Enhanced Activity of Transforming Growth Factor β1 (TGF-β1) Bound to Cartilage Oligomeric Matrix Protein

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Background: Cartilage oligomeric matrix protein (COMP) is a cartilage protein with a repeated modular structure that binds to and assembles extracellular matrix proteins but is not known to bind growth factors. We studied the binding interaction between COMP and TGF-β1 in vitro and determined the effect of COMP on TGF-β1-induced signal transduction in reporter cell lines and primary cells. Our results demonstrate that mature COMP protein binds to multiple TGF-β1 molecules and that the peak binding occurs at slightly acidic pH. These interactions were confirmed by dual polarization interferometry and visualized by rotary shadow electron microscopy. There is cation-independent binding of TGF-β1 to the C-terminal domain of COMP. In the presence of manganese, an additional TGF-β-binding site is present in the TSP3 repeats of COMP. Finally, we show that COMP-bound TGF-β1 causes increased TGF-β1-dependent transcription. We conclude that TGF-β1 binds to COMP and that TGF-β1 bound to COMP has enhanced bioactivity.

COMP monomer consists of an N-terminal coiled-coil domain, four EGF repeats, eight TSP3 (thrombospondin-3) repeats, and a thrombospondin C-terminal domain. The N-terminal coiled-coil domain is responsible for forming the pentameric mature COMP protein and contains two cysteine residues that covalently link adjacent chains (5, 6). The hydrophobic core formed by the coiled-coil domains in COMP pentamers can bind small molecules such as retinol and vitamin D (8). COMP has four EGF repeats, 13 TSP3 repeats, and a thrombospondin C-terminal domain (CTD), which together are responsible for binding interactions with other proteins and extracellular matrix components (9). Calcium-binding sites are located throughout COMP. Two of the four EGF repeats have calcium-binding sites, as do all of the TSP3 repeats and the CTD. Calcium, and presumably other cations as well, alters the conformation of COMP and affects the binding interactions between COMP and other proteins.

COMP interacts with multiple other cartilage matrix components, including type I, II, and IX collagens (10), proteoglycans such as aggrecan (11), non-collagenous matrix proteins such as fibronectin (12), and matrilins (13), as well as with glycosaminoglycans and heparin (11, 14). The repeated modular structure of COMP is critically important for its function as a “bridge” that assembles multiple extracellular matrix components (9); for example, the COMP pentamer (but not monomeric COMP) simultaneously binds several free collagen molecules to accelerate collagen fibrillogenesis (15). In addition to binding extracellular proteins, COMP also contains an integrin-binding RGD sequence (9, 16, 17), and it can interact with the thrombospondin receptor CD47 (18). The full implications of these cell-surface interactions in cartilage formation have not been determined.

The expression of COMP is detected early in chondrogenic differentiation during embryonic skeletogenesis (19). In the adult, COMP continues to be expressed in joint tissues, primarily in cartilage, but also at detectable levels in ligaments, tendons, and synovium (20). COMP is expressed in isolated chondrocytes, but it is rapidly down-regulated during monolayer...
COMP Enhances TGF-β1 Activity

The biological processes during which COMP expression is induced, including in vitro chondrogenic differentiation of stem cells and redifferentiation of passaged chondrocytes, are all heavily dependent on TGF-β signaling (23, 24). This suggests that there may be an interaction between COMP and TGF-β. Indeed, TGF-β up-regulates COMP mRNA expression and protein production in many systems (25, 26). However, a direct interaction between COMP and TGF-β proteins has not yet been identified.

The binding of COMP to cell-surface proteins and transmembrane receptors raises the intriguing possibility that, if COMP were able to directly bind to growth factors, it could act as a scaffold and influence the presentation of the growth factors to the receptors. Because of its repeated modular structure, COMP may bind several growth factors and increase their local concentration at the cell surface to enhance signaling.

Our goal in this study was to test the hypothesis that COMP directly binds to members of the TGF-β superfamily of growth factors. We further investigated the effects of COMP on TGF-β1-dependent transcriptional activation. We found that COMP bound to TGF-β1 and all of the bone morphogenetic protein (BMP) members we tested. When bound to COMP, both TGF-β1 and BMP-7 had increased signal transduction activity. These findings have implications for the role of COMP in regulating TGF-β activities in chondrogenesis and cartilage pathologies.

EXPERIMENTAL PROCEDURES

Recombinant Human COMP and TGF-β1—Human COMP cDNA in the pQE mammalian expression vector (Qiagen) was stably transfected into human 293T cells (American Type Culture Collection), which were cultured in serum-free medium. Recombinant human COMP was purified from the cell culture medium to near homogeneity by nickel-nitrilotriacetic acid column affinity chromatography. An N-terminally truncated COMP construct (COMP-ΔN) consisting of the TSP3 domains and the C-terminal domain (amino acids 269–757 of human COMP, shown in Fig. 4A) was similarly expressed and purified from 293T cell culture supernatants. The truncated COMP is missing the pentamerizing domain and EGF repeats and is secreted as a monomeric protein with an apparent molecular mass of ~80 kDa. Human TGF-β1 was purchased from PeproTech (Rocky Hill, NJ) in a carrier-free formulation. Its activity was confirmed in pellet culture chondrogenesis assays of human bone marrow-derived stem cells (BMSCs). Recombinant human BMP-7 was a generous gift from Dr. David Rueger (Stryker Biotech, Hopkinton, MA) to A. Hari Reddi. Human BMP-2 and BMP-4 were purchased from R&D Systems (Minneapolis, MN).

Solid-phase ELISA Binding Assays—TGF-β1 was coated onto ELISA plates at 0.5 or 1 μg/ml in 50 mM sodium carbonate (pH 9.8). In certain experiments, COMP constructs were similarly coated at 1 μg/ml. Unbound sites were blocked with PBS containing 1% heat-denatured BSA and 0.05% Tween 20 at pH 7.3. COMP protein was diluted in Tris-buffered saline with 1% heat-denatured BSA and 0.05% Tween 20 at pH 7.35 and allowed to bind overnight. COMP concentrations ranged between 0.06 and 20 μg/ml, which corresponds to 140 pm to 46 nm, using a calculated molecular mass of 433 kDa for the COMP pentamer (27). For lower pH experiments, PIPES buffer was used instead of Tris. Where indicated, the divalent cations magnesium, calcium, and manganese were added as chloride salts throughout all binding, washing, and incubation steps. Where indicated, COMP was preincubated with 2 μM retinoic acid or vitamin D, diluted at least 1:5000 from Me2SO stock. Bound COMP was detected with rabbit anti-COMP polyclonal antibody generated in-house. TGF-β1 was detected with monoclonal antibody MAB240 (R&D Systems), and BMP-7 with monoclonal antibody MAB3541. In all cases, detection of the primary antibody was performed with a peroxidase-conjugated anti-rabbit secondary antibody. For colorimetric detection, Turbo 3,3′,5,5′-tetramethylbenzidine substrate (Pierce) was added for 2 min, the reaction was stopped with 2 m sulfuric acid, and absorbance was measured at 450 nm on a 96-well microplate reader. For COMP binding to immobilized TGF-β1, the background of COMP binding to immobilized BSA was subtracted. Assays were performed in triplicate and reproduced.

Negative Staining/Electron Microscopy—TGF-β1 was labeled for negative staining with colloidal thioctyanate gold and allowed to form complexes with COMP by incubation for 30 min at room temperature in Tris-buffered saline (pH 7.4) with 10 mM manganese chloride. Complexes between TGF-β and COMP were analyzed by negative staining and electron microscopy as described previously (28). The specimens were finally examined in a JEOL 1200EX transmission electron microscope operated at 60 kV.

Dual Polarization Interferometry (DPI)—DPI analysis was performed using an AnaLight4D workstation (Fairfield Group, Manchester, United Kingdom), with COMP immobilized onto His-tagged AnaChip Plus chips. Soluble TGF-β1 was bound to the immobilized COMP at the indicated concentrations, and nonspecific sites were blocked with digested casein. Binding was performed in buffer containing 25 mM PIPES (pH 7.2), 150 mM NaCl, and no manganese. Data collection and analysis were performed using the AnaLight software suite and AnaLight Explorer, respectively. The DPI instrument provides absolute measurements of the change in the thickness and density of the molecular protein layer, which enabled a calculation of the mass of the immobilized COMP as TGF-β was added. These measurements are directly related to the structure and function of COMP and its interactions with TGF-β in close proximity to the measurement surface and provide the ability to determine binding constants and stoichiometry of binding.

Luciferase Assays for Transcriptional Activation—to test the effect of COMP on TGF-β-dependent transcriptional activation, we assayed the activation of a TGF-β-responsive promoter that had been stably transfected into a mink lung epithelial cell line (plasminogen activator inhibitor-1/luciferase cells kindly provided by Dr. Rifkin at New York University) (29). First, we determined that the mid-range of the TGF-β response was 4.6 ng/ml using our batch of TGF-β1 (data not shown).
COMP Enhances TGF-β1 Activity

Next, a constant amount of TGF-β1 (4.6 ng/ml) was mixed with increasing concentrations of COMP in a chemically defined low protein medium (Opti-MEM supplemented with 50 μM manganese chloride). After 30 h, luciferase activity was assayed in the cell lysate using the Dual-Luciferase assay reporter system (Promega) with readings normalized to cell seeding density.

Quantitative RT-PCR—Three strains of human BMSCs were obtained from different donors and maintained as stem cells in α-minimal essential medium supplemented with 10% FBS and 5 ng/ml FGF2. Cells at passage 2 were transduced with lentiviral constructs to express either human full-length COMP or GFP, and untransduced BMSCs were used as a control. COMP overexpression was confirmed by Western blotting of 20 μl of cell culture supernatant with anti-COMP antibody. Cells were seeded in 6-well dishes at 2 × 10^4 cells/well, allowed to attach in α-minimal essential medium with 10% FBS, and then treated with TGF-β1 for 48 h. Total RNA was isolated (RNasey micro reagents, Qiagen). The mRNA expression of the TGF-β-responsive gene TSP1 was quantified with TaqMan probe Hs00962908_m1 (Applied Biosystems) and TaqMan Fast Universal PCR reagents with a 7900HT Fast thermal cycler (Applied Biosystems). The relative expression of TSP1 was normalized to the level of 18 S RNA in each sample, and the -fold change in TSP1 expression between samples was calculated using the 2^ΔΔCt method.

Docking Simulation—Docking simulation was performed as described previously (30, 31) using AutoDock3 (32) and ADT (33). We performed 50 dockings of the COMP/TGF-β1 interaction, each one starting with a random initial position and orientation of TGF-β1 (Protein Data Bank code 3KFD, fragment A) with respect to the COMP C-terminal domain (amino acids 527–757; code 3FBY, fragment A).

Statistical Analysis—Experimental analysis was performed in triplicate, with key experiments repeated at least three times. Error bars represent S.D. Unless noted otherwise, statistical comparisons were made using JMP software (SAS Software, Cary, NC) and two-sided t tests with significance set at p < 0.05.

RESULTS

Binding of COMP to TGF-β and BMP Ligands—The TGF-β superfamily of proteins in humans includes three TGF-β isoforms and the BMPs. To determine whether COMP binds to members of the TGF-β family of ligands, we performed solid-phase binding assays using soluble COMP and immobilized TGF-β1, BMP-2, BMP-4, and BMP-7. We found that soluble COMP bound to immobilized TGF-β1, BMP-2, BMP-4, and BMP-7 (Fig. 1A). To confirm the specificity of the binding interactions, we repeated these assays with immobilized COMP and soluble TGF-β1 and BMP-7 (Fig. 1, B and C). We found that the binding interactions were reproducible when performed with either COMP or BMP-7/TGF-β in the soluble phase. These results demonstrate that COMP binds to TGF-β1, BMP-2, BMP-4, and BMP-7. In fact, COMP bound to every member of the TGF-β superfamily we tested. In the remainder of this study, we focused on the binding interaction between COMP and TGF-β1 in more detail.

Soluble COMP bound to immobilized TGF-β1 in a dose-dependent and saturable manner in the presence of calcium chloride (Fig. 1D). This was reproducible in the absence of calcium chloride (data not shown). Analysis of the binding curve showed that half-maximal binding occurred at ~1.4 μg of COMP bound to 1 μg of TGF-β1, which very roughly corresponds to 14 molecules of TGF-β1 bound to each pentameric COMP protein, and suggests that multiple TGF-β1 molecules interact with a single molecule of COMP.

pH Optimum of COMP/TGF-β1 Binding—We next determined the pH optimum at which COMP binds to TGF-β1. Solid-phase ELISAs were performed as described above but at varying pH values. When the pH of the binding buffers was adjusted between 6.5 and 7.5 in 0.25 increments, the maximal amount of TGF-β1 binding was found at pH 6.75 (Fig. 2A). The binding dropped off rapidly at pH 7.25 and higher.

Effect of Cofactors on COMP/TGF-β1 Interaction—The hydrophobic core formed by the COMP pentamerizing domains binds to hydrophobic molecules such as retinol and vitamin D. We used solid-phase binding assays to test whether the presence of these hydrophobic molecules affects COMP binding to TGF-β. We did not observe any change in the COMP/TGF-β1 interaction, even in the presence of the relatively high concentrations of retinol and vitamin D used in the assays (Fig. 2B).

In addition to binding hydrophobic compounds in its pentamerizing domain, there are up to 30 cation-binding sites in COMP, many of which are occupied by calcium ions (9). We therefore tested whether different cations or EDTA would affect the binding interaction between soluble COMP and immobilized TGF-β1 using ELISAs. A constant amount of COMP was allowed to bind the immobilized TGF-β1 in the presence of increasing concentrations of calcium or other divalent cations (calcium, magnesium, manganese, or EDTA). We found that manganese caused a dose-dependent increase in the amount of COMP bound to TGF-β1 (Fig. 2C). However, the binding of COMP to TGF-β1 was not affected by the addition of either calcium or magnesium to 40 mM. Surprisingly, EDTA up to 40 mM also did not affect the binding interaction (Fig. 2C). To further characterize the effect of manganese on the COMP/TGF-β1 interaction, increasing amounts of soluble COMP were bound to immobilized TGF-β1 in the presence or absence of 10 mM manganese. We observed a shift in the binding curve, indicating that higher affinity binding interactions occur in the presence of manganese (Fig. 2D).

Additional TGF-β1-binding Sites with Manganese—The observation that manganese increased the base-line binding of COMP to TGF-β1 raised the question of whether the presence of manganese unMASKS additional binding sites for TGF-β1 in each COMP monomer. Alternatively, manganese could simply enhance the affinity of a single TGF-β1-binding site. To discern between these possibilities, we obtained negative stained electron micrographs of gold-labeled TGF-β1 bound to COMP in the presence and absence of manganese. In the absence of manganese, TGF-β1 bound only to the CTD globule of COMP (Fig. 3, left panel). In the presence of 10 mM manganese, TGF-β1 bound to an additional site closer to the N terminus of COMP (Fig. 3, right panel). In the presence of manganese, TGF-β1...
occupied the N-terminal site, the CTD-binding site, or both sites on a single arm of the pentameric COMP protein. We also observed that TGF-β simultaneously bound to multiple arms of a single COMP pentamer. These results support the hypothesis that two TGF-β-binding sites exist on each COMP polypeptide and that manganese is a required cofactor for one of these sites.

Manganese-dependent TGF-β Binding of COMP-ΔN—From the electron micrographs, the manganese-dependent TGF-β-binding site could be located within either the EGF-like domains or the TSP3 repeats of COMP. To address this question, we generated and purified an N-terminally truncated COMP protein (COMP-ΔN) in which the secretion signal peptide was fused to C-terminal amino acids 269–757. The COMP-ΔN construct contains all TSP3 repeats and the COMP CTD but it is missing the pentamerizing domain and the four EGF domains (Fig. 4A). We found that soluble TGF-β1 bound to immobilized COMP-ΔN in a dose-dependent manner as measured by ELISA in the absence of manganese (Fig. 4B). Similarly, soluble COMP-ΔN bound to immobilized TGF-β1 in a dose-dependent manner (Fig. 4C). We tested whether the binding of COMP-ΔN to immobilized TGF-β1 was dependent on the presence of manganese. Although strong binding was observed in the presence of manganese, we did not detect binding in its absence in this particular assay.

Electron micrographs of TGF-β1 interacting with COMP-ΔN paralleled the observations made with full-length COMP. TGF-β1 bound to the CTD in a manganese-independent manner (Fig. 5, left panel), whereas binding to the more N-terminal region was observed only in the presence of manganese (right panel). Based on these electron micrographs, the manganese-dependent binding site is located within the TSP3 repeats of COMP. Taken together, these observations confirm that the CTD of COMP contains a manganese-independent TGF-β-binding site and that an additional manganese-dependent site is located within the TSP3 repeats. TGF-β1 bound to the CTD, the TSP3 repeats, or both sites on a single COMP-ΔN peptide.

Quantitative Measurement of Stoichiometry—A more quantitative analysis of binding stoichiometry was obtained using DPI performed on an AnaLight4D DPI instrument. This instrument provides a quantitative measurement of the change in the mass of immobilized COMP protein as TGF-β1 is added in solution. The number of TGF-β1 molecules bound to each COMP molecule, as well as the binding affinity of the interaction, can be calculated from these data. A binding stoichiometry of 11.5 molecules of TGF-β1 per COMP pentamer was determined, with the dissociation constant for the interaction roughly estimated to be in the nM range.

FIGURE 1. Binding of COMP to TGF-β and BMP by ELISA. A, soluble COMP binds to all TGF-β superfamily proteins tested, namely TGF-β1, BMP-2, BMP-4, and BMP-7. B and C, soluble TGF-β1 and BMP-7 bind to immobilized COMP in a dose-dependent manner. D, soluble COMP binds to immobilized TGF-β1 in a dose-dependent and saturable manner. For all assays, COMP, TGF-β1, and BMP-7 were coated at 1 μg/ml, and BMP-2 and BMP-4 were coated at 0.5 μg/ml. Blocking was with 1% denatured BSA. Binding was in 150 mM NaCl with 16 mM calcium chloride, 0.05% Tween 20, and 50 mM Tris (pH 7.35), and background binding to BSA-coated wells was subtracted.
range (Fig. 6). This stoichiometry measurement is in agreement with the ELISA estimate presented above. Using the same methodology for the monomeric COMP/H9004, the stoichiometry measurement for TGF-H9252 is 0.7, which suggests a 1:1 binding ratio for the COMP monomer and TGF-H9252 in the absence of manganese.

**COMP Enhances TGF-β1 Activity**

**Figure 2.** Cofactors affect COMP binding to TGF-β1. A, a pH of 6.75 is optimal for COMP binding to coated TGF-β1. B, the binding of COMP to TGF-β1 is not affected by hydrophobic compounds that bind the pentamerizing domain. VitD and VitA, vitamins D and A, respectively. C, increasing concentrations of manganese enhance the binding of COMP to TGF, whereas similar concentrations of calcium, magnesium, and EDTA do not affect the interaction. D, dose-dependent increase in the amount of COMP bound to TGF in the presence of manganese.

observed that the COMP-TGF-β1 complex elicited a greater transcriptional response than the same amount of unbound TGF-β1. The magnitude of the increased response was dependent on the amount of COMP used (Fig. 7A). COMP alone did not activate TGF-β-dependent transcription in this assay. These results demonstrate that COMP can enhance the cellular response to TGF-β1 in a cell line using a luciferase-based measure of transcription.

To confirm that COMP enhances the cellular response to TGF-β1 in primary human cells using endogenous gene activation, we tested the expression of TSP1 mRNA, a known TGF-β-responsive gene. COMP was overexpressed in BMSCs isolated from three individual donors, and the transcription of endogenous TSP1 was measured in response to TGF-β. Untransduced or GFP-transduced cells were used as a control. We found that COMP overexpression enhanced the transcriptional response to all doses of TGF-β (Fig. 7B). These cells did not express detectable levels of endogenous COMP (Fig. 7B, inset). Taken together, these results demonstrate that COMP enhances the response to TGF-β in cell lines using a luciferase reporter and in primary cells using an endogenous TGF-β-responsive mRNA.
DISCUSSION

In this study, we have demonstrated that COMP directly binds to members of the TGF-β family of growth factors, including BMPs, and characterized the interaction between TGF-β1 and COMP in greater detail. The binding interaction is optimal at slightly acidic conditions and enhanced by the presence of manganese but not other cations. At least two binding sites are present on each arm of the COMP pentamer, which can both be occupied simultaneously. The binding of TGF-β to COMP has the biological effect of enhancing TGF-β-dependent transcriptional activation in the mink lung cell system.

Our study has identified a direct interaction between COMP and the active 25-kDa TGF-β ligand. A large body of existing research shows that a fundamentally different interaction exists between TSP1 and TGF-β1 (34). TSP1 has specific sequences in its TSP1 domain that bind the TGF-β latency-associated peptide. The resulting conformational change in the latent complex then releases the active TGF-β peptide. The active TGF-β peptide itself does not interact directly with TSP1. COMP is a divergent member of the thrombospondin family and does not contain a TSP1 domain. Furthermore, COMP does not contain the specific amino acid sequence in TSP1 that binds the latent TGF-β peptide.
cy-associated peptide domain. To the best of our knowledge, COMP cannot activate latent TGF-β1. COMP is itself a transcriptional target of TGF-β1 signaling both in chondrocytes and in stem cells during chondrogenesis (25, 26, 35). We have shown recently that the induction of COMP by TGF-β1 occurs within 2 h in human stem cells (22). Furthermore, COMP is a primary response gene targeted directly by TGF-β1 signal transduction, and TGF-β1 stimulates COMP mRNA synthesis in the absence of additional protein synthesis. The observation that COMP enhances TGF-β1-dependent transcription may therefore indicate a positive feedback loop for the production of COMP protein by TGF-β1.

Cartilage matrix has a normal pH range of 6.6–6.9 (36). Synovial fluid, which has a normal pH of ~7.3, is primarily responsible for clearing the lactic acid resulting from chondrocyte glycolysis (37). The fixed negative charges in the cartilage proteoglycans cause the extracellular pH of cartilage to be ~0.5 units below that of the synovial fluid (38). The pH optimum for the COMP/TGF-β interaction is in the proper range for binding interactions that occur in the extracellular matrix of cartilage. In arthritic diseases, the pH of cartilage is thought to be somewhat more acidic. The pH of the synovial fluid can drop to as low as 6.5 (39), and chondrocytes respond to the inflammatory conditions and the presence of IL-1 by increasing their production of lactic acid (40). We found reduced binding of COMP to TGF-β1 below pH 6.75. This suggests that the interaction between these two molecules may be reduced in arthritis as a result of the acidification of the matrix and especially the pericellular matrix. However, the effect of the reduced pH on the biological response to the COMP-TGF-β complex remains unknown.

The binding of COMP to extracellular matrix and cell-surface proteins is often dependent on the presence of divalent cations such as calcium (41), magnesium, zinc, and manganese (12). EDTA changes the conformation of COMP, which alters its binding properties and increases its susceptibility to proteolytic degradation. In fact, the first step in purification of native COMP from cartilage is the extraction of COMP with EDTA in the presence of proteinase inhibitors (42). The recombinant COMP used in our experiments was synthesized and purified in the presence of calcium and magnesium. Given the large effect of cations on the conformation and binding properties of COMP. 

FIGURE 5. Electron micrographs of TGF-β1 bound to monomeric COMP-ΔN. A, TGF-β1 binds only to the C-terminal domain of COMP-ΔN in the absence of manganese. B, an additional TGF-β1-binding site is observed within the TSP3 repeats in the presence of 10 mM manganese. The schematics show that each COMP-ΔN protein can bind to a single molecule of TGF-β1 at either site or that both sites can be occupied.

FIGURE 6. Binding affinity and stoichiometry of COMP/TGF-β interaction by DPI analysis. Analysis indicated that full-length COMP bound ~11.5 TGF-β1 molecules. The apparent dissociation constant in the nM range is consistent in both full-length and truncated COMP. Full-length COMP and COMP-ΔN were coated onto His-tagged AnaChip Plus in an AnaLight4D DPI instrument. The binding of soluble TGF-β1 was performed at pH 7.35 without manganese.
COMP, we were somewhat surprised to find that the binding of COMP to TGF-β was not affected by additional calcium or by chelation of calcium with EDTA. Of the cations tested, only manganese increased the extent of interaction between COMP and TGF-β1. Manganese also enhances the interaction between COMP and fibronectin fragments (12). Together, these observations suggest that manganese causes a conformational molecular change in the COMP molecule that is required for the additional binding site to TGF-β1.

Genetic mutations of the COMP gene lead to protein misfolding and retention in the endoplasmic reticulum. The resulting chondrocyte apoptosis is the basis for pseudoachondroplasia and multiple epiphyseal dysplasia (43, 44). A COMP knockout mouse is viable with no obvious phenotype, although closer inspection revealed disturbances in the growth plate (45), and the knock-out mice are somewhat more prone to develop arthritis. Whether this is due to impaired mechanical properties of cartilage matrix assembled in the absence of COMP or to altered TGF-β-dependent pathways in cartilage without COMP remains to be determined.

We measured an intermediate binding affinity between COMP and TGF-β1. The estimated dissociation constants were in the nM range from both DPI and ELISA solid-phase binding experiments. This is in agreement with the affinity of COMP for other matrix proteins such as fibronectin (12) and matrilin-4 (13). However, this is at least an order of magnitude weaker than the binding of TGF-β1 with its type I, II, and III receptors, which have dissociation constants in the range of 50–300 pM (7).

Insight into how COMP may enhance TGF-β signaling is gained from molecular docking simulations performed with the crystal structures of COMP and TGF-β and its receptors (Fig. 8). This model suggests that a single C-terminal domain of COMP can be bound to the TGF-β active dimer and form a complex with the TGF-β receptors. According to this model, a possible mechanism through which pentameric COMP may enhance TGF-β signaling would be to increase the clustering of the TGF-β receptors.

The biological effect of the COMP/TGF-β interaction is to enhance the cellular response to TGF-β. The same amount of TGF-β1 elicited a much greater transcriptional response when precomplexed to COMP than when presented as free growth factor. Presented in a different way, in the presence of COMP, the amount of TGF-β1 required to elicit maximal transcriptional activation could be reduced by at least 50-fold. We are currently exploring the implications of this observation for TGF-β-induced chondrogenesis assays and cartilage tissue engineering applications.

In conclusion, COMP bound to all members of the TGF-β family of ligands we tested. In the case of TGF-β1, this interaction enhanced the biological response to TGF-β1. Our working model is that a COMP pentamer binds to multiple TGF-β ligands and presents these to cell-surface receptors. These data
provide a new insight into the function of COMP in cartilage and bone biology.

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