INTRODUCTION

Humankind has always been concerned with natural physiological problems of death (apoptosis) and immortality (we discussed the latter issue earlier [1]).

What is apoptosis? Elimination of unnecessary cells is vitally important and commonly occurs during individual development, particularly during embryogenesis, metamorphosis, renewal of cell composition in various tissues, and functioning of the immune system. This elimination is carried out through apoptosis, a phenomenon that is drawing increasing attention in molecular genetics of development.

Apoptosis is a genetically programmed cell suicide. This term is derived from the Greek apoptosis meaning a plant shedding leaves. Apoptosis is involved in the following processes: (1) form development; (2) precise regulation of the number of cells constituting certain cell assemblies; (3) removal of excessive of potentially dangerous cells, e.g., lymphocytes of some types; (4) tumor cells; and (5) virus-infected cells [2–4].

What are the differences of apoptosis from common necrotic cell death?

Apoptosis should be distinguished from common necrotic cell death. The latter, as a rule, is caused by acute cell damage, which involves rapid swelling and death of the cell. By contrast, apoptosis is characterized by specific phenomenology:

1. Condensation of the cell nucleus and degradation of nuclear DNA via endonuclear cleavage of chromosome DNA, which breaks first into large (50–300 kb) and then into very small fragments [5].

2. In the case of apoptosis, cell death is suicidal because cells execute an internal program of their death including activation of endogenous proteases, protein-degrading enzymes [6]. In view of this, apoptosis is often referred to as programmed cell death (PCD).

3. PCD is regulated by intercellular relationships; as a result, the organism can eliminate undesirable cells [7].

4. PCD is characterized by loss of the mitochondrial function, which implies a significant role of mitochondria in apoptosis regulation. Nevertheless, the dying cell maintains integrity of its plasmatic membrane [7].

5. Apoptosis-controlling genes have been identified [3, 4]. For instance, early studied of apoptosis have shown that drugs arresting protein synthesis prevent apoptosis. Hence, programmed cell death requires synthesis of specific proteins (in particular, the specific protein annexin, whose presence is diagnostic for apoptosis) and, consequently, depends on genes for these proteins. However, in some cases these substances can induce apoptosis, which testifies to the constant presence of apoptosis effectors in mammalian cells. Inhibitors of RNA or protein synthesis do not arrest apoptosis. Moreover, cells with nuclei removed by cytochalasin or centrifugation, all die showing characteristic signs of PCD.

6. In view of these data, it is generally thought that all genes required for apoptosis are constitutively expressed in mammalian cells. The requirement for synthesis of in RNA or proteins for induction of programmed cell death may reflect the requirement for synthesis of molecules activating the already existing PCD machinery rather than components producing cell death [2].

Apoptosis may appear as a result of a genetically regulated program switched by specific death signals. A universal signal has not been yet identified. Its stereotypical nature implies that a common evolutionarily conserved pathway underlie many, if not all, cases of apoptotic death. This view is confirmed by the existence of a family of structurally related proteins regulating apoptotic responses of stimuli in various types of cells [2, 4]. Recent years witnessed discovery of many novel genes expressing not only proteins from the BCL-2 family but also diverse proteins inhibiting or inducing apoptosis.

Components of the BCL-2 protein have been examined [5].
Fig. 1. Genetic pathway of programmed cell death in C. elegans. Fourteen genes affecting this process at different stages have been isolated. Mutations affecting the commitment to die act only in a small number of cells. By contrast, genes involved in the implementation of all consecutive stages of cell death, are common for all somatic cells of the given organism. Activity of genes ced-3 and ced-4 promotes cell death, while activity of gene ced-9 prevents this event saving the cell from dying. Epistatic interactions between these genes were established. Designations: → positive regulation; → negative regulation [2].

The mechanisms of apoptosis have long attracted attention of researchers but only recent studies yielded the results indicating that the main apoptosis mechanism actually exists [8–11].

The main aim of the present survey of molecular-genetic problems of apoptosis is attracting attention to the results of the modern fundamental studies of this problem, which indicate that apoptosis induction can be used as an approach to genetic treatment of cancer and other diseases.

PHASES OF APOPTOSIS AND GENES CONTROLLING IT

Apoptosis is a universal phenomenon occurring in diverse animal species. In the 1970s Robert Horvits and his colleagues (see review [12]) showed that in the nematode Caenorhabditis elegans, 131 out of constituting this animal 1090 cells die by apoptosis. In C. elegans, the process of programmed cell death is usually divided into four stages (Fig. 1).

(1) a decision that the cell will die or choose other fate;
(2) cell death;
(3) engulfment of the dying cell by a phagocyte;
(4) degradation of the engulfed cell.

Owing to the suitability of C. elegans, the first PCD-affecting mutations were isolated from its genome. Two of these genes, ced-3 and ced-4, proved to be death genes. Gene ced-9 is an anti-apoptosis gene whose activity prevents apoptosis. If the CED-9 protein having protease activity is defective, all 131 cells, which would otherwise all die by apoptosis, survive [2, 12].

In mammals, gene bcl-2 was discovered. This gene is identical to the oncogene ced-9 protecting immune and neural cells against apoptosis. Both genes were sequenced, and gene ced-9 was shown to be by 23% homologous to gene bcl-2 possessing a similar function. Gene bcl-2 can functionally replace gene ced-9 in C. elegans, thus saving mutants from death.

A screening of C. elegans mutants that showed an altered expression pattern of neurotransmitter serotonin revealed gene ces-2. Some genetic evidence indicates that this gene acts as a repressor of another gene, ces-1, thus inhibiting the activity of genes determining death of serotonin-containing neural cells. In animals that carry genes blocking the ces-1 function, the serotonin-containing cells preprogrammed for death does not undergo apoptosis.

Mutations with loss of ces-2 function induce the appearance of two additional serotonin-containing neurons in the throat of the developing nematode worm. These two cells are referred to as NSM neurons. They undergo apoptosis and die during normal development but stay alive in mutant ces-2 animals. Gene ces-2 is unique since its inactivation affects survival of only two cells. These genes belong to the genes coding for transcription factors with leucine zipper. These factors are capable of activating whole groups of genes responsible for cell differentiation in different directions. Another transcription factor is the so-called hepatic leukemia factor (HLF), which was discovered as a characteristic sign of acute leukocyte leukemia.

Chimeric proteins E2A-HLF produced as a result of this translocation prevent cell death in response to an apoptotic stimulus. Thus, these proteins increase the number of developing lymphocytes preventing their death [2, 4].

It seems likely that the genetic program of apoptosis is conserved and universal from worms to human (Fig. 2).

Owing to this, many homologous genes related to the implementation of the internal program of cell death (i.e., apoptosis) have been found. A search for ced-3 and ced-4 homologs was carried out by screening of gene banks of similar sequences. A novel gene coding for the protein termed ICE (interleukin-1β-converting enzyme) was thus discovered [13]. This protein is a protease activating interleukin-1β, an important mediator of inflammation.