NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Desperate Times Call for Desperate Measures: Sporulation in *Bacillus subtilis*

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Part I – Sporulation as a Survival Mechanism

As the lab meeting began, Susan struggled to pay attention and stay awake while Dr. Berg gave an update of the current status of her lab's work on bacterial development. It wasn't that Dr. Berg was boring; Susan loved working in her lab and was fascinated by the research they were doing. She was exhausted because she had stayed up late putting together the results of her recent experiments to present. She was also distracted by the figure in front of her, as she was still trying to make sense of her data. The longer she stared at the graph, the more confused she became. Soon, her weariness overcame her and she could no longer keep her eyes open. As Dr. Berg started discussing the pathway of sporulation in *Bacillus subtilis*, Susan drifted off to sleep.

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When she opened her eyes, Susan saw Dr. Berg and her lab mates John and Marcos, but they were no longer sitting around the table in the conference room, they were sitting in the sand on a small tropical island. While this locale might have been considered paradise under other circumstances, it was clear to Susan that this was no vacation. Out in the ocean, in the distance, she saw the remnants of a crashed plane. Judging by the ragged look of the group, their sun burnt skin, and tattered clothes, it seemed that they had been stranded for quite a while. Susan was sweating profusely and her skin sizzled from the intense heat, even though she sat in the shade beneath a palm tree. Her throat was dry and scratchy and her stomach ached with hunger, making her acutely aware of their lack of food and fresh water. They had managed to sharpen some sticks into crude spears, but were unable to find any fish or game on which to use them. They were worried that they might starve before they could be rescued.

As the group faced their struggle for survival, Susan could not help but think about the extreme survival mechanisms of the bacteria that they had been researching in the lab. If they were like the *B. subtilis* bacteria they studied, these conditions would not necessarily be a problem. The lack of nutrients and water would certainly inhibit normal growth, but *B. subtilis* could undergo a process called sporulation to transform itself into an endospore, a dormant structure that would allow it to withstand the harshest of conditions for extended periods of time until prospects improved and normal growth could resume. Susan and her lab mates had induced endospore formation in *Bacillus* by depriving cells of nutrients. They tested the hardiness of the endospores and found they were highly resistant to environmental stresses such as high temperature, strong acids, disinfectants, antibiotics, and irradiation. There were even rumors of bacterial endospores found trapped in amber for 25 million years that were still able to germinate into vegetative cells; it seemed they were the ultimate survival machines. The conditions the group was facing on the island, 90°F temperatures, UV rays from the sun, and a lack of food or water, would be a walk in the park for *B. subtilis* endospores.

Questions

1. Describe bacterial sporulation in your own words.
2. Why would it be beneficial for bacteria to form endospores?

Part II – To Sporulate or Not to Sporulate

As their situation on the deserted island grew more desperate, Susan thought about what she wouldn't give to be able to enter an endospore-like state. She could leave all of the pain, hunger, and thirst behind and shut down her body. She would be protected until she was rescued and could resume her life. She would awaken from her dormant state, safely back home in her air-conditioned house where she could drink glass after glass of cool water, and eat her way through her entire refrigerator. These thoughts distracted her from her worries for a moment, but she knew that even if she were a *Bacillus subtilis* cell that could undergo sporulation, it would not actually mean survival for her. . . but it could mean survival for her unborn child.

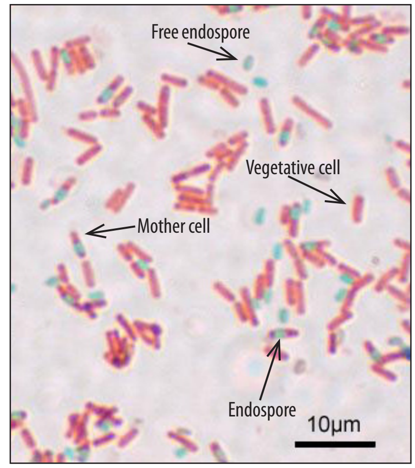

Figure 1. Endospore stain of *Bacillus subtilis* cells at 1000× magnification. Vegetative cells are stained pink and endospores are stained green. *Source:* Modified from http://commons.wikimedia.org/wiki/File:Bacillus_subtilis_Spore.jpg, Y tambe, CC BY-SA 3.0.

Susan was five months pregnant. She had just found out she was having a little girl. Susan's concerns for survival were more for her child than for herself. Now her plight truly did mirror that of the *B. subtilis* cell that undergoes sporulation. Once the sporulation pathway is triggered, the cell undergoes a cell division and becomes a mother cell, providing nutrients and directing the development of an endospore. The process is not easy; it takes a great deal of time and energy, is irreversible after a point, and ultimately leads to death of the mother cell to release the protective endospore.

Susan wondered what would happen if she could undergo "sporulation" to protect her child? It would mean she would have to give everything left of herself: her time, nutrients, energy, and ultimately her life. Surely, it would be worth the sacrifice to give her child a future. But what if she started the process of producing her own little "endospore" and a rescue ship showed up? By that time, it might be too late for her. She would already be committed to her fate of sacrificing herself to protect her little one. And once her endospore was formed, how long would it be before the child could resume her normal growth? Would she ever be able to fully recover from the traumatic experience? What if the child never came out of the resting state?

Questions

1. Describe in your own words the major steps that need to happen for *B. subtilis* to form an endospore.
2. When nutrients become available, what would have to happen for a bacterial endospore to transition from a dormant state to an actively growing cell during germination?
3. What is the cost to a bacterial cell undergoing sporulation and why might it be a disadvantage to form an endospore in response to a temporary shortage of nutrients in the environment?

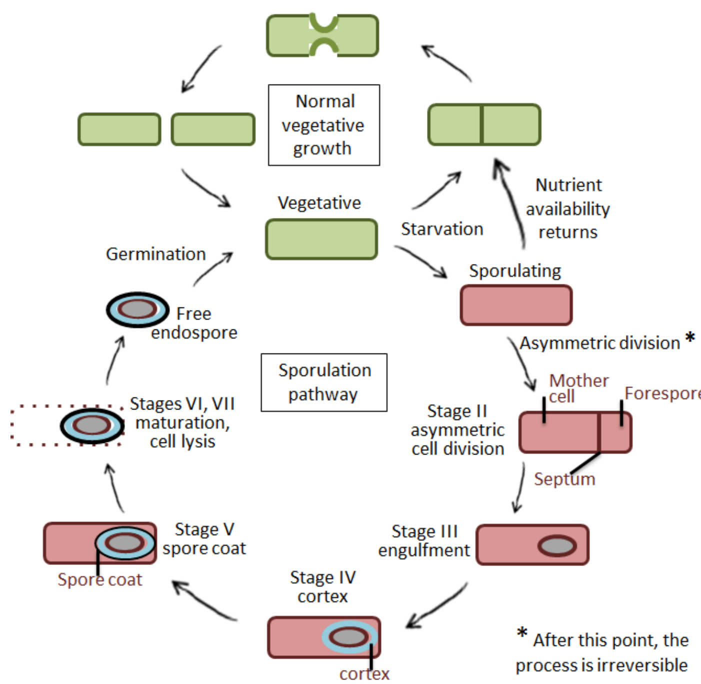

Figure 2. The vegetative and sporulation pathways of *Bacillus subtilis.* Key stages of the process are depicted. *Source:* Redrawn and modified from Gonzalez-Pastor (2010).

Part III – The Role of Killing Factors During Sporulation

Susan suddenly jerked awake and was back in the conference room just as her lab mate John began presenting his work. She tried to regain her bearing and forced herself to put the crazy dream behind her to focus on the presentation. John gave an overview of his project and then brought up some recent results that he found especially confusing. It was known that many bacteria produce weapons of chemical warfare called killing factors, which are antibiotic-like molecules produced in a cell and exported to the outside. The killing factors can target and kill other microorganisms in the environment, often by disrupting membrane structure and function, to reduce competition for important resources. The cells producing the killing factors also produce immunity factors, such as pumps that remove the factor or receptors that bind the factor and prevent it from disrupting its membrane, keeping the cells safe from the effects of their own killing factors.

They had discovered that *B. subtilis* produced two killing factors right after entering the pathway to sporulation. Their group had no clue why the bacteria would produce these killing factors in response to starvation, so John was doing some experiments to try to figure this out.

In his experiment, he was tracking the number of endospores formed (as a percent of the total number of viable cells) in four strains of *B. subtilis* over time (in hours). The experiment included a wildtype strain (circles) that produces all necessary proteins for endospore formation and survival, a strain that was lacking a gene encoding one killing factor Skf (triangles), a strain lacking a gene encoding a second killing factor Sdp (squares), and one lacking the genes for both Skf and Sdp (diamonds). He grew pure cultures of each strain in a nutrient rich medium then switched them to a medium that had limited nutrients and tracked endospore formation over time.

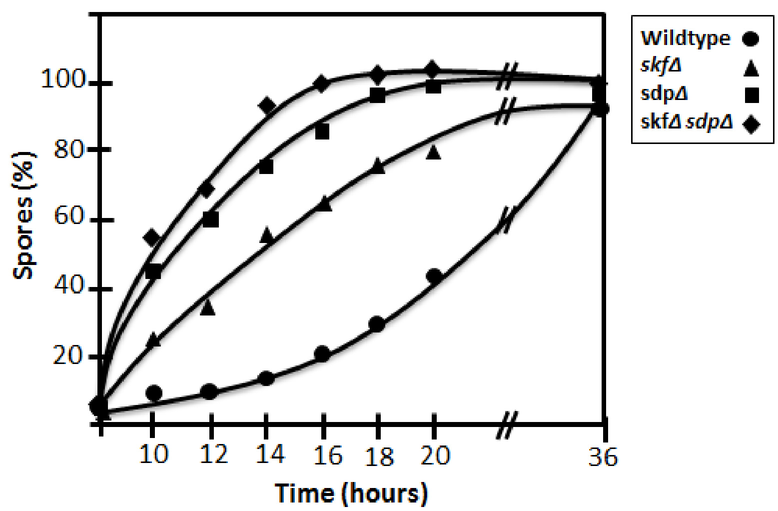

Figure 3. Rate of endospore formation over time with and without expression of killing factors. *Source:* Redrawn and modified from: Gonzalez-Pastor et al. (2003).

Questions

Refer to the graph (Figure 3) of John's data to answer the following questions:

1. What effect does preventing formation of the killing factors have on sporulation?
2. What does this indicate about the normal role of these killing factors in relation to sporulation?

Part IV – An Extreme Mechanism to Delay Sporulation

Finally, it was time for Susan to present the results of her recent experiment that was related to John's work. She was working with two strains of *Bacillus subtilis*, one wildtype strain (circles with solid line) and one that was unable to enter the pathway to sporulation due to a mutation (triangles with dashed line). She grew pure cultures of each strain in a rich medium then switched them to a medium completely devoid of nutrients and tracked their viability (colony forming units [CFU] per milliliter) over time (in hours). Susan noted that the medium used in this experiment was different from what John used in the last experiment (Figure 3). Because this medium contains no nutrients, cells were triggered to begin the sporulation pathway, but they could not complete the complex developmental steps and could not actually form endospores.

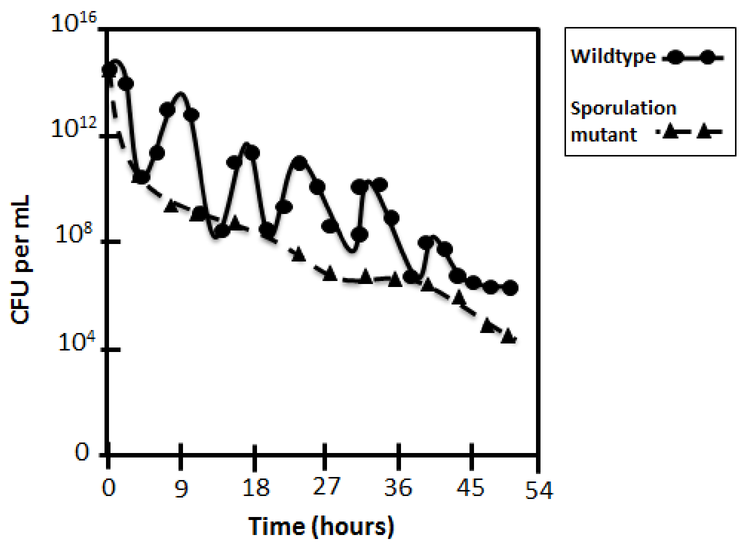

Figure 4. Viability of the population over time in response to extreme nutrient deprivation, with and without the ability to enter the sporulation pathway. *Source:* Redrawn and modified from: Nandy et al. (2007).

Questions

Refer to the graph (Figure 4) of Susan's data to answer the following questions:

1. Without the ability to enter the sporulation pathway (triangles with dashed line), what happens to the bacterial cells in response to extreme nutrient deprivation?
2. How can Susan explain why the curve of the wildtype *B. subtilis* strain (circles with solid line) seems to undulate over time?
3. Synthesize John and Susan's data from Figure 3 and 4 into a model explaining how *B. subtilis* delays sporulation. Draw a cartoon depicting what happens to a group of normal *B subtilis* cells over time when they are starved for nutrients. Make sure to clearly label all parts of your drawing.

Part V – Conclusion

John and Susan's experiments led the entire team down an exciting new avenue of research that ultimately led to them uncovering a mechanism by which *Bacillus subtilis* can delay endospore formation until conditions determine it is absolutely necessary. In response to nutrient limitation, only some cells trigger the pathway needed to form an endospore, dividing the population into sporulating and nonsporulating cells. The sporulating cells produce the killing factors Skf and Sdp as well as factors that make them immune to their effects. The nonsporulating cells, producing neither the killing factors nor immunity factors, are lysed. The nutrients released from the killed cells, are sufficient to allow the sporulating cells to resume normal growth and delay their commitment to sporulation.

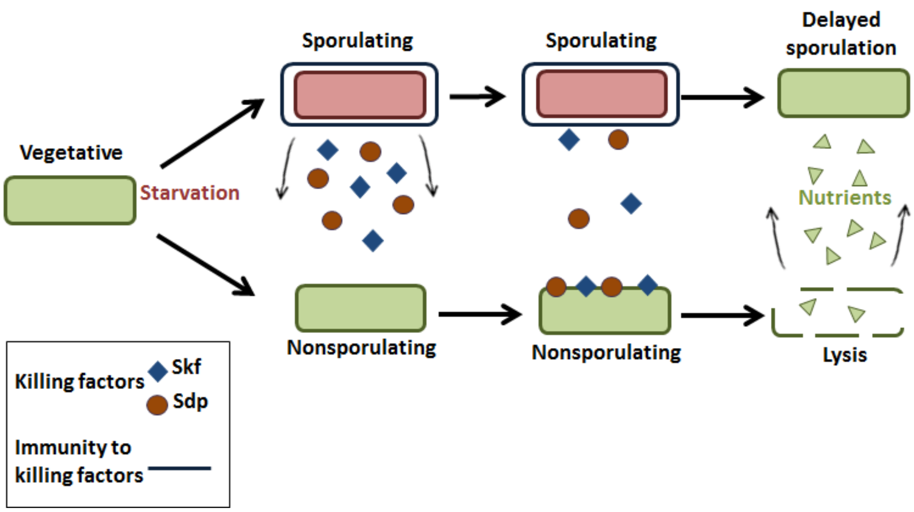

Figure 5. Cannibalism in *Bacillus subtilis* to delay sporulation. *Source:* Redrawn and modified from Gonzalez-Pastor (2010).

Questions

1. Why has the described mechanism been termed "cannibalism"?
2. How does cannibalism promote the survival of *B. subtilis?*

After presenting her work, Susan was satisfied knowing that her data was much more meaningful and interesting than she originally thought. As she sat down to let Marcos take his turn, she was finally able to take a deep breath and relax, but she still had a hard time paying attention. Her mind kept wandering back to her crazy dream. She wondered what she would have done if they were really trapped on that island. If they were unable to find food, they would certainly starve, but there was no reason why all of them should starve. There was a food source on the island, just not one she would normally ever consider. Would she have been able to keep herself alive, no matter what the cost? Would her decision be swayed by the fact that she was pregnant in the dream? She snapped back to reality and tried to push the crazy thoughts out of her head. She glanced across the conference table at John, who was stuffing his third donut down his throat, and she couldn't help picturing herself back on the beach, standing over him, clutching the crudely-fashioned spear, as he slept under a palm tree.