Eukaryotic Cell-free Systems:

Novel Strategies for the Synthesis of Membrane Proteins

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Abstract

Various genome sequencing projects have greatly accelerated the discovery of novel genes encoding membrane proteins. In contrast, the molecular analysis of membrane proteins lags far behind that of cytosolic proteins. Preparing high quality samples of functionally folded proteins represents a major bottleneck that restricts further structural and functional studies. Cell-free protein expression systems, in particular those of eukaryotic origin, have recently been developed as promising tools for the rapid and efficient production of a wide variety of membrane proteins. A large number of these proteins, however, require posttranslational modifications for optimum function. Several membrane 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. Based on these versatile properties of cultured cell lines, we have developed a technique for the standardized production of translationally active eukaryotic lysates from insect cells. Our homogenization procedure avoids any serious breakdown of membrane vesicles already existing in the cytoplasm of the prepared eukaryotic cells. We have demonstrated the functional integrity of these subcellular components by showing signal peptide cleavage as well as glycosylation of cell-free expressed membrane proteins. Moreover, we have expanded our cell-free protein expression system by the insertion of orthogonal tRNA/synthetase pairs to facilitate the cotranslational and site directed incorporation of non-canonical building blocks. These fluorescently labeled and chemoselective moieties enable the site-specific modification of *de novo* synthesized membrane proteins.
The development of this novel eukaryotic *in vitro* translation system now expands the possibilities of cell-free protein synthesis, since posttranslational modifications significantly alter the physical and chemical properties of membrane proteins, including their folding and conformational distribution and these modifications are frequently a fundamental prerequisite for functional activity.

Biography

Dr. Kubick is head of the department “Cell-free Bioproduction” at the Fraunhofer Institute for Biomedical Engineering, IBMT. He gained his PhD in Molecular Biology and Physiology from the University of Stuttgart-Hohenheim, Germany in 1997. During his postdoctoral research in the Institute of Pharmacology at the Free University of Berlin, he was involved in the characterization of cellular and biological functions of G protein-mediated signal transduction processes. In 2000 he moved to RiNA GmbH, Berlin, to work on the development and commercialization of novel solutions in the field of cell-free protein expression and labeling. In collaboration with Qiagen GmbH he developed a novel eukaryotic *in vitro* translation system. Since 2010 he has led a large group at the Fraunhofer Institute. His laboratory exploits cell-free protein synthesis as a versatile tool for functional genomics, e.g. cell-free synthesis of membrane proteins and glycoproteins, as well as chip-based protein synthesis and translational regulation. Dr. Kubick is also a lecturer at the Free University of Berlin and the University of Potsdam. He is an affiliate of the Technical University of Berlin and Lecturer at the University of Applied Sciences, Berlin, Germany.