PATTERN FORMATION IN THE BLUE-GREEN ALGA, ANABAENA

I. BASIC MECHANISMS

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SUMMARY

Filaments of Anabaena have a spaced pattern of differentiated cells called heterocysts, which is maintained as a filament grows by the regular determination of new heterocysts. By following the growth of every cell in a filament, we have identified proheterocysts (prospective heterocysts) at their earliest appearance, and described the sequence of events in the formation of the pattern. The determination of proheterocysts obeys 2 rules: (1) that there are inhibitory zones around pre-existing heterocysts, and (2) that only the smaller daughter of a division can become a heterocyst (all divisions are asymmetrical). There are, however, certain conditions in which these rules are over-ridden, where a pattern consisting of groups of consecutive proheterocysts is seen which resolves into a normal discrete pattern. This process is highly suggestive of interaction between developing cells. We have tested this hypothesis in normal growth conditions by breaking filaments near to early proheterocysts, on the assumption that this will cause a build-up of inhibitory effect of the cell upon itself. It is found that these cells regress, losing their differentiated character and dividing. We therefore propose an interactive model for pattern formation in Anabaena.

INTRODUCTION

Blue-green algae of the genus Anabaena produce specialized cells called heterocysts (Fritsch, 1951) which are probably concerned with nitrogen fixation. The filaments grow by division of (unspecialized) vegetative cells. Heterocysts, which do not divide, are found at fairly regular intervals along the filaments forming a spaced pattern. As the filament grows new heterocysts develop from vegetative cells in a position roughly midway between those already present. Fogg (1949) has put forward a model to account for this pattern. He has proposed that heterocysts produce a substance which diffuses along the filaments and inhibits the formation of other heterocysts. We assume that the postulated inhibitor is destroyed by vegetative cells, so that a gradient is set up around a heterocyst, and that there is a threshold level of inhibitor below which development begins (the above-threshold region defining an inhibitory zone). The simplest form of this model supposes that a cell embarks upon an irrevocable course of differentiation as soon as the concentration of inhibitor falls below threshold. This will be called the simple threshold model.

There is a difficulty with this model, in that considerable demands are made upon its accuracy if single cells, rather than groups, are to be picked from subthreshold regions. We have found (Mitchison & Wilcox, 1972) that only certain cells, the smaller
daughters of an asymmetrical division, can become heterocysts. This provides a mechanism for limiting the number of potential heterocysts in a subthreshold region. But there appears to be an additional mechanism, which ensures that close pairs of heterocysts do not arise. We shall present experimental evidence which shows that the early development of heterocysts is reversible, and implies the existence of interactions of a competitive kind: if two simultaneously developing cells are too close to one another, one must win through and the other regress. A model which incorporates competition will be proposed in the Discussion.

**MATERIALS AND METHODS**

Cultures of *A. cylindrica* (Cambridge Culture Collection strain 1403/28) and *A. catenula* (1403/1) were grown axenically in plugged 250-ml conical flasks containing 75 ml of a defined salts medium (Allen & Arnon, 1955). pH 8.0. Flasks were incubated at 24 ± 1°C in an orbital shaker (200 rev/min) illuminated by 2 x 30-W warm-white lamps at a vertical distance of 25 cm, and in an atmosphere of air containing 3% CO₂. For growth in the presence of ammonia, this medium was supplemented with 0.02% NH₄Cl. Inoculation procedures were as described earlier (Wilcox, 1970).

Growth was estimated by measuring the turbidity of cultures at 740 nm, following gentle blending to disperse the algal clumps. The generation times of *A. cylindrica* and *A. catenula* in shaken culture, both in the absence and presence of NH₄Cl, were 18–19 and 14–15 h, respectively. In this report young and old cultures are defined as having turbidities in the ranges 0.05–0.20 Aₗ 740 units and 0.60–1.50 Aₗ 740 units respectively. The pH of cultures containing NH₄Cl was adjusted, from the time when the turbidity reached about 0.4 Aₗ 740 units, by the daily addition of NaOH.

**Microscopy**

Filaments were observed and counted using a Zeiss standard RA microscope. Phase-contrast optics and a 400 x magnification were routinely employed. For cell counting a cell was defined as a compartment bounded by two complete septa (Mitchison & Wilcox, 1972).

**Transfer of filaments from ammonia-containing to ammonia-free medium**

We avoided centrifugation of cultures, which leads to variable lag periods in the growth and differentiation processes. For transfer, a culture was placed in a sterile conical flask containing, in its base, a fine porosity sintered glass disk. The medium was allowed to filter through this disk, without suction, and the filaments, retained in the flask, were washed twice and finally resuspended in ammonia-free medium to the required density (usually ca. 0.1 Aₗ 740 units). This procedure took 5–10 min.

**Setting up and observation of individual algal filaments**

Algal filaments in liquid culture, viewed through a dissecting microscope, were picked individually, using a finely drawn pipette. They were then transferred to plates of the salts medium solidified by the addition of 1% agar. A coverslip was subsequently placed over the filament and the plate kept on a shelf under 2 x 30-W lamps at a vertical distance of 20 cm. Observations of growth and differentiation were made every 2–6 h, using phase-contrast microscopy and a Phako IV Z7 long working distance condenser. The growth of the filament was followed by recording the stage of septum formation in each cell, consecutively along the filament. Such readings could be matched, cell by cell, for up to 4 generations.
Controlled breaking of filaments

We broke filaments, after transfer to plates, by puncturing a selected cell with a microelectrode, held in Zeiss (Jena) micromanipulators. Vegetative cells and proheterocysts (but not heterocysts) were easily punctured, bursting to leave little visible remains. A coverslip was then placed over the filament to improve optics. No ill effects were observed as a result of the breakage operation; in particular the cells adjacent to the break continued to grow and divide at a normal rate.

For regression studies, proheterocysts at an early stage of development were picked in older *A. cylindrica* filaments. To guard against any unconscious bias the experiments were done 'blind'. Suitable cells were selected by one experimenter who assigned numbers to them. From these numbers a proportion were chosen by a second experimenter who performed breakage operations on them, the rest being used as controls to assess the probability of spontaneous regression occurring.

RESULTS

The organisms and their heterocyst pattern

Two species of *Anabaena* have been used, *A. catenula* and *A. cylindrica*. Both these organisms can be grown either in liquid culture or on agar plates, the latter technique making it possible to follow the growth of every cell within an individual filament over several generations. The time between successive vegetative cell divisions in young cultures (see Materials and Methods) of *A. catenula* is 14–15 h, both in liquid and on plates, while in young *A. cylindrica* it is 18–19 h in liquid and 23–28 h on plates. Each organism has its peculiar experimental advantage, and experimental findings from both organisms were used in support of a pattern formation hypothesis. This is justifiable because the organisms have very similar patterns and responses to various experimental conditions. It is perhaps of interest that large-celled mutants of *A. cylindrica* which strikingly resemble *A. catenula* are easily obtained by nitrosoguanidine mutagenesis (Fig. 7).

In this section we shall describe the pattern seen when the organism is grown in the absence of ammonia. In these conditions, the organism has heterocysts interspersed with proheterocysts (presumptive heterocysts). The superficial features of this pattern will serve to show the similarity of the 2 organisms. The distributions of the number of vegetative cells between successive heterocysts and proheterocysts in young filaments of the organisms have a mean of 9.3 (standard deviation ±2.8) in *A. cylindrica* and 10.1 ± 2.5 in *A. catenula*. We would emphasize that distances of less than 3 cells are uncommon in both organisms. In particular, pairs of adjacent heterocysts are very rare, occurring with a frequency of less than 0.1%. Another parameter of the pattern is a measure of regularity defined by $R = 2\rho \bar{r}$, where $\rho$ = density of heterocysts and proheterocysts and $\bar{r}$ = mean nearest neighbour distance. $R$ lies between 0 and 2, with $R = 2$ for a perfectly regular pattern and $R = 1.07$ for a random pattern (Wilcox, 1970). For both organisms $R$ has a mean value of 1.70, signifying a fairly high degree of regularity.

More precise information was obtained by following the growth of single filaments of *A. catenula* on plates. We were then able to observe the position of newly formed proheterocysts in an *interval*, the set of vegetative cells bounded by 2 heterocysts.
M. Wilcox, G. J. Mitchison and R. J. Smith

The following description of pattern formation can be given: cell division is always asymmetrical, and heterocysts develop only from the smaller daughter of a division (Mitchison & Wilcox, 1972). There is an inhibitory zone of 4–5 cells around every heterocyst or proheterocyst. The asynchrony of cell division ensures that in an interval there will generally be a clearly observable first division of which the smaller daughter is outside the inhibitory zones. This smaller daughter will usually develop into a heterocyst, acquiring a characteristic proheterocyst appearance within 6 h of division, and reaching maturity some 15 h later. Later divisions in the interval outside the inhibitory zones will not yield proheterocysts unless the 2 smaller daughters are separated by at least 3 vegetative cells, when both may develop to give a pair of proheterocysts (Fig. 8). This we call a pair placing (as opposed to a single placing). The above description applies to most proheterocyst determinations (85%). We shall argue that the exceptional cases are best understood in terms of competition (Discussion).

Because of the asynchrony of cell division the number, N, of cells in an interval when a single placing is first visible varies over a wide range: 10–29 cells, with a mean of 18.2 ± 3.0. Despite this variation the relative positioning is strikingly central; 50% of new proheterocysts are placed exactly in the centre of their interval (Fig. 1 A, p. 711). Pair placings account for 4% of all determinations. In this case, N is usually larger, having a mean of 24.8 ± 3.9. The pair is separated by from 3 to 8 vegetative cells, with a mean of 5.1 ± 2.1. As in the case of single placings, the pair is symmetrically placed in the interval.

The pattern so far described is that found in young cultures; after a certain time the pattern shows a widening (Wilcox, 1970).

The effect of ammonia and the group pattern

Young cultures of A. cylindrica grown in the presence of ammonia have a pattern consisting mostly of proheterocysts with only a low proportion of mature heterocysts. The spacing of the pattern is nonetheless very similar to that of a culture grown in the absence of ammonia (Wilcox, 1970). We shall use the term transfer exclusively for the transfer of filaments from medium with ammonia to medium without. A normal heterocystous pattern appears within about 16 h after transfer of young filaments of A. cylindrica. The same phenomenon is found in young cultures of A. catenula, of a proheterocyst pattern in the presence of ammonia, and a heterocystous pattern after transfer which appears within 20 h. By following transferred single filaments of both organisms, we have shown that the original proheterocysts in these young filaments become mature heterocysts of the final pattern.

With older cultures of A. cylindrica the pattern in the presence of ammonia is far less distinct, and the appearance of heterocysts after transfer is slower, taking 20–24 h. Examination of the filaments 6–10 h after transfer reveals a pattern consisting of both isolated proheterocysts and groups of from 2–8 adjacent cells which have the appearance of proheterocysts under the light microscope (Fig. 9). This will be called the group pattern of transfer. It has been possible to examine such groups under the electron microscope, using correlation techniques (M. Wilcox et al. in preparation), from which
we have established that the cells of the groups show the ultrastructural characteristics of proheterocysts (Fig. 10). The proheterocysts in such a group may not all be smaller daughters of a division; the rule which applies in normal growth conditions (without ammonia) appears to be over-ridden in these older cultures (see Discussion).

![Histogram](image)

Fig. 1. Relative positions of new proheterocysts in intercalary, end and 'artificial end' *A. catenula* intervals, showing offset placing. The position of each proheterocyst is expressed as a percentage of the total interval (abscissa). To obtain an arbitrary orientation, intervals were read outwards from the centre to each end of the filament. Histogram (A) compares the placing of new proheterocysts in end (—) and intercalary (---) intervals. The data come from 470 end and 334 intercalary intervals. Histogram (B) shows the placing in 'artificial end' intervals (see Materials and Methods). Data are from 92 breakage experiments.

Single proheterocysts develop from these groups, while the other proheterocysts of the group regress, assuming the appearance of a normal vegetative cell and going into division. When the groups are small, 2-4 cells, one proheterocyst develops; when they are larger, 5 or more cells, two may develop, usually being separated by at least
3 vegetative cells. Adjacent heterocyst pairs are rare in the final pattern, occurring with a frequency of about 0.4%. A group pattern is not seen after transfer of older *A. catenula* cultures; we suggest a possible reason for this in the Discussion.

The group pattern and its subsequent resolution into a discrete pattern suggest that competition may be occurring. At a stage where most groups have been resolved, the remaining single proheterocysts must be assumed to be producing inhibitor. But when pairs and small groups of proheterocysts are closely matched they may continue to a much higher state of differentiation than these single proheterocysts before one is selected and the others regress (compare, for example, Figs. 11 and 12).

Direct evidence of competition is provided by the experiments of the following section. The assumption behind these experiments is that competition is not confined to transfer, but occurs under normal growth conditions to prevent close pairs of proheterocysts developing.

**Ends of filaments and micromanipulations**

Heterocysts only rarely arise at the ends of filaments in species of *Anabaena* under our conditions. These terminal heterocysts have only one junction, which takes the form of a polar structure, while intercalary heterocysts (with vegetative cells on either side) have two. Thus terminal heterocysts may be distinguished from those intercalary heterocysts which are sometimes found at the ends of filaments after breakage during growth.

The set of cells between one end (*T*) and the first heterocyst will be called an end interval (see Fig. 13). The placing of a new proheterocyst in an end interval shows a distribution (Fig. 1A) which differs from that of an intercalary interval. The new proheterocyst is displaced towards the end, the mean of the ratio \( m/n \) (Fig. 2) observed in a total of 100 ends of filaments grown on plates being 1.88 ± 0.85.

![Fig. 2](image)

The distribution shows that proheterocysts seldom appear in the last 20% (or 2–3 cells on average) of the end interval. This fact, together with the rarity of terminal heterocysts, is at variance with the simple threshold model. The terminal cell should have the lowest concentration of inhibitor of all cells in an end interval (or, equivalently, should be the first outside the inhibitory zone of the interval). Nor can the probabilistic element introduced by asynchrony of division explain the observed distribution.

It is perhaps helpful to take a different point of view. We are already assuming connections between the cells of a filament; let us assume further that the terminal cell of a filament is a vegetative cell which has only one connexion, and is otherwise bounded by a normal cell wall. Now, given a filament with mirror symmetry, there will be zero net flow of any diffusing (or transported) substance across the plane of symmetry, which may therefore be replaced by a barrier. From this it follows that the end will
behave exactly as though it were one half of a hypothetical interval made up from the original filament with its mirror image, joined at its terminal cell $T$ with a normal cell-cell connexion (Fig. 3).

Therefore, a terminal heterocyst arising from $T$ would be equivalent to an adjacent heterocyst pair in the interval $H-H'$. Because such a pair will be switched on simultaneously, the simple threshold model cannot avoid the occurrence of these artificial heterocyst pairs. But competition would prevent them developing and thereby provide an explanation for the absence of terminal heterocysts.

The finding of offset placing in end intervals offered the possibility of studying the readjustment of the pattern in an 'artificial end' produced by breaking a filament. The principle of these experiments was to pick an interval in which a proheterocyst would be expected to arise centrally, to break one end of this interval, and to see whether the position of the proheterocyst was displaced towards the newly formed terminal cell. The organism used was Anabaena catenula; the intervals picked for breakage were of the form $H-P$ (Fig. 4), $P$ being an early proheterocyst at a stage corresponding to 3-6 h of development from first appearing. The proheterocyst $P$ was punctured to give an end in which a new proheterocyst $P_1$ would be expected to arise in 8-12 h.

Control intervals, both end and intercalary, from filaments in which breaks were made showed the normal placing of proheterocysts. A total of 92 breakage experiments was performed; a typical experiment is shown in Fig. 14. The distance of new proheterocysts from the last heterocyst, in percentage relative to the total length of the interval, is shown in Fig. 1B. The distribution is indistinguishable from that of normal end intervals, implying that regulation takes place within 8-12 h of breakage. Differential cell-division rates cannot play a part in this regulation; an analysis has shown that the division rate of vegetative cells is constant at all points in end intervals.

The hypothesis of competition was further tested by breaking filaments next to, or a few cells away from, an early proheterocyst. This would be equivalent to producing a 'mirror image' proheterocyst close to the existing proheterocyst, which might then regress (Fig. 5).

The organism used for these regression experiments was Anabaena cylindrica. When the filaments were from young cultures there were few regressions. But in older cultures,
Table 1. Regression of A. cylindrica proheterocysts following filament breakage

<table>
<thead>
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<th>Experiment</th>
<th>Type</th>
<th>Number</th>
<th>Number</th>
<th>Percentage</th>
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<tr>
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<td>80</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
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<td>61</td>
<td>28</td>
<td>46.0</td>
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<tr>
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<td>55</td>
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<tr>
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<td>61</td>
<td>21</td>
<td>34.4</td>
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<tr>
<td>3-Cell</td>
<td></td>
<td>49</td>
<td>2</td>
<td>4.1</td>
</tr>
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Early proheterocysts were chosen and vegetative cells broken as described in Materials and Methods. In the first column of the table, 0-, 1-, 2- and 3-cell refer, respectively, to breaks leaving the proheterocyst as end cell or leaving 1, 2 or 3 vegetative cells next to the proheterocyst. Controls, to determine the rate of spontaneous regression, were carried out on proheterocysts picked when at the same stage of development as those used for breakage experiments.

where development of cells is slower, regressions were obtained with a very high statistical significance (Table 1 and Fig. 15). Spontaneous regressions were rare (3% frequency). Breaking the filaments to leave 1 cell next to the proheterocyst gave regressions with a 75% frequency; when 2 cells were left proportionally fewer regressions (34% frequency) occurred, as would be expected. When 3 cells were left, the proportion of regressions was not significantly different from that in controls.

But when the cell next to a proheterocyst was punctured, comparatively few cells regressed (46% frequency), an unexpected result which we return to in the Discussion.

DISCUSSION

Interpretations

We have seen that pattern formation in normal growth conditions may be reasonably well described by a modification of the simple threshold model (Introduction), which has the added assumption that a cell must be the smaller daughter of a division (as well as having subthreshold inhibitor concentration) before it can develop into a heterocyst. Evidence that this description is incomplete comes from the resolution of the group pattern seen on transfer, which would seem to imply the existence of interactions. In this section, we discuss the regression experiments which were designed to
Pattern formation in *Anabaena*
demonstrate these interactions in normal growth conditions. We argue that the simple threshold model cannot account for the results of these experiments, and then put forward an interactive model to take its place.

The simple threshold model would predict the occurrence of heterocysts at, or near to, the ends of *Anabaena* filaments, since the concentration of inhibitor should be at a minimum there. But we have observed that proheterocysts rarely arise in the last 2–3 cells at the end of a filament. We have explained this by assuming that the end of a filament is a barrier to the escape of inhibitor, and that the competitive control of development prevents a proheterocyst from arising close to such a barrier. This may be viewed as being due either to the build-up of inhibitor in a confined space or to the presence of a second, imaginary proheterocyst in a mirror image extension of the filament. The latter point of view makes it clear that a competitive mechanism which prevents adjacent heterocyst pairs from developing should also exclude terminal heterocysts. Although terminal heterocysts are rare in normal growth, they may be found when the organism is under unusual conditions such as temperature shift. At such times adjacent heterocyst pairs are also found, as we would predict.

In this interpretation we have assumed the existence of intercellular connexions. Dr Audrey Glauert (personal communication) has observed microplasmadesmata in cross-walls between vegetative cells in *A. cylindrica* which might serve as connexions, and in our electron microscopy studies we have recently found such structures in *A. catenula* (M. Wilcox et al. in preparation). However, examination of the end walls of terminal cells has shown no evidence of microplasmadesmata; the longitudinal wall appears to be continuous around the end of the cell. This supports our diffusion barrier hypothesis. Strictly, it is not necessary to assume that the normal cell wall is a diffusion barrier, but simply that any loss of inhibitor through it is not greater in the terminal cell than in other cells. Then the mirror image argument is valid.

There is an alternative explanation for end placings which postulates special properties for a terminal cell. Fritsch (1951) mentions the conical shape which terminal cells in *Anabaena* species frequently have, their lack of granularity and smaller amounts of photosynthetic pigments. He continues to suggest ‘that the specialized end cells (may) represent heterocysts which have not passed beyond the first stage in their differentiation’. With this hypothesis the simple threshold model can explain end placings. However, none of the above-mentioned characteristics is seen in the terminal cell of an ‘artificial end’ by the time when the first placing occurs, and such placings show the typical end distribution. Moreover, terminal cells divide (at the same rate as other cells), yielding normal vegetative daughter cells, which implies that any specialization must be reversible.

We have accounted for the regression of proheterocysts, when the filament is broken nearby as the inhibitory effect of a cell upon itself. This being granted, the concept of an irrevocable course of differentiation is untenable. But it may be suggested that these proheterocysts are in a ‘preliminary phase’ during which they are not producing inhibitor and are not committed to development. An increase of inhibitor concentration around such a proheterocyst might cause it to regress. For example, the change in configuration on breakage might lead to such an increase because of a greater effect
of the one neighbouring heterocyst ($H_1$ in Fig. 6) which remains. After reflexion, the image of this heterocyst ($H'_1$) could be closer to the proheterocyst $P$ than the other neighbouring heterocyst ($H_2$).

A comparison of the frequency of regressions with the value of $(a-b)$ showed no correlation: for breaks leaving 1 adjacent cell, the correlation coefficient (Hoel, 1966) $r$ is $-0.27$, which is not statistically significant ($|r| > 0.32$ for a significance level of 0.05). This makes it unlikely that such a mechanism plays an important part in causing regressions.

The fact that comparatively few regressions occur when the cell next to a proheterocyst is broken is contrary to our predictions. When this proheterocyst fails to regress, it frequently develops into a heterocyst with 2 junctions. Even when a second, unconnected, junction does not appear, the heterocyst generally shows signs of a structure at the broken end and differs in this from a true terminal heterocyst. We have observed that proheterocysts generally advance in their development before regressing and, in doing so, may reach a stage at which the beginnings of a specialized heterocyst junction may be formed. We therefore propose that such a junction may develop at the exposed end of a proheterocyst after the cell next to it is broken, and that this junction may serve as a leak for inhibitor. This would favour the development of the cell and explain the relative infrequency of regression in these experiments.

Assuming, then, that the regression experiments may be interpreted as evidence for competition, in what form do interactions appear in normal growth? Let us call a smaller daughter which appears outside the bounding inhibitory zones of an interval a candidate cell if no proheterocyst is yet detectable in that interval. Then the description given earlier may be rephrased to say that it is the first candidate to appear in an interval which generally develops into a proheterocyst. But there will often be several candidates in an interval before one has developed into a detectable proheterocyst, and these other candidates frequently show some signs of proheterocyst character before going into division (G. J. Mitchison & M. Wilcox, in preparation). This may be particularly clear in a proportion of cases (15%) where the proheterocyst which finally develops comes not from the first candidate but from a later one. In view of these observations, we consider that the determination of proheterocysts is not simply a matter of the first candidate inhibiting subsequent ones, but is rather an interactive process in which the first comer has an advantage.
An interactive model for proheterocyst development

The simple threshold model may be modified to permit competition between proheterocysts by assuming that, even after a proheterocyst has begun to produce inhibitor, it still remains susceptible to its effect. If inhibitor concentration exceeds a certain critical level, the cell begins to regress. This critical level rises as the proheterocyst develops, while, at the same time, the cell produces inhibitor at an increasing rate. The cell will continue developing only if the inhibitor concentration within the cell does not rise above the critical level. Now, the inhibitor must be destroyed by (or lost from) vegetative cells if a stable gradient is to be set up. The simplest assumption to make is that an enzyme system destroys it at a rate proportional to concentration, in which case a decreasing exponential gradient is set up from each heterocyst or proheterocyst. The effect of two or more sources of inhibitor at a given cell is simply the sum of their individual contributions. Thus, if two proheterocysts begin developing close together each will cause an increased level of inhibitor in the other by its contribution. Within a critical distance only one proheterocyst will be able to develop.

More formally, we suppose that there is a substance X whose concentration expresses the state of heterocyst development of a cell, and which does not diffuse between cells. Let the inhibitor be denoted by Y. Then the diagram shown below presents the essential features of the model.

\[ \frac{d[X]}{dt} = \alpha [X], \alpha \text{ a constant.} \]  

The synthesis of X has an autocatalytic component (ii) which effectively gives development its forward drive. The critical level will be an increasing function of [X]; comparison of [X] and [Y] by the combined effects of (ii) and (iii) determines the rate of synthesis or destruction of X. This might be carried out by a mechanism such as the competition of 2 chemical species for a site; a natural assumption in this case would be:

\[ \frac{d[X]}{dt} \geq 0 \quad \text{according to whether } [X] \geq \beta[Y], \beta \text{ a constant.} \]  

Development must be capable of beginning from the state of a vegetative cell, which can be achieved by having a lower bound L for [X], (a background level). The result
is that the concept of a threshold is retained: the competitive state commences when 
[Y] falls below this threshold (L/β in the case of equation (2)). The threshold will 
define an integer \( m \), the number of cells in the inhibitory zone on either side of a 
mature heterocyst. There will also be an integer \( n \) which defines the minimum distance 
within which 2 proheterocysts can develop simultaneously. In general, \( m \) and \( n \) will not 
be equal (although \( n \leq m \)). From our data suitable values would be \( m = 5, n = 3 \), 
the latter being given by the minimum distance apart of pair placings.

Why do heterocysts develop only from candidate cells? A candidate—a smaller 
daughter in the period immediately after a division—is a vegetative cell which has the 
smallest possible size. If the efficiency of a cell as a source of inhibitor (e.g. the constant 
\( \alpha \) in equation (1)) is an increasing function of its size, then a candidate will have an 
advantage over other cells, since it will produce relatively less \( Y \) for a given value of 
[X]. Such a correlation between cell size and source efficiency might derive from the 
volume of material available for synthesizing \( Y \). An indication that the selection of 
candidates is due to an advantage, rather than a rigorous ‘lineage’ rule, comes from 
the resolution of the group pattern of transfer. Here a non-candidate may emerge 
from a group if its geometrical position favours its development. However, such exceptions 
to the rule are not found in normal growth conditions.

We have considerable evidence for a complex interconnexion between division and 
development. Not only does a cell have to be in a certain part of its cycle (a candidate) 
before it can begin to develop, but there seems to be a level of development which has 
to be reached before division is stopped. When cultures are grown in the presence of 
ammonia the development of heterocysts is slowed down so that a pattern of pro-
heterocysts results. In the case of older cultures the process of competition may also 
be slowed down so that regressing proheterocysts retain their differentiated state for 
longer. These proheterocysts at a low level of development may go into division and 
give rise to the groups seen on transfer of such cultures of \( A. \) cylindrica. The fact that 
a group pattern is not seen in \( A. \) catenula under the same conditions may reflect a 
difference between the organisms in the precise relationship between development 
and cell division.

The existence of competition explains how a threshold model can be sufficiently 
accurate to select single cells. Competition may occur in the formation of other spaced 
patterns: Wigglesworth (1959) has proposed a threshold model for the 2-dimensional 
pattern of bristles on \( Rhodnius \) cuticle, where it is known (Lawrence, 1970) that pairs 
of bristles derived from neighbouring cells are very rare. The interactive model we 
have defined could be extended to 2 dimensions and applied here to ensure that 
isolated bristle precursor cells are picked. More generally, classical embryology pro-
vides abundant evidence for competition (cf. Waddington, 1956), but the cellular 
events underlying these interactions may be very complex in higher organisms. It 
appears that \( Anabaena \) may offer considerable advantages for studying such pheno-
mena, both because of the experimental control which is possible, and because of the 
geometrical simplicity of the organism.
Pattern formation in Anabaena

We wish to thank Sydney Brenner and Francis Crick for helpful discussions and John Phillips for a critical reading of the manuscript. We are particularly indebted to Peter Lawrence for his many suggestions and constant encouragement.

REFERENCES


(Received 11 August 1972)
Fig. 7. Phase-contrast light micrographs of filaments from ammonia-free cultures of A, *A. cylindrica*, B, *A. catenula*, and c, a nitrosoguanidine-induced mutant, L2, of *A. cylindrica*. Average sizes of vegetative cells in the 3 organisms (diameter x mean length) are 3.2 x 3.5, 6.2 x 7.2, and 5.4 x 6.2 μm, respectively. Early proheterocysts are arrowed in A and B. x 600.

Fig. 8. Pair placing in an *A. catenula* interval. Proheterocysts are arrowed. x 600.

Fig. 9. Group of early proheterocysts in an *A. cylindrica* filament 8 h after transfer. x 800.

Fig. 10. A, light micrograph of a proheterocyst group (marked) in a transferred *A. cylindrica* filament. x 1000. B, electron micrograph of the same group following fixing with glutaraldehyde-osmium. Part of the early fibrous layer of the proheterocyst envelope (Lang & Fay, 1971) is still visible around the cells (at arrow). × 4400.
Pattern formation in *Anabaena*
Fig. 11. Resolution of a proheterocyst group formed after transfer of an *A. cylindrica* filament. The group is marked and the surviving proheterocyst arrowed. The group was photographed at the following times after transfer: A, 10; B, 12; C, 15; and D, 19 h. ×800.

Fig. 12. As for Fig. 11 except that the group was photographed at the following times after transfer: A, 12; B, 14; C, 16; and D, 24 h. The material seen here around the group is a common finding in these circumstances and may be released fibrous layer (see Fig. 10). ×800.

Fig. 13. An *A. catenula* end interval showing an offset proheterocyst (arrowed). ×800.

Fig. 14. Sequence showing appearance of an offset proheterocyst (arrowed) in an *A. catenula* 'artificial end' interval formed by puncturing the proheterocyst indicated in A; B, C, D, E show the interval at 0, 4, 8, and 14 h, respectively, after breakage. ×500.

Fig. 15. Sequence showing regression of an early proheterocyst (arrowed) following breakage of an *A. cylindrica* filament to leave 1 vegetative cell adjacent to the proheterocyst. Photographs A, B, C, D were taken at 0, 9, 13, and 22 h, respectively, after breakage. ×500.