Published in final edited form as:

Q Rev Biol. 2017 June ; 92(2): 201–202. doi:10.1086/692218.

Review of Methods in Enzymology Volumes 551 and 552 Circadian Rhythms and Biological Clocks, Part A and B Edited by Amita Sehgal

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Circadian Rhythms and Biological Clocks, Part A and B, present the application of key biological systems and state-of-the-art methodologies to understanding the daily metabolic cycles encoded in genomes. Part A discusses genetics, organismal readouts, and techniques to investigate molecular clock components, whereas Part B focuses on the physiological aspects of biological clocks, including entrainment, the relationship between central clocks in the brain and those in periphery and approaches to the study of human circadian rhythms. Early chapters (Axelrod and Hurley) provide both a historical and a contemporary overview of the biological clocks found in two key model organisms: the fruit fly *Drosophila melanogaster* and the filamentous fungus *Neurospora crassa*. The former (Axelrod) also discusses the merits and drawbacks of mutagen and RNAi-based screens to detect genes that determine sleep behavior. As a compliment to genetics, Hirota describes chemical screening techniques for discovering interactions between clock components and novel small molecules. Methods for studying circadian behavior under conditions that more closely approximate the natural environment are related by Green, along with a defense of controversial findings from these studies that suggest clock behavior in the wild may differ from that in the laboratory. Many chapters provide the recipe-level detail needed to characterize clock components with molecular biology and biochemistry. Particularly beneficial are several sections that explain how to execute cell-wide analyses through phosphoproteomics (Price, Krahmer), chromatin immunoprecipitation sequencing (ChIP-seq), and RNA-seq in a variety of organisms (Cha, Takahashi, Zhou, Li, Abruzzi). These discussions emphasize that eukaryotic clocks are excellent systems for studying transcription owing to the fact that their core transcriptional-translational feedback loops are cyclic and responsive to entrainment. In this vein, detailed protocols are presented for applying next-generation-based sequencing methods (i.e. ChIP-seq and RNA-seq) in mammalian cells, flies and fungi (Cha, Takahashi, Zhou, Li, Abruzzi). For example, Abruzzi relates the power of the GAL4-UAS system in *Drosophila* to perform RNA-seq on small numbers of specific neurons. Additionally, several chapters explore methods to knock epitope-tagged clock components into cells so that interaction partners and large complexes can be isolated by immunoprecipitation (Price, Cha, Kim); Egli catalogs the biophysical approaches that can be potentially taken to understand such macromolecules once they are isolated in sufficient quantities.

Part B concerns the study of clock physiology in complex multicellular systems. The first section explores methods for dissecting the physiological responses of central clock circuits, with several chapters relating the use of gene expression reporters to monitor transcriptional responses (Herzog, Hughes). Later, Gaspar describes the application of lentiviral expression systems that report on clock gene expression in primary human cells – methods that have the promise of revealing individual responses to mutations, environmental variation and drugs. Protocols for investigating electrophysiological outputs and locomotor studies are given for a range of cell-types and organisms (Meijer, Schlichting, Hughes, Gaspar). Three central chapters of the volume concern the characterization of metabolic rhythms (Peek, Milev and McLoughlin). Of particular utility are discussions of special cell types and protocols for following cycles of oxidative stress, oxygen consumption and cardiovascular fitness. A key take-away is that different systems present distinct challenges for sample preparation and component analysis. The volume closes with a section on the study of circadian rhythms in humans. Roenneberg discusses the merits of questionnaires designed for self-reporting sleep patterns and how the resulting data have intriguing implications for human behavior (Roenneberg). Hsu then gives a textbook experimental design for discovering dominant mutations that affect human sleep. It is quite interesting to learn how alertness and performance tests can be given to humans (Goel) and mice (Tam) to evaluate relationships among sleep pressure, genetic background, circadian rhythms and diseases such as schizophrenia (Tan). Overall, these volumes provide a remarkable summary of the methods, experimental systems and findings of cutting-edge research into circadian rhythms. The breadth of systems presented and the depth of scrutiny, from molecular details to organismal behavior, forge an essential resource for all types of scientists studying chronobiology, as well as an informative read for anyone who wishes to understand better the ticking of their biological clock.