**Expression of TGF-β isoforms during first trimester human embryogenesis**

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**Summary**

We have studied the expression of the genes encoding transforming growth factors (TGFs) β1, β2 and β3 in human embryos ranging from 32 to 57 days post-coitum, using in situ hybridization. The spatial and temporal pattern of expression of each gene is distinct, though each occasionally overlaps. TGF-β1 is expressed in haematopoietic, endothelial and osteogenic tissues.

**Introduction**

The first member of the TGF beta family to be molecularly cloned was human TGF-β1 (Derynck et al. 1985), which has also been the most intensively studied in terms of biological function in vitro (Roberts and Sporn, 1990). This was followed fairly shortly by the cloning of TGF-β2 (Madisen et al. 1988) and TGF-β3 (ten Dijke et al. 1988). The gene family possesses many related members, including the chick and amphibian TGF-β1s 4-8 (Jakowlew et al. 1988a; Melton, 1990), and the more distantly related mammalian proteins, such as the inhibins/activins (Mason et al. 1985) and Müllerian inhibitory substance (Cate et al. 1986). In human, no more than three true TGF-βs have been identified to date.

The interspecies amino acid sequence conservation within the active domain of TGF-β1 is virtually complete (Derynck et al. 1985, 1987; Sharples et al. 1987; Van Obberghen-Schilling et al. 1987; Jakowlew et al. 1988b). The processed TGF-β1 proteins of human, bovine, chicken, porcine and simian are identical, and, between man and mouse, there is only one amino acid substitution in this region (Derynck et al. 1986). The same high degree of conservation is found for TGF-β2 and TGF-β3 (de Martin et al. 1987; Madisen et al. 1988; Cheifetz et al. 1987; Seyedin et al. 1985; Derynck et al. 1988; ten Dijke et al. 1988). Intraspecies divergence between the different TGF-β family members is much greater (70%) (Madisen et al. 1988; ten Dijke et al. 1988). On the basis of these observations, it is reasonable to suppose that the functions of each individual TGF-β are identical across the range of vertebrate species that possess them, and possibly, that each family member might be functionally distinct. However, there may be interspecies variation in the regulation and differential utilization of members of the TGF-β family during embryogenesis.

Interest in the role of TGF-β genes in mammalian development has been stimulated by homologies with developmentally important genes such as mammalian Müllerian inhibitory substance (Cate et al. 1986), the Decapentaplegic (DPP-C) gene of Drosophila (Padgett et al. 1987) and the Vgl cDNA clone of Xenopus (Weeks and Melton, 1987), and by the observation that exogenous mammalian TGF-β proteins can stimulate mesoderm induction in Xenopus tissue (Kimelman and Kirschner, 1987; Rosa et al. 1988). Furthermore, TGF-β has been detected in embryonal carcinoma cell lines, which have common features with early mammalian embryos (Rizzino et al. 1983, Rizzino, 1987; Mummery et al. 1990).

Expression of TGF-βs during murine embryogenesis has been studied immunohistochemically (Heine et al. 1987), by RNA gel blot analysis (Miller et al. 1989a, b; Denhez et al. 1990), by in situ hybridization (Wilcox and Derynck, 1988; Lehnert and Akhurst, 1988; Pelton et al. 1989; Akhurst et al. 1990a; Fitzpatrick et al. 1990) and by polymerase chain reaction (Rappolee et al. 1988). These studies have revealed that the expression patterns of TGF-β1, TGF-β2 and TGF-β3 RNA are distinct and that, in some cases, there is variation.
between the localization of TGF-β1 RNA and its mature protein. Both paracrine and autocrine modes of action of TGF-β1 and TGF-β2 have been proposed based on these models (Lehnert and Akhurst, 1988; Pelton et al. 1989). There is very little published data on murine TGF-β3 gene expression (Miller et al. 1989a; Denhez et al. 1990; Fitzpatrick et al. 1990) and data on the expression of TGF-β genes in human embryogenesis is limited to the work of Sandberg et al. (1988a, b), who examined TGF-β1 transcript distribution in the process of endochondral and intramembranous ossification of second trimester human fetuses.

An understanding of the molecular mechanisms controlling mammalian development is fundamental to our comprehension of the basis of congenital malformation. In order to validate the use of other mammalian species as models for human embryogenesis it is essential to determine whether key molecular developmental processes are similar across species barriers. The work presented in this paper is an investigation of the expression of TGF-β1, TGF-β2 and TGF-β3 in human embryogenesis using in situ hybridization. The results are discussed in the light of results of similar studies which have been performed on mouse embryos (Wilcox and Derynck, 1988; Lehnert and Akhurst, 1988; Pelton et al. 1989; Akhurst et al. 1990a, b; Fitzpatrick et al. 1990; Millan et al. 1990).

Materials and methods

Embryology

Intact human embryos 32–57 days post-conception (p.c.) were obtained following therapeutic abortion using mifepristone and gemeprost prostaglandin pessaries. These were processed for in situ hybridization according to the protocol of Wilkinson et al. (1987). Sex was determined by karyotype analysis. Embryonic stage was estimated by crown–rump length, embryo weight and morphological appearance; the estimated date of the last menstrual period was found to be less reliable. Staging relative to mouse embryos is difficult since different organ systems develop at different rates in the two species, however, we attempted to do this according to the observations of Rugh (1968). Supplementary material of 10 to 12 weeks gestational age (mainly limbs) were collected by suction termination and similarly processed. The embryos obtained were as follows:

RU113: 44 days post-last menstrual period (LMP). No chromosomal analysis was performed on this embryo, assessed as 32 days p.c. on the basis of morphology. Crown–rump length could not be measured due to distortion. The embryonic weight was 0.025 g. This embryo had a well-developed liver and the rudiments of limb buds. The heart was in the process of septation. The gut and mesonephros were distinguishable but the lung buds could not be identified. This is approximately equivalent to a 10 to 10.5 day p.c. mouse embryo.

RU74: Assessed chromosomally as Y-negative and morphologically as 43 days gestation. The crown–rump length was 12 mm and the weight was 0.213 g. The tail was torn off, and the embryo had already been sectioned sagitally for histological purposes and was therefore lacking its left side to a thickness of about 1/4 of its total. Lung buds were visible and the limb buds were well developed though there were no presumptive digits. The lens vesicle was closed and separate from the surface. This embryo is approximately equivalent to an 11 to 11.5 day p.c. mouse embryo.

RUI18: 58 days post-LMP. No chromosomal analysis was performed on this embryo, assessed as 47 days p.c. on the basis of fetal morphology. Crown–rump length was 19 mm and the weight was 0.614 g. The lungs, limbs and eyes were noticeably more advanced than in RU74. The tongue was readily distinguishable, although the pinnae were scarcely developed. This gives an approximate correspondence to a 12 to 13 day p.c. mouse embryo.

RU59: 71 days post-LMP. A chromosomally normal male fetus assessed as 57 days of gestation on the basis of morphology. The gut was herniated and there was a small degree of abrasion on the skin in many areas. Weight was 1.41 g and the crown–rump length was 25 mm. The fingers were separated distally, very primitive toothbuds were visible, and intramembranous ossification had begun. However, the eyelids and palate were unfused. This is approximately equivalent to a 14 to 15 day p.c. mouse embryo.

A summary of the biological material used is shown in Table 1.

Table 1. Quantitative characteristics of embryos studied

<table>
<thead>
<tr>
<th>Gestational age (days post-ovulation)</th>
<th>Crown-Rump Length (mm)</th>
<th>Embryonic wet weight (mg)</th>
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<tbody>
<tr>
<td>32</td>
<td>5*</td>
<td>25</td>
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<tr>
<td>43</td>
<td>12</td>
<td>213</td>
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<td>47</td>
<td>19</td>
<td>614</td>
</tr>
<tr>
<td>57</td>
<td>25</td>
<td>1410</td>
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* Estimated crown–rump length, according to Hamilton and Mossman (1972).

Probes

Full-length cDNA probes encoding human TGF-β1 (Derynck et al. 1985) and TGF-β2 (Madisen et al. 1988) were kindly supplied by Dr G. I. Bell (Howard Hughes Institute, Chicago) and Dr A. F. Purchio (Oncogen, Seattle) respectively. A probe for human cardiac actin was kindly provided by Dr P. Barton (Heart and Lung Institute, London), and Dr J. C. Fiddes (California Biotechnology Inc.) supplied the human chorionic gonadotropin gene probe. The TGF-β3 probe was described by ten Dijke et al. (1988).

Gene-specific probes were generated by subcloning sequences from the non-conserved 5' non-coding or precursor coding regions into Bluescribe or Bluescript plasmid vectors. The gene-specific TGF-β1 subclone was a 712 nucleotide EcoRI–PstI fragment from the 5' non-coding region, inserted antisense with respect to the T3 promoter. The gene-specific TGF-β2 subclone was a 450-nucleotide EcoRI–PstI fragment spanning amino acid residues 61 to 211, inserted antisense with respect to the T3 promoter. The gene-specific TGF-β3 subclone was a 463-nucleotide fragment spanning from 16 nucleotides upstream of the translation initiation site to amino acid 149, and inserted antisense with respect to the T7 promoter. This latter subclone also contained the conserved 30 amino acid coding region found at the N-terminus of the TGF-β precursor polypeptides.

Probe synthesis

35S-labelled single-stranded riboprobes were generated to a specific activity of 8×10⁶ disintegrations min⁻¹ μg⁻¹ using the Blue-
tissue or Bluescript T3 and T7 transcription system (Vector Cloning Systems). Probes were digested to an average length of 100 nucleotides by controlled alkaline hydrolysis (Cox et al. 1984). For the negative control probe, a human chorionic gonadotropin cDNA probe, kindly supplied by J.C. Fiddes (Fiddes and Goodman, 1980), was subcloned into the Bluescribe vector and the T7 promoter was used to generate antisense RNA. Both full-length cRNA probes and gene-specific subclone cRNA probes were utilized. No difference was found between these two categories, indicating that the in situ protocol is stringent enough to avoid cross-hybridization within the gene family.

In situ hybridization
In situ hybridizations were performed essentially according to the protocol of Wilkinson et al. (1987), except that the hybridizations were carried out at 52°C. Probe concentration was adjusted to 2 to 5×10⁴ disnts min⁻¹ μl⁻¹. Autoradiographic exposure times were between 1 and 2 weeks. After development, slides were counterstained in haematoxylin and eosin and examined using an Olympus BK2 microscope. Photomicrography was performed using Kodak Panatomic X film.

Results
TGF-β gene expression in the developing liver
TGF-β1 is the only member of the TGF-β gene family to be expressed at high level within the embryonic liver (Fig. 1 A,B). At 43 and 57 days p.c., intense expression of TGF-β1 RNA is seen in megakaryocytes (Fig. 1E–H) and, to a lesser extent, in certain cell clusters that are probably haematopoietic progenitors. These cells had not appeared at 32 days p.c. Primitive parenchymal cells do not express the gene at any time (Fig. 1E,F).

Chondrification and ossification
Sandberg et al. (1988a,b) demonstrated that TGF-β1 is expressed at high levels in osteoblasts, osteocytes and osteoclasts in areas of endochondral and intramembranous ossification of 17 week p.c. human fetuses. We have also confirmed that one of the most intense sites of TGF-β1 RNA expression is in areas of intramembranous ossification. This is observed in the 57 day p.c. embryo in the maxillary and palatine bones, and in the mandible (Fig. 2A,B). There is no endochondral ossification as early as 8 weeks p.c., although hypertrophic cartilage cells, which do not hybridize with any of the three TGF-β gene probes, are visible in the centres of the long bone cartilages (Fig. 2M,N). We have, however, seen expression of TGF-β1 in invading osteogenic cells at sites of endochondral ossification in the long bones of limbs from 10 to 12 week p.c. fetuses (data not shown).

Pelton et al. (1989) observed expression of TGF-β2 in osteoblasts and endothelial cells during endochondral, periosteal and intramembranous ossification in the mouse. In our study, although the TGF-β1 probe was seen to hybridize intensely to these sites, the TGF-β2 probe generated no autoradiographic signal (e.g. compare Fig. 2A,B and 2C,D). TGF-β2 RNA expression in the limb is, however, seen at the limb bud stage (43 days p.c.) in areas of mesenchyme that are morphologically indistinguishable from the surrounding tissue (Fig. 2K,L). We assume that these are early precartilaginous blastema.

At later stages of limb development, TGF-β2 RNA expression becomes limited to the actively proliferating chondroblastic zone at the diaphyseal/epiphyseal junction (Fig. 2M,N,O,P). There is also intense expression surrounding the digits at 57 days p.c. (Fig. 2O,P), in tendinous material, as observed by Pelton et al. (1989).

The earliest embryonic expression of TGF-β3 RNA seen in this study is in the intervertebral disc anlagen at 43 days p.c. (data not shown). This pattern persists at least to 57 days p.c. (Fig. 2G,H). TGF-β3 RNA is also expressed in the perichondria of cartilage models associated with the vertebral column, in particular the ribs (data not shown), but not those associated with the long bones of the limbs. A low level of expression is seen in the perichondria of non-ossifying cartilage, such as Meckel's cartilage and the tracheal cartilage rings, though there appears to be more TGF-β3 RNA in the surrounding mesenchyme than in the perichondria per se (Fig. 2E,F,I,J).

The cardiovascular system
Expression of TGF-β1 in the mouse cardiovascular system has been extensively studied by Akhurst et al. (1990a), who observed low levels of RNA in early endothelial cells and, at later stages, those specifically associated with septation and valve formation within the heart. TGF-β1 RNA expression is also detected in human fetal cardiac valve endothelia at 43 (Fig. 3A,B) and 57 days p.c. (data not shown), at a time when human heart development is virtually complete. Due to lack of suitably preserved specimens from very early embryos, we can make no comment as to the cardiac expression of TGF-β RNAs prior to 43 days p.c. Expression of TGF-β2 and TGF-β3 RNA in the heart at the later stages was not obvious, although there was some mesenchymal expression of TGF-β3 in the regions of the heart valves at 57 days p.c. (data not shown).

TGF-β1 RNA is also seen in the endothelia of large arteries (Fig. 3C,D), whereas TGF-β3 is expressed in the tunica intima underlying these endothelia (Fig. 3E,F).

TGF-β gene family expression in mesenchyme and mesothelia
TGF-β2 and TGF-β3, unlike TGF-β1, show widespread mesenchymal expression at the stages examined. In fact, the earliest expression of any of the TGF-βs detected in this study is that of TGF-β2 in the somatic mesoderm surrounding the liver at 32 days p.c. (Fig. 4A,B). TGF-β2 is also expressed extensively in mesenchymal areas at 43 and 57 days p.c., particularly in the non-ossifying regions of the head. Hybridization with a human cardiac actin gene probe (kindly supplied by Dr P. Barton), which hybridizes to fetal skeletal and cardiac actin (Sassoon et al. 1988), indicates that TGF-
Fig. 1. TGF-β expression in the fetal liver, 57 day p.c. fetal liver hybridized with the TGF-β1 specific (A,B,E,F,G,H) and TGF-β2 specific (C,D) probes. (A,C) Bright-field and (B,D) corresponding dark-field images. E and F (also G and H) show the same field in a different plane of focus to show cellular morphology (E,G), and autoradiographic silver grains above the cells (F,H). Arrowheads indicate positively hybridizing megakaryocytes and arrows indicate non-hybridizing parenchymal cells. Scale bar represents 200 μm (A,B,C,D), or 20 μm (E,F,G,H).
β2-positive tissue is adjacent to, but does not generally overlap, regions of myogenesis (data not shown).

Over the period of development examined in this study, the skin is at a very immature stage. At 47 days p.c. TGF-β2 RNA is present in the dermatome and by 57 days p.c. it is seen in the dermis immediately beneath the epidermis (Fig. 4C,D), as has been observed in the mouse by Pelton et al. (1989).

TGF-β3 is also expressed in mesenchymal tissue, particularly those surrounding perichondria (Fig. 2E, F,I,J). More striking, however, is the expression of this gene in mesothelia surrounding all the major organ systems, including the pericardium, diaphragm and viscera (Fig. 4E,F). In the diaphragm, the use of the human cardiac actin probe showed that TGF-β3 RNA is associated both with the mesothelial and the muscular layers (Fig. 4G,H).

**Lung development**

The lungs are formed by the proliferation and branching of the lung buds into the splanchnic mesoderm from the fifth week of development. The development of the lung occurs relatively late in gestation and, at the early stages examined in this study, the pulmonary epithelial cells are all columnar in morphology, the transition to a simple cuboidal cell occurring much later.

In the 43 day p.c. embryo, TGF-β2 can be found in the primitive lung epithelia (Fig. 5A,B). By 57 days p.c., the process of branching is fairly well advanced and only the growing tips of the developing bronchioles express TGF-β2 in their epithelia. (Fig. 5C,D). TGF-β3 shows a different pattern of expression in the lung. The RNA is seen submucosally in the proximal respiratory tract from the presumptive larynx downwards to a point that we believe represents the boundary between bronchi and bronchioles. Distal to this it has an epithelial expression pattern in the linings of the bronchioles but is also co-expressed with TGF-β2 in the terminal growing end buds (Fig. 5E,F,G,H).

**TGF-β gene family expression in other epithelia**

In previous studies, TGF-β1 RNA was detected in the epithelial component of the developing tooth, hair follicle, salivary gland and secondary palate of the mouse (Lehnert and Akhurst, 1988; Akhurst et al. 1990b; Fitzpatrick et al. 1990). The equivalent stages of development of these structures were not available for study here, so no conclusions regarding epithelial TGF-β1 expression can be made.

Intense epithelial expression of TGF-β2 was seen in the sensory epithelium, but not the simple cuboidal epithelium, of the developing inner ear at 47 and 57 days p.c. (Fig. 5I,J) and in a number of epithelial structures within the developing eye. At 43 days p.c., it is expressed in all the cells of the lens vesicle and the inner layer of the optic cup, which gives rise to the retina (Fig. 5K,L). By 57 days p.c., TGF-β2 RNA has become limited to the anterior germinal epithelium of the lens and is also seen in the most anterior part of the inner layer of the optic cup, which is destined to become the muscular iris (Fig. 5M,N).

**Expression of TGF-β2 in the nervous system**

There is no obvious high level expression of TGF-β1 or TGF-β3 in neuronal tissue in the embryos examined in this study, although the brain tissue tended to be less well preserved. However, the TGF-β2 probe hybridizes strongly with the ventral region of the nervous cord, demonstrated in a para-sagittal section in Fig. 6. This would be in agreement with the observations of Millan et al. (1990) who saw TGF-β2 expression in the ventral horns of the spinal cord in the mouse embryo at 10.5 days p.c.

**Discussion**

In this study, we report the spatial distribution of RNA transcripts for TGF-β1, TGF-β2 and TGF-β3 during early human embryogenesis. The results are summarised in Table 2.

We have made two assumptions in the interpretation of our data. First, that the gene-specific probes, when used for *in situ* hybridization, recognise *bona fide* mRNAs for the genes they represent. In general, we have found a good correlation between our *in situ* data and RNA gel blot analysis (e.g. Akhurst et al. 1988). Furthermore the gene-specific probes used in this study do recognise transcripts of the predicted size for each

| Table 2. Differential localisation of RNAs encoding TGF-β1, TGF-β2 and TGF-β3 during human embryogenesis |
|-----------------|-------|-------|-------|
|                 | β1    | β2    | β3    |
| Haematopoietic tissue | +     | -     | -     |
| Endothelia       | +     | -     | -     |
| Epithelia        |       |       |       |
| Early tooth bud  | -     | -     | -     |
| Bronchial epithelium | -   | +     | +     |
| Otic epithelium  | -     | +     | -     |
| Lens epithelium  | -     | +     | -     |
| Retina           |       |       |       |
| Immature epidermis | -   | -     | -     |
| Cartilage and Bone |     |       |       |
| Precartilaginous blastema | - | + (limb) | +(iv) |
| Growth zone of long bone | - | +     | -     |
| Perichondria      | -     | -     | +     |
| Hypertrophic cartilage | - | -     | -     |
| Osteoblasts, osteoclasts | + | -     | -     |
| Cardiovascular system |     |       |       |
| Valvular endothelium | + | -     | -     |
| Tunica intima of aorta | - | -     | -     |
| Neuronal tissue   |       |       |       |
| Ventral spinal cord | - | +     | -     |
| Mesothelia        | -     | -     | +     |
| Mesenchyme        | -     | +     | +     |

+, denotes that RNA is abundant at some stage between 32 and 57 days p.c.

-, is below the detection level of *in situ* hybridization.

1 observations from 12 week p.c. material.

iv, intervertebral disc anlagen.
Fig. 2. TGF-βs in cartilage and bone. (A,C,E,G,I,K,M and O) Bright-field images and (B,D,F,H,J,L,N and P) corresponding dark-field images. (A–F) Sagittal section through the mandible of a 57 day p.c. fetus hybridized with the TGF-β1 specific (A,B), TGF-β2 specific (C,D) and TGF-β3 full-length (E,F) probes. (G,H) Sagittal section through the spinal column of a 57 day p.c. fetus hybridized with a TGF-β3 specific probe. (I,J) Trachea of a 57 day p.c. fetus hybridized with a TGF-β3 full-length probe. (K,L) Limb bud of a 43 day p.c. fetus hybridized with a TGF-β2 specific
probe. (M,N) Longitudinal section through a long bone of a 57 day p.c. fetus hybridized with a TGF-β2 specific probe. (O,P) Cross section through the digits of a 57 day p.c. fetus hybridized with a TGF-β2 specific probe. m, Meckel’s cartilage; o, intramembranous ossification; tb, tooth bud; iv, intervertebral disc anlagen; v, vertebral anlagen; p, precartilaginous blastema; hc, hypertrophic cartilage; pz, proliferating chondroblast zone; t, tendon. Scale bar represents 200 μm.
gene and show no cross-reactivity on Northern blots (ten Dijke et al. 1988 and unpublished data). Specificity of hybridization is reinforced by the contrasting expression patterns of the three genes and the cross-species conservation of these expression patterns between mouse and man (see below). The second assumption, that the RNA is translated into biologically active protein, is discussed in detail by Millan et al. (1990).

It has been shown that both TGF-$\beta$1 and TGF-$\beta$2 show differential splicing in primate and porcine tissues. Alternative splicing of TGF-$\beta$2 is not reported to alter the nature of the mature TGF-$\beta$2 protein product (Webb et al. 1988), whereas, in the case of TGF-$\beta$1, the translation reading frame is shifted (Kondaiah et al. 1988). The probes used in this study, when used for in situ hybridization, would not differentiate between the various spliced forms. However, the alternatively spliced TGF-$\beta$1 and TGF-$\beta$2 transcripts are only minor components of the total TGF-$\beta$ mRNA (Webb et al. 1988; Kondaiah et al. 1988); therefore, although we cannot exclude the possibility that the transcripts detected in this study are non-translatable, we believe that this is unlikely, in the majority of cases.

Differential TGF-$\beta$ isoform distribution is conserved between man and mouse

Extensive accounts of differential gene expression patterns for TGF-$\beta$1 (Lehnert and Akhurst, 1988;
Fig. 4. Mesenchymal and mesothelial expression of TGF-βs. (A,C,E,G,H) Bright-field image and (B,D) corresponding dark-field images. (A,B) Coronal section through a 32 day p.c. fetus hybridized with a TGF-β2 specific probe. (C,D) Cross section through the skin of a 57 day p.c. fetus hybridized with a TGF-β2 specific probe. (E,F) Section through the heart and liver of a 57 day p.c. fetus hybridized with the TGF-β3 full-length probe. (G,H) Section through the diaphragm and adjacent liver hybridized with a cardiac actin probe (G) or the TGF-β3 full-length probe (H). e, epidermis; d, dermis; h, heart; m, mesothelium; l, liver; mc, muscle; s, somatic mesoderm. Scale bar represents 200 μm (or 50 μm in G and H).
Fig. 5. Epithelial expression of TGF-βs. (A,C,E,G,I,K,M) Bright-field images and (B,D,F,H,J,L,N) corresponding dark-field images. (A,B) 43 day p.c. fetal lung and (C,D) 57 day fetal lung hybridized with a TGF-β2 specific probe. (E,F) 47 day fetal lung hybridized with a TGF-β3 specific probe. (G,H) 57 day fetal lung hybridized with TGF-β3 full-length probe. (I,J) 57 day inner ear hybridized with a TGF-β2 specific probe. (K,L) 43 day and (M,N) 57 day fetal eye hybridized with a TGF-β2 specific probe. pe, proximal bronchial epithelium; de, distal bronchial epithelium; b, bronchus; s, sensory epithelium; c, simple cuboidal epithelium; l, lens; pr, pigmented retina; nr, neural retina; c, cornea. N.B. pigmented retina gives an artefactual dark-field signal due to reflection from the pigment granules. Scale bar represents 200 μm.
Wilcox and Derynck, 1988; Akhurst et al. 1990a,b; Fitzpatrick et al. 1990; Rappolee et al. 1988) and, to a lesser extent, for TGF-β2 (Pelton et al. 1989) have been reported during murine embryogenesis. It is therefore possible to compare interspecies divergence in expression patterns of these developmentally important genes. As yet, no extensive reports of embryonic expression of murine TGF-β3 have been published, but we have compared the data presented here with that of Millan et al. (1990).

In this study, no epithelial expression of TGF-β1 was observed. This reflects the inherent difficulties of working with human embryonic material. There is not an unlimited supply of well-preserved tissue and it can be difficult to obtain material covering critical developmental events. In the mouse, epithelial expression of TGF-β1 is associated with active morphogenesis, such as during the development of the tooth, salivary gland, hair follicle and secondary palate (Lehnert and Akhurst, 1988; Akhurst et al. 1990b; Fitzpatrick et al. 1990) and expression of this RNA is very transient. During murine secondary palate development, for example, medial edge epithelial expression of TGF-β1 is restricted to only a 24 h time period (Fitzpatrick et al. 1990). In the current study, the equivalent stages of development in the human were not available for study.

In general, the results presented in this paper are in broad agreement with those obtained from studying mouse embryogenesis (Heine et al. 1987; Wilcox and Derynck, 1988; Lehnert and Akhurst, 1988; Pelton et al. 1989; Akhurst et al. 1990a). Discrepancies between the data presented here and those of Pelton et al. (1989) include their report of TGF-β2 expression in areas of ossification, and an absence of this RNA in the epithelia of the lung and other organs. However, our data are entirely consistent with published reports on TGF-β1 RNA distribution in murine embryogenesis, and with our own studies on TGF-β2 and TGF-β3 transcripts.
Fig. 6. Neuronal expression of TGF-β2. (A,C) Bright-field and (B,D) corresponding dark-field images. (A,B,C,D) Parasagittal section through the spinal cord of a 57 day p.c. fetus, hybridized with TGF-β2 specific (A,B) or TGF-β3 specific (C,D) probe. d, dorsal; v, ventral. Scale bar represents 200 μm.
(Millan et al. 1990), at least for the organ systems that were examined. Very minor discrepancies are apparent, which could probably be explained by differential rates of development of different organ systems between mouse and man.

In a clinical context, these conclusions are significant, since it demonstrates a conservation of, at least some, molecular developmental mechanisms between mouse and man, thus reinforcing the use of the former as a suitable model for studying human embryogenesis.

**Differential function of TGF-β isoforms in embryogenesis**

It has been debated whether the different TGF-β proteins have different functions in vivo or whether they are interchangeable in different biological contexts (Roberts and Sporn, 1990). The conservation of the differential expression patterns of these genes during embryogenesis between man and mouse would argue in favour of the functional diversity of members of this gene family. This would also be supported by the fact that interspecies amino acid sequence conservation of individual genes between man and chick is much greater than intraspecies sequence conservation of gene family members (Jakowlew et al. 1988a; Wilcox and Derynck, 1988).

An alternative interpretation is that each gene encodes a protein of very similar function, but that the presence of several family members may have evolved to assist in modulation of gene regulation during development, where very precise temporal and spatial gene activity is required. Conservation of differential gene expression patterns would then reflect evolutionary pressures on maintenance of accurate gene regulatory machinery.

Comparative biological activity studies have mainly been restricted to analysis of TGF-β1 and TGF-β2. Indeed, it has been shown that, in several biological systems, not only are these proteins functionally distinct (Rosa et al. 1988; Jennings et al. 1988; Ohta et al. 1987), but their receptor-binding activities also vary (Segarini et al. 1987; Ohta et al. 1987; Cheifetz et al. 1988). Recent comparative biological activity studies on TGF-β1, TGF-β2 and TGF-β3, show that all three proteins have qualitatively similar, but quantitatively different, biological activities on keratinocyte, fibroblast, osteoblast and endothelial cells (Grayar et al. 1989; ten Dijke et al. 1990; Cheifetz, S., Hernandez, H., Laiho, M., ten Dijke, P., Iwata, K. K. and Massague, J. unpublished data).

The only member of the TGF-β gene family expressed at high levels in human endothelial and haematopoietic tissue is TGF-β1, which is entirely consistent with the TGF-β1 expression pattern of the mouse (Wilcox and Derynck, 1988; Lehnert and Akhurst, 1988; Akhurst et al. 1990a). This observation is particularly significant since comparative studies on the biological activities of TGF-β1 and TGF-β2 at physiological concentrations show that only the former is growth inhibitory to endothelial cells (Jennings et al. 1988). Controversial reports on the differential growth inhibitory activities of TGF-β1 and TGF-β2 on early haematopoietic progenitor cells have, however, been made (Ohta et al. 1987; Sing et al. 1988). Nevertheless, at least for the endothelial cell system, our data provides evidence that differential expression patterns within the embryo are related to functional specificities within this growth factor family. It also indicates that, in vivo, TGF-β1 is an endogenous autocrine growth regulator of both endothelial and haematopoietic cells.

The in vitro biological activity data, together with the possibility that the precursor domains of each TGF-β may confer biological specificity in tissue-targeting, receptor-binding and/or activation of the latent form (Roberts and Sporn, 1990), would support the idea that there is indeed functional specificity of members of the TGF-β family. Only when it is possible to switch gene promoters between the various TGF-β genes in vivo using animal models, will it be possible to unequivocally establish whether this is correct.

**Possible interaction of TGF-βs with other developmental control genes**

Glick et al. (1989) showed that some of the biological effects of retinoic acid on keratinocytes in vivo are mediated by TGF-β2, since they can be blocked by antibodies to this protein. In this context, it is a rather intriguing observation that the transcript distribution of the gamma retinoic acid receptor gene (Dollé et al. 1989; Ruberte et al. 1990) shows considerable overlap with those of TGF-β2 and TGF-β3 (Pelton et al. 1989; Millan et al. 1990 and this report). This includes not only suprabasal keratinocytes (Pelton et al. 1989), but head mesenchyme, precartilage condensations, such as the intervertebral disc anlagen and blastemae of the long bones, and perichondria of ossifying cartilage. It is tempting to speculate that the TGF-βs might be natural mediators of some of the biological effects of endogenous retinoic acid during development.

The predominant mesenchymal localisation of transcripts for TGF-β2 transcripts has been previously demonstrated by Pelton et al. (1989). In the current study, we have shown that, at the later stages of embrogenesis examined, the mesenchymal expression of TGF-β2 and TGF-β3 tends to be localised to specific structures, such as precartilaginous blastemae, tendons and proliferating chondroblasts. However, at earlier stages, a specific association with identifiable structures is less clear. The high transcript prevalence of TGF-β2 in head mesenchyme and somatic mesoderm might suggest a correlation with areas of mesenchymal cell proliferation and migration, both of which are known to be modulated by TGF-βs (Moses et al. 1985; Postlethwaite et al. 1987). Expression of TGF-β2 in the fronto-nasal mesenchyme of the mouse (Millan et al. 1990) and of TGF-β2 and TGF-β3 in condensing mesenchyme of the limbs and vertebral column would support such an hypothesis. If, indeed, some of the effects of retinoic acid were mediated by TGF-βs, one could easily imagine how deformities in facial structure...
might arise by administration of this substance at teratogenic doses.

Another gene that exhibits an overlapping expression pattern with one of the TGF-βs is Pax-1, a paired box-related gene of the mouse which is expressed specifically in the intervertebral disc anlagen in a very similar manner to TGF-β (Deutsch et al. 1988). undulated, a recessive mutation of the Pax-1 gene, leads to vertebral column deformities (Balling et al. 1988). The idea that centrally important developmental genes, such as Pax-1 or the HOX genes, are regulated by growth factors is not novel (Ruiz i Altaba and Melton, 1989). The correlation between TGF-β1 and HOX 7 gene expression during murine cardiac heart valve development has already been noted as one such possible interaction (Robert et al. 1989).

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