Development 106, 465-471 (1989)
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Tissue-specific expression of c-*jun* and *jun*B during organogenesis in the mouse

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Summary

c-*jun* and *jun*B are cellular genes related to the viral oncogene v-*jun* and encode members of the AP-1 transcription factor gene family. These genes have been implicated in the control of the G_0/G_1 transition in fibroblasts. Here, we have investigated the potential roles of c-*jun* and *jun*B during fetal growth and organogenesis in the mouse by *in situ* hybridization analysis of their expression patterns. c-*jun* expression is detected throughout organogenesis, and transcripts are detected in many tissues, although in restricted cell populations within developing cartilage, gut and the central nervous system (CNS). In cartilage, c-*jun* expression is associated with rapidly proliferating perichondrial cells, but occurs in postmitotic motor neurones in the CNS. jun*B* expression is initiated between 14·5 and 17·5 days of development, and is restricted to differentiating epidermal cells and endodermal gut epithelium. These data suggest that c-*jun* and *jun*B have distinct, tissue-specific roles in cell proliferation and differentiation during fetal development.

Key words: proto-oncogene, transcription factor, c-*jun*, *jun*B, mouse development.

Introduction

There is increasing evidence that proto-oncogenes implicated in the control of cell proliferation may also have other crucial roles in differentiation and development (Adamson, 1987; Nusse, 1988). This view is based on the finding that the normal developmental expression patterns of certain proto-oncogenes do not correlate with high rates of cell proliferation, but rather are expressed in a cell-type-restricted or region-specific manner. In addition, *in vitro* studies support the notion that some proto-oncogenes may have roles in cell differentiation (reviewed by Adamson, 1987).

A number of proto-oncogenes, including c-*jun* and c-*fos*, encode nuclear proteins that may be involved in transcriptional regulation (Bohmann *et al.* 1987; Angel *et al.* 1987; Chiu *et al.* 1988; Rauscher *et al.* 1988; Sassone-Corsi *et al.* 1988). c-*jun* and a related gene, *jun*B (Ryder *et al.* 1988), have substantial sequence homology with the v-*jun* oncogene and encode members of the AP-1 transcription factor family (Bohmann *et al.* 1987; Angel *et al.* 1987). Although AP-1 binding sites have been found in the promoter regions of a number of genes (Jones *et al.* 1988), little is known of the sequence specificity or physiological role of individual members of the AP-1 family. Both c-*jun* and *jun*B are strongly and rapidly upregulated (as is c-*fos*; Greenberg & Ziff, 1984; Kruijer *et al.* 1984; Muller *et al.* 1984; Bravo *et al.* 1987) on the addition of growth factors to quiescent fibroblasts (Ryder *et al.* 1988; Ryseck *et al.* 1988; Quantin & Breathnach, 1988), and thus these genes may be involved in specific gene transcription during the G_0 to G_1 transition in the cell cycle. Furthermore, the finding that AP-1 and c-*fos* proteins can form a complex suggests that they may act cooperatively in cells where they are coexpressed. It seems likely that c-*jun* has tissue-specific roles since RNA blot analysis detected transcripts only in certain tissues (lung, brain, thymus, intestine and testes) of the adult mouse (Ryseck *et al.* 1988).

To investigate whether c-*jun* and *jun*B might have roles in mouse development, we have analysed the pattern of their expression by *in situ* hybridization during fetal growth and organogenesis. We report a restricted expression pattern of these genes which is suggestive of multiple and distinct tissue-specific roles.

Materials and methods

RNA blots

Poly $(A)^+$ RNA was electrophoresed on agarose gels in the presence of formaldehyde and blotted onto Genescreen (Dupont-NEN) as described (Wilkinson *et al.* 1987*a*). Single-stranded RNA probes were prepared and used for hybridization in 50% formamide, 1 M-sodium chloride, 10 × Den-

hardt's, 10% dextran sulphate, 100 $\mu g \mu l^{-1}$ yeast RNA at 55°C, followed by washing at a final stringency of 50% formamide, 2×SSC at 65°C; the high stringency wash is under the same conditions as used for *in situ* hybridization. The sequences used for probes corresponded to *N*-terminal regions of c-*jun* (residues 1–451; Ryseck *et al.* 1988) and *jun*B (residues 335–903), which have less than 68% sequence similarity and do not include the conserved putative DNA-binding region (Ryseck *et al.* 1988). Previous studies have established the specificity of the c-*jun* (Ryseck *et al.* 1988) and *jun*B (Ryder *et al.* 1988) probes on RNA blots.

In situ *hybridization*

In situ hybridization of mouse embryo sections with ^{35}S-labelled RNA probes and high stringency washing was performed as described (Wilkinson *et al.* 1987*a*,*b*). Control hybridizations with sense-strand probes gave no signals above background except, on occasions, over liver.

Results

c-jun *and* junB *transcripts in mouse fetuses*

RNA blot analysis with *jun*B probe detected a ~2·0 kb transcript in 17·5 day mouse fetuses which comigrates with that present in serum-stimulated fibroblasts (Fig. 1, lanes 1,4). As anticipated, this RNA was not detected in quiescent fibroblasts (Ryder *et al.* 1988), but, suprisingly, was also not detected in RNA from 14·5 day fetuses. Thus, *jun*B RNA accumulation is strongly upregulated between 14·5 and 17·5 days of development. In contrast, c-*jun* transcripts are detected both in 14·5 day and 17·5 day fetuses, although the relative levels are upregulated during this period (Fig. 1, lanes 7,8). Two c-*jun* transcripts of ~2·7 and ~3·2 kb are detected in fetal RNA and serum-stimulated fibroblasts, but not in quiescent fibroblasts (Fig. 1, lanes 5–8). These multiple transcripts have been shown to be due to alternative polyadenylation sites in fibroblasts (Ryseck *et al.* 1988). These data suggest that similar c-*jun* and *jun*B transcripts exist in fetuses and serum-stimulated fibroblasts, and that they are subject to regulation during fetal growth and organogenesis.

To examine the localization of c-*jun* and *jun*B transcripts during fetal development, we have used *in situ* hybridization. c-*jun* expression was found to be relatively widespread, although found only in specific cell populations within certain tissues, whereas *jun*B transcripts were highly restricted. These sites of expression are described below.

c-jun *expression in developing cartilage*

c-*jun* transcripts were detected in perichondrial cells throughout the 14·5 day fetus, for example in developing limb (Fig. 2A–D) and ribs (Fig. 3A,B). These proliferating cells are the precursors to terminally differentiating cartilage which does not express c-*jun* (Fig. 2A–D, Fig. 3A,B). Expression also occurred in growth zones of cartilage at 17·5 days of development, although at this stage similar levels of c-*jun* transcripts were found in surrounding mesenchymal tissue, including mesodermal web cells (Fig. 2E,F).

c-jun *expression in developing muscle*

Examination of 14·5 and 17·5 day embryos hybridized with c-*jun* probe suggested that expression occurred in skeletal muscle. This was confirmed by revealing the presence of skeletal muscle by hybridization of adjacent sections with cardiac actin probe; c-*jun* transcripts were

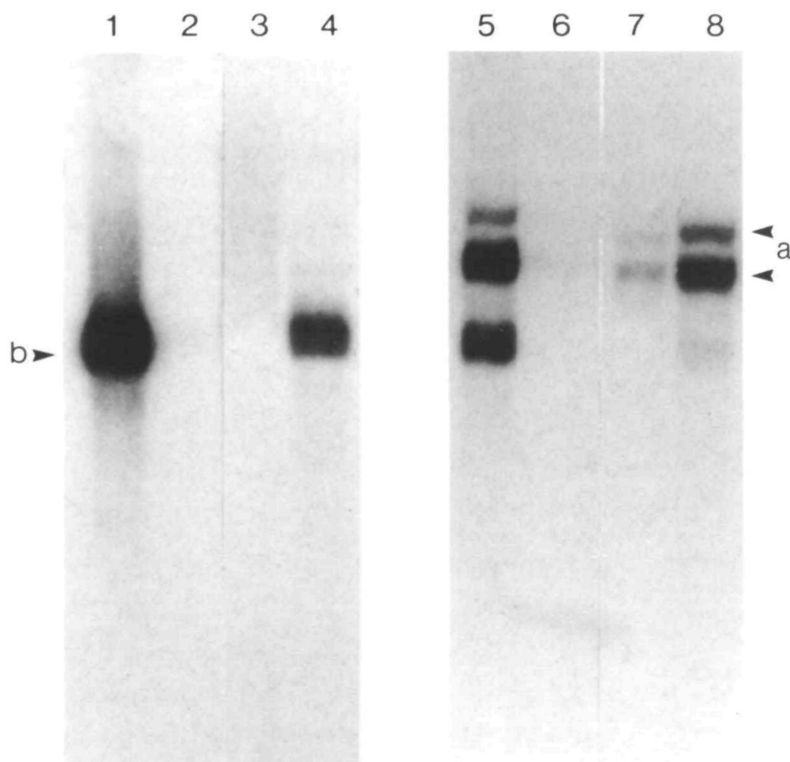

Fig. 1. Expression of c-*jun* and *jun*B RNA in mouse fetuses. RNA blots were hybridized with *jun*B probe (lanes 1–4), and then (without removal of *jun*B signal) with c-*jun* probe (lanes 5–8) to examine transcripts in mouse fetuses. RNA samples from serum-stimulated NIH3T3 fibroblasts (1,5), quiescent fibroblasts (2,6), 14·5 day mouse embryos (3,7) and 17·5 day embryos (4,8) were analysed. *jun*B probe detects a ~2·0 transcript, indicated as 'b'. c-*jun* probe detects two transcripts of ~2·7 kb and 3·2 kb, indicated as 'a'; previous work has shown these two transcripts to be due to alternative polyadenylation sites (Ryseck *et al.* 1988). Note that the autoradiographic exposure shown in lanes 1–4 is greater than that of lanes 5–8 in order to detect *jun*B transcripts, which are present in fetal RNA at lower relative levels than c-*jun* transcripts. Hybridization of the blots with actin coding region probe revealed similar levels of actin RNA in each of the fetal RNA samples (data not shown).

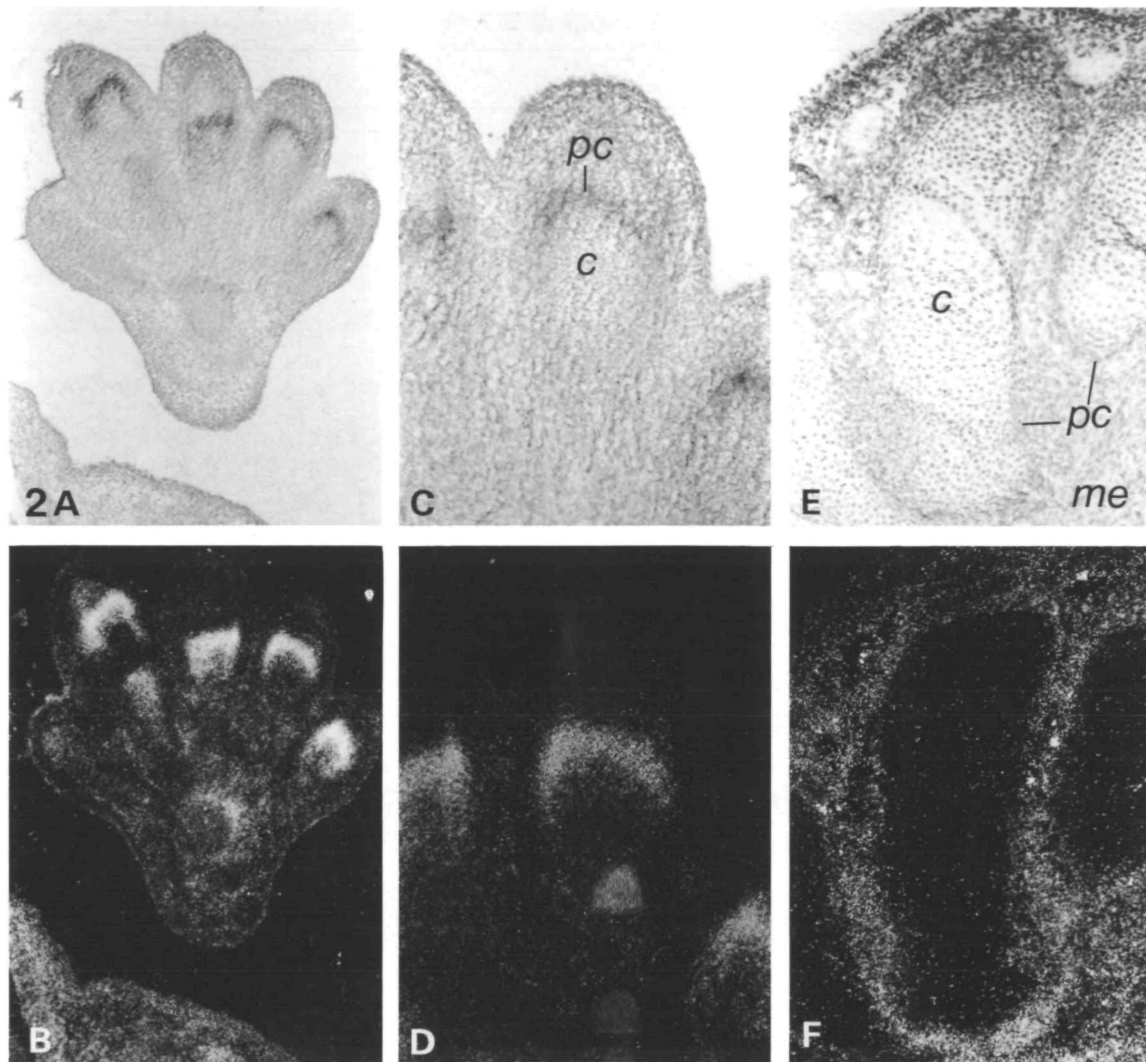

Fig. 2. c-*jun* expression in the developing limb. Tissue sections of 14·5 day (A–D) and 17·5 day (E,F) mouse fetuses were used for *in situ* hybridization with c-*jun* probe. Bright-field (A,C,E) and dark-field (B,D,F) photographs of forelimb are shown; C,D are higher-power photographs of the limb shown in A,B. pc, perichondrial cells; c, cartilage; me, mesenchyme.

detected in skeletal muscle throughout the embryo, for example the intercostal muscles of the ribs (Fig. 3A–C) and the diaphragm (Fig. 3D–F). In contrast, c-*jun* expression was not detected in smooth muscle in the developing gut (see below).

c-jun *expression in the nervous system*

Accumulation of c-*jun* transcripts was detected throughout the cranial and spinal ganglia (not shown), and the olfactory epithelium (Fig. 4A,B), components of the peripheral nervous system. However, c-*jun* expression was highly localized in the central nervous system. Transcripts were detected in proliferating neuroepithelial cells (the ventricular layer) in the telencephalon (Fig. 4C,D), but not in the adjacent zone of postmitotic cells. Lower relative levels of c-*jun* transcripts were detected in the ventricular layer of other regions of the central nervous system (Fig. 4E–H, and data not shown). A number of sites of c-*jun* expression were observed in the midbrain, hindbrain and spinal cord (Fig. 4E–H, and data not shown). The location of these sites suggests that they correspond to motor neurones, for example the oculomotor nucleus in the midbrain (Fig. 4E,F) and the somatic and visceral motor columns in the spinal cord (Fig. 4G,H).

Other sites of c-jun *expression*

c-*jun* transcripts were detected in a number of visceral organs of 14·5 and 17·5 day embryos, including lung (Fig. 3D,E), gut (see below), kidney, and adrenal gland (not shown). Notably, c-*jun* expression was not detected in liver (Fig. 3D,E).

jun*B and* c-jun *expression in developing gut and skin*

junB transcripts were not detected by *in situ* hybridization to 14·5 day fetuses (data not shown), and were found only in the developing gut and skin at 17·5 days. In the gut and oral cavity, *jun*B transcripts were

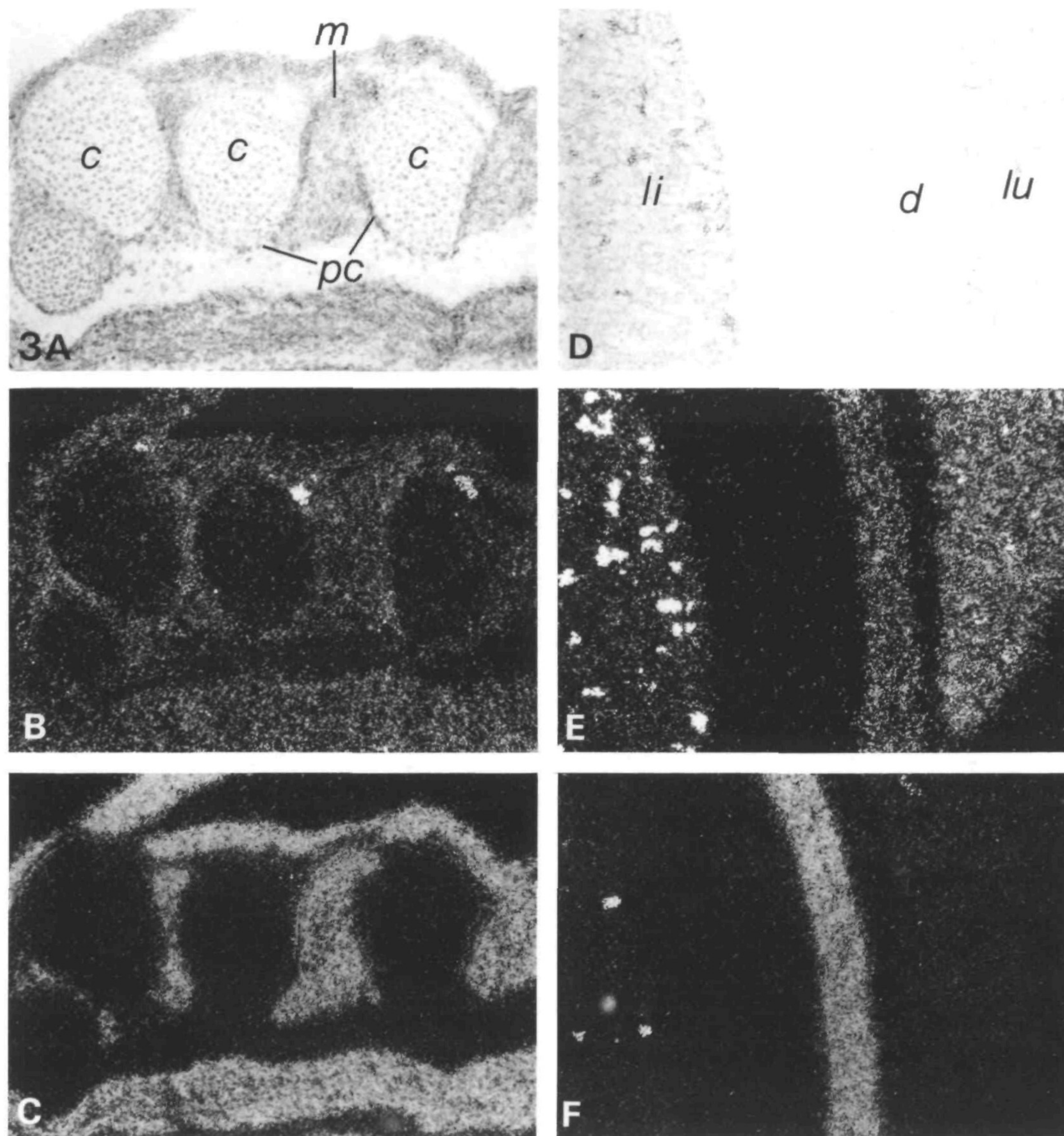

Fig. 3. *c-jun* expression in developing muscle. Tissue sections of 14·5 day fetuses were used for *in situ* hybridization with *c-jun* probe (A,B,D,E) or cardiac actin probe (Minty *et al.* 1982) to reveal developing muscle (C,F). Bright-field (A,D) and dark-field (B,C,E,F) photographs of ribs (A–C) and diaphragm (D–F) are shown. m, muscle; pc, perichondrial cells; c, cartilage; d, diaphragm; li, liver; lu, lung. Note that the intense localized signals over liver are due to refraction of light by erythrocytes, not silver grains.

restricted to the endoderm, for example, in the squamous epithelium of the forestomach (Fig. 5A,B). In contrast, *c-jun* expression was not found in the endoderm, or in the adjacent layer of smooth muscle, but rather was detected in the surrounding mesodermally derived mesenchymal tissue (Fig. 5C). In the skin, *jun*B expression was found in the outermost layer, the stratum corneum, but not in basal layers of proliferating epidermal cells (Fig. 5D,E). The stratum corneum consists of terminally differentiating cells and first arises at 16 days of development (Hanson, 1947), consistent with the absence of epidermal expression of *jun*B at 14·5 days. *c-jun* expression occurred in a distinct, punctate pattern in epidermis (Fig. 5F).

Discussion

Understanding of the normal function of *c-jun* and *jun*B requires knowledge of their expression patterns. In view of the presence of AP-1-binding sites upstream of a number of functionally unrelated genes, it seems likely that collectively AP-1-like proteins may influence gene expression in many tissues and be involved in multiple biological processes. However, it is possible that individual members of the AP-1 transcription factor family may have distinct, tissue-specific roles, and the developmental expression patterns reported here are consistent with this scenario. *c-jun* has a relatively widespread expression, although transcripts

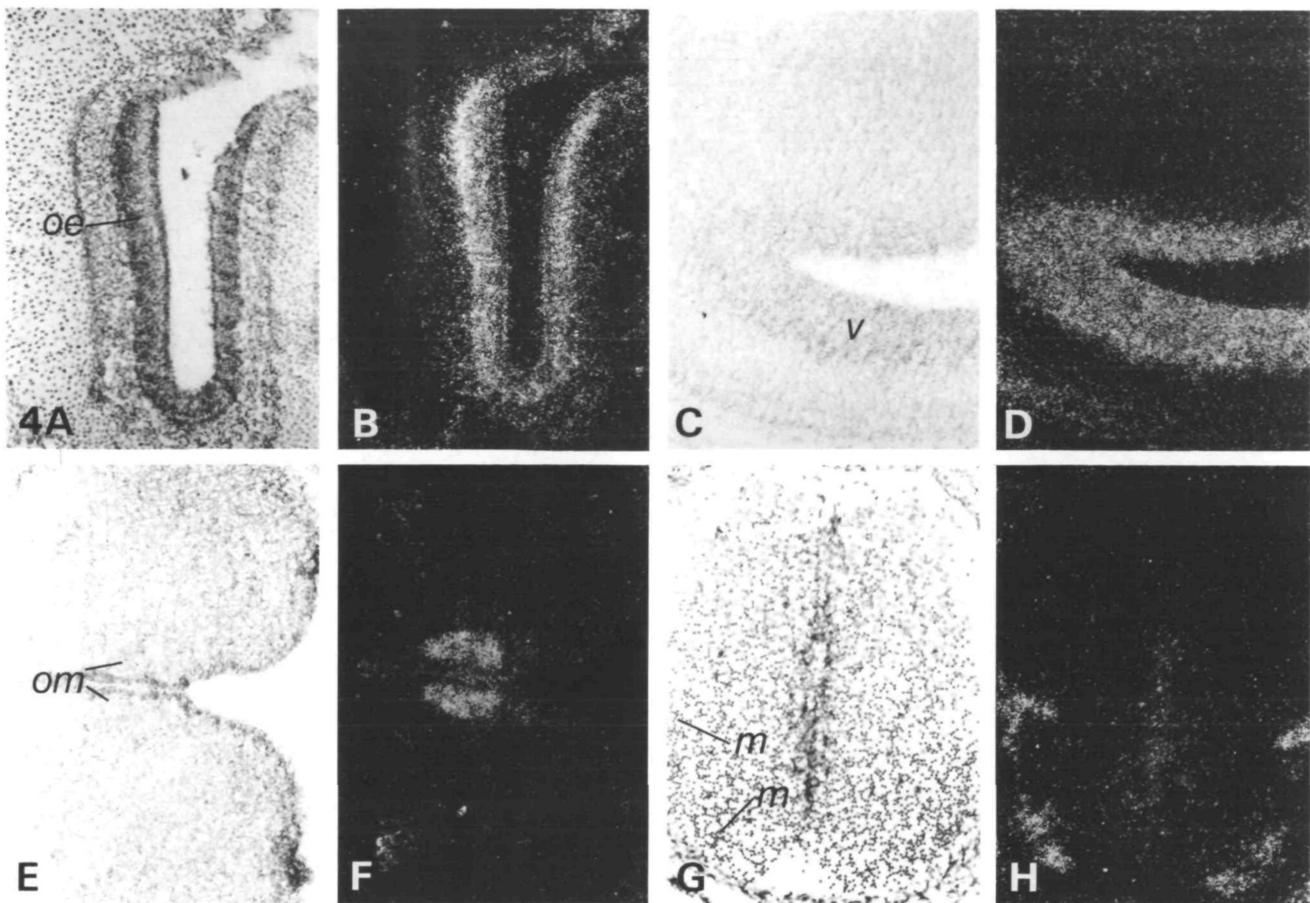

Fig. 4. c-*jun* expression in the developing peripheral and central nervous system. Tissue sections of 14·5 day mouse fetuses were used for *in situ* hybridization with c-*jun* probe. Bright-field (A,C,E,G) and dark-field (B,D,F,H) photographs of olfactory epithelium (A,B), telencephalon (C,D), midbrain (E,F) and spinal cord (G,H) are shown. A–D are longitudinal sections and E–H are transverse. oe, olfactory epithelium; v, ventricular layer of cerebral cortex; om, oculomotor neurones in midbrain; m, visceral and somatic motor neurones in the ventral spinal cord.

are restricted to specific cell populations in certain tissues. In contrast, *jun*B has a distinct and highly restricted expression pattern, suggestive of this gene having a more specialized role and regulating different target genes from c-*jun*.

The association of c-*jun* and *jun*B expression with the resumption of fibroblast cell growth (Ryder *et al.* 1988; Ryseck *et al.* 1988; Quantin & Breathnach, 1988) suggests that these genes could be involved in cell proliferation as part of a network of growth-factor-responsive genes. Indeed, such a role is consistent with the detection of c-*jun* expression in certain proliferating cell populations, notably in perichondrial cells and the ventricular layer of the telencephalon. Perichondrial cells are also sites of c-*fos* expression (Dony & Gruss, 1987), so, in view of the physical association of AP-1 and c-*fos* protein (Chiu *et al.* 1988; Rauscher *et al.* 1988; Sassone-Corsi *et al.* 1988), it is possible that these gene products could act cooperatively in tissue-specific cell proliferation. c-*fos* expression also occurs in mesodermal web cells (Dony & Gruss, 1987), and this tissue also expresses c-*jun*. However, c-*fos* transcripts have not been reported in many of the other sites of c-*jun* expression, so it seems likely that the latter may also act independently of c-*fos*. Furthermore, our data suggest that coexpression of *jun*B and c-*fos* does not occur during development.

The expression of c-*jun* in certain postmitotic cells, for example motor neurones, suggests a role of this gene in cell differentiation rather than in proliferation in at least this tissue. This restriction suggests that c-*jun* may be involved in the transcriptional regulation of genes expressed in motor, but not sensory, neurones. Similarly, although rapid proliferation of epidermal cells occurs in basal layers of the skin, *jun*B expression occurs in the superficial stratum corneum which consists of terminally differentiating cells. It is intriguing that the other site of *jun*B expression is the gut endoderm, since this epithelium is contiguous with, and shares a number of structural and functional properties with, the epidermis (Parakkal, 1967). In particular, it may be significant that these epithelial tissues are sites of keratin gene expression (reviewed by Fuchs, 1988), and it is pertinent to ascertain whether *jun*B might be directly involved in transcriptional control of these genes.

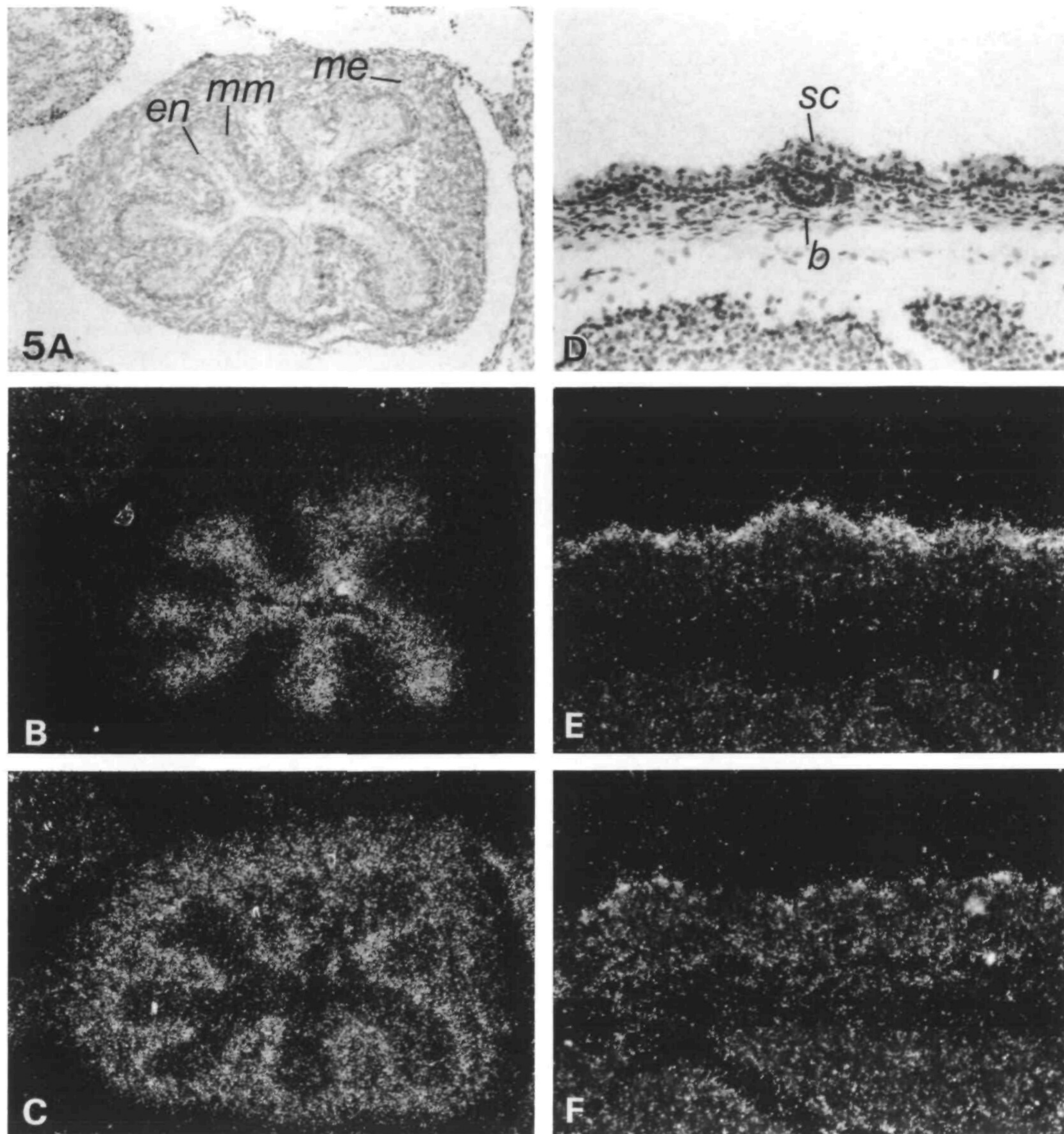

Fig. 5. *jun*B and c-*jun* expression in gut and skin. Tissue sections of 17·5 day fetuses were hybridized with *jun*B (A,B,D,E) or c-*jun* (C,F) probe. Bright-field (A,D) and dark-field (B,C,E,F) photographs of forestomach (A–C) and epidermis (D–F) are shown. en, endoderm; mm, muscularis mucosae; me, stomach mesenchyme; sc, stratum corneum; b, basal layers of epidermis.

Overall, our observations suggest that c-*jun* expression may be associated with tissue-specific cell proliferation, as also proposed for the c-*fos* proto-oncogene (Dony & Gruss, 1987). On the other hand, it seems likely that c-*jun* and *jun*B also have roles in cell differentiation: c-*jun* in motor neurones and *jun*B in skin and gut epithelia. An increasing number of other genes initially discovered in the context of cell growth and/or tumorigenesis, including c-*src* (Sorge *et al.* 1984) and *int*-2 (Wilkinson *et al.* 1989), have developmental expression patterns suggestive of roles in cell differentiation or phenotype. The rapid upregulation of c-*jun* and *jun*B RNA levels by growth factors in fibroblasts suggests that they mediate specific gene transcription in response to extracellular signals. We envisage that these members of the AP-1 family form part of a cascade of regulatory interactions during development that lead to appropriate gene transcription in specific cell populations, and that this may involve combinatorial action with other transcription factors. Thus, understanding of the significance of the patterns of c-*jun* and *jun*B regulation reported here will require analysis of the biological effects of perturbing their expression in appropriate cell types and identification of their presumed target genes.

We thank Patrick Charnay for the gift of fibroblast RNA samples, and David Sassoon for suggesting the use of cardiac actin probe. Rolf-Peter Ryseck was supported by an EMBL postdoctoral fellowship.

References

ADAMSON, E. D. (1987). Oncogenes in development. *Development* **99**, 449–471.

ANGEL, P., ALLEGRETTO, E. A., OKINO, S. T., HATTORI, K., BOYLE, W. J., HUNTER, T. & KARIN, M. (1987). Oncogene *jun* encodes a sequence-specific *trans*-activator similar to AP-1. *Nature, Lond.* **332**, 166–171.

BOHMANN, D., BOS, T. J., ADMON, A., NISHIMURA, T., VOGT, P. K. & TJIAN, R. (1987). Human proto-oncogene *c-jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science* **238**, 1386–1392.

BRAVO, R., MACDONALD-BRAVO, H., MULLER, R., HUBSCH, D. & ALMENDRAL, J. M. (1987). Bombesin induces c-*fos* and c-*myc* expression in quiescent Swiss 3T3 cells. *Expl Cell Res.* **170**, 103–115.

CHIU, R., BOYLE, W. J., MEEK, J., SMEAL, T., HUNTER, T. & KARIN, M. (1988). The *c-fos* protein interacts with *c-jun*/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* **54**, 541–552.

DONY, C. & GRUSS, P. (1987). Proto-oncogene c-*fos* expression in growth regions of fetal bone and mesodermal web tissue. *Nature, Lond.* **328**, 711–714.

FUCHS, E. (1988). Keratins as biochemical markers of epithelial differentiation. *Trends Genet.* **4**, 277–281.

GREENBERG, M. E. & ZIFF, E. B. (1984). Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature, Lond.* **311**, 433–438.

HANSON, J. (1947). The histogenesis of the epidermis in the rat and mouse. *J. Anat.* **81**, 174–197.

JONES, N. C., RIGBY, P. W. J. & ZIFF, E. B. (1988). *Trans*-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. *Genes Develop.* **2**, 267–281.

KRUIJER, W., COOPER, J. A., HUNTER, T. & VERMA, I. M. (1984). Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. *Nature, Lond.* **312**, 711–716.

MINTY, A. J., ALONSO, S., CARAVATTI, M. & BUCKINGHAM, M. E. (1982). A fetal skeletal muscle actin mRNA in the mouse and its identity with cardiac actin mRNA. *Cell* **30**, 185–192.

MULLER, R., BRAVO, R., BURCKHARDT, J. & CURRAN, T. (1984). Induction of *c-fos* gene and protein by growth factors precedes activation of c-*myc*. *Nature, Lond.* **312**, 716–720.

NUSSE, R. (1988). The *int* genes in mammary tumorigenesis and in normal development. *Trends Genet.* **4**, 291–295.

PARAKKAL, P. F. (1967). An electron microscope study of esophageal epithelium in the newborn and adult mouse. *Am. J. Anat.* **121**, 175–196.

QUANTIN, B. & BREATHNACH, R. (1988). Epidermal growth factor stimulates transcription of the *c-jun* proto-oncogene in rat fibroblasts. *Nature, Lond.* **334**, 538–539.

RAUSCHER, F. J., COHEN, D. R., CURRAN, T., BOS, T. J., VOGT, P. K., BOHMANN, D., TJIAN, R. & FRANZA, R. (1988). *fos*-associated protein p39 is the product of the *jun* proto-oncogene. *Science* **240**, 1010–1016.

RYDER, K., LAU, L. F. & NATHANS, D. (1988). A gene activated by growth factors is related to the oncogene v-*jun*. *Proc. natn. Acad. Sci. U.S.A.* **85**, 1487–1491.

RYSECK, R-P., HIRAI, S. I., YANIV, M. & BRAVO, R. (1988). Transcriptional activation of c-*jun* during the G_0/G_1 transition in mouse fibroblasts. *Nature, Lond.* **334**, 532–537.

SASSONE-CORSI, P., LAMPH, W. W., KAMPS, M. & VERMA, I. M. (1988). *fos*-associated cellular p39 is related to transcription factor AP-1. *Cell* **54**, 553–569.

SORGE, L. K., LEVY, B. T. & MANESS, P. F. (1984). $pp60^{c\text{-}src}$ is developmentally regulated in the neural retina. *Cell* **36**, 249–257.

WILKINSON, D. G., BAILES, J. A., CHAMPION, J. E. & MCMAHON, A. P. (1987*b*). A molecular analysis of mouse development from 8 to 10 days *post coitum* detects changes only in embryonic globin expression. *Development* **99**, 493–500.

WILKINSON, D. G., BAILES, J. A. & MCMAHON, A. P. (1987*a*). Expression of the proto-oncogene *int*-1 is restricted to specific neural cells in the developing mouse embryo. *Cell* **50**, 79–88.

WILKINSON, D. G., BHATT, S. & MCMAHON, A. P. (1989). Expression pattern of the FGF-related proto-oncogene *int*-2 suggests multiple roles in fetal development. *Development* **105**, 131–136.

(*Accepted 19 April 1989*)