experiments. That repeatedly gatecrash IP

Part of the long blacklist of proteins of fusion is His323.

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

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 unstable 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.

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.

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
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.

**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 cholesterol (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 excitosis-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.