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Supporting Material

Kinetics of genetic switching into the state of bacterial competence

Madeleine Leisner, Jan-Timm Kuhr, Joachim O. Rädler, Erwin Frey, and Berenike Maier

Supplementary information for

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Supplemental Text

Stochastic model and simulations

If molecular fluctuations are neglected, initial conditions determine the dynamics of the model and each cell develops towards one of the stable fixed points. Since numbers of M and K are low initially, a deterministic numerical simulation would end up with 0% cells in the K-state. To explain the heterogeneity of a cell culture molecular fluctuations are thus of prime importance.

To evaluate our model including stochastic fluctuations we implemented Gillespie's stochastic algorithm (14). All possible reactions and the corresponding transition rates are given in Supplemental Table 3.

To render our model more realistic, we further allowed parameter values to vary to incorporate cell-to-cell variability (size, variable number of cellular machinery, etc.), i.e. we introduced extrinsic noise. For each realization of the simulation every parameter was chosen out of a Gaussian distribution about its mean with a standard deviation of 5% of its mean, and then held constant for that run (γ_K was not varied, since its value is set by the topology of the model). The magnitude of variation in the parameters was chosen to lie in a realistic range, since no quantitative data on this subject could be obtained. The qualitative and quantitative influence of extrinsic noise can be investigated by stability and sensitivity analysis, respectively (see below). In each run the evolution of an individual cell was simulated for 6 hours.

The influence of the cell population as a whole is incorporated via S(t). Information on cell density and nutrient supply is integrated in this function by quorum sensing. For simplicity we assumed that there is a linear relation between cell density (which rises sigmoidal with time) and S(t). Thus a sigmoidal function as given by a Hill-function is appropriate. We empirically chose

$$S(t) = \frac{1500}{1 + \left(\frac{0.5h}{t}\right)^3} \qquad \textbf{(1)}$$

as shown in Supplemental Fig. 1. Realizations of our simulations are shown as ComK vs. time in Fig. 5a,b and in M-K phase space in Supplemental Fig. 2. In the former, one can see that saturation is achieved for most cells and that a band of saturation values is found, similarly to that of Fig. 2c.

We evaluated 1000 simulations and found that in 14.7% of all runs the K-state was reached, reproducing the known literature value. The K-state was defined as K > 900 at the end of the simulation period.

We also extracted the switching periods for our simulations. To this purpose we normalized the

timecourses of all cells that entered K-state to their maximal value and then shifted the time axis to overlay them at half maximal time (Fig. 5c). The temporal derivative of the mean of these curves was, in analogy with the experimental data, taken to obtain the switching rate. The switching period was defined as the time interval, where this rate was at least 0.1 of its maximal value. We found a mean switching period of 1.2h, in good accordance with experiment.

Motivation of reaction rates

Rates of the distinct chemical processes, as given in the terms of equations (1) and (2) of the main text are either well motivated from literature or generic representations of the molecular processes. Michaelis-Menten degradation of ComK is the primary mechanism which keeps protein numbers low in the vegetative state. Linear degradation, which is not catalyzed by protease complexes, is much slower (5), but establishes an equilibrium between degradation and production of ComK at high levels. Recent studies have postulated an inhibition of the *comS* promoter by high ComK concentrations on long timescales (6-8). This would lead to S becoming a function of not only t but also of K. Taylor expansion of this function S(K,t) in K then recovers to first order linear degradation.

Autocatalytic feedback in *comK* transcription is modeled by a Hill-function which is the generic function if formation of oligomers and promoter binding/unbinding are fast. It is known that four ComK proteins can bind to the *comK* promoter cooperatively (9), which gives $\gamma_K = 4$.

The small basal transcription rate is attributed to *comK* promoter leaking (10). The two linear terms for ComK production and mRNA degradation are the generic choices for these elementary processes. For parameter values of the model see Supplemental Table 2.

Rise of ComS facilitates activation of auto-feedback in two distinct manners.

Degradation of ComK follows Michaelis-Menten kinetics, catalyzed by the MecA/ClpC/ClpP protease complex (D). The reaction scheme is

$$K_f + D_f \underset{\lambda_{-1}}{\overset{\lambda_1}{\leftrightarrow}} [KD] \xrightarrow{\lambda_2} D_f \qquad \textbf{(2)}$$

where K_f, D_f, and [KD] refer to free ComK, free protease complex, and ComK bound to the protease complex, respectively. Reactions λ_1 and λ_{-1} are fast in comparison with the actual degradation λ_2.

Cells respond to high density by increasing ComS concentration which couples into the competence decision network ((9), (Fig. 1)). ComS is a small peptide that is degraded by the MecA/ClpC/ClpP protease complex (11). Protease complexes are thus partially occupied by ComS and degradation of ComK is slowed down. Furthermore K_f is increased in the presence of S and thus more K is available

for dimer formation and binding to the *comK* promoter, which has a prominent effect near the lower fixed point and threshold (Supplemental Fig. 3). Since degradation of ComS and ComK happens through the same processes (9) we assume identical rates,

$$S_f + D_f \underset{\lambda_{-1}}{\overset{\lambda_1}{\leftrightarrow}} [SD] \overset{\lambda_2}{\rightarrow} D_f \qquad \textbf{(3)}$$

Neither absolute affinities of ComK nor ComS towards the protease complex nor total ComS numbers are known. But since we are interested in the dynamics of K, only the number of protease complexes currently occupied by S is of importance. In this sense S is understood as an effective ComS number which gives, when assuming identical affinities towards the protease complex as for ComK, the right number of occupied MecA/ClpC/ClpP binding sites.

By the law of mass action one finds

$$K_f(K,S) = \frac{1}{2}\frac{K}{K+S}\left(K+S-D-\frac{\lambda_1}{\lambda_{-1}} + \sqrt{\left(K+S-D-\frac{\lambda_1}{\lambda_{-1}}\right)^2 + 4\frac{\lambda_1}{\lambda_{-1}}(K+S)}\right) \qquad \textbf{(4)}$$

which appears in eqn. (2) of the main text. Here particle conservation, i.e. $K = K_f + [KD]$, $S = S_f + [SD]$, and $D = D_f + [KD] + [SD]$, was used.

S as a function of time was set empirically as an external control parameter (see above).

Parameters of the model

Our model includes ten parameters (Supplemental Table 2) and the external control functions S(t) and $\alpha_M(t)$ (Supplemental Figure 1). All parameters were chosen consistently with results of earlier studies. δ_K, D and β_K/δ_M, could be obtained directly from literature. Further constraints were $M \approx 1$ in the vegetative state in stationary phase (12), a switching threshold of a few hundred ComK proteins and a saturation value of 10^4-10^5 proteins in the K-state (5).

In earlier studies (3) we found that basal expression increases about an hour before entrance to stationary phase and that after a 'switching-window' the percentage of competent cells stay constant. We incorporated that into the model by reducing α_M by a factor of r outside of that window in all simulations. $r = 0.5$ was chosen small enough so that switching was very rare for the reduced basal transcription rate $r \cdot \alpha_M$ (Supplemental Figure 1).

The rates of the degradation reaction λ_1, λ_{-1}, and λ_2 have not been addressed experimentally. However, this is not a drawback to the model, since λ_2 is incorporated in δ_K (Michaelis-Menten theory). λ_1 and λ_{-1} appeared explicitly in $K_f(K,S)$ and since both rates are fast, only their ratio is of interest. Varying λ_1/λ_{-1} has only minor influence on the model dynamics.

Sensitivity and Stability

The broad distribution of saturation levels was attributed to variability in the parameters of the model, known as extrinsic noise (13). To quantify its influence on the saturation value of ComK one can define the sensitivity $\Sigma(\mu)$ with respect to a given parameter μ as the relative change $\Delta K/K^*$ of the upper fixed point $K^*(\mu)$ due to a relative change $\Delta\mu/\mu$ in that parameter (Supplemental Table 2). An analytical expression is given by

$$\Sigma(\mu) = \frac{\Delta K / K^*}{\Delta \mu / \mu_0} = \frac{\mu_0}{K^*} \left. \frac{\partial K^*}{\partial \mu} \right|_{\mu=\mu_0} \qquad \textbf{(5)}$$

as follows by Taylor expansion up to linear order of the fixed point's position.

If parameters of the model are varied strongly the qualitative features of stationary states can change (bifurcation). This may for example be the case for non-wild type strains, for which the competence circuit (Fig. 1) is altered. Numerically we can compute the number and kind of fixed points of our model and thereby analyze its possible dynamics. An example is given in Fig. 4b, which also explains deterministic switching for the *rok*- strain. Note that the wild type parameters have to be set close to the bifurcation in which the lower stable and the instable fixed point annihilate to admit stochastically induced crossing of threshold.

Supplementary Tables

Strain	Genotype	Source
BD 630	*his leu met*	-
BD 2955	*his leu met rok- (spc[a]),*	(1)
BD 2711	*his leu met comK-gfp (CBL[b], cat[a])*	(2)
BM 101	*his leu met rok- (spc[a]), comK-gfp (CBL[b], cat[a])*	this study
BM 77	*his leu met comK-gfp (CBL[b], cat[a]), multicopy comS (kan[a])*	(3)
BM 2528	*his leu met, multicopy comS (kan[a])*	(4)

Supplemental Tab. 1. *B. subtilis* strains used in this study.
[a] kan, cat and spc stand for resistance to kanamycin, chloramphenicol and spectinomycin respectively.
[b] Inserted by Campell like integration.

Parameter	Significance	Mean value	Motivation/ Explanation	Sensitivity of upper fixed point Σ
β_K	Translation rate per mRNA	1 1/s	burst factor: $\beta_K/\delta_M \approx 50$; (12)	3.31
δ_K	Maximal Michaelis-Menten degradation rate of K	11.3 1/s	≈ 11.5 1/s; (5) Fig. 1A therein	-2.98
δ_0	Linear degradation rate of K	$1.5 \cdot 10^{-4}$ 1/s	Ration of saturation of *rok*- and wild type	-0.34
q_K	(K+S) at half-maximal enzymatic degradation	400	<9000; (5) Fig. 1A therein; sets position of fixed points	0.14
α_M	Minimal transcription rate	0.025 1/s	M ≈ 1 before switching; (12)	0.39
β_M	Maximal additional transcription rate by feedback	0.19 1/s	position of upper fixed points/ saturation	2.93
p_K	Half-maximal feedback	600	position of switching threshold	negligible
γ_K	Cooperativity/Hill Coefficient	4	four K proteins bind to the promoter cooperatively (9)	negligible
δ_M	Degradation rate per mRNA	0.022 1/s	burst factor: $\beta_K/\delta_M \approx 50$ and M ≈ 1 before switching; (12)	-3.31
D	Number of protease complexes	700	(5)	negligible
λ_1/λ_{-1}	Equilibrium constant of degradation reaction	1	variation of this parameter are of minor influence only	negligible

Supplemental Tab. 2. Parameters of the model. Besides the constraints given in the column motivation/explanation, parameters were chosen to be in line with our experimental findings.

Description	Reaction	Transition rate
protein production	$K \rightarrow K+1$	$\beta_K M$
protein degradation	$K \rightarrow K-1$	$\frac{\delta_K K}{q_K + S + K} - \delta_0 K$
mRNA production	$M \rightarrow M+1$	$\alpha_M + \frac{\beta_M}{1+(p_K / K_f)^{\gamma_K}}$
mRNA degradation	$M \rightarrow M-1$	$\delta_M M$

Supplemental Tab. 3. Reactions of the stochastic model. Processes and their corresponding transition rates are given. Note that the rates depend on the number of K and M. These transition rates are used in the stochastic simulations.

Supplementary Figure Captions

Supplemental Fig. 1. Empirically set development of the number of ComS molecules S(t) used for the simulations and of the basal transcription rate $\alpha_M(t)$. The latter gives rise to a window of opportunity.

Supplemental Fig. 2. Fluctuations in vegetative state and crossing of switching threshold. The lower left part of Figures 4a,b of the main text are shown. For a typical realization (black line) switching occurs after a long period of fluctuations about the lower fixed point. Note that only discrete molecule numbers are taken, which can be seen very well for the number of mRNAs. Nullclines (Blue: dM/dt = 0; red: dK/dt = 0) are plotted for S = 0 (dotted lines) and S = 1500 (straight lines).

Supplemental Fig. 3. Influence of [ComS] on the fraction of free ComK. ComS binds competitively to the MecA/ClpC/ClpP protease complex, thereby increasing the amount of free ComK (K_F), see eqn. (4) of Supplemental text. Dashed line: S = 0, straight line: S = 1500. Near the lower stable and unstable fixed points (K ≈ 200 and K ≈ 600, respectively) this has an important effect on free ComK concentration.

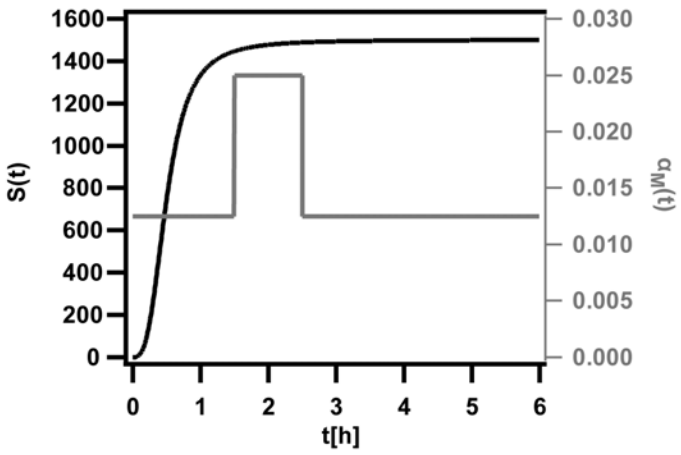
Supplemental Fig. 4. Comparison of switching kinetics between bulk and single cell measurements to confirm equal development under microscopic conditions and in liquid culture. Fraction of cells in the K-state. Black: cells grown in Erlenmeyer flask, grey: cells grown on a microscope slide. a) wt, b) *rok*-. Black and grey lines: best fit to a sigmoidal function.

Supplemental Fig. 5. Switching kinetics of individual *rok*- cells (BM101). a) Time course of fluorescence intensity of cells switching into the K-state. b) The fluorescence of 50 individual cells was normalized to the cumulative expression (maximum fluorescence intensity). The time axis was shifted by $\tau_{1/2}$, where cells had half maximum fluorescence intensity.

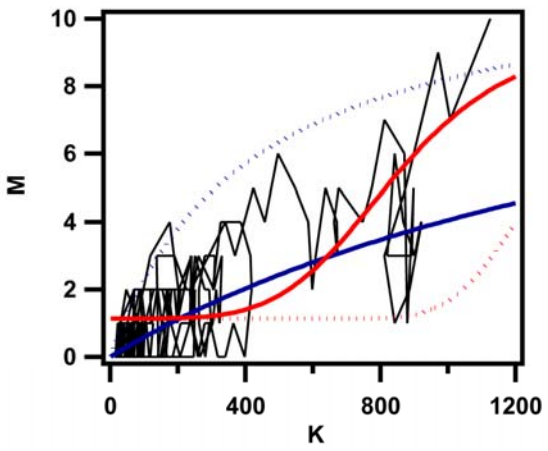
Supplemental Fig. 6. Switching kinetics of individual ComS overproducing cells (BM77). a) Time course of fluorescence intensity of cells switching into the K-state. b) The fluorescence of 50 individual cells was normalized to the cumulative expression (maximum fluorescence intensity). The time axis was shifted by $\tau_{1/2}$, where cells had half maximum fluorescence intensity.

Supplemental Fig. 7. Switching period is independent of growth phase. a) Histogram of switching

period ρ. Red line: best fit to a Gaussian function with $\langle\rho\rangle = 1.44 \pm 0.02$ and a width of 0.33 ± 0.02. b) Switching period ρ as a function of growth phase *T*.



Supplemental Fig. 1



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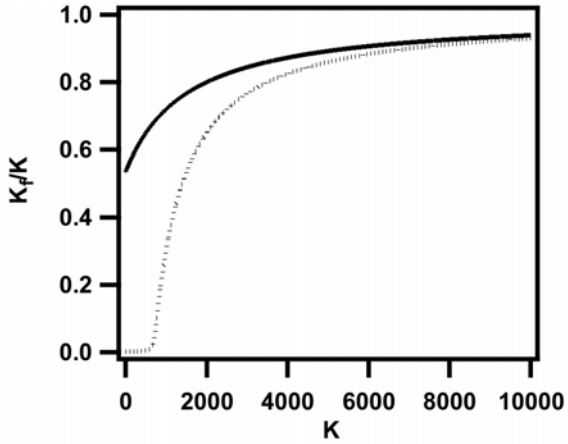
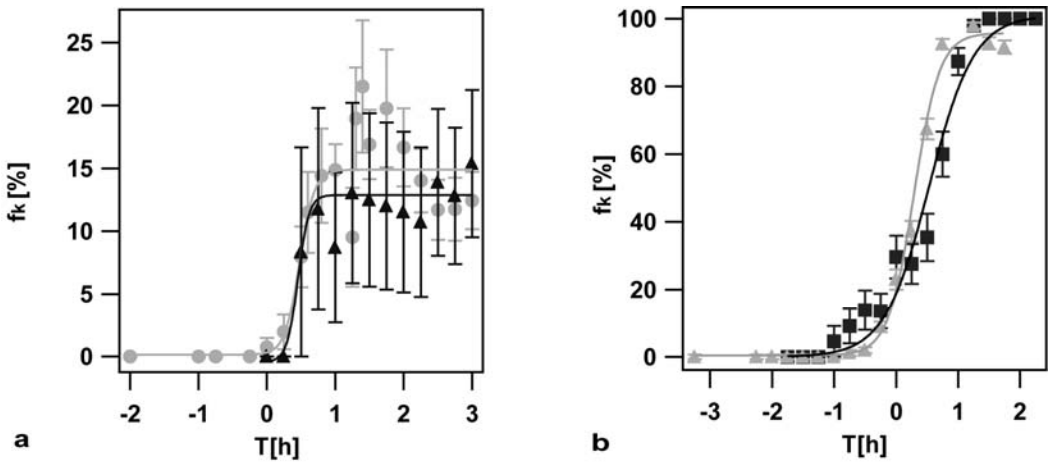
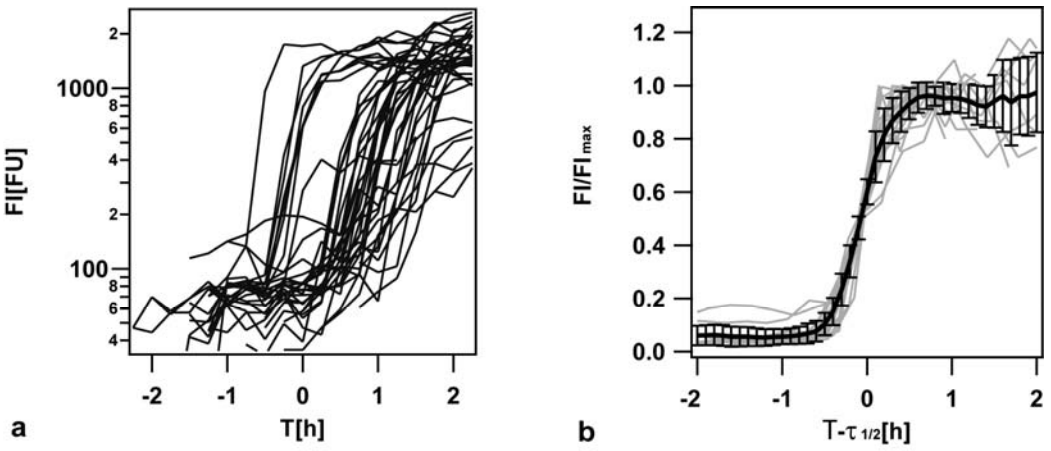


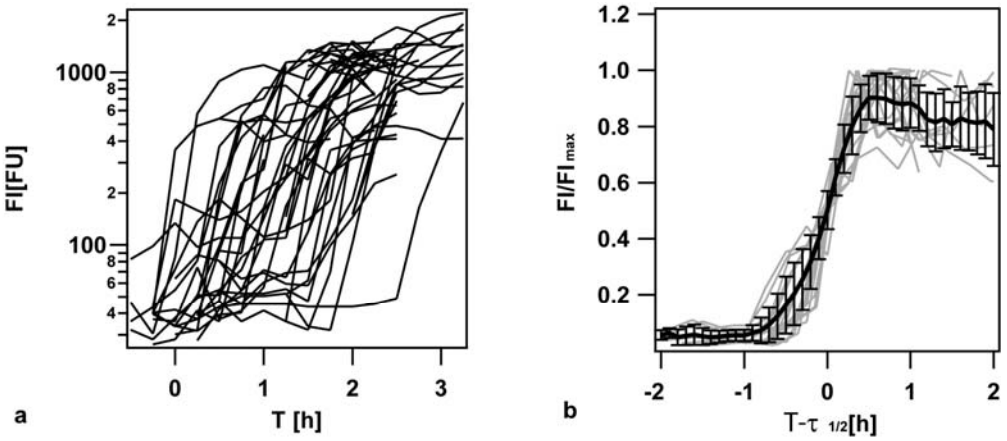
Supplemental Fig. 3



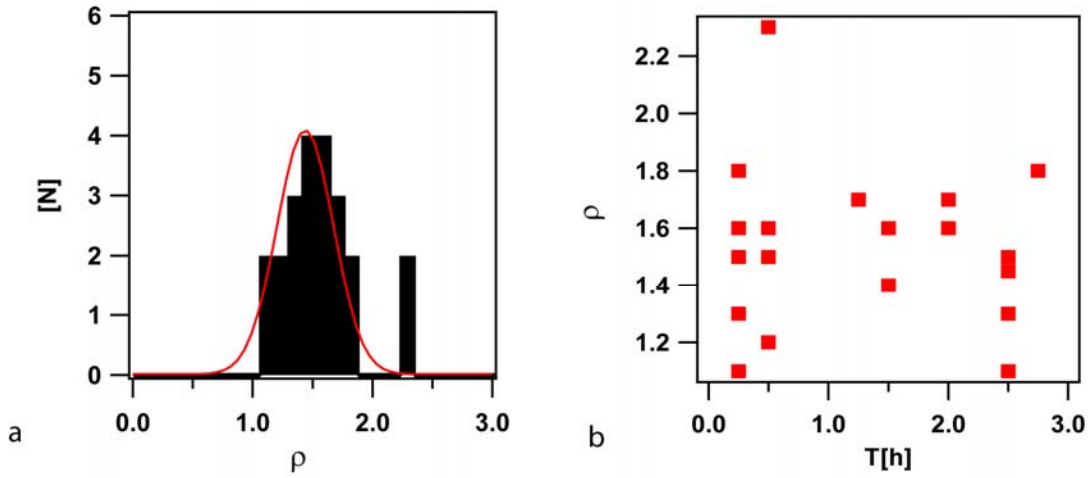
Supplemental Fig.4



Supplemental Fig. 5



Supplemental Fig. 6



Supplemental Fig. 7

Supplemental References

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