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Conditional gene targeting in the mouse nervous system: Insights into brain function and diseases

Claire Gavériaux-Ruff *, Brigitte L. Kieffer

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, UMR7104, Illkirch, France

Abstract

Conditional gene knockout represents an extremely powerful approach to study the function of single genes in the nervous system. The Cre-*LoxP* system is the most advanced technology for spatial and temporal control of genetic inactivation, and there is rapid progress using this methodology in neuroscience research. In this approach, mice with *LoxP* sites flanking the gene of interest (floxed mice) are bred with transgenic mice expressing Cre recombinase under the control of a selected promoter (Cre mice). This promoter is critical in that it determines the time and site of Cre expression. Cre enzyme, in turn, recombines the floxed gene and produces gene knockout. Here we review Cre mouse lines that have been developed to target either the entire brain, selected brain areas, or specific neuronal populations. We then summarize phenotypic consequences of conditional gene targeting in the brain for more than 40 genes, as reported to date. For many broadly expressed genes, brain-restricted knockout has overcome lethality of conventional knockout (KO) and has highlighted a specific role of the encoded protein in some aspect of brain function. In the case of neural genes, data from null mutants in specific brain sites or neurons has refined our understanding of the role of individual molecules that regulate complex behaviors or synaptic plasticity within neural circuits. Among the many developing functional genomic approaches, conditional gene targeting in the mouse has become an excellent tool to elucidate the function of the approximately 5000 known or unknown genes that operate in the nervous system.

Keywords: Knockout; Mouse transgenesis; Cre recombinase; Mouse genetics; Behavior

Abbreviations: AAV, adeno-associated virus; AgRP, agouti-related protein; ARC, arcuate nucleus of the hypothalamus; B-raf, Ser/thr kinase type-B; BAC, bacterial artificial chromosome; BDNF, brain-derived neurotrophic factor; BNST, bed nucleus of the stria terminalis; CA1, CA3, cornu ammonis fields 1 or 3 of the hippocampus; CamKII, calcium/calmodulin-dependent protein kinase II; CART, cocaine and amphetamine-related transcript; CAV, canine adenovirus; cGK1, cGMP-dependent protein kinase 1; CNS, central nervous system; CREB, cAMP-responsive element binding protein; CrePR, Cre recombinase progesterone receptor fusion protein; CRH, corticotropin releasing hormone; Crhr1, CRH receptor type 1; D1R, dopamine receptor type 1; D6, enhancer of mouse Dach1 (dachshund); DAT, dopamine transporter; Egr-2, early growth response gene 2; Emx1, mouse homologue of the *Drosophila* homeobox gene empty spirales; ES, embryonic stem cells; FLPe, enhanced FLP (flippase); Fmr1, Fragile X syndrome; Foxg1, Forkhead box G_1; $GABA_A$-R-alpha6, gamma-aminobutyric acid receptor subunit alpha6; GC, glucocorticoid; GR, glucocorticoid receptor; GFAP, glial fibrillary acidic protein; GluRdelta2, glutamate receptor delta 2 subunit; GluRepsilon3, glutamate receptor epsilon 3 subunit; 5-HT1AR, serotonin receptor type 1; Hap-1, Huntingtin-associated protein-1; HPA, hypothalamus–pituitary adrenal axis; iCre, codon improved Cre recombinase; KA1, kainate receptor subunit 1; KO, conventional knockout; L1-NCAM, L1 neuronal cell adhesion molecule; LHRH, luteinizing hormone-releasing hormone; LTD, long term depression; LTP, long-term potentiation; MAPK, mitogen associated protein kinase; MC4R, melanocortin-4 receptor; MeCP2, methyl-CpG binding protein 2; $Na_v1.7$, voltage-gated sodium channel type 1.7; $Na_v1.8$, voltage-gated sodium channel type 1.8; NCAM, neuronal cell adhesion molecule; NEX, neuronal helix-loop-helix protein; NGF, nerve growth factor; NLOT, nucleus of the lateral olfactory tract; NMDA, *N*-methyl-D-aspartate; NR1, NMDA receptor type 1; NR2, NMDA receptor type 2; NPY, neuropeptide Y; NSE, neuron-specific enolase; NTS, nucleus of the tractus solitarius; PAG, periaqueducal gray; PH, posterior hypothalamus nucleus; PLP, proteolipid protein; POMC, pro-opiomelanocortin; PS, presenilin; PVH, paraventricular nucleus of the hypothalamus; SERT, serotonin transporter; SF1, steroidogenic factor 1; Shp2, Src homology 2-containing tyrosine phosphatase; Sim-1, single minded-1; Socs3, suppressor of cytokine signalling-3; SON, supraoptic hypothalamus nucleus; STAT3, signal transducer and activator of transcription-3; SRF, serum response factor; TH, tyrosine hydroxylase; TrkA, tyrosine kinase A; TrkB, tyrosine kinase B; VMH, ventromedial hypothalamus; YAC, yeast artificial chromosome.

* Corresponding author. Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1 rue Laurent Fries BP 10142, 67404 Illkirch cedex, France. Tel.: +33 3 88 65 56 94; fax: +33 3 88 65 56 04.
E-mail address: gaveriau@igbmc.u-strasbg.fr (C. Gavériaux-Ruff).

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1. Introduction

One challenge of modern neurobiology is the identification of individual molecules that operate within neural circuits, regulate brain function and control synaptic plasticity. A unique approach to tackle the molecular basis of neuronal activity consists in manipulating the genome of higher organisms. In this context, the mouse represents an exceptional animal model which (i) is prone to targeted gene modifications, (ii) shares the complex genome and neuroanatomical organization of mammals and (iii) can be studied in paradigms which model the wide array of human neurological or psychiatric diseases.

The advent of gene targeting technology by homologous recombination (HR) in mouse has led to a first generation of so-called knockout animals (Capecchi, 1989). In these mutant mice, the gene of interest is inactivated following insertion of an antibiotic resistance gene within, or in place of, an essential exon (for detailed methods, see Dierich & Kieffer, 2004). Many genes expressed in the nervous system have been inactivated by HR. Phenotyping of these null mutant mice has provided invaluable information in the identification of key proteins involved in neural development and plasticity, as well as neurotransmission or drug activities in vivo. The conventional knockout (KO) technology, however, has limited utility in several situations. First, the gene of interest could be essential for development and survival, and the gene knockout lead to a lethal phenotype. Second, the targeted gene could be important for normal development and share functional redundancy with other genes. In this case the phenotype could be hardly detectable and would likely result from compensatory mechanisms that may be difficult to clarify. Third, some proteins are widely expressed both in the nervous system and peripheral tissues, and gene knockout throughout the body does not address their specific role in cerebral function. Additionally, and because of the high anatomic complexity of the nervous system, many neural proteins fulfill distinct functions depending on their site of expression within neurons and neural circuits. As a consequence, the complete deletion of a specific protein throughout the nervous system may prove ineffective towards understanding fine molecular processes in higher brain functions. Spatial and temporal control of the gene knockout, generally referred to as conditional gene knockout, was obviously the next step in the development of gene targeting technologies.

Among the many existing strategies that have been developed to modify the mouse genome (for extensive reviews, see Brusa, 1999; Lewandoski, 2001) the Cre-*LoxP* technology is the most popular approach to control targeted genetic inactivation in mice. The technique was first reported by Rajewski and colleagues to knockout the DNA polymerase beta gene specifically in T lymphocytes (Gu et al., 1994). Two years later, a similar approach was used in the field of neuroscience to address the role of *N*-methyl-D-aspartate (NMDA) receptor type 1 (NR1) subunit of the NMDA glutamate receptor in learning processes. In a pioneer set of studies, Tonegawa and coworkers circumvented the lethal phenotype of the NR1 null mutation by selective inactivation of the NR1 gene in the cornu ammonis field 1 of the hippocampus (CA1) after birth. Importantly, the data provided the first strong genetic evidence for a correlation between NMDA receptors, CA1 hippocampal long-term potentiation (LTP) and spatial learning (McHugh et al., 1996; Tsien et al., 1996a, 1996b). The successful targeting of this important gene in hippocampus launched the development of cell type or regional specific gene knockout to study gene function in behaviors, neurological disorders or psychiatric diseases.

Because of the technical complexity of the approach itself, as well as the need to refine the spatial and temporal control of the genetic recombination, several previous review articles have largely addressed the methodological aspect of conditional gene targeting (see Morozov et al., 2003; Beglopoulos & Shen, 2004; Branda & Dymecki, 2004; Sorrell & Kolb, 2005). Although certainly not comprehensive, the present review will (i) summarize progress of conditional gene targeting in the entire brain, in selected brain areas, or in specific neuron populations using the Cre-*LoxP* system and (ii) overview behavioral alterations triggered by the conditional inactivation of more than 30 genes in the brain. Gene function during brain development is out of the scope of this review, and only the consequences of genetic inactivation in the adult brain are discussed.

2. A key tool: the Cre mouse

Cre recombinase is an enzyme isolated from bacteriophage P1, which specifically catalyzes recombination between two 34-bp *loxP* recognition sites located in genomic DNA. The reaction results in the irreversible excision of the DNA segment comprised between the 2 *loxP* sites. This property of the enzyme is used to target recombination events in the mouse genome, endogenously devoid of *LoxP* sites. Another site-specific recombinase system used is the Flp-frt system where Flp, or flippase, recombines DNA flanked by 2 frt (Flp recombinase recognition target) sites.