

Illustration: Bryan Christie

**The tiny cells in our bodies harbor amazing internal communication networks. Understanding how those circuits are organized could help scientists develop new therapies for many serious disorders**

By John D. Scott and Tony Pawson

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**SUBTOPICS:** As anyone familiar with the party game "telephone" knows, when people try to pass a message from one individual to another in a line, they usually garble the words beyond recognition. It might seem surprising, then, that mere molecules inside our cells constantly enact their own version of telephone without distorting the relayed information in the least.

Refining the Question

On the Importance of

Actually, no one could survive without such precise signaling in cells. The body functions properly only because cells communicate with one another

Lego Block

Fail-Safe  
Features Aid  
Specificity

Scaffolds  
Abound

Many Payoffs

### **SIDEBARS:**

The Role of  
Modules in  
Signaling

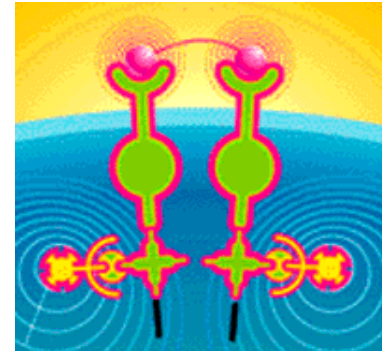
The Advantages  
of Adapters

Scaffolds Speed  
Signal  
Transmission

Getting a Line  
on Human  
Diseases

constantly. Pancreatic cells, for instance, release insulin to tell muscle cells to take up sugar from the blood for energy. Cells of the immune system instruct their cousins to attack invaders, and cells of the nervous system rapidly fire messages to and from the brain. Those messages elicit the right responses only because they are transmitted accurately far into a recipient cell and to the exact molecules able to carry out the directives.

But how do circuits within cells achieve this high-fidelity transmission? For a long time, biologists had only rudimentary explanations. In the past 15 years, though, they have made great progress in unlocking the code that cells use for their internal communications. The ongoing advances are suggesting radically new strategies for attacking diseases that are caused or exacerbated by faulty signaling in cells--among them cancer, diabetes and disorders of the immune system.

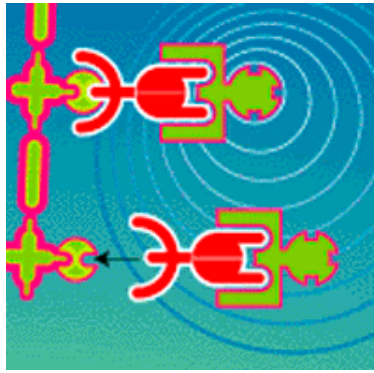


**THE ROLE OF MODULES IN  
SIGNALING**

### **Refining the Question**

The earliest insights into information transfer in cells emerged in the late 1950s, when Edwin G. Krebs and Edmond H. Fischer of the University of Washington and the late Earl W. Sutherland, Jr., of Vanderbilt University identified the first known signal-relaying molecules in the cytoplasm (the material between the nucleus and a cell's outer membrane). All three received Nobel Prizes for their discoveries.

By the early 1980s researchers had gathered many details of how signal transmission occurs. For instance, it usually begins after a messenger responsible for carrying information between cells (often a hormone) docks temporarily, in lock-and-key fashion, with a specific receptor on a recipient cell. Such receptors, the functional equivalent of antennae, are able to relay a messenger's command into a cell because they are physically connected to the cytoplasm. The typical receptor is a protein, a folded chain of amino acids. It includes at least three domains: an external docking region for a hormone or other messenger, a component that spans the cell's outer membrane, and a "tail" that extends a distance into the cytoplasm. When a messenger binds to the external site, this linkage induces a change in the shape of the cytoplasmic tail, thereby facilitating the tail's interaction with one or more information-relaying molecules in the cytoplasm. These interactions in turn initiate cascades of further intracellular signaling.



### THE ADVANTAGES OF ADAPTERS

order.

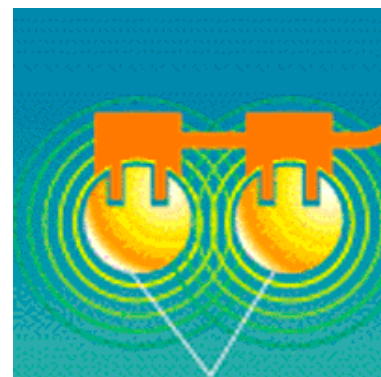
Yet no one had a good explanation for how communiqés reached their destinations without being diverted along the way. At that time, cells were viewed as balloonlike bags filled with a soupy cytoplasm containing floating proteins and organelles (membrane-bound compartments, such as the nucleus and mitochondria). It was hard to see how, in such an unstructured milieu, any given internal messenger molecule could consistently and quickly find exactly the right tag team needed to convey a directive to the laborers deep within the cell that could execute the

### On the Importance of Lego Blocks

Today's fuller understanding grew in part from efforts to identify the first cytoplasmic proteins that are contacted by activated (messenger-bound) receptors in a large and important family: the receptor tyrosine kinases. These vital receptors transmit the commands of many hormones that regulate cellular replication, specialization or metabolism. They are so named because they are kinases--enzymes that add phosphate groups to ("phosphorylate") selected amino acids in a protein chain. And, as Tony R. Hunter of the Salk Institute for Biological Studies in La Jolla, Calif., demonstrated, they specifically put phosphates onto the amino acid tyrosine.

In the 1980s work by Joseph Schlessinger of New York University and others indicated that the binding of hormones to receptor tyrosine kinases at the cell surface causes the individual receptor molecules to cluster into pairs and to attach phosphates to the tyrosines on each other's cytoplasmic tails. In trying to figure out what happens next, one of us (Pawson) and his colleagues found that the altered receptors interact directly with proteins that contain a module they called an SH2 domain. The term "domain" or "module" refers to a relatively short sequence of about 100 amino acids that adopts a defined three-dimensional structure within a protein.

At the time, prevailing wisdom held that messages were transmitted within cells primarily through enzymatic reactions, in which one molecule alters a second without tightly binding to it and without itself being altered. Surprisingly, though, the phosphorylated receptors did not necessarily alter the chemistry of the SH2-containing proteins. Instead many simply induced the SH2 domains to latch onto the phosphate-decorated tyrosines, as if the SH2 domains and the tyrosines were Lego blocks being snapped



SCAFFOLDS SPEED SIGNAL

modules were Lego bricks being snapped together.

## TRANSMISSION

By the mid-1990s groups led by Pawson, Hidesaburo Hanafusa of the Rockefeller University and others had revealed that many of the proteins involved in internal communications consist of strings of modules, some of which serve primarily to connect one protein to another. At times, whole proteins in signaling pathways contain nothing but linker modules.

But how did those nonenzymatic modules contribute to swift and specific communication in cells? One answer is that they help enzymatic domains transmit information efficiently. When a protein that bears a linker also includes an enzymatic module, attachment of the linker region to another protein can position the enzymatic module where it most needs to be. For example, the act of binding can simultaneously bring the enzymatic region close to factors that switch it on and into immediate contact with the enzyme's intended target. In the case of certain SH2-containing proteins, the linker module may originally be folded around the enzymatic domain in a way that blocks the enzyme's activity. When the SH2 domain unfurls to engage an activated receptor, the move liberates the enzyme to work on its target.

Even when a full protein is formed from nothing but protein-binding modules, it can function as an indispensable adapter, akin to a power strip plugged into a single socket. One module in the adapter plugs into a developing signaling complex, and the other modules allow still more proteins to join the network. An important benefit of these molecular adapters is that they enable cells to make use of enzymes that otherwise might not fit into a particular signaling circuit.

Nonenzymatic modules can support communication in other ways, too. Certain molecules in signaling pathways feature a protein-binding module and a DNA-binding module that meshes with, or "recognizes," a specific sequence of DNA nucleotides in a gene. (Nucleotides are the building blocks of genes, which specify the amino acid sequences of proteins.) James E. Darnell, Jr., of Rockefeller showed that when one of these proteins attaches, through its linker module, to an activated receptor kinase, the interaction spurs the bound protein to detach, move to the nucleus and bind to a particular gene, thus inducing the synthesis of a protein. In this instance, the only enzyme in the signaling chain is the receptor itself; everything that happens after the receptor becomes activated occurs through proteins' recognition of other proteins or DNA.

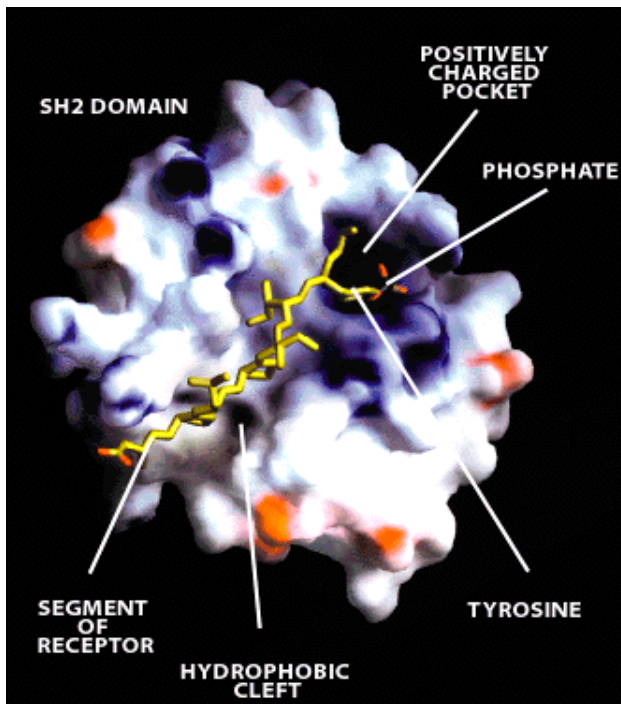


Illustration: Julie Forman-Kay, University of Toronto and Tony Pawson

**SH2 DOMAIN** (globular structure) in a signaling molecule is bound to a segment of a receptor (stick model). The two fit together in part because a positively charged pocket in SH2 is attracted to a negatively charged phosphate that has been added to the amino acid tyrosine in the receptor. Also, the nearby amino acids in the receptor fit snugly into a hydrophobic (water-hating) groove on SH2. All SH2 domains can bind to phosphate-bearing tyrosines, but they differ in their binding partners because they vary in their ability to lock onto the amino acids that lie next to tyrosine in a protein.

Studies of receptor tyrosine kinases and of SH2 domains have also helped clarify how cells guarantee that only the right proteins combine to form any chosen signaling pathway. Soon after SH2 domains were identified, investigators realized that these modules are present in well over 100 separate proteins. What prevented different activated receptors from attracting the same SH2-containing proteins and thereby producing identical effects in cells? For the body to operate properly, it is crucial that diverse hormones and receptors produce distinct effects on cells. To achieve such specificity, receptors must engage somewhat different communication pathways.

The answer turns out to be quite simple. Every SH2 domain includes a region that fits snugly over a phosphate-bearing tyrosine (a phosphotyrosine). But each also includes a second region, which differs from one SH2 domain to another. That second region—as Lewis C. Cantley of Harvard University revealed—recognizes a particular sequence of three or so amino acids next to the phosphotyrosine. Hence, all SH2 domains can bind to phosphorylated tyrosine, but these modules vary in their preference for the adjacent amino acids in a receptor. The amino acids around the tyrosine thereby serve as a code to specify which version of the SH2 domain can attach to a given

As these various discoveries were being made, work in other areas demonstrated that the cytoplasm is not really amorphous after all. It is packed densely with organelles and proteins. Together such findings indicate that high-fidelity signaling within cells depends profoundly on the Lego-like interlocking of selected proteins through dedicated linker modules and adapter proteins. These complexes assure that enzymes or DNA-binding modules and their targets are brought together promptly and in the correct sequence as soon as a receptor at the cell surface is activated.

### **Fail-Safe Features Aid Specificity**

phosphotyrosine-bearing receptor. Because each SH2 domain is itself attached to a different enzymatic domain or linker module, this code also dictates which pathways will be activated downstream of the receptor. Other kinds of linker modules operate analogously.

A pathway activated by a protein called platelet-derived growth factor illustrates the principles we have described. This factor is often released after a blood vessel is injured. Its attachment to a unique receptor tyrosine kinase on a smooth muscle cell in the blood vessel wall causes such receptors to cluster and become phosphorylated on tyrosine. This change draws to the receptor a protein called Grb2, which consists of a specific SH2 domain flanked on either side by another linker domain, SH3. Grb2 is a classic adapter; it has no enzymatic power at all, but its SH3 domains (which like to bind to the amino acid proline) hook an enzyme-containing protein called Sos to the receptor. There Sos activates a membrane-associated protein known as Ras, which triggers a series of enzymatic events. These reactions ultimately stimulate proteins in the nucleus to activate genes that cause the cells to divide, an action that promotes wound healing.

The signaling networks headed by receptor tyrosine kinases seem to rely on relatively small adapter proteins. Analyses of communication circuits in nerve cells (neurons) of the brain show that some proteins in neuronal pathways have an incredibly large number of linker domains. These proteins are often called scaffolding molecules, as they permanently hold groups of signaling proteins together in one place. The existence of such scaffolds means that certain signaling networks are hardwired into cells. That hardwiring can enhance the speed and accuracy of information transfer.

### **Scaffolds Abound**

One well-studied scaffolding protein goes by the name PSD-95. It operates primarily in neurons involved in learning. In nerve tissue, signals pass from one neuron to another at contact points called synapses. The first neuron releases a chemical messenger--a neurotransmitter--into a narrow cleft between the cells. Receptors on the second cell grab the neurotransmitter and then cause ion channels in the membrane to open. This influx of ions activates enzymes that are needed to propagate an electrical impulse. Once generated, the impulse travels down the axon, a long projection, to the axon's abundant tiny branches, inducing them to release more neurotransmitter. For the impulse to be produced, many components of the signaling system must jump into action virtually simultaneously.

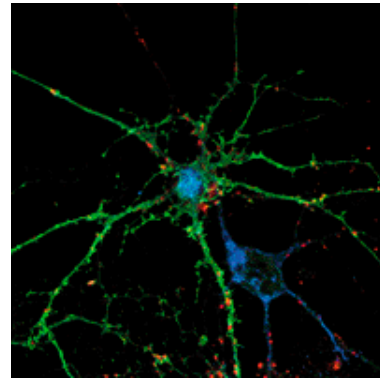
Among the multiple linker modules in PSD-95 are three so-called PDZ domains. One binds to the cytoplasmic tail of the receptor for the neurotransmitter glutamate. A second grabs onto a membrane-spanning ion channel (which controls the inflow of potassium), and a third clasps proteins in the cytoplasm (as does an additional module in the scaffold). PSD-95 thus yokes together several signaling components at once, enabling them to coordinate their activities. The eye of a fruit fly also relies on a PDZ-containing scaffolding protein-InaD-for the efficient relay of visual information from the eye to the brain.

Yet another preformed signaling complex has been found only recently, in mammalian neurons. The core is a scaffolding protein named yotiao. As one of us (Scott) and his colleagues have shown, this molecule grasps a dual-purpose, membrane-spanning protein that is both a glutamate receptor and an ion channel. It also clasps a kinase that adds phosphate to, and thereby opens, the ion channel when the receptor is activated by glutamate. And it anchors a phosphatase, an enzyme that removes phosphates from proteins. The bound phosphatase closes the ion channel whenever glutamate is absent from the receptor. This elegant arrangement ensures that ions flow through the channel only when glutamate is docked with the receptor.

Kinases and phosphatases control most activities in cells. If one kinase activates a protein, some phosphatase will be charged with inactivating that protein, or vice versa. Yet human cells manufacture hundreds of different kinases and phosphatases. Scaffolding proteins, it appears, are a common strategy for preventing the wrong kinases and phosphatases from acting on a target; they facilitate the proper reactions by holding selected kinases and phosphatases near the precise proteins they are supposed to regulate.

### Many Payoffs

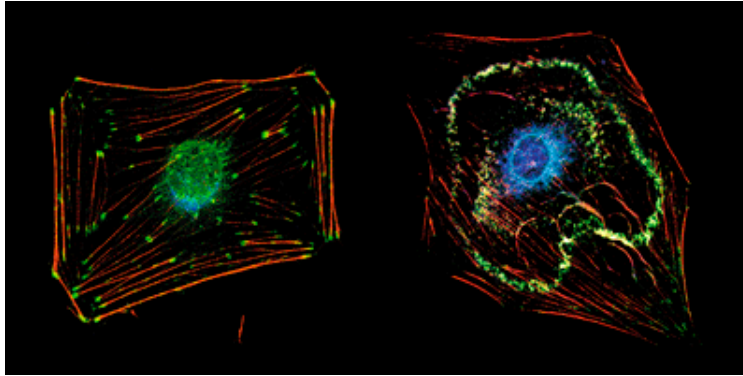
From an evolutionary perspective, the advent of a modular signaling system would be very useful to cells. By mixing and matching existing modules, a cell can generate many molecules and combinations of molecules and can build an array of interconnected pathways without having to invent a huge repertoire of building blocks. What is more, when a new module does arise, its combination with existing modules can increase versatility tremendously--just as adding a new area code to a city turns already assigned phone numbers into entirely new ones for added customers.



Micrograph: Lorraine Langeberg, Villum Institute

**TWO SCAFFOLDING PROTEINS** are highlighted in the larger nerve cell (neuron) in this micrograph. One, yotiao (green), tethers signal-relaying enzymes next to an ion channel involved in signal transmission. The other, PSD-95 (red), clusters a receptor and a different ion channel at selected synapses, the contact points between neurons. The blue in both neurons marks the location of a specific signaling enzyme.

For cell biologists, merely chipping away at the mystery of how cells carry out their myriad tasks is often reward enough for their efforts. But the new findings have a significance far beyond intellectual satisfaction.



Micrographs: Lorraine Langeberg, Vollum Institute

**FLOW OF MESSAGE** in a skin cell was made visible by coloring two components of a signaling pathway: a scaffolding protein (green) and one of two enzymes tethered to that scaffold (blue). Actin, a structured element in cells, is red. The top cell is quiet. Soon after an external messenger activated a signaling pathway in the bottom cell, the scaffolding protein moved its bound enzymes to their targets deeper in the cell. That movement is revealed by the yellow hue, which derives from the overlap of the colored signaling components with actin (to which the enzymes' targets were bound). The blue mass at the center reflects extra copies of the colored enzyme.

The much publicized Human Genome Project will soon reveal the nucleotide sequence of every gene in the human body. To translate that information into improved understanding of human diseases, those of us who study the functioning of cells will have to discern the biological roles of any newly discovered genes.

That is, we will need to find out what the corresponding proteins do and what happens when they are overproduced, underproduced or made incorrectly.

We already know the amino acid sequences and the functions of many modules in signaling proteins. Hence, we have something of a key for determining whether the nucleotide sequence of a previously unknown gene codes for a signaling protein and, if it does, which molecules the protein interacts with. When we have enough of those interactions plotted, we may be able to draw a wiring diagram of every cell type in the body. Even with only a partial diagram, we may uncover ways to "rewire" cells when something goes wrong--halting aberrant signals or rerouting them to targets of our own choosing. We might, for instance, funnel proliferative commands in cancer cells into pathways that instruct the cells to kill themselves instead of dividing.

By learning the language that cells use to speak to one another and to their internal "workers," we will be able to listen in on their conversations and, ideally, find ways to intervene when the communications go awry and cause disease. We may yet reduce "body language" to a precise science.

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### Further Information:

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The European Molecular Biology Laboratory SMART database Web site is at <http://smart.embl-heidelberg.de/>

The Howard Hughes Medical Institute News Web site is at [www.hhmi.org/news/scott.htm](http://www.hhmi.org/news/scott.htm)

The Oregon Health Sciences Vollum Institute Web site is at [www.ohsu.edu/vollum/faculty/scott/index.htm](http://www.ohsu.edu/vollum/faculty/scott/index.htm)

The Samuel Lunenfeld Research Institute at the Mount Sinai Hospital, Toronto, Web site is at [www.mshri.on.ca/pawson/research.html](http://www.mshri.on.ca/pawson/research.html)

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### **The Authors**

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