# DNA Chain Growth during Replication of Asynchronous L1210 Cells. Alkaline Elution of Large DNA Segments from Cells Lysed on Filters†

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ABSTRACT: The alkaline elution of DNA from cells lysed on filters depends on strand length. This procedure was calibrated on the basis of the dependence of elution on X-ray dose. The X-ray sensitivity appeared to be first order in X-ray dose and indicated an apparent target size of $1.3 \times 10^9$ daltons, under the conditions used for the assay. The assay permitted a kinetic study of the incorporation of newly replicated DNA strands into large segments, of the order of $10^9$ daltons in size. The kinetics conformed to a simple model of chain growth. It was estimated that the time required for the replication of a segment of this size was about 2.5 hr and that the steady-state fraction of incompletely replicated DNA was about 8%.

The growth of DNA strands from the shortest size class, analogous to Okazaki pieces (Okazaki *et al.*, 1968) to strands that correspond to the replicons observed by autoradiography (Huberman and Riggs, 1968), can be traced by alkaline sucrose gradient sedimentation (C. A. Friedman and K. W. Kohn, in preparation). The further course of events then involves joining of replicons to form much larger units that sediment as an aggregate in alkaline gradients. The anomalous sedimentation makes it difficult to study this last stage of replication by the alkaline sucrose gradient method.

In this report, we use an alkaline elution procedure to examine this last stage of replication. The procedure is based on a previously reported finding that DNA from mammalian cells lysed on filters is ordinarily eluted only very slowly by alkaline solutions, and that substantial increases in elution rates are produced by relatively small extents of single strand breakage (Kohn and Ewig, 1973). The methodology has been improved and is here applied to studies of X-ray sensitivity and replication of DNA in mammalian cells.

## Methods

*Cells.* Mouse leukemia L1210 cells (Moore *et al.*, 1966) were grown in suspension culture in RPMI 1630 medium with 20% heated fetal calf serum, plus penicillin and streptomycin. Stock cultures were maintained in static bottles without antibiotics and were used to initiate suspension cultures at weekly intervals. Cultures were periodically tested for PPLO and were free of contamination. Suspension cultures proliferated logarithmically up to $1.5–2.5 \times 10^6$/ml with a doubling time of 12–14 hr; cells harvested for the experiments were at a density of approximately $1.0 \times 10^6$/ml.

*Alkaline Elution.* Approximately $10^6$ cells were diluted in cold phosphate-buffered saline and filtered onto 25 mm diameter, 1.2 μm pore-size cellulose triacetate filters (Gelman 'Metricel'). After six washes with 5 ml of cold phosphate-buffered saline, the cells were lysed on the filters at room temperature with 10 ml of 2 M NaCl, 0.02 M $Na_3$-EDTA, 0.2% Sarkosyl, and 0.08% deoxycholate (pH 8.2) at a flow rate of approximately 3 ml/min. The salt and detergent were removed by washing with 5 ml of $10^{-3}$ M $Na_3$EDTA. The out-flow tube of the filter funnel (Gelman, catalogue number 1112, aluminum out-flow tube replaced by a 1-mm diameter stainless steel tube) was then connected by Tygon tubing to a peristaltic pump. The filter was then eluted with 0.018 M tetrapropylammonium hydroxide–0.02 M tetrapropylammonium salt of EDTA (pH 12.8) at a pump speed of 0.25–0.5 ml/min. The funnels were shaded against exposure of the alkaline lysates to room fluorescent lighting. Fractions were collected at 12-min intervals and were mixed for scintillation counting with 3.3 volumes of Aquasol (New England Nuclear) plus 0.3% acetic acid. Radioactivity remaining on the filters was determined by treating the filters with 0.4 ml of 1 N HCl at 70° for 1 hr, followed by 2.5 ml of 0.4 N NaOH at room temperature for 30 min and 10 ml of Aquasol. Radioactivity remaining in the funnel was recovered by three washes with 3 ml of 0.4 N NaOH and the counts were added to those remaining on the filter.

*Alkaline Sucrose Gradient Sedimentation.* DNA was eluted from filters as described above except that at intervals samples were pumped directly onto alkaline sucrose gradients, and the pump speed was reduced to 0.05 ml/min (1.5-mm diameter tubing) so as to minimize shearing. Gradients consisted of 5–20% sucrose in 0.12 M NaOH–0.9 M NaCl–0.01 M EDTA and were handled in the dark (Elkind, 1971). Total volume was 38 ml, including a 5 ml cushion of 60% sucrose. Gradients were centrifuged in an SW27 rotor at 10,500 rpm for 18 hr at 3° and were calibrated relative to a value of 32S for T7 DNA.

## Results

*Alkaline Elution of DNA from Cells Lysed on Filters.* The separation between newly synthesized DNA and bulk DNA by the alkaline elution method used is illustrated in Figure 1. In this experiment, L1210 cells were grown in the presence of [$^{14}$C]thymidine for 20 hr, by which time all of the radioactivity had been incorporated. The cells were then labeled with [$^3$H]thymidine for 2.5 hr. Most of the previously synthesized [$^{14}$C]DNA is seen to elute in a broad

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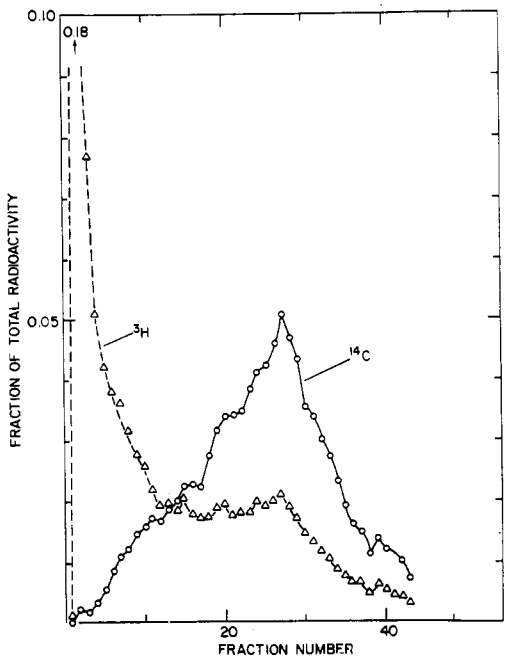


FIGURE 1: Alkaline elution patterns of DNA from L1210 cells labeled with [2-$^{14}$C]thymidine (0.01 $\mu$Ci/ml) (O) for 20 hr followed by [$^3$H]thymidine (0.5 $\mu$Ci/ml, $10^{-5}$ M unlabeled thymidine added) ($\Delta$) for 2.5 hr. Elution rate, 0.25 ml/min. (Fraction of radioactivity remaining on filter after elution was 5% of $^{14}$C and 2% of $^3$H.)

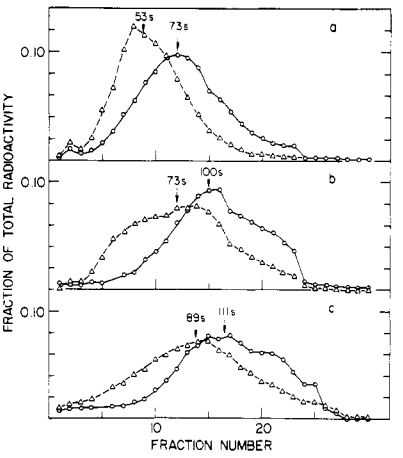


FIGURE 2: Alkaline sucrose gradient sedimentation of DNA eluted from filters at various times. Cells were labeled with [2-$^{14}$C]thymidine (0.1 $\mu$Ci/ml) (O) for 3 hr and with [$^3$H]thymidine (5 $\mu$Ci/ml) ($\Delta$) for 20 min. The collection times and total radioactivity on the gradients were (a) 30–60 min, $1.8 \times 10^4$ dpm $^{14}$C, $7.7 \times 10^5$ dpm $^3$H; (b) 74–105 min, $1.4 \times 10^4$ dpm $^{14}$C, $1.8 \times 10^5$ dpm $^3$H; (c) 120–150 min, $1.5 \times 10^4$ dpm $^{14}$C, $0.8 \times 10^5$ dpm $^3$H. (The initial 30 min of elution consisted mainly of dead space and therefore was not used.) The S values shown are for the median position of each distribution.

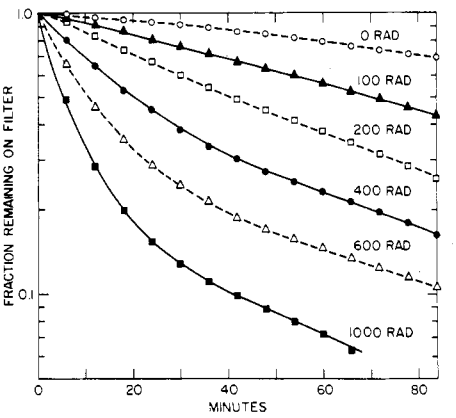


FIGURE 3: Effect of X-irradiation of L1210 cells on alkaline elution of DNA. Cells were labeled with [2-$^{14}$C]thymidine for 20 hr prior to X-irradiation at the indicated dose. Fraction of total cell DNA counts remaining on filter is plotted against elution time. Elution rate, 0.5 ml/min.

peak. The newly synthesized [$^3$H]DNA elutes rapidly, forming a sharp initial peak. When the pulse label was 20 min or less, 80–90% of the [$^3$H]DNA was in the initial peak (data not shown). After 2.5 hr of labeling, however, about 50% of the label eluted slowly, similarly to the main [$^{14}$C]DNA peak. About 15% of the [$^{14}$C]DNA appeared in a shoulder on the fast-eluting side, overlapping the rapid-elution region of the short-term label.

In order to determine the size range of DNA eluted at various times, the eluted solution was slowly pumped onto alkaline sucrose gradients and centrifuged. In the experiment illustrated in Figure 2, cells were labeled with [$^{14}$C]thymidine for 3 hr and [$^3$H]thymidine for 20 min. The elution rate was slowed to 0.05 ml/min in order to reduce the possibility for mechanical shearing. Figure 2 shows that all of the eluted DNA, even that labeled for only 20 min, is of high molecular weight. With increasing elution time, the sedimentation is seen to increase. The sedimentation of the DNA labeled for 2.5 hr was at all times greater than that labeled for 20 min. The major part of the eluted 20-min label sedimented with a peak at 53 S (Figure 2a), in agreement with the case of direct lysis of cells on the gradient (C. A. Friedman and K. W. Kohn, in preparation). The faster sedimenting distributions, seen especially in the 3-hr $^{14}$C label, could be influenced by breakdown of larger strands due to prolonged alkali exposure or shearing on passage through the filter.

*X-Ray Sensitivity of DNA in L1210 Cells.* The dependence of DNA elution on single-strand length was assessed on the basis of X-ray sensitivity. The general features of the time course of elution after various doses of X-ray are shown in Figure 3. X-Ray was delivered to intact cells that had been labeled for 20 hr with [2-$^{14}$C]thymidine. At the higher X-ray doses used, there is seen to be a rapid initial phase of elution, followed by a slower phase. After very low doses, on the other hand, the initial elution is very slow and gradually increases with time. The initial elution rate is strongly dependent on X-ray dose, whereas the later phase of elution becomes less dependent on X-ray dose.

The effect of X-ray can be quantitated by selecting a fixed time of elution. The dependence of extent of elution on X-ray dose usually approximated a first-order relation. This is shown in Figure 4 for an elution time of 48 min. Deviations from a first-order relation occur at high X-ray doses or long elution times, when the slope decreases with dose, and at very short elution times, when a small initial shoulder is sometimes seen. Under the conditions judged to be optimal (Figure 4), the X-ray sensitivity corresponded to an apparent one-hit dose of 340 rad. This dose corresponds to single-strand target size of about $1.3 \times 10^9$ daltons (see Discussion).

*Replication.* Shortly after its replication, DNA is in a rapidly eluting state (Figure 1). The kinetics of the conversion of this DNA to "mature" slowly eluting DNA was studied by pulse-chase and by continuous labeling experiments. Typical elution patterns at various times after a 15-

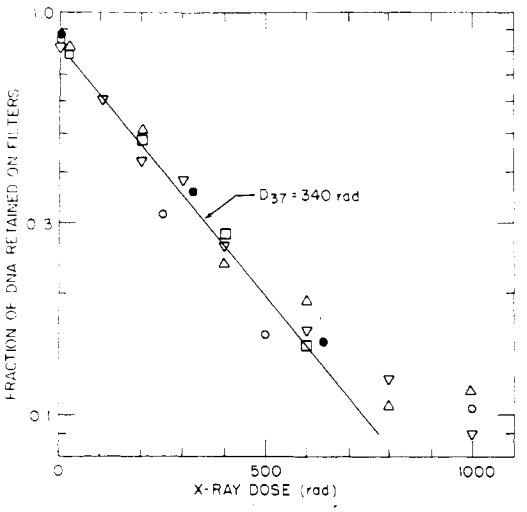


FIGURE 4: X-Ray sensitivity of total cell DNA as measured by alkaline elution. Experiments similar to those in Figure 3. Fraction of DNA retained on filter after 50 min of elution is plotted against X-ray dose. Different symbols refer to independent experiments.

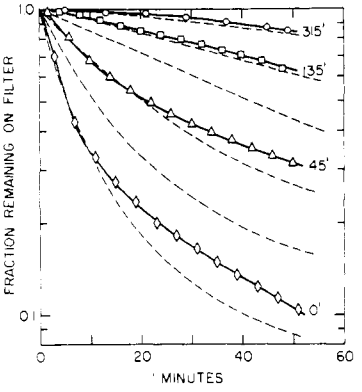


FIGURE 5: Elution of pulse-labeled DNA after various times of chase. L1210 cells were labeled with [$^3$H]thymidine for 10 min, then incubated without thymidine for the indicated times. Dashed lines are replotted from Figure 3 to show the similarity of the shapes of the curves to those produced by various X-ray doses.

min [$^3$H]thymidine pulse are shown in Figure 5. The shapes of the elution patterns were similar to those observed after various doses of X-ray (Figure 3), and a correspondence can be made between chase time in Figure 5 and X-ray dose in Figure 3. For example, the pattern obtained immediately after the [$^3$H]thymidine pulse corresponds to about 850 rad; the 45-min chase pattern corresponds to about 350 rad; and the 135-min chase pattern corresponds to about 100 rad. Thus, assuming that elution rate is a function of strand size, the size distributions of segments containing labeled DNA in various stages of chain growth can be associated with the size distributions produced by X-irradiation after uniform labeling of DNA.

The kinetics of the conversion of pulse-labeled DNA to "mature" DNA are shown in Figure 6. After 5 min of labeling, the cells were centrifuged and resuspended in fresh medium; the inset to Figure 6 shows the leveling-off of [$^3$H]thymidine incorporation with time after resuspension. The cells had also been uniformly labeled for 20 hr with [$^{14}$C]thymidine, so as to facilitate quantitation of the difference in elution between newly replicated ($^3$H-labeled) DNA and bulk ($^{14}$C-labeled) DNA. The fraction of the $^3$H label eluted divided by the fraction of the $^{14}$C label eluted during

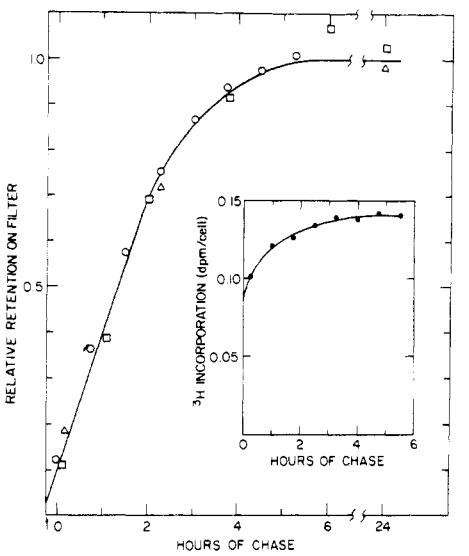


FIGURE 6: Change in elutability of pulse-labeled DNA with time of chase. L1210 cells were labeled with [2-$^{14}$C]thymidine (0.02 $\mu$Ci/ml) for 20 hr and with [$^3$H]thymidine (0.2 $\mu$Ci/ml) for 10 min, and then incubated in the absence of thymidine for various times. The inset shows the total incorporation of $^3$H at various times after resuspension in the absence of thymidine. Relative retention on filters is the fraction of $^3$H retained divided by the fraction of $^{14}$C retained, determined after 50 min of elution.

a standard 48-min elution period was taken to be the fraction of the newly replicated DNA that is "rapidly elutable." This is the fraction of newly replicated DNA that elutes more rapidly than bulk DNA. Figure 6 shows the increase in the fraction of slow eluting DNA as a function of chase time. The increase is seen to be initially linear. Extrapolation of the linear segment back to zero time of labeling indicated that more than 90% of newly synthesized DNA is rapidly elutable. The conversion of rapid eluting to slow eluting DNA was half-complete after 1.5 hr of chase.

The incorporation of [$^3$H]thymidine into rapidly eluting and slowly eluting DNA fractions was also examined by uniform labeling experiments (Figure 4). Labeling was carried out in the presence of $10^{-5}$ M thymidine so that the rate of incorporation into DNA was nearly constant over the time course of the experiment (Figure 5, inset). The $^3$H activity in rapid eluting DNA initially rises linearly and then levels off at a plateau. The $^3$H activity in slowly eluting DNA lags at first and then assumes a linear rise.

These kinetics are consistent with a precursor–product relation between rapid eluting and slow eluting DNA. The rapid eluting precursor becomes saturated with label in about 3 hr. This therefore is an estimate of the time required for the synthesis of slow eluting DNA.

## Discussion

We have outlined a procedure by which the synthesis of long DNA single strands in cells can be conveniently monitored. The procedure is based on the finding that bulk DNA in untreated cells elutes only very slowly when cells are lysed on filters and subjected to high pH, whereas the elution rate is greatly increased if the cell DNA has been broken by X-ray or if the labeled DNA is caught in the process of replication.

The elution patterns of DNA from cells exposed to various doses of X-ray (Figure 3) suggest that the elution is governed by two independent processes: (1) immediate elution of broken DNA segments at a rate that increases with

decreasing size of segments, and (2) the slow accumulation of new DNA breaks, resulting in the delayed elution of old DNA from control cells. This interpretation is further supported by more detailed studies of the kinetics of elution which are still in progress. The slowness of the elution of old DNA might be due simply to the large size of the strands or to an attachment to other cell structures that do not pass thru filters. Although we cannot rule out attachment, size clearly has a major effect on elution rate, possibly due to the time required for the disentanglement of long DNA double strands. In any case, slow elution of DNA strands from control cells indicates that these strands have achieved a large average size.

The alkaline elution assay involves a separation of cell DNA into rapid eluting and slow eluting fractions under defined conditions of elution time and pH. The choice of conditions is somewhat arbitrary and determines the sensitivity relative to the length of the DNA strands that are discriminated. The sensitivity of the conditions used was estimated from the dependence of DNA elution on X-ray dose, which was found to be approximately first order, at least over a reasonable range of conditions. Although the significance of the first-order relation requires further study, the data in Figure 3 indicate an apparent target size corresponding to 340 rad. This X-ray dose should produce about one single-strand break per $1.3 \times 10^9$ daltons, based on the relation between dose in rads ($r$) and number average molecular weight ($M_n$) derived by Lehmann and Ormerod (1970b): $1/M_n = 2.3 \times 10^{-12} r$. The slow eluting DNA component therefore appears to consist of strands of the order of $10^9$ daltons or 1000 $\mu$m in length.

The average size of strands at various times of replication can be roughly estimated by comparing the elution profiles with those obtained after various doses of X-ray. Figures 3 and 5 show that the elution profiles of pulse-labeled DNA after various chase times are similar in shape to the profiles obtained after various X-ray doses. It is therefore reasonable to associate the elution in a pulse-chase experiment with the X-ray dose required to produce similar elution. Since the number average molecular weight of DNA single strands can be calculated from the X-ray dose, the same molecular weight value is an estimate of the size of the labeled DNA in the pulse chase experiment.. Following a pulse label time of 10 min, chase times of 0, 45, and 135 min gave profiles that correspond to X-ray doses of 850, 350, and 100 rad, respectively. The corresponding number average molecular weights would be 0.5, 1.2, and $4.3 \times 10^9$, or in terms of length 500, 1200, and 4300 $\mu$m. This interpretation assumes that the X-ray sensitivity of newly synthesized DNA, as measured by elution, is as high as that of old DNA; this assumption was in accord with experiment. The length estimates may actually be somewhat too low because of end-labeling bias (Lehmann and Ormerod, 1970a). The sedimentation of eluted DNA in alkaline sucrose gradients (Figure 2) did not disclose strands of such high molecular weight, possibly because of inevitable breakage of extremely long strands during elution and layering on the gradients.

Within the approximations and limitations that have been discussed, we can now consider the kinetics of the production of slow eluting DNA strands. In the pulse-chase experiment of Figure 6, we see that the production of slow eluting DNA is initially linear and extrapolates back to an intercept of about 5% (experimental limits, 0–10%) slow eluting DNA at zero time. Two conclusions can be derived

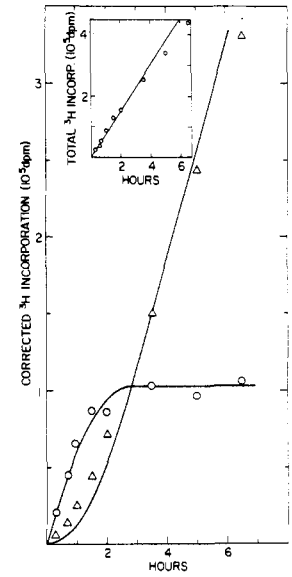


FIGURE 7: Incorporation of [$^3$H]thymidine into rapid eluting (○) and slow eluting (△) DNA during a uniform labeling period. Cells were labeled with [2-$^{14}$C]thymidine (0.02 $\mu$Ci/ml) for 20 hr, followed by [$^3$H]thymidine (0.5 $\mu$Ci/ml) plus $10^{-5}$ M unlabeled thymidine. The total incorporation of $^3$H is shown in the inset. The $^3$H incorporation values were corrected for the fraction of $^{14}$C eluted. The curves were derived from theory, as described in the Discussion.

from this. First, there is little or no incorporation directly into slow eluting strands. Hence all, or nearly all, of the DNA is synthesized initially as rapid eluting strands. By the time DNA has become slow eluting, there is little or no further growth of the strands. Secondly, the initial linearity indicates that there is no lag between the completion of labeling of a strand and its conversion to a slow eluting state. It can be concluded therefore that DNA strands become slow eluting at or about the time they stop replicating. If thymidine incorporation ceased before the DNA becomes slow eluting, there should be an initial lag before a pulse label begins to appear in the slow eluting fraction. This is contrary to the data within the limits of precision of the measurements. Conversely, the possibility that DNA becomes slow eluting before thymidine incorporation ceases would imply that some thymidine should be incorporated directly into slow eluting DNA. As explained above, such direct incorporation was less than 10% of the total incorporation. Hence, within experimental limits the two events are simultaneous.

The time required for the production of slow eluting DNA strands can be estimated from the experiments of Figures 6 and 7 to be about 3 hr. The X-ray sensitivity of such DNA was found to be similar to that of old DNA, indicating a size of about $10^9$ daltons; DNA segments of this length (1000 $\mu$) thus are generated in a period of about 3 hr. The average effective growth rate therefore is about 5 $\mu$/min. Since the elongation rate at a DNA growing point is probably not over 1 $\mu$/min (Painter and Schaefer, 1969) at least five growing points must contribute to the production of a 1000-$\mu$ length in 3 hr. If each replicon grows at both ends (Huberman and Tsai, 1973; Amaldi *et al.*, 1972), this means that at least three replicons must contribute to the production of these 1000-$\mu$ lengths. The autoradiograms of Huberman and Riggs (1968), however, suggest that perhaps as many as 25 replicons may contribute to a 1000-$\mu$ length. If the chain elongation rates are 1 $\mu$/min at each growing point, our figures would indicate that during the

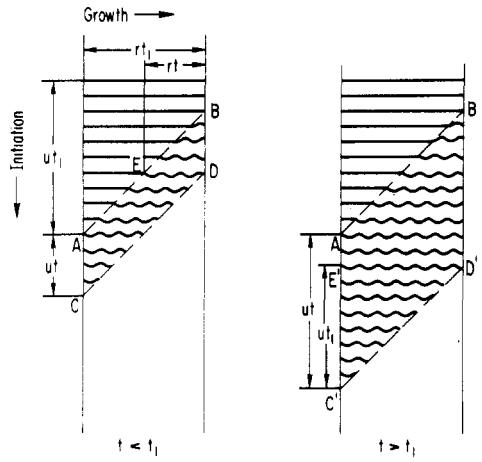


FIGURE 8: Diagrammatic representation of the simplified replication model (see text).

synthesis of a 1000-$\mu$ length in an exponential phase cell culture, there are an average of about five growing points active at any one time. The greater number of growing points seen in the autoradiograms can be attributed to cell synchronization.

*Replication Kinetics.* The observed kinetics are consistent with a simplified model based on the following assumptions. (1) Mature nuclear DNA is composed of equal-length "units" of slow eluting strands. (2) New units are synthesized by the joining of individual replicons; the total rate of incorporation of precursors into the replicons destined to make up a given unit is approximately constant and represented by $r$ dpm/min. (3) The replication of DNA units is initiated and completed at a fixed rate of $u$ units/min. (4) A unit becomes slow eluting at the same time it stops incorporating precursors.

This model predicts that the radioactivity in fast-eluting DNA is

$$urt(t_1 - (t/2))(t \leq t_1)$$

$$\frac{1}{2}urt_1^2 \qquad (t \geq t_1)$$

and the radioactivity in slow eluting DNA is

$$\frac{1}{2}urt^2 \qquad (t \leq t_1)$$

$$urt_1(t - (t_1/2))\,(t \geq t_1)$$

where $t$ is the incorporation time and $t_1$ is the time required for the complete replication of a DNA unit.

This model was applied to the data in Figure 7 to give the theoretical curves shown. The parameters used for fitting the data were (1) total incorporation rate ($urt_1 = 0.74 \times 10^5$ dpm/hr), derived from the slope of the incorporation curve shown in the inset of Figure 7, and (2) the steady-state level of radioactivity in rapid eluting DNA ($\frac{1}{2}\,urt_1^2 = 1.03 \times 10^5$ dpm), obtained from the plateau level in Figure 7. Considering the simplicity of the assumptions and the conservative nature of the method used to determine the values of the two parameters needed, the fit to the experimental data seems encouraging.

The time required for the synthesis of a slow eluting DNA unit, derived from Figure 7 is $t_1 = 2(\frac{1}{2}urt_1^2)/urt_1 = 2(1.03 \times 10^5)/0.74 \times 10^5 = 2.8$ hr. This is the time when equal radioactivity has been incorporated into rapid eluting and slow eluting DNA. This value indicates that a substantial fraction of a DNA synthetic phase of the cell cycle is required for the complete synthesis of a slow eluting unit.

The average amount of replicated DNA present in rapidly eluting form in an S-phase cell can be estimated from the level of the plateau in Figure 7. The amount of radioactivity at the steady-state level in rapid eluting DNA is equal to the amount incorporated by the exponential phase culture in $1.03 \times 10^5/0.74 \times 10^5 = 1.4$ hr. Since the doubling time was 12 hr, this represents $2^{1.4/12} - 1 = 8.4\%$ of the total DNA content of $G_1$ cells. Hence, an average S-phase cell in a random culture having a doubling time about twice the S-phase time would contain approximately 17% of a $G_1$ cell equivalent of DNA in rapidly eluting form. It will be of interest to compare these replication parameters in different cell types, under various physiological conditions, and in response to drug treatment.

## Appendix

*Kinetics of DNA Segment Maturation Based on a Simplified Replication Model.* The equations presented in the Discussion can be derived from a convenient pictorial representation of the model (Figure 8). The cell DNA is considered to be divided into equal segments that have the property of slow elution. Each such segment is formed during replication by the joining of shorter pieces such as replicons and Okazaki fragments. The nucleotides that have been incorporated by a given time into the pieces destined to become a given segment are represented by a horizontal line (Figure 8). The length of the line is proportional to the number of such nucleotides incorporated. Wavy lines are used to indicate radioactive nucleotides. It is assumed that the rate of incorporation of nucleotides, $r$ (*i.e.* the rate of elongation of a line), is constant up to the time when the segment is complete. Secondly, it is assumed that new chains are initiated at a constant rate $u$. Finally, we assume that a segment becomes slow eluting at the same time that it stops incorporating nucleotides. The time required for the growth of a complete segment is $t_1$. The number of nucleotides in a complete segment is $rt_1$. In the diagram, time moves from left to right as segments grow at a rate, $r$, and time moves downward on the vertical axis as new chains are initiated at a rate, $u$.

At a given time, the replication "front" is represented by the dashed diagonal line, AB, which delimits the growing "ends" of incomplete segments of various "lengths." We suppose that at this time incorporation of radioactive pre-

CHART I

| $t$ | Elution | Area | Radioactivity |
|---|---|---|---|
| $\leq t_1$ | slow | EBD | $\frac{1}{2}urt^2$ |
| $\geq t_1$ | slow | E'ABD' | $\frac{1}{2}urt_1^2 + urt_1(t - t_1) = urt_1(t - (t_1/2))$ |
| $\leq t_1$ | fast | AEDC | $\frac{1}{2}urt_1^2 - \frac{1}{2}ur(t_1 - t)^2 = urt(t_1 - (t/2))$ |
| $\geq t_1$ | fast | E'C'D' | $\frac{1}{2}urt_1^2$ |

cursors begins and continues for a time, $t$, at the end of which the replication front is at CD. Two cases must be considered: $t < t_1$ and $t > t_1$ (Figure 8). The radioactivity in fast and slow eluting DNA then corresponds to areas in Figure 8 as shown in Chart I.

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# Analysis of Isoaccepting tRNAs during the Growth Phase Mitotic Cycle of *Physarum polycephalum*†

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ABSTRACT: A reverse phase chromatography study of *Physarum polycephalum* isoaccepting tRNAs isolated during the growth phase mitotic cycle was undertaken. No significant quantitative or qualitative changes were noted in the 20 tRNA families during the mitotic cycle, although some question remains as to a possible quantitative change in the seryl tRNA population. These data combined with aminoacylation studies reported previously, which showed essentially complete quantitative stability in aminoacylation levels for 20 amino acids throughout the mitotic cycle (Merera, P. W., and Rusch, H. P. (1973), *Biochemistry 12,* 1307), strongly suggest that while under restrictive quantitative and qualitative control itself the possible involvement of tRNA in the active control of growth phase mitotic cycle events or growth phase protein synthesis appears to be minimal. The RPC-2 chromatograms revealed the presence of 44 isoaccepting tRNA species during the growth phase mitotic cycle. Single acceptors were found for seven amino acids, four of which, Asp, His, Ile, and Trp, are coded for by three or less codons, while the remaining three, Ala, Gly, and Val, are coded for by four codons. Only two isoacceptors were found for Leu.

The unique biology of the myxomycete *Physarum polycephalum, i.e.,* a naturally synchronous mitotic cycle during both growth phase and during starvation leading to differentiation, and the ability to maintain all stages of the life cycle in the laboratory (see Rusch, 1970, for a review), coupled with a general lack of information concerning transfer RNA metabolism during the eucaryotic mitotic cycle prompted us to undertake a study of tRNA in this organism.

Recently we reported the results of a quantitative study concerning the *in vitro* aminoacylation levels for 20 amino acids throughout the *Physarum* growth phase mitotic cycle (Melera and Rusch, 1973b). The results showed that during the cycle (1) the amount of tRNA per unit of total nucleic acid remained constant, (2) the level of *in vitro* aminoacylation for the total tRNA population remained constant, (3) the relative levels of *in vitro* aminoacylation for each of the 20 amino acids remained constant, and (4) the synthetase enzymes did not change in their ability to aminoacylate either homologous or heterologous tRNA. From these results it was suggested that a tight quantitative control on the amount of tRNA synthesized and aminoacylated was in effect during the mitotic cycle. It was mentioned, however, that constant *in vitro* aminoacylation levels could conceivably be maintained while considerable variations in isoacceptor profiles might be occurring. Such variations in isoacceptor profiles could be indicative of transcriptional changes in tRNA synthesis or post-transcriptional changes in tRNA maturation and/or modification at various mitotic cycle stages.

This report presents the results of a reverse phase chromatography study designed to characterize the isoaccepting tRNAs of *Physarum* during its growth phase and to compare isoaccepting profiles from early, mid, and late mitotic cycle times.

## Materials and Methods

*Maintenance of Cultures.* Stationary cultures of *Physarum polycephalum* subline $M_3C_7$ were grown as described previously by Mohberg and Rusch (1969) with the slight modifications of Melera and Rusch (1973b).

*Preparation of tRNA and Aminoacyl Synthetase.* Purified tRNA shown to be undegraded and uncharged, to possess in-

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