At present, there is an urgent need to speed-up the process of development of new drugs to fight old, re-emerging, and newly emerging diseases. In order to develop new drugs, pharmaceutical companies are aspiring to use faster and more effective methods of their development. In order to sustain the annual sales growth of 4–8%, companies have to increase the annual drug output, on average, from a current level of 1–1.5 drugs per year to a projected 3–5 drugs per year. Therefore, drug development programs involving screening of hundreds of thousands of potential compounds as candidates for drugs are expanding. Approaches relying on the detailed knowledge of the structure of the protein drug target play an important role. High-resolution determination of three-dimensional structure of drug target would serve as a basis for in silico modelling, virtual screening, and further development of pharmacologically suitable compounds with a predetermined or a new physiological activity.

In the case of membrane protein drug targets, crystals of which are difficult to obtain, solid-state nuclear magnetic resonance is in principle the only possible method of investigation. Structural—functional analysis using NMR spectroscopy allows obtaining detailed information on the three-dimensional structure of a receptor-bound ligand, its binding site, and receptor–ligand communication, including H-bonding and π-interactions, electrostatic effects, and charge redistribution. For these studies, milligram quantities of highly purified protein drug targets labelled with stable isotopes, such as $^{13}$C and/or $^{15}$N, are required. Such preparations are typically obtained using various microbiological approaches. Within this context, the selection of the expression system that is suitable for eukaryotic membrane protein expression on the milligram scale and at the same time allows achieving cost-effective labelling with stable isotopes plays an important role in labelling protein drug targets with stable isotopes.

The main bottleneck is that the use of heterologous expression systems does not lead to the desired results with the majority of drug targets belonging to the class of membrane proteins. Expression systems that ensure both production of functional proteins on the milligram scale and affordable stable-isotope labelling are based on bacteria (e.g., Escherichia coli) and yeast. Culture media with a relatively simple composition containing $^{15}$N-ammonium salts and $^{13}$C-labeled glucose, methanol, or acetate as a nitrogen and carbon source, respectively, are used for growing these host cells. However, these systems are usually not suitable for expression of eukaryotic membrane proteins. A cell-free expression system of soluble proteins is developing quite successfully, its use for production of eukaryotic membrane proteins is limited [1]. Expression of a G-protein-coupled receptor in a photosynthetic bacterium was recently described [2]; however, the functional level of expression in this case remains low as well.

Mammalian and insect cell lines are the most frequently used expression systems for obtaining eukaryotic recombinant proteins [3–5]; however, they require culture media with a fairly complex composition [6]. Direct replacement of amino acids and other metabolic precursors in commercially available media of known composition with corresponding stable isotope-labeled analogues is the most obvious approach, but in this case the price of cell culture medium ranges from 20000 to 100000 Eur per liter. This creates a
logical sources have been screened for their suitability as components of cell culture medium for stable-isotope labelling. This study was performed with the fall army worm *Spodoptera frugiperda* cell line (Sf9) from ATCC (CRL-1711), which was gradually adapted from the serum-free Insect-XPRESS culture medium (Lonza, Switzerland) to growth conditions in culture with 95% IPL-41 and 5% XPRESS in T25 culture flask (25 cm², Greiner Bio-One). The same conditions were used to maintain a continuous culture. Step-wise adaptation was performed first in an adhesion culture and then in spinner flasks as described in [5]. In order to develop effective culture media, a reduction in amino acid concentrations in IPL-41 with steps of 10% down to 10% of the original concentration was tested. During the adaptation process, samples were taken to measure the cell density and perform amino acid analysis. When the concentration of amino acids in the medium was less that 10–20% of their original content in IPL-41, cells continued growing but the recombinant protein production markedly decreased. The latter could be partly restored by adding 0.8% of yeast autolysates.

In order to simplify the formulation of the novel medium, we assessed the possibility to replace sucrose and maltose, which are present in the IPL-41 medium, with glucose and the role of vitamins and organic acids. The results showed that addition of vitamins to a concentration more than four times higher than the original concentration had no effect on the growth of Sf9 cell line and that sucrose and maltose could be completely replaced with 7.74 mM glucose without having significant effect on the osmolarity of the medium. Finally, it was shown that all organic acids of the IPL-41 medium could be omitted if at least 0.4% yeast autolysate is present.

As a proof-of-principle experiment for the human His-tagged H1R (Ht-H1R) production, the cell culture medium PLI-In1 was used, which contained 0.4% ¹⁵N-labeled yeast extract from the *Hansenula polymorpha*, 0.1% autolysate from the algae *Cyanidium caldarium*, and ¹⁵NH₄Cl as a ¹⁵N source as well as free amino acids in a concentration range of 10–20% of their original content in IPL-41. This culture medium was supplemented with the lipid extract of *C. caldarium* at a concentration of 5 ml per 1 l of medium; the extract was prepared by dissolving 50 mg of lipid fraction in 1 ml of ethanol as described in [10]. The pH of the medium was adjusted to 6.2 and the osmolarity was maintained at 340 mOsmol kg⁻¹ with NaCl. The culture medium was sterilized by passing through a 0.22-μm filter and stored at 4°C. The comparison of various cell culture media for the Sf9 insect cell line with one of the media developed by us is shown on Fig. 1. When testing various components for their use in culture media for growth of Sf9 insect cell line, a high-throughput screening protocol was developed; the WST-1 reagent (Roche Diagnostics, Switzerland) was used to measure cell densities.

**Fig. 1.** Effect of various cell culture media on growth of Sf9 insect cells: (1) Insect-XPRESS, (2) IPL-41, (3) IPL-41 without yeastolate, (4) PLI-In1, and (5) BioExpress 2000 (CIL).

Recently we published data on successful replacement of amino acids with yeast extracts in culture medium for insect and mammalian cell lines: Chinese hamster ovary cell (CHO) and human embryonic kidney (HEK 293) that allowed at least a five-fold cost reduction of culture medium [9]. This opens new possibilities for stable isotope labelling in mammalian cells. For stable isotope labelling of insect cells, the IPL-41 medium with a known composition [11] was selected as a basic nutrient medium. In the media for ¹⁵N, ¹³C or ¹⁵C, ¹³C labelling developed by us, the concentration of microelements, salts, and vitamins corresponded to that in the IPL-41 medium; in case of ¹³C labelling, NaH¹³CO₃ was used instead of the natural analogue. Over 500 extracts of various microbiological sources have been screened for their suitability as components of cell culture medium for stable-isotope labelling.