

# Transcriptional coactivators p300/CBP and Type I collagen gene expression

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Type I collagen is the major extracellular matrix protein in skin and other tissues. Excessive synthesis and deposition of collagen in the dermal region is the hallmark of skin fibrosis or scleroderma. The multifunctional cytokine transforming growth factor-beta (TGF- $\beta$ ) induces the Type I collagen synthesis and has been implicated in tissue fibrosis. The signal transducers Smads and transcriptional coactivators p300/CBP are major regulators in the TGF- $\beta$ -induced Type I collagen synthesis. In skin fibroblasts, p300 interacts with Smads and stimulates the Type I collagen synthesis. The intrinsic histone acetyltransferase activity of p300 is essential for maximal stimulation of collagen synthesis. The synthesis of Type I collagen is also negatively regulated by cytokines like interferon-gamma (IFN- $\gamma$ ) and tumour necrosis factor-alpha (TNF- $\alpha$ ). While the profibrotic cytokine TGF- $\beta$  induces the collagen synthesis, antifibrotic cytokines IFN- $\gamma$  and TNF- $\alpha$  abrogate the TGF- $\beta$ -induced collagen synthesis via activation of STAT1 $\alpha$  or STAT1 $\alpha$ -induced factor(s) and Jun family members respectively. The IFN- $\gamma$  and TNF- $\alpha$  activated molecules compete with TGF- $\beta$  signaling molecules Smads for limiting amount of cellular p300/CBP and suppress the TGF- $\beta$  signaling. The transcriptional coactivators p300/CBP are known to interact with numerous transcription factors and play a pivotal role in the transcriptional regulation of numerous genes whose products control the cell cycle, growth and development. Post-translational modifications of p300/CBP by phosphorylation and/or methylation play a significant role in their functional activities. Abnormality in p300 or CBP activity has been implicated in different diseases, also known as 'Coactivators Diseases'. The present review discusses the significance of transcriptional coactivators p300/CBP in the cytokine modulation of extracellular matrix protein, Type I collagen synthesis, and its relevance to tissue fibrosis like scleroderma.

SCLERODERMA is a connective tissue disease characterized by thickening and hardening of the skin due to excessive synthesis and deposition of collagen by the activated fibroblasts in the dermal region<sup>1-4</sup>. Type I collagen is the major component in the extracellular matrix of skin. In the last ten years, extensive studies have been

made to understand the molecular mechanism governing the transcriptional regulation of collagen synthesis in skin and other tissues. Several *cis*-acting regulatory elements and transacting protein factors, involved in basal as well as cytokine-induced or repressed collagen gene transcription, have been identified and characterized in human and other organisms<sup>5</sup>. Although it has been known for a decade that TGF- $\beta$  stimulates the Type I collagen synthesis and has been implicated in fibrosis, the exact molecular mechanism which causes hypertranscription of collagen gene in fibrotic tissues is not well understood. The transcription factors like Sp1, AP1 and Smads have been shown to be important in the TGF- $\beta$ -induced collagen synthesis<sup>5-10</sup>. Several recent studies provide strong evidence that p300/CBP act as transcriptional coactivators and adaptor molecules of Type I collagen gene transcription<sup>9,11-13</sup>. The intrinsic histone acetyltransferase (HAT) activity of p300/CBP play a major role in the transcriptional regulation of basal, TGF- $\beta$ -induced and IFN- $\gamma$ -inhibited Type I collagen synthesis<sup>9,11,13</sup>. In this review, we discuss the recent development on the role of transcriptional coactivators p300/CBP in basal as well as cytokine-modulated Type I collagen gene expression and its significance in tissue fibrosis research.

## p300/CBP transcriptional coactivators

p300 and CBP originally discovered as E1A associated 300 kDa protein and CREB binding protein respectively<sup>14,15</sup> and are present in all metazoans. p300 and CBP are products of two genes and have significant sequence and functional homology in different evolutionary conserved domains<sup>16,17</sup>. In spite of having significant homology, in many cases, one cannot completely substitute the other, indicating the functional distinctions of p300 and CBP<sup>18,19</sup>. It is now well documented that p300 and CBP are important components of transcriptional complex of several genes whose products are involved in cell cycle regulation, growth and development<sup>20</sup>. Deficiency of p300 or CBP causes several developmental abnormalities like open neural tube, heart defects, etc., as well as embryonic lethality in mice<sup>21,22</sup>. Akimura *et al.*<sup>23,24</sup> reported that in *Drosophila*, mutation in CBP causes embryonic lethality and hypomorphic CBP allele causes developmental abnormalities. Abnormalities in p300/CBP have also been linked with different human diseases. Single

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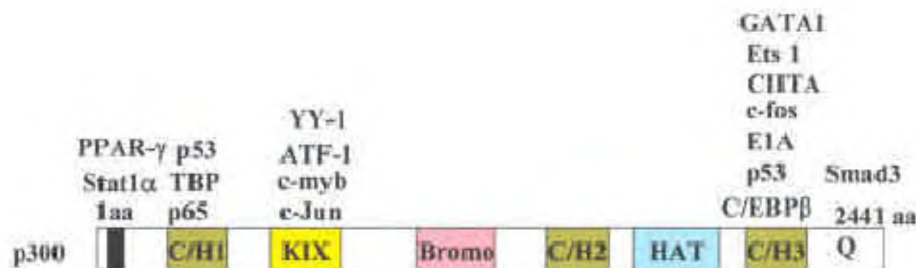
copy of CBP is not good enough for normal physical and mental growth as demonstrated in Rubinstein–Taybi syndrome<sup>25</sup>. Mutations and altered activity in p300 and CBP have also been reported in different cancer cells<sup>26</sup>. Nowadays, p300/CBP based diseases are known as ‘Coactivators Diseases’<sup>27</sup>.

Recently, several attempts have been made to understand the molecular mechanisms by which p300/CBP control the target gene transcription. It is now well documented that different evolutionary conserved domains like CH1, CH2, CH3 and KIX of both factors, interact with numerous transcription factors and receptors in a context-dependent manner (Figure 1), and act as adaptor molecules. Specific interaction of p300/CBP with specific transcription factor causes modulation of the factor’s transcriptional activity and thus p300/CBP are also known as transcriptional coactivators or corepressors, depending on their interaction with factors in response to different cellular signals, stresses or viral infections<sup>16,20</sup>. Besides interaction with transcription factors, p300/CBP possess intrinsic histone acetyltransferase activity (HAT) which acetylates histone and augments the activator-dependent transcription from reconstituted chromatin templates *in vitro*<sup>28</sup>. Recently, Kundu *et al.*<sup>29</sup> very elegantly demonstrated that VP-16- and SP1-mediated transcriptional activation from *in vitro* reconstituted chromatin template is dependent on the VP-16 or SP1 recruited p300 level, presence of acetyl-CoA and subsequent acetylation of targeted promoter proximal histone tails. Furthermore, activator-recruited HAT containing p300 acts synergistically with ATP-driven SW1/SNF chromatin remodeling complexes for activation of gene expression<sup>30</sup>. In addition, p300/CBP have also been shown to acetylate general transcription initiation factors<sup>31</sup> as well as several transcription factors like p53 (ref. 32), GATA-1 (ref. 33), NF-Y (ref. 34), HIV-1 Tat (ref. 35), and thus modulate the promoter region as well as factor’s activity and target gene expression<sup>36</sup>. Abnormal acetylation and deacetylation of nucleosomal histones and/or transcription factors have been implicated in different diseases<sup>37–39</sup>. Therefore, identification of natural or synthetic modulator that can control the level of coactivator-HAT activity will be a promising novel approach to control the coactivator-HAT related diseases. Recent discovery of p300/CBP HAT-specific synthetic (Lys-CoA) and natural (anacardic acid) small molecule modulators opens such a new field of biomedical research on different coactivator-HAT-related diseases<sup>40,41</sup>.

p300 and CBP are phosphoproteins and have been shown to be phosphorylated in a cell cycle-dependent manner<sup>42</sup>. Several recent reports suggest that the site-specific phosphorylation of p300/CBP by different kinases like PKA, PKB, PKC, MAPK, and cyclin E/Cdk2 causes the alteration of their functions<sup>16</sup>. Phosphorylation of CBP by PKA is required for activation of pituitary-specific factor, Pit-1 which represses or activates the ex-

pression of specific genes in response to signal transduction<sup>43</sup>. Swope *et al.*<sup>44</sup> demonstrated that PKA increases the ability of N-terminal domain of CBP to stimulate transcription. As this domain does not contain PKA-mediated phosphorylation consensus, authors predicted that the PKA-mediated activation of p300 may be indirect, i.e. through activation of other kinases that can phosphorylate CBP. p300 has also been shown to be phosphorylated by protein kinase B at 1834 aa in the domain 1752–1859 aa of p300 where C/EBP- $\beta$  and several other factors interact and control their target gene expression, and this phosphorylation causes disruption of C/EBP $\beta$  interaction with p300, leading to inhibition of IGFBP-1 gene promoter activity in response to insulin<sup>45</sup>. Therefore, the activity of transcription factors like Pit-1, C/EBP- $\beta$  are modulated (repressor or activator) by post-translational modification(s) of transcriptional coactivators p300/CBP in response to different signaling. Yuan and Gambe<sup>46</sup> demonstrated that the serine 89 residue of p300 undergoes phosphorylation by Protein Kinase C and represses the p300 activity as replacement of serine 89 residue with alanine increases the p300-induced ER and AP-1 mediated promoter activities. Furthermore, PKC- $\delta$ -mediated phosphorylation of p300 at serine 89 residue causes inhibition of its intrinsic HAT activity<sup>47</sup>. On the other hand, p300 and CBP can be phosphorylated by E2-CDK and this phosphorylation modulates the transcriptional activity of p300/CBP and also causes increased HAT activity of CBP as roscovitine, a specific inhibitor of E-CDK2 complex causes the inhibition of CBP phosphorylation and CBP HAT activity<sup>48,49</sup>.

Recently, See *et al.*<sup>50</sup> demonstrated that mitogen-activated/extracellular response kinase kinase 1 (MEKK1) stimulates p300-mediated transcription possibly through direct phosphorylation of different domains of p300. This MEKK1-mediated stimulation of p300 transcriptional activity is independent of JNK activation. Therefore, MEKK1 can control the p300 transcriptional activity through specific residue phosphorylation. Zanger *et al.*<sup>51</sup> demonstrated that growth factor-dependent phosphorylation of CBP at Ser 436 residue is required for recruitment of CBP in different specific promoters. Most importantly, this requirement is unique for transcriptional coactivator CBP. Therefore, it is a unique example where post-translational modification of p300 and/or CBP can alter the specificity of the coactivators for a particular transcriptional complex in a gene specific manner. Beside phosphorylation, methylation of p300/CBP also modulates their role in target gene expression. Methylation of specific arginine residue in the KIX domain of p300 by coactivator-associated arginine methyltransferase 1 (CARM1) inhibits the interaction between p300-KIX and CREB-KID and thus blocks the CREB-dependent transcription<sup>52</sup>. Therefore, along with the expression level of p300 and/or CBP, their post-translational modifications by phosphorylation or methylation under different



**Figure 1.** Molecular structure of p300 with different structural and functional domains. Different domains of p300 interact with numerous regulatory proteins. Only few interacting factors have been presented here. C/H, Cysteine/histidine rich domain; KIX, CREB interacting domain via its kinase inducible domain (KID); Bromo, bromodomain; HAT, histone acetyl-transferase domain; Q, glutamine-rich region.

physiological conditions may play a significant role in the control of their activities, and thus the target gene expression.

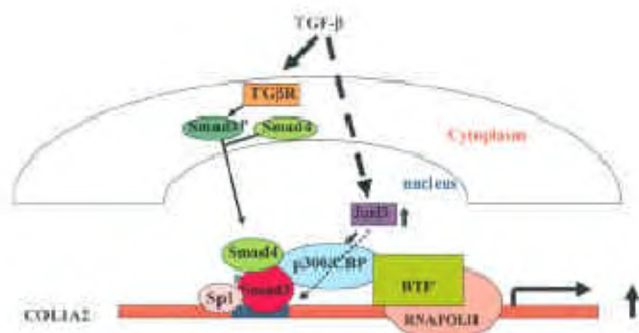
### p300 in TGF- $\beta$ /Smad signaling pathway and Type I collagen gene expression

TGF- $\beta$  is a very potent stimulus for extracellular matrix production by inducing the synthesis of many extracellular matrix proteins, and has been implicated in fibrosis<sup>4,5,53–55</sup>. TGF- $\beta$  activates TGF- $\beta$  receptors which transphosphorylate the R-Smads, Smad2 and Smad3. R-Smads heterodimerize with Co-Smad, Smad4 and translocate to the nucleus. Upon translocation, the Smads interact with Smad-binding element (SBE) and/or with transcriptional coactivators p300/CBP and regulate the transcription of TGF- $\beta$  target genes. On the other hand, the TGF- $\beta$ -induced inhibitory Smad, Smad7 translocates to cytoplasm and inhibits the TGF- $\beta$  signaling by direct interaction with TGF- $\beta$  receptors and blocking the receptor-mediated phosphorylation of R-Smads<sup>56–59</sup>. In human skin fibroblasts, while the overexpressed R-Smads, Smad2/3 stimulate the basal as well as TGF- $\beta$ -induced *COL1A2* gene transcription, the inhibitory Smad, Smad7 abrogates the TGF- $\beta$ -induced *COL1A2* gene transcription<sup>5,8,9,11,55</sup>. TGF- $\beta$  induces the translocation of Smad3 and Smad4 from cytoplasm to nucleus in primary culture of human skin fibroblasts. Furthermore, TGF- $\beta$  inhibits the level of Smad3/Smad4 and stimulates the anti-Smad, Smad7 suggesting TGF- $\beta$  activates the Smad-signaling in human skin fibroblasts<sup>60</sup>. Collectively, these results suggest the functional significance of activated Smad-signaling in Type I collagen gene regulation in human skin fibroblasts. The physical and functional interactions of Smad3/4 complex with Sp1 on *COL1A2* promoter have also been implicated in maximal stimulation of Type I collagen gene transcription in response to TGF- $\beta$ <sup>10</sup>.

The role of transcriptional coactivators p300/CBP in the Smad-dependent TGF- $\beta$ -induced Type I collagen syn-

thesis has been extensively studied. Overexpressed p300 stimulates the basal as well as TGF- $\beta$ -induced *COL1A2* promoter activity and endogenous Type I collagen mRNA, and protein levels in human skin fibroblasts. p300 also augments the Smad3-induced *COL1A2* promoter activity, suggesting the existence of functional interaction of Smads and transcriptional coactivators p300 in the regulation of collagen gene expression<sup>9</sup>. Wild-type adeno-E1A, but not mutant adeno-E1A $\Delta$ 2-36, abrogates the TGF- $\beta$ -induced *COL1A2* promoter activity, and overexpressed p300 rescues the TGF- $\beta$ -induced *COL1A2* promoter activity in presence of adeno-E1A, further supporting the involvement of p300/CBP in TGF- $\beta$ /Smad-induced Type I collagen synthesis in skin fibroblasts. It has also been demonstrated that complete Smad signaling is essential for the p300-mediated augmentation, as p300 can induce the *COL1A2* transcription in Smad4 deficient MDA-MB-468 breast carcinoma cell only in presence of exogenously expressed Smad4 (ref. 9). Recently, Zhu and Ting<sup>12</sup> demonstrated that p300-related CBP stimulates the *COL1A2* gene transcription in a NIH-3T3 cell. Collectively, these results strongly suggest the involvement of transcriptional coactivators p300/CBP in basal as well as TGF- $\beta$ -induced Type I collagen synthesis (Figure 2).

A recent report suggests that interaction of p300 with p53 is important in p53-mediated modulation of TGF- $\beta$  signaling and for suppression of TGF- $\beta$ -induced Type I collagen synthesis in human skin fibroblasts. The p53-mediated repression of TGF- $\beta$ -induced collagen gene transcription is not due to alteration of R-Smads or inhibitory Smad in p53 overexpressing cells. Overexpressed HAT containing p300 can significantly overcome the inhibitory action of p53 on TGF- $\beta$ -induced collagen gene transcription and p53 (22,23) mutant, which cannot interact with p300, fails to abrogate completely the TGF- $\beta$ -induced collagen gene transcription<sup>61</sup>. Taken together, these results strongly suggest the pivotal role of transcriptional coactivator p300 in the regulation of Type I collagen synthesis upon interaction with positive and negative regulators of Type I collagen gene.



**Figure 2.** Model demonstrating the mechanisms of TGF- $\beta$ -induced Type I collagen synthesis. TGF- $\beta$  activates the TGF- $\beta$  receptors which activate Smad signaling molecules or induce JunD. Activated TGF- $\beta$  receptors phosphorylate the R-Smad which heterodimerize with Co-Smad, Smad4 and translocate to nucleus. Activated Smads and or JunD stimulate the collagen gene transcription via interaction with *cis*-acting regulatory elements, transcription factor Sp1 and transcriptional coactivators p300/CBP. BTF, basal transcription factors; RNAPOLII, RNA polymerase II;  $\uparrow$ , up regulated; P, phosphorylated.

The intrinsic histone acetyltransferase (HAT) activity of p300 and CBP play a significant role in the alteration of target gene expression<sup>17</sup>. The HAT activity of p300 plays a significant role in the regulation of *COL1A2* gene transcription as HAT-deleted p300-mediated stimulation of *COL1A2* promoter activity is significantly less compared to stimulation by wild type p300. Thus, the intrinsic HAT activity of p300 is required for p300-mediated maximal induction of basal as well as TGF- $\beta$ -stimulated *COL1A2* gene transcription in human dermal fibroblasts<sup>9,13</sup>.

### p300 in IFN- $\gamma$ signaling pathway and Type I collagen gene expression

Interferon-gamma (IFN- $\gamma$ ) is a known anti-fibrotic cytokine which causes down regulation of Type I collagen synthesis<sup>62-65</sup>. In order to understand the molecular mechanism by which IFN- $\gamma$  imparts its negative influence on collagen synthesis, different laboratories reported the involvement of different *cis*-acting regulatory elements and transacting protein factors<sup>5</sup>. In this review, we will only discuss the possible mechanisms of IFN- $\gamma$ -mediated inhibition of collagen synthesis where p300/CBP are involved. IFN- $\gamma$  transmits the signal through the JAK-STAT pathway where activated JAK1 phosphorylates and activates STAT1 $\alpha$ . Activated STAT1 $\alpha$  dimerize and upon translocation to the nucleus control the IFN- $\gamma$ -responsive gene transcription by direct interaction with gamma-activated sequences or through interaction with other transcription factors or coactivators in the transcriptional complex<sup>66</sup>.

IFN- $\gamma$  inhibits the *COL1A2* promoter activity in human skin fibroblasts, but not in STAT1 $\alpha$  deficient fibrosarcoma cells U3A (A. K. Ghosh, unpublished observation) and in JAK1-deficient U4A cells. These results suggest that the JAK-STAT1 pathway is required for this IFN- $\gamma$ -

mediated inhibition<sup>11</sup>. In human skin fibroblasts, IFN- $\gamma$  strongly induces the interaction of STAT1 $\alpha$  with p300. Overexpression of p300 blocks the IFN- $\gamma$ -mediated inhibition suggesting that induction of p300-interacting factors like STAT1 $\alpha$ , and/or STAT1 $\alpha$ -induced factors sequester p300 from the basal transcriptional machinery and suppress the transcription<sup>11</sup>.

On the other hand, Zhu and Ting<sup>12</sup> recently reported that IFN- $\gamma$  suppresses the *COL1A2* gene expression via class II transactivator (CIITA), a master regulator of MHC class II gene<sup>67</sup>. A mutant cell line G3A which lacks the CIITA is unable to inhibit the *COL1A2* promoter activity in response to IFN- $\gamma$  and overexpression of CIITA in G3A causes inhibition in response to IFN- $\gamma$  suggesting the suppressive action of CIITA on *COL1A2* promoter activity. Interestingly, overexpression of a 36-amino-acid region of CIITA mimics the inhibitory effect of IFN- $\gamma$ . This 36-amino-acid domain of CIITA interacts with CBP, and overexpression of CBP blocks this CIITA-mediated suppression of *COL1A2* promoter activity. Involvement of CIITA in IFN- $\gamma$  inhibited *COL1A2* promoter activity was further supported by the observation that CIITA containing RFX5 complex is induced by IFN- $\gamma$  and inhibits the *COL1A2* promoter activity in rat fibroblasts and human lung fibroblasts<sup>68</sup>. Therefore, it is apparent that different factors like STAT1 $\alpha$  and/or STAT1 $\alpha$ -induced factor CIITA, or other factor(s) mediate the IFN- $\gamma$ -induced inhibition of *COL1A2* transcription in a cell type-dependent manner. Most importantly, IFN- $\gamma$ -activated factors interact with transcriptional coactivator p300/CBP and squelch it from the active transcriptional machinery to suppress the *COL1A2* transcription. These results, together further suggest the transcriptional coactivators p300/CBP and their interacting factors play a significant role in IFN- $\gamma$  inhibited Type I collagen synthesis (Figure 3).

### p300 in antagonistic effects of IFN- $\gamma$ and TNF- $\alpha$ on TGF- $\beta$ signaling in collagen synthesis

Type I collagen synthesis is highly regulated by different cytokines and plays a significant role in wound healing and fibrosis<sup>1,2,69</sup>. While TGF- $\beta$  enhances the Type I collagen synthesis, IFN- $\gamma$  and TNF- $\alpha$  abrogate the TGF- $\beta$  induced Type I collagen synthesis<sup>5,11,62-65</sup>. Although TGF- $\beta$ -induced collagen gene transcription is Smad3-dependent and inhibitory Smad, Smad7 abrogates the TGF- $\beta$ -induced *COL1A2* transcription. The mechanism of IFN- $\gamma$ -mediated abrogation of TGF- $\beta$  stimulation of collagen synthesis is not Smad7-dependent and therefore the inhibitory effect of IFN- $\gamma$  may not be at the receptor level<sup>11</sup>. Inhibition of ligand-independent Smad3-induced *COL1A2* promoter activity by IFN- $\gamma$  further supports the notion that inhibitory effect by IFN- $\gamma$  is at the downstream of receptor level or at nuclear level. IFN- $\gamma$  abro-

gates the TGF- $\beta$ -induced Type I collagen synthesis through activation of JAK-STAT pathway. The supportive evidence comes from the observations that IFN- $\gamma$  can block the TGF- $\beta$ -induced COL1A2 promoter activity in U4A cells stably transfected with JAK1, but not in JAK1-deficient U4A cells<sup>11</sup>.

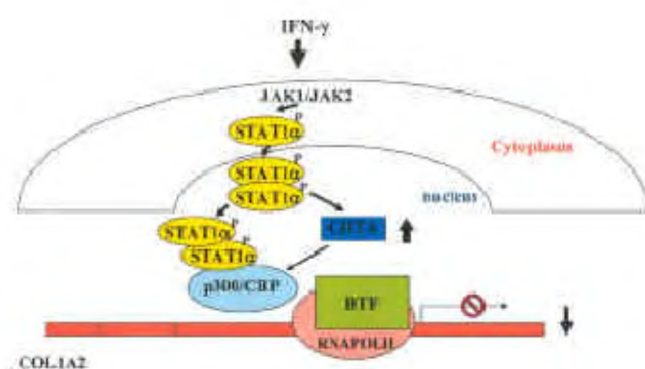
IFN- $\gamma$  induced inhibition of TGF- $\beta$ -stimulated COL1A2 promoter activity can be blocked by overexpression of transcriptional coactivator p300, suggesting the important role of p300 in the antagonistic action of IFN- $\gamma$  and TGF- $\beta$  on collagen synthesis. Both IFN- $\gamma$  activated STAT1 $\alpha$  and TGF- $\beta$ -induced Smad3 interact with transcriptional coactivator p300. IFN- $\gamma$  blocks the TGF- $\beta$ -induced interaction of Smad3 with p300, indicating that activated STAT1 $\alpha$ , and/or STAT1 $\alpha$  induced factor(s) sequester the endogenous p300 and reduce its interaction with Smad3, thus blocking the TGF- $\beta$ /Smad-induced collagen gene transcription<sup>11</sup>. The possible role of cytokines modulated p300 activity in this antagonistic action cannot be ruled out. Importantly, in the absence of HAT activity, p300 cannot overcome the IFN- $\gamma$  mediated inhibition of TGF- $\beta$ -induced COL1A2 promoter activity, further suggesting a significant role of HAT activity in the cytokine-modulated collagen gene expression (A. K. Ghosh, unpublished observation). Eickelberg *et al.*<sup>70</sup> demonstrated that TGF- $\beta$  stimulates collagen synthesis through induction of JunD/AP1 in human lung fibroblasts. IFN- $\gamma$  abrogates the TGF- $\beta$  induced collagen synthesis in human lung fibroblasts via IFN- $\gamma$  activated STAT1 $\alpha$ <sup>70</sup>. Both JunD/AP1 and STAT1 $\alpha$  are known to interact with transcriptional coactivators p300/CBP. It has been suggested that IFN- $\gamma$ -activated STAT1 $\alpha$  and TGF- $\beta$ -activated AP-1 compete for limiting amount of p300/CBP, where IFN- $\gamma$  activated STAT1 $\alpha$  interaction with p300 is stronger, and thus blocks the AP1-mediated activation. Therefore, both in

primary culture of skin fibroblasts and lung fibroblasts, IFN- $\gamma$ -induced STAT1 $\alpha$  dimers are able to abrogate the TGF- $\beta$ -induced collagen synthesis by squelching the transcriptional coactivator p300 from Smad/p300 or Jun/AP1/p300 complex. Therefore, transcriptional coactivator p300/CBP plays a pivotal role in the antagonistic action of antifibrotic cytokine IFN- $\gamma$  and profibrotic cytokine TGF- $\beta$  on Type I collagen synthesis<sup>11,70</sup> (Figure 4).

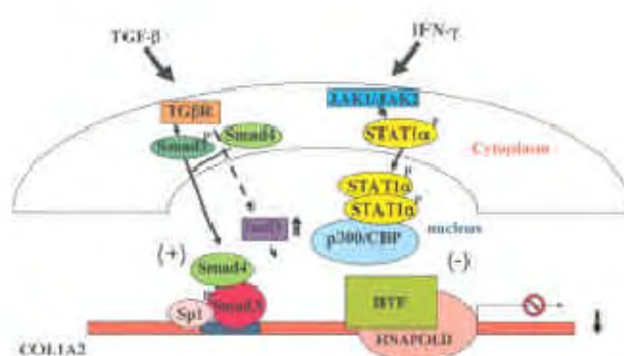
TNF- $\alpha$  also inhibits the basal as well as TGF- $\beta$ -induced COL1A2 promoter activity in dermal fibroblasts. TNF- $\alpha$ -mediated abrogation of TGF- $\beta$ -induced COL1A2 promoter activity is not due to altered Smad7. The TNF- $\alpha$ -induced Jun family members, c-jun and JunB block the TGF- $\beta$ /Smad3-induced collagen synthesis. Overexpression of p300 overcome the TNF- $\alpha$ -mediated inhibition where Jun competes with Smad3 for common transcriptional coactivator p300 (ref. 71). These results further suggest the significance of coactivators p300/CBP in the cytokine-modulated Type I collagen synthesis.

## Concluding remarks

Excessive deposition of extracellular matrix protein, Type I collagen in the dermal region causes skin fibrosis or scleroderma and the multifunctional cytokine TGF- $\beta$  has been implicated in the development of fibrosis. Although the transcription factors like Sp1 and AP-1 play significant role in TGF- $\beta$ -induced Type I collagen synthesis, recent discovery of Smad molecules, as substrate of TGF- $\beta$ -activated TGF- $\beta$  receptors, and as major signal transducers of TGF- $\beta$  signaling, opens a new dimension in the field of tissue fibrosis research. The importance and significance of HAT-containing transcriptional coactivators p300/CBP in the Smad-dependent



**Figure 3.** Model demonstrating the inhibitory action of IFN- $\gamma$  on Type I collagen synthesis. IFN- $\gamma$  activates the Janus kinase (JAK) which phosphorylates and activates the STAT1 $\alpha$ . Activated STAT1 $\alpha$  dimers translocate to nucleus and interact with p300/CBP. The inhibition is due to squelching of transcriptional coactivators p300/CBP from the transcriptional complex by activated STAT1 $\alpha$  and/or STAT1 $\alpha$ -activated CITA. BTF, basal transcription factors; RNAPOLII, RNA polymerase II;  $\uparrow$ , up regulated;  $\downarrow$ , down regulated; P, phosphorylated.



**Figure 4.** Model demonstrating the antagonistic action of IFN- $\gamma$  and TGF- $\beta$  on Type I collagen synthesis. IFN- $\gamma$  abrogates TGF- $\beta$ -induced Type I collagen synthesis via induction of STAT1 $\alpha$  or STAT1 $\alpha$ -induced factors which compete with TGF- $\beta$ -induced Smad molecules or JunD for transcriptional coactivators p300/CBP. Stronger interaction of STAT1 $\alpha$  with p300/CBP may suppress interaction of Smads or JunD with p300/CBP and thus abrogates the TGF- $\beta$ -induced Type I collagen synthesis. BTF, basal transcription factors; RNAPOLII, RNA polymerase II;  $\uparrow$ , up regulated;  $\downarrow$ , down regulated; P, phosphorylated.

TGF- $\beta$ -induced Type I collagen synthesis is now well documented. In addition, p300/CBP also play a significant role in the negative regulation of Type I collagen gene expression by tumour suppressor protein p53, or IFN- $\gamma$  induced STAT1 $\alpha$  and/or CIITA. Therefore, along with different positive and negative transcription factors, the transcriptional coactivators p300/CBP are key regulators in the basal as well as cytokine-modulated Type I collagen synthesis. However, to-date there is no strong evidence supporting the direct link of these factors with the development of fibrotic diseases.

In order to understand fully the molecular mechanism by which TGF- $\beta$  induces tissue fibrosis, characterization of the biochemical nature and functional activities of TGF- $\beta$  signaling molecules Smads, and transcriptional coactivators p300/CBP in fibrotic tissues are very important. Interestingly, recent studies suggest the post-translational modification of p300 by phosphorylation and methylation, control its functional activity, and thus control the expression level of many target genes. Furthermore, several reports suggest that alteration in activity of p300 or CBP causes different diseases, also known as 'Coactivator diseases'. Detection of any alteration of activators, repressors or coactivators, at the protein level, or post-translational modifications due to altered phosphorylation, acetylation or methylation in fibrotic tissues will help us to better understand the molecular causes of hypertranscription of collagen gene in fibrotic tissues. Therefore, it is interesting to assume that altered post-translational modifications of p300/CBP in fibrotic tissues lead to altered HAT activity or altered protein-protein interactions, and thus stimulate the collagen synthesis. The recent discovery of p300/CBP HAT-specific small molecule modulators opens a new possible avenue for the study and therapy of 'Coactivator diseases'. Specific inhibition of coactivator HAT activity by natural or synthetic small molecules may be a future novel approach for the treatment of tissue fibrosis. As the role and physiological significance of transcriptional coactivators p300/CBP in the regulation of Type I collagen synthesis in normal skin fibroblasts have been established, p300/CBP are attractive and important molecules for current and future study in the field of tissue fibrosis research.

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