37. Fornander T, Hellstrom AC, Moberger B. Descriptive clinicopathologic study of 17 patients with endometrial cancer during or after adjuvant tamoxifen in early breast cancer. *J Natl Cancer Inst* 1993, **85**, 1850–1855.
38. Fisher B, Costantino JP, Redmond CK, *et al.* Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP)B14. *J Natl Cancer Inst* 1994, **86**, 527–573.
39. Fornander T, Rutqvist LE, Cedermark B, *et al.* Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. *Lancet* 1989, **i**, 117–120.
40. Van Leuwen FE, Benraadt J, Coeberg JWW, *et al.* Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet* 1994, **343**, 448–452.
41. Boronow RC, Morrow CP, Creasman WT, *et al.* Surgical staging in endometrial cancer: clinical-pathologic findings of a prospective study. *Obstet Gynecol* 1984, **63**, 825–831.
42. Killackey MA, Hakes TB, Pierce VK. Endometrial adenocarcinoma in breast cancer patients receiving antiestrogens. *Cancer Treat Rep* 1985, **69**, 237–238.
43. Friedl A, Jordan VC. What do we know and what don't we know about tamoxifen and the human uterus. *Breast Cancer Res Treat* 1994, **31**, 27–39.
44. Smith LL. Personal communication, as reported at the MRC Tamoxifen Toxicology Meeting January 1994. Leicester, England.
45. Mühleman K, Cook LS, Weiss NS. The incidence of hepatocelluoar carcinoma in US white women with breast cancer after the introduction of tamoxifen in 1977. *Breast Cancer Res Treat* 1994, **30**, 201–204.

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Control of Oncogene Expression by Antisense Nucleic Acids

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INTRODUCTION

TRANSFORMATION OF normal cells into malignant cells is a multistep process involving the activation of proto-oncogenes and the inactivation of tumour-suppressor genes and of DNA repair genes [1]. The discovery of oncogenes, tumour suppressor and mutator genes has opened new areas of research in oncology aimed at discovering drugs that could selectively inhibit the biological effects of oncogene products and/or restore the function of tumour suppressor and DNA repair genes.

Most of the drugs presently available act at the level of proteins, the products of gene expression. Even DNA intercalating anticancer drugs exert their biological effect via inhibition of DNA processing enzymes such as topoisomerases. During the last decade, new approaches have been developed to selectively inhibit gene expression. The simplest way to control nucleic acids is to use nucleic acids themselves [2]. Short nucleic acid fragments, called oligonucleotides, can be designed to bind selectively to a complementary sequence on a single-stranded nucleic acid, for example, a messenger or a viral RNA, using the molecular code discovered by Watson and Crick in 1953, when they proposed the structure of the DNA double helix. Upon binding to the RNA target, these antisense oligonucleotides can block translation or reverse transcription. An oligoribonucleotide can also be designed to induce a catalytic cleavage of its RNA target. Such ribozymes bind to a complementary sequence on the RNA, as do the antisense oligonucleotides, but they contain an additional sequence that is responsible for the cleavage activity.

At the end of the 1980s, a new strategy was developed, which we called the antigene strategy [3], where the oligonucleotide is targeted to double-helical DNA to form a local triple helix. This triple-helical complex can block transcription, the first step of gene expression.

Oligonucleotides can also be used to control gene expression in the so-called sense approach. An oligonucleotide decoy can be designed to trap, for example, a transcription factor. It will therefore alter the expression of all genes which depend on this transcription factor for their activity. Compared with the antisense, ribozyme or antigene approaches, the sense strategy is expected to be less selective. However, the oligonucleotide decoy can be used to trap a viral or a parasitic protein which is involved in controlling the expression of viral or parasitic genes. Therefore, its effect should be selective for the virus or the parasite.

Another potential application of oligonucleotides has been more recently described. Oligonucleotides can be selected on the basis of their binding to proteins whose normal function does not involve any interaction with nucleic acids. This aptamer approach [4] leads to the design of oligonucleotides as a special class of ligands for enzymes, receptors, growth factors etc.

In all the approaches mentioned above, the oligonucleotide can be obtained through organic synthesis. This chemical approach has led to the development of oligonucleotide analogues, where the nucleic acid backbone or the bases are modified to confer upon the oligonucleotide additional properties when

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therapeutical applications are contemplated [5]. The oligonucleotide can be made resistant to nucleases in order to increase its lifespan after administration. Other modifications are aimed at increasing cellular uptake and modulating intracellular distribution to increase the oligonucleotide concentrations in the appropriate compartments, where the target nucleic acid is located. A large number of biotechnology and pharmaceutical companies have research programmes in this area of gene-specific pharmacology.

All the potential therapeutical applications of oligonucleotides can also make use of DNA constructs to generate RNA oligonucleotides within the cells. All strategies (antisense, sense, antigene, ribozyme, aptamers, etc.) are therefore amenable to gene therapy protocols. The selectivity of short synthetic oligonucleotides can be extended to RNA transcripts obtained from DNA vectors.

One of the important factors in determining the selectivity of oligonucleotides is the length that the oligomer should have in order to bind a single target within living cell. A minimum length is required if one wants to avoid binding to several mRNA species or to several genes [6]. A statistical calculation leads to the conclusion that an oligomer of 17 nucleotides should find a unique target within the human genome ($\sim 3 \times 10^9$ base pairs). This calculation assumes a random distribution of base pairs which is obviously not the actual situation, but we must await the sequence of the entire human genome before we can determine whether an oligonucleotide will find single or multiple targets. In the antisense strategy, the target sequence is contained within the RNA transcripts. Only a small fraction of the genome is transcribed in a living cell, depending on the cell function (tissue) and time in development. Therefore, an antisense oligonucleotide (targeted to RNAs) can be made shorter than an antigene oligonucleotide (targeted to DNA) without losing specificity. The oligonucleotide should not be made too long, otherwise partial complementarity with long-enough sequences will be found on other mRNAs or genes, and will lead to unexpected effects. These points will be illustrated below in the case of the *ras* oncogene family.

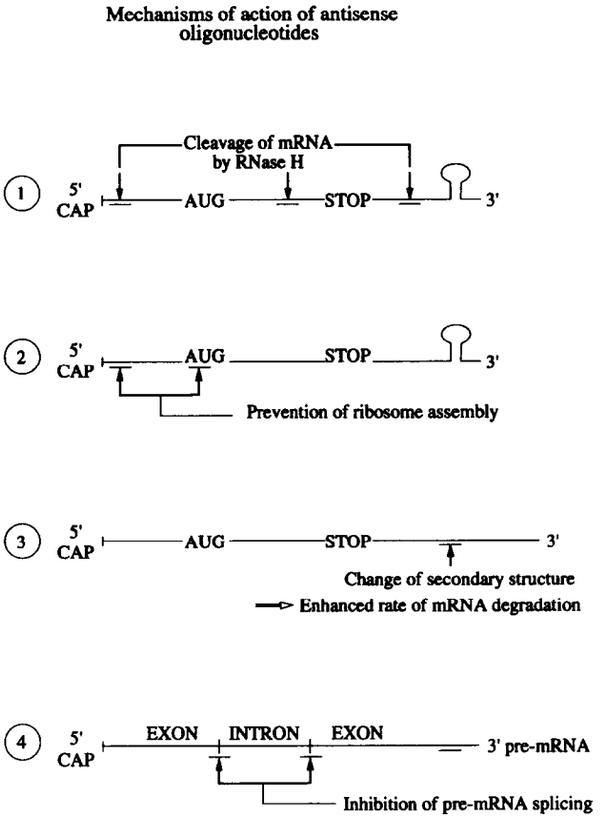

Figure 1. Mechanisms of action of antisense oligonucleotides (indicated by short bars). They can induce cleavage of the mRNA target via RNase H (1) or enhance RNA degradation by altering the secondary structure of the mRNA (3). Alternatively, they might block the correct assembly of the translation machinery (2) or splicing of pre-mRNA (4). (see [2] for a review).

GENE-SPECIFIC PHARMACOLOGY

I. Antisense oligonucleotides

(*a*) *Mode of action.* Antisense oligonucleotides exert their biological effects according to different mechanisms which are summarised in Figure 1. The chemical nature of the synthetic oligomer is important in determining its mode of action. For example, only natural phosphodiester or phosphorothioate backbones are able to induce site-specific RNase H cleavage of the mRNA. When the target sequence is located within the coding sequence of the mRNA, it is unlikely that the translation machinery wil be arrested by a reversibly bound oligonucleotide. An irreversible reaction (e.g. RNase H cleavage or chemical modification induced by a reactive group attached to the antisense oligonucleotide) or the formation of a more stable and bulky complex (e.g. a triple helix, see below) are required to block translation.

(*b*) *Selectivity.* There are many cases of tumour development where the activation of an oncogene creates unique target sequences for an antisense oligonucleotide [2]. This includes (i) point mutations as in the *ras* oncogene family; (ii) deletion of a small fragment of a gene; (iii) translocation which creates chimeric RNAs resulting from the fusion of two genes present on different chromosomes in normal cells; (iv) aberrant initiation of transcription within an intron, as observed in Burkitt lymphomas; and (v) alternative splicing which generates new junctions and/or new sequences in the mature mRNA.

In our laboratory, we have developed antisense oligonucleotides targeted to a mutant Ha-*ras* mRNA present in a human bladder carcinoma [7, 8]. This study will serve to illustrate some of the points alluded to in the introduction. The mutation (G-to-T transversion) occurs in the 12th codon of the oncogene. Antisense oligonucleotides were synthesised to be complementary to the mutated sequence and, therefore, have a mismatch with the normal mRNA. Will a single mismatch be sufficient to discriminate beween the two mRNA species? To answer this question, we synthesised oligonucleotides of different lengths and overlapping different sequences around the mutated base. An RNase H cleavage assay (using an HeLa cell extract) was used to determine the efficacy of each oligonucleotide to induce cleavage of the mutated and normal mRNAs. The highest discrimination was obtained when the mutation was located in the centre of the oligonucleotide complementary sequence (mismatches at the ends destabilise less than mismatches in the centre of a double helix). However, the oligonucleotide length had to be kept short enough (12–13 nucleotides) in order to maintain the specificity; oligonucleotides longer than 16 nucleotides did not discriminate between the two mRNAs [9].

(*c*) *Cell culture experiments*. Antisense dodecanucleotides were chosen for further studies on cell cultures. The antisense oligomer, complementary to the mutated mRNA, inhibited the proliferation of T24 cells (human bladder carcinoma) from which the Ha-*ras* mutation at codon 12 was first identified. The effect was sequence-specific 12-mer complementary to the normal mRNA or a 12-mer with a sequence in the opposite orientation compared with the antisense had no effect on cell growth under the same conditions. However, the concentration of the antisense 12-mer required to observe the biological response was in the 10 μM range, a concentration much too high to contemplate experiments on tumours in animals [8]. Therefore, we tried to develop an oligonucleotide vector that would enhance the efficacy but maintain the specificity. Polyalkylcyanoacrylate nanoparticles proved to be quite useful in this respect [8]. These nanoparticles have been previously used as vectors for a number of drugs. We recently showed that oligonucleotides could be adsorbed to hydrophobic nanoparticles provided the latter have incorporated hydrophobic cations, such as cetyl trimethylammonium bromide (CTAB) [9]. The oligonucleotide is then protected against nuclease degradation. Nanoparticle–cation–oligonucleotide complexes interact with cells in culture, and the oligonucleotide remains intact within cells for long periods of time (> 48 h), whereas an oligonucleotide which has penetrated in the absence of nanoparticles is degraded within hours.

The anti-Ha-*ras* antisense oligonucleotide targeted to the mutated mRNA was adsorbed to nanoparticles. An inhibition of tumour cell growth in culture was achieved at concentrations two orders of magnitude lower than with the free oligonucleotide, i.e. in the 100 nM range. The sequence specificity remained the same. An analysis of mRNA by reverse transcriptase polymerase chain reaction (RT-PCR) showed that the mutated Ha-*ras* mRNA was specifically ablated in antisense-treated cells compared with control-treated cells.

(*d*) *Animal studies*. Nanoparticle-adsorbed antisense oligonucleotides were used to treat tumours in nude mice. A human cell line (HBL100) transformed with the human Ha-ras gene mutated at codon 12, induced tumours in nude mice after subcutaneous injection. The nanoparticle-adsorbed antisense oligonucleotide was injected subcutaneously in the same region where the tumour-inducing cells had been grafted. An oligonucleotide with an inverted sequence was used as a control as in cell culture experiments. After 3 weeks, no tumour was detected in 70% of the antisense-treated animals, and the remaining 30% had very small tumours compared with control-treated animals, in which all had tumours with a mean size approximately 10 times larger. In these experiments, the animals received a total dose of 100 μg of oligonucleotide in eight injections. In a second series of experiments, we let the tumour grow first and then the nanoparticle-adsorbed oligonucleotide was injected directly within the tumour. Whereas the control-treated mice exhibited growth of tumours identical to untreated mice, the tumours did not grow or grew very slowly (less than 20% compared with the controls) in antisense-treated animals. Even though these experiments are only preliminary, they do show that tumour growth can be inhibited provided the antisense oligonucleotide reaches the tumour.

Other experiments on animal models have been recently reported (Table 1). Most of them made use of oligophosphorothioate or 3′-phosphorothioate-protected oligomers. Large quantities (> 1 mg per mouse) were usually required to observe a biological response. In our experimental model, much less oligonucleotide was used (< 100 μg per mouse) with natural phosphodiester linkages. Despite the nuclease-sensitivity of natural backbones, the adsorption to nanoparticle carriers strongly enhanced the biological activity of the antisense oligonucleotide without any loss in selectivity.

II. Antigene oligonucleotides

The recognition of base pair sequences in DNA can be achieved with oligonucleotides which bind to the major groove of the double helix where they form a triple helix. Even though the formation of triple helices by homopolynucleotides has been described long ago (1957), it is only recently (1987) that the possibility of triple helix formation by short oligonucleotides at specific sequences of DNA has been demonstrated [10, 11] (Figure 2). The target sequences remain, for the most part, limited to oligopurine·oligopyrimidine segments of double-heli-

Table 1. *Animal experiments using antisense oligonucleotides targeted to oncogenes or transcription factors and inhibiting tumour growth*

Target	Target gene	Animal	Route of administration	Oligonucleotide	Reference
Neuroblastoma (CHP-100)	N-*myc*	Nude mice	Subcutaneous, continuous infusion	Natural phosphodiester (15-mer)	28
Human leukaemia (K562)	c-*myb*	Scid mice	Subcutaneous, continuous infusion	Phosphorothiate (24-mer)	29
Transformed NIH 3T3 cells	Ha-*ras*	Mice	Treatment of cells by antisense oligo before injection into mice	Phosphodiester (15-mer)	30
ras-Transformed human epithelial cells (HBL 100)	mutated Ha-*ras* (codon 12)	Nude mice	Subcutaneous, repeated injections	Natural phosphodiester (12-mer) adsorbed to nanoparticles	8
Lymphocytes	c-*myc*	Transgenic mice Eμ-*myc*	Intravenous injection	Methylphosphonate (15 mer)	31
Mouse fibrosarcoma (induced by HTLV-1 tax in transgenic mice)	-p65 subunit of transcription factor NFκB -*tax* (HTLV1)	Syngenic mice	Intraperitoneal injection	3′-Terminal phosphorothioate (20 mer). (Phosphodiester with 3 P=S linkages at 3′-terminus)	32
Mouse fibrosarcoma (K-BALB)+melanoma (B16)	p65 subunit of NFκB	Mice	Subcutaneous injection	Phosphorothioate (18–24 mers)	33

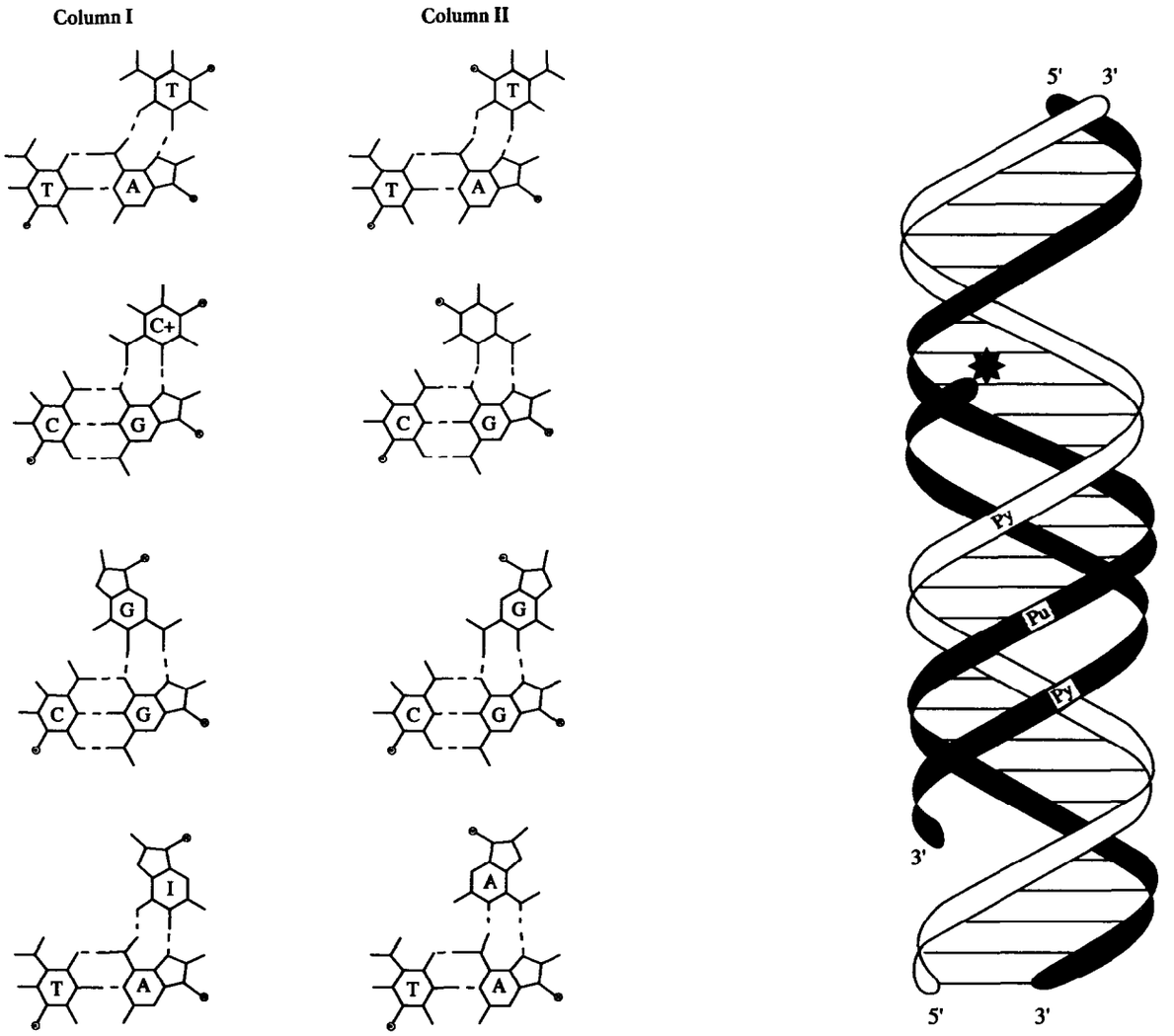

Figure 2. Formation of a triple helix when an oligonucleotide (black ribbon, right), binds to the major groove of the DNA double helix. The star attached to the third strand indicates a reagent covalently linked to the oligonucleotide end. Triple helix formation involves the formation of base triplets shown on the left part of the figure. Bases in the third strand form hydrogen bonds with the purines of the Watson-Crick base pairs. Hoogsteen hydrogen bonds (column I) are formed by oligonucleotides containing C and T which bind in a parallel orientation with respect to the oligopurine target sequence. Reverse Hoogsteen hydrogen bonds (column II) are formed when oligonucleotides containing G and A bind in an antiparallel orientation to the oligopurine sequence. Oligonucleotides containing G and T can form Hoogsteen or reverse Hoogsteen hydrogen bonds and bind parallel or antiparallel, respectively, to the target sequence, depending on base sequence (see [6] for a review).

cal DNA, even though an extension of recognition sequences has been described (for example, by switching from one strand to the other one at sequences where an oligopurine is followed by an oligopyrimidine, or by introducing base analogues or intercalating agents to "skipover" a pyrimidine interrupting a polypurine stretch). It remains a challenge to chemists to design base analogues that would allow the oligomer to recognise the four base pairs (A·T, T·A, C·G, G·C) when "reading" the major groove of DNA.

Triple helix formation on a gene promoter can compete with the binding of proteins which activate the transcription machinery [6]. Therefore, the transcriptional activity of a gene can be modulated by triplex-forming oligonucleotides. Binding of short oligonucleotides downstream of the transcription start site is unlikely to arrest RNA polymerase, unless the oligonucleotide is covalently substituted with a reactive group that can introduce irreversible reactions in the DNA template, or with an intercalating agent that will behave as a strong-enough obstacle to block the enzyme moving along the DNA.

There are several reports showing that antigene oligonucleotides can inhibit gene expression in cell cultures, although the actual demonstration that triple helix formation is the cause of the observed effect is, in most cases, indirect. Transcription of the c-*myc* gene [12] or of progesterone-responsive genes [13] has been shown to be inhibited by antigene oligonucleotides. A nuclease hypersensitive site in the *myc* gene promoter was protected by the oligonucleotide, suggesting that the oligonucleotide was indeed bound to its target DNA sequence. Antigene oligonucleotides were used to block the expression of the α-subunit of the interleukin-2 receptor. Using an oligonucleotide-intercalator conjugate, we have shown that triple helix formation inhibits binding of an essential transcription factor, named NFκB, to the promoter region [14]. The transcription factor and oligonucleotide binding sites had a four base pair overlap. A

mutation was introduced in the triple helix site which did not affect NFκB binding, but prevented triple helix formation. The expression of this mutant was not affected by the antigene oligonucleotide-intercalator conjugate in cell cultures, providing direct evidence that inhibition of the wild-type gene was due to triple helix formation [15, 16].

The development of the antigene strategy is still in its infancy. There are no animal studies yet reported. Even the number of reports on cell cultures is still limited. Part of the difficulty resides in the restricted number of DNA sequences that can form triple helices at appropriate positions within genes. Another problem arises from the association of DNA with histones and other proteins in the nucleus, which might make many sequences inaccessible to oligonucleotides. However, when a gene is actively transcribed, the chromatin structure must be "open" to give access to transcription factors and to RNA polymerase. The problems of target accessibility might not be more complex for antigene oligonucleotides than for antisense oligonucleotides, which must find access to their target sequence despite the presence of secondary and tertiary structures, and the binding of proteins to the mRNA or the pre-mRNA. One of the advantages of targeting genes in DNA is the limited number of targets (two alleles if the gene is not amplified) compared with mRNA, whose copy number can be very variable depending on transcription efficacy and metabolism.

III. Oligonucleotide clamps

Oligonucleotides can be designed to form a triple helix on a single-stranded nucleic acid. For example, an oligopurine sequence can first bind a complementary oligonucleotide (forming Watson–Crick base pairs) and then a second oligonucleotide that can form Hoogsteen (or reverse Hoogsteen) hydrogen bonds with the Watson–Crick double helix [17, 18] (see Figure 2). The two oligonucleotides can be linked together to form a clamp [18] or a circular molecule [19]. The binding energy of clamps or circular oligonucleotides is much larger than that of antisense oligomers. Moreover, they have a higher selectivity because an incorrect base in the target is recognised as a mismatch from both the Watson–Crick and the Hoogsteen sides. Oligonucleotide clamps can be substituted with intercalating agents that enhance their binding affinity and create strong road blocks for nucleic acid processing enzymes such as DNA polymerases, reverse transcriptase and ribosomes [18]. The potential applications of oligonucleotide clamps and circular oligonucleotides to control gene expression remain to be explored in depth.

GENE THERAPY APPROACHES

As mentioned in the introduction, antisense, antigene and sense oligonucleotides, clamps, ribozymes and aptamers can be produced within cells as RNA transcripts of DNA constructs.

A gene fragment can be introduced in a reverse orientation, compared with the gene itself under the control of, for example, an inducible promoter. The RNA transcript (the antisense RNA) will be complementary to the messenger RNA. Double helix formation between the antisense and the messenger RNA is expected to block translation of the latter. For example, an antisense RNA targeted against the mutated Ki-*ras* mRNA in a human colon tumour cell line inhibited cell proliferation [20]. Similarly, an antisense RNA against the growth factor IGF1 blocked proliferation of rat glioblastoma cell lines [21]. An episomal vector, based on the Epstein-Barr virus origin of replication and nuclear factor EBNA1, was used to produce large amounts of the antisense RNA. When injected into syngenic rats the antisense-treated glioblastoma cells induced an immune response targeted to the original tumour, so that a tumour growing in another part of the rat body (including brain) disappeared when the rat was treated with the antisense-expressing cells [21]. These results raise the possibility that a cell therapy approach could be used to cure or protect from tumour growth.

In addition to antisense, ribozymes can be generated *in situ* from DNA constructs [22]. The large amounts of antisense or ribozyme RNAs that must be produced in order to achieve a biological response might reflect the inappropriate folding of these RNAs or the inappropriate location of the antisense or ribozyme with respect to the target mRNA. Recent experiments with a ribozyme targeted to HIV have shown that co-localisation of the ribozyme and the viral RNA within a viral capsid leads to an efficient cleavage of the viral RNA, in contrast to the low efficiency observed when the ribozyme is produced at random within the nucleus [23].

CONCLUSION

The development of nucleic acids to control gene expression in a highly selective way has opened new possibilities for therapeutical applications, especially in the field of cancer [2] and AIDS [24]. The results obtained with antisense nucleic acids (synthetic oligonucleotides or RNA transcripts) targeted to the *ras* genes show that, even though *ras* gene activation (by mutation) is only one of the (early) events occurring during tumour development, tumour cells are still dependent on *ras* oncogene expression for proliferation. This might not be true of all oncogenes and some tumours might become independent of an activated gene that was responsible for an essential step in the transformation process leading from normal to malignant cells. Synthetic antisense oligonucleotides (oligophosphorothioates) have reached the stage of phase I clinical trials in the treatment of leukaemia [25] and AIDS patients [26]. *Ex vivo* treatments of CML cells with antisense oligonucleotides targeted to the *bcr-abl* junction arising from (t9,22) chromosomal translocation, are contemplated in bone marrow transplantation. However, there are still many problems to be solved before oligonucleotides reach the status of therapeutical drugs. It should be reminded that, despite their specificity for nucleic acid sequences, oligonucleotides can bind to targets other than nucleic acids, for example, proteins. After microinjection into the cytoplasm of cells, oligonucleotides rapidly move to the nucleus where they bind with high affinity to a large number of proteins [27]. Long-term effects might arise from binding to these (still) unidentified targets. Some oligonucleotides may also bind to an unexpected target and induce a biological response [34]. A large number of chemical modifications are being introduced in oligonucleotide backbones and bases [5]. Phosphorothioates represent only the first family of antisense oligonucleotides to be investigated from the points of view of pharmacokinetic, bioavailability and toxicology. Some modified oligonucleotides might exhibit a better tissue distribution, cellular uptake and intracellular compartmentalisation. Meanwhile, carriers and vectors of oligonucleotides are being tested. They might lead to the development of natural oligonucleotides as therapeutic agents.

Several protocols using antisense and ribozyme production are undergoing clinical trials as cellular therapy approaches. Antisense expression in tumour cells can be used to block an oncogene, a growth factor or a growth factor receptor. An immune response against the original tumour may be induced

when antisense-treated cells are reinjected into animal models. Clinical trials will demonstrate whether a similar situation prevails in human patients. Haematopoietic stem cells expressing an antisense or a ribozyme (or both) targeted to HIV RNA might become "immune" to HIV infection. As with synthetic antisense oligonucleotides, the results of the first clinical trials using cell therapy protocols should tell us how these approaches can be developed in the near future.

1. Vogelstein B,Kinzler K. The multistep nature of cancer. *Trends Genetics* 1993, **9**, 138–141.
2. Hélène C. Rational design of sequence-specific oncogene inhibitors based on antisense and antigene oligonucleotides. *Eur J Cancer* 1991, **27**, 1466–1471.
3. Hélène C. The anti-gene strategy: control of gene expression by triplex-forming-oligonucleotides. *Anti-Cancer Drug Design* 1991, **6**, 569–584.
4. Bock LC, Griffin LC, Latham A, Vermaas EH, Toole JJ. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature* 1992, **355**, 564–566.
5. Cook PD. Medicinal chemistry strategies for antisense research. In Crooke ST and Lebleu B, eds. *Antisense Research and Applications*, CRS Press, Boca Raton, 1993, 149–187.
6. Thuong NT, Hélène C. Sequence-specific recognition and modification of double-helical DNA. *Angew Chem Int Engl* 1993, **32**, 666–690.
7. Saison-Behmoaras T, Tocqué B, Rey I, Chassignol M, Thuong NT, Hélène C. Short modified antisense oligonucleotides directed against Ha-*ras* point mutation induce selective cleavage of the mRNA and inhibit T24 cells proliferation. *Embo J* 1991, **10**, 1111–1118.
8. Schwab G, Chavany C, Duroux I, Goubin G, Leabeau J, Hélène C, Saison-Behmoaras T. Antisense oligonucleotides adsorbed to polyalkylacyanoacrylate nanoparticles specifically inhibit mutated Ha-*ras*-mediated cell proliferation and tumorigenicity in nude mice. *Proc Natl Acad Sci USA* 1994, in press.
9. Chavany C, Le Doan T, Couvreur P, Luisieux F, Hélène C. Polyalkylcyanoacrylate nanoparticles as polymeric carriers for antisense oligonucleotides. *Pharm Res* 1992, **9**, 441–449.
10. Le Doan T, Perrouault L, Praseuth D, Habhoub N, Decout JL, Thuong NT, Lhomme J, Hélène C. Sequence-specific recognition, photocrosslinking and cleavage of the DNA double helix by an oligo-[α]-thymidylate covalently linked to an azidoproflavine derivative. *Nucl Acids Res* 1987, **15**, 7749–7760.
11. Moser HE, Dervan BP. Sequence-specific cleavage of double helical DNA by triple helix formation. *Science* 1987, **238**, 645–650.
12. Postel EH, Flint SJ, Kessler DJ, Hogan ME. Evidence that a triplex-forming oligodeoxyribonucleotide binds to the c-*myc* promoter in HeLa cells, thereby reducing c-*myc* mRNA levels. *Proc Natl Acad Sci USA* 1991, **88**, 8227–8231.
13. Nancy HI, Beekman JM, Kessler D, *et al. In vivo* transcription of a progesterone-responsive gene is specifically inhibited by a triplex-forming oligonucleotide. *Nucl Acids Res* 1993, **21**, 2789–2796.
14. Grigoriev M, Praseuth D, Robin P, *et al.* A triple helix-forming oligonucleotide-intercalator conjugate acts as a transcriptional repressor via inhibition of NFκB binding to IL-2 receptor α subunit regulatory sequence. *J Biol Chem* 1992, **267**, 3389–3395.
15. Grigoriev M, Praseuth D, Guieysse AL, Robin P, Thuong NT, Hélène C, Harel-Bellan A. Inhibition of interleukin-2 receptor α-subunit gene expression by oligonucleotide-directed triple helix formation. *CR Acad Sci Paris* 1993, **316** 492–495.
16. Grigoriev M, Praseuth D, Guieysse AL, Robin P, Thuong NT, Hélène C, Harel-Bellan A. Inhibition of gene expression by triple helix-directed site-specific cross-linking. *Proc Natl Acad Sci USA* 1993, **90**, 3501–3505.
17. Giovannangeli C, Montenay-Garestier Th, Rougée M, Chassignol M, Thuong NT, Hélène C. Single-stranded DNA as a target for triple helix formation. *J Am Chem Soc* 1991, **113**, 7775–7777.
18. Giovannangeli C, Thuong NT, Hélène C. Oligonucleotide clamps arrest DNA synthesis on a single-stranded DNA target. *Proc Natl Acad Sci USA* 1993, **90**, 10013–10017.
19. Prakash G, Koll ET. Structural effects in the recognition of DNA by circular oligonucleotides. *J Am Chem Soc* 1992, **114**, 3523–3527.
20. Mukhopadhyay T,Tainsky M,Cavender C, Roth JA. Specific inhibition of K-*ras* expression and tumorigenicity of lung cancer cells by antisense RNA. *Cancer Res* 1991, **51**, 1744–1748.
21. Trojan J, Johnson TR, Rudin SD, Ilan J, Tykocinski ML, Ilan J. Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I RNA. *Science* 1993, **259**, 94–97.
22. Yu M, Ojwang J, Yamada O, Hampel A, Rapapport J, Looney D, Wong-Staal F. A hairpin ribozyme inhibits expression of diverse strains of human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 1993, **90**, 6340–6344.
23. Sullenger BA, Cech TR. Tethering ribozymes to a retroviral packaging signal for destruction of viral RNA. *Science* 1993, **262**, 1566–1569.
24. Lisziewicz J, Sun D, Metelev V, Zamecnik P, Gallo RC, Agrawal S. Long-term treatment of human immunodeficiency virus-infected cells with antisense oligonucleotide phosphorothioates. *Proc Natl Acad Sci USA* 1993, **90**, 3860–3864.
25. Bayever E, Iversen PL, Bishop MR, *et al.* Systemic administration of a phosphorothioate oligonucleotide with a sequence complementary to p53 for acute myelogenous leukemia and myelodysplastic syndrome: initial results of a phase I trial. *Antisense Res Dev* 1993, **3**, 383–390.
26. Agrawal S, Tang JY. GEM* 91—an antisense oligonucleotide phosphorothioate as a therapeutic agent for AIDS. *Antisense Res Dev* 1992, **2**, 261–266.
27. Clarence JP, Lebleu B, Leonetti JP. Characterization of the nuclear binding sites of oligodeoxyribonucleotides and their analogues. *J Biol Chem* 1993, **268**, 56000–56004.
28. Whitesell L, Rosolen A, Neckers LM. *In vivo* modulation of N-*myc* expression by continuous perfusion with an antisense oligonucleotide. *Antisense Res Dev* 1991, **1**, 343–350.
29. Ratajczak MZ, Kant JA, Luger SM, Hijiya N, Zhang J, Zon G, Gewirtz AM. *In vivo* treatment of human leukemia in a scid mouse model with c-*myb* antisense oligodeoxynucleotides. *Proc Natl Acad Sci USA* 1992, **89**, 11823–11827.
30. Gray GD, Hernandez OM, Hebel D, Root M, Pow-Sang M, Wickstrom E. Antisense DNA inhibition of tumor growth induced by c-Ha-*ras* oncogene in nude mice. *Cancer Res* 1993, **53**, 577–580.
31. Wickstrom E, Bacon TA, Wickstrom EL. Down-regulation of c-*myc* antigen expression in lymphocytes of Eμ-c-*myc* transgenic mice treated with anti-c-*myc* DNA methylphosphonates. *Cancer Res* 1992, **52**, 6741–6545.
32. Kitajima I, Shinohara T, Bilakovics J, Brown DA, X Xu, Nerenberg M. Ablation of transplanted HTLV-I tax-transformed tumors in mice by antisense inhibition of NF-κB *Science* 1992, **258**, 1792–1795.
33. Higgins K, Perez JR, Coleman TA, Dorshkind K, McComas WA, Sarmiento UM, Roser CA, Narayanan R. Antisense inhibition of the p65 subunit of NF-κB blocks tumorigenicity and cause tumor regression. *Proc Natl Acad Sci USA* 1993, **90**, 9901–9905.
34. Fedoseyeva EV, Li Y, Huey B, Tam S, Hunt CA, Benichou G, Garovoy MR. Inhibition of interferon-γ-mediated immune functions by oligonucleotides. *Transplantation* 1994, **57**, 606–612.