

## SGM Prize Lecture

### Fred Griffith Prize Lecture 2009

#### Correspondence

Jeff Errington

jeff.errington@ncl.ac.uk

# From spores to antibiotics via the cell cycle

Jeff Errington

Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle-upon-Tyne, NE2 4HH, UK

Spore formation in *Bacillus subtilis* is a superb experimental system with which to study some of the most fundamental problems of cellular development and differentiation. Work begun in the 1980s and ongoing today has led to an impressive understanding of the temporal and spatial regulation of sporulation, and the functions of many of the several hundred genes involved. Early in sporulation the cells divide in an unusual asymmetrical manner, to produce a small prespore cell and a much larger mother cell. Aside from developmental biology, this modified division has turned out to be a powerful system for investigation of cell cycle mechanisms, including the components of the division machine, how the machine is correctly positioned in the cell, and how division is coordinated with replication and segregation of the chromosome. Insights into these fundamental mechanisms have provided opportunities for the discovery and development of novel antibiotics. This review summarizes how the bacterial cell cycle field has developed over the last 20 or so years, focusing on opportunities emerging from the *B. subtilis* system.

## Introduction

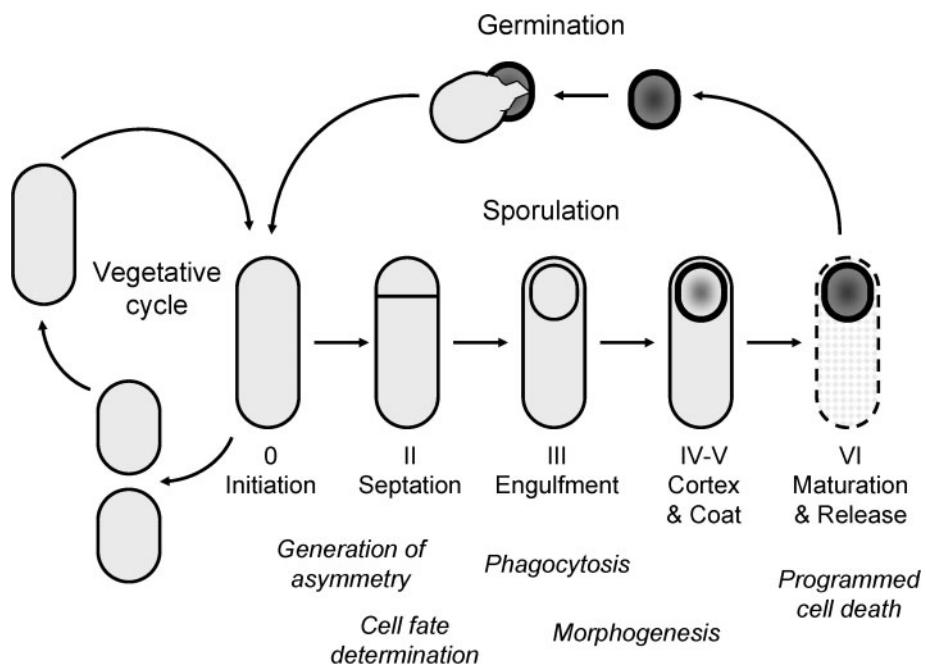
How a single relatively featureless fertilized egg gives rise to the spectacularly complex adult human is one of the central problems of biology. The adult contains a plethora of distinctive, highly differentiated cell types, and each cell needs to acquire its correct characteristics for the organism to function correctly. Differentiation and development are characterized by a range of loosely defined processes. These include, in no specific order and with no attempt to be comprehensive: the generation of asymmetry, cell fate determination, intercellular signalling, temporal and spatial control of gene expression, and programmed cell death. Patterns of differentiation need to be regulated both spatially and temporally. The complexity of development in humans is mind boggling, and the experimental tools needed to make headway in this problem remain rather inadequate.

As an undergraduate geneticist I was fascinated by the problems posed by the genetic control of development. After my PhD in 1981, I applied for various post-doc positions to study development, including that of *Dictyostelium*, *Drosophila* and *Mus*. The first offer I received (which I accepted) was to work on a bacterium, *Bacillus subtilis*, with Professor Joel Mandelstam at Oxford. This turned out to be a career-defining move and I still work on *B. subtilis* to this day.

## ***B. subtilis* as a model for cellular development and differentiation**

It had been recognized in the 1960s that spore formation in *Bacillus* exemplified, in simple form, several of the most

fundamental aspects of development. Fig. 1 shows a schematic of the life cycle of the organism, highlighting some of the interesting questions it poses (Errington, 2003; Hilbert & Piggot, 2004; Kroos, 2007). Sporulation is largely an adaptive response to starvation but the cell integrates a huge range of external and internal signals before making the decision to proceed. Having initiated sporulation, the rather symmetrical cell, which would normally divide precisely in the middle to generate two identical daughters, switches to a highly asymmetrical division, generating a small prespore cell destined to become the mature spore and a much larger mother cell. The generation of asymmetry is one of the hallmarks of development in most complex organisms, though it is poorly understood in any. Almost as soon as the prespore and mother cells are separated they initiate completely different programmes of gene expression, which specify their distinct cell fates. Next, the prespore is engulfed by the mother cell, through a mechanism that resembles phagocytosis in eukaryotes. Then, over a period of several hours, the prespore undergoes a dramatic morphological transition in which it is dehydrated and mineralized and coated with various protective layers. Finally, the mother cell undergoes programmed cell death, or apoptosis, to release the mature spore. Sporulation therefore encapsulates, in a relatively simple, two-cell system, many of the hallmarks of cellular development and differentiation in higher organisms. Importantly, *B. subtilis* is also a superb experimental system. The cells are easy to grow and the switch to sporulation is easy to induce by starvation of the cells. The standard protocol was developed in Mandelstam's lab (Sterlini & Mandelstam, 1969) and became his most highly cited paper (to Joel's dismay!) The standard lab strain of



**Fig. 1.** *B. subtilis* sporulation as a model for development and differentiation. Schematic overview of the sporulation cycle. The vegetative cycle is favoured under conditions supporting growth. Cells grow by elongating along their long axis and then divide medially to produce two identical daughter cells. Starvation induces the sporulation cycle. The sequence of key morphological stages is illustrated, labelled according to the classical nomenclature. For simplicity, Stage I has been omitted and Stages IV and V combined. Mature released spores can remain dormant for an almost indefinite period before undergoing germination and outgrowth to resume the vegetative cycle. Various events of general relevance to developmental biology are labelled below in italic font.

*B. subtilis*, 168, was also naturally transformable at high efficiency (Anagnostopoulos & Spizizen, 1961), so genetic analysis was very well developed. Finally, partly because *B. subtilis* and its relatives are important industrial organisms, a lot was known about the general biochemistry and physiology of *B. subtilis* and its relatives.

My arrival on the *B. subtilis* scene was timely because it was soon after the cloning and DNA sequencing revolution. Mandelstam and colleagues, together with a few other laboratories around the world, had spent about 15 years carefully isolating, classifying and characterizing mutations that specifically affected sporulation. Phenotypic characterization of the mutants, together with genetic mapping, had shown that there were 50 or more distinct genetic loci devoted to sporulation (Piggot & Coote, 1976). Moreover, it soon emerged that many loci probably contained more than one gene. The allocation of such a huge number of genes to such a simple, two-cell differentiation process was striking for someone who had been considering working on mouse development! Joel Mandelstam was coming to the end of his very successful career, and he was generous in allowing post-docs in his lab to run amusing side projects. Howard Jenkinson and I realized that the vast collection of *spo* mutants was going to be a fantastic resource if we could devise methods to clone the

corresponding wild-type genes and then characterize them in molecular terms. Howard did the first successful cloning experiments in the lab (Jenkinson & Mandelstam, 1983) and introduced me to the temperate bacteriophage  $\phi$ 105. I continued working with the phage as a vector and eventually cracked the cloning problem, obtaining libraries of recombinant phages from which we isolated virtually all of the known *spo* loci (Errington, 1984; Errington & Jones, 1987; Jones & Errington, 1987).

In 1985 I started my own lab at Oxford, and in 1987 Joel Mandelstam retired, leaving space in the field for me to build my own independent reputation. It was clear from an early stage that the initiation of sporulation was going to be an extremely complex problem, so we decided to focus on the later events that occur once the decision to initiate sporulation has been taken. We systematically worked through the collection of cloned sporulation genes to determine their sequences, and to examine their regulation and the effects of mutation on morphological phenotype and the expression of other sporulation genes. In the space of about 10 years, we and a few other laboratories had worked out the general pattern of gene expression, including the identification of a series of distinct and sequential classes of timing and a rather hierarchical pattern of interdependences (Errington, 1993).

## Asymmetry and the determination of cell fate

Our main interest was in understanding the basis for the generation of asymmetry and the specification of the separate fates of the prespore and mother cell, because these seemed to represent the most basic and fundamental questions posed by the system. Observations in various laboratories had shown that an early morphological event in sporulation was the switch from medial to polar division. Several classes of mutant shed light on the nature of this switch. First, mutants affected in the key initiator of sporulation, *spo0A*, simply behaved as if they were not starving and continued to divide at mid-cell to produce small but equally sized daughter cells (Dunn *et al.*, 1976). This suggested that an important early role for Spo0A~P was in diverting the division apparatus from its normal mid-cell position to the cell pole. Secondly, mutants affected in several so-called 'Stage II' genes, in the *spoIIA*, *spoIIE* and *spoIIG* loci, produced a curious 'abortively disporic' phenotype in which asymmetrical septa are formed near both cell poles. We showed that in these mutants, the two septa form sequentially, separated by a short time interval. Importantly, this suggested that in wild-type cells, the *spoII* loci are somehow needed to prevent a second 'symmetry-restoring' division from occurring soon after the critical first asymmetrical division (Lewis *et al.*, 1994).

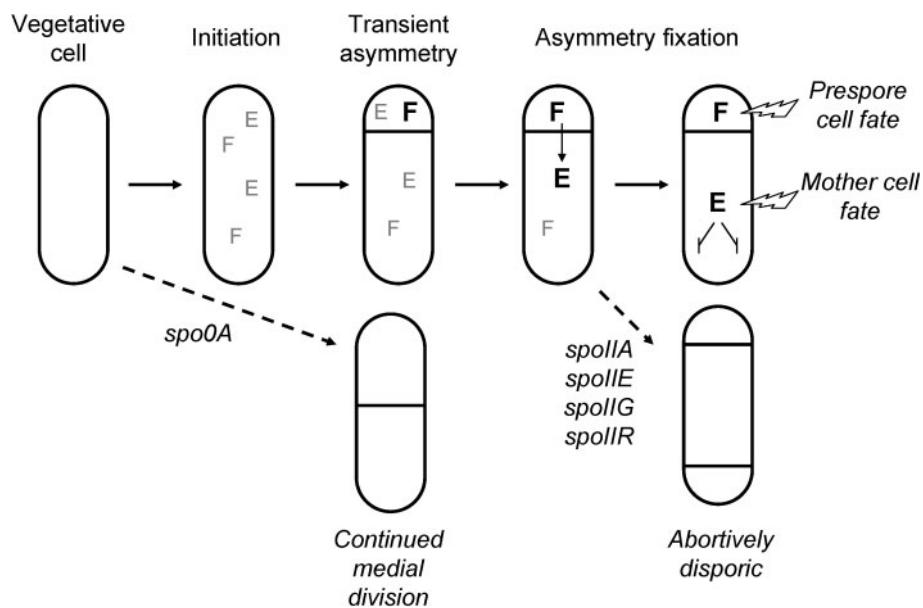
DNA sequencing had shown that the *spoIIA* and *spoIIG* loci both contained genes encoding sigma ( $\sigma$ ) factors (Fort & Piggot, 1984; Stragier *et al.*, 1984) – subunits of RNA polymerase that specify which promoters are recognized by the catalytic part of the enzyme. This was important because  $\sigma$  factors are powerful regulators that can turn on whole sets of genes in concert. The other proteins in the loci turned out to encode regulatory proteins that control the activity of their respective  $\sigma$  factor. We realized that  $\sigma^F$  and  $\sigma^E$ , which became active at about the same early time point in sporulation, were active in different compartments –  $\sigma^F$  in the prespore and  $\sigma^E$  in the mother cell (Errington & Illing, 1992). This realization provided the basis for understanding cell fate determination. Work in several laboratories including our own showed that activation of both factors was somehow coupled to asymmetrical division and that  $\sigma^E$  activation was dependent on that of  $\sigma^F$ . A model therefore emerged (Fig. 2) that explains, in outline, how asymmetry is generated and how the fates of the two cells are established. Many, though by no means all, of the molecular details of the signal transduction pathways involved in this process have been filled in since the mid-1990s (Errington, 2003; Hilbert & Piggot, 2004).

## Use of sporulation to probe the cell cycle and the discovery of the SpoIIIE/FtsK DNA translocator

By the mid-1990s I felt that several of the most interesting problems posed by sporulation had been solved, albeit in outline, and that it was becoming increasingly easy to study development in various higher organisms, so general

interest in the sporulation field was diminishing. Moreover, opportunities were emerging to tackle other fundamental and less well-explored problems, especially around the cell cycle. It was also apparent that further understanding of cell fate determination in the prespore and mother cell would be held up by our lack of understanding of the basic mechanisms of cell division, to which  $\sigma^F$  activation seemed to be coupled. From an experimental perspective we realized that asymmetrical division could be a powerful tool for studying certain key aspects of cell division, such as how the position of the division site is determined, and how chromosome segregation works. This latter problem was prompted by the simple observation that although the prespore is formed by an extremely polarly positioned division septum, the tiny cell always succeeds in acquiring a chromosome. Serendipity also played a part, because two of the sporulation loci we had been working on, *spo0J* and *spoIIE*, turned out to encode proteins with crucial roles in cell cycle control and chromosome segregation that are conserved in almost all bacteria, not just spore formers, so we had uncovered important leads into this fundamental area of biology.

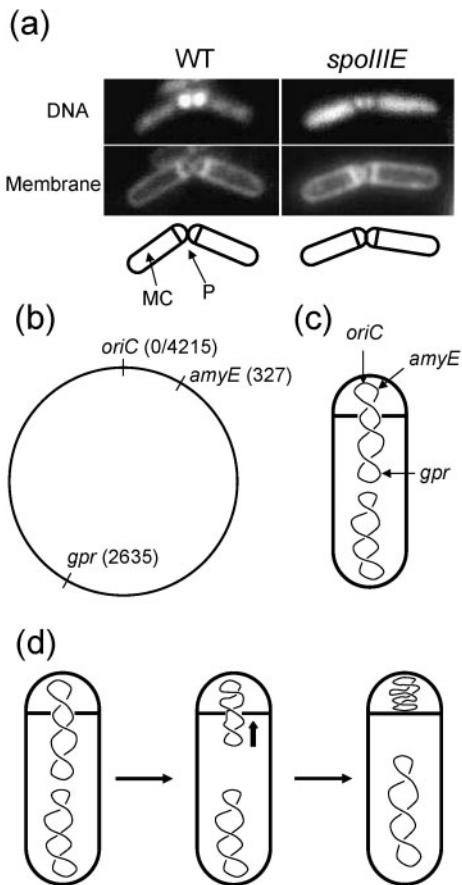
Our first major contribution emerged as soon as we began using fluorescence microscopy to look at chromosome organization, and this part of the story illustrates why subcellular imaging is now such an important part of the bacterial cell biologist's experimental tool box. We had been working on a protein called SpoIIIE for several years because we thought that it was a crucial regulator of  $\sigma^F$  activation. For example, mutation of *spoIIE* abolished expression of the  $\sigma^F$ -dependent *spoIIG* gene (Foulger & Errington, 1989). Mystifyingly, the Stragier lab had obtained contradictory results with a reporter gene located at the *amyE* locus, indicating no such role for SpoIIIE (Karmazyn-Campelli *et al.*, 1989). The Setlow lab then showed that the difference between our respective results was due to the chromosomal location of the *lacZ* reporter gene used to measure  $\sigma^F$  activation (Sun *et al.*, 1991). Ling Juan Wu's first fluorescence images of the chromosomes of a *spoIIE* mutant immediately provided the answer (although it was some time before we recognized this) (Wu & Errington, 1994). At first sight everything seemed normal in the *spoIIE* mutant. However, on closer inspection the mutants seemed to have a problem in capturing a full complement of chromosomal DNA in the prespore compartment (Fig. 3a). Quantification of the fluorescence signal suggested that the prespore compartments contained only about 30% of a chromosome equivalent of DNA, and that the missing 70% of a chromosome was probably located in the other (mother cell) compartment. We eventually realized that this could explain the chromosome location effect on reporter gene expression: perhaps expression of reporter genes such as *gpr* was abolished because they usually lay in the 70% of chromosome that failed to enter the prespore compartment and therefore did not gain access to active  $\sigma^F$ -RNA



**Fig. 2.** Generation of asymmetry and determination of cell fate during sporulation. Soon after the onset of sporulation, critical transcription factors  $\sigma^F$  (F) and  $\sigma^E$  (E) are synthesized, but they are initially held in an inactive state (grey font). After formation of the asymmetrical septum,  $\sigma^F$  activity is released (black font) specifically in the small compartment.  $\sigma^F$  turns on the prespore-specific programme of gene expression, effectively determining the prespore cell fate. One of the genes it activates, *spoIIR*, encodes a product that acts across the septum, in a vectorial manner, to trigger the release of  $\sigma^E$  activity specifically in the mother cell compartment.  $\sigma^E$  turns on a large number of genes comprising its own distinct programme of gene expression. Included among these genes are several that encode inhibitors of cell division and prevent the formation of a second prespore-like compartment at the other cell pole. The generation of asymmetry therefore occurs in two steps: first, a transient asymmetry in which a polar septum is formed at one end of the parent cell; then a fixation step in which a signal-transduction pathway recognizes the formation of a polar septum and triggers the synthesis of factors that prevent the formation of a symmetry-restoring second septum. Below are shown the effects of mutations in several genes that alter cell fate. Mutations in *spoOA* prevent the switch from medial to polar division. Mutations in any of the *spoIIA*, E, G or R genes prevent  $\sigma^E$  activity from appearing and lead to an 'abortively disporic' phenotype in which the transient asymmetry is lost and two prespore-like cells are formed.  $\sigma^F$  and  $\sigma^E$  therefore determine the respective fates of the two cells, by controlling both the establishment of asymmetry and the patterns of gene expression.

polymerase (Fig. 3b, c). According to this model, locations such as *amyE*, where reporter gene expression was normal in *spoIIIE* mutants, must usually lie in the 30% region of DNA that was correctly trapped in the small compartment (Fig. 3b, c). Thus, SpoIIIE was somehow acting on chromosome positioning, rather than affecting  $\sigma^F$  activation. Two important conclusions eventually emerged from this work. First, that the chromosome has a very precise orientation and organization in the cell, at least during sporulation, such that a region of about 1 Mbp roughly centred on *oriC* is always trapped in the small compartment in *spoIIIE* mutants (Wu & Errington, 1998; see below). The concept that chromosomes are precisely organized in bacterial cells has subsequently been confirmed and extended in diverse bacteria (Niki *et al.*, 2000; Viollier *et al.*, 2004; Wang *et al.*, 2005). Secondly, it revealed a remarkable mechanism for segregation of the chromosome into the prespore, in which it is initially bisected by the asymmetrical division septum; then, the

larger part (about 3 Mbp of DNA) of the chromosome is translocated or pumped from the mother cell into the prespore, in a SpoIIIE-dependent manner (Fig. 3d). This intermediate step in chromosome segregation was completely unexpected and only became evident in the *spoIIIE* mutant. Experiments with wild-type cells suggested this model by detecting the previously unseen intermediate state resembling that of *spoIIIE* mutant cells early in sporulation, before fully segregated prespore chromosomes appeared in the population (Wu *et al.*, 1995). Population counts and more recent direct time-lapse imaging showed that prespore chromosome segregation is completed in about 10 min (Lewis *et al.*, 1994; Poglano *et al.*, 1999), giving a remarkable rate of transfer through the septum of  $>1000$  bp per second. With Jon Bath and Jim Wang, we later showed that purified SpoIIIE protein could translocate on DNA, supporting the idea that SpoIIIE acts directly as the motor driving the DNA through the septum (Bath *et al.*, 2000). We now know, from work in numerous



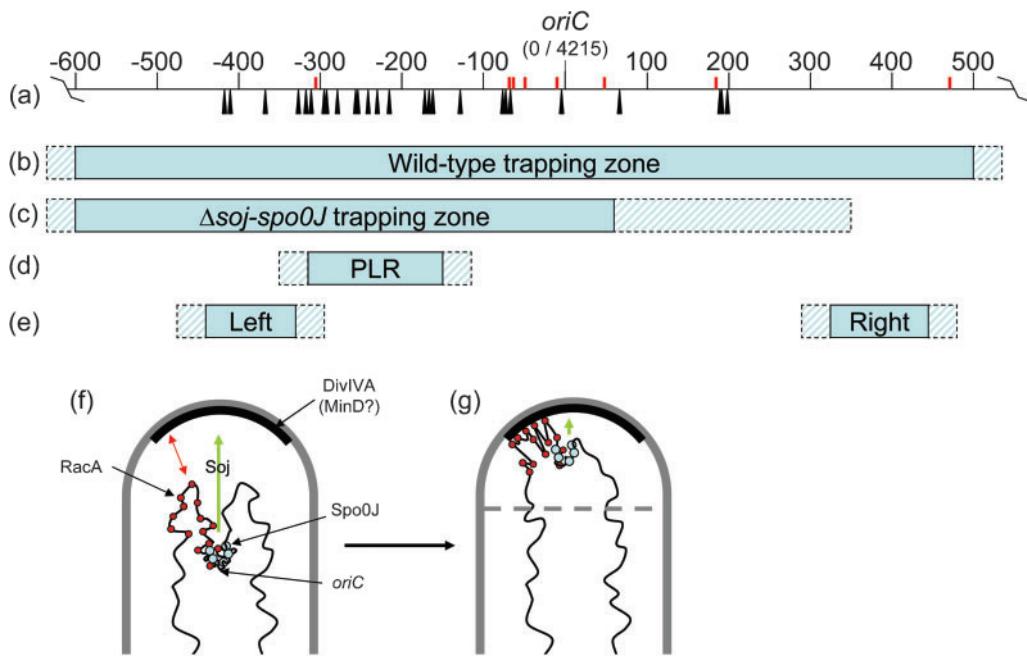
**Fig. 3.** Chromosome segregation into the prespore and the role of SpoIIIE/FtsK as a DNA transporter. (a) Images of DNA (DAPI) and membrane (FM5-95) stained cells of wild-type (left) and a *spoIIIE* mutant (right) during sporulation. The cartoons below show where the boundaries of the prespore (P) and mother cell (MC) compartments of these cells would lie. (b) Locations of reporter genes used to examine effects of sporulation mutations on  $\sigma^F$  activity on the circular *B. subtilis* chromosome. Numbers in parentheses give distance in kbp from the origin of chromosome replication (*oriC*). (c) Deduced typical organization of the chromosome in cells at the moment of polar septum formation. (d) Translocation of the prespore chromosome through the polar septum, driven by SpoIIIE.

laboratories, that SpoIIIE and its homologues (also frequently known as FtsK) are responsible for effecting or coordinating several important functions associated with the late stages of cell division. In addition to DNA translocation, these include regulation of chromosome dimer resolution, of chromosome catenation, and of cell division itself. There are also detailed structural models describing how SpoIIIE/FtsK carries out its remarkable DNA-pumping action, together with insights into questions such as how it can work out which direction to translocate the DNA, and how this protein also acts to seal off the two cell compartments between which the DNA traverses (Bigot *et al.*, 2007).

### Chromosome organization and the mechanism of prespore chromosome segregation

Although SpoIIIE/FtsK is clearly important and interesting in its own right, we became increasingly interested in using *spoIIIE* mutants to probe the mechanisms responsible for orientation of the chromosome. Ling Juan Wu developed a ‘chromosome trapping’ assay for chromosome orientation, in which a  $\sigma^F$ -dependent reporter gene was inserted at different sites around the chromosome, then reporter activity was measured in a *spoIIIE* mutant background. The results provided an estimate of the frequency with which that chromosomal site was trapped in the small compartment. She went on to show that the region of chromosome trapped was about 1 Mbp and centred just to the left of *oriC* on the standard chromosome map (Wu & Errington, 1998) (Fig. 4a, b). Over the years several approaches have been used to tease out the mechanisms responsible for orientation of the chromosome during sporulation. The first candidate genes we examined lay in a locus called *soj-spoOJ*. DNA sequencing had shown that these genes were closely related to a widespread family of genes involved in stable maintenance of low-copy-number plasmids in a wide range of bacteria. The *parAB* genes also turned out to be present and highly conserved in most bacteria, though curiously not in *Escherichia coli* and its close relatives (Livny *et al.*, 2007; Yamaichi & Niki, 2000). Importantly, the genes almost invariably lie close to the *oriC* site on the chromosome (Livny *et al.*, 2007), in the middle of the region of chromosome always trapped in the prespore. Alan Grossman’s lab had shown that the sporulation defect that formed the basis for discovery of the *spoOJ* gene in *B. subtilis* (Hranueli *et al.*, 1974) was suppressed if the *soj* gene was also mutated, though *soj* mutations themselves had little effect on sporulation (Ireton *et al.*, 1994). Thus, on the basis of this genetics, Soj might be an inhibitor of sporulation that is normally kept in check by SpoOJ (see below). A post-doc in my lab, Michaela Sharpe, tested whether chromosome organization or orientation was defective in *soj-spoOJ* mutants using the Wu ‘chromosome trapping’ assay. Although the effect was mild, Michaela found that chromosome orientation was indeed perturbed in the *soj-spoOJ* background. In particular, it was possible to detect trapping of sites distant from *oriC* that were normally completely excluded from the prespore (Sharpe & Errington, 1996). Ling Juan later showed that *soj-spoOJ* mutations had two effects: first, they resulted in a small general relaxation of the specificity of trapping; second, trapping of sequences to the right of *oriC* was considerably reduced, whereas the region to the left of *oriC* was largely unaffected (Wu & Errington, 2002) (Fig. 4c). Therefore, although *soj-spoOJ* clearly had a role in orientation of the chromosome, it was clearly redundant to at least one other system.

The next factor found to play a role in prespore chromosome segregation, called *divIVA*, was uncovered serendipitously, largely because it was already being studied in the lab through its role in division-site selection in



**Fig. 4.** Recruitment and binding of the *oriC* region of the *B. subtilis* chromosome to the cell pole during sporulation. (a) Locations of the binding sites for Spo0J (Lin & Grossman, 1998) and RacA (Ben-Yehuda *et al.*, 2005) in the *oriC* region of the chromosome. All of the sites lie within the *oriC*-proximal region shown (distances labelled in kbp). (b–e) Approximate limits of the chromosomal regions trapped in the prespore compartment of *spoIIIE* mutant cells under different conditions. The hatched edges to the boxes represent approximately the regions defined by reporter locations that were found to be in the 'on' and 'off' states. (b) Region trapped in otherwise wild-type cells. (c) Region trapped in *soj-spo0J* double mutants. (d) Region (PLR) responsible for the trapping effect in (c), as defined by the effects of chromosome inversions. (e) Regions to the left and right of *oriC* trapped in *divIVA\** or *racA soj* double mutants. (Wu & Errington, 2002, 2003). (f) Organization of the *oriC* region of the chromosome and the effectors involved in driving its movement to the cell pole. Two forces facilitate the movement: dynamic polymerization/depolymerization of Soj protein (green arrow) and direct interaction between RacA and a protein target at the pole (DivIVA or an associated factor) (red arrow). (g) Completion of origin movement, followed by polar septation (dashed line).

vegetative cells (see below). We noticed that *divIVA* mutants had a sporulation-deficient phenotype that could not easily be explained by its division dysfunction in vegetative cells. Marcelle Freeman succeeded in isolating a point mutation in *divIVA* that specifically affected sporulation, confirming that the vegetative and sporulation defects likely reflected distinct functions. Helena Thomaides then analysed the phenotype of the mutant in detail and found that the mutant cells were severely deficient in capturing DNA in the prespore (Thomaides *et al.*, 2001). Consistent with this observation, expression of a  $\sigma^F$ -dependent reporter gene was almost completely abolished in the mutant, irrespective of its location in the chromosome. Since DivIVA was known to be targeted to cell poles and to recruit other proteins to those sites (Edwards & Errington, 1997; Edwards *et al.*, 2000; Marston *et al.*, 1998; Marston & Errington, 1999a), we imagined that it also acted as the target in attracting the *oriC* region of the chromosome to the cell pole during sporulation (Thomaides *et al.*, 2001).

It was clear that the *divIVA* mutant had a much more severe sporulation chromosome segregation defect than

*soj-spo0J*. Therefore, there must be at least one more factor to be identified, which should act in parallel with *soj-spo0J*. As one approach to identifying this factor we tried to define the *cis*-acting DNA sequences that were responsible for orientation of the *oriC* region towards DivIVA at the cell pole in a *soj-spo0J* deletion background. Ling Juan Wu made a series of defined chromosome rearrangements and looked for sequences that retained their movement to the pole and those that did not. Surprisingly, she found that the *oriC* region and sequences up to 150 kbp from it were not required for the inclusion of the chromosome in the prespore. In fact, some chromosome inversions resulted in *oriC* being virtually excluded from the prespore. Instead, orientation of the chromosome was specified by a relatively dispersed region, which we called the polar localization region (PLR), located roughly 150 to 315 kbp to the left of *oriC* (Wu & Errington, 2002) (Fig. 4d).

The factor responsible for this effect was discovered independently in our lab and that of Richard Losick (Ben-Yehuda *et al.*, 2003; Wu & Errington, 2003). RacA protein is a sporulation-specific factor encoded by a gene that lies close to the region on which it mainly acts. RacA

has a classical helix–turn–helix motif and is a site-specific DNA-binding protein. Gratifyingly, when Losick's group later identified the specific DNA-binding sequences to which RacA binds *in vivo*, called *ram* sites (Ben-Yehuda *et al.*, 2005), the major cluster of sites coincided with the PLR identified by Ling Juan Wu as being required for polar orientation of the chromosome in the absence of Soj-Spo0J (Fig. 4a).

RacA protein is thought to interact with one or more proteins at the cell pole, though its specific target there has not yet been identified. Curiously, *racA* mutants have only a barely detectable defect in sporulation (Ben-Yehuda *et al.*, 2003; Wu & Errington, 2003). However, when the mutation was combined with disruption of *soj* (which, as mentioned above, also has only very mild phenotypic effects), a strong phenotype was obtained (Wu & Errington, 2003). Importantly, this phenotype was similar to that of the *divIVA* mutant, suggesting that Soj and RacA have redundant or overlapping functions in bringing the *oriC* region to DivIVA at the cell pole.

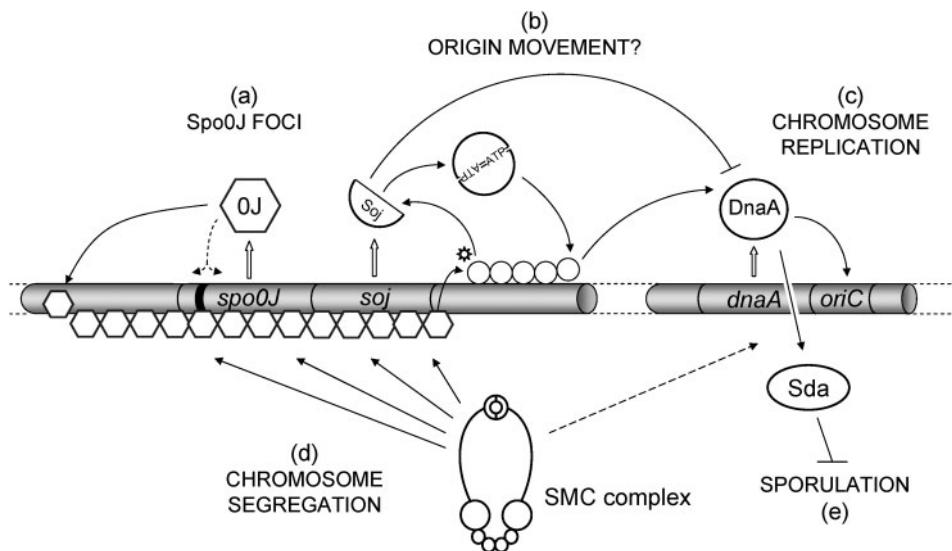
An intriguing feature of both the *divIVA* and *soj racA* phenotypes is that on close microscopic inspection the prespore compartments frequently contain tiny amounts of DNA. Furthermore, these DNA sequences are highly specific for narrow regions located about 300 kbp to the left and right of *oriC* (Fig. 4e). In contrast, the central *oriC* region is trapped with negligible frequency. We concluded that the chromosome retains a partially defined configuration of curious structure. This residual structure depends on *spo0J* because specific trapping of the left and right domains is lost when *spo0J* is also deleted (Wu & Errington, 2003). As we will see below, Spo0J binds mainly to sites located close to *oriC* (Breier & Grossman, 2007; Lin & Grossman, 1998) (Fig. 4a). Based on all of the data, our current model is that in vegetative cells the Spo0J/*oriC* region has a positioning system, as yet uncharacterized, which determines its normal subpolar position (Fig. 4f). The majority of the nucleoid lies centrally between segregating sister *oriC* domains but the sheer quantity of DNA forces DNA sequences either side of the constrained Spo0J/*oriC* domain to occupy space closer to the cell pole. During sporulation, in the absence of functioning Soj and RacA systems to drag the Spo0J/*oriC* domain towards the extreme cell pole, the left and right domains are the closest to the cell pole and only they have a chance of being trapped inside the polar compartment when the sporulation septum forms. The existence of these left and right domains may provide an experimental handle that can be used to probe the structure or organization of the Spo0J/*oriC* domain.

Although details remain to be resolved it is clear that RacA and Soj are important players in moving the *oriC* region to the cell pole during sporulation. Our current model (Fig. 4f, g) is that RacA and Soj use DivIVA as a polar target to which they deliver the origin region via their respective DNA-binding sites: RacA to its *ram* sites, and Soj probably via Spo0J *parS* domains (see next section). The

two proteins probably facilitate origin movement in different and complementary ways. Soj is a dynamic protein (Marston & Errington, 1999b; Quisel *et al.*, 1999) and by analogy to other ParA proteins it may actively move the origin towards the pole. RacA, in contrast, is thought to provide the 'glue' that attaches the origin to DivIVA at the pole (Ben-Yehuda *et al.*, 2003; Lenarcic *et al.*, 2009; Wu & Errington, 2003) when it arrives there (Fig. 4g). In the absence of RacA, active movement of Spo0J/*parS* towards the pole, driven by Soj, could be sufficient to ensure that the *oriC* region is captured inside the small compartment reasonably efficiently. Similarly, in the absence of Soj, RacA might drive *oriC* movement by a diffusion/capture mechanism: once one DNA-bound RacA protein binds to its polar anchor via diffusion it would raise the local concentration of adjacent *ram*-associated RacA molecules, driving a zipper-like cascade of binding events. In the absence of both systems, the default vegetative positioning of the *oriC* region would be retained (as in Fig. 4f), abolishing trapping of all but the small 'left' and 'right' domains flanking the Spo0J/*oriC* region.

### A general role for the ParAB/Soj-Spo0J system in coordinating chromosome replication and segregation?

In parallel with the above work we have continued to work on general aspects of the Soj/Spo0J system, particularly its role in vegetative cells. It has long been thought that the major role for the plasmid-borne systems lies in active DNA segregation, and it has been assumed that this would also be the role of the chromosomal systems. As alluded to above, Spo0J binds to specific sites (*parS*) located in close proximity to the *oriC* region of most eubacteria and spreads laterally from these primary binding sites to form domains that are readily visualized as foci by fluorescence microscopy of GFP fusion proteins (Glaser *et al.*, 1997; Lin *et al.*, 1997; Lin & Grossman, 1998; Murray *et al.*, 2006). As illustrated in Fig. 5(a), one of the *parS* sites is located in the coding region of the *spo0J* gene. Time-lapse observations of Spo0J foci provided some of the first experimental evidence for active segregation of chromosomal *oriC* regions (Glaser *et al.*, 1997). Early work on Soj in our lab and Grossman's lab suggested that it can undergo dynamic cooperative assembly on DNA, consistent with an active role in chromosome movement (Marston & Errington, 1999b; Quisel *et al.*, 1999). Similar properties were then described for plasmid ParA proteins and their respective plasmids (Møller-Jensen *et al.*, 2000). Recent work with chromosomal ParA homologues in *Vibrio* or *Caulobacter* has provided visual evidence for an active role in origin movement (Fogel & Waldor, 2006). The molecular basis for the dynamic behaviour of Soj has now been worked out in outline (Hester & Lutkenhaus, 2007; Leonard *et al.*, 2005; Murray & Errington, 2008) (Fig. 5b). Thus, monomeric Soj binds ATP and then dimerizes. In its dimeric form it has non-specific, cooperative DNA binding activity, arbitrarily shown here as binding in the vicinity of



**Fig. 5.** Coordination of chromosome replication and segregation by the Soj-Spo0J/ParAB system. Schematic summary of the interactions between the key proteins involved in coordination of chromosome replication, segregation and sporulation. Spo0J is a DNA-binding protein that binds to specific *parS* sequences, one of which lies in the coding sequence of its gene. From that site it spreads laterally, presumably by cooperative binding, to cover a region of several kbp (a). Spo0J promotes efficient chromosome segregation by recruiting the SMC complex to the origin region (d). Soj protein, which is encoded by the first gene of the *soj-spo0J* locus, binds ATP and in this state can dimerize. The dimer is a non-specific DNA-binding protein and is shown here arbitrarily as binding in the vicinity of its own locus, which is about 8 kbp from *oriC*. Soj binding is also cooperative and it polymerizes along the DNA. The N-terminal unstructured region of Spo0J can trigger the intrinsic ATPase activity of Soj, leading to dissociation of the dimer and loss of DNA-binding activity. In principle, this activity cycle of polymerization and depolymerization could be involved in origin separation (b), but supporting evidence for this idea remains elusive. The monomer and dimer forms of Soj act, respectively, as negative and positive regulators of DNA replication, via action on the key regulator of initiation, DnaA (c). DnaA regulates sporulation via transcription of the *sda* gene, which encodes an inhibitor of sporulation (e). Normally, synthesis of Sda when DnaA is activated at the time of initiation of DNA replication generates an 'eclipse' period, during which initiation of sporulation is prevented. Overinitiation of DNA replication, induced by the dimer form of Soj, results in a complete block in sporulation as a result of overproduction or inappropriate timing of Sda synthesis.

its own gene. The extreme N-terminus of Spo0J can trigger ATP hydrolysis (star), leading to dissociation of the dimer and release from DNA. This DNA-binding ATP-hydrolysis cycle may underlie the dynamic behaviour of Soj and its relatives seen *in vivo*, and be the basis for generating the force needed to actively move plasmids or chromosomal regions. It seems likely that this activity forms the basis for polar movement of *oriC*/Spo0J in sporulating cells, as described above. In vegetative growth gross defects in segregation have not been detected for *soj* mutants, though a subtle effect on separation of *oriC* regions has been reported (Lee & Grossman, 2006). *spo0J* null mutants do have a mild segregation defect but, based on our recent results, this does not seem to be connected at all to Soj function (see below). The situation is complicated by the fact that mutations in the *soj* (*parA*) and *spo0J* (*parB*) homologues in other bacteria generate a plethora of different phenotypic effects, including motility and virulence, as well as different aspects of the cell cycle (and sporulation) (Bartosik *et al.*, 2009; Kim *et al.*, 2000; Mohl *et al.*, 2001).

In the last 2 years we have broadened our thinking on the role of *soj-spo0J* in *B. subtilis*. As summarized in Fig. 5, we

think that a major role for this system lies in coordinating the replication and segregation of the chromosome. The new results show that Soj acts as both a negative and positive regulator of the initiation of chromosome replication, probably via direct interactions with DnaA protein (Murray & Errington, 2008) (Fig. 5c). DnaA is the key initiator of replication conserved throughout the eubacteria, and it is closely related to the ORC proteins that carry out similar roles in archaea and eukaryotes (Duncker *et al.*, 2009). In the ATP-dimer state in which Soj binds to DNA it acts as a positive regulator of replication initiation. In contrast, in its monomeric state, it acts negatively on initiation. Although we do not yet understand the function of the regulation of DnaA by Soj, it seems likely that it plays some role in fine-tuning of the timing of initiation, and/or coordinating replication with readiness for segregation.

In a separate development we (Gruber & Errington, 2009) and the Rudner lab (Sullivan *et al.*, 2009) recently found that the mild chromosome segregation defect of *spo0J* mutants is mainly or exclusively due to the failure to recruit a complex called Condensin (Smc, ScpA and ScpB

proteins), which is conserved from bacteria to man (Hudson *et al.*, 2009), to the origin region (Fig. 5d). The reason why Condensin needs to be recruited to the origin region is not yet clear, mainly because the precise function of this complex remains enigmatic. Nevertheless, mutations in genes encoding the SMC complex have a strong chromosome segregation defect, so recruitment of Condensin may explain the long-standing question of how Spo0J contributes to chromosome segregation in *B. subtilis*.

### The Sda checkpoint system coordinates the initiation of sporulation with the chromosome replication cycle

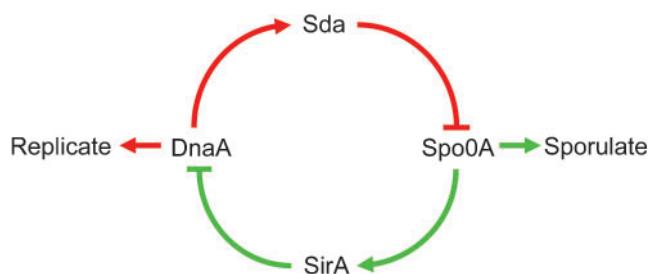
The enigmatic sporulation phenotype manifested by *spo0J* mutants was finally solved by realization that the over-initiation of chromosome replication that occurs in this mutant (due to accumulation of Soj in the ATP dimer state; see above) triggers an inhibitory system for sporulation called the Sda ‘checkpoint’ (Fig. 5e). Sda is a negative regulator of sporulation that works by inhibition of a kinase that promotes the accumulation of Spo0A~P, the ‘master regulator’ of the initiation of sporulation (Burbulys *et al.*, 1991). It had previously been shown that Sda is responsible for inhibition of sporulation in response to DNA damage or other factors perturbing DNA replication (Burkholder *et al.*, 2001). We found that inactivation of the *sda* gene more or less overcomes the sporulation defect of *spo0J* mutants (Murray & Errington, 2008), thus explaining the basis for the sporulation defect.

One of the most important contributions made by Mandelstam and colleagues was the discovery that regulation of the key decision to initiate sporulation is sensitive to cell cycle progression. They showed that there is a sensitive period in the cell cycle during which sporulation can be initiated. Once cells pass this window of opportunity, they must traverse another cycle before being capable of initiation, irrespective of their nutritional status (Dawes *et al.*, 1971; Dunn *et al.*, 1978; Hauser & Errington, 1995). For many years the nature of the regulatory mechanisms responsible for coupling of the initiation of sporulation with cell cycle progression remained unclear. We recently showed that the Sda system is not just brought into play when DNA replication is perturbed but that it is largely responsible for the cell cycle regulation of initiation. Under conditions of impending starvation, *sda* expression is upregulated, and it undergoes cell-cycle-dependent pulses of expression regulated by DnaA protein (Veening *et al.*, 2009). These pulses of Sda synthesis are generated each time a new round of DNA replication is initiated, and this results in a transient inhibition of sporulation. Ultimately, this mechanism helps to ensure that sporulating cells have the two completely replicated chromosomes (one for prespore and the other for mother cell) needed for successful sporulation (Veening *et al.*, 2009). In parallel with these findings, the Losick and Rudner labs found that

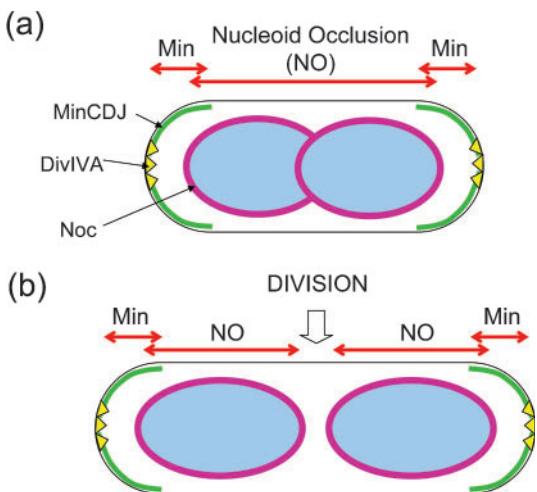
once sporulation has been initiated, the *sirA* gene is turned on, which reinforces the precision of cell cycle control by preventing further rounds of DNA replication from being initiated (Rahn-Lee *et al.*, 2009; Wagner *et al.*, 2009). These two systems acting in parallel can largely explain the long-standing problem of how the initiation of sporulation is coupled to cell cycle progression (Fig. 6). In cells that are early in the replication cycle when the sporulation stimulus is perceived, the high levels of Sda inhibit the accumulation of phosphorylated Spo0A, preventing sporulation (red pathway). Later in the cell cycle, when Sda levels have decayed, starvation leads to the accumulation of Spo0A~P, which triggers sporulation and also drives the synthesis of SirA, which inhibits DnaA, preventing reinitiation of chromosome replication (green pathway).

### Regulation of cell division – positioning and coordination with chromosome replication

This lab has been interested in cytokinesis for many years and has contributed particularly to understanding key elements of the mechanism responsible for directing the division septum to its correct mid-cell position. The division machine comprises a contractile ring made up of a large number of proteins that assemble at the site of cell division. At the heart of the machine is FtsZ, a homologue or ancestor of eukaryotic tubulin, which polymerizes in a manner regulated by GTP binding and hydrolysis. FtsZ polymerization generates the ring structure (hence called the ‘Z-ring’); it also recruits the other proteins, and probably contributes directly to the force of constriction. The other division proteins can be divided roughly into two groups: early proteins, which are largely cytosolic and concerned with regulation of Z-ring assembly or stability; and late proteins, which are largely transmembrane and thought to contribute directly to constriction of the membrane and synthesis of new cell wall material (Adams & Errington, 2009). We now know that the two main effectors required for positioning of the Z-ring are negative regulators that prevent assembly or activity of the division machine at incorrect sites (Fig. 7a). One of these



**Fig. 6.** Control of the replication/sporulation switch. The key effectors and outputs are indicated. Red and green arrows/bars represent alternative responses to the sporulation stimulus depending on the initial cell cycle status.



**Fig. 7.** Control of cell division by the nucleoid occlusion and Min systems of *B. subtilis*. Blue oval structures represent the replicating nucleoids, and their pink outline Noc protein. DivIVA protein (yellow triangles) targets to the cell poles, where it recruits the MinC, D and J proteins (green line). Red arrows above show the regions protected by the nucleoid occlusion and Min systems. In interdivision-state cells (a), division is prevented by the combined inhibitory systems. After replication and segregation of the chromosome (b), the DNA-free zone at mid-cell allows division to occur.

systems is called nucleoid occlusion, and the key (but probably not the only) player is a protein called Noc, which binds to sites over most of the chromosome and prevents division from occurring in its vicinity (Wu & Errington, 2004; Wu *et al.*, 2009). In normal cells in mid-cell cycle, the replicating chromosome occupies the middle of the cell and Noc acts to prevent mid-cell division. However, when replication has finished and the sister chromosomes have started to segregate, a DNA-free (and therefore Noc-free) space emerges in the middle of the cell, allowing the cytokinetic machinery to assemble there (Fig. 7b). This simple mechanism provides a means of regulating both the timing and location of division. The Min system probably fulfils two related roles that complement the function of Noc. There are always DNA-free spaces at the outer edges of the nucleoids, so one function for the Min system is to ensure that these sites, near the ‘old’ cell poles, are not substrates for division. Such polar divisions give rise to small anucleate ‘minicells’; hence the designation ‘Min’. The second role for the Min system seems to be in deactivating the division machinery after it has completed a mid-cell division (not shown). This would again give rise to a minicell, but at a ‘new’ pole rather than an ‘old’ pole.

The Min system is conserved throughout most rod-shaped bacteria, though some of the components are variably conserved. In *B. subtilis* four protein components are currently known. MinC is thought to be the actual division inhibitor, and it probably works at several levels to regulate

the division apparatus (Bramkamp *et al.*, 2008; Dajkovic *et al.*, 2008; Gregory *et al.*, 2008; Shen & Lutkenhaus, 2009). Its localization is determined by MinD protein (Hu & Lutkenhaus, 1999; Marston & Errington, 1999a; Raskin & De Boer, 1999), a Soj/ParA-like ATPase. MinD localization is in turn determined at least in part, by a recently discovered transmembrane protein, MinJ (Bramkamp *et al.*, 2008; Patrick & Kearns, 2008). Ultimately, polar localization of the Min complex in *B. subtilis* is determined by the DivIVA protein (Edwards & Errington, 1997; Lenarcic *et al.*, 2009; Marston *et al.*, 1998), which was mentioned above in the context of prespore chromosome segregation. DivIVA is turning out to play a plethora of roles in different Gram-positive bacteria, all associated with events that occur at cell poles. Of particular interest is its function in actinomycetes. These organisms are often filamentous and form a branched mycelium. Growth occurs specifically at the tips of the filaments. DivIVA seems to play a key role in tip growth and the establishment of new branches (Hempel *et al.*, 2008). Returning to the Min system, it is curious that MinCD localization is regulated in a quite different manner in bacteria outside the *Bacillus* group: in organisms such as *E. coli*, the protein complex oscillates from pole to pole in a manner regulated by the MinE protein, which is completely unrelated to MinJ or DivIVA (Lutkenhaus, 2007; Rothfield *et al.*, 2005). It is not clear why bacteria from these different groups use different protein-targeting strategies to regulate a common inhibitor of polar division.

### Discovery and development of cell division inhibitors and their efficacy as novel antibiotics

The negative regulators of division mentioned above act on a division machine that has as its core component the widely conserved bacterial tubulin homologue FtsZ (Adams & Errington, 2009). Like tubulin, FtsZ polymerizes into protofilaments and higher-order structures in a manner that is regulated by GTP binding and hydrolysis. Polymerized FtsZ forms a ring or tight helix at the impending division site. There it interacts with and recruits a plethora of proteins that together bring about constriction of the cell membrane and associated synthesis of wall material that forms the new poles of the daughter cells. Although there is still much to learn about the molecular mechanisms of cell division, the machinery includes several essential conserved proteins that, in principle, are excellent potential targets for novel antibiotics. One of the key challenges in antibiotic discovery is developing an assay that can detect specific inhibitors of the target function. In the mid-1990s we realized that because activation of  $\sigma^F$  during sporulation is dependent on formation of the asymmetrical division septum, and because that division uses essentially the same machinery as in vegetative cells of *B. subtilis* and most other bacteria, we should be able to use cells bearing a  $\sigma^F$ -dependent reporter gene to screen for inhibitors of cell division (Stokes *et al.*, 2005). A patent covering this idea was filed, and this and a family of similar

patents covering other potential antibiotic targets were used to found a spin-out company, Prolysis Ltd. Prolysis successfully used the  $\sigma^F$  assay to identify a novel class of FtsZ inhibitor (Stokes *et al.*, 2005), and recently, they showed that FtsZ inhibitors have efficacy in various model infection systems (Haydon *et al.*, 2008). Although there is some way to go before we know whether such drugs have clinical potential, the progress so far provides evidence that cell division is a valid target and that the approach to finding inhibitors works.

### Concluding remarks

All cells have basic fundamental requirements to ensure their evolutionary stability; including nutrition, growth, proliferation and survival. *Bacillus subtilis* represents a wonderful model system for studying fundamental aspects of the life of cells. In the space available it was not possible to mention other work on more general aspects of cell wall synthesis from this lab. Highlights of this work include the discovery that rod-shaped bacteria have homologues of actin that govern the spatiotemporal deposition of wall material (Daniel & Errington, 2003; Jones *et al.*, 2001). Very recently, the possibility that cells can exist, and even thrive, in the complete absence of a cell wall has been revisited, through studies of L-form bacteria (Leaver *et al.*, 2009), and this has opened up a new horizon, with important implications and opportunities.

There seems to be an impression, perhaps supported by simplifications in undergraduate textbooks, that the molecular cell biology of bacteria is essentially understood. This could not be further from the truth and indeed many of the key problems in the life of cells remain to be worked out. I hope that this review gives a flavour of the excitement of working in this area, as well as highlighting some of the important unsolved problems. The fact that a relatively small community of research groups has been able to support substantial progress across a range of important problems illustrates the importance of continuing to support research in this general area. I hope this in turn will help to convince new cohorts of researchers to take on the many outstanding issues.

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### References

- Adams, D. W. & Errington, J. (2009).** Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat Rev Microbiol* **7**, 642–653.
- Anagnostopoulos, C. & Spizizen, J. (1961).** Requirements for transformation in *Bacillus subtilis*. *J Bacteriol* **81**, 741–746.
- Bartosik, A. A., Mierzejewska, J., Thomas, C. M. & Jagura-Burdzy, G. (2009).** ParB deficiency in *Pseudomonas aeruginosa* destabilizes the partner protein ParA and affects a variety of physiological parameters. *Microbiology* **155**, 1080–1092.
- Bath, J., Wu, L. J., Errington, J. & Wang, J. C. (2000).** Role of *Bacillus subtilis* SpoIIIE in DNA transport across the mother cell-prespore division septum. *Science* **290**, 995–997.
- Ben-Yehuda, S., Rudner, D. Z. & Losick, R. (2003).** RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science* **299**, 532–536.
- Ben-Yehuda, S., Fujita, M., Liu, X. S., Gorbatyuk, B., Skoko, D., Yan, J., Marko, J. F., Liu, J. S., Eichenberger, P. & other authors (2005).** Defining a centromere-like element in *Bacillus subtilis* by identifying the binding sites for the chromosome-anchoring protein RacA. *Mol Cell* **17**, 773–782.
- Bigot, S., Sivanathan, V., Possoz, C., Barre, F. X. & Cornet, F. (2007).** FtsK, a literate chromosome segregation machine. *Mol Microbiol* **64**, 1434–1441.
- Bramkamp, M., Emmins, R., Weston, L., Donovan, C., Daniel, R. A. & Errington, J. (2008).** A novel component of the division-site selection system of *Bacillus subtilis* and a new mode of action for the division inhibitor MinCD. *Mol Microbiol* **70**, 1556–1569.
- Breier, A. M. & Grossman, A. D. (2007).** Whole-genome analysis of the chromosome partitioning and sporulation protein Spo0J (ParB) reveals spreading and origin-distal sites on the *Bacillus subtilis* chromosome. *Mol Microbiol* **64**, 703–718.
- Burbulys, D., Trach, K. A. & Hoch, J. A. (1991).** Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay. *Cell* **64**, 545–552.
- Burkholder, W. F., Kurtser, I. & Grossman, A. D. (2001).** Replication initiation proteins regulate a developmental checkpoint in *Bacillus subtilis*. *Cell* **104**, 269–279.
- Dajkovic, A., Lan, G., Sun, S. X., Wirtz, D. & Lutkenhaus, J. (2008).** MinC spatially controls bacterial cytokinesis by antagonizing the scaffolding function of FtsZ. *Curr Biol* **18**, 235–244.
- Daniel, R. A. & Errington, J. (2003).** Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell* **113**, 767–776.
- Dawes, I. W., Kay, D. & Mandelstam, J. (1971).** Determining effect of growth medium on the shape and position of daughter chromosomes and on sporulation in *Bacillus subtilis*. *Nature* **230**, 567–569.
- Duncker, B. P., Chesnokov, I. N. & McConkey, B. J. (2009).** The origin recognition complex protein family. *Genome Biol* **10**, 214.
- Dunn, G., Torgersen, D. M. & Mandelstam, J. (1976).** Order of expression of genes affecting septum location during sporulation of *Bacillus subtilis*. *J Bacteriol* **125**, 776–779.
- Dunn, G., Jeffs, P., Mann, N. H., Torgersen, D. M. & Young, M. (1978).** The relationship between DNA replication and the induction of sporulation in *Bacillus subtilis*. *J Gen Microbiol* **108**, 189–195.
- Edwards, D. H. & Errington, J. (1997).** The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division. *Mol Microbiol* **24**, 905–915.
- Edwards, D. H., Thomaides, H. B. & Errington, J. (2000).** Promiscuous targeting of *Bacillus subtilis* cell division protein DivIVA to division sites in *Escherichia coli* and fission yeast. *EMBO J* **19**, 2719–2727.

- Errington, J. (1984).** Efficient *Bacillus subtilis* cloning system using bacteriophage vector 10519. *J Gen Microbiol* **130**, 2615–2628.
- Errington, J. (1993).** *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. *Microbiol Rev* **57**, 1–33.
- Errington, J. (2003).** Regulation of endospore formation in *Bacillus subtilis*. *Nat Rev Microbiol* **1**, 117–126.
- Errington, J. & Illing, N. (1992).** Establishment of cell-specific transcription during sporulation in *Bacillus subtilis*. *Mol Microbiol* **6**, 689–695.
- Errington, J. & Jones, D. (1987).** Cloning in *Bacillus subtilis* by transfection with bacteriophage vector φ105J27: isolation and preliminary characterization of transducing phages for 23 sporulation loci. *J Gen Microbiol* **133**, 493–502.
- Fogel, M. A. & Waldor, M. K. (2006).** A dynamic, mitotic-like mechanism for bacterial chromosome segregation. *Genes Dev* **20**, 3269–3282.
- Fort, P. & Piggot, P. J. (1984).** Nucleotide sequence of sporulation locus *spoIIA* in *Bacillus subtilis*. *J Gen Microbiol* **130**, 2147–2153.
- Foulger, D. & Errington, J. (1989).** The role of the sporulation gene *spoIIIIE* in the regulation of prespore-specific gene expression in *Bacillus subtilis*. *Mol Microbiol* **3**, 1247–1255.
- Glaser, P., Sharpe, M. E., Raether, B., Perego, M., Ohlsen, K. & Errington, J. (1997).** Dynamic, mitotic-like behavior of a bacterial protein required for accurate chromosome partitioning. *Genes Dev* **11**, 1160–1168.
- Gregory, J. A., Becker, E. C. & Poglano, K. (2008).** *Bacillus subtilis* MinC destabilizes FtsZ-rings at new cell poles and contributes to the timing of cell division. *Genes Dev* **22**, 3475–3488.
- Gruber, S. & Errington, J. (2009).** Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. *Cell* **137**, 685–696.
- Hauser, P. M. & Errington, J. (1995).** Characterization of cell cycle events during the onset of sporulation in *Bacillus subtilis*. *J Bacteriol* **177**, 3923–3931.
- Haydon, D. J., Stokes, N. R., Ure, R., Galbraith, G., Bennett, J. M., Brown, D. R., Baker, P. J., Barynin, V. V., Rice, D. W. & other authors (2008).** An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. *Science* **321**, 1673–1675.
- Hempel, A. M., Wang, S. B., Letek, M., Gil, J. A. & Flärdh, K. (2008).** Assemblies of DivIVA mark sites for hyphal branching and can establish new zones of cell wall growth in *Streptomyces coelicolor*. *J Bacteriol* **190**, 7579–7583.
- Hester, C. M. & Lutkenhaus, J. (2007).** Soj (ParA) DNA binding is mediated by conserved arginines and is essential for plasmid segregation. *Proc Natl Acad Sci U S A* **104**, 20326–20331.
- Hilbert, D. W. & Piggot, P. J. (2004).** Compartmentalization of gene expression during *Bacillus subtilis* spore formation. *Microbiol Mol Biol Rev* **68**, 234–262.
- Hranueli, D., Piggot, P. J. & Mandelstam, J. (1974).** Statistical estimate of the total number of operons specific for *Bacillus subtilis* sporulation. *J Bacteriol* **119**, 684–690.
- Hu, Z. & Lutkenhaus, J. (1999).** Topological regulation of cell division in *Escherichia coli* involves rapid pole to pole oscillation of the division inhibitor MinC under the control of MinD and MinE. *Mol Microbiol* **34**, 82–90.
- Hudson, D. F., Marshall, K. M. & Earnshaw, W. C. (2009).** Condensin: architect of mitotic chromosomes. *Chromosome Res* **17**, 131–144.
- Ireton, K., Gunther, N. W. I. & Grossman, A. D. (1994).** *spoOJ* is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J Bacteriol* **176**, 5320–5329.
- Jenkinson, H. F. & Mandelstam, J. (1983).** Cloning of the *Bacillus subtilis* *lys* and *spoIIIB* genes in phage φ105. *J Gen Microbiol* **129**, 2229–2240.
- Jones, D. & Errington, J. (1987).** Construction of improved bacteriophage φ105 vectors for cloning by transfection in *Bacillus subtilis*. *J Gen Microbiol* **133**, 483–492.
- Jones, L. J. F., Carballido-López, R. & Errington, J. (2001).** Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* **104**, 913–922.
- Karmazyn-Campelli, C., Bonamy, C., Savelli, B. & Stragier, P. (1989).** Tandem genes encoding σ-factors for consecutive steps of development in *Bacillus subtilis*. *Genes Dev* **3**, 150–157.
- Kim, H. J., Calcutt, M. J., Schmidt, F. J. & Chater, K. F. (2000).** Partitioning of the linear chromosome during sporulation of *Streptomyces coelicolor* A3(2) involves an *oriC*-linked *parAB* locus. *J Bacteriol* **182**, 1313–1320.
- Kroos, L. (2007).** The *Bacillus* and *Myxococcus* developmental networks and their transcriptional regulators. *Annu Rev Genet* **41**, 13–39.
- Leaver, M., Dominguez-Cuevas, P., Coxhead, J. M., Daniel, R. A. & Errington, J. (2009).** Life without a wall or division machine in *Bacillus subtilis*. *Nature* **457**, 849–853.
- Lee, P. S. & Grossman, A. D. (2006).** The chromosome partitioning proteins Soj (ParA) and SpoOJ (ParB) contribute to accurate chromosome partitioning, separation of replicated sister origins, and regulation of replication initiation in *Bacillus subtilis*. *Mol Microbiol* **60**, 853–869.
- Lenarcic, R., Halbedel, S., Visser, L., Shaw, M., Wu, L. J., Errington, J., Marenduzzo, D. & Hamoen, L. W. (2009).** Localisation of DivIVA by targeting to negatively curved membranes. *EMBO J* **28**, 2272–2282.
- Leonard, T. A., Butler, P. J. & Löwe, J. (2005).** Bacterial chromosome segregation: structure and DNA binding of the Soj dimer – a conserved biological switch. *EMBO J* **24**, 270–282.
- Lewis, P. J., Partridge, S. R. & Errington, J. (1994).** σ factors, asymmetry, and the determination of cell fate in *Bacillus subtilis*. *Proc Natl Acad Sci U S A* **91**, 3849–3853.
- Lin, D. C.-H. & Grossman, A. D. (1998).** Identification and characterization of a bacterial chromosome partitioning site. *Cell* **92**, 675–685.
- Lin, D. C.-H., Levin, P. A. & Grossman, A. D. (1997).** Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. *Proc Natl Acad Sci U S A* **94**, 4721–4726.
- Livny, J., Yamaichi, Y. & Waldor, M. K. (2007).** Distribution of centromere-like *parS* sites in bacteria: insights from comparative genomics. *J Bacteriol* **189**, 8693–8703.
- Lutkenhaus, J. (2007).** Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu Rev Biochem* **76**, 539–562.
- Marston, A. L. & Errington, J. (1999a).** Selection of the midcell division site in *Bacillus subtilis* through MinD-dependent polar localization and activation of MinC. *Mol Microbiol* **33**, 84–96.
- Marston, A. L. & Errington, J. (1999b).** Dynamic movement of the ParA-like Soj protein of *B. subtilis* and its dual role in nucleoid organization and developmental regulation. *Mol Cell* **4**, 673–682.
- Marston, A. L., Thomaides, H. B., Edwards, D. H., Sharpe, M. E. & Errington, J. (1998).** Polar localization of the MinD protein of *Bacillus subtilis* and its role in selection of the mid-cell division site. *Genes Dev* **12**, 3419–3430.
- Mohl, D. A., Easter, J. & Gober, J. W. (2001).** The chromosome partitioning protein, ParB, is required for cytokinesis in *Caulobacter crescentus*. *Mol Microbiol* **42**, 741–755.

- Møller-Jensen, J., Jensen, R. B. & Gerdes, H. (2000).** Plasmid and chromosome segregation in prokaryotes. *Trends Microbiol* **8**, 313–320.
- Murray, H. & Errington, J. (2008).** Dynamic control of the DNA replication initiation protein DnaA by Soj/ParA. *Cell* **135**, 74–84.
- Murray, H., Ferreira, H. & Errington, J. (2006).** The bacterial chromosome segregation protein Spo0J spreads along DNA from *parS* nucleation sites. *Mol Microbiol* **61**, 1352–1361.
- Niki, H., Yamaichi, Y. & Hiraga, S. (2000).** Dynamic organization of chromosomal DNA in *Escherichia coli*. *Genes Dev* **14**, 212–223.
- Patrick, J. E. & Kearns, D. B. (2008).** MinJ (YvjD) is a topological determinant of cell division in *Bacillus subtilis*. *Mol Microbiol* **70**, 1166–1179.
- Piggot, P. J. & Coote, J. G. (1976).** Genetic aspects of bacterial endospore formation. *Bacteriol Rev* **40**, 908–962.
- Pogliano, J., Osborne, N., Sharpe, M. D., Abanes-De Mello, A., Perez, A., Sun, Y.-L. & Pogliano, K. (1999).** A vital stain for studying membrane dynamics in bacteria: a novel mechanism controlling septation during *Bacillus subtilis* sporulation. *Mol Microbiol* **31**, 1149–1159.
- Quisel, J. D., Lin, D. C.-H. & Grossman, A. D. (1999).** Control of development by altered localization of a transcription factor in *B. subtilis*. *Mol Cell* **4**, 665–672.
- Rahn-Lee, L., Gorbatyuk, B., Skovgaard, O. & Losick, R. (2009).** The conserved sporulation protein YneE inhibits DNA replication in *Bacillus subtilis*. *J Bacteriol* **191**, 3736–3739.
- Raskin, D. M. & De Boer, P. A. J. (1999).** MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in *Escherichia coli*. *J Bacteriol* **181**, 6419–6424.
- Rothfield, L., Taghbatalout, A. & Shih, Y. L. (2005).** Spatial control of bacterial division-site placement. *Nat Rev Microbiol* **3**, 959–968.
- Sharpe, M. E. & Errington, J. (1996).** The *Bacillus subtilis* soj-spo0J locus is required for a centromere-like function involved in prespore chromosome partitioning. *Mol Microbiol* **21**, 501–509.
- Shen, B. & Lutkenhaus, J. (2009).** The conserved C-terminal tail of FtsZ is required for the septal localization and division inhibitory activity of MinC(C)/MinD. *Mol Microbiol* **72**, 410–424.
- Sterlini, J. M. & Mandelstam, J. (1969).** Commitment to sporulation in *Bacillus subtilis* and its relationship to the development of actinomycin resistance. *Biochem J* **113**, 29–37.
- Stokes, N. R., Sievers, J., Barker, S., Bennett, J. M., Brown, D. R., Collins, I., Errington, V. M., Foulger, D., Hall, M. & other authors (2005).** Novel inhibitors of bacterial cytokinesis identified by a cell-based antibiotic screening assay. *J Biol Chem* **280**, 39709–39715.
- Stragier, P., Bouvier, J., Bonamy, C. & Szulmajster, J. (1984).** A developmental gene product of *Bacillus subtilis* homologous to the sigma factor of *Escherichia coli*. *Nature* **312**, 376–378.
- Sullivan, N. L., Marquis, K. A. & Rudner, D. Z. (2009).** Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation. *Cell* **137**, 697–707.
- Sun, D., Fajardo-Cavazos, P., Sussman, M. D., Tovar-Rojo, F., Cabrera-Martinez, R.-M. & Setlow, P. (1991).** Effect of chromosome location of *Bacillus subtilis* forespore genes on their *spo* gene dependence and transcription by E $\sigma$ <sup>F</sup>: identification of features of good E $\sigma$ <sup>F</sup>-dependent promoters. *J Bacteriol* **173**, 7867–7874.
- Thomaides, H. B., Freeman, M., El Karoui, M. & Errington, J. (2001).** Division-site-selection protein DivIVA of *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. *Genes Dev* **15**, 1662–1673.
- Veenig, J. W., Murray, H. & Errington, J. (2009).** A mechanism for cell cycle regulation of sporulation initiation in *Bacillus subtilis*. *Genes Dev* **23**, 1959–1970.
- Viollier, P. H., Thanbichler, M., McGrath, P. T., West, L., Meewan, M., McAdams, H. H. & Shapiro, L. (2004).** Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc Natl Acad Sci U S A* **101**, 9257–9262.
- Wagner, J. K., Marquis, K. A. & Rudner, D. Z. (2009).** SirA enforces diploidy by inhibiting the replication initiator DnaA during spore formation in *Bacillus subtilis*. *Mol Microbiol* **73**, 963–974.
- Wang, X., Possoz, C. & Sherratt, D. J. (2005).** Dancing around the divisome: asymmetric chromosome segregation in *Escherichia coli*. *Genes Dev* **19**, 2367–2377.
- Wu, L. J. & Errington, J. (1994).** *Bacillus subtilis* SpolIIIE protein required for DNA segregation during asymmetric cell division. *Science* **264**, 572–575.
- Wu, L. J. & Errington, J. (1998).** Use of asymmetric cell division and *spoIIIE* mutants to probe chromosome orientation and organization in *Bacillus subtilis*. *Mol Microbiol* **27**, 777–786.
- Wu, L. J. & Errington, J. (2002).** A large dispersed chromosomal region required for chromosome segregation in sporulating cells of *Bacillus subtilis*. *EMBO J* **21**, 4001–4011.
- Wu, L. J. & Errington, J. (2003).** RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating *Bacillus subtilis*. *Mol Microbiol* **49**, 1463–1475.
- Wu, L. J. & Errington, J. (2004).** Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. *Cell* **117**, 915–925.
- Wu, L. J., Lewis, P. J., Allmansberger, R., Hauser, P. M. & Errington, J. (1995).** A conjugation-like mechanism for prespore chromosome partitioning during sporulation in *Bacillus subtilis*. *Genes Dev* **9**, 1316–1326.
- Wu, L. J., Ishikawa, S., Kawai, Y., Oshima, T., Ogasawara, N. & Errington, J. (2009).** Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation. *EMBO J* **28**, 1940–1952.
- Yamaichi, Y. & Niki, H. (2000).** Active segregation by the *Bacillus subtilis* partitioning system in *Escherichia coli*. *Proc Natl Acad Sci U S A* **97**, 14656–14661.