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

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Summary

The mouse insulin-like growth factor II (IGF-II) gene encodes a polypeptide that plays a role in embryonic growth. We have examined the temporal and spatial pattern of expression of this gene in sections of the mouse conceptus between embryonic days 4.0 and 8.5 by in situ hybridization. Abundant IGF-II transcripts were detected in all the trophectodermal derivatives, after implantation. Labeling was then observed in primitive endoderm, but was transient and disappeared after formation of the yolk sac. Expression was next detected in extraembryonic mesoderm at the early primitive streak stage. Labeling in the embryo proper appeared first at the late primitive streak/neural plate stage in lateral mesoderm and in anterior–proximal cells located between the visceral endoderm and the most cranial region of the embryonic ectoderm. The position of the latter cells suggests that their descendants are likely to participate in the formation of the heart and the epithelium of the ventral and lateral walls of the foregut, where intense labeling was observed at the neural fold stage. Hybridization was also detected in cranial mesenchyme, including neural crest cells. The intensity of hybridization signal increased progressively in paraxial (presomitic and somitic) mesoderm, while declining in the ectoplacental cone. The neuroectoderm and surface ectoderm did not exhibit hybridization at any stage. Immunohistochemical analysis indicated co-localization of IGF-II transcripts, translated pre-pro-IGF-II, and the cognate IGF-II/mannose-6-phosphate receptor. These correlations are consistent with the hypothesis that IGF-II has an autocrine function.

Key words: mouse, embryo, insulin-like growth factor II, gene expression.

Introduction

Growth and differentiation of cells during embryogenesis are thought to occur by differential expression of hierarchies of gene sets functioning in a cascade fashion through complex developmental pathways. The branching of these diverse cascades is likely directed by combinations of products encoded by key regulatory genes. Good candidates for a subset of such pivotal elements are growth factor polypeptides that could function as autocrine and paracrine control signals during the developmental process (reviewed by Adamson, 1983; Milner and Hill, 1984; Underwood and D’Ercole, 1984; Froesch et al. 1985; Zapf and Froesch, 1986; Baxter, 1986; Daughaday and Rotwein, 1989), also plays a role in embryogenesis, as initially suggested by the developmental pattern of expression of the cognate gene. In rodents, the unique IGF-II gene utilizes at least three promoters (Soares et al. 1986; Frunzio et al. 1986; Ueno et al. 1987; Evans et al. 1988) and expresses multiple transcripts in many tissues during the embryonic and neonatal periods (Soares, 1985, 1986; Brown et al. 1986; Lund et al. 1986; Murphy et al. 1987; Gray et al. 1987; for reviews see Sussenbach, 1989; Gammeltoft, 1989), whereas in adult animals, expression is confined to the choroid plexus and the leptomeninges (Stylianopoulou et al. 1988a; Hynes et al. 1988). Previously, we localized IGF-II transcripts in rat embryos of embryonic days 10–16 (e10–e16) in the head mesenchyme, formed from both the mesoderm and the cephalic portion of the neural crest, and also in most tissues derived from somites, lateral plate and cardiac mesoderm (Stylianopoulou et al. 1988b; see also Beck et al. 1987). IGF-II gene expression in derivatives of the other germ layers was detected only in a few restricted sites, including the liver and the bronchial...
epithelium (endodermal derivatives) and also the choroid plexus, the pituitary and the auditory placode (ectodermal derivatives). Throughout mid-gestation, IGF-II transcripts were not detected in the ectoderm-derived central and peripheral nervous systems.

To examine directly the developmental role of IGF-II, we have disrupted one of the IGF-II alleles in cultured mouse embryonic stem (ES) cells by gene targeting and constructed chimeric animals by injecting cells of a selected line into host blastocysts (DeChiara et al. 1990). Germ-line transmission of the inactivated gene from male chimeras yielded growth-deficient heterozygous progeny; their body weight was approximately 60% of that of ES cell-derived wild-type littermates. However, these small animals were fertile and did not exhibit other obvious abnormalities. The growth-deficiency phenotype was evident in examined e16–e18 heterozygous embryos, demonstrating that IGF-II is involved in embryonic growth at a stage earlier than e16.

To assess the consequences of the IGF-II gene mutation in the heterozygous and homozygous states, it was crucial to obtain information about the onset and pattern of the IGF-II gene expression in the early mouse conceptus. Here we report our in situ hybridization results on IGF-II gene transcription during the interval between e4.0 and e8.5, inclusive. Our data demonstrate that the IGF-II gene is first expressed in trophoderm and its derivatives immediately after implantation. Subsequently, IGF-II transcripts are localized in the extraembryonic and embryonic mesoderm and in the lining of the foregut. We also report more limited data on the distribution of IGF-II precursor polypeptide and the cognate (IGF-II/mannose-6-phosphate) receptor that were detected by immunohistochemistry.

Materials and methods

In situ hybridization

For staging of mouse embryos, we considered the midnight before the day of vaginal plug appearance as time zero. However, because of the known asynchrony in development between embryos of the same litter, we also followed the appearance of characteristic embryonic features in our sections to assign standardized stages (Thélier, 1983, 1989). Embryos were sectioned and prepared for in situ hybridization as described (Pintar and Lugo, 1987). The sense and antisense 35S-labeled IGF-II riboprobes that we used have been described previously (Stylianopoulou et al. 1988b). They were synthesized from a construct containing the coding region of a rat IGF-II cDNA that differs from the corresponding mouse sequence in 10 of 545 nucleotides (1.8% mismatch). The specificity of the antisense probe that detects exclusively IGF-II transcripts has been documented (Stylianopoulou et al. 1988b). Hybridization, exposure to autoradiography, counterstaining and microphotography were as described (Lugo et al. 1989; Stylianopoulou et al. 1988b).

Immunocytochemistry

To detect translated pre-pro-IGF-II or one of its post-translational processing products (E-peptide), we used a polyclonal antibody provided by Dr D. Straus. This antibody was raised in rabbits (Hyldka et al. 1987) against a synthetic 40-residue peptide (Hyldka et al. 1985) corresponding to the last 39 amino acids of the carboxyterminal E-domain region of rat pro-IGF-II (positions 118–156; the N-terminal residue at position 117 was Phe instead of Met). For detection of the IGF-II/mannose-6-phosphate receptor, we used a rabbit antiserum provided by Dr C. Gabel, which cross-reacts with the receptor present in mouse cells (Goldberg et al. 1983). The immunocytochemical specificity of both the anti-E-domain and anti-receptor antibodies has been documented with appropriate pre-absorption controls using rat embryo sections (J. Pintar, unpublished data). Finally, for the detection of the blood group I-antigen, we used the C6 monoclonal antibody (Fenderson et al. 1988) provided by Drs B. Fenderson and E. M. Eddy. To expose C6-defined glycoconjugates, sections were pre-treated with neuraminidase as described (Fenderson et al. 1983). In all cases, immunocytochemistry was performed as described (Lugo et al. 1989), and the immunoreactivity was visualized by indirect immunofluorescence after incubation with a secondary rhodamine-conjugated antibody (swine anti-rabbit IgG for the first two antibodies, and goat anti-mouse IgM for the C6 monoclonal).

Results

In situ hybridization analysis

We have analyzed the expression of the IGF-II gene in sections of pre-implantation and early post-implantation mouse embryos by in situ hybridization using 35S-labeled, single-stranded, sense and antisense RNA probes that correspond to the coding region sequence of rat IGF-II cDNA. In all cases for which we describe patterns of positive hybridization with the antisense probe, incubation of adjacent sections with the control (sense) RNA generated only diffuse background grains (data not shown, except for one example in Fig. 2h). Background levels were also assessed from the appearance of the neuroectoderm (serving as an 'internal control') because of the complete absence of hybridization in this embryonic region at all stages. Below we present characteristic examples of our data documenting the time of appearance and the distribution of IGF-II transcripts during the early period of mouse embryogenesis.

Embryonic days 4.0–4.5 (Thélier stages 5/6)

Sections of blastocysts before implantation did not exhibit hybridization signal (data not shown). We conclude that either the IGF-II gene is not expressed during this early stage or the level of transcripts is below the detection limits of in situ hybridization. We note that in undifferentiated cultured ES cells, which are derivatives of the inner cell mass (ICM; Robertson and Bradley, 1986), the IGF-II gene is not transcribed at levels detectable by Northern analysis (data not shown).

Embryonic day 5.5 (Thélier stage 7/8; egg cylinder)

At this age of the conceptus, a strong hybridization...
signal was detected in both derivatives of polar trophectoderm (extraembryonic ectoderm and the ectoplacental cone), whereas IGF-II gene expression was not observed in the epiblast (Fig. 1a and b). We also noticed consistently the presence of scattered grains outlining the region of the mural trophectoderm, but the intensity of this signal was not convincingly above background levels (Fig. 1a and b; and data not shown). However, unequivocal hybridization in the mural trophectoderm that had differentiated into primary giant cells was clearly detected in subsequent stages (see below). Thus, in the conceptus, the IGF-II gene is first expressed in trophectoderm and all its derivatives before any activity in the embryo proper becomes evident.

**Embryonic days 6.5–7.0 (Theiler stages 9/10; early primitive streak)**

As in the previous stage, IGF-II transcripts were readily detectable in the extraembryonic ectoderm, the ectoplacental cone and the mural trophectoderm, including giant cells (Fig. 1c, d and h). Moreover, we clearly observed positive hybridization in parietal endoderm (lining the inner surface of the mural trophectoderm; Fig. 1c) and in a portion of the visceral endoderm (Fig. 1c and d). At this stage, the continuous layer of visceral primitive endoderm is composed of two morphologically distinct cell types (Bonnevie, 1950; Reinius, 1965; Snell and Stevens, 1966; Theiler, 1983, 1989). The cells at the free pole of the egg cylinder and those in the immediately adjacent distal region, which cover approximately half of the epiblast laterally, are flattened (squamous endoderm), whereas tall cells (columnar endoderm) cover the proximal epiblast and the extraembryonic ectoderm. Our results (Fig. 1c and d) indicated that the IGF-II gene is unequivocally expressed in columnar visceral endoderm. In contrast, occasional labeling in the region of squamous endoderm was never convincingly above background levels (Fig. 1c and d; and data not shown; see also Fig. 1e and f), possibly because of the thinness of this epithelium.

During these stages, the first mesodermal cells that delaminate from the newly formed primitive streak proliferate and migrate along two routes (Snell and Stevens, 1966; Poelmann, 1980; Tam and Meier, 1982; Theiler, 1983, 1989; Beddington, 1983; Bellairs, 1986; Tam and Beddington, 1987; Tam, 1989). Cells emerging from the most posterior end of the primitive streak move into the extraembryonic region and contribute to the progressive formation of the amniotic folds by pushing the overlining extraembryonic ectoderm. Other mesodermal cells move anteriorly in the embryo proper as two lateral sheets between the epiblast and the visceral endoderm (mesodermal wings). The embryonic and extraembryonic mesodermal cell populations exhibited a striking difference in IGF-II gene expression. The mesodermal cells in the embryo proper, located in the region of the primitive streak (Fig. 1c) and in the wings of lateral mesoderm (Fig. 1d), did not exhibit hybridization at this stage. In contrast, a very strong hybridization signal was detected in the forming amniotic folds (Fig. 1c; arrowhead). Because of this intensity and the low level of resolution, we could not assess from sagittal sections (e.g. Fig. 1c) whether the hybridizing transcripts in the prominent posterior amniotic fold were present exclusively in cells of the extraembryonic ectoderm or if there was also contribution from extraembryonic mesodermal cells. However, oblique transverse sections (e.g. Fig. 1d) clearly showed that the extraembryonic mesodermal cells lining the forming exocoelom were heavily labeled. Moreover, in the next stage very strong hybridization was detected in the allantois, which is a derivative of extraembryonic mesoderm. Therefore, we conclude that the IGF-II gene is expressed in extraembryonic mesodermal cells immediately upon their first appearance.

**Embryonic day 7.5 (Theiler stage 11; late primitive streak/neural plate)**

After amnion closure and division of the egg cylinder into three chambers (ectoplacental cavity, exocoelom and amniotic cavity), strong labeling was observed in all extraembryonic structures, including the allantois that protrudes into the exocoelom, the amnion, the chorion and the visceral yolk sac (Fig. 1e and f). The layer of extraembryonic mesoderm in the latter three bilaminar structures and also the ectodermal component of the chorion and the endodermal component of the visceral yolk sac were unequivocally labeled. However, because of the close apposition of the two amniotic layers, it was difficult to determine from in situ hybridization data whether the amniotic ectoderm, which is presumably an extraembryonic derivative of the epiblast (Bonnevie, 1950), contributed to the positive hybridization. On the basis of a rare unequivocal result showing exclusive localization of labeling in the mesodermal layer (Fig. 1i), we believe that the IGF-II gene is not expressed in amniotic ectoderm.

In the embryo proper, IGF-II hybridization signal appeared for the first time at this stage in a restricted region of embryonic mesoderm that did not include the primitive streak. In sagittal sections at or close to the midline, the signal was localized in the anterior-proximal embryonic mesoderm, and was continuous with the labeling in extraembryonic mesoderm, without visible demarcation at the anatomic embryonic/extraembryonic junction (Fig. 1e; arrow). The labeled segment of embryonic mesoderm occupied approximately one quarter of the distance between the extraembryonic/embryonic junction and the tip of the cylinder (Fig. 1e). Frontal sections revealed that a girdle of labeling was present in the proximal mesodermal wings (Fig. 1f). Thus, excluding the region of the primitive streak, which remains unlabeled, the subpopulation of embryonic mesodermal cells expressing the IGF-II gene at this stage constitutes a zone in the form of a horse-shoe surrounding the remaining proximal area of the epiblast. This distribution of hybridization signal was verified from transverse sections (Fig. 1g).
Embryonic day 8.0 (Theiler stage 12; neural folds)

At this stage, the amnion (Fig. 2a, d and i) and the walls of the ectoplacental cavity, which had almost completely collapsed (Fig. 2i), continued to exhibit a strong hybridization signal. The allantois, which in some specimens had made contact with the chorion, was also highly labeled (Figs 2a and i; 3d). In contrast, we observed a change in the hybridization pattern of other extraembryonic structures. Although hybridization was still detectable in the region of the ectoplacental cone, its intensity had diminished (Figs 2e and i; 3d). Complete elimination of IGF-II labeling was noted in parietal endoderm and in the outer endodermal layer of the visceral yolk sac, whereas the internal layer (extraembryonic mesoderm) continued to exhibit a positive signal (Fig. 2e and i). The blood islands that had appeared between the two layers of the visceral yolk sac did not exhibit hybridization (Figs 2e and i; 3d). These structures have been thought to arise from a thickening in regions of the visceral yolk sac mesoderm (Snell and Stevens, 1966), but recent evidence suggests that, at least in the human, they are probably derived from the endodermal layer (Takashina, 1989). If this is also the case in the mouse, our data would indicate that the blood islands are formed after extinction of the IGF-II gene transcription in the primitive extraembryonic endoderm.

In the embryo proper, the strongest hybridization signal was observed in the developing heart (Fig. 2a, c, e, i and j) and the lateral (somatic and splanchnic) mesoderm (Fig. 2d and f). Labeling of variable intensity also became evident for the first time in the mesoderm of other regions. This labeling was prominent in the head mesenchyme (Fig. 2a, g and i), which likely included neural crest cells destined to generate connective, skeletal and muscular tissues of the face and the mouth. The labeling was barely detectable in the paraxial (pre-somatic and somitic) mesoderm (Fig. 3a and b), and at background levels in the midline (Fig. 2a). The variable levels of hybridization signal presumably reflect regional differences in the level of IGF-II gene expression.

The lining of the foregut, which is a derivative of the definitive endoderm, also exhibited significant labeling. The hybridization signal was localized in the ventral (beneath the heart) and lateral walls, whereas the dorsal wall was practically unlabeled at this stage (Fig. 2a, c, e, i and j).

Embryonic day 8.5 (Theiler stage 13)

At this stage, the embryos had begun turning and neural tube closure was in progress (Fig. 3f). The pattern of IGF-II hybridization was very similar to that described for the previous stage, except that the somites were now strongly positive (Fig. 3c), and labeling had appeared in the dorsal wall of the foregut (Fig. 3e). While evaluating all of our in situ hybridization results (e5.5–e8.5), we noticed in some regions of the conceptus an interesting correlation between the distribution of IGF-II transcripts and the previously reported pattern of immunostaining with the monoclonal antibody C6 (Fenderson et al. 1988), which recognizes a specific cell-surface carbohydrate epitope (a blood group I-associated antigen). For a direct comparison, we used the C6 antibody to immunostain some sections adjacent to those hybridized with the IGF-II antisense probe (two examples of our results are shown in Fig. 2b and k). Our data, in conjunction with the reported observations (Fenderson et al. 1988), demonstrated that both molecular markers were absent from the region of the primitive streak, but present in the proximal zone of lateral mesoderm (see Discussion). In addition, the topography of the I-antigen distribution in the walls of the foregut was practically indistinguishable from that of IGF-II transcripts (Fig. 2b and k). Both the C6 immunoreactivity and the IGF-II gene expression increased in intensity during somitogenesis. A correlation was also noted in the heart, the head and trunk mesenchyme (Fig. 2b and k), and in extraembryonic tissues, including all the trophodermal derivatives and extraembryonic mesodermal structures (allantois and the mesodermal layer of the chorion, the amnion and the yolk sac). There were, however, two notable differences in the distribution of the two markers. The I-antigen was present in blood islands and the luminal surface of the neural tube (Fenderson et al. 1988; and
Fig. 2. Pattern of IGF-II gene expression and localization of the blood group I-antigen immunoreactivity at embryonic day 8.0. The sections in a, c-g, i and j were hybridized with IGF-II antisense probe, whereas the section in h was hybridized with sense (control) probe. The sections in b and k have been immunostained with the C6 monoclonal antibody (see Materials and methods); the immunoreactive cells appear yellow. The sections are sagittal (a-d; progressively more parasagittal in that order), oblique transverse (e, g, h, i and k), and oblique frontal (j). A transverse section (f) from a rat embryo at an equivalent developmental stage (e9.5) is also presented to complement the data. Comparison of a with c shows that IGF-II gene expression in embryonic mesoderm increases from medial to lateral; this is seen most convincingly in f. The contrast in IGF-II gene expression between ventral and dorsal foregut is seen in c, e, i and j. The similarity in the patterns of IGF-II gene expression and C6 immunoreactivity in mesoderm is evident from a comparison between a and b and between i and k. The additional abbreviations in this figure are: nf, neural folds; h, heart; fg, foregut; vf, ventral foregut; df, dorsal foregut; bi, blood island; hm, head mesenchyme; so, somatopleure; sp, splanchnopleure; l, luminal surface of the neural tube; se, surface ectoderm.
Fig. 3. Pattern of IGF-II gene expression at embryonic days 8.0 and 8.5. The sections are sagittal (a and e), oblique transverse (b and d); and (with respect to the long axis of the embryo at this stage) transverse (c) and frontal (f). The level of IGF-II transcripts in the ectoplacental cone (d) is significantly lower than that at earlier stages, whereas an increase in expression is observed in somites during their maturation (a–c). The additional abbreviations in this figure are: s, somites; prs, pre-somite mesoderm; nt, neural tube.
Fig. 2b and k), whereas the IGF-II gene was not expressed in these regions.

**Immunohistochemical detection of IGF-II precursor and IGF-II/mannose-6-phosphate receptor**

*In situ* hybridization analysis cannot provide information about the functional state of the detected IGF-II transcripts, which might be temporarily stored to be translated at a later stage. To address this question, we immunostained selected sections of e8.0 and e14 mouse embryos with an antibody that recognizes the E-domain of the IGF-II precursor polypeptide (see Materials and methods). We detected immunoreactivity at both ages.

At e8, intracellular E-peptide immunostaining was detected reproducibly in the allantois, the amniotic mesoderm, the mesodermal layer of the visceral yolk sac, the head mesenchyme, the heart, and the foregut (Fig. 4a–c). The distribution of this immunoreactivity was mostly perinuclear and, thus, consistent with the expected localization of the antigen in the Golgi apparatus.

At e14, the immunostaining in examined tissues (heart, cartilage and skeletal muscle; Fig. 4d–f) was more pronounced than in the earlier embryos, but we did not investigate whether this difference correlates with relative abundances of transcripts. Nevertheless, our overall qualitative results (Fig. 4a–f) demonstrate that pre-pro-IGF-II synthesis does occur in most, if not all, tissues that transcribe the IGF-II gene during embryogenesis (Figs 1–3; and Stylianopoulou et al. 1988b). However, we cannot ascertain from these data whether the precursor polypeptide is processed at these stages because the antibody can detect both precursor and cleaved E-peptide.

We also used a specific antiserum to examine the distribution of the IGF-II/mannose-6-phosphate receptor in other sections of the same e8.0 and e14 embryos. At e8, low but detectable receptor immunoreactivity was observed at least in some of the tissues that contain IGF-II transcripts, like the allantois, the chorion, the heart (Fig. 4g and h) and possibly the head mesenchyme and the foregut (data not shown). In mid-gestation embryos, intense immunostaining was detected in tissues like the heart, the tongue, and cartilage (Fig. 4i–k), which are known to contain abundant IGF-II transcripts at this stage (Stylianopoulou et al. 1988b).

**Discussion**

We have determined the spatial and temporal appearance of IGF-II gene transcripts during early mouse embryogenesis. The IGF-II gene is first expressed in all the trophodermal derivatives and then transiently in visceral and parietal primitive endoderm. Expression next appears in extraembryonic mesoderm and then in embryonic mesoderm, first in a zone of anterior and lateral mesoderm and subsequently elsewhere. The lining of the foregut also expresses the IGF-II gene, but the appearance of transcripts in the ventral and lateral walls precedes expression in the dorsal wall.

**IGF-II gene expression in the extraembryonic region of the conceptus**

The IGF-II gene is expressed in the mouse and the human trophoblast (Brice et al. 1989; Ohlsson et al. 1989a) only after implantation. Ultimately, all the trophodermal derivatives express the IGF-II gene, but our data suggest that the onset of transcriptional activity in the IGF-II locus might occur first in polar trophoderm and lag behind in mural trophoderm (see Results). Thus, opening of the IGF-II chromatin domain without immediate transcription could occur in the pre-implantation stage blastocyst, when the proliferating stem cells of polar trophoderm that are in contact with the ICM can still contribute some of their descendants to mural trophoderm (reviewed by Gardner, 1983; Rossant, 1986; Gardner and Beddington, 1988).

The first derivative of the epiblast that expresses the IGF-II gene is also an extraembryonic component of the conceptus, the extraembryonic mesoderm. At later stages, the IGF-II gene transcription persists in the entire extraembryonic mesoderm, which forms the allantois and lines the interior of the visceral yolk sac cavity, and in the derivatives of the extraembryonic ectoderm. Thus, the IGF-II gene is expressed intensely in all the components that eventually form the chorioniclantoic placenta, where IGF-II mRNA has been detected previously (Stempjen et al. 1986; Gray et al. 1987; Ohlsson et al. 1989b). Moreover, a functional role of IGF-II in placential growth has been indicated by the small size of the placentas in e16 growth-deficient heterozygous embryos with a mutated IGF-II gene (DeChiara et al. 1990).

After formation of the yolk sac, the IGF-II gene transcription becomes extinct in the primitive endo-
derm. This correlates with the observation that immunoprecipitable IGF-II precursor polypeptides were detected in radiolabeled culture supernatants of mouse e9.5 amniotic and yolk sac mesoderm, but not in the corresponding supernatants of visceral or parietal endoderm (Heath and Shi, 1986).

**IGF-II gene expression in the embryo proper**

The first IGF-II transcripts that we have detected in the embryonic part of the conceptus during the late primitive streak/neural plate stage are confined to a restricted anterior and lateral zone of mesoderm that occupies the proximal quarter of the mesodermal area, but does not include the region of the primitive streak. The cells in this region are also the first mesodermal components to express the I-antigen in the embryo proper (Fenderson et al. 1988). Thus, they transiently constitute a distinct mesodermal cell population recognized for the first time on the basis of expression of two unrelated molecular markers.

At the time we first observed the IGF-II-positive cell zone, the mesodermal wings have met and fused in the anterior midline completing the formation of the mesodermal layer. The mesoderm in the area of this fusion is likely to constitute the heart anlage (Tam and Meier, 1982). Our hybridization data and the immunostaining patterns of IGF-II and I-antigen in subsequent stages are certainly consistent with this suggestion. We infer from our results that the descendants of this particular cell pool also participate in the formation of the ventral and lateral walls of the foregut. If this interpretation is correct, the IGF-II-positive zone at the neural plate stage consists of lateral plate mesoderm at the two sides of the cylinder and a mixed population of mesodermal and endodermal cells in the area that is adjacent to the anterior–proximal midline. The mesodermal cells belonging to the lateral plate are thought to originate early from the primitive streak and to migrate anteriorly around the proximal part of the embryonic region (Snell and Stevens, 1966; Tam and Meier, 1982). However, the migratory route of the putative anterior–proximal mixed cell population, which we suggest is destined to form the foregut and the heart, could be different.

Recent evidence indicates that a subpopulation of cells emerges axially at the rostral end of the early primitive streak and spreads anteriorly, either displacing or replacing the visceral endoderm in the midline of the anterior half of the egg cylinder (Lawson et al. 1986; Lawson and Pedersen, 1987). These cells, which apparently constitute the first population of the definitive endoderm, reach their anterior destination at the late streak stage and their descendants colonize the ventral and lateral foregut walls. Cells arriving later, which could originate from the head process sometime between the midstreak and late streak stages, participate in the formation of the dorsal foregut (Lawson et al. 1986; Lawson and Pedersen, 1987). We propose that the cells that arrive at the presumptive foregut region during the first wave of migration begin expressing the IGF-II gene, either immediately or after the subsequent formation of precardiac mesoderm.

In the chick, the bilateral precardiac mesodermal cells emerge from the primitive streak about halfway between its rostral and caudal ends, and then migrate in an arc, first laterally and then anteriorly (DeHaan, 1963; Rosenquist, 1970). Arguments have been made that in the mouse the precardiac cells are not migratory, but are formed in situ in the most anterior embryonic region (Kaufman and Navaratnam, 1981; Navaratnam et al. 1986). However, microsurgery experiments have shown a posterior to anterior shift in the position of cardiogenic potential as the development of the egg cylinder progresses (Snow, 1981, 1985). These data are compatible with the idea that the precardiac cells migrate from a posterior–distal to an anterior–proximal position and then fuse in the midline above the precursors of the ventral foregut which have reached their final destination earlier. Notably, when labeled distal epiblastic cells were grafted to an anterior site, the only mesodermal derivative they ever colonized (but only in 3 of 11 embryos) was the heart (Beddington, 1982). Assuming that the fate (allocation) map of mouse epiblastic regions (Beddington, 1983, 1986; Snow, 1981, 1985; Tam, 1989) is similar to that of the chick (except for the timing of certain events), the mouse precardiac cells would be expected to follow a curved path in their migration, since gastrulation occurs in an egg cylinder rather than in a disk.

It remains to be seen whether a functional interrelationship, inductive or other, exists between the foregut and the heart primordia that form in close association, and what the exact role of IGF-II might be in the developmental programs of these structures. In this regard, we note that in the chick the prehepatic cells that are derivatives of the ventral foregut respond to the inductive influence of cardiac mesoderm and differentiate into hepatic epithelium (Fukuda-Taira, 1981).

**IGF-II gene expression and the autocrine hypothesis**

We think that the distinct temporal appearance of transcripts in specific embryonic regions, and also the waves of transient expression of the gene in parts of the primitive and definitive endodermal layers, could only reflect regulated, instead of adventitious, transcription. Moreover, our detection of pre-pro-IGF-II in the same embryonic cells that contain the corresponding transcripts suggests that at least some of the IGF-II mRNAs are indeed translated. Although we cannot ascertain from our data whether the precursor polypeptide is processed into mature IGF-II, which then plays an autocrine/paracrine role, we note that processing of pre-pro-IGF-II in tissues of late-stage (e19) rat embryos has been reported (Romanus et al. 1988). Most importantly, the growth deficiency observed in the e16 heterozygous embryos carrying a mutated IGF-II gene and in their placenta (DeChiara et al. 1990; see Introduction) has documented that the gene plays a physiological role in embryonic growth. Although the onset of mature IGF-II function remains to be determined exactly, we
consider as unlikely that the post-translational processing of the precursor differs between early and late embryonic stages, or that the mature IGF-II made early is bound to protein and remains inactive for a period of time. Thus, we suggest that the growth factor is formed throughout embryogenesis and is an active participant in the circuitry of some developmental pathways. In this regard, we think that the presence of the cognate receptor in the same tissues that transcribe the gene and also contain IGF-II precursor could hardly be coincidental. A striking correlation concerns cartilage-forming cells of late embryonic stages. The levels of IGF-II gene transcripts, translated pre-pro-IGF-II and receptor, which are very high in chondrocytes, decrease dramatically and co-ordinately upon hypertrophy prior to ossification (Fig. 4e and j; and Stylianopoulou et al. 1988b).

Although the above correlations are consistent with a hypothesis of IGF-II autocrine function, an element of this model remains ambiguous. There is no compelling evidence as yet to suggest that the IGF-II receptor participates in IGF-II signal transduction. This bifunctional molecule also serves as the cation-independent mannose-6-phosphate receptor (CI-MPR), which is involved in lysosomal enzyme targeting (reviewed by Roth, 1988; Kornfeld and Mellman, 1989). Since both the *Xenopus* and the chicken CI-MPR lack a high-affinity binding site for IGF-II (Canfield and Kornfeld, 1989; Clairmont and Czech, 1989), the possibility remains that the growth factor exerts its action via the IGF-I receptor to which it binds, albeit with lower affinity than IGF-I (Steele-Perkins et al. 1988). Interestingly, a gene was recently identified in both the human and the guinea pig that encodes a protein highly homologous to the insulin and IGF-I receptors (Shier and Watt, 1989). It remains to be seen whether this newly identified putative receptor could utilize IGF-II as a ligand.

Regardless of an autocrine and/or paracrine mode of IGF-II action, the distinct regionalization of IGF-II transcripts, particularly in the embryonic heart and foregut, at the beginning of organogenesis suggests that the encoded growth factor plays a mitogenic role in one of the earliest differentiation pathways. In this regard, it is notable that the first non- neural primordia to appear in the vertebrate body, which is progressively formed rostrocaudally, are those of the foregut and the heart.

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References


REINIUS, S. (1965). The morphology of the mouse embryo from the time of implantation to mesoderm formation. Z. Zellforschung 68, 711–723.


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