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Formation of Multicellular Fruiting Bodies by Myxobacteria and the Role of the *Espab* Locus

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Fruiting body development in *Myxococcus xanthus* consists of two separate but interacting pathways: one for aggregation of a group of cells to form a raised mound and the other for sporulation of individual cells into spherical myxospores. During early fruiting body development, a group of starved rod-shape cells move to a focal center to form a raised translucent mound of cells. Sporulation of individual cells requires the raised mound. Thus individual cells do not sporulate outside of the mound but sporulate only in the mound under normal conditions, resulting in the formation of mature fruiting bodies. However, the *espA* (early sporulation) mutant did not require the presence in the raised mound for sporulation and initiated sporulation much earlier than wildtype. In contrast, a mutant carrying a null mutation in an adjacent gene, *espB* showed delayed sporulation. These suggest that EspA and EspB might be a part of a mechanism that monitors aggregation status and regulates initiation of sporulation. The *espAB* genes compose a two-gene operon and their expression is translationally coupled. EspA contains an unusual number of interesting domains involved in signal transduction: a FHA domain, two PAS and PAC domains, and a histidine protein kinase and a receiver domain. In contrast, EspB appears to be a novel protein that is predicted to be localized in the cell membrane. The *espAB* genes are flanked by two Ser/Thr protein kinase genes, *pknD1* and *pknD2*, and a histidine protein kinase gene, *hpkA*. Genetic studies have suggested that the function of PknD1 and PknD2 might be similar to EspB, antagonizing the function of EspA. Null mutations in *pknD1* and *pknD2* caused delayed aggregation and defective sporulation, very similar to the *espB* mutant phenotype. HpkA appears to be a negative regulator for the expression of the *espAB* operon.

Introduction

M. xanthus has a complex life cycle and exhibits many social behaviors. When placed on a nutrient rich surface, rod-shape cells grow vegetatively and travel in large groups referred to as swarms. In the absence of nutrients, swarms of approximately 100,000 cells move towards an aggregation center and form raised mounds with a simple structure. The individual rod-shape cells within the mounds are then converted into spherical resting cells called myxospores. In contrast to endospore formation in *Bacillus* spp., spores of *M. xanthus* are formed by morphogenesis of whole cells. The myxospores are dormant and are resistant to various environmental stresses such as drying, heating to 50°C, detergent, and sonication. When spores are exposed to a nutrient rich environment, they germinate and become vegetative rod-shaped cells. In *M. xanthus*, mounds containing sporulated cells are called mature fruiting bodies (1-3).

Studies with mutants have revealed that some of the aggregation-defective mutants are also defective in sporulation. In contrast, some aggregation-defective mutants do not form raised mounds but still

sporulate. Furthermore, some of the sporulation-defective mutants still retain an ability to aggregate; forming raised translucent mounds filled with rod-shaped cells. (4). Based on these, it was proposed that the developmental program in *M. xanthus* contains two separate pathways, one for aggregation and the other for sporulation (4). However, sporulation is dependent on aggregation. Sporulation only occurs in raised mounds but does not occur without or outside of raised mounds. This suggests that *M. xanthus* should have a mechanism that recognizes progress towards aggregation and then couples it to the timing of sporulation initiation.

Results

We have identified the *espAB* locus that appears to regulate sporulation timing in response to aggregation status (5). The *espAB* genes compose a two-gene operon and their expression is translationally coupled. A null mutation in *espA* (early sporulation) caused sporulation to occur in the absence of aggregation and much earlier than wildtype. In contrast, a null mutation in an adjacent gene, *espB* delayed sporulation. These mutant phenotypes suggest that EspA inhibits sporulation until aggregation has been completed and EspB counters this inhibition. We propose that EspAB functions to ensure that sporulation occurs after aggregation is completed during fruiting body formation. We hypothesize that EspA acts as a repressor or inhibitor that delays sporulation until cells have aggregated into raised mounds. EspB would then function as a derepressor that relieves the sporulation inhibition after aggregation is completed. EspB may directly antagonize the function of EspA or may interact with other gene products to trigger sporulation. EspA contains an unusual number of interesting domains involved in signal transduction: a FHA domain, two PAS and PAC domains, and a histidine kinase and a receiver domain. FHA domains are associated with protein-protein interactions and known to bind to phosphoproteins (pSer/pThr-proteins). PAS and PAC domains are known to play a role in sensing energy and oxygen levels. Histidine protein kinases and receivers are components of two-component signal transduction systems. In contrast, EspB appears to be a novel protein that is predicted to be localized in the cell membrane. EspB is predicted to have at least 13 transmembrane domains.

The *espAB* genes are flanked by two Ser/Thr protein kinase genes, *pknD1* and *pknD2*, and a histidine protein kinase gene, *hpkA*. HpkA appears to be a negative regulator of *espAB*. Expression of *espA* is normally very low under vegetative conditions. However, the *hpkA* null mutant showed significantly increased *espA-lacZ* expression during vegetative growth. HpkA itself is expressed under stationary phase conditions and its expression increases markedly under developmental conditions. Null mutations in *pknD1* and *pknD2* caused delayed aggregation and defective sporulation (< 10% of wild-type sporulation levels), very similar to the *espB* mutant phenotype. The *espA-pknD1* and *espA-pknD2* double mutant phenotypes were also very similar to the *espA-espB* double mutant phenotype. Therefore, this suggests a possible role for these genes in the control of EspA and EspB interaction for the regulation of sporulation initiation. PknD1 is expressed only under developmental conditions and PknD2 is expressed only under stationary phase conditions.

References

1. Dworkin, M., and Kaiser, D. 1993. *Myxobacteria II*, ASM Press, Washington, D.C.
2. Reichenbach, H. and M. Dworkin. 1991. The myxobacteria, pp. 3416-3487. *In* Balows A. *et al.* (ed.), *The Porkayotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications*,

2nd ed. Springer-Verlag, New York.
3. Shimkets, L. J. 1990. Social and developmental biology of the myxobacteria. *Microbiol. Rev.* **54**: 473-501.
4. Morrison, C. E. and Zusman, D. R. 1979. *Myxococcus xanthus* mutants with temperature-sensitive, stage-specific defects: evidence for independent pathways in development. *J. Bacteriol.* **140**: 1036-1042.
5. Cho, K., and Zusman, D. R. 1999. Sporulation timing in *Myxococcus xanthus* is controlled by the *espAB* locus. *Mol. Microbiol.* **34**: 714-725.