

Making sense of latent TGF β activation

Justin P. Annes*, John S. Munger and Daniel B Rifkin

Departments of Cell Biology and Medicine, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

*Author for correspondence (e-mail: annesj01@med.nyu.edu)

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Summary

TGF β is secreted as part of a latent complex that is targeted to the extracellular matrix. A variety of molecules, 'TGF β activators,' release TGF β from its latent state. The unusual temporal discontinuity of TGF β synthesis and action and the panoply of TGF β effects contribute to the interest in TGF- β . However, the logical connections between TGF β synthesis, storage and action are obscure. We consider the latent TGF β complex as an extracellular sensor in which

the TGF β propeptide functions as the detector, latent-TGF β -binding protein (LTBP) functions as the localizer, and TGF- β functions as the effector. Such a view provides a logical continuity for various aspects of TGF β biology and allows us to appreciate TGF β biology from a new perspective.

Key words: Transforming growth factor- β , Activation, Sensor

Introduction

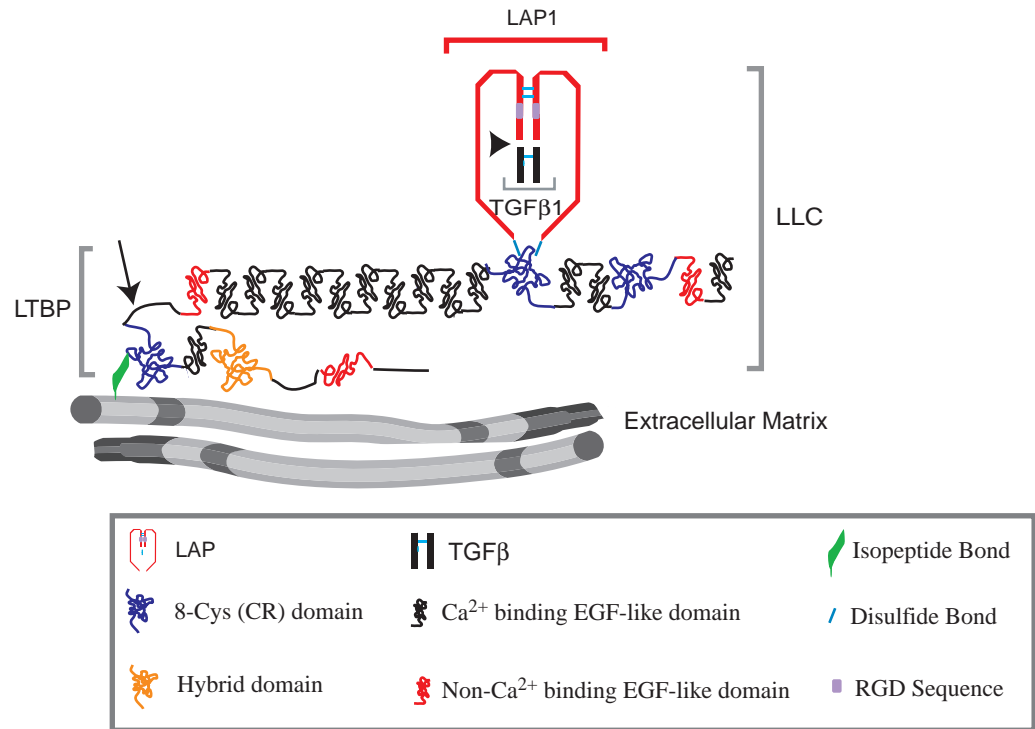
The transforming growth factors β (TGF β) are multipotent cytokines that are important modulators of cell growth, inflammation, matrix synthesis and apoptosis (Taipale et al., 1998). Defects in TGF β function are associated with a number of pathological states, including tumor cell growth, fibrosis and autoimmune disease (Blobe et al., 2000). The TGF β 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 β activity is primarily regulated by the conversion of latent TGF β to active TGF β ; tissues contain significant quantities of latent TGF β and activation of only a small fraction of this latent TGF β generates maximal cellular responses. Yet, despite this fact, many researchers overlook or misunderstand latent TGF β activation. This may be because TGF β biology is unusual: (1) the TGF β propeptide remains tightly bound to the cytokine after the bonds between the propeptide and mature TGF β are cleaved; (2) the interaction between TGF β and its propeptide renders the growth factor latent; (3) the TGF β s are secreted as a complex in which a second gene product is covalently bound to the TGF β propeptide; and (4) upon secretion, the TGF β large latent complex (LLC) may be covalently linked to the extracellular matrix (ECM) (Fig. 1). Moreover, the multiple activators of the latent TGF β complex comprise a seemingly unrelated group of molecules, and the three TGF β isoforms – TGF β 1, TGF β 2 and TGF β 3 – have similar properties in vitro, but distinct effects in vivo. Here, we present a model in which latent TGF β is considered to be a molecular sensor that responds to specific signals by releasing TGF β . 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 β in this manner unifies the processes of TGF β secretion, sequestration and activation and clarifies features of TGF β biology.

The components and assembly of the sensor

Before presenting the model, we must first describe the synthesis of TGF β and its latent complex. The three TGF β s are all synthesized as homodimeric proproteins (proTGF β) that have a mass of 75 kDa. The dimeric propeptides, also known as the latency-associated proteins (LAPs)[†], are cleaved from the mature TGF β 24-kDa dimer in the trans Golgi by furin-type enzymes. Early in the assembly of the TGF β LLC, disulfide linkages are formed between cysteine residues of LAP and specific cysteine residues in the latent-TGF β -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 LBTPs can disulfide bond to LAP (Saharinen and Keski-Oja, 2000); the other 8-cys domains may localize LBTPs to the ECM (Unsold et al., 2001). As part of the LLC, TGF β cannot interact with its receptors, because the TGF β 1, 2 and 3 prodomains (LAPs) function as inhibitors owing to their non-covalent, high-affinity association with TGF β (Lawrence et al., 1984; Dubois et al., 1995). We use the term 'TGF β activation' to refer to the liberation of TGF β from the latent complex. LTBP and its bound latent TGF β 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

[†]We refer to the N-terminal sequence of the TGF β proprotein (proTGF β) as either the TGF β propeptide or LAP. We also distinguish between two forms of LLC: LLC consists of LTBP, TGF β and LAP, whereas complexes that contain LTBP plus proTGF β are called proLLC.

Fig. 1. The TGF β large latent complex (LLC). The LLC comprises TGF β (black), LAP (red) and LTBP. TGF β and LAP are proteolytically separated at the site indicated by the arrowhead. After processing, TGF β remains noncovalently associated with LAP. LAP and LTBP are joined by disulfide bonds (light blue lines). The LLC is covalently linked to the extracellular matrix (ECM) through an isopeptide bond (green) between the N-terminus of LTBP (somewhere between EGF2 and the hinge domain) and a currently unidentified matrix protein. The hinge domain (arrow) of LTBP is a protease-sensitive region that allows LLC to be proteolytically released from the ECM.



been unambiguously identified. (Although our discussion is based primarily upon LTBP-1, the similar sequences and domain structures of the LTBP-1 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 –TGF β , LAP and LTBP– constitute a sensor (Fig. 1). This sensor consists of an effector (TGF β), a localizer (LTBP) and a detector (LAP). We consider TGF β 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 TGF β bioactivity (Gentry and Nash, 1990). The characterization of the mechanisms controlling the liberation of TGF β from the latent complex is central to the consideration of TGF β action because the release of TGF β determines the free TGF β levels. Several mechanisms for the activation of latent TGF β complexes are known (Munger et al., 1997; Koli et al., 2001), and a diverse group of activators, including proteases, TSP-1, the integrin $\alpha_v\beta_6$, reactive oxygen species (ROS) and low pH, can activate TGF β . However, the biological advantage of releasing TGF β 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 TGF β complex as a sensor

What general properties do sensors have and how do these properties relate to latent TGF β ? 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 TGF β complex is a sensor that responds to extracellular perturbations and couples these events with the activation of latent TGF β . As in the case of a smoke detector, the LLC must be appropriately assembled to function properly. The latent TGF β complex is formed intracellularly and proTGF β that fails to complex with LTBP is inefficiently secreted (Miyazono et al., 1991). Furthermore, failure to localize appropriately the latent TGF β complex in the extracellular milieu alters the effectiveness of activation of latent TGF β . 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 TGF β (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 TGF β signaling (Dabovic et al., 2002; Sterner-Kock et al., 2002). Specific LTBP isoforms may differentially localize the latent complex, and different

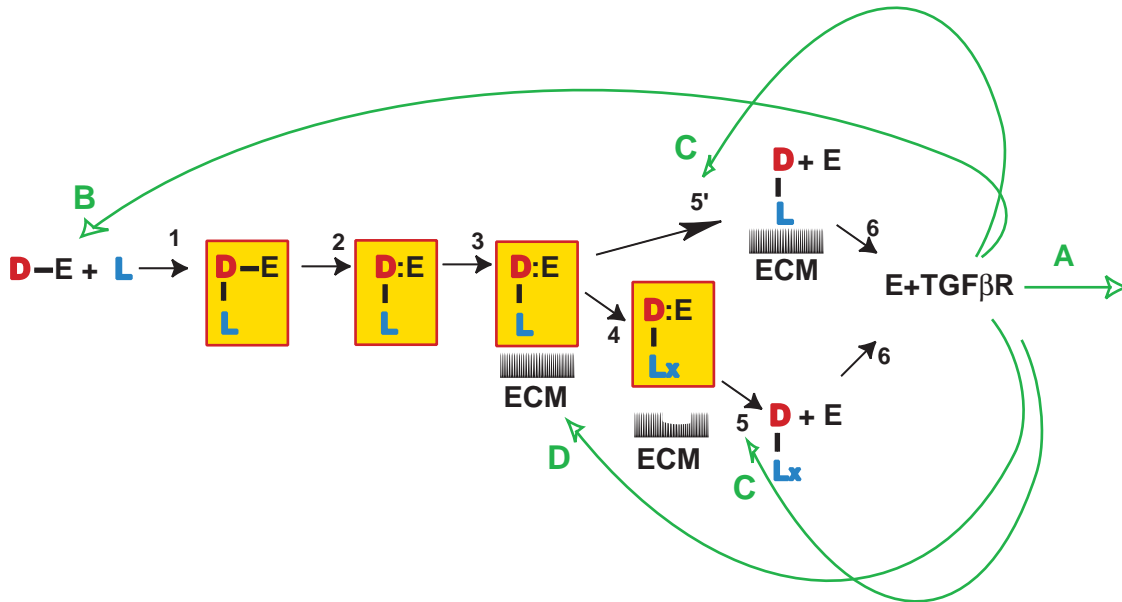


Fig. 2. The latent TGF β sensor model. The figure depicts the sequential events in the bioavailability of TGF β , from synthesis to signaling consequences, according to consideration of the TGF β LLC as a sensor. Sensor assembly (1) occurs cotranslationally when the localizer (LTBP; L) is covalently linked to pro-TGF β (D-E). As shown, the next step (step 2) is the proteolytic cleavage of the bonds between the detector (LAP; D) and the effector (TGF β ; E). This step turns the sensor 'on' or, in other words, makes the sensor competent (note that the timing of this step is variable and may occur after secretion). Once secreted, the sensor is stored in the ECM (step 3). Subsequently, the complex may be solubilized from the matrix (step 4) by cleavage of LTBP in the hinge region. This soluble form of TGF β is still latent and may be activated (step 5). Under other conditions, activation of the matrix-bound sensor occurs (step 5'). Binding of liberated TGF β (E) to its receptors (step 6) with subsequent signal transduction has multiple results (green arrows; A), including induction of TGF β expression (B), enhanced expression of transcripts encoding TGF β activators (C), and increased synthesis of ECM components (D).

LTBP isoforms may preferentially associate with specific TGF β isoforms. In fact, the third 8-cys domain of LTBP-4 is reported to bind only to TGF β 1 (Saharinen and Keski-Oja, 2000).

As with many sensing devices, the TGF β complex must be made competent to signal (i.e. turned on). Competence requires proteolytic separation of LAP from TGF β (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 proTGF β may occur in the Golgi, this is not always the case. For example, multiple glioblastoma cell lines primarily secrete unprocessed proTGF β as part of proLLC (Leitlein et al., 2001). To be a substrate for TGF β activation, this proTGF β 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 proTGF β processing. Once pro-TGF β is processed, the complex is 'on' (competent), and it can be activated. In our model, we distinguish between the processing of proTGF β (turning the sensor on or making it competent) and activating TGF β . Thus, processing of proTGF β is a regulated step affecting TGF β bioavailability. Furthermore, it is interesting to speculate that proTGF β performs a distinct signaling function from TGF β (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 TGF β complex resides mainly within LAP. This conclusion is

supported by several facts: (1) the known TGF β activators (e.g. plasmin, TSP-1 and $\alpha_v\beta_6$ integrin) interact directly with LAP (Lyons et al., 1988; Ribeiro et al., 1999; Munger et al., 1999); (2) the physical conditions that release active TGF β (e.g. heat and pH extremes) denature LAP but not TGF β (Lawrence et al., 1985); and (3) LAP adopts different conformations in unbound and TGF β 1-bound states (McMahon et al., 1996). Moreover, the relative lack of amino acid sequence conservation among LAP isoforms compared with TGF β isoforms may provide a mechanism for diversification of TGF β activation. For example, latent TGF β 1 and TGF β 3 can be activated by $\alpha_v\beta_6$, whereas TGF β 2 cannot (Annes et al., 2002; Munger et al., 1999). This is due to the presence of the integrin-binding sequence RGD in TGF β 1 and three LAPs but not TGF β 2 LAP. Sequence analysis reveals only 34-38% amino acid sequence identity among LAP isoforms (LAP β 1, β 2, β 3) compared with 75% identity among TGF β isoforms (TGF β 1, 2, 3). However, there is considerable conservation of LAP isoform sequences across species (Table 1). The amino acid sequence identity shared by human TGF β 1 LAP and chicken TGF β 1 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 TGF β in vivo, despite the overlapping expression patterns of the isoforms in vivo and their virtually identical functions in vitro. For example, TGF β 1 and TGF β 2 mRNAs are the predominant isoforms observed in the mouse heart during endocardial cushion and valvular genesis (Akhurst et

Table 1. The amino acid identities among the LAP isoforms of humans, mice and chickens

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

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 β 1 LAP (hLAP1; AAH01180); human TGF β 2 LAP2 (hLAP2; NP_003229); human TGF β 3 LAP (hLAP3; NP_003230); mouse TGF β 1 LAP (mLAP1; AAB00138); mouse TGF β 2 LAP2 (mLAP2; AAH11170); mouse TGF β 3 LAP (mLAP3; NP_033394); chicken TGF β 1 LAP (cLAP1; S01413); chicken TGF β 2 LAP2 (cLAP2; P30371); chicken TGF β 3 LAP (cLAP3; P16047).

al., 1990; Millan et al., 1991), and both recombinant TGF β 1 and TGF β 2 function in in vitro assays of endocardial cell transformation (Nakajima et al., 1997). However, TGF β 2^{−/−} but not TGF β 1^{−/−} mice have defects in endocardial and valvular genesis (Sanford et al., 1997). Structural differences in LAP may provide a mechanistic basis for activation of TGF β 2 and not TGF β 1 in this setting.

The paradigm of latent TGF β as a sensor also suggests that the response threshold of the latent TGF β complex might be modulated. Although no examples have been reported, the existence of molecules that either bind LAP and prevent an activator from binding or, conversely, alter the conformation of LAP to facilitate recognition by an activating molecule is a likely possibility.

TGF β activation, or tripping the TGF β sensor

A variety of molecules, from protons to proteases, have been described as latent TGF β activators (Fig. 2, steps 5,5'). 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 that the TGF β sensor detects may be alterations in the matrix. In this section we discuss some of the known TGF β activators.

Proteolytic activation of latent TGF β

A number of proteases including plasmin, MMP-2 and MMP-9 have been identified in vitro as latent TGF β activators (Sato and Rifkin, 1989; Yu and Stamenkovic, 2000). Plasmin and MMP2/9 belong to the serine protease and metalloprotease families, respectively. These protease families, along with the adamalysin-related membrane proteinases, are the primary enzymes involved in ECM degradation (Werb, 1997). The ability of these enzymes to activate the latent TGF β complex couples matrix turnover with the production of a molecule, TGF β , that has a primary role in maintaining matrix integrity and stability (Ignatz and Massague, 1986; Verrecchia et al., 2001). There are three ways in which proteases might facilitate the activation of latent TGF β . First, the protease-sensitive hinge region in LTBP is a potential target for the liberation of a still-latent remnant of the LLC, which would have to be further processed for activation (Taipale et al., 1994). Second, as discussed above, proteases can act in the extracellular

environment to convert proLLC to LLC and thereby render the latent complex activation competent. Third, proteolytic cleavage of LAP, resulting in destabilization of LAP-TGF β interactions, might release active TGF β from its latent complex (Lyons et al., 1988). Degradation of LAP is an attractive mechanism for sensor activation because heightened levels of proteases are associated with several processes that involve increased TGF β activation. However, thus far, mice that have null mutations in the genes that encode the known activating proteases do not demonstrate any phenotype consistent with TGF β deficiency. This may reflect redundancy among the activating enzymes or the fact that these mice have not been studied in the correct context.

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 (RKF) located between the first and second type 1 properdin-like repeats is believed to be responsible for latent TGF β activation. Surprisingly, a tetrapeptide (KRKF) 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 1-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, 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 (Iruela-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).

The first integrin to be identified as a TGF β activator was $\alpha_v\beta_6$ (Munger et al., 1999). The mechanism of activation depends upon a direct interaction between $\alpha_v\beta_6$ and the RGD amino acid sequence present in LAP β_1 and LAP β_3 (Fig. 1). The expression of $\alpha_v\beta_6$ 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 $\alpha_v\beta_6$ increases (Breuss et al., 1995; Miller et al., 2001). Therefore, epithelial cell upregulation of $\alpha_v\beta_6$ 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 β_6 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 $\beta_6^{-/-}$ mice have only a minor fibrotic response (Munger et al., 1999). In addition, global analysis of gene expression in the lungs of integrin $\beta_6^{-/-}$ 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 $\alpha_v\beta_6$ in response to the inflammatory stimulus. Since TGF β dramatically increases the generation of $\alpha_v\beta_6$ 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 $\alpha_v\beta_8$ can activate latent TGF β_1 (Mu et al., 2002). It is interesting that activation by $\alpha_v\beta_8$ requires protease (MT1-MMP) activity in addition to the integrin. Although the exact roles of MT1-MMP and $\alpha_v\beta_8$ 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 when ROS are produced in vitro (either by ionizing radiation or a metal-catalyzed ascorbate system) or in vivo after irradiation, latent TGF β_1 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 osteoblasts 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 (osteopetrosis) (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 (Kaartinen 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 TGF β 1-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 TGF β in a cell or tissue type, whereas the TGF β 1-null phenotype illustrates the result of a global loss of the cytokine.

A localization defect can occur not only when there is a defect 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 TGF β (Nanda et al., 2001; De Laurenzi and Melino, 2001). This may indicate the existence of redundant TGases. We suggest that closer examination will reveal TGF β -related changes in those tissues or cells that depend exclusively upon TGase2 for fixing of the sensor into the ECM.

An example of a human pathology related to altered TGF β latency is Camurati-Engelmann disease (CED). This autosomal dominant disease results from mutations in the TGF β 1 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 TGF β cDNAs indicated that proper disulfide bond formation is required to produce latent TGF β , because mutation of C223 and C225 yields constitutively active TGF β (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 TGF β 1 than do wild-type cells (Saito et al., 2001). Why the CED cells generate enhanced levels of active TGF β 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 TGF β .

There are curious differences between the TGF β produced by wild-type and CED fibroblasts. First, CED and normal cells produce similar amounts of total TGF β 1 as judged by TGF β 1 LAP immunoblotting; however, after acid activation of the latent TGF β , medium conditioned by CED cells contains five times the amount of active TGF β 1 compared with medium conditioned by normal cells (Saito et al., 2001). Thus, there is a discrepancy between the amounts of immunoreactive and biologically active TGF β 1 produced by the two cell types. Second, there is a difference in the degree of proteolytic processing of proTGF β 1 by CED fibroblasts compared with wild-type cells (Saito et al., 2001). Whereas wild-type cells produce substantial amounts of unprocessed proTGF β 1, CED cells process all of the proTGF β 1 to LAP and TGF β 1. 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 proTGF β 1 produced by wild-type cells. This form of TGF β is considered to be 'off' and cannot be activated by any known mechanism. Our definition of 'on' or competent latent TGF β clarifies why there is significantly more TGF β activity in CED, compared with wild-type, conditioned

medium following acid activation, despite the fact that the cells secrete equal amounts of the TGF β propeptide. Apparently, the CED mutation alters the susceptibility of LAP to proteolysis by furin and/or 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 TGF β 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 β 6 integrin, an activator important for generating TGF β 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 TGF β and TGF β signaling. It is likely that the defect in terminal alveolar septation in these mice is due to excess TGF β , because higher levels of TGF β activity were detected in the lungs of mutant animals, and the administration of TGF β -neutralizing antibodies reverses the pathology. The lack of fibrillin might result in defective localization of LLC and subsequent TGF β activation, because the LLC normally localizes with fibrillin-1 (Taipale et al., 1996). Thus, the abnormal distribution of LLC results in inappropriate activation. An additional explanation as to why fibrillin-1^{-/-} mice have altered TGF β levels is revealed through consideration of latent TGF β 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 TGF β activator.

Conclusion

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

The sensor model clearly separates two aspects of TGF β biology that are often misunderstood: the processing of the proTGF β (turning the sensor 'on') and the liberation of TGF β from the latent complex. Visualizing the latent TGF β complex as a sensor has offered insight into the somewhat confusing results reported for Camurati-Engelmann syndrome. Moreover, the consideration of active TGF β 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 TGF β activators is made apparent by representing TGF β activation as a process involving sensor detection: all identified TGF β activators are associated with ECM perturbation. Finally, the latent TGF β sensor could allow the activities of the three nearly identical TGF β cytokines to be distinguished, in part, through a diversity in LAP sequences that permits differential response to individual activators. By viewing latent TGF β as a matrix-localized sensor, we can understand TGF β assembly, latency, activation and activity as coordinated events rather than as disparate aspects of TGF β biology.

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