Gene and environment. The phenotype which develops in any organism is the resultant of two guiding influences, the genotype and the environment. Hence, the environment must be considered in any analysis of gene action, if it is desired to arrive at an understanding of how genes act toward the production of the phenotype. Practically, this is best done by keeping the environment as constant as possible while making studies of gene action. However, much also can be learned by varying the environmental conditions and keeping the genotype as constant as possible. *See* GENETICS.

The environment of genes is obviously a complex one. For convenience, two areas can be defined: (1) that immediately around the genes, the intracellular environment of the rest of the cell; and (2) the extracellular and extraorganismal environment. The intracellular environment can be changed by the mutation of other genes, which may then modify the action of a gene under study. Gene interaction is thus seen to be in part an aspect of the study of the internal environment of the cell.

Extracellular environmental factors, such as light and heat, may also influence the action of genes greatly. For example, in *Neurospora* there are some mutant strains which at one temperature have absolute growth factor requirements, but not at another temperature 18°F (10°C) removed. At the latter temperature they will grow as well as the wild type on the minimal medium with no supplement. In *Drosophila*, the eye-color mutant gene, blood, w^{bl}, permits the production of the eye pigments only at low temperatures. At temperatures above 77°F (25°C), little or no pigment is produced in the eyes. Even the types of antigens produced by an organism may be influenced by the environment. This has been demonstrated in *Paramecium*. In this protozoan, transformations in antigenic type can be brought about by a change in temperature or by several other environmental modifications. The transformation need not be permanent, for if the original environment is restored, the original antigenic type will reappear. Many of these simple temperature effects can be related to the changes in the conformation of proteins. For example, mutant allele a of the gene a^+ produces a functional protein, but at the nonpermissive temperatures it is inactive because the configuration of its active site is changed, or it may be inhibited by cellular substances that do not inhibit the wild-type protein.

Changes in phenotype which occur against a constant genetic background are in reality responses to the environment by the extragenic part of the living system. The genes themselves are not changed, as can be readily demonstrated by changing the environment back to the original condition, or by breeding the individual and showing that the offspring inherit the original parental genotype.

Phenocopies are particular types of environmentally induced changes which mimic in appearance mutant types produced by mutant genes. For example, by means of heat shock or chemical treatment of larvae of wild-type *D. melanogaster*, a number of different types of aberrant development may be induced that will result in the formation of adults with characteristics which superficially resemble those possessed by certain mutants. These environmentally induced modifications are not inherited, however, whereas those resulting from mutant gene action are inherited. *See* DEVELOPMENTAL GENETICS; GENE.

Robert P. Wagner

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Gene amplification

The process by which a cell specifically increases the copy number of a particular gene to a greater extent than it increases the copy number of genes composing the remainder of the genome (all the genes which make up the genetic machinery of an organism). It is therefore distinguished from duplication, which is a precise doubling of the genome preparatory to cell division, and endoreduplication, which leads to endopolyploidy.

Gene amplification results from the repeated replication of the deoxyribonucleic acid (DNA) in a limited portion of the genome, in the absence of or to a much greater extent than replication of DNA composing the remainder of the genome. Thus is formed a cell in which the genes composing a limited portion of the genome are present in relatively high copy number, while the genes composing the remainder of the genome are present in approximately normal copy number. *See* DEOXYRIBONUCLEIC ACID (DNA).

Genes coding for ribosomal RNA. Ribosomes are the site of cellular protein synthesis. They are particles which are composed of a specific type of ribonucleic acid (RNA), ribosomal RNA (rRNA), and some 30 specific proteins called ribosomal proteins. The genes coding for rRNA are multicopy genes (there are many identical or nearly identical copies in the genome of most organisms).

Gene amplification was first described in the oocytes of certain amphibians. The frog oocyte contains approximately 400,000 times as much rRNA as a normal frog liver cell in spite of the fact

that the amount of DNA in the oocyte nucleus is approximately the same as that of the liver cell. It has been estimated that, by using the normal genome number of rRNA genes, it would take the frog oocyte approximately 1000 years to synthesize the amount of rRNA found in the oocyte. By amplifying these genes approximately to the level known to occur (1000- to 2000-fold), the oocyte can synthesize this amount of rRNA in 6–9 months.

The developmental significance of such amplification lies in the fact that no rRNA is synthesized during early embryogenesis in amphibians. Proteins synthesized during early embryogenesis are synthesized on maternal ribosomes that were produced by the developing oocyte. Amplification of rDNA in the amphibian oocyte occurs extrachromosomally by a rolling-circle mechanism of DNA synthesis similar to that which occurs when certain bacteriophage viruses replicate their DNA.

Amplification of rDNA also occurs in the macronucleus (the vegetative nucleus) of certain ciliated protozoans. The macronucleus grows by polyploidization, but during macronuclear growth the genes coding for rRNA are replicated to a far greater extent than is the DNA composing the remainder of the genome; that is, the rRNA genes are amplified. *See* RIBONUCLEIC ACID (RNA); RIBOSOMES.

Single-copy genes. Since gene amplification increases the copy number of a specific region of the genome without altering the copy number of genes composing the remainder of the genome, it would appear to offer an alternative method for developmental control of gene expression. By increasing the number of copies of a particular gene, the number of gene copies available for transcription could thereby be increased.

In the ovary of insects, follicle cells produce the proteinaceous egg shell (or chorion). In the ovary of the fruit fly, *Drosophila melanogaster*, the chorion is produced during the final 5 h of egg-chamber development. Production of the chorion involves the synthesis of messenger RNAs (mRNAs) for several chorion proteins within a very brief period. The genes coding for the various chorion proteins are clustered within the genome, each gene present in single-copy number in germline cells. Amplification results in a 15- to 50-fold increase in the number of chorion gene copies per haploid genome. Chorion gene amplification appears to enable the genes to produce the amounts of mRNA necessary to build the egg shell.

Although the mechanism of chorion gene amplification has not been completely elucidated, the data suggest that each chorion gene cluster contains a specific origin of DNA replication. Multiple rounds of replication beginning at this origin give rise to a multiforked structure in which one strand branches into two, each of these branch again and again, and the branched structures contain copies of the chorion gene.

Another single-copy gene which appears to be amplified during development is one of the genes coding for the muscle protein actin during development of muscle cells (myogenesis) in the chick. The actin genes constitute a small family of genes which are present in single or very low copy numbers in the germ line. During myogenesis (formation of skeletal muscle cells), the actin genes coding for skeletal muscle cell actin are amplified approximately 85-fold.

Of course, not all single-copy genes which are responsible for the synthesis of a large amount of cell product at a particular stage of development are amplified. The silk gland of the larval silk moth, *Bombyx mori*, for instance, produces copious amounts of silk fibroin protein. Analysis of DNA from silk glands indicates that the silk fibroin genes are not amplified above the level of ploidy expressed by the silk glands. Similarly, amplification of globin genes is not a factor in the ability of the reticulocyte (precursor cell of the red blood cell) to synthesize large amounts of the protein hemoglobin.

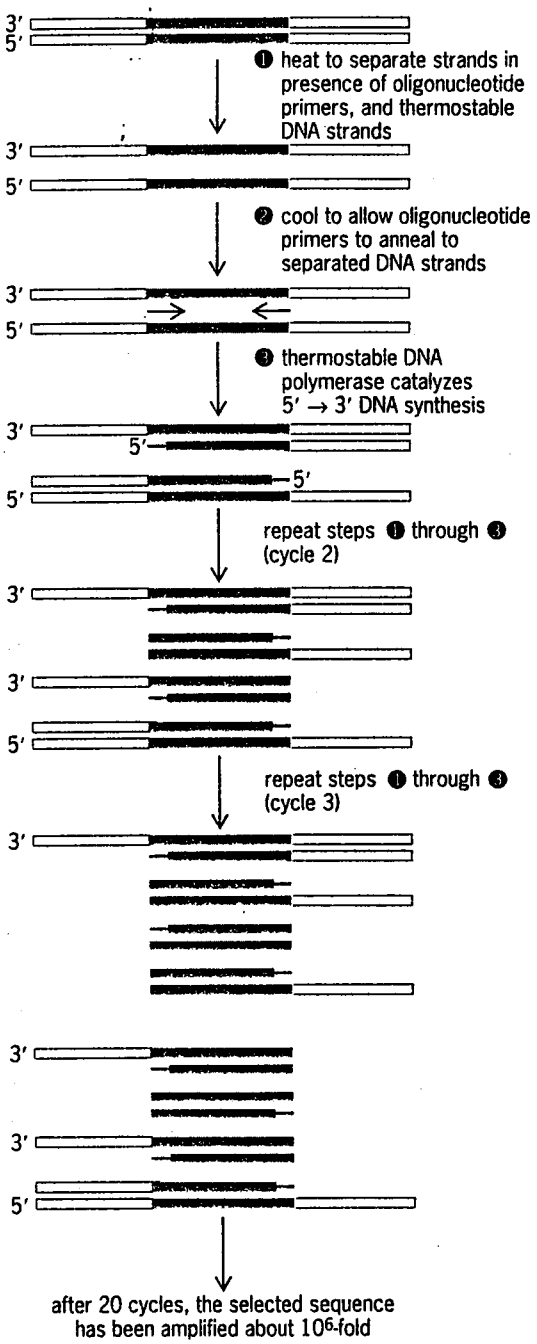
Other examples. In the above-mentioned examples of gene amplification, the amplification phenomenon appears to be developmentally regulated, and the amplified copies of the gene are subsequently lost from the cell. Studies on cells in culture have demonstrated "amplification" of genes involved in resistance to specific drugs. Resistance to the chemotherapeutic drug methotrexate by cultured mouse cells is associated with amplification of the dihydrofolate reductase gene. In this case the amplified genes are passed to daughter cells at the time of cell division. Such gene amplification can be either retained by cells or lost from cells when they are grown in the absence of methotrexate. *See* DRUG RESISTANCE.

Polymerase chain reaction. The polymerase chain reaction (PCR) is a technique that amplifies DNA sequences in laboratory cultures. Beginning with a sample that may contain only one DNA molecule, a selected sequence within that DNA molecule can be amplified millions or even billions of times.

To amplify a given segment of DNA, its entire sequence need not be known, but only short sequences near the ends of the segment. Short oligonucleotides, complementary to the end sequences and designed so that their 3′ ends orient toward the interior of the segment, are synthesized by conventional chemical means. The DNA in the sample is denatured by heating and then is cooled in the presence of the oligonucleotides, which anneal to the separated DNA strands. The annealed oligonucleotides are then utilized by DNA polymerase as primers for DNA synthesis, replicating the target DNA segment. These simple steps are repeated over and over again, doubling the amount of the target segment in each cycle (see **Illus.**). The use of thermostable DNA polymerase (isolated from bacteria that live naturally in hot springs) is an important refinement. Since the polymerase is not denatured when the DNA is

heated, it need not be added at every cycle. Amplification of DNA by the polymerase chain reaction is carried out conveniently by inexpensive machines that produce the amplified product DNA in a few hours. Target sequences between a few hundred and 10,000 base pairs in length are readily amplified. After 20 cycles, the target DNA is amplified more than a millionfold; after 30 cycles the amplification is over a billionfold.

The utility of polymerare chain reaction can be illustrated with a few examples. (1) For molecular biologists, this technique simplified the cloning of known genes, and led to the development of powerful methods to alter DNA sequences in laboratory cultures and detect alterations in DNA in organisms. (2) Certain parts of the human genome are sufficiently variable that they can be used to identify a particular individual unambiguously. With the aid of the polymerase chain reaction, DNA from samples as small as a single human hair can be amplified and analyzed to place an individual at the scene of a crime. Similar techniques are used to accurately establish paternity. Human DNA extracted from centuries-old burial sites and amplified by this technique is being used to trace prehistoric human migrations. (3) As the molecular genetic basis for more genetic diseases becomes known, polymerase chain reaction–based diagnostic tests for these diseases are possible. For example, prenatal diagnosis of genetic diseases can be carried out with minute samples of fetal tissue. (4) Polymerase chain reaction has also been used to amplify and study DNA from extinct species such as the quagga and the woolly mammoth. In addition, very small amounts of DNA added to the cargo hold of an oil tanker can be used to trace the origins of an oil spill. *See* FORENSIC MEDICINE; GENE; GENE ACTION; GENETIC ENGINEERING; MOLECULAR BIOLOGY; PRENATAL DIAGNOSIS. **Michael M. Cox**



Amplification of DNA using the polymerase chain reaction. The colored region is the target DNA to be amplified. The short arrows represent the oligonucleotide primers.

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General aviation

All aircraft activity not associated with major airlines or the military. Among all classifications of aviation in the United States, general aviation consists of the largest number of aircraft and pilots and accounts for the largest number of flying hours.

General aviation is an integral part of the transportation system of the United States, serving both business and pleasure travel. Business travel, which accounts for more flying hours than any other branch of general aviation, employs aircraft ranging in size from single-engine vehicles flown by one person to jet equipment with a professional crew of two. Approximately 70% of all general aviation is associated with some commercial