

# Preface

Two giants of nucleic acid drug (NAD) research, Dr. Paul O.P. Ts'o of the Johns Hopkins University and Dr. Paul Zamecnik of Harvard University, passed away in 2009. Both left big footprints on the path toward the discovery of NAD-based therapeutics. One of the Professor Ts'o's deepest imprints was the publication of his first paper on antisense technology, which described the synthesis of trinucleotides with triphosphate linkages to regulate tRNA function. He was well aware of the potential application of the technology, when predicting that the anionic plasma membrane may prevent the cellular uptake of NADs. He also realized that the removal of negative charges from NADs was essential for molecular design, and may increase the stability of hybrids formed between NADs and target RNAs. Ultimately, these effects were found to be limited, but the concept is highly appreciated. Dr. Ts'o then developed oligonucleoside methylphosphonate (OMP) which consisted of methylphosphonate linkages in place of the phosphodiester linkages in DNA. OMPs are resistant to cellular nucleases. Moreover, these compounds are not negatively charged, which facilitates their cellular uptake. However, stereoisomers of methylphosphonate linkages destabilized the hybrids between OMPs and the target RNAs. Thus, OMPs are disappearing from the front line of NAD research. On the other hand, the methylphosphonate linkage, when introduced at either of both termini of NADs, has found continued use in enhancing the exonuclease resistance of NADs. Dr. Zamecnik used oligodeoxyribonucleoside phosphodiester (ODNs) to specifically suppress the replication of Rous sarcoma virus. His experiments, using inactivated culture media, validated antisense technology and indicated a promising future for NADs. In the early 1980s, Dr. Paul Miller's group at the Johns Hopkins University confirmed that the antisense mechanisms regulate mRNA function. In the mid-1980s, Dr. Matsukura's group at the National Institutes of Health (NIH; USA) made the striking discovery that oligonucleoside phosphorothioate compounds (OPTs) could efficiently inhibit HIV replication. Dr. Fritz Eckstein from the Max Planck Institute in Göttingen, Germany, developed OPTs as innovative tools for studying the mechanisms of enzyme action. These reports attracted enormous attention by researchers working in diverse fields of life science and initiated intensive research aimed to develop

NADs as therapies for HIV/AIDS. Although inhibition of HIV replication did not result solely from antisense effects, the reports certainly impressed researchers. Because of their superior chemical, biochemical, and biological advantages, OPTs have been studied to a greater extent than other NADs and have more frequently found their way into clinical trials. OPTs, like OMPs, are mixtures of stereoisomers that can adversely affect antisense activity. It was later determined that the stereocontrolled synthesis of OPTs might produce more efficacious antisense ODNs. Dr. Wojciech Stec of the Polish Academy of Science developed the first method for stereospecific synthesis of OPTs and reported that their biological characteristic varies depending on which stereoisomers is used. Unfortunately, all clinical trials have utilized mixtures of stereoisomers.

Based on the efforts of these pioneers in NAD research, various NAD concepts have been presented. In this review issue, various types of NADs are introduced, and the recent status of NAD research is summarized by leaders in the field. This issue focuses on the following six aspects of NAD: (1) antisense oligonucleotides, (2) decoy DNA, (3) CpG-oligonucleotides, (4) aptamers, (5) NAD delivery, and (6) therapeutic applications. They have been involved in innovating new, particularly noteworthy synthetic routes for stereocontrolled synthesis of OPTs.

In Chap. 1, the basic concept of antisense and siRNA strategies is summarized by Dr. Yano of Nippon Shinyaku Co. The antisense strategy can be regarded as the striking NAD concept that was logically innovated in the mid-1970s. Originally, oligonucleotides complementary to mRNAs were thought to physically block translational steps in gene expression resulting in the blocking of protein synthesis. In the mid-1980s, it was reported that hydrolysis of the target mRNA by RNaseH was involved in the mechanism of antisense effects. RNaseH activity induced by certain types of NAD can drastically influence the effect of antisense molecules. Therefore, the type of NAD must be carefully chosen depending on the function of the target. For example, controlling alternative splicing by antisense molecules requires NADs that do not induce RNaseH activity. BNA (LAN) and morpholino-oligonucleotides are among the most suitable NADs for this purpose and have been used clinically to treat muscular dystrophy based on the exon-skipping concept.

In the mid-1990s, Fire and Mellow independently made the remarkable discovery that small concentrations of double-stranded RNAs suppress the function of mRNA by a quite small dose in a highly sequence-specific manner. This technique, RNA-interference (RNAi), serves as the basis for gene silencing protocols, and siRNAs are being studied intensively with regard to their potential as effective NADs. However, there are three major limitations. First, dsRNA is easily hydrolyzed in serum condition, and to induce RNAi by the molecules, they must acquire nuclease resistance. Thus, certain chemical modifications on siRNA are required. Second, siRNA (dsRNA) has to be transported into the target cells. Until date, several types of dsRNA analogs have been developed to address these problems, but certain drug stabilization materials and drug delivery systems (DDSs) should be innovated. This aspect is also discussed in Chap. 5. Third, it is quite difficult to chemically synthesize long RNAs for siRNA or miRNA studies. Dr. Yano's group have made great development in this aspect. It is important to note that it took a long

time to develop antisense drugs after the discoveries of Dr. Ts'o and Dr. Zamecnik. The discovery of the ability of siRNAs to specifically target mRNAs was made almost 25 years after RNA silencing was first described, and it may take as long or longer to develop efficacious siRNA-based drugs.

Chapter 2 reviews the decoy strategy. As the details of mechanisms of gene transcription were revealed, specific binding of transcription factors to dsDNA in a sequence-specific manner attracted researchers' attention with regards to the development of NADs for targeting this critical process. Thus, the function of certain transcription factors (TFs) could be regulated by dsDNA by binding to their DNA-recognition site. This is termed the dsDNA decoy mechanism. The advantage of the decoy strategy is that decoy molecules can potentially regulate gene expression at specific stages in gene regulation, and therefore, they can target physiological processes such as cell differentiation and proliferation. Dr. Morishita's group at Osaka University is a leader in pursuing the decoy strategy for drug development. They developed a nonchemically modified decoy DNA, the ribbon type decoy, and chimera decoy. Recently, a special AT-rich sequence binding protein-1 (SATB-1) was found to be a key factor in the regulation of various transcriptional processes. SATB-1 binds to chromatin in a sequence-specific manner, and this binding triggers the looping out of the double-stranded region, which facilitates the recruitment of TFs. This process may be a suitable target of the decoy approach. Decoy activity requires the accumulation of decoy molecules in nuclei; therefore, a suitable DDS is essential for clinical application.

In Chap. 3, immunostimulatory properties of synthetic oligonucleotides are reviewed by Dr. Agrawal of Idera Pharmaceuticals. In the 1960s, PolyI/PolyC was found to be immunostimulatory and was applied as a chemotherapeutic reagent. During studies of antisense strategy, some curious phenomena were observed. Some oligonucleotides containing a specific sequence, CpG (cytosine-phosphate-guanosine), stimulated immune functions of ODN-treated cells. Later, these phenomena were explained by the natural immunity imparted by Toll-like receptors (TLR 7, 8, 9). It has been revealed that single-stranded RNA is the ligand for TLR7 and TLR8, and that bacterial and viral DNA containing a CpG-motif are ligands for TLR9. Based on these findings, oligonucleotide-based antagonists against TLRs were developed. Dr. Agrawal's group now evaluating for candidates in clinical trials.

In Chap. 4, Dr. Nakamura's group at the University of Tokyo reviews the aptamer strategy with emphasis on RNA technology. The concept of the aptamer was first presented in the late 1980s, when it was reported that a DNA fragment could bind to a unique protein in a highly sequence-specific manner. Thus, thrombin activity is effectively inhibited by ODNs selected by the systemic evolution of ligands by exponential enrichment (SELEX) protocol from a pool of ODNs representing a huge library of unique sequences. This finding was remarkable for a number of reasons. The interaction between thrombin and ODN is thought to be the result of specific interactions involving the tertiary structures of both components. The discovery of aptamers was achieved by combinatorial chemistry, and can be compared to the fable "Finding a needle in a haystack."

This time-consuming protocol enabled the development of the novel NAD drug, Macugen. Variations of this concept are now being widely applied.

Dr. Nakamura's group has developed RNA-aptamers focusing on TNA's remarkable conformational plasticity.

Dr. Kataoka of the University of Tokyo reviews recent developments in polymer-based DDS technology in Chap. 5. As stated above, the optimization of NAD delivery systems is a key to a successful development of therapeutics; however, this goal has not been realized since the time Dr. Ts'o focused on the subject in the early 1970s. In this review, the challenges to the long-lasting issue are summarized with respect to polymer science, and the developments of polymer-based NAD carriers by Dr. Kataoka's group are introduced. His group has defined numerous factors required for effective delivery of NADs into cells and for the effective release of NADs into the cytosol.

In Chap. 6, recent developments in NAD clinical trials are reviewed by Dr. Gewirtz' group at the University of Pennsylvania. NADs have been evaluated in clinical trials for 40 years. Recent trials have been cited on the homepage of the NIH. In 2011, more than 300 NAD clinical trials were filed. In this review, recent clinical trials are introduced and reviewed in detail, and it should convince readers that NADs have significant therapeutic potential. To our deepest regret, one of the pioneers in this field, Dr. Alan M. Gewirtz, passed away in November 2010. However, his great achievements in the clinic and laboratory will be appreciated by young researchers who chose to pursue his research passions.

The overview of developments in NAD technology presented here should convince us that NADs could indeed be magic bullets to cure and control many diseases. Although number of trials were forced to withdraw at both preclinical and clinical stages, there must be ways to reach the final goal. I sincerely hope that the readers of this issue, mainly young polymer scientists and those working in other fields, will appreciate the encouraging messages for NAD research and development by our contributing authors.

Kyoto, Japan

Akira Murakami

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Murakami, A. (Ed.)

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