Mini-Review

Some Recent Advances in the Chemistry and Biology of Transforming Growth Factor-Beta

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WITHIN the past two years, there has been an exponential increase in research on transforming growth factor-beta (TGF-beta).[1] In this brief minireview, we cannot provide a detailed survey of this topic (see references 56 and 73 for other reviews). Rather, we will summarize some new results, indicative of the importance of this peptide as a multifunctional regulator of cellular activity. The term "multifunctional" implies that TGF-beta may either stimulate cell proliferation and growth, or inhibit cell proliferation and growth, or have numerous other actions having little relationship to either of these two processes. We will develop the theme that many of the actions of TGF-beta are related to the response of cells or tissues to stress or injury, and to the repair of resultant damage. However, it is clear that there is no one principal action for TGF-beta; moreover, the almost universal cellular distribution of its receptor encompasses a very broad spectrum of target tissues.

Chemical Structure of TGF-beta

Despite a common nomenclature, the purification, cloning, and sequencing of TGF-alpha and TGF-beta have made it clear that these are two entirely distinct peptides, each acting through its own unique receptor system. We will not discuss TGF-alpha in this minireview. TGF-beta was originally purified to homogeneity from human platelets (5), human placenta (24), and bovine kidney (58) and identified as a homodimeric peptide with a molecular mass of 25,000 D. The human cDNA clone sequence indicates that the monomer is synthesized as the COOH-terminal 112 amino acids of a 390-amino acid precursor (15). Recent cDNA cloning in our laboratory has shown that there is total sequence identity between the respective human, bovine, and porcine mature monomer sequences (Van Obberghen, E., and P. Kondaiah, personal communication); there is a single amino acid substitution in the mouse peptide (14).

The original purification of TGF-beta used the stimulation of anchorage-independent growth of normal fibroblast indicator cells as a functional assay (5, 24, 57, 58), and the name of the peptide is based on this activity. Since then, TGF-beta-like molecules have been purified to homogeneity from a variety of sources using other assays, reflecting the multifunctional nature of this set of molecules. Thus, two bovine cartilage-inducing peptides isolated from bone and a human immunosuppressive peptide isolated from glioblastoma cells, when purified and sequenced, were each found to be one or the other of two molecular forms of TGF-beta. These two distinct forms were identified first in bovine bone (66), from which they were isolated using an assay that measures induction of extracellular matrix proteins characteristic of cartilage, and hence were named cartilage-inducing factors A and B (66). Cartilage-inducing factor A is identical (67) to the form of TGF-beta that was originally isolated from human platelets (5), now known as TGF-beta 1 (11), while cartilage-inducing factor B represents a novel molecular form of TGF-beta (65), now called TGF-beta 2 (11). Both forms have subsequently been found in porcine platelets (11), and in both bovine bone and porcine platelets TGF-beta 2 is less abundant than TGF-beta 1, constituting only ~15–20% of the total recovered TGF-beta. This second type of TGF-beta also shows remarkable sequence conservation, in that no differences have yet been found in the respective bovine and porcine TGF-beta 2 sequences (11, 65), in spite of the fact that both have only a 69% homology with the first 36 residues of type 1 TGF-beta. The recent discovery of TGF-beta 2 in the conditioned medium of a human glioblastoma cell line (80) makes it apparent that both types of TGF-beta are also represented in the human genome. Interestingly, the human type 2 peptide was isolated and characterized by its immunosuppressive activity (many glioblastomas are strongly immunosuppressive to the patients with such tumors), and the purification was monitored with assays that measured the inhibition of mitogenesis in T-lymphocytes (80), another known activity of TGF-beta 1 (34). The exact chemical identities of two other peptides clearly related to TGF-beta, namely the growth inhibitor secreted by monkey BSC-1 kidney cells in culture (29, 74), and the myoblast differentiation inhibitor secreted by rat liver cells in culture (21, 45, 50), still need to be established.

In most assays, the two forms of TGF-beta are functionally indistinguishable. However, the data at hand indicate that there may be separate receptors for TGF-beta 1 and TGF-beta 2, some of which are cross-reactive (11, 64). All of these new findings suggest that there may be some unique function for

1. *Abbreviations used in this paper*: EGF, epidermal growth factor; TGF-beta, transforming growth factor beta.

the second form of TGF-beta, but as yet, none has been definitively shown. Is it possibly a protein with a unique embryonic or tissue-specific function? Why is it found in porcine platelets, but not human? It is conceivable that some of the intrinsic cellular activities that have been attributed in the past to "TGF-beta" are a reflection of the endogenous production of TGF-beta 2, as well as TGF-beta 1. Although greater amounts of TGF-beta 2, compared to TGF-beta 1, have not yet been reported in any tissue, this may be a possibility.

It is now also clear that both these forms of TGF-beta belong to a much larger gene family, of which most members have growth-regulatory functions (11, 51). This family includes two forms of inhibin (a gonadal protein that suppresses pituitary secretion of follicle stimulating hormone), three forms of activin (another gonadal protein that stimulates follicle stimulating hormone secretion), Müllerian inhibitory substance (a protein causing regression of the female rudiments in the developing male reproductive system), and a transcript from the decapentaplegic gene complex of *Drosophila*, which acts to control morphogenesis in the fly embryo. Although all of these peptides show strong homology with TGF-beta with respect to the position of seven of nine total cysteine residues, no cross-reactivity with TGF-beta with respect to receptor binding has been shown for any of these peptides.

Latent TGF-Beta

Many studies have shown that TGF-beta is secreted by virtually all cell types in a biologically inactive form (35, 37, 39, 40, 77). The biological latency appears to be due to an inability to bind to the TGF-beta receptor (77). In the laboratory, this latent form has generally been activated by transient acidification, although alkali or chaotropic agents can also activate, suggesting the process may involve disruption of a noncovalent complex (37, 39, 40). Recent studies have begun to identify the chemical nature of this complex and possible physiological mechanisms of activation (35, 76). The complex itself appears to be formed from the association of mature, dimeric TGF-beta, together with the "precursor remainder", which results from the proteolytic cleavage of mature TGF-beta from its precursor (at the Arg-Ala bond in position 278), plus a third component (76). By analogy with the epidermal growth factor- and nerve growth factor-binding proteins, the third component might be a processing protease, involved in cleavage of the TGF-beta precursor.

While the latent complex might be activated in vivo by exposure to acidic microenvironments such as are found in the vicinity of the osteoclast or in healing wounds, it seems more likely that it is activated by the action of exogenous proteases that disrupt the quaternary structure of the complex. Thus, plasmin and cathepsin D can activate latent TGF-beta in vitro (35). The exact chemical structure of latent TGF-beta and the physiological mechanism of activation of the complex are of great importance. Since the cellular receptor for TGF-beta appears to be essentially universally and constitutively expressed (77), the target specificity of TGF-beta action may be determined by the ability of a cell to activate the latent complex, and activation may be a critical regulatory step in TGF-beta action. Thus, for example, platelets release TGF-beta in the latent form (54, 76). This may provide an ideal mechanism to allow sustained action of TGF-beta during stress or injury, since the duration of action of many peptide hormones is very short if they are not protected by a binding protein. In another situation, unregulated epithelial cell growth may be a result of failure to activate the latent form of autocrine TGF-beta. Thus, the human A549 lung carcinoma cell, which has abundant receptors for TGF-beta and is strongly inhibited in its growth by exogenous active TGF-beta, secretes large amounts of TGF-beta in the latent form. However, this tumor cell appears to have lost the ability to activate latent TGF-beta and hence continues to proliferate in the presence of high concentrations of autocrine latent TGF-beta (77); in contrast, the parent normal cell type appears to be inhibited by the TGF-beta that it secretes (Wakefield, L., and T. Masui, unpublished data).

Proliferative Effects of TGF-Beta In Vitro

In most respects, the intrinsic role of TGF-beta in the in vivo physiology of the organism is an unknown. On the other hand, there are now many different actions that have been shown in various cell culture systems, which can arbitrarily be categorized as either proliferative, antiproliferative, or unrelated to proliferation. The original discovery of TGF-beta in an assay that measured promotion of anchorage-independent growth of fibroblasts clearly establishes TGF-beta as a bona fide growth factor, and there are many examples of cells of mesenchymal origin, in which proliferation is enhanced by TGF-beta. One such cell type that is the focus of intense interest at present is the osteoblast, because of the importance of this cell for the formation of new bone during fracture healing, as well as for the maintenance of existing bone to prevent osteoporosis. Two groups (10, 61) have recently shown that not only do cultured osteoblasts have high-affinity receptors for TGF-beta and respond to exogenous TGF-beta with a mitogenic response, they also produce and secrete TGF-beta, implying that there may be some autocrine growth control in bone, possibly related to bone remodeling; the highly acid microenvironment of the osteoclast may provide a mechanism to activate latent TGF-beta released by osteoblasts. The importance of TGF-beta for bone function is further emphasized by its key role in controlling formation of proteins of extracellular matrix, as will be discussed below.

Another cell type that has recently been shown to have a mitogenic response to TGF-beta is the Schwann cell of the peripheral nervous system, for which there are few known mitogens (Ratner, N., personal communication). The Schwann cell makes myelin and is involved in the repair of peripheral nerves after injury, although no role for TGF-beta in these processes is known at present. Like the osteoblast, the Schwann cell is highly specialized for synthesis of extracellular matrix (although the set of matrix proteins formed by Schwann cells is different from those formed by osteoblasts), and it will be of interest to determine whether TGF-beta has any unique role in promoting matrix formation in this particular cell.

Antiproliferative Effects of TGF-Beta In Vitro

TGF-beta is a potent inhibitor of the proliferation of many cells in vitro, particularly of epithelial cells (46, 48, 74). Moreover, these antiproliferative actions are not confined to epithelial cells, and strong antimitogenic effects are seen in mesenchymal cells such as embryonic fibroblasts (1), en-

dothelial cells (6, 22, 26), and T- and B-lymphocytes (33, 34). Based on these in vitro experiments, it appears that TGF-beta might be an important negative growth control for many cell types. Whether TGF-beta truly functions as a physiological negative autocrine or paracrine growth factor in vivo is still unproven. Enhanced levels of mRNA for TGF-beta have been shown in the regenerating liver at a time when DNA synthesis begins to diminish, and it has been suggested that TGF-beta may act as a regulatory signal to stop further cell replication as regeneration is completed (9, 19). Recent studies have also implicated TGF-beta as a negative growth control for mammary epithelium in vivo; minute plastic implants, containing TGF-beta in a sustained release form, were placed in the mammary glands of mice and found to cause marked suppression of the growth of epithelial ductal end buds (Daniel, C., and G. Silberstein, personal communication). However, this use of exogenous TGF-beta does not demonstrate that TGF-beta is an endogenous regulator in vivo, and further studies, using antibodies or antagonists, will be required to prove this point.

During carcinogenesis, the parent cells of the eventual malignant clone may lose their sensitivity to growth regulation by TGF-beta; mechanisms as diverse as failure to synthesize, process, or release TGF-beta; loss of receptors for TGF-beta; loss of ability to activate latent TGF-beta; or a failure in the intracellular TGF-beta signaling pathway have all been suggested as contributing to carcinogenesis. There is some experimental evidence for several of these possibilities (68; Wakefield, L., unpublished data). However, there are epithelial tumor cells that still retain some measure of growth regulation by TGF-beta, particularly the much studied hormonally responsive MCF-7 human breast cancer cell. These cells have functional receptors for TGF-beta and secrete small amounts of biologically active TGF-beta under basal conditions and much larger amounts of active TGF-beta when treated with growth-inhibitory concentrations of antiestrogens such as tamoxifen or its active metabolite, hydroxytamoxifen (36). Thus, in this system, TGF-beta has been shown to be a hormonally regulated growth inhibitor with a negative autocrine action on its producer cell.

Biological Effects of TGF-Beta Unrelated to Proliferation

TGF-beta has effects on many different cell types, unrelated to control of proliferation. These effects are so varied that they do not appear to conform to any particular pattern, although it is of interest that the action of TGF-beta on many cells is often uniquely related to the regulation of the specialized, critical function of a particular cell type.

Perhaps the most striking recent advance that has occurred in TGF-beta studies in the past two years has been the elucidation of an extensive role for TGF-beta in enhancing the formation of extracellular matrix, and this is undoubtedly of major importance with respect to embryogenesis and to repair of tissue injury. TGF-beta has many direct actions on fibroblasts. Thus, it is a potent chemotactic agent for these cells (55). Furthermore, it stimulates matrix formation and inhibits matrix degradation, as shown in Fig. 1. A primary effect of TGF-beta is its strong enhancement of the formation of both collagen and fibronectin in fibroblasts of human, rat, mouse, and chicken origin (30, 60). Within as little as 6 h, significant

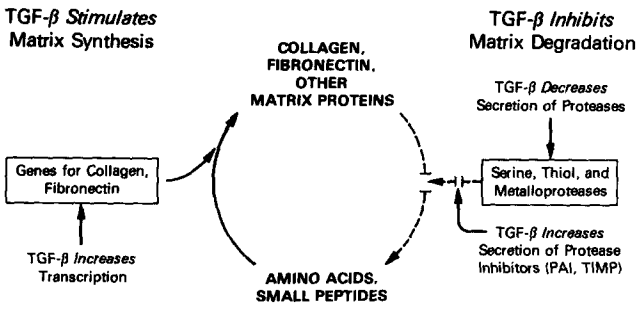

Figure 1. TGF-beta stimulates the formation of extracellular matrix and inhibits its degradation. See text for details.

increases in collagen can be measured; in normal rat kidney cells concentrations of TGF-beta below 100 pg/ml (4 pM) are stimulatory. Recent studies have shown that TGF-beta increases the level of mRNA for collagen (types I, III, and V) and fibronectin in normal rat kidney cells (31; Roberts, A., unpublished data). This increase in collagen and fibronectin mRNAs is, at least in part, the result of a stimulation by TGF-beta of the promoters for the type I collagen gene and the fibronectin gene, as measured in experiments in which the respective collagen or fibronectin promoters (Rossi, P., and B. de Crombrugghe, manuscript submitted for publication; Bourgeois, S., personal communication) have been linked to the reporter gene for chloramphenicol acetyltransferase; nuclear RNA runoff experiments still must be performed to confirm that TGF-beta induces an actual increase in gene transcription. The increased promoter activity for the collagen gene is mediated by a binding site for nuclear factor I (also known as transcription factor CTF), a DNA-binding protein that activates eukaryotic gene transcription (32, 69). These results have been obtained with specific mutants of the promoter of the alpha$_2$(I) collagen gene (63; Rossi, P., and B. de Crombrugghe, manuscript submitted for publication), which indicate that there is a functional binding site for nuclear factor I in this promoter, mediating the stimulatory effect of TGF-beta.

As shown in Fig. 1, a second action of TGF-beta directly related to enhanced formation of extracellular matrix is its ability to inhibit the proteolytic degradation of newly formed matrix proteins. This occurs by two distinct mechanisms, one of which involves an increase in the formation and secretion of protease inhibitors, and the other of which involves a decrease in the secretion of proteases themselves. In several cell types, TGF-beta increases the synthesis and secretion of a plasminogen activator inhibitor (38) (a member of the class of serine protease inhibitors, serpins); it has been suggested that such inhibitors function effectively to stabilize newly synthesized matrix proteins by protecting them from proteolytic degradation. Still another protease inhibitor whose synthesis and secretion is increased by TGF-beta is tissue inhibitor of metalloproteinases, a recently cloned (16) potent inactivator of yet another set of proteases involved in matrix degradation (Postlethwaite, A., and G. Stricklin, personal communication). In contrast to its stimulatory effects on protease inhibitors, TGF-beta decreases the formation or secretion by fibroblasts of three different types of proteases themselves, including a serine protease (plasminogen activator) (38), a thiol protease ("major excreted protein") (12), and a

metalloprotease (transin/stromelysin) (47). In this last case, it has been shown that TGF-beta can block the induction by EGF of the mRNA for transin/stromelysin, which is a major proteolytic enzyme of broad specificity, produced in large quantities by various fibroblasts (20, 23, 79). Thus, the concerted actions of TGF-beta, to increase synthesis of protease inhibitors and to decrease synthesis of proteases themselves, both serve to augment the accumulation of matrix proteins by TGF-beta.

Some of the effects of TGF-beta on extracellular matrix are indirect, and are mediated through an intermediate cell such as the macrophage. Thus, TGF-beta has been found to be an extremely potent chemotactic agent for monocytes (75); peak effects have been found at concentrations as low as 1 pg/ml, which makes TGF-beta the most potent known agent in this regard. At higher concentrations, TGF-beta increases levels of mRNA in these cells for a fibroblast mitogen, such as interleukin 1 (75). Furthermore, macrophages themselves are stimulated to secrete as much as 10-fold more TGF-beta when they are activated by an exogenous agent such as lipopolysaccharide (3). This increased secretion of TGF-beta by activated macrophages could further stimulate fibroblasts to make more matrix.

All of the above actions of TGF-beta are highly relevant to the problems of inflammation and tissue repair in response to injury; presumably they may also be of major importance with respect to the role of extracellular matrix in controlling embryonic development (25).

Mechanism of Action of TGF-Beta

As is true for most growth factors, the overall molecular mechanism of action of TGF-beta is unknown at present. However, the recent discovery of a nuclear factor I–binding site in the alpha$_2$(I) collagen gene, activated by TGF-beta (Rossi, P., and B. de Crombrugghe, manuscript submitted for publication) represents an important advance. This site is found in the promoter for many genes (8, 13, 32, 41, 63, 81), and its widespread occurrence may account, in part, for some aspects of the pleiotropic actions of TGF-beta. Several laboratories have reported that there is no detectable tyrosine kinase activity associated with TGF-beta receptors (18, 42). However, TGF-beta can antagonize the mitogenic actions of other growth factors that do act through a tyrosine kinase receptor, such as EGF (43, 48, 59, 68, 74), platelet-derived growth factor (1), fibroblast growth factor (6, 22), and insulin/insulin-like growth factor-I (43, 48). This antagonistic action of TGF-beta does not appear to occur directly at the respective tyrosine kinase receptors or at some other locus close to these receptors. Thus, it has been shown that although TGF-beta blocks the mitogenic effects of either EGF or insulin in mink lung epithelial cells, it does not block the elevation of ribosomal S6 kinase activity induced by either mitogen (43).

In a related system, it has been found that although mitogenic stimulation of hamster lung fibroblasts by fibroblast growth factor or thrombin is completely blocked by TGF-beta, the various early signals induced by these mitogens are not blocked; these include increases in phospholipid turnover and activation of protein kinase C, in Na^+/H^+ antiport activity, in expression of *myc* and *fos* in the nucleus, and in ornithine decarboxylase activity (Chambard, J., and J. Pouysségur, personal communication). These data all suggest that signals from the TGF-beta receptor probably do not involve pathways common to receptors with tyrosine kinase activity, but rather novel pathways, yet to be discovered, that converge in the nucleus to block DNA synthesis at some step distal to those already known. The further characterization of the structure and function of the TGF-beta receptor (or receptors) is thus one of the most critical problems in this entire field at the present time.

Another important mechanistic consideration is that many of the actons of TGF-beta on individual cell types may be indirect, that is mediated through another cell. This is particularly true for the effects of TGF-beta on angiogenesis: in vitro TGF-beta itself is a strong antimitogenic agent for capillary endothelial cells (6, 22, 26), yet, by virtue of its ability to act as a chemotactic agent for macrophages (75), and presumably by its ability to stimulate macrophages to secrete angiogenic peptides, it can act as a potent stimulator of angiogenesis in vivo (60).

Roles of TGF-Beta In Vivo

At present there is relatively little information about the intrinsic physiologic role of TGF-beta in vivo. Exogenous TGF-beta, prepared by acid-ethanol extraction of platelets (and therefore stripped of the protein that confers latency), has been used in a variety of in vivo studies, which have demonstrated that TGF-beta can stimulate wound healing and can induce the formation of the typical granulation tissue found in tissue repair (49, 60, 72). Two other situations in which TGF-beta has significant actions in cell culture, namely stimulation of osteoblasts and suppression of T- and B-lymphocytes, also have potentially important in vivo applications, but investigations along these lines are still in a preliminary state. The progress that has been made in wound healing studies has benefited greatly from the ability to apply TGF-beta directly to a wound site; better methods for complexing TGF-beta with a binding or a carrier protein will be required for in vivo studies of its potential application for use in bone formation or as an immunosuppressive agent.

At present, very little is known about the intrinsic physiological role of TGF-beta as an endogenous mediator of cell function in vivo. Immunohistochemical studies have begun to implicate TGF-beta as a mediator that may be important in controlling proliferation and differentiation in the developing embryo (17, 27), particularly in the differentiation of tissues of mesenchymal origin such as bone, muscle, blood vessels, and blood cells, as well as in the differentiation of various epithelial tissues. There is no question that TGF-beta functions as a mediator of inflammation and repair, since it is known to be released from platelets when they are degranulated with thrombin (2) or from activated macrophages (3) and T-lymphocytes (34). Whether there are disease states in which TGF-beta contributes to pathogenesis by virtue of provoking an excessive inflammatory response (such as overrecruitment of macrophages) or an excessive repair response (overproduction of matrix proteins such as collagen, characteristic of many proliferative diseases) remains to be determined.

The intrinsic roles of TGF-beta in the physiology of bone, the immune system, and many other tissues also remain to be determined. It is not yet clear why bone has such a high concentration of TGF-beta relative to most soft tissues (66),

and the possible roles of TGF-beta in bone remodeling or repair are important problems for the future. It has recently been found that agents that stimulate bone resorption, such as parathyroid hormone, 1,25-dihydroxyvitamin D_3, and interleukin 1, all increase TGF-beta activity in organ cultures of fetal rat or neonatal mouse calvaria (53). Whether TGF-beta is a direct mediator of the action of these agents, or whether the increase in its activity is a compensatory response to their presence, is not yet known. With respect to the immune system, the identification of TGF-beta 2 as a principal mediator responsible for the in vivo immunosuppression in a patient with glioblastoma suggests that this molecule might have an important intrinsic function in controlling cell kinetics and function in lymphocytes and monocytes (80). Finally, almost nothing is known about the physiological role of TGF-beta in adult tissues such as brain, heart, and kidney, in which there is little mitotic activity. In the initial description of TGF-beta as a new type of molecule, it was noted that the specific activity of TGF-beta–like peptides in acid-ethanol extracts of brain, heart, and kidney was almost the same as in extracts of sarcoma cells (57).

Future Directions

The numerous actions of TGF-beta in regulating epithelial cell proliferation, the growth and activity of immune cells, and the synthesis and degradation of extracellular matrix, all suggest that significant interactions will be found between the TGF-beta system and two other major classes of regulatory molecules, namely the glucocorticoids and the retinoids. There is a particularly impressive overlap in many of the actions of retinoids and TGF-beta, and it is attractive to suggest that the mechanisms of action of these two different molecules might be closely related (71). There are numerous loci at which one might suggest interactions, but this would be entirely speculative at the present time. The TGF-beta system can potentially be regulated by controlling synthesis of either the peptide itself or its receptor, and as we have noted above, regulation of the activation of the latent form also appears to be an important physiological process.

Little is known about promoters or enhancers of the genes for either TGF-beta 1 or 2, and we would suggest that activation of such promoters or enhancers might be useful targets for development of new pharmacological agents. There is good reason to believe that preneoplastic epithelial lesions, as compared with malignant cancers, should be more susceptible to control by an inhibitory growth factor (70) such as TGF-beta; therefore, an increase in TGF-beta gene transcription and translation in epithelial target cells might offer a new approach to chemoprevention of epithelial cancer, which is characterized in general by a very prolonged, premalignant, latency period. In any event, the elucidation of the regulatory elements that control transcription of the TGF-beta gene is a problem of paramount importance.

We have stressed the importance of TGF-beta in repair of tissue damage, and this suggests that there may be significant interactions between TGF-beta and another system that appears to be intimately involved in repair of cellular damage, namely the family of heat shock proteins (28, 52, 62). At present no such relationships are known; however, one might speculate that TGF-beta exerts some transcriptional or translational control over heat shock genes, as it is known to do for collagen and fibronectin genes. The occurrence of similar nuclear factor I binding sites in the promoters for collagen, fibronectin, and heat shock genes (8, 13, 41, 63, 81) suggests that this may be the case. Many of the roles of TGF-beta in tissue repair are suggestive of roles that it might also play in embryonic development, and studies in these two areas should complement each other. Indeed, cellular and biochemical mechanisms involved in embryogenesis may be reiterated during tissue repair in the adult (25); collagen, fibronectin, and heat shock gene products all play major roles in early embryonic development. In this regard, we would like to suggest that TGF-beta is a morphogenetic substance, one that "gives form to things." TGF-beta is a molecule that somehow regulates and organizes the activities of other growth factors (73), and at the cellular level even appears to be able to organize cells into functional units. Recent experiments showing that TGF-beta can induce disorganized capillary endothelial cells to form tubular structures in three-dimensional collagen gel cultures are germane to this concept (Madri, J., personal communication).

With respect to embryonic development, new studies have shown specific immunohistochemical localization of TGF-beta in early mouse embryos in both the notochord and somites (27), critical structures in embryonic morphogenesis. In a similar way, the product of the decapentaplegic gene complex, related to TGF-beta, is important in morphogenesis of the *Drosophila* embryo, particularly in establishing positional information and dorso-ventral patterning of embryonic structures (51). It will be of interest to determine whether TGF-beta might have a similar role, in the vertebrate embryo, in establishing the segmental pattern of somites, which in turn is responsible for the segmental nature of much of the musculoskeletal system in the adult (7). Although there is no known relationship between TGF-beta–like molecules and the function of the products of other patterning genes in *Drosophila* embryos, the known ability of TGF-beta to regulate the function of both EGF and its receptor (4, 44) in vertebrate cells and the known presence of EGF-like proteins in *Drosophila* (78), suggest that such relationships might exist. In this regard it will be of great interest to see if additional members of the TGF-beta gene family, beyond the decapentaplegic gene complex, will be discovered in *Drosophila*. There is no reason to believe that all members of the TGF-beta gene family have already been discovered, either in *Drosophila* or vertebrates. The evolutionary origin of this entire family is unknown at present.

Conclusions

At the rate that investigation of TGF-beta is proceeding, we have only seen the tip of the iceberg, especially in terms of understanding its physiology in vivo, in both the developing embryo and the adult animal. The discovery of a second form of TGF-beta offers a unique opportunity to investigate many problems relating to the specificity of the mechanisms of action of these substances. The unusual conservation of amino acid sequences across many species suggests that the functions controlled by both forms of TGF-beta are of critical survival value to the organism. The finding that TGF-beta activates a collagen gene promoter and that this activation is mediated by a functional binding site for the transcription

factor, nuclear factor I, represents a new advance in understanding the molecular mechanism of action of TGF-beta. The opportunities to use both TGF-beta 1 and 2 for practical therapeutic purposes remain an exciting challenge.

We dedicate this review to the memory of our colleague, George Khoury, who contributed so much to the knowledge of promoters and enhancers.

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References

1. Anzano, M. A., A. B. Roberts, and M. B. Sporn. 1986. Anchorage-independent growth of primary rat embryo cells is induced by platelet-derived growth factor and inhibited by type-beta transforming growth factor. *J. Cell. Physiol.* 126:312–318.
2. Assoian, R. K., and M. B. Sporn. 1986. Type-beta transforming growth factor in human platelets: release during platelet degranulation and action on vascular smooth muscle cells. *J. Cell Biol.* 102:1217–1223.
3. Assoian, R. K., B. E. Fleurdelys, H. C. Stevenson, P. J. Miller, D. K. Madtes, E. W. Raines, R. Ross, and M. B. Sporn. 1987. Expression and secretion of type beta transforming growth factor by activated human macrophages. *Proc. Natl. Acad. Sci. USA.* In press.
4. Assoian, R. K., C. A. Frolik, A. B. Roberts, D. M. Miller, and M. B. Sporn. 1984. Transforming growth factor-beta controls receptor levels for epidermal growth factor in NRK fibroblasts. *Cell.* 36:35–41.
5. Assoian, R. K., A. Komoriya, C. A. Meyers, D. M. Miller, and M. B. Sporn. 1983. Transforming growth factor-beta in human platelets. *J. Biol. Chem.* 258:7155–7160.
6. Baird, A., and T. Durkin. 1986. Inhibition of endothelial cell proliferation by type-beta transforming growth factor: interactions with acidic and basic fibroblast growth factors. *Biochem. Biophys. Res. Commun.* 138: 476–482.
7. Bellairs, R., D. A. Ede, and J. W. Lash, editors. 1986. Somites in Developing Embryos. Plenum Publishing Corp., New York. 320 pp.
8. Bienz, M., and H. R. B. Pelham. 1986. Heat shock regulatory elements function as an inducible enhancer in the Xenopus *hsp70* gene and when linked to a heterologous promoter. *Cell.* 45:753–760.
9. Carr, B. I., I. Hayashi, E. L. Branum, and H. L. Moses. 1986. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type-beta transforming growth factor. *Cancer Res.* 46:2330–2334.
10. Centrella, M., T. L. McCarthy, and E. Canalis. 1987. Transforming growth factor beta is a bifunctional regulator of replication and collagen synthesis in osteoblast-enriched cell cultures from fetal rat bone. *J. Biol. Chem.* 262:2869–2874.
11. Cheifetz, S., J. A. Weatherbee, M. L.-S. Tsang, J. K. Anderson, J. E. Mole, R. Lucas, and J. Massagué. 1987. The transforming growth factor-beta system, a complex pattern of cross-reactive ligands and receptors. *Cell.* 48:409–415.
12. Chiang, C.-P., and M. Nilsen-Hamilton. 1986. Opposite and selective effects of epidermal growth factor and human platelet transforming growth factor-beta on the production of secreted proteins by murine 3T3 cells and human fibroblasts. *J. Biol. Chem.* 261:10478–10481.
13. Dean, D. C., C. L. Bowlus, and S. Bourgeois. 1987. Cloning and analysis of the promoter region of the human fibronectin gene. *Proc. Natl. Acad. Sci. USA.* 84:1876–1880.
14. Derynck, R., J. A. Jarrett, E. Y. Chen, and D. V. Goeddel. 1986. The murine transforming growth factor-beta precursor. *J. Biol. Chem.* 261: 4377–4379.
15. Derynck, R., J. A. Jarrett, E. Y. Chen, D. H. Eaton, J. R. Bell, R. K. Assoian, A. B. Roberts, M. B. Sporn, and D. V. Goeddel. 1985. Human transforming growth factor-beta cDNA sequence and expression in tumor cell lines. *Nature (Lond.).* 316:701–705.
16. Docherty, A. J. P., A. Lyons, B. J. Smith, E. M. Wright, P. E. Stephens, and T. J. R. Harris. 1985. Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity. *Nature (Lond.).* 318:66–69.
17. Ellingsworth, L. R., J. E. Brennan, K. Fok, D. M. Rosen, H. Bentz, K. A. Piez, and S. M. Seyedin. 1986. Antibodies to the N-terminal portion of cartilage-inducing factor A and transforming growth factor beta. *J. Biol. Chem.* 261:12362–12367.
18. Fanger, B. O., L. M. Wakefield, and M. B. Sporn. 1986. Structure and properties of the cellular receptor for transforming growth factor type beta. *Biochemistry.* 25:3083–3091.
19. Fausto, N., J. E. Mead, L. Braun, N. L. Thompson, M. Panzica, M. Goyette, G. I. Bell, and P. R. Shank. 1987. Proto-oncogene expression and growth factors during liver regeneration. *Symp. Fundam. Cancer Res.* In press.
20. Fini, M. E., M. J. Karmilowics, P. L. Ruby, A. M. Beeman, K. A. Borges, and C. E. Brinckerhoff. 1987. Cloning of a cDNA for rabbit proactivator: a metalloproteinase that activates synovial cell collagenase, that shares homology with stromelysin and transin and that is co-ordinately regulated with collagenase. *Arthritis Rheum.* In press.
21. Florini, J. R., A. B. Roberts, D. Z. Ewton, S. L. Falen, K. C. Flanders, and M. B. Sporn. 1986. Transforming growth factor-beta. A very potent inhibitor of myoblast differentiation, identical to the differentiation inhibitor secreted by Buffalo rat liver cells. *J. Biol. Chem.* 261:16509–16513.
22. Fràter-Schröder, M., G. Müller, W. Birchmeier, and P. Böhlen. 1986. Transforming growth factor-beta inhibits endothelial cell proliferation. *Biochem. Biophys. Res. Commun.* 137:295–302.
23. Frisch, S. M., E. J. Clark, and Z. Werb. 1987. Coordinate regulation of stromelysin and collagenase genes determined with cDNA probes. *Proc. Natl. Acad. Sci. USA.* 84:2600–2604.
24. Frolik, C. A., L. L. Dart, C. A. Meyers, D. M. Smith, and M. B. Sporn. 1983. Purification and initial characterization of a type beta transforming growth factor from human placenta. *Proc. Natl. Acad. Sci. USA.* 80:3676–3680.
25. Hay, E. D. 1981. Collagen and embryonic development. *In* Cell Biology of Extracellular Matrix. E. D. Hay, editor. Plenum Publishing Corp., New York, 379–409.
26. Heimark, R. L., D. R. Twardzik, and S. M. Schwartz. 1986. Inhibition of endothelial regeneration by type-beta transforming growth factor from platelets. *Science (Wash. DC).* 233:1078–1080.
27. Heine, U. I., K. Flanders, A. B. Roberts, E. F. Munoz, and M. B. Sporn. 1987. Immunocytochemical localization of TGF-beta during embryonal development in mice. *Proc. Am. Assoc. Cancer Res.* 28:53.
28. Hightower, L. E., and F. P. White. 1981. Cellular responses to stress: comparison of a family of 71-73-kilodalton proteins rapidly synthesized in rat tissue slices and canavanine-treated cells in culture. *J. Cell Physiol.* 108:261–275.
29. Holley, R. W., P. Böhlen, R. Fava, J. H. Baldwin, G. Kleeman, and R. Armour. 1980. Purification of kidney epithelial cell growth inhibitors. *Proc. Natl. Acad. Sci. USA.* 77:5989–5992.
30. Ignotz, R. and J. Massagué. 1986. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J. Biol. Chem.* 261:4337–4345.
31. Ignotz, R. A., T. Endo, and J. Massagué. 1987. Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor-beta. *J. Biol. Chem.* 262:6443–6446.
32. Jones, K. A., J. T. Kadonaga, P. J. Rosenfeld, T. J. Kelly, and R. Tjian. 1987. A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. *Cell.* 48:79–89.
33. Kehrl, J. H., A. B. Roberts, L. M. Wakefield, S. B. Jakowlew, M. B. Sporn, and A. S. Fauci. 1986. Transforming growth factor beta is an important immunomodulatory protein for human B-lymphocytes. *J. Immunol.* 137:3855–3860.
34. Kehrl, J. H., L. M. Wakefield, A. B. Roberts, S. B. Jakowlew, M. Alvarez-Mon, R. Derynck, M. B. Sporn, and A. S. Fauci. 1986. Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *J. Exp. Med.* 163: 1037–1050.
35. Keski-Oja, J., R. M. Lyons, and H. L. Moses. 1987. Inactive secreted form(s) of transforming growth factor-beta: activation by proteolysis. *J. Cell Biochem. Suppl.* 11A:60.
36. Knabbe, C., M. E. Lippman, L. M. Wakefield, K. C. Flanders, A. Kasid, R. Derynck, and R. B. Dickson. 1987. Evidence that transforming growth factor-beta is a hormonally regulated negative growth factor in human breast cancer cells. *Cell.* 48:417–428.
37. Krycève-Martinerie, C., D. A. Lawrence, J. Crochet, P. Jullien, and P. Vigier. 1985. Further study of beta-TGFs released by virally transformed and non-transformed cells. *Int. J. Cancer.* 35:553–558.
38. Laiho, M., O. Sakesela, P. A. Andreasen, and J. Keski-Oja. 1986. Enhanced production and extracellular deposition of the endothelial-type plasminogen activator inhibitor in cultured human lung fibroblasts by transforming growth factor-beta. *J. Cell Biol.* 103:2403–2410.
39. Lawrence, D. A., R. Pircher, and P. Jullien. 1985. Conversion of a high molecular weight latent beta-TGF from chicken embryo fibroblasts into a low molecular weight active beta-TGF under acidic conditions. *Biochem. Biophys. Res. Commun.* 133:1026–1034.
40. Lawrence, D. A., R. Pircher, C. Krycève-Martinerie, and P. Jullien. 1984. Normal embryo fibroblasts release transforming growth factors in a latent form. *J. Cell Physiol.* 121:184–188.
41. Liau, G., M. Mudryj, and B. de Crombrugghe. 1985. Identification of the promoter and first exon of the mouse alpha$_1$(III) collagen gene. *J. Biol. Chem.* 260:3773–3777.
42. Libby, J., R. Martinez, and M. J. Weber. 1986. Tyrosine phosphorylation in cells treated with transforming growth factor-beta. *J. Cell Physiol.* 129:159–166.

43. Like, B., and J. Massagué. 1986. The antiproliferative effect of type beta transforming growth factor occurs at a level distal from receptors for growth-activating factors. *J. Biol. Chem.* 261:13426–13429.
44. Massagué, J. 1985. Transforming growth factor-beta modulates the high-affinity receptors for epidermal growth factor and transforming growth factor-alpha. *J. Cell. Biol.* 100:1508–1514.
45. Massagué, J., S. Cheifetz, T. Endo, and B. Nadal-Ginard. 1986. Type beta transforming growth factor is an inhibitor of myogenic differentiation. *Proc. Natl. Acad. Sci. USA.* 83:8206–8210.
46. Masui, T., L. M. Wakefield, J. F. Lechner, M. A. LaVeck, M. B. Sporn, and C. C. Harris. 1986. Type beta transforming growth factor is the primary differentiation-inducing serum factor for normal human bronchial epithelial cells. *Proc. Natl. Acad. Sci. USA.* 83:2438–2442.
47. Matrisian, L. M., P. Leroy, C. Ruhlmann, M.-C. Gesnel, and R. Breathnach. 1986. Isolation of the oncogene and epidermal growth factor-induced transin gene: complex control in rat fibroblasts. *Mol. Cell Biol.* 6:1679–1686.
48. Moses, H. L., R. F. Tucker, E. B. Leof, R. J. Coffey, J. Halper, and G. D. Shipley. 1985. Type beta transforming growth factor is a growth stimulator and a growth inhibitor. *Cancer Cells (Cold Spring Harbor).* 3:65–71.
49. Mustoe, T. A., G. F. Pierce, A. Thomason, P. Gramates, M. B. Sporn, and T. F. Deuel. 1987. Transforming growth factor type beta induces accelerated healing of incisional wounds in rats. *Science (Wash. DC).* In press.
50. Olson, E. N., E. Sternberg, J. S. Hu, G. Spizz, and C. Wilcox. 1986. Regulation of myogenic differentiation by type beta transforming growth factor. *J. Cell Biol.* 103:1799–1805.
51. Padgett, R. W., R. D. St. Johnston, and W. M. Gelbart. 1987. A transcript from a Drosophila pattern gene predicts a protein homologous to the transforming growth factor-beta family. *Nature (Lond.).* 325:81–84.
52. Pelham, H. R. B. 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. *Cell.* 46:959–961.
53. Pfeilschifter, J., and G. R. Mundy. 1987. Modulation of type beta transforming growth factor activity in bone cultures by osteotropic hormones. *Proc. Natl. Acad. Sci. USA.* 84:2024–2028.
54. Pircher, R., P. Jullien, and D. A. Lawrence. 1986. Beta-transforming growth factor is stored in human blood platelets as a latent high molecular weight complex. *Biochem. Biophys. Res. Commun.* 136:30–37.
55. Postlethwaite, A. E., J. Keski-Oja, H. L. Moses, and A. H. Kang. 1987. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. *J. Exp. Med.* 165:251–256.
56. Roberts, A. B., and M. B. Sporn. 1987. Transforming growth factor-beta. *Adv. Cancer Res.* In press.
57. Roberts, A. B., M. A. Anzano, L. C. Lamb, J. M. Smith, and M. B. Sporn. 1981. New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. *Proc. Natl. Acad. Sci. USA.* 78:5339–5343.
58. Roberts, A. B., M. A. Anzano, C. A. Meyers, J. Wideman, R. Blacher, Y.-C. E. Pan, S. Stein, R. Lehrman, J. M. Smith, L. C. Lamb, and M. B. Sporn. 1983. Purification and properties of a type beta transforming growth factor from bovine kidney. *Biochemistry.* 22:5692–5698.
59. Roberts, A. B., M. A. Anzano, L. M. Wakefield, N. S. Roche, D. F. Stern, and M. B. Sporn. 1985. Type beta transforming growth factor: a bifunctional regulator of cellular growth. *Proc. Natl. Acad. Sci. USA.* 82: 119–123.
60. Roberts, A. B., M. B. Sporn, R. K. Assoian, J. M. Smith, N. S. Roche, L. M. Wakefield, U. I. Heine, L. A. Liotta, V. Falanga, J. H. Kehrl, and A. S. Fauci. 1986. Transforming growth factor type-beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proc. Natl. Acad. Sci. USA.* 83:4167–4171.
61. Robey, P. G., M. F. Young, K. C. Flanders, N. S. Roche, P. Kondaiah, A. H. Reddi, J. D. Termine, M. B. Sporn, and A. B. Roberts. 1987. Osteoblasts synthesize and respond to TGF-beta in vitro. *J. Cell Biol.* 105:457–463.
62. Schlesinger, M. J. 1986. Heat shock proteins: the search for functions. *J. Cell Biol.* 103:321–325.
63. Schmidt, A., P. Rossi, and B. de Crombrugghe. 1986. Transcriptional control of the mouse alpha$_2$(I) collagen gene: functional deletion analysis of the promoter and evidence for cell-specific expression. *Mol. Cell. Biol.* 6:347–354.
64. Segarini, P. R., A. B. Roberts, D. M. Rosen, and S. M. Seyedin. 1987. Membrane binding characteristics of two forms of transforming growth factor-beta. *J. Biol. Chem.* In press.
65. Seyedin, P. R., P. R. Segarini, D. M. Rosen, A. Y. Thompson, H. Bentz, and J. Graycar. 1987. Cartilage-inducing factor-B is a unique protein structurally and functionally related to transforming growth factor-beta. *J. Biol. Chem.* 262:1946–1949.
66. Seyedin, S. M., T. C. Thomas, A. Y. Thompson, D. M. Rosen, and K. A. Piez. 1985. Purification and characterization of two cartilage-inducing factors from bovine demineralized bone. *Proc. Natl. Acad. Sci. USA.* 82:2267–2271.
67. Seyedin, S. M., A. Y. Thompson, H. Bentz, D. M. Rosen, J. M. McPherson, A. Conti, N. R. Siegel, G. R. Galluppi, and K. A. Piez. 1986. Cartilage-inducing factor-A. *J. Biol. Chem.* 261:5693–5695.
68. Shipley, G. D., M. R. Pittelkow, J. J. Wille, R. E. Scott, and H. L. Moses. 1986. Reversible inhibition of normal human prokeratinocyte proliferation by type beta transforming growth factor-growth inhibitor in serum-free medium. *Cancer Res.* 46:2068–2071.
69. Short, N. J. 1987. Regulation of transcription: are some controlling factors more equal than others? *Nature (Lond.).* 236:740–741.
70. Sporn, M. B. 1976. Approaches to prevention of epithelial cancer during the preneoplastic period. *Cancer Res.* 36:2699–2702.
71. Sporn, M. B., and A. B. Roberts. 1983. Role of retinoids in differentiation and carcinogenesis. *Cancer Res.* 43:3034–3040.
72. Sporn, M. B., A. B. Roberts, J. H. Shull, J. M. Smith, J. M. Ward, and J. Sodek. 1983. Polypeptide transforming growth factors isolated from bovine sources and used for wound healing in vivo. *Science (Wash. DC)* 219:1329–1331.
73. Sporn, M. B., A. B. Roberts, L. M. Wakefield, and R. K. Assoian. 1986. Transforming growth factor-beta: biological function and chemical structure. *Science (Wash. DC)* 233:532–534.
74. Tucker, R. F., G. D. Shipley, H. L. Moses, and R. W. Holley. 1984. Growth inhibitor from BSC-1 cells closely related to platelet type beta transforming growth factor. *Science (Wash. DC)* 226:705–707.
75. Wahl, S. M., D. A. Hunt, L. M. Wakefield, N. McCartney-Francis, L. M. Wahl, A. B. Roberts, and M. B. Sporn. 1987. Transforming growth-factor beta (TGF-beta) induces monocyte chemotaxis and growth factor production. *Proc. Natl. Acad. Sci. USA.* 84:5788–5792.
76. Wakefield, L. M., D. M. Smith, K. C. Flanders, and M. B. Sporn. 1987. Characterization of a latent form of transforming growth factor-beta secreted by human platelets. *J. Cell Biochem. Suppl.* 11A:46.
77. Wakefield, L. M., D. M. Smith, T. Masui, C. C. Harris, and M. B. Sporn. 1987. Distribution and modulation of the cellular receptor for transforming growth factor-beta. *J. Cell Biol.* 105:965–975.
78. Wharton, K. A., K. M. Johansen, T. Xu, and S. Artavanis-Tsakonas. 1985. Nucleotide sequence from the neurogenic locus notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell.* 43:567–581.
79. Whitham, S. E., G. Murphy, P. Angel, H.-J. Rahmsdorf, B. J. Smith, A. Lyons, T. J. R. Harris, J. J. Reynolds, P. Herrlich, and A. J. P. Docherty. 1986. Comparison of human stromelysin and collagenase by cloning and sequence analysis. *Biochem. J.* 240:913–916.
80. Wrann, M., S. Bodmer, R. de Martin, C. Siepl, R. Hofer-Warbinek, K. Frei, E. Hofer, and A. Fontana. 1987. T Cell suppressor factor from human glioblastoma cells is a 12.5 KD protein closely related to transforming growth factor-beta. *EMBO (Eur. Mol. Biol. Organ.) J.*, In press.
81. Wu, B. J., G. T. Williams, and R. I. Morimoto. 1987. Detection of three protein binding sites in the serum-regulated promoter of the human gene encoding the 70-kDa heat shock protein. *Proc. Natl. Acad. Sci. U.S.A.* 84:2203–2207.