SHORT COMMUNICATION

Effects of Chloramphenicol on Cell Division in Synchronized Cells of *Alcaligenes eutrophus*

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Chloramphenicol inhibited growth of asynchronous cells of *Alcaligenes eutrophus*. In synchronous cultures, different effects on cell division were observed, depending on the time of addition of chloramphenicol. The earlier the time of addition, the greater the inhibition of cell division, which indicates that protein necessary for cell division is synthesized at the beginning of the cell cycle.

INTRODUCTION

The cell division cycle of *Escherichia coli* has been proposed to consist of two parallel sequences of protein synthesis and chromosome replication (Jones & Donachie, 1973, 1974). During the sequence of protein synthesis it is postulated that proteins necessary for cell division are synthesized at specific times during the cell cycle. Similar schemes have been presented for *Streptococcus faecalis* (Shockman *et al.*, 1974) and *Bacillus subtilis* (Miyakawa *et al.*, 1980). These results contrast with the work of Lutkenhaus *et al.* (1979) who failed to detect any polypeptide which was synthesized at a specific stage of the cell cycle of *E. coli*.

In this communication we present preliminary evidence that protein necessary for cell division is synthesized early in the cell cycle of *Alcaligenes eutrophus*.

METHODS

Maintenance and growth of the organism. *Alcaligenes eutrophus* H16, ATCC 17699 was maintained on plates of nutrient agar. Growth in liquid salts medium, preparation of synchronous cultures by continuous-flow centrifugation and assessment of synchrony were as described previously (Edwards & Jones, 1977).

Analytical methods. Growth was followed by measuring A_{550} of the culture or by determining cell numbers using a Coulter counter model ZBI fitted with a 30 μm orifice tube (Coulter Electronics, Harpenden, Herts.) in culture samples which had been appropriately diluted in the electrolyte solution Isoton II (supplied by Coulter Electronics). Protein synthesis was inhibited by the addition of powdered chloramphenicol to growing cultures to give a final concentration of 100 $\mu\text{g ml}^{-1}$.

RESULTS AND DISCUSSION

Addition of chloramphenicol [to a final concentration of 100 $\mu\text{g (ml culture)}^{-1}$] to asynchronously growing cells of *A. eutrophus* (doubling time 75 min) resulted in an almost immediate cessation of growth. Cell numbers rose by only 15% and A_{550} by 12% during the subsequent 75 min incubation period after addition of the antibiotic (at time zero). Both growth and cell division were inhibited in similar fashion and to the same extent (Fig. 1a).

Synchronously growing cells were prepared by continuous-flow centrifugation. After size selection (see Edwards & Jones, 1977), the resultant cell suspension was used to establish three

Fig. 1. Effects of chloramphenicol on growth and division in *A. eutrophus*. (a) Chloramphenicol was added (time zero) to cells growing with a doubling time of 75 min; cell numbers (●) and A_{550} (○) were determined during the subsequent incubation period. (b) Synchronous growth of *A. eutrophus* in the absence (●) of chloramphenicol and when the antibiotic was added after 5 min (○) and 35 min (▲). (c) Percentage of initial cell population dividing (■) plotted against times of addition of chloramphenicol during synchronous growth. Data were pooled from four separate experiments.

cultures each of 100 ml volume. These were then incubated with shaking at 30 °C (time zero is taken as the point at which cultures were placed in the incubator). Chloramphenicol was added to one of the cultures after 5 min incubation and to another after 35 min incubation. The third culture remained untreated throughout and served as the control. Cell numbers were determined at intervals for all three cultures and the results are shown in Fig. 1(b). Cell numbers in the control increased synchronously at 35 min rising from 3.2×10^7 to 6.4×10^7 bacteria ml^{-1} . No cell division occurred in the culture to which chloramphenicol had been added at 5 min. However, cell numbers rose from 3.2×10^7 to 4.4×10^7 bacteria ml^{-1} in the culture to which the antibiotic had been added at 35 min. The action of chloramphenicol appeared to be cell cycle related. That is to say, the later its time of addition during synchronous growth the greater the extent of cell division. In order to locate more precisely the times during the cell cycle at which chloramphenicol exerted its greatest effect, a number of experiments similar to the one described in Fig. 1(b) were performed. The antibiotic was added at different times during synchronous growth and the percentage of cells dividing was calculated and plotted against the time of addition. These results are presented in Fig. 1(c). They show that chloramphenicol

inhibits cell division totally when added up to 10 min after the initiation of synchronous growth. Thereafter, the later the time of addition the greater the percentage of the initial population which divides. This corresponds to almost 40% division when chloramphenicol is added 35 min after the initiation of synchronous growth. Similar results have been reported for *E. coli* by Dix & Helmstetter (1973).

Our results for *A. eutrophus* imply that protein necessary for cell division is synthesized early in the cell cycle. We have also shown that DNA synthesis in this bacterium is confined to the first half of the cell cycle (Edwards & McCann, 1983). The early synthesis of division protein(s) suggested here may therefore be important either in initiation of chromosome replication or for elongation of newly synthesized DNA during the C period. Further work is necessary to distinguish between these possibilities and to determine whether another period of protein synthesis at termination of DNA synthesis is also important for cell division.

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