Neuroscience 134 (2005) 907–919

CELL TYPE-SPECIFIC AND SEXUALLY DIMORPHIC EXPRESSION OF TRANSCRIPTION FACTOR AP-2 IN THE ADULT MOUSE BRAIN

D. J. COELHO, D. J. SIMS, P. J. RUEGG, I. MINN, A. R. MUENCH AND P. J. MITCHELL*

Department of Biochemistry and Molecular Biology, 201 Life Sciences Building, Pennsylvania State University, University Park, PA 16802, USA

Abstract—Expression of transcription factor AP-2 family genes in adult mouse brain regions was examined at RNA and protein levels and in tissue sections. AP-2 family RNA transcripts, nuclear AP-2 DNA binding activity, and AP-2 immunoreactivity were greatest in hindbrain and midbrain regions. Cells expressing AP-2 were predominantly differentiated neurons and were abundant in the solitary tract nucleus, hypoglossal nucleus, locus coeruleus, cerebellar molecular layer, superior colliculus, mitral cell layers of the main and accessory olfactory bulbs, and in some divisions of the bed nucleus of the stria terminalis. Sexually dimorphic expression of AP-2 was seen in the bed nucleus of the stria terminalis, a forebrain region required for regulation of gender-specific reproductive and social behaviors. In males, AP-2 expressing neurons were present in supracapsular, lateral ventral, and medial ventral divisions of the bed nucleus of the stria terminalis. In contrast, females had AP-2 expressing neurons in the lateral ventral division, but not the supracapsular division, and AP-2 expression in medial ventral division neurons oscillated during the estrus cycle. With the exception of the bed nucleus of the stria terminalis, forebrain regions generally lacked cells with high levels of AP-2. However, a small population of cells co-expressing low levels of AP-2 and Notch1 was sparsely distributed in the cerebral cortex and hippocampal dentate gyrus subgranular zone. Based on their variable levels of NeuN, a marker for differentiated neurons, these cells may include nascent neurons. A subset of cerebellar Purkinje cells also co-expressed low levels of AP-2 and Notch1. Together, the adult brain regions with AP-2 expressing neurons are notable for their importance in pathways that integrate sensory and neuroendocrine information for regulation of reproductive, social, and feeding behaviors. Our data suggest that AP-2 transcription factors contribute at multiple levels to adult brain function including regulation of gender-specific behavior.

*Corresponding author. Tel: +1-814-865-5802; fax: +1-814-863-7024.
E-mail address: pjm23@psu.edu (P. J. Mitchell).

Abbreviations: AOB, accessory olfactory bulb; BST, bed nucleus of the stria terminalis (a.k.a. BNST); BSTLV, bed nucleus of the stria terminalis, lateral division, ventral; BSTM, bed nucleus of the stria terminalis, medial division; BSTMP, bed nucleus of the stria terminalis, medial division, posterior part; BSTMV, bed nucleus of the stria terminalis, medial division, ventral; BSTS, bed nucleus of the stria terminalis, supracapsular division; EMSA, electrophoretic mobility-shift analysis; GFAP, glial fibrillary acidic protein; hMTIIA, human metallothionein IIA; LC, locus coeruleus; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; MOB, main olfactory bulb; NGS, normal goat serum; NST, nucleus of the solitary tract; RT-PCR, reverse transcription–polymerase chain reaction; SGZ, subgranular zone of the dentate gyrus; SVZ, subventricular zone of the lateral ventricle; TH, tyrosine hydroxylase.

Key words: tfap2, Notch signaling, bed nucleus of the stria terminalis, olfactory mitral cell, locus coeruleus, solitary tract nucleus.

Transcription factor AP-2 family genes are expressed in restricted cell types during embryogenesis and have essential roles in development of multiple tissues, including the brain (Mitchell et al., 1991; Schorle et al., 1996; Zhang et al., 1996; Moser et al., 1997; Monge and Mitchell, 1998; Monge et al., 2001; Holzschuh et al., 2003). In humans and mice, AP-2 proteins (AP-2alpha, AP-2beta, AP2gamma, AP-2delta, and AP-2epsilon) are encoded by five genes; in mice, these are *tfap2a*, *tfap2b*, *tfap2c*, *tfap2d*, and *tfap2e*.

Expression in the developing brain is a feature shared by all known transcription factor AP-2 genes. The five murine AP-2 family genes are expressed in spatiotemporally distinct patterns along the anterior–posterior axis of the developing brain; and *tfap2a*, *tfap2b*, and *tfap2c* are also expressed in neural crest cells (Mitchell et al., 1991; Chazaud et al., 1996; Moser et al., 1997; Feng and Williams, 2003; Zhao et al., 2003; Wang et al., 2004a). Several studies have examined expression of AP-2 transcription factors in neural cell types (Philipp et al., 1994; Maconochie et al., 1999; Kramer et al., 2000a,b; Stewart et al., 2001; Holzschuh et al., 2003; Luo et al., 2003). Philipp et al. (1994) showed that AP-2alpha was expressed during retinoic acid-induced neurogenesis in P19 mouse embryonal carcinoma cell cultures, but not at late stages in P19 cultures when neurons died and astrocytes prevailed. AP-2 factors have been implicated in regulation of various neural specific genes, including pre-proenkephalin, acetylcholinesterase, and genes encoding catecholamine biosynthetic enzymes tyrosine hydroxylase (TH), dopamine beta-hydroxylase, and phenylethanolamine *N*-methyltransferase (Hyman et al., 1989; Ekström et al., 1993; Ebert et al., 1998; Kim et al., 2001). Recent genetic analysis in zebrafish has shown that AP-2alpha is required for differentiation and TH-expression of noradrenergic neurons in the locus coeruleus (LC) and medulla (Holzschuh et al., 2003).

With respect to AP-2 transcription factors in adult mouse brain, Kim et al. (2001) carried out double-immunostaining analyses which showed that nearly all noradrenergic neurons in the LC, nucleus of the solitary tract (NST), and area postrema and many adrenergic neurons in C1, C2, and C3 cell groups co-expressed AP-2 and TH. TH-expressing dopaminergic cells, however, did not express AP-2. Non-catecholaminergic AP-2-positive cells were also noted in the cerebellum, midbrain, and brain

0306-4522/05$30.00+0.00
doi:10.1016/j.neuroscience.2005.04.060

stem, but whether these were neurons, astrocytes, or other cell types was not examined.

Mice with targeted null mutations in *tfap2a* die perinatally with severe nervous system, craniofacial, and limb defects (Schorle et al., 1996; Zhang et al., 1996). Similarly, null mutations in *dAP-2*, the sole *AP-2* family gene in *Drosophila*, are lethal and result in CNS defects and significant undergrowth of proboscis and legs (Monge et al., 2001). In developing *Drosophila* legs (leg imaginal discs), *dAP-2* is activated by Notch signaling in epithelial cells at presumptive leg segment boundaries/future leg joints (Kerber et al., 2001). In wing imaginal discs, where dAP-2 is normally not expressed, ectopic expression of dAP-2 transforms wing vein epithelia into ectopic sensory bristles, a classic paradigm of Notch signaling-dependent neurogenesis (Monge et al., 2001). These findings suggest that AP-2 transcription factors mediate some of the effects of Notch signaling in specific epithelial and neuroepithelial cells.

The receptor Notch and its ligands Delta and Serrate/Jagged are evolutionarily conserved EGF-repeat-containing transmembrane proteins that regulate cell fate specification, stem cell maintenance, and initiation of differentiation in many embryonic and postnatal tissues, including the nervous system (de la Pompa et al., 1997; Lutolf et al., 2002) (reviewed by Artavanis-Tsakonas et al., 1999; Alvarez-Buylla and Lim, 2004; Schweisguth, 2004). More recently, mice with reduced levels of Notch signaling have been shown to exhibit impaired long-term potentiation at hippocampal CA1 synapses, implicating Notch in hippocampal synaptic plasticity (Wang et al., 2004b).

We provide here a comprehensive examination of regional and cell type-specific expression of AP-2 family transcription factors in the adult mouse brain. The region-specific expression levels of *AP-2* family genes, *Notch1*, *Notch2*, *Jagged1* and *Jagged2* were examined and double-immunostaining analyses were carried out with AP-2 and NeuN, GFAP, or Notch1 antibodies to determine AP-2 cell type specificity and to assess the potential for regulation of AP-2 by Notch signaling in adult brain cell types. A notable aspect of our study was the identification of sexually dimorphic AP-2 expression in neurons in the supracapsular and medial ventral divisions of the bed nucleus of the stria terminalis (BST), a forebrain region which gender-specifically integrates information between olfactory regions, amygdala and hypothalamus for regulation of social and reproductive behaviors. A second important finding was that AP-2 and Notch1 were co-expressed at low levels in a small population of cells which may include nascent neurons in the cerebral cortex and dentate gyrus, and in a subset of Purkinje cells. The latter findings are consistent with a potential role for Notch signaling in regulation of AP-2 in these cells. Overall, the pattern of AP-2 expression in neurons in restricted regions of the adult mouse brain is notable for highlighting a set of brain structures which include three regions known to be sexually dimorphic (BST, accessory olfactory bulb (AOB), and LC) and several additional regions that function in conjunction with these in sensory, arousal and stress response pathways affecting behavior.

EXPERIMENTAL PROCEDURES

Western blot analysis of sc-184 antibody

The anti-AP-2 antibody sc-184 from Santa Cruz Biotech, Inc. is an affinity-purified rabbit polyclonal antibody raised against a synthetic peptide corresponding to the C-terminal 18 amino acids of human AP-2alpha (TFAP2A). We carried out Western blot analysis to assess the relative sensitivity of sc184 antibody for detection of murine AP-2alpha, AP-2beta and AP-2gamma. These AP-2 family proteins conserve 17, 10 and seven amino acids, respectively, of the 18 amino acid epitope of human AP-2alpha used to generate sc-184. Murine AP-2alpha, AP-2beta and AP-2gamma proteins were *in vitro* synthesized from cDNA expression plasmids using the TNT Coupled Reticulocyte Lysate System (Promega, WI, USA) as described previously (Maconochie et al., 1999). Molar equivalents of each protein were subjected to SDS-PAGE, electroblotted to PVDF membrane, and incubated with sc-184 diluted 1:500. Immunodetection was carried out using HRP-labeled goat anti-rabbit secondary antibody and the chemiluminescent detection system (BioRad) according to the manufacturer's specifications.

Electrophoretic mobility-shift assay (EMSA) of brain region nuclear extracts

Care and handling of mice for experiments described here were according to local (IACUC) and international guidelines on ethical use of animals, including those to minimize numbers of animals used and their suffering. Adult female 129Sv/J mice were anesthetized by CO_2 inhalation. Brains were removed from decapitated heads, and dissected into six parts which were snap-frozen in polypropylene tubes in liquid nitrogen: 1) olfactory bulb, 2) cerebral cortex and hippocampus, 3) basal forebrain and diencephalon (striatum, thalamus and hypothalamus), 4) midbrain, 5) cerebellum, and 6) medulla and pons. Nuclear extracts were prepared by Dounce homogenization following the protocol of Schreiber et al. (1988). Nuclear protein concentration was quantified by Bradford assay. ^{32}[P]-labeled double-stranded oligonucleotide probe with the sequence 5′-GATCGAACTGACCGCCCGCGGCCCGT-3′ representing the AP-2 binding site from human metallothionein IIA (hMTIIA) promoter was used. DNA binding reactions contained 10 μg nuclear protein, 2×10^4 cpm [^{32}P]-end-labeled probe, 1 μg poly(dI-dC) non-specific competitor, 20% glycerol, 5 mM $MgCl_2$, 2.5 mM EDTA, 2.5 mM DTT, 250 mM NaCl, 50 mM Tris–HCl (pH 7.5) in 20 μl final volume. Reactions were incubated 1 h at 4 °C. Specific competition analysis was performed by including unlabeled binding site oligonucleotide at 50× molar excess relative to labeled probe in the binding reaction. Supershift analysis was performed by including 1 μl of sc184 anti-AP-2 antibody in the binding reaction. Samples were electrophoresed on 4% native acrylamide gels (20:1 acrylamide:bis-acrylamide) in 0.25× TBE for 3 h at 200 V at 8 °C. Gels were dried and exposed to X-ray film with an intensifying screen at −70 °C.

Reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA was prepared from five regions of the adult mouse brain. Brains were removed from decapitated heads of CO_2-anesthetized adult female 129Sv/J mice and dissected into five parts which were snap-frozen in polypropylene tubes in liquid nitrogen: 1) olfactory bulb, 2) cerebral cortex and hippocampus, 3) midbrain, basal forebrain, and diencephalon, 4) cerebellum, and 5) medulla and pons. Each brain region was homogenized in