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A transcriptional role for C/EBP β in the neuronal response to axonal injury

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The molecular mechanisms responsible for inducing gene expression following neuronal injury are not well understood. Here, we address this issue by focusing upon C/EBPβ, a transcription factor implicated in cellular injury and regeneration. We show that C/EBPβ mRNA is expressed in neurons throughout the mature brain and that levels of both C/EBPβ mRNA and phosphoprotein are increased in facial motor neurons following axonal injury. To determine the importance of these increases, we examined the regeneration-associated Tα1 α-tubulin gene which contains functional C/EBP binding sites in its promoter. In transgenic mice, expression of a minimal 176 nucleotide Tα1 α-tubulin promoter:nlacZ reporter gene was upregulated in injured facial motor neurons. This injury-induced transcriptional increase was inhibited in C/EBPβ −/− mice. A similar inhibition was observed in C/EBPβ −/− mice that carried a larger 1.1-kb promoter Tα1:nlacZ reporter construct. Moreover, in situ hybridization revealed that the injury-induced upregulation of the endogenous mouse α1 α-tubulin mRNA, and of a second regeneration-associated mRNA, GAP-43, was inhibited in C/EBPβ −/− mice. Thus, C/EBPβ is essential for the neuronal injury response, acting to transcriptionally activate regeneration-associated gene expression.

Introduction

Following axonal injury in the peripheral nervous system, neurons upregulate a number of growth-associated genes that are thought to be important for successful neuronal regeneration (Plunet et al., 2002). However, while numerous studies have examined the environmental factors that regulate this response (Selzer, 2003), little is known about the underlying transcriptional mechanisms. Thus, while transcription factors such as c-*fos* and c-*jun* are upregulated following axonal injury (Jenkins and Hunt, 1991; Jones and Evinger, 1991; Soares et al., 2001), and c-*jun* is necessary for appropriate neuronal regeneration (Raivich et al., 2004), the relationship between increased transcription factor expression and transcription of regeneration-associated gene has not been demonstrated. Moreover, studies of genes involved in axonal growth such as those encoding Tα1 α-tubulin and GAP-43 have shown that these genes contain injury-response elements in their promoters (Gloster et al., 1994; Goldman and Ding, 2000; Wu et al., 1997; Udvadia et al., 2001), but the transcription factors responsible for regulating them in vivo have not been identified.

In this regard, we have postulated that C/EBP transcription factors are candidates for regulating the neuronal injury response. The C/EBP family is composed of basic leucine zipper DNA-binding proteins (C/EBPs alpha, beta, gamma, delta, epsilon, and zeta) that recognize a common DNA-binding sequence (Johnson and Williams, 1994) and that are expressed in virtually all tissues, including the developing and adult brain (Sterneck and Johnson, 1998). The C/EBPs can either transactivate or repress gene expression, and within the nervous system, are known to regulate neurogenesis during development (Ménard et al., 2002) and neural plasticity in the adult (Alberini et al., 1994; Chen et al., 2003; Sterneck and Johnson, 1998). A number of intriguing findings suggest that this family of transcription factors might be involved in neuronal regeneration. First, the C/EBPs are well-known mediators of injury and inflammation responses in other tissues (Lekstrom-Hines and Xanthopoulos, 1998; Ramji and Foka, 2002) and potentially in the brain (Cortes-Canteli et al., 2004; Soga et al., 2003), where they act to couple numerous extrinsic cellular cues to the nucleus. Second, studies in injured neurons of nonmammalian species have implicated this family in neuronal regeneration; an aplysia C/EBP is activated via an ERK-related kinase following

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axonal injury (Sung et al., 2001), and leech C/EBP mRNA is increased during neuronal regeneration (Korneev et al., 1997). Finally, we have previously shown that one regeneration-associated gene, Tα1 α-tubulin, is a direct transcriptional target of the C/EBP family (Ménard et al., 2002).

The Tα1 α-tubulin gene (termed Tα1 in rat, Lemischka et al., 1981; α1 in fish, Hieber et al., 1998; Mα1 in mouse, Lewis et al., 1985; and bα1 in humans, Cowan et al., 1983) encodes a conserved α-tubulin isoform that is robustly expressed in neurons during early differentiation (Miller et al., 1987) and following axonal injury (Miller et al., 1989) where it likely provides the major pool of α-tubulin needed for the construction of nascent and regenerating axons. Studies on expression of the α1-tubulin gene suggest that the mechanisms responsible for its induction are likely conserved in vertebrates. We have previously demonstrated that a 1.1-kb promoter region upstream from the transcription start site of the rat Tα1 α-tubulin gene contains the transcriptional elements necessary to target reporter gene expression in mice as soon as neurons are born (Bamji and Miller, 1996; Gloster et al., 1994, 1999), as well as following peripheral nerve injury (Gloster et al., 1994; Wu et al., 1997). Interestingly, a 1.7-kb promoter region of the goldfish α1-tubulin isoform also confers developmental and injury-induced expression of a GFP reporter gene in the zebrafish nervous system, suggesting that this neuron-specific α-tubulin promoter contains transcriptional elements that are conserved across species (Hieber et al., 1998; Ménard et al., 2002).

In this regard, we have previously examined the transcriptional elements that are responsible for induction of the Tα1 α-tubulin gene in developing neurons, and have shown that the C/EBP family of transcription factors plays a key role in transcriptionally regulating this, and other neuron-specific genes, as progenitor cells become neurons (Ménard et al., 2002). We have therefore asked whether the C/EBP family might play an important role in transcriptional regulation of growth-associated genes following axonal injury. Here, we provide evidence that one C/EBP family member, C/EBPβ, is essential for transcriptional induction of the Tα1 α-tubulin gene following axonal injury, and thus may provide a direct downstream target of injury-induced signaling cascades that promote neuronal regeneration.

Results

C/EBPβ mRNA is expressed in neurons throughout the mature CNS and is increased in facial motor neurons following axonal injury

We initially defined the expression pattern of C/EBPβ mRNA in the mature brain using in situ hybridization. Every sixth section through the rostral–caudal extent of the adult mouse brain was hybridized with an antisense ^{35}S riboprobe specific for C/EBPβ mRNA, or, as a control, with a sense riboprobe. Hybridized sections were first exposed to X-ray film, and were then analyzed by emulsion autoradiography. This analysis revealed that C/EBPβ mRNA was broadly expressed throughout the CNS (Fig. 1). A semiquantitative analysis of the relative levels of C/EBPβ mRNA in different brain regions (Table 1) demonstrated that it was expressed at particularly high levels in, for example, the amygdala, hippocampus, and pyriform cortex, and that it was expressed in neurons in many of these regions (Fig. 1, Table 1).

To ask whether C/EBPβ was induced in injured mammalian neurons, we focused on the facial motor nucleus (7N), where this

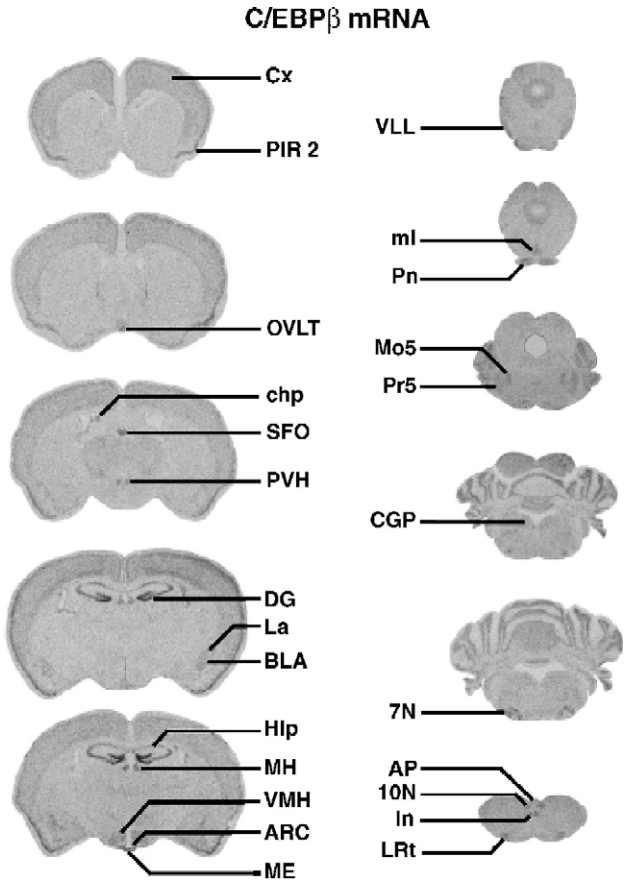

Fig. 1. Distribution of C/EBPβ mRNA in the adult mouse brain. Photographs of in situ hybridization X-ray film data showing the distribution of C/EBPβ mRNA in a representative series of coronal sections through the brain of an adult mouse. The most rostral section is at the top left of the series, while the most caudal is at the bottom right. Various brain nuclei are indicated by the abbreviations, which are defined in Table 1.

mRNA is expressed at detectable basal levels in uninjured motor neurons (Fig. 1, Table 1). The facial nerve was unilaterally transected as it exited the stylomastoid foramen, and in situ hybridization for C/EBPβ mRNA was performed 24 h later on coronal sections at the level of the facial nuclei. Autoradiography on X-ray films revealed that C/EBPβ mRNA levels were detectably increased in the injured facial motor neurons relative to the contralateral, uninjured facial motor neurons in the same sections (Fig. 2A). In contrast, levels of C/EBPβ mRNA were unaltered in sham-operated animals (Fig. 2A). Emulsion autoradiography confirmed this increase in C/EBPβ mRNA in injured facial motor neurons (Figs. 2B–D) and brightfield (data not shown) microscopy confirmed that this increase in C/EBPβ mRNA occurred in the large majority of injured facial motor neurons. Quantitation of the X-ray films revealed that, when normalized to the signal from the sense probe, C/EBPβ mRNA levels were increased approximately 3-fold in the injured versus uninjured facial motor neurons (Fig. 2E).

Following axonal injury in Aplysia, C/EBP protein is activated via an ERK-related kinase (Sung et al., 2001). To ask whether a similar activation of C/EBPβ occurs coincident with the injury-induced increase in C/EBP mRNA levels, we performed immunocytochemistry with an antibody specific for C/EBPβ phosphory-

Table 1
Basal expression of CEBPβ mRNA in adult mouse brain

Region	Abbreviation	Intensity
Area postrema	AP	–/+
Ambiguus nucleus	Amb	++
Arcuate hypothalamic nucleus	Arc	++
Basolateral amygdaloid nucleus, anterior part	BLA	++++
Choroid plexus	Chp	–/+
Central gray pons	CGP	++/+++
Cerebral cortex (layer 1)	Cx	–
Cerebral cortex (layer 2)	Cx	++++
Cerebral cortex (layer 3)	Cx	+
Cerebral cortex (layer 4)	Cx	+++
Cerebral cortex (layer 5)	Cx	+
Cerebral cortex (layer 6a, 6b)	Cx	++++
Dentate gyrus	DG	+++/++++
Dorsal motor nucleus of vagus	10N	++
Facial nucleus	7N	++/+++
Habenula nucleus	MH	–/+
Hipocampus (CA1)	Hip	+++/++++
Hipocampus (CA2)	Hip	+++
Hipocampus (CA3)	Hip	++++
Intercalated nucleus of the medulla	In	++
Lateral amygdaloid nucleus	La	++++
Lateral reticular nucleus	LRt	++
Medial lemnicus	ml	++
Median eminence	ME	–/+
Meninges	–	++
Motor trigeminal nucleus	Mo5	++/+++
Nucleus of roller	Ro	++
Paraventricular hypothalamic nucleus	PVH	+++
Pontine gray	Pn	++
Principal sensory trigeminal nucleus	Pr5	+
Reticulotegmental nucleus of the pons	RtT	+/++
Pyriform area 2	PIR 2	+++
Red nucleus	R	+++/++++
Subfornical organ	SFO	–/+
Vascular organ of the lamina terminalis	OVLT	–/+
Ventromedial thalamic nucleus	VMH	++/+++
Ventral nucleus of lateral lemnicus	VLL	+/++

The relative intensity of CEBPβ mRNA was assessed on X-ray film images and graded as undetectable (–), low (+), moderate (++), strong (+++), and very strong (++++). Anatomical identification was based on Paxinos and Franklin (2001).

lated at a previously-defined ERK site (Nakajima et al., 1993; Park et al., 2004). Double-label immunocytochemistry for phosphoC/EBPβ and βIII-tubulin revealed that phosphoC/EBPβ was present at low but detectable levels in the nuclei of uninjured facial motor neurons, and that levels were increased 24 h following axotomy (Fig. 3). Quantitation revealed that this increase in phosphoC/EBPβ was observed in more than 90% of the injured facial motor neurons. Thus, axonal injury causes a retrograde induction and activation of C/EBPβ.

A minimal Tα1 α-tubulin promoter of 176 nucleotides contains an injury-responsive transcriptional element

To ask about the functional relevance of this injury-induced increase in C/EBPβ, we examined a potential role for this transcription factor in the expression of genes known to be induced by axonal injury. In particular, we focused on the Tα1 α-tubulin gene, which is transcriptionally induced in injured facial motor neurons, and whose expression is maintained throughout the entire period of axonal regeneration (Gloster et al., 1994; Wu et al., 1997). Importantly, we have previously demonstrated that 1.1 kb of the Tα1 α-tubulin promoter is sufficient to target both appropriate

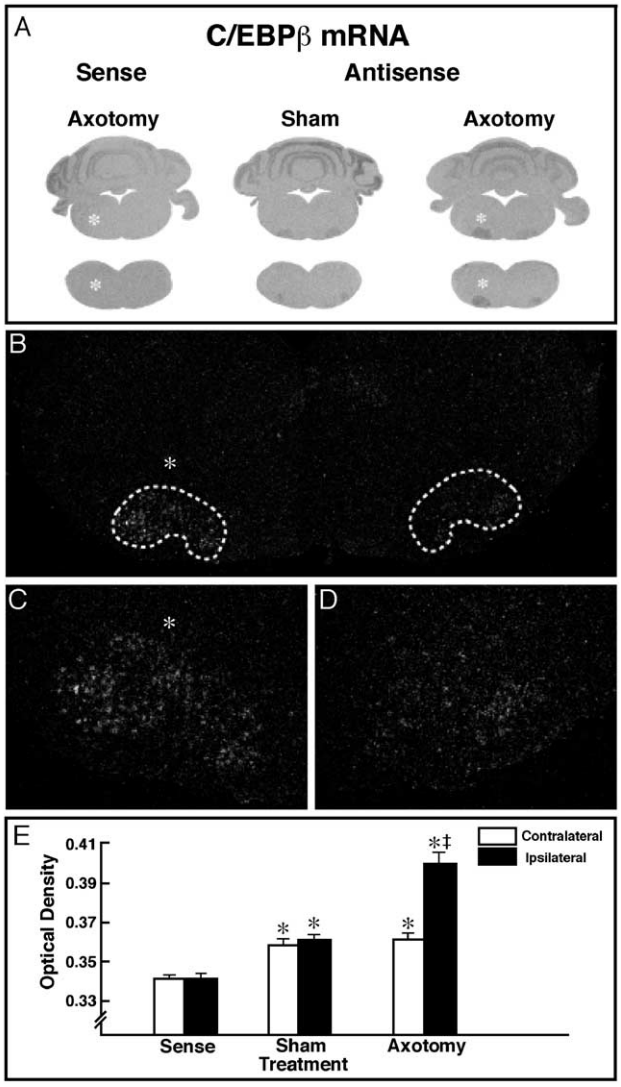

Fig. 2. Expression of C/EBPβ mRNA within facial motor neurons following unilateral axotomy of the facial nerve 24 h previously, as detected by radioactive in situ hybridization. (A) Photographs of X-ray films of coronal sections at the level of the facial nuclei that were axotomized and hybridized to a sense riboprobe (left sections), sham-operated and hybridized to an antisense riboprobe (middle sections), or axotomized and hybridized to an antisense riboprobe (right sections). The asterisk indicates the injured facial nucleus. (B–D) Darkfield photomicrographs of emulsion-dipped coronal sections at the level of the facial nuclei from an animal that was unilaterally axotomized 24 h earlier. The dotted lines indicate the facial nuclei, and the asterisk (B, C) indicates the injured facial nucleus. Panels C and D are higher-magnification photomicrographs of the injured (C) and uninjured (D) facial nuclei from the same section. (E) Quantification of images similar to those seen in panel A. The average transmittance for the C/EBPβ mRNA (sense and antisense) was measured on the contralateral, uninjured, and ipsilateral, injured sides. Each facial nerve nucleus (dashed line) was digitized and subjected to densitometric analysis. Four axotomized animals were analyzed (*$P < 0.05$ for the comparison between the sense and the antisense probes, and $^{\ddagger}P < 0.05$ for the ipsilateral, injured facial nucleus relative to the contralateral, uninjured nucleus).

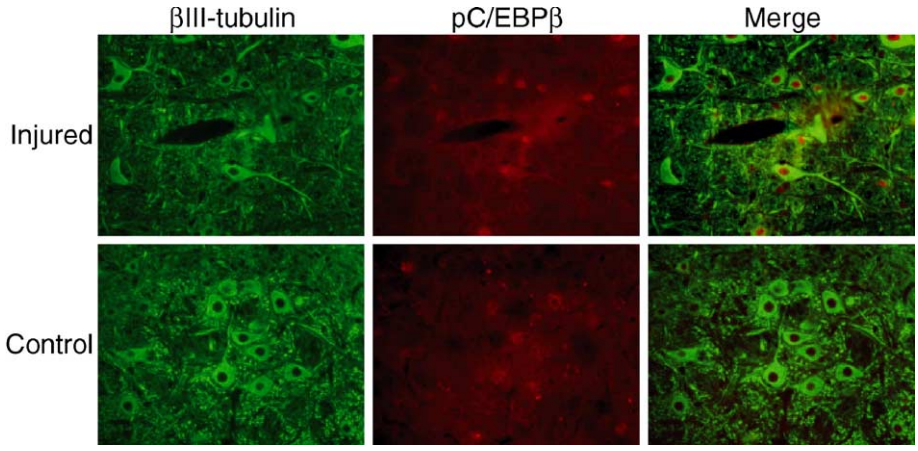

Fig. 3. Immunocytochemistry for C/EBPβ phosphorylated at a consensus ERK site in facial motor neurons following unilateral facial nerve axotomy. Fluorescence photomicrographs of injured and uninjured (control) facial nuclei from the same coronal section taken from mice that had a unilateral facial nerve crush 24 h previously. Sections were double-labeled for neuron-specific protein βIII-tubulin (green) and for phospho-C/EBPβ (red).

early neuronal gene expression and regeneration-associated gene expression in transgenic mice (Bamji and Miller, 1996; Gloster et al., 1994, 1999; Wu et al., 1997). Moreover, we have demonstrated that this promoter contains functional binding sites for the C/EBP family, and that these sites are important for neuronal transcription during development (Ménard et al., 2002).

Since the C/EBP binding sites in the Tα1 promoter are all found within a minimal promoter fragment of 176 nucleotides, we generated transgenic mice carrying this minimal promoter and the entire 5′ untranslated region linked to a nuclear lacZ reporter gene (Tα1MP:nlacZ). Four permanent transgenic lines carrying this construct in varying copy numbers were generated. Analysis of transgenic embryos ranging from E12 to E14 by staining with X-gal revealed that all four lines expressed the transgene, albeit at different levels of expression, and that in all cases transgene expression was limited to the nervous system (Fig. 4, data not shown), as was originally observed for the 1.1-kb promoter fragment (Gloster et al., 1994). Since the patterns of expression were similar, two lines with different expression levels, lines M and N, were chosen for more detailed analysis. Within the embryonic nervous system, in both of these lines, transgene expression was observed throughout the central nervous system, including the spinal cord. This widespread neural expression was confirmed in postnatal day 2 brains, although, as seen in the embryos, expression in line M was significantly lower than in line N (Fig. 4). Thus, the 176-nucleotide minimal Tα1 α-tubulin promoter was sufficient to target nervous-system-specific expression of a β-galactosidase transgene, as was previously seen with the 1.1-kb promoter (Bamji and Miller, 1996; Gloster et al., 1994).

Both the endogenous Tα1 α-tubulin mRNA in rats (Miller et al., 1989) and the 1.1-kb Tα1:nLacZ transgene in mice (Gloster et al., 1994) are upregulated in facial motoneurons following facial nerve injury. To determine whether the smaller Tα1 minimal promoter retained these injury-response elements, we performed similar experiments in lines M and N Tα1MP:nlacZ mice. The facial nerve was unilaterally crushed in adult animals from each line, and 3 days after surgery, brain slices at the appropriate level of the brainstem were analyzed for β-galactosidase activity using X-gal staining. This analysis revealed an induction of Tα1MP:nlacZ expression in the injured facial nucleus in both lines of transgenic animals (Figs. 5A, B). Thus, the Tα1 minimal promoter contains sequence elements that are transcriptionally activated following peripheral nerve injury, much as was previously seen with the larger Tα1 α-tubulin promoter fragment.

Transcription of the Tα1 α-tubulin promoter after neuronal injury requires C/EBPβ

To ask whether the C/EBPβ binding sites in the minimal Tα1 promoter play any role in the observed injury-induced transcription

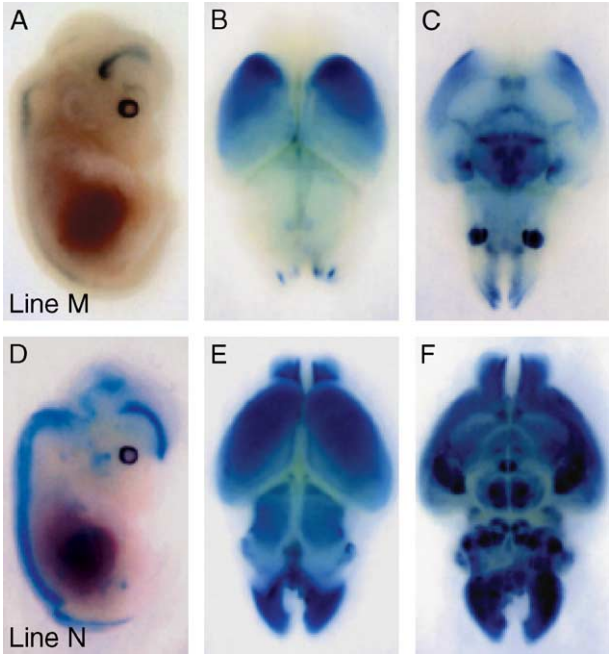

Fig. 4. Expression of a minimal promoter Tα1:nlacZ transgene in embryos and neonatal brains. Two independent lines of transgenic mice, lines M (top panels) and N (bottom panels), are illustrated. In all panels, the whole embryos or brains have been stained with X-gal. (A–C) Transgene expression in line M, with panel A being a side view of an E13 embryo, and panels B and C being staining of an entire P2 brain, with panel B being the perspective from the top, and panel C from the bottom. (D–F) Transgene expression in line N, with panel D being a side view of an E13.5 embryo, and panels E and F being staining of the whole brain of a P2 mouse, with panel E being a perspective from the top of the brain, and panel F the bottom.

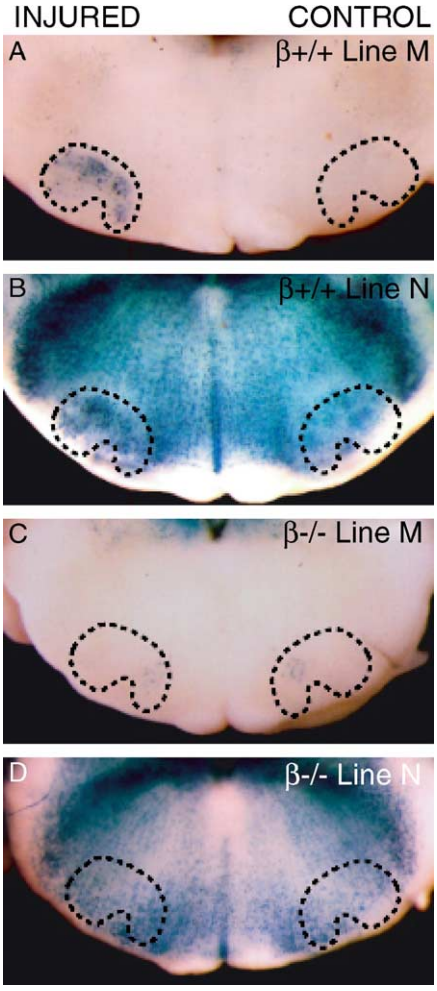

Fig. 5. (A, B) The Tα1MP:nlacZ reporter gene is induced in axotomized facial motor neurons in two lines of transgenic mice. Wild-type animals from line M (A) or line N (B) were subjected to a unilateral facial nerve crush, and 3 days later, thick coronal brainstem sections at the level of the facial nuclei were stained with X-gal to detect β-galactosidase expression. In both panels A and B, the injured facial motor neurons are on the left and the control on the right. The dashed lines outline the facial nuclei. Similar results were obtained with at least 3 animals from each line. (C–D) Injury-induced expression of the Tα1MP:nlacZ reporter gene is eliminated in C/EBPβ −/− animals. C/EBPβ −/− mice that were positive for the line M or line N Tα1MP:nlacZ reporter gene were subjected to a unilateral facial nerve crush, and 3 days later, thick coronal brainstem sections at the level of the facial nuclei were stained with X-gal to detect β-galactosidase expression. In both panels, the injured facial motor neurons are on the left and the control on the right. The dashed lines outline the facial nuclei. Note that for both lines, the transgene is not induced by axonal injury in C/EBPβ −/− animals (C, D). Similar results were obtained with at least 3 animals of each genotype.

of this promoter, we crossed the same two lines of Tα1MP:nLacZ transgenic mice (lines M and N) with mice carrying a null mutation for the C/EBPβ gene (Robinson et al., 1998). We then performed a unilateral facial nerve crush on animals that were transgene positive and that were homozygous for C/EBPβ deletion. As controls, we utilized heterozygote or wild-type littermates. Three days postaxotomy, slices at the level of the brainstem were stained with X-gal. This analysis revealed that while injury-induced Tα1MP:nlacZ transgene expression was observed in C/EBPβ +/+ or +/− animals (Figs. 5A, B; data not shown), this increase was never observed in C/EBPβ −/− mice (Figs. 5C, D). Thus, C/EBPβ is necessary for transactivation of the Tα1 minimal promoter following peripheral nerve injury.

While these data identify an injury-response element in the minimal Tα1 promoter, it is possible that additional, further-upstream elements might also be involved in the transcriptional activation that we previously observed with the longer, 1.1 kb Tα1 α-tubulin promoter (Gloster et al., 1994; Wu et al., 1997). To address this issue, we crossed transgenic animals carrying the longer Tα1:nLacZ construct (line K6; Bamji and Miller, 1996; Gloster et al., 1994, 1999; Wu et al., 1997) with the C/EBPβ +/− animals, and generated animals that were positive for the reporter transgene, and homozygous mutant for C/EBPβ. Analysis of transgene expression in the facial nucleus 3 days following unilateral facial nerve crush showed that Tα1:nlacZ transgene expression was not detectably upregulated in C/EBPβ −/− animals, but that it was robustly increased in C/EBPβ +/+ animals (Fig. 6), consistent with our previous observations in the CD1 mouse genetic background (Gloster et al., 1994; Wu et al., 1997). Thus, C/EBPβ is necessary for transcriptional activation of either the 176 or 1100 nucleotide Tα1 α-tubulin promoters following peripheral nerve injury.

C/EBPβ is necessary for appropriate induction of the endogenous Mα1 α-tubulin and GAP43 mRNAs following facial nerve injury

While these data support the idea that C/EBPβ provides a mechanism for transcriptional activation of the Tα1 α-tubulin gene following nerve injury, multiple mechanisms, including posttranscriptional mechanisms, are thought to play important roles in the

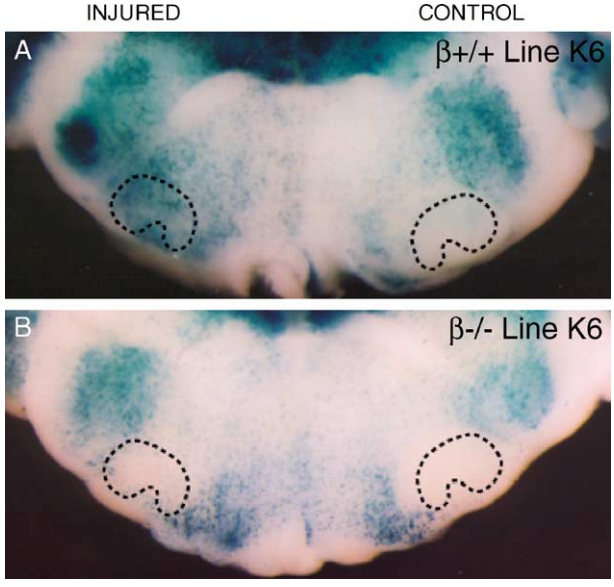

Fig. 6. Injury-induced transcription of the 1.1-kb Tα1:nlacZ transgene is decreased in C/EBPβ −/− mice. Animals from the previously characterized K6 line of transgenic mice carrying the 1.1-kb Tα1:nlacZ promoter were crossed into a C/EBPβ −/− background, and adult animals were then subjected to a unilateral facial nerve crush. Three days later, thick coronal brainstem sections at the level of the facial nuclei were stained with X-gal to detect β-galactosidase expression. In both panels, the injured facial motor neurons are on the left and the control on the right. The dashed lines outline the facial nuclei. Similar results were obtained with at least 3 animals of each genotype.

neuronal injury response. To ask whether the C/EBPβ-dependent increases in transcriptional activity that we defined in the reporter gene mice were necessary for the increased expression of the endogenous Mα1 α-tubulin (the mouse homologue of Tα1 α-tubulin) mRNA, we performed unilateral facial nerve crush on C/EBPβ +/+, +/−, or −/− mice and, 1 or 3 days later, performed in situ hybridization analysis. As was seen previously in rats (Miller et al., 1989), 1 day following injury, Mα1 mRNA was increased in injured versus uninjured, contralateral facial motor neurons in the C/EBPβ +/+ and +/− mice (Fig. 7). Emulsion autoradiography demonstrated that this increase occurred in the facial motor neurons, and quantitation demonstrated that the increase was significant relative to the contralateral, uninjured motor neurons (Fig. 7H). At 3 days, Mα1 mRNA levels had increased even further in the large majority of injured motor neurons (Fig. 7A) as observed by both darkfield (Figs. 7D, F) and brightfield (Fig. 7G) microscopy.

Quantitation revealed that, after normalization to the background hybridization seen with a sense probe (Figs. 7A, B), levels were increased approximately 3- to 4-fold (Fig. 7H), results similar to those previously documented in rats (Miller et al., 1989; Tetzlaff et al., 1991). In contrast, in the C/EBPβ −/− animals, this increase was largely inhibited (Figs. 7A, C, E, H). At 1 day postaxotomy, Mα1 mRNA levels were modestly increased in the injured versus uninjured, contralateral neurons (Fig. 7H). However, no further increase in Mα1 mRNA levels was observed at 3 days postaxotomy, so that, at this later time point, Mα1 levels were not significantly increased in the injured versus uninjured neurons in the absence of C/EBPβ (Figs. 7A, C, E, H). Thus, C/EBPβ is essential for the normal induction of the endogenous Mα1 α-tubulin mRNA following axonal injury.

To ask whether this lack of an appropriate injury response in C/EBPβ mice generalized to other regeneration-associated genes, we examined GAP-43 mRNA in similar experiments. At 1 day postaxotomy, GAP-43 mRNA levels were robustly increased in the injured facial motor neurons of C/EBPβ +/+ and +/− mice. Emulsion autoradiography and quantitation revealed that, after normalization to the background hybridization seen with a control sense probe, levels were significantly increased more than 5-fold (Fig. 8F). Levels were further increased by 3 days postaxotomy (Figs. 8A, B, D, F). In contrast, in the C/EBPβ −/− mice, while GAP-43 mRNA levels were increased significantly in injured versus uninjured, contralateral motor neurons at 1 day postaxotomy, at 3 days this response was blunted, so that no significant increase was observed at this later time point (Figs. 8A, C, D, F). Thus, in the absence of C/EBPβ, GAP-43 mRNA levels are increased immediately following axonal injury, but this increase is not maintained.

Discussion

The data presented in this paper support a number of conclusions. First, we show that C/EBPβ is expressed in neurons throughout the mature CNS, and that the levels of its mRNA and of phosphoC/EBPβ protein are increased following axotomy of facial motor neurons, suggesting a potential role in neuronal regeneration analogous to its role in regeneration of other tissues. Second, our analysis of the Tα1 α-tubulin minimal promoter indicates that 176 nucleotides of 5′ flanking sequence contains the sequence elements necessary to target nervous system-specific expression, as well as those necessary for transcriptional induction following axonal injury. Third, experiments reported here show that C/EBPβ is essential for transcriptional induction of the Tα1 promoter following axonal injury. Finally, our data showing that the lack of C/EBPβ disrupts the normal injury-induced upregulation and maintenance of the endogenous Mα1 α-tubulin and GAP-43 mRNAs indicate that C/EBPβ-mediated transcriptional activation is an essential component of the normal neuronal injury response. Thus, our findings identify a novel transcriptional mechanism for the upregulation of gene expression following axonal injury, and demonstrate a previously-unsuspected role for the C/EBPβ transcription factor during neuronal regeneration.

The finding that both the minimal and 1.1-kb Tα1 α-tubulin promoters, as well as the endogenous Mα1 α-tubulin gene, require C/EBPβ for their induction following axonal injury argues that this is a key component of the transcriptional regeneration response. However, since these studies have been performed in mice lacking C/EBPβ in all cell types, we cannot rule out the possibility of indirect effects as a consequence of perturbations in activated glia (Cardinaux et al., 2000; Kelicen and Tindberg, 2004), Schwann cells (Bermingham et al., 2002), or even inflammatory cells (Walton et al., 1998). Nonetheless, we believe that our previous data showing that the Tα1 α-tubulin gene is a direct target of the C/EBP transcription factor family (Ménard et al., 2002) argue strongly for a direct cell-autonomous transcriptional mechanism.

What pathways activate C/EBPβ-mediated transcription of the Tα1 α-tubulin gene following axonal injury? Various aspects of the peripheral neuron injury response have been attributed both to positive cues that are generated in the injury environment and to the loss of homeostatic factors following axonal injury. With regard to the Tα1 α-tubulin gene, it is clear that it is normally repressed in mature neurons by cues deriving from target contact and/or from the nerve itself (Mathew and Miller, 1993; Wu et al., 1993, 1997), but that it is also the target of growth-factor-mediated upregulation (Ma et al., 1992; Mathew and Miller, 1990). It is therefore difficult to know which of these two types of environmental cues initiates the signaling cascade that ultimately causes C/EBPβ-mediated transcription. However, recent studies have identified a number of candidate signaling pathways that could directly activate the C/EBPs following axonal injury. In particular, we (Ménard et al., 2002) and others (Sterneck and Johnson, 1998) have shown that tyrosine kinase receptors like TrkA and PDGF receptor activate C/EBPs in neurons and PC12 cells via a MEK–ERK pathway, and the MEK–ERK pathway is known to be important for growth-factor-mediated axonal growth (Atwal et al., 2000). In this regard, it is intriguing that in Aplysia, axonal injury causes retrograde transport of an ERK homologue that phosphorylates and activates aplysia C/EBP at the cell body (Sung et al., 2001). A recent study (Perlson et al., 2005) indicates that retrograde transport of phosphoERK also occurs in injured mammalian neurons, and data presented here demonstrate increased nuclear levels of C/EBPβ phosphorylated on a consensus ERK site in injured facial motor neurons. Together, these findings support a model where extrinsic cues generated at the injury site lead to a local activation of the MEK–ERK pathway, followed by enhanced retrograde transport of phosphoERK, increased phosphorylation and activation of C/EBPβ at the cell body, and, ultimately, enhanced transcription of regeneration-associated genes like α1-tubulin.

A number of alternative signaling pathways might also converge onto C/EBPβ following axonal injury. First, the JAK-

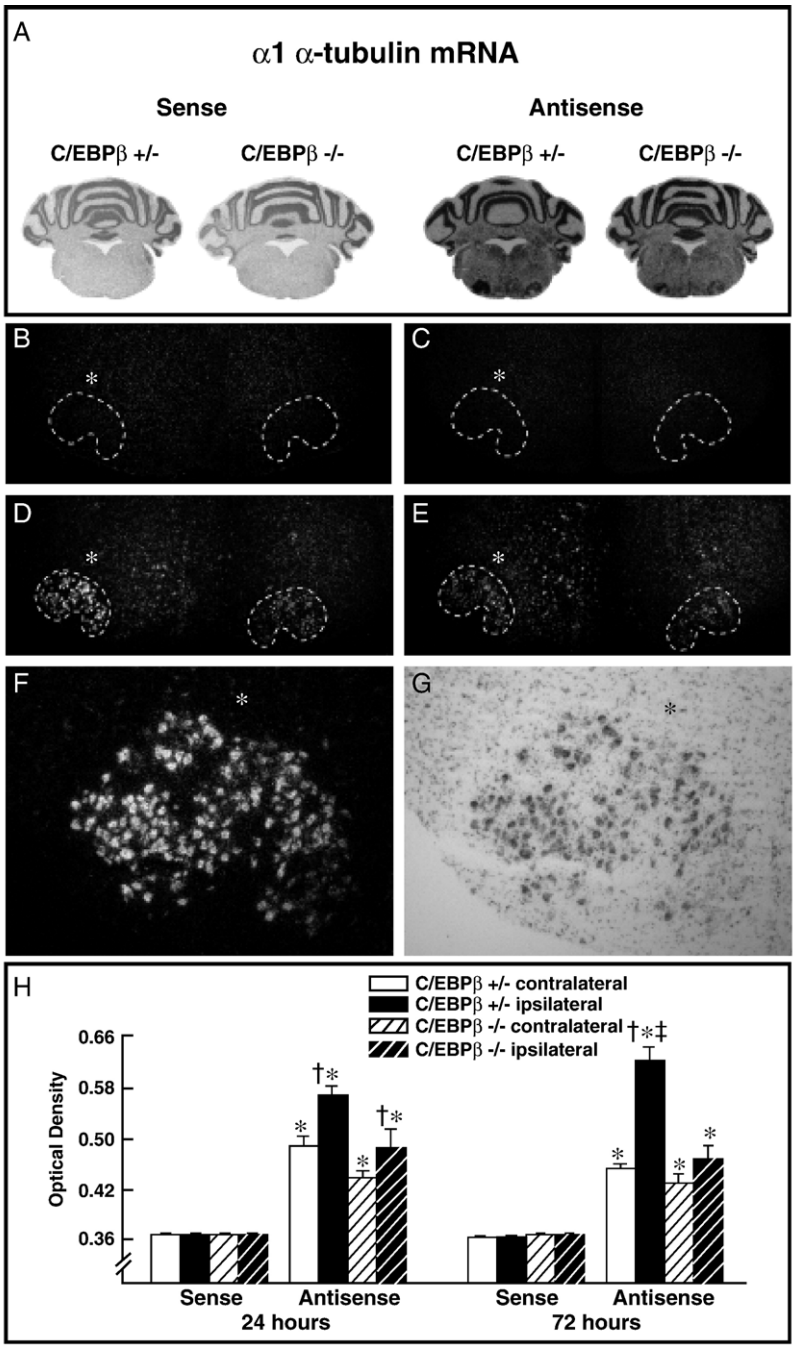

Fig. 7. Expression of Mα1 α-tubulin mRNA within facial motor neurons at 1 and 3 days following unilateral axotomy of the facial nerve in CEBPβ +/− and CEBPβ −/− adult mice. (A) Photographs of X-ray films of coronal sections at the level of the facial nuclei that were axotomized and hybridized to a sense riboprobe (left sections), or to an antisense riboprobe (right sections). The asterisk indicates the injured facial nucleus on each section. (B–G) Darkfield (B–F) and brightfield (G) photomicrographs of emulsion-dipped coronal sections at the level of the facial nuclei from C/EBPβ +/− (B, D, F, G) or C/EBPβ −/− (C, E) mice that were axotomized 3 days previously. The sections were hybridized to sense (B, C) or antisense (D–G) riboprobes for Mα1 mRNA. The dotted lines indicate the facial nuclei, and the asterisk indicates the injured facial nucleus (B–E). Panels F and G are photomicrographs of the same section of the injured facial nucleus, taken at higher magnification to demonstrate that induction of Mα1 mRNA occurs in neurons. (H) Quantification of images similar to those seen in panel A. The average transmittance for Mα1 α-tubulin mRNA (sense and antisense) was measured on the contralateral, uninjured, and ipsilateral, injured sides. Each facial nerve nucleus (dashed line) was digitized and subjected to densitometric analysis. Three axotomized animals of each genotype were analyzed (*$P < 0.05$ for the comparison between the sense and the antisense probes, $^{\ddagger}P < 0.05$ for the ipsilateral, injured facial nucleus relative to the contralateral, uninjured nucleus, and $^{\dagger}P < 0.05$ between the two genotypes).

STAT pathway, which is activated in injured facial motor neurons (Schwaiger et al., 2000), has recently been shown to be essential for sensory neuron regenerative axon growth (Liu and Snider, 2001), and directly regulates C/EBPβ (Jiang and Zarnegar, 1997). Second, C/EBPβ is activated in hippocampal neurons by cAMP (Yukawa et al., 1998), and cAMP has been shown to promote

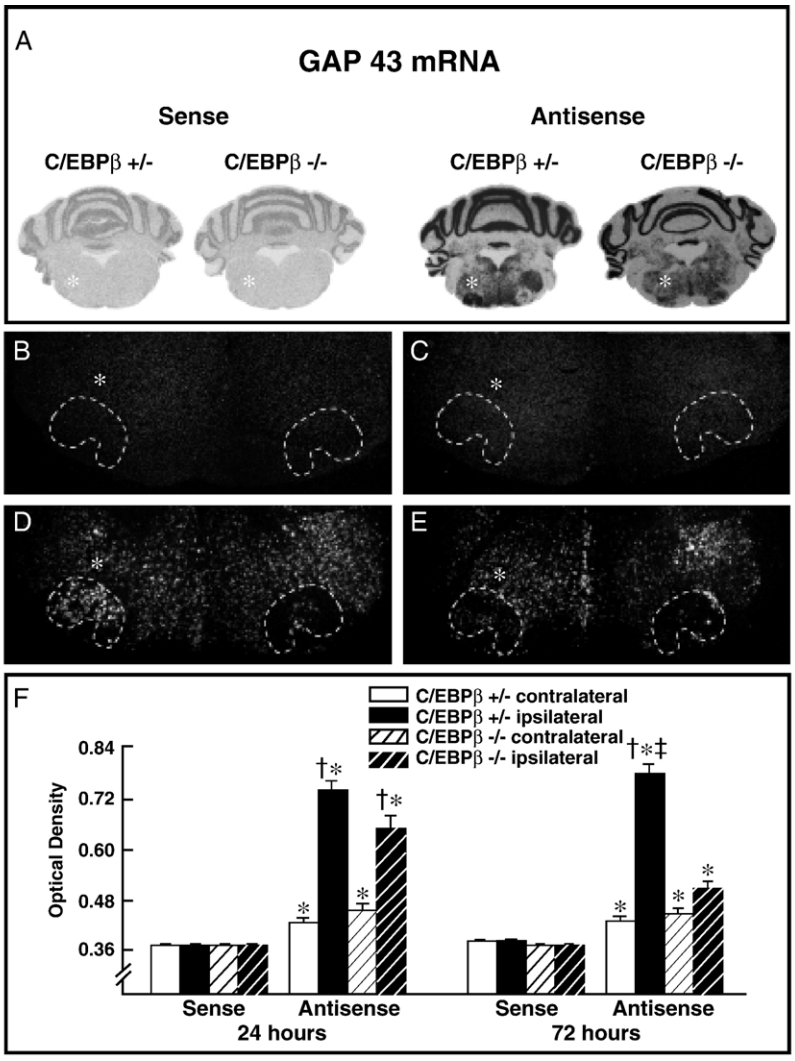

Fig. 8. Expression of GAP 43 mRNA within facial motor neurons at 1 and 3 days following unilateral axotomy of the facial nerve in CEBPβ +/− and CEBPβ −/− adult mice. (A) Photographs of X-ray films of coronal sections at the level of the facial nuclei that were axotomized and hybridized to a sense riboprobe (left sections), or to an antisense riboprobe (right sections). The asterisk indicates the injured facial nucleus on each section. (B–E) Darkfield photomicrographs of emulsion-dipped coronal sections at the level of the facial nuclei from C/EBPβ +/− (B, D) or C/EBPβ −/− (C, E) mice that were axotomized 3 days previously. The sections were hybridized to sense (B, C) or antisense (D, E) riboprobes for GAP-43 mRNA. The dotted lines indicate the facial nuclei, and the asterisk indicates the injured facial nucleus. (F) Quantification of images similar to those seen in panel A. The average transmittance for GAP-43 mRNA (sense and antisense) was measured on the contralateral, uninjured, and ipsilateral, injured sides. Each facial nerve nucleus (dashed line) was digitized and subjected to densitometric analysis. Three axotomized animals of each genotype were analyzed (*$P < 0.05$ for the comparison between the sense and the antisense probes, $^{\ddagger}P < 0.05$ for the ipsilateral, injured facial nucleus relative to the contralateral, uninjured nucleus, and $^{\dagger}P < 0.05$ between the two genotypes).

neuronal regeneration in a variety of paradigms (Qiu et al., 2002). Finally, injury-induced activation of the goldfish α1-tubulin promoter is inhibited by 6-thioguanine (Petrausch et al., 2000), and a purine pathway has been shown to be essential for neuronal regeneration (Benowitz et al., 1999). However, in the absence of data showing that this latter purine pathway directly activates C/EBPβ, it is possible that this represents a parallel, C/EBP-independent pathway. Thus, regenerating neurons may have multiple ways of activating C/EBPβ following neuronal injury, and the relative contribution of each of these pathways to the transcriptional response described here remains to be elucidated.

Our data showing that C/EBPβ is essential for appropriate induction and maintenance of GAP43 mRNA following axonal injury argues that this transcription factor may be important for regulating multiple regeneration-associated genes. While the GAP43 gene is not known to be a direct target of the C/EBP transcription factors, it does contain a functionally-important AP-1 site (Weber and Skene, 1998), two AP-1 transcription factors, c-*fos* and c-*jun* are induced following axonal injury (Jenkins and Hunt, 1991; Jones and Evinger, 1991; Soares et al., 2001), and c-*jun* is necessary for appropriate neuronal regeneration (Raivich et al., 2004). Since C/EBPβ can bind to and regulate both c-*fos* and c-*jun* (Hsu et al., 1994), then this would provide a mechanistic explanation for the decreased expression of GAP-43 that is seen in the C/EBPβ −/− mice. Thus, we suggest that the coordinate induction of C/EBPβ, c-*fos*, and c-*jun* in injured neurons provides a mechanism for the coordinate transcriptional regulation of multiple regeneration-associated genes. While such a transcriptional mechanism likely represents only one of several injury-response pathways, these findings nonetheless provide an impor-

tant window into the mechanisms that allow a peripheral neuron to "know" that it is injured and to mount an appropriate regenerative response.

Experimental methods

Generation and analysis of transgenic mice

The Tα1 α-tubulin minimal promoter construct was made from the previously-described 1.1-kb Tα1:nlacZ transgene (Gloster et al., 1994) by excising sequences upstream of −176, and leaving the remainder of the transgene, including the 5′ untranslated region, the reporter gene, intron and polyA addition site, intact. To make transgenic mice carrying the construct deleted to −176, the entire transgene fragment was isolated free of vector sequences. Presence of the transgene was confirmed by Southern blot analysis of tail DNA, and animals were routinely genotyped by PCR, as previously described (Gloster et al., 1994). Animals from four transgenic lines carrying this construct were characterized. To detect expression of the transgene, embryos or brains were dissected and fixed for 1 min at 4°C in fresh 4% paraformaldehyde in phosphate buffer (0.19 M NaOH, 0.24 M NaH_2PO_4). Tissue was rinsed three times for 30 min each in a solution of 0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, 1 M $MgCl_2$, 0.05% sodium deoxycholate, 0.1% NP40. Tissue was stained by incubating at 37°C in a reaction mix comprised of the same solution containing 1 mg/ml X-gal, 5 mM $K_3Fe(CN)_6$, and 5 mM $K_4Fe(CN)_6$ (pH 7.3–7.6). Staining was performed for 12 to 24 h. Embryos and brains were subsequently rinsed and stored in 4% paraformaldehyde.

For the analysis of transgene expression in C/EBPβ +/+, +/−, and −/− backgrounds, mice from Tα1MP:nlacZ lines M and N were crossed to C/EBPβ +/− mice (Robinson et al., 1998), and their progeny were then backcrossed for more than 8 generations into this C57BL/6 background to obtain animals that were Tα1MP:nlacZ+, C/EBPβ −/− for analysis. Littermates that were C/EBPβ +/+ or C/EBPβ +/− were used as controls in these experiments.

Animals and facial nerve lesion experiments

Adult male CD1 mice (Charles River Canada) were used for the C/EBPβ in situ hybridization studies. Mice were anesthetized by intraperitoneal injection with a saline solution containing ketamine (300 mg/kg) and xylazine (60 mg/kg). The facial nerve was exposed unilaterally, and both branches were crushed approximately 2 mm distal to the stylomastoid foramen, as previously described (Fernandes et al., 1998). Analysis of transgene induction was then performed by sacrificing the animals at various time points following injury, sectioning the brain into thick coronal slices as previously described (Gloster et al., 1994), and staining with X-gal as described above. All protocols were approved by the Montreal Neurological Institute or Hospital for Sick Children Animal Welfare Committees.

In situ hybridization and immunocytochemistry

Anesthetized mice were rapidly perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 100 mM borax buffer (pH 9.5 at 4°C). The brains were postfixed for 24 h and cryoprotected in 20% sucrose in 4% parformaldehyde-borax buffer. Brains were sectioned coronally at 30 μm on a freezing microtome, and sections were collected in a cold cryoprotectant solution (0.05 M sodium phosphate buffer, pH 7.3, 30% ethylene glycol, 20% glycerol) and stored at −20°C.

For immunocytochemistry, every sixth brain section at the level of the facial nuclei was mounted on a gelatin poly-L-lysine-coated slide. Double-label immunocytochemistry was performed using antibodies to neuron-specific βIII-tubulin (1:400, Covance Research Products) and phospho-C/EBPβ (Thr235, 1:100, Cell Signaling). Sections were incubated overnight at 4°C with the primary antibodies, rinsed in sterile PBS and incubated with a biotinylated anti-mouse IgG (1:250, Vector Laboratories) for 10 min at room temperature and with secondary antibodies Cy3-conjugated goat anti-rabbit (1:400, Jackson ImmunoResearch) and fluorescein-conjugated streptavidin (1:2000, Jackson ImmunoResearch).

For in situ hybridization, the pSPORT plasmid containing the full-length coding sequence for the mouse C/EBPβ was purchased from OPEN Biosystems (cat no. EMM1002-632216) (Huntsville, AL, USA). The plasmid was digested with *Sac*I and *Sma*I and the insert subcloned in pBlueScript SK+ vector containing the mice C/EBPβ fragment of 866 base pairs was linearized with *Sac*I and *Sma*I for the synthesis of sense and antisense probes, respectively. The plasmids containing the full-length coding sequences of the mouse GAP-43 (cat no. 7490850) and Mα1 α-tubulin (cat no. 9605113) cDNAs were purchased from American type culture collection (ATCC, Manassas, VA). The GAP-43 and Mα1 α-tubulin sense riboprobes were synthesized using the RNA polymerase Sp6 and T7, respectively, and linearized with *Not*I whereas the antisense riboprobes were obtained using the RNA polymerase T7 and T3 and linearized with *Sal*I and *Dra*III, respectively. For Mα1, this protocol results in the generation of a 3′UTR-specific probe that recognizes only Mα1 α-tubulin mRNA. In all cases, radioactive cRNAs were synthesized by incubation of 250 ng linearized plasmid, 200 μCi of ^{35}S-UTP (Dupont NEN), and either T7 or T3 RNA polymerase under standard conditions (Nadeau and Rivest, 2000). A concentration of 10^7 cpm probe was mixed with 1 ml of hybridization solution (500 μl formamide, 60 μl 5 M NaCl, 10 μl 1 M Tris pH 8.0, 2 μl 0.5 M EDTA pH 8.0, 50 μl 20× Denhart's solution, 200 μl 50% dextran sulfate, 50 μl 10 mg/ml tRNA, 10 μl 1 M DTT plus water to 1 ml). This solution was mixed and heated for 5 min at 65°C before application to slides.

In situ hybridization (performed as previously described in Nadeau and Rivest, 2000) was carried out on tissue sections mounted onto poly-L-lysine-coated slides that were desiccated overnight under vaccum, fixed in 4% paraformaldehyde for 30 min, and digested with proteinase K. Sections were then rinsed, treated with a solution of 0.1 M triethanolamine (TEA, pH 8.0), acetylated in 0.25% acetic anhydride in 0.1 M TEA, and dehydrated through graded alcohol solutions. After vacuum drying for a minimum of 2 h, 90 μl of hybridization mixture was spotted on each slide, sealed under a coverslip, and incubated at 60°C overnight in a slide warmer. Coverslips were removed, and slides were rinsed in 4× SSC at room temperature. Sections were digested with RNAse A (20 μg/ml, 37°C, 30 min), rinsed in descending concentrations of SSC (2×, 1×, 0.5× SSC), washed in 0.1× SSC for 30 min at 60°C, and again dehydrated. After drying for 2 h under vacuum, sections were exposed to X-ray film (Biomax, Kodak, Rochester, NY) at 4°C for 72 h. Slides were then defatted in xylene, dipped in NTB2 nuclear emulsion (Kodak; diluted 1:1 with distilled water), exposed for 20 days, developed in D19 developer (Kodak) for 3.5 min at 14–15°C, washed 15 s in

water, and fixed in rapid fixer (Kodak) for 5 min. Tissues were then rinsed in distilled water for 1–2 h, counterstained with thionine (0.25%), dehydrated in alcohol, cleared in xylene, and cover-slipped with DPX mounting media (BDH Laboratory Supplies, Poole, England).

Quantitative analysis

Quantitative analysis of mRNA hybridization signals were carried out on X-ray films. Brain sections were digitized with a D70 digital camera (Nikon) attached to a Sigma 105 mm macrolens (f2.8) under a white light transilluminator (Fisher scientific, FB-WLT-1417). Transmittance values (referred to here as optical density) for each pixel were calculated using a known standard of intensity and distance measurements from a logarithmic specter adapted from Bioimage Visage 110s (Millipore, Ann Arbor, MI). Sections through the facial nerve nuclei (bregma −6.12 and −6.36) from sham and axotomized animals were digitized and subjected to densitometric analysis, yielding measurements of mean density per area. All measurements were performed in triplicate. Data are reported as mean O.D. values and statistical analysis was performed by analysis of variance (ANOVA) for each transcript, followed by a Bonferroni/Dunn test procedure as post hoc comparisons with Statview software.

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References

Alberini, C.M., Ghirardi, M., Metz, R., Kandel, E.R., 1994. C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. Cell 76, 1099–1114.

Atwal, J.K., Massie, B., Miller, F.D., Kaplan, D.R., 2000. The TrkB-Shc site signals neuronal survival and local axon growth via MEK and PI3-kinase. Neuron 27, 265–277.

Bamji, S.X., Miller, F.D., 1996. Comparison of the expression of a Tα1:nlacZ transgene and Tα1 α-tubulin mRNA in the mature central nervous system. J. Comp. Neurol. 374, 52–69.

Benowitz, L.I., Goldberg, D.E., Madsen, J.R., Soni, D., Irwin, N., 1999. Inosine stimulates extensive axon collateral growth in the rat cortico-spinal tract after injury. Proc. Natl. Acad. Sci. 96, 13486–13490.

Bermingham Jr., J.R., Shumas, S., Whisenhunt, T., Sirkowski, E.E., O'Connell, S., Scherer, S.S., Rosenfeld, M.G., 2002. Identification of genes that are downregulated in the absence of the POU domain transcription factor pou3f1 (Oct-6, Tst-1, SCIP) in sciatic nerve. J. Neurosci. 22, 10217–10231.

Cardinaux, J.R., Allaman, I., Magistretti, P.J., 2000. Pro-inflammatory cytokines induce the transcription factors C/EBPbeta and C/EBPdelta in astrocytes. Glia 29, 91–97.

Chen, A., Muzzio, I.A., Malleret, G., Bartsch, D., Verbitsky, M., Pavlidis, P., Yonan, A.L., Vronskaya, S., Grody, M.B., Cepeda, I., Gilliam, T.C., Kandel, E.R., 2003. Inducible enhancement of memory storage and synaptic plasticity in transgenic mice expressing an inhibitor of ATF4 (CREB-2) and C/EBP proteins. Neuron.

Cortes-Canteli, M., Wagner, M., Ansorge, W., Perez-Castillo, A., 2004. Microarray analysis supports a role for ccaat/enhancer-binding protein-beta in brain injury. J. Biol. Chem. 279, 14409–14417.

Cowan, N.J., Dobner, P.R., Fuchs, E.V., Cleveland, D.W., 1983. Expression of human alpha-tubulin genes: interspecies conservation of 3′ untranslated regions. Mol. Cell. Biol. 3, 1738–1745.

Fernandes, K.J., Kobayashi, N.R., Jasmin, B.J., Tetzlaff, W., 1998. Acetylcholinesterase gene expression in axotomized rat facial motoneurons is differentially regulated by neurotrophins: correlated with trkB and trkC mRNA levels and isoforms. J. Neurosci. 18, 9936–9947.

Gloster, A., Wu, W., Speelman, A., Weiss, S., Causing, C., Pozniak, C., Reynolds, B., Chang, E., Toma, J.G., Miller, F.D., 1994. The Tα1 α-tubulin promoter specifies gene expression as a function of neuronal growth and regeneration in transgenic mice. J. Neurosci. 14, 7319–7330.

Gloster, A., El-Bizri, H., Bamji, S.X., Rogers, D., Miller, F.D., 1999. Early induction of Talpha1 alpha-tubulin transcription in neurons of the developing nervous system. J. Comp. Neurol. 405, 45–60.

Goldman, D., Ding, J., 2000. Different regulatory elements are necessary for alpha1 tubulin induction during CNS development and regeneration. NeuroReport 27, 3859–3863.

Hieber, V., Dai, X., Foreman, M., Goldman, D., 1998. Induction of α1-tubulin gene expression during development and regeneration of the fish central nervous system. J. Neurobiol. 37, 429–440.

Hsu, W., Kerppola, T.K., Chen, P.L., Curran, T., Chen-Kiang, S., 1994. Fos and jun repress transcription activation by NF-IL6 through association at the basic zipper region. Mol. Cell. Biol. 14, 268–276.

Jenkins, R., Hunt, S.P., 1991. Long-term increase in the levels of c-*jun* mRNA and jun protein like immunoreactivity in motor and sensory neurons following axon damage. Neurosci. Lett. 129, 107–110.

Jiang, J.G., Zarnegar, R., 1997. A novel transcriptional regulatory region within the core promoter of the hepatocyte growth factor gene is responsible for its inducibility by cytokines via the C/EBP family of transcription factors. Mol. Cell. Biol. 7, 5758–5770.

Johnson, P.F., Williams, S.C., 1994. CCAAT/enhancer binding (C/EBP) proteins. In: Yaniv, M., Tronche, F. (Eds.), Liver Gene Expression. R.G. Landes, Austin, TX, pp. 231–258.

Jones, K.J., Evinger, C., 1991. Differential neuronal expression of c-*fos* proto-oncogene following peripheral nerve injury or chemically-induced seizure. J. Neurosci. Res. 28, 291–298.

Kelicen, P., Tindberg, N., 2004. Lipopolysaccharide induces CYP2E1 in astrocytes through MAP kinase kinase-3 and C/EBPbeta and -delta. J. Biol. Chem. 279, 15734–15742.

Korneev, S., Fedorov, A., Collins, R., Blackshaw, S.E., Davies, J.A., 1997. A subtractive cDNA library from an identified regenerating neuron is enriched in sequences up-regulated during nerve regeneration. Invert. Neurosci. 3, 185–192.

Lekstrom-Hines, J., Xanthopoulos, K.G., 1998. Biological role of the CCAAT/enhancer-binding protein family of transcription factors. J. Biol. Chem. 273, 28545–28548.

Lemischka, I.R., Farmer, S., Racaniello, V.R., Sharp, P.A., 1981. Nucleotide sequence and evolution of a mammalian alpha-tubulin messenger RNA. J. Mol. Biol. 151, 101–120.

Lewis, S.A., Lee, M.G., Cowan, N.J., 1985. Five mouse tubulin isotypes and their regulated expression during development. J. Cell Biol. 101, 852–861.

Liu, R.Y., Snider, W.D., 2001. Different signaling pathways mediate regenerative versus developmental sensory axon growth. J. Neurosci. 21, RC164.

Ma, Y., Campenot, R.B., Miller, F.D., 1992. Concentration-dependent regulation of neuronal gene expression by nerve growth factor. J. Cell Biol. 117, 135–141.

Mathew, T.C., Miller, F.D., 1990. Increased expression of Tα1 α-tubulin mRNA during collateral and NGF-induced sprouting of sympathetic neurons. Dev. Biol. 141, 84–92.

Mathew, T.C., Miller, F.D., 1993. Induction of Tα1 α-tubulin mRNA during neuronal regeneration is a function of the amount of axon lost. Dev. Biol. 158, 467–474.

Ménard, C., Hein, P., Paquin, A., Savelson, A., Yang, X.M., Lederfein, D., Barnabé-Heider, F., Mir, A.A., Sterneck, E., Peterson, A.C., Johnson, P.F., Vinson, C., Miller, F.D., 2002. An essential role for a MEK-C/EBP pathway during growth factor-regulated cortical neurogenesis. Neuron 36, 597–610.

Miller, F.D., Naus, C.C., Durand, M., Bloom, F.E., Milner, R.J., 1987. Isotypes of alpha-tubulin are differentially regulated during neuronal maturation. J. Cell Biol. 105, 3065–3073.

Miller, F.D., Tetzlaff, W., Bisby, M.A., Fawcett, J.W., Milner, R.J., 1989. Rapid induction of the major embryonic α-tubulin, Tα1, during nerve regeneration in adult rats. J. Neurosci. 9, 1452–1463.

Nadeau, S., Rivest, S., 2000. Role of microglial-derived tumor necrosis factor in mediating CD14 transcription and nuclear factor kappa B activity in the brain during endotoxemia. J. Neurosci. 20, 3456–3468.

Nakajima, T., Kinoshita, S., Sasagawa, T., Sasaki, K., Naruto, M., Kishimoto, T., Akira, S., 1993. Phosphorylation at threonine-235 by a ras-dependent MAPK cascade is essential for transcription factor NF-IL6. Proc. Natl. Acad. Sci. U. S. A. 90, 2207–2211.

Park, B.-H., Qiang, L., Farmer, S.R., 2004. Phosphorylation of C/EBPβ at a consensus ERK/GSK3 site is required for the induction of adiponectin gene expression during the differentiation of mouse fibroblasts into adipocytes. Mol. Cell. Biol. 24, 8671–8680.

Paxinos, G., Franklin, K.B.J., 2001. The Mouse Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, New York.

Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R., Fainzilber, M., 2005. Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. Neuron 45, 715–726.

Petrausch, B., Tabibiazar, R., Roser, T., Jing, Y., Goldman, D., Stuermer, C.A., Irwin, N., Benowitz, L.I., 2000. A purine-senstivie pathway regulates multiple genes involved in axon regeneration in goldfish retinal ganglion cells. J. Neurosci. 20, 8031–8041.

Plunet, W., Kwon, B.K., Tetzlaff, W., 2002. Promoting axonal regeneration in the central nervous system by enhancing the cell body response to axotomy. J. Neurosci. Res. 68, 1–6.

Qiu, J., Cai, D., Filbin, M.T., 2002. A role for cAMP in regeneration during development and after injury. Prog. Brain Res. 137, 381–387.

Raivich, G., Bohatschek, M., Da Costa, C., Iwata, O., Galiano, M., Hristova, M., Nateri, A.S., Makwana, M., Riera-Sans, L., Wolfer, D.P., Lipp, H.P., Aguzzi, A., Wagner, E.F., Behrens, A., 2004. The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. Neuron 43, 57–67.

Ramji, D.P., Foka, P., 2002. CCAAT/enhancer-binding proteins: structure, function and regulation. Biochem. J. 365, 561–575.

Robinson, G.W., Johnson, P.F., Hennighausen, L., Sterneck, E., 1998. The C/EBPbeta transcription factor regulates epithelial cell proliferation and differentiation in the mammary gland. Genes Dev. 12, 1907–1916.

Schwaiger, F.W., Hager, G., Schmitt, A.B., Horvat, A., Hager, G., Streif, R., Spitzer, C., Gamal, S., Breuer, S., Brook, G.A., Nacimiento, W., Kreutzberg, G.W., 2000. Peripheral but not central axotomy induces changes in Janus kinases (JAK) and signal transducers and activators of transcription (STAT). Eur. J. Neurosci. 12, 1165–1176.

Selzer, M.E., 2003. Promotion of axonal regeneration in the injured CNS. Lancet Neurol. 2, 157–166.

Soares, H.D., Chen, S.C., Morgan, J.I., 2001. Differential and prolonged expression of Fos-lacZ and Jun-lacZ in neurons, glia, and muscle following sciatic nerve damage. Exp. Neurol. 167, 1–14.

Soga, Y., Yamanaka, R., Nishino, K., Tanaka, R., 2003. CCAAT/enhancer binding proteins are expressed in the gerbil hippocampus after transient forebrain ischemia. Neurosci. Lett. 337, 106–110.

Sterneck, E., Johnson, P.F., 1998. CCAAT/enhancer binding protein beta is a neuronal transcriptional regulator activated by nerve growth factor receptor signaling. J. Neurochem. 70, 2424–2433.

Sung, Y.J., Povelones, M., Ambron, R.T., 2001. RISK-1: a novel MAPK homologue in axoplasm that is activated and retrogradely transported after nerve injury. J. Neurobiol. 47, 67–79.

Tetzlaff, W., Alexander, S.W., Miller, F.D., Bisby, M.A., 1991. Response of facial and rubrospinal neurons to axotomy: changes in mRNA expression for cytoskeletal proteins and GAP-43. J. Neurosci. 11, 2528–2544.

Udvadia, A.J., Koster, R.W., Skene, J.H., 2001. GAP-43 promoter elements in transgenic zebrafish reveal a difference in signals for axon growth during CNS development and regeneration. Development 128, 1174–1182.

Walton, M., Saura, J., Young, D., MacGibbon, G., Hansen, W., Lawlor, P., Sirimanne, E., Gluckman, P., Dragunow, M., 1998. CCAAT-enhancer binding protein alpha is expressed in activated microglial cells after brain injury. Brain Res. Mol. Brain Res. 61, 11–22.

Weber, J.R., Skene, J.H., 1998. The activity of a highly promiscuous AP-1 element can be confined to neurons by a tissue-selective repressive element. J. Neurosci. 18, 5264–5274.

Wu, W., Mathew, T.C., Miller, F.D., 1993. Evidence that the loss of homeostatic signals induces regeneration-associated alterations in neuronal gene expression. Dev. Biol. 158, 456–466.

Wu, W., Gloster, A., Miller, F.D., 1997. Transcriptional repression of the growth-associated Tα1 α-tubulin gene by target contact. J. Neurosci. Res. 48, 477–487.

Yukawa, K., Tanaka, T., Tsuji, S., Akira, S., 1998. Expression of CCAAT/enhancer-binding proteins beta and delta and their activities are intensified by cAMP signaling as well as Ca2+/calmodulin kinases activation in hippocampal neurons. J. Biol. Chem. 273, 31345–31351.