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Poster Presentation

Increasing the quality of recombinant products – Higher attraction of ribosomes leads to suppression of secondary ribosome binding sites

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Background

In translation initiation the 3' end of the 16s rRNA binds to the complementary Shine Dalgarno (SD) sequence. Together with bound initiation factors translation can subsequently begin at the AUG start codon. However, there may be SD related sequences throughout the coding region of the mRNA. These secondary SD sequences can recruit ribosomes as well, in particular if they are embedded in purine rich regions [1]. The existence of such secondary ribosome binding sites can greatly reduce the expression efficiency since ribosomes recruited to the secondary SD site hinder elongating ribosomes in their progression. If a start codon is nearby a secondary SD site even truncated protein could build up in expense of full length protein [2].

Results

In our attempts to increase the production of recombinant Wnt4 protein in *E. coli* BL21(DE3) we optimised the 5' coding sequence by secondary structure modelling with silent mutations to promote the single stranded nature of the translation initiation region of the mRNA. Interestingly, a major result of this optimisation, which was performed to provide higher ribosome loading to the wnt mRNA, was the disappearance of a shorter variant of Wnt4, which was formed due a second internal ribosome binding site (nucleotides 90 to 97).

Conclusion

As no other properties of the expression system and conditions were changed we argue that a higher ribosome loading from the regular SD site raises the ribosome coverage of the mRNA such that the secondary ribosome binding site is obscured by translating ribosomes. As a result the production of truncated protein is reduced.

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