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Cover illustration: The cover depicts the character in Japanese for "kai", the name of the central circadian clock gene cluster in cyanobacteria. "Kai" means cycle or rotation number in Japanese, and is therefore apropos for a gene cluster that controls circadian cycles in cyanobacteria.

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Preface

Internal biological clock systems exist in nearly all organisms, including humans, rodents, insects, plants, fungi, and bacteria. These biological (circadian) rhythms allow for each system to maintain internal time and likely provide an adaptive advantage to those organisms. The discovery of circadian rhythms in the cyanobacteria was surprising to some who believed that bacteria were too "simple" to possess the machinery necessary for generating these internal rhythms; however, investigations into the basic biology of the temporal separation of oxygen-evolving photosynthesis and oxygen-sensitive nitrogen fixation demonstrated that this diverse group of bacteria was capable of generating and maintaining internal timing.

Since the discovery of a biological clock in cyanobacteria in the 1980s, the field has exploded with new information. The cyanobacterial model system for studying circadian rhythms, *Synechococcus elongatus* PCC 7942, has allowed for a detailed genetic dissection of the bacterial clock due to the methods in molecular biology and biochemistry that are currently available. Although the majority of research has been conducted using *S. elongatus*, work in other cyanobacterial species has been instrumental to our understanding of the bacterial biological clock. In addition, examination of the various, fully sequenced cyanobacterial genomes suggest that there may be several variations upon the same theme for producing internal rhythms in prokaryotes. Through mathematical modeling and generating synthetic oscillators in other bacterial strains, in conjunction with information derived from in vivo and in vitro oscillations, the mechanism for the generation of biological rhythms in a single cell can be better elucidated.

The rapid advancement in our understanding of the bacterial circadian clock is due to many different avenues of discovery and inquiry. The success in understanding bacterial circadian programs is due, in part, to the genetically malleable *S. elongatus* PCC 7942 system and the insightful investigations of geneticists, molecular biologists, evolutionary biologists, and biochemists. What cannot be overlooked when discussing the success of this model system is that the molecular work stands on the shoulders of hundreds of years of circadian insights into the physical, physiological, and chemical basis of rhythms defined by circadian biologists outside the prokaryotic arena. Currently the *S. elongatus* system is arguably one of the best characterized circadian clock systems of any model system, even though it is one of the newest model systems to be investigated.

Thanks to the many advances in our understanding of the bacterial biological clock, this book serves as a timely review of the fundamental process of circadian timing in prokaryotes. It is also organized as a compendium of the most current data on the circadian mechanism in prokaryotes. The chapters in this book are intended to address the history and background of the cyanobacteria and initial investigations and discovery of circadian rhythms in this diverse group of microorganisms (Chaps. 1, 2, 3, 4). The molecular basis and structure of the circadian clock system are reviewed (Chaps. 5, 6, 7), as well as entrainment of the oscillator with the environment (Chap. 8) and the downstream genes and behavioral activities that are controlled by the clock (Chaps. 9, 10, 11). A demonstration of the adaptive significance of the circadian clock in cyanobacteria (Chap. 12) and the prokaryotic clock's remarkable stability are also discussed (Chap. 13). Due to the great diversity of the cyanobacteria as a group, investigations have been conducted to address the evolution of cyanobacterial clock genes and whether those genes are involved in the generation of circadian rhythms in cyanobacterial strains other than the *S. elongatus* model system (Chaps. 2, 14, 15) and mathematical models for *S. elongatus* clock function and synthetic oscillator models are included (Chaps. 16, 17).

Our hope is that this book will serve many audiences, spanning from those who are currently expanding the studies discussed within, to those who are beginning their endeavor into the wonderful world of prokaryotic clock systems. We envision this text as a comprehensive reference of past accomplishments, but hopefully also a stepping stone for future work on this amazing group of microorganisms and timing. We are grateful to each of our colleagues and friends who contributed to this work. It is our hope that you enjoy reading each chapter as much as we enjoyed putting this combined work together.

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Chapter 1
Classic Circadian Characteristics: Historical Perspective and Properties Relative to the *Synechococcus elongatus* PCC 7942 Model

Jayna L. Ditty and Shannon R. Mackey

Abstract The purpose of this chapter is to introduce the basics of circadian biology relative to the cyanobacterial model system. It is meant to define the terms, characteristics, and rules that pertain to the study of circadian biology in the context of the cyanobacterial systems used to elucidate the mechanisms by which the prokaryotic circadian clock functions. In addition, its purpose is to serve as a conduit to the chapters in this book, which comprehensively review our most recent understanding about each of these canonical characteristics in the *Synechococcus elongatus* PCC 7942 model system as well as other cyanobacterial and prokaryotic systems.

1.1 Introduction

1.1.1 Overview

Our planet rotates about its axis every 24 h, which exposes the majority of plants and animals that inhabit the earth to sidereal fluctuations of light and temperature. This daily change in light and dark was a strong selective force (for those organisms that are subject to it) to devise physiological mechanisms with which to respond to, or better yet predict, when these daily changes were going to occur. As a result of this pressure, organisms have evolved internal timing mechanisms to anticipate the daily variations in light and temperature; this anticipatory behavior provides a selective advantage to the organism (DeCoursey 1961; Ouyang et al. 1998; Michael et al. 2003; Woelfle 2004; Johnson 2005).

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This daily clock phenomenon was termed "circadian" in 1959 by Franz Halberg using the Latin terms *circa* for "about" and *dies* "day". Therefore circadian phenomenon pertain to biological activities with a frequency of one activity cycle every 24 h (Halberg et al. 1977). The purpose of this chapter is to introduce the basics of "circadiana": to define the numerous terms, characteristics, and rules that pertain to the study of circadian biology in the context of the cyanobacterial systems that have been used to elucidate the mechanism by which the prokaryotic circadian clock functions. In addition, its purpose is to serve as a conduit to the chapters in this book, which comprehensively review the most recent understanding about each of these canonical characteristics in the *Synechococcus elongatus* PCC 7942 model system as well as other cyanobacterial and prokaryotic systems.

1.1.2 Historical Perspectives

Investigations into the mechanism that organisms use to relate and respond to diurnal fluctuations in light and temperature have been undertaken at least as early as the 1700s. One of the earliest reports that correlates behavior with specific times of day came from the French astronomer Jean-Jacques d'Ortous deMairan, who made the observation that the leaves of heliotrope plants move in response to changes in light. Even more importantly, he recognized that these leaves would continue to move in the same pattern when kept in constant darkness (DD), generating the first evidence that a behavioral activity could be regulated by an internal mechanism of the plant, and not a result of environmental light and dark cues (deMairan 1729). During the same period, the Swedish botanist Carl Linneaus developed his *horologium florae* or "flower clock," which could be used to tell the time of day based upon when particular plant species would flower (Freer 2003).

The modern field of chronobiology, or the study of biological timing processes in living things, was initiated in the mid-1950s by Colin S. Pittendrigh and Jürgen Aschoff. They were instrumental in defining and organizing the principles of a circadian system that mapped the course for circadian research, and these rules still hold true to the present time (Aschoff 1960, 1981; Pittendrigh 1961, 1981). While the characteristics and principles of circadian biology were being brought to bear by early circadian biologists, a particular question of interest was whether circadian activity was a learned behavior in organisms or had a genetic basis. The work of Erwin Bünning in 1935 alluded to the answer by providing evidence that period length was heritable in bean plants (Bünning 1935); however, it was not until the early 1970s that the first evidence for a genetic basis to circadian activity was brought to light by two independent groups working in fruit flies and fungus. Ronald Konopka and Seymour Benzer isolated *Drosophila melanogaster* mutants that had altered eclosion and activity rhythms. Each of the mutations was complemented by one genetic locus, termed the *period* gene (Konopka and Benzer 1971). Soon after, Jerry Feldman and Marian Hoyle identified the *frequency* gene, which

was shown to be essential for rhythms of asexual spore formation in *Neurospora crassa* (Feldman and Hoyle 1973).

The study of circadian clocks and rhythms was sequestered to eukaryotic models as historical circadian dogma dictated that nuclear structure, intercellular communication, and generation times longer than 24 h were required for rhythmic activity – characteristics that are lacking in prokaryotic cells and, at least in part, in unicellular eukaryotes (Edmunds 1983; Kippert 1987). However, in the 1980s, several lines of evidence were emerging to contradict the "eukaryocentric" circadian requirements. The cyanobacteria are a large and diverse group of microorganisms that are typically photoautotrophic and diazotrophic, and are responsible for a vast majority of the carbon and nitrogen fixation in the environment (see Chap. 2; Garrity 2001). Within several different cyanobacterial species, circadian activity in nitrogen fixation, amino acid uptake, and cell division were identified (see Chap. 3; Grobbelaar et al. 1986; Mitsui et al. 1986; Sweeney and Borgese 1989; Huang et al. 1990; Chen et al. 1991; Grobbelaar and Huang 1992; Schneegurt et al. 1994). While the physiological evidence drastically changed the manner by which scientists thought about circadian biology, a good model system for prokaryotic circadian research was lacking. Ultimately *S. elongatus* PCC 7942 became the model of choice in part because of the vast amount of molecular tools available in this strain (see Chap. 4; Golden 1987; Golden 1988; Kondo et al. 1993, 1994; Ishuira et al. 1998; Andersson 2000).

1.2 Properties of a Clock-Controlled Rhythm

Regardless of the model system one is using to understand the circadian process, the underlying mechanisms achieve a similar goal: maintain an internal, 24-h time. A circadian clock system is defined as an endogenous mechanism that allows an organism to temporally regulate biological activity as a function of the 24-h day. Such biological activities that are regulated by the circadian clock are therefore coined circadian rhythms (Pittendrigh 1981; Edmunds 1983; Dunlap et al. 2004; Koukkari and Sothern 2006). The rhythmic nature of daily activity can be described by three terms that correspond to the characteristic descriptions of a waveform: period, phase, and amplitude.

1.2.1 Period

The period of a rhythm is defined as the duration of one complete activity cycle (Fig. 1.1). Therefore, a circadian period would be an activity that completed its cycle (with a frequency of approximately 1) over a 24-h period of time (Dunlap et al. 2004; Koukkari and Sothern 2006). When measured under constant conditions (see Sect. 1.3.1) the period is defined as the free-running period (FRP),