CHAPTER 2

Modulation of Nucleic Acid Information Processing by PNAs:
Potential Use in Anti-Viral Therapeutics

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Introduction

Nucleic Acid Information Processing

The transfer of genetic information from one nucleic acid molecule (DNA or RNA) to another (replication, transcription) or to proteins (translation) is a fundamental life process. Although interference with this process is an important target for drug development, to date, drugs capable of targeting specific stages within this scheme (e.g., anticancer drugs and antiviral nucleoside analogs) have shown limited nucleic acid sequence specificity and therefore present consequent side effects. In contrast, short nucleic acid molecules are capable of binding to specific sequences of DNA or RNA and can, therefore, be tailor-designed to target specific genes. To what extent this exquisite targeting can be exploited to modulate information processing of these genes will be discussed in this review with special reference to antiviral strategies. Indeed, viruses are an ideal target in this respect as their genetic information is distinct from that of the host cell. In addition many mechanisms involved in the processing of nucleic acid information are specific to the viruses (reverse transcription, integration, certain stages of replication, transcription transactivation, nuclear transport, RNA dimerization).

PNAs as a Ligand for Nucleic Acids

PNAs are DNA analogs in which a 2-amino-ethyl-glycine linkage replaces the normal phosphodiester backbone[1] (Fig. 1). Nucleoside bases are attached to this backbone via methyl carbonyl linkers and stack in such a manner that they can hybridize to complementary DNA or RNA via hydrogen bonding. Watson-Crick hydrogen bonding allows hybridization to single stranded nucleic acid molecules in true antisense style[2] (Fig. 2A), whereas Hoogsteen hydrogen bonding allows formation of triple helices between two PNAs, which may be linked together, and a polypurine tract within a RNA (Fig. 2B) or DNA target (Fig. 2C).

The Pros and Cons of PNA as a Nucleic Acid Ligand

Short single stranded DNA oligomers have an extremely short half-life in biological systems, being rapidly degraded by nucleases. This major limitation to their use as therapeutic agents has led to the design of a large number of DNA analogues. PNAs stand apart from most analogues as their backbones have been completely replaced; as Nielsen has pointed out, it is more appropriate to refer to them as "mimics," as only the nucleobases are conserved. This structure endows them with a phenomenal stability with respect to nucleases.[3] In addition, the

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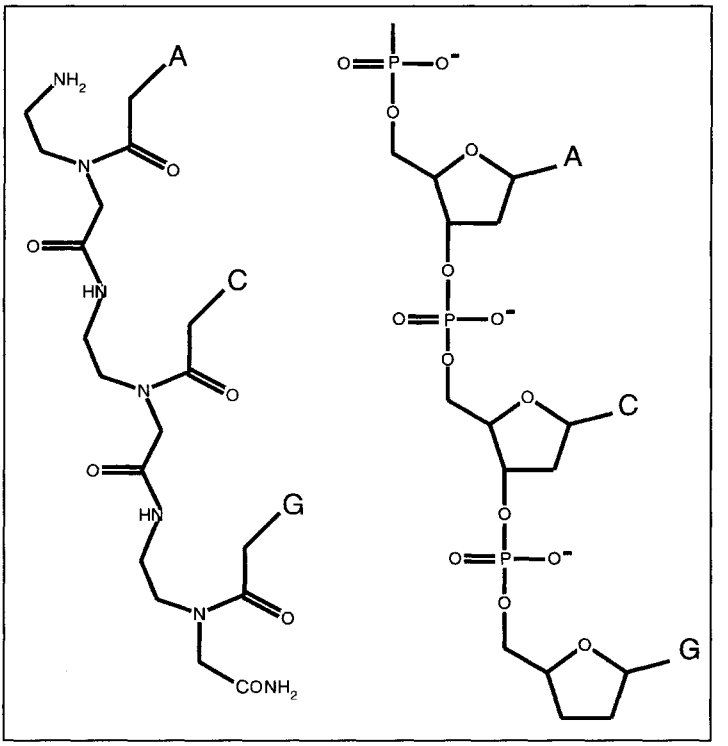

Figure 1. Chemical structure of PNA (left) and DNA (right).

uncharged nature of the pseudopeptide backbone confers a very high affinity for their target sequence. A tri-stranded (triplex) structure, involving both Watson-Crick and Hoogsteen hydrogen bonding (Fig. 2D), can form between two PNA molecules and a polypurine single stranded DNA or RNA target[1,4] (Fig. 2B). The formation of this complex can also occur on double stranded target molecules via an initial phase of strand invasion and Watson-Crick binding to the complementary strand, the other strand being displaced as a D-loop[5] (Fig. 2C).

Despite these obvious advantages of PNA, namely stability and versatility, two major limitations exist for some applications. First, PNA structure does not allow it to direct the activation of RNaseH.[6] This ubiquitous, cellular enzyme cleaves the RNA strand of DNA/RNA heteroduplexes and has been linked to the removal of the RNA primers of Okazaki fragments during DNA replication. This activity can be exploited to give catalytic activity to antisense DNA oligonucleotides, and RNaseH activity has been widely proposed as a major contributor to the efficacity of antisense oligonucleotides, although the exact contribution has never been clearly demonstrated. Secondly, the intracellular delivery of oligonucleotides in cell culture experiments has very often involved their complexation with vectors (e.g., cationic lipids) via the negatively charged backbone of DNA. Obviously, these techniques cannot be used (without modification) with the neutral backboned PNAs. Alternative vector strategies involving cell poration (electroporation, streptolysin-O), pinocytosis or physical membrane damage (scrape loading, syringe loading) are available, but have not been fully explored for PNA. Some success in cellular internalization has been achieved by chemically coupling the PNAs to receptor-binding ligands[7, 8] or to the cell-penetrating, Antennapedia peptide.[9-11]

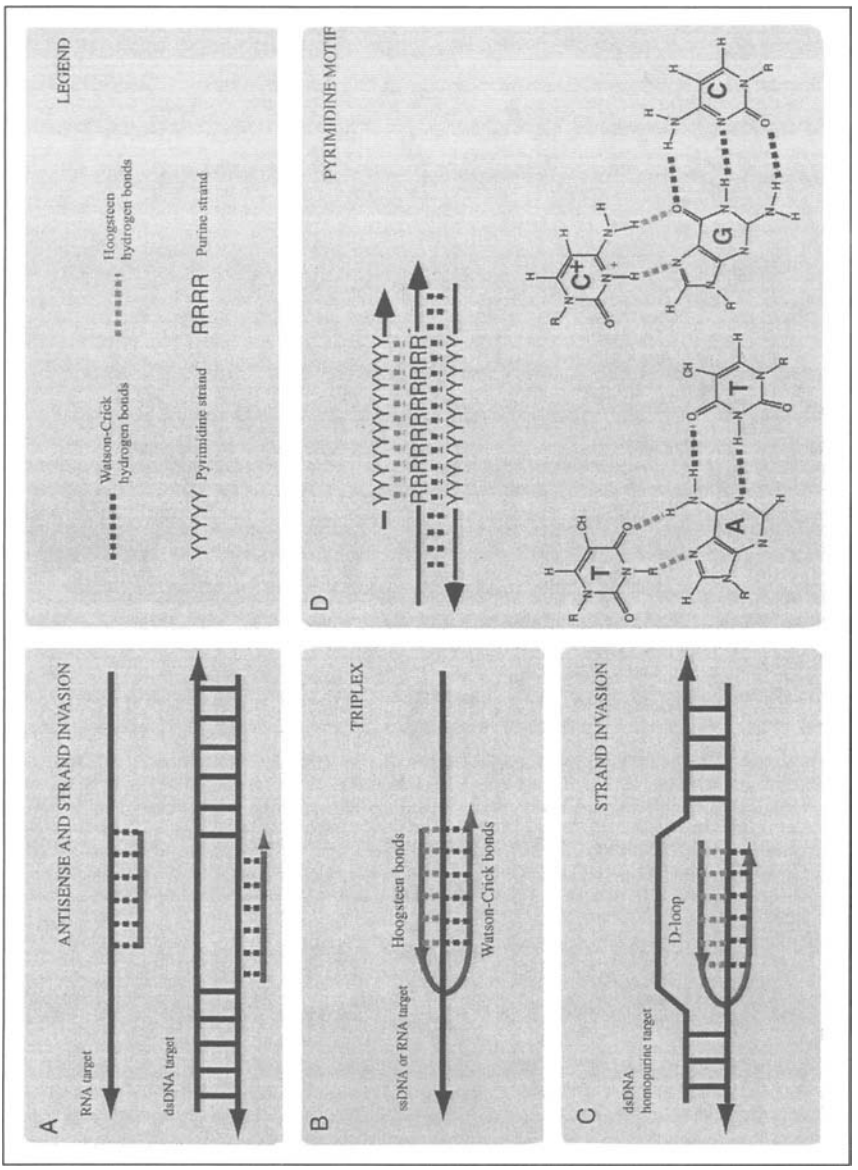

Figure 2. Schematic representation of PNA binding modes. A) Duplex formation. A homopurine PNA is required for duplex invasion. B) and C) Tripical helical structure formed by homopyrimidine bis-PNAs and polypurine targets. These structures contain both Watson-Crick and Hoogsteen base pairing. D) Canonical triads of the pyrimidine motif.

The Use of PNA to Modulate Nucleic-Acid Information Processing

In contrast to the classical scenario of RNase H-mediated cleavage of target RNA Molecules,[12] that lead to their destabilization and rapid elimination, PNAs induce no irreversible modification of their targets. Their high affinity, however, can possibly be exploited to interfere with different aspects of nucleic acid information processing. For this to occur, the hybridization of the PNA on its target must sterically block the processing mechanism. Many aspects of nucleic acid information processing can be targeted: replication, transcription, and translation. In this review we have taken a mechanistic approach to antisense or antigene PNA targeting and will analyze the following phases:

A. Initiation. The binding of specific nucleic acid sequences, in the midst of the vast excess of non specific sequences, by multiprotein complexes and the melting of the DNA duplex in the case of replication or transcription.
B. Elongation. The translocation along the template and chain extension.
C. Termination. The release of the template and of the elongated chain.
D. Coupling. Reactions which occur in parallel.

PNAs as Gene-Modulator Agents

Initiation

The first step of initiation for replication or transcription involves the recognition of a specific sequence (i.e., formation of a preinitiation complex), unwinding of the initiation site, and assembly of a primed holoenzyme complex. These holoenzyme complexes are large and involve many proteins and RNA in the case of translation. They are formed at specific sites on the nucleic acid via localizer proteins.

Replication initiation-point mapping in yeast and human cells has revealed well-defined start points at which DNA replication initiates; a scenario very reminiscent of transcription initiation. In higher eukaryotes, the origins of replication are recognized by a six-protein complex termed ORC (origin of replication recognition complex).[13]

In transcription the promoter/enhancer is recognized by DNA-binding transcription factors. Eukaryotic RNA polymerase II (pol II) is a large enzyme complex comprising at least 12 distinct subunits and possessing a molecular mass in excess of 500 kilodaltons. Transcription initiation by pol II is an elaborate, multistep process that requires, at a minimum, the five general initiation factors TFIIB, TFIID, TFIIE, TFIIF and TFIIH which together with the polymerase represent an aggregate molecular mass of nearly 2 megadaltons (2,000kD).[14]

In translation, the 5'-cap-modified extremity of all cellular and most viral mRNAs is recognized via a cap-binding complex. The 40S ribosomal subunit, carrying Met-tRNA, eIF2, GTP, and other factors (the 43S complex) then migrates through the 5' UTR until it encounters the first AUG codon, which is recognized by base pairing with the anticodon in Met-tRNA. When a 60S ribosomal subunit joins the paused 40S subunit, selection of the start codon is fixed.[15]

Replication

In all three processes (i.e., replication, transcription, translation) competition for binding sites by PNAs potentially inhibits the formation of initiation complexes. In this respect, replication of DNA is perhaps the most difficult process to inhibit. Indeed, no consensus ORC recognition sequence has yet been identified in higher eukaryotes, and indeed the sites bound may be broad. In contrast, many viruses of eukaryotic cells have retained a simpler initiation mechanism. For example, bovine papillomavirus type I (BPV-1)replication depends upon two virus-encoded proteins, El and E2. E2 is the sequence-specific localizer protein that binds the 12 bp palindromic sequence $ACC(N)_6GGT$ and assists the loading of the El helicase activity.[16] An 18-mer PNA complementary to this sequence was found to inhibit E2 binding and block replication of a plasmid containing it when the plasmid is transfected with the PNA precomplexed to it. However, this same PNA had no effect when transfected after plasmid transfection.[17] In

this study the nature of the complex formed with the double-stranded DNA is unclear, though strand invasion is probably involved. Simian virus-40 origin of replication localization is accomplished by the T-antigen (helicase) that binds to an AT-rich region. This origin has never been targeted by PNA, but an 8-mer T phosphodiester oligonucleotide, covalently linked to an intercalating agent (acridine), inhibits the cytopathic effect of SV-40 on CV-1 cells in culture.[18] It is unclear, however, whether the oligonucleotide has inhibited replication and/or bound to the mRNA transcribed from this sequence.

Transcription

Few studies have addressed the inhibition of transcription initiation with PNA. In one study, although a PNA targeting the NF-κB recognition site was capable of inhibiting NF-κB binding to double stranded DNA probes in gel retardation experiments,[19,20] inhibition of transcription initiation in vivo from a promoter containing multiple copies of this site was observed only when the promoter/PNA complex was preformed prior to transfection of the cell culture.[20] However, Praseuth et al[19] found no sequence-specific inhibition of transcription initiation in vivo using a preformed promoter/PNA complex.

Direct inhibition of TATA-binding protein by PNAs has not been tested yet. However, Helene et al[21] demonstrated a transcription block, in vitro, using a triple-helix forming DNA oligonucleotide in a prokaryotic system. The target site in this case was just outside of the footprint of the *E. coli* RNA polymerase and therefore it is unclear whether it was initiation or elongation of transcription that was inhibited.

Translation

Translation initiation in eukaryotes occurs in three phases. First, the 43S ribosome binds to the cap structure at the capped end of the mRNA. The 43S ribosome then scans to the first AUG start codon within a consensus Kozak sequence, where assembly of the complete 80S ribosome occurs.[15] Both duplex and triplex forming PNA are capable of inhibiting translation when targeted to the AUG initiator codon. Although the inhibition of 80S assembly has never been formally demonstrated, Mologni et al[22,23] have demonstrated an inhibition of translation by a mixture of three PNAs directed respectively against the 5' UTR, the first AUG codon, and a site within the coding region that includes the second AUG codon in cell-free extracts[23] and in cell culture.[22] In this case it is impossible to ascertain whether the effect involves only initiation. In another study conducted in intact cells, Doyle et al[24] targeted 27 PNAs to 18 different sites throughout the 5' UTR, the start site, and coding regions of luciferase mRNA. In contrast to PNAs targeted to other regions, even to the AUG site, only PNAs targeted to the 5' terminus were potent inhibitors. As emphasized by the authors, this suggests that PNAs can block binding of the translation machinery but are less able to block the progression of a ribosome.

PNAs have not been used to block translation initiation in viruses. This is surprising, as many viruses use an alternative initiation process that is cap-independent and involves the direct binding of the 40S ribosome to a highly structured 5'-untranslated region. This structure, named an internal ribosome entry site (IRES) is well characterized in the picornaviruses, but is also found in certain other viruses. It is found only rarely in normal cellular mRNA, making it a good target for an antisense PNA. In this regard, we have demonstrated the efficacy of PNAs directed to the IRES of hepatitis C virus (HCV) to block the assembly of the 43S ribosome and to specifically inhibit IRES-mediated translation (unpublished results).

Elongation

Once an initiation complex has formed, polymerization can start using a single stranded nucleic acid as a template. For this to occur, the double stranded DNA (in replication and transcription) or the structured RNA (in translation) must be melted by the action of helicases. Elongation is processive, involving the movement of large protein or ribonucleoprotein complexes with respect to the nucleic acid complex. Steric blocking of elongation, unlike initiation,

does not involve stopping a protein from docking, but rather the arrest of a large, dynamic, multi-subunit machine which has been engineered to be highly processive.

Replication

In vitro, PNA targeted to homopurine DNA sites is able to block both Taq DNA polymerase and the large fragment of *E. coli* DNA polymerase (Klenow).[25] The only published example of true replication-elongation arrest in cell-free extracts concerns mitochondrial DNA replication.[26] However, despite the accessibility of mitochondrial DNA to hybridization (as it is single-stranded during much of the replication process) and a clear demonstration of efficient uptake of oligonucleotide into mitochondria, no inhibition of replication has been observed in vivo.[27] Data from our laboratory indicate that the elongation activity of purified DNA polymerase or unfractionated replication-competent human cell extracts is significantly inhibited when a bis-PNA is bound to the template strand.

Transcription

Arresting transcription elongation by PNAs using purified RNA polymerase has been clearly demonstrated.[25,28] In all cases, triple helix formation was involved. Boffa et al[29] demonstrated an efficient inhibition of transcription elongation when targeting a tandem CAG repeat found in a number of binding sites of transcription factors with a 18-mer PNA in a permeabilized cell assay. The mechanism of hybridization was not proven, but probably involves strand invasion. In a recent study, a 17-mer c-myc anti-gene PNA has been shown to be effective in intact cells.[9] This PNA, when covalently linked to a basic nuclear localization signal (NLS) peptide, was able to specifically block the transcription of c-myc and inhibit some of its biological functions. Another indication that covalent linkage of PNAs to appropriate vectors is necessary for significant activity in intact cells is shown by a study of Boffa et al.[7] In that study, an anti-myc PNA linked to dihydrotestosterone was specifically internalized by a cell line bearing the receptor of this hormone and inhibited c-myc expression.

Translation

The capacity of duplex-forming PNAs to arrest ribosome progression is still unclear. In vitro, PNAs targeting the coding region of mRNA are, in most cases, incapable of inhibiting translation.[6,30] However, in other cases duplex-forming PNAs targeted to G+C-rich sequences can arrest polypeptide chain elongation.[31] In addition, a 10-mer pyrimidine-rich PNA complementary to a sequence in the coding region of SV40 T antigen mRNA could block translation in vitro and 15 and 20-mer pyrimidine-rich PNAs targeted to sequences in the same region inhibited T antigen expression when microinjected into Tsa 8 cells.[4] In contrast to duplex PNA, triplex-forming PNAs are clearly capable of arresting a ribosome.[6] Along these lines, a 10-mer pyrimidine-rich PNA complementary to a sequence in the coding region of SV40 T antigen mRNA could block translation in vitro and 15 and 20-mer pyrimidine-rich PNAs targeted to sequences in the same region inhibited T antigen expression when microinjected into Tsa 8 cells.[4] It should be noted, however, that PNA_2/RNA triplex use is somewhat restricted because it requires a homopurine target sequence.

Helicase Activity

An extrapolation of data obtained from cell-free assays to intact cells is difficult because cellular metabolic processes such as transcription, replication or translation are mediated by large 'protein machines' comprising many interacting polypeptides.[32] These complexes deal more efficiently with secondary structures and other obstacles than isolated polymerases. As an example, all transcription and replication complexes include helicases, which utilize the energy of nucleoside triphosphate hydrolysis to unwind the DNA double helix.[33] Helicases are thought to precede polymerases and their accessory proteins. It is therefore anticipated that helicases will most likely be the first proteins in these enzymatic complexes to encounter a triple-helical

structure. Several studies have shown that triple helices made with phosphodiester oligonucleotides are efficiently unwound by DNA helicases.[34, 35]

We have investigated the inhibitory effect of a triple helix forming bis-PNA on the helicase activity of herpes simplex virus type I, UL9 protein.[36] UL9 has been shown to be essential for DNA replication and its activity has been well characterized,[37] thus representing a particularly suitable model for our investigations. A bis-PNA triple helix formed on a synthetic DNA substrate significantly inhibits UL9 unwinding activity. The inhibitory effect of a bis-PNA on the template strand can be explained by a model in which translocation of the UL9 protein in the 3'→5' direction is impaired by bis-PNA. Another report indicates that hepatitis C virus helicase (nonstructural protein 3) activity was at least 25-fold slower for PNA/DNA than for the equivalent DNA/DNA substrate.[38] Interestingly, the same team has demonstrated that helicase from bacteriophage T4 was capable of unwinding DNA-PNA substrates at similar rates as DNA-DNA substrates.[39]

Termination

Transcription

Transcription termination is an important process as it enhances gene expression by facilitating polymerase recycling and thus maintains a pool of available polymerase. Mechanisms of termination have been described for genes transcribed by *E. coli* RNA polymerase and RNA polymerase I and III, but are poorly understood for RNA pol II which transcribes premRNA. The terminator element of eukaryotic class III genes is constituted by a run of thymidine residues on the coding strand and is expected to form highly stable triple-helix complexes with oligothymine peptide nucleic acids. Dieci et al[40] have analyzed the effect of a t_{10} PNA on in vitro transcription of three yeast class III genes. At nanomolar concentrations, the PNA almost completely inhibited transcription of supercoiled, but not linearized templates. This can reflect the fact that DNA supercoiling enhances PNA binding by influencing the dynamics of base-pair "breathing".[41]

Transcriptionally-Coupled RNA Processing

Polyadenylation

Eukaryotic genes often have long 3'-UTRs that contain more than one polyadenylation site. Choice of polyadenylation site can be critical to mRNA stability if destabilization signals are present in the longer, but not the shorter, form of the message. The site used may also influence translation efficiency or localization of a mRNA in a tissue- or disease-specific manner. Two sequence elements determine the precise site of 3'-end cleavage and polyadenylation of premRNAs: a highly conserved AAUAAA signal located 10-30 bases 5' of the cleavage site, and a variable GU-rich element 20-40 bases 3' of the site.[42]

Recently, 2'-O-methoxy-ethyl phosphorothioate oligonucleotides complementary to E-selectin polyadenylation sites and signals were tested for the ability to redirect polyadenylation from a site that results in an mRNA with many destabilization elements to sites resulting in a shorter message with fewer destabilizing elements.[43] These alternative transcripts had increased mRNA stability and altered protein expression. This was the first demonstration of the use of antisense oligonucleotides to increase stability of a targeted mRNA. Interestingly, such a mode of action requires RNase H incompetent oligonucleotides, thus making PNAs very attractive candidates to redirect polyadenylation.

Splicing

The precise removal of premessenger RNA introns from the premRNA in eukaryotic nuclei is a major step in the regulation of gene expression. RNA splicing provides a mechanism whereby

protein isoform diversity can be generated and whereby the expression of particular proteins with specialized functions can be restricted to certain cell or tissue types during development. For efficient splicing, most introns require a conserved 5' splice site, and a branch point sequence followed by a polypyrimidine tract and a conserved 3' splice site. Interestingly, it is now known that a significant fraction (15%) of mutations in mammalian genes that are implicated in disease states are thought to affect RNA-splicing signals.[44]

Antisense oligonucleotides that bind target premRNA with high affinity have been proposed to alter splicing patterns in human diseases.[45] The feasibility of such approaches has been demonstrated by a report of successful treatment of erythroid progenitor cells from thalassemic patients carrying mutations in the HBB (β-globin) gene.[46] The responsible mutations activate cryptic splice sites in β-globin premRNA, resulting in a deficiency of adult hemoglobin A. Steric blocking (RNase H incompetent) morpholino oligonucleotides were shown to block splicing at the targeted site and restore correct splicing, resulting in an increase in hemoglobin production.

Recently, Karras et al[47] also have demonstrated that PNAs can redirect constitutive and alternative splicing of the murine interleukin-5 receptor-alpha (IL-5Rα) chain premRNA in vitro as well as in intact cells.

Virus Specific Nucleic Acid Processing

Reverse Transcription

As is typical for all retroviruses, the first step of replication of human immunodeficiency virus (HIV) occurs through reverse transcription of the viral genomic RNA, upon entry of the viral core into the host cell. Synthesis of the first cDNA strand, the minus strand, is initiated at a tRNA primer, which binds to the primer-binding site located close to the 5'-end of the genomic RNA. Lee et al[48] demonstrated that PNAs as well as PNA-DNA chimeras complementary to the primer-binding site of the HIV-1 genome can completely block priming by tRNA Lys and consequently the in vitro initiation of reverse transcription by HIV-1 RT. An antisense PNA targeted against the TAR region, which is located at the 5'-end of HIV-1 RNA, is also a strong inhibitor of reverse transcription in cell-free assays.[49]

Integration

The integration of retroviral genomes into the host genome is mediated by the viral integrase protein, which binds to specific sequences located on both extremities of the DNA long terminal repeats (LTRs). The HIV U3 LTR end contains a short purine-pyrimidine sequence which has been selectively targeted by a 7-mer purine, triple helix-forming oligonucleotide coupled to the intercalating chromophore oxazolopyridocarbazole (OPC).[50] In another study, an 11-mer oligonucleotide-OPC was designed to form an alternate strand DNA triplex near the integrase-binding site of the U5 LTR HIV-1 end.[51] Both oligonucleotides were capable of inhibiting HIV integration in vitro. However, the 7-mer oligonucleotide and the noncanonical triplex forming 11-mer, lacked selectivity and affinity, thus preventing their use in vivo. It should be interesting to take advantage of the very high affinity of PNAs for targeting these short sequences.

Transactivation

Tat is an essential HIV-1 protein that interacts with the transactivation response element (TAR) and stimulates transcription from the viral long-terminal repeat (LTR). Blockade of Tat-TAR interaction halts viral transcription and hence replication. An anti-TAR PNA competes for TAR and prevents Tat-mediated stimulation of HIV-1 LTR transcription in vitro as well as in cell culture.[52]

HIV Gag-pol Ribosomal Frameshifting

The HIV gag and pol genes overlap by 241 nucleotides, with pol in the -1 phase with respect to gag. The gag-pol fusion is produced via a -1 ribosomal frameshifting event that brings the overlapping, out-of-phase gag and pol genes into translational phase. Frameshifting occurs 8-10 nucleotides upstream of a hairpin loop which may play a role in the regulation of frameshifting. Vickers et al have used 2'-O-methyl oligonucleotides designed to specifically bind sequences flanking the gag-pol hairpin stem loop. Ribosomal frameshifting is enhanced up to 6 fold by an oligonucleotide binding the region immediately 3' to the stem.[53]

Dimerization

Dimerization of two homologous strands of genomic RNA is an essential feature of the retroviral replication cycle. In HIV-1, genomic RNA dimerization is facilitated by a conserved stem-loop structure located near the 5' end of the viral RNA called the dimerization initiation site (DIS). The DIS loop is comprised of nine nucleotides, six of which define a self-complementary sequence flanked by three conserved purine residues. Base pairing between the loop sequences of two copies of genomic RNA is necessary for efficient dimerization.[54] Knowledge of the sequences involved in dimerization could provide the basis for development of PNAs targeted against this step during retroviral replication.

Polypurine Tract and Nuclear Import

During HIV-1 reverse transcription, initiation of the plus-strand DNA at the central polypurine tract (cPPT) and termination at the central termination sequence (CTS) leads to the formation of a three-stranded DNA structure: the HIV-1 central DNA flap. The DNA flap is a cis-acting determinant of HIV-1 genome nuclear import.[55] The PPT is highly conserved among the known HIV-1 retroviral isolates. Two PPTs occur within the genome, one within the coding region of integrase (cPPT) and the other adjacent to the 3' LTR. The PPT has been targeted by triplex forming oligonucleotides but no study has so far looked at their effects on nuclear import. Hiratou et al[56] have shown that triple-helix formation, using a foldback triplex-forming phosphorothioate oligonucleotide, inhibits primer extension in vitro. In HIV-1-infected MOLT-4 cells, the same oligonucleotide limits the replication of HIV-1. Faria et al[57] have demonstrated that oligonucleotide analogues containing N3'-P5' phosphoramidate linkages can form triplexes on their PPT target sequence integrated into cellular chromosomes and inhibit transcriptional elongation.

PNA-Tuning

The inability of PNA to cleave DNA targets or to form a substrate for RNase H when hybridized to RNA targets has often been seen as a drawback for its use as an antisense or antigene agent. This has led to a number of studies attempting to set up a lytic capacity to an otherwise nonlytic oligonucleotide.

RNase Competent PNAs

RNase H competence has been restored to PNA by creating either PNA-DNA chimeras or by creating a DNA window within the PNA (a gapmer). In the latter strategy, we have shown that a minimum window size of 8 DNA residues was required,[58] although this may be reduced to six by retargeting the oligonucleotide (unpublished data). PNA also has been used as a guide sequence to target specific RNAs to degradation by RNase L, a ubiquitous, but latent, endo-ribonuclease. This has been achieved by coupling the PNA to 2'-5' oligoadenylate, the unique activator of RNase L.[59,60,61,62]

Use as a Guide Sequence for Chemical Lysis of DNA

A cationic manganese porphyrin-peptide nucleic acid conjugate has been synthesized by Bigey et al[63] and used to cleave a double-stranded DNA target. Oxidative activation by this Mn

porphyrin-PNA conjugate leads to sequence-specific, 3'-staggered cleavage of both DNA strands near the strand displacement junction. Furthermore, the Mn porphyrin-PNA conjugates bind over 100-fold better to double-stranded DNA compared to the native PNA. When a Gly-Gly-His tripeptide is placed on either the Watson-Crick or Hoogsteen bis-PNA strand, nickel-mediated cleavage is detected at specific sites on the displaced and hybridized DNA strands as reported by Footer et al.[64] Armitage et al[65] have reported the synthesis of a bis-PNA that is covalently linked to an anthraquinone imide. This conjugate forms strand invasion complexes with duplex DNA and irradiation with near-UV light leads to selective patterned cleavage of the displaced DNA strand at the PNA binding site.

Conclusion

The capacity of short PNAs to selectively bind specifically-targeted nucleic acid sequences, either by Watson-Crick base pairing or by triple-helix formation involving Hoogstein bonding, confers on them an enormous potential to interfere with nucleic acid information processing. Their incapacity of forming a suitable substrate for RNase H has often been considered a major limiting factor to their capacity to regulate gene expression. It is now clear, however, that judicious targeting can lead to the disruption of specific and crucial steps of nucleic acid information processing. This is particularly true of many of the mechanisms that are specific to viruses.

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