

Genes, Cells, and Behavior

A View of Biology Fifty Years Later

by Norman H. Horowitz
Chairman of the Division of Biology

A symposium on "Genes, Cells, and Behavior: A View of Biology Fifty Years Later," marking the 50th anniversary of the founding of the Division of Biology, was held on the Caltech campus on November 1-3, 1978. Eighteen papers, covering topics ranging from molecular genetics of bacteriophage to human behavior, were presented in five sessions. The speakers were all alumni or former

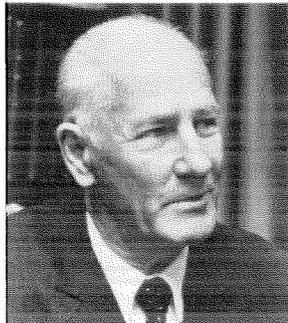
members of the Division. Over 700 alumni, students, and friends of the Division attended the symposium, which was moved to Beckman Auditorium after overflowing Ramo.

In the following article, we summarize the talks given in the last three sessions of the symposium. Summaries of the first two sessions appeared in the March-April issue of *Engineering & Science*.

Session III — Evolution, Genes, and Molecules

The Origin of Maize

Dr. George W. Beadle
Nobel Laureate
President Emeritus
University of Chicago



Maize was the most important food plant of pre-Columbian America, and it is today the third most important grain crop in the world, after wheat and rice. Its origin has long been a mystery. There are no wild representatives of maize; in fact, it is the sole member of its genus, *Zea*. Maize cannot survive without human intervention, owing to its lack of effective seed dispersal and its vulnerability to birds, rodents, and insects. In the early 1930's, R. A.

Emerson and Beadle concluded that the most probable ancestor of maize was *teosinte*, a relative that grows wild in Mexico and Nicaragua. *Teosinte* hybridizes with corn, and the hybrids are fertile. It has the same number of chromosomes (10 pairs), and Emerson and Beadle showed that crossing-over occurs normally between maize and *teosinte* chromosomes. Despite this evidence, the two plants are so different morphologically that some botanists questioned whether they are actually close relatives. Furthermore, *teosinte* has a tough seed case — so tough that it was doubtful whether primitive man would have found the plant edible and therefore valuable enough to cultivate.

In 1938, Mangelsdorf and Reeves proposed that *Tripsacum*, a more distant relative of corn, was the ancestor. This seemed unlikely on genetic grounds: *Tripsacum* and maize hybridize only with difficulty, and the hybrids are sterile. *Tripsacum* has 18 pairs of chromosomes, none of which pairs with those of corn.

Beadle decided to clarify the corn-*teosinte* relationship

by further study of their hybrids. He grew 16,000 F₂-backcross plants in a plot near Mexico City and found that parental phenotypes appeared in approximately one out of 500 plants. It was clear from this that there could not be a large number of major gene differences between maize and *teosinte*. Beadle also found that the seeds of *teosinte* can be made edible in several ways. They can be popped; they can be ground between stones with the seed cases and made into edible tortillas; or the shells can be separated from the seeds after grinding by flotation in water, and the seeds can be eaten. Thus, the Indians of Mexico would have had ample reason to cultivate *teosinte* and, by selecting random variants, gradually transform it into maize. Beadle considers this man's most impressive plant-breeding achievement.

This work has practical significance. *Teosinte* is an endangered species in Mexico because of overgrazing. Since it is the only wild relative of corn that can be exploited for desirable genetic traits, seed-banking and preservation of populations of *teosinte* should be carried out to save the species.

DNA Sequence Organization and Its Evolution in *Drosophila*

Professor M. S. Meselson
The Biological Laboratories
Harvard University



In this paper, Meselson described a study in his laboratory of certain segments of DNA that he termed "mobile genetic elements." These are short fragments of chromosomal DNA from *Drosophila* that Meselson and his co-workers find in some flies but not in others of the same or a closely related species. These fragments may be related to "transposable genetic elements" that have been known for some time in maize and in the bacterium *E. coli*. To detect the mobile elements, random pieces of *Drosophila* DNA (previously cloned in *E. coli* to obtain sufficient quantities) were made radioactive and then were reacted with giant salivary chromosomes under conditions that allowed the fragments to combine with their complementary sequences in the chromosomes. By autoradiography, the number and locations of the binding sites for each fragment could be determined.

Experiments were performed with *D. melanogaster* and

D. simulans, which are closely related species. They can be crossed, and their genetic maps and the banding patterns of their salivary chromosomes are very similar. Of 27 random pieces of DNA from these species that were reacted with the salivary chromosomes of both species, 19 combined with a single site (the same site) in both species. The other 8 combined with more than one site in the species of origin and with fewer sites (in some cases, none) in the other species. For example, one fragment from *D. melanogaster* combined with 23 sites in *melanogaster* chromosomes, but only 3 in *simulans*; of these 3, 2 were the same as the *melanogaster* sites, and one was different. These multisite fragments also showed differences within the species of origin. One fragment, called 232.2, was investigated in detail. It is a segment 1,500 nucleotides long from the Oregon-R race of *D. melanogaster*. It combines at five positions in one stock of Oregon-R, but at only four in another, and at none of these sites in *D. simulans*. Of particular interest is that it combines with a site of heat-shock puffing in the *melanogaster* chromosomes. (Puffs are enlargements of the chromosomes induced by temperature shocks; they are sites of intense transcription and translation of genes into RNA and proteins.)

It was hoped that this association would make it possible to identify the protein and the biological function of 232.2. By genetic methods, a portion of the puff region containing the binding site of 232.2 was deleted. The resulting flies were apparently normal in the production of all the heat-shock proteins. The function of the mobile element is still a mystery.

The Molecular Analysis of Genes in *Drosophila*

Professor David S. Hogness
Department of Biochemistry
Stanford University Medical Center



David Hogness and his students were the first to apply molecular cloning, or recombinant DNA techniques, to *Drosophila*. They isolated and cloned the first *Drosophila* gene in 1973, and since then they have cloned about two dozen more. Hogness discussed the state of the cloning art as applied to *Drosophila*, with the objective of showing both the possibilities and the limitations of this technology. One advantage that is not often emphasized is that

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Drosophila has one of the smallest genomes (total amount of nuclear DNA) of any higher organism. He briefly summarized the steps involved in producing a library of cloned genes — i.e., a collection of fragments of DNA, each one incorporated into the DNA of a self-replicating vector (a plasmid or a bacteriophage) that can be multiplied indefinitely.

Having produced a library that includes all the genes of an organism like *Drosophila*, the next problem is to select from among the many thousands of cloned fragments the one that includes the gene of interest. Current methods for solving this problem depend on the availability of a complementary copy of the desired gene. The copy is made radioactive and is allowed to combine with the gene by complementary base-pairing; the gene is then identified by its radioactivity. Such complementary copies exist in cells in the form of messenger-RNA, but usually in amounts too small to be useful for this purpose.

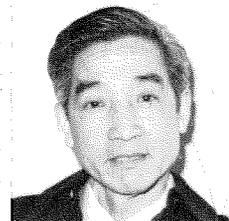
This was the case in the example that Hogness discussed in detail — that of the genes for the histones of *Drosophila*. (Histones are five proteins that are combined with DNA in chromosomes.) Histone messenger-RNA's had been obtained from the sea urchin, however, and it is known that histones have undergone little change in evolution. Taking advantage of this fact, Hogness and his co-workers used sea urchin RNA to identify clones bearing the histone genes of *Drosophila*. These were grown up, and the *Drosophila* genes were isolated from them. The usual procedure could now be reversed and, by use of the purified *Drosophila* genes, the messenger-RNA could be isolated from extracts of *Drosophila* cell cultures. It was found that the five histone genes are clustered on the second chromosome, and the cluster of five is repeated about 100 times. Studies on the "leader sequence" — the stretch of DNA immediately preceding the genes — are under way to identify the signals involved in translation of the genes.

More difficult cases are those in which it is not ever certain that the gene makes a messenger-RNA. One such example is the bithorax complex of genes. This is a cluster of eight or more genes that control the development of the thoracic and abdominal segments of *Drosophila*. These genes are typical of the majority of genes that have been studied in this organism in that the molecular mechanism of their action is unknown. This problem is approachable if a gene with an identified messenger-RNA exists in the neighborhood on the same chromosome, as is true in this case. By use of a series of overlapping DNA sequences, it should then be possible to reach and identify the gene (s) of interest.

Genetics of Bacterial Nitrogen Fixation

Dr. S.-C. Shen

Institute of Plant Physiology
Academia Sinica, Shanghai
People's Republic of China



The biological fixation of nitrogen is carried out by nitrogenase, an iron- and molybdenum-containing multiprotein complex found in certain bacteria; this enzyme complex catalyses the fixation and reduction of N_2 to NH_3 . Nitrogenase is specified by a set of nitrogen-fixation (*nif*) genes. (There is currently much interest in these genes, motivated by the desire to gain an understanding of the mechanism of nitrogen fixation and also by the possibility of applying genetic engineering methods to the *nif* genes of the bacterium *Klebsiella pneumoniae*.)

The *nif* genes of *K. pneumoniae* were first mapped by Dixon and Kennedy, who placed nine genes in two clusters in the neighborhood of the *his* operon (a group of genes controlling the synthesis of the amino acid histidine). The two clusters were separated by a "silent region" some 9,000 nucleotides in length. This result has been re-examined by Shen and his co-workers, using newly isolated as well as previously known *nif* mutants. No silent region was found. Seven different genes were identified among 21 mutants, and these all mapped in a single cluster in the *his* region. It is not yet known whether the *nif* cluster is organized into a single operon (a group of genes that are switched on and off as a unit).

Recently, Brill has identified 14 *nif* genes in seven operons in *K. pneumoniae*. It was shown that three of the genes determine the structure of three nitrogenase polypeptides, and four others determine the iron-molybdenum cofactor and an electron-transport factor. Results from Shen's laboratory show that one *nif* gene, N-120, is needed for the synthesis of the two components of nitrogenase. Whether N-120 is a 15th gene for N-fixation remains to be proved.

Shen also reported that *nif* mutant C-7 produces 60 percent of the normal level of glutamine synthetase activity and only 30 percent of the normal amount of glutamate synthetase activity. Extracts of the same mutant contain only traces of components I and II (major protein components of nitrogenase). C-7 is an example of a class of *nif* genes that exert a regulatory effect on other *nif* genes, besides specifying their own product. In view of such complexities, difficulties in the cloning of *nif* genes can be anticipated.

Session IV — Biology of Cells

Hormones and Tissue Culture: Basic and Health-Science Aspects

Professor Gordon H. Sato
Department of Biology
University of California
San Diego



Gordon Sato began his lecture with an autobiographical note. He recalled his youth in a tough neighborhood of Los Angeles, his job as a gardener in Pasadena, and his admission to Caltech as a probationary graduate student after he had a disabling fall from his truck. A guest in the audience — a physicist from the East Coast — wrote later to say that as long as Caltech remains flexible enough to admit students like Gordon Sato, it need not worry that it will suffer the decline he sees in some other elitist universities.

Sato then described the two major achievements that have been made in his laboratory in the last 20 years. The first of these was the discovery that the commonly observed overgrowth of animal-cell tissue cultures by fibroblasts was not caused by “de-differentiation” of differentiated cells, as was formerly believed, but by the fact that fibroblasts — which are ever-present in tissues — were selectively favored by the culture conditions then employed and outgrew all other cells. Having established this, the next step was to learn how to make the desired cell types grow. The accomplishment of this goal is the second achievement.

The key to the problem turned out to be hormones. It appears that each type of cell requires a particular set of hormones for growth. For example, a line of pituitary cells studied in Sato’s laboratory requires for optimal growth insulin, transferrin, thyroxin, parathyroid hormone, thyroid-releasing hormone, fibroblast growth factor, and somatomedin C. These hormones are usually provided in calf serum, which is customarily used in cell-culture media. The hormones can replace serum and thus make possible a chemically defined medium for this cell line.

Other cell lines require other hormones, and approximately 20 different kinds of cells have been cultured in this way in Sato’s laboratory. In some cases, growth is better in the defined medium than in serum-containing medium. The importance of the discovery that every cell is dependent on a specific complex of hormones is, first of all, that it constitutes new knowledge about the organization of the animal (including human) body. It makes it possible to es-

tablish new, primary cell cultures more readily than before. And it could lead to practical benefits, as in cancer biology, where knowledge of the specific hormone requirements of different kinds of cancer cells may offer, to quote Sato, “the possibility of manipulating the physiology of these tumors and combining them with chemotherapy to optimize treatment.”

Genetic and Functional Dissection of the Major Histocompatibility Complex

Professor Donald C. Shreffler
Chairman
Department of Genetics
Washington University
School of Medicine



Donald Shreffler’s subject was the major histocompatibility complex (MHC), which is a closely linked cluster of genes that is present in recognizably homologous form in mammals, birds, and amphibians. It contains the genes responsible for the classical histocompatibility, or self-recognition, function that is manifested in graft rejection. More recently, the MHC has been found to be involved in certain immune mechanisms and in susceptibility to various diseases. Shreffler’s talk focused on these newer aspects. He pointed out, for example, that there is a strong association between ankylosing spondylitis (a form of arthritis) and HL-A B27, an antigen determined by one form of a gene of the MHC of man. Over 90 percent of patients with ankylosing spondylitis have this antigen, but fewer than 10 percent of the unaffected controls show it. Other associations between particular MHC genes and disease are found in pharyngeal carcinoma in man and in virus-induced leukemia of mice.

Other recent findings relate to cell-recognition responses — for example, that involved in the interaction between so-called B- and T-lymphocytes, which cooperate in the production of antibodies. Shreffler and others have shown that, for this reaction to occur, the B and T cells must be identical in the I gene of the MHC. Similarly, in killing reactions against virus-infected cells, the target cell and the killing lymphocyte must share the same K and D regions of the MHC in order that the reaction may take place. Mutations in either genetic region lead to failure to kill virus-infected cells.

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Another interesting finding has been the identification of serum protein Ss (first detected by Shreffler when he was a student at Caltech and mapped by him to the MHC region of the mouse) with one of the components of complement, which is the name given to a group of proteins in the blood that act cooperatively to lyse foreign cells.

Shreffler also summarized evidence showing that considerable duplication of genes has occurred in the course of evolution of the MHC complex. Duplicate genes are common within the complex, and the proteins determined by these genes are very similar in structure. He concluded by observing that the MHC system is becoming one of the most useful in molecular genetics.

Simple Social Cells

Professor Dale Kaiser
Department of Biochemistry
Stanford University
Medical Center



Myxobacteria are rod-shaped bacteria about five microns in length. Although bacterial in structure, they exhibit multicellular behavior and may be the simplest social cells. Their social behavior is shown in their movements. Large groups of cells move together, in cell-to-cell contact, like rafts of logs. The mechanism of this movement, called "gliding," is unknown. It occurs only on solid surfaces and is always in the direction of the long axis of the bacteria, with frequent reversals. Individual cells are capable of movement, but movement is more frequent and continuous in groups. A moving group leaves a trail of slime

that is followed by other groups. When starved, myxobacteria aggregate to form stalked fruiting bodies that produce spores.

The social behavior of myxobacteria can probably be explained by the fact that they secrete enzymes into the external medium to digest the cells that form their food. Single cells cannot produce enough enzyme to support their growth at a maximal rate, but a large number of cells can do so by pooling their enzymes.

Kaiser and Hodgkin have isolated a large number of non-motile mutants. In many cases, the mutants complement one another; that is, they become motile when mixed together. This effect, called "stimulation," is phenotypic only. Re-isolated cells are still non-motile. There are six complementation groups of mutants, any two of which will show the stimulation response when mixed. Transduction crosses have revealed 22 loci, 17 of which belong to one of the six complementing groups. The other groups map to single loci.

Study of revertants has shown that there are two kinds of motile cells — those that can move as single cells and those that move only in groups. The former movement is determined by a set of genes called system A, the latter by system S. Wild type has both systems. System A consists of the genetic loci described above, and any cell with a complete set of these genes can move singly. System S contains at least nine genes. The two systems have one gene in common but are otherwise independent.

Social movement is associated with the possession of pili — long, hairlike processes growing out of one end of the cell. Pili production is controlled by the S genes. Pili apparently bind cells together and enable them to move as a group. Complementing S mutants stimulate one another to move, and — remarkably — they do so by inducing pili formation.

Session V — Neurons and Behavior

Variations in Human Brain Organization

Professor Jerre Levy
Department of
Behavioral Sciences
University of Chicago



It was discovered in 1836 that speech is localized to the left side of the brain. By the end of the 19th century it was

believed that all sensory understanding and cognition were the function of the left hemisphere. The right side of the brain was thought to receive and send information but not to process it. As recently as the 1940's, studies on split-brain patients (individuals whose corpus callosum — the large tract of fibers connecting the two hemispheres — had been severed to control intractable epilepsy) failed to detect any effects of hemispheric separation on brain function.

In the 1960's a series of split-brain patients of Drs. J. E. Bogen and P. J. Vogel were studied in Roger Sperry's lab-

oratory. These studies revealed a distinct syndrome. Sensory inputs directed to one hemisphere did not cross to the other. For example, an object placed in the left hand (hidden from view) was sensed by the right hemisphere but could not be named by the patient, since that hemisphere is speechless. The right hemisphere nevertheless recognized the object, as shown by the ability of the left hand to select a matching object.

Although the two hemispheres were completely isolated from each other, the performance of the patients in everyday life was normal, showing that the integrative function of each hemisphere had not been disrupted by the disconnection. This was one important result of these experiments. Another was the demonstration that the right hemisphere is a conscious, thinking half-brain. The cognitive functions of the hemispheres were explored by a variety of tests. The right hemisphere dominates in pattern recognition, especially facial or other complex patterns en-

countered for the first time. It is weak in verbal and phonetic abilities, but strong in extracting spatial and physical relationships nonlinguistically. Its memories are rich in the sensory qualities of the objects recalled. The left hemisphere understands logical causality; it is abstract and analytical; and its reasoning is propositional. These are all qualities associated with speech and language.

The foregoing applies to nearly all right-handed persons, but only 60-65 percent of left-handers have language in the left hemisphere. The others are lateralized in the opposite sense, or are only weakly lateralized. Those with left lateralization have the problem that when writing they need access to linguistic information from the left hemisphere, but normal motor pathways are crossed. In many cases it appears that ipsilateral (same-sided) motor pathways are used. Tests indicate a correlation between hand posture in writing (hand inverted above the line of writing) and the use of ipsilateral pathways.

Navigation by Honeybees

Professor James L. Gould
Department of Biology
Princeton University



Ethology is the study of animal behavior. It owes its existence as a scientific discipline to two basic discoveries. First, animals live in unique sensory worlds, separate from our own; and second, animals are to a large extent robots, pre-programmed by their genes. After outlining the evidence for these conclusions, Gould summarized the present state of knowledge of honeybee navigation.

Bees fly great distances in search of food, often 1 to 10 kilometers — the equivalent of a journey of 60 to 600 miles for a human being. To locate food and then find its way back to the hive without getting lost, the bee uses conspicuous landmarks and celestial navigation. The latter is performed with reference to the sun. Karl von Frisch, the founder of this field, showed that bees know the sun's position even when it is hidden behind trees or clouds or when it is below the horizon. This capacity resides in the ability of bees to perceive both ultraviolet (UV) and polarized light. (We are blind to both.) The advantage of polarized UV for navigation is that polarization (caused by Rayleigh scattering of sunlight in the atmosphere) contains

information concerning the position of the sun, and clouds are nearly transparent to UV.

In trying to learn how honeybees use polarized UV to locate the sun, Gould and a colleague first train bees to use an artificial food source. They then observe the bees when, after their return to the hive, they communicate the location of the food to the colony by means of their "dance language." This communication is performed under artificial sky patterns controlled by the experimenters.

Wavelength distribution, elevation, angular size, brightness, and polarization are varied, and the interpretation given by the bees to different patterns of sensory cues is deduced from their dance. Current evidence suggests that forager bees in the field somehow record a picture of the sky. On their return to the hive, they match the cues visible in the sky overhead with the recorded picture. They use a set of fixed rules to relate the visible cues to the location of the sun, and they then orient their waggle dance in the direction of the food. Since the attendant bees use the same rules to interpret the dance, it does not matter if, as can happen under the rules, the dance is in fact misoriented.

The goal of ethological research is to analyze behavior at all its levels of organization down to the genetic and molecular level. Another challenge for ethology, to quote Gould, "is to apply its insights to our own species — to discover how the unseen hands of evolution are directing or predisposing our behavior now that we have, at least overtly, left our hunter-gatherer heritage so very far behind."

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Plasticity of Transmitter Mechanisms in Sympathetic Neurons

Professor Edwin J. Furshpan
Department of Neurobiology
Harvard Medical School



One of the fundamental ideas of neurobiology is that neurons make connections only with a specific subset of other neurons. The resulting pattern of specific connections underlies the information processing that takes place in the nervous system. Another important idea is that neurons can be characterized by the specific neurotransmitter — or “flavor” — that they secrete. Thus, so-called cholinergic neurons secrete acetylcholine, and adrenergic neurons secrete norepinephrine. When the nervous system is formed in development, neurons have to make contacts with their proper target neurons, and the chemical transmitters have to be matched with the correct receivers. The heart provides a familiar example: Sympathetic neurons release norepinephrine onto the heart and cause its beat to increase, while parasympathetic neurons release acetylcholine and slow the beat. It is obviously important that each neuron secrete the appropriate transmitter.

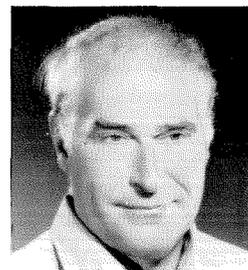
The experiments in Furshpan’s laboratory are designed to answer the question of what it is that determines transmitter flavor during the development of the nervous system. He and his colleagues worked with sympathetic neurons from the superior cervical ganglion of the rat. These neurons were grown in tissue culture. It was possible to use microelectrodes to stimulate and record action potentials from individual neurons in the cultures. When this was done, it was found that the responses were mostly cholinergic ones. Normally the majority of sympathetic neurons in the superior cervical ganglion are adrenergic. Only a small fraction — about 5 percent — are cholinergic. Further investigation showed that up to 75 percent of the cultured neurons were cholinergic, provided that the culture also contained non-neuronal cells. In pure neuron cultures, on the other hand, not over 2 percent were cholinergic. The rest were either adrenergic or were mixed. This effect of non-neuronal cells is due to the production of a diffusible chemical factor by these cells, and it does not require direct contact between neurons and non-neurons. The factor changes the fate of what would normally be adrenergic cells to cholinergic ones.

The factor is not the well-known nerve-growth factor, since experiments show that NGF simply increases the amount of transmitter the cell is already committed to make. Current information indicates that the factor is another large molecule, possibly a glycoprotein.

Finally, it has been found that if the cells in culture are stimulated electrically so as to imitate the nervous input they would normally receive in the animal, they fail to respond to the factor. This suggests that the timing of events in development may be important in determining the transmitter flavor of neurons.

The Leech Embryo

Professor Gunther S. Stent
Department of Molecular Biology
University of California
Berkeley



The last paper in the 50th Anniversary Symposium was, in the words of its author, “an old-fashioned, almost entirely descriptive” account of the embryology of the leech, a study that has its roots in a paper published 100 years ago by Charles Otis Whitman, who was one of the teachers of Thomas Hunt Morgan. The leech is a representative of those animals in which the cells of the early embryo are completely determined with respect to the role each will later play in development. In his 1878 paper on leech development, Whitman gave the first analysis of developmental cell lineages. Stent took up the account where Whitman left off.

Although his principal interest is in the neurobiology of the animal, Stent’s description of leech embryogeny is a general one. Points of special interest included the adaptation of the horseradish-peroxidase cell-staining technique to the problem of tracing cellular pedigrees in development. Another method involves the use of the proteolytic enzyme pronase to kill individual cells and, by observing the effect on subsequent development, deducing the normal role of the killed cell.

The paper includes a detailed description of the development of movement in the leech embryo, beginning with simple, irregular contractions of muscle fibers and ending with the highly coordinated movements of locomotion. Stent concluded with an expression of his belief that by the time the Division of Biology celebrates its 75th anniversary, “the leech will have become both the phage and the fruit fly of embryology.” □