Sensing the future of molecule detection and bioproduction

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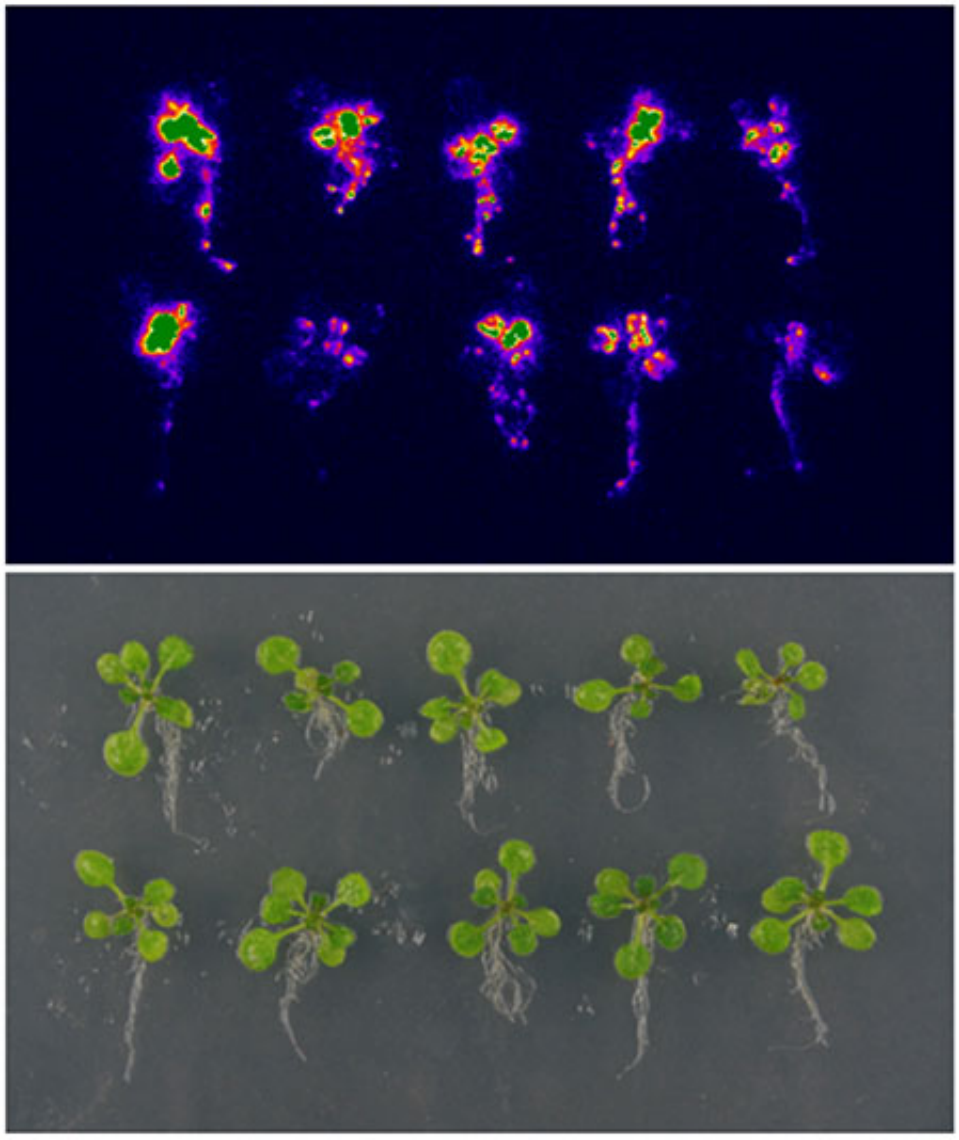

A team led by George Church has developed a methodology for turning eukaryotes into sensitive detectors of virtually any molecule of interest. Here,

Arabidopsis plants (bottom) have been engineered to detect the drug digoxin and emit fluorescence (top) to alert its presence. Credit: Wyss Institute at Harvard University

Synthetically engineered biosensors, which can be designed to detect and signal the presence of specific small molecule compounds, have already unlocked many potential applications by harnessing bacterial cells such as *E. coli* to sense toxins or enable bioproduction of valuable commodities including fuel, plastics, and pharmaceuticals. As of yet, however, scientists have been challenged to leverage biosensors for use in eukaryotic cells—which comprise yeast, plants and animals—because strategies-to-date are limited in the molecules they can detect and the signals they can produce.

But now, a team of researchers at the Wyss Institute for Biologically Inspired Engineering at Harvard University and Harvard Medical School (HMS) led by George Church, Ph.D., has developed a new method for engineering a broad range of biosensors to detect and signal virtually any desired molecule using living eukaryotic cells. Church, who is a Wyss Core Faculty member and the Robert Winthrop Professor of Genetics at HMS, and his team reported their findings in the journal *eLife*.

To test their new method, the team experimentally engineered yeast, plant, and mammalian cells to contain customizable ligand-binding domains (LBDs), which are receptors for hormones and other types of small molecules. These custom LBDs are tailored so that they only bind and "detect" a specific molecule of interest, such as the human hormone progesterone or the drug digoxin. Once the LBD binds to the target molecule, a secondary "signal" component fused to the LBD can be programmed to emit fluorescence or regulate gene expression. The components of this biosensor—the LBD in combination with the

fluorescent or genetic signal—degrade and fade away if the target molecule is not identified.

Strikingly, the team successfully engineered Arabidopsis plants to act as multicellular botanical biosensors, containing a custom LBD to recognize the drug digoxin and a luminescent signal protein to emit light when digoxin is "detected". These Arabidopsis biosensors gave off fluorescence when the plants were exposed to digoxin, proving that whole organisms can visually light up to signal detection of an arbitrary molecule.

"Like many eukaryotic organisms, plants are full of diverse hormones that make it challenging to sense and respond to a specific hormone of interest," said Dan Mandell, Ph.D., the study's co-first author and a Wyss Institute Technology Development Fellow and Postdoctoral Research Fellow at HMS. "But using our strategy, the Arabidopsis plants we engineered exhibited a 50-fold increase in luminescence in the presence of digoxin—very easily visualized—which could inspire exciting future applications involving trees or plants that detect harmful environmental pollutants or toxins and give off a visible indicator."

"Biosensors that can tell you about their environment are extremely useful for a broad range of applications," said Church. "You can imagine if they were used in agricultural plants, they can tell you about the condition of the soil, the presence of toxins or pests that are bothering them."

The team not only demonstrated its novel methodology in plants but also described its efficacy in turning yeast and mammalian cells into precise biosensors, which could one day be leveraged for use in industries that rely on the productivity of yeast or livestock, or for use as medical sensors. Overall, the method is extremely tunable and portable, meaning it can be used in a wide variety of organisms to detect a broad range of

small molecules.

An additional capability of the new biosensing methodology is the ability to connect it to gene regulators instead of fluorescent proteins. Such biosensors could precisely regulate gene transcription in order to improve yields of small molecules in organisms used for industrial bioproduction. Yeast, for example, could therefore be engineered to produce a desired molecule from a renewable feedstock, and furthermore programmed to self-identify the most efficient individuals within a population of producers so that only the highest producing yeast would survive. In this way, a population of organisms leveraged for bioproduction of pharmaceuticals or other valuable molecules could quickly self-evolve to become extremely efficient and productive. The team in fact used this strategy to evolve yeast that can produce the hormone progesterone with several-fold higher yield.

The biosensors could have a direct impact on human health as well, given that the team also used their method to tightly regulate the gene editing mechanism CRISPR-Cas9 inside living human cells, a step forward towards preventing unintended changes to the genome during gene therapy.

"These new reprogramming capabilities developed by the Church team open up an entirely new realm where ordinary organisms can be transformed into extraordinary living cellular devices that can sense specific signals and produce appropriate responses, whether its enhancing production of biofuels or secreting a therapeutic when the cells sense inflammation or infection. It's another great enabling capability that will undoubtedly advance the entire field of synthetic biology.," said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., who is the Judah Folkman Professor of Vascular Biology at HMS and the Vascular Biology Program at Boston Children's Hospital, as well as Professor of Bioengineering at the Harvard John A. Paulson School of

Engineering and Applied Sciences.

Provided by Harvard University