

# The No-Brainer That Wasn't

A SURPRISING RESULT POSES NEW QUESTIONS ABOUT THE ROLE OF AN "ESSENTIAL" BRAIN PROTEIN.

Imagine your mechanic yanked the engine out of your car. You buckle up, turn the ignition, and off you drive, undoubtedly with a shattered notion of how automobiles work. That's essentially what researchers led by HHMI investigator Pietro De Camilli experienced earlier this year when they eliminated a brain protein from mice thought to be an engine for transmitting nerve impulses.

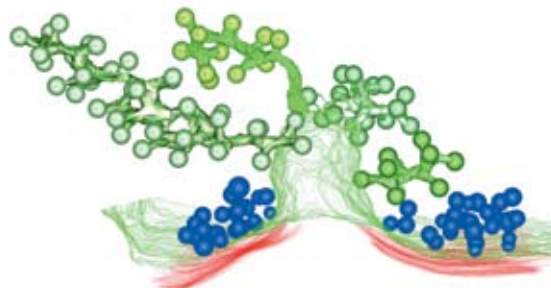
The Yale University School of Medicine team and their colleagues knocked out the *dynamain 1* gene, which encodes a protein implicated in pinching off budding synaptic vesicles from the plasma membrane of neurons—a process that recycles these vesicles once they have released their neurotransmitter content. Work in fruit flies and other species had led neurobiologists to presume dynamain 1 played an essential role.

But nobody had performed the definitive test—removing dynamain 1 from mice—to see what happened. “We thought it would be a no-brainer—no dynamain 1, no synaptic vesicles,” says De Camilli. Astoundingly, when his team created dynamain 1 knockout mice, the newborns appeared normal and lived for up to two weeks, as reported in the April 27, 2007, issue of *Science*.

Microscopic examination revealed that the neurons of the knockout mice contained plenty of synaptic vesicles, though they tended to be larger and less uniform in size, and numerous budding vesicles stayed attached to the plasma membrane in grape-like

clusters. Electrophysiology and other biophysical tests showed that the neurons behaved almost perfectly under normal stimulation, failing only under strong electrical stimulation.

Because mammals have two additional dynamain genes, producing slightly different forms of the protein, De Camilli suspects that one or both of these variants may compensate for dynamain 1 in the knockout mice. But given that these variants together represent less than 10 percent of the total dynamain in the brain, he wonders whether dynamain in any form is truly essential for synaptic transmission. The De Camilli lab plans to delete the other two forms to find out. ■ —PAUL MUHLRAD



A lack of dynamain 1 (green) causes synaptic vesicles (blue) and endocytic buds to arrest at the pre-fission stage, as revealed here by electron tomography.

## IN BRIEF

Researchers initially believed that analyzing a cross section of mutations in HIV could identify those that had arisen as a result of immune escape and pinpoint viral genome regions important for recognition by T cells.

HHMI investigator Bruce D. Walker at Harvard Medical School and others have now established that the accuracy of this type of analysis can falter because of the many HIV subtypes that circulate globally. Some of the mutations represent historical subtype or lineage differences rather than mutations that have arisen as a result of immune selection pressure.

The scientists found that identifying the presence of these multiple HIV lineages can greatly improve the accuracy of genetic analyses. Furthermore, statistical methods for elucidating such phylogenetic relationships among viral genome sequences will give virologists new insights into the evolution of viruses and how viruses mutate as they adapt to the immune system.

Their findings were published March 16, 2007, in *Science*.

### GENETIC "GANG OF FOUR" LINKED TO BREAST CANCER

Research by HHMI investigator Joan

Massagué at the Memorial Sloan-Kettering Cancer Center and colleagues suggests that abnormal activation of four genes drives the spread of breast cancer to the lungs. Their work, published April 12, 2007, in *Nature*, reveals that the aberrant genes work together to promote the growth of primary breast tumors, enabling cancerous cells to escape into the bloodstream and penetrate through blood vessels into lung tissues.

The researchers focused on genes coding for proteins called epiregulin, COX2, and matrix metalloproteinases 1 and 2. Various combinations of the four genes in human breast cancer cells that had metastasized to the lung were silenced by a technique called RNA interference. The cells were then tested in mice.

Silencing all four genes greatly reduced the tangled blood vessel growth typically seen in tumors. The tumor blood vessels that did form allowed fewer cancer cells to escape into circulation. But when these cells reached the lung capillaries, they got stuck. From this, Massagué's team concluded that these genes act to loosen up capillaries, allowing the cells to penetrate the lung and grow there.

### BEING BAD IS BEST FOR BACTERIA

Defying the old assumption that pathogens evolve to become less infectious, research by HHMI international research scholar B. Brett Finlay at the University of British Columbia, Vancouver, suggests that genes enhancing the virulence of bacteria are clearly favored for evolutionary survival.

Finlay's team created a model of natural selection that demonstrates how type III secretion system genes, a molecular complex that many virulent bacteria use to infect mammalian cells, contribute to an organism's fitness.

Using a strain of mouse that dies from infection by *Citrobacter rodentium* bacteria, the team exposed groups of mice either to the normal pathogen or to versions in which different type III secretion genes had been eliminated. The infected mice were exposed to uninfected mice, which in turn were exposed to more uninfected mice, thus producing various degrees of pathogenicity, ranging from mild to fatal. Bacterial strains with the greatest damage to their virulence genes were slowest at spreading from one host to another, suggesting that these swapped virulence genes are essential for spreading. The bacterial strain with all its virulence genes intact spread the fastest.