Gene Ther Mol Biol Vol 1, 467-474. March, 1998.

Sequence-specific control of gene expression by antigene and clamp oligonucleotides

Claude Hélène, Thérèse Garestier, Carine Giovannangeli, and Jian-Sheng Sun

Laboratoire de Biophysique, Muséum National d'Histoire Naturelle, INSERM U 201 - CNRS URA 481, 43 Rue Cuvier - 75231 Paris cédex 05, France.

Correspondence: Claude Hélène, Tel: (1) 40 79 37 08; Fax: (1) 40 79 37 05; E-mail biophy@mnhn.fr

Summary

Gene expression can be artificially controlled by synthetic oligonucleotides that bind either to the gene itself or to its messenger RNA. Binding of an antigene oligonucleotide to the major groove of DNA involves the formation of a triple helix. Covalent attachment of an intercalating agent to the third strand oligonucleotide strongly stabilizes the triple-helical complex. Oligonucleotide-intercalator conjugates inhibit transcription factor binding and transcription initiation. Introduction of N3' → P5' linkages into the oligonucleotide leads to stronger complexes that are able to arrest the transcription machinery at the elongation step. Cellular DNA is accessible to oligonucleotides within the chromatin structure of the cell nucleus as demonstrated for HIV proviral DNA in chronically infected cells. A triple helix can be formed on a single-stranded nucleic acid by an oligonucleotide made of two portions : one binds to the target sequence by forming Watson-Crick base pairs, the second one binds to this double-helical region to form a triple-helical complex. These clamp oligonucleotides are able to arrest replication of a single-stranded DNA or reverse transcription of a viral RNA. The antigene and clamp strategies can be adapted to a gene therapy protocol. A DNA expression vector is used to obtain a RNA transcript that can bind to DNA (antigene RNA) and block transcription.

I. Introduction

Tissue-specific regulation of gene expression is achieved through binding of sequence-specific transactivating factors to DNA sequences upstream of the transcription start site. Gene expression can be artificially controlled with oligonucleotides according to several strategies (for review see Hélène, 1994). Antisense oligonucleotides bind to complementary sequences on messenger RNAs and inhibit translation of the message into the coded protein. Ribozymes are also targeted to messenger RNAs (or viral RNAs) and induce a catalytic cleavage of the recognized RNA, thereby inhibiting translation of the mRNA (or expression of the viral RNA). An oligonucleotide decoy can be used to sequester a transcription factor and control the expression of genes that are regulated by this transcription factor. Several genes are expected to respond to the oligonucleotide decoy due to the involment of each transcription factor in the regulation of gene families. Oligonucleotide aptamers can be targeted to proteins involved at any step of gene control and expression .

Control of gene transcription can be achieved with antigene oligonucleotides that bind to double-helical DNA to form a local triple helix (Thuong and Hélène, 1993). Alternatively an oligonucleotide may inhibit transcription by strand invasion of a double-helical template, as observed with PNAs (Peptide Nucleic Acids) (Nielsen *et al.*, 1994). The targeted sequence may be located in the promoter or enhancer region of the gene or within the transcribed portion. A triple helix can also be formed on a single-stranded nucleic acid by clamp (Giovannangeli *et al.*, 1991, 1993) or circular oligonucleotides (Kool, 1991; Wang and Kool, 1995). If the target is a RNA, these oligonucleotides are expected to inhibit translation of a messenger RNA or reverse transcription of a viral RNA. This review will deal with triple helix-forming oligonucleotides and their gene regulatory activities at both the transcriptional and translational levels.

II. The antigene strategy

A. Sequence specific triple-helix formation on double-helical DNA

Triple helix formation involves the recognition of Watson-Crick base pairs by hydrogen bonding interactions within the major groove of the double helix (Thuong and Hélène, 1993). Oligonucleotides and oligonucleotide

analogues can wind around the double helix ; their orientation is dependent on base sequence (**Figure 1**).

Recognition of the purines in T.A and C.G base pairs may be achieved by T and protonated C (C^+), respectively, by forming Hoogsteen hydrogen bonds (as originally described by Hoogsteen). Pyrimidic oligonucleotides adopt a parallel orientation with respect to the oligopurine sequence. The two base triplets T. A x T and C. G x C^+ are isomorphous, i.e., the oligopyrimidine winds without any distortion of its backbone around the targeted double-helical sequence. The requirement for cytosine protonation to form a stable C. G x C^+ base triplet makes the stability of triple helices pH-dependent for (C,T)- containing oligonucleotides. However triple helices can be observed at pH7 if most cytosines have thymine and no cytosine neighbors.

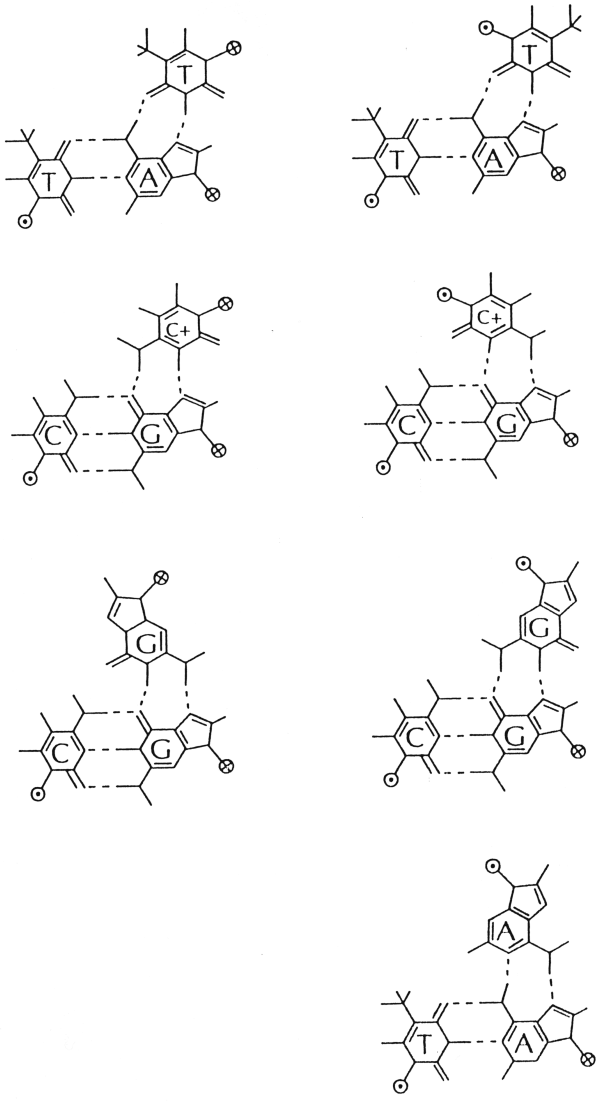

Figure 1: Base triplets formed by natural nucleosides with Watson-Crick T.A and C.G base pairs. The column on the left-hand side corresponds to the Hoogsteen configuration. The third strand runs parallel to the oligopurine target. The column on the right-hand side corresponds to the reverse Hoogsteen configuration. The third strand is antiparallel to the oligopurine strand of the double-helical target (Sun *et al.*, 1996).

Alternatively, the purines of T.A and C.G base pairs can be recognized by A and G, respectively. A purinic oligonucleotide binds in an antiparallel orientation with respect to the target oligopurine sequence. The two base triplets T. A x A and C. G x G are not isomorphous ; therefore an adjustement of the backbone conformation is required to form a triple helix.

The parallel orientation of (T, C) - containing oligonucleotides and the antiparallel one of (A, G) - containing oligonucleotides assumes that all nucleotides adopt an anti conformation (Beal and Dervan, 1991). Such orientations have been experimentally observed in all experiments reported to date. A syn conformation of the nucleosides would lead to a reverse orientation. It should be noted that T and C^+ can form base triplets with T. A and C. G base pairs, respectively, in a reverse Hoogsteen configuration that should lead to an antiparallel orientation of the (T, C) - containing third strand. This has never been observed with natural oligonucleotides, because T.A x T and C. G x C^+ base triplets are isomorphous and the free energy of base triplet formation and stacking may be higher in the Hoogsteen as compared to the reverse Hoogsteen configuration.

Oligonucleotides synthesized with G's and T's can also form triple helices with an oligopyrimidine•oligopurine sequence of double-helical DNA. The orientation of the (G,T)-containing oligonucleotide depends on base composition (number of 5' GpT 3' and 5' TpG 3' steps, length of G and T tracts). Parallel and antiparallel orientations involve Hoogsteen and reverse Hoogsteen configuration of the C•GxG and T•AxT base triplets, respectively (Sun *et al.*, 1996).

In order for the third strand oligonucleotide to wind smoothly around the major groove of DNA, all purines of the target sequence must be on the same strand of the double helix. Otherwise the backbone of the third strand would have to cross the major groove at the site where a pyrimidine interrupts the oligopurine tract. However it is possible to recognize a pyrimidine within an oligopurine sequence by a base forming a single hydrogen bond with the pyrimidine base. This possibility has been exemplified by introducing a guanine in a (C,T)-oligonucleotide to recognize a thymine in an oligopurine sequence, forming a non-canonical A•TxG base triplet. The energetics of this interaction depends on the flanking base triplets (Kiessling *et al.*, 1992). It is also possible to enhance the binding energy by attaching an intercalating agent at the site facing

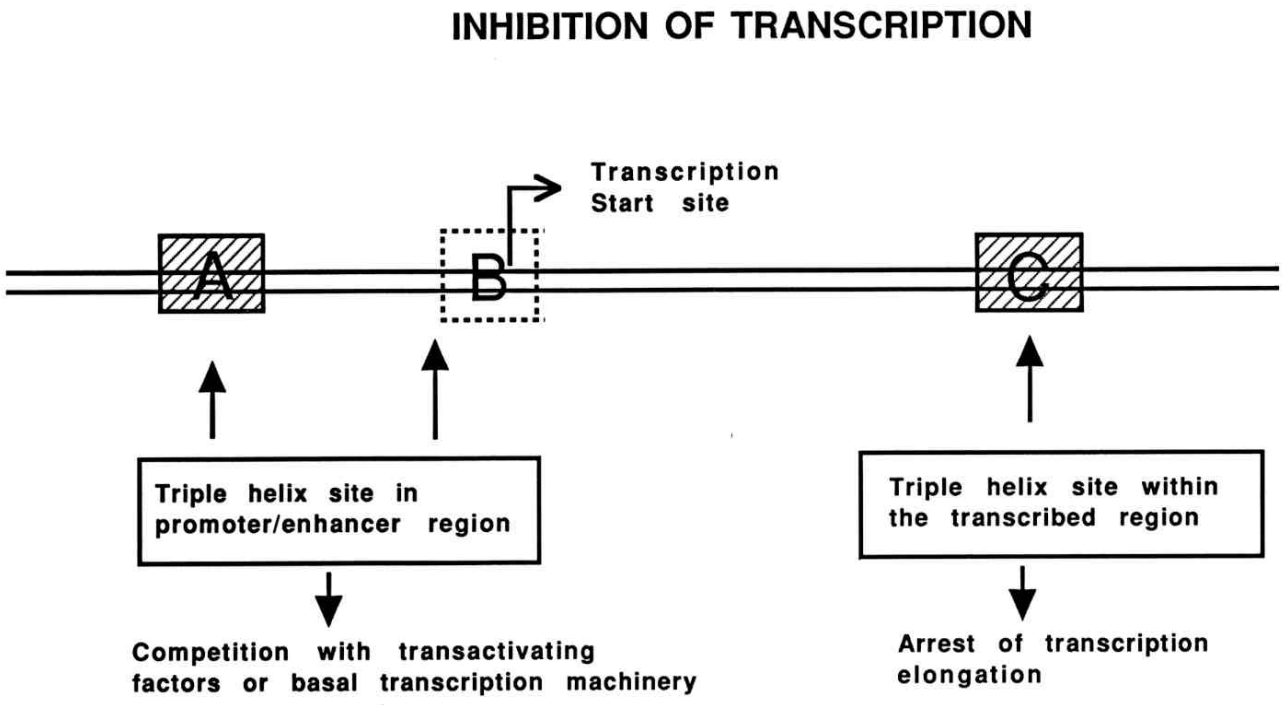

Figure 2: Location of binding sites of triplex-forming oligonucleotides on genomic DNA. In A the oligonucleotide compete with the binding of transcriptional activators, in B with the basal transcription machinery ; in C the oligonucleotide may arrest the transcription machinery during the elongation step.

the interruption of the oligopurine sequence (Zhou *et al.*, 1995).

The recognition of two oligopurine sequences alternating on the two strands of the DNA double helix can be achieved by two oligonucleotides linked to each other by a linker whose length and nature depends on the bases in the third strand and the site (5' PuPy 3' or 5' PyPu 3') where the third strand crosses the major groove (see Sun, 1995, for review).

B. Transcription inhibition by triple helix-forming oligonucleotides

The specificity of recognition of a double-helical target by an oligonucleotide provides the basis of the so-called "antigene" strategy to inhibit gene expression at the transcriptional level (Hélène, 1991, 1994). When the target is located within the control region (promoter, enhancer), the bound oligonucleotide may inhibit transcription factor binding. When the oligonucleotide binds downstream of the transcription start site it may inhibit the elongation step of the transcription process (**Figure 2**).

The possibility of inhibiting transcription by a (G,T)-containing oligonucleotide was first described in an *in vitro* transcription system (Cooney *et al.*, 1988). Binding of an oligonucleotide to a transcription factor binding site competes with the binding of the regulatory protein and, thereby, modulates transcription initiation (Cooney *et al.*, 1988, Maher *et al.*, 1992, Grigoriev *et al.*, 1992, Ing *et al.*, 1993). Several *in vitro* transcription systems have been used to demonstrate this competitive inhibition. However the elongation process is much more difficult to inhibit because the stability of the triple-helical complex is usually not sufficient to arrest the transcription machinery once it is launched on its double-helical template. Two strategies have been described to achieve such a transcription arrest :

i) the oligonucleotide can be covalently attached to an intercalating agent (**Figure 3**). The oligonucleotide-intercalator conjugate binds more tightly to its target DNA due to the additional binding energy provided by intercalation at the triplex-duplex junction or within the triple-helical region (Sun *et al.*, 1989 ; Giovannangeli *et al.*, 1996 ; Silver *et al.*, 1997) ;

ii) chemical modifications of the oligonucleotide may provide the analogue with a tighter binding affinity. PNAs do bind tightly to double-helical DNA but they involve a strand-displacement reaction where two PNA molecules binds to an oligopurine sequence on one strand of the

double helix, forming a local triple helix, whereas the second strand (an oligopyrimidine sequence) remains single-stranded (Nielsen *et al.*, 1994). Several other chemical modifications have been tested for their ability to form triple-helices (Escudé *et al.*, 1993). 2'-O methyl pyrimidine oligonucleotides form more stable complexes than DNA and RNA oligonucleotides. A (C,T)-containing RNA binds more tightly than the corresponding DNA oligonucleotide to a DNA double helix. In contrast neither 2'-O-methyl nor RNA purine oligonucleotides form stable triple helices when compared to a DNA oligonucleotide. Among all chemical modifications tested so far, N3' → P5' phosphoramidate linkages confer upon pyrimidine oligonucleotides a tighter binding than that of isosequential phosphodiester oligomers, even at pH 7 (Escudé *et al.*, 1996). Purine oligophosphoramidates do not appear to form stable triple helices.

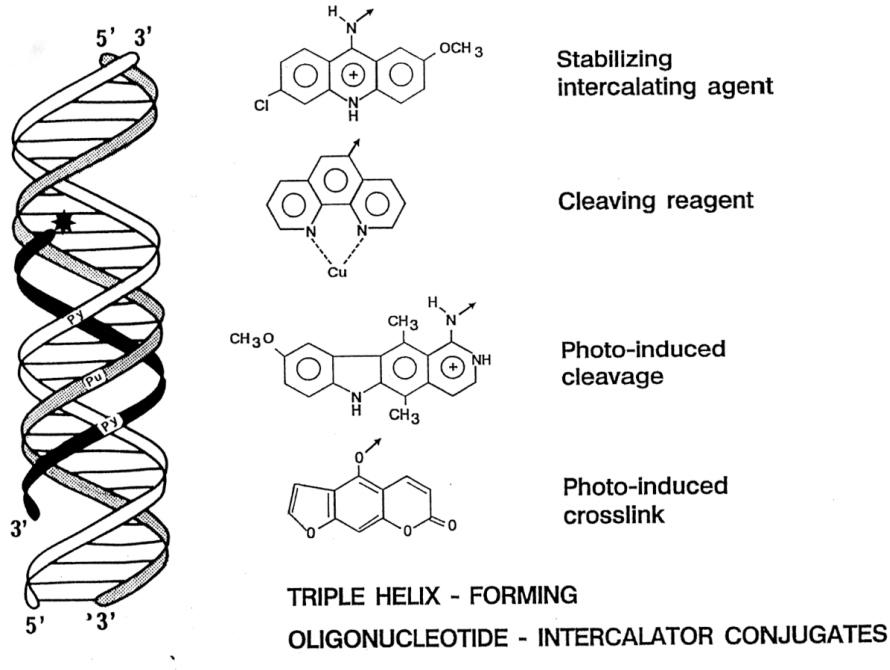

Figure 3 : Left : Schematic representation of a triple-helical complex where an oligonucleotide (black ribbon) wraps around the major groove of the double helix. The oligonucleotide can be covalently attached (star) to an intercalating agent that i) stabilizes the triplex (Sun *et al.*, 1989), ii) induces chemical cleavage of the target site (François *et al.*, 1989), iii) photo-induces cleavage of the target double helix (Perrouault *et al.*, 1990), iv) can be used to cross-link the two strands of DNA under UV irradiation (Takasugi *et al.*, 1991 ; Giovannangeli *et al.*, 1997).

C. Sequence specificity of transcription inhibition by antigene oligonucleotides

Oligonucleotide-intercalator conjugates, PNA and oligophosphoramidates have been shown to inhibit transcription *in vitro* in a sequence-specific manner. The data available within cells are more scarce. Very few studies provide evidence that the effect observed on gene expression is due to oligonucleotide binding to DNA to form a triple-helical complex. In some cases the observed effect on transcription might be due to binding of the oligonucleotide to a transcription factor rather than to DNA. When the gene of interest is carried by a plasmidic vector it is possible to provide evidence for the involvement of triple helix formation in the inhibi tion of gene transcription by introducing mutations in the target sequence. For example in the promoter region of the a-subunit of the interleukin-2 receptor (IL2Rα) there is a 15 base pair oligopyrimidine•oligopurine sequence which overlaps the binding sites of two transcription factors (NF-κB and SRF). When a pyrimidine oligonucleotide tethered to an intercalating agent binds to this sequence the triple-helical complex inhibits the binding of the transcription factors *in vitro* (Grigoriev *et al.*, 1992). After transfection of the plasmid into lymphocytic cell lines, the oligonucleotide-intercalator conjugate inhibits transcription of a reporter gene (CAT) placed downstream of the IL2Rα promoter. A mutant of the target sequence, where three pyrimidines interrupt the oligopurine target sequence, is unable to form a triple-helical complex with the oligonucleotide-intercalator conjugate but does not prevent transcription factor binding. The oligonucleotide-intercalator conjugate does not exhibit any inhibitory effect on transcription from the mutated IL2Rα promoter in contrast to the wild-type sequence (Grigoriev *et al.*, 1993). This experiment demonstrates that the effect of the oligonucleotide-intercalator conjugate is indeed due to binding to the targeted DNA sequence in the IL2Rα promoter and not to another cellular component (e.g, a transcription factor) involved in controlling transcription from this promoter.

In most cases, however, especially for an endogenous gene, it is difficult to construct a mutant of the target sequence. The control experiments rely upon changes of the oligonucleotide sequence with all the problems associated with this type of control since all potential interactions of the oligonucleotide (including self associaiton) may change upon sequence alterations.

D. Accessibility of DNA in cell nuclei to triple helix-forming oligonucleotides

One of the main questions raised by the development of the antigene strategy *in vivo* deals with the accessibiity of the target sequence within the chromatin structure in the cell nucleus. In order to answer this question we have developed a strategy based upon using oligonucleotide-psoralen conjugates. When such a conjugate forms a triple-helical complex with DNA, the psoralen moiety can be cross-linked to one or both strands of the double helix upon UV irradiation (Takasugi *et al.*, 1991). The cross-link arrests DNA replication when a restriction fragment containing the target sequence is used as a template for exponential (PCR) or linear amplification using primers flanking the target sequence. In linear amplification using a single primer a truncated product is obtained when replication is arrested at the cross-linked site. If PCR is used the inhibitory effect of the cross-link can be quantitated by using quantitative PCR methods. Alternatively if the site of triple helix formation and cross-linking overlaps a restriction site it is possible to reveal the inhibition of restriction enzyme cleavage at this particular site by using probes that overlap the targeted DNA region. The absence of inhibition at other restriction sites for the same enzyme provides an internal control of the sequence specificity of the cross-linking reaction and, therefore, of triple helix formation. The first strategy (linear amplification) has been used for a plasmidic vector carrying the IL2Rα promoter sequence (Guieysse *et al.*, 1996). The second (PCR) and third (cleavage inhibition) strategies have been used to demonstrate the accessibility of the proviral HIV sequence in chronically-infected cells (Giovannangeli *et al.*, 1997). The results presently available show that the DNA sequences that have been used as targets for oligonucleotide-psoralen conjugates are indeed accessible within the chromatin structure of cell nuclei. This might not be true of all targeted sequences due to the nucleosomal structure of chromatin. If the oligonucleotide interacts with a sequence where transcription factors bind to activate transcription it is likely that the oligonucleotide may have access to its target sequence as do transcription factors. Kinetic parameters might play an important role inasmuch as some triple-helical complexes exhibit a slow rate of formation as compared to protein binding (Maher *et al.*, 1990, Rougée *et al.*, 1992).

Oligonucleotide-psoralen conjugates have been used to induce site-specific mutations on plasmids. These mutations are located at the specific site where psoralen cross-linking is induced by UV irradiation after triple helix formation. They clearly indicate that the target site has been reached by the oligonucleotide within cells. However, until now the target sites have been limited to plasmidic vectors and not to endogenous genes. The yield of mutations reflects only a fraction of the cross-linked sites since it is expected that DNA repair systems remove part of the cross-links to restore the original sequence of DNA (Wang *et al.*, 1995, Sandor and Bredberg, 1994, Raha *et al.*, 1996).

III. Clamp oligonucleotides

An oligopurine sequence on a single-stranded nucleic acid can be recognized by a complementary (antisense) oligonucleotide. The short double helix with an oligopyrimidine•oligopurine sequence can, in turn, be recognized by a third strand oligonucleotide to form a triple helix. The two oligonucleotides can be linked to each other to form a unique molecule that can clamp the target sequence on the single-stranded template (Giovannangeli *et al.*, 1991) (**Figure 4**). The nature of the third strand (oligopyrimidine, oligopurine or (G,T)-oligonucleotide) determines its orientation with respect to the oligopurine target sequence. Therefore the linker between the antisense and the "antigene" portions will join a 3'- to a 3'-end or a 5'- to a 5'-end (for an antiparallel orientation of the third strand) or a 3'- to a 5'-end (for a parallel orientation). In the last case a circular oligonucleotide can also be synthesized (Kool, 1991). For (mostly) entropic reasons the circular oligonucleotide will bind more tightly than the clamp oligonucleotide which in turn binds much more tightly than two separate oligonucleotides (at least in the micromolar range of concentrations).

Clamp oligonucleotides have been shown to inhibit primer extension by DNA polymerase on a single-stranded template under conditions where antisense oligonucleotides are devoid of any inhibitory activity (Giovannangeli *et al.*, 1993). They can also arrest reverse transcription on a single-stranded RNA template. We have recently shown (C. Giovannangeli *et al.*,, unpublished results) that a clamp oligonucleotide is able to block HIV infection of CD4-positive cells at an early step after infection, most likely reverse transcription. No proviral DNA is detected after viral infection. Control experiments were carried out with a modified sequence of the clamp oligonucleotide and, more importantly (see above for antigene oligonucleotides), with a mutated version of the target sequence using the same clamp oligonucleotide. In both cases no inhibition of viral infection was observed indicating that the inhibitory effect on the wild-type sequence is likely due to clamp formation. An antisense oligonucleotide targeted to the same sequence exhibited no inhibition.

Clamp oligonucleotides can be covalently attached to an intercalating agent. If the antisense portion is made a little longer than the third strand portion, intercalation can lock the complex in place on the single-stranded target (Giovannangeli *et al.*, 1993).

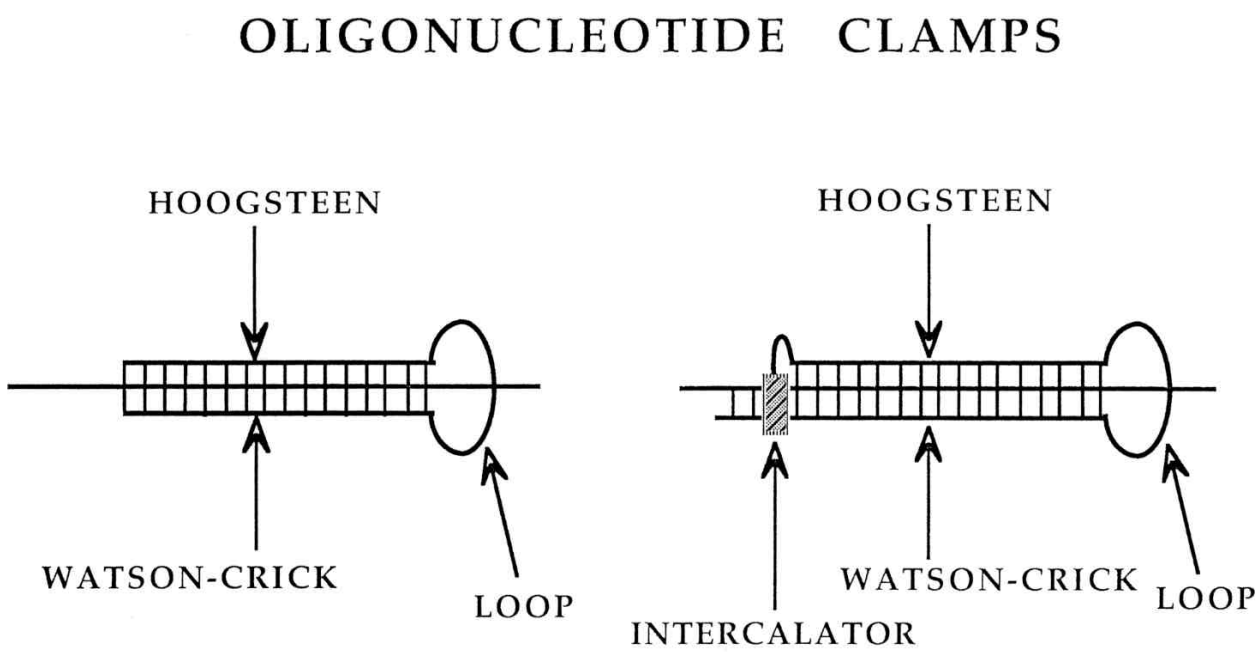

Figure 4: Oligonucleotide clamps formed of two portions connected by a loop ; they form both Watson-Crick and Hoogsteen hydrogen bonds with the single-stranded target. Attachment of an intercalator to one end of the clamp oligonucleotide stabilizes the complex (provided the Watson-Crick portion is made longer than the Hoogsteen portion) (Giovannangeli *et al.*, 1991, 1993).

IV. Gene therapy protocols based on the antigene strategy

Antisense RNAs can be generated in cells by transcription of appropriate DNA vectors. It is conceivable to use a DNA vector to express an RNA that could form a triple helix with a targeted sequence on cellular DNA. Recent experiments described by Judith and Joseph ILAN's group suggest that a potential triple helix-forming RNA can be obtained from an episomal vector. They observed the biological responses expected for transcription inhibition. The target gene was either IGF1 (Shevelev *et al.*, 1997) or its receptor IGF1-R (Rininsland *et al.*, 1997). In both cases an oligopyrimidine-oligopurine sequence was used as a target, in the 5'-untranslated region of the IGF1 gene or in the 3'-untranslated region of the IGF1-R gene.

Only the vector expressing the oligopurine third strand RNA sequence was shown to inhibit transcription of either gene. This oligopurine sequence was inserted in a much longer RNA transcript whose folding might play an important role in the biological effect. No evidence has been provided yet for the formation of a triple helix by the RNA transcript and the target gene. However several control sequences did not exhibit any activity. For example the RNA transcript inhibiting IGF1 did not show any activity on IGF1-R transcription.

Inhibition of IGF1 or IGF1-R by a potential antigene (triple helix) mechanism inhibits the tumorigenic potential of a rat glioblastoma cell line (C6) in nude mice and in syngenic rats. As observed with episomal DNA vectors expressing an antisense RNA, inhibition of IGF1 or IGF1-R expression induces an immune response in syngenic rats which leads to a shrinkage of grafted tumors (Trojan *et al.*, 1993). Further experiments are presently under way on chemically-induced hepatocarcinoma in rats (Frayssinet *et al.*, 1997). The antigene strategy could form the basis of a novel gene therapy approach to control the expression of specific genes, as does the antisense strategy.

V. Conclusion

Triple helix formation represents an alternative to antisense oligonucleotides to control gene function. Antigene oligonucleotides targeted to the DNA double helix can inhibit transcription. Clamp oligonucleotides targeted to a viral sequence can inhibit reverse transcription. They might also inhibit translation of a messenger RNA (even though there is no data yet available on translation inhibition). Circular oligonucleotides forming a triple helix with a single-stranded template might also be useful in both approaches. Strand displacement reactions as observed with PNAs (which involve triple helix formation by two PNAs on one of the two DNA strands) might represent an alternative to antigene oligonucleotides which bind to DNA without any opening of the double helix. Further

experiments with oligonucleotide analogues that form stable triple helices (e.g., oligophosphoramidates) will tell us whether the antigene or clamp strategies can be applied to biologically-relevant *in vivo* situations.

Antisense oligonucleotides have reached the stage of clinical trials in several pathological disorders. The information gained on bioavailability, pharmacokinetics, delivery, routes of administration... will be useful in any development of antigene oligonucleotides. Whether there is any advantage in targeting the gene rather than its messenger RNA (or pre-mRNA) remains to be determined in each particular case. Nuclease-resistant analogues (such as N3' → P5' oligophosphoramidates) could have long-lasting effects on gene transcription. The lower number of targets (two alleles for each gene) as compared to messenger RNAs might be an obvious advantage, especially for oligonucleotide analogues, such as oligophospho-ramidates, that do not bind strongly to cellular proteins. This should allow us to obtain a biological response at rather low oligonucleotide concentrations provided the target sequence is accessible within the chromatin structure of cell nuclei. Recent experiments have indeed shown this to be the case (Guieysse-Peugeot *et al.*, 1996 ; Giovannangeli *et al.*, 1997). In addition there might be proteins within cells that bind strongly to triple-helical complexes formed upon binding of antigene oligonucleotides to their target DNA sequence. Proteins with these charcteristics have been recently described (Kiyama *et al.*, 1991 ; Guieysse-Peugeot *et al.*, 1997).

Target sequences for antigene oligonucleotides remain limited to oligopyrimidine•oligopurine tracts of double-helical DNA. The design of nucleoside analogues or modifications of oligonucleotides involving, e.g., the insertion of intercalating agents should allow us to extent the range of triple helix-forming DNA sequences. Together with minor groove-binding ligands with an increased range of sequence-specific recognition (Gottesfeld *et al.*, 1997), major groove-specific ligands such as antigene oligonucleotides provide a new way of controlling gene transcription *in vivo*. The parallel development of a gene therapy approach based on triple helix formation opens new possibilities to control gene expression in pathological disorders.

References

Beal P.A & P.B. Dervan (**1991**) Second structural motif for recognition of DNA by oligonucleotide-directed triple-helix formation. **Science** 251, 1360-1363.

Cooney M., G. Czernuszewicz, E.H. Postel, S.J. Flint & M.E. Hogan (**1988**) Site-specific oligonucleotide binding represses transcription of the human c-myc gene *in vitro*. **Science** 241, 456-459.

Escudé C., J.C. François, J.S. Sun, G. Ott, M. Sprinzl, T. Garestier & C. Hélène (**1993**) Stability of triple helices containing RNA and DNA strands : experimental and molecular modeling studies. **Nucleic Acids Res**. 21, 5547-5553.

Escudé C., C. Giovannangeli, J.S. Sun, D.H. Lloyd, J.K. Chen, S. Gryaznov, T. Garestier & C. Hélène (**1996**) Stable triple-helices formed by oligonucleotide N3' → P5' phosphoramidates inhibit transcription elongation. **Proc. Natl. Acad. Sci. USA** 93, 4365-4369.

François J.C., T. Saison-Behmoaras, C. Barbier, M. Chassignol, N.T. Thuong & C. Hélène (**1989**) Sequence-specific recognition and cleavage of duplex DNA via triple-helix formation by oligonucleotides covalently linked to a phenanthroline-copper chelate. **Proc. Natl. Acad. Sci. USA** 86, 9702-9706.

Giovannangeli C., T. Montenay-Garestier, M. Rougée, M. Chassignol, N.T. Thuong & C. Hélène (**1991**) Single-stranded DNA as a target for triple helix formation. **J. Am. Chem. Soc**. 113, 7775-7777.

Giovannangeli C., N.T. Thuong & C. Hélène (**1993**) Oligonucleotide clamps arrest DNA synthesis on a single-stranded DNA target. **Proc. Natl. Acad. Sci. USA** 90, 10013-10017.

Giovannangeli C., L. Perrouault, C. Escudé, N.T. Thuong & C. Hélène (**1996**) Specific inhibition of *in vitro* transcription elongation by triplex-forming oligonucleotide-intercalator conjugates targeted to HIV proviral DNA. **Biochemistry** 35, 10539-10548.

Giovannangeli C., S. Diviacco, V. Labrousse, S.M. Gryaznov, P. Charneau & C. Hélène (**1997**) Accessibility of nuclear DNA to triplex-forming oligonucleotides : the integrated HIV1 provirus as target. **Proc. Natl. Acad. Sci. USA** 94, 79-84.

Gottesfeld J.M., L. Neely, J.W. Trauger, E.E. Baird, P.B. Dervan (**1997**) Regulation of gene expression by small molecules. **Nature** 287, 202-205.

Grigoriev M., D. Praseuth, P. Robin, A. Hemar, T. Saison-Behmoaras, A. Dautry-Varsat, N.T. Thuong, C. Hélène & A. Harel-Bellan (**1992**) A triple helix-forming oligonucleotide-intercalator conjugate acts as a transcriptional repressor via inhibition of NF KB binding to IL-2 receptor α regulatory sequence. **J. Biol. Chem**. 267, 3389-3395.

Grigoriev M., D. Praseuth, A.L. Guieysse, P. Robin, N.T. Thuong, C. Hélène & A. Harel-Bellan (**1993**) Inhibition of interleukin-2 receptor α-subunit gene expression by oligonucleotide-directed triple helix formation. **C. R. Acad. Sci. Paris, Sciences de la vie/Life Sciences** 316, 492-495.

Guieysse A.L., D. Praseuth, C. Hélène (**1997**) Identification of a triplex DNA-binding protein from human cells. **J. Mol. Biol.** 267, 289-298.

Guieysse A.L., D. Praseuth, M. Grigoriev, A. Harel-Bellan, C. Hélène (**1996**) Detection of covalent triplex inside human cells. **Nucleic Acids Res**. 24, 4210-4216.

Hélène C. (**1991**) The anti-gene strategy : control of gene expression by triplex-forming-oligonucleotides. **Anti-cancer Drug Design** 6, 569-584.

Hélène C. (**1994**) Control of oncogene expression by antisense nucleic acids. **Europ. J. Cancer** 30A, 1721-1726.

Ing N.H., J.M. Beekman, D.J. Kessler, M. Murphy, K. Jayaraman, J.G. Zendegui, M.E. Hogan, B.W. O'Malley & M.J. Tsai (**1993**) *In vivo* transcription of a progesterone-responsive gene is specifically inhibited by a triplex-forming oligonucleotide. **Nucleic Acids Res.** 21, 2789-2796.

Kiessling L.L., L.C. Griffin & P.B. Dervan (**1992**) Flanking sequence effects within the pyrimidine triple-helix motif characterized by affinity cleaving. **Biochemistry** 31, 2829-2834.

Kiyama R. & R.D. Camerini-Otero (**1991**) A triplex DNA-binding protein from human cells : purification and characterization. **Proc. Natl. Acad. Sci. USA** 88, 10450-10454.

Kool E.T. (**1991**) Molecular recognition by circular oligonucleotides : increasing the selectivity of DNA binding. **J. Am. Chem. Soc**. 113, 6265-6266.

Lafarge-Frayssinet C., H.T. Duc, C. Frayssinet, A. Sarasin, D. Anthony, Y. Guo, J. Trojan (**1997**) Antisense IGF I transfer into a rat hepatoma cell line inhibits tumorigenesis by modulating MHC-I cell surface expression. **Cancer Gene Therapy** 4, 276-285.

Maher III L.J., P.B Dervan & B. Wold (**1990**) Kinetic analysis of oligodeoxyribonucleotide-directed triple-helix formation on DNA. **Biochemistry** 29, 8820-8826.

Maher III L.J., P.B Dervan & B. Wold (**1992**) Analysis of promoter-specific repression by triple-helical DNA complexes in a eukaryotic cell-free transcription system. **Biochemistry** 31, 70-81.

Nielsen P.E., M. Egholm & O. Buchardt (**1994**) Peptide nucleic acids (PNA). A DNA mimic with a peptide backbone. **Bioconj. Chem**. 5, 3-7.

Perrouault L., U. Asseline, C. Rivalle, N.T. Thuong, E. Bisagni, C. Giovannangeli, T. Le Doan & C. Hélène (**1990**) Sequence-specific artificial photo-induced endonucleases based on triple helix-forming oligonucleotides. **Nature** 344, 358-360.

Raha M., G. Wang, M.M. Seidman & P.M. Glazer (**1996**) Mutagenesis by third-strand-directed psoralen adducts in repair-deficient human cells : high frequency and altered spectrum in a xeroderma pigmentosum variant. **Proc. Natl. Acad. Sci. USA** 93, 2941-2946.

Rininsland F., T.R. Johnson, C.L. Chernicky, E. Schulze, P. Burfeind, J. Ilan, J. Ilan (**1997**) Suppression of insulin-like growth factor type I receptor by triple-helix strategy inhibits IGF-I transcription and tumorigenic potential of rat C6 glioblastoma cells. **Proc. Natl. Acad. Sci. USA** 94, 5854-5859.

Rougée M., B. Faucon, J.L. Mergny, F. Barcelo, C. Giovannangeli, T. Montenay-Garestier & C. Hélène (**1992**) Kinetics and thermodynamics of triple helix formation : effects of ionic strength and mismatches. **Biochemistry** 31, 9269-9278.

Sandor Z. & A. Bredberg (**1994**) Repair of triple helix directed psoralen adducts in human cells. **Nucleic Acids Res.** 22, 2051-2056.

Shevelev A., P. Burfeind, E. Schulze, F. Rininsland, T.R. Johnson, J. Trojan, C.L. Chernicky, C. Hélène, J. Ilan, J. Ilan (**1997**) Potential triple helix-mediated inhibition of IGF-I gene expression significantly reduces tumorigenicity of glioblastoma in an animal model. **Cancer Gene Therapy** 4, 105-112.

Silver G.C., J.S. Sun, C.H. Nguyen, A.S. Boutorine, E. Bisagni, C. Hélène (**1997**) Stable triple-helical DNA complexes formed by benzopyridoindole- and benzopyridoquinoxaline- oligonucleotide conjugates. **J. Am. Chem. Soc.** 119, 263-268.

Sun J.S., J.C. François, T. Montenay-Garestier, T. Saison-Behmoaras, V. Roig, N.T. Thuong, C. Hélène (**1989**) Sequence-specific intercalating agents : intercalation at specific sequences on duplex DNA via major groove recognition by oligonucleotide-intercalator conjugates. **Proc. Natl. Acad. Sci. USA** 86, 9198-9202.

Sun J.S. (**1995**) Rational design of switched triple helix-forming oligonucleotides: extension of sequences for triple helix formation. In "**Modelling of Bimolecular Structures and Mechanisms**, A. Pullman *et al.* (Eds), Kluwer Academic Publishers, Netherlands, pp. 267-288.

Sun J.S. T. Garestier & C. Hélène (**1996**) Oligonucleotide directed triple helix formation. **Curr Opin Struct Biol** 6, 327-333.

Takasugi M., A. Guendouz, M. Chassignol, J.L. Decout, J. Lhomme, N.T. Thuong & C. Hélène (**1991**) Sequence-specific photo-induced cross-linking of the two strands of double-helical DNA by a psoralen covalently linked to a triple helix-forming oligonucleotide. **Proc. Natl. Acad. Sci. USA** 88, 5602-5606.

Thuong N.T. & C. Hélène (**1993**) Sequence-specific recognition and modification of double-helical DNA. **Angew. Chem. Int. Ed. Engl.** 32, 666-690.

Wang S. & T. Kool (**1995**) Relative stabilities of triple helices composed of combinations of DNA, RNA and 2'-O-methyl-RNA backbones: chimeric circular oligonucleotides as probes. **Nucleic Acids Res**. 23, 1157-1164.

Wang G., D.D. Levy, M.N. Seidman & P.M. Glazer (**1995**) Targeted mutagenesis in mammalian cells mediated by intracellular triple helix formation. **Mol Cell Biol** 15, 1759-1768.

Zhou B.W., E. Pugas, J.S. Sun, T. Garestier & C. Hélène (**1995**) Stable triple helices formed by acridine-containing oligonucleotides with oligopurine tracts of DNA interrupted by one or two pyrimidines. **J. Am. Chem. Soc.** 117, 10425-10428.