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Chromosome Replication and Cell Division in *Escherichia coli* at Various Temperatures of Growth

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The effect of temperature on the growth rate and the pattern of chromosome replication during the division cycle of *Escherichia coli* B/r growing in various media was investigated. The time between divisions, the time for a round of replication (C), and the time between completion of a round and cell division (D) were threefold longer at 21 C than at 37 C. At all temperatures and in all media, D equalled one-half C, suggesting that a common mechanism controls chromosome replication and the progression of the cell toward division after completion of a round of replication.

In *Escherichia coli*, the rate of cell division corresponds to the frequency of initiation of chromosome replication (15, 20, 21). In cultures growing exponentially at 37 C in media which support doubling times shorter than 60 min, chromosome replication is initiated upon the attainment of a critical cell size per chromosomal origin (7, 13). The time required for a round of replication (C) is 40 min (1, 2, 14), and the time between completion of replication and cell division (D) is 20 min (4-6, 14, 16). In cultures growing exponentially at 37 C in media which support doubling times between 60 and 120 min, chromosome replication is initiated at cell division (3, 12, 15) and replication is completed during two-thirds of the division cycle (1-3, 6, 12, 15). At all growth rates, C is approximately twice D (6, 12, 14, 15).

It has recently been reported that the processes of initiation and replication of the chromosome of slowly growing *E. coli* 15T$^-$ respond differently to changes in the temperature of incubation (27). A differential sensitivity to the temperature might indicate the existence of different modes of regulation of the kinetics of initiation and replication. To investigate this question, I have studied the temperature dependence of the processes of initiation and replication of the chromosome in *E. coli* B/r by measurement of the pattern of deoxyribonucleic acid (DNA) replication during the division cycle. The cell age at initiation and the times C and D were measured at 37, 30, and 21 C in media supporting various growth rates.

MATERIALS AND METHODS

Bacteria and growth conditions. The organism used was *E. coli* B/r (ATCC 12407). The minimal salts medium contained 2 g of NH_4Cl, 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 3 g of NaCl, and 0.25 g of $MgSO_4$ in 1 liter of distilled water. Glucose (1 g/liter), sodium succinate (1 g/liter), or sodium acetate (2 g/liter) was used as carbon source. Cells were grown with shaking in a constant-temperature water bath and maintained for at least 10 generations at a concentration of less than 1×10^8 cells/ml by dilution. Cultures were used in experiments approximately 16 hr after the last dilution. For experiments on cells growing at a rate faster than 1.5 doublings per hr, tryptophan at 50 μg/ml and Casamino Acids (Calbiochem, Los Angeles, Calif.) at 0.2% final concentration were added to exponentially growing cultures in glucose minimal medium at least 4 hr before an experiment.

Thymidine incorporation during the division cycle. Incorporation of ^{14}C- or ^{3}H-thymidine (35 mCi/mmole and 18.0 Ci/mmole, respectively; New England Nuclear Corp., Boston, Mass.) onto cold acid-insoluble material was taken as a measure of DNA synthesis (12). The pattern of incorporation of radioactive thymidine during the cell division cycle of *E. coli* B/r was essentially identical to the pattern of incorporation of radioactive thymine into an auxotroph *E. coli* B/r Thy$^-$ which required thymine for growth (Helmstetter, *personal communication*). Thus, although there may be preferential incorporation of thymidine into repair replication (28), in our experiments thymidine incorporation appears to reflect the rate of chromosome replication. Cultures containing 100 ml of approximately 10^8 cells/ml growing exponentially at various temperatures were pulse-labeled with ^{14}C-thymidine at 0.01 to 0.02 μCi/ml or ^{3}H-thymidine at 0.2 μCi/ml. Each culture

was filtered by suction onto the surface of a 0.22 μm grade GS membrane filter (Millipore Filter Corp., Bedford, Mass.). After being washed with minimal medium, the membrane was inverted and eluted with conditioned medium (12) at a rate of 2 to 3 ml/min. Samples of the effluent were collected continuously during constant time intervals, precipitated with cold trichloroacetic acid at 5% final concentration, and kept in an ice bath for at least 30 min. The acid-insoluble material was collected by filtration on grade HA membrane filters (25 mm diameter; Millipore Filter Corp., Bedford, Mass.), washed with cold 5% trichloroacetic acid containing 100 μg of thymidine/ml, and dried. The radioactivity on the membranes was determined in Liquifluor (Nuclear-Chicago Corp., Des Plaines, Ill.) with a liquid scintillation counter (Nuclear-Chicago Corp.).

Temperature. Exponentially growing cultures were incubated in shaking water baths at temperatures of 37.0 ± 0.5, 30.0 ± 0.5, and 21.0 ± 1.0 C to mid-logarithmic phase. During elution from a membrane filter, the temperature of the elution medium was maintained either at 37.0 ± 0.5 or 21.0 ± 2.0 C, as indicated in each experiment, in a Full View Incubator (Precision Scientific Co.).

Cell concentration and absorbance. Cell concentrations were determined with a Coulter counter model F (Coulter Electronics, Inc., Hialeah, Fla.). The absorbance of the cultures was determined at 450 nm (23) in 1-cm light path cuvettes with a Beckman model DB spectrophotometer.

RESULTS

Doubling times of exponentially growing cultures of E. coli B/r at various temperatures. The doubling times of cultures of *E. coli* B/r growing exponentially in different media at 21, 30, and 37 C are shown in Fig. 1. The doubling times at 21 and 30 C were 3-fold and 1.5-fold longer, respectively, than those at 37 C.

Pattern of DNA synthesis during the division cycle of E. coli B/r. The pattern of DNA synthesis during the division cycle of *E. coli* B/r was determined by briefly exposing an exponentially growing culture to radioactive thymidine, binding the population to a membrane filter, and measuring the radioactivity in the progeny cells which were eluted from the membrane. The number of cells and the radioactivity per cell in the effluent from a membrane-bound culture growing in succinate medium at 21.0 ± 1.0 C are shown in Fig. 2. Since progeny of the oldest through the youngest cells of the membrane-bound population are released consecutively during each generation of elution, the rate of incorporation of thymidine during the division cycle is seen by following the curve of radioactivity per cell from right to left (top of frame) in each generation. Abrupt increases in incorporation during

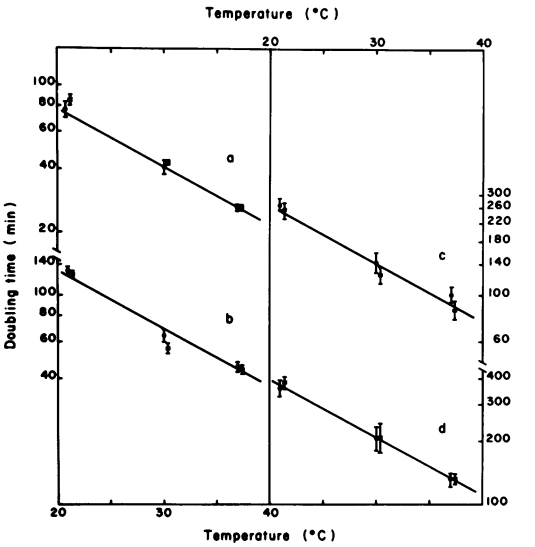

FIG. 1. *Doubling times of exponentially growing cultures at various temperatures. Doubling times (minutes) of the cell concentration (●) and the absorbance at 450 nm (×) of cultures growing in minimal salts medium containing glucose plus Casamino Acids and tryptophan (a), glucose (b), succinate (c), and acetate (d) are plotted against temperature of incubation. Values are the average of four to five determinations. Standard deviations of the mean are indicated. The straight lines have been drawn parallel to each other.*

the division cycle reflect initiation of a round of chromosome replication, and decreases reflect the completion of a round (12). In this experiment a round of replication was initiated at division, and it was completed after approximately two-thirds of a generation. A period devoid of DNA synthesis separated the end of the round from cell division. At the top of Fig. 2, the time (C) for a round of replication and the time (D) between completion of a round and division are indicated.

The patterns of chromosome replication during the division cycle of *E. coli* B/r growing in succinate media at 37.0 ± 0.5, 30.0 ± 0.5, and 21.0 ± 1.0 C are compared in Fig. 3. The patterns of DNA replication during the division cycle have been plotted versus generations rather than absolute time. At each temperature of growth, the DNA replication interval occupied the same portion of the cell division cycle. Altering the temperature of the elution medium did not alter the pattern of thymidine incorporation. Similarly, the temperature of growth did not alter the pattern of DNA replication of *E. coli* B/r growing in acetate minimal medium (Fig. 4).

As with slowly growing cells, the pattern of

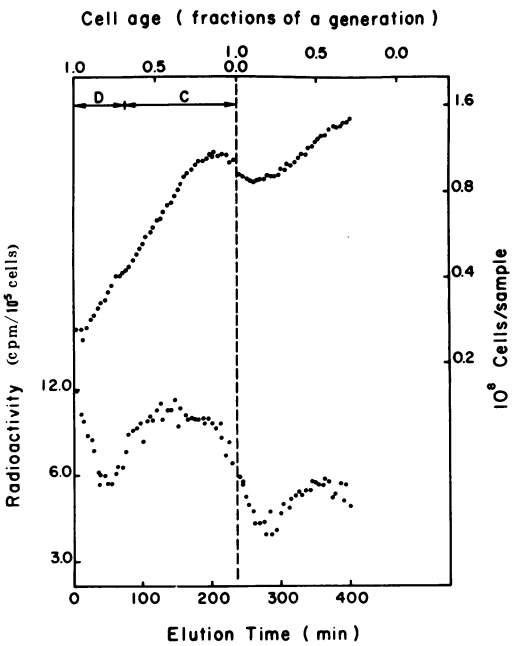

FIG. 2. *Cell number and radioactivity per cell in the effluent from a membrane-bound culture growing in succinate minimal medium at 21.0 ± 1.0 C. A 100-ml culture of 1.0 × 10⁸ succinate-grown cells per ml growing at 21.0 ± 1.0 C was exposed to 0.2 μCi of ³H-thymidine per ml for 10 min during mid-logarithmic growth. At the end of the labeling period, the culture was bound to a nitrocellulose membrane filter, washed, and eluted with conditioned (12) succinate minimal medium at 21.0 ± 2.0 C. Samples of the effluent were collected continuously during 5-min intervals, and the cell number (top curve) and radioactivity per cell (bottom curve) were determined. Elution time, increasing from left to right, is expressed in minutes. Ages of the ancestors of the cells in the effluent at the time of pulse-labeling are given at top of frame and increase from right to left. Vertical line indicates one generation of elution. The times C and D are shown at the top of the figure.*

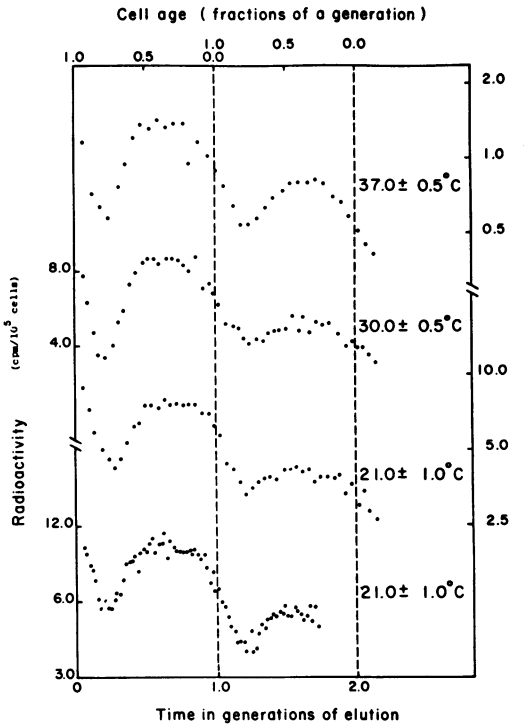

FIG. 3. *Radioactivity per cell in the effluent from membrane-bound cultures growing in succinate minimal medium at various temperatures. A 100-ml culture of 1.0 × 10⁸ cells/ml growing at 37.0 ± 0.5 C (top curve) was exposed for 8 min to 0.02 μCi of ¹⁴C-thymidine per ml and eluted with conditioned medium at the same temperature. Cultures of 100 ml of 1.0 × 10⁸ cells/ml growing at 30.0 ± 0.5 and 21.0 ± 1.0 C (second and third curves from the top) were exposed to 0.2 μCi of ³H-thymidine per ml for 8 min and eluted with conditioned medium at 37.0 ± 0.5 C. A 100-ml culture of 1.0 × 10⁸ cells/ml growing at 21.0 ± 1.0 C (bottom curve) was exposed for 10 min to 0.2 μCi of ³H-thymidine per ml and eluted with conditioned medium at 21.0 ± 2.0 C. Elution time is expressed in generations. The ages of the ancestor cells are indicated at top of frame. Vertical lines are drawn at 1.0 and 2.0 generations.*

radioactivity per cell during the division cycle of *E. coli* B/r growing in glucose minimal medium was similar at 37.0 ± 0.5 and 21.0 ± 1.0 C (Fig. 5). Although it was difficult to determine the exact cell age at initiation, no obvious difference was observable at the two temperatures. The rate of radioactive thymidine incorporation increased around the middle of the division cycle. This is consistent with the insertion of new replication points at a cell age of approximately 0.5 (12).

In contrast to the preceding examples, the pattern of DNA replication during the division cycle of cells growing in glucose medium supplemented with Casamino Acids and tryptophan was not the same at 21 and 37 C (Fig. 6). At 37 C new rounds appear to be initiated at approximately age 0.8, whereas at 21 C initiation was at age 0.4.

Chromosome replication and cell division. The results shown in Fig. 3 through 6 are summarized in Table 1. The generation time, the absorbance per 10⁸ cells at 450 nm, (C + D), and the ratio D/(C + D) are given for cultures growing at 37.0 ± 0.5 and 21.0 ± 1.0 C in various media. In all media, the temperature coefficients of (C + D) and the generation

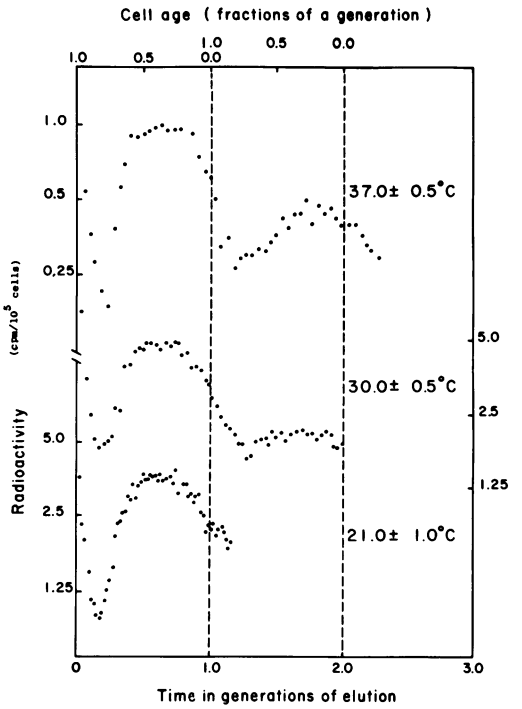

FIG. 4. *Radioactivity per cell in the effluent from membrane-bound cultures growing in acetate minimal medium at various temperatures. Cells growing in acetate medium at 37.0 ± 0.5, 30.0 ± 0.5, and 21.0 ± 1.0 C were exposed to 0.02 μCi of ^{14}C-thymidine per ml (37 C) or 0.2 μCi of ^{3}H-thymidine per ml (30 and 21 C) for 10 min, bound to a membrane filter, and eluted with conditioned medium at 37.0 ± 0.5 C.*

time were the same. The ratio D/(C + D) was independent of both media and temperature and equal to approximately 1/3.

Age at initiation. The times during the cell division cycle which corresponded to the midpoints of the decreases in the radioactivity curve were taken as the cell ages at initiation of chromosome replication. The ages were calculated by drawing straight lines through the experimental points of the plateau and the stepwise regions and determining the age corresponding to the midpoint of the step. Although the decreasing portions of the radioactivity curves in rapidly growing cells were broad and extended over as much as 50% of the generation time, the standard deviation of the calculated ages at initiation in repeated experiments was approximately ±10%.

The calculated cell age at initiation of chromosome replication was independent of temperature in cultures growing in succinate, acetate, and glucose minimal media. In contrast,

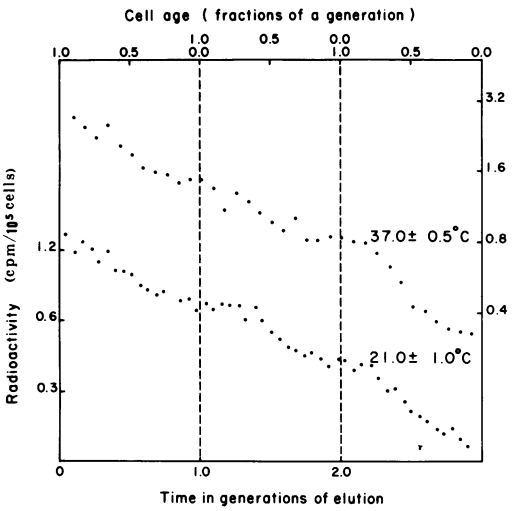

FIG. 5. *Radioactivity per cell in the effluent from membrane-bound cultures growing in glucose minimal medium at various temperatures. Cells growing in glucose minimal medium at 37.0 ± 0.5 and 21.0 ± 1.0 C were exposed to 0.01 μCi of ^{14}C-thymidine per ml for 4 min, bound to a membrane filter, and eluted with conditioned medium at 37.0 ± 0.5 and 21.0 ± 2.0 C, respectively.*

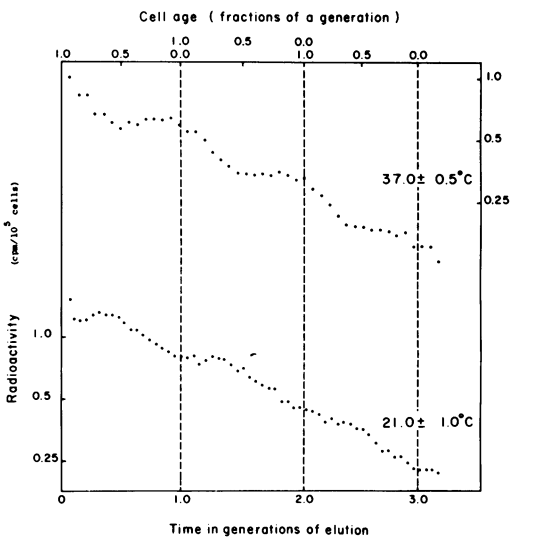

FIG. 6. *Radioactivity per cell in the effluent from membrane-bound cultures growing in minimal medium containing glucose plus Casamino Acids and tryptophan at various temperatures. Cells growing in glucose plus Casamino Acids and tryptophan at 37.0 ± 0.5 and 21.0 ± 1.0 C were exposed to 0.01 μCi of ^{14}C-thymidine per ml for 2 and 4 min, respectively, bound to a membrane filter, and eluted with conditioned medium. Elution media were at 37.0 ± 0.5 and at 21.0 ± 2.0 C, respectively.*

TABLE 1. *Growth characteristics at 37 and 21 C*

Growth medium	37 C					21 C				
	τ[a]	Absorbance[b]	Age at initiation[c]	(C + D)[d]	D/(C + D)	τ[a]	Absorbance[b]	Age at initiation[c]	(C + D)[d]	D/(C + D)
Succinate	85	0.10 ± 0.01	0.97	80	0.35	235	0.10 ± 0.01	0.93	235	0.32
Acetate	120	0.11 ± 0.01	0.92	110	0.32	(225)[e]	0.14 ± 0.01	0.97	(225)[e]	0.30
Glucose	47	0.24 ± 0.01	0.55	67		125	0.21 ± 0.01	0.53	187	
Casamino acids & glucose	27	0.44 ± 0.04	0.82 ± 0.04[f]	65	0.33	78	0.48 ± 0.07	0.32 ± 0.04[f]	200	

[a] Generation time in minutes on the membrane.
[b] Average absorbance at 450 nm per 10^8 cells of exponentially growing cultures ± standard deviation of the mean.
[c] Age at initiation in fractions of a generation.
[d] Length of (C + D) in minutes, determined on membrane.
[e] Culture was shifted to 37 C at the start of elution.
[f] Average age at initiation ± standard deviation of the mean.

the age at initiation in cultures growing in Casamino Acids-supplemented glucose medium was different at 37 and 21 C (Table 1). Since there is a relationship between cell size and cell age at initiation in rapidly growing cells at 37 C (7, 13), the observed variation in age at initiation at 37 and 21 C could be a consequence of a difference in the average cell size at the two temperatures. To determine whether initiation occurred at a critical size in rapidly growing cells, independent of the temperature, the age at initiation expected on the basis of the absorbance per cell at 450 nm was calculated. At the slow growth rates, initiation was assumed to occur at division (3, 12). A comparison between the theoretical ages at initiation and the experimental values (from experiments similar to those of Fig. 3–6) is shown in Fig. 7. If a round were initiated either at a fixed size or at division, independent of the temperature, the data points would lie along the line drawn at 45° from the origin. The data points generally lie along this line, but the dispersion of the values is so large that a firm conclusion concerning a relationship between initiation and cell size cannot be drawn.

DISCUSSION

In *E. coli* B/r, the interdivision times and (C + D) were, at 21 C, 2.9 ± 0.1- and 2.8 ± 0.1-fold longer than at 37 C. At all temperatures and in all growth media, C was twice D. Since C and D were equally affected by the temperature, it appears that they may be controlled by a common process which is temperature-dependent and is affected by the composition of the media only at the slow growth rates (1, 3, 12).

The process which may play a general regulatory function on DNA replication and cell division in *E. coli* could be the growth of the membrane envelope. A coupling between chromosome replication and membrane (17, 22, 24) was first suggested by Jacob et al. (18). It has since been shown that the chromosome is attached at the origin (26), the replication point (9–11, 25), and the terminus (26).

In contrast to the results reported here for *E. coli* B/r, a differential effect of the temperature on initiation and chromosome replication in *E. coli* 15T$^-$ has recently been reported by Urban and Lark (27). When cultures growing in aspartate and succinate media were shifted from 37 to 21 C, the period devoid of DNA synthesis between rounds of chromosome replication was greatly reduced or absent. At intermediate growth rates, the temperature did not have a differential effect.

Recently, Urban and Lark (27) have confirmed my observations with *E. coli* B/r. Succinate and acetate cells of *E. coli* B/r growing exponentially at 37 and 20 C were pulse-labeled with ^{3}H-thymidine and analyzed by autoradiography. The fractions of unlabeled cells at 20 C were 20.3% in succinate and 18.6% in acetate minimal media, which is not significantly different from the results at 37 C (Lark, *personal communication*). The reason for the different temperature response of the two strains of *E. coli* is unknown, but it seems unlikely that different mechanisms of regulation are the cause of this difference.

The results reported here are consistent with previous findings by Schaechter et al. (23) on *Salmonella typhimurium*. In this bacterium, the DNA and ribonucleic acid content were characteristic of the composition of the culture medium and independent of the temperature of growth.

My results indicate that chromosome replication extends throughout the first two-thirds of the division cycle of *E. coli* B/r growing in succinate and acetate medium (i.e., C + D = τ).

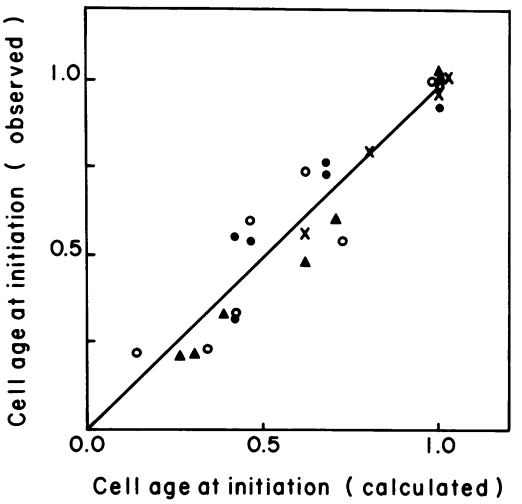

FIG. 7. *Cell age at initiation of chromosome replication. Cultures growing in Casamino Acids-supplemented glucose, Casamino Acids-supplemented acetate, and glucose, succinate, and acetate minimal media at 37.0 ± 0.5 (●), 30.0 ± 0.5 (×), and 21.0 ± 1.0 C (○, ▲) were pulse-labeled with radioactive thymidine, bound to a membrane filter, and eluted with media either at 37.0 ± 0.5 C (●, ×, ○) or at 21.0 ± 2.0 C (▲) (see Fig. 2–6 for details). Cell age at initiation was determined as shown in Fig. 2. The ages at initiation in cells growing in glucose plus Casamino Acids, acetate plus Casamino Acids, and glucose minimal media were calculated from the equation:*

$$\int_0^x 2^{1-x}dx = 2n(0.115)/\bar{m}$$

where x in the age at initiation in fractions of a generation, $\bar{m}$ is the absorbance at 450 nm per 10^8 cells of an exponentially growing culture, 0.115 is the absorbance at initiation per chromosomal origin per 10^8 cells (15), and (2n) is the number of origins per cell. When cell division occurred before the attainment of critical size, as at slow growth rates, division was considered to determine initiation, and the age was taken equal to 1.0. A similar relationship between experimental and theoretical age at initiation is obtained by assuming a linear increase in absorbance during the division cycle such that x = (ln 2) (2n)0.115/$\bar{m}$ at the faster growth rates, and x = 1.0 at the slower growth rates.

The results are in agreement with previous observations from this and other laboratories on the length (1–3, 6, 8, 12) and the location in the cell cycle (3–5, 12, 15) of the DNA synthetic interval. They are not consistent with the recent proposal of Kubitschek and Freedman (19) that (C + D) is constant and equal to 72 min in exponentially growing cultures with generation times as long as 120 min (i.e., C + D < τ). Their proposal was based on colorimetric determinations of the average amount of DNA per cell in exponentially growing cultures. However, the total DNA content per cell depends on (i) the length of the DNA synthetic interval, (ii) the location of the interval in the division cycle, and (iii) the rate of DNA synthesis during the interval. Their suggestion that DNA replication did not occupy the first two-thirds of the division cycle in slowly growing batch cultures was based on the assumption that the rate of DNA synthesis was constant during the interval. Although the different experimental approaches (1, 3, 12) give information on the length of a round of replication, they are inadequate for determining the rate of chromosome elongation during the interval. If the rate increased throughout the first two-thirds of the cycle, perhaps reaching the maximal level late in the interval, the average number of genomes per cell would be in the range of 1.4 to 1.5 which is in reasonable agreement with the values obtained by Kubitscher and Freedman (19).

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