Towards understanding molecular mechanisms of infradian rhythms

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The appropriate timing of various seasonal processes, such as reproduction, migration, and hibernation, is crucial for the survival of animals living in temperate regions. Although this phenomenon attracts great interest, its underlying mechanisms were not well understood. *Drosophila* and mouse are undoubtedly good models for understanding various aspects of physiology and behavior. However, these animals do not show clear seasonal responses. Therefore, it is better to choose "non-model animals" that show robust and dramatic seasonal responses, to understand the underlying mechanism of seasonality. With the use of non-model organisms that have highly sophisticated seasonal responses, we have uncovered the universality and diversity of the signal transduction pathway that regulate seasonal rhythms in vertebrates.

Although humans are not typically considered seasonal animals, some evidence suggests that seasonal variation in physiology and behavior also exists in humans. For example, the wavelength settings for the unique yellow hue are shifted to shorter wavelengths in summer when compared to those in winter. Seasonal affective disorder patients experience recurrent winter episodes of depressed mood, overeating, and hypersomnia along with electroretinogram changes in winter associated with lower sensitivity when compared to healthy subjects. These observations highlight the potential importance of the retina in seasonality. We have discovered dynamic plasticity in phototransduction regulates seasonal changes in color perception in Japanese medaka fish (*Oryzias latipes*), making it an excellent model for studying seasonal adaptation. Furthermore, we observed decreased sociability and increased anxiety-like behavior in medaka exposed to winter-like conditions. Chemical genomics approach uncovered molecular mechanisms underlying winter depression-like behavior in medaka. Moreover, we are studying seasonal changes in physiology of rhesus macaques kept under natural outdoor conditions. We believe that exploring the regulation of seasonal traits in animal models will provide insight into human seasonality and aid in the understanding of human diseases, such as seasonal affective disorder.

The existence of circannual and circalunar clock has been demonstrated in several organisms. However, their underlying molecular mechanisms remain mystery. We are currently attempting to uncover these mechanisms using medaka and grass puffer as model species.

CaMKIIα is involved in the coupling of the evening and morning circadian oscillators in mice

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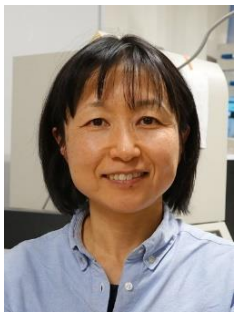

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The circadian pacemaker in mammals is located in the hypothalamic suprachiasmatic nucleus (SCN) and yields photoperiodic response to transfer seasonal information to physiology and behavior. Photoperiodic changes in behavior, especially in the length of activity time in nocturnal rodents, are ascribed to the phase-relation between two circadian oscillators which differentially respond to light, and respectively regulate the onset and offset of activity band of circadian behavior rhythms (Pittendrigh and Daan, 1976). The two oscillators are called the evening (E) for the onset and the morning (M) oscillators for the offset. We identified the regions corresponding to these two oscillators in the mouse SCN by using bioluminescence imaging (Yoshikawa et al. 2017). However, the dynamics and mechanism of coupling between the oscillators is not well understood.

Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) activity is essential for not only the cellular oscillation but also synchronization among oscillators in the SCN (Kon et al., 2014). The knock-in mouse with a kinase-dead mutation in CaMKIIα (K42R mouse) showed weakened behavioral rhythm and elicited decoupling between the E and M oscillators. Under prolonged DD, the activity time (α) was gradually expanded (α decompression) in association with an increase of circadian period of the activity offset. When α reached a critical length of about 18 hours, abrupt shortening of α (α compression) took place by phase-delay shifts of the activity onset and phase-advance shift of the activity offset. The amounts of the phase shifts were significantly larger in the activity offset than onset, suggesting that the internal coupling between the E and M oscillators is bidirectional but with different strength. Disrupted coupling of the E and M oscillator was also seen in the course of re-entrainment to a short photoperiod (LD6:18), where α decompression was accompanied. CaMKIIα was suggested to be involved in the coupling of the E and M oscillators. Possible mechanism of the coupling will be discussed.

Time to update the external coincidence model in *Arabidopsis*

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Many organisms developed seasonal responses in order to adopt to changing environment throughout a year. In plants, regulation of seasonal flowering is a representative example of photoperiodism and is important for successful propagation.

It is widely known that the expression of the gene that encodes florigen, *FLOWERING LOCUS T* (*FT*), is induced by specific photoperiod and promotes flowering in plants. Photoperiodic induction of *FT* expression is interpreted by a model called "external coincidence model", in which *FT* activation occurs when plants receive the light on the specific timing of the day. In long-day model plant, *Arabidopsis thaliana*, it was proposed that light- and clock-mediated activation of *CONSTANSE* (*CO*), one of the major transcriptional regulators of *FT*, is the actual mechanism of external coincidence model. *CO-FT* module fits well to the concept of "external coincident model", and has been widely validated in various plant species under laboratory conditions, where temperature remains constant and artificial light sources are used.

However, under natural long-day conditions, where plants are exposed to sunlight and daily fluctuation of temperature, *FT* is highly induced both in the morning and evening and promotes flowering. In different with the evening *FT*, the induction of morning *FT* requires both far-red signaling and long-day photoperiod. Interestingly, the induction levels of morning *FT* increased gradually as the day-length becomes longer. Since light exposure from dawn to the peak expression of morning *FT* (ZT4) is the same under any photoperiod condition, these results suggest that photoperiod information from the day before determines the induction levels of morning *FT*, and that current external coincidence model cannot fully explain long-day dependent *FT* induction in the morning.

To further analyze how day length affects *FT* expression, we applied transient jet-lag experiments and found that the timing of dawn and dusk determines the induction levels of morning and evening *FT*, respectively. Under this jet-lag condition, morning *FT* induction occurs even in the absence of far-red light, implying that far-red signaling and photoperiod independently regulate morning *FT*. In addition, the responses of each clock gene to the new light-dark cycle are slightly different. While morning-expressed gene *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) rapidly adjusted to the new light-dark cycle, evening-expressed *LUX ARRHYTHMO* (*LUX*) partially remains original phase, implying the transient decoupling between clock genes and *FT* expression.

Here, we discuss how the light-dark cycle affects the expressions of *FT* and circadian clock genes, and propose a possible model that explains photoperiodic flowering responses of *Arabidopsis* in nature.

Diet- and hibernation season- dependent enhancement of hepatic cold resistance in a mammalian hibernator Syrian hamster

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Mammalian hibernation is a strategy to survives during the harsh season with cold and little food by reducing basal metabolism and thermogenesis. In small mammalian hibernators, hibernation consists of multiday hypothermic deep torpor and normothermic periodic arousal. Deep torpor is characterized by the profound suppression of metabolism, body temperature, heart rate, and locomotive activity. The drastic alterations in physiology associated with hibernation can lead to multiple organ dysfunction and death in non-hibernators such as mice and humans, whereas hibernators tolerate these physiological extremes. The mechanisms responsible for such extremes remain poorly understood.

Syrian golden hamster (*Mesocricetus auratus*) is a facultative hibernator, in that they are induced to hibernate under a winter-like, short photoperiodic cold condition independent of circannual rhythm. When transferred from summer-like conditions to winter-like conditions, they begin to hibernate after several months of the pre-hibernation remodeling period. We found that hepatocytes from Syrian hamsters exhibited remarkable intrinsic resistance to prolonged cold culture, whereas murine hepatocytes underwent cold-induced cell death. The cold-induced cell death fulfills the hallmarks of a recently identified regulate cell death, ferroptosis, such as necrotic morphology, lipid peroxidation and prevention by an iron chelator. Unexpectedly, the resistance to cold in hepatocytes from Syrian hamsters was lost in a diet-dependent manner. The diet-dependent resistance in Syrian hamsters was attributed to their superior ability to retain dietary α-tocopherol (αT), a vitamin E analog in the liver and blood. We also found that Syrian hamsters also increased plasma αT concentration in a hibernation-season dependent manner. These results suggest that the cold resistance of Syrian hamsters is established by the ability to utilize αT effectively to prevent lipid peroxidation and ferroptosis, both of which can occurs by the repeated cooling and rewarming processes during hibernation.

Organizing complex seasonality: bird migration gives clues

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Over recent years, we have witnessed great strides in the neuroendocrine understanding and molecular decoding of photoperiodic stimulation of vertebrate reproduction, thanks in particular to research by the laureate Takashi Yoshimura. Apart from reproductive activation, the mechanisms driving further major transitions in the vertebrate annual cycle are still largely unknown. Yet ultimately, understanding the regulation of annual cycles requires knowledge of driving mechanisms of all phases, as well as of their integration.

Migratory birds are a particularly challenging, but potentially also rewarding, system for such an integrative understanding. The birds' change of location imposes photoperiodic change and uncertainty, but at the same time, major physiological transitions must be initiated well in advance. Thus, the birds' long-distance flights must be accurately timed relative to the anticipated environmental resources months later, at destination areas that can be several thousand kilometres away. Because of such high demands on biological time-keeping, bird migration has been a classical study system in chronobiology from its early days, including of photoperiodic pioneers such as William Rowan, and of circannual pioneers such as Eberhard Gwinner.

Given their complex environmental input and annual cycle, migratory birds require a timing system that selectively uses photoperiodic input during some phases of the annual cycle, and might individually time different phases of the annual cycle. Additionally, given the demands of their mobile life styles, it is likely that the fine-tuning of migratory timing involves integration of multiple physiological processes. Now, new data from multiple sources give clues to contributing factors. Circannual and photoperiodic studies of migratory birds suggest modular organization of the annual cycle, such that phases can be independently modified and may differ in free-running circannual period length. Data from wild migrants show good correspondence with captivity studies, and theory of migratory timing programs can accurately explain striking behaviour in the wild. Long-term data from wild birds confirm that through evolution and plasticity, different phases can be independently modified. Lastly, genomic studies of wild birds point to molecular components of the fine-tuning of annual timing. At present, these studies yield diverse, sometimes surprising, results, which however can inform hypotheses for future mechanistic studies.

Disentangling this multi-piece puzzle is a grand challenge, but bird migration offers unique pathways for relating complex rhythmic organization to the lives of free-living animals. New insights from migratory birds are likely to reveal fundamental regulatory mechanisms that may also play a role in less complex systems, where they might be less conspicuous and thus more easily overlooked.