14. Tomioka, N. *et al. J. Neurosci.* **20**, 5802–5812 (2000).
15. Ilgren, E. B., Saxton, D. G., Jones, A. E. & Duckworth, S. D. *J. Neurosci. Methods* **13**, 191–197 (1985).
16. Muneoka, K., Wanek, N. & Bryant, S. V. *J. Exp. Zool.* **239**, 289–293 (1986).
17. Gaiano, N., Kohtz, J. D., Turnbull, D. H. & Fishell, G. *Nat. Neurosci.* **2**, 812–819 (1999).

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Single neuron labeling and genetic manipulation

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Those who have observed brain sections stained by the Golgi method would agree with Ramon y Cajal[1]: "What an unexpected sight!…everything is simple, clear and unconfused. There is no longer any question of interpretation." The Golgi method labels a very small population of random neurons in their entirety in an otherwise unstained brain, allowing visualization of dendritic trees of individual neurons and tracing of long distance axonal projections[1]. It is difficult to overestimate the enormous contribution this method has brought to neuroscience.

Now imagine that one can use genetic manipulation to create, at will, singly-labeled neurons in intact brain tissue or *in vivo*, and moreover, knock out endogenous genes in only these labeled neurons. This will help us to assess the functions of genes in single clearly labeled neurons, increasing the power of phenotypic detection; avoid pleiotropic effects of genes by focusing on the tissue and developmental stages of interest; and determine cell-autonomy of gene action. The cellular and molecular mechanisms that ensure the elaborate connection and function of the nervous system can then be dissected with single neuron resolution.

How can one achieve this purpose? Genetically mosaic animals, in which a subset of somatic tissues have different genotypes compared to the rest of the organisms, have long been used to attack biological problems in *Caenorhabditis elegans*, *Drosophila* and mice. Traditionally, genetic mosaic animals were generated via spontaneous or X-ray-induced mitotic recombination, resulting in progeny homozygous mutant for a candidate gene of interest in the heterozygous (and therefore phenotypically normal in most cases) background. In mice, chimaeras can also be generated by mixing embryonic cells of different genotypes. With the introduction of sequence-specific recombination systems such as FLP/FRT or Cre/LoxP, not only can one dramatically increase the efficiency of generating mosaic animals, but also control where and when such recombination occurs by dictating the expression pattern of the FLP or Cre recombinase.

Mosaic analysis is only useful if one can tell apart mutant cells from wild-type cells. In *Drosophila*, for instance, traditional mosaic analysis relies on external markers such as body color or bristle shape. To make it versatile, a cell marking system was introduced in the highly efficient FLP/FRT system[2] by placing a marker gene on the chromosome arm in *trans* to the mutation of interest, both distal to the homologous FRT sites. After mitotic recombination, homozygous mutant cells become the only cells in mosaic animals that do not express the gene, and hence are uniquely unlabeled[3]. FRT transgenes have been inserted at the bases of all chromosome arms to allow mosaic analysis of vast majority of genes[3]. Although very useful in studying many developmental biology problems, it is not ideal to study complex neuronal morphogenesis, as one cannot visualize mutant neurons.

The MARCM system[4] (for mosaic analysis with a repressible cell marker) in *Drosophila* solved this problem. Taking advantage of the highly successful GAL4/UAS binary expression system in *Drosophila*[5], we introduced the GAL80 protein, an inhibitor of GAL4, into flies under the control of a ubiquitous promoter. The *GAL80* transgene was placed on the chromosome arm in *trans* to the mutation of interest. The generation of a homozygous mutant cell is therefore coupled with the loss of the *GAL80* transgene, and hence allows the marker gene expression. In this way, only homozygous mutant cells are uniquely labeled in the mosaic animal[4]. By controlling where and when the FLP recombinase is expressed, uniquely labeled single mutant neurons can be generated routinely. The MARCM system has been used to study cell lineage, analyze gene function in axon and dendrite development, isolate new mutants affecting complex neural developmental processes, and study neural network assembly[6–8].

The use of MARCM is not limited to studying neural development. For instance, a GAL4/UAS-based system has been described to conditionally inactivate synaptic transmission—by a simple temperature shift resulting in the expression of dominant temperature-sensitive mutant protein involved in synaptic vesicle endocytosis—in all neurons that express GAL4[9]. This has proven very useful to identify the roles of specific groups of neurons in specific behaviors[10–12]. It is conceivable that one can further map the function of individual neurons important for a specific behavior by combining this technique with the MARCM system.

How about mice? Through homologous recombination, endogenous genes can be removed in otherwise wild-type mice[13]. However, powerful, standard gene targeting has limitations: many genes have pleiotropic functions that impede the analysis of their nervous system functions. 'Conditional knockout' based on Cre/loxP site-directed homologous recombination has been used be to remove genes selectively in particular developmental stages or a subset of tissues[14,15]. FLP/FRT has also been used[16]. As discussed above, it will be very useful if one can delete genes in isolated neurons and simultaneously visualize these mutant neurons. Using the current technology[15], one can, in theory, uniquely label homozygous null mutant cells in the Cre/Lox system by inserting a marker gene after the transcription stop in the 'floxed' allele. Most Cre-mediated recombination is 'too efficient' to generate single-neuron mosaics. However, Cre-expressing mice generating very low efficiency recombination have been reported, capable of labeling isolated single neurons *in vivo* (personal communication, J. Huang, Cold Spring Harbor and S. Tonegawa, MIT). Still, there are several technical limitations in applying the above method other than the intense labor needed to generate these mice individually. Could a systematic way of generating mosaic animals be established by making use of mitotic recombination between homologous chromosomes in mice, as in *Drosophila*? The biggest unknown seems

to be whether one can achieve a sufficiently high recombination rate between homologous chromosomes in somatic cells of mice.

1. Cajal, S. R. *Histology of the Nervous System of Man and Vertebrates* (Oxford, Oxford, UK, 1911; translation, Oxford Univ. Press, 1995).
2. Golic, K. G. & Lindquist, S. *Cell* **59**, 499–509 (1989).
3. Xu, T. & Rubin, G. M. *Development* **117**, 1223–1237 (1993).
4. Lee, T. & Luo, L. *Neuron* **22**, 451–461 (1999).
5. Brand, A. H. & Perrimon, N. *Development* **118**, 401–415 (1993).
6. Lee, T. & Luo, L. *Trends Neurosci.* **24**, 251–254 (2001).
7. Lee, C. H., Herman, T., Clandinin, T. R., Lee, R. & Zipursky, S. L. *Neuron* **30**, 437–450 (2001).
8. Jefferis, G. S. X. E., Marin, E. C., Stocker, R. F. & Luo, L. *Nature* (in press).
9. Kitamoto, T. *J. Neurobiol.* **47**, 81–92 (2001).
10. Waddell, S., Armstrong, J. D., Kitamoto, T., Kaiser, K. & Quinn, W. G. *Cell* **103**, 805–813 (2000).
11. Dubnau, J., Grady, L., Kitamoto, T. & Tully, T. *Nature* **411**, 476–480 (2001).
12. McGuire, S. E., Le, P. T. & Davis, R. L. *Science* **293**, 1330–1333 (2001).
13. Capecchi, M. R. *Science* **244**, 1288–1292 (1989).
14. Gu, H., Marth, J. D., Orban, P. C., Mossmann, H. & Rajewsky, K. *Science* **265**, 103–106 (1994).
15. Joyner, A. L. (ed.) *Gene Targeting: A Practical Approach* 2nd edn. (Oxford Univ. Press, Oxford, UK, 2000).
16. Dymecki, S. M. *Proc. Natl. Acad. Sci. USA* **93**, 6191–6196 (1996).

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Tackling the brain's genetic complexity

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The evolving genomics technologies offer unprecedented opportunities for neuroscientists. Many questions now under investigation simply could not have been addressed with previous methods, in which the expression of only one or a handful of genes could be examined. At the heart of all the new methods is the ability to examine expression of tens of thousands of genes at the RNA and/or protein level; high throughput is envisioned such that analyses can be run on large numbers of samples, leading to statistical significance for even modest changes in expression levels. The new technologies enable characterization of the genes that are expressed by samples as small as a single cell[1] and as complex as the human brain. Neuroscientists are just beginning to apply genomics methods to problems such as measuring responses to chronic drug application[2] and characterizing developmental gene expression programs[3].

Many of the most popular methods rely upon microarrays or 'chips,' in which nucleic acids (and more recently, proteins) are attached to relatively small supports[4–6]. Fluorescently labeled cDNA probes are then used, which are taken from tissue or cells prepared in a variety of ways. A major advantage of chips is that they promise high throughput and are very easy to use. However, it is somewhat difficult to make one's own, and commercial arrays are currently expensive and limited in terms of the number of genes represented and the species from which they are taken. The sensitivity, accuracy and reproducibility of the microarray methods are currently being defined. However, with respect to sensitivity, the data to date are promising. For example, a probe prepared from the brain applied to a microarray representing approximately 18,800 genes revealed expression of a large percentage of those genes[7].

For a relatively small number of samples, serial analysis of gene expression (SAGE[8]) is an alternative to microarrays. SAGE does not require sophisticated hardware or an infrastructure to set up, but it is expensive and slow, as it requires large-scale sequencing (usually contracted to a sequencing facility). It involves making cDNA using standard molecular biological techniques and then sequencing 14–15 base pairs of each cDNA, which is referred to as a tag. The extensive collection of sequences in the public databases usually allows one to identify a tag as part of an expressed sequence tag (EST) or gene. By sequencing large numbers of tags, one can look for expression differences in samples from different conditions, such as normal versus diseased tissue. A major advantage of SAGE is that data-sharing is straightforward: each SAGE library is made in the same way, the data comprise simple frequencies of tags, and from anywhere in the world, the frequency of a tag in one sample can be directly compared to the frequency of that same tag in another sample (for example, see ref. 9). In contrast, microarray data are difficult to share, as chips themselves vary widely, probes are made using different protocols, data are expressed as relative differences among probes, and the methods of calculating signal versus noise vary significantly[10].

As the number of SAGE libraries from different tissues increases—SAGE libraries are now being analyzed from the cerebellum, hypothalamus, cerebral cortex, and retina—we are learning about the tissue specificity of expression of different genes. My laboratory has used SAGE to sequence over 700,000 tags from the developing, mature and diseased mouse retina (S. Blackshaw and C.L.C., unpublished observations). These data allow us to define a complete (or nearly complete) set of genes expressed in photoreceptor cells, including many newly characterized genes whose expression is enriched in or is specific to photoreceptor cells. Because previously characterized photoreceptor-specific genes comprise a significant fraction of the genes that cause blindness, the newly characterized set are anticipated to be involved in human disease as well. This method of disease gene discovery can be extended to other tissues where specificity of expression provides excellent candidates for disease genes.

Neuroscientists will be able to use both SAGE and microarrays to finish what Cajal began—that is, to make a catalogue of the cell types that comprise the brain. This catalogue will be based on the genes expressed in each area, ideally following examination of individual cells. The catalogue will provide for several important advances. First, we will know how many different types of cells we are dealing with. Second, on the basis of gene expression patterns, strains of mice bearing green fluorescent protein or other live reporters can be created to mark specific cell types[11,12]; combinatorial strategies will allow for unique marking in cases where a unique gene will not mark a specific cell type. Specific cell types can be isolated from these strains for applications