Formation and maintenance of nitrogen-fixing cell patterns in filamentous cyanobacteria

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Cyanobacteria forming one-dimensional filaments are paradigmatic model organisms of the transition between unicellular and multicellular living forms. Under nitrogen-limiting conditions, in filaments of the genus *Anabaena*, some cells differentiate into heterocysts, which lose the possibility to divide but are able to fix environmental nitrogen for the colony. These heterocysts form a quasiregular pattern in the filament, representing a prototype of patterning and morphogenesis in prokaryotes. Recent years have seen advances in the identification of the molecular mechanism regulating this pattern. We use these data to build a theory on heterocyst pattern formation, for which both genetic regulation and the effects of cell division and filament growth are key components. The theory is based on the interplay of three generic mechanisms: local autoactivation, early long-range inhibition, and late long-range inhibition. These mechanisms can be identified with the dynamics of *hetR*, *patS*, and *hetN* expression. Our theory reproduces quantitatively the experimental dynamics of pattern formation and maintenance for wild type and mutants. We find that *hetN* alone is not enough to play the role as the late inhibitory mechanism: a second mechanism, hypothetically the products of nitrogen fixation supplied by heterocysts, must also play a role in late long-range inhibition. The preponderance of even intervals between heterocysts arises naturally as a result of the interplay between the timescales of genetic regulation and cell division. We also find that a purely stochastic initiation of the pattern, without a two-stage process, is enough to reproduce experimental observations.

cyanobacteria | pattern formation | activator–inhibitor | heterocyst differentiation | gene-regulatory networks

Cyanobacteria were pioneer organisms to use oxygenic photosynthesis and are currently one of the most successful living groups, occupying a broad range of habitats across all latitudes and producing a large fraction of Earth's photosynthetic activity. Some types of cyanobacteria form colonies consisting of one-dimensional filaments of vegetative cells carrying photosynthesis. However, as a response to different environmental stresses, vegetative cells can differentiate into specialized cell types that perform important functions for the survival of the colony. This is a paradigmatic example of a prokaryotic form of life with differentiated cell types.

Bacteria and archaea are the only forms of life able to fix atmospheric nitrogen, making them crucial for all living forms on Earth. N_2 fixation is catalyzed by nitrogenase, and this enzyme is easily degraded by oxygen. Some filamentous cyanobacteria have developed a mechanism to protect nitrogenase from the oxygen produced by vegetative cells. When external nitrogen sources are scarce, specialized cells called heterocysts appear in a quasiregular pattern, with intervals of around 10 vegetative cells between consecutive heterocysts. Because cells can exchange metabolites and small peptides (1–6), the fixed nitrogen produced by heterocysts can reach vegetative cells. N_2 fixation requires high energy consumption. To maintain it, nutrients produced by photosynthesis in vegetative cells are shared with heterocysts (4, 7). Upon differentiation, heterocysts lose the possibility to undergo cell division. However, vegetative cells continue dividing, producing filament growth and increasing the distance between consecutive heterocysts. As a result, new heterocysts differentiate roughly in the middle of the intervals between previously existing heterocysts. This dynamic process of differentiation allows the overall pattern to conserve its properties over time.

The biology of heterocyst formation has been the subject of intensive work (4, 8). Most studies focus on the strain PCC 7120 of the genus *Anabaena*, which has become a model organism in the field. Recent quantitative experimental work has produced a wealth of data on vegetative cell intervals between heterocysts under a number of mutations and experimental conditions. Moreover, the one-dimensional nature of this pattern-forming system represents a very appealing system for theoretical and mathematical modeling (9–20).

Despite these efforts, many processes and genetic mechanisms involved in the regulation of heterocyst differentiation, pattern formation, and maintenance remain poorly understood. For example, it has not been clarified which particular vegetative cells differentiate into heterocysts and if this is related to some inherited predisposition (21, 22). Other open questions are whether the differentiating cells are selected stochastically, how the typical spacing between heterocysts is determined for cells differentiating at early and late times, or which mechanisms induce the appearance of multiple contiguous heterocysts, the so-called Mch phenotype (23), in some mutants.

Basic Genetics of Heterocyst Differentiation

There is a large number of processes involved in the regulation of heterocyst pattern formation. In addition to nitrogen levels and other environmental aspects, many genes and factors play a role (24). When nitrogen stress is perceived, the transcriptional regulator *ntcA* is important to trigger heterocyst differentiation (25, 26), directly or indirectly controlling the expression of several genes (27, 28), including *hetR*. The gene *hetR* is central to heterocyst differentiation. Its expression is the main positive regulatory factor in heterocyst development (29–32). The expressions

Significance

Cyanobacteria produce an important fraction of oxygen on Earth and, together with archaea, fix atmospheric nitrogen used by all other organisms. Some types live in colonies with specialized cells that perform different functions. In particular, the genus *Anabaena* forms filaments in which some cells differentiate, forming patterns to effectively provide nitrogen for the colony. We present a theory that combines genetic, metabolic, and morphological aspects to understand this prokaryotic example of multicellularity. Our results quantitatively reproduce the appearance and dynamics of this pattern and are used to learn how different aspects, like fixed-nitrogen diffusion, cell division, or stochasticity, affect it.

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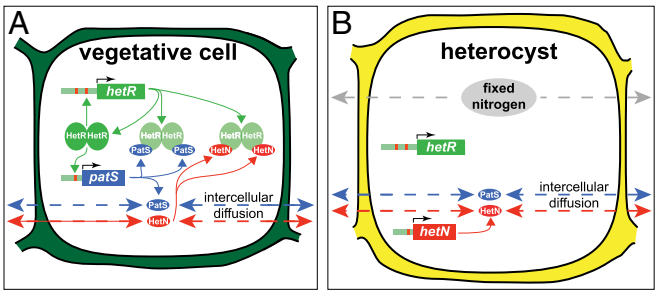

Fig. 1. Minimal model of the genetic network. (*A*) In vegetative cells, HetR dimers can activate the expression of *hetR* and *patS*. (*B*) In heterocysts, *hetN* is expressed constitutively and fixed-nitrogen products are produced. Active products of PatS and HetN, possibly the RGSGR pentapeptide, can diffuse between cells of any kind in the filament and bind HetR, preventing it from binding DNA. Fixed-nitrogen products also diffuse to other cells and contribute weakly to inhibit differentiation.

of *ntcA* and *hetR* are mutually dependent, and the latter seems to be necessary and sufficient for heterocyst development, even under conditions of excess of external nitrogen (33). Thus, positive autoregulation of *hetR* is required for differentiation and is particularly significant in developing heterocysts (30, 34). In addition to *ntcA*, other genes such as *patA*, *hetF*, and *hetP* also regulate heterocyst differentiation (35–38).

The gene *patS* is a negative regulator of *hetR* that suppresses differentiation when overexpressed and induces a Mch phenotype when deleted (23, 39, 40). PatS is a short peptide, predicted to be 13 or 17 aa, containing a carboxyl-terminal pentapeptide RGSGR that prevents DNA binding activity of HetR (41, 42) and inhibits differentiation when added to culture medium (23). The expression of *patS* in small groups of vegetative cells was shown to diminish the levels of HetR in adjacent cells (43), suggesting that a PatS-dependent signal can diffuse along the filament (44). It has been observed that *patS* is strongly expressed in developing heterocysts (23, 39), coming back to low levels after 24 h of nitrogen deprivation. Although lack of *patS* expression initially produces a pattern with frequent contiguous heterocysts and short intervals between separate ones, later this pattern tends to become more similar to a wild-type pattern (39), suggesting the presence of other patterning signals.

Thus, *hetR* and *patS* could be enough to obtain a minimalistic understanding of heterocyst pattern formation at early stages. However, other players have to be taken into account to explain pattern maintenance after an initial pattern of differentiated heterocysts appear. The key player for this role is the inhibitory factor *hetN*. Akin to PatS, HetN also contains a RGSGR motif, raising the possibility that a RGSGR-containing peptide derived from the full protein diffuses from cell to cell (45). In contrast to *patS* mutants, *hetN* mutants have a differentiation pattern similar to the wild type at the initial stages of nitrogen depletion and a Mch phenotype after 48 h (46), suggesting that *hetN* expression is activated later than that of *patS*. When both genes are suppressed, almost all cells along the filament eventually differentiate, causing lethal levels of heterocysts (47).

Metabolites have also been suggested to play a role in filament patterning, in particular the fixed-nitrogen products produced by heterocysts. It has been suggested that they inhibit heterocyst differentiation (39), although experiments with *Anabaena variabilis* (48, 49) did not find an observable effect.

Results

Minimal Model for the Heterocyst Differentiation Gene-Regulatory Network. Although the chemical and genetic regulation of heterocyst differentiation involves a great number of factors (4, 8, 24), we will focus on the core mechanisms involved: local autoactivation, early long-range inhibition, and late long-range inhibition. For concreteness, we will identify these generic mechanisms with the dynamics of three genes: *hetR*, *patS*, and *hetN*, and use what is known of their biochemistry to build a theoretical model. To first approximation, the effects of other genes like *ntcA* or *patA* could be included in a phenomenological way, as factors affecting the parameters. This approach will not capture all of the subtleties that the explicit inclusion of all possible effects would produce, but has the advantage of being generic, clear, and simple, allowing a more systematic analysis of the model.

Fig. 1 shows a diagram of the minimal genetic network considered in our theoretical description. Upon nitrogen deprivation, *hetR* is expressed, partly in a constitutive way (34). Its protein acts as a dimer (42, 50, 51), binding the *hetR* and *patS* promoter regions and activating expression. HetR has recently been observed also as a tetramer (52); including that information in our model would only change parameter values but not the dynamics of differentiation. A PatS product, the RGSGR pentapeptide, can bind HetR with a 1:1 stoichiometry, and RGSGR-tagged HetR molecules can no longer bind DNA (41, 53). Because HetN also contains the RGSGR motif, we assume that it binds and affects HetR analogously to PatS. PatS is only produced in vegetative cells, whereas HetN is exclusively produced in heterocysts. Because expression of HetR remains high in heterocysts (54), we model it through constitutive expression. We will show later that a second late inhibitor with fast diffusion and a weaker inhibition power than HetN is required to reproduce some experimental findings. A likely candidate for this inhibitor could be the effect of fixed-nitrogen products. Finally, we include intercellular diffusion of PatS, HetN, and fixed-nitrogen products. For simplicity, we do not represent RGSGR explicitly in our model, making instead its effect proportional to the concentration of PatS or HetN. Vegetative cells that maintain a threshold level of HetR during a predefined period are switched to heterocyst status.

In contrast to static patterns that are permanently defined once formed, the heterocyst pattern is a dynamic one: vegetative cells keep on growing and dividing, causing the intervals between contiguous heterocysts to continuously increase in size, until eventually a cell of the interval differentiates into a new heterocyst. Thus, we have included cell growth and division as a key ingredient of this model. Moreover, the stochastic nature of gene expression cannot be ignored, and especially the noise in the duration of the cell cycle can play a relevant role in defining the statistics of vegetative intervals between contiguous heterocysts. For this reason, noise should be included in a theoretical description, both in the genetic network and in cell division. The details of the mathematical formulation, analysis, and computer simulation of our model are described in *Supporting Information*.

From a physical perspective, the local (nondiffusive) character of the activator, the distinction between inhibitors acting at different times, and the dynamic character of the growing filament, are the features that set this problem apart from other reaction–diffusion pattern-forming systems.

The Theoretical Model Quantitatively Reproduces Wild-Type Patterns. A first feel of the properties of our model can be obtained by visual inspection of the patterns it produces (Fig. 2, Fig. S1, and

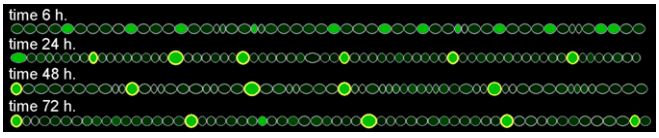

Fig. 2. Temporal evolution of a filament for the wild type. Heterocyst cells have a yellow membrane. The intensity of the green color shows the level of HetR concentration. Length of the cells represents its value in the simulation. See also Fig. S1 and Movie S1.

Movie S1). Cells with high levels of HetR appear early (55), frequently in clusters. Eventually only some isolated cells differentiate into heterocysts, whereas the others revert to a low HetR state. Once a heterocyst pattern is established, cell division increases the vegetative interval length between heterocysts. At some point, one or a few cells roughly in the middle of the interval increase their HetR levels, and, finally, just one vegetative cell differentiates into a new heterocyst, thus maintaining the quasiregularity of the pattern.

The properties of heterocyst patterns can be quantified making a histogram showing the relative frequency of vegetative intervals of each length. Changes in the shape of this histogram allow to characterize differences between different phenotypes, and also between different stages of filament growth after nitrogen deprivation. We have obtained these histograms and other statistical data averaging over 192 different filaments of 150 initial vegetative cells. The histograms in Fig. 3 are remarkably similar to experimental ones (23, 39, 43, 44, 47, 56–60), reproducing details such as the increase of the typical length of vegetative intervals with time, the gradual broadening of the shortest intervals observed on the filament, or the higher frequency of even intervals. The analysis can be pushed further from qualitative observation to quantitative characterization. This can be done using successive moments of the distributions given by the histograms: mean, variance, and skewness. The mean gives an estimation of the average interval length, and the variance, of the width of the intervals distribution. The skewness is a measure of the asymmetry of the distribution. Positive skewness indicates that the right tail of the distribution is heavier than the left tail, which is the situation generally found in experiments. We have compared the temporal evolution of these magnitudes in our simulations together with equivalent statistics extracted from experimental histograms in the literature (Fig. 4). A first observation for the wild type is that the mean interval length slightly increases from 10 cells at 24 h up to 12 cells after a few days.

In the following, we proceed to discuss other conditions. To start with, in experiments, all cells of *hetR* mutants remain in the vegetative state (61). In the same way, because HetR is necessary for differentiation in our model, no heterocysts form in that condition. The same is true for any double knockdown involving *hetR*. Overexpression of *hetR* induces differentiation of all of the cells in the filament.

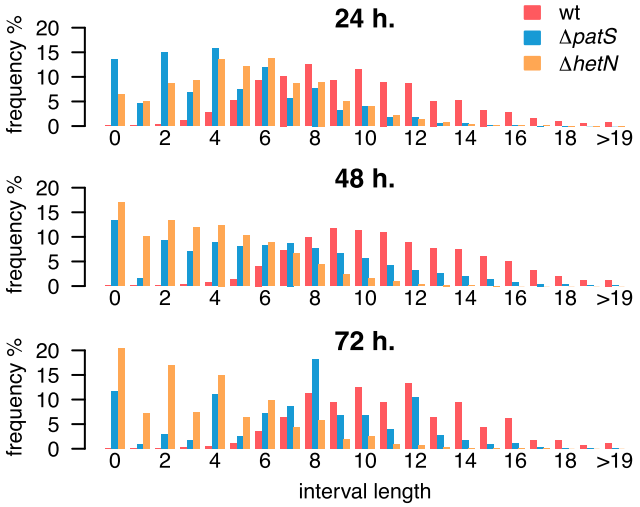

Fig. 3. Histograms of the number of vegetative cells between heterocysts. Conditions and times after nitrogen deprivation as indicated.

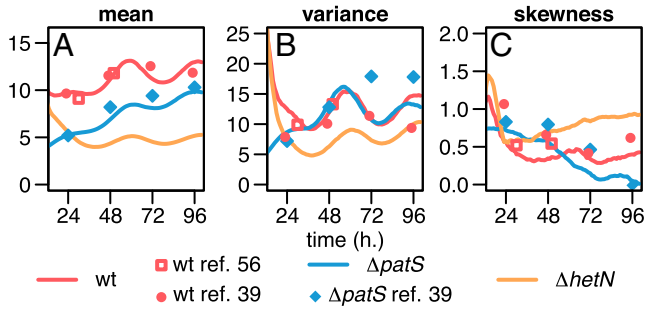

Fig. 4. Dynamics of heterocyst patterning. Time evolution of the (*A*) mean, (*B*) variance, and (*C*) skewness of the interval length distribution for our model (lines) and experimental results (symbols), as indicated. See Fig. S4*A* for a comparison of theory and experimental data on *hetN* loss of function.

PatS Promotes Early Pattern Formation. The early behavior of the *patS* mutant is quite different from the wild type (Fig. 5, Fig. S2, and Movie S2). Due to the lack of PatS, before differentiation there is no sequestration of HetR that can weaken its autoinducing feedback loop, so all cells build up high levels of HetR at short times. The first cells to differentiate start producing HetN that diffuses to their neighbors and prevents others from differentiating; however, an abnormally high number of cells, some of them contiguous, have already become heterocysts. This is the Mch phenotype observed in experiments (8, 23, 39, 47, 61). This high number of heterocysts inhibit differentiation until the vegetative interval between them becomes large enough. For this reason, at long times the Mch phenotype is attenuated and the pattern becomes more similar to wild type. Because new cells cannot appear between contiguous heterocysts, this phenotype persists as a hallmark of the irregular initiation of pattern formation in *patS* mutants. The histograms for the *patS* mutant (Fig. 3) show the predominance of contiguous heterocysts and short intervals at early times. Starting from mean intervals of five cells at 24 h, the mean converges to values similar to wild type after 4 d, as in experiments (Fig. 4*A*).

These results confirm that PatS is essential during the first stages of pattern induction to produce a regular pattern. At late stages, a regular pattern can be maintained even in the absence of PatS, pointing to the need of a subsequent inhibitor key for pattern maintenance. Continuing the study of the effect of PatS on filament development, we have also simulated PatS overexpression. As observed in experiments (23), we found a complete repression of heterocyst differentiation in the filament.

HetN Is Necessary, but Not Sufficient, for Pattern Maintenance: A Second Inhibitor, Probably Fixed-Nitrogen Products, Also Plays a Role. In the case of *hetN* loss of function, the presence of PatS assures a correct pattern formation until a first generation of heterocysts differentiates (Fig. 6, Fig. S3, and Movie S3). PatS produced in protoheterocysts inhibits the *hetR* feedback loop, but once those protoheterocysts differentiate into heterocysts, they cannot inhibit differentiation in their neighbors because they do not produce HetN. If the only mechanism of late long-range inhibition in the model is the *hetN* gene, in its absence, once a first pattern is established more and more cells continue to differentiate, until eventually the filament is almost entirely composed of heterocysts (Fig. S4*A* and Movie S4). This is far from experimental observations, where the loss of *hetN* condition does induce an increase in the number of heterocysts and a Mch phenotype that eventually stabilizes with time, forming a pattern with roughly 20% of heterocysts (46, 47, 61–63). This hints that a second late long-range inhibition mechanism must be at play to avoid the fatal differentiation of the whole filament. A natural candidate is the

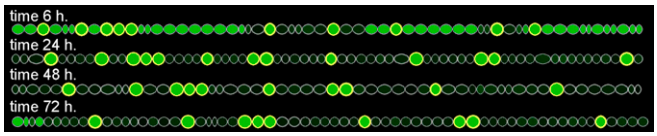

Fig. 5. Temporal evolution of a filament for the *patS* mutant. Details as in Fig. 2. See also Fig. S2 and Movie S2.

effect of fixed-nitrogen products produced by heterocysts (8, 39), although in principle it could be some other genetic or metabolic species. We have included it in our model explicitly, using a mechanism similar to that of HetN but with weaker inhibition power and larger diffusivity, which accounts for the smaller molecules involved (a detailed theoretical description of the effect of fixed nitrogen can be found in ref. 19). When the inhibition by fixed-nitrogen products is taken into account, the model reproduces quantitatively the experimental data from *hetN* loss of function (Fig. S4*A*). This suggests that, in a *hetN* loss-of-function background, fixed nitrogen plays a nonnegligible role in the maintenance of the pattern, a question that has been difficult to assess experimentally (8, 49). HetN and fixed-nitrogen products are not required for the appearance of the pattern but are essential for its maintenance at late stages. Because *hetN* is a much stronger inhibitor in our model, filaments in which fixed-nitrogen products have no function are identical to wild type (Fig. S5 and Movie S5), in agreement with experiments in *Anabaena variabilis* (48, 49).

As in experiments (46), overexpression of HetN in our simulations prevents heterocyst differentiation.

We have also simulated the double *patS*:*hetN* knockdown condition. Because in our model only fixed-nitrogen products are working as a weak inhibitor, most of the cells in the filament quickly differentiate into heterocysts, as experimentally observed (47), although as late as after 100 h there is still a small fraction of vegetative cells. Under PatS or HetN overexpression on a *hetN* or *patS* mutant background, respectively, no heterocysts form, as observed (47).

Variance and skewness from our simulations also quantitatively compare well with experimental data (Fig. 4 *B* and *C*). In contrast to the *hetN* mutant, for the wild type and the *patS* mutant skewness diminishes with time, as interval distributions become more symmetric.

Sequestration by PatS and HetN Forms Spatial Gradients of HetR. We have simulated the dynamics of gene expression in the model (Movies S1–S5). Because PatS and HetN (or more accurately, the pentapeptide RGSGR represented by them in our theory) can diffuse along the filament, not surprisingly their profiles can form gradients (64, 65). At early times after nitrogen deprivation, PatS levels increase in the whole filament (8, 23, 39). These levels remain high for some hours, but as some of these cells differentiate into heterocysts, system levels of PatS drop, and eventually high concentrations are observed only at new proto-heterocysts that appear between formed heterocysts when filament growth moves them apart (39). The dynamics of HetN are somewhat reversed. At early times, HetN is not observed (66) because there are no heterocysts that produce it in the filament. At late times, HetN cell concentrations form quasiregular gradients with peaks at heterocysts (46, 67).

HetR concentration levels have been found to form interesting spatial profiles. For instance, expression of *hetR* has been shown to be correlated between neighbor cells before nitrogen deprivation (68). Because HetR does not diffuse, any gradient of its concentration would be produced by its interactions with the gradients of PatS and HetN. In addition, this is precisely what occurs in our model: the expression of HetR shows higher values in cells far apart from heterocysts. The resulting HetR profile shows variability from cell to cell, just as observed experimentally (43). Another prediction of this model is that the dynamics of the border cell is different from those in the middle, due to the accumulation of diffusing peptides at the end of the filament (see Fig. S4*B* for more details).

Quasisynchronous Cell Division Induces Oscillatory Variation of Pattern Properties. The moments of the vegetative interval length distribution show an oscillatory behavior over time, especially the mean and the variance (Fig. 4). The interpeak time in these oscillations is related to the average time for cell division. When vegetative cells divide synchronously, the average distance (and variance) between heterocysts increases. When a new heterocyst appears in the middle of an interval, it divides in two intervals roughly one-half in length. If the cell cycle is roughly synchronous in the whole filament, this mechanism will produce a lengthening of the mean interval with each round of cell division, and a shortening with each round of differentiation. To test this idea, we have made simulations with different levels of noise in cell growth dynamics (Fig. 7*A*). Decreasing the noise, that is, synchronizing cell division, makes the oscillations of the mean interval distance more pronounced. Conversely, increasing the noise makes the oscillations disappear. The effects of the variation of the level of noise for other parameters are shown in *Supporting Information* (Fig. S6).

Quasisynchronous Cell Division Favors Intervals of Even Length. A characteristic property of the heterocyst pattern is the larger frequency of even-numbered vegetative cell intervals with respect to odd ones (58). This is apparent from experimental histograms of interval length in the literature (23, 39, 43, 56–58). Our theory reproduces this observation for wild type and mutants (Fig. 7*B*). The fraction of even intervals also shows an oscillatory behavior over time, a sign that it is also caused by the synchronous character of cell division along the filament. To test this, we plot (Fig. 7*C*) the percentage of even intervals for different levels of noise in cell growth. For weak noise levels in cell growth, synchronous cell division induces a large fraction of even intervals. This fraction drops when interval lengths are large and several differentiation events happen almost simultaneously, randomizing interval parity until a new round of cell division occurs. In contrast, for high noise, rounds of cell division are not even well defined, and the percentage of even intervals remains always close to 50%.

Our result supports previous suggestions (56) that the synchronous division of vegetative cells during the time that new heterocysts are formed is responsible for the higher fraction of even intervals, with no need of extra mechanisms.

Discussion

In this work, we have shown that a model based on a positive-feedback loop that promotes heterocyst differentiation, plus three diffusible inhibitors, an early one with source at protoheterocysts and two late ones with source at heterocysts, is enough to explain the formation and maintenance of heterocyst patterns up to quantitative detail. As suggested by the biochemistry of the system (41), inhibition works through a "multimer cloud" mechanism (69), in which the formation of complexes between activators and

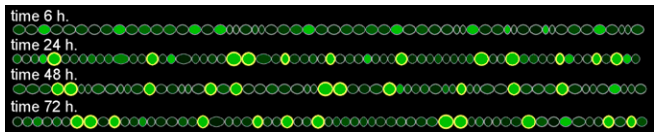

Fig. 6. Temporal evolution of a filament for the *hetN* mutant. Details as in Fig. 2. See also Fig. S3 and Movie S3.

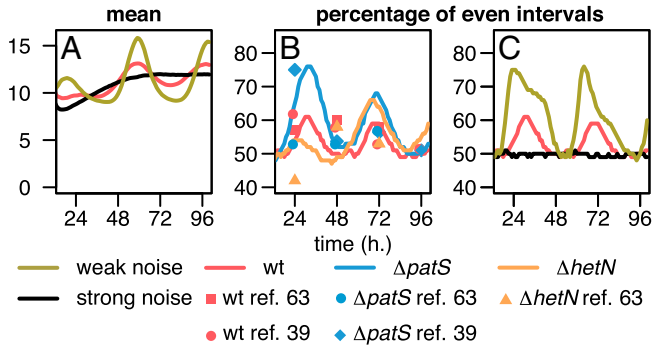

Fig. 7. (*A*) Effect of the noise in cell growth on the mean of the vegetative cells interval distribution. (*B*) Percentage of even intervals for the wild type and *patS* and *hetN* mutants in our model (lines) compared with experiments (symbols) as indicated. (*C*) Effect of the noise in cell growth on the percentage of even intervals. For noise strength parameters, see Tables S1 and S2.

inhibitors sequesters activator complexes and precludes them of realizing their activity. For clarity and concreteness, we have only considered the main genetic regulators of the system, *hetR*, *patS*, and *hetN*, as effective players for local activation, early long-range inhibition, and late long-range inhibition, respectively. A single late inhibitor would produce an overproduction of heterocysts in the *hetN* loss-of-function condition. This suggests the existence of, at least, a second late inhibitor, weaker and diffusing faster than HetN. A plausible candidate is the fixed nitrogen produced by heterocysts.

From a theoretical, more generic perspective, an interesting property of the model is that the main activator for heterocyst differentiation, HetR, is not diffusing. The main mechanisms for the pattern formation and maintenance are the positive-feedback loop for HetR production and diffusion of the inhibitor molecules between cells. The pattern shows a well-defined intrinsic wavelength, robust in time, that does not significantly change due to small variations in model parameter values (see *Supporting Information* for a sensitivity analysis, Fig. S7). This contrasts with the classical Turing continuum model for pattern formation in reactive-diffusive systems (70), where the linear wavelength is proportional to the square root of the product of the activator and inhibitor diffusivities (71). Another main difference with most Turing systems is that the conditions for pattern formation in Turing models need to be finely tuned, and patterns are generally nonrobust in the sense that small variations in parameters may alter the observed wavelength, and even move the system out of the Turing regime. Actually, frequently in the Turing regime different patterns arise at the same point in parameter space, simply owing to slight variations in initial conditions. Obviously, this is not the case in heterocyst pattern formation, where a similar pattern is found independently of environmental and initial conditions.

A key aspect of our work is bringing together regulatory aspects and filament growth through cell division. Without this last aspect, it is possible to create pattern-forming models, but something as important as the maintenance of the pattern cannot be reproduced. Moreover, we show that it is not necessary to consider any cross talk between the cell cycle and the regulation of heterocyst differentiation to reproduce experimental patterns. This cross talk, or some other mechanism, has been postulated as a part of a two-stage model (58), where this mechanism would form a coarse prepattern, from which in a second stage protoheterocysts are refined (22). Our model reproduces quantitatively experimental patterns with a purely stochastic pattern initiation. Both small irregularities in the initial condition or the dynamic noise in the simulations are enough by themselves to trigger the emergence of the pattern. Although a first stage is not needed to form a prepattern, we cannot exclude its existence. Evidence for it has been found in a different strain of cyanobacteria, *Nostoc punctiforme* strain ATCC 29133 (60).

The mechanisms contemplated in our model are only the backbone of this patterning process. The phenotypes of other genes such as *patA* and *hetF* (33, 35–37) show that there is more to the system than local activation and long-range inhibition. Future work should include modeling these finer mechanisms to be able to refine our understanding of how they work and discard hypothesis that do not pass the test of a theory–experiment comparison. Also, extension to different strains of cyanobacteria, and, importantly, use of live gene expression data as in ref. 68 are other main avenues for future work. A better understanding of biochemical kinetics and spatiotemporal aspects of gene expression and metabolite distribution would motivate the construction of models more experimentally constrained. This would allow to use these descriptions to obtain observable predictions and as guides to conduct novel experiments.

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