

Beyond Binary Fission: Some Bacteria Reproduce by Alternative Means

Some gram-positive bacterial symbionts form multiple offspring intracellularly in a process resembling endospore formation

Esther Angert

Many bacteria reproduce by binary fission, with each cell doubling in size, replicating and segregating its genetic material, and dividing to form two equivalent daughter cells. However, some bacteria follow alternative reproductive strategies, and researchers are steadily gaining insights about some of these other processes.

My collaborators and I study two groups of related low-G+C, gram-positive bacteria that have the extraordinary capacity to produce multiple offspring inside mother cells. Our findings suggest to us that symbiotic associations drove the development of reproductive strategies that appear to be based on, and certainly resemble, how endospores form.

Epulopiscium and Related Intestinal Symbionts from Surgeonfish

Epulopiscium spp. are large, bacterial symbionts found in the intestinal tracts of several species of tropical marine surgeonfish belonging

to the *Acanthuridae* family. These bacteria tend to be associated with herbivorous and detritivorous surgeonfish, and they probably help digest the food that their host fish consume. *Epulopiscium* cells are cigar-shaped and can reach lengths in excess of 600 μm , which is slightly bigger than the period at the end of this sentence.

The first examples of these unusual microbes were discovered in the brown surgeonfish, *Acanthurus nigrofuscus*, of the Red Sea, by researchers Lev Fishelson of Tel Aviv University in Tel Aviv, Israel, Linn Montgomery of Northern Arizona University (NAU) in Flagstaff, and Arthur Myrberg, Jr. at the University of Miami in Miami, Fla.

These early studies helped characterize this symbiotic association, as well as the peculiar biology and ultrastructure of these microbes. Subsequently, Linn Montgomery and Peggy Pollock of NAU named the intestinal symbionts *Epulopiscium fishelsoni* and described in detail their unusual mode of reproduction.

A later survey of intestinal biota of tropical reef fish from the South Pacific revealed a variety of microbes similar to *Epulopiscium*, all of which are specifically associated with surgeonfish, according to a team of biologists from James Cook University in Townsville, Australia, including Kendall Clements (now at the University of Auckland in New Zealand), David Sutton, and Howard Choat. They classified these intestinal symbionts based on cell shape, size, the number of internal offspring they produce, or whether they reproduce through binary fission. They assigned the “A” morphotype to large cells similar to *E. fishelsoni* that associate with several species of Pacific surgeonfish.

Esther Angert is an Assistant Professor in the Department of Microbiology at Cornell University, Ithaca, N.Y.

Summary

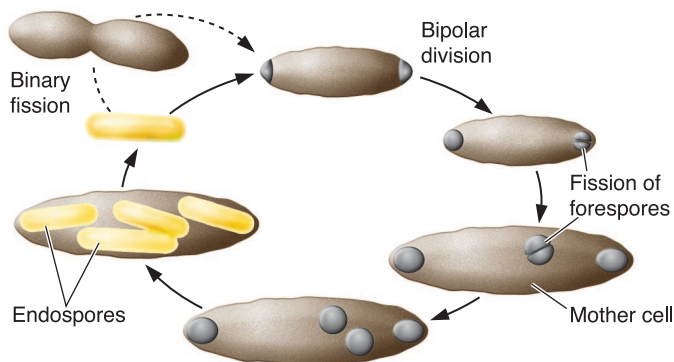
- Some microbes, including two groups of related low-G+C, gram-positive, typically symbiotic bacteria, reproduce not by binary fission but by producing multiple offspring inside mother cells.
- Viviparity in *Epulopiscium* resembles spore-forming steps in *Bacillus subtilis*.
- In general, growth, development, and reproduction in symbiotic *Epulopiscium* spp. appear to be coordinated with the feeding behavior of the host.



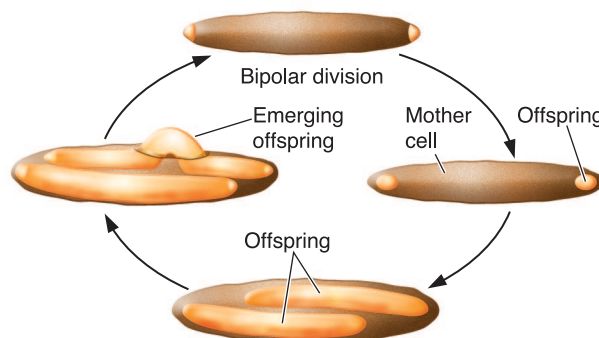
FIGURE 1

A *Metabacterium polyspora*

Limited binary fission; forms multiple endospores


B *Epulopiscium* sp. type B

No binary fission; shared mechanisms with sporulation


 Reproduction in the gram-positive symbionts *Metabacterium polyspora* and *Epulopiscium* sp. type B.

Putting *Epulopiscium* into a Phylogenetic Context

To place these unusual intestinal microbes in a phylogenetic context Clements, Norm Pace, who is now at the University of Colorado, Boulder, and I compared the sequences of the small-subunit ribosomal RNA genes of several large *Epulopiscium* morphotypes to sequences available in GenBank. According to our analysis, *Epulopiscium* spp. belong to the low-G+C, gram-positive bacteria, a surprising result in itself. Moreover, *E. fishelsoni* consists of at least two genetically distinct populations of closely related bacteria.

Because none of these diverse and unusual intestinal symbionts is available in culture, the only practical way to study their life cycles is to survey intestinal populations from fish that are sacrificed at different times. Based on such a survey, Montgomery and Pollack at NAU learned that large, morphotype A *Epulopiscium* symbionts produce offspring in a predictable circadian cycle. Thus, in fish that are collected early during a day, small internal offspring are found closely associated with the tips of an *Epulopiscium* mother cell. The average size of the offspring, relative to its mother cell, increases throughout the day. By late afternoon, the mother cell envelope splits, releasing the offspring and killing the mother cell. The average cell size decreases in *Epulopiscium* populations surveyed at night. Montgomery and Pol-

lack concluded that this was due to an interval of binary fission.

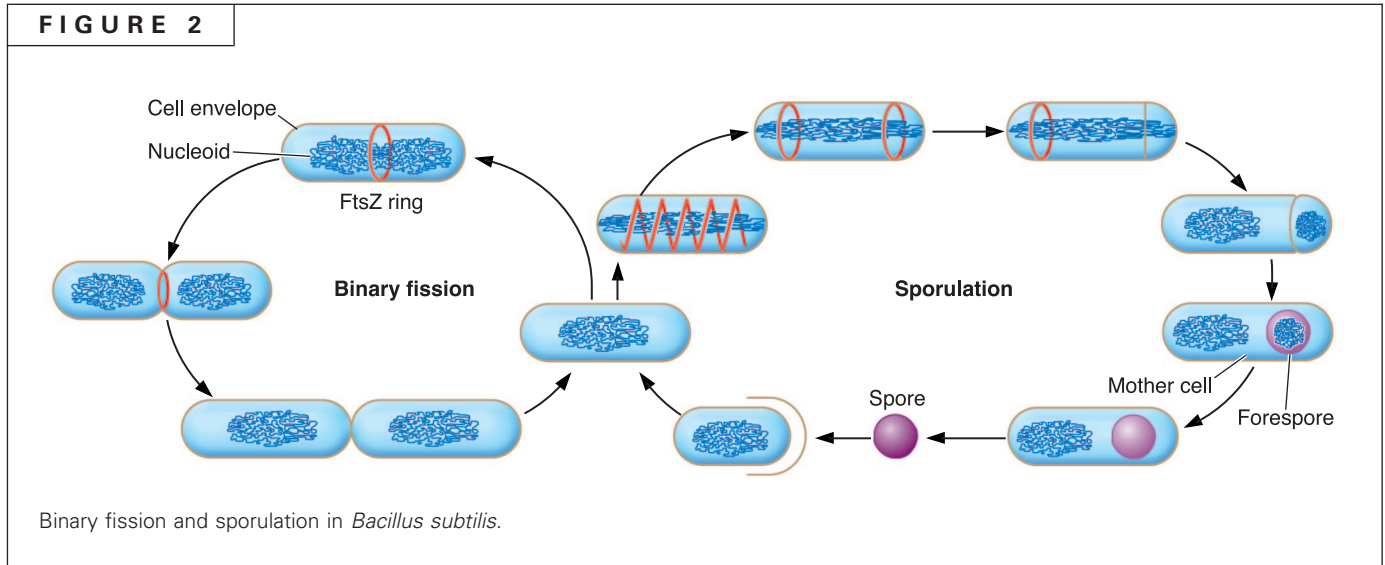
However, other genetically distinct *Epulopiscium* spp. and related surgeonfish symbionts follow a variety of other life cycles. We are focusing on how B morphotype *Epulopiscium*, which are symbionts within *Naso tonganus*, form offspring. These B morphotype cells attain lengths of 200 to 300 μm , produce as many as five intracellular offspring per mother cell, but never seem to reproduce by binary fission. Based on surveys of populations collected at different times of the day, B morphotypes also form offspring following a circadian cycle. Even though we collect symbionts from surgeonfish caught in the wild, the B morphotype appears to be genetically homogenous, particularly compared to other *Epulopiscium* communities.

Related Symbionts, *M. polyspora*, Reproduce by Forming Endospores

Clues about the origins of viviparity—a process by which active offspring are formed internally—in *Epulopiscium* come from analyzing *Metabacterium polyspora*, which are intestinal symbionts of guinea pigs. These bacteria are 12–35 μm long and produce as many as nine phase-bright endospores per mother cell. Based on small-subunit rRNA sequence comparisons, *M. polyspora* is one of the closest known relatives of *Epulopiscium* spp.

The striking resemblance between *M. poly-*

FIGURE 2



Binary fission and sporulation in *Bacillus subtilis*.

spora endospores and the internal offspring of *Epulopiscium* led us to suspect that these reproductive programs are related. For *M. polyspora*, which form spores as part of their life cycle, making multiple endospores is an important means of reproduction (Fig. 1A).

In 1998, Richard Losick of Harvard University in Cambridge, Mass., and I discovered that *M. polyspora* coordinates endospore development with its passage through the gastrointestinal (GI) tract of its host. Only mature endospores survive passage through the stomach, then germinate in the guinea pig small intestine.

For a brief period after *M. polyspora* spores germinate, they may undergo binary fission (Fig. 1A). However, many cells in a population bypass vegetative division and instead form endospores. From the small intestine, *M. polyspora* cells pass into the cecum, a blind sac at the junction between the small and large intestine, and the predominant organ of the guinea pig GI tract. Here the endospores develop further. Eventually *M. polyspora* cells pass from the cecum through the colon and exit the guinea pig.

Like many herbivores that eat high-roughage diets, guinea pigs are coprophagous, allowing them a second chance at extracting nutrients from feed. This process also provides *M. polyspora* a means of reentering the GI tract. As a dormant and resilient endospore, *M. polyspora* survives the world outside the guinea pig and passage through the upper GI tract.

Simple changes in the *M. polyspora* core sporulation program allow cells to form more

than one endospore. As *M. polyspora* begins to sporulate, it divides at both poles. Some cellular DNA is packaged in each of the forespores, while some remains in the mother cell. Both forespores are engulfed by the mother cell and gain the capacity to undergo binary fission. By combining bipolar division and forespore fission, *M. polyspora* produces multiple forespores, which grow and mature into multiple phase-bright endospores. These spores, like other endospores, contain dipicolinic acid and have a mineralized core, a cortex, and thick coat. Each sporulating *M. polyspora* cell contains at least three copies of its genome. In addition, the mechanisms that withhold division at the second pole in most endospore formers must be relieved in *M. polyspora*.

Viviparity in *Epulopiscium* Resembles Spore-Forming Steps in *B. subtilis*

Large, type-B *Epulopiscium* offspring cells begin to develop their offspring while they are still held within their mother cell (Fig. 1B). Asymmetric cell division forms tiny disc-shaped cells at the poles of each cell. These offspring primordia at first are approximately 4 μm in diameter and 1 μm thick, or about 1/45,000 the size of their mother cell. DNA is partitioned into the offspring cells during the division process. The mother cell then fully engulfs its polar offspring cells. Eventually, the offspring become free protoplasts within the mother cell cytoplasm, leav-



An Overview of Sporulation in *Bacillus subtilis*

When a *Bacillus subtilis* cell begins to sporulate, it forms an axial filament consisting of the replicated chromosome and associated proteins. At this stage, the axial filament extends the length of the cell and the origins of the two daughter chromosomes are fastened to opposite cell poles. The protein RacA, which is produced only during sporulation, mediates this remodeling and anchoring.

Next, the protein FtsZ travels from the midcell via a spiral intermediate, forming two rings, one near each cell pole. Although the division complex assembles at both locations, division occurs at only one. The division septum closes in around the axial filament, trapping about one-third of one chromosome in the smaller cell partition. The rest of the chromosome extends back through the division septum and into the larger compartment. A DNA translocase, SpoIIIE, in the division septum, assembles around the trapped chromosome, helping to pack it into the smaller cell. Each daughter cell now contains a chromosome, and the unused division apparatus soon dissipates.

The smaller cell formed by this asymmetric cell division, referred to as the “forespore” or “prespore,” is destined to become the mature spore. The larger “mother cell” also participates in subsequent spore development, engulfing the forespore after it receives its chromosome. The spore matures within the cytoplasm of the mother cell, where it is prepared for dormancy and to withstand environmental assaults it may encounter before being reanimated. Meanwhile, after supporting endospore development, the terminally differentiated mother cell dies.

ing substantial voids in the mother cell nucleoid network.

In collaboration with Kendall Clements, we are studying whether offspring development in type B *Epulopiscium* employs mechanisms like those in *Bacillus subtilis* (Fig. 2) when it begins to form an endospore. Conserved features include bipolar localization of rings of FtsZ, the remodeling of cellular DNA to accommodate development, and possibly the packaging of DNA in small polar cells. Key differences include division at both cell poles in *Epulopiscium*, its lack of a medial Z ring, and the presence of more than two copies of the *Epulopiscium* genome to accommodate the formation of multiple offspring cells. In B-morphotype *Epulopiscium* cells, DNA typically is found just under the cell membrane. Some of the DNA relocates to polar locations when offspring begin to form.

Rings of FtsZ form at both poles of the *Epulopiscium* mother cell. We have never observed a

medial FtsZ ring or Z spirals in *Epulopiscium*, although it is possible that spirals are present and we are unable to detect them using standard immunofluorescence techniques. Since a medial Z ring is not required in *Epulopiscium*, the way in which polar Z rings assemble may differ from that of *B. subtilis*.

Division routinely occurs at both poles in *Epulopiscium*, either simultaneously or in rapid succession. The division planes slice through the pole-associated DNA, trapping some of it in the newly formed cells. DNA rapidly accumulates within offspring cells. We do not know whether this process involves a DNA packaging mechanism homologous to that in *B. subtilis*, or whether the *Epulopiscium* mother cell DNA is being degraded while the offspring primordia DNA replicates.

Viviparity also Linked to Endospore-Forming, *Epulopiscium*-like Surgeonfish Symbionts

In collaboration with Linn Montgomery and Gordon Southam of the University of Western Ontario, Joe Flint, Dan

Drzymalski, and I are studying how endospores form in several *Epulopiscium*-like symbionts of surgeonfish caught off Hawaii. These findings further tighten the link between live offspring and spore formation.

Consider the life cycles of the C and J morphotypes of *Epulopiscium*-like symbionts of *Naso lituratus*. The C morphotype, which are cigar-shaped cells, 40 to 130 μm in length, appears never to use binary fission. The J morphotype, which are filamentous cells, 10 to 400 μm long, regularly uses binary fission.

Endospores form daily in both of these morphotypes—remarkably, only at night. The C morphotype produces two endospores, apparently its sole means of reproduction. This life cycle strategy may reinforce its association with surgeonfish by providing a means to transport symbionts safely to new hosts. Further, by forming spores, resident populations of *Epulopiscium*-like cells can survive in a dormant state when their hosts are not actively feeding. The deteriorating mother cells that are cast off early

each morning may also be providing nutrients to hosts. The J morphotype populations do not rely on the daily sporulation cycle for reproduction. Therefore, type-J populations may be better able to take advantage of fluctuations in nutrients.

Sometime during the evolution of low-G+C, gram-positive bacteria, cells began accumulating the genetic wherewithal to form dormant and highly resistant endospores. For many endospore-forming bacteria, this complex developmental program is invoked only after nutrient sources are exhausted. Because the endospore is metabolically quiescent and highly dispersible, it can survive harsh environmental conditions and may even be transported to other environments where conditions are more favorable for growth.

Some low-G+C, gram-positive bacteria that produce a single endospore exhibit many of the same morphological stages as does *B. subtilis*. Hence, many researchers believe that endospore-forming bacteria share a common evolutionary history. However, such bacteria do not carry all the genes that are essential for *B. subtilis* cells to form spores.

For example, activation of the highly conserved transcriptional regulator Spo0A triggers spore formation. In *B. subtilis*, a phosphorelay system, comprised of a network of kinases, integrates information about a cell's physiological state and environment. For sporulation to begin, these kinases must successfully phosphorylate

the Spo0A pool. Because spore-forming *Clostridium* spp. do not encode these phosphorelay kinases, it is likely that clostridia cells convey such information to Spo0A through different sensory proteins.

With *M. polyspora*, simple changes in the core sporulation program enable *M. polyspora* to form multiple endospores in a single mother cell, relying on this mechanism instead of binary fission as a primary means for reproduction. Endospore formation coordinates with cycling of the symbionts through the host GI tract. Some populations of surgeonfish symbionts develop endospores or active, intracellular offspring following a circadian cycle. In a host fish, such as *N. tonganus* that maintains a resident *Epulopiscium* population, the symbiont does not need to cycle through the upper GI tract on a regular basis and thus may not form resilient endospores. For other surgeonfish, endospore formation may enhance recurrent acquisition of symbiont populations. In general, symbiont growth and development appear to be coordinated with the feeding behavior of the host.

In these closely related intestinal symbionts, we can analyze the apparent emergence of alternative means for bacterial cells to reproduce. By comparing related organisms, we hope to understand how a key developmental program changes to support novel reproductive strategies that enhance or reinforce specific associations between intestinal symbionts and their hosts.

ACKNOWLEDGMENTS

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