A molecular mechanism regulating the circadian rhythmic expression of ATF4 and abcg2 in mice

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General conclusion

Biological rhythms are a fundamental feature of life, and most organisms living on the earth have an internal pacemaker, called the circadian clock. The discovery of clock genes and the understanding of a general principle of their oscillatory mechanism have stimulated research in chronobiology with a major impact in life sciences research field. In fact, the major function of the circadian clock consists in the adaptation of physiology to daily environmental changes and the accompanying stresses such as UV exposure and food-containing toxic compounds. In this way, most aspects of xenobiotic detoxification are subjected to circadian regulation. This phenomenon is now considered as the molecular basis for the time-dependent modulation of efficacy and toxicity of the drug. However, it remains poorly understood how the molecular components of circadian clock regulate the expression of genes responsible for the xenobiotic detoxification.

In the first chapter, we clarified the mechanism regulating the circadian expression of ATF4 in mice, and showed a molecular link between the core oscillation machinery of the circadian clock and cAMP signaling pathway. The central components of the molecular clock participate in the regulation of ATF4 expression. Consequently, ATF4 acts as a molecule in the circadian output pathway for regulating the CRE-mediated expression. These findings suggest that ATF4 transmits extracellular stimuli to the downstream events of the circadian clockwork.

In the second chapter, we also provide the molecular link between cAMP-signaling pathway and xenobiotic detoxification. BCRP is one of the most important transporter for controlling the xenobiotic detoxification and the development of cancer resistance. ATF4 time-dependently bound to the promoter region of exon1B isoform of *Abcg2* gene, leading to the circadian expression of Abcg2 mRNA and BCRP. The oscillation in the expression of BCRP ultimately causes the time-dependent change in the intestinal absorption of sulfasalazine (Scheme 2).

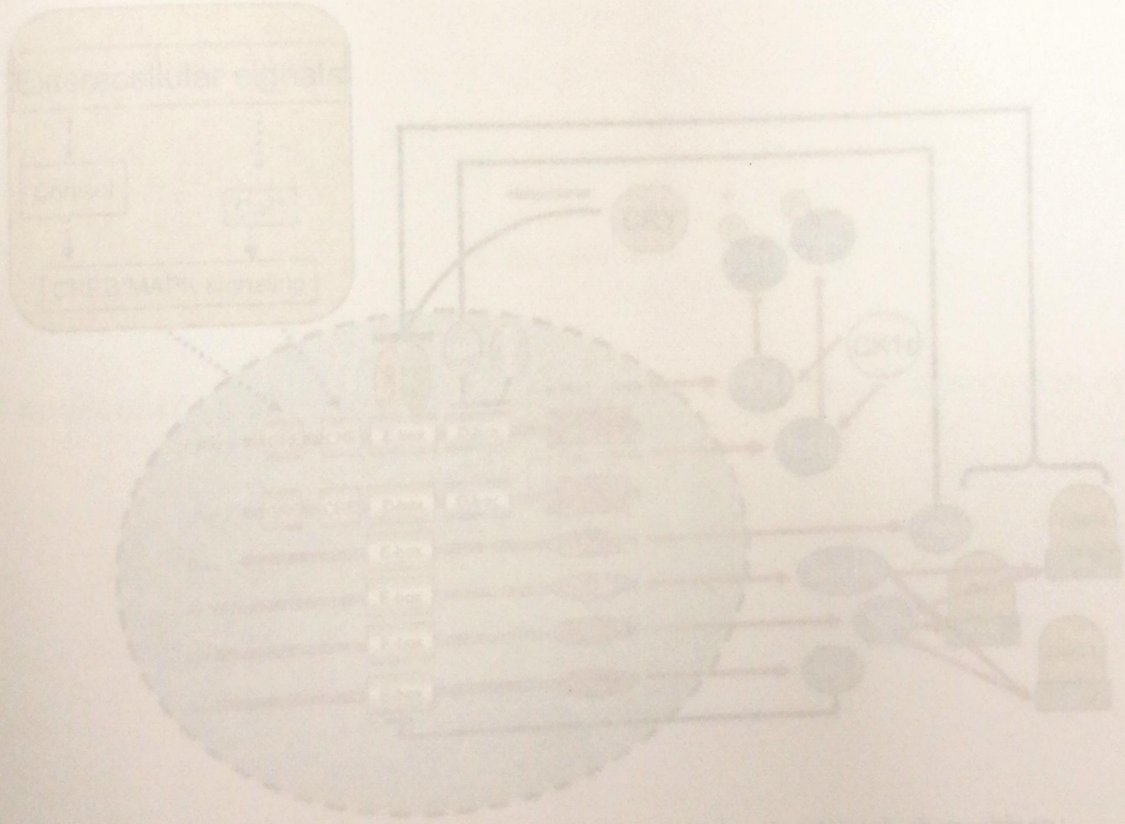
The individualization of pharmacotherapy has been achieved mainly by monitoring drug concentration. Consequently, dosage adjustment has been based on the inter-individual differences in drug pharmacokinetics. However, intra-individual variability should also be considered aiming at improving the rational pharmacotherapy; which we succeeded in this study to clarify the molecular link between the circadian clock and drug disposition.

expression of orphan nuclear receptor REV-ERBα, which in turn represses transcription of *Bmal1* (Preitner et al, 2002). This mechanism interconnects the positive and negative limbs of circadian clockwork circuitry and also regulates the 24-hr variations in output physiology through the periodic variation/repression of clock-controlled output genes (Jin et al, 1999, Maemura et al, 2000, Oishi et al, 2003). PAR bZIP proteins, HLF, TEF, and DBP are examples of such output mediators, because their expression is regulated by core oscillator components (Ripperger et al, 2000) (Scheme 1). The circadian-controlled output pathways include those that control the expression of many enzymes and regulators involved in xenobiotic detoxification, such as cytochrome P450, carboxylesterases, and xenobiotic transporters (Gachon et al, 2006). cAMP-dependent signaling is also involved in the output pathways of the circadian clock system, which in turn sustains the core oscillation mechanism and constitutes an additional, bona fide component of the oscillatory network (O'Neill et al, 2008). Intracellular accumulation of cAMP induces the cAMP response element (CRE)-mediated gene expression via activation of activating transcription factor (ATF)/CRE binding (CREB) proteins (Lin and Green, 1989). Although the circadian variation of phosphorelated state of CREB is detected in mouse body, the functional importance of the transcriptional factors for regulating the time-dependent changes in the pharmacotherapy remains to be clarified yet.

ATF4 is a member of ATF/CREB family. It is ubiquitously distributed amongst mice tissues. It is essential for the cellular signaling and proliferation. It shows high resemblance among different species in the developmental chain. Human and mouse ATF4 show great similarity on both the DNA sequence and protein levels.

The objective of this study is to characterize the circadian nature of a member of ATF/CREB family ATF4 in mice and to evaluate the role of ATF4 in the circadian clock machinery by focusing on the xenobiotic detoxification system. In chapter 1, I clarified the core clock proteins (CLOCK and BMAL1; the positive regulators and PER2 and CRY1; the negative regulators) to regulate the cyclic transcription of ATF4 through the conserved proximal E-box element. In Chapter 2, I evaluated the role of ATF4 on the circadian detoxification system though controlling the transcription of *abcg2* gene. Breast cancer resistance protein (BCRP), encoded by *Abcg2*, functions as an energy-dependent efflux pump for expelling cytotoxic substances. The molecular components of the circadian clock regulated the expression of an isoform of *Abcg2* gene through the mediation of ATF4. Since intestinal BCRP acts as a barrier to limit oral drug

absorption, the possibility was also tested whether the oscillation of BCRP function affected the pharmacokinetics of its substrates after oral administration.



Scheme 1. Schematic image of the molecular network of the circadian clock machinery.