Actinoplanes Swims into the Molecular Age

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ABSTRACT The survival strategy of Actinoplanes is fascinating from an evolutionary perspective, combining a short motile phase in an otherwise nonmotile, filamentous life cycle and the somewhat paradoxical concept of spores—normally thought of as a resting stage—that swim. In the first paper to report a molecular genetic analysis of development in Actinoplanes, the authors identify a key regulator of the entry into development (Y. Mouri, K. Konishi, A. Fujita, T. Tezuka, Y. Ohnishi, J Bacteriol 199:e00840-16, 2017, https://doi.org/10.1128/JB.00840-16).

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The rich variety and extraordinary beauty of the reproductive structures produced by different filamentous actinomycetes are wonderfully illustrated in the hundreds of scanning and transmission electron micrographs found in the Digital Atlas of Actinomycetes (http://www.actino.jp/DigitalAtlas/) (1). These reproductive structures vary considerably, from the single spores born on short hyphal side branches seen in Micromonospora, to the two parallel rows of banana-shaped structures seen in Planomonospora, each carrying a single large cylindrical spore inside, through to large saclike sporangia. Until the work published by Mouri et al. in this issue of Journal of Bacteriology (2), the only filamentous actinomycetes whose reproduction has been subject to molecular genetic analysis were the streptomycetes. Bacteria of the genus Streptomyces make reproductive structures called aerial hyphae. Each multigenomic aerial hypha undergoes a massive cell division event, synchronously laying down dozens of transverse sporulation septa at regular intervals along the filament to create a chain of unigenomic compartments that ultimately mature into rounded, thick-walled spores (3–5). The developmental biology of Streptomyces is widely studied. In contrast, other filamentous actinomycetes are usually only a focus for research if they produce an antibiotic or other natural product of potential medical relevance, and their developmental biology is not analyzed. It is for this reason in particular that the work reported by Mouri et al. (2) on reproduction in Actinoplanes is so refreshing.

Actinoplanes species are a rich source of natural products (6–10), including the clinically important antibiotic teicoplanin (11) and the alpha-glucosidase inhibitor acarbose, a potent drug used worldwide in the treatment of type 2 diabetes (12). The substrate mycelium of Actinoplanes colonies consists of branching vegetative hyphae. Each multigenomic aerial hypha undergoes a massive cell division event, synchronously laying down dozens of transverse sporulation septa at regular intervals along the filament to create a chain of unigenomic compartments that ultimately mature into rounded, thick-walled spores (3–5). The developmental biology of Streptomyces is widely studied. In contrast, other filamentous actinomycetes are usually only a focus for research if they produce an antibiotic or other natural product of potential medical relevance, and their developmental biology is not analyzed. It is for this reason in particular that the work reported by Mouri et al. (2) on reproduction in Actinoplanes is so refreshing.

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while in other species, like *Actinoplanes missouriensis*, the sporogenic hyphae seem to coil irregularly inside the envelope, leading to a more random arrangement of rounded spores (Fig. 1B). Within the sporangium, the spaces between spores are filled with an intercellular matrix, clearly visible in Fig. 1B. The spores are released when the sporangia burst open on contact with water, in a process known as dehiscence. However, pure water is not sufficient: additional undefined compound(s) present in soil extracts are required to induce dehiscence (18).

All of this is remarkable enough, but when the sporangia burst open, the spores they release are flagellated so-called “zoospores” that swim (http://www.actino.jp/DigitalAtlas/) (1, 19, 20) (Fig. 1C and F). These motile zoospores are chemotactic, swimming toward amino acids, sugars, aromatic compounds, and inorganic ions (21, 22). They have enough energy to swim for 2 to 3 h, during which time they must find nutrients or die. If the zoospores succeed, they germinate to regenerate a filamentous vegetative mycelium. In addition to *Actinoplanes*, bacteria belonging to at least four other genera of filamentous actinomycetes—*Catenuloplanes*, *Planomonospora*, *Kineospora*, and *Spiroillospora*—are known to produce motile, flagellated spores (http://www.actino.jp/DigitalAtlas/).

The group of Yasuo Ohnishi at the University of Tokyo has successfully taken up the challenge of studying the developmental biology of *Actinoplanes*, using *Actinoplanes missouriensis* as a model system (23, 24). In Mouri et al. (2), starting from a proteomics-based approach, they show that a transcriptional regulator called BldD plays a critical role in the *Actinoplanes* life cycle. By conjugating constructs from *E. coli* into *A. missouriensis*, they were able to construct a bldD null mutant and found that it entered development prematurely, making abundant sporangia much earlier than the wild type. These sporangia were abnormal, having distorted shapes, and occasionally, naked spores were seen that appear to have arisen from the differentiation of sporogenic hyphae without the formation of the sporangial envelope that would normally encompass them. In addition, on a medium that does not support differentiation of the wild type, the bldD mutant produced immature sporangiumlike structures. These results

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**FIG 1** Electron micrographs of the reproductive structures and motile zoospores of *Actinoplanes missouriensis* (courtesy of Yasuo Ohnishi, Aiko Hirata, and colleagues) (A to C) and *Actinoplanes regularis* (reproduced with permission from http://www.actino.jp/DigitalAtlas/), courtesy of Gernot Vobis, Naoki Muto, Ken-ya Ishizawa, Takao Okazaki, Ryuzo Enokita, and The Society for Actinomycetes Japan) (D to F). (A, D) Sporangia arising from the vegetative mycelium. (B, E) Thin sections through the sporangia showing immature zoospores. (C, F) Motile zoospores with numerous flagella originating from a small patch of the cell surface. The scale bars are 2 μm (A to C) and 5 μm (D).
suggest that BldD functions to inhibit entry into development in Actinoplanes. Consistent with this conclusion, Mouri et al. (2) go on to use chromatin immunoprecipitation sequencing (ChIP-seq) to identify a large regulon of genes directly under the control of BldD, and their transcriptional profiling shows that many of these target genes are repressed by BldD during vegetative growth. Strikingly, some of these BldD-repressed genes encode orthologs of transcriptional regulators known to be required for sporulation in Streptomyces. In addition, another target of BldD repression in Actinoplanes is ssgB, an actinomycete-specific protein involved in recruiting FtsZ to the sites of sporulation septation in Streptomyces (25).

BldD is present not just in Actinoplanes but in all filamentous actinomycetes (Fig. 2) (26), and it has been the subject of extensive study in Streptomyces, where it also serves to inhibit entry into development by repressing a large regulon of sporulation genes during vegetative growth (27–30). In Streptomyces, BldD is a cyclic di-GMP (c-di-GMP) effector protein, and it represses its targets in a manner that depends on its binding to c-di-GMP (29, 30), one of the most important and widespread second messengers in bacteria (31). Thus, it is not BldD but a BldD–c-di-GMP complex that blocks differentiation, and the crystal structure of this complex has been determined. Remarkably, Streptomyces BldD binds not one but four molecules of c-di-GMP, tightly packed together in a tetrameric cage (29). Binding to this c-di-GMP tetramer is specified by just four key amino acid residues in BldD, two arginines and two aspartates, arranged in a bipartite RXD-X8-RXXD motif.

Mouri et al. (2) noted that the RXD-X8-RXXD c-di-GMP-binding signature observed in the Streptomyces BldD–c-di-GMP crystal structure is conserved in Actinoplanes. As a consequence, they used electrophoretic mobility shift assays (EMSA) to see if c-di-GMP might also control the activity of Actinoplanes BldD and found that it greatly enhanced the binding of Actinoplanes BldD to its target DNA. In fact, the bipartite RXD-X8-RXXD c-di-GMP-binding signature is present not just in Actinoplanes and Streptomyces BldD, it is perfectly conserved in all BldD proteins, which are found throughout the filamentous actinomycetes. In addition, all filamentous actinomycetes have c-di-GMP (the unicellular actinomycetes like mycobacteria, corynebacteria, and rhodococci have c-di-GMP but not BldD) (Fig. 2). Together, these observations suggest that c-di-GMP is likely to signal through BldD to control entry into development, not just in Actinoplanes and

**FIG 2** Distribution of BldD and c-di-GMP in the Actinobacteria. Based on available genome sequence information, both BldD and c-di-GMP are present in all the sporulating actinomycetes (shaded green). In contrast, the unicellular actinomycetes, such as mycobacteria (pink), corynebacteria (beige), and rhodococci (blue), have c-di-GMP but lack BldD. The image shows a cladogram in the form of an unrooted binary tree.
Streptomyces but in all the filamentous actinomycetes. It will be exciting to see if engineering an increase or decrease in c-di-GMP levels can be used to, respectively, block or accelerate entry into sporangium formation in Actinoplanes.

A. missouriensis encodes 58 enzymes involved in c-di-GMP metabolism (28 c-di-GMP biosynthetic enzymes, 3 degradative enzymes, and 27 multidomain proteins predicted to have both activities), a very large number in comparison to the 10 such enzymes found in Streptomyces venezuelae. Perhaps c-di-GMP has a wider role in Actinoplanes that extends beyond development to the control of zoospore motility. In this regard, it is striking that one gene in A. missouriensis, AMISS55850, encodes a predicted methyl-accepting chemotaxis protein that also has a PilZ domain. PilZ domains bind c-di-GMP, and PilZ domain-containing proteins are the best-characterized class of c-di-GMP effector proteins (31). It is therefore tempting to speculate that the Actinoplanes life cycle might combine c-di-GMP-mediated regulation of differentiation, as seen in the streptomyces, with c-di-GMP-mediated regulation of motility, as seen in the gamma-proteobacteria.

In most actinomycetes, the production of antibiotics is temporally and genetically coordinated with morphological differentiation. Bearing this in mind, it is important to note that in another filamentous actinomycete, Saccharopolyspora erythraea, BldD not only controls morphological differentiation, it also directly regulates the expression of the gene cluster that specifies the biosynthesis of the clinically important antibiotic erythromycin (32, 33). Given that members of the genus Actinoplanes are the source of teicoplanin and acarbose, it will be exciting to find out in future if the developmental processes regulated by BldD in Actinoplanes extend to the control of their secondary metabolism.

Finally, for those of us who work on Streptomyces, it is fair to say that bacterial movement is not often central to our thinking. Already this year, however, the exciting discovery of explorer cells in Streptomyces (34) and now this ground-breaking analysis of zoospore-producing Actinoplanes will force us to broaden our thinking in a very welcome way.

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REFERENCES


