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Temporal Control of Retroviral Transgene Expression in Newborn Cells in the Adult Brain

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SUMMARY

Neural stem/progenitor cells (NSPCs) generate new neurons throughout life in distinct areas of the adult mammalian brain. Besides classical transgenesis-based approaches, retrovirus-mediated genetic manipulation is frequently used to study mechanisms that regulate neurogenesis in the nervous system. Here, we show that fusion of a tamoxifen-regulatable estrogen receptor (ER^{T2}) motif to transcription factors (i.e., ASCL1 and NEUROD1) enables temporal control of transgene expression in adult mouse NSPCs in vitro and in vivo. Thus, the approach described here represents a versatile strategy for regulating gene expression to study gene function in dividing cells and their progeny.

INTRODUCTION

Neural stem/progenitor cells (NSPCs) generate new neurons throughout life in distinct areas of the adult mammalian brain, including the subventricular zone (SVZ) lining the lateral ventricles from which newborn cells migrate toward the olfactory bulb and the hippocampal dentate gyrus (DG) (Zhao et al., 2008). Due to the relative sparseness of NSPCs and their progeny in relation to pre-existing neural structures, there is a need to selectively manipulate gene activity in NSPCs and their progeny to address their functional significance during the course of development from a dividing NSPC to a fully mature and synaptically integrated neuron (Dhaliwal and Lagace, 2011).

One approach to test the functional significance of genes/pathways is to use transgenic mice carrying floxed alleles of genes of interest, together with Tamoxifen (TAM)-regulatable Cre-recombinase controlled by NSPC- or immature neuron-selective promoters, to genetically recombine and delete genes (Ihrie et al., 2011; Sahay et al., 2011). In addition, retroviral vectors derived from Moloney murine leukemia viruses have proved to be an important tool to visualize newborn cells through the expression of fluorescent proteins, as well as to manipulate gene expression using both gain- and loss-of-function strategies (Ge et al., 2006; van Praag et al., 2002). The use of retroviruses has the advantage that it is highly selective for dividing cells (i.e., neurogenic cells when injected into the SVZ or DG) and is a very fast method because extensive breeding to obtain correct genotypes, as is the case for classical transgenesis, is not required (Zhao and Gage, 2008).

However, retroviral vectors do not target bona fide NSPCs that are largely quiescent but integrate into highly proliferative neural progenitors, which restrict virus-mediated genetic manipulations to later steps of neurogenesis. Furthermore, current vectors do not allow inducible or temporally controlled expression of the virus-driven transgene. Temporal control of transgene expression would be advantageous for studying gene function during distinct steps in the course of neuronal development. This is especially true for transcription factors (TFs) that may exert stage-specific functions depending on the age of a given newborn cell (Iwano et al., 2012). We reasoned that fusion of a TAM-regulatable estrogen receptor (ER^{T2}) motif (Indra et al., 1999; Jiang et al., 2010) to expression constructs of TFs involved in neurogenesis would enable TAM-induced transgene expression, allowing for temporal control of virus-mediated gene expression (Figures 1A and 1B; Supplemental Experimental Procedures available online).

RESULTS

It was previously shown that *Ascl1* overexpression in cultured adult NSPCs results in robust neuronal differentiation (Jessberger et al., 2008). To analyze whether fusion of *Ascl1* to the ER^{T2} motif leads to functional ASCL1 expression upon TAM treatment, we transduced NSPCs isolated from adult mice with *Ascl1*-ER^{T2}-IRES-GFP (hereafter called *Ascl1*-ER^{T2})-expressing retroviruses in vitro. To induce translocation of the ASCL1-ER^{T2} fusion protein, we treated the cells with hydroxy-TAM (OH-TAM) and analyzed them 7 days after the onset of differentiation. Whereas *Ascl1*-ER^{T2}-expressing cells cultured without OH-TAM did not show any difference in their rate of neuronal differentiation compared with cells transduced with a control

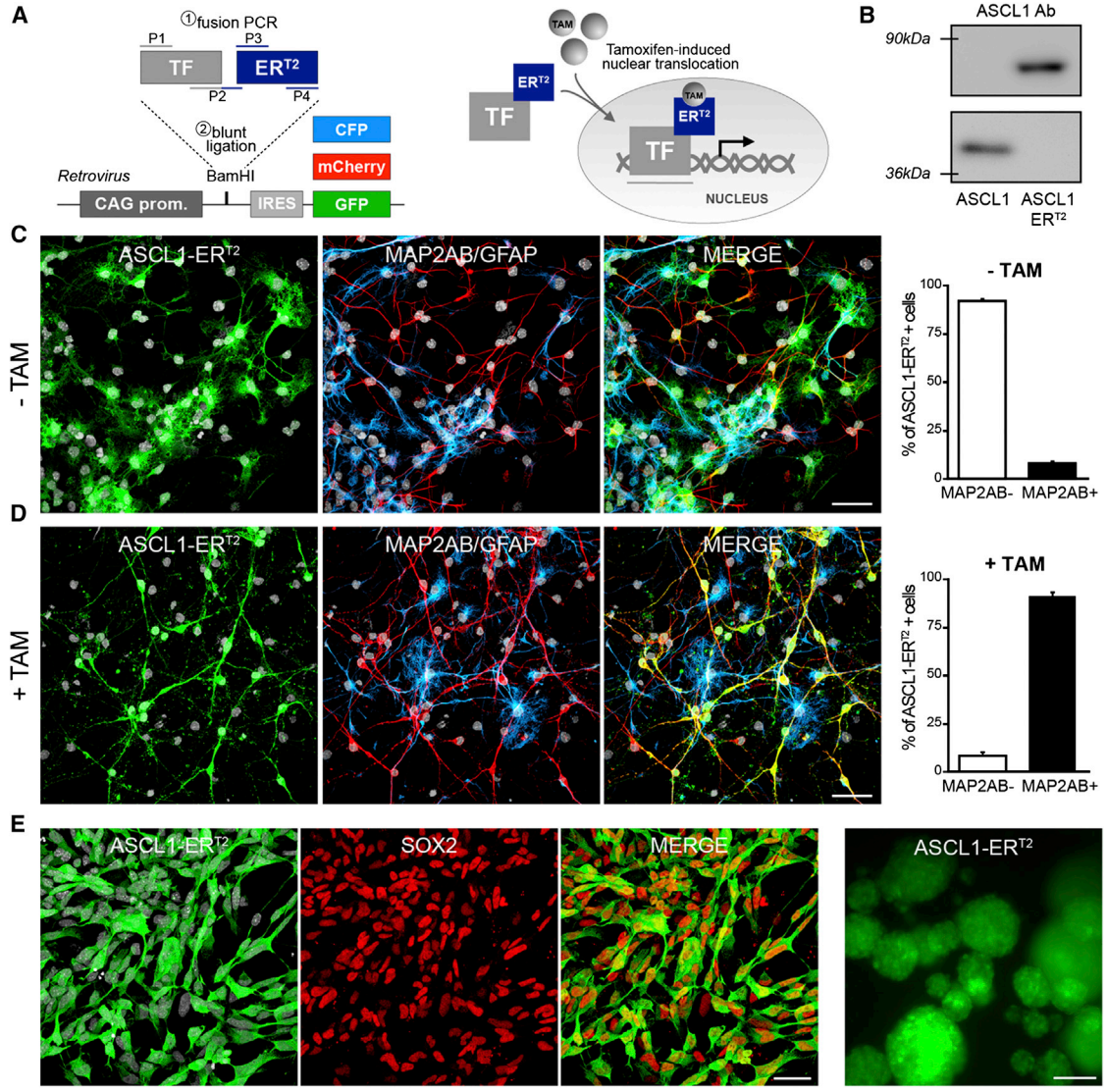

Figure 1. Temporal Control of Retrovirus-Mediated *Ascl1*-ER^{T2} Activity In Vitro

(A) Two-step PCR cloning strategy to fuse an ER^{T2} motif to TFs expressed from a retroviral vector. Upon binding of TAM to the ER^{T2} motif, the TF translocates to the nucleus and regulates target gene transcription.

(B) Western blot analysis reveals the fusion of an ER^{T2} motif to ASCL1, using an ASCL1 antibody to visualize the WT and fusion proteins.

(C) Differentiation of adult NSPCs transduced with *Ascl1*-ER^{T2}-expressing virus (green) without OH-TAM yields only a minority of neuronally differentiated cells expressing MAP2AB (red), whereas most cells differentiate into glial cells expressing GFAP (blue). Right bars show quantifications.

(D) Exposure of *Ascl1*-ER^{T2}-expressing cells (green) to OH-TAM for 4 days leads to the dramatic induction of neuronal differentiation as measured with MAP2AB (red) and a reduction in GFAP-positive cells (blue). Right bars show quantifications. −TAM: 8.04% ± 1.53% neurons; +TAM: 91.58% ± 2.14% neurons; *$p < 0.001$; $n = 3$, biological replicates.

(E) NSPCs transduced with *Ascl1*-ER^{T2}-expressing virus (green) can be properly propagated as monolayers expressing SOX2 (red, left panels) or floating neurospheres in the absence of OH-TAM (right panel), indicating the tight control of virus-mediated gene expression with TAM. Images were taken 10 days after viral transduction.

Error bars represent mean ± SEM. Scale bars represent 40 μm (C, D, and E, left panel) and 100 μm (E, right panel). Nuclei were stained with DAPI (gray).

See also Figures S1 and S3.