
Preface

It seems fitting that this book on therapeutic oligonucleotides should begin by mentioning the name of Paul Zamecnik. A great gentleman of science, Paul died in 2009, thirty-one years after reporting that an oligonucleotide complementary to the end of Rous sarcoma virus could interfere with its replication (1, 2). Despite his suggestion that oligonucleotides might be used to treat various diseases, the publication aroused little interest at the time. Even for those who shared his vision, it was prohibitively difficult or expensive for most workers to acquire sufficient amounts of oligonucleotides in the 1970s. In any case, few RNA sequences were known that could be used as drug targets.

During the 1980s and 1990s, improvements in synthesis and sequencing of nucleic acids led to an increased use of “antisense” oligonucleotides complementary to “sense” mRNAs to prevent their translation into proteins. This helped to assign functions to the growing number of identified genes. There were also a handful of people who, like Paul Zamecnik, believed that antisense oligonucleotides offered a general approach for treating disease by modulating the levels of relevant proteins.

These early aspirations did not anticipate the problems that emerged during attempted implementation. Antisense inhibition of translation is not as straightforward as was hoped and has still not produced any important drugs. Yet, 30 years on, there is still intense interest in therapeutic oligonucleotides that justifies the present volume. Why is that? I would like to propose two reasons.

The first is that the nucleic acids and RNA, in particular, continue to surprise us. New discoveries have refreshed enthusiasm for oligonucleotide therapeutics by suggesting new applications. Most recently, small RNAs such as siRNA and miRNA have become the focus of interest.

The second reason is the unique predictability of base pairing that offers an alluring shortcut to drug design. There is ample proof of principle for the therapeutic potential of oligonucleotides but this has not yet been reduced to practice in a generally applicable manner. However, it is still reasonable to suggest that one success could lead to many others using the same approach.

Why has this not happened already? Early hopes were naïve due to ignorance of the chemical and biological complexity of nucleic acids. Only the base pairing properties of oligonucleotides were considered and not the fact that they are complex molecules in their own right. The icon of the double helix illustrates base pairing beautifully but it ignores the effects of sequence on structure. It was tempting to assume that all antisense oligonucleotides would behave similarly and would differ only in hybridizing to different sequences of RNA. However, a single-stranded nucleic acid is densely packed with functional groups that can participate in secondary and tertiary structure formation. For example, the nucleoside guanosine alone contains 15 lone pairs of electrons and six hydrogen atoms able to form hydrogen bonds. Its heterocyclic base can stack with other bases and is also capable of tautomerization. In addition, each nucleotide has an ionized phosphate

group that can recruit metal ions of varying charge. Even small oligonucleotides have abundant capacity to form higher order structures.

This book illustrates many different ways that oligonucleotides might be applied as therapeutics. It contains a selection of established and emerging methods that have been chosen for their potential to change the field. Often, the same problems are encountered in different approaches; cellular uptake is probably the single most important problem that must be addressed in all of them. So, for example, a method for getting siRNA into cells of one type might be used with ribozymes or aptamers in other types of cells. All types of oligonucleotide must survive the same onslaught of nucleases long enough to be effective; modifications that stabilize antisense oligonucleotides might be used with aptamers or any of the other classes of therapeutics.

Similarly, several protocols describe methods for optimizing or improving cell uptake that might be tried with other classes of oligonucleotide. They include photochemical internalization, modified cell penetrating peptides, antibody conjugates, and nanoparticles. Other contributions address quantitation of RNA therapeutics in cells, assaying gene knockdown, selecting the best target site, and synthesis of various modified oligonucleotides. The chapter on agRNA contains much useful information on control experiments and off-target effects, selection of RNA targets, validation of experiments, preparation of cells for successful transfection, and the effects of stress due to transfection. This is valuable reading for anybody using oligonucleotides.

The hope remains that approaches for uptake and delivery of one oligonucleotide might work with others, that developing one successful drug might lead to others. It is possible that we already know all the methods needed to solve the problem of oligonucleotide delivery. It could be that we just need to find the right combination of some of the methods in the literature – such as those reported here. Maybe a 3'-S-phosphorothioate oligonucleotide combined with an antibody or cell penetrating polypeptide or light activated uptake would be ideal. Nobody has yet tried such combinations. Perhaps drawing together some of the most promising new approaches in a book such as the one you are reading will help someone to find a winning combination.

John Goodchild

References

1. Zamecnik, P. C., and Stephenson, M. L. (1978) Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proceedings of the National Academy of Sciences USA* **75**, 280–284.
2. Stephenson, M. L., and Zamecnik, P. C. (1978) Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxynucleotide. *Proceedings of the National Academy of Sciences USA* **75**, 285–288.



<http://www.springer.com/978-1-61779-187-1>

Therapeutic Oligonucleotides

Methods and Protocols

Goodchild, J. (Ed.)

2011, XI, 340 p. 70 illus., Hardcover

ISBN: 978-1-61779-187-1

A product of Humana Press