

PREFACE

In the 10 years since a previous circadian volume of *Methods in Enzymology* was published, the circadian field has evolved with the introduction of new concepts, new approaches, and, of course, many new investigators. As the previous volume was preceded by an explosion of discoveries and information about how clocks work in diverse organisms, from cyanobacteria to humans, one might have predicted a gradual plateauing of activity, but this was clearly not the case. Importantly, the new discoveries have continued to come from species across the phylogenetic tree, and so the field remains truly interdisciplinary, which is reflected in two volumes, *Circadian Rhythms and Biological Clocks Part A* and *B*.

We continue to see advances in the methods used to measure and analyze rhythms, to identify new circadian genes, and to characterize known clock components. In fact, clocks are now being modulated with a goal of therapeutic application. At the same time, there is considerable focus on the molecular mechanisms through which rhythms are transmitted by the clock. Major clock proteins are transcription factors that effectively drive rhythmic expression of many genes, which generally vary from tissue to tissue. The mechanism by which such rhythmic transcription occurs is a subject of intense investigation, and sophisticated techniques have been brought to bear on it. However, it is also evident that posttranscriptional or even post-translational mechanisms alone can drive the cycling of RNA or protein. Indeed, proteins may not even need to cycle in terms of levels as their activity can be regulated through cyclic modifications, such as phosphorylation. Several clock proteins are kinases, and in cyanobacteria, the entire clock can be reconstituted as a rhythmic phosphorylation cycle. In addition, 24-h oscillations that are independent not only of transcription but also of known clock elements have been proposed for redox pathways in the cell. Thus, while genome-wide analyses are allowing identification of RNAs expressed rhythmically in different tissues, and even in small groups of brain neurons, proteomic studies have been initiated to pinpoint rhythms at this level. All of these have required development and use of the appropriate, anatomical, molecular, biochemical, and statistical tools described here.

Rhythms in molecules lead to rhythms in cellular and organismal physiology. As clocks are found throughout the body, they control many physiological processes and behaviors. In the brain, rhythms of electrical activity

are synchronized across clock cells and transmitted across circadian circuits, likely comprised of neurons and glia, to generate rhythmic behavior. Cellular rhythms, such as in mitochondrial respiration, may occur in every cell in mammals. In addition, each organ/system, such as the cardiovascular system, has its own circadian physiology that can be measured through application of specific methods. Interestingly, while the light:dark cycle is the most powerful entraining stimulus for the clock in the brain, which then synchronizes other body clocks, rhythmic gene expression in peripheral tissues like the liver responds most strongly to the time of feeding. Thus, aberrant feeding schedules can have adverse effects on metabolic function, but the time of feeding can even be manipulated to produce beneficial consequences.

Overall, it is increasingly evident that circadian rhythms are critical for organismal fitness. Several approaches are now being used to assess circadian function in humans, its control by genetic factors, and its relevance to human health. Disrupted rhythms have been associated with neurological and psychiatric disorders and studies are now underway to address the significance of such association. These volumes provides methodological insight into circadian physiology and behavior in model organisms and in humans and touches upon the pathological implications of circadian dysfunction.

While I took on the job of putting these volumes together, the actual credit should go to all the contributing authors, who took time out of their extremely busy lives to make the volume representative of the best work in the circadian field. I am incredibly grateful for their efforts and their cooperation. Also, all of this was made possible by the constant help of Editorial Project Manager, Sarah Lay, who was a real pleasure to work with throughout the process.

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