

GENOTYPIC AND PHENOTYPIC VARIATIONS

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Contents

1. Brief Introduction to Historical Background
 2. Brief Introduction to the Genetics of the Nematode *Caenorhabditis*
 - 2.1. Cell Lineage and Genome Sequence
 - 2.2. Genetics and Mutants
 3. Uncoordinated Mutants of *C. elegans*
 - 3.1. General Introduction to Uncoordinated Mutants
 - 3.2. Structural Protein of Muscle
 - 3.2.1. Thick Filament (Myosin Heavy Chain, Paramyosin, and Twitchin)
 - 3.2.2. Thin Filament (Actin and Tropomyosin, Troponins)
 - 3.3. Transcription Factors
 - 3.4. Receptors and Channels
 - 3.5. Kinases and Enzymes
 - 3.6. Neuron-Related Proteins
 4. Other Mutants
 - 4.1. Vulva Formation
 - 4.2. Sex Determination and Dauer Formation
 - 4.3. Chemical Response and Signal Transduction
 - 4.4. Cell Death and Life Span
 5. Future Scope
 - 5.1. Gene Duplication and Genome Organization
 - 5.2. Interaction Among Different Genes
 - 5.3. Economic Estimate
- Acknowledgments
Glossary
Bibliography
Biographical Sketch

Summary

In this article studies of the relation between genotype and phenotype, especially of the motility of the soil nematode *Caenorhabditis elegans*, are described. First, the background of the study of motility is briefly reviewed. We focus on the results of the isolation of mutants that are useful for understanding the gene function of other animals. A brief overview of recent research follows. As details on *C. elegans* have already appeared in two monographs, the principal approach to the study of this animal is explained only briefly. A typical genetic analysis of the motility mutant gene known as *unc* (uncoordinated) is described. More than one hundred *unc*-genes have been isolated and classified into five groups. *unc* genes are related in various ways to muscle and other proteins like cytoskeleton proteins, transcription factors, ion channels of trans-

membrane proteins, kinases or enzymes, and neuron-related proteins. Interestingly, these proteins are known to be abundant in the multicellular organisms from the results of genome sequencing. Other mutant genes are also described. Strategies for the genetic analysis of genes involved in heterochronicity, vulva formation, chemotaxis, and cell death are briefly presented. Finally, an overview of the post genome research is discussed. The reasons why *C. elegans* research has progressed so rapidly and the strategy for experiments in progress are also explained.

1. Brief Introduction to Historical Background

Before G. Mendel discovered the rules of inheritance, genetic knowledge was confined to blood lineage, hair color, and facial features. It was not so much scientific as cultural. Marriage to a close relative was prohibited to avoid producing homozygous offspring caused by the overlapping of chromosomes with a mutation, although the scientific reasons were not known. One famous example of genotypic and phenotypic variation is the lip morphology in the royal family of Hapsburg. After rediscovery of the Mendelian rules in 1900, genetic knowledge accumulated quickly and continuously. In 1929, T.H. Morgan chose *Drosophila* as a model for studying phenotypic variation.

The gene served as a functional unit for genetic markers in classic genetics. A central dogma was established in the mid-1960s; the gene encodes an amino acid sequence governed by the genetic code. This means that a single gene divides into unit nucleotides. This may correspond to the finding in quantum physics that a unit particle divides into many smaller units, a reminder that visible phenomena on a macro scale stem from a step-by-step construction of much smaller units. Genes consist of DNA, a polynucleotide having four different bases—adenine, guanine, thymine, and cytosine—as explained in *Molecular Biology*. It is helpful to remember that even the term “gene” can mean different things at different levels. This is now common knowledge but earlier works, even those by famous scientists, could not distinguish among the various meanings, as the information was not available in their era.

Parallel to the genetic studies of F. Jacob and J. Monod on the lactose operon in *Escherichia coli*, studies on protein chemistry and structure also progressed. The three-dimensional (3D) structure of hemoglobin (Hb) was determined by X-ray diffraction analysis on protein crystals together with myoglobin in the early 1960s. This was the first time the structure of a biomolecule had been determined and a model structure produced, and the achievement won Max Perutz and John Kendrew the Nobel Prize in 1962. The double-strand model of DNA reported by Watson and Crick in 1953 helped to reveal how a gene divides and a characteristic is inherited by offspring. Since then, the many functional bases in biology of this huge chemical molecule have become apparent. Although the 3D structure of DNA is not directly connected to biological function, the 3D structure of Hb helps to reveal the function of the molecule. Abnormal hemoglobin is determined from a mutant gene of hemoglobin, and amino acid substitution can be located on the model structure.

Many tissue-specific proteins and alternative gene products have been isolated from heart muscle defects, Alzheimer’s disease, and others. This approach can now be applied to more complex molecules to develop the human genome project (and drug

therapy along with it). A body consists of many cells that have identical genetic information but different biomolecules. Each cell is formed after gene expression in a tissue-specific or lineage-specific manner. The simplest living organisms are bacteria; even bacteria as small as one micron meter can grow, divide and move. Finally it was established that the central dogma is true for all species from elephants to *E.coli*.

Motility is the most important characteristic of living organisms, together with division and growth. Single-cell bacteria move in a medium using flagella. Bacterial flagella consist of three parts: the filament, a screw; the hook, a universal joint; and the basal body, a motor. More than 50 genes are involved in making up a single flagellum. Some gene defects cause a change in motility and an abnormal morphology. Each of the genes is controlled by transcriptional machinery. If the basal body gene is defective, bacteria with a filament gene are not motile because they lack a motor. Bacteria can also not move if the energy supply system is disrupted, even if structure is perfect. The structural gene for the formation of filaments is controlled by a promoter, transcription factor, and other factors. As mentioned in the previous section, a single molecule in the subunit structure is important for function. Protein relies on many characteristics of the assembled molecules to maintain its biological functions. We cannot solve the biological function by the time when the interaction mystery between assembled molecules is known. It is accepted that the central dogma of genetics applies to all living organisms. Living organisms are constructed of building blocks. Each of these building blocks interacts to form complex parts. This is the reason why genetic analysis of *Escherichia coli* is relevant even to the study of elephants. This will be even truer after the human genome sequence is completed in the twenty-first century.

2. Brief Introduction to the Genetics of the Nematode *Caenorhabditis*

Sydney Brenner first used the soil nematode *Caenorhabditis elegans* to study development and the nervous system in 1967. *C. elegans* feeds bacteria and grows on nutrient growth medium at 20 °C. The hermaphrodite grows into an adult in three days and lays about 200 eggs after self-fertilization. Genes can be transferred from rare arising males to the hermaphrodite by mating. This is probably the most convenient animal model for genetics and biochemistry. In *C. elegans*, mutant phenotypes include Unc (uncoordinated), Sma (small), Lon (long), and Dpy (dpy). Changes in morphology and other visible characteristics also arise from mutant genes. Bacterial motility is observed only by an indirect approach: swarm formation in semi-solid agar medium on a petri dish. Worm motility can be observed directly by dissecting microscopy, which is an advantage when isolating a mutant. Worms have muscle tissues: a pharynx for feeding, a body wall for locomotion, a vulva for laying eggs, and an anus for defecation. Interestingly, the pharynx is similar to the heart muscle of vertebrates. The analysis of worm muscle genes is therefore applicable to vertebrates. Molecular biology can provide an understanding of one organism through study of another by comparing amino acid sequence homology or cell proliferation. Two monographs are available for *C. elegans*: “The nematode *Caenorhabditis elegans*” (1987) and “*C. elegans* II” (1997). These describe conventional methods for handling and basic knowledge developed through many research studies. In the later monograph, more recent studies into subjects such as cell death and signal transduction are explained more precisely. Completion of the genome sequencing of *C. elegans* in 1998 accelerated study of the relation between

genes and functions by using transposon insertion, RNA interference, and transgene techniques.

2.1. Cell Lineage and Genome Sequence

How fertilized eggs divide and cells proliferate is fundamental to our understanding of development. The use of a dissecting microscope and visible light easily reveals the cell division in *C. elegans* without any pretreatment. In 1983, after seven years of study, John Sulston reported the process of cell proliferation. There are 959 cells after the programmed cell death of 131 cells. This is made clear by recording the position of the nucleus. This information is available at the web site AceDB (<<http://eatworms.swmed.edu/>>). The most important conclusion from completing the cell lineage is that cell proliferation is divided into two stages: cell division and morphogenesis. Most of the cell division is complete by the 500-cell stage, with only a limited number of cells continuing to undergo asymmetrical division to form special muscle, cuticle, neurons, and vulva. This indicates that, even in higher animals, characteristic appearances come from a limited cell lineage. We should note that many genes are conserved in species from the nematode and *Drosophila* to mice, and even humans. This conclusion is also true for genome analysis. Higher organisms have transcription factors, a cytoskeleton, signal transduction molecules, and kinase/phosphatase. These molecules specialize the cells of multi-cellular organisms. This means that the conclusions drawn from a simple model like the nematode or *Drosophila* are also true for mice and humans, as is known from molecular genetic studies on bacteria. Genome structure and gene numbers are discussed in more detail in the next section.

2.2. Genetics and Mutants

The nematode *Caenorhabditis elegans* grows as a hermaphrodite and lays eggs after self-fertilization. It is therefore easy to isolate a mutant from a single worm that has a heterozygous chromosome. In 1974, Sydney Brenner established the genetics of the nematode, and since then many mutants have been isolated from numerous phenotypes. The first mutants to be identified were Sma (small body), Lon (long body), and Mab (male abnormal), together with Unc (uncoordinated), these phenotypes being easy to recognize. It is also possible to isolate a mutant that has no phenotypic variation under normal conditions but a novel phenotype under a specific set of conditions. Drug or temperature sensitivity is used as a conditional marker. It is possible to isolate a mutant that has a novel phenotype under specific conditions after treatment of some mutagens, which is why so many important mutations have phenotypic variations. Recently, another genetic approach has been used to isolate mutations based on motility in the genome, applying transposons such as Tc1 in the nematode and P-element in *Drosophila*. The insertion of a transposon into a gene disrupts its function. Knock-out of the gene by inserting Tc1 in *C. elegans* produces an increase in genomic size, as detected by Southern analysis. If a mutant is isolated by a transposon insertion, a mutant gene can be isolated by the transposon fused fragment. Designed transposons are also used in *Drosophila* for studying promoter activity. When a P-element is inserted into downstream of a gene's promoter, *lac-Z* gene activity can be detected by staining the mutant fly. This approach allows one to know where the gene was expressed and which

gene was disrupted by transposon insertion. These transposon functions are essentially similar from bacteria to maize.

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Bibliography

Brenner S. (1997). *Loose Ends*. London: Current Biology. [An essay on modern biology and new fields of science.]

C. elegans sequencing consortium (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018. [Presents the entire genome sequence of the first example of a multicellular animal.]

Ellis R.E., Yuan J., and Horvitz H.R. (1991). Mechanisms and functions of cell death. *Annual Review of Cell Biology* 7663–7698. [Presents a genetic approach to the study of programmed cell death.]

Lawrence P.A. (1992). *The Making of a Fly*. Oxford, UK: Blackwell Scientific. [Explains the principal mechanisms of morphogenesis in development, as well as the background and topics of molecular biology in the fruit fly. It is applicable to the worm.]

Medawar P.B. (1979). *Advice to a Young Scientist*. London and Sydney: Pan. [Max Perutz presented this to Fred Sanger on his retirement in 1984.]

Perutz M. (1992). *Protein Structure*. Oxford, UK: W.H. Freeman. [Presents protein structures, especially those obtained by X-ray crystallography, and includes an explanation of why three-dimensional structure is useful for understanding human disease.]

Riddle D., Blumenthal T., Meyer B., and Priess J. (1997). *C. elegans II*. New York: Cold Spring Harbor Laboratory Press. [Presents more current results in the nematode but does not overlap the first monograph *The Nematode Caenorhabditis elegans*, published in 1988.]

Wood W. (1988). *The Nematode Caenorhabditis elegans*. New York: Cold Spring Harbor Laboratory Press. [Presents fundamental information accumulated since 1974 on the handling, genetics, and morphology of the nematode.]

Biographical Sketch

Hiroaki Kagawa is Professor of Molecular Biology, Graduate School of Natural Science and Technology, Division of Biomolecular Science, and Department of Biology, Faculty of Science, Okayama University. He was born in Kagawa Prefecture of Japan in 1942. He received his B.A. in agricultural chemistry from Okayama University in 1964. His bachelor thesis was on Terminal electron transfer of sulfur oxidation in *Thiobasillus thiooxidans* (cytochrome c). He obtained his Ph.D. (Biophysics, Nagoya University) in 1972, with Professors Fumio Oosawa and Sho Asakura, on the Structure and function of Bacterial flagella hook assembly. He was assistant professor, Institute of Molecular Biology, Nagoya University from 1972–1979; lecturer, Faculty of Science, Okayama University, from 1979–1985, and scientific staff (1st grade) MRC Laboratory of Molecular Biology, Cambridge, UK from 1983–85. He achieved the cloning of *unc-15*, the paramyosin gene and a model of thick filament assembly of *C. elegans* with Dr. Jon Karn under the director Dr. Sydney Brenner. He was associate professor at Okayama University from 1986 to 1991, and has been Professor since 1991. He is a member of the Japan Biophysics Society, Japan Genetic Society, Japan Molecular Biology Society, and GSA. His research interests are: calcium signaling in excitation-

contraction coupling of *C. elegans*; muscle gene expression and muscle filament assembly; muscle gene mutation and animal behavior; and the evolution of muscle genes.

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