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Summary

The first investigations of cell-to-cell heterogeneity in isogenic populations date back decades, with the early investigation of bi-modal β-galactosidase expression in batch cultures being one example. As a result of recent technological developments such as time-lapse microscopy, fluorescent labeling, and image-analysis tools, cell-to-cell heterogeneity has recently received an increased interest.

In this thesis we report the results from our investigation of two different processes. First, we examined the cell-to-cell heterogeneity of *Escherichia coli* cell cycle and DNA replication process. Second, we investigated the phase variation of *Escherichia coli* type 1 fimbrial expression.

In chapter 2 we report the experimental procedures that were developed to obtain the results presented in later chapters. To follow DNA replication within the cell cycle, SeqA protein was fused to fluorescent mCherry protein. SeqA is a DNA sequestering protein involved in negative regulation of DNA replication initiation by binding to nascent and hemimethylated DNA. *In vivo*, SeqA forms visible discrete foci when fluorescently labeled, and allows one to track the location of DNA replication forks. To follow the phase varying type 1 fimbrial expression dynamics, we place *gfpmut2* gene under the control of the chromosomal *fim* switch. Time-lapse microscopy combined with fluorescent labeling allowed us to follow the growth of single cells into microcolonies as well as their DNA replication and *fim* switching dynamics.

In chapter 3 we focus on cell-to-cell heterogeneity of the division cycle and on DNA replication process in individual slow growing *E.coli* cells. In *E.coli*, DNA replication starts at the origin of the replication site *oriC*, and finishes at the termination site *ter*. Traditionally, individual events within the division and DNA replication cycle are studied separately and correlations between the two processes are inferred from modelling. This approach lead to the concept of initiation mass, in which DNA replication initiation is proposed to occur at a constant critical mass per origin of replication. However, separate investigations do not allow one to correlate two or more events or quantities pertaining to a single cell cycle. The ability to follow DNA replication and cell growth simultaneously offers a unique opportunity to investigate such correlations directly.

The investigation of SeqA dynamics in slow growing cells has shown both the appearance and disappearance of SeqA foci within one cell cycle according to the following regular pattern in the number of foci: 0-1-2-1-0. This pattern is consistent with the absence of replication at the start and end of the cell cycle as

well as bidirectional movements of two replication forks in between. We concluded that the ratio of SeqA foci to the number of replication fork is 1:1.

We observed a strong anticorrelation between cell interdivision time and its elongation rate; a relationship that has not, to our knowledge, been previously reported. Interestingly, we find that the larger cells in the population predominantly exhibit smaller interdivision times, while the smaller cells predominantly exhibit larger interdivision times. Furthermore, there is only a small variance in cell size at the start of DNA replication. Together with the observation of large variation in the length of the B period (the period between cell birth and the start of replication) and a correlation between the B period and interdivision time, our results support the critical mass model. Finally, we observed that the C period is relatively invariant, as opposed to the very variable B period and D period (the period between the end of replication and cell division).

In chapter 4 we report the results of the first real-time study of type 1 fimbrial phase variation in *E. coli* at the single cell level. Phase variation of antigens and of other proteins expressed on the cell surface largely occurs in pathogens. Classically viewed as a mechanism to avoid host immune system, phase variation involves a heritable and reversible 'all-or-nothing' expression of one or multiple sets of genes. Switching between states is considered to be stochastic in nature, resulting in a mixed population of different states.

In *E.coli*, the expression of type-1 pili (fimbriae) is phase variable. The promotor for *fim* expression is positioned in the invertible 314 bps DNA fragment (*fimS)*. Upon inversion, the promoter sequence in *fimS* changes orientation, and hence acts as a reversible genetic switch. In the ON orientation, *fimS* drives the expression of multiple fimbrial structural genes. In the inverted OFF orientation *fim* genes are not expressed. The inversion of *fimS* is performed by two site-specific recombinases, FimB and FimE. While FimB is able to switch in both directions, FimE has an extreme bias to switch towards the OFF orientation. Bulk studies have produced a detailed understanding of the regulation of the *fim* system. However, this approach cannot capture the switching dynamics nor the correlation between switching events and other cell processes (such as DNA replication).

Bulk experimental methods on the *fim* system monitor the ratio of the *E.coli* population in different states to infer the occurrence of switching events. Here, we place GFP (Green Fluorescent Protein) under the control of the *fim* switch, and perfome timelapse phase contrast and fluorescence microscopy of growing microcolonies. We directly followed individual switching events and observed distinct GFP expression patterns following such an event among genealogically

related cell lineages. In addition to the expected monotonic increase in brightness, we also see lineages with transient brightness. By simultaneously monitoring the progress of replication, we show that these patterns arise from the inheritance of different *fim* states by daughter cells. Our data is consistent with multiple replication forks as described by the Cooper-Helmstetter model. Furthermore, we find a dependency of OFF to ON switching on cell age, with a higher probability of switching at the beginning of the cell cycle, suggesting a possible correlation between *fim* switching and chromosome replication.

In chapter 5 we explore how *fim* switching is influenced by the cell history. Early investigations on phase variation have assumed that phase variation is a purely random process that follows Poissonian statistics. However, this assumption has never been tested directly. Recent phase variation studies suggest various possible mechanisms that would lead to non-Poissonian switching behaviour. One example is the dependence of FimE expression on the state of *fimS*. Such regulation has been postulated to affect the OFF switching probability in time due to the time required for FimE to achieve steady state level.

We followed a single cell in a growing microcolony that just switched to the ON state and measured the time it remained ON. The data indicates that the OFF switching rate is constant over time and is independent of the switching history, thus verifying the Poissonian nature of *fim* switching.