HOLIDAY LECTURES ON SCIENCE

TEACHER AND STUDENT GUIDE

CLOCKWORK GENES

DISCOVERIES IN BIOLOGICAL TIME

An activity and content guide on the topic of biological clocks based on lectures by

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Presented as a public service by the Howard Hughes Medical Institute

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Viewing and Participating in This Year's Lectures

To view the lectures—The 2000 lectures will be available live starting at 10:00 a.m. EST on December 4 and 5 via satellite in the United States, and worldwide via webcast from WWW.HOLIDAYLECTURES.ORG/WEBCAST. If you plan to view the live satellite broadcast, please refer to the back inside cover of this guide for information on satellite coordinates and telephone numbers in case of technical problems. If you plan to view the live webcast, make sure that RealPlayer has been successfully installed on your computer ahead of time. After December 5, the webcast will be available on demand. For more information about the webcast, visit the Holiday Lectures website at WWW.HOLIDAYLECTURES.ORG/WEBCAST. You will find instructions and a link to the "Technical Tips" page, which is also available directly at WWW.HHMI.ORG/GRANTS/HELP.HTM.

To ask questions—Students and teachers will have the chance to ask questions directly to Drs. Rosbash and Takahashi by participating in live Q&A sessions to be held each day from 1:30 p.m.–2:30 p.m. EST on both the satellite broadcast and the webcast. Questions may be phoned in live to 1-800-223-9050, e-mailed in advance to asklive@hhmi.org, or asked via our chat software at WWW.HOLIDAYLECTURES.ORG/ASKLIVE2000.

To view the January rebroadcast—If your school receives its satellite programming via the Channel One Network and you have no other satellite capacity, you will not be able to receive the Holiday Lectures at the times and coordinates listed on the inside back cover of this guide. However, Channel One Connection will rebroadcast Lectures 1 and 2 on January 17, 2001, and Lectures 3 and 4 on January 24, 2001. Please ask your school's media specialist to tape the Channel One rebroadcast. See your January Channel One TeachWorld.com poster for more information.

To order educational materials—Videotapes of the lectures and a detailed index to the lectures will be available a few months after the live event. In addition, the interactive demonstrations are available on the Web and later on CD-ROMs. To order these materials from the 2000 or earlier Holiday Lectures, visit the HHMI website at WWW.HOLIDAYLECTURES.ORG/ORDER and fill out an order form.

Joseph Perpich (far left), vice president for grants and special programs, and Dennis Liu (far right), senior grants program officer, with Donald Ganem and Brett Finlay, the two scientists who delivered the 1999 Holiday Lectures on Science.

Dear Educator:

Each December the Howard Hughes Medical Institute (HHMI) presents the Holiday Lectures on Science, a lecture series that brings the latest developments in a rapidly moving field of research into the high school classroom. The Institute selects some of the world's leading biomedical researchers to deliver the lectures. This year, two Institute investigators who have done groundbreaking work in biological clocks will present the four-part lecture series "Clockwork Genes: Discoveries in Biological Time."

Ever since the series began in 1993, we have added important new features that make it easier for you and your students to make the best use of the lectures. Although the lectures began as a local event for students in the metropolitan Washington, D.C., area, we now make them available to a national and international audience through a live satellite broadcast and simultaneous webcast. The lectures are also rebroadcast over the Channel One Network, which reaches 12,000 schools that might not otherwise have cable access. Once the live lectures are over, they are available on our website as on-demand streaming video, as well as on videotape.

Using information from evaluations and conversations with hundreds of teachers and students, we continue to add new elements to our Teacher and Student Guide that are aimed at helping you integrate the lectures into your classroom curriculum. This year, we are pleased to announce a new feature, "Teacher-Directed Classroom Activities," developed with the advice of high school teachers who serve on the Institute's teacher advisory committee. Three activities are designed for general and honors biology. Because we also know that Advanced Placement biology students are a major audience for this guide, we have included a laboratory exercise that enhances a required AP biology laboratory. The guide also contains a page correlating lecture content to the AP curriculum. In addition, we have supplemented "Challenge Questions," a feature we began last year, with guidelines for helping your students answer the questions.

We know that you and your students enjoy reading the lecturers' biographies. Therefore, we have expanded the "Lives in Science" section. You'll learn why the scientists—Michael Rosbash and Joseph S. Takahashi—chose science as a career and what challenges they overcame to pursue their research. Takahashi describes why mentors are so valuable to scientists, and Rosbash explains the importance of learning to balance work with outside interests.

The core of the guide remains the lecture summaries and key concepts, which this year provide more details about the lectures. The publications and websites listed in the guide can direct students to avenues for further exploration. The guide's description of the features of the Holiday Lectures website (**WWW.HOLIDAYLECTURES.ORG**) should also prove valuable to your classroom. Our website contains a wealth of materials that not only supplement the

lectures but also enhance science education in many other ways. We encourage you to explore the animations, multimedia activities, virtual laboratories, and virtual exhibits on the site. We believe that you will also find the “Ask a Scientist” feature—which enables students to e-mail questions and receive prompt answers from scientists who volunteer for the program—an important classroom tool.

Please continue to check the website at **WWW.HOLIDAYLECTURES.ORG** for program updates and topical resources. We hope you enjoy the 2000 Holiday Lectures on Science and all the lectures in the years to come, with the new features that we continue to add based on your good counsel and advice.

Sincerely,

Dennis Liu, Ph.D.
Senior Grants Program Officer

Joseph Perpich, M.D., J.D.
Vice President, Grants and Special Programs

Ever since the scribe Androsthenes, traveling on campaigns with Alexander the Great, observed that the leaves of certain trees open during the day and close at night, people have recorded their fascination with the intrinsic biological rhythms that characterize virtually every species in the plant and animal kingdoms. Intriguing internal clocks keep pace with the 24-hour cycle of day and night and light and dark, enabling organisms to adapt their behavior accordingly. Whether these daily—or circadian—rhythms help a single-celled organism anticipate the first rays of morning light or a rodent prepare for a busy night foraging for food, they are a ubiquitous feature of life on earth.

Circadian clocks govern human activities as well. From sleep-starved students to active octogenarians, the body intrinsically observes a daily regimen of wakefulness and sleep. Our internal clocks keep us mentally alert during the day and prompt us to rest and recuperate during the night. The pattern continues even in the absence of external cues from the rising and setting sun. Research volunteers living in a bunker in constant dim light naturally maintain a roughly 24-hour cycle of activity and rest. Researchers have concluded that sunrise can reset the clock, but that the clock does not depend on sunrise and sunset to keep time.

We generally take our biological clock for granted, but as anyone who has experienced the misery of jet lag or worked the night shift knows, disturbing our natural rhythms exacts a toll. In rare cases, severe disturbances in the body clock can be inherited, with problems affecting members of particular families. Individuals suffering from advanced phase sleep syndrome, for example, routinely fall asleep in the late afternoon or early evening, only to wake up in the middle of the night.

Despite a long-standing fascination with biological clocks, only in recent years have scientists made major progress in understanding the molecular mechanisms that regulate them. The circadian pacemaker, or control center, in humans is located in the brain's hypothalamus. A cluster of only several thousand neurons called the suprachiasmatic nucleus (SCN) governs a wide range of 24-hour physiological variations in our body, ranging from changes in hormonal levels and body temperature to susceptibility to disease. Understanding the detailed workings of the circadian clock may explain why heart attacks occur more often in the morning and why the incidence of asthma is more common at night, for example.

The breakthrough in unraveling the molecular basis of circadian rhythms came, as genetic advances so often have, using the fruit fly, *Drosophila melanogaster*. In the late 1960s and early 1970s, a student working in the laboratory of the famous *Drosophila* geneticist Seymour Benzer identified three mutant flies that exhibited abnormal patterns of circadian behavior. These mutant flies were named *period* or *per* mutants, and all of their circadian timekeeping problems were traced to different changes—mutations—in a single gene named *per*. It took some 20 years from the initial discovery to clone and determine the nucleotide sequence of the *per* gene, the first "clock gene."

Over the past five years, the pace of activity in circadian rhythm research has accelerated. Studies using cyanobacteria, fungi, fruit flies, mice, plants, and humans have identified a small but growing family of genes that control circadian rhythms. Many of the most important recent discoveries in this rapidly advancing field have come from the laboratories of this year's lecturers, Michael Rosbash and Joseph S. Takahashi. Their studies and those of their colleagues have unveiled the molecular choreography of circadian rhythms. The molecular players, with names like *timeless*, *Clock*, and *cycle*, share a remarkable degree of sequence similarity across different species. The emerging picture is of exquisitely sensitive molecular mechanisms in which the levels of specific circadian genes ebb and flow in harmony with daily light and dark cycles.

The significance of the latest research into circadian rhythms goes beyond our efforts to understand how fungi, fruit flies, and even humans track time or to devise effective new treatments for various sleep disorders. The flurry of new insights offers one of the best examples in modern biology of how complex patterns of behavior can be scripted by interactions at the molecular level. These insights are a portal into the fascinating world of genes and behavior.

Lives In Science

Meet this year's lecturers, Michael Rosbash, Ph.D., and Joseph S. Takahashi, Ph.D.

MICHAEL ROSBASH, PH.D.

For a decade, Michael Rosbash's research was as basic as fundamental research gets: the metabolism and processing of RNA, which forms the molecular link between gene and protein. But in the early 1980s, he found himself increasingly interested in a more sweeping—even philosophical—question: the genetics of human intelligence and personality. That's why today Rosbash is one of the world's leading experts on circadian rhythms—the internal body clock—in the fruit fly *Drosophila melanogaster.*

Flies? Aren't the waking and sleeping patterns of insects far removed from the genetics of human personality?

"I've asked myself the same question," Rosbash says. But noting that personality involves an unknown (but presumably vast) number of unidentified genes, he explains: "Once you have multiple factors, with variable genetic expression, which then also interact with the environment, it becomes a very complex problem."

"My taste in doing science," Rosbash says, "is heavily skewed toward asking questions where I think there will be substantial answers—and where you can solve the problem to some degree of satisfaction within your lifetime."

Rosbash, 56, a Howard Hughes Medical Institute (HHMI) investigator at Brandeis University in Waltham, Massachusetts, already has plumbed the mystery of the circadian clock to a considerable degree of satisfaction, although he says lots of questions still remain. He and his major collaborator, Jeffrey Hall, also of Brandeis, cloned the first *Drosophila* circadian clock gene, *period*, in 1984, and in 1990 they proposed the mechanism—a transcriptional negative feedback loop—by which the clock works. A decade later, after many additional gene discoveries, their model and mechanism still hold up. Moreover, the mechanism appears to apply not just to flies but also to mammals—including people—making clear that the workings of *Drosophila*'s clock are indeed relevant to human neuroscience.

"BEING A RESEARCHER IS NO DIFFERENT FROM DOING ANYTHING WITH UNBRIDLED ENTHUSIASM"

When Rosbash entered the field, several behavioral mutations had been identified in *Drosophila* through phenotypic studies that identified mutations by their effects on fly behaviors. "Seymour Benzer and his students were pioneers in these important early studies," says Rosbash. But scientists were unable to determine how those genes actually functioned until the advent of modern recombinant DNA techniques—the ability to clone the genes themselves, identify and analyze their proteins, and transfer a mutated or a normal gene into an organism. "That broke open the field," Rosbash says. "It allowed one to understand what the genes might do and then to make links to the same processes in other organisms."

Rosbash was well positioned to apply cutting-edge molecular genetics techniques to the problem of circadian clocks. Since his days as a graduate student at the Massachusetts

Institute of Technology in 1966–1970, his research had centered on the role of RNA in the many steps between the transcription of the DNA sequence that forms a gene to the assembly of a finished protein—one of the most fundamental processes in biology.

He continues his RNA research today. The graduate students, postdoctoral students, and technicians in his lab are divided into two groups, one studying circadian clocks and the other RNA synthesis, processing, transport, and function. Most scientists, although they may have multiple projects running, engage in only one specialized field; Rosbash balances two.

Unlike some researchers, Rosbash doesn't trace his interest in science back to early childhood. In fact, he recalls attending a symposium several years ago during which "one guy would say, 'When I was six years old, I was fascinated with the problem of consciousness.' The next guy would get up and say, 'When I was eight, I built an artificial intelligence computer in the basement.'" Somewhat skeptically, Rosbash says, "When I was that age, I was playing with toy trains and watching the Red Sox." (He remains a long-suffering "pathological and pathetic" Boston Red Sox fan.)

His childhood wasn't as idyllic as that may sound, however. Rosbash's parents fled Nazi Germany in 1938, "and my mother went from a wealthy family to cleaning toilets in New York City before my father found a job." His father was a cantor, a Jewish clergyman who sings or chants prayers; his mother had earlier been forced out of medical school when the Nazi government expelled Jews from German universities.

BALANCING ACT

As a graduate student at the Massachusetts Institute of Technology (MIT), Michael Rosbash learned to balance his personal passions with his commitment to scientific research. The need for making hard choices is a lesson he continues to share with his laboratory team.

During the mid- to late 1960s, the Vietnam War escalated, and Rosbash became "heavily involved" in antiwar activities, protesting and organizing against the war at high schools and colleges around the Boston area. Finally, he reached the point where he was spending more time on antiwar activities than in the laboratory, and his lab chief, Sheldon Penman, stepped in.

Penman asked Rosbash either to start putting in reasonable time in the laboratory or to consider a change to another lab. Rosbash sought the advice of friends, including David Baltimore, then an assistant professor at MIT, who also was deeply involved in antiwar efforts. "And David said to me, 'You know, it's really his right. If you want to change labs and can find a place where they'll let you do almost nothing for six months to a year, go ahead.' "

Although Rosbash was surprised by Baltimore's answer, he respected his opinion. So after more thought, he told Penman the next day, "Fair enough. My first obligation is to be a grad student." Rosbash scaled back his antiwar campaigning.

Now, as a lab chief, he says he would handle a graduate student who spent most of his time and energy outside the lab just the way Penman did. "Business first," he says. "I like to think I'm a little more tolerant about what people do in their spare time—or if they have a problem, that they can come in and tell me. If it's truly temporary, I'll tolerate it. If it's semipermanent, I won't," he says.

"You know, when you spend 60 hours a week at a job and everyone else does, too, it becomes a bit like family," he adds. "The younger people look to you for advice and stability. So it's like being a parent. You have to be sympathetic, understanding, and tolerant, and yet firm on occasion."

Rosbash was born in Kansas City, Missouri, but the family moved to the Boston area when he was about two years old. When he was 10 and his younger brother was six, his father died of a heart attack at the age of 42. His mother, who had just registered at Boston University Medical School to resume her training, had to abandon medicine again and go to work—first as a technician in a cytology laboratory and ultimately as the chief technician there—to support her family.

In high school, Rosbash was "reasonably interested" in science, mostly math, "but I didn't work very hard. I wasn't inspired by anything." At the California Institute of Technology, he originally planned to major in math, but "I found myself not very interested in math or, I might add, very outstanding at that level of competition with abstract math."

During his sophomore year, he found his first biology course electrifying. The genetic code was just being cracked, and there were weekly updates. In the summer after his sophomore year, he worked in the laboratory of Norman Davidson, a renowned chemist who was transitioning into the nascent science of molecular biology. Davidson was "a personal inspiration."

"This guy was not only a brilliant scientist, but he also absolutely loved his work," Rosbash says. "He was happy as a clam in the lab. Now and then, Davidson wore a tee shirt bearing the motto 'I'd rather be in the lab (or maybe playing tennis).' And I thought, 'Gosh, if anybody can enjoy work so much, there must be something to this stuff—not just as a student researcher but as a life's pursuit,'" Rosbash says.

Caltech was good for Rosbash in other ways, too. For the first time in his life, he made a mark in sports. He played on the freshman baseball team and throughout college as a tackle on the varsity football team. "I was sort of a semi-jock in college, albeit the definition at Caltech was a very low standard," he says. "It was very strange to go to Caltech and fulfill your desire to be a reasonably competent athlete."

Rosbash is married to a fellow scientist, Nadja Abovich, who came to Brandeis in 1979 and worked in his laboratory for almost 20 years. He has a 25-year-old stepdaughter, Paula, who is studying to become a graphic artist, and a 14-year-old daughter, Tanya, who is in ninth grade and "a good athlete as well as a good student."

"We spend a lot of time with our 14 year old and her athletic exploits," Rosbash says. He helps out with her basketball team, and his wife resigned from the lab late last year, in part to spend even more time with Tanya.

Combining a research career with a family is difficult, Rosbash agrees, but he doesn't think this situation is unique to research. "Being a researcher is no different from doing anything with unbridled enthusiasm," Rosbash says. "People who are obsessed by what they do, or very ambitious, or both, work very hard. But I think people who are healthy and well balanced, and have a spouse who helps them maintain a little more balance, are well served to take that time and build a family and enjoy it," he adds. "Someone once said to me, 'No scientist ever looks back on his career and says I wish I'd spent more time in the lab.' They all say, 'I wish I'd spent more time with my kids.'"

Michael Rosbash, Ph.D.

Career Highlights

Born March 7, 1944, in Kansas City, Missouri

EDUCATION

Undergraduate Degree: B.S., Chemistry, California Institute of Technology, Pasadena, California, 1965

Graduate Degree: Ph.D., Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, 1970

POSTDOCTORAL TRAINING

1971–1972: Research Fellow in Zoology, University of St. Andrews, Fife, Scotland

1972–1974: Research Fellow in Genetics, University of Edinburgh, Edinburgh, Scotland

"France and Scotland were very active in molecular biology at the times I went to these places, especially in 1966 and the early 1970s, respectively. But I was also lured by an interest in foreign cultures, language, and travel. My mother has always had a bit of the same tendencies; so whether through genetics or environment, I've probably inherited these same interests."

KEY APPOINTMENTS

1980 and 1986: Associate Professor and Professor, Department of Biology, Brandeis University, Waltham, Massachusetts

1989: HHMI Investigator at Brandeis University

1989: Adjunct Professor, Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts

SELECTED HONORS, AWARDS, AND FELLOWSHIPS

1965–1966: Fulbright Fellow, Institut de Biologie Physico Chimique, Paris, France

1971–1974: Helen Hay Whitney Fellow

1988–1989: Guggenheim Fellow, Pasteur Institute, Paris, France

1997: Elected Fellow of the American Academy of Arts and Sciences

SELECTED PROFESSIONAL ACTIVITIES

Editorial board member of *RNA* and *Cell*

Member of NIH Sleep Disorders Research Advisory Board

Chair, Department of Biology, Brandeis University

PUBLICATIONS

More than 200 original research articles in peer-reviewed journals such as *Cell, Genes and Development, Journal of Embryology, Neuron, Science,* and *Nature*

JOSEPH S. TAKAHASHI, PH.D.

As a child, Joseph S. Takahashi lived in "some unusual places where there were a lot of exotic animals." His father, an economist, had a number of overseas assignments, taking the family first to Burma and then to Pakistan. In Burma, Takahashi remembers riding elephants and taking boat trips up the Irrawaddy River. In Pakistan, it was riding camels and deep-sea fishing in the Arabian Sea. "I always had many pets, and our house was a menagerie of dogs, cats, rabbits, pigeons, and tropical fish," he says.

His interest in animals eventually led him to major in biology at Swarthmore College thinking he would likely pursue a career in medicine. But Kenneth Rawson, a Swarthmore professor who was Takahashi's first mentor (see sidebar), taught a course with a "remarkable field and laboratory section on the physiological basis of animal behavior," Takahashi recalls. It was Rawson who set Takahashi on the research path he now vigorously pursues—circadian rhythms.

As a Howard Hughes Medical Institute (HHMI) investigator and professor of neurobiology and physiology at Northwestern University in Evanston, Illinois, Takahashi, 48, leads an effort to sort out the genes and proteins that make up the mammalian circadian clock—and then to figure out how they work together ultimately to control the behavior of the animal. In 1997 Takahashi's lab discovered the first circadian rhythm gene in mammals, the mouse gene *Clock*, an acronym for "circadian locomotor output cycles kaput." Since then, researchers have identified eight other mammalian circadian genes, enabling scientists to study the interplay of proteins that make up the clock's negative-feedback mechanism at the most basic molecular level.

Most recently, in a major paper in the April 21, 2000, issue of *Science*, Takahashi and his colleagues reported cloning the gene responsible for the *tau* mutation in hamsters, a spontaneous mutation first recognized in 1988 by Takahashi's Ph.D. mentor, Michael Menaker. When present in two mutant copies, the gene shortens the animal's daily cycle to 20 hours rather than 24. It codes for an enzyme that is involved in regulating several other circadian-clock proteins. It is closely related to a *Drosophila* gene known as *doubletime* and to a human gene known as *casein kinase I epsilon*—another demonstration of what Takahashi calls "a striking level of evolutionary conservation" of the clock mechanism in animals.

"WE ARE JUST BEGINNING TO UNDERSTAND THE KINETICS OF CIRCADIAN MECHANISMS"

Takahashi's genetic circadian discoveries have given him the reputation in some quarters of being a hard-core molecular geneticist. In fact, his Ph.D. training at the University of Oregon in Eugene in 1981 was in neuroscience. It was in the laboratory of Michael Menaker—"a remarkable person who provided vast resources and an unusually stimulating environment for research"—that Takahashi's appreciation for neuroscience, evolutionary biology, and large-scale experiments began in earnest.

Later, Takahashi did postdoctoral work as a pharmacology research associate trainee at the National Institute of Mental Health. At Northwestern, whose faculty he joined in 1983, most of his early research was conducted at the cellular level, and he didn't begin to work on mouse genetics until the early 1990s. But all through his career, Takahashi has pursued the mechanism of circadian rhythms as a model for understanding how the brain controls behavior.

"Since graduate school, I have always been focused on the mechanism of circadian clocks," he says. "And I will do anything it takes to understand that problem—whatever task we have to do, whether it's molecular biology, genetics, or electrophysiology."

This attitude will probably lead him to another discipline, biophysics, before he is done. Takahashi says that a biological mechanism, such as the circadian clock, can't be called "solved" until it can be expressed in the analytical, quantitative language of biophysics. "The beauty of biophysics is that you can write equations which describe the behavior of particular molecules in quantitative terms," he says. "We're not there for rhythms. We are just beginning to understand the kinetics of circadian mechanisms."

THE VALUE OF MENTORS

When Joseph S. Takahashi recounts his own scientific background, he does so in terms of the people—mentors and role models—who have influenced him along the way.

He chose science as a career in his junior year at Swarthmore College because of courses taught by Ken Rawson. Rawson had been trained in the European tradition of ethology, studying the natural behavior of animals in their own environments. His courses highlighted the link between innate behavior and genes. Evolution, Rawson made clear, has driven the selection of particular forms of animal behavior, or of extreme adaptations such as bat echolocation, which scientists can then use as models. "I was just fascinated," Takahashi says.

He chose circadian rhythms as a field after working for a year with Patricia DeCoursey of the University of South Carolina, who was the first scientist to demonstrate that light pulses can reset the circadian clock in mammals. She also gave him some "extremely good advice" about how to pick a Ph.D. mentor. "Not only do you have to work for someone who is good," Takahashi recalls her saying, "but you need to be able to really get along. You could go to work for a very prominent person, but if you don't click with that person, it's not necessarily going to help you down the road."

Takahashi's Ph.D. mentor, Michael Menaker—who was then at the University of Texas in Austin but moved later to the University of Oregon—fitted that prescription. "Menaker is an unusually creative and optimistic person," Takahashi says. "His laboratory was particularly appealing because it had a massive satellite facility"—an old factory building with huge animal rooms to record the circadian behavior of rodents and birds. "It had all these components that I love: machinery, the requirement to build things and fix things on site, and scale," Takahashi says.

Takahashi was also strongly influenced by Colin Pittendrigh and Jürgen Aschoff, who were founders of the field of circadian rhythms. He admires Eric Kandel, an HHMI investigator at Columbia University in New York, as "an incredible scholar, not just in science but in history and the arts." Kandel's research into learning and memory "spans the entire dimension of the field, from behavior to genes and all the steps in between," Takahashi says. "That is the approach we try to emulate." As he begins to turn his attention toward biophysics, Takahashi finds that Denis Baylor of Stanford University is "a very clear role model in biophysics because of his rigor and special insights into understanding mechanisms."

Although today his lab is largely a molecular genetics lab, “we’ve never lost our behavioral and physiological roots,” he says. “We started with the behavior. And the behavior is what’s taken us to genes. Understanding those genes was really the key that we needed to understand the behavior.”

Takahashi emphasizes that this approach is hardly unique in neuroscience. His lab is, if not unique, at least highly unusual in another respect. Harking back to his days at Richard Montgomery High School in Rockville, Maryland—when he and a friend rebuilt the V8 engine in a 1959 Ford Thunderbird as a senior project—he insists that the people in his lab not only know how to operate complex laboratory instruments but also understand how those instruments work. “I strongly believe that one must begin with first principles,” he says.

If someone uses a microscope, Takahashi says, he or she ought to be familiar with optics and should know how the instrument functions. “Otherwise, they’re not going to use the microscope properly.” If they use a scintillation counter—a device that measures the presence and concentration of radioactively labeled substances such as proteins—“they really should understand how the instrument works.” Otherwise, they will just have to take the machine’s numbers at face value. “If they don’t know how it works, they cannot make a judgment about whether those numbers are valid or not, or what kind of artifacts compromise those numbers,” he says.

Takahashi may be a hard taskmaster, but he apparently is a generous collaborator. Although many scientists are reluctant to share credit, Takahashi believes collegiality is “very important” in science, and he seeks out collaborations in which each partner can bring special strengths to a project. For example, he recruited Menaker and Martin R. Ralph as collaborators in the effort to find the gene responsible for the hamster *tau* mutation.

Menaker, now at the National Science Foundation Center for Biological Timing at the University of Virginia in Charlottesville, and Ralph, who is at the University of Toronto, made the original discovery of the *tau* mutation, Takahashi explains. “I felt it was important for them to participate in the ultimate discovery of the gene,” he says. “I think that’s the way it should be.”

His work keeps Takahashi incredibly busy. “The schedule for professionals is just brutal,” he says. He tries to get home by 6 p.m. each evening—he lives just 10 minutes away from his lab—but he often puts in some work at night, linking with his lab’s computers remotely.

His wife, Barbara Takahashi, a medical doctor who also holds a master’s degree in public health, stopped practicing medicine to look after their children, Erika, 9, and Matthew, 6. “She felt it was more important to raise the children,” Takahashi says. “This is a very complicated decision—career versus family. I feel incredibly fortunate to have such a wonderful and supportive family.” He likes to give Matthew a ride to school in the morning in his bright blue Miata sports car and to handle other small chores. “I can pitch in a little bit,” he says. “What I would call very minor, though.”

Perhaps surprisingly, because science is typically thought of as a stay-in-the-lab endeavor, Takahashi finds that the biggest drain on his family time is travel. He makes about 25 trips a year to scientific conferences, advisory committee meetings, and symposia of one sort or another. Recently, his travel pace has been accelerating. “That’s because the field is sort of hot right now,” Takahashi says. “Clocks are hot.”

Joseph S. Takahashi, Ph.D.

Career Highlights

Born December 16, 1951, in Tokyo, Japan

EDUCATION
Undergraduate Degree: B.A., Biology, Swarthmore College, Swarthmore, Pennsylvania, 1974

Graduate Degree: Ph.D., Institute of Neuroscience, Department of Biology, University of Oregon, Eugene, Oregon, 1981

POSTDOCTORAL TRAINING
1981–1983: Pharmacology Research Associate Training Program, Laboratory of Clinical Sciences and Laboratory of Cell Biology, National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland

KEY APPOINTMENTS
1983, 1987, 1991: Assistant Professor, Associate Professor, and Professor, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois
1996: Walter and Mary Elizabeth Glass Professor in the Life Sciences, Department of Neurobiology and Physiology, Northwestern University
1997: HHMI Investigator at Northwestern University
1997: Professor of Neurology, Northwestern University Medical School

SELECTED HONORS, AWARDS, AND FELLOWSHIPS
1983: Alfred P. Sloan Research Fellowship in Neuroscience
1985: NSF Presidential Young Investigator Award
1985: Searle Scholars Award, The Chicago Community Trust
1986: Honma Prize in Biological Rhythms Research, Honma Life Science Foundation, Sapporo, Japan
1987–1997: MERIT Award, National Institute of Mental Health
1995: Sixth C.U. Ariëns Kappers Award, Netherlands Society for the Advancement of Natural Sciences, Medicine and Surgery
1995–1999: Bristol-Meyers Squibb Unrestricted Grant for Neuroscience Research
2000: Fellow, American Academy of Arts and Sciences

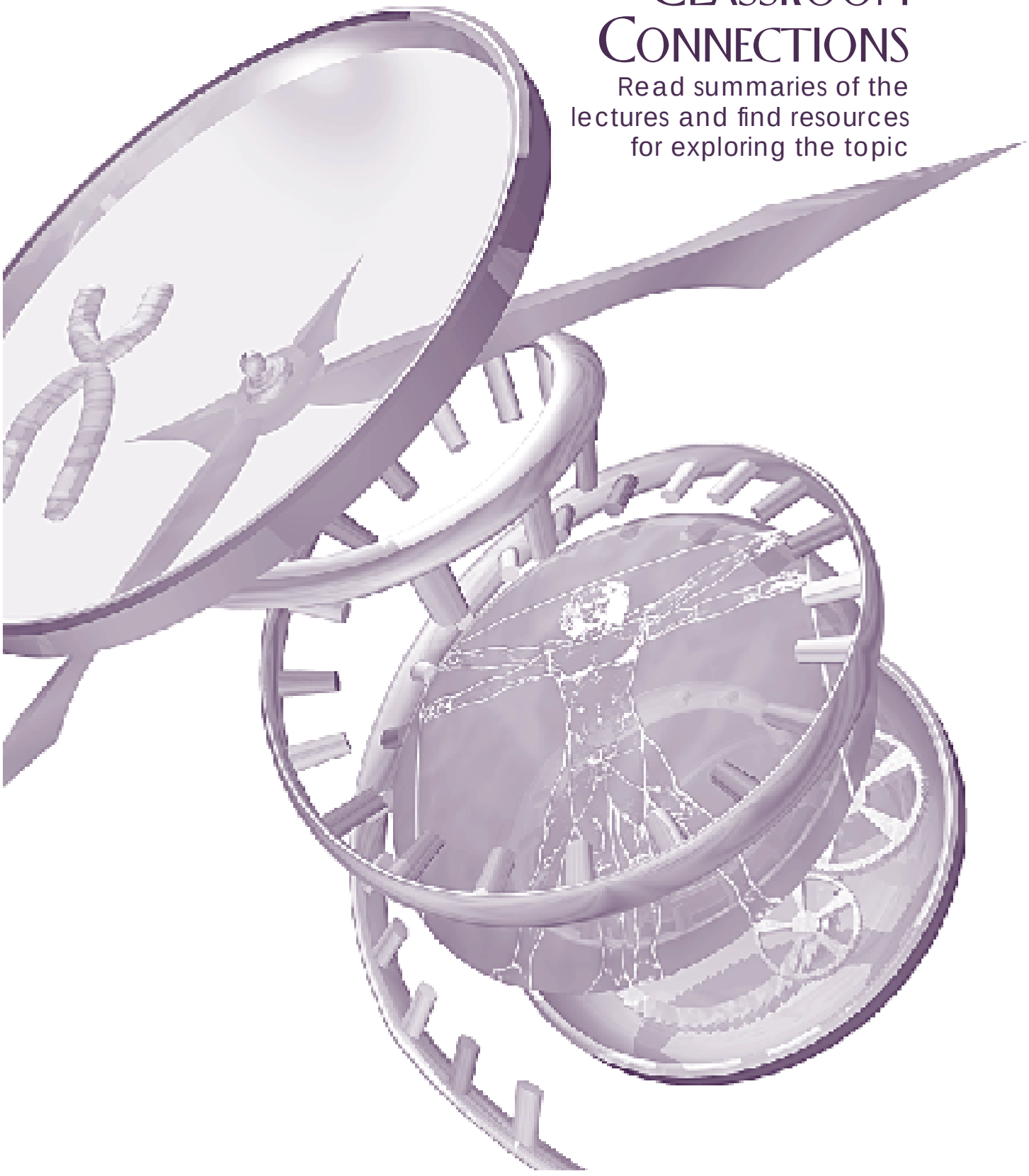
"What's my secret? I love to read and have a good memory. It is easy to do something that you love. It's hard for me to imagine having a more interesting career. I feel incredibly lucky to be in science today."

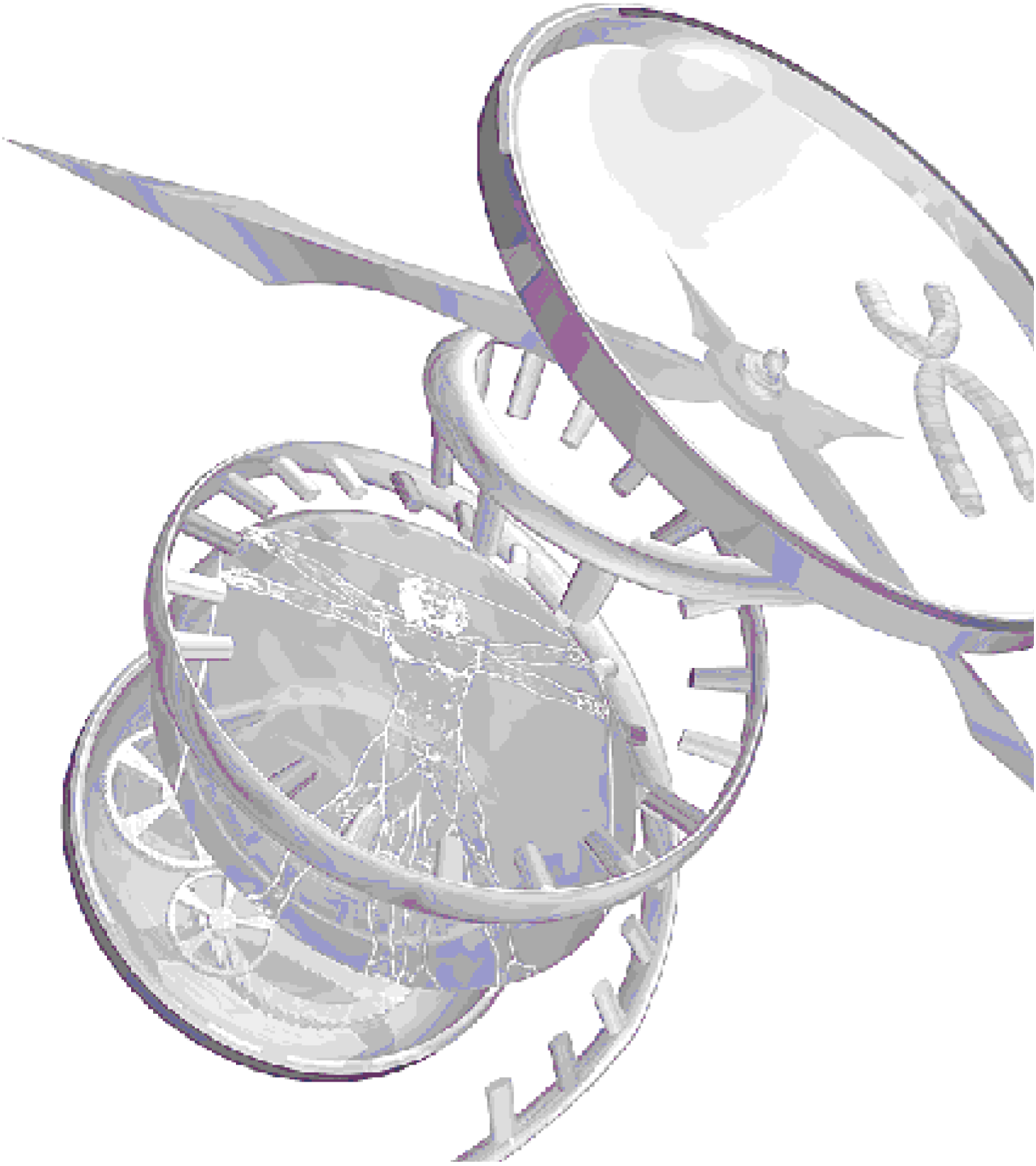
SELECTED PROFESSIONAL ACTIVITIES
Neuroscience Advisory Committee, The Klingenstein Fund
National Advisory Mental Health Council, National Institute of Mental Health
Editorial Board, *Physiological Genomics*
Scientific Advisory Board, Restless Legs Syndrome Foundation
Associate Editor, *Neuron*, 1995–1999
Associate Director, Northwestern University Center for Circadian Biology and Medicine
Member and Advisory Committee, NSF Science and Technology Center for Biological Timing, University of Virginia, Northwestern University, and Rockefeller University
Reviewer for nearly three dozen science journals and organizations

PUBLICATIONS
More than 100 original research articles in peer-reviewed journals such as *Science*, *Nature*, *Cell*, *Neuron*, and *Journal of Neuroscience*

Classroom Connections

Read summaries of the lectures and find resources for exploring the topic

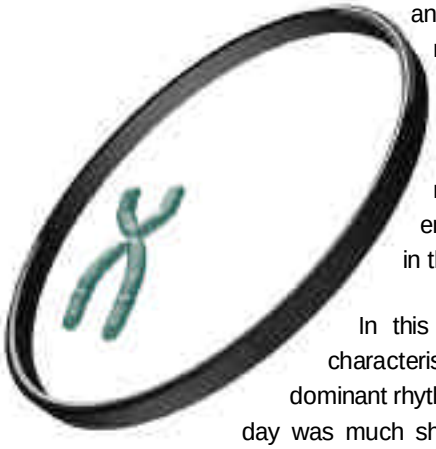




Biology in Four Dimensions

Joseph S. Takahashi, Ph.D.

The world we live in is a rhythmic place—the rising and setting of the sun, the ebb and flow of ocean tides, the changing seasons. Nearly every organism must adapt to these rhythms to thrive. It's advantageous to be able to anticipate changes in the environment, and most organisms, from humans to cyanobacteria, have internal mechanisms that track time. Such mechanisms have many uses. They may regulate daily activity patterns, trigger reproduction, and even aid in navigation. Biological timekeepers that operate on a daily cycle are called circadian clocks. The word *circadian* means "about a day." Genuine circadian rhythms are generated endogenously, or within the organism—that is, the rhythms persist in the absence of external time cues.



In this lecture, Joseph S. Takahashi will begin by discussing the characteristics and neural basis of circadian rhythms. Although the dominant rhythm on Earth is the 24-hour day generated by Earth's rotation, the day was much shorter in the distant past when circadian clocks first evolved, perhaps as short as six hours. Since organisms as diverse as plants and mammals have clocks, is it possible that all clocks arose from a common ancestral mechanism, or are clocks so important to survival that they evolved independently in different organisms?

Daily rhythms in nature have been noted throughout human history. In 1729 Jean Jacques d'Ortous deMairan, a French astronomer, had a sharp insight into how to test whether a rhythm is internal or completely dictated by the environment. He kept a heliotrope in the dark and reported that its leaves kept opening and closing on its usual daily schedule. DeMairan did not publish any further studies on the puzzle of the internal clock. In the 1950s Karl von Frisch, Gustav Kramer, and Colin Pittendrigh each independently discovered compelling evidence for internal clocks in animals. Their investigations marked the beginning of the modern field of circadian rhythms research. Scientists undertook intensive research aimed at understanding the nature of the circadian clock, including its possible influence on human health, particularly sleep.

In the 1960s Jürgen Aschoff, working in Germany, initiated studies of human circadian rhythms under controlled laboratory conditions. He built a bunker in which volunteers lived for several weeks. For some periods they were isolated from the outside world, and at other times the door to the real world was opened. Aschoff found that even when the subjects were in isolation and under constant light conditions, their sleep-wake cycles persisted. He also found that they drifted out of synchronization with the real world

because their endogenous cycles were a bit longer than 24 hours. When the door was reopened, however, the subjects readjusted, demonstrating not only that humans were paced by a circadian clock but also that the clock could be reset by environmental cues.

The properties of the clock were now well understood in several different organisms. But how did this clock work? Could it be located in a particular part of the body? It was generally assumed that the nervous system contained the clock in animals.

In the 1970s scientists demonstrated that the mammalian circadian clock is located in a part of the brain called the hypothalamus, specifically in a set of neurons on each side of the brain called the suprachiasmatic nucleus (SCN). The hypothalamus governs many bodily functions, including sleep, hormone production, and metabolism. In critical experiments, researchers removed the SCN from rats and hamsters, and the animals lost their normal rhythms of sleeping and running on exercise wheels. But when SCN tissue was transplanted back into hamsters, normal rhythms were restored. More recently, experiments from many laboratories have shown that the SCN can be completely isolated from the animal and still function as a clock, generating rhythmic chemical and physiological signals that can be experimentally measured. Indeed, we now know that each neuron in the SCN is an autonomous cellular circadian clock. Normally, these cellular clocks in the SCN act in unison as a master pacemaker for the organism in a manner analogous to the sinoatrial node pacemaker of the heart, which triggers coordinated rhythmic heartbeats.

While some scientists were describing the anatomical organization of circadian systems, others were making breakthroughs from different directions. Genetic approaches were providing confirming and complementary evidence and opening up the possibility of understanding the molecular underpinnings of the clock.

CLASSROOM CONNECTIONS

KEY CONCEPTS

- Biological rhythms are tied to the physical environment in which organisms have evolved.
- Humans have long observed and studied natural rhythms, including the daily activity patterns of plants and animals.
- Biological rhythms that occur on the scale of a day are called circadian rhythms, and they are driven by an internal clock.
- It is advantageous to organisms to anticipate changes in the environment, such as the rising and setting of the sun, and an internal clock can be used to anticipate important environmental events.

- Nearly every organism studied, including humans, has a circadian clock.
- Experiments in which individuals were kept in isolation in caves or bunkers have shown that humans have circadian clocks.
- Circadian rhythms affect human physiology and activity and thus influence many aspects of health.
- The approximately 24-hour internal clock can be reset, or entrained, by environmental factors—usually light—to track day length more accurately.
- The clock in the brain uses specific biochemical and neural pathways for passing its timing message along to other parts of the body, such as the liver or kidneys.
- Chemical reactions and physical properties are dependent on temperature. Circadian clocks have mechanisms to keep accurate time even when the temperature varies.
- In mammals, including humans, the clock resides in a part of the brain called the hypothalamus, specifically in a small cluster of cells on each side of the brain called the suprachiasmatic nucleus (SCN).

CHALLENGE QUESTIONS

These questions are intended to stimulate class discussions before and after the lectures. Teacher guidelines for helping students answer these questions begin on page 59.

1. Many living things have adapted to the rhythms of Earth by means of an internal timekeeping mechanism, a biological clock. Using humans, fruit flies, and plants as examples, explain what role the biological clock might have had in ensuring their evolutionary success.

2. Biological clocks are endogenous, meaning they can function without external environmental cues such as temperature or light changes. Design a scientific experiment to determine the role of the biological clock in governing motor activity in fruit flies. Choose one variable to test, such as temperature, light duration, or feeding times. Explain how you would measure the duration and frequency of activity. Describe the results you would expect, and explain why you would expect these results.

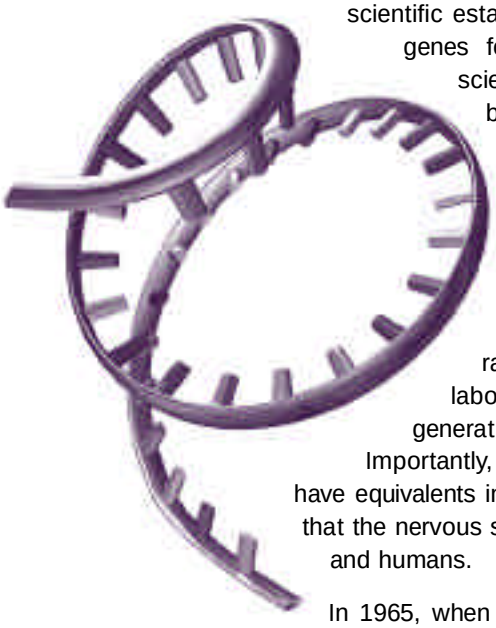
3. Think about the rhythmic patterns of Earth such as light and darkness, temperature, tides, and seasons. Describe the clock-driven adaptations that have evolved in organisms to enable them to survive.

4. Circadian rhythms are physiological cycles approximately 24 hours in duration that appear to be present in all eukaryotic organisms. These rhythms persist without external cues and are governed by an internal clock. This internal clock can be entrained (reset) by environmental factors, usually light. Discuss the role of light as a possible remedy for jet lag or winter depression.

Unwinding Clock Genetics

Michael Rosbash, Ph.D.

The 20th century, beginning with the rediscovery of Mendel's seminal experiments with peas and ending on the verge of sequencing all human genes, was in many ways the century of genetics. As Michael Rosbash will explain in the second lecture, a vast scientific establishment has developed the tools to study and manipulate genes for many traits, including various human diseases. Some scientists are using genetic research to provide insights into behavioral traits, such as temperament, appetite, and the ability to learn, and to identify the molecules that affect behavioral patterns.



The fruit fly, *Drosophila melanogaster*, has been central to genetic research, and it is the laboratory animal with which Rosbash works. Fruit flies breed quickly—a new generation every 10 days—and they're easy to maintain because they are raised in small bottles and fed simple chow. The DNA of laboratory flies can be intentionally mutated, and then resulting generations of flies can be screened for specific genetic changes. Importantly, a large number of genes and biochemical pathways in humans have equivalents in the fly. Many basic biological processes—including the way that the nervous system functions—are essentially unaltered between fruit flies and humans.

In 1965, when Seymour Benzer decided to look for mutations that altered brain function and behavior in *Drosophila*, most of the modern tools of molecular genetics were not yet available. Benzer was interested in identifying genes and molecules affecting behavioral patterns such as circadian rhythms, courtship movements, and even learning. Benzer's initial approach was very much like that of the classic fly geneticist Thomas Hunt Morgan—induce mutations by subjecting adult flies to radiation or chemicals and look for anomalous properties in their offspring.

In a series of experiments in the late 1960s and early 1970s, Benzer and one of his graduate students, Ronald Konopka, discovered the first circadian rhythm gene and mapped it to a specific location on the X chromosome of flies. They named it *period*, often shortened to *per*. This gene was identified by causing random mutations to occur in the eggs of parent flies and then looking at the behavior of the offspring. The Benzer laboratory had built a variety of clever devices that could rapidly identify flies with altered behavioral patterns. Flies carrying mutations in the *period* gene were recognized as having abnormal circadian rhythms.

A given gene in a species can exist in different variant forms, called alleles. For example, one allele might lead to white eye color, while another contributes to red eye color. In the case of the *period* gene, three variants were identified, each of which

produced a very different behavioral pattern. One type of mutant fly appeared to have a clock that kept a short day, a period of about 19 hours. The second type of mutant appeared to have a clock with a long period of about 29 hours. And the third type of mutant appeared to have no clock at all; its activity rhythms were random. These three changes in the way the clock ran resulted from different changes made to this single *period* gene, a very good candidate for being at the heart of the internal clock.

In 1984, using the new tools of molecular genetics, Rosbash and his collaborator Jeffrey Hall cloned and sequenced the *period* gene, as did Michael Young of Rockefeller University. Both research teams proved they had the right gene by inserting the wild-type—that is, normal—gene into *period* mutant flies and restoring the flies' normal day-night pattern of activity.

The identification of the *period* gene and its DNA sequence raised the possibility of answering key questions in the circadian mystery. The fly *period* gene could be used to look for related genes in other animals, including humans. Could close relatives of that gene be central to the generation of circadian rhythms in all animals? Although the *period* gene is central to the function of the circadian clock, no gene acts alone, and the *period* gene could be used to bootstrap to other genes with equally important roles in the generation and control of circadian rhythms. Research throughout the 1990s has indeed shown that the control of circadian rhythms at the molecular level is very similar in all animals studied. Scientists have also succeeded in identifying new genes that play important roles in the function of the circadian clock.

KEY CONCEPTS

- The fruit fly, *Drosophila melanogaster*, has been important in the history of genetic research, including the area of behavioral genetics.
- Fruit flies are advantageous for genetic research because they are small and have a short generation time. Mutations can be induced in specific genes to rapidly identify molecules important for a particular biological process.
- The community of *Drosophila* geneticists has developed naming conventions for the genes and proteins that they discover. Gene names are italicized and often abbreviated to three letters, which are also italicized—for example, *period* or *per*. The protein that is made from a gene is designated by all capital letters; the *per* protein is also referred to as PER. The conventions for other organisms are different (see the Key Concepts for Lecture Four).
- The fruit fly has about 100,000 cells in its nervous system, including a group of neurons near the eyes that makes up the circadian clock.

- Although flies and humans don't look alike, they share many similarities at the molecular level. Lessons learned in flies can help direct research in mammals, including humans.
- The first gene to be identified as having an important role in the function of the internal clock was found in fruit flies and named *period*, or *per*.
- Different variants, or alleles, of the *per* gene exist: those that cause flies to have a short-period day; those that cause a long-period day; and those that appear to have random rhythms.
- Once the *period* gene was mapped to a specific chromosomal location and cloned, and its DNA sequence was determined, its function could be studied in molecular detail.
- The identification of the *period* gene in flies enabled the identification both of similar genes in other animals and of other genes that contribute to making a circadian clock.

CHALLENGE QUESTIONS

These questions are intended to stimulate class discussions before and after the lectures. Teacher guidelines for helping students answer these questions begin on page 60.

1. Fruit flies have been important in the history of genetic research and are playing an important role in the quest to understand the genetics of the biological clock. Discuss the advantages and disadvantages of using fruit flies to gain an understanding of human biological clocks.

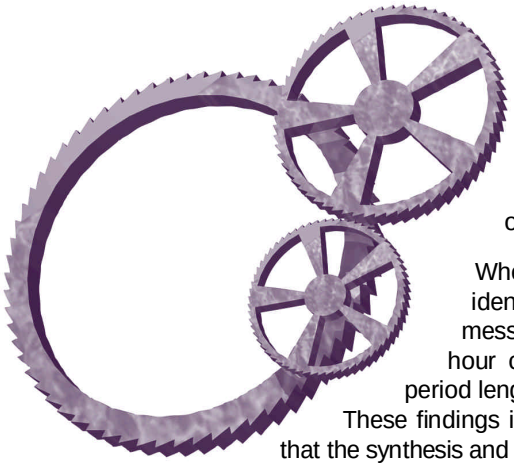
2. The first gene to be identified as having an important role in the function of the circadian clock was found in fruit flies and named *period* (*per*). Describe the process researchers used to identify this gene and current efforts to determine its function.

3. The expression of each gene is regulated by its own precise set of rules. To understand gene regulation, a common approach is to identify mutations in the targeted gene. Discuss how the study of mutations in the gene in flies led to our understanding of the length of cycle in a circadian rhythm.

PERFECT TIMING

MICHAEL ROSBASH, PH.D.

During the 1990s researchers identified key molecular components of the circadian clock and greatly advanced understanding of the internal timekeeper and how it adjusts to the outside world. Drawing from his own groundbreaking research and that of other scientists, Michael Rosbash will explain how the clock works at the molecular level in the fruit fly, *Drosophila melanogaster*.



Detailed studies of the fruit fly's *period* gene, known as *per*, gave Rosbash and his colleagues their first insight into how the clock actually functions and allowed them to propose a molecular model for clock function. Today, the essentials of that model still hold, but the details of *Drosophila*'s clock are considerably more complicated. At least seven additional key genes have been identified, and most researchers believe that others will be found.

When *per* was still the only circadian rhythm gene that had been identified and cloned, Rosbash and colleagues noted that both the messenger RNA (mRNA) and the *per* protein itself oscillated on a 24-hour cycle in normal flies. Moreover, the *per* mutants that altered period length also similarly altered the period of the molecular oscillations. These findings indicated that the clock mechanism was a biochemical circuit, that the synthesis and degradation of the *per* protein was an intimate part of the circuit, and that feedback of the protein on its own metabolism was central to the clockworks.

While the details of *per*'s role continue to be explored, researchers have been isolating additional clock genes in *Drosophila*. Many of these have been identified by the same basic strategy—namely, screening for mutant flies with altered circadian rhythms. All of these new circadian rhythm genes appear to have counterparts in mammals as well. The new genes *timeless*, *doubletime*, and *vrille* were identified by Michael Young's laboratory between 1994 and 1999; *Clock* and *cycle* were cloned by Rosbash's team in 1998. That year, Rosbash's laboratory also cloned the first photoreceptor gene involved in resetting the *Drosophila* clock by light—a *cryptochrome* (*cry*) gene that is related to blue-light photoreceptor genes in plants.

In 1999 Rosbash and his colleagues began tracing the pathway by which the *Drosophila* master clock communicates with other tissues to drive the fly's daily activity rhythms. A study led by Paul Taghert and Jeffrey Hall showed that the *pdf* gene, which codes for a peptide (a small protein of only a few amino acids) called pigment-dispersing factor, was essential for clock function. The *pdf* protein is secreted by a cluster of neurons where the fly's master clock resides, which is near the brain's visual centers.

The current model of *Drosophila*'s timekeeping mechanism presents a detailed description of how the known genes and their proteins work together to produce the functions of a clock (see diagram). The *Clock* and *cycle* proteins are transcription factors—the positive limb of the feedback loop. They combine in the cell's nucleus to

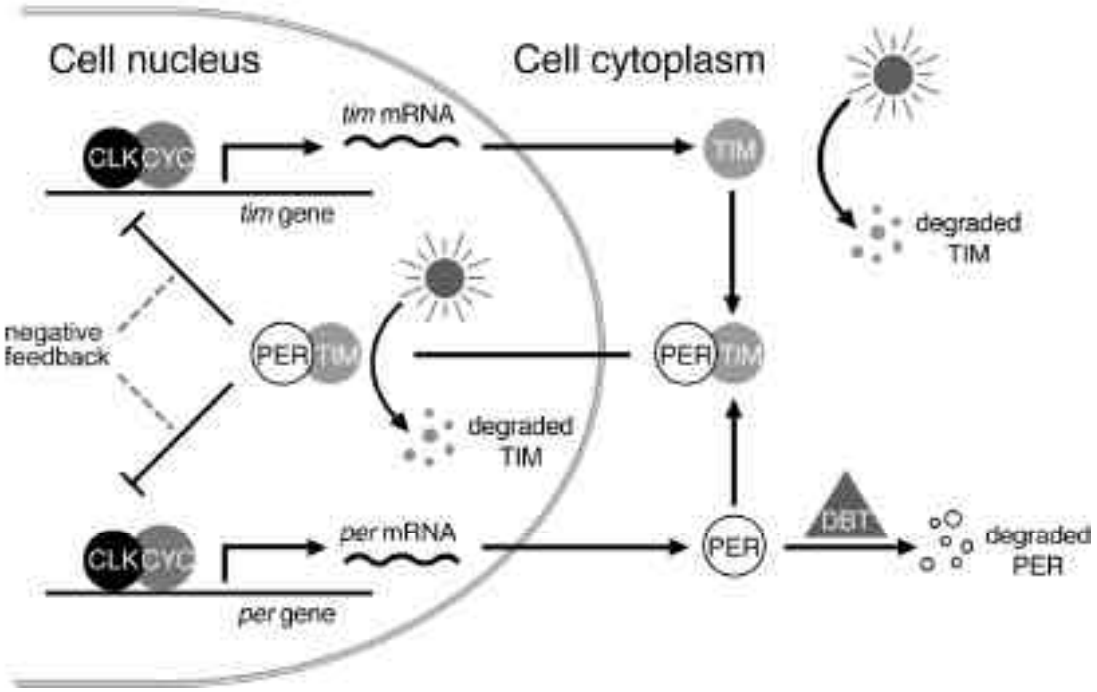
drive transcription of the *per* and *tim* proteins (PER and TIM). Then, in the cell's cytoplasm, PER and TIM bind together to form a heterodimer—a protein complex made of two different proteins. As evening progresses, the PER/TIM heterodimer enters the cell's nucleus in sufficient amounts to inhibit the functioning of *Clock* and *cycle*, thus leading to the shutting down of further PER and TIM production. As PER and TIM turn over during the late night and early part of the morning, *Clock* and *cycle* go back to work, and a new cycle begins. The *doubletime* protein (DBT) is believed to phosphorylate PER and thereby increase PER degradation.

Light causes the *Drosophila* clock to reset, or entrain, to synchronize with the 24-hour day-night cycle. Photoreception by cryptochromes appears to trigger the rapid degradation of TIM, and since TIM stabilizes PER, PER also disappears.

This picture is not yet complete. There is reason to believe that additional proteins may modify the action of the various proteins that have already been identified, and there may be additional transcription factors regulating gene activity. In addition, many of the details of clock input and output—of entrainment and of passing the timing signal to various body tissues—have yet to be completely worked out.

KEY CONCEPTS

- The circadian clock operates on the principle of a negative feedback loop with a period of about 24 hours.
- The *per* protein acts to inhibit its own production via a negative feedback loop. The *per* protein shuts down the transcription of the *per* gene, halting the production of further *per* protein.



- The levels of both the mRNA transcripts produced from the *per* gene and the *per* protein oscillate on a 24-hour cycle.
- Mutations in the *per* gene that produce shorter behavioral cycles also produce shorter oscillations in mRNA and protein levels. Likewise, mutations that produce longer behavioral cycles produce longer cycles in molecular levels.
- Other circadian rhythm genes have been identified in addition to *per*. The key clock genes are *period*, *timeless*, *doubletime*, *vrille*, *Clock*, and *cycle*.
- The *Clock* and *cycle* proteins are transcription factors—proteins that bind to DNA to regulate the transcription of genes, such as *per* and *tim*.
- The discovery of additional circadian clock genes led to a more elaborate model of the negative feedback loop that underlies the clock mechanism. In general, genes and their protein products are either part of the positive part of the feedback loop (*Clock* and *cycle*), or part of the negative part of the feedback loop (*period* and *timeless*).
- The *doubletime* gene makes an enzyme that phosphorylates PER, increasing PER degradation and thus contributing to maintaining the appropriate levels of PER for a particular time of day.
- Light leads to an increase in TIM degradation.
- A *cryptochrome* (*cry*) gene identified in flies probably makes a photoreceptor protein important for resetting the clock by light.
- The *pdf* gene codes for a peptide called pigment dispersing factor, which is probably responsible for communicating the clock's timing message to the parts of the nervous system that are directly responsible for controlling the fly's movement.

CHALLENGE QUESTIONS

These questions are intended to stimulate class discussions before and after the lectures. Teacher guidelines for helping students answer these questions begin on page 61.

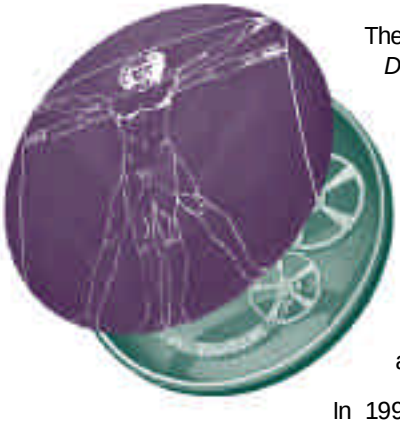
1. Negative feedback loops are used by organisms to maintain homeostasis—for example, to keep temperature constant. Circadian rhythms appear to be based on the principle of a negative feedback loop. Discuss the role of the negative feedback loop in the regulation of the *per* protein. How is regulating a clock function similar to or different from maintaining homeostasis?

2. Scientists have discovered that cells near the eyes of fruit flies have photoreceptor proteins that respond to blue and ultraviolet light and play a role in regulating the biological clock. Discuss the implications of these findings in evolutionary terms and predict other organisms that might have similar photoreceptor proteins.

THE MAMMALIAN TIMEKEEPER

JOSEPH S. TAKAHASHI, PH.D.

As more circadian clock genes are found—among diverse species—our picture of the underlying mechanisms of circadian clocks grows more complicated. In this lecture, Joseph S. Takahashi will discuss recent circadian gene discoveries and highlight the similarities and differences between the circadian clock in *Drosophila* and the clock in mammals.



The circadian genetics story for the mouse is similar to that for *Drosophila*—at least at the start. Takahashi and his colleagues created random mutations in mice by exposing them to mutation-causing chemicals, screened the mice for defective circadian behavior, mapped the suspect mutant gene to a specific chromosome, and, in 1997, cloned the first mammalian circadian gene, which they called *Clock*, for "circadian locomotor output cycles kaput." Up to that point, only three other circadian rhythm genes had been cloned and sequenced: *period* (*per*) and *timeless* (*tim*) in *Drosophila* and *frequency* (*frq*) in the fungus *Neurospora*. But the pace of gene discovery was about to take off.

In 1998 Takahashi's laboratory in collaboration with Chuck Weitz at Harvard University identified *Clock*'s partner in the mouse, *Bmal1*—the counterpart to *Drosophila*'s *cycle* gene—and determined that CLOCK and BMAL1 form a heterodimer that activates *period* gene expression in mice. In collaboration with Steve Kay at the Scripps Research Institute, they also showed that the PER/TIM heterodimer inhibits transcription of the *period* gene by interacting with CLOCK and BMAL in flies, pinpointing the negative feedback step in the circadian loop.

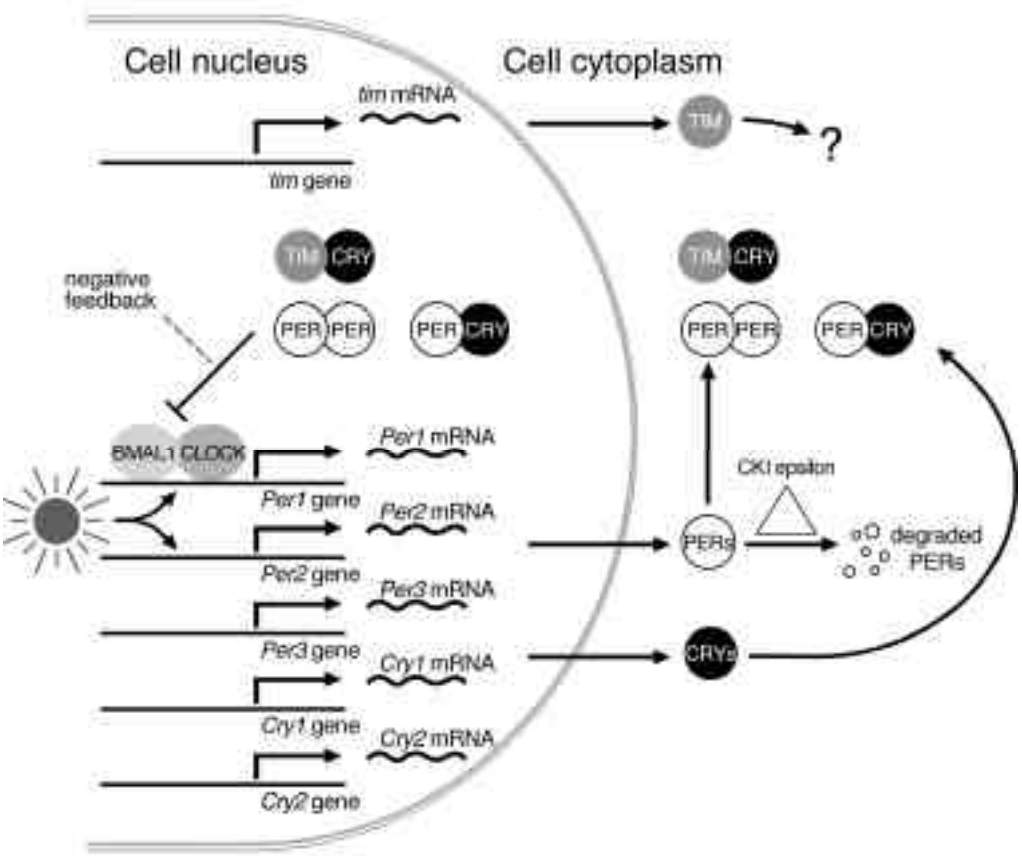
Meanwhile, other laboratories cloned not one but three mammalian *Period* genes in mice—now known as *Per1*, *Per2*, and *Per3*. This research provided the first evidence that *Drosophila* and mammalian circadian genes are related. Then, five laboratories, including Takahashi's, identified the mouse equivalent of the *Drosophila*'s *timeless* gene. Two mouse *Cryptochrome* genes, *Cry1* and *Cry2*, which are involved in photoreception, were characterized, bringing the total number of known clock genes in animals to eight. A ninth circadian rhythm gene made its appearance this year. Takahashi's laboratory identified the gene responsible for a circadian rhythm mutation in hamsters known as *tau*. The gene disrupted in *tau* mutants encodes for the enzyme casein kinase I epsilon and is the counterpart of the *doubletime* gene in *Drosophila*, discovered by Michael Young.

Many of these genes were found by using the increasingly important research tools of genomics and computer-based bioinformatics rather than mutagenesis screening. Researchers zeroed in on these genes by using the DNA sequences of known clock genes and searching large databases of DNA sequence information.

The mouse circadian machinery appears more elaborate than the fly's, and its detailed operations are somewhat different (see diagram). The mouse *Clock* and *Bmal1* genes are the activators, like *Clock* and *cycle* in flies, launching transcription of other circadian genes. But the negative feedback loop has more elements and thus more complex molecular interactions. For example, the three mouse *Period* genes—*Drosophila* has only one—

display different expression patterns. The *Period* proteins may be interacting with each other or with other components of the negative feedback loop. The photoreceptor proteins CRY1 and CRY2 appear to play more central roles than *Drosophila*'s *cryptochrome* protein; they can directly inhibit CLOCK and BMAL1, thus shutting down transcription.

Experiments with knockout mice have demonstrated the importance of *Cry* genes. Mice lacking *Cry1* have shortened circadian periods, while mice lacking *Cry2* have lengthened periods. Mice lacking both have no rhythm at all, and their *Period* genes do not oscillate.



Recent discoveries have given scientists a better idea about how the mammalian circadian clock works. Much, however, is still largely unknown: the biochemical pathways by which the clock is entrained, or reset; the degradation half-life of each protein involved; details of various protein interactions; and the process that sets proteins moving from the cytoplasm of the cell into the nucleus to block their own transcription. In addition, researchers are just beginning to explore the output side—how the clock sends its signals to the rest of the body, and what those signals are.

How relevant is such research to human health? Every circadian gene that has been found in flies or mice appears to have a counterpart in humans. Takahashi and Phyllis Zee at Northwestern University and Louis Ptacek, an HHMI investigator at the University of Utah, are already investigating at least one inherited human disorder as the possible result of a damaged circadian rhythm gene—advanced sleep phase syndrome, in which people fall asleep too early and wake up too early.

KEY CONCEPTS

- Recent research has identified at least nine genes that play key roles in the function of mammalian circadian clocks.
- The naming convention for mammalian genes and proteins is slightly different than that for *Drosophila*. In the case of mammalian genes like the *Clock* gene, the first letter is capitalized and the gene name italicized. The protein made by the *Clock* gene is written in all capital letters, CLOCK, and is not italicized.
- In contrast to pioneering genetic work in fruit flies, much research on circadian genes in mammals has been aided by recently developed tools—genomics and bioinformatics—that facilitate using information about existing genes to identify new genes and similar genes in different species.
- Although there are differences in clock functions between *Drosophila* and mammals, the similarities are fundamental and indicate a common evolutionary origin.
- Although the issue is still being explored, circadian clocks may have arisen independently four times during evolution, highlighting the importance of the timekeeping mechanism for the survival of organisms.
- The first circadian gene identified in mice, *Clock*, was discovered by making mutations in mice and looking for animals with altered rhythms.
- The partner of CLOCK, encoded by the *Bmal1* gene, was identified by biochemical interaction screening. BMAL1 in mice does not function on its own but forms a heterodimer with CLOCK.
- Unlike fruit flies, mice have three *Per* genes rather than one: *Per1*, *Per2*, and *Per3*. Mice also have their own version of *Drosophila*'s *timeless* gene, as well as two *Cry* genes.
- A new circadian gene, first identified in the *tau* hamster mutant, was recently cloned in mammals. It codes for the enzyme casein kinase 1 epsilon.
- The overall structure and function of the human clock is likely to be very similar to the structure and function of the mouse clock.
- Circadian rhythms are very significant in human health, most obviously as they affect sleep. There are genetic disorders in humans that apparently disrupt circadian rhythms and may represent mutations in genes related to those identified in mice and fruit flies.

CHALLENGE QUESTIONS

These questions are intended to stimulate class discussions before and after the lectures. Teacher guidelines for helping students answer these questions begin on page 61.

1. Bioinformatics involves the use of powerful computers and huge databases to compare gene sequences. Once a new gene is discovered, a first step investigators often take is to use bioinformatics to determine if the same or a similar gene is present in other organisms. Discuss why this is important information to acquire.

2. Genetic analysis indicates that circadian clocks in today's living organisms probably do not trace back to a single ancestor but may have evolved independently as many as four times. Discuss the process researchers used to arrive at this hypothesis and the significance of the independent appearance of genes over time.

3. An understanding of how the mammalian clock works will allow researchers to study how the clock sends signals to the rest of the body. At least one inherited human disorder, advanced sleep phase syndrome, appears to be the result of an altered circadian rhythm gene. How could this knowledge be used in the treatment of sleep disorders?

Selected Websites

Center for Biological Timing provides links to a directory of publications in biotiming research, an online experiment, a useful glossary of terms, classroom activities, and a tutorial on biological timing. www.cbt.virginia.edu/

Simon Fraser University Circadian Rhythms Lab provides an overview of biological rhythms, details of current research at SFU, and links to rhythms societies, journals, and other relevant sites. www.sfu.ca/~mcantle/rhythms.html

Society for Research on Biological Rhythms provides information about its meetings and links to other online resources. www.srbr.org/

Circadian Technologies provides news briefs and feature articles on circadian rhythms and related subjects. The "Learning Center" link has tutorials and more useful links. www.circadian.com

NASA's Fatigue Countermeasures Group discusses NASA research into fatigue, sleep, circadian rhythms, and performance in flight operations. olias.arc.nasa.gov/zteam

Society for Neuroscience includes a "Brain Briefing" on biological clocks. Discusses how molecular clocks create cycles in body systems and offers early and current biological cycle research data. www.sfn.org/briefings/bio_clocks.html

Biological Clock Site, designed by the biological sciences department of Manchester University, provides a useful introduction to the study of biological clocks and also includes a reference list of relevant texts, journals, and websites. www.teaching_biomed.man.ac.uk/student_projects/1999/sanders/start.htm

National Wildlife Federation, What Makes Us Tick? is a detailed article about the internal devices that keep track of time. www.nwf.org/natlwild/2000/ustick.html

Sleep and Circadian Rhythms, designed for a course offered by the University of Washington, discusses sleep, circadian rhythms, synchronization, different types of rhythms, and more. courses.washington.edu/nurs308/sleep/sleepslide/

San Diego Sleep and Rhythms Society links to many relevant sites. Be sure to check out the San Diego hamsters! varesearch.ucsd.edu/klemfuss/sdsrs.htm

Red River Sleep Center discusses circadian rhythms, insomnia, narcolepsy, and differences in sleeping patterns in children and the elderly, provides a printable sleep diary, and has useful links. www.redriversleepcenter.com/circadian_rhythms.htm

Molecular Genetics of Circadian Rhythms provides links to 15 laboratories around the country performing circadian rhythms research. www.csa.com/hottopics/circad/webpage.html

Selected Publications

Scientific

Konopka, R.J., and Benzer, S. 1971. Clock mutants of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 68:2112–2116.

Ralph, M.R., and Menaker, M. 1988. A mutation of the circadian system in golden hamsters. *Science* 241:1225–1227.

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General Audience

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Teacher-directed Classroom Activities

CHARTING STUDENTS' KNOWLEDGE: THE KWL CHART

The KWL chart (What do you **k**now? What do you **w**ant to know? What did you **l**earn?) is a useful strategy to give teachers quick information about the current knowledge level of their students and the kinds of questions that most interest them. After students have watched the Holiday Lectures on Science, they can complete the last section of the chart.

What do you know about biological clocks? For the first section of the chart, individual students list what they know about biological clocks and circadian rhythms. Teachers can compile this information into a class list.

What do you want to know about biological clocks? For this section, students can work individually, form pairs, or work in small groups to write questions about biological clocks.

What did you learn about biological clocks? After watching the Holiday Lectures, answering the challenge questions in this publication, and completing some or all of the other activities, students should summarize their knowledge about biological clocks.

KWL CHART	
What do you already know about biological clocks and circadian rhythms?	Example: Our eating and sleeping patterns are governed by a biological clock.
What questions do you have about biological clocks and circadian rhythms?	Example: Do all organisms have a biological clock?
What did you learn about biological clocks and circadian rhythms?	Example: Biological clocks are genetically programmed.

Biological Clock Scavenger Hunt

Overview

Biological clocks drive behavioral cycles or rhythms that repeat at regular intervals. A 24-hour cycle is called a circadian rhythm, which comes from the Latin meaning “about a day.” Circadian rhythms, such as the sleep-wake cycle, have been found in all eukaryotic organisms. The rhythms continue even when all external environmental conditions remain constant. They originate within the organism itself—that is, they are endogenous.

Shorter rhythms, such as the rhythm of a beating heart, are termed “ultradian.” Cycles longer than 24 hours are called “infradian.” If they last a year, such as migration and hibernation, they are called “circannual” rhythms. Biological clocks play an important role in synchronizing internal and external events of organismal physiology.

In this activity, students will record and explain several examples of different repeating behaviors or physiological functions that they have read about or observed in themselves and other organisms.

Objective

To discover the pervasiveness of biological clock evidence in eukaryotic organisms.

BIOLOGICAL CLOCK SCAVENGER HUNT

Fill out the chart by using as many examples of repeating behaviors or physiological functions as you can think of. Give an explanation of each example.

BEHAVIOR	EXPLANATION

Possible Answers

Photosynthesis rate	Stomata open and close in response to guard cells interpreting various environmental stresses during the day.
Auxin production	Growth regulation by hormones in plants, including cell elongation, varies according to time of day.
Photoperiodism	Response to relative day and night length in 24 hours.
Pollination	Programming of pollination by certain pollinators to release nectar in flower.
Body temperature	Peaks at about 4 p.m. and lowest at about 4 a.m.
Heart rate	Variation of about 20 beats per minutes during day in humans.
Hormone secretions	Corticosteroids peak between 4 a.m. and 8 a.m., growth hormone rises an hour after falling asleep, and testosterone peaks about 9 a.m.
Birth	Most likely to occur between 3 a.m. and 4 a.m.
Tolerance to pain	Lowest at about 9 p.m.
Blood pressure	Varies as much as 20 percent in course of a day.
White blood cell count	Varies as much as 50 percent in a 24-hour period.
Alertness	Daily patterns affect most mammals.
Sex drive	Variations during the day and season that affect various animals.
Sleep patterns	Individual daily and seasonal patterns.
Hibernation	Seasonal variances that affect animals.
Migration	Seasonal variances that affect animals.
Reproduction	Seasonal variances that affect organisms.
Cell cycle	Varies during the day.

Body Temperature and Reaction Time Laboratory

This activity was developed by the Center for Biological Timing at the University of Virginia, a National Science Foundation-sponsored consortium of researchers who work together to solve the mysteries of biological timing, based on an idea by Rebecca Prosser at the University of Tennessee. The center's website at http://www.cbt.virginia.edu contains numerous classroom activities for high school and middle school students, as well as a tutorial on biological timing.

Be sure to follow local law and your school district's rules for obtaining consent for students to participate in this activity. Teachers should also keep the following points in mind:

- Since the range of temperature change is likely to be just two or three degrees at most, a good thermometer is essential.
- The two most important time points in this experiment are immediately upon waking up and any time in the two hours before going to sleep. Therefore, if students do not wish to set their alarm clocks for the 4 a.m. temperature reading, they may omit that measurement.
- Students should gain some mastery of jacks before the experiment begins so that they do not become more skillful simply from the constant repetition of the game.

Objective of the Laboratory

By studying their own rhythms, students may gain a better understanding of what circadian rhythms are, their prevalence, and their importance. The students will monitor their body temperatures and reaction times over the course of two days.

Methods

Equip each student with a thermometer and a game of jacks. Over one weekend, have the students maintain a regular schedule (for example, no sleeping late, no staying up all night). Beginning at 8 a.m. on Saturday, they should measure their temperatures orally once every four hours. While they are taking their temperatures, they are to play three games of jacks. Then they record the time, their temperatures, and the number of jacks they picked up in each of the three trials. For the night measurements, the students will have to set their alarms for the correct time and take the same measurements. They should continue taking these measurements for two days to get a good range of data. The students could also be asked to keep track of when they slept, ate meals, and engaged in vigorous activities.

Having collected the measurements, the students can plot the results in class, calculating means and standard deviations for the results of the three trials for jacks at each time point. Assuming they observe some sort of variation, they can note the times when their temperatures and reaction times peaked and troughed, and determine whether there is a correlation. Comparisons can also be done between students. If they kept track of other behaviors, they could investigate whether the changes are in any way related to the other behaviors.

Discussion Topics

What confounding variables might be affecting these measurements, and how could they be eliminated experimentally?

How could you determine whether these rhythms are endogenously controlled?

Do you think these rhythms would continue under unusual circumstances, such as during space travel or while living in a submarine?

What other aspects of our behavior and physiology vary rhythmically?

How might the changes in reaction time relate to people who work at night (for example, police, medical personnel, pilots, workers in nuclear power plants)?

Do these types of rhythms occur in other organisms?

BODY TEMPERATURE AND REACTION TIME LABORATORY

TIME	TEMPERATURE	GAME OF JACKS		
		TRIAL 1	TRIAL 2	TRIAL 3
DAY 1				
8 a.m.				
12 noon				
4 p.m.				
8 p.m.				
12 midnight (optional)				
4 a.m. (optional)				
DAY 2				
8 a.m.				
12 noon				
4 p.m.				
8 p.m.				
12 midnight (optional)				
4 a.m. (optional)				

Correlations to Advanced Placement Biology Curriculum

The unifying themes and constructs that underlie the Advanced Placement (AP)* biology curriculum have been organized in the *AP Biology Course Description* (referred to by many teachers as the "acorn book") to correlate the eight major themes with the three major areas of the topic outline. The chart included in the course description provides blank lines that allow teachers to write their own examples of applied themes. Provided here are some examples of themes applied to the topic of biological clocks. There were no correlations for themes III. "Energy Transfer," V. "Relationship of Structure and Function," or VII. "Interdependence in Nature."

The AP Biology Course Description is available in PDF format on the College Board's website at **www.collegeboard.org/ap/library** or by linking from **www.collegeboard.org/ap/biology.**

MAJOR THEMES	MAJOR AREAS		
	I. Molecules and Cells	II. Heredity and Evolution	III. Organisms and Populations
I. Science as a Process	Experiments using light of specific colors (frequencies) have yielded information about the photoreceptors that supply input to the clock, since photoreceptors are tuned to specific frequencies of light.	The identification, cloning, and sequencing of circadian rhythm genes, first in *Drosophila*, have led to the understanding that the clocks in diverse species have a common evolutionary origin controlled by a related set of genes.	Experiments dating to before the modern era of molecular genetics established that many organisms, including bacteria, plants, and animals, have an internal clock that affects many biological functions, such as sleep, flower opening, and migration.
II. Evolution	An important molecule for light signals to reach the clock in animals is a type of photopigment called a cryptochrome protein. The animal protein is similar to cryptochrome proteins found in plants, where they are sensitive to blue and ultraviolet light frequencies.	Nearly every organism studied has been found to have a circadian clock. Evidence may indicate that circadian clocks arose independently four times during evolution.	Biological rhythms are tied to the physical environment in which organisms have evolved. Internal clocks enable organisms to anticipate and adapt to changes in the environment.
IV. Continuity and Change	Studies of mutation in the *per* gene revealed the relationship between molecular and behavioral oscillations.	The genes that regulate circadian rhythms and the biological clock that drives them have been identified in a wide variety of organisms.	The biological clock in mammals resides in the hypothalamus as a small set of cells called the suprachiasmatic nucleus (SNC), while the master clock in birds resides in the pineal gland.
VI. Regulation	Circadian rhythms affect many aspects of cellular physiology and activity and are often regulated at the molecular level by biochemical negative feedback loops.	The *Drosophila* and mammalian circadian clocks appear similar at the molecular level. However, *Drosophila* has a single *per* gene, while mice have three different *per* genes.	Circadian rhythms can be entrained (reset) by environmental cues, such as light, to adjust for more accurate tracking of day length.
VIII. Science, Technology, and Society	Understanding the molecular details of how circadian rhythms are controlled may help in the development of drugs to treat sleep disorders and jet lag.	The sequencing of the entire genomes of many organisms, including *Drosophila* and humans, will likely lead to the identification of new circadian genes.	Understanding circadian rhythms has significance in human health, for example, sleep disorders, shift work, and jet lag.

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Circadian Rhythms and the Circulatory System

This activity is intended to serve as an extension to the required AP Biology Laboratory #10, Physiology of the Circulatory System.

Teachers who decide to use this activity should keep the following points in mind:

- Be sure that students can comfortably take their pulses and blood pressure so that anxiety does not become a factor.
- Encourage students to record the data with no expectations. Although blood pressure, in theory, changes by 20 percent over 24 hours, no results can be guaranteed. Many factors such as stress or recent exercise can affect blood pressure.
- Urge students to be consistent in taking measurements at similar times during the day.
- This experiment is probably best done during school days rather than on the weekend, since school days are more likely to have more regular routines.

Overview

Students should be familiar with the proper procedures for taking blood pressure and pulse rate. In this activity, students will measure blood pressure and pulse rate at several set times each day for at least three days. An analysis of results should show how these physiological functions relate to circadian rhythms.

Objective

Before doing this laboratory, students should understand

- the relationship between circadian rhythms and the rates of physiological processes.

After completing this laboratory, students should be able to

- describe how blood pressure and pulse rate relate to circadian rhythms;
- discuss and explain the relationship between circadian rhythms and the body's biological clock; and
- discuss how entrainment might affect their circadian rhythms.

Procedure

Students should measure both blood pressure and pulse rate for at least four specific times each day—immediately upon waking, just before going to sleep, and at two other times (for example, 6 a.m., noon, 4 p.m., and 10 p.m.). They should take these measurements for at least three days and record the data in the chart provided, graph the results, and analyze the data by using the questions provided.

BLOOD PRESSURE AND PULSE RATE

DAY/DATE	TIME	BLOOD PRESSURE	PULSE RATE

GRAPH BLOOD PRESSURE AND TIME OF DAY FOR THREE DAYS

For this graph students will need to determine the following:

a. The *independent* variable:

_____

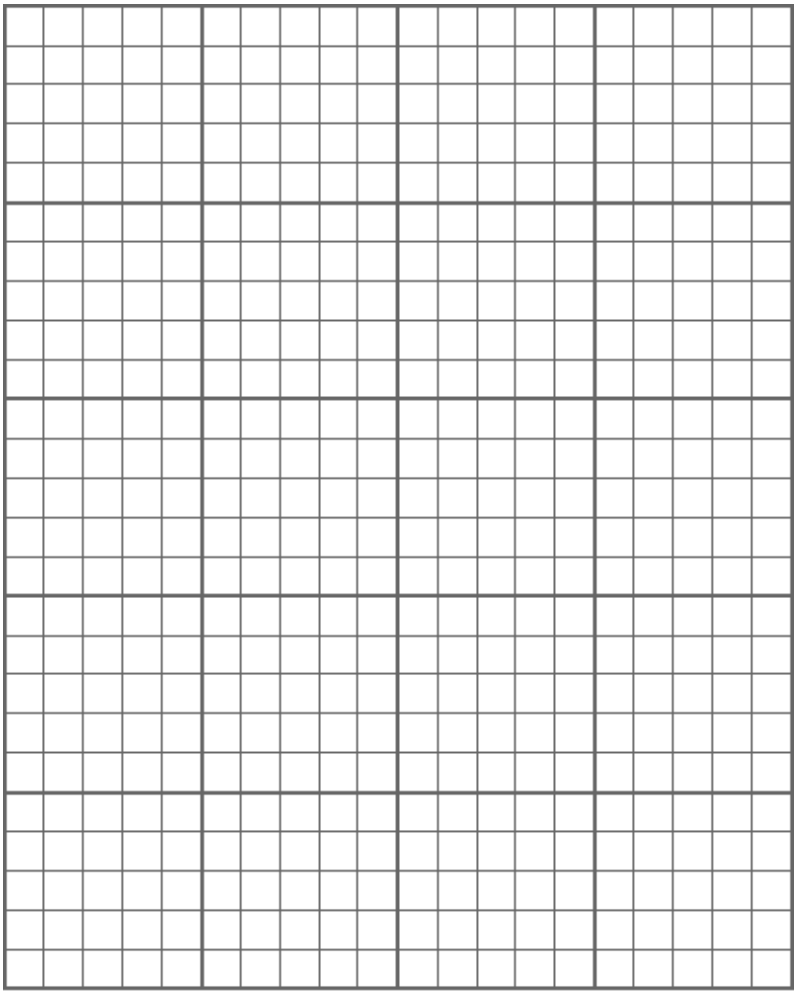
Use this to label the horizontal (X) axis.

b. The *dependent* variable:

_____

Use this to label the vertical (Y) axis.

GRAPH TITLE: _____



GRAPH PULSE RATE AND TIME OF DAY FOR THREE DAYS

For this graph students will need to determine the following:

a. The *independent* variable:

_____

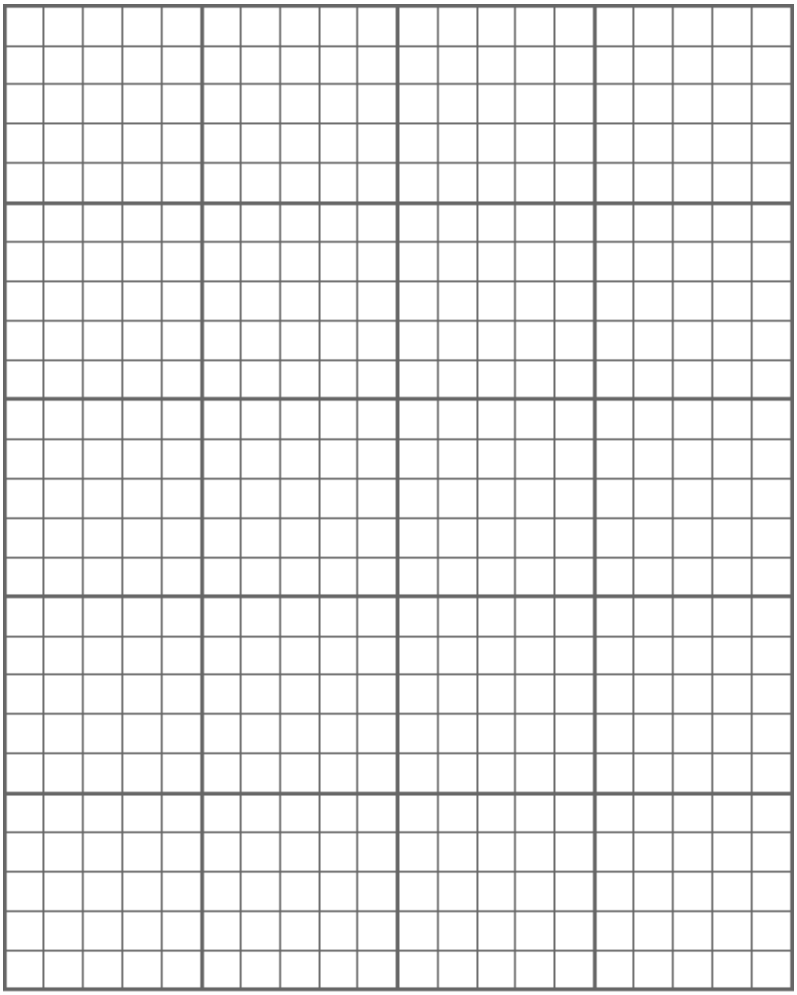
Use this to label the horizontal (X) axis.

b. The *dependent* variable:

_____

Use this to label the vertical (Y) axis.

GRAPH TITLE: _____



TOPICS FOR DISCUSSION

1. How does the time of day affect your blood pressure and pulse rate?

2. Explain why it is necessary to keep an accurate record at exact times for several days when studying the circadian rhythm of blood pressure and pulse rate.

3. Discuss how you might use entrainment to change your circadian rhythm relating to blood pressure and pulse rate.

4. List five other circadian rhythms that occur within your body systems.

5. Where is the the body's biological clock located? What is its neural basis?

6. How is jet lag related to the biological clock? How can this knowledge be used to help prevent it?

Lecture One

Question 1: Many living things have adapted to the rhythms of Earth by means of an internal timekeeping mechanism, a biological clock. Using humans, fruit flies, and plants as examples, explain what role the biological clock might have had in ensuring their evolutionary success.

Discussion Points: Ask students to think about the selective advantages of foraging for food in daylight, of sleeping when it is cold and dark, or of having seasonal mating periods timed so the birth of young animals occurs in spring, providing time to mature before the winter months. Remember that the early bird gets the worm. Also think of the value of anticipation. Most organisms are prepared for daylight when it comes. Plants, for example, are ready to start photosynthesizing immediately at daybreak.

Question 2: Biological clocks are endogenous, meaning they can function without external cues such as temperature or light changes. Design a scientific experiment to determine the role of the biological clock in governing motor activity in fruit flies. Choose one variable to test, such as temperature, light duration, or feeding times. Explain how you would measure the duration and frequency of activity. Describe the results you would expect, and explain why you would expect these results.

Discussion Points: Remind students to keep all conditions (for example, amount of food and habitat conditions) constant except the variable being manipulated (temperature, light duration, or feeding times).

Question 3: Think about the rhythmic patterns of Earth such as light and darkness, temperature, tides, and seasons. Describe the clock-driven adaptations that have evolved in organisms to enable them to survive.

Discussion Points: Some of the adaptations students might mention are hibernation, migration, and feeding patterns, as well as the timing of sexual/mating behaviors, which results in the birth of the young at a good time of year for food.

Question 4: Circadian rhythms are physiological cycles approximately 24 hours in duration that appear to be present in all eukaryotic organisms. These rhythms persist without external cues and are governed by an internal clock. This internal clock can be entrained (reset) by environmental factors, usually light. Discuss the role of light as a possible remedy for jet lag or winter depression.

Discussion Points: Travelers experiencing a delayed sunset caused by traveling from New York to San Francisco in the evening readjust to the new time zone more quickly than travelers who experience a premature sunrise caused by traveling from San Francisco to New York in the early morning hours. This fact indicates that light is a major entraining cue for humans at specific times of day.

Lecture Two

Question 1: Fruit flies have been important in the history of genetic research and are playing an important role in the quest to understand the genetics of the biological clock. Discuss the advantages and disadvantages of using fruit flies to gain an understanding of human biological clocks.

Discussion Points: This is an opportunity to help students consider the use of model organisms such as fruit flies. Fruit flies are easy to raise, and they reproduce rapidly. Genetic screens can be used to look for mutants in a complex process such as circadian behavior. The gene that has been mutated can be identified, and the mutant flies can be used to understand the function of the mutated gene. The past several decades of molecular biology research have confirmed that gene functions are conserved to a surprising extent between humans and fruit flies. Without first identifying the *period* gene in a genetic screen in *Drosophila*, researchers would have had very little idea of the function of the human *per* genes when their sequence was found. While the human and the fly genes involved in circadian rhythms are very similar, the anatomy of humans and flies is quite different. Flies, for example, do not have a hypothalamus or a suprachiasmatic nucleus. Since humans are complex organisms with feelings and emotions that can alter physiological responses, fruit flies may provide a simpler system for understanding basic circadian biology. Many of these research findings in fruit flies will hold true for humans. However, humans will be more complex than flies.

Question 2: The first gene to be identified as having an important role in the function of the circadian clock was found in fruit flies and named *period* (*per*). Describe the process researchers used to identify this gene and current efforts to determine its function.

Discussion Points: Suggest that students develop an outline of the lecture content that covers key stages in the process of identifying a new gene by inducing random mutations in fruit flies and studying the activity patterns in the offspring. The evidence of mutation in the *period* gene was abnormal circadian rhythms. Current efforts to determine *per* function may include looking for what other proteins *per* binds; understanding how the *per* protein's half-life is regulated; discovering where the *per* protein is found in the fly and within the cells; learning which genes the *per* protein regulates; and understanding how a protein like PER makes the link from acting within a cell to regulating fly behavior.

Question 3: The expression of each gene is regulated by its own precise set of rules. To understand gene regulation, a common approach is to identify or induce mutations in the targeted gene. Discuss how the study of mutations in the *per* gene in flies led to an understanding of the length of cycle in a circadian rhythm.

Discussion Points: The process of identifying the *per* gene involved inducing random mutations in fruit flies and studying the activity patterns in the offspring. Evidence of mutation in the *period* gene was abnormal circadian rhythms. Some mutations in the *per* gene caused the period length of the fly to alter. *Per*-short flies have a 19-hour day, and *per*-long flies have a 29-hour day. Studies of these mutants found that the molecular cycles had also changed to 19 and 29 hours. These data established that the length of the molecular cycle of *per* protein regulates the length of the behavioral circadian rhythm. This question also provides an opportunity to discuss the cellular changes in the *per* protein cycle. In *per* short, the protein rapidly disappears from the nucleus. In *per* long, the protein enters the nucleus several hours later than normal. Both of these results show that *per* has a critical function in the nucleus that makes the cycle last 24 hours.

Lecture Three

Question 1: Negative feedback loops are used by organisms to maintain homeostasis—for example, to keep temperature constant. Circadian rhythms appear to be based on the principle of a negative feedback loop. Discuss the role of the negative feedback loop in the regulation of the *per* protein. How is regulating a clock function similar to or different from maintaining homeostasis?

Discussion Points: Consider a home thermostat as an analogy to help students conceptualize the principle of a negative feedback loop. Air conditioning systems pump cool air until the air becomes colder than the thermometer's set point, causing the system to shut off. Once the air conditioning is off, the temperature gradually rises to a set point, and the air conditioning comes back on. Similarly, as the levels of the *per* protein rise, the concentration causes a shutdown on the production of *per* protein until nearly all of the per protein degrades. Once the amount drops to a certain level, *per* protein production switches back on. This whole loop takes 24 hours. Unlike homeostatic systems, where oscillations are damped by rapid feedback within narrow set points, the negative feedback oscillations are used by biological clocks to generate a time signal. Because there is a long delay in the feedback—with *per* protein shutting down further *per* mRNA production—the whole system oscillates, with the peaks of *per* mRNA and protein occurring at very different times. To return to the home thermostat analogy, such a feedback delay would cause large oscillations in the temperature inside the house.

Question 2: Scientists have discovered that cells near the eyes of fruit flies have photoreceptor proteins that respond to blue and ultraviolet light and play a role in regulating the biological clock. Discuss the implications of these findings in evolutionary terms and predict other organisms that might have similar photoreceptor proteins.

Discussion Points: Help students understand that when the same gene is located in organisms as distantly related as flies and humans, the likelihood is very high that the gene codes for a protein essential to a wide variety of living things.

Lecture Four

Question 1: Bioinformatics involves the use of powerful computers and huge databases to compare gene sequences. Once a new gene is discovered, a first step investigators often take is to use bioinformatics to determine if the same or a similar gene is present in other organisms. Discuss why this is important information to acquire.

Discussion Points: When a great number of organisms share the same gene, it suggests that the gene has an important function. Essential genes tend to be highly conserved. As a result, scientists can make strong predictions about the function of a gene in one organism on the basis of prior knowledge of that gene in a different organism. This knowledge greatly helps scientists design their experiments.

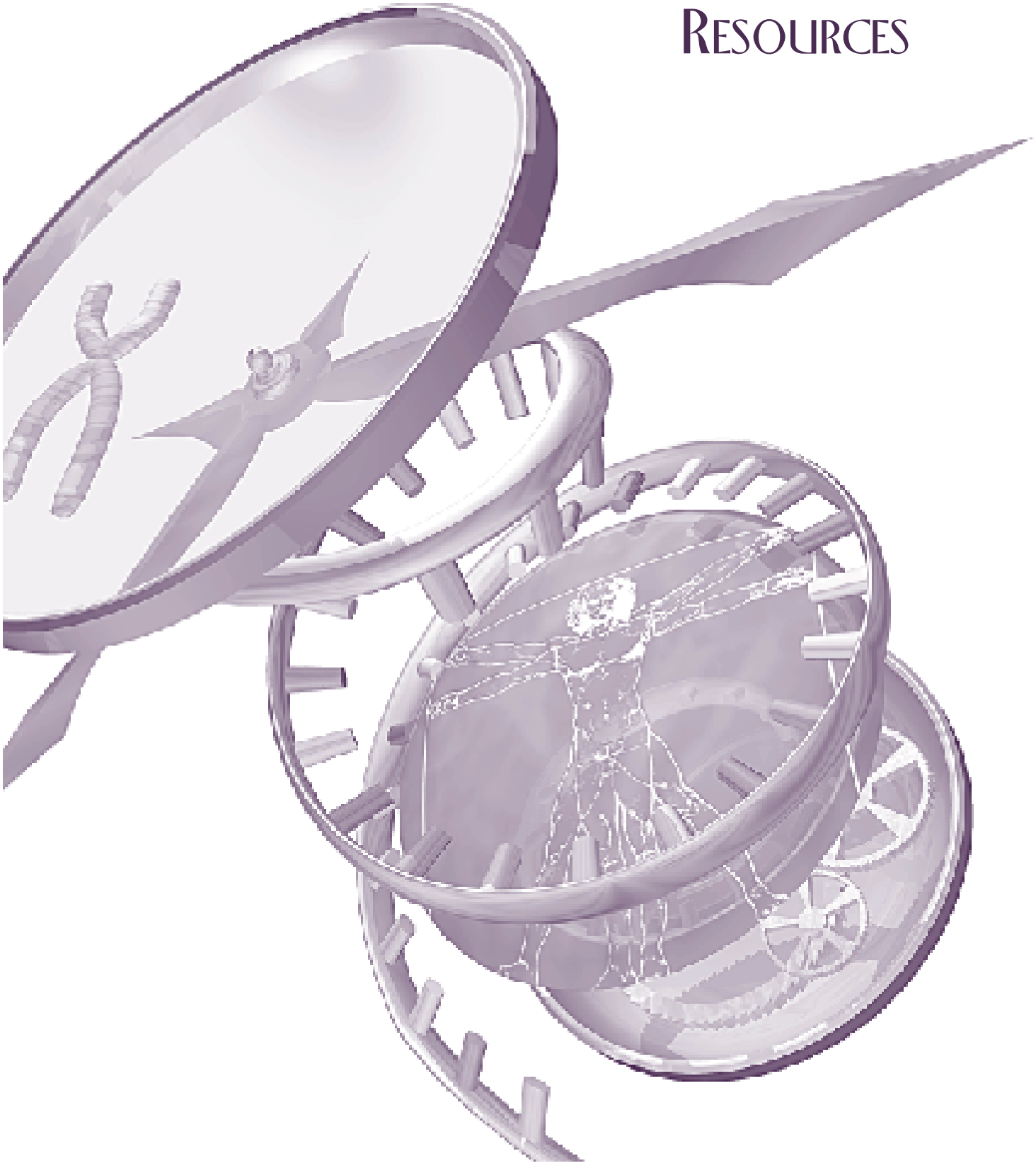
Question 2: Genetic analysis indicates that circadian clocks in today's organisms probably do not trace back to a single ancestor but may have evolved independently as many as four times. Discuss the process researchers used to arrive at this hypothesis and the significance of the independent appearance of genes over time.

Discussion Points: Investigators have found that unrelated genes function in circadian clocks in bacteria, plants, and animals. The gene sequences have been very informative for gaining clues about how the genes function, often by making proteins that regulate gene transcription. The fact that clock genes have independently evolved multiple times shows that circadian rhythms must be advantageous for an organism.

Question 3 : An understanding of how the mammalian clock works will allow researchers to study how the clock sends signals to the rest of the body. At least one inherited human disorder, advanced sleep phase syndrome, appears to be the result of an altered circadian rhythm gene. How could this knowledge be used in the treatment of sleep disorders?

Discussion Points: We may be able to design and manufacture drug treatments that specifically correct sleep disorders in people. Care would have to be taken to make sure that the drug was specific and did not interfere with another aspect of the body's metabolism. Alternatively, we may be able to correct the gene in people before birth. However, a technique to correct genes before birth is not yet possible. It carries the risk of artificially changing the DNA of another gene and raises serious ethical concerns about manipulating the human gene pool.

Holiday Lectures Resources



RESOURCES ON THE HOLIDAY LECTURES WEBSITE

The Holiday Lectures website contains a wealth of materials to make complex biology topics easier for high school students to understand. Please visit WWW.HOLIDAYLECTURES.ORG often for lecture updates and additional materials, or go directly to WWW.BIOINTERACTIVE.ORG for a variety of multimedia activities.

Webcast—The 2000 Holiday Lectures on Science will be broadcast live over the Web starting at 10 a.m. EST on December 4 and 5, 2000. Using a Web browser, students, teachers, and other visitors around the world can view the lectures with streaming video and audio. To watch the webcast, you will need to download RealPlayer. We recommend that you do this a few days before the lectures to make sure that the software works. The Holiday Lectures website at WWW.HOLIDAYLECTURES.ORG/WEBCAST and the "Technical Tips" page at WWW.HHMI.ORG/GRANTS/HELP.HTM offer instructions for downloading software. You may also e-mail technical questions to WEBCAST@HHMI.ORG.

AskLive2000—Students and teachers will be able to send questions to Michael Rosbash and Joseph S. Takahashi online at WWW.HOLIDAYLECTURES.ORG/ASKLIVE2000. The lecturers will respond to the questions during real-time, online Q&A sessions that will take place from 1:30 p.m. to 2:30 p.m. EST on December 4 and 5, 2000. Please visit the website for instructions.

Interactive Web Resources—The website will contain at least three animations related to circadian rhythms. In one animation, the viewer will see a three-dimensional model of a human brain showing the suprachiasmatic nucleus (SCN).

Using evidence obtained from *Drosophila* and mammalian models, the second animation provides a more detailed view of the negative feedback loop. This loop demonstrates how certain genes are activated by transcription factors and how the protein products of these genes can block their own gene activation.

In another animation, the SCN is shown as a master clock regulating the oscillations of peripheral organs. Normally, the SCN and peripheral organs oscillate synchronously. However, the SCN, muscles, liver, and lung are thought to adjust to time changes, such as those associated with air travel, at varying rates. Asynchronous oscillations are adjusted over time in this animation based on a mouse model.

One of the most valuable tools available in research today is the ability to manipulate genes to learn about their functions in organisms. A virtual laboratory will be available on the Web that will teach the user how to tag a gene important in circadian rhythms

with the reporter gene luciferase. Luciferase was originally isolated from fireflies and is now widely used as a marker for active genes, both in transgenic models and in cell culture models of gene function. By observing luciferase glowing in cells, the user will explore oscillations of circadian genes.

Ask a Scientist—This feature enables Web visitors to e-mail questions on the current topic, biological clocks, and on a range of other biological topics to scientists associated with HHMI-funded programs. Most questions receive a personal e-mail response within two to three weeks. Each week an interesting question and answer is posted on the website at WWW.HHMI.ORG/ASKASCIENTIST. Visitors to the site can also read profiles of some of the scientists who volunteer their time to Ask a Scientist.

Virtual Exhibit—Each year, a museum-quality exhibit, mounted at HHMI headquarters in Chevy Chase, Maryland, complements the lecture series and provides an interactive experience for students. The exhibit, described on page 67, will be available in virtual format on the Web. QuickTime Virtual Reality technology allows Web visitors to view the exhibit hall and browse artifacts and explanatory text.

Holiday Lectures Exhibit 2000

The exhibit will focus on the biological mechanisms by which organisms judge time on a 24-hour basis. These particular biological rhythms are termed circadian. Historical and modern artifacts will trace how researchers became aware of the timekeeping ability of plants and animals, including humans. In centuries past, people observed that the daily activities of organisms coincided with the rising and setting of the sun and moon. Today we recognize that organisms maintain a sense of time even in the absence of any environmental clues. The research and thinking that led to a shift in our understanding of activity rhythms is a key focus of the exhibit.

Artifacts from the National Museum of Health and Medicine and the Smithsonian Institution in Washington, D.C., and from other individual and corporate contributors will depict advances in our understanding of circadian rhythms. Students will see devices by which the circadian activity patterns of organisms—whether plant, fungus, fly, crab, sparrow, or mouse—can be monitored automatically. Images of human experiments to test circadian rhythms aboard modern spacecraft in flight will be shown. Mechanical and chemical oscillators that model biological timekeeping systems will also be presented. Graphics will depict how the circadian cycle modulates our patterns of sleep.

Visitors to the exhibit will be able to test whether their own circadian rhythms indicate that they are "night owls" or "morning larks." Visitors will also be able to measure their temperature and compare their readings to a chart that reveals how the body's core temperature fluctuates during the day. An interactive display will allow participants to see and hear a renowned scientist explain the circumstances behind the discovery of the first gene known to be part of the biological clock mechanism.

Previous Lectures

The Holiday Lectures on Science have covered a wide range of cutting-edge research topics since the series began in 1993. Webcasts of most previous lectures can be viewed on demand at WWW.HOLIDAYLECTURES.ORG/WEBCAST. These lectures are indexed by topic, so Web visitors can go directly to the portions of the lectures that most interest them. Web resources such as virtual laboratories, virtual exhibits, animations, and multimedia demonstrations are available at WWW.BIOINTERACTIVE.ORG.

The Institute also makes available free videos and educational materials to supplement the lectures. To order videos and other resources, go to WWW.HOLIDAYLECTURE.ORG/GRANTS/LECTURES/VIDEO.

1999—"2000 and Beyond: Confronting the Microbe Menace." Topic: Infectious disease. Speakers: Donald Ganem, M.D., HHMI investigator at the University of California—San Francisco, and

B. Brett Finlay, Ph.D., HHMI international research scholar at the University of British Columbia. Web resources include a virtual bacterial identification laboratory, animations on infection by *E. coli* and *Salmonella*, and a primer on antibiotics and antibiotic resistance. A virtual exhibit on infectious disease, "When Worlds Collide: Micro Versus Macro," will soon be available on the Web.

1998—"Of Hearts and Hypertension: Blazing Genetic Trails." Topic: The genetic basis of heart disease and hypertension. Speakers: Richard P. Lifton, M.D., Ph.D., HHMI investigator at Yale University, and Christine E. Seidman, M.D., HHMI investigator at Harvard Medical School. Web resources include the virtual cardiology lab; a virtual exhibit; "Vital Signs: Understanding Cardiovascular Diseases;" and the human heart in 3-D animation.

1997—"Senses and Sensitivity: Neuronal Alliances for Sight and Sound." Topic: How the eye and the ear enable humans and other animals to see and hear. Speakers: A. James Hudspeth, Ph.D., HHMI investigator at Rockefeller University, and Jeremy H. Nathans, M.D., Ph.D., HHMI investigator at Johns Hopkins University. Web resources include "The Cochlea," an illustration of how hearing happens; a virtual exhibit entitled "Hearing and Seeing: Models for Thought"; and a virtual neurophysiology laboratory.

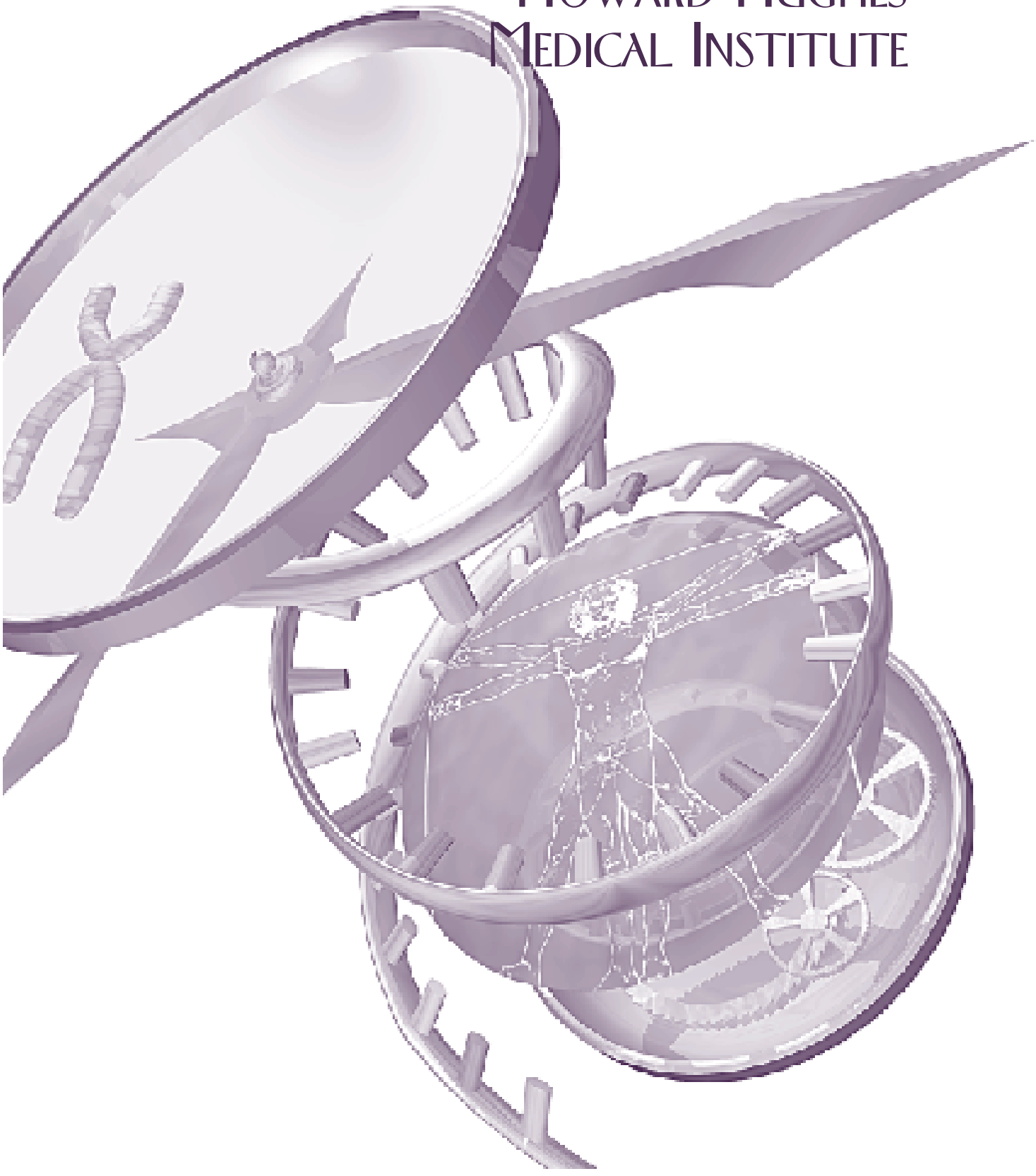
1996—"The Immune System: Friend and Foe." Topic: The functioning and occasional malfunctioning of the immune system. Speakers: John Kappler, Ph.D., and Philippa Marrack, Ph.D., both HHMI investigators at the National Jewish Center for Immunology and Respiratory Medicine in Boulder, Colorado. Web resources include a virtual laboratory on the ELISA assay.

1995 —"The Double Life of RNA." Topic: The discovery of a catalytic function of RNA that may help fight viruses, cancer, and genetic disease. Speaker: Nobel laureate Thomas R. Cech, Ph.D., now president of the Howard Hughes Medical Institute.

1994—"Genes, Gender, and Genetic Disorders." Topic: Current findings on sex determination in mammals and other animals. Speakers: Shirley M. Tilghman, Ph.D., HHMI investigator at Princeton University, and Robert L. Nussbaum, M.D., of the National Human Genome Research Institute at the National Institutes of Health. (For this series, brief lecture summaries are on the Web, but no webcast, videos, or educational resources are available.)

1993—"DaVinci and Darwin in the Molecules of Life." Topic: The three-dimensional structure of biologically important molecules and their role in health and disease. Speakers: Stephen Burley, M.D., D.Phil., and John Kuriyan, Ph.D., both HHMI investigators at Rockefeller University. (For this series, brief lecture summaries are on the Web, but no webcast, videos, or educational resources are available.)

Howard Hughes Medical Institute



OVERVIEW OF PROGRAMS

The Howard Hughes Medical Institute was founded in 1953 by aviator-industrialist Howard R. Hughes. Its charter, in part, reads: *The primary purpose and objective of the Howard Hughes Medical Institute shall be the promotion of human knowledge within the field of the basic sciences (principally the field of medical research and medical education) and the effective application thereof for the benefit of mankind.*

BIOMEDICAL RESEARCH PROGRAM

The Howard Hughes Medical Institute (HHMI) is a nonprofit medical research organization dedicated to basic biomedical research and education. Its principal objectives are the advancement of fundamental knowledge in biomedical science and the application of new scientific knowledge to the alleviation of disease and the promotion of health.

Through its program of direct conduct of medical research, it employs about 350 independent investigators based at locations throughout the country. HHMI conducts research in five broad areas: cell biology, genetics, immunology, neuroscience, and structural biology.

To aid these research efforts, HHMI is involved in the training of graduate and postgraduate students in its investigators' laboratories, has given substantial support to the international genome mapping program, provides research training to medical students through the Research Scholars Program (conducted jointly with the National Institutes of Health), and organizes scientific conferences, workshops, and program reviews.

GRANTS AND SPECIAL PROGRAMS

To complement its research activities, HHMI has a grants program committed to strengthening education in the biological and related sciences at all levels—from kindergarten through postgraduate training. Other important objectives of HHMI's grants program are to advance public understanding and appreciation of science and to broaden access to science for all persons, including women and members of underrepresented minority groups.

HHMI grants are administered by the Office of Grants and Special Programs and focus on two major areas: 1) graduate science education and research and 2) undergraduate and precollege science education. HHMI's grants programs are supported by an array of interrelated Web-based data-collection and reporting initiatives that facilitate grants management, communication and outreach, and HHMI grants program assessment. HHMI's home page, with direct links to grantee home pages, is at **WWW.HHMI.ORG/GRANTS**.

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