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CONTROL OF MEMBRANE PROTEIN SYNTHESIS IN *BACILLUS SUBTILIS*

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SUMMARY

In synchronous cultures of *Bacillus subtilis* 168/S grown on succinate as a sole carbon source (mean generation time 115 min), chromosome initiation occurs at the beginning of the cell cycle but the rate of membrane protein synthesis doubles in mid-cycle more or less coincident with nuclear segregation. In glucose-grown cultures, the doubling in rate of membrane protein synthesis occurs at about the same time as nuclear segregation and DNA initiation at the beginning of the cycle. Control of the rate of membrane synthesis by the chromosome has been demonstrated by inhibiting DNA synthesis using thymine starvation and showing that membrane protein synthesis continues at a constant rate, whereas the rate of cytoplasmic protein synthesis almost doubles.

I suggest that the replication of a region at or close to the chromosome terminus is required to allow the doubling in rate of membrane synthesis.

INTRODUCTION

Although continuous extension in length of rod-shaped micro-organisms is one of the principle manifestations of the cell cycle, the relationship between surface extension and the chromosomal cycle has received relatively little attention. Many attempts have been made to investigate surface extension by studies of the location of newly inserted material [1] and by attempts to describe the overall kinetics of surface growth in relation to the cell cycle [2]. Studies of location of sites of synthesis have provided conflicting data [1], in some cases showing zonal growth and in others growth from many sites. The lack of discrete zones of growth shown in some studies may not be incompatible with those which show discrete zones if there is randomisation after insertion into the surface or if the site of insertion is not the major growth zone of the surface. For example, the location of newly synthesised phospholipid would not necessarily indicate their site of synthesis as they are known to be mobile within the membrane. The rate of turnover of peptidoglycan in bacilli is substantial so that the rate and site of incorporation may not correspond with net growth [3]. Experiments in which zonal growth has been demonstrated in rod-shaped micro-organisms have employed methods which show net growth [4] or in which there is reduced turnover of the component studied [5]. Kepes and Autissier [6] have provided

evidence that some membrane proteins in *Escherichia coli* may be the most strongly conserved components of the cell surface during growth, and may be the best available indicator of the number of growth zones per cell.

The time course of surface extension in rod-shaped microorganisms has been extensively, although inconclusively, studied [2]. Growth of rod-shaped bacteria undoubtedly involves continuous lengthwise extension without change in cell width, though it is not certain whether length extension is linear or exponential. Differentiation between these possibilities can probably be made only by measuring the rate of synthesis of a surface component that corresponds to the surface area. Of the candidates available, cell wall and phospholipid synthesis may not be suitable, as in both cases turnover during growth would mean that the measured rate of incorporation of precursors (during pulse incorporation) would include a component which was not concerned with increase in surface area. Membrane proteins may be a more attractive candidate as turnover is not a serious consideration [7].

A previous study of membrane synthesis in synchronous cells of *Bacillus subtilis* [7] has indicated that during the cell cycle a large part of the membrane protein is synthesised initially at a constant rate, which increases sharply at one time in the cycle to a new constant rate approximately double the initial rate which is maintained for the rest of the cycle. Although the bulk of membrane proteins is incorporated by a continuous linear process, a small proportion of proteins is added relatively slowly to the membrane from the cytoplasm. In contrast to the continuous linear incorporation of pulse-labelled proteins into the membrane with doublings in rate at one point in each cycle, the activity of succinic dehydrogenase increases discontinuously (i.e. in only one short period of the cell cycle). Discontinuous increases in the activity of a number of membrane components have been observed by others (8–10). One interpretation of the linear synthesis of membrane is that, during a period of linear increase, the number of sites of membrane synthesis is not changing and at the time of the doubling in rate the number of sites doubles. The pattern of incorporation of individual polypeptides is at present a matter of speculation. However, to account for the observed kinetics of bulk labelling, a large proportion of proteins would probably be incorporated linearly and continuously at each growth site. The observed cases of discontinuously increasing activities [7–10] may be reconciled with this scheme if the polypeptide is synthesised continuously and activated at one time in the cycle. However, a small proportion of protein that is actually synthesised discontinuously may not contribute significantly to the bulk labelling pattern and this could occur at sites distinct from the cells' major growth zones.

Studies of the variation in length of rod-shaped bacteria analysed on the basis of a linear growth model suggest that the number of growth zones doubles at, or close to, chromosome termination in both *E. coli* and *B. subtilis*, and that the rate of lengthwise extension per growth zone is proportional to the growth rate of the culture [11, 12]. Length extension in bacteria in which DNA synthesis is inhibited is linear with time, suggesting that DNA synthesis is required to generate new growth zones [12, 13]. The purpose of this communication is to show whether the controls on surface growth suggested by studies of the control of cell size can be seen at the level of membrane protein synthesis, by showing if (a) the change in rate of membrane protein synthesis during the cell cycle correlates with termination and (b) if DNA synthesis is required to allow an increase in rate of membrane synthesis. The timing

of chromosome replication in relation to the cell cycle in *B. subtilis* has not been extensively studied. Chromosomal replication is clearly sequential and the rate of synthesis is governed by the frequency of chromosome initiation [14]. There is now evidence that replication proceeds at a constant velocity per fork as in *E. coli* [15] and bidirectionally, with the two forks proceeding at similar rates terminating simultaneously [16].

METHODS

Bacteria

An asporogenic derivative of *B. subtilis* 168 *thy*$^-$ *tryp*$^-$ able to grow on succinate as sole carbon source was isolated (168/S). This strain grows on succinate and glucose with doubling times of 115 and 60 min, respectively. The parent strain had a doubling time of 70 min using glucose as a carbon source and was unable to grow on succinate. *B. subtilis* Nil, a PBSX$^-$ (a defective phage) derivative of *B. subtilis* 168 *thy*$^-$ *tryp*$^-$ was kindly supplied by D. Karamata. This strain is similar to its parent strain, except that it does not lyse during thymine starvation.

Media

Basal medium was as described previously [17], with either glucose (0.4 %) or sodium succinate (0.4 %, pH 7.0) as carbon source. Cultures were grown at 35 °C.

Synchronous cultures

Synchronous cultures were prepared as described previously [17]. However, cells of strain 168/S were retained less strongly by glass fibre filters than those of strain 168 and a slight modification of the method has been made. Five layers of GF/C filters (Whatman) were used with a starting negative pressure of 8 cm Hg rising to 20 cm Hg. The filtrate was then collected at a negative pressure of 40 cm Hg on 1 GF/F filter. This filter was then turned over and eluted at low negative pressure (2 cm Hg) with an appropriate volume of medium.

As with the earlier studies [17], the selected population of cells was enriched at least 2.5-fold with cells from the smallest size quartile of the exponential phase population. The number of cells per ml of culture was determined using a Coulter electronic particle counter. Samples were collected in an equal volume of 10 % formalin and were counted in 2 % saline. All bacterial numbers represent particle numbers. No correction for the number of septate cells has been made.

Nuclear stain

Bacteria were heat fixed onto glass slides, hydrolysed with 1 M hydrochloric acid for 7 min at 60 °C, washed in phosphate buffer (0.1 M, pH 7.0) and then stained with Giemsa's stain (B.D.H.), which was diluted 1 : 10 with phosphate buffer and filtered. The average number of nuclei per bacterial unit was determined by counting the nuclei in at least 200 units in two separate preparations. A bacterial unit may include cells with septa [17]. The time elapsing between nuclear division and cell separation (y) is given by the relationship $N = 2^{y/g}$ where N is the average number of nuclei per bacterial unit in an exponential phase population and g is the generation time [12].

Pulse labelling

Samples (1 ml) of a synchronous culture were transferred to tubes prewarmed to 35 °C (diameter 1.5 cm) containing 1 μCi of [^{3}H]tryptophan or [^{3}H]thymine (labelled in the methyl group) in 0.1 ml of water. (Specific activities 10–25 Ci/g or 0.1–0.2 mCi/μg, respectively.) [^{3}H]Histidine (1.25 Ci/g) was also used at 0.05 μCi per ml of sample. Samples were incubated at 35 °C with shaking for 9 min, after which the pulse was terminated by addition of 1 ml of an ice-cold solution containing chloramphenicol (200 μg/ml), sodium azide (0.002 M), tryptophan (200 μg/ml) or thymine (200 μg/ml), after which there was no significant increase in radioactivity incorporated. Cells were sedimented and resuspended in 0.25 ml of citrate/phosphate (0.01 M sodium citrate, 0.1 M sodium phosphate, pH 7.3), containing lysozyme (200 μg/ml). When lysis was complete, samples were mixed vigorously and were diluted with 2 ml of citrate/phosphate. Thymine-labelled samples were precipitated with an equal volume of ice-cold 10 % trichloroacetic acid and collected on Whatman GF/C filters. Carrier membranes (50 μg protein) were added to tryptophan-labelled samples which were then sedimented at 15 000 rev./min for 15 min and washed once more. Sediments and the pooled supernatants were precipitated with trichloroacetic acid (5 % final concentration), heated at 70 °C for 15 min with bovine serum albumin (100 μg per sample) and collected on Whatman GF/C filters. These filters were then counted in toluene-based scintillation fluid (0.4 % 2,5-diphenyloxazole and 0.1 % 1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene at an efficiency of about 25 %. During growth of the *thy*$^-$ *tryp*$^-$ strain the exogenous concentration of thymine and tryptophan falls continuously throughout the experiment. Specific activities of the isotopes used in pulse labelling were therefore increasing during the course of the experiment. Standard curves were prepared showing the amount of thymine or tryptophan consumed plotted against culture absorbance. This standard curve was then used to calculate the amount of thymine and tryptophan present in the medium at the time of the pulse. During the course of the experiment shown in Figs 1 and 2, approximately 10 % and 30 %, respectively, of the thymine and tryptophan present initially were consumed (10 and 2 μg/ml).

RESULTS

Synchronous cultures of B. subtilis 168/S

Synchronous cultures of *B. subtilis* 168/S were prepared by isolating small cells from an exponential phase population. In previous studies [7] with *B. subtilis* 168 *thy*$^-$ *tryp*$^-$, the mass growth rate of selected cells was usually 15 % lower than before selection. In the new strain the post-selection mass growth rate on both succinate and glucose media is not significantly reduced below the preselection rates (mean generation times 115 and 60 min, respectively). The strain 168/S also usually exhibits greater synchrony than the parent when comparing selected populations with the same degree of enrichment for small cells. The time courses of cell division for glucose- and succinate-grown synchronous cells are shown in Figs 1 and 2. The beginning and end of each cycle has been defined operationally as the point at which the highest rate of division occurs. In the glucose-grown cells, the fastest rates of cell division occur at about 40, 100 and 160 min (± 10 min). In succinate-grown cells, cell division occurs at 80 and 200 min (± 20 min). These points are marked by dashed vertical lines in

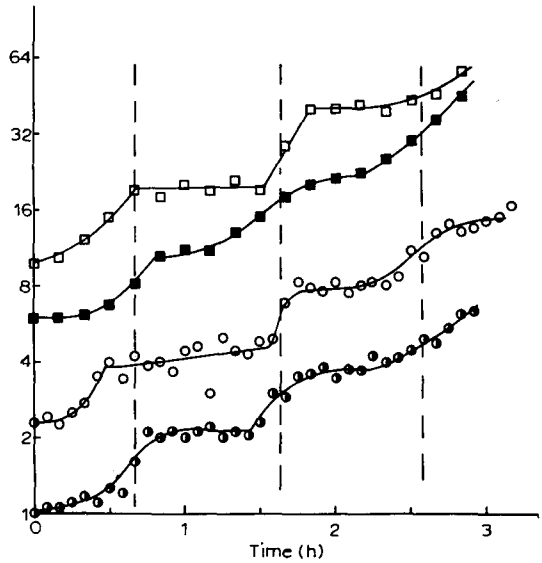

Fig. 1. Membrane and DNA synthesis in synchronous cultures of 168/S grown on glucose. ◑, rate of DNA synthesis × 2 ng of thymine incorporated/min per ml of culture; ○, rate of membrane protein synthesis, ng of tryptophan incorporated/min per ml; ■, cell numbers, $\times 10^{-7}$ Coulter units/ml; □, number of nuclei per ml $\times 10^{-7}$ (cell number × average number of nuclei per cell). Vertical dashed lines represent the end of each cycle.

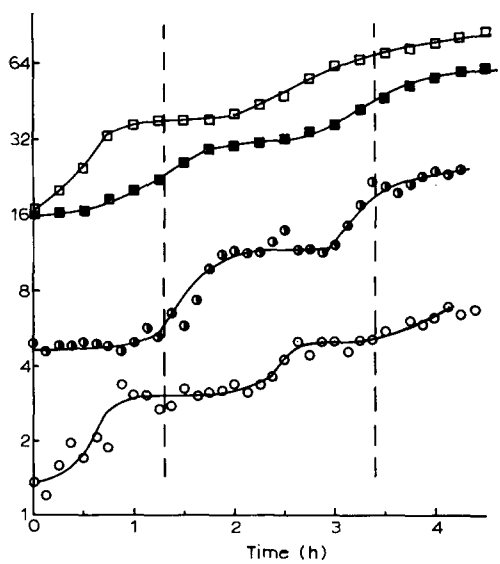

Fig. 2. Membrane and DNA synthesis in synchronous cultures of 168/S grown on succinate. Symbols used are as for Fig. 1, except ◑, rate of DNA synthesis × 1 ng thymine/min per ml of culture.

Figs 1 and 2. Division occurs at a substantial rate over about one third of a cycle, but the high accuracy with which cell numbers can be counted allows a more precise estimate of the beginning and end of each cycle.

In studies of timing of cell-cycle events, described below, the time at which the fastest rate of DNA synthesis or membrane protein synthesis occurs (to the nearest 10 min) has been used. The period of increase between plateaus is in most cases about one third of a cycle in length. This is a reflection of the lack of perfect synchrony so that an error of one third of a cycle is attached to each figure.

In both experiments, the number of nuclei per cell was determined using the Giesma stain (as described above). These appear as discrete entities and can be enumerated unambiguously, as there are very few apparent intermediate forms [18]. The time course of nuclear division is shown in Figs 1 and 2 by plotting the average number of nuclei per ml (i.e., average number of nuclei per cell $\times$ cell number per ml of culture). In both glucose- and succinate-grown cultures, the time course of nuclear division is more synchronous than that for cell division.

In glucose-grown cultures]Fig. 1], nuclear division occurs at 30 ± 10 min, this being more or less the time of cell separation after the first division, and at 100 ± 10 min, slightly after the second period of cell separation (i.e. about 60 min before cell separation). In succinate-grown cells, nuclear division occurs at 30 ± 20 and 160 ± 20 min, which in both cycles is about 40–50 min before cell separation. The average number of nuclei per cell in exponential phase cultures of 168/S grown on succinate and glucose is 1.37 and 1.8 per cell, respectively [9]. The time elapsing between nuclear division and cell separation (i.e. nuclear separation time) can be calculated from these figures (as shown above) and gives values of 52 and 49 min [12], respectively. There is therefore reasonable agreement between the nuclear separation time in exponential phase cultures and in synchronous cultures, suggesting that the selection process does not distort this relationship and that growth is moderately well balanced in the two growth media. The interval between each cell division is close to the mean mass doubling time and each step is within 20 % of a doubling in number.

DNA replication in synchronous cells

The rate of DNA synthesis in glucose- and succinate-grown cells has been measured by pulse incorporation of radioactive thymine. In both glucose- and succinate-grown cultures there are abrupt increases in rate, followed by plateaus of constant rate (Figs 1 and 2). Each plateau is approximately 2-fold greater than the previous one. In the glucose-grown cells (Fig. 1) the sharp increase in rate occurs at 40 ± 10 and 100 ± 10 min, more or less coincident with the end of each cell cycle. In succinate-grown cells, the rate increases at 90 ± 20 and 200 ± 20 min. The first increase occurs slightly after cell separation in the first cycle, whereas the latter occurs almost simultaneously with separation in the second. These observations can be interpreted in terms of the Cooper-Helmstetter model of DNA synthesis [19, 20] (see Discussion). The abrupt changes in rate of DNA synthesis probably represent the time of chromosome initiation in each cycle, but termination is not evident in either set of data.

Membrane synthesis in synchronous cells

The rate of synthesis of membrane protein has been measured using pulse incorporation of radioactive tryptophan, as described previously [7]. In that study,

magnesium-stabilised membranes isolated from protoplasts by osmotic shock were extracted with citrate/phosphate to give a reproducible sedimentable fraction that contained all the cells' succinic dehydrogenase. The citrate/phosphate extraction removed almost all the membrane-bound ribosomes and some loosely attached proteins (NADH-dehydrogenase), but left behind a virtually intact ghost. To simplify this procedure, pulse-labelled bacteria have been lysed directly in citrate/phosphate and washed once. The yields of membrane obtained by the new method and the specific activities of succinic dehydrogenase in them are similar to those obtained with the previously described method. The rate of membrane protein synthesis increases in steps in both glucose- and succinate-grown cells. Thus in glucose-grown cells an increase in rate of membrane synthesis occurs at about 25 ± 10 min and 95 ± 10 min, and there are indications of a third burst at about 150 min. The first burst occurs slightly before cell separation and the second occurs at cell separation. In succinate-grown cells, the rate of membrane synthesis increases most sharply at 40 ± 20 min and again at 150 ± 20 min.

These data are shown in Table I, together with the times of DNA initiation, nuclear division and cell division. In succinate-grown cells, the maximum rates of nuclear division and of membrane synthesis occur almost simultaneously, about 40–50 min before cell separation, whereas DNA initiation is clearly temporally separated from these events, occurring at a maximum rate at or just after the time of cell separation. In glucose-grown cells, the maximum rates of DNA initiation, nuclear division and membrane synthesis occur at about the time of cell separation. There is, therefore, a clear qualitative difference between succinate- and glucose-grown cells. The data shown in Table I are derived solely from the experiments in Figs 1 and 2, but similar conclusions have been reached in at least three experiments with each medium. As in

TABLE I

TIMING OF CELL CYCLE EVENTS IN SYNCHRONOUS CULTURES OF *BACILLUS SUBTILIS* 168/S

Time of events (in min) are from Figs 1 and 2. Each time represents the point in each cycle at which the rate changed fastest. In each case, the period of most rapid change occurs over one third of a cell cycle, so that the error in these figures is ±20 and ±10 for succinate and glucose, respectively.

	Time (min)	
	Succinate medium	Glucose medium
Generation time	115	60
1st cycle		
Nuclear division	30	30
Doubling in rate of membrane protein synthesis	40	25
DNA initiation	90	40
Cell separation I	80	40
2nd cycle		
Nuclear division	160	100
Doubling in rate of membrane protein synthesis	150	95
DNA initiation	200	100
Cell separation II	200	100

the previous study of membrane synthesis in synchronous cells [7], the rate of cytoplasmic protein synthesis increased exponentially (data not shown), throughout the cell cycle at the same doubling time as total mass increase.

Effect of thymine starvation on rate of membrane synthesis

The effect of inhibition of DNA synthesis on the rate of membrane synthesis was investigated in *B. subtilis* Nil, a strain derived from *B. subtilis thy⁻ tryp⁻*, believed to be free of the defective phage PBSX (Karamata, D., personal communication). Thymine starvation of the parent strain causes lysis.

Strain Nil grown on glucose minimal medium was collected on a Millipore filter (pore size 0.45 μm), washed with fresh medium lacking thymine and was resuspended in medium without thymine. The rates of synthesis of membrane and cytoplasmic proteins (Fig. 3) were measured using 9-min pulses of [^{3}H]histidine, as described above. The rate of cytoplasmic protein synthesis continued to rise after the start of thymine starvation, and reached a constant rate after one generation, at approximately twice the initial rate. On the other hand, there was no increase in rate of membrane protein synthesis during thymine starvation, indicating that an increase in rate of membrane protein synthesis requires DNA synthesis. Similar results were obtained using tryptophan labelling and correcting for changes in specific activity as described above [7].

During thymine starvation, cell division continues for about one generation time (Fig. 3) and then stops, giving a 67 % increase in particle number. During this time, a number of septa are completed and the cells containing them fail to separate

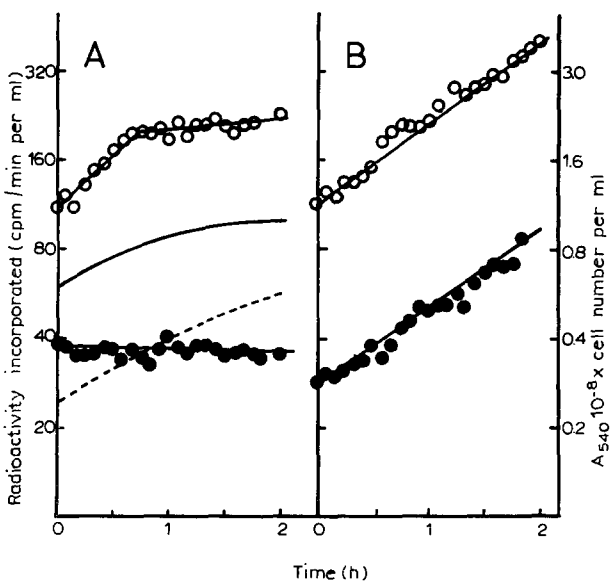

Fig. 3. Rate of membrane synthesis during thymine starvation. Strain Nil was collected on a millipore filter (0.45 μm) and resuspended in fresh medium lacking thymine (A) or containing thymine (B). 1 ml samples were pulse labelled with [^{3}H]histidine for 9 min. ○, Cytoplasmic protein; ●, membrane protein. (—) Cell number per ml; (- - -) A_{540}.

(i.e. 20 % of the cells after 2 h contain a septum). Cells in which the chromosomes are not complete are unable to divide during thymine starvation, whereas those in which chromosomes are complete are committed to division [19]. The experiment also shows, therefore, that no further increase in the rate of membrane synthesis is required for division of those cells which have completed their chromosomes.

DISCUSSION

Cooper and Helmstetter [19, 20] have shown that the reference point of cell-cycle events, such as DNA initiation and termination, should be the cell division to which these events contribute. Thus, they have shown that a fixed time may elapse between such events and cell separation over a range of growth rates and that the actual cell ages at which these events occur under various conditions can be variable. The qualitative difference between succinate- and glucose-grown cells, in the temporal relationship between the doubling in rate of membrane protein synthesis, DNA initiation, nuclear division and cell separation, can be interpreted in this way.

Nuclear division occurs at about the time of cell separation in glucose-grown cells (i.e. 60 min before the next division) and at mid-cycle (40–60 min) before cell separation in succinate-grown cells. In exponential phase cells on both media, nuclear division occurs about 50 min before cell separation, thus demonstrating that the synchronous cultures are not seriously perturbed by the selection procedure. The time taken for nuclear division during the synchrony experiments reflects principally the scatter of cell ages in a culture of less than perfect synchrony. The length of time during which any individual nucleus is dividing is apparently short]18]. Using the Giemsa stain [12] under the conditions described above, nuclei appear as discrete spherical entities either in the centre of a mononucleate cell or at 25 % from either end of each cell in binucleate cells, with very few intermediate forms. The number of intermediate forms between these two extremes is very small and can be used to calculate the time to complete a division (about 6 min [18]). As this is small compared with the generation time of the organism, this factor is unimportant in the interpretation of the synchrony experiments. The Giemsa-stained nucleus does not, however, represent the true morphology of the nucleus as seen with electron microscopy and it is possible that the actual process of division takes longer than this estimate. I have suggested elsewhere that the stained nucleus represents a condensation of the nuclear body about each chromosomal terminus so that, once termination has occurred, two nuclei are observed [12, 18].

It is widely believed that only those cells which have completed their chromosomes are able to divide during inhibition of DNA synthesis. The number of nuclei present during exponential growth is equal to the amount of residual division during inhibition of DNA synthesis in *B. subtilis* 168/S [12]. Therefore the number of nuclei per cell can be used as an estimate of the number of chromosomal termini per cell.

The acceleration in rate of membrane protein synthesis occurs approx. 60 min before cell separation in glucose-grown cultures and 40–50 min before cell separation in succinate-grown cultures. The qualitative difference in the time of acceleration in rate relative to the age of the cell found in the two media suggests that, during the cell cycle, the time elapsing between the increase in rate and the subsequent cell division tends to be constant at different growth rates (40–60 min), rather than pro-

portional to growth rate. In the experiments shown, the time at which the acceleration in rate of membrane synthesis occurs is quite well correlated with the time of nuclear division. In glucose-grown synchronous cells, although the time between nuclear segregation and cell separation is slightly longer than in exponential phase cells, the acceleration in rate of membrane synthesis is still correlated with nuclear division. I suggest, therefore, that the acceleration in rate of membrane synthesis occurs at or close to nuclear segregation in exponential-phase cells.

In reconstructing the time course of membrane synthesis in individual cells from the labelling pattern of the less than perfectly synchronous cells described above, the simplest interpretation of the discontinuous change in rate of membrane synthesis is that in each cell cycle it is linear with a doubling at a specific time in each cycle. The variation in cell age within the population of synchronous cells used would obscure certain more complex schemes of membrane synthesis. Thus, if at one point in the cell cycle the rate of membrane protein synthesis increased greatly and then fell, a period of relatively low synthetic rate could be obscured by the scatter in cell age.

The doubling in rate of membrane protein synthesis may represent a doubling in the number of sites of insertion of membrane protein. This view is supported by Ryter [4] who has shown that sites of surface extension (shown by segregation of flagella) are found only around the nuclei in exponential-phase *B. subtilis*. Furthermore, an analysis of the factors determining cell length in rod-shaped bacteria suggests that, if the bacteria grow linearly, the number of sites of length extension double at or close to nuclear segregation (chromosome termination) [4, 11, 12]. Discontinuous changes in rate of membrane protein synthesis support linear rather than exponential growth models.

In both succinate- and glucose-containing media, chromosome initiation occurs close to the time of cell separation. Using the Cooper-Helmstetter nomenclature [19, 20], this indicates that $(C+D)$ (where C is the chromosomal replication time and D is the time elapsing between termination and cell separation) is approximately equal to the generation time or a multiple thereof. Independent estimates of $C+D$ can be obtained from the time taken to reach a new steady state of cell division during a nutritional shift [21]. Approximate values of $(C+D)$ of 230 and 130 min for succinate and glucose, respectively, have been obtained (ref. 12 and Sargent, M. G., unpublished observations). This suggests that, in the synchrony experiments, $C+D$ extends over two cycles on both media. Studies of DNA synthesis during the nutritional shift experiments also suggest that termination and nuclear segregation occur simultaneously and that the values of C are approximately 80 and 180 min for glucose and succinate, respectively (Sargent, M. G., unpublished observations). Ephrati-Elizur and Borenstein have shown that the C period in thymine-requiring mutants of *B. subtilis* extends considerably at low growth rates [22]. Chromosome termination has not been identified successfully in these experiments by radioactive labelling. However, as suggested above, nuclear segregation is probably a marker for termination.

If nuclear segregation and chromosome termination occur simultaneously, the correlation between the times of nuclear segregation and the doubling in rate of membrane synthesis suggests that replication of the chromosome terminus may be required for the doubling in rate of membrane protein synthesis. Evidence that DNA synthesis is required to allow an increase in rate of membrane synthesis has been

obtained by showing that, during thymine starvation, the rate of membrane synthesis is maintained at a constant rate. In contrast, the rate of cytoplasmic protein synthesis increases during thymine starvation. The rate of synthesis of a number of cytoplasmic enzymes is known to be constant during inhibition of DNA synthesis, but examples of increases in rate of synthesis of cytoplasmic protein after inhibition of DNA synthesis have been reported [23, 24]. These results indicate that a large proportion of cytoplasmic protein synthesis can be derepressed during thymine starvation, whereas there is a clear-cut requirement for DNA synthesis to give an increase in rate of membrane synthesis.

The lack of an increase in rate of membrane synthesis in thymine-starved exponential-phase cells also indicates that division of those cells which have completed their chromosomes is not dependent on the doubling in rate of membrane protein synthesis.

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