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PROSPECTS

How Is Type I Procollagen Synthesis Regulated at the Gene Level During Tissue Fibrosis

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Abstract In response to tissue injury connective tissue synthesis occurs either normally or abnormally, which is mediated by transforming growth factor-β (TGF-β) and other growth factors. This article will be primarily concerned with the response of injured tissues at the gene level of Type I procollagen synthesis in response to TGF-β. This leads to provisional repair, which in turn may lead to involution, remodeling, regeneration, and ultimately repair. Alternately, continuation of provisional repair may lead to fibrosis and ultimately scarring. Scarring of internal organs such as the liver and the lung leads to loss of function and ultimately death. In the case of scarring of skin, this is a cosmetic problem and can be rectified by surgery. Type I procollagen is synthesized by two genes, *proα1* (Type I) and *proα2* (Type I) collagen genes. This article will focus on DNA binding sites on these two genes, which regulate the transcription of the specific gene. This article will also define specific cell signaling pathways for the turning on of the *proα1* and *proα2* (Type I) collagen genes. This article will address several questions. First, what is the major cytokine acting extracellularly which stimulates the transcription of the *proα1* and *proα2* (Type I) collagen genes during tissue fibrosis? Secondly, how are the signals transmitted by the extracellular profibrotic cytokine TGF-β from the cellular membrane to the nucleus for transcription of the *proα1* (Type I) and *proα2* (Type I) collagen genes? Thirdly, what signaling pathways cross-talk with the signaling pathways resulting in the expression of the Type I collagen genes? Fourthly, how does TGF-β affect extracellular matrix homeostasis? Fifthly, what are the nuclear factors corresponding to the DNA elements required for the promotion of the *proα1* (Type I) and *proα2* (Type I) collagen genes? Finally, how are the *proα1* (Type I) and *proα2* (Type I) collagen genes coordinately regulated? Strategies will also be presented for reducing fibrosis, which is the result of overexpression of TGF-β. J. Cell. Biochem. 90: 1–5, 2003.

Key words: wound healing; TGF-β; Proα1(I) collagen gene transcription; Proα2(I) collagen gene transcription; Smads; TGF-β activator protein; nuclear factors; cross-talk

Soft and connective tissue homeostasis requires the orchestrated regulation of the synthesis and the degradation of the components of the extracellular matrix (Fig. 1). Of all the collagens, Type I collagen is the most fibrous form and comprises approximately 84% of the collagen synthesized by fibroblasts. Therefore, the large deposition of Type I collagen leads to skin and internal organ fibrosis. Fibrosis of the skin, the largest organ of the body, leads to scarring which is a cosmetic problem. However, scarring of soft internal organs such as liver and lung leads to loss of function and ultimately death. Upon tissue injury inflammation occurs with platelets, fibroblasts, myofibroblasts, and esinophils releasing transforming growth factor-β (TGF-β) which stimulates fibroblasts and other reparative cells to proliferate and synthesize extracellular matrix components (Fig. 2). This leads to provisional repair, which under normal conditions results in involution, maturation, remodeling, reorganization, and regeneration. A continuation of provisional repair results in fibrosis and ultimately scarring. Table I lists strategies for reducing fibrosis. Our laboratory has shown that glucocorticoids have pronounced effects on procollagen and TGF-β (Table II).

Type I procollagen is composed of two polypeptide chains, proα1(I) and proα2(I) in the ratio of 2:1, respectively. The synthesis of these two polypeptide chains is under the control of

This article is dedicated to the memory of Dr. Edward Bresnick.

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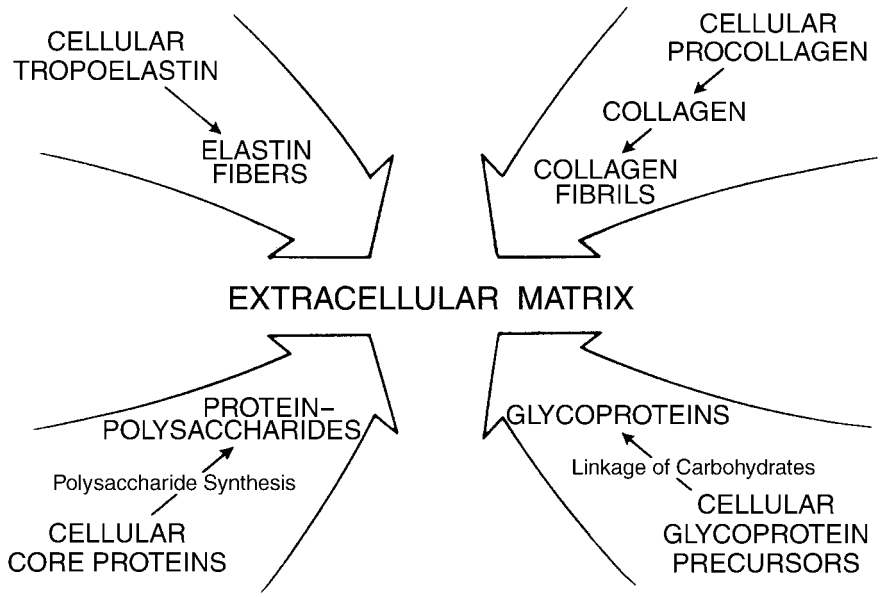

Fig. 1. The components of the extracellular matrix.

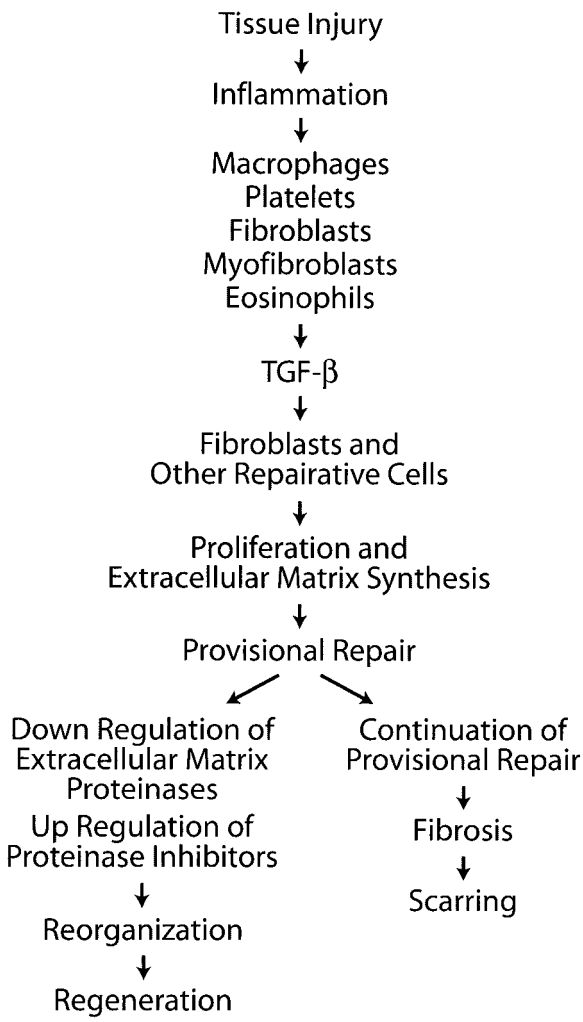

Fig. 2. Tissue response to injury.

two separate genes. This article will address several questions. What is the major cytokine acting extracellularly which stimulates the transcription of the *proα1(I)* and *proα2(I)* collagen genes during tissue fibrosis?

How are signals transmitted by the extracellular profibrotic cytokine, TGF-β, from the cellular membrane to the nucleus for the transcription of the *proα1(I)* and *proα2(I)* collagen genes? What signaling pathways cross-talk with these signaling pathways? How does TGF-β affect extracellular matrix homeostasis? What are nuclear factors and corresponding DNA elements required for the promotion of the *proα1(I)* and *proα2(I)* collagen genes? Finally, how are the *proα1(I)* and *proα2(I)* collagen genes coordinately regulated?

The major cytokine leading to tissue fibrosis is TGF-β [Shukla et al., 1999; Varga, 2002]. In the case of the *proα1(I)* collagen gene after TGF-β reacts with extracellular receptors, signals are transmitted to the *proα1(I)* collagen gene through the TGF-β activator signaling pathway

TABLE I. Strategies for Reducing Fibrosis

Transforming growth factor-β (TGF-β) antibodies
Soluble receptors
Receptor antagonists
Proteoglycans (decorin or biglycan)
Latent TGF-β1 binding proteins
TGF-β3 isoform
Glucocorticoids
Anti-sense oligodeoxynucleotides
Sense oligodeoxynucleotides
Smad 7

TABLE II. Glucocorticoid Effects on Type I Procollagen Synthesis and TGF-β1

↓ Type I collagen synthesis
↓ Procollagen Type I mRNA levels
↓ Procollagen gene transcription
↓ TGF-β1 secretion
↓ TGF-β1 mRNA levels
↓ TGF-β1 gene transcription

[King et al., 1994; Cutroneo, 2000] (Fig. 3). The *proα2(I)* collagen gene is regulated by TGF-β signaling through the Smad pathway (Fig. 4). After TGF-β binds to the TGF-β receptor I (TGF-βRI) and the TGF-β receptor II (TGF β R II), the intrinsic serine/threonine kinase activity of TGF-β RI phosphorylates Smad 2 and 3 which combine and forms a heteromeric complex with unphosphorylated Co-Smad 4. This complex then translocates into the nucleus. This complex regulates target gene transcription either by directly binding DNA sequences or by complexing with other transcription factors or coactivators [Cheng and Grande, 2002]. TGF-β signaling is regulated positively and negatively. Positive regulation amplifies signals to a level sufficient for biological activity. Negative regulation occurs at the extracellular membrane receptors, cytoplasmic and (or) nuclear levels [Miyazono, 2000]. For example, we have shown that when the Smad 3 binding to the *Smad 7* gene is decreased by knockout of *Smad 7* gene transcription by sense phosphorothioate oligos,

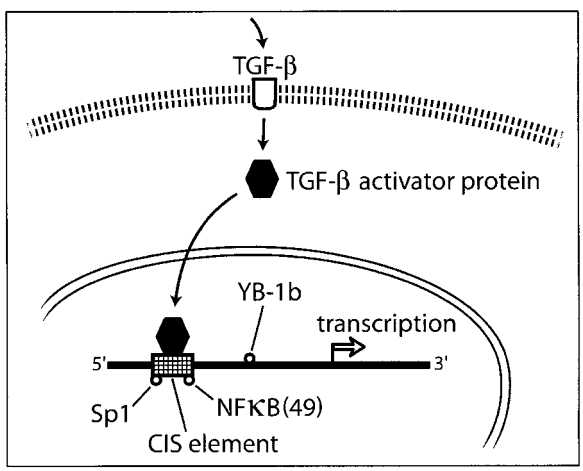

Fig. 3. Promotion of the *proα1(I)* collagen gene by the transforming growth factor-β (TGF-β) activator protein signaling pathway and other cellular factors. The TGF-β *cis*-binding element is in the distal promoter region of the *proα1(I)* collagen gene.

this enhances the action of TGF-β and produces a marked increase in TGF-β1 secretion [Cutroneo and Phan, 2003].

Smad signal transduction does not proceed in a linear fashion but involves a network of intracellular proteins that influence the TGF-β signaling pathway. There is much cross-talk with other cellular signaling pathways [Lutz and Knaus, 2002]. The integration of the TGF-β/Smad signaling pathway with other cellular signaling networks contributes to the many cell and tissue-specific effects of TGF-β. Smads regulate through cooperative and physical interactions with other transcription factors, which might be targets for regulation by other cellular signaling pathways (Fig. 4). There is cross-talk between the TGF-β/Smad signal pathway and G proteins [Visser and Themmen, 1998] and the Ras/mitogen-activated protein kinase pathways [Kretzschmar and Massague, 1998; Visser and Themmen, 1998; Cheng and Grande, 2002]. The cross-talk amongst a variety of cellular pathways is necessary for the maximal collagen gene expression during tissue fibrosis. A basic unraveling of the interplay between these signaling pathways will reveal new targets for precise action on the TGF-β/Smad signaling pathway. There is also evidence in the literature for cross-talk between retinoic acid and TGF-β signal transduction pathways [Nugent and Greene, 1994].

TGF-β affects extracellular matrix homeostasis. TGF-β is a potent inducer of metal metalloproteinase-13 gene expression requiring activation of the p38 mitogen-activated protein kinase pathway, which involves activation of Smad 3 by p38 alpha [Leivonen et al., 2002]. The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of these enzymes (TIMPs) is essential in remodeling the extracellular matrix (Fig. 4). TGF-β exerts effects on extracellular matrix homeostasis by balancing gene expression of MMPs and TIMPs. The promoter-proximal activator protein 1 (AP1) site is necessary for TGF-β mediated induction of TIMP-1 and the repression of MMP-1 [Hall et al., 2003].

The promotion of the *proα1(I)* (Fig. 3) and *proα2(I)* (Fig. 4) collagen genes requires two different signaling pathways, the TGF-β activator protein for the *proα1(I)* collagen gene and the Smad signaling pathway for the *proα2(I)* collagen gene. Besides these proteins, there are other cellular factors and DNA binding

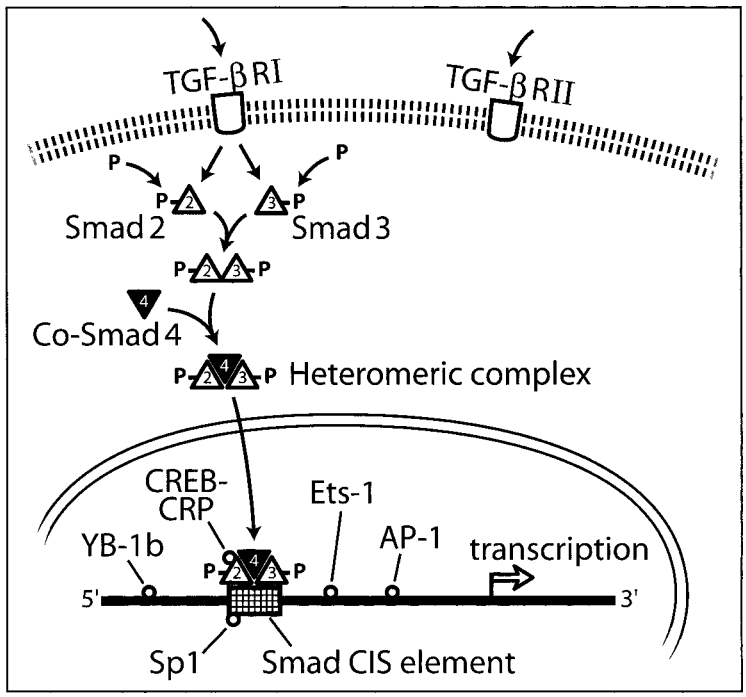

Fig. 4. Promotion of the *proα2(I)* collagen gene through the Smad signaling pathway and other cellular factors. The Smad *cis*-binding element is in the proximal promoter region of the *proα2(I)* collagen gene.

elements required for the transcription of these Type I procollagen genes. The 5′ flanking region of the rat proα1(I) collagen containing the TGF-β element (TGCCCACGGCCAG) binds human SP1 and human NF-κB (49) but not human AP-1, AP-2, c-fos, and NF-κB (50) [Cutroneo, 2000]. YB-1β, a Y-box protein is thought to be involved in the coordinate expression of both the *proα1(I)* and *proα2(I)* collagen genes since the chicken YB-1B binding site is one of the few sites conserved in the proα1(I) and and proα2(I) gene promoters (Figs. 3 and 4) from chickens to humans [Dhalla et al., 1998]. TGF-β enhances *proα1(I)* and *proα2(I)* collagen gene expression through a functional interaction between SP-1 and other components of the TGF-β response element complex [Inagaki et al., 1994a]. Furthermore, this same group demonstrated by the use of a tyrosine kinase inhibitor, increased proα2(I) collagen gene expression, whereas a tyrosine phosphatase inhibitor decreased gene expression. Thus, changes in proα2(I) collagen gene expression occur by post-translational modification of a protein(s) directly or indirectly interacting with SP-1 [Greenwel et al., 1995]. It has been shown that a SP-1 GC box binding factor binds to Smad 3 and determines the cell lineage-specific interaction, accounting for why activated hepatic stellate cells mainly produce Type I collagen while the parenchymal hepatocytes produce little of this protein [Inagaki et al., 2001b]. At the level of the *proα2(I)* collagen gene, differential proα2(I) transcription and TGF-β responsiveness occur in hepatic stellate cells and parenchymal hepatocytes. Transient and stable cell transfection assays indicate an AP-1 binding site on the proα2(I) gene promoter which is competed by AP-1, but not NF-1 or NF-κB or AP1 oligonucleotides [Chung et al., 1996].

Ets-1 stimulates proα2(I) collagen gene promoter activity [Czuwara-Ladykowska et al., 2001]. Fli-1 inhibits proα2(I) promoter activity by competing with Ets-1. A corepressor may be required for maximal inhibition after recruitment to the Fli-1–SP-1 complex. The ratio of Fli-1 to Ets-1 and the presence of coregulatory proteins ultimately controls collagen proα2(I) gene transcription.

Cross-talk exists between TGF-β and cyclic AMP signal transduction pathways at the level of transcriptional complex formation [Warner et al., 2003]. These investigators demonstrated that treatment of cells with a stimulator of adenylate cyclase or TGF-β increased the amount of phosphorylated CAMP response element binding protein (CREB) and the coactivator,

CREB binding protein (CBP) that were bound in a complex to the Smad binding element.

In conclusion, tissue injury may lead to either regeneration of normal tissue or tissue fibrosis leading to scarring. Type I collagen is the most fibrous form of collagen and is synthesized predominantly by fibroblast, myofibroblast, and other reparative cells. Type I collagen is synthesized under the direction of two genes; *proα1* (Type I) and *proα2* (Type I) collagen genes. These genes regulate the synthesis of two propolypeptides in the ratio of 2:1, 2 proα1 and 1 proα2. There are various strategies for inhibiting fibrosis induced by the cytokine TGF-β, the most potent profibrotic growth factor released during tissue injury. The transcription of the *proα1* (Type I) and *proα2* (Type I) collagen genes is under the control of the various signaling pathways. Proα1 (Type I) is under control of the TGF-β activator protein signaling pathway and the *proα2* (Type I) collagen gene is under the control of the Smad signaling pathway. Besides these signaling pathways, these genes are under control of other cellular signaling pathways which cross-talk with the above-mentioned signaling pathways and various nuclear factors which bind in cooperation with the signaling pathways and other elements involved in these signaling pathways.

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