Making sense of latent TGF\(\beta\) activation

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Summary

TGF\(\beta\) is secreted as part of a latent complex that is targeted to the extracellular matrix. A variety of molecules, ‘TGF\(\beta\) activators,’ release TGF\(\beta\) from its latent state. The unusual temporal discontinuity of TGF\(\beta\) synthesis and action and the panoply of pathological states, including tumor cell growth, fibrosis and apoptosis, are relayed to the sensor by a number of different molecules, and, perhaps, cell growth. Changes in the cell’s environment are relayed to the sensor by a number of different molecules, including proteases, integrins and thrombospondin (TSP). We propose that consideration of latent TGF\(\beta\) in this manner unifies the processes of TGF\(\beta\) secretion, sequestration and activation and clarifies features of TGF\(\beta\) biology.

The components and assembly of the sensor

Before presenting the model, we must first describe the synthesis of TGF\(\beta\) and its latent complex. The three TGF\(\beta\)s are all synthesized as homodimeric preproteins (proTGF\(\beta\)) that have a mass of 75 kDa. The dimeric propeptides, also known as the latency-associated proteins (LAPs)\(^1\), are cleaved from the mature TGF\(\beta\) 24-kDa dimer in the trans Golgi by furin-type enzymes. Early in the assembly of the TGF\(\beta\) LLC, disulfide linkages are formed between cysteine residues of LAP and specific cysteine residues in the latent-TGF\(\beta\)-binding protein (LTBP) (Fig. 2, step 1) (Saharinen et al., 1996; Gleizes et al., 1996; Miyazono et al., 1991). LTBP is a member of the LTBP/fibrillin protein family, which comprises fibrillin-1, fibrillin-2 and fibrillin-3, and LTBP-1, LTBP-2, LTBP-3, and LTBP-4 (Ramirez and Pereira, 1999). These proteins contain multiple epidermal-growth-factor-like repeats as well as unique domains containing eight cysteine residues (8-cys domains) (Fig. 1) (Kanzaki et al., 1990; Tsuji et al., 1990; Sinha et al., 1998). LTBP-1, LTBP-3 and LTBP-4 form a subset within the family based on their ability to bind LAP. Only the third of the four 8-cys domains within each of the LAP-binding LTBPs can disulfide bond to LAP (Saharinen and Keski-Oja, 2000); the other 8-cys domains may localize LTBPs to the ECM (Unsold et al., 2001). As part of the LLC, TGF\(\beta\) cannot interact with its receptors, because the TGF\(\beta\)1, 2 and 3 prodomains (LAPs) function as inhibitors owing to their non-covalent, high-affinity association with TGF\(\beta\) (Lawrence et al., 1984; Dubois et al., 1995). We use the term ‘TGF\(\beta\) activation’ to refer to the liberation of TGF\(\beta\) from the latent complex. LTBP and its bound latent TGF\(\beta\) are found primarily as components of the matrix. Indeed, the N-terminal region of LTBP-1 is covalently cross-linked to ECM proteins by transglutaminase (tTGase) (Fig. 1; Fig. 2, step 3) (Nunes et al., 1997). However, an LTBP binding partner in the ECM has not

Introduction

The transforming growth factors \(\beta\) (TGF\(\beta\)) are multipotent cytokines that are important modulators of cell growth, inflammation, matrix synthesis and apoptosis (Taipale et al., 1998). Defects in TGF\(\beta\) function are associated with a number of pathological states, including tumor cell growth, fibrosis and autoimmune disease (Blobe et al., 2000). The TGF\(\beta\) signal transduction pathway is a topic of intense investigation, and much progress has been achieved in characterizing the proteins involved. The extracellular concentration of TGF\(\beta\) activity is primarily regulated by the conversion of latent TGF\(\beta\) to active TGF\(\beta\); tissues contain significant quantities of latent TGF\(\beta\) and activation of only a small fraction of this latent TGF\(\beta\) generates maximal cellular responses. Yet, despite this fact, many researchers overlook or misunderstand latent TGF\(\beta\) activation. This may be because TGF\(\beta\) biology is unusual: (1) the TGF\(\beta\) propeptide remains tightly bound to the cytokine after the bonds between the propeptide and mature TGF\(\beta\) are cleaved; (2) the interaction between TGF\(\beta\) and its propeptide renders the growth factor latent; (3) the TGF\(\beta\)s are secreted as a complex in which a second gene product is covalently bound to the TGF\(\beta\) propeptide; and (4) upon secretion, the TGF\(\beta\) large latent complex (LLC) may be covalently linked to ECM proteins by LTBP (Fig. 1). Moreover, the multiple activators of the latent TGF\(\beta\) complex comprise a seemingly unrelated group of molecules, and the three TGF\(\beta\) isoforms – TGF\(\beta\)1, TGF\(\beta\)2 and TGF\(\beta\)3 – have similar properties in vitro, but distinct effects in vivo. Here, we present a model in which latent TGF\(\beta\) is considered to be a molecular sensor that responds to specific signals by releasing TGF\(\beta\). These signals are often perturbations of the ECM that are associated with phenomena such as angiogenesis, wound repair, inflammation and, perhaps, cell growth. Changes in the cell’s environment are relayed to the sensor by a number of different molecules, including proteases, integrins and thrombospondin (TSP). We propose that consideration of latent TGF\(\beta\) in this manner unifies the processes of TGF\(\beta\) secretion, sequestration and activation and clarifies features of TGF\(\beta\) biology.

\(^1\)We refer to the N-terminal sequence of the TGF\(\beta\) proprotein (proTGF\(\beta\)) as either the TGF\(\beta\) propeptide or LAP. We also distinguish between two forms of LLC: LLC consists of LTBP, TGF\(\beta\) and LAP, whereas complexes that contain LTBP plus proTGF\(\beta\) are called proLLC.
been unambiguously identified. (Although our discussion is based primarily upon LTBP-1, the similar sequences and domain structures of the LTBPs suggest that most of our statements are generally applicable.) LTBP-1 exists in a range of sizes (125-210 kDa) owing to the use of two independent promoters as well as differences in splicing and glycosylation (Koski et al., 1999). Most forms of LTBP-1 have two protease-sensitive regions; proteolysis at the more N-terminal site can release a truncated form of LTBP-1 (or LLC) from the ECM (Fig. 2, step 4) (Taipale et al., 1994). The functions of LTBP-1 may vary depending on its size. For example, LTBP-1 that contains an N-terminal extension (LTBP-1L) generated by use of the upstream promoter associates more readily with the ECM than does LTBP-1 (LTBP-1S) formed by use of the downstream promoter (Olofsson et al., 1995).

In our model the three components of the LLC – TGFβ, LAP and LTBP, constitute a sensor (Fig. 1). This sensor consists of an effector (TGFβ), a localizer (LTBP) and a detector (LAP). We consider TGFβ to be the effector because it is the output of the sensor, LTBP to be the localizer because it interacts with the ECM, and LAP to be a detector because any activation mechanism must act on LAP, since LAP is sufficient to inhibit TGFβ bioactivity (Gentry and Nash, 1990). The characterization of the mechanisms controlling the liberation of TGFβ from the latent complex is central to the consideration of TGFβ action because the release of TGFβ determines the free TGFβ levels. Several mechanisms for the activation of latent TGFβ complexes are known (Munger et al., 1997; Koli et al., 2001), and a diverse group of activators, including proteases, TSP-1, the integrin α6β4, reactive oxygen species (ROS) and low pH, can activate TGFβ. However, the biological advantage of releasing TGFβ as a latent complex and the relationships between the various activators are obscure. By considering the LLC as a sensor, we think that the role of the latent complex and its activators is clarified.

The latent TGFβ complex as a sensor

What general properties do sensors have and how do these properties relate to latent TGFβ? Consider, as an example, a smoke detector. Before it is used, it must be assembled correctly, placed in an appropriate location and put into a competent state (turned on). The sensor can then change in response to a stimulus (smoke) above a certain threshold, and this change relays information about the environment in the form of an effector (an alarm). Modification of the assembly or the location of the device can alter its effectiveness to respond to smoke.

These features of a smoke detector have analogies in the structure/function of the LLC. The latent TGFβ complex is a sensor that responds to extracellular perturbations and couples these events with the activation of latent TGFβ. As in the case of a smoke detector, the LLC must be appropriately assembled to function properly. The latent TGFβ complex is formed intracellularly and proTGFβ that fails to complex with LTBP is inefficiently secreted (Miyazono et al., 1991). Furthermore, failure to localize appropriately the latent TGFβ complex in the extracellular milieu alters the effectiveness of activation of latent TGFβ. Evidence to support this supposition derives from the ability of both inhibitors of tTGase (Kojima and Rifkin, 1993) and antibodies raised against LTBP-1 to block the activation of latent TGFβ (Flaumenhaft et al., 1993; Dallas et al., 1995; Nakajima et al., 1997; Gualandris et al., 2000). In addition, mice that are null for LTBP-3 or LTBP-4 demonstrate failure to localize appropriately the latent TGFβ complex in the extracellular milieu alters the effectiveness of activation of latent TGFβ. Evidence to support this supposition derives from the ability of both inhibitors of tTGase (Kojima and Rifkin, 1993) and antibodies raised against LTBP-1 to block the activation of latent TGFβ (Flaumenhaft et al., 1993; Dallas et al., 1995; Nakajima et al., 1997; Gualandris et al., 2000). In addition, mice that are null for LTBP-3 or LTBP-4 demonstrate phenotypes consistent with altered TGFβ signaling (Dabovic et al., 2002; Sterner-Kock et al., 2002). Specific LTBP isoforms may differentially localize the latent complex, and different
Latent TGFβ activation

LTBP isoforms may preferentially associate with specific TGFβ isoforms. In fact, the third 8-cys domain of LTBP-4 is reported to bind only to TGFβ1 (Saharinen and Keski-Oja, 2000).

As with many sensing devices, the TGFβ complex must be made competent to signal (i.e. turned on). Competence requires proteolytic separation of LAP from TGFβ (i.e. processing of proLLC into LLC; Fig. 2, step 2). ProLLC cannot be activated by any known mechanism, including heat (85°C for 10 min) or pH (1.5). Although proteolytic cleavage of proTGFβ may occur in the Golgi, this is not always the case. For example, multiple glioblastoma cell lines primarily secrete unprocessed proTGFβ as part of proLLC (Leitlein et al., 2001). To be a substrate for TGFβ activation, this proTGFβ must be processed at the furin protease site by a plasma-membrane-bound furin or another extracellular protease, such as plasmin ([Lyons et al., 1988] our own observation). Indeed, the addition of furin inhibitors to glioma cultures blocks proTGFβ processing. Once pro-TGFβ is processed, the complex is ‘on’ (competent), and it can be activated. In our model, we distinguish between the processing of proTGFβ (turning the sensor on or making it competent) and activating TGFβ. Thus, processing of proTGFβ is a regulated step affecting TGFβ bioavailability. Furthermore, it is interesting to speculate that proTGFβ performs a distinct signaling function from TGFβ (perhaps through integrin ligation) similar to the separate signaling capacities of proNGF and NGF (Lee et al., 2001).

We propose that the sensing function of the latent TGFβ complex resides mainly within LAP. This conclusion is supported by several facts: (1) the known TGFβ activators (e.g. plasmin, TSP-1 and αβ6 integrin) interact directly with LAP (Lyons et al., 1988; Ribeiro et al., 1999; Munger et al., 1999); (2) the physical conditions that release active TGFβ (e.g. heat and pH extremes) denature LAP but not TGFβ (Lawrence et al., 1985); and (3) LAP adopts different conformations in unbound and TGFβ-bound states (McMahon et al., 1996). Moreover, the relative lack of amino acid sequence conservation among LAP isoforms compared with TGFβ isoforms may provide a mechanism for diversification of TGFβ activation. For example, latent TGFβ1 and TGFβ3 can be activated by αβ6, whereas TGFβ2 cannot (Annes et al., 2002; Munger et al., 1999). This is due to the presence of the integrin-binding sequence RGD in TGFβ1 and three LAPs but not TGFβ2 LAP. Sequence analysis reveals only 34-38% amino acid sequence identity among LAP isoforms (LAPb1, β2, β3) compared with 75% identity among TGFβ isoforms (TGFβ1, 2, 3). However, there is considerable conservation of LAP isoform sequences across species (Table 1). The amino acid sequence identity shared by human TGFβ1 LAP and chicken TGFβ1 LAP is 90% (Table 1). We suggest that the relative lack of conservation between LAP isoforms allows LAPs to act as isoform-specific detectors. The divergence between LAP amino acid sequences may explain, in part, the isoform-specific functions of TGFβ in vivo, despite the overlapping expression patterns of the isoforms in vivo and their virtually identical functions in vitro. For example, TGFβ1 and TGFβ2 mRNAs are the predominant isoforms observed in the mouse heart during endocardial cushion and valvular genesis (Akhurst et
In this section we discuss some of the known TGF-β activators. A variety of molecules, from protons to proteases, have been described as latent TGF-β activators (Fig. 2, steps 5, 5Δ). A commonality among these activators is that they are all indicative of ECM perturbations. Indeed, given the profound effects of TGF-β on matrix homeostasis, the primary change of tissues, where it may function as a TGF-β activator in vitro and in vivo (Crawford et al., 1998). TSP-1 facilitates wound repair in several ways: modulation of cell adhesion, promotion of angiogenesis, and reconstruction of the matrix (Frazier et al., 1998). This peptide probably acts by disrupting the non-covalent interactions between LAP and TGF-β. Interestingly, TSP-1 null mice demonstrate a partial phenotypic overlap with TGF-β-null animals, thereby supporting the contention that TSP-1 is an in vivo activator of latent TGF-β (Crawford et al., 1998). TSP-1 facilitates wound repair in several ways: modulation of cell adhesion, promotion of angiogenesis, and reconstruction of the matrix (Frazier et al., 1991). The correlation between wounding and enhanced TSP-1 expression suggests that TSP-1 is an appropriate molecule for activation of the latent complex, since TGF-β plays a prominent role in wound healing (Border and Ruoslahti, 1992). TSP-1 is also expressed throughout development in a number of tissues, where it may function as a TGF-β activator (Irulna-Arispe et al., 1993; Majack et al., 1987).

**Activation by thrombospondin-1**

The matricellular protein TSP-1 activates latent TGF-β (Schultz-Cherry and Murphy-Ullrich, 1993). The mechanism involves a direct interaction between TSP-1 and LAP (reviewed by Murphy-Ullrich and Poczatek, 2000). A short amino acid sequence (RFK) located between the first and second type 1 properdin-like repeats is believed to be responsible for latent TGF-β activation. Surprisingly, a tetrapeptide (KRFK) also functions as a TGF-β activator in vitro and in vivo (Crawford et al., 1998). This peptide probably acts by disrupting the non-covalent interactions between LAP and TGF-β. Interestingly, TSP-1 null mice demonstrate a partial phenotypic overlap with TGF-β-null animals, thereby supporting the contention that TSP-1 is an in vivo activator of latent TGF-β (Crawford et al., 1998). TSP-1 facilitates wound repair in several ways: modulation of cell adhesion, promotion of angiogenesis, and reconstruction of the matrix (Frazier et al., 1991). The correlation between wounding and enhanced TSP-1 expression suggests that TSP-1 is an appropriate molecule for activation of the latent complex, since TGF-β plays a prominent role in wound healing (Border and Ruoslahti, 1992). TSP-1 is also expressed throughout development in a number of tissues, where it may function as a TGF-β activator (Irulna-Arispe et al., 1993; Majack et al., 1987).

**Activation by integrins**

Integrins are dimeric cell surface receptors composed of α and β subunits (reviewed by van der Flier and Sonnenberg, 2001).

| xxxxx | hLAP1 | hLAP2 | hLAP3 | mLAP1 | mLAP2 | mLAP3 | cLAP1 | cLAP2 | cLAP3 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| hLAP1 | –     | 35    | 36    | 85    | 36    | 36    | 90    | 34    | 36    |
| hLAP2 | –     | 46    | 34    | 96    | 47    | 47    | 88    | 48    | 36    |
| hLAP3 | –     | –     | 35    | 47    | 97    | 36    | 46    | 85    | 36    |
| mLAP1 | –     | –     | –     | 35    | 35    | 83    | 35    | 35    | 35    |
| mLAP2 | –     | –     | –     | –     | 48    | 35    | 87    | 48    | 48    |
| mLAP3 | –     | –     | –     | –     | 36    | 48    | 84    | 38    | 38    |
| cLAP1 | –     | –     | –     | 39    | 39    | 48    | 48    | 48    | 48    |
| cLAP2 | –     | –     | –     | 39    | 39    | 48    | 48    | 48    | 48    |
| cLAP3 | –     | –     | –     | 39    | 39    | 48    | 48    | 48    | 48    |

The amino acid sequences of TGF-β LAP 1, 2 and 3 from human (h), mouse (m) and chicken (c) were compared by Blast P: BLOSUM62 without a filter. The signal sequences of these proteins were determined using a weighted matrix program (Nielsen et al., 1997) (http://www.cbs.dtu.dk/services/SignalP/#submission) and were not included in the analysis. Accession numbers: human TGF-β1 LAP (hLAP1; AAH01180); human TGF-β2 LAP2 (hLAP2; NP_003229); human TGF-β3 LAP (hLAP3; NP_003230); mouse TGF-β1 LAP (mLAP1; AAB00138); mouse TGF-β2 LAP2 (mLAP2; AAH1170); mouse TGF-β3 LAP (mLAP3; NP_033394); chicken TGF-β1 LAP (cLAP1; S01413); chicken TGF-β2 LAP2 (cLAP2; P30371); chicken TGF-β3 LAP (cLAP3; P16047).
The first integrin to be identified as a TGF-β activator was αvβ6 (Munger et al., 1999). The mechanism of activation depends upon a direct interaction between αvβ6 and the RGD amino acid sequence present in LAP β1 and LAP β3 (Fig. 1). The expression of αvβ6 is restricted to epithelia, and in most epithelia the integrin is normally expressed at low levels (Breuss et al., 1993). In response to wounding or inflammation, the expression of αvβ6 increases (Breuss et al., 1995; Miller et al., 2001). Therefore, epithelial cell upregulation of αvβ6 and subsequent TGF-β activation is a situation in which the cellular response to a process (inflammation) produces a potent suppressor of that process. Consistent with both the ability of β6 integrin to activate latent TGF-β and the pro-fibrotic effects of TGF-β (Border and Ruoslahti, 1992) is the observation that wild-type mice develop pulmonary inflammation followed by fibrosis in response to the inflammatory and profibrotic drug bleomycin, but integrin β6−/− mice have only a minor fibrotic response (Munger et al., 1999). In addition, global analysis of gene expression in the lungs of integrin β6−/− mice treated with bleomycin compared with similarly treated wild-type mice demonstrates a pronounced failure to induce expression of TGF-β-regulated genes in the mutant mice. These results indicate that fibrosis is the result of excess TGF-β produced by heightened expression of αvβ6 in response to the inflammatory stimulus. Since TGF-β dramatically increases the generation of αvβ6 by primary airway epithelial cells in vitro (Wang et al., 1996), it is likely that bleomycin triggers a feed-forward mechanism for coordinately up-regulating integrin expression and TGF-β generation. We suggest that fibrosis is the result of a failure to interrupt this feed-forward loop that is perpetuated by persistent ECM perturbation after wounding or inflammation.

Recently, Mu et al., reported that the integrin αvβ8 can activate latent TGF-β1 (Mu et al., 2002). It is interesting that activation by αvβ8 requires protease (MT1-MMP) activity in addition to the integrin. Although the exact roles of MT1-MMP and αvβ8 in this activation mechanism remain to be elucidated, the authors suggest that the integrin concentrates latent TGF-β on the cell surface, where it is subsequently activated by MT1-MMP. A cooperative interaction between different classes of latent TGF-β activator has been suggested previously (Yehualaeshet et al., 1999): the cell-surface-associated proteins (CD36 and TSP-1) concentrate latent TGF-β on the membrane where it is subsequently activated by plasmin.

Activation by reactive oxygen species (ROS)
Barcellos-Hoff and her co-workers showed that ROS are produced in vitro (either by ionizing radiation or a metal-catalyzed ascorbate system) or in vivo after irradiation, latent TGF-β1 is activated (Barcellos-Hoff et al., 1994; Barcellos-Hoff and Dix, 1996). This is probably a result of scissions and side group modifications caused by hydroxyl radicals that disable LAP. The response of the TGF-β sensor to certain types of oxidative stress may reflect a need to produce TGF-β during processes such as inflammation and apoptosis that can cause ECM damage through the production of ROS.

Activation by pH
Latent TGF-β present in conditioned medium is activated by mild acid treatment (pH 4.5) (Lyons et al., 1988), which probably denatures LAP, thereby disturbing the interaction between LAP and TGF-β. In vivo, a similar pH is generated by osteoclasts during bone resorption when an integrin-dependent sealing zone is generated between the bone and the cell (Teitelbaum, 2000). Since the bone matrix deposited by osteoclasts is rich in latent TGF-β, the acidic environment created by osteoclasts in vitro might result in latent TGF-β activation (Oreffo et al., 1989; Oursler, 1994).

TGF-β biology and the role of the sensor
The evidence that TGF-β is released in a latent form and must be activated is derived primarily from in vitro studies. There is little in vivo evidence demonstrating a requirement for latent TGF-β activation for several reasons, including the fact that measurement of changes in active TGF-β levels in tissues or animals is extremely difficult. In this section we discuss the in vivo evidence supporting the importance of extracellular TGF-β activation by examining the phenotypes of animals or people in whom specific steps in the post-translational assembly and/or processing of latent TGF-β are defective (Fig. 2). By incorporating the sensor model into our analysis, we have arrived at new interpretations of these phenotypically complex situations.

The effect of improper LLC assembly is illustrated in the phenotypes of mice that have null mutations in the LTBP-3 or LTBP-4 genes. LTBP-3−/− mice display bone phenotypes including osteoarthritis and osteopetrosis (Dabovic et al., 2002), which also occur in mice that have defective TGF-β signaling pathways resulting from either mutations in Smad3 (osteoarthritis) (Yang et al., 2001) or the expression of a dominant negative type II TGF-β receptor in osteoblasts (osteoopetrosis) (Filvaroff et al., 1999). LTBP-4−/− mice develop pulmonary emphysema, cardiac myopathy and colorectal cancer (Sterner-Kock et al., 2002). It is interesting that the defects in LTBP-4−/− animals are consistent with both increased and decreased TGF-β activity: (1) emphysema has been associated with both increased and decreased TGF-β activity (Kaatinen et al., 1995; Zhou et al., 1996); (2) cardiac myopathy is associated with increased TGF-β activity (Schultz Jel et al., 2002); and (3) colorectal cancer is associated with a lack of TGF-β activity (reviewed by Gold, 1999). Thus, the phenotypes displayed by the LTBP-mutant mice are not necessarily described by a simple deficit in TGF-β.

Does consideration of TGF-β biology in terms of the sensor model clarify aspects of this situation? In the absence of a specific LTBP, TGF-β may be (a) inefficiently secreted and unable to localize to the ECM or (b) secreted in a complex with a different LTBP, presuming the cell expresses more than one LTBP isoform. According to the sensor model, these scenarios have varying effects on TGF-β activity. Whereas decreasing TGF-β secretion results in less TGF-β activity, eliminating or changing the isoform of LTBP is predicted to modulate the localization and/or activation pattern of the complex in a context-dependent manner. Therefore, it is not accurate to say that there is more or less TGF-β in these LTBP-null mice; rather, the distribution and timing of TGF-β activities may be modified. For instance, LTBP-3-null mice have increased bone density, which is similar to transgenic mice expressing a dominant negative type II TGF-β receptor under control of the osteocalcin.
promoter, but TGFβ1-null mice become osteoporotic rather than osteopetrotic as they age (Geiser et al., 1998). It is likely that the LTBP-3–/– phenotype emphasizes the effect of altered local distribution of a TGFβ in a cell or tissue type, whereas the TGFβ1-null phenotype illustrates the result of a global loss of the cytokine.

A localization defect can occur not only when there is a deficit in LLC assembly but also if there is an alteration in ECM binding. This might occur if the binding partner for LTBP is missing or defective or if tTGase, which cross-links LLC to the matrix, is absent. However, mice with a null mutation in the Tgase2 gene do not display a phenotype consistent with a global deficit in TGFβ (Nanda et al., 2001; De Laurenzi and Melino, 2001). This may indicate the existence of redundant TGases. We suggest that closer examination will reveal TGFβ-related changes in those tissues or cells that depend exclusively upon Tgase2 for fixing of the ECM.

An example of a human pathology related to altered TGFβ latency is Camurati-Engelmann disease (CED). This autosomal dominant disease results from mutations in the TGFβ1 LAP sequence and is characterized by hyperostosis and sclerosis of the base of the skull and long bones, respectively (Janssens et al., 2000; Kinoshita et al., 2000; Nishimura et al., 2002). Most of the mutations in CED occur at or close to the cysteine residues involved in the interchain bonds of the LAP dimer. Earlier work with mutated TGFβ cDNAs indicated that proper disulfide bond formation is required to produce latent TGFβ, because mutation of C223 and C225 yields constitutively active TGFβ (Brunner et al., 1989). Studies with fibroblasts from three patients with CED mutations at or close to C225 indicate that the mutant cells produce substantially more active TGFβ1 than do wild-type cells (Saito et al., 2001). Why the CED cells generate enhanced levels of active TGFβ is not clear, since disulfide bonds between the appropriate cysteine residues do form; however, the answer to this question may be clarified by consideration of the available data on CED in terms of the sensor model of latent TGFβ.

There are curious differences between the TGFβ produced by wild-type and CED fibroblasts. First, CED and normal cells produce similar amounts of total TGFβ1 as judged by TGFβ1 LAP immunoblotting; however, after acid activation of the latent TGFβ, medium conditioned by CED cells contains five times the amount of active TGFβ1 compared with medium conditioned by normal cells (Saito et al., 2001). Thus, there is a discrepancy between the amounts of immunoreactive and biologically active TGFβ1 produced by the two cell types. Second, there is a difference in the degree of proteolytic processing of proTGFβ1 by CED fibroblasts compared with wild-type cells (Saito et al., 2001). Whereas wild-type cells produce substantial amounts of unprocessed proTGFβ1, CED cells process all of the proTGFβ1 to LAP and TGFβ1. According to the sensor model, all of the latent complex produced by CED, but not wild-type, cells is in an activation-competent state (i.e. the CED LLC is ‘on’ because it has been proteolytically cleaved) (Fig. 2, step 2). This is in contrast to the primarily proTGFβ1 produced by wild-type cells. This form of TGFβ is considered to be ‘off’ and cannot be activated by any known mechanism. Our definition of ‘on’ or competent latent TGFβ clarifies why there is significantly more TGFβ activity in CED, compared with wild-type, conditioned medium following acid activation, despite the fact that the cells secrete equal amounts of the TGFβ propeptide. Apparently, the CED mutation alters the susceptibility of LAP to proteolysis by furin and other processing proteases. It is interesting to speculate that this same conformational change might make LAP more sensitive to activating proteolytic events. Therefore, we suggest that the latent TGFβ complex of CED individuals is assembled and localized normally but is hyper-responsive. Two reports indicate that altered expression of molecules that activate the latent complex result in pathologies. The first report describes lung fibrosis after bleomycin treatment (Munger et al., 1999). In this example, fibrosis is impaired in mice missing β6 integrin, an activator important for generating TGFβ during inflammatory states (Munger et al., 1999). A second example is the developmental pulmonary emphysema observed in fibrillin-1-hypomorphic mice (E. R. Neptune, P. A. Frischmeyer, D. A. Arking et al., personal communication). These animals have a defect in the terminal septation of the alveoli that correlates with excess of both TGFβ and TGFα signaling. It is likely that the defect in terminal alveolar septation in these mice is due to excess TGFβ, because higher levels of TGFβ activity were detected in the lungs of mutant animals, and the administration of TGFβ-neutralizing antibodies reverses the pathology. The lack of fibrillin might result in defective localization of LLC and subsequent TGFβ activation, because the LLC normally localizes with fibrillin-1 (Taiapale et al., 1996). Thus, the abnormal distribution of LLC results in inappropriate activation. An additional explanation as to why fibrillin-1–/– mice have altered TGFβ levels is revealed through consideration of latent TGFβ as a sensor. We propose that the altered ECM of the mutant mice cues cells to remodel the matrix and that this remodeling is associated with the inappropriate and persistent expression of a TGFβ activator.

Conclusion

We have conceptualized latent TGFβ as an ECM-localized sensor in order to unify our current understanding of TGFβ biology. In our model, the sensor comprises a localizer (LTBP), a detector (LAP) and an effector (TGFβ). Failure to localize latent TGFβ appropriately results in altered TGFβ activity. The role of the latent TGFβ complex in coordinating ECM perturbation with ECM reorganization is emphasized if one considers latent TGFβ as a sensor that responds to ECM damage or other extracellular perturbations. The storage of latent TGFβ in the ECM provides a mechanism for spatially and temporally linking perturbation with restructuring. The sensor model provides a framework for understanding the complex and varied nature of TGFβ activity: the primary role of TGFβ is to ‘report’ an alteration of the extracellular milieu and initiate a response.

The sensor model clearly separates two aspects of TGFβ biology that are often misunderstood: the processing of the proTGFβ (turning the sensor ‘on’) and the liberation of TGFβ from the latent complex. Visualizing the latent TGFβ complex as a sensor has offered insight into the somewhat confusing results reported for Camurati-Engelmann syndrome. Moreover, the consideration of active TGFβ formation in terms of a matrix-localized sensor makes it easier to imagine the existence of accessory molecules that interact with the sensor.
and either potentiate or dampen activation as well as the context-specific use of or localization by specific LTBP forms.

In addition, a commonality of TGFβ activators is made apparent by representing TGFβ activation as a process involving sensor detection: all identified TGFβ activators are associated with ECM perturbation. Finally, the latent TGFβ sensor could allow the activities of the three nearly identical TGFβ cytokines to be distinguished, in part, through a diversity in LAP sequences that permits differential response to individual activators. By viewing latent TGFβ as a matrix-localized sensor, we can understand TGFβ assembly, latency, activation and activity as coordinated events rather than as disparate aspects of TGFβ biology.

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