Rac1 Signaling Stimulates N-cadherin Expression, Mesenchymal Condensation, and Chondrogenesis*

Anita Woods1, Guoyan Wang, Holly Dupuis, Zhuhong Shao, and Frank Beier2

From the CIHR Group in Skeletal Development and Remodeling, Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario N6A 5C1, Canada

The molecular mechanisms controlling differentiation of mesenchymal precursor cells into chondrocytes (chondrogenesis) are not completely understood. We have recently shown that the small GTPase RhoA inhibits this process. Here we demonstrate that a different Rho GTPase family member, Rac1, promotes chondrogenesis. Pharmacological inhibition of Rac1 expression in micromass culture resulted in reduced mRNA levels of the chondrogenic markers collagen II and aggrecan, and decreased accumulation of glycosaminoglycans. Expression of the essential chondrogenic transcription factors Sox9, Sox5, and Sox6 was also reduced upon inhibition of Rac1 signaling. In contrast, overexpression of Rac1 in the chondrogenic ATDC5 cell line increased mRNA transcripts of Sox9, 5, and 6, collagen II, and aggrecan. Inhibition of Rac1 resulted in a reduction in the number, size, and organization of cellular condensations and decreased expression of N-cadherin. Overexpression of Rac1 resulted in an increase in N-cadherin expression levels. Furthermore, genetic ablation of Rac1 in primary micromass cultures resulted in reduced expression of chondrogenic markers. Additionally, we provide evidence that Cdc42 also promotes chondrogenesis. Overexpression of Cdc42 in ATDC5 cells resulted in increased expression of Sox5, Sox9, and collagen II but not Sox6, aggrecan, or N-cadherin. Therefore, we demonstrate that Rac1 and Cdc42 are positive regulators of chondrogenesis, but act at least in part through different cellular and molecular mechanisms.

Chondrocytes are the cellular component of cartilage, responsible for generating and maintaining its extracellular environment (1). Cartilage has multiple functions such as providing a cushion on the articular surfaces of joints (2), providing a template for the formation of endochondral bone (3), and contributing to fracture repair (4). The formation of cartilage templates in endochondral ossification begins with condensation of mesenchymal cells, increased expression of the cell adhesion molecules N-cadherin and N-CAM, and therefore increased cell-cell interactions (5–7). As cells become chondrogenic, the expression of these adhesion molecules is decreased (8). Cells within these condensations commit to the chondrogenic lineage, acquire a spherical cell morphology and induce expression of the essential chondrogenic transcription factor Sox9 (9, 10). Sox5 and Sox6 cooperate with Sox9 to control chondrogenesis and are themselves under the transcriptional control of Sox9 (9, 11, 12). Together, these transcription factors activate transcription of the major chondrogenic matrix genes, collagen II and aggrecan (11, 13–15). Furthermore, increased glycosaminoglycan (GAG) content is another marker of the chondrogenic extracellular matrix (1). The production of glycosaminoglycans is partially regulated by the enzymes chondroitin 4-sulfotransferase 11 (Chst11) and chondroitin 6-sulfotransferase 3 (Chst3), which have been determined to be important for proper cartilage and bone formation (16, 17).

Although many molecular mechanisms have been shown to regulate the commitment and differentiation of cells to the chondrogenic lineage (3, 18, 19), gaining a better understanding of signaling pathways that regulate these molecules is essential. Such knowledge would aid, for example, in the development of therapeutics for the replacement of damaged cartilage in osteoarthritis.

As stated above, it is well known that chondrocytes display distinctive spherical cell shape (20). Forcing spherical cell shape by pharmacological inhibition of actin polymerization is sufficient to stimulate dedifferentiated cells to re-express chondrogenic matrix molecules (21–24) and promotes mesenchymal cells to commit to the chondrogenic lineage (24, 25). We therefore speculated that signaling pathways that regulate cell shape also regulate chondrocyte differentiation.

Rho GTPases are a family of small G-proteins that have multiple roles in cells including the regulation of the cytoskeleton (26, 27), cell cycle control (28), and regulation of transcription factor activity (29); however, their specific roles are cell type-specific (30–33). Rac1 and Cdc42 have been shown to regulate cortical actin organization (34, 35) and to be involved in the formation of lamellipodia and filopodia, respectively (36). In contrast, RhoA acting through Rho-kinase (ROCK) promotes the formation of stress fibers and spindle-shaped cells (32, 37).

We have recently demonstrated that RhoA/ROCK signaling inhibits the commitment of mesenchymal cells to the chondro-
genic lineage (32, 38). In this study we wanted to determine the role of Rac1 and Cdc42 signaling in early chondrogenesis. There is strong evidence that Rac1/Cdc42 and RhoA signaling have antagonizing roles in later stages of chondrocyte differentiation (39), as well as in other cell types (40, 41), but the roles of Rac1 and Cdc42 in the differentiation of mesenchymal precursor cells to early chondrocytes have not been described. Furthermore, it has been determined in other cell types that Rac1 and Cdc42 regulation of cortical actin is essential in the stabilization of cadherin dependent cellular junctions (34, 42–44). Because the formation these junctions is essential for the commitment of cells to the chondrogenic lineage (6, 7), we hypothesized that Rac1/Cdc42 signaling promotes chondrogenesis, in part through modulation of cell-cell interactions. For our studies we used a Rac1- and Cdc42- overexpressing ATDC5 cells that we described previously (39), a Rac1-specific inhibitor, NSC23766 in primary micromass cultures and genetic ablation of Rac1 in micromass cultures treated with an adenovirus expressing Cre recombinase.

**EXPERIMENTAL PROCEDURES**

**Materials**

Time-impregnated CD1 mice were purchased from Charles River Laboratories. All medium components were purchased from Invitrogen or Sigma unless otherwise stated. The Rac1 inhibitor NSC23766 (45) and the ROCK inhibitor Y27632 were purchased from Calbiochem. The following antibodies were purchased from Calbiochem: N-cadherin, cat. no. ab-12221, and β-actin cat. no. A5441 (Sigma).

**Methods**

**Micromass and ATDC5 Cell Cultures**—Mesenchymal limb buds cells were obtained from E11.5 CD1 mice, and cultured in micromass cultures as described (32, 46, 47). Briefly, cells were suspended in micromass media, 60% F12, 40% Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum (Invitrogen), 0.25% penicillin/streptomycin (Pen/Strep), and 0.25% L-glutamine, at a density of 2.5 × 10^7 cells/ml and plated in 10-μl droplets to simulate the high density of chondrogenic condensations. One hour after plating, medium (as above, supplemented with 1 mM β-glycerol phosphate and 50 μg/ml of ascorbic acid) was added to the cultures and supplemented with ddH_2O (vehicle), 50 μM of NSC23766 (Rac1 inhibitor) or Me_2SO (vehicle) and 10 μM Y27632 (ROCK inhibitor). Medium and inhibitors were changed every 24 h until harvesting. Rac1-, Cdc42-, and pcDNA3 vector-transfected ATDC5 cells were cultured and induced to differentiate as described (39, 48).

**Real-time PCR**—RNA was extracted using the Qiagen RNeasy kit according to the manufacturer’s instructions. 25 ng of collected RNA was used for real time PCR. Relative gene expression was determined by measuring collagen II mRNA levels of collage II (A) and aggrecan (B) were decreased compared with control cultures upon inhibition of Rac1 signaling, as determined by real-time PCR. C, accumulation of glycosaminoglycans as demonstrated by alcian blue staining was decreased in Rac1 inhibited cultures in comparison to control. Real-time data shown are an average of three independent trials run in triplicate ± S.E.; *, p < 0.05. The alcian blue image is a representative picture from three independent trials.

**Alcian Blue Staining**—Treated micromass cultures were harvested on day 4 of culture and fixed in 100% ethanol for 2 min at −20 °C and then incubated with 0.1% HCl-alcian blue for 2 h (32, 46). Excess stain was washed off with double distilled water, and pictures were taken.

**Lectin Peanut Agglutinin (PNA) Staining**—Visualization of cellular condensations was achieved by growing micromass cultures on glass coverslips with treatments as specified. Cultures were fixed on day 4 of culture in 4% paraformaldehyde (PFA) at 4 °C for 30 min. Coverslips were rinsed with PBS and then incubated for 2 h in 50 μg/ml PNA diluted in PBS. Cultures were washed again with PBS, and the PNA was detected colorimetrically by DAB AEC. Brightfield images were taken with a Leica DMRA2 microscope using OpenLab 4.0 software with 10- and 40-fold magnification.

**Western Blot Analysis**—Protein was isolated in radioimmune precipitation assay lysis buffer and concentration was calculated as described (38). 40 μg of total protein was loaded on
non-denaturing gels for determination of N-cadherin levels, and 40 μg of total protein were loaded on SDS-PAGE-denaturing gels for analyses of β-actin or Cre recombinase. Proteins were transferred to nitrocellulose (Schleicher and Schull) and blocked in 5% bovine serum albumin in Tris-buffered saline plus 0.01% Tween 20 (BSA-TBST) for 1 h at room temperature. The membrane was incubated overnight at 4 °C with 1.0 μg/ml anti-N-cadherin, or 0.5 μg/ml anti-β-actin. After washing in TBST, horseradish peroxidase-conjugated secondary antibodies were diluted 1:200 in 5% BSA-TBST and incubated with the membrane for 1 h at 4 °C. Excess secondary antibody was rinsed off the membrane with TBST, and signal was detected by the addition of Western blotting detection reagents (ECL, Amersham Biosciences) according to the manufacturer’s protocol and visualized on a ChemiImagerTM 5500 (AlphaInnotech Inc).

Immunofluorescence—ATDC5 cells were plated at a density of 16,000 cells/well in a 24-well dish (Falcon) on glass coverslips. Cells were transfected to differentiate by the addition of insulin-transferrin-selenium (ITS) (Sigma), and media was changed every other day as described by (32, 48). On day 3 of culture, cells were harvested and fixed in 4% PFA for 30 min at 4 °C. Cells were washed in PBS, incubated for 5 min with 0.1% Triton X in PBS and rinsed again in PBS. Cells were then incubated in blocking solution containing goat serum (Sigma) in PBS, 1:20, for 30 min at room temperature. Primary antibodies directed to N-cadherin were diluted in blocking solution at a concentration of 1:200 and incubated with coverslips for 1 h at room temperature. Coverslips were rinsed in PBS and then incubated with a fluorescein isothiocyanate-conjugated secondary antibody diluted 1:200 in PBS for 1 h at room temperature in the dark. Following another wash in PBS, coverslips were mounted in VectaShield anti-fade mounting media containing 4′,6-diamidino-2-phenylindole. Images were taken with a Leica DMRA2 fluorescence microscope with 63-fold magnification and analyzed using OpenLab 4.0 software.

Mouse Breeding and Genotyping—Rac1<sup>fl/fl</sup> (49) mice were exposed to a 12-h light-dark cycle and fed tap water and regular chow ad libitum. All procedures involving animals were approved by the University of Western Ontario Animal Care and Use Committee. For PCR genotyping, tail snips were used to prepare DNA for PCR analysis. PCR genotyping was performed by simultaneous amplification of the wild-type (<sup>Rac1</sup>), conditional (<sup>Rac1</sup>fl/fl), and null (<sup>Rac1</sup>−/−) alleles (49) as described. PCR fragments were analyzed by agarose gel electrophoresis. Homozygous Rac1<sup>fl/fl</sup> mice were time impregnated, and mesenchymal cells were isolated at E11.5 mice as described above.

Adenovirus Infection—Adenovirus encoding Cre recombinase was obtained as a generous gift from Drs. Collins and Scacheri (National Institutes of Health) through Dr. Dagnino (London, Canada). Adenovirus expressing Cre Recombinase was purified using the Adeno-XTM virus mini-purification kit (Clontech) and incubated with mesenchymal cells from limb buds of CD1 control mice or Rac1<sup>fl/fl</sup> mice in high density cell suspension (2.5 × 10<sup>7</sup> cells/ml) at a multiplicity of infection (MOI) of 1. Cells were plated in 10 μl droplets as described above. Four hours after plating, media was added as above.

Statistical Analysis—Data collected from real-time PCR are an average of three independent trials of samples run in quadruplicate. Means were quantified relative to GAPDH, and then data were normalized to day 1 of control-treated RNA per trial. Statistical significance was determined by a one or two-way analysis of variance with Bonferroni post test using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA.
RESULTS

Inhibition of Rac1 Signaling Decreases mRNA Levels of Chondrogenic Matrix Genes and GAG Accumulation—Our recent studies suggested inhibition of chondrogenesis by RhoA (32, 38) and antagonism between RhoA and Rac1/Cdc42 at later stages of chondrocyte differentiation (39, 48). However, the roles of Rac1 and Cdc42 in early chondrogenesis have not been described. In previous studies, we confirm the overexpression of Rac1, Cdc42, and RhoA in stable transfectants of the ATDC5 cell line, which were used for these studies (39, 48). We therefore asked whether similar antagonistic mechanisms control chondrogenesis. We first tested the effects of a pharmacological inhibitor of Rac1, NSC23766 (45), on chondrogenesis in primary micromass culture. These studies revealed a decrease in the transcript levels of the chondrogenic matrix molecules collagen II and aggrecan upon NSC23766 treatment (Fig. 1, A and B). Real-time PCR demonstrated a strong decrease of these mRNAs by day 2 of culture, and expression remained low until day 4 of culture in comparison to the control. Micromass cultures were stained with alcian blue to visualize GAG content after the fourth day in culture with vehicle or NSC23766 (Fig. 1C). Rac1 inhibition resulted in a strong decrease in the amount of alcian blue staining, suggesting a decrease in GAG accumulation.

Inhibition of Rac1 Signaling in Micromass Culture Reduces mRNA Levels of Sox5, 6, and 9—We next examined the effects of Rac1 inhibition on the expression of Sox9, Sox5, and Sox6 to investigate whether down-regulation of these transcription factors could be responsible for the observed attenuation of chondrogenesis. Micromass cultures were grown for up to 4 days with vehicle control or 50 μM NSC23766. Inhibition of Rac1 signaling caused a significant decrease in the expression of Sox9 mRNA levels compared with control cultures from day 3 on (Fig. 2A). Sox5 and Sox6 also demonstrated reduced mRNA levels in cultures treated with the Rac1 inhibitor (Fig. 2, B and C). mRNA levels for the transcription factor ATF3 increased strongly during the time course, but were not affected by the Rac1 inhibitor (Fig. 2D). Cycle thresholds for both GAPDH and 18 S rRNA also remained constant (relative to RNA concentration) under all conditions (Fig. 2, E and F), confirming that both probes are suitable as loading controls. Therefore we employed GAPDH throughout this study. These data suggest that Rac1 signaling is required for chondrogenesis through stimulation of Sox9, Sox5, and collagen II levels.

Overexpression of Rac1 and Cdc42 Increases Markers of Chondrogenesis—ATDC5 cells stably transfected with empty vector for control or expression vectors for Rac1 or Cdc42 (32, 39) were stimulated to differentiate in culture for a 9-day period. Analysis of isolated RNA by real-time PCR revealed that the expression of Sox9 increases until day 9 in control cultures (Fig. 3A). Overexpression of Rac1 or Cdc42 resulted in a higher expression of Sox9 over each day of culture, with significant...
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increases demonstrated on day 9 of culture (Fig. 3A). Sox5 and 6 also demonstrated an increase in expression over the 9-day period of control cultures (Fig. 3, B and C). Overexpression of Rac1 or Cdc42 further increased the expression of Sox5, but only Rac1 increased Sox6 mRNA levels (Fig. 3, B and C). The expression of collagen II similarly increased in the control cells over the culture period and was further enhanced by overexpression of Rac1 or Cdc42 (Fig. 3D). Interestingly, only Rac1 overexpression resulted in significant increase of aggrecan mRNA expression, while Cdc42 overexpression did not affect aggrecan gene expression (Fig. 3E). ATF3 and GAPDH levels were not affected by overexpression of Rac1 or Cdc42 (Fig. 3, F and G).

Inhibition of Rac1 Signaling Reduces Mesenchymal Condensations and the Expression of N-cadherin—Because our data demonstrated that Rac1 promotes chondrogenesis, we next asked whether it also controls cellular condensation, the first step in chondrogenesis. Micromass cultures that were fixed and stained with PNA on day 4 of culture demonstrate decreased and diffuse staining upon Rac1 inhibition, suggesting a decrease in cellular condensations in comparison to control cultures (Fig. 4A). Analysis of expression levels of a marker of chondrogenic condensations, N-cadherin, showed a significant decrease of transcripts upon inhibition of Rac1 signaling as determined by real-time PCR (Fig. 4B). In contrast, Rac1 inhibition did not affect expression of another cell adhesion molecule, N-CAM (Fig. 4C). These data suggest that Rac1 signaling is essential for normal formation of cellular condensations, an essential determinant of chondrogenesis.

Inhibition of RhoA/ROCK Signaling Does Not Affect Cellular Condensations—In previous work, we had demonstrated a negative role for RhoA/ROCK signaling in chondrogenesis (32). We therefore asked whether ROCK signaling would affect cellular condensations, potentially antagonizing Rac1 effects. However, we did not see a change in mesenchymal condensations in response to ROCK inhibition by Y27632 (10 μM), as confirmed by the absence of differences in PNA staining (Fig. 4D) or levels of N-cadherin mRNA or protein (data not shown). These data suggest that RhoA/ROCK signaling regulates chondrogenesis at a stage later than cellular condensations.

N-cadherin mRNA and Protein Levels Are Increased by Rac1 Signaling—We next examined the effects of Rac1 on N-cadherin expression in more detail. ATDC5 cells stably overexpressing Rac1 displayed increased mRNA levels of N-cadherin (Fig. 5A) but did not affect levels of N-CAM (Fig. 5B). Western blotting also demonstrated increased levels of N-cadherin protein by day 3 of culture in cells overexpressing Rac1 (Fig. 5C). Immunofluorescence demonstrated that Rac1 overexpression increases staining for N-cadherin, (Fig. 5D). Negative controls reveal minor background fluorescence of cells incubated without the N-cadherin antibody. In contrast to Rac1, Cdc42 overexpression did not affect N-cadherin expression markedly.

Genetic Loss-of-function of Rac1 Reduces N-cadherin Expression and Other Chondrogenic Markers—Micromass cultures of wild-type CD1 or Rac1fl/fl mesenchymal cells infected with an adenovirus expressing Cre Recombinase demonstrated ~70% decrease of Rac1 mRNA transcripts by day 3 of culture (Fig. 6A), thus demonstrating efficient inactivation of the Rac1 gene.

Chondrogenic markers were analyzed by real-time PCR, demonstrating that genetic ablation of Rac1 inhibits the increase in mRNA levels of N-cadherin (Fig. 6B) and Sox9 (Fig. 6C), in comparison to wild-type cells. However, expression of the transcription factor ATF3, a known regulator of chondrocyte differentiation and limb development (50, 51), did not change between control and Rac1-deficient cultures (Fig. 6D). Furthermore, the endogenous control GAPDH did not change between control cells and Rac1 fl/fl infected with the Cre-expressing adenovirus (Fig. 6E). These data further confirm the importance of Rac1 in the regulation of chondrogenesis.

Matrix Molecules and GAG Enzymes Are Decreased in Response to Genetic Ablation of Rac1—Analysis of Rac1-ablated micromass cultures demonstrated significantly reduced levels of collagen II and aggrecan transcripts (Fig. 7, A and B), simi-
larly to our pharmacological data. In addition, transcripts encoding chondroitin sulfotransferase enzymes Chst3 and Chst11 were both up-regulated during differentiation in micro-mass culture, but showed decreased levels in response to Rac1 knockdown (Fig. 7, C and D).

**DISCUSSION**

We demonstrate in the present study that Rac1 and Cdc42 signaling pathways are positive regulators of chondrogenesis. In primary micromass culture of limb mesenchyme, cultures differentiate along the chondrogenic lineage and express the classical chondrogenic markers (46, 52). In this study, we show that inhibition of Rac1 signaling reduces transcript levels of collagen II and aggrecan, and the amount of alcian blue stain indicating a decrease in glycosaminoglycan accumulation. The reduction in GAG staining is accompanied by reduced expression of the two sulfotransferases Chst3 and Chst11. We also demonstrate a decrease in the transcript levels of the Sox family members Sox9, 5, and 6. Genetic ablation of Rac1 also results in a decrease in collagen II, aggrecan, and Sox9 mRNA levels. Because the effects of pharmacological inhibition and genetic ablation of Rac1 are so similar, these data suggest that the effect of the inhibitor is specific to Rac1 activity. Conversely, overexpression of Rac1 in the chondrogenic cell line ATDC5 stimulates an increase in transcript levels of Sox 9, 5, 6, collagen II, and aggrecan, while overexpression of Cdc42 results in an increase in Sox9 and 5 and collagen II. Interestingly, Rac1 overexpression stimulates all markers of chondrogenesis, while Cdc42 overexpression stimulates only some markers of chondrogenesis, indicating that these two GTPases have different modes of action in chondrogenesis. Because the overexpression of RhoA, Rac1, and Cdc42 all result in affecting the expression of different sets of genes, this suggests that the effects seen are not due to unspecific effects of overexpression. In addition, other genes known to be involved in chondrocyte differentiation such as N-CAM and ATF3 were not affected by our manipulations, providing further evidence that the observed effects are specific.

We wanted to explore earlier stages of chondrogenesis through the analysis of cellular condensations. The formation of cellular condensations precedes the commitment of mesenchymal cells to the chondrogenic lineage (6, 7). Inhibition of Rac1 signaling resulted in decreased mesenchymal condensation, as confirmed by decreased PNA staining and reduced expression of N-cadherin. Genetic loss-of-function of Rac1 also demonstrates a decrease in N-cadherin mRNA levels. Conversely, overexpression of Rac1 in ATDC5 cells increases N-cadherin transcripts and protein. On the other hand, Cdc42 overexpression did not significantly change N-cadherin expression at both the mRNA and protein level, and ROCK inhibition did not affect PNA staining or N-cadherin expression. Together, these data suggest that Rac1 signaling regulates chondrogenesis by exerting its effects (at least some of them) at the level of forming cellular condensations, while Cdc42 and RhoA regulate chondrogenesis at stages later than condensations (Fig. 8).

It is well established that cadherin junction formation is dependent on the actin cytoskeleton, due to the interaction of the cytoplasmic portion of cadherins with the actin cytoskeleton, mediated by catenins (53). Keratinocytes are similar to chondrocytes in that their differentiation depends...
on adherens junctions and that keratinocyte cell shape undergoes drastic changes during differentiation. However, differentiated keratinocytes are fibroblastic in cell shape, whereas chondrocytes display spherical cell shape once committed to the chondrogenic lineage (54, 55). Inhibition of Rac1 signaling results in removal of cadherins from cellular junctions in keratinocytes and MDCK cells (42, 44), possibly due to the rearrangement of the cortical actin cytoskeleton. It has also been described that RhoA signaling regulates cadherin-dependent cellular junction formation in keratinocytes (42), whereas we demonstrate that ROCK signaling does not affect condensations nor expression of N-cadherin in chondrocytes. Furthermore, the involvement of N-cadherin has also been demonstrated in myoblast differentiation where activation of N-cadherin junction formation results in decreased Rac1 activity and increased RhoA activity (56). Thus, the interplay between Rho GTPases and cadherin junctions appears to be cell type-specific, a conclusion further supported by our data from chondrocyte differentiation. Although there is a fair amount of data suggesting a role of RhoA and Rac1 in the localization of N-cadherin to cellular junctions and in activation of both GTPases by N-cadherin in other cell types, we are (to our knowledge) the first to demonstrate that Rac1 signaling regulates N-cadherin at the level of mRNA expression. It is also noteworthy that while all three prototype RhoA GTPases regulate chondrogenesis, only Rac1, but not RhoA/ROCK or Cdc42, achieves this through regulation of mesenchymal condensations and N-cadherin expression.

We demonstrate a disparity of Sox 5, 6, and matrix gene regulation by Rac1 and Cdc42 overexpression. Sox9 is a required factor for chondrogenesis, and expression of Sox5 and Sox6 are required for maximal activity of Sox9 (11, 38, 57). In our studies, overexpression of Rac1 increases Sox9, Sox5, and Sox6 expression, matched by an increase in collagen II and aggrecan. However, Cdc42 overexpression only causes an increase expression of Sox9 and Sox5, and not Sox6. Similarly, only collagen II expression is increased upon Cdc42 overexpression, but no change in aggrecan is seen. These data suggest that it is not essential that
both Sox5 and Sox6 be increased to stimulate collagen II expression. On the other hand, Sox6 induction might be required for the stimulation of aggrecan expression.

In conclusion, we have provided evidence that Rac1 and Cdc42 are positive regulators of chondrogenesis. Additionally, our studies and others demonstrate that it is essential that the role of GTPase signaling is studied in cell type-specific manners. We have demonstrated that Rac1 signaling is a required signaling pathway to generate normal N-cadherin-dependent cellular junctions that are known to be essential for chondrogenesis.

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