Periodic cell cycle changes in the rate of CO₂ production in the fission yeast *Schizosaccharomyces pombe* persist after a block to protein synthesis

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Summary

CO₂ production has been followed by manometry in synchronous and asynchronous control cultures of *Schizosaccharomyces pombe* prepared by elutriation from the same initial culture. Earlier results showed a periodic change in the rate of production, which took place once per cell cycle. These changes were most clearly shown as oscillations in the difference between values of the second differential (acceleration) for the synchronous and asynchronous cultures. This paper shows that the oscillations continue for at least three cycles in the presence of cycloheximide (with and without chloramphenicol). Protein synthesis is virtually absent and there is no cell division. The control of this metabolic oscillation is therefore not directly dependent on translation. The period of the oscillation under these conditions is about 60% of the normal cycle time.

Key words: cell cycle, CO₂ production, cycloheximide, fission yeast, *Schizosaccharomyces pombe*.

Introduction

We have shown in an earlier paper (Novak & Mitchison, 1986) that the rate of CO₂ production follows a linear pattern in synchronous cultures of *Schizosaccharomyces pombe* with a rate change once per cycle. This rate change is at the time of division except in wee mutants. The rate changes persist after a block to the DNA-division cycle produced by shifting a *cdc* mutant to the restrictive temperature. We examine here the effect of a different block, one to protein synthesis, and we show that in this case also the periodic rate changes persist though against a background of falling CO₂ production.

Materials and methods

These were described in detail by Novak & Mitchison (1986). In summary, wild-type cells (strain 972h−) were grown in a minimal medium, EMM3, at 35°C. A synchronous culture was made with an elutriating rotor together with an asynchronous control culture that had also been through the rotor. CO₂ production was then measured in both cultures by Warburg manometry. Fitted polynomial curves were used to determine the rate of production and the acceleration (rate of the rate). Cycloheximide and chloramphenicol came from the Sigma Chemical Co. Ltd.

Results

Fig. 1 shows the effect of adding cycloheximide (100 µg ml⁻¹) on the rate of CO₂ production in a synchronous and an asynchronous culture that had just been prepared by the elutriating rotor. In both cases there was an overall decline but there were two marked fluctuations in the synchronous culture (Fig. 1, curve A) in contrast to the steady fall-off in the asynchronous culture (Fig. 1, curve B). The cell-number curves show that cycloheximide stopped division in both cultures (Fig. 1, curves D,E; cf. Polanshek, 1977). A control without cycloheximide showed a typical stepwise rise in numbers in the synchronous culture (Fig. 1, curve C) and a smooth exponential rise in the asynchronous culture (data not shown).
Fig. 3. Timing map of the oscillations in synchronous cultures treated with cycloheximide. The upper triangles show the mid-rise points in the difference curves and the lower diamonds show the troughs in these curves. The arrows give the mean values. The values in parentheses are the differences between successive mid-rise points and troughs.

A timing map from a number of similar experiments is given in Fig. 3. In most experiments, only one oscillation could be timed accurately and it had a period of 81 min on average. There was some indication of an earlier oscillation with a shorter period of 67 min but this depended on the results from only two experiments. In two of the experiments, it was clear that the oscillations continued for at least a third cycle but it was impossible to get accurate timings because the manometers could not be read with precision at very low values.

Cycloheximide at 100 µg ml⁻¹ reduces the rate of protein synthesis in Schizosaccharomyces pombe, as judged by [³H]leucine incorporation, to 3–5% of the normal rate after 30 min (Mitchison & Creanor, 1969; Polanshek, 1977). It was possible, however, that the oscillations might be associated with mitochondrial protein synthesis, which was relatively insensitive to cycloheximide. We therefore added chloramphenicol, an inhibitor of mitochondrial protein synthesis at 2 mg ml⁻¹ (Goffeau et al. 1972), together with the cycloheximide, but the oscillations were unaffected.

Discussion

The most important conclusion from these experiments is that oscillations in CO₂ production that are normally entrained to the cell cycle can continue in the virtual absence of protein synthesis. They are not therefore under direct translational control. The clearest parallels are the cell cycle oscillations in cytoplasmic pH in Dictyostelium, which also continue after cycloheximide treatment (Aerts et al. 1985). On the other hand, cycloheximide stops continuing oscillations of maturation promoting factor in amphibian oocytes (Gerhart et al. 1984) and of nucleoside diphosphokinase activity in S. pombe (Creanor & Mitchison, 1986).

Two points should be made about the oscillations. The first is that the changes in the rate of CO₂ production shown in Fig. 1, curve A, are much more conspicuous in this non-growing situation than they are in the normal growing situation before any block (Novak & Mitchison, 1986). The absence of growth seems to enhance the pattern. The second is that the period of the oscillations (81 min) is only 60% of the normal cycle time (134 min). Stopping the DNA-division cycle shortens the period to 85% of the normal cycle time (Novak & Mitchison, 1986) and stopping growth shortens it even further. There may perhaps be an analogy with the very short cell cycle times in non-growing early embryos.

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References


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