A MecA Paralog, YpbH, Binds ClpC, Affecting both Competence and Sporulation

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Received 14 November 2001/Accepted 26 January 2002

ComK, the master regulator of competence, is degraded by the general stress-related protease ClpCP but must be targeted to this protease by binding to the adapter protein MecA. The genome of Bacillus subtilis contains a paralog of mecA, ypbH. We show in the present study that YpbH, like MecA, binds ClpC and that its elimination or overproduction affects competence and sporulation.

The development of genetic competence, the ability to bind and internalize free DNA from the environment, is tightly regulated in Bacillus subtilis. The competence transcription factor ComK is necessary and sufficient to activate the transcription of late competence genes, which encode components of the DNA uptake machinery, as well as many other operons (6, 25). It has been suggested that this massive reorganization of the transcriptional program defines a unique physiological condition, the K state (3). ComK is regulated both transcriptionally and posttranscriptionally. Five transcription factors, including ComK itself, control the level of comK transcription of late competence genes, which encode components of the DNA uptake machinery, as well as many other operons (6–8, 19, 21). ComK acts as a positive autoregulator, thus imposing a switch-like behavior on K-state development. These mechanisms ensure a low level of comK transcription during exponential growth and a postexponential burst of ComK synthesis.

Another layer of control, regulated proteolysis, also keeps the concentration of active ComK at the basal level during exponential growth. Two key players in this process are MecA and ClpC. MecA binds and targets ComK for degradation by the ClpCP protease (22, 23). ClpC is composed of two heat shock proteins, the protease subunit ClpP and ClpC. ClpC, an ATPase belonging to the family of Clp/HSP100 proteins (18), is widespread among bacteria. The role of ClpC is complex: cells deficient in clpC are affected in sporulation, competence, and growth at high temperatures (4, 10, 11, 14). ClpC probably forms a hexameric structure similar to ClpAP or ClpXP in Escherichia coli (5). By binding to both ComK and ClpC, MecA adapts the general stress-related proteolytic machine for the degradation of the competence transcription factor ComK. Upon entry into the stationary phase, the small protein ComS is synthesized in response to the quorum-sensing phenomenon ComX and CSF (12, 13, 20). ComS binds to MecA, causing the release of ComK and protecting it from degradation. MecA consists of two domains: its N-terminal domain recognizes ComK and ComS while its C-terminal domain binds ClpC (16). There are other phenotypes associated with mecA knockout or overexpression in Bacillus. For instance, MecA-overproducing cells are sporulation deficient (9), and the inactivation of mecA causes a rough colony shape (Persuh and Dubnau, unpublished). These phenotypes are not dependent on ComK, and MecA probably targets proteins other than ComK for degradation by ClpCP.

YpbH is a paralog of MecA. The search of sequence databases with mecA as a query revealed the presence of a gene, ypbH, the product of which shows high similarity to MecA. The B. subtilis MecA paralog shares 26% sequence identity and 52% sequence similarity with the B. subtilis MecA on the amino acid level. MecA consists of two domains, the N- and C-terminal domains, with a linker region between them (16). The similarity of YpbH to MecA extends over both domains, but YpbH is 24 amino acids shorter than MecA and lacks the linker region. ypbH is present in two other sequenced Bacillus species: Bacillus anthracis and Bacillus halodurans. It seems that ypbH is present only in the genus Bacillus while MecA, with a much broader distribution, is found in essentially all low-GC gram-positive bacteria (Bacillus, Listeria, Staphylococcus, Streptococcus, Lactococcus, and Enterococcus) (16).

YpbH binds to ClpC. Cells overexpressing YpbH are very deficient in sporulation, and we have observed that colonies of the overexpressing strain lyse on plates within 48 h. The introduction of a clpC knockout in the YpbH-overproducing strain reversed this lysis phenotype. Although clpC knockout cells are themselves sporulation deficient, the expression of the spoII genes is not affected and rapid lysis does not occur (15). These results demonstrated that YpbH needs ClpC for its effect on sporulation. The strong similarity of YpbH to MecA further implied that YpbH, like MecA, might bind to ClpC. We used surface plasmon resonance to detect this interaction. The His-tagged YpbH protein was purified as described previously for MecA (23). A clpC-intein construct (a gift from M. Nakano) in pTYB2 (New England Biolabs) was used for expression, and ClpC was purified according to the manufacturer’s instructions. Protein concentrations were determined by using the Bio-Rad reagents with bovine serum albumin as the protein standard. His-tagged YpbH (800 response units) was noncovalently immobilized to the surface of a nitrilotriacetic acid chip by the injection of a 150 nM solution of YpbH-His in eluent buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 50 μM EDTA, and 0.005% polysorbate 20) at flow rate of 10 μl/min.
To follow the binding of ClpC, a 200 nM solution of ClpC in buffer A (20 mM Tris, pH 8, 150 mM KCl, 10 mM MgCl₂) was passed over the chip. After each run, the chip was regenerated with 0.3 M EDTA and recharged with a 200 μM solution of NiCl₂. This experiment demonstrated that ClpC was able to bind YpbH (Fig. 1). In the same experiment, a ComK fusion to the maltose binding protein was passed over the chip and no binding to YpbH was detected (data not shown), indicating the specificity of the YpbH-ClpC interaction. Like MecA, YpbH was able to stimulate the ATPase activity of ClpC in vitro (K. Turgay, personal communication), confirming its interaction with ClpC.

**YpbH plays a role in sporulation and competence.** To explore the role of YpbH, we constructed a deletion-insertion mutant of ypbH as well as a ypbH multicopy construct in the vector pUB110 and examined the associated phenotypes. The phenotype of the ypbH knockout was not due to polarity since the downstream gene, gudB, codes for an inactive protein (2). To make the ypbH knockout, the regions 200 bp upstream and 500 bp downstream from ypbH were amplified with the primers ypbH-EcoRI1 (5′-CGG GAT CCT GTA TGG TGC TTG TCT TAC-3′) and ypbH-BamHI1 (5′-CGG GAT CCA CGT CTT TGT CTC GGC-3′). The resulting 2.1-kb fragment was cut with EcoRI and BamHI and cloned into pUCCm18. The recombinant plasmid was cut with SpeI and BglII, which removes nearly all of ypbH, and a spectinomycin cassette was cloned between the sites. The resulting plasmid was transformed into B. subtilis to make strain BD3349, and transformants were checked for chloramphenicol sensitivity (the plasmid contained a chloramphenicol marker which is lost upon the double crossover). The construct in the chromosome was verified with a PCR. To make the overproducing strain BD3350, the ypbH gene was amplified with the primers ypbH-BamHIFI (5′-CGG GAT CCT GTA TGG TGC TTG TCT TAC-3′) and ypbH-EcoRI1 (5′-CGG GAT CCT GTA TGG TGC TTG TCT TAC-3′). The PCR fragment was then cut with BamHI and EcoRI and cloned between the corresponding sites in pUB110 to produce pMB1. The growth of B. subtilis strains in competence medium and transformation were carried out as described previously (1). The morphology of colonies lacking or overexpressing ypbH suggested the involvement of ypbH in sporulation. Measurements of sporulation frequency, carried out as described previously (17), confirmed this impression. A ypbH knockout formed 20-fold fewer spores than the wild type while a strain overexpressing ypbH on a multicopy plasmid completely lacked the ability to sporulate (a sporulation frequency of less than 10⁻⁸). We tested the same mutant strains for the expression of a spoIIE-lacZ reporter and found that the ypbH strain showed reduced spoIIE-lacZ activity while the overexpressing strain was completely deficient in this activity (data not shown). The effect of YpbH overproduction was not due to titration of ClpC, since ClpC deficiency does not affect the expression of spoIIE (15). The sporulation deficiency of the YpbH overproducing strain was not due to the titration of a regulatory molecule by the ypbH promoter, since a multicopy plasmid with a partially deleted YpbH coding region had no sporulation phenotype. Since active Spo0A is required for spoIIE transcription (26), YpbH may affect the transcription of spo0A or the phosphorylation of its gene product. We tested the competence phenotype of the ypbH mutant strain by measuring the expression of ComK-dependent genes. β-Galactosidase assays were carried out as described previously (16). comG-lacZ transcription was delayed in the strain lacking ypbH and increased in the overexpressing strain (Fig. 2A and B). Similar results were obtained with other late competence genes (comF, comE, and comC) (data not shown). The results shown in Fig. 2 demonstrate that in the YpbH-overproducing strain, the mechanisms that regulate the timing of competence gene expression are still functional. The effect of YpbH overproduction is to increase competence gene expression at all time points but particularly during exponential growth. In this respect, the effect of YpbH overproduction is what would be expected from a decrease in MecA. Western blot analysis suggests that this is the case (data not shown). Since ComK is needed for the transcription of late competence genes, we measured comK-lacZ expression and found it was elevated in a strain overproducing YpbH, particularly before T₈₅ (0 h after the end of log-phase growth) (Fig. 2C), suggesting that the effect of YpbH overproduction on late competence gene expression is due to the increased synthesis of ComK.

**The transcription of ypbH is growth-stage regulated.** We constructed a transcriptional ypbH-lacZ fusion and measured its activity in Luria-Bertani (LB), sporulation, and competence media. The vector pMutin2 (24) was used to make a transcriptional fusion to ypbH. pMB1 was cut with SpeI, the ends were filled in, and the fragment was cut with BglII. This treatment generated an N-terminal fragment of ypbH. pMutin2 was cut with HindIII, the ends were filled in, and the fragment was cut with BamHI. The N-terminal ypbH fragment was cloned into the vector fragment. The resulting plasmid, pMB3, was used to transform B. subtilis to make strain BD3531. Proper insertion in the chromosome was checked by PCR. The results are shown in Fig. 3. The transcription of ypbH increased at the end
FIG. 2. The effect of ypbH knockout on comG-lacZ expression (A) and of ypbH overproduction on comG-lacZ (B) and comK-lacZ (C) expression. The expression of β-galactosidase from a lacZ fusion to the promoters of comG or comK was measured as a function of the growth stage. In panel B, the strains carried either the vector pUB110 or the ypbH plasmid, pMB1. The time scale refers to hours before and after the transition from the exponential to the stationary growth phase (T₀). wt, wild type.

FIG. 3. Expression of ypbH-lacZ in competence (□), LB (♦), and sporulation (○) medium. The expression of β-galactosidase from a lacZ fusion to the promoter of ypbH (BD3351) was measured as a function of the growth stage. The time scale refers to hours before and after the transition from the exponential to the stationary growth phase (T₀).

of exponential growth in both LB and competence medium and reached approximately the same level in both. In contrast, lacZ activity slowly decreased after T₀ in sporulation medium and was at least fourfold lower than in LB or competence medium. These results are consistent with the effects of YpbH on competence and sporulation and suggest that this molecule may play a regulatory role in vivo. In an additional experiment we found that the overproduction of YpbH had no effect on ypbH-lacZ expression (data not shown).

The effect of YpbH on late competence gene transcription can be explained by the modulation of comK transcription (Fig. 2C). There is a striking parallel between MecA and YpbH regarding their effects on sporulation. Both of these proteins eliminate sporulation when overproduced, and in both cases this effect is dependent on ClpC (9, 16), indicating that YpbH-ClpC hetero-oligomers are likely the functional regulatory complexes inside the cell. These observations and the patterns of ypbH transcription in competence and sporulation media suggest that YpbH is a pleiotropic regulator affecting both competence and sporulation.

We thank K. Turgay for the gift of pY8, T. Msadek for contributing the mecA-lacZ transcriptional fusion, and M. Nakano for the clpC intein construct. We thank all the members of our lab for valuable discussions.

This work was supported by NIH grant GM57720 and a fellowship from the Slovene Ministry of Science and Technology awarded to M.P.

REFERENCES


