The circadian rhythm in intervertebral disc degeneration: an autophagy connection

Tai-Wei Zhang¹, Ze-Fang Li¹, Jian Dong¹ and Li-Bo Jiang¹

Abstract

There is one circadian clock in the central nervous system and another in the peripheral organs, and the latter is driven by an autoregulatory molecular clock composed of several core clock genes. The height, water content, osmotic pressure and mechanical characteristics of intervertebral discs (IVDs) have been demonstrated to exhibit a circadian rhythm (CR). Recently, a molecular clock has been shown to exist in IVDs, abolition of which can lead to stress in nucleus pulposus cells (NPCs), contributing to intervertebral disc degeneration (IDD). Autophagy is a fundamental cellular process in eukaryotes and is essential for individual cells or organs to respond and adapt to changing environments; it has also been demonstrated to occur in human NPCs. Increasing evidence supports the hypothesis that autophagy is associated with CR. Thus, we review the connection between CR and autophagy and the roles of these mechanisms in IDD.

Introduction

Approximately 2/3 of adults suffer from lower back pain (LBP), for which age-related degenerative processes in intervertebral discs (IVDs) and intervertebral disc herniation are the most common reasons. Each IVD is composed of a cartilage end-plate (CEP), a nucleus pulposus (NP) and an annulus fibrosus (AF). All three of these components are involved in intervertebral disc degeneration (IDD). It has long been known that diurnal shifts in mechanical loading lead to osmotic pressure changes, and these two types of alterations together with other microenvironmental factors stimulate nucleus pulposus cells (NPCs), contributing to the stress of NPCs. Recently, an autoregulating circadian rhythm (CR) was identified in IVDs, the abolition of which led to IDD. The abolition of CR is also observed in osteoarthritis (OA) cartilage, and the degeneration of cartilage can be induced by BMAL1 or REV-ERBα knockdown with loss of TGF-β signaling.

Circadian clocks are time-measuring devices that are present in most light-sensitive organisms. The central nervous system interacts with surrounding tissues with a 24-h oscillation period, controlling nutrient metabolism, energy balance, redox status and organismal behavior and maintaining homeostasis through complicated pathways. Autophagy is responsible for a significant connection between CR and homeostasis, as will be discussed below. Autophagy (macroautophagy) is a fundamental cellular process in eukaryotes that is essential for individual cells or organs to respond and adapt to changing environments in which cells digest themselves to generate life-supporting substances. Autophagy plays prominent roles in determining the life spans of many model organisms and in pathological processes in many organs. Additionally, autophagy maintains the homeostasis, inhibits the apoptosis and prevents the senescence of NPCs.

Recently, evidence of an interaction between CR and autophagy has emerged. Many genes involved in different steps of the autophagy pathway, such as Atg14, UNC51-like kinase 1 (Ulk1), GABA(A) receptor-associated protein-like 1 (Gabarap1), microtubule-associated protein 1 light chain 3 B (LC3B), and BCL2/adenovirus E1B-
interacting protein 3 (Bnip3), have been found to be associated with CR. In this review, we discuss how autophagy is connected to IDD and CR and what we can do as a next step to clarify the mechanisms by which CR and autophagy are involved in IDD by summarizing evidence from both in vivo and in vitro studies.

**Overview of the molecular circadian clock**

There are two kinds of circadian clocks: one in the central nervous system and another in surrounding tissues. The former is controlled by suprachiasmatic nucleus neurons following light stimulation, and the latter is controlled in every cell by the expression of clock genes. The cell-autonomous molecular clock in mammals is generated by two interlocking transcription/translation feedback loops (TTFLs) that function together to produce robust 24 h rhythms of gene expression. The core TTFL is driven by four integral clock proteins, including two activators (circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein-1 (BMAL1, also known as ARNTL)) and two repressors (Period (Per) and cryptochrome (Cry)) as well as by kinases and phosphatases that regulate the phosphorylation (P) and thereby the localization and stability of these integral clock proteins (kinases: CKIα, CKIβ, and CKIε; phosphatases PP1, PP5). The fundamental principle of the molecular clock in mammals relies on a transcriptional activator inducing the transcription of a repressor, which results in the accumulation of the latter over time until it reaches a sufficient level to repress its own activation. The basic helix-loop-helix PER-ARNT-SIM (bHLH-PAS) proteins CLOCK and BMAL1 are the primary transcriptional activators within the circadian clock mechanism of mammals. The CLOCK and BMAL1 transcription factors act as heterodimers to activate the Per and Cry gene families through interaction with E-box regulatory sequences. As a result, the Per and Cry protein products form heterotypic complexes that accumulate over time in the cytoplasm. The levels of Per/Cry complexes increase until they reach a threshold, marking the beginning of the repressive phase, in which the Per/Cry complexes translocate back to the nucleus and repress CLOCK/BMAL1 transcriptional activation. REV-ERBa/β and retinoic acid-related orphan receptor α (RORa) are major regulators of cyclic transcription within the positive limb of the mammalian circadian oscillator, although their roles are nonessential. Postranslational regulation mechanisms, such as phosphorylation, acetylation, methylation, sumoylation, and glycosylation, then modulate protein turnover, intracellular localization, and DNA-binding affinity. CR then regulates NAD biosynthesis, amino acid and carbohydrate metabolic pathways, and nucleotide biosynthesis, thus contributing to glucose homeostasis, energy homeostasis, hematopoietic cell homeostasis, epidermal stem cell homeostasis, and many other vital activities.

**The circadian rhythm in intervertebral discs**

Many characteristics of the relationship between IVDs and CR have long been known. Body height changes during the day. Over 50% of the height loss in a day occurs within the first hour of rising, and 80% occurs within 3 h of rising; the rate of creep decelerates throughout the remainder of the waking day. One of the reasons for these changes is pressure-dependent fluid shifting in the IVDs. When an IVD is subjected to a diurnal cycle involving 16 h of loading followed by an 8-h recovery period, the osmotic pressure of the nucleus becomes much higher than that of the annulus, which is why fluid is drawn back into the disc after the end of loading. A decrease in osmotic pressure in the central nucleus region through degeneration leads to an inability to draw fluid back into the disc. The range of lumbar flexion is increased during the day compared to that during the night and increases after creep, suggesting that forward bending movements subject the lumbar spine to higher bending stresses in the early morning than later in the day; this increase is ~300% for the discs and 80% for the ligaments of the neural arch. Discs appear to be very sensitive to their prevailing osmotic environment (at least in vitro), which has a powerful effect on matrix synthesis and, hence, ultimately on the structure and composition of the discs during each diurnal cycle. Penetration through the end-plate increases with diurnal loading.

The CR of IVDs is dampened with aging in mice and can be abolished by treatment with interleukin (IL)-1β. The genetic disruption of the mouse IVD molecular clock, specifically through the disruption of BMAL1, predisposes mice to IDD. This finding suggests that the disruption of CR may be a risk factor for degenerative IVD disease and LBP. Some upstream factors and downstream pathways that interact with CR in IVDs have also been found. Passive cigarette smoking changes the CR of clock genes in rat IVDs, with most genes showing a phase shift of ~6 to ~9 h and some clock-related genes showing abolished oscillation in the NP. BMAL1 and RORα regulate hypoxia-inducible factor (HIF)-1 activity in NPCs and play important roles in the overall adaptation of NPCs to their hypoxic niche; the dysregulation of these proteins affects normal tissue homeostasis and function. HIFs coordinate cellular adaptations to low-oxygen stress by regulating transcriptional programs in erythropoiesis, angiogenesis, and metabolism.

**Overview of autophagy**

The canonical process of autophagosome formation, which is evolutionarily conserved, involves the stages of initiation, nucleation, elongation and closure, recycling.
and degradation. It is usually promoted by the direct activation of Ulk1 through Ulk1 phosphorylation by adenosine 5′-monophosphate (AMP)–activated protein kinase (AMPK). The mammalian target of rapamycin kinase complex 1 (mTORC1) is incorporated into the Ulk1–Atg13–FAK family kinase-interacting protein of 200 kDa (FIP200) complex, and mammalian target of rapamycin (mTOR) kinase phosphorylates Ulk1 Ser 757, disrupting the interaction between Ulk1 and AMPK and suppressing autophagy directly. AMPK also phosphorylates Raptor to suppress the inhibitory effect of mTORC1 on the Ulk1 autophagic complex.

When autophagy is induced, Ulk1 phosphorylates AMBRA1, releases the Beclin1 complex and produces phosphatidylinositol 3-phosphate (PtdIns3P or PI3P) from dynein. PI3P is recognized by WD-repeat protein Interacting with Phosphoinositides (WIPI) proteins in the endoplasmic reticulum and is rapidly recruited to the site of autophagosome formation. Beclin1 forms a complex with ER-associated Bcl-2 under nutrient-rich conditions and is released upon the phosphorylation of Bcl-2 by c-Jun N-terminal kinase 1 (JNK 1).

Atg8 (in yeast)/microtubule-associated protein 1 light chain 3 (LC3) is cleaved at its C terminus by ATG4 to generate cytosolic LC3-I with a C-terminal glycine residue, which is conjugated to phosphatidylethanolamine (PE) in a reaction that requires ATG7 and the E2-like enzyme ATG3. The LC3-binding protein p62 is a specific substrate for autophagy, and the Atg12–Atg5–Atg16L1 complex is also required for the formation of the covalent bond between LC3 and PE. The lipidated form of LC3 (LC3-II) is attached to both faces of the phagophore membrane but is ultimately removed from the autophagosome outer membrane; this removal is followed by the fusion of the autophagosome with a late endosome/lysosome. Autophagy occurs at low basal levels in virtually all cells to perform homeostatic functions such as protein and organelle turnover.

**Autophagy in intervertebral discs**

The end-plate chondrocytes (EPCs) of cervical spondylosis patients show decreased autophagy compared with those of fracture and dislocation patients. During the aging process, the expression of the autophagy-related
genes LC3 and Beclin-1 significantly decreases with the decreasing activity of EPCs\(36\). In contrast, autophagy has been demonstrated to occur and even to increase during normal aging in NPCs\(37\). Autophagy has also been shown to be upregulated in AF cells (AFCs) in IDD patients\(38\). The current findings regarding the conditions influencing autophagy in IVDs and their associated pathways and effects are summarized in the Supplementary information (Fig. 2).

Among these factors, tumor necrosis factor-α (TNF-α) and IL-1β regulate autophagy\(39,40\). However, some studies have shown that neither TNF-α nor IL-1β can regulate autophagy in rat NPCs and that IL-1β alone cannot induce autophagy in AFCs\(41,42\). In rats, IL-1β does not induce autophagy in AFCs by itself but augments the autophagy induced by serum deprivation; such autophagy may contribute to delays in apoptosis and IDD\(42\). Hyperosmotic stress may activate the autophagy of NPCs via the Ca\(^{2+}\)-dependent AMPK/mTOR pathway\(43\). However, this is still a controversial theory\(44\). Specifically, mechanical loading is one of the factors that causes IDD\(45\).

Although the mTOR pathway is involved in many of the conditions listed in the Supplementary information, the pharmacological inhibition of only mTORC1, and not mTORC2, protects against human disc apoptosis, senescence, and extracellular matrix catabolism through Akt and autophagy induction\(46\).

The circadian rhythm of autophagy

The presence of autophagic vacuoles and the atrophy of the liver was found to follow a diurnal pattern in meal-fed rats in comparison with nutrient-deprived controls using electron microscopy\(47\). This was the first evidence of a link between autophagy and circadian regulation, which was established in the early 1970s\(47\). Additional evidence of autophagy regulation by CR in the kidney, heart, liver, brain, and retina has been found via electron microscopy, autophagic flux measurements and fluorescence measurements, not only in mammals but also in Drosophila and zebrafish\(13,48–50\).

The autophagic rhythm varies from tissue to tissue. In the convoluted tubules of the kidney, analyses of the number of autophagic vacuoles per area unit and the total amounts of segregated material have shown that the minimum values occur during the night, while the maximum values occur during the day\(51\). The volume and numeric density of autophagic vacuoles in the heart peak during the late-light phase and later decline toward the early dark period\(51\). Autophagy flux has been found to reach its peak in the afternoon, rapidly decrease at night and rise again throughout the light phase in the mouse liver\(13\). The daily rhythms of liver autophagy might not directly arise from the molecular circadian oscillator but may instead be indirectly coupled to the molecular clock via feeding or other normally circadian-gated behaviors\(56\). This evidence suggests that autophagy can be determined by the internal environment of a specific organ regardless of the regulation of the central nervous system.

The circadian rhythm regulates autophagy

Integration between CR and behavior or the environment is vital for normal physiological processes. The feeding/fasting rhythm has been found to regulate autophagy in muscle, adipose and liver tissue and to play a protective role in metabolism and life span\(58\). Intermittent fasting (IF) has been shown to restore autophagic function, thereby preserving organelle quality\(61\). The autophagy-associated protein LC3-II has been shown to exhibit a CR of its protein expression in the hippocampus, which is dampened by sleep fragmentation (SF)\(51\). However, recovery sleep did not return LC3 expression to the basal oscillating level\(51\). The basal levels of autophagy within the outer retina change in a dynamic manner during the course of the day and night and are regulated by the circadian light input\(59,62\). In the neural retina, cyclic illumination is one of the factors that activates autophagy\(60\).

Recently, it has been demonstrated that autophagy levels are controlled by several clock genes, especially Period2 (Per2) and BMAL1. Per2 in the liver functions as a scaffold protein to tether tuberous sclerosis complex 1 (TSC1), Raptor, and mTOR together to specifically suppress the activity of the mTORC1 complex\(61\). Knockdown of Per2 downregulates autophagy levels while leaving core clock oscillations largely intact\(55\). Knockdown of Per2 also reduces cellular levels of the Ulk1 protein without affecting Ulk1 mRNA levels, consistent with the rhythmic Ulk1 protein levels and nonrhythmic Ulk1 mRNA levels in these cells\(65\). The transient overexpression of Per2 results in the downregulation of the PI3K Class 1/Akt pathway in vitro and increases autophagic flux\(65,66\). Tor, ATG5 and ATG7 exhibit rhythmic expression in the brains of wild-type flies under day/night conditions (LD, 12:12), which is abolished in Per1 clock mutants\(59\). C/EBPβ is a transcription factor that is regulated by CR and induces the expression of autophagy genes such as Ulk1, Gabarap1l, LC3B, and Bnip3 and the degradation of proteins\(13\). However, its expression and the expression of the autophagy-related genes LC3B, GABA(A) receptor-associated protein a (Gabarapa), ATPase H\(^+\)-transporting lysosomal V1 subunit D (Atp6v1d), ATG4a, ATG4d, Beclin1, Bnip3, Ulk1a, and Ulk1b are upregulated without rhythmicity in Per1b mutant fish\(50\). These findings suggest the indispensable role of Per in maintaining the rhythmicity of autophagy.

In the heart, BMAL1 protects cardiomyocytes under hyperglycemic conditions by inducing autophagy through...
mTORC1 signaling downregulation. BMAL1−/− mice exhibit increased LC3A and decreased p62 levels in muscle, whereas Beclin-1 levels are unchanged. AMPK activation has been indicated to be involved in the regulation of BMAL1 in autophagy. Cardiomyocyte-specific BMAL1 knockout (CBK) and cardiomyocyte-specific Clock mutant (CCM) mice exhibit hyperactivation of the PI3K Class 1/Akt/mTOR signaling axis, which likely contributes to attenuation of autophagy and the augmentation of protein synthesis. Thus, the expression of BMAL1 may be associated with the activation of autophagy.

In vitro, REV-ERB agonism blocks autophagy and induces apoptosis. In vivo, REV-ERB agonism has been found to downregulate the mRNA and protein levels of Ulk3, Ulk1, Beclin1, and ATG7 and to significantly improve survival in two glioblastoma models. In zebrafish, REV-ERBα has been found to bind directly to the ROR-responsive element (RORE) sites in the Ulk1a promoter, thereby repressing Ulk1a in vivo, and another autophagy-related gene, Atg6v1d, is a direct target of REV-ERBα. However, it has also been shown that REV-ERBβ does not seem to be directly involved in autophagy regulation but likely acts as a cytoprotective factor downstream of a blockade of autophagy.

Other core clock proteins also show the ability to influence autophagy. AMPK activity and nuclear localization are rhythmic and are inversely correlated with Cry1 nuclear protein abundance. The rhythmic regulator CLOCK may potentially affect tumor cell resistance to cisplatin by inducing autophagy. ATG14 gene expression, which is controlled by CLOCK/BMAL1 binding to its E-box, also exhibits a CR.

**Autophagy regulates the circadian rhythm**

Although there is relatively less evidence that autophagy regulates CR than that CR regulates autophagy, findings related to the core components of autophagy and CR are
included in such evidence. The silencing of Tor in Per-expressing cells shortens the period of the locomotor activity rhythm of flies\cite{59}. The stimulation of AMPK destabilizes cryptochromes and alters CR, and mice in which the AMPK pathway is genetically disrupted show alterations in their peripheral clocks\cite{72}. The circadian proteins BMAL1, CLOCK, REV-ERBα, and Cry1 are lysosomal targets, and the selective autophagic degradation of Cry1 occurs in a diurnal window when rodents rely on gluconeogenesis, suggesting that Cry1 degradation is time imprinted for the maintenance of blood glucose\cite{75}. In addition, high-fat feeding accelerates autophagic Cry1 degradation and contributes to obesity-associated hyperglycemia\cite{75}. A CLOCK mutant attenuates BMAL1 degradation through both proteasomal and autophagic pathways under high-fat diet feeding, providing evidence of autophagic regulation of a core clock component\cite{76}.

**Indirect regulation between autophagy and the circadian rhythm in intervertebral discs**

The circadian regulator CLOCK is a histone acetyltransferase (HAT) that also acetylates a nonhistone substrate: its own partner, BMAL1\cite{77}. Both nonhistone acetyltransferase and HAT activities are essential to the circadian rhythmicity and activation of clock genes\cite{77}. Sirt1 is a circadian deacetylase for core clock components\cite{78}. The NAD(+)-dependent enzyme Sirt1, which functions as a histone deacetylase whose activity is also regulated by the redox states of NAD cofactors, counteracts the activity of CLOCK\cite{77}. Additionally, Sirt1 binds to CLOCK-BMAL1 and Per2 in a circadian manner and supports the deacetylation and degradation of Per2\cite{79}. In the absence of Sirt1, constitutively high protein levels of Per2 may lead to the repression of Per1, Per2, Cry1, and retinoic acid-related orphan receptor γ (RORγ) mRNA expression\cite{79}. On the other hand, Sirt1 may activate autophagy through the Sirt1-LKB1-AMPK pathway and by deacetylating ATG5, ATG7, LC3, and tuberous sclerosis complex 2 (TSC2), a component of the mTOR inhibitory complex upstream of mTORC1\cite{80–83}. Taken together, these findings suggest that Sirt1 serves as a link between autophagy and CR and between the redox state and the circadian clock.

Melatonin, an endocrine hormone synthesized and secreted by the pineal gland in the brain that helps to maintain CR, significantly enhances protective effects in different systems, including the central nervous, cardiovascular, gastrointestinal and endocrine systems, through the enhancement or inhibition of the autophagy process\cite{84}. It is believed that oxidative stress can activate autophagy; for this reason, the antioxidant activity of melatonin could account for its inhibitory effects on autophagy\cite{85}. If so, melatonin may affect mechanisms that stimulate autophagy, rather than affecting the process itself\cite{85}. Melatonin seems to inhibit autophagy triggered by either mTOR activation or JNK/Bcl-2/Beclin1 pathway signaling\cite{86,87}. Cyclosporine A is known to induce autophagy via ER stress\cite{88}. Melatonin suppresses cyclosporine-induced autophagy in rat pituitary GH3 cells through the MAPK/ERK pathway, an effect that is due either totally or in part to the antioxidant properties of melatonin\cite{85,88}. Deficiency of the nuclear melatonin receptor RORα aggravates autophagy dysfunction in diabetic hearts and myocardial ischemia/reperfusion injury in mice\cite{89–91}. However, in regimen 1-treated N2a/APP cells, only a slight increase in cellular autophagy has been found using flow cytometry, and there is no significant alteration in the expression of the autophagy-associated markers Beclin-1 and LC3-I/II\cite{92}. These results suggest that autophagy may play a negligible role in the beneficial effects of caffeine, melatonin, and coffee on AD\cite{92}.

The circadian regulation of C/EBPβ and the autophagy disruption observed in mice lacking a functional liver clock suggest that C/EBPβ is a key factor that links autophagy to the biological clock and maintains nutrient homeostasis throughout light/dark cycles\cite{13}. In zebrafish, a CLOCK-BMAL1 heterodimer binds to E-boxes to regulate the transcription of C/EBPβ, which in turn controls the transcription of autophagy-related genes indirectly\cite{90}. Fus1, a tumor suppressor protein residing in mitochondria, maintains mitochondrial homeostasis and is highly expressed in the brain\cite{93}. One study revealed that KO mice showed sleep/wake disturbances compared to WT mice and that the autophagy marker LC3-II was decreased in both the olfactory bulbs and hippocampi, suggesting an early onset of autophagy dysregulation in Fus1 KO mice\cite{93}. Heme oxygenase (Ho), whose silencing results in the downregulation of autophagy-related genes in both light and dark phases, is expressed in a circadian manner\cite{94}. FoxOs are tightly controlled by fasting/feeding cycles and can upregulate the expression of ATG14\cite{94}. Casein kinase 1α (CK1α) exhibits dual functions in autophagy regulation\cite{95}. CK1α-mediated phosphorylation stimulates the degradation of Per1, suggesting a function in CR\cite{95}.

The expression of the core clock genes Per2 and REV-ERBα is increased after weight loss\cite{96}. CLOCK gene expression levels and their weight loss-induced changes are tightly correlated with each other and with the expression of genes involved in autophagy (LC3A and LC3B)\cite{96}. Folic acid deprivation increases autophagic activity in hippocampal neuron cells, an effect that is associated with the activation of autophagy- and circadian-related genes\cite{97}.

In the brain, acrylamide (ACR), a chronic neurotoxin, substantially attenuates spontaneous alternation. ACR dampens the oscillatory amplitudes of clock genes
unsuitable nutrient status induces autophagy in an ROS-renewal in IVDs. ACR deprivation can activate autophagy and lead to cell dysfunction of autophagy. Limited nutritional oscillation, as observed in the context of diabetes, leads to autophagy and their interaction, while a lack of nutritional availability is a periodic lack of nutrition favors the molecular CR, particularly interested in this field. As we described above, a periodic lack of nutrition favors the molecular CR, autophagy and their interaction, while a lack of nutritional oscillation, as observed in the context of diabetes, leads to dysfunction of autophagy. Limited nutritional deprivation can activate autophagy and lead to cell renewal in IVDs. Thus, we can infer that nutrition is an incentive for CR, leading to the further regulation of autophagy. Redox status is also a prevalent factor involved in many metabolic processes. Not only H$_2$O$_2$ but also an unsuitable nutrient status induces autophagy in an ROS-dependent manner in IVDs. Sirt1 and melatonin are bridges between redox status and autophagy; one of them regulates clock-related molecules, while the other is regulated by CR. It has long been known that night shift work is a significant risk factor for the development of IDD and its progression. SF has been indicated to blunt CR and damage the expression of autophagy-related proteins in the CNS, perhaps permanently. Given all of these findings, we can ask whether sleep rhythms regulate autophagy in IVDs. Night shift work also involves abnormal loading/resting cycles. In these cycles, IVDs undergo changes in the mechanical loading and activation of autophagy, which can result in IDD. As the most recent research has shown, inflammation is a significant factor in IDD, as IL-1β abolishes the normal molecular functions of clock-related components, participates in multiple pathological processes during disc degeneration (including inflammatory responses, matrix destruction, angiogenesis and innervation, apoptosis, oxidative stress and cellular senescence), and activates protective autophagy in IVDs. However, despite this contradictory evidence, one thing is clear: clock dysfunction is a key factor associated with inflammation-related autophagy in IDD. The water content varies in IVDs during the loading/resting cycle together with changes in the osmotic environment. The osmotic environment is also associated with autophagy in IVDs. Whether this phenomenon affects the molecular CR in IVDs could be investigated in the future. Given the core circadian regulation observed in IVDs and the autophagy regulation mediated by components, such as Per, BMAL1, and REV-ERBα, in other models, we can hypothesize that there is a fairly close interaction between CR and autophagy.

We can infer that limited autophagy coordinated by CR protects IVDs from degeneration, while excessive autophagy and autophagic dysfunction induced by abnormal CRs under different clinical conditions accelerate IDD. Since an intrinsic CR has already been found in IVDs, further research should investigate the molecular mechanism by which CR influences autophagy in the process of IDD.

Discussion and prospects
To the best of our knowledge, there are few studies linking autophagy and CR in IVDs. However, the findings reported to date provide some insights and clues that CR induces IDD at least partially in an autophagic manner. Nutrient status, oxidative stress, inflammation, the osmotic environment and mechanical loading are the most common factors that induce changes in autophagy and CR in IVDs. Considering the great deal of evidence regarding nutritional status, we are also particularly interested in this field. As we described above, a periodic lack of nutrition favors the molecular CR, autophagy and their interaction, while a lack of nutritional oscillation, as observed in the context of diabetes, leads to dysfunction of autophagy. Limited nutritional deprivation can activate autophagy and lead to cell renewal in IVDs. Thus, we can infer that nutrition is an incentive for CR, leading to the further regulation of autophagy. Redox status is also a prevalent factor involved in many metabolic processes. Not only H$_2$O$_2$ but also an unsuitable nutrient status induces autophagy in an ROS-dependent manner in IVDs. Sirt1 and melatonin are bridges between redox status and autophagy; one of them regulates clock-related molecules, while the other is regulated by CR. It has long been known that night shift work is a significant risk factor for the development of IDD and its progression. SF has been indicated to blunt CR and damage the expression of autophagy-related proteins in the CNS, perhaps permanently. Given all of these findings, we can ask whether sleep rhythms regulate autophagy in IVDs. Night shift work also involves abnormal loading/resting cycles. In these cycles, IVDs undergo changes in the mechanical loading and activation of autophagy, which can result in IDD. As the most recent research has shown, inflammation is a significant factor in IDD, as IL-1β abolishes the normal molecular functions of clock-related components, participates in multiple pathological processes during disc degeneration (including inflammatory responses, matrix destruction, angiogenesis and innervation, apoptosis, oxidative stress and cellular senescence), and activates protective autophagy in IVDs. However, despite this contradictory evidence, one thing is clear: clock dysfunction is a key factor associated with inflammation-related autophagy in IDD. The water content varies in IVDs during the loading/resting cycle together with changes in the osmotic environment. The osmotic environment is also associated with autophagy in IVDs. Whether this phenomenon affects the molecular CR in IVDs could be investigated in the future. Given the core circadian regulation observed in IVDs and the autophagy regulation mediated by components, such as Per, BMAL1, and REV-ERBα, in other models, we can hypothesize that there is a fairly close interaction between CR and autophagy.

We can infer that limited autophagy coordinated by CR protects IVDs from degeneration, while excessive autophagy and autophagic dysfunction induced by abnormal CRs under different clinical conditions accelerate IDD. Since an intrinsic CR has already been found in IVDs, further research should investigate the molecular mechanism by which CR influences autophagy in the process of IDD.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (no. 81702177; no. 81772855; no. 81572629) and the Shanghai Sailing Program (17YF1402300).

Conflict of interest
The authors declare that they have no conflict of interest.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Supplementary information accompanies this paper at https://doi.org/10.1038/s12276-019-0372-6.

Received: 16 July 2019 Revised: 1 September 2019 Accepted: 17 September 2019.
Published online: 27 January 2020

References

1. Deyo, R. A. & Weinstein, J. N. Low back pain. N. Engl. J. Med. 344, 363–370 (2001).
2. Pattappil, G. et al. Diversity of intervertebral disc cells: phenotype and function. J. Anat. 221, 480–496 (2012).
3. Rey, G. J. P., Smith, A. B. & Fairbank, J. C. Nutrition of the intervertebral disc. Spine (Phila. Pa 1976) 29, 2700–2709 (2004).
4. Yuan, W. et al. Establishment of intervertebral disc degeneration model induced by ischemic sub-endplate in rat tail. Spine 15, 1050–1059 (2015).
5. Massey, C. J., van Donkelaar, C. C., Vreislovic, E., Zavaliangos, A. & Marcolongo, M. Effects of aging and degeneration on the human intervertebral disc during the diurnal cycle: a finite element study. J. Orthop. Res 30, 122–128 (2012).
6. Sivan, S., Neildinger-Wille, C., Wurtz, K., Marounds, A. & Urban, J. P. Diurnal fluid expression and activity of intervertebral disc cells. Biochemistry 43, 283–291 (2006).
7. Numaguchi, S. et al. Passive cigarette smoking changes the circadian rhythm of clock genes in rat intervertebral discs. J. Orthop. Res 34, 39–47 (2016).
8. Dudek, M. et al. The intervertebral disc contains intrinsic circadian clocks that are regulated by age and cytokines and linked to degeneration. Ann. Rheum. Dis. 76, 576–584 (2017).
9. Akagi, K. et al. Dysregulated circadian rhythm pathway in human osteoarthritis: NR1D1 and BMAL1 suppression alters TGF-beta signaling in chondrocytes. Osteoarthr. Cartil. 25, 943–951 (2017).
10. Green, C. B., Takahashi, J. S. & Bass, J. The meter of metabolism. Cell 134, 728–742 (2008).
11. Rubinstein, D. C., Marino, G. & Kroemer, G. Autophagy and aging. Cell 146, 682–695 (2011).
12. Ito, M. et al. Selective interference of mTORC1/RAPTOR protects against human disc cellular apoptosis, senescence, and extracellular matrix catabolism with Akt and autophagy induction. Osteoarthr. Cartil. 25, 2134–2146 (2017).
13. Ma, D., Panda, S. & Lin, J. D. Temporal orchestration of circadian autophagy rhythm by C/EBPbeta. EMBO J. 30, 4642–4651 (2011).
14. Dibner, C., Schibler, U. & Albrecht, U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu. Rev. Physiol. 72, 517–549 (2010).
15. Parth, C. L., Green, C. B. & Takahashi, J. S. Molecular architecture of the mammalian circadian clock. Trends Cell Biol. 24, 90–99 (2014).
16. Reddy, A. B. & Rey, G. Metabolic and nontranscriptional circadian clocks: eukaryotes. Annu. Rev. Biochem. 83, 165–189 (2014).
17. Yan, C. & Reddy, A. B. Connecting cellular metabolism to circadian clocks. Trends Cell Biol. 23, 234–241 (2013).
18. Janich, P., Meng, Q. J. & Benitah, S. A. Circadian control of tissue homeostasis and adult stem cells. Curr. Opin. Cell Biol. 31, 8–15 (2014).
19. Reilly, T., Tyrell, A. & Troupp, J. D. Circadian variation in human stature. Chronobiol Int 1, 121–126 (1984).
20. Kramer, J. & Gritz, A. (Changes in body length by pressure dependent fluid shifts in the intervertebral discs (author's transl) J. Zahn. Zahnheilk. 118, 161–164 (1980).
21. Adams, M. A., Dolan, P. & Hutton, W. C. Diurnal variations in the stresses on the lumbar spine. Spine (Phila. Pa 1976) 12, 130–137 (1987).
22. Gantenbein, B. et al. An in vitro organ culturing system for intervertebral disc explants with vertebral endplates: a feasibility study with ovine caudal discs. Spine (Phila. Pa 1976) 31, 2665–2673 (2006).
23. Suyama, K. et al. Circadian factors BMAL1 and RORalpha control HF-1/Alpha transcriptional activity in nuclear pulposus cells: implications in maintenance of intervertebral disc health. Oncotarget 7, 23056–23071 (2016).
24. Wu, D., Portlin, N., Lu, J., Kim, Y. & Rastinejad, F. Structural integration in hypoxia-inducible factors. Nature 524, 303–308 (2015).
50. Huang, G., Zhang, F., Ye, Q. & Wang, H. The circadian clock regulates autophagy directly through the nuclear hormone receptor Nrl1/1Rev-
      erbalpha and indirectly via Celrbp/C/eifbpieta in zebra fish. Autophagy. 12, 1292–1309 (2016).

51. He, Y. et al. Circadian rhythm of autophagy proteins in hippocampus is
      blunted by sleep fragmentation. Chronobiol Int 33, 553–560 (2016).

52. Ryzhikov, M. et al. Diurnal rhythms spatially and temporally organize
      autophagy. J Neurosci 37, 1454–1467 (2017).

53. Rabinovich-Nikitin, I., Lieberman, B., Martin, T. A. & Kirshenbaum, L. A.
      Circadian-regulated cell death in cardiovascular diseases. Circulation 139, 965–980 (2019).

54. Frost, L. S. et al. The contribution of melanoregulin to microtubule-associated
      protein 1 light chain 3 (LC3) associated phagocytosis in retinal pigment
      epithelium. Mol Neurobiol 52, 1135–1151 (2015).

55. Kijak, E. & Pyza, E. TOR signaling pathway and autophagy are involved
      in the regulation of circadian rhythms in behavior and plasticity of L2
      interneurons in the brain of Drosophila melanogaster. PLoS ONE 12, e0171848 (2017).

56. Stockman, C. M., Thomas, D., Burke, J. & Apovian, C. M. Intermittent fasting
      is the light response of rat retinal disk-shedding and autophagy. Brain Res 369, 356–360 (1986).

57. Chen, X., Kondo, K., Motoki, K., Homma, H. & Okazawa, H. Fasting activates
      macroautophagy in neurons of Alzheimer’s disease mouse model but is
      insufficient to degrade amyloid-beta. Sci Rep 5, 12115 (2015).

58. Reme, C. & Wirz-Justice, A. [Circadian rhythm, the retina and light].

59. Pfeifer, U. & Scheller, H. A morphometric study of cellular autophagy
      during diurnal variations in kidney tubules of normal rats. J Cell Biol 64, 609–621 (1975).

60. Lamia, K. A. et al. AMPK regulates the circadian clock by cryptochrome
      phosphorylation and degradation. Science 326, 437–440 (2009).

61. Sun, Y., Jin, L., Sui, Y. X., Han, L. L. & Liu, J. H. Circadian gene CLOCK Affects
      drug-resistant gene expression and cell proliferation in ovarian cancer
      SKOV3/DDP Cells lines through autophagy. Cancer Biol Ther 32, 139–146 (2017).

62. Xiong, X., Tao, M., Teng, R. & DePinho, R. A. & Dong, X. C. The autophagy-related gene 14
      (Atg14) is regulated by forkhead box O transcription factors and circadian
      rhythms and plays a critical role in hepatic autophagy and lipid metabolism.
      J. Biol. Chem. 287, 39107–39114 (2012).

63. Toledo, M. et al. Autophagy regulates the liver clock and glucose metabolism
      by degrading CRY1. Cell Metab 28, 268–281 e264 (2018).

64. Jeong, K. et al. Dual attenuation of prosomatostatin and autophagic
      BVAL1 degradation in Clock Delta19/+ mice contributes to improved glucose
      homeostasis. Sci Rep 5, 12801 (2015).

65. Nakahara, Y. et al. The NAD+-dependent deacetylase SIRT1 modulates
      CLOCK-mediated chromatin remodeling and circadian control. Cell 134, 329–340 (2008).

66. Belden, W. J. & Dunlap, J. C. SIRT1 is a circadian deacetylase for core clock
      components. Cell 134, 212–214 (2008).

67. Asher, G. et al. SIRT1 regulates circadian clock gene expression through PER2
decaylation. Cell 134, 317–328 (2008).

68. Ghosh, H. S., McBurrey, M. & Robbins, P. D. SIRT1 negatively regulates the
      mammalian target of rapamycin. PLoS ONE 5, e9199 (2010).

69. Lan, F., Caicedo, J. M., Ruderman, N. & Idso, Y. SIRT1 modulation of the
      acetylation status, cytosolic localization, and activity of LKB1. Possible role in
      AMP-activated protein kinase activation. J. Biol. Chem. 283, 27628–27635 (2008).

70. Lamia, K. A. et al. A role for the NAD-dependent deacetylase Sirt1 in the
      regulation of autophagy. Proc. Natl Acad. Sci. USA 105, 3374–3379 (2008).

71. Chung, S. et al. Regulation of SIRT1 in cellular functions: role of polyphenols.
      Arch. Biochem. Biophys. 501, 79–90 (2010).

72. RoohiBaliksh, A., Shamszadeh, A., Hayes, A. W., Reiter, R. J. & Kamini, G. M.
      Melatonin as an endogenous regulator of diseases: the role of autophagy. Pharmacol. Res. 133, 265–276 (2018).

73. Mothia, V., Garcia-Maurino, S., Talero, E. & Illanes, M. New paradigms in
      chronic inflammatory disorders and cancer: role of melatonin. J. Pineal Res 51, 44–60 (2011).

74. Kongsuphol, P., Mukda, S., Nopparat, C., Villarroel, A. & Govitrapong, P. Mel-
      atonin attenuates methamphetamine-induced deactivation of the mam-
      malian target of rapamycin signaling to induce autophagy in SK-N-SH cells. J.
      Pineal Res 46, 199–206 (2009).

75. Nopparat, C., Porter, J. E., Ebadi, M. & Govitrapong, P. The mechanism for the
      neuroprotective effect of melatonin against methamphetamine-induced
      autophagy. J. Pineal Res 49, 382–389 (2010).

76. Yoo, Y. M. & Jeung, E. B. Melatonin suppresses cyclosporine A-induced
      autophagy in rat primary GH3 cells. J. Pineal Res 48, 204–211 (2010).

77. Jerwinehues, A., Nopparat, C., Mukda, S., Wongchitrat, P. & Govitrapong, P.
      Melatonin regulates aging and neurodegeneration through energy meta-
      bolism, epigenetics, autophagy and circadian rhythm pathways. Int J Mol.
      Sci. 15, 16848–16884 (2014).

78. Zhao, Y. et al. Novel protective role of the circadian nuclear receptor retinoic
      acid-related orphan receptor-alpha in diabetic cardiomypathy. J Pineal Res 62, https://10.1111/jpi.12378 (2017).

79. He, B. et al. The nuclear melatonin receptor RORalpha is a novel endogenous
      defender against myocardial ischemia/reperfusion injury. J Pineal Res 60, 313–326 (2016).

80. Zhang, L. F. et al. Coffee and caffeine potentiate the antiamyloidogenic
      activity of melatonin via inhibition of Abeta oligomerization and modulation
      of the Tau-mediated pathway in N2a/APP cells. Drug Des. Devel Ther. 9, 241–272 (2015).

81. Coronas-Samano, G., Baker, K. L., Tan, W. J., Ivanova, A. V. & Verhas, J. V.
      Flu1 KO mouse as a model of oxidative stress-mediated sporadic Alzheimer’s
      disease: circadian disruption and long-term spatial and olfactory memory impairments. Front Aging Neurosci. 8, 268 (2016).

82. Damulewicz, M. et al. Daily regulation of phototransduction, circadian clock,
      and circadian oscillations in Drosophila. J. Pineal Res 62, 1212–1219 (2017).

83. Sull, G. et al. Pharmacological activation of REV-ERBsi is lethal in cancer and
      oncogene-induced senescence. Nature 553, 351–355 (2018).

84. De Mei, C. et al. Dual inhibition of REV-ERBbeta and autophagy as a novel
      pharmacological approach to induce cytotoxicity in cancer cells. Oncogene 34, 2597–2608 (2015).

85. Sun, Y. et al. Circadian gene CLOCK regulates autophagy-related gene 14 (Atg14)
      is regulated by forkhead box O transcription factors and circadian
      rhythms and plays a critical role in hepatic autophagy and lipid metabolism.
      J. Biol. Chem. 287, 39107–39114 (2012).

86. Sun, Q. et al. Folate deprivation modulates the expression of autophagy- and
      circadian-related genes in HT-22 hippocampal neurons through GR-
      mediated pathway. Steroids 113, 12–19 (2016).

87. Tan, X. et al. Acylcarnitine aggregates cognitive deficits at night period via
      the gut-brain axis by reprogramming the brain circadian clock. Arch. Toxicol. 93, 467–486 (2019).
99. Bretin, A. et al. Activation of the EIF2AK4-EIF2A/eIF2alpha-ATF4 pathway triggers autophagy response to Crohn disease-associated adherent-invasive Escherichia coli infection. *Autophagy* 12, 770–783 (2016).

100. Chang, H. et al. Early-stage autophagy protects nucleus pulposus cells from glucose deprivation-induced degeneration via the p-eIF2alpha/ATF4 pathway. *Biomed. Pharmacother.* 89, 529–535 (2017).

101. Chen, J. W. et al. Hypoxia facilitates the survival of nucleus pulposus cells in serum deprivation by down-regulating excessive autophagy through restricting ROS generation. *Int J. Biochem Cell Biol.* 59, 1–10 (2015).

102. Elfering, A. et al. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. *Spine (Phila. Pa 1976)* 27, 125–134 (2002).

103. Yang, W. et al. Interleukin-1beta in intervertebral disk degeneration. *Clin. Chim. Acta* 450, 262–272 (2015).