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Poster Presentation

Production of cysteine-rich proteins in *E. coli* – the challenge of Wnts

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Background

The Wnt family consists of secreted extracellular glycoproteins that induce an intracellular signaling pathway involved in many events during embryogenesis and adult tissue maintenance. As they are involved in a large variety of cellular processes, errors in the Wnt-signaling pathways can lead for example to degenerative diseases and cancer and in embryos to developmental defects. So far there are 19 genes encoding for Wnt-proteins found in both mouse and human. Wnt-proteins are highly conserved, both with each other as well as between species. They all consist of about 350–400 amino acids, have multiple cysteines, are hydrophobic (possibly due to a conserved palmitoylation) and have a high pI.

Recombinant Wnt proteins are needed as important tools for example in basic research concerning developmental biology and Wnt function as well as for medical applications (e.g. therapeutics, stem cells). However, production of recombinant Wnt proteins in *E. coli* is very challenging, because their hydrophobicity and several disulfide bonds make their proper folding extremely difficult. In this study, strategies for the expression of Wnt proteins in *E. coli* are investigated.

Results

A process for production of active murine Wnt1 in *E. coli* has recently been developed in our laboratory [1]. This was the first time a Wnt protein has been produced in an active form using a prokaryotic expression system. Here Wnt1 was targeted to the periplasmic space which has more oxidative conditions for proper folding than the bacterial cytoplasm. Currently, processes for the expression of other Wnt proteins are developed.

Two different expression strategies are being followed. First, since the folding in the bacterial cytosol for disulfide bond-containing proteins is very difficult, one option is to let the recombinant proteins aggregate as inclusion bodies and refold them later *in vitro*. Advantages of using inclusion bodies for expression include for example larger yield of recombinant protein and fewer purification steps.

The second strategy utilizes cytoplasmic expression and thioredoxin reductase deficient host strains. In these host strains the cytoplasm is more oxidative than in wild type *E. coli* facilitating disulfide bond formation and folding.

For both strategies some encouraging preliminary results have been obtained. The expression conditions are currently optimized followed by protein purification (and refolding) as well as activity tests.

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References

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