Two-step Doubling of Synchronous Cultures of *Escherichia coli* K12

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INTRODUCTION

In experiments designed to study the division cycle of *Escherichia coli* we have observed an unusual response of cultures of strain K12 to the induction of synchrony by a single change of temperature. We report the characteristics of that response, which suggests the existence of two cold-sensitive stages in the cell division cycle of *E. coli* K12.

METHODS

Strains. *Escherichia coli* B and K12 c600 (Appleyard, 1954) were obtained from S. Luria. The lysogenic K12 c600 (λ) strain has been isolated in our laboratory using phage λ .K12, obtained by u.v. induction. *Escherichia coli* K12 wild type was provided by L. R. Caldas of this Institute.

Culture medium. Cultures of bacteria were grown with aeration at 37 °C in a glucose-containing minimal medium (Smith & Pardee, 1970) prepared in deionized water and supplemented with L-leucine, L-threonine (20 µg/ml each) and thiamine (1 µg/ml).

Techniques of synchronization. (i) Single change of temperature (modified from Scott & Chu, 1958). Overnight cultures of the various *E. coli* strains were diluted 1:100 with fresh glucose-minimal medium and grown at 37 °C with aeration to about 10⁸ organisms/ml. The suspensions were then incubated at 11 to 12 °C for 90 min and quickly brought back to 37 °C (zero time) by dilution into fresh pre-heated medium. Samples were thereafter taken every 5 to 10 min, diluted as needed in 0.05 M-phosphate buffer pH 7.2 containing 2 % (w/v) formaldehyde, and counted under a phase-contrast microscope (Phasestar model, American Optical) in a Petroff-Hausser bacterial counting chamber. (ii) Stationary phase method (modified from Cutler & Evans, 1966). An overnight culture was diluted 60-fold in fresh medium and incubated at 37 °C. Growth was followed by extinction measurements with a Klett-Summerson photoelectric colorimeter. Stationary phase was reached after 240 min; 25 min later (one-half the mean generation time) the culture was diluted sevenfold in fresh medium. This procedure was repeated, stationary phase then being reached after 180 min. Samples from the second dilution culture were taken and processed as described for method (i). (iii) Gradient separation (as described by Mitchison & Vincent, 1965, using a 5 to 30 %, w/v, sucrose gradient). Growth was followed by plating samples on nutrient agar for colony counting.

RESULTS AND DISCUSSION

Initial experiments were performed using the lysogenic strain K12 c600 (λ). Synchronous growth was observed following a 90 min shift to 11 to 12 °C (Fig. 1c). The culture started dividing after about 20 min and bacterial concentration doubled once but by two steps, each

Fig. 1. Synchronization of *Escherichia coli* K12 cultures by various methods. (a) Strain c600, early stationary phase method. (b) Strain c600 (λ), sedimentation technique; after centrifuging, a fresh culture was started using as inoculum a sample (0.1 ml) from the top of the bacterial layer (about 0.5 % of the original load). (c) Strain c600, cold shock procedure (90 min, 11 to 12 °C).

corresponding to approximately 50 % of the population in the culture at the time of its return to 37 °C. The first step took place at around the 23rd minute, the second about the 60th minute. In both cases division was spread over some 10 min. Similar results were obtained with a cold shift of 45 min and with temperatures as low as 6 °C. These features were observed for two successive cycles. The generation time of the synchronous culture, taken as the time that elapsed from the mid-point of the first step to the mid-point of a similar step in the next cycle, averaged 51 min, while the mean value for the random exponential-phase culture is 50 min. Successful induction of synchrony in cultures of the c600 strain seems to depend on the presence of a lambda prophage (Cox & Strack, 1972). To investigate the possible interference of the prophage with the synchronous response described here, the induction of synchrony by a single temperature change was tried with the corresponding non-lysogenic strain as well as the wild-type K12S. These strains responded with similar two-step increments in numbers, an indication that the cold-sensitivity is neither dependent on nor influenced by the presence of a lambda prophage.

The double step is not found when synchronization techniques other than a single chilling and warming cycle are applied to the K12 strain and related mutants. A single rise for the doubling in number is observed whenever cultures of strain K12 are synchronized after physical separation of organisms by centrifugation through a sucrose gradient (Mitchison & Vincent, 1965) or by the stationary phase method (Cutler & Evans, 1966) (Fig. 1). Single step doublings have also been described for K12 in liquid cultures synchronized either by fractional filtration (Nagata, 1963; Masters, Kuempel & Pardee, 1964) or by the polyvinyl chloride membrane technique (Cummings, 1970).

Other strains of *E. coli* so far tested did not behave like K12. Cultures of strain B synchronized by the cold shock procedure showed a single doubling step per division cycle, confirming previous reports by Scott & Chu (1958) and Perry (1959). The same held for strain B/r, according to Doudney (1960). Thus the double step derived from the cold treatment is a characteristic of the K12 strain. The results can be interpreted as due to the existence of two separate sensitive stages in the division cycle of *E. coli* K12, each of which becomes growth-limiting in response to the cold shock. The cold-sensitive stages determine two points in the division cycle to which organisms are brought, depending on the part of the cycle in which they were when the cold shock was applied. The number of organisms found in each group was approximately equal. The conditions described seem to provide an interesting example for the study of the bacterial division cycle.

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