IMAPPROT

Integrated, multi-host approach for the improved microbial production of high quality therapeutic enzymes and proteins

Partners involved in the IMAPPROT project

Project coordinator

Prof. A. Villaverde
Universitat Autonoma de Barcelona - Spain

Project leaders

Dr. M. Bollok
Bioingenium - Spain

Dr. S. Schwartz
Vall d'Hebron University Hospital - Spain

Dr. S. Markku
VTT Technical Research Centre - Finland

Ms L. Tutino
University of Naples Frederico II - Italy

Prof. D. Mattanovich
University of Natural Resources and Applied Life Sciences - Austria

Contact information

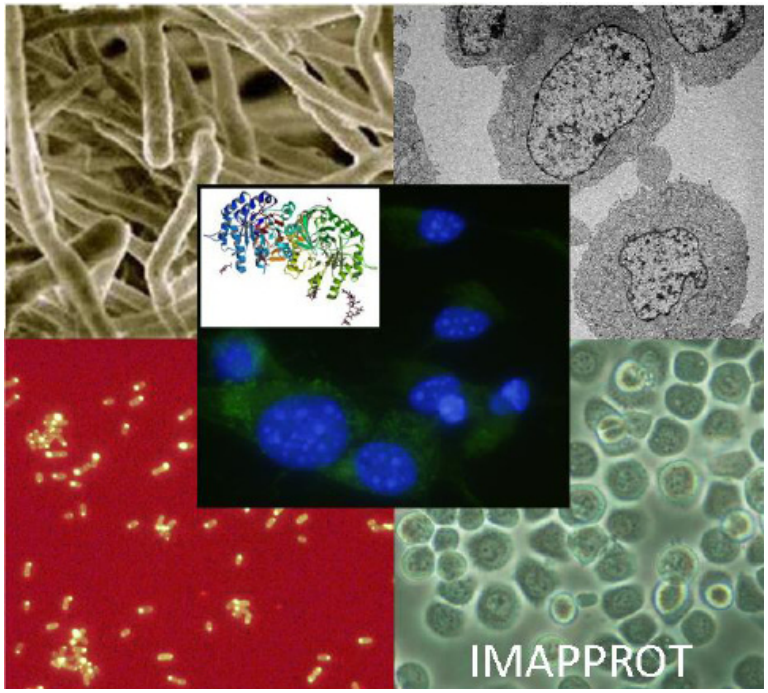
A. Villaverde.
Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona
Spain
antoni.villaverde@uab.cat

An important sector in the biopharma market is based on the industrial-scale production of therapeutic proteins for administration to humans as drugs. The production of this type of biopharmaceuticals imposes a major load on the national health systems (or, alternatively, on patients), since the production processes, which are increasingly based on mammalian cells as convenient factories, are highly expensive.

Furthermore, the stability of protein drugs in the receiving organism is generally low, so that most of the product does not reach the appropriate organ or cells as a consequence of poor targeting, thereby resulting in low therapeutic effect. This fact tends to encourage the increase of the amount of protein in the administered doses, which again impacts on production costs and raises the risk of unwanted side effects.

The IMAPPROT ERA NET-IB project has been designed to explore two overlapping strategies as general platforms for a more efficient production of protein drugs. The first of these strategies involves the use of alternative and/or emerging microbial systems for the production of therapeutic proteins.

The second strategy aims to confer cell-targeting properties on the recombinant drug by convenient rational or semi-rational protein engineering. With these aims in mind, we have focused on the human enzyme alpha-galactosidase A, which is used for the treatment of Fabry disease, a rare lysosomal storage illness caused by a deficiency in the production of this enzyme. The lack of alpha-galactosidase A results in the accumulation of globoside in the lysosomes of many tissues (including kidney, heart and blood vessels) and is an important factor in the reduction of life expectancy.



The current protein replacement therapy uses two versions of the recombinant enzyme, administered through intravenous injection.

The enzymes in question are Replagal® (from Transkaryotic Therapies), approved in Europe in 2002, and Fabrazyme® A (from Genzyme) also licensed in 2002 for Europe and the USA. Both products are purified from cell culture media, and the cost of the annual treatment of a 70-kg person is estimated at approximately € 150,000 for Fabrazyme and £170,000 for Replagal.

The IMAPPROT project has put together a European consortium, consisting of recognized experts in the fields of protein production and engineering in bacteria, yeast and fungi, as well as engineering in mammalian and insect cells (as convenient references). In particular, we have explored the production of native and modified forms of human alpha-galactosidase A in Escherichia coli K-12, Pseudomonas haloplanktis TAC125, Trichoderma reesei, Pichia pastoris, HEK 293F and CHO DG44 mammalian cell lines and also in different insect cell lines. More than 25 engineered versions of human alpha-galactosidase A have been produced in these systems, and they have been characterized with regard to productivity, stability, enzymatic activity, cytotoxicity and therapeutic potential in vitro. Convenient protocols for clone selection, protein production and purification have been implemented in respect of the different biological systems in which they have been produced.

For the in vitro analysis, different cell models, in which the expression of the human alpha-galactosidase A had been silenced, were explored in detail and optimized. Finally, endothelial cells defective in alpha-galactosidase A production and derived from aorthic rings of Fabry mice (KO animals) were used as a standard system for in vitro studies of the therapeutic potential. Among the different versions of the enzyme produced in the different hosts, at least four have already passed stability, toxicity and activity tests; they also perform efficiently in the in vitro cell culture models and are now being tested in Fabry mice models in pre-clinical studies. The in vivo Fabry model has been largely characterized in this project and adapted for the fine determination of pharmacokinetics, toxicity and for the evaluation of the therapeutic effects of the protein drugs generated within it.

The IMAPPROT project has been coordinated from Spain by A. Villaverde (Universitat Autònoma de Barcelona), and the consortium is composed of other leading world experts in the field of protein production; namely, D. Resina from the Bioingenium, Luisa Tutino company from the University of Naples, Markku Saloheimo from VTT Technical Research Centre, Finland, and Diethard Mattanovich from the University of Natural Resources and Applied Life Sciences, Vienna. The IMAPPROT team is completed by S. Schwartz from the Molecular Biology and Biochemistry Research Centre for Nanomedicine at Vall d'Hebron University Hospital, Barcelona, who is responsible for the enzyme testing at both in vitro and in vivo levels.