

SIGNAL TRANSDUCTION

2016: Signaling Breakthroughs of the Year

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Signaling breakthroughs of 2016 clustered mainly in the areas of neuroscience, immunology, and metabolism, with excursions into plant hormone signaling and bacterial manipulation of host signaling pathways. Perhaps reflecting the growing maturity of the discipline of cell signaling, many of this year's breakthroughs have implications for the pathogenesis or treatment of human disease.

The editors of *Science Signaling* welcome you to 2017 with the 15th edition of *Signaling Breakthroughs of the Year*. The breakthroughs list is compiled from nominations provided by members of the *Science Signaling* Editorial Board, along with nominations of studies that caught the eyes of the *Science Signaling* editors. The 2016 nominations clustered mainly in the areas of neuroscience, immunology, and cellular metabolism, with intriguing excursions into mechanisms of plant hormone receptor signaling and bacterial manipulation of host cell signaling pathways. More specifically, this year's breakthroughs include studies of ligand-receptor interactions, an unexpected mechanism of synaptic plasticity, a unique way to inhibit a kinase, insight into the consequences of cellular nutrient deprivation, and a quantitative analysis of the mitochondrial metabolome. Perhaps reflecting the growing maturity of the discipline of cellular signal transduction, many of this year's nominations are relevant to the pathogenesis or have application to the treatment of human disease. This edition of *Signaling Breakthroughs of the Year* comprises research nominated by Ivan Dikic (Goethe University Medical School, Germany), Henrik Dohlman (The University of North Carolina Chapel Hill, USA), James Faeder (University of Pittsburgh School of Medicine, USA), Johannes W. Hell (University of California, Davis, USA), Kevin Janes (University of Virginia, USA), Rune Linding (University of Copenhagen, Denmark), Li Ma (The University of Texas MD Anderson Cancer Center, USA), Samuel Miller (University of Washington, USA), Klaus Okkenhaug (Babraham Institute, UK), and Norbert Perrimon (Harvard Medical School, USA).

Two nominations concerned the application of insights into G protein-coupled receptor (GPCR) signaling to disorders involving the nervous system. Dohlman drew our attention to a study that used a computational approach to build a better opioid (Fig. 1A)

(1). Although the use of opioids to relieve pain dates back thousands of years, their pain-relieving properties are counterbalanced by serious side effects, such as addiction and potentially fatal respiratory depression, and their abuse has become a serious global problem (1, 2). Morphine and related opioid analgesics act as agonists of the μ -opioid receptor, a GPCR that activates both $G\alpha_i$ - and β -arrestin-dependent signaling pathways; μ -opioid receptor signaling through $G\alpha_i$ is thought to mediate analgesia, whereas signaling through β -arrestin is thought to mediate the undesirable side effects. Manglik *et al.* (1) used the recently reported crystal structure of the μ -opioid receptor to computationally dock more than 3 million compounds to the ligand-binding pocket, in the hopes of identifying compounds that engaged the receptor in new ways to stabilize unique conformations. The authors then used structure-based optimization to synthesize PZM21, a compound that displayed $G\alpha_i$ -biased signaling and, remarkably, potently elicited long-lasting relief from pain in mice. Moreover, PZM21 did not cause respiratory depression or addiction-like behaviors. Dohlman's second nomination concerned a study identifying the cellular prion protein PrP^c as an agonistic GPCR ligand (3). Although the crucial role of misfolded prions in the neurodegenerative prion diseases is well known, the physiological function(s) of PrP^c has remained unclear. Noting that ablation of neuronal PrP^c leads to chronic demyelinating polyneuropathy in mice, Küffer *et al.* (3) determined that the amount of cAMP (cyclic adenosine monophosphate) in sciatic nerves of mice lacking PrP^c was lower than that in nerves from wild-type mice, suggesting that PrP^c might signal through a GPCR. Further analysis revealed that PrP^c acted as a ligand of Adgrg6 (adhesion G protein-coupled receptor G6; also known as Gpr126) on Schwann cells to promote myelin homeo-

stasis. In addition to providing insight into the physiological role of PrP^c, this work may have implications for the treatment of demyelinating peripheral neuropathies, as well as for the potential consequences of strategies attempting to deactivate PrP^c as an approach to treating prion disease.

Like GPCRs, protein kinases are implicated in various disease states and are therefore promising targets for therapy. Excess activity of glycogen synthase kinase 3 (GSK-3) may contribute to various neurological disorders, including Alzheimer's disease. Like other protein kinases, however, developing an inhibitor that is both potent and selective has been a challenge. Kinase inhibitors that target the adenosine 5'-triphosphate (ATP) binding site tend to lack specificity, whereas competitive inhibitors derived from kinase substrates tend to be weak. The *Science Signaling* editors nominated a study by Licht-Murava *et al.* (4) describing the development of a substrate-derived GSK-3 inhibitor with a unique mechanism of action (Fig. 1B). Unlike other substrate-derived inhibitors, this compound, L807mts, is subject to GSK-3 phosphorylation and binds tightly to the enzyme after undergoing phosphorylation, thereby undergoing conversion from a selective substrate to a potent inhibitor at the catalytic site. L807mts showed promise in a mouse model of Alzheimer's disease and may herald the development of a new class of kinase inhibitors that are both selective and effective.

The final neuroscience-themed nomination, from Hell, concerned an intriguing twist on the mechanisms underlying long-term depression (LTD) of synaptic transmission in the CA1 region of the hippocampus (5). Long-lasting changes in the efficacy of excitatory transmission, such as long-term potentiation (LTP) and LTD, have been implicated in learning and memory. In the CA1 region, where these processes have been extensively studied, LTP depends on an increase in the function of and localization of postsynaptic AMPA-type glutamate receptors (AMPA),

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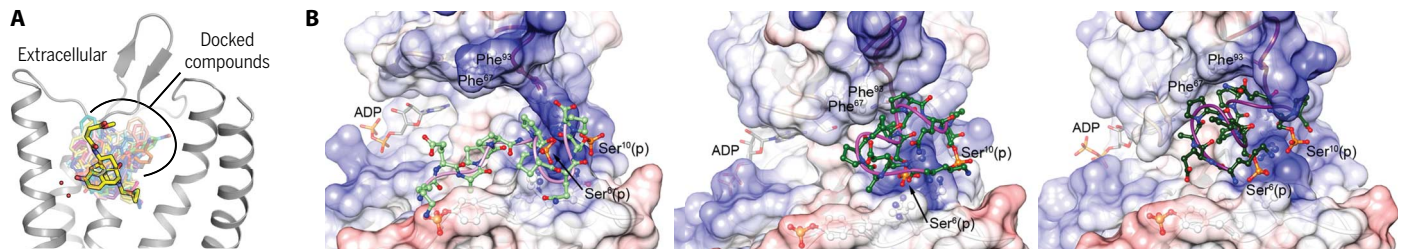


Fig. 1. Breakthroughs in drug discovery. Manglik *et al.* (1) and Licht-Murava *et al.* (4) both used computational approaches to discover either new drugs targeting the opioid receptor (A) or a new mechanism of inhibition of the kinase GSK-3 (B). [Panel (A) is reprinted with permission from *Nature* from Manglik *et al.* (1). Panel B is from Licht-Murava *et al.* (4)]

whereas LTD depends on decreased postsynaptic function and localization of AMPARs. In a study Hell described as “fascinating,” Sanderson *et al.* [(5); see associated commentary (6)] showed that, unexpectedly, induction of LTD depends on the transient recruitment of Ca^{2+} -permeable AMPARs to the postsynaptic membrane. The authors determined that recruitment occurred in response to phosphorylation of a serine on the AMPAR GluA1 subunit by AKAP-anchored protein kinase A and was followed by their removal subsequent to dephosphorylation of this same serine by the Ca^{2+} -activated phosphatase calcineurin.

The immune system rivals the nervous system in complexity, and the organization of signaling proteins in receptor complexes of cells of the immune system is as crucial to their function as the organization of postsynaptic proteins is to the networks that mediate plasticity. The site of contact between antigen-presenting cells and T cells is referred to as the immune synapse, in analogy to the neuronal synapse. Both are highly organized and dynamically regulated sites where two cells communicate. Activation of T cell receptor (TCR) signaling involves the reorganization of the receptor into multiprotein signaling complexes that recruit downstream signaling molecules forming clusters. The organization of the immune synapse has been extensively studied because it can be recapitulated *in vitro*. Faeder and Linding both nominated a study by Su *et al.* [(7); see associated commentary (8)] that used an *in vitro* system with model membranes and 12 components of the TCR signaling pathway to explore the phosphorylation-dependent assembly of the transmembrane protein LAT (linker for activated T cells) and its binding partners. The authors found that their assembly resulted in a phase separation that served as the driving force for the formation of liquid-like clusters that promoted downstream signaling, including mitogen-activated protein kinase (MAPK) signaling

(Fig. 2). The idea that proteins can organize dynamically into ordered liquid-like macromolecular structures to promote signal transduction has been theorized, and this study provides clear experimental support for this model of phase transition-mediated signal propagation within the cytosol. As Faeder explained, “This paper uses the combination of an *in vitro* reconstruction assay using recombinant proteins and experiments in Jurkat T cells to show how multivalent interactions involving LAT and other signaling molecules can give rise to microclusters that are essential for T cell activation. These experiments are important for several reasons: (i) they demonstrate that multivalent protein-protein interactions at the plasma membrane can nucleate key signaling complexes that are necessary for signaling processes and that have up to now been hypothesized but not directly confirmed; (ii) they provide a mechanistic explanation for the phenomenon of TCR microclusters that have not been adequately explained up until now; (iii) they confirm predictions of theory and modeling (9) that such interactions lead to the formation of a distinct clustered phase and that the propensity of this phase to form would...exhibit a biphasic response with respect to the amount of input signal; (iv) the novel reconstitution systems that the Vale lab has pioneered the use of to study TCR signaling offer a powerful new tool for the systematic study of receptor-mediated signaling.” He further noted that another study by Huang *et al.* (10) used a similar reconstitution system to provide an additional mechanistic basis for promotion of MAPK signaling by LAT clustering.

Productive T cell activation depends not only on the stimulation of the TCR by its cognate antigen but also on costimulatory signals. CD28, which colocalizes with the TCR in the immune synapse, is a well-studied costimulatory protein; the mechanism whereby CD28 transmits costimulatory signals to T cells, however, remains controversial. K. Okkenhaug nomi-

inated a pair of papers that shed light on the role of RLTPR (RGD, leucine-rich repeat, tromopomodulin, and proline-rich-containing protein), a cytosolic protein previously implicated in CD28 signaling (11), in lymphocyte function. Wang *et al.* (12) found that people lacking RLTPR were immunodeficient, with CD4^+ T cells that failed to respond to CD28 stimulation and with B cells that had dysfunctional B cell receptor signaling. Roncagalli *et al.* (13) showed that RLTPR acts as an adaptor to link the CD28 C-terminal to the CARD11/CARMA1 complex and activation of nuclear factor κB (NF- κB), a transcription factor stimulated in activated T cells. In making his nomination, K. Okkenhaug noted, “More work is needed to map the precise molecular interactions, but this body of work finally defines a unique CD28-dependent signaling pathway in T cells.”

A deeper understanding of the signaling pathways by which cancer cells evade destruction by the immune system can be expected to lead to more effective approaches to cancer immunotherapy. One such mechanism involves the interaction between PD-1 (programmed cell death protein 1), a receptor found on T cells, and PD-L1 (programmed death ligand 1), found on macrophages and dendritic cells, as well as various cancer cells. Engagement of PD-1 by tumor cell PD-L1 stimulates immune checkpoint signaling, leading to T cell inhibition and suppression of the immune response, thereby providing a mechanism whereby the tumor cells can escape the immune response. L. Ma nominated a pair of papers that explored mechanisms involved in regulation of PD-L1 stability and thereby abundance (14, 15). Various forms of cancer are associated with chronic inflammation, and a study by Lim *et al.* (14) investigated how the proinflammatory cytokine tumor necrosis factor- α (TNF- α) promoted PD-L1 stability in cancer cells. As L. Ma explained, “[The authors] showed that the macrophage-secreted inflammatory cytokine TNF- α upregulates PD-L1 expression in breast cancer cells by inducing the

expression of the fifth component of the mammalian COP9 signalosome complex (CSN5), leading to deubiquitination and stabilization of PD-L1." Inhibiting CSN5 with curcumin, a compound derived from turmeric, decreased PD-L1 abundance and sensitized mouse models of several cancers to immune checkpoint antibody therapy. In the second paper, by the same research group, Li *et al.* (15) studied the regula-

tion of PD-L1 abundance by N-glycosylation and ubiquitination.

As the name suggests, ubiquitin—and ubiquitination—is found in all eukaryotic cells and has been implicated in the regulation of nearly all cellular processes. Ubiquitination involves a three-enzyme cascade, in which ubiquitin is activated by the E1 enzyme, in a process that requires ATP; activated ubiquitin is transferred to the E2 ubiquitin-conjugating enzyme and finally covalently linked to the substrate by the E3 ubiquitin ligase. Although prokaryotic signaling does not use ubiquitination, various bacterial pathogens have evolved effector proteins, including deubiquitinases and E3 ubiquitin ligases, that modulate ubiquitin signaling in their eukaryotic targets (16–19). Dikic nominated a paper by Qiu *et al.* (16) revealing an unexpected addition to the pathogen toolbox mediated by a parallel ubiquitin pathway independent of ATP and the E1 and E2 enzymes. Rather, the *Legionella pneumophila* SdeA effector protein catalyzes an NAD⁺ (nicotinamide adenine dinucleotide)-dependent ubiquitination process, in which ubiquitin undergoes adenosine 5'-diphosphate (ADP) ribosylation before its transfer to host Rab proteins. Following up on this surprising development, Bhogaraju *et al.* (19) determined that SdeA catalyzed the ubiquitination of target proteins on serine residues (rather than lysines, as in the classical ubiquitination pathway) and that ADP-ribosylated ubiquitin was converted to phosphoribosylated ubiquitin, which inhibited the eukaryotic polyubiquitination cascade.

Whereas the receptors of T cells and B cells are exquisitely tuned to specific antigens, pattern recognition receptors, such as those in the Toll-like receptor (TLR) family, sense less specialized "danger signals" that arise in the context of pathogen invasion or tissue injury. Janes nominated a study by Piccinini *et al.* (20) investigating how a signal associated with bacterial infection and one associated with

tissue injury act through the same TLR to elicit completely different macrophage responses (Fig. 3). As Janes explained, "[The] work provides an interesting analysis of how two completely different ligands (lipopolysaccharide and tenascin-C) signal downstream of a common surface receptor (TLR4). The authors report striking differences in cytokine secretion and metalloproteinase activation...which are accompanied by more quantitative and time-dependent differences in canonical TLR4 signaling (p38, JNK, and NF- κ B). It is intriguing to think about how qualitatively different outcomes may be [determined] by the magnitude and kinetics of TLR4 signaling or by crosstalk with other receptors that recognize individual TLR4 ligands. Interesting signaling questions remain for matricellular proteins such as tenascin-C and biglycan, which also acts as a ligand for TLR4."

Although pattern recognition receptors provide an important mechanism for alerting cells of the immune system to dangerous conditions, the immune system may also sense and respond to conditions associated with cellular stress signals, such as amino acid starvation or endoplasmic reticulum stress. S. Miller nominated research by Ravindran *et al.* (21) showing that, during amino acid starvation, the kinase GCN2 (general control nonderepressible 2), a component of the integrated stress response that senses amino acid deficiency, inhibits activation of the inflammasome to suppress gut inflammation. Moreover, mice fed a low protein diet (to elicit the amino acid starvation response) were partially protected from colitis; as S. Miller noted, "This breakthrough allows a precedent for the effects of specific diet on intestinal inflammation." This connection between nutrient deficiency, induction of the integrated stress response, and inhibition of the inflammasome is unexpected, because many other stresses that stimulate the integrated stress response activate inflammatory signaling, including the inflammasome. Amino acids are critical activators of mTORC1 (mechanistic target of rapamycin 1), a protein complex that regulates cell growth in response to nutrient availability and growth factor signaling. 2016 was the year that two amino acid sensors for mTORC1 were identified: one for arginine and one for leucine. In the first of two nominations, Perrimon proposed a study by Chantranupong *et al.* (22) identifying a previously uncharacterized protein the authors renamed CASTOR1 (cellular arginine sensor for mTORC1) as an arginine sensor crucial to arginine-dependent mTORC1 activation (22–24). In the absence of arginine,

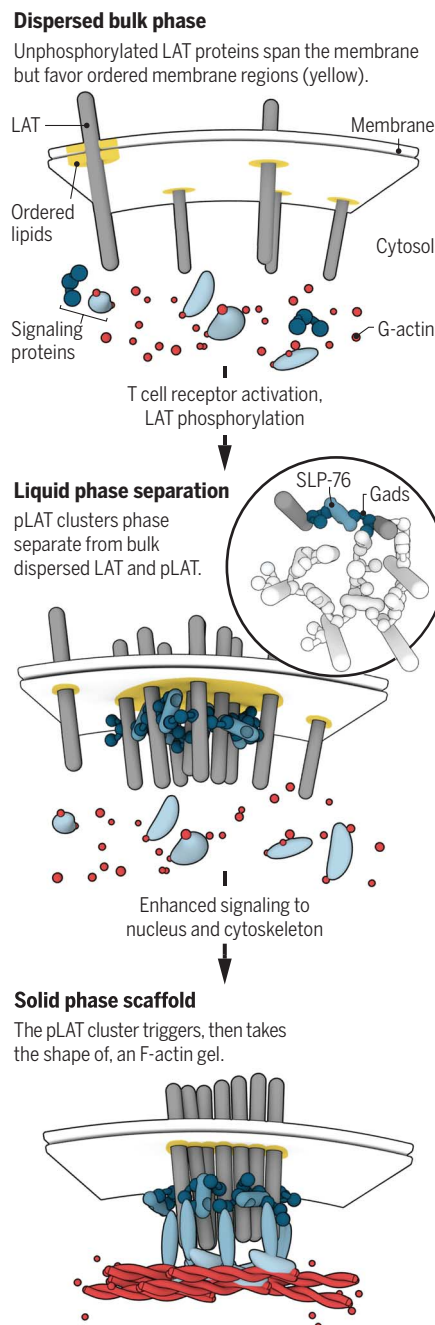


Fig. 2. The formation of liquid-like clusters promotes downstream signaling from TCRs. [Reprinted with permission from Dustin and Muller (8)]

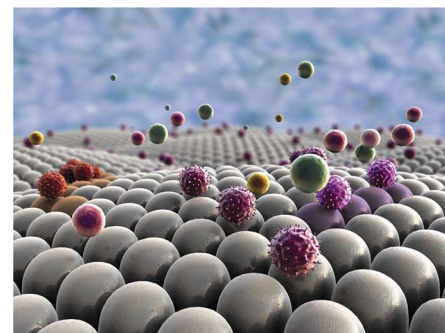


Fig. 3. Macrophages can produce specific signals from the same receptor.

CASTOR1 binds to the GATOR2 protein complex to inhibit mTORC1; arginine binds to CASTOR1, disrupting its interaction with the GATOR2 protein complex, and thereby activating mTORC1. This research group also identified the cytosolic protein SESTRIN2 as a leucine sensor involved in mTORC1 regulation. Like CASTOR1, SESTRIN2 interacts with GATOR2 in the absence of its amino acid ligand to inhibit mTORC1 (25, 26), suggesting that the GATOR2 complex represents a key hub in mTORC1 regulation by amino acids.

When mTORC1 is inhibited by amino acid deprivation, autophagy is induced. This catabolic response to cell stress provides molecular building blocks to help cells cope with nutrient deficiency. Glucose starvation also induces autophagy. The disaccharide trehalose can stimulate cellular autophagy through a mechanism independent of mTORC1 signaling, a property that may be beneficial in mitigating various neurodegenerative conditions associated with pathological protein aggregation (27, 28). An intriguing study by DeBosch *et al.* (27), nominated by the *Science Signaling* editors, provided a mechanism for trehalose-dependent autophagy, showing that trehalose promoted AMPK (adenosine 5'-monophosphate-activated protein kinase)-dependent hepatocyte autophagy and mitigated hepatic steatosis by inhibiting glucose uptake by SLC2A family transporters.

Many crucial metabolic processes, including β -oxidation of fatty acids, the tricarboxylic acid cycle, and oxidative phosphorylation, take place in the mitochondria (Fig. 4). Obtaining a quantitative analysis of mitochondrial metabolites under various conditions—or in response to perturbations associated with signaling—has been challenging, because of the small size of mitochondria relative to total cellular volume and the challenges of rapidly and specifically isolating intact mitochondria from cells. Perrimon's second nomination was for a study

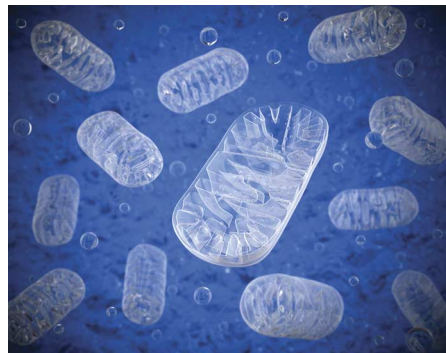


Fig. 4. Isolated mitochondria reveal their metabolic secrets.

by Chen *et al.* [(29); see associated commentary (30)] that used epitope tagging to rapidly and specifically isolate mitochondria, combined with mass spectrometry analysis using a library of all predicted mitochondrial metabolites, to quantify mitochondrial matrix metabolites under various conditions. As Perrimon noted, "This method will help compare mitochondria in different conditions including disease states." Moreover, the general approach should be applicable to the study of other organelles that influence—or are influenced by—cell signaling.

Last year's edition of *Signaling Breakthroughs of the Year* featured a series of papers investigating the evolution, identity, and structure of the receptors that enable the parasitic weed *Striga* to locate hosts by detecting minute amounts of strigolactones, a class of plant hormones involved in growth and development. The strigolactones and their receptors make a reappearance for this year's final signaling breakthrough, in a pair of studies nominated by the *Science Signaling* editors that revealed a most unusual mechanism of interaction between strigolactone and the DWARF 14 (D14) strigolactone receptor (31–33). Whereas most hormones bind reversibly and noncovalently with their cognate receptors, Yao *et al.* (31), investigating the *Arabidopsis* receptor, and de Saint Germain *et al.* (32), investigating the pea receptor, found that D14 hydrolyzes strigolactone to create its own ligand. The active ligand then binds covalently to the D14 receptor, triggering a major conformational change that enables it to trigger downstream strigolactone signaling.

We thank all of the scientists who provided nominations, making *Signaling Breakthroughs of the Year* possible, and T. Dietzel and A. Mushegian for their help with compiling the papers.

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Perspective

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