RESEARCH NEWS

Detecting drug-resistant tuberculosis: Beacons in the dark

Harvey Bialy

The control of tuberculosis remains one of the more elusive goals of medical science. With the disease reaching epidemic proportions in Africa, Asia, Eastern Europe, and South and Central America, few new treatments on the horizon, and new vaccine development still the subject of sessions at Keystone symposia with the word *prospects* in their titles, the emergence of drug-resistant strains of *Mycobacterium tuberculosis* has become a major public health concern¹.

Given this somber situation, the need for improved detection of *M. tuberculosis* antibiotic resistance has become increasingly important. As the number of antibiotic-resistant cases increases, rapid susceptibility testing will help in choosing an appropriate therapy for individual patients. Because incorrect treatment of drug-resistant tuberculosis can lead to the development of resistance to additional drugs, rapid susceptibility testing will contribute the prevention of new multidrug-resistant disease. A simple method to screen for drug resistance in developing countries will also be useful for monitoring the efficacy of tuberculosis control strategies and will make it possible to formulate treatment protocols that are appropriate for each geographic area.

In this issue, David Alland and colleagues² report a major advance in producing such a tool. Their work extends the use of molecular beacons³, a new type of reporter designed to interrogate short regions of DNA accurately, rapidly, and simply. Molecular beacons are single-stranded nucleic acids of 25–30 nucleotides that possess a stem-loop structure, the loop of which is complementary to a target sequence of up to 20 nucleotides. Attached to the 5' and 3' ends of the stem, which is formed by the annealing of complementary sequences flanking the probe sequence, are a matched pair of fluorescent dye and quencher. When the beacon encounters an appropriate target sequence, the ensuing probe–target hybrid, which is stronger than the hairpin stem, separates the dye–quencher combination, resulting in an easily detectable fluorescence.

Because molecular beacons can detect PCR reaction products that are perfectly complementary to the probe in sealed tubes and can be made with many colors⁴, they offer the possibility of designing a relatively

simple and inexpensive test for drug-resistant *M. tuberculosis*. There is, however, one important catch. To date, the demonstration of allele discrimination by molecular beacons has been limited to only the central regions of the probe–target hybrid. To be more generally useful for sequence analysis it is essential that molecular beacons are able to distinguish mismatches at all positions within the duplex. It is this demonstration, in the context of a pressing real-world problem, that the paper by Alland and colleagues provides.

Figure 1. Detection of point mutations in the *rpoB* gene of *M. tuberculosis* by visual inspection under ultraviolet light. The tubes contain, from left to right, PCR products from reactions initiated with wild-type and mutant DNAs, and from a control reaction lacking template. In addition, each tube contains a pair of beacons that fluoresce either red (wild type) or green (mutant) when perfectly hybridized to their target.

The rationale for their experiments is that as almost all of multidrug resistant tuberculosis cases are resistant to the antibiotic rifampicin⁵, and 96% of rifampicin resistance is confined to a single 81-bp region of the *rpoB* gene⁵, sequence analysis of this region could form the basis of a rapid diagnostic test for drug-resistant tuberculosis in a format suitable for use in those countries that most need it. As described lucidly in the paper, they were able to show that a set of only seven molecular beacons (five to interrogate the target sequences paired with a species-specific internal control, and another complemen-

tary to a species-specific multicopy gene) was sufficient to correctly identify all 52 rifampicin-resistant and all 23 rifampicin-sensitive clinical isolates in a blinded study. The real excitement, however, in terms of extending this work beyond laboratories that have access to spectrofluorometric thermal cyclers, stems from their last experiment, in which they show that fluorescence resulting from allele-discriminating molecular beacons can be detected by visual inspection of closed PCR tubes illuminated by an ultraviolet light source (Fig. 1).

This is the first documentation of in-tube detection of PCR products by any method that does not rely on instrumentation. In principle, therefore, it should now be possible to produce and field-test an affordable system for rapidly assessing (in hours, instead of days to weeks) *M. tuberculosis* drug resistance. The major expenses, a thermal cycler and Taq polymerase, are not prohibitive, as the former is a one-time purchase, and it is likely that a license to produce the polymerase for developing world applications could be negotiated with the owners of the patents.

Somewhat paradoxically, such a PCR-based assay would often be more practical than conventional susceptibility-testing methods in developing countries, as conventional testing is based on the ability to culture and subculture large numbers of *M. tuberculosis* isolates—work that is fastidious and must be performed under biological containment hoods. Although improved susceptibility testing is far from an adequate response to the tuberculosis pandemic, realizing even this limited promise would nonetheless make a significant impact on current and future disease control strategies and contribute to lessening the severity of the disease until genomic-based therapeutic and vaccine development strategies can fulfill their own larger, but more distant, goals.

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