Summary

The goal of this work, which was designed to be applied in the field of biotechnology, was to improve the properties and the construction of templates with regard to the efficiency in an Escherichia coli *in vitro* translation system. Limitations of the expression efficiency on the level of the secondary structure, the translational initiation, the codon usage and the stability of a mRNA were planed to be exhibited and reduced. Finally a time saving method based on the polymerase chain reaction for the generation of templates for the protein synthesis was planed to be developed.

It was shown, that the expression efficiency of a protein at the crossing point from the translational initiation to the elongation strongly depends on the second amino acid codon. In this connection, some evidence was found to support the suggestion that the synthesis of the fatty acid binding protein (H-FABP) depends on the strength of a secondary structure of the mRNA within the translational initiation region. Effects based on the kind of the incorporated amino acid and the corresponding tRNA could be ignored as a consideration.

By means of the expression of heterogeneous fusion proteins consisting of H-FABP and dehydrofolate reductase (DHFR) as well as multimeres of these proteins it was shown that in contrast to cell culture systems the *in vitro* translational initiation of optimized mRNAs is unlikely to be the rate limiting step of the protein synthesis. In comparison, the expression of DHFR is strongly limited by the rare $tRNA^{ArgU}$. The synthesis of the protein could by clearly improved by supplementation of the system with *in vitro* transcribed tRNA. A similar effect was observed in the expression of the NusA protein, which has a high content of lysyl codons. The concentration of the corresponding aminoacyl-tRNA could be identified as a limiting factor.

The stability of the mRNA coding for H-FABP increases with increasing expression of the corresponding protein. The stability is increased in the same way by blocking the ribosomes by means of the addition of chloramphenicol. In comparison, stripping the ribosomes of the mRNA leads to reduced stability. These results show, that just the covering of the translated region of the mRNA has a protective effect. The stability of the mRNA also increases with the strength and an increasing length of the 3'-untranslated region. A previously published translation-independent effect of chloramphenicol could be confirmed. However, some of the suggested mechanisms in this connection could be ignored as a consideration.

Finally, a method for the fast amplification of small amounts of gen sequences out of complex DNA mixtures including a simultaneous introduction of procaryotic regulatory elements for the efficient cell-free protein synthesis was developed and optimized. Illustrated by the example of the expression-polymerase chain reaction for H-FABP it was shown, that a PCR product produced in this way can be as eficiently expressed as a an optimized plasmid template. The method described here represents a time saving for the expression of newly found genes. Moreover the method has the advantage, that there is no need for working with genetic engineered organisms.