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Description of the three parts of the simulation

A simplified description of the MP assembly mechanism (digitization module). This module requires three parameters that describe the relative differences between SPBs. It generates a digital output (zero to four) based on one input, which is the amount of expressed MP components. This module allows digitization of a graded input (amounts of MP components). An example is given in Fig. 6 C.

A description of how acetate regulates the amounts of MP components. We assumed that the shape of this function (called the acetate → protein function; Fig. 6 D) is approximated by the relationship of acetate → sporulation efficiency (Fig. 6 B). We assume that the amplitude of this function follows a Gaussian distribution to consider individual responses to acetate. This module requires two parameters that describe this distribution.

A description that considers heterogeneities present in the population of sporulating cells that cause individual cells to perform meiosis with different speeds (Fig. 1). Because this directly affects the uptake of acetate, this description was solely used to convolve the output of the first module. We assumed that heterogeneities follow a symmetric Gaussian distribution with a constant width. As a result of this assumption, no additional parameters needed to be introduced into the simulation.

Deterministic simulation of this process, in combination with descriptions for the translation of the stimulus (acetate) into the production of MP components, enabled us to obtain a mathematical model for SNC on the level of populations. Because their behavior can be measured precisely on large numbers of cells, we were able to calibrate the model using wild-type cells. We demonstrated the value of the simulation to predict and understand mutant behavior.

The simulation is based on the idea that the starting situation on each SPB is different. This was implemented by simply assuming different start sizes for crystals. Feedback provided by size generates a strong amplification of these differences and leads to a digital outcome. This digitization module needs three parameters that describe the relative initial crystal sizes. In the simulation, input into the digitization module is generated by descriptions for how much acetate each individual cell is able to take up and how this is converted into MP components. Experimental measurement of this correlation is not feasible on a precise quantitative level, as the different timing of MP component production and MP assembly in different cells in a culture makes it impossible to obtain samples that reflect exactly how many MP components are produced. The method we chose to compare produced protein amounts under different acetate concentrations (Fig. 2 C) partially accounts for these limitations. Thus, it enables the qualitative conclusion that the produced amounts of MP components depend on acetate. To solve this problem for the simulation, we assumed that the sporulation efficiency must be a valid approximation for the correlation between acetate and amounts of MP components produced in the wild type (Fig. 6, B and D). To simulate populations, we assumed that this acetate → protein function is valid for all cells but that the amplitude of the function is subject to cellular variations. We assumed that a Gaussian distribution could approximate this. It describes the distribution of the amplitude of acetate → protein functions for all of the cells in a population. The two values of this distribution (G_w and a_{av}), in addition to the three parameters from the digitization module, were the only other parameters that were entirely open. Proper adjustment of these five parameters yielded the sporulation profile of the wild-type strain. The fact that we were able to use this simulation to generate profiles of the gene dosage mutants by only changing the two parameters that describe the amplitude of the acetate → protein function validated the simulation. The obtained values for G_w and a_{av} for these mutants can thus be seen as a result obtained from the simulation. Both values (Fig. 6 D) correlate with the gene dosage. The values for a_{av} indeed suggest increasing amounts of expressed protein according to the gene dosage. One plausible explanation for the proportional increase of G_w could be that this behavior reflects gene noise (Elowitz et al., 2002; Blake et al., 2003) that accumulates with increasing amounts of copies for the MP component genes. The simulation is validated by the fact that it was not necessary to adjust the digitization module. This provides strong support that the SPBs provide constitutive functionality toward regulation of MP assembly, which rules out the previously proposed model that they are selectively regulated in dependency on external acetate (Nickas et al., 2004). For the simulation of the *Δady2* mutants, the shape of the acetate → protein function and a_{av} needed to be changed, which is in contrast to the gene dosage mutants, but the values for G_w still correlated with the gene dosage (Fig. 6 E). This means that Ady2p solely functions in the pathway that regulates MP component abundance in response to acetate and is not involved in the regulation of other processes that might affect the functioning of the digitization module.

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