BBA Report

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Segregation of membrane markers during cell division in *Escherichia coli*. II. Segregation of Lac-permease and Mel-permease studied with a penicillin technique

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SUMMARY

A rapid method is described for the detection of heterogeneity in a bacterial population with respect to a membrane marker. The technique involves treatment with penicillin in the presence of a carbon source, the utilization of which is limited by the inducible membrane marker, usually a permease. The part of the population containing the marker is lysed rapidly, and the cells devoid of the marker can be separated.

The distribution of Lac-permease and Mel-permease in the progeny of fully induced populations during growth and cell division in the absence of inducer was examined using this technique. A non-synchronous population gives rise to approximately 50% of permease-less segregants after three generation times in mineral medium with glycerol or glucose as sole carbon source. These results can be interpreted in terms of a simple model of membrane growth.

A localized growing zone in bacterial membranes has been postulated[1] to account for the equal distribution of genomes during cell division in the absence of a mitotic apparatus. A number of attempts to demonstrate a regular growth pattern utilized cell wall markers[2-9] with contradictory results. Recent reports on synchronized *Escherichia coli* carrying a lipid density label[10] and on a mini-cell-producing strain of *E. coli*[11] conclude that membrane growth is diffuse.

If *E. coli* is induced for the lactose operon for several generation times, Lac-permease is presumably distributed along the whole surface area of the membrane, which contains approximately 10^4 protein copies per cell[12]. When these bacteria are transferred into a non-inducing growth medium, the new membrane will not contain

Abbreviations: Lac, lactose; Mel, melibiose; IPTG, isopropyl-D-thiogalactoside.

permease. If new membrane appears in definite coherent growing zones in small number, permease in the old membrane also will remain in coherent zones which will be fragmented into a small number of zones by the appearance of new growing zones. In this hypothesis, only a small number of bacteria from the progeny will inherit permease.

Penicillin at a high concentration is known to cause lysis of growing bacteria[13]. Non-growing bacteria survive penicillin treatment. This method, widely utilized in genetics to select mutants which, in a given condition, are unable to grow, is applied to Lac-permease-less bacteria unable to grow on lactose as sole carbon source. Since lactose will, in a short time, induce the synthesis of new permease, the discrimination provided by penicillin is transitory. In order to obtain fast lysis of the part of the population containing permease, cells were submitted to EDTA treatment[14] prior to incubation with penicillin and lactose.

Fig. 1 shows the time course of lysis of bacterial populations grown on minimal medium[15] with or without IPTG, and a mixture of the two in penicillin lactose medium, with or without prior treatment with EDTA.

Fig. 2 shows the time course of lysis of bacterial populations, (a) non-induced, (b) fully induced, (c) deinduced for one generation time, (d) two generation times, (e) three generation times. All except the last behave as a homogeneous population with respect to the test applied. The population which had been deinduced three generation times lysed in two distinct steps; the proportion of survivors remained nearly constant during 20 min after about 50% of the population had been lysed. A similar time course of lysis is observed when survivors are measured by colony counts after plating on nutrient agar.

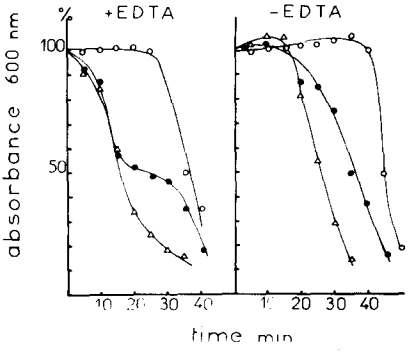
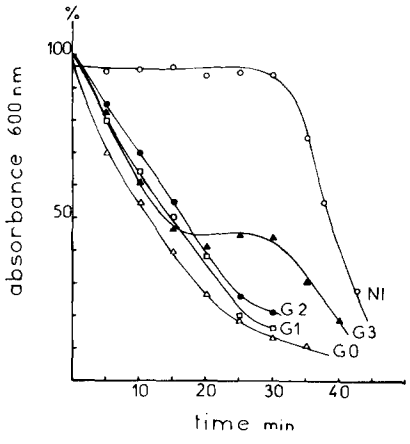


Fig. 1. Comparison of the time course of lysis of bacteria, induced or non-induced for Lac-permease and a 1:1 mixture of the two. *E. coli* K12 strain 3000 was grown for 6 generation times in Medium 63 glycerol B1 (ref. 15), with or without 0.2 mM IPTG. When absorbance at 600 nm reached 1.0, bacteria were filtered on a millipore filter, 0.45 μm pore size, washed with 0.12 M Tris-HCl buffer, pH 7.6, and resuspended with the same buffer in one-tenth of the original volume. 1 mM EDTA was added to one part of each suspension and after 1 min of vigourous shaking at 37° the suspensions were diluted in 9 vol. of Medium 63 containing penicillin (1000 units/ml) and 5 mM lactose, and placed on a reciprocating shaking bath. At intervals, absorbance readings at 600 nm were made in a Zeiss PM QII spectrophotometer in cuvettes of 1 cm light path. Left: bacteria treated with EDTA; right: without EDTA. o——o, non-induced; Δ——Δ, induced; •——•, mixture.



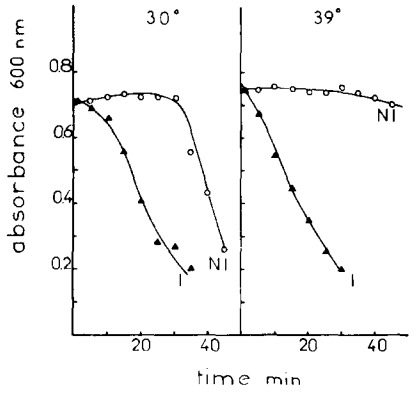


Fig. 2. Time course of lysis of populations of *E. coli* 3000 after various times of deinduction. Fully induced bacteria were centrifuged and washed with Medium 63, then transferred into Medium 63 glucose B1 at 37°. After 0, 1, 2, 3 generation times (G0, G1, G2, G3), EDTA treatment and lysis in Medium 63 penicillin lactose were performed as described in the legend of Fig. 1. Non-induced bacteria were used as control (NI).

Fig. 3. Comparison between time course of lysis of *E. coli* 300 P pregrown on Medium 63 glycerol B1 with or without added melibiose (4 g/l) at 30°, when treatment with penicillin and melibiose is carried out at 30° or 39°. Techniques of EDTA treatment and lysis were as described in legend for Fig. 1, except that lactose was replaced by melibiose.

Mel-permease is known to be induced by a number of α-galactosides including melibiose, but this sugar also induces the Lac operon, and therefore a Lac-permease-deficient strain 300 P was used. The synthesis of Mel-permease is heat sensitive[16] but the activity of the previously synthesized transport system is fairly stable at 39°. Accordingly, Fig. 3 shows that bacteria not preinduced for Mel-permease fail to lyse in melibiose penicillin medium at 39°.

Fig. 4 shows the time course of lysis in penicillin melibiose medium at 39° of cell populations of *E. coli* 300 P, non-induced, induced with melibiose, and deinduced for one, two and three generation times; the heterogeneity of the population at the third generation here results in the prolonged survival of the permease-poor half of the population.

Fig. 5 shows that the heterogeneity of the population after deinduction for three generation times is due to the unequal distribution of Mel-permease, while β-galactosidase is evenly distributed.

The simplest pattern of membrane growth and cell division compatible with these results is represented in Fig. 6. Since the cell population was not synchronized, in this figure a cell midway between two cell divisions has been taken as representative of the population. This cell will give rise to 8 cells after two and a half doubling times, and out of these only four carry permease, from the mother cell. Only the growing zone active at the time of deinduction and the two growing zones in daughter cells, initiated around the first cell division, will insert new membrane within permease-containing old membrane, the growing zone initiated at the time of the second cell division arises at the frontier between parental membrane and membrane synthesized after deinduction, giving no further fragmentation of the former.

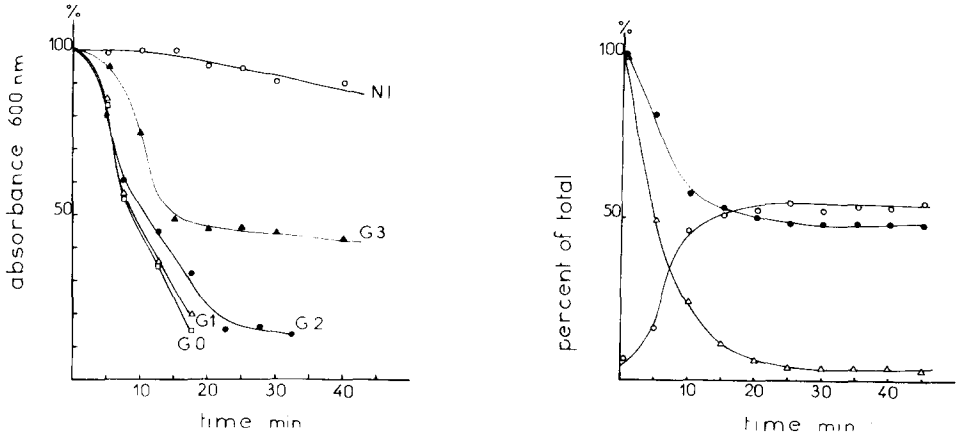


Fig. 4. Time course of lysis at 39° of populations deinduced for Mel-permease 0, 1, 2, 3 generation times (G0, G1, G2, G3).

Fig. 5. Fate of Mel-permease and β-galactosidase during segregation. During the lysis at 39° of a 3 generation times deinduced population, besides absorbance readings at 600 nm (●——●), samples were removed and treated with penicillinase (10 μg/ml). After centrifugation, Mel-permease content was measured in the survivors, resuspended in Medium 63 with chloramphenicol, by the initial velocity of uptake of [^{14}C] thiomethyl-β-D-galactoside at 25°, according to Kepes[20] (△——△). β-Galactosidase was estimated in the supernatant by the rate of *o*-nitrophenyl-β-D-galactoside hydrolysis[21] (○——○). 100% control was measured after toluene treatment of the original population.

Some other models, such as two polar growing zones per cell would also result in a maximum of four permease-containing descendants from one fully induced mother cell, the one pictured in Fig. 6 is the easiest to correlate with the postulated role of the membrane in the distribution of the genome between daughter cells[1], when the cells are carrying two nuclear bodies, *i.e.* between two and four full complements of DNA.

In a rich medium, the heterogeneity of the population appears only after four generation times (results not shown), suggesting that a correlation exists between the number of nuclei[17] and the number of growing zones in the cell membrane.

Microscopic observations of cell growth and localization of the growing zone as the place of the first hernia of cytoplasm appearing upon penicillin treatment[19], are consistent with a model of a unidirectional growing zone starting from a pole of the cell to

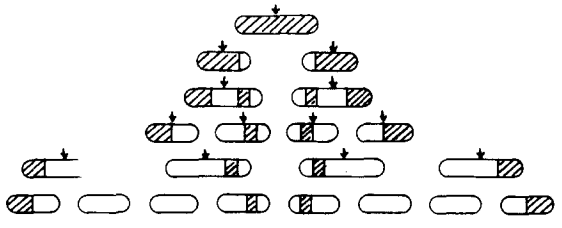


Fig. 6. Expected distribution of permease during deinduction among the progeny of an individual bacterial cell. The case of a bacterial cell midway between two cell divisions is considered as the most representative of a non-synchronized population. The part of the membrane containing permease protein is represented as a hatched area. The growing zone (arrow) is supposed to be median and to cease activity after septum formation. A new growing zone is supposed to appear in the median plane of the daughter cells.

end up in the median zone where it is divided in two by septum formation (in minimal medium). If applicable to the membrane, this model would predict segregation of permease-less progeny one generation earlier than in our results.

In a previous report[18] using a chloramphenicol phage technique, we also observed segregation of permease-less progeny after two generation times, instead of three.

These different observations might reflect a common mechanism of coordination of membrane growth and nuclear division with a difference, according to the conditions of growth, in the delay of cell division *versus* nuclear division of zero, one, two or more generation times. This point should be checked by the observation of the number of nuclear bodies in each case.

With the technique described above, during the plateau of lysis, cells having only new membrane can be physically separated from the lysate of cells containing parental membrane, and if different cell wall and membrane components are specifically labeled in the parental cell, the distribution among the progeny of non-selective markers can be examined.

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