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Previews

Adding Broccoli to the Biosensor Menu

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Small-molecule metabolites regulate many cellular processes but are often present at low concentrations, confounding studies of their signaling networks. In this issue of *Cell Chemical Biology*, You et al. (2019) describe RNA "integrators" that measure the concentrations of metabolites in live cells to enable in-depth studies of signaling pathways.

Small molecule metabolites and second messengers regulate biological processes through signaling pathways. Alterations to this delicate balance can mediate the difference between a healthy and disease-affected state (Kholodenko et al., 2010). Therefore, sensitive and accurate tools are required to detect metabolites intracellularly with real-time resolution to study their function. Fortunately, a number of techniques, mostly involving fluorescent protein or RNA molecules, are available for such measurements in live cells.

Genetically encoded fluorescent biosensors allow for long-term, localized, live imaging without interrupting cellular functions. Protein-based sensors generally consist of a target-binding protein domain bordered by two fluorescent proteins that upon ligand binding, allow the visualization of dynamic processes through perturbation of a fluorescence state by Förster resonance energy transfer (FRET) (Ni et al., 2018). The lack of availability and the complexities of generating protein domains that bind to specific metabolites, however, can significantly limit the scope of this technique.

As an alternative to protein-based techniques, two types of RNA biosensors have been developed. The first employs riboswitches, which change conformation upon metabolite binding. This conformational "switch" alleviates translation repression of a downstream fluorescent protein, which serves as a proxy for metabolite concentration. However, temporal resolution is limited, as this method requires the translation and maturation of nascent fluorescent proteins prior to signal production (Garst et al., 2011). The second type of sensor is based on genetically encoded fluorogenic RNAs, such as Spinach and Broccoli. A typical RNA aptamer sensor is comprised of the fluorogenic Spinach/Broccoli domain, a transducer domain, and a target-binding aptamer domain, which is tuned to the metabolite of interest. Metabolite binding to the aptamer is relayed through the transducer domain to induce a conformational change in Spinach or Broccoli that allows it to bind 4-(3,5-difluoro-4-hydoxybenzylidene)-2-methyl-1-(2,2,2-trifluoroethyl) imidazolinone (DFHBI-1T), a cell-permeable small molecule that only fluoresces upon binding to the fluorogenic detector (Jaffrey, 2018). Although it is relatively straightforward to develop aptamers against various biomolecules, metabolites, and small molecules, this approach is limited by the intracellular concentration of the metabolite. Metabolites and other signaling small molecules are often present in cells at nanomolar or low micromolar concentrations, well outside the detection limit of the biosensor (typically micromolar to millimolar concentrations). That is, a fluorescence signal is generated in a stoichiometric manner; even if all metabolites are bound by an RNA-based sensor, a fluorescent signal cannot be detected by standard fluorescence microscopy. Such signal-to-noise issues exist in the protein biosensor domain as well (Christen et al., 2010).

To overcome the problems associated with currently available protein- and RNA-based biosensors, You et al. (2019) describe RNA integrators, an innovative, modularly designed, purely RNA-based biosensor that allows for the live-cell detection of metabolites in low abundance. By marrying the idea of a ribozyme, a catalytic RNA that can perform self-cleavage at specific positions, with the release of a Broccoli aptamer, Jaffrey and colleagues address a challenge in metabolite detection by endowing biosensors with signal amplification that allows for an accumulation of fluorescent signal (Figure 1) (You et al., 2019). These "integrators" thus enable the detection and quantification of low abundance metabolites with temporal resolution.

Taking advantage of the well-studied natural catalytic RNA the hammerhead ribozyme (HHR) (Perreault et al., 2011), the authors created a modular design strategy consisting of a metabolite-binding aptamer fused to sequences encoding an HHR and Broccoli, both of which are folded in an inactive conformation (Figure 1A). A target molecule binding to the aptamer induces proper folding of the HHR, which undergoes self-cleavage to release Broccoli RNA. The released Broccoli folds and binds DFHBI-1T to produce a fluorescent signal. Once the metabolite is released (dependent on its k_{off}), it can bind to sensors and produce additional fluorescent signals. This results in a time-dependent accumulation of Broccoli fluorescent signals, improving the detection limit of genetically based RNA biosensors.

The authors first integrated the RNA Integrator Broccoli components into a theophylline-regulated HHR in which theophylline allosterically induces HHR activity (You et al., 2019). Addition of the cognate ligand (theophylline) caused self-cleavage of HHR and released the Broccoli fragment, resulting in an increase in fluorescent signal upon addition of DFHBI-1T *in vitro*, thus validating the RNA Integrator design. The authors then asked if the RNA integrator can work with other small molecules. By applying

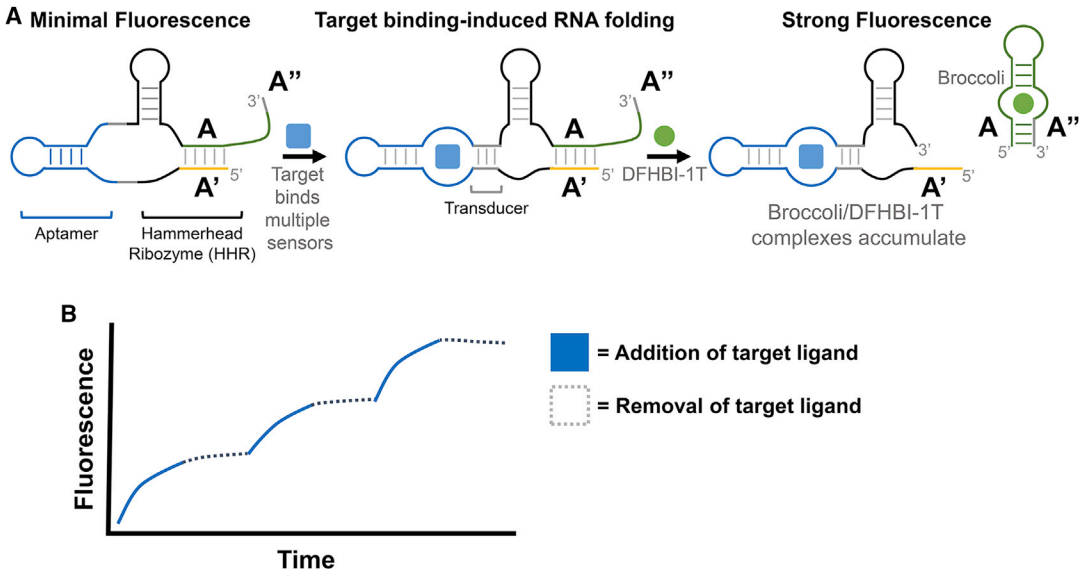

Figure 1. Design of RNA Integrators

(A) Schematic of RNA integrator strategy based on a target binding aptamer attached to a transducer domain that upon target binding, induces conformational change to the hammerhead ribozyme (HHR), which cleaves and releases the inactive Broccoli precursor (A-A′) that subsequently forms an essential duplex (A-A″) to enable binding to DFHBI-1T and fluorescence enhancement. Thus, the signal is catalytic and amplified, allowing the detection of low abundance metabolites. (B) RNA integrator signal accumulates upon the addition and removal of target ligand.

the RNA integrator design principles to aptamers for second messenger molecules, ci-di-GMP and cAMP, the authors observed fluorescence enhancement upon addition of the cognate ligand, demonstrating the modularity of this technique. To demonstrate the generality of the approach, the authors showed that their RNA integrator design could be applied to other classes of ribozymes. Indeed, a cleavage-induced fluorescence signal was observed in five of eight classes of ribozymes (some of which have never been used as an allosteric biosensor before), as applied to the theophylline-binding aptamer module. Importantly, the RNA integrator design was also applied to a pseudoknot HHR ribozyme scaffold that functions at physiological levels of divalent Mg^{2+} ions.

Additional *in vitro* studies confirmed important features of RNA integrators: (1) the irreversible self-cleavage of HHR allows for signal amplification (You et al., 2019); (2) optimized RNA integrators have a dynamic range of three orders of magnitude, a marked improvement from a dynamic range of traditional genetically encoded RNA or protein biosensors, and (3) integrators can model the dynamic flux of signal molecule synthesis and degradation in the cell life cycle. That is, re-activation of a fluorescence signal was observed upon multiple additions of the target ligand while no further enhancement of a fluorescence signal was observed upon metabolite removal (Figure 1B). Indeed, an RNA integrator detected endogenously generated thiamine pyrophosphate (TPP) in *Escherichia coli*, with an 8-fold improved sensitivity compared to a previously developed, non-integrating TPP-targeting Spinach riboswitch.

As discussed by the authors, while a cleavage-based RNA integrator allows for higher sensitivity to detect low abundance metabolites, the irreversible nature makes detection of minute fluctuations more difficult. Reversible allosteric riboswitch sensors display a decrease in intracellular target concentration corresponding with a decrease in fluorescence signal, while an RNA integrator would only maintain a constant fluorescence expression, potentially concealing minor changes in target concentration. This limitation necessitates analyzing the change in slope in continuous measurements to gain insight into the dynamics of intracellular metabolite concentrations. In the future, reversible switches that can sensitively detect marginal changes in concentration and can also amplify low abundance molecules may be desirable.

In summary, You et al. (2019) present an alternative, purely RNA-based target-induced signal amplified biosensor. This approach adds to the toolbox of genetically encoded fluorescent reporters. Given the modular design, improved detection limit, and broader dynamic range of the RNA integrator over current biosensors, these studies contribute a valuable resource in the field of cellular imaging. As mammalian cells have lower steady-state levels of RNA expression (Jaffrey, 2018), the use of an RNA integrator that catalytically amplifies the signal can potentially enable the use of these genetically encoded RNA biosensors in human-disease-relevant contexts.

DECLARATION OF INTERESTS

M.D.D. is a founder of Expansion Therapeutics.

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Ubiquinone Biosynthetic Complexes in Prokaryotes and Eukaryotes

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Ubiquinone (UQ) is a conserved polyprenylated lipid essential to cellular respiration. Two papers, one in this issue of *Cell Chemical Biology* (Hajj Chehade et al., 2019) and another in *Molecular Cell* (Lohman et al., 2019), identify lipid-binding proteins that play crucial roles in chaperoning UQ-intermediates.

Ubiquinone, also known as Coenzyme Q or UQ, is an essential redox-active lipid that functions in cellular energy metabolism. The reversible reduction and oxidation of the quinone ring to the hydroquinone ($UQ + 2\ e^- + 2\ H^+ \Leftrightarrow UQH_2$) allows UQ to function as an acceptor and donor of electrons and protons in respiratory electron transport chains. Such transport establishes the H^+ gradient used to produce ATP in prokaryotes and eukaryotes. The redox activity of UQ also enables it to serve as an electron acceptor in the synthesis of pyrimidines and in the oxidation of sulfide, choline, dimethylglycine, sarcosine, glycerol-3-phosphate, proline, and fatty acyl-CoA substrates in beta-oxidation (Alcázar-Fabra et al., 2018).

The redox active ring of UQ is decorated with a long and extremely hydrophobic polyisoprenoid tail (Figure 1). The number of isoprene units in the tail of UQ_n is species dependent (UQ_6 in *Saccharomyces cerevisiae*, UQ_8 in *Escherichia coli*, and UQ_{10} in humans). Membrane biophysical and modeling studies indicate that the polyisoprenoid tails of UQ_n isoforms of UQ_6 or greater are positioned at the mid-plane of the membrane bilayer (Quinn, 2012). This location enables reduced UQH_2 to function as a lipid-soluble antioxidant that chain-terminates lipid peroxidation reactions and preserves membrane fluidity and function (Bentinger et al., 2010). Two papers, one in this issue of *Cell Chemical Biology* (Hajj Chehade et al.,

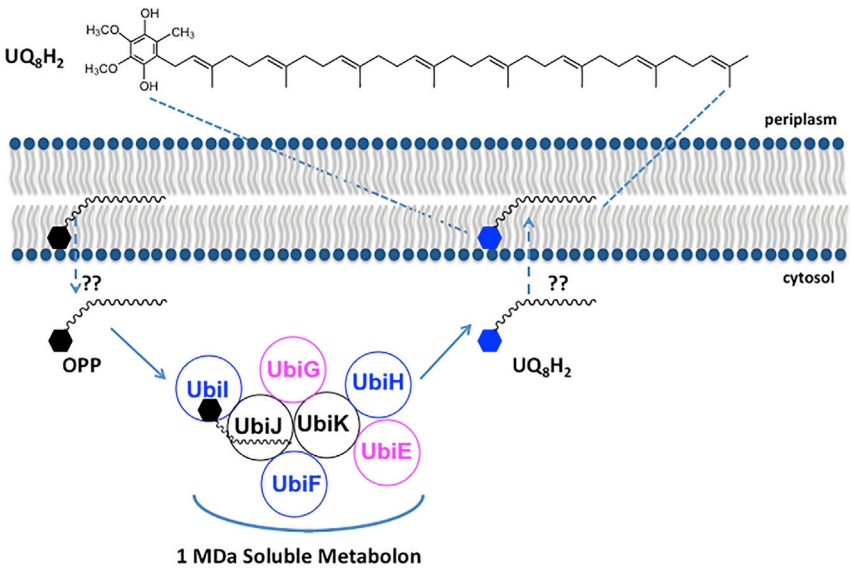

Figure 1. A 1 MDa Soluble Metabolon Synthesizes UQ_8 in *E. coli*
The long octaprenyl tail of UQ_8 and UQ_8 intermediates in *E. coli* are thought to reside at the midplane of the membrane bilayer. Hajj Chehade et al. (2019) show that octaprenyl phenol (OPP), an early intermediate in *E. coli* UQ_8 biosynthesis, is converted in six steps to the final product UQ_8H_2, by a high molecular mass soluble metabolon located in the cytosol. Five Ubi enzymes (shown in blue and pink) are organized around the accessory proteins UbiJ and UbiK. UbiJ contains a sterol carrier protein 2 (SCP2) domain responsible for binding the UQ_8 intermediates. The steps whereby OPP is extracted from the membrane and the UQ_8H_2 product is delivered back to the membrane are designated by the dashed arrows and represent intriguing and unknown trafficking steps ("??").