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ON THE PARADIGM OF ALTRUISTIC SUICIDE IN THE UNICELLULAR WORLD

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Altruistic suicide is best known in the context of programmed cell death (PCD) in multicellular individuals, which is understood as an adaptive process that contributes to the development and functionality of the organism. After the realization that PCD-like processes can also be induced in single-celled lineages, the paradigm of altruistic cell death has been extended to include these active cell death processes in unicellular organisms. Here, we critically evaluate the current conceptual framework and the experimental data used to support the notion of altruistic suicide in unicellular lineages, and propose new perspectives. We argue that importing the paradigm of altruistic cell death from multicellular organisms to explain active death in unicellular lineages has the potential to limit the types of questions we ask, thus biasing our understanding of the nature, origin, and maintenance of this trait. We also emphasize the need to distinguish between the benefits and the adaptive role of a trait. Lastly, we provide an alternative framework that allows for the possibility that active death in single-celled organisms is a maladaptive trait maintained as a byproduct of selection on pro-survival functions, but that could—under conditions in which kin/group selection can act—be co-opted into an altruistic trait.

KEY WORDS: **Adaptive role, co-option, evolution, maladaptive trait, programmed cell death.**

The Problem of Self-Induced Death: An Evolutionary Conundrum

> *"Natural selection will never produce in a being any structure more injurious than beneficial to that being, for natural selection acts solely by and for the good of each. No organ will be formed . . . for the purpose of causing pain or for doing an injury to its possessor." (Darwin)*

Typically, evolutionary theory has been concerned with explaining life. Within this framework, selection is expected to promote the evolution of various molecular, physiological, and behavioral mechanisms (i.e., adaptations) that increase the individual's ability to avoid death. In this view, death is seen as the failure to survive and should not be selected for. Hence, conditions that promote an individual's own death—and the evolution of active mechanisms of self-destruction—are more difficult to envision. However, because of the hierarchical organization of biological systems, selection can act at different levels (Lewontin 1970), and death can occur at multiple levels.

Self-induced death is best known in the context of programmed cell death (PCD) in multicellular individuals (Box 1). PCD is an active, genetically regulated cell death process that contributes to the development and functionality of multicellular organisms by, for instance, removing superfluous, damaged, or mutated cells (e.g., Danial and Korsmeyer 2004). In this context, self-destruction of individual cells can be understood as an

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Box 1: Glossary of terms

Addiction modules	Encode both long-lived toxins and short-lived antitoxins. Following cell division, bacterial daughter cells lacking the plasmid but still containing the long-lived toxin are killed, thus indirectly favoring the plasmid's own propagation (Jensen and Gerdes 1995).
Altruism	A behavior that is costly to the actor (i.e., the focal individual who performs the behavior) and beneficial to the recipient; costs and benefits are defined in terms of the lifetime direct fitness consequences of the behavior (West et al. 2007c)
Apoptosis	A PCD morphotype characterized by specific morphological and biochemical features, including mitochondrial depolarization, reduction of cellular volume, chromatin condensation, nuclear fragmentation, little or no ultrastructural modifications of cytoplasmic organelles, and plasma membrane blebbing (but maintenance of its integrity until the final stages of the process) (Kroemer et al. 2009)
Autophagy	A PCD morphotype characterized by massive cytoplasm vacuolization, accumulation of autophagic vacuoles, and lack of chromatin condensation (Kroemer et al. 2009)
Direct fitness	The component of fitness gained through the consequences of an individual's behavior on the production of offspring (West et al. 2007c)
"Greenbeards"	Genes that code for a conspicuous phenotype that can be used to discriminate between carriers and noncarriers of the gene, and that induce a carrier to behave altruistically toward another carrier, irrespective of the genetic relatedness at other loci between the two partners. This mechanism emphasizes that, in terms of relatedness, what is most important for altruism to evolve is genetic relatedness at the altruism locus (i.e., the probability that interacting partners have the same allele) as opposed to genealogical relationship over the entire genome (Gardner and West 2010)
Group selection	A concept proven difficult to define. The most popular definitions include Price's "between-group selection" (Price 1972) and the contextual-analysis approach (Heisler and Damuth 1987). The former partitions total evolutionary change into within- and between-group components, with between-group selection (i.e., group selection) being responsible for group-level adaptations. The latter concerns the effect of the group phenotype upon an individual's fitness
Inclusive fitness	The effect of one individual's actions on its genetic contribution to future generations through its direct descendants and those of its relatives
Interactor	An entity that interacts, as a cohesive whole, directly with its environment in such a way that its replication is differential; in other words, an entity on which selection acts directly (Hull 1980)
Kin selection	Traditionally, a process by which traits are favored because of their beneficial effects on the fitness of related individuals (West et al. 2007c)
Level of selection	A hierarchical level at which Darwinian principles (heritable variation in fitness) apply
Multi-level selection	The concept that selection can occur at multiple levels of biological organization, for example genes, cells, individuals, groups, populations etc.
Programmed cell death (PCD)	An active and genetically regulated type of cell death, expressed as several distinct morphotypes, including apoptosis, paraptosis, and autophagy
Necrosis	A form of cell death characterized by cytoplasmic and organelle swelling, rupture of the plasma membrane, and the absence of features of apoptosis or autophagy (Kroemer et al. 2009)
Relatedness	Measure of the statistical association among the genes of interacting individuals

extreme form of cooperation that is costly to the lower level (the cell) but that benefits the higher level (the multicellular individual). This altruistic behavior is analogous—in terms of the associated loss of direct fitness (Box 1)—to the reproductive altruism generally displayed by somatic cells in multicellular organisms (e.g., Queller 2000; Michod and Nedelcu 2003).

During the last 15 years, PCD-like processes have also been reported in many single-celled eukaryotic lineages (Table 1), and the available data suggest that at least some elements of these processes are evolutionarily related to PCD in multicellular lineages (e.g., Bidle and Falkowski 2004; Gordeeva et al. 2004; Deponte 2008; Nedelcu 2009a). In addition, several (albeit more divergent) PCD-like processes have also been described in prokaryotes (Table 1). Nevertheless, unlike in multicellular individuals, in unicellular lineages cell death results in the complete loss of the individual. Furthermore, although the conditions required for the evolution of altruistic behaviors are thought to be met for cells in a multicelled individual (e.g., high relatedness, group structure), it is not clear to what extent similar conditions can be invoked in the case of self-induced death in single-celled lineages. Yet, although some believe that there are no reasons to assume that unicellular organisms would have evolved a program for self-destruction,

Table 1. Unicellular lineages in which active death processes have been reported.

Division/lineage	Genera (examples)	Inducing factors
Prokaryotes		
Gram-positive	*Myxococcus, Streptococcus, Staphylococcus* (Engelberg-Kulka et al. 2006; Regev-Yochay et al. 2007; Rice et al. 2007; Nariya and Inouye 2008; Sogaard-Andersen and Yang 2008)	Nutrient stress, ROS
Gram-negative	*Pseudomonas, Escherichia* (Hazan et al. 2004; Kolodkin-Gal and Engelberg-Kulka 2008)	Quorum-sensing signals, phages
Cyanobacteria	*Trichodesmium, Microcystis* (Berman-Frank et al. 2004; Ross et al. 2006)	P and Fe starvation, light stress, ROS
Eukaryotes		
Chromalveolata		
Apicomplexa	*Plasmodium* (Al-Olayan et al. 2002; Meslin et al. 2007)	Pro-apoptotic drugs
Stramenopiles	*Blastocystis* (Nasirudeen et al. 2001)	Antibiotics, pro-apoptotic drugs; surface-reactive antibodies
Ciliates	*Tetrahymena, Blepharisma, Euplotes* (Christensen et al. 1998; Cervia et al. 2009; Takada and Matsuoka 2009)	Staurosporine, light-stress
Diatoms	*Thalassiosira, Ditylum, Skeletonema* (Montsant et al. 2007; Chung et al. 2008)	Iron starvation, nitrogen and phosphorus starvation
Dinoflagellates	*Peridinium, Symbiodinium, Amphidinium, Prorocentrum* (Franklin and Berges 2004; Zhang et al. 2006)	CO_2 depletion, light-deprivation, heat stress
Haptophytes	*Emiliania* (Bidle et al. 2007)	viruses
Excavata		
Diplomonads	*Giardia* (Chose et al. 2003)	Pro-apoptotic drugs, ROS
Kinetoplastids	*Leishmania* (Das et al. 2001; Lee et al. 2002; Bera et al. 2003)	Heat shock, ROS, UV, nutrient depletion
Trichomonads	*Trichomonas, Tritrichomonas* (Chose et al. 2003; Mariante et al. 2006)	Pro-apoptotic drugs, nutrient depletion
Trypanosomatids	*Trypanosoma* (Welburn et al. 1996; Ridgley et al. 1999)	Neuropeptides, human serum, nutrient depletion, ROS
Plantae		
Chlorophytes	*Chlamydomonas, Chlorella, Dunaliella, Micrasterias* (Segovia et al. 2003; Moharikar et al. 2006; Nedelcu 2006; Zuppini et al. 2007; Darehshouri et al. 2008; Affenzeller et al. 2009b; Jimenez et al. 2009; Murik and Kaplan 2009)	Extended darkness, salt-stress, UV, heat, H_2O_2
Unikonts		
Amoebozoa	*Entamoeba, Dictyostelium* (Cornillon et al. 1994; Arnoult et al. 2001; Tatischeff et al. 2001; Mendoza et al. 2003; Ramos et al. 2007; Villalba et al. 2007)	Starvation and signaling molecules, starvation and inhibition of development, prolonged stationary phase, nitric oxide, antibiotics
Fungi	*Candida*, *Saccharomyces* (Madeo et al. 1997; Gourlay et al. 2006; Ramsdale 2008)	UV, acetic acid, oxidative stress, DNA damage, pheromone, amino acid starvation, defects in cellular processes, chronological aging

reports invoking a multitude of benefits and beneficiaries for this type of death are common (e.g., Bidle and Falkowski 2004; Buttner et al. 2006; Duszenko et al. 2006; Deponte 2008; Cervia et al. 2009). The concept of active self-induced death in unicellular lineages is thus considered a matter of "ongoing debate" and a "controversial and obviously confusing" issue (Deponte 2008; Jimenez et al. 2009).

Here, we critically evaluate the most common suggestions for the role of PCD-like processes in unicellular lineages and propose new perspectives. Our goal is to provide a more analytical view that may trigger the re-interpretation of previous findings and direct new experiments. Overall, we argue that unreservedly importing the paradigm of altruistic cell death from multicellular organisms to explain self-destruction in unicellular lineages can

limit the types of questions we ask and bias our understanding of the nature, origin, and maintenance of this trait. This is not to say that the analogies with PCD in multicellular organisms are not justified in some settings. However, we think that the concept of altruistic suicide in unicellular lineages needs further experimental validation in a sound evolutionary context to justify its position as the dominant conceptual framework for all research on cell death in single-celled lineages.

Self Destruction in the Unicellular World

Cells can die in many ways. Several types of cell death have been described under the "PCD" umbrella, including apoptosis, paraptosis, necroptosis, and autophagy (Box 1). On the other hand, only one type of death, necrosis (Box 1), is considered accidental (Kroemer et al. 2009). In this essay, our focus is not on the morphological and biochemical differences between various cell death modes (a detailed discussion of the different death morphotypes is provided in Kroemer et al. 2009). Rather, we focus on why active death processes have evolved and are maintained in unicellular lineages.

For a long time unicellular lineages were considered "immortal," in the sense of only being subjected to accidental and predator-related death. In the last years, however, many different death modes have been described in both prokaryotic and eukaryotic unicellular lineages (e.g., Engelberg-Kulka and Glaser 1999; Lewis 2000; Rice and Bayles 2003, 2008; Bidle and Falkowski 2004; Deponte 2008; Jimenez et al. 2009). Active death processes (usually referred to as PCD or PCD-like death) have been described in response to many types of environmental stress, DNA damage, parasitic agents, or secondary metabolites and toxins (Table 1). In unicellular eukaryotes—depending on species and/or type and intensity of stimulus—several types of PCD-like processes have been reported with similarities to distinct multicellular PCD morphotypes. In some cases, different types of PCD and even various combinations of markers associated with distinct death morphotypes were found in the same species or even the same cell (Affenzeller et al. 2009a; Jimenez et al. 2009). In prokaryotes, PCD-like types of death (with fewer similarities to eukaryotic PCD) have also been observed, including autolysis and "rapid cell death" (e.g., Rice and Bayles 2003).

In a multicellular context, the term "programmed" has been used to imply two different issues. The first implication is that some cells are destined to die; that is, they are physiologically or developmentally programmed to die in a time- and position-dependent manner, for instance to maintain tissue homeostasis or shape organs during development. Second, "programmed" implies that cells die following an internal, genetically encoded death program that ensures an organized death in response to either stress or developmental factors. The difference between the concept of PCD and that of a cell death program was previously noted (Ratel et al. 2001) but is still largely underappreciated. In the former, the cell is one of the constitutive elements of a system, and the death of the cell is involved in the formation or functioning of the higher-level system. In the latter, the cell is the system, whose constitutive elements are involved in its own demise. As in unicellular lineages, the cell level is also the individual level, PCD in single-celled lineages is better thought of as simply death following the activation of a cell death program (Ratel et al. 2001; Franklin et al. 2006). Exceptions could include cases in which death might be "programmed" with respect to a higher level such as during the formation of multicellular fruiting bodies in myxomycetes (e.g., Nariya and Inouye 2008; Sogaard-Andersen and Yang 2008) and slime molds (e.g., Kessin 2001), or during the development of bacterial biofilms (e.g., Bayles 2007). Thus, to avoid confusion associated with the two types of "programs," we use the term active cell death (ACD) to refer to any cell death process that is genetically determined, energy dependent, and proceeds through a series of organized steps. Our use of ACD is independent of whether such death is "programmed" or not with respect to a higher level; that is, ACD refers to any death process that follows a cell death program (i.e., it is genetically regulated).

Generally, a cell death process is deemed active if in addition to being energy dependent and involving changes in gene expression, it can also be blocked (or delayed) by the inhibition of a signal or activity within the cell (e.g., Leist and Jaattela 2001). In these cases (e.g., apoptosis), cells that still have the potential to survive activate a death program in response to sublethal factors. In other words, cells die prematurely, and this is reflected in the use of the term "cell suicide." Therefore, the term "ACD" also encompasses two aspects: (1) the issue of premature death, and (2) the issue of a controlled demise. However, some types of death referred to as active do not appear to be premature. For instance, autophagic cell death (induced under severe bioenergetic stress) is thought to be the consequence of failed active attempts to survive (e.g., Levine and Kroemer 2009), and in many cases the inhibition of autophagy does not result in increased survival but in death via necrosis (e.g., in yeast and the slime mold *Dictyostelium discoideum*; Kourtis and Tavernarakis 2009). The distinction between premature and natural active death (and between apoptosis and autophagy) is important when interpreting the potential benefits of death in unicellular lineages, especially as many of the reported examples of active death in unicellular lineages are autophagy (e.g., Bera et al. 2003; Affenzeller et al. 2009b; Delgado et al. 2009).

When thinking about death, several questions are relevant: Can dying ever be a "better" strategy than living? If so, when and why? Is there an "optimal" way to die? And, who would benefit

from one's death? In multicellular organisms, where ACD was first described, all four questions have been answered with respect to the multicellular individual. Active (vs. passive) and premature (vs. natural) cell death—both during development and in response to stress—are thought to have been selected for because they benefit the multicellular individual.

After the realization that PCD-like processes can also be induced in unicellular lineages, the same questions and similar answers (i.e., involving adaptive roles for PCD) have been subsequently imported into the field of active death in unicellular lineages. This view has become more plausible with the realization that many unicellular individuals live part or most of their lives in multicellular-like communities (e.g., yeast colonies, slime mold and myxomycete fruiting bodies, and bacterial biofilms) (e.g., Cornillon et al. 1994; Engelberg-Kulka and Glaser 1999; Webb et al. 2003a; Vachova and Palkova 2005; Engelberg-Kulka et al. 2006; Kolodkin-Gal et al. 2009). Accordingly, most efforts directed toward understanding active death in unicellular lineages have followed a "top-down" approach—importing the PCD framework into the unicellular world and focusing on who the recipients of this altruistic behavior are (i.e., kin, group, population, or species) and how they can benefit from this type of death (Table 2). As in the case of PCD in multicellular lineages, the proposed benefits are related to both the premature and the organized aspects of ACD in single-celled species. For instance, dying sooner than later is thought to avoid the unnecessary use of resources by unhealthy or aged individuals (thus allowing the healthy and young individuals to take advantage of limited resources) (e.g., Fabrizio et al. 2004) or to prevent the replication of intracellular parasites or the transmission of mutations to offspring (e.g., Engelberg-Kulka et al. 2006). Similarly, a controlled self-destruction would both avoid the release of toxic intracellular components that could hurt neighboring individuals (Vachova and Palkova 2007) and provide the surviving individuals with nutrients released during the active processing of the dying individuals (Gonzalez-Pastor et al. 2003; Vachova and Palkova 2005). The implicit assumption in these views is that because it could provide benefits to kin/group, active death in unicellular lineages is an adaptive trait that has evolved and is maintained through kin/group selection.

Levels of Selection, Benefits, and Adaptation

Natural selection is conventionally thought to act on individuals, and adaptations are regarded as occurring at the level of the individual where they function to maximize its fitness. In this view, the focal level is the individual, which is not only the "interactor" (i.e., the target of selection; Box 1), but also the "manifestor" and the beneficiary of adaptations (Lloyd 2001). However, an interactor may be at any level of biological organization, from a gene to a group of organisms, and thus selection can act at multiple levels (Lewontin 1970). Evolution by selection can benefit the particular level of entity under selection by producing adaptations at that level (Williams 1966). Yet interaction at a particular level does not require attributing adaptations to the interactor. In particular, groups can act as interactors and benefit from selection without manifesting group-level adaptations (see discussion in Lloyd 2001).

The current literature on cell death in unicellular lineages often reflects difficulties in discriminating among the conceptual issues mentioned above. For a thorough analysis of the evolutionary role of active death in unicellular lineages several questions need to be addressed. Is active death an adaptation in the sense of having evolved and being maintained by selection? If so, which is the level on which selection acts and that manifests this adaptation? Could ACD benefit another level of organization without being an adaptation at that level?

While there is no doubt that unicellular organisms can be seen as interactors, the presence of a trait with such a negative impact on the fitness of the manifestor is inconsistent with active death being an adaptation maintained by traditional individual-level selection. Consequently, ACD in unicellular lineages is most often thought to be an adaptation that benefits others (kin, population or even species; Table 2) and that evolved and is maintained by either kin (e.g., Bidle and Falkowski 2004; Vachova and Palkova 2005; Fabrizio and Longo 2008; Gomes et al. 2008) or group selection (e.g., Franklin et al. 2006) (the latter being used in its traditional sense of group selection for group advantage).

Yet, for a behavior to be cooperative (including altruistic) it is required not only to provide a benefit to other individuals, but also to have evolved (at least partially) because of this benefit (West et al. 2007b,c; Gardner and Grafen 2009). For instance, under a kin selection model, ACD would be an individual-level adaptation that benefits kin and has evolved to increase the actor's inclusive fitness (Box 1). Similarly, under a model of selection at the group level, ACD would be a group adaptation. Note that although some consider as group adaptations any traits favored by selection at group level (e.g., Sober and Wilson 1998), others (Gardner and Grafen 2009) require that such group characters have been selected "according to the design principle of group-fitness maximization"; under this latter view, group adaptations are thought to evolve only when within-group selection is completely abolished (see further discussion in Gardner and Grafen 2009). However, this is not to say that groups cannot benefit from ACD, because individual-level traits can inadvertently improve group reproductive success without having evolved as adaptations at that level; this is the case for the so-called cross-level byproducts, "fortuitous group benefits" or characters for 'group

Table 2. Proposed roles for active death in unicellular lineages; "+" indicates cases where evidence for the proposed role was reported.

Proposed role	Examples	Evidence
Development	Bacteria (*Bacillus*, myxomycetes) (Lewis 2000)	
Release of nutrients when resources are limiting	Yeast (Frohlich and Madeo 2000; Fabrizio et al. 2004; Vachova and Palkova 2005; Fabrizio and Longo 2008)	+
	Bacteria (myxobacteria, *Bacillus*, *Pseudoalteromonas*) (Lewis 2000; Segovia et al. 2003; Mai-Prochnow et al. 2006; Schmitt and Breinig 2006)	+
Regulate population size in limiting environments	Dinoflagellates (Vardi et al. 1999; Dunn et al. 2004)	
Removal of mutated/damaged cells	Yeast (DNA-damaged cells) (Vachova and Palkova 2005; Gomes et al. 2008)	+
	Yeast (motility-impaired cells) (Leadsham and Gourlay 2008)	+
	Bacteria (defective cells) (Lewis 2000; Engelberg-Kulka et al. 2006)	+
	Dictyostelium (abnormal cells) (Tatischeff et al. 2001)	
	Green algae, dinofalgellates (Murik and Kaplan 2009)	
Removal of weak, unhealthy or sterile cells	Yeast (Fabrizio et al. 2004; Fabrizio and Longo 2008)	+
	Trypanosoma (Debrabant and Nakhasi 2003; Seed and Wenck 2003)	+
Protection (or survival stimulation) of surviving cells	*Chlamydomonas* (Moharikar et al. 2006)	+
	Yeast (Frohlich and Madeo 2000; Fabrizio et al. 2004; Herker et al. 2004; Vachova and Palkova 2005)	+
Lower population mutational load	Bacteria (Lewis 2000)	
Limit spread of viral infection	Bacteria (Lewis 2000; Hazan et al. 2004; Engelberg-Kulka et al. 2006)	+
	Diatoms (Parker et al. 2008), *Emiliania* (Frada et al. 2008)	
Avoiding host's death	*Plasmodium* (Deponte and Becker 2004; Hurd and Carter 2004)	+
Bloom termination control	Diatoms (Vardi et al. 2006)	
Facilitate adaptation to new/changing environments	Yeast (Herker et al. 2004), *Dunaliella* (Segovia et al. 2003)	
	Gram-negative bacteria (Mai-Prochnow et al. 2006, 2008)	
Evasion strategy to circumvent killing by host	*Leishmania* (van Zandbergen et al. 2006)	
	Entamoeba (Villalba et al. 2007)	
	Green algae (Segovia et al. 2003)	
Dispersal	*Dictyostelium* (Kessin 2001)	+
	Myxococcus (Nariya and Inouye 2008; Sogaard-Andersen and Yang 2008)	
	Biofilm-forming bacteria (Webb et al. 2003b, 2004; Mai-Prochnow et al. 2004; Engelberg-Kulka et al. 2006)	+
Stabilization of bacterial biofilms	Bacteria (Bayles 2007; Kolodkin-Gal et al. 2009)	+

optimality' (see discussion in Okasha 2006; Gardner and Grafen 2009). Thus, a distinction needs to be made between the "benefit" provided by a trait and its "evolutionary/adaptive role."

As mentioned earlier, in multicellular organisms, the term "programmed" is generally meant to imply not only that the activity is regulated but also that it serves a purpose (i.e., it is inherently adaptive). Its use to describe active death processes in unicellular lineages has thus artificially extended the mechanistic similarities (which reflect proximal causes) between the two processes to imply that PCD-like processes in the unicellular world are adaptive as well (that they have the same ultimate explanation). Yet, the fact that a trait is genetically regulated and requires energy does not necessarily mean, by itself, that it is also adaptive. Nonadaptive or maladaptive traits (e.g., aging—under the antagonistic pleiotropy hypothesis of aging; Williams 1957) can also be genetically regulated.

Separating and correctly distinguishing among these issues is likely to provide a different perspective on active death in unicellular lineages. Below, we critically evaluate the most frequent suggestions regarding the evolutionary role of ACD, focusing on

the issues highlighted above. In the next sections, we then argue that the possibility that active death in single-celled species has evolved and is maintained as a byproduct of selection on other levels or traits should also be considered, especially when the evolutionary origin of ACD is taken into account (the "bottom-up" approach discussed in the last section).

Kin-Selection, Altruism, and Active Death in Unicellular Populations

> *"Worn-out individuals are not only valueless to the species but they are even harmful, for they take the place of those, which are sound . . . I consider that death is not a primary necessity but it has been secondarily acquired as an adaptation." (Weismann)*

The idea that death is an altruistic adaptation can be traced back to August Weismann (1889). However, the evolution of costly forms of cooperation, including altruistic death, posed a major problem to evolutionary theory because traits that benefit others while decreasing the actor's reproductive success were thought to be unfavored by natural selection. Several theories and approaches including kin selection/inclusive fitness and group/multilevel selection (Box 1) have been put forward to explain the evolution and maintenance of altruistic behaviors (e.g., Hamilton 1964a,b, 1975; Okasha 2005; West et al. 2007c; Wilson and Wilson 2007). Although there is still controversy as to the equivalence or usefulness of these views (e.g., West et al. 2007c, 2008; Wilson and Wilson 2007; Wilson 2008; Leigh 2010; Traulsen 2010; Wade et al. 2010), kin selection in its more general form of inclusive fitness is the explanation most often invoked for altruistic behaviors.

(1) CONDITIONS

The appeal of kin selection as a mechanism to explain active death in unicellular lineages is based in part on three assumptions: (i) due to their predominantly asexual reproduction, unicellular populations are clonal (or have a high level of relatedness); (ii) because the relatedness is very high in clonal populations altruism should evolve easily; and (iii) relatedness (genealogical relatedness in particular) is the main component of kin selection/inclusive fitness theory.

Yet, contrary to the frequently held view for asexually reproducing single-celled species, in nature, unicellular populations are not necessarily clonal. For instance, the genetic structure of phytoplankton blooms has been shown to be in fact "heterogeneous" (Medlin et al. 2000; Thornton 2002; Rynearson and Armbrust 2005), and genetic diversity can also arise at local scales. Specifically, clonal diversity has been reported in aquatic protists (in small ponds and lakes) as well as in terrestrial species (e.g., Fortunato et al. 2003). Nevertheless, the relevance of relatedness is a function of the scale at which social interactions occur, and we know almost nothing about relatedness at the relevant scale in most systems. This can vary between species, and is dependent on the trait and the benefit associated with the altruistic behavior (for a discussion, see West et al. 2007a).

Furthermore, for any altruistic behavior to evolve via kin selection, it is not the average genetic similarity of the population that is important. Rather, what is important is the relatedness between an actor and a recipient compared to the relatedness between an actor and a random member of the population (e.g., Grafen 2006). Consequently, the population-wide average genetic similarity is meaningless in the absence of mechanisms or conditions that can promote "nonrandom associations between genotypes (assortment)" (Hamilton 1971). These mechanisms can include (1) kin recognition/discrimination, (2) population genetic structure due to low rates or short ranges of dispersal (although widespread dispersal can occur at some stage of the life-cycle; e.g., spore dispersal in *D. discoideum*), such that the interacting partners are more likely to be genealogically related (population viscosity), or (3) the so-called "greenbeard" effect (Hamilton 1964b; Dawkins 1976). The relative importance of these mechanisms is different in unicellular lineages (and among distinct unicellular lineages) compared to complex multicellular lineages. In the former, the "greenbeard" effect (Box 1) and population viscosity are believed to be more important than kin recognition (West et al. 2007a, but see Benabentos et al. 2009 and Chaine et al. 2010).

More recently, it has been argued that altruistic suicide can evolve without the need for the benefits to be exchanged between genetically similar individuals. This is because what is most important for the evolution of altruism is the assortment between individuals carrying the cooperative genotype and the helping behaviors of others with which these individuals interact (Fletcher and Doebeli 2009). Thus, cooperation can evolve simply due to assortment between phenotypic cooperators—even when coded by distinct cooperative genes—in the absence of kin selection (Fletcher and Doebeli 2009). In fact, the most general requirement for the evolution of any altruistic trait, including active death, is that carriers of altruistic genes must accrue more of the benefits of cooperation, on average, than noncarriers.

Overall, our point is that in the absence of mechanisms that can promote nonrandom associations among related genotypes (or between cooperative genotypes and the helping behaviors of others with which these individuals interact), a high level of genetic similarity (genealogical or not) in unicellular populations by itself is not enough to support the notion that unicellular ACD evolved by kin selection. Yet, little is known about the nature and generality of mechanisms that can promote such nonrandom associations in the unicellular world. Such conditions might be met for some lineages and in some settings (e.g., bacterial biofilms)—and some types of ACD might be in fact genuine kin/group-selected

adaptations. However, the possibility that these conditions are not met in all unicellular lineages (e.g., planktonic species), and thus that not all ACDs are altruistic, should not be disregarded—and will be discussed later.

A number of other factors can also limit cooperative behaviors or affect their stability. These include kin competition (but see Gardner et al. 2007 and discussion in Platt and Bever 2009), ecological factors, demography (i.e., habitat saturation), patch lifetime, rates of dispersal, and mutation rates (West et al. 2007a; Lion and Gandon 2009). The latter in particular can have a strong influence on relatedness and cooperative behaviors in unicellular populations. Due to short generation times in single-celled species, mutations can lead to a decrease in relatedness over time, which would allow selfish cheaters to spread. For instance, it is thought that mutations in populations with low dispersal can result in the loss of cooperative traits (see West et al. 2007a for a discussion).

(2) BENEFITS

Inclusive fitness theory is based not only on relatedness but also on the costs and benefits of the altruistic behavior. The idea that unicellular ACD is an altruistic adaptation has, naturally, triggered many speculations on the nature of the benefits (which need to be high enough to offset the obvious large cost) and the evolutionary role of this trait. Most explanations for active death in unicellular lineages invoke direct or indirect benefits to kin, group, population, or species (Table 2). Among the most frequently proposed kin benefits are the sparing of nutrients by, and the release of nutrients from, dying cells. However, in the absence of a specific mechanism that can direct these benefits to related individuals, they are also likely to be available to nonkin, especially in unstructured populations (Bidle and Falkowski 2004). In this context, it is noteworthy that the collapse of phytoplankton populations during bloom demise was found to lead to a marked increase in bacteria numbers and the establishment of a new opportunistic eukaryotic community (Brussaard et al. 1995; Castberg et al. 2001), suggesting that the released nutrients were available (mostly) to nonkin. Close kin might still be able to obtain enough benefit (on average) to offset the cost of ACD, but this is not usually addressed (either theoretically or experimentally) when such benefits are invoked. Moreover, strictly photosynthetic species would likely not be able to make immediate use of the organic matter released by the dying cells (Segovia et al. 2003). Lastly, these benefits will be provided only by some active types of death; autophagic death, in particular, might not provide such benefits due to both its slow progression and the use of existing resources during attempts to survive that precede death.

In addition to direct short-term kin benefits, several indirect long-term population benefits have also been envisioned. These include removing damaged or mutated individuals (which is alleged to improve the "genetic stability" of the population and lower the mutational load), limiting the spread of viral infections, regulating population size, and adapting to new environments (Table 2). As discussed earlier, although these processes might provide advantages to the populations exhibiting ACD, by themselves they are not sufficient to argue for ACD being an adaptation that evolved and is maintained for these benefits. For instance, if ACD is triggered by stress it is conceivable that damaged or mutated individuals would be more susceptible to stress. Consequently, ACD would be preferentially induced in these individuals, which in turn would result in a "healthier" population. However, this process would simply reflect individual-level selection (i.e., the "elimination of the less fit") without the need to invoke kin/group selection and consider ACD an altruistic adaptation. Of course, the question of why "less fit" cells would follow an active death process—as opposed to passive death—still requires an explanation (see discussion in the next section).

Ultimately, what we want to emphasize is that even in cases in which ACD does provide direct benefits to others, we still need to distinguish between two possibilities: (i) whether ACD evolved and is maintained because of its benefit to others, or (ii) whether ACD inadvertently benefits others (i.e., the benefits to others are fortuitous). In other words, statements such as ACD benefits a group/population should not be automatically interpreted to mean that the evolutionary/adaptive role of ACD is to provide that benefit. Similar distinctions were recently made between form/phenotype and function/"purpose" in bacterial biofilms (Monds and O'Toole 2009), and, more generally, between functional observations and adaptive explanations (Nielsen 2009).

(3) EXPERIMENTAL EVIDENCE

Some of the proposed benefits of active death in unicellular species have been addressed experimentally (Table 2). However, laboratory experiments are not always performed in conditions that reflect natural environments (e.g., they are usually performed with single-species cultures). Furthermore, in some cases the results allow for alternative interpretations. For instance, the inhibition of ACD through gene deletions or mutations resulted in yeast cell populations with decreased fitness, and this was interpreted as evidence for role of ACD in removing damaged cells (Herker et al. 2004; Gomes et al. 2008). Yet, the effects observed in these experiments can also be interpreted in terms of the disrupted genes having pleiotropic effects on other cellular activities. Below, we discuss several such examples.

Many experiments directed toward investigating ACD in unicellular organisms involve interfering with metacaspases; these are homologs of caspases, which are the main players in the execution phase of apoptosis in animals. Metacaspases are widespread in plants, fungi, and unicellular eukaryotes (e.g., Nedelcu 2009a),

and distant homologs have also been identified in prokaryotes (e.g., Bayles 2003; Bidle and Falkowski 2004). In yeast, metacaspase deletion prevented death under conditions that induce ACD in wild-type yeast, including oxidative stress and chronological aging (Madeo et al. 2002; Khan et al. 2005; Vachova and Palkova 2005). However, aged yeast metacaspase mutants lost their ability to regrow when transferred to fresh medium, accumulated more mutations than the wild-type, and although they had a short-term advantage, ultimately lost in competition with the wild-type (Vachova and Palkova 2005). Because these mutants did not undergo ACD during aging, the accumulation of mutations and the decreased fitness in this population relative to the corresponding wild-type population was interpreted to argue for ACD having a role at the population level—that is, in removing stress-induced damaged or mutated cells. Yet the inactivation of the metacaspase gene could have (in addition to its short-term effect on preventing ACD) a long-term negative effect on individual fitness, especially in stressful environmental conditions such as during chronological aging. Thus, the observed decreased fitness could be a direct effect of metacaspase loss on individual fitness, not a consequence of the inability to remove damaged cells through ACD. Consistent with this possibility, yeast metacaspase mutants have been found to have a higher content of oxidized proteins relative to the wild-type, even in the absence of stress (Khan et al. 2005).

A similar example is provided by the glutaredoxin 2 gene. In yeast, glutaredoxin 2 mutant populations avoided cadmium stress-induced ACD but showed higher mutation rates (as suggested by the accumulation of "petite" cells) relative to the surviving wild-type population (Gomes et al. 2008). These data were interpreted to suggest that ACD is a "mechanism for elimination of mutated and impaired cells" (Gomes et al. 2008). However, the apparent accumulation of "petite" cells in the mutant population can be due to an increased mutation rate in the glutaredoxin 2 mutant, and not to the inability to remove stress-induced mutants in the absence of ACD. This is consistent with the finding that the glutaredoxin 2 mutant exhibited high mutation rates (Gomes et al. 2008).

Lastly, in *Escherichia coli*, the isolation of mutations conferring increased resistance to autolysis induced by various factors was interpreted to indicate that although *E. coli* has "a way to dramatically increase survival in the presence of a variety of unrelated lethal factors, the majority of cells 'choose' to die" (Lewis 2000). Furthermore, the fact that no such mutants have been found in natural isolates of *E. coli* was viewed as an indication that "improved survival to lethal factors is a deleterious trait" because "the ability to eliminate defective cells (through programmed death) provides a clonal population with a significant competitive advantage" (Lewis 2000). Yet the absence of such mutants in natural isolates can also be interpreted as evidence for the genes whose disruption would result in death avoidance also having pro-survival functions under different environmental settings. Such ACD-free mutants would be outcompeted by the wild-type, and thus eliminated by individual-level selection.

Our main point in discussing these studies is to stress the fact that the blocking of ACD in experiments aimed at addressing its benefits should be performed in ways that are not likely to interfere with other cellular activities. Furthermore, experimental studies focusing strictly on the potential benefits conferred by ACD cannot, by themselves, determine the evolutionary role of ACD in unicellular lineages and the selective forces responsible for the evolution and maintenance of this trait. As we discussed above, because of potential pleiotropic effects, interfering with the cell death program by inactivating ACD genes might be problematic in terms of inferring the role of ACD. Thus, different types of experimental strategies, including experimental evolution, should be considered. For instance, relaxing or removing selection on ACD should result in the loss or deterioration of the trait. This could be achieved by either (i) removing the benefit (e.g., if populations are grown for many generations under conditions that do not trigger ACD, mutations in the cell death program should accumulate), (ii) providing the benefit externally (e.g., if the benefit is in terms of additional nutrients, providing the population with nutrients should remove the advantage of the trait), or (iii) removing the selection pressure (e.g., if selection acts at the kin/group level, growing the population under conditions that do not allow nonrandom associations should relax the selection on ACD; decreased relatedness should have a similar effect). If under such conditions the ACD machinery is preserved, explanations in terms of pleiotropy would have to be considered.

Unicellular ACD as a Byproduct of Selection

> *"The principle of spandrels provides a more radical version of the principle of cooptation for a "widely different purpose" because the exapted structure originated as a byproduct and not as an explicit adaptation at all. Therefore, structures that may later become crucial to the fitness of large and successful clades may arise nonadaptively (whatever their subsequent, coopted utility) . . ." (Gould)*

In the previous section, we argued for the possibility that in some unicellular lineages active death might not be an altruistic adaptation. If this were the case, why would a trait with such a negative effect on individual fitness be maintained? Generally, traits become fixed and are maintained in a population either as a consequence of natural selection (these are adaptive traits that increase fitness) or through genetic drift (these are neutral, nonadaptive traits). Traits that have a negative impact on fitness (i.e., maladaptive traits) are usually expected to be ultimately eliminated (although they could be fixed by drift when the detrimental effect

or the effective population size is small). However, traits that have the potential to negatively impact fitness could also be maintained if they are disadvantageous under some conditions but advantageous in some other circumstances, or if they are collateral to (or byproducts of) selection on a different trait.

Although they are not usually given much consideration, suggestions that ACD might be an unavoidable outcome of metabolic imbalances have been made (e.g., Bidle and Falkowski 2004). In actively growing cells, when growth is arrested by some form of sublethal stress, energy utilization becomes uncoupled from energy production, and this can lead to an oxidative burst resulting in cell death (Aldsworth et al. 1999). This scenario is consistent with the observation that under the same ACD-inducing conditions, cells from exponentially grown cultures (or nonquiescent cells) are more likely to undergo ACD compared to cells from stationary phase (or quiescent cells). For instance, in aging yeast cultures, nonquiescent cells (i.e., those that continue to divide after the exhaustion of glucose in the medium) are much more likely to develop apoptotic markers than the quiescent/resting cells (Allen et al. 2006). Similarly, *E. coli mazEF*-mediated cell death occurs during exponential but not stationary phase (Hazan et al. 2004, but see Kolodkin-Gal et al. 2007). In this view, ACD is thought to have no beneficial value at any level—it is simply maladaptive. The obvious problem with such a scenario is that maladaptive traits are expected to be eliminated by natural selection.

Nevertheless, one can envision that ACD is maintained because genes whose products are involved in the activation and/or execution of the cell death program have pleiotropic effects on other cellular activities that are under strong selection. Consequently, the loss of ACD-related genes would have a detrimental effect on individual fitness under non ACD-inducing conditions. Although ACD-free mutants would survive a sublethal stress, their fitness would be negatively affected in the long-term, and they would be outcompeted by the wild-type. As most genes affect more than one trait, pleiotropy is thought to be a major constraint on evolution because adaptive changes in one trait may be opposed to by selection on another trait affected by the same gene (e.g., Barton 1990; Otto 2004). Such pleiotropic effects are thought to underlie many biological phenomena, including senescence (the antagonistic pleiotropy hypothesis; Williams 1957). Is there any evidence to support the possibility that this might also be the case with active death in unicellular lineages?

Many components of the cell death machinery are known to perform in vital cellular functions as well, and mutations in the genes coding for these components affect the fitness of the individual under normal conditions. As discussed earlier, the cases reported as evidence for the role of ACD at the population level can also be interpreted as evidence for the ACD-associated genes having pleiotropic effects on other vital functions. To argue further for such effects, in addition to increasing the levels of oxidized proteins, metacaspase gene deletions also affect cell cycle progression and growth rates in several unicellular lineages (Khan et al. 2005; Ambit et al. 2008; Lee et al. 2008; Cao et al. 2009). In addition to metacaspases, caspase-like activities have also been reported in many unicellular lineages, and in some cases their inhibition prevents death under ACD-inducing conditions (Vardi et al. 1999; Segovia et al. 2003; Berman-Frank et al. 2004; Jimenez et al. 2009; Segovia and Berges 2009). However, lower levels of caspase-like activities can also be detected under normal conditions, suggesting that these proteins have additional house-keeping activities (Segovia et al. 2003; Berman-Frank et al. 2004; Bidle and Falkowski 2004) that are thought to "offset the liability in terms of mortality" (Segovia et al. 2003).

In eukaryotes, many components of apoptosis are released from, or associated with, mitochondria and have vital functions in mitochondrial activities (e.g., cytochrome *c*, mitochondrial fission factor Drp1, apoptosis inducing factor, endonuclease G) (Kroemer 1997). ACD-related genes are also involved in cell cycle progression or various responses to stress and DNA damage (e.g., tumor suppressor genes); mutations in such genes will affect not only ACD but also the ability to avoid or properly repair DNA damages.

In prokaryotes, toxin–antitoxin modules, such as the *relBE* and *mazEF* loci in *E. coli*, are often associated with active death processes (Hazan et al. 2004). However, because during nutritional stress *relBE* and *mazEF* are directly involved in quality control of gene expression at the translation level, it has been suggested that the primary function of these modules is in adapting to nutrient stress, not in ACD (Pandey and Gerdes 2005). This possibility is consistent with the observation that these loci have been lost in obligate intracellular bacterial species (which are living in a more constant environment), but are found in large numbers of copies in free-living slowly growing bacteria (Pandey and Gerdes 2005).

Altogether, although we do not know enough about the genes involved in active death processes in single-celled species, it is conceivable that many ACD genes are maintained because of strong selection on ACD-unrelated activities in which they also participate. This scenario is analogous to the antagonistic pleiotropy hypothesis of aging, whereby genes with negative effects late in life are maintained because of having beneficial effects early in life (Williams 1957). But why would cell death genes be pleiotropically linked to pro-survival mechanisms? The strong link might be as old as the program itself.

From Selfish, to Maladaptive, to Altruistic Traits

"If we look at the sting of the bee, as having originally existed in a remote progenitor . . . and which has been modified but

not perfected for its present purpose, with the poison originally adapted to cause galls subsequently intensified, we can perhaps understand how it is that the use of the sting should so often cause the insect's own death: for if on the whole the power of stinging be useful to the community, it will fulfill all the requirements of natural selection, though it may cause the death of some few members." (Darwin)

(1) ORIGINS: FIGHTING DEATH

Several hypotheses have been proposed to address the origin of PCD. Ameisen (1998) has developed a detailed picture of how PCD could have originated and evolved throughout the history of life (note that PCD is used here to refer to self-induced premature types of ACD such as bacterial autolysis and apoptosis). Ameisen envisioned a multistep scenario for the evolutionary origin of PCD in which the altruistic program regulating PCD may have initially emerged from the propagation of selfish genetic modules that were selected through their ability to addict or manipulate their host cells. Such entities include the plasmid-encoded addiction modules (Box 1) in bacteria and the ancestor of mitochondria in eukaryotic cells.

The idea that PCD has evolved from host–pathogen interactions has been proposed by many others (e.g., Frade and Michaelidis 1997; Kroemer 1997; Blackstone and Green 1999; Kourtis and Tavernarakis 2009). In all of these views, the PCD machinery evolved from the pathogen's killing mechanisms activated in response to changes in the metabolic state of the host cell. For instance, in the case of the mitochondria, a drop in ATP levels (indicative of low catabolic rates in the host, and thus low levels of substrates for the pathogen) would initiate a cascade of events including the activation of proteases that would allow the pathogen to exit the dying cell and/or use the liberated nutrients (Frade and Michaelidis 1997). The stabilization of the initially antagonistic interaction and the realization of the benefits associated with this enforced cooperation required both control over the replication of the selfish element and the repression of its killing strategies. This control may have been achieved through the integration of plasmid-encoded addiction modules in the bacterial chromosome and the transfer of genes from mitochondria into the host genome and the evolution of anti-death mechanisms (e.g., anti-apoptotic factors).

Generally, ACD is a complex trait involving many genes and proteins whose expression and regulation is integrated with other cellular activities. A rather unappreciated prerequisite for the spread and maintenance of ACD is conditionality. That is, the expression of ACD (whether maladaptive or altruistic) must be restricted to a fraction of the population. Scenarios for the origin of ACD based on pathogen–host interactions provide a potential explanation for both the complexity and the conditionality of ACD. In these views, the complex cell death machinery evolved from a set of adaptive traits designed to increase the fitness of the selfish element, and the conditionality of the cell death program reflects the conditional expression of the pathogen's killing machinery as a function of the host's metabolic state. The clear evolutionary connections between the apoptosis machinery and mitochondria, and between some forms of prokaryotic ACD and selfish elements (e.g., Kroemer 1997; Ameisen 1998), are also consistent with such scenarios.

(2) MAINTENANCE: LEARNING TO LIVE WITH IT

The question that follows is whether these initially selfish killing programs have been specifically maintained because they also provided some benefits at the kin/group level (scenario (i) in Fig. 1A) or whether the observed ACD processes in unicellular lineages simply reflect the inability to fully repress the pathogen's killing machinery (scenario (ii) in Fig. 1A). Note that the latter scenario does not exclude the possibility that ACD can provide fortuitous kin/group benefits, or that in specific settings the cell death program could be co-opted into an altruistic trait (discussed in the next section).

The first scenario implies that selfish elements can be maintained if they induce an altruistic behavior in their host. Several examples of independent selfish elements whose killing mechanisms are thought to provide kin/group-level benefits are known. For instance, in *Pseudomonas aeruginosa* and *E. coli*, the nutrient-induced lytic cycle of integrated phages is induced in biofilms to generate a predictable cell death pattern that contributes to the proper development and dispersal of the biofilm (Webb et al. 2004; Rice et al. 2009; Wang et al. 2009). Previous selfish elements are also thought to have been integrated and maintained because they favor altruistic behaviors at the individual level (see discussion in Ameisen 1998). For instance, in *E. coli*, in response to various types of stress, the chromosomally integrated addiction module *mazE/mazF* triggers cell lysis. The role of this *mazEF*-mediated cell lysis is thought to be in either eliminating damaged bacterial cells (thus contributing "to the maintenance of genomic stability of the whole population"), defending against the spread of phages, or providing nutrients for the surviving cells (Engelberg-Kulka et al. 2006). The stress-induced *cid/lrg* system in *Streptococcus aureus* might also be a remnant of a selfish element whose maintenance is thought to have been favored by the benefits that cell death provides in terms of the proper development and stability of biofilms (Bayles 2007; Rice et al. 2007).

The alternative scenario implies that ACD has been maintained as a byproduct of selection on prosurvival functions that have been provided by the "domesticated" selfish entity—and not because of potential benefits at the kin/group level. In this view, the occasional expression of ACD is triggered by metabolic imbalances that evoke the initial stages of the conflict between the host and its parasite. For apoptosis, these could be imbalances between the cytosolic and mitochondrial compartments that would trigger

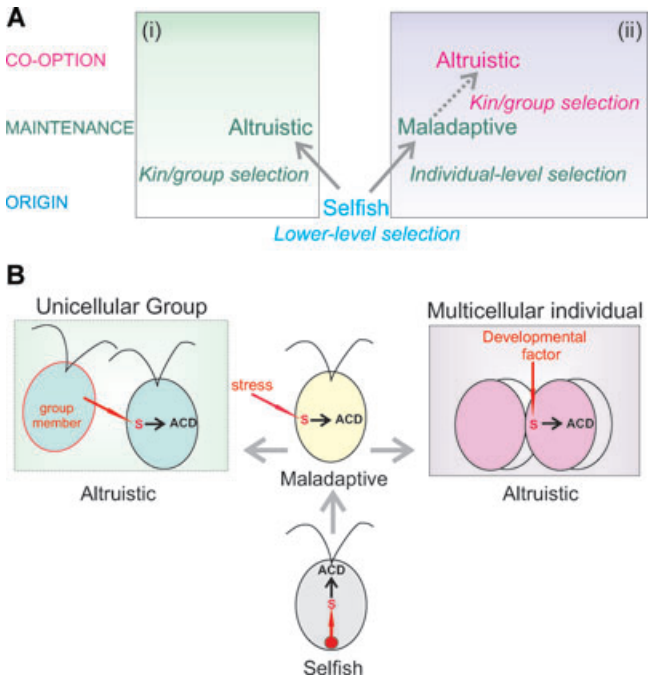

Figure 1. **The evolution of active death (ACD). (A) Two alternative scenarios (boxes) for the evolution of altruistic ACD from death induced by selfish elements (see text for discussion): (i)—without, and (ii)—with the possibility that in some unicellular lineages ACD is not altruistic and is maintained as a byproduct of individual-level selection on pro-survival traits (dashed arrow indicates the proposed co-option of maladaptive ACDs into altruistic types of death in settings in which kin/group selection can act). The type of death (selfish, maladaptive, altruistic) and the level of selection responsible for the origin, maintenance and co-option of ACD in both scenarios are also indicated. (B) A mechanistic model for the evolution of ACD by co-opting killing mechanisms employed by selfish elements (see text for discussion). The ancestral signal (S) induced by previously independent selfish elements (solid circle) to exit an unsuitable host can be evoked by environmental or metabolic stress factors. In contexts in which kin/group selection can act, the same signal can also be induced by either other group members (in unicellular groups) or developmental factors (in multicellular individuals).**

the overproduction of reactive oxygen species (ROS) (Blackstone and Green 1999). Consistent with this scenario is the fact that mitochondria plays the central executioner role in apoptosis (e.g., Kroemer 1997; Wang and Youle 2009), and the fact that most environmental types of stress that induce ACD also result in the overproduction of ROS (e.g., Halliwell and Gutteridge 1999; Mittler 2002). In this view, stress-induced ACD is strictly a maladaptive trait maintained by individual-level selection acting on prosurvival functions in which ACD-related genes are also involved. As long as the potential costs are lower (on average) than the benefits, ACD could be stable even in the absence of any kin/group benefits. Its facultative nature and its conditionality are likely to contribute to the maintenance of this trait.

These two scenarios differ in several important ways. In the former scenario, sociality/group-living is responsible for the evolution of ACD; that is, the advantages of group-living provide the pressure for the evolution of ACD (ACD is an evolutionary consequence of group-living). On the other hand, in the latter scenario ACD is a factor that can promote the evolution of sociality; in other words, an existing maladaptive cell death program can facilitate the evolution of complex group behaviors (ACD is a cause). Although the two alternatives are not necessarily mutually exclusive when all types of independently evolved ACD are considered, they are evocative of other "egg-chicken" dilemmas, including the question of whether high relatedness is a cause or a consequence of eusociality (Wilson and Holldobler 2005), and—more generally—whether mechanisms of conflict resolution are a cause, rather than a consequence, of group adaptation (Gardner and Grafen 2009). The two scenarios also make different predictions: although the former predicts that under conditions in which ACD is not beneficial at the kin/group level the cell death program should be lost, the latter predicts that the cell death program should be maintained regardless of the strength of selection on ACD.

(3) CO-OPTION: MAKING THE BEST OF IT

As discussed above, the second scenario allows for the possibility that maladaptive stress-induced ACD can be co-opted into a genuine social trait and be selected for specific kin/group benefits. How can this occur? If social/group-living signals (either chemical or position-dependent signals) can simulate the ancestral ACD-inducing signal (e.g., ROS; Fig. 1B), and if this group-induced signal-dependent death is beneficial (at the kin/group level), such types of ACD might be selected for and evolve into genuine altruistic adaptations. A similar suggestion has been made for the evolution of reproductive altruism; specifically, the temporary repression of reproduction in response to environmental stress in unicellular individuals is suggested to have been co-opted into reproductive altruism by simulating the environmental stress-induced intracellular signal in a group context (Nedelcu 2009b).

But how can group-related signals simulate environmental or metabolic stress? In addition to environmental or metabolic changes, biotic factors such as toxins or secondary metabolites can also increase ROS production and can trigger ACD (Curtis and Wolpert 2004). In this context, it is noteworthy that in the slime mold *D. discoideum* the factor responsible for the initiation of cell death in the stalk cells is a doubly chlorinated hexanone that acts as a cellular poison (Masento et al. 1988), and thus it could be triggering an increase in ROS levels in the cells that respond to this factor (note that the response to the inducing

factor is conditional on the metabolic state of the cells; Giusti et al. 2009). The same is likely true for the death of diatoms at the end of a bloom, where toxic aldehydes have been identified as the signaling molecules that trigger ACD (Vardi et al. 2006). Prokaryotes provide several analogous examples. For instance, the marine bacterium *Pseudoalteromonas* produces a toxin that provides a competitive advantage during the colonization of marine environments by inhibiting the growth of other surface-settlers. However, in the late stages of biofilm development, this toxin also kills cells of its own strain; this behavior is thought to benefit the surviving cells by supplying nutrients for the continuous development and dispersal of the biofilm (Mai-Prochnow et al. 2004, 2006). Furthermore, it is possible that some of the prokaryotic types of ACD that involve a quorum-sensing signal are in fact derived traits evolved via co-option of maladaptive traits. This might be the case for the *E. coli mazE/mazF*-mediated ACD that is dependent on a quorum sensing molecule (Kolodkin-Gal et al. 2007), especially if—as discussed earlier—the *mazEF* locus is maintained as a byproduct of selection on pro-survival functions.

In this view, the cases in which ACD is induced by social/group-living signals can be seen as analogous to developmentally induced PCD in multicellular lineages (Fig. 1B). Different types of group-induced ACD can evolve independently in distinct unicellular lineages in the same way as various forms of developmentally induced PCD evolved independently in multicellular lineages. Such co-option events could simply entail tinkering with the induction component of the cell death program, as a function of the social setting and the selective pressures specific for each lineage. Furthermore, they can involve either apoptotic or autophagic types of death; note that the group-induced ACD in *D. discoideum* is of the autophagic type (Giusti et al. 2009), although apoptosis-like ACD have been reported in solitary individuals (Tatischeff et al. 2001).

This co-option scenario allows for the possibility that maladaptive traits maintained as byproducts of selection on prosurvival traits could be co-opted into traits that can maximize inclusive or group fitness. The advantage of such a strategy is that the resulting co-operative behavior has a built-in safety system against cheaters. That is, if the cell death program is pleiotropically linked to pro-survival functions, ACD-free mutants will have a short-term benefit, but their fitness will be negatively affected in the long term. Pleiotropy has been previously suggested to be important for the stabilization of cooperation (Foster et al. 2004). For instance, in *D. discoideum,* "cheater mutants" that avoid death associated with differentiation into stalk cells are later excluded from the spores because of a pleiotropic linkage between stalk and spore formation (Foster et al. 2004). Although these findings were interpreted to argue for the role of pleiotropy in stabilizing cooperation (i.e., in a framework that assumes ACD as a cooperative act that evolved via kin/groups selection), the data also suggest that the loss of ACD can have a negative effect on individual fitness. Thus, it is possible that ACD is in fact maintained by individual-level selection, but can be co-opted into an altruistic behavior in response to group-living signals. More generally, the possibility that maladaptive traits can be maintained as byproducts of selection and be co-opted into genuine adaptations could add to recent proposals that many aspects of biological diversity are rooted in nonadaptative processes (Lynch 2007).

(4) MANIPULATION: TAKING ADVANTAGE OF IT

Under a co-option scenario, the cell death program could also be susceptible to manipulation for competitive or selfish purposes, and such examples do exist. For instance, the ciliate *Euplotes crassus* releases a toxin that induces apoptosis in nonproducer *Euplotes* species (Cervia et al. 2009). Similarly, in yeast, strains that harbor killer viruses produce a toxin that triggers ACD in uninfected cells (Ivanovska and Hardwick 2005). Likewise, in *Bacillus subtilis*, the *skf* operon codes for a killing factor that, under nutrient deprivation, causes the lysis (and release of nutrients) of sensitive *B. subtilis* cells; note that this behavior was deemed cannibalistic by some (Gonzalez-Pastor et al. 2003), but altruistic—that is, beneficial for the population as a whole—by others (Engelberg-Kulka et al. 2006). A rather different example is provided by the bacteriocins that reduce competition by killing nonproducer-sensitive strains. In this case, because the release of the toxic compound requires the lysis of the producer cell, the behavior is considered either spite or indirect altruism (West et al. 2007a). Selfish elements can also take direct advantage of existing cell death programs. For instance, viruses that infect the haptophyte *Emiliania huxleyi* induce ACD as part of their replication strategy by actively recruiting host metacaspases (Bidle et al. 2007).

Conclusions

> "We can't solve problems by using the same kind of thinking we used when we created them" (Einstein)

The presence of active death processes in single-celled organisms is a particularly puzzling problem that is in great need of both an evolutionary explanation and a framework with which to address it. Until relatively recently, ACD was considered an altruistic trait that evolved and only makes sense in multicellular lineages. This paradigm greatly influenced, and at times hindered, investigations of ACD in unicellular lineages (Ameisen 1998). As data supporting a cell death program in single-celled organisms have accumulated, the paradigm of altruistic cell death from multicellular lineages has been extended and adjusted to include the observed active death processes in unicellular lineages. Consequently, the concept of altruistic suicide has become entrenched in the literature on ACD in unicellular lineages, and current research

on ACD in single-celled organisms has been largely driven by attempts to fit experimental evidence into this extended paradigm.

But is this paradigm general enough to serve as the conceptual framework for all research on cell death in single-celled lineages? Here, we questioned some of the theoretical and experimental arguments generally used to argue for altruistic suicide in unicellular lineages. In particular, we pointed out several misconceptions regarding the required conditions for the evolution of altruism, especially with respect to genetic relatedness and assortment. We noted that although in some instances these conditions might be met, there is not enough information to assume that they are generally met in all unicellular lineages. In addition, we highlighted the sometimes unappreciated distinction between the selective forces behind the origin of a trait and the forces responsible for its further evolution and maintenance, as well as between the benefits and the evolutionary/adaptive role of a trait. Specifically, we stressed the fact that experimental studies indicating that ACD provides benefits to kin/group should not be automatically interpreted to mean that the evolutionary/adaptive role of ACD is to provide that benefit. Furthermore, we showed that much of the experimental evidence that has been put forward to argue for ACD in unicellular lineages having a role at the population level is equally compatible with components of the ACD machinery being pleiotropically linked to prosurvival traits. Because many ACD genes might have pleiotropic effects, we suggest that additional types of experimental strategies, including relaxing or removing selection on ACD, need to be employed when addressing the adaptive role of ACD.

Lastly, using a "bottom-up" approach that takes into consideration the evolutionary history of the cell death program, we provided an alternative framework that allows for the possibility that ACD is a maladaptive trait maintained as a byproduct of selection on prosurvival functions at the individual level. Nevertheless, this maladaptive trait could—under conditions in which kin/group selection can act—be co-opted into an altruistic trait. We hope that this framework will provide a "null hypothesis" against which the current models of altruistic death can be tested.

Overall, we think that the concept of altruistic suicide in unicellular lineages needs further experimental validation in a sound evolutionary context to justify its position as the dominant conceptual framework for all research on cell death in single-celled lineages. Considering alternative frameworks and explanations when asking questions about the adaptive role of ACD in unicellular lineages has the potential to provide us with new perspectives that could be extended to other evolutionary phenomena and processes.

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