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Signals of growth regulation in bacteria

Christopher S Hayes and David A Low

A fundamental characteristic of cells is their ability to regulate growth in response to changing environmental conditions. This review focuses on recent progress toward understanding the mechanisms by which bacterial growth is regulated. These phenomena include the 'viable but not culturable' (VBNC) state, in which bacterial growth becomes conditional, and 'persistence', which confers antibiotic resistance to a small fraction of bacteria in a population. Notably, at least one form of persistence appears to involve the generation of nongrowing phenotypic variants after transition through stationary phase. The possible roles of toxin–antitoxin modules in growth control are explored, as well as other mechanisms including contact-dependent growth inhibition, which regulates cellular metabolism and growth through binding to an outer membrane protein receptor.

Address
Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, CA 93106, United States

Corresponding author: Low, David A (low@lifesci.ucsb.edu)

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Introduction

This review focuses on recent advances in our understanding of bacterial growth regulation, with an emphasis on the mechanisms that control entry and exit from a slow growth or nongrowth (dormant) state, excluding spore formation. This topic has relevance to a number of important aspects of bacterial biology including resistance of a small fraction of a bacterial population to killing by an antibiotic, termed 'persistence'. The maintenance of bacterial viability without growth impacts human health in a number of ways including maintenance of pathogen reservoirs and chronic infections such as tuberculosis and melioidosis. This has been a difficult area of research, in part due to phenotypic variability in which only a small fraction of bacteria are within a dormant state in a population, making it hard to isolate and study dormant cells. Moreover, since many genes influence cell growth, it has been a challenge to identify those that constitute specific pathway(s) for dormancy/antibiotic resistance. Our aim in this review is to delineate some of the key findings and concepts in growth control, bringing together new developments in different fields of research that may impinge on one another.

The viable but not culturable (VBNC) state

Colwell and coworkers first reported that bacteria can fail to grow on laboratory media but still appear viable on the basis of outer membrane integrity and the ability to recover growth through temperature shifts [1]. This phenomenon, termed 'viable but not culturable' (VBNC), has now been described for over 50 bacterial species using various criteria for viability including propidium iodide exclusion, redox activity, and green fluorescent protein reporter expression, but of course none of these assays proves that cells are actually viable, only that they retain some function(s) of living cells. The word 'viable' indicates that under some condition the VBNC bacteria must be able to resuscitate and grow, and thus it has been pointed out that VBNC is a misnomer [2]. Despite these nomenclature problems, it appears that bacteria can become nonculturable under certain conditions while maintaining at least some metabolic activity (i.e. not dormant) but can be recovered under other conditions [3]. This poses a problem for water-borne pathogen monitoring in estuarine and marine environments (such as *Vibrio* spp.) as pointed out by Colwell and coworkers [1]. Although many papers have been published on the VBNC phenomenon, no specific mechanism has been identified. Indeed, because many environmental conditions induce the VBNC state in different bacterial species, it seems likely that there is no single underlying mechanism. A well-characterized VBNC system is in *Vibrio vulnificus*, in which prolonged incubation at 5°C leads to the VBNC state. Work from several groups suggests that hydrogen peroxide could play a role in VBNC because a fraction of VBNC cells grow on media containing antioxidants such as catalase. Although growth in the presence of catalase slowed the formation of VBNC cells, eventually virtually all cells became VBNC, indicating that sensitivity to oxidation is only one factor that limits growth [4]. Recent data from Abe *et al.* [5] support this hypothesis. Chemical mutagenesis of *V. vulnificus* yielded VBNC suppressor mutants that retain culturability after low temperature stress. One VBNC suppressor mutant expressed glutathione *S*-transferase to higher levels than wild type at low temperature and was found to be more resistant to hydrogen peroxide. However, this mutant almost certainly contains additional uncharacterized mutations, as indicated by its increased expression of CspA (cold-shock protein) homologs. In summary, the

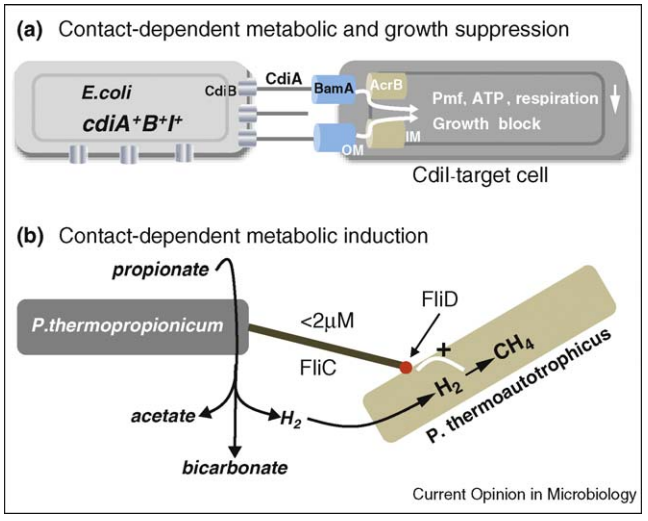
term VBNC likely encompasses several distinct mechanisms by which bacteria become unable to grow on certain media. Whether there is any commonality to bacterial VBNC mechanisms remains to be determined.

Contact-dependent growth inhibition

Recently a phenomenon called 'contact-dependent growth inhibition' or CDI was described, in which cell growth is controlled by direct cell-to-cell contact mediated by the CdiA–CdiB two-partner secretion (TPS) system. CdiA–CdiB is present in certain *Escherichia coli* strains, and homologous proteins are found in many bacterial species [6]. By homology with other TPS systems, CdiB appears to be an outer membrane protein required for the transport and assembly of CdiA at the cell surface. The CDI receptor has been identified as BamA [7], also known as YaeT, which is an essential, highly conserved outer membrane protein required for the biogenesis of beta-barrel proteins in Gram-negative bacteria [8]. Concomitant with a block in cell growth, CDI induces significant reductions in respiration, proton-motive force (pmf), and ATP levels [9]. The mechanism that connects interaction with BamA at the cell surface to downregulation of metabolism is unclear, but it may involve the inner membrane multidrug resistance protein AcrB since *acrB* mutants are resistant to CDI [7]. One possibility is that CdiA interacts with AcrB through BamA to modulate its activity. AcrB exploits the pmf to couple proton import to the export of small toxic molecules. Thus, CdiA could induce AcrB to open its proton channel and dissipate pmf. This speculative model for the CDI mechanism is outlined in Figure 1A.

Another important aspect of the CDI mechanism involves an open reading frame that overlaps with *cdiA* and encodes a small protein termed CdiI that confers immunity to CDI [9]. Expression of CdiI in target cells is sufficient for protection from CDI$^+$ inhibitor cells (Figure 1A). CdiI is also necessary to protect CDI$^+$ cells from inhibiting their own growth. By removing CdiI from the regulatory control of CdiBA, an autoinhibition system was developed in which CDI can be induced in a uniform population of cells. This system was used to demonstrate that CDI is a reversible process, at least under laboratory conditions. Autoinhibited cells resume growth within two hours after induction of the CdiI immunity protein. Recovery also requires an energy source, which could re-establish the proton gradient via the electron transport chain. The proton gradient increases in recovering cells before respiration and cell division, suggesting that it plays a critical role in mediating CDI. CDI shares features with the VBNC phenomenon in that CDI-inhibited cells exclude propidium iodide, and their growth can be induced under specific conditions [9]. This raises questions about the function of CDI systems, which appear to be widespread among Gram-negative species [9]. Because CDI-inhibited cells are metabolically downregulated, CDI could be a counter-surveillance mechanism, allowing bacteria to hide-out within host tissues or the environment. Alternatively, CDI could be used as a bacterial warfare system to inhibit growth of neighboring cells, for example in a biofilm.

Figure 1



Examples of contact-dependent alteration in metabolism and growth. **(A)** *Escherichia coli* containing the *cdiBAI* genes express CdiB, which is predicted to reside in the outer membrane and function in export of CdiA to the cell surface. CdiA is postulated to mediate binding to the BamA receptor in the outer membrane (OM) of target cells, and reduce the proton gradient, respiration, ATP, and cell growth/division of target cells. CdiI provides immunity to autoinhibition by cognate CdiA effector. The CDI mechanism may involve interaction with the multidrug efflux pump AcrB located in the inner membrane (IM) [6,7,9]. **(B)** *Pelotomaculum thermopropionicum* strain S1 expresses flagella that bind to its syntrophic partner *Methanothermobacter thermoautotrophicus strain ΔH*, keeping the cells close by tethering. Binding of the FliD cap protein of the flagellum to an unidentified receptor on *M. thermoautotrophicus* alters cellular metabolism including induction of methane formation. Hydrogen gas from *P. thermopropionicum* is used for methane production, and serving to keep H_2 levels low and enabling continued fermentation of propionate by *P. thermopropionicum* [12•].

Other growth control mechanisms

Other interesting phenomena involving control of cell growth and metabolism have been recently described. An evolved variant of *E. coli* K-12 was shown to inhibit its ancestral form in stationary phase, through a contact-mediated process called stationary contact-dependent growth inhibition or SCDI [10]. In eight independent cultures the *glgC* gene involved in glycogen metabolism was affected, with resulting overexpression of glycogen. In parallel with CDI, there is an immunity component to SCDI but it is the mutated form of *glgC*! Thus, glycogen overexpression appears to induce SCDI and provides immunity to SCDI, by an unknown mechanism. An 'identification of self' (*ids*) locus in *Proteus mirabilis* was recently described that regulates growth at boundaries