

Improved Protein Production from Mammalian Cells

Potential of Targeting Signals

There is a general consensus that although non-mammalian systems can be effectively employed in the manufacture of many proteins, mammalian systems will have to be chosen for the bulk production of a large number of proteins to be used in therapeutics. The main reason being that only the latter type of system can assure the correct form of post-translational modification that many proteins require to be biologically active. In comparison to, for example, microbial systems those based on mammalian cells are extremely expensive. It is therefore evident that an intense effort is required in order to greatly improve the efficiency of mammalian cell factories such that they can become competitive with their non-mammalian counterparts. A new approach in genetic engineering is now being employed to make vast improvements in protein production using mammalian cells.



Rapid advances in disease-related research are continuously leading to the production of an increasing number of protein therapeutics and protein diagnostics. Many of these are on the market, some are currently in clinical trials whilst others are further back in company development pipelines. The market for protein therapeutics is worth some US-\$ 41 billion and has been growing at a CAGR of 21% over the past 5 years. More than 120 protein drugs are at present on the world market, including nine blockbuster

drugs (i.e. with annual sales > US-\$ 1 billion). In the field of cancer therapy 370 protein drugs are currently in company pipelines, including 47 monoclonal antibodies (mAbs) [1]. An analysis of the general market for mAbs in May 2004 showed that there were 376 preclinical-to-market products under development in 95 key companies [2]. Opportunities for the development of “biogenerics” are also rising, as US-\$ 16 billion worth of protein medicines are coming off patent during the course of the next five years.

Biopharmaceutical firms are often challenged by difficulties in producing proteins in amounts large enough to allow for their proper characterization/evaluation and for scale-up before market launch. Furthermore, a potential manufacturing shortfall and failures in achieving economically viable production yields threaten to put a brake on the rapid growth of this market. There are a number of current industrial bottlenecks which include the following: 1) failure to achieve laboratory scale production, impeding

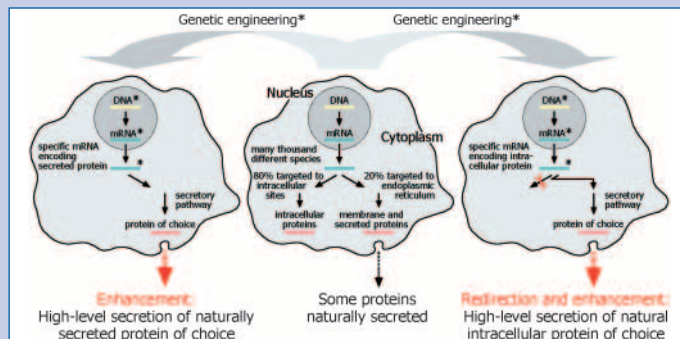


Fig. 1: Conversion of a mammalian cell into a secretory cell factory by genetic engineering.

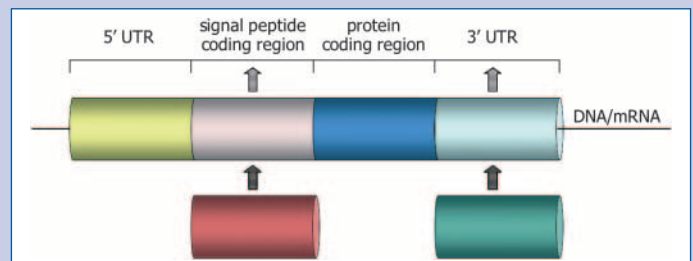


Fig. 2: Generation of constructs using seamless cloning for sequence element insertion/substitution.

R & D success (drug lead candidates frequently falter due to an inability to produce enough quality protein for preclinical and clinical trials), 2) commercially unviable yield levels, halting product development or hurting margins (failure to produce quality protein at yields that are economically viable is a common problem occurring in the development of novel biopharmaceuticals as well as generic protein drugs), 3) high investment requirements in production capacity (current expectations are that the available production facilities will not suffice to meet the rapidly growing demand for the supply of protein pharmaceuticals).

Within the space of a few years it is anticipated that the market supply of mAbs will represent a major challenge due to the fact that patients require long-term treatment with relatively high doses of antibody. There are forecasts suggesting that already from 2006 there will be a serious lack of production capacity for proteins derived from mammalian cell cultures [3]. Another important factor is that of high cost: the production of 1g mAb in CHO cells costs about US-\$ 500. It is thus quite evident that a major effort is required to vastly improve current protein production systems based on the use of mammalian cell factories.

Traditional Approaches to Improve Protein Production

Using traditional approaches, companies utilizing mammalian systems to produce recombinant proteins have attempted to scale up production by strategies focusing mainly on the modulation of growth conditions (media composition and process control) or on increasing transcriptional activity of the recombinant gene within the host genome (e.g. utilisation of strong promoters/enhancers in the expression vector, amplification of gene copy number) [4]. Although leading to higher cellular levels of messenger RNA (mRNA) this in itself may not be sufficient

to result in optimal protein production. An important consideration that has hitherto been poorly addressed is that of attempting to achieve the maximal efficiency of translation of the mRNA.

Genetically Engineered Cell Factories

A completely new approach is currently being developed in our laboratory (Fig. 1) where our focus is on aspects of post-transcriptional events (mRNA/protein targeting and trafficking). Genetically engineered cell factories containing selected targeting "instructions" for the recombinant mRNA and the encoded protein of interest are generated.

The move toward boosting the yield of high-quality proteins by the implementation of this unique genetic engineering technology is based on the finding that the efficiency of targeting mRNA to the endoplasmic reticulum (ER) is heavily dependent on the presence of, and correct interplay between, specific key elements (signal peptide and the 3' untranslated region (3' UTR)) [5]. A "seamless" cloning methodology has been developed to insert these elements on either side of the coding region of the protein of interest, thus avoiding the use of linker sequences that may perturb the biological activity of the protein (Fig. 2). The protein encoded by the engineered mRNA then being efficiently directed to the cell's secretory pathway for transport out of the cell. Depending on the protein in question production has been improved by a factor of 3-50 in laboratory scale experiments.

The signal peptide is an intrinsic targeting signal located on the nascent polypeptide chain that has the property of directing the synthesis of membrane proteins and proteins destined for secretion to the ER [6]. It has been demonstrated that different signal peptides vary greatly with respect to the efficiency by which they direct synthesis/secretion of a reporter protein in transfected CHO cells. A particular signal peptide derived from a

marine organism was found to be surprisingly efficient when compared to well known signal peptides of mammalian origin (e.g. albumin, Fig. 3), [7]. Interestingly, although similar amounts of mRNA were detected when the signal peptides of the marine organism and human albumin were used (results not shown), the levels of recombinant protein differed by a factor of 50. Having previously shown that mRNA targeting to the ER is affected by the 3' UTR [5], we now demonstrate that the nature of the 3' UTR engineered into the mRNA molecule also plays an important role in enhancing the amount of the final product secreted by transfected CHO cells. Figure 4 exemplifies this and in addition shows that a signal peptide and 3' UTR originating from the same gene do not necessarily form the most effective "doublet". Efficient doublets have been identified and it appears in principle that synthesis/secretion of any naturally secreted protein can be boosted through the cooperate action of the two components. The technology is currently being evaluated in collaboration with various pharmaceutical companies for the improved production of protein therapeutics (mAbs, erythropoietin).

Production of Intracellular Proteins

The technology has another area of application, namely the production of proteins that normally reside within the cell. Intracellular proteins are commonly recovered after lysing the cell membrane to generate an extract from which the molecule in question is purified. Such proteins often represent only a minor percent of the total cell content. It has been predicted that approximately 30,000 different proteins are produced by human cells, probably >90% being synthesized for internal use. Methodology is currently being developed such that cells can be engineered to efficiently secrete large quantities of intracellular proteins of potential commercial interest

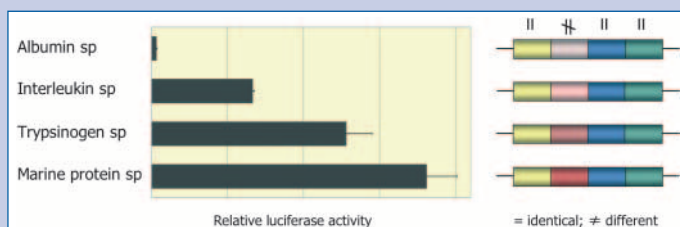


Fig. 3: Efficiencies of signal peptides (sp) derived from different proteins in secretion of a reporter luciferase.

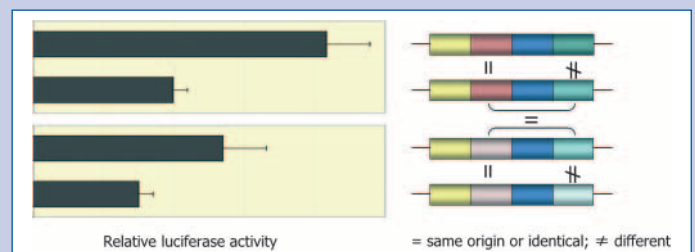


Fig. 4: Importance of correct choice of signal peptide/3' UTR doublet on reporter luciferase secretion.

(such as toxic proteins, protein targets for drug design and protein diagnostics).

Complementarity of the Technology

The technology outlined here has proven to be complementary with other related forms of methodology aiming at producing protein in high yield. In a feasibility study performed together with Selexis (www.selexis.com), focusing on transcriptional enhancement by using specific DNA elements binding to nuclear scaffolding (MAR), it was shown that when the two technologies were combined then the level of protein production could be further enhanced. A similar effect was seen in collaborative experiments with Inovio Biomedical Corporation (www.inovio.com), a company engaged in gene therapy aiming at increasing protein production *in vivo* using electroporation-mediated DNA delivery. A major increase in recombinant protein in mice serum was observed.

Conclusion

We consider that our technology represents a new and novel approach to enhancing protein synthesis/secretion and is set to offer a significant improvement

in the protein production process based on the use of mammalian cell culture. This is manifested by the fact that a number of key global pharmaceutical companies involved in therapeutic and diagnostic protein production are currently evaluating the technology. It is expected that the implementation of this new approach will assist companies to improve the technological process currently available and enable them to increase both the quantity and quality of the protein being produced.

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