SYNTHETIC BIOLOGY

# Let's start talking

**Synthetic networks in mammalian cells can regulate cellular behavior.**

Biological processes in multicellular organisms are not based on monologues. "Cells talk to each other," says Martin Fussenegger of the ETH Zurich. He surmises that what is needed to establish synthetic networks is not ever-more-sophisticated circuits in individual cells but rather networks of cells in communication with each other.

Fussenegger and his team have a rich history of reproducing cellular functions with synthetic switches and designer networks. His team's efforts culminated earlier this year with a programmable biocomputer, a synthetic transcription-translation control device that performed basic molecular arithmetic in response to a two-molecule input. But it did so in the context of a single cell. "We had seven different expression units which we assembled in a single mammalian cell," Fussenegger recalls, "and there will be a limit to the complexity that can be put into one cell." Spreading control systems over several cells will allow increases in complexity.

Other researchers have been engineering communication between yeast or bacterial cells, but Fussenegger was looking for a way to instigate a back-and-forth dialogue between mammalian cells.

His team engineered HEK-293 cells as sender, processor and receiver cells. The sender cell expresses tryptophan synthase, which converts indole in the medium to tryptophan that the cell secretes. The tryptophan acts as a signal that works on the processor cell and induces expression of alcohol dehydrogenase, which converts alcohol into acetaldehyde, which in turn signals to the receiver cell to trigger the expression of a reporter, such as alkaline phosphatase.

The first challenge was that the signaling molecules used in this system are part of the normal metabolism of a cell. A cell will secrete excess tryptophan, but a neighboring cell may consume and metabolize it, leaving none to trigger a signal. Of course, one way to avoid interference of endogenous pathways with signaling molecules is to use molecules that do not appear in mammalian cells, such as bacterial metabolites. But Fussenegger cautions that those may have unforeseen side effects, and the dependency on foreign molecules is a problem for any downstream therapeutic application.

To develop mathematical models for how the system works with endogenous and ectopically expressed molecules, Fussenegger collaborated with Jörg Stelling, also at the ETH Zurich. By determining the optimal levels of overexpressed tryptophan synthase, Stelling concluded that "for the tryptophan system, communication between mammalian cells can indeed be engineered in a quantitative and predictable manner."

Models in hand, the team was ready to build multicellular assemblies. First they wired the cells in daisy chain–like fashion: sender talks to processor, which talks to receiver. Such communication is seen in nature: for example, in pancreatic cells. To achieve two-way communication, the researchers combined sender and receiver elements into one cell, creating a closed-loop feedback circuit. The signal from the sender-receiver cells triggers signal expression in the processor cell, which feeds back to the sender-receiver cell and triggers expression of a gene of interest.

The researchers showed the utility of this circuit by regulating the expression of vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang1), two factors with complementary effects on blood vessels. Whereas VEGF promotes vessel growth and leakiness, Ang1 tightens and stabilizes a vessel. VEGF and alcohol dehydrogenase were expressed by the processor cells in response to tryptophan from the sender-receiver cell, and Ang1 was expressed in the sender-receiver cell in response to acetaldehyde produced by the alcohol dehydrogenase. With this communication system, the scientists could program the permeability of cocultured endothelial cells.

To reach the goal of engineering synthetic mammalian networks for human therapies, Fussenegger sees the need for yet more complex systems. The tryptophan system proved remarkably robust, but more systems that are compatible with endogenous metabolites are needed. Fussenegger sums it up: "[We need] different designer cells, each of which makes different metabolites to communicate to other cells to achieve logic operations."

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**RESEARCH PAPERS**

Bacchus, W. *et al.* Synthetic two-way communication between mammalian cells. *Nat. Biotechnol.* **30**, 991–996 (2012).