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Development of an innovative protein production system using second-generation mRNA export proteins created by domain engineering

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Research aims

Proteins intended for use in clinics have to be produced in mammalian cells because accurate post-translational modifications such as glycosylation, adequate disulfide-bonds, acylation, and phosphorylation are essential for their activity in the human body. Proteins, peptides, their analogs, and drugs that contain such components produced using recombinant protein technology are called biotechnology-based drugs. These drugs exist in various forms; their protein-based forms include recombinant proteins whose sequence is originally derived from an endogenous protein and a genetically modified antibody, the nucleic acid-based forms include an aptamer and a peptide-based form. The global sale of protein-based biomedicines reached 86.8 billion in 2007 and an estimated 160.1 billion in 2014.

Unlike the existing drugs formed by low-molecular weight molecules, biotechnology-based drugs are known to have specific effects on cancer and rheumatism.[1, 2] Around 60% of the biotechnology-based recombinant proteins currently used in the clinics are produced in CHO cells. However, protein production in CHO cells is more expensive than that in bacteria.[3] Generally, a strong promoter like the CMV promoter supports the transcription and protein production in mammalian cells. This promoter efficiently transcribes pre-mRNA from the template DNA and achieves high rates of protein production. Recent studies have led to the whole genome sequencing of various species, including humans and bacteria, and these updates make it difficult to obtain better promoters than the CMV promoter. In addition, several attempts have been made to produce proteins effectively in translation process.[4] In the present study, an mRNA export process[5] was created to establish a novel protein production system in mammalian cells (Fig. 1).

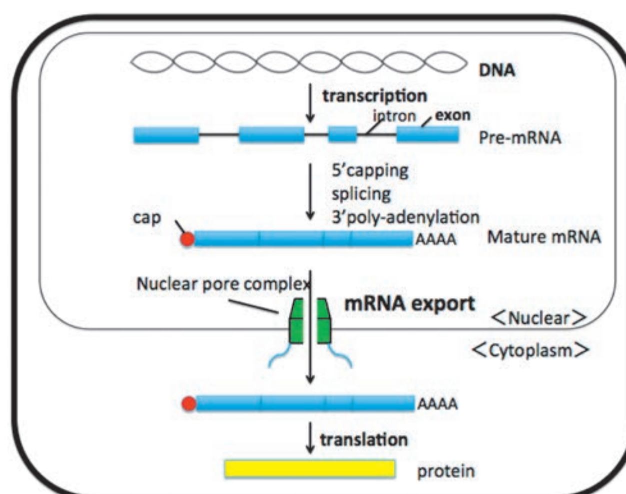

Fig. 1. Schematic representation of gene expression in higher eukaryotes

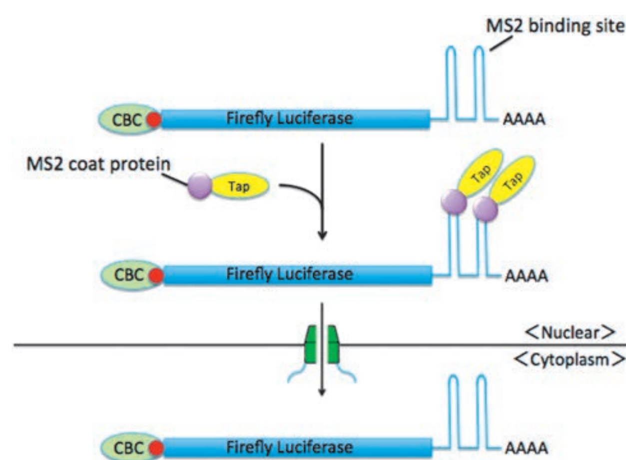

Fig. 2. Export of mRNA by MS2-Tap

Methods

A novel expression system, which efficiently exports a specific mRNA to the cytosol using the MS2 binding site and MS2 coat protein, was developed. Two expression plasmids were constructed: one containing the luciferase gene followed by the MS2 binding sites, and the other expressing MS2 coat protein fused with Tap, an mRNA exporter. When MS2-Tap is expressed, it binds specifically to the MS2 binding sites and efficiently exports its mRNA by means of Tap. In this way, mRNAs containing the MS2 binding site can be specifically exported into the cytosol (Fig. 2).

Cells (CHO, HeLa, and HEK293) were seeded in 12-well plates and cultured for 24 h. Plasmids were transfected

into the cells using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. The transfection efficiency was standardized with the plasmid phRL-TK expressing renilla luciferase. After culturing for 24 h, the cells were lysed and the luciferase activity was measured using a Luciferase Assay Kit (Promega), according to the manufacturer's instructions.

Results

When the plasmid containing the MS2 binding sites was co-transfected with that expressing MS2-Tap, the luciferase activity was enhanced, compared to co-transfections with a control MS2-GFP plasmid. In particular, the luciferase activity was enhanced 5.2 fold, 2.5 fold, and 2.5 fold in the HeLa, HEK293, and CHO cells, respectively. These results indicate that the protein expression system containing MS2 binding sites and MS2-Tap fusion protein achieved an efficient increase in the luciferase activity in all the cells examined. Notably, the number of MS2 binding sites can change the efficiency of the luciferase activity.

Conclusions

Luciferase activity was efficiently increased by the protein expression system containing MS2 binding sites and MS2-Tap fusion protein. CHO and HEK293 are the most frequently used mammalian cell lines in protein production. Our evidence suggests that enhancing mRNA export can serve as a useful strategy for protein production in mammalian cells. In the future, additional research is required to develop an optimum combination of the MS2 binding sites and the amount of MS2-Tap, for increasing the efficiency of protein production in cells.

References

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