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Quantal Behavior of a Diffusible Factor Which Initiates Septum Formation at Potential Division Sites in *Escherichia coli*

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Analysis of nucleated cell size in a minicell-producing strain of *Escherichia coli* and in its parental strain shows that the two distributions are considerably different. A model is proposed to account for this difference. The model states that: (i) in the mutant population, the cell poles are available as potential division sites in addition to the normally located division sites; (ii) the probability of a division occurring at any of the potential division sites is equal; and (iii) only enough "division factor" arises at each unit cell doubling to permit a single division. This factor is utilized entirely in the formation of a single septum. Thus, the occurrence of a polar division with the production of an anucleate minicell (which occurs only in the mutant strain) prevents the occurrence of a non-polar division, with the result that the average nucleated cell length is increased in minicell-producing strains. The model has been used to construct a theoretical population, and a number of parameters of the real and theoretical populations have been compared. The two populations are very similar in all of the parameters measured.

In the normal cell cycle of *Escherichia coli*, the cell volume doubles, the cylindrical cells doubling in length without any apparent change in diameter. The cells then divide in the middle of the long axis. Donachie and Begg (6) have shown that a potential division site, detected by its sensitivity to penicillin, is formed very early in the cell cycle. The number of potential sites increases at each doubling of a constant unit cell volume.

In this paper we present an analysis of the pattern of cell growth and division in *E. coli* strain K-12 P678-54, a mutant strain isolated by Adler and Hardigree (1). In this strain, cell division can take place not only in the normal way, by septation between two nucleated regions in the interior of the cylinder, but it can also take place very close to the cell poles. If division takes place at a polar site, a very small, anucleate "minicell" is cut off from the parent cell (Fig. 1). In the wild-type strain, each potential division site is used only once, giving rise to two cell poles. The mutant strain behaves as if potential division sites, once formed, are never inactivated. Hence, the cell poles, which represent the sites of preceding divisions, can serve as sites for further divisions, giving rise to minicells.

MATERIALS AND METHODS

E. coli K-12 P678 (Thr$^-$Leu$^-$Thi$^-$) and the minicell-producing derivative P678-54 were used in these experiments. Minicell production has been reported to be the result of two mutations at different loci (K. Roozen and R. Curtiss III, Abstract no. 4607A of Oak Ridge National Laboratory, 1969). The genotype of P678-54 is therefore Thr$^-$Leu$^-$Thi$^-$MinA$^-$MinB$^-$. Both strains were obtained from H. Adler.

Media and experimental techniques. Cells were grown at 37 C in either L broth (10 g of tryptone [Difco], 5 g of yeast extract [Difco], and 10 g of NaCl in 1 liter) or minimal medium (007 salts [3] plus 0.2% glucose [wt/vol], 50 μg of threonine per ml, 50 μg of leucine per ml, and 10 μg of thiamine per ml). The generation time for both strains was 26 min in L broth and 65 min in minimal medium.

The distribution of cell lengths in exponentially growing populations was obtained by spreading the cells on thin layers of 1.2% agar containing 0.05% sodium azide, and photographing the cells with a Zeiss Ultraphot microscope by using phase contrast. In some experiments, observations were made on living cells growing on a thin layer of 1.2% agar containing growth medium. Measurements were made on enlarged projections of the negatives.

For electron microscopy, cultures (10 ml) were grown in exponential phase to approximately 5×10^7 cells/ml, fixed with glutaraldehyde (10), and centrifuged at 13,000 × *g* for 30 min. The cells were

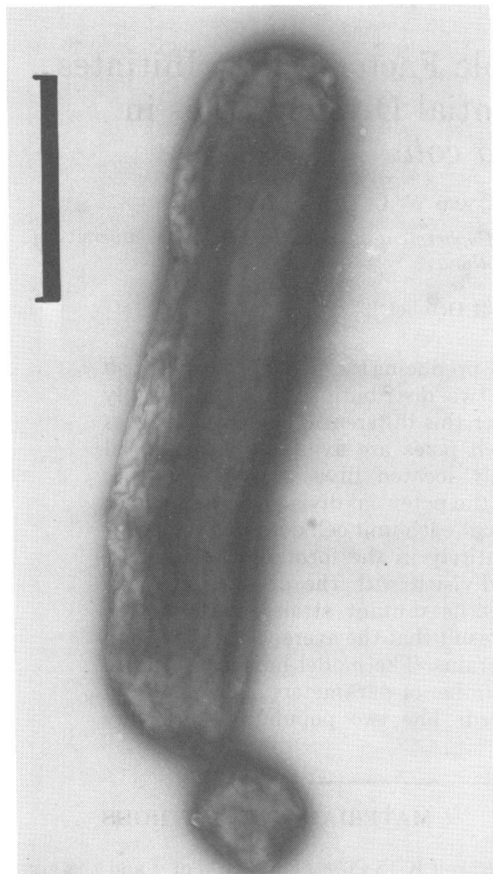

FIG. 1. *Polar division of strain P678-54. Cells were grown at 37 C in L broth, fixed with glutaraldehyde, washed, placed on grids, and stained with uranyl acetate. The photographs were taken with a Siemens Elmiskop 1A electron microscope. Bar equals 1 μm.*

washed twice with 10 ml of 1% ammonium acetate and resuspended in 0.5 ml of 1% ammonium acetate. Samples were placed on freshly prepared grids and stained with uranyl acetate (10). Photographs were taken with a Siemens Elmiskop 1A electron microscope.

RESULTS

The length distribution of 350 cells from an exponential population of wild-type strain P678 is shown in Fig. 2a. Over 95% of the cells lie within a twofold range. This corresponds very well with the size distribution expected from an ideal population in exponential growth.

The cell length distribution of minicell-producing strain P678-54 under the same growth conditions is shown in Fig. 2b. In this case, the range in size of a sample of 400 cells is at least ninefold. The presence of long cells in populations of P678-54 has been reported previously (1). The minimal length for cells is approximately the same for both strains (excluding minicells from the measurements), but the average cell length of P678-54 is 1.68 times that of P678. Cells of both strains grown in minimal medium were smaller, but the relative range of cell size and the shape of the length distributions were unchanged.

Two other minicell-producing strains isolated in this laboratory (Teather, unpublished observations) show a similar proportion of long cells in the population.

A model was devised to account for the length distributions shown in Fig. 2. The assumptions of the model are as follows. (i) Potential sites for cell division arise during cell growth in the same way in both strains. Such sites are active for only one division in strain P678, but they remain active indefinitely in strain P678-54. A division site at which a division has occurred forms two cell poles. In strain P678-54, subsequent divisions can occur at these sites and will produce minicells. (ii) The probability of division is equal at all potential division sites, whether polar or nonpolar. (iii) If a newborn cell in an exponentially growing wild-type popula-

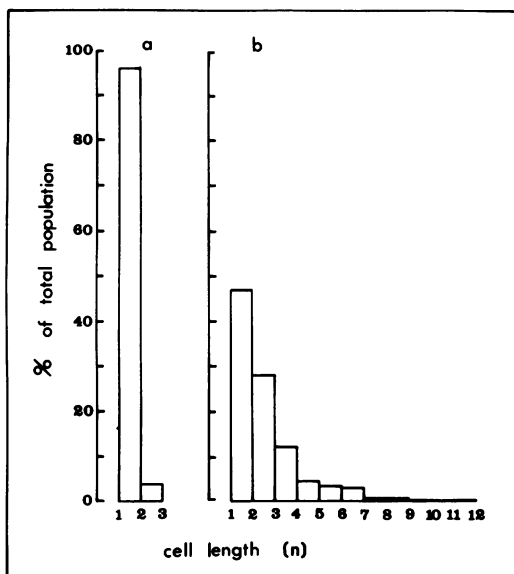

FIG. 2. *Size distributions of strains P678 (a) and P678-54 (b). Cells were grown in L broth, spread on a thin layer of agar containing 0.05% sodium azide, and photographed in a Zeiss Ultraphot photomicroscope using phase contrast. Measurements were made on enlarged projections from the negatives. The size classes were then grouped in multiples (n) of the minimal length (c) of strain P678.*

tion has the length c, then division will not occur until a length of $2c$ is reached. At this size, there is only enough of some specific factor (division factor) for a single division. This division factor is consumed entirely in the formation of a single septum. If there is more than one division site available (as in the minicell-producing strain), the division factor is expressed solely at one of the available sites.

As a consequence, the length of a daughter cell in a minicell-producing strain may be a multiple of the newborn cell size (c) of a wild-type strain. If the length of a particular newborn cell is $n \cdot c$ (where n is an integer), the cell after one doubling in mass will be $2n \cdot c$ in length and will have produced sufficient division factor to undergo n divisions. The number of available division sites will be the normal number of internal division sites ($2n - 1$) plus the two cell poles, giving $2n + 1$ division sites.

Using these assumptions, it is possible to predict the length distribution of a minicell-producing population (Table 1). For any newborn cell of length $n \cdot c$., the table shows the expectation of producing a daughter cell of a particular length one mass doubling later. (The mathematical procedures used to generate these expectations are discussed in the appendix to this paper.)

These expectations have been used to predict the length distribution of an exponentially growing population of cells. The length distribution generated in this way is shown in Fig. 3a. Figure 3b shows the observed and predicted distributions after the predicted distribution was altered by shifting 10% of each size class to each adjacent size class. This transformation of the theoretical distribution was carried out because there is a significant probability of classification errors in the observed distribution when the length of the projected image is within 1 mm of a class boundary. The χ^2 value (10 degrees of freedom) was 3.5, indicating a satisfactory measure of agreement ($P > 95\%$).

The length distribution of the progeny of any cell should approach the predicted distribution within a few generations, even if the initial cell was abnormally long. To test this, individual cells growing on L-broth agar slides were observed and photographed at frequent intervals. Cells at least two times the average length were

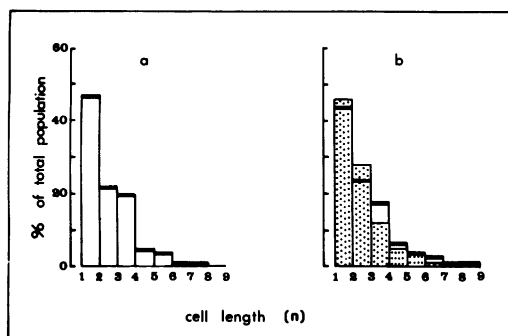

FIG. 3. *Theoretical size distribution according to the model. (a) Predicted distribution. (b) Predicted distribution after "smearing" to allow for probable experimental classification errors (heavy bar). Smearing was accomplished by shifting 10% of the total in each size class to each adjacent size class. Superimposed on this distribution is the observed distribution for strain P678-54 (shaded area).*

TABLE 1. *Predicted pattern of daughter cell lengths*

Newborn cell size	Properties of the cell at division			Relative frequency of newborn cells of sizes shown[a]								
	Size	No. of division sites available	Division factors available	Mini	1c	2c	3c	4c	5c	6c	7c	8c
nc	2nc	$2n + 1$	n									
1c	2c	3	1	0.67	0.67	0.67						
2c	4c	5	2	0.80	1.00	0.70	0.40	0.10				
3c	6c	7	3	0.86	1.43	0.91	0.51	0.23	0.06			
4c	8c	9	4	0.89	1.89	1.15	0.63	0.30	0.11	0.02		
5c	10c	11	5	0.91	2.36	1.39	0.76	0.37	0.15	0.05	0.01	
6c	12c	13	6	0.92	2.85	1.64	0.88	0.43	0.19	0.07	0.02	
7c	14c	15	7	0.93	3.33	1.89	1.01	0.50	0.22	0.09	0.03	0.01
8c	16c	17	8	0.94	3.82	2.14	1.13	0.56	0.26	0.10	0.04	0.01

[a] The expectations given represent the relative frequency with which parental cells, when they divide, will give rise to daughter cells of the sizes shown. The sum of the expectations in any given case is equal to $n + 1$, the number of daughter cells produced from one cell. For example, a normal *E. coli* cell, dividing at length $2c$, gives rise to two similar daughter cells of length c. The expectation for cells of length c arising is therefore 2.

selected for this analysis. Figure 4 shows an example of a life history of such a cell (initial length, 5c) and its progeny. Because the predicted distribution applies only to exponentially growing cells, the rate of growth of individual clones was measured. Figure 5 shows that the growth of the cells under such conditions is in fact exponential. A combined size distribution for the progeny of these large cells after two to three generations is shown in Fig. 6, superimposed on the predicted distribution from Fig. 3b. The value of χ^2 (10 degrees of freedom) was 3.0, again indicating a satisfactory measure of agreement ($P > 97\%$).

Other predictions of the model were tested. An exponentially growing population of wild-type cells which range in length from c to $2c$, where c represents the normal newborn cell size, should have a mean length of $1.44c$ (12). If the normal, minimal, newborn cell length in P678-54 is also c under the same growth conditions, then the predicted mean cell length for P678-54 is $2.53c$ (see Appendix for details of these calculations). The mean lengths observed for P678 and P678-54 were $1.46c$ and $2.46c$, respectively.

Another prediction of the model is that there should be a constant ratio (if the minicells are stable) of 0.75 minicells per nucleated cell in an exponential population (see Appendix). A direct count of minicells, with an electron microscope, gave a ratio of 0.67 ± 0.08. This method may possibly underestimate the proportion of minicells; first, because minicells may be preferentially lost during preparation for electron microscopy and, second, because the length of time for which minicells persist after formation is unknown. Measurement of the relative frequencies of polar and nonpolar divisions was therefore made by microscope observation of living cells growing on nutrient agar. The ratio obtained by this method was 0.72 ± 0.11.

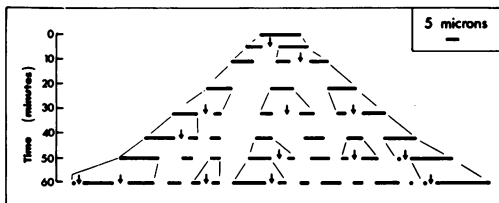

FIG. 4. *Growth and division of a large cell of strain P678-54 growing on L broth plus 1.5% agar (Davis) at 37 C. Arrows show divisions that have occurred since the previous observation. The growing cells were photographed by phase contrast, and measurements were made on enlarged projections of the negatives.*

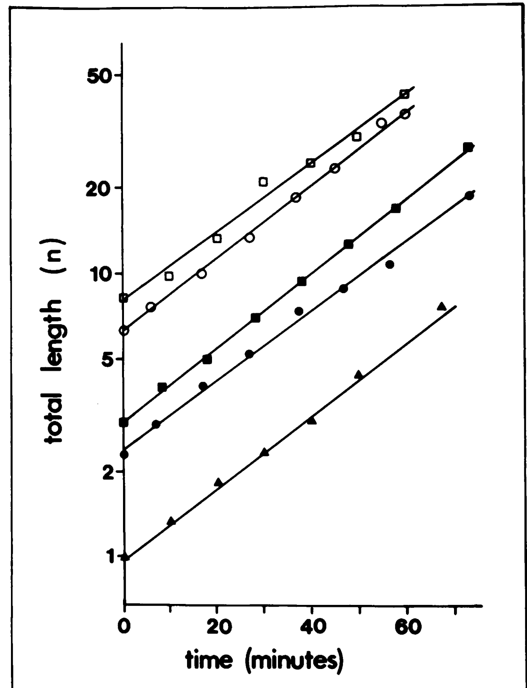

FIG. 5. *Growth of strain P678-54 on L broth plus 1.5% agar (Davis). Each symbol represents one cell (zero time) and its progenies. The log of the sum of the length of all cells in a clone (ordinate) is plotted against time. Measurements were made as described in the legend to Fig. 4. Growth is clearly exponential.*

Among cells which divide at a length of $2c$, the predicted frequency of polar divisions is 0.67. Direct observation of cells of this length growing on nutrient agar gave a frequency of polar division of 0.61 ± 0.08. Minicells were also observed to be produced at equal frequency from both "new" and "old" cell poles, confirming another assumption of the model.

DISCUSSION

It can be concluded from the observations made that our model provides an accurate description of the growth and division of this minicell-producing mutant. An effort was made to devise alternative models consistent with the known biology of *E. coli* that would fit the experimental data. For example, if the location of division sites were random within a cell, then cells of all lengths would be produced, including both very long and very short cells. This possibility can be excluded because no cells intermediate in length between minicells and the normal newborn cell are found in the mutant population. (Although this model therefore clearly does not apply to P678-54, mutants

of this kind may exist and would appear superficially similar to this strain.) Another model which might be considered states that the amount of division factor produced at each mass doubling is equal to the number of nonpolar, potential division sites. In this case, the predicted average cell size would be much less than that observed in P678-54. Other models which we considered initially have also been ruled out by the experimental findings reported here.

The pattern of division observed in the mutant suggests that the substance responsible for triggering division at any given site behaves as a quantum. Specific cell histories can be used to show that this factor could not be distributed in association with, or as a consequence of, localized cell envelope growth. Figure 7 shows one such history that has been observed. The "division factor" therefore behaves as a diffusible entity.

The nature of this diffusible substance which is responsible for triggering division is unknown. However, termination of a round of chromosome replication is known to be required for each cell division (2, 11). It also seems possible that the chromosome can "diffuse" within the cell (5, 13). It is therefore possible that the attachment and replication of the chromosome at a potential division site is required for division at that site after replication. However, the triggering event might not be termination itself but the attendant production of some diffusible substance, such as the "termination protein," described elsewhere (7, 9). In this case, the location of the replicating chromosome(s) within the cell would not be a factor in the determination of the localization of the next division.

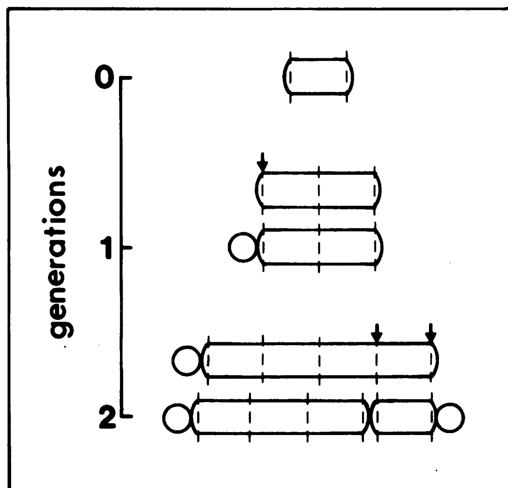

FIG. 7. *One observed division pattern for strain P678-54 growing on L broth plus 1.5% agar. Dotted lines show the presumed location of potential division sites. Arrows show the sites at which division is initiated.*

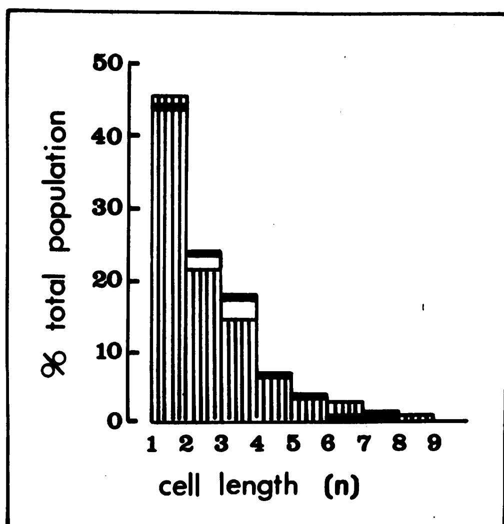

FIG. 6. *Size distribution for the progeny of large cells ($n \geq 5$) of strain P678-54 after two to three generations of growth on L broth plus 1.5% agar (Davis) at 37 C (shaded area). Superimposed on this distribution is the predicted distribution from Fig. 3b. Measurements were made as described in the legend to Fig. 4.*

The model proposed contains only one assumption that is specific for the mutant strain: that potential division sites persist. The other statements apply equally well to the mutant and to normal strains. If the model is correct, then in *E. coli* there are distinct processes controlling the localization and the number of cell divisions.

APPENDIX

Normal and abnormal cell length distributions. The distribution of cell lengths observed in an exponentially growing population of rod-shaped organisms depends primarily on two characteristics: (i) the distribution of the cell lengths at birth and (ii) the pattern of length increase during the cell cycle (4). The parental strain of *E. coli* P678 shows an almost ideal length distribution consistent with cells born at a single length and growing linearly with time until they divide at the end of the cell cycle. The mean cell size (see above) is very close to the theoretical value of 1.44 times the newborn cell size (12).

Interpretation of cell length distributions in mutants of increased average length can help reveal the physiological basis of the mutation. For instance, if there were an increase in the size of the newborn cell, the length distribution should resemble the parental

one, drawn on a larger scale, and should retain the same relationship between the newborn cell size (c) and the mean size ($1.44c$) as the parental strain.

An alternative cause for increased length in a mutant could be the occasional failure of division to occur at a potential division site (PDS). The relative efficiency with which the division process occurs at a PDS and the increased size are related in the following manner. Consider a large number of organisms, N, of total length, L, of the parental strain, in which we assume that every PDS is used when formed with an efficiency of 1. After one doubling time, there will be $2N$ organisms of total length $2L$. N divisions have occurred, and the necessary N PDS were provided with the increase in length, L; that is, one PDS is formed for every increase in length of L/N units. Now consider the mutant strain; N organisms have a total length kL, where k is the observed average increase in length over the parental strain. After one doubling time, there will be $2N$ organisms with a total length $2kL$. The length increase is kL, and kN new PDS will have been formed. In addition, there were $(k - 1)N$ unused PDS present in the original organisms, equal to the total original number present (kN) less those already used in forming the N organisms (N). The N new divisions are distributed among the unused sites, which total $(2k - 1)N$. The probability of a division at an unused site is therefore $N/(2k - 1)N$, or $1/(2k - 1)$. The proportion, p, of used to total sites remains constant at N/kN, or $1/k$. If the distribution of the used sites among the total sites along a hypothetical chain of organisms is random, the probability that n segments between adjacent PDS are included in a new organism is $h(n)$, where

$$h(n) = (1 - p)^{n-1}p \quad \text{which reduces to}$$

$$h(n) = (k - 1)^{n-1}k^{-n}$$

The length of the segment between adjacent PDS is constant in newborn cells (6, 7). The observed population in each length class will consist of cells which may have been born at different sizes and are thus at different stages of the cell cycle. In a stable, exponentially growing population of organisms born at length a, dividing at length $2a$, and increasing in length linearly, the fraction of the population of length l is $2^{(2 - l/a)} \cdot \ln(2)/a$ (12). The proportion of the population between lengths a and l is

$$\int_a^l 2^{(2 - l/a)} \cdot \ln(2)/a \cdot dl = 2(1 - 2^{(1 - l/a)})$$

The observed population of length less than or equal to l, $Pop(l)$, can be expressed as the sum of the contributions corresponding to each birth size (nc) in proportion to the frequency of such births, $h(n)$.

$$Pop(l) = \sum_{n=1}^{n=\infty} h(n) \cdot 2(1 - 2^{(1 - b/nc)}) \qquad (1)$$

where the variable $b = 2nc$ when $2nc \leq l$, and $b = 1$ when $2nc > l$. The fraction of the population lying between lengths l and $l + x$ is calculated as the difference between $Pop(l + x)$ and $Pop(l)$.

Such a model does not, however, include any statement about the production of minicells by polar division. The simplest model that combines the modified division property with the occurrence of minicells among the population assumes that the synthesis of each new unit of cell length c leads to the accumulation of one "division factor" which has an equal probability of being used at any PDS, whether internal or at either pole; polar septation produces a minicell. For each initial cell size, there will be a variety of possible patterns for the ensuing division process. The distribution of progeny sizes and the rate of minicell production can be calculated from the possible arrangements into groups of indistinguishable particles which have Bose-Einstein statistical characteristics (8). An organism of initial length nc will grow to length $2nc$, and n division factors will be formed. These will be distributed among the $(2n - 1)$ internal PDS and two polar PDS. n septa will be formed in the dividing organism, and $(n + 1)$ progeny will be produced. Septation is possible at either or both poles, but at least $(n - 2)$ septations must occur at internal PDS, which will result in $(n - 1)$ organisms each of minimal length c. $(n + 1)$ units of length c remain to be distributed among the progeny in a random fashion, and all the possible distributions define all the possible ways in which the dividing organism can complete n septations. The probability of any of the progeny receiving x additional units of length c is $p(x)$, where:

$$p(x) = \frac{(2n - x)!\,(n + 1)!\,n!}{(2n + 1)!\,(n + 1 - x)!\,(n - 1)!}$$

(see reference 8, p. 59, equation 5.1). The addition of any units of length c to either of the two potential minicell progenies results in nonappearance of the minicell and formation of an additional viable organism in its place. The expectation of progenies of length ac arising from an organism of initial length nc at the end of its growth and division cycle is $Pr(n,a)$, where $Pr(n,0) = 2p(0)$ (the probability of minicell production) and $Pr(n,a) = 2p(a) + (n - 1)p(a - 1)$ when $a \neq 0$. Computed values are given in Table 1. Since the progenies from any one size of initial cell are varied in length, the distribution of newborn organisms that maintains a stable length distribution as the population grows (an essential feature of a stable exponentially growing population [4]) is such that the number of progenies formed at any size after one generation is twice the number of newborn organisms of that size initially present. If the frequency of newborn organisms of length nc is $f(n)$, and of length mc is $f(m)$, then

$$\sum_{n=1}^{n=\infty} f(n) \cdot Pr(n,m) = 2f(m)$$

The values of $f(n)$ were computed by simulating the growth of a single organism of length c through many generations till the relative proportions of each size class stabilized. This distribution of initial cell sizes was converted into the length distribution that should be observed in an exponentially growing population (Fig. 3a) by equation 1, substituting $f(n)$ for $h(n)$. The average length of the population is 2.52 c, where c is

the smallest newborn cell size, and minicells are produced in the ratio 0.748 per cell per doubling time. If the minicells are stable, this ratio should be constant.

A variant of this model in which only one end of a dividing organism retains the capacity to undergo a division process with the formation of a minicell has been similarly analyzed. An organism of initial length nc doubles in length to $2nc$, and then has the capacity for n divisions. These will form n septa, giving $(n + 1)$ progenies. Septation is possible at one pole only, so at least $(n - 1)$ septa must be formed at internal PDS, producing n organisms of minimal length c. n units of length c remain to be distributed among the progenies in a random fashion. The probability of any of the progenies receiving x additional units is given by $p(x) = [(2n - x - 1)!\, n!\, n!]/[(n - x)!\, (n - 1)!\, 2n!]$. The expectation of new organisms of length ac arising from the divison after doubling of an organism originally nc units long is $Pr(n,a)$ where

$$Pr(n,0) = p(0) \quad \text{(the rate of minicell production)}$$

and

$$Pr(n,a) = p(a) + n \cdot p(a - 1)$$

when $a \neq 0$. The average initial cell length is 1.5 c, and the mean observed cell length in an exponentially growing population is 2.16 c. Minicells will be generated at a rate 0.5 per organism per doubling time. Both the mean size and minicell production level distinguish this model from the previous one in which both ends of the organism were possible sites of minicell production, although direct observation of division in cells of various sizes can provide a more direct test.

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