Conceptual Foundations of Cell Mortality

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Contents

Abstract *The new era of microbial cell death* stems from a flood of new information emanating from the mechanistic and evolutionary life sciences, philosophy, and even sociology. In the shifting landscape, longstanding cell death terminologies and concepts have rightfully been questioned. There is currently very little consensus on how these concepts should be defined. One result of this is that similar findings often prompt different explanations because of the diversity of meanings associated with the terms. In this chapter, we review terms and concepts in microbial cell death that are key to understanding cell mortality. We discuss concepts like cell death, mortality, and the distinction between endogenous and exogenous death. We examine the contentious problem of defining programmed cell death (PCD) and argue that an evolutionary concept of PCD is foundational and applies to all cells across the tree of life, including microbial taxa. Alternative conceptions that define PCD in mechanistic, developmental, and ecological terms are useful tools for dissecting the

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molecular mechanisms, environmental triggers, and functions of PCD, but they do not define what PCD fundamentally is. Finally, we emphasize the importance of being clear on such concepts in order to achieve an overarching cell mortality framework.

1 Introduction

As the title of this edited volume indicates, we have entered a *new era of microbial cell death*. The view that most microbial taxa have few, if any, dedicated self-induced cell death mechanisms is increasingly called into question by new empirical evidence. Nevertheless, the nature and status of self-induced microbial death as a bona fide evolved function remains a contentious issue. How should cases of such death be detected in different microbial taxa? How should these measures be interpreted? And what do the new discoveries in microbial death mean for the broader field of cell mortality? One of the main reasons behind such questions is the inconsistent use of key terms. The field lacks a general, agreed-upon conceptual framework, and the same terms are often used for different concepts. The confusion and inconsistencies this brings about bear on our interpretations of empirical data. Divergent assessments of the same empirical findings are common, which leads investigators to find themselves at cross purposes.

Over the last decade, there has been some progress in our conceptual analysis of cell death. However, the conflicting interpretations of cell death—its mechanisms and meaning—are an ongoing problem, making it imperative to establish a unified conceptual foundation of cell death. This is especially true in microbiology (Ameisen 2002; Nedelcu et al. 2011; Bayles 2014), but the need for greater clarity on cell death is far-reaching. Cell death is studied in astrobiology (Hammond et al. 2020), origin of life research (Jodder et al. 2025), ecology and evolutionary biology (Durand 2021; Ndhlovu et al. 2021), history and philosophy of biology (Morange 2010; Durand and Ramsey 2023; Huneman 2023), and sociology (Landecker 2003; Reynolds 2014).

It is beyond the scope of this chapter to propose an overarching conceptual framework of cell mortality across the life sciences. That is a larger project. Instead, we critically analyze terms, concepts, and definitions in cell death studies and aim for conceptual unity in microbial cell death. We begin by examining common terms like "microbial," "cells," "death," and "mortality." Next, we highlight the distinction between endogenous and exogenous cell death, before discussing the contentious concept of programmed cell death (PCD), regulated cell death (RCD), and other terms.

For PCD, which is a central concept foundational to many problems in cell death research, we analyze its diverse conceptualizations and conclude that an evolution-based PCD concept is foundational. Such a concept is applicable across the tree of life, including microbial taxa. However, this does not mean that other conceptions of PCD—ones based on mechanisms or triggering conditions rather than a taxon's

evolutionary history—are misguided. Mechanistic approaches are, of course, essential for disentangling the cellular processes responsible for cell death. Elucidating the mechanisms, irrespective of their evolutionary histories, can be helpful. This is especially the case in microbial cell death studies where most of the conceptual challenges currently lie. Nevertheless, we hold that while understanding PCD mechanisms is central to the study of PCD, the basic definition of what PCD is requires an evolutionary framework.

2 Microbes, Cells, Death, and Mortality

Let us begin by considering the meaning of "microbial," since this term carries considerable ambiguity. Is every microscopic organism a microbe, or is the term restricted to single-celled organisms like prokaryotes and unicellular eukaryotes? This is important because the word "microbe" draws a line between microbiota (whose observation requires a microscope) and macrobiota (organisms that can be observed with the naked eye). Understood in this way, "microbe" is not restricted to unicellular organisms; although, of course, most microbes are microscopic and unicellular. This is a point worth emphasizing because some concepts of microbial cell death are tied to the assumption that we are dealing exclusively with unicellular organisms. In this chapter, by labeling an organism "microbial," we do not make any assumptions about its cellular organization. It could be a prokaryote, a unicellular eukaryote, or a facultative or obligate multicellular eukaryotic microbe. As microbiologists acknowledge, the model organisms in microbial cell death studies represent diverse taxa (Ameisen 2002), including fungi, amoebae, chlorophytes, and myxobacteria, some of which may be multicellular at various stages in their life cycles.

Despite the diversity across the tree of life, a universal feature is that organisms are all built from cells. A major tenet in biology is the "Unified Cell Theory of Life," articulated by Schleiden (1839) and Schwann (1839), which states that all organisms have cells as their functional units. One may reasonably assume, therefore, that in establishing the foundations of cell death, we are including all organisms. Any overarching conceptual framework of cell death should thus be appropriate for all cells irrespective of the organisms in which they find themselves. As we indicated earlier, the organization of cells in organisms varies across taxa, which is known to play a role in the evolution of cell death mechanisms. However, it is important to remember that the focal units in cell death studies are the cells, irrespective of their number and organization in organisms.

Next, consider the usage of "mortality" and "death." The main difference between the two terms lies in their contextual uses. In the mechanistic (e.g., molecular-cellular) disciplines, where individual cells are usually the target of inquiry, the term "cell death" is more commonly used. Death refers to a permanent loss of cellular viability, which may result from a gradual shutdown in metabolic activities (e.g., aging or poisoning) or a catastrophic disruption of the cell's structural

and functional integrity (e.g., predation or physicochemical insults). Mortality, by contrast, is typically used in reference to groups or populations, e.g., the mortality rate of cells in algal blooms or organismal/microbial mortality in ecology (Franklin et al. 2007). Mortality is also used in reference to organisms or cell lines to draw a contrast with immortality.

In the evolutionary literature, both "death" and "mortality" are employed, with "mortality" often being preferred in broader contexts. For example, mortality may be used to denote the extinction of evolutionary lineages, or preferred when cell mortality is discussed in relation to the levels and units of selection. In the evolutionary literature, cellular mortality includes phenomena like cell lineage senescence and aging, which are not always included when the molecular mechanisms of cell death are discussed. An obvious example is the Gompertz-Makeham law of mortality (the calculated likelihood of death with age), which is used in multiple contexts and applies to all lineages of cells and organisms (Kirkwood 2015).

From our discussion, there is no definitive boundary in the microbial cell literature between the terms "death" and "mortality." It is prudent, however, to acknowledge that there are usually different usage contexts and that the term mortality is often preferred when the discussion goes beyond individual cells or organisms. In this chapter, the terms "mortality" and "death" are mostly interchangeable, although we recognize that more significant conceptual differences between the two terms may emerge as the field advances.

3 Two Kinds of Microbial Cell Death: Endogenous and Exogenous

The two main categories of cell death trace their origins to reflections on the causes of mortality in plants and animals that long preceded the discovery of cells. The causes of organismal mortality were classified by Aristotle as arising internally or externally. (He labeled the two types "exhaustion" and "extinction," respectively; see Durand and Ramsey 2023.) The distinction between these two kinds of death (endogenous/exhaustion and exogenous/extinction) was incorporated into the cell death literature once it became clear that cells are the functional units of living systems. The distinction was also applied to unicellular organisms when it was discovered that they are not immortal and, under some circumstances, self-destruct by activating internal genetic mechanisms. It was only in the last few decades that the transition from the concept of cell death by exhaustion to the concept of endogenous cell death was accepted. Prior to this, most researchers considered microbial cell death a question of aging (ref to follow Vaux et al. 1988).

As with the cells in plants and animals, microbial cell death is due not only to external causes like predation, infection by foreign nucleic acids and viruses (which trigger host cell death mechanisms), or physicochemical stress but also to internal processes like aging and programmed cell death. In the shifting landscape of the

study of microbial cell death, a host of additional terms have been suggested, such as self-destruction, suicide, and programmed organismal death, also called phenoptosis. However, at the center of these new labels remains an emphasis on the causes of death that arise from within the cell and those that originate outside the cell's plasma membrane or wall. As our understanding of cell death advanced, the terms "endogenous" and "exogenous" gradually came to dominate, especially in the evolution literature. They have now largely replaced "internal" and "external" and are currently used to represent a fundamental distinction in the causes of microbial cell death.

3.1 The Challenge of Distinguishing Endogenous from Exogenous Causes

While the endogenous-exogenous distinction is conceptually useful, it is not always easy to make a clean distinction in practice. For illustration, consider a compound that diffuses into a microbial cell and causes a damaging genetic mutation. The cell's internal genome surveying mechanisms recognize the genetic pathology and activate a molecular program for death. How should we understand the complex causal dynamics in such a case vis-à-vis the endogenous-exogenous distinction? The answer depends on the specific research question at hand and the potential hypotheses to be tested. As Lloyd (2015) argues in her analysis of causality in evolutionary biology, what is really at stake is the logical structure of the research question. In the example above, one may wish to know how the molecular compound diffuses into the cell and induces DNA damage. But one may also want to understand how the mutation is detected and how its detection activates a molecular pathway, leading to cell death. Both investigations are valid in that they are investigating real causal paths, but they suggest different answers to this question: What was the cause of the cell's death? Focusing on the diffusion of the mutagenic compound will tend to make one see the cell's death as exogenous, whereas focusing on the protein that detects the mutation suggests that its death is endogenous.

Does this imply that causation is somehow observer dependent and that there is no fact of the matter whether the death is endogenous or exogenous? While some authors suggest that in view of the "fuzzy" picture of cell death and its causes it may be helpful to "renounce the existence of stable categories in favor of temporary and local clustering of molecular components for a transient function" (Morange 2010, p. 179), we resist this response and hold that the question—What was *the* cause of the cell's death?—is itself misleading in its simplicity.

There is seldom a single cause that brings about PCD. Instead, death is the result of a complex nexus of individually necessary and jointly sufficient causes. Some of these causes are outside of the cell, and others are inside. In order to focus on *the* cause, one must highlight one of the causes and place others in the background. One can do so by focusing on the hypotheses in question and on the evolutionary history

of the cell. For instance, if a cell is exposed to a damaging substance that leads to death not just of that cell but of any kind of cell, then we can safely place the cause external to the organism. On the other hand, if a variety of cells are exposed to a substance but only some of them respond to it by dying, and if the molecular pathway leading to death involves an evolved mechanism, then it is this mechanism that is the salient cause of death. In such a case, death is properly labeled endogenous. Consider, for example, the case of a ligand binding to a cell receptor like TNFR1, which may trigger apoptosis. There are many such mechanisms across the tree of life.

3.2 Endogenous Microbial Cell Death

Until the middle to late 1900s, the prevailing belief was that unicellular organisms (especially prokaryotes) that escaped exogenous death were potentially immortal because they either reproduced clonally or exhibited negligible aging (Florea 2017). In some taxa, it was known that indeterminate growth and indefinite reproduction are constrained by physical and physiological limits (e.g., the scarring of the cell membrane in budding yeast or the metabolic shutdown in physiologically aged cells), but it was widely assumed that most microbial organisms, in the absence of exogenous harm, were capable of reproducing or maintaining viability indefinitely. The concept of endogenous microbial death, therefore, challenged the prevailing dogma of immortality in the unicellular world. As in cells in multicellular tissues, single-celled microbes harbored molecular mechanisms that actively induced their own death. This "suicidal" behavior is now known to have widespread evolutionary and ecological consequences (Durand and Ramsey 2018). It poses a challenge to the paradigm of organism-level selection in the unicellular world and is one of the main drivers behind the new era of microbial cell death.

The recognition that unicellular organisms are capable of endogenous death precipitates a range of new questions: Are the mechanisms underlying death selected for, or are the cells simply malfunctioning? What is the role of self-destruction in microbial ecology and evolution? And what will the answers to these and related questions mean for the applied sciences like medical microbiology and environmental science? As we alluded to earlier, one of the ongoing frustrations in engaging with these questions results from unicellular cell death being poorly defined. As Nedelcu et al. (2011) emphasized, there is a tendency to import concepts from studies in multicellular organisms without thinking critically about what they may imply in the microbial world. A good example is Kerr et al.'s (1972) conceptualization of "apoptosis" in animal tissues. While this represented a landmark for new thinking about animal cell death, it is also problematic for microbes because it conveys two ideas: First, apoptosis describes a specific form of cell death, the molecular basis of which was later uncovered, which redefined the word "apoptosis" for those working in the field. Second, its conceptualization was coupled with tissue kinetics that was known to exist only in the stratified tissues of multicellular organisms. Kerr observed

that dying cells exhibited distinct morphological features and eventually fragmented into apoptotic bodies. He noted that such bodies were engulfed by other cells in the tissues, thereby inhibiting an inflammatory response, transporting materials between cells, and playing a central role in tissue homeostasis, which is unique to animals and plants. There are functional analogies of tissue homeostasis in social microbes (e.g., yeast and social bacteria), but these are transient stages in the organism's life cycle.

Apoptotic cell death was, subsequently, irrevocably tied to animal development and protection against neoplasia in multicellular organisms. (It is, thus, always interpreted as an adaptation in evolutionary biology.) The term "apoptosis" found favor in the microbial literature largely because apoptosis-like morphologies and homologous elements of apoptosis genes were identified in both prokaryotes and unicellular eukaryotes. The first part of the apoptosis concept, as outlined by Kerr et al., is, therefore, not problematic because similar phenotypes are observed in unicellular and multicellular organisms. However, the second idea from Kerr et al. relies on a conceptual link to cellular kinetics and tissue homeostasis, properties considered unique to multicellularity. This focus on tissues, of course, excludes the occurrence of apoptosis in unicellular organisms. Such an exclusion calls into question the validity of using concepts from the multicellular literature to describe cell death in the microbial world. The conflicting usage of terms like apoptosis (and many others) has led to more fundamental questions concerning the broader conceptualization of endogenous death in the unicellular world. The problem extends beyond the word "apoptosis" itself, and beyond the need to more narrowly define and distinguish apoptosis from other molecularly defined cell death pathways discovered in animals. That is, while different fields can successfully use the same or similar terms to mean different things, a problem arises when the transfer of a word from one field to another also carries with it assumptions about specific biological processes that may or may not occur in both animals and microbial cells.

One term that has risen to be of key importance in cell death studies is programmed cell death (PCD). As with apoptosis, there is some disagreement on how it should be defined and whether a single definition can apply to both unicellular and multicellular organisms.

4 Conceptualizations of Programmed Cell Death

Programmed cell death is a highly disputed concept in the field of microbial cell death (Durand and Ramsey 2023). The term was coined to differentiate cells dying in a genetically encoded, regulated fashion from those dying haphazardly or being killed, described as necrosis, which was considered an antonym of PCD (Lockshin and Williams 1964). A key source of dispute relates to PCD's status as a bona fide mechanism of cell death in unicellular organisms as opposed to in multicellular organisms where PCD was discovered. These disputes can be traced back to the origin of the term and its relationship with other related concepts. A detailed historical overview of PCD and its relationship with other cell death labels is beyond

the scope of this chapter, but see Clarke and Clarke (1996) and Durand and Ramsey (2023).

Debates about unicellular PCD arise largely because of its ecological and evolutionary significance. In some cases, there is irrefutable evidence for microbial cell death being an evolved function—an adaptation (e.g., Fukuyo et al. 2012; Refardt et al. 2013). In others, there are endogenous molecular mechanisms that cause death via pleiotropic side effects (e.g., Proto et al. 2013). In such cases, death is not an evolved function and is considered nonadaptive. Such nonadaptive death is especially common in stressful environments in which biochemical activities are more susceptible to dysregulation. Cell death is the outcome, but death is spuriously associated with other evolved molecular functions unrelated to cell death. To further complicate the issue, adaptive cell death is also sometimes an environmental stress response (Mata et al. 2019). Disentangling adaptive from spurious cell death is thus challenging experimentally, especially in stressful environments.

Lockshin and colleagues employed the term "PCD" to describe cells that "followed a sequence of controlled (and thereby implicitly genetic) steps towards their own destruction" (Lockshin and Zakeri 2001, p. 546). Similarly, in the microbial literature, PCD is defined by Berman-Frank et al. (2004) as "active, genetically controlled, cellular self-destruction driven by a series of complex biochemical events and specialized cellular machinery" (p. 997). There are several examples of such gene-based definitions of PCD that underpin experimental research aiming to disentangle the molecular pathways. However, defining PCD based on genetic (or other biochemical) mechanisms has an important limitation: it fails to distinguish between cases of adaptive and spurious cell death.

It has been argued that to draw a clear line between adaptive and nonadaptive cell death, an evolutionary PCD concept is needed (Durand and Ramsey 2018). The reason is that without taking into account the trait's evolutionary history, one cannot distinguish mistakes from adaptations. An evolutionary definition takes PCD to be an adaptation. It is an altruistic phenomenon that is selected for because of the fitness advantages it provides to others (e.g., Durand et al. 2011; Fukuyo et al. 2012; Refardt et al. 2013; Orellana et al. 2013).

If the mechanisms generating cell death are not adaptations, we label the death "ersatz PCD," not true PCD. This distinction between PCD and ersatz PCD is especially relevant in microbial cell death because the evolutionary history of PCD has not been investigated in most microbial taxa. By defining PCD in terms of its evolutionary history, one can test hypotheses that PCD evolved as an environmental stress response (e.g., Durand et al. 2014; Barreto Filho et al. 2022) or as a protective mechanism against invading viral parasites (e.g., Fukuyo et al. 2012; Refardt et al. 2013). PCD may also exhibit a history of phenotypic plasticity (Zeballos et al. 2023) in response to changing environments (Miller et al. 2024).

To understand the evolution of PCD in microbial species, it is important to consider its ecological role and the potential advantages it provides for population-level immunity to viruses (Fukuyo et al. 2012; Refardt et al. 2013), orchestrating population-level responses in constrained habitats (Vardi et al. 2007), or being a keystone species in communities (Ndhlovu et al. 2021). To investigate some of these

Table 1 Conceptualizations of programmed cell death in five categories

Concept	Definition of PCD	Key references
Mechanistic	PCD is an active, genetically controlled, cellular self-destruction driven by a series of complex biochemical events and specialized cellular machinery	Berman-Frank et al. (2004, p. 997)
Evolutionary	PCD is an adaptation across all scales of life (this contrasts with ersatz PCD or nonadaptive cell death)	Durand and Ramsey (2018)
Developmental	PCD is a physiological developmental cell death that occurs at a stage in the life cycle of the cell	Cornillon et al. (1994), Hosoya et al. (2007), and Wireman and Dworkin (1977)
Ecological	PCD is a stress response and a mechanism for nutrient recycling within and between trophic levels	Franklin et al. (2007)
Immunological	A cell death-inducing immune response across all taxa	Berngruber et al. (2013), Débarre et al. (2012), Fukuyo et al. (2012), Koonin and Zhang (2017), and Refardt et al. (2013)

The proposed conceptualizations of programmed cell death (PCD) arose from different disciplinary approaches. Whether explicitly stated or not, the definition applies to all domains of life (adapated from Durand and Ramsey 2023)

issues, many authors have, sometimes unintentionally, proposed alternative interpretations of PCD. We have organized the interpretations into five conceptualizations of PCD (Durand and Ramsey 2023): mechanistic, evolutionary, developmental, ecological, and immunological (Table 1). A mechanistic conceptualization of PCD was proposed at the time of its discovery (Lockshin and Zakeri 2001). Developmental, ecological, and immunological concepts followed once PCD was demonstrated in unicellular organisms. The latter three conceptualizations are based on proximate causes and, at least in the case of the immunological account, evolution.

However, these five conceptualizations differ in what each considers to be the essential elements of PCD and how they relate to fundamentally different definitions of PCD. The mechanistic concept defines PCD in terms of genes and regulated protein pathways. The evolutionary concept is based on ultimate causes—selection pressures—underlying the origin and maintenance of PCD (Durand and Ramsey 2018). The developmental account conceptualizes PCD in terms of a developmental stage, whereas the ecological account focuses on the ecological triggers of death. The immunological account considers PCD to be an (autonomous and nonautonomous) immunological response. While the research behind these accounts is valuable, one must be careful not to conflate the definition of PCD with its origins and mechanisms. For instance, we view the evidence currently available for the immunological account to be better understood as articulating a possible role of PCD, not as defining what unicellular PCD fundamentally is.

As we alluded to in Sect. 3.1, the mechanistic concept of PCD, defined in broad terms as organized and regulated processes, contrasts with haphazard mechanisms of cell death. It is important to emphasize that the mechanistic conceptualizations include a range of mechanisms and cellular morphotypes. It is argued elsewhere (Morange 2010; Durand and Ramsey 2023) that the tendency to equate any specific mechanism or cell death phenotype with PCD is misguided. The difference between PCD and apoptosis is a good example and highlights the superimposed meanings of a mechanistic subtype with PCD. This is especially problematic in microbial cell death and doing so is a continual source of confusion. In some taxa or microbial species, a standardized nomenclature has been proposed to avoid further confusion. In yeast cell death, for example, there is a range of cell death mechanisms and terms have been proposed to classify the diverse subtypes of PCD based on their cellular and biochemical features (Chaves et al. 2025). These are extensively debated, and there are many critics of the labels (Hardwick 2018).

5 Endogenous Death, Programmed Cell Death, and Microbial Cell Fate

Are microbial and animal PCD the same thing as endogenous death? At first glance, it may appear that they are synonymous since both are forms of endogenous cell death. However, we hold that PCD is best seen as a type of endogenous death, that there can be endogenous death that is not PCD, just as there can be PCD that is not apoptosis, e.g., autophagy or necroptosis (see Fig. 1). To support this claim, we will discuss two examples, chosen because of the challenging questions they raise concerning the intracellular origin of cell death.

The first example is the case of microbial cell death induced by autonomous genetic elements (replicons). Such cases are labeled PCD in the literature because the cause is a well-defined genetic program that codes exclusively for cell death. An important feature of replicons is that the death-inducing genetic elements are structurally and functionally independent of the cell's genome. They are autonomous and considered molecular parasites. (Examples of this include some plasmids; see Singh et al. 2021.) Cell death occurs via toxins synthesized by the genetic parasites, which

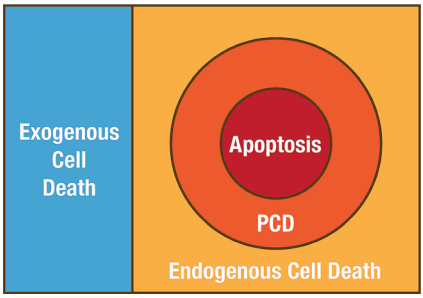

Fig. 1 Apoptosis is nested inside of PCD, which in turn is nested within endogenous cell death

poison host cells attempting to purge themselves of the invading replicons. In a purely mechanistic sense, cell death is caused by an intracellular genetically encoded molecular pathway. Our evolutionary view, which understands PCD to be a kin/group-level adaptation, excludes cell death induced by molecular parasites from PCD (unless, of course, the death is an evolved immunological response triggered by the invading parasite). We have previously argued that such death is more accurately labeled "forced cell death" rather than PCD (Durand and Ramsey 2023) because the replicon and the cell are evolutionarily distinct individuals, and the former forces death upon the latter. Forced cell death is endogenous, but it is not PCD. This illustrates an important distinction between endogenous death and PCD: The former includes any causes of death that arise intracellularly, while PCD concerns the cell as an evolutionary individual and includes only death that is an adaptation for the cell or for the group of cells to which it belongs.

The second example comes from cell fate theory, which describes a cell's fate in terms of the type of cell it "chooses" to become. Totipotent cells select one of four possible trajectories: replication, differentiation, quiescence, and PCD (Casey et al. 2020). Quiescence is one kind of endogenous death since it involves the cell shutting down metabolically and losing viability. The choice of trajectory and mode of death originate endogenously, but these are unrelated to the other three cell type trajectories—one of which is PCD. The cell fate theoretical framework draws a sharp line between two kinds of endogenous death—quiescence and PCD.

As we suggested above (Sects. 3 and 4), it is justified to treat endogenous death and PCD as distinct. Endogenous death is a broader category that includes PCD but also includes cell death induced by intracellular parasites or nonadaptive metabolic exhaustion. Next, we turn to the terms and concepts of cell death that are sometimes posited as alternatives to PCD.

5.1 Alternative Terms for Programmed Cell Death in Microbes: Is Anything Gained?

Cell death plays a central role in the evolution of different life history strategies in unicellular and multicellular organisms. To differentiate the nature of cell death between these two groups, alternative terms for PCD (or PCD-like phenomena) in unicellular organisms are sometimes proposed. We will discuss the three most frequently used labels: *Cell Death Program* (CDP) (Ratel et al. 2001), *Active Cell Death* (ACD) (Nedelcu et al. 2011), and *Regulated Cell Death* (RCD) (Proto et al. 2013). RCD is often favored in the microbial cell death literature, and several authors may use the term in this book. It is also noted that RCD is also used in multicellular literature—where the term originated, but it is often not explicitly stated why the authors prefer RCD over PCD in their particular context. Because of the significance attached to whichever terminology is preferred, it is necessary to examine the

reasons for proposing alternative terms in further detail. There are three main arguments against the PCD term, which we will consider in turn.

In the first argument against PCD, the differences in genetic mechanisms for cell death between microbes and multicellular organisms are emphasized. Most authors agree that some molecular/evolutionary elements of the PCD pathways are shared between multicellular and unicellular organisms, but there are also substantial differences. A new term is sometimes used to reflect these differences. However, it is difficult to justify a new term based merely on such differences. The original conception of PCD was quite broad, encompassing endogenous cellular mechanisms without reference to taxonomic groups or specific genetic toolkits. If PCD applies across the tree of life, introducing a new term for the same concept is misguided. In addition, by suggesting that new terms should be introduced for different mechanisms because of the heterogeneous mechanisms underlying multicellular PCD, this implies that new terms would also need to be introduced for the varieties of multicellular PCD. Furthermore, as we indicated in Sect. 2, unicellular organisms sometimes have multicellular stages in their life cycles, which is a problem for drawing a clear line between taxa based on their cellular organization.

A second reason for proposing alternative terms stems from the close association between PCD and developmental cell death in multicellular organisms. While PCD was first identified in multicellular development, this is not a sufficient reason to restrict the term to multicellular organisms. The vague boundary between multicellularity and unicellularity is thus a further reason to question the value of using an alternative term.

The third motivation for substituting the PCD term with something else, which is the one most commonly offered, relates to the issue of adaptive versus spurious PCD. When the PCD concept was first used in the microbial literature, there were valid reasons for questioning its suitability because of the entrenched idea that microbes are immortal. If unicellular organisms were immortal, then what appeared to be PCD would simply be a pleiotropic side effect (e.g., Nedelcu et al. 2011; Proto et al. 2013). The motivation for labeling the process as *regulated* or *active* as opposed to *programmed* was to draw attention to the nonadaptive property of microbial cell death. It emerged later, however, that death in unicellular organisms can be both adaptive in a current setting by providing a fitness advantage to others (e.g., Durand et al. 2011; Orellana et al. 2013; Zeballos et al. 2023) and an evolved adaptation by demonstrating that the trait is due to its selection history (e.g., Fukuyo et al. 2012; Refardt et al. 2013; Durand et al. 2016). Thus, genuine PCD is a feature of the microbial world. It is also important to note that the assumption that ersatz PCD is not present in multicellular organisms is mistaken. In some instances, one of the most prominent cell death subtypes—autophagy—is a nonadaptive side effect of starvation.

Our analysis suggests that there is very little, if anything, to be gained by introducing alternative terms for PCD in microbial cell death. Doing so introduces an additional (unnecessary) layer of complexity and, in some cases, creates confusion. The justification for the new terms is further undermined by the fact that the

authors often do not indicate why the new terms are being introduced, or even what they mean.

While we are suggesting that PCD remains a well-defined concept that is appropriate across the tree of life, we recognize that the concept should be scrutinized. There are, for instance, legitimate reasons for questioning the use of the term "programmed." The branching, probabilistic nature of biological systems may not be well captured by the term, with its deterministic connotations (Durand and Ramsey 2018). However, this issue represents a general question that concerns biological programs across the tree of life. Furthermore, many programs are branching, not linear—and the branch points can involve probabilities. We suggest, therefore, that despite this controversy, the PCD label is helpful.

Some authors argue for maintaining the PCD term purely because of its metaphorical value. Reynolds (2014) argues that scientific metaphors like PCD are valuable "conceptual 'tools' which fulfill various roles, from highlighting a phenomenon as of particular interest, situating it in a particular context, or suggesting explanatory causal mechanisms" (Reynolds 2014, p. 175). Reynolds describes PCD as a "machine metaphor" and cell suicide an instance of a "social agent metaphor" and suggests that these terminologies have great value for understanding the field. He argues that introducing alternative terminologies may be counterproductive and illustrates that the cell death field is replete with metaphors such as cell "choice," "decision-making," and "individuality" that enrich our understanding of the biology.

6 Toward an Overarching Framework of Cell Mortality

In this chapter, we have examined some of the foundational concepts in cell death and suggest that in order to address the current inconsistencies and ongoing debates in microbial cell death, it is essential that a consensus be reached on these and other fundamental questions. Lastly, in striving for a universal framework of cell mortality, there is a final observation worth emphasizing.

From our analysis of the conceptualizations of PCD, we have concluded that an evolutionary PCD concept is universally applicable and that the purely mechanistic and discipline-specific definitions are more valuable as part of a conceptual toolkit for empirical investigations. Similarly, in a recent article, *Cell Fate: What's evolution got to do with it?* (Ramsey and Durand 2023), we argued that some explanations in biology—we used the example of choice in cell fate theory—are inaccessible to purely mechanistic approaches. Our analysis of the nature of PCD and its conceptualization resonates with these arguments and motivates us to emphasize the importance of including evolutionary concepts as we work toward an overarching framework of cell mortality.

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