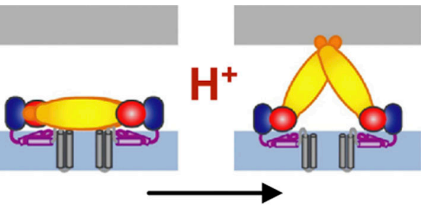
In This Issue



The fusion protein (yellow, red, blue) on the viral membrane (pale blue) changes shape at low pH and attaches to the endosome membrane (gray). The pH sensor for this first step of fusion is His323.

Flavivirus reveals its access code

Fritz et al. have identified an amino acid switch that flaviviruses flip to gain access to cells.

Flaviviruses such as tick-borne encephalitis virus (TBEV), yellow fever, and dengue are dangerous human pathogens. These membrane-encircled viruses enter cells by being gobbled up into endosomes and fusing their membrane with that of the endosome.

Fusion is triggered by the endosome's acidic environment. Low pH prompts the aptly named fusion protein, on the virus's outer membrane, to change shape and grab hold of the endosome membrane, bringing the two membranes together. In their search for possible pH sensors, researchers have focused on five highly conserved histidine residues in the flavivirus fusion protein. The chemical properties of histidines make them prime candidates—they switch from uncharged to having a double positive charge upon acidification of their environment, such as that in endosomes.

Fritz et al. replaced each of the five histidines of the TBEV fusion protein with alternative residues and observed the virus's fusion ability. Given the conservation of the five histidines, the team was surprised, that mutation of one of the histidines, His323, was sufficient to completely abolish fusion. Individual mutation of three of the others had no effect on fusion whatsoever, and mutation of the fourth led to an untestable ill-formed fusion protein. The team went on to show that mutation of the crucial His323 interfered with the pH-induced shape change of the fusion protein.

Fritz, R., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806081.

AHNAK	AHNAK nucleoprotein isoform 1	54.5	28.6	55.6
ALB	Albumin	100.0	45.5	57.1
ANXA1, 2	Annexin 1, A2	100.0	100.0	77.8
ASCC3L1	Activating signal cointegrator 1 complex subunit 3-like 1	63.6	0.0	22.2
ASS1	Argininosuccinate synthase	0.0	57.1	55.6
ATAD	ATPase family, AAA domain containing protein	27.3	0.0	22.2
ATP5A, 5B	ATP synthase, H+ transporting, mitochondrial F1 complex	81.8	14.3	77.8
BAG2	BCL2-associated athanogene 2	27.3	0.0	11.1
BOLA2B	BolA-like protein 2B	22.2	36.4	28.6
CAD	Carbamoylphosphate synthetase 2	0.0	85.7	33.3
CAND1	Cullin-associated NEDD8-dissociated protein 1	9.1	28.6	33.3
CAPRIN1	Cytoplasmic activation- and proliferation-associated protein 1	33.3	45.5	0
CCT	Chaperonin containing TCP1	45.5	28.6	55.6
CD180	Elongation factor 1-alpha	45.5	57.1	33.3
CFL1	Cofilin	63.6	85.7	66.7
CHD3, D4	Chromodomain-helicase-DNA-binding protein 3, 4	18.2	0.0	11.1
CLEC2D	C-type lectin domain family 2, member D	36.4	0.0	0.0
CLTC	Cathrin heavy chain 1	36.4	85.7	77.8
COPA, B1	Coatomer protein complex, subunits A, B1	18.2	28.6	44.4
CORO1C	Coronin, actin binding protein 1C	66.7	27.3	14.3
CPS1	Carbamoyl-phosphate synthetase 1	54.5	71.4	44.4
CRKL	Crk-like protein	22.2	9.1	28.6
CSDA	Cold shock domain-containing protein A	55.6	36.4	71.4
CSRP2	Cysteine and glycine-rich protein 2	54.5	28.6	11.1
DBN1	Drebrin 1 (developmentally regulated brain protein)	66.7	36.4	28.6
DHRS2	Dehydrogenase/reductase (SDR family) member 2	36.4	14.3	22.2
DUT	dUTP pyrophosphatase	22.2	18.2	14.3
DYNLL1	Dynein light chain 1	27.3	28.6	11.1
EDARRAD	EDAR-associated death domain	9.1	0.0	66.7
ELAVL1	ELAV-like 1	63.6	0.0	22.2
EMD	Emerin	36.4	28.6	0.0
ENO	Enolase 1	9.1	14.3	77.8
EWSR1	Ewing sarcoma breakpoint region 1	27.3	14.3	33.3
FARSA,B,FASN	Phenylalanyl-tRNA synthetase; Fatty acid synthase	36.4	85.7	88.9
FBL	Fibrillarin	63.6	14.3	22.2
FKSG30	FKSG30	36.4	57.1	66.7
FUS	Fus-like protein	36.4	14.3	33.3

Part of the long blacklist of proteins that repeatedly gatecrash IP experiments.

IP blacklist

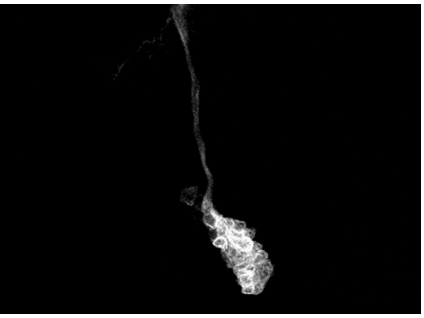
Like gatecrashers at a celebrity party, there are proteins that turn up in immunoprecipitation (IP) experiments despite having no real association with the A-list protein of interest. But now, researchers-cum-bouncers, Trinkle-Mulcahy et al. are armed with a list of repeat gatecrashers to look out for.

One way to lessen the chance of uninvited proteins turning up in your IP is to increase the stringency of purification methods; however, this is also likely to remove low affinity, low abundance and yet genuine interaction partners. To keep stringency low but cope with the large number of co-precipitating proteins, researchers have developed a mass spectrometry approach called SILAC (stable isotope labeling with amino acids in culture) that identifies and quantifies all precipitated proteins.

Trinkle-Mulcahy and colleagues now describe an optimized methodology for the technique with the important addition of a "bead proteome"—a blacklist of proteins that bind nonspecifically to the three most commonly used affinity matrices: magnetic, sepharose, and agarose beads. The authors identified the culprits by examining all proteins that repeatedly precipitated at similar quantities in test and control samples.

If a protein appears on the list, it should not be automatically discounted—these gatecrashers have parties at which they are genuine invitees. And of course, proteins not on the list should not be assumed to be bona fide, either. The authors have so far compared commonly observed nonspecific binding proteins for two different cell types, three different matrices, and one affinity tag, and so are quick to point out that the blacklist is only a guide. That said, with the time and expense that goes into confirming possible interaction partners, the blacklist now enables researchers to focus their attentions on the most likely VIPs of IP.

Trinkle-Mulcahy, L., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200805092.



A central brain neuron grows a perfectly good axon even in the absence of APC.

APC ain't always necessary for axons

Like many neuroscientists, Rusan et al. considered that the mechanism controlling axonal positioning and outgrowth might be the same in all neurons. As they now show, however, one neuron's must-have axon-promoting protein is virtually dispensable in other neurons.

In a young neuron, multiple mini neurites protrude until one is chosen to become the axon. A microtubule-associated protein called APC was thought to be required for axon outgrowth, as dominant-negative APC expression in cultured neurons reduced axon growth dramatically. Other reports had suggested that APC delivers a polarity protein called Par3, necessary for axon growth, to the chosen neurite.

Despite the strong evidence for APC's axon-promoting role, definitive knockout experiments in neurons were limited to one study, which showed that medulla neurons behaved much as expected—no APC, no axon extension. Now, the same lab have found that neurons from the central brain, mushroom body, and lobular plug will happily grow axons without APC. Cells from these parts of the brain were

Text by Ruth Williams
ruth.williams@rockefeller.edu

not entirely normal: precursor neuroblasts had slightly longer cell cycles and some had bent spindles. Though, neither problem prevented mitosis.

The need for APC in some neurons but not others seems to reflect a requirement for Wnt signaling. As well as its cytoskeletal role, APC is a downstream regulator of Wnt, and while medulla neurons need Wnt for axon growth, the other types of neurons did not. It will be of interest to check whether hippocampal neurons, where the dominant-negative experiments were carried out, are also Wnt responsive.

Rusan, N.M., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200807079.

Astrocyte's clean up job

By mopping up excess neurotrophic factor from neuronal synapses, astrocytes may finely tune synaptic transmission to affect processes such as learning and memory, say Bergami et al.

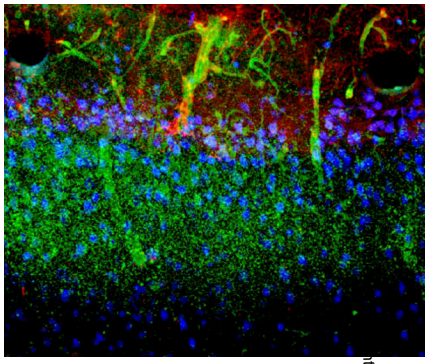
The major cellular events of learning and memory are long-term potentiation (LTP) and long-term depression (LTD), both of which affect neurons' ability to communicate with one another. Neurons that have undergone LTP display a stronger electrical response to the same level of a stimulus, whereas neurons that have gone through LTD display a weaker response. These changes are thought to result from modifications of the neuronal synapses, such as alterations in the density of postsynaptic receptors, or downstream signaling events.

Secretion of the neurotrophic factor BDNF (brain-derived neurotrophic factor) has been implicated in long-term synaptic modification, and the function of BDNF on synaptic strength depends on its particular form: in its pro-BDNF form it is believed to promote LTD, and in its mature form it prompts LTP. Neurons were thought to secrete pro-BDNF, which then matured into BDNF in the synaptic space. However, a recent study suggests that only mature BDNF is secreted, pro-BDNF being processed intracellularly.

To get to the bottom of things, Bergami et al. investigated the fate of both forms after LTP induction in brain slices from the rat cortex. By fluorescent immunohistochemistry they showed that that neurons indeed secrete both mature and pro-BDNF, but that a large amount of the pro-BDNF is immediately taken up by astrocytes.

Astrocytes, previously thought to be unimportant in neuronal transmission, have recently been implicated in long-term modulation of neuronal synapses. For example, they release the neurotransmitter glutamate into the synapse prompting LTP. By specifically mopping up pro-BDNF, astrocytes seem to have another means to assist in LTP. However, while it's likely that most pro-BDNF gets degraded inside astrocytes, say the authors, some gets recycled and re-released, suggesting that astrocytes in fact fine-tune synaptic plasticity.

Bergami, M., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806137.



Upon LTP, neurons (blue) release pro-BDNF (green), much of which gets mopped up by astrocytes (red).

Microdomain switching is a bad move for APP

Amyloid precursor protein (APP), whose cleavage product, amyloid-β (Aβ), builds up into fibrous plaques in the brains of Alzheimer's disease patients, jumps from one specialized membrane microdomain to another to be cleaved, report Sakurai et al.

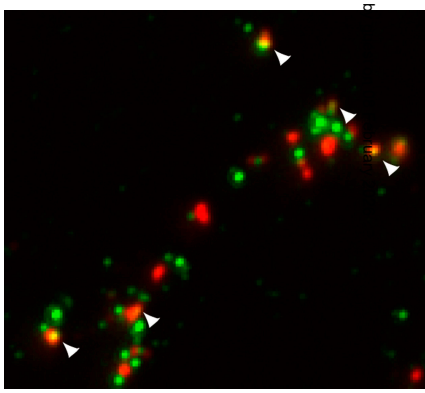
Although there is no definitive evidence that Aβ plaques are the direct cause of Alzheimer's disease, there is much circumstantial evidence to support this. And working on this hypothesis, scientists are investigating just how the plaques form and what might be done to stop or reverse their formation.

APP, a protein of unknown function, is membrane associated and concentrates at the neuronal synapse. Certain factors such as high cellular cholesterol and increased neuronal or synaptic activity are known to drive APP cleavage, and Sakurai and colleagues' paper pulls these two modes of Aβ regulation together.

APP associates with membrane microdomains high in cholesterols (lipid rafts). These lipid rafts can also contain the enzyme necessary for APP cleavage, BACE. Synaptic activity is known to involve a very different type of membrane microdomain high in an excytosis-promoting factor called syntaxin. Sakurai et al. now show that although APP preferentially associates with syntaxin microdomains, upon neuronal stimulation APP instead associates with microdomains that contain BACE.

It's unclear why APP should be associated with syntaxin, though it might suggest a role for APP in vesicle trafficking and exocytosis. Also unclear is why neuronal activity should cause APP to jump from syntaxin domains to BACE domains. What is clear, however, is that the process is an active one, requiring a kinase called cdk5. Furthermore, treating neurons with a cdk5 inhibitor called roscovitine, which is currently in trials for cancer treatment, reduced APP's association with BACE microdomains and reduced APP cleavage.

Sakurai, T., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200804075.



APP (green) in the safety of syntaxin domains (red). This association is disrupted upon high neuronal activity and leads to APP's association with and cleavage by BACE.