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Does the eclipse limit bacterial nucleoid complexity and cell width?

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ABSTRACT

Cell size of bacteria *M* is related to 3 temporal parameters: chromosome replication time *C*, period from replication-termination to subsequent division *D*, and doubling time τ. Steady-state, bacillary cells grow exponentially by extending length *L* only, but their constant width *W* is larger at shorter τ's or longer *C*'s, in proportion to the number of chromosome replication positions n ($= C/\tau$), at least in *Escherichia coli* and *Salmonella typhimurium*. Extending *C* by **thymine limitation** of **fast-growing *thyA* mutants** result in continuous increase of *M*, associated with rising *W*, up to a limit before branching. A set of such puzzling observations is qualitatively consistent with the view that the actual cell mass (or volume) at the time of replication-initiation *Mi* (or *Vi*), usually relatively constant in growth at varying τ's, rises with time under thymine limitation of fast-growing, thymine-requiring *E. coli* strains. The hypothesis will be tested that presumes existence of a minimal distance l_{min} between successive moving replisomes, translated into the time needed for a replisome to reach l_{min} before a new replication-initiation at *oriC* is allowed, termed Eclipse *E*. Preliminary analysis of currently available data is inconsistent with a constant *E* under all conditions, hence other explanations and ways to test them are proposed in an attempt to elucidate these and other results. The complex hypothesis takes into account much of what is currently known about Bacterial Physiology: the relationships between cell dimensions, growth and cycle parameters, particularly nucleoid structure, replication and position, and the mode of peptidoglycan biosynthesis. Further experiments are mentioned that are necessary to test the discussed ideas and hypotheses.

Contents

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1. Introduction

1.1. The bacterial cell cycle: temporal and spatial aspects

The conventional Bacterial Cell Division Cycle (BCD) is defined by four parameters, 3 of which are temporal: mass doubling time τ, chromosome replication time C and the time D between replication-termination and cell division, the latter two are relatively constant (about 40 and 20 min, respectively) under steady-state exponential growth at fast rates (τ < 70 min) in 37 °C, modulated by nutritional conditions [20]. The 4th parameter, also relatively constant, is the strain-dependent cell mass *Mi* (or volume *Vi*, since density does not change with τ_m [27] per *oriC* at which chromosome replication is initiated [2,13,39,47], synchronously at all existing *oriC*s [7]. Together, these 4 parameters couple cell size *M* to the linear processes of the BCD: cells are larger at shorter τ's [46,57] because they grow (exponentially so) more during the fixed time ($C + D$) between replication-initiation (at *Mi*) and the consequent division (reviewed in Ref. [72]). Put in an equation, average cell size $<M> = Mi \times (\ln 2) \times 2^{(C+D)/\tau}$. Size-control is thus coupled to temporal aspects (rates) of mass growth and nucleoid replication leading to division [67]. Regulation of replication-initiation has largely been resolved [29]; the molecular meaning of the constant *D* period, on the other hand, is still enigmatic (and see below). Under faster growth rates $\mu (= \tau^{-1})$, initiation occurs in the mother or grandmother cell when $\tau < (C + D)$. Furthermore, when $\tau < C$, a replication cycle starts before the previous one has terminated [20,22], to form a multi-forked replicating nucleoid with a higher complexity *NC* [23]. *NC* is defined as the culture-average amount of DNA in genome equivalents associated with a single *terC* [$NC = (2^n - 1)/n \ln 2$] [60,67,76] where $n = C/\tau$ (the number of replication positions; [49], irrespective of the value of *D*.

During steady-state of exponential growth conditions [16], cells enlarge by elongation and divide in a perpendicular plane; cell width *W* is strikingly constant, in the culture and during individual cycle [51]. The simple prediction that the larger, faster-growing cells in richer media are proportionately longer is not fulfilled: they are wider as well (Fig. 1)! A fundamental question thus arises: **how does cell width change during transfer to a richer medium**, so-called nutritional shift-up [24]; Fig. 2)? This question interfaces the major spatial aspects of the cell (placing the FtsZ-ring exactly in mid-cell, fixing and changing cell dimensions under different growth conditions) with the temporal aspects (rates of growth, DNA replication and division processes). The long-standing puzzle of the crucial coordination between nucleoid structure and FtsZ-ring assembly to form the divisome is elusive because "several partially redundant mechanisms to achieve this task" seem to be involved [31] as safeguards for species survival. The *primary signal* delivered from the nucleoid to assemble a divisome for cell division

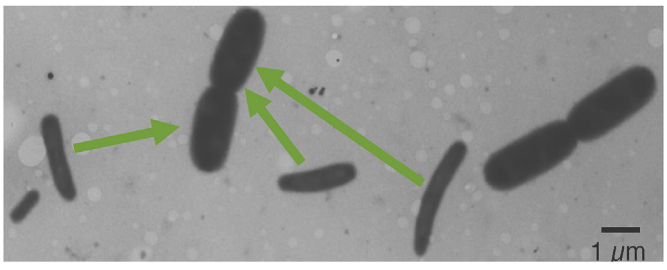

Fig. 1. Electron micrograph of a mixture of two *E. coli* B/r cultures on agar filters. The big cells were grown with a doubling time τ = 22 min; the small cells, with τ = 150 min. Adapted from Zaritsky & Woldringh [72]. Arrows indicate the transition enigma.

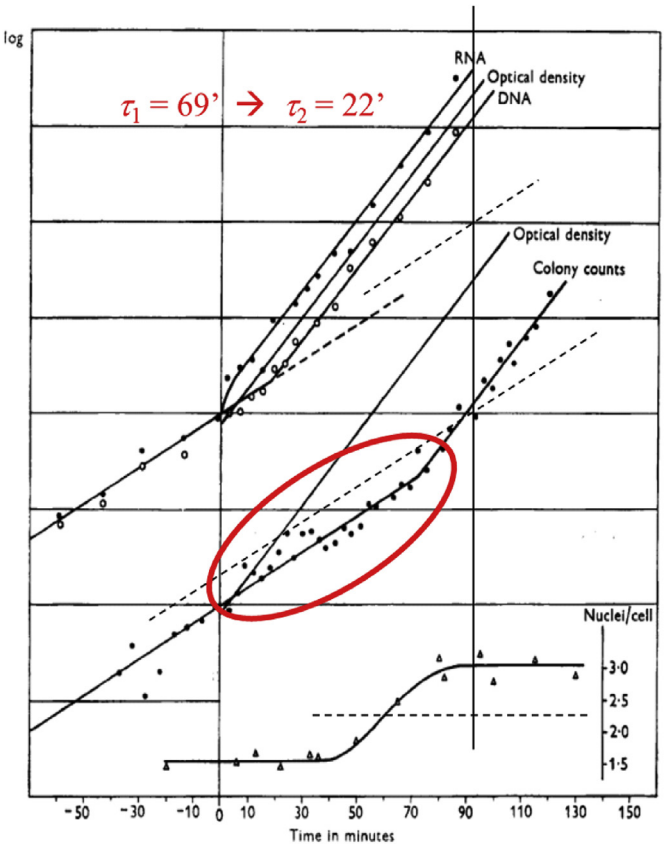

Fig. 2. The classic nutritional shift-up experiment (Adapted from Ref. [24].). The red oval depicts the maintenance period (~65 min) of cell division rate.

in the right place and time cannot be simply a protein-set because the question of their expression is analogous to the "enzyme-cannot-make-enzyme paradox" [48]. As discussed by Kirschner et al. [25]: "This picture of self-organization to a thermodynamic minimum at steady state is likely applicable to many, perhaps all, cellular assemblies". - Isn't the divisome one? A physics-based mechanism for division site-selection was therefore proposed [41]; and see below). Repeating waveform pattern of cell surface undulations along the long axis was just observed in mycobacteria that lack both Min and NO systems [14], but a mechanism for coordinating the FtsZ-ring assembly with the nucleoid is missing. Here, we succinctly summarize the current knowledge about this sought for signal.

The classical upshift experiment (Fig. 2) discovered the then-enigmatic "rate maintenance" phenomenon: cell divisions remain at pre-shift rate for ~65 min before abruptly soaring to the post-shift rate. This exciting observation was resolved by a series of experiments with the so-called "baby machine" [19] to yield the BCD Dogma (reviewed in Ref. [72]). This rate maintenance time roughly equals the period ($C + D$) thus resulting in a corresponding change of average cell size <*M*> (= total mass/cell number in a withdrawn sample). This understanding however does not answer the main question posed here about the *primary* signal(s) for cell division and width determination.

1.2. Cell dimensions under steady-state growth and during nutritional shift-up

The BCD Dogma, which explained the rate maintenance phenomenon (Fig. 2) and resolved the temporal aspects of the cell