The circadian clock: a central mediator of cartilage maintenance and osteoarthritis development?

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ABSTRACT

The circadian clock is a specialised cell signalling pathway present in all cells. Loss of clock function leads to tissue degeneration and premature aging in animal models demonstrating the fundamental importance of clocks for cell, tissue and organism health There is now considerable evidence that the chondrocyte circadian clock is altered in osteoarthritis (OA). The purpose of this review is to summarise current knowledge regarding the nature of the change in the chondrocyte clock in OA and the implications of this change for disease development.

Expression of the core clock component, BMAL1, has consistently been shown to be lower in OA chondrocytes. This may contribute to changes in chondrocyte differentiation and extracellular matrix turnover in disease. Circadian clocks are highly responsive to environmental factors. Mechanical loading, diet, inflammation and oxidative insult can all influence clock function. These factors may contribute to causing the change in the chondrocyte clock in OA.

Key messages:

Expression of the circadian clock transcription factor, BMAL1, is reduced in chondrocytes in OA.

Reduced BMAL1 levels have been linked with aberrant control of chondrocyte differentiation and cartilage turnover.

The chondrocyte clock may be regulated by lifestyle-associated factors such as diet and exercise.

Key words: Chronobiology, cartilage degradation, cartilage turnover, biological clocks, BMAL1, ARNTL

Almost all cells contain a circadian clock; a specialised cell-signalling circuit that synchronises biological activity with rhythmic changes in the external environment(1). Highly conserved across species and phyla, clocks provide an adaptive advantage, allowing regular environmental changes to be anticipated, and cellular activity modified in advance, to maximally exploit favourable changes and minimize the impact of negative changes.

(2).

The most well-known circadian clock is the central clock located in neurons of the suprachiasmatic nucleus (SCN) in the hypothalamus. Sensitive to light, the SCN clock synchronises diurnal rhythms in physiology and behaviour, such as the sleep/wake cycle and hormone secretion, with the external day/night cycle(3). Circadian clocks in other tissues (known as "peripheral" clocks) are integrally involved in controlling the timing of the cell cycle, energy metabolism, cell differentiation and have recently emerged as potential key regulators of extracellular matrix synthesis(4-12). Just as chronic disruption to the SCN clock has been associated with increased risk of disease (e.g. obesity, diabetes and cancer), peripheral clock disruption has also been implicated in several diseases including cancer, asthma and rheumatoid arthritis(13-16). Recently, disruption to the chondrocyte peripheral clock has been observed in osteoarthritis (OA)(17-19).

The cartilage loss in OA is driven in part by abnormal activity of cells within various joint tissues, including chondrocytes(20). The purpose of this review is to evaluate the evidence for a role of chondrocyte clock disruption in contributing to the disease-associated changes in chondrocyte behaviour in OA.

The molecular circuitry of the circadian clock

The core circadian clock proteins are BMAL1, CLOCK, Period (PER) and Cryptochrome (CRY)(21-23). BMAL1 and CLOCK dimerise to form a transcription factor(23) that drives expression of several target genes including those of the *PER* (*PER1*, *PER2*, *PER3*) and *CRY* (*CRY1*, *CRY2*) families. PER and CRY proteins form heterodimers which in turn repress the transcriptional activity of BMAL1 and CLOCK(24). Other transcriptional and translational regulators also contribute to controlling PER/CRY and BMAL1/CLOCK expression such that levels of BMAL1 and CLOCK oscillate with those of PER and CRY in a self-sustaining cycle(25-27) (Figure 1). Although clock cycling can continue in the absence of any external input (known as "free-running" (Table 1)), this seldom occurs *in vivo*. Instead clocks are sensitive to, and will synchronise with, rhythmic changes in the environment. For the SCN clock, the most powerful entraining rhythm or "zeitgeber" is the daily light/dark cycle and the cycling of BMAL1:CLOCK/PER:CRY in the SCN is synchronised with the 24h day/night cycle(1, 27).

Most peripheral tissues are not light-sensitive. Instead, they are entrained by various rhythms in their local environment. This includes rhythms in circulating hormone levels e.g. cortisol which are regulated by the SCN clock(28). Originally it was assumed that peripheral clocks were entirely regulated by the SCN. However this notion was dispelled in 2000-2001, when two groups independently demonstrated that clocks in peripheral tissues preferentially entrain to daily rhythms in food availability over light/dark cycles(29, 30). Since this time, other factors such as mechanical loading have also been shown to regulate peripheral clocks(31). Peripheral clocks are therefore controlled by both systemic and

localised factors(31, 32). Although usually these cues would align e.g. in a diurnal animal cortisol levels peak in the morning which corresponds with the time the animal is active and consuming food, misalignment of these cues may lead to discordant clock control.

The chondrocyte circadian clock

Chondrocytes within the growth plate, articular cartilage and secondary cartilage all contain circadian clocks(33-35) and there is evidence that chondrocyte differentiation is under circadian control(34, 35).

Transcription of *IHH*, a master controller of chondrocyte differentiation, is directly regulated by BMAL1 in chondrocytes (34, 35). *Ptch1* expression (encoding the IHH receptor) has also been shown to oscillate over a 24h period in chondrocytes(34). Other transcription factors critical for chondrocyte differentiation may also be under circadian control including *Sox9*(15), *Nfatc2*(17) and *Sox6*(36).

Expression of several genes involved in matrix turnover have been shown to oscillate over the course of a day in chondrocytes including *ACAN*, *MMP13*, and *COL2A1*(17, 18, 33). Whether this is due to direct circadian control or a consequence of circadian regulation of transcription factors such as IHH and SOX9 is unclear.

At present, the most well understood mechanism by which peripheral clocks control cell activity is through the regulation of gene transcription. However, it is likely that the circadian clock influences chondrocyte behaviour through mechanisms that extend beyond transcriptional control. For instance, both BMAL1 and PER2 have been found to associate with components of the mTOR complex and provide temporal control of protein translation(37, 38).

The SCN (central) clock in OA pathogenesis

Although SCN clock disruption is implicated as a risk factor for other chronic conditions(39), to date there is minimal evidence to support a link with OA. No difference in cartilage was observed in transgenic mice bearing the *clock*$^{\Delta 19}$ mutation (which results in a lengthened SCN circadian period >24h(40)) or the *csnk1e*^{tau} mutation(41) (which results in a shortened SCN circadian period <24h(42)). Two further studies found that mice exposed to a simulated shift-work regimen (induced by shifting the light/dark cycle by 12h every week), had reduced proteoglycan content, increased immune cell infiltrate and signs of fibrillation in articular cartilage within the knee (but not other joints) and these effects were exacerbated by a high fat diet(41, 43). A link between shift-work and increased risk of knee OA has also been reported in humans(44). However, shift-work has many effects other than just SCN clock disruption. Whether SCN clock disruption itself has adverse effects on cartilage remains to be established.

The chondrocyte (peripheral) clock in OA pathogenesis

There is considerable evidence that the chondrocyte circadian clock is altered in OA. Expression of *BMAL1* has been consistently shown to be lower in OA chondrocytes(17-19, 45, 46). Reduced *Bmal1* levels have also been observed in a mouse model of TMJ OA(47) but not in the DMM mouse where *Bmal1* expression was instead found to be elevated(33).

Differences in expression of other clock genes have also been observed in OA(18, 19, 45, 46, 48, 49), however results have been inconsistent. This is likely a reflection of the difficulty in investigating the circadian clock in human disease. The dynamic nature of the clock means that both the magnitude and direction of differences in clock gene expression between healthy and diseased tissue may differ at different timepoints throughout a 24h period. This is an important consideration when interpreting the results of studies investigating potential differences in the circadian clock in OA particularly as two different experimental approaches have been used for these studies, often yielding different results.

One approach is to measure clock gene expression directly in cartilage of individuals with and without OA at the single timepoint at which tissue is collected. The second approach is to isolate chondrocytes from OA and normal cartilage and use a technique such as serum shock (an *in vitro* zeitgeber(50)) to synchronise cell clocks. Chondrocytes are then cultured *in vitro,* and clock gene expression measured at regular intervals over the circadian cycle.

The two approaches provide slightly different information regarding changes in the clock in OA. Whilst the first approach only allows identification of differences in clock gene expression at a single timepoint, it will capture differences in expression due to both intrinsic factors i.e. a change in the epigenetic control of clock genes, as well as those due to extrinsic factors i.e. as a result of a change in clock-regulating cues in the diseased environment. In contrast, the second approach allows comparison of clock gene expression throughout the circadian cycle, however, it is likely that only intrinsic differences in clock gene expression will be detected as differences due to exposure to different clock regulating cues in disease may be lost during *in vitro* culture. The second approach will detect whether clocks in OA chondrocytes respond in the same way as normal chondrocytes to a given zeitgeber. Combining data obtained from the two approaches can provide insight into the mechanisms involved in causing changes in the clock in OA.

Reduced *BMAL1* expression in OA chondrocytes has been observed in studies using both approaches suggesting epigenetic control of *BMAL1* is altered in disease. However, the two approaches have often yielded different results when expression of other clock components have been assessed. It is likely that these changes are a result of differences in the types of clock regulating stimuli to which chondrocytes are exposed to in OA, and/or a change in the responsiveness of chondrocytes to clock-resetting cues in OA.

Potential mechanisms leading to alteration of the chondrocyte circadian clock in OA

There is evidence that factors present in the OA disease environment can induce changes in the chondrocyte circadian clock. Oxidative stress, IL-1β and basic calcium phosphate crystals alter expression of circadian clock genes in chondrocytes with all causing a reduction in *BMAL1* expression, (51, 52).

Various studies have identified signalling pathways involved regulating the chondrocyte clock. Reduced sirtuin 1 (sirt1) activity(19) and altered NMDA receptor signalling(48), (the latter a major pathway involved in re-setting the SCN clock to light) have been implicated in the change in the chondrocyte clock in OA. Increased NF-κB(52) and reduced sirt1 activity(19) have also been implicated in

IL-1β induced effects on the chondrocyte clock. It is plausible that these three pathways may act in concert to regulate the clock. In other cell types, NMDA receptor signalling leads to reduced levels of sirt1(53) and increased NF-κB activation(54, 55). Increased NF-κB activation also drives a reduction in sirt1 levels(56). Both sirt1 and NF-κB are epigenetic regulators. Sirt1 is a histone deacetylase and whereas NF-kB binds chromatin and regulates chromatin remodelling and histone acetylase recruitment (57, 58). Whether either are involved in epigenetic control of clock gene expression, particularly *BMAL1*, remains to be determined.

Consequences of changes in the chondrocyte clock for cartilage integrity

The effects of reduced BMAL1 levels

Global *Bmal1* knockout mice display a premature aging phenotype, developing multiple morbidities including cataracts and reduced bone mass(59). However, although extensive ectopic calcification of tendons, ligaments and intervertebral discs was observed in *Bmal1*-null mice, articular cartilage appeared normal(59). Other types of cartilage though do appear to be compromised in BMAL1-null animals. Levels of aggrecan and types II and X collagen were reduced in mandibular condyle cartilage during both embryonic as well as postnatal development in global *Bmal1* knockout mice (35) suggesting that Bmal1 is involved in mandibular cartilage development(35).

In contrast to the global *Bmal1* knockout, articular cartilage has been shown to be compromised in cartilage-specific *Bmal1* knockout mice. Chondrocyte number was reduced, chondrocyte apoptosis increased and extracellular matrix loss apparent in cartilage-specific *Bmal1*-knockout mice(17). Data from the global and cartilage-specific *Bmal1* knockout models suggest that it may be asynchrony of the chondrocyte clock relative to other tissue clocks, or reduced *Bmal1* levels in chondrocytes relative to other tissues, that drives cartilage lesion formation. This is an interesting concept given that OA is a disease affecting multiple joint tissues.

Intriguingly, although cartilage-specific *Bmal1* ablation resulted in loss of cartilage integrity in knee joints, hip cartilage was unaffected(17). This indicates that loss of *Bmal1* alone may be insufficient to cause substantive changes in cartilage. Rather it may sensitise tissue to other cartilage-damaging agents such as mechanical loading. Understanding why hip cartilage was preserved following *Bmal1* knockout may provide insight into understanding the risk factors for OA development between different joints.

It should be noted that *reduced* levels of *BMAL1* rather than complete BMAL1 ablation are seen in patients with OA. (17, 18, 48). The effect of reduced *BMAL1* levels in human cartilage are likely to be less profound than those observed in *Bmal1* knockout animals. Nevertheless, findings in *Bmal1* knockout animals provide compelling evidence that a change in the chondrocyte clock can profoundly impact cartilage integrity.

The effects of Bmal1 on the growth plate has also been studied in knockout animals(34, 60). Both body length and long bone length is reduced in cartilage-specific *Bmal1* knockout mice(34, 60) suggesting growth plate function is compromised. Effects were only apparent when *Bmal1* knockout was induced postnatally not during embryonic growth(60), differing from effects seen in mandibular cartilage(35). This indicates that unlike mandibular cartilage, *Bmal1*

is not involved in growth plate cartilage development but rather has a role in post-natal maintenance of the tissue. Whether the same is true for articular cartilage is unclear.

Mechanisms by which BMAL1 affects chondrocyte activity and cartilage integrity

Chondrocyte proliferation and viability

In growth plates from cartilage-specific *Bmal1* knockout mice, number of proliferating chondrocytes was lower whereas number of apoptotic chondrocytes higher compared to wildtype controls(60). This is consistent with findings in articular cartilage(17), indicating that *Bmal1* deletion reduces chondrocyte viability. However, a reduction in *BMAL1* levels in human chondrocytes appears to have less dramatic effects than complete BMAL1 ablation in animal models. . A small increase in cell proliferation was reported in one study following approximately 50-60% knockdown of BMAL1 in human chondrocytes isolated from macroscopically-normal cartilage from OA joints(18) whereas no effect on cell number was observed in a second study following BMAL1 knockdown in chondrocytes obtained from patients without OA (19). The level of BMAL1 knockdown achieved was not reported in the latter study therefore it is unclear whether a comparable level of knockdown was achieved in the two studies. Differences in the sensitivity of the assays used and/or the source of primary chondrocytes may also have contributed to the difference in effects observed between these studies. . Both studies involved only a transient reduction in *BMAL1* expression. Whether a chronic reduction in BMAL1 levels has more pronounced effects on cell number is unknown.

Total *Bmal1* knockout leads to complete loss of circadian clock cycling. However, this does not occur following just a partial reduction in BMAL1 levels and oscillations in the chondrocyte clock appear to be retained in OA chondrocytes (17, 18, 48)It It is possible that the anti-proliferative and pro-apoptotic effects observed in *Bmal1* knockout mice are due to loss of overall clock cycling rather than a direct effect of loss of *Bmal1* itself.

There is some indication that BMAL1 levels may naturally be lower in proliferating compared to non-proliferating chondrocytes. By immunohistochemistry, Takarada et al(34) found BMAL1 staining was noticeably less intense in proliferating cells compared to pre-hypertrophic or hypertrophic chondrocytes within growth plate cartilage. This raises the possibility that BMAL1 knockdown has different effects in proliferating compared to differentiating chondrocytes.

Chondrocyte differentiation

There is evidence that reduced BMAL1 levels result in altered expression of chondrocyte phenotype markers conducive with changes seen in OA. In articular chondrocytes from cartilage-specific *Bmal1* knockout mice, phosphorylated smad 2/3 levels were lower and phosphorylated smad 1/5 levels higher (17).. Likewise, expression of *Nfatc2* and *Sox9* have also been shown to be reduced following cartilage-specific *Bmal1* ablation(15).

However, mRNA levels of *Ihh*(34) as well as protein levels of Hif1α and Hif2α and their downstream effector *Vegf,* were found to be reduced in growth plate chondrocytes in cartilage-specific *Bmal1* knockout mice(60). These findings are somewhat disparate with the changes in these factors observed in OA(61-63). The

number and type of genes regulated by the circadian clock is known to differ between different tissues(33). Whether BMAL1 has differing effects in the growth plate than it does in articular cartilage remains to be determined.

Extracellular matrix turnover
Expression of *Col2* and *Acan* has been found to be reduced in chondrocytes from cartilage-specific *Bmal1* knockout mice consistent with the reduced *Col2a1* and *Acan* expression observed at certain stages of OA (34). However, in growth plate cartilage, *Col10* expression was also found to be reduced in *Bmal1* knockout mice suggesting that loss of *Bmal1* inhibits chondrocyte hypertrophy(64). These data may suggest that the contribution of reduced BMAL1 levels to OA pathogenesis may differ depending on the stage of disease.

Transient knockdown of *BMAL1* (50-60% reduction) in chondrocytes isolated from macroscopically-normal regions of cartilage from patients with OA resulted in elevated *MMP13* expression in one study(18) but had no effect in a second study involving chondrocytes isolated from patients without OA (19). As for cell number, the level of BMAL1 knockdown was not reported in this second study. In both studies, gene expression was only measured at a single time point. Given that expression of *MMP13* differs across the course of a day (suggesting it may follow a circadian rhythm)(18), the effects of *BMAL1* knockdown may differ depending on the timepoint measured. Interestingly in the second study, although knockdown of *BMAL1* alone had no effect on *MMP13* expression(19), it exacerbated the effects of IL-1β(19). This is conducive with the notion that reduced BMAL1 levels may sensitise chondrocytes to other factors in the disease environment. It is possible that chondrocytes within macroscopically normal regions from OA joints are more vulnerable to the effects of reduced BMAL1 levels than chondrocytes from non-OA joints and this may be a factor in the difference in effects observed following BMAL1 knockdown between the two studies. The potential causes and consequences of reduced BMAL1 levels in OA chondrocytes are summarised in Figure 2.

The effects of other clock components

BMAL1 is not the only clock component with altered expression in OA and hence may not be the only means by which an altered circadian clock influences cartilage integrity in OA.

Global *Clock* mutant mice (*Clock*$^{\Delta 19}$ mutation) exhibit pronounced cartilage degradation which is further exacerbated by anterior cruciate ligament destabilisation(65). CLOCK regulates NF-κB activity by physically interacting with the p65 NF-κB subunit(66). Increased NF-κB activation was found to contribute to the cartilage damage in *Clock* mutant mice(65). At present there is little evidence that *CLOCK* expression is altered in OA. However, since CLOCK is the dimer partner of BMAL1, reduced BMAL1 levels in OA may lead to increased levels of free CLOCK, enabling dimerization of CLOCK with p65 and promoting NF-κB activation.

Other clock components have also been shown to influence chondrocyte activity and cartilage integrity. Cartilage thickness is greater in global *Cry2* knockout mice compared to wild type controls(64). However, pathological changes in cartilage, subchondral bone and synovium were more pronounced in *Cry2* knockouts following surgical OA induction by medial meniscus destabilisation(64).

Knockdown of *NR1D1* in normal chondrocytes resulted in altered expression of components of the TGF-β pathway(49) whereas knockdown of *PER2* in OA chondrocytes led to increased *SOX9* and modest reductions in both *ADAMTS5* and *MMP13*(51).

Circadian clocks in other joint tissues in osteoarthritis

In addition to chondrocytes, cells within other musculoskeletal tissues including synovial fibroblasts, tenocytes, osteoblasts, myocytes and cells of the immune system also contain functional circadian clocks(67-72). Clocks in these tissues have important roles in contributing to joint tissue health (Figure 3). For instance, clocks in T cells are involved in joint inflammation, the osteoblast clock is involved in bone remodelling, and the tenocyte clock regulates collagen secretion (4, 73, 74). At present there is a paucity of data as to whether clocks in joint tissues other than cartilage are altered in OA. Differences in clock gene expression have been observed between synovial fibroblasts and immune cells in RA compared to OA(69, 70, 75). However, in the absence of normal controls, this data is difficult to interpret.

The circadian clock as a therapeutic target in OA

A number of existing drugs are known to modulate the circadian clock(76). For instance, both synthetic glucocorticoids and general anaesthetics cause phase shifts in the clock (although whether this contributes to their clinical efficacy is unclear)(77, 78). In addition, novel small molecule inhibitors targeting specific clock components are also in development(79). However, the ubiquity of circadian clocks throughout the body may limit their utility as direct drug targets. Targeting the clock in one tissue whilst avoiding effects in non-target tissues may be problematic. There is some indication that there are tissue specific differences in the responsiveness of clocks to regulators. For instance, TNF-α, IL-6 and IL-1β have all been shown to regulate the synovial fibroblast clock(69, 70) whereas IL-1β but not TNF-α was shown to regulate the chondrocyte clock(52). This is an important avenue for further investigation as it will not only aid in determining how clocks in individual tissues become disrupted in disease but may also inform drug discovery studies aimed at achieving tissue-specific targeting of the clock.

Aside from pharmacological approaches, circadian clocks are likely to be particularly amenable to modification through lifestyle interventions. Peripheral clocks in general are known to be regulated by feeding cycles, mechanical loading as well as diurnal rhythms in hormone release. At present, how these different cues converge to ultimately control the chondrocyte clock is unclear. It is possible that the timing of exercise itself, or in relation to meal intake, may be important for retaining normal chondrocyte clock cycling. Further studies investigating these factors may enable the development of targeted advice regarding exercise and diet for those with early-stage OA or those at risk of developing OA.

Technical considerations for circadian clock studies in human tissue

There are several important factors that need to be considered when interpreting data from studies investigating circadian clocks in human tissue:

1. Differences between study participants in lifestyle and chronotype can lead to profound inter-individual variation in circadian clock cycling. Tissue obtained at the same time of day from different individuals may not be at the same

circadian time. This has implications when comparing the circadian clock between normal and diseased tissue obtained from different patient donors.

2. The effects of regulators of the circadian clock often vary depending on the time during the circadian cycle at which exposure occurs. For instance, dexamethasone and forskolin have been shown to invoke phase advances, phase delays or have no effect on the chondrocyte clock depending on the time of day at which they are administered(80). The time at which a treatment is administered needs to be considered when assessing the effects of potential regulators of the chondrocyte clock. One approach to overcome these issues is to use an *in vitro* zeitgeber such as serum shock to synchronise cell clocks before treatment with a clock regulator. The timing of treatments relative to zeitgeber exposure can then be kept consistent between patient samples.
3. It is difficult to determine whether apparent changes in the level of clock gene expression in disease are due to a true change in the magnitude of gene expression or are caused by a change in the phase or period of the circadian clock. Establishing whether a change in phase or period has occurred requires near continual monitoring of clock cycling over several days, a process which is problematic in primary cells due to the need to minimise *in vitro* manipulation to preserve *in vivo* characteristics. However, assessing clock gene expression at more than one timepoint during the circadian cycle will provide more comprehensive insight into the nature of a change in clock gene expression.

Conclusions and Future Directions

There is compelling evidence that the chondrocyte circadian clock is altered in OA. . Changes in the type of clock regulating cues chondrocytes are exposed to and changes in the responsiveness of chondrocytes to clock regulating signals may both contribute to driving the change in the clock in OA.

There is consensus that *BMAL1* levels are reduced in OA chondrocytes. Whilst studies in cartilage-specific *Bmal1* knockout animals clearly show that complete loss of *Bmal1* in chondrocytes leads to a loss of cartilage integrity, the effects of a partial reduction in *BMAL1* levels are less clear. Further studies exploring the effects of long-term, rather than transient, reductions in *BMAL1* levels in human chondrocytes are required.

A surprising finding from animal studies is that detrimental effects of BMAL1 knockout on articular cartilage are only apparent with cartilage-specific, not global knockout and are restricted to the knee not the hip joint. Determining whether the circadian clock is altered in other joint tissues in OA and how clocks in different joint tissues interact would aid in understanding how changes to the chondrocyte clock contribute to disease.

Circadian clocks are attuned to environmental change. Understanding how factors such as exercise and meal timing impact clocks in tissues such as cartilage, may provide an opportunity for more targeted lifestyle-based strategies to aid in preventing or slowing the progression of OA.

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References

1. Albrecht U. Timing to Perfection: The Biology of Central and Peripheral Circadian Clocks. Neuron. 2012;74(2):246-60.
2. Ouyang Y, Andersson C, Kondo T, SS G, CH J. Resonating Circadian Clocks Enhance Fitness in Cyanobacteria. Proc Nat Acad Sci. 1998;95(15):8660-4.
3. Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M, Bittman EL. Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. J Neuroscience. 1987;7(6):1626-38.
4. Chang J, Garva R, Pickard A, Yeung C-YC, Mallikarjun V, Swift J, et al. Circadian control of the secretory pathway maintains collagen homeostasis. Nature Cell Biol. 2020;22(1):74-86.
5. Brown SA. Circadian clock-mediated control of stem cell division and differentiation: beyond night and day. Development. 2014;141(16):3105-11.
6. Chatterjee S, Nam D, Guo B, Kim JM, Winnier GE, Lee J, et al. Brain and muscle Arnt-like 1 is a key regulator of myogenesis. J Cell Sci. 2013;126(10):2213-24.
7. Malik A, Kondratov RV, Jamasbi RJ, Geusz ME. Circadian Clock Genes Are Essential for Normal Adult Neurogenesis, Differentiation, and Fate Determination. Plos One. 2015;10(10).
8. Yu X, Rollins D, Ruhn KA, Stubblefield JJ, Green CB, Kashiwada M, et al. TH17 cell differentiation is regulated by the circadian clock. Science. 2013;342(6159):727-30.
9. Farshadi E, Yan J, Leclere P, Goldbeter A, Chaves I, van der Horst GTJ. The positive circadian regulators CLOCK and BMAL1 control G2/M cell cycle transition through Cyclin B1 Cell Cycle. 2019;18(1):16-33.
10. Khapre RV, Samsa WE, Kondratov RV. Circadian regulation of cell cycle: Molecular connections between aging and the circadian clock. Ann Med. 2010;42(6):404-15.
11. Liu C, Li S, Liu T, Borjigin J, Lin JD. Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. Nature. 2007;447:477.
12. Sancar G, Brunner M. Circadian clocks and energy metabolism. Cell Mol Life Sci. 2014;71(14):2667-80.
13. Ehlers A, Xie W, Agapov E, Brown S, Steinberg D, Tidwell R, et al. BMAL1 links the circadian clock to viral airway pathology and asthma phenotypes. Mucosal Immunology. 2018;11(1):97-111.
14. El-Athman R, Relogio A. Escaping Circadian Regulation: An Emerging Hallmark of Cancer? Cell Systems. 2018;6(3):266-7.
15. Masri S, Sassone-Corsi P. The emerging link between cancer, metabolism, and circadian rhythms. Nature Medicine. 2018;24(12):1795-803.
16. Gibbs JE, Ray DW. The role of the circadian clock in rheumatoid arthritis. Arthritis Res Therapy. 2013;15(1):205.
17. Dudek M, Gossan N, Yang N, Im HJ, Ruckshanthi JPD, Yoshitane H, et al. The chondrocyte clock gene Bmal1 controls cartilage homeostasis and integrity. J Clin Invest. 2016;126(1):365-76.
18. Snelling SJ, Forster A, Mukherjee S, Price AJ, Poulsen RC. The chondrocyte-intrinsic circadian clock is disrupted in human osteoarthritis. Chronobiology Int. 2016;33(5):574-9.
19. Yang W, Kang XM, Liu JL, Li HX, Ma ZM, Jin XX, et al. Clock Gene Bmal1 Modulates Human Cartilage Gene Expression by Crosstalk With Sirt1. Endocrinology. 2016;157(8):3096-107.

20. Goldring MB. Articular cartilage degradation in osteoarthritis. HSS J. 2012;8:7-9.
21. Zheng B, Albrecht U, Kaasik K, Sage M, Lu W, Vaishnav S, et al. Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. Cell. 2001;105(5):683-94.
22. Van Der Horst GTJ, Muijtjens M, Kobayashi K, Takano R, Kanno SI, Takao M, et al. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature. 1999;398(6728):627-30.
23. Hogenesch JB, Gu Y-Z, Jain S, Bradfield CA. The basic-helix–loop–helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. Proc Nat Acad Sci. 1998;95(10):5474.
24. Kume K, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X, et al. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell. 1999;98(2):193-205.
25. Preitner N, Damiola F, Luis Lopez M, Zakany J, Duboule D, Albrecht U, et al. The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell. 2002;110(2):251-60.
26. Schmutz I, Ripperger JA, Baeriswyl-Aebischer S, Albrecht U. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. Genes and Development. 2010;24(4):345-57.
27. Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves I, Zheng BH, et al. Interacting molecular loops in the mammalian circadian clock. Science. 2000;288(5468):1013-9.
28. Kamagata M, Ikeda Y, Sasaki H, Hattori Y, Yasuda S, Iwami S, et al. Potent synchronization of peripheral circadian clocks by glucocorticoid injections in PER2::LUC-Clock/Clock mice. Chronobiology Int. 2017;34(8):1067-82.
29. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev. 2000;14(23):2950-61.
30. Stokkan K-A, Yamazaki S, Tei H, Sakaki Y, Menaker M. Entrainment of the Circadian Clock in the Liver by Feeding. Science. 2001;291(5503):490.
31. Kanbe K, Inoue K, Xiang C, Chen Q. Identification of clock as a mechanosensitive gene by large-scale DNA microarray analysis: downregulation in osteoarthritic cartilage. Mod Rheumatol. 2006;16(3):131-6.
32. Yang N, Williams J, Pekovic-Vaughan V, Wang PB, Olabi S, McConnell J, et al. Cellular mechano-environment regulates the mammary circadian clock. Nature Comm. 2017;8.
33. Gossan N, Zeef L, Hensman J, Hughes A, Bateman JF, Rowley L, et al. The Circadian Clock in Murine Chondrocytes Regulates Genes Controlling Key Aspects of Cartilage Homeostasis. Arthritis Rheum. 2013;65(9):2334-45.
34. Takarada T, Kodama A, Hotta S, Meida M, Shimba S, Hinoi E, et al. Clock genes influence gene expression in growth plate and endochondral ossification in mice. J Biol Chem. 2012;287(43):36081-95.
35. Yu SL, Tang QM, Xie MR, Zhou X, Long YL, Xie YL, et al. Circadian BMAL1 regulates mandibular condyle development by hedgehog pathway. Cell Proliferation.11.
36. Nguyen Quynh L, Nguyen Thanh B, Takarada T, Takarada-Iemata M, Hinoi E, Yoneda Y. A Negative Correlation Between Per1 and Sox6 Expression During Chondrogenic Differentiation in Pre-chondrocytic ATDC5 Cells. J Pharmacol Sci. 2013;122(4):318-25.

37. Lipton JO, Yuan ED, Boyle LM, Ebrahimi-Fakhari D, Kwiatkowski E, Nathan A, et al. The Circadian Protein BMAL1 Regulates Translation in Response to S6K1-Mediated Phosphorylation. Cell. 2015;161(5):1138-51.
38. Wu R, Dang FB, Li P, Wang PF, Xu Q, Liu ZS, et al. The Circadian Protein Period2 Suppresses mTORC1 Activity via Recruiting Tsc1 to mTORC1 Complex. Cell Metabolism. 2019;29(3):653-+.
39. Walton ZE, Altman BJ, Brooks RC, Dang CV. Circadian Clock's Cancer Connections. Ann Rev Cancer Bio. 2018;2(1):133-53.
40. Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, et al. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science. 1994;264(5159):719-25.
41. Kc R, Li X, Voigt RM, Ellman MB, Summa KC, Vitaterna MH, et al. Environmental Disruption of Circadian Rhythm Predisposes Mice to Osteoarthritis-Like Changes in Knee Joint. J Cell Physiology. 2015;230(9):2174-83.
42. Meng QJ, Logunova L, Maywood ES, Gallego M, Lebiecki J, Brown TM, et al. Setting clock speed in mammals: the CK1 epsilon tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. Neuron. 2008;58(1):78-88.
43. Kc R, Li X, Forsyth CB, Voigt RM, Summa KC, Vitaterna MH, et al. Osteoarthritis-like pathologic changes in the knee joint induced by environmental disruption of circadian rhythms is potentiated by a high-fat diet. Sci Rep. 2015;5:16896.
44. Zhou M, Yang S, Guo Y, Wang D, Qiu W, Wang B, et al. Shift work and the risk of knee osteoarthritis among Chinese workers: a retrospective cohort study. Scandinavian J Work, Environment Health. 2019.
45. Soul J, Dunn SL, Anand S, Serracino-Inglott F, Schwartz JM, Boot-Handford RP, et al. Stratification of knee osteoarthritis: two major patient subgroups identified by genome-wide expression analysis of articular cartilage. Ann Rheum Dis. 2018;77(3).
46. Fisch KM, Gamini R, Alvarez-Garcia O, Akagi R, Saito M, Muramatsu Y, et al. Identification of transcription factors responsible for dysregulated networks in human osteoarthritis cartilage by global gene expression analysis. Osteoarthritis Cartilage. 2018;26(11):1531-8.
47. He D, An YX, Li YH, Wang J, Wu GY, Chen L, et al. RNA sequencing reveals target genes of temporomandibular joint osteoarthritis in rats after the treatment of low-intensity pulsed ultrasound. Gene. 2018;672:126-36.
48. Kalev-Zylinska ML, Hearn JI, Rong J, Zhu M, Munro J, Cornish J, et al. Altered N-methyl D-aspartate receptor subunit expression causes changes to the circadian clock and cell phenotype in osteoarthritic chondrocytes. Osteoarthritis Cartilage. 2018;26(11):1518-30.
49. Akagi R, Akatsu Y, Fisch KM, Alvarez-Garcia O, Teramura T, Muramatsu Y, et al. Dysregulated circadian rhythm pathway in human osteoarthritis: NR1D1 and BMAL1 suppression alters TGF-β signaling in chondrocytes. Osteoarthritis Cartilage. 2017;25(6):943-51.
50. Balsalobre A, Damiola F, Schibler U. A Serum Shock Induces Circadian Gene Expression in Mammalian Tissue Culture Cells. Cell. 1998;93(6):929-37.
51. Rong J, Zhu M, Munro J, Cornish J, McCarthy G, Dalbeth N, et al. Altered expression of the core circadian clock component PERIOD2 contributes to osteoarthritis-like changes in chondrocyte activity. Chronobiology Int. 2018;36(3):319-31.

52. Guo B, Yang N, Borysiewicz E, Dudek M, Williams JL, Li J, et al. Catabolic cytokines disrupt the circadian clock and the expression of clock-controlled genes in cartilage via an NF kappa B-dependent pathway. Osteoarthritis Cartilage. 2015;23(11):1981-8.
53. Yang X, Sun X, Wu J, Ma J, Si P, Yin L, et al. Regulation of the SIRT1 signaling pathway in NMDA-induced Excitotoxicity. Toxicology Lett. 2020;322:66-76.
54. Shen W, Zhang C, Zhang G. Nuclear factor kappaB activation is mediated by NMDA and non-NMDA receptor and L-type voltage-gated Ca(2+) channel following severe global ischemia in rat hippocampus. Brain Res. 2002;933(1):23-30.
55. Lipsky RH, Xu K, Zhu D, Kelly C, Terhakopian A, Novelli A, et al. Nuclear factor kappaB is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. J Neurochem. 2001;78(2):254-64.
56. Kauppinen A, Suuronen T, Ojala J, Kaarniranta K, Salminen A. Antagonistic crosstalk between NF-kappaB and SIRT1 in the regulation of inflammation and metabolic disorders. Cell Signal. 2013;25(10):1939-48.
57. Zhang L, Xiao X, Arnold PR, Li XC. Transcriptional and epigenetic regulation of immune tolerance: roles of the NF-κB family members. CellMol Immunology. 2019;16(4):315-23.
58. Bosch-Presegue L, Vaquero A. Sirtuin-dependent epigenetic regulation in the maintenance of genome integrity. Febs Journal. 2015;282(9):1745-67.
59. Bunger MK, Walisser JA, Sullivan R, Manley PA, Moran SM, Kalscheur VL, et al. Progressive arthropathy in mice with a targeted disruption of the Mop3/Bmal-1 locus. Genesis. 2005;41(3):122-32.
60. Ma Z, Jin X, Qian Z, Li F, Xu M, Zhang Y, et al. Deletion of Clock Gene Bmal1 Impaired the Chondrocyte Function due to Disruption of the HIF1alpha-VEGF Signaling Pathway. Cell Cycle. 2019.
61. Qing L, Lei P, Liu H, Xie J, Wang L, Wen T, et al. Expression of hypoxia-inducible factor-1alpha in synovial fluid and articular cartilage is associated with disease severity in knee osteoarthritis. Exp therap med. 2017;13(1):63-8.
62. Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Ohba S, et al. Transcriptional regulation of endochondral ossification by HIF-2α during skeletal growth and osteoarthritis development. Nature Med 2010;16(6):678-86.
63. Asik MD, Gursoy S, Akkaya M, Kozaci LD, Dogan M, Bozkurt M. Microarray analysis of cartilage: comparison between damaged and non-weight-bearing healthy cartilage. Connective Tiss Res.9.
64. Bekki H, Duffy T, Okubo N, Olmer M, Alvarez-Garcia O, Lamia K, et al. Suppression of circadian clock protein cryptochrome 2 promotes osteoarthritis. Osteoarthritis Cartilage. 2020;28(7):966-76.
65. Yuan G, Xu L, Cai T, Hua B, Sun N, Yan Z, et al. Clock mutant promotes osteoarthritis by inhibiting the acetylation of NF kappa B. Osteoarthritis Cartilage. 2019;27(6):922-31.
66. Spengler ML, Kuropatwinski KK, Comas M, Gasparian AV, Fedtsova N, Gleiberman AS, et al. Core circadian protein CLOCK is a positive regulator of NF-κB-mediated transcription. Proc Nat Acad Sci. 2012;109(37):E2457-E65.
67. Fu LN, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. Cell. 2005;122(5):803-15.
68. Andrews JL, Zhang X, McCarthy JJ, McDearmon EL, Hornberger TA, Russell B, et al. CLOCK and BMAL1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. 2010;107(44):19090-5.

69. Becker T, Tohidast-Akrad M, Humpeler S, Gerlag DM, Kiener HP, Zenz P, et al. Clock gene expression in different synovial cells of patients with rheumatoid arthritis and osteoarthritis. Acta Histochemica. 2014;116(7):1199-207.
70. Haas S, Straub RH. Disruption of rhythms of molecular clocks in primary synovial fibroblasts of patients with osteoarthritis and rheumatoid arthritis, role of IL-1 beta/TNF. Arthritis Rese Therapy. 2012;14(3).
71. Early JO, Menon D, Wyse CA, Cervantes-Silva MP, Zaslona Z, Carroll RG, et al. Circadian clock protein BMAL1 regulates IL-1β in macrophages via NRF2. Proc Nat Acad Sci. 2018.
72. Adrover JM, Aroca-Crevillen A, Crainiciuc G, Ostos F, Rojas-Vega Y, Rubio-Ponce A, et al. Programmed 'disarming' of the neutrophil proteome reduces the magnitude of inflammation. Nature Immun. 2020:30.
73. Hand LE, Hopwood TW, Dickson SH, Walker AL, Loudon ASI, Ray DW, et al. The circadian clock regulates inflammatory arthritis. Faseb J. 2016;30(11):3759-70.
74. Takarada T, Xu C, Ochi H, Nakazato R, Yamada D, Nakamura S, et al. Bone Resorption Is Regulated by Circadian Clock in Osteoblasts. J Bone Miner Res. 2017;32(4):872-81.
75. Kouri VP, Olkkonen J, Kaivosoja E, Ainola M, Juhila J, Hovatta I, et al. Circadian Timekeeping Is Disturbed in Rheumatoid Arthritis at Molecular Level. Plos One. 2013;8(1).
76. Tamai TK, Nakane Y, Ota W, Kobayashi A, Ishiguro M, Kadofusa N, et al. Identification of circadian clock modulators from existing drugs. Embo Molecular Medicine. 2018;10(5).
77. Cheeseman JF, Winnebeck EC, Millar CD, Kirkland LS, Sleigh J, Goodwin M, et al. General anesthesia alters time perception by phase shifting the circadian clock. Proc Nat Acad Sci. 2012;109(18):7061-6.
78. Mavroudis PD, Scheff JD, Calvano SE, Lowry SF, Androulakis IP. Entrainment of peripheral clock genes by cortisol. Physiological Genomics. 2012;44(11):607-21.
79. Sulli G, Manoogian ENC, Taub PR, Panda S. Training the Circadian Clock, Clocking the Drugs, and Drugging the Clock to Prevent, Manage, and Treat Chronic Diseases. Trends Pharmacol Sci. 2018;39(9):812-27.
80. Okubo N, Minami Y, Fujiwara H, Umemura Y, Tsuchiya Y, Shirai T, et al. Prolonged Bioluminescence Monitoring in Mouse Ex Vivo Bone Culture Revealed Persistent Circadian Rhythms in Articular Cartilages and Growth Plates. PloS one. 2013;8:e78306.

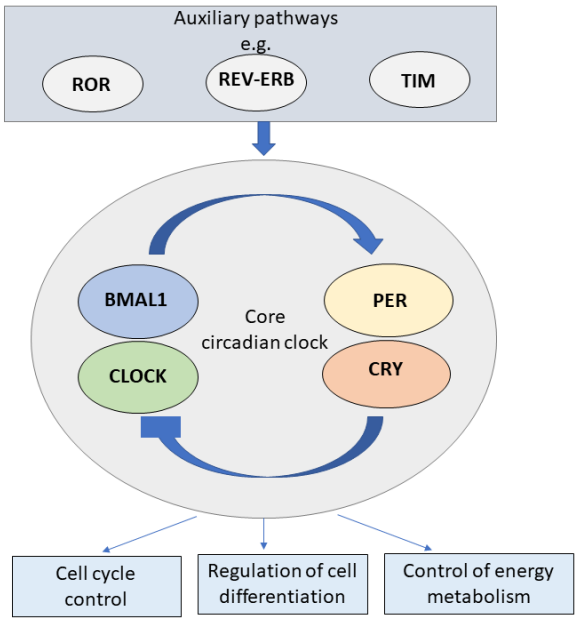

Figure 1 The molecular circuitry of the circadian clock. In nucleated cells, the circadian clock consists of interconnecting transcription/translation feedback loops. At its core, the clock consists of a repressing arm made up of the proteins PER (Period) and CRY (cryptochrome) and an activating arm made up of the proteins BMAL1 and CLOCK. BMAL1 and CLOCK form a dimer which promotes the transcription of PER and CRY. As PER and CRY protein levels build up, these too form a dimer which feeds back to inhibit the activity of BMAL1:CLOCK. Auxiliary loops (such as those involving REV-ERBs, RORs and TIM) also act on BMAL1:CLOCK/PER:CRY cycle resulting in the creation of a self-sustaining oscillating molecular circuit. The circadian clock regulates fundamental cell processes such as the cell cycle, cell differentiation and energy metabolism.

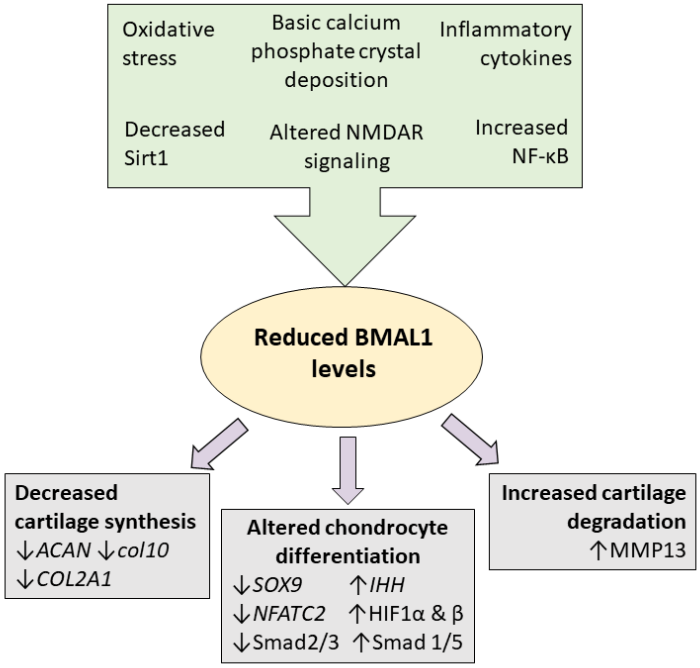

Figure 2 The causes and consequences of reduced BMAL1 levels in chondrocytes in OA. Changes in the joint environment in OA such as increased levels of inflammatory cytokines, oxidative stress and basic calcium phosphate crystal deposition have been implicated as possible causes of the change in the chondrocyte circadian clock in disease. Mechanistically, decreased sirtuin 1 levels, increased NF-κB activity and altered NMDA receptor signalling may combine to drive the reduction in BMAL1 expression in OA chondrocytes. Reduced BMAL1 levels in chondrocytes have been linked with altered expression of chondrocyte differentiation markers, altered TGF-β signalling activity, decreased synthesis of extracellular matrix (ECM) components and increased expression of the cartilage degrading enzyme MMP13.

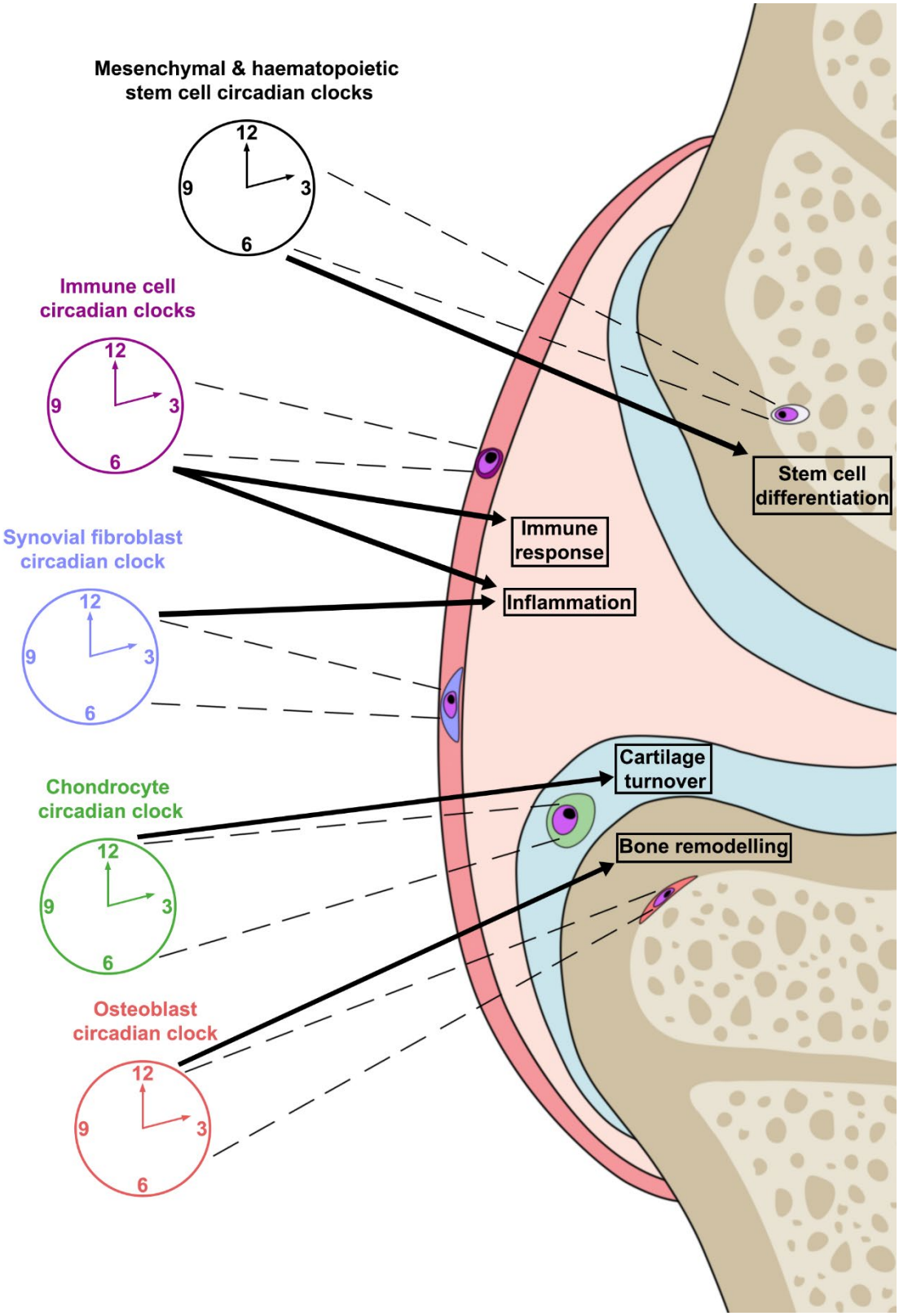

Figure 3 The involvement of circadian clocks in the maintenance of joint tissues. Circadian clocks are present within cells in all joint tissues. They contribute to the regulation of tissue turnover and the control of cell differentiation, immune responses and inflammation. Although the chondrocyte circadian clock is known to be altered in OA, it is currently unknown whether clocks in other joint tissues are also altered in disease.