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MINIREVIEW

Genetic and Physiological Regulation of Bacterial Endospore Development

KRYSTYNA I. WOLSKA,* ANNA M. GRUDNIAK and ANNA KRACZKIEWICZ-DOWJAT

Faculty of Biology, Institute of Microbiology, Department of Bacterial Genetics, Warsaw University

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Abstract

Bacterial endospores are complex structures residing inside endospore-forming, mainly gram-positive bacteria. The process of sporulation is considered a simple example of cell differentiation. Endospores enable the organism to resist environmental stresses. Sporulation can be divided into several stages, from axial DNA filamentation to mother cell lysis. The structure and formation of an endospore is an attractive model for the assembly of complex macromolecular structures during development. The expression of genes involved in sporulation is compartmentalized and different sets of genes are expressed in the prespore and mother cell, this being associated with the subsequent activation of four sporulation-specific σ factors. Their synthesis and activity are tightly regulated and the regulatory mechanisms have overlapping roles.

K e y w o r d s: alternative sigma factors, compartmentalization, endospores, sporulation

Introduction

Certain bacteria produce specific intracellular structures, endospores; the process of their formation is called sporulation. Endospore formation can be considered a primitive system of cell differentiation and has become a paradigm for the study of this phenomenon in prokaryotes. Bacterial endospores are complex structures, whose basic architecture is conserved across species (Errington, 2003). Endospore formation is preceded by asymmetric cell division in which sister cells undergo dissimilar fates (Horvitz and Herskowitz, 1992).

Endospores enable an organism to resist extreme environmental conditions such as: temperature, drying, ultraviolet radiation, strong acids and bases, oxidizing agents, extremes of both vacuum and ultrahigh hydrostatic pressure (Nicholson *et al*., 2002). These highly resistant structures survive heating to 150°C although the endospores of most species are killed at 121°C in moist heat (Madigan and Martinko, 2006a).

Biogenesis of endospores is initiated mainly by extracellular conditions, of which nutrient deprivation and high cell density are the most important (Grossman and Losick, 1988; Stragier and Losick, 1996). Intracellular environment is also monitored, *e.g*. damage of DNA and blocking of replication prevent the initiation of sporulation causing that only cells with undamaged replicating chromosomes can proceed to spore formation (Lemon *et al*., 2000).

A chemical substance characteristic of endospores is dipicolinic acid, complexed with calcium ions. This complex functions to reduce water availability within the endospore and thus helps to dehydrate it and also, due to its ability to intercalate in DNA, stabilized this compound to heat denaturation. Endospores contain also high level of unique, small acids-soluble proteins (Madigan and Martinko, 2006b). Recently it was shown that membrane-bound, thiol-disulfide oxidoreductases are required for efficient production of *Bacillus subtilis* endospores (Möller and Hederstedt, 2006). The signals stimulating sporulation are involved in phosphorelay, a complex version of two-component system which activates the master sporulation regulator, Spo0A (Burbulys *et al*., 1991). The problem is discussed in the next chapter of this review.

All endospore formers show phylogenetic affiliation with the "low GC" Gram-positive *Bacteria*, among which the most frequently studied are *Bacillus* and *Clostridium*. The major genera of endospore forming bacteria include: *Bacillus*, *Paenibacillus*, *Sporolactobacillus*, *Desulfotomaculum*, *Clostridium*,

* Corresponding author: K.I. Wolska, Faculty of Biology, Institute of Microbiology, Dept. of Bacterial Genetics, Warsaw University, Miecznikowa 1, 02-096 Warsaw, Poland; **e-mail: izabelaw@biol.uw.edu.pl**

Thermoanaerobacter, *Sporomusa*, *Sporohalobacter*, *Anaerobacter*, *Alicyclobacillus*, *Amphibacillus*, *Helicobacterium*, *Heliophilum*, *Heliorestis*, *Syntrophospora*, *Desulfitobacterium* and *Sporosarcina* (Madigan and Martinko, 2000b). It can be mentioned here that endospore preparations derived from *Bacillus thuringiensis* and *Paenibacillus popillae* are commercially available as biological insecticides. The unique feature of *Sporosarcina ureae* sporulation is the position of sporulation septa, which is medially located with respect to the cell poles, in contrast to the gross asymmetry of its localization for bacilli and clostridia (Zhang *et al*., 1997).

Recently several excellent reviews were published focusing various aspects of bacterial sporulation (Henriques and Moran, 2000; Hilbert and Piggot, 2004; Yudkin and Clarkson, 2005). This review focuses endospores formed by *B. subtilis*.

Stages of sporulation

The process of sporulation can be divided into eight stages designated 0 to VII. In *B. subtilis* sporulation takes about 7 h at 37°C. Spores purified at 9, 24 and 48 h after the onset of sporulation appear structurally equivalent when examined by electron microscopy. More than 400 genes are involved in sporulation, they govern the synthesis of endospore-specific proteins and cessation of the synthesis of many proteins involved in vegetative cell functions. A schematic representation of the stages of spore formation is presented in Fig. 1.

The vegetative cell represents stage 0, at this stage two copies of cellular chromosome become more dense. During stage I DNA filament stretching across the long axis of the cell is formed (Bylund *et al*., 1993). Then the cell divides at the subpolar site and two unequally sized daughter cells – mother cell and forespore (prespore) are formed (stage II). SpoIIE and FtsA proteins play a major role in polar (instead of mid-cell) formation of Z ring determining the future division site which is composed of protein FtsZ – the homologue of prokaryotic tubulin (Ben-Yehuda and Losick, 2002). Originally the prespore contains the origin-proximal one third of the chromosome, subsequent efficient pumping of DNA to the prespore by translocase SpoIIIE results in the two daughter cells having identical genomes (Bath *et al*., 2000). After migration of the septal membranes around both sites of the prespore and their fusion at the cell pole, the prespore becomes engulfed by the mother cell in a phagocytosis-like process – stage III (Piggot *et al*., 1994). Recently the possibility of ratchet-like mechanism of engulfment has been postulated which involves zipper-like interactions between the forespore protein SpoIIQ and its mother cell ligand SpoIIIAH (Broder and Poligano, 2006). During stage IV two murein (peptidoglycan) layers, primordial germ cell wall and cortex, are formed in the space between the membranes surrounding the prespore. Then (stage V) the prespore is covered by the coat composed by various proteins (Henriques and Moran, 2000). During the following stage VI the spore acquires resistance to UV radiation and high temperature in a process called spore maturation (Nicholson *et al*., 2000). At the last step VII, mature spore is released to the environment after mother cell lysis. Sporulation is coupled to profound changes in gene expression executed by RNA polymerase containing the alternative s factors, which will be described later.

Endospore structure

The structure of endospore is more complex than that of vegetative cell. Inside the spore there is a core (spore protoplast), Cr, containing cytoplasm, nucleoid and ribosomes. The core of the mature endospore has

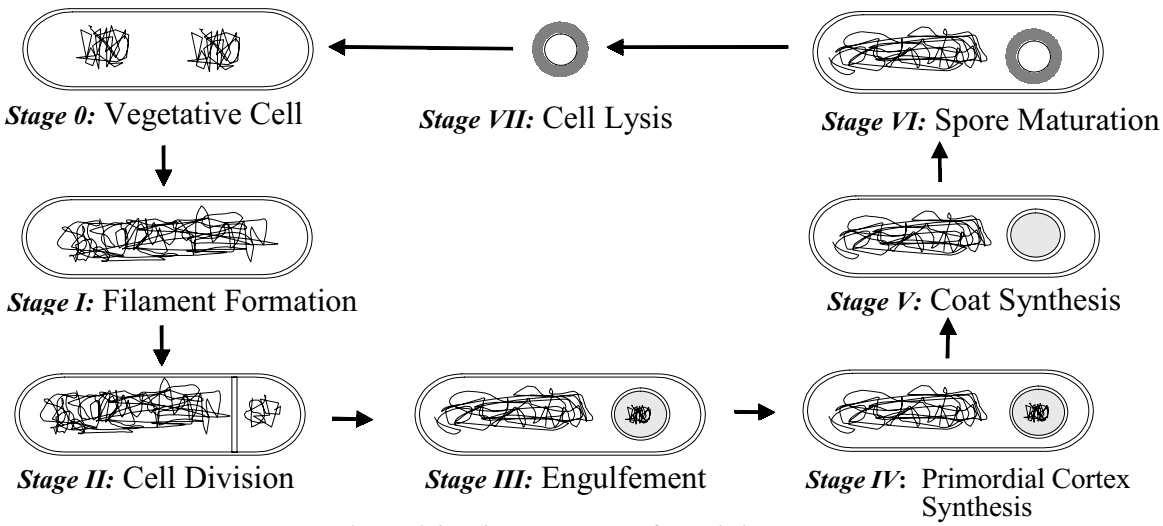

Fig. 1. Eight subsequent stages of sporulation.

only 10–25% of the vegetative cell water content what increases its resistance to heat and chemicals. The pH of the core is about one unit lower than that of the vegetative cell cytoplasm and the core contains a high level of small acid-soluble proteins, SASPs, able to bind DNA and to protect it from potential damage (Madigan and Martinko, 2006a). The basic endospore structure is depicted in Fig. 2. The core compartment is separated from the mother cell cytoplasm by two membranes of opposing polarity-inner and outer forespore membrane, respectively IFM and OFM. Between them thin primordial germ cell wall (PGCW) and cortex (Cx) composed of murein are deposited. Cortex murein has several unique structural modifications, the most dramatic is the removal of the peptide side chains from approximately 50% of the N-acetylmuraminic acid residues and their conversion to muramine δ-lactam (Warth and Strominger, 1972). The cortex is critical for maintaining spore dormancy, heat resistance and protection from lytic enzymes (Jenkinson *et al*., 1980). The endospore is covered with a coat composed of several protein layers (Henriques and Moran, 2000).

The process of coat assembly depends on the sequential interactions among specific components and on their secondary modification (Zhang *et al*., 1993). The most inner layer of the coat comprises amorphous material called undercoat (Zheng *et al*., 1988). Recent analysis characterized the complex interactions between 32 coat proteins in which low-affinity interactions are abundant. The assembly of most of the coat is directed by a small subset of proteins (Kim *et al*., 2006). The inner coat (IC) is surrounded by the undercoat (UC) from the inside and from the outside by the outer coat (OC). The latter is organized in a pattern of closely aligned rods and bars positioned along the longitudinal axis of the endospore (Aronson and Fitz-James, 1976). The whole structure is usually covered by a thin additional surface layer (Zilhão *et al*., 1999).

The coat contains 50–78% of total spore proteins which are conventionally denoted Cot and can be divided into two groups – alkali-soluble and alkali-insoluble. Genes *cot* are transcribed by RNA polymerase with σ^E and σ^K subunits, regulatory proteins SpoIIID and GerE are also involved in their transcription (Henriques and Moran, 2000; Kuwana *et al*., 2004). Around 20 *cot* genes have been identified by reverse genetics, they encode coat structural components and also proteins participating in coat assembly (Beall *et al*., 1993). Many coat proteins are posttranscriptionally modified by glycosylation, proteolytic processing and crosslinking (Henriques and Moram, 2000). Following septation, the CotE protein starts to assemble in a ring-like structure that completely encircles the prespore during engulfment, The formation of the CotE ring is guided by SpoIVA (Driks *et al*., 1994; Roels *et al*., 1992). During the next steps of coat formation, other coat components are expressed and assembled due to the sequential interactions between specific Cot proteins (Seyler *et al*., 1997).

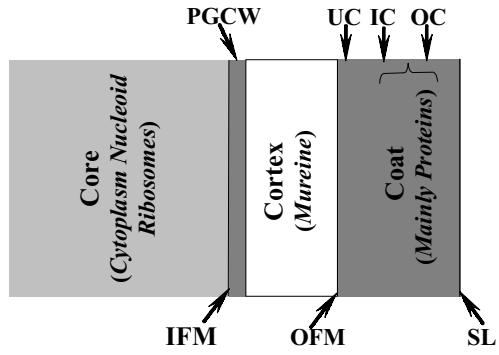

Fig. 2. Endospore structure (acc. to Henriques and Moran, 2000, modified). Symbols explanations in the text.

The proper formation of coat layers is of great importance for spore germination induced in response to the presence of several nutrients and also non-nutrient agents such as heat activation (Bourne *et al*., 1991; Popham *et al*., 1995). Nutrient germinants bind to receptors in endospore inner membrane, causing the release of dipicolinic acid and cations from the core, this being followed by hydrolysis of the murein cortex (Setlow, 2003). The ultrastructural analysis of germinating spores reveals that the coat is cracked at the discrete locations which may reflect the site of assembly of specific lytic enzymes.

Genetics of endospore formation

In response to yet unidentified stimuli at least five kinases involved in phosphorelay (KinA, KinB, KinC, KinD and KinE) autophosphorylate and then transfer their phosphate groups to the response regulator Spo0F (Hilbert and Piggot, 2004). The phosphotransferase Spo0B transfers the phosphate from Spo0F-PO_4 to Spo0A (Burbulys *et al*., 1991). Active Spo0A and alternative σ factor, σ^H, are involved in axial filamentation and asymmetrically located sporulation division. The remodeling of two complete chromosomes or partially replicated chromosome is executed trough the action of DivIVA, RacA and Soj proteins (Cha and Steward, 1997; Ben-Yehuda *et al*., 2003; Martson and Errington, 1999). In turn Spo0A-PO_4 activates the transcription of genes encoding the early compartmentalization s factors: σ^F and σ^E. The transcription patterns differ in the prespore and mother cell, σ^F and σ^G factors are active in the prespore, σ^E and σ^K are active in the mother cell in early and late stages of prespore development, respectively. It is claimed that successful sporulation depends mainly on the regulation of σ^F which is activated only in the prespore,

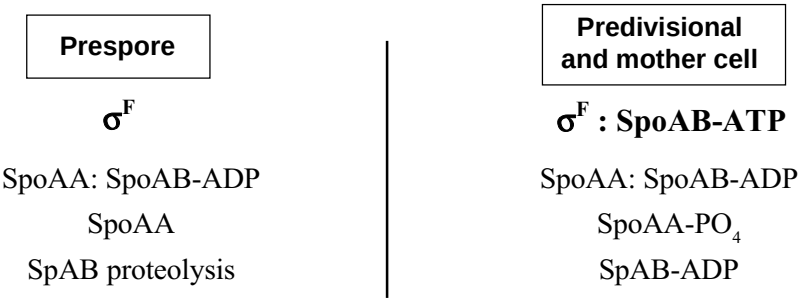

Fig. 3. Forms of σ^F and its regulators present in predivisional (and mother) cell and prespore.

immediately after asymmetric division (Yudkin and Clarkson, 2005) where they remain active for at least an hour. The fluorescence microscopy of cells expressing transcriptional fusions of *gfp* (gene encoding green fluorescent protein) demonstrated that σ^F and σ^E are active very soon after completion of septum formation and σ^G and σ^K become active after engulfment, respectively in prespore and mother cell (Harry *et al*., 1995; Zhang *et al.*, 1996). Genome analysis of temporally regulated and compartment-specific gene expression in sporulating cells of *B. subtilis* revealed 55 genes expressed under σ^F, 154 – under σ^E, 113 under σ^G and 132 under σ^K control (Steil *et al*., 2005). In *B. subtilis* the main σ factor, σ^A, is active in the prespore and the mother cell throughout entire process of sporulation (Li and Piggot, 2001).

It should be mentioned that the substitution of the main s factor by the alternative ones results in the global changes in gene expression. This strategy is utilized by various bacteria, mainly under stress conditions. The best known examples, except factors involved in bacterial sporulation, include: σ^{32} which governs the expression of heat shock regulon (Yura *et al*., 1996), σ^{54} regulating a variety of functions, *e.g.* the expression of nitrogen regulon in *Enterobacteriaceae* (Kustu *et al*., 1989; Wolska, 1996a) and σ^{38} important for the gene expression in the stationary growth phase (Eisenstark *et al*., 1996). The negative regulation of transcription by anti-σ factors (see below) was also described in the systems other than these involved in the control of sporulation (Helman, 1999; Wolska, 1996b).

σ^F and its regulon

σ^F is encoded by *spoIIAC* gene, the third gene in the *spoIIA* operon. The other two products of this operon, SpoIIAA and SpoIIAB regulate σ^F activity. Briefly, regulation relies on the interactions of four proteins: σ^F, its anti-sigma factor, SpoIIAB, having protein kinase activity, anti-anti-sigma factor, SpoIIAA and phosphatase SpoIIE (Yudkin and Clarkson, 2005; Schmidt *et al*., 1990). Before asymmetric division and also in mother cell σ^F is inactivated by forming the complex with SpoIIAB-ATP; only the phosphorylated form of anti-sigma factor is active. Anti-anti-σ SpoIIAA also remains phosporylated on Ser58 due to SpoIIAB kinase activity (Najafi *et al*., 1995). Thus SpoIIAB inhibits σ^F both directly and indirectly by inactivating the anti-anti-σ factor SpoIIAA. Membrane-bound serine phosphatase, SpoIIE, which is localized to sites of asymmetric septum assembly dephosporylates SpoIIAA in prespore, first leading to the formation of SpoIIAA-SpoIIAB-ADP complex and then free SpoIIAA what is simultaneous to the disruption of σ^F-SpoIIAB-ATP complex.

Asymmetric division increases the level of dephosphorylated SpoIIAA in prespore either by the activation of SpoIIE phosphatase activity or by the regulation of its interactions with the division proteins. Sequestration and proteolysis of SpoIIAB in the prespore can also be involved in σ^F activation (Pan *et al.*, 2001). Released σ^F initiated the temporal sequence of sporulation-specific gene expression (Barák and Youngman, 1996; Duncan *et al*.,1995). Partner switching by SpoIIAB from σ^F to SpoIIAA is crucial for σ^F activation (Alper *et al*., 1994).

The possible forms of σ^F and its regulators present in the predivisional cell and in the prespore are listed in Fig. 3. It should be mentioned that this regulation is very efficient, moreover it is executed with very limited number of regulatory proteins and at low cost in ATP (Yudkin and Clarkson, 2005).

σ^F regulon comprises around 70 genes that were active during the middle part of sporulation (Fawcett *et al*., 2000). The primarily function of σ^F are: 1) to couple prespore and mother-cell specific gene expression, *e.g*. s*poIIR* and *spoIVB* genes expressed under control of σ^F are involved in the regulation of early σ^E and late σ^K factors in mother cell (Cutting *et al*., 1991; Karow *et al*., 1995) and 2) to direct synthesis of late prespore transcription factor σ^G encoded by *spoIIG* gene (Sun *et al*., 1991).

σ^E and its regulon

σ^E is activated in the mother cell following the asymmetric septation, after receiving a signal from the prespore. It was the first purified sporulation factor (Haldenwang *et al*., 1981). Original pro-σ^E, a product

of *spoIIGB* gene is processed into mature σ^E by the proteolytic removal of 27 residues from N terminus (Stragier *et al*., 1988) by membrane-bound SpoIIGA protease. SpoIIGA is activated only in mother cell by SpoIIR protein which expression is govern by σ^F and which acts from the prespore (Hilbert and Piggot, 2004). Expression of pre-σ^E depends mainly on Spo0A-PO_4 which is present in both cellular compartments but its activity is largely confined to the mother cell (Fujita and Losick, 2002). Pro-σ^E synthesized in the prespore is efficiently degraded (Hilbert and Piggot, 2004).

σ^E governs the transcription of genes encoding functions needed for 1) preventing a second asymmetric division in the mother cell (Eichenberger *et al*., 2001), 2) triggering the engulfment of the prespore (Abanes-De Mello *et al*., 2002), 3) initiating the spore coat assembly (Beall *et al*., 1993) and 4) directing the synthesis of the late mother cell-specific σ factor, σ^K (Kunkel *et al*., 1990).

σ^G and its regulon

Transcription of *spoIIIG* gene encoding σ^G is directed in the prespore by RNA polymerase containing σ^F (Sun *et al*., 1991). Its activation requires the activity of σ^E in the mother cell. The main function of σ^E-directed regulation appears to coordinate σ^G activation with the completion of engulfment (Chary *et al*., 2006). Transcription of *spoIIIG* in the prespore depends also upon expression of *spoIIQ* in the prespore (Sun *et al*., 2000). σ^G is originally inhibited by anti-anti-σ factor SpoIIAB (Coppolecchia *et al*., 1991), this inhibition can be relieved by SpoIIIJ which is localized to the prespore membrane and expressed in vegetative cell but its activity is needed only in the prespore (Errington *et al*., 1992). It remains to be documented if SpoIIIJ is involved in the reception of signal from the mother cell which communicates that the engulfment is completed. Except SpoIIIJ at least one product of *spoIIIA* operon is needed for σ^G activation (Hilbert and Piggot, 2004).

σ^G regulon comprises genes involved in: 1) spore formation, *e.g. spoVA* operon which is required for dipicolinic acid uptake into prespore from the mother cell (Moldover *et al*., 1991); 2) germination, for example *gerA* and *gerB* operons needed for germination in response to alanine and other germinants, respectively (Paidhungat and Setlow, 2002); 3) protection the spore from DNA damage, *e.g. splB* which encodes spore photoproduct lyase (Fajardo-Cavazos and Nicholson, 2000).

σ^K and its regulon

Full-length σ^K is encoded by composite *sigK* gene formed during sporulation, after excision of *skin* element (signal intervening element) localized between loci coding for N and C-terminal parts of σ^K. The excision demands activity of SpoIVCA protein which shows substantial similarity to Hin family of site-specific recombinases (Stragier *et al*. 1989). σ^K is synthesized in inactive form, its processing depends on both the mother cell and prespore components and occurs in the outer prespore membrane (Lu *et al*., 1995). Pro-σ^K is cleaved by SpoIVFB protein, BofA and SpoIVFA negatively regulate this process by forming the inactive SpoIVFA-SpoIVFB-BofA complex (Rudner and Losick, 2001). Processing of pro-σ^K into mature σ^K needs prespore signaling. Serine protease SpoIVB inserted into the inner prespore membrane undergoes autoproteolysis and then diffuses across the inter-membrane space and interacts with the inactive complex, leading to SpoIVFA degradation and triggering pro-σ^K processing (Rudner and Losick, 2002). It was proposed that the regulated membrane proteolysis of σ^K involves a three-step proteolytic cascade in which SpoIVB first cleaves SpoIVFA, another serine protease CtpB cleaves BofA and finally SpoIVFB cleaves pro-σ^K (Zhou and Kroos, 2005).

The σ^K regulon is involved in: 1) formation of the spore coat (Henriques and Moran, 2000); 2) spore maturation (Fan *et al*., 1992) and 3) regulation of σ^K-dependent transcription (Kunkel *et al*., 1989).

The mother cell and prespore communicate with each other by influencing the activity of σ factors throughout the intermediate and late stages of sporulation. The main regulators are: SpoIIR, SpoIIGA, SpoIIIA, SpoIIIJ, SpoIVFB (Hilbert and Piggot, 2004). This intercommunication is crucial for compartmentalization and temporal control of gene expression. σ^F factor is absolutely confined to the prespore and its activity is indispensable for subsequent activation of σ^E in mother cell. It is executed through σ^E-dependent prespore SpoIIR protein which activates SpoIIGA membrane protease processing inactive pro-σ^E to active σ^E in the mother cell. In turn, σ^G is expressed in the prespore under the control of σ^F but is activated after transcription of SpoIIIA operon in the mother cell. σ^G causes the expression of SpoIVB which triggers the processing of pro-σ^K to σ^K by mother cell protein SpoIVFB.

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