DATABASES

Gene Expression Nervous System Atlas (GENSAT)

Nathaniel Heintz

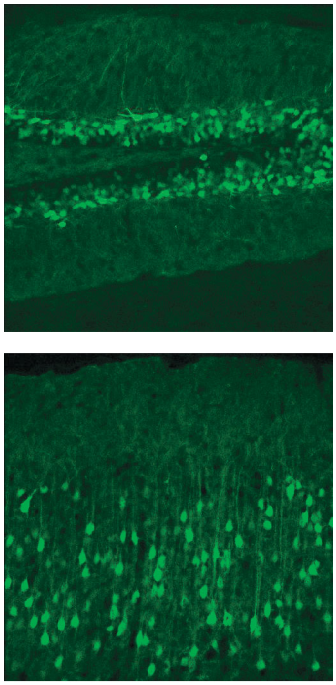
Our understanding of the molecular mechanisms that contribute to the formation and function of the brain must include information about the precise distributions of specific genes and proteins throughout development, and the ability to identify, visualize and genetically manipulate each of the major central nervous system (CNS) cell types. The aims of the GENSAT project are to use results from a variety of studies to identify and map the expression of ~5,000 of the most important CNS-expressed genes throughout development, to create a characterized library of bacterial artificial chromosome (BAC) clones that provide genetic access to each of the major cell populations in the mammalian brain, and to establish a collection of BAC transgenic mouse lines carrying fluorescent reporter genes that allow further anatomical and physiological studies of these cells. The large chromosomal segments carried in the BACs ensure that reproducible expression is retained for most genes after integration into the mouse genome. The GENSAT project is the first large-scale effort to combine *in situ* hybridization and BAC transgenic methods to create an atlas of gene expression in the mammalian brain, while improving genetic access to all classes of CNS cells.

The project uses two approaches to gene-expression mapping. First, a high-throughput isotopic *in situ* hybridization prescreen for gene expression is done to map the expression of over 1,000 genes per year on sections from developing and adult mouse brain (at St. Jude Children's Research Hospital, Memphis, Tennessee under the supervision of T. Curran, S. Magdaleno and P. Jensen). The genes analyzed as part of the prescreen are chosen by advisory committees representing a broad spectrum of the neuroscience community, by project sponsors at the National Institute of Neurological Disorders and Stroke (NINDS) and by the GENSAT investigators.

Nathaniel Heintz is at The Rockefeller University and Howard Hughes Medical Institute, 1230 York Avenue, New York, New York 10021, USA.
e-mail: heintz@rockvax.rockefeller.edu

Second, 250 genes are chosen each year from the prescreen data to be analyzed at high resolution using enhanced green fluorescent protein (EGFP) reporter genes incorporated into BAC transgenic mice (at The Rockefeller University under the direction of N. Heintz, M.B. Hatten and A. Joyner). This two-stage approach takes advantage of the sensitivity, dynamic range and efficiency of isotopic *in situ* hybridization methodology to allow parallel analysis of large numbers of CNS-expressed genes, while exploiting BAC reporter gene technology to allow systematic analysis and high-resolution visualization of each cell type expressing a gene of interest. In addition to the atlas of gene expression created by the GENSAT team, the BAC transgenic mice are being used in a number of laboratories for advanced imaging studies, cell isolation by fluorescence-activated cell sorting, electrophysiological analysis of defined CNS cell populations, and additional anatomical studies. Furthermore, the BAC vectors identified by GENSAT provide a rich resource for additional genetic studies of specific CNS cell types through expression of site-specific recombinases, dominant-activating or dominant-negative proteins and RNAs, and other molecules created to alter the properties of cells. We anticipate that the gene expression atlas, as well as the *in situ* probes, the BAC transgenic mice and the BAC vectors generated by GENSAT, will be important tools for a broad range of neuroscientists.

The first description of results from the GENSAT project and the use of this information to formulate specific hypotheses regarding CNS development and function[1] was accompanied by online publication of data collected from the BAC transgenic reporter genes (www.gensat.org). This site is public, and it is continually updated with detailed expression data from the BAC transgenic mice and with appropriate technical detail to exploit the expression data for additional genetic studies. The methods used for the *in situ* hybridization prescreen, the first sets of results from this screen, and visualization tools for mining the data are currently being prepared for publication and data release. Furthermore, a database is being constructed at the National Center of Biotechnology Information (NCBI) under the direction of M. DiCuccio, S. Kurdin, B. Jones and M. Inata that will incorporate all of the GENSAT data, fully integrated into the Entrez family of databases for presentation of scientific information.



Direct fluorescence of EGFP reporter gene in the cerebral cortex and hippocampus of adult *Cx3cl1* BAC transgenic mice.

Immunohistochemistry of EGFP reporter gene expression in adult brain from *Cx3cl1* BAC transgenic mice.

1. Gong, S. *et al. Nature* **425**, 917–925 (2003).