Whole Genome Transplantation Becomes A Reality

Microbiologists at the J. Craig Venter Institute (JCVI) in Rockville, Md., transferred the genome from one bacterium to another, “resulting in new cells with both the genotype and phenotype of the input genetic material,” says Carole Lartigue, lead scientist on the project. “The recipient genome is completely replaced by that of the donor cell—a clean change of one bacterial species into another.” Moreover, unlike Oswald Avery’s experiments involving transformed pneumococcal bacteria during the 1940s, she adds, “There isn’t any recombination between incoming and outgoing chromosomes during genome transplantation.”

JCVI researchers began stripping down bacterial genomes several years ago, viewing genomic transplantation as part of a far wider and highly ambitious program in microbial genomics, according to Venter, who is president and chairman of JCVI. “The successful completion of this research is a key proof of synthetic genomic principles, allowing us to realize our ultimate goal of creating organisms with the ability to manufacture biofuels, clean up pollution, and synthesize new medicines,” he says.

Earlier, the JCVI transplant team focused on Mycoplasma genitalium, a genomically parsimonious human urogenital pathogen. However, in a last-minute switch, the researchers chose two other mycoplasmas to use for their genome transplant experiments. The bacteria in that chosen pair are phylogenetically close, but distinct species of mycoplasma. The genome donor was Mycoplasma mycoides subspecies mycoides Large Colony (LC), while the recipient was Mycoplasma capricolum subspecies capricolum—a strain known affectionately as “the California kid.” These diminutive microbes were already close to being minimal bacterial cells before the JCVI team began paring them down. Both strains are fast growing, dividing every 90 minutes or so, and adapt readily to nutrient-rich media.

Mycoplasma lack cell walls, enabling the JCVI researchers “to adapt established eukaryotic nuclear transplantation methods to prokaryotic DNA transfer,” Lartigue says. The process has three key phases: first, intact circular donor genomes are isolated from M. mycoides LC; second, M. capricolum cells are prepared to receive the molecules; and third, the isolated donor genome is installed in the recipient cells. Additional details describing the JCVI experiments can be found in Science online, 28 June 2007;10.1126/science.1144622 and, in print, 317:632–638.

Manipulating bacterial chromosomes includes a particularly daunting technical challenge—avoiding breakage due to shear forces. Thus JCVI microbiologists designed novel ways of gently removing intact DNA molecules from M. mycoides LC and into capricolum. The scientists also needed a way to recognize those recipient cells that contained functional, implanted genomes. They monitored this process by incorporating tetracycline resistance marker genes into donor bacteria, which turned colonies of capricolum with mycoides DNA blue when placed in antibiotic-laced media. “Once identified,” says Lartigue, “We
Examined both the phenotypes and genotypes of the transplanted clones to confirm whole genome transfer and rule out any possible recombination with resistance fragments from *M. mycoides* LC."

The ability to change one bacterium into another seems to delight other researchers working on synthetic biology projects. Chris Voigt of the University of California, San Francisco, calls whole genome transplantation “an extraordinarily useful tool.” Moreover, he says, “It’s particularly exciting that the DNA used was ‘naked.’ Without attached proteins, all the information required to convert the host cell was encoded in the DNA. It’s amazing that a dead, static polymer can spontaneously generate all the dynamics of life.”

“This is obviously an important technical milestone on the path to creation of highly modified bacteria,” says Chris Somerville of Stanford University in Stanford, Calif. “I don’t think it raises any concerns that are not already part of the broad discussion about synthetic genomics,” he adds, when asked about potential harmful consequences of such experiments.

Marcia Stone
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### Engineering Phage To Target Biofilm Matrices Enhances Disruption

Bacteriophage can be genetically engineered to produce enzymes that dissolve polysaccharide matrices surrounding and protecting bacterial biofilms. These enzymes in turn enhance the ability of phage to break down such biofilms, according to bioengineers James Collins of Boston University in Boston, Mass., Timothy Lu of nearby Massachusetts Institute of Technology in Cambridge, and their collaborators. Although their efforts are at the proof-of-principle stage, they anticipate such engineered phage someday helping to control bacterial biofilms in the lungs of patients with cystic fibrosis or other diseases, on catheters and other medical devices, and in other settings where biofilms cause fouling of surfaces, such as ship hulls or water mains.

Collins and Lu engineered bacteriophage T7, which infects *Escherichia coli*, by inserting the gene encoding dispersin B, an enzyme produced by *Actinobacillus actinomycetemcomitans*. This enzyme can hydrolyze a crucial glucosamine-containing adhesin that is an important ingredient of some biofilms, including those formed by *E. coli* and *Staphylococcus* spp.

When tested on laboratory-grown *E. coli* biofilms, the T7-dispersin phage removes biofilm bacteria significantly better than does T7 alone. The biofilms had an average cell density of 6.4 colony forming units (CFU) that was reduced to 3.2 CFU after 24 hours of treatment with the T7-dispersin phage, compared to 5.1 CFU after treatment with unmodified T7. Additional details of these proof-of-principle experiments are reported in the July 3, 2007 issue of the *Proceedings of the National Academies of Science*.

The T7-dispersin phage starts to destroy biofilms within five hours of infection, and the killing persists for up to 48 hours. Biofilm destruction appears dose-dependent—higher doses of the T7-dispersin phage cause more biofilm disruption. “We did not add ‘naked’ dispersin B . . . to the biofilm,” Lu says. “Encoding the gene for dispersin B within the bacteriophage allows rapid production of high levels of enzyme near the biofilm.” Despite the biofilm barrier, Lu says, “phage can penetrate biofilms to a certain extent on their own due to their small size and the heterogeneity of the matrix.” He further notes that, when other researchers, led by Tony Romeo and Yoshikane Itoh at Emory University in Atlanta, Ga., tested the enzyme...

### Helicase Mutants Greatly Boost West Nile Virus Levels in Crows

A mutation in the West Nile virus (WNV) gene encoding helicase seems to account for major changes in virulence of this virus, enabling such mutants to replicate at least 10⁴ more efficiently than usual, according to Aaron Brault of the University of California, Davis (UCD), and his collaborators there and at several other institutions. The single mutation is “under positive selection,” and it “dramatically modulates viral replication in birds,” he says. The mutation markedly affects WNV behavior in American crows, which are monitored as the sentinel species for WNV outbreaks. Further, the mutation is “not located at a position in the protein where it would affect helicase activity, [although] it may affect binding to other proteins,” he adds. “Our hypothesis is that the mutation allows for greater replication of the virus in birds, leading to higher infection rates in mosquitoes, which will translate into greater numbers of infections in humans. Although this could explain more severe cases of disease, we can’t discount that it’s more virulent in humans, but so far we have no data [for how the mutant virus behaves] in mammalian cells.” Details of the UCD study appear in the August 12 online issue of *Nature Genetics*. 
by itself against biofilms, they proved that it is disruptive for biofilms produced by several bacterial species.

The next step is to show that this prototype phage can effectively destroy more complex biofilms, such as those that consist of mixed colonies of bacteria instead of a single species such as the E. coli biofilms that were tested, according to Collins. His collaborators are engineering phages to express additional enzymes that target different components of biofilm matrices, anticipating that they can destroy biofilms containing more than a single species.

Eventually, an array of species-specific, biofilm-degrading phage could be assembled through use of DNA sequencing and synthetic biology techniques. Different combinations could be tested against multiple biofilms composed of many different species. “We’re confident that we can go after complicated, multispecies biofilms with a cocktail approach,” Collins says.

“The new technology is exciting because there are not great therapies for treating biofilms,” says microbiologist George A. O’Toole, Jr., at Dartmouth University in Hanover, N.H. The technology seems ideal for solving industrial biofilm problems, such as contaminated water lines, which now are controlled with toxic chemicals. A bioengineered phage, however, may prove a harder sell as a therapy for human biofilm infections. In addition to “overcoming the technical hurdles of targeting a range of disease-causing organisms,” O’Toole says, “you have to convince a patient to swallow a living organism to cure an infection.” In other words, the public relations issues may prove as challenging as the scientific ones.

E. coli L Forms Do Not Conform to Cell Wall-Less Dogma

For ordinary bacteria, cell division is tightly linked with cell wall synthesis. Bacterial L forms, however, which are amorphous and penicillin-resistant, supposedly grow without making cell wall, which also is known as peptidoglycan or murein. Not so—at least for Escherichia coli L forms, according to Richard D’Ari of the Jacques Monod Institute in Paris, France, and his collaborators there and in Spain. Thus, contrary to long-held belief, E. coli L forms unexpectedly continue to synthesize at least small amounts of peptidoglycan. “There is residual murein, which presumably is necessary for constriction site development at cell division—otherwise, L forms would not produce progeny,” points out Anthony Pugsley of the nearby Pasteur Institute in Paris, who was not involved in this research.

D’Ari and his collaborators developed a protocol for efficiently converting ordinary E. coli strains to grow as L-forms. When viewed by electron microscopy, these and other L forms show no cell walls, making them resemble mycoplasmas. E. coli are by no means the only bacteria that can produce L forms, and there are many other examples of bacterial species producing L forms since they were first observed during the 1930s. “It is not known whether L forms in nature are an accident or an evolutionarily selected variation which might, for example, enable bacteria to escape the immune system and migrate to a new location in their animal host,” D’Ari says, “Questions such as this should now be amenable to study.”

By genetically manipulating E. coli, D’Ari and his collaborators developed a series of slightly different strains, each of which is selectively blocked at distinct steps in cell division because particular enzyme-catalyzed steps no longer act properly. With these strains, the researchers could determine which functions are specifically required during L form division.

To their surprise, however, the researchers could not get several of those specific E. coli strains to grow as L forms. “When we finally succeeded in making L forms, we understood why,” says D’Ari. “We had been trying to impose an absolute genetic block on cell wall synthesis, but we found, to our great surprise, that our L

“Social” Dictyostelium Shows Immune-Like Behaviors

The soil-dwelling amoeba Dictyostelium discoideum, although heralded for its social behavior, also has what might be considered an antisocial side—displaying immune-like activities among a subset of cells that protect against bacterial or other threats encountered in its immediate environment, according to Adam Kuspa and his collaborators at Baylor College of Medicine in Houston, Texas. In particular, during the slug phase of development, the amoebae contain numbers of novel, circulating sentinel (S) cells that, like neutrophil cells within mammalian immune systems, can efficiently engulf bacterial cells, including pathogens such as Legionella pneumophila. Moreover, some of these protective functions depend on a functional Toll-like-interleukin-1 receptor domain protein, TirA, that also is required for the amoebae to feed on live bacteria. Details appear in the 3 August 2007 issue of Science.

Carol Potera
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forms still required a residual amount of cell wall.” Under some conditions, the L forms make as much as 7% of the amount of peptidoglycan made by ordinary cells. “We also obtained evidence strongly suggesting that this residual cell wall is required for division of the L forms, meaning that their division mechanism is likely to be very similar to that of normal bacteria,” he says. Thus, he also wonders whether L forms of other bacterial species also require small amounts of cell wall to grow and divide.

“As with any truly satisfying discovery, [D’Ari’s] work . . . raises more questions than it answers while at the same time making it possible to attack them,” notes Kevin D. Young of the University of North Dakota School of Medicine, Grand Forks, in his commentary on that work. Both items are published in the September issue of the Journal of Bacteriology (189: 6512–6520).

Answers to one of those questions, namely why the L forms that D’Ari and his collaborators were studying could “grow on an agar surface or in liquid media, while classical L forms grow most easily embedded in agar. . . might also inform the longstanding issue of how the cell wall evolved in the first place,” Young points out. Because it is unlikely that a “rigid exoskeleton [could] spring into place fully formed,” Young proposes that early during evolution, precursors to E. coli and other bacterial species with rigid walls produced “a semistable intermediate.” Moreover, he adds, an “obvious candidate” for playing that role “would be a secreted carbohydrate that stabilized the cells in some environments.”

“I think L forms need to be looked at from an ecological perspective,” says Rita Colwell of the University of Maryland, College Park. “It would seem to me that there is probably a powerful ecological explanation for L forms as they have evolved.”

“[D’Ari’s] contention that cell division proteins like FtsZ, PBP3, and others can perform physical constriction together with a very limited amount of peptidoglycan is important,” says Conrad L. Woldringh of the University of Amsterdam in Holland. “The finding could serve as an intermediate step in the development of a, so far, elusive system of in vitro cell division.”

“One interesting lesson from our work, an old lesson that nevertheless seems to need repeating, is that dogmas should not be accepted at face value,” he says. “We read an enormous number of old papers on L forms, paying special attention to cell wall studies, and nowhere did we see any evidence that there is not 10% of the normal amount of cell wall. Often the lower limit of detection wasn’t even specified.”

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

Microbial Trace Fossils Dated at 3.35 Billion Years Raise Questions

Using pillow lava samples from the Euro Basalt of Pilbara Craton in western Australia, Canadian geochemist and petrologist Neil R. Banerjee from the University of Western Ontario and his collaborators identified microbial tunnels that they date to the Archean period. If this evidence holds up it has important implications for evolution. It not only suggests that microbial life was abundant in the deep sea 3.35 billion years ago, but also that it was endolithic and relatively advanced in terms of structure and physiology.

Endolithic, or rock-boring, microorganisms presumably ate their way into the glassy rims of pillow lavas in the deep Archean oceans in search of nutrients such as iron and manganese, leaving those tunnels as a record of their foraging forays, according to Banerjee. “What this unequivocally proves is that these are very old microfossils, Archean in age, older than 3 billion years,” he says. He and his team analyzed the samples using a newly acquired instrument called a laser ablation multicollector-inductively coupled plasma mass spectrometer (LA-MC-ICP-MS) that is housed at the University of Alberta, Canada. Details appear in the June issue of Geology.
Micropaleontologists are determined to firmly establish a date for early microfossils, but this pursuit is hotly contested. Some argue that such tunnels are artifacts that resulted from abiotic processes, whereas others consider them genuine evidence of microbial life. For example, Banerjee and his colleagues reported in *Science* in 2004 that they had found microbial tunnels in pillow lava samples, which have an accepted age of 3.5 billion years, from the Barberton Greenstone Belt in South Africa.

The microbial trace fossils that Banerjee and his team described in 2004 and earlier this year closely resemble those found in recently formed pillow lavas. Could it be that the trace fossils found in Archean pillow lavas are made by modern endolithic microorganisms? Banerjee says the dating of titanite crystals within the samples through use of the LA-MC-ICP-MS instrument rules out this explanation. Moreover, the tunnels in the Archean pillow lavas have the same morphology as their modern counterparts, proving microorganisms made them, he says.

The LA-MC-ICP-MS instrument was used to ablate 1-to-2-μm-thin shards of titanite crystals found inside the 1-to-5-μm-diameter fossilized tunnels in the pillow lava samples. Titanite is a calcium-silicon-titanium mineral that incorporates small amounts of uranium when it crystalizes. The titanite crystals are stable over a wide range of temperatures and pressures, and thus can preserve microfossil tubules for billions of years. More importantly, the uranium in titanite (U-238) has a half-life of 4.5 billion years. Because it gradually decays to lead, geochronologists can calculate when the titanite was incorporated into the tunnel by measuring the ratio of uranium to lead in the crystals. Although geochronologists previously used uranium-lead ratios to date Archean zircons in rocks, this is the first time that an LA-MC-ICP-MS instrument was applied to date microbial trace fossils.

Once crystals are pulverized into submicrometer particles, the instrument then sends the titanite dust into the mass spectrometer where a uranium-lead isotope ratio is used to determine the age of the crystals. “Although any geochronologist would point out that the margin for error on our analysis could be off by 100 million years, when you are dealing with Archean rocks, it is not that much” Banerjee says. “The importance of our findings is that microbial life in volcanic rocks requires only two ingredients, water and basaltic lavas, and I might add that those two ingredients are quite likely on the planet Mars as well,” Banerjee says.

“The challenge, of course, is proving that the fossils were contemporary with the formation of the pillow lavas,” cautions microbial ecologist James Fredrickson with the Pacific Northwest National Laboratory in Richland, Washington. “If the interpretations are correct,” he adds, “they suggest that early microbial inhabitants may have been lithoautotrophs, assuming that such organisms are able to gain sufficient energy from materials to support cell growth.”

The results “look pretty good to me,” adds Roger Buick, an astrobiologist at the University of Washington in Seattle. “Of course, the dating isn’t very precise, but it does confirm that the microborings are indeed ancient and not modern contamination. Given the imprecision of the dating, this can’t be taken as the oldest evidence of life, but it does suggest that the endolithic habitat was occupied early in Earth’s history.

“The interesting question now is why the bloody hell would early organisms want to do this,” Buick continues. “In more modern examples, it is presumed that the bugs are micromining for metal nutrients, but in anoxic early oceans, these metals should
have been more readily available and not something that would require . . . a great deal of energy to get a hold of. Perhaps they were after some other critically limiting nutrient, but the geological distribution of the microboring doesn’t offer strong hints as to what this might be.”

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HIV Disrupts Antimalarial Protective Effects of Pregnancy

Women who are pregnant for the first time are highly susceptible to malaria, a condition known as pregnancy-associated malaria (PAM). However, during subsequent pregnancies women typically build up resistance to PAM, except for those who become infected with HIV. Although pregnant women typically make more of the immunoglobulins that help to clear malaria parasites with each successive pregnancy, HIV disrupts this protective response, according to Kevin Kain and his collaborators at the University of Toronto in Toronto, Ontario, Canada.

Plasmodium falciparum cells concentrate in red blood cells of the placenta, where the parasites become coated with novel variant surface antigens of PAM (VSA-PAM) that specifically stimulate the production of IgG1 and IgG3 antibodies. These immunoglobulins, in turn, direct host macrophages to devour the parasite-pooled red blood cells via opsonic phagocytosis. The researchers determined levels of circulating antibodies as a way of estimating their activity in placentas.

Kain and his collaborators from Toronto and a malaria-infected area of Kenya in Africa collected plasma samples soon after women gave birth. In an in vitro assay, plasma samples from women who have had more than one child typically show high phagocytosis scores. Other samples, including those from women giving birth for the first time, men, and individuals who were never exposed to malaria, score about threefold lower.

HIV infections change this picture. HIV infections do little to drive already low scores lower for first-time pregnant women, according to Kain. However, for women on their second or subsequent pregnancies, HIV infections drive down their phagocytosis scores by about 50%. Levels of VSA-PAM specific IgG1 and IgG3 also are significantly lower in such HIV-positive women. Thus, HIV appears to impair the immunoglobulin-driven phagocytic clearance that otherwise protects such women, Kain and his colleagues report in the May 2007 PLoS Medicine.

Just how HIV intervenes to undermine this protective effect against malaria remains to be determined. Kain suspects that the susceptibility of HIV-positive women to PAM increases because they cannot clear parasites in the placenta. “HIV is known to meddle with normal B cell function,” he says, preventing the formation of antibodies or accelerating the loss of antibodies. “Either way, you end up with fewer antibodies.”

PAM kills 10,000 women and 200,000 infants yearly in malaria-infested regions of Africa and contributes to maternal anemia and low-birth-weight infants. About 1 million pregnancies yearly are complicated by coinfections of malaria and HIV. One goal among researchers is to design a vaccine to enhance production of VSA-PAM-specific IgG1 and IgG3, according to Kain. The vaccine would be used mainly among young females to boost antibody responses before any of them become pregnant. “We should be able to come up with a few reasonable antigens to stimulate IgG1 and IgG3 antibodies that would be protective,” he says.

Infectious disease specialist Julie Moore at the University of Georgia, Athens, agrees that it makes sense to develop specialized vaccines for specific population groups such as pregnant women. “It’s very unlikely that we’ll find a one-shot vaccine for everyone,” she says. Kain’s study “beautifully identifies a way to evaluate in the laboratory an indicator of protection against malaria in pregnant women,” she says. However, the pathogenesis of malaria and interactions with the immune system are very complicated, and “we’re just beginning to scratch the surface of how it all works.”

Carol Potera

Congress Proposes User Fees on Imports To Bolster Food and Drug Safety

With little fanfare last August, Representative John Dingell (D-Mich.) proposed sweeping legislation to reform the safety of imported foods and drugs. Dingell chairs the House Committee on Energy and Commerce, which has held a series of hearings on the safety of the food and drug supply. The new legislative proposal seeks to create a user fee on imported food and drug shipments, and funds generated would be used to hire additional personnel to conduct inspections at U.S. borders and to do tests in laboratories that are run by the Food and Drug Administration (FDA). Those user fees would also be used to test imported samples and to conduct research to develop new testing techniques. If passed, the legislation also would give FDA authority to issue mandatory product recalls and would require country-of-origin labeling of products.