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Non-random distribution of macromolecules as driving forces for phenotypic variation

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Clonal populations employ many strategies of diversification to deal with constraints. All these strategies result in the generation of different phenotypes with diverse functions. Events like cell division are major sources of phenotypic variability due to the unequal partitioning of cellular components. In this review we concentrate on passive and active mechanisms cells employ to distribute macromolecules between their offspring. Different types of segregation are described, addressing both metabolically pertinent molecules such as PHA/PHB or polyphosphates, and components that adversely affect cells by promoting aging, such as damaged protein complexes or extrachromosomal rDNA circles. We also refer to mechanisms generating plasmid copy number (PCN) variation between cells in a population, and how elaborate partitioning systems counteract partitioning errors and ensure equal distribution. Finally, we demonstrate how simple differences in chromosomal copy number determine the fate of a cell, in this case the effect of gene dosage on the onset of sporulation in *Bacillus subtilis* or on a functional trait in *Sinorhizobium meliloti*.

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Introduction

In nature, bacteria participating in microbial communities are an essential driving force for biogeochemical cycles. A high phylogenetic diversity guarantees the functional stability of a community, with related traits often being shared by different phylotypes. Thus, stable microbial ecosystems are shaped that can more easily withstand external perturbations [1].

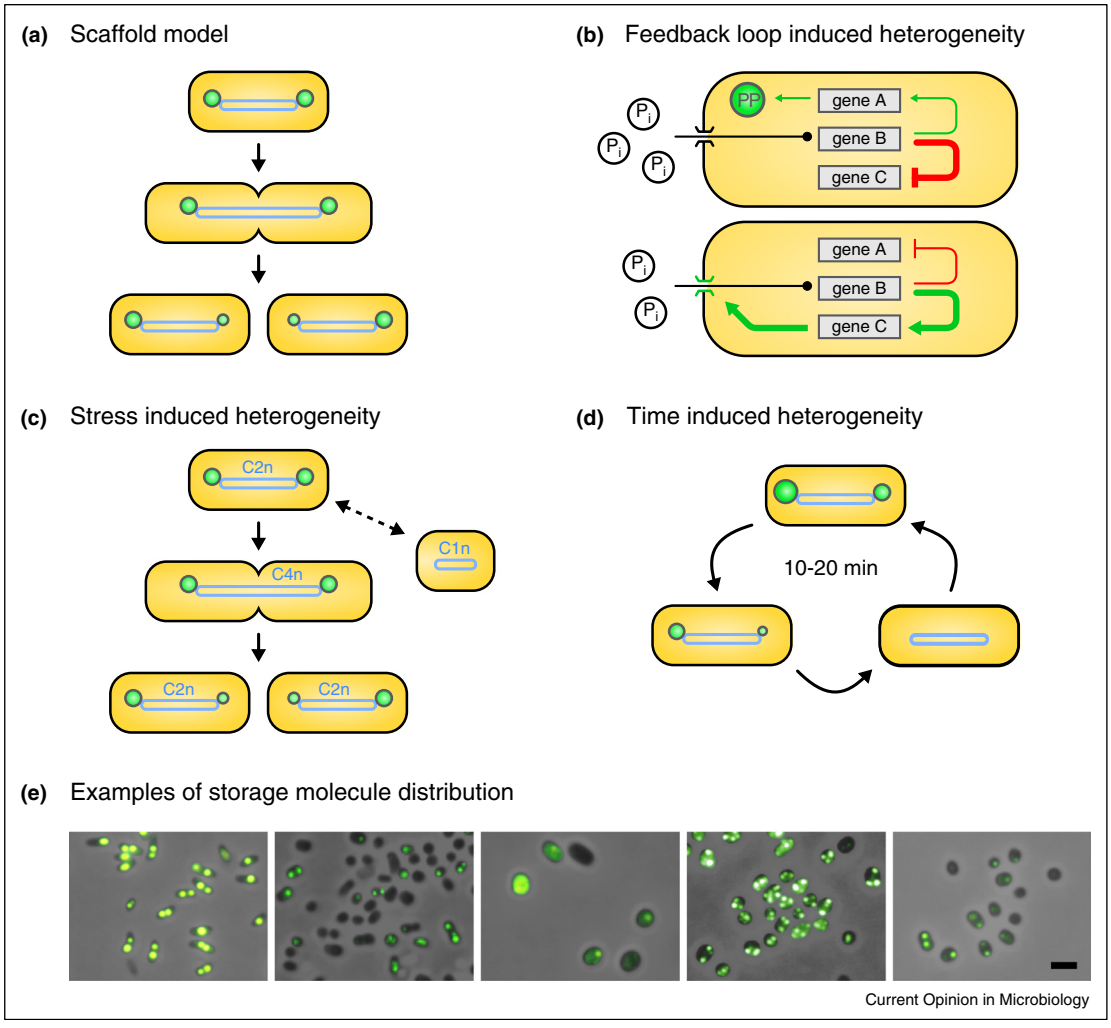
When applying this concept to clonal cultures under stress conditions, the range of options to choose from becomes much smaller. In principle, clonal populations have an identical genome and are therefore bound to create diversity in other ways. Mechanisms like epigenetic modification [2], differential gene expression [3–5], or cellular noise produce phenotypic variability [6]. In addition to these basic principles, processes connected to cell cycle and cell division [7•] contribute to phenotypic variation [8,9,10••]. Though recently the proteome composition was found to be nearly identical in replicating cells of different chromosomal copy numbers in *Pseudomonas putida* under balanced conditions [11], cell division processes seem to be a major source of phenotypic variation, for example, by producing daughter cells of different sizes or even function [12,13] or by an unequal distribution of cell components [14•]. Here we review mechanisms of unequal partitioning of macromolecules and how these generate heterogeneity, including components like cellular storage materials (e.g. PHA/PHB), cytoplasmic proteins in the context of aging, plasmids and even whole chromosomes.

Phenotypic variation due to segregated macromolecule distribution

Apart from DNA and proteins, many microbial cells synthesize macromolecules for the storage of energy or carbon. Formation and degradation of these macromolecules occur within minutes to hours. For polyphosphates, turnover rates of less than 12 min have been reported ([15], Figure 1d) and polyhydroxyalkanoate or polyhydroxybutyrate (PHA/PHB) granules were described to be produced within 10 min after transfer of cells to a fresh medium [16]. It is still unclear what compels individual cells to synthesize or degrade their storage compounds while others maintain granules, when all are kept under identical conditions. Yet, the presence or absence of a macromolecule can critically change the phenotype of a cell [17].

Prokaryotic cells are not compartmentalized and therefore synthesized macromolecules have for a long time been assumed to be stochastically distributed throughout their cytoplasm [18••]. Three models have been suggested for the distribution of PHA/PHB granules during cell division [18••]. The micelle model [19,20] describes a cytoplasmic PHB synthase starting on hydrophobic precursors, possibly resulting in a random distribution of granules during cell division. According to the budding model [20], granule formation at the cytoplasmic membrane results in a localized but random distribution of macromolecules within the cytoplasm. The scaffold model ([18••], Figure 1a) is currently the preferred model.

Figure 1



Macromolecules (in green) are either not (a) or seemingly stochastically distributed (b–d). **(a)** According to the scaffold model, discussed for PHA/PHB and polyphosphate granules, the macromolecules in the dividing mother cell bind to the nucleoid (in blue) via proteins or via yet unknown binding factors and are uniformly distributed to the daughter cells. **(b)** Transport regulation system induced heterogeneity. High external phosphate (P_i) concentrations lead to expression of low affinity phosphate transporters (gene A) and to the formation of polyphosphate granules. Under low external P_i concentrations polyphosphate stores are degraded and high affinity transporters (gene C) are expressed. Both transporter types are regulated by the PHO pathway (summarized as gene B). **(c)** Stress induced cell cycle related heterogeneity. Cells normally double their chromosomal content in the mother cells and then divide to form daughter cells with equal chromosome numbers. Under stress conditions, some cells leave the cell cycle and become dormant, a situation associated with low DNA and storage molecule concentrations. **(d)** (Observation-) Time induced heterogeneity. Short half-lives have been reported for polyphosphates ($\leq$12 min [15]) and PHB (formed within 5–20 min in previously PHB free cells [16]). **(e)** Microscopic pictures from left to right: *Pseudomonas putida*, *Acinetobacter calcoaceticus*, *Alcaligenes* sp., *Paracoccus* sp., and *Microlunatus phosphovorus* with tetracycline stained polyphosphate granules (bar 2 μm, staining procedure in [70]).

The synthase is suggested to be tethered to a scaffold molecule which is supposed to be the nucleoid as various phasins (PhaF in *P. putida* KT2442, PhaM in *Ralstonia eutropha* H16 [21,22]) have been shown to interact with DNA. Thus, population heterogeneity might be minimized by combining the macromolecule distribution with the nucleoid occlusion mechanism [23] during cell division (Figure 1). However, cell division events frequently result in an unequal distribution of basic cellular components [7•,12,24] and may also account for an unequal distribution of macromolecules [25]. Independent of basic cell division mechanisms, external constraints may induce intrapopulation variation by degradation of macromolecules in only a part of the individuals. Excessive and therefore toxic carbon concentrations have been shown to lead to the development of subpopulations with reduced DNA and depleted PHB contents in *Cupriavidus necator* ([26], Figure 1c). By contrast, very low carbon concentrations have been shown to result in asymmetric PHB granule distribution during cell division in the symbiotic