Winter 1989

Genespeak: [Dr. Michael W. Young]

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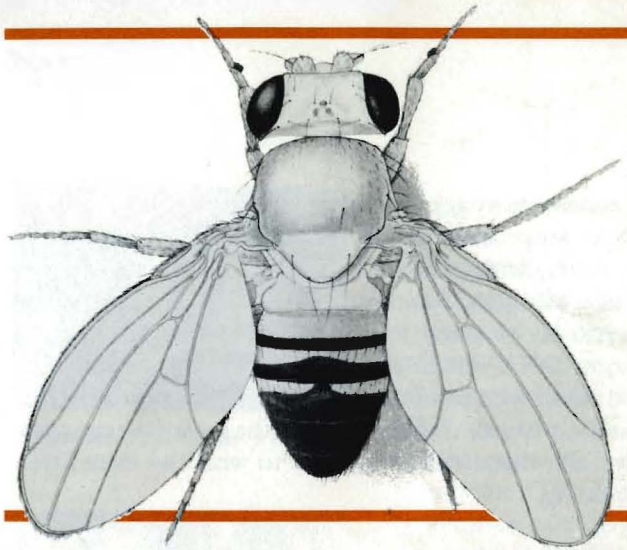


Male Drosophila

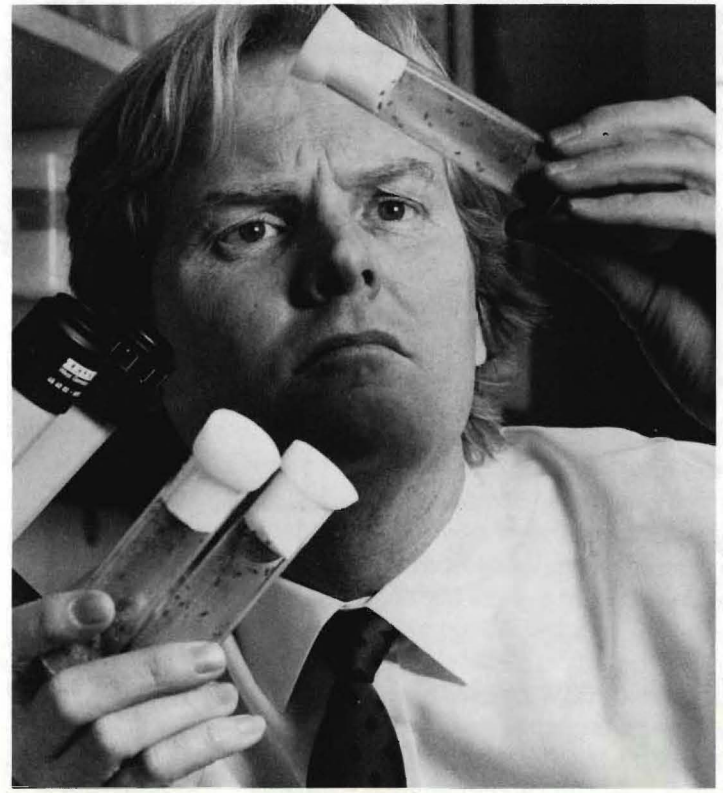
THE ROCKEFELLER UNIVERSITY

RESEARCH PROFILES

WINTER 1989-90

Genespeak

According to Arabian myth, in heaven every desire is satisfied, only to be followed by other desires, which are fulfilled in turn—*ad infinitum*. Michael W. Young, Professor of Genetics at The Rockefeller University and Investigator with the Howard Hughes Medical Institute on campus, has been dwelling in what could be thought of as Arabian heaven's terrestrial equivalent. In Dr. Young's case, desire translates into a thirst for answers to elemental questions about the nature of genes and chromosomes, which, once acquired, give rise to deeper questions. As a result of his persistent and often tortuous decade-and-more quest, he is closing in on two of the most fundamental mysteries in biology: cell differentiation—how do cells "know" what to become—and biological rhythms—what makes the "biological clock" tick?



Michael W. Young

ELEPHANTS AND *E. COLI*

In the 1960s and early 1970s, much of what had been learned about the organization of genetic machinery had come from research on prokaryotes (pro-kar-EE-oats)—single-celled, nucleus-free microorganisms. Specifically, much research had centered on *E. coli*, a harmless bacterium found predominantly in the intestine. The prevailing belief at the time was that whether the organism was the simple prokaryote with all life processes confined to a single cell or a highly differentiated eukaryote (you-kar-EE-oat) possessing an organized nucleus and elaborating a complex body structure with many specialized cells, the

Mapping Genes:
A. *Messenger (m)RNA is isolated from cells of a* Drosophila *and radioactively labeled.*
B. *Bacterial colonies are produced containing sections of the fly's entire DNA. The labeled (m)RNA is then placed onto the colonies where individual RNA strands selectively bind to corresponding segments of DNA.*
C. *From one bacterial colony, many copies of the same DNA are replicated in a solution.*
D. *The replicated DNA is introduced onto the fly chromosome where it binds with its corresponding DNA segment at a specific location.*
E. *The photomicrograph shows radioactively labeled DNA as clusters of black dots adhering to each site on the chromosomes where a copy of the gene resides.*

genetic machinery of the two was essentially the same. As a geneticist-cum-aphorist of the time put it: "What is true of *E. coli* is also true of elephants."

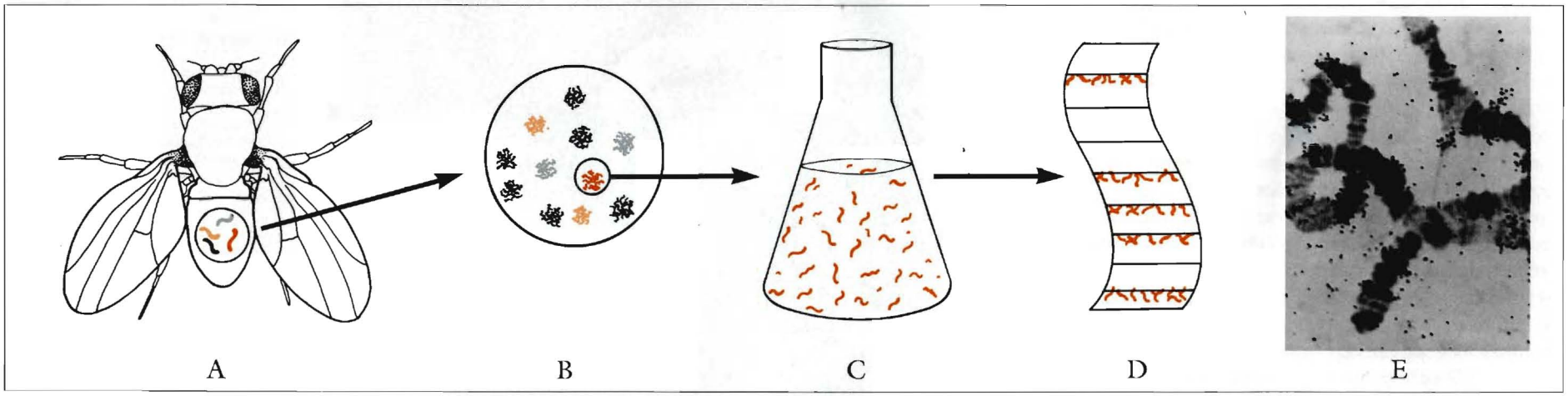
Indeed, existing genetic research seemed to say that multicellular organisms might have roughly the same numbers and kinds of genes as could be found in a typical single-celled bacterium. As a doctoral candidate at the University of Texas, Young set out to get a new count of the number of genes required to create a eukaryote. His inventory-taking studies were conducted on the fruit fly *Drosophila*, a long-time darling of genetics research. The fly was easy to handle in the lab; its short breeding time translated into quick, efficient crossings of many varieties of lab-made mutants. Another of the fly's assets was the larval salivary gland, which housed a naturally amplified, easy-to-observe version of the fly's complete genome—four large chromosomes, each made up of a thousand identical copies stacked one atop the other. Thus, gene mutations can often be detected as minute changes in the chromosome material with an ordinary microscope.

The early genetic studies that stimulated Dr. Young's new work had concluded that the fruit fly's full gene complement totaled between four and five thousand, roughly equal to the gene count in the far-less-complex, single-celled prokaryote *E. coli*! New biochemical research made the count even more surprising because the fly genome was found to possess forty times more DNA. Suspecting that the usual inventory-taking system might be faulty, he devised a way to measure the sizes of genes packed in the salivary chromosomes. He found that most of the genes previously counted took up only a small part of the chromosome DNA. Much of the extra DNA was between the genes and could even be removed from the chromosome without causing any apparent abnormality in the growth or structure of the fly. Still, there wasn't a clue as to what the extra DNA might be doing.

A NOMADIC DETOUR

As prelude to further defining the anatomy of the eukaryotic genome, Dr. Young, then a postdoctoral fellow at Stanford University, wished to produce another map of the chromosome. This map would distinguish those stretches of DNA that were actively instructing the synthesis of proteins from the large amount of DNA that presumably did not.

It was known that a gene contained a continuous stretch of double-stranded, helical DNA located on the chromosome. There is a sequence of chemical bases (four in all) arranged like rungs on a ladder between each strand, creating a code for the manufacture of a specific protein. Another long molecule called ribonucleic acid (RNA) copies the code using the same four



bases and, as m (messenger) RNA, travels to the cell's protein manufacturing center, the ribosome, where the surrogate code is "read" and the gene-prescribed protein produced.

Using newly invented gene-splicing techniques, Young was able to propagate (clone) different short pieces of the *Drosophila* chromosome DNA in each of thousands of bacterial colonies. He then took a heterogeneous soup composed of all messenger RNA molecules made by a particular kind of *Drosophila* cell, and tagged all of these molecules with a microscopic amount of radioactive material. Each radioactively labeled mRNA, he knew, would bind only to its parental segment of DNA where it coded for protein. By allowing the radioactive mRNA mix to react with a sheet of paper spotted with the bacterial colonies, he was able to sort the individual, component mRNA molecules by affinity to their parental "genes". Every responding bacterial colony was a factory for producing copies of one parental gene. By allowing these copies of the purified genes to bind to their original sites on the salivary chromosome—using a process called *in situ* hybridization—their locations could be mapped on the chromosome.

Volumes of genetic research indicate that no matter what the organism, each gene usually occupies a single, unique location in the chromosome. Dr. Young and his collaborators at Stanford were surprised, therefore, when they saw most of the purified "genes" attaching to multiple locations on the chromosome. It was a discovery that triggered a detour in his research focus.

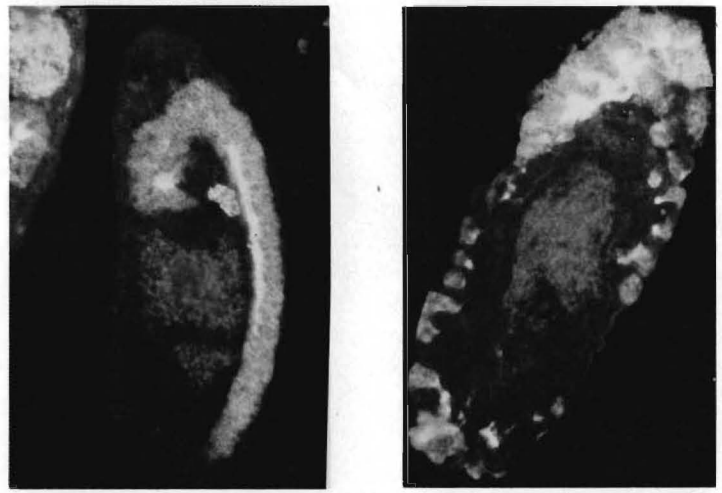
"I got sidetracked," he admits. "But it was something that demanded attention and explanation."

In 1978, Dr. Young elected to pursue this finding at The Rockefeller University when he joined the University Fellows Program. The program had been previously established in order to broaden the university's research focus by providing support for gifted young researchers whose area of interest was not already under investigation in existing university laboratories.

Over the next three years, Dr. Young conducted a series of experiments which demonstrated that the new repetitive genes were a part of the extra DNA separating "traditional" genes on the chromosome. It also became apparent why the new repetitive genes were missed in the early gene counts: The repeat fragments were "nomadic" (also known as transposons or transposable elements). They are, in effect, genetic prowlers, with the molecular wherewithal to jump from place to place in the genome. Dr. Young showed that the nomads, which could be arranged into 50 to 100 different families, together compose nearly a sixth of the total chromosome DNA. In collaboration with Andrew Dowsett, a postdoctoral fellow, he also discovered that different numbers and kinds of nomads could be found in different fruit fly populations, suggesting that they had no life sustaining role. Instead of providing a code for the fly, the mRNA that the nomads produce functions in their movement and self-propagation.

BACK TO THE TRADITIONAL GENE

In the early '80s, Dr. Young once again turned his attention to the question of eukaryotic gene architecture. He set his sights on the fruit fly gene called *Notch*. It was a long-known fact that mutations in *Notch* were at the root of a dazzling spectrum of abnormalities, or phenotypes, involving fly wings, bristles, eyes, or a lethal failure of cells to differentiate properly into nerve and skin cells in the embryonic stage. Young wondered: How could one gene affect such a diverse mix of organs, such a multiplicity of function? *Notch* must be providing critical instructions for building the fly. What was the secret of this developmental blueprint?



The Notch *gene is critical to the development of the fruit fly's nervous system. Left, the discrete, lightly stained areas of the normal fly embryo show the nervous system growing in an organized fashion. Right, the absence of the* Notch *gene product results in a disorganized nervous system shown by the diffuse, lightly stained areas.*

To launch his study, Young acquired samples of the many *Notch* mutant flies and, together with postdoctoral fellows Simon Kidd and Trevor Lockett, isolated recombinant DNA clones corresponding to each of the mutant genes. Their work, reported in 1983, showed that, ironically, the array of different mutations affecting eye, bristle, and wing stemmed from the insertion of nomadic DNA within the boundaries of the *Notch* gene: On rare occasions nomads were moving from spaces between the genes into the genes themselves.

They discovered that mutations can be caused by transposable elements landing in the *Notch* gene in two different ways: Some genes, such as *Notch*, are composed of a patchwork of protein coding and non-coding DNA, and the nomads can interrupt both.

As Dr. Young explains, in the late '70s molecular biologists discovered that the eukaryotic gene is often composed of two different types of DNA sequences arranged in a series of split or discontinuous segments. One type, called exons, codes for a portion of protein; and exons are separated by non-coding sequences of the second type, called introns. When a gene is "turned on" or "being expressed" to produce a specific protein, an unbroken stretch of RNA is manufactured in correspondence to the chemical code contained in all the DNA bases. This complementary RNA, containing both introns and exons, is called a "primary transcript". Then, the molecule undergoes biological editing, such that only those fragments that bear the code of the exons are spliced to form active mRNA. This abridged RNA molecule travels out of the nucleus to the cell's protein-making machinery.

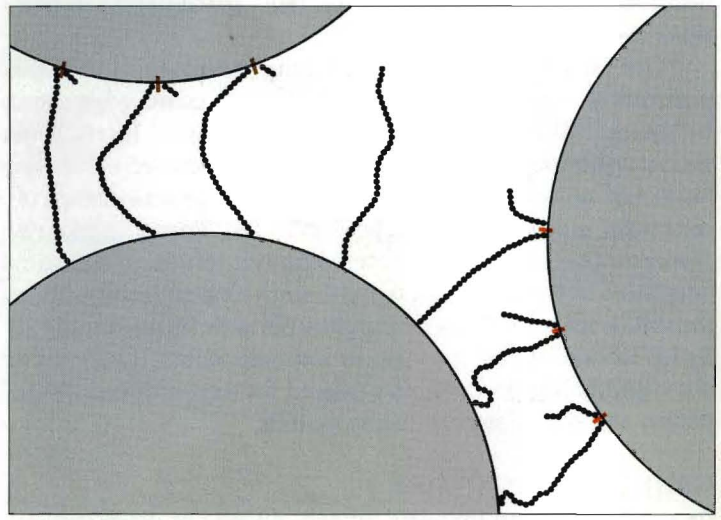
Dr. Young and his colleagues found that nomads landing in coding parts of the *Notch* gene always caused the same abnormality: embryonic death. By contrast, transposons landing in non-coding regions produced the wing, eye, and bristle abnormalities. In a paper published in 1986, Drs. Young and Kidd demonstrated that the particular non-coding abnormality seemed to depend on the "species" of transposable element that had dropped in. Unlike the nomads that destroyed the coding structure of exons and therefore eliminated the protein, nomads in non-coding parts of *Notch* caused abnormalities by instructing the gene to make its protein in the wrong place or at the wrong time. Each species of nomad could misregulate *Notch* in a unique way. "The ostensible complexity of *Notch*," says Dr. Young, "was really due to the transposable elements. Without them, a much simpler picture of gene architecture and function emerged."

INSTRUCTIONS FOR IDENTITY

How do cells, which are identical in the early embryonic stages of life, know when to modify their structure in order to perform specialized life–producing functions? For Dr. Young, abnormalities in embryonic development caused by mutational changes in the *Notch* protein pointed the way.

Three hours after fertilization, the fly embryo reaches the stage in which a yolk-filled ball is surrounded by about 8,000 cells. At that point, about a quarter of the cells, clustered on the ventral surface, start to take on either the characteristics of nerve cells (which will move into the embryo interior) or skin cells (which will remain on the surface). In the absence of a normal functioning *Notch* gene, all 1,800 cells in that ventral aggregate, rather than just 400 in normal circumstances, develop into nerve cells and the embryo eventually dies.

"Cells begin to talk to each other early in development," says Dr. Young. "The information they exchange allows the cells to



The protein produced by the Notch *gene is thought to play an important role in the cell's ability to communicate to other cells and to orchestrate normal nerve growth. The protein strands (dotted lines) extend beyond the cell surface and are composed of 36 hormone-like units. Nearby cells may come into contact with the protein at different places along its length to receive different instructions regarding subsequent nerve cell development.*

Graduate Fellow Mary Baylies

Dr. Lino Saez

do what they cannot do on their own." The protein encoded by the *Notch* gene is a crucial component in this decision-making chatter when it comes to making skin and nerve.

To get at the details of this signaling system, Dr. Young had to determine the composition of the *Notch* protein. He and his colleagues worked out its base-by-base coding sequence contained in the DNA master molecule. From this molecular profile it was concluded that the *Notch* protein is anchored to the cell that produces it and that it extends beyond the cell's surface membrane. This extracellular extension was found to comprise an uninterrupted array of 36 copies of a hormone-like molecule. Young and his colleagues speculated that such a string of hormones tethered to the cell surface might allow especially intimate chatter since only cells that touch each other could communicate through the protein. More support for this picture has come from research by Drs. Young and Kidd and graduate fellows Mary Baylies and Gregory Gasic (now at Yale University), who used gene-splicing strategies to successfully follow production of the *Notch* protein in developing embryonic cells.

Why so many hormone repeats? Actually, every hormone repeat in the *Notch* protein is a little bit different, and with postdoctoral fellows Mark Kelley and Simon Kidd, and visiting professor Andrew Deutsch, Dr. Young found that the different hormones composing the *Notch* protein carry different instructions about cell development.

"Our current thinking," Dr. Young explains, "is that this protruding portion of the *Notch* protein represents what could be thought of as an information-rich paragraph. Interactions with neighboring cells at varying times may involve a different 'sentence' or 'phrase'. The likelihood is that a series of such interactions guides cell development." Dr. Young thinks the *Notch* protein may allow a developing cell to talk to changing neighbors from the time of its birth until its final incorporation into adult tissue. He hopes experiments now being conducted by his laboratory will produce an intimate, detailed portrait of the communication network required for nerve/skin differentiation and cell differentiation in general.

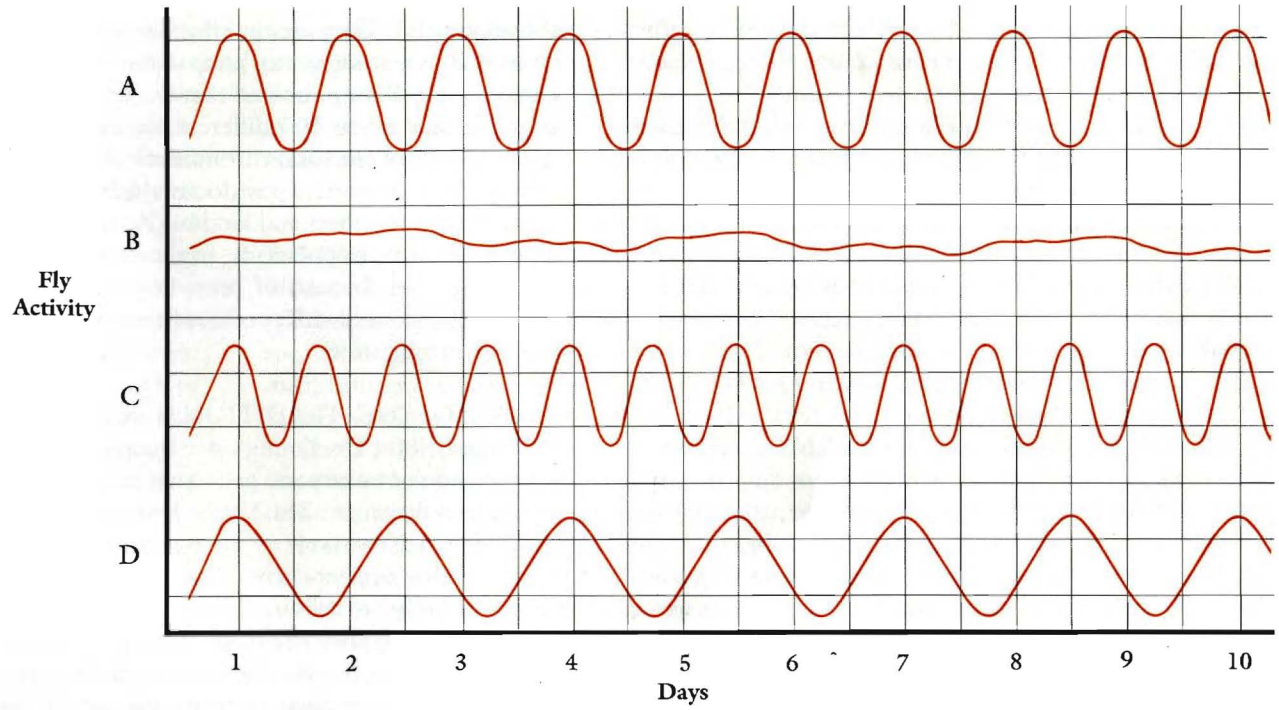
GENETIC CLOCKWORK

As an outgrowth of his work on nervous system development, Dr. Young became interested in how genes affect the dynamic function of the nerve cells, particularly how genes govern behavior. For this new line of study, Dr. Young set his sights on *per*, one of several genes involved in regulating biological rhythms. He was already familiar with the gene, having helped map it to a region of the chromosome that lay very near to *Notch* when he was a student in Texas.

Studies in the fruit fly revealed that the *per* gene regulates at least three timed events: the circadian (24-hour) wake-sleep cycle, the courtship song of the male fruit fly, and when the adult fly will hatch from its pupal cocoon. In one mutant fruit fly strain, called per^s (s for short), the biological clock runs faster than normal, reducing the wake-sleep cycle to 19 hours, the rhythm of the wingbeat courtship song from 60 seconds to 40 seconds, and the once-in-a-lifetime hatching from a dawn to a predawn event. By contrast, the flies with per^l (l for long) have a slower-than-normal clock, manifesting itself in a 30-hour sleep-wake cycle, a courtship song lasting 80 seconds, and a later-in-the-morning hatching time. The third mutant strain, per^o (o for nothing, or null) offers altogether unpredictable, arrhythmic, essentially random timing patterns on all three events.

Subsequent studies made it clear that no single, centralized pacemaker—no single tissue—was responsible for these various timed functions. Rather, says Dr. Young, there seems to be a series of independent timers that use the same protein product of *per* again and again. Control of circadian rhythm, for instance, has been mapped to brain tissue, while the courtship song has been linked to action in a nerve bundle in the chest.

Dr. Young and his associates, Drs. Thaddeus Bargiello and Rob Jackson, had cloned the *per* gene in 1984 and had worked out its code or sequence two years later. Hence, they were able to deduce *per's* protein product. However, knowledge of the overall structure of the protein offered little insight into how it might work to regulate time. So Young took another approach. He introduced into fruit fly embryos specially engineered *per* genes which would generate normal protein but at varying concentrations. When these flies reached adulthood, he found that the more protein the animal produced, the faster its biological clock ticked. This held for daily rhythms and for rhythms of the minute-long courtship song.



The per *gene helps regulate biological rhythms in the fruit fly.*
GRAPH A. *When present in normal amounts, the* per *gene helps promote a rhythmic wave of alternating activity and inactivity.*
GRAPH B. *The arrhythmic timing pattern of the mutant fly strain,* per°, *which lacks a working* per *gene.*
GRAPH C. *The* per^{s} *mutation causes a faster running biological clock with peaks of activity every 18 rather than the usual 24 hours.*
GRAPH D. *The amount of* per *protein a fly makes affects the speed of its biological clock. Specially engineered* per *genes have been introduced into the fly generating protein in varying concentrations. Shown is the activity of a fly making 20 times less protein than normal. It has about half as many cycles as the hyperactive mutant fly,* per^{s} *in Graph C.*

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Continuing to focus at the molecular level, Dr. Young wondered how does the protein, at whatever dosage, regulate the biological clock? Clues were provided from a surprising source: the fruit fly's salivary glands.

Dr. Young and co-workers Thaddeus Bargiello, Lino Saez, Mary Baylies, and Gregory Gasic began a collaborative effort with Dr. David Spray, an electrophysiologist at the Albert Einstein College of Medicine. The idea was to measure some of the electrical and chemical communication properties of cells in mutant fruit flies. By looking at the entire clock mutant series, evidence was uncovered for altered communication through small channels, called gap junctions, connecting the cytoplasm of certain adjacent cells. Salivary gland cells of short-period mutants were intensely communicative; those of long-period mutants less so, and arrhythmic mutations were found to cause lowest levels of cell-cell connectivity. Since gap junctions are involved in transmission of electrical signals across some synapses in the brain, Young and his associates have speculated that the *per* mutations may alter biological rhythms by modifying rates of electrical conductance among nerve cells specifically devoted to timing. Where might these "pacemaker" cells be? For sleep-wake cycles, Drs. Saez and Young find cells composing the eye and optic lobes of the brain make the protein, and thus may hold the key.

A new series of experiments is now underway, in collaboration with researchers at the University of Virginia, to determine just what precisely it is the *per* protein is doing to affect transmission among cells. Is the protein working directly on the gap junctions, prompting more of them to remain open? Or is it acting on something else first, which in turn leads to alterations in conductance?

There seems to be no end to the questions still awaiting resolution, including, most assuredly, questions yet to be posed. How best to describe this perpetual experimentation with uncertainty, this seemingly endless peeling of proverbial layers?

In a word: heavenly. □