On...off...on...off... The circuitry of insulin-releasing cells

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A myriad of inputs can indicate a body's health bombard pancreatic beta cells continuously, and these cells must consider all signals and "decide" when and how much insulin to release to maintain balance in blood sugar, for example. Reporting in *Nature Chemical Biology* last month, researchers at the Johns Hopkins University School of Medicine have teased out how these cells interpret incoming signals and find that three proteins relay signals similar to an electrical circuit.

"Pancreatic beta cells are influenced by hormonal, metabolic and electrical signals and something must be integrating all of these inputs to determine how to generate the cell's output," says Jin Zhang, Ph.D., an associate professor of pharmacology and molecular sciences at Johns Hopkins. "We have discovered a tunable circuit that may control the behavior of the cell."

According to Zhang, typically PKA (protein kinase A) acts as a switch and turns on and does what it needs to do until it's done and turns off. The team initially noticed that PKA was switching on and off while observing fluorescently tagged live cells under a microscope. "It was so interesting and uncharacteristic we had to study it further," says Zhang.

So the team started by again recording video of live cells over the course of an hour. They took advantage of so-called biosensors that they engineered, which are protein tags that glow one color when turned off and another color when turned on. By inserting a PKA biosensor into these cells, they were able to see when PKA was turned off and on. They

found that PKA does turn on and off in regular intervals—about three cycles every 10 minutes.

"We already knew that calcium levels in these cells oscillate and this controls the release of insulin," says Zhang. "So we were curious to see how the PKA oscillation we observed was linked to calcium."

Using a dye that changes color when calcium levels are high, the team again observed live cells and found that PKA oscillations and calcium oscillations were in register with each other—every time PKA turned on, calcium peaked a short while later, and PKA would turn off almost immediately, overlapping with a decrease in calcium. "This too was surprising because turning off PKA in other types of cells normally is slow, on the order of tens of minutes, but in these cells it was fast, on the order of just a few minutes," says Zhang.

The team then turned to colleagues in biomedical engineering at the Johns Hopkins Whiting School of Engineering to build a mathematical model of this circuit to better study and predict how these oscillating signals are used in a cell. Culling everything that is known about PKA, calcium and another chemical in the cell that affects PKA activity, Levchenko's team came up with a model where all three components are closely linked by cross-talk so that the oscillatory behavior of each was determined by the activity of the other two. "Human engineers have figured out a long time ago that oscillating signals can carry more information than the steady ones, and it was fascinating to see that cells might have arrived at the same solution, too," says Andre Levchenko, Ph.D., an associate professor of biomedical engineering at Johns Hopkins.

The model predicted that blocking PKA activity would stop calcium levels from oscillating as well; so the team treated cells with a chemical that blocks PKA and found that indeed, calcium levels stopped changing.

The model also predicted that increasing PKA activity would change the frequency of calcium oscillations; again, adding a different chemical that increases PKA activity in turn increased calcium oscillation frequency. "The mathematical model enabled us to do more informed experiments and uncover even more about the activity of these molecules in the cell," says Zhang.

So what does this all mean? According to Zhang and Levchenko, they may have come up with an explanation for a long-standing mystery in the field. Low PKA oscillation frequency tunes PKA to act locally, in the immediate region where it is anchored in the cell. And high PKA oscillation frequency tunes PKA to work more globally throughout the cell, to generate a different response.

The discovery of PKA's oscillating activity and its involvement in this protein circuit in pancreatic beta cells is intriguing to Zhang, who hopes that this finding can lead to repairing deficient cells in treating diabetes. "This type of circuit-like control also may be more widespread among different kinds of cells," says Zhang. "We're eager to see what our new biosensors can teach us."

More information: *Nature Chemical Biology*: www.nature.com/nchembio/index.html

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