The Induction of a Shortened, Synchronous Cell Cycle by Removal of Chloramphenicol from an Inhibited Culture of the Fission Yeast *Schizosaccharomyces pombe*

By ANTHONY QUINTON* AND ROBERT K. POOLE†

Department of Microbiology, Queen Elizabeth College
(University of London), Campden Hill, London W8 7AH

(Received 21 June 1977)

Adding chloramphenicol to cultures of *Schizosaccharomyces pombe*, growing exponentially on glycerol as carbon source, resulted in a heterogeneous population of abnormally large cells. After removal of the inhibitor and resuspension in fresh medium, the cells increased in size and the cell volumes became more normally distributed. Cells then underwent two synchronous divisions separated by 5 h; this is significantly shorter than the doubling times for culture extinction and total cell volume which are both about 7 h. There was an overall decrease in mean cell volume. The chloramphenicol-induced cell cycle was shorter than the first complete cell cycle in synchronous cultures prepared by size-selection.

INTRODUCTION

When exponentially growing cultures of the fission yeast *Schizosaccharomyces pombe* are treated for 3 to 4 h with 2 mM-2'-deoxyadenosine, DNA synthesis and cell division are temporarily inhibited, but the synthesis of RNA and the accumulation of dry mass are virtually unaffected (Mitchison & Creanor, 1971). The abnormally large cells that accumulate subsequently complete two synchronous divisions in only 60% of the normal cycle time. This can be explained, teleologically, as an attempt to restore the volumes of cells growing in exponential culture. Shortened cell cycles such as these are frequently encountered when abnormally large cells result from inhibition of division by heat shocks (Zeuthen, 1964), ultraviolet irradiation (Sachsenmaier *et al.*, 1970) and a variety of chemotherapeutic agents (Ross, 1976). Addition of chloramphenicol, an inhibitor of mitochondrial protein synthesis (Lloyd, 1974), to an exponential culture of *S. pombe* growing with glycerol (a non-fermentable carbon source) allows almost a doubling in cell numbers in the following 24 h. Cell volume, however, continues to increase after cessation of division, resulting in a population of cells whose mean cell volume is about 1.5-fold greater than that before addition of chloramphenicol (Poole, 1977*a*). This paper describes the synchronous division of these cells induced by removal of the inhibitor.

METHODS

Organism and growth conditions. *Schizosaccharomyces pombe* strain 972 h⁻ was maintained and grown on defined medium containing 1% (w/v) glycerol as carbon source (Poole & Lloyd, 1974). Cultures (200 ml medium in 1 l conical flasks) were inoculated from starter cultures that had been inoculated with cells from an agar slope culture 48 to 72 h previously. Initial cell concentrations were 1×10^5 to 7.5×10^5 organisms

* Present address: Woodhouse Hume Ltd, Bicester Road, Aylesbury, Buckinghamshire HP19 3AX.

† To whom all correspondence should be addressed.

Fig. 1. Changes in *S. pombe* following resuspension in fresh medium of (a) cells 20 h after adding chloramphenicol (2 mg ml⁻¹) to a culture containing 1.52×10^7 cells ml⁻¹, and (b) cells that had entered stationary phase after 60 h growth. In both experiments the defined medium contained 1% (w/v) glycerol as carbon source. Cell numbers (●) and mean cell volumes (○) were determined using a Coulter counter Z_{BI} and Channelyzer C1000 at the following settings: 1/amplification, 1; 1/aperture current, 1; edit switch, on; count range, 1000. Window width and base channel threshold were varied and were appropriate for the distribution. Total cell volume (□) is [no. of cells ml⁻¹] × [mean cell volume (μm³)]. Cell plate index (■) was measured microscopically on formaldehyde-fixed cells.

ml⁻¹. Chloramphenicol was added as a powder (final concentration 2 mg ml⁻¹) to exponentially growing cultures; treated cultures were incubated for a further 20 to 24 h. The inhibitor was removed by centrifuging the culture to 5600g (r_{av}) followed by immediate deceleration at ambient temperature, and then washing twice with prewarmed fresh medium (lacking chloramphenicol) under the same conditions. Cells were finally resuspended in 200 ml fresh medium lacking chloramphenicol.

Cell numbers and volumes. Culture samples were treated ultrasonically for 10 s (8 μm peak to peak) using a titanium probe (end diam. 3.2 mm) of an MSE 100 W Ultrasonic Disintegrator to break up cell clumps that form during growth on glycerol, and then diluted into electrolyte prior to analysis using a Coulter counter model Z_{BI} (Poole, 1977b). The probe of the counter had an aperture diameter of 50 μm. Statistical analysis of cell volume distributions and the plotting of normalized distributions on microfilm (Bazin, Richards & Saunders, 1977) was done by a CDC 6600 computer.

Cell plate index. This is the percentage of cells showing cell plates (Mitchison, 1970). It was determined by examination of culture samples, which had been fixed with formaldehyde (final concn 2%, w/v), using a Nikon phase contrast microscope (total magnification 1500). Between 400 and 700 randomly selected cells were scored in each sample.

Chemicals. D(-)-threo-Chloramphenicol was purchased as Chloromycetin from Parke-Davis.

RESULTS AND DISCUSSION

Figure 1(a) shows some effects of chloramphenicol treatment of *S. pombe*. Cell numbers remained almost constant for about 4 h after resuspension in fresh medium. During this time the cell plate index, which had fallen to < 1% during the treatment with chloramphenicol, rose sharply to a peak value of 12% at 4.5 h. This peak slightly preceded an

Fig. 2. Changes in the distribution of volumes of cells of *S. pombe* on resuspension in fresh medium (a) following 24 h chloramphenicol treatment (2 mg ml^{-1}) of a culture that contained 1.2×10^6 cells ml^{-1} , or (b) after entering the stationary phase of growth. The cells in (b) are the same as those whose growth is shown in Fig. 1(b), but the cells in (a) are from a third experiment. Numbers accompanying individual distributions are sampling times (h). Volume distributions were obtained using a Coulter counter and Channelyzer C1000 as described in the legend to Fig. 1. Distributions were transferred to paper tape using a Coulter Teleprinter Interface and analysed and plotted on microfilm by a CDC 6600 computer. All volume distributions are normalized with respect to volumes under the curves.

increase in cell numbers and a sharp drop in mean cell volume. Mean cell volume continued to decrease during the rest of the experiment, decreasing more rapidly after the second lower peak in the cell plate index at 9.5 h than in the 3 h prior to this peak. The interval between the two peaks in the cell plate index (5 h) is similar to the doubling time for cell numbers (5.5 h) indicating a partially synchronous cell cycle of this length.

The peak values for the cell plate index are considerably lower than those reported for glucose-grown cultures synchronized by induction (Mitchison & Creanor, 1971) or size-selection (for example, Mitchison, 1970). This reflects firstly a lower degree of synchrony after chloramphenicol treatment, and secondly, the fact that the index determined using our technique in exponential cultures containing glycerol is lower (about 5 to 7%) than that obtained in glucose-containing exponential cultures (about 10%; Mitchison, 1970).

This cycle is significantly shorter than the first complete cell cycle (7.08 h, s.d. 0.63, 6 experiments) observed in synchronous cultures prepared by selection of the smallest cells from an exponential culture using glycerol density gradients (Poole, 1977a; R. K. Poole, unpublished results). The total volume of cells in the culture (Fig. 1a) and extinction at 600 nm (measured in a separate experiment, not shown here) increased less rapidly than cell numbers (doubling time about 7 h in the period 6 to 16 h), reflecting the progressive decrease in mean cell volume.

Further evidence for the existence of two synchronous divisions after removal of chloramphenicol came from the changing form of distributions of cell volumes (Fig. 2). Volume distributions were normalized with respect to the area enclosed by the curve to allow

comparison of the frequencies of different volume classes. Chloramphenicol was added at an earlier stage of growth in the experiment illustrated in Fig. 2(a) compared with that in Fig. 1(a); thus, since cell volume decreases during growth in batch culture on media containing either glucose (Johnson, 1967; Johnson & Rupert, 1967) or glycerol (R. K. Poole, unpublished results), the cell volumes are smaller in Fig. 1(a). Cells incubated for up to 24 h with chloramphenicol showed heterogeneous volume distributions (Fig. 2a; zero time). In this experiment, cell numbers did not increase during the first 6 h of incubation in chloramphenicol-free medium. During this period, the modal cell volume increased from $70 \mu\text{m}^3$ to about $90 \mu\text{m}^3$ and the relative positive skewness of the distribution decreased from 0.65 to 0.33 (Croxtan & Cowden, 1939). After 7 to 7.5 h, a new population of cells, modal volume about $45 \mu\text{m}^3$, was distinguishable. The frequency of this population, relative to that of the population of larger cells, gradually increased. At 10.5 to 11 h, the volume distributions showed the presence of a further population, modal volume 22 to $23 \mu\text{m}^3$, indicating a second synchronous division. This pattern of events was confirmed in another similar experiment.

It could be argued that the synchronous divisions described above were induced, not by the removal of chloramphenicol, but by the transfer to fresh medium of cells that had stopped growing or by the centrifugation and washing procedures. A stationary-phase culture was harvested, washed and resuspended in fresh medium (Fig. 1b) to give a cell population similar to that in Fig. 1(a). After a short lag, cell numbers increased gradually. Mean cell volume fluctuated by about 15% in the first 6 h after resuspension and then remained relatively constant. The curve for total cell volume closely followed that of cell numbers. Volume distributions throughout this experiment remained positively skewed and unimodal (Fig. 2b) confirming the absence of synchronous cell divisions.

Under certain conditions cells failed to recover on transfer to chloramphenicol-free medium. When medium containing chloramphenicol (2 mg ml^{-1}) was inoculated with a stationary-phase starter culture to give an initial cell population of 1×10^6 organisms ml^{-1} , and then incubated for 72 h, cell numbers increased fourfold but the resulting large cells failed to recover on transfer to fresh medium lacking chloramphenicol. However, when this procedure was applied to cells growing in complex medium that contained yeast extract, peptone, phosphate and glycerol, cells recovered, dividing synchronously 5 to 8 h after resuspension in fresh medium. Furthermore, in defined medium, using the procedure described in Methods, recovery and synchronous division was reproducible only when the cell population at the time of adding chloramphenicol exceeded about 1×10^6 organisms ml^{-1} . The factors involved in the varying ability of cells to recover from chloramphenicol treatment have not been investigated further. Resumption of growth occurs after removal of chloramphenicol from cultures of *Tetrahymena pyriformis* (Turner & Lloyd, 1971; Gleason *et al.*, 1975) but the details of this recovery have not been reported.

The abnormal volumes of cells grown with chloramphenicol result from continued increase in cell volume in the absence of cell division (Poole, 1977a). This may be due to the direct requirement for a product of mitochondrial protein synthesis for division. Alternatively, chloramphenicol may inhibit DNA synthesis (Shah, 1975), thus indirectly inhibiting division since these two processes may be closely linked in a 'DNA-division' cycle (Mitchison, 1973). The shift in volume distribution preceding the first synchronous division in chloramphenicol-free medium is of particular interest. All cells increase in volume equally after addition of chloramphenicol, but only those cells prior to the early transition point in the cell cycle are incapable of division (Poole, 1977a), leading to two populations of cells. The results in Fig. 2(a) are consistent with the hypothesis that on removal of chloramphenicol the smaller cells (modal cell volume $70 \mu\text{m}^3$) increase in volume to attain the modal volume ($90 \mu\text{m}^3$) of the second population. This results in a population that is almost normally distributed (skewness close to zero) and divides synchronously.

REFERENCES

- BAZIN, M. J., RICHARDS, L. & SAUNDERS, P. T. (1977). Automated data processing of microbial size distributions. In *Haematological and Biological Applications of the Coulter Counter (Proceedings of a Conference held at the Tara Hotel, London, April 14-16, 1975)*, pp. 426-435. Harpenden, England: Coulter Electronics Ltd.
- CROXTON, F. E. & COWDEN, D. J. (1939). Dispersion, skewness and kurtosis. In *Applied General Statistics*, pp. 234-264. New York: Prentice Hall.
- GLEASON, F. K., OOKA, M. P., CUNNINGHAM, W. P. & HOOPER, A. B. (1975). Effect of chloramphenicol on replication of mitochondria in *Tetrahymena*. *Journal of Cellular Physiology* **85**, 59-72.
- JOHNSON, B. F. (1967). Growth of the fission yeast, *Schizosaccharomyces pombe*, with late, eccentric, lytic fission in an unbalanced medium. *Journal of Bacteriology* **94**, 192-195.
- JOHNSON, B. F. & RUPERT, C. M. (1967). Cellular growth rates of the fission yeast, *Schizosaccharomyces pombe*, and variable sensitivity to 2-deoxyglucose. *Experimental Cell Research* **48**, 618-620.
- LLOYD, D. (1974). Mitochondrial protein synthesis in eukaryotic microorganisms. In *The Mitochondria of Microorganisms*, pp. 397-435. London: Academic Press.
- MITCHISON, J. M. (1970). Physiological and cytological methods for *Schizosaccharomyces pombe*. *Methods in Cell Physiology* **4**, 131-165.
- MITCHISON, J. M. (1973). The cell-cycle of a eukaryote. *Symposia of the Society for General Microbiology* **23**, 189-208.
- MITCHISON, J. M. & CREANOR, J. (1971). Induction synchrony in the fission yeast *Schizosaccharomyces pombe*. *Experimental Cell Research* **67**, 368-374.
- POOLE, R. K. (1977a). Termination of the cell cycle of *Schizosaccharomyces pombe* by inhibition of mitochondrial protein synthesis. *Proceedings of the Society for General Microbiology* **4**, 75.
- POOLE, R. K. (1977b). Fluctuations in buoyant density during the cell cycle of *Escherichia coli* K12: significance for the preparation of synchronous cultures by age selection. *Journal of General Microbiology* **98**, 177-186.
- POOLE, R. K. & LLOYD, D. (1974). Changes in respiratory activities during the cell-cycle of the fission yeast *Schizosaccharomyces pombe* 972h⁻ growing in the presence of glycerol. *Biochemical Journal* **144**, 141-148.
- ROSS, D. W. (1976). Cell volume growth after cell cycle block with chemotherapeutic agents. *Cell and Tissue Kinetics* **9**, 379-387.
- SACHSENMAIER, W., DÖNGES, K. H., RUPFF, H. & CZIHAK, G. (1970). Advanced initiation of synchronous mitosis in *Physarum polycephalum* following ultraviolet irradiation. *Zeitschrift für Naturforschung* **25b**, 866-871.
- SHAH, V. C. (1975). Effects of some antibiotics on cell cycles of cultured and meristematic cells. In *Regulation of Growth and Differentiated Function in Eukaryote Cells*, pp. 41-55. Edited by G. P. Talwar. New York: Raven Press.
- TURNER, G. & LLOYD, D. (1971). The effect of chloramphenicol on growth and mitochondrial function of the ciliate protozoon *Tetrahymena pyriformis* strain ST. *Journal of General Microbiology* **67**, 175-188.
- ZEUTHEN, E. (1964). The temperature-induced division synchrony in *Tetrahymena*. In *Synchrony in Cell Division and Growth*, pp. 99-158. Edited by E. Zeuthen. New York: Interscience.