Genes, Junctions, and Disease at Cell Biology Meeting

SAN FRANCISCO—Some 7500 scientists from 50 countries sloshed through the rain to discuss 4000 talks and posters at the combined Sixth International Congress on Cell Biology and the 36th Annual Meeting of the American Society for Cell Biology, held here from 7 to 11 December. Two highlights focused on development gone awry.

Connexins and Disease

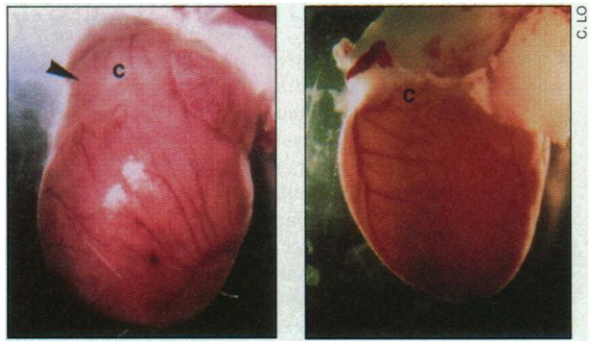
For cells to be good neighbors, they need gap junctions, communication channels formed of proteins on the outer membrane that allow adjacent cells to coordinate their activities and movements. New research from several teams is now showing that abnormalities in the proteins that form gap junctions, which are called connexins, can lead to a wide range of defects in mice, including heart malformations, infertility, and cataracts. Similar defects occur in humans, and researchers hope that understanding these abnormalities in animals will lead to ways to prevent the problems in people. "The mouse experiments tell you where to go to look for problems [in humans]," says neurobiologist David Paul of Harvard Medical School in Boston.

One gap-junction defect already shown to cause a human developmental problem affects the protein called connexin43. Last year, William Fletcher, a molecular biologist at the Loma Linda University School of Medicine in California, and his colleagues established that the connexin43 gene is mutated in some children who need heart transplants because their hearts failed to develop the asymmetry needed to supply enough blood to the lungs. Another research group showed that knocking out this gene in mice also causes malformations in that part of the heart, which kill the animals at birth.

More recent work, described at the meeting by developmental biologist Cecilia Lo of the University of Pennsylvania, Philadelphia, has helped pinpoint just where connexin43 comes into play during heart development. She used DNA from cytomegalovirus to cause an extra copy of the connexin43 gene to become active in the neural crest, which gives rise to the peripheral nervous system, but also has cells that migrate to what becomes the heart's right ventricle and the pulmonary artery. They seem to help the heart become asymmetrical, Fletcher notes.

At birth, Lo's mice have defects, such as an obstructed pulmonary artery, that are "strikingly similar to those that have occurred in people," says Fletcher. Preliminary data from Lo's group suggest that the heart defects in the mutant mice arise because the neural crest cells move too fast to the heart. Thus, they may not be in the right place at the right time to get, or give, the signals needed for normal heart growth.

Gap-junction disruption can also lead to abnormalities of other organs. At the meeting, Paul's Harvard colleague Alexander Simon described experiments in which his team made mice that lack the gene for the gap-junction protein called connexin37, which forms junctions between the oocytes and surrounding tissue. The resulting females were infertile: It seems their oocytes never received the right signals to mature into eggs and leave the ovary.



Bulging heart. Extra connexin43 led to excess tissue at "c" in heart at left. The heart on the right is normal.

Also in these mice, there was a premature change in the tissue surrounding the oocyte, which normally transforms into the progesterone-secreting corpus luteum after ovulation occurs. Because this change occurred in the connexin37 knockouts in the absence of ovulation, Simon concludes that immature oocytes need connexin37 to inhibit this transformation. The ovaries in these infertile mice resemble those of women with spontaneous premature ovarian failure syndrome, a problem leading to premature menopause, he notes. "It would be nice to look at the DNA of [these women]," Simon says, to see if they too have abnormal connexin37.

The effects of lacking another connexin, connexin46, show up in the eye, as Xiaohua Gong, a graduate student in Norton Gilula's lab at the Scripps Research Institute in La Jolla, California, reported. Eliminating the gene for this protein does not inhibit the formation of the lens, but does cause cataracts to begin to develop in mice about a month after they are born. "The connexin is important for maintaining lens transparency," says Gong. Learning more about what happens in the animals may help explain cataracts in people, he adds.

And Paul thinks the connexin connection to human disease is just beginning. "I bet we'll see a variety of disease-associated [connexins]," he predicts.

Gene Linked to Down Syndrome Retardation

People born with Down syndrome suffer a grim spectrum of problems, including mental retardation, immune and endocrine system abnormalities, and skeletal, heart, and digestive system defects. Because those affected—about one in 800 newborns—usually have an extra copy of all or part of human chromosome 21, sorting out which of the many extra genes cause these symptoms has been difficult. But now several research teams have homed in on a gene that may contribute to one aspect of Down syndrome, the brain defects.

The work suggests that having either too much or too little of the gene's protein product, an enzyme called DYRK for dual specificity tyrosine phosphorylation-regulated kinase (Y denotes tyrosine), disrupts brain development. "This is a gene [for which] dosage plays an important role in how the neuronal pathways are put together," says geneticist Edward Rubin of the Lawrence Berkeley National Laboratory in Berkeley, California.

Woo-Joo Song and David Kurnit, molecular biologists at the University of Michigan Medical Center in Ann Arbor, linked the *DYRK* gene to the mental symptoms of Down's while following up on a discovery made last year by Olaf Pongs and his colleagues at the Center for Molecular Neurobiology in Hamburg, Germany. Those researchers had identified a strain of mutant fruit flies that have smaller than normal brains and impaired learning as a result of a defect in a gene they called *minibrain*. That gene's sequence indicated that its protein product is a new type of kinase enzyme that may control cell division.

Thinking that a similar human gene might be involved in the mental retardation of Down syndrome, Song used the sequence of *minibrain* to probe for its human equivalent on a region of chromosome 21 that appears critical for Down syndrome, as it is the only extra genetic material found in some patients. He reported at the cell biology meeting that he indeed found the gene there. Other

teams in Japan, Spain, and the United States have come up with the same result, Song adds.

By itself, that finding doesn't prove that excess DYRK causes Down's problems; the critical chromosome 21 region contains about 50 genes in all. But the Song team also found that in mouse embryos, the *DYRK* gene is expressed in the same places—areas such as the gray matter of the brain, the spinal cord, and the retina—that are defective in Down syndrome.

Instead of doing a genetic search for key Down syndrome genes, Rubin and colleague Desmond Smith at the Lawrence Berkeley lab homed in on *DYRK* by looking at the behavior of several new strains of mice, each carrying a part of the crucial human chromosome 21 region. One strain, which did poorly in simple learning tests, contained a stretch of human DNA 180 kilobases long that includes *DYRK*, Smith and his colleagues reported in late October at the American Society of Human Genetics meeting in San Francisco. "It's like a pincer movement," he says. "We both landed on the same gene."

The two prongs of the pincer show that any departure from the gene's normal activity level can be dangerous. The mice's problems, like those of Down's patients, were caused by an extra copy of the *DYRK* gene, while the mutant fruit fly's were caused by having too little of *minibrain*'s protein product. "You need exactly two copies of the gene," Smith concludes. He cautions, though, that more work is needed to confirm that *DYRK* is the gene at fault in the 180-kilobase region.

Assuming that it is, it's unlikely to work alone. Using mouse genetic engineering studies similar to those of Rubin and Smith, a French team, led by Dahmane Nadia of the Hospital Necker in Paris, has found that another chromosome 21 gene, which lies outside the 180-kilobase region and is the human equivalent of the fruit-fly *single-minded* gene, seems to lead to learning problems in mice with an extra copy. Sorting out how these two genes, and possibly others, contribute to Down's and to normal neural development, says Smith, is going to make "for a very exciting next few months."

–Elizabeth Pennisi

CHEMICAL SYNTHESIS

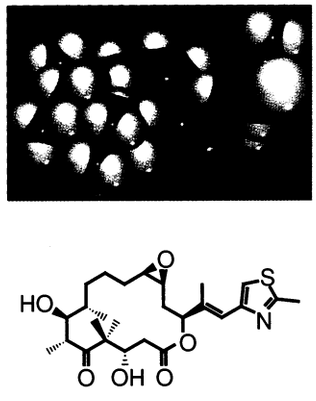
Tumor-Killer Made; How Does It Work?

The promising anticancer properties of taxol and its relatives have a way of sparking hot competitions. In 1994, two groups tied in the race to complete the first total synthesis of taxol, a name trademarked by Bristol-Myers Squibb (*Science*, 18 February 1994, p. 911). Now, in a contest to synthesize a related compound, two groups have again crossed the finish line in a virtual dead heat. In this sprint, the groups, one led by Samuel Danishefsky at the Memorial Sloan-Kettering Cancer Center in New York, the other by K. C. Nicolaou of the Scripps Research Institute in La Jolla, California, synthesized a bacterial compound known as Epothilone A, which kills cultured tumor cells in a manner similar to taxol, thwarting their ability to divide.

Although the structure of Epothilone A is less complicated than that of taxol, its synthesis represents "a great achievement," says Gerhard Höfle, an organic chemist at the National Biotechnology Research Institute (Gesellschaft für Biotechnologische Forschung) in Braunschweig, Germany. The ability to build these molecules from scratch will let chemists alter their structures and possibly improve their properties, says Höfle. "No drug is so good that you can't improve upon it," he says. Analogs of the compound could also help researchers conclude yet another race, which is still under way: a competition to understand these compounds' exact modus operandi.

To synthesize the molecule, both groups started with commercially available materials and then used about 20 synthetic steps to build these compounds up into separate fragments, which they then linked together to form the compound's double-ring–shaped structure. The specific fragments and the chemistry by which the two groups linked them differed, however. Danishefsky and his colleagues report their findings in the December issue of *Angewandte Chemie, International Edition in English*. Nicolaou and his colleagues, who synthesized the molecule using two different schemes, will publish their results in two separate issues of the same journal early next year.

Epothilone, which was discovered just last year by researchers at Merck to kill cells like taxol, is not alone in that ability. Researchers are also tinkering with another compound known as discodermolide, which was synthesized in 1995 and shown earlier this year to kill cells in much the same way. Epothilone may have some advantages over the competition, however. Unlike taxol and its close relatives, epothilone is water-soluble, which could make it easier to administer to patients. And unlike both taxol and discodermolide, it can be produced cheaply in large quantities by bacterial fermentation.



C. BODDY AND K. C. NICOLAOU/SCRIPPS RESEARCH INSTITUTE

Model killer. The synthesis of Epothilone A, which kills like taxol, may help make it even more potent.

All three molecules appear to have one critical feature in common: the ability to prevent cells from reproducing by paralyzing the microtubules that ordinarily pull chromosomes to opposite poles of a dividing cell. Now, researchers hope to learn just how they do so. Past work with taxol and its analogs has shown that three regions of the molecule—known as a C-13 side chain, a C-2 benzoate, and a hydroxyoxetane ring—are all critical to the molecules' activity, says Jeffrey Winkler, an organic chemist at the University of Pennsylvania, Philadelphia. Although epothilone contains atomic building blocks similar to those that make up these critical regions, x-ray images of the two molecules indicate that "the structures are very different," says Danishefsky.

To see how the two different structures could have similar biological effects, Winkler and his Penn colleague Paul Axelsen compared computer models of taxol and epothilone. In an upcoming report in *Bioorganic and Medicinal Chemistry Letters*, the researchers report that the molecules could change their shapes and adopt similar stable conformations in which 13 of the 15 atoms in the large ring in epothilone line up with corresponding atoms on taxol, including the C-13 and C-2 groups. This match, says Winkler, suggests that these conformations are the ones responsible for the molecules' biological activity. If so, Winkler and Axelsen believe that the C-13 and C-2 groups may fold together to represent one recognition site that taxol uses to bind to its target on microtubules, and that the hydroxyoxetane ring either represents an independent site or a structural beam that holds the C-13 and C-2 groups in place so they can do their job. Next, the researchers hope to look at models of discodermolide to see if one of its conformations fits with this model as well.

Höfle notes that other teams, including his own, are now testing these ideas in the laboratory by analyzing how epothilone, discodermolide, and their analogs bind to the protein target on the microtubules. In the end, researchers hope, all these many races will lead to a single kind of prize: a way to design new compounds that will make more potent and safer anticancer drugs.

–Robert F. Service