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 \( \sigma \) factors. Their synthesis and activity are tightly regulated and the regulatory mechanisms have overlapping roles.

Key words: 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, Desulfootomaculum, 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, Helioresistis, 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 SpoIIE and its mother cell ligand SpoIIA (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 σ 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
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 σE and σK 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 posttranslationally modified by glycosylation, proteolytic processing and crosslinking (Henriques and Moran, 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).

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-P to Spo0A (Burbuly et al., 1991). Active Spo0A and alternative σ factor, σH, 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-P activates the transcription of genes encoding the early compartmentalization s factors: σF and σE. The transcription patterns differ in the prespore and mother cell, σE and σG factors are active in the prespore, σF and σK 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 σF which is activated only in the 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 $\sigma^F$ and $\sigma^E$ are active very soon after completion of septum formation and $\sigma^G$ and $\sigma^K$ become active after engulfment, respectively in prespore and mother cell (Harry et al., 1995; Zhang et al., 1996). Genotype analysis of temporally regulated and compartment-specific gene expression in sporulating cells of B. subtilis revealed 55 genes expressed under $\sigma^F$, 154 – under $\sigma^E$, 113 under $\sigma^G$ and 132 under $\sigma^K$ control (Steil et al., 2005). In B. subtilis the main s factor, $\sigma^K$, 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: $\sigma^{32}$ which governs the expression of heat shock regulon (Yura et al., 1996), $\sigma^{34}$ regulating a variety of functions, e.g. the expression of nitrogen regulon in Enterobacteriaceae (Kustu et al., 1989; Wolska, 1996a) and $\sigma^{38}$ 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 those involved in the control of sporulation (Helman, 1999; Wolska, 1996b).

$\sigma^F$ and its regulon

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

Asymmetric division increases the level of dephosphorylated SpoIIBA 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 SpoIIB in the prespore can also be involved in $\sigma^F$ activation (Pan et al., 2001). Released $\sigma^F$ initiated the temporal sequence of sporulation-specific gene expression (Barák and Youngman, 1996; Duncan et al., 1995). Partner switching by SpoIIB from $\sigma^F$ to SpoIIBA is crucial for $\sigma^E$ activation (Alper et al., 1994).

The possible forms of $\sigma^F$ 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).

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

$\sigma^E$ and its regulon

$\sigma^E$ 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-$\sigma^E$, a product
of spoIIGB gene is processed into mature $\sigma^K$ 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 $\sigma^K$ and which acts from the prespore (Hilbert and Piggot, 2004). Expression of pre-$\sigma^K$ depends mainly on SpoA-PO$_4$ which is present in both cellular compartments but its activity is largely confined to the mother cell (Fujita and Losick, 2002). Pre-$\sigma^K$ synthesized in the prespore is efficiently degraded (Hilbert and Piggot, 2004).

$\sigma^K$ 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 $\sigma$ factor, $\sigma^K$ (Kunkel et al., 1990).

$\sigma^G$ and its regulon

Transcription of spoIII gene encoding $\sigma^G$ is directed in the prespore by RNA polymerase containing $\sigma^K$ (Sun et al., 1991). Its activation requires the activity of $\sigma^K$ in the mother cell. The main function of $\sigma^K$-directed regulation appears to coordinate $\sigma^G$ activation with the completion of engulfment (Chary et al., 2006). Transcription of spoIII in the prespore depends also upon expression of spoIIO in the prespore (Sun et al., 2000). $\sigma^G$ is originally inhibited by anti-anti-$\sigma$ factor SpoIIB (Coppolecchia et al., 1991), this inhibition can be relieved by SpoIII 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 SpoIII is involved in the reception of signal from the mother cell which communicates that the engulfment is completed. Except SpoIII at least one product of spoIII operon is needed for $\sigma^G$ activation (Hilbert and Piggot, 2004).

$\sigma^G$ 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. spIB which encodes spore photoproduct lyase (Fajardo-Cavazos and Nicholson, 2000).

$\sigma^K$ and its regulon

Full-length $\sigma^K$ is encoded by composite sigK gene formed during sporulation, after excision of skin element (signal interving element) localized between loci coding for N and C-terminal parts of $\sigma^K$. The excision demands activity of SpoIVCA protein which shows substantial similarity to Hin family of site-specific recombinases (Stragier et al. 1989). $\sigma^K$ 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-$\sigma^K$ is cleaved by SpoIVFB protein, BofA and SpoIVFA negatively regulate this process by forming the inactive SpoIVFABoomIVFAB complex (Rudner and Losick, 2001). Processing of pro-$\sigma^K$ into mature $\sigma^K$ 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-$\sigma^K$ processing (Rudner and Losick, 2002). It was proposed that the regulated membrane proteolysis of $\sigma^K$ involves a three-step proteolytic cascade in which SpoIVB first cleaves SpoIVFAB, another serine protease CtpB cleaves BofA and finally SpoIVFB cleaves pro-$\sigma^K$ (Zhou and Kroos, 2005).

The $\sigma^K$ 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 $\sigma^K$-dependent transcription (Kunkel et al., 1989).

The mother cell and prespore communicate with each other by influencing the activity of $\sigma$ 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. $\sigma^K$ factor is absolutely confined to the prespore and its activity is indispensable for subsequent activation of $\sigma^K$ in mother cell. It is executed through $\sigma^K$-dependent prespore SpoIIR protein which activates SpoIIGA membrane protease processing inactive pro-$\sigma^K$ to active $\sigma^K$ in the mother cell. In turn, $\sigma^K$ is expressed in the prespore under the control of $\sigma^K$ but is activated after transcription of SpoIIIA operon in the mother cell. $\sigma^K$ causes the expression of SpoIVB which triggers the processing of pro-$\sigma^K$ to $\sigma^K$ by mother cell protein SpoIVFB.

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Literature


