Trends in Microbiology

CellPress

Opinion

Step by Step, Cell by Cell: Quantification of the Bacterial Cell Cycle

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The *Escherichia coli* cell cycle is a classic, but we are still missing some of its essential aspects. The reason is that our knowledge is mostly based on population data, and our grasp of the behavior of single cells is still very limited. Today, new dynamic single-cell data promise to overcome this barrier. Existing data from single cells have already led to findings and hypotheses that challenge standard views, and have raised new questions. Here, we review these recent developments and propose that a systematic exploration of the correlation patterns between 'cell-cycle intervals' defined by key molecular events measured in many single cells could lead to a quantitative characterization of the cell cycle in terms of inherent stochasticity and homeostatic controls.

Trends

The cell cycle is stochastic due to intrinsic cellular noise, affecting decision making related to key steps (i.e., initiation of replication, chromosome segregation, Z-ring contraction, and septation).

Recent high-throughput single-cell measurements of growing *E. coli* show a constant average added size between consecutive cell divisions.

Similar measurements allowing the full stochastic unraveling of the *E. coli* cell cycle will likely become available in the coming years.

These data will open new perspectives and challenge classic views, starting from the long-standing hypothesis that a critical mass per origin triggers replication initiation.

The Cell Cycle Is a Feature of Single Cells

Based on all the research on the cell cycle of *Escherichia coli*, we might think that we know a great deal about this problem. To some extent, this is true. The discovery of a key role of **replication initiation** (see Glossary) dates back to work from the late 1960s [1,2], and by the early 1990s most of the molecular players that we consider relevant today had been identified [3].

However, it is not difficult to convince ourselves that we are missing some essential aspects of the problem. The reason is that most of the information in our possession comes from bulk **population measurements** and indirect inference. In this Opinion piece, we argue that today it is the right moment to revisit the *E. coli* cell cycle exploiting dynamic **single-cell measurements**. Such experiments are challenging, as they require efficient imaging and cell segmentation-tracking methods, but are now possible at high throughput and with microfluidic control of nutrient exchange. Used in combination with reporters of molecular events and new theoretical models, they provide a unique opportunity to build a new basis for understanding the cell cycle.

This data will be more and more accessible in the coming years, but the recent literature leaves us with a wealth of conclusions and models that seem incompatible and incoherent. The main hypotheses that we put forward in this opinion article are that (i) this apparent incoherence can be overcome and is not due to intrinsic limitations of the approach, and (ii) it is a smoking gun for the need of more comprehensive and precise quantitative methods in both data analysis and theoretical descriptions. Such tools are necessary to properly handle the large amount of data and, most importantly, to formulate and test falsifiable models of cell-cycle control. This approach can be applied broadly, including eukaryotes [4–6].

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The Average Cell Is Not the Typical Single Cell

From the pioneers of quantitative bacterial physiology of the 'Copenhagen School' [7], empirical observations in terms of quantitative relations between physiology-related variables averaged over large populations have been used to infer specific control mechanisms of the cell cycle [7,8]. The problem is that the average cell behavior does not correspond necessarily to the typical behavior of single cells. Therefore, models based on population averages have limitations and ought to be revisited and tested with single-cell data. The classic example is the model proposed by Donachie [2] in the 1960s, stating that DNA replication is initiated at a critical mass per replication origin. As we will discuss, although appealing and describing properly the measured cell-cycle response to changes in growth media, this model may be insufficient to describe recent single-cell measurements. In other words, there are unique specific behaviors of single live cells that are obscured if we average everything.

For the cell cycle to progress, events related to DNA replication and **segregation**, metabolism, growth, and cell division must occur in a specific time order for each cell (across many divisions along lineages [9,10]), despite considerable molecular noise and variability of parameters. This hierarchy has a major impact on single cells, but may be missed when drawing data from population averages. For example, a missed **septation** due to late segregation may lead to failure of cell division, formation of a filamentous cell, and subsequent rescue, which can be accompanied by a nonsymmetric division, with consequences on the balance of cell size, DNA amounts, and cell-cycle regulators observable over several generations. However, these events typically affect a small fraction of the population and their consequences are not observable in population averages unless most of the population becomes filamentous. Therefore, dissecting such a cascade of errors and controls is impossible without a single-cell view, since the complex temporal interplay of several concurrent processes and the important role of **stochasticity** are hidden by population averages.

From Phenomenological to Mechanistic Models

A clear sign that the application of high-throughput single-cell techniques is effective comes from recent work. These measurements have already unveiled the stochastic nature of metabolism and resulting growth [11], intriguing universal properties of the joint distribution of cell size and interdivision times [12,13], as well as an effective principle where cells add, on average, a constant volume to their initial one every cell division (sometimes called the **adder mechanism**) [12,14–16], which is consistent with long-term **homeostasis** of the cell-size distribution in a population.

However, the molecular basis of this observation remains unknown. Indeed, several phenomenological models can in principle reproduce the empirical observation of a constant average added size. For example, a 'concerted' control of cell division based on cell size and on time [13,17–19] as well as a completely different mechanism based on the ratio between cell surface and volume [20] can both reproduce this behavior. Thus, the recent papers leave us with a rather obscure picture. Clearly, part of such differences may be due to sensitivity of measurements to differences in growth conditions, genetic background, experimental and analysis methods, and so on. However, we argue that insufficient integration of theoretical approaches and data also plays a large role, and the lack of a general theoretical framework to interpret the data (and compare and falsify models) also limits the experiments. Therefore, it is necessary to link more closely theoretical models to data on one side and to molecular mechanisms on the other.

Cell-Cycle Intervals

To move our mathematical descriptions towards more specific biological mechanisms, a first step is to focus the analysis on specific cell-cycle events that have been directly linked to molecular controls. Indeed, a common way to qualitatively describe the progression of the cell

Glossary

Adder mechanism: the hypothesized mechanism by which *E. coli* cells tend to add a constant volume or mass to the initial size to decide the moment of cell division. This mechanism enforces size homeostasis [12,15].
Cell-cycle interval: defined here as the period of time between two key events in the cell cycle (Figure 1). For example, three cell-cycle intervals are classically defined with respect to DNA replication: the B, C, and D periods, which are separated by replication initiation and the end of replication.
DnaA: ATP-ase protein that accumulates in its active ATP-bound form to a threshold value during the cell cycle inducing DNA melting by binding cooperatively to the origin(s) and thus triggering initiation of DNA replication [26].
Homeostasis: the process through which single cells control key variables (such as size and total protein concentration) in order to ensure their stability along lineages. There is, in general, a difference between homeostasis in fixed conditions and the average response to a perturbation.
Population measurements: measurements of average quantities over large cell populations. Most of growth-related laws in bacterial physiology are based on such measurements [8], typically for exponentially growing populations. For example, the typical population estimate of the average cell size consists in a measurement of optical density divided by a cell count [7].
Replication initiation: the start of DNA replication, defining the end of the B period in bacteria and corresponding to the G1/S transition in mammalian cells.
Segregation: the process of disentanglement and separation of duplicated chromosomes necessary to ensure a chromosome copy to each daughter cell.
Septation: formation of a cell wall that constricts the cell (approximately in the middle for symmetrically dividing bacteria like *E. coli*) and leads to new cell poles.
Single-cell measurements: experiments following dynamically many cells with single-cell resolution, monitoring size, shape, and fluorescent probes, and allowing to