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c-*fos* Proto-Oncogene Expression in the Nervous System during Mouse Development

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I show, by in situ hybridization, that c-*fos* is expressed in the nervous system during mouse development. This expression was found to be restricted to specific regions at late stages of development (day 16 postcoitum), particularly to the spinal cord, dorsal root ganglia, and olfactory lobe. The c-*fos* protein may play a role in the maturation of these structures by activating specific genes.

The c-*fos* proto-oncogene product is a nuclear phosphoprotein thought to be involved in both differentiation and growth control processes (34). The c-*fos* protein is able to form a complex with other proteins, one of which was recently identified as the v-*jun* oncogene product (24). This Fos complex has DNA-binding properties (27, 29), and its DNA target sequence was recently shown to be the activator protein 1-binding site (13, 25). The strong induction of c-*fos* expression after growth factor stimulation and at different stages of development suggests that the c-*fos* protein may be involved in the regulation of specific sets of genes controlling growth and differentiation. Moreover, the presence and induction of the c-*fos* protein in the adult nervous system suggest that the protein may play a role in neuronal plasticity. In the brain, the level of c-*fos* protein is low (8–11, 20) but increases markedly after seizures (9, 10, 20). A specific pattern of c-*fos* immunoreactivity was observed after cortical stimulation of the adult brain (28) and after sensory stimulation in the spinal cord (18). I show here that the c-*fos* proto-oncogene is expressed at high levels in the nervous system during mouse development, as determined by in situ hybridization.

In situ hybridization analysis of c-*fos* and c-*myc* expression during mouse development. Transcription of c-*fos* was studied in parallel with that of c-*myc*, a nuclear proto-oncogene induced with c-*fos* in many situations (15, 23), on adjacent sections. Embryos at various stages of development (between days 12 and 18 postcoitum) were frozen after dissection, within 5 to 10 min of cervical dislocation of pregnant mice. Serial cryostat sections (20 μm) were fixed for 1 h with 4% paraformaldehyde–0.3% glutaraldehyde in phosphate-buffered saline and then in 70% ethanol–2.3% paraformaldehyde overnight at 4°C. Murine c-*fos* exon IV *Sac*I-*Pvu*II (26) and murine c-*myc* *Hin*dIII-*Sac*I cDNA (32) fragments were subcloned into the Gemini vector system (Promega Biotec), and ^{35}S-labeled single-stranded RNA probes (specific activity, 1.5×10^8 cpm/μg) were prepared. RNA generated from a bacteriophage lambda fragment and the α-globin *Hpa*II-*Apa*I cDNA fragment (22) were used as controls. Hybridization was carried out in 50% formamide–4× SSC (0.6 M NaCl, 0.06 M sodium citrate)–10% dextran sulfate–10 mM dithiothreitol at 50°C for 3 h. A total of 10^6 cpm of probe and 1 μg of tRNA in 30 μl of hybridization buffer were used per slide. Slides were rinsed three times for 10 min each in 50% formamide–2× SSC–5% dextran sulfate–10 mM dithiothreitol at 52°C and three times for 10 min each in 2× SSC at 52°C. Slides were dehydrated in ethanol, exposed to X-Omat film (Eastman Kodak Co., Rochester, N.Y.) for 20 h,

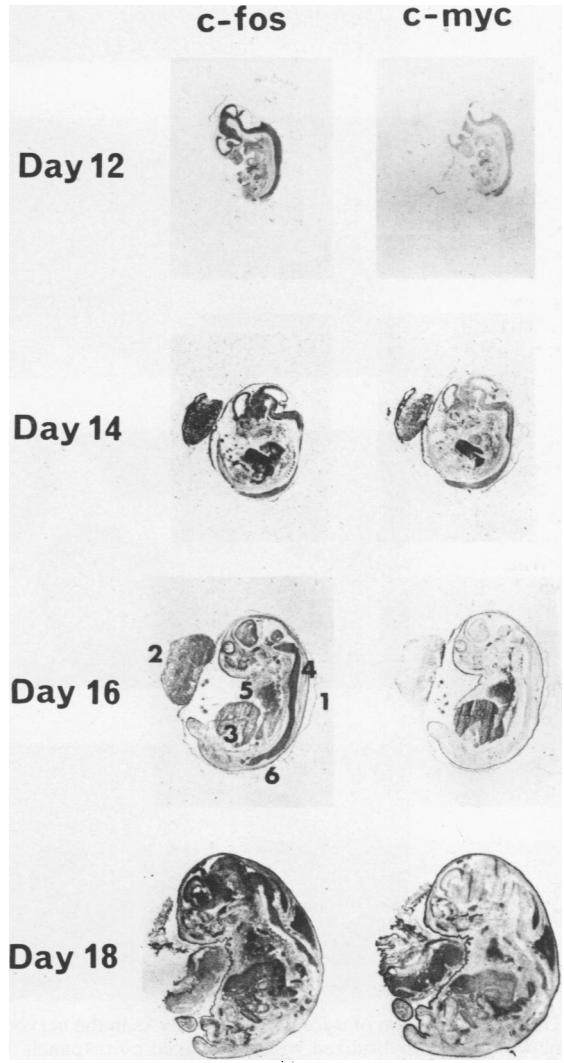

FIG. 1. In situ hybridization analysis of c-*fos* and c-*myc* expression on sagittal sections at different stages of mouse development (magnifications, ×1.4). 1, Extraembryonic membranes; 2, placenta; 3, liver; 4, spinal cord; 5, blood cells within the heart and vessels; 6, skin.

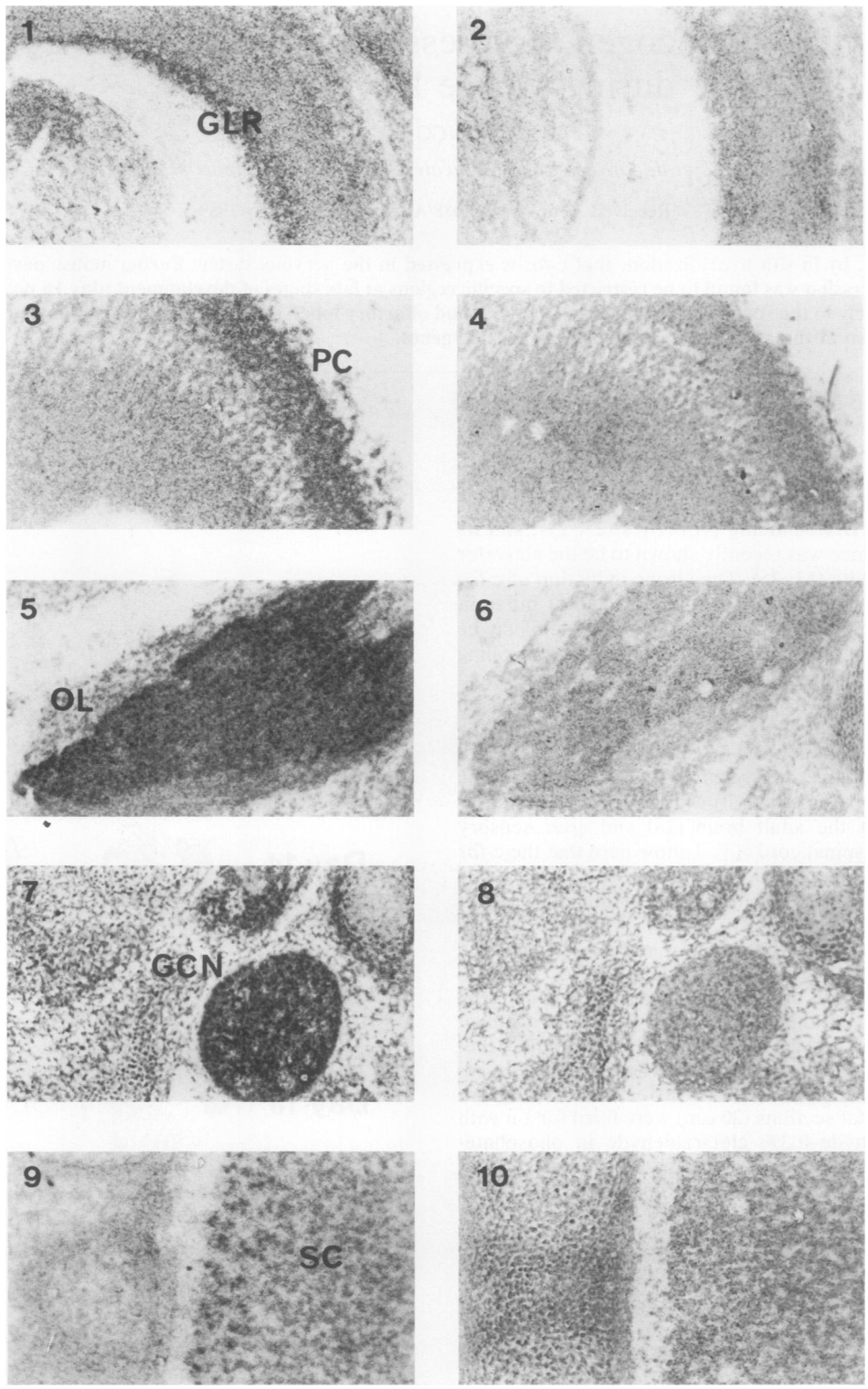

FIG. 2. Localization of c-*fos* and c-*myc* RNAs in the nervous system of day 16 postcoitum mouse embryos. Shown are photomicrographs of embryo sections hybridized with ^{35}S-labeled c-*fos* (panels 1, 3, 5, 7, 9, 11, and 13), c-*myc* (panels 2, 4, 6, 10, and 14), or phage lambda fragment (panels 8 and 12) probes, autoradiographed for 5 to 10 days with NTB3 Kodak emulsion, and stained with hematoxylin and eosin. Identification of the ganglia was often facilitated by examining nerve connections on adjacent sections. Magnification: panels 1 through 6, ×90; panels 7 through 14, ×150. GLR, Ganglionic layer of the retina; PC, primary cortex; OL, olfactory lobe; GCN, ganglion of the cranial nerves (Gasser's ganglion); SC, spinal cord; DRG, dorsal root ganglion.

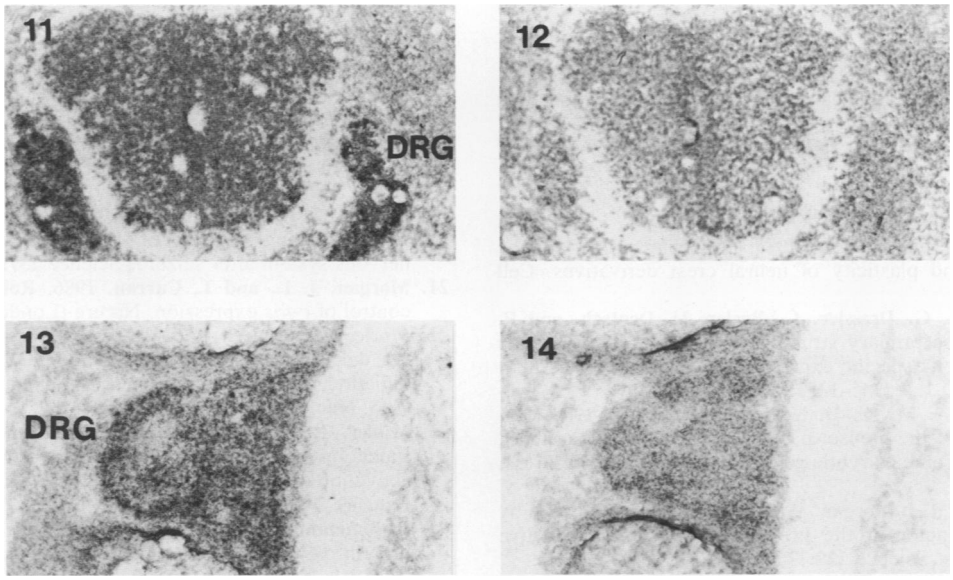

FIG. 2—*Continued.*

autoradiographed for 5 to 10 days with NTB3 Kodak emulsion, and stained with hematoxylin and eosin.

Assays of embryos at various stages of development. Studies of mouse embryos at different stages of development showed that high levels of c-*fos* mRNA were expressed in the nervous system (Fig. 1). The signal, found throughout the brain and spinal cord on day 12 postcoitum, was localized in specific regions on days 16 (Fig. 2) and 18. In day 16 embryo sections, the strongest *fos*-specific signal was observed in the anterior part of the spinal cord, dorsal root ganglia, olfactory lobe, ganglion layer of the retina, cerebellum, and cortex (Fig. 2). To confirm that c-*fos* RNA was not induced during preparation of the embryos, I performed in situ hybridization of embryos directly frozen in the maternal uterus within 5 min of cervical dislocation of the pregnant mice. A strong signal was also observed in the nervous system under these conditions.

Other tissues contained c-*fos* mRNA on day 16 of mouse development. The liver, which has hematopoietic activity from day 12 to birth, and the blood cells circulating in the vessels and in the cardiac cavities contained high levels of c-*fos* mRNA. Signals on the bone and tooth-forming regions, growth regions of the cartilaginous skeleton (5, 7, 30), and skin have also been found. The extraembryonic tissues (amnion) showed a strong signal, as expected. With the c-*myc*-specific probe, expression in the nervous system was low except in the spinal ganglia, where I found a signal significantly different from background (Fig. 2). A signal was detected on the liver, skin (at a higher level than the c-*fos* signal), blood cells, and placenta (Fig. 1). Dissociation between c-*fos* and c-*myc* expression in the nervous system after day 14 postcoitum suggests that c-*fos* plays a role in maturation rather than proliferation of neural cells. In addition, autoradiographic studies of terminal mitosis performed in the nervous system of the rat embryo indicate that c-*fos* expression in the spinal cord and peripheral ganglia occurs after the neural cells stop dividing (see reference 12 for a review).

Immunohistochemical studies found low levels of c-*fos* protein in the adult nervous system (8–11, 20). Induction of c-*fos* expression was observed during Metrazol-induced seizures (11, 20) and after electrical stimulation of the hippocampus (10). Recently, topographic-specific expression of the c-*fos* proto-oncogene was reported in the adult brain after cortex stimulation or water deprivation (28) and in the dorsal horn of the spinal cord after cutaneous stimulation (18). Moreover, PC 12 phaechromocytoma cells express c-*fos* mRNA after stimulation with nerve growth factor (4, 19) and depolarization-induced calcium influx (16, 21). The c-*fos* proto-oncogene is therefore expressed in many situations involving neuronal differentiation and stimulation, which suggests that the c-*fos* protein plays a role in regulation of the genes involved in neuronal organization and function, such as those encoding cytoskeletal proteins, enzymes, and neurotransmitters. Analysis of expression of these genes in the developing nervous system and during stimulation of the adult brain may provide further evidence for common events at the transcriptional level between neuronal differentiation and plasticity (14). In this respect, a cDNA clone isolated from a sympathetic neuron-specific library was shown to be expressed in the neural tube and in cranial ganglia during development and in PC 12 cells after exposure to nerve growth factor (1).

High expression of homeotic and zinc finger genes, which code for nuclear proteins associated with chromatin, has also been observed in the central nervous system during mouse development (2, 3). As with the c-*fos* proto-oncogene, zinc finger *Egr-1* gene expression is induced in PC 12 cells by nerve growth factor treatment and membrane depolarization (33), and the *Hox 1-1* gene is induced in fibroblasts by growth factor stimulation (31).

Coregulation of the c-*fos* gene and homeotic and zinc finger genes in neural cells suggests that the encoded nuclear proteins cooperate in gene regulation, which, in view of the DNA-binding properties of the proteins (6, 13, 17, 25), may involve the formation of a transcriptional regulatory complex. The high expression of these genes in neural cells during development may be correlated with major changes in their metabolism or organization during differentiation. Detailed identification of the regions expressing the c-*fos* proto-oncogene at different stages of pre- and postnatal develop-

ment should be of assistance in studies of the maturation of the developing brain.

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