Deciding fate in adverse times: sporulation and competence in *Bacillus subtilis* - Supporting Information

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1 The Competence switch

1.1 The ComK switch

As is mentioned in the main article, the competence module controls the transitions from the sporulation path into (and back from) the competence state [1, 2, 3, 4, 5, 6]. This stochastic switch module consists of a self-activator master regulator ComK and a degradation complex MecA/ClpP/ClpC. The active degradation of ComK is regulated by a peptide ComS which is also degraded by the MecA/ClpP/ClpC complex and thus competes with ComK for binding to the complex. The concentration of ComK is usually kept at low levels by rapid degradation, but can cross the threshold for self-activation when the concentration of ComS is sufficiently high. The operation of this ComK-MecA-ComS circuit has been studied in detail by modeling it as a dynamical system with two variables - the concentrations of ComK and ComS,

and was proposed to act as an excitable system, a bi-stable system or both [7, 8, 9, 10, 11]. Either excitable or bi-stable, the module acts as a stochastic switch, the action of which can be described as activation over an effective energy barrier which depends on the concentration of ComS.

Overexpression of ComK also has a negative effect on ComS, in a mechanism which is important for the exit from competence and essential for the excitable model. Here, we focus on the decision to enter competence; that is, we focus on the regulation of the escape time into competence. Hence, we treat the concentration of ComS as a control parameter (figure S1) and describe the switch operation by the following one-dimensional dynamical equation of ComK:

$$\frac{dk(t)}{dt} = F(k,s) \tag{1}$$
$$F(k,s) = g_0 + g_1\left(\frac{k^n}{k_0^n + k^n}\right) - \frac{\Lambda k}{1 + s/\Gamma_s + k/\Gamma_k}$$

where g_0 and g_1 are the basal and activated ComK synthesis rates, Λ is the maximal degradation rate of ComK, k_0 is the ComK concentration for half-activation of the feedback loop and n its cooperativity (Hill coefficient), Γ_s and Γ_k are concentrations of ComS and ComK for half-maximal degradation.

Here, since ComS is taken as a control parameter, the competent state can be stable. However, while the stability of the competent state affects the return time from competence, it does not affect the transitions into competence which is the focus here. We use a set of parameters corresponding

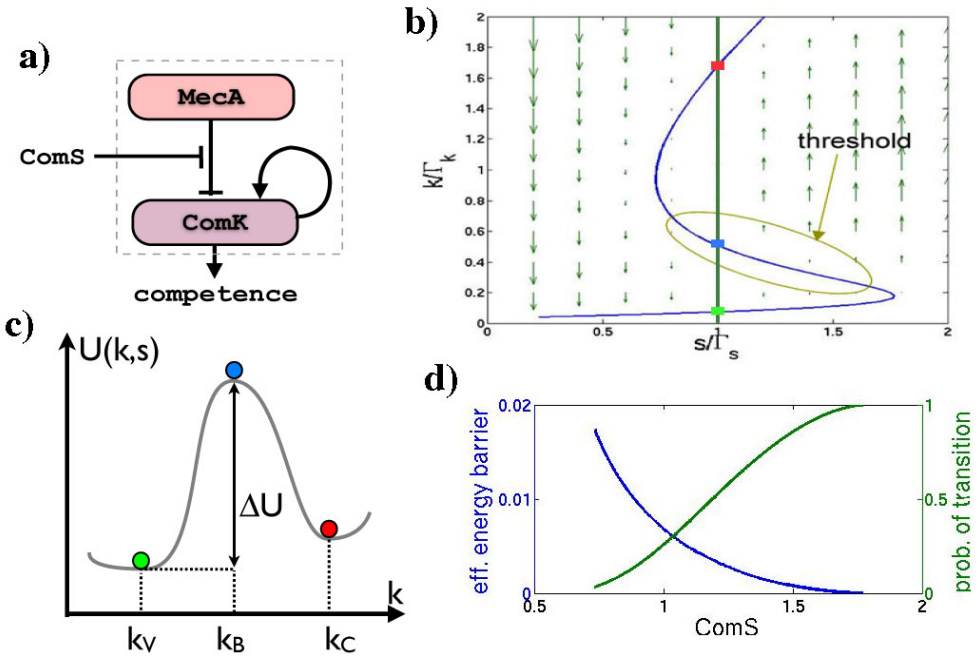

Figure S1: Function of the competence switch described by equation 1 when ComS acts as a control parameter. **(a)** Scheme showing the competence circuit with ComS acting as a control parameter interfering with the active degradation of ComK. This can possibly lead to overexpression of ComK, due to the positive feedback loop, and entrance into competence. **(b)** A typical nullcline and flux in the phase diagram defined by the concentrations of ComK and ComS - equilibrium solutions $k^*(s)$, such that $F[k^*(s), s] = 0$ as a function of the control parameters. For a fixed value of ComS, the intersections with the nullcline indicate the vegetative (green), barrier (blue) and competence (red) values of k. **(c)** Taken from figure 2 in the article, illustrates the effective potential $U(k, s) = \int F(k, s)$ along the perpendicular solid line in (b). **(d)** The potential barrier $\Delta U(s)$ and probability of transition τ^{-1} as functions of ComS. Note that for a given value of s, $\Delta U(s)$ is the difference $U(k, s) = k_B - k_V$ between the barrier point and the vegetative state. The parameters used are the same as figure 2 in the main text, typical of excitable systems: $g_0/\Lambda\Gamma_k = 0.03$, $g_1/\Lambda\Gamma_k = 0.5$, $k_0/\Gamma_k = 0.7$.

to bistable behavior in k, which could correspond to either the bistable or excitable model in the two-variable problem. The corresponding nullcline and flow for the ComK equation are shown in figure S1.

From the plot we see that below a certain concentration ComS_1, vegetation (low concentrations of ComK) is the only stable state, and the system cannot enter competence. Above this limit, the competent state, with high concentrations of ComK, is stabilized. There is an interval between ComS_1 and ComS_2 where the system is bistable. Above this limit the vegetative state is no longer stabe, and the cell necessarily goes into competence. However, usual concentrations of ComS in the cell are well below this limit. Fluctuations in ComK concentration play a crucial role in the transition from vegetation to competence by allowing the crossing of the threshold for self-activation [12, 13, 14]. The threshold itself is a function of ComS, regulating the probability of transition into competence. The threshold can be represented by an energy barrier $\Delta U(s) = U(k_B) - U(k_V)$, where the potential is defined by $U(k,s) = \int F(k,s)dk$. $k_B(s)$ and $k_V(s)$ are the ComS-dependent typical ComK value for vegetation and the unstable fixed point in dk/dt representing the top of the energy barrier. The probability of transitions into competence is then proportional to $exp[-\Delta U/\epsilon]$, ϵ being the effective fluctuation in ComK concentration.

1.2 The Quorum Sensing Mechanism

The concentration of ComS, the control parameter of ComK, is regulated by the ComP-ComA two-component sensing system [15, 16, 17, 18, 19], as illustrated in figure S2. A general analysis of the function of two-component sensing systems is presented in section 2. In particular, we show that this sensing system acts as a gate. The concentration of ComA* is low in an isolated cell, but reaches high levels above a threshold concentration of the quorum sensing pheromone ComX. In other words, activation of ComA is only reached above a certain colony density. We also show that the threshold level is further regulated by the Rap system discussed in section 5. ComA* acts as the transcription factor that activates the competence inducer ComS, with a Hill function dependence.

As is explained in the main article and further discussed in SI6, the transcription of ComK is inhibited by AbrB, the master regulator of the decision system, and by Rok (which is also regulated by AbrB), as is shown in figure S2. To model these effects we modify equation (1) to include a transcription inhibition by $H(x)$ - a Hill function ranging from 1 to 0 representing the repression of ComK by a signal x composed of AbrB and Rok. Since the binding sites of these proteins in the ComK promoter overlap, the signal x can be considered an addition of the AbrB and Rok concentrations normalized by the binding constants. The extended equation of the competence switch including the effects of the decision system is given by

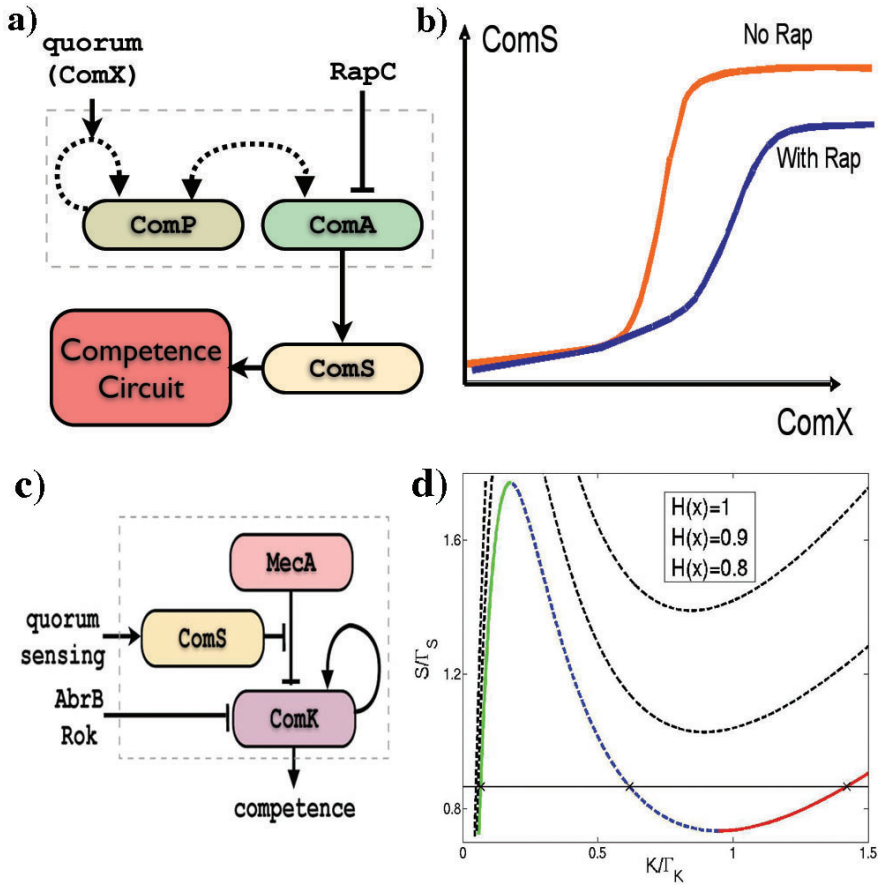

Figure S2: Schematic representation of **(a)** the ComP-ComA module and **(b)** its quorum sensing gate characteristics, as discussed in the text. **(c)** The combined description of the competence switch corresponding to equation (2), with schematic presentation of the circuit including the effect of the AbrB-Rok system. **(d)** Nullclines showing the effects of repression of ComK. When ComK is repessed, higher levels of ComS are required to make competence possible. Parameters are the same as figure 1 and figure 2 in the main text.

$$F(k,s) = \frac{dk(t)}{dt} = H(x)\left[g_0 + g_1\left(\frac{k^n}{k_0^n + k^n}\right)\right] - \frac{\Lambda k}{1 + s/\Gamma_s + k/\Gamma_k} \qquad (2)$$

We note that this is equation (1) in the article. Illustration of the effect of AbrB+Rok on the stochastic switch is presented in figure S2.

2 The operation of two-component systems

The sporulation and competence key regulatory modules of the signal transduction decision making network are controlled by inputs from two-component sensing systems. The ComP-ComA which senses the colony density via ComX in the case of competence and the Kin-Spo0F which senses cell stress in the case of sporulation. Two-component systems are a widespread signal transduction mechanism used by bacteria for sensing information signals. In general, a two-component system operates by a histidine kinase being phosphorylated in response to a signal and then transfering the phosphate to an aspartate in the response regulator [20, 21, 22, 23]. The latter can act either as a kinase or a transcription factor. The binding of phosphate to a histidine residue is more unstable than the binding to an aspartate residue. Therefore, the transfer of phosphate to the response regulator happens relatively quickly. Some details might vary depending on the case, such as the mechanism of phosphorylation of the histidine kinase or the dephosphorylation of the response regulator by specific dephosphatases.

2.1 General Description

Here we model and analyze the operation of the general two-component system presented in figure S3. The dynamics of the system is governed by the following set of equations in which h, r are the concentrations of the histidine kinase and response regulator and h^*, r^* are the concentrations of the phosphorylated histidine kinase and response regulator:

$$\begin{aligned} h + P_i &\overset{s}{\rightleftarrows} h^* \\ h^* + r &\overset{K}{\rightleftarrows} h + r^* \\ r^* &\overset{q}{\rightleftarrows} r + P_i \end{aligned} \tag{3}$$

s is the signal controlling the influx of phosphate in the system through the histidine kinase - ComX in the case of the ComP-ComA system and the cell stress signals in the case of the Kin-Spo0F system. q is the signal controlling the output of phosphate through the dephosphorylation of the response regulator.

It is important to note that phosphate can flow both ways in the system, the subscripts i and o in s and q representing inflowing and outflowing phosphate in the circuit. H and R being the total concentrations of histidine kinase and response regulator, the most general case has the following reactions:

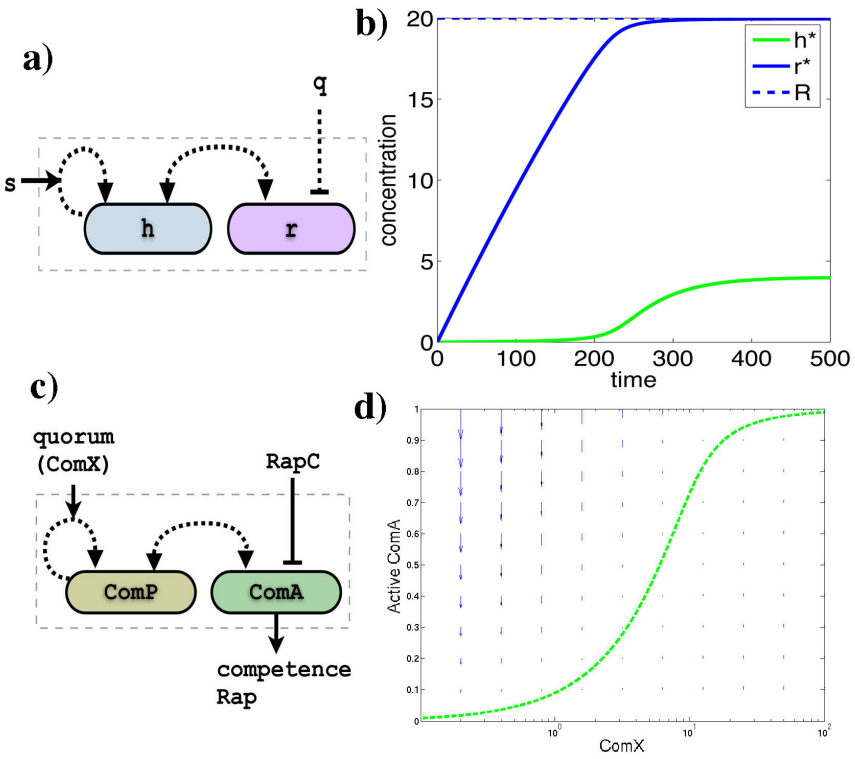

Figure S3: The operation of two-component systems. **(a)** Scheme representing the circuit of a generic two-component system. The histidine kinase is phosphorylated in response to a signal and rapidly transfers the phosphate to a response regulator. The response regulator can be a target of dephosphorylation. **(b)** Typical time dependence of the circuit modeled by equation (3), showing the concentrations of the histidine kinase and the response regulator. The rapid transfer of phosphate from the histidine kinase to the response regulator is reflected in the fact that there is a linear increase in r^* in response to the signal, while h^* only increases after saturation of r^*. Therefore, the concentration of histidine kinase does not play a big role, and its transcription is usually not regulated. Parameters are $R = 20$, $H = 5$, $s = 0.2$, $q = 0.01$, $K = 100$, $q_i = s_o = 0$. **(c)** Scheme description of the quorum sensing system of the competence module. ComP is autophosphorylated when bound to ComX and rapidly transfers the phosphate to ComA. ComP can dephosphorylate ComA when unbound. ComA is inactivated when bound to RapC. **(d)** Curve showing the level of active (phosphorylated) ComA* as a function of ComX. Parameters are $A = 1$, $P = 1$, $K_p = 0.1$, $K_d = 1$.

$$\frac{dh^*}{dt} = (s_i + r^*)(H - h^*) - Kh^*(s_o + R - r^*) \quad (4)$$
$$\frac{dr^*}{dt} = (q_i + Kh^*)(R - r^*) - r^*(q_o + H - h^*)$$

The constant K reflects the equilibrium constant of the phosphate transfer between h and r, by which the other rates and signals are normalized. In figure S**??** we see the time dependance of the species involved. We see first the increase of r^* to high levels, and then an increase in h^* levels. The equilibrium concentration of r^* is therefore not dependent in the concentration of histidine kinase H. This might be the reason why the transcription of these proteins are not regulated in the phosphorelay, while the response regulators are subject to transcriptional control - Spo0F and Spo0A are regulated by the sigma factor σ^H.

2.2 The ComP-ComA quorum sensing system

We proceed to apply the model of the generic two-component system to the specific case of the ComP-ComA sensing system of the competence module. The functional role of this system is to sense the colony density by measuring the external concentration of the quorum sensing pheromone ComX. The system then regulates the competence switch by transcription activation of the competence inducer ComS by ComA*, according to the level of ComX and input from the Rap system. Schematic presentation of this circuit is shown in figure S3.

The genes involved in the process (ComQ, ComX, ComP, ComA) are co-transcribed in the *com*QXPA cassete, regulated by a single promoter. The peptide ComX is transcribed in a precursor form, which is modified and exported out of the cell by ComQ. ComP is an intramembrane histidine kinase which autophosphorylates upon binding to ComX, quickly transfering the phosphate to the response regulator ComA. ComP can also dephosphorylate ComA in the absence of ComX. ComA acts as a transcription activator of the competence inducer ComS and many Rap genes. ComA is inactivated upon binding to several Rap proteins. Here we consider the specific case of RapC.

The operation of the system is described by the following model:

$$
\begin{aligned}
&ComP_0 + ComX \rightleftarrows ComP_1 \\
&ComP_1 + P_i \rightarrow ComP^* \\
&ComP^* + ComA \rightarrow ComP_1 + ComA^* \\
&ComP_0 + ComA \rightarrow ComP_1 + ComA^* + P_i
\end{aligned}
\tag{5}
$$

where ComP_0 and ComP_1 are unbound and bound to ComX. Applying the two-component model to the quorum sensing system, we would have the ComP (p) autophosphorylation rate depending on the fraction of ComP which is bound to ComX (x). The fraction of ComP which is not bound to ComX will act as a dephosphatase. If we consider the signal x as the concentration of ComX normalized by its binding constant, the binding/unbinding

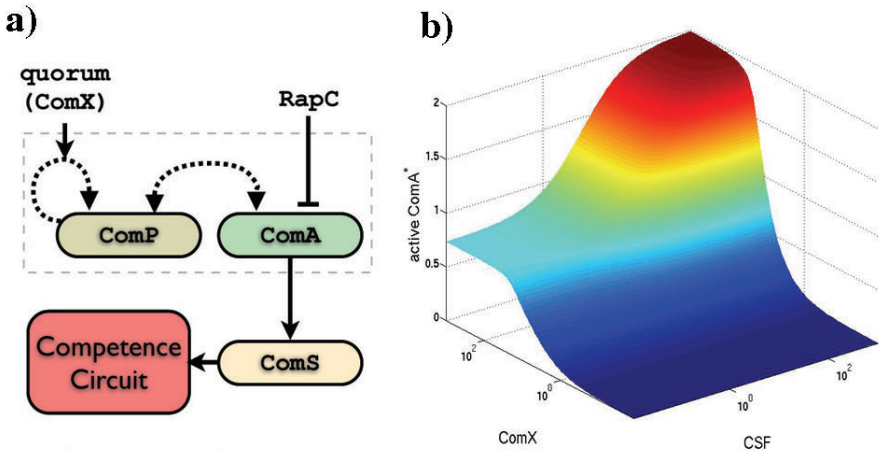

Figure S4: The gating of ComS by the ComP-ComA sensing system. **(a)** Schematic representation of the circuit including the effect of the Rap system (here we consider specifically the effect of RapC). **(b)** Active ComA as a function of the pheromones ComX and CSF. ComX is essential for ComA activation, while CSF increases its activity by protecting ComA* against Rap dephosphorylation. Since ComA* acts as a transcription activator of ComS, the signal inducing competence will be the level of ComA* times the Hill function representing ComS activation. Parameters are the same as figure 4 in the main text: $A = 2$, $K_d = 1$, $K_p = 0.1$, $K_b = 5$ for the quorum sensing mechanism and the parameters for the Hill function regulating RapC production are $a_0 = 1$ and $m = 2$.

of ComX to ComP in equilibrium, K_p and K_d the phophorylation and dephosphorylation rates normalized by the transfer rate between ComP and ComA, P and A the total concentration of the proteins, we have:

$$\begin{aligned} \frac{dp^*}{dt} &= K_p \left(\frac{x}{1+x} P - p^* \right) - p^*(A - a^*) \\ \frac{da^*}{dt} &= p^*(A - a^*) - K_d \frac{x}{1+x} P a^* \end{aligned} \tag{6}$$

Typical dependence of ComA* on the level of ComX is shown in figure S3.

The ComP-ComA module operates as a quorum sensing gate that activates the production of ComS only above a threshold concentration of ComX. We also note that the production of ComS is limited to guarantee the existence of the vegetative state (kept below $ComS_2$, as defined in SI1). The response regulator ComA interacts with the Rap module: while ComA* activates the production of some of the Rap proteins, it becomes inactive when bound to RapC. The binding is independent of the phosphorylation state. Hence, high levels of RapC can modulate the gate characteristics of the ComP-ComA sensing module, and levels of ComA* are highly dependent on the concentration of RapC associated pheromone PhrC (CSF). We model this as a reduction of the total concentration of ComA A by a factor of $\frac{1}{1+c}$, where c is the level of RapC normalized by its binding constant K_b. In figure S4 we show a schematic presentation of the effect of RapC on the gating effect of the ComP-ComA system and the ComA* dependence on ComX and CSF.

3 The Sporulation Module

As is mentioned in the article, the sporulation module, shown in figure S5, acts as a timer that controls the cell progression towards sporulation [24, 25, 26, 27, 28, 29]. The module is composed of a sensing two-component system - the Kin-Spo0F which measures the cell stress, and a regulatory two-component system - the Spo0B/A which determines the entry into sporulation. This happens when the level of the sporulation master regulator Spo0A* exceeds a certain threshold level. As phosphate is transferred down

the phosphorelay, it leads to an accumulation of Spo0A* (phosphorylated Spo0A), which induces the production of a sporulation specific sigma factor σ^H. Since Spo0A itself has its transcription activated by σ^H, Spo0A is more rapidly produced when its concentration crosses a certain threshold level S_{Act}. The Rap system regulates the Kin-Spo0F sensing system by dephosphorylation of Spo0F*, while Spo0B/A is regulated by Spo0E, which dephosphorylates Spo0A*.

Further below we present detailed model and simulation results of the entire sporulation module. But we start with heuristic description of the activity of the Kin-Spo0F sensing system based on the results for general two-component system presented in SI2. This sensing system is similar to that of the ComP-ComA two-component system, although more elaborate, since it is comprised of five different histidine kinases (KinA-KinE), each responding to (autophosphorylated by) a different stress signal. Spo0F, the response regulator, is regulated by the Rap system and has its transcription activated by the sigma factor σ^H. In figure S5 we show the circuit of Kin-Spo0F representing the five different histidine kinases by one protein. In the absence of input from the Rap system or Spo0A* (via σ^H), the dependence of Spo0F* on the stress signal s is similar to the dependence of ComA* on ComX. The rate of production of Spo0F*

$$\omega = \frac{d\, Spo0F^*}{dt} \tag{7}$$

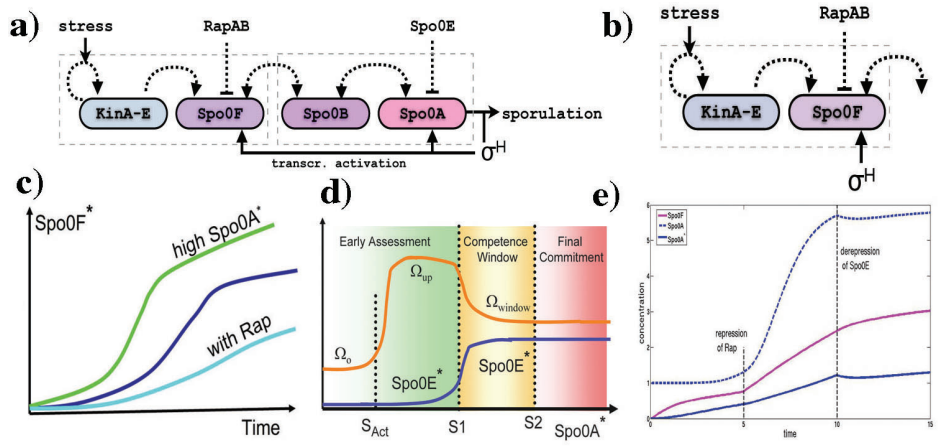

Figure S5: **(a)** Schematic description of the sporulation module, composed of two two-component systems acting in series. The integration of the stress signals starts with the stress-induced autophosphorylation of KinA-E, followed by a phosphate transfer to Spo0F. Spo0B quickly transfers phosphate between Spo0F and Spo0A, the direction of the flux depending on the regulation of both response regulators. Spo0A* activates the production of σ^H, a transcription activator of many sporulation specific promoters including Spo0A, Spo0F and PhrC. **(b)** Kin-Spo0F sensing system used in (c). **(c)** Schematic description of the circuit operation showing the level of Spo0F* as a function of time for some stress level s. The dark blue curve shows no regulation. The green curve corresponds to transcription activation of Spo0F by Spo0A* (σ^H). The light blue curve shows Spo0F* dephosphorylation by the Rap system. **(d)** Ω - the rate of the sporulation timer. Below S_{Act}, $\Omega = \Omega_0$. Above this level, transcription activation of Spo0F and Spo0A increase the rate to Ω_{up}, determined by the stress level. As the level of Spo0E increases, the timer rate slows down to Ω_{window}. **(e)** The dynamics of the sporulation timer. We show results of simulations of the kinetic rate equations (9). At time $t = 5$ we imitate repression of Rap by CSF (PhrC), the concentrations of both Spo0F* and Spo0A* increasing rapidly. At time $t = 10$ we imitate the effect of dephosphorylation of Spo0A* by Spo0E. As a result, Ω decreases (can even be negative). Parameters are: $s = 0.5$, $r = 0.4$ (before $t = 5$), $e = 0.3$ (after $t = 10$), $K_1 = 10, K_1' = 0, K_2 = 0.5, K_2' = 10, K_3 = 10, K_3' = 1$. $N = 1, B = 1$ and the total concentrations of Spo0A and Spo0F are $A = A_0 + A_1(a^{n_a}/(a_{0a}^{n_a} + a^{n_a}))$ and $F = F_0 + F_1(a^{n_a}/(a_{0f}^{n_a} + a^{n_a}))$, where $F_0 = 1, F_1 = 5, A_0 = 1, A_1 = 5, n_a = 5, a_{0a} = 0.7, a_{0f} = 0.7$.

has a linear dependence on the stress above a certain threshold level. Input from the Rap system, which dephosphorylates Spo0F*, decreases ω as is illustrated schematically in figure S5. We also illustrate in this figure the effect of the transcription activation by σ^H which leads to an increase in ω.

The function of the entire phosphoryley path shown in figure S5 is goverened by the following equations:

$$
\begin{aligned}
&Kin + P_i \xrightarrow{s} Kin^* \\
&Kin^* + Spo0F \overset{K_1}{\rightleftarrows} Kin + Spo0F^* \\
&Spo0F^* + RapA/B \rightarrow Spo0F + RapA/B \\
&Spo0F^* + Spo0B \overset{K_2}{\rightleftarrows} Spo0F + Spo0B^* \\
&Spo0B^* + Spo0A \overset{K_3}{\rightleftarrows} Spo0B + Spo0A^* \\
&Spo0A^* + Spo0E \rightarrow Spo0A + Spo0E
\end{aligned}
\tag{8}
$$

The first equation describes aoutophosphorylation of the histidine kinases. The rate of the reaction is proportional to the input signals represented by s. The second equation describes the exchange of phosphate between the histidine kinases andSpo0F. Note that the transfer can be in both directions. For this reason, Kin-Spo0F functions as a gate similar to ComP-ComA, transfering phosphate down the phosphoryley path only when the stress is sufficiently high. The third equation describes the dephosphorylation of Spo0F* by the Rap system (represented here by the two Rap proteins RapA and RapB). The fourth and fifth equations describe the exchange of phosphate between-

Spo0F and Spo0B and between Spo0B and Spo0A respectively. The last equation describes the dephosphorylation of Spo0A* by Spo0E.

The kinetic rate equations that correspond to equations (7) are:

$$\begin{aligned}
\frac{dn^*}{dt} &= (s + K_1' f)(N - n^*) - K_1(F - f^*)n^* \\
\frac{df^*}{dt} &= (K_1 n^* + K_2' b^*)(F - f^*) - [K_1'(N - n^*) + K_2(B - b^*) + r]f^* \\
\frac{db^*}{dt} &= (K_2 f^* + K_3' a^*)(B - b^*) - [K_2'(F - f^*) + K_3(A - a^*)]b^* \\
\frac{da^*}{dt} &= K_3 b^*(A - a^*) - [K_3'(B - b^*) + e]a^*
\end{aligned} \tag{9}$$

Where n^*, f^*, b^* and a^* are the concentrations of phosphorylated Kin, Spo0F, Spo0B and Spo0A respectively.

The rates K_i and K_i' refer to the forward and backward fluxes of phosphate through the system. s, r and e are the stress, RapA/B and Spo0E signals normalized by the rates of the reactions involving them. We note that the rate of accumulation of Spo0A*, $\Omega = d(Spo0A^*)/dt$ (the clock rate of the sporulation timer), is given by $d(a^*)/dt$ in the fourth equation. Simulations of the model are shown in figure S5.

4 The Rap System

The Rap early assessment module acts as the central communication and information possessing system of the decision-making network. It sends and receives to and from other cells peptide pheromones that carry information about the cells stress, progress towards sporulation and inclination to make

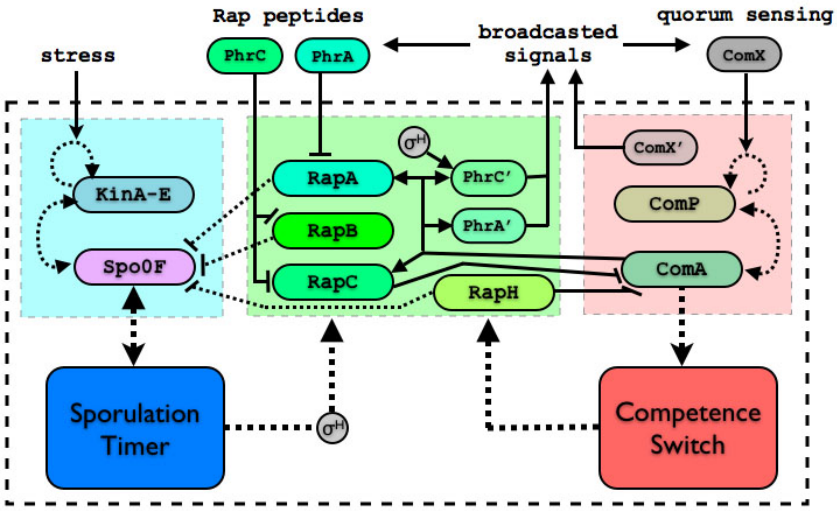

Figure S6: Generic description of the Rap early assessment system. The dashed grey lines indicates the modules and the dashed bold black line indicates the cell membrane. We include ComP-ComA and Kin-Spo0F as part of the general early assessment system. The Rap communication and information processing module is the module with green background. The system input signals include the cell stress, the quorum sensing pheromone ComX, a variety of active pheromones containing information about the neighboring cells' status and σ^H, which provides information about the level of Spo0A* - the measure of the sporulation progression. Recently it was found that ComK also acts as an input signal of the Rap system, being a transcription activator of RapH.

competence transitions [30, 31, 32]. The ComP-ComA and Kin-Spo0F two-component systems then use this information sent to, received from other cells and processed by the Rap system. Generally speaking, the Rap decreases the clock rate of the sporulation timer and increases the waiting time of the competence switch by dephosphorylation of Spo0F and inactivation of ComA, respectively. The dephosphatases are in general up-regulated by

the quorum sensing signal (via ComA) and down-regulated by the external peptide pheromones secreted by the neighboring cells and the cell itself. Rap is also regulated by Spo0A* which enhances the production of some of the pheromones (e.g. PhrC) via σ^H. More recently, it was discovered that the Rap system is regulated by ComK, enhancer of RapH which dephosphorylates Spo0F*, possibly to prevent sporulation during competence. A generic description of the Rap integrated system with its input and output signals, including RapA, RapB, RapC and RapH and the two two-component sensing systems of the competence and sporulation modules, is shown in figure S6.

More specifically, the Rap communication and information processing module consists of 11 Rap proteins identified in *B. subtilis*, sometimes with redundant motifs. They act as a response regulator inhibitor either by dephosphorylation or by interfering with DNA binding. Most Rap proteins were shown to target either Spo0F or ComA. Rap proteins are usually co-transcribed with their respective pheromones. The pheromones are produced as precursors, modified and exported out of the cell, then reimported to act as antagonists to their own associated Rap protein. Many of the Rap proteins are induced by ComA*, a signal coming from the quorum sensing mechanism.

Many of the pheromones have an additional promoter that can be activated by σ^H, a sigma factor induced by Spo0A*. We focus here on the first three Rap proteins, since the mechanisms of other Rap proteins are analogous to these. RapA, a Spo0F* dephosphatase, RapC, which inactivates ComA upon binding, and RapB, which also acts as a Spo0F* dephosphatase

but is not regulated by ComA and does not have a pheromone associated to it. Instead, RapB is inhibited by the pheromone PhrC associated to RapC, making this single pheromone an important part of the regulation of both sporulation and competence.

We show a typical example of information processing by Rap proteins by detailed study of the RapA-PhrA pair, co-transcribed through activation by ComA* and shown in figure S7. As it is illustrated in the figure, the pheromone PhrA is first produced as a precursor PhrA', which is modified into its active form PhrA and exported out of the cell. The external active signal PhrA is imported into the cell and acts as antagonist which binds to its own associated protein RapA inhibiting its activity. The external concentration of the active pheromone PhrA is composed of both the pheromone produced by the cell and also PhrA produced by other cells around it. Hence, the RapA-PhrA circuit acts as a comparator element - it provides information about the individual concentration of ComA* in the cell vs. the group average level of ComA* in the vicinity.

The modeling and analysis of the operation characteristics of the RapA-PhrA element are detailed as follows: r_A - the steady state concentration of the RapA, is given by

$$r_A = \frac{g}{1 + K_{bind} p_A} \tag{10}$$

where p_A is the cell concentration of the active form of the pheromone

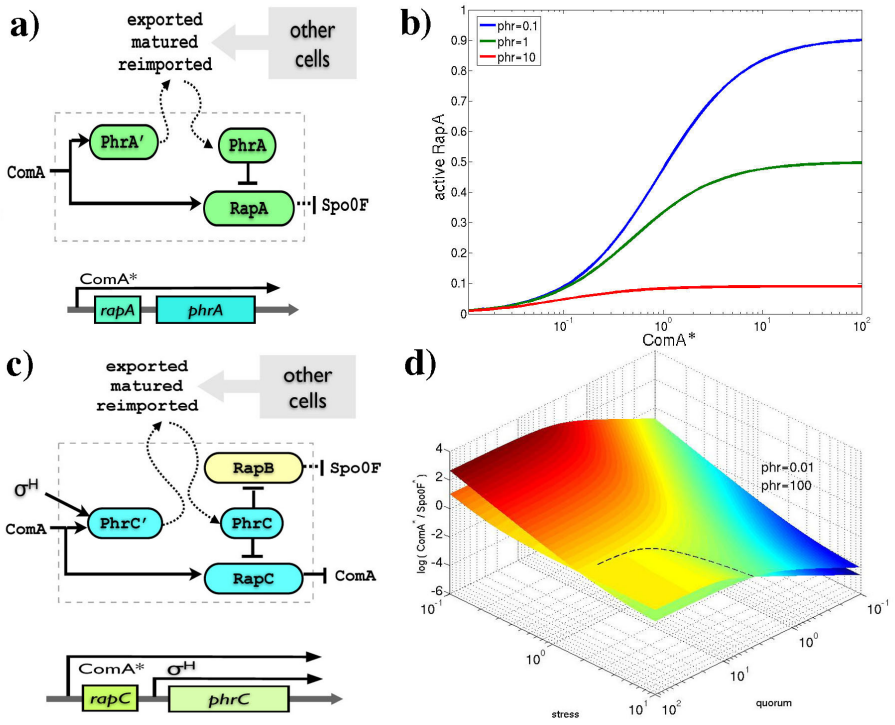

Figure S 7: **(a)** Schematic representation of the RapA-PhrA unit, showing its regulation, communication and information processing. **(b)** Level of active RapA as a function of ComA* for different values of imported pheromone (indicated here as *phr*). The amount of imported pheromone includes contributions by the cell itself and also from other cells in the vicinity. The concentration of ComA is normalized by its value for half-activation of RapA/PhrA, pheromone PhrA concentration is normalized by its binding constant to RapA and RapA activity is represented by fraction of the total concentration. **(c)** Schematic representation of the RapC-PhrC unit, showing its regulation, communication and information processing. **(d)** Effect of PhrC (CSF) on the decision between competence or sporulation. In the presence of CSF cells with different levels of stress have similar tendencies towards competence, here measured by $ln(ComA/Spo0A^*)$, while in the absence of CSF cells with higher levels of stress are more inclined to enter competence. Parameters are: $K_p = 0.1, K_d = 1, M = 1, P = 1$ for the quorum sensing, $K_1 = 10, F = 1, N = 1$ for the Kin/Spo0F, $\gamma = 5, a_0 = 0.3, n = 1, K_b = 2$ and the normalized dephosphorylation of Spo0F by Spo0B is $q_0 = 0.2$. Pheromone PhrA concentration is normalized by its binding constant to RapA.

PhrA, K_{bind} is the binding rate of PhrA to RapA, g is the synthesis rate of RapA and PhrA. This rate is given by

$$g = \gamma \frac{a^n}{a_0^n + a^n} \tag{11}$$

where a is the ComA concentration, a_0 is the ComA concentration for half-activation, n is a Hill exponent, and γ is the synthesis rate in units of the degradation rate.

The synthesis of the pheromone precursor p'_A is the same as the one for RapA g. The pheromone precursor is exported and matured at a combined rate K_{exp}. A fraction f of the matured pheromone is readily reimported and does not escape the vicinity of the cell. $1 - f$ of the pheromone reaches the environment and adds to the amount of pheromones coming from neighboring cells $p_A^e = K_e(1 - f)g + p_A^{diff}$, where p_A^{diff} is the rate of diffusion of PhrA^A from the other cells. K_{imp} being the import fraction of pheromones from the environment, the amount of pheromones present inside the cell is $p_A = K_{imp}p_A^e + fK_{exp}g$.

The aforementioned RapA-PhrA circuit represents a typical example of Rap proteins whose transcription is induced only by ComA*. Other Rap proteins, like RapC shown in figure S7, present more complex computational elements. In this case, in addition to the co-transcription of the Rap protein and its associated pheromone by ComA*, the transcription of the pheromone can be further enhanced as the cell progresses towards sporulation. The

pheromone has an additional promoter that can be activated by the sigma factor σ^H induced by Spo0A*. Hence, the RapC-PhrC circuit integrates two internal input signals (the levels of ComA* and Spo0A*) to set the output broadcasted signal - the rate of pheromone secretion. The model of the RapC-PhrC unit is similar to that of that of the RapA-PhrA unit with the additional effect of the transcription regulation of PhrC by σ^H. The pheromone precursor synthesis rate is $g + g_\sigma$, accounting for the promoter activated by σ^H. The amount of pheromone in the environment is $p_C^e = K_e(1-f)(g+g_\sigma) + p_C^{diff}$, and the pheromone concentration in the cell is $p_C = K_{imp}p_C^e + fK_{exp}(g+g_\sigma)$.

While the RapA-PhrA comparator regulates the sporulation module by dephosphorylation of Spo0F*, the RapC-PhrC gated comparator regulates the competence modules by inactivation of ComA. PhrC also regulates the sporulation module via inhibition of RapB, as shown in figure S6. Consequently, the pentapeptide PhrC is especially important in sporulation and competence control. High concentrations of this pentapeptide, which is widely termed competence and sporulation factor (CSF), promotes both competence and sporulation by protecting ComA* from the inhibiting effect of RapC and Spo0F* from the inhibiting effect of RapB.

5 The AbrB, Rok and SinI/SinR repressors

The AbrB and Rok proteins are two essential elements of the AbrB-Rok decision module shown in figure S8. As is explained in the main article, both

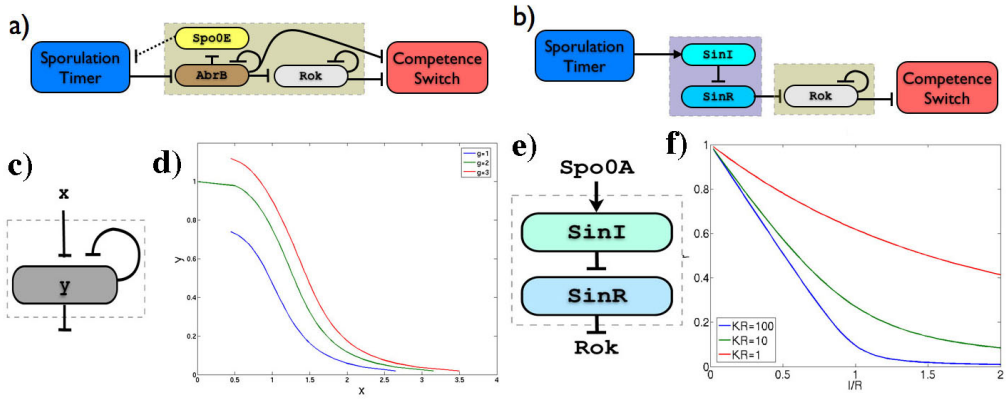

Figure S8: **(a)** Schematic description of the AbrB-Rok decision module and **(b)** the SinI-SinR final commitment circuit, described in the text. **(c)** The typical characteristics of AbrB and Rok, with a schematic circuit of an inhibited self-repressor gene. We note thyat the negative feedback loop prevents the level of the protein from being too high. **(d)** Curve showing the repression for different values of the synthesis rate g. **(e)** The Sin regulation, with a schematic description of the Sin circuit and its function, which is based on cascade of inhibitions - SinI binds to and inactivates SinR which in turns inhibits Rok. **(f)** Curve showing the fraction of SinR which is active as a function of the total concentration of SinI I in relation to the total concentration of SinR R. For high values of the binding constant the repression is linear until $I \approx R$.

act as transcription inhibitors of ComK and thus allow competence transitions only for specific values of Spo0A*. They are also both self-inhibitory proteins which are also inhibited by other genes: Spo0A* inhibits AbrB and AbrB inhibits Rok.

The transition state regulator AbrB is an unstable protein involved in the repression of a wide variety of genes [33, 34]. AbrB is repressed by the sporulation master regulator Spo0A* and also by itself, in a negative feedback loop that prevents overexpression. Due to the instability of the protein,

AbrB concentration responds quickly to transcriptional repression, dropping its levels quickly in the presence of Spo0A*. This drop derepresses ComK and ComS, allowing competence to take place. Lower AbrB concentrations also allow the expression of Spo0E, a dephosphatase that acts directly in Spo0A*, slowing down its accumulation. Further repression of AbrB by Spo0A* can have a negative effect on competence, since minimal levels of AbrB are still important in the repression of competence downregulator Rok.

Rok is a protein that acts as a competence downregulator by transcriptionally repressing the ComK promoter [35]. Rok also binds to its own promoter and represses its own transcription, in a negative feedback loop preventing its overexpression. Rok is repressed by AbrB, SinR and ComK (figure S8), integrating these signals into competence regulation. Absence of AbrB and SinR, due to high levels of Spo0A*, cause a rise in Rok concentration that will prevent ComK from reaching the threshold for self-activation. High levels of ComK also repress Rok, functioning indirectly as a second positive feedback loop of ComK.

5.1 An inhibited self-inhibitory gene

In figure S8 we show a general circuit and the gate characteristics of a generic circuit of an inhibited gene that is also self-inhibitory [36]. In other words a circuit that corresponds to both AbrB and Rok. Considering a self-repressor y subject to repression by a signal x, both signals normalized by their half-repression concentration, with a normalized synthesis rate g and the cooper-

ativities of the repressions, n_y and n_x, we have the kinetic rate equation:

$$\frac{dy(t)}{dt} = g\left(\frac{1}{1+x^{n_x}}\right)\left(\frac{1}{1+y^{n_y}}\right) - y \tag{12}$$

Typical n equilibrium characteristics of the circuit is shown in figure S**??**, in which we plot the response function y to the signal x for different values of the maximum synthesis rate g. We see that even for much higher values of g the gene is not overexpressed.

5.2 The SinI-SinR circuit

SinR is a pleiotropic regulator involved in several late growth processes in *B. subtilis*. It acts positively in competence through the repression of competence downregulator Rok. Higher levels of Spo0A* induce the production of SinI, a SinR antagonist. These two proteins bind to each other forming an inactive complex SinI::SinR, allowing Rok to repress competence master regulator ComK. SinR is an essential gene for the development of competence, and its inactivation at higher levels of Spo0A* closes the time window in which competence can happen.

Consider the equilibrium $i + r \overset{K}{\rightleftarrows} i :: r$ with constant K. Let R and r be the total concentration of SinR and the fraction of R which is free. I being the total concentration of SinI, we have the response function as the solution of:

$$KRr^2 - [K(R - I) - 1]r - 1 = 0 \tag{13}$$

Typical simulation results are shown in figure S8. By plotting the response function, we can see that if the affinity of SinI and SinR is high enough, the total concentration of SinI has to drop below the total concentration of SinR for derepression. After crossing this threshold, the derepression is linear.

6 Spo0A*-AbrB-Spo0E decision repressilator

The repressilator is a well studied network motif consisting of three genes that repress each other in sequence and in a loop - A represses B, B represses C, C represses A [37]. This circuit, when implemented experimentally in a cell, showed oscillatory behavior. We have noticed that the circuit formed by Spo0A*, AbrB and Spo0E (figure S10) is a special variant of the repressilator motif, where one of the repressions is a dephosphorylation, rather than a transcriptional repression, and two of the components show regulatory feedback loops, one positive and one negative. Spo0A receives a flux of phosphate at a certain rate. The phosphorylated Spo0A* transcriptionally represses AbrB. Lowering the levels of AbrB causes the derepression of dephosphatase Spo0E, while higher levels of Spo0E dephosphorylates Spo0A* [38]. AbrB shows a negative feedback loop repressing its own transcription, while Spo0A* activates its own transcription indirectly through σ^H.

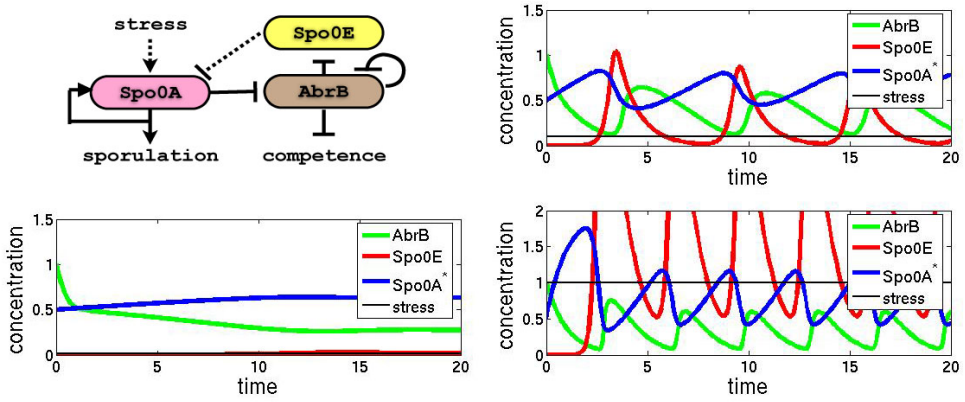

Figure S9: Scheme showing the repressilator-like circuit and concentrations of the repressilator components in time for different levels of constant stress. System can show oscillations with a constant signal, and different levels of stress can produce different frequencies in the response. Parameters: $m = 6, g_b = 100000, b_{0b} = 0.5, b_{0e} = 0.075, g_e = 50, a_0 = 0.075$.

Let s be the flux of phosphate into Spo0A normalized by the Spo0E dephosphorylation rate, a, b and e be the concentrations of Spo0A*, AbrB and Spo0E normalized by their binding constants, A be the total concentration of Spo0A and g_a, g_b and g_e their synthesis rates normalized by their degradation rates. The following equations can capture the general behavior of the system:

$$\begin{aligned} \frac{da}{dt} &= s(A-a) - ea \\ \frac{db}{dt} &= g_b \left(\frac{a_0^n}{a_0^n + a^n} \right) \left(\frac{b_{0b}^m}{b_{0b}^m + b^m} \right) - b \\ \frac{de}{dt} &= g_e \left(\frac{b_{0e}^m}{b_{0e}^m + b^m} \right) - e \end{aligned} \tag{14}$$

The total concentration of Spo0A A is subject to indirect regulation by Spo0A*. If this regulation happens at the same timescale as the other reactions considered here, we can model this regulation by a similar Hill function $\frac{dA}{dt} = g_a \left(\frac{a^n}{a_0^n + a^n} \right) - A$. In figure S10 we see the the concentrations of the proteins varying in time for different levels of input phosphate. The system can show oscillations for a signal with no noise, and can show great variations in frequency for different levels of input. Since the levels of Spo0A* and AbrB regulate the decision and final commitment we refer to the Spo0A*-AbrB-Spo0E as the decision repressilator, which can be responsible for phenotypical variation [39, 40, 41, 42, 43].

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Figure S10: Scheme showing the repressilator-like circuit and concentrations of the repressilator components in time for different levels of constant stress. System can show oscillations with a constant signal, and different levels of stress can produce different frequencies in the response. Parameters: $m = 6, g_b = 100000, b_{0b} = 0.5, b_{0e} = 0.075, g_e = 50, a_0 = 0.075$.

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