BOOK REVIEWS

Circadian Clockwork

Molecular Genetics of Biological Rhythms. MICHAEL W. YOUNG, Ed. Dekker, New York, 1993. xiv, 319 pp., illus. $125. Cellular Clocks, 4.

Rhythms of plant and animal behavior have been known since antiquity, especially those daily rhythms that are prominently synchronized to the periodic alternation of light and darkness. A more recent discovery is that many of these biological rhythms are innately generated by endogenous clock mechanisms that continue to oscillate with circadian (about a day) periods even in environments free of external timing cues. Such timekeeping systems are of profound importance to all organisms forced to adapt to the Earth's rotation about its axis. They function both to recognize local time and to measure elapsed time; provide an "internal temporal order" for the flexible scheduling of various chemical and physiological events; allow for anticipatory responses to periodic environmental challenges; and participate in seasonal behaviors and navigational strategies that rely on the sun as a celestial compass. From a clinical perspective, they may underlie the pathophysiology of certain diseases; influence the efficacy, side effects, and toxicity of drugs; and dictate the performance capabilities of shift workers. Although much is known about the formal properties, localization, and physiology of these clocks, the molecular processes that make up the actual oscillatory machinery have not been fully understood. This situation is changing quickly through the application of the powerful techniques of genetics and molecular biology, and readers of *Molecular Genetics of Biological Rhythms* can now learn what all the excitement is about.

Much of the volume is devoted to the results of experiments in which mutations are induced and the surviving mutants screened for altered clock phenotypes. Single-gene mutations with abnormal circadian rhythms were described about 20 years ago by Ronald Konopka and Seymour Benzer in fruit flies (*Drosophila*), Jerry Feldman and Marian Hoyle in fungi (*Neurospora*), and Victor Bruce in algae (*Chlamydomonas*) and only 5 years ago by Martin Ralph and Michael Menaker in hamsters (*Mesocricetus*). The most intensively studied loci, *period* in *Drosophila* and *frequency* in *Neurospora*, each comprise a series of alleles with both short– and long–period-length mutants as well as strains with behaviors so disorganized that they appear to be arrhythmic. Remarkably, the cloned *per* and *frq* clock genes show a region of sequence similarity, although under closer analysis this putative homology is questionable and the resemblance may be fortuitous. The *per* mutations map to single-base substitutions of the gene's coding region, with an abnormally truncated protein accounting for the arrhythmic phenotype. The function of *per* is not well understood and the genetics is complex, but some tantalizing features are reviewed in the book. Both *per* mRNA abundance and protein levels exhibit circadian rhythms (with the peak of the latter lagging the former by 6 to 8 hours), and there are reasons to propose that the *per* gene product might act as a transcriptional regulator with a delayed negative feedback on the transcription of its own mRNA. The *per* mutations alter not only circadian locomotion and pupal eclosion rhythms but also the approximately 1-minute courtship song cycle, suggesting that circadian and ultradian oscillatory mechanisms might include a common molecular substrate. It turns out, however, that the situation is far from straightforward; analyses of complementation tests, genetic dosage effects, and flies molecularly transformed with *per* fragments all indicate that the *per* gene must be controlling 24-hour and 1-minute rhythmicities differently. Meanwhile, although *Drosophila*'s photoperiodic timer involves a circadian mechanism, this clock still seems to function to discriminate day length in *per* mutants. So it is likely that we have only reached the end of the beginning—not the beginning of the end—of the evolving *per* story.

Also discussed are mutants that affect clock properties other than period (for example, phase or temperature compensation), as well as the design of new schemes to select for altered phenotypes rather than just screen for them. It seems possible that some critical clock genes may still escape detection if the null mutants represent lethal phenotypes. Nevertheless, the collection of mutant phenotypes available to us now already serves as an invaluable resource for physiological studies of circadian organization. Mosaic analysis and tissue transplantation using long– and short–period-length mutants in insects and hamsters have helped to localize endogenous pacemaker sites and to assess their relative contributions to overt rhythmicity. In *Drosophila*, photoreceptor mutants have shown that retinal input is not necessary for entrainment of circadian rhythms to the environmental light-dark cycle, and rhythm-specific mutants (altering locomotion but not eclosion, and vice versa) should prove useful in dissecting the pathways and processes responsible for the overt expression of clock outputs.

Another research strategy covered in the book is the use of subtractive hybridization and other techniques to isolate genes whose expression varies as a function of time of day. These studies aim to demonstrate how the clock is coupled to its downstream effectors and may ultimately identify rhythmic components of the timekeeping apparatus itself. It is already clear that such clock-controlled genes are regulated at multiple levels: transcriptional (as with the genes for the chlorophyll a/b binding proteins [*cab*] in higher plants, a fungal hydrophobin in *Neurospora*, and the cone pigment iodopsin in chicken retina), post-transcriptional (the translational rhythm of luciferin binding protein synthesis in the marine alga *Gonyaulax*), or both (the expression of the *per* gene product in *Drosophila* and probably vasopressin in rodent suprachiasmatic nuclei). The clock also acts to temporally gate the induction of light-activated genes (for example, c-*fos* in suprachiasmatic nuclei and *cab* in plants) that may be part of the clock's own entrainment (input) pathway; these findings are stimulating the search for clock-controlled *trans*-acting transcription factors. Given this expanding array of nested loops, researchers may encounter increasing difficulty in separating the causal oscillatory loop from others governed by it.

The unspoken promise of these molecular analyses is that they will be able to address some of the most fundamental questions in chronobiology. Are the clock's state variables to be found as circadian rhythms of intracellular regulatory molecules, or does rhythmicity instead emerge from coupled ultradian processes or even from chaotic intercellular interactions? How does the oscillator exert phase control over all the disparate rhythms that it drives? Are "clock genes" used exclusively for clocks, or do they function differently in distinct tissues and compartments? Which molecules are used to build

the clock and which ones to run it? Is the clock conserved through evolution, or do functional similarities in diverse organisms represent an adaptation to the same geophysical cycle rather than a reflection of a common molecular mechanism? How are tidal and annual clocks to be explained? Even further challenges await an accounting of the circadian rhythms of prokaryotic cyanobacteria and the possibility of multiple clocks in individual *Gonyaulax* unicells.

It is apparent that a genuine understanding of circadian clocks will require the integration of molecular genetics with other approaches. At present, the study of rhythmicity is weakest in some of the best genetic models (yeast, *Caenorhabditis elegans*, *Arabidopsis*) whereas some of the best cellular circadian systems (*Gonyaulax*, isolated eyes of the marine mollusks *Aplysia* and *Bulla*, retina of the frog *Xenopus*, pineal gland of the chicken) are among the poorest systems for molecular genetics and so are not discussed in this book. There is much fertile ground here, and it is easy to share the enthusiasm of the contributors to this book that the molecular dissection of circadian clocks, in concert with cellular and network analyses, is among the most attractive problems in behavioral biology today.

William J. Schwartz
Department of Neurology,
University of Massachusetts Medical School,
Worcester, MA 01655

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Structural Drug Design

Nucleic Acid Targeted Drug Design. C. L. PROPST and THOMAS J. PERUN, Eds. Dekker, New York, 1992. xvi, 619 pp., illus. $165.

The success of molecular biology in furthering our understanding of cellular physiology and pathophysiology is recorded weekly in the scientific literature. Articles presenting new insights into the mechanism of viral infectivity or growth derangement in neoplastic cells frequently conclude with an expression of hope that our improved understanding of pathogenic processes at the molecular level will lead to the development of novel therapeutics. What is actually involved in the path that leads from biological insight to the development of a new therapeutic agent? Several years ago Propst and Perun sought to provide an overview of the topic of structure-based drug design for all those interested in the emerging technology. Their 1989 *Computer-Aided Drug Design* explores the design of drugs targeted at proteins (for example, enzymes and receptors). *Nucleic Acid Targeted Drug Design*, an interdisciplinary work containing contributions by chemists, biologists, and computer scientists, takes up where *Computer-Aided Drug Design* leaves off, covering the design of drugs targeted at nucleic acids.

Traditionally, new classes of drugs have been identified by randomly screening microbial broths, plant extracts, or preexisting libraries of small organic compounds for a desirable biological activity. In fact, most of the compounds described in *Nucleic Acid Targeted Drug Design* were first identified in this way. More recently, structural biologists have convinced the drug discovery and development community that studying the structure of specific molecules as well as the mechanics of their interactions with known therapeutics can make the search for new and better drugs much more efficient. In their introductory chapter Perun and Propst present a balanced discussion of the drug design process that acknowledges the role of serendipity while providing a protocol for structure-based, nucleic acid–targeted drug design.

Like its predecessor volume, *Nucleic Acid Targeted Drug Design* is divided into two sections: Methods and Applications. In the first of these Wang and Robinson provide a comprehensive overview of the use of x-ray crystallography and nuclear magnetic resonance spectroscopy in exploring drug-DNA interactions. Biologists will be pleased to find a minimum of mathematics in this chapter, which places emphasis on the practicalities of structural studies.

Many antineoplastic agents bind to DNA, and some covalently modify the nucleic acid, often introducing breaks into the chromosome. Potent examples include the DNA intercalator adriamycin, a drug that has some efficacy in the treatment of solid tumors including breast cancer, and *cis*-platinum, a DNA cross-linking agent that plays a central role in the therapy of testicular cancer. Many other such agents are described. Unfortunately, all of these compounds have a common problem: the line between efficacy and toxicity is thin and easily crossed. This poor therapeutic index is not surprising given the limited ability of these compounds to distinguish DNA sequences much longer than dinucleotides. It is refreshing, then, to find in this book an exceptional chapter on the molecular basis of sequence-specific protein–nucleic acid interactions. Zinc finger proteins, the leucine zipper, and the helix-turn-helix motif are described, and detailed examples show how certain structural motifs bind to the major groove of DNA, recognizing specific nucleotide sequences and DNA conformations.

In the section on applications, the therapeutic merits of interrupting protein-DNA interactions are described in a chapter on quinolones, an important group of broad-spectrum antibiotics. Although a high-resolution picture of the structure of the DNA gyrase alone or complexed to duplex DNA is unavailable, a structure-activity model is developed for this group of compounds from a combination of spectroscopic studies and activity data on more than 500 quinolone analogs. Nalidixic acid, a first-generation quinolone, was found useful only in the treatment of urinary tract infections, but third-generation agents such as ciprofloxacin are readily absorbed when administered orally and demonstrate significant activity against Gram-positive organisms, including *Staphylococcus aureus*, and Gram-negative bacteria, including stubborn hospital-acquired pathogens such as *Pseudomonas*.

The book concludes with two chapters on antisense therapy—a topic popular with readers of the *Wall Street Journal* as well as those who keep up with the scientific literature. Clearly, antisense therapies, whether catalytic ribozymes or simpler code blockers, offer the prospect of tremendous specificity with (presumably) little toxicity. Yet enthusiasm for these approaches should be tempered by an awareness of the problems that this class of therapeutics has encountered: poor bioavailability, sensitivity to endogenous nucleases, difficulty in penetrating cell membranes, and the high cost of synthetic scale-up. Although progress has been made in each of these areas, the hurdles that remain make it unlikely that this type of compound will find its way into clinical medicine any time soon.

Should nucleic acids be targets for drug design? Given that antineoplastic agents that exert their therapeutic effect through intercalation with DNA seem to strike a Faustian bargain and that antisense therapy still faces formidable pharmacokinetic problems, protein–nucleic acid interactions may provide the best targets for drug design (especially in the area of viral and bacterial diseases, where the human homolog of the protein target should have diverged substantially from its microbial counterpart). In any case, with this valuable volume Propst and Perun have opened a window on the challenge of applying the tools of molecular biology to the search for safe and effective therapeutic agents.

Fred E. Cohen
Departments of Medicine, Pharmaceutical Chemistry, and Biochemistry and Biophysics,
University of California,
San Francisco, CA 94143–0446