Dinosaur ancestor’s vision possibly nocturnal
240-million-year-old protein created in test tube

Call it “Trassic Park”—with statistics, instead of amber-pre-
served DNA, researchers at the Howard Hughes Medical
Institute at The Rockefeller University and Yale University
recreated in the test tube a func-
tional pigment that would have
characterized the eyes of
archosaurs (“ruling reptiles”) and
allowed these direct ancestors of
dinosaurs to see in dim light.
The pigment, rhodopsin, was
recreated based on the scientists’ “inferring” its protein sequence.
Their findings, reported in the
September issue of Molecular
Biology and Evolution, offer the
first look at a protein that has
ever been seen in 240 million
years, and pave the way for sci-
entists to study how the struc-
ture and function of vision pig-
ments — and ultimately other
biologically important molecules — have changed over the
course of evolutionary time.

“Visual pigments trigger the
critical first step in the bio-
chemical cascade of vision in
humans and other animals and
obviously were present in now
extinct species,” says senior
author Thomas P. Sakmar, head of
the Laboratory of Molecular
Biology and Biochemistry.

When Nature published a
research paper by Professor
Vincent Fischetti and his colla-
agues, postdoctoral researchers
Raymond Schuch and Daniel
Nelson, that identified a phage
enzyme that kills the deadly
anthrax bacteria, the journal
showcased the article on its
August 22 cover. The research
also attracted international press
attention, with reports on the
findings appearing on the front
pages of The New York Times and
USA Today.

“I can’t remember the last time a
microbiology research paper made
the cover of Nature, let alone
the front pages of two major
dailies,” Fischetti says.

“Microbiology is now looked
upon as playing a particularly
important role, both in fighting
tuberculosis, as well as emerg-
ing and re-emerging infectious
bugs.”

Scientific and media interest in
phage myxom is not new — it
attracted attention in the early
20th century (a 1925 New York
Times story is headlined, “The
Virus that Eats Bacteria”), and phage therapy was moderately
successful in the Soviet Union in
the 1930s. However, bacteria
develop resistance to phage as they
do to antibiotics, eventually ren-
dering the treatment ineffective.
Fischetti, however, is using
phage in a new way.

Phage (for bacteriophage —
“bacteria-eating” virus) has been
battling anthrax and other
species of bacteria for billions of
years. By isolating one of their
primary weapons, an enzyme
called a lysozyme, the scientists
developed a powerful new agent
that can specifically target and
wipe out millions of anthrax
bacteria in seconds.

“We’re looking at a new plat-
form: nature vs. nature,” he says.

“Rather than engineering an
antibiotic, we’re using nature to
control the environment.”

Working with Rockefeller’s
Bioinnovation Resource Center,
headed by Alison North, the
Fischetti lab produced a stun-
ing video of the phage enzyme
destroying a noninfectious
cob of Bacillus anthracis (left to right), the offspring strain of anthrax used in this
study.

Belinda S.W. Chang, first author
and research assistant professor
at Rockefeller: “We are doing
further biochemical studies on
this recreated pigment to clarify
this issue.”

Chang turned to existing
databases and employed sophisti-
cated statistical methods to infer
continued on page 2
What inspires yeast cells to divide? New findings by Rockefeller scientists shift focus to new model

Often in science a novel set of experiments comes along that forces researchers to abandon old models for new ones that better fit their observations. This is the case in a recent *Nature* report by Rockefeller University researchers, which finds that past models of cellular division in the simple yeast organism were focused on the wrong protein.

Until now, scientists thought that yeast cells began dividing into two separate cells upon the destruction of a “cyclin” protein called Clb5. But the new research shows that a related protein called Clb2 is in fact the real trigger.

“To our surprise, the current model of cyclins and cellular division in yeast does not appear to hold true,” says Ralph Wein, a postdoctoral researcher at Rockefeller and first author of the paper. “We found that replicating cells do divide in the absence of Clb5, which means that its destruction cannot be the signal for division. What’s more, we show that replicating cells cannot divide in the presence of Clb2.”

In addition to providing fundamental insight into the “cell cycle,” the process by which all cells from yeast to human create exact duplicates of themselves, the findings have implications for treating cancer — which is characterized by a cell cycle gone awry.

“Yeast and human cells share many of the same cell cycle mechanisms,” says Frederick R. Cross, head of the Laboratory of Yeast Molecular Genetics and principal author of the paper. “Because of this and because they are easier to work with, yeast organisms are ideal models for studying how the cell cycle may normally work in humans, as well as how it might malfunction in cancer.”

How cells reproduce

All eukaryotic cells (cells that contain a nucleus) must undergo some form of a cell cycle to grow and reproduce. During this process, two crucial events must occur within a cell’s nucleus: replication of the DNA, called S-phase, and separation of the daughter chromosomes into two groups, called mitosis or M-phase. Complicating this process are two periods of rest, which take place just before both S- and M-phase, and are called G1 and G2, respectively.

Only when the cell senses that these events have transpired without error will it exit mitosis and divide into two daughter cells. At this point, the process either begins anew, or a cell enters a state of dormancy, called G0.

How a cell moves from one phase to the next depends on periodic waves of cyclins: low levels prepare DNA for replication, higher levels trigger S-phase and mitosis, and a drastic drop in cyclin number signals the cell to begin dividing. Equally important to this process are the proteins that cyclins bind to and activate, called cyclin-dependent kinases (CDKs). Once activated, CDKs carry out the specific cellular tasks required for growth and division.

Cancer arises when the body fails to properly regulate this process. For example, healthy cells respond to DNA-damaging agents, such as sunlight or cigarette smoke, by halting their cell cycle while the damage is repaired, or by committing a type of cell suicide called apoptosis. But cancerous cells have lost this system of checks and balances, resulting in uncontrolled cell growth, DNA damage and eventually tumors. This breakdown in the cell cycle is caused by genetic mutations that lead to abnormal amounts of cell cycle proteins, such as the cyclins.

Cellular oscillators

The latest findings also suggest a new way of thinking about a yeast cell’s “oscillators.” Oscillators are protein complexes that control the ebb and flow of cyclins within a cell’s nucleus, thereby ensuring an orderly progression through the cell cycle. During mitosis, they signal the cell to destroy certain cyclins, which then forces it to exit mitosis and begin division.

In both human and yeast cells, there are two oscillators: the Cdc20 oscillator and the Cdc1 oscillator. Previously, scientists thought that the Cdc20 oscillator controlled chromosome separation as well as mitotic exit via elimination of Clb5, while the Cdc1 oscillator was thought to complete exit from mitosis by destroying Clb2.

But the new *Nature* report tells a different story. It shows that the Cdc20 oscillator dictates exit from mitosis via elimination of Clb5, not Clb2.

“Previous experiments showing the destruction of Clb5 to be the primary trigger for cell division were not flawed,” says Wein. “Rather, the conclusions drawn from them were incorrect. We can now go back and reinterpret those experiments as meaning only that the elimination of Clb5 can act as a trigger for mitotic exit under experimental conditions. But we now know that the essential trigger is the direct destruction of Clb2 by Cdc20.”

The researchers say that the destruction of Clb5 may instead be required for proper chromosome separation and mitotic exit, while the second mainly oversees the break between cycles of growth and division. G1. Because G1 provides higher organisms with the ability to create different types of cells, the researchers speculate that this second oscillator may represent a necessary step in the evolution of both yeast and humans.

— Whitney Clavin

Dinosaur’s ancestor

The most likely DNA sequences that the ancestral archosaur would have had for its rhodopsin.

“From the databases, we pulled rhodopsin gene sequences for such animals as dogs, rats, cows, birds, teleost fish, eels and amphibians. Then we aligned them,” says Chang. “Using our knowledge of how these vertebrates are related to each other, we inferred a clade and a model of how often certain types of genetic changes occur over time, we calculated the most likely gene sequence.”

In her calculations, she used maximum likelihood phylogenetic statistical methods. Chang and her colleagues next took the inferred DNA sequence for archosaur rhodopsin and reconstructed a gene, which they then inserted into mammalian tissue cell cultures — a standard method for producing rhodopsin in the lab.

As expected, the gene instructed the cells to generate rhodopsin in the mammalian tissue. But, did the protein structure and biological function of the artificially produced archosaur rhodopsin resemble “natural” rhodopsin? To answer this question, the researchers showed that it binds to a molecule called 11 cis-retinal, gives a characteristic absorption spectrum in the visible range and activates in response to light.

To see color, humans have three types of visual receptors, sensitive to red, green and blue light, but the primary molecule involved in all of these is a form of vitamin A called 11-cis-retinal.

“We found it does bind 11-cis-retinal and produces a very beautiful absorption spectrum with a minimal sensitivity to slightly red-shifted wavelengths when compared with our control in the laboratory, which is bovine rhodopsin,” says Chang. “Although we don’t know why the archosaur rhodopsin is shifted toward the red end of the spectrum, it is closest to the spectrum measured for bird rhodopsin.”

The final piece of evidence that the researchers had produced a functional rhodopsin was that the activated form of rhodopsin triggered the rest of the signal transduction cascade in the photoreceptor cell in other words, it interacted with the “second messenger,” the G protein transducin.

“Characteristics of rhodopsin determine characteristics of vision directly, so from this, we can infer things about how archosaurs actually saw at night and under dim-light conditions,” says Chang. “We can infer that their night vision was, at least on the level of their rhodopsin and its activation of G protein, basically as good as mammalian rhodopsin, which is surprising, since mammals went through a nocturnal phase.”

— Joseph Bonner

Beibind S. W. Duong

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— Joseph Bonner
A year later, communications head looks back ... and to the future

Pheromones — chemical signals that influence social and reproductive behaviors — have been studied since the 1950s, but the molecules in the mammalian nervous system that actually detect pheromones have remained elusive.

Now, a research team led by Associate Professor Peter Mombaerts and graduate student Karina Del Panta provides the first functional evidence for molecular receptors for pheromones in mammals. Their findings contribute to understanding the brain’s function in orchestrating social and reproductive behavior as well as helping explain why sexual reproduction typically occurs only within a species.

Ultimately, the findings may explain how species form.

In the September 5 issue of Nature, Mombaerts, Del Panta and colleagues report that intruding or invading animals’ vomeronasal organ (VNO) is a part of the olfactory system thought to specialize in the detection of pheromones. A third test focused on male-male sexual behavior. Socially inexperienced mice are often observed to exhibit sexual behavior toward other males until they become more experienced and learn to distinguish males from females. Socially inexperienced mutant mice, surprisingly, made fewer sexual advances toward males, suggesting that the mutants are better at distinguishing between sexes without prior experience, or that their sex drive is reduced.

The fourth behavioral test analyzed sexual behavior of males toward females, also dependent on a functioning VNO. Compared to their normal counterparts, the mutant males tended to mount females fewer times, and the more they were exposed to females, the less they mounted. The Rockefeller flame is very close to home, principally because my high school science classes were dreadful. While a reporter in the early 1970s for The Atlanta Constitution I covered women’s health. I soon realized that great science is behind great medicine and that biomedical research has advanced civilizations.

Why should any institution communicate?

People identify closely with their places of work. Thus, informing them about what is accomplished and how good the business is. A robust internal communications program contributes to employee morale, productivity and retention. And, open lines of communication help redress rumors. In the absence of information, people tend to speculate. That’s why Tom Sakmar’s interview on the trustees’ decision about construction (see News&Notes, May 24) was so helpful.

Communications to the external world — the public — is essential. Too it helps ‘build good will.’ People tend to support organizations about which they know. George Goodwin, a public relations pro and former Pulitzer Prize-winning reporter, told me in 1983, ‘people are down on what they are not up on.’ His advice helped me cope with the first animal rights campaign targeted at the Yerkes Primate Center where I then worked. The public, including the news media, were not up on or informed about the rationale for animal research and thus were vulnerable to animal rights- ists’ misinformation campaigns. That’s why it is and is still important to engage in public outreach via the news media, tours, lectures and community relations. At one time or another, bad luck affects most institutions, even great ones, in the form of events such as a major protest, an explosion that injures or kills employees or visitors, or accusations of misconduct. An organization needs ‘good will’ even if it hasn’t done anything wrong. If good will exists, people will give the institution the benefit of the doubt — allow it time to gather and communicate the facts about the situation.

In addition to ‘buying’ good will, effective external communications contribute to recruitment of patients for clinical trials, recruitment of students, faculty and staff, and fundraising.

Cathy Yarbrough says robust communications ‘generates public “good will.”’

Rockefeller researchers provide first functional evidence for mammalian pheromone receptors

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The role of pheromones in human behavior also is not clear. The Mombaerts team had shown earlier that the human genome harbors five putative pheromone receptor genes that could be functional. The mouse genome, by contrast, has at least 140 receptor genes of this type, and the mutant strain of mice described in the Nature paper makes precisely 16 of these.

“…”

For more information visit http://www.rockefeller.edu/ pubsinfo/090402.php.

— Joseph Bonner

Art reflects on 9/11

“South Tower, North Tower,” a commemorative work of art reflecting on the September 11 attack on the World Trade Center, will be on display in the Abby Aldrich Rockefeller Dining Room beginning the week of September 16. Currently on display is the Winus Research Building lobby, “South Tower, North Tower,” created in Paris by New York City-based artist Robert Lambert.