News
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*****Researchers use gene probes to study brain function at UTHSCD.

DALLAS -- Researchers at The University of Texas Health Science Center at Dallas can now probe single cells of the brain to see what proteins they are producing. In a new combination of techniques, Dr. Marcelle Morrison and Dr. Sue Griffin have become the first team to analyze individual brain cells in the laboratory not only for the presence of a specific protein but also for the presence of instructions -- in the form of messenger ribonucleic acid (mRNA) -- to make that protein.

With a single slice of brain tissue from a 10-day-old rat, the researchers first looked for specific proteins in the cells, using a complex procedure of tagging the proteins with specially treated fluorescent antibodies.

Then on the same slice of tissue, they successfully probed the brain cells for mRNAs by use of recombinant DNA (deoxyribonucleic acid), a spliced gene, tagged with radioactive molecules.

Griffin, associate professor of Cell Biology, recently returned from a two-month stint as visiting scientist in the Department of Neurobiology at Weizmann Institute in Rehovot, Israel. She collaborated with scientists there, demonstrating her technique for "in situ hybridization" (combining specific recombinant DNA probes with their mRNAs) and using DNA probes synthesized at the Weizmann for mRNAs encoding different proteins.

"The cerebellum provides an excellent model for our studies because the timing of developmental events such as neuronal cell division, differentiation and migration have been well characterized. The layered architecture is geometric, and the neuronal circuitry is well documented."

The cerebellum is the part of the brain that controls fine muscle movement. Griffin compares the learning of a motor skill to "programming a computer."

"You can see a baby programming the cerebellum when you put a toy on its high chair tray. The first time it tries to pick it up, the hands oscillate and it knocks the toy off. Presently, the cerebellum learns to judge how far to move the hand, how much to expand or contract the grasp."

In the adult, the cerebellum is calculating other fine measurements, for example in tennis playing: "Here comes the ball. How fast is it coming? Where should the racket be when it gets here? Do I need to move? What angle should I use?"

"When the skill is learned, the cerebellum just says, 'Okay, this is playing tennis,' and plugs in the almost infinitely modifiable program for playing tennis," says Griffin. "Outstanding athletes probably have an exceptional cerebellum or exceptional synapses (junctions between neurons)." If a person's cerebellum is damaged through disease, he or

she must consciously think through every fine movement. And the movements are never as good as when the cerebellum is working properly.

Different parts of the brain have different specific times of development. With the cerebellum, development begins before birth and continues at least through the first year of life in the human. In the rat, cerebellar development is compressed into a much shorter period and lasts for about three weeks after birth.

Development of the cerebellum begins with massive cell division followed by migration of the newly formed neurons to their permanent adult site. This developmental sequence was first documented in human cerebellar tissue early in this century by Ramon y Cajal.

What Morrison, associate professor of Neurology and assistant professor of Biochemistry, and Griffin are now working toward is "the characterization of these developmental events at the molecular level."

Brain cells contain the information that makes us what we are. Brain cells, like every other cell of the body, contain the genetic code in their DNA to produce every protein that is produced in the body. Every life process that takes place within a cell is mediated by a specific protein. But specific chemical events trigger the production of only the proteins that a cell needs to make -- when things go normally.

Cells operate like miniature factories, producing whatever proteins they need to carry out their particular function. The DNA is the computer that contains the instructions for making a particular protein. Tubulin, for example, is a protein involved in cell division and cell structure. When the cell needs tubulin, some unknown trigger causes a section of double-stranded DNA (a gene) to unwind into two single strands. With the help of specific enzymes, mRNA for tubulin is formed along one of the single strands of DNA. Through a series of reactions, the "message" sent from the DNA is translated, that is, the "raw materials," amino acids, are lined up according to the message, and the needed protein is synthesized.

So if a neuron contains the specific mRNA for tubulin, the cell is making tubulin.

Morrison, a molecular biologist, and Griffin, a developmental neurobiologist, have previously found that immature brain contains more tubulin mRNA than mature brain does.

The brain contains many different kinds of cells that carry out different functions. They also mature at different times. But until recently researchers could find out only the total amount of tubulin present in tissue by grinding up a brain or a portion of a brain. With these new techniques, they can look at specific cells in brain tissue and see which individual cells are making tubulin. "We can now study the regulation of protein synthesis at the level of gene expression within a specific cell type and actually quantitate specific mRNAs on a per cell basis," says Griffin.

To obtain DNA probes for this work, they have constructed "libraries" of genes that code for all the cerebellar proteins of the human as well as of the rat. The individual gene copies (clones) can now be labelled with a radioactive molecule to become cellular probes in tissue from the cerebellum. Since the gene clones will pair only with their "matching" mRNAs, the presence of a "hybrid" of a gene clone for tubulin with the mRNA for tubulin, seen under the microscope, indicates that the cell was synthesizing tubulin. Using this in situ hybridization technique, Morrison and Griffin have shown that cells that are dividing and using more tubulin show more radioactive hybrids than others.

Now that they have perfected the technique, they can study other developmental activity of individual cells. Eventually, they want to know how the various events in brain

development are triggered. It may be that each cell has its own internal switch, or the impetus may come from outside the cell. After a neuron has migrated to its adult site, input from other cells turns on the gene expression for the synthesis of enzymes responsible for the production of neurotransmitters.

Studies like these will some day enable researchers to attack previously unapproachable questions about abnormal brain development in the fetus and in the infant as well as brain dysfunction in senility.

"Neurons are now thought to synthesize more than 100,000 different proteins," says Morrison. "If the synthesis of any one of these proteins is not properly regulated, the correct function of that neuron will be compromised.

"The isolation of gene probes to 'tag' mRNAs synthesizing important neuronal proteins now makes it possible to study human and rat brain to learn how individual cells respond to stimuli by altering the synthesis of these mRNAs. The gene probes can be used to study single processes in brain cells and thus identify a normal cellular function gone awry. At the same time, the gene responsible for the abnormal cell function can be identified. Once researchers identify it in the brain, the "bad" gene can be tested for clinically in any cell of the body -- even in fetal cells through amniocentesis."

Some inherited degenerative neurological diseases such as Huntington's chorea show up in middle age after people have had their children. Recent developments suggest that soon family members of patients can be tested for the abnormal gene. Those with the gene can then choose not to have children. And, eventually, this disease will die out.

Another frightening disorder, Alzheimer's disease, is not clearly a genetic disease, but there is a higher incidence among family members of patients.

"Now we are using in situ hybridization to look at the brain cells of people who died with Alzheimer's disease. We want to see why specific cells die and why there is such a rapid and progressive loss of memory in these patients," says Griffin. If an abnormal gene is found to start the death of brain cells in this disease, the gene responsible could be tested for in the fetus.

"These clinical developments are a long way off," says Griffin. "But they are now possibilities."

"DNA probes are about the only way to study anything as complex as the nervous system," says Morrison. "Obtaining a gene for a specific protein and using it as a cellular probe for one mRNA out of 100,000 provides the magnet to pull the needle out of the haystack."

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