The underlying mechanism of the circadian expression of concentrative nucleoside transporter 2 in the intestinal epithelial cells

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論文題名　：The underlying mechanism of the circadian expression of concentrative nucleoside transporter 2 in the intestinal epithelial cells
（マウス消化管における核酸輸送トランスポーター CNT2 の概日リズム制御機構）

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Many biological processes are subject to daily oscillations, and some of these are generated by a self-sustained oscillation mechanism called “circadian clock”. Molecular studies of the circadian clock system have revealed that oscillations in the expression of specific clock genes and their products play central role in the generation of 24-hour rhythms in physiology and behavior. In mammals, the master circadian pace maker is located in the hypothalamic suprachiasmatic nucleus (SCN). The circadian oscillation in the SCN are entrained to a 24-hour period by daily light input from visual neural system and produce output signals for coordinating the phase of independent oscillator in peripheral tissues. The peripheral oscillators generate daily rhythms in output physiology through the periodic activation/repression of clock-controlled output genes.

The concentrative nucleoside transporters (CNTs) are mainly expressed in the apical membrane of the intestinal epithelial cells and are responsible for the active uptake of nucleosides and their analogs from the intestinal lumen into the epithelial cells. In mammals, nucleosides metabolism is timely orchestrated in the liver. Several data already showed that the activities of de novo and salvage pathway enzymes exhibit a circadian oscillation and are under the control of the circadian clock. Furthermore, there was a time dependent variation of nucleosides and free bases abundance. These rhythms are not only influenced by the circadian clock system, but are under the control of other factor such as food intake. Therefore, day and night difference in food intake produces the circadian changes in the uptake of nucleoside from the small intestine. However, understanding of the role for CNTs in the circadian changes in the intestinal uptake of nucleosides is still limited. The objective of the study is to explore whether CNTs are involved in the circadian regulation of intestinal uptake of nucleosides in mice.

In the first chapter, the underlying mechanism of the circadian expression of CNTs was investigated by focusing on its plasmalemmal localization process. Two types of concentrative nucleoside transporters, CNT1 and CNT2 have been identified in mammals. The expression of CNT2, but not of CNT1, in the plasma membrane of intestinal epithelial cells of mice exhibit circadian oscillation without

changing its protein levels in whole cell lysate. The scaffold protein PDZK1 interacted with CNT2, thereby stabilizing its membrane localization. The expression of PDZK1 was under the control of molecular components of circadian clock. Temporal accumulation of PDZK1 protein in the intestinal epithelial cells enhanced the membrane localization of CNT2 during certain times of the day. The enhancement of CNT2 membrane localization by PDZK1 also facilitated the uptake of adenosine from the intestinal lumen into the epithelial cells. These results suggest a novel molecular mechanism of circadian expression of cell surface transporters and also provide new insight into the understanding about biological clock system generating obvious physiological rhythms.

In the second chapter, the influence of food intake on the expression of the scaffold protein and membrane expression of CNT2 were investigated by manipulation of feeding schedule. The lighting cycle is the most powerful “Zeitgeber” for the center of mammalian circadian clock system located in the SCN, but the act of feeding generates dominant cues for biological rhythms in the peripheral tissues so that under certain conditions, they can override the entraining signals coming from the SCN. Mice are nocturnal animals, so that they mainly consume their food during the dark phase. The time restricted feeding that allow mice to access the food during the dark phase had little effect on the rhythmic phase of PDZK1 and CNT2 in the plasma membrane of intestinal epithelial cells, but both protein levels were markedly changed by manipulation of feeding schedule that allow mice to access the food during the light phase. The 24-hour fasting disrupted the circadian rhythm of PDZK1 at transcriptional levels without changing the rhythmicity in the expression of clock genes in the intestinal epithelial cells. The fasting induced disruption of PDZK1 rhythms also affected the time dependency of CNT2 membrane localization. These results suggest the importance of feeding schedule for the circadian regulation in the function of scaffold protein. The feeding and fasting cycle seems to have a direct effect on PDZK1-mediated circadian localization of CNT2 in the intestinal epithelial cells.

The present findings in this doctoral dissertation showed that PDZK1-mediated circadian regulation of plasmalemmal localization of CNT2 caused the time-dependent change in the intestinal absorption of nucleosides. Furthermore, this membrane localization process was not only under the molecular circadian clock but was also regulated by feeding schedule. PDZK1 can interact with various cell surface proteins and stabilize their membrane expression. Consequently, other membrane transporters may also exhibit circadian oscillations that are time dependently anchored by PDZK1. Hereafter, it should be made a strategic arrangement to predict the absorption of the nucleoside analogues after its oral administration.