Transient gene expression from mammalian cells – a new chapter in animal cell technology?

A report on the First European Technology Workshop on: Current Status and Trends in Mammalian Transient Protein Expression, Ittingen, Switzerland, October 2000

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Transient protein expression in mammalian cells has been used for more than 20 years in biomedical research in order to rapidly access a gene product after DNA transfection. Generally a small number of cells, not exceeding 10^7 cells, are exposed to a DNA transfer vehicle, and, upon successful transfection, these cells begin to synthesize the product of a gene (or several thereof) during a short period of time (days). The rapid introduction of exogenous DNA in as many cells as possible assures that its consequences becomes measurable or detectable instantly. Transient protein expression is based on a short residence time of transferred DNA molecules within the nuclear environment of cells. This is in contrast to the main mode of use of animal cells in the pharmaceutical biotech industry. Here, a gene of interest is integrated into the chromosome of a cell and creates a stable cell clone – for the eventual industrial scale-up for manufacturing purposes and theoretically indefinite usefulness of such cells.

Maybe, the interest in the recently held workshop in the picturesque monastery in the Swiss pre-alps emphasizes the emergence of a new chapter in animal cell technology. The all-Swiss organizers of the workshop were surprised themselves that the (ex) Carthusian monastery in Ittingen near Winterthur hosted for two and half days more than 60 scientists and engineers, mainly industry based, from a representation of the major European countries and even a good number of scientists from the USA and from Canada participated. The workshop addressed just one theme: *Transient gene expression from mammalian cells*, not only from small-scale cell culture, but also from cultures that maintained 10^9 to 10^{11} cells. We hesitate to use the term ‘large scale’ here. The standard for large scale with mammalian cells are the manufacturing plants that can cultivate 1000 liter or more of cells in a single

reactor ($>10^{12}$ cells). However, considering the scope of this workshop, it becomes evident that first steps of operations of transient gene expression at least for pilot scale operations are being aggressively explored.

The organizers, Dr. Horst Blasey and his team (Dr. Martin Jordan, Dr. Sabine Geisse, Dr. Ernst-Jürgen Schläeger, Dr. Martin Fusseneger and Dr. Kenneth Lundström) were able to put together a high-level program that was covering the various challenges of the field. The workshop comprised balanced and representative presentations on the techniques and systems, used for transiently expressing genes (DNA, RNA and viral vectors), the various purposes this technology is used for and the various scales at which genes are successfully expressed (from petry dish to fermentor).

In the session ‘The cellular level: the basis for successful transient gene expression’, talks by Tony Mulcahy (Cobra Therapeutics, UK), and Martin Jordan (EPFL, Switzerland) addressed some of the molecular and cellular bottlenecks in gene transfer to animal cells. Studies at Cobra have provided regions of DNA, associated with ubiquitously expressed house-keeping genes (UCOE: Ubiquitously-acting Chromatin Opening Elements). Inserts of these 4–8 kb fragments into vectors provided high-level expression, both from transiently and stably transfected mammalian cells. Work of the EPFL group showed that intracellular plasmid copy number after transfection is positively correlated with expression levels, but negatively correlated with survival. This work also showed quite conclusively, for the first time, that calcium-phosphate transfected cells take up to 100 000 plasmid molecules, mostly into endosomal compartments.

The session on ‘Expression: tools and applications’ featured a presentation of Ernst-Jürgen Schläeger, Hoffman-LaRoche, on the Ro1530 gene transfer system that delivers with high efficiency DNA to cells,

both in suspension and in an adherent mode. The advantage of this system is its low toxicity and the possibility to work with low DNA to cell mass ratios. Usually only 0.2 μg DNA/ml of culture medium are required, which is one fifth or so of the DNA quantity required by other systems. An interesting new cell host for transient expression was presented by Sam Cho of Bayer, USA. The cell line, HKB, combines, as a fusion product, the advantageous feature of high transfectability of HEK 293 cells with the single cell suspension growth of Burkitt lymphoma cells. A presentation by Randal Goffe, founder of Genespan Corporation, USA, dealt with an unfortunately undisclosed reagent Vectorstat[®], capable of prolonging the expression of protein from transfected DNA through enhancement and stabilization. In the 3rd and 4th session on technologies and scale-up, a very stimulating talk, highlighted by a large number of colorful microscopic images from transfected COS-7 cells, was given by Ingrid Caras of Eos Biotech, USA. The selective labeling of tag-sequence modified recombinant proteins from libraries allowed, from the staining intensity of the endoplasmic reticulum and the Golgi apparatus, to judge whether a novel protein faces processing problems within the cell. Also, this approach allows to categorize, in a rather efficient way, the subcellular localization (membrane, nuclear, cytoplasmic or secretory) of an unknown protein entity. A very stimulating talk was given by Tina Etcheverry from Genentech, demonstrating routine use of transient gene expression for the production of 10s to 100s of milligrams of research proteins at the 5 to 10 liter scale. The Genentech group uses a 'self-made' lipid-based DNA carrier and CHO cells as hosts. They also reported the use of transiently produce protein, in the context of preclinical studies, in toxicity studies and efficacy studies in chimps. Interesting to note: in a very general assessment of screening of random DNA sequences, is the observation that, from more

than 1000 proteins expressed so far, about one third appeared to be poorly expressed, for reasons to be studied eventually and individually.

The most exciting presentation was probably given by the invited keynote lecturer Jean-Paul Behr, Université de Strasbourg – 'Towards artificial viruses'. Behr, the inventor of the very first lipid and polymer based transfection vehicles, some of them marketed, extended his work towards smaller and more virus like particles. Most interesting, condensation of a polymerizable cation with a single plasmid molecule, allowed to generate complexes of 20 to 30 nm. These, together with nuclear localization signals, may eventually behave very similar to viruses, and thus may improve by a factor 100 to 1000 the efficiency of artificial DNA vehicles in order to approach the nucleic acid delivery potency of viruses.

In another session, 'Technologies and Methods', Georg Schmid described the way DNA is extracted and purified for transient expression applications. This subject clearly requires more attention to understand exactly which level of DNA purity is required. Some poster results coming from the EPFL lab (J. Wright et al.) indicate that 'crude' preparations made in controlled conditions are actually equivalent to and sometimes even more efficient than pure ones at transfecting some mammalian cells.

Overall, the First European Workshop on Transient Gene Expression provided a very stimulating atmosphere for the exchange of ideas and concepts, thorough session discussions and personal interchange, since enough time in-between the talks was held open for that purpose. It is to be hoped that there will be a second workshop again soon, because it was felt that the attendees represented only the 'tip of an iceberg' of interest. It demonstrates that this highly relevant and fast moving field does address the tremendous need of the biotechnology community for rapid protein expression these days.