Current Topics

Species-Jumping Viruses Raise Core Questions about Mutation Rates

The human B19 erythrovirus appears to be evolving more rapidly than expected, according Edward C. Holmes of the Pennsylvania State University, University Park, and his collaborators, who report their findings in the April Journal of Virology (80:3666–3669). Their work challenges conventional wisdom about how quickly viruses evolve and raises core questions about mutation rate differences among different classes of microorganisms.

In earlier work, Holmes and Laura A. Shackelton, a graduate student at the University of Oxford, in Oxford, United Kingdom, studied molecular changes in a carnivore parvovirus that occurred when it switched from one host species to another. It “is one of the few viruses for which we have sequence data from before and after it crossed host species,” says Shackelton, who plans to move to Penn State this fall. After the virus jumped from cats to dogs, probably in the late 1960s, it “showed a great increase in growth rate, causing a true epidemic, as it further adapted to its new host.”

This virus has an exceptionally high rate of mutation, raising the question of whether this trait is general among parvoviruses or specific to the carnivore parvovirus. The question was especially intriguing because microbes with DNA genomes tend to have mutation rates of about 0.003 changes per genome replication, giving large genomes a much lower rate of mutation per gene than small genomes, according to other researchers, notably John W. “Jan” Drake of the National Institute of Environmental Health Sciences in Research Triangle Park, N.C.

However, the mutation rate that Holmes and Shackelton measured in carnivore parvovirus appears to be several orders of magnitude faster—a rate that is closer to that seen for some kinds of RNA viruses. “What we are measuring is the fixation rate, how long it takes for a mutation to get fixed in a population, which does not necessarily reflect the mutation rate,” Holmes cautions. Nonetheless, he adds, “If you see a very high substitution rate like we’re observing in these parvoviruses, that strongly suggests that [their] underlying mutation rate is also high.”

The B19 is related to carnivore parvoviruses, but only distantly. When it infects humans, it targets bone marrow progenitor cells, is associated with heart complications, and infects hosts through routes different from those used by the carnivore parvoviruses. Regardless, however, the B19 virus also shows the same surprisingly high rate of mutation, according to Holmes and Shackelton.

That result makes it unlikely that the speedy genomic changes observed in the carnivore parvoviruses are due to mutator genes, which boost mutation rates and are common to “very roughly 10% of microbes that have recently adapted to a new host,” Drake says.

It also blurs mutation rate differences among RNA- and DNA-containing viruses and bacteria. For instance, the simian foamy RNA-containing virus has a remarkably slow substitution rate, which is comparable to that of mitochondrial DNA. Some microbiologists reasoned that RNA-containing viruses generally mutate several orders of magnitude more quickly than do DNA-containing viruses because the fidelity of DNA polymerases is far greater than that of RNA polymerases. However, Drake notes, evolutionary forces determine fidelity rates, and “if an RNA virus wanted a lower rate, it could easily improve insertion accuracy, as already observed in a few cases.”

Amid this blurring of long-held views about differences in mutation rates among classes of viruses and bacteria, so far these exceptions are, well, exceptional. A core question is why there is a standard rate at all, according to Drake. Any particular organism cannot tolerate having an increased mutation rate if it leads to “more unfit offspring,” he points out. “On the other hand, you can’t drive the rate to zero, because you have to pay a substantial cost in resources to get the rate lower. But what’s really amazing is the tradeoff is the same for upwards of eight or nine really different organisms with different life histories. So the balancing forces are very deep and mysterious.” Holmes and Shackelton thus provide valuable information that may help uncover answers to those questions, he says.

David Holzman
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In Yeast, Genetic Redundancies Help To Ensure Genomic Integrity

Thousands of pairs of genes in the yeast Saccharomyces cerevisiae mutually back up one another, one com
penning when the other member of a pair develops a defect, according to Jef D. Boeke and his collaborators at Johns Hopkins University School of Medicine in Baltimore, Md. This gene network contains at least 16 previously unrecognized genetic pathways for guarding against lethal DNA damage. Moreover, they say, a database describing this yeast network will expedite efforts to analyze and identify disease-causing combinations of mutated human genes.

“A lot of human diseases are caused by multiple gene mutations that are difficult to identify,” says Boeke. Because 25% of human disease genes are also found in yeast, it serves as a useful model. Of the 6,000 documented genes in yeast, defects in any of about 1,000 of those genes are lethal by themselves. However, defects in the other 5,000 genes are not lethal, in part because of backup pairings. To find them, the investigators used a microarray technique that enables them to analyze large numbers of gene combinations simultaneously. Additional details of this research appear in the March 10, 2005, Cell.

The researchers used that information to identify pathways that help to maintain genome integrity in yeast. The genes encode proteins involved in pathways of DNA replication and other cellular processes, and it is the pathways that perform the compensating activities.

Boeke offers the following analogy: “a bus and the subway are transporting school kids to the museum. A power outage (loss of one pathway) takes the subway out of commission, but the children on the subway can still crowd into the bus. But if the bus also gets sidelined, due to a flat tire, bad brakes, an electrical problem, or a broken axle (loss of one gene in one of the many pathways that keep the bus going), there will be no field trip.” As Boeke’s analogy implies, an individual gene can have backup relationships not only with one gene, but with many. Thus, the subway can back up the bus’s brakes, axle, electrical system, and tires.

Consider DNA replication, which is not a single pathway, but a complex process involving several pathways. “If you mess up DNA replication by mutating some gene that contributes to its fidelity, it can lead to lots of errors and breaks in the DNA,” Boeke says. Without backup pathways, the cell would die. But two kinds of pathways provide backup. One, the DNA replication checkpoint, “arrests the cell cycle so that there is time to repair the damage before the cell tries to divide. Another, the DNA damage checkpoint, accomplishes the same cell cycle arrest via a different set of proteins.”

The same pathways can also arrest the cell cycle when environmental forces—chemicals, for example—damage cellular DNA.

In the study, one identified gene has backup relationships with 278 other genes. Not surprisingly, some sets of backup genes overlap extensively. A lot of overlap, or congruence, between two sets of backup genes puts the genes that they back-up in the same pathway. However, such pairs of genes, with overlapping sets of backup genes, cannot themselves form backup pairs.

Besides identifying 16 unrecognized genetic integrity pathways, Boeke and his colleagues also confirmed several previously known pathways, which “shows our method is robust and works well,” he says. Additionally, they found that the Golgi apparatus is involved in maintaining DNA integrity, a suprise since this organelle serves mainly to direct molecules to their proper places within a cell.

“The work provides a number of important insights into the function and interactions among DNA integrity proteins,” says Jonathan S. Weissman of the University of California, San Francisco. It also “illustrates the power of large-scale synthetic lethal studies in revealing functional relationships among genes.”

RNA Interference Appears a Promising Means for Blocking Prions

Experimental RNA interference (RNAi) techniques—although still cumbersome—appear capable of reducing prion proteins in livestock and possibly of eliminating prion-related diseases in such species, according to Gregory J. Hannon and Michael C. Golding at Cold Spring Harbor Laboratory (CSHL) in Cold Spring Harbor, N.Y., and Mark E. Westhusin of Texas A & M University in College Station. Their experiments involved goats, which are susceptible to the prion disease scrapie, and thus required less stringent biosafety precautions than are required when studying bovine spongiform encephalopathy (BSE) in cattle. After identifying appropriate genetic sequences, the researchers made and then introduced a gene for the interfering RNA into goat fibroblast cells, which were used to produce goat embryos. The prion protein in the brain of the cloned fetal goat was reduced by more than 90%, lowering it enough to prevent prion-related symptoms from developing in the animals, according to the researchers. In other experiments, they confirmed that the viral transfer technique also is effective in fertilized eggs of cattle, meaning it should be applicable for BSE. Details of their findings appear in the March 20, 2006, online edition of the Proceedings of the National Academy of Sciences.
“The evolutionarily conserved pathways provide excellent genetic targets to develop therapies for cancer,” says Keshav Singh, of the Roswell Park Cancer Institute, Buffalo, N.Y. “The lethality defect should guide us in identifying and quickly testing potential cancer drugs in yeast.”

David Holzman

Confronting, Combating Plant Diseases at the Molecular Level

Plants react to viral and bacterial pathogens through mechanisms that researchers are learning to exploit on the molecular level, according to several experts who spoke last April at a conference, “From Functional Genomics of Model Organisms to Crop Plants for Global Health,” convened by the National Academy of Sciences in Washington, D.C.

In some cases, the goal is to broaden very specific host defense mechanisms, with the aim of protecting commercially valuable plants against viral pathogens that otherwise cause problematic crop losses, according to David Baulcombe of the Sainsbury Laboratory in Norwich, United Kingdom. For instance, the Rx protein of potatoes recognizes specific viral coat protein motifs, including one carried by potato virus X, and responds to this pathogen by unfolding and also either recruiting or releasing additional host resistance factors, he says.

This ordinarily narrowly focused resistance mechanism of potato plants can be broadened, Baulcombe continues. Thus, particular mutant plants become resistant to the poplar mosaic virus, which is only distantly related to potato virus X. Other mutant plants show reduced recognition capacity, but heightened responsiveness to viral pathogens, he says. These findings suggest an approach that he calls “molecular breeding of disease resistance.” Although it might entail exchanging genes between plant species to ensure “durable resistance,” he and his collaborators also are determining whether ordinary mutagenesis, which consumers may find “more acceptable,” could also provide a successful path to disease-resistant, commercially useful potato plants.

Pneumococcal Vaccine Use Is Lowering Drug-Resistant Infection Rates

The rates of antibiotic-resistant invasive pneumococcal infections decreased among young children and older persons in the years after a conjugate vaccine that guards against seven prevalent types of pneumococcal infections came into use, according to Cynthia Whitney and her collaborators from the Active Bacterial Core Surveillance group at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. Because this vaccine, which is marketed as Prevnar by Wyeth, is administered only to children and by no means on a universal basis, it appears to be exerting a significant herd effect, indirectly protecting part of the adult population while significantly protecting both young and old against drug-resistant serotypes of Streptococcus pneumoniae. However, the CDC group reports, there was some increase in infections caused by serotypes that are not included in the vaccine after it was introduced in 2000. Details are reported in the April 6, 2006 issue of the New England Journal of Medicine.
The 147 known single-stranded DNA gemini viruses, although small and equipped with a mere four to eight genes, are capable of infecting a wide range of tropical plants and are responsible for huge losses in crops such as tomatoes and cassava each year, according to Claude Fauquet of the Donald Danforth Plant Science Center in St. Louis, Mo. There is no known chemical way of blocking these viruses, and efforts to control the white flies that spread these viruses have also proved futile because of their resistance to insecticides, he says.

Gemini viruses are on the move in Africa, headed from Uganda toward Nigeria, a major producer of cassava, according to Fauquet. When two different types of gemini mosaic viruses infect cassava plants, the plants collapse because these two particular viruses act synergistically. Each acts on and suppresses a separate gene-silencing mechanism that is an inherent part of plant defenses against such pathogens, he says. One of the viruses works within 48 hours and apparently deregulates developmental genes in the plants, while the other works more slowly to attack another gene-silencing scheme within this host.

Some gemini viruses carry a single-stranded DNA satellite molecule that confers on them yet another means for disrupting resistance to these viruses in host plants, according to Fauquet. “If this satellite is added to plants, it breaks their natural resistance to gemini viruses very quickly,” he says. “But we don’t know the mechanism.” Even so, it is a target for research efforts, and one approach involves introducing the M13 phage into plants where its G5 protein binds to and combats gemini viruses, he adds. However, the G5 protein is by no means 100% effective, and is “not yet a magic bullet to offer Africa.” Obtaining higher expression of G5 and combining this approach with gene-silencing mechanisms may improve the effectiveness of these approaches for protecting cassava crops.

Meanwhile, bacterial pathogens also tap into and circumvent inherent plant protection mechanisms, according to Pamela Ronald of the University of California, Davis, and her collaborators, who study rice, and Brian Staskawicz of the University of California, Berkeley, and his collaborators, who study tomatoes and peppers. Ronald says that researchers in China who are working on a receptor kinase, which confers resistance to many pathogens of rice plants, could soon introduce this gene into commercially grown rice in that country. In general, pathogen resistance mechanisms are complex and involve many pathways with many molecular components in rice and other crop plant species, she adds.

The bacterial pathogens that attack crops such as tomatoes and peppers are complex in terms of the virulence factors—both proteins and smaller molecules—that they draw upon while confronting the also-complex defense mechanisms of their hosts, according to Staskawicz. Plant breeders sort through domestic and wild cultivars to find disease-resistant combinations, but those breeding efforts are not always so straightforward.

For example, tomatoes are susceptible to a devastating “spot disease” that induces premature ripening and is caused by a Xanthomonas sp., to which some peppers but no known tomato varieties show “natural” resistance, according to Staskawicz. However, this resistance, which depends on a specific protein encoded by the BS2R gene, can be cloned from pepper plants and transformed into tomatoes, rendering them highly resistant to spot disease, he says.

Staskawicz calls these modified tomatoes the “poster child” for disease resistance, predicting that even those consumers who are squeamish about using genetically modified foods will find them acceptable because the gene transfers occur between such similar plant types and involve a gene and gene product that already are part of the diet. Moreover, no antibiotic resistance markers are needed, and there are no closely related weed species into which this trait is likely to spread, he adds. “This strategy should reduce the use of chemicals to control [spot] disease in tomatoes, making this a strong candidate for an environmentally acceptable genetically engineered plant.”

Jeffrey L. Fox
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Yes, We Got No Bananas: from Edible to Extractable Plant-Based Vaccines

Through the 1990s, Charles Arntzen traveled the lecture circuit, describing technologies for producing edible vaccines in the form of antigen-carrying bananas or other tissues from green plants. Now the Arizona State University (ASU) researcher calls himself guilty of fostering this “oddball” idea, but still sees a bright future in efficiently producing in green plants antigenic proteins for use in vaccines or other medicinal products —and then purifying and formulating them in ways more suited to traditional regulatory review and dispensing modes.

The technology for making edible vaccines was “gaining traction” by the end of the 1990s, says Arntzen, codirector of the ASU Center for Infectious Diseases and Vaccinology, who spoke in April at a conference, “From Functional Genomics of Model Organisms to Crop Plants for Global Health,” convened by the National Academy of Sciences in Washington, D.C. The technology for using plants to produce immunologically active antigens, including for the hepatitis B and Norwalk viruses, proved successful, leading to early-phase clinical trials. In those cases, however, volun-
Researchers Report First Well-Documented Cases of Mouse Retrovirus Infecting Humans

Patients with a rare type of prostate cancer carry a retrovirus that is closely related to a cancer-causing virus found in mice, making these the first documented cases of human infection with a retrovirus that is native to rodents, according to Robert Silverman of Cleveland Clinic in Cleveland, Ohio, and Joseph DeRisi and Don Ganem at the University of California, San Francisco. The virus from humans is closely related by genomic sequence to murine leukemia virus (MuLV), and is being called xenotropic MuLV-related virus, or XMRV. Calling their findings “the first really solid example of an authentic xenotropic retroviral infection in a human being,” Ganem says that assuming any causal link between XMRV and prostate cancer is “tenuous.” Rather, the virus appears to infect a small group of men with prostate cancers who also happen to have a mutation of the RNASEL gene, which likely is ordinarily involved in protecting against such infections. However, chronic inflammation from infection of nearby stromal tissues “may play a role in triggering such cancers,” the researchers note. Details appear in the March 31, 2006, issue of the Public Library of Science Pathogens.

Speculate with Genomics but Test, then Test again with Experiments

A bacterial protein called WrbA, although long considered a repressor-binding protein, instead protects cells for producing antigenic materials or monoclonal antibodies (MAbs) in green plants ran out of money and were forced to declare bankruptcy. Besides, he adds, there was no shortage of other means for producing protein antigens and MAbs. Bacteria, yeast, and mammalian cell systems prove efficient for production and are familiar to regulatory officials, whereas green plant-based systems are still considered “prototypes.”

Nonetheless, there is a “compelling point” in favor of using green plants to produce such materials, according to Arntzen. Using plants offers opportunities for “lower capital outlay” during the early stages of development and production of candidate vaccines or therapeutic products, and this approach can be readily scaled up for producing those materials cheaply and in bulk. For such reasons, plants continue to loom as attractive means for production to entrepreneurs in countries such as India, Brazil, China, and South Korea, he says. More generally, these countries are “ramping up and want to expand their home markets beyond the middle class, and want cheaper materials. I have high hopes for plant-derived [vaccines and therapeutics] in the developing world.”

Although such hopes are temporarily depressed across some segments of the U.S. scene, Arntzen describes domestic pharmaceutical companies as “closet supporters” of this plant-based technology. Moreover, the Defense Department has emerged from the closet at least tentatively to embrace this technology.

For example, Arntzen and his collaborators at ASU are using tobacco plants to produce several antigenic proteins from Yersinia pestis en route to a vaccine for protecting troops against plague, with principal support from the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID). “These antigens are as good as anything they’ve seen,” he says. It takes about 100 tobacco plants to yield about 1 g of purified protein antigen, and a greenhouse covering about 1 acre of land can be built for about $13 million to produce about 150 tons of biomass per year. These and other figures lead to production estimates of about $100 per gram of purified protein antigens.

“People at USAMRIID said that can’t be right, so I should have made it higher,” Arntzen says. But there is a good deal of “capital cost avoidance” following this approach, at least in its early stages. Admitting that he was “consistently wrong so far” about this technology’s prospects, he also admits to renewed optimism stemming from this modified approach.

Jeffrey L. Fox
Vaccine that Blocks Access to Key Nutrient Proves Effective against H. ducreyi

Immunizing pigs with the outer-membrane, receptor protein for hemoglobin from Haemophilus ducreyi protects such animals against becoming infected by this gram-negative pathogen and blocks recovery of viable bacteria from “this highly relevant animal model,” according to Christopher Elkins of the University of North Carolina, Chapel Hill, and his collaborators. Because the vaccine induces antibodies that block binding of hemoglobin to its specific receptor protein in this pathogen, this strategy of blocking access to nutrients that pathogens specifically require may be applicable to other bacterial diseases, he points out. H. ducreyi causes chancroid, one of the genital ulcer diseases in humans, and is a risk factor for transmitting HIV in Asia and Africa. Details of these findings appear in the April 2006 issue of Infection and Immunity (74:2224–2232).

against oxidative stress, according to J. Greg Ferry of The Pennsylvania State University (PSU) in University Park and his graduate student Eric V. Patridge. Their findings underscore the importance of conducting experiments rather than relying on bioinformatics and genomics, notes Robert H. White of Virginia Polytechnic Institute, Blacksburg, whose guest commentary appears in the May Journal of Bacteriology (188:3431–3432) along with the research report from PSU (188:3498–3506).

WrbA (“W” stands for tryptophan) was mistakenly identified as a tryptophan repressor-binding protein because it copurifies with the tryptophan repressor protein. “If you read in depth the original paper, you understand that this was speculation,” Ferry says. “The paper didn’t go into any experiments to support that speculation.” Now it appears merely a coincidence that WrbA copurifies with the tryptophan repressor protein. Nonetheless, despite the lack of experimental evidence, when it turned up in subsequent genomic analyses, it was assumed to have the repressor-binding role, which spread the misinformation far and wide.

Ferry became interested in WrbA in a roundabout way. Before he began studying this protein, he was investigating methane formation for its role in the global carbon cycle. One gene that is involved in making methane encodes a protein called iron-sulfur flavoprotein. Its unusual amino acid sequence piqued Ferry’s curiosity. Moreover, he adds, it “showed up in every genomic sequence of every anaerobic microbe.”

Similarly, the gene for WrbA is found “in almost every prokaryote genome that has been sequenced,” Ferry says, suggesting that it, too, plays an important role. Moreover, there are hints that this protein is useful to bacterial cells when they are coping with stress, he adds. “When proteomics and microarrays are used to investigate the abundance of proteins under certain stressful growth conditions, this protein shows up quite often as being elevated.” WrbA has an amino acid sequence similar to that of the widely distributed iron-sulfur flavoprotein that is involved in making methane.

Meanwhile, looked at in another way, the WrbA sequence resembles that of a plant quinone reductase—an enzyme that is important for enabling cells under oxidative stress to neutralize potentially harmful quinones, which in its absence are readily converted to chemical free radicals. “The radical is extraordinarily reactive, and breaks bonds right and left,” Ferry says, explaining how it can damage molecules in cells. Quinone reductases reduce quinones without making radicals out of them. The iron-sulfur flavoprotein also reduces oxidative stress—but by converting oxygen, which is harmful to anaerobes, to water.

When Ferry and Patridge tested WrbA, they found that it has quinone reductase activity. “It was our first attempt in understanding what the function might be,” he says.

That finding “settles a long-standing mystery about the role of a protein [WrbA] that is ubiquitous in nature,” says Ronald Somerville of Purdue University in West Lafayette, Ind., one of the researchers who first found WrbA and mistook it for a tryptophan repressor-binding protein. “As the co-discoverer of this protein, I am delighted to know that it remains an object of scientific investigation,” he says, praising Ferry’s efforts to uncover its role as a quinone reductase in bacterial cells.

In his commentary, White notes that slight changes in protein structure “can have large catalytic consequences” for enzymes that might be missed if analysis were left to mere sequence comparisons. He also complains that in the era of genomics and bioinformatics, fewer scientists are continuing to fall by the wayside and are being replaced by computer-generated experiments.”

David Holzman