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Molecular mechanisms of regulation of type I collagen biosynthesis

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Abstract. Type I collagen is a heterotrimeric protein composed of two $\alpha1$(I) and one $\alpha2$(I) polypeptide chains, each encoded by a unique gene. Both collagen genes are coordinately regulated in response to a variety of endogenous and exogenous stimuli. Collagen genes are mainly regulated by transcriptional mechanisms, although post-transcriptional regulatory mechanisms have occasionally been documented. To understand the molecular basis of transcriptional control, we have been studying the *cis*-regulatory sequence motifs of human Pro$\alpha1$(I) collagen gene and *trans*-acting factors with which these elements interact. The major elements include TATA and CCAAT boxes, Ap-1, Ap-2, NF-1 and Sp1, and a unique TGFβ1-activating element. Transcription factor Sp1 is obligatory for the activation of the Pro$\alpha1$(I) promoter since it failed to be activated in *Drosophila* SL2 cells that lack Sp1. Of the six putative Sp1 motifs in the Pro$\alpha1$(I) collagen promoter and the first intron, the most proximal Sp1 element located at –87·to –82 bp was sufficient for its Sp1-dependent activation. Additional *cis*-acting motifs in the intron of the Pro$\alpha1$(I) gene, including an Ap-1 site, participated in its regulation by TGFβ1 and okadaic acid. Thus, activation of Pro$\alpha1$(I) promoter involves combinatorial actions of multiple ubiquitous and unique *cis*-regulatory elements.

Keywords. Type I collagen; gene regulation; constitutive and inducible transcription; *cis*- and *trans*-acting factors.

1. Introduction

The formation and deposition of extracellular matrix (ECM) is a prerequisite for proper assembly and compartmentalization of cells to generate tissues and organs. In addition to serving as a scaffold and a physical barrier between cells, ECM is a key determinant of cell shape and locomotion. Recent observations suggest that ECM may also modulate vital parameters of phenotypic differentiation of many cells including their rates of proliferation and apoptosis. Many protein and non-protein molecules constitute ECM, collagens being a major protein constituent of many tissue-specific matrices. Collagens are characterized by their triple-helical conformation that may be either continuous or interrupted by globular domains, depending on the specific type of collagen. Type I

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collagen, the most abundant and best characterized of all collagens, is composed of two $\alpha 1$(I) and one $\alpha 2$(I) chains. The individual polypeptide α chains are processed from larger precursors, procollagens, each of which is encoded by a unique gene. The biosynthesis, post-translational processing and assembly of type I procollagen chain is tightly regulated during development and in response to a host of biological and pharmacological stimuli. An excessive production of type I collagen accompanies a number of common fibrotic conditions. In contrast, declined rates of collagen synthesis and accumulation are often associated with neoplastic transformation of cells. Expression of type I collagen genes is mainly controlled by transcriptional mechanisms, although post-transcriptional and translational mechanisms have also occasionally been observed. In this review, we outline the generally accepted paradigms of regulation of synthesis and turnover of type I collagen and role of these processes in the pathophysiology of fibrogenesis, particularly pulmonary fibrosis. We attempt to synthesize an overview of the mechanistic features of type I collagen gene expression and review the literature pertaining to the putative interactions among the known *cis*-acting elements and transcription factors that orchestrate the constitutive and inducible transcription of collagen genes. We particularly focus on the regulatory pathways of type I collagen gene expression in response to transforming growth factor $\beta 1$ (TGF-$\beta 1$) due to an intimate relationship of this cytokine to the fibrotic process. We fully recognize that in the light of extensive and sometimes controversial observations reported in the literature, our discussion is intended to be neither comprehensive nor free of our own particular bias. Of course, we are solely responsible for both these deficiencies.

2. Type I collagen in health and disease

Type I collagen, the most abundant protein constituent of the mammalian extracellular matrix, is involved in cell migration, differentiation and tissue morphogenesis during embryogenesis [1,2], postinflammatory regeneration and repair of injured tissues [3–5] and in neoplastic transformation[6,7]. Abnormal biosynthesis and/or processing of collagens, resulting from heritable lesions in the genomic loci which encode them, are known to cause osteogenesis imperfecta, abnormally hyper-flexible joints, and some forms of osteoarthritis. Down-regulation of type I collagen gene expression is associated with neoplastic transformation of cells [6,7], whereas the excessive synthesis and accumulation of type I collagen is a hallmark of scleroderma, keloids formation, cirrhosis of liver, and pulmonary fibrosis [3,4,8,9].

2.1 Structure of type I collagen

Type I collagen belongs to a super-family of about twenty closely related yet genetically distinct proteins, encoded by more than two dozen unique genes [10–12]. Collagens may be broadly classified into fibrillar (e.g., types I, II and III) and the so-called FACIT (fibril-associated collagens with interrupted triple helices) collagens (e.g., type IX). The fibrillar collagens, as represented by type I collagen, are rod-like molecules composed of three polypeptides coiled about each other in a triple helix. The helical conformation of collagens is facilitated by the existence of the amino acid glycine at every third position in the polypeptide chain; thus collagen α chains consist of a repeated tri-peptide glycine-X-Y. Although X and Y positions may be filled by many different amino acids, they are frequently occupied by proline and hydroxyproline. Assumption of triple-helical

conformation by collagen α chains can only be initiated after a number of post-translational modifications have taken place. These include hydroxylation of lysine and proline residues, which are involved in the formation of intermolecular cross-links between collagen chains [13].

Although collagens are highly conserved in their structural organization, they differ from one another in their primary amino acid sequences and relative degrees of hydroxylation and glycosylation, and in the locations of the disulfide- and lysine-derived covalent cross-links. While the triple-helical collagen molecules tend to form fibrils through their end-to-end alignment in quarter-stagger arrays, their diameter increases by side-to-side associations between fibers. The structure of collagen fibrils is stabilized by covalent cross-links within and between the adjacent collagen molecules. Collagens may be grouped based on the size of their α-chains and the number of triple helical regions they contain [14]. Collagen types I, II, III, V, and XI are composed of α chains of Mr 95,000 or greater with a single long triple-helical domain, approximately 300 nm in length which represent the major interstitial fibrillar group of collagens. Individual procollagen α chains are encoded by unique genes which are dispersed throughout the genome. Genomic organization of a number of collagen genes has been extensively studied. The two genes encoding the α chains of type I procollagen typify this organization; these genes are large, consisting of up to 50 exons, interspersed with introns of varying sizes. Each of the polypetides is synthesized as a larger precursor, the procollagen, which contains both amino- and carboxyl-terminal extensions or propeptides; removal of the propeptide termini is carried out by specific proteinases [15]. A failure of the amino or carboxyl extensions to be removed from procollagen molecules or imperfections in the triple helix formation due to mutations result in either partial or complete inhibition of fibrillogenesis [16]. The propeptide extensions are believed to serve a number of roles, including initiation of chain association, stabilization of helical conformation of the nascent molecules and retardation of premature molecular aggregation of collagens. In addition to intra- and inter-molecular associations, collagens are capable of interacting with a number of other molecules of ECM (e.g., elastin, proteoglycans, fibronectin, and laminin) to form a three-dimentional lattice-work. These qualitative and quantitative permutations of interactions among the collagenous and noncollagenous molecules generate an impressive variety of ECM uniquely suited for different tissues and organs.

2.2 *Role of collagen in the lung*

Much like the ECM of other tissues, the pulmonary ECM is composed of many protein and non-protein constituents; however, 60–70% of the connective tissue mass in an adult human lung is represented by collagens [17,18]. Although the alveolar interstitium of the normal lung contains at least five types of collagen, the vast majority of these are collagen types I and III, normally found in a ratio of 2:1. Type I collagen forms a continuum throughout the lung in such a way that the collagen arrays of interlobular septa, blood vessels and airways are connected through the alveolar interstitium. The type III collagen is preferentially distributed in the alveolar interstitium and in the intima and media of the arteries [19,20]. The remaining ~5% of the total lung collagen is represented mainly by type IV collagen which is distributed in the alveolar basement membranes underneath the epithelial and endothelial cells.

2.3 Biosynthesis of type I collagen

Collagen biosynthesis occurs in a series of sequential and well-coordinated steps which can be outlined as follows: (1) transcription of the collagen gene(s) into a primary transcript or precursor RNA; (2) post-transcriptional modification and processing of the primary transcript into mature mRNA; (3) translocation and translation of mRNA in the cytoplasm and (4) post-translational enzymatic processing of procollagen α chains (e.g., proteolytic cleavage, hydroxylation of the correct prolyl and lysyl residues, and glycosylation). Thereafter, the processed collagen molecules spontaneously align to form microfibrillar structures that ultimately form collagen fibers. Collagen fibers are stabilized by covalent lysine- and hydroxylysine-derived intermolecular cross-links. The formation of cross-links occurs after the oxidative deamination of some lysyl and hydroxy lysyl residues by lysyl oxidase, leaving aldehyde moieties that are responsible for the cross-linking of collagen through condensation.

Cross-linking of collagen chains results in the formation of rigid fibrillar structures composed of triple-helical collagen molecules. The covalently-bonded configuration of collagen is resistant to proteolytic degradation by many proteases. However, there is one group of enzymes, known as the matrix metalloproteinases (MMPs) that readily degrade collagen fibrils [21–23]. The MMPs, produced mainly by connective tissue cells, neutrophils, and macrophages, are synthesized and secreted in a latent form that is activated by proteolysis. The activities of the various MMPs are subject to precise regulation by specific inhibitors, the most prominent of which are α2-macroglobulin and the tissue inhibitor of metalloproteinase (TIMP [24]). The inhibitors of MMPs are enriched in the serum and occasionally in the extracellular microenvironment (e.g., TIMP3 [21–23]). The native, interstitial collagen types I, II and III are cut at a specific site within the triple helix to yield two fragments, named TC^A and TC^B, representing 75% and 25% of the collagen molecule, respectively. The fragments of collagen generated by MMPs denature at body temperature and become susceptible to additional proteolysis by other enzymes. Different MMPs preferentially cleave individual collagen types; for instance, polymorphonuclear leukocyte-derived collagenase (MMP-8) selectively digests type types I > II > III collagens [25]. Type III collagen is more rapidly degraded by MMP-13 than by either MMP-8 or -9 [26]. Conversely, MMP-1 from fibroblasts and macrophages degrades type III collagen preferentially, cutting types I and II collagens much more slowly [27]. Finally, IV collagen is not degraded by MMPs 1, 8 or 13 but is subject to degradation by other MMPs. It is believed that the activity of specific MMPs is the rate limiting step for the proteolysis of collagen types I, II, and II.

2.4 Regulation of genes encoding type I collagen

Type I collagen biosynthesis is a highly regulated process as evident from its preferential activation in bones, teeth, tendons, and the dermis of skin, where collagen is synthesized by osteoblasts, odontoblasts, and fibroblasts, respectively [28]. The rates of biosynthesis and deposition of type I collagen in the ECM of a particular tissue reflect the types and numbers of mesenchymal cells *in situ* and the sum of the synthetic and degradative mechanisms operating in these cells. Under normal conditions, the pathways of synthesis and degradation of type I collagen are tightly coordinated. However, a large influx of inflammatory cells to the site of damage and release of potent cytokines/growth factors during tissue repair and regeneration can profoundly alter this balance.

A variety of transcriptional and post-transcriptional mechanisms have evolved to cope with the ever-changing demand of tissues for collagen homeostasis under physiological and pathological conditions. Although mechanisms of transcriptional control predominate under most conditions, occasionally, steady-state levels of Proα1(I) collagen mRNAs may also be altered by factors that affect rates of messenger RNA turnover[3]. For instance, the rate of Proα1(I) collagen mRNAs turnover was shown to be dramatically lower in the activated hepatic stellate cells. This altered stability of mRNA was determined by the binding of a protein to a cytidine-rich sequence in the 3' untranslated region of Proα1(I) collagen mRNA[29]. Similarly, interferon γ not only altered Proα1(I) collagen mRNA stability but also modulated its rate of translation[30]. Additional potential sites of regulation of type I collagen biosynthesis may involve the many post-translational modifications needed for the processing and assembly of the procollagen α chains. There is evidence to suggest that collagen secretion may also be potentiated by decreasing the rates of its intracellular degradation[31,32]. Finally, feedback mechanisms, capable of modulating the rate of collagen biosynthesis by amino- and carboxyl-terminal propeptides have also been demonstrated[33–36].

2.5 Cis- and trans-acting factors which regulate transcription of type I collagen genes

As a result of the recognition that transcription plays a paramount role in the regulation of type I collagen biosynthesis, recent effort has been focused on the transcriptional control of many collagen genes. Using a variety of *in vitro* and *in vivo* techniques, the precise molecular topography of genes encoding various collagens has been defined. These studies have uncovered a number of major *cis*-acting elements and the *trans*-acting factors which bind to these sequences. However, with much recent progress notwithstanding, the identities of the key elements required to regulate constitutive, tissue-specific and inducible expression of collagen genes are far from completely clear. The regulatory sequences of collagen genes are distributed on either side of the transcription start point (TSP) and may encompass 100–150 kb of DNA, depending on the specific gene and the assay system used to investigate its expression. The 5'- and 3'-flanking and intronic elements of collagen genes are modularly arranged and interactions among these modules determine the tissue-specific and inducible promoter activation. Transient and stable expression of reporters (chloramphenicol acetyl transferase, CAT or luciferase) driven by type I collagen-specifying genomic DNA have unraveled the existence of sequence elements upstream of the TSP and in the intron. The exact contribution of the individual *cis*-acting motifs towards cell-specific or inducibe activation of the promoter remains to be firmly established[37–40]. In addition to the more proximal sequences found in the promoters of several collagen genes, a number of distal elements have been characterized. Significantly, a potent enhancer was identified in the mouse Proα2(I) collagen gene between 15–17 kb upstream of TSP[41]. Recent analyses suggest that *cis*-acting sequences located in the 3'-flanking region of the murine Proα1(I) collagen gene may also be involved in the regulation of its transcription[42].

The promoters of all known Proα1(I) and Proα2(I) collagen genes contain TATA boxes which are located 25–30 bp upstream of TSP. Analysis of the murine Proα1(I) and Proα2(I) collagen promoters revealed a conserved CCAAT box at –80 bp; a heterodimeric CCAAT binding factor, CBF, binds to this sequence and mutations that abolish binding of CBF to the cognate motif severely reduce promoter activity[43–45]. Immediately upstream of the CCAAT box there are binding sites for an inhibitory factor,

IF1, in both murine Proα1(I) and Proα2(I) collagen genes; presumably, these elements are involved in a coordinate regulation of the two collagen genes[46]. The murine Proα1(I) collagen promoter also contains a binding site for another inhibitory factor, IF2, which interacts with sequences on both sides of the CCAAT box[45,47]. Binding of IF2 is blocked by occupation of the CCAAT sequence by CBF. Three other putative *cis*-acting sites have been identified in the mouse Proα2(I) collagen gene, located at 250, 300, and 400 bp upstream of the TSP[43,48]. The binding site at –300 bp is a nuclear factor 1 (NF1)-like site which binds to recombinant NF1 protein[49] as well as to histone H1[50]. It has been claimed that NF1 mediates TGFβ1-induced enhancement of collagen gene transcription[51]. A TGFβ1-responsive NF1 like element was also located in the rat Proα1(I) collagen promoter, 1600 bp upstream of TSP[40].

Cis-regulatory sequence elements located upstream of TSP and in the first intron of the human Proα1(I) collagen gene have also been studied extensively[52–56]. However, studies to examine the role of the first intron in regulating the Proα1(I) collagen gene, using transient or stable expression of promoter-reporter constructs in cells in culture and in transgenic mice have revealed inconsistent information and these data are somewhat contentious[52,57,58]. As determined by DNase footprinting, at least 10 protein binding sites are located in the intron, between + 900 and + 1400 bp of the human Proα1(I) collagen gene. The intronic regulatory elements include a nuclease-hypersensitive site, two enhancer core consensus sequences, and two adjacent Sp1 motifs[52].

The orientation-dependent enhancer of the intron apparently contains both positive and negative *cis*-acting elements[52,53]. While the presence of the first intron enhanced activity of the human Proα1(I) promoter constructs in the lungs of transgenic mice, intronic sequences were apparently inconsequential in the skin and in cultured dermal fibroblasts isolated from these animals[59]. In contrast, most of the first intron was found to be dispensable for tissue-specific expression of the transgene, as long as adequate 5' flanking sequences were present[58]. In transient transfection assay in NIH 3T3 cells, the presence of the intron was shown to be necessary for the okadaic acid (OA)-induced suppression of the human Proα1(I) promoter[60]. In dermal fibroblasts cultured from systemic sclerosis patients, the presence of the intron up-regulated the activity of the Proα1(I) promoter[61]. It has been speculated that the intronic DNA may act as a transcription factor reservoir, enabling a high local concentration of transcription factors to compete with histones for binding to DNA, especially during its replication[62].

The concept of a modular arrangement of the *cis*-acting elements in collagen promotera was further corroborated by analyzing the structure and activation of a 220 bp long minimal promoter of the Proα1(I) collagen gene[63]. It was demonstrated that the minimal promoter contained sufficient information for basal tissue-specific transcription in transgenic mice[63], and even responded to TGFβ1 as seen in transient expression assays[64]. The basal promoter contains two moderately conserved GC-rich sequences with bona fide binding sites for Sp1[65–68]. Experiments carried out in our laboratory suggest that Sp1 is an obligatory activator of the full-length human Proα1(I) collagen promoter; we also showed that a single Sp1 motif located in close proximity of the TATA box, was sufficient to activate the minimal promoter[69]. Binding of Sp1 and its ability to activate the Proα1(I) collagen promoter has been corroborated by others[65,70]. The question of whether other members of Sp1 family interact with collagen genes and are involved in their regulation has also received recent scrutiny[70]. Expression of Sp3, which binds to the same sites as Sp1 stimulated promoter activity but only at higher concentrations; in contrast, Sp2 failed to transactivate the Proα1(I) promoter. Furthermore, coexpression of Sp1 and

Sp3 greatly reduced Sp1-induced activation of the Proα1(I) collagen promoter[70]. Sp1-binding activity has been implicated in the enhanced activity of Proα1(I) promoter seen in the activated Ito cells[71] and in the activation of Proα1(I) collagen promoter by TGFβ1[68]. It has been reported recently that the reduced bone density and osteoporosis in a subset of humans is associated with a polymorphic Sp1 binding site in the Proα1(I) collagen gene[72]. The precise significance of these data remains unknown at present and awaits more in-depth observations.

3. Regulation of collagen biosynthesis by phoshorylation/dephosphorylation pathways

Reduced synthesis of several cell surface-associated and ECM proteins, including fibronectin and type I collagen, is a common consequence of transformation *in vitro*[6,7]. Significantly, the tumorigenicity of Ras-transformed fibroblasts could be suppressed by exogenous expression of collagen in the transformed cells[73]. The molecular mechanisms involved in the altered regulation of ECM after transformation are only beginning to be elucidated. A number of biological and pharmacological agents modulate expression of their downstream target genes through a cascade of signal transduction elicited by sequential phosphorylation- and dephosphorylation of proteins[74]. These signaling mechanisms involve reversible modifications of proteins at tyrosine and serine/threonine residues and are catalyzed with specific kinases and phosphatases. A role for protein serine/threonine phosphatases in the control of cell growth has been inferred from the actions of a known tumor-promoter, okadaic acid (OA), that is also a potent inhibitor of serine/threonine phosphatase 2A[75–77].

While investigating the potential relationship between OA-induced cellular proliferation and collagen synthesis, we found that OA was a potent suppressor of type I collagen biosynthesis in NIH 3T3 cells[60]. The reduced rates of α1 and α2 procollagen synthesis in OA-treated cells were accompanied by lower steady state levels of their corresponding transcripts. Additionally, we discovered that expression of the luciferase reporter driven by Proα1(I) promoter was also downregulated by OA. Co-transfection of cells with Proα1 1(I) promoter-luciferase DNA with a PP2A expression vector strongly stimulated the collagen promoter. In contrast, coexpression of phosphatase PP1 did not affect collagen promoter activity significantly under identical conditions[60]. Based on these data, we hypothesized that OA acted via PP2A-mediated dephosphorylation of factor(s) that are essential for the activation of the human Proα1(I) collagen promoter. We also speculated that the reduced collagen biosynthesis observed in transformed cells could be mediated by mechanisms that were similar to those elicited by OA treatment of cells.

Phosphatase PP2A is known to dephosphorylate and inactivate several growth factor-stimulated protein kinases *in vitro*, suggesting that PP2A normally functions as a suppressor of cell growth. This is accomplished by attenuating the activity of a kinase(s) involved in cell proliferation. Specific binding of PP2A to SV40 small tumor antigen inhibits dephosphorylation of a number of substrates, including SV40 large tumor antigen and the growth suppressor protein p53[77]. A possible consequence of interaction between SV40 small tumor antigen and PP2A may be stimulation of mitogen-activated protein kinase pathway and induction of cell proliferation[78]. Inactivation of PP2A by viral oncoproteins may therefore be directly responsible for collagen down-regulation in SV40-transformed cells. On the other hand, since activities of PP2A and PP1 enzymes are also

regulated by protein kinases commonly elicited in transformed cells[79], suppression of collagen production may result from an indirect inactivation of a phosphatase(s).

3.1 Regulation of collagen gene expression by TGFβ1

Transforming growth factor β1(TGFβ1) is one of the most potent modulators of ECM synthesis, including type I collagen; it has been postulated to be a key cytokine involved in fibrotic disorders[3–5,80]. TGFβ1 belongs to very large family of peptides that are involved in the regulation of a multiplicity of growth and differentiation processes. The TGFβ subgroup consists of five closely related members found in the genomes of vertebrates and have been named TGFβ1 through 5. TGFβ1, the best-studied member of this group, is produced and secreted primarily by inflammatory cells and platelets. Transforming growth factor β1 is required during embryogenesis and wound repair and is considered a key modulator of the immune response. The varied and profound effects of TGFβ1 on the processes of developmental vasculogenesis and cardiogenesis in the embryo and in regulating the immune system have been elegantly demonstrated through detailed analyses of TGFβ1 knock-out mice[81,82]. The embryonic fibroblasts from the TGFβ1 null mice corroborated the notion that TGFβ1 acts as a negative autocrine regulator of growth and a positive autocrine enhancer of ECM biosynthesis[83].

The regulation of ECM in response TGFβ1 occurs via pathways of synthesis and degradation. While TGFβ1, TGFβ2 and TGFβ3 all exert profibrotic effects *in vitro*, TGFβ1 appears to be the predominant isoform expressed during pulmonary fibrosis[84]. Treatment with TGFβ1 leads to enhanced transcription and increased steady-state accumulation of Proα1(I), Proα1(III), and Proα1(IV) collagen mRNAs in cells in culture[85]. In addition to stimulating transcription of several procollagen-encoding genes[80,86–88], TGFβ1 has been shown to alter the stability of Proα1(I) collagen mRNA[88,89], and to decrease intracellular degradation of collagen[90]. The excessive collagen deposition seen in idiopathic pulmonary fibrosis, systemic sclerosis, renal interstitial fibrosis, and postoperative wound adhesions may be directly mediated by TGFβ1[3–5,80].

A central role of TGFβ1 in collagen deposition in lungs has been demonstrated in a number of animal models of bleomycin-induced pulmonary fibrosis[80,91,92]. Enhanced accumulation of TGFβ1 mRNA and protein in bleomycin-treated lungs was shown to precede the peak of synthesis of collagen, and overt fibrosis[80,93–97]. Immunohistochemical staining of bleomycin-treated lungs initially showed a patchy localization of TGFβ1 in the sub-epithelial matrix and later at the edges of the tissue undergoing repair, characterized by hyper-cellularity and enhanced collagen deposition[97]. Antagonizing the activity of TGFβ1, either with neutralizing antibodies or by treating the tissues with TGFβ1-binding proteoglycan decorin, was shown to ameliorate bleomycin-induced lung fibrosis[98,99]. TGFβ1 plays a central role in the excessive production and pathological deposition of ECM in the liver as well. The hepatic Ito cells respond to TGFβ1 by increasing synthesis, secretion and deposition of collagen and fibronectin into ECM[100]. The level of TGFβ1 mRNA in liver biopsy specimens from patients with chronic fibrosing liver disease was positively correlated with fibrosing activity[101].

The molecular mechanisms of TGFβ1-mediated enhancement in collagen gene expression have been analyzed in a number of laboratories. These observations suggest that the *cis*- and *trans*-acting interactions needed for TGFβ1 response may not be as simple as originally anticipated. The earliest known element claimed to be involved in the TGFβ1 response was NF1, identified in the mouse Proα2(l) collagen promoter[51]. The

promoter of rat Proα1(I) collagen gene contains a similar TGFβ1-responsive element 1600 bp upstream of the TSP[40]. Involvement of an AP-1-binding sequence in the action of TGFβ1 was shown for the human Proα1(I) and Proα2(I) collagen promoters[102,103]. However, a later report concluded that response of the human Proα2(I) collagen promoter to TGFβ1 was more likely to be dependent on an Sp1 element[68]. In summary, based on the information gleaned from the published literature, it is not difficult to conclude that a consensus TGFβ1-responsive element remains elusive at present.

4. Conclusion

The examination of the biochemical, genetic and molecular mechanisms of collagen biosynthesis have been extremely rewarding. These studies have shed light on the tissue-specific and inducible sequence motifs of Proα1(I) and Proα2(I) collagen promoters. These multidisciplinary efforts have unravelled the complexity of the modular organization of the tissue-specific elements of various collagen promoters. The precise interactions among the tissue-specific *cis*-acting modules and the transcription factors binding to these sequence motifs remain incompletely elucidated and are of great interest. We believe that a detailed mechanistic understanding of collagen gene expression is a prerequisite for devising rational therapeutic interventions for a host of serious diseases caused by abnormal collagen metabolism.

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