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REVIEW ARTICLE

Running on time: the role of circadian clocks in the musculoskeletal system

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The night and day cycle governs the circadian (24 hourly) rhythm of activity and rest in animals and humans. This is reflected in daily changes of the global gene expression pattern and metabolism, but also in the local physiology of various tissues. A central clock in the brain co-ordinates the rhythmic locomotion behaviour, as well as synchronizing various local oscillators, such as those found in the musculoskeletal system. It has become increasingly recognized that the internal molecular clocks in cells allow a tissue to anticipate the rhythmic changes in their local environment and the specific demands of that tissue. Consequently, the majority of the rhythmic clock controlled genes and pathways are tissue specific. The concept of the tissue-specific function of circadian clocks is further supported by the diverse musculoskeletal phenotypes in mice with deletions or mutations of various core clock components, ranging from increased bone mass, dwarfism, arthropathy, reduced muscle strength and tendon calcification. The present review summarizes the current understanding of the circadian clocks in muscle, bone, cartilage and tendon tissues, with particular focus on the evidence of circadian rhythms in tissue physiology, their entrainment mechanisms and disease links, and the tissue-specific clock target genes/pathways. Research in this area holds strong potential to advance our understanding of how circadian rhythms control the health and disease of the musculoskeletal tissues, which has major implications in diseases associated with advancing age. It could also have potential implications in sports performance and sports medicine.

Key words: cartilage entrainment, circadian rhythm, homoeostasis, muscle.

INTRODUCTION

The mammalian circadian system is organized in a hierarchical manner. The central clock [SCN (suprachiasmatic nuclei)] in the hypothalamus controls rest/activity rhythms, and is entrained on a daily basis by the LD (light–dark) cycle, whereas clocks in peripheral tissues were thought to be slave oscillators, subject to the neuronal or hormonal control by the SCN. However, the emerging paradigm is that SCN synchronizes peripheral tissue clocks, but is not an absolute master [1]. The autonomous peripheral clocks contain identical clock genes/proteins and can be entrained by external time cues, such as feeding/fasting and physical exercise, independently of the SCN and the LD cycle. This means that the LD cycle can be overridden in peripheral tissues by other zeitgebers (time cues), allowing animals greater flexibility in responding to demands of the environment [2–4].

In mammals the circadian clock relies on a transcriptional/translational feedback loop consisting of the transcriptional activators Bmal1 (brain and muscle ARNT-like 1)/Clock and the repressor complex Per1–Per2 (period circadian clock 1/2) and Cry1/Cry2 (cryptochrome circadian clock 1/2). The Bmal1/Clock complex activates the expression of the *Per* and *Cry* genes. The Per/Cry complex periodically feeds back to suppress the activity of Bmal1/Clock, inhibiting their own transcription. Nuclear hormone receptors *Rev-Erb* (repressor) and *Ror* (activator) form an additional stabilizing loop with *Bmal1* to fine-tune the precision of the clock (Figure 1). The interaction of these core components and modulation by additional regulators results in an approximately 24-h period. In addition to the autoregulation, the core clock transcription factors rhythmically control the expression of other CCGs (clock-controlled genes) through specific regulatory elements [E-box, D-box and RORE (ROR/REV-ERB-binding element)] in their promoters [5]. Despite the same core clock mechanism operating in different tissues, the circadian transcriptome of peripheral tissues is strikingly different with only a small overlap [6]. Many of the oscillating genes are key tissue-specific transcription factors, characteristic structural proteins or are involved in metabolic pathways fundamental for that particular tissue [7–10]. Conditional ablation of the peripheral clocks in liver, pancreas, adipose tissue and skin revealed profound and diverse disorders and pathologies [11–13], highlighting the importance of the local clocks in tissue physiology and diseases. In this regard, the musculoskeletal system is particularly relevant to the daily rest/activity cycles. Coupling of the local gene expression and physiology to the daily loading/unloading and related metabolic changes could be an important part of the musculoskeletal biology. Here we review the current developments in the field of circadian biology with respect to the musculoskeletal system and the

Abbreviations: ADAM, a disintegrin and metalloproteinase; Adrb2, β2-adrenergic receptor; Bmal1, brain and muscle ARNT-like 1; BMP, bone morphogenetic protein; BMSC, bone marrow-derived stem cell; CCG, clock-controlled gene; Creb/CREB, cAMP-responsive-element-binding protein; Cry, cryptochrome circadian clock; ECM, extracellular matrix; Fbxo32, F-box protein 32; Gilz, glucocorticoid-induced leucine zipper; Grem2, gremlin 2; HBM, high bone mass; Ihh, Indian hedgehog; KO, knockout; LD, light–dark; Mmp, matrix metalloproteinase; Mrf4, muscle-specific regulatory factor 4; MSC, mesenchymal stem cell; Myf, myogenic factor; MyoD, myogenic differentiation; Noc, nocturnin; Nr1d1, nuclear receptor subfamily 1, group D, member 1; Per, period circadian clock; Pgc1, Pparg co-activator 1; Pparg, peroxisome-proliferator-activated receptor γ; PTH, parathyroid hormone; RORE, ROR/REV-ERB-binding element; SCN, suprachiasmatic nuclei; Sirt1, sirtuin 1; TGF-β, transforming growth factor-β; WT, wild-type.

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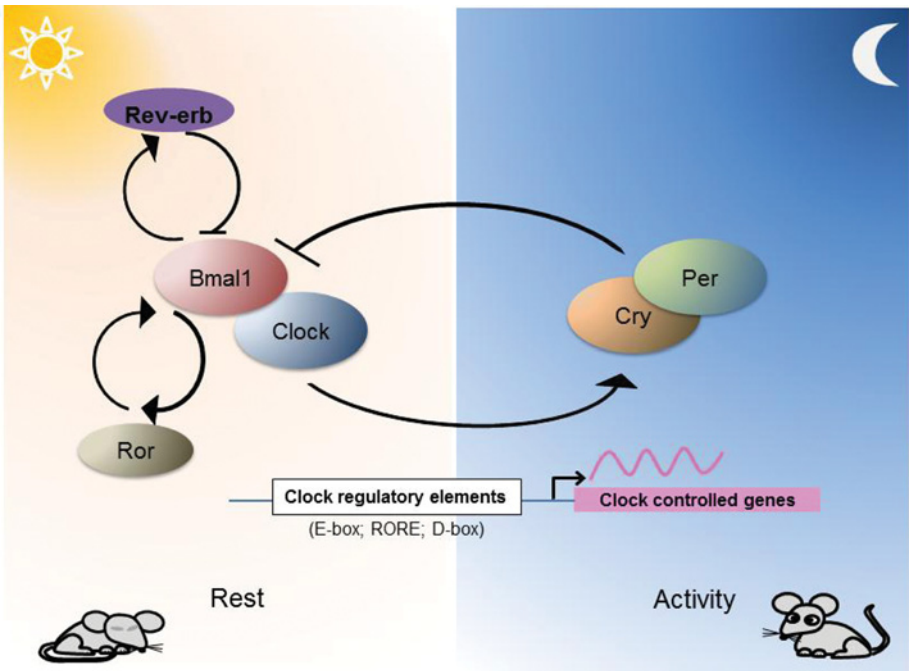

Figure 1 A simplified model of the core clock components of the mammalian circadian oscillator

Bmal1/Clock complex activates transcription of genes containing E-box sequences in their promoters. Among these are *Per1/2* and *Cry1/2*, which following dimerization can inhibit the activity of the Bmal1/Clock complex. Rev-Erb and Ror provide an additional stabilizing loop to fine-tune the expression of *Bmal1*. In addition to E-boxes, other clock regulatory elements (such as ROREs and D-boxes) are commonly found in the promoters of CCGs.

implications of the circadian rhythms in tissue homoeostasis and disease.

MUSCLE CLOCK

Skeletal muscle is the most abundant tissue in the human body comprising approximately 40 % of body weight. Accompanied by bones, cartilage and tendons it provides the means of locomotion, but also plays an important role in metabolic homoeostasis of the whole organism. The muscle is a malleable organ with the ability to adapt its mass to the different pathophysiological conditions through regulation of pathways responsible for protein and cellular turnover. External stimuli, such as exercise and nutrients, can increase the rate of muscle protein synthesis and result in hypertrophy (increase in muscle mass). Conversely starvation, immobilization and aging increase the rate of protein degradation and result in muscle atrophy (reduction in mass) [14].

The daily pace of light and dark, rest and activity, is reflected in the physiology of the muscle tissue. There has been ample evidence for the time of day-dependent variation in muscle physiology and neuromuscular performance [15–18]. Early evidence for the diurnal expression of clock genes in skeletal muscle came from studies in humans [19]. That study also showed that exercise up-regulated the expression of *Bmal1*, *Per1* and *Per2*. However, the first comprehensive studies of the muscle circadian transcriptome were performed by Miller et al. [20] and McCarthy et al. in 2007 [7]. More than 200 rhythmic genes were identified in skeletal muscle. Interestingly, both studies reported a large cluster of genes to be peaking at ZT18 (zeitgeber time 18), corresponding to the middle of the active phase in mice. These findings further reinforce the notion that locomotor activity may play a role in entraining the rhythmic expression of genes in skeletal muscle. The largest Gene Ontology clusters consisted of genes involved in biosynthesis and metabolism (18 %) and regulation of gene transcription (17 %). The specificity of the muscle circadian transcriptome was underscored by the presence of skeletal muscle characteristic genes such as *MyoD* (myogenic differentiation), *Ucp3* (uncoupling protein 3), *Fbxo32* (F-box protein 32; Atrogin1), *Pdk4* (pyruvate dehydrogenase kinase 4) and *Myh1* (myosin heavy chain I). Comparison with arrhythmic *Clock*Δ*19* mutant mice showed that a large proportion of the muscle circadian genes either lost their rhythmicity or shifted their phase. The majority was found to be down-regulated, including genes involved in muscle contraction (such as actin, dystrophin, titin and myosin heavy chain IIb), protein synthesis and energy metabolism [7].

Consistent with the key pathways controlled by the circadian clock, various muscle phenotypes were reported in *Clock*- or *Bmal1*-deficient mice (summarized in Table 1). *Bmal1*$^{-/-}$ mice exhibit reduced muscle mass accompanied by decreased diameter and number of muscle fibres [21,22]. Myoblasts isolated from *Bmal1*$^{-/-}$ mice exhibited impaired formation of organized myotubes and a considerably lower percentage of MHC-positive myonuclei. Lower expression levels of myogenic markers such as *Myf5* (myogenic factor 5), *Mrf4* (muscle-specific regulatory factor 4/*Myf6*), *MyoD* and *Myf4* (myogenin), as well as MHC3, may partially explain the phenotype. Further, expression of several genes involved in canonical Wnt signalling was markedly reduced in isolated myotubes from *Bmal1*$^{-/-}$ mice, including *Wnt10a*, *Dvl2* (dishevelled segment polarity protein 2), β-catenin and *Tcf3* (transcription factor 3). The KO (knockout) cells also remained unresponsive to Wnt3a stimulation *in vitro* as evidenced by the lack of nuclear accumulation of β-catenin after treatment. Overexpression of *MyoD* and *Wnt3a* in the *Bmal1*$^{-/-}$ myoblasts

Table 1 Summary of musculoskeletal phenotypes found in mice deficient in various core circadian clock components

	Tissue phenotype			
Circadian mutant	Muscle	Bone	Cartilage	Tendon
Cry KO or mutants	Not reported	High bone mass [41,78] Increase in osteoblast number [41] Normal osteoblast number, but decreased osteoclast activity [78]	Not reported	Not reported
Per KO or mutants	Not reported	High bone mass and increase in osteoblast number [41,78]	Not reported	Not reported
$Per^{-/-}/Cry^{-/-}$	Not reported	Normal phenotype [78]	Not reported	Not reported
$Bmal1^{-/-}$	Reduced diameter and number of muscle fibres at 40 weeks [21,22] Disorganized myofilaments [24] Abnormal mitochondria [24]	Thicker bones [41] Increase in osteoblast number [41]	Calcification of ribcage cartilage [62] Affected growth plate (shorter bones) [62] Decreased expression of *Col Xlhh* and *Alp* in the growth plate [61]	Ectopic calcification [62]
Rev-Erb$\alpha^{-/-}$	Impaired myogenic differentiation [22] Misalignment of Z lines [29] Abnormal mitochondria [29] Impaired muscle regeneration [29] Increased autophagy [29]	Not reported	Not reported	Not reported
Clock mutant	Disorganized myofilaments [24] Abnormal mitochondria [24]	Not reported	Not reported	Ectopic calcification [77]

resulted in improved myogenic differentiation, but failed to fully rescue the myotube formation defect, indicating involvement of other Bmal1-controlled pathways in the process [22].

Both *Clock* mutant and $Bmal1^{-/-}$ mice exhibit decreased muscle force and disorganized arrangement of the thin and thick filaments. Interestingly, a similar phenotype was found in $MyoD^{-/-}$ mice. *MyoD* is a muscle specific basic helix–loop–helix transcription factor which, along with other myogenic regulatory factors such as *Myf5* and *Mrf4*, controls the expression of a myriad of genes during myogenesis [23]. *MyoD* was one of the rhythmic genes identified in the muscle which is a direct target of Clock/Bmal1 through a functional E-box in its promoter [24,25]. Also of interest is the muscle-specific rescue model of the global $Bmal1^{-/-}$ mice. Although the activity level and longevity of the global mutants were restored by muscle Bmal1 overexpression, the behavioural rhythmicity remains abolished, and the tendon calcification phenotype is still present. These findings further support the concept of tissue-specific functions of clocks in driving a diverse range of physiology [26].

Clock mutant and *Bmal1*-KO mice were found to have a dramatically smaller number and volume of mitochondria in muscles with aberrant morphology. The mitochondrial abnormalities are possibly due to decreased expression of *Pgc1a* [Pparg (peroxisome-proliferator-activated receptor γ) co-activator 1α] and *Pgc1b* [7,27]. *Pgc1* genes are a family of co-activators that activate the expression of mitochondrial genes and are major regulators that determine the type of muscle fibre [28]. Interestingly, the *Rev-Erb-α* [*Nr1d1* (nuclear receptor subfamily 1, group D, member 1)]-KO mouse also shows decreased mitochondrial content and lower respiratory chain function in isolated organelles, and lower muscle endurance. The mitochondria also displayed abnormal morphology and the muscles showed a slight misalignment of Z lines and vacuolated fibres. The ATP, NAD^+ and NADH concentrations were lower in skeletal muscle from $Nr1d1^{-/-}$ mice, revealing dysregulation of muscle energy metabolism. Notably, the expression of the *Sirt1* (sirtuin 1) gene was reduced in the $Nr1d1^{-/-}$ muscle, associated with heavy acetylation (and activation) of Pgc1a [29]. SIRT1 is an NAD^+-dependent deacetylase involved in the control of muscle mitochondrial function and Pgc1a is one of its targets [30].

Feeding/fasting is considered as one of the zeitgebers (time cues) to synchronize the skeletal muscle clock. It was found that although the core clock components are unaffected in mice fasted for 24 h, the circadian rhythm of *MyoD* and *Myf4* was disrupted and their expression was reduced. Furthermore, the amplitude of rhythmic expression of *MuRF1* [Trim63 (tripartite motif-containing 63)] and *Fbxo32* genes, E3 ubiquitin ligases involved in muscle wasting, was increased [31]. This suggests that, at least in the muscle, it is not the food consumption, but rather the fasting which exhibits an inhibitory effect on muscle specific rhythmic genes, leading to resetting of the muscle clock. Another candidate zeitgeber is locomotor activity. Scheduled exercise entrains the muscle peripheral clock, while leaving the SCN cycle unaffected [32–34]. Further research is clearly needed to define the entrainment mechanisms by physical activity.

BONE CLOCK

Bones provide the scaffold for skeletal muscles and bear the weight of the organism. They also play an important role in mineral balance as a reservoir of calcium and phosphorus. Bone homoeostasis depends on two opposing processes, resorption and synthesis. Normal bone remodelling requires strict temporal control of these two processes to guarantee maintenance of the bone mass and structural quality [35]. These processes are regulated by two distinct types of cells, the osteoclasts and osteoblasts. First, there is a rapid phase of resorption of old mineralized bone by osteoclasts which is followed by a relatively slow phase of new bone formation by osteoblasts [36,37]. Diurnal variations in serum markers of bone metabolism, such as calcium, C-telopeptide, osteocalcin, skeletal ALP (alkaline phosphatase), PTH (parathyroid hormone), tartrate-resistant acid phosphatase and calcitonin have long been recognized [38–40].

Characterization of bone diurnal transcriptome from mice kept in 12:12 h LD cycle revealed that 26 % of genes expressed in calvarial bone exhibit near 24-h oscillations. Among the rhythmic

genes found were core clock genes as well as genes involved in BMP (bone morphogenetic protein) and FGF (fibroblast growth factor) signalling, Wnt signalling, multiple ADAM (a disintegrin and metalloproteinase) proteases and various pro-collagen isoforms, underscoring the scope of clock influence on bone physiology [8].

Studies of the skeletal phenotypes of various circadian gene KO mice (summarized in Table 1) established the circadian system as an important regulator of bone homoeostasis. Mice lacking the *Per1* gene or the PAS domain (Per/Arnt/Sim domain) of *Per2* show normal bone morphology up to 6 weeks of age. After this time increase in bone mass starts to be evident and progresses with age. Similar HBM (high bone mass) phenotype can be seen in *Per1*/ *Per2* and *Cry1*/*Cry2* double-KO mice. Biochemical and histomorphometric analyses found a significant increase in the number of osteoblasts in bones of these mutant mice, which correlated with increased mineral apposition rate and bone-formation rate [41]. Somewhat surprisingly, *Bmal1*-KO mice also exhibit increased bone-formation rate, mineral apposition rate and osteoblast numbers similar to *Per*- and *Cry*-KO mice. These phenotypes point to a mechanism in which the circadian clock negatively regulates bone formation by inhibition of osteoblast proliferation.

Another mechanism through which the circadian clock could affect bone formation is the differentiation of osteoblasts from BMSCs (bone marrow-derived stem cells). *Noc* (nocturnin), a deadenylase that regulates gene expression post-transcriptionally [42], has been shown to be a direct rhythmic target of Bmal1–Clock heterodimer [43]. *Noc* is down-regulated during differentiation of osteoblasts *in vitro* and its overexpression inhibits osteoblastogenesis. *Noc*-KO mice display a HBM phenotype and reduced numbers of adipocytes in the bone marrow, suggesting a shift in BMSC differentiation favouring the osteoblastic route [44,45]. Pparg2 has a well-established role in MSC (mesenchymal stem cell) fate determination. Pparg2 activation favours MSC differentiation along the adipogenic lineage over osteogenic differentiation, and suppresses osteogenic signalling pathways such as BMP, TGF-β (transforming growth factor-β), Wnt, and osteoblast-specific transcription factors such as *Runx2* (runt-related transcription factor 2), *Sox9* (SRY-box 9) and *Sp7* (osterix) [46]. *In vivo* silencing of Pparg2 in bone marrow was found to result in reduction in intramarrow adiposity and increased trabecular bone formation [47]. Interestingly, Noc can interact with Pparg2 and promote its nuclear translocation [44]. Expression of *Pparg2* was significantly decreased in *Noc*-KO brown adipocytes [44]. Further *in vivo* studies are clearly warranted to test this interesting hypothesis.

Another potential mechanistic link between circadian disruption and bone phenotype could be *Gilz* (glucocorticoid-induced leucine zipper/*TSC22D3*), a glucocorticoid-responsive transcriptional regulator. *Gilz* displays a robust circadian rhythm in brown and white adipose tissue, liver, cartilage, tendon and calvarial bone [8,10]. Shi and colleagues found that *Gilz* expression can be induced by dexamethasone in 2T3 osteoblasts and 3T3-L1 pre-adipocytes. Gilz was shown to block transcription of *Pparg2* by competing with C/ebpα (CCAAT/enhancer-binding protein α) [48,49]. Overexpression of *Gilz* in MSCs *in vitro* leads to inhibition of adipogenesis and enhancement of osteogenesis [46,50].

The output signals from the SCN can be transmitted to the local bone clocks through β-adrenergic (sympathetic), glucocorticoid signalling and feeding/fasting [51]. It has been shown that leptin regulates the bone clock through sympathetic nervous system and Adrb2 (β2-adrenergic receptor) present on the osteoblasts, thus linking global metabolism and the bone peripheral clock. Adrb2 acts through Creb1 (cAMP-responsive-element-binding protein 1; a cell-cycle regulator) to stimulate the expression of clock genes such as *Bmal1* and *Clock* [36]. This implies that osteoblast activity is coordinated by both the external stimuli and the internal osteoblast clock to maintain the diurnal rhythms in bone formation and resorption, and consequently the maintenance of bone mass. In osteoclasts, glucocorticoids were shown to be a more potent time cue than sympathetic signalling. Circadian expression of genes in osteoclasts can be stimulated *in vitro* with dexamethasone but not with isoprenaline [52]. Dexamethasone was also shown to induce rhythmic bone resorption in an *in vitro* pit assay [52]. Adrenalectomy of mice reduces the amplitude of *Per1* and *Per2* genes in cancellous bone, and completely abolishes rhythmic expression of osteoclast specific genes *Ctsk* (cathepsin K) and *Nfatc1* (nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1). Glucocorticoid injection was able to restart the rhythmic expression of these two osteoclast markers *in vivo* [52]. Similarly to the muscle, the diurnal variations in the bone metabolism seem to be governed by timing of food intake and fasting, providing an additional entrainment mechanism [53,54].

CARTILAGE CLOCK

Cartilage in the growth plate or the end of long bones (articular cartilage) consists of abundant ECM (extracellular matrix), sparsely populated by chondrocytes. The growth plate is responsible for the longitudinal growth of bones whereas the articular cartilage provides a smooth, resilient surface for the movement of joints. Isolated from the vasculature and lacking innervations, the fine balance between anabolic and catabolic activities is critical to maintain cartilage tissue homoeostasis. Disruptions to this balance are implicit in the degeneration and destruction of the articular cartilage tissue, which is frequently seen in diseases such as osteoarthritis and rheumatoid arthritis [55].

Evidence for diurnal variations in cartilage metabolism has been well described. Based on measurements of phosphorus and calcium fractions from rat tibial growth plate it was shown that cartilage mineralization occurs at night [56]. Moreover, measurements of chondrocyte mitotic index and height of the growth plate showed that chondrocytes proliferate most actively in the early morning. The proliferation phase is followed by expansion of the growth plate, reaching peak height at noon in rats [57]. The peak height of growth plate coincides with the highest collagen matrix synthesis as evidenced by incorporation of [^{3}H]proline [58]. Diurnal variations in serum levels of COMP (cartilage oligomeric matrix protein), hialuronic acid, keratan sulphate, aggrecan, collagen type II and TGF-β have also been reported [59,60].

Deletion of the core clock gene *Bmal1* in mice provided genetic evidence for the role of the molecular clock in cartilage physiology. Although the *Bmal1*-KO mice are shorter than the WT (wild-type) and the overall weight of the skeleton is lower, *Bmal1* appears to have opposing effect on osteoblasts and chondrocytes. Closer inspection of the skeleton reveals that the KO mice have higher bone formation rate, mineral apposition rate and osteoblast number, but their bones are significantly shorter [21,41,61,62]. It was proposed that the *Bmal1*$^{-/-}$ phenotype in bone length is caused, at least partially, by the altered circadian expression of *Ihh* (Indian hedgehog) in the growth plate, a master regulator of endochondral ossification [61].

In contrast, the circadian clock in articular cartilage has only recently been described. Long-term imaging of the long bone from clock gene reporter mouse revealed strong rhythmic

bioluminescence signals from the articular cartilage and growth plate, with limited signal in the ossified bones [63]. More recently, autonomous circadian rhythms in mouse articular cartilage tissues (and human chondrocyte-derived cell lines) have been unequivocally demonstrated using real-time bioluminescence photon counting of explant cultures of xiphoid and femoral head cartilage [9]. Both types of cartilage display robust circadian oscillations. Dexamethasone and temperature cycles were able to entrain the cartilage circadian rhythm [9]. The temperature response could provide a mechanism by which the central clock can synchronize cartilage rhythms. This is particularly interesting because cartilage is avascular and not innervated. Cartilage clock could also potentially be entrained by the daily rhythm of activity as the *Clock* gene was identified as a mechanosensitive gene that is down-regulated in cartilage by mechanical stress [64].

Time series microarrays revealed hundreds of rhythmic genes in murine cartilage, including catabolic extracellular proteases [*Adamts4/9* and *Mmp14* (matrix metalloproteinase 14)], anabolic or ECM genes (e.g. *Timp4*, elastin and fibrillins), and genes involved in apoptosis [*Xiap* (X-linked inhibitor of apoptosis)] and oxidative stress pathways [9]. Adamts4 is one of the main aggrecanases responsible for remodelling the aggrecan network in articular cartilage and has been implicated in progression of osteoarthritis [65]. Mmp14 is a membrane-tethered protease with multiple roles within cartilage, including activation of other matrix proteases, such as Mmp13 and Mmp9 [66,67], modulation of TGF-β signalling [68,69], articular cartilage homoeostasis and chondrogenic differentiation [70,71]. The circadian rhythmicity of many cartilage specific genes found in mouse is supported by microarray studies in rat cartilage under diurnal (LD cycle) conditions [72].

PTH, which exhibits diurnal variation in serum concentration [38], could be another factor with the ability to entrain the circadian clock in growth plate chondrocytes. PTH, which signals through the PPR (PTH-related protein receptor), was shown to induce *Per1* expression in osteoblasts and chondrocytes. It appears that PTH can induce *Per1* expression by a mechanism related to cAMP/PKA (protein kinase A)/CREB signalling in both types of cells [73,74]. Interestingly, Takarada et al. [61] showed that overexpression of *Per1 in vitro* suppresses chondrocyte differentiation by binding and blocking a functional E-box in the *Ihh* promoter. Therefore the circadian clock could serve as a modulator of the interplay between Ihh and PTHrP (PTH-related protein) signalling which regulates differentiation of chondrocytes in the growth plate [75].

Interestingly, in aged cartilage tissue, the amplitude of circadian oscillations is significantly reduced [9]. This reduction could have a substantial impact on the susceptibility to joint diseases such as osteoarthritis, due to the loss of rhythmic expression of the downstream cartilage genes controlling tissue homoeostasis. Expression of core clock genes was found to be affected early on in an experimental mouse model of osteoarthritis, further supporting a role of clock genes in the initiation and progression of osteoarthritis [9]. Future studies should address the functional significance of the cartilage rhythm in relation to joint degeneration and repair.

TENDON CLOCK

Tendons are an essential component of the musculoskeletal system that provides transmission of forces and an attachment point of muscles to bones. Uniform collagen fibrils arranged parallel to each other are the main component of the tissue with a small population of fibroblasts called tenocytes responsible

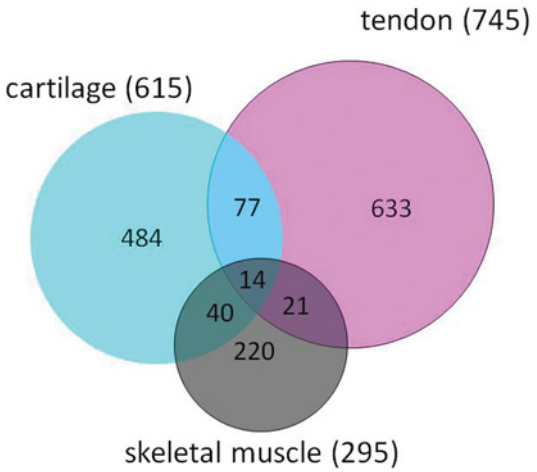

Figure 2 Venn diagram comparing the circadian transcriptome of mouse cartilage, tendon and skeletal muscle

Although all the tissues share the same core molecular clock mechanism, there is limited overlap in the rhythmic CCGs between tissues. Circadian time series microarrays of the musculoskeletal system showed that the circadian clock controls tissue specific sets of target genes, many of which were hallmark transcription factors, key signalling pathways or structural components of the particular tissue. Please note that the transcriptome study in calvarial bone [4] was performed on diurnal animals (kept under LD conditions instead of constant darkness), and therefore is not directly comparable with the circadian transcriptomes listed in the present review. Adapted from Yeung, C.Y., Gossan, N., Lu, Y., Hughes, A., Hensman, J.J., Bayer, M.L., Kjaer, M., Kadler, K.E. and Meng, Q.J. (2014) Gremlin 2 is a BMP antagonist that is regulated by the circadian clock. Sci. Rep. **4**, 5183 [77].

for the formation and maintenance of the vast ECM [76]. The daily loading/unloading of tendons during the rest/activity cycle suggests potential circadian control of tissue physiology. In fact, the *Bmal1*$^{-/-}$ mice showed an extensive ectopic tendon calcification phenotype, although the underlying mechanisms remain unknown [62]. Recently, Yeung et al. [77] described an autonomous circadian clock in mouse tendon tissue and human tenocytes. The authors reported the first tendon circadian transcriptome and the age-associated changes in the expression of clock genes in mouse tendon. Importantly, the tendon transcriptome study used tissue samples from the same animals used for the cartilage studies [9], and performed the analysis using identical algorithms and stringency criteria. Therefore it is possible to compare appropriately the clock target genes between two skeletal compartments in the joint, i.e. the cartilage and tendon. This comparison revealed only ~15 % overlap between the two closely related tissues, highlighting the tissue-specific adaptations of the circadian system (see Figure 2). Glucocorticoid signalling was shown to be capable of entraining the tendon clock [77]. However, additional mechanisms, such as mechanical loading, feeding/fasting or temperature cycles may well be at play for synchronizing this particular local clock.

Ectopic calcification of the tendons in *Clock* mutant mice could be observed at as early as 18 weeks of age. Because the calcified tendon phenotype was observed in both global *Bmal1*-KO mice [62] and *Clock*Δ*19* mutant mice [77], these data strongly suggest a role of the core circadian clock complex (Bmal1–Clock) in regulating tendon physiology. *Grem2* (gremlin 2), an antagonist of BMP signalling, was among the rhythmic genes identified by the microarray study and was shown to oscillate in antiphase to that of the BMP signalling activities. Importantly, BMP signalling is known to play a role in calcification of tendons. Recombinant Grem2 protein was demonstrated to inhibit the activation of Smad signalling by BMP2 and prevent deposition of calcium

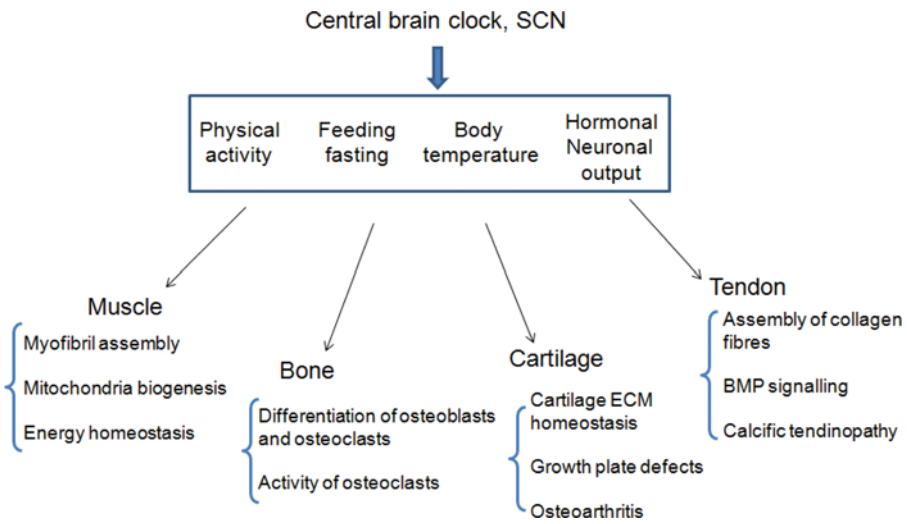

Figure 3 The circadian control of tissue homoeostasis within the musculoskeletal system

Light and other environmental zeitgebers entrain the master clock in the SCN. The SCN then generate endogenous circadian time cues (e.g. hormonal, neuronal and body temperature cycles) to synchronize the peripheral oscillators. The musculoskeletal clocks can also be entrained by other time cues, such as food intake and physical activity, either directly or indirectly (through the SCN). The circadian rhythms control a variety of genes and pathways crucial for the correct functioning of the particular musculoskeletal tissue. Mutations of the core clock genes can result in a range of pathologies affecting structural components of the tissues, energy metabolism and differentiation along the tissue specific lineages.

in human tenocytes cultured in an osteogenic medium. The importance of the circadian clock in tendon is further supported by the finding that aged WT mice show dampened circadian rhythm in their tendons, with disturbed expression of *Grem2*, and spontaneous calcification [77]. Together, these data support a hypothesis that the tendon circadian rhythm plays a critical role in tissue homeostasis. Future studies should be directed towards understanding the contribution of the local tendon oscillator to the tissue homeostasis, and to explore whether a similar clock-controlled pathway (BMP signalling) also operates in human tendinopathies.

SUMMARY

Our understanding of the influence of the circadian clock on the physiology of the musculoskeletal system is still in an early phase (see Figure 3). Although diurnal variations in serum markers of bone and cartilage metabolism and time of day dependent neuromuscular performance were known to exist for a long time, only several studies in the last decade addressed the roles of the peripheral clocks in homoeostasis of the musculoskeletal system. In tissues that are under heavy load the structural components deteriorate with use and age. It may provide an advantage to temporally divide the clean-up and the rebuilding phases so that the two opposing processes, degradation and synthesis, do not interfere with each other. Loss of rhythm could make both phases less efficient and more costly for the organism, but could also result in structurally inferior ECM in bone, cartilage and tendon or myofibrils in muscle. This view is supported by the phenotype of circadian mutant mice which display disorganized ultrastructure of myofibrils in the muscle. The coupling of the circadian phase in gene expression and metabolism with the animal's activity rhythm may also be beneficial. For example, in cartilage, the majority of catabolic genes peaked early in the day, following the night time activity of mice. Meanwhile the anabolic genes peaked 12 h later, early at night. Similar peaking time of anabolic genes was observed in the muscle. Chronic decoupling of the metabolic phases and the animal's daily activity could lead to degenerative changes in cartilage and overall reduced performance in muscles. On the other hand, synchrony among different tissues could play critical roles in physiology. In long bones, the rhythm of growth plate cartilage expansion needs to be in tune with the activity of osteoclasts and osteoblasts. Indeed, loss of circadian rhythm in cartilage results in shorter bones in *Bmal1*-KO mice.

So far, studies of the circadian clocks in the musculoskeletal system provide a glimpse into the rhythmicity of the physiology of tissues involved. Nevertheless, the findings point to an essential role of the molecular clock in the homoeostasis of the muscle, bone, cartilage and tendon. Future studies should test the hypothesis that chronic circadian disruption (during ageing or in shift workers) contributes to the increased susceptibility to diseases of the musculoskeletal system.

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