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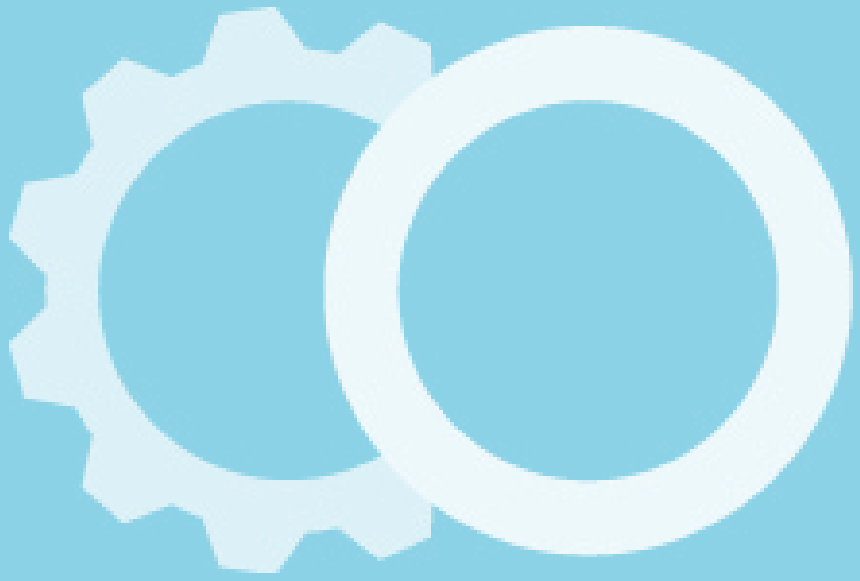
SIMPLIFYING RECOMBINANT PROTEIN PRODUCTION: BREAKING DOWN THE CORYNEX® PROTEIN EXPRESSION SYSTEM

The rise of biologics is having a dramatic impact on how doctors treat many serious and chronic illnesses. While these drugs have great promise, biologics are complex and, as a result, are very expensive to manufacture and subject to technical pitfalls. Pharmaceutical companies looking to take a piece of the biologics pie must come up with innovative ways to approach drug development and cut the high costs associated with it.

One way to do this is to simplify the processes to produce and purify recombinant proteins. Simpler processes result in reduced production costs and a faster time to market. Yasuhiro Takenaka, senior director of technical development at Althea, explains how the Corynex® Protein Expression System uses an extensive toolbox to improve the levels of protein secretion and overall success rate.



What are some of the biggest challenges of producing and purifying recombinant proteins?

Manufacturing highly purified recombinant proteins requires 1) an upstream with high titer and recovery of active, correctly folded proteins, 2) a downstream with multiple steps of purification with high yield, and, therefore, 3) significant time and cost. Recombinant proteins are often expressed in *E.coli* as insoluble aggregates called inclusion bodies. Active protein can be obtained after dissolving the inclusion bodies and refolding the protein, which generally returns quite low yield. Expression using mammalian systems requires long cycles for cell breeding, screening, and cultivation. Further, even one amino acid substitution can cause significantly different levels of expression in any system. This results in researchers using trial and error approaches to look for the appropriate expression system for each of their proteins of interest.

What is Corynex®?

Corynex® is a novel protein/peptide expression system using a gram-positive, non-sporulating soil bacterium, *Corynebacterium glutamicum*. This nonpathogenic bacterium has successfully been used for the industrial production of amino acids, such as glutamate and lysine and for use in human food, animal feed, and pharmaceutical products for more than 50 years. Corynex® has the advantage of a scalable, high-cell-density fermentation process that can be used to manufacture commercially valuable proteins, such as biopharmaceuticals, drug targets, and enzymes. Corynex® secretes soluble, properly folded, and biologically-active recombinant proteins directly into culture media with high purity.

How is the purification process used by Corynex® different from traditional processes?

Corynex® has the ability to:

- **Secrete with high purity (Figure 1):** Because target proteins/peptides are directly secreted into culture media, there is no need for cell disruption. Purity of the secreted protein is quite high since *Corynebacterium* secretes a limited amount of host cell proteins.

  Furthermore, *Corynebacterium* produces no endotoxins. These characteristics make it possible to construct simple processes with reduced purification steps. High purity of secreted proteins/peptides can be seen in SDS-PAGE analyses of culture supernatant from expression tests.

FIGURE 1. High purity of secreted proteins/peptides (unpurified culture supernatants)

- **Express active proteins (Figure 2):** Proteins having complex disulfide bonds (S-S bonds) can be secreted in active form using the Corynex® system. There is no need for inclusion body recovery or complicated and unwanted refolding processes. In the expression of human epidermal growth factor (hEGF), which has three intramolecular S-S bonds, it was secreted as a single band in SDS-PAGE analysis and confirmed to be properly folded and functional with correct S-S bonds. The hEGF produced using the Corynex® system had a similar biological activity as endogenous hEGF.

Because there are many fewer steps than required with other bacterial platforms, there is less protein loss during purification and overall yield is increased. This results

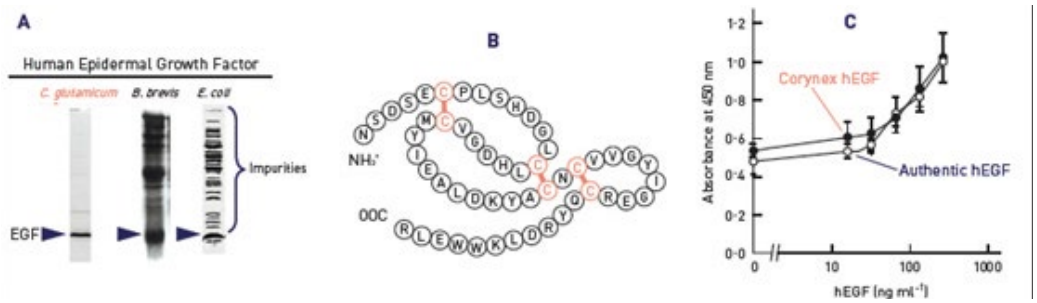


FIGURE 2. A. Fermentation broth of *Corynebacterium glutamicum* contains fewer host proteins when compared to lysates from other systems. B. Structural conformation of hEGF with three internal disulfide bonds. C. Biological activity of hEGF expressed by the Corynex® system versus endogenous hEGF.

in a reduction of costs and time in the development of protein therapeutics.

What tools are used during strain and process development?

First, there are two secretory pathways by which proteins can be targeted for secretion: the Sec and Tat pathways. When comparing the Sec and Tat pathways, proteins are secreted in the Sec pathway through the Sec machinery in unfolded form and subsequently fold extracellularly. On the other hand, the more recently identified Tat secretory pathway is able to transport folded proteins, including those that are large and heterologous, which are sometimes poorly secreted by the Sec pathway (Figure 3). Secretion of properly folded recombinant proteins has been achieved with both pathways including hundreds with disulfide bonded and dimerized structures. Next, a library of 154 signal peptides can be screened to further increase secretion levels. The optimum signal peptide is determined empirically for each target protein. Secretion levels of transglutaminase, insulin-related protein, and IGF-1 related protein could be increased by screening for optimal signal peptides (Figure 4). Finally, Nitrosoguanidine (NG)-induced mutagenesis and high throughput screening allows the Corynex® team to quickly and efficiently select for optimal host strains tailored to target proteins. More than 2,000 strains can be generated and tested within three months. With optimized host strains, protein expression for target A and B were significantly increased (Figure 5).

What makes Corynex® different from other similar technologies?

Microbial expression systems, such as *E. coli*, are inexpensive and easy to handle in upstream, while in most cases require refolding and removal of host cell proteins and endotoxins in complex downstream operations. Mammalian cells, such as CHO cells, have highly sophisticated protein modification and maintenance mechanisms, which make downstream processes simpler than many microbial systems; however, creation of stable cell lines and bioreactor production require substantial time and cost. Corynex® possesses the best of both worlds: simplified upstream and downstream processes.

What is the track record of the Corynex® platform?

In a recent announcement, Althea reported that a biologics candidate developed by a Japanese global pharmaceutical company and manufactured at Althea

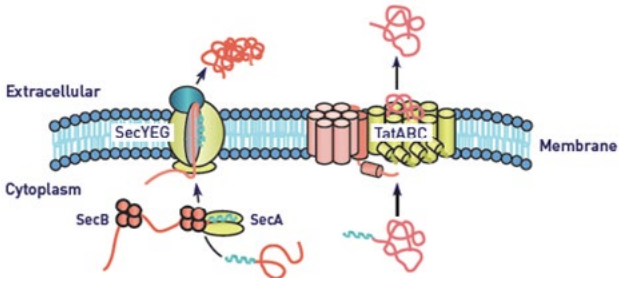


FIGURE 3. Illustration of the Corynex® system's two different secretory pathways

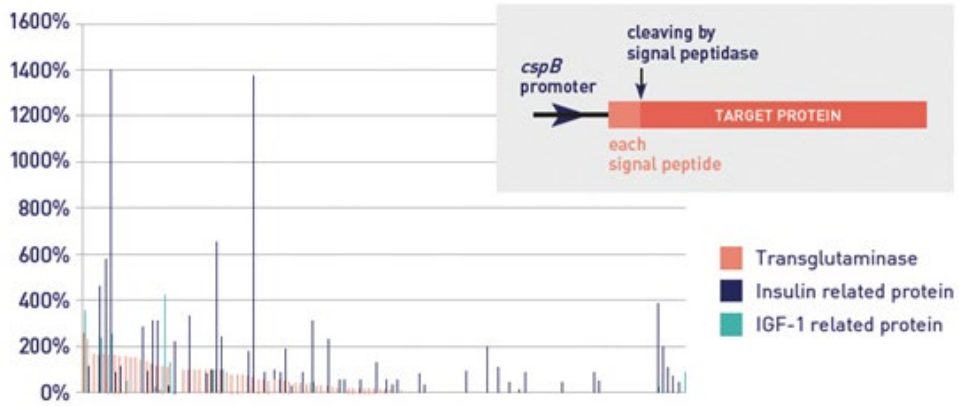


FIGURE 4. Screening of signal peptide library to determine best expression level of three target proteins

using Corynex® has entered Phase I clinical trials. After feasibility studies were successfully performed by Ajinomoto Co., the pharma company engaged with Ajinomoto Co. and its subsidiary Althea to scale up and produce GMP material at the 1000-liter scale to support a Phase 1 clinical study. Althea has produced successful GMP batches and the first human subjects have been dosed.

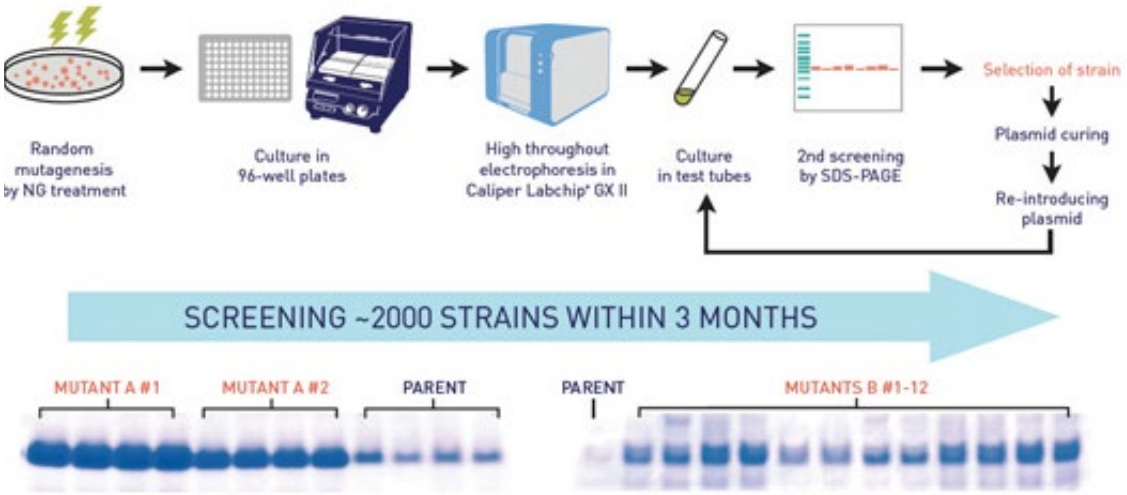


FIGURE 5. Key steps in generation and identifying optimal Corynex® host strains

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