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MicroReview

How mathematical modelling elucidates signalling in *Bacillus subtilis*

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Summary

Appropriate stimulus perception, signal processing and transduction ensure optimal adaptation of bacteria to environmental challenges. In the Gram-positive model bacterium *Bacillus subtilis* signalling networks and molecular interactions therein are well-studied, making this species a suitable candidate for the application of mathematical modelling. Here, we review systems biology approaches, focusing on chemotaxis, sporulation, σ^B-dependent general stress response and competence. Processes like chemotaxis and Z-ring assembly depend critically on the subcellular localization of proteins. Environmental response strategies, including sporulation and competence, are characterized by phenotypic heterogeneity in isogenic cultures. The examples of mathematical modelling also include investigations that have demonstrated how operon structure and signalling dynamics are intricately interwoven to establish optimal responses. Our review illustrates that these interdisciplinary approaches offer new insights into the response of *B. subtilis* to environmental challenges. These case studies reveal modelling as a tool to increase the understanding of complex systems, to help formulating hypotheses and to guide the design of more directed experiments that test predictions.

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Introduction

Bacillus subtilis is one of the best-studied prokaryotes and serves as the main model organism for Gram-positive bacteria. Research related to *B. subtilis* has provided substantial information regarding the organization of bacterial life cycles. This knowledge provides an excellent basis for mathematical modelling of cellular processes. Indeed, *Bacilli* have been investigated in theoretical biology for a long time. In the 1970s, Sargent compared different models for the control of cell length (Sargent, 1975), which have since then been further refined (e.g. Koch, 1992; Grover *et al*., 2004). Espinosa *et al*. (1977) examined the acquisition of competence in cultures, while Jeong *et al*. (1990) presented a mathematical model for growth processes including sporulation and central metabolism. Particularly during the last decade, there has been increased interest in systems biology, a discipline encompassing the interaction of experimental approaches, mathematical modelling, and computer simulations (Wolkenhauer *et al*., 2003). *B. subtilis* has gained increasing attention due to its capacity for developmental responses and population heterogeneity.

In this review, we summarize recent results in the modelling of signalling systems and survey how mathematical modelling provides a better understanding of sophisticated cellular responses. The first models we review are related to chemotaxis. They have been made to predict adaptation mechanisms of the rotational orientation of flagella in response to changes in concentrations of external substances. Furthermore, we discuss models describing protein localization which is an important factor for chemotactic signalling. We review spatial models of MinCD and the early sporulation factors Spo0J/Soj and summarize mathematical interpretations of the initiation of sporulation that attracted attention because of parallels to developmental processes in eukaryotes. Additionally, we show how signalling processes that include proteins of the *spoIIA* operon resemble the general stress response

mediated by sigma factor σ^B with respect to the use of the so-called partner switch mechanism. Recent studies demonstrate how the operon organization of *spo0A* and *sinIR* supports their function during sporulation. We also discuss models regarding another important developmental process: competence. Different investigations have revealed the mechanism by which the excitable response is induced and how the cell exits competence. Finally, an outlook to future developments of the application of mathematical models is given.

Chemotaxis

During colony growth B. subtilis *can display two distinct phenotypes namely motile cells that respond to chemotactic signals and non-motile cells growing as connected chains* (Kearns and Losick, 2005). Expression of chemotactic proteins in the *che-fla* operon is controlled by the sigma factor σ^D (Marquez *et al*., 1990). Analysis of chemotaxis is interesting from two perspectives: first, how cells in a population 'decide' whether to become motile or not; second, how the information of a chemotactic signal is transmitted from the receptor to the flagella to result in a directed movement. Here, we focus on the latter aspect since no mathematical models for the genetic regulation of σ^D expression have been published to date.

The chemotactic behaviour of various organisms has been studied intensively in the past and a thorough overview of the mathematical approaches is given by Tindall *et al*. (2008). Mathematical modelling of chemotaxis started in the 1970s using *Escherichia coli* as a model organism (Tindall *et al*., 2008). Investigations in *B. subtilis*, notably by Ordal (e.g. Garrity and Ordal, 1995), uncovered that although the molecular machinery is conserved between *E. coli* and *B. subtilis*, the mechanism of chemotaxis is surprisingly different (Bischoff and Ordal, 1991; Rao and Ordal, 2009). A simplified scheme displaying the mechanism of chemotactic signalling in *B. subtilis* is shown in Fig. 1. Once a ligand binds to a methyl-accepting chemotaxis protein (MCP) receptor, CheR methylates while CheB demethylates-specific glutamate residues of the receptor. This change in methylation leads to the activation of CheA, which phosphorylates CheY. CheY~P binds to the flagellar motor protein FliM reversing the spin of the flagellum from clockwise to counterclockwise rotation (Garrity and Ordal, 1995). Instead of tumbling, the cell now performs a directed movement along the concentration gradient. Dephosphorylation of CheY~P is accomplished largely by FliY, which is located at the base of the flagellum. An additional player is the CheCD heterodimer that has three functions: (i) CheCD binds CheY~P and thus competes with with binding to FliM, (ii) CheC displays weak CheY~P phosphatase activity and (iii) CheD increases CheA-receptor affinity by deamination of a glutamine residue on the receptor (Kristich and Ordal, 2002). Surprisingly, in contrast to *B. subtilis,* the *E. coli* CheY~P induces clockwise rotation of the flagellum, resulting in smooth runs (Garrity and Ordal, 1995). The tumbling frequency will resume its pre-stimulus activity even if the attractant concentration remains constant, a phenomenon called adaptivity.

Rao *et al*. (2004) presented a model that includes the previously mentioned signalling mechanisms. The authors assumed a mechanism by which CheY~P enhances the transition of an active to an inactive receptor conformation. This assumption is experimentally testable as it requires an affinity of CheY~P to the receptor complex. The authors examined their model with respect to a *cheBCDR* quadruple mutant to compare it with published data. The adapted model hints at causes for the observed oscillatory phenotype of the mutant. CheV, an adaptor protein that mediates the interaction between CheA and the receptor, is assumed to generate a positive feedback loop concerning CheA activation while CheY~P stimulates CheA deactivation. The authors also gave an explanation for the population heterogeneity regarding chemotactic oscillations. Variations in the concentration of CheV by just a factor of two, achievable by gene expression noise, can determine the rise of oscillations (Rao *et al*., 2004). However, there is a caveat in the model assumptions because not CheV is inhibiting CheA–receptor association but CheV~P (Aizawa *et al*., 2002; Rao and Ordal, 2009).

Rao *et al*. concluded that the *B. subtilis* system is more robust, i.e. CheY~P steady-state levels and adaptation time are relatively independent of CheB and CheR. This is thought to buffer against genetic mutations and probably reflects the more variable and hostile environment in which *B. subtilis* lives. However, although the regulation of the chemotactic systems of *B. subtilis* and *E. coli* differ, the motility of both organisms is similar in effectiveness over five orders of magnitude of stimulus concentration (Rao *et al*., 2004).

A very important aspect of chemotaxis is that the receptors are located at the poles, while the flagella are evenly distributed on the cell surface. This implies that protein localization is an integral part of the signal transduction and needs to be considered. The signalling molecule CheY~P has to diffuse from the poles throughout the cell volume to act on the flagellum motor (Szurmant *et al*., 2003). Although the switching decision at a given time is stochastic, the frequency of switching is a crucial parameter in controlling motility and is ultrasensitive to the concentration of CheY~P. If spatial gradients of CheY~P concentration exist along the cell, chemotaxis could be disrupted because motors receive conflicting signals as examined by Rao *et al*. (2005) using reaction–diffusion equations. Again, they compared

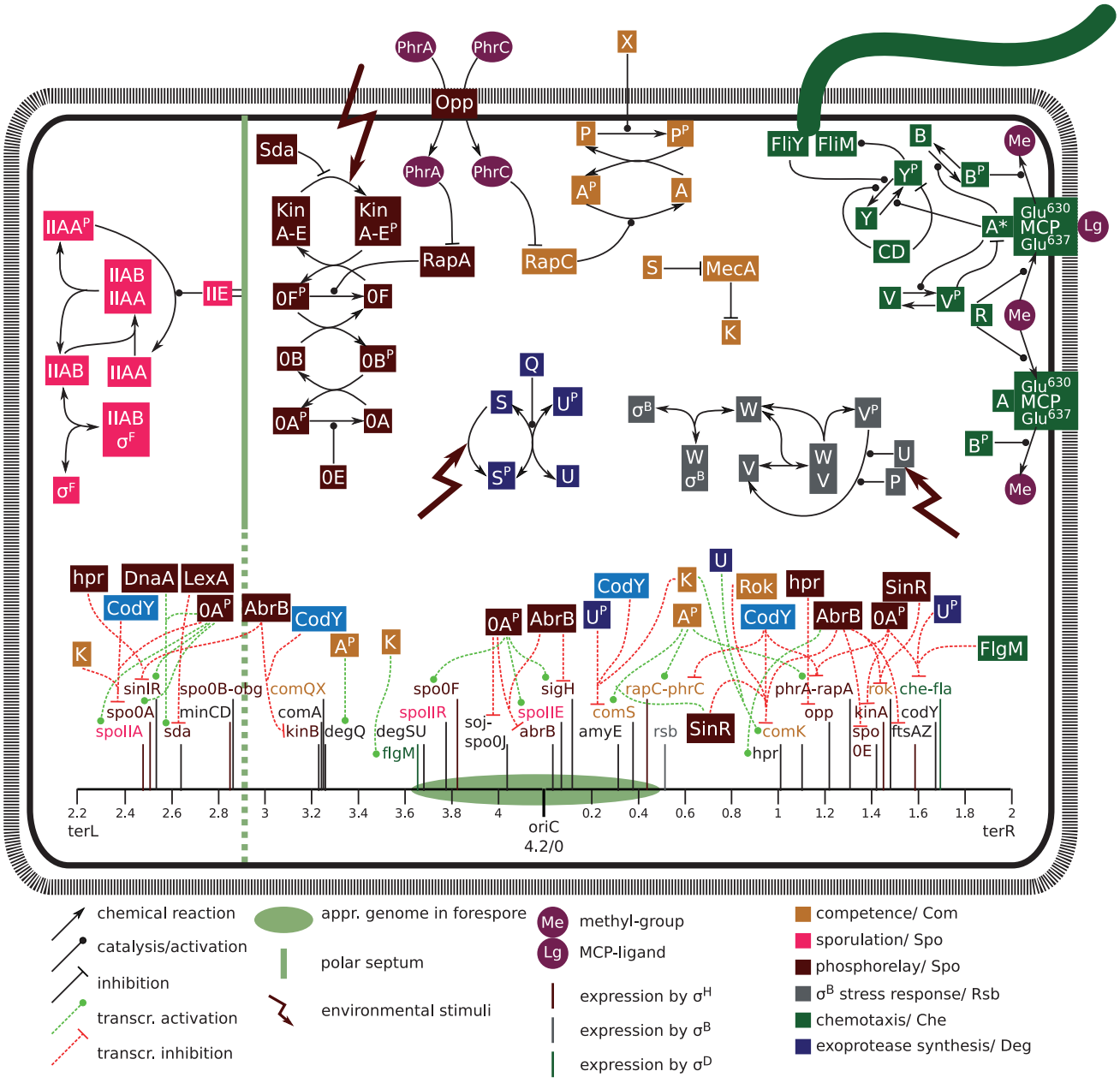

Fig. 1. Reaction diagram for the main signalling cascades discussed in this review. The figure shows the signal transduction that leads to switching of flagella rotation after binding of a ligand (Lig) (green) (Rao and Ordal, 2009), regulation of competence development (yellow) (Hamoen *et al.*, 2003), the switch of the response regulator DegU to DegU~P (dark blue) (Murray *et al.*, 2009), activation of σ^B-mediated general stress response (grey) (Hecker *et al.*, 2007), phosphorylation of Spo0A via the phosphorelay (dark red) (Piggot and Hilbert, 2004) and the reactions in the SpoIIA network towards commitment to sporulation (pink) (Errington, 2003). The upper part shows only interactions in the cytoplasm while the lower part indicates the genomic interconnections of the transcription factors (derived from DBTBS at http://dbtbs.hgc.jp). The environmental signals that lead to the activation of KinA-E, DegS and RsbUP are mostly unknown.

B. subtilis with *E. coli*. In *E. coli* the phosphatase for CheY~P is located at the chemosensing receptor while in *B. subtilis* phosphatases are located both at the receptor (CheC) as well as the flagellum motor (FliY) (Szurmant *et al.*, 2003). The model shows, that *E. coli* can establish a homogeneous CheY~P concentration throughout the cell, because the kinase and the phosphatase are located close to each other. In contrast, for *B. subtilis* a linear decrease of CheY~P concentration with increasing distance to the receptor is predicted. However, simulations for *B. subtilis* indicate the presence of circular concentration gradients around each flagellum motor that render the CheY~P levels comparable at each motor base. The function of CheC could not be

determined by the simulations. CheC did not have an effect on the CheY~P gradient (Rao *et al*., 2005). The authors speculated that the phosphatase network of *B. subtilis* optimizes signal processing of both membrane bound as well as soluble receptors, which have been found for aerotaxis (Hou *et al*., 2000; Rao *et al*., 2005).

Protein localization

Protein localization increases signal transduction speed, specificity and sensitivity not only for chemotaxis (Lewis, 2004; Shapiro *et al*., 2009; Vescovi *et al*., 2010). Preceding cell division proper arrangement of the 'divisome' is critical (Graumann, 2007). The GTPase FtsZ determines the location of the division site as it assembles into a ring-like structure at the midcell, thereby providing the frame for subsequent separation processes. The targeting of FtsZ to the midcell is controlled by the MinCD/DivIVA system. DivIVA is located at the cell poles, presumably because of its affinity for negative membrane curvature (Huang and Ramamurthi, 2010). The proteins MinCD associate with DivIVA and inhibit polymerization of FtsZ (Errington and Daniel, 2002). The hypothesis that the membrane binding equilibrium depends on membrane curvature and leads to MinCD clustering was tested by Howard (2004). The significant finding of this study is not that MinCD pole localization could be reproduced eventually, but rather to uncover the conditions and parameter values that were necessary *in silico*. In the simulation the diffusion of membrane bound MinCD and DivIVA was very restricted (no diffusion was assumed for MinCD), DivIVA binds to the edges of MinCD and binding of MinCD is heavily influenced by geometric effects. Indeed, it seems it is DivIVA not MinCD that is the driving force for membrane curvature sensitivity (Huang and Ramamurthi, 2010).

Another localization phenomenon is chromosome segregation during cell division in conjunction with Spo0J/Soj interactions. Spo0J condenses at nucleoids to compact foci. This process is catalysed by Soj, a protein that performs irregular oscillatory relocations from pole to pole and nucleoid to nucleoid. The large fluctuations in the relocation process are likely to be caused by the low copy numbers of Spo0J/Soj with each being present at about 1500 molecules per cell (Doubrovinski and Howard, 2005). To examine the nature of the fluctuations, Doubrovinski and Howard (2005) formulated a stochastic reaction–diffusion model. They assumed cooperative binding of Soj and Spo0J to nucleoids. Depending on the level of bound Soj, Spo0J can switch to its condensed form causing Soj to diffuse from the foci. After being released, Soj has to reacquire catalytic activity at the cell pole involving interaction with MinD (Doubrovinski and Howard, 2005). The model was tested using the Spo0J19 mutant, which displays a higher frequency of Soj relocations (Autret *et al*., 2001). Analysis of the model indicates that two different modifications could reproduce the mutant phenotype: either (i) Soj is capable of getting reactivated in the cytoplasm without the need of MinD or (ii) Soj is more rapidly expelled from the condensed Spo0J foci. Doubrovinski and Howard (2005) went on to simulate a hypothetical *ftsZ-soj* double mutant. In a cell carrying only an *ftsZ* mutation Soj relocations are suspended. This Soj dysfunction can be suppressed *in silico* with an additional Spo0J19 mutation.

Phosphorelay

The phosphorelay provides a decision device for various phenotypic adaptation reactions like competence, motility, biofilm formation and cannibalism or even the return to vegetative growth (Fawcett *et al*., 2000; Fujita *et al*., 2005; Lopez *et al*., 2008). To distribute risk and benefit of any of the developmental responses, only part of an isogenic population enters any of them (Dubnau and Losick, 2006; Smits *et al*., 2006; Veening *et al*., 2008a). The five histidine kinases KinA-E are the environmental sensors that lead to an activation of the phosphorelay. Among the signals sensed are nutritional stress, cell density, Krebs cycle, DNA damage and presence of extracellular matrix in biofilms (Claverys and Havarstein, 2007; Aguilar *et al*., 2010). The phosphorylated kinases transfer their phosphate group to the Spo0F protein (Sonenshein, 2000; Errington, 2003; Piggot and Hilbert, 2004). The phosphate group of Spo0F~P is then sequentially and reversibly relayed to Spo0B and Spo0A respectively. The response regulators Spo0F and Spo0A are dephosphorylated by the phosphatases RapA and Spo0E respectively. These phosphatases are used for additional environmental regulation (RapA activity inhibited by PhrA) and genomic negative feedback regulation (Spo0E expression activated by Spo0A~P). Phosphorylated Spo0A (Spo0A~P) is the response regulator that directly or indirectly controls the expression of over 500 genes (Fawcett *et al*., 2000). The genes under control of Spo0A~P can be classified according to their affinity to the response regulator (Fujita *et al*., 2005). Genes with high affinity are activated at early stages of phosphorelay activation, e.g. competence, cannibalism and biofilm formation, while genes with low affinity are only activated once sufficiently high levels of Spo0A~P have accumulated, e.g. sporulation genes like the *spoIIA* operon (Fujita *et al*., 2005).

The processes outlined above have attracted various modelling efforts since the interactions within the system are well known and supported by a large body, albeit mostly qualitative, experimental data. Because of the complexity of the phosphorelay network a prediction of its behaviour is

difficult, if not impossible, without the help of computational analysis. Next, we give a short integration of the modelling approaches with respect to the activation of the phosphorelay, followed by a more detailed discussion of the respective models. Jabbari *et al*. (2010) examined how environmental and cellular conditions shape the decision for sporulation. While Jabbari *et al*. (2010) focussed on the elucidation of the contributions of factors external to the phosphorelay, de Jong *et al*. (2003) investigated the dynamics of proteins regulated by Spo0A~P following activation of the phosphorelay. A stability analysis of a simplified model of the phosphorelay was performed by Morohashi *et al*. (2007) while Bischofs *et al*. (2009) went a step further by asking how different environmental signals are integrated by phosphatase activities on top of the phosphorelay kinases. Within a given population the output of the phosphorelay is highly heterogeneous, enabling the population to follow several distinct phenotypes, a finding of investigations by de Jong *et al*. (2010) and Chastanet *et al*. (2010). As indicated, the activation of the phosphorelay is not just a preparation to sporulate but the starting signal for a variety of responses. Schultz *et al*. (2009) started to additionally consider competence, aside from sporulation, being activated by Spo0A~P. For their study on the activation and dynamics of extracytoplasmic protease synthesis Veening *et al*. (2008b) neglected the phosphorelay dynamics instead using AbrB, a Spo0A~P regulated repressor, as the input signal.

The main goal of the modelling work by Jabbari *et al*. (2010) was to elucidate which environmental and cellular conditions allow the activation of sporulation (accumulation of Spo0A~P). Their model can be subdivided into several modules, namely the regulation of:

(i) KinAB activity;
(ii) the phosphorelay;
(iii) expression of SinIR proteins; and
(iv) the activity of RapA by PhrA.

The KinA/B activity controls the initiation of the phosphorelay and sensitivity to environmental conditions. The phosphorelay controls how much Spo0A~P can be generated eventually (Sonenshein, 2000). SinR is a repressor of Spo0A, other late sporulation genes, as well as genes for motility and competence and is inhibited by SinI (Bai *et al*., 1993). PhrA is a phosphatase regulator that inhibits the activity of the receptor aspartyl phosphatase RapA. PhrA is secreted to the medium and re-imported by the oligopeptide permeases (Opp, Spo0K) (Piggot and Hilbert, 2004). The phosphorelay leads to the phosphorylation of Spo0A that inhibits the expression of AbrB. The drop in AbrB concentration results in: (i) an elevated expression of σ^H, and a subsequent increase in Spo0F and Spo0A concentrations, (ii) higher concentrations of KinB, (iii) lower levels of AbrB with the subsequent reduction in the concentration of the transcription factor Hpr and increased SinIR expression and (iv) a reduced level of Hpr leads to derepression of *opp* genes thus increasing the role of quorum sensing by Phr proteins. The environmental signals and cellular states that Jabbari *et al*. (2010) investigated are:

(i) population density sensed via PhrA;
(ii) cellular nutrient and energy availability sensed via CodY-GTP;
(iii) competence decision sensed via the level of ComA; and
(iv) condition of the DNA sensed via Sda.

The authors transformed these four cellular states into yes/no decisions and assigned a priori whether sporulation is desirable or not. Contradictions of simulations with the a priori assigned sporulation decisions was observed for the condition of a cell in a large population (high PhrA level), no nutrients available (no CodY-GTP), no competence (no ComA) but damaged DNA (high Sda level). Contrary to expectations, the model induced sporulation even with damaged DNA, albeit after a significant time delay compared with cells without damaged DNA. In the model, this delay is caused by the sporulation positive signal of PhrA emitted from neighbouring cells. Eventually, PhrA and nutrient limitation are stronger than inhibition of KinA by Sda. Thus, PhrA not only acts as a quorum sensing molecule, as shown by Bischofs *et al*. (2009), but also as a timer allowing cells to repair the DNA. Significantly, the authors conclude that activation of PhrA and RapA transcription by ComA serves to heighten the sensitivity of the phosphorelay towards the input signals (Jabbari *et al*., 2010). This increase in phosphorelay sensitivity might well be a cause for the heterogeneity in the phosphorelay output as observed by de Jong *et al*. (2010) and Chastanet *et al*. (2010).

The model of de Jong *et al*. (2003) is relatively similar to that of Jabbari *et al*. (2010) with respect to the biological scope. But in contrast to Jabbari *et al*. who tested the input–output completeness of our understanding, de Jong *et al*. compared their model with a dozen sporulation mutants. This allowed them to test whether our understanding of the internal structure of the initial sporulation network is correct. Furthermore, the model of de Jong *et al*. is based on a different modelling framework compared with Jabbari *et al*. De Jong *et al*. used discrete time and protein concentration steps. This model allows predictions about relative steady-state concentrations of the components considered, but a comparison with the dynamic simulations of Jabbari *et al*. is not possible. One outcome of the simulations by de Jong *et al*. is that activation of the phosphorelay can result in two steady-state solutions with or without increased levels of Spo0A~P. The reason is a competition of activating KinA and inhib-

iting Spo0E activity in the sporulation network. The system is extremely sensitive with respect to environmental variation and noise in gene transcription, providing an explanation for the observed phenotypic variations in experiments. These findings were further corroborated by Morohashi *et al*. (2007) who performed a stability analysis with a simple model of the phosphorelay. Their model only considers phosphorylation of Spo0A~P by an entity called phosphorelay and its dephosphorylation by Spo0E. They conclude that the feedback of Spo0E influences the distribution of sporulating to non-sporulating cells.

A more detailed examination of the phosphorelay mechanism has been conducted by Bischofs *et al*. (2009). The authors focused particularly on the integration of starvation signals from the medium by quorum sensing mechanisms involving Raps and Phrs. The authors examined the steady-state level of Spo0A~P in response to varying ratios of kinase activity (the environmental signal) to phosphatase activity by the Raps (the population signal). Four different phenotypes are possible: 1. Spo0A~P is not affected by changes in kinase and phosphatase activity; 2. and 3. Spo0A~P is either sensitive to changes in kinase or phosphatase activity; 4. Spo0A~P is sensitive to changes of both kinase and phosphatase activity. Only mechanisms underlying the fourth phenotype can properly integrate the different signals termed by the authors 'signal integration regime'. Interestingly, Spo0B, the second phosphotransferase of the phosphorelay, is devoid of feedback regulations by Spo0A~P. Bischofs *et al*. (2009) showed that if a positive feedback from Spo0A~P to Spo0B would be present, the cell would not be able to properly integrate nutrient level and population density and thus not being able to measure the 'food per cell'.

Even though the goal of systems biology is to increase our understanding of the behaviour and dynamics of complex systems, most models discussed in this review focused on supposedly separate and simplified functional modules of signal transduction. However, we can only understand *B. subtilis* in greater detail if we gain more insight in the interplay and cross-talk of the different environmental response strategies. A step towards dealing with this challenge was taken by Schultz *et al*. (2009). They studied interactions between the processes of sporulation, competence and quorum sensing. Their work showed that small noise levels in many environmental- and community-related signals transmitted by Phrs and Raps resulted in a great variability in the concentration of Spo0A~P, which in turn eventually lead to phenotypic diversity in isogenic populations. The authors related the mutual inhibition of Spo0A~P by AbrB and Spo0E to the synthetic genetic regulatory network called 'repressilator' that was designed by Elowitz and Leibler (2000) to display oscillations. It is an intriguing question whether the early

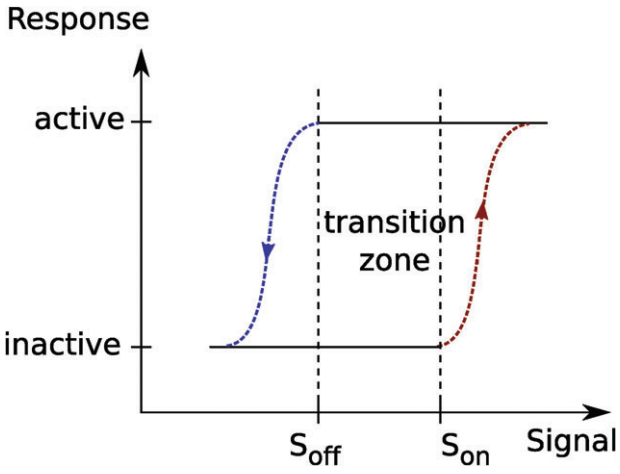

Fig. 2. Hysteretic signal-response curve that can give rise to bistability. In the study of Igoshin *et al*. (2006), the authors tested dynamical properties of the availability of σ^F (response) as a function of the dephosphorylation rate of AA~P (signal). For particular parameter region of the dephosphorylation, the system becomes bistable. Under such conditions, the inactive state can easily switch to the active state characterized by a high σ^F availability, at latest at a signal strength S_{on} (AA~P dephosphorylation rate threshold). However, the active state is robust against deactivation (decrease in AA~P dephosphorylation), since the signal strength S_{off} is reached at lower value compared with S_{on}. In the transition zone, the response is highly sensitive to changes in the signal, with a sufficient perturbation the system can switch easily from the inactive to the active state.

phase of sporulation should be composed of a regulatory network that could generate oscillations and how those detrimental oscillations could be suppressed.

An overarching conclusion for most of the discussed articles investigating the phosphorelay concerns the generation of variability in the Spo0A~P output. Jabbari *et al*. (2010) as well as Schultz *et al*. (2009) observed that Phr and Rap proteins sensitize the output to the input. de Jong *et al*. (2003) and Morohashi *et al*. (2007) detected the competition between Spo0E and KinA as a source for variability and bistability. Further information comes from studies by de Jong *et al*. (2010) and Chastanet *et al*. (2010) who examined the heterogeneity in gene expression after activation of Spo0A. Because of the experimental classification of cells in sporulators and non-sporulators as well as the positive and negative feedback regulations with respect to phosphorylation and dephosphorylation of Spo0A, it was tempting to view the phosphorelay as a bistable switch. Bistability is a property that describes the switching of the system between an activated and deactivated state (Millat *et al*., 2008). Under such a regime, the system can be sensitive to a signal, leading to a switch-like transition into a new steady state. Once it is activated, the system can resist deactivation (see Fig. 2). Bistability is particularly interesting for biological systems as it provides the cell a way for fast yes/no decisions as well as enabling a heterogeneous population

with only some cells being activated (Veening *et al*., 2008a).

Bistability is implicated with several of the *B. subtilis* signalling networks, including competence (ComK) (Maamar and Dubnau, 2005), production of exoproteases (DegU) (Veening *et al*., 2008b) or biofilm formation (SinR) (Chai *et al*., 2007). However, the data by de Jong *et al*. and Chastanet *et al*. show that there is no bistability in Spo0A~P, instead Spo0A~P induced expression is highly heterogeneous. Neither is σ^H, providing the positive feedback via KinA, necessary for establishing a heterogeneous Spo0A~P signal. To reproduce a sufficient accumulation in Spo0A~P using a computational model Chastanet *et al*. had to increase the concentration of all phophorelay proteins. This modelling outcome is surprising since Spo0B concentration remains constant during stationary phase (de Jong *et al*., 2010) and since the modelling of Bischofs *et al*. (2009) showed that Spo0A~P driven *spo0B* expression would mean a violation of the signal integration of nutrients and community density. Sporulation is an all-or-nothing process and surely has to be controlled with switch-like dynamics. It seems however, that the phosphorelay is not the sporulation switch but prepares the cell for a variety of phenotypic diverse responses (Lopez *et al*., 2008).

Sporulation

One of the most conspicuous phenotypes of B. subtilis *is sporulation. The final commitment to this developmental process is established by* σ^F*-dependent gene expression* (Dworkin and Losick, 2005). Spo0A~P-mediated expression of *sigF* is crucial for establishing compartment-specific gene expression during sporulation. Two studies thoroughly investigated the regulation of σ^F activity using ordinary differential equation models. One study focused on molecular processes that lead to asymmetrical differentiation (Iber *et al*., 2006) while the other primarily aimed to uncover the principles of irreversibility of the σ^F activation (Igoshin *et al*., 2006). A simplified graphical description of the regulation of σ^F activity is shown in Fig. 1. Its activity is negatively regulated by the formation of a heterodimer with SpoIIAB (AB), upon which the binding of the sigma factor to its target DNA is prevented. SpoIIAA (AA) is able to competitively bind to AB to release σ^F. However, in non-sporulating conditions AA is predominantly phosphorylated by the kinase activity of AB. Thus, the steady-state ratio of phosphorylated to non-phosphorylated AA determines the level of free σ^F. This level is additionally regulated by the rate of dephosphorylation via the phosphatase SpoIIE (IIE). Iber *et al*. (2006) modelled in detail the different states that exist for AB: (i) its basic form of a homodimer, (ii) bound with σ^F and (iii) bound with one or two molecules of AA (phosphorylated or non-phosphorylated). Each of these configurations harbours combinations of ATP and ADP in the nucleotide binding pockets of the dimer. Finally, the number of states doubles since a central aspect of the model is the allosteric functionality of AB. In any configuration AB is either in a relaxed or in a tense conformation that affects its enzymatic activity (Iber *et al*., 2006). Ultimately, the authors determined 50 states connected by 150 reactions and 25 rate constants. The model was successful in approximating qualitative results of a number of published experiments. A quantitative demand of the model regarding the reaction rate constant of IIE phosphatase was that it is 75–150 times lower compared with *in vitro* rates. In order to resolve this paradox, IIE activity was measured by the authors in an assay with supposedly more *in vivo*-like conditions (switching from manganese to magnesium dominated solutions) and indeed the phosphatase activity matched the model predictions. Iber *et al*. (2006) modelled the higher activity of σ^F in the forespore by assuming that the IIE phosphatase associates with FtsZ homogeneously over the septum. The forespore volume is about four times smaller than that of the mother cell, thus the concentration of phosphatase facing the forespore is four times larger compared with the mother cell (Iber *et al*., 2006). This concentration difference leads to an effective increase in the ratio of IIE to the substrate AA in the forespore and is the primary developmental trigger. The model did not include alternative triggers for the activation of σ^F-like effectors that are compartment-specific expressed due to the genetic asymmetry (Feucht *et al*., 2002) and thus cannot judge these effects. The allostery of the AB kinase activity further amplifies the different AA~P dephosphorylation dynamics in the two compartments. Furthermore, the result implies that the allosteric system is optimized to reduce the need of ATP (Iber *et al*., 2006).

A similar study has been performed by Igoshin *et al*. (2006), who examined the same regulation system with more or less the same intermediate complexes. However, instead of the allosteric nature of AB their model focused on the so-called 'dead-end complex' of AA~P–AB–ADP. The dead-end complex serves to buffer the concentration of AB such that AB is unable to titrate σ^F. Igoshin *et al*. (2006) constructed a model with 27 states, 55 reactions and 12 independent parameters. Analyses of the steady-state concentration of σ^F under various conditions revealed that for certain physiologically feasible circumstances the system shows a hysteretic response, i.e. activation of the system is more easily achieved than deactivation. The hysteretic behaviour necessitates a higher concentration of AA over AB (considering monomers) in the model, a situation that could arguably take place in the forespore since AB is much more unstable than AA (Dworkin, 2003; Igoshin *et al*., 2006). The authors

suggest that the dead-end complex of AA~P–AB–ADP is effectively causing increased σ^F activity in the forespore and that the stability of the complex serves to conserve ATP. A saving of ATP was also implicated by Iber *et al*. (2006) with respect to the allosteric forms of AB. However, how the submicromolar concentrations of the AB–AA complex may contribute to the conservation of ATP present in millimolar concentrations is not discussed. Both studies by Iber *et al*. (2006) and Igoshin *et al*. (2006) explain the compartment-specific developments during sporulation, however, they assumed different mechanisms, Iber *et al*. with AB allostery and Igoshin *et al*. with AB–AA dead-end complex.

Competence

Besides sporulation, the development of competence is one of the best-studied phenotypic adaptations of B. subtilis *and is a widely used example for stochasticity in survival strategies* (Leisner *et al*., 2008; Raj and van Oudenaarden, 2008). During late exponential growth when nutrient availability decreases and the population density increases, about 10% of the individuals in a *B. subtilis* population become competent (Hamoen *et al*., 2003). Competence development is governed by ComK, a transcriptional factor that regulates the expression of more than 100 genes including those required for DNA binding and uptake (Berka *et al*., 2002; Hamoen *et al*., 2002; Ogura *et al*., 2002). As shown in Fig. 1, *comK* expression is controlled by a positive feedback loop, since ComK binds to its own promoter, and by a negative feedback loop via ComS. ComS protects ComK from degradation by the MecA/ClpC/ClpP proteolytic complex. Nevertheless, ComK inhibits expression of *comS* (Maamar and Dubnau, 2005; Süel *et al*., 2006). Development of competence is tightly connected with the activation of the phosphorelay (Lopez *et al*., 2008). The expression of *comK* is inhibited by AbrB and thus *comK* expression can only be effectively activated if the concentration level of AbrB is sufficiently reduced by inhibition via Spo0A~P (Hamoen *et al*., 2003). However, further increases in concentration of Spo0A~P are leading to a derepression of *rok*, an inhibitor of *comK* expression, and thus again development of competence is blocked (Hamoen *et al*., 2003). Development of competence is additionally regulated via pheromones and quorum sensing (Lopez *et al*., 2008). The pheromone ComX activates autophosphorylation of ComP, which activates the transcription factor ComA by transfer of the phosphate group (Hamoen *et al*., 2003). A second pheromone PhrC (also: competence stimulating factor) promotes competence by inhibition of RapC, the ComA~P phosphatase (Lopez and Kolter, 2009). ComA~P induces the expression of ComS, thus stabilizing ComK but also induces expression of PhrA-RapA (Lopez *et al*., 2008). ComA~P as an input to the phosphorelay was examined by Jabbari *et al*. (2010) while Schultz *et al*. (2009) simulated the dynamic sequential activation of competence and sporulation respectively.

The competence system is an example for excitability: a small perturbation induces a significant developmental response which, however, is only transient and the cell eventually returns to vegetative growth (Lindner *et al*., 2004; Süel *et al*., 2007). Positive autoregulation of ComK was found to be the most important factor for the transition to competence (Maamar and Dubnau, 2005; Smits *et al*., 2005). Süel *et al*. (2006) assembled a model to investigate the importance of ComS for switching to competence. They added a noise term to the equation of ComS generation and simulated the concentrations of ComK and ComS. Their model predicted that if ComK positively affects transcription of *comS* then the competence state becomes much more stable without affecting the probability to enter this stress pathway. Experiments with mutants, in which ComS is positively controlled by ComK, revealed that 4.2% of the mutant cells entered competence, similar to wild-type cells with a percentage of 3.6%. In accordance to the simulations, 88% of the mutant cells were locked in the competent state compared with 39% of wild-type cells. Next, Süel *et al*. (2007) have examined the factors controlling entry to competence and the duration of that state. They found that the higher the *comK* expression rate, the higher the probability to enter competence. These findings apply until an oscillation-like regime with successive enter and exit cycles is reached. ComS in turn determines the duration of competence that finally leads to a bimodal distribution of competent cells. Additionally, they showed that after sensitization of the cell by environmental signals, it is noise that stimulates activation of competence. They used an *ftsW* mutant, which develops long filamentous cells that are connected via a common cytoplasm. In this mutant noise is reduced due to the averaging affect implied by diffusion while the physiological mean concentrations are not affected. Indeed, it turned out that the probability to develop competence becomes lower with decreasing noise.

Maamar *et al*. (2007) employed a stochastic simulation approach, using the Gillespie algorithm (Gillespie, 2007), to address the question whether the noise is of transcriptional or translational origin. They performed experiments in which transcription is improved and translation of ComK is reduced, resulting in conditions with relatively constant ComK levels. The analysis revealed that fewer cells became competent in the engineered strains, showing that increased levels of transcription result in less competence. The authors argue that the initiation of competence is controlled by noise, and that the source of the

noise can be attributed to irregularities in transcription. An interesting condition of competence is that the phenotype can only be developed within a certain time window in culture conditions (Leisner *et al*., 2007; Maamar *et al*., 2007). This idea requires that the system is robust most of the time to become sensitive and excitable to gene expression noise under specific conditions.

Leisner *et al*. (2009) examined the system from a different perspective by addressing the question under which condition bistability arises. They ignored the negative feedback loop of *comS* transcriptional regulation by ComK and used ComS as an external parameter that represents quorum sensing signals. Their results imply that during exponential growth, when ComS levels are low and ComK degradation is high, the system is monostable, which indicates that variations in the protein concentrations are not sufficient to activate competence. Only if ComK levels increase due to reduced degradation the system can enter the transition state leading to bistability as response to noise in expression (Leisner *et al*., 2009).

Production of extracytoplasmic proteases

One of the alternative responses following Spo0A activation is the increase in expression of the extracellular protease AprE (subtilisin) and Bpr (bacillopeptidase) (Lopez *et al*., 2008; Lopez and Kolter, 2009; Murray *et al*., 2009). Initiation of sporulation can be delayed by the production of extracellular proteases, which break down proteins in the environment to provide the cells with additional nutrients. The pivotal regulator is DegU. In its phosphorylated form as DegU~P the expression of exoproteases, among them AprE, is stimulated while competence is suppressed (Murray *et al*., 2009). DegU~P is phosphorylated by DegS~P, which in turn autophosphorylates in response to as yet unknown environmental signals. Regulation of DegU is integrated in the phosphorelay network as well. DegQ, an activator for DegU phosphorylation by DegS~P, is activated by ComA~P (Murray *et al*., 2009). Thus, DegU is connected with the cell-density measurement via ComX (Murray *et al*., 2009). Veening *et al*. (2008b) conducted several experiments and used mathematical modelling to detect the original signals and the mechanisms that regulate the dynamics of AprE expression. Transcription of the proteases is additionally inhibited by AbrB. This inhibition is compensated upon phosphorylation of Spo0A at early stages in the preparation of sporulation (Veening *et al*., 2008b). Veening *et al*. (2008b) have built a mechanistic model of the DegSU two-component system and used experimentally measured AbrB levels to empirically include regulation through sporulation signals. Deterministic analyses uncovered bistability of DegU depending on the ratio of phosphorylated/non-phosphorylated protein. The model predicted an increase in AprE levels until 20 h of growth. Indeed that prediction was subsequently verified by the authors in microculture experiments (Veening *et al*., 2008b).

σ^B-response – partner switch mechanism

The partner switch mechanism, including proteins on the spoIIA *operon, is based on exclusive mutual interaction of an anti-sigma factor with both a sigma factor and an anti-anti-sigma factor* (Hecker and Völker, 2001; Price, 2002; Hecker *et al*., 2007). In addition to the irreversible initiation of sporulation, the principle of partner switching mechanism observed for σ^F is also seen in other adaptation responses. One of them is the general stress response, which is mediated by σ^B and activated by a whole collection of environmental challenges including the transition from exponential to stationary phase (Price, 2002; Hecker *et al*., 2007). Although both share a similar regulation scheme, they display critical mechanistic differences that reflect the different physiological needs of the cell (Price, 2002). The anti-anti-sigma factor RsbV (V) is homologous to SpoIIAA and the anti-sigma factor RsbW (W) corresponds to SpoIIAB. Comparable with the *spoIIA* interaction network the phosphorylation status of V regulates the available pool of free σ^B. However, while there is only one phosphatase of SpoIIAA, SpoIIE, which is activated following the formation of the polar septum (Feucht *et al*., 2002; Dworkin, 2003), two phosphatases dephosphorylate V~P in a stress-dependent manner (Hecker *et al*., 2007). RsbU (U) reacts largely to physical stress while RsbP reacts to nutritional stress (Price, 2002; Hecker *et al*., 2007). The main difference in the structures of the sporulation and general stress response is the dead-end complex of AA~P–AB–ADP, which does not exist for V~P–W–ADP because the latter complex can quickly exchange nucleotides (Price, 2002). Since the dead-end complex is missing, the general stress response is readily reversible. This reversibility is necessary since the physiological task of σ^B is to respond to temporary cues from the environment. The second difference is the transcriptional feedback loop since all three proteins, V, W, σ^B, are arranged in an autoregulated operon (Price, 2002). Following σ^B activation by energy stress, the increased expression of σ^B and V provides the potential for further amplification of σ^B activity. In contrast, σ^B driven W expression on the operon counteracts the positive feedback loop since W deactivates σ^B by dimerization. Based on the analysis of the *spoIIA* operon, Igoshin *et al*. (2007) compared the differences of σ^F and σ^B. Simulations showed that this negative feedback by W results in a two stage response, i.e. the full activity of σ^B is not abruptly achieved as it would be without negative feedback. The positive transcriptional feedback increases

the capacity for regulation, i.e. it maximizes the differences in free σ^B before and after stress activation (Igoshin *et al*., 2007). While Igoshin *et al*. (2007) included RsbX, which is involved in negative regulation in response to environmental challenges (Hecker *et al*., 2007), they did not include the partner switch that controls the activity of the phospatase RsbU, which is responsible for environmental stress response activation of σ^B.

Operon organization of stress responses

Operon organization can improve the performance of stress response strategies. This was examined by Iber (2006) or the *spoIIA* network and by Voigt *et al*. (2005) for the phosphorelay with respect to the SinI/R dynamics. The implications of the co-regulation hypothesis of the operon theory by Jacob and Monod (1961) has been tested by Iber (2006) based on her model of the dynamics of the *spoIIA* network during sporulation (Iber *et al*., 2006). The central question addressed with the existing and validated model was how sporulation efficiency is affected if noise in protein expression is either coupled or uncoupled among the proteins of the *spoIIA* operon (Fig. 1). This coupling can, to a certain degree, be justified by the assumption that ribosomes can continue protein synthesis on one mRNA to a following protein coding region without dissociation and re-association rounds. These conditions are met for the mRNA of AA and AB, which have an overlap of four bases. Simulations of sporulation efficiency showed that the detrimental effects of expression noise are more pronounced if protein expression is uncoupled. An operon organization therefore reduces noise by means of coexpression (Iber, 2006; Tabor *et al*., 2008). This implies that operon organization would be disadvantageous for regulation of competence, in which noise plays a purposeful role (Süel *et al*., 2006).

A conceptually related study has been published by Voigt *et al*. (2005), in which the authors investigated possible dynamics regarding the co-regulation of *sinI* and *sinR* with a special focus on evolutionary implications. As described earlier and shown in Fig. 1, SinR is a sporulation inhibitor and controls biofilm formation and SinI is the antagonist that deactivates SinR (Bai *et al*., 1993) A σ^A-dependent internal promoter upstream of *sinR* (P3) establishes an excess of SinR over SinI molecules during vegetative growth. In the model, SinR represses activation of the promoter upstream of *sinI* (P1/2) that transcribes the whole operon (*sinI* + *sinR*). These mutual negative feedback relations can generate a variety of dynamics in SinI, ranging from a graded response to bistability, oscillation and pulse response. The dynamics are most sensitive to the production rate of SinR and indeed a sequence comparison of several *Bacillus* genera shows a pronounced conservation of the P3 promoter region. The sporulation probability is determined by the efficiency of the P1 promoter as well as the SinI-R protein–protein interaction. Since different *Bacilli* are adapted to distinct environments, it seems likely that their tendency to enter sporulation evolved differently. Sequence comparison reflects this drift since the P1 promoter is very diverse and SinI accumulated mutations that could potentially affect the dimerization rate of SinI and SinR while still allowing for dimerization (Voigt *et al*., 2005). However, new experimental findings challenge two model assumptions, namely that SinR inhibits the *sinI* (Chu *et al*., 2005) and the *spo0A* promoter (Kearns *et al*., 2004). These inhibitions are necessary for the development of bistability; thus, either the SinIR network is not intrinsically bistable or there are of yet unknown negative feedbacks. Nonetheless, the article by Voigt *et al*. (2005) expands our understanding of sigma-factor anti-sigma-factor interactions and depicts the potential to understand evolutionary tendencies that take place over years based on the dynamic events of protein concentrations that occur within minutes at most.

Conclusion

The complexity of signalling in *B. subtilis* has motivated numerous studies that used mathematical modelling to elucidate principles and mechanisms of the cell's response to changing environmental conditions. Despite the apparent gap between the complexity of cell signalling networks and the simplicity of their models, many positive examples exist in which mathematical modelling has offered additional insights and in which the models provided guidance for the design of experiments.

For example, analyses of the phosphorelay by Bischofs *et al*. (2009) convincingly showed how the regulation is organized to optimize the information of available nutrient per cell. The combination of model and experiments by Maamar *et al*. (2007) could elegantly explain that temporal regulation of transcription controls the frequency of transition to the competent state.

The formation of heterogeneous subpopulations within isogenic populations (Dubnau and Losick, 2006; Smits *et al*., 2006) and the question of how cell responses are determined by past experiences (Veening *et al*., 2008a; Wolf *et al*., 2008) provide further challenges that motivate the application of mathematical modelling. Rather than studying individual responses in isolation, it is also important to address questions about the interplay of different environmental response strategies. An example in this direction is the work of Schultz *et al*. (2009) that looked at sporulation and competence. Following on from this, future studies should consider signalling between genetically identical individuals and eventually address interspe-

cies interactions (Bassler and Losick, 2006; Little *et al*., 2008).

The knowledge of many regulatory mechanisms can be transferred from *E. coli* to *B. subtilis*. In some cases, however, due to their evolutionary distance these two model organisms have developed different environmental response strategies. Spore formation in *B. subtilis* is one example for a strategy that exists in this organism, but not in *E. coli*, while in other cases even protein homologues function in a surprisingly different way. An example is CheY~P, which induces completely different chemotactic responses in *E. coli* and *B. subtilis*. This suggests that exciting problems remain that have to be addressed specifically for *B. subtilis.* No doubt, this Gram-positive model organism provides plenty of challenges and exciting opportunities for mathematical modelling.

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