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Oral Presentation

Antisense RNA based control of detrimental factors for recombinant gene expression in *Escherichia coli* – down-regulation of RNase E

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Background

Messenger RNA decay is an important mechanism for controlling gene expression in all organisms. The rate of the mRNA degradation directly affects the steady state concentration of mRNAs and therefore influences the protein synthesis. It is assumed that RNase E is the initial point for general mRNA decay in *E. coli*. RNase E is starting the mRNA decay by performing the initial cut. The resulting fragments are then further degraded by endo- and exonucleolytic activity in the cell. During recombinant protein production mRNA instability may be the major bottleneck for a successful product formation. While RNase E initiates the degradation of most mRNAs in *E coli*, it is likely that the enzyme is also responsible for the degradation of recombinant RNAs. Therefore a system which allows the controlled reduction of RNase E might lead to a higher product formation in recombinant production processes. As RNase E is essential for cell viability and knockout mutants can be not cultured, we investigated the possibility for down-regulation of the level of RNase E by antisense RNAs.

Results

During this study the antisense RNA based approach could be established and indicated that the reduction of the intracellular level of RNase E in *E. coli* is possible. The expression of antisense RNAs showed no influence on the cell growth. In a fluorescence based sandwich hybridisation assay the amount of antisense RNAs was sequence specifically quantified. We could prove that the induction of antisense RNAs was followed by a 25-fold increase of the detectable antisense RNA molecules per cell. The antisense RNA level was maintained above 400 molecules per cell. When the cells passed into the stationary phase the level of expressed antisense RNAs decreased markedly. Western blot experiments revealed the strongest reduction in the RNase E protein level 90 min after the induction of antisense RNAs. The RNase E level could be decreased to a maximum of 35 % of the wild type level. When the growth entered the stationary phase the RNase E level was maintained at 50 to 60 % of the wild type level.

Conclusion

In contrast to eukaryotic cells, where the RNAi technology is widely used, this technology is nearly unexplored in bacteria, although different natural systems use antisense RNA based silencing and antisense RNA mechanisms have bee used to control plasmid stability. Only recently few studies have indicated that antisense based strategies may be a way to regulate the biosynthesis of global regulatory proteins and metabolites and may be beneficial for the expression of recombinant target proteins as well [1-4]. Our initial results clearly indicate the possibility to control the expression of RNase E, a key enzyme for mRNA degradation, by antisense RNAs in *E. coli*. This was challenging and surprising due to the feed-back control

mechanism which keeps the RNase E concentration in the cell constant independent on the growth stage. Investigations to study the effect of the down-regulation of RNase E on the stability of heterologous genes are ongoing.

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