Activation and function of immediate-early genes in the nervous system

Beatriz Pérez-Cadahía, Bojan Drobic, and James R. Davie

Abstract: Immediate-early genes have important roles in processes such as brain development, learning, and responses to drug abuse. Further, immediate-early genes play an essential role in cellular responses that contribute to long-term neuronal plasticity. Neuronal plasticity is a characteristic of the nervous system that is not limited to the first stages of brain development but persists in adulthood and seems to be an inherent feature of everyday brain function. The plasticity refers to the neuron’s capability of showing short- or long-lasting phenotypic changes in response to different stimuli and cellular scenarios. In this review, we focus on the immediate-early genes encoding transcription factors (AP-1 and Egr) that are relevant for neuronal responses. Our current understanding of the mechanisms involved in the induction of the immediate-early genes is presented.

Key words: neuronal plasticity, immediate-early gene expression, AP-1, Egr-1, mitogen- and stress-activated kinase.

Introduction

The nervous system has been studied since well before the 20th century (Kandel and Squire 2000). Currently it is described as a highly sophisticated and complex system. The neuron, the hallmark of communication, is one of the most specialized cell types. However, the complexity is acquired through the existence of a large variety of neuronal subtypes that are anatomically, biochemically, and physiologically integrated via complex circuits that constitute the structural axis for brain function. The existence of hundreds of types of synapses and over a hundred known neurotransmitters, many of them having multiple types of receptors, is also necessary to yield normally functioning neurological processes.

Neuronal plasticity is another outstanding characteristic of the nervous system. It is not limited to the first stages of brain development but persists in adulthood and seems to be an inherent feature of everyday brain function (Loebrich and Nedivi 2009). The plasticity refers to the neuron’s capability of showing short- or long-lasting phenotypic changes in response to different stimuli and cellular scenarios (Hughes and Dragunow 1995; Loebrich and Nedivi 2009). Neuronal responses to stimuli can be classified as early and late responses. The early response occurs rapidly after stim-
ulation and lasts from milliseconds to minutes. During this process, a first messenger interacts with cell surface receptors and activates the second messenger system composed of protein kinases that phosphorylate certain neuronal proteins to execute the appropriate neuronal response. Phosphatases act in conjunction with the kinases to ensure the neuronal response is short-lived. Conversely, the late responses last from hours to days and can even result in permanent changes under certain circumstances within the neuron. Importantly, the late neuronal response requires changes in gene expression and has been related to processes such as learning, memory formation, and drug tolerance and sensitization (Hughes and Dragunow 1995).

The number of proteins involved in transcriptional activation in mammalian neurons has been estimated to be between 1000 and 10,000, and a substantial fraction of them are inducible transcription factors (Herdegen and Leah 1998). Their role in regulating transcription within the neuronal system is crucial both during development and in the adult. Development is accompanied by the expression of a large number of genes, more than half encoding transcription factors, required to regulate cellular replication and differentiation in precise temporospatial order (He et al. 1989). In the adult nervous system there is a constant expression of inducible proteins. Many effector genes are up- or down-regulated, with some of these being rapidly induced (e.g., immediate-early genes, IEGs) while others are expressed with a delay (Herdegen and Leah 1998). It has been proposed that these fine-tuned mechanisms couple the cell surface events to the long-term coordinated changes in gene expression that give rise to alterations in neuronal function. Evidence to support this model came from studies reporting synaptic rearrangements involving changes in gene expression during long-term memory formation and consolidation, conditioning, imprinting, and learning (Friedman et al. 1992; Robertson 1992; Kaczmarek 1993). Stress, neurodegeneration and neuronal cell death, and the behavioral changes associated with drug administration have also been related to marked and long-term changes in neuronal chemistry being mediated by increased production of neuropeptides and receptor proteins (Besse et al. 1992; Noguchi et al. 1992; Pellegrini-Giampietro et al. 1992). Therefore, all of the above-mentioned processes require reprogramming of gene expression and de novo protein synthesis (Davis and Squire 1984; Matthes 1989). The first genes activated, linking membrane events and the nucleus, are the IEGs (Beckmann and Wilce 1997). Since their discovery, an increasing number of studies have demonstrated important roles for this class of genes in regulating the dramatic phenotypic changes in neurons (Dijkmans et al. 2009). Alterations in the expression of IEGs were at first considered a part of a general neuronal response to natural stimuli — a result of normal synaptic activity. However, it has become clear that each IEG-encoded protein can be individually regulated in different brain regions via different stimuli (Beckmann and Wilce 1997).

IEGs are a class of genes rapidly and transiently induced by a large number of stimuli. Induction occurs within minutes in the absence of de novo protein synthesis. Regulated expression of IEGs is essential for the cell, as IEG products can in turn activate downstream targets and typically function as part of a network of constitutively expressed proteins. Indeed, mutation of some of these IEGs, like their viral relatives, can cause cell transformation (Herdegen and Leah 1998; Lanahan and Worley 1998). IEGs encode many functionally different products such as secreted proteins (e.g., cytokines and chemokines), cytoplasmic enzymes (the phosphatase 3CH134/MKP-1/Erp and the cyclooxygenase COX-2), ligand-dependent transcription factors (NGFI-B/Nur77/N10), and inducible transcription factors (Jun, Fos, Krox, Myub, Myc, NGFI-C, SRF). A library of neuronal rapid response genes was cloned and comprised more than 500 genes, including some already-characterized IEGs and genes encoding inducible transcription factors (Nedivi et al. 1993). Most attention has been paid to the IEG products acting as transcription factors, as they regulate the expression of the so-called delayed response genes (Yamamoto and Alberts 1976).

Therefore, it is clear that IEGs play an essential role in cellular responses that contribute to long-term neuronal plasticity, and their study at the molecular and biochemical levels will provide information on the processes leading to modifications of synaptic function (Lanahan and Worley 1998). Numerous studies have focused on different brain sections and different cellular environments and stimuli (Mokin and Keifer 2005), but the literature is quite disperse. In this review we focus on the IEGs encoding transcription factors that are relevant for neuronal responses.

**Transcription factors**

Transcriptional regulation by IEGs is quite complex. IEGs themselves encode a diverse array of factors that act in a combinatorial fashion to differentially affect a distinct second wave of gene expression (Loebrich and Nedi 2009). However, their activation of a late response transcriptional program can vary according to the differentiation and physiological state of the stimulated cell and is not necessarily restricted to proliferative genes. Moreover, multiple signals and intracellular pathways can influence IEG-mediated transcriptional activation via an assortment of cis- and trans-acting elements, adding more layers of complexity to IEG regulation of transcriptional activation.

**AP-1**

The AP-1 (activating protein 1) was identified more than two decades ago (Lee et al. 1987; Angel et al. 1988), and retroviral homologs of some of its components were found even earlier (van Straaten et al. 1983). However, the biological relevance and physiological functions of AP-1 and its components are still being elucidated (Shaulian and Karin 2001). Although AP-1 transcription factors are uniquely positioned in the mitogenic signal transduction cascade, they also transduce multiple signals from cellular stress, growth factors, steroid hormones, and inflammatory cytokines (Eferl and Wagner 2003).

At the molecular level, the transcriptional regulator AP-1 is a large family of dimeric protein complexes. The canonical AP-1 members are Jun proteins (c-Jun, JunB, and JunD) and Fos proteins (c-Fos, Fra-1, Fra-2, and FosB) (Chinenov and Kerppola 2001; Mechta-Grigoriou et al. 2001), which share the common feature of two adjacent highly conserved domains: the leucine zipper (mediating di-
merization) and a basic DNA-binding region (Basbous et al. 2008).

Jun family members can form both homo- and heterodimers among themselves, with Fos, with members of the ATF family, and with other transcription factors such as C/EBP, NFAT, MyoD, and c-rel (Herdegen and Leah 1998; Raivich 2008). Individually, c-Jun exhibits the highest activation potential, while JunB homodimers are almost inactive and only activate transcription from promoters containing multiple AP-1/TRE sites (Ryseck and Bravo 1991). Fos family members are not able to form homodimers. Besides the basic leucine-zipper region for dimerization and the DNA-binding region, c-Fos and FosB harbor a C-terminal transactivation domain. Fra-1, Fra-2, and FosB2 lack this region and therefore an inhibitory function of these factors on AP-1 activity has been proposed (Wisdom et al. 1992). However, it has been suggested that these Fos proteins, especially Fra-1 and Fra-2, might be involved in the progression of many tumor types (Milde-Langosch 2005). Therefore, mechanisms underlying transactivation seem to be more complex, and AP-1 functions and cellular consequences are not completely understood. On the other hand, because of the apparently widespread induction of AP-1 and its contribution to both basal and stimulus-activated gene expression (Young and Colburn 2006), many researchers initially questioned the specificity and importance of AP-1 for neuronal function. Currently, it is assumed that the way in which a small number of IEGs (AP-1 members) can be involved in such a large number of processes with high specificity is through the combinatorial control that leads to the formation of a vast number of different homo- and heterodimeric combinations with distinct regulatory properties and transcriptional abilities (Angel and Karin 1991; Hughes and Draganow 1995; Whitmarsh and Davis 2000). Differential dimerization of AP-1 family members results in great differences in the transactivation potential owing to different DNA binding affinities, dimer stability, and transactivation capacities (Shaulian and Karin 2001). Dimerization of c-Jun with c-Fos further increases c-Jun's transcriptional capacity through formation of a more stable dimer (Kouzarides and Ziff 1988), while heterodimerization with JunB attenuates it (Chiu et al. 1989). Thus, it is the dimer composition together with the promoter architecture, as well as the interaction with other transcription factors and co-activators, that provides the high degree of specificity (van Dam and Castelazzi 2001).

Regulation in the nervous system

The constitutive expression of AP-1 members within the central nervous system is low (Hughes et al. 1992), but AP-1 expression can be induced by various agents such as neurotransmitters, growth factors (e.g., EGF, NGF, FGF, PDGF), and depolarization caused by KCl (Curran and Morgan 1985; Greenberg et al. 1985; Bravo et al. 1987). Several layers of regulation act coordinately to ensure the tight control of AP-1 expression. The activity of Jun and all Fos family members is modulated by posttranslational phosphorylation, connecting the transcription factors to the cellular signaling cascades. MAPK, CDK, PKA, and PKC influence AP-1 phosphorylation, which in turn influences dimer stability, DNA-binding activity, and the transactivation potential (Hurd et al. 2002).

Upstream promoter region architecture is also an important source of regulation, mainly because of the existence of different AP-1-like binding sites at the upstream promoter region of many genes. However, dimer composition seems to be the major factor in regulation of gene expression in the nervous system. Most of the evidence comes from studies in the last decade showing that JunB and JunD are poor transactivators and may act to dampen the response to the potent transactivator c-Jun (for review see Angel and Karin 1991; Herdegen and Leah 1998; Young and Colburn 2006). Furthermore, the formation of Jun–Fos heterodimers appears to be mostly responsible for initiating the induction response, while c-Jun homodimers are involved in its maintenance (Angel and Karin 1991). It is currently believed that dimer composition is not determined by a single change in the AP-1 binding proteins available for potential dimerization, but is rather the result of a complex and continually evolving group of changes in these proteins and their expression pattern. Through this temporal variation, different networks of target genes may be hierarchically activated and (or) repressed, ultimately having effects on the progressive and long-lasting changes required for the correct functioning of the nervous system (Moratalla et al. 1996).

AP-1 activation and functions in the nervous system

Since Morgan and Curran (1987) reported the seminal observation that c-Fos is rapidly induced in brain neurons by seizures, IEGs have been considered the vehicle leading to long-lasting changes in neurons. Overall, it appears that the different IEG-encoded proteins appear only during specific periods and (or) in specific cell types during embryonic and postnatal neural development. Their levels generally then decline to low basal levels in adults, although in a few areas of the nervous system they remain elevated (Herdegen and Leah 1998).

The main scenarios in which AP-1 members seem to play a role are cell death and apoptosis, memory formation, and behavioral changes related to drug exposure and withdrawal. The role of c-Jun with regard to cell death and apoptosis is not easy to envision. Some studies have related c-Jun expression with UV-induced cell death through promotion of the exit from p53-imposed cell cycle arrest (Shaulian et al. 2000). In the nervous system, strong expression of the c-Jun gene and protein is known to precede or coincide with periods of intense cell death during development or following trauma, brain ischemia, and seizures (Gall et al. 1990; Wessel et al. 1991; Raivich et al. 2004; Sun et al. 2005). Similar induction also occurs in human neurodegenerative diseases such as Alzheimer’s dementia and amyotrophic lateral sclerosis and following exposure to neurotoxic chemicals such as MPTP, which causes degeneration of dopaminergic neurons in the substantia nigra and thus provides a model of Parkinson’s disease (Migheli et al. 1997; Oo et al. 1999; Pearson et al. 2006). In vitro, withdrawal of trophic support through removal of neurotrophins or high, depolarizing concentrations of potassium causes rapid neuronal cell death. Withdrawal of nerve growth factor (NGF) leads to a rapid
accumulation of N-terminal phosphorylated c-Jun protein and Jun-dependent up-regulation of the Bel2-proapoptotic family member Bim (Whitfield et al. 2001) and ATF3 (Mei et al. 2008). Overexpression of c-Jun induces cell death in PC12 cells (Raivich 2008). However, conflictive results on the role of c-Jun have been obtained, as other researchers studying axotomy of peripheral neurons have suggested that Jun and Fos proteins may play a role in initiating a regeneration program in neurons (Leah et al. 1991; Jenkins et al. 1993). Peripheral neurons regrow axons after axotomy and this process has been reported to be initiated by c-Jun and JunD, eventually leading to the expression of regenerative proteins (Hughes and Dragunow 1995). Expression of Jun proteins may be initiated by the absence of transported growth, survival, or maintenance factors. When axons are regenerated and target cells are re-innervated, Jun expression disappears (Leah et al. 1991). Supporting evidence for this is the observation that NGF reduces c-Jun expression in axotomised peripheral neurons (Raivich 2008). In this scenario it has been suggested that the biological effect of AP-1 members might depend on the different time courses of AP-1 expression in dying versus surviving neurons, as prolonged but not brief expression of c-Jun was required for the DNA-activating ability of Fos (Hughes and Dragunow 1995).

A remarkable feature is the induction of c-Fos in response to noxious stimulation in the nervous system (Hunt et al. 1987). Since this discovery, considered a landmark in neuroscience, c-Fos protein expression has been used for mapping nociceptive pathways and for examining factors that modulate their activity. The current conception is that long-term changes in neuronal chemistry and electrophysiology after noxious stimuli are mediated by inducible transcription factors that direct the increased production of neuropeptides and receptor proteins (Ruda et al. 1988; Rukenstein et al. 1991; Noguchi et al. 1992). In this regard it has been shown that c-Fos antisense oligonucleotides reduce noxious stimulus-induced synthesis of both Fos and enkephalin and produce a behavioral hyperalgesia (Hunter et al. 1995).

AP-1 proteins are also induced after seizures, although with a pattern of expression that depends on the seizure-provoking agent. After systemic application of a seizure-mimicking agent, many genes are induced in the rat brain (more than 3000) encoding membrane-bound ion channels, differentiation- and growth-related proteins, and membrane-, vesicle-, and synapse-related proteins (Nedivi et al. 1993). The first increases in inducible transcription factor mRNAs are detectable in the dentate gyrus, with the strongest appearance of JunB, c-Fos, and Krox-24 (Lanaud et al. 1993) occurring within 10–15 min. Within the next 30 min mRNA signals are enhanced in the hippocampus and cortex (White and Gall 1987; Cole et al. 1990). In general, the amount of mRNA declines to basal levels within 1–4 h. The effect of repetitive seizures is striking, with a reduction in the expression of c-Fos, c-Jun, JunB, and Krox-24 after chronic daily electroconvulsive seizures ranging from 20%–30% to almost complete disappearance (Cole et al. 1990). Another interesting observation is that with repeated seizures the affinities and the composition of AP-1 bound proteins change compared with those after an acute seizure, as there is an increase in Fra proteins (Hope et al. 1994a). Thus, the behavior of inducible transcription factors in these processes is complex. Not much is known about the mechanisms underlying their induction. Studies have shown the involvement of JNK-2 and ERK cascades (Asanuma et al. 1995), but more research is needed to elucidate the exact roles of IEGs.

Experimental evidence has shown that both constitutive and inducible transcription factors mediate the long-term alterations in gene activity necessary for conditioning, imprinting, learning, and memory (Robertson 1992; Kaczmarek 1993). Expression of the IEGs is induced by behavioral training and is thought to play an important role in long-term memory. Furthermore, it has been known for decades that protein synthesis inhibitors can affect memory retention in mammals (Flexner et al. 1963). IEG expression even under resting conditions is not random (Marrone et al. 2008). c-Fos is induced in about 50 different brain areas. However, once again little is known about the mechanisms acting in this cellular context and studies developed to date have shown conflicting results. Long-term potentiation has been reported to be accompanied by the expression of inducible transcription factors that interact with promoter regulatory elements of downstream effector genes (Cole et al. 1989; Richardson et al. 1992; Davis et al. 2000). Conversely, other reports have shown no correlation (Abraham et al. 1991) or even an inverse correlation between them (Schreiber et al. 1991; Herdegen and Leah 1998). One of the few studies focusing on the underlying mechanisms found that forced swimming induced c-Fos expression in dentate neurons through the activity of MSK1 (Chandra-mohan et al. 2008). The effect of MSK1/2 gene deletion on c-Fos induction was specific to the dentate gyrus, as c-Fos induction was normal in other parts of the brain. A similar requirement of MSK1 phosphorylating histone H3 at serine 10 was observed for Jun induction in striatal neurons in response to glutamate treatment and through the ERK signaling pathway (Brami-Cherrier et al. 2007) (Fig. 1). Learning-related phenomena induced c-Fos by 3-fold, though basal levels were restored after 2 h (Tischmeyer and Grimm 1999); similar observations were made for Fos and FosB proteins (Jeffery et al. 1990; Demmer et al. 1993). The fact that the majority of neurons (96.5%) that express Fos also express the gamma protein kinase C iso-enzyme (PKCγ) suggests that a connection may exist between learning and Fos expression (Ambalavanar et al. 1993).

Another cellular scenario where IEGs have been shown to play a major role is during the processes derived from exposure to psychostimulants. The long-lived behavioral abnormalities associated with drug addiction are characterized by stable changes in neuronal plasticity. At the molecular level, AP-1 members have been proposed to confer permanence to drug-induced changes in gene transcription, RNA and protein processing, and synaptic structure (Nestler 2001). Induction of Fos family members (together with c-Jun) after acute cocaine and amphetamine exposure (proteins peaking at 2–3 h after drug administration and returning to control levels by about 4–8 h) (Malaplate-Armand et al. 2005) has been proposed to be one of the prime events of a complex signaling cascade leading to cocaine-induced neuronal adaptation and behavioral alteration (Brami-Cherrier et al. 2009). Strikingly, IEGs seem to be more tightly regulated than genes en-
coding proteins participating in the pathways activated after drug exposure. Hope and co-workers showed that an acute challenge with cocaine 1 day after chronic administration no longer induces the mRNAs encoding c-Fos, the Fras, JunB, and Egr-1, while it does induce almost all of the proteins involved in the pathways leading to IEG induction (Hope et al. 1992; Hope 1998). This might indicate either down-regulation of an activating pathway (i.e., via the serum response element or SIS-inducible element sites in the promoter of c-fos) or up-regulation of a repressing pathway (Hope 1998). On the other hand, this observation also indicates that IEGs respond differently after acute and chronic psychostimulant administration, and the alterations in brain function are different. Behavioral sensitization by chronic psychostimulant administration is accompanied by changes in gene expression leading to neuronal adaptation (Nestler et al. 1993). Interestingly, after repeated daily administration of amphetamine or cocaine, the induction of Fos and Jun mRNAs and proteins is attenuated (Nestler et al. 1993), but the AP-1 binding remains unchanged (Cole et al. 1995). In addition, the half-life of the AP-1 complex seems to be increased by 50-fold, coinciding with an increase in synthesis of Fos-related antigen (Fra) (Moratalla et al. 1996). This specific behavior of IEGs gave rise to the concept of the chronic AP-1 complex, associated with long-lasting changes in synaptic efficacy and structural changes. Chronic exposure to psychostimulants results in a gradual long-term accumulation of novel Fos-related proteins, termed “chronic Fras”. A gradual change in the composition of the AP-1 complex would replace the earlier, shorter-lasting Fra proteins with less rapidly induced, longer-lasting Fras (Turgeon et al. 1997; Valjent et al. 2000). Such accumulation has been observed for cocaine, morphine, amphetamine, alcohol, nicotine, and phencyclidine, as well as natural reinforcers such as food, drink, sex, and social interactions (Hope et al. 1998).
After chronic treatments the chronic Fras were found only in those regions where the same acute treatment induced c-Fos and the acutely induced Fra proteins (Hope 1998). Studies trying to characterize the chronic AP-1 complex identified that there are at least four chronically induced 35–37 kDa Fra-immunoreactive proteins (Hope et al. 1994b). A later study postulated that the chronic 35 kDa Fra is immunologically related to the spliced variant of FosB, ΔFosB (Nye and Nestler 1996). ΔFosB has a low induction during acute administration of drugs but it accumulates after chronic treatment and remains elevated even after weeks of withdrawal owing to its unusually high stability (Hope et al. 1994b; Nestler et al. 2001; Brami-Cherrier et al. 2009). The ΔFosB isoform is considered the longest-lived adaptation known to occur in the adult brain, lasting 1–2 months after drug withdrawal. Its stability, mainly due to its predominant dimerization with JunD (Chen et al. 1995), provides a novel molecular mechanism mediating the long-lived changes in neuronal morphology and synaptic structure associated with chronic drug exposure, such as the increase in the density of dendritic spines that parallels the enhanced behavioral sensitivity to cocaine (Robinson and Kolb 1997). However, many questions are still unsolved. ΔFosB was originally established as a transcriptional repressor, but later studies in cell culture showed that it can either induce or repress AP-1-mediated transcription, depending on the AP-1 site used (Nestler et al. 2001). Depending on the promoter, full-length FosB can exert the same transactivation effect as ΔFosB or the opposite effect. Although it has been shown that JunB and JunD are preferentially complexed with the chronic Fras during long-term adaptive processes, the exact composition of the dimers and their dynamics need further investigation (Chen et al. 1995).

Conflicting results related to the AP-1 response to chronic drug exposure have been obtained for Fra-2. Beauvais and colleagues showed Fra-2 induction upon both methamphetamine and cocaine treatment in the mouse striatum, supporting previous observations (Nye et al. 1995; Pennypacker et al. 2000; Beauvais et al. 2010). Conversely, another study identified Fra-2 as the common component in the response of U373 MG cells to chronic amphetamine and cocaine treatments but showed a decrease rather than an increase in its basal levels (Malaplate-Armand et al. 2005). The correlation between the decrease in Fra-2 levels and an increase in AP-1 DNA-binding activity was suggested to be due to the constitutive Fra-2 protein antagonizing AP-1 transcriptional activity, as Fra-2 complexes have low transcription potential and could act as a negative regulator (Nankova et al. 2000). The possibility that factors such as brain region, cell type, or stimuli influence Fra-2 behavior still awaits to be elucidated.

Egr

Early growth response (Egr) proteins constitute another well-characterized family of IEG-encoded transcription factors. Egr proteins are classified as Cys2His2 zinc finger motif-containing proteins (Beckmann and Wilce 1997). The family comprises five members: Egr-1 (also known as NGFI-A, Krox-24, Zif268, and TIS8), Egr-2 (also known as Krox-20), Egr-3, Egr-4 (also known as NGFI-C and pAT133), and the product of the Wilms’ tumor gene, WT-1 (Beckmann and Wilce 1997). All the members of the Egr family recognize the GSG motif (GCG(G/T)GGGCG) and through its binding they exert their transactivation effects (Crosby et al. 1991; Chavrier et al. 1988).

AP-1 and Egr transcription factors are often activated in parallel and are capable of interacting with each other in the regulation of late response genes. However, they are considered two different “gateways” to discrete programs of late response gene expression, as they differ in terms of structural properties and regulatory mechanisms. The coordinated induction of transcription factors and the differences in their temporal profiles and the sets of genes they activate allow for the ordered and complex array of transcriptional control signals necessary for the induction and repression of late response genes over time (Beckmann and Wilce 1997).

Egr-1 was first isolated from NGF-treated PC12 cells (Milbrandt 1987). Subsequent studies reported its high basal expression in the brain, although with regional differences (Waters et al. 1990; Mack et al. 1992). Nevertheless, Egr-1 induction showed a good correlation with c-Fos induction, indicating that its expression also reflects neuronal activity (Herdegen et al. 1995; Hiroishi et al. 1999). Dopamine D1, AMPA, and NDMA receptors have been implicated in the basal control of Egr-1 mRNA expression (for review see Beckmann and Wilce 1997). Egr-2, Egr-3, and Egr-4 are also present at different levels of basal expression in several regions of the rat brain. Egr-2 shows a different pattern of expression than the other family members both in the cerebral cortex and at the cellular level (Egr-2 present not only in the nucleus but also in the cytoplasm) (Mack et al. 1992). Expression of WT-1 has not been detected in the brain (Call et al. 1990).

Several factors have been described that modulate the transactivating activity of Egr family members. The first one is the presence of activation–repression domains. Four regions capable of stimulating transcription have been described for Egr-1 and two have been described for Egr-2, while no studies have yet reported activation domains for Egr-3 or Egr-4 (Beckmann and Wilce 1997). Furthermore, two repression domains for Egr-1 and Egr-2 have been described as functional and proposed to play an important role in the regulation of the function of Egr-1 and Egr-2 in vivo (Svaren et al. 1996). A second regulatory mechanism, considered the main control step, is Egr posttranslational phosphorylation. Egr phosphorylation increases the half-life of the protein (Cao et al. 1992) and its DNA-binding activity (Cao et al. 1993). Casein kinase II and tyrosine kinase are the enzymes proposed to mediate Egr-1 phosphorylation (Russo et al. 1993). Egr-1 phosphorylation by casein kinase II reduces its transactivating ability without modulating its DNA-binding activity (Jain et al. 1996). This occurs through protein kinase C phosphorylation of an inhibitory domain that disrupts the binding of a cellular inhibitory factor (Russo et al. 1993). N-glucosylation in the second zinc finger, regulating the DNA-binding activity, has also been reported for Egr proteins (Müller et al. 1991). Another layer of regulation is related to Egr cross-talk with AP-1 members. Fos is able to regulate Egr-1 by interacting with the serum response element (SRE) in the regulatory region of the
Egr-1 gene (Gius et al. 1990). In addition, the presence of a sequence similar to an AP-1 binding site immediately adjacent to the 3’ SREs of the Egr-1 and Egr-2 genes, at exactly the same relative position as the AP-1 binding site located close to the c-fos SRE, has been reported (Piette and Yaniv 1987). Since this AP-1 binding site is likely to be involved in c-fos auto-repression (Sassone-Corsi et al. 1988), it is possible that genes for Egr-1, Egr-2, and c-Fos, and maybe other immediate-early serum response genes, may share not only positive regulatory elements but also negative ones (Janssen-Timmen et al. 1989).

With regard to Egr induction, studies have reported changes in Egr expression in response to a wide variety of stimuli. Physiological stimulation, stress, brain injury, and ischemia have all been shown to stimulate Egr-1 mRNA expression (Mack et al. 1992; Beckmann and Wilce 1997). Egr family member involvement in synaptic plasticity during memory consolidation and reactivation of consolidated fear memories has also been reported (Thomas et al. 2002; Bozon et al. 2003). Numerous studies have shown Egr-1 to be regulated by seizure activity, electroconvulsive shock, kindling and pathological stimuli, brain injury, neurodegeneration, and apoptosis (reviewed in Hughes and Dragunow 1998). Parallel induction of Egr-2, Egr-3, and Egr-4 has also been reported (Honkaniemi et al. 1995). Interestingly, structural differences of Egr-2 with regard to the other family members seem to entail differences in function (for review see Beckmann and Wilce 1997). During long-term potentiation, Egr-2 mRNA follows a time course of up-regulation and decay similar to that of Egr-1 mRNA, although the Egr-2 protein product outlasts that of Egr-1 (Williams et al. 1995). In addition, data regarding Egr-2 induction in response to injury and ischemia are not conclusive, with Egr-2 induction being dependent on the cell type (nerve vs. non-nerve cells) and some studies showing no induction of Egr-2 (Herdegen et al. 1993; Collaço-Moraes et al. 1994).

A close relationship between drug intake or withdrawal and Egr induction has also been reported. Amphetamine and cocaine increase Egr-1 mRNA expression through dopamine D1 receptors in several brain regions (Moratalla et al. 1992). Cocaine injection has also been reported to induce Egr-3 in the striatum (Yamagata et al. 1994) and amphetamine or alcohol withdrawal induces the expression of Egr-2 and Egr-1, mediated by NMDA and GABA_A receptors (Beckmann and Wilce 1997).

Although the exact physiological roles of Egr family members remain unclear, the lethality of Egr-2 deletion in the germ line suggests an essential role for Egr-2 during development. It was proposed that the knockout lethal phenotype was due to a failure of the late events in myelination of peripheral nerves, indicating the first defined absolute requirement for a member of the Egr transcription factor family in vivo (Beckmann and Wilce 1997). Conversely, homozygous deletion of Egr-1 does not lead to any abnormality in embryonic stem cells or mice carrying a knockout germ line (Lee et al. 1995). It has been suggested that Egr-2 may compensate the lack of Egr-1. Indeed, Egr-1 was proposed to play a role in plasticity events in the adult rather than during development, as it was shown to be essential for expression of the low-affinity NGF receptor after injury (Nikam et al. 1995). Further, knockout studies of Egr-3 and Egr-4 are needed and may provide information on the possible redundancy of the Egr family members.

Among some other functions, a pro-survival role for Egr-1 has been recently proposed. During processes of somatic neurodegeneration, reductions in the level of Egr-1 were observed; this suppression was confined to dying neurons. Conversely, increased levels of FosB or ΔFosB were found in degenerating axonal endings and synaptic contacts (de Olmos et al. 2009). Suppression of Egr-1 during neuronal death suggests that Egr-1 is required for cell survival, through regulation of downstream target genes (de Olmos et al. 2009). To date, only a few neuron-specific genes, such as neurofilament phenylethanolamine N-methyltransferase and synapsin I genes, have been shown to be under the control of Egr proteins, although the list is likely to be enlarged shortly with data coming from microarray experiments (Beckmann and Wilce 1997).

**MSK and chromatin remodeling of the IEG regulatory region**

Nuclear DNA is packaged into nucleosomes, the basic repeating structural units in chromatin. The nucleosome consists of a histone octamer, arranged as an (H3–H4)_2 tetramer and two H2A–H2B dimers, around which DNA is wrapped. The core histones (H2A, H2B, H3, H4) have a similar structure with a basic N-terminal domain, a globular domain organized by the histone fold, and a C-terminal tail. The N-terminal tails emanate from the nucleosome in all directions and are available to interact with linker DNA, nearby nucleosomes, or other proteins. Core histones undergo a variety of reversible posttranslational modifications (PTMs), including acetylation, methylation, and phosphorylation. Core histone PTMs function to alter chromatin structure and (or) provide a “code” for recruitment or occlusion of nonhistone chromosomal proteins to chromatin (Delve et al. 2009; Espino et al. 2005; Hake et al. 2004; Jenuwein and Allis 2001; Taverna et al. 2007). Thus the core histone PTMs are involved in the remodeling of chromatin to either facilitate or hinder transcription.

In postmitotic neurons the ERK signaling pathway is essential to synaptic plasticity and long-term potentiation, two of the major cellular mechanisms underlying learning and memory (Sweatt 2004; Thomas and Huganir 2004). Activated ERK will phosphorylate and activate MSK1, which in turn will result in the transient phosphorylation of the H3 tail (at serine 10 or serine 28) and IEG induction (Fig. 1). The mechanism by which MSK-induced H3 phosphorylation leads to IEG transcription has been recently reported (Drobic et al. 2010).

MSK1 is in a complex with BRG1 (the ATPase subunit of the SWI/SNF chromatin remodeling complex), PCAF (an H3 lysine acetyltransferase), Jun, the p65 subunit of NF-κB, and 14-3-3 (Davie et al. 2010). The MSK1 complex is recruited to IEG promoters by transcription factors such as ELK-1, which recruits MSK1 to Fos and Egr-1 gene promoters (Zhang et al. 2008; Shimada et al. 2010). The recruited MSK1 complex will phosphorylate histone H3 at either serine 10 or serine 28 of nucleosomes positioned over...
the upstream promoter regions of IEGs. The 14-3-3 protein, particularly the isoforms ε and ζ, binds to H3S10ph and H3S28ph, with the affinity of binding being S28ph > S10phK14Ac > S10ph (Macdonald et al. 2005; Winter et al. 2008). 14-3-3 in the MSK complex may facilitate the recruitment or retention of the MSK complex to the phosphorylated H3 tails of IEG promoter nucleosomes (Macdonald et al. 2005; Vicent et al. 2008). The recruited SWI/SNF remodels nucleosomes at the promoters of IEGs, enabling the binding of transcription factors and RNA polymerase II and the initiation of transcription. Inhibition of MSK activity with H89 or knockdown of MSK expression reduces or eliminates the induced expression of the IEGs, demonstrating the importance of MSK1 in IEG expression (Drobic et al. 2010).

Concluding remarks

Since the first identification of stimulus- and region-differential induction of IEGs in the nervous system, numerous studies have investigated their function and association with neuronal plasticity. Over 500 rapid response genes have been reported in the neural system, and investigators have proposed that the encoded proteins act as mediators, linking nerve cell membrane events to the neuronal genome. Nowadays, it is clear that not only short-term but also long-term changes in the brain occurring in response to alterations in the cellular and extracellular environment are dependent on new protein synthesis. Activation of IEGs is involved in this process, as they code for proteins at very different cellular levels: several IEGs encode transcription factors, which are key factors in the reprogramming of gene expression, while several others connect signaling cascades to cellular structural machinery.

IEGs have important roles in processes such as brain development, learning, and responses to drug abuse. Some IEG products such as Arc, Fos, and Egr-1 are now widely used as markers of neuronal activation and plasticity during memory formation (Lanahan and Worley 1998; Loebrich and Neubacher 2003). However, further research on IEG products will provide major insights into the program of gene expression leading to the long-term effectors of neuronal stimulation, the molecular mechanisms engaged in distinct forms of learning, and the molecular pathways leading to abnormal neuronal morphology and function associated with neurodegenerative processes and mental disorders.

Acknowledgements

This work was supported by the Canadian Institutes of Health Research (ROP-100446), CancerCare Manitoba Foundation, Inc., Canadian Cancer Society Research Institute, Canada Breast Cancer Foundation, Manitoba Health Research Council, a Canada Research Chair to J.R.D., a “Programa Angeles Alvaríno” from Xunta de Galicia award to B.P., and a Canadian Cancer Society Research Institute Terry Fox Foundation studentship to B.D.

References


Piette, J., and Yaniv, M. 1987. Two different factors bind to the α-domain of the polyoma virus enhancer, one of which also inter-
acts with the SV40 and c-fos enhancers. EMBO J. 6(5): 1331–1337. PMID:3038517.


This article has been cited by:


