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Identification of σ^V-dependent genes of *Bacillus subtilis*

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Abstract

The chromosome of *Bacillus subtilis* codes for seven extracytoplasmic function sigma factors the activity of which is modulated normally by a cognate anti-sigma factor. While inducing factors and genes for four of them (σ^M, σ^W, σ^X, and σ^Y) have been identified, those of the remaining three sigma factors including σ^V remain elusive. The objective of the present study was the unequivocal identification of its anti-sigma factor and of genes controlled by σ^V. In many cases reported so far the gene coding for the anti-sigma factor is located immediately downstream of the gene coding for the sigma factor, and both form a bicistronic operon. We could show by two different experimental approaches that this is also the case for *sigV* and *rsiV*. Under conditions of overproduction of σ^V, 13 genes could be identified being induced several-fold by the DNA macroarray technique. Induction of three of them was confirmed by Northern blots, and the potential promoter of *sigV* was identified by primer extension. This led to the deduction of a consensus sequence recognized by σ^V.

Keywords: ECF sigma factor; DNA macroarray; Anti-sigma factor

1. Introduction

All bacterial cells are equipped with genetic programs allowing adaptation to a variety of environmental stresses (see book edited by Storz and Hengge-Aronis [1]). These programs result in the transient expression of a subset of genes to cope with the stressful situation. One possibility to express these stress genes is to replace the housekeeping sigma factor by an alternative one. The Gram-positive soil bacterium *Bacillus subtilis* codes for a total of 16 alternative sigma factors participating in the sporulation process (σ^H, σ^E, σ^F, σ^G, and σ^K) [2], in chemotaxis, motility, and production of autolysins (σ^D), amino acid catabolism (σ^L), one coding for an unknown function (σ^{ykoZ}) and in the general stress response (σ^B) [3]. Ten years ago a subfamily of sigma factors involved in responses to environmental challenges, the so-called extracytoplasmic function (ECF) subfamily [4,5], was defined from sequence comparisons [6]. Completion of the *B. subtilis* genome uncovered seven hitherto unknown alternative sigma factors belonging to the ECF subfamily [7]. ECF sigma factors share common features such as being cotranscribed with and modulated by an anti-sigma factor. In most cases, this anti-sigma factor is an integral membrane protein with one transmembrane helix where the C-terminal domain is exposed on the outside of the cytoplasmic membrane and supposed to be involved in stress sensing and the N-terminal domain is present in the cytoplasm

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sequestering the cognate sigma factor thereby preventing its interaction with the RNA polymerase core enzyme. Upon sensing a signal generated on the outside of the cytoplasmic membrane by the C-terminal domain, the sigma factor is released and now capable to interact with the RNA polymerase core enzyme. This in turn leads to transcription initiation of the appropriate genes [5,8]. Another common feature is that the promoter of the operon encoding the ECF sigma factor and its anti-sigma factor is often recognized by its own sigma factor resulting in autoregulation.

From the seven identified ECF sigma factors in *B. subtilis* (σ^M, σ^V, σ^W, σ^X, σ^Y, σ^{YlaC}, and σ^Z) most studies concentrated on σ^M, σ^W, σ^X, and σ^Y. σ^M has been reported to play an essential role for growth and survival in response to high concentrations of salt, cell wall antibiotics, heat, ethanol, acid stress and paraquat [9–12]. Genes controlled by σ^W can be induced by alkaline shock, by high concentrations of sodium chloride, by phage infection and certain antibiotics such as vancomycin [10,13,14]. σ^X controls cell envelope modification processes [15] and is involved in detoxification, production of and resistance to antimicrobial compounds [16–18]. Nitrogen starvation was reported to induce the σ^Y operon [19].

Our present study focused on another ECF sigma factor, σ^V. The gene *sigV* (coding for σ^V) together with its downstream gene *yrhM* (renamed *rsiV*, see below) is induced during outgrowth of *B. subtilis* endospores, but a *sigV* knockout did not impair outgrowth [20]. Using an IPTG-inducible *sigV* gene, 98 *B. subtilis* genes exhibited increased expression [21]. Furthermore, it could be shown, using the yeast two-hybrid system that σ^V interacts with the N-terminal domain of RsiV, the presumed anti-sigma factor [22].

2. Materials and methods

2.1. Strains, plasmids, and culture conditions

The strains and plasmids used in this work are listed in Table 1. Strains were routinely grown in Luria broth (LB) at 37 °C with vigorous shaking. Antibiotics were added when appropriate (100 μg ml^{-1} ampicillin, 1 μg ml^{-1} erythromycin, 10 μg ml^{-1} chloramphenicol, 10 μg ml^{-1} tetracycline, or 10 μg ml^{-1} neomycin). *E. coli* strain DH10B was used as a recipient for cloning experiments.

2.2. DNA manipulation and PCR

DNA manipulation was performed according to standard procedures [23]. For PCR reactions, high-fidelity Deep Vent^R DNA polymerase (New England Biolabs) was used.

2.3. Construction of strains

Plasmid pMUTIN–FLAG [24] was used to fuse the FLAG epitope tag in-frame to the 3′ end of the *sigV* gene and, at the same time, to put the downstream gene *rsiV* under the control of an IPTG-inducible promoter (Fig. 1A). To accomplish this goal, about 350 bp of

Table 1
Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Sources or references
E. coli		
DH10B	*mcrA Δ(mrr hsdRMS mcrBC) 80d lacZM15 ΔlacX74 deoR recA1 araD139 Δ(ara leu)7697 galU galK rpsL endA1 nupG*	Bethesda Research Laboratories, Inc.
B. subtilis		
1012	*leuA8 metB5 trpC2 hsrM1*	[31]
CH01	1012 *sigV*–FLAG P_{spac}–*rsiV*	This work
CH05	1012 *amyE::*P_{sigV}–lacZ	This work
CH07	1012 *sigV*- FLAG P_{spac}–*rsiV amyE::*P_{sigV}–*lacZ*	This work
ST01	1012 pX2*sigV*	This work
ST03	ST01 with *rsiV* replaced by a spectinomycin resistance gene	This work
ST04	ST03 *amyE::*P_{sigV}–*lacZ*	This work
ST06	ST03 *amyE::*P_{yuaF}–*lacZ*	This work
ST09	ST01 *amyE::*P_{sigV}–*lacZ*	This work
Plasmids		
pLacZ	*bgaB* in pBgaB replaced by *lacZ*	[32]
pX2	Plasmid for xylose regulatable expression of target genes	[25]
pX2*sigV*	Plasmid for xylose regulatable expression of the *sigV* operon	This work
pBR322	standard cloning vector	[33]
pBRΔ*rsiVspc*	Plasmid for *rsiV* deletion	This work
pSigV–*lacZ*	Plasmid with transcriptional fusion of the *sigV* promoter and *lacZ*	This work
pMutin–FLAG	Plasmid for epitope tagging	[24]
pSigV–FLAG	FLAG epitope fused to *sigV*	This work