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Transforming Growth Factor-<latex>$\beta $</latex>: Multifunctional Regulator of Differentiation and Development

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Transforming growth factor-β: multifunctional regulator of differentiation and development

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Transforming growth factors-β (TGF-β) are 25 kilodalton (kDa) homodimeric peptides with multifunctional actions controlling the growth, differentiation and function of a broad range of target cells of both epithelial and mesenchymal derivation. They are expressed early in embryogenesis and their tissue-specific and developmentally dependent expression is strongly suggestive of an essential role in particular morphogenetic and histogenetic events. Five distinct TGF-βs have been characterized so far, with 65–80% homology to each other. By using both molecular biological and immunohistochemical techniques, we are currently attempting to define specific sites of expression of the different TGF-βs and to determine whether TGF-βs 1–5 might have unique functions in development and in the mature organism. Comparative study of the promoter regions for the different TGF-βs and for any particular TGF-β in different species is also underway. Mechanistically, TGF-βs act to control gene expression of their target cells, many of their actions converging on a complex, multifaceted scheme of control of matrix proteins and their interactions with cells; these effects on matrix are thought to mediate many of the effects of TGF-β on development.

Introduction

In recent years, there has been an exponential increase in understanding of the chemistry and biology of the family of peptides called transforming growth factor-β (TGF-β). The original narrow definition of TGF-β, in terms of induction of a transformed phenotype in mesenchymal cells (Roberts *et al.* 1983), has now been supplanted by the knowledge that this protein affects many functions in nearly all cells. Its broad spectrum of cellular targets as well as its multifunctional actions suggest that it has a pivotal control function in many physiological and pathological processes. The finding of five distinct, highly conserved, yet functionally similar TGF-βs presents a challenge to unravel the specific roles of each of these closely related, developmentally relevant peptides.

In this article, we review current knowledge on the family of TGF-β peptides, with particular emphasis on their role in developmental processes. The reader is referred to several recent reviews on TGF-β for a more comprehensive overview of field (Roberts *et al.* 1988; Roberts & Sporn 1989); these reviews will often be referred to in lieu of the original references.

The family of TGF-β peptides

The original isolation of TGF-β from human platelets resulted in the identification of a single form of the peptide, a homodimer of relative molecular mass 25000, now called TGF-β1 (Assoian *et al.*, 1983). A few years later, a second form of the peptide, called TGF-β2 was purified from several sources including porcine platelets, bovine bone, as well as cultured cells; although only 71 % homologous, TGF-β2 is interchangeable with TGF-β1 in most biological assays (for review see Roberts & Sporn (1989)). Recently, three new forms of the peptide, called TGF-βs 3, 4, and 5 have been identified by screening of complementary deoxyribonucleic acid (cDNA) libraries (Ten Dikje *et al.* 1988; Derynck *et al.* 1988; Jakowlew *et al.* 1988*a*, *b*; Kondaiah *et al.* 1989). All of the five TGF-βs have 64–82 % homology and share essential structural features such as synthesis from a long precursor and conservation of all nine cysteine residues in the processed peptide (reviewed in Roberts & Sporn (1989) (figure 1). To the extent that it has been examined, each of these different TGF-βs is more than 98 % conserved between species; thus, for example, TGF-β1 is identical in man, monkey, pig, cow and chicken, and TGF-β3 has only one conservative amino acid substitution between man and chicken.

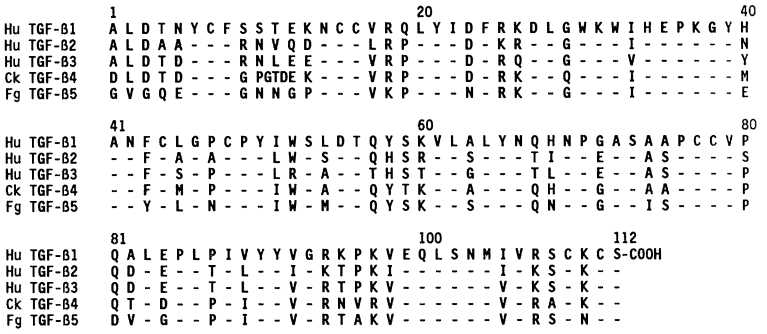

Figure 1. Amino acid sequences of the mature, processed TGF-βs 1–5. A dash indicates that all five peptides share the identical residue.

In addition to the TGF-βs, many other proteins have now been found to belong to the TGF-β supergene family by virtue of amino acid homologies, particularly with respect to conservation of seven out of the nine cysteine residues of TGF-β (see Roberts & Sporn 1989). These proteins have only 30–40 % homology to the TGF-βs and are functionally distinct. A unifying feature of the biology of all of the peptides of the supergene family is their ability to regulate developmental processes. Thus Mullerian inhibiting substance (MIS) induces regression of the female rudiments of the developing male reproductive system; the inhibins and activins regulate the activity of the gonadotropin, follicle stimulating hormone; the bone morphogenetic proteins (BMPs) are though to play a role in the formation of cartilage and bone *in vivo*; the putative product of the decapentaplegic gene complex (DPP-c) in *Drosophila* directs dorsal–ventral patterning in the developing fly embryo, and *Vg*1, an amphibian gene expressed in frog oocytes, is postulated to be involved in the process of induction of mesoderm from ectoderm during gastrulation in the amphibian embryo. The chemical and biological relation of these proteins and the TGF-βs raises the possibility that this superfamily of proteins diverged early in evolution from a common ancestral gene encoding a protein essential to the development of very primitive organisms.

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Expression of recombinant TGF-β3 (S. Watanabe, personal communication) and isolation of TGF-β5 from medium conditioned by *Xenopus* tadpole cells (Roberts *et al.* 1988) have demonstrated that these two TGF-βs also bind to TGF-β receptors and have activity equivalent to TGF-βs 1 and 2 in a standard assay of growth inhibition (Danielpour *et al.* 1989). In addition, like TGF-βs 1 and 2, TGF-βs 3 and 5 are secreted from cells in a biologically latent form that must first be activated before the peptide can bind to signalling receptors; the latent form of TGF-β is a non-covalent complex of the processed peptide and the remainder of the TGF-β precursor (Wakefield *et al.* 1988; Miyazono *et al.* 1988). TGF-β4 has not yet been expressed. It is unique among the TGF-βs in that it lacks a signal peptide sequence, has a shorter precursor (308 amino acids compared with 382–412 for the other TGF-βs), and has an insertion of two amino acids in the processed coding region (Jakowlew *et al.* 1988*b*). Whether TGF-β4 might play a unique intracellular role is currently being investigated.

The discovery of these different forms of TGF-β raises several questions. Why are there so many different forms of TGF-βs? Do the different forms of TGF-β have unique biological activities *in vivo*? Do the different forms of TGF-β bind to common or distinct cellular receptors? Is the expression of the five TGF-βs differentially regulated? And finally, is activation of the latent forms of the different TGF-βs independently regulated?

Developmental roles of the TGF-βs

Evidence obtained so far suggests that expression of the five TGF-βs is regulated differently in development of different species and in specific tissues of any particular organism. Thus, for example, based on both molecular biological and immunohistochemical techniques, it has been shown that TGF-βs 1–3 are prominantly expressed in the developing mouse embryo, but only TGF-βs 2 and 3, not TGF-β1 are expressed in developing nervous tissue (K. Flanders, unpublished data). In contrast, expression of messenger ribonucleic acids (mRNAs) for TGF-βs 1 and 4 are not detectable in any tissues examined in the developing chicken, and expression of TGF-βs 2 and 3 mRNAs follows a similar pattern in certain tissues such as striated muscle, but is differentially regulated in other tissues such as the heart and brain (S. Jakowlew, unpublished data). Moreover, expression of TGF-β5 has been detected only in the frog where it is prominantly expressed in the developing embryo beginning at the neurula stage and sustained in adult tissues (Kondaiah *et al.* 1989).

Although investigations into the temporal and tissue-specific patterns of expression of TGF-βs 2–5 are still in the preliminary stages, there is now an extensive literature on the expression of TGF-β1 during embryogenesis in the mouse, based on both *in situ* hybridization techniques and immunohistochemical staining. The results suggest both autocrine and paracrine modes of action. Expression of TGF-β1 mRNA first appears after fertilization (Rappolee *et al.* 1988) and remains high throughout the remainder of the development of the mouse embryo (Heine *et al.* 1987) and on into neonatal and adult life (Thompson *et al.* 1989). Using *in situ* hybridization, Wilcox & Derynck (1988) have demonstrated prominent expression of TGF-β1 mRNA in haematopoietic cells of early mouse embryos, in agreement with the immunolocalization in foetal bovine liver described by Ellingsworth *et al.* (1986) as well as with the staining of megakaryocytes in adult bone marrow (Thompson *et al.* 1989). In later mouse embryos, Lehnert & Akhurst (1988) have shown *in situ* hybridization of a TGF-β1 probe in foetal bone in both perichondral osteocytes and osteocytes involved in

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intramembraneous ossification; these same cells stain for TGF-β1 protein, which suggests autocrine action (Ellingsworth *et al.* 1986; Heine *et al.* 1987). Similar patterns of *in situ* hybridization have been observed in developing human long bones and calvaria (Sandberg *et al.* 1988*a*, *b*). These data are in agreement with the observations of Robey *et al.* (1988) demonstrating secretion of and response to TGF-β1 by foetal bovine osteoblasts. TGF-β1 mRNA and immunohistochemical staining also co-localize in the submucosa of developing intestine and in the cushion tissue of developing heart valves. The latter is in agreement with recent data of Potts & Runyan (1989), who have demonstrated that TGF-β plays a role in the process of epithelial-mesenchymal cell transformation to yield valve progenitor cells in early development of the chicken heart.

In contrast to these potential examples of autocrine action of TGF-β, in most differentiating tissues that have both epithelial and mesenchymal components, TGF-β1 mRNA is expressed in the epithelial components, whereas the protein is localized to the underlying mesenchymal elements (Heine *et al.* 1987; Lehnert & Akhurst 1988; Flanders *et al.* 1989); examples of such tissues are the developing hair follicles of the snout, the developing tooth bud and the submandibular gland. The simplest interpretation of these data is that TGF-β1 is synthesized by the epithelial cells of these tissues, secreted and localized in the mesenchyme. This concept is also substantiated by recent observations of the staining pattern of two different antibodies to TGF-β1 (Flanders *et al.* 1989) in ectodermal branching in the developing mouse lung (Heine *et al.* 1989). Staining of an antibody that recognizes intracellular TGF-β1 is restricted to epithelial cells of the developing bronchiolar ducts, whereas an antibody, which recognizes preferentially the secreted form of TGF-β1, stains principally mesenchyme, particularly basement membranes surrounding the developing ducts and in clefts of the branches (Heine *et al.* 1989).

The studies of Heine *et al.* (1987) clearly establish that TGF-β1 is localized in a unique pattern, not only spatially, but also temporally, in the developing mouse embryo, correlating with specific mosphogenetic and histogenetic events. For example, the pattern of TGF-β1 staining in the developing somites changes as the somites mature, demonstrating that TGF-β1 contributes to segmentation of the axial skeleton: staining is uniform throughout the primitive somite, but subsequently localizes in the sclerotome and dermatome as development progresses, and finally in the area defining the centrum of the future definitive vertebrae. The rapidly changing staining patterns for TGF-β1 that accompany maturation of the hair follicles (Heine *et al.* 1987) and endodermal branching in the lung and kidney (Heine *et al.* 1989) also suggest a dynamic role for the peptide in control of epithelial–mesenchymal interactions.

Clearly, parallel studies of the expression of TGF-βs 2–5 are now required before we can assign specific developmental roles to these peptides. The extensive sequence conservation of the five TGF-βs makes design of specific antibodies and interpretation of their respective staining patterns difficult (see figure 1). For example, a similar neuronal staining pattern has been observed in mouse embryos by using antibodies raised against either TGF-β2 or TGF-β3 (K. Flanders, unpublished data). Each antibody appears specific as assessed by Western blotting against the respective TGF-βs. The data leave open the questions whether both of these peptides are coordinately expressed in neural tissue, or whether the antibodies are cross-reactive to either TGF-β2 or TGF-β3 on the fixed sections. Future use of antibodies raised against the less highly conserved precursor sequences of the different TGF-βs coupled with *in situ* hybridization under stringent conditions will help resolve such problems.

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Mechanisms of action of TGF-β in embryogenesis

Mechanisms operative in embryonic development have long been thought to be recapitulated in the processes of wound healing and carcinogenesis in the adult. The well-documented central roles of TGF-β in both wound healing and carcinogenesis (reviewed in Roberts *et al.* (1988)), the almost universal distribution of the TGF-β receptor (Wakefield *et al.* 1987), and the potent effects of the growth factor in control of cell migration, growth, differentiation and function, as well as its ability to regulate extracellular matrix, all shed light on the mechanisms of action of the TGF-βs in embryogenesis. Analysis of the role of TGF-β in wound healing provides an excellent example of coordination of seemingly unrelated effects of TGF-β in a complex physiological process. For example, TGF-β1 stimulates chemotaxis of macrophages and fibroblasts, suppresses proliferation of lymphocytes and antibody secretion by B-cells, activates macrophages to secrete other growth factors and stimulates fibroblasts to elaborate connective tissue proteins; all of these actions augment wound healing. Some of the other physiological processes in which TGF-β participates are outlined in figure 2; all of these clearly relate to roles of TGF-β in embryogenesis.

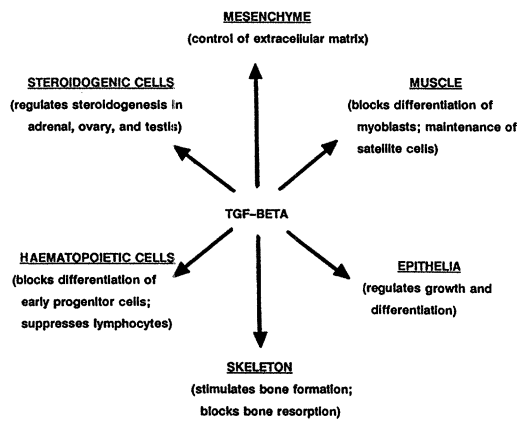

Figure 2. Diagrammatic representation of some of the major physiological systems regulated by TGF-β.

Any analysis of the roles of the TGF-βs must also take into account the multifunctional actions of these peptides. Thus Hill *et al.* (1986) have observed that the growth of very early human embryonic fibroblasts is stimulated by TGF-β1, whereas that of later stage cells is inhibited. Along similar lines, TGF-β1 and TGF-β2 stimulate primitive mesenchymal cells to differentiate and express a cartilaginous phenotype, but treatment of mature chondrocytes with TGF-βs leads to suppression of cartilage markers, such as synthesis of type II collagen (Rosen *et al.* 1988). These examples underscore the plasticity of the cellular response; TGF-β is acting merely as a cellular switch to initiate a new programme of gene expression, which is dependent ultimately on the environment and state of differentiation of the target cell. It should be emphasized that the actions of TGF-β are distinct from those of most other growth factors whose principal effects are mitogenic. TGF-β inhibits the growth of most epithelial cells as well as early haematopoietic progenitors and lymphoid cells, often interfering with the actions of mitogenic growth factors. None the less, it is mitogenic for a select subset of cells including osteoblasts (Robey *et al.* 1988). Any consideration of its role in embryogenesis must take into account the full range of its activities.

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Whereas the receptor-signalling mechanisms of TGF-β are still a mystery, it is certain that the ultimate response to TGF-β action on a cell is a change in the pattern of target cell gene transcription (figure 3). The best studied examples of control of gene transcription by TGF-βs are found in study of its multifaceted actions on connective tissue.

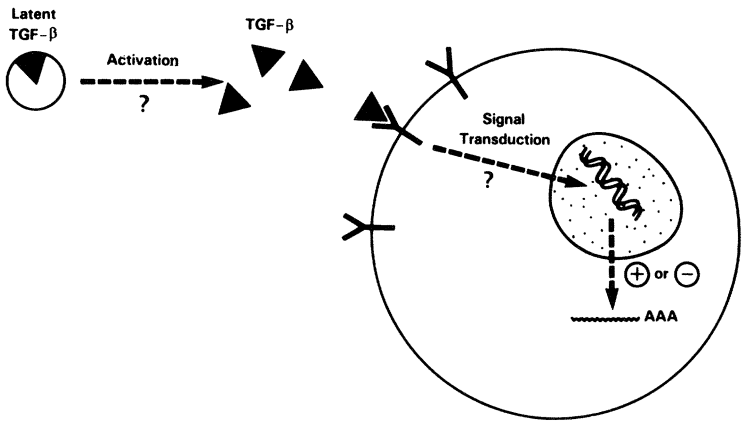

FIGURE 3. Diagrammatic representation of the proposed mechanism of action of TGF-β on cells. The peptide is secreted from cells in a latent form, which must first be activated before it can bind to signalling cellular receptors. Although the signalling pathways are unknown, there is now substantial evidence that TGF-β regulates gene transcription of its target cells.

EFFECTS OF TGF-β ON EXTRACELLULAR MATRIX

Extracellular matrix (ECM) is in a dynamic state of synthesis and degradation, and small changes in the balance between these two processes will lead to selective accumulation or removal of its components, with resultant modulation of its composition. The composition and organization of ECM is an important determinant of cellular behaviour in that it regulates cellular adhesion, migration, proliferation and differentiation (Ekblom *et al.* 1986; Ruoslahti & Pierschbacher 1987). Thus ECM has been implicated in morphogenesis and more generally in embryogenesis, tissue repair and normal as well as pathological physiology.

Many of the effects of TGF-β in embryogenesis are probably mediated by its effects on extracellular matrix (for reviews see Roberts *et al.* (1988); Roberts & Sporn (1989)). Thus TGF-β has been shown to: (i) activate gene transcription and increase synthesis and secretion of many different matrix proteins including proteoglycans; (ii) decrease synthesis of proteolytic enzymes that degrade matrix proteins and increase synthesis of protease inhibitors that block the activity of these enzymes; and (iii) increase both the transcription, translation and processing of cellular receptors for matrix proteins. The multiple levels at which TGF-β acts suggest that control of matrix interactions of cells represents one of the principal mechanisms by which the peptide controls growth, differentiation and function of cells.

Mechanistically, it has been shown that TGF-β increases mRNA levels for most of the matrix proteins for which this has been examined. At a molecular level, it has been shown that TGF-β treatment of cells results in stimulation of the transcription of the mouse α(2)I collagen gene via a binding site for the transcription factor, nuclear factor 1, located in the promoter of that gene (Rossi *et al.* 1988). This same site accounts for part, but not all, of the stimulatory action of TGF-β on transcription of the fibronectin gene (Dean *et al.* 1988, 1989). In addition to these direct enhancing effects of TGF-β on transcription, it has also been shown to stabilize

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both collagen and fibronectin mRNAs (Raghow *et al.* 1987; Penttinen *et al.* 1988). The effects of TGF-β to decrease synthesis and secretion of matrix-degrading proteases and increase that of their inhibitors also occur at the levels both of gene transcription and of stabilization of the respective mRNAs.

Control of expression of receptors for cell adhesion proteins (integrins) is also critical to movements of cells in embryogenesis (Ruoslahti & Pierschbacher 1987). These receptors, which constitute a family of glycoproteins, represent the discrete sites on the cell membrane through which cells both attach to ECM and also link with cytoskeletal elements on the cytoplasmic side (Hynes 1987). Massagué and co-workers are rapidly accumulating evidence for specific and selective regulation of the different α and β subunits of integrin receptors by TGF-β, as demonstrated by analysis of the relative expression of $\alpha_{1-6}\beta_1$ receptors in several different normal and neoplastic cell lines (Ignotz & Massagué 1987; Heino *et al.* 1989). These results suggest that TGF-β might control migration and differentiation of embryonic cells not only through regulation of the composition of ECM, but also by specifically modulating the ability of the cell to adhere to different components of the extracellular matrix.

Clearly, the *in vitro* data suggest that many of the effects of TGF-β on cellular growth and differentiation and, in a broader sense, on embryonic development could be mediated by its effects on regulation of ECM and the integrin family of receptors. Whereas mechanisms are more elusive *in vivo*, the data are nonetheless highly suggestive of the association. Thus immunohistochemical examination of the developmental expression of TGF-β1 and types I and III collagen, fibronectin, and proteoglycans during endodermal branching and cleft formation during lung formation in the 9–15-day-old mouse embryo demonstrates a temporal pattern of expression of TGF-β1 and ECM proteins that is consistent with a role for TGF-β in control of ECM expression (Heine *et al.* 1989).

Specifically, throughout the developmental period resulting in differentiation of the ducts into their bronchiolar and alveolar components, the staining of the ECM components, particularly type III collagen and less pronounced for fibronectin, co-localized with that of TGF-β1. In no instance was staining for TGF-β1 or any of the ECM components ever found to be associated with the tips of terminal buds. Similar localization of TGF-β1 and ECM components has been reported in branching morphogenesis of the mammary gland (G. Silberstein and C. Daniel, personal communication). Collectively, these data suggest that TGF-β could be controlling the pattern of ductal development by local regulation of ECM, and that *in vitro* models of control of ECM by TGF-β are relevant *in vivo*.

Mechanisms of control of TGF-β expression

Ultimately, understanding of the molecular mechanisms governing differential expression of TGF-βs 1–5 during embryogenesis as well as in other situations will require comparative analysis both of the promoter elements of each of the respective genes and of the promoter regions of the same TGF-β in different species. So far, only the human TGF-β1 promoter has been cloned and analysed, although we have recently cloned the human promoters for TGF-βs 2 and 3 as well (T. Noma and R. Lechleider, personal communication).

The human TGF-β1 gene contains two major transcriptional start sites, 271 nucleotides apart (Kim *et al.* 1989*a*). Two distinct promoter regions have been characterized, one extending 1400 base pairs (b.p.) upstream of the first transcriptional start site and the second

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one located between the two start sites. Both of these promoter elements are responsive to autoinduction by TGF-β1 (Kim *et al.* 1989*b*). Within the upstream promoter, two different negative regulatory regions, an enhancer-like region, and a positive regulatory region extending from −453 to −323 have been identified (Kim *et al.* 1989*a*). The negative regulatory regions correspond to the presence of FSE2 negative elements (Distel *et al.* 1987), while the positive regions contain several binding sites for known transcription factors, including nuclear factor 1, SP1 and AP-1, which binds to the region of several promoters responsive to phorbol esters (TRE element).

These studies are laying the groundwork for what will become a very important area of research in terms of understanding, at a molecular level, the various cellular signals regulating TGF-β expression. Recent investigations demonstrating that TGF-β1 is selectively induced in human embryo fibroblasts treated with tamoxifen (Colletta *et al.* 1989) and that TGF-β2 is selectively induced in mouse keratinocytes stimulated to differentiate by elevated calcium concentrations (Glick *et al.* 1989) certainly point to specific control elements in the promoters of the different TGF-βs. It is not yet known whether, through analysis of the promoter elements for these peptides, we will be able to determine why TGF-β1 is expressed in mouse and human, but not in chicken embryos, what signals first turn on and then turn off TGF-β1 expression in the developing hair follicle, or what signals selectively stimulate expression of TGF-β2 and TGF-β3 in neural tissue. The answers to these questions represent important frontiers in advancing our understanding of the role of the TGF-βs in embryogenesis.

Conclusion

Data from both *in vitro* and *in vivo* studies demonstrate that the family of TGF-β peptides plays a specific and unique role in regulation of elaboration of ECM and in control of cell–matrix and cell–cell interactions mediated by integrin receptors. The mechanisms of action of TGF-β in embryonic development, tissue repair, immune response and certain diseases are clearly dependent, in part, on this particular aspect of its biological activity. An exciting area for future research will be to determine whether the various members of the TGF-β family, which now comprises five distinct peptides, will be found to have selective effects on expression of ECM components and integrin receptors *in vivo*.

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