News and Commentary

Looking for death at the core of life in the light of evolution

JC Ameisen*,1

1   EMI-U 9922 INSERM/Université Paris 7, Faculté de Médecine Xavier Bichat, Hôpital Bichat-Claude Bernard, AP-HP, IFR 02, 16 rue Henri Huchard, 75870 Paris cedex 18, France
*   Corresponding author: JC Ameisen EMI-U 9922 INSERM/Université Paris 7, Faculté de Médecine Xavier Bichat, Hôpital Bichat-Claude Bernard, AP-HP, IFR 02, 16 rue Henri Huchard, 75870 Paris cedex 18, France.
    Tel: + 33 1 44 85 61 50; Fax: + 33 1 44 85 61 49;
    E-mail: ameisen@wanadoo.fr

'Nothing makes sense in biology, but in the light of evolution' wrote Theodosius Dobzhansky, one of the founders of the New Synthesis that led to the unification of evolutionary theory and genetics in the midst of the 20th century. During the last 3 years, the Nobel Committee has provided strong support to this view by highlighting the importance of seminal discoveries identifying in various, early diverging model organisms, ancestral evolutionary conserved molecular mechanisms of crucial importance for our own survival and fitness. In 2000, the Nobel prize for physiology and medicine recognized the contribution of the Aplysia model to the deciphering of the molecular processes allowing the emergence and maintenance of long-term memory. In 2001, it highlighted the contribution of the Yeast model to the identification of the basic, conserved, machinery driving the cell cycle. And last but not least, in 2002, the Nobel prize awarded to Sydney Brenner, John Sulston and Bob Horvitz singled out the extraordinary scientific adventure that led in the *Caenorhabditis elegans* model to the elucidation of cell fate during embryonic development, and in particular to the first identification of the existence of a genetic control of developmental cell death.[1–5] The latter provided the first proof of concept that the term and idea of programmed cell death (PCD)[6] had indeed a genetic correlate, in the form of a basic genetic module operating in most – if not all – cells during the development of at least one living organism. It also provided support to the hope – raised by the previous discovery of the often conserved phenotype of PCD, apoptosis[7] – that a search for a similar form of genetic control might one day prove fruitful in other animal species more closely related to us. Because genetically regulated PCD has today become textbook knowledge, it is difficult to recapture and convey the feeling of substance, coherence and simplicity that these findings conveyed at the beginning of the 1980s to the fascinating but somehow fleeting notions of developmentally regulated cell death, PCD, cell suicide, self-destruction or apoptosis.[6–10] 'What is true for the bacteria is true for the elephant' had written Jacques Monod. But could it be that what was true for PCD in *C. elegans* might also be true for PCD in us? This was far from being a predominant idea.

Walking Along the Evolutionary Tree Upwards from the *C. elegans* Divergence

Between 1992 and 1994, Bob Horvitz and his students JunYing Yuan and Michael Hengartner reported the sequence of the three major components – *ced3*, *ced4* and *ced9* – of the genetic module involved in the control of PCD in *C. elegans*. This led to the surprising finding that the core machinery of PCD in *C. elegans* had evolutionary conserved counterparts in mammals,[11–14] including humans, in drosophila,[15] and in other animal species.[16] Such a striking conservation across a phylogenetic divergence range of around 700 million years reinforced the view that PCD may have been essential for animal survival. However, consistent with the notion that evolution of crucial molecular pathways usually involves both genetic conservation and diversification, at least 14 homologues of the Ced3 executioner – the caspases – were progressively identified in mammals, together with at least 20 homologues of the Ced9 protector – the antagonistic Bcl2/Bax family,[11,12,14] whose first members had already been discovered in humans[13,17] prior to the *C. elegans* Ced9 sequencing. For the last 10 years, *C. elegans* has provided a blueprint for the identification and ordering of the complex and diverse PCD pathways operating in our cells, while at the same time research performed on our cells, and then on drosophila cells, revealed the involvement in mammals and insects of several molecular actors, signaling pathways and intracellular organelles whose implication had not been predicted by the *C. elegans* studies. These included the tumor necrosis factor (TNF) superfamily of death ligands and receptors,[18] the inhibitors of apoptosis (IAPs),[19] the IAP inhibitors, such as Reaper in drosophila[15] and Smac/Diablo in mammals,[19] the mitochondria and their intermembrane proteins, such as Cytochrome *c* in mammals,[20,21] and more recently, in drosophila cells, small noncoding micro RNAs (miRNA) sharing similarities with the interfering RNAs (RNAi).[22] Moreover, during the last 5 years, mammalian research and *C. elegans* research have begun to cross-fertilize in more intricate ways. This turn began in 1998 with the identification by Bob Horvitz's group of the Egl1 homologue of the mammalian proapoptotic BH3-only members of the Bcl2/Bax family,[23] and of its important role in *C. elegans* PCD induction. More recently, a new molecular effector of PCD, the mitochondrial protein Endonuclease G, has been simultaneously identified in *C. elegans*[24] and mammalian cells,[25] and a *C. elegans* homologue of the mammalian effector of PCD, the mitochondrial apoptosis-inducing factor (AIF) has been found to participate to *C. elegans* PCD,[26] suggesting that mitochondria, which play a crucial role in mammalian PCD, might also participate, in a still poorly understood way, in the regulation of *C. elegans* PCD.

The pioneering work of Bob Horvitz and colleagues did not only lead to the first identification of a genetic control of developmental PCD, but also to the discovery of a genetic control of the mechanisms allowing neighboring cells to ingest the dying cells.[5,14,27] Although this process of ingestion was believed, during almost 20 years, not to influence the process of self-destruction itself, recent independent findings by Bob Horvitz's and Michael Hengartner's groups have revealed that the molecular mechanisms controlling engulfment by neighboring cells may also enforce 'from without' the destruction process operating 'from within'.[28,29] This has broadened the persistent and elusive question of the 'point of no-return' in PCD, by implying that irreversibility does not only depend on the nature and intensity of the initial cell response to pro-death signaling, but also on the subsequent response of neighbor cells to the behavior of the cell that has entered the pathway towards self-destruction. A cell may thus oscillate for some time between life and death, its ultimate fate resulting from its interactions with nearby cells.[30,31] Hence, the important concept of a 'social control' of cell survival and cell death[32] may not be restricted, as initially proposed, to the availability of extracellular survival signals, but should be extended to include the behavior of surrounding, competitor cells, thereby introducing a component of evolutionary ancient 'predator/prey' context in the long predominant 'altruistic' view of the regulation of PCD.

The scientific adventure of the molecular deciphering of developmental PCD has become progressively associated with another adventure, resulting from the view that PCD dysregulation may also play a major role in the pathogenesis of several major human diseases.[17,33–37] A series of experimental approaches in various animal models of acute and chronic human diseases has recently confirmed the promises of artificial modulation of PCD as a potential therapeutic strategy with broad implications.[37–39] Thus, from essential actors of development, the molecular mechanisms of PCD have moved towards being recognized as crucial actors of both health and disease over the entire lifespan of several animal species, including humans. And the Nobel Committee appropriately underlined the potential contribution of the understanding of *C. elegans* PCD to both physiology and medicine.

But there has been more to the rich relationships between PCD and evolution than this walk along the last 700 million years, upwards from the *C. elegans* phylogenetic divergence. Since the beginning of the 1990s, various forms of PCD have been identified in a wide range of phylogenetically diverging branches of the evolutionary tree, which have radiated long before the emergence of the most recent common ancestor we share with *C. elegans*.[16,40] PCD has been identified in the plant kingdom, that emerged around 1 billion years ago, near the period of the emergence of the first animals. In many plants, PCD plays a major role in development, sexual reproduction, and resistance to infection.[41,42] Moreover, diverse forms of regulated cell death processes have also been identified in several unicellular eukaryote species, that emerged between 1 and 2 billion years ago, and in several bacterial species, whose ancestors are believed to have represented one of the earliest forms of life on our planet, and emerged around 4 billion years ago.

The Less Traveled Road: Walking Along the Evolutionary Tree Downwards from the *C. elegans* Divergence

Unicellular eukaryotes in which regulated processes of cell death have been identified range from kinetoplastids[43] and ciliates[44] to slime molds,[45] dinoflagellates[46] and yeasts.[47] PCD induction involves intercellular signaling in response to environmental changes, and may participate in various important functions[16,40] including enforcement of cell differentiation, selection of the fittest cells in a given environment,[43] or the building of transient multicellular bodies made up of dead cell corpses favoring the persistence of long-lived, resistant, resting spores.[45] The involvement of ancestral, evolutionary conserved molecular mechanisms of PCD has been suggested by the identification of a potential role for cysteine proteases in kinetoplastids[48] and dinoflagellates,[46] for an AIF homologue in slime molds,[49] and for a metacaspase in yeast.[50] But it is the bacteria that may provide today the most fascinating model for addressing the question of the possible emergence and selection of PCD in a broad evolutionary perspective.[16,40,51–54] The genetic and molecular mechanisms participating in the control of cell death in bacteria are very diverse, and blur most of the usual conceptual frontiers between death 'from within' and 'from without', 'altruism' and 'selfishness', cooperation and competition, outside environment and intercellular signaling, infection and symbiosis, and unicellular and multicellular behaviors. The toxin/antidote modules harbored by numerous infectious mobile genetic elements, such as plasmids and bacteriophages, enforce both the extent and irreversibility of their colonization of bacterial preys by enforcing the death of uninfected cells. Some of these genetic modules encode paracrine killers which induce death 'from without' by releasing a toxin that kills uninfected or 'cured' neighbor cells, while the infected cells are protected by the antidote that they retain. Other modules – the 'addiction modules' – encode a toxin and an antidote that are both retained by the infected cell. The antidote is constantly cleaved by a bacterial protease, coupling the survival of the infected cell to the continuous synthesis of the antidote, and hence to the continuous expression of the toxin/antidote genetic module. If a cell happens to inactivate the plasmid or to escape its segregation during cell division, the 'cured' cell stops producing both the toxin and the antidote. The remaining antidote is cleaved, freeing the remaining long-lived toxin which then executes the cell 'from within'. Thus, a vast array of toxin/antidote modules involved in evolutionary arms races between infectious predators and their bacterial preys may have provided the reservoir from which emerged the molecular tools (the executioners and protectors) allowing the emergence of regulated, 'altruistic' PCD. In particular, the 'addiction modules' that induce death 'from within' suggest a potential role for enforced symbiosis – an extreme form of infection resulting in irreversible association between heterogeneous genetic entities – in the emergence of regulated self-destruction. Accordingly, I have previously proposed a model in which successive steps of symbiotic events – between bacteria and the addiction modules of plasmid origin, and between eukaryote cells and their mitochondria of bacterial

origin – might have accounted for the continuous selection and progressive radiation and evolution of regulated PCD throughout life kingdoms.[16,40,51] Such an evolutionary scenario extends the concept of 'social control' of cell survival and cell death by considering each cell itself as an evolving society in which competition and cooperation between heterogeneous genomes, compartments and organelles will influence the cell fate in terms of life and death. The blurring of the frontiers between killing and self-destruction, cooperation and competition, predators and preys, can also be observed in bacteria in situations that do not involve infection. Most bacterial species organize into multicellular groups, who rely on intercellular signals, such as density-dependent quorum factors, which control multiple gene expression.[55] Some of these bacterial species, when confronted with adverse environmental conditions, undergo differentiation into long-lived spores. Such differentiation is coupled with the induction of premature death in part of the colony, with dead cells either dismantling, or remaining as aggregated corpses which form complex multicellular structures protecting the spores. Very recent findings suggest that these developmental programs involve a succession of several different steps of symmetry breaking in the colony. For example, in *Bacillus subtilis*, a decrease in nutrient availability will induce, in some cells – the future survivor cells – the expression of a differentiation factor (the sporulation factor, SpoA), that causes the production of at least three molecules.[56] The first one is a released, extracellular factor that increases energy production in both the future survivors and their neighbors. The second one is a released pseudotoxin, that requires cooperation with the first released factor to induce cell death. The third one is retained by the future survivors and protects them against the effect of the pseudotoxin. The cells that have not expressed SpoA will die and provide newly available nutrients to the cells that have expressed SpoA.[56] Thus, SpoA can be viewed as both an armor and a sword, acting both as an executioner 'from without' for the cells that do not express it, and as a protector 'from within' for the cells that have synthesized it. Once the population is entirely composed of the survivors that have initially responded to the adverse environment by expressing SpoA, and if the environmental conditions continue to be detrimental, another step of radical form of reciprocal differentiation will further break symmetry in the survivor cells, leading to the formation of spores.[57] The SpoA-expressing cells initiate an incomplete process of asymmetric division. A criss-cross exchange of transcription factors through the intercellular membrane that links the big mother cell and the small daughter cell will allow the differentiation of the daughter cell into a spore, while leading to the death of the mother cell.[57,58] Should these successive processes of symmetry breaking – coupling survival of a part of the colony with the death of another part – be viewed as examples of 'murders' by which some cells survive by killing their neighbors, or rather as examples of cooperative forms of 'altruistic' self-destruction regulated by intercellular signaling and allowing the survival of a part of the colony at the expense of the sacrifice of another part?

Genuine forms of self-destruction seem indeed to exist in bacteria, as suggested by the identification of 'addiction modules' that reside in the bacterial chromosomes in the absence of any other plasmid components.[59] In such cases, the repression of the expression of the 'addiction module' – leading to the cleavage of the remaining antidote, the freeing of the remaining toxin and the induction of death 'from within' – is triggered by intracellular signaling in response to adverse environmental conditions such as nutrient shortage.[52,54,59] Thus, in the face of future starving, that will cause inescapable death 'from without', the induction of premature death 'from within' in a part of the colony will favor the survival of another part, that will not only be surrounded by a greater rate of nutrient per cell, but will also benefit from feeding on the self-destructing neighbor cells.

Interestingly, such adverse environmental conditions might also trigger a process of chromosomal DNA rearrangement and mutations operating 'from within' through the induction of a SOS-stress response.[60] Hence, it is all the more striking that despite the existence of such potent mechanisms of genetic diversification, self-destruction escape mutants do not rapidly emerge and overtake the whole colony. Death-escaping 'cheater' mutants, biasing differentiation towards spores, have indeed been identified in some myxobacteria species,[61] but their persistence depends on the 'presence of ready-to-die neighbors, implying the existence of constraints limiting the spread of such escape mutants. The emergence and evolution of regulated cell death processes, including self-destruction might only represent a particular and extreme example of the recently studied emergence of several other cooperation processes in several bacterial species,[62,63] suggesting that cooperation may be under strong selection pressures and may have represented a somehow stable evolutionary strategy despite its high individual costs.[62,63]

The 'Original Sin' Hypothesis for the Emergence and Evolution of Programmed Cell Death

I have previously proposed that an additional factor which might have been critical for the persistent selection of self-destruction mechanisms is a potential ancestral pleiotropy of the molecular tools allowing the execution of self-destruction – a multifunctional involvement in both pro-life and pro-death activities.[16,40,51] In such an evolutionary context, the advantages that such tools might have provided at the level of the colony as a whole, in terms of improved survival of part of the cells at the expense of the premature death of another part, would have been strongly reinforced by the selective advantages that such tools might have provided at the level of each cell from the colony, in terms of improved individual survival as long as self-destruction is not induced. Such a multifunctionality might not only provide an explanation for the continuous selection of molecular tools of PCD during evolution, but also for its very evolutionary origin, in the framework of a model that I have termed 'the original sin' hypothesis.[16,40,51] Briefly, the hypothesis postulates that most molecular tools – architects – required for vital functions such as metabolism, differentiation or cell cycle, will induce stochastic self-destruction in any cell (including in the first cells that might have emerged on our planet) if their activity is not regulated by other molecules that act as partial antago-

nists. In such a view, the potential executioners of PCD are already present, from the onset, among most architect molecules involved in various vital functions, and the potential protectors are already present among most architect partial antagonists which are themselves involved in other vital functions. Accordingly, the capacity to self-destruct would be an 'original sin' of the earliest cells, an ancestral cost paid for their very capacity to self-organize, produce and use energy, persist and reproduce. As previously discussed elsewhere, this view predicts that as long as the executionary tools that become progressively selected (in host/pathogen wars, for example) for their killing properties retain at least some of their architect, vital functions, such persistent pleiotropy will strongly favor further positive selection. This view also links the evolution of mechanisms which control death 'from within' to those which control genetic diversification 'from within'. Finally, this view makes several testable predictions. In their most extreme formulation, these predictions are that in any species, there should be more than a single PCD molecular pathway, and, more importantly, that there should be no effector involved in the execution of cell death that does not also participate in some vital function.[16,40,51] But can such a view be reconciled with the implications of the *C. elegans* paradigm of PCD?

C. elegans: the Paradigm and the Paradox

Two of the most important, wide ranging conceptual implications of the initial studies of PCD in *C. elegans* were derived from the exploration of *C. elegans* mutants with Ced3 or Ced4 loss of function, and with both Ced3 or Ced4 and Ced9 loss of function. These studies implied (1) that there is only one molecular pathway of PCD; and (2) that the molecular effectors of PCD (Ced3 and Ced4), as well as the protector (Ced9) had no other possible function than the execution and repression of cell destruction.[5,27,64] This led to the concept of the existence of specific, *bona fide* death genes (executioners and protector) that emerged and became selected during evolution for their sole capacity to induce or repress self-destruction. Because of their obvious diversification in drosophila and mammalian cells, these *C. elegans* death genes were long considered as being close to their genetic ancestors that may have emerged in the first metazoan confronted with the problem of a multicellular body becoming the unit of selection, instead of each individual cell.

In a somewhat paradoxical manner, however, cell death seemed to play no significant role in the development and adult life of *C. elegans*,[5,27,64] raising the very question of why genes whose sole apparent role was to trigger cell death may have been conserved if they did not make any contribution to the fitness of the organism. During a long period, this puzzling problem was rarely raised, probably because, as happens with most seminal advances, the explicative power of the paradigm and its illuminating implications by far outweighed this cryptic paradox.

However, while simple ancestral models may reveal hidden simplicity in more complex models, various levels of unexpected complexity are often discovered in seemingly simple ancestral models, which, as ourselves, have evolved and

been subjected to selection since their initial divergence. Accordingly, a recent series of findings has suggested that pleiotropic functions of at least some of the gene products involved in the induction of cell death might indeed be a common feature in phylogenetically diverging branches of the evolutionary tree ranging from mammals to bacteria and including *C. elegans* itself.

Selective 'Death Programs' or Pleiotropic 'Life Programs'?

In mammals, including humans, several findings have suggested (1) that several PCD pathways may coexist in parallel and operate simultaneously or alternately; and (2) that a wide range of gene products involved in the induction or execution of PCD also have important vital functions, such as energy production, metabolism, differentiation or cell cycle. Examples include various upstream inducers of PCD such as the TNF family of death ligands and receptors, the initiator caspase 8, the mitochondrial intermembrane protein cytochrome *c* and the p53 tumor suppressor, but also, more surprisingly, downstream executioners of self-destruction such as caspase 3[65–67] and AIF.[68] Most recently, Bad, a proapoptotic BH3-only member of the Bcl2/Bax family was shown to play an unexpected critical role in the regulation of metabolism at the whole body level.[69] Lack of Bad expression *in vivo*, in mice, resulted in a form of diabetes. Thus, Bad not only allows a coupling of the sensing of extracellular environmental conditions to the induction of cell death, but also actively participates in the shaping of this extracellular environment.

Appropriate environment (appropriate blood glucose levels) inactivates the killing potential of Bad, through Akt-mediated Bad phosphorylation, thereby enabling Bad to regulate this environment by modifying the uptake and usage of glucose by cells in the body. Conversely, poor environment (low blood glucose levels), to which phosphorylated Bad itself may have contributed, will trigger its killing activity, by inducing its dephosphorylation.[69] But intercellular communication can compensate for such poor environmental conditions. Other molecules (survival growth factors) expressed or released by neighboring cells can induce the expression of the Bcl2/BclXL protectors that will neutralize dephosphorylated Bad, allowing cells to survive in a state of lower metabolic activity despite glucose shortage.[70]

An important question that remains to be addressed is whether the two essential[71] proapoptotic Bcl2 family actors of mitochondria-mediated death – Bax and Bak – also have other still unsuspected pro-life activity, as Bad, or whether they might represent true examples of a selection during evolution of specific, *bona fide* death genes.

Pleiotropic functions of molecular executioners of PCD may not be a particular evolutionary feature related to mammalian complexity. Recent findings in bacteria also suggest an unsuspected degree of bifunctionality for the molecular actors – toxins – previously considered as having no other possible function than inducing death.[72–74] Indeed, the repression of the expression of at least two different chromosomal toxin/ antidote modules – triggered in part of the colony in response to nutrient deprival, and resulting in antidote degradation and

free toxin availability – can, depending on the circumstances, have two opposite outcomes in terms of life and death. It can either trigger bacterial death 'from within',[59,74] or paradoxically favor the survival of the free toxin-containing cell,[72,73] for example, through selective toxin-mediated inhibition of protein synthesis at the ribosomal level, and thus inhibition of energy consumption. If nutrients subsequently become available, the re-expression of the toxin/antidote module will allow the *de novo* synthesis of the antidote, leading to the neutralization of the toxin, and allowing the bacteria cell to resume normal metabolism and activity.[72,73] Thus, in adverse environmental conditions, bacteria-encoded toxins acting 'from within' can favor the survival of the colony in two opposite ways: either by precipitating the 'altruistic' death of the cells that have lost their antidote, providing increased nutrients for the cells that have continued to synthesize the antidote, or alternately by enhancing the 'selfish' survival capacity of the very cells that have lost their antidote.

In such a broad evolutionary context, spanning from bacteria to mammals, I think that the most interesting question may not be whether such a pleiotropy might have resulted from the selection of pro-life activity among initial pro-death effectors or from a reverse process – to identify the exact pattern of exaptation, in the sense proposed by Stephen Jay Gould[75] – but rather to explore to what extent pleiotropy may be a general feature in most – if not all – organisms endowed with the capacity to undergo PCD. Concerning *C. elegans* itself, recent findings suggest that things may also be more complex than initially believed. First, there may be more avenues towards death than the single Egl1/Ced9/Ced4/Ced3 pathway. For example, the *C. elegans* CEP-1 homologue of the mammalian p53 tumor suppressor can induce cell death in a Ced3/Ced4-independent manner.[76] More recently, ICD-1, a new inhibitor of *C. elegans* PCD has been identified, which is not redundant with Ced9 and represses a Ced4-dependent death pathway that does not require Ced3, suggesting the involvement of other *C. elegans* caspases in the execution of PCD.[77] Concerning pleiotropy, the *C. elegans* p53 homologue has been found to play a crucial role in meiosis, independent of its pro-death function, and to be required for whole animal survival in stressful environmental conditions.[76] Also, Ced3 and Ced4 have been found to play a role in the whole body fitness of *C. elegans* by allowing resistance to infectious pathogens.[78] Whether such an involvement of Ced3 and Ced4 in anti-infectious defenses directly results from the induction of post-developmental cell death in a way similar to the hypersensitivity response in plants, or whether it reflects a broader, more complex involvement of Ced3 and Ced4 in the immune response awaits to be assessed. Meanwhile, the *C. elegans* model, by revealing a hidden level of complexity, will remain as useful as before in the still ongoing quest towards making sense of PCD.

Premature Death: from Programmed Cell Death to Aging

The choice and use of the *C. elegans* model has resulted in seminal advances not only in the understanding of the genetic control of PCD but also, more recently, in the understanding of the genetic control of whole organism aging. Minor genetic mutations in genes involved in *C. elegans* development can result in significant extension of youth, fecundity and longevity. The main aging pathway identified in *C. elegans* has been found to operate in widely diverging species, including drosophila and mouse.[79–81] And pleiotropy, as initially proposed 50 years ago by George Williams in his evolutionary theory of aging,[82] is obvious for several of the major gene products recently identified in this conserved aging pathway, which participate in metabolism, and in particular in insulin and IGF signaling. Because *C. elegans* mutants in the *ced3*, *ced4* and *ced9* genes involved in the PCD pathway were not found to have particular whole organism longevity phenotype, and because mutants in the *age1*, *daf2*, *daf16* genes involved in the aging pathway showed no particular PCD phenotype, it has been suggested that there is no relationship between genes involved in the regulation of cell death and genes involved in the regulation of whole organism aging. However, the recent findings indicating that Ced3 and Ced4, and the CEP-1 p53 homologue influence *C. elegans* longevity in adverse environmental conditions[76,78] suggest that such a crosstalk may not be impossible. In mice, p66shc and maybe p53, which are both involved in PCD, may also play a role in whole organism aging and longevity.[83–85] This could either suggest a direct relationship between PCD and aging, or rather point towards additional levels of pleiotropic functions for the gene products involved in the control of PCD or aging.

As PCD, aging – at both levels of the whole body and of the cell – has long been considered as a specific feature of multicellular organisms – an evolutionary price paid for the emergence of complexity. However, obligate aging has now been shown to exist in some unicellular organisms, including yeast[79,86] and at least one bacterial species,[87] in which asymmetric cell division has allowed a discrimination between mother cells and daughter cells. For example, in *Saccharomyces cerevisiae*, a mother cell will give birth to around 20 daughter cells, and will then become sterile and die.[79,86] Thus, the apparent eternal youth and fecundity of a yeast colony in fact results from endless successive generations of short-lived cells. Aging in yeast, as in several metazoan species, seems related to glucose metabolism.[79] It also depends on mitochondrial oxidative activity, and is repressed by the Silent information regulator (Sir2) deacetylase,[88] that also extends whole organism life span in *C. elegans*.[89] Interestingly, aging in yeast appears to result from an asymmetric distribution of some molecular components (such as damaged proteins and circular ribosomal DNA minicircles) whose accumulation in the mother cell precipitates aging, while their initial lack at birth in daughter cells endows them with youth and fecundity. As with PCD, it is difficult to decide whether mother cells undergo an 'altruistic' premature death favoring their daughters survival, or whether the daughter cells enforce deleterious molecule retention in their mother cell. Whatever the answer, the important implication is that the premature dismissal of the mother cells is one of the basic mechanisms that may allow the generation of the paradoxical molecular phenomenon that we call youth, which endows cells, which are ever older in terms of their genealogical age of several hundreds of million or billion years, to begin their existence with the same life expectancy and fecundity that their youngest, long-gone

ancestors. It may be that this process of symmetry breaking operates in most – if not all – unicellular organisms, and that the often apparently symmetric process of cell division usually masks subtle intercellular segregation mechanisms that allow the propagation of life. Are similar processes operating in our stem cells, with asymmetric division leading to the production of an aging mother cell that will undergo differentiation, and of a young daughter cell that will become a new stem cell? Are in contrast some of our other cell populations, such as fibroblasts, aging as a whole population, ending up reaching the Hayflick limit[90] for the very reason that they might lack this symmetry breaking mechanism?[91]

It is tempting to speculate that further investigations of the intricate mechanisms which link cell metabolism, differentiation, cycle and aging will also reveal unsuspected relationships with the molecular mechanisms involved in the regulation of PCD.[91]

Natural Selection 'from within'?

Random variations at each generation resulting from genetic diversification and death 'from without' resulting from the confrontation with the environment represent the main features of the Darwinian theory of natural selection and of species evolution. In such a conceptual framework, PCD and aging may represent two particular instances in which the selection of a regulated enforcement of premature death 'from within' may have provided enhanced fitness and survival in the face of outside environmental conditions inhospitable to the propagation of life and of inner damages that metabolism causes to the components of cells and bodies. In parallel, the emergence and selection of mechanisms allowing the regulated induction of genetic diversification 'from within' has endowed cells – and organisms – with a capacity to change identities in the face of ever changing, deleterious environments. An initial level of pleiotropic functions of the molecular actors controlling death, aging and genetic diversification 'from within' might have favored their initial selection, their constant availability for *de novo* selection, and their progressive propagation in most – if not all – species.

Almost 150 years ago, Charles Darwin concluded on *The Origin of Species* by stating: 'Thus, from the war of nature, from famine and death, the most exalted object which we are capable of conceiving, namely the production of higher animals, directly follows. There is grandeur in this view of life…'[92]

Part of this grandeur might also reverberate in the view that premature death, operating from within, may have been progressively recruited as a mechanism allowing life to persist in the face of the inescapable threat of destruction inflicted by the outside world.

The choice, 30 years ago, of *C. elegans* as a model organism paved the way for crucial conceptual advances in the understanding of death at both levels of the cell and the whole body. This model, and the spirit that animated the pioneers that conquered it will undoubtedly continue to open many more unexpected avenues, and should unveil unsuspected riches in the ways in which a blind and increasingly intricate and complex game with its own end – death – has

allowed life to propagate for such a long period, and, in Charles Darwin's words, to evolve 'from so simple a beginning endless forms most beautiful and most wonderful'.[92]

1. Sulston JE (1976) Philos. Trans. R. Soc. Lond. B Biol. Sci. 275: 287–297
2. Sulston JE and Horvitz HR (1977) Dev. Biol. 56: 110–156
3. Hedgecock EM *et al.* (1983) Science 220: 1277–1279
4. Horvitz HR *et al.* (1983) Cold. Spring Harb. Symp. Quant. Biol. 48 (Part 2): 453–463
5. Ellis HM and Horvitz HR (1986) Cell 44: 817–829
6. Lockshin RA and Zakeri Z (2001) Nat. Rev. Mol. Cell. Biol. 2: 545–550
7. Kerr JFR *et al.* (1972) Br. J. Cancer 26: 239–257
8. Glucksman A (1951) Biol. Rev. Camb. Philos. Soc. 26: 59–86
9. Saunders JWJ (1966) Science 154: 604–612
10. Levi-Montalcini R (1987) Science 237: 1154–1164
11. Hengartner M (2000) Nature 407: 770–776
12. Meier P *et al.* (2000) Nature 407: 796–801
13. Vaux DL and Korsmeyer J (1999) Cell 96: 245–254
14. Horvitz HR (1999) Cancer Res. Suppl. 59: 1701–1706
15. Song Z and Steller H (1999) Trends Cell Biol. 9: 49–52
16. Ameisen JC (2002) Cell Death Differ. 9: 367–393
17. Vaux DL *et al.* (1988) Nature 335: 440–442
18. Krammer P (2000) Nature 407: 789–795
19. Goyal L (2001) Cell 104: 805–808
20. Kroemer G and Reed J (2000) Nat. Med. 6: 513–519
21. Martinou JC and Green D (2001) Nat. Rev. Mol. Cell. Biol. 2: 63–67
22. Brenneke J *et al.* (2003) Cell 113: 25–36
23. Conradt B and Horvitz HR (1998) Cell 93: 519–529
24. Parrish J *et al.* (2001) Nature 412: 90–94
25. Li LY *et al.* (2001) Nature 412: 95–99
26. Wang X. *et al.* (2002) Science 298: 1587–1592
27. Ellis RE *et al.* (1991) Ann. Rev. Cell. Biol. 7: 663–698
28. Hoeppner DJ *et al.* (2001) Nature 412: 202–206
29. Reddien PW *et al.* (2001) Nature 412: 198–202
30. Green DR and Beere HM (2001) Nature 412: 133–135
31. Chemini G (2002) Nature 418: 139–140
32. Raff MC (1992) Nature 356: 397–400
33. Umansky S (1982) J. Theor. Biol. 97: 591–602
34. Ameisen JC and Capron A (1991) Immunol. Today 12: 102–105
35. Thompson CB (1995) Science 267: 1456–1462
36. Evan G and Littlewood T (1998) Science 281: 1317–1322
37. Nicholson DW (2000) Nature 407: 810–816
38. Gurney M *et al.* (2000) Science 288: 283–284
39. Kaspar B *et al.* (2003) Science 301: 839–842
40. Ameisen JC (1996) Science 272: 1278–1279
41. Greenberg JT (1996) Proc. Natl. Acad. Sci. USA 93: 12094–12097
42. Beers EP (1997) Cell Death Differ. 4: 649–661
43. Ameisen JC *et al.* (1995) Cell Death Differ. 2: 285–300
44. Christensen ST *et al.* (1995) Cell Death Differ. 2: 301–308
45. Cornillon S *et al.* (1994) J. Cell. Sci. 107: 2691–2704
46. Vardi A *et al.* (1999) Curr. Biol. 9: 1061–1064
47. Madeo F *et al.* (1999) J. Cell. Biol. 145: 757–767
48. Arnoult D *et al.* (2002) Cell Death Differ. 9: 65–81
49. Arnoult D *et al.* (2001) Mol. Biol. Cell 12: 3016–3300
50. Madeo F *et al.* (2002) Mol. Cell 9: 911–917
51. Ameisen JC (1998) In When Cells Die Lockshin R, Zakeri Z, Tilly J, eds New York: Wiley-Liss, Inc., pp. 3–56

52. Engelberg Kulka H and Glaser G (1999) Annu. Rev. Microbiol. 53: 43–70
53. Kobayashi I (1998) Trends Genet. 14: 368–374
54. Hayes F (2003) Science 301: 1496–1500
55. Kaiser D (1996) Science 272: 1598–1599
56. Gonzalez-Pastor J (2003) Science 301: 510–513
57. Losick R and Stragier P (1992) Nature 355: 601–604
58. Nugroho FA *et al.* (1999) J. Bacteriol. 181: 6230–6237
59. Aizenman E *et al.* (1996) Proc. Natl. Acad. Sci. USA 93: 6059–6063
60. Bjedov I *et al.* (2003) Science 300: 1404–1409
61. Velicer GJ *et al.* (2000) Nature 404: 598–601
62. Rainey P and Rainey K (2003) Nature 425: 72–74
63. Vencer G and Yu Y (2003) Nature 425: 74–78
64. Avery L and Horvitz HR (1987) Cell 75: 641–652
65. Elkon K (1999) J. Exp. Med. 190: 1725–1727
66. Los M *et al.* (2001) Trends Immunol. 22: 31–34
67. Perfettini JL and Kroemer G (2003) Nat. Immunol. 4: 308–310
68. Klein JA *et al.* (2002) Nature 419: 367–374
69. Danial N *et al.* (2003) Nature 424: 952–956
70. Plas DR *et al.* (2002) Nat. Immunology 3: 515–521
71. Wei MC *et al.* (2001) Science 292: 727–730
72. Pedersen K *et al.* (2002) Mol. Microbiol. 45: 501–510
73. Pedersen K *et al.* (2003) Cell 112: 131–140
74. Sat B *et al.* (2003) J. Bacteriol. 185: 1803–1807
75. Gould SJ and Vrba ES (1982) Paleobiology 8: 4–15
76. Derry WB *et al.* (2001) Science 294: 591–595
77. Bloss T *et al.* (2003) Nature 424: 1066–1070
78. Aballay A and Ausubel FM (2001) Proc. Natl. Acad. Sci. USA 98: 2735–2739
79. Guarente L and Kenyon C (2000) Nature 408: 255–262
80. Patterson G (2003) Curr. Biol. 13: R279–R280
81. Holzenberger M *et al.* (2003) Nature 421: 182–187
82. Williams GC (1957) Evolution 11: 398–411
83. Migliaccio E *et al.* (1999) Nature 402: 309–313
84. Tyner SD *et al.* (2002) Nature 415: 45–48
85. Garcia-Cao I *et al.* (2002) EMBO. J. 21: 6225–6235
86. Jazvinsky SM (1996) Science 273: 54–59
87. Ackermann M *et al.* (2003) Science 300: 1920
88. Lee S and Ruvkun G (2002) Nature 418: 287–288
89. Tissenbaum H and Guarente L (2001) Nature 410: 227–230
90. Hayflick L (1965) Exp. Cell. Res. 37: 614–636
91. Ameisen JC (1999) 4th ed. 2003 La Sculpture du vivant. Le suicide cellulaire ou la mort créatrice. Paris: Editions du Seuil
92. Darwin CR (1859) On the Origin of Species. London: John Murray