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A novel *in vitro* translation system based on insect cells

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Background

Various genome sequencing projects are identifying many new protein sequences but it is key to attribute functions to these proteins. A huge number of proteins have been expressed *in vivo* to date, most of them being functionally, antigenically and immunogenically similar to their authentic counterparts. This is mainly due to the properties of cultured eukaryotic cells, which are able to carry out many types of posttranslational modifications such as addition of N- and O-linked oligosaccharides, but also palmitoylation, myristylation and phosphorylation.

Results

Based on the versatile properties of cultured cell lines, e.g. insect cells, we have developed a novel eukaryotic *in vitro* translation system [1]. Our homogenization procedure maintains the functional integrity of subcellular components, thus allowing the cell-free synthesis of membrane proteins and posttranslationally modified proteins, e.g. glycoproteins. An indispensable prerequisite for the reliable high-throughput expression of different proteins in cell-free systems is the generation of efficient templates. Therefore, we have developed a PCR-based methodology which allows the user to introduce regulatory elements as well as tags for protein purification into the desired gene. Additionally, we have shown that recombinant viral mRNA is a suitable template in this system and we have also demonstrated activity of the *Rhopalosiphum padi* virus (RhPV) 5'-UTR IRES in our lysates [2].

Conclusion

The standardized large-scale production of lysates from various cell-lines is a powerful tool for the development of novel eukaryotic *in vitro* translation systems with individual cell type- and tissue-specific properties.

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