INTERNATL MICROBIOL (2000) 3:153–158

REVIEW ARTICLE

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Streptomycetes: a new model to study cell death

Received 8 May 2000
Accepted 5 July 2000

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Summary Colonies of streptomycetes are now viewed as multicellular entities containing morphologically and biochemically differentiated cell types which have specific functions and precise spatial relationships to one another. Like multicellular organisms, colony development in streptomycetes is also maintained by a tight balance between cell proliferation and cell death processes. This review describes the current state of knowledge concerning cell death in streptomycetes.

Key words *Streptomyces* · Programmed cell death · Physiological cell death · Colony development · Apoptosis

Introduction

A major feature of streptomycetes is their ability to carry out a complex developmental life cycle that, phylogenetically, can be considered as one of the probably several evolutionary attempts at multicellularity [9]. They form highly structured multicellular colonies composed of physiologically distinct hyphae (whose development is controlled by a complex system of intercellular communication). Besides, they have evolved separated somatic and germ-line functions through two distinct cell lineages: the substrate and the aerial mycelium. As in higher multicellular systems, the final architecture of the streptomycetes colony is the integrated result of two major physiological processes: those promoting growth and differentiation and those causing senescence and hyphal death. Much of what is currently known on the life cycle of streptomycetes comes largely from studies addressed to identify the mechanisms responsible for growth and hyphal differentiation. By contrast, there has been little or no exploration of the mechanisms underlying cell degeneration and hyphal death.

It is not the purpose of this article to present a comprehensive review of all that is known about colony development in streptomycetes. There are many excellent reviews that can be consulted for such detailed information [8, 9, 10, 20, 29]. Here, we focus on *Streptomyces* colonies considered as multicellular organisms. We will describe some of the key features involved in their development such as the coexistence of different cellular types (each playing a specific function in colony building), and how (to carry out the program of morphological development) the cells communicate with each other by emitting extracellular signals and responding to them. We will summarize the current knowledge about the process of cell death that accompanies mycelial growth, with particular emphasis on the function of this process on colony formation. Finally, we will discuss the similarities between cell death in streptomycetes and in higher eukaryotic systems and will speculate on the possibility that they could have a common evolutionary origin.

The developmental cycle of *Streptomyces*: an overview

The colony growth cycle of the streptomycetes initiates when a spore germinates and produces one or more long multinucleoid filaments [17]. These filaments, which elongate by apical growth [5, 15] and branch repeatedly, originate a vegetative mycelium (substrate mycelium) that develops both on the culture medium and into it. This mycelium grows attached to its substrate, forming an intrincate network of hyphae that penetrates the medium solubilizing organic molecules by the action of extracellular hydrolytic enzymes. Such a filamentous morphology allows full utilization of the solid materials in the soil, and enables the streptomycetes to colonize solid substrates more efficiently than non-motile, unicellular microorganisms. As the colony ages, and the nutrients become exhausted, specialized branches emerge from the surface of the colony, originating the reproductive aerial mycelium that grows upwards, vertically into the air [34].

While the substrate mycelium has a primarily vegetative function, the role of the aerial mycelium appears to be mainly reproductive, forming spores and placing them in a favourable position to be dispersed, thus solving the problem raised by the immobility of the substrate mycelium. By the end of the colony growth cycle, the aerial hyphae undergo multiple

septation which originates chains of uninucleoid compartments which, finally, metamorphose into thick-walled spores [18, 30, 31].

Distinct from the substrate mycelium, the aerial hyphae have been reported to be thicker and less branched, to contain a pigment not found in the substrate mycelia, and to be hydrophobic [20, 29]. Moreover, aerial hyphae develop under quite peculiar environmental conditions. As they grow in a nonaqueous environment, they must be protected from dehydration. The hydrophobic sheath that surrounds these hyphae most likely helps to avoid this problem [12, 29]. Furthermore, the hydrophobic nature of the outer envelope of aerial cells might be relevant to the initial orientation of these mycelial cells and their emergence through the gas-liquid interphase. In fact, a small hydrophobic protein called SapB (which has analogies with the hydrophobins that coat the aerial parts of fungal hyphae [7, 42, 49]), secreted from colonies at the onset of differentiation, coats hyphae at the colony surface, thus helping them to escape from the aqueous environment [47, 48].

As a consequence of the previous growth of the substrate mycelium, aerial hyphae develop on a nutrient-depleted medium, which raises questions about how these hyphae obtain nutrients for growth and sporulation. It has been reported that aerial mycelium develops mainly by cannibalizing the vegetative mycelium [9], the degeneration of which provides the necessary nutrient supply for the aerial hyphae to develop far from the surface of the colony [32, 36]. At this vulnerable stage in development, when the degenerating substrate mycelium might be consumed easily by invading motile microorganisms, chemical defense mechanisms are employed. Indeed, most antibiotics are produced by streptomycetes coincidently with both aerial mycelium formation and degradation of substrate mycelium [6, 11].

An elaborate system of intercellular communication controls when and where hyphal differentiation will occur

Interactions between cells are crucial for an efficient coordination of cell differentiation in higher multicellular organisms. Therefore, the key to bacterial multicellularity lies in the ability of each individual cell to receive and interpret information from its neighbours and to respond to it. In prokaryotes, multicellular development is controlled by means of small, diffusible signalling molecules (sometimes referred to as microbial “hormones”, “pheromones” or “autoinducers” [40, 46]), which inform individual bacterial cells about the presence and physiological status of other members of the same species. Such a signalling mechanism closely resembles that operating in higher organisms, and indicates that bacteria have patterns of multicellular behaviour more complex than might be predicted for unicellular organisms.

Cell interactions are also involved in governing multicellular development and cellular differentiation in the *Streptomyces* colony [23, 27, 47]. Most streptomycetes produce a small range of γ-butyrolactones (analogues of the homoserine lactones), which have been recognized to play a role as signals for the onset of morphological and/or physiological differentiation [3, 11, 23, 24]. Among them, the best known is the A-factor, present in many (but not all) streptomycetes. This γ-butyrolactone was the first streptomycete sporulation factor identified and was first characterized as a stimulator of streptomycin production and spore formation in *S. griseus*. A cytoplasmic protein binds A-factor efficiently, and it has been concluded that morphological, physiological differentiation in this organism is repressed by the A-factor-binding-protein, and the repressor activity is eliminated when the protein binds A-factor [35].

The ability of certain genes to restore aerial mycelium in some *S. griseus bld* mutants (which fail to develop aerial mycelium) defective in production of A-factor suggests that the phosphorylation of two regulatory proteins by cognate membrane-bound protein kinases is important for the A-factor-dependent initiation of aerial growth in *S. griseus* [22]. Beppu [3] has pointed out parallels between the use of protein kinases by *S. griseus* in the γ-butyrolactone response and in eukaryotic signal transduction systems. The identification of a specific receptor for A-factor and an A-factor-controlled promoter sequence in *S. griseus* indicate the close similarity of this system to eukaryotic hormonal control.

Another well-known signal is factor-C of *S. griseus* [41], which restores sporulation of a mutant strain in the submerged culture. Factor-C, produced at a late stage of growth, is a protein that accumulates mainly in the membrane fraction [4]. In addition, the production of the small morphogenetic protein SapB (see above) by *S. coelicolor* and, hence, the formation of aerial hyphae, depends on a series of extracellular molecules that are exchanged between cells by diffusion [9]. Intercellular signalling mechanisms seem to control also antibiotic production. Antibiotic synthesis has been reported to be dependent on γ-butyrolactone accumulation [3, 24].

Two distinct, major groups of physiological processes operate during colony development: those promoting growth and differentiation and those causing senescence and hyphal death

Like most, if not all, multicellular biological systems, colony development in streptomycetes depends on the proper relationships between cell proliferation, cell differentiation, and cell death. Over the colony growth cycle, many hyphae (including the original substrate hyphae and any portion of the aerial mycelium which does not differentiate into spores) degenerate and die. Hyphal death, which primarily provides

nutrients for aerial mycelium development [32, 36], was first described as an indiscriminate process of autolysis [45]. Recent observations, however, have revealed that during the life cycle of the streptomycetes the hyphae die by following two quite distinct sequences of degenerative events: they either autolyse or undergo physiological cell death [33]. Autolytic hyphal death, which affects a minority of the hyphae throughout the colony, is characterized by the early degradation of the cell wall as a consequence of an uncontrolled, lytic action of murein hydrolases (Fig. 1A). Therefore, it is usually violent and presumably very rapid since it results from the physical trauma caused by the rupturing of the plasma membrane and the release of the cellular contents to the extracellular medium.

In physiological cell death, however, the hyphae undergo an orderly process of internal cell dismantling that takes a long time: progressive disorganization of the nucleoid and degradation of DNA and cytoplasmic constituents followed by shrinkage and distortion of the hyphal shape (Fig. 1B). Plasma membranes maintain their integrity for a long time and there is little or no leakage of cellular contents to the extracellular medium, which minimizes the risk of adjoining cells being exposed to potentially harmful substances. Finally, dead hyphae, although empty of cellular contents,

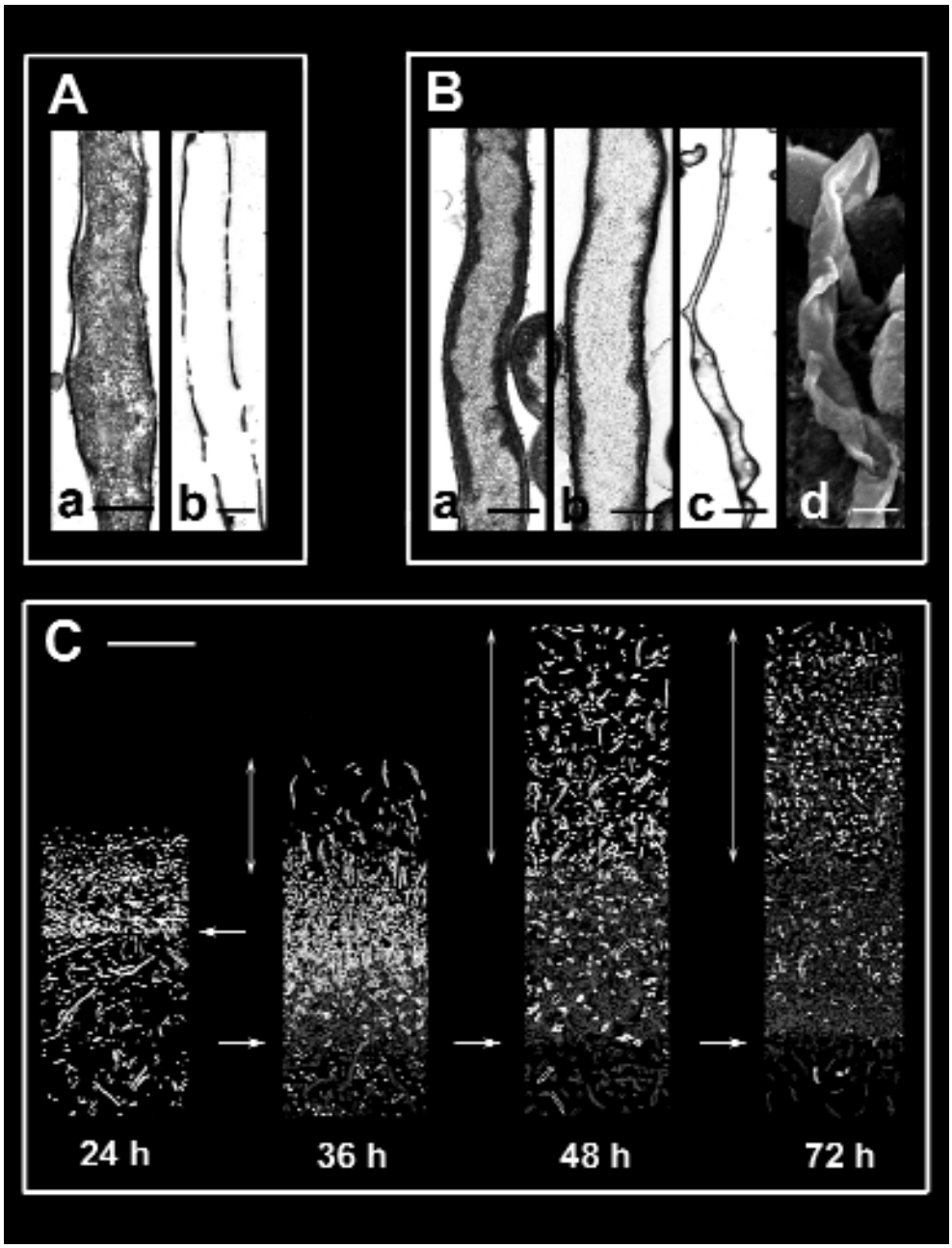

Fig. 1 Morphological features of autolysis (A) versus physiological hyphal death (B). A: (a) early stage of autolysis illustrating rupture of the cell wall and leakage of cellular contents; (b) final stage of autolysis showing large zones of wall rupture. B: (a and b) early stages of hyphal degeneration showing loss of electron density in the cytoplasm and disorganization of the nucleoid substructure; (c and d) final stages of hyphal degeneration showing collapsed hyphae and aberrant shapes. (a-b in A and a-c in B) Transmission electron micrographs. (d) Scanning electron micrograph. Bars: (a-b in A and a, b and d in B) = 0.5 μm; (c in B) = 1 μm. (C) Course of hyphal death during colony development. Vertical ultrathin sections of colonies at different developmental stages showing hyphae undergoing autolysis (hatched cells) and hyphae undergoing physiological cell death (gray cells). Numbers indicate the age of the colony. Vertical arrows mark the aerial mycelium. Horizontal arrows point to the surface of the culture medium. Bar = 20 μm

retain an apparently intact cell wall. Maintenance of cell wall integrity allows the cellular contents to be degraded and reused for growth, and allows the doomed cells to be eliminated without disturbing the general organization of the colony. Another major feature of physiological hyphal death that distinguishes it from autolysis is that it affects most hyphae and occurs in predictable places and at predictable times during development, which suggests that this process is somehow programmed into the developmental plan of the microorganism (Fig. 1C). In fact, there are two rounds of physiological death during colony development [33]. The first round coincides with development of the aerial mycelium, and causes massive death in the substrate mycelium, but has no apparent effect on the emergent aerial hyphae. The second round is not triggered until sporulation has been initiated and is more selective, since it only affects the basal nonsporulating parts of the aerial hyphae.

Physiological hyphal death serves many functions during colony development

Physiological cell death provides an efficient mechanism to eliminate unnecessary cells, either because they may have been produced in excess or because they may have served a function at some time, but are no longer needed [25, 37, 38]. Like multicellular organisms which have separate germ cells and somatic cells, streptomycetes have found different uses for physiological hyphal death.

For example, it may help to adjust population sizes continuously in accordance with the availability of nutrients and to select the most appropriate. In this respect, hyphal death not only provides a source of nutrients [32], but also results in more availability of the nutrients present in the culture medium to the survivors. Therefore, it would ensure continued efficient growth of the mycelium as a whole. This could be crucial for a densely-packed, hyphal population that grows firmly attached to the substratum (as is the case of the substrate mycelium), where cells are forced to compete with one another for a limited amount of nutrients. What is more, dead hyphae of streptomycetes do not completely disappear, but remain as part of the colony structure, where they still could potentially perform two additional roles: they can provide mechanical support for aerial hyphae to develop far from the surface of the culture medium and, as dead hyphae retain the cell wall, they could serve to conduct water and solutes through the colony (a process of considerable importance for the aerial mycelium which develops in the absence of a surrounding liquid medium).

Hyphal death in streptomycetes compared to cell death in higher eukaryotic systems

Whereas cell death phenomena in streptomycetes have some traits of their own [33], they also display striking similarities with those occurring in the higher eukaryotic developmental systems. Thus, whereas physiological hyphal death mimics many aspects of apoptosis, autolysis shows similarities to necrotic cell death. In fact, like apoptosis in higher organisms, physiological hyphal death appears to be a developmentally controlled mechanism that allows many unneeded, aged or damaged bacterial cells to be eliminated without loss of cell contents to the exterior and, therefore, with little or no apparent toxic effect on the immediate neighbouring cells. Autolysis, on the other hand, resembles necrotic cell death in that it is rapid and violent and seems to be solely the outcome of severe and acute cell injury. A comparison between these two forms of hyphal death is given in Table 1.

The mechanism responsible for physiological cell death in streptomycetes still remains unknown. A number of plasmids carrying genes capable of killing their host have been reported to be responsible for phenomena of programmed cell death in bacteria [13, 26, 50]. Such plasmid-encoding killer-systems consist of a pair of genes that code for two components: a stable toxin and a labile antitoxin that prevents the action of the former. As the unstable antitoxin is degraded faster than the more stable toxin, they cause selective killing of the plasmid-free population of cells [13]. Note that two such killer plasmids seem to be present in streptomycetes [21]. However, whether programmed bacterial death is similar to physiological hyphal death remains to be established.

Table 1 Physiological hyphal death versus autolysis: a comparison

Physiological hyphal death	Autolysis
Cell wall integrity maintained	Cell wall destroyed early
Plasma membrane maintains integrity for a long time	Early rupture of the plasma membrane
Distinctive pattern of nucleoid disorganization	Nuclear changes are unremarkable
Little or no leakage of cellular content	Leakage of cellular content
Shrinkage and distortion of hyphal shape	Loss of cellular integrity
Proceeds over a long period of time	Usually very rapid
Occurs predictably at specific sites and times	At random throughout the colony
Programmed or developmentally controlled	Accidental or poorly regulated

Regardless of the exact mechanism which causes hyphal death, the present observations reveal that multicellular behavior of streptomycetes resembles that of higher organisms much more than had been assumed previously. They add new support to the hypothesis that the basic structure of the cell death processes has been preserved and extended throughout evolution [2, 19, 25, 43, 44]. A process of this kind in an ancestral prokaryote may have provided the starting point from which death programs in animal cells eventually evolved. The finding that mitochondria could have a role in the control of apoptosis in eukaryotes opens new perspectives on the evolution of the process of cell death in biological systems [1, 14, 16, 28].

Conclusions and future directions

A new scenario for the bacterial cell death phenomenon is emerging, and the exciting results now raise major questions that should be addressed in future investigations. For example, what triggers physiological bacterial death and what are the components of cell death machinery? How is cell death controlled in time and space? Do the survivors represent cells that enter a "death resistant program"? Are specific signalling molecules involved in controlling which cells die? If so, could such hypothetical signalling molecules be useful to interfere with the apoptosis signalling pathway of higher cells? If cell death is a corollary to multicellularity [25, 37] and multicellular development seems to be the rule among bacteria [39], are physiological cell death processes also present in other, simpler bacterial systems as for example *Escherichia coli*?

The colony growth cycle of the streptomycetes provides an excellent prokaryotic experimental system for the study of the mechanism of cell death and its role in development. Such studies may provide insights both into the role of cell death in more complex eukaryotic systems and into the evolution of this phenomenon. Moreover, a better understanding of prokaryotic cell death could have major implications for research areas ranging from bacterial physiology to pathogenesis and to industrial microbiology.

Acknowledgments This work was supported by grant PM97-0197 from the CICYT, Spain.

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