Identification of a 3Kbp Mechanoresponsive Promoter Region in the Human Cartilage Oligomeric Matrix Protein Gene

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Expression of chondrocyte-specific genes is regulated by mechanical force. However, despite the progress in identifying the signal transduction cascades that activate expression of mechanoresponsive genes, little is known about the transcription factors that activate transcription of mechanoresponsive genes. The DNA elements that confer mechanoresponsiveness within a cartilage gene promoter have yet to be identified. We have established an experimental system to identify the DNA elements and transcription factors that mediate the mechanoresponse of a promoter to nominal compressive stress in primary human chondrocytes and stem cells in a three-dimensional culture system. Our results demonstrate that the proximal 3Kb of the human cartilage oligomeric matrix protein promoter is sufficient to mediate a mechanoresponse in human articular chondrocytes and stem cells, and that the magnitude of mechanoresponse correlates to the regulation of the endogenous gene at the RNA and protein level. This information is critical to understanding how mechanical force regulates the transcriptional activation of cartilage genes in three-dimensional culture.

Introduction

IN 1862, RICHARD von Volkmann postulated that the growth of long bones in children is affected by mechanical compression and tension at the epiphyseal plate.¹ Subsequent studies confirmed that growth is slowed or arrested when large static compressive forces are applied.^{2–4} In contrast, relatively moderate dynamic compressive force enhances longitudinal bone growth by stimulating matrix synthesis and hypertrophy in growth plate chondrocytes.⁵ Orthopedic surgeons accept the paradigm that the growth plate aligns itself with the axis of compressive mechanical force. In other words, proper joint alignment during growth is effectively maintained by the interplay between compressive force and the biological response of growth plate chondrocytes.⁵ In adults, compressive forces continue to affect gene expression in articular chondrocytes. It is clinically accepted that mechanical overloading of adult joints ultimately contributes to osteoarthritic cartilage degradation, whereas extended disuse (i.e., mechanical unloading) leads to cartilage atrophy. Between these two extremes lies a range of beneficial compressive stimuli that promote articular cartilage homeostasis through the enhanced expression of cartilage matrix genes.^{6,7} An inappropriate response to mechanical forces can contribute to cartilage degradation.⁸

Identifying the fundamental mechanisms of how compressive forces regulate chondrocyte gene transcription is critically important as we try to alleviate the tremendous burden of joint diseases in our aging society.

The appropriate cellular response to compressive force must be invoked to maintain healthy cartilage. Despite progress in identifying the signal transduction cascades, the transcription factor complexes that mediate the chondrocyte mechanoresponse remain largely unknown, and DNA elements within a cartilage gene promoter that confer mechanoresponsiveness to the gene have yet to be identified. In part, this may be due to the difficulties in adapting traditional assays used for promoter analysis, performed on monolayer cultures of immortalized cell lines, to the study of cells in three-dimensional culture systems. Stretch assays and fluid shear assay systems on monolayer cells are tremendously useful in endothelial cell studies, but have only limited relevance to cartilage in which chondrocytes, which have little cell–cell contact, and rapidly alter their phenotype in monolayer culture.⁹

The objective of this study was to establish an experimental system to identify the DNA elements and transcription factors that mediate the mechanoresponse of a promoter to nominal compressive stress in primary human chondrocytes and stem cells in a three-dimensional culture. The

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second objective was to identify a cartilage gene promoter that responds to compressive mechanical loading, both in human articular chondrocytes and in stem cells during chondrogenesis. Our previous microarray data suggested that the cartilage oligomeric matrix protein (COMP) is one of the most highly responsive cartilage matrix genes induced by compressive loading of human articular chondrocytes, and this was substantiated by several independent studies.^{10–16} Here, we report that the proximal 3Kb of the human COMP promoter is sufficient to mediate a transcriptional response to mechanical forces in human articular chondrocytes and stem cells, setting the stage for defining the role of joint loading in cartilage homeostasis and pathology.

Methods

COMP promoter cloning and lentiviral transduction

To enable continuous and nondestructive measurements of promoter activity in cells, we designed a secreted luciferase reporter construct driven by the 3Kb proximal COMP promoter. The 3Kb sequence upstream of the transcription start site of the human COMP promoter was amplified from the pGL3-COMP plasmid (Dr. Karen L. Posey, University of Texas, Houston, TX) by polymerase chain reaction (PCR) (5'-ACTGAGATCTCTACACCAGTCCAGGCC-3' and 5'-TTGATATCGGTGGCTGCTCGCTTTCTA-3') and subcloned into the BglII/EcoRV site in the *Gussia princeps*-secreted luciferase reporter plasmid, pGLuc (New England Biolabs, Ipswich, MA). This construct was sequenced and determined to be free of mutation (designated hCOMP-pGLuc). We chose a secreted luciferase protein to overcome the need for cell lysis and enable repeated nondestructive measurements of promoter activity. The hCOMP-pGLuc cassette was subcloned into a lentiviral vector pCCLc (designated pCCLc-hCOMP-pGLuc). Infectious, but replication-defective lentiviral particles were packaged in 293T cells via cotransfection of pCCLc-hCOMP-pGLuc with pMDG-VSVG envelope and pCMV-d8.9 packaging vectors. Lentiviral particles were titered using a PCR protocol at the Vector Core Facility of UC Davis Medical Center. A lentiviral deliver system was chosen to provide stable integration of the constructs into the host genome, to avoid complications inherent in transient non-integrated plasmid transfection.

To ensure that the measured COMP-luciferase response accurately represents the response of the entire cell population, it was necessary to develop a highly efficient lentiviral transduction protocol. Primary human articular chondrocytes or bone marrow-derived stem cells (BMSCs) were seeded at 1×10^5 cells/mL and allowed to adhere for 4h before transduction. The media was replaced with 1 mL of respective culture media with 1 μ g/mL polybrene and lentivirus. A multiplicity of infection of ten was used for all transductions. The cells were allowed to incubate with virus overnight, the media was changed to the respective culture media, and the cells were cultured until approximately 80% confluent before passaging or embedding in alginate three-dimensional culture. Primary cells were routinely transduced with greater than 90% efficiency, which was verified using the same lentiviral system expressing a green fluorescent protein. This high efficiency minimizes the time between infection and three-dimensional culture in alginate, as well as the need for antibiotic selection after transduction.

Primary human cell isolation and culture

This study was performed using only primary human cell strains. Primary chondrocytes in the monolayer dedifferentiate rapidly, but at low passages this is reversible and the cells redifferentiate when placed into three-dimensional culture. Cells were used before passage 4 for primary chondrocytes and 8 for BMSCs. Experiments were performed on chondrocytes from two donors to account for donor variability, and confirmed in stem cells. Primary chondrocytes and BMSCs were isolated from human total knee replacement patients utilizing institutional review board-approved deidentified waste tissues as previously described.^{17–19} Briefly, cartilage was dissected from the bone and digested overnight with collagenase to release chondrocytes. Bone marrow was washed with buffered saline, strained through a 70- μ m filter to remove clotted blood and bone chips, centrifuged to remove fat, then layered on a Percoll cushion. After centrifugation, multipotent cells were removed from the top of the Percoll and further selected for adherence on tissue-culture-treated plastic. Cells were cultured until approximately 80% confluent before passaging or transduction. Cells isolated in this manner have been characterized by flow cytometry to confirm the presence of stem cell surface antigens (CD73, CD90, CD105) and the absence of hematopoietic stem cell (CD14, CD34) and monocyte markers (CD45).²⁰

COMP promoter activity analysis by luciferase activity

To first systematically establish that the luciferase activity secreted into the cell culture medium in 24 h is representative of the total amount produced by the cells, the measurement of luciferase activity in the media was added to the measurement of that in the cells plus alginate beads (Fig. 2). The hydrogel with embedded cells was treated with 50 mM EDTA in buffered saline to dissolve the alginate, then cells were mechanically disrupted, and DNA sheared by repeated aspiration through a 25-gauge needle before assay in a luminometer. Having established that the luciferase activity secreted into the culture media accurately represents the total amount produced in 24 h, for subsequent experiments, we measured the secreted luciferase activity in the media. At each 24-h time point during compression and in free swell controls, the cell culture media was completely exchanged and the conditioned media was centrifuged and the supernatant frozen at -80°C . After collection of all time points, the conditioned media supernatant was thawed, and 2 μ L was assayed in a luminometer (20/20 Glomax; Promega, Madison, WI) using 50 μ L of the BioLux *Gussia* Luciferase Assay substrate (New England Biolabs, Ipswich, MA) and presented as relative light units.

Control promoter luciferase activity analysis

A nonresponsive promoter-driving expression of secreted luciferase (pSV40-CLuc; New England Biolabs, Ipswich, MA) was cloned into our lentiviral expression vector and transduced into primary chondrocytes as described above. To ensure that cell seeding was equal and compression does not influence cell proliferation or viability, dsDNA was quantified at the end of compression using the Quant-iT dsDNA Assay Kit (Molecular Probes, Eugene, OR) and viability assessed using a Live-Dead stain (Molecular Probes).

FIG. 1. (A) Three-dimensional assembly of the aluminum top and bottom aligned by four guide posts and bushings. The aluminum top connects to the polysulfone pistons (yellow) and aligns the pistons into the wells of the tissue culture tray. The aluminum bottom houses the tissue culture tray. The guide posts force the lid assembly and culture dish to remain parallel, to ensure that uniaxial translational motion occurs during compression and to avoid applying shear stress to the sample. **(B)** Full assembly of the dynamic compression (DC) apparatus.

Three-dimensional culture in alginate hydrogels

For three-dimensional culture of primary human chondrocytes and BMSCs, primary cells were cast into 2% alginate hydrogel beads or discs 3 mm × 6 mm in size (height × diameter) as described previously.^{19,21,22} Each alginate disc contained approximately 66,000 cells and weighed 66 ± 4 μg, which was equivalent in volume and cell number the combined weight of 3 beads (65 ± 3 μg). To confirm that the alginate material properties were sufficient to transmit the nominal compressive strain to the cell cytosol, the stiffness of the alginate was estimated from the slope of the equilibrium stress versus strain curves between 2% and 20% strain, as gels were subjected to unconfined compression at 3% strain intervals. The unconstrained Young's modulus of the alginate was measured as approximately 9 kPa, which is stiffer than the chondrocyte cytosol.²¹

Bioreactor design

To apply precisely controlled dynamic forces, a custom bioreactor was built essentially similar to one described previously, with improvements in accuracy and ease of use.¹⁷ The bioreactor was designed to accept a four-well culture dish (without the lid) made for *in vitro* fertilization (Catalog #176742; Nunc, Roskilde, Denmark). Manual manipulation of the hydrogels is required to place hydrogels into each four-well culture dish. However, subsequent compression experiments can be simultaneously performed on four hydrogels without additional manipulation. An LAL-90 electric moving-coil linear actuator (SMAC, Carlsbad, CA) was connected to a lid assembly machined from autoclavable medical-grade polysulfone (Fig. 1). The lid assembly was designed with 10-mm pistons centered over each well of the four-well tissue culture dish. Four parallel guide posts were designed for parallel alignment of the dish and lid assemblies to eliminate shear stress in the sample by ensuring uniaxial translation motion during compression. The actuator was controlled using a custom LabView VI (LabView 6.5 National Instruments, Austin, TX) interfaced with a 1-axis Galil DMC controller (Galil Motion Control, Rocklin, CA). A 5-lb tension-compression load cell (Sensotec-Honeywell, Columbus, OH) was placed in-line to determine the position at which the polysulfone lid established contact with the alginate gels. The actuator, load-cell,

and compression chamber were housed in a 37°C humidified incubator with a 5% CO₂ atmosphere. We confirmed that the force applied at each well position was evenly matched using a 7 psi Tekscan force sensor (Tekscan, Boston, MA) and pressure mapping software (data not shown). The combined variability in the mechanical stimulation of samples within an experiment was less than 30 μm, which corresponds to less than 1% of the thickness of the 3-mm alginate discs.

Dynamic compression

To apply dynamic compressive forces to the alginate-embedded stem cells and chondrocytes, the alginate/cell constructs were first precultured for 24 h in chondrogenic media in the culture dish, then the discs were placed into the bioreactor. Cyclic dynamic compression from 5% to 15% strain, following the path of a sinusoidal waveform, was applied to the gels at a frequency of 0.5 Hz for up to 5 days. Uncompressed control gels were in the same incubator next to the bioreactor. Exactly 1.0 mL of media was used for each well, which was completely exchanged every 24 h during the course of the experiments. Luciferase activity was assayed from the cell culture media as described.

FIG. 2. The luciferase activity in a 2-μL sample of cell culture media (y-axis) is linearly correlated to the total amount of luciferase activity in combined homogenate of cells, alginate hydrogel, and media (x-axis). Conditioned media was sampled at 24 h. Please note that 1 light unit on the x-axis is 1 × 10⁶ light units on the y-axis. Errors are reported as standard deviation.

Endogenous COMP protein analysis

To test whether changes in hCOMP mRNA correspond with similar changes in COMP protein production, 10 μ L samples of the cell culture supernatants were assayed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, blotted to polyvinylidene fluoride membranes, and probed with a polyclonal rabbit anti-COMP antibody characterized previously.²³

Quantification of mRNA by quantitative reverse transcription-polymerase chain reaction

To confirm that the changes in luciferase activity from the hCOMP promoter accurately represent the changes in hCOMP mRNA levels, quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed. Cells were released from the alginate hydrogel with 50 mM EDTA in buffered saline and collected by centrifugation. Total RNA was isolated from the cell pellet using the RNeasy Lipid Tissue Mini Kit according to the recommended protocol with the optional on-column DNase digestion step (Qiagen, Valencia, CA). Quantitative RT-PCR was performed using TaqMan Gene Expression chemistry and Assays-on-Demand probe sets on an Applied Biosystems 7700 instrument (Life Technologies Corporation, Carlsbad, CA). Expression was normalized to the housekeeping gene GAPDH, and relative expression levels (fold change) were calculated using the $2^{-\Delta\Delta C_t}$ method, with the uncompressed sample at day 5 serving as baseline.

Statistical analysis

Statistical analysis was performed in JMP software (SAS, Cary, NC) using the Student's *t*-test, analysis of variance

with Tukey–Kramer *post hoc* correction for multiple comparisons, or linear regression, as appropriate. *p*-values less than 0.05 were considered significant.

Results

The first objective was to ensure a reproducible experimental setup. It was essential to confirm that the luciferase activity measured from a small sample of cell culture media was linearly correlated to the combined total amount of luciferase activity present in the media, alginate gel, and intracellularly, in other words that the secreted luciferase was not simply trapped in the alginate gel and released more efficiently due to the dynamic compression. We found that the amount of secreted luciferase activity in the media correlated linearly ($r^2=0.9982$) with the total luciferase activity present in the homogenized sample (Fig. 2). Since clusters of cells may respond differently to mechanical stimuli with respect to single cells, and may represent local macro-discontinuity in the alginate matrix, homogenous cell distribution with no evidence of proliferation or cluster formation was confirmed by brightfield microscopy (Fig. 3A), which is consistent with our previous experience using alginate gels.²⁴ Together, the uniform cellular distribution and strong correlation between secreted and total luciferase indicates that the luciferase activity in the media alone is an excellent indicator of total luciferase production by the cells, and enables repeated nondestructive monitoring of COMP promoter activity over the course of our experiments.

To determine whether the 3Kb of the human COMP promoter proximal to the transcription start site contained a mechanoresponsive element, luciferase activity in the media was monitored daily as alginate gels containing chondrocytes transduced with pCCLc-hCOMP-pGLuc construct

FIG. 3. (A) Brightfield Microscopy of alginate embedded cells. (B) DNA Quantification after 5-days of DC and free swell (FS), *t*-test was not significant ($p>0.9$). (C) Accumulation of control SV40 promoter-driven luciferase activity. Two tailed *t*-test was not significant ($p>0.6$) for comparisons between FS and DC with the nonresponsive promoter. Error bars are reported as standard error.

were subject to continuous cyclic dynamic compression. We found that the transfer from monolayer to three-dimensional culture resulted in increased luciferase activity in both chondrocytes and BMSCs. Dynamic compression slightly activated the *COMP* promoter within 1 day when compared to the uncompressed controls, and there was a statistically significant increase in secreted luciferase activity after the second day of dynamic compression that was maintained until the fifth day (Fig. 4A). A similar result was obtained when lentiviral-transduced BMSCs were subject to continuous dynamic compression (Fig. 4B). Under these conditions, a maximal induction of approximately two- to threefold was observed. We confirmed that there was no difference in the viability of chondrocytes or BMSC subject to 5 days of dynamic compression and their respective uncompressed controls (data not shown). Furthermore, we found no difference in the amount of luciferase activity retained by the alginate scaffold between dynamic compression or free swell gels at 5 days. To confirm that the *COMP* reporter contained a mechanoresponsive element, a lentiviral construct coding for secreted luciferase from an unrelated promoter (SV40) was similarly transduced into chondrocytes, and we observed no response to dynamic compression (Fig. 3C). To ensure that the net increase in luciferase is due to promoter activity rather than an increased baseline cell number or proliferation, we quantified total DNA after 5 days of dynamic compression and free swell controls (Fig. 3B). Assuming an average of 6.6pg dsDNA per cell, we found that DNA quantification approximates 51k cells in free swell samples and 52k in dynamically compressed samples, corresponding to approximately 86% of the seeding density. We observed no difference in the DNA isolated from the compressed and free swell gels. Taken together, these results suggest that the 3-Kb proximal promoter of the human *COMP* gene contains a mechanoresponsive element that is activated by dynamic compression, both in chondrocytes and in stem cells.

To determine whether the changes in *COMP* promoter-driven luciferase activity accurately represent the changes in endogenous *COMP* mRNA transcription, we quantified *COMP* mRNA in compressed and uncompressed chondrocytes by qRT-PCR. We found a significant 2.8-fold increase in *COMP* mRNA after 5 days of dynamic compression when compared to uncompressed samples over the same duration. This increase is of a similar magnitude as the change in the *COMP* luciferase reporter activity. In addition, we examined the mRNA expression of *COLII*, a gene known to respond to compressive forces in cartilage, and found a significant twofold increase after 5 days of dynamic com-

pression when compared to uncompressed samples over the same duration. The mRNA levels of the superficial zone protein, which is more responsive to shear forces than compressive forces in cartilage, remained unchanged after 5 days of dynamic compression when compared to uncompressed samples over the same duration (Fig. 5). These results confirm the anabolic effect of dynamic compression on chondrocytes and indicate that the 3 Kb hCOMP luciferase reporter and the endogenous *COMP* mRNA transcription level both respond similarly to dynamic compression.

Finally, to determine whether the changes in *COMP* promoter activity and mRNA transcription translated to an increase in *COMP* protein production, Western blots were performed on the conditioned cell culture media. We found a significant increase in the amount of pentameric *COMP* protein in the media in both articular chondrocytes and BMSCs after 5 days of dynamic compression compared to the free swell controls (Fig. 6).

Discussion

Using the human *COMP* as a model mechanoresponsive gene, we have developed a system to repeatedly and non-destructively quantify the activation of the *COMP* promoter in response to compression of human chondrocytes and stem cells in three-dimensional culture. To accomplish this, we generated a lentiviral construct containing the human *COMP* promoter driving the expression of a secreted *Gausea* luciferase protein, and optimized the transduction to obtain greater than 90% efficiency of both human BMSCs and primary human articular chondrocytes. We have previously shown lentiviral gene transduction into chondrocytes and stem cells provides long-term stable gene expression and protein production.¹⁸

Cartilage is constantly subjected to mechanical forces, including compression, tension, and shear. These forces are converted into physiological responses that are critical to cartilage matrix composition, cellular survival, and progenitor cell differentiation. Cellular responses to mechanical forces vary greatly, and can range from proliferation, to differentiation, to apoptotic cell death, and from matrix synthesis to enzymatic matrix degradation. The appropriate cellular response to compressive force must be invoked for maintenance of health cartilage tissue. An inappropriate cellular response to mechanical loading contributes to pathological outcomes during growth and in aging. A clearer understanding of the mechanotransduction pathway has tremendous potential in many clinical applications.

FIG. 4. DC increases cartilage oligomeric matrix protein (*COMP*) reporter activity. Accumulation of *COMP* promoter-driven luciferase activity over the course of 5 days of DC compared to FS for (A) chondrocytes and (B) bone marrow-derived stem cells. *Statistical significance. Errors are reported as standard error of the mean.

FIG. 5. RT-polymerase chain reaction quantification of the expression of *COMP*, *COL2*, and superficial zone protein (*SZP*) in chondrocytes after 5 days of DC. *Statistical significance. Errors are reported as standard deviation. RT, reverse transcription.

Our reporter construct allows the observation of *COMP* expression during dynamic compression. Stable expression of the reporter gene is critical. The lentiviral reporter system confers genomic integration and long-term stable expression of the constructs in primary human cells. This system over-

FIG. 6. Western blot analysis for secreted pentameric *COMP* expression in the conditioned media after 5 days of DC compared to FS over the same duration for articular chondrocytes (AC) and bone marrow-derived stem cells (BMSC).

comes the obstacles of transient plasmid transfections that produce an initial peak of activity followed by a rapid decline of both copy number and activity over time. Additionally, we have not found a human chondrocytic cell line suitable for three-dimensional culture, making the use of primary human chondrocytes and BMSCs critical. Additionally, our reporter construct offers multiple measurements of reporter activity over time from the same cells. The choice of secreted luciferase reporter enables nondestructive measurement of luciferase activity from a small (2 μ L) sample of cell culture media. Upon transfer from monolayer expansion culture to hydrogel, we observed that *COMP* promoter activation increased even in free swell conditions. An increase in *COMP* mRNA expression is expected when cells (both chondrocytes and BMSCs) are transferred from monolayer expansion culture to a three-dimensional hydrogel culture, as this is itself a chondrogenic stimulus.²⁴ The increased luciferase activity indicates that the 3 kb *COMP* promoter accurately reports this. The secreted luciferase activity over a 5-day period of dynamic compression faithfully represents both h*COMP* mRNA expression and secreted h*COMP* pentameric protein production in both primary human articular chondrocytes as well as in BMSCs.

Study of the *COMP* promoter has revealed several candidate proteins that may mediate mechanotransduction of physical force to gene expression. Our recent data show that *COMP* expression is an early marker of chondrogenic differentiation in bone marrow stem cells.²⁵ Analysis of the h*COMP* promoter in transgenic reporter mice suggest that the proximal 375 bp are sufficient for expression in chondrocytes, but that the remaining 1.7 kb contain enhancer elements that increase *COMP* expression *in vivo*.²⁶ Study of the proximal regulatory region of the *COMP* promoter has revealed that, despite the location of a TATA box in the mouse *COMP* promoter at -70 bp, the h*COMP* promoter contains no TATA or CAAT box. Instead, the h*COMP* promoter contains two SP1- and two AP2-binding sites typically found in TATA-less promoters.^{27,28} Four h*COMP* transcripts have been identified -47, -58, -69, and -108 bp upstream from the translational start codon with the -108 transcript being specifically expressed in articular cartilage.²⁸ SP1 has been shown to bind to the h*COMP* promoter at -171 bp in EMSA. However, mutation of the SP1- or the AP2-binding sites did not alter *COMP* promoter activity in chondrocytes.²⁸ Potentially, recruitment of other factors (i.e., histone acetylases, transcriptional coactivators, etc.) to the SP1 platform occurs during the activation of *COMP* transcription by mechanical compression.

Sox9 is a master regulator of chondrocyte extracellular gene expression, including many mechanoresponsive genes. We have previously shown that Sox9 is phosphorylated and activated by dynamic compression of chondrocytes, and that extended dynamic compression increases the Sox9 protein levels.¹⁷ Potential Sox9-binding sites have been identified in the proximal m*COMP* and h*COMP* promoter, but the binding of Sox9 to these sites has not been demonstrated and the human *COMP* promoter is not activated by Sox9 in co-transfection experiments.²⁶ Clearly, the mere presence of Sox9 alone is not enough to drive chondrogenesis or *COMP* transcription, since Sox9 is present in many other tissues and cells that do not express *COMP*. Furthermore, not all genes with Sox9 response elements are regulated to the same

extent, indicating other transcriptional modulators are required. The implications of Sox9 phosphorylation during dynamic compression on the assembly and activity of the larger multiprotein transcriptional complex remain unknown.

Currently, it is not possible to clinically regulate the cellular function of chondrocytes or affect the differentiation of stem cells to alter the progression of osteoarthritis. In articular cartilage repair and cartilage tissue engineering, it is important to stimulate stem cell chondrogenesis and to enhance extracellular matrix synthesis in existing chondrocytes. Both are profoundly affected by the mechanical environment at the cellular level through unknown transcription factors and DNA promoter elements. A mechanoresponsive transcription factor complex has not been identified, nor have mechanoresponsive promoter elements been identified until now. Identifying and mapping such pathways will enhance our fundamental understanding of how cellular responses can be manipulated to enhance endochondral bone growth, increase stem cell chondrogenesis, create tissue-engineered cartilage constructs, and promote the maintenance of healthy articular cartilage tissue in adults. In this study, we have established that a mechanoresponsive element exists in the proximal 3-Kb promoter of hCOMP, and studies are ongoing to map the exact location of these elements. We predict that the mechanoresponse regulatory elements in the COMP promoter will be conserved in other genes.

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Disclosure Statement

No competing financial interests exist.

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