SHORT COMMUNICATION

Oscillatory Accumulation of Catalase during the Cell Cycle of *Acanthamoeba castellanii*

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The accumulation of catalase in synchronously dividing cultures of *Acanthamoeba castellanii*, prepared by size selection techniques involving minimal perturbation, was discontinuous. During the cell cycle of 8 h both catalase activity and immunologically determined catalase protein oscillated with a periodicity of about 1 h and a mean trough-to-peak amplitude of 21% of the minimal values. These patterns of enzyme accumulation were not observed in control asynchronous cultures after exposure to identical experimental conditions. The results are discussed suggesting that periodic biosynthesis and degradation occurs during growth and division of this organism.

INTRODUCTION

Studies of the patterns of enzyme expression during the cell cycle provide basic information on control processes in growing cell cultures. Extensive lists show that examples of both continuous and discontinuous accumulation have been observed (Mitchison, 1971). It has been suggested that some of the periodic cases may reflect perturbation during the establishment of synchronous cultures (Mitchison, 1977). A major shortcoming of almost all investigations is that enzyme *activity* measurements are assumed to give valid assessments of amounts of enzyme protein present in cell-free extracts. Immunological confirmation of this assumption has only occasionally been presented (Klevecz, 1969; Martin *et al.*, 1969). Alternatively, individual proteins may be identified by two-dimensional gel electrophoresis and their rate of synthesis estimated by a dual-labelling technique (Elliott & McLaughlin, 1978, 1979).

The present work demonstrates that in synchronous cultures of *Acanthamoeba castellanii* which have experienced essentially no perturbation the accumulation of catalase activity (and of the enzyme protein as assayed by an immunological method) oscillates with a periodicity of about 1 h so as to give about seven maxima per cell cycle of 8 h.

METHODS

Organism and culture conditions. *Acanthamoeba castellanii* was grown axenically in complex medium in shaken flasks (1 litre culture in 4 litre flasks) at 30 °C and counted as described previously (Edwards *et al.*, 1977; Edwards & Lloyd, 1980). Synchronous cultures were prepared by centrifugation of exponentially growing cultures in sterile 1 litre centrifuge pots at 800 rev. min⁻¹ (150 g; r_{av} 21 cm) for 2 min in an MSE Mistral centrifuge. After centrifugation, about 70% of the supernatant was carefully decanted; the slowly sedimenting cells in this suspension accounted for about 10% of the original exponentially growing population and provided the synchronous culture. The degree of synchrony was assessed as described previously (Edwards & Lloyd, 1980).

Preparation of cell-free extracts and enzyme assays. Cell-free extracts were obtained by treatment of cells with ultrasound (Branson Sonicator, 10 s) after resuspension in 50 mM-Tris/H₂SO₄ buffer (pH 7.4); breakage was

100%. Catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase; EC 1.11.1.6) activity was assayed by the method of Lück (1963). It was established that the rate of disappearance of substrate was directly proportional to the amount of extract in the assay and that there was no detectable change in enzyme activity from the time of preparation of the extract to the time of assay; experiments in which extracts of different enzyme activity were mixed showed that there was no naturally occurring catalase inhibitor present in the extracts. The coefficient of variation [$100 \times (\text{standard deviation})/(\text{mean})$] of 16 assays was 4%.

Immunological determinations of catalase protein utilized titration of cell-free extracts against anti-catalase serum in the complement fixation test (Wilkinson, 1962). Rabbit anti-catalase serum was prepared by injecting rabbits with 40 mg dialysed bovine liver catalase suspension (6×10^4 U mg⁻¹) intramuscularly once a week for 4 weeks and test bleeding 10 d after the final injection. The recovered serum was de complemented and tested for immunological activity against extracts of *A. castellanii* by complement fixation. Lyophilized guinea-pig complement and rabbit haemolytic serum were obtained from Wellcome and the defibrinated sheep erythrocytes without preservative were from Difco. One unit of immunological activity (U) was expressed as the reciprocal of the end-point titre per mg protein in each sample. Protein was estimated by the Lowry method.

RESULTS AND DISCUSSION

In a synchronous culture the catalase activity increased during the cell cycle from 23 to 46 mU ml⁻¹, but this overall doubling in enzyme activity was achieved by a discontinuous increase (Fig. 1*b*). The oscillation showed a mean trough-to-peak amplitude of 21% of the minimal values; over the first 6 h of the experiment the period was exactly 1 h but then increased to 1.25 and 1.5 h. We have previously shown that the total cellular protein of *A. castellanii* also oscillates with an hourly periodicity during the growth of synchronous cultures (Edwards & Lloyd, 1980). Nevertheless, the oscillation in catalase was still evident when results were expressed as specific enzyme activity (Fig. 1*c*). An independent immunological assay gave a similar profile through the cell cycle (Fig. 1*c*). Since each sample was assayed against fixed dilutions of antiserum, overall profiles of enzyme activity and enzyme protein may be similar but direct comparisons of percentage changes are not possible. Differential proteolytic degradation of catalase after cell breakage does not contribute to this phenomenon, since the oscillatory accumulation of catalase was also observed in an experiment in which organisms were disrupted in the presence of the different protease inhibitors (2 mM-phenylmethylsulphonyl fluoride, 5 mM-*p*-aminobenzamidine.HCl and 5 mM- ϵ -aminocaproic acid).

It should be stressed that the degree of synchrony assessed by cell counts is underestimated in these cultures; DNA replication is completed in 1 h (Edwards & Lloyd, 1980). Oscillatory expression of catalase activity has also been observed (Chagla, 1975) in synchronous cultures which were prepared using a different technique for cell size selection (the continuous flow method; Lloyd *et al.*, 1975).

In order to show that the oscillatory expression of catalase did not originate from metabolic perturbation induced by the synchronization procedure, an asynchronous culture was subjected to an identical centrifugation procedure, but the sedimented cells were resuspended together with the non-sedimented population before their subsequent growth. Cell numbers then increased exponentially (Fig. 2), as did total protein and catalase activity [expressed as mU (ml culture)⁻¹]; the smoothly continuous increases indicate that the oscillatory accumulation of catalase previously observed in synchronous cultures was not a consequence of possible metabolic disturbance. The discrepancies in activities of catalase per cell in Figs 1 and 2 are explained by the fact that during exponential growth activity varies considerably (Edwards & Lloyd, 1977) and it is impossible to produce synchronous and control cultures under identical conditions.

These experiments indicate that in synchronous cultures of *A. castellanii*, in common with the majority of cell proteins (Edwards & Lloyd, 1980), the accumulation of a peroxisomally located protein, catalase (Müller & Møller, 1969; Morgan *et al.*, 1973), is oscillatory. The cell cycle of this protozoon is organized into seven subcycles, and in each of these subcycles, a

Fig. 1. Changes in cell numbers, catalase activity and catalase protein during synchronous growth of *Acanthamoeba castellanii*. An exponentially growing culture (4.5×10^6 cells ml^{-1}) was centrifuged at $800 \text{ rev. min}^{-1}$ for 2 min and the supernatant (containing 7% of the original culture) was grown as a synchronous culture. At 15 min intervals samples were removed for determination of (a) cell numbers, with synchrony index, F (Blumenthal & Zahler, 1962), (b) catalase activity [expressed as $\text{mU (ml culture)}^{-1}$], and (c) catalase activity [expressed as $\text{mU (mg protein)}^{-1}$] (●) and immunologically determined catalase protein [$\text{mU (mg protein)}^{-1}$] (○).

Fig. 2. Changes in cell numbers, protein content, catalase activity and catalase protein after centrifugation of an exponentially growing culture of *Acanthamoeba castellanii*. An exponentially growing culture was centrifuged at $800 \text{ rev. min}^{-1}$ for 2 min; the pellet and supernatant were then re-mixed and returned to the growth vessel. At 15 min intervals samples were removed for determination of cell numbers (●), protein content (○), catalase activity [expressed as $\text{mU (ml culture)}^{-1}$] (■) and $\text{mU (mg protein)}^{-1}$] (□), and immunologically determined catalase protein [$\text{mU (mg protein)}^{-1}$] (▲).

phase of synthesis of catalase precedes its partial degradation as indicated by loss of both enzyme activity and immunological determinants. These observations thus indicate rapid turnover of catalase protein even when the organism is growing under optimum conditions. That this turnover is separated into temporally discrete 'compartments' in which *either* biosynthesis *or* degradation predominates suggests that time-resolution of the kind achieved in synchronous cultures is necessary for a further understanding of the control of protein biosynthesis, accumulation and degradation during cellular growth.

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REFERENCES

- BLUMENTHAL, A. K. & ZAHLER, S. A. (1962). Index for measurement of synchronization of cell populations. *Science* **135**, 724.
- CHAGLA, A. H. (1975). *The biochemistry and physiology of encystation in amoebae*. Ph.D. thesis, University of Wales.

- EDWARDS, S. W. & LLOYD, D. (1977). Changes in oxygen uptake rates, enzyme activities, cytochrome amounts and adenine nucleotide pool levels during growth of *Acanthamoeba castellanii* in batch culture. *Journal of General Microbiology* **102**, 135–144.
- EDWARDS, S. W. & LLOYD, D. (1980). Oscillations in protein and RNA content during synchronous growth of *Acanthamoeba castellanii*. *FEBS Letters* **109**, 21–26.
- EDWARDS, S. W., CHAGLA, A. H., GRIFFITHS, A. J. & LLOYD, D. (1977). The cytochromes of *Acanthamoeba castellanii*. *Biochemical Journal* **168**, 113–121.
- ELLIOTT, S. G. & MCLAUGHLIN, C. S. (1978). Rate of macromolecular synthesis through cell cycle of yeast, *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America* **75**, 4384–4388.
- ELLIOTT, S. G. & MCLAUGHLIN, C. S. (1979). Synthesis and modification of proteins during the cell cycle of the yeast *Saccharomyces cerevisiae*. *Journal of Bacteriology* **137**, 1185–1190.
- KLEVECZ, R. R. (1969). Temporal order in mammalian cells. I. The periodic synthesis of lactate dehydrogenase in the cell cycle. *Journal of Cell Biology* **43**, 207–219.
- LLOYD, D., JOHN, L., EDWARDS, C. & CHAGLA, A. H. (1975). Synchronous cultures of micro-organisms: large-scale preparation by continuous-flow size selection. *Journal of General Microbiology* **88**, 153–158.
- LÜCK, H. (1963). Catalase. In *Methods of Enzymatic Analysis*, p. 885. Edited by H. U. Bergmeyer. New York: Academic Press.
- MARTIN, D., TOMKINS, G. M. & GRANNER, D. (1969). Synthesis and induction of tyrosine aminotransferase in synchronized hepatoma cells in culture. *Proceedings of the National Academy of Sciences of the United States of America* **62**, 248–255.
- MITCHISON, J. M. (1971). In *The Biology of the Cell Cycle*, pp. 159, 180. Cambridge: Cambridge University Press.
- MITCHISON, J. M. (1977). Enzyme synthesis during the cell cycle. In *Cell Differentiation in Microorganisms, Plants and Animals*, pp. 377–401. Edited by L. Nover & K. Mothes. Jena: Gustav Fischer Verlag.
- MORGAN, N. A., HOWELLS, L., CARTLEDGE, T. G. & LLOYD, D. (1973). The acid hydrolase-containing organelles of eukaryotic microorganisms. In *Methodological Developments in Biochemistry*, vol. 3, pp. 219–232. Edited by E. Reid. London: Longmans.
- MÜLLER, M. & MØLLER, K. M. (1969). Urate oxidase and its association with peroxisomes in *Acanthamoeba*. *European Journal of Biochemistry* **9**, 422–430.
- WILKINSON, A. E. (1962). *Association of Clinical Pathologists Broadsheet*, no. 41.