

Meeting Report

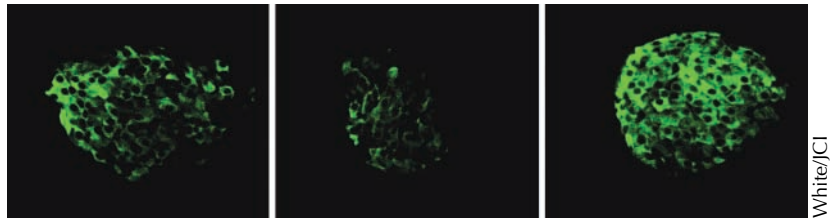
It's the signaling, stupid

Morris White (Harvard Medical School, Boston, MA) had a message that went down well at a signaling conference. Two major misconceptions about diabetes, he said, can be corrected by putting more emphasis on signaling pathways.

White talked primarily about type II (late-onset) diabetes, which has traditionally been ascribed to insulin resistance in peripheral tissues, as insulin is still detected in the blood. "That's ingrained in the field: you get fat; you get old; you make your [pancreatic] β cells work too hard," said White. "But the global view of our work is that β cells don't get tired if the signaling is right."

White contests the vague notion that pancreatic β cells—the body's only source of insulin—get exhausted from trying to pump out enough insulin to meet the demands of the peripheral tissues. As one of his counter-examples, he cites the rare patients with mutant insulin receptors, who always have severe peripheral insulin resistance but develop diabetes only late in life.

This may be because a normal pancreas can meet even excessive requirements for insulin. In contrast, in most type II diabetics some of the pathways that are failing to mediate insulin signaling in muscle and liver might also be essential for β cell survival, glucose sensing, and insulin secretion. The IRS branch of the insulin/IGF signaling pathway seems to be the key here, and if you put these signaling components back into the β cells, at least in mice, "you can restore β cell function and diabetes goes away," said White. Thus, it might be useful to design drugs or



Insulin secretion (green) is lost without IRS2 (middle) but restored by Pdx1 (right).

pretransplant treatments to up-regulate this pathway.

White's signaling theory also extends to side effects. Poorly controlled blood sugar levels have been blamed for the life-threatening side effects of diabetes, as tight control of blood sugar by frequent monitoring and administration of insulin results in fewer side effects. But, says White, "it's always seemed to me that the other thing you are doing [in these studies] is normalizing insulin signaling." When White fixes only the pancreatic signaling, by turning up a pancreatic transcription factor called Pdx1, he gets good insulin-based control of blood sugar. But side effects such as loss of sight and neuropathy remain in the periphery, presumably because the signaling components in these areas are still obliterated. (In the human subjects, by contrast, residual peripheral signaling components can respond to increased pancreatic signaling.)

This should not change the way that patients approach their therapy, says White, as good blood sugar control remains the best readout for good control over the signaling pathways. But the findings may prompt future drug company efforts to focus more on the peripheral signaling pathways that are doing the real damage. ■

Reference: Kushner, J.A., et al. 2002. *J. Clin. Invest.* 109:1193–1201.

Integration goes modular

Sequencing gazers can act as though proteins are Lego creations—an assemblage of domains that adds up to a predictable whole. And sometimes, said Wendell Lim (University of California, San Francisco, CA), that simplistic view might just be right. Lim studies signaling proteins that bind to two input molecules and act as integrative switches. In at least some cases, he has found that the two inputs add together cooperatively simply because the two binding domains occur next to each other. That simple construction can apparently be reproduced, and perhaps exploited, by biologists wanting to

manipulate signaling pathways.

Lim's protein of choice is N-WASP, which turns on the actin polymerizing activity of the Arp2/3 complex. N-WASP shuts itself down unless Cdc42 and phosphatidylinositol(4,5)-biphosphate (PIP₂) are around to disrupt two autoinhibitory interactions. Lim has mapped these two interactions and found that they form a cooperative switch because binding of either single activator destabilizes both autoinhibitory interactions. The two activators therefore cooperate to stabilize the open or active state.

The apparent simplicity of N-WASP regulation suggests that it may be manipulable. Lim is replacing the

domains that bind Cdc42 and PIP₂ with other interaction domains such as PDZ and SH3 domains, thus creating proteins that respond to different signals. So far, he said, "it seems to be relatively easy to make these integrating switches." The switches may be useful as readouts or activators of specific signaling pathways.

According to Lim, a good switch has two primary characteristics. It is sensitive to inputs because its autoinhibitory structures involve low-affinity interactions that can be disrupted easily. And it is highly cooperative in response to two inputs because the two interacting domains are tightly coupled by a short and rigid linker. The linker ensures

Networking is the future

Connecting the dots is what dominates the life of many signaling researchers today. Starting with a known component of a pathway, the investigator moves up and down, one component at a time, by using anything from two-hybrid and *in vitro* kinase assays to immunoprecipitation.

But soon enough the era of making those links will come to a close, and standard interaction data, like restriction maps and cloning strategies before them, will be relegated to the talks of distant memory. What will replace them? If Tobias Meyer (Stanford University, Stanford, CA) is right, we will be thinking about much more than one protein binding to another. "Seventy percent of the papers in ten years," he said, "will tackle systems questions."

Meyer is starting out on that path by investigating classes of proteins, such as all proteins with a particular domain. He reported in March that the calcium-sensing C2 domain found in many proteins (including protein kinase C) translocates to the plasma membrane in a discrete, step-like manner, and that the translocation is transient or sustained depending on the source of calcium. "You have a fundamental bistability—

a switch-type behavior," he said. "That's a different type of outcome than a graded type of translocation."

If similar decision points are common, Meyer believes that the interpretation of signaling networks can be simplified. "Instead of having to know all the parameters you just need to know if this state has been reached," he said.

Meyer is also interested in the organization of signaling networks. "The pathway idea has been quite powerful but it is not sufficient," he said. "How signaling systems evolved is by keeping modules together, with weaker connections to other modules. There will be some fragmentation of the structure, but it won't be as simple as single pathways."

The idea of linear pathways has worked well for directed and conserved pathways such as those in the cell cycle, he said, but most signaling pathways are more flexible and less linear, as they are used in different ways in different cell types.

This is where modules—proteins

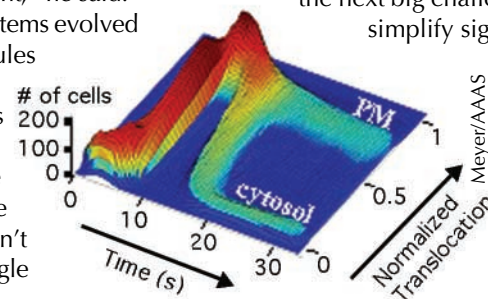
grouped into feedback systems and signaling complexes—come into play. Meyer hopes to determine the degree of connectedness between those modules by using transient expression and perhaps chemically activated proteins. The single molecule version of a module he terms a node, with examples including calcium, diacylglycerol, and MAP kinase.

Naming things modules and nodes doesn't solve anything, but it might provide the intellectual framework for the next big challenge. "How can we simplify signaling systems to

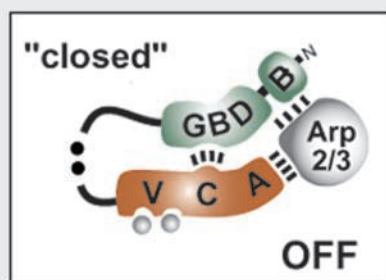
understand how decisions are being made?" asked Meyer. "All these [protein–protein] interactions will be in databases. The big challenge is not anymore to find what binds to what.

The big challenge is how you put all this together, and understand how the systems are dynamically connected." ■

Reference: Teruel, M.N., and T. Meyer. 2002. *Science*. 295:1910–1912.



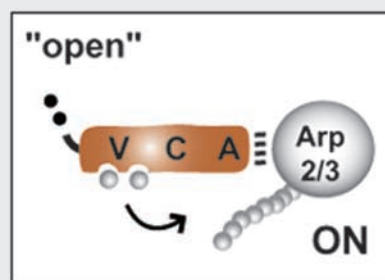
Translocation to the membrane is an all or none affair in individual cells.



WASP autoinhibition (left) is relieved by two activators (right).

that the switch is locked into either an all-on or all-off state.

Not all integrating switches have such a two-state mode of action. The cell cycle kinase Cdk2, for example, activates partially in response to either cyclin binding or phosphorylation, although



both inputs are absolutely required for full activation. Cdk2's multiple activity states are achieved through a complex series of allosteric motions that are not easily generalizable to other proteins.

That lack of generalizability may be just the reason why the N-WASP style

of regulation turns out to be the more common variety. What is easier for molecular biological tinkering is also easier for the random tinkering that is evolution. Lim pointed out that transcriptional circuits can be rewired relatively easily, either *in vitro* or during evolution, by swapping promoter sequences. "If you want to do that for cytosolic signaling it's a more daunting problem," he said. "But, even though it is not quite the same, there is the same type of modularity using these [autoinhibitory] domains. Nature can use this to evolve different networks because of the flexibility." ■

Reference: Prehoda, K.E., et al. 2000. *Science*. 290:801–806.