Lighting up hybridization

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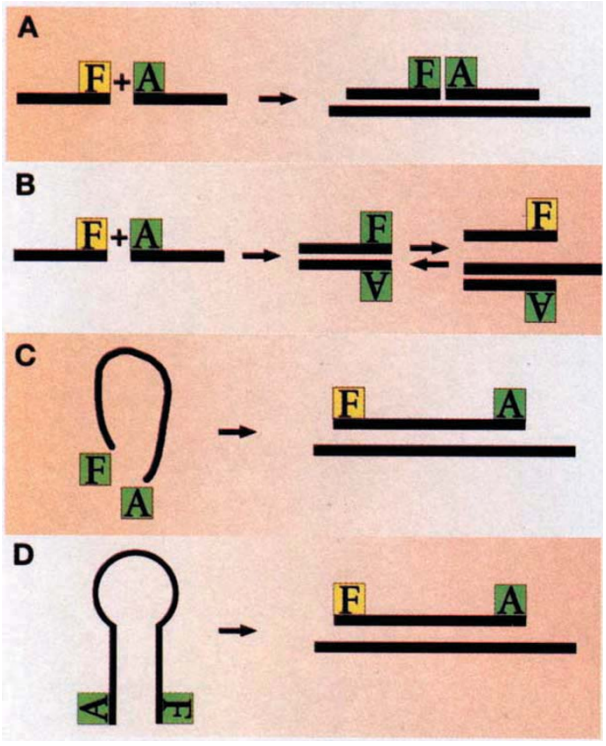

Figure 1. Improving the detection of hybridization. A: Dual probes. Hybridization quenches fluorescence signal[2]. B: Dual probes, competitive assay format. Hybridization removes quenching[3,4]. C: Single flexible probe. Hybridization removes quenching[6]. D: Hairpin stabilized single probe. Hybridization removes quenching[1].

The path from a successful laboratory assay to an effective, automated, clinical diagnostic test is often tortuous. The polymerase chain reaction provides an excellent example of this. An older tool, hybridization, is still one of the most effective laboratory methods for the analysis of specific DNA or RNA sequences. Many have sought to make hybridization a clinically useful tool. Originally developed as a homogeneous phase solution technique, most effective hybridization formats today use an immobilized nucleic acid strand to capture a labeled complementary strand for subsequent detection. However, most preferred automated, clinical diagnostic formats are based on the development of a color or a fluorescent signal in homogeneous solution. Thus, the challenge for those who wish to configure nucleic acid analyses for such diagnostic tests is to develop a signal generation system that depends on a specific hybridization reaction. In this issue of *Nature Biotechnology* (see p.303), Tyagi and Kramer report the latest such system, arguably the most general and practical one to date[1].

In hybridization, two (or more) nucleic acid strands are brought into physical contact. A natural way to exploit this change in strand proximity to generate a color signal is fluorescence resonance energy transfer (FRET). This method has been used as a biological research tool for almost 30 years and, indeed, the same optical phenomenon is exploited by biological systems to increase their effectiveness in harvesting light energy. In FRET, light energy absorbed by a higher energy chromophore is transferred by a nonradiative coupling process to a nearby, lower energy chromophore, providing that certain geometric and spectroscopic constraints are met. The key experimental variable is the distance between chromophores. The challenge in adopting this very powerful spectroscopic tool is placing appropriately chosen chromophores in the system so as to achieve the maximum possible dynamic signal range without interfering with the assay process itself.

The earliest effort to develop a homogeneous hybridization assay system based on FRET was described in a 1982 European patent application by Morrison, Heller, and others[2] working at Amoco (Napierville, IL). This was a true homogeneous solution, phase assay (Fig. 1A), but two weaknesses are immediately apparent. Two different labeled probes must be complexed simultaneously with the target, and a positive hybridization result is indicated by the disappearance of the fluorescent signal. This is clearly an undesirable feature, since many types of artifacts (for instance, probe not added) could compromise such an assay by leading to false positives. In an improved procedure from the same group (Fig. 1B)[3,4], two different fluorescent-labeled probes are still needed, but a competition assay format ensures that a positive result is indicated by a positive fluorescent signal.

A hairpin structure is used to ensure efficient energy transfer in the isolated probe.

Several recent advances in FRET-detected hybridization have led to protocols that are much more general and that require only one labeled DNA species. In each of these, both chromophores are positioned on a single nucleic acid probe. Hybridization leads to a change in the intramolecular distance between these probes, and this in turn leads to the change in fluorescence signal detected. The earliest approach used a rather complex probe design[5]. This was simplified (Fig. 1C) by exploiting the fact that, in a flexible single strand, terminal probes can approach close enough to lead to energy transfer, which is not possible in the much more rigid and extended double-strand formed after hybridization[6]. How general such an approach will turn out to be remains to be seen.

The advance contained in the new results of Tyagi and Kramer is shown in Figure 1D. A hairpin structure is used to ensure efficient energy transfer in the isolated probe, but this is eliminated in a long duplex after hybridization. The method is appealing and should be quite general. Inadvertently, it has introduced another potential advantage. The energetics of the hairpin structure will modulate the energetics of duplex formation; hence, the probes can be designed to enhance the specificity of hybridization detection, as in the method of stringency clamping developed for a slightly different purpose by Roberts and Crothers[7].

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