that they couldn't have just "hopped out" of the single original *HOX* cluster one by one. Instead, he concluded, "the two gene clusters originated suddenly, by duplication of a primordial cluster."

What's more, the team found that the genes' order of appearance on the chromosome corresponds to the head-to-tail order of their activity in the body. However, they differ from other *HOX* genes in one key respect: They are expressed only in the endoderm, the innermost of the three embryonic germ layers, which gives rise to the gut and other organs of the viscera. Other *HOX* genes—including the first *HOX* gene cluster of *Amphioxus* identified by the Holland team in 1992—are active only in the embryonic ectoderm, the outermost germ layer, which produces tissues including the skin, nervous system, and sense organs. "What we've got here are two gene clusters, each obeying spatial colinearity but in different ... layers," Holland said.

To back his contention that the genes of the new cluster were key to the development of the inner germ layer, Holland used data from other laboratories to show that analogous genes in mice and frogs are expressed in the gut. One of the *Amphioxus HOX*-like genes, for instance, is analogous to the *IPF* gene in mammals which, gene knockout experiments show, is crucial for development of the pancreas in mice. Putting this all together, Holland proposed that doubling of the primordial *HOX* cluster occurred before an ancestor of *Amphioxus* acquired an endoderm and was, in fact, instrumental in its creation.

Other meeting participants view this suggestion cautiously. Michael Akam of the University Museum of Zoology in Cambridge, U.K., says that while Holland's data linking the three genes "look ironclad," his hypothesis that one *HOX* cluster took over the patterning of the inner body layer and the other the outer one is "almost too clean a model to be probable." Diethard Tautz of the University of Munich in Germany agrees. *Amphioxus*, he says, "is only one data point. You need to have more than one organism to form a complete picture."

The additional data points may come soon, as the search for *HOX* genes and their relatives in primitive organisms such as acorn worms is heating up. The implications are so important, says participant Axel Meyer, an evolutionary biologist at the University of Konstanz in Germany, that the race to find these genes "is a gold rush." But the "gold" in this case will be a clear idea of how evolution really happened.

–Steven Dickman

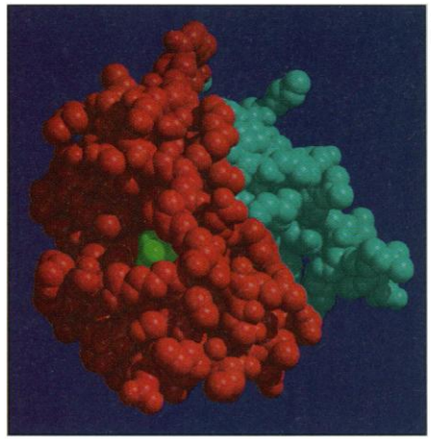
Steven Dickman is a writer in Cambridge, Massachusetts.

CELL BIOLOGY

Pinning Down Cell Division

The events in the cell just before it divides are some of the most dramatic in biology. The chromosomes condense, the nuclear membrane disappears, and the cell starts to build its mitotic spindle—a set of fibers that will eventually pull the chromosomes to the opposite poles of the dividing cell. How the cell choreographs these complex changes is unclear, but on page 1957, molecular biologist Kun Ping Lu of Beth Israel Deaconess Medical Center in Boston and Harvard University and his colleagues report evidence for a new mechanism that may play a key role.

Cell biologists have long known that the cell's progress toward division is controlled by a group of kinases, enzymes that add phosphate groups to a variety of cell proteins. For the most part, though, they've had few clues to what those phosphate additions actually do. That's where the Lu team's work comes in. It suggests that the phosphates serve as a sort of tag for attracting an enzyme called Pin1, which may cause the phosphorylated proteins to change their shapes. Researchers don't yet know exactly what this accomplishes, although they point to several possibilities, such as turning off an active enzyme, directing a protein to a new place in the cell, or targeting a protein for degradation. Whatever the precise result, however, the work provides "a new function for phosphorylation," says molecular biologist Tony Hunter of the Salk Institute in La Jolla, California.



LU ET AL.

Twister. The Pin1 protein may help regulate mitosis by binding to phosphorylated proteins. The bright green region at left is the phosphate binding site.

Lu and Hunter first discovered Pin1 2 years ago as a protein that interacts with and inhibits another critical cell regulator, called NIMA, which helps turn on mitosis. Pin1 itself is an isomerase enzyme that changes the configuration of the peptide bond preceding proline, an amino acid that is an important determinant of protein structure because it can put kinks into a protein chain. Previous studies also showed that Pin1 is crucial for both yeast and human cells to divide properly. Without it, for example, cells can't complete mitosis. But its precise role in the cell remained a mystery.

Researchers got a clue earlier this year, however, when Joseph Noel of the Salk Institute solved Pin1's three-dimensional structure. It showed that the enzyme has a pocket for binding phosphate next to the site where it binds its proline target, says molecular biologist Lewis Cantley of Harvard, a coauthor on the *Science* paper. That suggested Pin1 might bind phosphorylated proteins.

To confirm that hunch, the team searched through a library of protein fragments for peptides that bind to the enzyme. Sure enough, says Cantley, Pin1 preferentially picked out peptides that have a phosphate attached to an amino acid adjacent to a proline. With some sequences, in fact, the phosphorylated version bound thousands of times better than the unphosphorylated peptide.

Other unpublished work suggests that Pin1 might help orchestrate cell division by interacting with other proteins involved in mitosis. When members of Lu's team went "fishing" through the contents of ruptured cells for proteins that bind to Pin1, they landed at least a dozen that are also targeted by an antibody, called MPM-2, that binds to proteins involved in mitosis in a wide range of cells. These proteins, too, contain a proline and an adjacent phosphate.

Taken together, say Lu and his colleagues, the experiments suggest that Pin1 helps regulate a two-step process that governs cell division. Adding phosphates to proteins involved in mitosis creates binding sites for Pin1, which can then latch onto them and twist the peptide bond next to the prolines it contacts. That might, in turn, change the shape of the whole protein, perhaps altering its ability to interact with still other proteins, its location in the cell, or its life-span.

Whatever the binding does, Cantley suggests that it might enable Pin1 to serve as a sort of checkpoint on the way to cell division. He notes that while cells lacking the protein can't divide—indeed, they die instead—manipulations that increase Pin1 production delay the onset of mitosis. Based on that, he proposes that by binding to phosphorylated proteins, Pin1 may slow down the activity of any proteins that are getting

ahead of the rest of the cell. Hunter agrees. The properties of the protein suggest it might work as "some sort of threshold device," he says, preventing premature functioning of certain proteins. If so, cells lacking the protein may die, because events get so out of order that they go into mitotic arrest.

Other researchers aren't convinced that the story is that straightforward, however. Cancer pharmacologists Sally Kornbluth and Tony Means of Duke University have evidence that Pin1 can bind to NIMA without the help of phosphate, and that it binds to other proteins that do not bind MPM-2. "The mechanisms that govern the effects of Pin1 in the cell ... have yet to be defined," Means says.

Indeed, Cantley cautions that no one has yet pinned down exactly what the protein does when it binds: "We have no proof that isomerization is what's required for physiological function." It is possible, he says, that simply binding to a protein is enough to slow it down. Because researchers can now identify Pin1's partners, they hope they will soon be able to sort out its role.

But even before that happens, the protein is attracting drug companies' interest. Because blocking the enzyme kills cells as they attempt to divide, drugs that inhibit the enzyme should target fast-dividing cancer cells without affecting the majority of cells in the body that divide only occasionally. "At least three or four companies are interested in looking for inhibitors," Lu says.

–Gretchen Vogel

CANCER THERAPY

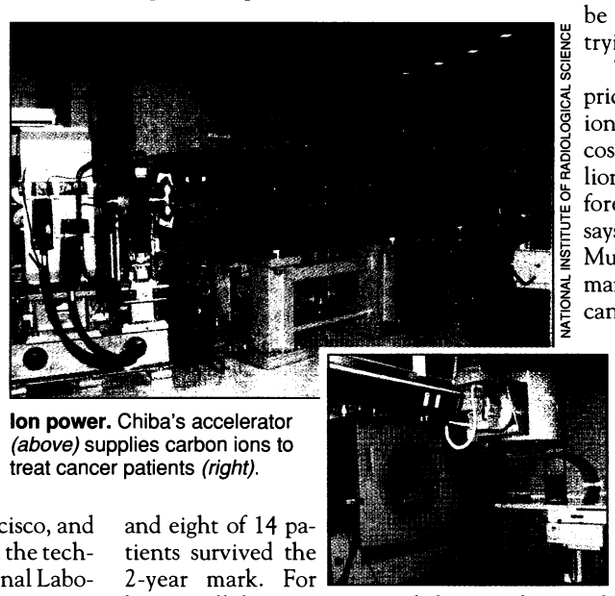
Heavy Ions Pack Powerful Punch

CHIBA, JAPAN—A high-stakes experiment in cancer therapy appears to be paying significant short-term dividends for dozens of patients. A team of researchers presented preliminary results at a recent conference here* indicating that expensive treatments with beams of carbon ions showed promise against a variety of tumors that had been considered untreatable or had resisted previous treatments. But questions remain about the long-term efficacy of the approach, as well as about its value for money.

The data were the most complete results yet from Japan's Heavy-Ion Medical Accelerator in Chiba (HIMAC), which began operating in 1995 at the National Institute of Radiological Sciences (NIRS) (*Science*, 12 May 1995, p. 797). HIMAC was built on the premise that because beams of heavy ions can be focused more precisely than x-rays, the larger mass and charge of the particles will result in greater damage to the tumor and less injury to surrounding healthy tissue. "And that's what they're seeing," says Inder Daftari, a radiation oncology physicist at the University of California, San Francisco, and a member of a team that pioneered the technique at Lawrence Berkeley National Laboratory in California until the lab's aging accelerator was shut down in 1993. "The results are very encouraging," adds John Munzenrider, a radiation oncologist at Massachusetts General Hospital and Harvard Medical School, both in Boston.

The new results come from clinical studies to evaluate both toxicity and effectiveness in patients with head and neck, lung, liver, and cervical cancers. Treatments typically consisted of periods of irradiation with carbon-12 ions, two to four times a week for 4 to 6 weeks. In all cases, alternative treatments had failed or been ruled out.

Twenty-four of 34 patients with advanced head and neck cancers had complete or substantial regression of the tumors after 6 months. And seven of nine patients treated more than 2 years ago are still alive. Just over half (23 out of 44) of the patients with non–small cell lung carcinomas showed complete or partial response 6 months after treatment, and eight of 14 patients survived the 2-year mark. For hepatocellular carcinoma with liver cirrhosis, 18 of 25 tumors showed complete or partial regression after 6 months, and three of four patients survived beyond 2 years of treatment. There were also preliminary indications of a good response for cervical cancer. "It's very impressive, especially for lung cancers," says William Chu, a radiation physicist at the Lawrence Berkeley lab.



NATIONAL INSTITUTE OF RADIOLOGICAL SCIENCE

Ion power. Chiba's accelerator *(above)* supplies carbon ions to treat cancer patients *(right)*.

Those results were achieved with dosages that started at around 2 grays and were raised to as much as three times that level in subsequent trials following an evaluation one to several months after treatment. (One gray equals 1 joule of radiation energy deposited in 1 kilogram of tissue.) But some researchers think that such high per-session dosages could cause harmful, long-term side effects. Yasuyuki Akine, director of the University of Tsukuba's Proton Medical Research Center, worries that possible damage to surrounding tissue may not surface for several years. "Before escalating [the dosage], you need to evaluate late injury 3 to 4 years after treatment," he says. Hirohiko Tsujii, head of NIRS's department of medicine, admits that the NIRS's team debated the timing but decided it would be impractical to wait 3 to 4 years before trying higher doses.

These results come, however, with a hefty price tag. HIMAC, the world's only heavy-ion accelerator dedicated to medical use, cost $326 million to build and takes $58 million a year to operate. U.S. scientists don't foresee restarting heavy-ion experiments, says Munzenrider, "because of the expense." Munzenrider and others hope, however, that many of the benefits of heavy-ion therapy can be achieved with beams of protons, which require less powerful—and much cheaper—accelerators than those needed to generate heavy-ion beams. But Daftari believes that each of the different therapies will eventually find its own niche in treating different cancers. "If effective, [heavy-ion therapy] would justify the cost," he says.

A group in Germany is waiting for government approval for a 5-year trial of up to 350 patients. It will be the first human trials for the team, which is based at GSI, the German national lab for heavy-ion research in Darmstadt. "We were very pleased to hear the [HIMAC] results," says Gerhard Kraft, a physicist who heads the group. And Japan's Hyogo Prefecture is already building a $230 million, medical-use accelerator capable of delivering both protons and heavy ions. Even so, therapy is likely to be restricted to protons until HIMAC produces recommended protocols for heavy ions. Researchers say such protocols could be ready in 4 to 5 years.

–Dennis Normile

* Proton Therapy Coordinating Group semiannual meeting PTCOG27, 17 to 19 November in Chiba, Japan.