Preface

Rhythmicity is a pervasive feature of life. Most organisms, from bacteria to humans, have the ability to interpret and predict the daily cycles of our world, which indicates the presence of a timing device, a circadian (from the Latin *circa diem*, "about a day") clock, able to synchronize the endogenous functions with the external environment. Furthermore, the ability to manipulate the temporal dimension offers ground to complexity, as the organisms have the opportunity to separate competing or even incompatible functions within the same cell. Thus, it is not surprising that natural selection is operating on the circadian clock, an additional reminder of the importance of this regulatory pathway. Selection has been shown directly by competition experiments between clocks with different periodicities, and indirectly by studying the molecular evolution of clock genes.

In the last 20 years, the molecular mechanisms underlying the functioning of the circadian clock have been actively investigated for several model systems. It has emerged that circadian timing affects every kind of organism and, in multicellular organisms, many different cell types. Basic and specialized cell functions are regulated by the clock through multiple molecular events. Furthermore, although the major divisions of life use different molecular cogs in the building of the pacemaker, there is a common design based on interlocked negative feedback loops. Many components and molecular functions can feed into the loops at different levels, making the architecture of the clock intrinsically robust and open to a wide range of interactions with other major regulatory pathways. This has become even more apparent after microarray studies have shown that key regulators of metabolic pathways, cell cycle components, ion channels, and immuno-response genes are all transcribed in a rhythmic fashion. Further developments have extended the description of the interconnection between the circadian and cell cycles and sketched a role for clock dysfunctions in cancer development. Although we have begun to understand the basic mechanisms of the clock, we still do not have a definitive answer to many questions. We still ask ourselves how the clock generates rhythmic phenotypes in the model systems we have studied for so long. Moreover, we start asking with more insistence how the circadian clock is regulated in other organisms, especially those also showing robust rhythmicity in other temporal domains.

To answer those questions, we have at our disposal a large arsenal of methodologies. These range from a whole organism approach, analyzing physiology and behavior, to a more reductionist attitude using genetics, molecular and cellular biology, and post-genomics technologies. The power of this multilevel approach is visible in the huge progress achieved by the chronobiology field in the last 20 years. However, the variety of methods, further multiplied by the peculiarities of each model system, and the hitches added by the temporal dimension, might have a hard impact on the novice.

The aim of *Circadian Rhythms: Methods and Protocols* has been to provide a resource that can be adopted by several types of users: those who are new to circadian biology, those who are already active in the field but are interested in learning new techniques, and researchers who are considering moving to a new model system or undertaking comparative studies and would like to consult protocols applied to different organisms before starting the study of new species. This task has been achieved by collecting a full range of methods, many provided by leading experts in the field, that should satisfy the needs of the novice, by illustrating procedures that have been recently introduced in circadian studies, and by presenting, for many basic techniques, variations to take into account the peculiarities of different model systems.

Finally, I would like to express my gratitude to the contributors who have shared their protocols and experience with the community, making the realization of *Circadian Rhythms: Methods and Protocols* possible.

Ezio Rosato