7. Summary

The introduction of unnatural amino acids into proteins can be achieved by the use of an *amber* suppressor tRNA, that has been chemically acylated with the desired amino acid and that is not a substrate for the natural aminoacyl tRNA synthetases. The addition of the charged *amber* suppressor tRNA to the protein biosynthesis reaction results in site specific incorporation of the amino acid into the protein. However very often the usually applied methodology does not lead to sufficient amounts of mutant protein. The goal of this work was to expand our knowledge of *in vitro amber* suppression expecting that the strategy mentioned above can be applied more general.

Within the presented work the site specific incorporation of an unnatural amino acid could be demonstrated by the introduction of ε-dansyl lysine into FABP (fatty acid binding protein). As expected the incorporation of the unnatural amino acid was very inefficient. A further limitation of the methodology proved to be the preparation of large amounts of shortened tRNA molecules, which are necessary to produce the chemically acylated tRNA. The high level of 3'-end heterogeneity of the *in vitro* transcription products strongly impedes the purification of homogenous tRNA. The homogeneity and the amount of the tRNAs during T7-transcription could clearly be improved by increasing the reaction temperature from 37°C to an optimum temperature of 44°C. Additionally, various preparations of T7-RNA-Polymerase showed different n+1-activities.

To investigate the activities of *amber* suppressor tRNAs expressed *in vivo*, total tRNA from 10 different *Escherichia coli amber* suppressor strains (Kleina et al. 1990) was prepared and employed in cell-free translation. The relations between the suppression activities of the *amber* suppressors for leucine, histidine, tyrosine and serine *in vitro* corresponded to the *in vivo* results of other authors. In contrast to those four the suppression activities of other *amber* suppressors were decreased *in vitro* indicating that the activities of the corresponding amino acyl tRNA synthetases may be reduced.

Processing, repair and aminoacylation of *in vitro* transcribed tRNAs and the relation of these processes to each other were investigated in-depth. Incubation of 3'-shortened or 3'-prolonged heterogenous *in vitro* transcription products in the S100 enzyme fraction of the total translation system resulted in homogenous tRNA populations with correct 3'-terminal CCA-ends. Processing of prolonged tRNAs and repair of shortened tRNAs in the whole translation system were shown not to be limiting for protein biosynthesis *in vitro*.

The suppression activities of the transcripts of seven different *amber* suppressor tRNA species ($tRNA^{Ser}_{CUA}$ {su^{+}_{1}}, $tRNA^{Tyr}_{CUA}$ {su^{+}_{3}}, $tRNA^{Leu}_{CUA}$ {su^{+}_{6}}, $tRNA^{Leu5}_{CUA}$, $tRNA^{Phe}_{CUA}$, $tRNA^{His}_{CUA}$ and $tRNA^{Ala1}_{CUA}$) were shown not to be limited by aminoacylation. Therefore the suppression activities of these tRNAs reflects structural properties of their aminoacylated

counterparts. Suppression efficiency was defined as the frequency of ribosomal tRNA selection divided by the frequency of RF1 selection. The anticodon loops of all tRNAs contained the sequence $^{5'}C_{34}U_{35}A_{36}A_{37}A_{38}{}^{3'}$ which is ideal for efficient suppression as known from other studies. Still, in the presence of this sequence suppression efficiencies varied over a large range. The rate of tRNA selection was 20 times higher for the strongest suppressor, $tRNA^{Ser}_{CUA}$, compared to the efficiency of the weakest one, $tRNA^{Ala1}_{CUA}$, which represents the suppression efficiency of the actual tRNAs used for chemical aminoacylation. In general the more the sequence of the *amber* suppressor tRNA reflected the sequence of the original wild type tRNA, from which it was deviated, the better the suppression efficiencies became. With increasing number of nucleotide exchanges, that were necessary to get the sequence $^{5'}C_{34}U_{35}A_{36}A_{37}A_{38}{}^{3'}$ into the anticodon loop of the *amber* suppressor, suppression efficiencies decreased, indicating that tRNA sequences have been evolved to support optimal interaction between codon and anticodon, as it is postulated in the "Extended Anticodon" (Yarus 1982). The two best *amber* suppressors by far, $tRNA^{Ser}_{CUA}$ and $tRNA^{Tyr}_{CUA}$, both contain the base C_{32} inside their anticodon loop. There is also some evidence, that certain structural features important for suppression are localized outside the anticodon arm and that these structural features are found mainly in typeII-tRNAs.

The most important conclusion resulting from this work within the field of biotechnology is, that the actual *amber* suppressor tRNAs used for chemical aminoacylation are comparably weak suppressors. A logical step from this work is the construction of new *amber* suppressor tRNAs with higly improved suppression efficiences for chemical aminoacylation. Therefore the present work should allow a much improved incorporation of unnatural amino acids into proteins in the future.