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"Division Potential" in *Escherichia coli*

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The phenotype of a *minC* mutant has been reexamined and found to correspond closely to the quantitative predictions of Teather et al. (R. M. Teather, J. F. Collins, and W. D. Donachie, J. Bacteriol. 118:407–413, 1974). We confirm that the number of septa formed per generation per cell length is fixed and independent of the number of available division sites and that "division potential" is directly proportional to cell length. In the *minC* mutant, septa form with equal probabilities at cell poles, cell centers, and cell quarters. In addition, we show that the time to next division is inversely related to cell length while division is asynchronous in long cells, suggesting that a single cell can form only one septum at a time.

In 1967, Adler et al. described a mutant of *Escherichia coli* that produced minute, DNA-free cells (minicells) from the cell poles (1). Teather et al. (19) proposed that the mutation(s) in this strain had abolished a system that prevented cell division from taking place at cell poles. They also claimed that the total number of cell divisions (polar plus internal) per cell length was identical in *min* mutant and *min*$^+$ strains. In consequence, minicells were produced at the expense of normal (nonpolar) divisions so that the *min* mutant population consisted of a mixture of normal cells, minicells, and abnormally long cells. The proportion of minicells and the distribution of cell lengths were consistent with a model in which each cell had a number of potential division sites (PDS), one at each pole plus a number of internal sites in proportion to the total cell length (i.e., 1 PDS per pair of complete unit cell lengths), and in which a fixed number of quanta of "division potential" (equal to one quantum per pair of complete unit cell lengths) were assigned at random among the available PDS. Each quantum of division potential was proposed to be inactivated in the formation of a single septum.

Davie et al. (6) showed that the Min phenotype in the original minicell-producing mutant was due to a mutation at a single locus (*minB*), and de Boer et al. (7) showed that the mutation was in a gene, *minC*, that encoded a protein inhibitor of cell division (MinC) and that this gene was part of a three-gene operon including *minD*, encoding an ATPase required for the activation of MinC, and *minE*, coding for a protein that localizes the action of the MinC inhibitor to the cell poles. Bi and Lutkenhaus (3) showed that increasing amounts of FtsZ protein in a *minC* mutant increased the total number of divisions per cell length so that, if enough FtsZ was produced, every PDS, both polar and nonpolar, was used for division in every cell cycle. They therefore proposed that FtsZ protein was the limiting division factor that had been proposed in the Teather model (19). Bi and Lutkenhaus (5) also showed that FtsZ, now known to be a tubulin-like GTP-binding protein that, in normal cells, forms a ring around the inside of the cell membrane in the center of the cell at the initiation of division (4), formed a ring at the pole in *minC* cells that were producing minicells.

Despite the general agreement between these experimental observations and the original model of Teather et al. (19), other suggestions about the basic defect in *minC* mutants have been made. Jaffé et al. (14) reported that *minB* mutants produced minicells of a variety of sizes (unexpected if polar division sites are at fixed locations), together with a small proportion of normal-sized cells without DNA. Using 4′,6-diamidino-2-phenylindole (DAPI)-stained fixed cells, they reported that some *minC* cells had abnormalities in DNA localization. They suggested that the mutation had affected chromosomal DNA partition within the cell and that the abnormal localization of division sites could be the consequence of the appearance of DNA-free areas (especially near the cell poles) at which division could take place. Mulder et al. (16) confirmed that the DNA segregation in *min* mutants was abnormal and also reported that plasmids in these cells showed decreased negative supercoiling. Åkerlund et al. (2) also reported that minicells varied in size, although they did not report any normal-sized cells without DNA, and that the distribution of DNA within long *minC* cells (and cells with the whole *min* operon deleted) was sometimes abnormal (in comparison with DNA distribution in elongated *min*$^+$ cells). Because of the reports that minicells were of various sizes, Donachie (8) proposed a modification of the original model (19) in which the cell poles were not inactivated division sites but simply DNA-free parts of the cell that would be usable as PDS, except when blocked by the action of the *min* genes. This modification would allow for the formation of minicells in a narrow range of sizes.

Åkerlund et al. (2) questioned the claim (19) that an amount of division potential sufficient for one septum was produced per unit cell length doubling. Thus, according to Teather et al., if a cell reached two unit lengths it could make one septum; if it then used this to make a polar division, the cell would have to grow to four unit lengths before it would have twice the amount of potential to use at two of the five PDS, and so on. A *minC* cell of n units (i.e., with n chromosomes) would have one amount of division potential for every new pair of sister chromosomes, which would then be used at the $(n + 1)$ PDS. However, Åkerlund et al. (2) found that divisions in clones of *minC* cells could take place at intervals that were much less than one cell length doubling time. On this basis, they questioned the idea that fixed amounts of division potential were produced at fixed times in each cell cycle. Because of this report, we have reexamined the behavior of *minC* mutant cells. Our new measurements confirm that both *minC* and wild-type cells have the same division potential that is used up to make the same integral number of septa. However, we also confirm the observation of Åkerlund et al. (2) that the average interval

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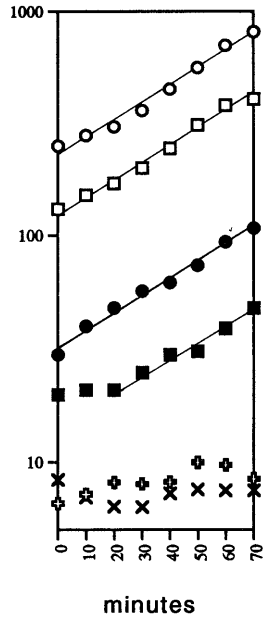

FIG. 1. Growth of C600 and C600*minC* cells on agar. At 0 min, cells from exponential-phase cultures in liquid NB were placed on thin layers of NB agar on microscope slides, covered with coverslips, and photographed at 10-min intervals. (Cells were maintained at 37°C throughout.) A total of 20 C600 cells and 24 C600*minC* cells were photographed, and the total number of cells (F, C600*minC*; ■, C600) and the total length of the cells (E, C600*minC*; □, C600) in all the clones of each strain are plotted against time. Nota bene: in the C600*minC* microcolonies, the lengths of minicells were ignored, because they made little difference to the summed cell lengths, and the total number of cells at each time interval was corrected by adding a number equal to 0.75 × the number of rod-shaped cells present at time zero. This is the best estimate of the number of minicells that were present in the field at time zero (see text and Fig. 3). The estimated doubling times for length and number, respectively, were 40 and 39 min for C600 and 38 and 39 min for C600*minC*. The logarithmic ordinate shows the summed cell number for all clones, the summed cell length for all clones (1 unit = 0.567 μm), and the ratio of summed length to summed number („, C600; ×, C600*minC*).

between cell divisions is less than the length-doubling time in the *minC* mutant. (This is possible because a proportion of the products of division, the minicells, do not divide again.) To explain this, we propose a minor modification of our original model (19), namely, that a cell cannot form more than one septum at a time. Long cells with a large amount of division potential therefore express this successively, at intervals of 10 min or less, over the following cell cycle.

MATERIALS AND METHODS

Strains. The wild-type strain *E. coli* K-12 C600 was transduced with a P1 lysate grown on a strain derived from P678-54 (1) in which a Tn*10* had been inserted close to the *minCDE* locus (2a). Among the tetracycline-resistant transductants, ~60% showed the typical *minC* phenotype (production of minicells, together with a broad range of cell lengths). One of these transductants, C600*minC*, was used in our experiments.

Culture and measurements. Cells were grown in Oxoid Nutrient Broth no. 2 (NB) at 37°C with shaking. Aliquots of mid-log-phase cultures were spread on thin layers of NB agar, covered with a coverslip, and photographed under phase-contrast at 10-min intervals with a Zeiss Photomicroscope. The slides were maintained at 37°C during this period. The negatives were projected, and the cells were measured with a ruler.

RESULTS

Growth rates in mass and cell numbers are similar in isogenic *minC*$^+$ and *minC* mutant strains. Cells of both strains (C600 and C600*minC*) were grown at 37°C in NB, transferred to the surface of a thin layer of NB agar on a prewarmed microscope slide, covered with a coverslip, and maintained at 37°C. Photographs were taken every 10 min. Enlarged projections of the negatives were used to measure the growth and division of clones derived from single cells. Summed cell lengths and numbers were used to construct growth curves. Figure 1 shows that after a variable initial lag the cells grew exponentially and that the generation times for both strains are similar (38 to 40 min at 37°C on NB agar). Growth rates of cells in liquid cultures were also identical for both strains (data not shown).

Minimum cell length after division is the same in *minC*$^+$ and *minC* mutant strains. Figure 2A shows the distribution of cell lengths in clones of *minC*$^+$ or *minC* mutant cells after 100 min of growth on agar. The range of cell lengths in C600*minC*

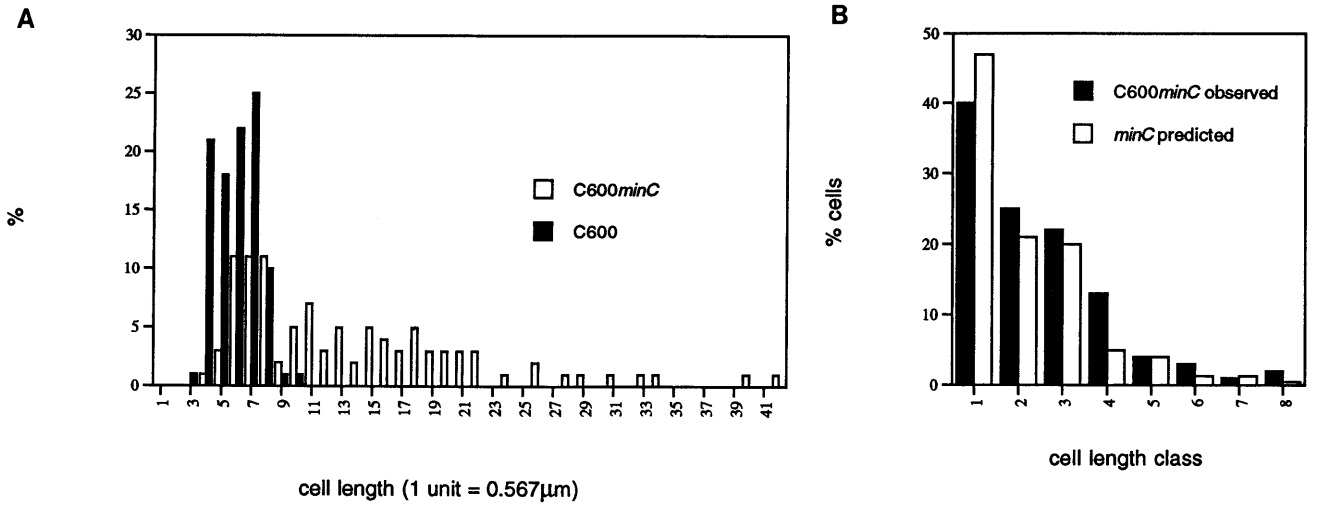

FIG. 2. Distribution of cell lengths in exponential populations of C600 and C600*minC* growing on NB agar at 37°C. (A) Cell lengths in the microcolonies arising from single cells after 100 min were measured (lengths of minicells were not measured). (B) Distribution of cell lengths in C600*minC* compared with the distribution predicted by Teather et al. (19). To make this comparison, cells were grouped into length classes; a length class equals the twofold length range (2.27 to 4.54 μm) within which 96% of C600 cells lie (see panel A).

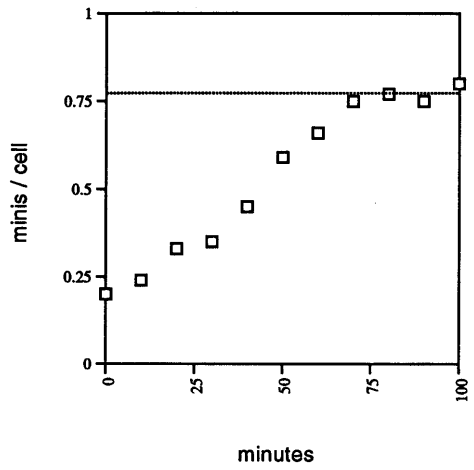

FIG. 3. Proportion of minicells to normal cells during growth of C600*minC* clones. The ratio of minicells to normal cells was measured during growth of clones arising from 20 single cells on NB agar at 37°C. Because minicells do not give rise to clones and are difficult to score separately the initial proportion of minicells is underestimated (with only those minicells actually attached to normal cells being scored). However, as the number of cells in the clones increases, scorable minicells accumulate and approach the ratio for a population of infinite size growing on agar.

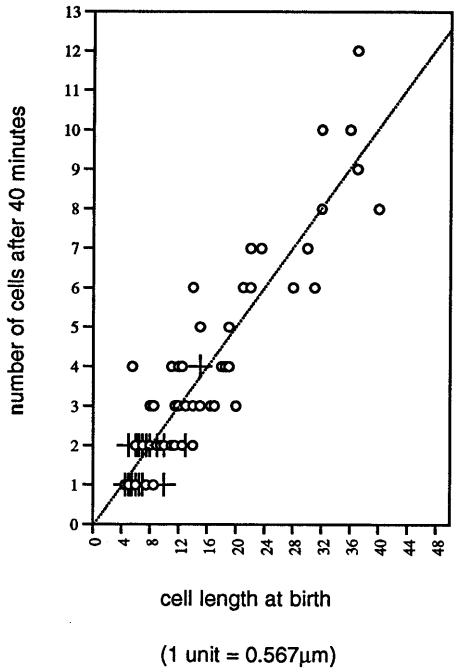

FIG. 4. Division potential is a linear function of cell length in C600*minC*. The lengths of C600*minC* cells in microcolonies were measured immediately after division (cell length at birth) and plotted against the number of daughter cells produced after 40 min (E). A total of 64 newborn cells, which gave rise to 236 daughter cells (including minicells) after 40 min, were measured. A linear regression of number of daughter cells after 40 min (y) on mother cell length at 0 min (x [in micrometers]) gave $y = 0.444x - 0.045$ (dotted line), with a regression coefficient of 0.916. (Cells with the same length that gave rise to the same number of progeny are superimposed on the graph but entered separately for regression analysis.) Similar measurements for C600 cells are entered in the same graph (+) but not used in the regression analysis.

is much greater than that in C600 (giving an average cell length, when minicells are excluded, roughly twice that of the wild type, i.e., 7.6 versus 3.4 μm), but the smallest cells (again excluding minicells) are about the same size in both populations (~2.3 μm). Thus, the mutant cells never divide to give cells shorter than those produced by the wild type (except for divisions at or near the cell poles, giving minicells).

Taking the range 2.27 to 4.54 μm as one to two "newborn cell lengths," the length distributions for C600*minC* can be compared with those predicted by the model of Teather et al. (19). Figure 2B shows that the observed distribution of cell lengths corresponds closely to this theoretical prediction, as previously found for the original P678-54 strain (19).

The ratio of minicells to normal cells is about 0.75 in the *minC* strain. The model of Teather et al. (19) predicts that the ratio of minicells to nucleated cells will be 0.75 in an exponential population. It is difficult to accurately identify all the minicells in a sample of a culture because they are so small (similar in size to cell debris and other small detritus). Thus, in the experiments described here in which a small sample of an exponential culture is spread on an agar surface, it is not easy to estimate the number of minicells visible in a microscope field at time zero. However, it is easy to recognize a minicell when it is being divided off from a cell end. Therefore, we have scored only those minicells that were seen to arise from cell ends during growth of the nucleated cells on the agar. In consequence, the proportion of minicells identified per nucleated cell rises asymptotically as the microcolony grows. Figure 3 shows that the initial ratio of minicells (i.e., those physically attached to the end of a cell at time zero) to normal cells does indeed rise during subsequent growth of the clones to plateau at about 0.75.

The number of septa per cell length is the same for *minC*$^+$ and *minC* mutant strains. We used this number (0.75) to correct the total cell number (minicells + normal cells) and the average cell length [i.e., total cell length/(total cells + minicells)] during the growth of clones. Figure 1 shows that the values for total cell length/total cell number (including minicells) are closely similar for wild-type and *minC* mutant strains. The fluctuations in the calculated value are probably due in part to the small sample sizes and in part to variations in growth rate which themselves affect cell length at division (9–11), but it is nevertheless clear that the two strains have closely similar division potentials. (After 100 min of growth on agar, the average cell lengths were 3.4 and 4.3 μm for C600 and C600*minC*, respectively.)

Division potential is a linear function of cell length. The model of Teather et al. (19) explains the presence of long cells in *minC* populations by supposing that random choice among PDS over a number of past generations has resulted in a higher-than-average number of polar divisions in the clone of which the long cell is a member. Consequently, these long cells are themselves fully capable of further division, giving numbers of progeny cells that are directly proportional to their lengths. To test this, the length of every cell in every clone was measured after division, and then the number of progeny cells arising from each measured cell was determined after a fixed time interval. Figure 4 shows the relationship between a cell's length after division and the number of cells to which the cell then gave rise after 40 min. The points in this graph show a large scatter, because measurements were made at 10-min intervals; thus, each division must have occurred between 0 and 10 min before it was scored. Nevertheless, there is a clear linear relationship between a cell's length 0 to 10 min after "birth" and the number of progeny to which it gives rise exactly 40 min later. The calculated linear regression is $y = 0.444x - 0.045$ (regression coefficient, 0.916), where y equals the number of cells arising after 40 min from an initial cell of length x (in

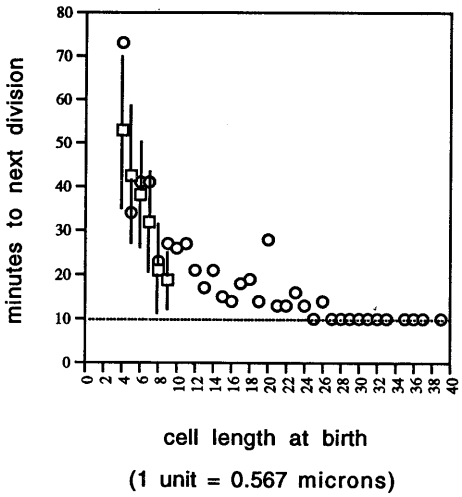

FIG. 5. The longer the cell at birth, the shorter the time until the next division. The lengths of all C600 (□) and C600*minC* (E) cells in microcolonies were measured immediately after division and plotted against the time until their next division. Standard deviations (bars) are shown only for C600 cells. A total of 148 C600 cells and 253 C600*minC* cells were measured. (Nota bene: because photographs were taken at intervals of 10 min, this is the shortest observable interval between divisions. The true minimum interval is therefore probably shorter.)

micrometers). Because 40 min is almost exactly the doubling time for cell number and length (Fig. 1), we can calculate the length of a cell that will give rise to two daughter cells one generation time later (i.e., the average length of a "newborn" cell): this is 2.35 μm. The average cell length in the wild-type strain C600 is 3.4 μm (see above), and therefore the approximate length of a newborn cell can be estimated (17) as $3.4 \times \ln 2 = 2.36$ μm.

The longer the cell, the shorter the time until the next division. The data in Fig. 4 show that as many as 12 progeny cells can be formed from a single long C600*minC* cell in one length-doubling time. Therefore, in this case 11 septa were formed in 40 min. These divisions could have taken place simultaneously or at short intervals. In fact, even a long cell is seldom observed to have formed more than a single septum during the 10-min interval between observations. (After the first division, each sister cell can of course form a single septum during the next 10 min, and so on.) Figure 5 shows the measured interval between one division and the next for cells of different lengths during the growth of microcolonies of C600 and C600*minC*. Each datum point is the average interdivision time for all cells in that size class. The number of cells in each size class is very variable (with most cells of C600 dividing at about the same length), and standard deviations for the C600 points are shown. There is a clear negative correlation between the cell length and the time to the next division, with cells of normal newborn cell length (~2.8 μm) showing the average interdivision time of about 40 min. This confirms the original observation of Schaechter et al. (18) that formed part of their evidence that cell size determines the probability of cell division (15).

The shorter cells in the C600*minC* microcolonies showed a similar relationship between length and time to division, but the longer cells, whose lengths fall outside the range of those for C600, showed progressively shorter interdivision times, down to the minimum observational interval of 10 min. Thus, the average division interval is much shorter for populations of C600*minC* because of the presence of long cells (although the doubling time for the whole population is unchanged from that for C600 [Fig. 1]; this comes about because minicells, which are included in the estimated cell number, do not themselves divide).

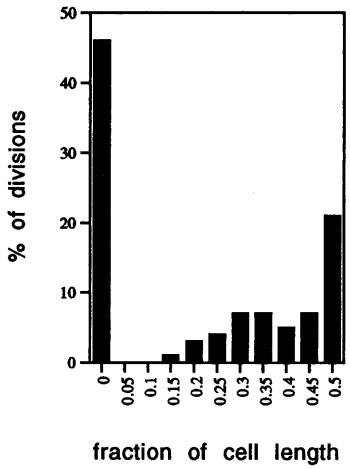

FIG. 6. Frequency of division at different locations along the length of C600*minC* cells. The locations of 141 divisions were measured during the growth of C600*minC* microcolonies on NB agar. (The predicted proportions and locations for a C600*minC* cell of twice the average length of a C600 cell at division are 40% at 0 [poles], 20% at 0.5 [center], and 40% at 0.25 [quarter].)

Septal position in C600*minC* is not random. The locations of division sites in C600 and C600*minC* were measured. Divisions in C600 were always very close to the cell center (data not shown). In C600*minC* cells, however, about 46% of divisions were polar and about 21% took place near the cell center (Fig. 6). The remainder formed a broad peak around 25 to 35% of the cell length. Because the average cell length in C600*minC* is roughly twice that in C600 (see above), the model of Teather et al. (19) would predict that the average C600*minC* cell would have one PDS at the cell center, two PDS at the cell quarters, and two PDS at the cell poles. If each of these PDS had an equal chance of being used for septum formation but the average number of septa formed was limited to two (as predicted by the model), the proportions of polar to central to nonpolar-noncentral septa would be 0.4:0.2:0.4. Thus, the observed locations and frequencies (0.46:0.21:0.33) are in good agreement with the predictions of the model.

DISCUSSION

The original model of Teather et al. (19) was an attempt to explain the peculiar behavior of the P678-54 mutant (1), which produced a mixture of minicells and rod-shaped cells in a wide range of lengths. The model had two main features: that septum formation at cell poles was normally blocked by the action of the *min*$^+$ gene product and that some substance ("division factor") was produced in exactly the amount required for the formation of a single septum per pair of sister chromosomes. More recent work has confirmed that the products of the *min* operon do indeed block septation at the cell poles (7) and that FtsZ protein has many of the properties of the hypothetical division factor (3). A corollary of the hypothesis was that the available division factors were used in packets, or quanta, because in cells in which there was more than one PDS, the number of septa that formed was equal to the number of pairs of sister chromosomes that had been completed during the last cell cycle; thus, the available division factors were not distrib-

uted evenly among all available PDS, because this should lead to delayed or permanently inhibited division.

The alternative explanations that have been suggested for the *min* mutant phenotype have not been formulated in such a way as to provide quantitative predictions about parameters such as the relative frequencies of polar and nonpolar divisions, cell length distributions, etc., but they have been proposed because of observations that were seen to be at odds with the model of Teather et al. (19). Such observations include the following: that minicells are of variable size (14), that DAPI-stained *min* mutant cells sometimes show what appears to be abnormal localization of DNA (or even no DNA [2, 14, 16]), that *min* mutant cells show altered plasmid supercoiling (16), and that the intervals between successive cell divisions in *min* mutants are erratic (2). The essence of the alternative proposals for *min* function seems to be that the *min* genes are somehow required for proper DNA segregation in cells and that the mislocation of DNA in turn causes the mislocation of septa. Thus, if DNA is not localized so precisely in *min* mutants, the absence of DNA near the cell poles in some cells might allow these sites to be used for septum formation, thus accounting for the variable size of DNA-less minicells. According to such models, PDS are simply DNA-free areas, a concept for which there is experimental support (e.g., see references 8, 12, and 20). However, if DNA (chromosomes) were randomly localized in *min* mutant cells, septa would also be randomly located. Our measurements of septal positions show that this is not so (Fig. 6): septa in *minC* cells are located mainly at the cell poles (46%) and close to the cell center (21%) and intermediate positions (33%). These locations and frequencies are close to those predicted by our original model, i.e., 40, 20, and 40% at poles, centers, and quarters, respectively, for cells that average twice the length of normal cells (C600), such as the cells in our C600*minC* populations. In contrast to others, we have seen no DNA-less cells (except minicells) in DAPI-stained preparations of C600*minC* (data not shown). Furthermore, all cells except minicells were able to grow and divide on agar, demonstrating that all cells larger than minicells contained a full complement of DNA. In addition, although minicells may differ in size over a small range, there are no cells of lengths intermediate between those of minicells and normal newly divided cells (Fig. 2). Similarly, the ratio of minicells to DNA-containing rods was shown in this study to be close to 0.75 (Fig. 3), exactly as predicted by our model. We therefore conclude that, if there is any irregularity in DNA localization in *min* mutant cells, then this does not affect septum localization or the frequency of division at different PDS.

If divisions are localized to the predicted locations in *minC* cells, there remains the question of how many divisions take place per cell cycle. Teather et al. (19) suggested that the number of divisions per total cell mass was exactly the same in wild-type and *minC* populations, thus explaining why the production of minicells (polar divisions) was concomitant with the appearance of abnormally long cells. Our present measurements, with the original *minC* mutation in a new genetic background (C600), show that this must indeed be true, because the final ratios of total cell length to total cell number (including minicells) in a number of microcolonies are closely similar for C600 and C600*minC* strains. This is probably the most accurate estimate of this parameter, because minicells are easy to identify and score at the time that they are formed at the cell poles and can be identified thereafter because they remain in position while the parent cells continue to elongate past them. Minicells therefore accumulate along the lengths of the rod-shaped cells in the growing microcolony and, once formed, appear to be quite stable. The idea that cells produce a limited amount of division potential, sufficient for only one septum per pair of sister chromosomes, is therefore fully confirmed.

If a fixed amount of division potential per chromosome replication is produced, long, multichromosomal cells should produce n times this amount (where n is the number of newly replicated pairs of sister chromosomes). According to the original model (19), this amount should then all be consumed in the formation of n septa. Septum formation in a clone would therefore be expected to take place at intervals of one generation time. Åkerlund et al. (2) found that this was not so, because the interval between divisions was often much less than the doubling time for cell length. Our measurements confirm their finding, and therefore we agree that the original model needs modification. There seem to be two possibilities: either division potential is produced continuously, until enough is present to trigger a division, or it is indeed produced all at once but the cell is incapable of forming more than a single septum at a time. (Nota bene: in both these cases, division potential is used quantally, which is what our original model actually proposed. We did not say that it must be produced in packets.) What we have shown here is that the longer the cell is, the higher its division potential is (Fig. 4) and the shorter the time to division is (Fig. 5). This is consistent with an inability to form more than one septum at a time, something that we had already noticed during the rapid division of long cells after a period of growth when division was prevented (either by an inhibitor such as penicillin, by induction of SulA, or, in the case of *fts* mutants, by being at a restrictive temperature [unpublished observations]).

What can it mean that division potential appears to be used quantally? One possibility is that when some protein or proteins have accumulated in sufficient amount, rapid autocatalytic self-assembly is triggered at a single PDS (some localized aggregate of membrane proteins?), thus depleting the pool of division potential proteins by an amount equal to one quantum. After division, these proteins might be either inactivated (e.g., in a complex with other septal proteins) or distributed equally between each sister cell (including minicells, if any), so that synthesis of an equal amount of new division potential would be required for each unit cell doubling.

The main finding that argues against the original formulation of our model is that of Jaffé et al. (13), who found that filamentous cells, including those in *min* mutant populations, always divide to give progeny cells with 2^n chromosomes (where n is a positive integer). Thus, cells with other numbers of chromosomes ($\neq 2^n$) are rare or absent. This implies that PDS are used not at random but in some kind of pairwise symmetry. In our observations of cells dividing in microcolonies, asymmetric division (e.g., 1:3, 1:7, etc.) was often recorded, in apparent contradiction to that report. Assuming that there is no trivial explanation for this difference, such as strain differences, then part of the solution to the paradox could be that no more than one division can take place at a time within the same cell but that rapid consecutive divisions follow the pattern described by Jaffé et al. The reason for the pattern itself is another matter: perhaps it has something to do with a hierarchy of PDS of different ages, with those of a particular age being used preferentially but not exclusively.

Our new observations are in good agreement with the quantitative predictions of the model (19) in which the production of division potential is limited to an amount sufficient for the formation of only one septum per pair of newly completed sister chromosomes and in which division potential is used quantally to make septa, with the additional fact that divisions are asynchronous in cells with lengths greater than the normal division length (and which therefore divide more than once

during the next length-doubling time). This observation probably applies to all cells that are longer than normal but that have full division potential (e.g., recovering *fts* filaments). By being so close to the quantitative predictions of the model of Teather et al. (19), our measurements do not favor models in which aberrant localization of DNA causes the aberrant localization of septa in *min* mutant cells.

ACKNOWLEDGMENT

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