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Gene-Expression Atlas Will Provide New Direction for Brain and Spinal-Cord Studies

Using a technique to insert fluorescently labeled genes into live mice, researchers have created a new atlas that will quite literally light the way for neuroscientists to explore the maze of connections between cells in the central nervous system (CNS).

The researchers who developed the atlas said it would enable scientists to determine when and where specific genes are switched on in the CNS. Researchers can use such clues to explore the molecular machinery that coordinates neural development and to chart the functional circuitry of the brain and spinal cord. All data from the Gene Expression Nervous System Atlas (GENSAT) BAC Transgenic Project, will be available online to researchers worldwide at <http://www.gensat.org>.

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Data derived from the project could have a have major impact on the understanding of neurological disorders, according to the project's leaders, Howard Hughes Medical Institute (HHMI) investigator Nathaniel Heintz and Mary E. Hatten at The Rockefeller University. The researchers reported the first results from the project in an article published in the October 30, 2003, issue of the journal *Nature*.

Heintz and Hatten collaborated with HHMI investigator Alexandra Joyner at the New York University School of Medicine, as well as with scientists from East Tennessee State University, Roswell Park Cancer Institute and the National Institute of Neurological Disorders and Stroke.

To track gene expression, the researchers developed bacterial artificial chromosomes (BACs) that contained a segment of a mouse chromosome representing a single gene found in the central nervous system—including the regulatory segments that determine when and where it will be switched on and off. Instead of inserting only the mouse gene into the BAC, the researchers also spliced in a gene that expressed a green fluorescent protein.

When the researchers introduce the BACs into CNS cells, the cells emit a green fluorescence if the genes the BAC contains are switched on. In addition, since the natural, or endogenous, gene remains undisturbed, the labeled cells are otherwise normal and viable.

The GENSAT BAC Transgenic Project will make available an atlas of micrographic images depicting the gene expression in the labeled cells, the “library” of BACs, and the transgenic mice that contain the BACS.

So far, Heintz, Hatten and their colleagues have applied the technique to gather data on some 400 genes expressed in the CNS and they expect the database to grow at a rate of several hundred genes per year.

“This project will give researchers an atlas of gene expression at a cellular resolution, which allows them to visualize and characterize novel cell populations and subpopulations that express a given gene,” said Heintz. “This information will allow investigators to form very precise hypotheses about a gene's function based on from where and when it is expressed in the brain, which consists of thousands of types of cells.”

The project is also unique because it will provide experimental materials for neuroscientists. “The library of BAC vectors that we are developing will enable them to identify and access any major cell type in the brain. And the transgenic mice themselves will give neuroscientists animals in which specific living cells of interest are fluorescently labeled, so that they can image, separate or electrophysiologically record from them,” said Heintz. “This access to mice with precisely labeled cell populations will stimulate the whole area of neurobiology that studies cell physiology and connections,” he said.

Joyner, who helped direct the group that produced the transgenic mice for the project, said, “those of us who study a particular area of the brain can use these mice to discover what genes are expressed in that area. We may well see new expression patterns—in which different genes are expressed in different subsets of cells—that tell us these areas are not as homogeneous as we believed. This tells us that there is some new biology going on that we didn't suspect. Thus, these mice will be an invaluable resource for further exploration.”

In the *Nature* article, the researchers presented data on a variety of CNS genes to illustrate the utility of the atlas. The genes included those that code for “lineage markers” that reveal the location and development of specific subpopulations of CNS cells; guidance molecules that govern the wiring of neural circuitry during embryonic development; and molecules that enable

researchers to trace the migration of neural cells during development of the brain and spinal cord.

Heintz and his colleagues also hope that data from the atlas will enable better functional analyses of neural circuitry by permitting researchers to manipulate individual circuits in the brain. “It’s well known that the nervous system works by forming and using very specific circuits,” said Heintz. “And, in some cases, those circuits have been mapped out in cellular detail, so that investigators know which neurons in the brain actually contribute to a circuit controlling a specific behavior. Although this is somewhat futuristic, several laboratories are working on genetic methods to control the electrical activity of neurons. The GENSAT project will enable these strategies to be targeted to specific cells in a circuit, to better understand how that circuit operates and controls behavior.”

For researchers studying neurological disorders, the atlas will enable dissection of molecular mechanisms and neural circuitry underlying those disorders, said Heintz. “There are now many mouse models of neurodegenerative disease, such as Huntington’s disease, that fairly accurately reproduce the symptoms seen in humans,” he said. “Yet, in most cases, it’s hard to know where the primary pathology lies—which cells are responsible for contributing to the primary effects in the disease. Based on data provided by this atlas, investigators can express a mutant protein that causes a human disease in each of the cell types implicated in the disease, and find out which ones are primarily affected by this mutant gene product. Such studies will enable to dissect mechanisms of degeneration at a much more precise level in the intact brain.”