E. coli uses the growth arrest to reshape its proteome under starvation

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It is widely believed that, owing to the limitation of nutrients in natural environment, bacteria spend most of their life in a non-growing state. However, despite its major clinical and ecological implications, very little is known about what determines the phenotype of starved bacteria, in particular what controls the concentration of different gene products inside the cells. Using a newly designed microfluidic device in combination with fluorescence time-lapse microscopy and automated image analysis, we quantified growth and gene expression in many independent lineages of single *E. coli* cells as we switched them from exponential growth conditions to a carbon-free medium. We observed that single cells undergo a remarkably reproducible program in which growth arrests immediately in all cells, and no further elongation or cell death occurred in any cell over a period of two days. At the same time, gene expression dynamics, which we measure by quantifying volumic production of proteins across time in single cells, undergoes dramatic remodeling upon entry in stationary phase. Some promoters, including ribosomal protein promoters, immediately arrest gene expression, others show a slow exponential decay of expression on a 10 hour time scale, while a third subset exhibits a transient burst in activity before decaying exponentially. Moreover, these patterns are highly homogeneous, with only moderate variability in the time dynamics across single cells. Furthermore, control experiments show that protein decay rates are themselves also time-dependent, decreasing exponentially with time as well. Combining the observed time-dependent protein production and decay rates we show how protein concentrations deep into stationary phase are determined mainly by the expression dynamics in the first 10 hours of starvation. Finally, we have also shown that this expression program at the start of stationary phase is crucial for cell viability. In particular, using perturbation experiments in which gene expression is inhibited for different periods during stationary phase we show that the ability of cells to withstand stress deep into stationary phase depends strongly on the gene expression during the first 10 hours.

In summary, we are the first to comprehensively quantify gene expression dynamics of single bacterial cells during starvation, and uncover a highly reproducible expression program that is activated at the onset of starvation, dramatically remodels the proteome during the first 10 hours of stationary phase, and which is crucial for cell viability during starvation. These results provide a starting point for a quantitative study of cell maintenance and the emergence of specific phenotypes when nutrients become scarce.