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Review

Antisense Pharmacodynamics: Critical Issues in the Transport and Delivery of Antisense Oligonucleotides

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This review critically examines current understanding of the kinetics and biodistribution of antisense oligonucleotides, both at the cellular level and at the level of the intact organism. The pharmacodynamic relationships between biodistribution and the ultimate biological effects of antisense agents are considered. The problems and advantages inherent in the use of delivery systems are discussed in the light of further enhancing *in vivo* pharmacological actions of oligonucleotides.

KEY WORDS: antisense; oligonucleotides; drug delivery; pharmacodynamics; biodistribution.

INTRODUCTION

This is a critical juncture in the development of antisense oligonucleotides as pharmaceutical agents (1). Antisense is rapidly moving from being a laboratory technology to becoming a full-fledged strategy for therapy of human diseases. That antisense can work effectively in cell culture has been quite clear for some time; more recently, promising results in animal models have led the way to the first round of human clinical trials. At present, there are at least 11 clinical trials underway using antisense compounds directed at various targets that play a role in cancer (2–4), viral diseases (4), and inflammatory disorders (5). Virtually all clinical trials now in progress utilize first generation phosphorothioate oligonucleotides administered either systemically, or locally into diseased areas. However, a variety of chemically modified and potentially more effective oligonucleotides are in the development pipeline, while increasing consideration is also being given to means for more efficiently delivering oligonucleotides to their therapeutic targets in the body.

It has often been pointed out that the Chinese character for "crisis" incorporates the symbol for "danger" as well as the symbol for "opportunity." In like manner, this critical time for antisense research clearly has elements of both danger and opportunity. The danger may lie in blindly forging ahead with the development of antisense molecules as drugs while ignoring a number of serious issues that have emerged as antisense research has proceeded from the tissue culture dish, to animals, to the clinic. Opportunity will likely come from a concerted elucidation of fundamental aspects of oligonucleotide biodistribution, interactions with their biological targets, and relationships to therapeutic effects.

The intent of this mini-review is to identify and analyze some of the key issues, problems and controversies in the field of antisense pharmacology, particularly in relation to the biodistribution and pharmacodynamics of oligonucleotides. It is not intended as a survey or overview, and thus we will inevitably fail to cite many important contributions to the field; our apologies in advance to any we may slight. Over the last several years there have been several excellent reviews concerning both chemical (6–9) and biological aspects (10–15) of antisense oligonucleotides; the reader is referred to these for a broader overview of the antisense field. The current article will try to present an objective consideration of the issues addressed, but it also clearly reflects the biases and enthusiasms of the authors.

In our minds, one of the major problems with current antisense pharmacology is a lack of full understanding of the *pharmacodynamics* of antisense molecules, that is the relationship between the level and duration of drug at the target site and the therapeutic effect. One way to highlight this issue is to compare current understanding of antisense oligonucleotides with that of more conventional drug candidates. For low molecular weight drugs, the development process is accompanied by an extensive analysis of biodistribution, metabolism, and drug interactions with targets (16). Information is assembled on blood clearance kinetics, on re-distribution to tissues and persistence therein, on the formation of altered metabolic products including both anabolites and catabolites, and most importantly, on the relationship between drug levels at the target site and therapeutic outcome. Powerful analytical technology is available that readily allows quantitation of the drug and its products in various body compartments. In addition, elegant receptor-binding and biochemical or physiological measurements permit a careful evaluation of the relationship between drug levels at the

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target and therapeutic effect. In other words, the pharmacodynamics of the drug candidate is fully understood before the candidate advances into the clinic. In contrast, the level of understanding of the biodistribution and target interactions of oligonucleotides is substantially less sophisticated, as we will discuss below. This is not surprising since antisense compounds are an entirely new class of therapeutic agents, and are larger, more complex molecules than conventional drugs. However, these problems do not diminish the importance of striving for a fuller understanding of antisense oligonucleotide pharmacodynamics.

UPTAKE AND ACTIONS OF ANTISENSE OLIGONUCLEOTIDES IN CELL CULTURE

Over the last decade antisense oligonucleotides have emerged as an important tool for laboratory investigation. In theory, one should be able to use an appropriate antisense molecule to selectively ablate the expression of the RNA and protein products of a single gene. However, it has become clear that the biological actions of oligonucleotides are quite complex, and attainment of a pure, selective, antisense effect is sometimes difficult. Indeed, much of the early work on oligonucleotide effects in cell culture is probably flawed, with investigators erroneously interpreting biological actions of oligonucleotides as being antisense effects when indeed they were not. A growing consensus (17,18) concerning the pitfalls of antisense experiments and the definition of appropriate control experiments have helped to reduce the number of problematic studies. There are a number of ways in which oligonucleotides can exert biological and biochemical effects in a non-antisense manner (14,19). Perhaps the most common relates to so-called aptameric effects of oligonucleotides (20), based on the fact that these molecules can fold into complex 3-dimensional structures. Oligonucleotide aptamers can bind to receptors, enzymes and other proteins and affect their function; this can occur in an sequence-specific fashion, thus tending to confound simple control strategies such as comparing antisense to sense or scrambled controls. Oligos containing so-called "G-quartets" are particularly prone to engage in specific interactions with proteins (21,22). In addition, oligonucleotides with a phosphorothioate backbone are known to be non-specifically "sticky" with a propensity to interact with a wide variety of proteins in a sequence-independent manner (14). Another important non-antisense action of oligonucleotides relates to their effects on the host defense system. Thus, certain types of oligonucleotides, particularly those with CpG motifs, are potent B-cell mitogens (23), while phosphorothioates are known to affect the clotting and complement systems (24). Finally, one should remember that oligonucleotides can eventually break down into nucleotides, nucleosides, and bases, and that these entities can have potent pharmacological effects in their own right (14,18,25). Despite these problems, there are now numerous examples of antisense studies in cell culture where all currently accepted standards have been met and where it seems extremely likely that the observed effects are due to a true antisense action (reviewed in (14)).

In order to exert their pharmacological and biological effects, antisense molecules must enter cells and interact with pre-mRNA and mRNA in the nucleus and cytoplasm. In many instances, the pharmacological activity is due to binding of the antisense molecule to the target RNA and subsequent degradation of the RNA by RNaseH (14,26); however, there are also some well documented examples of non-RNaseH-dependent antisense mechanisms (27–29). Most studies have found that oligonucleotides are initially taken up by cells through endocytosis and accumulate in an endosomal-lysososomal compartment (30); this is true for both charged and uncharged backbone oligonucleotides (31,32). Thereafter, some of the oligonucleotide is released from endosomes and enters the cytoplasm by an as yet undefined mechanism; once in the cytoplasm the oligonucleotide can rapidly enter the nucleus and accumulate there (33,34), presumably permitting interactions with nuclear RNA species. Thus, oligonucleotides initially accumulate in an endosomal-lysosomal compartment that represents a pharmacologically non-productive site, a sort of detour. Because of this, agents or techniques that promote the release of oligonucleotides from endosomes (or that bypass the endosomal compartment) should enhance the pharmacological effectiveness of antisense molecules (see Fig. 1). This seems to be the case, since in most cell culture studies, free antisense oligonucleotides are ineffective but become effective in the presence of a facilitator or delivery agent (14,30,35). In some cases it may be possible to attain antisense effects without a delivery agent by utilizing high concentrations of stable oligonucleotides (36), but this clearly seems to be the exception rather than the rule.

A variety of "adjuvants" or delivery agents have been used to enhance the cytoplasmic and nuclear accumulation of antisense oligonucleotides; this includes cationic lipid complexes, polypeptides, dendrimers, surfactants, liposomes and other agents (14,35,37). The "gold standard" and most widely used delivery approach involves complexation of the oligonucleotides with cationic lipids such as Lipofectin® as originally described by Bennett et al (38). The mechanism involved in antisense delivery mediated by such cationic lipid complexes is still not fully understood (39) although new insights are beginning to emerge (40). Recent work has shown that the lipid-oligonucleotide complex is internalized by endocytosis; thereafter the complex probably induces a flip-flop of anionic phospholipids in the endosome membrane, leading to neutralization of the cationic lipid charge, displacement of the bound oligonucleotide, and release from the endosome (41). Current

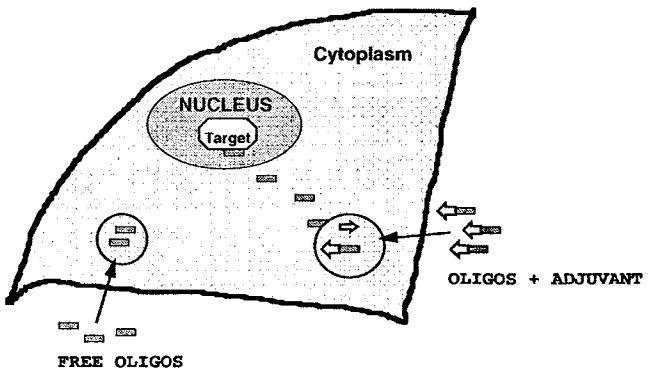

Fig. 1. Oligonucleotide delivery adjuvants. Free oligonucleotides (dark rectangles) are initially taken up into endosomal/lysosomal compartments. Use of a delivery "adjuvant" such as a peptide, polymer, or lipid carrier (represented by arrows) can enhance both total uptake, and release of the oligonucleotide from endosomes to the cytoplasm and nucleus.

observations suggest that the pharmacologically active antisense oligonucleotides are released from the cationic lipid complexes and enter the nucleus as free oligonucleotides prior to the onset of the antisense effect (42). Modified forms of cationic liposomes are under consideration for *in vivo* delivery of antisense molecules (43,44). However, the very large size (45) and high surface charge density of cationic lipid complexes clearly present some major obstacles in terms of systemic use.

Another popular approach has been to couple antisense molecules to various types of peptides designed to enhance intracellular delivery. For example, some workers have coupled oligonucleotides to peptides intended to promote interactions with cell surface receptors, thus potentially targeting the oligonucleotides to specific cell types an/or enhancing uptake by receptor mediated endocytosis. This has included use of polypeptides that interact with growth factor receptors (46), and RGD containing peptides that bind to integrins (47). Another approach has been to use peptides that de-stabilize membranes, thus either promoting release from endosomes or passage across the plasma membrane (48,49). Yet another approach has been to link oligonucleotides to peptides that modify the intracellular retention and distribution of the oligonucleotides (50). Thus far the pharmacological results with peptide-oligonucleotide conjugates have not been impressive; however, there is increasing interest in the use of various peptide "delivery modules" for intracellular delivery of both proteins and nucleic acids (51–53), and thus this technology is likely to evolve rapidly.

Dendrimers, that is, repetitively branched polymers, have also attracted some interest as deliver agents for both genes and oligonucleotides (54–56). One promising aspect of dendrimers is that the delivery complex can be of relatively modest molecular size (55), as is also true of peptide-oligonucleotide conjugates, but not of cationic lipid complexes. A number of other approaches for enhanced oligonucleotide delivery *in vitro* have also been employed; this includes novel surfactants (35), conjugation with lipophiles such as cholesterol (57), use of toxins capable of permeabilizing membranes, and even electroporation (58). A particularly promising new technology is the use of biodegradable nanoparticles as carriers of antisense molecules. Several interesting reports on this approach have appeared recently suggesting that nanoparticles can enhance oligonucleotide effects in biological systems (59–61).

Many questions remain unresolved concerning the uptake, sub-cellular distribution and site of action of antisense oligonucleotides. For example, the exact intracellular site where the major pharmacological effects of oligonucleotides occur remains undefined. However, several lines of evidence suggest that accumulation in the nucleus is important if not essential for antisense activity. The major improvement in effectiveness of antisense oligonucleotides produced by cationic lipid delivery systems coincides with up to a 1000 fold increase in the nuclear concentration of the oligonucleotides (38). As mentioned above, free oligonucleotides rapidly migrate to the nucleus when microinjected to the cytoplasm (34) or when released from the lipid complexes (41,42). The fact that pre-mRNA splicing, a nuclear event, can be targeted by antisense oligonucleotides also points to the nucleus as an important site of action (62). Further, there is no real information on the concentration of antisense oligonucleotide at the intracellular target site that is actually required for a given pharmacological effect (i.e., little information on pharmacodynamics at the cell level).

There is also a paucity of information about the delivery agents currently being employed. In many cases it is not even clear whether a particular agent will work in the presence of the blood plasma proteins found *in vivo*. Many of the agents used to date interact strongly with the cell membrane. Thus they are likely to have profound effects on membrane receptors and signal transduction systems, and consequently on many downstream aspects of cell function; this has not yet been studied with great care.

One important issue to note is the high degree of variability of cellular uptake of oligonucleotides. Thus, there are major cell-type specific differences in the uptake of "free" oligonucleotides, and in the response to delivery agents; this seems to be particularly true for hematopoietic cells (63,64). In addition, even within a given cell type, there are often major cell-to-cell differences in oligonucleotide accumulation that do not seem to have any obvious basis in terms of cell morphology or growth state (56).

IN VIVO ACTIONS OF OLIGOS

During the last few years many interesting examples have emerged concerning the use of antisense oligonucleotides in the *in vivo* setting. This has included oligonucleotides directed against genes involved in tumor growth (65–68), inflammatory conditions (69,70), cardiovascular disease (22,71,72), and in regulation of CNS functions (73). In some cases, the *in vivo* pharmacological action of the administered oligonucleotides was clearly due to a non-antisense mechanism (22). In many other cases, while an antisense mechanism was presumed, there was insufficient data to fully support this presumption. However, there are also a number of reports where most accepted criteria for documenting an antisense effect have been met, suggesting that true antisense actions are attainable *in vivo* (65,69,74,75).

It is important to note that there is currently a major dichotomy between *in vitro* and *in vivo* studies of antisense effects. As mentioned in Sec 1 above, in the *in vitro* setting the attainment of antisense effects almost always involves use of a delivery agent of some type; "naked" oligonucleotides rarely produce an effect. However, the *in vivo* examples mentioned above all involve the administration of free oligonucleotides without use of any delivery entity. Further, initial clinical trials, some of which seem promising (3,5), also utilize free oligonucleotides. There are a number of potential explanations for this odd dichotomy between *in vitro* and *in vivo* observations. One possibility is that there are fundamental differences in oligonucleotide uptake and transport processes between cells in culture and cells in a tissue environment. In support of this notion, there is abundant evidence that cells engaged in an appropriate interaction with a 3-dimensional extracellular matrix (as is the case in tissues) can have very different patterns of gene expression and differentiation as compared to cells in a 2-dimensional culture environment (76,77). Further, cells can change their ability to respond to certain drugs as they are taken from the tissue environment and placed in culture (78). However, the dichotomy between antisense effects *in vitro* and *in vivo* seems to apply to all gene targets tested thus far, and to all cell and tissue types examined thus far. It seems rather

remarkable that transfer from the *in vivo* to the cell culture environment would universally select for loss of oligonucleotide transport capabilities. Another possibility, however, is that the dichotomy between *in vivo* and *in vitro* observations is more apparent than real. One issue is the different design of *in vitro* versus *in vivo* studies, with the latter usually being much more protracted. Thus, in animal experimentation the oligonucleotides are often administered using a sustained infusion or by multiple injections over a period of days. By contrast, in cell culture experiments the cells are exposed to the oligonucleotides for a period of a few hours and the result assessed a few hours later. In the *in vivo* setting, the prolonged period of drug administration may allow build-up of oligonucleotide in the endosomal-lysosomal compartment and gradual transfer to cytoplasm and nucleus, where a sustained effect on the target message can be attained. In contrast, the transient uptake of free oligonucleotides by cells in culture may not be sufficient to permit significant antisense actions, whereas use of a delivery agent not only increases total cell uptake and release from endosomes, but probably also enhances the persistence of the oligonucleotides in the cell. In other words there may be a pharmacodynamic basis for the dramatic differences observed in the actions of oligonucleotides *in vitro* versus *in vivo*; this is certainly a testable concept.

PHARMACOKINETICS, METABOLISM AND TISSUE DISTRIBUTION OF OLIGONUCLEOTIDES

Our knowledge of the pharmacodynamics of oligonucleotides *in vivo* is rapidly growing, but is still limited. Over the last few years there have been a number of careful studies of the blood clearance kinetics and organ distribution of oligonucleotides administered to animals and to patients. Many of these studies have been discussed in recent review articles (11–13,79). Most of the *in vivo* observations on the biodistribution of oligonucleotides have dealt with molecules having a phosphorothioate background; in this case, the following general statements can be made. (a) The biphasic plasma half lives of phosphorothioate oligonucleotides are in the range of minutes for $t1/2\alpha$ (distributional phase) and many minutes to several hours for $t1/2\beta$ (elimination phase). (b) The oligonucleotides distribute widely in the body and are accumulated in most tissues, particularly the liver, but excepting the CNS. (c) Although phosphorothioate oligonucleotides are significantly protein bound, particularly at low doses, the primary route of elimination is via the kidneys. (d) Metabolism is complex, but breakdown by 3′-exonucleases is important (80). (e) In general, the pharmacokinetic behavior of phosphorothioate oligonucleotides in humans is similar to that in lower animals (12,81).

An interesting recent finding is that so-called scavenger receptors on liver cells and other cell types may play an important role in oligonucleotide clearance (82,83). Another interesting new development concerns initial studies of the pharmacokinetics and biodistribution of oligonucleotides other than phosphorothioates (84). In particular, a substantial amount of work is being done on various mixed backbone oligonucleotides and on "gapmers" having a phosphodiester or phosphorothioate central region protected by 2′-O-alkyl modified residues (85–87). There has also been considerable interest recently in the enteric uptake and hepatic processing of oligonucleotides (12,88–90). In that context, it seems clear that very stable forms of oligonucleotides have an appreciable permeation rate across the GI epithelium, are cleared or degraded to only a modest degree during passage through the portal circulation and liver, and thus these stable compounds have some degree of oral bioavailability.

An issue that has only been addressed to a limited degree is the cellular and subcellular distribution of oligonucleotides *in vivo*. It is to be expected that different types of cells will have different affinities and uptake capacities for various types of oligonucleotides. In addition, the patterns of cellular uptake may also reflect concentration gradients of oligonucleotides between blood and extracellular fluid or between well perfused and poorly perfused regions of an organ. Recent careful immunofluorescence, immunochemical, and autoradiographic studies have shown a great variability in oligonucleotide uptake into different cells types (91). For example, little uptake into muscle cells was noted, while certain phagocytic cells, kidney proximal tubule cells, and liver endothelial cells showed significant uptake. A substantial amount of oligonucleotide is also accumulated in the extracellular matrix (92), but this may turn over more quickly than intracellular accumulations (91). An interesting sidelight is provided by the findings that liver endothelial cells show a very high level of oligonucleotide accumulation when these compounds are administered either systemically (82,91) or by perfusion of the portal system (88) (see Fig. 2). An important point to note is that studies thus far (e.g., (88)) have failed to detect significant amounts of oligonucleotide in the nuclei of cells *in vivo*. This is somewhat disconcerting since it seems likely that true antisense effects (at least those due to RNase H) occur within the nucleus. Further, in cell culture studies there is usually a good correlation between the magnitude of the antisense effect in a cell population and the presence of observable accumulation of oligonucleotides in the nucleus (56). Hence the subcellular distribution of oligonucleotides observed thus far in the *in vivo* setting is somewhat at variance with the several well documented examples of true *in vivo* antisense pharmacological effects.

IN VIVO DELIVERY SYSTEMS FOR OLIGOS?

An important question to ask at this point is whether the notion of developing drug delivery systems for antisense oligonucleotides is supported by current insights into oligonucleotide biodistribution. A strong reason not to invest in delivery technology is provided by several clear examples of the attainment of antisense effects in animals using "free" oligonucleotides, in the absence of any delivery modality. If the drug itself works, why bother with the complexities of a delivery system? Another, more hypothetical, issue is that any delivery moiety will add to the size and complexity of the antisense pharmacophore, thus possibly limiting its access to certain body compartments, and perhaps opening new avenues for sequestration and degradation. Against this line of argument, one might cite some of the classic reasons for employing a delivery modality, including protection against degradation, increased cell uptake, reduced toxicity, and the possibility of "targeting" the drug to specific cells or tissues. In the case of antisense oligonucleotides, it seems likely that the effective work of oligonucleotide chemists will soon provide compounds of such rock-like stability that the protective role of a delivery system will be a moot

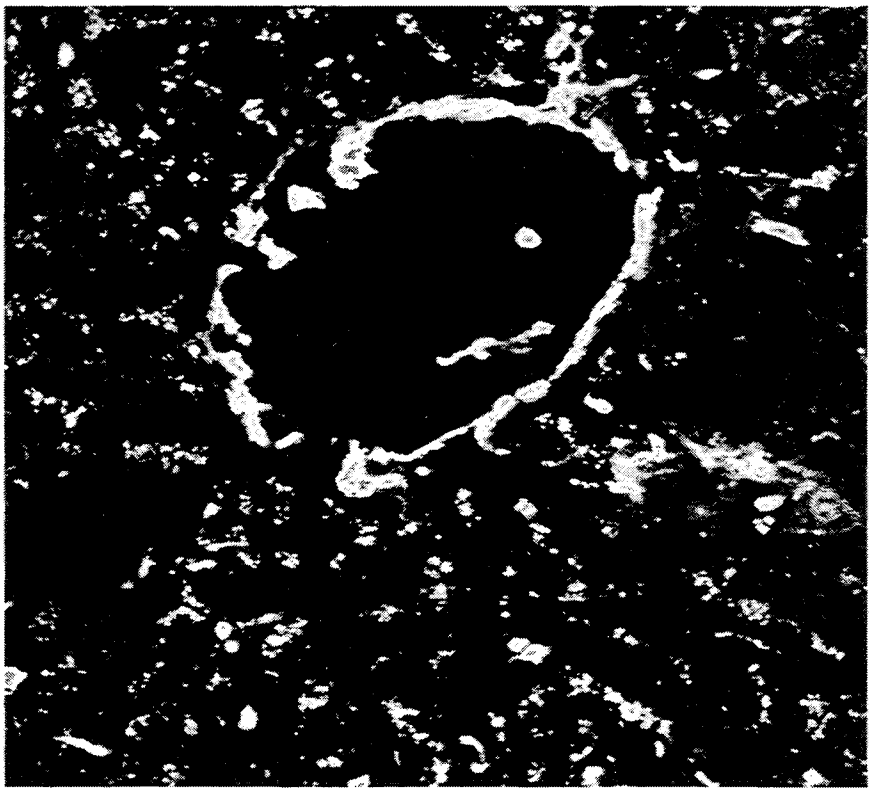

Fig. 2. Tissue distribution of oligonucleotides. Phosphorothioate oligonucleotides labeled with a cyanine fluor were complexed with lipofectin and perfused through a rat liver (88). Sections of the organ were examined by confocal fluorescence microscopy. The image shows substanital uptake by the endothelial lining of a blood vessel (center of field) and by occasional interstitial cells that may be Kupffer cells; little uptake by hepatocytes was noted.

issue. In like manner, although oligonucleotides are not devoid of toxicity, even first generation phosphorothioates are relatively non toxic, and newer chemically modified oligonucleotides, which display reduced non-specific protein binding, will likely be even less toxic (12). However, the problem of poor cellular uptake and nuclear accumulation of antisense oligonucleotides seems to remain as a potentially important task for delivery systems. The fact that antisense effects have been attained *in vivo* with free oligonucleotides does not exclude the possibility that more robust effects might be attained through use of a delivery modality. The persistent dichotomy between *in vitro* and *in vivo* studies concerning the need for agents such as cationic lipids in attaining antisense effects also argues for continuing to explore the role of delivery systems in antisense therapeutics.

If one is to use a delivery entity in conjunction with antisense oligonucleotides, what sort of entity should be used? As discussed above, there has been a great deal of *in vitro* experimentation using liposomes, cationic lipid complexes, polymers and other agents that give rise to oligonucleotide delivery complexes that are large macromolecules or supramolecular in scale (i.e., the complex has a mass of 10^6 daltons or greater). Aside from concerns about toxicity, such large complexes are clearly going to compromise one of the real advantages of antisense oligonucleotides; that they are relatively small in size and can thus penetrate widely into tissue compartments. This line of discussion suggests that efforts should be concentrated on delivery moieties that are of modest molecular size, for examples peptides or lipophilic adductants. Use of this approach should result in an oligonucleotide-delivery agent complex or conjugate that is still of relatively low molecular weight and thus potentially capable of broad biodistribution.

THE NEED FOR NEW TOOLS

Further progress in antisense therapeutics would be facilitated by the availability of several types of improvements in oligonucleotides and the means to study their actions and behavior. Obviously improvements in the chemical and biological characteristics of antisense compounds themselves will be important. Rapid progress is currently being made in the creation of new chemical entities that provide increased resistance to degradation, reduced spurious interactions with proteins, and increased affinity for RNA targets. Nucleic acid chemists are exploring a variety of modifications that enhance the desirable properties of antisense compounds; among the promising approaches are morpholino-based oligonucleotides having an uncharged backbone (6) and 2-O-alkyl modified antisense "gapmers" (85,93). Recent studies have also open the way to side-step the issue of the role RNaseH in antisense action. To date the ability of an antisense oligonucleotide to attain a pharmacological effect has usually (though not universally) been correlated with its ability to activate RNaseH; activation of this enzyme requires a "DNA-like" oligonucleotide, thus

only such chemistries could be considered for antisense development. However, recent work has identified a novel mammalian double stranded RNase activity that cleaves RNA duplexes (94); this potentially opens the door to the design of new "RNA-like" antisense oligonucleotides thus increasing the range of possible chemistries.

One of the key problems in antisense therapeutics is the identification of optimal sites for oligonucleotide complexation on the target RNA under study. In general, computer predictions of RNA folding patterns and of oligonucleotide-RNA melting temperatures do not provide an accurate prediction of the pharmacological efficacy of antisense compounds. Thus, to date, therapeutically directed antisense research has relied upon a "shotgun" type initial screen in cell culture using a large number of antisense compounds that blanket the target RNA. This is a rather inefficient way to proceed, and there is a great deal of interest in developing cell-free screens that will accurately predict accessible sites on RNA thus permitting the design of efficacious antisense molecules (19). Recently there have been reports of successful identification of potent antisense oligonucleotides using heteroduplex formation in a combinatorial oligonucleotide array anchored on a surface (95), and using an oligonucleotide combinatorial library in solution coupled with RNase H mapping (96). These are promising developments, but their general applicability remains undefined. It is important to remember that RNA *in vivo* is decorated with a complex array of proteins that may affect the binding of oligonucleotides (97).

In the context of antisense pharmacodynamics, an important issue is the accurate measurement of oligonucleotides and their metabolic products in biological samples. A very promising development in this regard is the rapidly evolving using of mass spectrometry to detect and identify nucleic acids (98). Both electrospray (99,100) and laser assisted desorbtion (101,102) techniques have been used to quantitate oligonucleotides. Capillary electrophoresis provides another important new tool for analysis of antisense molecules (103–106). However, in the long run the conjoint use of HPLC and mass spectrometry (98) promises to provide unrivaled precision and sensitivity.

Another important issue involves improved means for evaluating the intracellular delivery and pharmacological efficacy of antisense compounds. An important development in this regard is the discovery that antisense molecules can be used to correct splicing of pre-mRNA, rather than to degrade RNA. Thus, Kole and colleagues have found that non-RNaseH activating 2-O-alkyl or morpholino oligonucleotides can force the alternative selection of splice sites, for example correcting the abnormal splicing of thalassemic hemoglobin RNA (28). An intriguing aspect of this for antisense oligonucleotide pharmacodynamics is that the antisense effect now provides a positive readout (more correct message) rather than an inhibition; this permits more sensitive and more accurate determination of the antisense action. This aspect has now been converted into an elegant tool for measurement of the delivery and efficacy of oligonucleotides by inserting an abnormal intron into the luciferase gene; effective delivery of the oligonucleotide then results in splicing out of the intron and upregulation of luciferase activity (107) (see Fig. 3). Assays of this type with luciferase or with other reporter genes set the stage for more precise evaluation of the *in vivo* delivery and efficacy of antisense compounds. One drawback of this approach is that it only pertains to oligonucleotides that do not activate RNaseH; however, this development may trigger other clever approaches for more precise analysis of antisense activity.

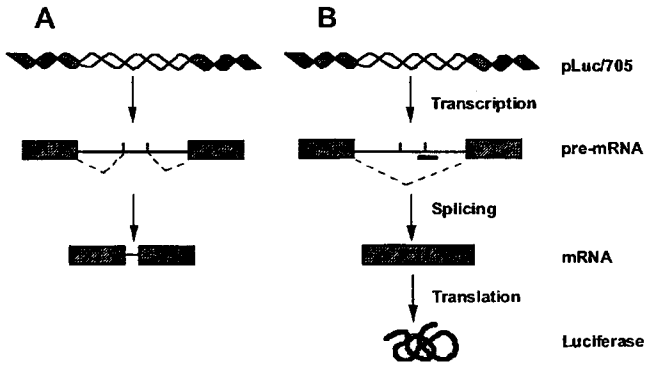

Fig. 3. Activation of luciferase expresion by an antisense oligonucleotide that modifies splicing. (A) An abnormal intron inserted into the luciferase gene results in the production of a non-translatable RNA. (B) An antisense oligonucleotide to a cryptic splice site allows the intron to be spliced out, leading to expression of normal luciferase mRNA and protein product. Thus, the effect of the antisense oligonucleotide can be monitored by read-out of the luciferase.

Thus, a number of new tools are emerging that will increase the scope and precision of antisense research. Hopefully, this will result in a firmer intellectual base for antisense therapeutics.

SUMMARY

It is hoped that this brief review will indicate the current state of research on antisense transport and delivery. Clearly, many questions remain about the biodistribution and pharmacological effects of oligonucleotides. It seems likely that the evolution of sophisticated new tools to study the pharmacokinetics and pharmacodynamics of these molecules will permit increasingly rapid progress in this area.

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