as a consequence of their relationship to the Calendar Round, it is also possible that these long numbers were designed at least in part to serve astronomical ends (Table 1). That the Xultun numbers are divisible by integral and half-integral multiples (give or take additive or subtractive small multiples of 52 and 260) is reminiscent of the character of numbers one finds in the upper portions of multiplication tables that accompany astronomical reckonings in the codices. The latter were developed to place canonical events in closer proximity to the occurrence of actual sky phenomena, e.g., an eclipse, the start of a retrograde loop of Mars, or a heliacal rise of Venus.

Though the Dresden Codex dates to ca. the 15th century, there are Long Count entry dates to the Eclipse table on the same page as the multiplication table dated to 755 C.E. (19), which corresponds well with the 800 C.E. date for Xultun 10K-2. Codical tables were likely copied and re-copied over many generations, with updates, based on observational data, incorporated periodically. Thus, the known Postclassic written documents derived from others of classical origin.

One goal of the Maya calendar keepers, gleaned from studies of the codices, was to seek harmony between sky events and sacred rituals. The Xultun paintings may represent an expression of the same ambition several centuries earlier. The Xultun paintings may represent an expression of the same ambition several centuries earlier.

References and Notes


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Over 70% of Americans between the ages of 55 and 70 are affected by osteoarthritis (OA), which is characterized by the progressive breakdown of articular cartilage (1) and ultimately leads to the functional failure of synovial joints. OA is mediated by several pathogenic mechanisms, including enzymatic degradation of extracellular matrix, deficient new matrix formation, cell death, and abnormal activation and hypertrophic differentiation of cartilage cells (2). The only current therapeutic options for OA are pain management and surgical intervention (3). Mesenchymal stem cells (MSCs), which reside in bone marrow and many adult tissues, are capable of self-renewal and differentiation into a variety of cell lineages, including chondrocytes, osteoblasts, and adipocytes (4, 5). MSCs have been identified in healthy and diseased cartilage and appear to retain at least some potential to regenerate cartilage in vivo (6, 7).

A Stem Cell–Based Approach to Cartilage Repair
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Osteoarthritis (OA) is a degenerative joint disease that involves the destruction of articular cartilage and eventually leads to disability. Molecules that promote the selective differentiation of multipotent mesenchymal stem cells (MSCs) into chondrocytes may stimulate the repair of damaged cartilage. Using an image-based high-throughput screen, we identified the small molecule kartogenin, which promotes chondrocyte differentiation (median effective concentration = 100 nM), and induces chondrogenesis by regulating the CBFβ-RUNX1 transcriptional program. This work provides new insights into the control of chondrogenesis that may ultimately lead to a stem-cell–based therapy for osteoarthritis.
describe the identification and characterization of a small molecule that selectively directs MSC differentiation into chondrocytes for cartilage regeneration.

An image-based high-throughput screen was established with primary human bone marrow MSCs [CD29 CD44 CD166 CD105 CD45, termed hMSCs (5)] in a 384-well format. Chondrogenic nodules containing cartilage-specific matrix components (proteoglycans and type II collagen) were positively stained by rhodamine B to identify the early cell condensation phenotype associated with the induction of chondrogenesis (Fig. 1B). Of 22,000 structurally diverse, heterocyclic, druglike molecules screened, one compound, kartogenin (KGN) (Fig. 1A), promoted chondrocyte differentiation from hMSCs in a dose-dependent manner [median effective concentration (EC50) = 100 nM, fig. S1]. Lineage-specific differentiation in the presence of KGN was further confirmed by fluorescent immunostaining of chondrocyte-specific proteins, including type II collagen, SOX9, and aggrecan under both monolayer and high-density conditions (fig. S2). Reverse transcriptase polymerase chain reaction (RT-PCR) with mRNA isolated from differentiated cells confirmed the expression of gene products associated with chondrocytes, including lubricin (superficial zone protein), aggrecan, and type II collagen (2). There were only slight changes in the expression of gene products associated with chondrocyte hypertrophy and calcification, such as osteocalcin, alkaline phosphatase, discoidin domain receptor, Indian hedgehog (IHH), and type X collagen, in either hMSCs or chondrocytes (Fig. 1C and figs. S2 to S4). In a three-dimensional 21-day hMSC pellet culture, up-regulation of tissue inhibitor of metalloproteinase I, type II collagen, and aggrecan was observed upon KGN treatment (Fig. 1D), indicating retention of the cartilage phenotype and inhibition of further matrix breakdown by matrix metalloproteinases (MMPs). Increased production of collagen is often associated with an increase in MMP activity in osteoarthritic cartilage (8). However, KGN did not alter either MMP-3, MMP-13, or aggrecanase expression in primary chondrocytes and MSCs (figs. S3 to S5), nor did it show any inhibition of aggrecanase or MMP13 in vitro (fig. S6). In addition, no toxicity was observed with KGN at 100 μM in hMSCs, chondrocytes, osteoblasts, and synoviocytes (fig. S7).

We next evaluated the effects of KGN on articular chondrocytes under pathophysiological conditions. Primary bovine articular chondrocytes and cartilage explants were grown in the presence of tumor necrosis factor-α (TNF-α) and oncostatin M to mimic cytokine-induced damage during OA pathogenesis (9). When treated with this cytokine cocktail, cultured primary chondrocytes released four to five times (50 μM) more nitric oxide (NO) than did untreated chondrocytes, as measured by the Greiss reaction. Upon treatment with 1 to 5 μM KGN, NO release was significantly inhibited (up to 70%). In cartilage explant organ cultures treated with 5 μM KGN, the cytokine-induced release of glycosaminoglycans (GAGs) was also reduced by up to 60% (Fig. 1E). These data suggest that KGN also protects existing chondrocytes under the pathological conditions of OA.

OA is considered to be the consequence of a variety of etiologic factors, including abnormal biomechanical stress, genetics, and abnormalities of the articular cartilage or bone (10). To evaluate the efficacy of KGN in vivo, we took advantage of two widely used rodent models: the collagenase VII-induced chronic joint injury model (11) and the acute surgical model, involving transection of three of the major ligaments in the joint (12). In the collagenase VII-induced model, a 3-day period of mild inflammation is followed by collagenase-induced destabilization of the joint, resulting in mild to moderate cartilage destruction based on pathological alteration of joint morphology (Fig. 2, A and C). Intra-articular (IA) administration of KGN (10 μM in 4 μl of saline on days 7 and 21) and subsequent histological analysis and grading of the medial tibial plateau [based upon the Osteoarthritis Research Society International scoring system (13)] revealed regeneration in the cartilage matrix as indicated by a decrease in the fibrillations in the superficial and midzone of the articular cartilage (Fig. 2, C and D). Peripheral blood was collected during the 8-week period to measure the serum level of cartilage oligomeric matrix protein (COMP), which is elevated in the plasma of patients with OA and correlates with

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**Fig. 1.** KGN induces chondrocyte differentiation from hMSCs and protects articular chondrocytes in vitro. (A) Structure of KGN. (B) KGN induces chondrocyte nodule formation in primary hMSCs. Cells were grown in serum-free DMEM under high-density conditions for 3 days and stained with rhodamine B (1 μg per milliliter of phosphate-buffered saline). Scale bars, 100 μm. (C) KGN increases chondrocyte-specific gene expression in hMSCs. Cells were treated with KGN as indicated for 72 hours. Relative mRNA levels were determined with Taqman gene-specific assays (n = 3). Fold change relative to a dimethyl sulfoxide (DMSO) control is shown. DDR, discoidin domain receptor. (D) Long-term hMSC pellet cultures treated with KGN exhibit cartilage properties. Cells were treated with compound for days 1 to 3 of the 21-day differentiation period, fixed, and stained to assess chondrocyte differentiation. Scale bars, 400 μm. (E) KGN inhibits NO and GAG release induced by cytokines. Primary bovine articular chondrocytes were induced to release NO by stimulation with 20 ng/ml TNF-α and 10 ng/ml oncostatin M (OSM) with and without KGN. After 48 hours, NO released into the media was determined by the Griess reaction (n = 12 experiments). Bovine cartilage (femoral groove only) explants were treated as above for 72 hours. GAG content was determined by the dimethylene blue reaction (n = 6 experiments). Relative levels of NO and GAG, normalized to that of DMSO-treated controls, are shown.
the severity of disease \(14\). At both days 13 and 56, there were significantly lower levels of COMP in the mice treated with KGN (Fig. 2E).

In the acute surgical model, the majority of the lesions were located on the lateral tibial plateau (Fig. 2, B and F). After KGN treatment, there was a 50% reduction in the joint score (Fig. 2G) as compared to that with vehicle treatment alone. Furthermore, there was a 1.8-fold reduction in the levels of circulating type II collagen alone. Furthermore, there was a 1.8-fold reduction of joint damage and four weekly drug treatments (days 7, 21, 28, and 35), treatment with as little as 1 μM KGN produced significant improvement as compared to the vehicle-treated injured knees (Fig. 2I). Although we do not know the IA exposure of KGN, systemic exposure of KGN is minimal after IA injection (half-life = 2 hours, maximum concentration = 0.1% of IA dose). This result suggests that KGN acts at the diseased joint, and systemic exposure is minimal. Consistent with this result, no obvious adverse effects (weight loss, significant swelling, or signs of pain or distress) were observed in either animal model. Collectively, the in vitro and in vivo data strongly support the notion that directed differentiation of cartilage resident MSCs leads to improvements in the damaged joints when given at early stages of the disease. The overall in vivo efficacy of KGN may be attributable to a combination of both a regenerative/repair effect and a protective effect; further in vivo studies will be required to analyze the relative contribution of each.

To elucidate the biological mechanism of KGN, we performed a focused structure-activity-relationship study. Among a group of derivatives of KGN, we found that cyano-KGN (KGN-CN, fig. S1) has very similar chondrocyte differentiation activity \(EC_{50} = 137 \text{nM}\) to that of the parent molecule. We therefore synthesized a close structural analog of KGN-CN as an affinity probe that contains a biotin moiety and a phenyl azide photo-cross-linker [biotin-kartogenin-azide (BKA), fig. S1]. We incubated the probe with hMSCs and cross-linked it to cellular targets by ultraviolet irradiation. Western blotting with antibodies to biotin identified 90- and 280-kD bands in an SDS–polyacrylamide gel electrophoresis gel of ammonium sulfate–precipitated fractions of the cell lysates (fig. S8). The intensity of both bands decreased in the presence of 100 μM free KGN (50 times in excess of BKA). The two bands were identified as filamin A (FLNA) by mass spectrometry. FLNA, a member of the filamin family of proteins, is a 280-kD protein that can be cleaved in certain cells to afford a 90-kD C-terminal fragment (\(\text{EC}_{50} \approx 16\)). Knockdown of FLNA in hMSCs using lentiviral particles expressing short hairpin RNAs (shRNAs) targeting FLNA (>70% knockdown, fig. S9) resulted in up to a fivefold increase in chondrocyte formation as compared to the control virus (expressing nontargeting shRNA)–infected cells (Fig. 3A), suggesting that KGN induces MSC differentiation through its association with FLNA.

FLNA is an actin-binding protein that crosslinks actin filaments, thereby regulating cytoskeletal network organization and dynamics \(17\). Previously, cytoskeleton rearrangement has been shown to induce chondrocyte differentiation \(18\). However, treatment of hMSCs with KGN revealed no significant effects on either the G-actin (monomeric) or F-actin (filamentous) fractions, nor altered distribution of FLNA in the two fractions (fig. S10), which is consistent with binding of KGN distal to the actin-binding domain at the N terminus of the protein. Three C-terminal fragments of FLNA (Fig. 3B) were then cloned into pEGFP and transfected into hMSCs and cross-linked it to cellular targets by ultraviolet irradiation. Western blotting with anti-FLNA antibodies to biotin identified 90- and 280-kD bands in an SDS–polyacrylamide gel electrophoresis gel of ammonium sulfate–precipitated fractions of the cell lysates (fig. S8). The intensity of both bands decreased in the presence of 100 μM free KGN (50 times in excess of BKA). The two bands were identified as filamin A (FLNA) by mass spectrometry. FLNA, a member of the filamin family of proteins, is a 280-kD protein that can be cleaved in certain cells to afford a 90-kD C-terminal fragment (\(\text{EC}_{50} \approx 16\)). Knockdown of FLNA in hMSCs using lentiviral particles expressing short hairpin RNAs (shRNAs) targeting FLNA (>70% knockdown, fig. S9) resulted in up to a fivefold increase in chondrocyte formation as compared to the control virus (expressing nontargeting shRNA)–infected cells (Fig. 3A), suggesting that KGN induces MSC differentiation through its association with FLNA.

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FLNA is core-binding factor β subunit (CBFβ), which has recently been shown to bind to the hinge 2 and immunoglobulin-like repeat 24 region of FC-1 (19). We confirmed the association between CBFβ and FC-1 (but not FC-2 or FC-3) in an in vitro pull-down experiment and observed that KGN blocks this interaction at its active concentrations (Fig. 3, D and E). The specific interaction between FLNA and CBFβ was further confirmed in hMSC cell lysates by a coimmunoprecipitation assay (Fig. 3F) and could again be disrupted by KGN.

CBFβ is the regulatory subunit of the heterodimeric core-binding factor transcription complex; the other subunit is one of the runt-related transcription factor (RUNX) family members, which include RUNX1, RUNX2, and RUNX3 (20). In the resting state, CBFβ is sequestered in the cytoplasm by binding to its cytoplasmic partner, FLNA; upon activation, it dissociates from FLNA and translocates into the nucleus, where it binds the RUNX factors and regulates transcription (19). When hMSCs were treated with 5 μM KGN, CBFβ nuclear localization was significantly increased (Fig. 4, A and B). We next overexpressed CBFβ in hMSCs and saw that cells overexpressing CBFβ showed increased chondrocyte differentiation as compared to empty vector–transfected cells (Fig. 4C and fig. S11). Moreover, knockdown of CBFβ expression by shRNAs blocked KGN’s effect on chondrocyte differentiation in hMSCs (Fig. 4D and fig. S11). Thus, KGN appears to induce chondrogenesis by regulating the nuclear localization of CBFβ.

The RUNX proteins are highly regulated by complex signaling networks [IHH, fibroblast growth factor (FGF), WNT (21), and RUNX themselves (22)] in a context-dependent manner that correlates with their distinct roles in musculoskeletal development and maintenance. RUNX1 has been shown to play a critical role in chondrogenesis, chondrocyte proliferation, and survival (23, 24). RUNX2 is a key factor in osteogenesis (25–27) and also plays a pivotal role in chondrocyte hypertrophy (the terminal differentiation of chondrocytes often associated with OA). Knockdown of RUNX2 diminishes bone formation and blocks chondrocyte hypertrophy, cartilage calcification, and the development of OA (28). Recently, evidence has also suggested a possible role for RUNX3 in chondrocyte differentiation (29).

Microarray gene expression analysis revealed that 5 of the 39 genes affected by KGN (>1.5 fold at 6 or 24 hours) are associated with the RUNX1 network, including DOX5, SFN, and THRAP3 (fig. S12) (30). In addition, ANKRD1, a downstream target gene of RUNX2, was downregulated upon KGN treatment in hMSCs; and altered transcription of shared downstream target genes of RUNX1 and RUNX2 [e.g., anaphase-promoting complex subunit 10 (ANAPC10)] was also observed. Quantitative RT-PCR analysis showed an interesting pattern of expression for RUNX family members and CBFβ in hMSCs, chondrocytes, and osteoblasts: RUNX1 is highly expressed in all cell types, with slight upregulation in chondrocytes; RUNX2 and RUNX3 are expressed at low levels in hMSCs, significantly up-regulated in osteoblasts, and almost

![Fig. 3. KGN binds to the FC-1 fragment of FLNA and regulates its interaction with CBFβ.](image)

(A) Knockdown of FLNA with shRNA-expressing lentiviral particles increased chondrocyte differentiation from hMSCs. Type II collagen expression levels were normalized to nontargeting shRNA control virus-infected cells (n = 6 experiments). (B) Human FLNA structure and fragment cloning. Fragments: FC-1, nucleotides 7264 to 7941 (relative to ATG start codon), contains immunoglobulin-like (Ig) repeat 23, hinge 2, and Ig repeat 24; FC-2, nucleotides 6391 to 7566, contains Ig repeats 20 to 23; FC-3, nucleotides 5275 to 6390, contains partial hinge 1 and Ig repeats 16 to 19. ABD, actin-binding domain. (C) Western blotting shows the specific labeling of the FC-1 fragment but not the other two FLNA fragments by BKA (anti-biotin). (D) The specific interaction between the FC-1 fragment and CBFβ was demonstrated by pull-down of purified recombinant CBFβ (400 ng) using FLNA fragments fused to glutathione S-transferase. (E) KGN treatment disrupts the interaction between FC-1 and CBFβ. (F) KGN disrupts the interaction between FLNA and CBFβ in hMSC cell lysates. The FLNA-CBFβ complex was immunoprecipitated with antibody to CBFβ and probed with antibodies to FLNA and CBFβ.

![Fig. 4. KGN enhances the nuclear localization of CBFβ and induces hMSC chondrocyte differentiation.](image)

(A and B) KGN treatment induces CBFβ nuclear localization in hMSCs, as demonstrated by (A) nuclear fractionation followed by Western blotting and (B) fluorescent immunostaining. Scale bars, 20 μm. (C) CBFβ overexpression mimics the effect of KGN in hMSCs (n = 6 experiments). (D) CBFβ knockdown in hMSCs blocks chondrocyte differentiation induced by KGN with shRNA-expressing lentiviral particles (n = 6 experiments).
Differential Diffusivity of Nodal and Lefty Underlies a Reaction-Diffusion Patterning System

Patrick Müller, Katherine W. Rogers, Ben M. Jordan, Joon S. Lee, Drew Robson, Sharad Ramanathan, Alexander F. Schier

Biological systems involving short-range activators and long-range inhibitors can generate complex patterns. Reaction-diffusion models postulate that differences in signaling range are caused by differential diffusivity of inhibitor and activator. Other models suggest that differential clearance underlies different signaling ranges. To test these models, we measured the biophysical properties of the Nodal/Lefty activator/inhibitor system during zebrafish embryogenesis. Analysis of Nodal and Lefty gradients revealed that Nodals have a shorter range than Lefty proteins. Pulse-labeling analysis indicated that Nodals and Leftys have similar clearance kinetics, whereas fluorescence recovery assays revealed that Leftys have a higher effective diffusion coefficient than Nodals. These results indicate that differential diffusivity is the major determinant of the differences in Nodal/Lefty range and provide biophysical support for reaction-diffusion models of activator/inhibitor-mediated patterning.

In 1952, Alan Turing put forward the reaction-diffusion model, in which two interacting and diffusing species of molecules can generate complex patterns (1). Gierer and Meinhardt postulated that pattern formation in reaction-diffusion models requires a short-range activator that enhances both its own production and that of a long-range inhibitor (2) (Fig. 1A). Despite the prominence of reaction-diffusion models and the widespread occurrence of short-range activators and long-range inhibitors in development (3–10), it is unclear how differences in activator and inhibitor ranges arise in vivo. The classic reaction-diffusion models postulate that the inhibitor is more diffusive than the activator (text S1), but more recent studies suggest that differential signal clearance might be a major determinant of differences in signaling range (11–18) (Fig. 1B). This question has not been resolved because the biophysical properties of diffusion and clearance have not been measured for any activator/inhibitor pair.

The transforming growth factor-β superfamily signals Nodal and Lefty constitute an activator/inhibitor-based system in animals as different as sea urchin and mouse (3–5, 16, 18–22) (text S2). Nodals activate signaling during mesendoderm induction and left-right patterning,


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