THE GENETIC CONTROL OF PROGRAMMED CELL DEATH

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First, I would very much like to thank the Paul Ehrlich Foundation for selecting me to share the Paul Ehrlich and Ludwig Darmstaedter Prize 2000.
It is a great honor. I am particularly delighted to be recognized in conjunction with Professor John Kerr, as Professor Kerr and I clearly have both spent many years sharing a fascination with the problem of how and why cells die. It is our studies of cell death that are being recognized by this Paul Ehrlich and Ludwig Darmstaedter Prize. Why should there be a prize for studies of dying cells?

First, let me say that it is not a significant accomplishment to make cells die or to observe that cells can die. What has proved significant from our work is the finding that in many cases in which cells die, this cell death is a manifestation of a normal biological process. More specifically, cells can die in an orderly, controlled, reproducible way, in fact in a process that has proved to involve an endogenous program of cellular suicide. This process is known as "programmed cell death," or, as suggested by Professor Kerr, as "apoptosis."

Let me say a few words about programmed cell death/apoptosis. To me, programmed cell death is synonomous with naturally-occurring cell death.
Let me explain how it is that cell death can be naturally occurring.

During the development of an animal from a single cell, the fertilized egg, many things happen. The fertilized egg divides, and then its daughters divide, over and over again, generating large numbers of cells, as many as 10^{13} -- ten million million -- in humans. Then each of these cells must differentiate, i.e. take on specific characteristics, such as becoming a nerve cell or a muscle cell or a skin cell. Furthermore, all of these cells must interact so as to form groups of cells with the proper structures -- say an arm or a leg -- and proper interconnections -- as in the highly complex brain. These processes of cell division, cell differentiation and morphogenesis constitute the basic events of development and define the basic problems of developmental biology.

In addition to these processes, there is another process that appears to be universal among developing animals -- the process of cell death. Quite remarkably, many of the cells that are generated as animals develop do not survive to form part of the animal, but instead die, often before they have had a chance to do anything whatsoever. It is this naturally-occurring cell death that is often refered to as programmed cell death.

That cell death occurs during the normal course of animal development has long been known by developmental biologists. For example, the regression of a tadpole's tail as it undergoes metamorphosis to become a frog involves the programmed deaths of essentially all of the cell types found in the tail. Similarly, the formation of digits, such as our fingers and toes, occurs by the removal of interdigital webbed regions by programmed cell death. Chicken feet are formed by such interdigital programmed cell death, whereas, by contrast, the webbed feet of a duck are generated because this process does not occur.

So far, what I have told you about programmed cell death is a normal, biological process. Not surprisingly, where there is a biology, there is a pathology. In other words, the disruption of any biological process can lead to disease, and programmed cell death is

no exception. For example, certain human cancers -- notably follicular lymphoma -- have been found to be diseases of cell death: cell number is defined by an equilibrium between cell addition and cell loss; it has long been known that too much cell division can lead to cancerous growth; it has now been found that too little cell death can do so as well.

It also seems that just as too little cell death can lead to disease, so could too much cell death. Many human diseases, particularly neurodegenerative disorders such as Alzheimer's, Parkinson's, Huntington's and Lou Gehrig's Disease (also known as amyotrophic lateral sclerosis) involve cell death: in each case, specific classes of neurons die, leading to the particular clinical features of each of these neurological disorders. The clinical features of other neurological disorders -- such as stroke and traumatic brain injury -- are also characterized by the deaths of neurons. One current hypothesis is that at least some human neurodegenerative disorders are caused by ectopic programmed cell deaths, i.e., by cell deaths that are mechanistically similar to those that occur in normal development but that are for some reason being expressed by the wrong cells or at the wrong time.

In short, there is a biology of cell death that is important for normal development and that, in addition, when disrupted in humans can lead to disease. How do we know that there is such a biology? This, I think, is where the findings of Professor Kerr and myself come in.

As you have just heard Professor Kerr, in the 1960s and 1970s, was working as a pathologist and studying cells that die using an electron microscope, the most powerful type of microscope available. What he and his colleagues discovered is that many cells that die look the same, i.e., undergo the same series of changes in appearance as they die. They first found this was true for cells in different types of human tumors. Then they examined cells that were caused to die by anti-tumor agents, and these dying cells looked the same. Finally, they studied cells that die during animal development, i.e., programmed cell deaths. They, too, looked the same. From these observations, Professor Kerr concluded that there was likely to be an active, inherently controlled biological process responsible for this ultrastructurally conserved form of cell death, which he called "apoptosis."

Meanwhile, in the middle 1970s I, too, began studies of cell death. I encountered this problem while working in the field of developmental biology. Specifically, I was interested in the basic developmental biology of a microscopic roundworm, a nematode, called *Caenorhabditis elegans*. In studies I did in collaboration with John Sulston at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, I helped trace the complete pattern of cell divisions that occurred while a *C. elegans* egg developed into an adult animal. *C. elegans* is a very simple animal, with only 959 cells. What we found was that the generation of these 959 cells was accompanied by the generation of an additional 131 cells. These 131 cells are not found in the adult. Rather, these 131 cells die soon after they are formed, i.e., they undergo programmed cell death. When we studied what these deaths looked like, they all looked very much the same. In fact, they looked very much like the apoptotic deaths that had been described by Professor Kerr.

That specific cells from distinct points of the cell lineage undergo a specific series of morphological changes during programmed cell death suggested that we could think about programmed cell death as a cell fate. Now, it was known for other cell fates, such as becoming a muscle cell or a gut cell or a nerve cell, that the actions of specific genes

were required. For this reason, we postulated that the fate of programmed cell death should be no different, i.e., that there should be genes that control the cell fate of programmed cell death.

We sought such genes, and we found them. To date, we have analyzed in detail 16 different genes that control programmed cell death. These efforts have defined a genetic pathway for programmed cell death in *C. elegans.* Basically, this pathway consists of four sequential steps. In the first step, each cell has to decide whether it is to live or to die by programmed cell death. The second step is the killing step, in which a happy, healthy cell is made dead. The third step is "get rid of the corpse." The genes that act in this step are involved in the engulfment of dying cells by their neighbors. The fourth step is "destroy the evidence." One gene has been identified that functions in the degradation of the macromolecules of the cell corpse.

We have studied these genes in detail, and we have learned how many of them work. In this way, we have defined the molecular genetic mechanisms responsible for programmed cell death or apoptosis in *C. elegans*. In work both by us and by others, our findings about *C. elegans* have been extended to other organisms, including humans. The remarkable conclusion has been that the molecular genetic program for programmed cell death we have established for *C. elegans* seems to be conserved in many other animals, including humans. Thus, we have helped establish a mechanistic understanding of the process of programmed cell death and apoptosis not only in *C. elegans* but also in ourselves.

To summarize what I have told you, our studies of programmed cell death in *C. elegans* have helped establish two major findings: (1) programmed cell death/apoptosis is a normal, biological process that involves specific genes and proteins; and (2) the genes and proteins responsible for programmed cell death/apoptosis appear to be universal, conserved among organisms as distinct as microscopic roundworms and human beings. In addition, our discoveries concerning programmed cell death have now been moved into the pharmaceutical world, as both major drug companies and smaller biotechnology companies are using as potential drug targets some of the proteins we have revealed to be key in regulating programmed cell death. What I very much hope is that through these efforts our findings will help pave the way toward the development of novel pharmaceutical agents that can be used to treat human disorders as diverse as follicular lymphoma, neurodegenerative disorders, AIDS and autoimmune disease.