

“Pathway Modulator” - Novel Productivity-Boosting Technology

“Pathway Modulator Technology”: A permanent modifier of animal cell lines for substantially improved protein yields.

In eukaryotic cell culture any increase in volumetric productivity has a direct impact on cost of goods for complex biopharmaceuticals and is, thus, desirable from a commercial and healthcare socioeconomic perspective. The producer cell itself offers potential for improvement. The expression and secretion of heterologous proteins in non-polarized eukaryotic cells is a complex regulated multi-step process with several actuating variables. Every single step such as transcription, RNA transport, translation, post-translational modification and protein transport has a strong impact on the specific productivity. As a logical consequence of this complexity, the elimination of one bottleneck at early process stages is likely to lead to the creation of other bottlenecks either further downstream in the process chain or even in other crucial parallel sidelines of the secretion process.

Many cell engineering approaches have used single enzymes and regulator proteins in the past. They often resulted in increased specific productivity while compromising growth properties.

To address several potential bottlenecks at once, ProBioGen investigated regulator proteins that are known for pleiotropic effects on cellular function and identified CDC42 as a key regulator of multiple cellular and transgene related pathways.

The CDC42 protein is a small GTPase of the Rho-subfamily and exists in an inactive GDP-bound and active GTP-bound form. It is involved in regulation of many important cellular processes such as actin organization, cell cycle progression, transcription, cell-cell adhesion, cell motility, vesicle shuttling, secretion, endocytosis, phagocytosis, mitogenesis, and/or apoptosis.

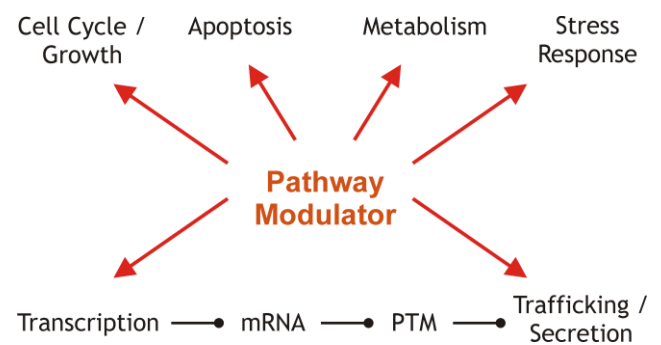


Figure 1: ProBioGen’s modulator targets several host cell and transgene-related pathways.

Activation of the CDC42 protein is mediated by Guanine nucleotide exchange factors (GEFs), particularly by dbp proteins, which catalyze the replacement of GDP by GTP.

ProBioGen uses a mutant of the murine CDC42 to achieve a concerted boosting effect on recombinant protein yield.

Compared to the native CDC42 protein this mutant exhibits several changes of the amino acid sequence allowing it to switch between its GDP-bound inactive form and GTP-bound active form without Guanine nucleotide exchange factors.

ProBioGen has shown that adjusted expression of the CDC42 mutant repeatedly enhances cell specific productivities and also boosts the titers for several different glycoproteins. The technology is highly flexible: Substantial yield improvement has been demonstrated for both CHO DG44 and K1 derivatives. The modulator is effective regardless of its route of application: Cell line development starting from a CDC42 pre-modified host, engineering of an existing producer clone with the modulator transgene and co-transfection of the target product and the modulator gene into a blank starter cell all result in a yield-enhanced final producer clone.

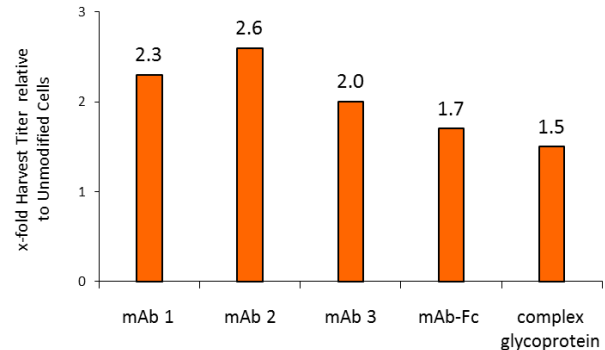


Figure 2: Application of the Pathway Modulator Technology resulted in 1.5 to 2.6-fold yield enhancement for several glycoproteins.

Advantages of ProBioGen's "Pathway Modulator Technology":

- Targets several potential bottlenecks at once
- Requires no culture additives
- Rapid & Flexible: applicable to **new** and **pre-existing** producer cells
- Substantial yield enhancement for monoclonal antibodies, Fc-fusion proteins or other glycoproteins
- **Royalty-Free!**