Nanostructured Probes for RNA Detection

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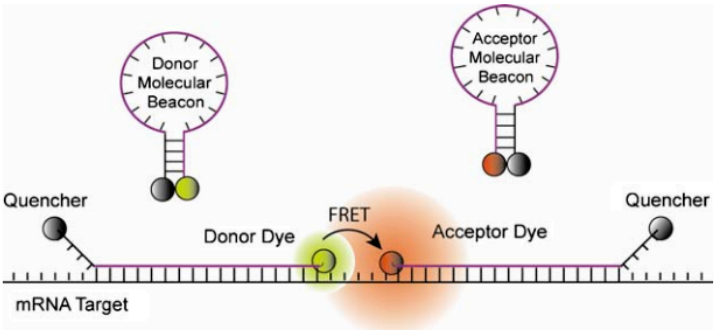
Being able to visualize the expression level and localization of certain RNAs in living cells in real-time has the potential for remarkable opportunities in biological studies. Recently, nanostructured probes have been developed that have the ability to detect living cell RNAs. These will be able to help provide important information on RNA synthesis, processing, transport, and localization, thereby leading to progress in disease pathophysiology and medical diagnostics. RNAs are essential to development as they are the messenger which allows DNA to be translated into proteins. It has been suggested in the past 10 years that RNA molecules have a much more broad range of functions in living cells; some being interpreting genetic information, essential catalytic roles, and providing structural support for molecular machines. These functions were found through control of the expression level as well as stability (in both time and space) of specific RNAs in a cell. The next step is to discover the dynamics and localization of these RNA molecules in living cells.

Many methods have been developed to provide a comparative measure of gene expression level of cells using purified DNA or RNA. A few of these methods include Northern blotting, expressed sequence tag, and DNA microarrays. Though these are impressive methods for understanding human disease, they have proved to be difficult. Due to a low abundance of diseased cells in blood, sputum and stool samples, it is challenging to detect foreign nucleic acids. Also, they cannot reveal the spatial and temporal variation of RNA in a single cell. By studying past methods used, researchers concluded that in order to successfully detect specific RNAs, probes must have high specificity, sensitivity, and signal-to-background ratio. Also, it is necessary that cellular delivery be efficient.

Hairpin nucleic acid probes (when designed correctly) have proven to provide the necessary levels of sensitivity and specificity. Molecular beacons, one class of this type of probe, are dual-labeled oligonucleotide (ODN) probes consisting of a fluorophore at one end and a quencher at the other. They work by hybridizing with a target



nucleic acid which then emits a fluorescence signal upon excitation that can be analyzed. Since the target recognition is transduced directly into such a signal, this allows multiple analyte detection, protein-DNA interactions, gene typing and mutation detection, and cancer cell detection.

A standard molecular beacon has four basic components including the loop, stem, flurophore and quencher (see figure). They are able to recognize targets with very high specificity, and are even be able to distinguish targets that are different by just a single nucleotide. They are simple to use and a very promising tool for diagnosing genetic disease. The most efficient way to deliver the probes into cells has proved to be using cell penetrating peptides. When the probes are linked to certain peptides, they internalize into all cell compartments within 30 minutes with almost 100% efficiency. This peptide-based approach has proved much better performance than any other method suggested thus far.

The notion of being able to detect genes in living cells is an exciting application. The molecular beaconds discussed potentially can provide an extremely powerful tool for laboratory and clinical studies of gene expression, human health and disease. Though extremely challenging, the perfection and development of nanostructured ODN probes will have a significant impact on research in the future.

References

Philip Santangelo, Nitin Nitin, Gang Bao. "Nanostructure Probes for RNA Detection in Living Cells." Annals of Biomedical Engineering (2006): 39-50.