Fos–Jun and the Primary Genomic Response in the Nervous System

Possible Physiological Role and Pathophysiological Significance

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Introduction

A rapidly growing body of literature has accumulated in the last five years on the role of gene expression as a target of stimulus transduction in cells of the nervous system. Understanding the role of receptor-mediated generation of intracellular messengers and events in the coupling of cell stimulation to gene activation is currently an active area of research, particularly as the problem applies to neurobiology. Genomic targets of neurotransmitters, or of depolarizing stimuli, include a set of genes that are characterized by rapid, stimulus-mediated induction of expression. These genes are called *immediate-early* or *primary response* genes, and the most prominent and best-studied genes of this group include the *fos* and *jun* proto-oncogenes. It is our purpose in this review to establish a common ground for molecular neurobiologists interested in stimulus-induced gene expression in the nervous system. In this process, we additionally intend to discuss the possible role of metabolites accumulated at the onset of brain ischemia and convulsions as mediators of inducible gene expression in the nervous system. In particular, our recent finding that the glycerophosphocholine platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3 phosphocholine), a cellular metabolite that accumulates in experimental neural injury, activates transcription of the primary response genes *fos* and *jun* in neuronal cell culture suggests that this lipid may participate in the coupling of neuronal membrane events to modulation of gene expression. Moreover, since PAF is accumulated in the brain during ischemia and convulsions, we discuss in this review the possible involvement of this bioactive lipid in transcriptional activation during pathophysiological conditions.

The Primary Genomic Response

When cells are stimulated by growth factors, differentiation-inducing agents, or by depolarization, signal transduction mechanisms converge on their cytoplasmic and nuclear substrates, and generate and amplify an appropriate cellular event or response. In the nucleus, these second messengers' activities converge on and stimulate expression of a small group of genes called primary response genes (PRGs; reviewed in Herschman, 1989). The PRGs are characterized by rapid induction of their respective mRNAs and proteins. Induction of PRG mRNA transcription occurs in the presence of the protein synthesis inhibitor cycloheximide, indicating that the transcriptional machinery necessary for rapid PRG induction is poised, awaiting stimulation through the transduction of extracellular stimuli. The regulatory sequences necessary for induction through second messenger systems have been identified for some of the PRGs (as will be described for *fos* and *jun* in the following sections), and the occurrences of some regulatory sequence motifs in different PRGs are indicators of coordinate regulation. We shall refer to a cell's complement of inducible PRGs as the *primary genomic response*. Genes of the primary genomic response are the first targets of the stimulus transduction machinery of the cell, and activities of their protein products are presumably responsible for secondary stimulation of the genome, and the consequent and specific long-term response to stimulation.

Stimulus–Transcription Coupling

A number of PRGs have been cloned to date, and the predicted peptide structures of the majority of these genes contain structural motifs that suggest their role as modulators of transcription. The rapid, inducible expression of such transcriptional modulators as a response to ligand–receptor interactions led Curran and Morgan (1987; Curran et al., 1989) to formulate the idea of *stimulus–transcription coupling*, the transduction of extracellular signals to long-term genomic responses and cellular adaptation. The characteristic rapid induction of the primary genomic response and synthesis of its

transcription factor PRG products following cell stimulation are hypothesized to act as a physiological generation of *third messengers*, coupling cell surface phenomena to the regulation of genes ultimately responsible for the cell's phenotypic long-term response to stimulation. In the nervous system, long-term responses of cells may contribute to such phenomena as differentiation, plasticity, learning, and regeneration.

The Fos–Jun Paradigm

Expression of *fos* is associated with the traversal of cells from quiescence into the cell cycle, the induction of terminal differentiation in cells and withdrawal from the cell cycle, and the increased activity of mature, nondividing cells, such as macrophages and neurons. These phenomena have been referred to collectively as *cell activation* (Kaczmarek and Kaminska, 1989).

Expression of *fos* is characteristically rapid and transient in response to various cell activating agents (reviewed in Cohen and Curran, 1989). *fos* mRNA and protein (Fos) coordinately appear and disappear within hours after cell activation. Fos is a 55 kD protein that localizes in the cell nucleus and binds chromatin (Renz et al., 1987). Fos is highly and variably posttranslationally phosphorylated, and the degree of phosphorylation is inversely related to its ability to bind DNA (Muller et al., 1987). In vitro, Fos stimulates transcription of genes that possess the AP-1 transcription factor binding site (consensus sequence 5'-TGACTCA-3'; Rauscher et al., 1988), and presumably Fos functions in this manner at the genomic level. This transcriptional regulatory function is imparted upon Fos by its heterodimeric association with the 39 kD protein product of the *jun* proto-oncogene (Jun; Chiu et al., 1988). Fos and Jun are integral components of AP-1 transcription factor binding complexes, as are members of a large group of proteins called *fos*-related antigens (FRAs), nuclear peptides that are immunoreactive with Fos antisera (Franza et al., 1988). To date, genes for several FRAs have been cloned: *fra*-1 (Cohen and Curran, 1988), *fra*-2 (Matsui et al., 1990), and *fos* B (Zerial et al., 1989), all of which are inducible at the mRNA level with kinetics similar to that of *fos*, and at least the protein products of *fra*-1 (Fra-1; Cohen et al., 1989) and *fos* B (Zerial et al., 1989) are capable of heterodimeric interactions with Jun. Two cloned *jun* homologues, *jun* B (Ryder et al., 1988) and *jun* D (Ryder et al., 1989), are expressed with different kinetics than *jun*: whereas *jun* B is inducible, *jun* D is actually a constitutively expresssd member of the *jun* family. In addition, the protein products of *jun* B and *jun* D can form heterodimers with Fos, but the Fos–Jun B heterodimer, unlike other Fos–Jun combinations, is a transcription repressor (Chiu et al., 1989; Schutte et al. 1989). In addition, members of the Jun family of PRGs can form homodimeric transcription factor complexes in vitro, whereas members of the Fos family must heterodimerize with members of the Jun family for activity. Thus, Fos and Jun are prototypical, stimulus-inducible transcription factors, and the apparent combinatorial mechanism of Fos and Jun proteins suggests a differential specificity at the genomic level in response to cell activation.

Second Messenger Stimulation of fos and jun

The immediate transcriptional induction of *fos* and *jun* is mediated by nuclear substrates of intracellular signal transduction activities (Fig. 1). The 5' regulatory region of human fos has been extensively characterized (reviewed in Prywes et al., 1989). Sequences regulating *fos* transcription include: a serum-responsive element (SRE) that mediates the induction of *fos* by growth factors and proteins of the Ha-*ras* (Fukumoto et al., 1990) and *raf* (Jamal and Ziff, 1990) proto-oncogenes; an AP-1-like sequence that mediates induction by phorbol esters, presumably through protein kinase C; and a cyclic AMP-responsive element (CRE) that is coincident with the nucleotide sequence necessary

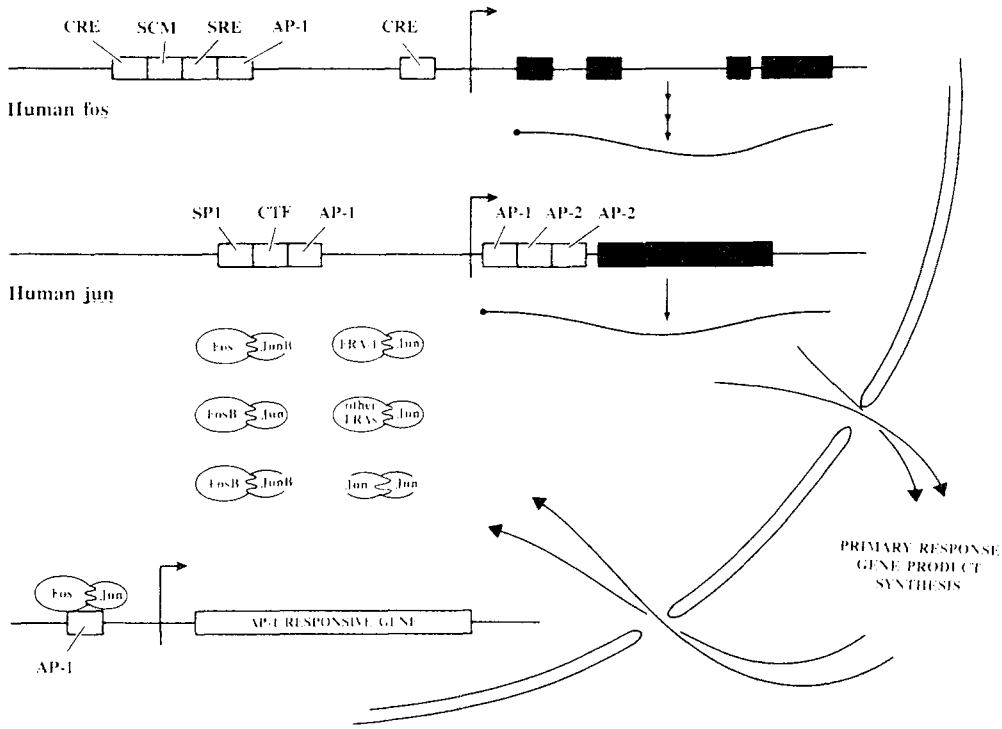

Fig. 1. Transcriptional regulation of and by human *fos* and *jun* primary response genes. Sequences conferring inducibility of human *fos* and *jun* are represented schematically. Some regulatory elements are common to other PRGs in addition to *fos* and *jun*. Comparison between human sequences and sequences of rat and mouse, animals used in the experiments described in the text, reveals a general conservation of these regulatory sequences, suggesting that regulation of these PRGs in different species is similar. Transcriptional induction by substrates of second messenger activities is followed by rapid cytoplasmic messenger RNA translation, protein synthesis, and return transport to the nucleus. The diagram includes examples of different Fos and Jun family complexes that have been described in the literature. FRA-1 and Fos B have not been described in neural tissue to date. The Fos and Jun complexes contribute to transcriptional regulatory activity at AP-1 regulatory elements. CRE, cyclic AMP responsive element; SCM, *sis*-conditioned medium responsive sequence; SRE, serum-responsive element; AP-1, activator protein-1 binding sequence; SP1, binding site for cellular transcription factor Spl; CTF, CAAT-sequence binding factor; AP-2, activator protein-2 binding site. Sequence information derived from Prywes et al., 1989 (*fos*) and Angel et al., 1988 (*jun*).

for induction by Ca^{2+}. Transcription factor footprint analysis of the human *jun* regulatory region (Angel et al., 1988) has revealed in vitro binding sites for several cellular transcription factors, including Spl; CAAT sequence transcription factor, CTF; AP-1; and AP-2, a factor that mediates induction through phorbol esters and stimulators of cyclic AMP. Interestingly, one AP-1 site and two AP-2 sites occur downstream of the transcription start site in untranslated sequence. Transcription of *jun* is neither serum-responsive nor cyclic AMP-responsive, and corresponding SRE and CRE sequences are not apparent in the *jun* promoter. However, *jun* B and *fos* B are serum-responsive (Ryder et al., 1988).

Permutations of Fos–Jun Complexes

Since both *fos* and *jun* possess the necessary regulatory sequences to which their protein

products bind as components of the AP-1 complex, the possibility of autoregulation of these two genes has been the focus of several studies. Jun has been shown to regulate expression of its own gene positively through its most upstream AP-1 site (Angel et al., 1988). This contrasts with the presumptive autoregulation of *fos* expression, which is negative (Sassone-Corsi et al., 1988; Schonthal et al., 1989; Shaw et al., 1989; Konig et al., 1989; Lucibello et al., 1989) and which involves an AP-1-like sequence near the SRE. The AP-1 site in *fos* is capable of imparting positive regulation of the gene when factor binding to the SRE is abolished (Shaw et al., 1989).

As mentioned previously, members of the Fos and Jun families function as transcriptional regulators by forming DNA-binding complexes that are dimeric in nature; whereas members of the Jun family can form homodimers as well as heterodimers with Fos, Fra-1, and Fos B, those members of the Fos family require dimerization with Jun proteins for function. Two common structural features of these proteins are important for transcriptional regulatory activity. First, a *leucine zipper* structure, comprised of regularly spaced leucine residues on a common α-helical surface, provides an interface for dimerization. The amino acid sequences of members of the Fos and Jun families predict leucine zipper structures, and the specificity of dimerization and DNA-binding affinity is presumably imparted by differences in primary structure between these leucine residues and beyond the zipper structure on the dimerization interface (Kouzarides and Ziff, 1989). Second, a basic, presumably α-helical region adjacent to the leucine zipper imparts the interaction of dimers to nucleotides of AP-1 sequences (Neuberg et al., 1989; Kouzarides and Ziff, 1989). The sequence information of respective leucine zippers allows formation of Fos–Jun heterodimers and Jun homodimers (Halazonetis et al., 1988). Both Fos and Jun participate in the DNA-binding activity of the heterodimer (Abate et al., 1990; Hirai et al., 1990).

Other Components of the Primary Genomic Response in Neuronal Tissues

Expression of several other PRGs has been examined in neural tissue. The sequence of NGFI-A, cloned from PC12 cells induced with nerve growth factor and cycloheximide (Milbrandt, 1987) predicts Zn^{2+}-finger domains common to certain transcriptional regulatory proteins. Identical cDNAs have been cloned as *zif/268* (Christy et al., 1988), *Egr-1* (Sukhatme et al., 1988,), and *Krox24* (Lemaire et al., 1988). NGFI-B (Milbrandt, 1988) is a member of the steroid–thyroid hormone receptor family, and is also known as *nur77* (Hazel et al., 1988).

Lim et al. (1987) have cloned a family of PRGs inducible by the phorbol ester TPA. Some of these TPA-inducible sequences (TIS) are identical to other named PRGs. Namely, TIS28 is identical to *fos*, as is TIS8 to NGFI-A and TIS1 to NGFI-B.

As with Fos and Jun, the protein products of these other PRGs are predicted to have nuclear functions as transcriptional regulators. In the sections to follow that describe the demonstration of components of the primary genomic response in various tissues and cells of neural origin, we will refer to the PRG sequences (e.g., NGFI-A vs *Egr-1*) as they occur in the cited references.

The Primary Genomic Responses of Neural Tissues

In the following sections, we summarize studies published since the first demonstrations of NGF induction of *fos* in PC12 cells (Curran and Morgan, 1985; Greenberg et al., 1985; Krujier et al., 1985). The larger portion of the reviewed data derives from *fos* hybridization and immunocytochemical analysis of its protein product, since *fos* DNA probes and Fos antisera were in use before the cloning of *jun* or other

PRGs. Very recent reports involve detection of FRA peptides and *jun*-like mRNAs as well as mRNAs of other PRGs expressed as a response to neural activity.

Membrane Depolarization and Receptor-Mediated Events Trigger fos Expression

Expression of the *fos* proto-oncogene can be induced by a variety of membrane-associated events, including depolarization and induction of mitogenesis and differentiation (Curran and Morgan, 1987). Induction of PC12 pheochromocytoma cell differentiation by nerve growth factor (NGF) is associated with an early and transient increase in *fos* expression (Curran and Morgan, 1985; Greenberg et al., 1985; Krujier et al., 1985). This increase can be dramatically enhanced in the presence of certain benzodiazepines (Curran and Morgan, 1985). Induction of *fos* is also stimulated by either elevated K^+ or veratridine-induced, Ca^{2+}-sensitive Na^+ channel activity (Morgan and Curran, 1986). The NGF-benzodiazepine superinduction of *fos* is apparently Ca^{2+}-independent. Induction of *fos* in PC12 cells by NGF and other growth factors results in a Fos protein with extensive posttranslational modifications, whereas calcium channel-mediated induction gives rise to a less extensively modified protein (Curran and Morgan, 1986). A zinc finger-encoding primary response gene, *Egr-1*, is transcriptionally induced in PC12 cells by NGF, K^+-induced depolarization, and benzodiazepines with kinetics similar to that of *fos*, suggesting that *Egr-1* and *fos* may share common transcriptional regulatory elements (Sukhatme et al., 1988). Nicotine activation of acetylcholine receptors in differentiated PC12 cells also stimulates *fos* expression in a Ca^{2+}-dependent manner (Greenberg et al., 1986). The upstream regulatory sequence that confers Ca^{2+} inducibility upon the *fos* gene has been localized to a region between nucleotides -62 and -54 relative to the transcription start site (Sheng et al., 1988). PC12 cells induced to differentiate by the interaction of interleukin 6 with its specific cell surface receptor similarly exhibit induction of *fos* (Satoh et al., 1988).

Bartel et al. (1989) have demonstrated that membrane depolarization and growth factors activate distinct patterns of PRG expression in PC12 cells. NGF or epidermal growth factor induces *fos*, *jun*, and *jun* B in PC12 cells, but membrane depolarization by nicotine or by K^+ activates expression of *fos* and *jun* B only. These findings suggest that the *fos* protein may form transcriptional complexes with different Jun cofactors in response to different cell surface stimulation and that the specificity of the genomic response to stimulation may be transduced by these differential combinations of Fos and Jun proteins.

Expression of *fos* is elicited in vivo in the rat hippocampus by intracerebral injection of either glutamate or noradrenaline (Kaczmarek et al., 1988). Acute morphine treatment induces *fos* mRNA and protein in rat caudate-putamen, presumably through μ-type receptors, and induction is completely abolished by the morphine antagonist naloxone (Chang et al., 1988). L-DOPA activates *fos* in the rat striatum, nucleus accumbens, and parts of the neocortex ipsilateral to a 6-hydroxydopamine lesion of the substantia nigra (Robertson et al., 1989). Peripherally, β-adrenoreceptor stimulation by isoproterenol in the mouse submandibular gland results in an induction of *fos* mRNA and protein (Barka et al., 1986).

Gubits et al. (1989) have observed that intraperitoneal injection of vehicle in the absence of drug induced a transient, fourfold increase in rat brain *fos* mRNA levels, and this response was potentiated by the injection of the presynaptic α_2-adrenoreceptor antagonist yohimbine. Both α_2-agonist clonidine and β_2-antagonist propranolol inhibited the induction of *fos* by yohimbine, suggesting (1) that β-adrenergic stimulation mediates the induction of *fos* in response to the stress of intraperitoneal injection, and (2)

that the prolonged synaptic presence of norepinephrine potentiates the induction.

The etherphospholipid platelet-activating factor (PAF; reviewed in Braquet et al., 1987), enhances Ca^{2+} influx in neural cells (Kornecki and Ehrlich, 1988) and accumulates in brain following injury (Kumar et al., 1988). PAF presumably acts as an extracellular paracrine or autocrine factor, but some evidence suggests that PAF synthesized by activated cells may have an intracellular function (Henson, 1987; Marcheselli et al., 1990b). In SH-SY5Y neuroblastoma cells, exogenous PAF induces the expression of both *fos* and *jun* as well as transcription from a poly-AP-1 promoter, and the induction of *fos*, *jun*, and the AP-1-mediated transcription is abolished by a PAF antagonist (Squinto et al., 1989a).

In primary cultures of rat cerebellar granule cells, L-glutamate elicits a dose-dependent increase in the steady-state level of *fos* mRNA followed by an accumulation of Fos antigen in cell nuclei (Szekely et al., 1987, 1989). This induction is inhibited by the isosteric glutamate receptor antagonist 2-amino-5-phosphonovalerate and by two noncompetitive, allosteric NMDA-sensitive glutamate receptor antagonists, Mg^{2+} and phencyclidine. Glycine, the positive allosteric modulator of NMDA-sensitive receptors, potentiates the glutamate response. Kainate and quisqualate failed to induce *fos* in these cells. However, kainate- and quisqualate-type receptors are associated with *fos* expression in other cells: kainate induces seizure-associated *fos* expression in rat hippocampus (Popovici et al., 1988), and quisqualate induces differentiation-associated *fos* expression in primary glial cell cultures (Condorelli et al., 1989).

Areander et al. (1989a) have used muscarinic and adrenergic agonists in cultured rat astrocytes to stimulate the expression of several TIS genes (Lim et al., 1987). Carbachol induces the rapid induction of *fos* and five other TIS genes in an atropine-sensitive manner that is potentiated by lithium. Both β- and α_1-adrenergic agonists, but not α_2 agonists, stimulate expression of the TIS genes through cyclic AMP and protein kinase C mechanisms, respectively. The *fos* gene possesses regulatory sequences necessary for transcriptional induction by both of these second messengers. Induction of TIS genes by dibutyryl cyclic AMP and forskolin in these cells is associated with rapid stellation resembling the in vivo differentiated phenotype, whereas TIS gene induction by EGF, FGF, TPA, and ganglioside GM_1 is associated with cell proliferation (Areander et al., 1989b). Interestingly, GM_1, which induces biological and morphological alterations in PC12 cells similar to those induced by NGF, could not induce TIS gene expression in PC12 cells. EGF and FGF apparently saturate a common mechanism for inducing TIS expression and DNA synthesis, and this mechanism is distinct from the TPA-responsive mechanism (Areander et al., 1989c). Both cycloheximide and benzodiazepines superinduce *fos* and the other TIS genes in astrocytoma cells (Areander et al., 1989c), and similar superinduction is observed in PC12 cells (Curran and Morgan, 1985; Kujubu et al., 1987). Thus, in rat astrocytes, the expression of *fos* and TIS genes is associated both with induction of proliferation, and induction of growth inhibition, and seems to be a transcriptional response to specific ligand-receptor or second messenger activity.

Nerve Growth Factor and fos

The association of *fos* expression with the neurotrophic agent nerve growth factor (NGF) was demonstrated in 1985 (Curran and Morgan, 1985; Greenberg et al., 1985; Krujier et al., 1985): *fos* mRNA and protein in rat PC12 cells are inducible by NGF. PC12 cells can be induced to differentiate by NGF into nondividing cells resembling sympathetic neurons, phenotypically characterized by neurite extension and increased electric excitability and neurotransmitter release (Krujier et al., 1985). Stimulation of *fos* occurs rapidly, peaking at 30 min after NGF

treatment, and may be a consequence of protein kinase C activation in these cells (Chan et al., 1989). Interestingly, *fos* induction by NGF was enhanced 100-fold in the presence of benzodiazepines, and this superinduction resulted in an increase in posttranslational modification of *fos* protein. Although the mechanism through which benzodiazepines enhance the effect of NGF is unclear, the concentrations of the drug used to superinduce *fos* were sufficient to modify NGF-induced phenotypic changes in PC12 cells, suggesting that *fos* is important in mediating induction of PC12 cell differentiation. NGF action in PC12 cells is apparently cell-cycle specific, with induction of differentiation occurring concomitantly with *fos* stimulation in the G_1 phase (Rudkin et al., 1989). Transcription of *jun* is also induced in PC12 cells by NGF (Wu et al., 1989). High levels of expression of the *fos* oncogene, on the other hand, block NGF-induced PC12 cell differentiation (Ito et al., 1989).

Induction of *fos* by NGF in vivo closely parallels the anatomical distribution of *fos* induced by tissue lesioning. Cortical lesions or injections of NGF induce Fos immunostaining in neuronal nuclei of the ipsilateral neocortex and amygdala, but not adjacent structures such as the hippocampus (Sharp et al., 1989). That NGF mimics the effect of cortical lesioning in these experiments supports the idea that trophic factors, such as NGF, released at sites of injury in the brain, diffuse throughout the hemisphere and induce Fos in distant cells. Interestingly, NGF receptors have not been described on neurons of the neocortex, but have been described in the hippocampus where no *fos* protein was detected. Therefore, the response of *fos* expression to cortical injury may mediate the effect of trophic factors on recovering neurons.

NGF regulates gene expression by at least two mechanisms: rapid induction of transcription and posttranscriptional increases in mRNA half-life (Cho et al., 1989). In PC12 cells, the former mechanism predominates, and regulatory sequences upstream of the *fos* gene that confer responsiveness to NGF have been defined (Visvader et al., 1988). Distinct nucleoprotein complexes bind the SRE and a second element, the SRE-2, which occurs 20 base pairs downstream of the SRE. These two sequences mediate induction of *fos* expression by NGF, and confer inducibility independently of one another, suggesting that distinct intracellular mechanisms may transduce the response to NGF and converge on the *fos* promoter to activate transcription.

In contrast to the inducing effect of NGF on *fos* expression, Fos may be involved in inducing the expression of NGF (Mochetti et al., 1989). Stimulation of β-adrenergic receptors in rat C6-2B astrocytoma cells by the agonist isoproterenol induced *fos* mRNA within 15 min and NGF mRNA by 3 h. Intense Fos immunoreactivity was observed *in situ* at 3 h. The accumulation of Fos protein inhibited by the protein synthesis inhibitor cycloheximide also inhibited the accumulation of NGF mRNA. 2-Aminopurine, a protein kinase inhibitor that inhibits induction of *fos* mRNA, reduced the accumulation of NGF mRNA. These results suggest that Fos regulates NGF transcription. The production of cyclic AMP stimulated by isoproterenol may be responsible for the induction of *fos* through the CRE. The temporal relationship between *fos* and NGF mRNA syntheses suggests that the NGF gene is a target of an inducible Fos-AP-1 complex in astrocytoma cells. Further evidence that NGF is a target of Fos is provided by Hengerer et al. (1990). In fibroblasts from transgenic mice carrying an exogenous, inducible *fos* gene, an AP-1 site in the first intron of the NGF gene is occupied coordinately with induction of *fos* expression; additionally, induction of *fos* results in increased NGF transcription. These data provide direct evidence that Fos regulates transcription of the NGF gene; however, it remains to be demonstrated whether this type of interaction occurs in neuronal tissue in vivo, and whether unnatural levels of exogenous Fos induce aberrant transcriptional regulation.

fos and jun Expression in Differentiation and Development of the Nervous System

In PC12 cells, expression of *fos* is concomitant with induction of sympathetic neuron-like differentiation by NGF (Curran and Morgan, 1985; Greenberg et al., 1985; Krujier et al., 1985) or interleukin 6 (Satoh et al., 1988). However, agents without differentiating activity in PC12 cells, such as epidermal growth factor, phorbol esters, and elevated K^+, induce transcription of *fos* (Greenberg et al., 1985; Krujier et al., 1985), suggesting that *fos* expression may be necessary but not sufficient for expression of the differentiated phenotype. Dexamethasone-induced differentiation of PC12 cells into growth-arrested chromaffin-like cells is not accompanied by *fos* expression (Krujier et al., 1985). In addition, a 14-base oligodeoxynucleotide, complementary (antisensical) to a sequence of *fos* mRNA, was effective in reducing NGF-induced synthesis of the *fos* protein by about 50% in PC12 cells, but the extent and time frame of neurite outgrowth was unaffected (Kindy and Verma, 1988). These results suggest that *fos* expression is not an essential event in the induction of PC12 differentiation, but is a function of the type of cell surface signal involved in the stimulus to differentiate. Evidence that Jun–Jun and FRA-1-Jun dimers recognize AP-1 sites (Franza et al., 1988) suggests that the action of Fos can be compensated for by other inducible PRG products in cells where the expression of Fos is specifically antagonized. Molecular cloning of the remaining FRAs, determination of their transcriptional regulatory sequences, and, ultimately, selective antagonism of the expression of Fos, Jun, the FRAs, and other gene products with AP-1 activity will furnish the necessary answers. Furthermore, observation of neurite outgrowth as a phenotypic indicator of differentiation without regard to genotypic events is probably insufficient to exclude a role for Fos in differentiation.

An association of *ras* proto-oncogenes with *fos* expression and PC12 cell differentiation has been demonstrated in several studies. Micro-injection of activated *ras* protein into PC12 cells induces differentiation similar to the NGF-induced phenotype (Bar-Sagi and Feramisco, 1985; Hagag et al., 1986), and transient expression of a transforming *ras* gene, but not the normal c-Ha-*ras* gene, induces *fos* expression concomitant with PC12 differentiation (Sassone-Corsi et al., 1989). Transforming *ras* proteins increase transcription from and binding to an AP-1 sequence in transfected PC12 cells, and these activities are enhanced by transient coexpression of *jun* (Sassone-Corsi et al., 1989). In a PC12 subline stably transfected with a glucocorticoid-inducible *N-ras* gene, expression of *N-ras* induces NGF-like phenotypic differentiation without induction of *fos* expression (Guerrero et al., 1988). This suggests that the protein products of deregulated *ras* proto-oncogenes are capable of inducing neuronal differentiation of PC12 cells and, presumably through regulation of *fos*, induce differentiation through different AP-1 activity requirements.

Neuroblastoma SH-SYSY cells induced to differentiate to growth-arrested, neurite-expressing cells by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) also exhibit rapid induction of *fos* (Hammerling et al., 1987; Jalava et al., 1988). The TPA-induced expression of *fos* is inhibited by the protein kinase C inhibitor H-7 without inhibiting differentiation, and dioctanoylglycerol induces *fos* without inducing differentiation (Jalava et al., 1988). Thus, *fos* expression and neuroblastoma differentiation can be dissociated. Moreover, retinoic acid also induces SH-SY5Y differentiation without inducing *fos* (Hammerling et al., 1987). Therefore, it seems that in these neuroblastoma cells, as is the case in PC12 cells, *fos* expression is associated with certain second messenger stimulations and is apparently independent of neuron-like differentiation.

Measles virus in vitro and in vivo causes persistent infections specifically in cells of neuronal

origin, and the specificity of persistent infection suggests specific molecular interactions between viral and cellular macromolecules. In NS20Y neuroblastoma cells, persistent infection leads to an elevation of protein kinase C gene transcription and protein activity, and induces a considerably higher level of *fos* expression compared to uninfected cells (Wolfson et al., 1989). It is tempting to speculate that persistence of viral infections in the CNS and their episodic resurgences are caused by differential deregulation of *fos* or other genes. Indeed, some viruses encode gene regulatory activities that suggest regulation by AP-1 activity. For instance, both human immunodeficiency virus-1 (HIV-1; Franza et al., 1988) and Epstein-Barr virus (EBV; Flemington and Speck, 1990a) possess regulatory AP-1-like sequences that bind Fos–Jun complexes; the sequence for EBV occurs within a region that regulates the lytic switch. Furthermore, autoregulation of the lytic switch sequence occurs through binding of a viral protein homologous to Fos (Flemington and Speck, 1990b).

In primary rat glial cell cultures, *fos* expression is associated with activation of several second messenger systems (Condorelli et al., 1989). Epidermal growth factor, coupled to its receptor's intrinsic tyrosine kinase activity, and TPA, a potent activator of protein kinase C, both induce proliferation and DNA synthesis in these cells concomitant with induction of *fos* expression. In contrast, isoproterenol, the β-adrenoreceptor agonist that stimulates adenylate cyclase activity, and the glutamate receptor agonists quisqualic and ibotenic acid, which are coupled to inositol lipid hydrolysis, inhibit cell proliferation, induce cell morphological changes that suggest differentiation, and stimulate the expression of *fos*. Hence, in this study, expression of *fos* is associated with specific receptor stimulations, and is either independent of the proliferation induced by different receptor agonists or is a mediator of both proliferation and differentiation processes.

Inducers of differentiation and proliferation in cultured rat astrocytes are capable of inducing the expression of *fos* and several other TIS genes (Areander et al., 1989b,c). Agonism of the cyclic AMP mechanism in astrocytes transiently induces *fos* and TIS expression coordinate with morphological differentiation. Interestingly, ganglioside GM_1, which is mitogenic in astrocytes and induces TIS gene expression, induces neurite outgrowth in PC12 cells, but is incapable of inducing TIS expression.

Expression of *fos* is associated with stimulation of cell growth in vitro, and aberrant (constitutive) expression of *fos* is associated with several tumors of neural origin. Accumulation of *fos* mRNA was demonstrated in two benign tumors, a cystic cerebellar astrocytoma, and a ganglioma, and no *fos* mRNA was detected in the malignant glioblastomas and a medulloblastoma, whereas high levels of mRNA for the *myc* proto-oncogene are associated with all five tumors (Fujimoto et al., 1988). Similarly, adrenal pheochromocytomas (Goto et al., 1990) and thyroid carcinomas (Terrier et al., 1988) were found to exhibit constitutive levels of *fos* and *myc*. Thus, *fos* is associated with the maintenance or proliferative growth of these neuroectodermal tissues, and the apparent continual induction of *fos* may be responsible for the aberrant cell growth in these tumors.

Barka et al. (1986) injected isoproterenol intraperitoneally in mice, as an in vivo model to investigate the involvement of *fos* in tissue development, and determined *fos* expression in the submandibular gland. Development and function of the acinar compartment of the parotid and submandibular gland in the mouse are largely regulated by the autonomic nervous system, and adrenergic agonists stimulate DNA synthesis and cell division in these glands. Isoproterenol induces a rapid, transient increase in *fos* expression, but the expression is apparently independent of drug-induced cell proliferation and growth, since doses that induce maximal *fos*

expression are insufficient to induce DNA synthesis. The antagonist propranolol blocks the isoproterenol induction of *fos*, demonstrating that *fos* expression is a response to a specific ligand-receptor interaction.

Ruppert and Willie (1987) demonstrated dramatic inducibility of *fos* expression by simple mechanical disaggregation of mouse brain tissue. A maximal 40-fold increase in *fos* mRNA levels occurs 1 h after disaggregation of cerebella. Interestingly, *fos* could be induced only in disaggregated neonatal tissue and not in adult tissue. The age-dependent inducibility of *fos* expression corresponds to a neonatal phase of neural cell differentiation and proliferation, and these steady-state levels of *fos* may be contributed by both differentiating and proliferating populations of cells. A typical rapid and transient increase in *fos* expression occurs at birth in several tissues from mouse, but in brain, *fos* mRNA levels continue to increase for the first 5 d of neonatal development (Kasik et al., 1987). The methylation pattern of the *fos* gene locus in mouse changes dramatically at 1 mo after birth (Uehara et al., 1989), and these nucleotide modifications could be responsible for the apparent age-dependent expression of *fos* in mouse brain.

Elegant *in situ* studies by Caubert (1989) illustrate *fos* expression in the nervous system during different stages of development. Hybridization *in situ* with an ^{35}S-labeled, single-stranded RNA probe demonstrates high levels of *fos* throughout the brain and spinal cord on day 12 postcoitum. By day 16, the strongest *fos*-specific signals were observed in the anterior spinal cord, dorsal root ganglia, olfactory lobe, ganglion layer of the retina, cerebellum, and cortex. The presence of high levels of *fos* in the developing nervous system, as well as in other models of neural cell proliferation and differentiation, provides evidence for the importance of *fos* in regulating neuronal growth activities. Wilkinson et al. (1989) have demonstrated a localization of *jun* transcripts in the central nervous system of the developing mouse fetus; *jun* transcripts were detected by *in situ* hybridization in proliferating neuroepithelial cells of the ventricular layer of the telencephalon, but not in the adjacent layer of postmitotic cells. The location of *jun* transcripts in midbrain, hindbrain, and spinal cord sites suggests an association restricted to motor neurons. Expression of *jun* B, on the other hand, is limited to nonneural tissue in these experiments, and does not seem to be coexpressed with *fos*, indicating that the functional adjuncts of Fos exhibit tissue specificity, and may act independently with Fos through homodimerization.

Ashkenazi et al. (1989) have shown that muscarinic receptor activation stimulates DNA synthesis in primary astrocytes from perinatal rat brain, and is mitogenic in certain brain astrocytomas and neuroblastomas. This mechanism is apparently mediated by phosphoinositide hydrolysis, and the associated intracellular Ca^{2+} influx could conceivably be a trigger for the expression of growth-related genes such as *fos*. Induction of *fos* is a well-characterized phenomenon in cells stimulated with growth factors (Muller et al., 1984; Curran, 1988), and specific blockage of *fos* by antisense expression or antibody microinjection has been shown to result in cell cycle blockage (Riabowol et al., 1988; Nishikura and Murray, 1987). In addition, both differentiation and *fos* expression induced by NGF in PC12 cells are apparently G_1-specific (Rudkin et al., 1989). Persistent K^+-induced depolarization or NMDA receptor stimulation enhances both survival and maturation of murine cerebellar granule cells in culture, and these apparent neurotrophic effects correlate with increased Fos expression (Didier et al., 1989). The dual role of neurotransmitter receptor stimulation in both regulating cell growth and neurotransmission predicts a bimodal mechanism for ligand–receptor stimulation of *fos* expression in differentiating embryonic cells and, subsequently, in quiescent mature neuronal and glial cells.

Convulsions and Experimental Epilepsy Activate Expression of fos and Other Primary Response Genes

A dramatic and specific induction of *fos* in certain neuronal populations takes place in the brains of mice undergoing convulsions induced by Metrazole (Morgan et al., 1987). This surge in *fos* gene expression coincides with an increase in intracellular Ca^{2+} during seizure (Siesjö and Bengtsson, 1989). The anticonvulsants diazepam and sodium pentobarbital completely block the effects of Metrazole on *fos* expression, suggesting that seizure is the stimulus responsible for *fos* induction. Three hours after Metrazole injection, Fos was immunocytochemically localized in specific brain areas, including the pyriform cortex, the anterior olfactory nucleus, the dentate gyrus, and the hippocampus. Dragunow and Robertson (1987a) similarly find Fos immunoreactivity in the pyriform cortex and dentate gyrus, and induction is abolished by the benzodiazepine midazolam. Using immunoelectron microscopy to localize Fos after a single injection of Metrazole, Mugnaini et al. (1989) have localized Fos to areas of euchromatin in granule cell nuclei of the dentate gyrus. This contrasts with the localization of Fos immunoreactivity in hormone-stimulated nonneuronal cells in culture, where both euchromatin and heterochromatin regions are positively stained (Squinto et al., 1989b,c). Localization of Fos with such dispersed areas of chromatin in neuronal cells suggests that Fos associates with actively transcribed DNA sequences in the differentiated neuron and, presumably, participates in the regulation of those sequences. The high content of Fos immunoreactivity in dentate granule cell nuclei suggests that the protein can regulate expression of numerous genes within a single neuron. In addition to *fos*, mRNAs of three other PRG transcription factors, *jun*, *jun* B, and *zif/268*, accumulate in the hippocampus, dentate gyrus, and pyriform and cingulate cortices following Metrazole-induced seizures (Saffen et al., 1988). The local coexpression of Fos and different Jun-like peptides (e.g., in the dentate gyrus) suggests that composition of the AP-1 complex in response to seizure may be heterogeneous. The zinc finger-encoding PRG *Egr*-1 (identical to *zif/268*) is stimulated in brain following Metrazole treatment with kinetics similar to *fos* induction (Sukhatme et al., 1988). In seizures induced by lesion of the dentate gyrus, an 18-fold induction of preproenkephalin mRNA occurs and is preceded by a rapid induction of *fos* (White and Gall, 1987), suggesting that *fos* protein may be participating in the upregulation of preproenkephalin expression in response to seizure.

Sonnenberg et al. (1989a) have shown that total AP-1 DNA-binding activity increases, and remains elevated for 4–6 h in response to Metrazole-induced seizure, and combinations of the constituent Fos, Jun, and FRAs change during this period. Fos immunoreactivity appears for 2 h after induction in the rat hippocampus, and, subsequent to the disappearance of Fos, two FRAs, of masses 46 kD and 35 kD, are present for up to 8 h after seizure. Fos antisera, which recognize both Fos and the FRAs, disrupt AP-1 DNA-binding activity at all time points, indicating that the consecutive increases in Fos and the FRAs contribute to the protracted AP-1 activity in response to seizure. *Jun* mRNA levels are similarly increased over the 8-h postseizure period, suggesting that *jun* is a constant constituent of AP-1 that dynamically complexes with Fos and FRAs as they appear in the nucleus. In addition, benzodiazepine anticonvulsants are unable to block the increase in AP-1 DNA-binding activity *after* induction by Metrazole, suggesting that the program of Fos, Jun, and FRA expression induced by seizure is independent of ongoing neuronal activity. Similar studies using whole brain preparations from mouse (Sonnenberg et al., 1989b) have demonstrated that glutamate receptor agonists NMDA and kainate both stimulate expression of Fos and

FRAs as well as AP-1 activity, although with different kinetics; the NMDA response mimicked Metrazole induction, but the response of Fos to kainate was protracted. The large increase in *fos* mRNA in response to kainate corresponded to relatively low levels of Fos protein. This suggests that both kainate- and NMDA-type glutamate receptors stimulate coordinate induction of Fos and the FRAs in brain, but the induction at least of Fos protein occurs independently, as is evidenced by the protracted kinetics of its expression in response to kainate. The kinetics of Fos expression in response to both Metrazole and picrotoxin were similar, suggesting a mechanism in which Metrazole stimulates the picrotoxin site on the GABA receptor. Additionally, since NMDA antagonists partially block Metrazole-induced Fos expression, Metrazole-induced seizure may stimulate Fos expression through the temporal stimulation of different receptors, in this case GABA and NMDA receptors.

Brief, high-frequency stimulation of the perforant path in the rat, sufficient to induce long-term potentiation, selectively induces the immediate-early gene *zif/268*, and inconsistently induces *fos*, *jun*, and *jun*-B (Cole et al., 1989). This dissociation of *fos* expression from perforant stimulation and long-term potentiation has been studied by others (Douglas et al., 1988). The mRNA increases of these PRGs occur ipsilateral to stimulation, and are sensitive to NMDA-receptor antagonism.

Electrically induced seizure activity, which "kindles" or leads to a sustained increase in the response of the brain to seizures, rapidly induces Fos immunoreactivity in the nuclei of granule cells of the rat dentate gyrus (Dragunow and Robertson, 1987b). Kindling, however, does not seem to be maintained by aberrant expression of Fos (Dragunow et al., 1988). These findings were reinforced by the results of Douglas et al. (1988), which showed that long-term potentiation of dentate granule cells failed to induce the Fos expression elicited by repetitive electrical discharges of these cells. A strong Fos-like immunoreaction is also seen in granule cells of the dentate gyrus 3 h after treatment with the seizure-inducing glutamate analog kainic acid (Popovici et al., 1988). A single electroconvulsive shock in rat leads to widespread induction of *fos* in the limbic structures, hippocampus, and cerebellum (Daval et al., 1989).

Vitamin B_6 plays a vital role in neuronal cell function. *fos* mRNA and protein were induced in mouse brain in response to seizure induced by deoxypyridoxine, a B_6 antagonist that competes with the cofactor pyridoxal phosphate (Mizuno et al., 1989). Other antagonists, such as hydrazine, thiosemicarbazide, and penicillamine, which bind and derivatize pyridoxal phosphate, were not effective in inducing seizure-associated *fos* induction.

In an effort to help understand and standardize the use of Fos immunoreactivity as a measure of activity-stimulated brain metabolism, Dragunow and Faull (1989) have recognized a number of problems with immunolocalization of Fos:

1. Basal levels of Fos exist in neurons, and can be nonspecifically induced after behavioral stress.
2. Neuronal bursting is required to induce Fos levels in anesthetized animals.
3. Ketamine and barbiturates can block induction of Fos levels.
4. The time course of Fos appearance varies in different regions of the brain, and may not be inducible in some regions.

Cerebral Ischemia and Other Models of Neurotrauma Result in Enhanced fos Expression

Rats subjected to transient forebrain ischemia exhibit induction of *fos* mRNA in the cerebral cortex within 30 min of reperfusion (Onodera et al., 1989). A similar study (Jorgensen et al., 1989) demonstrates increased *fos* mRNA in the dentate hilus and in hippocampal area CA1 from 24

to 72 h after initiation of reperfusion. The findings in the latter study correlate well with the known time course of Ca^{2+} accumulation in CA1 following ischemia (Dienel, 1984), suggesting that accumulation of the cation is involved in *fos* induction.

In the Onodera et al. (1989) study, contribution from glial cells in response to ischemia could not be excluded. Dragunow and Robertson (1988) have shown that glial cells in white matter surrounding ischemic margins express Fos maximally 12 to 24 h after injury. Expression of *fos* is also observed in nerve cells of the damaged cortex. Rabiolabeled thymidine incorporation showed that the response of *fos* was followed by glial cell proliferation. NGF treatment can mimic the induction of *fos* in ipsilateral neurons of the amygdala and cortex following injury (Sharp et al., 1989), perhaps suggesting that a trophic response to injury occurs and is associated with the expression of *fos*.

A massive increase in *fos*-like immunoreactivity occurs in brains of anesthetized rats in response to whole-body heat shock (Dragunow et al., 1989). The time course of Fos expression is 1 to 24 h. The induction of Fos and Fos-like antigens following thermal stress is localized in white and gray matter regions of the brain, and is not detectable in neurons.

Antagonism of NMDA receptors by MK-801 prevents accumulation of Fos in dentate granule cells and neurons of the pyriform cortex ipsilateral to hippocampal lesions in rats (Dragunow et al., 1990). Inhibition of Fos expression was time- and dose-dependent, and MK-801 did not affect Fos accumulation in glia or ependyma. This positive correlation between Fos induction and NMDA receptor stimulation contrasts with the finding that MK-801 stimulates *fos* expression in thalamic and neocortical areas of the brain (Dragunow and Faull, 1990). One possible explanation for this *fos* induction is disinhibition of inhibitory neurons through NMDA receptors, resulting in cell activation.

These correlations between NMDA receptors and Fos expression are potentially valuable indicators of a role for Fos in neuronal survival. Didier et al. (1989) have demonstrated increased survival of cerebellar granule cells by K^+ and NMDA, and activation by both of these stimuli is associated with induction of a *fos* response. Competitive and noncompetitive NMDA antagonists protect against selective cell loss in the CA1 region of the hippocampus following transient forebrain ischemia (Swan and Meldrum, 1990) and against neuronal dysfunction following brain injury (Faden et al., 1989). Taken together, these results allude to an important role for Fos in mediating stimuli triggered through the NMDA receptor.

Sensory Stimulation Results in Transcriptional Activation of fos

Stimulation of cutaneous sensory afferents via application and injection of the irritant mustard oil or application of radiant heat to rat hindlimb results in Fos expression in the superficial layers of the dorsal horn of the spinal cord, which receive the primary afferents (Hunt et al., 1987). The induction of Fos by sensory input is apparently dependent upon the synaptic activity, the postsynaptic propensity for induction, or both, since axon depolarization is insufficient to induce expression. Noxious mechanical stimulation of rat hindlimb induces Fos in the ipsilateral superficial layers of the medial and dorsal horn, in the midline thalamus, the contralateral reuniens, and submedial and paraventricular nuclei (Bullitt, 1989).

Similar to the nociceptive stimulation described above, experimentally induced inflammation in the rat hind paw was followed by a rapid increase in mRNA levels of *fos*, preprodynorphin, and preproenkephalin in the ipsilateral dorsal spinal cord (Draisci and Iadarola, 1989). The increase in *fos* mRNA levels

is rapid and transient (30 min), and is followed by an increase in preproenkephalin mRNA at 2 h and preprodynorphin mRNA at 4 h. Preprodynorphin mRNA levels are sustained at about fourfold for 4 d. This temporal relationship suggests that *fos* may be involved in the stimulation of transcription of proenkephalin and prodynorphin genes during neuronal response to peripheral inflammation.

Sagar et al. (1988) demonstrated Fos immunoreactivity in the rat thalamus, cerebellum, pontine nuclei, and the globus pallidus following electrical stimulation of the rat hindlimb motor/sensory cortex. A 24 h, water deprivation induces Fos in the paraventricular and supraoptic nuclei, hypothalamic areas known to be activated by dehydration. Therefore, induction of Fos provides a postsynaptic marker of activated neurons.

A Membrane-Derived Lipid Mediator, Platelet-Activating Factor (PAF) Activates Transcription of fos and jun in Neuroblastoma Cells

Neuronal signal transduction often involves degradation of membrane phospholipids through selective receptor-mediated events, giving rise to metabolites that function as second messengers. The biologically active lipid mediator PAF accumulates during tissue injury, ischemia, inflammation, and immune reactions (reviewed in Braquet et al., 1987). In the nervous system, PAF seems to be involved in neuronal differentiation and with events linked to increased cellular calcium (Kornecki and Ehrlich, 1988). PAF biosynthesis is stimulated by certain neurotransmitters (Bussolino et al., 1986), and PAF affects release of neuropeptides and other hormones (Junier et al., 1988; Rougeot et al., 1990). PAF accumulates in several pathophysiological responses of the nervous system, and is implicated in neuronal degradation associated with injury or damage. PAF accumulates in the brain during seizures and during ischemia, and PAF antagonists decrease hippocampal neuron damage (Oberpichler et al., 1990), brain damage (Panetta et al., 1987), and neurochemical changes in ischemia (Spinnewyn et al., 1987; Panetta et al., 1987; Birkle et al., 1988) and convulsions (Birkle et al., 1988; Kumar et al., 1988). The increased concentration of PAF in brain under these conditions reflects early brain responses to stimulation or damage, and may be related to the accumulation of free polyunsaturated fatty acids (Bazan, 1970; Bazan et al., 1986). Additional studies are needed to establish whether these changes are coincidental, or whether there are common or sequentially affected metabolic steps. The physiological PAF precursor, alkyl-acyl-glycerophosphocholine, is enriched in arachidonoyl groups at *sn*-C_2 (Braquet et al., 1987). Because the major free fatty acids accumulated in brain during ischemia are arachidonic acid and docosahexaenoic acid, phospholipase A_2 activation is believed to be responsible for increase in both PAF and free arachidonate (Bazan, 1970; Bazan and Rodriguez de Turco, 1980; Bazan et al., 1986). Moreover, PAF is an activator of phospholipase A_2 (Braquet et al., 1987).

Although PAF receptors have not been clearly identified as molecular entities, it was recently found that the cerebral cortex, in addition to containing a synaptosomal PAF-binding site, contained two intracellular binding sites recoverable from the microsomal fraction (Marcheselli et al., 1990b). One of the intracellular sites displays the highest affinity for PAF of all cells and membranes studied to date. These observations, taken together with evidence of uptake and intracellular retention of PAF (Henson, 1987; Homma et al., 1987; Bussolino et al., 1986; Sisson et al., 1987), indicate a possible intracellular role of PAF.

At high concentrations, PAF exhibits detergent-like properties on membranes (Sawyer and

Anderson, 1989), and it is possible that local increases in PAF concentrations or local depletions in membrane-bound PAF precursors are responsible for altering membrane-mediated events through a nonreceptor mechanism. Such membrane events could include alteration of ion fluxes across membranes, both intra- and extracellular. Thus, the release of PAF from cellular membranes in response to stimulation or injury may function bimodally, through specific receptors or through membrane perturbations. Moreover, neuronal damage during epileptic and ischemic incidents or other injuries, and the corresponding cytoplasmic and genomic responses, may be partially accounted for by such dramatic alterations in cell membrane structure.

When PAF is added to SH-SY5Y neuroblastoma cells, it promotes a rapid and transient activation of *fos* and *jun* (Squinto et al., 1989a). The PAF antagonist BN 52021 inhibits this response, suggesting receptor involvement. The expression of these PRGs in MOLT-4 human T-lymphocytes is only minimally increased by PAF (Squinto et al., 1989a), in spite of the fact that *fos* transcription has been found to be activated in circulating leukocytes (e.g., monocytes; Ho et al., 1987).

In SH-SY5Y cells, PAF and phorbol esters seem to induce *fos* mRNA accumulation synergistically. The effects of PAF in these cells can be attributed to an effect at the transcriptional level, and transcriptional induction of *fos* by PAF requires both SRE and CRE sequences. Moreover, PAF activates transcription from an AP-1-linked reporter gene (Squinto et al., 1989a). Therefore, PAF may couple the responses to injury of the nervous system to gene expression, leading to the activation of PRGs and, subsequently, to activation of gene cascades involved in survival of certain neural cells.

PAF may effect induction of the primary genomic response through regulating calcium fluxes (Kornecki and Ehrlich, 1988) or, conceivably, through a membrane perturbation event (Sawyer and Anderson, 1989) that affects receptor and ion channel efficiency. In addition, PAF may act synergistically with stimulation of neurotransmitter and neuropeptide receptors to elicit a primary genomic response during neuronal cell activation. Possible in vivo stimulation of neuronal cells by PAF could result from an interplay between neurons and resident (microglia) or infiltrating leukocytes responding to injury. This concept is suggested by the interplay of leukocytes and vascular endothelia during trauma (Braquet et al., 1989). A direct effect of PAF on *fos*, *jun*, and AP-1-mediated transcription in SH-SY5Y cells suggests that PAF can act through a paracrine mechanism. However, we have recently demonstrated the importance of the high-affinity intracellular PAF-binding site in mediating the *fos* response to ECS (Marcheselli et al., 1990a).

It is possible that, by way of an interaction with a receptor, either on the cell surface or within the cell, PAF activates Fos and Jun expression along with a consequent activation of AP-1-responsive target genes. By such a mechanism, PAF and perhaps other lipid messengers may, by activating Fos–Jun/AP-1 transcriptional complexes, either positively or negatively affect the expression of key neuronal genes that participate in long-term changes in neural function, particularly during injury conditions when synthesis and accumulation of these lipids is a characteristic of pathophysiological events.

Physiological Role of fos and jun in the Nervous System: Targets of the AP-1 Complex

The usefulness of Fos–Jun expression in the study of cell activation in the nervous system is firmly established. In fact, an overview describing problems with, and suggesting remedies for, Fos immunocytochemistry has recently appeared (Dragunow and Faull, 1989). At the genomic level, determining patterns of gene expression activated by Fos–Jun provides an in-

creasingly provocative area of research. The recognition of and transcriptional activation from AP-1 sequences by protein products of the Fos and Jun families seem to provide a wide level of specificity essential for the fine regulation of genomic responses elicited in neuronal and glial cells by growth and maturation factors, neurotransmitters, and ionic conductivity. Results from several laboratories suggest that each combination of Fos, Jun, FRAs, and other AP-1 constituents imparts its own degree of specificity to recognition of AP-1-like sequences, and, alternatively, each complex may recognize different AP-1-like sequences. DNA sequences recognized by purified AP-1 activity vary, accommodating selective base changes and insertions at the center of the AP-1 site's dyad symmetry. In fact, an in vitro Fos-binding, negative-regulatory sequence of the *myc* proto-oncogene regulatory region is comprised of a 7-nucleotide, AT-rich segment flanked by 5'TGA and 3'TCA halves of the AP-1 consensus sequence (Hay et al., 1989). Several laboratories have demonstrated molecular interaction between Jun and the cyclic AMP-response element binding protein (CREB; Hoeffler et al., 1989; Benbrook and Jones, 1990; Ivashkiv et al., 1990; Sassone-Corsi et al., 1990). CREB, which mediates gene induction though cyclic AMP (Montimy and Bilezikjian, 1987; Yamamoto et al., 1988), can dimerize with Jun and recognize either cyclic AMP-responsive elements (CREs; 5'-TGACGTCA-3') or AP-1 sites (5'TGACTCA-3'). This convergence of second messenger systems at the level of transcriptional induction exposes the potential for those genes known to be cyclic AMP-responsive, including genes for preproenkephalin (Comb et al., 1986), somatostatin (Montimy et al., 1986), and tyrosine hydroxylase (Lewis et al., 1986), to be targets of inducible AP-1 complexes, and suggests an entirely new set of inducible transcription factor complex permutations as part of the primary genomic response. Inspection of the cloned 5' regions of both the tyrosine hydroxylase and preproenkephalin genes reveals the presence of sequences similar to the AP-1 consensus sequence in addition to respective CREs. In fact, Fos and Jun synergistically bind to and activate transcription from the proenkephalin promoter in vitro (Sonnenberg et al., 1989c). The regulatory regions of a number of neuropeptide transmitters contain the GCTGA sequence, which is recognized by AP-1 complexes (Hyman et al., 1988).

Interestingly, genes whose protein products are associated with neurodegenerative diseases, the Alzheimer's A4 amyloid precursor gene and the Scrapie PrP gene, contain AP-1 consensus sequences in their 5' regulatory regions (Salbaum et al., 1988; Basler et al., 1986). However, sequences of the promoters of A4 and PrP are characteristic of constitutively expressed genes, and hence, expression of these two genes may not be responsive to the inducible Fos–Jun signals. The promoter for the human NGF receptor gene also resembles a constitutively expressed promoter (Shimizu et al., 1988), and an AP-1-like sequence occurs. The long terminal repeats (LTRs) of several retroviruses contain potential AP-1 sites, including a negative-regulatory region in the LTRs of Human immunodeficiency virus-1 (HIV-1; Franza et al., 1988), a virus known to persist in brain (Kristensson and Norrby, 1986). We have recently demonstrated PAF-specific activation of transcription from the HIV-1 LTR in SH-SY5Y neuroblastoma cells (Squinto et al., 1990).

Temporal relationships between *fos* expression and other genes in neuronal cells suggest that these genes are part of an AP-1-mediated genomic program. PAF induces expression of the calcyclin gene coordinately with expression of *fos* in SH-SY5Y cells (Allan and Bazan, 1989). In F-98 rat glioma cells induced to differentiate with sodium butyrate, the rapid induction of *fos* is followed at 24–48 h by increases in mRNA levels of fibronectin, collagen, and the *sis* proto-oncogene (Tang et al., 1990); a consensus AP-1 sequence occurs upstream of the *sis* gene (van

den Ouweland et al., 1986). Ornithine de-carboxylase (ODC) is an activity-dependent gene (Kaczmarek and Kaminska, 1989) often correlated with *fos* expression; in PC12 cells (Guerrero et al., 1988) and in rat brain following ECS, ODC mRNA levels rise coordinately following a transient rise in *fos* mRNA levels. The rat ODC gene contains consensus AP-1 sites (Wen et al., 1989). A number of genes expressed in PC12 cells following activation are potential targets of the products of the primary genomic response, as is suggested in a recent review by Sheng and Greenberg (1990), and one of these, the gene for the metalloproteinase transin, contains an AP-1 site. Temporal relationships between *fos* and proenkephalin, prodynorphin, and NGF suggest a stimulus–transcription couple perhaps designed to increase synthesis of these peptides following their vesicular release and depletion during nerve activity.

In fibroblasts from transgenic mice carrying an exogenous, inducible *fos* gene, Hengerer et al. (1990) have found an AP-1 site in the first intron of the NGF gene that is occupied coordinately with induction of *fos* expression; additionally, induction of *fos* results in increased NGF transcription. These data provide direct evidence that Fos regulates transcription of a neurotrophic factor; however, it remains to be demonstrated whether this type of interaction occurs in neuronal tissue in vivo.

Indeed, a complex regulatory relationship exists between the components of the primary genomic response. Deregulated Fos expression in PC12 cells results in differential suppression of *jun*, *jun* B, *egr*-1, and endogenous *fos* induced by NGF, cyclic AMP, and Ca^{2+} (Ito et al., 1990). *Egr*-1 (*zif/268*), NGFI-B, and *fos* contain CRE-like sequences (Christy et al., 1988; Changelian et al., 1989). The regulatory role of Fos at the CREs and AP-1 sites of the other PRGs remains to be established, but, taken together with the AP-1-mediated autoregulation of *fos* and *jun*, an intimate and precise self-regulation of the primary genomic response is indicated.

Conclusion: The Primary Genomic Response in Neural Injury

Expression of the Fos and Jun proto-oncogenes, described as an immediate-early response to cell activation, is associated with various cellular activities in the nervous system. Expression of *fos* is associated with differentiation of PC12 and SH-SY5Y cells and differentiation and proliferation of glial cells in culture. Expression can be likewise generated in response to sensory stimuli. Fos is implicated in mediating cellular responses to NGF as well as mediating the transcription on the NGF gene, and it seems to be a general genomic response to seizure, cerebral ischemia, and neural injury. The temporal relationships observed between expression of *fos*, proenkephalin, prodynorphin, calcyclin, and nerve growth factor genes suggest that these neuromodulatory peptides are genomic targets the inducible Fos–Jun complexes.

Expression of *fos* in the nervous system is apparently intimately involved with cellular Ca^{2+} concentrations (reviewed in Morgan and Curran, 1988), as is evidenced by both in vitro and in vivo studies; this correlates with calcium fluxes during neuronal injury (reviewed in Siesjö, 1990). In fact, PAF is apparently a mediator of intracellular calcium increase in neuronal cells (Kornecki and Ehrlich, 1988). Alternatively, the initial enzymatic step in PAF generation, activation of phospholipase A_2, is likely to be sensitive to changes in Ca^{2+}. We have demonstrated PAF-specific induction of *fos* and *jun* expression and of AP-1-mediated transcription in a neuronal cell line. Considering the evidence of PAF involvement in neuronal injury, we hypothesize

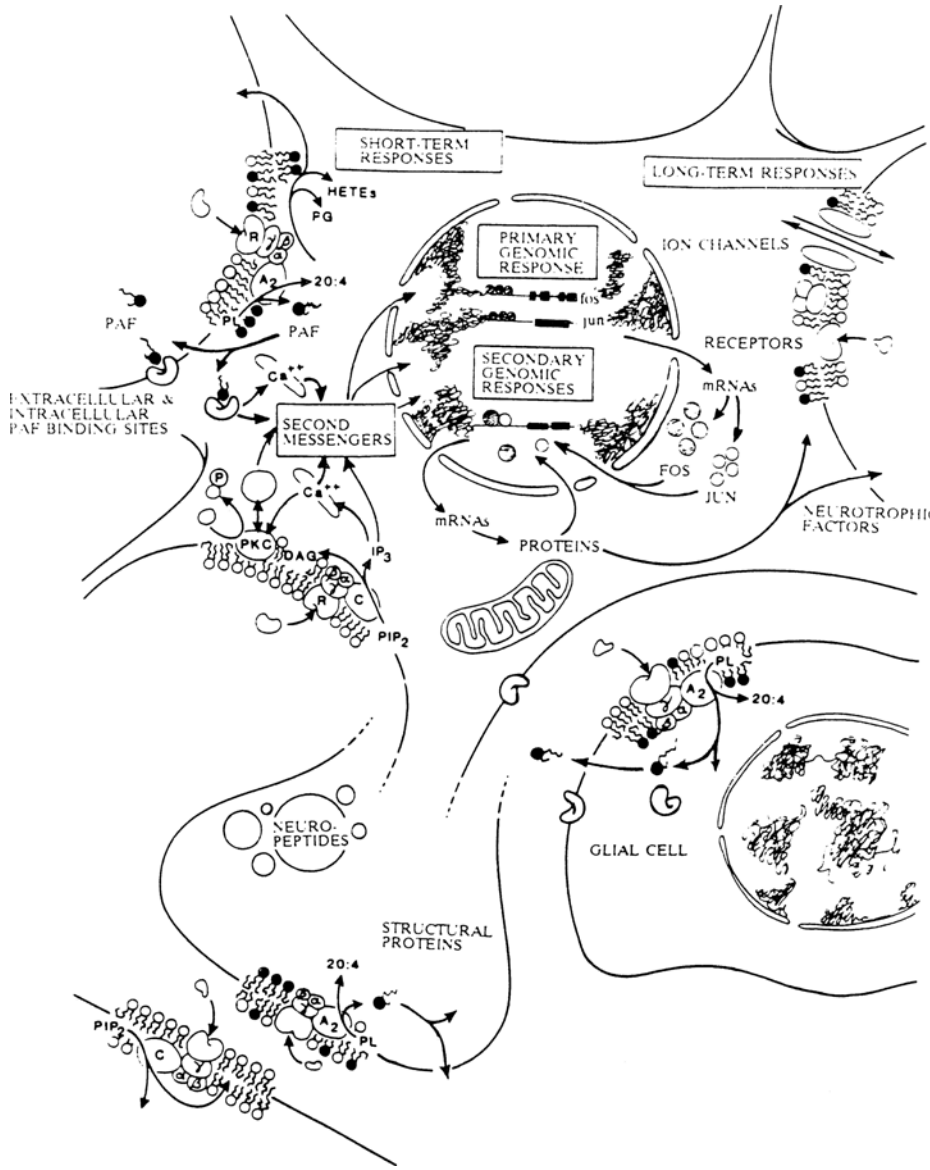

Fig. 2. Participation of PAF in induction of a primary genomic response in cells of the nervous system. This scheme depicts a neuron stimulated through receptor mechanisms that elicit short-term responses as intracellular messenger activity. Stimulation of membrane phospholipase A_2 releases arachidonate and PAF from membrane stores of 1-*O*-alkyl-2-arachidonyl-PAF. PAF acts either through an extra- or an intracellular mechanism, as is demonstrated by antagonism at each of these sites. PAF may alter cellular calcium levels through either mechanism. The second messenger signals are transduced into the nucleus where transcription of primary response genes, such as *fos* and *jun*, is induced. Fos and Jun function to activate secondary genomic responses that presumably mediate the neuron's long-term response to stimulation. The molecular products of secondary genomic responses may include neurotrophic factors, structural proteins, neuropeptides, other nuclear factors, or receptors and ion channels. In addition, possible PAF-mediated synaptic and neuronal-glial responses are shown. (Modified from Bazan, N. [1990] from S. Karger AG, Basel, Switzerland, *with* permission.)

that PAF may be involved in inducing the primary genomic response in vivo. As a logical extension of these studies, we have demonstrated, using a PAF antagonist against the high-affinity intracellular binding site, a repression of *fos* in rat hippocampus following ECS treatment (Marcheselli et al., 1990b). We now envision a general model (Fig. 2) in which, either alone or in concert with neurotransmitter release, PAF modulates the plastic response of the nervous system through the primary genomic response. Furthermore, gene regulation in neurons by PAF through a pathway involving PAF synthesis as a result of tissue injury suggests that a PAF-responsive program of genomic events exists that could involve upregulation of gene products required for repair or maintenance of damaged neurons. Alternatively, the increased synthesis of *fos* and PAF observed during experimental ischemia, epilepsy, and injury may be simply a function of the tissue damage through deregulated control of receptor stimulation or ion fluxes. Nevertheless, we have demonstrated that, at least to some extent, PAF participates in the induction of *fos* in vivo in response to ECS.

Although the significance of *fos* and PRG induction during nervous system damage is unknown, the sheer number of experimental models in which *fos* is induced suggests a real, if general, role for the Fos protein as a transducer of cellular stimuli to genomic targets. The coupling of *fos* and the primary genomic response to pathophysiological events such as the synthesis of PAF and other membrane metabolites provides a hint that the physiological response to trauma in the nervous system may have a genomic component. Although in vivo genomic targets of the transcription-activating function of Fos in the nervous system have not yet been discovered, elucidation of the genes regulated by Fos and other PRG products is essential to understanding the function of the genetic components of neural stimulation and injury, with which the primary genomic response is associated.

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