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DISSERTATION SUMMARY

Adaptation of synthetic oligonucleotide-based inhibition in plant systems

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Antisense oligonucleotides have gained an ever-increasing significance during the last few years. Beyond a therapeutic application, antisense oligonucleotides have proven to be a very useful research tool in the molecular level analysis of life processes, as it is possible to selectively decrease the production of the targeted protein via sequence-specific inhibition.

The aim of this research was to set up an experimental system that enables us to trace the slightest change in the activity of the reporter gene – the luciferase of the firefly, *Photinus pyralis*. First of all, the cellular system and the factors influencing gene expression were optimized. The oligonucleotides exhibited the strongest effect after overnight incubation following transfection.

Our strategy for selecting an oligonucleotide was to choose some functional element of gene expression, such as an ATG-region. The next level of selection was searching for a single stranded region of the mRNA that is accessible for the oligonucleotides. This was optimized with the use of computer modelling.

The following steps were used in designing the oligonucleotides:

First of all, suitable genes were selected (EMBL GenBank database). The applied marker genes were firefly luciferase and GUS. Afterwards, the sequences were sent to a server (mfold 2.3), which calculates the three-dimensional structures of the mRNA. Then matches were located among the open structures, and sections of 18-21 base pairs, suitable for antisense oligonucleotide application were selected. To ensure specificity of the oligonucleotides, homologue sequences were excluded (BLAST 2.0). Next, the T_m points were optimized to physiological environment. Loop and self-complementary sequences were discarded. To increase stability, we used chemically modified oligonucleotides. However, as thioesther bonds are toxic beyond a certain threshold, it was necessary to optimize the number of such bonds within the molecules. In the application step, the uptake of the molecules was first checked, and then measurements were made to investigate the inhibitory effect on the marker genes.

We have found that antisense oligonucleotide-based inhibition is applicable to plants; however, the efficiency of inhibition is somewhat lower as compared to other systems.

Since antisense oligonucleotides exert their inhibitory effect specifically even on the level of the organism, therefore we have attempted to apply the technique to intact plants. Our results show that the inhibition is inducible, but again with less efficiency.

Our other approach gene silencing was through the use of small inhibitory RNAs (siRNAs), which were synthesized artificially with the Expedite system (Applied Biosystem).

RNA interference was discovered a few years ago, and as of yet it has never been applied in plant systems. si RNAs have sense and antisense strands of about 21 nucleotides that form 19 base pairs to leave overhangs of two nucleotides at each 3' end. A double stranded RNAs matching a gene sequence is synthesized in vitro and introduced into a cell. si RNA are thought to provide the sequence information that allows a trigger-specific mRNA to be targeted for degradation.

The non specific pathway is triggered by dsRNA of any sequence as long as it is at least 30 base pairs long. Currently the adaptation of the protocols to plant systems is being tested, simultaneously on protoplasts, cell suspensions and intact organisms.

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