ABSTRACT SUBMISSION GUIDELINES

Abstracts must be submitted by March 1, 2009.

Abstract submissions must include a Title, List of Authors, and Affiliations with a line space between each of them. The name of the presenter should be underlined. Font should be Times New Roman, size 12, with one inch margins. The abstract text should be single spaced. Do not edit font size or margins to accommodate the length of your abstract. Entire abstract submission is not to exceed 32 lines including the title. If references are included, they must fit within the line limit. Please refer to the example below.

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(ex. Perego Abstract.doc) and emailed to mperego@scripps.edu.

Negative feedback loop on competence development and sporulation initiation by a ComK-activated dual specificity Rap-Phr system in *Bacillus subtilis*

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The establishment of genetic competence in *Bacillus subtilis* is controlled by a complex regulatory circuit that is highly interconnected with the developmental pathway for spore formation. The two pathways seem to be mutually exclusive and strategies have been developed to ensure that a cell may become competent or sporulate, but not both at the same time. Members of the Rap family of proteins, RapA and RapE, were previously shown to play a role in the cell decision process between competence and sporulation because they are activated by the early competence transcription factor ComA. As a consequence they limit the accumulation of phosphoryl groups within the sporulation phosphorelay and thus inhibit spore formation while allowing competence to develop. Here we show that another member of the Rap family, RapH, plays a dual role by inhibiting sporulation initiation and early competence gene expression in response to activation by the late competence transcription factor ComK. RapH has a dual specificity and acts by dephosphorylating the Spo0F~P response regulator intermediate of the phosphorelay and by inhibiting the DNA-binding activity of the ComA transcription factor. Thus, RapH generates a negative feedback regulatory loop that prevents sporulation initiation while competence is in full development but also contributes to the exit from the competent state in order to ensure a return to vegetative growth and cell survival. By means of *in vivo* and *in vitro* analysis, we report that while overexpression of RapH inhibited both competence development and sporulation initiation, a deletion of the *rapH* gene induced both pathways and interfered with their temporal separation. The results indicate that RapH is part of a multifactorial regulatory circuit affecting the cell's decision between distinct developmental pathways.

All abstracts will later be reduced to fit the format below (3.5 x 5.125 inches or 8.8 x 13 cm) for publication in the Conference Book of Abstracts.

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Characterization of a new BlaI like repressor included in the *Bacillus cereus BC1074-75-76* divergeon encoding protein of unknown function

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In *Bacillus cereus* ATCC14579, as well as in *Bacillus anthracis* A2012, the *BC1075* gene that probably codes for a BlaI like repressor, is included in a divergeon that has a similar structure to the *bla/mec* divergeons of *Bacillus licheniformis* and *Staphyloccocus aureus*. In the last both strains, the regulated gene (that codes for the β-lactamase BlaP in *B. licheniformis* and for the β-lactamase BlaZ or the penicilin binding protein 2a (PBP2a) in *S. aureus*) is translated in the opposite direction of the regulatory genes which code for a transcriptional repressor (BlaI/MecI) and a membrane receptor specific to β-lactam antibiotics (BlaR/MecR).

In *B. cereus* 14579, the three proteins coded by the *BC1074-75-76* divergeon are BC1074, BC1075 and BC1076. The potential regulated protein, BC1074, has no similarity with β-lactamase or PBP. BC1075 exhibits 41% identity with the *B. licheniformis* BlaI repressor and the N-terminus domain of BC1076 is homologous to the corresponding domain of BlaR/MecR receptors, instead, its C-terminus domain has no significative similarity with known proteins. Therefore, we postulate that this regulation system could operate like the ones of *B. licheniformis* and *S. aureus* but the trigger stimulus and functions remain to be established.

To know if the system is regulated in a similar way in *B. cereus* and *B. licheniformis*, the *blaI* gene has been substituted by the *BC1075* gene in the *B. licheniformis bla* divergeon. Then, BlaP β-lactamase induction assays were done by using this construction in *B. subtilis* that contain no *bla* divergeon. This experiment has shown that BC1075 is able to repress *blaP* expression. However, in presence of antibiotics, BC1075 isn't inactivated and the induction of the BlaP β-lactamase is not achieved.

To confirm that BC1075 acts as a DNA-binding protein, band shift assays had been performed with a *B. licheniformis blaI* operator. As expected, BC1075 is able to bind to that operator, even if its binding seems to be weaker compared to the one of the BlaI repressor. Further studies are ongoing to pursue the functional characterization of this divergeon in *B. cereus* ATCC14579.

1. Clarke et al. (2001). The signal transducer (BlaRI) and the repressor (BlaI) of the Staphylococcus aureus beta-lactamase operon are inducible. Microbiology **147**(Pt 4): 803-10.
2. Salerno et al. (1988). Differential transcription of the bla regulatory region during induction of beta-lactamase in Bacillus licheniformis." FEBS Lett **227**(1): 61-5.

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The program of oral presentations at the meeting will be drafted, as usual, based on the abstracts received. All the abstracts received that will not be chosen by the organizing committee for an oral presentation will be automatically accepted for a poster presentation. You will be notified by the end of April if your abstract has been chosen for an oral presentation.