JB Journals.ASM.org

Biofilm Research Uncovers a Novel Nonenzymatic Signal Peptidase Function in *Bacillus*

Jan Maarten van Dijl

Department of Medical Microbiology, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands

The Gram-positive soil bacterium *Bacillus subtilis* is highly adaptable to environmental changes and insults. This adaptability is dearly needed since the soil is an ever-changing environment in terms of humidity, temperature, and nutrient availability. To respond appropriately to changing conditions, *B. subtilis* employs fast stress responses plus a series of relatively slower developmental programs that lead to cell differentiation as encountered in multicellular organisms. For example, subpopulations within a larger population of *B. subtilis* cells can become motile to move toward more attractive environments, or they can differentiate into spores that are highly resistant to harsh conditions. Another very effective survival strategy is the formation of thick multilayered biofilms in which cells are protected by a matrix of extracellular proteins and other macromolecules. *B. subtilis* can form such biofilms at air-liquid interfaces (i.e., pellicle biofilms) and on solid surfaces. Importantly, all these protective and developmental programs involve the transport of particular proteins from the cytoplasm across the cytoplasmic membrane to the membrane-cell wall interface, the cell wall, or the extracellular environment. Apparently, there has been a high selective pressure for protein export in *B. subtilis* and related bacilli, as all of them have a very high capacity for protein export (17).

Most exported *Bacillus* proteins are synthesized with a signal peptide that is needed for their targeting to a translocation machinery in the membrane (17, 18, 19). Once the membrane translocation process is initiated or shortly thereafter, the signal peptide is proteolytically removed by so-called signal peptidases (8, 22). This is a prerequisite for the release of the translocated protein from the *trans* side of the membrane. During the evolution of *Bacillus* species, there must have been a particularly high selective pressure on efficient processing of exported proteins, as the signal peptidase-encoding (*sip*) genes have been multiplied several times. Thus, the *B. subtilis* genome contains five different *sip* genes, and up to seven *sip* genes are present in genomes of species belonging to the *Bacillus cereus* group (18, 22). Additional *sip* genes are found on otherwise cryptic endogenous plasmids of *B. subtilis* (6, 21). Quite unexpectedly, sequencing of the *B. subtilis* genome revealed that one of the five chromosomal *sip* genes, denoted as *sipW*, encodes a signal peptidase that is atypical for bacteria. Instead, SipW closely resembles the signal peptidases encountered in the plasma membrane of archaea and the endoplasmic reticular (ER) membrane of eukaryotes (18). The ER-type signal peptidase SipW differs from other prokaryotic (P)-type signal peptidases in several respects. First, it employs a Ser-His catalytic dyad or Ser-His-Asp catalytic triad, whereas the P-type signal peptidases employ a Ser-Lys catalytic dyad (8, 20). Second, SipW has both N-terminal and C-terminal membrane anchors, whereas the majority of P-type signal peptidases have N-terminal membrane anchors only. Subsequent studies and, especially, large-scale genome sequencing efforts have shown that homologues of SipW can be found in various species belonging to the *Actinobacteria* (e.g., *Arthrobacter*, *Rhodococcus*, and *Xylanimonas*), *Firmicutes* (e.g., *Bacillus*, *Clostridium*, *Desulfitobacterium*, *Eubacterium*, and *Ruminococcus*), and *Mollicutes* (e.g., *Sphaerobacter*).

The signal peptidases of *B. subtilis* have different but overlapping specificities. Accordingly, they are functionally redundant in the general secretion of proteins (18). However, SipW has a rather specific nonessential role in protein export (1, 3, 4, 18). The two major substrates processed by SipW are the precursors of the TapA (YqxM/YqhD) and TasA (CotN/YqhF) proteins, which are encoded by the *tapA-sipW-tasA* operon (13, 14). TasA was initially identified as a spore-associated protein with antibacterial activity and roles in the assembly and function of the endospore coat (11, 13, 14). Consistently, processing of the TasA precursor by SipW is required for the incorporation of mature TasA into spores (20). Subsequent studies revealed that SipW and its substrates TapA and TasA are also required for the formation of biofilms (2, 3, 4, 7). TasA is in fact a major protein component of *B. subtilis* biofilms that provides structural integrity through the formation of amyloid fibers (1, 10).

In the present issue of the *Journal of Bacteriology*, Terra et al. (16) describe the intriguing observation that the SipW protein but not its enzymatic activity is needed for the formation of surface-adhered biofilms. Specifically, they show that the 20 C-terminal residues of SipW, which localize to the cytoplasmic side of the membrane, are critically needed for solid-surface biofilm formation, whereas the catalytic Ser, His, and Asp residues are dispensable for this process. On the other hand, the 20 C-terminal residues turn out to be dispensable for SipW-catalyzed processing of TasA, showing unambiguously that these residues are needed for the second function of SipW in solid-surface biofilm formation. The first evidence for a second role of SipW in biofilm formation came from the earlier observation that strains lacking SipW were more strongly inhibited in solid-surface biofilm formation than strains lacking TasA and TapA (1, 4). While this might suggest a role for other, as-yet-unidentified substrates of SipW in solid-surface biofilm formation, Terra et al. entertained the more interesting hypothesis that SipW might have a second function specifically required in this process. This is now shown to be correct. A subsequent screen for *sipW* suppressors revealed that mutations in

Published ahead of print 16 March 2012

Address correspondence to j.m.van.dijl01@umcg.nl.

doi:10.1128/JB.00270-12

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.

the *sinR* gene can compensate for the absence of SipW. SinR is a repressor of the *eps* genes for the synthesis of extracellular polysaccharides and the *tapA-sipW-tasA* operon. Indeed, induced expression of the *eps* genes was shown to bypass the requirement for SipW in surface-adhered biofilm formation, while TapA and TasA are not needed for this process. Based on these observations, Terra et al. conclude that the SipW protein, especially its cytoplasmic C terminus, is required for the expression of genes needed in the formation of biofilm matrix components when the cells are on a solid surface. Importantly, the enzymatic signal peptidase activity is dispensable for this process.

The findings reported by Terra et al. create a new dimension in the research on signal peptidase function, and this applies not only to bacterial signal peptidases but also to the signal peptidases in the archaeal plasma membrane and the ER membrane of eukaryotes. First, SipW and related ER-type signal peptidases are now placed in the group of bifunctional proteases. Other well-known representatives of this group are the mitochondrial Lon and AAA proteases, as well as the DegP (HtrA) protease of *Escherichia coli*, which all have chaperone activities in addition to their proteolytic activity (9, 12, 15). Second, homologues of SipW in other bacteria and eukaryotes may have similar dual functions, and these need not be limited to the regulation of biofilm formation. While the C terminus of *B. subtilis* SipW is fairly well conserved in some bacilli, like *B. atrophaeus*, *B. amyloliquefaciens*, and *B. licheniformis*, it is less well conserved in other *Bacillus* species, like *B. cereus* and *B. anthracis*. And yet, a set of core conserved residues are detectable in the 20 C-terminal residues of *B. subtilis* SipW, which have the sequence 170-RE**IERKTKALE**TDT**KD**ST MST-190 (the conserved residues are marked in bold, and the best-conserved residues are underlined). This raises the question of whether these residues in SipW proteins from other bacilli or other bacterial genera have related gene regulatory functions. Third, the observations of Terra et al. may provide a lead for explaining the enigmatic observation that certain *Firmicutes*, such as *Staphylococcus aureus*, produce signal peptidase homologues that lack the catalytic Ser and Lys residues (22). Such noncatalytic signal peptidases might perhaps serve other functions, for example in pilus formation (23) or gene regulation. Finally, a major challenge for direct follow-up on the studies of Terra et al. will be to define how the SipW protein is positioned in the signal transduction pathway that is needed for *B. subtilis* cells to sense the presence of solid surfaces. Do the extracytoplasmic or transmembrane domains of SipW have a sensing role in this? If so, is this a direct role relating to physical/mechanical forces acting on the cell wall or membrane, or are there other membrane proteins or extracytoplasmic proteins involved? Equally important, it needs to be tested whether the C-terminal residues of SipW interact directly or indirectly with SinR, and if there is a direct interaction, whether SipW serves some sort of "antirepressor" function in relation to SinR. In this case, the newly identified second function of SipW could be analogous to that of the anti-sigma factors that sequester the so-called extracytoplasmic function (ECF) sigma factors until they are needed for the cell's responses to externally imposed stresses (5). Altogether, the novel observations of Terra et al. have generated a completely new perspective for the research on signal peptidases, which was until now focused on their enzymology and structure.

ACKNOWLEDGMENTS

J.M.V.D. acknowledges support through the CEU projects PITN-GA-2008-215524 and -244093.

REFERENCES

1. **Branda SS, Chu F, Kearns DB, Losick R, Kolter R.** 2006. A major protein component of the *Bacillus subtilis* biofilm matrix. Mol. Microbiol. **59**: 1229–1238.
2. **Branda SS, et al.** 2004. Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. J. Bacteriol. **186**:3970–3979.
3. **Chu F, Kearns DB, Branda SS, Kolter R, Losick R.** 2006. Targets of the master regulator of biofilm formation in *Bacillus subtilis*. Mol. Microbiol. **59**:1216–1228.
4. **Hamon MA, Stanley NR, Britton RA, Grossman AD, Lazazzera BA.** 2004. Identification of AbrB-regulated genes involved in biofilm formation by *Bacillus subtilis*. Mol. Microbiol. **52**:847–860.
5. **Hughes KT, Mathee K.** 1998. The anti-sigma factors. Annu. Rev. Microbiol. **52**:231–286.
6. **Meijer WJJ, et al.** 1995. The endogenous *Bacillus subtilis* (natto) plasmids pTA1015 and pTA1040 contain signal peptidase-encoding genes: identification of a new structural module on cryptic plasmids. Mol. Microbiol. **17**:621–631.
7. **Murray EJ, Strauch MA, Stanley-Wall NR.** 2009. SigmaX is involved in controlling *Bacillus subtilis* biofilm architecture through the AbrB homologue Abh. J. Bacteriol. **191**:6822–6832.
8. **Paetzel M, Karla A, Strynadka NC, Dalbey RE.** 2002. Signal peptidases. Chem. Rev. **102**:4549–4580.
9. **Rep M, et al.** 1996. Promotion of mitochondrial membrane complex assembly by a proteolytically inactive yeast Lon. Science **274**:103–106.
10. **Romero D, Aguilar C, Losick R, Kolter R.** 2010. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. Proc. Natl. Acad. Sci. U. S. A. **107**:2230–2234.
11. **Serrano M, et al.** 1999. A *Bacillus subtilis* secreted protein with a role in endospore coat assembly and function. J. Bacteriol. **181**:3632–3643.
12. **Spiess C, Beil A, Ehrmann M.** 1999. A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell **97**:339–347.
13. **Stöver AG, Driks A.** 1999. Secretion, localization, and antibacterial activity of TasA, a *Bacillus subtilis* spore-associated protein. J. Bacteriol. **181**: 1664–1672.
14. **Stöver AG, Driks A.** 1999. Control of synthesis and secretion of the *Bacillus subtilis* protein YqxM. J. Bacteriol. **181**:7065–7069.
15. **Suzuki CK, et al.** 1997. ATP-dependent proteases that also chaperone protein biogenesis. Trends Biochem. Sci. **22**:118–123.
16. **Terra R, Stanley-Wall NR, Cao G, Lazazzera BA.** 2012. Identification of *Bacillus subtilis* SipW as a bifunctional signal peptidase that controls surface-adhered biofilm formation. J. Bacteriol. **194**:2781–2790.
17. **Tjalsma H, et al.** 2004. Proteomics of protein secretion by *Bacillus subtilis*: separating the "secrets" of the secretome. Microbiol. Mol. Biol. Rev. **68**: 207–233.
18. **Tjalsma H, et al.** 1998. Functional analysis of the secretory precursor processing machinery of *Bacillus subtilis*; identification of a bacterial homolog of archaeal and eukaryotic signal peptidases. Genes Dev. **12**:2318–2331.
19. **Tjalsma H, Bolhuis A, Jongbloed JDH, Bron S, van Dijl JM.** 2000. Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome. Microbiol. Mol. Biol. Rev. **64**:515–547.
20. **Tjalsma H, et al.** 2000. Conserved serine and histidine residues are critical for activity of the ER-type signal peptidase SipW of *Bacillus subtilis*. J. Biol. Chem. **275**:25102–25108.
21. **Tjalsma H, et al.** 1999. The plasmid-encoded signal peptidase SipP can functionally replace the major signal peptidases SipS and SipT of *Bacillus subtilis*. J. Bacteriol. **181**:2448–2454.
22. **van Roosmalen ML, et al.** 2004. Type I signal peptidases of Gram-positive bacteria. Biochim. Biophys. Acta **1694**:279–297.
23. **Zähner D, Scott JR.** 2008. SipA is required for pilus formation in Streptococcus pyogenes serotype M3. J. Bacteriol. **190**:527–535.