tween one loop and another," Oppenheim says. "The way they are spaced, they are all facing the same direction." This geometric arrangement determines how the DNA interacts with the protein and what happens once bound. The DNA, says Oppenheim, "is more or less straight, but it becomes curved when Sp1 binds to it."

The specific binding between Sp1 and the capsid proteins is maintained even in the face of a 1,000-fold excess of cellular DNA, "providing strong support for the recruitment hypothesis," Oppenheim and her collaborators note. Apparently, the capsid protein "building blocks are recruited by Sp1 to *ses*, where they form the nucleation center for capsid assembly. By this mechanism, the virus ensures that capsid formation is initiated at a single site around its mini-chromosome." They also note that Sp1 "enhances the formation of SV40 pseudovirions in vitro, providing additional support for the model."

The strength of binding between Sp1 and the capsid proteins is maintained even in the face of a 1,000-fold excess of cellular DNA

"What [Oppenheim] has now shown, I think very beautifully, is what's required for encapsulation forming the nidus on which the capsid grows is this Sp1 complex, and so all she needs is the region around the origin where the Sp1 sites are," says Robert Martin, a research molecular biologist at the National Institutes of Health (NIH) in Bethesda, Md. "She can put any piece of cellular DNA and get fairly efficient encapsidation—which is a considerable advance towards using this thing for gene therapy."

However, an important drawback to Oppenheim's system is its limited packaging capacity, says James M. Mason, who is director of the gene therapy vector laboratory at North Shore-Long Island Jewish Research Institute, Manhasset Campus, North Shore University Hospital in New York. The viral genome is 5.2 kb, which sets a low upper limit on the size and number of genes that an SV40-based vector can transfer into target cells. The vector, which is now prepared in vitro, has been modified to hold 15 kb, with no need for an SV40 DNA sequence.

Another drawback is that the SV40-based vector lacks precision delivery, as do most vectors. "There is no way to deliver the gene to the right chromosome, the right segment, the right position," Martin says. The lack of precision delivery is a major problem where precision production is required, he adds. However, "Where all you need is a smidgeon and not every cell has to have it, there is a reasonable chance gene therapy will work."

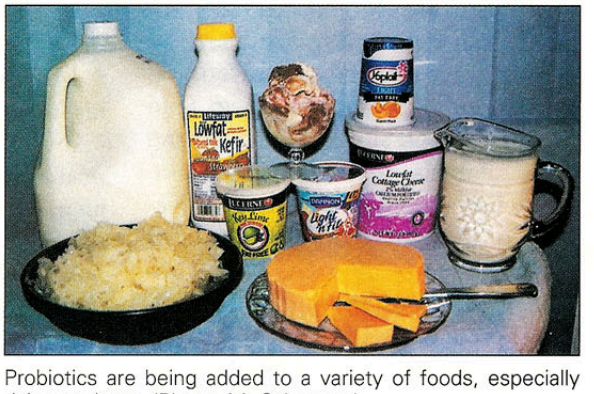
On the plus side, SV40 vectors packaged both in vivo and in vitro are very efficient transducers of human hematopoietic and liver cells, according to Oppenheim. Moreover, this vector is relatively safe, offering a means for delivering DNA into cells that does not deliver genes that could cause illness or cancer because everything is produced in vitro and because very little viral DNA is involved. For instance, the capsid proteins can be manufactured in insect cells, and the plasmid that carries the therapeutic gene, along with the little bit of viral DNA that is required for binding Sp1 is produced in *Escherichia coli*.

David Holzman
David Holzman writes from Lexington, Mass.

Much Ferment on the Probiotics Front

Researchers have launched the first scientific society and established the first institute in North America dedicated to studying probiotics—live microorganisms consumed or applied to specific anatomic sites for their purported health benefits. The International Scientific Association for Probiotics and Prebiotics (ISAPP) held its inaugural conference 3–5 May in London, Ontario, Canada, attended by 70 researchers from five continents. A day earlier, researchers at the Lawson Health Research Institute and the University of Western Ontario (UWO) in London, Ontario, inaugurated the Canadian Research and Development Centre for Probiotics (CRCDP).

More than two dozen strains of bacteria, mostly in the genera *Lactobacillus* and *Bifidobacterium*, are used as dietary supplements, according to a recent report from the Swiss market-research firm Giract. Moreover, probiotic-containing foods and supplements, including yogurts, fermented milk, capsules, and powders, now make up a worldwide product sector that is worth hundreds of millions of dollars in sales per year. And prebiotics—otherwise indigestible food ingredients that, when consumed, fuel the growth of beneficial gut microbes—represent a similar product sector.



Probiotics are being added to a variety of foods, especially dairy products. (Photo: M. Schwartz.)

For researchers in these fields, however, respect has been elusive, in part because some companies make unsupported health claims about these products. What's more, mainstream medical researchers have been slow to recognize the potential health value of these microbe-containing products, according to Gregor Reid, director of CRCDP and UWO professor of immunology and microbiology. "This field has not been trendy and has had next to no funding, but it has major implications for the health of people," he says.

Hence, a group of microbiologists, dairy scientists, geneticists, immunologists, and gastroenterologists formed the multidisciplinary ISAPP. "We wanted to provide an opportunity for everybody to get together without relying on [a single company] to do it," says Mary Ellen Sanders, the first president of the new association, who is an adjunct research professor at California Polytechnic State University in San Luis Obispo, Calif., and runs a consulting business in Centennial, Colo., that advises food, dairy, and dietary supplement companies. ISAPP's main financial support so far has come from dairy and food industry groups, with some help from the government of the United Kingdom. CRCDP was funded initially by $1.7 million from the Ontario provincial government and $4.2 million from industry groups, UWO, and the University of Guelph.

Despite skepticism in some circles, available evidence indicates that probiotics can treat or prevent diarrhea, urinary tract infections, and food allergies, and could one day help hold off gut diseases, surgical wounds, and other ailments, Reid says. For example, when administered as a supplement or in fermented milk, a purified form of *Lactobacillus* GG (LGG) appeared effective in double-blinded, placebo-controlled clinical trials against different types of diarrhea, including rotavirus-induced diarrhea in children, antibiotic-associated diarrhea, and traveler's diarrhea.

This strain also helps to prevent a recurrent food-associated allergic condition called atopic eczema in infants, according to a double-blind, placebo-controlled clinical trial reported last year in *The Lancet* by Erika Isolauri of Turku University in Turku, Finland [357:1076–1079, 2001)]. In addition, earlier this year Reid and several colleagues reported that probiotics appear effective for treating vaginal infections in humans. In a separate study, *Lactobacillus* RC-14 prevented surgical wound infections in rats. The bacterium or the biosurfactant that it produces kept *Staphylococcus aureus*, a potentially life-threatening pathogen, from sticking to cells and causing subcutaneous abscesses, according to Jeffrey Howard of UWO, Reid, and their colleagues.

Such studies are just the beginning, says Todd Klaenhammer of North Carolina State University in Raleigh. The genomes of five probiotic *Lactobacillus* and *Bifidobacterium* strains are already sequenced, and genome sequences of 12 additional probiotic strains are on the way. With such extensive genomic data becoming available, researchers plan to design chips with which to pinpoint genes critical for colonizing, interacting with, and functioning in human tissues, such as the gut and vagina, and to knock out specific bacterial genes to evaluate their roles in these host-related interactions. "The field is now positioned to do the right microbiology and the right clinical trials," he says.

Meanwhile, regulators are searching to define some standards for the many products that are marketed as probiotics. The influential Codex Alimentarius, an advisory commission of the U.N. Food and Agriculture Organization and World Health Organization that recommends food-safety standards to individual countries, accepted new guidelines for probiotic products in June. Tighter standards and better science are needed to improve the credibility of the probiotics field, which could have a big impact on human health, according to Reid. "Science should be driving this, not quackery or industry," he says.

Dan Ferber
Dan Ferber writes from Urbana, Ill.

Insights May Explain High Sensitivity of *E. coli* Chemoreceptors

Chemical receptors form clusters at the poles of *Escherichia coli* cells and act like highly sensitive "noses," responding to the faintest whiff of particular chemicals. How the receptors react depends on the company they keep, perhaps explaining how bacteria sense even slight fluxes in local nutrients, sometimes responding as if a mere morsel were a veritable feast. Typical *E. coli* cells carry five different types of chemoreceptors for responding to different types of nutrients. These sensors elicit a signal cascade that directs the cell's flagella to rotate counterclockwise, enabling the bacterium to swim toward those nutrients. However, interactions between nutrient molecules and their corresponding receptors are fleeting, with each contact lasting a fraction of a second. How, then, are such brief encounters amplified into a loud call to dinner?

In addressing that question, John S. Parkinson, Peter Ames, and colleagues at the University of Utah in Salt Lake City, Utah, investigated interactions between two of the five types of chemoreceptors—one set responding to the amino acid serine and the other to aspartate. As part of their approach, they created mutant receptors that are less proficient at forming clusters. They then asked how the mutant receptors affect signaling by normal receptors in the cell. Their findings are published in the May 14 issue of the *Proceedings of the National Academy of Sciences* (PNAS) (99:7060–7065).

"We've known for a long time that