RESEARCH PAPER ANALYSIS

REPLICATING THE REPORTER: LIFE AFTER PCR

In this issue of *Bio/Technology*, Paul Lizardi and his colleagues at the Research Center for Genetic Engineering and Biotechnology (Cuernavaca, Mexico) and Fred Kramer (Public Health Research Institute, New York City) describe a new approach to nucleic acid amplification based on the replication system of bacteriophage Qβ. Studied initially by Sol Spiegelman in the mid 1960s, the RNA polymerase (replicase) of this phage possesses some unique features, which have been elegantly exploited by Lizardi *et al.* to exponentially amplify *probe* sequences rather than *target* sequences as in the polymerase chain reaction (PCR).

RNA synthesis by the replicase is literally autocatalytic. Highly specific single-stranded RNAs serve as templates for the synthesis of complementary single-stranded products. After the completion of product-strand synthesis, the template and product are released from the replication complex. Both strands can therefore serve as templates in the next round of synthesis. Consequently, as long as there is an excess of replicase, the number of RNA strands increases exponentially. As little as one molecule of template can initiate replication.

To take advantage of these properties, the researchers designed a bifunctional RNA that is both a specific hybridization probe and a template for the replicase. They accomplished this by inserting the probe sequence within a hairpin loop that occurs on the exterior of MDV-1 RNA, a natural template for the Qβ polymerase. Such recombinants hybridize specifically to DNA containing sequences complementary to the probe portion of the molecule, despite topological constraints on the probe domain, and are replicated at the same rate as MDV-1 RNA despite their additional length. A replicase reaction initiated with 0.14 femtograms of recombinant RNA (1,000 molecules) produces 129 nanograms of recombinant RNA product (a billion-fold amplification) in 30 minutes. The amount of product generated is large enough to be measured by simple colorimetric techniques.

An additional kinetic feature of the reaction—particularly attractive for designing multiple-diagnostic assays—is that after enough product has been generated to saturate the replicase, the amount of RNA synthesized is linearly proportional to the logarithm of the initially bound RNA. This dramatically extends the dynamic range of any assay system. For example, after hybridization with a recombinant probe, two targets initially present in amounts differing by 1,000 would, following amplification, generate reporter-RNAs whose amounts differ by only three-fold.

Although not yet reduced to practice, such an assay is easy to envision. MDV-1 RNAs containing different probe sequences would be mixed with a sample DNA. Following hybridization, unbound probe would be removed by washing, and after amplification the product RNAs would be hybridized to a strip containing excess target DNAs. Given the large amounts of RNA generated, detection might be as straightforward as dipping the strips into a solution of ethidium bromide.

There is however one obstacle that must be overcome before this potential can be realized. As with any signal amplification scheme, the replication of *non-specifically* bound probes remains a problem. But methods involving DNA capture, such as sandwich hybridization, or those based on a conformational change of the probe-containing MDV-1 that is coupled to capture or signal-generation, have the potential to dramatically reduce the background caused by such non-specific replication.

This problem notwithstanding, the Qβ amplification system is extremely sensitive and rapid, and represents a new and powerful approach to nucleic acid probe-based diagnostics.

—Harvey Bialy

PROTEIN ENGINEERING

DESIGNING ANTIGEN-BINDING FRAGMENTS—THE CREATIVE WAY

NEW YORK—Monoclonal antibodies show great promise as *in vivo* diagnostics and therapeutics. Their clinical usefulness, however, tends to be limited by their persistence in the circulation and their immunogenicity. Smaller versions of whole antibodies, it is predicted, should be as specific for target sites, but less immunogenic and shorter-lived.

Scientists at Creative BioMolecules (Hopkinton, MA) have engineered a single chain biosynthetic antibody binding site (BABS™) that is only one-sixth the size of the parent, but retains its antigen-binding specificity. The biosynthetic single chain protein binds to digoxin, a cardiac glycoside. According to James Huston, a senior scientist at Creative, the synthetic binding site incorporates the natural antibody's heavy and light chain variable regions (V_H and V_L) connected by a 15-amino acid linker.

Creative scientists designed the synthetic gene for the variable region fragment plus linker, derived the DNA sequence, assembled the gene, and expressed it as a fusion protein in *Escherichia coli*. (For details see J. S. Huston et al., *PNAS* **85**:5879, '88.)

Huston says they chose the anti-digoxin antibody as a model because it is particularly well-characterized and families of analogs are available. This simplifies analysis of the binding site's structure. Anti-digoxin antibody has also found clinical utility: it is used to reverse acute toxicity in humans.

Genex scientists (Gaithersburg, MD) have also designed and patented a single chain antibody (see *Bio/Technology* **4**:1041, Dec. '86). According to Creative's Thomas G. Tachovsky, Genex scientists used a computer model and amino acid data base to fit existing amino acids into the designed linker region betweeen the V_H and V_L genes; Creative scientists, on the other hand, generated the linker sequence from first principles. "We designed a synthetic linker, rather than looking at a natural analog for the linker," explains Tachovsky.

The next step is to alter individual amino acids in the BABS fragment to see what effects this would have on its binding specificity. Among the family of anti-digoxin molecules already available, says Tachovsky, is one containing a single amino acid change in the binding site that results in a three-log difference in its binding affinity.

"By engineering these molecules, one can rapidly change their characteristics to suit a particular purpose," explains Tachovsky. For instance, BABS fragments might be at least as effective in immunoaffinity purifications as the monoclonals used today. By engineering the binding site to bind its ligand a little less tightly, it might be easier to remove a purified entity from the column.

—Jennifer Van Brunt