MOLECULAR BIOLOGY

A Faster Walk Along the Genome

If there's to be any hope of completing the Human Genome Project on schedule and within budget, geneticists agree they need more efficient ways to sequence the genome. But many experts doubt that the currently favored "shotgun" method—which relies on sequencing a random selection of overlapping DNA fragments, then piecing them together—will ever become much faster and cheaper. Larger gains could come, say some sequencers, from a new twist on a technique called primer walking, in which geneticists move steadily along a longer strand of DNA, sequencing contiguous stretches of it.

So far, the time and expense needed to make the primers—short DNA sequences that define the starting point for each sequencing run—have handicapped the technique. Now, however, several groups claim to have eased that step. If the technique lives up to its promise, says one of the researchers, Mathias Uhlén at the Royal Institute of Technology in Stockholm, it could lower the cost of sequencing by a factor of 10 or more and produce similar gains in speed.

The problem with the shotgun method is that it contains two bottlenecks. The first is the initial step of cloning the many different fragments of a DNA strand into sequencing vectors—typically circular strands of DNA from a phage virus. The fragments are sequenced by adding a short primer, usually consisting of 15 or more bases, that binds to a known sequence in the vector and serves as the starting point for producing a series of strands complementary to the unknown DNA, which can be analyzed by gel electrophoresis to read the sequence. Then comes the second bottleneck: figuring out where each fragment fitted on the original, continuous DNA.

Primer walking, in contrast, has only one cloning step: A single stretch of DNA is inserted in its entirety into a larger vector. And there's no need to piece together random fragments of DNA, either. Instead, after their first sequencing run, geneticists make a new primer that binds near the end of the stretch of DNA they've just sequenced—and repeat the process over and over until they've sequenced the whole of their unknown DNA.

Large-scale sequencers have turned their backs on primer walking in the past, however, because making a custom-designed primer for each sequencing run introduces lengthy delays. Now, four groups are testing libraries of ready-made modules, which join up into effective primers at the right spot on the template.

In 1990, Waclaw Szybalski of the University of Wisconsin proposed in a paper in *Gene* that the ideal modules might be six-base sequences called hexamers, which come in only 4096 possible unique forms. Molecular geneticist William Studier of the Brookhaven National Laboratory was the first to put that suggestion to the test. As he reported late last year in *Science* (11 December 1992, p. 1787), he and two colleagues coated a DNA template with a DNA binding protein, then added the three hexamers that, combined, would form the desired primer sequence. Studier found that the hexamers displaced the protein only where they could bind to the template side by side, mimicking an orthodox 18-base sequencing primer.

In the 1 May *Proceedings of the National Academy of Sciences*, Levy Ulanovsky and his colleagues at the Weizmann Institute in Rehovot, Israel, report a similar success—only these researchers managed to prime sequencing reactions using strings of three hexamers even without shielding the template with binding protein. Meanwhile, the two remaining groups, one led by Uhlén and another led by Szybalski, have added the extra step of covalently bonding their hexamers together after they've bound to the template.

All four groups are now working furiously toward the same goal: automation. That should make it possible, Szybalski says, for a group of fewer than 10 people to sequence the entire genome of the bacterium *E. coli* within a few weeks—a task that would take several years with today's technology.

Most big-time shotgun sequencers are much more cautious, however. "It's interesting," says John Sulston, head of the Sanger Center in Cambridge, England, home of one of the world's largest sequencing initiatives. "But it's yet to be proved on a large scale." He's particularly worried that the frequent repeated sequences found in the genomes of higher organisms will pose a major obstacle, causing the hexamers to bind at multiple positions along the same template.

The primer walking enthusiasts acknowledge the problems but remain confident that they can be solved. "This technology is at the stage those people were at 3 to 5 years ago," says Studier. Within a year or two, he's confident that primer walking will be a "bread-and-butter technique" for the world's large-scale sequencers.

–Peter Aldhous

RESEARCH IN JAPAN

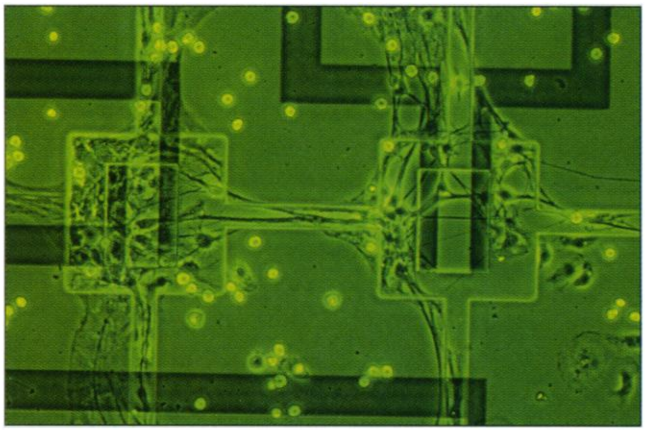
Computer Firms Look to the Brain

In a cramped laboratory in the western suburbs of Tokyo, researcher Akio Kawana sends pulses of electricity across his specialized culture dishes and measures the faint electrical signals given off by nerve cells as they communicate with one another. The scene itself is not unusual. Scientists doing similar work can be found in many neuroscience labs at universities and research institutes around the world. But Kawana doesn't work in an academic laboratory. His employer is Nippon Telegraph and Telephone (NTT), the world's second largest telecommunications company (AT&T is first). Why would a former optical fiber engineer at an electronics giant like NTT be interested in nerve cell behavior? And here's a better question: At the very moment in scientific history when the premier U.S. corporate research labs are shutting down basic research efforts, why would NTT encourage work so seemingly unrelated to its business?

Neural learners. Brain researchers *(from left)* Fukunishi, Sekiguchi, and Tanaka.

And NTT is not the only Japanese company to which the question applies. Rather than relying just on academic research, several of Japan's corporate giants are betting that pure research today will guarantee leadership tomorrow in computers. Since present-day computers are bad at higher forms of information processing, such as recognizing spoken language, a person's face, or making logical inferences about what they are told—abilities that children have but which elude multimillion-dollar supercomputers—Japanese corporate research directors are looking to mimic the human brain's information processing capabilities. Enter Kawana—who hopes that what he learns from small networks of nerve cells will help him understand how information is processed in the brain—and a raft of Japanese researchers at more than four of Japan's major electronics corporations, all directing portions of their R&D budgets toward neurobiology research. "We want to elucidate the coding of the brain, its grammar. We want to develop new computers with new architectures using new algorithms," says Sanyo Electric bioresearcher Tatsuhiko Sekiguchi.

Indeed, the companies' neuroscience efforts belie the popular perception in the United States that Japan is a copy-cat nation that improves on technology discovered elsewhere rather than inventing it. To the contrary, Japan's corporate giants are willing to take a long-term business view and put money into research that might not have an immediate payoff. NEC Corp., for example, established research laboratories back in 1939, and company scientists have been performing neurobiology research since the late 1970s. NEC also runs a research lab in Princeton, New Jersey, that has a team in theoretical neuroscience.


NTT

Computer of the future? Studies of networks of neurons like these may provide information that leads to a new computer era.

Unlike much of the secretive R&D done in Japan, the corporate neuroscientists publish their results in international journals, including *Science*, winning plaudits from their colleagues abroad. "Several people have international reputations," says neuroscientist Alan Gelperin of Bell Labs. "They operate as members of the international community and are open to communications in both directions." Michael Stryker, a physiology professor at the University of California, San Francisco, agrees: "I think that in many areas, particularly in integrative neuroscience, in the kind of neuroscience of studying hard things that the brain does, like perception, there are several laboratories in Japan doing work that is absolutely as good as the best in the world." Which is why *Science* embarked on a 2-week tour of several labs to see for itself what was going on. What did we find?

High spirits, for one thing. Unlike their colleagues at IBM (*Science*, 23 April, p. 480) or Bell Labs, who are dispirited with the plummeting corporate commitments to basic research, Japanese company researchers have taken to comparing their facilities to the old Bell Laboratories. From manicured laboratory grounds, well-equipped labs, and good company cafeteria food to ample travel budgets and contacts with colleagues overseas, the researchers have few complaints. Take, for example, Shigeru Tanaka, who works at NEC's Fundamental Research Laboratories in Tsukuba, the government-sponsored "science city" north of Tokyo. Backed by an ¥80 million (about $720,000) annual budget, Tanaka can recount times when he has been the only user on-line to NEC's own top-of-the-line supercomputers—an enviable situation since some of his calculations require several hours of computing time to complete. He's doing this number-crunching as part of his efforts to model the structure of the brain's gray matter.

Neurobiologists have known for years that neurons in the visual cortex, which is the part of the brain where nerve impulses from the eyes are assembled into images, become grouped into ocular dominance columns. The columns are believed to be the brain's processing centers for visual information. Using his company's SX-2 supercomputer and ideas borrowed from theoretical physics, Tanaka has developed a mathematical model that closely simulates how ocular dominance columns form in primate brains.

Tanaka is now trying to learn how visual stimulation regulates the formation of the columns. He believes that by developing an understanding of one small area of the visual cortex a model of the entire brain's functions can be created one day. "Anatomists say there are quite different architectures and shapes of neurons in different areas [of the brain]. But from the viewpoint of information processing, we feel the structure of the brain is quite uniform and similar," explains Tanaka.

If this sounds far from the real world, NEC research managers report that they are already beginning to get some return from Tanaka's work, although in the realm of computer software, rather than hardware. His computer simulations of the organization of neurons have led to a new mathematical formulation of neural network systems that is being tested by NEC's software engineers.

Over at Hitachi's Advanced Research Laboratory in the woodlands of Saitama Prefecture, 2 hours from the smog and crowding of Tokyo, Kohyu Fukunishi is trying to discover how the brain hears. The pride of his well-furnished lab is a special 128-channel high-speed optical recording system that he uses to videotape the movement of electrical currents on the surface of the auditory cortex region of guinea pig brains as the animals listen to sounds. Fukunishi first places a voltage-sensitive dye on the surface of the auditory cortex and then uses a sensitive camera to pick up the rapid changes in the dye's fluorescence during sound stimulation. Using slow motion replays he is able to view the propagation of the electrical currents across the auditory cortex.

Fukunishi has been able to identify neuron centers that react only to certain audio frequencies. It is a small step toward creating a thinking computer, but he is convinced that further analysis of the brain's information processing system will pay off. "We can use hints of how the brain works to help computation [theory]....If we don't use knowledge of the brain until we understand the whole brain, it will take a long time (to create thinking machines)," he says.

Fukunishi would next like to study human brain activity using ultrasensitive SQUID (for superconducting quantum interference device) magnetic detectors placed on the surface of the head. But he has run into one drawback of working for a private company in Japan. A custom-built $5 million room specially insulated against radio waves and other forms of electromagnetic interference stands empty in a corner of the lab since the American company from which he would like to buy SQUIDs has refused to sell them to Hitachi. According to Fukunishi, the company is afraid Hitachi will copy the devices and market them. The firm's position may be shortsighted since Fukunishi has now asked Hitachi's Central Research Laboratory independently to develop SQUIDs for his work. He expects to get them in 2 years.

Tatsuhiko Sekiguchi has been trying to understand one of the highest levels of brain function, memory and learning ability. At Sanyo Electric's Tsukuba Research Center, Sekiguchi has spent 5 years teaching slugs new tricks. Slugs (*Limax flavus*) are well-known research animals with a love for carrot juice and a distaste for garlic. By quickly chilling the animals to induce amnesia immediately after teaching them to avoid the smell of carrots by giving them a bitter taste, Sekiguchi has been able to identify distinctive short and long-term memory states in the slugs.

His colleague, Tetsuya Kimura, has also measured the continuous, coordinated electrical oscillations of neurons on the slug brain surface. He has found they are modulated up and down depending on whether the slug smells carrots or garlic. The reasons for the neurons' oscillating electrical activity are not yet understood, but the research points to an information processing system very different from any invented so far by the computer industry. Sekiguchi says: "There have been no models for neural processing showing oscillating activity." While the work seems unconnected with Sanyo's bread-and-butter electronics business, the goal of Sekiguchi's 12-person biosystems group is one day to develop a model of biological information processing that could be used to build computers.

And finally, let's come back to NTT's Kawana. His measurements of the electrical communication that goes on within small networks of neurons seem to reveal the rudiments of organized behavior among the neurons. "The entire network, about every 10 seconds, fires and then is silent," he says. Understanding the spontaneous and coordinated firing of the networks of neurons is now the focus of his lab's research. At this early stage of the research, no final answers have yet emerged, and it's still too early to tell whether the information will help in computer design.

Indeed, the same can be said of the other industrial lab-based neuroscience programs, raising a question about just how promising they are. One answer might come from the fact that none of these private labs has yet surpassed the best research being done elsewhere in the world.

Given the long road that will have to be traveled before the companies see any fruits of their basic neuroscience research, there's also the question of how long the companies will continue to fund the research, especially considering the fact that after enjoying a booming economy for much of the 1980s, Japan is now in a recession. But so far, at least, bioscience researchers say that they have not experienced cutbacks, although some feel pressure. "Bio-research is expected to have a great impact on Sanyo's future business, so it seems very important to continue small-scale research despite the severe economic conditions," says Shigeru Mackawa, the head of Sanyo's Tsukuba Research Center.

If Japan's corporate neuroscientists can persevere, theoretical neuroscientist Christof Koch of the California Institute of Technology is optimistic about their prospects: "Look at Bell Labs. They developed the transistor, they developed the laser. They have a spectacular record. I don't see why it should be different in Japan."

–Toomas Koppel

Toomas Koppel is a science writer based in Tokyo.

PHYSICS

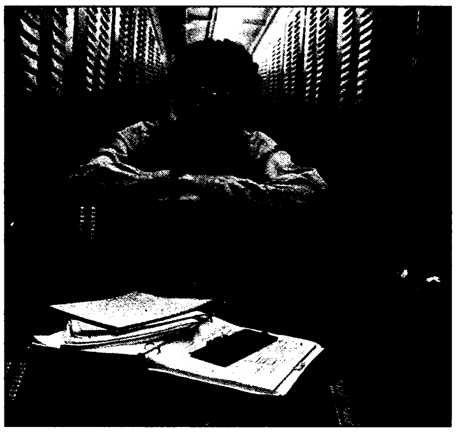
Mass Calculations Add Weight to QCD

Theories of fundamental particles and forces aren't easy to put to the test. Physicists usually do so by whirling protons or electrons at high speeds in particle accelerators, smashing them together, then analyzing the debris for telltale traces that would confirm their ideas. But a group of IBM researchers has now taken another tack to make one of the most stringent tests yet of quantum chromodynamics (QCD), a theory that is a key part of physicists' picture of the subatomic world.

Rather than depending on the energies achieved in a particle accelerator, the researchers, led by physicist Don Weingarten, relied on another kind of brute force: a year of intense calculations in a special-purpose supercomputer. By showing that QCD correctly predicts the masses of the proton, neutron, pion, and others of the fundamental particles called hadrons, the calculations give additional support to QCD, says Daniel Zwanziger, a theoretical physicist at New York University. They also provide an impressive demonstration of what some researchers are calling the "third branch of science"—using superpowerful computers to simulate physical processes with such fidelity that many researchers think of them as more like experiments than theoretical calculations.

QCD is the theory that describes the "strong force," the fundamental force that, among other things, holds together protons and neutrons in the atomic nucleus. The theory posits that protons, neutrons, and other hadrons (the particles that respond to the strong force) are made up of quarks and antiquarks, all held together by gluons. A proton, for instance, consists of three quarks; a pion comprises a quark and an antiquark. Over the past two decades, physicists developing QCD have applied the theory to predicting what happens at extremely high energies such as those achieved in particle accelerators.

But because of the theory's mathematical structure, QCD is much harder to work with at low energies than high. Calculating the rest mass of the proton, for instance, involves not only knowing the masses of the three quarks in the proton but also computing the binding energy of the gluons that hold the quarks together. Because these gluons interact with each other, the calculations are nonlinear, and it's impossible to reach an analytical solution. As a result, scientists have to approximate a solution through complex numerical calculations aimed at reproducing the behavior of the gluons.


IBM

The strong force was with him. Weingarten.

Weingarten has worked for more than a decade cooking up algorithms to do just that. His approach, called lattice QCD, makes the calculations more tractable by breaking up continuous space-time into a finite four-dimensional lattice of points. To home in on a solution, though, Weingarten had to expand the lattice and shrink its spacing, which increases the number of lattice points that must be considered and makes the number of computations mushroom. As a result, Weingarten has devoted much of his effort to finding ways of streamlining these calculations.

At the same time, Weingarten was also developing a computer that was up to the task. In 1982, he left a tenured position at Indiana University to join IBM's Thomas J. Watson Research Center in Yorktown Heights, New York, where he and other IBM workers designed and built the GF-11, a massively parallel computer with 566 processors intended specifically for QCD calculations. Then, with Frank Butler, Hong Chen, Jim Sexton, and Alessandro Vaccarino, he set it loose on calculating the masses of hadrons—a task that eventually took a year and more than one hundred quadrillion (10^{17}) arithmetic operations.

As reported in last week's issue of *Physical Review Letters*, Weingarten's team computed ratios among the masses of 11 hadrons, including the proton, pion, kaon, delta, omega, and other members of a Greek alphabet soup. Because the QCD formulation includes three unspecified parameters (the quark charge and the masses of the normal and strange quarks), Weingarten fixed three of the hadron masses according to experimental measurements, which left him with predictions for the masses of the eight remaining hadrons. All agreed with experiment to within 6%, and most were a good deal closer.

Besides vindicating the theory, Weingarten says, the calculations demonstrate just how important the computer has become in theoretical physics. Other theories of the subatomic world can be solved analytically. But for exploring the predictions of QCD, says Weingarten, computer simulation—what he calls "experimental theoretical physics"—has become an essential implement in the physicist's toolbox.

–Robert Pool