A simple and rapid strategy for cloning and expression of „toxic“ human ABC-transporter genes in yeast

Philipp Ellinger, Jan Stindt, Sander H.J. Smits and Lutz Schmitt

Institute of Biochemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

Roughly sixty percent of human drugs are targeting against membrane proteins. The major bottleneck in eukaryotic membrane protein research lies in their overexpression to obtain sufficient amounts for biochemical and biophysical experiments. For expression, the genes of interest first have to be cloned into a suitable expression system like for example yeast like *Pichia* or bacterial systems.

The initial cloning is sometimes hampered due to a toxicity of the encoding DNA against *E. coli*. Thereby, the standard cloning procedures for heterologous overexpression of these mammalian membrane proteins cannot be used. So the first step in investigating these transporters already encounters a major obstacle. This holds true for example for two human ATP binding cassette-transporter (ABC-transporter) genes, BSEP (Bile Salt Export Pump) and MDR3 (Multidrug Resistance Protein 3) both expressed and localized in the liver. In both proteins mutations are associated with severe hereditary diseases of the liver. Due to their strong toxic effect on *E. coli* either on DNA or protein level, biochemical studies on these transporters have been very limited. We developed a simple and rapid cloning strategy to create and manipulate any construct of interest which exhibit these „toxic“ effects via homologous recombination in *Saccharomyces cerevisiae*, exemplified for both ABC transporters, BSEP and MDR3. As outcome of our approach, we report the first-time heterologous expression of two human ABC-transporters BSEP and MDR3 in *S. cerevisiae* as well as *Pichia pastoris*. Our cloning method allows cloning of toxic gene *in S. cerevisiae* without the need of *E. coli* as shuttle organism. Furthermore, we show that this method can even be used for rapid site directed mutatgenesis with the same efficiency as the normally used *E. coli* protocols.