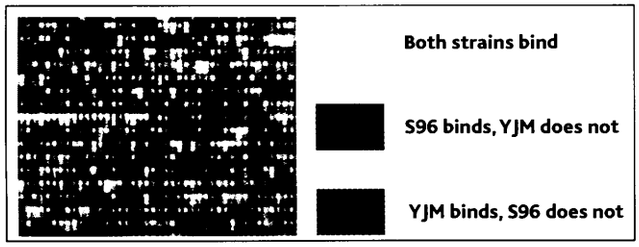
GENOMICS

DNA Chips Survey an Entire Genome

Rack up another jackpot for DNA chips. Already these postage stamp–sized arrays of DNA snippets have proven themselves adept at providing snapshots of all the genes expressed in particular tissues. Now they appear poised to reshape the future of gene mapping, the effort of tracking down which genes contribute to different traits. Earlier this year, one U.S. team used DNA chips to map thousands of commonly varying sites in DNA fragments sampled throughout the human genome (*Science*, 15 May, p. 1077). And now, in work described on page 1194, researchers report for the first time that they have used chips to map an entire genome—that of yeast—in one fell swoop.



On display. Composite computer image of two DNA chips reveals binding patterns of DNA fragments from yeast strains S96 and YJM.

FROM WINZELER *ET AL.*

The success, say genetics researchers, could pave the way to a better understanding of conditions, such as virulence in pathogenic microorganisms and susceptibility to heart disease in humans, thought to be caused by the contributions of several genes. "This is a brand-new age. It's really exciting," says Jasper Rine, a geneticist at the University of California, Berkeley. Still, because current chips offer complete surveys only of relatively small genomes, or samples of larger ones, advances in identifying the microbial genes are expected to come much faster.

Pinpointing the location of the genes for traits controlled by single genes is relatively straightforward. Researchers simply compare the DNA of families that share the trait in question to see how often it's inherited together with "markers" at known locations along the chromosomes. But conventional gene-mapping techniques can look only at how a relatively small number of genetic markers differ between individuals, and it becomes unwieldy when tracking so-called quantitative traits that may be caused by the combined workings of several genes scattered throughout the genome.

To circumvent that problem, geneticists Elizabeth Winzeler, Dan Richards, and Ronald Davis, along with their colleagues at Stanford and Duke universities, turned to the DNA chip technology currently under development at the biotech firm Affymetrix, in Santa Clara, California. Affymetrix researchers had previously designed chips composed of over 150,000 snippets of yeast DNA dotted across a 2-centimeter-square silicon wafer. These snippets correspond to overlapping DNA fragments from a complete yeast genome sequenced in 1996. Researchers at Affymetrix and elsewhere have shown that they can create an instant snapshot of all the proteins being made in given cells by exposing such chips to the cell's RNA molecules—each of which corresponds to an active gene—and seeing where the RNAs bind to the known sequences on the chip (*Science*, 3 June 1994, p. 1400).

To map yeast genes, the Stanford, Duke, and Affymetrix researchers took advantage of the same basic chip technology. Their strategy consisted of two parts. First, they created a map of the yeast genome with known genetic landmarks along the way. Next, they showed they could use this map to track the inheritance patterns of markers—and thus of genes—through generations of yeast.

For the mapping phase, the researchers obtained DNA from two different yeast strains, called S96 and YJM789, and used enzymes to break them into small pieces. After tagging the pieces with a fluorescent compound that allows them to be detected, the team applied the fragments to the chips, one yeast strain at a time. If the two sequences matched exactly, the pieces would bind to those in the array. The result was two chips, each displaying different patterns of bright spots showing where the DNA fragments being tested correspond to those on the chip.

Because the S96 genome is virtually identical to that of the strain used to prepare the chip, almost all of the S96 fragments matched those on the chip. But tests on the second strain—YJM789—revealed over 3000 spots on the array with little fluorescence, denoting mismatches, or differences, with the reference strain. The researchers have not yet determined what most of those differences are. But even without that information, Winzeler says, the spots can still serve as genetic markers.

To make a map, the researchers had to figure out where each marker belonged on the yeast genome. That was straightforward, because the full sequence of the yeast genome is known along with the sequence of each of the snippets on the chip. By comparing the sequences of the snippets that didn't bind to any of the test yeast fragments with the genome sequence, the researchers could determine where each of their genetic landmarks fell on the genome.

In the next step in their study, the researchers used this map to locate—simultaneously—four control genes whose positions in the yeast genome were already known, plus a previously unidentified gene involved in yeast's resistance to an antifungal compound known as cycloheximide. Strain YJM789 is susceptible to the drug, while S96 is not. So the researchers mated the organisms and tested their progeny. By looking for genetic markers shared by all the drug-resistant progeny, the researchers were able to pinpoint the location of the drug resistance gene to a 57,000–base pair region on chromosome 15. The researchers also found that the control genes mapped to their expected locations. The team hopes to extend this demonstration to track down the multiple genes that contribute to virulence, says Duke team member John McCusker.

Rine says the work is "a terrific demonstration" of how researchers can now analyze entire genomes in a single step—an achievement that should aid efforts to map multigene traits. But Kenneth Weiss, a geneticist at Pennsylvania State University in University Park, cautions that identifying the genes responsible for complex human traits may still be difficult. He notes, for example, that some 50 genes may influence susceptibility to heart disease and that many different combinations of mutations in those genes may all lead to the same outward manifestation: heart trouble. That's likely to frustrate efforts to link the condition to just a few key genes. But at least the chips will allow researchers to try something that was previously impossible.

–ROBERT F. SERVICE

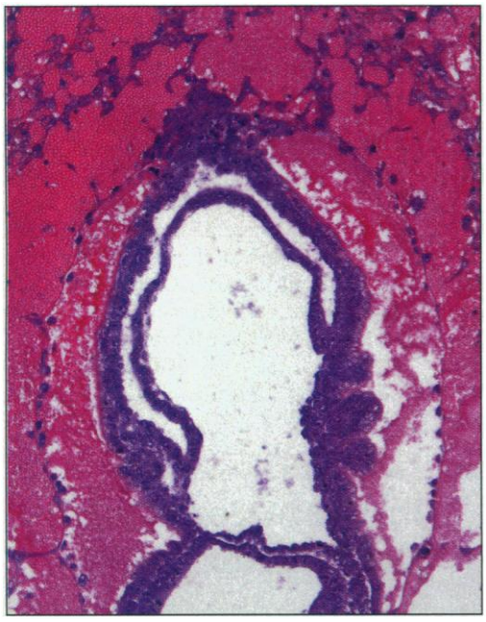
IMMUNOLOGY

How Embryos May Avoid Immune Attack

The portrait of an infatuated mother coddling her newborn baby is a fixture in most family photo albums. But before such a touching picture could be taken, the baby had to survive a potentially fatal conflict with its mother: developing as an essentially foreign tissue within the womb without triggering a hostile immune attack. How the fetus does that has long been a mystery, but new results in this issue may provide an answer.

Previous explanations have included the possibility that the mother somehow suppresses her immune responses to the baby, or that the placenta acts as an anatomical barrier to her immune cells. But on page 1191, Andrew Mellor, David Munn, and their colleagues at the Medical College of Georgia in Augusta report evidence indicating instead that the embryo actively shuts down the mother's natural defenses. In their scenario, embryonic cells in the placenta manufacture

MUNN *ET AL.*



PHOTO, RIGHT: NASA

Target. The micrograph shows an embryo coming under immune attack after the mother was treated with an IDO inhibitor.

an enzyme known as indoleamine 2,3-dioxygenase (IDO), which destroys an amino acid, tryptophan, that the mother's immune sentries, known as T cells, need to do their job. Immunologist Phillipa Marrack of the National Jewish Hospital in Denver, Colorado, describes the observations as "very striking, interesting, and provocative."

In addition to explaining, as Mellor puts it, "why we, as mammals, survive gestation," the results—if they hold up—might help women with a history of spontaneous miscarriages. If those miscarriages are due to failure of the fetal cells to produce enough IDO, then drugs might be developed that mimic the enzyme's T cell–dampening effects. Such drugs might even be useful for preventing transplant rejection and treating autoimmune diseases. And conversely, compounds that inhibit IDO might lead to abortifacients that work by boosting the mother's innate rejection response.

Munn, a pediatric oncologist, did not set out to determine what protects the fetus from immune attack. He was searching for ways in which immune cells known as macrophages might activate the tumor cell–killing potential of T cells. Instead, Munn's team found that in their cell culture system, the macrophages were sedating the T cells, apparently because they were somehow destroying tryptophan. Since T cells, like other human cells, can't make their own tryptophan—it has to be supplied in the diet—they were unable to replicate, as they usually do when activated. To try to figure out whether tryptophan depletion might have a role in living animals, the oncologist teamed up with Mellor, a reproductive immunologist, and together the two groups showed that the amino acid declined in the cultures because the macrophages were making IDO.

Other researchers had shown in the early 1990s that the enzyme, in addition to being made by macrophages, is also produced in the placenta by fetus-derived cells called syncytiotrophoblasts. That finding suggested another possibility. We hypothesized, Mellor recalls, "that IDO prevents the maternal T cell response to [genetically foreign] fetuses, and inhibiting the enzyme would cause the mothers to abort."

That is exactly what the researchers have now found. For their experiments, Munn, Mellor, and their colleagues used two groups of pregnant mice. One set had been bred to genetically identical fathers of the same inbred strain while the other was bred to fathers from a genetically different strain. When the researchers implanted time-release capsules containing either the IDO inhibitor 1-methyl-tryptophan or a control substance under the skin of the pregnant animals, they found fetal rejection in only one group: mice that had been given the inhibitor and were carrying genetically foreign fetuses. The embryos developed normally at first, but then inflammatory cells moved in and extensive hemorrhaging occurred around the embryos. "The mother is rejecting the placenta and eventually the embryo chokes off and dies," Munn says.

Other experiments pointed to the mothers' T cells as instigators of the attack. The researchers mated mice that were genetically identical, except that the males had been engineered to carry a gene for an immune system protein that induces potent T cell responses. Treating the pregnant females with 1-methyl-tryptophan caused them to reject their fetuses, indicating that that single antigen was all it took to get the response. More direct proof for T cell involvement came when the researchers added T cells that recognize the antigen to mice that can't produce T or B cells and found that this could restore the response to the inhibitor.

From these results, Munn and Mellor propose that once the embryo implants and begins establishing connections with the mother's blood supply, fetal-derived cells located in the placenta begin making IDO. By destroying tryptophan, the enzyme then suppresses the activity of maternal T cells that would otherwise make their way through the placenta and attack the fetal blood supply.

Some researchers have reservations about this scenario. As immunologist Joan Hunt of the University of Kansas in Kansas City points out, for example, since the embryo can't make the tryptophan it needs to

ScienceScope

EARTH PROBES EARTHBOUND

It is proving harder for NASA to explore Earth than to send spacecraft millions of kilometers to Jupiter. Launches of two Earth observation satellites are again on hold due to technical problems, hobbling researchers' efforts to gather data on everything from land-use changes to the carbon cycle.

Landsat 7 and EOS-AM1 were slated for launch earlier this summer, but NASA officials now say neither will be ready for orbit until next year. During a recent thermal vacuum test, key components failed on the $444 million Landsat 7, according to James Irons, the project's deputy scientist. Meanwhile, problems with the flight operations software for the $1.2 billion EOS-AM1, the first major spacecraft in the Earth Observing System series, likely will set that launch back until next summer, according to agency officials.

Landsat image of Virginia.

INTERNET'S FAST TRACK

The faster Internet being built by the federal government is supposed to help knit the research community more tightly together. But scientists working in academic outposts worry that it may actually make them second-class cybercitizens. That's because most rural researchers can't afford to pay the higher tolls needed to get onto the Internet's fast lane, says Joe Thompson of Mississippi State University, who has invited academic, government, and industry leaders to his campus next month to hash out the problem.

"Universities in nonurban states are at a significant disadvantage," says the engineer, who notes it can cost up to 10 times more for rural institutions to hook up to high-speed computer connections, which rely on infrastructure typically installed first in urban areas. Under pressure from Congress, the National Science Foundation last year began offering extra hookup funds to nonurban researchers (*Science*, 13 June 1997, p. 1639), but Thompson claims the bonus has proved "an order of magnitude too small" to cover real costs.

Contributors: Dennis Normile, Li Hui, Jennifer Couzin, Andrew Lawler, and David Malakoff

produce proteins and grow, it's hard to understand how it could survive if the amino acid is destroyed in the placenta.

Munn and Mellor concede that more work will be required to show that loss of tryptophan, and not some currently unsuspected consequence of IDO action, is behind the embryo's ability to ward off an immune attack. They say they intend to pursue this issue in further mouse studies. And the investigators also want to see if possible defects in IDO production or action in the placenta might be linked to the repeated miscarriages experienced by some women.

In addition, immunologists will want to explore hints that IDO might have a broader role in immune regulation. The Georgia team has evidence in lab animals that the enzyme also suppresses the activity of T cells that might otherwise attack the body's own tissues. If so, then the researchers may have tapped into a new arena from which to look at the immune system's checks and balances, especially in patients with autoimmune illnesses. "We have come up with a natural immunosuppressive mechanism that is linked to an evolutionarily ancient mechanism: nutrient depletion," Mellor says. "And placental mammals have adapted it in a dramatic way to protect their fetuses."

–TRISHA GURA

Trisha Gura is a science writer in Cleveland, Ohio.

PLANETARY SCIENCE

Neptune's Hasty Moon Poses Celestial Puzzle

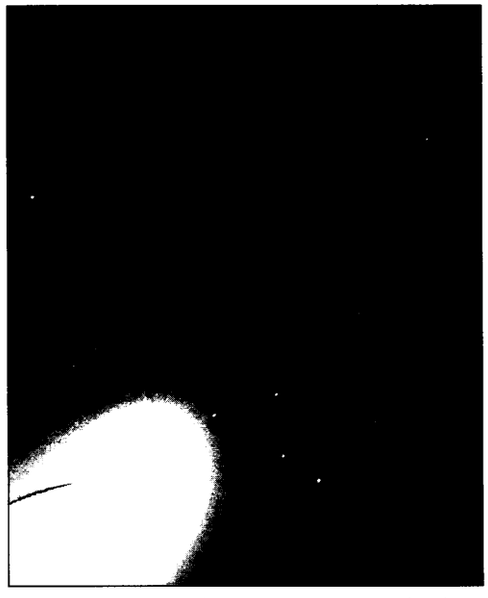
Ever since Newton, astronomers have been calculating the orbits of planets and moons and getting them exactly right. But last week, a team of observers reported that Galatea, a small satellite of the planet Neptune, is a few minutes ahead of schedule. To explain this puzzling haste, astronomers are blaming everything from the gravitational tug of Neptune's mysterious Adams ring to the pull of other, undiscovered moons to an error in the original orbital predictions.

A team led by Claude Roddier of the Institute for Astronomy of the University of Hawaii, Honolulu, learned that the 160-kilometer moon was straying from its orbital timetable on 6 July, when they tracked it down with the 3.6-meter Canada-France-Hawaii Telescope on Mauna Kea. The observations—the first in the 9 years since Galatea was discovered by the Voyager 2 spacecraft—showed that Galatea was 5±1 degrees ahead of its predicted position, or 8.6 minutes ahead of schedule. The difference, they said in an 11 August circular of the International Astronomical Union, is "possibly due to [Galatea's] interaction with Neptune's Adams ring."

The Adams ring, lying a mere 1000 kilometers outside Galatea's orbit, has a strange, arclike appearance, indicating that its dust particles aren't spread evenly around its full circumference. Galatea's gravity is presumably sweeping the particles into clumps, as Carolyn Porco of the Lunar and Planetary Laboratory of the University of Arizona, Tucson, showed in 1991 (*Science*, 30 August 1991, p. 995). But for the ring to pull back strongly enough to affect the satellite's orbit, Porco says, it "would have to have substantial mass." She speculates "that there are bigger bodies within [the arcs], which are the source of the dust that we actually see."

Brian Marsden of the Harvard-Smithsonian Center for Astrophysics in Cambridge, Massachusetts, isn't so sure that there's a deviation to explain. For Galatea's orbit to accumulate five degrees of drift in 9 years, its half-day period would have to differ from its predicted value by a mere 0.07 second. "My own inclination is that the prediction is off simply because the observations used for it were only [a limited number of images] from Voyager," he says. Porco disagrees. "There were lots of observations of Galatea by Voyager," she says. "I doubt they are in error."

If the prediction isn't at fault, Marsden says, the gravitational effects of other satellites, or of Neptune's own oblate shape, could have skewed Galatea's orbit, as could a perturbation from a small unknown satellite in nearly the same orbit as Galatea. "Perhaps," agrees Porco, "[but] it would have to be small enough to have escaped detection by the Voyager cameras," which, she says, could spot a 6-kilometer object.



NASA

Orbital mystery. Do bright arcs on Neptune's Adams ring hold clues to Galatea's quickness?

She notes, however, that there's a problem even if the Adams ring is to blame. An interaction between satellite and ring could speed up Galatea, but only if the objects and particles in the ring are colliding with one another "because otherwise the gravitational interaction is not 'shared,' so to speak, among all the bodies in the ring." But, Porco adds, "if there are colliding particles, then the arcs wouldn't stick around very long. The net result: a faster Galatea leaves us with a big puzzle, and I wonder if [the new observation] will stand the test of time."

–GOVERT SCHILLING

Govert Schilling is an astronomy writer in Utrecht, the Netherlands.

SCIENCE IN SOCIETY

Institute Copes With Genetic Hot Potato

A premature warning about the potential dangers of transgenic potatoes sparked a global media frenzy last week and appears to have ended the career of a food safety expert at the Rowett Research Institute in Aberdeen, Scotland. In a press statement, the institute said it regretted "the release of misleading information about issues of such importance."

The incident is the latest high-profile setback for agricultural biotechnology, which in Europe is still struggling to gain consumer acceptance (*Science*, 7 August, p. 768). Indeed, activists have torn up dozens of trial plots in Europe over the last year, and in a June interview with the *Daily Telegraph*, Prince Charles declared that tinkering with genes for food production "takes mankind into realms that belong to God and God alone."

That was the backdrop for the 10 August British TV show "World in Action," on which Rowett researcher Arpad Pusztai announced findings on rats fed potatoes containing the gene for concanavalin A, or Con A, a compound found in jack beans. Con A is a member of the lectins, a huge family of insecticides that occur naturally in plants. Biotech companies have spliced lectin genes into various crops, to try to get them to resist insect pests. Pusztai warned, however, that rats in his experiments suffered from stunted growth and suppressed immune function. He said more safety research was needed, adding: "If you gave me the choice now, I wouldn't eat it."

Even before the show aired, the institute was flooded with calls from journalists who had received a press release touting Pusztai's comments. In most of the ensuing coverage, reporters failed to distinguish between genetic engineering and the specific use of lectins, making it appear that Pusztai warned against eating anything transgenic.