

What history tells us XXI. Apoptosis and programmed cell death: when biological categories are blurred

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1. Introduction

The description of programmed cell death (PCD) (Lockshin and Williams 1964), the characterization of its most popular form, apoptosis (Kerr *et al.* 1972), and its rapid molecular description thanks to the model organism *Caenorhabditis elegans* (Ellis *et al.* 1991) might appear as a paradigmatic example of the success of what the Norwegian philosopher of science Nils Roll-Hansen has called “biological reductionism” (Roll-Hansen 1996): biological phenomena are first described in organisms and cells, in structural and functional terms; then, molecular mechanisms and components involved in their realization are described. Nils Roll-Hansen opposed this form of reductionism, characteristic of present-day biology, with what he called physicalist reductionism, in which the existence of biological phenomena is denied.

In the first part of this article, I will argue that, for many reasons, PCD and apoptosis were models for the whole of molecular and cell biology. But in the second part, I will show that the picture has become fuzzy. The frontiers with other forms of death and other cellular processes have been blurred. I will describe the notions which are used to explain why the frontiers of these phenomena have been blurred, and why their molecular description has become more and more complex. The difficulties originate in the complex evolutionary history of the systems under study. I will argue that these explanations are insufficient to face the difficulties. With the increasing role of evolutionary questioning in functional biology, some of the categories used in the latter will have to be transformed, or will even disappear.

2. Apoptosis: a model process

The visibility of the phenomena of apoptosis and PCD, and the impact they had on the biological community have their origins in the rapidity with which these phenomena were characterized after their discovery. In fact, precise descriptions of PCD were given in the 19th century, but the phenomenon as such was not recognized (Clarke and Clarke 1996). Similar observations were made during the first half of the 20th century, especially in studies of the development of the nervous system (Glücksmann 1951). One had to wait until 1964 and the introduction of the term “PCD” to distinguish this form of death, visible for instance during metamorphosis, from the accidental form of death, necrosis (Lockshin and Williams 1964). In 1972, Kerr used the term apoptosis to describe a special form of programmed cell death characterized by the morphology of the dying cell, and the absence of inflammation resulting from it (Kerr *et al.* 1972).

The genes involved in apoptosis were characterized in *C. elegans*, in which a complete pattern of cell fate, including cell division but also cell death, had been defined before (Ellis *et al.* 1991). The characterization of the genes involved in cell death was used to establish a list of molecular components necessary for this form of death. Among them, caspases, proteases involved in the degradation of the cells, received most attention. They participate in the initiation of cell death, as well as in its realization and last steps (Thornberry and Lazebnik 1998). One of these caspases is indirectly responsible for the regular extra-nucleosomal cleavage of DNA, which is characteristic of apoptosis.

Most of all, the functions of these forms of cellular death appeared clearly distinct from those of other forms of death

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such as necrosis. Apoptosis and PCD are responsible for the sculpture of the organism, the elimination of useless and misplaced cells, and the design of an efficient and safe immune system.

Evolutionary scenarios were rapidly proposed to explain how this form of death might have been introduced in eukaryotes from the mechanisms of prokaryotic cell death involved, for instance, in quorum sensing, through the endosymbiosis of bacteria, and the formation of mitochondria (Ameisen 1996, 2002, 2004). These scenarios explained the central role played by mitochondria in apoptosis. The last reason for the overwhelming importance of apoptosis was that its understanding could open up therapeutic opportunities in diseases where cell death plays a major role, as in neurodegenerative diseases.

3. The picture becomes more and more fuzzy

The fact that the picture was so clear in the 1980s-1990s enables us to see, better than in other fields, how subsequent discoveries made it more and more fuzzy.

Consider for instance caspases. It was quite rapidly shown that some members of the caspase family are involved in other functions, such as the triggering of the inflammatory response. The pruning of axons during the morphogenesis of the central nervous system is due to caspases (Nikolaev *et al.* 2009). A large number of additional vital functions of caspases have recently been described (Garrido and Kroemer 2004; Launay *et al.* 2005; Lamkanfi *et al.* 2007) and they concern members of the family involved in apoptosis. Caspases are also involved in the regulation of the pluripotent state of embryonic stem cells (Zwaka 2010).

More puzzling was the observation that a cellular rescue process was recently considered as the second form of programmed cell death: autophagy. Autophagy was described as a way for cells to maintain their supply of nutrients in situations of starvation, by digesting some of their own components. Autophagy also participates in the elimination of useless organelles and of protein aggregates which form, in particular, in neurodegenerative diseases. But autophagy is also a form of death. In addition, it crosstalks with apoptosis (Sandoval *et al.* 2008), and can replace apoptosis when the latter has been experimentally inactivated. The authors of many articles have argued that the “true” function of autophagy is not cell death. It is only an “impostor” of cell death (Ceconi and Levine 2008; Levine and Kroemer 2009). Obviously, autophagy also has functions other than cell death. But how is it possible to say that its “true” function is not cell death, when so many studies demonstrate that autophagy is involved in physiological cell death?

The existence of a clear boundary between programmed cell death and accidental death, necrosis, has recently

been questioned (Golstein and Kroemer 2006). As in the case of apoptosis, there are molecular components which are characteristic of necrosis; necrosis can participate in physiological processes; and it can replace apoptosis when the latter is blocked, for instance in the formation of fingers in mammals.

More generally, new forms of cell death are progressively being described (Bredesen *et al.* 2006; Golstein and Kroemer 2007). The distinction between programmed and accidental cell death is becoming increasingly fuzzy, and the efforts made to classify the different forms of cell death look as desperate as those of Sisyphus (Kroemer *et al.* 2009).

4. Saving appearances: the way to interpret these difficulties

Different concepts and notions have been introduced by biologists to account for this fuzziness. The first, due to Darwin, found new life through its reintroduction by François Jacob (1977). To evolve, organisms tinker with what they have to hand. They use pre-existing pieces to generate new structures and new functions. Two other notions are frequently used: recruitment and its corollary pleiotropy. They are also not newcomers in biology, at least in the case of the second, and they tell the same story. They have the advantage of being neutral, whereas the use of tinkering suggests that the result might have been better if the work had been done by an engineer. Another notion, used by evolutionary biologists, and only exceptionally by cell and molecular biologists, describes a similar process: exaptation. Nevertheless, exaptation consists more of the transformation of pre-existing functions than of the addition of new functions, as suggested by the notion of recruitment. The reason is that it does not generally concern molecular functions, but more elaborate ones.

When one considers a process like apoptosis, tinkering and recruitment might have participated in its formation, by using pre-existing molecular functions, or in its blurring by attributing new functions to its components.

A second, different explanation for the difficulty of delineating precise boundaries and of defining a process such as apoptosis originates in the recurrent action of evolution to increase regulation and robustness. Such action limits the consequences of the impairment of one functional system, and coordinates the different processes within cells and organisms. It perfectly explains what is observed in the case of apoptosis: the molecular connections between different forms of death, the capacity for one form of death to replace another.

These explanations propose that a process does exist, but its frontiers and definitions have been blurred. It remains

possible, by filtering out the noise that evolution has introduced into the system, to restore the “pure” process of apoptosis. But is this the case? Before answering in the negative, I will discuss the difficulties encountered by researchers in an apparently unrelated field, the establishment of phylogenies for early organisms in evolution, archaea, eubacteria and eukaryotes. One major difficulty is the existence of horizontal gene transfer, the exchange through conjugation and bacteriophages of genetic information between organisms belonging to different lineages. Specialists initially considered that it would be possible to overcome this difficulty by improving computer programs and selecting a group of genes (those involved in the transfer of information) less likely to be exchanged. This core of conserved genes has become smaller and smaller. What is the significance of a tree of life established by sequence comparisons of less than 1% of the genes? Some specialists consider that the structural category “tree” ought to be abandoned in favour of other categories such as “rhizomes”, thus investing this horizontal genetic exchange with its full significance.

The situation is less dramatic in the case of PCD and apoptosis, but difficulties accumulate at a rapid pace, and it becomes reasonable to question the distinctions so far made between accidental and programmed cell death, and to stop discussing whether autophagy and apoptosis are vital or death processes.

The vanity of the efforts made to justify the difficulties by the dialogue between the different processes may also be illuminated by a comparison. When a dialogue becomes highly active, the individual discourses no longer exist. This is visible in the case of active collaboration of scientists leading to important breakthroughs: it becomes impossible for the participants themselves to estimate their personal contribution to the discovery (for a case study of such difficulties, see Olby 2009).

5. Taking evolution seriously, and renouncing the present categories

All the difficulties described previously are the consequence of the blurring action of natural selection. As mentioned earlier, some of the discoverers of PCD and apoptosis elaborated scenarios to explain the emergence of these forms of death (Ameisen 1996, 2002, 2004, 2005; Golstein and Kroemer 2005). One might imagine that they were well prepared to face the subsequent difficulties. Such was not the case, because their scenarios had all the characteristics of evolutionary scenarios elaborated by functional biologists, and clearly differed from the scenarios elaborated by evolutionary biologists (Morange 2009). Evolution is spontaneously identified with complexification and progress (Golstein and Kroemer 2005). Evolution has no costs:

instead of considering a trade-off between costs and benefits, the different steps leading to the formation of an apoptotic process are seen as beneficial whatever the environment. And the scenarios are elaborated retrospectively, with the obvious objective of explaining the present situation. All these characteristics are at odds with the efforts made by evolutionary biologists, and limit the value of most of these scenarios.

What has to be abandoned to have a more adequate vision of these processes? What is most difficult apparently is to give a precise molecular description of them, because the macromolecules and the mechanisms involved are not specific to the processes under study. Some biologists would probably consider that this simply highlights the failure of the reductionist programme of molecular biology. But do these processes resist better at the cellular and functional levels? We have seen that such is not the case.

There are two ways to overcome the present difficulties. The first is to renounce the present classifications and categorizations, and to replace them by new ones, better adapted to the recently accumulated observations. Such re-categorizations frequently occur in biology. An interesting example can be found in protein chemistry, and the functions of chaperones (Morange 2005). These proteins were first described as part of a machinery involved in protein folding. Later, it was admitted that their major role is to prevent improper folding and aggregation. But these proteins were subsequently also shown to be required for protein degradation. A new functional category has emerged, quality control, in which both chaperones and proteases play roles. Similar functional categories, such as “cell quality control”, might perhaps better explain the vital and lethal roles of autophagy and apoptosis.

A second possibility would be to renounce the existence of stable categories in favour of temporary and local clustering of molecular components for a transient function. Such clusterings would be adapted to one organism, to one process in an organism at a given time of evolution. In the vague category of PCD could be included, by convention and for the sake of simplicity, many different processes. These vague categories would only be an artificial way of classifying the different processes under study.

6. Conclusion

These difficulties in establishing structural and functional categories on firm molecular and cellular grounds are far from being limited to studies on cell death. In the 1980s, development was also explained by the existence of a recently identified group of genes, developmental genes

(Morange 2000). It would have seemed that, thanks to this precise molecular definition, it would be possible to give development precise boundaries in time and space. Such is not the case: whereas for certain biologists, development is completed when the organisms are able to reproduce, for others development continues throughout the life of the organism. This disagreement is not solved by molecular and cellular descriptions, because neither the action of developmental genes nor the specific cellular mechanisms involved in development are limited to a specific period of time in the life of the organism. In the case of developmental biology, the existence of specific molecular and cellular mechanisms was never unanimously accepted by all biologists, whereas in the case of apoptosis, the definition of this process by a core of specific molecular and cellular mechanisms was widely accepted. This makes the latter field of research a better example of the present, new difficulties.

Many biologists would probably consider that the previous difficulties are not so serious. They do not prevent the therapeutic use of the knowledge accumulated on apoptosis. The opposite is true: the distinction between different forms of death, and the complex relations between them provide opportunities for new therapeutic approaches, and a guide on how to avoid side effects and treatment failure.

More generally, vague concepts have been shown to be useful in science, because they can circulate between different domains of research: such is the case of the famous notion of gene, the precise definition of which is nevertheless so difficult.

It is true, but up to a certain point! Too much confusion can create obstacles to the progress of knowledge. In particular, as we have seen, it may orient researchers towards questions that have no answers and to efforts that have no sense.

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