

has been elucidated in animals and the internalization of ricin is an elegant example of how this mechanism works in animal cells [21]. Another possibility might be that perhaps the signal sequence is cleaved in the cytoplasm before the Lt-B is ever moved into the ER lumen, then the mature Lt-B protein might move directly into the amyloplast. All of these are testable hypotheses.

### Conclusion

Even though several targeting studies with recombinant proteins have been conducted in transgenic plants, and their expression levels monitored, the localization of those proteins might not be in the expected place. Tissue-specific localization is often correct [22], but the jury is still out on subcellular localization. The article by Chikwamba *et al.* [1] is a beautiful example of how biotechnology has raised interesting biological hypotheses and at the same time offered the means to address those hypotheses – a potential benefit not often recognized.

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## Surface-attached molecular beacons light the way for DNA sequencing

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**Rapid testing of DNA and RNA nucleotide sequences is required for various research protocols including wide-scale genetic testing, diagnostics, fast detection of biological warfare agents, environmental testing and forensic medicine. At present many laboratories are interested in research and development of an inexpensive, easy-to-use, fast-response device for this purpose.**

**Various methods based on acoustic, electronic and optical detection of the DNA hybridization event have been reported.**

The so-called molecular beacons have attracted great attention for their use as sensors for DNA sequencing. They are composed of an oligodeoxynucleotide (ODN) hairpin-like stem-loop structure with a fluorescent label and its quencher at each end. When the molecular beacon

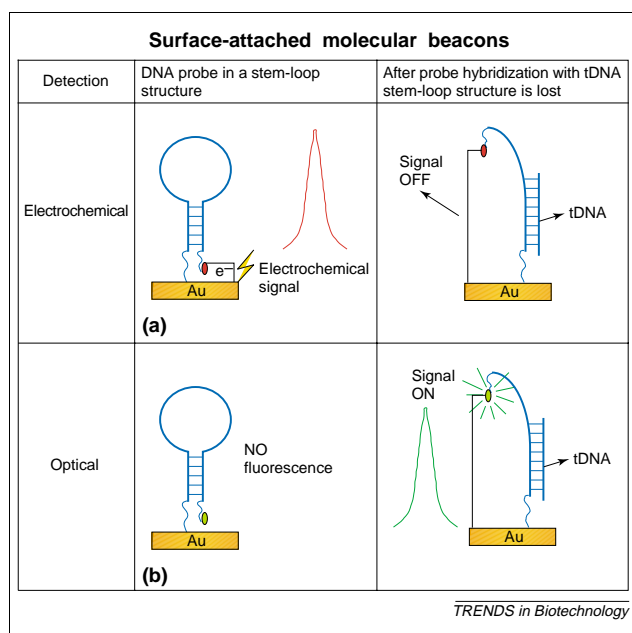
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is in its folded stem-loop configuration the ODN termini are held in close proximity and fluorescence is quenched. In the presence of a complementary target DNA strand the stem-loop structure is converted into a rigid linear DNA duplex, thus removing the fluorophore from the quencher proximity and inducing strong emission enhancement.

In an attempt to create a molecular beacon that is potentially useful for arrayable chip-based optical detection [1], Du *et al.* [2] introduced a solid-state version. Development of electrochemical transducer-based devices began slowly compared with devices based on optical transducers but progress in the research of electrochemical DNA (E-DNA) sensors in recent years is encouraging [3–9]. Advantages of electrochemical devices include sensitivity, speed, low cost and low power requirements, simple design and small dimensions. Fan *et al.* [10] proposed an electrochemical variant of the molecular beacon and developed a new strategy for the sensitive, reagentless transduction of DNA hybridization into an electrochemical signal (Figure 1a).

#### Electrochemical and optical molecular beacons

Both Du *et al.* [2] and Fan *et al.* [10] immobilize the probe ODN on gold surfaces; however their strategies for achiev-



**Figure 1.** Scheme of surface-attached optical and electrochemical molecular beacons. A stem-loop oligodeoxynucleotide (ODN) with a terminal thiol is immobilized at a gold surface (Au) forming a self-assembled monolayer. The other end of the ODN contains either (a) electroactive label (ferrocene) or (b) a fluorophore (e.g. rhodamine). On hybridization with the complementary target (t) DNA the stem-loop structure is lost and a rigid rod-like DNA duplex is formed. In the electrochemical DNA (E-DNA) sensor (a) the stem-loop structure holds the ferrocene label in close proximity with the gold electrode ensuring efficient electron transfer, manifested by an electrochemical signal. As a result of duplex DNA formation, the ferrocene label is moved away from the electrode and the electrochemical signal is decreased or eliminated. In the optical sensor (b) the fluorophore is silent in the stem-loop structure because of its location close to the gold surface, which serves as a quenching agent. On DNA hybridization, which induces removal of the fluorophore from the surface neighborhood, a strong fluorescence enhancement is observed.

ing this are different. Du's group applies the principles of the solution molecular beacon but instead of an ODN end-attached quencher, it uses the gold surface as a quenching agent. Gold nanoparticles covalently attached to a molecular beacon at its 5'-end were previously shown to be an efficient quencher [11]. Du and coworkers used a sensor in which DNA hybridization results in a strong fluorescence signal [2] (Figure 1b). By contrast, in the E-DNA sensor proposed by Fan and coworkers [10] the DNA hybridization decreases or eliminates the strong signal produced by the stem-loop structure before hybridization (Figure 1a). In this sensor the 27-mer single-stranded ODN labeled with ferrocene at its 5'-end is immobilized on the gold electrode via its sulfhydryl group at the 3'-end, forming the stem-loop structure (Figure 1a). Ferrocene is located close to the electrode surface within this structure, producing a distinguished cyclic voltammetric redox pair in the absence of the complementary target (t) DNA. The voltammetric signals are caused by electron tunneling between ferrocene and the electrode. Hybridization of the tDNA with the 17-base loop of the 27-mer ODN induces a conformational change resulting in the formation of a rigid, rod-like duplex DNA structure and causing the ferrocene label to move away from the electrode surface (Figure 1a). Increasing the distance of the label from the electrode surface results in a decrease or elimination of the ferrocene electrochemical signal because efficiency of electron tunneling decreases exponentially with increasing distance. This E-DNA sensor is reagentless, reusable, sensitive and simple, offering significant advantages over previously published electrochemical sensors. It is well suited to the continuous monitoring of the flow of analyte.

No significant changes in the E-DNA signal occur in the presence of 10  $\mu\text{M}$  noncomplementary ODN, suggesting a high selectivity of this sensor. The detection limits of the E-DNA sensor (10 pM or 5 fmol) are comparable with other sensors requiring DNA electroactive labels [3,6,7,9]. These are substantially more sensitive than the 10 nM sensitivity reported by Du *et al.* for the optical sensor [2]. Improvement of the optical sensor sensitivity might be possible through surface enhancement provided by roughened metal substrates and further improvement can be expected with the replacement of dye molecules with semiconductor quantum dots. Like other electrochemical DNA hybridization systems, the E-DNA sensor can be conveniently adapted to be consistent with chip-based electrode arrays, giving a promising alternative to traditional fluorescence-based gene arrays.

#### Comparison with other electrochemical DNA sensors

Recently several E-DNA techniques have been developed (reviewed in [3–5,7,9,12]). They usually use gold, carbon or indium tin-oxide electrodes with an immobilized capture probe that does not yield any electrochemical signal. This DNA probe is hybridized with complementary tDNA that is end-labeled with an electroactive moiety, or in label-free methods, direct or mediated oxidation of guanine is used to detect tDNA at the electrode. Alternatively an end-labeled reporter (or signaling) probe complementary to the tDNA can be used for this purpose.

### Single- and double-surface methods

The requirements for the optimum hybridization surface differ significantly from requirements for the surface used for electrochemical detection. For example, the hybridization surface should be relatively large and nonspecific DNA adsorption should be minimized, whereas the detection electrode should be small, and adsorption of DNA (after its selective capture) can be beneficial for increasing the sensitivity of the electrochemical analysis. Optimization of both hybridization and detection at a single surface can therefore be difficult, particularly when long tDNA molecules are analyzed. These problems prompted the development of a new approach, the so-called double-surface (DS) technique [5]. Commercially available magnetic beads with attached probe ODN served as the hybridization surface and high selectivity of the DNA hybridization was achieved because of minimum nonspecific DNA adsorption on the beads. Optimum detection electrodes could be chosen only with respect to the given electrode process. One of the most important advantages of the DS method is that relatively long tDNAs (>1000 base pairs) can be analyzed and with this method lengths of repetitive sequences can be determined. It is noteworthy that the sensitivity of the label-free DS method [5] increases with the length of tDNA molecules so fmoles of 25-mer tDNA are detectable but a 1kb tDNA fragment can be analyzed at the amole level [13]. This method requires acid treatment of the captured tDNAs and is therefore more complicated than that of Fan *et al.* [10]. Moreover, to create a practical high-throughput device, integration of a microfluidic system into a chip would be necessary. Using the DS technique and the so-called electroactive beads Wang *et al.* [14] reported determination of a biotinylated 27-mer target ODN at levels as low as  $5 \times 10^{-21}$  mol (~31 000 molecules).

### Signal-off (and signal-on) methods

To date the only reported reagentless sensors [7] based on the guanine oxidation signal require probe DNA without guanine, and sensitivity in these methods is significantly lower than that of the E-DNA sensor [10]. Being a signal-off device, this sensor is susceptible to false-positive responses because of various sample contaminants, for example contaminants that degrade DNA. However, false-positive signals can be detected by simultaneously monitoring both sensor elements and control sequences at a single electrode using 'multicolor' redox markers that produce signals at different potentials [14–16]. Because of its signal-off nature, false-negatives (caused by binding of electroactive species, which yield signals at the same or similar potentials as those of ferrocene) can be easily recognized. Another ferrocene-based signal-off device, the Electrically Detected Displacement Assay (EDDA) is advertised by the German company FRIZ Biochem ([www.frizbiochem.com/en/0830\\_idx.htm](http://www.frizbiochem.com/en/0830_idx.htm)). In this device the capture probe DNA is not labeled and weakly bound ferrocene-labeled signaling probes are removed from the capture probe by the DNA hybridization, thus decreasing the ferrocene electrochemical signal. However, the company does not mention any 'multicolor' redox markers, which could help to eliminate false-positive signals.

### Sensors for amplified and non-amplified DNAs

At present the various E-DNA sensors appear to be sufficiently sensitive for detecting amplified tDNAs. However, electrochemical detection of non-amplified tDNA (for which the picomolar sensitivity is too low) has not been reported because of insufficient sensitivity and selectivity of the current methods. Detection of non-amplified tDNA represents a challenge that could be met by the E-DNA sensor as well as by some other single- and double-surface methods employing amplification of the electrochemical signals, such as electrocatalysis. DS techniques could be particularly successful in attempts to significantly increase the selectivity of DNA and RNA detection.

### Transfer of basic research in biotechnology

The tendency of denatured (single stranded) DNA to reform its double-helical structure was discovered >40 years ago by Julius Marmur and Paul Doty (reviewed in [17]). Knowledge of this ability of DNA to reform the double helix helped with the development of several DNA methods and biotechnologies, including the amplification of DNA by PCR. The electroactivity of nucleic acids was discovered around the same time [18] and it was shown that DNA renaturation can be traced electrochemically with mercury electrodes (reviewed in [19]). At this point we can hope that combination of these two findings will help in creating new technologies and devices useful in medicine and other fields for improving human life.

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## Tracking the 'General': tagging skin-derived dendritic cells

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**Dendritic cells (DCs) are the sentinels of the immune system; their migration, maturation and mobilization are fundamental to immunity and tolerance. The recent tracking of DCs from the skin to lymph node (LN) and their enumeration using a Cre/loxP system demonstrate the recruitment of a higher than expected number of DCs to the draining LN after cutaneous administration of DNA-coated gold particles. The longevity of the migrated DCs was also longer than previously reported.**

Whereas T cells are effector 'soldiers' of the immune system, professional antigen presenting cells (APCs), namely DCs, are the 'Generals' that command and dictate to the T cells. APCs process and present peptide antigens to T cells through molecules of the major histocompatibility complex (MHC) – HLA (human leukocyte antigen) in the human and H-2 in the mouse. The antigen presenting molecules are classified into two major classes, class I molecules, which present antigens to killer T cells or CD8<sup>+</sup> T cells, and class II molecules, which present antigens to helper T cells or CD4<sup>+</sup> T cells. Being professional APCs, DCs efficiently prime naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells and have crucial roles in immunity and tolerance [1]. Also, their cytokine profiles might help shift an immune response (Th1/Th2). Hence the study of dendritic cell biology is important in the understanding of immune recognition in health and disease.

DCs are no longer believed to be a homogenous population of cells. There are at least five different subtypes of DCs in the LN of a mouse (described in [2]). They are differentiated by the presence or absence, or difference in level of expression of different cell surface markers such as CD4, CD8 $\alpha$ , DEC205, MHC class II and CD11b. DCs migrate from nonlymphoid tissues to lymphoid tissues and this trafficking is

important in the context of infection, inflammation and active immunization. For example, when a mouse is immunized with an antigen and adjuvant, DCs take up the antigen and migrate to the draining LN to efficiently present the antigen to specific T cells to elicit an antigen-specific immune response.

### Tracking DCs

Tracking and enumeration of DCs has been carried out by conventional methods using fluorescent contact sensitizers or DNA vaccines encoding reporter genes [3–7]. Much of the focus of natural DC trafficking has been on the movement of epidermal Langerhans cells from skin to draining LNs in response to exogenous stimulus, such as contact sensitizers applied to the shaved skin of a mouse [8]. Such studies have shown that DC influx into LNs occurs as soon as 4 h after antigen exposure, peaking at 2 days after exposure and then gradually declining [3,4]. Subsequently, plasmid DNA, or naked DNA, was successfully delivered to skeletal muscle and skin to express the encoded proteins [6,7]. Recently, one group used a gene gun to deliver gold particles coated with DNA (a plasmid encoding the *Escherichia coli* LacZ gene under the control of the human cytomegalovirus intermediate-early promoter) resulting in substantial expression of the encoded protein in the epidermal layer and detectable expression in DCs in the draining LNs [9]. The group found that LN cells directly expressing the DNA-encoded antigen are rare and that the number of these cells does not exceed 50–100 cells in an individual draining LN [9]. These approaches have yielded low numbers of DCs migrating from the site of sensitization to the draining LN, leading to speculation that extremely low numbers of these APCs could stimulate rare antigen-specific T cells. A recently published manuscript by Garg *et al.* provides a new dimension to the study of the dynamics of DCs in terms of their longevity and frequency *in-vivo* by revisiting this issue [10]. Garg *et al.*

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