What is a Gene?

1. A Question With Variable Answers

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Although the term *gene* is very commonly used, there is no *typical* gene, thanks to the recent exciting developments in the field of genetics. Genes are now known to exist in all possible designs and to function in many different ways. As the following historical survey reveals, the concept of gene has undergone remarkable changes since its birth less than 100 years ago.

The exciting developments in the field of genetics in recent years and their implications for human society have prompted newspapers and magazines to regularly carry news items and articles relating to genetics and this has brought the term *gene* to almost every household. As a result, a layman may easily define the term gene without much hesitation and say that genes determine how and what we are and that they are made up of DNA. However, the specialist may find it difficult to give a precise definition. At best, the specialist may list properties and features expected of a gene. The basis for this paradoxical situation lies in the fact that the concept of a gene has undergone remarkable changes with the progress of the subject of genetics and unexpected properties/features of this common, yet somewhat abstract, biological entity continue to be discovered. The following provides a brief historical survey of how the concept of a gene has changed during the evolution of genetics.

The Mendelian Factor

Gregor Johann Mendel, while experimenting with inheritance of characters in the garden pea, first formulated a formal concept of genes, which he designated *factors*. Mendel assumed that for each trait, the body had a pair of factors and that each parent

contributed one of the pair. Members of the pair could be identical (homozygous) or different (heterozygous). Mendel's factors were presumed to be particulate such that during gamete formation, each gamete received only one of the pair (*segregation*) and the segregation of different pairs of factors was independent of their parental (maternal or paternal) origin (*independent assortment*). Since Mendel did not encounter any instance of pairs of factors that did not assort independently, in his concept, each factor was kind of free-floating, each of which through some unknown yet ordered mechanism regularly segregated or separated from its partner and this separation of one pair of factors occurred independent of the other pairs of factors. With the rediscovery of Mendel's principles of inheritance by Hugo de Vries, E von Tschermark and C Correns in the 1900s, the science of genetics was formally born. By this time, the processes of cell division by mitosis and meiosis were fairly well known and the chromosome behavior during meiosis was seen to be strikingly parallel to the proposed principles of segregation and independent assortment of factors. This led W S Sutton and T Boveri to suggest that Mendel's factors reside on chromosomes. The first major change in the Mendelian concept was the discovery in the first few years of this century by W Bateson and R C Punnet and others of linkage between the factors controlling certain characters so that the inheritance of such characters did not show the expected independent assortment. This was also to be expected on the basis of the chromosomal theory of inheritance (see *Box 1*) since the number of chromosomes (the N or the haploid chromosome number) in any given species was far fewer than the number of characters or traits which were determined by the factors.

The production of new combinations of genes or alleles by independent segregation or through the exchange of genetic material between homologous chromosomes by crossing over during meiosis is generally termed *recombination*.

Classical Concept of Gene as a Unit of Function, Mutation and Recombination

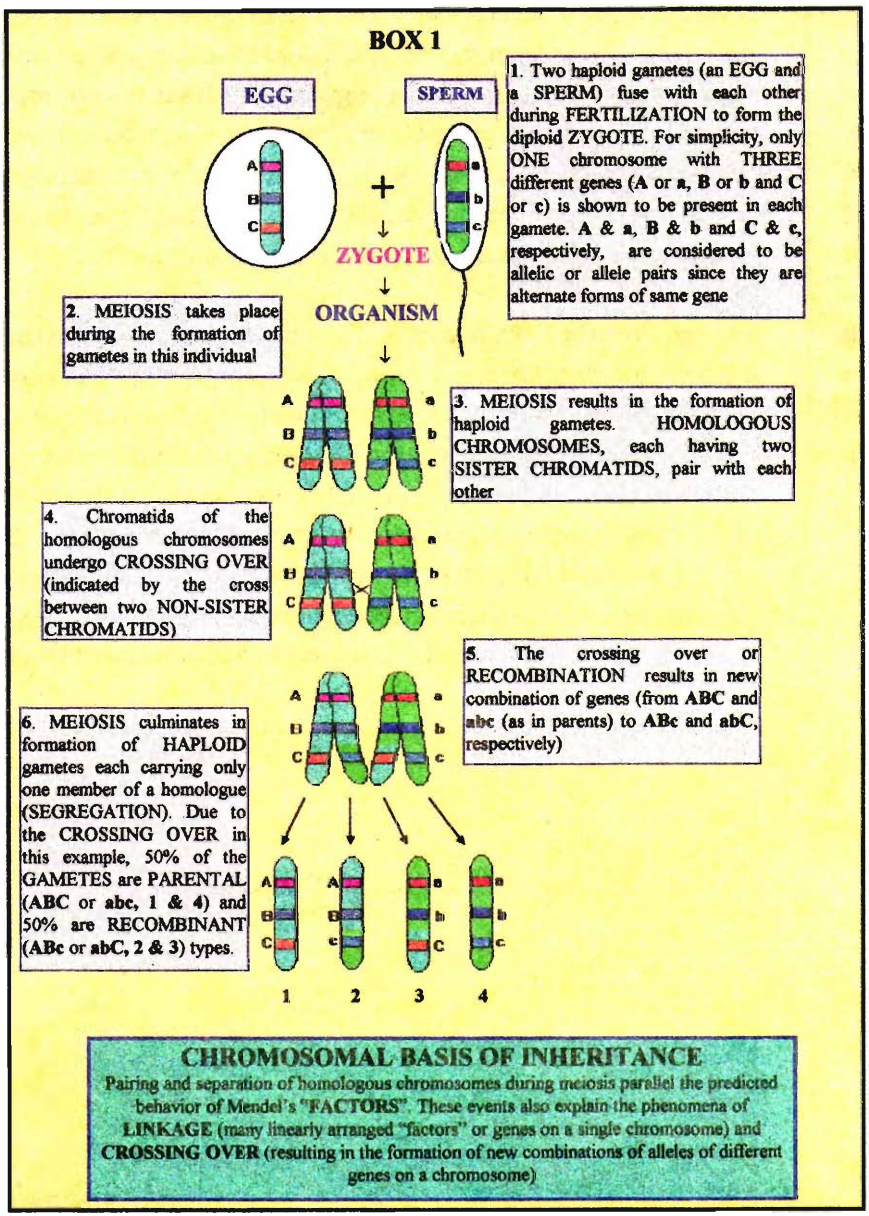
Wilhelm Johannsen introduced the term gene in 1909. He preferred it to remain free of any hypothesis about its physical or chemical nature, just as the numbers used in counting have no

The linear order of genes on a chromosome and distances between them deduced from genetic recombination is called *linkage* or *genetic map*.

Existence of more than two alleles (alternative forms) of a gene in a population of individuals is designated as *multiple allelism.*

physical or chemical units of their own. Introduction of the tiny fruit fly or *Drosophila melanogaster* for genetic studies by Thomas Hunt Morgan within a few years of the rediscovery of Mendel's laws was a turning point in the young field of genetics since inheritance patterns could now be studied in a much shorter time than was possible with the plant systems used by most others. *Drosophila* completes its life cycle within two weeks and could be very easily reared in large numbers in the laboratory, a pre-requisite for any genetic study. The fruit fly has ever since continued to be the young 'Cinderella' of genetics.

The elegant analysis of inheritance patterns of different traits in *Drosophila* by Thomas Hunt Morgan and his group led to a better definition of a gene. These and other studies also established that different genes controlling different traits are arranged in a linear order on chromosomes. The establishment of this linear order, called linkage maps, in *Drosophila*, maize and other organisms and the discovery of new mutant alleles lent support to the view that the gene is an indivisible unit of transmission, recombination, mutation and function. The view that genes were something like 'beads on a string' also appeared to be supported by microscopic visualization of chromomeres during the pachytene stage of meiosis and by the appearance of special chromosomes, the polytene chromosomes, in the salivary glands of larvae of insects like *Drosophila.* Although the molecular and physical nature of the gene remained unknown, a concrete picture of the gene as a unit of heredity with chromosomes as the material counterpart of genes, emerged in this period. This classical view of a gene was summarized by D Raffel and H J Muller in 1940 in the following words: "In genetic theory, genes have been considered as (1) crossover units hypothetical segments within which crossing over does not occur; (2) breakage units again hypothetical segments within which chromosome breakage and reattachment do not occur (at any rate not without destruction of one or both fragments); (3) mutational and functional units those minute regions of the chromosomes, changes within one part of which may be so



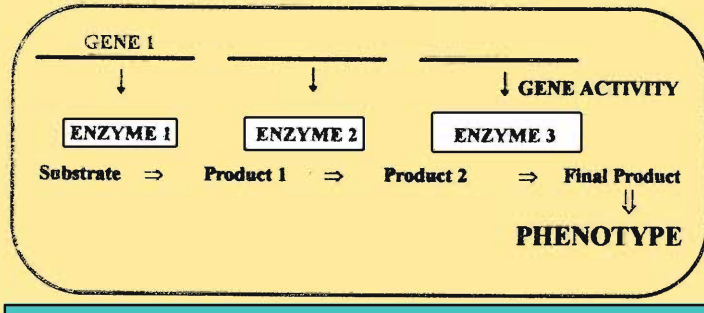
connected with changes in the functioning of the rest of that region as to give rise to the phenomenon of (multiple) allelism; or (4) reproductive units the smallest blocks into which, theoretically, the gene-string could be divided without loss of power of self-reproduction of any part". In simpler terms, this implied that many genes were arranged in a linear order on a

The gene was proposed to be an indivisible unit of transmission, recombination, mutation and function with the different linked genes believed to be arranged on a chromosome like beads on a string.

given chromosome and that a given gene controlled a specific function; a given gene was believed to mutate as a whole rather than its sub-parts mutating separately; finally and most importantly, during meiosis, the chiasmata or crossing over (recombination) was believed to occur *between* two adjacent genes (inter-genic recombination) rather than *within* the 'boundary' of a given gene (intra-genic recombination).

George Beadle and Edward Tatum initiated interesting experiments with the bread mold, *Neurospora crassa*, with a view to correlate mutations in genes with specific biochemical changes in cells. They analyzed mutants of *Neurospora* that needed the culture medium to be supplemented with a specific nutrient for growth. Such mutants are termed as auxotrophs compared to the wild type prototrophs which can grow without such nutrient supplements. The well known 'one gene one enzyme' hypothesis of Beadle and Tatum (see *Box 2*) also reflected the classical view

BOX 2

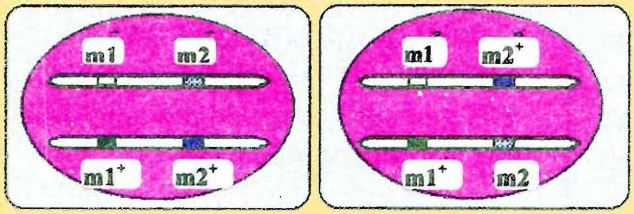


ONE GENE ONE ENZYME HYPOTHESIS

Suppose three genes, 1, 2 and 3, all affect the same phenotype so that mutation in any of the three results in a similar phenotypic change. The "**ONE GENE ONE ENZYME**" hypothesis states that these three genes determine the synthesis of three specific enzymes, **Enzyme 1**, 2 and 3, respectively. These enzymes catalyze stepwise conversion of a **Substrate** through intermediates (**Product 1** and **Product 2**) to the **Final Product** which is responsible for the given **PHENOTYPE**. Non-functioning or improper functioning of any of these enzymes due to mutation in the corresponding gene results in a block in one of the intermediary steps: the final consequence at the phenotypic level, however, is similar.

BOX 3

cis-trans TEST FOR ALLELISM & COMPLEMENTATION



cis-heterozygote *trans*-heterozygote

PHENOTYPE

WILD TYPE irrespective of the m1 and m2 being allelic or non-allelic

WILD TYPE (if m1 and m2 are allelic)

MUTANT (if m1 and m2 are non-allelic)

Conventional genetic test to determine if two linked mutations affecting a given phenotype are allelic or non-allelic compares the phenotype of heterozygous individuals that carry both the mutant genes, (e.g., *m1* and *m2*) in *cis* (i.e., both the mutant genes on one chromosome and both the wild type alleles ($m1^+$ and $m2^+$) on the other homologue, left panel) or in *trans* (i.e., each of the two homologues carries one mutant and one wild type gene, right panel). If the two mutants are *non-allelic* (do not affect the same "gene"), the trans-heterozygote shows wild type phenotype. On the other hand, if they affect the same "gene" (i.e., they are *allelic*), the trans-heterozygote shows a mutant phenotype

of a gene as the unit of function, recombination, mutation and transmission.

With a gene being the unit of function, an individual heterozygous for two mutant alleles of the same gene was expected to have only a mutant, but not a wild type phenotype due to the absence of complementation between alleles (*Box 3*). Likewise, heterozygotes for two mutant alleles were not expected to generate a wild type allele by recombination due to the absence of recombination within a gene. However, some developments in the 1940s and 50s were already becoming incompatible with the above classical view since intra-genic recombination (i.e., recombination between two mutant alleles) and intra-genic

Asking the right question is the most important step in any search or research.

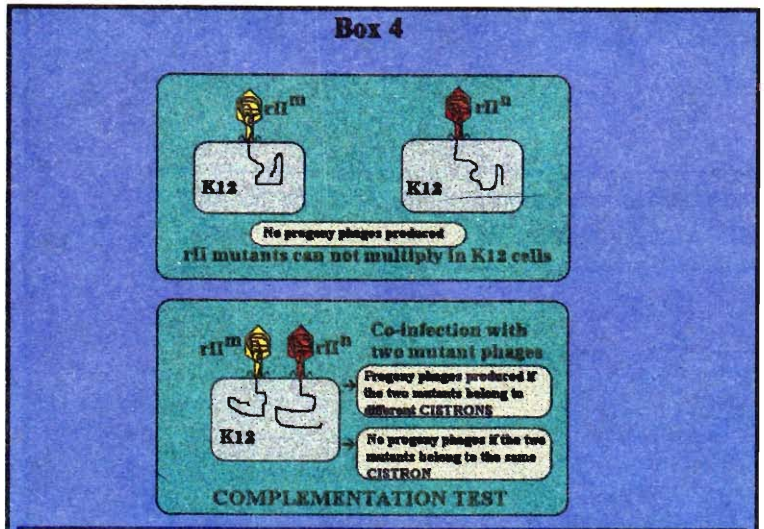
Seymour Benzer's elegant studies on the *rII* locus of T4 bacteriophage finally established that a gene may contain more than one functional unit and as many units of recombination and mutation as the bases comprising that 'gene'.

complementation (i.e., production of a wild type phenotype when an individual carried two different mutant alleles of the same gene) were demonstrated to occur in at least some cases of multiple allelic series. These suggested that a gene was further divisible. Such results led E B Lewis in 1948 to coin the term *pseudoalleles* for those alleles that could recombine to generate a wild type allele but could not complement functionally to give rise to wild type phenotype.

Fine Structure of Gene Reveals it to be Divisible in Sub-parts

A very significant change that happened around the 40s was the increasing interest of physicists and chemists in biological processes with a view to explain the biological world in terms of the laws of material sciences. Now the genes had to be viewed in terms of their physical and chemical dimensions and properties rather than remain merely as conceptual entities like arithmetic numbers. The discovery of DNA as the genetic material through the experiments of O T Avery , C MacLeod and M McCarty and later of A D Hershey and M Chase provided the needed boost in the quest for the physical and chemical bases of the biological inheritance. Within a few years, J D Watson and F H C Crick proposed their famous double helix model for the DNA structure. Now specific questions about the mechanistic aspects of a gene's function like their duplication, control of phenotypic characters, mutation and recombination and other properties could be asked. As is often stated, asking the right question is the most important step in any search or research; scientists did not miss available opportunities and obviously very rapid progress was made in the following years.

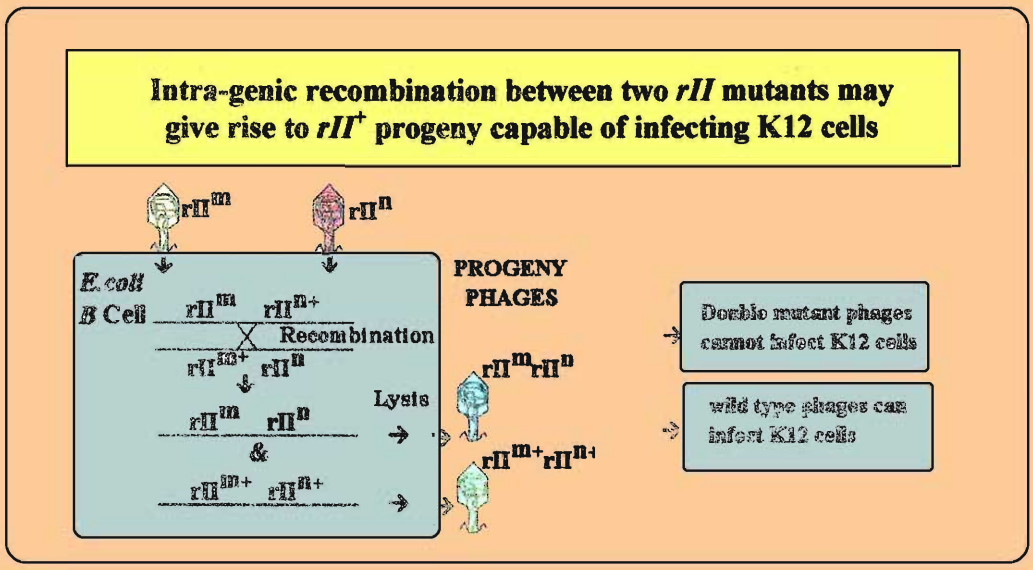
Seymour Benzer, working at the California Institute of Technology in USA, with his elegant studies on the *rII* locus of T4 bacteriophage finally established in the 50's that the gene is divisible in all of its three major properties. Benzer took advantage of the rapid multiplication of bacteriophages to obtain large



The complementation test employed by S. Benzer to define cistrons in the *rII* locus of T4 bacteriophage. The *rII* mutant phages are unable to multiply in the K12 strain of *E.coli*. However, if two phages that carry different *rII* mutant genes (*rII*^{m} and *rII*^{n}) are made to infect (co-infection) the same K12 *E.coli* cell, the infection may be successful if the two *rII* mutations affect different *cistrons* (A and B, respectively) of the *rII* gene because of *complementation*. Since each cistron is the unit of function, the unaffected cistron A of one phage (mutant for the B-cistron) and the unaffected cistron B of the other (mutant for the A-cistron) produce normal A and B products, respectively, so that both the mutant phages can multiply in the otherwise non-permissive K12 host.

numbers of progeny phages and a very convenient selection system to identify even extremely rare phenotypes. Benzer collected a very large number of mutants (point mutations as well as deletions) at the *rII* locus that displayed rapid lysis (causing lysis of more bacterial cells in a given unit of time and therefore, producing larger plaques on a bacterial lawn) and limited host-range phenotypes (the mutants are able to infect the B strain of *E. coli* but not the K12 strain while the wild type phages can multiply in both strains). To find out if two different *rII* mutant alleles could complement each other's defects, he infected the same K12 cell of *E. coli* with the two *rII* mutant phages and examined if the K12 cell was lysed. Benzer argued that if the cell was lysed, the two mutants affected different functional units of the *rII* gene but if the cell was not lysed they belonged to the same functional unit (*Box 4*).

A mutation event does not affect a gene as a whole but only a sub-part of it and parts of two defective genes can be recombined to generate a functional wild type allele.



Demonstration of intra-genic recombination by S. Benzer in the *rII* locus of T4 bacteriophage. *E.coli* cells of B-strain were simultaneously infected with two phages (rII^m and rII^n), each carrying a different mutation in the *rII* gene. Rare recombination between the DNA of the two phages in the interval between the two mutant sites generates a DNA molecule that carries both the mutations (rII^m and rII^n) and another molecule which is wild type at both the sites (rII^{m+} and rII^{n+}). The progeny phages that carry the latter type DNA can successfully infect the K12 cells of *E.coli*.

Benzer coined the term *cistron* for such functional units within a gene. To ascertain if two different mutants mapping within the *rII* locus could recombine, he allowed infection of a B strain cell of *E. coli* with the two mutant phages: if the two mutants affected different locations within the same gene and if intra-genic recombination could occur between these locations, Benzer argued that such recombination would generate 50% phages that are wild type (*Box 5*).

Benzer demonstrated the occurrence of such recombination by allowing the progeny phages to infect K12 cells. He found that

some of the progeny phages were indeed wild type since they successfully infected K12 cells and caused their lysis. These experiments thus clearly established that a mutational event did not affect a gene as a whole but only a sub-part of it and that parts of two defective genes could be recombined to generate a functional wild type allele. These results with *rII* mutants thus showed that with respect to the function as well as mutation and recombination, a 'classical' gene could be subdivided. Such unambiguous results with phage and other bacterial systems vindicated the views expressed by geneticists working with higher organisms, that a gene may after all be divisible into subunits. Thus, a gene may include more than one functional unit or cistron (leading to the 'one gene one enzyme' hypothesis being replaced by the 'one cistron one polypeptide' concept) while for recombination and mutation, the units (recon and muton, respectively) were shown to correspond almost to single nucleotides.

Since, during 1950s, the DNA was well established as the genetic material and was known to be a linear molecule, a polymer of a long chain of nucleotides, the divisibility of a gene into smaller units was now not difficult to imagine or understand.

In the next part, we will see how the advent of molecular biology led to a revolution in the understanding of a gene. Interestingly, although it is more than four decades since the structure of DNA was deciphered, we continue to discover something new about genes.

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Suggested Reading

- **Peter Portin. The concept of the gene: short history and present status. *Quarterly Review of Biology*. Vol. 68. 173-223, 1993.**
- **Benjamin Lewin. *Genes V*. Oxford Univ. Press. Oxford, New York, Tokyo,1994.**

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