Electrophysiological dynamics during sporulation in *Bacillus subtilis*

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Introduction

Sporulation is a complex cellular differentiation process during which a large amount of cations accumulate in the core of prespores. Calcium is the most abundant cation in mature spores and is essential for production of viable and resistant spores. However, the dynamics and role of calcium during the sporulation process are poorly understood.

We examined dynamics of calcium in sporangia and the effect of alterations of calcium dynamics on the sporulation process at single-cell level in *B. subtilis*. We also characterised profiles of potassium, pH and membrane potential during sporulation both in the prespore and mother-cell compartment. In addition, we compared the profiles of these ions in σ^K mutant and in calcimycin-treated (calcium ionophore) wild type sporangia.

Methods

Sporulation was induced by standard resuspension method (1) and *B. subtilis* bacterial cultures (PY79) were prepared for live time-lapse microscopy. Sporulating cultures were transferred from liquid media onto agarose pads. Images were acquired with Leica DMi8 and analysed using Fiji/ImageJ software.

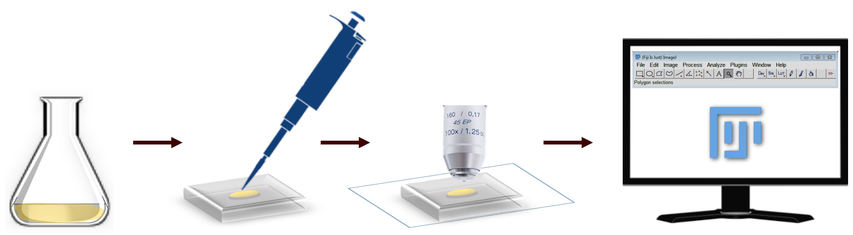

Figure 1: Schematic representation of the experimental procedure.

Results

Sporulation strongly relies on electrochemically active compounds.

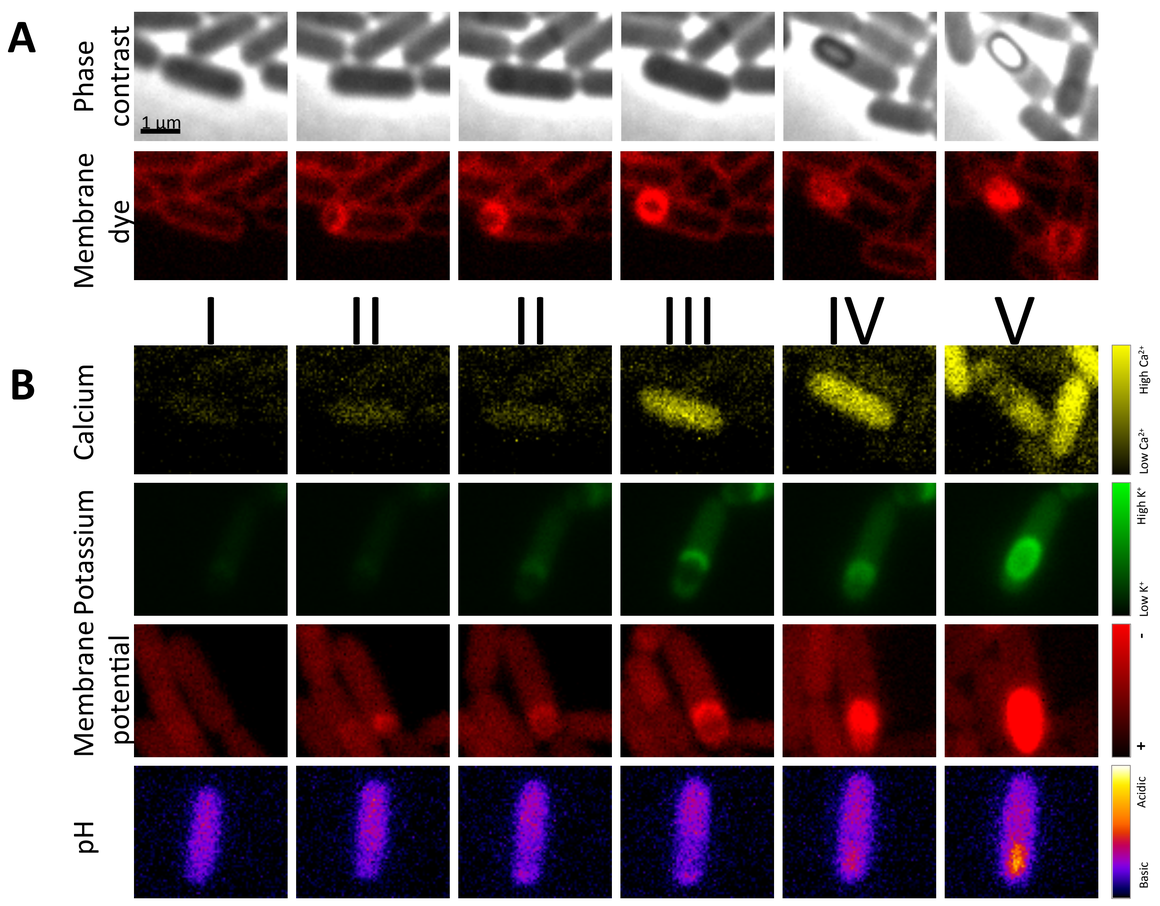

Figure 2: A) Film strip shows phase contrast and membrane dye (FM4-64) images of sporangia in different developmental stages. B) For tracking of calcium, potassium, membrane potential and pH dynamics in sporangia between stages I – V, we used specific fluorescent reporters GCaMP6s (2), APG2-AM, TMRM and pHluorin (3), respectively.

- Calcium accumulates within the sporangium in phase III of sporulation. In phase V calcium signal in prespore diminishes due to precipitation with dipicolinic acid (DPA).
- Potassium accumulates in the mother-cell cytoplasm in phases IV-V.
- Prespore membranes and mother cell membrane are polarized gradually during the sporulation process.
- pH decreases in prespore simultaneously with appearance of a phase-bright spore in phase IV-V.

Results

Calcium levels drastically increase in phase III of sporulation (Figure 2B). To narrow the list of genes potentially involved in calcium regulation, we tested mutants in sporulation specific sigma factors to verify regulon dependence.

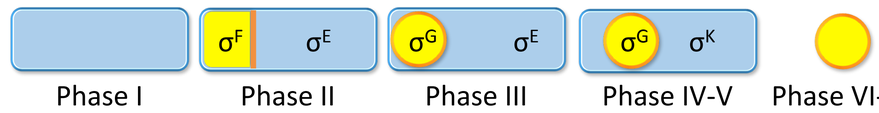

Figure 3: Schematic representation of genetic control in prespore and mother cell during sporulation.

We used an antibiotic calcimycin (4), which increases the ability of calcium ions to cross biological membranes. We treated wild type sporulating cultures with calcimycin to assess the effect of disrupted calcium dynamics on sporulation process.

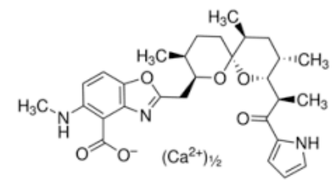

Figure 4: Molecular structure of calcium ionophore calcimycin.

Regulation of calcium permeability is crucial for successful spore formation.

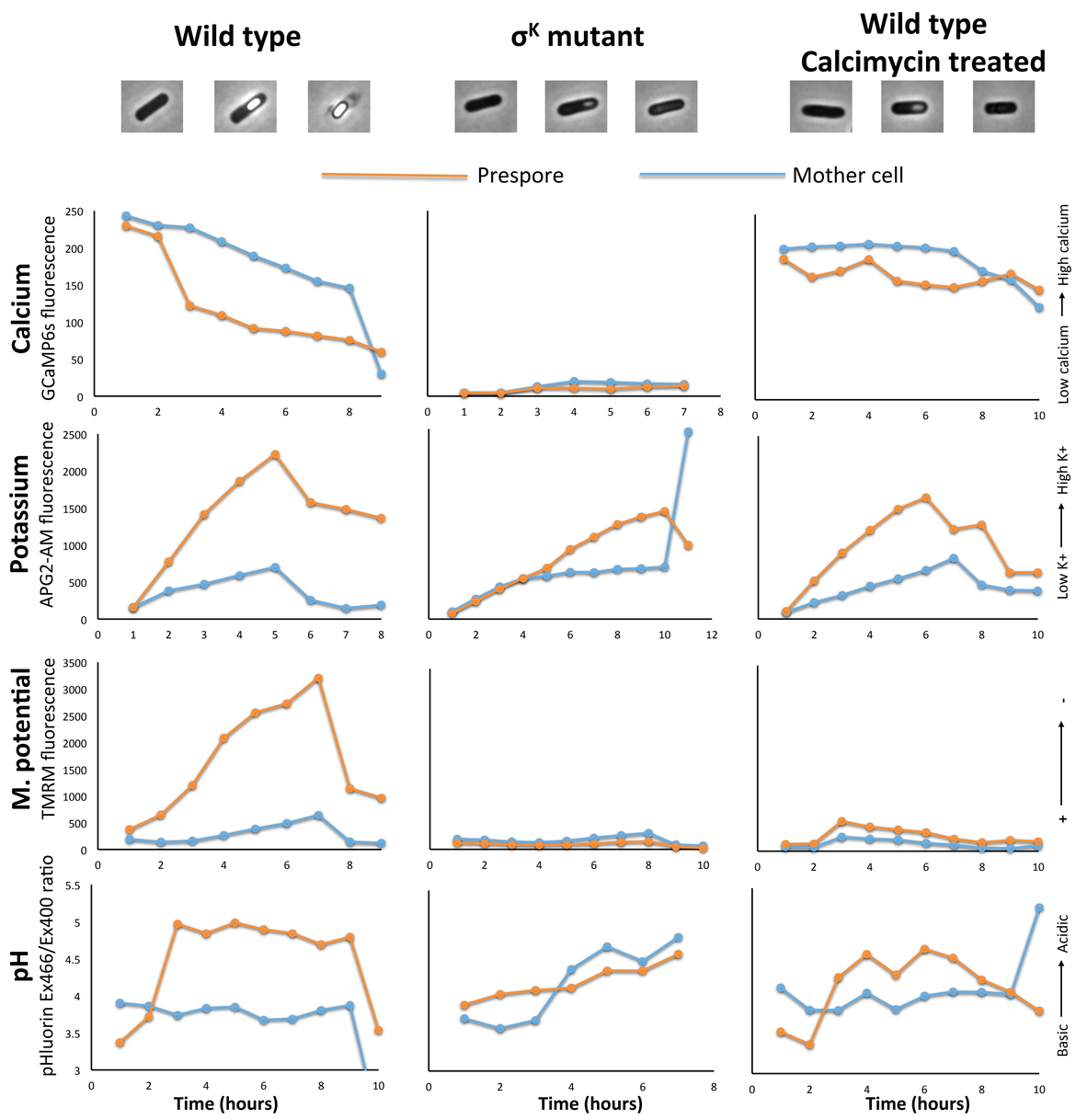

Figure 5: Measurements of calcium, potassium, membrane potential and pH during sporulation in wild type, σ^K mutant and calcimycin-treated wild type sporangia.

σ^K mutant and calcimycin-treated wild type sporangia failed:

- to produce viable spores, since they lyse in mother cell during the sporulation
- to create a calcium gradient between the prespore and mother cell
- to establish a membrane potential across membranes in sporangium
- to establish and maintain a pH gradient between the prespore and mother cell.

Conclusions

Regulation of calcium permeability across membranes is:

- crucial for production of viable spores
- crucial for establishment of membrane potential across membranes
- crucial for establishment and maintenance of pH gradient in sporangium
- regulated by σ^K or σ^K-controlled factor.

Future goals

We are currently working towards identification of molecular mechanism for calcium uptake and understanding how calcium regulates membrane potential and pH during the sporulation process.

References:
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AMR Tackling Antimicrobial Resistance

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