Pattern of the insulin-like growth factor II gene expression during rat embryogenesis

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Summary

The rat insulin-like growth factor II (IGF-II) gene, encoding a fetal somatomedin, expresses a family of transcripts in embryonic/fetal tissues, and also in the adult choroid plexus and the leptomeninges. We have localized IGF-II gene transcripts in sections of rat embryos of embryonic days 10–16 by performing in situ hybridization. These transcripts are present in the head mesenchyme, formed from both the mesoderm and the cephalic portion of the neural crest, and also in the majority of other tissues of mesodermal origin, predominantly those derived from the somites and the lateral mesoderm. Intense labelling was detected in muscle cells, and their precursors, throughout the examined stages, whereas in chondrocytes the initial high level of hybridization declined substantially prior to ossification. IGF-II gene transcripts are also present in derivatives of other germ layers, but in restricted sites. Thus, from the derivatives of the endoderm, only the liver and the bronchial epithelium yielded hybridization signals. Ectoderm-derived tissues, including the central and peripheral nervous system, were negative for hybridization, with the exception of the choroid plexus, the newly forming pituitary rudiment and, to a lesser extent, the auditory placode. The pattern of IGF-II gene expression during embryogenesis overlaps significantly with the reported distribution of immunohistochemically detected TGF-β1. A paracrine/autocrine role for IGF-II in the developmental process is discussed.

Key words: fetal somatomedin, in situ hybridization, mesoderm, neural crest, insulin-like growth factor II, IGF-II, gene expression.

Introduction

The somatomedins or insulin-like growth factors I and II (IGF-I and IGF-II) are mitogenic polypeptides that have a structural similarity to proinsulin (for recent reviews see Froesch et al. 1985; Zapf & Froesch, 1986; Baxter, 1986).

In the rat, IGF-II is an embryonic/fetal somatomedin (Moses et al. 1980), although its exact developmental role is still unknown. The single-copy IGF-II gene uses at least three promoters (Soares et al. 1986; Frunzio et al. 1986; Ueno et al. 1987; Evans et al. 1988) and expresses multiple transcripts during the embryonic, fetal and neonatal period in many tissues that have been examined by RNA blot hybridization (Soares et al. 1985, 1986; Brown et al. 1986; Lund et al. 1986; Murphy et al. 1987; Gray et al. 1987). These transcripts disappear from most adult rat tissues, with the exception of the choroid plexus and the leptomeninges (Stylianopoulou et al. 1988).

The identification of IGF-II gene transcripts in rat embryonic tissues suggested that the growth factor is produced locally and, thus, might exert paracrine and/or autocrine effects. In this regard, it is notable that IGF-II, in addition to its mitogenic activity (stimulation of DNA synthesis and cell proliferation), promotes the differentiation of cells that are primarily of mesodermal origin (reviewed by Hill & Milner, 1981; Milner & Hill, 1984; Underwood & D’Ercole, 1984; Froesch et al. 1985; Zapf & Froesch, 1986; Baxter, 1986).

To provide information about IGF-II gene activity during rat embryogenesis, we studied the cellular
localization of IGF-II transcripts by performing in situ hybridization on sections of carefully staged rat embryos.

**Materials and methods**

**Materials**

Restriction enzymes were from New England Biolabs; SP6 and T7 polymerases were from Bethesda Research Laboratories; placental ribonuclease inhibitor (RNasin), and the plasmid vector pGEM-1 (a derivative of plasmid pSP64, containing the promoters of the bacteriophage SP6 and T7 polymerases) were from Promega Biotec (Madison, WI); α-[³⁵S]thio-UTP (1200 Ci mmol⁻¹) was from New England Nuclear; NTB2 nuclear track emulsion, D19 developer and fixer were from Kodak.

**In situ hybridization**

Spague-Dawley rat embryos were staged as described (Christie, 1964), considering the day of vaginal plug appearance as day zero. Embryos were sectioned and prepared for in situ hybridization as described (Pintar & Lugo, 1987). To generate hybridization probes, we first subcloned into the BamHI and EcoRI sites of the pGEM-1 vector polylinker a 545-base pair fragment from the rat IGF-II cDNA clone 27 (Soares et al. 1986). This fragment extends between a BamHI site, located one nucleotide downstream from the ATG initiator, and an EcoRI linker present at the end of the fragment, three nucleotides downstream from the TGA terminator. Transcription with T7 polymerase from BamHI-linearized recombinant plasmid generates sense RNA probe. Thus, transcription with SP6 polymerase from EcoRI-linearized plasmid generates sense (control) probe. Transcription reactions (10 µl each) with these polymerases were performed according to the manufacturer's specifications, using 25 µM [³⁵S]UTP, 500 µM each of the other three nucleotide triphosphates, 20 units of RNasin, 1 µg of linearized template and 20 units of polymerase. For certain control in situ hybridization experiments (see Results), we used sense and antisense RNA probes of rat IGF-I coding region sequence. The corresponding templates were generated by subcloning into pGEM-1 a fragment of a rat IGF-I cDNA clone (J. Welsh and A. Efstratiadis, unpublished data). Hybridization, followed by washing under stringent conditions, autoradiography, exposure to photoemulsion, development and counterstaining with haematoxylin/eosin, was as described (Fremeau et al. 1986; Pintar & Lugo, 1987). Low-power, dark-field photographs were taken using a Wilde-M5 stereomicroscope and Kodacolor 400 film. Photomicrographs were obtained using a Leitz microscope equipped with a polarizing cube and epifluorescence.

**Results**

**In situ hybridization**

Complete sets of sagittal, transverse or frontal sections were prepared from rat embryos at embryonic days 10 through 16 (E10–E16) and hybridized to [³⁵S]-labelled, single-stranded, rat IGF-II coding-region RNA probes (sense and antisense; see experimental procedures). The hybridization patterns were first grossly evaluated by X-ray film autoradiography, followed by histological examination of the sections after exposure to nuclear track emulsion. In all cases, hybridization with control (sense) RNA yielded very low background hybridization (data not shown, except for one example in Fig. 3j). We note that Northern analyses indicated that the antisense RNA probe is specific for IGF-II transcripts (absence of cross-hybridization with IGF-I sequences; data not shown). Moreover, when we used an IGF-I antisense RNA probe, the in situ hybridization patterns of adjacent sections from the same embryos were different (unpublished data).

Below we present characteristic examples of our data and analyse our results by tracing the distribution of the IGF-II gene transcripts in the derivatives of each germ layer. An overall summary of our data is shown in Table 1.

**Distribution of IGF-II transcripts in mesodermal derivatives**

The following regions of mesoderm (in addition to the transient notochord) can be discriminated in the developing embryo: dorsal (somitic), intermediate and lateral. There is also mesodermal contribution to head mesenchyme together with cells from the cephalic neural crest. The distribution of IGF-II gene transcripts in derivatives of the head mesenchyme will be presented in the next section. Below, we describe the hybridization patterns of the other mesodermal derivatives during embryogenesis.

Somites consist of blocks of dorsal mesodermal cells that lie along each side of the neural tube. The ventral—medial somitic region (sclerotome) gives rise to chondrocytes that will form the axial skeleton; the dorsolateral layer (dermatome) will form the dermis of the skin; and the medial layer (myotome) the striated muscles. At E10, intense hybridization with the antisense IGF-II RNA probe was detected in all regions of intact somites, which at this age are restricted to the caudal region of the embryo, but not in the neuroectoderm of the head or the trunk (Fig. 1, A–C and e–i). IGF-II gene expression continued during later stages of somite morphogenesis (Figs 2–5). Thus, intense hybridization was detected in all of the somite-derived tissues, including dermatome (Fig. 2f), myotome (Fig. 3j), and newly forming cartilage (Figs 3h, 4A').

Comparative assessment of grain density in many sections suggested that, immediately following somite breakdown, the myotomes were labelled relatively
Table 1. Germ layer derivatives expressing the rat IGF-II gene*

ECTODERM

Surface ectoderm
- Epidermis, hair and nails, cutaneous and mammary glands
- Placodes
  - Optic (eye lens)
  - Otic or auditory (membranous labyrinth)
  - Olfactory (olfactory epithelium and nerve)
  - Nasal
  - Rathke’s pouch (anterior and intermediate pituitary)
- Oral epithelium

Neuroectoderm
- Central nervous system
  - Cranial motor nerves, spinal motor nerve roots
  - Optic vesicles (retina and optic nerve)
  - Posterior pituitary
  - Pineal gland
  - Choroid plexus
- Neural crest
  - Cranial sensory ganglia and nerves
  - Spinal dorsal root ganglia
  - Cranial sensory nerve roots
  - Sympathetic ganglia
  - Adrenal medulla
  - Pigmented cells
- Contribution to head mesenchyme (face and mouth)
  - Cartilages, bones (maxilla, mandible, palate), muscles, connective tissues, leptomeninges

MESODERM

Head mesoderm
- Contribution to head mesenchyme (dorsal and caudal)
  - Skull, cephalic muscles and connective tissue

Dorsal or paraxial (Somites)
- Sclerotomes (axial skeleton)
- Myotomes (striated muscles of trunk)
- Dermatomes (dermis)

Intermediate
- Urogenital system (including ducts, gonads and accessory glands)

Lateral
- Somatic (pleura, pericardium, peritoneum, limb buds)
- Splanchnic
  - Connective tissue and smooth muscles of viscera and blood vessels
  - Heart (epicardium, myocardium)
  - Hemangioblastic tissue (blood cells, endotheilia, endocardium)
  - Adrenal cortex
  - Spleen

ENDODERM

Epithelial parts of liver, pancreas, urinary bladder, larynx, trachea, bronchi, lungs, gastrointestinal tract, salivary glands, pharynx, thyroid, middle ear, tonsils, thymus, parathyroids

* Underlining indicates those derivatives of the germ layers that were intensely or moderately labelled by in situ hybridization using an antisense IGF-II RNA probe. A dashed line indicates low level of hybridization.

more intensely than the other somite derivatives (see Figs 2f, 3e). The labelling of myoblasts remained high even during their differentiation into myotubes (E16; Fig. 6e). In contrast, the abundance of IGF-II transcripts in cartilage-forming cells changed with developmental time, regardless of their location (sclerotome, limb buds or head mesenchyme). Thus, the level of transcripts first increased as mesodermal cells differentiated into cartilage during E12 (Fig. 3c–i), remained high in chondrocytes during E13–E16 (Figs 4A’–D’, 5A’–C’, 6b and c), and then decreased dramatically upon hypertrophy prior to ossification (E16; Figs 5A’–C’, 6a). Specifically, limb bud mesoderm, including precartilagenous cells and muscle primordia, was uniformly labelled during E12, both in the forelimb (Fig. 3e and f) and in the hindlimb (Fig. 3c–e). Newly formed cartilage of the vertebral column (Figs 4A’–D’) and cranial cartilage
IGF-II gene expression in rat embryonic tissues

(Fig. 4C' and D') were intensely labelled in E13. However, hybridization was near background levels in the regions of cartilage that were undergoing ossification, for example in the ribs, the vertebral column and the long bones of the forelimb during E16 (Figs 5A' and C', 6a), in contrast to neighbouring regions in which hypertrophy had not yet begun.

Expression of the IGF-II gene in tissues derived from the intermediate mesoderm (urogenital system, including the gonads) was detectable, but at significantly lower levels than in the tissues derived from the other mesodermal regions (Fig. 4C and C').

In addition to the limb bud mesoderm, other derivatives of lateral mesoderm, also expressed the IGF-II gene. Thus, the cardiac mesoderm (pericardium, epimyocardium and endocardium) of both the atrial and ventricular regions exhibited relatively high levels of labelling, at all stages that we examined (Figs 1A–D and h, 2B', 4A'–D', 5A'). We also observed labelling in the mesoderm lining the coelom (Fig. 1h), and in endothelial cells lining blood vessels, especially in the cranial region (Fig. 3a and b).

Distribution of IGF-II transcripts in ectodermal derivatives

The parenchyma of the developing central and peripheral nervous system was unlabelled at all stages (Figs 1–5, 6c and g–i). Thus, the IGF-II gene is not expressed in derivatives of the neural tube, with the exception of the epithelium of the choroid plexus. This structure, which originates from the neuroepithelial cells lining the internal surface of the neural

![Image](500)

Fig. 2. (Above and opposite) Pattern of IGF-II gene expression at embryonic stage 11. The sections are near mid-sagittal (A), parasagittal (B), transverse (a–e and g), and frontal (f). The levels of the cross sections are shown in A. A', B' and g' are X-ray autoradiograms of the stained sections in A, B and g, respectively. IGF-II gene expression remains high in the tissue types identified in Fig. 1, including all regions of head mesenchyme (a–c) and the heart (B'). Hybridization levels are particularly high in the branchial arches (A', B', d, e and g'). In cross section, the medial (Me) mesenchymal cells of the arches are more intensely labelled than those in lateral (La) regions (d, e, g'). In the trunk, the density of labelling of the dermamyotome is relatively higher than that of the sclerotome (f). The liver rudiment is labelled at this stage (B' and f). Endothelial cells, particularly those in the cranial region (a), also express the IGF-II gene. The neuroepithelium and the V ganglion are unlabelled (b). Abbreviations are BA, branchial arches (I–IV); MA, mandible; VT, ventricle of the heart; L, liver; V, blood vessels; GA, ganglion; DR, dorsal root ganglion; SC, spinal cord; DM, dermatome. Other abbreviations are as in Fig. 1.
Fig. 1. Pattern of IGF-II gene expression at embryonic day 10. (A–D) Oblique sagittal sections. (a–i) Transverse sections at the levels indicated in C. The sections were hybridized with $^{35}$S-labelled, antisense RNA probe, followed by emulsion autoradiography. Labelled areas appear white following dark-field photography. IGF-II gene transcripts are distributed throughout the head mesenchyme (A–C and a–c), the branchial arches (A–C and d–g), all regions of cardiac mesoderm (A–C and g–i) and the somitic mesoderm of the trunk (A–C and e–i). Labelling of Rathke’s pouch is also evident (c; see also Figs 4A, 6i). The neuroepithelium is unlabelled at all levels, as is foregut endoderm (h and i). The abbreviations are BA, branchial arch; HE, heart; NT, neural tube; S, somites; HM, head mesenchyme; T, telencephalon; M, myelencephalon; OV, optic vesicle; RP, Rathke’s pouch; MP, maxillary process; AP, auditory placode; FG, foregut; and LM, lateral mesoderm lining the coelum.
Fig. 3. Pattern of IGF-II gene expression at embryonic stage 12. Midsagittal section (A), and cross sections (a–j) at the levels indicated in A. The next two sections after j in the series are j' (the only one hybridized with sense probe) and k. An X-ray autoradiogram of the stained section k' is shown in k. Widespread labelling of mesodermal derivatives continues at this stage. Segmented somitic regions are heavily labelled (k; arrowheads). The intensity of hybridization in sclerotome contributing to vertebral cartilage is beginning to increase (d, e, h and i). The hindlimb bud (c–e) and forelimb bud (e and f) are relatively uniformly labelled. Medial portions of the fused mandibular arches remain more intensely labelled than the lateral regions (c). Endothelial cells in the cranial region are labelled (a and b). Cranial ganglia V (b) and VII & VIII (d), dorsal root ganglia (i and j) and the neuroepithelium (all sections), are unlabelled. Abbreviations are MY, myotome; FL, forelimb; HL, hindlimb; CA, cartilage; MS, muscle. Other abbreviations are as in previous figures.
Fig. 4. Pattern of IGF-II gene expression at embryonic day 13. Stained midsagittal (A) and parasagittal sections (B–D), and their corresponding X-ray autoradiograms (A′–D′). Newly forming tongue (A′ and B′) is intensely labelled. Cells beginning to form the choroid plexus of the fourth ventricle express the IGF-II gene (B′), as does the pituitary (A′). Both the atrium (A′) and the ventricle (A′–D′) of the heart are labelled. Labelling of the liver is apparent in all sections. Cartilage of the vertebral column (A′–D′) and cranial cartilage (C′ and D′) are labelled. Low to moderate labelling of the mesonephros and gonad is also discernible (C′). All neuroepithelial cells remain unlabelled. Abbreviations are MS, mesencephalon; MT, metencephalon; MN, mesonephros; GO, gonad; FT, pituitary; TO, tongue; and CP, choroid plexus. Other abbreviations are as in previous figures.

tube, exhibited intense hybridization during embryogenesis. Figs 4B′ and 6g show labelling of the primordial choroid plexus of the fourth ventricle (E13) and the lateral ventricle (E16), respectively. Expression of the IGF-II gene in the cells of the choroidal epithelium continues during adult life, in contrast to other tissues in which the gene is switched off after the early neonatal period (Stylianopoulou et al. 1988; Soares et al. 1986).

In contrast to the endogenous derivatives of the neural tube, some derivatives of the neural crest in the cephalic region, but not in the trunk, apparently express the IGF-II gene. At stage E10, labelling was observed in the mesenchyme of the diencephalic region surrounding the optic vesicles (Fig. 1b and c). IGF-II gene expression was also noted in the mesenchyme surrounding the myelencephalon (Fig. 1a–d), including the mesenchyme that contributes to the formation of the pia and arachnoid membranes (see Fig. 6h).

Head mesenchyme contributing to the maxillary process (Fig. 1e), branchial arch mesenchyme (Figs 1d–g, 2A′,B′,d,e and g′), and the orbital cartilage (Figs 4C′ and D′, 5B′ and C′), were intensely labelled. In the first and second branchial arches, the intensity of such labelling was very high at stage E11, which is the time when the medial parts of these processes are approaching each other, prior to fusion. In cross section (Fig. 2d, e, and g′), more medially positioned mesenchymal cells of the arches were labelled more intensely than those in the lateral regions. This pattern of hybridization persists after fusion (Fig. 3c). The tongue (both the portions derived from the tuberculum impar and the lateral thickening of the mandibular arch) was intensely labelled from the earliest stages of its morphogenesis (Fig. 4A). Labelling persisted in later stages (see Fig. 5A′). We have already mentioned that the abun-
dance of IGF-II transcripts in crest-derived cartilage-forming cells declined upon hypertrophy, in parallel with the observed decrease in the corresponding somitic derivatives.

In contrast to the head mesenchyme, extending from the diencephalon to the myelencephalon, which was heavily labelled (Figs 1–3), the crest-derived cranial sensory ganglia remained unlabelled (see Fig. 3b and d). The trunk neural crest derivatives, including sensory (dorsal root) and sympathetic ganglia and the adrenal medulla, were unlabelled at all stages that were examined (see Figs 3j, 5A′, 6c).

Interestingly, some other ectodermal derivatives also express the IGF-II gene. Thus, the primordium of the anterior and intermediate pituitary (Rathke’s pouch) was heavily labelled even at the earliest stages of its formation (Figs 1c, 6i). However, as the presumptive anterior and intermediate lobes of the pituitary developed, the labelling decreased, and it was quite low by embryonic day 16 (data not shown). The ectoderm-derived auditory placode was also labelled, but not very intensely, during the time of its invagination (Fig. 1f).

Distribution of IGF-II transcripts in endodermal derivatives

Most of the endoderm-derived tissues did not exhibit hybridization signals that were convincingly over background. In the lung, however, labelling was observed in the cuboidal epithelia surrounding the bronchioles (Fig. 5A and A′, and B; and data not shown). The liver was labelled at all stages that we examined (Figs 2f, 3f, 4A′–D′, 5A′). Upon histological examination (see Fig. 6f) the distribution of grains appeared diffuse. Han et al. (1987) reported that in human embryonic liver the hybridization signal is localized in perisinusoidal cells, but not in hepatocytes. The resolution we attained does not allow us to draw a firm conclusion about the identity of the hybridizing cells. Nevertheless, after examining the hybridization pattern in sections from several embryos, we believe that even if perisinusoidal cells, which are less abundant than hepatocytes in E16, are labelled, the latter also contribute to the hybridization signal (Fig. 6f). We note that a species difference cannot be excluded.

Derivatives of the pharyngeal pouches, including the thymus (Fig. 5A′) and submaxillary gland (Fig. 5B′), were unlabelled.

Discussion

Growth and differentiation of fetal tissues during embryogenesis proceeds through complex developmental pathways, in which polypeptide growth factors presumably play pivotal roles by providing necessary autocrine/paracrine control signals (see Adamson, 1983, 1986, 1987; Milner & Hill, 1984). However, it is difficult at present to support this view experimentally in whole embryos. Nevertheless, two types of indirect arguments can be made that are consistent with the autocrine/paracrine growth control hypothesis, especially in regard to the rat IGF-II somatomedin.

First, classical hormones (endocrine gland products) that are involved in growth processes postnatally do not seem to play key roles during embryogenesis. For example, the absence of growth hormone does not impair prenatal development, as evidenced by the normal size of human neonates with congenital absence of the pituitary, or of experimental animals, including rats, that have been decapitated or hypothysectomized in utero (reviewed by Underwood & D’Ercole, 1984; Milner & Hill, 1984). Apparently, after birth, IGF-I is produced by target tissues in response to growth hormone stimulation, and then mediates, at least in part, the growth-promoting effects of growth hormone on skeletal tissue (reviewed by Froesch et al., 1985; Baxter, 1986). It has been suggested that IGF-II might be under the influence of placental lactogen, but not growth hormone (Adams et al. 1983). However, the involvement in fetal growth of placental hormones (members of the placental lactogen family, for example) is questionable; there is no evidence to suggest that these hormones cross the placenta–blood barrier. In addition, it is known that at least the human placental lactogen (chorionic somatomammatropin) is not involved in embryogenesis; several cases of gene deletion of both alleles have been described in which the fetus developed normally (see Simon et al. 1986).

A second indirect argument suggesting involvement of the IGF-II somatomedin in rodent embryogenesis can be based on the developmental expression of the cognate gene. The gene is expressed during the embryonic period and in neonates, but is switched off in most adult tissues. Indirect evidence suggests that the gene is probably active in even earlier embryonic stages than those we have examined; IGF-II transcripts were detected in cultured pluripotent mouse embryonic stem cells by S1 analysis (T. DeChiara and A. Efstratiadis, unpublished results). These cell lines (EK or ES cells; see Robertson & Bradley, 1986, for a review), which have been derived from the inner cell mass of mouse blastocysts, have a normal karyotype and high differentiation ability, can integrate into the embryonic stem-cell pool following injection into blastocysts and differentiate into most tissue types of a chimeric adult animal.

The developmental expression of the IGF-II gene is apparently different in humans. Although IGF-II
Fig. 5. Pattern of IGF-II gene expression at embryonic day 16. Sagittal (A) and parasagittal (B and C) sections exposed to X-ray film after hybridization. Areas of A–C are shown in the dark-field photographs A’–C’. Regions of cartilage in the ribs, vertebral column and long bones of the limbs remain intensely labelled (A–C and A’–C’). Closed arrows and open arrows indicate, respectively, different regions of the same cartilage before and after hypertrophy. Transcription in the latter regions is practically undetectable (see also Fig. 6a). Labelling of bronchial epithelium is evident in the lung (A and A’). Labelling of the kidney is barely above the background levels seen in the adrenal and the stomach (A’). Endodermal components of the thymus (A’) and submaxillary gland (B’), the spinal cord, dorsal root ganglia, and all brain regions remain unlabelled. Abbreviations are TH, thymus; AT, atrium; RB, ribs; LU, lung; ST, stomach; AD, adrenal; KI, kidney; and LE, lens. Other abbreviations are as in previous figures.
Fig. 6. Distribution of IGF-II gene transcripts in embryonic tissues at the microscopic level. All sections are from embryos at E16, except for i. (a) Rib cartilage undergoing hypertrophy. The chondrocytes of the cartilage (CA) are intensely labelled, in contrast to the low hybridization signal of hypertrophied (HY) cells. (B,C) Intensely labelled vertebral cartilage (CA). The cells of a dorsal root ganglion (DR) in c are unlabelled. (d) Unlabelled peripheral nerve fibre (NF) with Schwann cells, in contrast to surrounding mesenchyme (MN); V, blood vessel. (e) Myocytes (MY) in developing rib cage are labelled more intensely than rib cartilage (CA) beginning to hypertrophy. (f) Liver. The distribution of grains is diffuse. This microscopic field includes primarily hepatocytes. (g) Labelling is evident in both the epithelial layer and the mesenchyme of the choroid plexus (CP) of the lateral ventricle (LV). The neural parenchyma is unlabelled. (h) Intense labelling is detected in mesenchymal cells that are precursors of the leptomeninges, in the region of the pontine flexure, separating the diencephalon from the metencephalon. (i) Cross section of the pituitary (PT) rudiment at E13. Labelling is intense in both the presumptive anterior lobe (AL) and intermediate lobe (IL), whereas the diencephalon (DE) and the infundibulum (IN) are unlabelled.
transcripts have also been detected in several human fetal tissues (Scott et al. 1985; Han et al. 1987), the circulating factor is present in serum at lower concentrations during fetal development than in adults (Ashton et al. 1985). It is notable that IGF-II transcripts are certainly synthesized in adult human liver as a consequence of the operation of an adult gene promoter (de Pagter-Holthuizen et al. 1987), which might not be present in the corresponding rat gene.

The notion that IGF-II is involved in paracrine/autocrine developmental control is further strengthened by two correlations. First, as shown in this study, the IGF-II gene is expressed primarily in mesoderm-derived tissues, which are known to respond to this mitogen in tissue culture (reviewed by Froesch et al. 1985; Zapf & Froesch, 1986; Baxter, 1986). Second, the distribution of IGF-II receptor molecules, detected immunohistochemically, closely parallels that of the IGF-II transcripts in embryonic head mesenchyme, cartilage and heart (J. Pintar, unpublished data). We note that the autocrine control hypothesis is consistent with the observation that retinoic-acid-induced, differentiating murine embryonal carcinoma cells express high affinity plasma membrane IGF receptors, a portion of which seems to be occupied by secreted molecules that are immunoprecipitable by an anti-IGF-II antibody (Heath & Shi, 1986).

One of the striking results from our in situ hybridization analysis is the detection of IGF-II transcripts not only in mesoderm-derived tissues in both the trunk and the head, but also in cephalic tissues derived from neural crest mesenchyme (for reviews, see Le Douarin, 1982; Noden, 1982, 1984; Weston, 1982; Newgreen & Erickson, 1986). In contrast to the trunk mesenchyme, which in vertebrate embryos is primarily mesodermal in origin, there are two sources of mesenchymal cells with musculoskeletal potential in the head region; ectodermal mesenchymal cells from the neural crest form the connective, muscular and skeletal tissues of the face and mouth, whereas the corresponding tissues that are located dorsally and caudally are derived from mesoderm (Le Douarin, 1982; Noden, 1984). However, primordia formed on each side of this interphase between the two mesenchymal cell populations can fuse at more advanced developmental stages to form some of the tissues, for example the frontal bone and the otic capsule (Noden, 1984). Intense hybridization at stage E10 was observed in the mesenchyme of the diencephalic region surrounding the otic vesicles, which is the route followed by mammalian neural crest cells migrating to the maxillary process (Tan & Morriss-Kay, 1986). Expression of the IGF-II gene was also observed in the mesenchyme of the myelencephalon, which is the route taken by crest cells contributing to the most posterior branchial arches (Tan & Morriss-Kay, 1986). In the latter, the more medial mesenchymal cells were labelled more intensely than those in the lateral regions. Although neural crest cells contribute to all regions of the branchial processes, the crest cells in more lateral regions (which are neurofilament positive) eventually enter the foregut and give rise to the enteric mesoderm, whereas those in more medial regions form cartilage (Le Lièvre & Le Douarin, 1975). We note that despite the dual origin of the mesenchyme (mesoderm-derived and crest-derived cells) in the cranial and branchial areas we discussed above, the uniformity of labelling suggests that crest cells are actively expressing the IGF-II gene.

The cephalic mesenchyme that contributes to the formation of the pia and arachnoid membranes was also labelled. In addition to the choroid plexus, these are the only structures that continue to express the IGF-II gene in adult rats (Stylianopoulos et al. 1988). The embryological origin of the leptomeninges is still controversial. It is believed that they are derived from the neural crest or from mesoderm or from both. At least partial contribution of neural crest cells to the leptomeningeal anlage is strongly supported by evidence derived from avian systems (see Noden, 1978; Le Douarin, 1982).

Interestingly, the presence of IGF-II transcripts (implying, but not proving, local production of the growth factor) in rat embryonic tissues parallels closely the distribution of transforming growth factor \(\beta1\) (TGF-\(\beta1\)) detected immunohistochemically in tissues of developing mouse embryos (Heine et al. 1987). TGF-\(\beta1\) is a multifunctional, positive and negative, regulator of cell proliferation and differentiation, acts by both paracrine and autocrine mechanisms, and enhances the formation of extracellular matrix, which is important in regard to embryogenesis (reviewed by Sporn et al. 1987; Massague, 1987).

TGF-\(\beta1\) was present in somitic derivatives of the trunk, and also in the cephalic mesodermal and crest derivatives, including the leptomeninges, whereas localization in tissues of endodermal or ectodermal origin was not evident. However, there are notable differences between the distributions of IGF-II and TGF-\(\beta1\). Although TGF-\(\beta1\) was homogeneously distributed in each caudal somite, only the sclerotome and the dermatome, but not the myotome, stained positively for the factor after somite differentiation. Moreover, in contrast to IGF-II, ossification centres and calcified cartilage continued to exhibit TGF-\(\beta1\) immunoreactivity. Despite these differences, and the inability to demonstrate from simple localization data that either factor is causally related to morphogenetic phenomena, the correlation in the distribution of IGF-II and TGF-\(\beta1\) in developing mesenchyme
suggests that a functional relationship might exist between the two growth factors during embryogenesis.

An in vitro result indicates that a functional relationship between TGF-β and IGF-II can exist: either TGF-β or IGF-II acting alone cannot induce phenotypic transformation (anchorage-independent proliferation) of mouse 3T3 cells. However, when the two factors are assayed together they induce significant colony growth (Massague et al. 1985).

We note that these interesting correlations simply imply, and only indirectly, that IGF-II and TGF-β are potential active participants in developmental pathways of some cell lineages. It is likely that the pathways themselves are quite complex and proceed with the participation and interplay of additional autocrine and paracrine growth factors, possibly in various combinations. For example, a maternal mRNA (Vg1), localized to the vegetal hemisphere of Xenopus eggs, was shown to encode a TGF-β-like factor (Weeks & Melton, 1987), and synergistic induction of mesoderm by TGF-β and basic fibroblast growth factor was demonstrated in amphibia (Kimelman & Kirschner, 1987).

After completion of this work, a paper appeared (Beck et al. 1987) describing in situ hybridization results for IGF-II transcripts in rat embryos. Although these authors did not describe expression of the gene in neural crest derivatives of the cephalic region and in the embryonic pituitary, their other less-detailed results are overall similar to ours.

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