THE TIMING OF DEOXYRIBONUCLEIC ACID SYNTHESIS IN THE CELL CYCLE OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Randomly dividing cultures of *Saccharomyces cerevisiae* were briefly exposed to radioactive adenine and then treated successively with dilute acid, ribonuclease, buffered formaldehyde, and NaOH. This treatment was shown to remove virtually all the radioactivity of the labelled cells other than that in DNA. Thus, in subsequent autoradiographs, only cells which had been synthesizing DNA during exposure to the precursor were labelled. The ages of these individuals within the cell cycle were estimated by measuring their sizes. This revealed that incorporation into DNA occurred almost exclusively during the first quarter of the cell cycle, starting with the initial appearance of the bud. This behaviour agreed closely with that of cells growing in artificially synchronized cultures.

The synthesis of deoxyribonucleic acid (DNA) is restricted in most types of growing cell to a relatively short part of the reproductive cycle (3, 7, 10). The first suggestion that this was so in budding yeasts was made by Ogur *et al.* (5) who observed that the appearance of the first generation of buds in a young culture of *Saccharomyces cerevisiae* was accompanied by a sharp rise in the DNA content of the cells. This observation was confirmed by Williamson and Scopes (14) using cultures which had been synchronized by an "environmental" procedure. In these cultures the DNA content of the cells doubled in a short period as soon as the buds emerged, and no further DNA was synthesized during the rest of the cycle. However, since the induction of synchronous division may have distorted the "normal" timing of the synthesis (3), and since it is difficult to determine the duration of synthesis accurately from the data obtained using synchronized cultures, it was desirable to determine the pattern of DNA synthesis in the cell cycle of unsynchronized individuals.

The method adopted for this purpose involved briefly exposing a randomly dividing culture to radioactive adenine and then treating the cells so as to remove virtually all their radioactive constituents other than DNA. In subsequent autoradiographs, only those cells which had been synthesizing DNA during the period of exposure to the precursor were labeled, and their positions within the cell cycle were estimated by measuring their sizes. The results indicate that in a randomly dividing culture the cell synthesizes DNA only during the first quarter of its reproductive cycle.

A preliminary account of this work has been published elsewhere (12).

METHODS

ORGANISM AND CULTURAL METHODS: A strain of *Saccharomyces cerevisiae* (National Collection of Yeast Cultures No. 239) was used throughout. Synchronously dividing cultures in a semi-defined medium (9) were prepared as described elsewhere (15) and incubated with vigorous aeration at 25°C. Randomly dividing cultures growing under the same

conditions were obtained by inoculating with between 5 and 20 × 10^3 cells per ml, using cells from a fully grown culture in double strength malt-extract medium (11). Under these conditions, the culture had a mean generation time of 115 to 120 minutes and passed through a well defined logarithmic growth phase of about 24 hours' duration. This ended when the cell density had reached about 30 × 10^6 per ml; the stationary phase was entered after about 42 hours, at a density of about 90 × 10^6 cells/ml. For the experiments described below, the cultures were used during the mid-logarithmic phase when they had reached densities of 1 to 12 × 10^6 cells/ml.

CELL COUNTING: All counts of cells in suspension were performed with a Coulter particle counter, the procedure being as described previously (9) except that the ultrasonic treatment needed to separate pairs of adhering individuals was carried out by exposure for about 90 seconds in an ultrasonic "cleaning bath" (M.S.E. Ltd., Crawley, England; 40 kc/second, 500 watts).

CHEMICAL ANALYSES: Protein was estimated by the Folin-phenol method of Lowry *et al.* (4). Analyses for DNA and ribonucleic acid (RNA) were performed using, respectively, the diphenylamine and orcinol reagents on extracts of cells subjected to a modified Ogur-Rosen preliminary extraction routine (14). In order to estimate separately the amounts of radioactivity in DNA and RNA, labelled cells were fractionated by a modification of the Schmidt and Thannhauser (8) procedure. After subjection to the modified Ogur-Rosen preliminary extraction routine (14), the cells were extracted for 30 minutes at 37°C with N NaOH, then rapidly cooled and acidified by adding ice-cold 6 N perchloric acid. The resulting precipitate, removed by centrifugation and washed in ice-cold 0.2 M perchloric acid, constituted the DNA fraction, whilst RNA was assumed to remain wholly in the initial supernatant fluid.

ENZYMIC DIGESTIONS: All digestions with enzymes were performed at 37°C. Crystalline deoxyribonuclease I (DNase) (Worthington) was used at a concentration of about 100 μg/ml in 0.05 M "tris" buffer (pH 7.5) containing 0.002 M $MgSO_4$. Crystalline pancreatic ribonuclease (RNase) (Worthington Biochemical Corp., Freehold, New Jersey) was used at a concentration of about 250 μg/ml in 0.05 M phosphate buffer (pH 7.6). Digestion of protein was accomplished using a mixture of trypsin and chymotrypsin (both Worthington products), each at a concentration of 250 μg/ml in 0.05 M "tris" buffer (pH 8.0). Snake venom (*Crotalus adamanteus*) was obtained from Ross Allen's Reptile Institute, Silver Springs, Florida.

CHROMATOGRAPHY: Descending paper chromatography of cell extracts containing nucleosides was carried out on washed Whatman No. 1 paper using butanol:ammonia solution (S.G. 0.88):water (86:1:4). Ultraviolet-absorbing areas were located with the aid of a "Chromatolite" lamp (Hanovia). Radioactive regions were detected by autoradiography on Kodak Blue Brand x-ray film.

MEASUREMENT OF RADIOACTIVITY: (8-^{14}C) Adenine (specific activity 28.3 mc/mM) and (2,8-^{3}H) adenine (specific activity 243 mc/mM) were obtained from the Radiochemical Centre, Amersham, England. The radioactivity of suspensions of cells and of solutions was estimated by drying down 40-μl aliquots onto discs of lens tissue mounted on glass coverslips and counting in a thin end-window counter (Nuclear Chicago model D-47). No correction was applied for self-absorption.

CELL AUTORADIOGRAPHY: Smears were made by freeze-drying drops of cell suspension on "subbed" slides. This had no noticeable effect on the morphology of the cells, but largely prevented their clumping during drying. Autoradiographs were prepared using Kodak V 1042 stripping film and exposure time of between 14 and 30 days (2). After photographic processing, they were stained for 15 minutes in Giemsa solution (G. T. Gurr, Ltd.) diluted 1:10 in 0.05 M phosphate buffer, pH 7.0.

PROCEDURE FOR SELECTIVELY LABELLING CELLS ENGAGED IN DNA SYNTHESIS: Twenty ml of a randomly dividing culture containing 5 to 10 × 10^6 cells/ml was rapidly transferred to a tube held in the water bath and containing a small volume of a concentrated solution of the appropriate radioactive precursor (see below). After aeration for 15 minutes, this subculture was rapidly mixed with an excess of ice-cold 0.2 M perchloric acid. The cells were rapidly collected on a membrane filter (Oxoid Ltd, London), and suspended in 0.2 M perchloric acid at 0°C for 30 minutes. After washing in water, they were digested for an hour at 37°C in about 2 ml of RNase solution (see above) and thereafter suspended first in 0.1 M phosphate buffer (pH 7.0) containing formaldehyde (*ca.* 0.3 per cent, w/v) for 30 minutes at room temperature and finally in N NaOH for 1 hour at 25°C, each step being followed by thorough washing in water. Appropriate autoradiographic or other analyses were then carried out.

When cell autoradiographs were to be made, tritiated adenine was used at a final concentration in the medium of 10 μc/ml, but when the amounts of radioactivity in whole samples of suspensions were to be measured by end-window counting, 0.1 μc/ml of ^{14}C-adenine was employed.

The validity of this process forms the subject of many of the experiments described below. For ease of reference, the extraction routine will be termed the "ribonuclease extraction procedure," and the radioactivity remaining in the cells after treatment the "residual radioactivity."

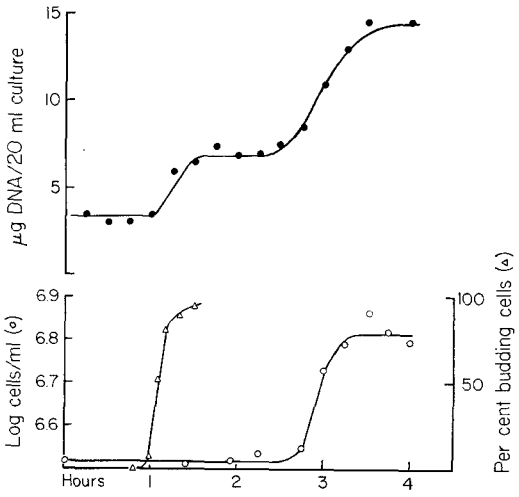

FIGURE 1 The course of DNA synthesis and cell division in a synchronously dividing culture. The appearance of the first generation of buds, denoting the end of the lag phase and the start of the first generation (13), is also shown.

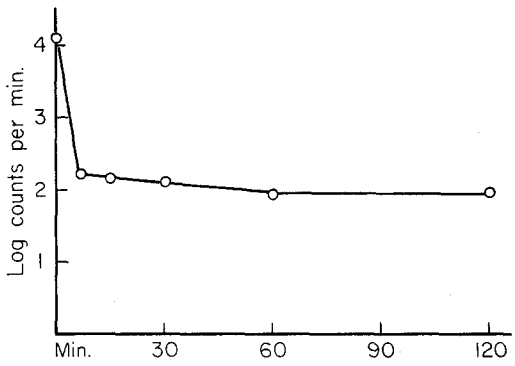

FIGURE 2 The effect of incubation with RNase on the radioactive content of labelled cells from a synchronously dividing culture. The culture was exposed to ^{14}C-adenine for 15 minutes during a period when no net synthesis of DNA took place. The cells were extracted for 30 minutes in ice-cold 0.2 M perchloric acid and washed before being suspended in the enzyme solution.

RESULTS

Trials with radioactive compounds showed that, in common with other tested strains of *Saccharomyces cerevisiae*, the chosen strain failed to incorporate exogenous thymine or thymidine into its nucleic acids. This was also the case with orotic acid, but adenine, guanosine, uracil, uridine, and cytidine were all incorporated to some extent, and of these adenine appeared to be the most effective precursor of DNA. Nevertheless, it was a very non-specific precursor. This was shown by an experiment in which a randomly dividing culture was exposed to ^{14}C-adenine for 15 minutes, extracted for 30 minutes in ice-cold 0.2 M perchloric acid, and then examined for its radioactive contents of DNA and RNA. Only about 1 per cent of the total radioactivity was associated with the DNA fraction. Moreover, exposure of the washed DNA precipitate to DNase for several hours rendered only about 40 per cent of its radioactive content soluble in dilute acid. Thus DNA probably accounted for about 0.4 per cent of the total radioactivity incorporated into acid-insoluble compounds. Consequently, in order to identify autoradiographically cells containing radioactive DNA, it would clearly be necessary first to submit the samples to a highly efficient procedure for selective removal of radioactive cell constituents other than DNA.

Development of the Ribonuclease Extraction Procedure

The efficiency of various procedures in removing "non-DNA" radioactivity was next examined. Synchronously dividing cultures were used for this purpose since, by labelling them during a period when no net synthesis of DNA was taking place (Fig. 1), cells free of radioactive DNA were obtained, and only procedures which proved highly efficient in removing radioactivity from these cells were examined further. The course of loss of acid-insoluble radioactivity from one such labelled culture on treatment with RNase is shown in Fig. 2. In other tests the course of events was similar, though the efficiency of the enzyme was somewhat variable and the radioactivity remaining after an hour's treatment ranged between 1.0 and 0.4 per cent. The remaining radioactive material was not identified, though it was apparently not an RNase-resistant RNA fraction, for it proved refractory to extraction with N HCl at 60°C for 15 minutes. However, about 75 per cent of this residual radioactivity could be removed without undue loss of DNA by first exposing the RNase-treated cells to buffered formaldehyde and then extracting them with NaOH. The formaldehyde had no value as an extracting agent, but helped in preserving the nucleus and in reducing the rate of loss of DNA during subsequent alkaline extraction. It was found necessary to use formalde-

hyde after, rather than before, digestion with RNase, since this enzyme acted less efficiently on formaldehyde-treated cells. The end result of applying this procedure to these cells containing no labelled DNA was to reduce their radioactive content to between 0.25 and 0.1 per cent of the initial level.

TABLE I

The Effect of the Ribonuclease Extraction Procedure on the Nucleic Acid Contents of Cells from a Randomly Dividing Culture

	Working suspension		
	Before treatment	After treatment	Loss
	μg/ml	*μg/ml*	*Per cent*
DNA	5.4	3.7	32
RNA	259	<1.4	>99.6
DNA:RNA	1:48	>2.6:1	—

Validity of the Ribonuclease Extraction Procedure

Table I shows that the ribonuclease extraction procedure removed essentially all the RNA from a randomly dividing culture, but only a third of the DNA. Experiments with both synchronized and randomly dividing cultures indicated that if the culture had been previously exposed to radioactive adenine, the extraction procedure removed all but a small fraction of the cells' radioactivity, and this residual fraction was mainly in the form of DNA.

SYNCHRONOUS CULTURES: A typical example of the results obtained on labelling synchronously dividing cultures with radioactive adenine is shown in Fig. 3. Samples withdrawn at intervals from a culture were incubated for 15 minutes in the presence of ^{14}C-adenine, taken through the RNase extraction routine and assayed for residual radioactivity. A further series of samples not so treated was analyzed for nucleic

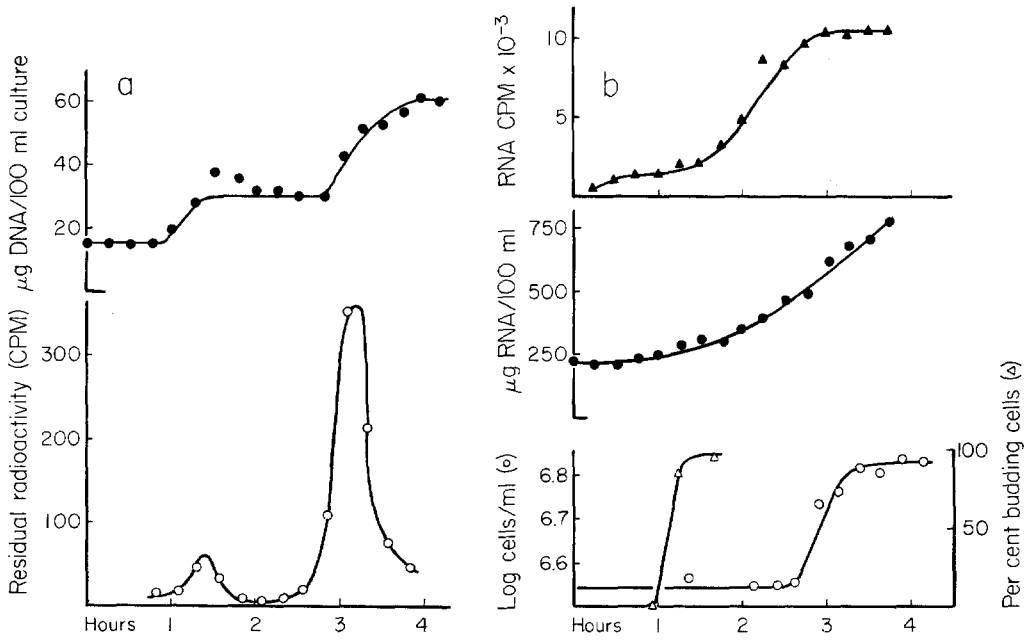

FIGURE 3 Changes in the incorporation of ^{14}C-adenine during the growth of a synchronously dividing culture. Samples were withdrawn at intervals from the culture, incubated for 15 minutes with the precursor and taken through the ribonuclease extraction procedure. *a*, Changes in the amount of residual radioactivity and in the total DNA. *b*, The emergence of the first generation of buds, the course of cell division, and changes in the total RNA content of the culture. The uppermost curve in *b* shows the results obtained on measuring the amounts of radioactivity entering the RNA fraction. They were obtained with a separate, but similar, culture.

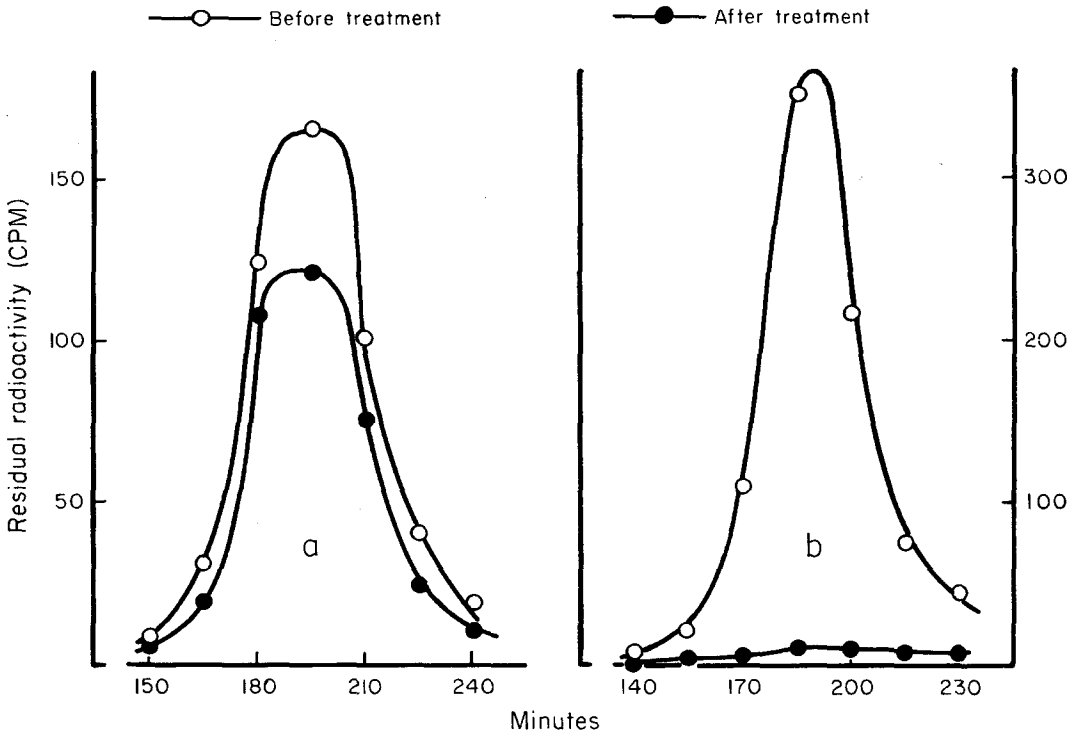

FIGURE 4 The effects of *a*, proteases, and *b*, DNase on the residual radioactive contents of samples from synchronously dividing cultures labelled with ^{14}C-adenine during periods of DNA synthesis and subsequently taken through the ribonuclease extraction procedure. The time scale refers to the age of the cultures.

acids. Fig. 3 also shows typical results obtained on incubating a series of sequential samples with ^{14}C-adenine and measuring the amount of radioactivity entering the RNA fraction.

The most striking feature of these results is that incorporation of radioactivity into the residual material was almost entirely confined to the periods when the culture was synthesizing DNA. This strongly suggested that the residual radioactive material was DNA. Nevertheless, the possibility could not be ignored that it consisted of a specific minor RNA fraction synthesized at the same time as DNA and small enough to escape detection in the gross RNA measurements (Fig. 3 *b*). However, two pieces of evidence indicated that this was not the case.

In the first place, digestion with DNase (Fig. 4) released more than 98 per cent of the residual radioactivity appearing in the cells during DNA synthesis. In contrast, extraction with proteases (Fig. 4) under conditions which removed at least 99 per cent of the protein remaining in the treated cells, reduced the residual radioactivity by only about 20 per cent; even this reduction might have been due to loss of DNA retained in the cells only by the presence of protein. Secondly, samples withdrawn from a synchronously dividing culture and labelled while synthesizing DNA were examined for radioactive nucleosides. The samples were pooled, taken through the RNase extraction procedure, and incubated overnight at 37°C in an aqueous solution of snake venom (2 mg/ml) adjusted to pH 8.5 with ammonia. This released most of the residual radioactivity in a soluble form. After cooling, protein was precipitated by adding HCl, and removed by centrifugation. The pH value of the supernatant fluid was adjusted to neutrality with ammonia and the nucleosides adsorbed on acid-washed charcoal. They were eluted with aqueous ethanol (50 per cent, v/v) containing ammonia (about 3.5 per cent, w/v), and the eluate examined chromatographically. The distribution of radioactivity on the chromatogram is shown in Fig. 5. There were two distinct

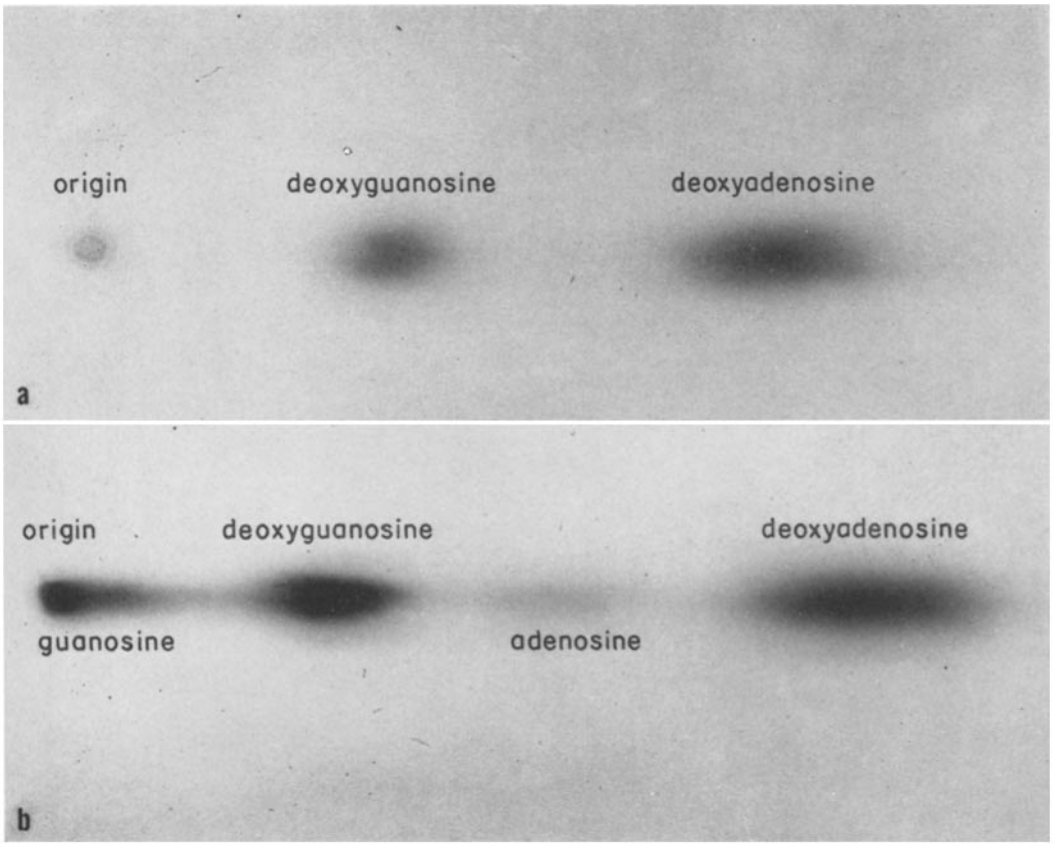

FIGURE 5 The distribution of radioactivity on the chromatograms obtained on examining for nucleosides samples taken from *a*, a synchronously dividing culture labelled with ^{14}C-adenine while synthesizing DNA, and *b*, a randomly dividing culture labelled with ^{14}C-adenine. The identities of the unknown components were established by conventional use of known standards, and in certain cases confirmed by elution and determination of UV-absorption spectra.

radioactive spots with the R_F values of deoxyadenosine and deoxyguanosine, but no radioactivity was detectable in the areas in which the corresponding ribonucleosides would be expected. This directly confirmed the presence of radioactive DNA in the residual material and its freedom from radioactive RNA.

RANDOM CULTURES: When a randomly dividing culture was labelled with radioactive adenine and taken through the RNase extraction procedure, 0.45 per cent of the total acid-insoluble radioactivity was found in the residue. This is comparable with the amount found in the DNA (about 0.4 per cent, see above) and is consistent, therefore, with the view that the residual radioactivity comprised mainly labelled DNA.

The presence of radioactive DNA and an estimate of the relative amount of labelled RNA was established by examining the residual material for radioactive nucleosides. In order to extract all the radioactivity, the material was incubated for 4 hours first with DNase and then with the mixture of trypsin and chymotrypsin. After precipitating protein by acidification, the supernatant fluids from these digestions were pooled and the pH value adjusted to 8.5. Incubation with snake venom and subsequent purification and chromatography followed just as with the samples from the synchronous cultures. The results are shown in Fig. 5. It will be seen that in addition to the two large spots corresponding to radioactive deoxyguanosine and deoxyadenosine, small

TABLE II

The Effect of Enzymes and of Acid on the "Residual Radioactivity" in Labelled Cells from a Randomly Dividing Culture*

Exposure to	"Residual radioactivity"* extracted‡		
	per cent	*per cent*	*per cent*
Desoxyribonuclease§	67	(92)	(92)
Ribonuclease§	6	—	—
5 per cent trichloracetic acid at 90°C for 30 minutes (twice)	98.5	(98)	—

* Defined in text.

‡ Figures in parentheses were from separate experiments.

§ Control treatment with buffer only gave no loss.

amounts of radioactivity were detectable in the areas corresponding to guanosine and adenosine. Thus DNA was the major nucleic acid component of the radioactive residue, but a small proportion of RNA was also present. The total radioactivity in the adenosine spot was 14 per cent of that in the deoxyadenosine, which indicated that there was about seven times as much labelled DNA as RNA.

An estimate of the proportion of the residual radioactivity attributable to labelled DNA was obtained by digestion with DNase (Table II). Although the effectiveness of the enzyme varied on different occasions, at least 67 per cent of the radioactivity was removed by this treatment. The solubility of the radioactive material in hot acid ruled out the presence of appreciable amounts of labelled protein, whilst the material's resistance to digestion with RNase confirmed the above observation of the presence of only small amounts of RNA. It seems reasonable to conclude, therefore, that at least 67 per cent of the radioactivity in the residue left by the RNase extraction procedure was in the form of DNA.

Estimation of Cell Age

The age of a cell (*i.e.*, its position within the cell cycle) was estimated from the ratio of the length of the parental part of the cell to that of the bud ("relative bud length," Fig. 6). The measurements were made on a *camera lucida* projection and the relative bud length was converted to an estimate of age by means of a calibration curve (Fig. 7).

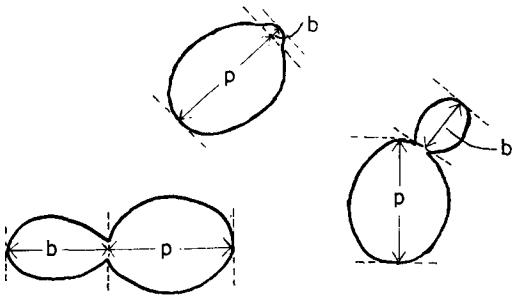

FIGURE 6 Diagrammatic illustration of the measurement of relative bud length, calculated as the ratio *b/p*.

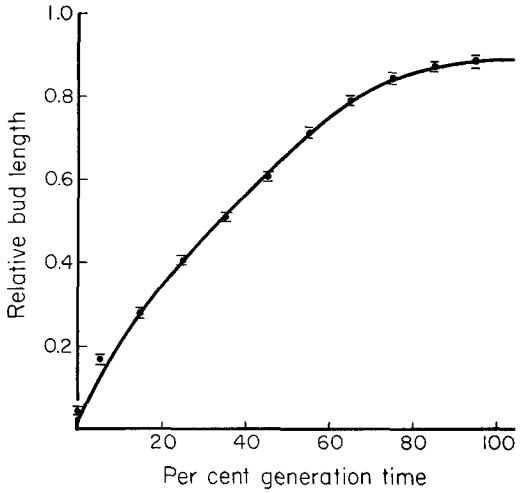

FIGURE 7 Calibration curve relating relative bud length to the age of a cell within the cell cycle. The horizontal lines denote ± Standard Error.

This curve was based on measurements made on thirteen individuals while growing in a specially made slide culture chamber. With the help of this chamber (which is to be described elsewhere), cells growing in a fast-moving stream of fresh aerated medium could be photographed using normal phase-contrast oil-immersion optics. The medium was the semi-defined one used throughout these studies, and the chamber was inoculated with cells taken from a randomly dividing culture during the logarithmic phase of growth. They were incubated in the chamber for at least one generation time before any photographs were taken.

Theoretical reasons for believing that the

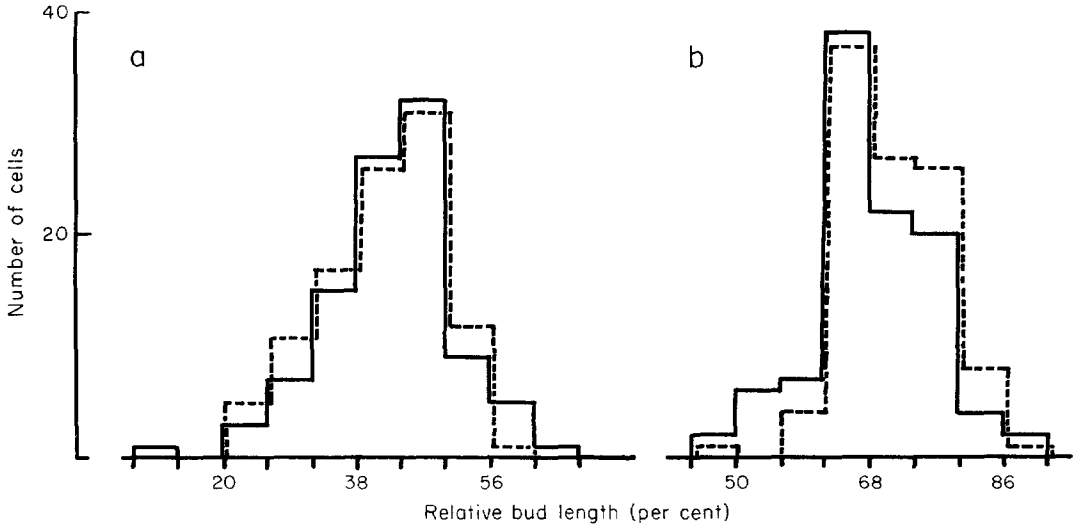

FIGURE 8 Frequency distributions of relative bud length for two separate samples, *a*, and *b*, taken from synchronously dividing cultures at different times in the first generation period. The broken lines refer to "untreated" cells, and the continuous lines to the cells prepared for autoradiography.

relative bud length of a cell in an exponentially dividing population is, in general, a valid measure of its age will be discussed below. In order to check that preparation for autoradiography did not distort the relative bud lengths of individual cells, synchronously dividing cultures were sampled at two stages in the cell cycle, thus providing two groups of cells with widely different mean ages. A portion of each sample was exposed briefly to dilute formaldehyde to arrest growth, and photomicrographs prepared of the wet mounted cells. A further portion of each sample was subjected to the RNase extraction . procedure and dummy (unexposed) autoradiographs prepared and processed in the usual fashion. About 100 cells in both the treated and control portions of each sample were then measured. From Fig. 8 it will be seen that the frequency distributions of relative bud length in the two groups were not at all affected by the treatment involved in preparing the autoradiographs. This treatment, therefore, did not invalidate the proposed use of relative bud length as a measure of cell age.

Cell Autoradiography

The results obtained on making autoradiographs of cells treated in the manner outlined above are illustrated in Figs. 9 to 11. Fig. 9 shows the actual grain counts plotted against cell age of 200 randomly chosen individuals from one such culture, whilst some idea of the reproducibility of the procedure is afforded by Fig. 10 which compares the condensed data of this and one other culture. The data were condensed by averaging the grain counts per cell in each of a number of small arbitrarily chosen age classes. Cells without buds, constituting 8 to 10 per cent of the populations, were counted as one such class, since they deserve special consideration. About 4 per cent of the population fell outside the oldest class, but they have been included within it in determining the averages. These averages, but not the original grain counts, have been corrected by subtracting the average background count (less than one grain per cell) determined by counting about 100 randomly chosen areas of background of about the size of an average cell. Experience showed that the manipulations involved in preparing the cells for autoradiography eliminated all tendencies of daughter cells to remain adherent to their parents after division, so that only single cells were present in the finished smears.

The results show that incorporation of radioactivity into the residual material was limited almost entirely to cells exposed to the precursor during the early part of their reproductive cycle, the maximum incorporation during this period

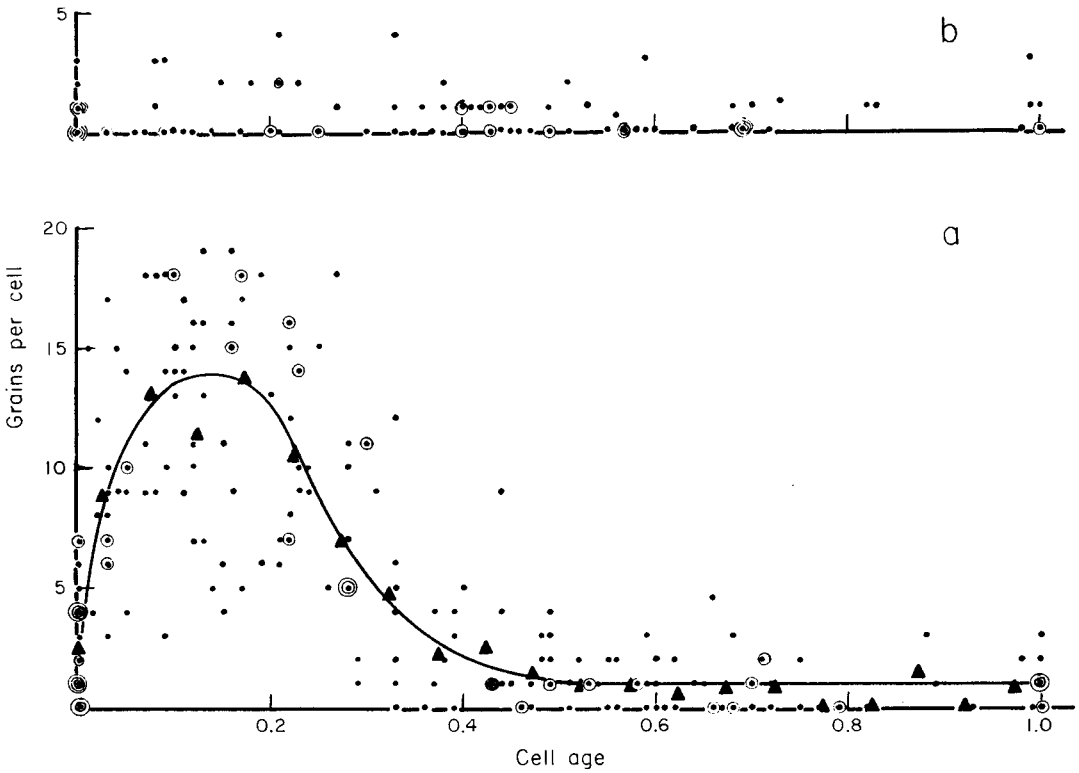

FIGURE 9 *a*, Relationship between grain count and cell age in an autoradiograph of cells from a randomly dividing culture labelled for 15 minutes with ^{3}H-adenine and taken through the ribonuclease extraction procedure. The triangles denote the averages. 200 cells are recorded. *b*, A sample of the same culture after incubation with DNase. 100 cells are recorded.

being nearly twenty times the average value for the older cells.

Fig. 9 *b* illustrates the results obtained on making autoradiographs of extracted cells after incubating them with DNase for 4 hours. Whilst control slides of cells exposed to buffer only remained unaffected, the DNase-treated cells had lost practically all their residual radioactivity. Corrected for background, the resistant fraction amounted to only 8 per cent of the total, and appeared to be equally distributed amongst cells of different ages. Most of the loss of radioactivity was, of course, incurred by the heavily labelled younger cells, but it is worth pointing out that the older cells apparently lost about 65 per cent of their low radioactive content.

A similar test was also carried out using a mixture of trypsin and chymotrypsin instead of DNase. The morphology of the cells was grossly distorted by incubation in this mixture and their relative bud lengths could not be accurately determined. However, this did not prevent grain counting, and it was found that the treatment with proteases had not materially altered the frequency distribution of radioactivity.

DISCUSSION

Just over 90 per cent of the grains in the autoradiograph recorded in Fig. 9 *a* were located over cells younger than 0.4 generations. Since at least two-thirds of the total residual radioactivity in the culture was shown to be in the form of DNA, it follows that the majority of these grains over the young cells must have been attributable to DNA. This was confirmed by comparing the autoradiographs made before and after digestion with DNase, for this treatment removed 94 per cent of the radioactive content of the young cells in this particular culture. The status of the grains over the older cells is less certain; the fact that exposure

to DNase removed about two-thirds of their radioactivity suggests the possible occurrence of incorporation into a minor DNA fraction distinct from the bulk of the cell's chromosomal DNA. However, the level of radioactivity in these cells was barely above the background, and further study is needed before a definite conclusion can be reached. In any event, the incorporation into DNase-sensitive material in the older cells accounted for only about 3 per cent of the total incorporation into the whole culture, so it is fair to conclude that synthesis of DNA was restricted almost entirely to the younger cells.

In passing, it should be pointed out that the resolution obtained with this type of autoradiograph is insufficient to permit detection of the site of the labelled material in these cells. Therefore, the dispersion of the grains over the labelled cells should not be taken as evidence that the radioactive DNA was extranuclear. In fact, an intranuclear location was indicated by experiments (unpublished) with a cylindrical yeast having dimensions of about $3 \times 10\ \mu$, for in these cells the concentration of grains in the nuclear area was readily discernible.

The accuracy with which the period of incorporation into DNA can be located within the cell cycle is largely governed by the accuracy of the estimates of cell age. The choice of relative bud length as the criterion of age was based on the fact that when a yeast cell grows, the parental part of the cell remains sensibly constant in size, all the expansion in volume being accounted for by the growth of the bud (1). Consequently it should be possible, in principle, to assess the age of a cell by comparing the size of the bud with that of the parental part of the cell. Length was the most easily measured parameter of size, and experience in this laboratory, coupled with the observations by Bayne-Jones and Adolph (1), indicated that the pattern of growth of the cell in terms of bud length was reproducible and did not vary substantially with differences in environmental conditions. However, the use of relative bud length as a measure of age has the limitation that it is only applicable to cells bearing buds. Consequently, the cell cycle defined by its use starts with the appearance of the bud and ends when the fully grown bud separates from the parental part of the cell. It does not include any interval that may occur between the separation of parent and daughter and the emergence of the next generation of buds. However, a lag period of this type only develops in poor growth conditions

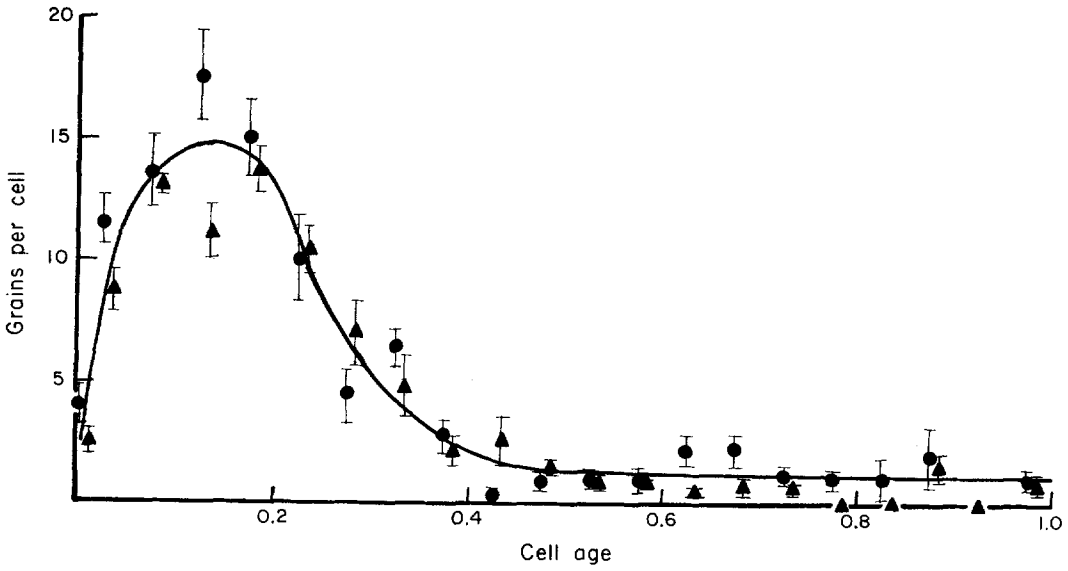

FIGURE 10 The relationship between grain count and cell age in autoradiographs of cells from two cultures labelled with ^{3}H-adenine and treated as described in the text. The symbols denote the average values. For clarity, one set has been displaced slightly to the right. The vertical lines denote ± Standard Error.

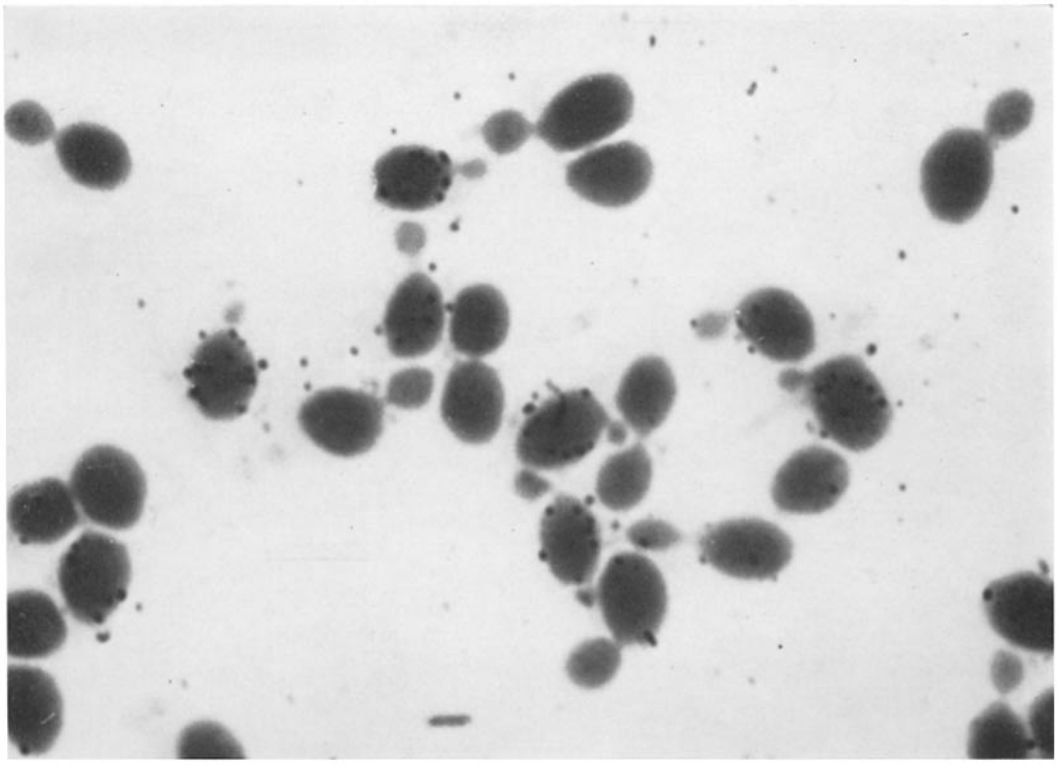

FIGURE 11 An autoradiograph of cells from a randomly dividing culture labelled with ^{3}H-adenine and treated as described in the text. Only the cells with small buds are labelled.

(13). It was, therefore, not displayed in the slide cultures used in constructing the calibration curve relating age to bud length, for these were continuously perfused with fresh medium. On the other hand, a slight lag between division and bud formation was detectable in the bulk randomly dividing cultures, for 8 to 10 per cent of the organisms from these cultures were free of buds. Taking into account the approximate distribution of cell ages in the cultures (6), it was calculated that the average length of this lag period for any given cell was about 6 per cent of the generation time. Moreover this is probably an over-estimate, since a proportion of the cells recorded as lacking buds may, in fact, have possessed small ones which were hidden from view. In any event, little error is involved in neglecting this period.

Turning to the final interpretation of the autoradiographs, it is as well to point out that, strictly speaking, the data indicate a period of incorporation of the precursor into DNA; they can be interpreted in terms of net synthesis only on the assumption that negligible breakdown of DNA takes place in the growing cell. A somewhat more serious consideration is that any changes in the amount of radioactivity incorporated at different stages of the cell cycle may only reflect changes in the kinetics of uptake of precursor from the medium, or in the size and composition of internal precursor pools. There is, however, no reason to suppose that any such effects in the randomly dividing cultures would be more pronounced than in the synchronously dividing ones, and in these they did not obscure the close temporal relationship between the periods of net synthesis of DNA and the appearance of radioactivity in the residual material. Nevertheless, minor variations in the apparent rate of synthesis or in its timing might result from this cause.

Bearing in mind these limitations, the individual grain counts may be regarded as providing a comparison between the relative amounts of DNA synthesized by cells of different ages during the period of exposure to the precursor. They are

only a poor guide to the rates of synthesis in these individuals for, on each side of the peak in particular, the rates may change markedly within the labelling period. However, interpretation of the curve relating grain counts to cell age is straightforward. It can easily be shown that the peak in this curve should span an interval equal to the sum of the labelling and synthetic periods. This is so regardless of the relative lengths of these periods. It is also immaterial that the experimental curve is asymmetrical, having a pronounced "tail" on the older side of the peak. Such asymmetry may result from a change in rate during the later stages of the synthetic process or from a change in the kinetics of incorporation of the labelled precursor, as mentioned above. It would also result if synthesis started at the same time in every cell (*e.g.*, as the bud appeared), but finished at different times in different cells. The present results do not indicate which of these conditions is operative, but in any event, none of them should alter the above-mentioned relationship between the span of the curve and the lengths of the labelling and synthetic periods. The labelling period was about 13 per cent of the generation time, and, taking the span of the peak in Fig. 10 as 40 per cent of the generation time, it is, therefore, deduced that the first 27 per cent of the cell cycle was occupied by synthesis of DNA.

This pattern of synthesis is in general agreement with that observed in synchronously dividing cultures, in which synthesis also starts as soon as the bud appears, and lasts for only a short part of the reproductive cycle of the cell. Unfortunately, for reasons that need not be discussed here, it is difficult to estimate the length of the synthetic period accurately from the results obtained with synchronized cultures. Nevertheless, it seems clear that in this respect the behaviour of the synchronized cell does not differ greatly, if at all, from that of the unsynchronized individual. A more important conclusion is that, with regard to its pattern of DNA synthesis, the behaviour of *Saccharomyces cerevisiae* is comparable with that of the cells of higher organisms.

It is a pleasure to acknowledge the helpful advice and criticism of many colleagues, particularly Drs. J. W. Watts and J. McLeish.

Received for publication, July 27, 1964.

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