthetic rewiring of bacterial metabolic pathways presents a promising approach for optimizing recombinant protein production. By integrating computational and experimental techniques, we can enhance yield, maintain protein integrity, and improve host cell viability, ultimately advancing the biopharmaceutical industry.

REFERENCES:

1. Keasling, J. D. (2010). Manufacturing molecules through metabolic engineering. *Science*, 330(6009), 1355-1358.
2. Paddon, C. J., & Keasling, J. D. (2014). Synthetic biology for production of natural products. *Nature*, 510(7503), 395-402.
3. Chen, X., & Zaro, J. L. (2011). Recombinant protein expression in *Escherichia coli*: advances and challenges. *Current Opinion in Biotechnology*, 22(5), 675-682.
4. Nikerel, E., van Winden, W. A., & van Gulik, W. M. (2012). Regulation of metabolic fluxes in *Escherichia coli*. *Metabolic Engineering*, 14(1), 66-73.
5. Krishnan, K. M., & Swartz, J. R. (2009). Engineering bacterial cells for synthesis of membrane proteins. *Biotechnology and Bioengineering*, 102(2), 342-349.
6. Mairhofer, J., Wittwer, A., & Grabherr, R. (2015). Synthetic biology in bacteria: From genetic circuits to novel pathways and products. *Biotechnology Advances*, 33(8), 1494-1503.
7. Rosano, G. L., & Ceccarelli, E. A. (2014). Recombinant protein expression in *Escherichia coli*: advances and challenges. *Frontiers in Microbiology*, 5, 172.
8. Baneyx, F. (1999). Recombinant protein expression in *Escherichia coli*. *Current Opinion in Biotechnology*, 10(5), 411-421.
9. Choi, J. H., & Lee, S. Y. (2004). Secretory and extracellular production of recombinant proteins using *Escherichia coli*. *Applied Microbiology and Biotechnology*, 64(5), 625-635.
10. Gupta, P., Shukla, P. (2016). Advanced applications of microbial expression systems for recombinant protein production. *Frontiers in Microbiology*, 7, 135.
11. Xu, P., Rizzoni, E. A., Sul, S. Y., et al. (2017). Improving metabolic pathway efficiency by dynamically regulating pathway component concentrations. *Nature Communications*, 8(1), 1-10.
12. Kwon, S. K., Kim, S. K., Lee, D. H., et al. (2015). Evolutionary engineering of *Escherichia coli* for improved recombinant protein production. *Applied Microbiology and Biotechnology*, 99(14), 5679-5688.
13. Tyo, K. E., Alper, H. S., & Stephanopoulos, G. (2007). Expanding the metabolic engineering toolbox: more options to engineer cells. *Trends in Biotechnology*, 25(3), 132-137.
14. Du, J., Shao, Z., Zhao, H. (2011). Engineering microbial factories for synthesis of value-added products. *Journal of Industrial Microbiology & Biotechnology*, 38(8), 873-890.
15. Wei, C., & Zhang, F. (2019). Synthetic biology approaches to metabolic pathway engineering in bacteria. *Biotechnology Advances*, 37(6), 107455.