Cell Polarity in *Bacillus subtilis*: Effect of Growth Conditions on Spore Positions in Sister Cells

BY G. DUNN AND J. MANDELSTAM

Microbiology Unit, Department of Biochemistry, University of Oxford,
Oxford OX1 3QU

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INTRODUCTION

In bacilli, sporulation begins by formation of a septum at one end of the bacterium rather than at the centre. This raises the question of whether the cell should be regarded as an unpolarized structure in which the position of the spore is a result of a random event, or whether it possesses polarity which specifies that the spore is to be formed at one end of the cell rather than at the other. It is possible to test this because in cultures of *Bacillus subtilis*, as well as in those of other species, the two bacteria resulting from a division tend to remain attached, often for many hours. Furthermore, sporulation in such sister cells is highly synchronized (Dawes, Kay & Mandelstam, 1971). It is then easy to determine by phase-contrast microscopy whether a spore has been formed proximally or distally in relation to the newest division septum. The possible patterns for spore positions in sister cells are as follows:

where S indicates a spore, and — indicates no spore.

If the probability that the spore will be formed at the proximal end is 0.5, i.e. if the position of sporogenesis is determined by a random event, then each of the four possible combinations should be equally represented in the population. Since the two possibilities represented under B are indistinguishable, cell pairs of this type should constitute 50 % of the population with A and C each accounting for 25 %. By contrast, if there is complete polarization in favour of one end, class B should be absent altogether. Such a result has been reported for spore distributions in *Bacillus megaterium* (Hitchins, 1975).

The experiments to be described show that the existence or non-existence of polarity in *B. subtilis* sporangia is determined by the medium in which vegetative growth occurs. In addition, experiments were carried out to determine whether polarity was correlated with the number of chromosome replication forks present in the cell at the onset of starvation.

METHODS

Strain and culture conditions. *Bacillus subtilis* 168 (*trpC2*) was grown either in a hydrolysed casein medium (CH) (Sterlini & Mandelstam, 1969), or in brain-heart infusion broth with 0.2 % yeast extract (BHIB) (Difco). Cultures were harvested while in exponential growth (0.25 mg dry wt ml⁻¹) and sporulation was induced by transferring the cells to a resuspension medium containing glutamate, tryptophan and inorganic ions (Sterlini & Mandelstam, 1969). Incubations in the growth media and in the sporulation medium were either at 37 or 42 °C as specified.

Microscopy. The intracellular positions of phase-bright spores were scored in sister cells 6 to 8 h after resuspension.

DNA estimation. DNA was estimated by the method of Burton (1956).

Cell number estimation. Cell numbers were determined using a Coulter counter (model ZF), as described previously (Dunn, Torgersen & Mandelstam, 1976).

RESULTS

Distribution of spore positions in sister cells after growth in CH. Bacteria were grown in CH at 37 °C and then transferred to resuspension medium also at 37 °C. The distribution of spore positions was measured 7 h after resuspension, at which time more than 80% of the cells were still in pairs. The following counts were obtained from 223 pairs (i.e. 446 cells): A, 141 (63%); B, 67 (30%); and C, 15 (7%). The total number of cells in which a spore appeared at a distal end is the sum of all those in class A and half of those in class B, i.e. $282 + (\frac{1}{2} \times 134) = 349$, or 78% of the total. The observation that under these conditions 75 to 80% of spores are formed at the distal end was confirmed in a number of experiments in which the scoring was done independently by several individuals.

Effect of sister cell separation on pattern of spore positions. The proportion of *B. subtilis* sporangia that persist as linked sister cells during starvation varies from day to day. Chains of cells are invariably formed soon after transfer to resuspension medium. Then, as sporulation proceeds, there is a tendency for the chains to break up. It could, therefore, be argued that the results are due to a decrease in 'stickiness', produced for some unknown reason, whenever a spore is generated in the proximal position. However, in the experiments already described only 20% of the sporangia were free, and even if all of these single sporangia had been exclusively derived from cell pairs of either class B or class C it would still be insufficient to upset the conclusion that spore formation occurs preferentially at the distal end – though the estimate of the degree of polarization would be different.

Nevertheless, to check this point counts were also done on days when the incidence of single cells was very high (about 90%); the relative counts did not differ significantly from those obtained previously.

Effect of temperature on pattern of spore positions. In the first experiments incubations in both the growth and the sporulation media were carried out at 37 °C. The effect of temperature was examined by increasing the sporulation temperature to 42 °C. This did not affect the incidence of distal spores, nor was there any significant effect produced by raising the growth temperature to 42 °C, although growth and sporulation were both accelerated at 42 °C.

Effect of growth medium on pattern of spore positions. We considered the possibility that the polarization within the sporulating cell might be influenced by the number of chromosome replication forks at the time of resuspension. We therefore grew the bacteria in BHIB in which the doubling time is 25 to 30 min, compared with 40 to 45 min in CH. An increase in the growth rate of the culture could be expected to increase the number of replicating forks in the chromosomes (Cooper & Helmstetter, 1968), and perhaps change the subsequent pattern of chromosome segregation on termination of the final round of replication after resuspension.

Bacteria were grown in BHIB at 42 °C, and then transferred to the sporulation medium at the same temperature. The use of this growth medium resulted in the complete disappearance of the polarity effect; only 51% of the sporangia contained spores at their distal ends. Similar changes in polarity as a result of changing the growth medium have been observed in cultures of *Bacillus cereus* (Hitchins, 1976).

The next step was to establish how long growth had to be maintained in BHIB to destroy the polarity effect. Bacteria were therefore grown first in CH for many generations at 42 °C, i.e. from a small inoculum to a density of 0.25 mg ml⁻¹. The culture was centrifuged and the bacteria were suspended to the same density in BHIB. One portion was removed immediately and the bacteria were transferred to resuspension medium for sporulation. Further portions

Fig. 1. Effect of medium changes on polarity in *Bacillus subtilis*. Bacteria were first grown in either CH or BHIB for many generations at 42 °C. The cultures were then centrifuged and the bacteria were suspended to the same density in the other medium. One portion was removed from each of these cultures and bacteria were transferred to resuspension medium for sporulation. Further portions of each culture were diluted 2-, 4-, 8-, 16-fold etc. in the relevant new medium. The diluted cultures were grown at 42 °C. As each attained a density of about 0.25 mg ml⁻¹, it was centrifuged and the bacteria were placed in resuspension medium for sporulation. The patterns of spore positions were determined 6 h after the induction of sporulation, and the proportion of cells with spores at their distal ends (% polarity) was then estimated. (a) Change in polarity after transferring the cells from CH to BHIB (150 pairs scored for each point). (b) Change in polarity after transferring the cells from BHIB to CH (600 pairs scored for each point). In each graph the error limits are given by $\pm 1.96\sqrt{P(100-P)/N}$, where P is the estimated % polarity, and N the total number of pairs scored.

of the culture were diluted 2-, 4-, 8-, 16-fold etc. with BHIB. These diluted cultures were grown at 42 °C. As each attained a density of about 0.25 mg ml⁻¹, it was centrifuged and the bacteria were placed in resuspension medium for sporulation. The patterns of spore positions were determined 6 h after the induction of sporulation. At least 150 cell pairs were counted for each sample.

The portion of cells exhibiting spores in the distal position was, as before, taken to comprise all those in class A and half of those in class B. The initial value, for cells that had just been grown in CH, was about 73 %. This value fell during growth in BHIB and was about 45 % after four generations (Fig. 1a).

Experiments of the reverse type were then done, i.e. bacteria grown in BHIB for many generations were transferred to CH and tested periodically to establish their sporulation pattern. As expected, after growth in BHIB the bacteria were unpolarized, and the acquisition of polarization, like its loss, took place over several generations (Fig. 1b).

DNA synthesis after the onset of starvation. The amount of DNA in resuspended cultures was also measured. Bacteria were grown in CH or BHIB, resuspended in sporulation medium,

and then the amount of DNA in these cultures up to 4 h after resuspension was determined. The number of bacteria in each of the cultures was also determined so that the amount of DNA per sporangium could be estimated. This was done because when sporulation has been induced, chromosome replications that are in progress go to completion, the cell divides, and the daughter cells then sporulate (Dawes *et al.*, 1971). We assumed that the faster-growing cells in BHIB (mean generation time about 30 min) would contain more replication forks than those in CH (mean generation time about 40 min) and that the increase in DNA would reflect this. In fact the DNA content in both cultures increased by about 40% to yield sporangia containing about 1.1×10^{-8} μ g, which is equivalent to that contained in two complete chromosomes (Dennis & Wake, 1966). We have no explanation for this result.

DISCUSSION

These results indicate that polarity in starving cells depends on the previous history of those cells. After resuspension, those grown in CH produce prespore septa which are predominantly at the distal end of the cell, while in those grown in BHIB the spore positions are randomly distributed. Although cells growing in BHIB presumably contain more replicating forks in their chromosomes, since they are growing faster, this does not appear to be the controlling factor in the determination of cell polarity. That this is so is indicated by the kinetics of changes in cell polarity after transfer of bacteria from one growth medium to the other. It takes about four generations for the polarity to change to that characteristic of the new growth medium, a period which appears to be far too long to be explained by cell-cycle mediated phenomena. After growth in either medium, nuclear segregation and cell division result in the formation of sporangia containing two chromosomes, and there is no reason to believe that the mechanisms of nuclear segregation differ in the two situations. It is necessary, therefore, to seek some other determining factor in the control of the positioning of the prespore septum.

It is possible under certain conditions to detect polarity in the *B. subtilis* wall using phage SP50 as a marker (Archibald & Coapes, 1976). A similar type of polarity, presumably associated with a particular structure or conformation in the wall, may determine the position of the prespore septum. If it does, there is an implied difference between the structure of walls in bacteria grown in CH and those grown in BHIB. Furthermore, it is known that wall turnover occurs at about 10% per generation (Pooley, 1976*a, b*). On this basis one would expect that after bacteria are transferred from one growth medium to another it would take several generations before the whole population had acquired or lost the structures that determine spore position.

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