MOLECULAR GENETICS OF INHERITED DISORDERS

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Summary

The genetic information stored in the DNA molecules is not absolutely stable, and spontaneous and induced mutations causing genetic disorders are currently occurring. Types of mutations are discussed and, as an example, disorders in the beta-globin gene are taken up. Mechanisms of the inheritance of genetic disorders are also discussed.

1. Introduction

The genetic information is stored as sequences of nucleotides in deoxyribonucleic acid (DNA) molecules, and is the basis of life. The genetic information directs the development of an organism and is transmitted to the succeeding generations. The

genetic information should be stable to achieve these biological functions. On the other hand, the genetic information must be changed to allow living organisms to evolve. Indeed, genetic information is not absolutely stable, and changes in the genetic information—mutations—can be induced by a number of agents. The mutations affect the functions of genes so that they cause production of traits or derangement of normal gene functions. These traits or disorders (phenotypes) have been valuable tools for the investigations of the mechanisms of inheritance and gene function. In this chapter, how the mutations affect gene function and how the traits are transmitted are briefly described.

2. Mutations

2. 1. Causal agents of mutations

Mutations can be induced spontaneously from errors during DNA replication, but the proofing mechanisms for DNA replication permit the occurrence of mutations only at very low frequencies. In addition to the spontaneous mutations, a number of environmental agents, so called mutagens, contribute to induce mutations. Mutagens can be categorized into three groups; physical, chemical and biological agents. Physical agents include UV light and ionizing radiation. Chemical agents include chemicals which interact with DNA and modify it and nucleotide analogs. Transposition or insertion of biological agents such as transposable elements and some viral genomes also contribute to alter the genetic information.

Mutations can occur both in germline cells and somatic cells, but only mutations in the germline are transmitted to the succeeding generations. Mutations in somatic cells may cause various diseases, such as cancers, but are not heritable.

2. 2. Types of mutations

The minimum unit of mutation is a single nucleotide, and a number of different types of mutations have been reported. They vary from substitutions, deletions or insertions of single or several nucleotides to more complicated alterations of DNA molecules such as rearrangements between or among DNA molecules. When the sequences involved in the rearrangements are large enough, the rearrangements can be recognized with a light microscope as chromosomal aberrations such as deletions, insertions, inversions, and translocations.

3. Effects of mutations on gene function

3. 1. Gene structure and function

Mutations can affect various functional aspects of a gene and the features can be better understood when we consider the structure and function of a gene and how mutations affect a gene at the molecular level. The basic structural features of a typical gene producing a protein product and the steps for its expression are schematically represented in Figure 1. Each step can be prone to mutation.

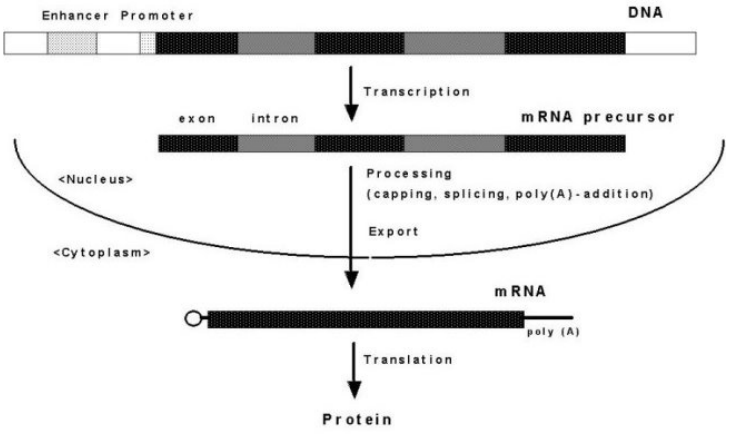

Figure 1. The basic structural features of a gene and steps for its expression.

A gene is composed of two major parts, a sequence transcribed into RNA and sequences not transcribed but which is necessary for the expression of the gene activity. The transcribed sequence can be further subdivided into the coding and non-coding sequences. The coding sequence directs the sequence of amino acid residues of a protein. In eukaryotes, the transcribed sequence is usually split into several segments—exons—by one or several intervening sequences—introns. The introns are precisely spliced out within the nucleus, so that a continuous coding sequence is established in the mature mRNA. In addition to splicing, the transcribed RNA molecule undergoes multiple processing steps before a functional mRNA is produced. After initiation of transcription, nascent RNA molecules are capped with 7-methylguanosine at their 5' ends. After termination of transcription, the RNA molecule is cleaved at 15 to 35 nucleotides downstream of a poly-adenylation (poly(A)) signal sequence and about 100 to 250 nucleotides of poly-adenine are added at the 3' end. The modifications at both ends of the RNA molecule are important for the stability and efficient translation of the mRNA in the cytoplasm. Sequences necessary for all these processing events reside in the transcribed sequence. After or in concomitance with these processing events, the mature RNA is exported from the nucleus to the cytoplasm. The exported mRNA is, then, employed as a template for directing the synthesis of a chain of amino acids, a polypeptide or a protein, in the factory for protein synthesis—the ribosome. The mRNA also contains sequences necessary for proper initiation of translation. In some genes, a sequence involved in the instability of mRNA also resides on the transcribed sequence, usually in the 3'-untranslated region (3'-UTR) of the mRNA.

Sequences involved in transcriptional regulation include those for the proper initiation and termination of transcription and those for the transcription at proper levels in a tissue- and stage-specific manner. The sequence necessary for the initiation of transcription at a proper site is called promoter, and usually locates at the 5'-side of the initiation site. Enhancers are responsible for increasing the levels of transcription in specific tissues at certain developmental stages independent of the orientation and distance from the transcription initiation site. Enhancers locate either outside or/and inside, usually within the introns, of the transcribed sequence.

Mutations can affect either one or several of these sequences or even all of them and

produce a wide variety of phenotypes.

3. 2. Alleles may have different phenotypes

Each gene can be affected in a number of ways by mutations as discussed above. These alternative forms of the gene are called alleles. The mutant alleles can be categorized into five groups according to their effects on the gene functions, and this classification is useful to studies of gene function through their mutant phenotypes.

1. **Amorphic alleles** produce no gene products at all or produce completely inactive gene products, so that they completely lack function. They are also called 'null alleles'.
2. **Hypomorphic alleles** exhibit a reduced gene activity by either producing a lesser amount of normal gene product or a product with lowered activity.
3. **Hypermorphic alleles** exhibit an increased gene activity by either over-producing a gene product or making a gene product with increased activity.
4. **Antimorphic alleles** produce a product with an effect antagonistic to that of the wild-type. Dominant negative mutations, which produce a non-functional gene product competing with the normal gene product, are included in this category.
5. **Neomorphic alleles** exhibit a function different from the wild-type. They make either a gene product that is functionally different from that of the wild-type or a normal gene product at the wrong time or in the wrong place during development.

Amorphic and hypomorphic alleles are often referred to as 'loss-of-function' (lof) mutations, and are usually recessive to their wild-type allele. On the other hand, hypermorphic, antimorphic and neomorphic alleles are referred to as 'gain-of-function' (gof) mutations and are usually dominant to their wild-type allele.

These variant forms of a gene can be produced by various types of mutations. For example, the amorphic mutations can result from either deletion of a part of or an entire gene, insertion or deletion of a single nucleotide causing a frame-shift of the coding sequence, or even a single nucleotide substitution in the coding sequence causing a premature termination of translation.

4. Molecular pathology of the human β-globin gene.

As an example, mutations in the human β (beta)-globin gene is discussed in this section, since the molecular pathology of this gene has been best characterized with protein and recombinant DNA techniques, and has led the studies on the molecular biology of inherited disorders. This should help readers to understand how a particular gene can be affected by mutations and produce a large variety of phenotypes with a gradation of severity.

4.1. The structure of the human β-globin gene cluster.

Hemoglobin (Hb) is the carrier of oxygen in vertebrate red blood cells, and its adult form (Hb A) is a tetramer containing two α (alpha)-globin chains and two β (beta)-

globin chains. Each of the four globin chains contains a heme group, which is an iron-containing pigment and gives the hemoglobin molecule its oxygen-transporting ability. Defects in hemoglobin cause anemias.

The α- and β-globin chains resemble one another both in amino acid sequence and in three-dimensional structure, indicating that both molecules have a common ancestral origin. Molecular cloning of the α- and β-globin gene loci revealed that both of the globin genes have been duplicated several times during evolution, and each has organized a gene family. For example, the human β-globin gene is located on the short arm of chromosome 11 (11p15.5) and the locus contains multiple β-like globin genes and an inactive pseudogene in addition to the β-globin gene (Figure 2A). On the upstream of the ε-chain gene, there is a locus control region (LCR) which allows the high level expression of the β-family globin gene locus specifically in erythrocytes.

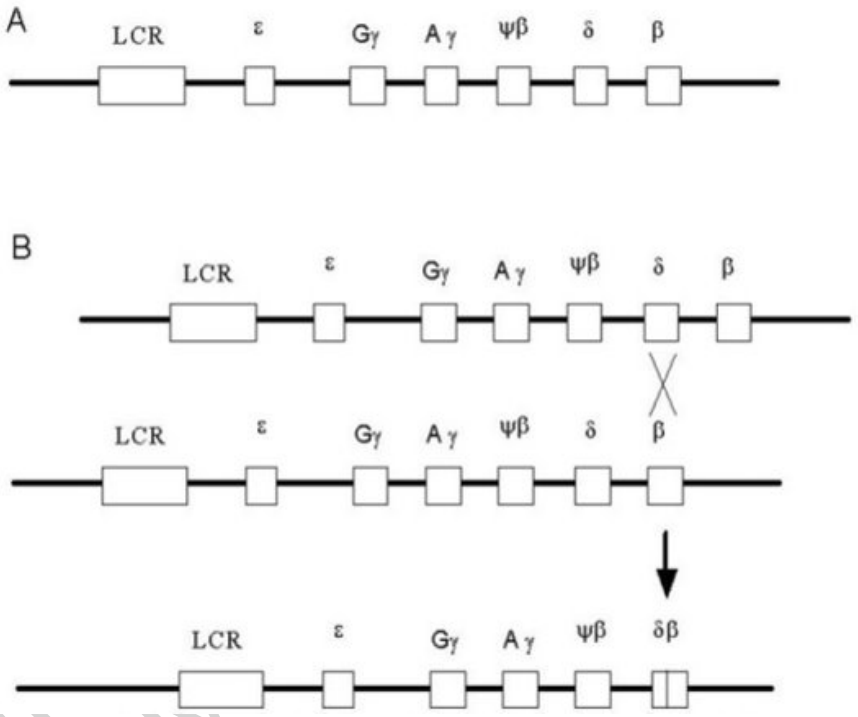

Figure 2. The structure of the human -globin gene cluster (A), and possible mechanism for the generation of Hemoglobin Lepore (fusion) by unequal recombination (B).

The globin genes are expressed at different times and in different amounts during human development. During embryonic development (first six weeks), the hemoglobin consists of two α-like ζ-chains and two β-like ε-chains.

The major hemoglobin of the fetus (weeks 6 to 35 of gestation) consists of two α-chains and two β-like γ-chains. In the adult, Hb A which consists of two α-chains and two β-chains is the major component (about 98 %), and the minor component, Hb A_2, consists of two α-chains and two β-like δ-chains. Expression of the β-globin gene starts after birth, and a deficiency of β-chains may cause symptoms after several months of age.

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Biographical Sketch

Yasuyoshi Nishida was born in June 1948 in Osaka City, Japan. He graduated from the Department of Biology, School of Science, Kyushu University, Fukuoka, Japan in 1971. He obtained a Ph.D. from Osaka University Medical School, Japan, in 1982, and was Assistant professor of Osaka University Medical School from 1976 to 1985. His career has been as follows: Research Fellow at The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, USA (1983~1985); Unit Head of the Molecular Biology Unit, Aichi Cancer Center Research Institute, Nagoya, Japan (1985~1990); Chief of the Laboratory of Experimental Radiology, Aichi Cancer Center Research Institute, (1990~1994); Professor of Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya, Japan (1994~present). His major research area is molecular genetics of *Drosophila* development. He won a Scientific Award from the Kihara Memorial Foundation in 1998.