Proposal for a Research Project on Control of Growth in Bacteria

Increasing the replication time (C) of the bacterial chromosome without changing the doubling time (τ) of a steady state exponentially-growing culture (Pritchard & Zaritsky, 1970, Nature, 226: 126) increases the average number of forks per chromosome ($F = 2^{C/\tau}-1$; Zaritsky & Pritchard, submitted to J.Mol.Biol.) in the culture. This, in turn, will increase the dosage of the structural genes for the ribosomal proteins in any bacterial strain in which the chromosomal location of these genes is such that they are replicated soon after the start of a round of DNA replication (e.g. Gorelic, 1970, Molec. Gen. Genetics, 106, 323).

If this gene dosage is one of the important factors determining αr (the fraction of r-proteins of total proteins in the culture) due to a permanent derepression of the r-protein cistrons (Maaløe, 1969, Symp. Soc. Developmental Biol.) then an increase in C will raise αr. Since this parameter is inversely proportional to the doubling time of the culture (Maaløe, ibid; Cooper, 1970, J. Theoret. Biol. 28: 151; Koch, 1970, J. Theoret. Biol. 28: 203) the consequence of increase in αr will, in the absence of compensating factors, be a reduction in τ, thus increasing F still further (if C remains unaffected).

However, if the assembly of repressions (described by the multi-variable partition function; Maaløe, 1969, ibid) indeed stabilises αr during steady-state growth and is determined only by the nutrients present in the medium, then it must have a "buffering" effect on the gene dosage, hence cancelling this apparent "vicious circle". Since it is possible to vary the replication time of the chromosome of thy$^-$ strains of E.coli over a threefold range just by varying the thymine concentration in the medium without affecting significantly the growth rate of the culture (Pritchard & Zaritsky, ibid; Zaritsky & Pritchard, ibid), this system can be a very useful tool to analyse the validity of this assumption. In a culture, where $C = \tau$ the relative number of copies of an early replicating gene (presumably like the r-proteins' cistrons) is two-fold higher than the number of copies of late-replicating markers, whilst in a culture where $C = 3\tau$ this ratio is raised to 8.

In my work with E.coli 15T$^-$ (555-7) growing in M9-glucose ($\tau = 40 \pm 5$ minutes) or M9-glycerol ($\tau = 60 \pm 6$ minutes) a slight decrease in the doubling

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time was observed when the culture was supplied with a lower concentration of thymine. Unfortunately, the differences in values of τ were variable from one day to another.

Recording the cell sizes and their distributions as well as several other observations led me to the hypothesis (Ph.D. Thesis, in preparation) that in glucose grown cultures of this strain there exist cells in which an irreversible event has occurred. These cells are "dead" in the sense that although they continue to synthesize macromolecules and to increase their mass for some time they cannot divide nor form colonies, They are being diluted in the culture by growth of normal cells. Their fraction in the population is higher when the medium is supplied with lower thymine concentrations(i.e. the probability of such a "lethal" event to occur is proportional to the reciprocal of the thymine concentration).

If these cells exist, then the measured doubling time of the culture (mass or cell numbers) will be longer than it really is for the normal cells alone. However, this "masking" could presumably be revealed by means of measurement of αr (Schleif, 1967, J.Mol.Biol., 27: 41) since the metabolism of cytoplasmic RNA and proteins is probably normal (or near normal).

A refined method for revealing differences in τ may be to calculate the rate of synthesis of ribosomal proteins, since this is proportional to the square of the growth rate.

A series of measurements of αr (or of the rate of synthesis of the r-proteins) may, therefore, provide more information about: (a) the nature of the control of growth in bacteria, and (b) the nature and quantitation of the presumed "lethal" event occurring in fast growing *thy*$^-$ strains.

By stepping-up a steady-state culture grown on low thymine concentration to higher concentration of thymine the rate of chromosome replication increases without any delay, thus changing the relative gene dosages. This will have an immediate effect on αr, if the controlling system stabilizing it is slower to adjust to the change in gene dosage than the response to the gene dosage itself. If this were the case then this technique can be used in order to locate the genes specifying the r-proteins in relation to the origin of chromosome replication.

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