ABOUT THE CARNEGIE INSTITUTION

Andrew Carnegie founded the Carnegie Institution of Washington in 1902 as an organization for scientific discovery. Since then, Carnegie scientists have pioneered many fields. The institution is headquartered in Washington, D.C., and has five departments around the country devoted to research in plant biology, developmental biology, earth and planetary sciences, and astronomy.

Mr. Carnegie’s intention was for the institution to be home to the “exceptional” person—an individual with imagination and dedication who worked at the cutting edge of a specialty. Some of the institution’s exceptional individuals include Nobel laureate geneticists Barbara McClintock and Alfred Hershey, and Mount Wilson astronomer Edwin Hubble, for whom the Hubble Space Telescope is named.

Scientists at Carnegie today are free to investigate their specific areas of interest under the broad goals of an individual department. Researchers are given the support and equipment they need in a nurturing environment. This arrangement has produced unexpected benefits to society, among them hybrid corn and radar.

The organization is an endowed, independent, nonprofit institution. Significant additional support comes from federal grants and private donations. A board of trustees, consisting of leaders in business, the sciences, education, and public service, oversees Carnegie’s operations. An appointed president presides over day-to-day administration. Each of the five departments is independently managed by a director, aided by support staff. In addition to the scientists on staff, there is a constantly changing roster of pre- and postdoctoral fellows and associates, plus visiting investigators at each facility.

Carnegie is also involved in education at the lower levels. In 1989 President Maxine Singer launched the Saturday science school, First Light. The school encourages Washington, D.C., children to explore the world around them with the aid of a unique, hands-on curriculum. The success of First Light led to CASE, the Carnegie Academy for Science Education, which is a training ground for elementary school teachers in the art of teaching science. Recently CASE expanded its program to the study of mathematics.

Carnegie’s legacy of pioneering scientific research is vibrant today, and its unique founding principles ensure that Carnegie researchers will continue to extend the frontiers of science for decades to come.
Introduction .......................... 3
A Brief History ......................... 3
Research at the Department ........ 4
Facilities ............................... 5
The New Building ...................... 5
Life at Embryology .................... 6
Research Staff Scientists ............. 7
Glossary ............................... 20
Staff Directory ....................... 24

Pictured from top are three of the organisms used in the department’s laboratories: a frog, a rat, and an immature zebrafish.

Images of the scientists and facilities are courtesy Kris Belschner of the Department of Embryology.
This image from Erika Matunis’s laboratory shows photomicrographs of two Drosophila testes. A control is at top. See page 14 for more details about the study.
The department has become recognized worldwide as one of the premier research centers in cellular, developmental, and genetic biology.

A Brief History

Carnegie’s Department of Embryology was founded in 1913 in affiliation with the department of anatomy of Johns Hopkins University in Baltimore, Maryland. It was established to study human embryo development. Over the next four decades, the scientists procured and studied a collection of some 10,000 human embryos, developing a fundamental description of human development and conducting pathbreaking experimental studies. In 1960 the department moved from its location at the Hopkins medical school to its current site on the Homewood Campus. The move initiated a close relationship with the university’s Department of Biology and bolstered a new research focus on understanding fundamental developmental mechanisms at the cellular and molecular levels. In 1987 faculty members were first appointed Investigators of the Howard Hughes Medical Institute. Currently three of the eight regular faculty members are so affiliated.

Since the 1960s, investigators at Embryology have focused their efforts on seeking answers to some of the fundamental questions in biology. How do molecular processes acting at the level of promoters, chromatin, and whole chromosomes ensure that genes turn on and off appropriately during development? How are the many sub-assemblies of the cell put together from specific gene products, and how do they work? How do embryonic cells communicate with each other, and how do they differentiate into many different kinds of cells? A solid understanding of these issues will not only advance basic knowledge but is also likely to help solve human health problems such as cancer, birth defects, and developmental disorders. In addition, departmental scientists have periodically invented widely used experimental methods, including the first procedure for genetically engineering a multicellular organism (Drosophila), which have significantly advanced the progress of research.

The unique atmosphere and research style fostered at Embryology have allowed this small enterprise to have a disproportionately large impact on science. The department’s philosophy is based on the premise that scientific leadership requires exceptional individuals with the insight, resources, and courage to investigate the margins of what is feasible and respectable. We make every effort to hire the most creative and skilled researchers, paying relatively little attention to their area of current research. We encourage bold ventures by stimulating intellectual development, providing material resources, and trusting that each faculty member’s interests will evolve.

Several other factors reinforce scientific creativity. The department offers no tenure; instead, faculty members are evaluated at five-year intervals. We emphasize originality and the long-term significance of a research program rather than its...
level of funding or professional visibility. Communication, a key to stimulating new ideas, is extensive and is promoted in several ways. First, we encourage a steady flow of new associates, fellows, students, and visitors. In addition, research groups are kept small—to fewer than 10 members—to enhance communication between faculty and lab staff and to make it easier for faculty to remain engaged in experimentation. We revere this atmosphere as the source of our inspiration and strive to improve it as the department evolves within the current milieu.

Research at the Department

Genetic studies devoted to entire genomes, a field now known as genomics, are attracting widespread public interest. Genomics has several goals: to discover all of an organism’s genes, to determine their location on a chromosome and what their structure is, and to learn how they are regulated, expressed, and function.

Genomic research is viewed by many people as a relatively new development. In reality, though, the Carnegie Institution and the Department of Embryology have been deciphering gene structure and function for most of the last hundred years. Carnegie became involved in the genomics of the fruit fly Drosophila almost 90 years ago through its support of researchers in the Thomas H. Morgan laboratory at Columbia University. The intellectual program of classical genetics, as reflected in Morgan’s research, set many of the same goals of gene discovery, mapping, and analysis that are today being realized with DNA sequences.

In 1918 Drosophila genomic information was compiled into one of the first genome databases, and from 1918 to 1992 it was distributed to the public in a series of publications produced by the Carnegie Institution of Washington.

Identifying genes and describing their activity is unquestionably important, but analyzing gene function remains the critical element in gene-centered biology. Research from this department, on several occasions, has accelerated this aspect of genomic research. Classically, genes required for a particular physiological process are discovered by identifying mutations that prevent the process from occurring normally. To complete this “forward” genetic approach, it is necessary to decipher the molecular identities of the altered genes. In contrast, by first purifying genes, transcribing them in vitro, and assessing the effects of chemically produced mutations, Don Brown’s group at Embryology forged one of the original templates for an alternative or “reverse genetic” approach to understanding gene function. Today, with a plethora of genes defined only by their DNA sequence, reverse genetics is on its way to becoming the norm.

One of the most versatile new methods for discovering what genes do was developed recently by Staff Member Andrew Fire in collaboration with Dr. Craig Mello of the University of Massachusetts, Amherst. Their method of “RNA interference” (RNAi) allows gene transcripts to be inactivated within individual cells or in whole invertebrate organisms following uptake of complementary double-stranded RNA. The discovery by Fire and postdoc Lisa Timmons that a gene’s activity can be shut down when Caenorhabditis elegans worms simply ingest bacteria containing the appropriate double-stranded RNA (dsRNA) has made it possible to use RNAi to functionally screen large numbers of C. elegans genes. Several laboratories and startup companies are currently racing to study all of the known Caenorhabditis genes using this method.

Several other departmental members, including Staff Member Yixian Zheng, are using RNAi to down-regulate specific Drosophila genes in tissue culture cells. Another group, led by Staff Associate Jim Wilhelm, is preparing to expand this approach so that the entire roster of fruit fly genes can be scanned for their effects on any cellular process of interest. In addition to its speed and simplicity, RNAi promises to allow experimenters to shut off multiple genes located in different chromosomes simultaneously. This has been difficult to do using traditional methods and greatly facilitates the discovery of gene interactions and pathways.

The need for detailed information on genome structure and for tediously acquired mutant collections has caused biologists to focus on a relatively small number of “model” organisms. These include flies, worms, mice, zebrafish, and yeast, all of which are studied at the department. One unfortunate side effect of this trend has been to discourage gene-based research into problems that are better studied in species lying outside this narrow group. However, advancing genomic technology is now creating opportunities for gene-based studies within nontraditional organisms.
Staff Associate Alejandro Sánchez and postdoc Phil Newmark have taken the flatworm planaria, an organism that lacked modern genomic resources, and in just three years have generated the tools needed for sophisticated genetic studies, including genome-wide surveys of gene expression. Planaria exhibit unparalleled powers of regeneration. Sánchez and Newmark discovered that planarian genes can be inactivated with RNAi and have used their new tools to identify candidate genes that seem to be important for regeneration. Their work, along with other ongoing projects in the department, continues the Carnegie Institution’s long tradition of genomic studies and expands it to encompass an ever broader collection of organisms and phenomena.

Facilities

Currently, the department’s laboratories and seminar rooms are housed in a building located on the northwestern corner of the Johns Hopkins University campus. However, plans are under way to construct a new home for the department just a few blocks away on a three-acre site south of the Space Telescope Science Institute along San Martin Drive. The new facility will enhance the department’s current interactions with the Department of Biology at Johns Hopkins University and the Howard Hughes Medical Institute. A primary purpose of this new structure is to help the department evolve in such a manner as to fully exploit the unprecedented opportunities that currently exist for gaining fundamental new understanding of biological processes. The new building is expected to be completed in 2004.

The current building, constructed in 1960, remains highly functional but can no longer fully accommodate all the department’s activities. It includes a large number of advanced microscopes as well as biotechnology and computational facilities. Other laboratories support cell and tissue culture studies, or house analytical and preparative ultracentrifuges and spectrophotometric, electrophoretic, and chromatographic instruments. Research is advanced by a number of service facilities, each staffed by experienced personnel. These include an instrument-making shop and centers for histology, computing, housing animals, and administrative support. The departmental library receives about 45 key periodicals locally, and many others are available by electronic subscription. The libraries and campus of Johns Hopkins University, within easy walking distance, are open to staff, fellows, and associates of the department.

The New Building

The new building will be located within a long, narrow stream valley containing large wooded areas and steep rocky slopes. To the east of San Martin Drive is the Johns Hopkins University. To the west of the stream is a city park that is heavily used by Hopkins students and local residents. The creative integration of the new building into this spectacular setting may well result in the creation of a new landmark, both on the Hopkins campus and in Baltimore.

The building is envisioned to have three levels, totaling about 70,000 gross square feet. The first floor, which will be mostly underground, is planned to house animal-care facilities and other functions that do not require windows.
The rest of the structure will contain a mixture of biochemistry-type laboratories suitable for cell and developmental genetics research, support space located near the laboratories, core facilities, and public areas for meetings and seminars. The building will also house state-of-the-art genomic research, with its attendant equipment. The actual wet laboratories will take up approximately 40% of the space, a smaller fraction than is found in older-style laboratory buildings. However, the structure will be flexible to allow for changes in the percentages of laboratory and support space and to accommodate new research directions, experimental organisms, and core activities.

**Life at Embryology**

The department’s successes over the years are in large part due to an atmosphere of creative interaction. A variety of regular scientific meetings are held each week. In the most important meeting, every researcher presents the results of his or her most recent investigations. Questions are asked throughout the presentation, and discussion may continue long afterward. Ideas and novel solutions to problems are often born from these exchanges. And a common sense of our intellectual standards is developed.

Other general meetings are held to present recent research papers and to listen to outside speakers. Smaller groups of 4 to 15 persons meet regularly as well; they consist of researchers in individual laboratories, and of mixed groups from several labs that share particular research systems or an interest in particular problems. Two or three noon seminars are held each week. All these events are open to scientists in the Baltimore community interested in developmental problems. In addition, the department sponsors a daylong minisymposium once a year, organized by graduate students and postdoctoral fellows.

Through its academic program, the Department of Embryology offers a unique training ground for research scientists. In appointing postdoctoral and predoctoral fellows and associates, the department gives first consideration to those who appear likely to contribute to the advancement of a field in biology or medicine. The previous training of an applicant, whether in embryology, molecular biology or genetics, pediatrics or physics, may thus be of secondary importance.

The department offers opportunities to about 30 outside scholars each year. Of this number, the majority are postdoctoral fellows and associates, senior visiting investigators, and independent Staff Associates. Staff Associates are supported at the department for five years. Most postdoctoral fellows and associates spend four years in the program.
Jimo Borjigin is interested in the molecular mechanisms of circadian rhythms, the predictable daily variations in our bodies that influence sleep and behavior. Using the rat, her lab is studying the pineal, a small organ within the brain that rhythmically secretes the hormone melatonin at night. The goal of the lab is to identify the molecules that control pineal rhythms and the genetic processes involved in melatonin production.

Researchers in the lab have been investigating the function of several previously identified night-specific pineal genes. In one area, they cloned the gene that encodes serotonin NAT (N-acetyltransferase), a key enzyme in melatonin synthesis, and discovered that melatonin production is controlled by the night-specific transcription of the NAT gene. A chief objective of the lab is to understand the molecular basis behind the circadian production of this enzyme and the regulation of melatonin secretion. As part of this work, the scientists found that the neurotransmitter serotonin, implicated in a variety of human disorders, is produced at much higher levels at night and may influence the production of melatonin. Further studies of serotonin activity in this system may help researchers understand other serotonin-related functions of the brain, which could be important to the treatment of depression and migraine headaches.

Another night-activated gene currently under investigation is PINA (Pineal Night-specific ATPase). The scientists discovered that PINA is a shorter form of ATP7B, a gene involved in copper transport that is defective in patients with Wilson’s disease, affecting their ability to excrete copper. The Borjigin lab has found that PINA does not influence melatonin synthesis and release, suggesting that the pineal may have other yet unidentified circadian functions. As the investigators unravel the function of PINA in the rat, more may be learned about the role of the gene in Wilson’s disease.

The scientists could not conduct their work without integrating molecular techniques with physiological approaches. One valuable tool recently perfected in the Borjigin lab is online pineal microdialysis. This technique allows the scientists to measure hormone production of the pineal in a single rat, in real time, over many circadian cycles. Another procedure they developed is the delivery of recombinant viral vectors into live animals, allowing them to express genes in the pineal. This permits the researchers to bypass other time-consuming and costly manipulations and, when coupled with online microdialysis, provides an opportunity to analyze circadian gene function in real time in live animals.

SELECTED PUBLICATIONS


Borjigin is a recipient of the Life Sciences Research Foundation Fellowship. In addition, since 1999 she has received the John Merck Scholars Award, given for research “underlying developmental disabilities.”
The thyroid gland secretes thyroxine (TH), a hormone essential for the growth and development of all vertebrates including humans. To understand TH action, Donald Brown studies one of the most dramatic roles of the hormone, the control of amphibian metamorphosis—the process by which a tadpole turns into a frog. He studies the frog *Xenopus laevis*, from South Africa, because of the ease with which it can be reared in the laboratory. Events as different as the formation of limbs, the remodeling of organs, and the resorption of tadpole tissues such as the tail are all directed by TH. How can a simple molecule control so many different developmental changes? The hormone works by regulating the expression of groups of genes. It instructs some genes to absorb the tail and gills and others to start new tissues and organs.

To understand how a simple hormone triggers different responses in different tadpole tissues, Brown and colleagues first cloned two forms of thyroid hormone receptors. They were then able to identify many of the genes that are regulated by TH, including those involved in resorption of the tail, those that control the growth and differentiation of new limbs, and those that remodel the intestine. The researchers discovered the exact cell types that express these genes and identified the protein that each gene encodes. Knowing the identity of a gene and where and when it is expressed provides clues to the possible role it has in metamorphosis.

Recently, the researchers have been using a technique called transgenesis, in which any gene can be introduced into the genome of a *Xenopus* embryo before first cleavage—the stage when the fertilized egg becomes multicellular. This method guarantees that all the cells of the embryo and tadpole will contain the gene. The tadpole that is expressing the "transgene" then grows to metamorphosis and the influence of this genetic alteration on metamorphosis is assessed. This powerful protocol has allowed Brown’s group to test the effect of many *Xenopus* genes on metamorphosis. They have also been able to clarify the role of pituitary hormones on metamorphosis by forcing tadpoles to overproduce these hormones.

**SELECTED PUBLICATIONS**


Brown, D. D. 1994. Some genes were isolated and their structure studied before the recombinant DNA era, *BioEssays 16*, 139-143.
The mouse is a traditional model organism for understanding physiological processes in humans. Chen-Ming Fan uses the mouse to study the underlying mechanisms involved in human development and genetic diseases. He concentrates on identifying and understanding the signals that direct the musculoskeletal system to develop in the mammalian embryo. Skin, muscle, cartilage, and bone are all derived from a group of progenitor structures called somites. Various growth factors—molecules that stimulate the growth of cells—in the surrounding tissues work in concert to signal each somitic cell to differentiate into a specific tissue type. The Fan lab has identified four types of growth factors that have profound effects on somite development. The goal of the lab is to identify more factors, the pathways in which they operate, and the genes that control the processes. Taking advantage of the genome information and DNA chip technology, the lab has surveyed 15,000 genes (more than half of the total mouse genes) for their expression in the somites and is currently investigating the functions of these genes.

The scientists have identified several genes, which they call Sim1, Sim2, and Gas1, and are currently determining their roles in musculoskeletal development. They mutated the genes to study what happens in their absence and found that the Sim2 and Gas1 genes play important roles in the formation of skeletal and craniofacial structures. This finding suggests that Sim2 may contribute to aspects of Down’s syndrome because the human SIM2 gene is located within the Down’s syndrome “critical region” of chromosome 21. Future work in this area will help to understand aspects of this condition.

As a bonus, the researchers found that Sim1 controls the developing hypothalamus—the part of the brain that governs the function of the pituitary by integrating multiple brain signals and regulating the release of essential neuroendocrine hormones. Recently, they found that the Sim2 gene also contributes to this process. As a result, mice lacking the Sim1 and Sim2 genes cannot keep the body’s endocrine system in balance. The mouse Sim genes are similar in their protein structure to the sim gene in the fruit fly, which also controls the developing central nervous system. Because of this similarity, the scientists propose that this pathway is inherent to many species, from fly to human, in generating the neuroendocrine system.

SELECTED PUBLICATIONS


Scientists in the Fan lab found to their surprise that Sim1-heterozygous mice become obese from an uncontrollable appetite (an obese Sim1 is shown on the left next to a control). Human patients that are heterozygous for the Sim1 gene also display very early onset obesity. Many of the genetic components involved in mouse and human obesity have been identified. Since the hypothalamus has been implicated in the control of satiety, the researchers are currently investigating how Sim1 fits into the known genetic pathway involved in the obese condition.
Scientists in the Fire lab are interested in mechanisms by which cells choose their fates during development. They use the nematode *Caenorhabditis elegans* for their investigations because of the ease with which it can be manipulated and studied under a microscope.

The earliest choices that cells make during development are between germ line fates, leading to immature eggs (oocytes) and sperm, and somatic fates, yielding other tissue types. The Fire lab is investigating how germ line/soma decisions are made and executed, and how the two cell populations later differ in their capacities for gene expression. It appears that germ line cells have highly active mechanisms to limit the expression of genes that are turned on in somatic cells. Some of these mechanisms act at the level of the protein complexes that decorate chromosomes, while others reflect gene context—the nature of DNA sequences surrounding a specific gene. The scientists are taking a variety of genetic and cytological approaches toward understanding the components that allow these initial decisions.

A second project in the Fire lab addresses distinctions made between different somatic fates. For this project the researchers have chosen to focus on a small number of muscle types that are formed during embryonic and postembryonic development. This work has led the investigators to analyze a multiple-step process, starting with the initiation of the identities of early embryonic cells called blastomeres, and followed by steps that maintain these early identities, provide a memory of lineage information, induce the decision to differentiate into a muscle cell, and mediate subsequent distinctions between different classes of muscle cells.

The biochemical mechanisms by which genes are stably maintained in “on” or “off” states form a basis for much of biology. Analyses of developmental processes in the Fire lab have unexpectedly illuminated a variety of control mechanisms that are used as general “off” switches. These “gene silencing” processes seem to augment standard (and more transient) mechanisms that regulate gene expression. An “off” state can be triggered by a variety of different signals, the most common being aberrant aspects of RNA or DNA structure (e.g., extended regions of double-stranded RNA and extensively repeated DNA sequence). These mechanisms appear to benefit the organism by expanding the available repertoire of developmental control strategies, and by providing a general defense against foreign or unwanted gene expression.

**SELECTED PUBLICATIONS**


The first step in gene expression is the formation of an RNA copy of its DNA. This step, called transcription, takes place in the cell nucleus. Transcription requires an enzyme called RNA polymerase to catalyze the synthesis of the RNA from the DNA template. This, in addition to other processing factors, is needed before messenger RNA (mRNA) can be exported to the cytoplasm, the area surrounding the nucleus. Although the biochemical details of transcription and RNA processing are known, relatively little is understood about their cellular organization. Joseph Gall is interested in how the structure of the nucleus is related to the synthesis and processing of RNA—specifically, what changes occur in the chromosomes and other nuclear components when RNA is synthesized, processed, and transported to the cytoplasm.

Much of the lab’s work is carried out with unlaid eggs removed from the female frog *Xenopus*. These eggs, called oocytes, are giant cells up to 1.5 millimeters (mm) in diameter with a nucleus, or germinal vesicle (GV), 0.4 mm in diameter. The large GV permits scientists to examine the contents and structure of the nucleus in unprecedented detail.

It has generally been thought that RNA polymerase, and other factors involved in RNA synthesis, travel separately to active genes on the chromosomes for processing. Researchers in the Gall lab, however, recently proposed a different model of RNA synthesis in which the RNA machinery is preassembled as a completely separate particle called a transcriptosome. In this model, the transcriptosome brings the entire transcription machinery as a single packet to the genes on the chromosome for processing.

To study what goes on in the GV, the scientists inject transcription and processing factors into the cell and observe where they go and where they end up. They have determined that structures in the GV called Cajal bodies contain many, if not all, factors required for transcription and processing of messenger RNA. The scientists believe that all of the transcription machinery exists in the Cajal bodies, and that a significant fraction of it is assembled there.

Gall hopes to learn how various factors are recruited to the Cajal bodies and how they assemble into the large transcriptosome complexes. He and his colleagues are also trying to isolate transcriptosomes from oocytes to determine their composition and structure in more detail.

SELECTED PUBLICATIONS

The embryo of the tiny zebrafish *Danio rerio* is entirely clear. This feature makes it an ideal model for understanding the genetics behind the formation and patterning of the vertebrate central nervous system. From the moment of fertilization, researchers in Marnie Halpern’s laboratory can observe the fish’s development, identify the genes involved, and learn how they function.

Halpern is studying genes that activate the series of signals that regulate neural differentiation and correct patterning of left-right differences in the brain. She, in collaboration with others, discovered that a zebrafish gene called *cyclops* (*cyc*) encodes a protein involved in this signaling during early neural development. The scientists are examining how Cyclops activity promotes the formation of the ventral midline of the neural tube—a structure that contains cells that play a central role in guiding impulse-transmitting nerve fibers called axons and influence the differentiation of the spinal cord and brain.

Although it has been known that asymmetric positioning of internal organs in vertebrate embryos is mediated through a similar signaling pathway, the researchers have for the first time implicated it in regulating differences between the left and right sides of the forebrain. Three genes in the pathway are transiently expressed on the top of the developing forebrain, but only on the left side. This asymmetry localizes to the region that will give rise to the pineal organ, which is involved in circadian rhythms. In the brains of adult fish that lacked left-sided gene expression as embryos, the pineal stalk is displaced from its normal left-medial position, suggesting that these genes are required for its correct positioning. More recently, Halpern and co-workers have discovered additional anatomical and gene expression asymmetries in this region of the brain.

The Halpern lab has also produced transgenic zebrafish lines with the green fluorescent protein (GFP). These fish allow scientists to label discrete regions of the brain and follow the fates of cells. The transgenic strains will be extremely useful for identifying other genes that regulate forebrain left-right asymmetry.

The lab’s work on zebrafish is providing new insights into neurobiology, and comparative studies on similar genes in other vertebrates will further reveal whether activity of this pathway in the left brain is a feature found in most species. These studies could provide a framework for exploring higher cognitive functions and behavior and shed light on how hemispheric specialization arose in the evolution of the brain.

SELECTED PUBLICATIONS


All organisms are made up of cells, which in turn house the organism’s genetic material as chromosomes. Chromosomes are long, threadlike molecules analogous to the magnetic tape of an audiocassette. To ensure proper inheritance during cell division, the cell first replicates its chromosomes into paired copies called sister chromatids. The chromatids are “glued” together along their length and then condensed, much the way a magnetic tape is spooled within the cassette housing. During mitosis the chromatids “unglue,” separate into two groups, and move away from each other. This segregation is accomplished by the attachment of a specialized “hook” on the chromosomes (the centromere) to a winchlike structure called the mitotic spindle. The cell then divides to produce two new cells, each of which encapsulates one of the two groups of separated chromatids. When chromosome segregation goes awry, cell death or cancer can result. Douglas Koshland and colleagues are trying to understand the structure and molecular control of these mitotic chromosomes. They want to identify the molecules in the centromeres and learn how they move the chromosomes along ropelike structures of the spindle called microtubules. They also want to determine how the “glue” is regulated, and what goes on when sister chromatids condense during cell division.

Koshland uses the simple single-celled yeast *Saccharomyces cerevisiae* for his research. He and his group are also developing new ways to analyze mitotic chromosomes in the yeast. One method focuses on centromere-microtubule interactions, and another uses a fluorescent technique to follow sister chromatid pairing and condensation. Using tools such as these, the group has been able to identify and characterize proteins necessary for centromere function and others important for sister chromatid pairing and condensation. The first of these proteins, called Pds1p, regulates the sister chromatid “glue.” The second is a family of chromosomal proteins known as Smc proteins; they are found in organisms ranging from bacteria to humans and are essential for such processes as chromosome condensation, sister chromatid cohesion, and recombination repair.

Over half of the genes associated with human diseases are similar to ones in the yeast. As a result of the work in the Koshland lab, a human gene similar to one encoding the yeast Pds1p has been discovered and is implicated as a potential important contributor to pituitary cancer. As the researchers learn more about mitotic chromosomes in yeast, other connections to cancer are likely to be discovered.

**SELECTED PUBLICATIONS**


---

In 2000 Douglas Koshland was elected to the American Academy of Arts and Sciences. He is also an Associate Investigator with the Howard Hughes Medical Institute, an appointment he received in 1997.
Stem cells are the undifferentiated cells that have the unique capacity to both renew themselves and form other specialized cells. Because they regenerate, researchers are interested in their potential in treating a variety of human disorders. However, little is known about how stem cells behave because they are rare and reside in complex environments. Erika Matunis studies the stem cells that sustain the production of sperm in the fruit fly *Drosophila melanogaster*. Her goal is to use genetic studies to identify the cues that regulate stem cells in the *Drosophila* testis.

In most cases, stem cells cannot be distinguished from their daughter cells. However, germ line stem cells (gscs)—cells that produce gametes—in the *Drosophila* testis can be identified because they are attached to a small cluster called the hub, which is made up of somatic cells—cells that form nongamete structures. When a germ line stem cell divides, one daughter stays attached to the hub, remaining a stem cell, while the other daughter (called a spermatoblast) moves away from the hub and differentiates into a bundle of sperm. This suggests that cells nearest the hub receive signals to maintain their stem cell fate, while spermatoblasts lose their stem cell character and differentiate.

The Matunis researchers are searching for molecules that regulate these stem cells. Through genetic mutations, they remove candidate signaling molecules from the hub cells to determine if there is a corresponding change in the number of stem cells. They have found that a signaling pathway regulated by a gene called *Jak-STAT* is required to maintain germ line stem cells. This signaling pathway is found in many different organisms. In it, extracellular signaling chemicals called ligands bind to a cell’s receptor and activate an enzyme, which in turn activates transcription factors—genes that control the expression of other genes. The latter are in the Signal Transducer and Activator of Transcription (STAT) family. The scientists have identified a ligand that activates this pathway during sperm production, and found that it is localized to the hub. When the ligand is overproduced, a dramatic increase in the number of stem cells results, suggesting that this signaling instructs stem cell fate.

These data support the researchers’ hypothesis that the hub defines a stem cell niche, and that localized activation of the Jak-STAT pathway instructs the self-renewal of nearby stem cells. The researchers are currently asking if the Jak-STAT pathway is activated directly in the gscs, or in nearby somatic cells.

**SELECTED PUBLICATIONS**


The primary purpose of cell division is to provide each daughter cell with a complete set of chromosomes, the containers for all of the genetic material. To accomplish this task, the cell must first replicate the chromosomes, and then segregate the two copies into new daughter cells using an elaborate structure called the mitotic spindle. The spindle forms from two poles that extend filament-like microtubules toward the chromosomes. The microtubules attach to each pair of duplicated chromosomes at a single site called the centromere. One chromosome copy attaches to each pole, and ultimately the microtubules pull them to the poles on the opposite sides of the cell. Terence Murphy uses the fruit fly Drosophila to study the genes and proteins that control how chromosomes are properly segregated in this manner.

Scientists in the Murphy lab are taking a two-pronged approach to identify the genes and proteins important for centromere function. First, they are systematically testing candidate genes using the method of RNA interference (RNAi), whereby double-stranded RNA is introduced into a cell, silencing the expression of a specific gene. The researchers are trying to determine if the loss of a gene’s product disrupts centromere function. Promising candidates are then tested to see if they interfere with the ability of chromosomes to attach to microtubules, or with another process such as spindle formation. Second, the researchers are developing a technique to measure the frequency at which centromeres malfunction by analyzing the behavior of chromosomes that have been mutated to contain two centromeres. These methods will help identify the genes that specify the centromere and those connected with centromere function.

Murphy also studies how the spindle poles are formed. Animal cells contain an organelle outside the nucleus called the centrosome, which duplicates once and forms the two spindle poles during mitosis. Murphy found that in Drosophila cells a mutant of the gene skpA, a component of the protein degradation machinery, duplicates additional centrosomes that can then disrupt chromosome segregation. These findings suggest that an unknown protein is accumulating in skpA mutant cells, resulting in additional rounds of centrosome duplication. Centrosome overduplication is a common feature of many cancer cells, which might mean that the unknown target of skpA is an important cancer-causing component.

**SELECTED PUBLICATIONS**


From simple wound healing to the complete replacement of lost body parts, regeneration is fundamental to all living things. Although the mechanism of regeneration has been a puzzle in biology for more than 250 years, it has failed to attract the attention of modern molecular biology until recently. Scientists in the Sánchez Alvarado lab are intrigued with this phenomenon and are studying its genetic components, using both vertebrate and nonvertebrate model organisms. They use tadpoles to study regeneration in vertebrate organisms and the planarian, or flatworm, Schmidtea mediterranea for invertebrate investigations.

Research into the regenerative abilities of the planarian date back to the work of the German naturalist Peter Simon Pallas (1741-1811). But it has been in just the last few years that researchers in the Sánchez Alvarado lab have been able to create the tools required to carry out sophisticated genetic studies to understand this phenomenon. Lab scientists have shown that gene expression in these animals can be silenced by using the technique of RNA interference, whereby double-stranded RNA is introduced into a cell, inactivating a target gene. They have also succeeded in labeling planarian stem cells called neoblasts, a feat that has eluded many scientists before them. Neoblasts are the foundation of the flatworm’s regenerative abilities; they are undifferentiated stem cells that migrate to the site in need of repair, undergo differentiation, and regenerate the missing tissues. Labeling these cells assists the investigators in tracing this activity.

The Sánchez Alvarado team has also generated several clonal lines of S. mediterranea, from which it has prepared cDNA libraries. These are collections of expressed genes that are used to obtain genetic sequences. To date, the investigators have procured sequences for close to 4,000 different S. mediterranea genes; the tissues expressing the genes are currently being identified by in situ hybridization. The scientists are also able to simultaneously analyze thousands of DNA fragments using DNA microarrays. This technology is greatly speeding the process for determining gene function. The collection of genes the group is assembling will form part of a Web-based genomic database developed and maintained in the Sánchez Alvarado lab; it will be made accessible to the entire scientific community.

Using other methods, Sánchez Alvarado has made significant strides toward the creation of transgenic planarians using neoblasts as vectors to carry the foreign DNA into the animals. Ultimately, the progress the lab makes to identify regeneration-related genes and understand their function will allow researchers to integrate what they learn into understanding the process of regeneration in higher organisms.

**SELECTED PUBLICATIONS**


Large animals such as humans must regularly replace tissue cells that have worn out or become damaged. Populations of special, relatively undifferentiated “stem cells” located at strategic positions continually revitalize tissues by dividing and giving rise to replacement cells exactly when, where, and in the amounts needed. This remarkable ability is akin to the ordered development of the embryo itself and has long attracted the interest of developmental biologists. However, unlike embryonic cells, stem cells are rare, difficult to recognize, and, consequently, hard to analyze. Allan Spradling investigates the stem cells that give rise to Drosophila eggs. Here, powerful genetic methods can be used to understand stem cell biology.

Spradling’s studies have reinforced the old idea that stem cells are regulated by a special environment known as a stem cell niche. His group has identified three sets of nondividing cells that form habitats for stem cells and send signals that control their behavior. Specific genes and mechanisms have begun to emerge that hold stem cells (but not daughter cells) in the niche, keep the stem cells from differentiating prematurely, and control cell division. In contrast, stem cells themselves may be relatively passive players and may, to some extent, be interchangeable between niches within different tissues. Interestingly, niches appear to use the same growth-promoting signals as developing embryonic tissues, like small embryonic zones persisting in the adult.

How can germ line cells remain healthy over millions of generations, while other cell types age and die? Spradling believes that small clusters of interconnected germ cells that arise immediately after stem cell division hold the answer. The membrane-rich cytoplasmic organelles of the cell, including mitochondria and endoplasmic reticula, reorganize and move between cells within these clusters. A unique germ cell cytoskeleton known historically as the fusome develops as the clusters grow, and may guide a sorting process. Spradling proposes that damaged organelles are sent through the junctions into cells fated to die, while pristine organelles move into cells that will become new oocytes.

Finally, Spradling and his lab members continue to develop new tools for Drosophila research through participation in the Drosophila genome project. Now that the genome has been fully sequenced, the current goal of the lab is produce the key tools needed to understand gene function by isolating insertional mutations in all of the 13,600 genes that have been identified.

SELECTED PUBLICATIONS


One of the central questions in cell biology is how cells organize their interiors. While a lot is known about how proteins are sorted to various membrane-bound compartments within the cell, little is known about how proteins are sorted to particular domains within the cytoplasm—the area that surrounds the nucleus and contains other cell structures. One mechanism cells use to distribute proteins within the cytoplasm is to send the instructions for making proteins to particular locations within the cell. Localizing these instructions, in the form of mRNAs, ensures that the proteins they encode are synthesized only at those sites where the mRNAs are located. It has long been suspected that mRNAs are transported to specific places within the cytoplasm by motors that move along microtubules—tiny filaments within the cell. However, the identity of these motors and how they work has remained elusive. James Wilhelm is interested in identifying the cellular machinery that is responsible for mRNA localization.

One of the best-characterized examples of mRNA localization is during the production of eggs in the *Drosophila* ovary. Two mRNAs, called *oskar* and *bicoid*, contain the messages that are responsible for establishing the head-tail axis of the embryo. The *oskar* mRNA is sent to the posterior pole of the egg, while the *bicoid* mRNA remains at the anterior pole. The molecular basis for this sorting event is completely unknown and is a major focus of the Wilhelm lab’s research.

The scientists in the lab have isolated a complex of seven proteins associated with the protein Exuperantia (Exu), which is genetically implicated in *bicoid* mRNA transport to the anterior pole. Surprisingly, the researchers discovered that this complex also contains the posteriorly localized mRNA, *oskar*. When the *exu* gene is inactivated, *oskar* mRNA is not properly positioned, suggesting that this complex may play a role in both the anterior and posterior signaling pathways that regulate mRNA placement.

A number of proteins in the Exu complex are expressed outside the ovary. Wilhelm believes that these proteins might be part of a core-transport complex that is used to localize different mRNAs. To test this, the lab’s researchers developed a screening method for identifying new localized mRNAs. This should allow them to decipher previously unknown localized messages in the ovary and other tissues, and to test if the components of the Exu complex play a role in mRNA localization in other parts of the body.

SELECTED PUBLICATIONS


Cell division is essential for all organisms to grow and live. During a specific time in a cell’s cycle the elongated apparatus consisting of string-like microtubules called the spindle is assembled to move the chromosomes into two cells. Another structure near the cell’s nucleus, the centrosome, is important for nucleating, or creating, the microtubules and for assembling the spindle. Researchers in the Zheng lab are trying to understand the regulation of the spindle assembly, the structure of the centrosome, and how it organizes the microtubules and participates in spindle assembly. They use the frog *Xenopus* and the fruit fly *Drosophila* for their research.

The centrosome consists of a pair of cylinder-shaped structures called centrioles, which are surrounded by a material called pericentriolar material (PCM). Microtubules are nucleated from this PCM. Upon examination of both the frog and the fruit fly, the Zheng scientists discovered a ring complex containing an essential protein component of microtubules called γ-tubulin. They found that the ring complex, named γTuRC, is essential for centrosomes to form microtubules. The scientists hypothesize that γTuRC is the major microtubule activator at the PCM.

The ring complex consists of approximately six proteins in addition to γ-tubulin. The Zheng team is using a combination of molecular, biochemical, and genetic approaches to understanding the complex. They are focusing on how γTuRC is involved in regulating the microtubule-nucleating activity of the centrosome, how it is recruited and assembled at the PCM, and whether (and how) it is involved in centrosome duplication.

The researchers are also investigating the signals that regulate spindle assembly during mitosis. As the cell divides, the reorganization of the microtubule array into a highly dynamic mitotic spindle requires more than just the presence of centrosomes. Several studies have shown that when the nuclear membrane breaks down during cell division, nuclear signals are released that exert many different effects on microtubule arrays. Recently, the group discovered that a protein, GTPase Ran, made in the cell’s nucleus, can stimulate microtubule and spindle formation in the absence of both centrosomes and chromosomes. These findings suggest that Ran is the nuclear signal that regulates microtubule assembly in mitosis.

Future projects in the lab will involve understanding the steps of the Ran signaling pathway during mitosis. To this end, the researchers are developing tests to help identify the downstream targets of this protein.

**SELECTED PUBLICATIONS**


Every cell in an organism contains all of the organism’s genes. Each gene is a specific portion of the two-stranded molecule of DNA (deoxyribonucleic acid), which is arranged in chromosomes in the cell’s nucleus. Although the number of genes and the number of chromosomes vary from species to species, research today is revealing how much genetic similarity there is among different plants and animals.

Each gene, or piece of DNA, is a code that instructs the cell to make one protein. It is these proteins that drive every function in every cell of an organism, and are handed down to subsequent generations. Not all of the genes packed into every cell are activated in every cell. Much genetic research focuses on what makes certain genes become active at specific times, and in different types of cells.

When it is time for a gene to be expressed; that is, for the gene to instruct the cell to make a unique protein to accomplish a specific job, the cell receives a signal telling it to begin a process called transcription. First, the two strands of DNA unwind. One strand becomes a template with the code for the protein. The code is then copied, or transcribed, to a messenger RNA (ribonucleic acid) molecule (mRNA), which leaves the cell’s nucleus. Another molecule of RNA called tRNA (transfer RNA) attaches to the mRNA and makes the protein at a structure called the ribosome. Once the new protein is made, it is ready to perform its function.

Cells are continually being replenished. At a specific point in a cell’s cycle the cell is programmed to split; this process is called mitosis. Each new daughter cell has the same complement of genes as the original cell. In the first step of mitosis, the paired chromosomes inside the nucleus are copied. The membrane surrounding the nucleus dissolves and fibers called spindles emerge. The duplicated chromosomes, called chromatids, are pulled to opposite ends of the cell along the spindles and the cell splits down the middle, forming two new cells. Much of developmental biology looks at the genetic programming behind this process.

Some useful terms in the study of genetics and developmental biology follow.

**Allele**—One of the two alternative forms of a gene coming from each parent.

**Antisense strand**—The non-coding strand of DNA that binds with a strand of messenger RNA (mRNA).

**Assay**—A test, experiment, or trial.

**cDNA (complementary DNA)**—An artificially made section of DNA using an mRNA sequence as a template. It is often a first step in cloning particular genes.

**cDNA library**—A group of cell clones made from cDNA.

**Centriole**—A structure in an animal cell that is involved in cell division. As the cell divides, these cylinder-like components move to opposite sides of the nucleus to form the ends of filament structures called spindles, which assist in separating the material into the two new daughter cells.

**Centromere**—A section of a chromosome that attaches to a structure called a spindle when the cell divides.

**Centrosome**—An area of a cell near the nucleus that contains a pair of centrioles. It is the primary organizing center where filaments called microtubules are created for cell division and is called the microtubule organizing center.
Chromatid—Paired replicas of chromosomes formed during cell division to ensure that all of the organism’s genetic material is properly inherited. The paired replicas are often referred to as sister chromatids.

Chromatin—The material that makes up chromosomes.

Chromosome—The elongated structure in the nucleus of a cell consisting of DNA (groups of genes). When the cell is not dividing, the chromosomes cannot be identified.

Clone—Genetically identical cells or individuals, formed by asexual reproduction.

Cytoplasm—The gelatinous material around the nucleus of a cell.

Diploid—Having two sets of chromosomes, one from each parent in all cells except reproductive cells.

DNA (deoxyribonucleic acid)—A long-chain molecule arranged in a double helix that, with other proteins, makes up chromosomes inside every cell. The two strands of the double helix are made of sugars and phosphates and are linked together via chemical bonds between pairs of four different bases: adenine, guanine, thymine, and cytosine. The sequence of the bases makes up the genetic code.

DNA amplification—A process that creates many copies of a piece of DNA.

DNA microarray—A technology that allows the analysis of thousands of genes simultaneously. A gene, or fragment of DNA, is adhered to a specific location on a glass surface and analyzed to determine information about gene expression and function. About 10,000 DNA fragments can be placed on a 3-square-centimeter area to make up a microarray.

Endoplasmic Reticulum—A network of membranes within a cell’s cytoplasm important to biosynthesis and support.

Expressed sequence tag (EST)—A short DNA sequence derived from a cDNA library. Its location on the chromosome is known and thus represents a “tag” of an expressed gene, which is useful for gene mapping.

Fusome—A structure specific to germ line cells, which is seen only during germ line cyst formation. Cysts are batches of egg or sperm. The fusome may be involved in cell-cycle regulation.

Gamete—A reproductive cell, such as egg or sperm, which joins with another gamete from the opposite sex to produce a zygote.

Gene—A fragment of DNA that is the fundamental unit of heredity.

Gene amplification—The process that produces many copies of a DNA sequence that makes up a gene.

Gene expression—The result of the process whereby a gene is “turned on” and produces its specific protein, polypeptide, or RNA.

Gene library—An assemblage of cloned DNA fragments, which includes all of an organism’s genetic information.

Genome—All of an organism’s genes.
**Glossary**

**Germ line cell**—Any one cell in a series that eventually results in reproductive cells, such as eggs or sperm.

**Heterozygote**—A plant or animal with two different alleles for a given trait.

**Homologue**—Similar genes among different species.

**Insertion mutation**—An alteration in sequence of the bases in a DNA molecule originating from the random integration of DNA from another source.

**Jumping gene**—A gene that moves from one gene to another or from one place on a chromosome to another; also known as a transposon.

**Kinetochores**—During cell division, these bush-like filaments located in the centromere of a chromosome attach chromatids to the spindle.

**Messenger RNA (mRNA)**—An RNA molecule that conveys the information from a DNA sequence to the ribosomes in a cell. The information is used to create specific proteins.

**Microtubule**—Threadlike structures in a cell made of the protein tubulin. They are involved in moving cellular components.

**Mitochondrion**—The organelle of a cell that powers all its processes.

**Neural tube**—In an embryo, the tube that differentiates into the brain and spinal cord.

**Nucleosome**—The basic unit of chromatin, the material that makes up chromosomes.

**Nucleotide**—An organic compound whose long chains make up DNA and RNA.

**Nucleus**—The membrane-enclosed part of a cell that contains chromosomes and other cellular constituents.

**Oocyte**—An immature egg.

**Oogenesis**—The process of egg formation in female animals.

**Organelle**—Any number of structures that are encased within a cell.

**Promoter**—A section of DNA that initiates transcription.

**Protein**—An organic molecule made of long-chain amino acids. Proteins are fundamental to all biological processes. Genes are encoded to make proteins.

**Recombinant DNA**—DNA with genes from different sources created with recombinant DNA technologies.

**Recombination**—The process by which genes are rearranged during the formation of gametes.

**Regulator gene**—A type of gene that controls another gene.

**Reverse genetics**—A process that takes a cloned segment of DNA, or a protein sequence, to introduce a mutation into a genome. This technique is used to determine gene function.
Ribosome—A complex cell part that translates the coded information on mRNA into an amino acid sequence that makes up a protein.

RNA (ribonucleic acid)—A compound found in the nucleus and cytoplasm of cells. It is structurally similar to DNA and is involved in protein synthesis and other chemical activities. RNA molecules include messenger RNA, transfer RNA, and ribosomal RNA; they each serve a different purpose.

RNA interference—A process that inactivates a gene by inserting complementary double-stranded RNA into an organism. By inactivating the gene and observing the effects, scientists can learn how the inactivated gene functions.

RNA polymerase—An enzyme that starts the synthesis of an RNA strand from a DNA template.

Sense strand—The strand of DNA that is the template with specific genetic information.

Sequencing—Determining the order of nucleotides in a molecule of DNA or RNA.

Somatic cells—Nongamete cells, such as those that make up skin, bone, and muscle.

Spindle—The set of fibers that move chromosomes during cell division.

Telomere—The end portion of a chromosome that ensures proper replication.

Transcription—The first step in protein synthesis in which the information on a sequence of DNA is transferred to mRNA.

Transcription factor—A gene that controls the transcription of other genes.

Transgenic—An organism that has been modified by having foreign DNA inserted into its genome.

Transposable genetic element—An element of DNA that can move from one location in the genome to another. Also called a jumping gene or transposon.

Transposon—A gene that moves spontaneously from one chromosome to another, or from one position to another in the same chromosome; also known as a jumping gene or a transposable element.

Vector—A vehicle, such as a virus, used to carry a foreign DNA segment into an organism.
Director, Allan C. Spradling (Ph.D. 1975, Massachusetts Institute of Technology)  
  e-mail: spradling@ciwemb.edu  
  Phone: (410) 554-1221

Jimo Borjigin (Ph.D. 1994, Johns Hopkins University)  
  e-mail: borjigin@ciwemb.edu  
  Phone: (410) 554-1231

Donald D. Brown (M.D. 1956, University of Chicago)  
  e-mail: brown@ciwemb.edu  
  Phone: (410) 554-1252

Chen-Ming Fan (Ph.D. 1991, Harvard University)  
  e-mail: fan@ciwemb.edu  
  Phone: (410) 554-1222

Andrew Z. Fire (Ph.D. 1983, Massachusetts Institute of Technology)  
  e-mail: fire@ciwemb.edu  
  Phone: (410) 554-1234

Joseph G. Gall (Ph.D. 1952, Yale University)  
  e-mail: gall@ciwemb.edu  
  Phone: (410) 554-1217

Marnie Halpern (Ph.D. 1990, Yale University)  
  e-mail: halpern@ciwemb.edu  
  Phone: (410) 554-1218

Douglas E. Koshland (Ph.D. 1982, Massachusetts Institute of Technology)  
  e-mail: koshland@ciwemb.edu  
  Phone: (410) 554-1216

Erika Matunis (Ph.D. 1992, Northwestern University)  
  e-mail: matunis@ciwemb.edu  
  Phone: (410) 554-1243

Terence Murphy (Ph.D. 1998, The Salk Institute/University of California at San Diego)  
  e-mail: tmurphy@ciwemb.edu  
  Phone: (410) 554-1255

Alejandro Sánchez Alvarado (Ph.D. 1992, University of Cincinnati)  
  e-mail: sanchez@ciwemb.edu  
  Phone: (410) 554-1256

James Wilhelm (Ph.D. 2000, University of California at San Francisco)  
  e-mail: wilhelm@ciwemb.edu  
  Phone: (410) 554-8192

Yixian Zheng (Ph.D. 1992, Ohio State University)  
  e-mail: zheng@ciwemb.edu  
  Phone: (410) 554-1232