

Oligonucleotide derivatives: Biologically active compounds targeted to specific genes

Valentin V. Vlassov

Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, 8 Lavrentiev ave, Novosibirsk 630090, Russia, tel. (3832)353162, Fax (3832)353459

Abstract. Oligonucleotides and their analogs can form specific complexes with RNA, DNA and various proteins interacting with nucleic acids. Results of *in vitro* studies evidence that the compounds may affect expression of specific genes and can be considered as potential efficient and specific biologically active compounds of therapeutic value. Studies on antiviral activity of oligonucleotides are strongly suggestive that they can suppress transcription and translation of viral nucleic acids and may interfere with the process of the virus - cell interaction. In this paper results of recent studies on oligonucleotide derivatives as gene-targeted drugs are surveyed.

INTRODUCTION

Fragments of nucleic acids, oligonucleotides, possess a unique capability of specific interactions with the most important biopolymers responsible for storage, processing and realization of genetic information - nucleic acids and proteins interacting with nucleic acids. Due to their unique targeting potential, oligonucleotides and oligonucleotide analogs are considered as promising structures for design of novel biologically active compounds that will be able to inactivate foreign genetic information of infectious agents and regulate expression of the inappropriately functioning cellular genes, e.g. overexpressing genes causing cancer. An important advantage of oligonucleotide derivatives is that in contrast to conventional drugs they affect directly genetic programs of organisms. The number of mRNA molecules is far less than the number of the protein molecules synthesized on the messengers and DNA is present at only a few copies per cell in the case of endogeneous genes or latent viruses. Therefore, in theory, only small amount of oligonucleotides is required to affect a given biochemical process. In contrast to the protein targeted drugs which can cause only temporal biochemical effects by inactivating specific proteins which will be produced again by the cell, the nucleic acid targeted oligonucleotides will inactivate or destroy the corresponding genetic material and stop completely production of the disease making protein. Therefore oligonucleotides offer the prospect of long acting therapeutics.

Since first attempts to develop reactive derivatives of oligonucleotides capable of modifying nucleic acids in sequence specific way (ref. 1), and to use oligonucleotides to control viruses proliferation (ref. 2), considerable progress in design and biological studies of oligonucleotide analogs and conjugates has been achieved (for reviews see refs. 3-10). Recent experimental studies demonstrate that chemical modifications of the natural oligonucleotides and synthetic analogs can improve pharmacological properties of the compounds and lead to the development of efficient therapeutics.

OLIGONUCLEOTIDES AS TARGETING STRUCTURES

A major advantage of oligonucleotides as targeting structures is the relatively simple rational design of the compounds capable of binding only to specific target nucleic acids. The principles of interaction of oligonucleotides with polynucleotides are well understood. Oligonucleotides can be directed in a Watson-Crick fashion toward complementary single-stranded nucleic acids or via triple-helix formation toward double-stranded DNA targets. Calculations show that oligonucleotides need to be only 15-18 nucleotides in

length to specify an unique target in human cell. Therefore, in principle, any sequenced nucleic acid unique to a disease can be targeted with corresponding oligonucleotides of reasonable length which form sufficiently stable complementary complexes in physiologic conditions and can be easily synthesized. It is important, that oligonucleotide derivatives targeted to different nucleic acids can be prepared in a rational way from a standard set of chemical precursors which provides the possibility to use universal technology to synthesize drugs for treatment various diseases. Chemical methods for synthesis of oligonucleotides have undergo dramatic improvement in the last years. Natural oligonucleotides and some analogs can now be synthesized using automated synthesizers in amounts and at prices which open up the possibility of large scale animal and human tests.

There are two types of nucleic acid targets for oligonucleotides. The first type is single stranded RNAs of which the most important are the viral RNAs and messenger RNAs. Since they are coding, sense nucleic acids, the complementary oligonucleotides have been given the name "antisense" oligonucleotides. The RNAs are located in cell cytoplasm (mRNA, viral RNA) and nucleus (new synthesized mRNA precursor species). It should be mentioned, that physiologic conditions, single-stranded RNAs are folded in compact structures and many of the sequences are not accessible for oligonucleotides because they are buried within the RNA structure. Therefore, for efficient targeting RNA, knowledge of the secondary structure of the molecules is needed. Experimentally it was found, that efficient targeting is usually achieved with oligonucleotides complementary to the 5'-terminal part of mRNAs and to the splice junctions of the mRNA precursors.

The second type of the nucleic acid targets for oligonucleotides is the double stranded DNA. It was found that homopurine-homopyrimidine sequences in dsDNA can be targeted with natural purine and pyrimidine oligonucleotides capable of forming triple-stranded complexes with DNA by binding into the wide groove of the corresponding homopurine-homopyrimidine sequences (for recent reviews see refs. 11-20). The possibility to use natural pyrimidine oligonucleotides as the targeting structures is limited because for the triple-stranded complex formation, protonation of cytidines is required and they bind to DNA only below pH 6. Purine oligonucleotides form triple-stranded complexes with DNA in physiologic conditions. Under controlled conditions binding of oligonucleotides to DNA can be very specific (e.g. ref. 19). However the possibility of specific targeting cellular DNA using the triple helix approach is questionable because of the existence of a few recognition schemes within the triple helices (ref. 11,12) and the possibility of formation of nonperfect triple-stranded complexes (refs. 21,22). To develop oligonucleotides capable of interacting in a sequence-specific fashion with mixed DNA sequences in physiologic conditions, a number of oligonucleotide analogs containing unnatural bases have been synthesized (refs. 13,17,23,24). It remains to be investigated also, to what extent cellular DNA is available for the triple helix formation in the chromatin structure.

The triple-stranded complex formation is not the only approach to target DNA. It was found, that oligonucleotides can form complementary complexes with some sequences in cellular DNA (ref. 25) which may be either true open by nature sequences or sequences which open up in the presence of oligonucleotides due to some yet unknown mechanism. Development of oligonucleotide analogs capable of opening double-stranded DNA structure due to formation of tight complementary complexes may provide a general approach to affect specific cellular genes and proviruses. One successful attempt to develop such compounds was the synthesis of oligomers consisting of thymine-linked aminoethylglycyl units. These analogs containing polyamide backbone form extraordinarily stable complementary complexes and can recognize their complementary target in dsDNA by strand displacement (ref. 26).

A number of oligonucleotide analogs was designed in order to obtain compounds with favourable therapeutic properties. Stability of oligonucleotides toward nucleases can be improved by modifications of the phosphate linkages or the sugar. The most commonly used approaches to prepare nuclease-resistant oligonucleotides were to substitute one of the non-bridging oxygen atoms on the phosphate groups with either a sulfur atom or a methyl group or to conjugate some bulky group to the 3'-end of oligonucleotides (for review see refs. 27-29).

Since the efficacy of oligonucleotide action will depend on concentration of the compounds in the cells, modifications were introduced in oligonucleotides to improve their cellular uptake. An example is the electroneutral methylphosphonates which enter cells more easily than the natural oligonucleotides. The problem is that each phosphorus atom of these compounds is a chiral center and the oligonucleotides are mixtures of isomers, which have different abilities to form complementary complexes. The methods for stereospecific synthesis of the compounds need further development to yield uniform population of the active molecules. To anchor oligonucleotides to the cell membrane and facilitate their uptake, oligonucleotides were conjugated to various lipophilic groups, e.g. cholesterol (refs. 30,31) and polymers known to be taken up by cells efficiently, e.g. polylysine (ref. 32). Receptor mediated delivery was used to

direct oligonucleotides to tumor cells overexpressing transferrin receptors. The targeted vehicle was the transferrin-polylysine conjugate; oligonucleotides were bound to the polylysine (33).

To enable oligonucleotides to modify the target nucleic acids covalently or to destroy them, various reactive groups were conjugated to the compounds (for reviews see refs. 5-7,34). Oligonucleotides were conjugated also to relatively nonspecific DNA-binding ligands such as planar aromatic structures intercalating themselves between the bases of nucleic acids and strengthening the complementary complexes (7). This allows to use short oligonucleotides as targeting structures in physiologic conditions, where the parent unmodified oligomers do not form stable complexes.

Although the most advantageous targets for oligonucleotides are nucleic acids, it should be noted, that the compounds can be used as specific inhibitors of certain proteins, in first line, polymerases of nucleic acids and regulatory proteins which recognize DNA and RNA substrates.

Recently developed combinational approaches imitating the natural process of evolution at the molecular level, allows to obtain oligonucleotides capable of recognizing specific proteins and small molecules. These oligomers have been given the name "aptamers"(ref. 35). The method resides on chemical synthesis of a very large random pool of oligonucleotides, each having a different sequence, selection of the molecules capable of binding to specific molecules by affinity chromatography or filter binding and enzymatic amplification of these oligonucleotides and their sequencing to identify the motif, providing the structure needed for the recognition.

PHARMACOLOGY OF OLIGONUCLEOTIDE DERIVATIVES

It was considered unlikely that natural negatively charged oligonucleotides could enter cells because they can not cross lipid membrane of the cell wall. However experiments have shown that incubation of cells with oligonucleotide derivatives leads to accumulation of the compounds in the cytoplasm and nucleus of cells (for reviews see refs. 36,37). The distribution pattern varied according to the cell system and oligonucleotide derivative used. The compounds were shown to be taken up by the endocytosis mechanism. Specific proteins at the surface of various cells have been detected which bind nucleic acids and may be specific receptors mediating the nucleic acids uptake (38,39). A number of modifications mentioned in the preceding section facilitate uptake of oligonucleotides by mammalian cells. Encapsulation of oligonucleotides in liposomes, viral envelopes and erythrocyte ghosts allowed efficient intracellular delivery of the compounds (36).

Natural oligonucleotides are rapidly degraded by nucleases present in cells and blood serum. 3'-endonucleases were identified as a principal source of the degradation. Conjugation of various bulky groups to oligonucleotides termini and modifications of the phosphate linkages (40-43) extended the serum half-life of oligonucleotides from minutes to hours and days.

Natural oligonucleotides and the methylphosphonate and phosphorothioate analogs showed low toxicity in animal tests (28,36,37). When injected into animals intravenously or intraperitoneally, oligonucleotides rapidly distribute themselves throughout all tissues and organs, the brain being the less accessible (36,44,45). Normal phosphodiester oligonucleotides and the methylphosphonates are excreted rapidly by kidney. Half-life of the compounds in the serum of mice was of the order of 20 min. However a part of the compounds was retained in tissues and a few hours post injection some material still was found there undegraded. A few injections per day can sustain concentration of the compounds in an organism compatible with that needed for the antiviral effect of oligonucleotides since in some cases oligonucleotide derivatives are efficient in tissue culture at a concentration of 0.1 μM which corresponds to a dose of 0.5 mg/kg body weight. In experiments with mice it was found that oligonucleotides can be introduced in organism by application to mucosa (the most efficient was the intranasal administration, ref. 46). Distribution of oligonucleotides in an organism and their fate depend on their interactions with proteins in the blood stream and at the cell surface. In addition to the above mentioned nucleic acids binding receptors, oligonucleotides and the phosphorothioate analogs bind to cellular CD4 receptors and to immunoglobulins present in the blood (ref. 47,48). These interactions are relatively nonspecific and occur at some anion binding areas of the proteins. The cholesterol-conjugated oligonucleotides bind to the CD4 receptor more tightly than the parent oligonucleotides. In the bloodstream, these modified oligonucleotides bind to the blood cells which results in longer circulation time of the undegraded compounds in the organism (36,49).

OLIGONUCLEOTIDES AS AFFINITY REAGENTS FOR MODIFICATION OF NUCLEIC ACIDS, SPECIFIC INHIBITORS OF GENE EXPRESSION AND ANTIVIRAL AGENTS

A great number of reactive derivatives of oligonucleotides have been synthesized (refs. 5-8,34), which are able to form crosslinks with nucleic acids (e.g. alkylating derivatives of oligonucleotides), or to

cleave nucleic acids (e.g. oligonucleotides bearing the groups producing free radicals). In experiments *in vitro* it was found that the derivatives can bring about sequence specific modification of both single stranded and double stranded nucleic acids. One application of these sequence specific reagents can be coupling of reporter groups to specific positions of nucleic acids for investigation of folding and interactions of the macromolecules. Another potential application is the sequence specific fragmentation of nucleic acids. The reagents are specific enough to consider them as potential tools for cleavage of DNA to large fragments for the purposes of mapping and sequencing of large genomes (ref. 19).

The main goal in design of oligonucleotide derivatives is the development of specific inhibitors of gene expression. In some cases oligonucleotide binding *per se* affects functions of the target nucleic acid by masking of a sequence needed for binding of a specific protein. The formation of triple stranded complexes of oligonucleotides with specific sequences in DNA recognized by some enzymes or regulatory proteins inhibits binding of the proteins to the DNA and can arrest transcription initiation (ref. 14,16,20). Oligonucleotides complementary to the 5'-terminal part of mRNAs arrest efficiently translation of the messengers in the cell free systems by interfering with binding of the initiation factors and ribosomes to the mRNAs. However oligonucleotides complementary to the central part of the messengers can be displaced by the translating ribosomes. They are only active when their complexes with RNA are substrates for ribonuclease H, an enzyme cleaving the RNA component in the DNA-RNA complexes, and the enzyme is present in the system (for reviews see 5,7).

A general approach to affecting functions of specific nucleic acids consists in chemical modification of the targets. Reactive oligonucleotide derivatives which bind to mRNA covalently, block the mRNA translation efficiently irrespective of the ribonuclease H presence.

For the therapeutic purposes efficient and nontoxic reactive derivatives of oligonucleotides need to be developed. To this end, attempts are made to design efficient catalytic groups which would react with nucleic acids repeatedly like the catalytic centers of enzymes. For cleaving RNA, the groups may be simple imitations of the catalytic center of ribonucleases or ribozymes or complexes of metals known to cleave RNA. Oligonucleotides equipped with such groups might be used for efficient temporal arrest of synthesis of specific cellular proteins and for inactivation of viral nucleic acids. For irreversible silencing of specific cellular genes, some modifications should be introduced in the DNA. This can be achieved by using oligonucleotide derivatives capable of covalent crosslinking to the regulatory region of the genes at the sites recognized by transcription factors. However the response of the repair system to such modifications should be investigated to predict long term effects.

Experiments with oligonucleotides targeted to specific cellular mRNAs or viral RNAs in infected cells evidence, that the compounds reach their targets in cytoplasm and can down regulate protein synthesis in cells. Oligonucleotides have been reported to inhibit growth of different viruses in cell culture, including such important viruses as human immunodeficiency virus (HIV) associated with AIDS, influenza, herpes and hepatitis B (3,4,8,10). Modifications of the oligonucleotides facilitating cellular uptake of the compounds, protecting them from nucleases and providing with ability of covalent or tight noncovalent binding to the target nucleic acids improved efficacy of the compounds.

In many cases the antiviral activity seemed to be the result of the complementary interactions of the compounds with target nucleic acids. There are also evidences that oligonucleotides may interfere with virus multiplication via some other mechanisms. The most thorough studies were carried out with HIV-1 (28,37). In experiments with the cells chronically infected with the virus, it was found that perfectly matched oligonucleotides are superior to the mismatched ones in inhibition of the HIV replication. However, in the case of the freshly infected cells, activity of oligonucleotides was relatively sequence independent. Thus, the phosphorothioate oligomers inhibited HIV replication even though mismatched oligonucleotides or homooligonucleotides were used. It was found that the compounds inhibit essential viral enzyme, reverse transcriptase (for reviews see 28,37,29). Another source of the antiviral activity of oligonucleotides arises from the interference of oligonucleotides with the virus-cell interaction. It was already mentioned, that oligonucleotides bind to CD4 receptors. It was also shown that they can compete for the receptor with the HIV-1 envelope glycoprotein gp120 (29,47) which targets the virus to the cells bearing CD4 receptors. The above mentioned mechanisms of the antiviral action of oligonucleotides explain why in many cases multiplication of different viruses was suppressed by "control" oligonucleotides and polynucleotides which are not supposed to form stable complexes with viral nucleic acids.

The results of a few reported animal tests on antiviral activity of oligonucleotides (3,4) are quite promising and suggest the existence of a few mechanisms for antiviral action of oligonucleotides. Oligonucleotides were reported to have protective effect in animals infected by different RNA viruses. The compounds showed substantial therapeutic activity at low doses and the effect was not strictly sequence specific. Although these effects might be rationalized as result of competition among the oligonucleotides

and viral nucleic acids for some proteins essential for virus development, stimulation of the immune system or some other defence mechanisms could not be excluded.

CONCLUSION

Development of the gene targeted oligonucleotide derivatives capable of affecting functions of nucleic acids in the living cell opens up unusual possibilities for rational design of therapeutics and offer the potential to revolutionize the pharmaceutical industry. The present state of development of the oligonucleotide derivatives allows one to hope for the development of the compounds for treatment of diseases for which there are presently no effective therapy. The key problems to be solved in the near future is the development of approaches for efficient delivery of the compounds into cells and design of the efficient nontoxic reactive groups which would allow the oligonucleotides to inactivate the genetic programs responsible for the disease irreversibly.

REFERENCES

1. A.M. Belikova, V.F. Zarytova and N.I. Grineva, *Tetrahedron Lett.* 37, 3557-3562 (1967).
2. P.C. Zamewcnik and M.L. Stephenson, *Proc. Natl. Acad. Sci. USA* 75, 280-285 (1978).
3. E. Wickstrom, ed., *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, Willey-Liss, New York, (1991).
4. J.S. Cohen, ed., *Oligonucleotides: Antisense Inhibitors of Gene Expression*, Macmillan Press, London (1989).
5. J. Goodchild, *Bioconjugate Chem.* 1, 165-174 (1990).
6. E. Uhlman and A. Peyman, *Chem. Rev.* 90, 543-584 (1990).
7. C. Helene and J.J. Toulme, *Biochim. Biophys. Acta* 1049, 125 (1990).
8. D.G. Knorre and V.V. Vlassov, *Genetica* 85, 53-63 (1991)
9. D.G. Knorre and V.V. Vlassov, *Biomed. Sci. (USSR)*, 1, 334-343 (1990).
10. E. Wickstrom, *Trends Biotechnol.* 10, 281-287 (1992).
11. J.M. Chubb and M.E. Hogan, *Trends Biotechnol.* 10, 132-136 (1992).
12. L.J. Maher III, P.B. Dervan and B. Wold, in E. Wickstrom, ed., *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, pp. 227-242, Willey-Liss, New York, (1991).
13. M.D. Matteucci and N. Bischofberger, *Annu. Reports Med. Chem.* 26, 287-296 (1991).
14. L.J. Maher III, *Biochemistry* 31, 7587-7594 (1992).
15. C. Giovannangeli, N.T. Thuong and C. Helene, *Nucl. Acids Res.* 20, 4275-4281 (1992).
16. W.M. McShan, R.D. Rossen, A.H. Laughter, J. Trial, D.J. Kessler, J.G. Zenguei, M.E. Hogan and F.M. Orson, *J. Biol. Chem.* 267, 5712-5721 (1992).
17. S.H. Krawczyk, J.F. Milligan, S. Wadwani, C. Moulds, B.C. Froehler and M. Matteucci, *Proc. Natl. Acad. Sci. USA* 89, 3761-3764 (1992).
18. P.A. Beal and P.B. Dervan, *J. Am. Chem. Soc.* 114, 4976-4982 (1992).
19. T.J. Povsic, S.A. Strobel and P. Dervan, *J. Am. Chem. Soc.* 114, 5934-5941 (1992).
20. M. Grigoriev, D. Praseuth, P. Robins, A. Hemar, T. Saison-Behmoaras, A. Dautry-Varsat, N.T. Thuong, C. Helene and A. Harel-Bellan, *J. Biol. Chem.* 267, 3389-3395 (1992).
21. D. Pei, H.D. Ulrich and P.G. Shultz, *Science* 253, 1408-1441 (1992).
22. E.B. Brossalina, E. Demchenko and V.V. Vlassov, *Antisense Res. Devel.* in press (1992).
23. P.S. Miller, P. Bhan, C.D. Cusman and T.L. Trapane, *Biochemistry* 31, 6788-6793 (1992).
24. J.S. Koh and P.B. Dervan, *J. Am. Chem. Soc.* 114, 1471-1478 (1992).
25. V.V. Vlassov, N.D. Kobetz, E.L. Chernolovskaya, S.G. Demidova, R.G. Borissov and E.M. Ivanova, *Mol. Biol. Reports* 14, 11-15 (1990).
26. P.E. Nielsen, M. Egholm, R.H. Berg and O. Buchardt, *Science* 254, 1497-1500.
27. P.S. Miller, *Biotechnology* 9, 358-361 (1992).
28. S. Agrawal and P.S. Sarin, *Adv. Drug Delivery Rev.* 6, 251-270 (1991).
29. C.A. Stein, J.L. Tonkinson and L. Yakubov, *Pharmac. Ther.* 52, 365-384 (1991).
30. A.S. Boutorin, L.V. Gus'kova, E.M. Ivanova, N.D. Kobetz, V.F. Zarytova, A.S. Rytel, L.V. Yurchenko and V.V. Vlassov, *FEBS Lett* 254, 129-132 (1989).
31. F. Farooqui, P.S. Sarin, D. Sun and R.L. Letsinger, *Bioconjugate Chem.*, 2, 422-426 (1991).

32. J.-P. Leonetti, G. Degols and B. Lebleu, *Bioconjugates Chem.* 1, 149-153 (1990).
33. G. Citro, D. Perrotti, C. Cucco, I. D'Agnano, A. Sacchi, G. Zupi and B. Calabretta, *Proc. Natl. Acad. Sci. USA*, 89, 7031-7035 (1992).
34. D.G. Knorre and V.F. Zarytova, in E. Wickstrom, ed., *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, pp. 195-218, Willey-Liss, New York, (1991).
35. A.D. Ellington and J. Szostak, *Nature* 346, 818-822 (1990).
36. V.V. Vlassov and L.A. Yakubov, in E. Wickstrom, ed., *Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS*, pp. 243-266, Willey-Liss, New York, (1991).
37. S. Agrawal and P.S. Sarin, M. Zamecnik and P.C. Zamecnik, in R.P. Erickson and J.G. Izant, eds., *Gene Regulation : Biology of Antisense RNA and DNA*, pp. 273-283, Raven Press, Ltd., New York (1992)
38. S.L. Loke, C.A. Stein, X.H. Zhang, K. Mori, M. Nakanishi, C. Subasinghe, J.S. Cohen and L.M. Necker, *Proc. Natl. Acad. Sci. USA* 86, 3474-3478 (1989).
39. L.A. Yakubov, E.A. Deeva, V.F. Zarytova, E.M. Ivanova, A.S. Ryte, L.V. Yurchenko and V.V. Vlassov, *Proc. Natl. Acad. Sci. USA* ?, 6454-6438 (1989).
40. T.V. Abramova, V.V. Vlassov, V.F. Zarytova, E.M. Ivanova, E.A. Kuligina and A.S. Ryte, *Nucleosides Nucleotides*, 10, 639-640 (1991).
41. A.S. Boutorine, C. Boiziau, T. LeDoan, J.J. Toulme and C. Helene, *Biochimie*, 74, 485-489 (1992)
42. S. Akhtar, R. Kole and R.L. Juliano, *Life Sci.*, 49, 1793-1801 (1991)
43. J.-P. Shaw, K. Kent, J. Bird, J. Fishback and B. Froehler, *Nucl. Acids Res.*, 19, 747-750 (1991)
44. J. Goodchild, B. Kim and P.C. Zamecnik, *Antisense Res. Devel.*, 1, 153-160 (1991)
46. V.N. Karamyshev, L.A. Yakubov and V.V. Vlassov, *Antisense Res. Devel.*, in press (1992)
47. L. Yakubov, L. Yurchenko, M. Nechaeva, E. Rykova, V. Karamyshev, J. Tonkinson, V. Vlassov and C.A. Stein, *Nucl. Acids Res.*, Symp. Ser. 24, 311 (1991).
48. L.V. Pautova, E.Y. Rykova, L.A. Yakubov and V.V. Vlassov, *Mol. Biol. (Russian)*, 43, in press (1992)
49. P. C. Smidt, T.L. Doan, S. Falco and T.J.C. Berklei, *Nucl. Acids Res.* 19, 4695-4700 (1991).
50. C. Boiziau, R. Kurfurst, C. Cazenave, V. Roig, N.T. Thuong and J.-J. Toulme, *Nucl. Acids Res.*, 19, 1113-1119, (1991)
51. L.W. Whiesell, A. Rosolen and L.M. Neckers, *Antisense Res. Devel.*, 1, 343-350 (1991)