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Development of Efficient Recombinant Protein Expression System via mRNA Export Mechanism

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Research aims

The proteins that is modified with sugar chain for its *in vivo* activity like drugs are essential to be produced in mammalian cells. Protein production in mammalian cells, however, is much less efficient production compared with in bacteria such as *E. coli*. The use of virus mRNA containing CTE can bypass the cellular mRNA pathway without splicing and the transfer of this virus export pathway to mammalian protein production will improve the protein production than ever tried. Recently, the mechanism why mRNA with CTE is independent of splicing was found.[1] CTE binds specifically to mRNA export cargo, Tap/p15 heterodimer, thereby mRNA containing CTE does not need splicing for its export. The method used in this project is expected to achieve a highly efficient protein production in mammalian cells because this method enhances the export pathway and it is different from promoter enhancement in cell. The final goal of this project is to make the SUPERCELL for the protein production in mammalian cells.

Methods

Luciferase expression plasmid containing CTE(plucCTE) was a gift from Dr. Kimura T.[2] CTE was removed from plucCTE to make control plasmid(pluc). pCMVHATap was from Dr. Ferber, BK.[3] pCMVflagp15 was constructed by using p15 full length PCR product amplified from HeLa cDNA library. The pcDNA5/FRT/HATap was constructed by inserting HATap into pcDNA5/FRT. DNA was transfected into 293T cells using lipofectamine2000. Luciferase activity was measured with luminometer. 293T cells were cultured as standard method. HATap was detected with western analysis.

Results

The analysis of a novel expression system was done by measuring luciferase activity. Luciferase expression in the presence of CTE enhanced its activity by 30% than in the absence of CTE. Its enhancement, however, was not as expected. It is suggested that transient expression of large amount of mRNA may cause the lack of mRNA export cargo, Tap/p15, thereby the luciferase expressed in the nucleus was not export efficiently. To solve the lack of export receptor, inducible expression of Tap/p15 was examined. First, Ha tagged Tap was inserted into pcDNA5/FRT vector to make pcDNA5/FRT/HATap whose expression was induced by *Tet* and/.or *Dox*. pcDNA5/FRT/HATap was introduced into 293Trex cells with pOG44 which express Frp recombinase to make 293HATap cell. 293Trex cell can integrates 1 copy of target DNA into its genome. As a result, HATap expression was very similar between 15 clones obtained. Second, using one of these clones (No1), flag tagged p15 was introduced and now the best clone is selecting using flag antibody.

Conclusion

It becomes obvious that the validity of CTE mediated mRNA export system to produce protein whose activity is dependent of mammalian cell production, may be difficult from the data of transient expression trial in the absence of exogenous expression of Tap/p15. Then, the way to better estimation is to make a clone that express Tap/p15 export cargo proteins. After obtaining the clone, validity of CTE mediated mRNA export system will bwe examined. CTE. The position of CTE, then, will be examined to obtain the information where CTE is the best for the expression. It is not described in this report, there is another method to enhance the protein production using the export system. This uses the export pathway in its cell. The TREX complex is responsible for the global mRNA export pathway,[4,5] then if the expression of TREX was well tuned, the mRNA export pathway will be activated than the standard condition. TREX consists of 8 proteins. The complete information of TREX takes 3 years of time.[5] The tight regulation of these TREX complex protein expression, then, may have a great difficulty to set the efficient protein production system up in a short period. The best way to develop the efficient protein production system using the mRNA export patway is focusing on the construction of virus CTE system and looking for the production rate by changing the condition of Tap/p15 expression.

References

1. Gruter, P., *et al.*, TAP, the human homolog of Mex67p, mediates CTE-dependent RNA export from the nucleus. *Mol. Cell*, **1**, 649–659, 1998.
2. Kimura, T., *et al.*, CRM1-dependent, but not ARE-mediated, nuclear export of IFN-α1 mRNA. *J. Cell. Sci.*, **117**, 2259–2270.

3. Bear, J., *et al.*, Identification of novel import and export signals of human Tap, the protein that bind to constitutive transport element of the type D retrovirus mRNAs. *Mol. Biol. Chem.*, **19**, 6306–6317, 1999.
4. Stresser, K., *et al.*, TREX is a conserved complex coupling transcription with messenger RNA export. *Nature*, **417**, 304–308, 2002.
5. Masuda, S., *et al.*, Recruitment of the human TREX complex to mRNA during splicing. *Genes Dev.*, **19**, 1512–1517, 2005.