

## *C. Elegans* Clock Genes Characterized as Researchers Investigate Aging Processes: Implications for Humans?

Research into the mysteries of aging has heated up dramatically in recent months. A report in the April 12 issue of *Science* presented the gene responsible for Werner's syndrome, an ailment that results in an accelerated aging process.

More recently, Siegfried Hekimi and Bernard Lakowski of McGill University have reported the isolation of several new genes that play an important role in the aging of the nematode *Caenorhabditis elegans* (*Science*, May 17, p. 1010).

Mutations in the new genes, termed clock genes (*clk*), slow down many of the life processes of the worms. These findings suggest that there may be an underlying biological master clock that determines when an organism dies.

As Hekimi told *Science*, "It shows that there is such a thing as a central biological clock which puts into synchrony everything that has a temporal component."

Before the isolation of the new genes it was already known that mutations in the dauer formation (*daf*) genes

can result in constitutive or defective formation of a larval stage known as dauer, in which the organism does not eat.

These larvae arise under conditions of overcrowding or insufficient food supply and represent a hibernation-like state that allows survival until environmental factors improve.

Lakowski and Hekimi's genes appear to affect lifespan by a different mechanism than the dauer genes. Double mutants of the new genes and *daf-16* (a dauer defective mutation) have the same lifespan as the *clk* single mutants.

While the *daf* mutations increase lifespan by extending a stage of development, the *clk* mutations slow down everything from the rate of cell division to the rate of food intake and swimming. Thus, both development and adult life take longer to complete.

The most drastic increase in lifespan occurs in a *daf-2* (a dauer constitutive mutation) *clk-1* double mutant which lives nearly five times longer than a wild type worm. This is the most dra-

matic increase in lifespan ever witnessed in any species.

What does this imply about aging in higher organisms such as humans? Obviously, until *clk* homologs are found



The nematode worm *C. elegans*.

## Varmus Speaks at Commencement

On June 6, Nobel laureate Harold E. Varmus was awarded an honorary degree and delivered the Commencement Address at Harvard University's 345<sup>th</sup> Commencement ceremony.



NIH Director Harold E. Varmus delivered this year's Commencement Address.

In his speech, the current Director of the National Institutes of Health (NIH) said the federal government could be a "powerful source for public good."

At the same time, Varmus cautioned that "We should not try to balance the budget by robbing the future to pay for today's medical services. Rather, we should provide enough money for both."

Varmus noted that significant challenges were in store for the Class of 1996, including Alzheimer's Disease, HIV and Ebola, as well as demographic transitions and rising medical costs.

He also offered a solution: new talent, enthusiasm for science, money, and strong institutions.

Furthermore, according to Varmus, other countries have now surpassed America's spending on basic research. Nevertheless, the NIH director was still optimistic about the future if taxpayers, citizens, and scientists remain committed to addressing the needs of the future in the present.

in humans, little can be said. However, Hekimi has reportedly discovered a similar gene in yeast indicating that the mechanism through which the *clk* genes influence aging may be conserved across many species. The next step is to isolate these genes and perform detailed biochemical analysis on the proteins they encode.

One hint the authors point out is that a homozygous mutant animal born by a heterozygous mother shows almost none of the altered aging effects. This means that the genes have a maternal effect and that their translated product is passed on by the mother in the egg.

Thus it seems likely that the *clk* genes somehow control the rate of metabolism, acting much like a conductor of an orchestra. Only time will tell whether or not this is true, but with the new findings, research into this oldest of mysteries will surely heat up.

—Jeffrey A. Ferrell

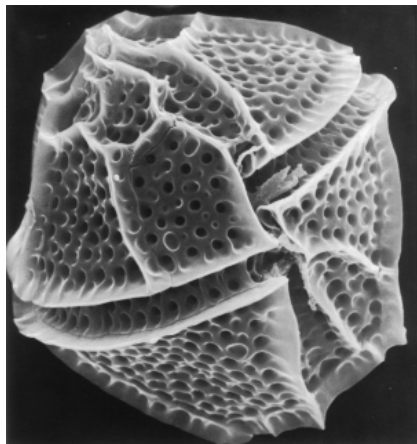
—Maximilian Diehn

# Professor Profile: J. Woodland Hastings

For J. Woodland Hastings, the Paul C. Mangelsdorf Professor of Natural Sciences, bioluminescence is not only at the core of his research, but is also part of his personal life. “Fireflies use light emitting reactions for courtship, and so did I,” said Hastings. “That’s how I met my [future] wife—I invited her to accompany me catching fireflies.”

The Hastings Lab is devoted to the study of the mechanisms of bioluminescence and the biological clock. Using the model organism *Gonyaulax polyedra*, a single-celled algae best known for causing “red-tide” in coastal waters, Hastings’ lab investigates the biochemistry of circadian rhythms—so named because they revolve around a cycle of about one day.

Hastings and his colleagues have cloned two genes that play key roles in this process. One of these—luciferase—



Scanning electron microscope image of *Gonyaulax polyedra*, the model organism used in the Hastings laboratory.

combines with another protein called luciferin to give off visible light. The other confines the luciferin protein to little sacs called scintillons.

The biological clock, as Hastings has discovered, does not revolve strictly around a 24-hour cycle. “By shining different colors of light on the algae, we found the length of a day can vary anywhere from 21 to 27 hours,” Hastings said.

Even more interestingly, Hastings discovered that the schedule of the clock can be reset by exposure to light at certain times.

Current research in the laboratory

is focusing on the regulation of the proteins involved in maintaining the circadian rhythm. While they have discovered that there is a cyclic synthesis and destruction of two key proteins in the cycle, Hastings and his colleagues have also found that the levels of mRNA for each protein remains constant throughout the cycle. This implies that the regulation is at the translational level, differentiating this system from many others which are controlled at the level of the gene.

To carry out these extensive studies, Hastings maintains two rooms, each filled with flasks of the bioluminescent algae from floor to ceiling. One room is lit from 8:00 am to 8:00 pm, while the other is lit from 8:00 pm to 8:00 am.

“When the algae are disturbed,” Hastings said in the pitch-black culture room, “the organisms generate an action potential that causes luminescence.”

As Hastings picked up a culture flask, a burst of blue light filled the room, then quickly subsided.

As a scientist, Hastings emphasizes the value of basic research. “Success in science comes from the study of simple organisms and the study of simple things,” Hastings said.

He also encourages students to get involved in a laboratory. “I feel [research] is very important, even at the undergraduate level,” said Hastings, who has about four students in his lab each year.

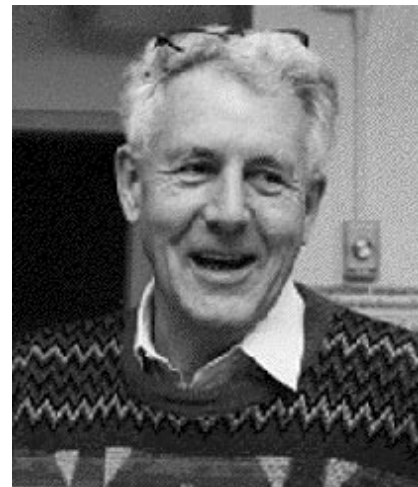
“I’ve tried to help get students interested,” he said, “but research is not for everybody—you shouldn’t do it as an undergraduate just because you’re told to.”

“You have to do what you’re good at, otherwise it won’t be fun,” said Hastings.

Apart from his busy life in the labo-

ratory, Hastings and his wife Hanna have served as masters of Pforzheimer House for the past 20 years. Having just retired this year, Hastings and his wife now plan to move—but only down the street.

“House life has been wonderful,”



Professor J. Woodland Hastings.

Hastings said. “It’s been interesting and fun to help students work through college.”

During their tenure at Pforzheimer, Hastings and his wife brought computers to the house before Harvard University had begun to invest in such programs. They also introduced sports facilities as well as music and art rooms. “House life has been very fulfilling,” Hastings said of his twenty years at Pforzheimer.

Whether in the world of science or the realm of college house life, Professor Hastings is enlightening all those around him.

— Vivek Jain

## New Primate Species Discovered

Researchers in the central Amazonian rain forests of Brazil have recently discovered a new species of marmoset, the 18<sup>th</sup> such primate.

Known as the Satere marmoset (*Callithrix seterei*) after a Native Amazonian group with whom it shares a part of its range, the new addition to the Primate order brings Brazil’s primate count to 75. There are 250 species worldwide.

The marmoset’s range has not yet been clearly defined, but it does not appear to be threatened, according to primatologist Russell Mittermeier, president of Conservation International.

Marmosets are among the smallest primates and are endemic to the tropical forests of the New World. Most tend to be canopy dwellers, living on a diet of insects and soft fruits. →

## Internet Conference Draws Over 1,000; Gates, McNealy Outline Competing Plans of Future Technology

From May 28-31, more than 1000 scientists, businessmen, lawyers and professors descended on Cambridge to attend the Harvard Conference on the Internet and Society—a gathering devoted to the growing importance and potential of the Internet.

The conference attendees were addressed by several keynote speakers including Bill Gates, Chairman and CEO of the Microsoft Corporation, Scott McNealy, Chairman, CEO and President of Sun Microsystems, Larry Tesler of Apple Computer and Neil Rudenstine, Harvard's President.

Speaking to a packed Sanders Theatre, Gates explained Microsoft's intent to lead the industry in developing software for the Internet. At the same time, however, Gates tried to focus his speech on broader societal issues raised by the Internet, rather than detail Microsoft's specific plans. His speech even included a comic video segment which mocked IBM's and AT&T's recent ad campaigns.

"It's good to be back [in Sanders Theatre]," said Gates, the richest man in the world. "One of the few classes I actually attended was held here," he

said, referring to his brief career as a Harvard undergraduate.

Scott McNealy, whose company has pioneered one of the Internet's hottest products—the computer language Java—spoke of the potential of such languages to transform both the Internet and the way we think about computing.

In a speech which featured several jokes—most of which were pointed directly at Bill Gates—McNealy, too, spoke of his days as a Harvard undergraduate. "I want you to know I graduated from here. I didn't drop out and I'm still trying to make up for his two and a half year head start," he joked.

McNealy spoke of Java's potential to deconstruct and decentralize computing. According to McNealy, instead of using large cumbersome applications, users will have Java "applets" which will perform smaller sets of functions.

President Rudenstine, addressing

the conference on the third of four days, spoke about how the Internet has transformed undergraduate education at Harvard. Using statistics from Harvard's computer network, he described how connectivity among undergraduates has soared, as well as new ways students and faculty are interacting via the Internet.

"The Internet has distinctive powers to complement many of our most powerful traditional approaches to learning," said Rudenstine.

In between the keynote speeches, several panels were held in the science center lecture halls. Topics included security encryption, wireless technologies, online commerce and banking, the transformation of the health industry, new educational approaches using the Internet, censorship and government regulation.

—Vivek Jain



Microsoft CEO Bill Gates.

### *New Primate cont'd*

They are polyandrous, meaning that females mate with more than one male. The males participate in raising young and carrying offspring on their backs as they leap among the twigs of the canopy to forage for food.

*C. seterei* adds a curious element to marmoset reproductive biology: both sexes feature fleshy appendages on the genitalia whose function is as yet unknown. Other distinctive features of the species include its mahogany-colored fur and unpigmented facial skin.

Mittermeier expressed optimism about the future of the new species, saying that marmosets are generally quite adaptable to the ongoing destruction of primary rainforests. They can thrive in both abandoned slash-and-burn plots and secondary forest growth.

Unfortunately, other marmosets, including the golden lion tamarin, rank among the world's most endangered animals.

—Daniel M. Paskowitz

## Cyclospora Outbreaks Concern Health Officials in U.S., Canada

Over the past several weeks, outbreaks of *Cyclospora cayotamensis* (a protozoan parasite which causes severe diarrhea, abdominal pain, nausea, fever, and extreme fatigue) have been reported in Houston, Florida, and Toronto.

Health officials in Houston and Toronto reported a statistical association with strawberries, prompting California health officials to take preventive testing precautions.

The results of the initial tests were negative, and major hospitals have reported no cases of *Cyclospora*-related illness contracted in the state.

*Cyclospora* is treatable with antibiotics sold under the name Bactrim and Septra and is generally not fatal.

*Cyclospora* is an extremely difficult organism to identify which has made it difficult to pinpoint the cause of the recent outbreaks.

The parasite, once thought to be

linked to blue-green algae, has been identified as a distinct protozoan parasite and is a relative of *Cryptosporidium*. It passes from an infected individual in an unsporulated state and requires one to two weeks to become infectious.

Moisture and temperatures of at least 25°C are necessary for the organism to sporulate and become infectious. Virtually every outbreak in the past has occurred in an area near water.

The Alliance for Food and Fiber and the California Strawberry Commission announced on June 21 the formation of a panel of experts to study the situation.

The panel included *Cyclospora* expert Dr. Charles Sterling of the University of Arizona, who is credited with identifying the parasite and determining its maturation process.

—Jeffrey A. Ferrell

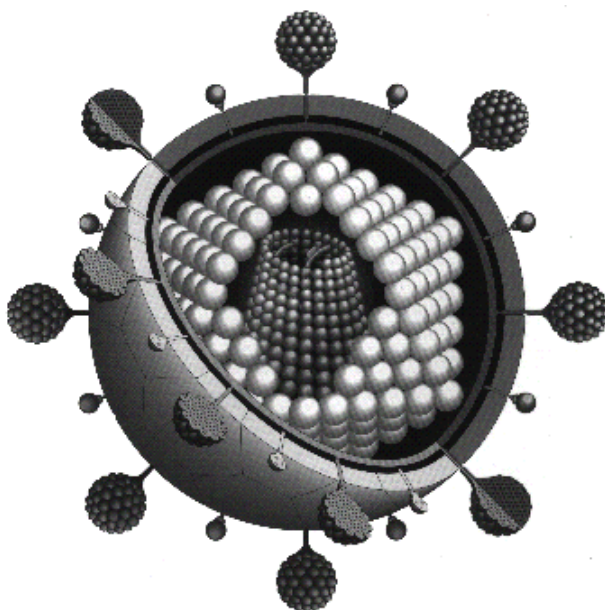
## SIV Orally Transmitted; Viral Dose Required is 6,000 Times Smaller: A New Risk Factor for Spread of HIV?

Ever since human immunodeficiency virus (HIV) was demonstrated to be the cause of AIDS, public health researchers have attempted to understand the ways in which HIV is transmitted. Clinical interviews and laboratory studies have shown the sharing of needles by intravenous drug users and vaginal and anal intercourse are the major modes of HIV transmission.

Although it is theoretically possible that HIV can be transmitted through lesions in the mouth or throat, it has been difficult to assess the risk of oral transmission.

Recently, however, researchers at Tulane and Boston Universities have published a report in the June 7 issue of *Science* showing that monkeys can be orally infected by simian immunodeficiency virus (SIV), a close relative of HIV.

In the study, headed up by oncologist Ruth Ruprect of the Dana Farber Cancer Institute, various concentrations of SIV were administered to monkeys orally, rectally and intravenously.



A rendition of the human immunodeficiency virus (HIV) particle.

When they placed the virus on the tongues of the monkeys, six of the seven animals became infected. The dose of virus needed to orally infect the monkeys was 6000 times lower than the amount needed to infect the monkeys via rectal exposure. However, the viral dose needed for oral infection is still 800 times

lower than the amount needed for intravenous infection.

The report indicates that casual contact such as kissing is unlikely to transmit HIV, but the amount of fluid exchange associated with oral-genital contact could lead to high enough doses to transmit the virus.

Several factors must be considered before extrapolating the conclusions of this study to HIV transmission in humans. This study involved infecting monkeys with cell-free virus.

Oral transmission among humans would most likely involve infected cells rather than free virus; in addition, other research has shown that viral particles can be attenuated by human saliva.

Nevertheless, this study has alerted researchers and the public to the possibility of oral transmission of HIV and has laid the groundwork for further research.

— Michael P. Dybbs

## Combinatorial Chemistry: A High-Speed Pathway to Discovery of Pharmaceutical Compounds

The development of newer, safer and more effective pharmaceutical agents stands at the forefront of medicinal chemistry. This process begins with the identification of so-called "lead" compounds which display desirable biological activity.

Once characterized, these compounds are thoroughly assayed for activity, tested for safety, and, if necessary, modified chemically to enhance their activity and reduce any undesirable side-effects they might exhibit.

Traditionally, pharmaceutical companies discover lead compounds by extracting them from organisms, particularly plants. Numerous molecules possessing highly valuable medicinal properties such as taxol, an anti-tumor agent produced by the yew tree, have

been discovered through this process.

However, the random nature and intense time and effort involved in this operation hinders its popularity.

Combinatorial chemistry represents an increasingly popular alternative method to finding lead compounds. It has been defined as the systematic and repetitive, covalent connection of a set of different "building" blocks of varying structures to each other to yield a large array of diverse molecular entities. Although conceptually simple, combinatorial chemistry is profoundly important in that the evolution of all life hinges upon combinatorial synthesis.

Nucleic acids, proteins, and oligosaccharides, the base components of all biomass, are synthesized from nucleotide, amino acid, and monosac-

charide building blocks, respectively. Without coincidence, these same building blocks were used in the early development of industrial combinatorial methods.

The industrial process, in short, involves submitting building block molecules to a series of bond forming and "deprotection" reactions. As shown in Figure 1, many building blocks are first covalently attached to a solid support. These molecules are then allowed to contact a solution of a mixture of other building blocks, each of which contains two reaction sites. The molecules bound to the support react with the "activated" site of the molecules in solution to form covalent bonds.

No reactions occur at the "protected" site of the solution bound  $\rightarrow$

**Combinatorial Chemistry** *cont'd*

building blocks so as to prevent building block polymerization. Once all reactions have finished, excess reagents are washed away leaving only the linked building blocks covalently bound to the solid support. Subsequent "deprotection" of the support bound compounds followed by exposure to another solution of building blocks elongates the chain by one more unit. This process is repeated until the desired chain length of building blocks has been assembled.

Extremely large numbers of different compounds can be created quickly and easily through this process. Numerically, the number of different compounds  $N$  which can be formed in an

say for biological activity involves dividing compound libraries into smaller pools, portions of which are subsequently exposed to fluorescently labeled receptors.

The pools of compounds which demonstrate the greatest levels of binding to the receptor are then subdivided and the process repeated. In this way, pools are enriched for the desired substance, which can then be identified through spectroscopy.

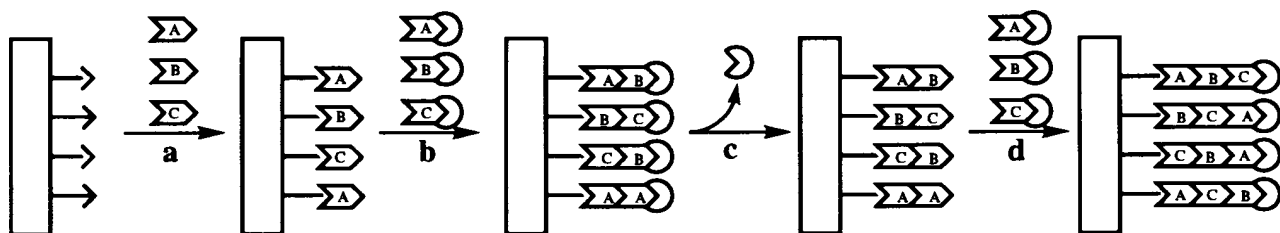
It must be emphasized that identification of compounds which demonstrate biological activity represents the key step in the combinatorial process. While modern spectroscopic methods are excellent, they do have limitations. Thus, other strategies for discovering bioactive compounds have recently been developed.

bind leads to the immediate identification of the biologically active compounds.

Although proven effective, this technique contains a large drawback in that the receptors must be able to bind to the synthesized compounds while still covalently attached to the silicon wafer. This condition almost certainly hinders the activity of many compounds which would be biologically active in solution.

Since the vast majority of biological interactions between compounds and receptors occur at cell surfaces, new combinatorial techniques have been developed in which artificial proteins are displayed on cell membranes.

Random oligonucleotide cassettes are synthesized and placed in appropriate genetic vectors for the produc-



**Figure 1.** Combinatorial chemistry enables the assembly of a large number of different molecules in a short period of time following a simple protocol: (a) initial attachment of building blocks to a solid support; (b) exposure of these building blocks to a solution of protected molecules; (c) subsequent deprotection; (d) re-exposure to another solution of building blocks, etc. This process is repeated until the desired chain length of building blocks has been assembled.

ideal combinatorial synthesis is determined by the number of building blocks  $b$  available for each step and the number of synthetic steps  $x$  in the reaction scheme. If in each step in the synthesis the same number of building blocks is used, the number of possible compounds which could be made is  $N=b^x$ . Thus, if 100 different building blocks are available for each step in the synthesis, 10 billion different compounds can be formed after only five steps!

Once chemical libraries are produced, the compounds in these libraries are assayed for biological activity. The sheer number of compounds generated, coupled with their minute quantities, makes this process a formidable task.

Most often, the biological activity of compounds sought through combinatorial chemistry involves binding to a specific receptor target. While many methods exist, one popular way to as-

One such strategy relies on the spatial segregation of molecules produced in the reactions. A very effective method uses solid supports of small silicon wafers and building blocks containing photolabile protecting groups. Using lasers, very fine patterns of the silicon wafers can be illuminated, removing only the protecting groups of molecules present in these patterns. The wafers are then exposed to a solution of a known compound.

By successively and systematically illuminating different patterns and exposing the wafers to different chemicals, the wafers become coated with a chemical library of extremely high density (40,000 oligomers/cm<sup>2</sup>). The compound libraries, still covalently attached to the wafers, are then exposed to fluorescently labeled receptors.

Because the pattern of illumination and reactions with compounds is systematic, the position(s) where receptors

tion of membrane surface proteins. Mutant cell colonies are then screened for bioactive surface proteins by exposing the colonies to appropriately labeled receptors. The specific peptides which display activity can then be isolated and subsequently identified via well established peptide degradation processes.

The number of different compounds which can be produced via combinatorial chemistry is astounding. Statistically, the chances of finding lead compounds through combinatorial synthesis are much greater than traditional methods. In addition, combinatorial chemistry is fast, efficient, and cost effective, rendering it highly suitable for industrial applications.

Combinatorial chemistry clearly represents a significant advance in the continuing quest to discover pharmaceutical compounds.

—Matthew B. Oak

