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The level of synthesis and secretion of *Gaussia princeps* luciferase in transfected CHO cells is heavily dependent on the choice of signal peptide

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Abstract

There is a great demand for the improvement of mammalian cell production systems such that they can compete economically with their prokaryotic counterparts. Of a number of parameters that need to be explored to accomplish this we have tested the effects of different signal peptides on the synthesis and secretion of *Gaussia princeps* luciferase in mammalian cells. A series of plasmids were transfected into CHO cells where the coding region for the marine luciferase was fused to the signal peptide coding regions derived from different sources. Both cell extracts and medium samples were analysed for luciferase activity. When the native *Gaussia* luciferase signal sequence in the vector was substituted by that from human interleukin-2 or albumin then the amount of active recombinant protein produced was substantially reduced, both in transiently and stably transfected cells. Western blotting showed that enzyme activity and protein levels mirrored one another. The major decrease in luciferase activity was shown not to be a result of decreased mRNA levels, indicating the involvement of a post-transcriptional event. When the coding region of human endostatin was fused to that of the *Gaussia* luciferase signal peptide then an elevated level of secreted endostatin was observed compared to when that of the albumin signal peptide was used. Stable transfection of HepG2 cells with the different signal peptide constructs gave essentially the same results as seen in CHO cells. The overall results indicate that the choice of signal peptide can be imperative to ensure an optimal synthesis and secretion of a recombinant protein in a mammalian cell culture system.

Keywords: Albumin; Cell leakage; Interleukin-2; Mammalian protein production; Secreted luciferase; Signal sequence

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1. Introduction

Although prokaryotic cells grow more rapidly, are cheaper to maintain and are easier to handle in the laboratory than mammalian cell cultures, they have some important disadvantages when it comes to producing mammalian proteins. A major problem is that the prokaryotic cell is deficient with respect to machinery for post-translational modification of the synthesised protein e.g. glycosylation. Bacteria also lack appropriate chaperones, and there is, therefore, a certain risk of misfolding of the protein. These two factors may cause a recombinant protein, produced in a bacterial expression system, to differ severely from the native protein, both when it comes to biological activity and immunogenicity. Another difficulty is that of solubility of the recombinant protein since it may be incorporated into inclusion bodies, which would complicate its purification. In addition there is a problem if the recombinant protein is to be used for therapeutic purposes in humans, in view of the fact that many bacteria have a high endotoxin content that may be pathogenic in nature. For patients, especially those to be subjected to long term treatment with repeated injections of a recombinant protein, there is a certain danger of transmission of toxins and infectious particles. Based on the above it is evident that considerable effort is necessary to bring mammalian systems to a level that will make them economically competitive with their prokaryotic counterparts.

A lot of work concerning the optimisation of the production of a recombinant protein by a transfected mammalian cell line has concentrated on modulating parameters affecting cell growth such as pH, CO_2, ionic strength, and cell density (Wurm, 2004). Genetic engineering approaches have predominantly focused on increasing protein production by raising the level of transcription through e.g. the incorporation of a strong promoter region, an activator and/or enhancer (Darzacq et al., 2005; Fiering et al., 2000; Lee and Young, 2000; Verrijzer et al., 1995). A novel and very different approach would be to identify specific signal peptides mediating a high level of protein production. This has not yet been extensively explored in mammalian host systems, but is addressed in the present study.

As a reporter protein luciferase from the marine copepod *Gaussia princeps* was chosen because of the sensitivity of the assay system. In nature, after the luciferase is synthesised by the copepod, it is packed into secretory vesicles for storage. The enzyme is released in bulk into the sea water along with its substrate following an appropriate stimulus such as pressure waves generated by a potential predator. The flash of light produced confuses the predator enabling the copepod to escape. Signal peptides from human interleukin-2 (Il-2) and human albumin were adopted as alternatives to the *Gaussia* luciferase signal peptide because both proteins are known to be secreted with great efficiency in nature. Another reason was that they exhibit different patterns of secretion. Albumin is constitutively expressed and its secretion occurs as a constant flow into the blood from hepatic cells to replace that removed from circulation. Interleukin-2, on the other hand, is produced and secreted in a "burst-like" manner upon appropriate stimulation of leukocytes. A further motivation for choosing the albumin signal peptide was the fact that as described by Partridge et al. (1999) its coding region was known not to affect mRNA stability when present in a chimaeric construct.

To our knowledge this is the first study reporting significant differences in synthesis/secretion of a recombinant protein in mammalian cells due to substitution of the signal peptide. The overall goal being to optimise vectors to be used for high-yield production of medically important proteins such as monoclonal antibodies.

2. Materials and methods

2.1. Vector construction

All constructs in this study were made based on the pTRE2hyg expression vector (Clontech Laboratories) and using a seamless cloning strategy (Chen et al., 2000). This is a PCR-based restriction site-free cloning method, used here to assemble the "secretion cassettes" containing selected untranslated regions (UTRs) and coding regions fused without any linker sequences. The cassettes were introduced into pTRE2hyg at the multiple cloning site and the constructs termed p followed by a four-letter code. The first, third and fourth letters refer to the 5′UTR, the coding region and the 3′UTR of *Gaussia* luciferase cDNA (GenBank accession no. AY015993), respectively. The second letter refers to