Cannibalism by Sporulating Bacteria

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Introduction

• Some bacteria form spores.
• Scientists are intrigued by them.
• *Bacillus subtilis* has been the model bacterium for spore-forming bacteria.
• Spo0A protein controls several genes involved in spore formation.
• Two operons (*skf* and *sdp*) are induced at start of sporulation.
Operons *skf* and *sdp* are induced at the start of sporulation

Fig. 1. (A) Gene organization of the *skf* and the *sdp* operons.

Fig. 2. *skf* and *sdp* transcription at start of sporulation depends on Spo0A. A) WT Pskf-lacZ (circles). spo0A mutant (squares) B) WT Psdp-lacZ (circles). spo0A mutant (squares).
Inactivation of \textit{skf} and \textit{sdp} accelerate spore formation

\textbf{(1B)} Colonies of \textit{skf} and \textit{sdp} mutants, as well as the double \textit{skf sdp} mutant were brighter.

\textbf{(1C)} Time course of spore formation in solid medium by the wild-type (\_), and the \textit{skf} (\_), \textit{sdp} (\_) and \textit{skf sdp} (\_) mutants.
Inactivation of *skf* and *sdp* accelerate spore formation (1D). Cells from *skf, sdp* and *skf sdp* mutants contained more spores than WT cells.
skf operon products are similar to peptide antibiotics.

- **SkfA**-small peptide, characteristic of operons involved in peptide antibiotics production.
- **SkfB**-similar to protein involved in production of antilisterial protein.
- **SkfD**-has a domain characteristic of amino terminal proteases.
- **SkfE** **SkfF**-resemble ATP binding cassette transport complex (ABC transporter).
Are WT Cells Killing Mutant Cells?

Fig. 3. (A) *skf* mutant cells harboring a *lacZ* fusion and wild-type cells (PY79) (▲). Wild-type cells were mixed with wild-type cells that carried a *lacZ* fusion (▲) and *skf* mutant cells were mixed with *skf* mutant cells that carried a *lacZ* fusion (▲).

(B) Cells harboring the *skf* operon under the control of an IPTG-inducible promoter spotted on a lawn of wild-type or *skf* mutant cells..
Are WT Cells Killing Mutant Cells?

**Fig. 3 (C).** Cells lacking the *skf* operon but containing a copy of *skfE* and *skfF* under the control of an IPTG-inducible promoter were with wild-type cells that carried a *lacZ* fusion in the absence (○) or in the presence (■) of the inducer (IPTG).

**Fig. 3 (D).** Number of viable cells was measured in cultures of wild-type cells (○), and cells of *skfA* (■) and *skfABCDEF* (■) mutants.
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**Fig. 3 (D).** Live/dead staining of wt and skf mutant cells during sporulation.
Conclusion

- WT produces mix population in which Spo0A is active (skf) in some cells and not in others.

Fig. 4. *skf* operon is expressed only in some cells of the population.
So Far....

Fig. 5. *skf* operon model.

So Far...

Spo0A

Induces

SkfA

SkfB

SkfD

SkfE/F

*No sporulation signal*
Don’t Forget about \textit{sdp}. How does it delay sporulation?

(1B) Colonies of \textit{skf} and \textit{sdp} mutants, as well as the double \textit{skf sdp} mutant were brighter.

(1C) Time course of spore formation in solid medium by the wild-type (\_), and the \textit{skf} (\_), \textit{sdp} (\_) and \textit{skf sdp} (\_) mutants.
Microarrays

Fig. 6. Microarrays
Fig. 7. Plot of spot intensities. **Red circle:** \textit{sdpC}; **Green oval:** \textit{yvbAZ}; Blue oval: \textit{yusLJK, atpIBEFHAGDC}, and \textit{ysiAB etfBA} operons.
What do these genes do?

- *yvbA* inferred product could be a transcriptional regulator.
- *yvbZ* product could have multiple transmembrane segments.
Transcription of *yvbAZ* is dependent on *sdp*, and *sdp* operon encode extracellular protein

Fig. 8. A. WT and *sdp* mutant cells containing *PyvbAZ-lacZ* fusion growing on medium with X-gal. B. *sdp* mutant cells containing *PyvbAZ-lacZ* fusion (white arrows) setraked next to either WT or *sdp* mutant cells.
Transcription of \( yvbAZ \) is dependent on \( sdp \)

Fig. 8. C. A \( \sim 5 \) kDa protein seems to be the extracellular factor. Sequence revealed that it is the product of \( sdpC \).

D. \( sdp \) mutant cells containing \( PyvbAZ-lacZ \) fusion. Supernatant of cells with \( sdp \) operon under IPTG inducible promoter was added. + IPTG (▲), - IPTG (●).
Conclusions

• Spo0A activates $sdp$ operon.
• SdpC is excreted and activates $yvbAZ$.

• Still do not know how is sporulation delayed.
Fig. 9. A. 1) _sgp_ _yvb_, 2) _sgp_ _yvb_/ _yvbAZ_-IPTG inducible, 3) _sgp_ _yvb_/ _yvbA_-IPTG inducible, 4) _sgp_ _yvb_/ _yvbZ_-IPTG inducible.
How does yvbA do it?

Fig. 7. **Blue oval:** yusLJKJ (*inferred lipid catabolism enzymes*), atpIBEFHAGDC (*ATP synthase operon*), and ysiAB etfBA operons.
Expression of yusLKJ is dependent on YvbA

Fig. 9. (B) Time course of accumulation of -galactosidase from PyusLKJ-lacZ in a wild-type strain (●), and in a strain mutant for sdp and yvbA yvaZ and harboring Pspac-hy-yvbA. The cells were grown in the absence (▲) or presence of 1 mM IPTG (◆).
SdpC role

Spo0A

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No sporulation signal

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YvbA is needed for \textit{skf} (killing factor) to do it’s job

\text{(C)} Time course of the number of viable cells during sporulation of a strain (EG526) mutant for \textit{sdp} and \textit{yvbA yvaZ} and harboring \textit{Pspac-hy-yvbA (\_)} and of a derivative of EG526 that was additionally mutant for \textit{sfk} (EG528) (\_) grown in the absence (open symbols) and in the presence of 1mM IPTG (filled symbols).
Conclusions

• Sporulating cells of B. subtilis kill their siblings and feed on them to delay spore formation.
• Delaying spore formation as long as possible would be beneficial to cell.
H. Engelberg-Kulka and R. Hazan.
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More questions

• What do SkfB, SkfD do?
• Is SkfE/F really ABC transporter?
• What do SdpA and SdpB do?
• Is yus operon used for lipid catabolism?
• Does really yvbA induces atp operon?
Questions?

Eat your vegetables!

What kind of sick bastard are you?!