CMLS, Cell. Mol. Life Sci. 59 (2002) 403–409
1420-682X/02/030403-07 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Spore germination

A. Moir*, B. M. Corfe† and J. Behravan‡

Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN (United Kingdom), Fax +44 114 272 8697, e-mail: a.moir@sheffield.ac.uk
† Current address: School of Biological Sciences, University of Manchester, Manchester M13 9PT (United Kingdom)
‡ Current address: Department of Pharmacy and Pharmaceutics, Mashhad University of Medical Sciences, Mashhad 91775-1365, Iran

Abstract. Despite being relatively insensitive to environmental insult, the spore is responsive to low concentrations of chemical germinants, which induce germination. The process of bacterial spore germination involves membrane permeability changes, ion fluxes and the activation of enzymes that degrade the outer layers of the spore. A number of components in the spore that are required for the germination response have been identified, including a spore-specific family of receptor proteins (the GerA family), an ion transporter and cortex lytic enzymes. The germinant traverses the outer layers of the spore and interacts with its receptor in the inner membrane to initiate the cascade of germination events, but the molecular details of this signal transduction process remain to be identified.

Key words. Germination; *Bacillus*; receptor; membrane; transporter.

Introduction

In order to initiate germination and restore vegetative growth when conditions become favourable, bacterial spores must be able to monitor their external environment. The complex structure of the spore, already discussed in this issue, maintains the dormant state and protects the cellular compartment from environmental challenge. The relative dehydration of the spore core or protoplast, the intracellular compartment of the structure, is the feature that distinguishes this type of cell from all others, and is the most important contributor to heat resistance and long-term quiescence of the spore. Despite its resistance to environmental insult, and its metabolic dormancy, the spore must be able to recover the characteristics of a metabolically active cell in response to an appropriate environmental trigger, such as a simple amino acid or riboside.

This germination process is essentially a biophysical and degradative one [1] – the spore's inner membrane increases in fluidity [2, 3] and ion fluxes resume; monovalent cations, potassium and sodium, move across the spore membrane, and calcium ions and dipicolinate are excreted. The peptidoglycan of the spore cortex is degraded, and the coat layers are partially degraded [4, 5]. ATP synthesis and oxidative metabolism resume [6], DNA damage is repaired [7] and the DNA-complexing small acid-soluble proteins (SASPs) are degraded by a specific protease [8], providing a source of amino acids for outgrowth. As germination events precede any de novo synthesis of macromolecules, the apparatus required for spore germination must be already present in the mature spore. We now recognise a number of these components – prospective germinant receptors, cortex lytic enzymes, one or two germination specific proteases – but there are many others that remain to be identified.

Early events in germination: the signal transduction process

The process of germination involves interaction of chemical germinants with presumed specific receptors in the spore, and the transduction of this signal in some way. There is no evidence of bulk transport or metabolism of germinant [9].

* Corresponding author.

One hypothesis to explain the germination-associated changes is that the earliest events in germination would involve membrane changes that alter permeability properties, leading to a redistribution of ions and water in the spore, and thereby activate lytic enzymes [10]; evidence of inhibition of spore germination by ion channel blockers supports this [11], as does the likely membrane association of *gerA* gene products [1].

If spores are permeabilised to lytic enzymes by removal of the spore coat, then the cortex digested with lysozyme, they will germinate [12]. Thus, the hypothesis that the direct effect of germinant binding is to activate germination specific cortex lytic enzymes and initiate the earliest stages of germination [13] was appealing. However, in recent years, several independent lines of evidence have demonstrated that the early events of germination, including loss of heat resistance, ion movements and partial rehydration of the core, are not dependent on prior cortex hydrolysis. In a *cwlD* mutant, the spore peptidoglycan lacks the normal muramic δ-lactam, and is not recognised by the cortex-specific lytic enzymes. Nevertheless, spores are still able to complete the early events of spore germination, losing heat resistance, releasing ions and the core partially rehydrating [12]. Cortex lysis is important – in fact essential for the spore to complete germination, to release the spore protoplast from the spore structure – and it does remain possible that small changes to cortex, resulting in a change in its local conformation, but not detected by bulk analysis procedures, could be involved at an earlier stage. Cortex lytic enzymes in *Bacillus subtilis* have been identified (reviewed in [14]); double mutants in lytic enzyme genes *sleB* and *cwlJ* are unable to hydrolyse the cortex to a detectable extent, and, like the *cwlD* mutant, are completely blocked in germination after the stage of loss of heat resistance and release of DPA [15].

Triggering spore germination: the germinant receptors

Mutational analysis of *B. subtilis* identified several classes of spore germination mutants defective in their response to particular germinants. *gerA* mutants, for example, are unable to germinate in L-alanine as sole germinant [16, 17], and the *gerB* [16, 18] and *gerK* [19] mutants are defective in their response to a combination of an amino acid that is incapable of acting as sole germinant, such as L-asparagine, with glucose and fructose. The germinant-specific nature of the defect in these mutants suggested that they represented the genes encoding germinant specific components – probably the receptor in the spore for germinant. The *gerA* and *gerB* genes, at least, are expressed in the developing forespore [20, 21] under the control of σG-containing RNA polymerase and of SpoVT [22], a regulatory protein homolog of AbrB [see the accompanying review by Strauch].

The *gerA* operon represents the first described of a large family of related operons in spore formers. All three genes of the *gerA* operon are likely to encode membrane-associated components (fig. 1) – the GerAA protein has a predicted domain of five or six membrane-spanning segments, as well as a large N-terminal hydrophilic domain; the GerAB protein resembles an integral membrane protein with 10 transmembrane helices; and the GerAC protein, a hydrophilic gene product, is predicted to have a prelipoprotein signal sequence [23], suggesting that it is transported across a membrane, the signal peptide cleaved, and the protein then anchored to the outer surface of the membrane via an N-terminally attached lipid moiety. As the *gerB* and *gerK* operons encode homologs of *gerA* [18, 19], the response of spores to different germinants has evolved as a consequence of gene and operon duplication and divergence; the requirement for all three protein components encoded by a particular operon for the function of that particular receptor, and their lack of functional interchangeability between operons, implies that the genes within an operon have co-evolved, and therefore that the gene products are likely to interact.

Genome sequencing revealed five *gerA* operon homologs in the *B. subtilis* genome [24] – the *gerA*, *B* and *K* operons have functions already identified by mutant phenotypes, whereas the two others, *yndDEF* and *yfkQRT*, which have been inactivated [25], are either nonfunctional or respond to germinants as yet unrecognised. The loss of the three operons *gerA*, *gerB* and *gerK* is sufficient to reduce the germination of spores, as measured by colony formation, by 1000-fold on rich agar, and deletion of the other two has no additional effect [25].

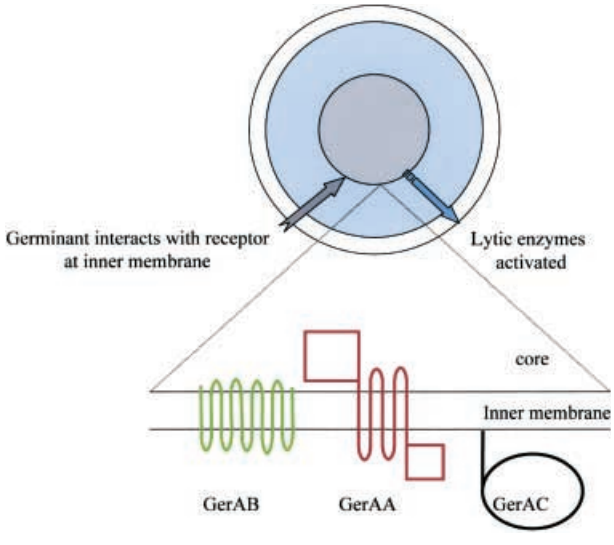

Figure 1. A representation of the *B. subtilis* spore, showing the likely organisation of components of a receptor complex in the inner membrane.

Other bacilli, including *B. halodurans*, *B. megaterium*, *B. cereus* and *B. anthracis*, also contain *gerA*-like genes in their genomes, as determined by BLAST searches of microbial DNA sequences. Direct phenotypic evidence for specific germination functions exists for three operons in *B. cereus*, whose spores can germinate in inosine or in alanine. The *gerI* [26] and *gerQ* [27] operons are both required for inosine germination, and the *gerI* and *gerL* operons contribute to L-alanine germination [27]. *B. cereus*, *B. anthracis* and *B. thuringiensis* are very closely related [28]; the latter two organisms may be considered to represent strains within the wider species of *B. cereus*, their major distinguishing characteristics deriving from the plasmids they carry. In *B. anthracis*, a spore germination operon (*gerX*) is part of the virulence gene cluster in the pXO1 plasmid, and is required for optimal spore germination in the macrophage in a mouse model [29]. Clostridia, too, encode germination genes – e.g. *C. pasteurianum* [30] – though the gene organisation may sometimes differ from the tricistronic operon organisation in *B. subtilis.*

Evidence for receptor function

The *gerAB38* and *gerAB44* mutant alleles of the *gerAB* gene [17, 31] have been sequenced [B. M. Corfe and A. Moir, unpublished]; they would each alter a residue (L24F and T192I respectively) within predicted membrane-spanning elements of the hydrophobic protein. In these mutants, the concentration of L-alanine or its analogs required for germination is increased, and the ratio of D- to L-alanine required for competitive inhibition is altered [17]. It is therefore probable that the mutations affect the ability of the protein to bind L-alanine and related germinants. The GerAB protein and its homologs form a subfamily of the APC (amino acid/polyamine/organocation) superfamily of integral membrane transporters [32, 33]. The binding site(s) for L-alanine germinant is thus predicted to lie within the membrane-spanning regions of a protein that has at least an at least evolutionary, and possibly functional, relationship to membrane transporters.

D-alanine competes with L-alanine for binding and competitively inhibits germination mediated by the *gerA* proteins. It has no effect on *gerB/K*-dependent germination, although *gerB*, too, is likely to encode an alanine-reponsive receptor component [34]. Paidhungat and Setlow [35] have recently identified *gerB** mutations that allow germination in D-alanine plus glucose, as well as in L-alanine plus glucose; the spores will even germinate in D-alanine alone, though it requires 24-h exposure to germinant for all the spores to respond. These mutations also allow spore germination in L-asparagine as sole germinant, without glucose. Two of the mutations that each are capable of permitting D-alanine as an alternative germinant lie in the *gerBA* gene, and one in the *gerBB* gene. A combination of mutations in both *gerBA* and *gerBB* genes is yet more effective, increasing the ability of the spores to respond to D-ala, and resulting in spontaneous germination of a proportion of the spores (~20%) during spore-washing procedures. The intermediate phenotype of a $gerB^{+}/gerB^{*}$ mutant merodiploid suggests that protein subunits may interact in a receptor complex, affecting each other's potential function. The mutant alleles have been sequenced; all lie in predicted hydrophobic regions of these proteins – one lies in *gerBA*, in a region that is likely to encode a membrane span in the protein; another is in a proline-rich region, conserved in all the homologs, that might be important to the structure. In the case of the *gerBB** mutation, it lies in a region that is again strongly conserved and is likely to be at the outer end of a membrane helix. The mutations may affect the binding site directly, or may affect the conformation of the proteins, resulting in an increased ability to accommodate a potential germinant, or favouring an activated conformation.

Evidence for localisation of the GerA proteins has been conflicting: immunochemical studies suggested a location for the GerAA, AB and AC proteins in the outer layers of the spore [36, 37], but these studies lacked the necessary controls to demonstrate that the antibodies were specific for the GerA proteins. The forespore location of expression, and the predicted integral association of GerAA and GerAB proteins with the membrane, made this a surprising observation. Preliminary reports of location of GerAA in a membrane fraction [32], and a more detailed analysis of the localisation of GerAA and GerAC proteins in spore fractions, controlled with null mutants [38] demonstrates that the proteins are associated with a membrane fraction that contains only inner, and not outer, spore membrane, and that they remain present in coat-extracted spores. An inner membrane location is more appealing conceptually, and fits with the retention of the specificity of germinant response in coat-defective [39] or coat-stripped [40, 41] spores. Other research has led to the same conclusion for the GerBA protein [41a].

The role of ions in spore germination

In general, monovalent cations are required in combination with nutrient germinants [42]. Tani and coworkers [43] demonstrated that a sodium proton antiporter homolog, named GrmA, was important for the germination of spores of *B. megaterium* ATCC12872 in any of its germinants (glucose, proline, leucine or KNO_3). Because germination in all germinants was affected, it was impossible to be sure whether this was a direct or pleiotropic effect. *B. megaterium* germination also requires receptors

of the *gerA* type identified in *B. subtilis* (Genbank, accession no. U61380).

A homolog of *grmA* called *gerN* has been detected and inactivated in *B. cereus*; germination in inosine is affected, but germination in alanine remains normal in rate, unless the concentration is reduced to suboptimal levels [44]. The stronger effect on inosine germination is particularly interesting, as it implies a specific role for a particular ion transporter in spore germination via a particular receptor. GerN protein is a Na^+/H^+-K^+ antiporter [44a], but its role is not yet clear – it could be directly involved in the signal transduction process, changing local membrane behaviour by localised ion transport, or its activity might be required to restore ion or charge balance if the germinant association with the receptor itself involved linked movement of the germinant with or counter to an ion species [44]. There are differences in the ion requirements for spore germination by different germinants in *B. cereus* – for example, inosine germination is stimulated by sodium ions, but unusually strongly inhibited by potassium ions [26]. There is no close *gerN*/*grmA* homolog in *B. subtilis*, and mutants in either of the more distant homologues of this sodium transporter family, *yhaU* or *yjbQ* of *B. subtilis*, germinate normally [44].

Other *ger* genes

A number of other loci were originally identified as involved in germination on the basis of altered germination properties of mutants: amongst these, the *gerC* locus [16, 45, 46] encodes enzymes of menaquinone biosynthesis; *gerCA* and *gerCC* encode the heptaprenyl diphosphate synthase, and *gerCB* the 2-heptaprenyl-1,4-naphthaquinone methyltransferase. A single *gerCC* point mutant was barely viable and accumulated suppressor mutations that restored viability; although the nature of these was not identified, the resulting mutants could have a variable degree of defect in placement of the vegetative septum, and of spore germination [46], therefore, the GerC proteins are not directly concerned with spore germination, although an abnormality in menaquinone biosynthesis may indirectly affect membrane-associated events in germination.

The *gerF* gene has been demonstrated [47] to correspond to *lgt*, encoding the prelipoprotein diacylglyceryl transferase; the consequent defect in prelipoprotein processing will affect spore germination, as a number of proteins, including GerAC and its homologs, and GerD, are all predicted lipoproteins.

Reports of a germination defect in a *fruB* mutant remained as evidence that metabolism of a germinant might be important [48]; more recent analysis [A. Moir, unpublished], failed to confirm the previously reported germination defect – despite the confirmation of the metabolic defect in vegetative cells, the mutant's spores germinated normally in fructose-containing germinant mixtures.

The *gerD* protein is required in *B. subtilis* for germination in both *gerA* and *gerB/K*-mediated responses, but its role has not yet been clearly defined [1]. There are close homologs of *gerD* in the genomes of other bacilli. Although a lipoprotein like GerAC, and expressed in the forespore compartment [49], it is not located in the inner membrane; instead, it is likely to be cortex located, as it is found in the integument fraction of mature spores and released from it by lysozyme treatment [50].

Other genes affecting the progress of sporulation, and pleiotropically affecting germination, include *gerJ* and *gerM* [1]. The *gerM* gene has been sequenced [24, 51], but has no homologs of known function. The *gerJ* locus has not yet been correlated with a sequenced ORF.

Spore coats and germination

As discussed elsewhere in this issue and in [52], mutants in spore coat assembly may have defects in spore germination. The first such mutant recognised was the *gerE36* mutant [39], which has a defect in the regulation of expression of a number of late mother cell expressed genes, including coat proteins [52]. Some mutants with coats that are not intact are unable to complete late stages of germination, such as the *cotE* mutant [41]. Some coat mutants may lack coat- or cortex-associated enzymes required for late stages of germination if these were either not correctly assembled or subsequently lost. Other coat mutants may have coats whose misassembly affects the passage of germinants to their inner membrane target. A new germination locus, *gerP*, which focuses attention on the issue of spore coat permeability, has been described in both *B. cereus* and *B. subtilis* [41]. Mutants are unable to germinate efficiently in nutrient germinants. They are, however, able to complete early stages of germination normally if the coats are removed by chemical treatment or if the coats are very incomplete, as in a *cotE gerP* double mutant. The *gerP* operon comprises six genes, expressed (like coat proteins) from a σ K-dependent promoter in the mother cell, and negatively regulated by GerE; but the level of expression is too low for these to represent major spore coat proteins. They could contribute a structural feature of the spore coat that is important in facilitating transfer of germinant molecules across the coat.

Spore cortex lytic enzymes and their activation

Genes encoding several enzymes concerned with cortex peptidoglycan processing during germination have been described; these include *sleB* gene products in *B. subtilis*

and *B. cereus* [53–55] and *cwlJ* in *B. subtilis* [15]. Although there was some speculation that germination-specific cortex lytic enzymes were covalently attached to peptidoglycan, at least in *B. megaterium* KM [56] this has not been demonstrated. In general, proteolytic activation of the *Bacillus* enzymes during germination is not probable, as these enzymes are present in the spore in their mature form; how they are held in an inactive state and activated during germination remains a mystery. There is a significant literature demonstrating that protease inhibitors can interfere with germination, at early and late stages [57], and this area deserves more attention. There is evidence of proteolytic cleavage of a clostridial lytic enzyme during germination [58, 59].

Germination by nonnutrient germinants

Nonnutrient germinant stimuli [60] include dodecylamine, dipicolinc acid and pressure. The role of calcium dipicolinate (DPA) has recently been addressed [25]; mutants blocked in all the germinant receptor operons were recoverable as colony-forming organisms by incubation in calcium DPA. DPA-induced germination may involve a different germination process from nutrient germination, or may activate the process at a later step in the pathway. Coat-depleted spores, still entirely able to complete early stages of nutrient-stimulated germination, such as loss of heat resistance, were not responsive to DPA. Presumably the heat and alkali treatment had inactivated or removed some component essential specifically for DPA-induced germination.

Inactivation of spores by high-pressure treatments will be increasingly important to the food-processing industry. Wuytack et al. [61] demonstrated that pressure induces germination at relatively low pressures (100 MPa), and that 600 MPa results in an incomplete germination response – at the higher pressure, early events were approximately normal, but late stages such as degradation of SASP proteins and ATP synthesis were delayed or inhibited. The germination of spores in response to pressures of 100 MPa is dependent on the nutrient germination pathways, as it is defective in *gerA*, *gerB* double mutants or in a *gerD* mutant [62], and a pressure-resistant mutant was also defective in germination in nutrient germinants [62]. Inhibitors of nutrient germination were able also to inhibit pressure-induced germination; more surprisingly, even at 600 MPa these inhibitors could affect germination. This suggests that even at these high pressures, the germination-like changes reflected not a mere biophysical redistribution of water in the spore, but a protein-mediated process that might well be related to a normal germination pathway.

Overview of the signal transduction process

The molecular details of signal transduction in spore germination are not yet clear, but reasonable hypotheses can be constructed with the available information (fig. 1). The chemical germinant must traverse the outer layers of the spore (possibly through specific pores in the structure, contributed by GerP proteins), to interact with the GerA-like receptor protein(s) at the inner membrane. This interaction, which may also involve local ion transfer, stimulates membrane-associated changes and bulk ion movements. Water moves into the core, partially rehydrating it. A signal whose nature is unknown is transmitted to the outer layers of the spore, activating cortex and coat lytic enzymes; cortex hydrolysis allows complete rehydration of the spore core, and consequent full metabolic activity, macromolecular synthesis resumes and the spore outgrows to form a vegetative bacterial cell.

Acknowledgments. This work was supported by the BBSRC.

1 Moir A. and Smith D. A. (1990) The genetics of bacterial spore germination. Annu. Rev. Microbiol. **44:** 531–553
2 Stewart G. S. A. B., Eaton M. W., Johnstone K., Barratt M. D. and Ellar D. J. (1979) An investigation of membrane fluidity changes during sporulation and germination of *Bacillus megaterium* KM measured by electron spin and nuclear magnetic resonance spectroscopy. Biochim. Biophys. Acta **600:** 270–290
3 Skomurski J. F., Racine F. M. and Vary J. C. (1983) Steady-state fluorescence anisotropy changes of 1,6-diphenyl-1,3,5,-hexatriene in membranes from *Bacillus megaterium* spores. Biochim. Biophys. Acta **731:** 428–436
4 Atrih A., Zollner P., Allmaier G., Williamson M. P. and Foster S. J. (1998) Peptidoglycan structural dynamics during germination of *Bacillus subtilis* 168 endospores. J. Bacteriol. **180:** 4603–4612
5 Atrih A., Bacher G., Korner R. Allmaier G. and Foster S. J. (1999). Structural analysis of *Bacillus megaterium* KM spore peptidoglycan and its dynamics during germination. Microbiology **145:** 1033–1041
6 Otani M., Ihara N, Umezawa C. and Sano K. (1986) Predominance of gluconate formation from glucose during germination of *Bacillus megaterium* QMB 1551 spores. J. Bacteriol. **167:** 148–152
7 Nicholson W. L., Chooback L. and Fajardo-Cavazos P. (1997) Analysis of spore photoproduct lyase operon (*splAB*) function using targeted deletion-insertion mutations spanning the *Bacillus subtilis* operons *ptsHI* and *splAB*. Mol. Gen. Genet. **255:** 587–594
8 Nessi C., Jedrzejas M. J. and Setlow P. (1998) Structure and mechanism of action of the protease that degrades small acid-soluble spore proteins during germination of spores of *Bacillus* species. J. Bacteriol. **180:** 5077–5084
9 Scott I. R. and Ellar D. J. (1978) Metabolism and the triggering of germination of *Bacillus megaterium*; use of L-[^{3}H]alanine and tritiated water to detect metabolism. Biochem. J. **174:** 635–640
10 Keynan A. (1978) Spore structure and its relations to resistance, dormancy and germination, in Spores VII, pp. 43–53, Chambliss G. and Vary J. C. (eds), American Society for Microbiology, Washington, DC

11 Mitchell C., Skomurski J. F. and Vary J. C. (1986) Effect of ion channel blockers on germination of *Bacillus megaterium* spores. FEMS Lett. **34:** 211–214
12 Popham D. L., Helin J., Costello C. E. and Setlow P. (1996) Muramic lactam in peptidoglycan of *Bacillus subtilis* spores is required for spore outgrowth but not for spore dehydration or heat resistance. Proc. Natl. Acad. Sci. USA **93:** 15405–15410
13 Foster S. J. and Johnstone K. (1990) Pulling the trigger; the mechanism of bacterial spore germination. Mol. Microbiol. **4:** 137–141
14 Atrih A. and Foster S. J. (1999) The role of peptidoglycan structure and structural dynamics during endospore dormancy and germination. Antonie Leeuwenhoek **75:** 299–307
15 Ishikawa S., Yamane K. and Sekiguchi J. (1998) Regulation and characterization of a newly deduced cell wall hydrolase gene (*cwlJ*) which affects germination of *Bacillus subtilis* spores. J. Bacteriol. **180:** 1375–1380
16 Moir A., Lafferty E. and Smith D. A. (1979) Genetic analysis of spore germination mutants of *Bacillus subtilis* 168: the correlation of phenotype with map location. J. Gen. Microbiol. **111:** 165–180
17 Sammons R. L., Moir A. and Smith D. A. (1981) Isolation and properties of spore germination mutants of *Bacillus subtilis* 168 defective in the initiation of germination. J. Gen. Microbiol. **124:** 229–241
18 Corfe B. M., Sammons R. L., Smith D. A. and Mauel C. (1994) The *gerB* region of the *Bacillus subtilis* 168 chromosome encodes a homologue of the *gerA* spore germination operon. Microbiology **140:** 471–478
19 Irie R., Fujita Y. and Kobayashi M. (1996) Nucleotide sequence and gene organisation of the *gerK* spore germination locus of *Bacillus subtilis* 168. J. Gen. Appl. Microbiol. **42:** 141–153
20 Feavers I. M., Foulkes J., Setlow B., Sun D., Nicholson W., Setlow P. et al. (1990) The regulation of transcription of the *gerA* spore germination operon of *Bacillus subtilis*. Mol. Microbiol. **4:** 275–282
21 Corfe B. M., Moir A., Popham D. and Setlow P. (1994) Analysis of the expression and regulation of the *gerB* spore germination operon of *Bacillus subtilis* 168. Microbiology **140:** 3079–3083
22 Bagyan I., Hobot J. and Cutting S. (1996) A compartmentalized regulator of developmental gene expression in *Bacillus subtilis.* J. Bacteriol. **178:** 4500–4507
23 Zuberi M. A., Moir A. and Feavers I. M. (1987) The nucleotide sequence and gene organisation of the *gerA* spore germination operon of *Bacillus subtilis* 168. Gene **51:** 1–11
24 Kunst F., Ogasawara N., Moszer I., Albertini A. M., Alloni G., Azevedo V. et al. (1997) The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. Nature **390:** 249–256
25 Paidhungat M. and Setlow P. (2000) Role of Ger proteins in nutrient and non-nutrient triggering of spore germination in *Bacillus subtilis*. J. Bacteriol. **182:** 2513–2519
26 Clements M. O. and Moir A. (1998) Role of the *gerI* operon of *Bacillus cereus* 569 in the response of spores to germinants. J. Bacteriol. **180:** 6729–6735
27 Barlass P. J. (1998) Characterisation of the L-alanine spore germination response of *Bacillus cereus*. PhD thesis, University of Sheffield
28 Helgason E., Okstad O. A., Caugant D. A., Johansen H. A., Fouet A., Mock M. et al. (2000) *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* – one species on the basis of genetic evidence. Appl. Env. Microbiol. **66:** 2627–2630
29 Guidi-Rontani C., Pereira Y., Ruffie S., Sirard J.-C., Weber-Levy M. and Mock M. (1999) Identification and characterization of a germination operon on the virulence plasmid pXO1 of *Bacillus anthracis*. Mol. Microbiol. **33:** 407–414
30 Meyer J. (1995) Sequence of a 10.5kbp fragment of *Clostridium pasteurianum* genomic DNA encompassing the hydrogenase-1 gene and 2 spore germination genes Anaerobe **1:** 169–174
31 Zuberi M. A., Feavers I. M. and Moir A. (1985) Identification of three complementation units in the spore germination *gerA* locus of *Bacillus subtilis* 168. J. Bacteriol. **162:** 756–762
32 Moir A., Kemp E. H., Robinson C. and Corfe B. M. (1994) The genetic analysis of bacterial spore germination. J. Appl. Bacteriol. **76:** 9S–16S
33 Jack D. L., Paulsen I. T. and Saier M. H. (2000) The aminoacid/polyamine/organocation APC superfamily of transporters specific for amino acids, polyamines and organocations. Microbiology **146:** 1797–1814
34 McCann K. P., Robinson C., Sammons R. L., Smith D. A. and Corfe B. M. (1996) Alanine germination receptors of *Bacillus subtilis.* Lett. Appl. Microbiol. **23:** 290–294
35 Paidhungat M. and Setlow P. (1999) Isolation and characterization of mutations in *Bacillus subtilis* that allow spore germination in the novel germinant D-alanine. J. Bacteriol. **181:** 3341–3350
36 Sakae Y., Yasuda Y. and Tochikubo K. (1995) Immunoelectron microscopic localization of one of the spore germination proteins, GerAB, in *Bacillus subtilis* spores. J. Bacteriol. **177:** 6294–6296
37 Yasuda Y., Sakae Y. and Tochikubo K. (1996) Immunological detection of the GerA spore germination proteins in the spore integuments of *Bacillus subtilis* using scanning electron microscopy. FEMS Lett. **139:** 235–238
38 Hudson K. D. , Corfe B. M., Kemp E. H. Feavers I. M., Coote P .J and Moir A. (2001) Localisation of GerAA and GerAC spore germination proteins in the *Bacillus subtilis* spore. J. Bacteriol. **183:** 4317–4322
39 Moir A. (1981) Germination properties of a spore coat-defective mutant of *Bacillus subtilis* 168. J. Bacteriol. **146:** 1106–1116
40 Kutima P. M. and Foegeding P. M. (1987) Involvement of the spore coat in germination of *Bacillus cereus* T spores. Appl. Environ. Microbiol. **53:** 47–52
41 Behravan J., Chirakkal H., Masson A. and Moir A. (2000). Mutations in the *gerP* locus of *Bacillus subtilis* and *Bacillus cereus* affect the access of germinants to their target in the spore. J. Bacteriol. **182:** 1987–1994
41a Paidhungat M. and Setlow P. (2001). Localization of a germinant receptor protein (GerBA) to the inner membrane of *Bacillus subtilis* spores. J. Bacteriol. **183:** 3982–3990
42 Foerster H. F. and Foster J. W. (1966) Response of *Bacillus* spores to combinations of germinative compounds. J. Bacteriol. **91:** 1168–1177
43 Tani K., Watanabe T., Matsuda H., Nasu M. and Kondo M. (1996) Cloning and sequencing of the spore germination gene of *Bacillus megaterium* ATCC12872: similarities to the NaH-antiporter gene of *Enterococcus hirae*. Microbiol. Immunol. **40:** 99–105
44 Thackray P. D., Behravan J., Southworth T. W. and Moir A. (2001) GerN, an antiporter homologue important in the germination of *Bacillus cereus* endospores. J. Bacteriol. **183:** 476–482
44a Southworth T. W., Guffanti A. A., Moir A. and Krulwich T. A. (2001) GerN: an endospore germination protein of *Bacillus cereus* is a Na^+/H^+-K^+ antiporter. J. Bacteriol. **183:** 5896–5903
45 Yazdi M. A. and Moir A. (1990) Characterisation and cloning of the *gerC* locus of *Bacillus subtilis* 168. J. Gen. Microbiol. 136: 1335–1342
46 Leatherbarrow A. J. H., Yazdi M. A., Curson J. and Moir A. (1998) The *gerC* locus of *Bacillus subtilis*, required for menaquinone biosynthesis, is concerned only indirectly with spore germination. Microbiology **144:** 2125–2130
47 Robinson C., Rivolta C., Karamata D. and Moir A. (1998) The product of the *yvoC* (*gerF*) gene of *Bacillus subtilis* is required for spore germination Microbiology 144: 3105–3109

48 Prasad C., Diesterhaft M. and Freese E. (1972) Initiation of germination in glycolytic mutants of *Bacillus subtilis.* J. Bacteriol. **110:** 321–328
49 Kemp E. H., Sammons R. L., Moir A., Sun D. and Setlow P. (1991) Analysis of transcriptional control of the *gerD* spore germination gene of *Bacillus subtilis* 168. J. Bacteriol. **173:** 4646–4652
50 Robinson C. (1996) A molecular analysis of the *gerD* and *gerF* spore germination genes of *Bacillus subtilis* 168. PhD thesis, University of Sheffield
51 Slynn G. M., Sammons R. L., Smith D. A., Moir A. and Corfe B. M. (1994) Molecular genetical and phenotypical analysis of the gerM spore germination gene of *Bacillus subtilis* 168. FEMS Micro. Lett. **121:** 315–320
52 Driks A. (1999) *Bacillus subtilis* spore coat. Microbiol. Mol. Biol. Rev. **63:** 1–21
53 Moriyama R., Hattori A., Miyata S., Kudoh S. and Makino S. (1996) A gene (*sleB*) encoding a spore cortex-lytic enzyme from *Bacillus subtilis* and response of the enzyme to L-alanine-mediated germination. J. Bacteriol. **178:** 6059–6063
54 Moriyama Kudoh S., Miyata S., Nonobe S., Hattori A. and Makino S. (1996) A germination-specific cortex-lytic enzyme from *Bacillus cereus* spores: cloning and sequencing of the gene and molecular characterization of the enzyme. J. Bacteriol. **178:** 5330–5332
55 Boland F. M., Atrih A., Chirakkal H., Foster S. J. and Moir A. (2000) Complete spore-cortex hydrolysis during germination of *Bacillus subtilis* 168 requires SleB and YpeB. Microbiology **146:** 57–64
56 Foster S. J. and Johnstone K. (1988) Germination-specific cortex lytic enzyme is activated during triggering of germination of *Bacillus megaterium* KM spore germination. Mol. Microbiol. **2:** 727–733
57 Boschwitz H., Gofshtein-Gandman L., Halvorson H. O., Keynan A. and Milner Y. (1991) The possible involvement of trypsin-like enzymes in germination of *Bacillus cereus* T and *Bacillus subtilis* 168. J. Gen. Microbiol. **137:** 1145–1153
58 Urakami K., Miyata S., Moriyama R., Sugimoto K. and Makino S. (1999) Germination-specific cortex lytic enzymes from *Clostridium perfringens* S40 spores: time of synthesis, precursor structure and regulation of enzyme activity. FEMS Microbiol. Lett. **173:** 467–473
59 Okamura S., Urakami K., Kimata M., Aoshima T., Shimamoto S., Moriyama R. et al. (2000) The N-terminal prepeptide is required for the production of spore cortex-lytic enzyme from its inactive precursor during germination of *Clostridium perfringens* S40 spores. Mol. Microbiol. **37:** 821–827
60 Gould G. (1969) Germination, In: The Bacterial Spore, pp. 397–444, Gould G. W. and Hurst A. (eds), Academic Press, London
61 Wuytack E. Y., Boven S. and Michiels C. W. (1998) Comparative study of pressure-induced germination of *Bacillus subtilis* spores at low and high pressures. Appl. Environ. Microbiol. **64:** 3220–3224
62 Wuytack E. Y., Soons J., Poschet F. and Michiels C. W. (2000) Comparative study of pressure- and nutrient-induced germination of *Bacillus subtilis* spores. Appl. Env. Microbiol. **66:** 257–261