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A new type of transforming growth factor-β, TGF-β3

Rik Derynck, Patricia B.Lindquist, Angela Lee, Duanzhi Wen, Joseph Tamm, Jeannette L.Graycar, Lucy Rhee, Anthony J.Mason, Duncan A.Miller[1], Robert J.Coffey[1], Harold L.Moses[1] and Ellson Y.Chen

Departments of Developmental Biology and Molecular Biology, Genentech, Inc., 460 Pt San Bruno Boulevard, South San Francisco, CA 94080 and [1]Department of Cell Biology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

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A new type of TGF-β, TGF-β3, has been identified by cDNA characterization. The amino acid sequence of mature TGF-β3 and its precursor has been derived from porcine and human cDNA sequences. The human TGF-β3 gene is spread over seven exons as in the case of the TGF-β1 gene. Comparison with TGF-β1 and -β2 indicates a strong conservation of the mature sequences, but a relaxed homology in the precursor segments. TGF-β3 mRNA is mainly expressed in cell lines from mesenchymal origin, suggesting a biological role different from the other TGFs-β.

Key words: transforming growth factor-β/proliferation/growth inhibition/growth factor

Introduction

Transforming growth factors-β (TGFs-β) are polypeptides that influence the proliferation and differentiation of many cells types (Massagué, 1987; Sporn *et al.*, 1986, 1987). Two distinct homodimeric TGF-β polypeptides have been identified. TGF-β1 was the first polypeptide characterized and is present in high concentrations in platelets (Childs *et al.*, 1982; Assoian *et al.*, 1983). The mature form contains two identical chains of 112 amino acids that are each synthesized as the C-terminal part of a 390 amino acid precursor (Derynck *et al.*, 1985, 1986). TGF-β2 has been recently identified in bone extracts (Seyedin *et al.*, 1987), porcine blood platelets (Cheifetz *et al.*, 1987) and the medium of some cell lines (de Martin *et al.*, 1987; Marquardt *et al.*, 1987; Hanks *et al.*, 1988). The amino acid sequences of these two mature TGF-β polypeptides are equal in length and are ~70% similar (Cheifetz *et al.*, 1987; de Martin *et al.*, 1987; Marquardt *et al.*, 1987; Seyedin *et al.*, 1987; Hanks *et al.*, 1988; Madisen *et al.*, 1988), but the precursor moieties have a higher degree of structural divergence (de Martin *et al.*, 1987; Hanks *et al.*, 1988; Madisen *et al.*, 1988). Relatively few comparative data on the biological activities of TGF-β1 and -β2 have been published. It appears that both species are about equally potent in some activities such as inhibition of cell proliferation and adipogenic differentiation (Cheifetz *et al.*, 1987). However, there are some striking differences in the activities of both TGF-β species as shown by their differential effect on multipotential hematopoietic progenitor cells (Ohta *et al.*, 1987) and on mesoderm induction in *Xenopus laevis* embryos (Rosa *et al.*, 1988). We report here the existence of a third type of TGF-β and propose the name TGF-β3. The structure of mature TGF-β3 and its precursor has been deduced from cDNAs isolated from porcine and human cDNA libraries. The TGF-β3 gene is spread over seven exons, similarly to the TGF-β1 gene. Comparison with the precursor sequences of TGFs-β1 and -β2 indicates a strong conservation of the mature sequences but a relaxed homology in the precursor segments. Expression of TGF-β3 mRNA is more restricted than in the case of TGF-β1.

Results and discussion

Characterization of porcine and human TGF-β3 precursor cDNAs

A cDNA library in the λgt10 vector was constructed using mRNA derived from a porcine ovary, induced for superovulation (Mason *et al.*, 1985). A 1050 bp human TGF-β1 cDNA insert from λβC1, that comprises the entire sequence for mature TGF-β1 and most of the precursor sequence (Derynck *et al.*, 1985), was hybridized to the phage plaques under low stringency conditions. Approximately 300–600 of the 600 000 plaques displayed a hybridization signal of variable intensity. Some of these plaques presumably corresponded to the porcine TGF-β1 cDNA (Derynck and Rhee, 1987) since they also hybridized under high stringency conditions. Sequence analysis of five cDNA inserts revealed that four of these contained a segment of very high G-C content, yet lacked similarity at the amino acid sequence level with the TGF-β1 sequence. A low stringency hybridization of these cDNAs with the TGF-β1 cDNA probe is presumably due to the high G-C content of the TGF-β1 cDNA sequence (Derynck *et al.*, 1985; Derynck and Rhee, 1987). The fifth cDNA analyzed contained a sequence of ~300 bp with structural similarity at the amino acid level with mature TGF-β1. This cDNA was hybridized to the same ovarian cDNA library (~1.5×10^6 phages) under high stringency conditions. The longest two of the 25 isolated cDNAs were analyzed by nucleotide sequence analysis. Using a porcine cDNA as a hybridization probe, several libraries derived from human cell sources were screened. Three hybridizing cDNAs, H-4, G3-7 and β3-2000, were isolated from an ovary, an A172 glioblastoma and a placenta cDNA library respectively. The combined nucleotide sequence analyses resulted in the cDNA and derived amino acid sequences for the human homologue. The cDNA and deduced amino acid sequences for the human and porcine cDNAs are shown in Figure 1.

The amino acid sequences predicted from the human and porcine cDNA sequences are 410 and 409 amino acids long respectively and have a C-terminal sequence that resembles the previously established sequences for mature TGFs-β. The

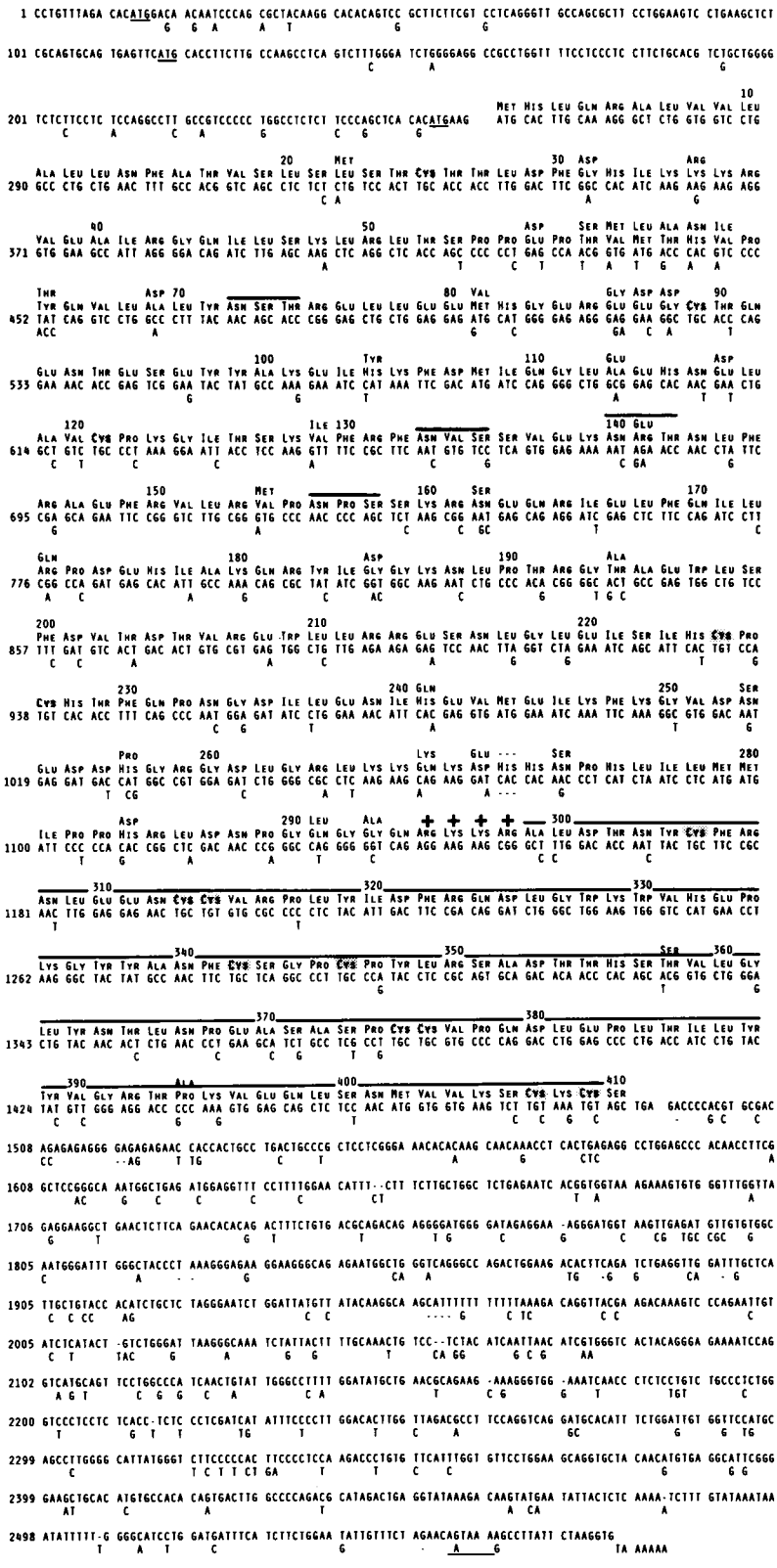

Fig. 1. Nucleotide sequence and deduced amino acid sequence of the human and porcine TGF-β3 precursor cDNAs. The human cDNA and amino acid sequences are shown in full. The porcine sequences differ only in the residues shown below the human nucleotide sequence or above the human amino acid sequence. Dashes indicate the absence of a residue. The 112 amino acid sequence of mature TGF-β3 (overlined) constitutes the C terminus of the porcine and is preceded by four basic residues (+). The precursor segment contains four overlined potential N-glycosylation sites. All cysteine residues are shaded. The AATAAA (porcine cDNA) and the related AGTAAA (human cDNA) sequence close to the 3′ end of the cDNA and preceding the polyadenylation site are underlined.

C-terminal 112 amino acid sequence has ~80% similarity to the porcine (Derynck and Rhee, 1987) and human (Derynck *et al.*, 1985) TGF-β1 sequence and shares a similar degree of homology with the sequence of TGF-β2 (Cheifetz *et al.*, 1987; de Martin *et al.*, 1987; Marquardt *et al.*, 1987; Hanks *et al.*, 1988; Madisen *et al.*, 1988). On the other hand,

```
                        20                  40
hTGF.β3   MHLQRALVVLALLNFATVSLSLSTCTTLDFGHIKKKRVEAIRGQILSKLR
          ********************.********.***.***************
pTGF.β3   MHLQRALVVLALLNFATVSLSMSTCTTLDFDHIKRKRVEAIRGQILSKLR

                60                  80                  100
hTGF.β3   LTSPPEPTVMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENTESEYYA
          *****.*......* *** ***********.**** ..************
pTGF.β3   LTSPPDPSMLANIPTQVLDLYNSTRELLEEVHGERGDDCTQENTESEYYA

                        120                 140
hTGF.β3   KEIHKFDMIQGLAEHNELAVCPKGITSKVFRFNVSSVEKNRTNLFRAEFR
          *** ******** ***.***********.********** *********
pTGF.β3   KEIYKFDMIQGLEEHNDLAVCPKGITSKIFRFNVSSVEKNETNLFRAEFR

                160                 180                 200
hTGF.β3   VLRVPNPSSKRNEQRIELFQILRPDEHIAKQRYIGGKNLPTRGTAEWLSF
          ***.*******.**********.**********.********.******
pTGF.β3   VLRMPNPSSKRSEQRIELFQILQPDEHIAKQRYIDGKNLPTRGAAEWLSF

                        220                 240
hTGF.β3   DVTDTVREWLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIKFKG
          ****************************************.*********
pTGF.β3   DVTDTVREWLLRRESNLGLEISIHCPCHTFQPNGDILENIQEVMEIKFKG

                260                 280                 300
hTGF.β3   VDNEDDHGRGDLGRLKKQKDHHNPHLILMMIPPHRLDNPGQGGQRKKRAL
          **.*** **********.*. *.**********.****** *.********
pTGF.β3   VDSEDDPGRGDLGRLKKKKE-HSPHLILMMIPPDRLDNPGLGAQRKKRAL

                        320                 340
hTGF.β3   DTNYCFRNLEENCCVRPLYIDFRQDLGWKWVHEPKGYYANFCSGPCPYLR
          **************************************************
pTGF.β3   DTNYCFRNLEENCCVRPLYIDFRQDLGWKWVHEPKGYYANFCSGPCPYLR

                360                 380                 400
hTGF.β3   SADTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRTPKVEQLS
          *******.**********************************.******
pTGF.β3   SADTTHSSVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRTAKVEQLS

hTGF.β3   NMVVKSCKCS
          **********
pTGF.β3   NMVVKSCKCS
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Fig. 2. Homology between the amino acid sequences of the human (h) and porcine (p) TGF-β3 precursors. The asterisks mark identical residues while a dot indicates a conservative replacement. The mature TGF-β3 sequences are boxed.

the sequence comparison indicates that this is a new species of TGF-β, distinct from porcine and human TGF-β1 and -β2. We therefore propose the name TGF-β3.

The ATG we propose as the initiator codon for the porcine TGF-β3 precursor is located at nucleotide position 260 in the human sequence (Figure 1) and is in moderate agreement with the proposed consensus sequence for initiation codons (Kozak, 1984). The second codon encodes a His residue which is also the case in the simian (Hanks *et al.*, 1988) and human (de Martin *et al.*, 1987; Madisen *et al.*, 1988) TGF-β2 precursor sequences. In addition, the presumed initiator methionine is closely followed by a hydrophobic amino acid sequence that probably corresponds to the core of the signal peptide (Perlman and Halvorson, 1983; von Heyne, 1986) as in the case of TGF-β1 (Derynck *et al.*, 1985, 1986; Derynck and Rhee, 1987) and TGF-β2 (de Martin *et al.*, 1987; Hanks *et al.*, 1988; Madisen *et al.*, 1988). However, it cannot be excluded that translation initiates six nucleotides upstream at an in-frame ATG which does not conform to the consensus sequence. Another ATG at position 118 is in the same reading frame as the porcine TGF-β3 precursor sequence, but is in a different frame in the human TGF-β3 cDNA sequence, indicating that a single nucleotide deletion took place in the porcine 5′ untranslated cDNA sequence. Yet another ATG is present at position 14 but is followed by an in-frame stop codon (position 92 in the human sequence, Figure 1).

It is not known at what residue the signal peptide is cleaved from the rest of the TGF-β3 precursor. In the case of the TGF-β1 precursor, this cleavage precedes the Leu-Ser-Thr-Cys quadruplet at positions 30−33 (Miyazono *et al.*, 1988). This sequence can also be found in positions 22−25 in the

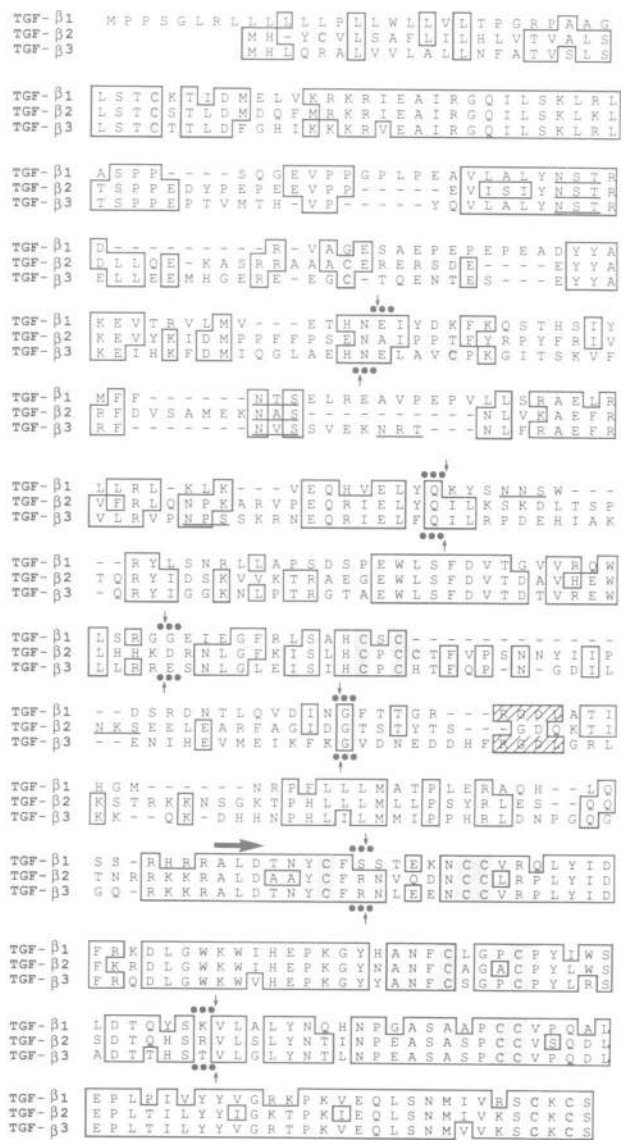


Fig. 3. Polypeptide sequence similarity between the human TGF-β1, -β2 and -β3 precursors at the amino acid level. Dashes are introduced for maximal alignment. Identical amino acids are boxed. The heavy horizontal arrow indicates the start of the mature TGF-β sequences. The cysteine residues are shaded, while the RGDL sequences are cross-hatched. The potential sites for N-glycosylation are underlined. The position of the introns in the TGF-β1 or -β3 genes are indicated above the TGF-β1 or under the TGF-β3 sequence. The three dots represent the nucleotides of the codon for the corresponding amino acid and the arrow marks the insertion point of the intron.

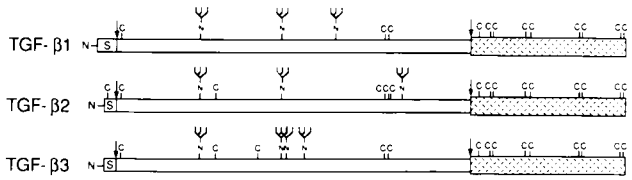
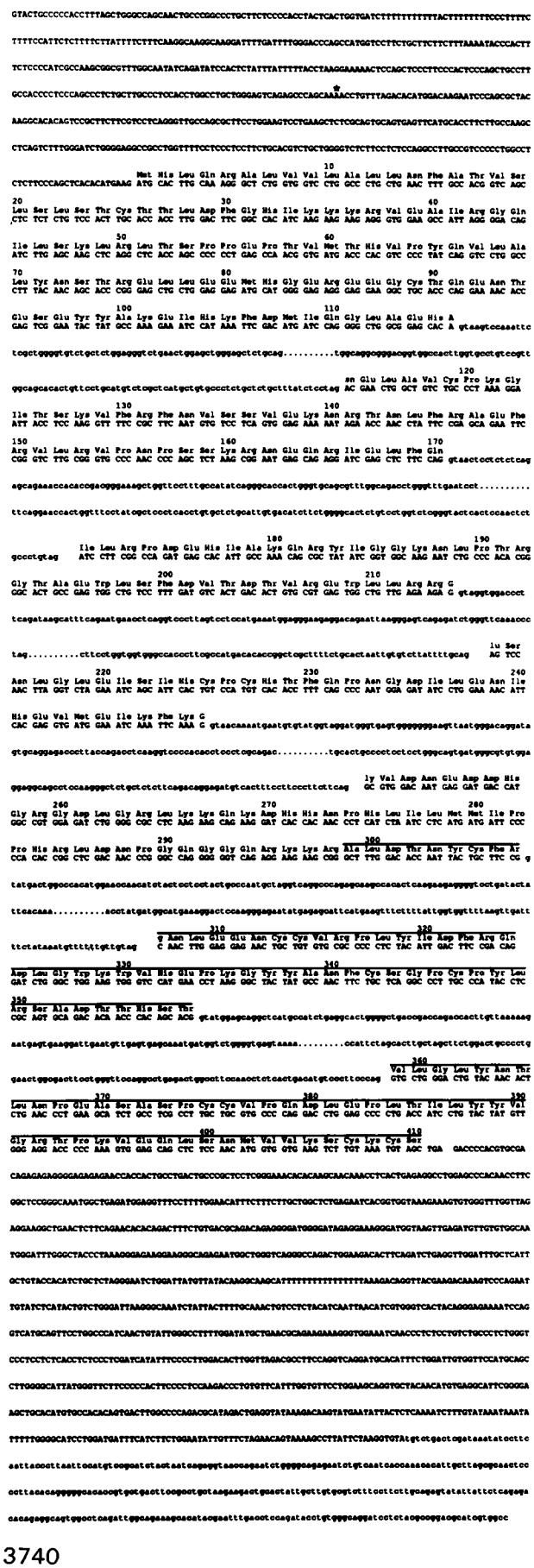


Fig. 4. Schematic representation of the three human TGF-β precursors with the relative positions of the N-glycosylation sites and cysteines (C). The vertical arrows indicate the proposed cleavage sites following the signal peptide (S) and preceding the C-terminal mature TGF-β sequence.

human TGF-β3 precursor (Figure 1). The TGF-β3 precursor sequence contains four potential N-glycosylation sites (Asn-X-Ser or -Thr; Winzler, 1973) and five cysteine residues.

The 112 amino acid TGF-β3 sequence is preceded by four basic residues, as in the case of TGF-β1 (Derynck *et al.*, 1985, 1986; Derynck and Rhee, 1987) and -β2 (de Martin *et al.*, 1987; Hanks *et al.*, 1988; Madisen *et al.*, 1988). By analogy with the other two TGFs-β, one can assume that proteolytic cleavage following this basic tetrapeptide generates the mature TGF-β3 homodimer.



Comparison of the porcine and human TGF-β3 precursor sequences reveals a 90% amino acid identity. The porcine sequence is one amino acid residue shorter due to a single deletion in the precursor segment (position 271, Figure 2). The sequence conservation between both mature TGF-β3 sequences is much stronger since only two conservative amino acid differences are present. The high degree of sequence conservation is also apparent at the cDNA level. However, it is remarkable that both the 5′ and 3′ untranslated sequences are also very highly conserved (Figure 1). This feature is rather uncommon among the known mRNA sequences and may reflect a relevant biological or regulatory role for these non-coding sequences.

Sequence comparison of the three human TGF-β precursors

The establishment of the sequence of this third type of TGF-β and of its precursor allows a comparison of all three human TGF-β precursor sequences (Figure 3). The sequence conservation of the mature TGFs-β includes all nine cysteines which determine the disulfide bridge formation. TGF-β3 is ~80% similar to TGF-β1 and to TGF-β2, while TGF-β2 is 72% similar to TGF-β1. In contrast, the precursor sequences for the three TGFs-β are remarkably dissimilar. Relatively large gaps have been introduced in the sequence in order to achieve maximal similarity. However, some structural features and sequences are conserved in all three precursors, presumably due to their biological significance. Both the TGF-β1 and TGF-β2 precursor contain three potential N-glycosylation sites, in contrast to the TGF-β3 precursor that has four sites (Figures 3 and 4). Two of these are found in all three precursors in corresponding positions. Also conserved in the TGF-β1 and TGF-β3 precursors is the tetrapeptide RGDL (residues 259−262 in Figure 1), which has been detected in several extracellular matrix proteins that are involved in interaction with the cells (Pierschbacher and Ruoslahti, 1984a,b; Ruoslahti and Pierschbacher, 1987). This tetrapeptide is absent in the TGF-β2 precursor. A major difference between the three precursor sequences is their number of cysteine residues. The TGF-β3 precursor segment contains five cysteines versus three in the corresponding TGF-β1 precursor sequence and six in the TGF-β2 precursor segment. Three of these are in corresponding positions in all three precursors (Figures 3 and 4). Recently, it has been shown that, following cleavage from the mature TGF-β1 sequence, the TGF-β1 precursor segment remains hydrogen-bonded with mature TGF-β1 (Miyazono *et al.*, 1988; Wakefield *et al.*, 1988). The 'latent' or inactive TGF-β1 stored in platelets (Pircher *et al.*, 1986) and presumably also the 'latent' TGF-β1 secreted by cells in culture may correspond to this complex. If TGF-β2 or TGF-β3 are also made in 'latent' form, it is possible that these inactive complexes are significantly different from each other and from 'latent'

Fig. 5. Partial nucleotide sequence of the human TGF-β3 gene. The asterisk marks the 5′ most residue of the cDNA (Figure 1b). The incomplete intron sequences are in small letter type. The last nucleotide of the 3′ untranslated region in capitals marks the presumed polyadenylation site. The mature TGF-β sequence is overlined.

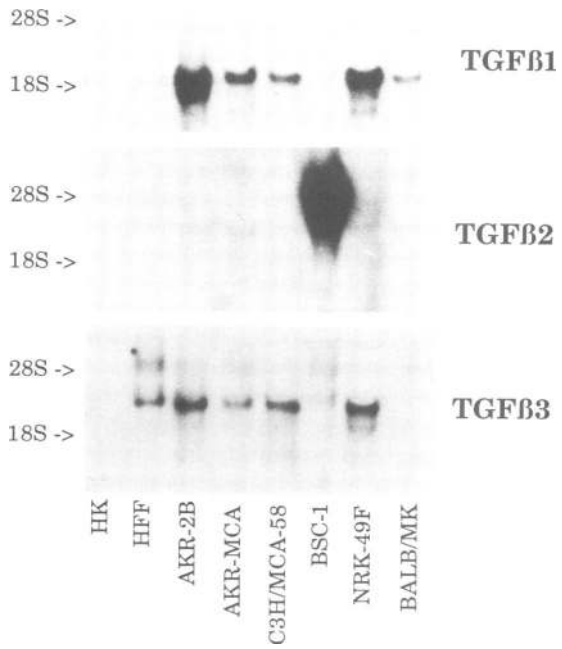


Fig. 6. Northern blot analysis of TGF-β1, TGF-β2 and TGF-β3 mRNA from cultured cells. The cell types are: HK, secondary cultures of human foreskin keratinocytes; HFF, secondary cultures of human foreskin dermal fibroblasts; the rodent fibroblastic cell lines AKR-2B, AKR-MCA, C3H/MCA58 and NRK49F; BALB/MK, a murine keratinocyte cell line and BSC-1, a monkey epithelial cell line.

Table I. Differential expression of TGF-β mRNAs in tissues and cultured cells

	TGF-β1	TGF-β2	TGF-β3
Epithelial cell lines			
A431	+++	+	−
SW480	+++	−	−
SW620	+	−	−
BSC-1	+	++++	−
BALB/MK	++	±	−
Mesenchymal cell lines			
HT-1080	+++	++	++
NRK-49F	+++	+	++
AKR-2B	++	±	++
AKR-MCA	++	±	+
C3H/MCA-58	++	±	+
Cell strains and tissues			
Human keratinocytes (HK)	+	±	±
Human foreskin fibroblasts (HFF)	+	±	+
Rat liver	++	−	−
Dog kidney	++	+	−

The cell lines examined and not illustrated in Figure 6 are derived from a human bronchio-alveolar carcinoma (A-431; Moses *et al.*, 1981), human colon carcinomas (SW480 and SW620; Coffey *et al.*, 1986) and human fibrosarcoma (HT1080; Moses *et al.*, 1981). Estimation of band intensities were made relative to positive controls, which were AKR-2B cells (TGF-β1 and TGF-β3) and BSC-1 cells (TGF-β2) and range from a weak (+) to an exceptionally strong (++++) signal. The rating of ± indicates the presence of definite bands only after prolonged (2-week) exposures, but not with shorter (1−4 day) exposures.

TGF-β1. This is suggested by the structural dissimilarity between the three TGF-β precursor sequences. It is conceivable that several of the sequences conserved in all three precursors may play an important role in the inactivating interaction between the precursor segment and the mature TGF-β. This could certainly be the case for the largest conserved continuous sequence in the precursor segment, located at positions 34−55 in the TGF-β3 sequence (Figures 1, 2 and 3). This sequence is rich in basic residues. The TGF-β3 stop codon in the cDNA sequence is followed by ~1080 bases of 3′ untranslated sequence (Figure 1). The porcine sequence ends with a poly-A tail which is preceded by the hexanucleotide AATAAA that presumably functions as the polyadenylation signal (Proudfoot and Brownlee, 1976). The stop codon of the TGF-β1 cDNA sequence is immediately followed by a very G-C rich sequence that may play a role in transcriptional or translational control (Derynck *et al.*, 1985). A similar sequence also follows the stop codons for inhibin-β_A and -β_B, which are structurally related to TGF-β (Mason *et al.*, 1985). Such a G-C rich sequence is absent in the 3′ untranslated region of the TGF-β3 and -β2 precursor cDNAs.

The human TGF-β3 gene: intron−exon structure

Using the porcine TGF-β3 cDNA as hybridization probe, we have isolated several recombinant genomic phage from a human genomic liver DNA library (Lawn *et al.*, 1978). Detailed analysis and nucleotide sequence determination led to the characterization of the exons and intron−exon junctions in the TGF-β3 gene (Figure 5). As shown in Figures 3 and 5, the TGF-β3 gene contains seven coding exons, while there are no introns in the 3′ untranslated region. It cannot be excluded that there may be one or more introns in the 5′ untranslated region, which is incomplete in the cDNA. We have previously reported that the human TGF-β1 gene is also spread over seven exons (Derynck *et al.*, 1987b). Comparison of the sequences of both the TGF-β1 and -β3 genes indicates that all intron−exon junctions are localized at exactly corresponding nucleotide positions, with the exception of the first intron (Figure 3). The location of this first intron−exon boundary in the coding sequences of the TGF-β1 and -β3 precursors differs by only three nucleotides. This striking conservation of the splice junctions stands in marked contrast to the relatively low degree of sequence similarity of the precursor segments. Areas with high sequence conservation are not encoded in separate exons, as is best illustrated in the fifth exon which contains a segment of the divergent precursor sequence and the beginning of the conserved mature TGF-β. The conserved exon configuration indicates that the existence of genes for both TGF-β1 and -β3 (and also TGF-β2) is a result of an ancestral duplication of the entire gene. Recent chromosomal mapping studies (Brissenden *et al.*, 1985; Barton *et al.*, 1988) have localized the three TGF-β genes to three different chromosomes. These data rule out a tandem duplication as has been suggested for many gene clusters. It is likely that the TGF-β genes have been duplicated in very early times and that the structural similarities at the polypeptide level have been maintained by functional constraints.

Synthesis of mRNA for the three TGF-β precursors

In order to evaluate possible cell sources for TGF-β3

better, Northern hybridizations were done using the human TGF-β1, -β2 and -β3 cDNAs as probe (Figure 6, Table I). Under the stringency conditions used, there was no cross hybridization of the different cDNA probes (data not shown). The major TGF-β1 mRNA species was ~2.5 kb long and could be found in all cell lines tested. The ubiquitous presence of TGF-β1 mRNA in cell lines is consistent with previously reported data (Derynck *et al.*, 1985, 1987a). TGF-β2 transcripts were present in several but not all cell lines and had major transcript sizes of ~4 and 6 kb. These mRNA sizes conform with the previously reported sizes of TGF-β2 mRNA (de Martin *et al.*, 1987; Madisen *et al.*, 1988). TGF-β3 is encoded by a single mRNA species of ~3.2 kb, indicating that the cDNA sequences shown in Figure 1 are close to full length. TGF-β3 mRNA could be detected in several of the cell lines tested, mainly cell lines of mesenchymal origin (Table I). No TGF-β3 mRNA was detected in many cell lines from epithelial origin [Table I; also the cell lines KB, HBL100, MDA-MB 436 and T24 (for references see Derynck *et al.*, 1987a)] and in the hematopoietic cell lines CEM and Raji (data not shown). In related studies, TGF-β3 mRNA has been detected in relatively high abundance (++) in freshly isolated rat testicular myoid and Sertoli cells (M.Skinner *et al.*, unpublished). TGF-β3 mRNA was also present in freshly isolated bovine ovarian theca cells (+), but not in granulosa cells (M.Skinner *et al.*, in preparation). Our data may reflect the possibility that tissues of mesodermal origin are the primary source for TGF-β3 synthesis *in vivo*. Many of the cell lines tested contain several species of TGF-β mRNA.

We have thus established the existence and the structure of a third type of TGF-β mRNA and have derived the amino acid sequence of the mature form and its precursor. TGF-β3 mRNA is synthesized by various cell lines, chiefly of mesenchymal origin. Considering the structural differences among these three types of TGF-β, it will be important to explore their differential expression and their regulation in cell populations *in vivo*. Such evaluation will require specific tools, due to the high degree of sequence conservation of the mature TGFs-β and it is unlikely that detection based on polyclonal antisera will discriminate between the different species. Additional studies will also be needed to evaluate the biological role of all three TGFs-β in relation to each other, especially since the various cell sources may secrete several types of TGF-β.

After submission of this manuscript, ten Dijke *et al.* (1988) reported the human TGF-β3 cDNA sequence. Their sequence is in agreement with ours, although they chose the ATG at position 254 (Figure 1) as initiator codon. There are several differences in the alignment of the sequence conservation between the three TGF-β precursors by ten Dijke *et al.* (1988) and by us (Figure 3).

Materials and methods

Isolation and characterization of cDNAs and gene fragments

About 6 × 10^5 plaques of a λgt10 based porcine ovary library were hybridized with the ^{32}P-labelled (Taylor *et al.*, 1976), 1050 bp long human TGF-β1 cDNA from λβC1. The hybridization took place in 5 × SSC, 20% formamide, 50 mM sodium phosphate pH 6.8, 0.1% sodium pyrophosphate, 5 × Denhardt's solution, 50 μg/ml salmon sperm DNA at 42°C for 15 h. The filters were washed at increasing stringency: 2 × SSC, 0.5 × SSC, 0.2 × SSC and 1 × SSC (all at 42°C). Autoradiography was performed following the washes at a given stringency. The human cDNAs were obtained by hybridizing a human ovary, an A172 glioblastoma and a human placenta library (~1 × 10^6 plaques each) with the porcine TGF-β3 cDNA. Hybridizations were at 42°C in 50% formamide, 5 × SSC, 50 mM sodium phosphate pH 6.8, 0.1% sodium pyrophosphate, 5 × Denhardt's solution, 50 μg/ml salmon sperm DNA and subsequent washes were in 0.2 × SSC at the same temperature. Using the same hybridization conditions and probes, 1.5 × 10^6 recombinant phage from a human genomic liver DNA library (Lawn *et al.*, 1978) were screened. This led to the isolation of 48 phage which were further characterized by hybridization to different TGF-β3 cDNA restriction fragments. Restriction fragments containing all human TGF-β3 precursor exons were derived from the two phage λβ3-24 and λβ3-5. The DNA fragments were subcloned into M13 phage derivatives (Messing *et al.*, 1981) and were sequenced using the dideoxy sequencing methods (Smith, 1980).

Northern hybridization

RNA was prepared from the following cell sources: HK, secondary cultures of human foreskin keratinocytes (Tucker *et al.*, 1984); HFF, secondary cultures of human foreskin dermal fibroblasts (Tucker *et al.*, 1984); AKR-2B, continuous line of nontransformed mouse embryo-derived fibroblastic cells (Moses *et al.*, 1981); AKR-MCA, a chemically-transformed derivative of the AKR-2B cells (Moses *et al.*, 1981); C3H/MCA-58, a chemically-transformed derivative of the C3H/10T mouse embryo derived fibroblastic line (Moses *et al.*, 1981); BSC-1, African green monkey kidney epithelial cell line (Hanks *et al.*, 1988); NRK-49F, rat kidney-derived fibroblastic cell line (Assoian *et al.*, 1983); and BALB/MK, a mouse skin keratinocyte cell line (Coffey *et al.*, 1988). HT1080, a human fibrosarcoma cell line (Moses *et al.*, 1981); A431, a human bronchio-alveolar carcinoma (Moses *et al.*, 1981) and the human colon carcinomas SW480 and SW620 (Coffey *et al.*, 1986). RNA was extracted as described (Schwab *et al.*, 1983). Polyadenylated RNA (4 μg) was electrophoresed into a formaldehyde/1.2% agarose gel (Dobner *et al.*, 1981) and blotted onto nitrocellulose (Thomas, 1980). The nitrocellulose filters were hybridized with ^{32}P-labelled (Taylor *et al.*, 1976) cDNA probes in 50% formamide, 5 × SSC, 0.1% SDS, 1 × Denhardt's, 250 μg/ml salmon sperm DNA and 50 μg/ml poly(A) at 43°C for 18–24 h. Washings were done in 0.1 × (TGF-β1) or 1.0 × (TGF-β2 and TGF-β3) SSC, 0.1% SDS, and 1 mM EDTA at 43°C. The TGF-β1 probe consisted of the 1050 bp cDNA of λβC1 (Derynck *et al.*, 1985). The 2.2 kb *Eco*RI fragment of human TGF-β2 cDNA (Madisen *et al.*, 1988) was used to probe for TGF-β2 mRNA. The human TGF-β3 cDNA, shown in Figure 1b but starting at position 712, was used in Northern hybridization for the TGF-β3 mRNA.

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