Close encounters of the PNA kind

Peter M. Lansdorp

Recognition of specific DNA sequences is of major importance for the identification and analysis of any genome. Oligonucleotide probes are convenient and versatile tools for such recognition, but require single-strand molecules that are not always available or informative. In this issue, Smulevitch et al.[1] describe novel ways to create stretches of single-stranded DNA in genomic DNA that are likely to greatly expand the use of oligonucleotide probes for genome research and diagnostic purposes.

Current methods to detect point mutations and other sequence alterations typically involve PCR amplification of the DNA of interest followed by sequencing of the amplified product. This is a relatively labor-intensive and time-consuming approach that has nevertheless proven its value and is likely to remain the gold standard in the foreseeable future. The paper of Smulevitch et al. suggests, however, that alternative, more direct methods are on the horizon that may result in considerable savings in time and cost.

The strategy is based on alterations of the backbone charge of oligonucleotides and focuses on the unique chemical properties of peptide nucleic acid (PNA) in which the charged phosphate sugar backbone of DNA or RNA oligonucleotides has been replaced by an uncharged *N*-(2-aminoethyl) glycine peptide backbone[2,3]. The uncharged nature of the peptide backbone in PNA results in a set of remarkable hybridization properties that are exemplified by recent papers on the use of PNA in antisense applications[4,5], fluorescence in situ hybridization[6], and strand invasion[7,8].

In this latest work, PNA specific for selected inverted repeat sequences was allowed to invade double-stranded target DNA and form a DNA–PNA duplex. PNA oligomers, but not regular DNA or RNA oligomers, can perform this trick because the absence of the charged phosphate group allows sufficient access to specific nucleotide sequences to replace existing nucleotides and destabilize the double helix at selected sites. Once a PNA–DNA duplex has formed, the displaced single-stranded DNA becomes accessible for secondary sequence specific probes, which can then be used to tag such sequences or initiate sequencing reactions.

Taken together, the observations by Smulevitch et al. point to novel ways to insert unique restriction sites in genomes or tag selected sites in ways that could be helpful in dissecting and organizing (map) genome sequences. For detection of point mutations, it is conceivable that PNA targeted to suitable sequences in the vicinity of possible sequence alterations may allow access to single-stranded DNA with or without such alterations. Secondary DNA or PNA probes labeled with suitable reporter molecules could subsequently be detected without further manipulations.

PNA appears particularly attractive to "report" such mutations because another very useful property of these molecules is their intolerance to base pair mismatches relative to regular oligonucleotide probes[3]. Of course, it helps for the proposed applications that PNA molecules are extremely stable and appear ideally suited for deposition on high-density oligonucleotide arrays or "sequence chips."

Do we perhaps expect too much from PNA? Don't count on it. What needs to be established are the exact sequence requirements needed to achieve PNA strand invasion and the frequency of suitable sequences in various genomes. However, given the potential of these novel ways to access genetic code, this information should become available in the near future.

Peter M. Lansdorp is senior scientist at the Terry Fox Laboratory, British Columbia Cancer Research Centre, 601 W. 10th Avenue, Vancouver, BC V5Z 1L3, Canada (peter@terryfox.ubc.ca).

1. Smulevitch, S.V., Simmons, C.G., Norton, J.C., Wise, T.W., and Corey, D.R. 1996. *Nature Biotechnology* **14:**1700–1704.
2. Nielsen, P.E., Egholm, M., Berg, R.H., and Buchardt, O. 1991. *Science* **254:**1497–1500.
3. Egholm, M. et al. 1993. *Nature* **365:**566–568.
4. Knudsen, H. and Nielsen, P.E. 1996. *Nucleic Acids Res.* **24:**494–500.
5. Norton, J.C., Piatyszek, M.A., Wright, W.E., Shay, J.W., and Corey, D.R. 1996. *Nature Biotechnology* **14:**615–619.
6. Lansdorp, P.M. et al. 1996. *Hum. Mol. Genet.* **5:**685–691.
7. Nielsen, P.E. and Christiansen, L.J. 1996. *Am. Chem. Soc.* **118:**2287–2288.
8. Demidov, V.V. et al. 1995. *Proc. Natl. Acad. Sci. USA* **92:**2637–2641.

Big news for plant transformation

Richard Michelmore

The plant parasitic bacterium *Agrobacterium* spp. provides a remarkably efficient method for introducing DNA into a wide range of plant species. As a critical part of its natural pathogenicity, *Agrobacterium* transfers a 15–25 kb T-DNA section from the large Ti plasmid into the plant chromosome[1]. To generate vectors for transformation, the T-DNA has been engineered to remove the bacterial genes, and a variety of sequences have been added to allow routine transfer of a wide range of recombinant transgenes. Most constructs contain less than 25 kb between the T-DNA borders, although inserts up to at least 35 kb have been known to be transferred with reasonable efficiency. Infrequent transfer of large inserts has been demonstrated: 185 kb of the wild-type Ti plasmid was transferred when the orientation of the right T-DNA border sequence was reversed[2]. However, routine transfer of large recombinant T-DNAs has not been reported.

Hamilton et al. have transferred a 150-kb fragment of human DNA from a bacterial artificial chromosome (BAC) vector into tobacco[3] and tomato (personal communication). This has been made possible by the development of BAC technology for the cloning of large genomic fragments in *Escherichia coli*[4] and the development of a vector that functions as a binary T-DNA as well as a BAC vector (BIBAC; C. Hamilton, unpublished data).

There are several potential uses for this technology, but its most significant impact will be on map-based cloning efforts. A rate-limiting step has been the identification of clones encoding the targeted gene by complementation[5]. Previous complementation experiments involved binary vectors with up to 30-kb inserts. Transformation with numerous overlapping genomic clones has often been required. The capability to introduce over 100-kb genomic fragments will greatly reduce the number of transformation experiments necessary to localize the gene and allow subsequent complementation efforts to be focused on clones likely to carry the targeted gene. The increase in size provided by the BIBAC vector is particularly significant, as it provides for the first time an overlap between the size of fragments used for complementation and the average spacing between molecular markers that can be readily achieved using AFLPs[6]. The rate-limiting step will now be the genetic resolution

Richard Michelmore is a professor at the department of vegetable crops, University of California, Davis, CA 95616 (rwmichelmore@ucdavis.edu).