System-level Identification of Mammalian Circadian Clocks

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Mammalian circadian clocks consist of complexly integrated regulatory loops, making them difficult to elucidate without both the accurate measurement of system dynamics and the comprehensive identification of network circuits. Toward a system-level understanding of this transcriptional circuitry, we identified clock-controlled elements on 16 clock and clock-controlled genes in a comprehensive surveillance of evolutionarily-conserved cis-elements and measurement of their transcriptional dynamics using an *in vitro* validation system[1], in which cultured fibroblasts transiently transfected with clock-controlled reporter vectors exhibited robust circadian bioluminescence.

Here we demonstrate the roles of E-boxes/E'-boxes, DBP/E4BP4 binding elements(D-boxes) and RevErbA/ROR binding elements(RREs) on nine, seven and six genes, respectively. Our results indicate that circadian transcriptional circuits are governed by two design principles: E-box/E'-box and RRE regulation follows a "repressor precedes activator" pattern, resulting in delayed transcriptional activity, while D-box regulation exhibits a "repressor antiphasic to activator" mechanism, which generates high-amplitude transcriptional activity. Our analysis further suggests that E-box/E'-box regulation represents a topological vulnerability in mammalian circadian clocks, which has been functionally verified using *in vitro* phenotype assay systems[2].

In this conference, we will report a current progress in system-level identification of mammalian circadian clocks, and also present a development of "molecular-timetable" methods for detection of body time and rhythm disorders[3].

1. Hiroki R. Ueda et al. "A transcription factor response element for gene expression during circadian night" Nature, 418, 534-539, 2002.

2. Hiroki R. Ueda et al. "System-level Identification of Transcriptional Circuits Underlying Mammalian Circadian Clocks" Nature Genetics, 37, 187-192, 2005.

3. Hiroki R. Ueda et al. "Molecular-Timetable Methods for Detection of Body Time and Rhythm Disorders from Single-time-point Genome-wide Expression Profiles" PNAS, 101, 11227-11232, 2004.