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CRISPR-Cas9 Mediated Modification of *Escherichia coli* for Enhanced Production of Therapeutic Proteins: A Narrative Review

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ABSTRACT

Insulin, interferons, and antibodies are examples of therapeutic proteins that are essential for the treatment of cancer and diabetes. Because of its affordability, quick growth, and genetic tractability, *Escherichia coli* is the preferred host for their production. However, its effectiveness is restricted by issues like low yields, protein misfolding, aggregation, and inclusion body formation. *E. coli* engineering has been transformed by the precise genome-editing tool CRISPR-Cas9, which allows for targeted modifications to improve protein production. In order to increase the yield of therapeutic proteins, this review examines CRISPR-Cas9 techniques such as chaperone regulation, metabolic pathway engineering, and codon optimization. Examples of CRISPR's ability to get around production bottlenecks include insulin and nanobodies. Advanced variants like dCas9 and base editors offer further precision. *E. coli* is a reliable platform for the scalable production of therapeutic proteins, despite obstacles such as off-target effects. CRISPR-driven innovations hold promise for advancements in synthetic biology.

INTRODUCTION

In contemporary medicine, therapeutic proteins such as hormones, cytokines, enzymes, and antibodies are essential for treating ailments ranging from cancer to diabetes (1). These biologics, which are frequently made recombinantly, have transformed medicine by providing highly specific targeted therapies. Because of its quick growth, inexpensive cultivation costs, and well-characterized genetics, *Escherichia coli* continues to be a key component for the production of recombinant proteins, making it perfect for large-scale industrial applications (2). Low protein yields, misfolding, aggregation, and inclusion body formation are some of the major obstacles that conventional genetic engineering in *E. coli* must overcome to produce functional proteins efficiently (3). *E. coli's* prokaryotic state restricts its capacity to carry out intricate post-translational modifications, such as glycosylation, which makes the manufacturing of some biologics even more challenging (4).

By facilitating accurate, effective, and multiplex genome editing, the CRISPR-Cas9 system, which was initially identified as a bacterial immune mechanism, has revolutionized microbial engineering (5). CRISPR-Cas9 facilitates targeted modifications by using guide RNA (gRNA) to direct the Cas9 nuclease to particular DNA loci, in contrast to more conventional techniques like homologous recombination (6). Key production bottlenecks in *E. coli* have been addressed by adapting CRISPR-Cas9 to optimize codon usage, rewire metabolic pathways, and regulate chaperone expression (7). With an emphasis on techniques like gene integration, promoter engineering, and pathway optimization, this review attempts to investigate how CRISPR-Cas9 improves the production of therapeutic proteins in *E. coli*. We assess the transformative potential, difficulties, and prospects of

advanced CRISPR variants in biotechnology through in-depth case studies and discussion.

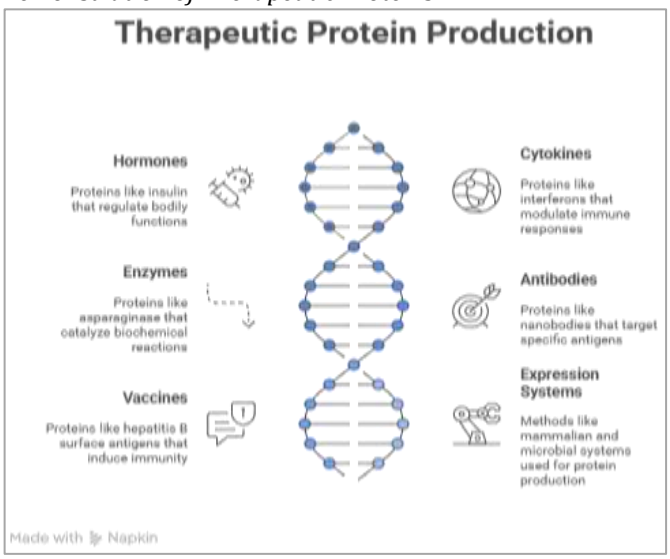
Therapeutic Protein Production: Global Perspective

Hormones (like insulin), cytokines (like interferons), enzymes (like asparaginase), antibodies (like nanobodies), and vaccines (like hepatitis B surface antigens) are all examples of therapeutic proteins (8). With different benefits, these biologics are made in mammalian systems like CHO cells or microbial systems like *E. coli* (9). Although mammalian cells are excellent at producing complex proteins that need to be glycosylated, their scalability is limited by their high cost, complicated media requirements, and slower growth rate (10). Because of its low cost, quick doubling time (about 20 minutes), and ease of genetic modification, *E. coli* is preferred for the production of simpler proteins like growth factors and insulin (11). Its industrial appeal is further enhanced by its established fermentation processes (12).

But *E. coli* has problems with protein misfolding, aggregation, and proteolysis, which frequently lead to inclusion bodies that need expensive refolding procedures (13). Its application to complex proteins, such as monoclonal antibodies, is limited by the lack of glycosylation machinery (14). Notwithstanding these drawbacks, *E. coli's* significance has been maintained by developments in genetic engineering, especially CRISPR-Cas9, which allow for precise adjustments to get around these obstacles and increase its contribution to the production of therapeutic proteins worldwide (15).

Figure 1

Demonstration of Therapeutic Proteins



CRISPR-Cas9 Technology: Principles and Applications

Derived from bacterial adaptive immunity, CRISPR-Cas9 creates double-strand breaks that are repaired by homology-directed repair (HDR) or non-homologous end joining (NHEJ) by using a gRNA to direct the Cas9 nuclease to particular DNA sequences. It was discovered in 2012 and outperforms less versatile and labor-intensive earlier tools such as transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) in terms of simplicity, specificity, and versatility (16). CRISPR-Cas9 facilitates effective multiplex genome editing in *E. coli*, enabling the simultaneous targeting of multiple genes to improve protein production. The programmable gRNA, which guarantees precise DNA cleavage and reduces off-target effects, is the source of its high precision (17).

Codon optimization, promoter engineering, and metabolic pathway redesign are some of the CRISPR applications in *E. coli* that greatly increase the yields of recombinant proteins. Because of *E. coli*'s simpler genome and absence of NHEJ, HDR-based editing is much more effective than in eukaryotic systems, allowing for precise modifications for the production of therapeutic proteins. Because of these properties, CRISPR-Cas9 has emerged as a game-changing instrument in microbial biotechnology, propelling advancements in the production of biologics (18).

CRISPR-Cas9 Engineering Strategies in *E. coli*

Codon Optimization and Gene Integration

By precisely optimizing codons to match the tRNA pool of *E. coli*, CRISPR-Cas9 improves translation efficiency. CRISPR has enhanced codon usage for insulin, resulting in yield increases of up to 30%. Plasmid-related variability is decreased by stable gene integration into high-expression genomic loci, which guarantees consistent production (19).

Metabolic Pathway Engineering

CRISPR reroutes metabolic flow to the synthesis of precursor proteins, such as interferons and growth factors. CRISPR increases the availability of precursors by blocking competing pathways, which redirects carbon flux and increases interferon yields. For high-value biologics, this method maximizes cellular resources (20).

Promoter and Regulatory Element Modification

Strong promoters created with CRISPR improve expression levels by enhancing the transcription of cytokines such as IFN-α and IFN-β. Reducing cellular stress and avoiding toxicity from overexpression are achieved by fine-tuning regulatory elements to balance expression (21). Under industrial conditions, this approach guarantees strong protein production (22).

Knockout of Competing or Degrading Pathways

Proteins such as asparaginase are stabilized by CRISPR-mediated knockout of proteases, which decreases degradation and raises yields by 25%. Protein stability is further improved by removing competing metabolic pathways, which is essential for industrial scalability (23).

Multiplex Genome Editing for Multigene Pathways

By simultaneously altering genes involved in secretion and folding, CRISPR's multiplex capability optimizes intricate pathways for nanobodies and scFv antibodies. This method reduces the time and expense of developing high-value treatments by streamlining the production of multigenic proteins (24).

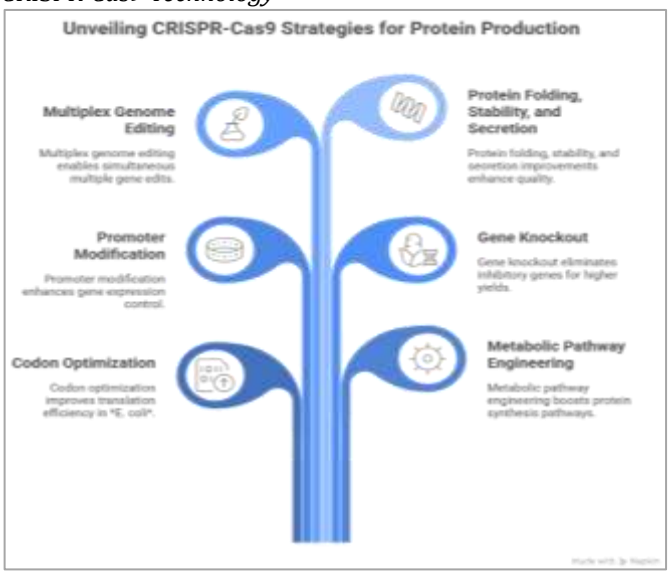
Enhancing Protein Folding, Stability, and Secretion

In *E. coli*, protein misfolding and aggregation pose serious problems. In order to improve the folding of complex proteins like human growth hormone (hGH), CRISPR-Cas9 upregulates molecular chaperones such as GroEL and DnaK. Functional yields are increased by engineering disulfide bond isomerases, like DsbA, which improve stability for proteins that need disulfide bonds (25).

Additionally, CRISPR removes genes that are prone to aggregation, which lowers inclusion bodies and increases the recovery of soluble proteins by up to 40%. By using CRISPR to optimize periplasmic transport pathways, antibody fragments and nanobodies are secreted more readily, making purification from the periplasm simpler. When combined, these tactics overcome the drawbacks of *E. coli* and enhance the yield and quality of therapeutic proteins for use in clinical settings (26).

Figure 2

CRISPR-Cas9 Technology



Case Studies of CRISPR-Enhanced Therapeutic Proteins in E. coli

The production of important medicines by *E. coli* has been transformed by CRISPR-Cas9. CRISPR-mediated codon optimization and stable gene integration into high-expression loci have reduced production costs and increased yields by 30–40% for human insulin, the first recombinant therapeutic. CRISPR-driven chaperone regulation improves the folding and solubility of interferons (IFN-α, IFN-β, and IFN-γ) by 25%, which is important for antiviral and anticancer therapies (27). Through CRISPR-mediated protease knockouts, asparaginase, which is used to treat leukemia, achieves greater stability, reducing degradation and improving therapeutic efficacy. CRISPR-optimized periplasmic transport enhances the production of human growth hormone (hGH), decreasing aggregation and boosting yields by 20%. Multiplex editing of folding and transport pathways streamlines production and improves secretion and solubility of nanobodies and scFv antibodies (28). Through CRISPR-driven promoter optimization, recombinant vaccine antigens like HPV proteins and hepatitis B surface antigen achieve higher yields while enhancing immunogenicity and scalability. These case studies demonstrate how CRISPR can be used to overcome production bottlenecks, thereby establishing *E. coli* as a reliable platform for a variety of biologics (29).

Advanced CRISPR Variants in *E. coli*

Advanced versions of CRISPR-Cas9 increase the usefulness of *E. coli* beyond the standard version. Dead Cas9 (dCas9) modulates the expression of folding and secretion pathways for proteins such as antibodies by enabling transcriptional regulation without DNA cleavage (30). CRISPR activation (CRISPRa) and interference (CRISPRi) optimize the production of complex biologics by fine-tuning gene expression (31). Base editors improve codon optimization without double-strand breaks by enabling accurate single-nucleotide modifications (32). For complex pathways such as the production of vaccine antigen, prime editors provide scarless edits. Development timelines are shortened by multiplex editing systems, which simplify multigene modifications and allow for the effective engineering of enzyme and antibody biosynthesis pathways (33). These developments increase the accuracy and adaptability of CRISPR, propelling breakthroughs in the synthesis of therapeutic proteins.

Challenges and Limitations

CRISPR-Cas9 in *E. coli* has difficulties despite its promise. Even though they are less common in prokaryotes, off-target effects can cause unwanted mutations, so careful gRNA design is necessary (34). *E. coli* has a lower HDR efficiency than eukaryotes, which limits complex modifications and makes precise edits more difficult. The production of complex proteins, such as monoclonal antibodies, by E. coli is limited by protein misfolding and the lack of glycosylation machinery. Environmental stressors can cause yield and stability variability when CRISPR-modified strains are scaled in industrial bioreactors (31). Challenges also arise from biosafety issues, such as the possible release of modified strains into the environment, and ethical issues related to genome editing. For CRISPR to be widely used in industrial biotechnology, these restrictions must be addressed (32).

Future Perspectives

Exciting opportunities for improving *E. coli* strains are presented by the combination of CRISPR with metabolic modeling and synthetic biology (35). The range of protein production may be increased by new CRISPR-Cas12 and Cas13 systems that allow for RNA targeting and more extensive microbial applications (33). By anticipating the best gRNA sequences and edit sites and minimizing off-target effects, AI-driven CRISPR design may improve accuracy. CRISPR-enhanced cell-free *E. coli* systems have the potential to overcome cellular constraints, increasing their scalability and consistency for industrial uses. These developments could revolutionize the production of therapeutic proteins and propel personalized medicine by enabling customized biologics with reduced costs and increased efficacy (35).

CONCLUSION

By tackling important issues like low yields, protein misfolding, and aggregation, CRISPR-Cas9 has made *E. coli* a very effective platform for therapeutic protein production. The production of insulin, interferons, asparaginase, human growth hormone, nanobodies, and vaccine antigens has been greatly enhanced by techniques like codon optimization, metabolic pathway engineering, promoter modification, and chaperone regulation. Complex pathway engineering and fine-tuned expression are made possible by advanced CRISPR variants, such as dCas9, CRISPRi, CRISPRa, base editors, and prime editors,

which provide previously unheard-of precision and flexibility. The combination of CRISPR with artificial intelligence, synthetic biology, and developing Cas systems has enormous potential, despite obstacles like off-target effects, reduced HDR efficiency, and scale-up problems. By overcoming these obstacles, CRISPR-driven breakthroughs have the potential to transform biotechnology and make it possible to produce therapeutic proteins in a way that is affordable, scalable, and customized. This would open the door to improvements in precision medicine and healthcare around the world.

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