Systems analysis of context-dependent epidermal growth factor receptor signaling in the circadian pacemaker

Daniel E. Zak[1,2], Haiping Hao[1,2], Gregory M. Miller[1,2], Rajanikanth Vadigepalli[2], Babatunde A. Ogunnaike[1], and James S. Schwaber[2]

(1) University of Delaware, Department of Chemical Engineering, Newark, DE 19716.

(2) Daniel Baugh Institute for Functional Genomics and Computational Biology, Department of Pathology, Cell Biology and Anatomy, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107.

Circadian rhythms are activity cycles with periods of roughly twenty-four hours (*circa-dies*) that are ubiquitous throughout all complex forms of life (Young and Kay, 2001). The suprachiasmatic nucleus (SCN) of the hypothalamus is the central coordinator of circadian rhythms in mammals (Reppert and Weaver, 2002); it integrates physiological signaling inputs to produce a coherent rhythm that is suited to environmental and homeostatic conditions (Yannielli et al., 2004; Mendoza et al., 2005). SCN integration is “gated” or “circadian context-dependent” because the response to a signaling input depends on the circadian time when that signal is initiated (Gillette and Mitchell, 2002).

In the present work, we have undertaken a systems-level analysis of context-dependent signaling through the epidermal growth factor receptor (EGFR) in the SCN. EGFR signaling has been implicated in the mechanism by which the SCN regulates locomotor activity (Kramer et al., 2001) and has been suggested to play a role in the autosynchronization of SCN rhythms (Jobst et al., 2004). We collected gene expression profiles for SCN microdissected from brain slices treated with EGF during the circadian day and the circadian night. We employed mixed-model analysis of variance (Pinheiro and Bates, 2000; Wolfinger et al., 2001) to identify genes with circadian context-dependent regulation by EGFR. By performing a transcriptional regulatory network analysis (Vadigepalli et al., 2003) on the regulated gene groups, we generated hypotheses about the transcription factors responsible for the clock-dependent regulation of EGFR target genes. Established EGFR responsive transcription factors (ex: Ets1, c-Jun) and transcription factors involved in the entrainment of the circadian clock (ex: CREB) were identified by the analysis. Measurement of the transcription factor expression levels by quantitative real-time PCR revealed different mechanisms by which they may mediate circadian context-dependent EGFR gene regulation in the SCN.

Our results provide insights into the mechanisms behind functional input integration in the SCN. They also provide a framework for further analysis of this important physiological process, demonstrating the power of integrating factorial experimental design with gene expression profiling and promoter analysis to generate testable hypotheses about gene regulatory mechanisms.

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