



# Activating transcription factor 3 and the nervous system

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Activating transcription factor 3 (ATF3) belongs to the ATF/cyclic AMP responsive element binding family of transcription factors and is often described as an adaptive response gene whose activity is usually regulated by stressful stimuli. Although expressed in a number of splice variants and generally recognized as a transcriptional repressor, ATF3 has the ability to interact with a number of other transcription factors including c-Jun to form complexes which not only repress, but can also activate various genes. ATF3 expression is modulated mainly at the transcriptional level and has markedly different effects in different types of cell. The levels of ATF3 mRNA and protein are normally very low in neurons and glia but their expression is rapidly upregulated in response to injury. ATF3 expression in neurons is closely linked to their survival and the regeneration of their axons following axotomy, and that in peripheral nerves correlates with the generation of a Schwann cell phenotype that is conducive to axonal regeneration. ATF3 is also induced by Toll-like receptor (TLR) ligands but acts as a negative regulator of TLR signaling, suppressing the innate immune response which is involved in immuno-surveillance and can enhance or reduce the survival of injured neurons and promote the regeneration of their axons.

**Keywords:** ATF3, c-Jun

## INTRODUCTION

It has long been known that the ability of peripheral axons to regenerate is fundamentally greater than that of axons in the brain, spinal cord, or optic nerves (early literature was reviewed in Ramon y Cajal, 1928). One factor that contributes to the successful regeneration of the axons in peripheral nerves is the complex cell body response the neurons show to axotomy. That transcription factors must play an important role in enabling neurons to regrow their axons is implicit to the observation that several hundred genes are regulated in neurons during axonal regeneration (Costigan et al., 2002; Boeshore et al., 2004). In addition, similarly large numbers of genes are regulated in the non-neuronal cells present in injured peripheral nerves, especially Schwann cells (Barrette et al., 2010). Of the transcription factors that regulate these changes in gene expression, the function of c-Jun is the best understood. However, activating transcription factor 3 (ATF3), also known as LRF-1, LRG-21, CRG-5, and TI-241, is also upregulated in most of the neurons (Figure 1) as well as in Schwann cells that express c-Jun. Indeed, ATF3 has become a standard marker for neurons axotomized by peripheral nerve injury (Tsuzuki et al., 2001; Yamanaka et al., 2005; Yano et al., 2008; Linda et al., 2011) and its expression by injured neurons is closely correlated with a regenerative response. None the less, surprisingly little is known about the functions of ATF3 in neurons or glia within the injured nervous system, especially when compared with those of its potential binding partner, c-Jun.

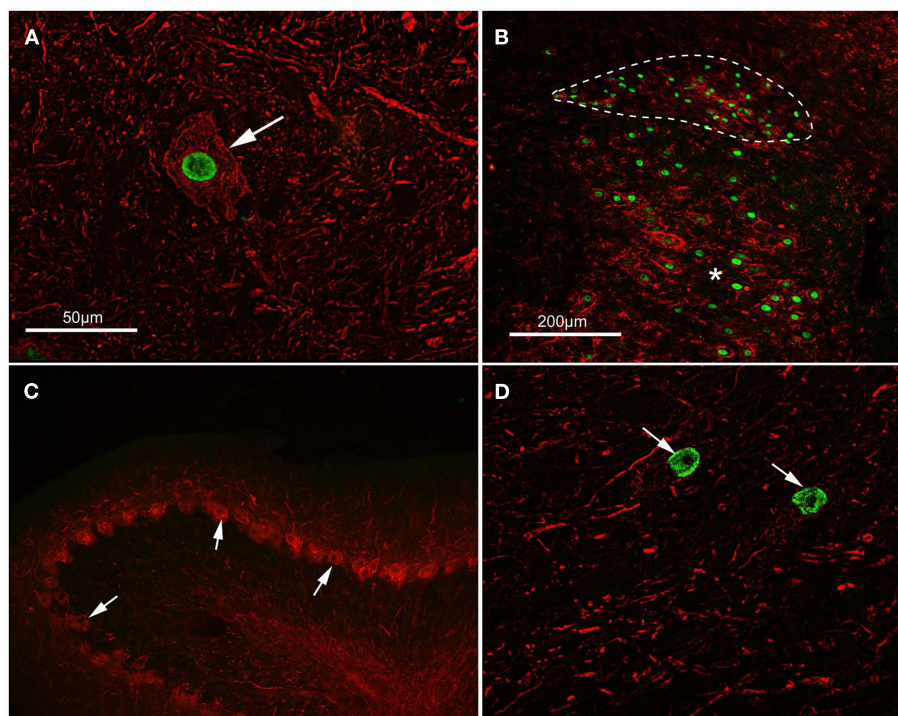
## THE ATF/CREB FAMILY

Activating transcription factor 3 belongs to the ATF/cyclic AMP responsive element binding (CREB) family of transcription

factors, characterized by a basic region leucine zipper (bZip) motif. The basic region is necessary for binding to the ATF/CRE promoter DNA sequence (TGACGTCA), and the leucine zipper element for homo- and hetero-dimerization with other bZip proteins to differentially regulate transcription. Within this relatively large family there are a number of subgroups which have been classified according to their level of homology both within and outside the bZip domain. Between subgroups, however, there is very little similarity outside the bZip domain (Hai and Hartman, 2001). None the less, some ATF/CREB members can form heterodimers with members of other families (e.g., C/EBP and Maf families), as well as with various other ATF/CREB proteins (Thompson et al., 2009).

## The ATF3 gene

Full-length ATF3 was originally isolated from a serum-induced HeLa cell cDNA library using a DNA probe (Hai et al., 1989). The ATF3 gene is on chromosome 1q32.3 and spans ~56 kb, technically comprising six exons (see Figure 2). *In vitro* experiments suggest that the human ATF3 gene has two promoters, with the most recently identified alternate promoter (labeled P1 in Figure 2) being some ~43.5 kb upstream from the established canonical promoter (labeled P2 in Figure 2). This novel promoter appears to be highly conserved between human and mouse (Miyazaki et al., 2009). There are multiple P1 transcription start sites. Using RNAi against P1 and P2 transcripts, Miyazaki and colleagues were able to show that P1 activity was dominant in cancer cells expressing ATF3 in response to serum, whilst P2 activity was dominant in cells expressing oncogenic hRas. This alternate promoter usage is intriguing, not least because the variant transcripts give rise to



**FIGURE 1 | Activating transcription factor 3 (ATF3) is expressed in axotomized, regeneration-competent neurons. (A)** ATF3 (green) is concentrated in the nucleus of a lumbar motor neuron (arrowed) from an adult rat 14 days following sciatic nerve transection. This type of cell regenerates its axon vigorously following nerve injury. Neurofilament immunoreactivity (red) has been used to show neuronal cytoplasm. The scale bar also applies to (D). **(B)** ATF3 (green) is present in the nuclei of preganglionic parasympathetic neurons in the dorsal nucleus of the vagus (outlined with a dashed line) and in motor neurons of the hypoglossal nucleus (asterisk) 5 days after crush injury to the vagus and hypoglossal nerves in an adult rat. These neurons also regenerate axons following

injury. CD11b immunoreactivity (red) shows reactive microglia around the axotomized neurons. The scale bar also applies to (C). **(C,D)** Show the results of an experiment where a peripheral nerve graft was implanted into the cerebellum of an adult rat, axotomizing both deep nucleus neurons and Purkinje cells. Fourteen days after grafting, ATF3 (green) can be seen in the nuclei (arrows) of neurons within a cerebellar deep nucleus in (D) but not in the nuclei of Purkinje cells, arrowed in (C). Deep nucleus neurons regenerate axons into grafts in the cerebellum but Purkinje cells do not do so; ATF3 is expressed only by the neurons which mount a regenerative response to axotomy. Neurofilament and calbindin immunoreactivity (red) has been used to identify Purkinje cells.

the same protein. The variant 5'UTR sequences may be important in the regulation of translation; Miyazaki et al. (2009) reported that the structure of the 5'UTRs of the P1 transcripts, as well as the differential use of transcription start sites appears to affect the level of translation in response to stress. There was evidence for the constitutive activation of P1 in two cancer cell lines, LNCaP and Hodgkin L428.

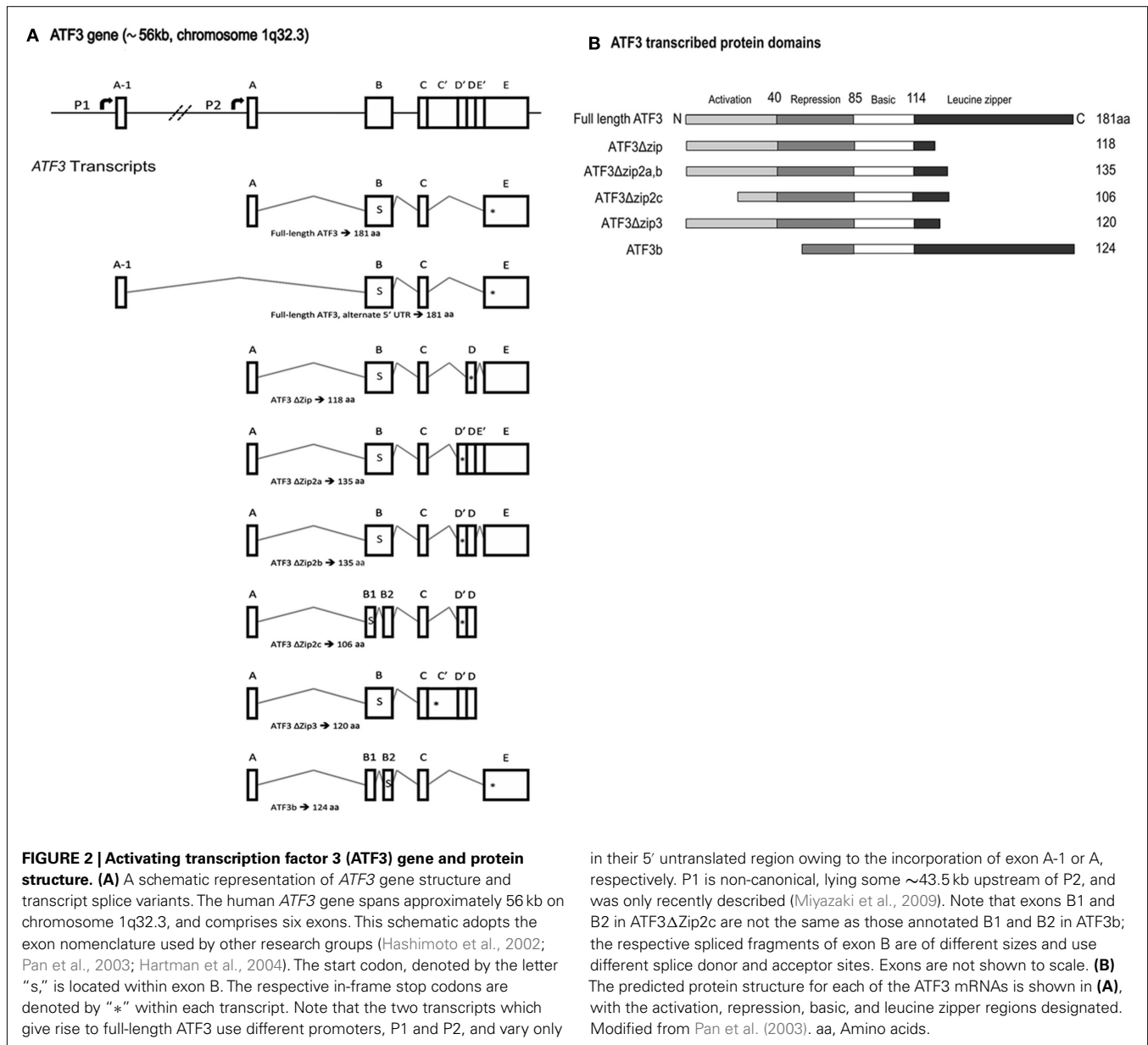
**Alternative mRNA splicing and protein isoforms**

Numerous splice variants have been reported which give rise to truncated forms of ATF3 (see Figure 2). The first described variant results in a protein termed ATF3Δzip owing to its loss of the carboxy-terminal leucine zipper dimerization domain (Chen et al., 1994). The resultant protein is truncated at 118 aminoacids (118aa). The expression of two other splice variants of ATF3 was demonstrated by using RT-PCR on cDNA from primary human umbilical vein endothelial cells treated with the calcium ionophore A23187 (Hashimoto et al., 2002). Somewhat surprisingly, although these two variant transcripts were abundant, ATF3Δzip was not detectable in this system. Subsequent analysis of the two novel variant transcripts revealed that they both gave rise to the same

protein isoform, truncated at the carboxy terminus and lacking the leucine zipper dimerization domain. The authors have termed the variant transcripts ATF3Δzip2a and ATF3Δzip2b, and their common 135aa protein ATF3Δzip2 (which shares its first 115aa with full-length ATF3).

Two novel ATF3 mRNA isoforms have been described in human HepG2 hepatoma cells undergoing metabolic stress (Pan et al., 2003); both were detected by RT-PCR. The first- ATF3Δzip2c – gives rise to a 106aa protein and is identical to the ATF3Δzip2 isoform except for a further truncation of 29aa near the N terminus. The second splice variant, termed ATF3Δzip3, is similar to the ATF3Δzip2b splice variant. However, it retains the sequence between exons C and D', which the authors term C'; this introduces an early in-frame stop codon, giving rise to a 120aa protein truncated at the carboxy terminus. Interestingly, Pan et al. did not detect expression of ATF3Δzip, ATF3Δzip2a, or ATF3Δzip2b in HepG2 cells.

Finally, an ATF3 mRNA splice variant termed ATF3b has been identified in αTC1.6 cells, which results from splicing of 106 bases within exon B (Wang et al., 2003). An early in-frame stop codon is introduced at the site corresponding to position 10 in the ATF3



amino acid sequence. However, there is another in-frame start codon downstream which, if used for initiation of translation, gives rise to a 124aa ATF3 isoform. The authors report that this protein, which is truncated at the amino terminus, can be detected in whole pancreas homogenate.

**Structure/function relationship for ATF3 protein isoforms**

Full-length ATF3 comprises 181aa, with a molecular mass of 22 kDa (Hai et al., 1989; Chen et al., 1994; Thompson et al., 2009). The region between 40aa and 84aa is thought to exert transcriptional repression (Hsu et al., 1992), whilst the basic region leucine zipper domain, spanning the region from 88 to 147aa is necessary for dimerization and DNA binding (Hashimoto et al., 2002). ATF3Δzip and ATF3Δzip2 are truncated at 118aa and 135aa, respectively.

Somewhat paradoxically ATF3Δzip, a 14-kDa protein, is thought to have the capacity to activate transcription despite retaining the putative transcriptional repression domain and lacking most of the basic region leucine zipper domain. This may be achieved by ATF3Δzip sequestering inhibitory co-factors (Chen et al., 1994). Hua et al. (2006) also reported that ATF3Δzip2 displaces the positive cofactor CBP/300 from a protein complex involved in the transcriptional control at nuclear factor-κB (NF-κB) sites in the anti-apoptotic gene *cIAP2*. The same ATF3Δzip2 protein modulates the activity of the full-length ATF3 protein during stress responses and cellular proliferation (Hashimoto et al., 2002). ATF3Δzip3 stimulates transcription from the asparagine synthetase promoter (Pan et al., 2003); as with the transcriptional activation effects of ATF3Δzip, this may be mediated through a sequestration of co-repressors away from the promoter.

Finally, the 124aa ATF3b isoform (with N-terminal truncation) can still form homodimers, despite lacking the weak activation region and part of the repression region. These ATF3 homodimers can bind to the CRE/ATF site of the proglucagon promoter and stimulate transcription (Wang et al., 2003). It is worth noting that the prevalence and significance *in vivo* of the products of the numerous splice variants of ATF3 have not yet been fully established. In particular, almost nothing is known about the expression in the nervous system of the protein products of splice variants.

### ATF3 INDUCTION AND REGULATION

Activating transcription factor 3 has been variously described as an immediate early gene, a stress inducible gene and an adaptive response gene (Thompson et al., 2009; Zmuda et al., 2010). It appears to play an important role in mediating the cellular response to injury and stress, but there are microarray data to show that ATF3 also enables cells to cope with intracellular and extracellular changes which are not directly harmful (Hai, 2006). In most healthy tissues ATF3 is expressed at a very low level, if at all (Hsu et al., 1991; Chen et al., 1994; Allen-Jennings et al., 2001).

The regulation of ATF3 appears to take place mainly at the translational level: there is no evidence that its activity is regulated by phosphorylation as is that of c-Jun or STAT3, for example, whose expression by neurons is also correlated with axonal regeneration. Analysis of the ATF3 promoters reveals an array of transcription factor binding sites consistent with its expression being induced by stressful stimuli and other factors. P2 contains a canonical TATA box, inducible transcription factor binding sites (e.g., for ATF/CRE, AP-1, and NF- $\kappa$ B), and cell cycle dependent sites (e.g., for E2F and Myc/Max). The alternate promoter P1 contains a non-canonical TATA box as well as ATF/CRE, AP-1, p53, E2F, and NF- $\kappa$ B binding sites. As would be expected, c-Jun/AP-1, ATF2, ATF4, ATF6, CREB, Myc, C/EBPB, Erg-1, and other transcription factors have all been shown to induce ATF3 expression (Tamura et al., 2005; Huo et al., 2006). In addition, ATF3 represses its own expression (Wolfgang et al., 2000), limiting the period of its expression in response to stressful stimuli.

The co-expression of c-Jun and ATF3 in the nervous system following traumatic injury and other stressful stimuli makes c-Jun a prime candidate for controlling ATF3 transcription. ATF3 expression is upregulated by c-Jun in rabbit reticulocytes (Weidenfeld-Baranboim et al., 2009), human vascular endothelial cells (Weidenfeld-Baranboim et al., 2009), and neuronal cell lines (Jankowski et al., 2009) but elevation of c-Jun does not appear to be a prerequisite for ATF3 upregulation in human skin fibroblasts (Abe et al., 2003) and primary sensory neurons (Tsujino et al., 2000). Another factor in the control of ATF3 induction is Jun Dimerization Protein 2 (JDP2), a ubiquitous bZip protein closely related to ATF3, which exerts its effect at the ATF/CRE and two non-canonical auto-regulatory sites, at -90 and -20, of the ATF3 promoter (Kim et al., 2010b). JDP2 represses ATF3 expression and its ablation results in the potentiation of ATF3 expression by stressful stimuli (Weidenfeld-Baranboim et al., 2009). Interestingly, phosphorylation of JDP2 has been observed in response to such stimuli, but the significance of this remains unclear.

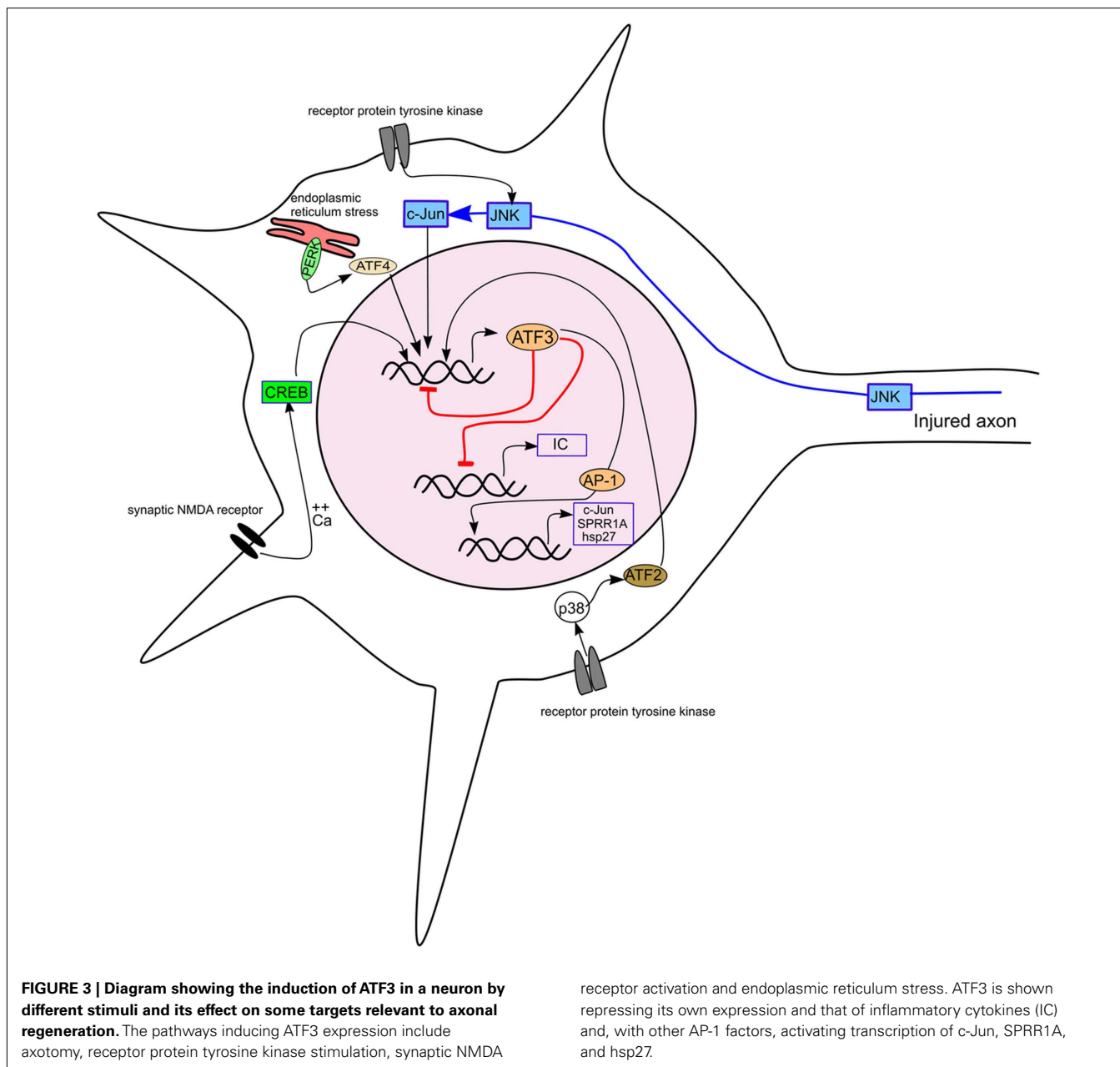
Studies with siRNA have shown that the transcription factor SOX11 (Jankowski et al., 2009), a member of the group C SOX

(SRY-related HMG-box) transcription factor family, positively regulates ATF3 expression in axotomized sensory neurons *in vivo*. ATF3 expression in astrocytes is also upregulated at the transcriptional level by NF-E2-related factor 2 (Nrf2), a transcription factor which binds to the antioxidant response element (ARE) in promoters. Treatment with Nrf2 activators and oxidants provokes ATF3 induction in astrocytes, and ATF3 expression is reduced in Nrf2-depleted cells (Kim et al., 2010b). Phosphorylation of the  $\alpha$ -subunit of the eukaryotic translation initiation factor 2 (eIF2) in lung cells in response to hypoxia leads to nuclear translocation of ATF4 and a subsequent increase in ATF3 expression (Konsavage et al., 2012).

If extracellular signals are to increase ATF3 expression, signal transduction mechanisms between the cell membrane and the DNA must be activated. Mitogen-activated protein kinase (MAPK) pathways are involved in the cellular response to many external stimuli. Of these MAPK pathways, the p38 pathway, extracellular signal-regulated kinase (ERK), and the c-Jun N-terminal kinase (JNK) pathways are known to affect ATF3 (Figure 3). ATF3 expression is upregulated by both ATF2 and c-Jun, and both of these are phosphorylated by MAPK pathways. For example, the induction of ATF3 expression in colonic cancer cells treated with tolfenamic acid, a pro-apoptotic drug, is mediated by p38 activation leading to phosphorylation of ATF2 and involves the ATF/C/EBP-binding site in the ATF3 promoter (Lee et al., 2010). The p38 pathway, but not the ERK nor the JNK pathways, was also required for ATF3 expression in several cell lines exposed to anisomycin (Lu et al., 2007). However, JNK activation may also be associated with ATF3 induction in cultures of primary sensory neurons (DRG and nodose ganglion cells); inhibitors of JNK reduced c-Jun phosphorylation, ATF3 upregulation, and neurite outgrowth (Lindwall et al., 2004). As those authors pointed out, it is tempting to construct a pathway involving JNK activation leading to c-Jun phosphorylation and the ATF3 upregulation but some neurons showed elevated ATF3 without pc-Jun or pJNK (Lindwall et al., 2004). The retrograde axonal transport of JNK by neurons appears to be an important part of the signaling mechanism by which an injured axon induces a cell body response, including the upregulation of ATF3 (Lindwall and Kanje, 2005). However, MAPK activation is not essential for upregulation of ATF3, at least in non-neuronal cells (Konsavage et al., 2012).

Activating transcription factor 3 expression is induced by activation of the innate and adaptive immune systems. Toll-like receptor (TLR)3, TLR4, and TLR9 agonists are potent inducers of ATF3 expression in macrophages and epithelial cells (Gilchrist et al., 2006; Sukanami et al., 2009; Roussel et al., 2011) as is TCR ligation in CD4 lymphocytes (Gilchrist et al., 2008). TLR activation stimulates the JNK and p38 MAPK pathways and NF- $\kappa$ B activation.

Apart from transcriptional regulation, there is now evidence that the expression of ATF3 is regulated at the post-transcriptional level by selective degradation. Double minute 2 (MDM2) is an E3 ubiquitin ligase for ATF3 and regulates ATF3 expression by promoting its degradation, at least in HCT116 (colorectal carcinoma) cells and mouse embryonic fibroblasts (Mo et al., 2010). It is not yet clear how significant this form of regulation is in injured neurons,



however, where there is a close correlation between ATF3 mRNA levels and nuclear ATF3 protein (Tsujino et al., 2000).

### INTERACTIONS WITH OTHER TRANSCRIPTION FACTORS AND GENERAL FUNCTIONS OF ATF3

The interaction of ATF3 with many other transcription factors dramatically alters its effects on transcription (reviewed by Hai, 2006). ATF/CREB members can form heterodimers with members of other families (e.g., C/EBP and Maf families), as well as with other ATF/CREB subgroup members. c-Jun is a particularly important partner for ATF3 in the nervous system; as well as being a potential inducer of ATF3 expression, c-Jun binds to ATF3. c-Jun co-immunoprecipitates with ATF3 in extracts of reticulocytes (Hai and Curran, 1991), PC12 cells (Nakagomi et al., 2003;

Pearson et al., 2003), Neuro-2a cells (Pearson et al., 2003), suggesting that these two transcription factors can form heterodimers, at least in non-neuronal cells and “neuronal” cell lines. Importantly, both ATF3 and c-Jun are present in hypoglossal neurons after their axon was cut (Kiryu-Seo et al., 2008). Here, an unconventional mechanism of action for ATF3 and c-Jun has been proposed, in which the constitutively expressed transcription factor Sp1 (a member of the Sp/KLF family) acts as scaffold protein to bring about the association of ATF3, c-Jun, and STAT3 at the Sp1 binding sites. This notion is supported by the coimmunoprecipitation of ATF3 and c-Jun, ATF3 and Sp1, c-Jun and Sp1, and STAT3 and Sp1, but not STAT3 and c-Jun (or ATF3; Kiryu-Seo et al., 2008). A transient upregulation of JunB has been reported in axotomized neurons (Kenney and Kocsis, 1997),

making ATF3/JunB heterodimers another possible combination regulating axonal regeneration.

It has been suggested that in regenerating liver, ATF3 in combination with c-Jun activates specific promoters containing AP-1 and CRE sites, but in combination with JunB it potentially represses activation of AP-1 sites (Hsu et al., 1992). ATF3 has been implicated in the cellular response to many varied insults, including ischemia/reperfusion of the kidney or heart (Chen et al., 1996; Yin et al., 1997), liver damage (Chen et al., 1996), ventilation-induced lung damage, and many types of insult to cultured cells including hypoxia (Ameri et al., 2007) and other treatments (Lerner et al., 1996). Many circumstances that cause ATF3 expression also lead to cell death (Kim et al., 2010a; Liu et al., 2011) but ATF3 has also been found to be constitutively expressed in a number of human cancers and it is said to be associated with metastatic potential. The interaction of ATF3 with p53 is thought to facilitate apoptosis (Yan et al., 2005). It is believed that ATF3 stabilizes the p53 protein through blocking MDM2-mediated ubiquitination, nuclear export, and degradation. Protein deletion studies reveal that the 102–139aa region of ATF3 is necessary for p53 binding.

Activating transcription factor 3 is believed to have at least two key roles: as a mediator of the cellular stress response, and as a regulator of cellular proliferation. However, because the functions of ATF3 depend on the transcriptional milieu within which it is found, ATF3 can have opposite effects on different types of cell (Yin et al., 2008). Both gene delivery (Mashima et al., 2001) and gene inactivation experiments (Nawa et al., 2002) have attributed pro-apoptotic effects to ATF3, yet anti-apoptotic effects have also been described (Kawauchi et al., 2002). Similarly, ATF3 has been reported to suppress or promote cell cycle progression (Fan et al., 2002; Lv et al., 2011). In addition, in recent years its role in immune response pathways has received much attention, especially as a negative regulator of TLR family signaling in the inflammatory response (reviewed by Thompson et al., 2009). Network analysis predicts that ATF3 is part of a transcriptional complex containing members of the NF- $\kappa$ B family of transcription factors and that ATF3 can regulate the expression of inflammation-related genes including IL1 $\beta$ , IL6, IL12, TNF $\alpha$ , and CCL4 (Gilchrist et al., 2006). Hai (2006) proposed that ATF3 induction by various stimuli in different cell types contributes to the control of the cell death and cell cycle machineries in a context-dependent manner. The nervous system appears to be a special context in that while ATF3 tends to produce tissue dysfunction and disease progression in many types of non-neuronal cells (Hai, 2006), in the nervous system ATF3 appears to contribute to the regenerative response (Campbell et al., 2005).

One adaptive pathway in which ATF3 has been implicated is the Endoplasmic Reticulum Stress Response (ERSR; also known as the Unfolded Protein Response) which occurs in response to the pathological accumulation of unfolded or misfolded proteins within the lumen of the endoplasmic reticulum. In essence, this pathway attempts to restore normal ER protein folding and processing through several different actions including the inhibition of translation and reduction in translocation of new proteins into the ER, promotion of the export of aberrant proteins from the ER lumen to the cytosol for degradation, and the recruitment of more molecular chaperones to assist in protein folding. Ultimately, if the

ER remains stressed, the pathway will instead default to apoptosis. The ERSR is complex and is mediated by a tripartite system of transmembrane molecular sensors within the endoplasmic reticulum, namely ATF6, PERK, and IRE1 alpha. Each of these has a fairly well defined pathway, becoming activated following the detection of inappropriately folded protein within the ER lumen. ATF3 has been implicated in the PERK-mediated arm (**Figure 3**), which is initially protective but also has a role in inducing apoptosis via the ATF4–ATF3–CHOP cascade (Mungrue et al., 2009).

Pan et al. (2003) have presented evidence to show that ATF3 expression was enhanced in HepG2 hepatoma cells in which the ERSR pathway had been activated. There are many examples of ERSR activation in neurodegenerative diseases and following traumatic injury to the nervous system, including Parkinson's disease (Bellucci et al., 2011) and *in vitro* models of that condition (Holtz et al., 2006), amyotrophic lateral sclerosis (Kanekura et al., 2009), Alzheimer's disease (Salminen et al., 2009), and following peripheral nerve injury (Penas et al., 2011). In many of these cases (but not apparently Alzheimer's disease) ATF3 expression is also found in the affected neurons. However, a causal link between the ERSR and ATF3 expression usually has not been established and the effect of ATF3 within the stressed cells is uncertain. Cultured midbrain neurons treated with 6-OHDA (a model of the neurodegeneration found in Parkinson's disease) showed an ERSR and upregulated ATF3 expression but neurons from ATF3 “knockout” mice were not protected from cell death following 6-OHDA treatment (Bernstein et al., 2011). This was interpreted as indicating that the induction of an ERSR and consequent ATF3 expression was not the pathway leading to cell death in this model (it was proposed to be neuroprotective instead). However the value of this mutation for investigating ATF3 function in the nervous system has been questioned (see below).

## ATF3 IN NORMAL AND INJURED NEURONS INTACT NEURONS

Activating transcription factor 3 is either not expressed or expressed at very low levels in most intact neurons *in vivo*. In some studies ATF3 could not be detected in the neurons of uninjured animals. For example, it has been reported that there is no immunohistochemically detectable ATF3 in the facial motor neurons of intact mice (Parsadanian et al., 2006) and that there is no signal in Western blots for ATF3 in adult rat cerebral hemisphere (Yano et al., 2008). Although ATF3 has been detected in the nuclei of a very small percentage of primary sensory neurons in DRG of uninjured rats in some studies (Averill et al., 2002), others have failed to find such cells (Tsuzuki et al., 2001; Takahashi et al., 2003; Carlton et al., 2009). A similar low number of sympathetic neurons with ATF3-positive nuclei have been found in the superior cervical ganglia of intact rats (Hyatt-Sachs et al., 2007). It seems likely that ATF3 expression by sensory and postganglionic sympathetic neurons in intact animals is infrequent enough to be overlooked in most studies and may reflect a low level of axonal injury in populations of neurons that project to exposed sites.

Cytoplasmic ATF3 has been reported in von Economo neurons in the anterior cingulate gyrus of primates including man (Stimpson et al., 2011); this is quite different from the normal pattern of ATF3 expression in which the gene is only expressed at times of

changes in the intracellular or extracellular environment and the protein is found predominantly in the nucleus. In our experience cytoplasmic immunohistochemical signal for ATF3 in neurons is more common in mice than in rats, whereas nuclear staining is injury-specific and similar in both mice and rats. Delivery of the ATF3 gene with an amplicon vector to rat DRG neurons in culture resulted in both cytoplasmic and nuclear localization of the protein (Seiffers et al., 2006) in contrast to the nuclear localization of endogenous ATF3. The same combined pattern was also observed *in vivo* with neuronal ATF3 overexpression, with majority neurons showing strong cytoplasmic staining, and a minority, also nuclear translocation (Seiffers et al., 2007). It has been reported that STAT1 is necessary for nuclear expression of ATF3 in hepatocytes and pancreatic beta cells (Kim et al., 2009, 2010a). It is to be hoped that there will be similar studies involving neurons and glial cells.

### **ATF3 IS UPREGULATED IN NEURONS PROJECTING INTO INJURED PERIPHERAL NERVES**

The most consistent observation on ATF3 expression in the nervous system is that ATF3 is upregulated by neurons with axons projecting into injured peripheral nerves, including spinal motor neurons (Tsujino et al., 2000; **Figure 1A**), facial motor neurons (Parsadanian et al., 2006; Shokouhi et al., 2010), primary sensory neurons (Tsujino et al., 2000; Tsuzuki et al., 2002), postganglionic sympathetic neurons (Hyatt-Sachs et al., 2007), and preganglionic parasympathetic neurons (Peddie and Keast, 2011; **Figure 1B**). ATF3 immunoreactivity in these regenerating peripheral neurons is concentrated in the nucleus, but not the nucleolus, but cytoplasmic immunoreactivity is sometimes detectable. All DRG neurons with injuries to their peripheral axon appear to express ATF3 mRNA (Tsujino et al., 2000). ATF3 mRNA appears in DRG neurons by 6 h after spinal nerve transection but takes several hours longer after a more distal sciatic nerve transection (Tsujino et al., 2000). These observations on the effects of distance between the site of axotomy and the cell bodies of the injured neurons have been confirmed (Linda et al., 2011). There are two obvious explanations of the reduced time delay following spinal nerve injury: the shorter time taken for a retrograde signal to reach the cell bodies after axotomy or the more intense cell body reaction seen after more proximal axotomy (Lieberman, 1971). In addition, ATF3 is retrogradely transported in axons of peripheral nerves, as demonstrated by its accumulation proximal to a ligation (Lindwall and Kanje, 2005). Transection and crush injuries to peripheral nerve have been most often used to upregulate ATF3 in peripheral neurons but tetanic stimulation of rat sciatic nerve, which produces Wallerian degeneration in the nerve, also induces ATF3 expression in DRG neurons (none was found in naïve rats; Liang et al., 2010). Less intensive stimulation did not upregulate ATF3 in DRG neurons (Tsujino et al., 2000; Tsuzuki et al., 2001).

A key feature of the neuronal response to axotomy in DRG neurons is that this response is much more muted following injury of the central process of their axons. In the case of ATF3, this response is weaker, confined to larger neurons and more transient, disappearing by 7 days after injury (Huang et al., 2006). None the less, ATF3 upregulation is one of the more obvious effects of injury to the central processes of primary sensory neurons.

Neuronal ATF3 is also upregulated in peripheral neuropathy. Paclitaxel, a cytotoxic drug used in the treatment of cancer is associated with a peripheral neuropathy side-effect, also produces the upregulation of ATF3 in primary sensory neurons in DRG and trigeminal ganglia in experimental animals (Jimenez-Andrade et al., 2006; Peters et al., 2007a). However, ATF3 expression remained low in animals where no axonal degeneration was detected (Flatters and Bennett, 2006). A rat model of diabetes, which induces a peripheral neuropathy, also caused ATF3 upregulation in some DRG neurons. Injection of resiniferatoxin into the paws of rats causes a dying-back neuropathy involving nociceptive axons and is associated with ATF3 upregulation in the DRG neurons during the subsequent period when axonal regeneration occurs (Mitchell et al., 2010). Similarly, ATF3 is upregulated in motor neurons in models of amyotrophic lateral sclerosis (Malaspina et al., 2010) which also involve dying-back of axons. A plausible explanation of all these results is that each of the procedures that produce upregulation of ATF3 in neurons, with the possible exception of NGF-deprivation of sympathetic neurons, also involves some degree of axonal injury or separation from targets which, in turn, signals to the cell body to upregulate ATF3.

### **ATF3 UPREGULATION BY PERIPHERAL NEURONS IN THE ABSENCE OF DIRECT INJURY**

Several treatments that do not involve direct injury to nerve trunks have been found to induce ATF3 in peripheral neurons. Loss of NGF support induces ATF3 expression in postganglionic sympathetic neurons (Hyatt-Sachs et al., 2007). In addition, some treatments which produce peripheral inflammation induce ATF3 in neurons. For example, injection of formalin into the footpad of rats induced an inflammatory response in the foot and induced ATF3 expression in some DRG neurons and even a few motor neurons in the spinal cord (Tsujino et al., 2000). However, Freund's complete adjuvant, when injected into the footpad, did not produce ATF3 upregulation despite the profound inflammation produced at the injection site (Tsujino et al., 2000; Segond von Banchet et al., 2009; Braz and Basbaum, 2010). Injections of capsaicin, formalin, mustard oil, or menthol into the hind paw of mice induced expression of ATF3 in distinct subpopulations of sensory neurons, an observation which was interpreted as showing that the different agents injured different populations of sensory axons (Braz and Basbaum, 2010). ATF3 was also upregulated in DRG neurons in rat models of arthritis where the extent of axonal injury is unknown (Ivanavicius et al., 2007; Christianson et al., 2010).

### **ATF3 IS UPREGULATED BY SOME TYPES OF CNS NEURONS FOLLOWING INJURY**

Unlike neurons which project into peripheral nerves, most injured CNS neurons do not regenerate their axons although many produce local sprouts from their injured axons (Fishman and Mattu, 1993; Zeng et al., 1994; Hill et al., 2001). ATF3 is upregulated by axotomized intrinsic CNS neurons, but only by some and only under certain conditions. Among the critical factors that can reduce ATF3 expression after axotomy appear to be the distance between the site of axotomy and the neuronal cell body and the abundance of collaterals proximal to the injury site. These factors can be difficult to separate in the case of CNS neurons which

give off collaterals at several points along their length. Cerebellar Purkinje cells which are particularly poor at regenerating axons (Anderson et al., 1998; Chaisuksunt et al., 2000) do not upregulate ATF3 after axotomy (**Figure 1C**).

### **Cerebral neocortex and thalamus**

Activating transcription factor 3 is upregulated in neurons around cortical ischemic infarcts and in cortical neurons thought to project through the damaged region (Ohba et al., 2003, 2004). The same studies also find a transient (lasting a few days) upregulation of ATF3 mRNA and protein in some thalamic neurons. ATF3, and several other growth-associated molecules including GAP43, were not upregulated by corticospinal neurons after spinal cord injury but they were upregulated after intracortical axotomy (Mason et al., 2003). Together these results suggest that ATF3 is upregulated by thalamic projection neurons and by neocortical projection neurons following axotomy, but only if the injury is sufficiently proximal to the cell body.

### **Hippocampus**

Strong ATF3 expression was identified in the nuclei of dentate gyrus neurons following injection of kainate, an excitotoxin (Francis et al., 2004). Interestingly, the CA1 pyramidal neurons, which are more susceptible to kainite neurotoxicity, do not upregulate ATF3 so strongly as dentate neurons. This may suggest that ATF3 expression following excitotoxicity is involved in the survival of neurons rather than cell death.

### **Retina**

After optic nerve injury (Takeda et al., 2000), retinal ganglion cells show a stronger and more prolonged upregulation of ATF3 (and other growth-associated molecules; Berry et al., 2008) than most other CNS neurons. This response may be the result of the relatively proximal axotomy and/or the lack of collaterals of the injured axons. Retinal ganglion cells also show a greater capability for axonal regeneration and a greater susceptibility to cell death following axotomy than most other CNS neurons (Allcutt et al., 1984; Campbell et al., 1999, 2003), and these findings are compatible with ATF3 having a role in regulating axonal regeneration and/or in cell death in response to axonal injury. Raised intraocular pressure in a rat model of glaucoma also elevates ATF3 expression in retinal ganglion cells to an extent that correlates linearly with the degree of injury to their axons (Guo et al., 2009, 2011).

### **Brainstem**

Pontocerebellar neurons show a prolonged (weeks) upregulation of ATF3 after axotomy (Lovas et al., 2010). The same applies to dopaminergic neurons in the substantia nigra pars compacta (Song et al., 2008), and olivocerebellar neurons following axotomy within the cerebellum (our unpublished observations).

### **ATF3 IN TREATMENT-STIMULATED, REGENERATING CNS NEURONS**

Since most intrinsic mammalian CNS neurons do not regenerate their axons (Ramon y Cajal, 1928; Bolsover et al., 2008) the relationship between ATF3 expression and regeneration can usually only be studied in models with an artificially induced regenerative response. One exception is the mammalian optic nerve where a very small percentage of axons can regenerate after

injury even without further stimulus (Campbell et al., 1999). The many treatments claimed to induce axonal regeneration by CNS neurons include antibodies to myelin proteins (Raineteau et al., 1999), administration of neurotrophins (Bradbury et al., 1999), administration of chondroitinase (Bradbury et al., 2002), and the implantation of olfactory glia (Li et al., 1997). However, a particularly successful way to induce regeneration of axons of intrinsic CNS neurons involves the implantation of peripheral nerve grafts (Richardson et al., 1980), which provide good pro-regenerative environment.

None the less, even nerve grafts only induce the regeneration of axons of some types of CNS neurons (Anderson et al., 1998; Anderson and Lieberman, 1999). The neurons that can be induced to regenerate their axons have always been found to show a prolonged upregulation of ATF3. Following the implantation of nerve grafts into the thalamus more than 90% of the neurons that regenerate axons into the graft come from the thalamic reticular nucleus (TRN; Morrow et al., 1993), whereas just few thalamic projection neurons and no corticothalamic neurons regenerate their axons into the grafts. Interestingly, at early time-points, such grafts induced the upregulation of ATF3 protein in many nearby thalamic neurons. However, after 7 days or longer most of the ATF3-positive nuclei were in TRN neurons that had regenerated their axons into the grafts (Campbell et al., 2005). Although the grafts must have axotomized many cortical neurons near their terminations in the thalamus, cortical neurons were never ATF3-positive.

Other CNS neurons that can be induced to regenerate axons into peripheral nerve grafts include retinal ganglion cells, forebrain cholinergic neurons, rubrospinal neurons, and those in the cerebellar deep nuclei. ATF3 upregulation has been reported in rubrospinal neurons regenerating axons into peripheral nerve grafts in the cervical spinal cord (Shokouhi et al., 2010) and is also found in cerebellar deep nucleus neurons that have been stimulated to mount a regenerative cell body response by the presence of a peripheral nerve graft (**Figure 1D**). In marked contrast, Purkinje cells, which never regenerate into such grafts, do not upregulate ATF3 (**Figure 1C**) ATF3 upregulation has been reported in retinal ganglion cells of Zebrafish during axonal regeneration following optic nerve crush (Saul et al., 2010).

### **ATF3 UPREGULATION IN NEURONS WITHOUT DELIBERATE AXOTOMY**

#### ***Trans-synaptic effects of nerve injury***

Chronic constriction injury (CCI) of peripheral nerves is a model of chronic pain (Bennett and Xie, 1988; Vos et al., 1994) which involves inflammation – microglial activation and T-cell invasion – in the central target regions of the primary nociceptive neurons, the superficial dorsal horn of the spinal cord or the spinal nucleus of the trigeminal nerve. Sciatic nerve CCI elevates ATF3 expression in L4 and L5 dorsal root ganglion neurons (Obata et al., 2003; which could be due to neuropathic pain and/or axotomy) but also in the dorsal horn (Latremoliere et al., 2008). At both sites, ATF3 expression and inflammation were attenuated by the neuroinflammation-suppressing minocycline.

#### ***Stress and amphetamine administration***

The limbic part of the striatum, the nucleus accumbens, is important in the responses to psychological stress and the entire striatum



is an important site of amphetamine action. A nearly 20-fold increase in ATF3 mRNA expression was produced in the striatum of adult rats by the stress induced by restraint within a polythene cone for 60 min. A non-toxic dose of amphetamine, which increases dopamine levels in the striatum, also upregulated ATF3 mRNA in the striatum but to a lesser extent (Green et al., 2008). Methamphetamine also raised ATF3 levels in the striatum of rats (Jayanthi et al., 2009).

#### **Non-axotomy signals for ATF3 expression in neurons**

Activating transcription factor 3 expression may be upregulated in neurons in response to stimuli similar to those that increase its expression in non-neuronal cells (see above) including the MAPK pathways and the ERSR, i.e., extracellular signals and endoplasmic reticulum stress. Treatment with an antiserum against NGF induces ATF3 expression in rodent postganglionic neurons (Hyatt-Sachs et al., 2007), a result which could be explained by retrograde transport of NGF suppressing ATF3 expression. A similar effect had been demonstrated previously in differentiated PC12 cells (Mayumi-Matsuda et al., 1999).

*In vitro*, stimulation of synaptic NMDA receptors led to a neuroprotective effect and ATF3 expression by the postsynaptic hippocampal neurons. Both effects were blocked by the NMDA antagonist MK801. Transfection of the neurons with inhibitors of CREB and CaMKIV showed that this expression of ATF3 was regulated by a nuclear calcium signaling pathway involving these molecules (Zhang et al., 2011).

Demyelination of DRG/glial cultures also leads to ATF3 upregulation by the neurons (Kiryu-Seo et al., 2010). Treatment with shRNA directed against ATF3 reduced ATF3 expression, decreased the velocity of mitochondria within demyelinated axons and increased oxidative damage in the neurons.

#### **ATF3 IN GLIAL CELLS**

Injury to the peripheral nervous system induces axonal regeneration-associated with profound phenotypic changes in glial and other non-neuronal cells including satellite cells and Schwann cells. ATF3 is strongly upregulated in peripheral glia following various types of injury. Schwann cells in the distal stump of injured sciatic nerves in rats and mice upregulate ATF3 (Hunt et al., 2004; Isacson et al., 2005; Kataoka et al., 2007; Dahlin et al., 2008; Saito and Dahlin, 2008) in addition to c-Jun. Glial cells including Schwann and satellite cells in the superior cervical ganglion expressed ATF3 after decentralization (section of the cervical sympathetic trunk); this procedure induces the anterograde degeneration of preganglionic fibers and some of the ATF3 expression was probably around degenerating fibers (Zigmond and Vaccariello, 2007). There was a suggestion that satellite cells in DRG upregulate ATF3 following injury to the sciatic nerve (Isacson et al., 2005; Hu et al., 2007) but this has not been generally reported, perhaps because the expression is weaker than in the sensory neurons.

Satellite cells and Schwann cells upregulated ATF3 following paclitaxel treatment (Peters et al., 2007a,b) which induced a peripheral neuropathy. Such expression may, of course, be the result of damage to the axons or direct effects on the glia. An early and significant observation was that, following dorsal root

injury, ATF3 upregulation by glia around the degenerating axons within the CNS was less than that by glial cells around the same degenerating axons within the dorsal root (Hunt et al., 2004), i.e., ATF3 was upregulated by glia where axonal regeneration normally occurs but upregulated to a much lesser extent where axonal regeneration does not occur. The implication of this is that the changes in gene expression mediated by ATF3 in glia may be crucial to providing an environment suitable for regenerating axons.

The specific role of glial ATF3, upregulated after axonal injury, is subject of ongoing debate. In transformed mammary epithelial cells ATF3 enhanced fibronectin expression (Yin et al., 2010), which may exert a growth-promoting effect (Vogelezang et al., 1999). In untransformed chick fibroblasts ATF3 also repressed the expression of decorin (Perez et al., 2001; Yin et al., 2010), a TGF $\beta$ -binding glycoprotein which may reduce scarring and thus promote regeneration (Davies et al., 2004). But to determine a specific role for ATF3 in glia, one would probably need a cell type-specific deletion of most of the ATF3 gene encompassing exons C to E, which encode all the bZip domain and large part of the repressor region.

#### **ATF3 in neuroinflammation**

Inflammation in the CNS provides a stimulus for glial ATF3 upregulation. ATF3 can be induced in macrophages and microglia by the cytokine, IL6 (Dominguez et al., 2010). Lipopolysaccharide, a highly inflammatory bacterial product, applied to the motor cortex induced ATF3 expression in the nuclei of many glial cells in the subcortical white matter (Hossain-Ibrahim et al., 2006). ATF3 is a negative regulator of TLR4, the receptor for lipopolysaccharide in such cells (Gilchrist et al., 2006) and as such might be expected to dampen down the inflammatory response of microglia.

Activating transcription factor 3 is also induced in neurons in some inflammatory states: ATF3 is upregulated in the inflamed spinal cord of animals with CCI of the sciatic nerve (see above and Dominguez et al., 2010) and in the pontine nuclei following demyelination of the pontocerebellar tract. In a patient with Marburg's variant of multiple sclerosis ATF3 was found in neurons within the pontine nuclei and neocortex (Lovas et al., 2010). A caveat is that the extent of axonal injury in such cases of demyelination remains unknown.

Neuroinflammation has been hypothesized to play a role in the genesis of some psychiatric conditions (Smith, 1992). ATF3 expression by monocytes was increased in schizophrenia and bipolar disorder (Drexhage et al., 2010; Weigelt et al., 2011). ATF3 binds to the triggering receptor expressed on myeloid cells 1 (TREM-1) promoter and may be a negative regulator of the inflammatory response (Weigelt et al., 2011) but it is not clear how this would fit in to the pathology of psychoses.

#### **WHAT IS THE FUNCTION OF NEURONAL ATF3?**

Activating transcription factor 3 has no known role in neural development or the intact nervous system; its expression appears to be to a response stimuli which are mainly stressful or pathological (Tables 1 and 2). Most direct evidence in terms of functional role comes from experiments with transgenic mice. Transgenic mice that overexpress ATF3 in sensory dorsal root ganglion neurons show enhanced rate of sensory axon regeneration following

**Table 1 | Genes and signaling pathways related to axonal regeneration that are regulated by ATF3.**

Upregulated by ATF3	Downregulated by ATF3	Significance for axonal regeneration
	Fibronectin (Yin et al., 2008) in mammary epithelial cells	Fibronectin is produced by Schwann cells and promotes neurite outgrowth (Tucker and Mearow, 2008)
	Toll-like receptor signaling (Gilchrist et al., 2006; Litvak et al., 2009) in immune cells	Inflammation around neuronal cell bodies is correlated with and may stimulate axonal regeneration (Leon et al., 2000; Shokouhi et al., 2010)
	Decorin (Perez et al., 2001) in embryonic chick fibroblasts	Decorin reduces scar formation in the CNS and enhances axonal regeneration (Davies et al., 2004; Minor et al., 2008)
	Thrombospondin 2 (Perez et al., 2001) in embryonic chick fibroblasts	Thrombospondin-deficient mice show axonal sprouting deficits (Liau et al., 2008)
SPRR1A (Seiffers et al., 2007) in neurons		Promotes neurite outgrowth (Bonilla et al., 2002)
Hsp27 (Seiffers et al., 2007) in neurons		Expression is associated with axonal regeneration (Williams et al., 2006)
c-Jun (Seiffers et al., 2007) in neurons		Important regulator of axonal regeneration (Raivich et al., 2004)
DINE (Kiryu-Seo et al., 2008) in neurons		Required for neuromuscular junction formation and sprouting of motor axons (Nagata et al., 2010)

sciatic nerve injury (Seiffers et al., 2007). This is accompanied by increased expression of the genes for the regeneration-associated molecule SPRR1A (which, like ATF3, is not found in neurons during developmental axonal growth), hsp27, c-Jun, and CAP-23 but not for the prototype growth-associated molecule GAP43 or the regeneration-related transcription factor, STAT3. *In vitro*, ATF3 overexpression induces the growth of longer, less branched neurites from DRG neurons (Seiffers et al., 2006). Unlike peripheral injury, ATF3 overexpression alone also does not produce a pronounced cell body response comparable to that observed following injury to the peripheral axon.

Activating transcription factor 3 frequently exerts a supplementary function. Co-transfection of ATF3 and c-Jun into neuronal cell lines produced greater neurite outgrowth than transfection with individual genes (Pearson et al., 2003). Using Sp1 as a scaffold, ATF3 interacts with at least three other transcription factors, C/EBP, c-Jun, and STAT3 (Kiryu-Seo et al., 2008), each of which has been shown to be important in axonal regeneration (Raivich et al., 2004; Nadeau et al., 2005; Bareyre et al., 2011) and plays an important role for the induction of expression of damage-induced neuronal endopeptidase (DINE) in a neuroblastoma cell line (Kiryu-Seo et al., 2008). DINE is necessary for the branching of motor neuron axons within muscle during development and the formation of adequate neuromuscular junctions (Nagata et al., 2010).

In the absence of other transcription factors found in regenerating neurons (e.g., c-Jun, STAT3), the overexpression of ATF3 has relatively modest effects. For example, it is not sufficient to allow DRG axons to regenerate in the presence of CNS myelin *in vitro* or in the injured spinal cord *in vivo*. Moreover, deletion of gp130, a component of the common neurokinine receptor, upstream of STAT3 phosphorylation (Habecker et al., 2009), abolishes the conditioning lesion response in explanted, sympathetic neurons, even though ATF3 is still upregulated (Hyatt-Sachs et al., 2010).

One apparent anomaly is that inflammation-related genes, which are typically downregulated by ATF3 in cells of the immune system, are upregulated in sensory and autonomic ganglia containing axotomized (and regenerating) neurons (Costigan et al., 2002; Boeshore et al., 2004). Inflammatory responses around neurons are positively correlated with axonal regeneration (Shokouhi et al., 2010) and the generation of inflammation around neuronal cell bodies has been shown to enhance regeneration of axons (Lu and Richardson, 1991; Yin et al., 2003). It is possible that either the transcriptional network in axotomized neurons ameliorates the effects of ATF3 on inflammatory genes or that one function of ATF3 expression in regenerating neurons is to limit the inflammatory response, preventing neuronal cell death.

Overexpression of ATF3 is frequently neuroprotective, probably by acting as a transcriptional repressor. *In vitro*, delivery of ATF3 to sympathetic neurons with a viral vector (Nakagomi et al., 2003) reduced cell death resulting from growth factor deprivation and enhanced neurite outgrowth, by a pathway involving Hsp27 and Akt activation, and requiring c-Jun. Similar neuroprotective effect, this time from kainite-induced excitotoxic cell death, is also observed in the hippocampal CA1 region (Francis et al., 2004). ATF3 reduces brain damage following a cerebral ischemic insult in mice, acting as a transcriptional repressor (Zhang et al., 2011). Overexpression of ATF3 had neuroprotective effects on PC12 cells expressing mutant Huntingtin, whereas knock down of ATF3 increased toxicity (Liang et al., 2009).

An ATF3 “knockout” mouse has been used in many experiments to help determine the function of ATF3 in biological processes, particularly stress responses and cancer (Hartman et al., 2004; Khuu et al., 2007; Akram et al., 2010; Zmuda et al., 2010). The mouse was generated by replacement of exon B, containing the start codon, with a neomycin resistance gene expressed from the reverse strand (Hartman et al., 2004). The animal has no reported defects in the development of the nervous system, a result which concurs with

**Table 2 | Genes and signaling pathways regulated by ATF3 that are potentially related to neuronal death/neuroprotection.**

Upregulated by ATF3	Downregulated by ATF3	Significance for neuroprotection
Death receptor 5 (DR5) in colonic cancer cells (Taketani et al., 2011)		Binding of its ligand causes DR5 to trigger cell death
DR5 and BBC3/PUMA in stressed colorectal cancer cells (Tanaka et al., 2011)	TNFRSF10B/DR5 and BBC3/PUMA in prostate cancer cells (Tanaka et al., 2011)	DR5 and BBC3/PUMA are pro-apoptotic genes (Tanaka et al., 2011)
STAT1 in pancreatic beta cells is induced and stabilized by ATF3 (Kim et al., 2010a)		STAT1 is involved in beta cell apoptosis in response to streptozotocin (Rasschaert et al., 2005)
	Inhibition of differentiation-1 (Id-1; Kashiwakura et al., 2008)	ID-1 downregulation is associated with cell death in cancer cells (Wong et al., 2008)
Hsp27 (Seijffers et al., 2007) in neurons		Protects neurons from cell death following various insults (Lewis et al., 1999)
CHOP/GADD153 in mouse fibroblasts subject to leucine starvation (Jiang et al., 2004)		CHOP/GADD153 expression is known to induce cell cycle arrest and apoptosis in response to cellular stress factors and is also known to promote apoptosis of cancer cell lines (Maytin et al., 2001)
	CHOP/GADD153 (Wolfgang et al., 1997) in HeLa cells	See above
CHOP/GADD153 levels in 6-OHDA – treated dopaminergic neurons are partially dependent on ATF3 (Bernstein et al., 2011)		See above
p53 is stabilized in fibroblast cell lines by ATF3 (Yan et al., 2005)		P53 can activate DNA repair or initiate apoptosis. Its expression is linked to cell death in ischemic brain injury (Hong et al., 2010).
Axonal transport of mitochondria (Kiryu-Seo et al., 2010)		The knockdown of ATF3 increased signs of mitochondrial damage in axons (Kiryu-Seo et al., 2010). Mitochondrial dysfunction is important for axonal pathology in multiple sclerosis (Smith et al., 1999)
	IRS2 in pancreatic beta cells (Li et al., 2008)	IRS2 is anti-apoptotic in beta cells (Li et al., 2008)
	NRF2 in mouse mammary epithelial cells	NRF2 helps cells from Parkinson's disease patients to resist oxidative stress (Cook et al., 2011) and its absent enhances the damage caused by MPTP (Innamorato et al., 2010)
c-Jun (Seijffers et al., 2007) in neurons		Important regulator of neuronal survival following axotomy (Raivich et al., 2004)
	ATF3 $\Delta$ Zip2 indirectly attenuates the anti-apoptotic effects of NF- $\kappa$ B signaling in rodent cell lines by displacing a positive cofactor from target genes (Hua et al., 2006)	

Sample citations to indicate the significance of individual genes/pathways are given. As can be seen from the cases of CHOP, DR5, and BBC3/PUMA, ATF3 can regulate genes in opposite directions in different types of cells and under different conditions. Unfortunately, few of the data refer to neurons or glial cells.

the finding that ATF3 is not expressed in the developing mouse cortex at embryonic day 15.5 (Dr S. Rakic, UCL, unpublished microarray data) when such events are underway.

Preliminary experiments on sciatic nerve regeneration using ATF3 mutants (Hossein-Ibrahim, unpublished) showed no significant difference in the time taken for the return of the toe spreading reflex or the return of the response to the von Frey hair stimulation of the footpad compared to that in control animals. Studies of neurite outgrowth from DRG neurons *in vitro* also show

no obvious defect (Seijffers et al., 2006). Interestingly, ATF3 can still be detected in the nuclei of denervated Schwann cells and injured neurons using a commercially available antibody from Santa Cruz Biotechnology (Seijffers et al., 2006; Hossein-Ibrahim, Mason and Anderson, unpublished). This suggests that parts of the ATF3 protein, including those required for nuclear localization, are still expressed and coincides with a presence of a 17-kDa ATF3-immunoreactive protein on Western Blots (Seijffers et al., 2006). However, it is uncertain whether this was a truncated form

of ATF3 and, if so, whether it retains biological activity. It is not clear how an in-frame sequence can be generated when exon B is eliminated from the mouse ATF3 gene. The mutation has effects in non-neural cells (Hartman et al., 2004; Jiang et al., 2004; Whitmore et al., 2007; Akram et al., 2010). In some of these studies ATF3 has been reported to be absent from Western blots of the mutant cells, even with the Santa Cruz antibody (Hartman et al., 2004; Jiang et al., 2004). However, to our knowledge, no studies which have reported a phenotype for the mutant cells have reported immunohistochemically detectable ATF3 in cell nuclei, as is the case for injured neurons and Schwann cells. There is one report (Bernstein et al., 2011) of effects of the mutation on cultured neurons (CHOP levels were reduced in 6-OHDA – treated dopaminergic neurons) but the preliminary data make it unlikely that any effects of this mutation on axonal regeneration will be profound.

## CONCLUSION

Although ATF3 is now widely used as marker for axotomized neurons, the complex interactions of ATF3 with other transcription factors make an assessment of its role in the injured nervous system difficult. ATF3 has been shown to be an adaptive response gene which allows cell death and cell cycle pathways in non-neuronal cells to be modulated to produce an appropriate response to mainly

stressful stimuli. Its expression in many tissues is associated with tissue dysfunction and disease progression (Hai, 2006) but in neurons its expression is usually correlated with the responses to injury that lead to axonal regeneration and neuroprotection. One problem with understanding the role of ATF3 in the nervous system is that most studies of ATF3 function have been carried out on cultured non-neuronal cells or neuronal cell lines. It is not all clear whether ATF3 will have the same functions in such cells as in post-mitotic neurons.

The difficulties with the existing strain of knockout mice (Seiffers et al., 2006) have delayed a full evaluation of the role of ATF3 in the nervous system *in vivo* and the status of ATF3 expression in those animals requires clarification. Other gain of function and loss of function studies have demonstrated a modest role for ATF3 in driving the expression of other growth-related and neuroprotective genes but the generation of more sophisticated conditional knockouts in neurons, glial cells, and immune system cells will be required to elucidate all the functions of ATF3 in the nervous system.

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- could be construed as a potential conflict of interest.

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