In one study, also published online on 24 October in *Annals of Neurology*, Schiff and colleagues examined EEG recordings taken from 44 people who had severe brain injury and found that four patients who showed very little or no responsiveness nevertheless had EEG patterns that looked similar to those of healthy controls. When the researchers tested a subset of the people on an fMRI test similar to the tennis task used in Owen's original study, only the four who had more normal-looking EEGs were able to communicate.

Owen points to another recent study, on which he was a co-author, as support for the potential of EEG recordings. A research group led by Srivas Chennu of the University of Cambridge performed a complex mathematical analysis on the EEG signals of healthy and vegetative people to determine how well their different brain regions were working together. In three of the 32 brain-injured patients, the EEG analyses resembled those of healthy people, the team reported on 16 October in *PLOS Computational Biology*. Follow-up studies showed that all three of those patients were conscious and able to communicate with researchers through the fMRI technique that involves imagining playing tennis.

These findings suggest that EEG may work "much better" as a screening tool for locked-in patients than fMRI, which has been shown to miss telltale signs of awareness in many patients, Schiff says. Ultimately, he adds, the goal is to come up with a bedside EEG screening test that could be administered in just 30 minutes. If patients passed the EEG test, physicians might be able to help them communicate by monitoring their brain activity with fMRI, he says.

Cheap and practical solutions such as EEG "have to be the answer" in the long term, Owen agrees, although they will need refining to detect levels of consciousness that lie somewhere between a vegetative state and full awareness. One promising approach, he says, is to apply strong magnetic pulses to the brain and use EEG to measure the electrical "echo" that comes back. Marcello Massimini, a neuroscientist at the University of Milan in Italy, has recently found that these echoes are highly complex in healthy people. But in people with severe brain damage, the echoes often fail to propagate throughout the brain, or they create uniform "ripples" of activity that don't convey much information, "like dropping a stone in a pond," Massimini says.

As EEG proves its value as a diagnostic tool for locked-in patients, Schiff says that one advantage could be decisive: "You can get an EEG test anywhere in the world." ■

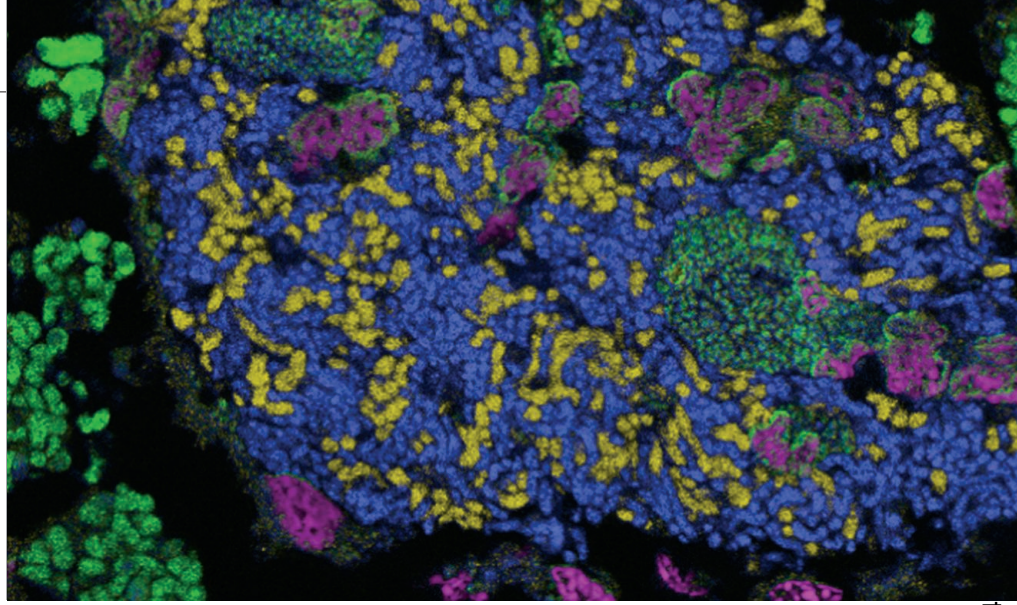


IMAGE: JAMES VAN LEUVEN AND JOHN MCCUTCHEON, PUBLISHED IN *CELL* 158:1270–1280

MICROBIOLOGY

Modern symbionts inside cells mimic organelle evolution

Long-term partnerships can result in extremes in genome reduction or expansion

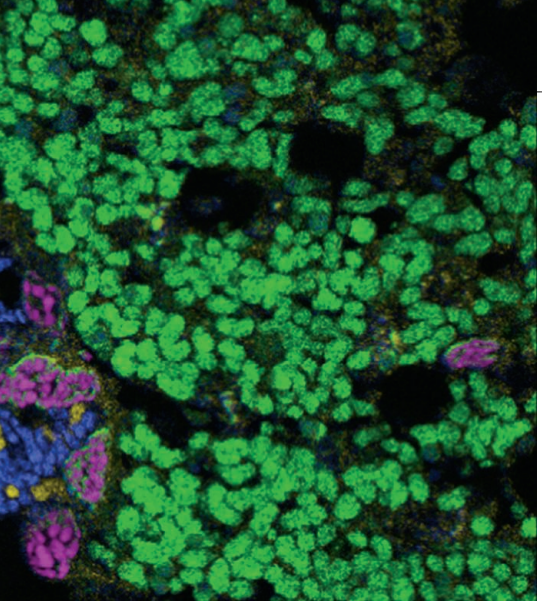
By **Elizabeth Pennisi**, *in Irvine, California*

Some 2 billion years ago, primitive cells took in guests—and life was changed for good. A once free-living bacterium took up residence in a cell and gave rise to the organelles called mitochondria, which generate energy for their host cells by oxidizing sugars and also equipped some early life to survive Earth's increasing levels of oxygen. Another intracellular guest microbe became the ancestor of chloroplasts, the photosynthesizing organelles that made plants and algae possible.

Those momentous events, investigators are realizing, were not one-of-a-kind. Endosymbiosis, as a mutually beneficial relationship between an organism and a guest living inside its cells is called, is alive and well today, and has lessons to offer for how the process played out early in life history. "The line separating endosymbiont from organelle is very unclear indeed," said John McCutcheon, a biologist at the University of Montana, Missoula, earlier this month at "Symbioses becoming permanent," a meeting held here by the National Academy of Sciences and the Canadian Institute for Advanced Research.

At the meeting, biologists described how they are exploring those parallels. They are probing how insects such as cicadas and other multicellular organisms set up partnerships inside their cells with microbes that, like their ancient forerunners that became organelles, help provide essential nutrients and other services. Along the way, they are learning about the complex bargains host cells strike with their microbial partners—trade-offs that can explain some of the features of mitochondria and chloroplasts today.

Although DNA studies have convinced biologists that mitochondria evolved just once, from a type of microbe called an α-proteobacterium, the organelles have diversified wildly since then. Whereas modern free-living relatives of this bacterium harbor about 2000 genes across several million bases, its mitochondrial descendants all have far fewer genes—sometimes as few as three—and a wide range of genome sizes and shapes. The smallest mitochondrial genome is just 6000 bases long; human mitochondrial DNA stretches 16,000 bases. Some plants, in contrast, greatly expanded the genomes of their mitochondria, padding them with apparently inessential DNA: The biggest known, at 11 million bases, belongs to a flower called *Silene*, and it's divided into many circular chromosomes, some of which have no genes at all on them. Mitochondria have "been an endless reservoir of unconventional genomes," says David Smith, an evolutionary biologist at the University of Western Ontario in London, Canada. "They break all the rules."



Inside a specialized cicada organ, one endosymbiotic microbe has split into two species (yellow and blue). They are surrounded by a third (green). Insect nuclei are purple.

But many modern endosymbionts break those same rules, McCutcheon noted at the meeting, drawing on his studies of cicadas. These sap-sucking insects derive amino acids missing from their diet from bacterial partners that reside in specialized cells. The cicada *Diceroprocta semicincta*, for example, harbors two such partners. One, called *Hodgkinia*, supplies the insect with two amino acids it cannot provide on its own, while the other, *Sulcia*, provides another eight. In another cicada species, however, *Hodgkinia* has doubled the amount of its DNA, diverged into two distinct genomes, and divided up the task of supplying the two amino acids, McCutcheon and his colleagues reported at the meeting and online on 28 August in *Cell*. One *Hodgkinia* provides some of the genes and the other fills in the gaps in amino acid production, making both "species" essential to the endosymbiosis.

McCutcheon's team has more recently looked at the endosymbionts of a *Magicicada* cicada, which emerges on a 13- or 17-year cycle and may harbor scores of distinct *Hodgkinia* genomes, some that seem to carry very few or no functional genes. The finding parallels what happened to the plant mitochondrial genome in *Silene*, McCutcheon suggests. It seems that in both these cases, unchecked mutation rates and DNA amplification led to greatly expanded but marginally functional, fragmented genomes. "When things go wonky, they really go wonky," Smith says.

The cicada's unusual lifestyle—long dormancy, followed by a brief burst of activity—may play a role in this "genomic insanity," McCutcheon proposes. The species with a single *Hogkinia* genome can take 3 years to fully develop, and the one with many *Hodgkinia* genomes can take 17. Although cicada nymphs are basically dormant during most of that long cycle, the endosymbionts might be free to replicate with no survival pressures acting to keep their genomes stable. "Some cicada life histories seem to allow slop and chance to take over, or maybe just slop," McCutcheon told the audience.

Investigators are also examining modern analogs to the complex evolution of the chloroplast, which seems to have emerged once, but then was lost and regained in different ways in various modern photosynthetic organisms. The photosynthetic organelle was originally a cyanobacterium that was engulfed by a eukaryote. Plant biologists have long recognized that in some branches of the plant and algal family tree, this initial chloroplast was lost, but a new one was acquired when a host cell swallowed up an alga that in turn had its own chloroplast.

At the meeting, Patrick Keeling, a protistologist at the University of British Columbia, Vancouver, in Canada, described his studies of a more recent example of this process, called tertiary endosymbiosis. Some dinoflagellates, single-cell aquatic protists, no longer have their original chloroplast, relegating some of its light-sensing apparatus to a cellular component dubbed an eyespot. But they have replaced it by taking in a diatom, a single-celled alga that has its own photosynthetic machinery. The dinoflagellate still carries the diatom's nucleus and mitochondria, but "I would challenge anyone to say this is not an organelle," Keeling says.

> ***"The line separating endosymbiont from organelle is very unclear indeed."***
>
> **John McCutcheon**, University of Montana, Missoula

The meeting highlighted other similarities between endosymbionts and organelles. Mitochondria are typically passed only from the mother to offspring, and some endosymbionts similarly depend on maternal transmission, dwelling in eggs and perhaps even promoting female progeny over male to perpetuate themselves, says Steve Perlman of the University of Victoria in Canada.

All these results "make organelles not so special," says W. Ford Doolittle, a molecular evolutionary biologist emeritus at Dalhousie University in Halifax, Canada. Embracing endosymbionts as good models for the evolution of organelles makes for "an interesting paradigm shift in the field." ■