Molecular Neurobiology

ISSN0893-7648/95/10(2–3): 219–229/$6.20

Brief Overview of Control of Genetic Expression by Antisense Oligonucleotides and In Vivo Applications

Prospects for Neurobiology

Gerald Zon

Lynx Therapeutics, Inc., 3832 Bay Center Place, Hayward, CA 94545

Abstract

Over the past several years, the use of synthetic oligonucleotides and functional analogs thereof as a possibly general means of controlling genetic expression has received widespread attention. Following a brief overview of some of the basic principles and strategies for this approach, attention is focused here on summarizing some recent reports of in vitro and, in particular, in vivo investigations in various animal models using phosphorothioate analogs of 2'-deoxyoligonucleotides. In view of these findings, which include studies related to neurobiology, this field should find significant utility in applications of the antisense method for controlling genetic expression.

Index Entries: Genetic expression; antisense; oligonucleotides; phosphorothioate analogs; in vitro activity; animal models; neurobiology.

Introduction

Antisense research employing oligonucleotides has rapidly generated a number of reviews (Zon, 1988; Uhlmann and Peyman, 1990; Bishofberger and Wagner, 1992; Neckers and Whitesell, 1993; Stein and Cheng, 1993) and several comprehensive edited books (Cohen, 1989; Mol and van der Krol, 1990; Wickstrom, 1991; Crooke and Lebleau, 1993), all of which should be consulted to obtain a complete picture of the current scope and directions of this new interdisciplinary technology. What follows is purposely very brief and is intended as an introductory overview that may be of some use before proceeding to a synopsis of some of the current state-of-the-art applications of this exciting methodology. Although these applications are largely in the context of potential therapeutic drugs, it is important to recognize the utility of the antisense approach for elucidating gene function in many areas of basic research.

Control of genetic expression by "antisense oligonucleotides" refers to use of synthetic oligonucleotides or functional analogs thereof to

bind in a sequence-specific manner to preselected RNA targets and thus block translation of the corresponding protein. The underlying physical-chemical principle for this interception process is hydrogen bonding of the type first described in the 1950s by Watson and Crick for A-T and G-C basepairs in antiparallel ("reverse") complementary nucleic acid sequences (Fig. 1). Given that the "sense" or coding strand of a gene by definition gives rise to the complementary "sense" RNA sequence that is, in turn, translated into the protein encoded by that particular gene, it is evident that interception of sense RNA requires the reverse complementary or, by definition, "antisense" oligonucleotide sequence. These sense and antisense relationships are depicted in Fig. 1 for interception of sense RNA by a 2'-deoxyoligonucleotide antisense agent. This is in contrast to a ribooligonucleotide antisense agent or vector-encoded antisense RNA transcript used in a related but different methodology discussed elsewhere (Mol and van der Krol, 1990). A 2'-deoxy or DNA-like antisense oligonucleotide offers the significant potential advantage of cleavage of the sense RNA target by RNase H, and, hence, more effective inhibition than that achievable by the latter ribo- or RNA-like oligomer, which can form a complex but is not cut by RNase H. In this regard, it is worth noting that only certain structurally modified 2'-deoxyoligonucleotide analogs *(see the following)* retain sufficient resemblance to DNA to allow formation of a complex with target RNA that is a substrate for RNase H.

In principle, this interception process may involve targeting either unspliced RNA in the nucleus or spliced mRNA in cytoplasm. Although any RNA or mRNA sequence can in theory be targeted, it is expected *a priori* (and found experimentally) that only certain target RNA sequences allow for successful interception by the antisense oligonucleotide. RNAs have complex secondary and tertiary structures that make many possible sequences unavailable for binding owing to their being involved in either a basepaired "stem" structure or some other structural element that in some way precludes effective hydrogen binding of the target RNA to the antisense oligonucleotide.

That there is functionally adequate uptake of synthetic oligonucleotides by cells was initially viewed by many with surprise; however, it is now generally accepted that such cellular uptake does take place. What is not currently understood, however, is the mechanistic detail for either the internalization process(es) or intracellular trafficking. These subjects, that are well beyond the scope of the present brief overview, are obviously of fundamental importance and should be considered by consulting relatively recent reviews (Crooke and Lebleau, 1993; Stein and Cheng, 1993) as well as more recent primary literature.

Antisense Oligonucleotide Sequence

The specificity, or lack thereof, of antisense oligonucleotide binding to RNA that is theoretically achievable is largely controlled by thermodynamic parameters akin to those that are operative for the well-known use of synthetic 2'-deoxyoligonucleotides as "hybridization probes," "sequencing primers," "PCR primers," and related molecular biological techniques. An approx 13-mer antisense oligonucleotide has, from a statistical viewpoint, adequate sequence "information" to bind uniquely to a specific target RNA, although most studies generally employ 18- to 21-mers, perhaps by analogy to what is used for probes and primers. However, although relatively long oligonucleotides offer statistically greater probability of specific hybridization to the particular target RNA, as well as tighter binding that could lead to increased efficacy, thermodynamic equilibrium may not be achievable in cells, where kinetics of reaction may instead dominate. Hybridization conditions for use of probes and primers can generally be optimized by adjusting temperature, salt concentration, and the ratio of probe or primer to target. By contrast, the stringency for hybridization of antisense oligonucleotides in cells can only

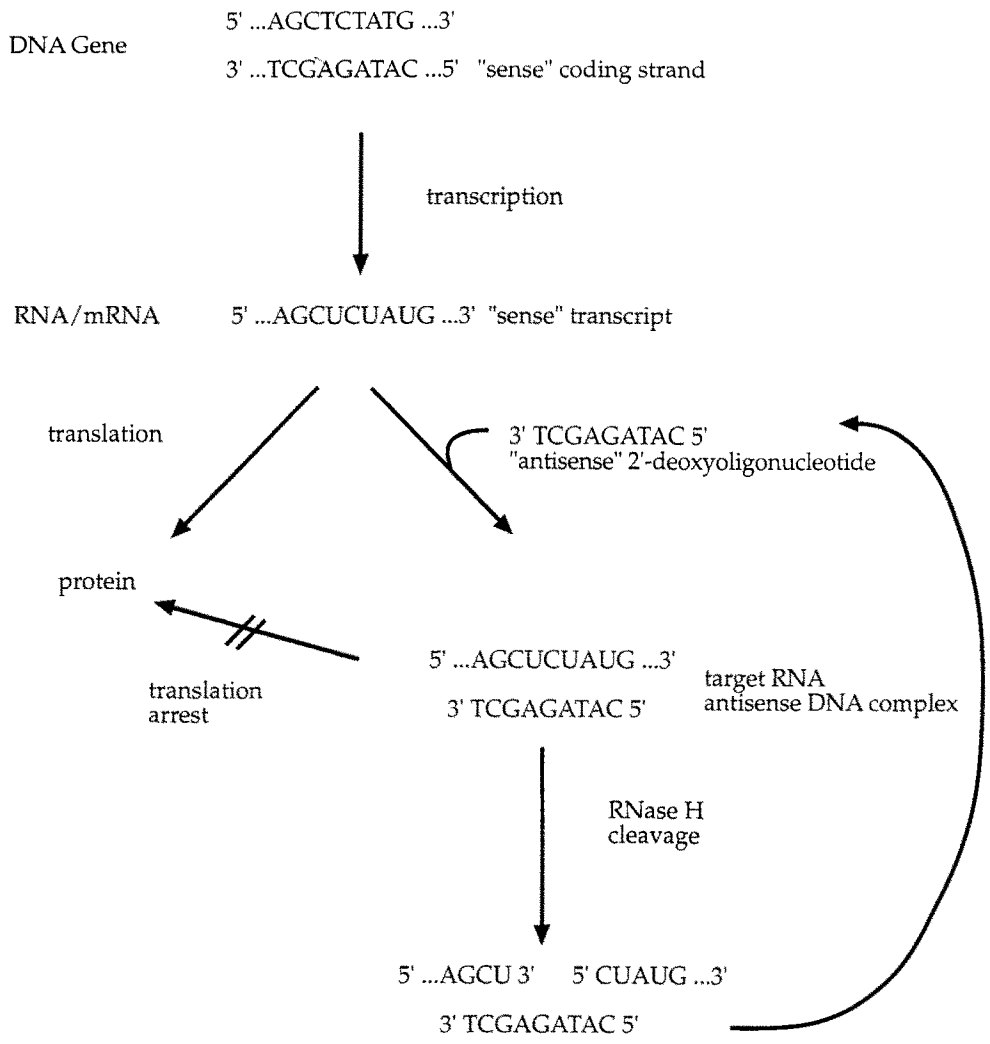


Fig. 1. Schematic representation of the mechanism of action for sequence-specific inhibition of genetic expression by use of an "antisense" 2'-deoxyoligonucleotide to hybridize to the reverse complementary sequence of mRNA encoded by the "sense" strand of DNA, and thus form a complex that prevents translation of a preselected protein and/or leads to cleavage of the mRNA strand by RNase H.

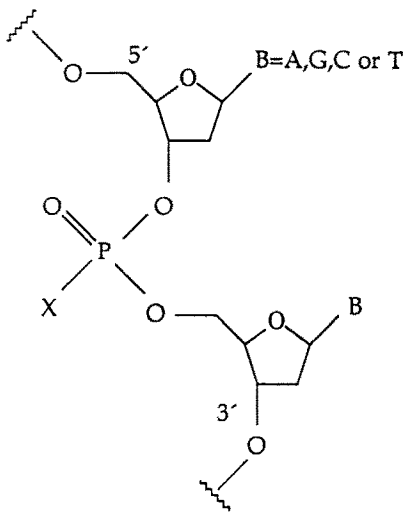
be controlled by shortening chainlength to decrease the binding affinity and thereby giving up, statistically, target-sequence uniqueness relative to "background," i.e., all other RNA that are expressed per unit of time in a given cell type. Because of these obvious complexities, and the already mentioned inability to reliably predict which RNA sequences will be, in fact, both accessible and mechanistically productive, when complexed with an intended antisense agent, designing and using antisense oligonucleotides is therefore largely an empirical process with few rules at this time. On the other hand, most antisense reports have generally used 15- to 24-mers targeted to the translation start site.

Antisense Oligonucleotide Analogs

A cardinal feature of an antisense agent is that it have adequate resistance to degradation by exo- and endonucleases that are ubiquitous in genetically-based systems, i.e., all living

things. This was recognized by Zamecnik in his seminal studies (Stephenson and Zamecnik, 1978; Zamecnik and Stephenson, 1978) with Rous sarcoma virus on the antisense approach, which investigated protection of an antiviral 2'-deoxyoligonucleotide by use of 3', 5'-terminal modification as an isourea derivative. Although there are now available many types of 3' and 5' modifications reported to afford some degree of protection against degradation by exonucleases, evidence for endonuclease activity suggests that more extensive, if not complete, modification of an antisense oligonucleotide analog is required. This was originally achieved by Agris et al. (1986) who pioneered the use of 2'-deoxyoligonucleotide methylphosphonate analogs (Fig. 2, X = CH3), which also allowed investigation of nonionic, relatively hydrophobic, antisense agents that were originally envisaged as being able to possibly better penetrate the lipid bilayer of cellular membranes. However, it now appears that this potential advantage may not, in fact, be significant generally.

Shortly following these early studies by Zamecnik and Ts'o and Miller, several reports (Marcus-Sekura et al., 1987; Matsukura et al., 1987, 1988) appeared on the use of 2'-deoxyoligonucleotide phosphorothioate analogs (Fig. 2, X = S). Subsequently, a large number of other types of possible antisense structures have been investigated. 2'-Deoxy- and ribooligonucleotide analogs with either phosphate modifications or nonphosphate linkages, 2'-0-alkyl analogs, α-anomers, and many more, have been discussed elsewhere. (Cohen, 1989; Uhlmann and Peyman, 1990; Wickstrom, 1991; Crooke and Lebleau, 1993; Neckers and Whitesell, 1993). Probably because of the fact that they are relatively easy to synthesize, purify, analyze, handle, store, and investigate both in cell culture and in animals as possible inhibitors of gene expression, phosphorothioate analogs of 2'-deoxyoligonucleotides have become the leading first-generation antisense oligonucleotide analogs, based on the number of reported studies to date. Methods of preparation (Zon and Geiser, 1991; Zon and Stec, 1991), uniform [^{35}S]-labeling (Stein et al., 1990), and analysis (Zon and Geiser, 1991; Zon and Stec, 1991) of phosphorothioate analogs have been described in detail elsewhere.



phosphodiester, X = O^-
methylphosphonate, X = CH_3
phosphorothioate, X = S^-

Fig. 2. Schematic partial structure of the generalized repeating unit of a 2'-deoxyoligonucleotide with either phosphodiester (X = O^-), methylphosphonate (X = CH_3), or phosphorothioate (X = S^-) linkages.

Antisense Phoshorothioate Studies in Cell Culture

Investigations of the control of genetic expression in cell culture using antisense phosphorothioate oligonucleotide analogs have been compiled recently by Cohen (1994) for most of the published literature through much of 1993, and for this reason will not be reiterated here. Of these 31 reports, there are 14 that deal with viruses and 13 that deal with cancer; 30 additional reports not included in Cohen's compilation are listed in Table 1 and

Table 1
Additional Reports
of Antisense Phosphorothioate Investigations
in Cell Cultures Not Compiled in Cohen (1994)

Antiviral	Reference
HIV-1 *vpr*	(Balotta et al., 1993)
HIV-1 *rev*	(Balotta et al., 1993; Zelphati et al., 1993)
HIV-1 *rev* responsive element	(Li et al., 1993)
Anticancer	
bcr-abl	(McGahon et al., 1994; Smetsers et al., 1994)
p53	(Bayever et al., 1993; Li et al., 1993, 1994)
Ha-*ras*	(Monia et al., 1993)
c-*myc*	(Rosolen et al., 1993)
HPV-18	(Steele et al., 1993)
p65 Subunit of NF-κB	(Higgins et al., 1993)
c-*raf*	(Riedel et al., 1993)
Other	
IL-1b	(Doken et al., 1993)
IL-4	(Benbernou et al., 1993)
Wnt-1 and *Wnt*-3a	(Augustine et al., 1993)
c-*myc*	(Paria et al., 1992; Bennett et al., 1994; Takeda et al., 1994)
Procollagen	(Colige et al., 1993)
N-methyl-D-aspartate	(Wahlestedt et al., 1993)
Integrin	(Lallier Bronner-Fraser, 1993)
cdc2	(Lapidot-Lifson et al., 1992)
Regulatory factor RFX-1	(Siegrist and Mach, 1993)
Catalytic subunit of PKA	(Sugiyama et al., 1992; Yokazaki et al., 1993)
PKC	(Bennett and Dean, 1993)
Phospholipase A_2	(Barbour and Dennis, 1993)
Gephyrin	(Kirsch et al., 1993)

therefore bring the total for phosphorothioates to more than 60. Viral diseases and cancer represent applications that obviously have very important therapeutic potential and, if achievable, would open up an exciting new era in modem molecular medicine. It is also evident from Table 1 that other applications are receiving considerable attention; neurobiology is addressed in the last section of this work.

The direct association between the presence of an exogenous ("foreign") viral genome in host cells and the resultant virally related disease simplifies the design of a potentially sequence-specific antisense agent as a possible antiviral drug. The sequence of the viral genome limits the sequence selection, and thus provides the basis for using further selection criteria such as choosing critical viral gene-product sequences, oligonucleotide length, elimination of self-complementary sequences, and elimination (if possible) of homology to known human gene sequences.

With regard to antiviral activity, it is important to note that sequence-specific antisense phosphorothioate analogs offer, as a possible practical advantage, additional sequence-independent mechanisms of antiviral activity. These sequence-independent antiviral effects were first evidenced for phosphorothioate analogs in the early studies on HIV-1 (Matsukura et al., 1987, 1989), and have been discussed recently by Stein and Cheng (1993).

In contrast to the cause-and-effect relationship between viruses and human viral diseases, human cancers are inherently more complex with regard to whether antisense inhibition of a single oncogene, proto-oncogene, mutant gene, amplified gene, or some other "inappropriately expressed" gene will necessarily lead to a clinically significant anticancer effect. This situation, despite the rapid, major advances being made in cancer molecular biology, gives the antisense (or any other) approach to attacking cancer cells an inherently more uncertain outcome. However, the possible higher specificity of antisense agents compared to relatively nonspecific cellular toxins now in clinical use is an attractive proposition in studies of improved cancer treatment; moreover, results to date have been promising. Recent cancer-related reports not included in Cohen's (1994) compilation of antisense stud-

ies in cell culture using phosphorothioate analogs are listed in Table 1.

Antisense Phoshorothioate Studies in Animal Models

Among the major milestones that have been reached in antisense oligonucleotide control of genetic expression is demonstration of in vivo efficacy in animal models. Virtually all of these proof-of-concept experiments have been conducted with phosphorothioate-modified 2'-deoxyoligonucleotide analogs. An up-to-date list of such studies is given in Table 2. The listed report by Rosenberg and coworkers in 1992 on the use of an antisense compound targeted to c-*myb* to inhibit intimal arterial smooth muscle cell accumulation in rat carotid artery, as a model for prophylactic treatment of stenosis following coronary artery angioplasty, was historically the first to claim an in vivo effect using a phosphorothioate analog. Restenosis has received considerable attention since then with regard to other RNA targets and alternative modes of delivery of antisense phosphorothioates (Table 2).

Although this early antirestenosis work using local delivery of the antisense phosphorothioate was a landmark with regard to in vivo applications, the listed report by Gewirtz and coworkers published shortly thereafter at the end of 1992 on an antileukemic effect targeting c-*myb* was equally important as the first demonstration of an apparent antisense effect in an animal using systemic infusion of a phosphorothioate agent. In this SCID mouse model of human chronic myelogenous leukemia (CML), a daily dose of only 100 μg was continuously administered by means of a subcutaneously implanted osmotic (Alzet) pump for a period of 2 wk, which has been recently estimated (P. Iversen, personal communication) to be equivalent (on a mg/m^2 basis) to an approx 30 mg daily dose for an adult, which is a reasonable amount from various practical considerations.

As is evident from Table 2, these pioneering in vivo experiments have been followed by a relatively large number of additional reports published in a relatively short period of time, especially considering the degree of technical difficulty of these types of experiments. One can reasonably expect this pace to continue and presumably expand as interest grows and antisense phosphorothioates as well as other effective analogs become more readily available.

Human Clinical Trials with Antisense Phosphorothioates

All of the current clinical trials with antisense agents as investigational new drugs are being conducted with phosphorothioated 2'-deoxyoligonucleotides, and are listed in Table 3. Although it is apparently too early for conclusions to be drawn regarding efficacy of these investigational new drugs for the indicated diseases, as yet unpublished results (P. Iversen, personal communication) from the only study involving continuous iv infusion have shown that administration of a phosphorothioate analog targeted to *p53* mRNA at doses up to 0.25 mg/kg/h for 10 d is well tolerated by patients with either acute myelogenous leukemia or myelodysplastic syndrome. For a 75-kg patient, this infusion rate translates to 4.5 g of the investigational antisense agent, much of which is slowly excreted and low levels (~0.5 μ*M*) of that are maintained in blood plasma. As might be expected, there is binding of phosphorothioate analogs to serum albumin and other serum proteins (Cossum et al., 1993).

Prospects for Antisense Studies Related to Neurobiology

Several years ago, any comments regarding the prospects for applications of antisense oligonucleotides to the field of neurobiology, in a broad sense, would necessarily be purely speculative owing to the absence of such reports at that time. Now, it is possible to cite several recent reports of phosphorothioate analogs in cell culture systems that give realistic

Table 2
In Vivo Animal Model Efficacy Studies Using Antisense Phosphorothioate

Application	Gene target	Model and mode of treatment	Reference
Restenosis	c-*myb*	Ratcarotid artery; local delivery to exterior of artery by pluronic gel	(Simons et al., 1992)
	cdc2 Kinase and PCNA	Rat carotid artery; local intraluminal delivery by infusion	(Morishita et al., 1993)
	c-*myc*	Pig coronary artery; local intraluminal delivery by catheter	(Shi et al., 1994)
Leukemia	c-*myb*	SCID-huK526;subcutaneously implanted osmotic pump	(Ratajczak et al., 1992)
	bcr-abl	SCID-hu BV173 mouse; repeated intravenous injections	(Skorski et al., 1994)
B-cell lymphoma	*BCL-2*	SCID-hu DoHH2 mouse; subcutaneously implanted osmotic pump	(Pocock et al., 1993)
Colon cancer	PKA/RIα	HU LS-174T cells in nude mouse; repeated subcutaneous injections	(Cho-Chung, 1993)
Malignant melanoma	c-*myb*	SCID-hu Hs294T mouse; subcutaneously implanted osmotic pump	(Hijiya et al., 1994)
Malignant melanoma	NF-κB/p65	Hu B-16 cells in nude mouse; repeated subcutaneous injections or subcutaneously implanted osmotic pump	(Higgins et al., 1993)
	p120	Hu Lox cells in nude mouse; repeated intraperitoneal injections	(Perlakey et al., 1993)
Fibrosarcoma	NF-κB/p65	HTLV-I Tax-transformed mouse	(Kitajima et al., 1993)
	NF-κB/p65	Hu K-BALB cells in nude mouse; repeated subcutaneous injections or subcutaneously implanted osmotic pump	(Higgins et al., 1993)
Hypertension	Angiotension II type-1	Spontaneously hypertensive rat; single intracerebroventricular injection	(Gyurko et al., 1993)
Neuronal function	c-*fos*	D-Amphetamine-induced rat; infusion into opposing striata by implanted cannulae	(Heilig et al., 1993)
	Kinesin	Rabbit; intravitreous injection	(Amaratunga et al., 1993)
Ischemic infraction	*N*-Methyl-D-asparate receptor	Spontaneouslyhypertensive rat; repeated intracerebroventricular injections	(Wahlestedt et al., 1993)
Axonal growth	SNAP-25	Chick embryo; repeated intravitreous injections	(Osen-Sand et al., 1993)
Neuropsychiatric disorders	D_2-dopamine receptor	6-Hydroxydopamine-induced mouse; repeated intracerebroventricular injections	(Weiss et al., 1993)
Memory inhibition	Ependymin	Task-conditioned goldfish; injections	(Schlingensiepen, unpublished)
Hepatitis	Duck HBV	Pekin ducks; repeated intravenous injections	(Whitesell et al., 1993)

Table 3
Human Clinical Trails with Antisense Phosphorothioates

Disease	Gene target	Status	Investigator(s)
Acute myelogenous leukemia (systemic infusion)	p53	Phase I	University of Nebraska Medical Center/Lynx
Acute myelogenous leukemia (marrow purge)	p53	Phase I	University of Nebraska Medical Center/Lynx
Chronic meylogenous leukemia (marrow purge)	c-*myb*	Phase I	University of Pennsylvania Cancer Center/Lynx
Chronic meylogenous leukemia (systemic infusion)	c-*myb*	Phase I (planned for 1994)	University of Pennsylvania Cancer Center/Lynx
Genital warts (local application)	HPV	Phase II	Isis
AIDS-related retinitis (intraocular)	CMV	Phase I	Isis
AIDS (subcutaneous infusion)	HIV	Phase I	Hybridon

indications of what such prospects can include. For example, the following reports are listed in Tables 1 and 2. Antisense oligonucleotides were used to attenuate *Wnt-1* and *Wnt-3*a proto-oncogenes expression and thus revealed roles for these genes in craniofacial, spinal cord, and cardiac morphogenesis (Augustin et al., 1993). In another study, (Wahlestedt et al., 1993), an antisense phosphorothioate to *N*-methyl-D-aspartate receptor channel was reported to protect cortical neurons from excitotoxicity and reduce focal ischemic infarctions produced by occlusion of the middle cerebral artery in the rat. Another investigation (Lallier and Bronner-Fraser, 1993) on the neural crest, which is a population of cells that arises in the dorsal neural tube and migrates throughout the embryo along defined pathways, found that neural crest cell attachment was inhibited by antisense targeting of integrin. Other investigators have studied (Kirsch et al., 1993) a phosphorothioate antisense to the message for the peripheral membrane protein gephyrin, which copurifies with the postsynaptic inhibitory glycine receptor on affinity chromatography. It was found (Kirsch et al., 1993) that treatment of rat spinal neurons in culture with gephyrin antisense phosphorothioate prevented the formation of glycine receptor clusters in the dendritic plasma membrane, and thus concluded that gephyrin is essential for localization of this receptor to presumptive postsynaptic plasma membrane specializations. c-*Fos* has been targeted to study the role of immediate-early genes in specific sites of mammalian brain (Heilig et al., 1993). Other studies (Osen-Sand et al., 1993) include inhibition of axonal growth by a SNAP-25 antisense agent, which led the investigators to suggest that high levels of this nerve terminal protein in specific areas of the adult brain may contribute to nerve terminal plasticity.

Investigators (Amaratunga et al., 1993) have also injected an antisense phosphorothioate targeted to the translation start site of rat kinesin heavy chain into the vitreous of anesthetized rabbits in order to asses the effects on transport in the retinal ganglion cells whose axons form the optic nerve. The results were said by these investigators to provide direct evidence for the specific role of kinesin in rapid anterograde transport in vivo and indicate the utility of antisense oligonucleotide analogs to explore neuronal dynamics in a specific neuronal cell type in a living animal.

Another promising and quite different investigation involved a phosphorothioate antisense to the mouse D_2 dopamine receptor mRNA, which when administered to mice by repeated intraventricular injections caused a dose-dependent inhibition of quinpirole-induced rotational behavior (Weiss et al., 1993).

This behavioral modification was reversible, with normal quinpirole responsiveness returning within 2 d after cessation of antisense treatment, and was not seen in animals treated with vehicle or a random-sequence phosphorothioate oligonucleotide. Furthermore, this antisense compound did not inhibit the rotational response induced by a D_1 receptor against (SKF 38393) or a muscarinic cholinergic against (oxotremorine). Envisaged applications for such antisense compounds include treatment of behavioral and motor disorders associated with dopaminergic hyperactivity, such as schizophrenia, Huntington disease, certain forms of drug abuse, and tardive dyskinesia, as well as certain eating disorders and blood regulation where dopamine may also be involved.

Neckers and coworkers (Whitesell et al., 1993) have explored the possible utility of the antisense approach for therapeutic applications within the central nervous system (CNS), such as treatment of viral and neoplastic disorders within the CNS. To this end, these investigators have reported (Whitesell et al., 1993) that, although there is apparently little intrinsic nuclease activity in cerebrospinal fluid (CSF), unmodified oligonucleotides (Fig. 2, X = O^-) are rapidly degraded by brain-associated exonuclease activity. Phosphorothioates, however, were apparently found to be resistant to degradation in the CNS and, after intraventricular administration, were cleared in a manner consistent with CSF bulk flow. In addition, continuous infusion of phosphorothioates at 1.5 nmol/h by a minosmotic pump could maintain potentially therapeutic concentrations (micromolar) of the intact oligonucleotide analog in CSF for at least 1 wk without obvious neurologic or systemic toxicity. Also, after such infusion, there was extensive brain penetration and marked cellular uptake, especially by astrocytic cells.

In summary, there have been a flurry of reports of antisense experimentation dealing with neurobiology that suggest that this new methodology will continue to be of use within this area of the life sciences.

Acknowledgment

The skilled assistance of Pamela Dal Bozzo in the preparation of this work is gratefully acknowledged.

References

Agris C. H., Blake K. R., Miller P. S., Reddy M. P., and Ts'o P. O. P. (1986) *Biochemistry* **25,** 6268.

Amaratunga A., Morin P. J., Kosik K. S., and Fine R. E. (1993) *J. Biol. Chem.* **268,** 17,427.

Augustine K., Liu E. T., and Sadler T. W. (1993) *Dev. Gen.* **14,** 500.

Balotta C., Lusso P., Crowley R., Gallo R. C., and Franchini G. (1993) *J. Virol.* **67,** 4409.

Barbour S. E. and Dennis E. A. (1993) *J. Biol. Chem.* **268,** 21,875.

Bayever E., Haines K. M., Iversen P. L., Ruddon R. W., Pirrucello S. J., Mountjoy C. P., Arneson M. A., and Smith L. J. (1994) *Leukemia Lymphoma* **12,** 223.

Benbernou N., Matsiota-Bernard P., and Guenounou M. (1993) *Eur. J. Immunol.* **23,** 659.

Bennett F. C. and Dean N. (1993) PCT Pat, Appl. No. WO93/19203.

Bennett M. R., Anglin S., McEwan J. R., Jagol R., Newby A. C., and Evan G. I. (1994) *J. Clin. Invest.* **93,** 820.

Bi S., Lanza F. and Goldman J. M. (1993) *Leukemia* **7,** 1840.

Bi S., Lanza F. and Goldman J. M. (1994) *Cancer Res.* **54,** 582.

Bischofberger N. and Wagner R. W. (1992) *Semin. Virol.* **3,** 57.

Cho-Chung Y. S. (1993) *Int. J. Oncol.* **3,** 141.

Cohen J. S. (1994) *Adv. Pharm.* **25,** 319.

Cohen J. S., ed. (1989) *Oligodeoxynucleotides Antisense Inhibitors of Gene Expression,* CRC, Boca Raton, FL.

Colige A., Sokolov B. P., Nugent P., Baserga R., and Prockop D. J. (1993) *Biochemistry* **32,** 7.

Cossum P. A., Sasmar H., Dellinger D., Truong L., Cummins L., Owens S. R., Markham P. M., Shen J. P., and Crooke S. (1993) *J. Pharm. Exp. Ther.* **267,** 1181.

Crooke S. T. and Lebleau B., ed. (1993) *Antisense Research and Applications,* CRC, Boca Raton FL.

Doken K., Higaki M., Shoji Y., Shimada J., Nishioka K., and Mizushima Y. (1993) *Drug Del. Syst.* **8,** 257.

Gyurko R., Wielbo D., and Phillips M. I. (1993) *Reg. Pep.* **49,** 167.
Heilig M., Engel J. A., and Söderpalm B. (1993) *Eur. J. Pharmacol.* **236,** 339.
Higgins K. A., Peres J. R., Coleman T. A., Dorschkind K., McComas W. A., Sarmiento U. M., Rosen C. A., and Narayanan R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9901.
Hijiya N., Zhang J., Ratajczak M. Z., Kant J. A., DeRiel K., Herlyn M., Zon G., and Gewirtz A. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4499.
Kirsch J., Wolters I., Triller A., and Betz H. (1993) *Nature* **366,** 745.
Kitajima I., Shinohara T., Bilakovics J., Brown D. A., Xu X., and Nerenberg M. (1992) *Science* **258,** 1792.
Lallier T. and Bronner-Fraser M. (1993) *Science* **259,** 692.
Lapidot-Lifson Y., Patinkin D., Prody C. A., Ehrlich G., Seidman S., Ben-Aziz R., Benseler F., Eckstein F., Zakut H., and Soreq H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 579.
Li G., Lisziewicz J., Sun D., Zon G., Daefler S., Wong-Staal F., Gallo R. C., and Klotman M. E. (1993) *J. Virol.* **67,** 6882.
Lisziewicz J., Sun D., Metelev V., Zamecnick P., Gallo R. C., and Agrawal S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3860.
Marcus-Sekura C. J., Woerner A. M., Shinozuka K., Zon G., and Quinnan G. V. Jr. (1987) *Nucleic Acids Res.* **15,** 5749.
Matsukura M., Shinozuka K., Zon G., Mitsuya H., Reitz M., Cohen J. S., and Broder S. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7706.
Matsukura M., Zon G., Shinozuka K., Robert-Garoff M., Shimada T., Stein C. A., Mitsuya H., Wong-Staal F., Cohen J. S., and Broder S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4244.
Matsukura M., Zon G., Shinozuka K., Stein C. A., Mitsuya H., Cohen J. S., and Broder S. (1988) *Gene* **72,** 343.
McGahon A., Bissonnette R., Schmitt M., Cotter K. M., Green D. R., and Cotter T. G. (1994) *Blood* **83,** 1179.
Mol J. N. M. and van der Krol A. R., eds. (1990) *Antisense Nucleic Acids and Proteins, Fundamentals and Applications,* Marcel Dekker, New York.
Monia B. P., Johnston J. F., Ecker D. J., Zounes M. A., Lima W. F., and Freier S. M. (1992) *J. Biol. Chem.* **267,** 19,954.
Morishita R., Gibbons G. H., Ellison K. E., Nakajima M., Zhang L., Kaneda Y., Ogihara T., and Dzau V. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8474.
Neckers L. and Whitesell L. (1993) *Am. J. Physiol.* **265,** (*Lung Cell. Mol. Physiol.* **9**) LI.
Offensperger W.-B., Offensperger S., Walter E., Teubner K., Igloi G., Blum H. E., and Gerok W. (1993) *EMBO J.* **12,** 1257.
Osen-Sand A., Catsicas M., Staple J. K., Jones K. A., Ayala G., Knowlesk J., Grenningloh G., and Catsicas S. (1993) *Nature* **364,** 445.
Paria B. C., Dey S. K., and Andrews G. K. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10,051.
Perlakey L., Saijo Y., Busch R. K., Bennett C. F., Mirabelli C. K., Crooke S. T., and Husch H. (1993) *Anti-Cancer Drug Des.* **8,** 3.
Pocock C., Al-Mahdi N., Hall P., Morgan G., and Cotter F. (1993) American Society of Hematology Meeting abstract no. 784.
Ratajczak M. Z., Kant J. A., Luger S. M., Hijiya N., Zhang J., Zon G., and Gewirtz A. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11,823.
Riedel D., Brennscheidt U., Kiehntopf M., Brach M., and Herrmann F. (1993) *Eur. J. Immunol.* **23,** 3146.
Rosolen A., Kyle E., Chavany C., Bergan R., Kalman E. T., Crouch R., and Neckers L. (1993) *Biochimie* **75,** 79.
Schlingensiepen K.-H., unpublished results.
Shi Y., Ford A., Galeo A., Hutchinson H. G., Vermani P., Dodge G. R., Hall D. J., Shaheen F., and Zalewski A. (1994) *Circulation* **90,** 944.
Siegrist C.-A. and Mach B. (1993) *Eur. J. Immunol.* **23,** 2903.
Simons M., Edelman E. R., DeKeyser J.-L., Langer R., and Rosenberg R. D. (1992) *Nature* **359,** 67.
Skorski T., Nieborowska-Skorska M., Nicolaides N. C., Szczylik C., Iversen P., Iozzo R. V., Zon G., and Calabretta B. (1995) *Proc. Natl. Acad. Sci. USA* **91,** 4505.
Smetsers T. F. C. M., Skorski T., van de Locht L. T. F., Wessels H. M. C., Pennings A. H. M., deWitte T., Calabretta B., and Mensink E. J. B. M. (1994) *Leukemia* **8,** 129.
Steele C., Cowsert L. M., and Shillitoe E. J. (1993) *Cancer Res.* **53,** 2330.
Stein C. A. and Cheng Y.-C. (1993) *Science* **261,** 1004.
Stein C. A., Iversen P. L., Subasinghe C., Cohen J. S., Stec W. J., and Zon G. (1990) *Anal. Biochem.* **188,** 11.
Stephenson M. L., and Zamecnik P. C. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 285.
Sugiyama H., Chen P., Hunter M., Taffs R., and Sitkovsky M. (1992) *J. Biol. Chem.* **267,** 25,256.
Takeda A., Norris J. S., Iversen P. L., and Ebadi M. (1994) *Pharmacology* **48,** 119.

Uhlmann E. and Peyman A. (1990) *Chem. Rev.* **90,** 544.
Wahlestedt C., Golanov E., Yamamoto S., Yee F., Ericson H., Yoo H., Inturrisi C. E., and Reis D. J. (1993) *Nature* **363,** 260.
Weiss B., Zhou L.-W., Zhang S.-P., and Qin Z.-H. (1993) *Neuroscience* **55,** 607.
Whitesell L., Geselowitz D., Chavany C., Fahmy B., Walbridge S., Alger J. R., and Neckers L. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4665.
Wickstrom E., ed. (1991) *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS,* Wiley-Liss, New York.
Yokazaki H., Budillon A., Tortora G., Meissner S., Beaucage S. L., Miki K., and Cho-Chung Y. S. (1993) *Cancer Res.* **53,** 868.
Zamecnik P. C. and Stephenson M. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 285.
Zelphati O., Zon G., and Lesserman L. (1993) *Antisense Res. Dev.* **3,** 323.
Zon G. (1988) *Pharm. Res.* **5,** 539.
Zon G. and Geiser T. G. (1991a) *Anti-Cancer Drug Design* **6,** 539.
Zon G. and Stec W. J. (1991b) in *Oligonucleotides and Analogs, A Practical Approach* (Eckstein F., ed.), IRL, New York, pp. 87–108.