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論文題目	Identification of Common and Separate Mechanisms Governing Circadian Locomotor Activity and Body Temperature （行動と体温の概日変動を支配する共通および個別メカニズムの同定）		

（論文内容の要旨）

Almost all organisms, including humans, display daily rhythms of behaviour and physiology generated by an endogenous mechanism called circadian clock. In mammals, these rhythms are established by transcription/translation-based autoregulatory feedback loops, in which non-coding *cis*-regulatory elements on the promoter of the clock genes are believed to play a key role in generating circadian transcription. Genetic evidence supporting this model, however, is still exclusively based on the effects of mutations in the protein-coding sequences of the clock genes. Therefore, while non-coding circadian *cis*-elements are assumed to be important for daily maintenance of behaviour and physiology, there is currently no direct evidence to corroborate this notion.

Because locomotor activity rhythms (LAR) and body temperature rhythms (BTR) are both robust and easy to be measured, they have been used to detect circadian rhythms in living animals. Interestingly, BTR is separately regulated from LAR, and BTR has essential roles in maintaining circadian energy homeostasis and entrainment of peripheral tissue clocks. A previous study in which subsets of neurons in the brain of rats were ablated suggests that LAR and BTR are controlled by different output pathways that originate from the suprachiasmatic nucleus (SCN). However, molecules involved in regulating BTR have not been identified so far.

Methodologically, there are several critical problems in measurements of BTR. In many studies, BTR measurements were performed by implanting a thermal sensor into abdominal cavity of animals. This requires surgery. Moreover, a sensor device is often large for small laboratory animals such as mice, making these experiments highly invasive. In addition, long-term measurement of BTR with high time-resolution over several days is difficult due to limited data storage performance of thermal sensor devices. LAR is usually detected by infrared sensor, but this conventional method also has several limitations. Firstly, it is difficult to accurately quantify locomotor activities because the sensitivity differs between infrared sensor devices, and the sensitivity changes gradually over time due to mechanical degradation. Secondly, mice often show non-locomotor activities such as feeding, grooming, and postural adjustments, but these subtle changes in activity cannot be distinguished via a conventional infrared sensor. These limitations have hampered elucidation of whether and how BTR and LAR are temporally related to each other.

In Chapter 1, using mice with a point mutation of the *cis*-regulatory element E′-box in the promotor of *Period2* (*Per2*), I showed that circadian transcription of *Per2* and other clock genes was drastically attenuated in cells extracted from the mutant mice, indicating that circadian core clock cycling is achieved through the *Per2* E′-box. Furthermore, these mutant mice cannot maintain proper LAR and BTR. **In Chapter 2**, I showed that the calcitonin receptor, a G-protein coupled receptor (GPCR) that is abundantly expressed in the SCN, is involved in BTR control without affecting LAR. **In Chapter 3**, I established a new method for simultaneous measurement of body movements and body surface temperature (BST) using an infrared camera to elucidate their detailed temporal relationship.

Chapter 1: Non-coding *cis*-element of *Period2* is essential for maintaining organismal circadian behaviour and body temperature rhythmicity.

To determine whether *cis*-element-mediated transcription/translation-based feedback loops is required for the formation of the circadian clock, I focused on the E'-box located near the transcriptional start site of *Per2*. I found that the mutation of the *Per2* E'-box attenuated the circadian rhythms of cultured cells and tissues, and prevented mice from maintaining proper circadian rhythms in locomotor activity and body temperature. These results provide the first genetic evidence that non-coding element-based *Per2* transcription is essential for generating cell-autonomous clock and for maintaining organismal circadian behaviour and body temperature rhythmicity.

Chapter 2: Calcitonin receptor modulates body temperature rhythms in mammals.

The neurons in the hypothalamic SCN, the center of the body circadian clock, are known to projects to various brain regions and involved in shaping circadian rhythms of many behavioral and physiological states. However, their molecular mechanisms are largely unknown. The calcitonin receptor (*Calcr*) is a GPCR that is abundantly expressed in the SCN, but its contribution to BTR has been completely unknown. To elucidate the role of *Calcr*, I simultaneously measured BTR and LAR of *Calcr*-deficient mice. LAR of *Calcr*-deficient mice were comparable to that of wild-type mice. However, BTR of *Calcr*-deficient mice are significantly different from that of wild-type mice. BTR of wild-type animal normally displays bimodal two peaks, one in the early night and at dawn, with a deep trough at midnight, but BTR of *Calcr*-deficient mice lost this characteristic dip at midnight and remained relatively unchanged throughout the night. Thus, I have identified for the first time a molecule involved in mid-night BTR control without affecting LAR.

Chapter 3: Temporal relationships between body temperature and behaviour revealed by thermographic imaging.

To elucidate the temporal relationship between behaviour and body temperature in mice, I developed a method able to trace simultaneously body movements and body surface temperature (BST). To this end, I used infrared video camera. It is known that locomotor activity and body temperature are highly correlated; basically, body temperature is high when animals are locomotorily active. As a result, locomotion is considered to elevate body temperature. However, I found that changes in BST are not always associated with locomotor activity changes. Interestingly, video analysis revealed that mice exhibit non-locomotor activities just before start of locomotion and that BST is increased in association with these non-locomotor activities. I also found that significant BST variations occur even when animals are at rest. Thus, my thermographic video imaging identified thermoregulation which is independent of locomotor activities.

（続紙 ２ ）

（論文審査の結果の要旨）

人類を含む地球上のほぼ全ての生物は体内時計をもち、地球の自転に伴う環境変化への適応として活動量や体温を24時間周期で自発的に変化させる。このような自発的なリズムは、個々の細胞に存在する時計遺伝子の5’上流ノンコーディング域のシスエレメントを介した転写フィードバックループによって成立すると考えられている（2017年ノーベル生理学・医学賞：体内時計を生み出す遺伝子機構の発見）。しかし当モデルの論理的根拠は時計遺伝子の蛋白質コーディング領域を欠落させた遺伝学的見地に基づいており、実際にノンコーディング域のシスエレメントが仲介するフィードバックが生体リズム形成に不可欠であるかどうかは未解明であった。シアノバクテリアにおいては転写を阻害しても時計蛋白質のリン酸化が概日変動を示すことが示され、従来のモデルに合わない分子時計機構の存在が議論され始めている。個体レベルにおいて、体内時計の中枢は、脳の視床下部にある視交叉上核が担う。視交叉上核のニューロン群は運動や体温などのすべての体内リズムを支配するが、これらの複数の異なる生理活動リズムがどのように協調的かつ統合的に制御されるのかはいまだほとんど明らかにされていない。このような中、嶋谷 寛之氏は本論文の第一章において、哺乳類の体内時計の中核振動子である*Per2*遺伝子の5’上流ノンコーディング配列に存在するシス制御エレメントE’-boxに点変異を導入したマウスを駆使して、本エレメントを介したフィードバックが個体の自発活動および基礎体温の安定的な日内変動の維持に不可欠であることを明らかにした。さらに第二章では、視交叉上核の一部のニューロンに発現するカルシトニン受容体に着目し、本受容体ノックアウトマウスを用いて、カルシトニン受容体が体温の正常な日内変動パターン形成に必要であるが、活動リズムの制御には関わらないことを明らかにした。第三章では、赤外線サーモカメラを用いたマウスの体温と活動の同時計測系を樹立し、マウスの体温が歩行行動とは完全に相関しないこと、歩行行動を伴なわない背伸びや毛づくろいなどの姿勢の変化によってマウスの体温が大きく変動し、これが体温の日内リズムの形成に大きく寄与していることを示した。これら一連の研究成果は、行動と体温の概日変動を支配する共通および個別メカニズムの同定を報告するものである。ヒトにおいても活動リズムと体温・代謝リズムの解離による健康障害が確認されており、本論文は体内時計の基本機構の解明を通じて医学・薬学に貢献する重要な知見をもたらしたといえる。よって、本論文は博士（薬科学）の学位論文として価値あるものと認める。また、２０２１年2月１９日に、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。なお、本論文は、京都大学学位規程第１４条第２項に該当するものと判断し、公表に際しては、（令和６年３月２２日までの間）当該論文の全文に代えてその内容を要約したものとすることを認める。

要旨公表可能日：　　年　　月　　日以降