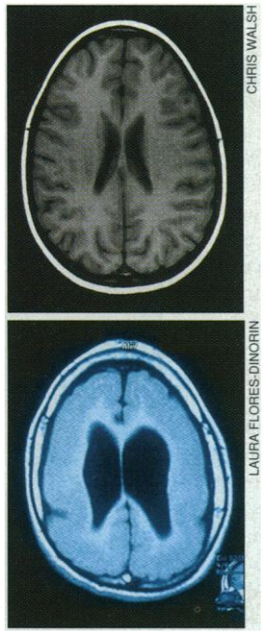
Guiding Neurons to the Cortex

Mutations that disrupt cortical development are helping researchers identify the biochemical signals that neurons use to find their final destinations in the brain's cortex

The woman whose brain image appeared on the cover of last January's issue of *Neuron* is outwardly normal. A recently married college graduate with a high-powered job, she complains only of occasional epileptic seizures. But inside her brain, the magnetic resonance image had revealed a striking abnormality. During embryonic development, large numbers of neurons had never made it to the woman's cerebral cortex, the neuron-rich outer layers of the brain, but instead remained jumbled in the neuron nurseries deep in the brain where they were born. This woman has one of several genetic mutations that seem to derail neurons on their journey to their proper locations in the highly ordered cortex. But she is one of the lucky ones. Most people with such mutations not only have epilepsy, but are mentally retarded as well.



Underdeveloped. A lissencephalic brain *(below)* appears smooth compared to the normal brain above.

Until recently, neurobiologists knew little about the molecular causes of these rare conditions. But that has begun to change, and as researchers have gained insight into these human tragedies, they are also finding clues to a fundamental question about normal brain development: What molecular signals guide immature neurons in their complex migrations from their place of birth to their proper locations in the cortex?

In the past 3 years, researchers who study these human conditions as well as several similar genetic mutations in mice have isolated two genes apparently at fault in the cortical malformations, and have mapped the chromosomal locations of three more. While they do not yet know what the proteins encoded by these genes do, they hope that the genes will ultimately help them piece together the signaling pathways that help brain neurons find their way. "Having a gene allows you to jump into a genetic pathway," says neuroscientist Chris Walsh of Harvard Medical School, who studies two of the human conditions. The promise of this approach, says neuroscientist Verne Caviness, also of Harvard Medical School, means "people are going to work on this like a dog on a bone. They aren't going to let it go. It is too interesting."

Researchers have marveled for decades over the orderly migrations of young cortical neurons that give rise to the neatly layered gray matter of the cortex. By tracking the neurons in animal embryos, they found that they travel from the areas deep in the brain where they are born, following roadways formed by support cells called radial glia.

The radial glia lead to a layer of cells near the outer surface of the developing brain called the preplate which the migrating neurons enter, arranging themselves in the middle "like the filling in a sandwich," says developmental neurobiologist Carla Shatz of the University of California, Berkeley. That simple sandwich eventually becomes a multilayer club special, as subsequent generations of young neurons continue to stream in from below, passing through the layers of neurons already in place and arranging themselves in overlying layers, until there are six in all.

A jumbled brain

Despite all they had learned about the neurons' migration, researchers still didn't know the identity of the protein components of the molecular machinery that gets them to their destination. The best entree to those proteins would be through their genes, which when mutated should cause the process to go awry. The first such mutation came along nearly 50 years ago, when D. S. Falconer, of the Institute of Animal Genetics in Edinburgh, U.K., described a mutant strain of mice known as *reeler* for their reeling gait, whose brains lack the normal cortical layers and have a jumbled-up collection of neurons instead. As researchers learned more about brain development, they discovered that normal cortical layers don't form in *reeler* because the migrating neurons never enter the preplate, but pile up below it. "This gene seemed to be indispensable to the conduct of the migration process," says Caviness.

But to learn what the *reeler* gene actually does, researchers needed to find the gene and its protein product. Finally, in the spring of 1995, that goal was achieved with two remarkable findings that re-energized the field: A research group led by Masaharu Ogawa at Kochi Medical School in Japan reported that it had made antibodies to the *reeler* protein and used them to identify the cells that express the gene, and a team led by Tom Curran, then at the Roche Institute of Molecular Biology in New Jersey, cloned the *reeler* gene itself.

The Ogawa team made its antibodies by immunizing *reeler* mutant mice with the brains of normal mice. The idea was that because the mutants don't make the normal protein, they would react to it as foreign and make antibodies to it. But says Curran, now at St. Jude's Children's Hospital in Memphis, Tennessee, while such an experiment makes sense on paper, no one expected that it would work in reality: "What they did was really amazing."

The Ogawa team's next result was also "a big surprise," says Caviness. Using their antibodies, the researchers identified the cells that

SOME MUTATIONS LINKED TO ABNORMAL CORTICAL DEVELOPMENT

Mutation or Condition	Chromosomal Location	Protein Product	Symptoms	Brain Alterations
Reeler	5 (mouse)	Reelin	Unsteady gait	Normal cortical layers don't form
Scrambler	4 (mouse)	??	Unsteady gait	Normal cortical layers don't form
CDK5	5 (mouse)	CDK5	Die before birth	Apparent cortical layering defect
Lissencephaly	17 (human)	PAF acetyl-hydrolase	Seizures, severe retardation	Smooth brain; normal cortical layers don't form
Double cortex	Xq21.3–24 (human)	??	Mental retardation, seizures	Thinner cortex with jumbled neurons below
Periventricular heterotopia	X28 (human)	??	Mental retardation, seizures	Thin cortex; mature cortical neurons in ventricles

make Reelin: the so-called Cajal-Retzius (C-R) cells, which are found in the part of the preplate that becomes the marginal zone, the top layer of the ever-growing club sandwich. Because loss of Reelin interferes with the migration of the cortical neurons up the radial glia, Caviness and others expected that the protein would be made by the cortical cells themselves, or perhaps by the glia.

No less amazing was the Curran group's serendipitous cloning of *reeler*, while they were studying a different gene, called *fos*. They had introduced a mutant form of *fos* into mice to study its function. But one of their transgenic mouse strains "looked crazy," says Curran. "[The animals] kept falling over. When we looked at their brains, my colleague Richard Smeyne said 'That looks just like *reeler*.' " Sure enough, the introduced *fos* gene had accidentally disrupted and inactivated the *reeler* gene. Using molecular probes for *fos*, postdoc Gabriela D'Arcangelo and graduate student Graham Miao pulled out the *reeler* gene, which codes for a large (400-kilodalton) protein they named Reelin.

Based on Reelin's structure, Curran predicts that the protein is secreted by the C-R cells and sticks to the molecular matrix surrounding the cells. This extracellular location suggests, he says, that Reelin may be signaling the migrating neurons to insert between the marginal zone at the top of the sandwich and the so-called subplate neurons at the bottom.

But the nature of that signal is anyone's guess. Kazunori Nakajima, a neuroscientist at RIKEN in Tsukuba, Japan, who as a graduate student conceived of and worked on the antibody experiment with Ogawa, proposes that Reelin acts as a stop signal for each wave of arriving cortical neurons, telling them to get off the radial glia fibers and develop into a layer of mature neurons just under the marginal zone. But there are other possibilities, Curran notes: "Does [Reelin] allow the insertion because it creates a space, by repelling the subplate neurons? Or does it provide an attractive signal to the migrating cortical neurons?"

One way to test those possibilities is to track down the receptor that allows cells to respond to Reelin and see which cells wear it on their surfaces. Curran's group and Nakajima are working hard to find proteins that bind Reelin and might therefore be its receptor. And there is the possibility of even more serendipity: Researchers at the Jackson Laboratories in Bar Harbor, Maine, have identified a new mutant mouse called *scrambler*, which has characteristics similar to *reeler*. Curran's group, in collaboration with Dan Goldowitz of the University of Tennessee, found that the *scrambler* mutation doesn't affect the Reelin protein. That, says Curran, means the *scrambler* protein "could potentially be a receptor" for Reelin.

Another newly created mutant mouse may also help researchers tease out the Reelin signaling pathway. In the 1 October *Proceedings of the National Academy of Sciences*, a research group led by Ashok Kulkarni at the National Institute of Dental Research reported that it had made a mutant mouse lacking the gene for the catalytic subunit of a neuron-specific kinase called cyclin-dependent kinase type 5 (Cdk5). Although these animals suffer more serious defects—unlike *reeler* mice, they die before birth—their brains seem to lack cortical layers, as those of *reeler* mice do. Developmental neurobiologist Karl Herrup, of Case Western Reserve University in Cleveland, is now studying the mutant mice in collaboration with Kulkarni's group, to see how close their resemblance to *reeler* mice really is. It may be that Cdk5 is involved in the intracellular signaling triggered by Reelin, he says. But he cautions that the apparent similarity may also "turn out to be a red herring."

Missing ridges and valleys

Mutant mice aren't the only guides to the genes that orchestrate cortical neuron migration. Human mutations that disrupt cortical development are offering more candidates. "What is really exciting" about the human conditions, says Berkeley's Shatz, "is that you see these abnormal cells, and they

D'ARCANGELO *ET AL.*, *NATURE* 374, 719 (1996)

On the edge. Reelin *(bright areas)* is made in the marginal zone of the developing mouse brain.

are reminiscent of *reeler*." That suggests that the genes responsible for the human conditions may be involved in the very same neuronal migration process.

The most severe of the human conditions is lissencephaly, which means "smooth brain," so named because those stricken with it have a cerebral cortex that lacks the ridges and valleys characteristic of a normal human brain. Lissencephalic children are severely retarded; many can't respond to the world or communicate at all, and most suffer from seizures and die in the first few years of life. Their brains show abnormal cortical layering, reminiscent of that in *reeler* mice, says Orly Reiner, who studies lissencephaly at the Weizmann Institute in Israel.

Three years ago, Reiner cloned the gene mutated in lissencephaly, and it turned out to code for a subunit of an enzyme that inactivates a phospholipid signaling molecule called platelet-activating factor. At present, researchers have no idea about how the enzyme might influence neuronal migration.

To try to resolve the mystery, Reiner and her colleagues have cloned the same gene from mice and found that it is expressed in the migrating cortical neurons and in the newly forming cortical layers. That, she says, suggests that it is indeed involved in cortical development. Her group is making conditional knockouts of the gene so that she can turn the gene off in developing mice and study its effect on development.

Even less is known about the genes mutated in periventricular heterotopia (PH), the condition that afflicts the woman whose brain scan appeared on the cover of *Neuron*, and in a disorder called double cortex (DC), whose symptoms—epilepsy, usually with mental retardation—resemble those of PH. Both genes are located on the X chromosome. As a result, virtually all PH and DC patients are women because males, who have only one X, are so severely affected if they inherit the mutant genes that they die before birth or at an early age.

Like the lissencephaly mutation, those in PH and DC seem to disturb cortical neuron migration, says Harvard's Walsh, who is trying to clone the genes. Women with DC have a thinner than normal cortex with the correct layering system, and underneath it a wide band of cortical neurons that Walsh says appear to have "left the region where they were formed, migrated about halfway [to their destination beneath the marginal zone], and then stopped dead right there." PH "is very similar to double cortex," says Walsh, except that the affected neurons never leave the brain's ventricles, where they were born.

Walsh cautions that although DC and PH look like migration defects, his group hasn't proven that is true. To do that will require neuron-tracing experiments that can't be done in humans. As soon as the human genes are cloned, says Walsh, the next task will be to clone their counterparts in mice, make mouse mutants, and trace the paths of the mutant neurons as the rodents' brains develop.

These won't be the last genes for researchers in this field to add to their list; at last year's Society for Neuroscience meeting in San Diego, Kevin Lee of the University of Virginia reported his discovery of a mutant rat with a double cortex similar to that of human DC patients. The as-yet-uncloned gene appears different from the gene that causes the human disorder. With genes accumulating at this rate, the mechanisms that guide newborn neurons are starting to look almost as intricate as the cortical architecture their journeys create.

–Marcia Barinaga