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MOLECULAR GENETICS OF MOUSE SEROTONIN NEURONS ACROSS THE LIFESPAN

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Abstract—New molecular genetics approaches have been developed over the past several years to study brain serotonin (5-HT) neuron development and the roles of 5-HT neurons in behavior and physiology. These approaches were enabled by manipulation of the gene encoding the *Pet-1* ETS transcription factor whose expression in the brain is restricted to developing and adult 5-HT neurons. Targeting of the *Pet-1* gene led to the development of a mouse line with a severe and stable deficiency of embryonic 5-HT-synthesizing neurons. The *Pet-1* transcription regulatory region has been used to create several new 5-HT neuron-type transgenic tools that have greatly increased the experimental accessibility of the small number of brain 5-HT neurons. Permanent and specific marking of 5-HT neurons with *Pet-1*-based transgenic tools have now been used for flow cytometry, whole cell electrophysiological recordings, progenitor fate mapping, and live time lapse imaging of these neurons. Additional tools provide multiple strategies for conditional temporal targeting of gene expression in 5-HT neurons at different stages of life. *Pet-1*-based approaches have led to advances in understanding the role of 5-HT neurons in respiration, thermoregulation, emotional behaviors, maternal behavior, and the mechanism of antipsychotic drug actions. In addition, these approaches have begun to reveal the molecular basis of 5-HT neuron heterogeneity and the transcriptional mechanisms that direct 5-HT neuron-type identity, maturation, and maintenance.

Key words: serotonin, Pet-1, raphe, transcription, transgenic.

Contents

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Abbreviations: DRN, dorsal raphe nucleus; Fev, Fifth Ewing Variant; ROb, raphe obscurus; Tph, tryptophan hydroxylase; VGlut3, vesicular glutamate transporter 3.

Brain serotonin (5-HT) neuron signaling is a well known form of neuromodulation that shapes many behaviors and physiological processes through interactions with at least 14 broadly distributed postsynaptic receptor subtypes (Berger et al., 2009; Filip and Bader, 2009). The seemingly ubiquitous neuromodulatory role of the 5-HT system is effected by about 26,000 neurons in the rodent brain (Ishimura et al., 1988). These small numbers of neurons are sparsely intermingled with many non-serotonergic neurons in most of the raphe nuclei. In addition, while many 5-HT neurons are clustered in the midline raphe about 35% of them are scattered off the midline in disparate regions of the midbrain, pons, and medulla (Steinbusch, 1981). Similar to most neuronal types, this experimentally unwieldy neuroanatomical organization has made it very difficult to access 5-HT neurons for molecular and cellular studies of their development and their impact on postnatal behavior and physiology. Although genetic loss and gain of function studies have implicated the broadly expressed serotonin transporter gene (*Sert*) in numerous behavioral and physiological processes (Murphy et al., 2008), genetic-based methods to selectively and stably alter 5-HT neuron gene expression at different stages of life have been lacking. The identification of *Pet-1* (Pheochromocytoma 12 ets) (Fyodorov et al., 1998), an ETS (E26-specific) transcription factor gene, provided a solution to this methodological shortcoming. This review describes how the unique expression pattern of *Pet-1* has been exploited to create 5-HT neuron-type genetic based experimental approaches. The power of these approaches is then illustrated by highlighting some of their recent applications to questions that have been impossible or difficult to address with previously established experimental approaches: (1) transcriptional mechanisms that control 5-HT neuron identity, (2) the impact, in the intact animal, of 5-HT neuron perturbation at different stages of life on behavior, physiology, and pharmacology, and (3) 5-HT neuron molecular heterogeneity.

Pet-1 encodes a class 1 ETS factor whose human ortholog is Fifth Ewing Variant (Fev), an ETS gene whose fusion to the Ewing's sarcoma gene locus in a chromosomal translocation was identified in a subset of Ewing's sarcoma family of tumors (Peter et al., 1997). A degener-

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ate RT-PCR screen, aimed at identifying ETS factors expressed in the nervous system, led to the discovery of *Pet-1* sequences in PC12 cell total RNA. RNase protections assays then revealed extremely weak expression of *Pet-1* in total RNA isolated from whole brain. Consistent with the weak protection signal, a follow-up *in situ* hybridization study indicated that *Pet-1* transcripts were restricted to the raphe nuclei. *In situ* hybridization combined with anti-tryptophan hydroxylase (Tph) immunostaining showed that *Pet-1* is expressed in what appeared to be all 5-HT-synthesizing neurons of the rodent brain (Hendricks et al., 1999). Expression of rat and mouse *Pet-1* in the brain is induced specifically in postmitotic 5-HT neuron precursors about 0.5 days before the initiation of brain 5-HT synthesis (Hendricks et al., 1999; Pfaar et al., 2002). Thus, *Pet-1* is induced and maintained specifically in this single neuronal type across the lifespan. *Pet-1* is unique among the known factors that constitute the transcriptional network that specifies serotonergic phenotype, as it is the only one expressed specifically in 5-HT neurons (Scott and Deneris, 2005). Significantly, the spatiotemporal pattern of expression of the zebrafish ortholog, *zPet-1*, in the zebrafish brain is similar as it is restricted to raphe 5-HT neurons and is induced about 5 h before the onset of *zTph2*, the gene encoding the isoform of the rate-limiting enzyme responsible for zebrafish hindbrain 5-HT synthesis. Interestingly, *zPet-1* is not expressed in other zebrafish 5-HT-synthesizing neurons present in the diencephalon of this species but not in that of rodents (Lillesaar et al., 2007, 2009). In support of its functional orthology, *Fev* is expressed in the human and primate raphe in a pattern that suggests it is restricted to 5-HT neurons (Maurer et al., 2004; Iyo et al., 2005; Lima et al., 2009). A recent study (Kriegebaum et al., 2010) reported detection of *Fev* transcripts by RT-PCR in various dissected human forebrain regions. However, the cellular source of the RNA template in these seemingly weak amplifications was not identified. *Pet-1* is also expressed in small number of peripheral cell types, including the 5-HT-synthesizing enterochromaffin cells of the intestine, adrenal medulla, and pancreatic islets (Fyodorov et al., 1998; Ota et al., 2005; Wang et al., 2010).

Pet-1-BASED APPROACHES

Manipulation of *Pet-1* has enabled the development of diverse mouse molecular genetic approaches for the study of 5-HT neurons (Table 1). The first approach, simple germline targeting of *Pet-1*, provided insight into the transcriptional induction of 5-HT neuron identity as acquisition of serotonergic phenotype failed to occur in most *Pet-1*$^{-/-}$ embryonic 5-HT neuron precursors (Fig. 1). Second, unlike 5-HT synthesis inhibitors and serotonergic neurotoxins whose effects may not be specific to 5-HT and whose physiological and behavioral effects may not depend on

Table 1. *Pet-1*-based transgenic and targeted mice

Name	Background	Description	Application	References
Pet-1$^{-/-}$	C57BL/6*129sv; SJL congenic, n=10; C57BL/6J congenic, n=10	Germline-targeted *Pet-1* null	5-HT neuron differentiation	Hendricks et al., 2003; Erickson et al., 2007; Bonnin et al., 2011
Pet-1 floxed allele	C57BL/6*129Sv	*Cre recombinase* conditional allele	Temporal conditional targeting of *Pet-1*	Liu et al., 2010
ePet-Cre	C57BL/6*SJL	*Cre recombinase* controlled by *Pet-1* enhancer sequences	Gene targeting and optogenetic viral gene expression in 5-HT neurons	Scott et al., 2005; Hodges et al., 2008; Samaco et al., 2009; Liu et al., 2010; Depuy et al., 2011
ePet1::Flpe	Unknown	*Flp recombinase* controlled by *Pet-1* enhancer sequences	Intersectional/subtractive fate mapping of 5-HT neurons	Jensen et al., 2008
ePet::CreER^{T2ascend}	C57BL/6*129	Tamoxifen-inducible *Cre recombinase* controlled by *Pet-1* enhancer sequences	Temporal control of gene expression in ascending 5-HT neurons	Liu et al., 2010
Pet1-CreER^{T2}	Unknown	Tamoxifen-inducible *Cre recombinase* controlled by *Pet-1* enhancer sequences	Temporal control of gene expression in adult 5-HT neurons	Song et al., 2011
eFev::LacZ (Fev60Z)	C57BL/6*129Sv	*LacZ* controlled by human *Fev* enhancer sequences	5-HT neuron marker	Krueger and Deneris, 2008
ePet-EYFP	C57BL/6*SJL	Enhanced yellow fluorescent protein controlled by *Pet-1* enhancer sequences	5-HT neuron flow cytometry, electophysiology, cell culture, live cell imaging	Scott et al., 2005; Wylie et al., 2010; Hawthorne et al., 2010; Hawthorne et al., 2011
ePet::mycPet-1	C57BL/6*129sv	Myc epitope-tagged Pet-1 cDNA controlled by *Pet-1* enhancer sequences	Chromatin immunoprecipitation	Liu et al., 2010
Pet1-tTS	129S6Sv*C57BL/6*CBA	*Tetracycline-dependent transcriptional suppressor* controlled by *Pet-1* enhancer sequences	Inducible suppression of Htr_{1A} autoreceptor	Richardson-Jones et al., 2010; Richardson-Jones et al., 2011