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Programmed Cell Death in Protozoa

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About the Editor...

JOSÉ MANUEL PÉREZ MARTÍN was born in Madrid, Spain in 1960. In 1983, he obtained a bachelors degree in Biochemistry and Molecular Biology at the Autonomous University of Madrid (Spain) and completed his molecular biology training in 1985. In 1992, he obtained his Ph.D. degree in Molecular Biology at the Autonomous University of Madrid. Dr. Pérez Martín had a postdoctoral position from 1992 to 1996 conducting clinical trials with antitumor drugs under the sponsorship of the Bristol Myers-Squibb Pharmaceutical Research Institute (Wallingford, Connecticut, USA, and Madrid, Spain). From 1997 to 2006 he was Associate Profesor at the Faculty of Sciences of the Autonomous University of Madrid (Spain), teaching inorganic and bioinorganic chemistry. At present, he is Head of Laboratory of the Farmacia "Castilla" in Madrid (Spain). His research is focussed on the interactions between drugs and DNA or proteins and the pharmacological modulation of antitumor drugs. He is a co-author of more than 90 scientific papers including reviews and book chapters and is co-editor of the book *Metal Compounds in Cancer Chemotherapy* (Research Signpost, 2005). He is also a member of the Advisory Board of the scientific journals: *Current Medicinal Chemistry*, *Medicinal Chemistry*, *Anticancer Agents in Medicinal Chemistry*, and *Recent Patents on Anticancer Drug Discovery*.

Dedication

This book is dedicated to the memory of my late father and also to my mother, wife and son.

As an amateur guitarist, I would also like to dedicate this book to the virtuoso Spanish guitarists Fernando Sor, Dionisio Aguado, Francisco Tárrega, Andrés Segovia, Narciso Yepes and Paco de Lucia.

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PREFACE

Under the name of programmed cell death (PCD) are included diverse molecular mechanisms of cell suicide which play an essential role in the development of multicellular organisms. The best known PCD mechanism in multicellular organisms is called apoptosis. However, recent studies indicate that PCD is also present in protozoa and unicellular eukaryotes. The twelve chapters of this book give the reader a comprehensive update of the progress in the understanding of the mechanisms of PCD in protozoa. The chapters have been written by experts in this field of research and are arranged following an evolutionary point of view starting with PCD in protists and ending with PCD in ciliated protozoa.

Chapter 1 is an overview of the current knowledge about PCD in protozoa using the example of kinetoplastid parasites as unicellular eukaryotic (mitochondriate) organisms that inherited PCD from ancestral prokaryotic protozoa. Chapter 2 deals with the intriguing fact that in amitochondriate organisms where only hydrogenosomes and mitosomes subsist as mitochondrial relics, recent findings show that PCD also occurs. This exciting discovery is presented here in the light of sequencing in various species as well as recent findings about mitochondrial derivates and ancestral viruses, contributing to a better understanding of the life tree as well as to the future discovery of new molecules of interest. In Chapter 3 the phenomenon of apoptosis, one of the types of PCD, is reviewed in three vector-borne trypanosomatids (*Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania spp*) responsible for diseases of great medical and veterinary importance. Chapter 4 summarizes the most obvious findings regarding programmed cell death in African trypanosomes. In Chapter 5, the use of topoisomerase inhibitors as tools to disentangle the molecular mechanisms of PCD in the kinetoplastid parasite *Leishmania* is presented. Chapter 6 reports on the uses of metal complexes as leishmanicidal drugs, especially those having leishmanicidal activity which could be linked to their interaction with the parasitic DNA. In addition, PCD-inducing drugs used clinically against Leishmaniasis and those currently in the experimental and evaluation phases are reviewed. Chapter 7 deals with the fact that in recent years there has been an increasing awareness of the role of PCD in the malaria parasite's infection of its vertebrate host and mosquito vector. In fact, a significant body of research now indicates that PCD of both vertebrate host and mosquito vector cells plays an important, if still incompletely understood, role during infection with this parasite. The understanding of this role may have medical applications in the treatment of malaria. In Chapter 8 the hypothesis that some stages of malaria parasites (*Plasmodium*) are able to undergo a form of PCD is supported by available data. Moreover, this chapter presents the current knowledge on *Plasmodium* metacaspases; these putative proteases are the most promising candidates that might be essential for the execution of PCD in malaria parasites. Chapter 9 is mainly devoted to the important study

of PCD in *Trichomonas foetus*, a cattle parasite, and *Trichomonas vaginalis*, a human parasite. *Trichomonads* do not possess mitochondria but harbor another type of membrane-bounded organelle, an unusual anaerobic energy-producing organelle known as the hydrogenosome. Studies of cell death in trichomonads are under way in order to establish whether the hydrogenosome could represent an alternative to mitochondria and whether these organisms possess all caspase activities and which conditions lead *Trichomonads* to cell death. The presence of a cell death program in *Trichomonads* suggests the existence either of a dependent or independent caspase-like execution pathway in such organisms. In Chapter 10, PCD in the enteric protozoon parasite *Blastocystis hominis* is described. Chapter 11 presents PCD in *Dinoflagellates* which are protists ecologically important as components of phytoplankton. The acquisition of PCD genes in *Dinoflagellates* goes back to ancient times where endosymbiotic events took place. Chapter 12 reports on the fascinating phenomenon of programmed nuclear death (PND) in ciliated protozoa. The main objective of this PND is to remove the old macronucleus while a new recombinant vegetative nucleus develops in each conjugating cell. The mechanism of PND is still not elucidated, but we know that it involves caspase-like proteins, an intense acid phosphatase activity and an autophagic process.

Finally, I would like to thank Landes Bioscience and all the authors participating in this book for the patience they have had with the Editor during the two years that have passed since the original project of this book was conceived.

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CHAPTER 1

Programmed Cell Death in Protozoa: An Evolutionary Point of View. The Example of Kinetoplastid Parasites

Miguel A. Fuertes, Paul A. Nguewa, Josefina Castilla, Carlos Alonso and José Manuel Pérez Martín*

Abstract

Programmed cell death (PCD) is a molecular event which plays an essential role in the development of multicellular organisms. However, recent studies indicate that PCD is a mechanism also present in protozoa and unicellular eukaryotes. For instance, it has been recently proposed that some *Trypanosomatid* parasites have a PCD mechanism descendant from an ancient life form that has actually evolved. Thus, two hypotheses may explain the existence of PCD in protozoa such as *Trypanosomatids*. First, PCD could simply be a process without a defined function inherited through cell evolution, which is triggered in response to diverse stimuli and stress conditions. Alternatively, PCD might be used by *Trypanosomatids* as a control mechanism to maximize their biological fitness.

Introduction

Figure 1 shows that diverse forms of programmed cell death (PCD) have been recently described in at least nine species of protozoa, whose phylogenic divergence is believed to range from around two to one billion years ago.[1] Some of these PCD forms have been reported in the kinetoplastid parasites of the genuses *Trypanosoma* and *Leishmania* that are believed to be amongst the earliest diverging eukaryotes. These kinetoplastid parasites are the agents responsible for trypanosomiasis and leishmaniasis, tropical illnesses that suffer approximately 30 million people around the world.[2] It is interesting to know that the cell death phenotype of the kinetoplastid parasites shares several features with apoptosis which is the mechanism of cell death shown by multicellular organisms. In fact, PCD in kinetoplastid parasites includes cytoplasmic blebbing and vacuolization, chromatin condensation and DNA fragmentation. These findings indicate that PCD may have evolved together with the endosymbiotic incorporation of aerobic bacteria (the precursors of mitochondria) into ancestral unicellular eukaryotes.[3] Hence, two hypothesis may account for the existence of PCD in single-celled organisms such as *Trypanosomatids*. On the one hand, PCD in *Trypanosomatids* could simply be a remnant process of eukaryotic cell evolution without a particular function, which is induced in response to diverse stimuli (for example, serum removal, oxidants such as H_2O_2 and chemotherapeutic agents).[4] On the other hand, PCD could have a defined biological role for *Trypanosomatids* as a way to maximise the biological fitness of these parasites facilitating their adaptation to a digenic life cycle (mammalians-insect-mammalian). Therefore, of particular interest is the question of the origin and nature of PCD in protozoa as well as its important role in the

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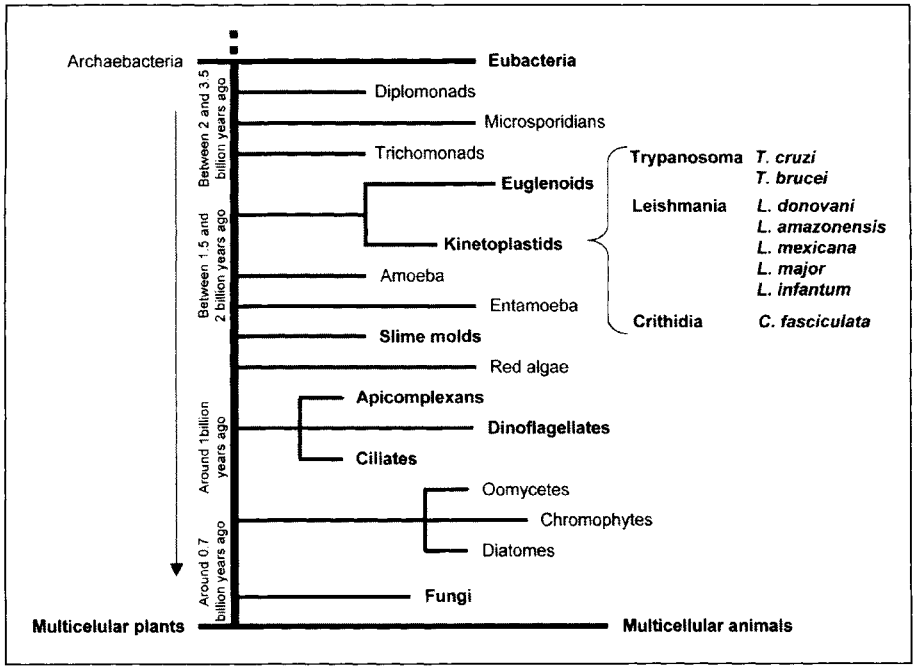

Figure 1. Programmed cell death (PCD) during evolution. Within the phylogenic tree, PCD has been identified in various members (in bold) of the tree including *Trypanosomatids*.

regulation of the complex interactions between unicellular and multicellular organisms which allow, for instance, the establishment and persistence of stable host/parasite interactions. Last but not least, the extent of overlapping between effectors and regulators of PCD among *Trypanosomatids* and mammalian hosts PCD pathways may determine whether or not the pathways leading to cell suicide in these parasites may be pharmacologically exploited as a parasite control strategy.

The Example of PCD in Kinetoplastids as an Heritage of Evolution

Much evidence accumulated over the last years indicates that PCD in protozoa comes from an ancestral death machinery.[5] As mentioned above, *Kinetoplatid* parasites are eukaryotic protozoa that belong to one of the most ancient diverging branches of the eukaryotes phylogenic tree.[6,7] These single-celled organisms are reported amongst the first mitochondrial eukaryotes and contain only one giant mitochondrion, called the kinetoplast.[8]

First of all, we shall discuss about the evolutionary implications of mitochondrial acquisition in life/death regulation. Thus, we should take into account that programmed cell death may be particularly useful when cells are interacting. For instance, in trypanosomes PCD may control communication between unicellular and multicellular organisms, which allows the establishment of a stable host-parasite relationship.[9,10] But, how and when did protozoa choose genes allowing cell suicide? Firstly, it has been hypothesized that a common PCD mechanism arose before the evolution of multicellularity.[11] Secondly, the hypothesis that the eukaryote cell is a symbiont that emerged from the fusion of different bacteria species suggests that PCD may have evolved from a resolution of conflict between heterogeneous genomes within a cell, which subsequently led to an enforced cooperation.[1] Hence, it is likely that PCD may have evolved together with the endosymbiotic incorporation of aerobic bacteria (the precursors of mitochondria) into ancestral unicellular eukaryotes.[3] In fact, it is gaining recognition the hypothesis that eukaryotic cells are descendants of ancient anaerobic organisms that survived, in a world that had become rich in

oxygen, by engulfing aerobic bacteria, keeping them in symbiosis for the sake of their ability to consume atmospheric oxygen and produce energy as ATP. So, mitochondria would originate from Krebs-cycle-containing eubacteria (promitochondria) invading a fermentative anaerobe.[3,12] Then, during eukaryotic evolution, most of the genetic information contained in the promitochondrial genome was incorporated into the nuclear genome.[13] This symbiotic process is believed to have helped the ancestral eukaryotic cells to utilize the oxidative metabolism of the symbiotic bacteria to gain energy as the eukaryotes adapted to the change from an anaerobic environment to an increasing oxygen-rich surrounding.[14] As a result, over the next few hundred million years, the symbiotic bacteria lost most of their essential genes except some of those required for oxidative respiration and ATP synthesis, giving some of the genes to the host nucleus whose proteins would still target the symbiont for activity. This process is hypothesized to have directed the ancestral bacterial symbionts to become obligate endosymbionts and a de facto eukaryotic organelle known as mitochondrion.[15-17] Additional evolutionary divergence led to different eukaryotic organisms having different numbers of mitochondria.

It has been recently reported that some present day prokaryotes liberate redox proteins that induce apoptosis in eukaryotic cells through stabilization of p53 protein.[14] Thus, it has been proposed that the parents of the present day prokaryotes released redox proteins to kill the ancestors of the eukaryotes. Subsequently, during the evolution of mitochondria as obligate endosymbionts, mitochondrial ancestors offered some useful functions to their hosts, which in turn provide the formers with physical protection and essential nutrients. In this way, the mitochondria adapted their original "killing" functions to programme their host cell death. As it is known for mitochondria in multicellular organisms, kinetoplasts might not only be powerhouses for generation of cellular energy but also organelles, which play a major role in inducing PCD of the parasite through the release of redox proteins.[14]

Although apoptosis or developmentally regulated programmed cell death is probably only present in multicellular organisms,[18,19] it seems that some forms of PCD may have evolved at the same time as did endosymbiosis. Hence, it is of crucial importance to know the reasons whereby the basic mechanisms of PCD were established in protozoa, particularly in a number of single-celled eukaryotes, such as *Trypanosoma cruzi*,[9] *Trypanosoma brucei rhodesiense*[10] and *Leishmania amazonensis*,[20] *L. donovani*[21,22] and *L. major*.[8] The understanding of those reasons would also help to explain PCD phenomena described in fungi and plants.[3]

The Hypothesis of PCD in *Trypanosomatids* as an Adaptative Mechanism and a Defence Strategy

As we have discussed in the previous section, PCD in *Trypanosomatids* may be a remnant process of cellular evolution without a specific function. However, it is gaining recognition the hypothesis that considers PCD in *Trypanosomatids* as a pathway to maximise their biological fitness. In this context, PCD in *Trypanosomatids* might serve as a molecular mechanism of adaptation and defence against the host.

One of the proposed functions of the PCD pathway in unicellular protozoa is to control cell population, as is the case of multicellular organisms.[11,23-25] On the other hand, it is well-known that programmed cell death takes place during the digenic life cycle of *Trypanosomatids*. In fact, it has been hypothesized that PCD might act during the digenic cycle of *Leishmania*. So, some individuals may be programmed to infect and suffer "terminal differentiation", while others are ancillary to the former and still others are present to maintain a certain population density for the infection.[20,26] It is interesting to point out that procyclic trypanosomes displaying morphological features of apoptosis have been found in the midgut of flies.[27] It is known that for their survival within the sand fly gut (or within the hosts), *Trypanosomatids* must have a thorough control to restrict individuals; otherwise death of the insect vector (or the macrophages) may occur prematurely. In this scenario, PCD could be triggered to accomplish this required control because of the competition of the parasites for the limited resources in the sand fly gut (or within the hosts). For example, in *Trypanosoma brucei*, PCD could be an important event in the tsetse fly, where careful

parasite population size control operates.[28] Since parasites and flies compete for the aminoacid proline, the maintenance of such an equilibrium, in which parasite multiplication is compensated by parasite death, can be mutually helpful.[29]

Recently, it has been proposed that mammalian host cells can trigger apoptosis rather than necrosis of some *Trypanosomatids*.[30] Thus, in the three severe human disease agents, *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania amazonensis*,[31] PCD could be regulated by signals from their multicellular hosts, such as temperature and lectins, as well as by components of the host immune system including complement proteins and cytokines.[32] Massive death of kinetoplastid parasites has also been postulated as an evolutionary process in which the non-adapted parasites die by apoptosis, probably, for the maintenance of an immunological silent state of the host during the infection process.[30] On the other hand, it has been reported that phagocytosis of apoptotic cells favours the intracellular growth of *Trypanosoma cruzi*.[33] So, PCD may be a suitable strategy of defence, which permits the parasitic infection to "go-ahead". This mechanism of cell death may also result beneficial for other *kinetoplastid* parasites allowing not only their adaptation to the external condition and their development but also protecting them against possible damages from the hosts.

PCD might also serve as a cell sorting mechanism to select specific parasitic forms (for example the metacyclic form from the procyclic one). This mechanism of cell sorting would ensure the selection of the infectious parasitic form, since uninfectious forms no longer contribute to the perpetuation of parasite life cycle and however may compete with the differentiated *Trypanosomatids* for available nutrients.

Altogether the above-mentioned data suggest that in *Trypanosomatids* the regulation of PCD might allow a careful coupling of appropriate cell differentiation and cell survival. In this context, PCD induction through complex parasite-mammalian host interactions would be in agreement with the central role that has been attributed to apoptosis in multicellular organisms, namely, the thorough control of cell differentiation, the matching of cell numbers to their environment and the defence against genetic damage and infections, leading to the elimination of abnormal and infected cells.

Future Prospects

When dealing with programmed cell death in protozoa we need to keep two things in mind for the future. First, we need to be aware of the differences, as well as the similarities with multicellular organisms. Hence, convergence and divergence of primitive characteristics of PCD in protozoa and mammalians cells might be useful fingerprints to establish evolutive relationships. In fact, studies in *Trypanosomatids* suggest the existence of effector and regulator molecules as caspase-like, poly(ADP-ribose) polymerase-like (PARP-like), GSH-like (trypanothione) that exhibit similar activities to the observed in mammalian PCD phenomena (Fig. 2). Moreover, *Trypanosomatids* have a phylogenetically mitochondrial-originated protein called metacaspase,[34,35] which might have evolved to the today-known caspases of multicellular organisms. Second, from a pharmacological point of view, we have to take into account that depending on whether infectious single-celled organisms share some or all of the effectors and regulators common to multicellular apoptosis or have evolved their own divergent pathways, we will have to use different therapeutic approaches to induce specific killing by PCD.[11] For example, it has been found that the lipophilic drug o-naphthoquinone β-lapachone inhibits a PARP-like enzyme isolated from the *Trypanosomatid Crithidia fasciculate*.[36] Because PARP enzymes are involved in recognition of DNA damage and induction of cell death, inhibition of PARP activity might be used in the future to increase the antiparasitic effects of DNA-binding drugs such as pentamidine.

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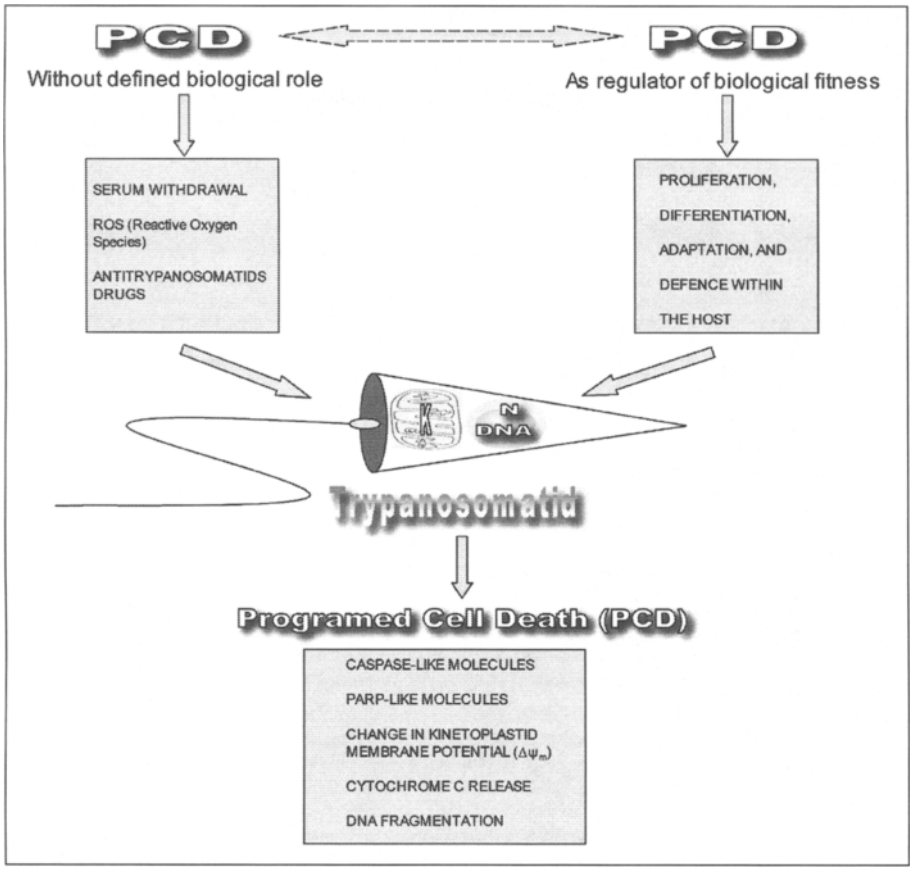

Figure 2. Two hypotheses for the existence of PCD in *Trypanosomatids*. PCD in *Trypanosomatids* may be a remnant process of cell evolution without a defined function, which is activated in response to diverse stimuli (serum deprivation, ROS, cytotoxic drugs, etc.). Alternatively, PCD may serve as a way to maximise the biological fitness of the parasites by controlling proliferation, differentiation, adaptation and defence within the host. In this latter case, PCD will be also triggered in response to stress conditions (discontinuous arrow). K = kinetoplastid, N = nucleus.

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