Synopses of Research Articles

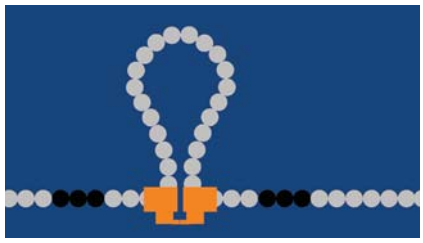
Looping Out Introns to Help Splicing

Richard Robinson | DOI: 10.1371/journal.pbio.0040041

One of the most surprising discoveries in molecular biology was that a gene's coding region is broken up into smaller pieces (the exons) interrupted by noncoding portions called introns. After the DNA is transcribed into RNA, and before the RNA can leave the nucleus, the introns must be cut out and the exons spliced together.

Since introns were discovered in 1977, the details of the splicing operations have been a major object of study. For splicing to occur, the ends of an intron must be brought into close proximity, and a number of proteins have been identified that aid this process. However, the function of one group of these proteins, called the hnRNP proteins, which are known to associate with prespliced RNAs, has not been clear. To date, the most accepted role for a subgroup of these proteins, the hnRNP A/B proteins, has been a negative one, since binding of these proteins to certain exons can prevent their inclusion in the mature RNA. In this issue, Rebecca Martinez-Contreras, Benoit Chabot, and colleagues show that when hnRNP A/B or hnRNP F/H proteins bind to intron sequences near splicing signals, they can stimulate splicing.

The authors began by making long artificial RNA segments, which are poorly spliced due to the more than 1,000 nucleotides separating the two ends of their introns. By inserting hnRNP A/B–binding sites in the intron near the future splice junctions, they could increase splicing efficiency 4-fold. The binding sites did not have to be on the RNA itself, as long as they stayed close to the ends of the intron, as shown when the authors tethered short pieces of RNA to each end of the intron. These short RNAs contained the binding sites on their tails, which hung loose in approximately the right place next to the intron. This allowed the hnRNP A/B proteins to take up position near the ends of the introns, and splicing efficiency was increased. When binding sites were placed well into the interior of the intron, either on the intron itself or on an RNA tail, splicing was inefficient. Similar results were found when binding sites were inserted for a different binding protein group, hnRNP F/H.



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Two hnRNP proteins interact to connect the two ends of an intron, forming a loop to help the cell's splicing machinery remove the intron.

The authors propose a model for these results in which the bound hnRNP proteins interact with one another, clasping the two ends of the intron together, forming a loop to help the splicing machinery remove the intron. The authors further support their model by showing that splicing could also be stimulated just by inserting complementary RNA sequences at each end of the intron. These have the ability to bind to one another, forming the intron into a loop.

However, they note that in some introns, only one hnRNP A/B site, positioned on the upstream end, is needed to promote splicing, and it does so nearly as well as when sites at both ends are present. The reason may lie in the particular introns that display this behavior—they contain a sequence which may itself bind an hnRNP A/B protein, thus providing the missing binding site and leading to loop formation. Confirmation or refutation of this hypothesis will have to await future experiments. Notwithstanding, the model is generally appealing because the two ends of many human introns are enriched in binding sites for these proteins. Overall, this mechanism suggests that hnRNP proteins can remodel the structure of prespliced RNAs, a property, the authors suggest, that could be important for both splicing and alternative splicing in a wide variety of genes.

Martinez-Contreras R, Fisette JF, Nasim FH, Madden R, Cordeau M, et al. (2006) Intronic binding sites for hnRNP A/B and hnRNP F/H proteins stimulate pre-mRNA splicing. DOI: 10.1371/journal.pbio.0040021

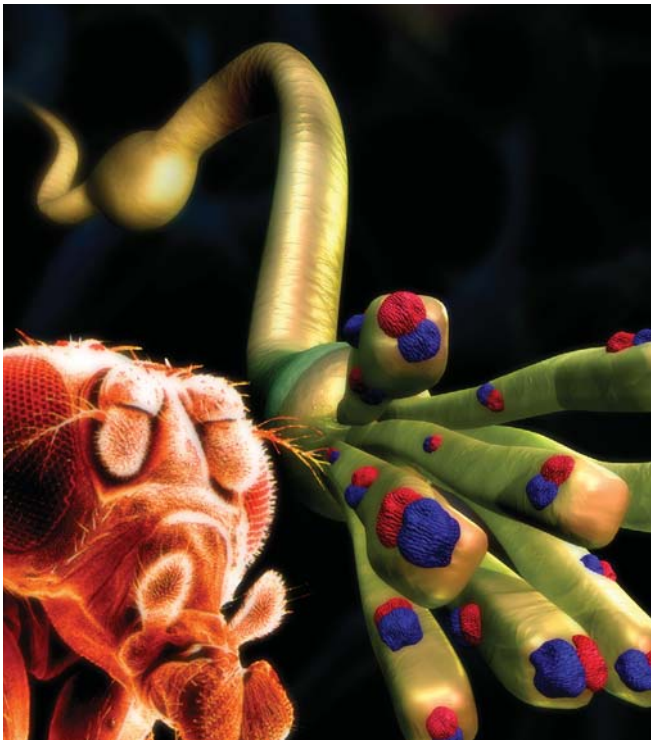
A Novel Design Principle for the Insect Odorant Receptor

Liza Gross | DOI: 10.1371/journal.pbio.0040034

From flies to lions, smell figures prominently in survival, pointing to food, predators, and mates. For humans, smell plays mostly an aesthetic role, prompting poetic ruminations on evocative aromas and intoxicating bouquets of fine wine. Insects and mammals have evolved common anatomical and physiological features and similar strategies to discriminate among thousands of aromas, using a large repertoire of odorant receptors.

Odorant receptors in mammals belong to a family of signaling proteins called G protein–coupled receptors (GPCRs). Since insects share the GPCR's characteristic seven-transmembrane domain structure (which has an extracellular N-terminus), it has been suggested that insect odorant receptors are a divergent class of GPCRs. But little is known about the signaling pathway activated by the insect receptors. In a new study, Richard Benton, Leslie Vosshall, and colleagues examined the molecular basis of insect olfaction in the fruit fly, *Drosophila melanogaster*, and show that insects use a "completely different molecular solution to detect odors."

Odorant receptors are localized to the ciliated tips of olfactory sensory neuron dendrites. In mammals, olfactory sensory neurons express just one odor-specific receptor while insects express these conventional receptors along with a broadly expressed, evolutionarily conserved receptor called



DOI: 10.1371/journal.pbio.0040034.g001

Olfactory receptors localized to the cilia of sensory neurons in *Drosophila*.

odorant receptor 83b (OR83b). It's thought that OR83b collaborates with conventional receptors, based on evidence that odor responses are impaired in *Or83b* mutant fruit flies and that levels of the conventional receptors, such as OR22a/b, drop markedly in OR83b-deficient neurons.

With little evidence of OR83b's molecular modus operandi, Benton et al. analyzed the subcellular localization, interactions, and function of OR83b and conventional odorant receptors in *Drosophila* sensory neurons. By visualizing the localization of OR22a/b in *Or83b* mutant neurons in relation to organelles involved in protein trafficking, the authors found that conventional receptors got stuck in the endoplasmic reticulum (where proteins are synthesized) in the absence of OR83b. OR83b, on the other hand, can reach the cilia on its own, suggesting that OR83b is required to link conventional odorant receptors to the transport machinery. The authors confirmed this by showing that OR83b can promote the localization of odorant receptors to the cilia of neurons that are not normally involved in the detection of odors, converting them into new olfactory neurons. Benton et al. went on to show that OR83b forms protein complexes with conventional odorant receptors, indicating it forms a direct physical link between the transport machinery and the odorant receptor.

Which part of the odorant receptor mediates these interactions? Though previous computational analyses predicted a seven-transmembrane domain in *Drosophila* odorant receptors, fly sequences resemble no known GPCRs. Benton et al. ran their own computational analysis, comparing fly and mouse odorant receptor sequences, which predicted that fly odorant receptors have seven-transmembrane domains but with an intracellular N-terminus. To experimentally test this surprising prediction, they developed a novel "topology sensor"—using fluorescent proteins designed to sense extracellular versus intracellular domains—and found that the N-terminus of both OR83b and a conventional odorant receptor was indeed located inside the cell, opposite to that of GPCRs. The authors go on to show that conventional odorant receptors interact with OR83b through conserved parts of the proteins that are located inside the cell.

While both insects and mammals transform odors into an internal neural representation, this study reveals that insects use different molecular mechanisms, relying on a novel family of transmembrane proteins that form a complex of two different receptor types: the conventional receptor recognizes odor molecules while OR83b helps to stabilize and transport this receptor to the part of the cell exposed to odors. Since the nature and configuration of the odorant receptor complexes appear unique to insect olfaction, the authors suggest the complexes could prove effective targets against insect vectors of human disease. Future studies can further explore the evolution of this divergence and the molecular basis of odor recognition and olfaction.

Benton R, Sachse S, Michnick SW, Vosshall LB (2006) Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. DOI: 10.1371/journal.pbio.0040020

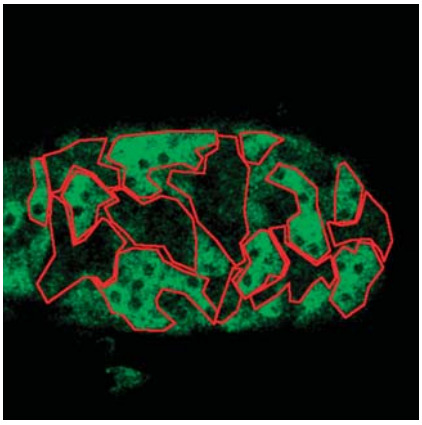
Potassium Channels Rule over Insulin Release with an Ion Fist

Françoise Chanut | DOI: 10.1371/journal.pbio.0040053

Diabetes is characterized by abnormally high sugar concentration in the blood and urine. The condition can eventually cause kidney and heart failure, blindness, and poor circulation, often requiring amputation of an afflicted limb. In the late 1800s, scientists studying digestion removed the pancreas of a dog and noticed that the dog's urine was laced with sugar. What they and others eventually established is that the pancreas secretes insulin, a hormone that helps tissues pump sugar out of the blood to use as fuel or store for later use. The discovery led to an essentially overnight recovery for millions of diabetics.

Insulin is secreted by β-cells, which are nestled inside small ball-shaped pockets of the pancreas known as the islets of Langerhans. β-cells respond to rising levels of glucose in the blood by releasing insulin, which prevents hyperglycemia. Conversely, when glucose levels fall below a particular threshold, β-cells stop secreting insulin, which prevents the equally dangerous hypoglycemia. In a healthy individual, blood glucose levels stabilize around one gram per liter.

The mechanisms that couple insulin production to blood sugar levels in normal individuals are not fully understood. In a new study, Jonathan Rocheleau, David Piston, and their colleagues show that the free circulation of electric charges among adjacent β-cells, a phenomenon known as electrical coupling, allows the cells to



DOI: 10.1371/journal.pbio.0040053.g 001

GFP fluorescence within an intact islet marks β-cells that do not have active potassium channels. Regions are outlined that contain cells with either normal or inactive channel activity.

coordinate their response to changing glucose concentrations.

All cells in the body maintain an uneven distribution of electric charges—mostly carried by ions such as potassium, sodium, and chloride—across their outer membranes, which are therefore polarized. Changes in membrane polarization act as signals for various cell functions. In β-cells, a reduction of the transmembrane charge difference, called depolarization, triggers insulin release. The molecule that links membrane polarization to insulin release is the ATP-dependent potassium channel. The channel sits at the β-cell's outer membrane and keeps the membrane polarized by maintaining a sharp gradient of potassium distribution across the membrane. As long as the membrane is polarized, β-cells keep insulin trapped inside secretory vesicles. But as β-cells take up glucose, they transform the sugar into ATP, a small energy-carrying molecule that closes the potassium channel. The resulting membrane depolarization causes a massive influx of calcium inside the cells, which in turn allows the vesicles to release insulin to the outside.

When analyzed in culture dishes, isolated β-cells display a wide range of sensitivity to glucose, whereas in the pancreas they release insulin coordinately above a specific glucose threshold. β-cells are tethered together in an islet by gap junctions, areas of their outer membrane that are riddled with intercellular pores through which small molecules circulate freely. This arrangement led to the proposal that ions crossing the gap junctions could harmonize the distribution of electric charges among adjacent β-cells, thereby coordinating their membrane polarization and insulin secretion.

To test this proposal, Rocheleau et al. used transgenic mice whose islets contained a mixture of β-cells with normal potassium channels and β-cells with channels that can't transfer potassium ions. When dispersed in culture, cells carrying the deficient potassium channels were permanently depolarized, and secreted insulin regardless of glucose concentration. But within the islets, they behaved exactly like their normal counterparts: in low glucose concentrations they were polarized, and when glucose concentration reached one gram per liter they became depolarized and took up calcium to the same extent as their normal neighbors. In the presence of a chemical that disrupts gap junctions, however, cells with normal and mutated channels regained their independent responses to glucose.

The authors conclude that the cells carrying an active potassium channel impose their polarized state on neighboring cells, presumably via the free circulation of ions through gap junctions. In a normal pancreas, they propose that a few glucose-resistant cells could clamp others in a polarized state, thereby stamping out natural variations in glucose sensitivity and reducing noise in insulin release.

Rocheleau JV, Remedi MS, Granada B, Head WS, Koster JC, et al. (2006) Critical role of gap junction coupled K_{ATP} channel activity for regulated insulin secretion. DOI: 10.1371/journal.pbio.0040026

Self-Generated Touch: A Neural Perspective

Liza Gross | DOI: 10.1371/journal.pbio.0040048

Why can a person go into near hysterics when tickled by another, yet feel barely a trace of that sensation at the flutter of their own fingers? While there's little doubt that we perceive externally generated touch more acutely than we do our own, the underlying mechanisms for this attenuated response have remained unresolved. In a new study, Daniel Wolpert, J. Randall Flanagan, and Paul Bays present experimental evidence that offers new insight into this long-standing question.

Neuroscientists have long distinguished two possible mechanisms to explain this attenuated response. One mechanism relies on prediction: when a motor command is generated—touch face with right hand, for example—the brain predicts what the sensory consequence will be, based on previous experience. This predicted effect is removed from sensory signals sent to the brain, reducing the response. But attenuation could also result from processing that arises after the sensation is received, but before it is perceived. In such a nonpredictive, or postdictive, mechanism, sensory cues persist after the initial touch and undergo additional processing that is modified by inputs occurring around the same time.

Using a device outfitted with force sensors and a torque motor (to generate a stable force), the authors studied the neural mechanisms of self-generated touch in 20 volunteers. With their left hand palm-side up, participants rested their left index finger in a molded support, beneath a force-sensing device mounted on a lever attached to the torque motor. The apparatus allowed investigators to distort the force and sensations experienced when one finger touched the other. Specifically, subjects moved their finger to tap a sensor, which caused a motor to initiate taps to a second passive finger.

The authors designed three trials: contact, no contact, and a delay between the tap and the force generated. In a previous study, the authors had established that a 300-millisecond delay does not cause attenuation, so they used a 500-millisecond delay as a baseline measure. The perceived magnitude of taps during contact trials was, as the authors expected, significantly less than that reported for the baseline trials.

In no-contact trials—given just once out of every six trials—the top sensor was surreptitiously moved prior to a "go" signal (both sensors were hidden), so the person didn't touch the sensor. The test tap was triggered when the person's finger reached the flexion angle associated with contact. Again, the perceived magnitude of the test tap was "substantially reduced" compared with the delay trials. This result would be expected for a predictive mechanism: because the test taps in both contact and no-contact trials were given at the same time, a predictive mechanism would expect the sensation and so attenuate it. But a postdictive mechanism, which integrates sensory input from both fingers, would not recognize the test tap in no-contact trials as self-generated and, thus, not cause attenuation.

Still, it is possible, the authors explain, that a postdictive mechanism might rely on other cues, such as finger motion or position. To explore this possibility, they repeated their experiment with a second group of volunteers. But in these trials, the tapping finger never touched the sensor and the test tap was triggered as the tapping finger reached the position at which contact would have been made, or after a 500-millisecond delay. These participants perceived little difference between the two sets of trials, indicating that motion or location cues alone did not cause attenuation.

Since attenuation was perceived only for those participants given mostly contact trials whether or not contact occurred, these results argue for a predictive mechanism. What advantage could a predictive mechanism offer? It might allow us to rehearse movements in our mind before we carry them out, the authors suggest, compensating for irregularities in sensory processing to ensure an environmentally appropriate sensory response. And by heightening our sensitivity to external sensory cues, it may help focus our attention on those things more likely to affect our well being.

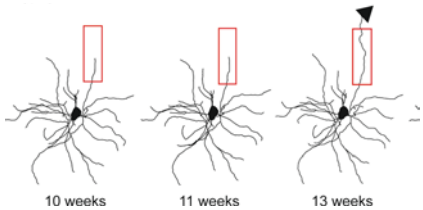
Bays PM, Flanagan JR, Wolpert DM (2006) Attenuation of self-generated tactile sensations is predictive, not postdictive. DOI: 10.1371/journal.pbio.0040028

A New Window into Structural Plasticity in the Adult Visual Cortex

Liza Gross | DOI: 10.1371/journal.pbio.0040042

The developing human brain is a hotbed of activity that continues well beyond the first year. During early postnatal development, we manufacture some 250,000 neurons per minute, then spend the next few years building the connections that underlie brain function. It has long been assumed that the neural plasticity of youth eventually settles down by adulthood. Though experimentally induced lesions in the adult cat and monkey cortex can produce anatomical changes, these findings are based on inferences from statistical evidence across different populations rather than on direct observation. And while neuroscientists have known for decades that the adult brain can reorganize neural pathways in response to new experiences—by changing the firing pattern and responses of neurons, for example—it has remained an open question whether structural changes accompany this functional plasticity.

In a new study, Wei-Chung Allen Lee and Elly Nedivi, along with Hayden Huang and Peter So, and their colleagues, take advantage of recent advances in imaging technology and single-cell genetic labeling techniques to investigate this question in mice. Continuous observations of the mouse adult visual cortex over the course of a few months revealed that the adult brain can indeed rewire its circuits under normal conditions. These rearrangements appear to follow neuron-specific rules, with one type of neuron undergoing a range of structural modifications while another maintains its original architecture.



DOI: 10.1371/journal.pbio.0040042.g001

One of the dendritic branch tips of this inhibitory neuron grew out of the microscope's imaging area in just two weeks.

Many studies have focused on pyramidal neurons—excitatory neurons that promote neuron firing—but few have focused on the possible structural dynamics of a range of different neuron types. In this study, Lee et al. focused on a cross-section of neurons, imaging every neuron they saw. The only neurons they saw growing were the inhibitory, nonpyramidal neurons, which inhibit the activity of cortical neurons and lack the classic pyramid structure that so easily identifies their excitatory brethren. Since these neurons can help adjust the brain's internal maps by inhibiting signaling in response to new stimuli or learning, the authors wondered if they could be involved in structural changes as well.

The authors focused on the surface layers of the neocortex. (The neocortex consists of six cell layers, with layer 1 closest to the cortical surface; the authors focused on layers 2 and 3.) To allow direct observation of the area, they implanted a glass window over the two areas of the visual cortex in four- to six-week-old mice. These mice express fluorescent protein in neocortical neurons, allowing Lee et al. to track the location and morphology of these neurons using two-photon microscopy. Time-lapse images of six pyramidal neurons and eight nonpyramidal neurons in 13 mice were taken over the course of four to ten weeks. The length of dendritic branch tips were measured over time to evaluate physical changes in the neurons.

The pyramidal neurons showed no structural changes in individual branch tips, but the nonpyramidal neurons showed dynamic changes, with one branch tip undergoing dramatic remodeling. "Within as little as two weeks," the authors note, "this branch tip more than doubled its length and exited the imaging volume." One nonpyramidal neuron even showed a few new branch tip additions.

All of the nonpyramidal neurons showed at least one and up to seven dynamic branch tips, with an average of about 14% showing structural modifications. (The authors monitored up to 50 branch tips from a single neuron.) This remodeling occurred both incrementally and in short bursts, and involved both branch tip growth and shrinking. Lee et al. confirmed that these nonpyramidal neurons were in fact inhibitory interneurons by showing that they expressed gamma-aminobutyric acid (GABA)—a neurotransmitter that inhibits neuron firing—while the pyramidal neurons did not.

Since the laws of probability suggest that given the changes observed in the nonpyramidal neurons, at least one pyramidal branch tip of 124 monitored should change if all things are equal, the authors argue that the pyramidal and nonpyramidal neurons have different dynamic properties. The branch tips of nonpyramidal cells in the adult neocortex can grow, retract, and sprout new additions—without experimental manipulations. Many studies support the idea of a relatively stable adult neocortical structure, but as Lee et al. point out, they focused on pyramidal neurons, while this study focused on nonpyramidal neurons. Both may be right. Under normal conditions at least, pyramidal structural modifications are far less obvious than those seen in the nonpyramidal neurons.

It remains to be seen whether the structural plasticity seen here underlies observed functional reorganizations. Probing this question will depend on determining what kinds of structural changes might be expected, figuring out how to detect them, and then interpreting the changes. Studying the responses of axonal arbors connected to the nonpyramidal dendrites, for example, may prove instructive. Based on these results, direct observation of specific neurons in a local pathway should yield promising results.

Lee WCA, Huang H, Feng G, Sanes JR, Brown EN, et al. (2006) Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex. DOI: 10.1371/journal.pbio.0040029

Protein Complexes Help Point Migrating Cells in the Right Direction

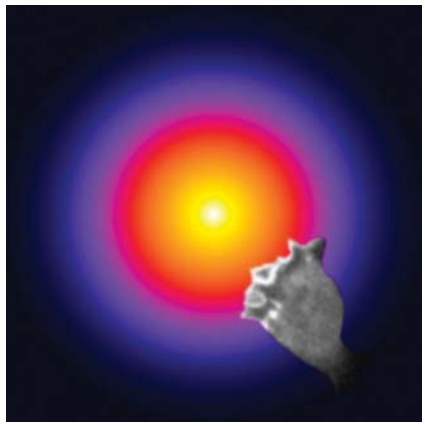
Liza Gross | DOI: 10.1371/journal.pbio.0040058

Self-generated movement is a key aspect of cellular life. From a single-celled amoeba hunting for food or mates to a human neutrophil finding and killing an infectious microbe, cells use many of the same molecular components to move.

Directed motility results from asymmetric activities in the cell, as localized signaling networks produce different physical and morphological changes at a cell's leading and trailing edge. This type of polarity occurs when chemical signals—associated with food, pheromones, bacteria, or antigens, for example—trigger and amplify internal signaling cascades that recruit proteins at polar ends of the cell to organize different actin structures at each end.

To modify its actin cytoskeleton in response to these chemoattractants, a fast-moving cell like the neutrophil must make one set of actin assemblies in front and another, completely different set in the rear, using different signaling pathways at each site. At the front, actin-rich structures form protrusions that project the cell forward. At the rear, actin–myosin contractile complexes retract the cell's trailing edge, helping it keep pace with the leading front edge. These structures and signaling pathways must be segregated so the assemblies can move in a coordinated manner. Segregation is reinforced by signaling pathway feedback loops, which in turn are likely controlled by specifically targeted regulatory protein complexes. Many questions remain, however, about the identity and function of the proteins that generate and maintain this polarity during chemotaxis. In a new study, Orion Weiner, Henry Bourne, Marc Kirschner, and their colleagues describe a family of protein complexes that regulate polarity circuits at the leading edge of migrating neutrophils.

One important regulator of polarity and cytoskeletal remodeling during chemotaxis is a protein called Rac. Neutrophils use Rac to control the positive feedback loops that stabilize its leading edge and Rho to oversee the myosin-driven contractions that bring along the rear. Rac induces actin polymerization under the direction of WAVE proteins, which mediate actin rearrangements and cell migration in processes as diverse as development and tumor metastasis. Rac and WAVE indirectly interact through a multicomponent protein complex called the WAVE regulatory complex.



DOI: 10.1371/journal.pbio.0040058.g001

A neutrophil migrating toward a chemical gradient.

Working with a neutrophil-like cell line, the authors focused on one of these components of the WAVE regulatory complex, Hem-1. The authors tagged Hem-1 with fluorescent protein to monitor its movements. In the absence of a chemical signal, Hem-1 is seen throughout the cytosol. But when a chemoattractant stimulates the cell, the proteins rapidly travel to the periphery, then accumulate at the leading edge as the cells polarize.

The authors suspected that Hem-1 complexes might regulate other proteins in addition to WAVE. If so, Hem-1 should exist in higher concentrations than WAVE2 (the WAVE protein most abundant in neutrophils), exist in distinct biochemical milieus, and associate with other proteins. Biochemical assays confirmed these predictions and showed that over 60% of Hem-1 did not associate with WAVE2, making a strong case that Hem-1 targets other proteins.

To find out what those targets might be, Weiner et al. isolated complexes containing Hem-1 to identify their components. As expected, they retrieved other members of the WAVE

regulatory complex, but also found "excellent candidates" for regulating the positive and negative feedback loops required for neutrophil polarity, including proteins that could keep myosin complexes from acting in the front of the cell. The most abundant candidates likely associate with Hem-1 complexes that do not contain WAVE2, the authors concluded, because the majority of Hem-1-containing complexes do not associate with WAVE2.

Reducing Hem-1 concentrations in the neutrophils produced defects in actin polymerization, cell polarity, and morphology in the depleted cells, demonstrating Hem-1's essential role in these processes. Given Rac's importance in actin polymerization and cell polarity orchestration, the authors analyzed its activity in Hem-1-depleted cells. They found that Hem-1 complexes, which act downstream of Rac, are also required for Rac activation. This is consistent with a Rac positive feedback loop in which Rac increases its own activation via Hem-1 complexes. Positive feedback loops are highly conserved elements in cell polarity circuits from yeast to slime molds to neutrophils. Hem-1 supports this feedback loop through mechanisms that operate independently of and in addition to WAVE-regulated actin assembly. The authors go on to show that Hem-1 also protects the leading edge from the contractile influence of myosin.

Altogether, these results suggest that Hem-1 protein complexes function to organize the leading edge of migrating neutrophils. Here, these versatile complexes steer multiple inputs, such as Rac, to the appropriate output at the leading edge—whether it be stimulating WAVE-regulated actin polymerization, promoting feedback loops that stabilize the protrusions, or segregating activated myosin to ensure proper chemotaxis. The authors' next round of experiments will investigate the upstream signals that regulate the targets of Hem-1 complexes and help the cell reach its destination.

Weiner OD, Rentel MC, Ott A, Brown GE, Jedrychowski M, et al. (2006) Hem-1 complexes are essential for Rac activation, actin polymerization, and myosin regulation during neutrophil chemotaxis. DOI: 10.1371/journal.pbio.0040038

A New Tile in the Biochemical Puzzle of Insulin Biology

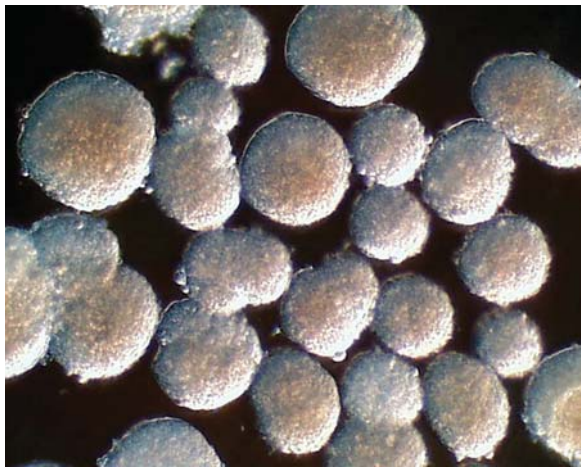
Françoise Chanut | DOI: 10.1371/journal.pbio.0040059

Molecular biologists are like obsessive jigsaw-puzzle lovers. They won't stop until they have found a spot for every molecule in their favorite cell. But their obsession rarely amounts to just fun and games, particularly when that cell is linked to a serious disease. By patiently fitting individual proteins into simple biochemical pathways, and biochemical pathways into complex cell functions, molecular biologists establish solid scaffolds to support effective therapies.

One such highly scrutinized cell is the pancreatic β cell. These cells produce insulin, a key hormonal regulator of metabolism and blood sugar content. Their malfunction or premature loss causes type I—or juvenile onset—diabetes, which affects millions of people worldwide. Type I diabetics respond well to regular injections of synthetic insulin, but this lifelong treatment is not without shortcomings. Therapies based on β cell replacement, using stem cells for instance, are promising but depend on engineering replacement cells that mimic β cells closely, which require an in-depth understanding of β cell biology.

β cells store insulin and release it when needed: for instance, when post-meal glucose levels rise far above one gram per liter. A rapid return to normal blood sugar level ensues, because insulin stimulates glucose uptake by muscles and other tissues. But once insulin stores are depleted, they must be replenished, which the β cell accomplishes by keeping its insulin gene active. The expression level of any gene usually reflects the combined influence of a variety of activating or inhibitory biochemical pathways. Cells use molecular signals to modulate the pathways' outputs according to their needs. In a new study in *PLoS Biology*, Nora Smart, Seung Kim, and their colleagues show that one of the pathways that regulates insulin gene expression in β cells is the TGF-β signaling cascade. Furthermore, they find that TGF-β signaling is crucial for the maintenance of mature β cell identity.

TGF-β is the founding member of a large family of extracellular proteins that control a variety of cell decisions.



DOI: 10.1371/journal.pbio.0040059.g001

TGF-β signaling is essential for maintaining the identity and function of pancreatic islet β-cells. (Image: Hainan Chen and Seung Kim)

All TGF-β family members act by binding to a receptor in the cell membrane. This triggers an enzymatic cascade that adds phosphate tags to a series of intracellular proteins called Smads. Some Smads transmit the TGF-β signal; others interrupt it by preventing the phosphorylation of activating Smads. Eventually, a phosphorylated activating Smad enters the cell's nucleus, where it controls the expression of various target genes.

Work from several labs, including Kim's, has shown that TGF-β signaling is important for the formation of β cells within the developing pancreas. But mature, adult β cells also display telltale signs of active TGF-β signaling, such as phosphorylated Smads, which suggest that TGF-β signaling could also control the normal functioning of pancreatic β cells. Smart and her colleagues interrupted TGF-β signaling in β cells with one of

the inhibitory Smad family members, Smad7. The difficulty was to express Smad7 in mature β cells only, and not at earlier stages, when it would prevent β cell formation. The researchers engineered mice with a modified Smad7 gene (transgene) that expressed Smad7 specifically in β cells and could be turned off at will with the drug doxycycline. By simply adding or eliminating doxycycline in the animals' diet, the researchers controlled the timing of Smad7 expression.

Mice fed doxycycline from embryonic to adult stages were normal, as expected if the Smad7 transgene is silent and unable to block signaling. But a few weeks after doxycycline was removed from their food, the mice became diabetic: their blood insulin levels dropped and glucose accumulated to abnormally high levels. Looking inside the β cells, the researchers found that Smad7 expression caused the near disappearance of MafA, a protein known to directly activate the insulin gene. Indeed, the pancreas of mice expressing Smad7 contained far less insulin than normal, confirming that Smad7 prevented insulin synthesis. Smad7 also prevented the expression of key markers of mature β cell identity, such as Menin and $p27^{Kip1}$, two proteins that prevent β cell proliferation. Of note, when the mice were given doxycycline again, their diabetes disappeared in a few days, and expression of MafA, Menin, and $p27^{Kip1}$ returned to normal. Therefore, Smad7 did not simply destroy the cells, but reversibly altered their β cell identity.

Having thus firmly established the importance of TGF-β signaling for the maintenance of β cell characteristics, the researchers can now look for the precise TGF-β molecules that act in mature β cells, and continue piecing together the β cell puzzle.

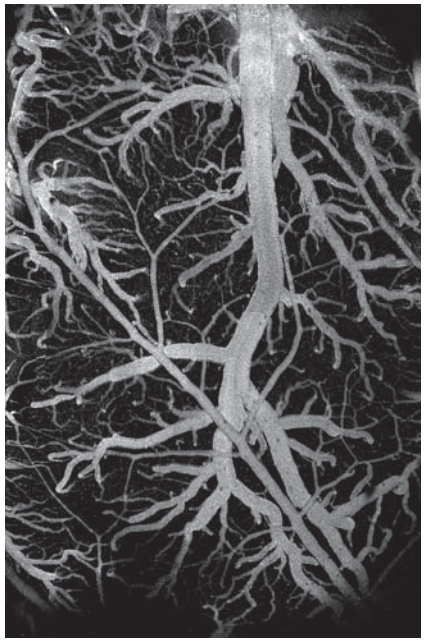
Smart NG, Apelqvist ÅA, Gu X, Harmon EB, Topper JN, et al. (2006) Conditional expression of Smad7 in pancreatic β cells disrupts TGF-β signaling and induces reversible diabetes mellitus. DOI: 10.1371/journal.pbio.0040039

Redundancy in Cortical Surface Vessels Supports Persistent Blood Flow

Liza Gross | DOI: 10.1371/journal.pbio.0040043

While examining the brain of a man who had died of a mesenteric tumor, Sir Thomas Willis was shocked to find a nearly totally blocked right carotid artery, the brain's main blood source. In his 1664 book, *Anatomy of the Brain*, the Oxford physician and neuroanatomist reported that given the "influx of blood being denied by this route, it seemed remarkable that this person had not previously died of an apoplexy." A stroke was averted, Willis explained, because the circular arrangement of the arteries on the base of the brain—later named the circle of Willis—created redundant connections in the circulatory network.

Scientists are still elucidating the details of vascular resilience—and how stroke can result when it is compromised. Much as the circle of Willis protects large-scale blood flow, it's been a matter of conjecture if a similar mechanism ensures microvessel flow to local brain regions. Until recently, scientists have lacked the appropriate methods to test this hypothesis. In a new study, Chris Schaffer, David Kleinfeld, and their physics and medical school colleagues take advantage of cutting-edge techniques in microscopy and laser-induced clotting (photothrombosis) to directly measure the resilience and dynamics of cortical blood flow. By manipulating and monitoring blood flow in the surface vasculature of rat parietal cortex, they show that the arteriole network rapidly reestablishes blood flow following a targeted occlusion to a surface vessel.



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The surface arteriole communicating network in the rat.

To analyze flow dynamics among the interconnected arterioles on the cortical surface, Schaffer et al. labeled blood plasma with a dye that allowed them to map the vessel architecture and also measure the flow of individual blood cells, using two-photon microscopy. Candidate vessels were selected for photothrombotic clotting, a technique that uses photosensitive molecules and focal laser beams to produce free radicals and damage the vessel wall, ultimately triggering an occluding blood clot. This enabled the authors to pinpoint a single vessel for occlusion without harming any neighboring vessels. They further used two-photon microscopy to determine the direction and speed of red blood cells by repetitively scanning the axis of each vessel, which allowed them to monitor the pattern of blood flow throughout the surface vascular network in real time.

Schaffer et al. first tested the brain's vascular resilience by inducing targeted photothrombotic clots in an individual surface arteriole upstream of a branch point. Despite the blockage, blood flow was maintained in both downstream branches because of a reversal in the direction of flow through one of the two branches—just one second after occlusion. Of a total 47 clots in 34 rats, all showed the same result. The redistribution in flow was sufficiently robust so that little change in flow occurred in vessels farther downstream from the occlusion.

A second test of the brain's vascular resilience involved a more traditional method to block flow that uses a fine filament threaded through the carotid to partially obstruct the middle cerebral artery, the major source of blood to the parietal cortex. While the flow is reduced throughout the entire surface network of communicating arterioles, a pattern of reversal in flows was also seen. Thus, reversals are a common

feature in the redistribution of blood across the cortex.

Schaffer et al. show that the architecture of the cortical surface arterioles, with redundant connections between branches of the middle cerebral artery, ensures persistent blood flow and protects against localized occlusions. This extends the concept of redundant connections from a single loop in the circle of Willis, at the base of the brain, to the network of communicating arterioles on the cortical surface. Since humans and rats share a similar surface vasculature, these results could help identify potential links between vascular topology and stroke vulnerability in different regions of the brain.

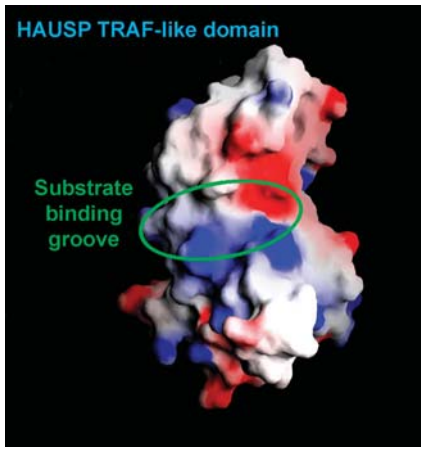
Schaffer CB, Friedman B, Nishimura N, Schroeder LF, Tsai PS, et al. (2006) Two-photon imaging of cortical surface microvessels reveals a robust redistribution in blood flow after vascular occlusion. DOI: 10.1371/journal.pbio.0040022

Structural Insights into the Regulation of a Key Tumor Suppressor

Liza Gross | DOI: 10.1371/journal.pbio.0040040

The most common mutation in many human cancers disables p53, a key cell-growth regulator and tumor-suppressor protein. When a cell sustains DNA damage or some other stress, p53 activates genes involved in programmed cell death, cell cycle arrest, DNA repair, or other stress-induced responses. In healthy cells, p53 keeps a low profile, its numbers minimized by MDM2, an enzyme that marks p53 for rapid degradation with a ubiquitin tag through a process called ubiquitylation (also known as ubiquitination). As it happens, p53 also engineers its own destruction by including MDM2 in its list of transcriptional targets. How does the cell counteract this negative feedback loop and rescue p53 during times of stress?

Recent studies identified a deubiquitylating enzyme called HAUSP (herpesvirus-associated ubiquitin-specific protease) that can bind to p53, stabilize the protein, and promote cell death and cell growth arrest. But HAUSP can also deubiquitylate and stabilize MDM2. How can it stabilize both p53 and p53's nemesis? In a new study, Min Hu, Yigong Shi, and their colleagues used structural and mutational approaches to explore this paradox, and discovered that both p53 and MDM2 bind to the same location on the HAUSP protein domain in a mutually exclusive manner. Analysis of the molecular basis of their differential binding revealed that



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HAUSP TRAF-like domain contains a shallow surface groove, which corresponds to the region where the receptor peptides bind.

MDM2 binds HAUSP with a much higher affinity, and suggests how HAUSP may regulate the critically important p53–MDM2 pathway.

Having determined that HAUSP recognizes p53 through a region of N-terminal residues within the TRAF-like domain in a previous study, the next step was determining the domain's structure. Proteins consist of linear sequences of amino acid residues joined end-to-end through peptide bonds. A polypeptide chain starts with an N-terminus (the domain at this end is called the N-terminal domain), and terminates at the C-terminus (so designated for its chemical properties). Hu et al. discovered that the HAUSP TRAF-like domain (named after a common domain in the TRAF family of cell signaling proteins) contains a shallow groove like the TRAF domain but lacks many of the same amino acids.

To study the structural details of HAUSP–p53 interactions, the authors mutated different amino acid residues in the p53 binding site and identified a short stretch of five amino acids required for binding. As efforts to obtain crystals of the p53–HAUSP complex were not successful, they generated protein chimeras—made of half p53 peptides and half HAUSP TRAF-like domain—to determine the complex's structure and mechanism of binding. This structure shows that the p53 peptide binds to HAUSP's shallow groove and reveals how many of the amino acids previously identified as important for HAUSP–p53 binding interact with specific p53 residues.

Hu et al. followed the same approach to reveal the mechanism of HAUSP–MDM2 interactions, and identified a ten–amino acid fragment in MDM2 as key to HAUSP recognition. Though this fragment bore little resemblance to the p53 peptide, HAUSP recognizes MDM2 with the same mechanism it uses to recognize p53—the N-terminus TRAF-like domain in the same shallow groove. As both p53 and MDM2 bind to the same groove on HAUSP, the authors realized their binding must be mutually exclusive and so staged a binding competition. And because MDM2 consistently formed stable complexes with HAUSP despite the presence of ten times more p53 peptides, it was clear that MDM2 binds to the deubiquitylating enzyme with a higher affinity.

Next, Hu et al. superimposed the two bound complexes. Though both peptides bind to the same shallow groove in the TRAF-like domain with the same orientation, MDM2's configuration allows more extensive interactions with HAUSP, which could explain its competitive binding edge. Interestingly, HAUSP's TRAF-like domain lacks most of the peptide-binding residues found in other TRAF proteins, suggesting that the HAUSP domain provides a new binding motif in the TRAF family. Though the HAUSP binding sites of p53 and MDM2 do not have obvious sequence similarity, the authors did find a common four-residue motif within them that is also present

in a different HAUSP-binding protein (EBNA1). With knowledge of this motif, researchers can scan sequence databases for additional candidate HAUSP targets.

But how does recognition and binding mediate deubiquitylation? After solving the structure of a larger HAUSP fragment (including the catalytic domain, responsible for deubiquitylation, and the peptide-binding domain), the authors constructed a model of how HAUSP might recognize a ubiquitylated MDM2 protein. A small peptide fragment of MDM2 stabilizes binding to the TRAF-like domain while a separate HAUSP domain binds ubiquitin, and then cleaves the ubiquitin tag, promoting deubiquitylation.

These results suggest that HAUSP likely targets MDM2 under normal physiological conditions, and provide a valuable framework for probing the function of the p53–MDM2 pathway. The differential binding properties of p53 and MDM2 also suggest promising drug-screening targets. Given MDM2's negative impact on p53, it may be that inhibiting HAUSP, and thus MDM2, could counteract mutations that interfere with p53 function, and give this tumor suppressor the boost it needs to do its job.

Hu M, Gu L, Li M, Jeffrey PD, Gu W, et al. (2006) Structural basis of competitive recognition of p53 and MDM2 by HAUSP/USP7: Implications for the regulation of the P53–MDM2 pathway. DOI: 10.1371/journal.pbio.0040027

Genetic Evidence that Humans Have Pushed Orang-utans to the Brink of Extinction

Liza Gross | DOI: 10.1371/journal.pbio.0040057

Humans' capacity to alter the environment has grown exponentially over the past century, challenging other species to adapt to a transfigured landscape. Over some 15,000 plants and animals face extinction, the overwhelming majority threatened by increasingly fragmented, polluted, and deteriorating habitats. These forces are most acute in Africa and Asia, where 25% of all primate species are threatened. Chimpanzees, bonobos, orang-utans, and gorillas could vanish from the wild completely within a matter of decades.

In Africa, deforestation, illegal hunting, and disease outbreaks are decimating chimp, bonobo, and gorilla populations. Orang-utans, the only non-African great ape, face similar threats, compounded by agricultural conversion of logged forests. But because orang-utans spend most of their time in trees and avoid open spaces, they may suffer most from deforestation. A new study by Benoit Goossens, Lounès Chikhi, Michael Bruford, and their colleagues shows that the case for orang-utans is in fact even more desperate than previously believed. Using computer simulations and the largest genetic dataset ever collected from wild orang-utans, the authors build up a picture of alarming—and very recent—declines of orang-utan populations of over 95%.

Orang-utans, Malaysian for "man of the forest," once ranged from Indonesia to southern China and northeastern India, but now struggle to survive in isolated forest patches in northern Sumatra and Borneo. Over 66% of the Sumatra orang-utan population was killed between 1993 and 2000; 33% of the Borneo population may have succumbed to drought and fire between 1996 and 1997 alone. When a 1987 study estimated that over 20,000 orang-utans persisted in Sabah, Malaysia, in the 1980s, it was challenged as too optimistic.

Developing effective conservation and recovery programs depends on determining when the decline began, its trajectory, and the original population size. To shed light on these questions, Goossens and Chikhi used three statistical methods to mine the genetic material of wild orang-utans in Eastern Sabah, scarred by over 50 years of large-scale commercial logging and agriculture.

For the genetic analysis, the authors and their local assistants collected hair from tree nests—some nearly 100 feet above the ground—and feces found under nests or near orang-utans encountered along the Kinabatangan River. Two hundred orang-utans were identified using genetic markers

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Ongoing deforestation and palm oil plantations leave no room for the orang-utan.

called microsatellites, tandem repeats of short DNA motifs that are sometimes used as genetic fingerprints. The authors used 14 microsatellites from each animal in an analysis that compares observed genetic diversity with that expected for a stable population, based on the number of alleles (gene pairs, or genetic motifs, at one chromosomal location) and expected heterozygosity (the alleles in the pair differ).

When population size drops significantly, genetic diversity decreases as well, but in a very specific way: rare alleles are lost whereas heterozygosity remains relatively little affected. And it is this signature that the authors were looking for. The authors first simulated what the expected heterozygosity would be, based on the number of alleles and sample size (nine forest blocks were sampled) for each population and microsatellite locus, and compared these values to those

seen in the collected genetic data. Three different mutation models were used to simulate variations of microsatellite repeat number under different mutational processes and demographic histories to detect evidence of a decline. Even the most conservative model produced the same "strong and significant signal for a population bottleneck."

They next used two computer-intensive statistical approaches to characterize the extent and timing of the decline, focusing on the two largest populations. The first statistical model (called the Beaumont method) makes specific demographic and mutation assumptions that can quantify the relative change in population size. Again, this model yielded the same result: orang-utan populations have declined by at least a factor of 50 (with 95% probability) or 100 (with 90% probability).

The second model (the Storz and Beaumont method) allowed the authors to estimate past and present population sizes and timing of the collapse. They found strong genetic evidence of a collapse for both Sabah populations, and a likely time frame within the past decades—coinciding with accelerated deforestation in the region in the 1950s and 1970s. This result also supports the 1987 estimate of 20,000 and the current estimate of 11,000 Sabah orang-utans—which means the decline was far more recent, and far more drastic, than previously assumed.

Normally, the genetic effects of such recent events would be obscured by ancient demographic events, such as those following major climatic changes, or the arrival of the first hunter-gatherers or farmers. But humans have so devastated orang-utan populations that the genetic signature of the recent bottleneck may have overshadowed any previous population fluctuations. These results demonstrate that genetic data can provide independent and extremely detailed information by detecting and quantifying the consequences of human impacts on endangered species. They also underscore the need to act now to protect the long-term survival of the species. The animals show enough genetic diversity to stabilize, the authors argue, if immediate steps are taken to reconnect remnant forest patches and halt further deforestation. Otherwise, humans will have restricted the fabled forest man to the realm of memory—or a life behind bars in a zoo.

Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Andau P, et al. (2006) Genetic signature of anthropogenic population collapse in orang-utans. DOI: 10.1371/journal.pbio.0040025

The *Sirt1* Gene Promotes Insulin Secretion in Accord with Diet

Françoise Chanut | DOI: 10.1371/journal.pbio.0040044

Those seeking the fountain of youth would do well to watch their calories. Indeed, caloric restriction has been known for many years to increase longevity in laboratory animals. A simple explanation for this effect is that reducing food intake slows down the body's metabolism, which reduces the formation of toxic byproducts that cause tissue damage and aging.

Whatever the mechanisms, the benefits of caloric restriction hold from furry mammals to single-celled fungi. In yeast, nematodes, and fruit flies, caloric restriction increases the activity of the *Sir2* gene, which in turn changes the expression of genes related to metabolism. One of the key regulators of mammalian metabolism is the pancreatic hormone insulin. In a new study, Laura Bordone, Leonard Guarente, and their colleagues show that the mammalian homolog of *Sir2*, called *Sirt1*, modulates insulin production in response to diet.

In times of famine, the body taps into its own resources to provide energy for its working tissues. For instance, it mobilizes the lipid molecules stored in fat, and coaxes the liver into producing the simple sugar glucose. Cells take up glucose and lipids from the blood, and extract their chemical energy. In times of plenty, glucose and lipids come from food. As their levels rise in the blood, the pancreas secretes insulin, which stimulates the uptake of glucose by muscles and lipids by fat. An important function of insulin is to regulate glucose levels in the blood; its secretion is therefore tightly controlled by glucose concentration. But during fasting—and starvation—insulin secretion dips to very low levels, an adaptation that increases glucose availability for the brain.

Bordone et al. asked whether Sirt1 influenced insulin production. They disrupted the *Sirt1* gene of mice, and found that these mice produced very little insulin, regardless of whether they were well fed or starved. These results suggested that Sirt1 is necessary for glucose to induce insulin production.

The authors next asked at what step of insulin production Sirt1 acts. Insulin is made by specialized cells of the pancreas, called β cells. β cells can only secrete insulin when they accumulate enough ATP. This happens when glucose levels rise in the blood, after a meal for instance, because β cells metabolize glucose into ATP. Bordone and her colleagues found that β cells with an inactive *Sirt1* gene did not secrete as much insulin in response to glucose as normal β cells. Nor did they convert glucose into ATP as efficiently as normal β cells. This last observation led the authors to examine the activity of a type of protein known as uncoupling protein (UCP), which diverts glucose breakdown from ATP synthesis. In β cells, the UCP2 protein is known to inhibit insulin secretion by routing glucose metabolism toward a molecule called NADH, rather than toward ATP.

The authors demonstrate that Sirt1 inhibits the production of UCP2 by directly preventing the expression of the *UCP2* gene. How does the interaction between Sirt1 and UCP2 relate to caloric restriction? The authors find that in starved mice, UCP2 levels increase in β cells. This suggests that caloric restriction induces a decrease in Sirt1 activity in mice. This result is somewhat surprising since in yeast and other organisms, caloric restriction increases Sir2 expression.

Because Sirt1 and insulin have many roles in mammals, it is at present unclear how they mediate the effect of diet on lifespan. An intriguing hypothesis stems from the fact that UCP2 dampens the formation of toxic metabolic byproducts that precipitate aging. If the relationship between Sirt1 and UCP2 holds in more tissues than just β cells, Sirt1 may open a simple path to a longer life.

Bordone L, Motta MC, Picard F, Robinson A, Jhala US, et al. (2006) Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic β cells. DOI: 10.1371/journal.pbio.0040031