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Deoxyribonucleic Acid Synthesis During Exponential Growth and Microcyst Formation in *Myxococcus xanthus*

EUGENE ROSENBERG, MARY KATARSKI, AND PETER GOTTLIEB

Department of Bacteriology and Institute of Molecular Biology, University of California, Los Angeles, and Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California

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Myxococcus xanthus in exponential phase with a generation time of 270 min contained a period of 50 min during which deoxyribonucleic acid (DNA) synthesis did not take place. After induction of microcysts by the glycerol technique, the DNA content increased 19%. Autoradiographic experiments demonstrated that the DNA made after glycerol induction was not evenly distributed among the microcysts. The distribution of grains per microcyst fits the following model of chromosome replication: in exponential phase, each daughter cell receives two chromosomes which are replicated sequentially during 80% of the divison cycle; after microcyst induction, no chromosomes are initiated. Mathematical formulas were derived which predict the kinetics and discrete probability distribution for several chromosome models.

The bacterium, *Myxococcus xanthus*, characteristically undergoes a morphogenetic change during its life cycle. There occurs an intracellular differentiation in which motile vegetative rods convert to nonmotile, refractile, spherical bodies called microcysts. This process differs from the well-studied endospore formation in that the entire vegetative cell is transformed into the microcyst (4). The formation of microcysts can quantitatively be induced experimentally in a liquid medium in about 120 min (3). *M. xanthus*, therefore, presents a simple system for the study of biochemical events during differentiation.

In the present study, we have investigated the kinetics of deoxyribonucleic acid (DNA) synthesis during microcyst formation, and the cell distribution of this newly synthesized DNA in the microcysts. To interpret these data, it was also necessary to investigate the possible existence of a DNA cycle during the exponential phase and to develop certain mathematical formulas for several hypothetical models presented.

We have included an appendix which describes general mathematical treatment of the kinetics and discrete cell probability distribution for several chromosome models. The derived formulas are pertinent to exponentially growing systems which complete the cycle of chromosome synthesis but do not initiate another.

MATERIALS AND METHODS

Bacterial growth and microcyst formation. The characteristics of *M. xanthus* strain FB have been described (2). The organism was grown at 30 C in aerated NZ medium: 1% N-Z-Case (Sheffield Chemical, Norwich, N.Y.), 0.008 M $MgSO_4$, adjusted to *p*H 7.5 with NaOH. Under these conditions, the culture has a doubling time of 270 min and reaches a final optical density at 560 mμ (OD_{560}) of over 2.0. Experiments with exponentially growing cells were conducted when the OD_{560} = 0.3. Microcysts were induced by the following modification of the glycerol technique: prewarmed 50% glycerol was added to an exponentially growing culture of *M. xanthus* (OD_{560} = 0.3) to yield a final glycerol concentration of 0.5 M. Morphologically, the process is synchronous and over 95% of the cells are converted to microcysts in 120 min.

Determination of rate of DNA synthesis. To determine the kinetics of DNA synthesis, 10 μc (per ml) of thymidine-methyl-^3H (6 c/mmole; New England Nuclear Corp., Boston, Mass.) or 1 μc (per ml) of thymidine-2-^{14}C (6 mc/mmole; Schwarz Bio Research Inc., Orangeburg, N.Y.) was added to the medium; 1-ml samples were removed at timed intervals and mixed with 1 ml of ice-cold 10% trichloroacetic acid containing 1 mg (per ml) of thymidine. After standing in the cold for at least 30 min, the precipitate was collected on a membrane filter (Millipore Corp., Bedford, Mass.; 0.45-μ pore diameter) and washed successively with four 5-ml portions of 5% cold trichloroacetic acid and two 5-ml portions of absolute ether. After air-drying, the membranes were placed

in counting bottles to which was added 10 ml of toluene containing 40 mg of 2,5-diphenyloxazole and 0.5 mg of *p*-bis (2,5-phenyloxazdyl) benzene. Radioactivity was measured in a liquid scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill).

DNA was also determined chemically by the following procedure. An equal volume of cold 10% trichloroacetic acid was added to the washed-cell suspension. After standing in the cold for 30 min, the precipitate was collected by centrifugation at 10,000 × *g* for 15 min, dissolved in 0.2 M NaOH, and reprecipitated with 1 N trichloroacetic acid. After centrifugation, the precipitate was again dissolved, reprecipitated, and recentrifuged. The final precipitate, dissolved in 0.1 M NaOH, was analyzed for DNA by the diphenylamine reaction (1) by use of trichromic readings (2 $OD_{595} - OD_{645} - OD_{545}$). To correct for losses during the procedures, predetermined radioactive DNA was mixed with the cells prior to the first addition of trichloroacetic acid. The values for DNA were then corrected according to the amount of radioactivity recovered in each sample.

Autoradiography of thymidine-methyl-^3H labeled cells. To 1.0 ml of aerated culture was added 0.1 mc of thymidine-methyl-^3H (6 c/mmole) without carrier; however, the NZ medium used in these experiments contain as contaminants approximately 5 μg (per ml) of thymine or thymidine (or both).

Radioautographs were prepared by spreading a drop of the culture on slides, fixing these with 2% formaldehyde, washing successively with cold 5% trichloroacetic acid and water, and then dipping the slides in diluted Kodak NTB-2 photographic emulsion. The emulsion was prepared and tested, and the slides were dipped two at a time by standard techniques (10). After the emulsion was dry, the slides were stored in Bakelite boxes containing Drierite until development. After an appropriate exposure time, the slides were developed for 2 min at 25 C in Kodak D19 developer and fixed for 5 min before washing. Radioautographs were evaluated by scoring the number of grains per cell under 1,280 × magnification in a phase-contrast microscope. Although most fields contained well-isolated cells, occasional clumps of microcysts were observed. No attempt was made to score these clumps. Both vegetative and microcyst cells could easily be observed without staining. The observed data were corrected for background grain counts (0.094 grain per cell).

RESULTS

Kinetics of DNA synthesis during microcyst formation. Figure 1 shows the rate of thymidine-methyl-^3H incorporation into DNA by an exponentially growing culture and by a glycerol-induced culture. The labeled thymidine was incorporated into the exponentially growing cells at a rate which was directly proportional to the OD of the culture for over two generations. The glycerol-induced culture continued to synthesize DNA but at a decreasing rate for approximately

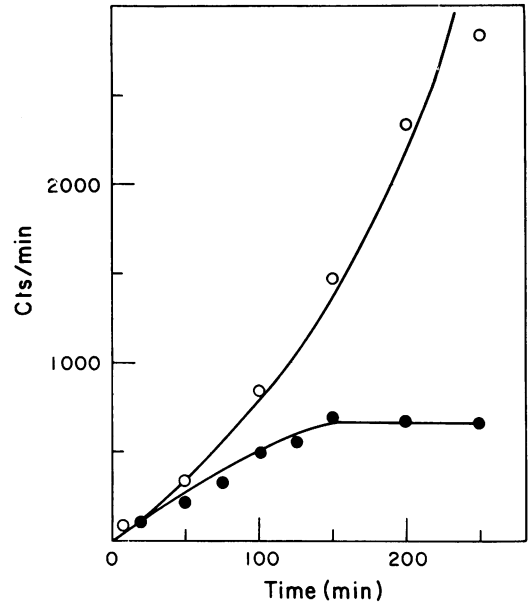

FIG. 1. *Kinetics of thymidine incorporation into DNA during microcyst formation. After adding thymidine-methyl-^3H (time zero), to 10 μc/ml, the exponentially growing culture of Myxococcus xanthus was divided into two equal parts. One part was induced to form microcysts (●) by addition of glycerol to a final concentration of 0.5 M; the other (○) received water instead of glycerol. At timed intervals, 1.0-ml samples were removed and the radioactivity in their DNA determined.*

140 min, resulting in a 19% increase in DNA. After 140 min, no DNA synthesis or turnover was detected.

The approximate 20% increase in DNA following induction of microcysts, determined chemically or calculated from both ^3H and ^{14}C incorporation data, is consistent with at least the following three models. Model A: two chromosomes per cell, synthesized sequentially; after microcyst induction, chromosomes are completed but no chromosomes are initiated (i.e., two initiation points per cell). Model B: one chromosome per cell, but DNA synthesized during only one-half of the division cycle; chromosomes are completed but not initiated after microcyst induction. Model C: all cells continue to synthesize DNA at a decreasing rate for 120 min, stopping anywhere along the chromosome(s).

Model A is identical to the model proposed by Lark and Lark (7) for succinate-grown *Escherichia coli* after amino acid starvation. Their model predicts an 18.4% increase in DNA after amino starvation. Model B would also predict an ap-

proximate 20% increase in DNA, the precise value depending on how the DNA cycle coincided with the division cycle.

The observed rate of DNA synthesis after microcyst induction fits closely that predicted by model A (Fig. 2). The theoretical curve for model B would be very similar to that of model A, the exact curve depending on how the DNA cycle and division cycle coincided. Model C is too general to specify uniquely a kinetic curve. Thus, autoradiographic studies were performed to distinguish the three models.

Autoradiographic experiments. Table 1 presents the data from four radioautograms. Experiment A was a control in which an exponential culture was incubated with radioactive thymidine for one generation. For a simple Poisson distribution, a straight line is obtained when the log $[P_{(n)}]$ is plotted against n. As presented in Fig. 3, experiment A closely fits a straight line, indicating that each cell incorporated the same amount of radioactivity during one generation. The only class which deviated significantly from a Poisson distribution was the zero grains per cell class. The predicted probability for the zero class for a population with a mean of 5.01 is 0.005, whereas we observed 0.035. Thus, 3% of the cells (presumably dead cells) did not incorporate any thymidine during a generation.

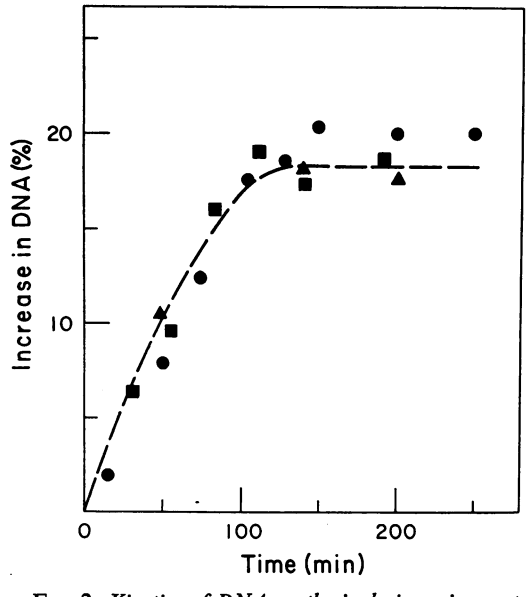

FIG. 2. *Kinetics of DNA synthesis during microcyst formation. The percentage increase in DNA after glycerol addition was determined chemically (■), or was calculated from thymidine-methyl-^3H (●) and thymidine-2-^{14}C (▲) incorporation data. The dashed line is obtained from the theoretical rate equation,*

$$M(t) = (2+\sqrt{2})\lambda\left[t - \left(\frac{t_0}{\sqrt{2}\ln 2}\right)\left(2^{t/t_0}-1\right)\right],$$

which is derived in the Appendix (equation 6). The equation describes the increase in DNA. M, as a function of time, t, assuming that there are two chromosomes per cell which are synthesized sequentially, and that, after t = 0, DNA synthesis continues until the chromosome is completed, but no new chromosomes are initiated (model A). The constant, λ, was determined for a generation time, t_0, of 270 min, (Appendix, equation 6).

TABLE 1. *Autoradiographic data*

Grains per cell	Experiment[a]			
	A	B	C	D
0	35	372	505	332
1	34	249	299	184
2	59	152	193	96
3	110	91	110	64
4	156	25	67	25
5	219	10	40	9
6	164	1	14	7
7	92	0	9	2
8	65	0	7	1
9	37	0	0	0
10	15	0	1	0
11	10	0	0	0
12	6	0	0	0
Cells scored	1,002	900	1,245	718
Mean	5.010	1.090	1.363	1.078

[a] In experiments A and B, exponentially growing cells were labeled with thymidine-methyl-^3H for 1.0 and 0.1 generation, respectively. Experiments C and D both represent incorporation of the label for 150 min after induction of microcyst formation by glycerol.

Experiment B was a typical autoradiogram from a series in which exponentially growing cells were exposed to radioactive thymidine for 27 min (0.1 generation). Except for the zero class, the data closely fit a Poisson distribution. The predicted Poisson probability for the zero class with a mean of 1.09 is 0.337, whereas we observed 0.413. The data from experiment B are consistent with the assumption that approximately 20% of the cells did not synthesize any DNA during the 27-min incorporation period, while the remaining 80% of the cells became uniformly labeled. When the data are recalculated based on this assumption, a Poisson distribution with a mean of 1.35 is obtained (Fig. 3).

Experiments C and D, performed on different days, both represent incorporation of thymidine-methyl-^3H into DNA during microcyst formation. Analyzed graphically, neither experiment yielded a Poisson grain distribution, regardless of

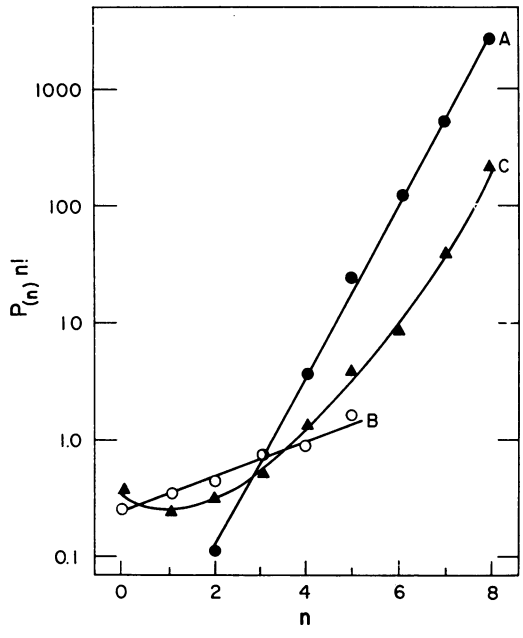

FIG. 3. *Graphical presentation of the data from Table 1. The frequency function, log [P(n)n!], is plotted as the ordinate. The abscissa indicates the number of grains per cell. The data from experiments B and C have been recalculated with the assumption that 20% of the cells are not synthesizing any DNA.*

whether the zero class was corrected for possible resting cells. Therefore, the following equation, which describes the distribution expected from model A, was derived (see Appendix, equation 12):

$$P_k(n) = \frac{\ln 2}{m\gamma(2^{1/k}-1)}\left[\gamma^{-n} - 2^{1/k}\sum_{j=0}^{n} P_j\left(\frac{m}{k}\gamma^{j-n}\right)\right]$$

where $\gamma = 1 - (\ln 2)/m$; $P_j(m/k)$ is the ordinary Poisson distribution; and k, the number of chromosomes, is 2.

This distribution is essentially a normalized summation of Poisson distributions for the various age classes predicted from model A. If we assume that 20% of the cells do not synthesize any DNA (consistent with experiment B), then m = observed mean/(0.253) (0.8) (see Appendix, part A5).

Table 2 compares the observed grain distribution in experiments C and D with that expected from the Poisson distribution and new distribution. Both expected distributions have been calculated with the assumption that 20% of the cells do not synthesize any DNA. The observed data clearly fit our derived distribution better than the Poisson distribution. The broader distribution observed and predicted from model A is most evident in the four to six grains per cell classes.

The expected grain distribution obtained from equation 12 and shown in Table 2 assumed that the individual cells were synthesizing DNA at a constant rate. Only a slightly better fit to the data was obtained if we assumed that DNA synthesis proceeded at an exponential rate until cell division.

DISCUSSION

DNA cycle during exponential growth. The data presented indicate that *M. xanthus*, growing in NZ medium with a generation time of 270 min, contains a period of approximately 50 min during which DNA synthesis does not take place. Since the time sequence of this period relative to the division cycle is not known, its exact duration cannot as yet be determined.

The conclusion of a DNA cycle with a rest phase is based solely on autoradiographic experiments in which exponentially growing cells were labeled for 0.1 generation. The zero grains per cell class was consistently too high and could only be reconciled with the assumption that 20% of the cells were not synthesizing any DNA. The possibility that the zero class was too high because of dead cells is unlikely since parallel experiments in which the incorporation period was extended to 1.0 generation indicated that only 3% of the cells did not produce grains.

TABLE 2. *Analysis of grain distribution during microcyst formation*

Grains per cell	Experimentally observed[a]		Expected for Poisson's distribution[b]		Expected for new distribution[c]	
	C	D	C	D	C	D
0	505	332	503	293	510	330
1	299	184	347	201	246	163
2	193	96	235	135	196	111
3	110	64	107	61	133	65
4	67	25	36	20	77	32
5	40	9	9	5	38	14
6	14	7	2	1	14	6
χ^2			170	59	15.8	9.9
P			<0.001	<0.001	0.05	0.20

[a] From Table 1.
[b] Assuming 20% of the cells synthesize no DNA.
[c] From equation 12, derived in the appendix, where $\gamma = 1 - \ln 2/m$; $P_j(m/k)$ is the ordinary Poisson distribution; k, the number of chromosomes is 2; and, assuming that 20% of the cells synthesize no DNA, m = observed mean/(0.253) (0.8) (see Appendix, part A5).

The existence of a DNA cycle, although well established in eucaryotic cells (6), is presently not clear in bacteria. Early studies, with use of temperature shifts to synchronize *Salmonella typhimurium*, indicated a DNA cycle (8). Subsequently, it was demonstrated that the temperature shift employed upset the normal sequence of cellular events, and that, in *E. coli* and *S. typhimurium* grown in glucose or succinate media, DNA is synthesized throughout at least 90% of the division cycle of an individual cell (12). Recently, it has been reported that *E. coli* growing on "poor" carbon sources, with abnormally long generation times, has a distinct DNA cycle (5). In cultures with generation times of 180 and 270 min, some 40% of the cells have been found not to incorporate thymine in pulse-labeling experiments. It should be emphasized that in our experiments *M. xanthus* was growing at its maximal rate (generation time, 270 min), whereas *E. coli* with a generation time of 180 min is growing abnormally slowly.

DNA synthesis during microcyst formation. Based on chemical analysis, ^{14}C- or ^{3}H-thymidine incorporation, and autoradiographic analysis, there is a 19% increase in DNA after glycerol induction. Using the same organism, Sadler and Dworkin (11) reported that, after glycerol induction, DNA increases 25% in a two-step manner. These workers chilled, washed, and centrifuged the cells prior to glycerol induction. In our experiments, we avoided centrifugation steps and temperature shifts so as not to select cells or possibly induce synchrony.

The DNA synthesized after glycerol induction is clearly not evenly distributed among the microcysts. The observed distribution fits reasonably well the distribution predicted from the following hypothetical model: in exponential phase, each daughter cell receives two chromosomes which are replicated sequentially during 80% of the division cycle; after microcyst induction, no chromosomes are initiated.

The model is a modification of that proposed by Lark and Lark (7) for DNA synthesis in *E. coli* 15T$^-$ growing in a succinate medium after amino acid starvation. However, the DNA made during amino acid starvation was abnormal in that it was subsequently replicated at a slower rate than DNA synthesized in the presence of the amino acid (9). It should, thus, be interesting to examine the replicative fate of the DNA synthesized after glycerol induction.

APPENDIX

In recent years, there has been an attempt to describe in more detail the mode of chromosome replication during the normal division cycle and after environmental shifts. The experimental approach to this problem has primarily been a study of the incorporation of a specific radioactive precursor, usually thymidine, into the DNA. Evidence has accumulated that inhibition of protein synthesis in bacteria by addition of chloramphenicol or deprivation of an essential amino acid results in the completion of the DNA replication cycle in which it is engaged, but that no further chromosomes are initiated. A similar proposal has been presented for chromosome synthesis following induction of microcysts. We present here a mathematical treatment of the rate and discrete probability distribution for DNA synthesis (thymidine incorporation) for an *exponentially growing culture after inhibition of chromosome initiation.* The derived probability distribution is particularly pertinent to the interpretation of radioautographic experiments.

A1. *Rate equation for DNA cycle = cell division cycle.* The rate of DNA synthesis at any given time is proportional to the number of actively synthesizing cells. Our model assumes that all cells remain equally active from the time of inhibition of chromosome initiation to the time of completion of all chromosomes. If the generation time is t_0, and there are k chromosomes produced sequentially at a uniform rate, then the maximal time taken to complete a chromosome is t_0/k. For any time in this interval, $0 \leq t \leq t_0/k$, the number of active cells can then be written as

$$N(t) = N(0)\left[Pr\left(0 \leq \text{AGE} \leq \frac{t_0}{k} - t\right) + Pr\left(\frac{t_0}{k} \leq \text{AGE} \frac{2t_0}{k} - t\right) + \cdots Pr\left(\frac{t_0 k - t_0}{k} \leq \text{AGE} \leq t_0 - t\right)\right] \quad (1)$$

where $N(0)$ is the number of active cells at time, t, = 0, and where $Pr(t_1 + \leq \text{AGE} \leq t_2)$ is the probability that a cell has an age between t_1 and t_2 at $t = 0$.

If we use the distribution of ages in an exponential culture,

$$Pr\{t - dt/2 \leq \text{AGE} \leq t + dt/2\} = 2(\ln 2)2/t_0^{-t/t_0}\, dt, \text{ for } dt \ll t_0,$$

then the cumulative age distributions of interest can be written as

$$Pr\left[\frac{(j-1)t_0}{k} \leq \frac{jt_0}{k} - t\right] = \frac{2 \ln 2}{t_0} \int_{((j-1)t_0)/k}^{(jt_0/k)-t} e^{-(t'/t_0)\ln 2}\, dt' \quad (2)$$

$$= 2^{1-j/k}\,[2^{1/k} - 2^{t/t_0}]$$

We can then substitute into equation 1 to obtain

$$N(t) = 2N(0)(2^{1/k} - 2^{t/t_0}) \sum_{j=1}^{k} 2^{-j/k} \quad (3)$$

evaluating the geometric series gives

$$N(t) = \frac{N(0)}{2^{1/k} - 1} (2^{1/k} - 2^{t/t_0}) \tag{4}$$

This result is seen to give $N(t) = N(0)$ at $t = 0$, and $N(t) = 0$ at $t = t_0/k$, as would be expected. If we denote by λ the rate of incorporation of thymidine per cell per unit time, then the total rate of incorporation can be written as

$$R(t) = \lambda N(t) \tag{5}$$

A2. *Rate equation for DNA cycle = cell division cycle.* If we assume that a certain fraction of the cells are in resting phase at $t = 0$, and that these cells never start to make DNA, then the total number of active cells at time $t = 0$, $N(0)$, should be reduced by that fraction. In addition, t_0 should not be the cell generation time, but only the time required for DNA replication. Thus, t_0 = cell generation time − resting time.

In this case, λ is the rate of DNA production for just the active cells.

A3. *Total increase in DNA.* The amount of DNA formed up to any given time, t, is found by integrating equation 5 from 0 to t. This yields

$$M(t) = \frac{\lambda N(0)}{2^{1/k} - 1} \left[t 2^{1/k} + \frac{t_0}{\ln 2} (1 - 2^{t/t_0}) \right] \tag{6}$$

The total amount of DNA formed is obtained from equation 6 where $t = t_0/k$, the maximum possible time.

For reference purposes we note that, for an exponential culture running a full generation time, the increase in DNA is

$$\lambda N' \int_0^{t_0} 2^{t/t_0}\, dt = \frac{\lambda N' t_0}{\ln 2} \tag{7}$$

where N' is the total number of cells at time, t, = 0, and is not corrected for the fraction of resting cells. For a full generation time, the DNA content must double; thus, the constants N' and λ can be experimentally determined.

It should further be noted that when the DNA cycle $\neq$ generation time, we must use the corrected generation time, t_0, because the cell makes DNA for only that amount of time.

A4. *Discrete probability distribution after chromosome completion.* If all the cells produced the same amount of radioactive DNA, the number of radioactive decays would be Poisson-distributed. Our model, on the other hand, assumes that each cell makes DNA only during the time remaining for the completion of a chromosome. A cell which makes DNA for a time, τ, will have a probability of giving n decays (in an exposure time, T, for an isotope with a decay rate, $\wedge$) given by

$$\left(\frac{m\tau}{t_0}\right)^n \frac{\exp\left(-\frac{m\tau}{t_0}\right)}{n!} \tag{8}$$

where m is a constant depending on T, $\wedge$, λ, t_0, and the counting efficiency, and will be related below to the experimentally observed mean.

Now we divide the age distribution over t_0 of the initial culture into k intervals of length t_0/k. For cells with initial ages falling in the j'th interval (those cells working on the j'th chromosome when inhibition of chromosome initiation commences), the active time remaining will be $\tau = j/k\, t_0 - t$, where t is the age of the cell at the beginning of the experiment. Averaging the distribution of equation 8 over all ages then gives

$$P_k(n) = 2 \ln 2 \sum_{j=1}^{k} \int_{\frac{(j-1)t_0}{k}}^{\frac{jt_0}{k}} \frac{m^n \left(\frac{j}{k} - \frac{t}{t_0}\right)^n}{n!} \cdot \exp\left[-m\left(\frac{j}{k} - \frac{t}{t_0}\right) - \frac{t}{t_0} \ln 2\right] \frac{dt}{t_0} \tag{9}$$

This equation can be simplified by changing variables in each of the integrals, $z = j/k - t/t_0$, to yield

$$P_k(n) = 2 \ln 2 \int_0^{1/k} \frac{(mz)^n}{n!} \cdot e^{-mz + z \ln 2}\, dz \sum_{j=1}^{\gamma} 2^{-j/k} \tag{10}$$

summing the geometric series gives

$$P_k(n) = \frac{\ln 2}{2^{1/k} - 1} \int_0^{1/k} \frac{(mz)^n}{n!} e^{-mz + z \ln 2}\, dz \tag{11}$$

which can be integrated by parts, to yield the final expression

$$P_k(n) = \frac{\ln 2}{m\gamma(2^{1/k} - 1)} \cdot \left[\gamma^{-n} - 2^{1/k} \sum_{j=0}^{n} p_j\left(\frac{m}{k}\right) \gamma^{j-n}\right] \tag{12}$$

where $\gamma = 1 - (\ln 2)/m$, and $p_j(m/k)$ is the ordinary Poisson distribution given by

$$p_j(m/k) = (m/k)^j \frac{e^{(-m/k)}}{j!}.$$

The difficulty with this form of the probability distribution is that there is no convenient way to measure m directly. The simplest procedure for obtaining m is to calculate the probability generating function

$$\pi_k(s) = \sum_{n=0}^{\infty} P_k(n) s^n = \frac{\ln 2}{2^{1/k} - 1} \cdot \int_0^{1/k} e^{zm(s-1) + z \ln 2}\, dz \tag{13}$$

which can be integrated to give

$$\pi_k(s) = \frac{\ln 2\,[1 - e^{\frac{m}{k}(s-1)} 2^{1/k}]}{(2^{1/k} - 1)(m - ms - \ln 2)} \tag{14}$$

The experimentally observed mean number of counts, $\bar{n}_k$, can then be written as

$$\bar{n}_k = \sum_{n=0}^{\infty} nP_k(n) = \left.\frac{d\pi_k(s)}{ds}\right|_{s=1} = m\left[\frac{2^{1/k}}{k(2^{1/k}-1)} - \frac{1}{\ln 2}\right] \quad (15)$$

Hence, m can be expressed as a numerical constant times the mean number of counts per cell at chromosome completion, and the probabilities of equation 12 are uniquely specified.

A5. *Discrete probability distribution following chromosome completion when DNA cycle ≠ generation time.* If a certain fraction of the cells are resting initially, and never begin to incorporate DNA, then the probabilities of equations 12 and 14 retain the same form because they do not depend explicitly on t_0 or $N(0)$. However, to equate theoretical and experimental results, we will have to make the following adjustments. (i) If the fraction x of cells are initially resting, use the corrected experimental mean, $\bar{n}_k$ = observed mean/$(1 - x)$. The experimentally observed probabilities are then obtained by modifying the probabilities calculated from equation 12 in the following manner: $(1 - x)P_k(n)$ for $n \neq 0$; $x + (1 - x)P_k(0)$ for $n = 0$.

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