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Regulation of procollagen genes. From forces to factors

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Abstract. Collagens are the most abundant vertebrate proteins. Their primary role is to provide a supportive scaffolding to which cells attach but other actions in cell communication and cell function are now recognized. The work of pioneers of collagen research, of whom G N Ramachandran is a giant, have provided us with a detailed understanding of collagens' structure and function. In many of the inherited disorders (i.e., osteogenesis imperfecta) specific molecular lesions have been identified in collagen genes but in the common diseases, such as fibrotic disorders or rheumatoid arthritis, it is an imbalance in the rates of synthesis and breakdown which are critical. *In vivo* studies have shown that collagen turnover occurs at rapid rates in body tissues and that fibroblasts are dynamic cells actively synthesizing and degrading collagens. These cells are central to normal wound repair and the pathogenesis of fibrotic diseases. They organise and respond to their extracellular milieu and produce cytokines which exert autocrine and paracrine effects. They react to a variety of stimuli, including feedback from procollagen breakdown products, mechanical forces and polypeptide mediators. Mediators which regulate procollagen turnover; include the *TGFβ family* of homodimeric peptides which act via partially described signaling systems involving G-protein linked pathways. Elements of the coagulation cascade, including the serine protease *thrombin,* also promote collagen production and it is likely that these agents are part of a primitive system of haemostasis and tissue repair. For example, thrombin promotes procollagen production and gene expression via a recently characterized proteolytically activated receptor (PAR-1). Inhibitory molecules, such as *prostaglandin E2,* are also vital to collagen homeostasis and there is evidence that loss of this inhibitory control occurs in fibrotic conditions. The existence of multiple mediators regulating collagen deposition provides important questions and challenges for the future. For example, which are the key regulatory molecules *in vivo* and in which physiological and pathological settings are they playing roles? We also need to ascertain whether or not the different mediators are acting via common signaling pathways, or common transcription factors that may be appropriate targets to promote or inhibit collagen deposition? Answers to these questions are being sought using disparate technologies. For example, techniques of molecular genetics are being applied to the above diseases and should be instructive in the identification of key mediators in disease. The use of genetically manipulated animals, such as gene knock-outs and gene over-expressors will continue to be useful in defining the important mediators that regulate collagen deposition in normal developmental growth and disease states.

Keywords. Collagen; thrombin; prostaglandin E_2.

1. Introduction

I first heard of G N Ramachandran as an undergraduate student in biochemistry. I knew nothing about collagen, and cared even less, but it was clear to me that protein structures

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had an intricate beauty and the collagen triple helix, proposed by Ramachandran[1], was part of that beauty. Ramachandran also frightened undergraduates with his theoretical analyses, as elegant as they might be. I remember the "Ramachandran Plot" ranking with "Kreb's Cycle" as one of those things on which I wavered, preferring to focus on the elegance of the concepts rather than the tedium of the details. Some years later I got enticed to investigate some of these details, examining the affects of mechanical forces on muscle collagen turnover[2].

Ramachandran remains a giant in collagen's scientific evolution. His studies are the bedrock on which others have built. Our recognition of heterogeneity in the collagen superfamily, has been vital, as has our understanding of the way collagens interact with surrounding cells and regulate their function. Collagen is more than just a "glue of life", it is the very stuff of life – binding cells together and providing a conduit between them, influencing behaviour via mechanical and cell surface cues (figure 1).

Since Ramachandran's elucidation of collagens structure, another development in collagen biochemistry has been our understanding of the role of collagens in a number of pathological states. In many of the inherited disorders (i.e., osteogenesis imperfecta) specific molecular lesions have been identified in collagen genes. However, in many more disorders, such as lung fibrosis, and degenerative conditions, such as rheumatoid arthritis, it is an imbalance in the rates of collagen synthesis and breakdown that are likely to be critical[2–4]. For this reason, an appreciation of the key determinants of the rates of collagen turnover and their cell signaling pathways is of importance.

We now recognize that most body cells are actively synthesising a host of matrix components as well as the proteinases that are capable of degrading these components. The rates of matrix synthesis and degradation are also recognised to be more rapid than we previously thought. Thus fibroblasts make and degrade up to 3·5 procollagen molecules per hour[5] and some body tissues synthesize and degrade between one-tenth and to one-fifth of their collagen mass every day[6,7]. Furthermore, these rapid rates are

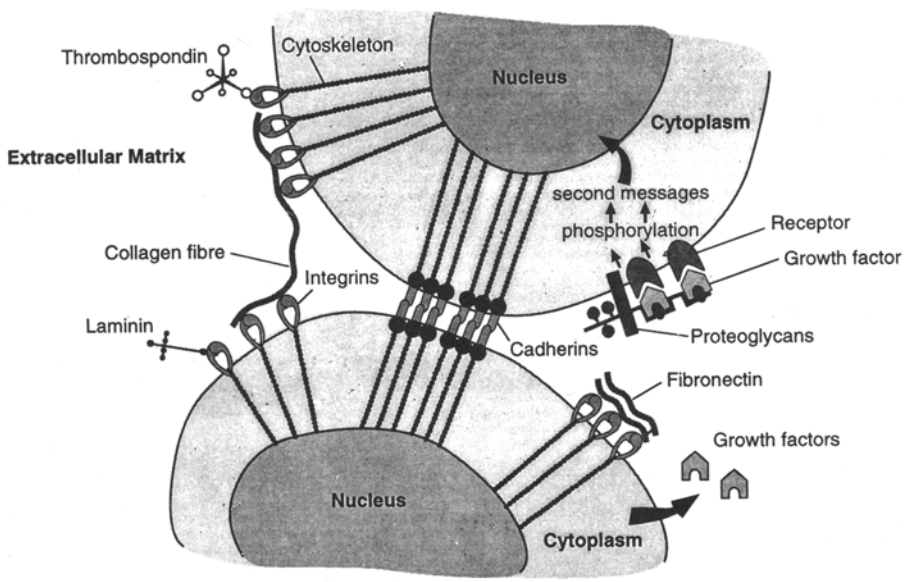

Figure 1. Matrix molecules in cell-cell interactions. Collagen can bridge between cell surface receptors and thus facilitate and influence signaling through to the nucleus.

maintained throughout life, although their magnitude varies greatly between tissues [6]. It is also recognised that degradation of collagen may occur at both intra-cellular and extra-cellular locations with intra-cellular degradation occurring within minutes of synthesis [6,8]. Extracellular degradation can also proceed rapidly and is mediated by a family of metalloproteinases, including collagenases, gelatinases and stromolysins [9,10].

Despite advances in our understanding of fibroblast biology as well as the pathways of collagen synthesis and degradation, many questions remain. What dictates whether or not these cells proliferate or reduce their population via apoptosis (see figure 2)? Which, amongst the myriad of factors, are the key regulators of procollagen turnover, and how are they regulated in different phases of growth and in disease states?

2. Regulation of collagen synthesis – the promoters and the inhibitors

Figure 3 lists some of the factors and processes known to regulate the production of procollagen. There is great diversity in these influences – from cytokines, lipids, coagulation cascade elements, physical forces as well as feed back from amino acids or

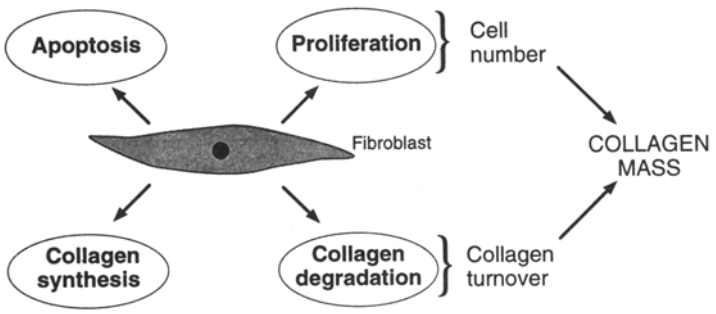

Figure 2. Fibroblasts regulate the collagen content of tissues through dynamic homeostasis regulating cell number and their rates of turnover.

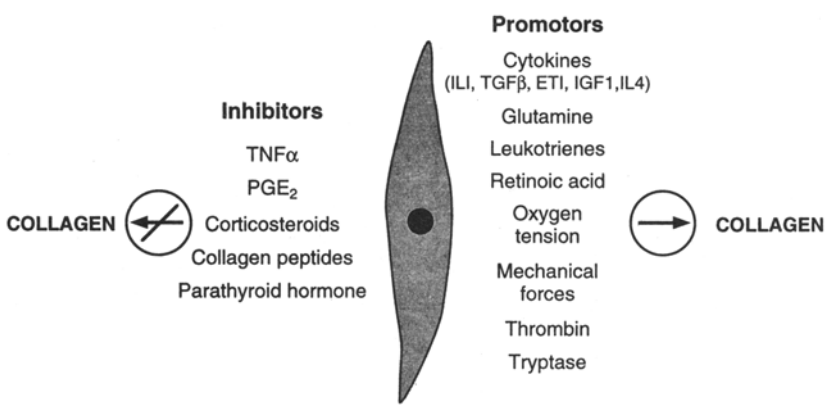

Figure 3. Procollagen synthesis is controlled via a network of promoters and inhibitors. This multiplicity provides us with challenges as we try to devise ways to influence collagen deposition but many opportunities to limit collagen production by inhibiting the affects of promoters or amplifying the actions of inhibitors.

procollagen breakdown products. Such diversity likely reflects the evolutionary pressures for multiple pathways in processes vital for survival. In this mini-review I will focus on several prototypic mediators which demonstrate this diversity through differences in their structure, their mechanism of generation and their mode of action. These mediators are *TGFβ*, a product of platelets and inflammatory cells; the serine proteinase *thrombin*, generated in the coagulation cascade; and *prostaglandin* E_2, a small molecular weight lipid mediator generated from aracadonic acid, which is an inhibitor of procollagen synthesis. All these mediators act via very different receptor systems (see figure 4); providing those of us trying to devise strategies to interfere with collagen deposition in disease states with a formidable challenge. However, as usual, the facts, as they come in, will bring solutions. For example, it is tempting to speculate that mediators regulating collagen deposition may act via common signalling pathways with common transcription factors (figure 4).

3. The TGFβs

3.1. The TGFβ superfamily and its receptors

The isolation of $TGF\beta_1$, the first member of this large family of secreted signalling molecules was reported almost twenty years ago[11]. The ability of this molecule to

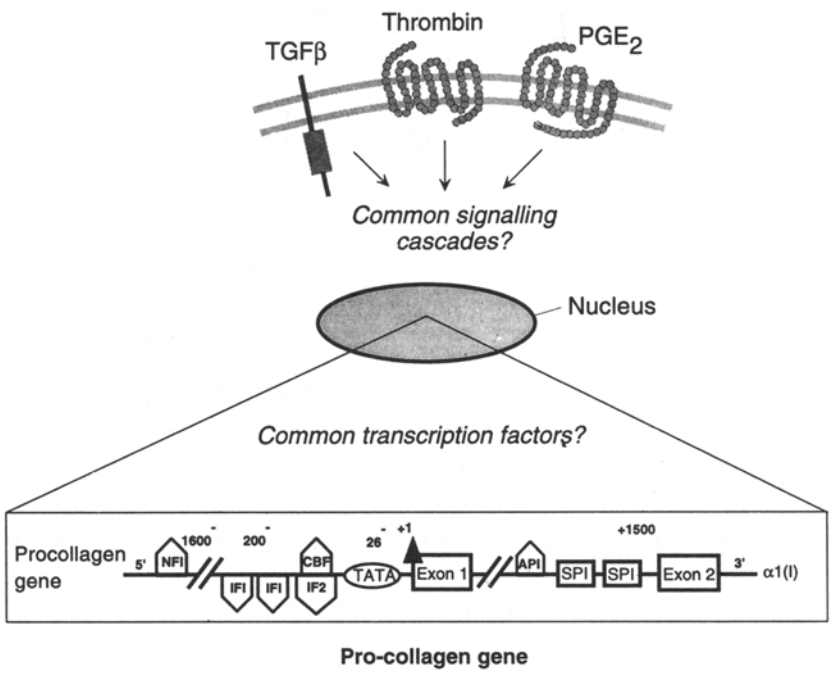

Figure 4. Three prototypic pathways regulating procollagen genes via polypeptides (thrombin and TGF-β) or lipids (PGE_2) acting via different receptors. Current efforts are focusing on the identification of common signaling cascades and common transcription factors which may provide us with novel targets to influence procollagen production.

promote matrix production was recognised early and it may be this property which is vital to TGFβ1's ability to induce anchorage-independent growth [12]. The TGFβ superfamily consists of more than 30 ligand proteins and is divergent in its effects [13]. It includes the five members of the TGFβ subfamily (TGFβ_{1-5}) of which three (TGFβ_{1-3}) are found in man. All of these isoforms are capable of increasing procollagen synthesis [14]. TGFβs$_{1-3}$ are all capable of binding to the two main receptors involved in signalling – receptors 1 and II [15]. In addition, they can all bind β glycan, a cell surface proteoglycan that may modulate the binding of TGFβs to their receptors.

3.2 TGFβ promotion of procollagen production

It has been recognized for some time that TGFβ_1 was capable of promoting production of a variety of matrix proteins, particularly fibronectin and collagen [12,16,17]. The activity of TGF-β appears to operate via concerted actions at various levels of collagen regulation and include increased levels of steady state procollagen mRNA [17] and increased mRNA stability [18]. TGF-β_{1-3} are now recognized to be potent stimulators of procollagen production; with significant activity at concentrations less than 50 picomolar [14]. In addition, TGF-β_1 reduces the proportion of newly synthesized collagen that is degraded [5]. Others have shown that TGF-β decreases extracellular degradation both by decreasing metalloprotease production and increasing expression of their inhibitors [19].

The intracellular signalling downstream of the TGF-β serine/threonine kinase receptor interaction is at present poorly understood. In lung fibroblasts, TGF-β has been reported to induce a number of signalling pathways including cyclin dependant kinase (CDK) inhibitors [20] and the RAf–MEK–MAPK pathway [21] but the pathways mediating TGF-β's effects on collagen synthesis remain to be elucidated.

3.3 Role of prostaglandin E_2 in modulation of TGFβ's effects on procollagen production

PGE_2 inhibits procollagen production. Salzman and co-workers [22] first demonstrated that PGE_2 suppressed collagen production by human foetal lung fibroblasts. This was accompanied by a concomitant increase in cAMP which was shown to increase the proportion of newly synthesized collagen degraded intracellularly [23]. PGE_2 also down-regulates lysyl oxidase gene expression and suppresses the enhancing effects of TGF-β and IL-1β on expression of this gene [24,25].

Interestingly, several mediators that stimulate procollagen production by fibroblasts, also induce fibroblasts to synthesize PGE_2. These include PDGF, TGF-β, TNF-α, EGF and IL-1β [26–30]. In the case of IL-1β, upregulation of cyclooxygenase-2 was suggested as the mechanism for enhanced PGE_2 synthesis [31].

TGF-β has long been implicated in the pathogenesis of pulmonary fibrosis (see Coker and Laurent [32] for review) and recently, *in vivo* gene transfer of active TGF-β_1 was shown to produce a sustained and severe fibrosis in rat lungs [33]. TGF-β is one of the agents which stimulates PGE_2 production by cells and we have proposed that this provides an important feedback loop whereby TGFβ's effects on collagen synthesis and fibroblast proliferation are limited by autocrine production of PGE_2 [27,34] (also see figure 5). The observation that many profibrotic mediators are capable of inducing PGE_2 synthesis highlights the importance of positive and negative feedback in the control of fibroblast function. Such interactions may be relevant in fibrotic conditions where reduced levels of PGE_2 could de-repress fibroblast proliferation and collagen synthesis. This concept is

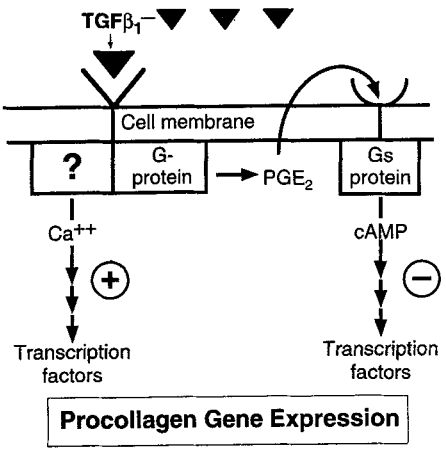

Figure 5. The TGF-β-PGE_2 system for regulating procollagen gene expression. TGF-β promotes procollagen production via signaling pathways which are currently poorly understood. It also stimulates release of PGE_2 which exerts inhibitory effects.

supported by data demonstrating reduced levels of PGE_2 in fluids taken from lungs of patients with pulmonary fibrosis compared with normal subjects[35]. Further, Wilborn and colleagues have shown that fibroblasts from patients with pulmonary fibrosis synthesized less PGE_2 than control cells in response to IL-1 or lipopolysaccharide[31].

4. Thrombin

4.1 Thrombin and the coagulation cascade

The primary function of the coagulation cascade is to limit blood loss and to form a temporary matrix that provides a scaffold for fibroblasts. However, it has recently become clear that products of this cascade exert a number of cellular effects including the regulation of matrix production. One of the key molecules exerting such effects is thrombin. It promotes recruitment and proliferation of inflammatory and immune cells and enhances trapping of these cells by inducing expression of adhesion molecules on endothelial surfaces. It also promotes platelet aggregation with subsequent release of powerful fibroblast growth factors such as PDGF and TGFβ. Finally, thrombin may promote repair through its action on fibroblasts; it is a chemoattractant[36] and mitogen for these cells[36–39] and effects on procollagen synthesis have been reported[40–42].

4.2 Thrombin promotes procollagen production via activation of proteolytically activated receptor-1

In 1991 Coughlin and colleagues reported the cloning and sequencing of the first thrombin receptor in platelets[43] and fibroblasts[44]. This was named proteolytically activated receptor-1 (PAR-1) reflecting its unique activation mechanisms whereby thrombin binds via its anion binding site (residues 53-64) and cleaves an Arg–Ser bond at residue 41-42 in the human receptor (figure 6, left hand side). This reveals a new

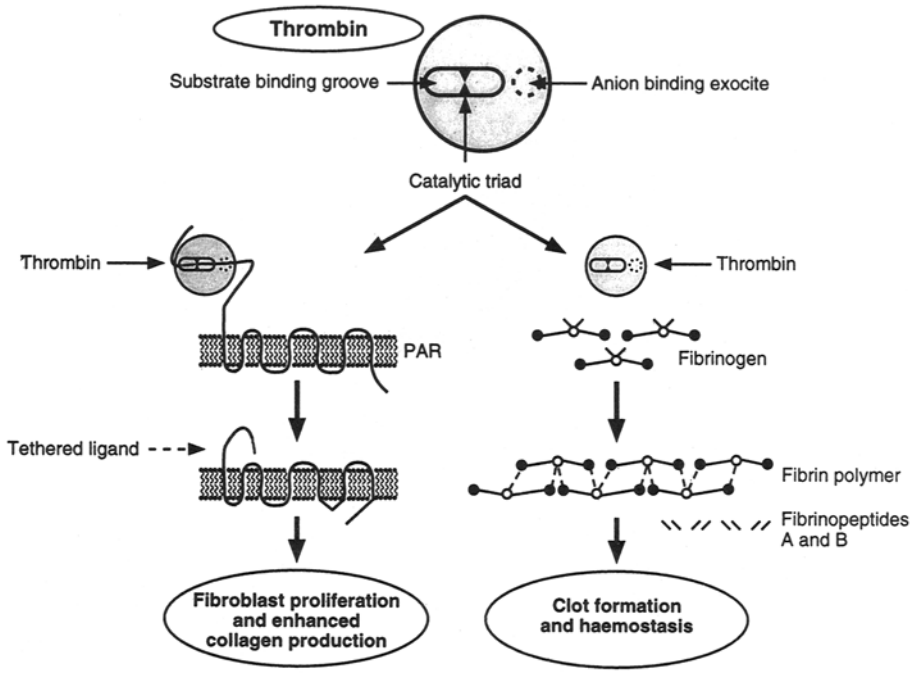

Figure 6. Thrombin affects disparate events via proteolytic cleavage of various substrates. Cleavage of fibrinogen leads to fibrin formation whereas cleavage of proteolytically activated receptor, PAR1, leads to enhanced procollagen production[41,42]. Figure adapted from Goldsack *et al*[49].

N-terminus which then acts as a "tethered ligand", interacting with the second extracellular loop of the 7-trans membrane receptor (see Derry *et al*[45] for review). Since the discovery of PAR-1, three further receptors activated in a similar manner (PAR-2, PAR-3 and PAR-4) have been reported[46–48].

The activation of fibroblast procollagen production by fibroblasts occurs via cleavage of PAR-1. The key evidence for this is threefold: first, thrombin's proteolytic activity is required for stimulation of procollagen production[41]; second, the peptide sequence SFLLRN, identical to the new N-terminal of the thrombin receptor revealed at cleavage (the so-called thrombin receptor activating peptide, TRAP), can activate procollagen production[41]; and, finally, activation of procollagen production is not seen in fibroblasts derived from animals in which PAR-1 has been deleted using gene transfer technology (Chambers *et al*, unpublished information).

5. Role of thrombin, TGFβ and PGE_2 in fibrosis

There is now evidence that thrombin and the TGFβ-PGE_2 system are playing key roles in fibrotic disorders (see figure 7).

TGFβ is present at sites of fibrosis (see Coker and Laurent[32] for review) and is produced by a variety of cell types including macrophages and epithelial cells[14]. Pulmonary fibrosis develops when TGFβ is overexpressed in the lungs of rats using

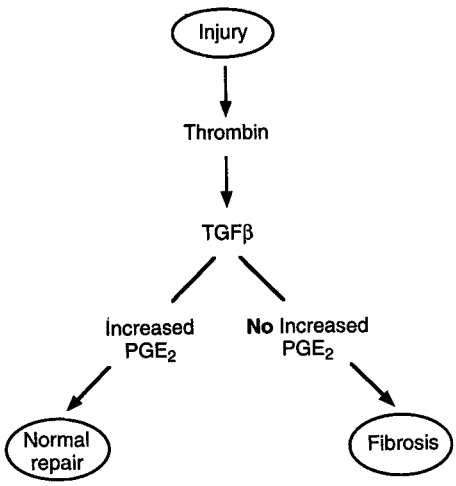

Figure 7. Hypothesis proposing central roles for thrombin and the TGF-β-PGE_2 system in fibrotic disorders. Thrombin is present at the earliest stages of tissue damage and can influence vascular permeability and inflammation. TGF-β is released at sites of injury where it activates collagen production by fibroblasts. In normal repair there is an increase in PGE_2 which limits this increase but in fibrotic disorders there is a defect in this pathway and uncontrolled and progressive collagen deposition occurs.

adenoviral vectors in which TGFβ is linked to a SV40 promoter. Finally, if the actions of TGFβ are blocked, either with antibodies or binding molecules, such as decorin the extent of fibrosis is reduced.

Thrombin is also a major candidate as an important profibrotic mediator. The coagulation cascade is activated in most inflammatory diseases associated with fibroproliferation and again inhibitors have been shown to partially block the development of fibrosis.

Similarly, a deficit in the PGE_2 pathway has been implicated in the development of fibrosis. There is evidence, reviewed above that the amounts of PGE_2 are reduced in fibrotic tissues and PGE_2 production is diminished in cells derived from tissues of patients with fibrosis.

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