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ISOPYCNIC ANALYSIS OF INTACT CELLS - I: Escherichia coli OVER ITS GROWTH CURVE

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Two computer models for the decline of ribosomal RNA in late exponential phase E. coli are tested isopycnographically. The model in which the excess r-RNA is scavenged to support the last stages of cell division is discarded, whereas the experimental data support the model in which r-RNA production is halted and the r-RNA is diluted among the cells as they continue their final divisions. This plus the pycnotic profile of E. coli relA⁻ support previous work pertaining to control of the genome by guanosine -3'-diphosphate-5'-diphosphate (ppGpp). Other evidence suggests the possibility that part of the genome is also under separate control.

The beauty of the method of equilibrium density gradient centrifugation is that it facilitates observations of heterogeneity in a population of discrete units. Although molecular biologists have long taken advantage of this fact, its application to whole cells has received scant notice. Among the few studies made, it is seen that most were concerned with sporulation (1,2) because the methods as used then for other types of cells were fraught with uncertainties due to the infant state of knowledge about cell membranes, plasmolysis and the penetrability of the various highly soluble compounds used in gradient making (3). In recent years, these questions have been sufficiently well answered to allow us now to proceed with confidence (4).

Hence, in this first paper we wish to report on some fundamental findings concerning gross changes in the cellular makeup of E. coli B using refinements of the sodium bromide gradients as used by Kaney, et al (5).

Because the RNA concentration in an E. coli is elevated to 30% of the dry weight of the cell in exponential phase (versus only about 10% in stationary phase) (6,7), and because RNA is intrinsically dense, the over-

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all density of the cell should be markedly greater during rapid growth as opposed to stationary phase cells. Furthermore, it should not be unexpected that possibly most of the cell's change in density would be due to the variation in just the ribosomal component alone. If this is true, then by observing how the cell's density shifts during transitions from one phase of growth to another, one should be able to gain insights into the mode of control regulating the synthesis of ribosomes. It is on this basis that we wish to focus upon the transition between late exponential phase and early stationary phase because of the currency of guanosine-3'-diphosphate-5'-diphosphate (ppGpp) models for genomic control (8).

Two models are hypothesized for a cell's losing RNA and becoming less dense during the slow shift-down. One model holds that as the cell depletes its food source it turns inward and scavenges its excess dense components, which by shear mass is RNA. This model holds little credence, however, as it has long been known that the great bulk of the RNA is quite stable within such a time-frame of a few hours (9). Nevertheless, this heuristic model will be useful for comparative purposes. The second model holds that at some point in the slow shift-down, transcription of RNA is halted and the existing RNA molecules are from then on merely diluted out among the cells during the final stages of growth. This dilution model can be seen to be compatable with the scheme proposed for ppGpp (10).

MATERIALS AND METHODS

E. coli B and *E. coli* relA$^-$ (CA274) (11) were grown in tryptone broth (0.7% glucose + 0.7% NaCl + 0.8% Bacto-Tryptone) at 37C. Heat shocked cells for the aggregation test were produced by taking 5 ml of an overnight culture to 50C in 3 seconds using 1100 watts of 2.47 bhz microwave, and then instantaneously cooling by dilution into 5 ml of cool broth. For the time-course experiments, overnight cultures were diluted 20 fold with fresh broth, and the absorbance at 620 nm was followed. Periodically, aliquotes were taken for cellular specific gravity measurements. These aliquotes were immediately made to 1.3 NaBr* to stop further growth or development (12).

* NaBr solutions here are abbreviated to indicate their specific gravities. Water saturated with NaBr has a value of 1.5 gm/cc, and is thus written 1.5 NaBr.

Isopycnography was done by using a centrifuge equipped with a fixed angle rotor and thick walled glass tubes and spun at 12,000 x G for 10 minutes. The linear gradients ranged from 1.3 NaBr (mentioned above) to 1.5 NaBr. Samples of the bands were withdrawn from the centrifuged gradients using a capillary pipet, and these were read on a refractometer and compared against NaBr solutions of known densities.

The computer assisted mathematical models were constructed such that two parameters were held in common: (a) the "generation time" of the RNA within a cell was a constant 20 minutes, which is the rate needed in the most rapidly dividing real cell at any time, and (b) that the generation time of the cells is a reciprical of the concentration of the RNA, which is reasonable since slower growing cells require less protein synthetic machinery (13). In both models, the RNA begins at 15% of the cell's dry weight at the beginning of lag phase, and then climbs to 50% during exponential phase. From there it is made to decline according to whichever model is being considered. In the scavenging model, no other carbon sources are available except for the RNA itself which is consumed back down to the original 15% as the cells continue to slowly divide. In the dilution model, only the production of RNA is halted and the cells are allowed to continue using the "broth." In both cases, the cellular doubling rate remains tightly coupled with the RNA concentration. (Annotated copies of the APL programs will be sent upon request.)

RESULTS

It is seen that the specific gravity of E. coli changes markedly over its growth curve - going from 1.375 to about 1.41 and returning (Figure 1). In all cases the bands were narrow indicating little heterogeneity. Aggregation was also ruled out (Figure 4).

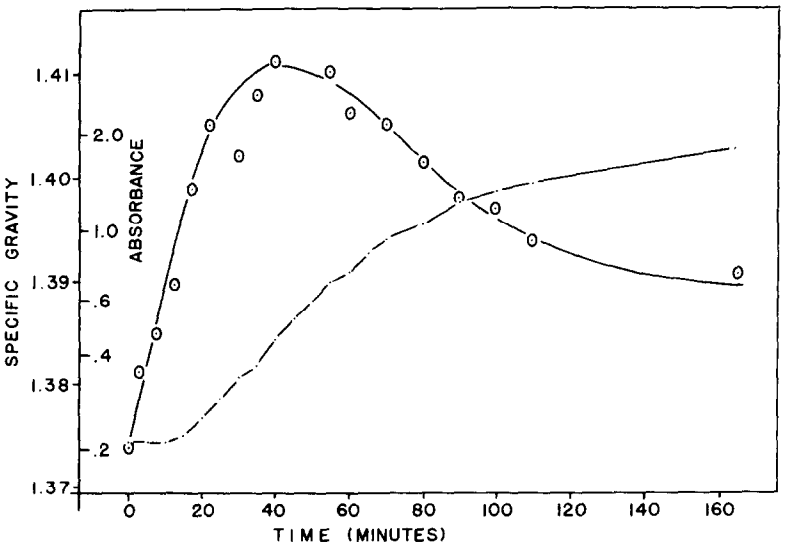

Figure 1. Wild-type E. coli B exhibits a marked change in specific gravity (-0-) as it progresses through its growth curve (-·-).

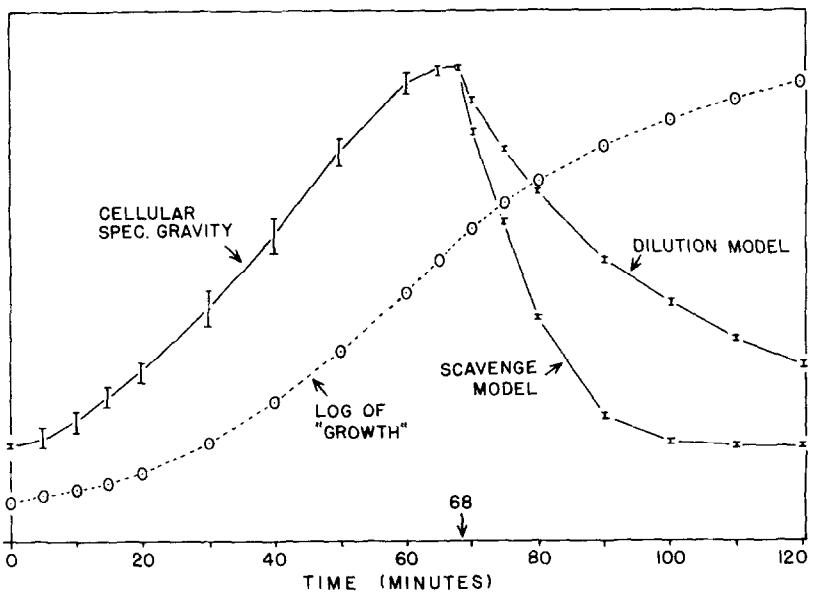

Figure 2. A synopsis of a mathematical modelling of the E. coli growth curve superimposed upon hypothetical profiles of cellular specific gravity. The cellular growth rate is coupled with the concentration of a single, changing dense component. The scavenging model is an illustration of the cell's consuming the excess dense material upon nutrient exhaustion, and the dilution model shows the cessation of synthesis of the dense material with subsequent dilution among the population during the final stages of cultural growth.

The ascent of the specific gravity curve begins several minutes prior to the first signs of accumulation of matter that scatters visible light from which the growth curve is derived. The peak values of specific gravity occur in mid-exponential phase and then rapidly fall back as the cells enter the very earliest stages of stationary phase.

The computer profile that incorporates the dilution model is remarkably similar to the observed experimental data in almost all respects (Figure 2).

The specific gravity profile of the relaxed mutant (Figure 3) ascends to levels approximating those of the normal cells. Then, however, the curve falls back only 15% towards its starting level in the first hour after reaching its maximum. By comparison, the normal cells lose 40% during that time period. Although not shown, after 12 hours the normal cells have returned to their "light" constitution of 1.375 gm/cc whereas the relA$^-$ cells descend only to 1.398 gm/cc and remain thereafter at that level.

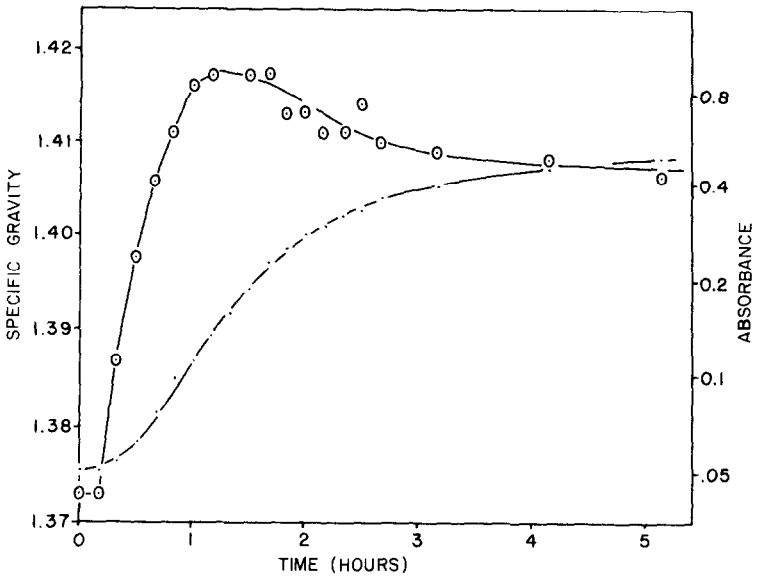

Figure 3. The specific gravity (-0-) of a relA⁻ strain of E. coli HfrH remains high after the cells enter the stationary phase of their growth curve (-·-).

One final observation is that the pycnotic bands of the mutant indicate great heterogeneity especially in the post-peak period. Only the initial few samples and a 24 hour sample (not shown) were narrow bands.

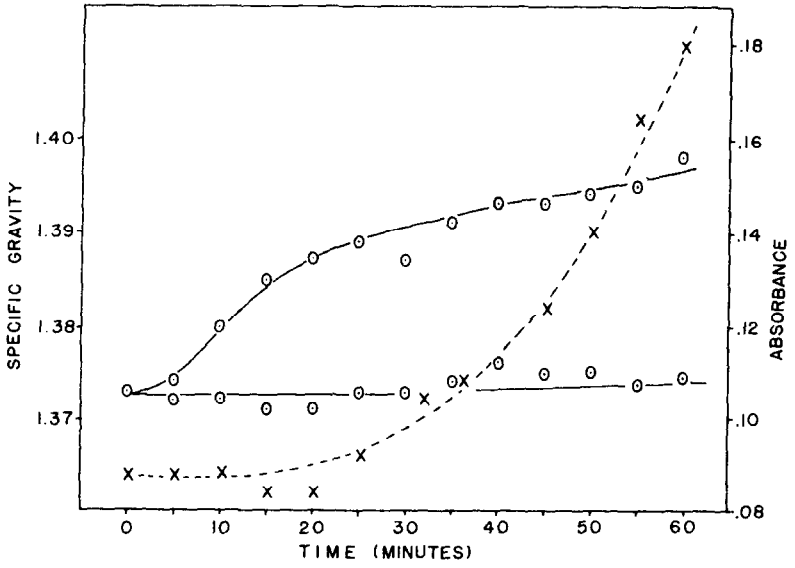

Figure 4. A mixture of both untreated and heat-shocked E. coli B demonstrates that the cells do not aggregate under the conditions reported here.

DISCUSSION AND CONCLUSION

Isopycnography, which allows a glimpse beyond the mythical statistically average cell, affords an overall view of a population as its members move through their growth cycles with, as we have seen, a rather high degree of homogeneity - at least as far as normal E. coli are concerned. This is of no small consequence as one might suspect that individual cells may have slightly different thresholds when genomic controls are imposed that are due to either cellular idiosynchrasies or to problems of statistical mechanics operating at very low concentrations in the cell. However, such suspicions are not upheld. On the contrary, the data from the relaxed strain suggests that only in the absence of stringent control does diversity manifest itself. This would thus, in turn, suggest that normal genomic control is exerted before the concentrations of intermediates drop so low that statistical mechanic considerations would play an observable role.

Furthermore, we have seen by a computer model that our data supports a scheme of genomic control of the type being proposed for guanosine tetraphosphate repression as the medium approaches exhaustion. However, the data also show that this control is not complete in that cell wall growth, and possibly that of other molecular species, follows a separate profile - especially with regard to the later times in the growth cycle when the dense component is merely diluted out while the light scattering component of the culture continues to increase. Certainly further study is warranted in order to ascertain whether or not wall growth continues because it is under genomic controls that are separate from those which regulate bulk RNA synthesis or is completely independent of direct genomic control and subject merely to the presence of residual active enzyme activities.

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