Tough nuts to crack: encouraging progress in peptide nucleic acid hybridization to structured DNA/RNA targets

Sequence-specific targeting of DNA and RNA by oligonucleotide analogues are of significant interest to molecular pharmacology and in vitro testing for the development of new medicines (‘magic bullets’) and robust diagnostics. On the one hand, the oligonucleotide-like probes and drugs have been brought to life by the discovery of duplex forms of nucleic acids and by finding the complementarity principle that governs their formation. On the other hand, the same double-helical structures, intrinsic in almost all DNA and RNA targets, impose severe obstacles for hybridizing the mixed-base oligonucleotides [1]. Therefore, modified oligonucleotides and their mimics with enhanced binding affinities for DNA/RNA have to be developed to overcome the thermodynamic and kinetic barriers to probe hybridization within structured polynucleotides.

Peptide nucleic acids (PNAs), synthetic nucleobase oligomers with a pseudopeptide backbone, have this ability. PNAs have several other advantages, but this important feature makes PNAs one of the most potent tools in molecular diagnostics [2,3]. The future design of PNA-based gene therapeutics also looks very promising [4,5].

A recent paper by Armitage [6] presents an informative overview of the basics and current status of PNA hybridization to a variety of DNA and RNA complex structures, including DNA duplexes, DNA and RNA hairpins and DNA quadruplexes. Potential therapeutic and diagnostic targets for PNAs might be the gene-regulatory sites, triple-repeated sequences and telomeric regions of chromosomes, as well as a variety of folded RNAs, such as mRNAs, ribosomal RNAs and the RNA component of telomerase.

Recently, there have been significant advances in PNA cellular delivery and aqueous solubility, and this progress has reduced much of the initial scepticism regarding the use of PNAs as drugs. In fact, some companies and clinics, such as PantheoA/S (Denmark) and the Mayo Clinic (USA), have initiated pilot pharmacokinetic studies on PNA constructs. Moreover, these recent advances have broadened the range of PNA applications, taking the field in novel drug-related directions. For example, PNAs have been used as agents for target validation studies [7,8], and PNA-directed mutagenesis/recombination has been used to correct defective genes [9,10]. My optimistic view of this field is also supported by the dramatic extension of the sequence repertoire of duplex DNA targets available for PNA oligomers through the employment of their pseudocomplementary modifications [11,12].

There is much work ahead, of course. Further mechanistic and molecular modeling studies must be carried out to clarify more the importance of the PNA steric fit, backbone neutrality and achirality, and the effect of end-tagged cationic residues and modified bases on PNA binding affinity for DNA/RNA. Another important question that needs to be addressed in more detail is how the increased binding affinity of various PNA constructs safely coexists with a high sequence specificity, because for ordinary probes these characteristics usually anticorrelate each other [13]. The answers to all these problems might help to shed new light on why PNAs are able to break DNA and RNA secondary structures. They might also be applied to other recently designed high-affinity probes, such as locked nucleic acids (LNAs) [14], and would certainly be helpful in the rational search for new probes of that type.

References