Complementary Deoxyribonucleic Acid Cloning of a Novel Transforming Growth Factor-β Messenger Ribonucleic Acid from Chick Embryo Chondrocytes

Sonia B. Jakowlew, Pamela J. Dillard, Paturu Kondaiah, Michael B. Sporn, and Anita B. Roberts

Laboratory of Chemoprevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20892

Transforming growth factorβ 1 (TGFβ 1) has been purified and the mRNA cloned from a number of mammalian species including human, murine, bovine, porcine, and simian. Using a human TGFβ 1 cDNA probe, we have detected two distinct TGFβ RNAs in cultured primary chick embryo chondrocytes. One of these RNAs, migrating at about 1.7 kilobases, shows similarity to mammalian TGFβ 1. The second RNA, migrating at about 3 kilobases, is a novel TGFβ mRNA which we have named TGFβ 3. Clones corresponding to each of these RNAs were isolated from a cultured primary chick embryo chondrocyte cDNA library. Two cDNA clones for TGFβ 3, pTGFB-ChX17 and pTGFB-ChX25, contained a 39 nucleotide-long 5′-untranslated region, a 1236 nucleotide-long coding region, and a 911 nucleotide-long 3′-untranslated region. The predicted protein includes a signal peptide of 20–23 amino acids as in human TGFβ 1 and 2, and a precursor protein consisting of 412 amino acids, which can be cleaved at a lys-arg site to produce a 112 amino acid processed peptide containing nine cysteine residues in the same positions as in human TGFβ 1 and 2. At the nucleotide level, the processed coding region of TGFβ 3 shows 72% and 76% identity with the processed coding regions of human TGFβ 1 and TGFβ 2, respectively; at the amino acid level, TGFβ 3 shows 76% identity with TGFβ 1 and 79% identity with TGFβ 2. RNA Northern analysis shows that TGFβ 3 RNA is expressed at high levels in primary chick embryo chondrocytes that have been cultured for 1–3 days, but is not detected in uncultured chick embryo chondrocytes. TGFβ 3 RNA is also expressed in cultured primary chick embryo fibroblasts and Rous sarcoma virus-transformed chick embryo fibroblasts and in whole chick embryo tissue. (Molecular Endocrinology 2: 747–755, 1988)

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INTRODUCTION

Transforming growth factorβ (TGFβ) is the prototype of a large family of structurally related multifunctional dimeric proteins (for recent reviews see Refs. 1–3). The similarities in this family of peptide effectors reside in the C-terminal cysteine-rich portion of the precursor proteins, and all family members share the identical carboxyl terminus, Cys-X-Cys-X-COOH. TGFβ has been shown to have widespread effects on growth, differentiation, embryogenesis and tissue repair (see refs. 1–3). Thus far, direct functional homologs of TGFβ have been described only in mammalian species; these include two forms of TGFβ called TGFβ 1 and TGFβ 2 which, although only 71% similar at the amino acid level, are interchangeable in most biological assay systems (4). TGFβ 1 and TGFβ 2 display receptor cross-reactivity (4). Each of these TGFβs is highly conserved between mammalian species: the processed 25,000 mol wt dimer of TGFβ 1 is identical in human (5), porcine (6), bovine (7), and simian (8) species, and different in only one amino acid in murine species (9), while that of TGFβ 2 is identical in human (10) and simian species (11). The high degree of evolutionary conservation of these two proteins suggests that they play essential roles in mammalian physiology. TGFβ 1 is synthesized by a variety of normal and malignant cells and is found in relatively high amounts in blood platelets (12, 13). TGFβ 2 has been isolated from bovine bone (14, 15), porcine platelets (4), and from media conditioned by human glioblastoma cells (16), human PC-3 adenocarcinoma cells (17), and BSC-1 African green monkey kidney epithelial cells (11).

In the adult, one of the principal actions of TGFβ is to mediate tissue repair and remodeling (3). However, recent immunohistochemical studies of TGFβ staining in the developing mouse embryo demonstrate that it also plays an important role in organogenesis and mesenchymal-epithelial interactions during periods of mor-

phogenesis (18). Molecules resembling TGFβ also appear to play a role in the very early embryo in the establishment of the germ layers (19). In this case, TGFβ 2, but not TGFβ 1, can induce ectodermal explants from *Xenopus* blastocysts to express mesoderm-specific genes (20), and a mesoderm-inducing activity in the media of tadpole-derived XTC cells (21) has been shown to be related immunologically to TGFβ 2. Other members of the TGFβ family of polypeptides also play roles in embryogenesis. Thus Müllerian inhibitory substance (22), the putative product of the decapentaplegic gene complex in *Drosophila* (23), and the putative product of the Vg1 gene in *Xenopus* (24) have been implicated in embryonic remodeling of the developing male reproductive system of mammals, dorsal-ventral patterning of the fly embryo, and mesoderm induction in amphibian embryogenesis (25), respectively.

Because avian embryogenesis has been so extensively studied, we chose to characterize and clone avian TGFβ homologs to facilitate more detailed analysis of the developmental roles of TGFβ. Using a human TGFβ 1 cDNA probe, two distinct TGFβ cDNA clones were isolated from a chick embryo chondrocyte cDNA library. One clone appears to encode a novel TGFβ that is different from either mammalian TGFβ 1 or 2, while the other clone shows similarity to mammalian TGFβ 1 mRNA. The novel TGFβ mRNA, which we have named TGFβ 3, is expressed in primary cultures of chick embryo chondrocytes that have been cultured for 1–3 days, but is not expressed in uncultured chick embryo chondrocytes. It is also expressed in cultured primary chick embryo fibroblasts, in cultured Rous sarcoma virus-transformed chick embryo fibroblasts and in whole chick embryo tissue.

RESULTS AND DISCUSSION

Northern Analysis of Chick Embryo Chondrocyte RNA

Immunohistochemical analysis of TGFβ expression in the developing mouse embryo has shown that staining of TGFβ 1 is closely associated with the mesenchyme *per se*, or with tissues derived from mesenchyme, such as connective tissue, cartilage, and bone (18). Based on these results, RNA isolated from primary cultures of chondrocytes derived from sterna of 16-day-old chick embryo (hereafter referred to as chondrocytes) was screened for the expression of TGFβ RNA on an RNA Northern blot using a ^{32}P-labeled human TGFβ1 cDNA probe, consisting of a 218 nucleotide single-stranded sequence complementary to the 3′-carboxyl region of the human TGFβ cDNA coding region (7, 26); this region of TGFβ 1 has been shown to be highly conserved in mammalian species (5–9). As a positive control, the probe hybridized to a 2.5 kilobase (kb) TGFβ 1 mRNA from human HT1080 fibrosarcoma cells (Fig. 1A, lanes 1 and 2) (5). The human TGFβ 1 probe hybridized to two distinct chondrocyte RNA species of 1.7 and 3 kb in RNA prepared from cultured primary chick embryo chondrocytes (Fig. 1A, lane 3). Both of these RNAs

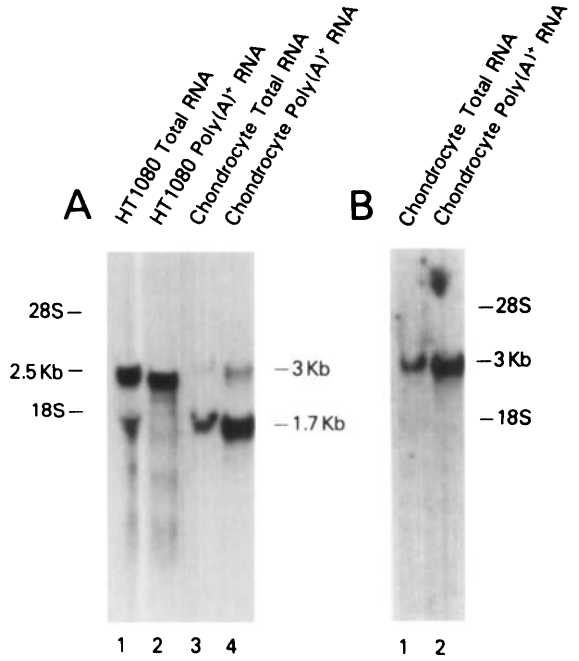

Fig. 1. Northern Analysis of HT1080 and Chondrocyte RNA

A, Total RNA (15 μg) and poly(A)$^+$ RNA (5 μg) isolated from exponentially growing subconfluent HT1080 cells or cultured primary chondrocytes extracted from 16-day-old chick embryo sterna and poly(A) selected using oligo d(T)-cellulose, was electrophoresed on a 1% agarose-formaldehyde gel, and transferred to a Nytran filter as described in *Materials and Methods*. Hybridization was performed with a ^{32}P-labeled probe specific for the mature processed coding region of human TGFβ 1 (7, 26). The sources used are labeled above each lane of RNA. The position of human TGFβ 1 mRNA is shown as 2.5 kb and the position of chick TGFβ 3 is shown as 3 kb. B, Total RNA (15 μg) and poly(A)$^+$ RNA (5 μg) isolated from chondrocytes, electrophoresed, and transferred as in panel A, was hybridized to nick-translated ^{32}P-labeled plasmid pTGFB-ChX17.

appeared to be polyadenylated since they bound to oligo d(T)-cellulose (27) (Fig. 1A, lane 4). Chondrocyte poly(A)$^+$ RNA was used to construct a cDNA library and cDNA clones corresponding to the 3 kb TGFβ mRNA were isolated from this library using the single stranded human TGFβ probe (Fig. 1B). We present evidence that the 3 kb mRNA encodes a novel TGFβ mRNA, which we propose to name TGFβ 3. We have also isolated cDNA clones corresponding to the 1.7 kb mRNA from the same library; DNA sequencing of these clones shows them to be about 85% similar to mammalian TGFβ 1 mRNA at the nucleotide level (Jakowlew, S. B., manuscript in preparation).

Isolation and Characterization of Chondrocyte TGFβ 3 cDNA Clones

Chondrocyte poly(A)$^+$ RNA obtained from one cycle of oligo d(T)-cellulose chromatography (27) was used to make cDNA and double stranded cDNA (ds-cDNA) according to the method of Gubler and Hoffman (28). The ds-cDNA was G-tailed and annealed to plasmid

pUC19 C-tailed at its *PstI* site, and the material was used to transform *E. coli* DH5 cells to ampicillin resistance (29). Twenty six positive cDNA clones were isolated from a sub-library containing 25,000 independent recombinants using the human single stranded 218 base pair (bp) probe (7, 26). The 26 cones contained inserts which ranged in size from 800 to 2200 bp. Six of the cDNA clones contained inserts of about 2200 bp and five cDNA clones contained inserts of about 1400 bp. Restriction enzyme mapping of two of the 2200 bp cDNA clones indicated that these clones were different from the 1400 bp cDNA clones (data not shown). To sequence the 2200 bp cDNA clones, cDNA inserts from two representative clones were subcloned into phagemid Bluescript SK and KS and sequenced using the dideoxy chain termination method of Sanger *et al.* (30).

The cDNA sequence of chondrocyte TGFβ 3 mRNA and the derived amino acid sequence is presented in Fig. 2. The sequence was determined on both DNA strands of each of the two clones to ensure accuracy. The two TGFβ 3 ds-cDNA clones, pTGFB-ChX17 and pTGFB-ChX25, each contain 2220 nucleotide inserts including 20 and 14 dCMP residue tails at the 5′- and 3-ends, respectively. Both clones contain a relatively short 5′-untranslated region consisting of 39 nucleotides, a 1236 nucleotide-long coding region extending from nucleotide 40 to 1275, and a 3′-untranslated region consisting of 911 nucleotides. None of the clones contain a full 3′-untranslated region, as judged by the absence of a poly(A) homopolymer at their 3′-ends as has been reported for cDNA clones for TGFβ 1 and 2 (5, 6, 8–11). Primer extension experiments are currently in progress to determine whether there is a longer 5′-untranslated region.

The deduced amino acid sequence shown in Fig. 2 represents the longest open reading frame that can be obtained from the possible 1236 nucleotide coding region. The translational initiation site was tentatively assigned to the methionine ATG codon at nucleotide position 40 because it is the first methionine codon in the sequence, and the first ATG triplet, by virtue of its position, is usually the preferred initiation codon (31). Translation initiation could also start at the methionine codons at nucleotide positions 46 or 286. While the methionine codon at nucleotide position 286 has flanking nucleotides that conform more to the general eukaryotic initiation codon consensus sequence C-C-G/A-C-C-A-T-G-G (32) than the methionine codons at positions 40 or 46 do, it is doubtful that this could be the true translation initiation codon for the following reasons. First, it is the third ATG codon in the sequence and is relatively far into the proposed coding region. Second, its use as a translation initiation codon would negate both a 21 amino acid similarity with both mammalian TGFβ 1 and 2 that would exist N-terminal to this methionine codon (to be discussed later) and a proposed signal peptide. If the first ATG codon is used for translation initiation, the 2186 bp cDNA codes for a predicted precursor protein of 412 amino acids with a mol wt of 47,180.

An examination of the N-terminal sequence of the putative TGFβ 3 protein suggests that it contains a signal peptide typical of secreted proteins (33). A number of signal peptides have been analyzed in terms of both the amino acid sequences and functions and these peptides typically consist of 15–30 amino acid residues with a highly hydrophobic central core (34–37). As indicated in Fig. 2, the sequence starting with the first methionine at nucleotide 40 exhibits features that are characteristic of a signal peptide. There are two basic residues (Lys and Arg at amino acid positions 2 and 7) followed by nine nonpolar amino acids (at amino acid positions 8–18) interrupted by only two polar amino acids (both being serines) which probably represents the secretory signal peptide core. A Von Heijne (36) analysis predicts that the signal peptidase cleavage site is likely to occur after alanine at position 18 or 23, consistent with the analysis devised by Perlman and Halvorson (37) and this results in a secreted protein of 394 or 389 amino acids; hydropathy plots of human TGFβ 1 and 2 and of chick TGFβ 3 support this conclusion. A lys-arg dibasic cleavage site is found at positions 299 and 300 of chick TGFβ 3 which would result in cleavage of the precursor to a processed 112 amino acid C-terminal peptide in analogy to TGFβ 1 and 2. In this respect, chick TGFβ 3 resembles human TGFβ 2 rather than human TGFβ 1, which is cleaved at an arg-arg site (5, 10, 11).

As with human TGFβ 1 and TGFβ 2, the sequence of the proposed processed coding sequence of chick TGFβ 3 contains nine cysteine residues in the same positions as in the mammalian TGFβ (5–11). The cysteine residues in the precursor region of TGFβ 3 are not as highly conserved when compared to the corresponding regions of human TGFβ 1 and TGFβ 2, although there are striking similarities. The cysteine residues at positions 27, 91, 227, and 229 of chick TGFβ 3 are positioned similarly to the cysteines at 24, 89, 226, and 228 of human TGFβ 2 (10, 11) and cysteines at 33, 224, and 226 of human TGFβ 1 (5–9). The cysteine residue at position 123 of chick TGFβ 3 does not have a counterpart in human TGFβ 1 or 2 and the cysteine at position 91 of chick TGFβ 3 does not have a counterpart in human TGFβ 1. Conversely, the cysteines at positions 4 and 230 in human TGFβ 2 do not have corresponding counterparts in chick TGFβ 3.

Other features of the TGFβ 3 sequence include four potential N-linked glycosylation sites (Asn-X-Thr) which are over-lined in Fig. 2 at positions 74, 135, 142 and 158. Human TGFβ 1 and TGFβ 2 each have three potential N-linked glycosylation sites at positions 82, 136, and 177, and at positions 72, 140, and 241 in human TGFβ 1 and 2, respectively (5, 10). There is a potential fibronectin cellular recognition site (Arg-Gly-Asp) (38) at positions 261–263 in the chick TGFβ 3 precursor. This sequence is also found in the human TGF-B 1 precursor at positions 245–247; however, human TGFβ 2 has no such site.

Comparison of Chick TGFβ 3 with Human TGFβ 1 and 2

A comparison of chick TGFβ 3 protein precursor with human TGFβ 1 and 2 protein precursors is shown in

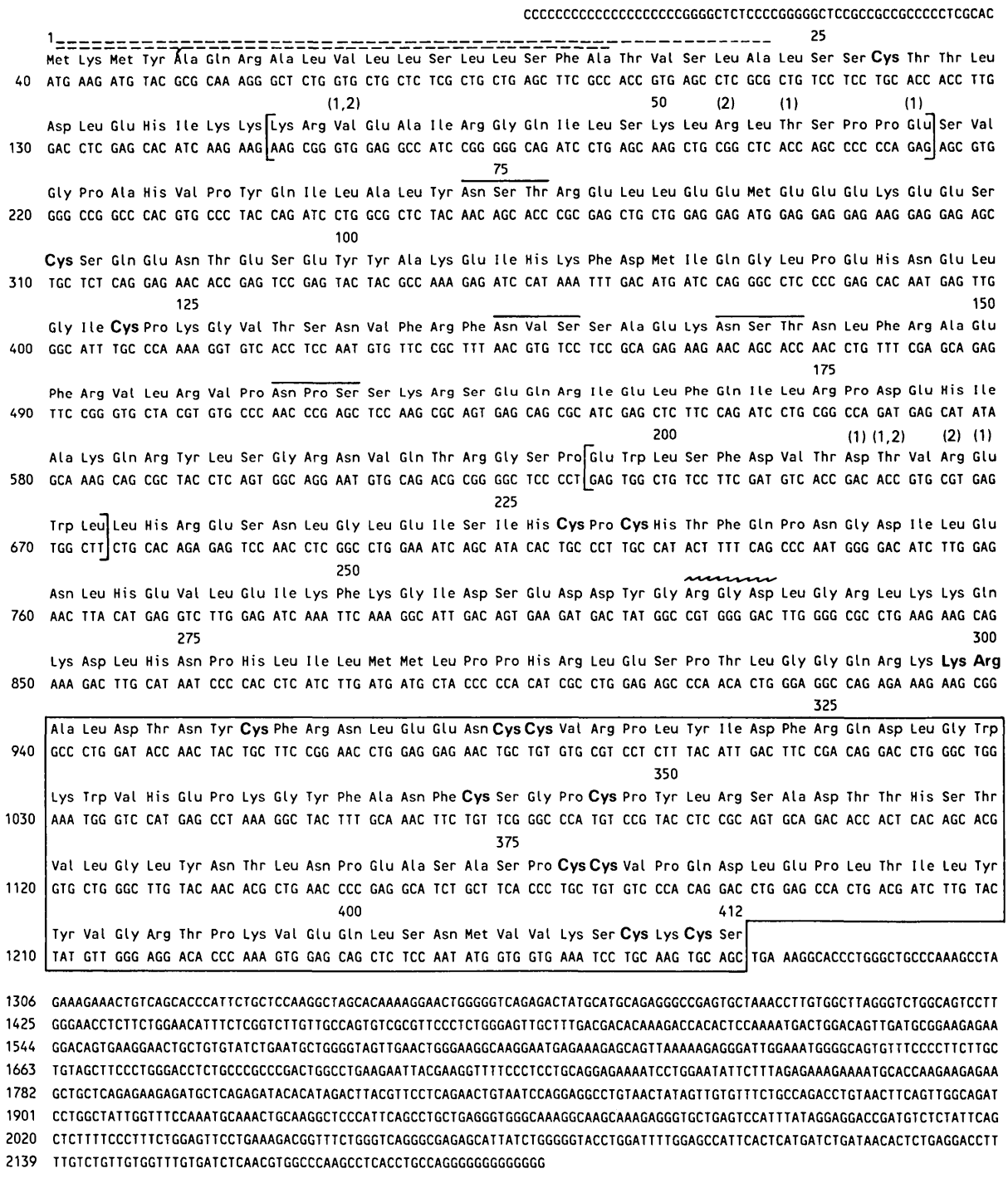

Fig. 2. Nucleotide and Predicted Amino Acid Sequence of Chick Embryo Chondrocyte TGFβ 3

Nucleotide and deduced amino acid sequence of the chick embryo chondrocyte TGFβ 3 cDNA with nucleotides numbered at the beginning of each line designating nucleotide no. 1 as the first G after the C tail. The amino acid residues are numbered above the amino acid sequence in multiples of 25, starting with methionine no. 1 as the first in-frame amino acid. Two potential signal peptide sequences are designated by *dashed lines* at the beginning of the sequence. Potential N-linked glycosylation sites are overlined with a *solid line*. A potential fibronectin cellular recognition site (Arg-Gly-Asp) is designated by a *jagged overline*. The 112 amino acid residues of mature TGFβ 3 (*boxed*) are preceded by a proteolytic processing site, highlighted in *bold* letters. Cysteine residues are outlined in *bold* letters. *Brackets* in the precursor region indicate regions of homology between TGFβ 1, 2, and 3, and *numbers in parentheses* above amino acid residues in these regions indicate an amino acid difference from TGFβ 1 (1) or TGFβ 2 (2).

Fig. 3. Numerous gaps have to be inserted in order to align the three sequences. All of these gaps are located in the precursor portion of the sequence; there is complete alignment of all three processed coding sequences beginning at amino acid 301 (denoted by the *heavy arrow*). More gaps are required to align the sequence of TGFβ 3 with TGFβ 1 than with TGFβ 2 because TGFβ 2 and 3 both contain 412 amino acids

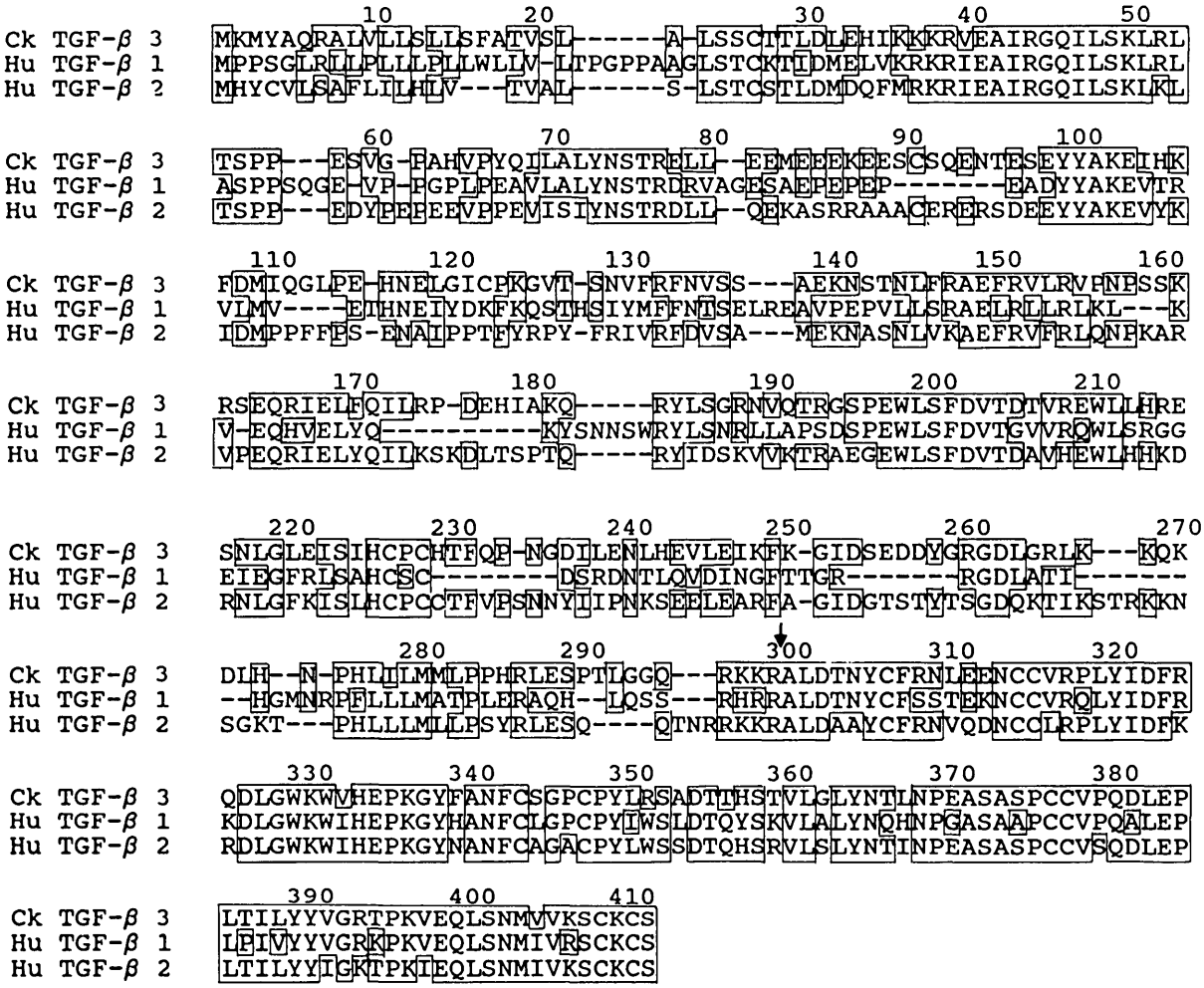

Fig. 3. Comparison of Chick TGFβ 3 and TGFβ 1 and 2 Precursor Polypeptides

Amino acid sequences of chick TGFβ 3 precursor (Ck TGFβ 3), human TGFβ 1 precursor (Hu TGF-β 1) (5), and human TGFβ 2 precursor (Hu TGFβ 2) (10) are aligned for maximal homology. Gaps, designated by *dashed lines*, are introduced into the sequences for alignment. Residue *numbers* are indicated *above the one letter* amino acid *codes*. Positions of identity are *boxed*. The *bold arrow* indicates the beginning of the C-terminal 112 amino acids comprising mature processed TGFβ 3.

while TGFβ 1 contains only 390 amino acids. Alignment of the amino acid sequences of chick TGFβ 3 precursor and human TGFβ 1 precursor shows that 212 amino acids are matched while alignment of TGFβ 3 precursor with human TGFβ 2 precursor shows that 233 amino acids are matched. There is a region of similarity extending from amino acids 38–58 in the precursor sequences of TGFβ 1, 2, and 3 (see also Fig. 2). In this region, there is 86% and 91% identity of chick TGFβ 3 with TGFβ 1 and TGFβ 2, respectively, at the nucleotide level. At the amino acid level, there are only three and two amino acid differences between chick TGFβ 3 and human TGFβ 1 and TGFβ 2, respectively. There is a shorter region of similarity extending from amino acids 198–212 in the precursor sequences of the three TGFβ. Here, there are only three and two amino acid differences between TGFβ 3 and human TGFβ 1 and TGFβ 2, respectively.

There is a striking degree of similarity in the processed coding portion of the TGFβ sequences. Overall, there is 76% identity of chick TGFβ 3 with human TGFβ 1, and 79% identity with human TGFβ 2. There are long stretches of similarity interrupted by differences of only one to three amino acids. At the nucleotide level, the processed coding region of chick TGFβ 3 shows 72% and 76% identity with the processed coding regions of human TGFβ 1 and 2, respectively. The conservation in all three TGFβ of the amino acid sequences in distinct regions of the peptides is particularly striking (see for example amino acids 96–105); most often the regions of substitutions are identical in all three TGFβ, although the substitutions are often unique. Often the amino acid residues of TGFβ 3 are identical to those of TGFβ 1 and 2 at positions that are substituted between TGFβ 1 and 2; however, there are 10 residues that are unique to TGFβ 3 at a substituted position. In addition, there are four residues that are unique to TGFβ 3 at a position conserved between human TGFβ 1 and 2.

Expression of Chick TGFβ 3 mRNA in Chondrocytes

Expression of the 2.5 kb TGFβ 1 mRNA has been observed in a wide variety of normal and transformed cells in culture (for reviews see Refs. 39–41). The fact that many cultured cells constitutively express TGFβ may be related to its stimulatory action on synthesis of matrix proteins which facilitate adaptation to growth on plastic (42–44). Using RNA Northern blot analysis, we compared the expression of TGFβ 3 RNA by uncultured chick embryo chondrocytes with that of chondrocytes cultured for 1, 2, or 3 days (Fig. 4). Probing with nick-translated TGFβ 3 cDNA, a 3 kb RNA band was detected in RNA prepared from the cultured chondrocytes (Fig. 4, lanes 4–6) but not in the uncultured cell preparation (Fig. 4, lane 3); no hybridization was detected to heterologous RNA prepared from human HT1080 cells (Fig. 4, lane 2). As a control, TGFβ 1 RNA was detected in HT1080 cells that were probed with human TGFβ 1 cDNA (Fig. 4, lane 1). The 3 kb TGFβ 3 RNA was also expressed in cultured primary chick embryo fibroblasts, in RSV-transformed chick embryo fibroblasts, and in RNA prepared from whole 16-day-old embryos at 2-, 0.25-, and 0.5-fold, respectively, the level in chondrocytes (Fig. 5). There is evidence that TGFβ 3 RNA is also expressed in some adult chicken tissues (Jakowlew, S. B., manuscript in preparation).

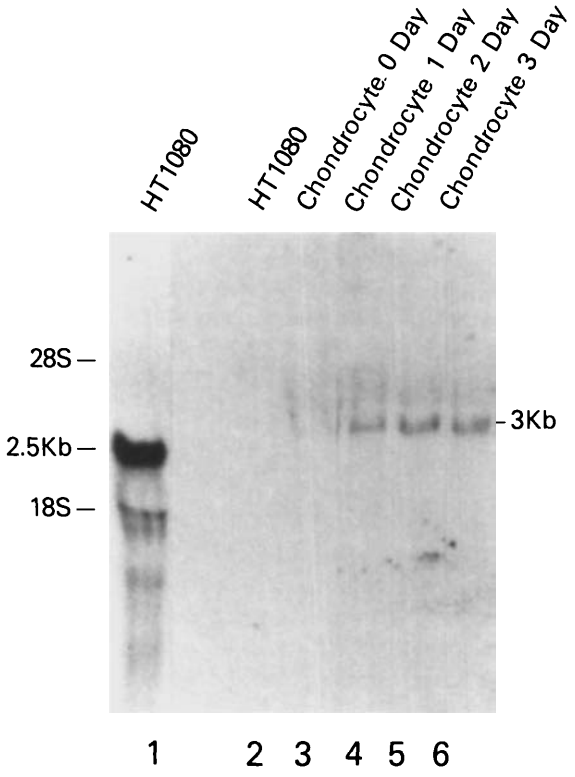

Fig. 4. Northern Analysis of Chondrocyte RNA

Total RNA was isolated from HT1080 cells and chick embryo chondrocytes cultured for 0, 1, 2, or 3 days, electrophoresed using 15 μg RNA and transferred as described in Fig. 1 and in *Materials and Methods*. Hybridization was performed with a ^{32}P-labeled probe specific for the mature processed coding region of human TGFβ 1 (lane 1) (7, 26) or with nick-translated ^{32}P-labeled plasmid pTGFB-ChX17 (lanes 2–6). Human TGFβ 1 RNA is designated as 2.5 kb and chick TGFβ 3 RNA is designated as 3 kb.

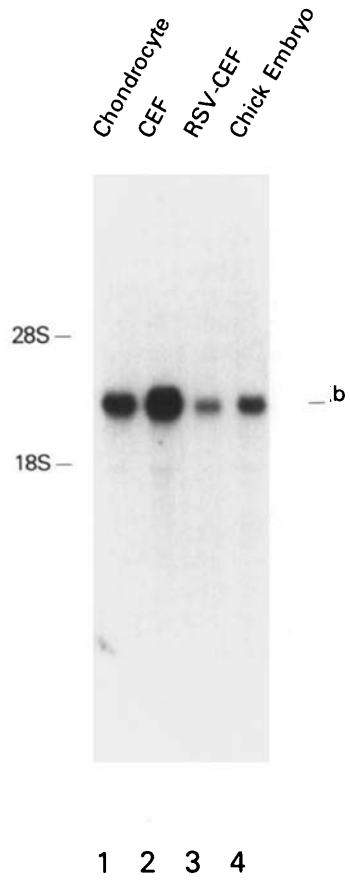

Fig. 5. Northern Analysis of Chick RNA

Total RNA was isolated from 3-day-old cultured primary chick embryo chondrocytes, from exponentially growing subconfluent primary chick embryo fibroblasts (CEF), from Rous sarcoma virus-transformed chick embryo fibroblasts (RSV-CEF), and from 16-day-old whole chick embryo, electrophoresed using 15 μg RNA and transferred as described in Fig. 1 and *Materials and Methods*. Hybridization was performed with nick-translated ^{32}P-labeled plasmid pTGFB-ChX17.

The TGFβ Family and Chick TGFβ 3

A new family of polypeptides that regulate cell growth and differentiation has recently been defined, of which TGFβ is the prototype. Though functionally distinct, these peptides are structurally related to TGFβ and include activins (45, 46), inhibins (47, 48), Müllerian inhibiting substance (22), the putative decapentaplegic gene complex protein in Drosophila (23), and the putative Vg1 protein in *Xenopus* (24). All of these peptides are synthesized as part of larger precursors, as deduced from their cDNA sequences. The similarities in these proteins are restricted to the C-terminal regions of the precursors, which are cleaved to generate the

mature bioactive dimers. The positional conservation of seven of the nine cysteine residues of TGFβ in all of the family members is particularly striking; only the β chains of inhibin contain all nine cysteine residues in similar positions (47, 48).

In contrast to the more distantly related family members, the different TGFβ, TGFβ1 and TGFβ 2, are functionally interchangeable in most assay systems (4). They share a number of similarities with each other and with the chick TGFβ 3 we have described here, that are not common to other members of the family. These include strict conservation of both the number of amino acids in the processed peptide (112) and the number and position of all nine cysteine residues in the processed peptide. In order for the more distantly related peptides to be maximally aligned with the TGFβ, a number of gaps must be introduced; in contrast, no gaps are required to align the amino acid sequences of the processed peptides of human TGFβ 1 and 2 and chick TGFβ 3. TGFβ 1, 2, and 3 show 71–79% identity to each other, whereas, each of these three TGFβ shows only approximately 40% identity with the other members of the family. In addition, a characteristic pattern of conserved regions is found in all three TGF-betas (see Fig. 3) and all terminate in the identical sequence Ser-Cys-Lys-Cys-Ser at the C-terminus. There is a region of similarity unique to the precursor regions of TGFβ 1, 2, and 3, and not found in other members of the family, where there is 86% and 91% identity of TGFβ 3 with TGFβ 1 and 2, respectively. Based on this comparison, it is clear that, although functional similarity cannot yet be demonstrated, this newly described family member is indeed a TGFβ; sequence comparisons suggest that it is a secreted, dimeric peptide like TGFβ 1 and 2. The nearly 100% identity that has been demonstrated thus far for all mammalian TGFβ 1 and 2 suggest that chick TGFβ 3 is a novel TGFβ. Whether it might have a human counterpart and whether it might have a specific developmental role remains to be demonstrated. Experiments are currently in progress to determine more about the properties of this newest member of the TGFβ family.

MATERIALS AND METHODS

General Methods

All methods were as described by Maniatis *et al.* (29) unless otherwise stated.

Preparation of Primary Chick Embryo Chondrocytes

Primary chondrocytes were prepared from the sterna of 16-day-old white Leghorn chick embryos (Truslow Farms, Chestertown, MD) by digestion of excised, muscle-free sterna with 0.5% collagenase in PBS containing calcium and magnesium at 37 C for 3 h. Collagenase was inactivated by the addition of Minimal essential medium containing fetal calf serum. Chondrocytes were centrifuged at low speed (1000 rpm), resuspended in Minimal essential medium containing 10% fetal calf serum and 1% penicillin-streptomycin using 0.5 ml media/sternum, plated in 100-mm plastic dishes, and grown at 37 C in 95% air-5% CO_2 for 1–3 days.

Complementary DNA Synthesis and Library Screen

Construction of the cDNA library from poly(A)$^+$ cultured primary chick embryo chondrocyte RNA was performed according to the method of Gubler and Hoffman (28) using Moloney murine leukemia virus reverse transcriptase. Colony hybridization was performed as described by Maniatis *et al.* (29) using a 218 nucleotide single-stranded probe complementary to the mature protein-coding region of human TGFβ 1 cDNA (7, 26). Moloney murine leukemia virus reverse transcriptase and Klenow large fragment were purchased from BRL (Gaithersburg, MD), oligo d(T)-cellulose (type 3) was from Collaborative Research (Waltham, MA), unlabeled nucleotides, oligonucleotides, and RNAse H were from Pharmacia (Piscataway, NJ), terminal deoxynucleotidyl transferase were from Boehringer-Mannheim (Indinapolis, IN), restriction enzymes were from New England Biolabs (Boston, MA), and labeled dCTP (3000 Ci/mmol) was from Dupont-New England Nuclear (Boston, MA).

DNA Sequence Analysis

Complementary DNA restriction fragments were subcloned into Bluescript plasmid vectors and sequenced according to the Sanger dideoxy chain-termination method (30) modified to use [^{35}S]dATP (49). The Bluescript KS and SK M13 plasmid vectors were obtained from Stratagene (San Diego, CA) and the Sequenase DNA sequencing kit was from United States Biochemicals (Cleveland, OH). Data were analyzed using the IBI-Pustell DNA and protein sequence analysis system.

RNA Northern Analysis

Total RNA was extracted from human HT1080 fibrosarcoma cells (American Type Tissue Culture Collection, Rockville, MD) and chick embryo chondrocytes by the guanidinium thiocyanate-cesium chloride gradient method of Chirgwin *et al.* (50) as modified by Freeman *et al.* (51) and poly(A)-selected by oligo d(T)-cellulose affinity chromatography (Collaborative Research) (27). RNA was separated for Northern RNA blot analysis on 1% agarose gels containing 0.66 M formaldehyde and transferred to Nytran (Schleicher & Schuell, Keene, NH). Hybridization was carried out according to Maniatis *et al.* (29) using 50% formamide, 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50 mM sodium phosphate (pH 7.0), 1% glycine, 0.05% each of BSA, Ficoll, and polyvinylpyrrolidone, 1 μg/ml yeast tRNA, and 100 μg/ml sonicated, denatured calf thymus DNA at 42 C for 48 h. The blots were washed one time in 2× SSC-0.1% sodium dodecyl sulfate (SDS) at 42 C for 15 min, four times in 0.2× SSC-0.1% SDS for 30 min each time at 42 C, two times in 0.2× SSC-0.1% SDS for 0 min each time at 65 C.

Note Added in Proof

Since submitting this manuscript, we have learned that a human TGF-β 3, differing from chick TGFβ 3 only in the substitution of a phenylalanine for a tyrosine at position 340 has been described by Dijke, P., P. Hansen, K. K. Iwata, C. Pieler, and J. G. Foulkes (Identification of a new member of the TGβ gene family. Proc Natl Acad Sci USA, in press).

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Address requests for reprints to: Sonia B. Jakowlew, Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Building 41, Room C629, Bethesda, Maryland 20892.

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