Review

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Feature Review

Molecular neuroanatomy: a generation of progress

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The neuroscience research landscape has changed dramatically over the past decade. Specifically, an impressive array of new tools and technologies have been generated, including but not limited to: brain gene expression atlases, genetically encoded proteins to monitor and manipulate neuronal activity, and new methods for imaging and mapping circuits. However, despite these technological advances, several significant challenges must be overcome to enable a better understanding of brain function and to develop cell type-targeted therapeutics to treat brain disorders. This review provides an overview of some of the tools and technologies currently being used to advance the field of molecular neuroanatomy, and also discusses emerging technologies that may enable neuroscientists to address these crucial scientific challenges over the coming decade.

From gene-centric to cell-centric strategies

Progress in neuroscience over the past decade has relied heavily on gene-centric strategies, such as the genetic or pharmacological manipulation of gene function, affecting multiple cell types and tissues in the nervous system. Although progress in the gene-centric realm has been substantial, the fundamental organizing principle of the nervous system is the cell and not the gene. Transmission of information and the generation of behavior are directly determined by cell type and by the connectivity among various cell types. Improved cell-centric strategies, such as those permitting functional manipulation of specific neuronal cell types and circuits, are crucial for understanding the nervous system, and may be essential for both a full mechanistic understanding of important brain disorders and the eventual development of next-generation cell type-targeted therapeutics for these disorders. Furthermore, the development of nanoparticles that are targetable to specific cell types (as defined by molecular phenotype and neuronal circuit) could enable non-invasive mapping, monitoring, and manipulation of the activity of millions of neurons at the single cell and millisecond resolution, as conceived by projects such as the Brain Activity Map (BAM) [1–3].

This review will describe several of the most important gene-centric technologies and resources that have been developed, and will describe the ways in which they provide a firm foundation for the further development of new and improved cell-centric strategies for analysis of the nervous system in the coming decade. These technologies and resources include gene expression atlases of the brain, gene expression profiling, knockouts and transgenic animals, Cre driver lines, viral vectors, connectivity maps, genetically encoded biosensors and modulators, and molecular phenotype datasets [4]. Cell-specific genetic manipulation has been inhibited by: (i) the limited number of cell type-specific promoters, (ii) the very few genes that are selectively expressed in a given cell type, and (iii) our still limited knowledge of the mechanisms that specify cell type. Emerging single-cell technologies used to profile cell types and synapses in heterogeneous tissues, such as cell-specific barcoding strategies, provide a means to overcome this barrier.

Brain atlases

Eighty percent of the 20 000 genes in the mammalian genome are expressed in the central nervous system [5]. These distinct patterns of gene expression underlie neuronal identity, anatomical boundaries, and the specification of neuronal circuits. Characterization of changes in neuronal gene expression has provided key insights into neural development and the response of the nervous system to the environment and drugs of abuse. The Allen Brain Gene Expression Atlas and the GENSAT atlas were developed with the expectation that gene expression arrayed in either 2D or 3D would identify cell type-specific molecular markers. This would then provide targets to facilitate the delivery of genes and gene products to these various cell types for the analysis of cellular development, connectivity, and function, as well as casting light on the principles by which genes organize the nervous system. Early advances in mouse genome sequencing and in manipulating the mouse genome through transgenesis and homologous recombination led to a strong preference for the mouse over other organisms such as the rat. However, targeted mutation in the rat can now be

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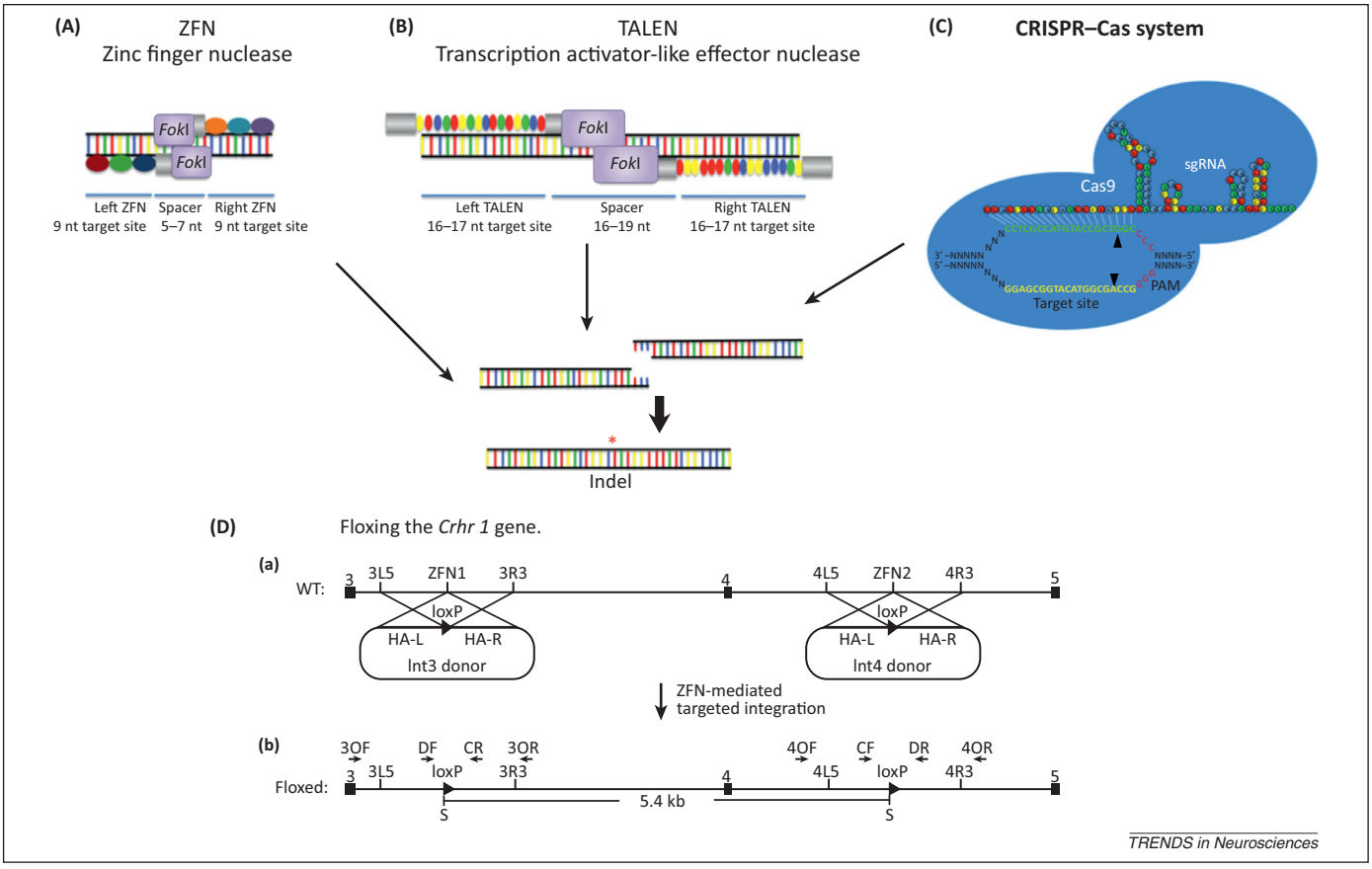

Figure 1. Genome engineering: creating a Cre/*loxP* rat using ZFN (zinc-finger nuclease), TALEN (transcription activator-like effector nuclease), and CRISPR (clusters of regularly interspaced short palindromic repeats) technologies for inducible gene knock-in or knockout. **(A)** ZFN links an engineered DNA-binding zinc-finger (ZF) domain with a DNA-cutting nuclease domain, which contains a *FokI* restriction enzyme site. Each ZF recognizes and binds to three targeted nucleotides. The pairing of left ZFN and right ZFN acts like a pair of genomic scissors to produce a double-strand (ds) DNA incision in the spacer region. The ZF domains are often extended, doubled, or tripled for longer sequence recognition and increased specificity on each side. ZFN-induced chromosomal breaks are then randomly reconnected by endogenous cellular DNA repair mechanisms ('non-homologous end-joining'), leading to gene knockout. Nonetheless, if manipulated DNA strands ending with nucleotides on each side can bind via homologous pairing to the DNA break during ZFN incision, homology-dependent repair will take place, resulting in gene knock-in. Adapted, with permission, from [140]. **(B)** TALENs bind to DNA using transcription activator-like effector (TALE) repeat domains derived from *Xanthomonas* that recognize individual nucleotides. These TALE repeats are ligated together to create binding arrays that recognize extended DNA sequences. The coupled nuclease domain of TALEN cleaves the DNA, in the same fashion as ZFN, within the intervening spacer region. Adapted, with permission, from [140]. **(C)** CRISPRs were first found in the *Escherichia coli* genome and are probably involved in immune defense against foreign DNA. CRISPR loci are surrounded by a cohort of conserved CRISPR-associated genes (*Cas* genes) adjacent to the cluster repeats. The processed CRISPR RNAs (crRNAs) serve as sequence-specific guides, bringing the Cas proteins/nucleases to the target and generating a ds break (DSB) in the DNA. In CRISPR genome editing, the crRNA and the transactivating crRNA (tracrRNA) form a dsRNA structure that directs Cas9 to generate DSBs in the target DNA. At the genomic site complementary to the crRNA, the Cas9 HNH nuclease domain cleaves the complementary strand, and the Cas9 RuvC-like domain cleaves the non-complementary strand. Reprinted, with permission, from [141]. **(D)** Floxing the *Crhr1* gene. In conditional knock-in rats, a plasmid DNA containing *Cre* or *loxP* is used together with the genomic editing methods. The plasmid DNA contains homologous binding sequences to bind to the DSB. Insertion of the plasmid DNA is achieved through a mechanism termed homologous end-joining during DNA repair. This allows *Cre/loxP* to be inserted at desired genomic sites to create inducible mutations and to generate *Cre/loxP* rats for specific neural circuitries in the brain. Figure courtesy of Dr Xiaoxia Cui, Sage Labs, St. Louis, MO, USA.

achieved using transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), and clusters of regularly interspaced short palindromic repeat (CRISPR) technologies (Figure 1).

The Allen Mouse Brain Atlas

The Allen Mouse Brain Atlas is a high-resolution 2D and 3D digital atlas of the C57BL/J mouse brain populated by 20 000 transcripts [5–7]. This effort led to the development of high-throughput methods for performing *in situ* hybridization, a standardized coordinate system for displaying mouse brain gene expression, and an informatics framework for data integration and analysis. The standardized coordinate system is particularly important for correlating the expression of multiple genes to infer the number of cell types in a given brain region, delineate anatomical boundaries, and ultimately correlate gene expression patterns with neuronal connectivity [8]. Using data from the Allen Mouse Brain Atlas, Wolf *et al.* [9] suggest that regional gene expression predicts neuronal connectivity. Recent work by Ko *et al.* [10] suggests that the anatomical boundaries within a mouse brain can be defined by the clustering of only 170 neuron-specific genes (Figure 2). Work by Grange *et al.* [11] also suggests that anatomical boundaries for cortex, hippocampus, striatum, ventral midbrain, medulla, and cerebellum can be delineated based on the cell type density and gene expression profiling for 64 cell types from these different regions. Experiments in which these gene expression patterns are subtracted from each voxel suggest that many more mouse brain cell types may exist. Expression profiling of many additional neuronal cell types is necessary to provide combinatorial gene expression data that can be used to characterize better the anatomical boundaries and cell type-specific organization.