INVITED COMMENTARY

Sense, Antisense, and Common Sense

The central dogma of molecular biology stipulates that DNA molecules are the genetic storehouse that contains the entire repertoire to build the cells and tissue of an organism. In higher eukaryotes, a large portion of all genomic material, perhaps well over 90%, does not encode any precursor. Protein-encoding DNA regions, also called genes, lie amid this expanse of nonfunctional portion of the genome. Proteins, a cell's working molecules that perform the structural, regulatory, and other programs of activities encoded by genes, are synthesized through a complex, molecular 2-step process. The genetic information stored in DNA is first copied into messenger RNA (mRNA) molecules in a process called transcription. mRNA molecules are then translated into proteins by the ribosomal machinery through a remarkably accurate, stepwise assembly of peptides and proteins. Through this process, the nucleic acid sequence of mRNA is translated into the language of amino acid molecules.

DNA consists of 2 antiparallel and complementary base-pair polynucleotide strands that wind together through space to form a double helix. In the transcription process, 1 of the DNA strands (the template strand) is copied into a complementary molecule (mRNA) whose base sequence is arranged in triplet (group of 3 nucleotides), or codon. Each codon is then read and translated into a specific amino acid, which is incorporated into the elongating polypeptide. Because the sequence of bases along the mRNA determines the series of amino acids that will string together to make a protein, the mRNA sequence is said to make sense. To produce a molecule that recognizes and binds to the sense strand, a string of nucleotides that are complementary to the sense sequence must be constructed. Hence, the name *antisense* is given to a short strand of DNA or RNA molecule that is complementary to a specific mRNA sequence.

The idea of using antisense oligodeoxynucleotides (ODNs) was proposed in 1967 to specifically inhibit gene expression through the formation of an mRNA–DNA duplex suppressing or preventing the translation of the targeted message into protein (*1*). At the time, no automated method was available to produce oligomers of more than 4 bases and in sufficient amounts. Over the last 20 y, progress in nucleotide chemistry, the automation of syntheses, and the development of a nuclease-resistant DNA backbone have made DNA (and RNA) antisense molecules readily available. Antisense molecules have been used to regulate, knock down, or express genes in culture (*2*); to engineer plants (*3*); and to design chemotherapeutic agents against viruses (*4,5*).

With the identification of genes responsible for cell growth, development, and malignant transformation (oncogenes and tumor suppressor genes) has come the desire to translate this information into new strategies for the treatment of cancer, cardiovascular diseases, and other common ailments. The concept of "silencing" oncogene expression in malignant diseases is now being tested in multiple clinical trials. Antisense oligonucleotides targeting Ha-*ras*, c-*raf*, *bcl*-2, c-*myb*, *bcr-abl*, and *erb*-B2 oncogenes are being tested in various types of cancer (*6*). Modulation of intimal smooth muscle hyperplasia after coronary artery angioplasty and cardiac allografting is also being tested in vivo with ODNs (*7,8*). Patients with Crohn's disease have experienced successful remission with a phosphorothioate ODN that inhibits the human endothelial adhesion molecule ICAM-1 (*9*). The most important potential application of antisense in viral diseases is HIV infection. A recent phase I trial in HIV-infected patients using an ODN directed at the gag site of the HIV gene showed minimal side effects (*10*). Antisense ODNs are also being targeted against cytomegalovirus retinitis, a severely disabling disease seen in AIDS patients (*11*).

Antisense DNA molecules have also been proposed by a few researchers as a tool to image tumors expressing various oncogenes (*12,13*). The requirements for the use of antisense molecules in cancer therapy are well defined (*14*) and have served as criteria for their use in imaging (*15*). In contrast to therapeutic applications, in which a single antisense molecule can eventually knock down the expression of an oncogene, imaging techniques require a high concentration of antisense molecules inside the target cells. For successful antisense imaging, target cells should have a sufficient amount of mRNA oncogene product and should specifically and selectively retain DNA antisense probes. Various methods for labeling antisense molecules with single photons and positron emitters have been published (*16,17*).

In a state-of-the-art study reported in this issue of *The Journal of Nuclear Medicine*, Zhang et al. (*18*) investigated the effect of the mercaptoacetyltriglycine/linker group on the hybridization of an 18mer phosphorothioate DNA antisense to the intracellular growth regulatory molecule subunit Iα (RIα) of the cyclic adenosine monophosphate–dependent protein kinase

Received Jul. 15, 2001; revision accepted Jul. 17, 2001.

For correspondence or reprints contact: Jean-Luc C. Urbain, MD, PhD, Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195.

A, type I. They also tried to show an antisense effect on various cell lines expressing different levels of RIα.

Several factors play a critical role in the design of antisense molecules for imaging. The first factor is the specificity of the antisense DNA molecule to its mRNA target. With the assumption that a eukaryotic cell contains about 10^4 different mRNA species of 2-kb average length, the total RNA sequence complexity is about 2×10^7 bases. The expected number of occurrences for a given sequence of N bases within the RNA pool is given by dividing the complexity of the pool by 4^N. Therefore, the shortest sequence likely to be unique among an RNA pool of 2×10^7 is 13 bases (*19*).

The second parameter is optimization of the duplex formation between the antisense molecule and its mRNA target by varying the sequence of the DNA probe. To inhibit gene expression, many investigators have chosen to target the translation initiation site of an mRNA on the assumption that this region is important and accessible. Although efficient inhibition of translation usually occurs with DNA oligonucleotide sequences complementary to this region, most regions of an mRNA molecule are in fact accessible to ODNs, except those with many antiparallel duplex structures, called hairpins. Steps at which antisense molecules have been shown to inhibit mRNA also include 5′-capping, splicing, and mRNA stability. The overall extent of base pairing is reflected in the biophysical properties of each RNA molecule and can eventually be predicted by rules that govern the interaction of base pairs. Thermodynamic algorithms are now available to predict the secondary structure of a particular RNA and can be used, to a certain extent, to select optimal target sites on the mRNA to be imaged (*20*).

Third, to be used as an in vivo imaging agent, an ODN sequence should be able to recognize and bind tightly to its complementary sequence in the target nucleic acid at 37°C. This means, on the one hand, that the temperature required for dissociation of the hybrid (i.e., the hybrid melting temperature [Tm]) should be well above 37°C and, on the other hand, that at 37°C the dissociation constant of the complex should be low. Because the Tm of the hybrid is highly dependent on the nature of the bases involved in the bond pairing, it is essential that the target site and, consequently, the ODN sequence be selected with respect to the Tm (*21*).

ODNs containing phosphodiester linkages are rapidly degraded in serum by exo- and endonuclease enzymes, with a half-life of approximately 20 min. Over the past decade, the greatest successes in developing nuclease-resistant ODNs have been achieved by substituting a sulfur atom for an oxygen atom—one not involved in the sugar–sugar bridge—of the phosphate group (phosphorothioate ODN). This substitution increases the stability of nucleases, retains solubility in water, and improves cellular uptake (*22*,*23*). Various strategies are being developed to produce ODN analogs with better bioavailability and better metabolic, therapeutic, and imaging properties by modifying the structure of the internucleotide linkages, bases, and sugars (*24*,*25*).

Zhang et al. (*18*) have to be commended for using the most advanced molecular biology techniques such as spectrophotometry, surface plasmon resonance, reverse transcriptase polymerase reaction, and microautoradiography to compare the hybridization, melting temperatures, and cellular accumulation of labeled and unlabeled sense, antisense, and scrambled ODN sequences. The absence of significant changes in hybridization of the labeled versus the unlabeled ODN sequence, the specific retention of the antisense strands, and the decrease in targeted mRNA content suggest that radiolabeled antisense molecules can successfully be used for imaging and, eventually, for therapy. Antisense ODNs interfere with gene expression by several mechanisms. RNA–DNA dimers formed inside the cell immediately attract the endogenous nuclease RNase-H, which catalyzes cleavage of the RNA species of the duplex, leaving the DNA sequence intact and able to interact with the same sequence in other mRNA molecules. This mechanism likely renders for the decrease in cell growth and proliferation observed in vitro and in preclinical and clinical trials. The observation, in this study, that synthesis of the targeted mRNA is increased suggests a negative feedback mechanism between the amount of mRNA in the cell and synthesis of mRNA. This is a new concept and is interesting and intriguing.

Naked nucleic acids have a strong negative charge, which can prevent their passage through the bilipid layer of the cell membrane. However, cellular uptake of ODNs appears to be a natural phenomenon, and a receptor-like mechanism is probably involved. Chemical modifications to make ODNs more electrically neutral; their association with carriers such as lipid vesicles, liposomes, and adenovirus vector; and the preparation of antisense nanoparticles (*26–29*) seem to significantly improve cellular uptake of ODNs.

Like the specific delivery of gene reporters to target cells, the delivery of antisense to target cells will need to be refined in greater detail (*30*). This factor may also potentially interfere with hybridization and will require further study. The methodology of Zhang et al. (*18*) can serve as a template for validation of these new ODN probes.

A new avenue to exploit the exquisite specificity of the Watson-Crick nucleic acid base pairs is being developed in nuclear medicine. Antisense DNA technology, although still in its infancy for therapy, is now being actively conceptualized and investigated for imaging. I believe that this innovative approach will mature rapidly and result in a new class of unique radiopharmaceutical agents that can characterize the phenotype and genotype of cells and organs.

Jean-Luc C. Urbain
Cleveland Clinic Foundation
Cleveland, Ohio

REFERENCES

1. Belikova AM, Zarytova VF, Grineva NI. Synthesis of ribonucleosides and diribonucleoside phosphates containing 2-chloroethylamine and nitrogen mustard residues. *Tetrahedron Lett.* 1967;37:3557–3562.
2. Helene C, Toulme JJ. Specific regulation of gene expression by antisense, sense and antigene nucleic acids. *Biochem Biophys Acta.* 1990;1049:99–125.
3. van der Krol AR, Mur L, Delange P, Mol JNM, Stuitje AR. Inhibition of flower pigmentation by antisense CHS genes: promoter and minimal sequence requirements for the antisense effect. *Plant Mol Biol.* 1990;14:457–466.
4. Stephenson ML, Zamenick PC. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxynucleotide. *Proc Natl Acad Sci USA.* 1978; 75:285–288.
5. Matsukura M, Shinozuka K, Zon G, et al. Phosphorothioate analogs of oligodeoxynucleotides: inhibitors of replication and cytopathic effects of human immunodeficiency virus. *Proc Natl Acad Sci USA.* 1987;83:7706–7710.
6. Green DW, Roh H, Pippin J, Drebin JA. Antisense oligonucleotides: an evolving technology for the modulation of gene expression in human disease. *J Am Coll Surg.* 2001;191:93–105.
7. Suzuki J, Isobe M, Morishita R, et al. Prevention of coronary arteriosclerosis by antisense cdk2 kinase oligonucleotide. *Nat Med.* 1997;3:900–903.
8. Poston R, Ennen M, Pollard J, et al. Ex vivo gene therapy prevents chronic graft vascular disease in cardiac allografts. *J Thorac Cardiovasc Surg.* 1998;116:386–396.
9. Yacyshyn BR, Bowen-Yacyshyn MB, Jewell L, et al. A placebo controlled trial of ICAM-1 antisense oligonucleotide in the treatment of Crohn's disease. *Gastroenterology.* 1998;114:1133–1142.
10. Sereni D, Tubiana R, Lascoux C, et al. Pharmacokinetics and tolerability of intravenous trecovirsen (GEM 91), an antisense phosphorothioate oligonucleotide, in HIV-positive subjects. *J Clin Pharmacol.* 1999;39:47–54.
11. Perry CM, Balfour JA. Formivirsen. *Drugs.* 1999; 57:375–380.
12. Dewanjee MK, Ghafouripour AK, Kapadvanjwala M, et al. Noninvasive imaging of c-myc oncogene messenger RNA with indium-11-antisense probes in a mammary tumor-bearing mouse model. *J Nucl Med.* 1994;35:1054–1063.
13. Urbain JL, Shore S, Vekemans MC, et al. Scintigraphic imaging of oncogenes with antisense probes: does it make sense? *Eur J Nucl Med.* 1995; 22:499–504.
14. Stein CA, Cheng YC. Antisense oligonucleotides as therapeutic agents: is the bullet really magical? *Science.* 1993;261:1004–1012.
15. Piwnica-Worms D. Making sense out of anti-sense: challenging of imaging gene translation with radiolabeled oligonucleotides. *J Nucl Med.* 1994; 35:1064–1066.
16. Hnatowich DJ. Antisense and nuclear medicine. *J Nucl Med.* 1998;40:693–703.
17. Kuhnast B, Dolle F, Terrazzino S, et al. General method to label antisense oligonucleotides with radioactive halogens for pharmacological and imaging studies. *Bioconjug Chem.* 2000;11:627–636.
18. Zhang Y-M, Wang Y, Liu N, Zhu Z-H, Rusckowski M, Hnatowich DJ. In vitro investigations of tumor targeting with ^{99m}Tc-labeled antisense DNA. *J Nucl Med.* 2001;42:1660–1669.
19. Woolf TM, Melton DA, Jennings CGB. Specificity of antisense oligonucleotides in vivo. *Proc Natl Acad Sci USA.* 1992;89:7305–7309.
20. Patzel V, Sczakiel G. Theoretical design of antisense RNA structures substantially improves annealing kinetics and efficacy in human cells. *Nat Biotech.* 1998;16:64–68.
21. Tidd DM. A potential role for antisense oligonucleotide analogues in the development of oncogene targeted cancer chemotherapy. *Anticancer Res.* 1990;10:1169–1182.
22. Agrawal S, Temsamani J, Tang JY. Pharmacokinetics, biodistribution and stability of oligodeoxynucleotide phosphorothioate in mice. *Proc Natl Acad Sci USA.* 1991,88:7595–7599.
23. Temsamani J, Kubert M, Tang J, Padmapriya A, Agrawal S. Cellular uptake of oligodeoxynucleotide phosphorothioates and their analogs. *Antisense Res Dev.* 1994;4:35–42.
24. Tavitian B. In vivo antisense imaging. *Q J Nucl Med.* 2000;44:236–255.
25. Shi N, Boado RJ, Pardridge WM. Antisense imaging of gene expression in the brain in vivo. *Proc Natl Acad Sci USA.* 2000;97:14709–14714.
26. Akhtar S, Hughes MD, Khan A, et al. The delivery of antisense therapeutics. *Adv Drug Deliv Rev.* 2000;44:3–21.
27. Semple SC, Klimuk SK, Harasym TO, et al. Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionizable aminolipids: formation of novel small multilamellar vesicle structures. *Biochim Biophys Acta.* 2001;1510:152–166.
28. Junghans M, Kreuter J, Zimmer A. Phosphodiester and phosphorothioate oligonucleotide condensation and preparation of antisense nanoparticles. *Biochim Biophys Acta.* 2001;1544:177–188.
29. Im SA, Kim JS, Gomez-Manzano C, et al. Inhibition of breast cancer growth in vivo by antiangiogenesis gene therapy with adenovirus-mediated antisense-VEGF. *Br J Cancer.* 2001;84:1252–1257.
30. Urbain JL. Reporter genes and imagene. *J Nucl Med.* 2001;42:106–109.