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ALTERED PATTERNS OF RIBONUCLEIC ACID SYNTHESIS DURING THE CELL CYCLE: A MECHANISM COMPENSATING FOR VARIATION IN GENE CONCENTRATION

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SUMMARY

In the fission yeast *Schizosaccharomyces pombe*, a series of diploid mutants divides at smaller cell sizes than wild type. In these smaller strains, the mean gene concentration (defined by previous authors as the DNA to protein ratio) is higher than in wild type. Such an increase in gene concentration should also increase the concentration of those components such as messenger and ribosomal RNA, whose rate of synthesis is determined by gene dosage. We show that the mean concentrations of these 2 RNA species in the small cells are not increased, but are the same as in wild type. The small mutant cells are thus able to compensate for changes in gene concentration. This compensation is shown to operate through differences in the patterns of synthesis of RNA during the cell cycle. In all the strains of the diploid series, the rates of synthesis of messenger and ribosomal RNA double as steps once in each cell cycle. The timings of the steps in the cell cycle appear to be cell-size related, since the smaller the cell at division, the later are the steps in the cell cycle. In contrast, there is comparatively little variation in the timing of DNA replication in the cycles of cells of different sizes. We propose that after DNA replication, there is a delay before doubling in the rate of transcription. Such a cell mass-related delay is all that is required to compensate for increased gene concentration, and results in the same mean functional DNA concentration in all strains. This mechanism will maintain the same mean messenger and ribosomal RNA concentrations in cells dividing at different sizes. Ways in which the cell size-related control over transcription may operate are discussed.

INTRODUCTION

Gene concentration can vary between cells of the same species. There are 3 ways in which this variation can arise. The mean number of copies of a gene per cell can vary, an example being the genes carried on the X chromosome. In a cell of the homogametic sex (usually the male) with one X chromosome, there are half as many copies of these genes as in a cell of the heterogametic sex (usually the female) with 2 X chromosomes. Secondly, gene concentration will be altered if the mean number of gene copies per cell remains the same, but if mean cell size varies. Thus a cell smaller than normal will have a higher mean gene concentration. Thirdly, any alteration in the timing of DNA replication during the cell cycle will alter the average gene concentration of cells in steady-state growth.

Variation in gene concentration poses questions about the regulation of synthesis of

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gene products. For genes regulated autogenously or by feedback effectors, variation in gene product concentration will be less than corresponding variation in gene concentration, and may become negligible if the power of control in the regulatory circuit is sufficiently high (Chandler & Pritchard, 1975). A contrasting case is where the rate of synthesis of a gene product is determined by gene dosage, that is by the number of copies of the gene in the cell. In both prokaryotes and eukaryotes, there is evidence that gene dosage can be a major component of the controls limiting rates of synthesis of certain proteins (Hilger *et al.* 1973; Reichart & Winter, 1975; Kuempel, Masters & Pardee, 1965; Donachie, 1965; Helmstetter, 1968) and of messenger and ribosomal RNAs (Pfeiffer & Tolmach, 1968; Klevecz & Stubblefield, 1967; Fraser & Carter, 1976; Fraser & Moreno, 1976). In the absence of other levels of control, the mean concentration of those gene products regulated at the gene dosage level will reflect variation in gene concentration. It may be difficult for the cell to accommodate such changes in concentration of gene products and to achieve balanced, steady-state growth, particularly if the activity of a significant number of genes is regulated solely at the gene dosage level.

In this paper we explore the consequences of varying gene concentration in the simple eukaryote *Schizosaccharomyces pombe*. In this organism the rates of both ribosomal and polyadenylated messenger RNA synthesis double as steps just after DNA replication in each cell cycle (Wain & Staatz, 1973; Fraser & Moreno, 1976). The experimental evidence suggests that the rates of RNA synthesis may be determined by gene dosage (Fraser & Moreno, 1976; Fraser & Nurse, 1978). Gene concentration can be conveniently altered in *S. pombe* by making use of a mutation in the gene *wee* 1 which determines the cell size at which nuclear division is initiated. Haploid cells of a mutant strain *wee* 1–50 divide at approximately half the size of haploid wild type cells (Nurse, 1975). We have used diploid lines derived from these haploid parents. The diploid *wee* 1–50 strain divides at about half the size of the diploid wild type, while the heterozygous *wee* 1–50/wild type diploid is intermediate in cell size at division (P. Nurse & P. Thuriaux, unpublished results).

We have studied the control of RNA synthesis in these 3 strains, to find how they accommodate their different gene concentrations. Our results suggest that in cells dividing at a smaller mean size than wild type, the step doublings in rates of RNA synthesis occur much later in the cell cycle than DNA replication. The point at which the step doubling occurs seems to be cell mass related, since the smaller the mean cell size, the greater the delay. We suggest that this phenomenon may compensate for the observed variation in gene concentration.

A brief description of some of our conclusions from experiments with haploid and diploid strains has been published (Fraser & Nurse, 1978). The purpose of the present paper is to present full experimental results for the diploid strains, and discuss further aspects of the control mechanism. A theoretical analysis of the consequences of a size control over transcription for the control of balanced exponential growth is given in the following paper (Barnes, Nurse & Fraser, 1978).

MATERIALS AND METHODS

Strains and genetical procedures

We used the standard genetical procedures for *Schizosaccharomyces pombe* Lindner as described by Gutz, Heslot, Leupold & Loprieno (1974). Derivation of strain *wee* 1–50 from wild type strain 972 h^- was as described by Nurse (1975). Diploid strains were constructed as described by Nurse, Thuriaux & Nasmyth (1976) using the mating-type allele *mei* 1–102 as described by Egel (1973).

Culture conditions

Cultures were grown with stirring at 35 °C in a minimal medium EMM2 (Mitchison, 1970) as modified by Nurse (1975). Synchronous cultures of 1000 to 1300 ml with a starting density of $1–2 \times 10^6$ cells/ml were prepared by selection of small cells from 15 l. of exponentially growing asynchronous culture with a density no greater than 5×10^6 cells/ml. Selection of small cells was by sedimentation through a 7·5 to 30 % gradient of lactose in culture medium using a M.S.E. Type A zonal rotor (Mitchison & Carter, 1975). Changes in cell number were monitored using a Coulter Counter as described by Mitchison (1970).

Radioactive labelling

Samples of 50 to 80 ml synchronous culture, containing $1–2 \times 10^8$ cells, were withdrawn at 20-min intervals. These samples were pulse-labelled for 10 min with 2·5 μCi/ml [2-^{3}H]adenine (Radiochemical Centre, Amersham, U.K.). The total adenine concentration was adjusted to 1 or 3 μg/ml with non-radioactive adenine. Under these conditions, less than 10 % of the isotope supplied was taken up during the labelling period. At the end of the pulse, crushed ice was added to stop further incorporation. Cells were harvested by filtration on an Oxoid membrane filter with 0·45-μm pore diameter. Adenine uptake by the cells was measured and total nucleic acid was extracted by a detergent-phenol procedure (Fraser & Moreno, 1976). The rate of synthesis of ribosomal RNA (rRNA) was determined from the radioactivity of the 25 S plus 18 S rRNA peaks after fractionation of samples of total nucleic acid on polyacrylamide gels (Loening, 1967) as explained by Fraser & Carter (1976). The rate of synthesis of polyadenylated messenger RNA (poly(A)$^+$mRNA) was measured by the radioactivity of that fraction of total RNA binding to oligo(deoxythymidylic acid)-cellulose at high salt concentration. Details of the assay and characteristics of the poly(A)$^+$mRNA fraction isolated are given elsewhere (Fraser, 1975). Changes in DNA content during synchronous culture were determined as described by Fraser & Moreno (1976).

To measure the rate of total RNA synthesis in asynchronous, exponentially-growing cultures, 1-ml samples containing $1–2 \times 10^6$ cells were incubated with 2·5 μCi/ml [2-^{3}H]adenine at a total adenine concentration of 1 μg/ml for 5 min. Incorporation of adenine into cold-acid-insoluble material was measured as explained by Fraser & Moreno (1976).

Mean DNA, protein and total RNA contents of cells in asynchronous, exponential culture were measured as described by Nurse (1975) and Nurse & Thuriaux (1977). Mean poly(A)$^+$mRNA content was calculated from the ultraviolet absorption spectrum of the poly(A)$^+$mRNA fraction isolated as above.

Asynchronous control cultures

Cells harvested from an asynchronous exponential culture were exposed to conditions similar to those used for preparation of synchronous cultures. The cells were resuspended in medium: the suspension was slowly diluted, and the lactose concentration increased, to mimic the zonal centrifugation treatment used to prepare synchronous cultures. The final lactose concentration to which control cells were exposed equalled that in the region of the zonal rotor from which the inoculum for a synchronous culture was taken. The total time of exposure to high cell density and lactose was similar to the time required for a zonal rotor fractionation of cells. A sample of the lactose-treated cells, containing cells of all sizes and hence of all stages in the cell cycle, was used as inoculum for the asynchronous control culture.

Table 1. *Generation times, volume at division and mean protein and DNA contents of 3 diploid strains of Schizosaccharomyces pombe*

Strain	Mean generation time, min	Mean volume at division, μm^3	Protein, pg/cell	DNA, fg/cell
Wild type/wild type	140	283	22·6	62·8
wee 1–50/wild type	160	231	18·5	60·2
wee 1–50/*wee* 1–50	205	144	13·0	50·1

Mean generation time, DNA and protein contents were measured in asynchronous cultures in the early phase of exponential growth. Mean cell volume at division was measured on samples of 200 cells with cell plates as described by Nurse (1975).

Table 2. *Concentrations (expressed as μg per μg total protein) of DNA, total RNA and poly(A)⁺mRNA in 3 diploid strains of S. pombe*

Strain	DNA/ protein	Total RNA/ protein	Poly(A)⁺mRNA/ protein
Wild type/wild type	$2{\cdot}78 \times 10^{-3}$	0·219	$0{\cdot}72 \times 10^{-3}$
wee 1–50/wild type	$3{\cdot}25 \times 10^{-3}$	0·223	$0{\cdot}72 \times 10^{-3}$
wee 1–50/*wee* 1–50	$3{\cdot}85 \times 10^{-3}$	0·225	$0{\cdot}72 \times 10^{-3}$

RESULTS

Characteristics of the yeast strains

Table 1 shows that at division, *wee* 1–50 diploid cells are approximately half the volume of wild type diploid cells, while the heterozygous diploid is intermediate between its 2 homozygous parents. As *S. pombe* cells are slightly irregular in shape, cell volume is difficult to measure accurately, especially if changes in volume through the cell cycle are to be considered. We will therefore use total protein per cell as a more reliable and easily measurable index of cell size. Table 1 shows that by this criterion also, *wee* 1–50 diploid cells are about half the size of wild type diploid cells, with the heterozygous diploid again intermediate between its 2 parents. In exponentially growing, asynchronous cultures, the mean generation time of the heterozygous diploid is close to that of wild type diploid cells. The mean generation time of *wee* 1–50 diploid cells is appreciably longer than that of wild type diploids. This difference will be discussed in more detail later.

Using total protein content as a measure of cell size, we have calculated mean cell concentrations (i.e. per unit total protein) of DNA, poly(A)⁺mRNA and total RNA (Table 2). It is clear that there is considerable variation in gene concentration (DNA to protein ratio) between the 3 strains. In contrast, poly(A)⁺mRNA and total RNA concentrations are identical in all 3 strains. Thus cells are able to compensate for variation in gene concentration. To investigate further the mechanisms maintaining poly-(A)⁺mRNA and total RNA concentrations at the same levels in all strains, we followed the patterns of RNA synthesis during the cell cycle for each strain.

Patterns of macromolecular synthesis through the cell cycle

Fig. 1 shows results obtained from a synchronously dividing culture of wild type diploid cells. Cell number per ml increased in a series of steps. The mid-points of 2 successive step-doublings in number are taken to mark 0·0 and 1·0 of the cell cycle. The time during the cell cycle at which a particular event occurs is expressed as a fraction of the cell cycle using this scale.

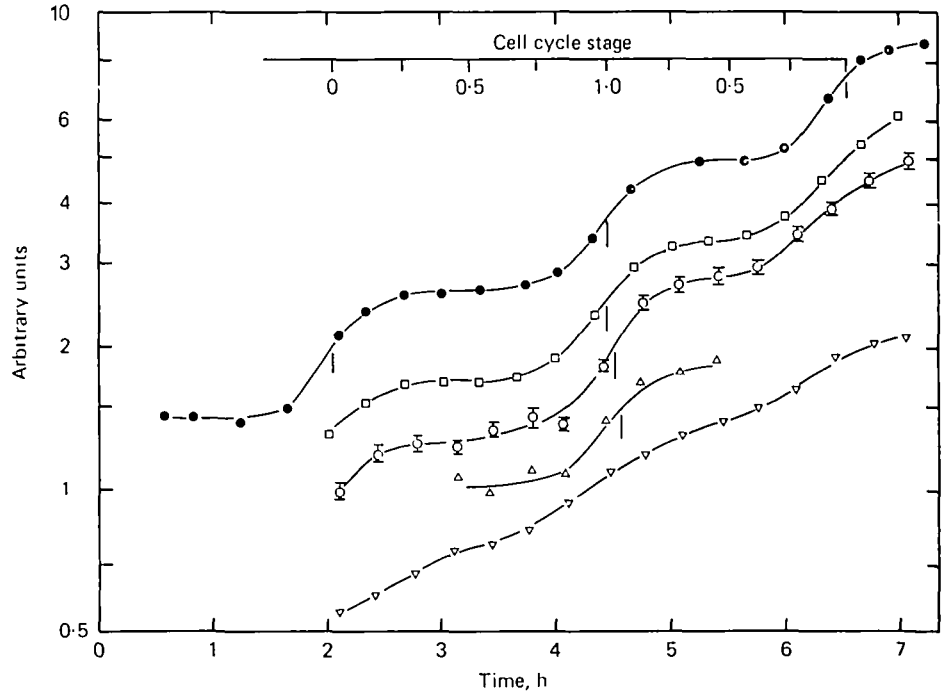

Fig. 1. Changes during growth of a synchronous culture of wild type/wild type diploid cells of *Schizosaccharomyces pombe*. The vertical scale is a log scale marked in arbitrary units; the absolute value of 1 scale unit is indicated in brackets for each parameter. ●, cells/ml (1 scale unit equals 10^6 cells/ml); □, DNA content per ml culture (relative units); ○, rate of poly(A)$^+$mRNA synthesis per ml culture (1 scale unit equals 2500 cpm/ml culture); △, rate of ribosomal RNA synthesis per ml culture (relative units); ▽, rate of adenine uptake per ml culture (1 scale unit equals 2×10^5 cpm/ml). Values for poly(A)$^+$mRNA synthesis are means ± standard errors of 6 determinations. The vertical bars on each curve show the mid-point of a stepwise increase in that parameter, measured as half-way between the plateaux levels before and after the step.

In wild type diploid cells, DNA replication occurred very early in the cycle, at about the same time as cell division (Fig. 1). A similar early timing of DNA replication has been reported for haploid wild type cells (Mitchison & Creanor, 1971). The rate of poly(A)$^+$mRNA synthesis rose discontinuously during growth of the culture, with a stepwise doubling in rate early in each cell cycle. The mid-point of the doubling was 0·0 to 0·1 of a cycle later than the mid-point of DNA accumulation. Although fewer

observations were made, the rate of synthesis of rRNA also appeared to double in a stepwise manner during the cell cycle, at about the same time as the doubling in rate of poly(A)$^+$mRNA synthesis. Similar early doublings in the rates of rRNA and poly-(A)$^+$mRNA synthesis have been reported in the cell cycle of haploid cells of wild type size (Fraser & Moreno, 1976; Fraser & Nurse, 1978).

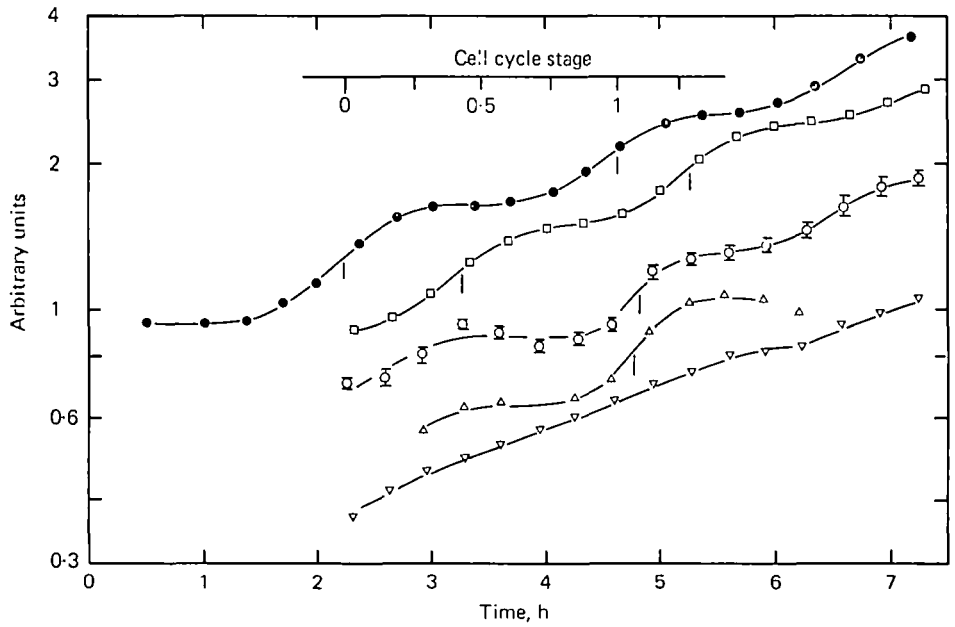

Fig. 2. Changes during growth of a synchronous culture of *wee* 1–50/*wee* 1–50 diploid cells of *S. pombe*. The vertical scale is a log scale marked in arbitrary units: the absolute value of 1 scale unit is indicated in brackets for each parameter. ●, cells/ml (1 scale unit equals 10^6 cells/ml); □, DNA content per ml culture (relative units); ○, rate of poly(A)$^+$mRNA synthesis per ml culture (1 scale unit equals 1250 cpm/ml); △, rate of ribosomal RNA synthesis per ml culture (relative units); ▽, rate of adenine uptake per ml culture (1 scale unit equals 2×10^5 cpm/ml). Values for poly(A)$^+$mRNA synthesis are means ± standard errors of 6 determinations. The vertical bars on each curve show the mid-point in a stepwise doubling in that parameter.

Fig. 2 shows that in a synchronous culture of the homozygous *wee* 1–50 diploid, the mid-point of DNA replication was at 0·3 to 0·4 of the cycle. This is later than in wild type diploid cells (Fig. 1) and a little later than in haploid *wee* 1–50 cells (Nurse, 1975). The late occurrence of DNA replication in the *wee* 1–50 diploid is consistent with its mean DNA content per cell in asynchronous, exponential culture being lower than that of wild type diploid cells (Table 1). The rates of poly(A)$^+$mRNA and rRNA synthesis in synchronous cultures of *wee* 1–50 diploid cells increased periodically, with stepwise increases in rate once per cell cycle (Fig. 2). However, in contrast to wild type, the mid-points of the steps were more than half a cycle later than the mid-point of DNA replication, and were close to the end of the cycle. The stepwise increases in rates of RNA synthesis in homozygous *wee* 1–50 diploid cells were not doublings, but

were between 1·5 and 1·7 times. Similarly, at each division the increase in cell numbers was within the range 1·5 to 1·8 times. This lack of doublings will be discussed later.

In synchronous cultures of the heterozygous *wee* 1–50/wild type diploid (Fig. 3) the mid-point of DNA replication was at the start of the cycle, as in wild type diploid cells (Fig. 1). Poly(A)$^+$mRNA and rRNA synthesis each showed a single stepwise doubling in rate during each cell cycle. The mid-points of the rate doublings occurred at about mid-cycle, considerably later than the corresponding rate doublings in wild type cells, and about half a cycle later than the mid-point of DNA replication in the heterozygous diploid.

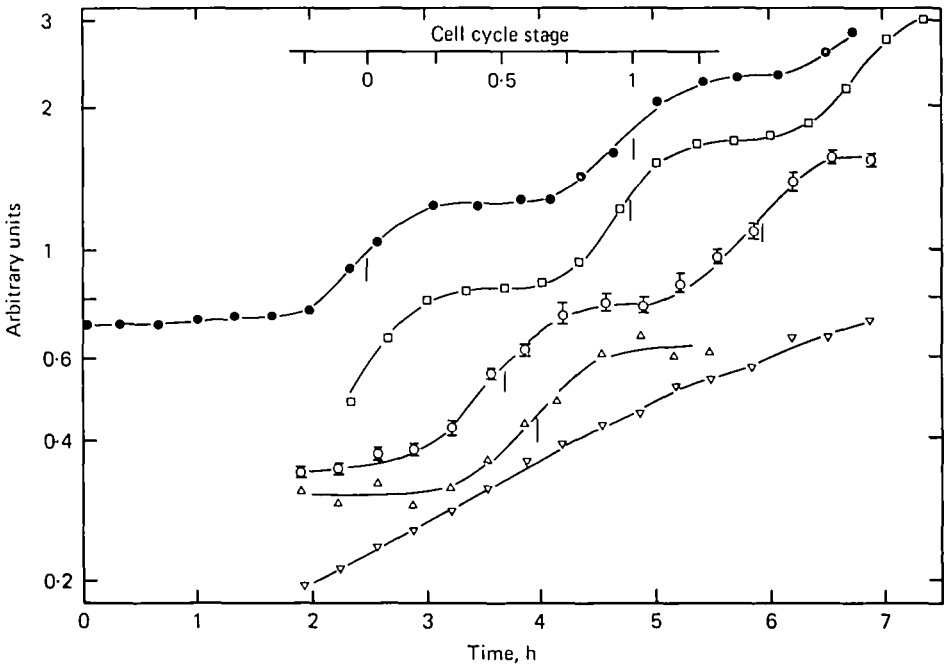

Fig. 3. Changes during growth of a synchronous culture of *wee* 1–50/wild type heterozygous diploid cells of *S. pombe*. The vertical scale is a log scale marked in arbitrary units; the absolute value of 1 scale unit is indicated in brackets for each parameter. ●, cells/ml culture (1 scale unit equals 10^6 cells/ml); □, DNA content per ml culture (relative units); ○, rate of poly(A)$^+$mRNA synthesis per ml culture (1 scale unit equals 2500 cpm/ml); △, rate of ribosomal RNA synthesis per ml culture (relative units); ▽, rate of adenine uptake per ml culture (1 scale unit equals 3×10^5 cpm/ml). Values for rate of poly(A)$^+$mRNA synthesis are means ± standard errors of 6 determinations. The vertical bars on each curve show the mid-point of a stepwise doubling in that parameter.

We have interpreted changes in the rate of incorporation of adenine into RNA as reflecting changes in the rate of RNA synthesis, rather than merely changes in the rate of adenine uptake by the cells. Figs. 1–3 show changes in the rate of adenine uptake during synchronous cultures of the 3 strains. In each case, the rate of adenine uptake increased continuously, or close to continuously. Therefore changes in the rate of adenine uptake are unlikely to have accounted for the sharp changes in rate of incorporation of adenine into RNA.

Before considering the stepwise changes in rates of rRNA and poly(A)$^+$mRNA synthesis as cell cycle-related events, it is necessary to establish that they are not merely artifacts produced by the conditions required to prepare a synchronously dividing culture. In control experiments, changes in the rate of poly(A)$^+$mRNA and rRNA synthesis were examined in cultures inoculated with cells which had been exposed to the conditions used for preparation of a synchronous culture, but in which the inoculum consisted of cells from all stages of the cell cycle. Fig. 4 shows an

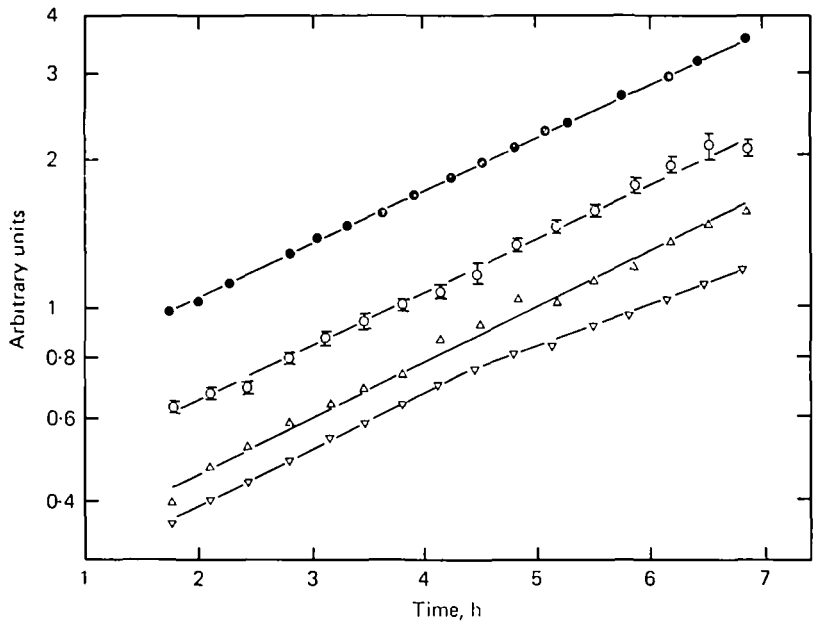

Fig. 4. Changes during growth of an asynchronous control culture of *S. pombe*. The vertical scale is a log scale marked in arbitrary units. The absolute value of 1 scale unit is indicated in brackets for each parameter. ●, cells/ml (1 scale unit equals 10^6 cells/ml); ○, rate of poly(A)$^+$mRNA synthesis per ml culture (1 scale unit equals 3000 cpm/ml); △, rate of ribosomal RNA synthesis per ml culture (relative units); ▽, rate of adenine uptake per ml culture (1 scale unit equals 3×10^5 cpm/ml). Values for rate of poly(A)$^+$mRNA synthesis are means ± standard errors of 6 determinations.

example of a control culture. Cell number, and rates of adenine uptake, poly(A)$^+$mRNA synthesis and rRNA synthesis all rose continuously, with no suggestion of discontinuous changes in rate. We conclude that the stepwise changes in rates of RNA synthesis found in synchronously dividing cultures are cell cycle-related events.

Relationship between DNA replication and changes in rate of RNA synthesis

Earlier experiments with haploid cells of wild type size (Fraser & Moreno, 1976) suggested that the stepwise doubling in rate of poly(A)$^+$mRNA synthesis depended on the occurrence of the immediately preceding round of DNA replication. In the case of the heterozygous *wee* 1–50/wild type diploid and homozygous *wee* 1–50 diploids, there was a long delay between DNA replication and the doubling in rate of RNA synthesis

in synchronous culture. To test whether these late changes in rate of RNA synthesis depended on the preceding round of DNA replication, we followed the rate of total RNA synthesis in asynchronous cultures after inhibition of DNA synthesis by hydroxyurea. At a concentration of 11 mM, this inhibitor depresses DNA synthesis to less than one quarter of the control rate within 5 min of addition (Mitchison & Creanor, 1971; Fraser & Nurse, 1978). Fig. 5 shows that in wild type diploid cells, the rate of RNA synthesis became constant within 10 to 15 min of addition of the inhibitor. In homozygous *wee* 1–50 diploid cells, the delay between addition of inhibitor and onset of a constant rate of RNA synthesis was 80–100 min, or about 0·6 of a cell cycle.

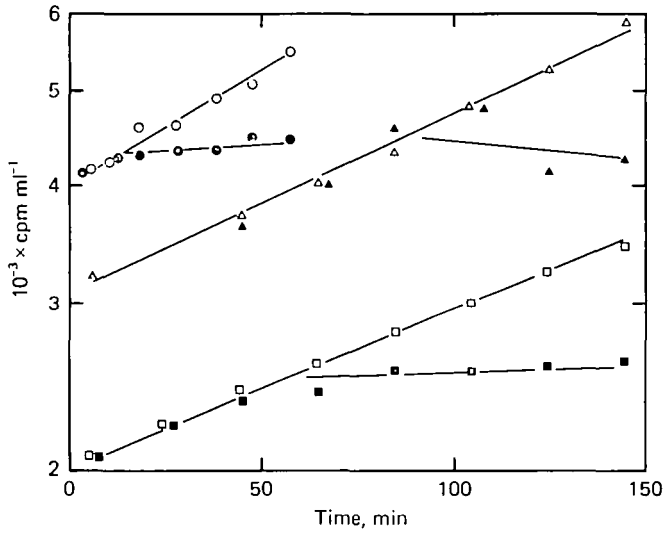

Fig. 5. Changes in rates of total RNA synthesis in asynchronous exponential phase cultures of 3 diploid strains of *S. pombe*, after inhibition of DNA synthesis. Initial density of cultures was between 0·2 and 1 × 10^6 cells/ml. ○, ●, wild type/wild type diploid cells; □, ■, *wee* 1–50/wild type diploid cells; △, ▲, *wee* 1–50/*wee* 1–50 diploid cells. Cultures were split at time 0; hydroxyurea was added to one half of each culture to a final concentration of 11 mM. Closed symbols show rates of RNA synthesis in hydroxyurea-treated subcultures. Open symbols show rates of RNA synthesis in control, untreated cultures.

In heterozygous *wee* 1–50/wild type cells, there was a delay of 50 to 70 min, or about 0·45 of a cell cycle, between addition of hydroxyurea and the onset of a constant rate of RNA synthesis.

These results are consistent with the following model: the stepwise doublings in rates of rRNA and poly(A)$^+$mRNA synthesis in each cell cycle are dependent on the previous round of DNA replication. However, in the case of the homozygous *wee* 1–50 and heterozygous *wee* 1–50/wild type diploids, there is a fraction of the cell cycle during which the cell has completed replication of its DNA, but has yet to double its rate of transcription. In asynchronous cultures (Fig. 5), there will be a proportion of cells in this state: these account for the period after addition of hydroxyurea, during which the rate of RNA synthesis rose as in the controls. It is consistent with this

argument that for each of the 3 strains, the length of the delay between addition of hydroxyurea and the onset of a constant rate of RNA synthesis was similar to the period between the mid-points of DNA replication and the following step-doublings in rates of RNA synthesis in synchronous cultures.

Hydroxyurea is the only inhibitor of yeast DNA synthesis known to produce the very rapid inhibition of DNA synthesis required for these experiments. It does, however, suffer from the drawback that it has been shown to inhibit cell growth and precursor uptake at concentrations only a little higher than required for inhibition of DNA synthesis (Mitchison & Creanor, 1971). In our experiments, the rate of adenine uptake in hydroxyurea-treated cultures did not deviate from that in control cultures until well after the onset of a constant rate of RNA synthesis. There was also no effect on general growth of the culture, as measured by optical density, until long after the effects on RNA synthesis had been noted. However, because of possible side effects, we cannot entirely exclude the possibility that the constant rates of RNA synthesis came about indirectly, through inhibition of some other aspect of metabolism by hydroxyurea.

DISCUSSION

The 3 diploid strains had different mean sizes and consequently different mean DNA concentrations (Tables 1, 2). DNA replication in the *wee* 1–50 diploid occurred later in the cell cycle than in the wild type or heterozygous diploid strains (Figs. 1–3). The timings of DNA replication in the 3 strains are consistent with the hypothesis that DNA replication cannot take place in cells which have not completed mitosis and which are beneath a minimum size (Nurse & Thuriaux, 1977). Further evidence for this hypothesis will be discussed elsewhere (K. Nasmyth, P. Nurse & R. S. S. Fraser, in preparation). The delayed DNA replication in *wee* 1–50 diploid cells served partly but not completely to compensate for the increased DNA concentration caused by the small mean size of cells of this strain.

Despite the 1·4-fold variation in gene concentration in the 3 diploid strains, the concentrations of total RNA and poly(A)$^+$mRNA were the same in all 3 lines. In all 3 strains, the rates of rRNA and poly(A)$^+$mRNA synthesis increased as a step once in each cell cycle, but the timing of the steps differed in the different strains. The smaller the cell at division, the later in the cell cycle were the steps in rates of rRNA and poly(A)$^+$mRNA synthesis.

The following paper (Barnes *et al.* 1978) shows that the observed differences in time of these rate doublings in the 3 strains are sufficient to maintain the same mean total RNA and poly(A)$^+$mRNA concentrations in cells of these different sizes. In this discussion we are concerned with 2 questions: what determines the cell cycle stage at which the doubling in rate of synthesis occurs, and what mechanism fixes the synthetic rate before and after the rate doubling.

Cell cycle control of transcription

Cell size-monitoring mechanisms have been proposed as controls of initiation of DNA synthesis and mitosis (Fantes *et al.* 1975). The observed correlation between cell

size and cell cycle stage of doubling in rate of RNA synthesis suggested that some aspect of cell size might also be involved in the control of initiation of the rate increase. We have examined this quantitatively by taking total protein as an index of cell size. Knowing the mean protein content per cell in exponential, asynchronous culture, and knowing that total protein increases close to exponentially during the cell cycle (Stebbing, 1971), we have calculated the protein per cell at the time of the mid-point of each doubling in rate of poly(A)$^+$mRNA synthesis (Table 3). For the 3 diploid strains, the doublings in rate of poly(A)$^+$mRNA synthesis occurred when the cells had very similar protein contents.

Table 3. *Mean protein per cell values at various stages of the cell cycles of* 3 *diploid and* 2 *haploid strains of S. pombe, and cell cycle stage of mid-point in doubling in rate of poly(A)$^+$mRNA synthesis in each strain*

Strain	pg protein/cell at		Cell cycle stage of mid-point in doubling of rate of poly(A)$^+$mRNA synthesis	Protein content at mid-point of doubling of rate of poly(A)$^+$mRNA synthesis	
	Start of cycle	End of cycle		pg/cell	pg/haploid genome
Diploids					
Wild type/wild type	16·3	32·6	0·03 ± 0·02	16·6 ± 0·2	8·3 ± 0·1
wee 1–50/wild type	13·3	26·6	0·50 ± 0·03	18·9 ± 0·3	9·5 ± 0·2
wee 1–50/*wee* 1–50	9·0	18·8	1·04 ± 0·04	19·0 ± 0·6	9·5 ± 0·3
Haploids					
Wild type	8·8	17·5	0·11 ± 0·04	9·5 ± 0·2	9·5 ± 0·2
wee 1–50	5·0	10·0	0·81 ± 0·06	8·7 ± 0·4	8·7 ± 0·4

Protein values were calculated from the measured mean protein content of cells in asynchronous exponential growth, using the cell age-distribution equation, and knowing that protein rises close to exponentially during the cell cycle. Time of mid-point of doubling in rate of poly(A)$^+$mRNA synthesis was measured in synchronous cultures (Figs. 1–3) and expressed in cell cycle units. Protein content per haploid genome for the diploid strains was obtained by dividing the value per cell by 2. Values are means ± standard errors. Values for haploid strains are from Fraser & Nurse, 1978.

The wild type and *wee* 1–50 haploid lines, for comparison, doubled their rates of poly(A)$^+$mRNA synthesis when their protein contents were quite different from the three diploid lines (Table 3; haploid data from Fraser & Nurse, 1978). However, if the protein contents per cell at the time of rate doubling are expressed per haploid genome, i.e. by dividing the diploid values by 2, it is clear that all 5 strains, diploid or haploid, doubled their rate of poly(A)$^+$mRNA synthesis when at a very similar protein content (Table 3). Similarly, the doubling in rate of rRNA synthesis also occurred in cells of a similar protein content per haploid genome, although the data available are less extensive. These results suggest that the rate doublings are triggered when the cells reach a threshold size, and that the controlling aspect of size is monitored on a per haploid genome basis. The comparison of haploid and diploid lines suggests that monitoring mechanisms based on surface area to volume ratios can be excluded.

One type of mechanism which would fit our results is the inhibitor-dilution model, such as has been proposed for the control of initiation of DNA replication in *Escherichia coli* by Pritchard, Barth & Collins (1969). A pulse of inhibitor is synthesized at a particular point in the cell cycle, for example at DNA replication or nuclear division. When diluted by cell growth to below a threshold level, the doubling in rate of transcription is initiated. If the amount of inhibitor synthesis per cell is constant, and the inhibitor is stable, then all cells will have to grow to the same size to reduce the inhibitor concentration to the same threshold level. Comparison of diploid and haploid lines suggests that the amount of inhibitor synthesized must be a constant amount per haploid genome. This would occur if inhibitor synthesis were gene-dosage dependent, and if the inhibitor genes were only available for transcription for a limited period during each cell cycle.

An alternative mechanism is that some controlling component is synthesized at a rate proportional to cell mass (Sompayrac & Maaløe, 1973). When a certain amount of this component has been accumulated per cell the events leading to the doubling in rates of RNA synthesis are set in motion. If the amount that has to be accumulated is double in a diploid then the doubling in rate of RNA synthesis will take place in cells of a constant size per haploid genome.

We do not know the biochemical mechanism by which a cell mass-related control could alter the rates of RNA synthesis. It is even possible that the mass control acts on some separate component of cell metabolism, and that the induced cyclic behaviour of that component entrains the periodic changes in rates of RNA synthesis (Goodwin, 1963).

Mechanisms setting the rates of RNA synthesis

The cell cycle patterns of RNA synthesis in the 3 diploid strains may be interpreted in terms of a model outlined briefly elsewhere (Fraser & Nurse, 1978). This model proposes that the rates of synthesis of rRNA and the majority of polyadenylated mRNAs are under a general control, perhaps analogous to stringent control of RNA synthesis in bacteria (Cashel & Gallant, 1974). This control sets the rates at a certain level at the beginning of the cycle, and at twice that rate when the cell attains a threshold size.

The dependency of the doubling in rates on the previous round of DNA replication suggests that the setting of the rate at each level may be based ultimately on gene dosage. However, the rate cannot depend solely on gene dosage, as in *wee* 1–50 diploid and heterozygous diploid cells, a considerable delay occurs between DNA replication and doubling in the rate of RNA synthesis. There must be some mechanism in these strains to keep the rate of RNA synthesis at the lower level during the period between replication of the genome and the attainment of a critical cell size.

One possibility would be that half of the copies of each gene present after DNA replication might be completely inactivated until the cell reached the threshold size. Such inactivation could be caused by DNA modification (Holliday & Pugh, 1975), by chromosomal folding (Vaughan, 1977) or by chromosomal proteins (Paul & Gilmour, 1968). An analogous form of control exists over those genes situated on the X chromosome in mammals. In females, with 2 X chromosomes and hence twice as many X

chromosome genes as males, compensation for the doubled concentration of these genes is by complete inactivation of one of the 2 X chromosomes (Lyon, 1961, 1972). In this context, it is interesting that in the early female mammalian embryo both X chromosomes are active (Monk & Kathuria, 1977). Only later, during cell cleavage when the cells get smaller is one of the X chromosomes inactivated. Perhaps when the cells are reduced beneath a certain critical size, a mechanism similar to that observed in *S. pombe* operates to inactivate one of the X chromosomes.

Alternatively, the rate of transcription might be maintained at the pre-DNA replication rate if the 2 copies of each gene present after replication were transcribed, on average, at half the rate of transcription of the single copy present before replication. On reaching the threshold size, the rate of transcription of both copies would double, to equal that of the single copy present before replication. A similar mechanism exists in dosage compensation of the X chromosome in *Drosophila* (Lucchesi, 1973; Courtright, 1976). In this organism, the 2 genes present on the 2 X chromosomes in the female are each expressed at half the rate of the single gene present on the single X chromosome in the male. The means by which the rate of transcription per gene could be restricted in *S. pombe* to half of the rate before DNA replication are not clear. The mechanism could involve limited availability of the gene, or control by altered activity of RNA polymerase or some other component of transcription.

We have discussed 3 examples from eukaryotes of mechanisms compensating for increased gene concentrations. All operate by effectively reducing the mean concentration of active DNA. In the prokaryote *Escherichia coli*, a mechanism compensating for a reduced gene concentration has been proposed. Chandler & Pritchard (1975) suggested that when gene concentration is reduced, the rate of total protein synthesis is maintained by lowering the level of repression of those genes subject to specific controls at the transcriptional level.

Elsewhere, we have considered how a cell size-controlled doubling in rate of RNA synthesis may be of widespread importance in the control of enzyme accumulation and balanced exponential growth of *S. pombe* cells (Fraser & Moreno, 1976; Fraser & Nurse, 1978; Barnes *et al.* 1978). It should be emphasized, however, that the proposed general size control over rate of messenger RNA synthesis is obviously not the only mechanism controlling cell growth and enzyme synthesis in *S. pombe*. Some genes, such as those associated with periodic events like DNA replication, may be transcribed periodically. Transcription of some is induced by alteration in nutrient conditions (Mitchison & Creanor, 1969). There is also evidence that synthesis of some enzymes may be regulated at the translational level (Creanor, May & Mitchison, 1975; Fraser, 1975). The proposed cell size-related general control over gene activity also does not exclude the possibility that genes are individually subject to further, specific controls.

Limits of the compensation mechanism in S. pombe

If the doubling in rate of RNA synthesis is dependent on DNA replication, there are limits to the cell size range over which the compensation mechanism will function effectively. In cells of wild type size, DNA replication and thus the doubling in rate of RNA synthesis cannot occur significantly earlier in the cycle, as DNA replication

cannot take place before completion of mitosis (Nurse & Thuriaux, 1977). Any increase in mean cell size at division of such cells would reduce the gene concentration and the steady-state RNA concentration.

The mechanism of the size control initiating the doubling in rate of RNA synthesis may also set a limit to how small a cell can be. For example, if the rate doubling is triggered when a certain amount of component has been accumulated per cell, since division will halve that amount, a cell which has not reached the threshold level of component at division will never do so. With this mechanism the smallest cell which could maintain a constant mean concentration of RNA would be about half the size of wild type. A similar minimum cell size would also apply to the inhibitor-dilution model if the inhibitor were made at a constant time in the cell cycle, say just after cell separation. However, if the inhibitor were made at the time of DNA replication, which takes place later in the cycle in small cells, the minimum cell size would be less than half that of wild type.

The nature of the mechanism limiting the rate of transcription between DNA replication and doubling in rate of RNA synthesis may also have a bearing on minimum cell size. If one copy of each gene is inactivated until the threshold size is reached, and the cell undergoes nuclear division beneath this size, then by independent segregation of sister chromatids each daughter nucleus would have an incomplete complement of active genes. This is likely to be a non-viable situation, and would place a lower limit on viable cell size.

In this context, it is interesting to return to the behaviour of the *wee* 1–50 diploid in synchronous culture. The interval between successive divisions in synchronous culture of *wee* 1–50 diploid cells was the same as in wild type diploids, while in asynchronous culture the mean cell number doubling time of the *wee* 1–50 diploid was much longer than that of wild type diploids. The increase in cell number at each division in synchronous cultures of *wee* 1–50 diploids was less than a doubling. These results show that in *wee* 1–50 diploid synchronous cultures, a fraction of cells lost viability in each cell cycle. Cells at the end of their cell cycle will show a variation in size: it is possible that the smallest cells were too small to activate the doubling in rate of RNA synthesis before division, and thus became non-viable. These cells would also explain the failure of synchronous cultures of *wee* 1–50 diploids to double their rates of rRNA and poly(A)+mRNA synthesis in each cycle. It may be relevant to this argument that mutants smaller than *wee* 1–50 have not yet been found.

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