

VIROLOGY

Evolution on Life's Fringes

Fresh evidence that viruses have existed for billions of years has scientists wondering what role these stripped-down microbes played in evolution

TOURTOUR, FRANCE—In the beginning, there was the land, the sky, and a sterile sea. After hundreds of millions of years in that primordial soup, molecules with carbon backbones began replicating and eventually begat cells. The cells were fruitful and multiplied, giving rise to life's three domains: Bacteria, Archaea, and Eukarya.

This creation story, or variations on the theme, is an article of faith to most scientists. But there is one embarrassing omission. Where did viruses come from? Most scientists agree that viruses are life-forms. They are not cells, but they have their own DNA or RNA genomes and can reproduce with the unwilling help of a cellular host. Yet although viruses are able to hijack organisms of all sorts, they have long been consigned to a taxonomic ghetto that had little to do with the origins of the three domains.

Earlier this summer, two dozen scientists gathered here in southern France's Var region, at the forested estate of the Les Treilles Foundation—a family-run organization that promotes scientific exchange—to take another crack at the question of viral origins and evolution.* They had some powerful new findings to charge the discussion. Despite the difficulties of reconstructing events that may have taken place more than 3 billion years ago, recent work using the structure of viral genes and proteins to infer relationships between organisms has sparked some provocative ideas. Among them is the notion that viruses arose very early—perhaps before the three domains diverged—and the hypothesis that viruses, rather than being an aberrant branch on the tree of life, have played a major role in the evolution of multicellular organisms. “I hope we will dismiss the notion that they are somehow alien, like the Andromeda Strain,” virologist Stephen Morse of Columbia University in New York City told the meeting attendees.

Even before the first electron

microscopes in the 1930s opened a window onto the shadowy world of viruses—which scientists in the late 19th century had recognized only as infectious particles that could pass through filters fine enough to ensnare bacteria—scientists were speculating on how these mysterious microbes originated. In 1924, French-Canadian microbiologist Félix d’Herelle proposed that viruses were the ancestors of cells. Echoing that idea, Nobel Prize-winning biologist Salvador Luria suggested in the 1960s that modern viruses are relics of the precellular primordial soup. Others have argued that viruses came into being more recently as the vestiges of bacteria that lost their cell walls and degenerated from a free-living to a parasitic existence. Currently, the most widely held view, inspired by research in the 1970s by Nobel laureate Howard Temin and others, is that viruses trace their heritage to cellular genes that somehow broke free and spun a protein sheath that enabled them to survive briefly outside a cell’s protective environs.

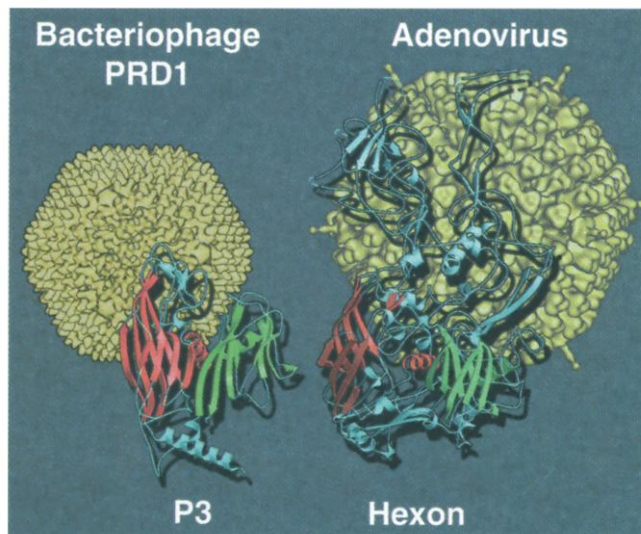
It is only in the last few years that researchers have been able to put these ideas to the test. The enormous genetic diversity of viruses—whether a result of being around for billions of years, from mutating rapidly, or

both—makes it difficult to trace evolutionary lineages by comparing gene sequences from one species or strain to another. Researchers find many nearly identical genes in organisms as diverse as fruit flies, mice, and humans. But bacteriophages—viruses that infect bacteria—seldom bear more than a trifling resemblance when their genomes are lined up side by side, even when outwardly they appear very similar.

So instead of simply lining up genomes, virologists have been hunting for other measures of evolutionary relatedness—and they have come up with some surprising pairings. Two unlikely cousins are the herpes virus HSV-1, which infects people, and the bacteriophage T4. The two viruses are remarkably similar in the shapes of their outer coats, the complicated steps they take to assemble their coat proteins, and how their DNA molecules are packaged inside—even though they are very dissimilar genetically. At the meeting, molecular biologist Dennis Bamford of the University of Helsinki described an even more compelling parallel between the coat protein structure of the bacteriophage PRD1—which was discovered nearly 30 years ago in the sewers of Kalamazoo, Michigan—and that of human adenovirus, blamed for many chronic respiratory and intestinal infections.

Bamford’s group, in collaboration with structural biologist Roger Burnett’s team at the Wistar Institute in Philadelphia, worked out the x-ray crystal structure of P3, the main protein of PRD1’s outer coat. Like many viral coat proteins, P3 features a compact “jelly roll,” made up of eight parallel polypeptide strands (see diagram). A comparison of P3 with the coat protein of human adenovirus type 2, called hexon, revealed that the configurations of each virus’s jelly rolls are remarkably similar. Moreover, both PRD1 and adenovirus arrange their protein subunits in the same way during viral assembly, in a fashion atypical of other viruses. “The parallels are striking,” says Stanford University geneticist Allan Campbell, a pioneer in bacteriophage research, because P3 and hexon lack any detectable similarity in their amino acid sequences. Campbell and others agree that the structures must have been conserved even though the amino acid combinations changed radically during the course of evolution.

Indeed, meeting participants interpreted these results—as well as other fresh data showing that genetic similarities can sometimes be found, for example, between viruses that infect bacteria and those that infect archaea—as evidence that



Striking parallels. Only distant cousins, the sewer-dwelling bacteriophage PRD1 and the human adenovirus have similar “jelly roll” configurations and share a peculiar arrangement of protein subunits that hints at an ancient origin of viruses.

* The Origin and Evolution of Viruses, 25 to 29 July.

viruses arose before life's three domains diverged. "It is difficult to imagine this could be convergent evolution," says Campbell. Adds molecular biologist Roger Hendrix of the University of Pittsburgh, "The similarities are great enough that it is unlikely they rose independently." But because the molecular clock used to unravel genetic relationships of other organisms doesn't work well for viruses, researchers admit that it's impossible for now to trace the viral family tree back to its roots. The best guess, says Hendrix, is that "their divergence must have been really far back in time." If so, that would be gratifying to some researchers. "There must have been something that existed before cellular life, assembling subunits but having no membrane," says molecular biologist Henry Krisch of the Laboratory of Microbiology and Molecular Genetics in Toulouse, France. "What else would you call it other than a virus?"



Worth their weight in carbon? Cited as evidence that viruses are key players in evolution is their influence on the carbon cycle: Seagoing viruses, including bacteriophages (right), lock up at least 20 times more carbon than contained in all the whales on Earth.

A "moronic" hypothesis

Another theory of viral origins, the "moron accretion hypothesis," was put forward at the meeting by Hendrix. He and Pittsburgh colleagues have been sifting the genomes of bacteriophages for clues to how they evolved. Earlier this year, they reported the complete DNA sequences of two bacteriophages that infect *Escherichia coli*, HK97 and HK022. When the sequences were compared to each other as well as to those of two other outwardly similar bacteriophages, it became clear that all four have some DNA stretches in common. But these stretches are interspersed with longer sequences that vary greatly from one bacteriophage to the next. Such genetic mosaicism has often been taken as evidence that viral strains swap genes.

But the Pittsburgh group also found extra genes, apparently capable of acting independently of the rest of the viral genome—because they carry their own instructions for the initiation and termination of gene expression—inserted into the genomes of some bacteriophages. The function of these extra genes is unclear, but they do not appear to act in concert with adjacent genes. The researchers whimsically dubbed these genes "morons," says Hendrix, because they represent "more DNA." Some morons are similar to known bacterial genes.

Extrapolating from these observations, Hendrix laid out an intriguing, albeit assump-

tion-laden, scenario. If the DNA of early primitive cells was not yet organized into chromosomes but floating free, and if a gene mutation led to a protein that self-assembled into the kind of icosahedrally shaped shell typical of many virus coats (a penchant of a number of proteins under certain conditions, such as the bacterial enzyme lumazine synthase), the result could have been a DNA segment trapped in protein—a "primitive virus," as Hendrix says. Over time, the viral genome

could have grown moron by moron, particularly if the addition of a new gene made it more likely to survive in its environment. "This would allow you to build a virus from the ground up," Hendrix says. "The morons are simple, but if you put them together over time you might get something that is pretty smart genetically."

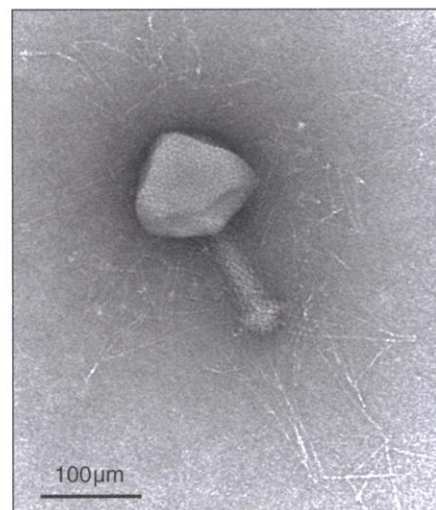
Hendrix's proposal generated debate at the meeting. "I'm not convinced that viral genomes have been built up by starting with one gene and then adding genes until we get where we are now," says Campbell. And while agreeing that the wholesale defection of genes or groups of genes from early cells may well have given rise to viruses, Krisch argues that the moron accretion idea would require the viral coat to get bigger and bigger as new genes are added, to accommodate the growing genome. "That part of the theory leaves me a little ill at ease," he says.

The whale and the virus

Although the origins of viruses remain obscure, their potential role as forces shaping the early evolution of life is itself taking life. Underscoring the fact that viruses are much more than a sideshow in the biosphere, marine microbiologist Curtis Suttle of the University of British Columbia in Vancouver offered data on the influence of viruses on the carbon cycle in the oceans. A typical

milliliter of seawater contains on average some 10 million virus particles; extrapolating from that figure, seagoing viruses lock up as much as 270 million metric tons of carbon, more than 20 times the top estimate for the amount stored in all the whales on Earth. "With all those numbers of [viruses], it is hard to imagine that they were not a significant force in evolution," says Krisch.

In an essay in the June 1998 issue of the *Proceedings of the National Academy of Sciences*, microbiologist Carl Woese—who is credited with discovering the Archaea—suggested that early life was a hotbed of "lateral gene transfer" between cells, and that this gene swapping was the key driver of evolution. Only after the domains of life diverged, he argued, did the barriers to such promiscuity grow formidable. Woese didn't



touch upon the question of how viruses originated. But building on this idea, molecular biologist Patrick Forterre of the University of Paris's Orsay campus suggested at the meeting that viruses might have been important early vehicles for such gene exchange. If so, Forterre argues, it might explain puzzling relationships between the proteins involved in DNA replication in each domain. Many such proteins in the bacteriophage T4, for example, are more similar to their eukaryotic counterparts than to those in bacteria. Indeed, many viral proteins are more closely related to viral or cellular proteins from organisms in domains other than that of their own host.

If viruses did play an active gene-shuffling role in life's early days, then their reputation as being somehow alien to other life-forms would be way off the mark. "These viruses have been having a global orgy of gene swapping for 3.5 billion years," Hendrix says. Indeed, Krisch submits, "if there hadn't been all these things around exchanging genetic information with their hosts, we wouldn't be what we are today."

—MICHAEL BALTER