On Chip Protein Dynamics in Single Bacteria Cells with Spatio-Temporal Resolution

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ABSTRACT

High resolution single cell microscopy on bacterial cells is a sophisticated task, mostly due to the small size which makes it difficult to gain insight into the subcellular processes. We designed a new technique for cell immobilization on a microfluidic chip which allows manipulation and imaging of single bacterial cells and collected time lapse fluorescence imaging (TLFI) data for monitoring the dynamics of proteins in individual cells and the cell cycle.

Exemplarely, we investigated the intracellular protein GcrA as well as the asymmetric localization of the protein DivK in daughter cells of *S.meliloti* over at least two cell division cycles.

KEYWORDS: Single Cell Analysis, TLFI, Spatio-Temporal Protein Dynamics

INTRODUCTION

The dynamics of biological processes at a single cell level is an important topic for systems nanobiology, where single cell processes are quantified and ensemble averaging effects can be excluded [1, 2]. Quantitative, statistical, and standardised *in situ* data are required for modelling approaches that allow a better understanding of such processes in space and time.

With typical length scales of only 0.5 to 3 microns it is a demanding task to handle bacterial cells in order to obtain spatial and temporal insight into subcellular processes allowing for example the tracking of the dynamic regulation of proteins during cell division. We demonstrate a novel microfluidic method to immobilize cells with a gradient coating technique. As a first application, we studied the regulation of bacterial cell division of the alpha-proteobacterium *S.meliloti*, a highly organized process that is precisely coordinated in space and time and which has not be adequately addressed to date, by collecting time lapse fluorescence imaging (TLFI) data.

Exemplarily, we present observations of the intracellular protein transcriptional master regulator GcrA as well as the asymmetric localization of the protein DivK, which is part of the phosphorelay system.

EXPERIMENTAL

Methodically, a high numerical aperture optical setup with a sensitive CCD camera and a microfluidic chip was used for cell imaging and handling. The microfluidic chip was fabricated in poly(dimethysiloxane) (PDMS) because of its biocompa-

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tibility and gas permeability, assuring a very good cell viability. For cell immobilization a poly(ethylenimine) concentration gradient was used for the first time known to the authors to prevent clogging of the channel entrance with bacteria while loading the chip. The observed proteins were genetically modified with the enhanced green fluorescence protein (EGFP) and the original vitally important proteins within the bacterium were replaced by this fusion constructs.

RESULTS AND DISCUSSION

The simple chip layout is shown in Figure 1. A lateral rotation of the bacteria is avoided because of the channel height of only 3 microns.

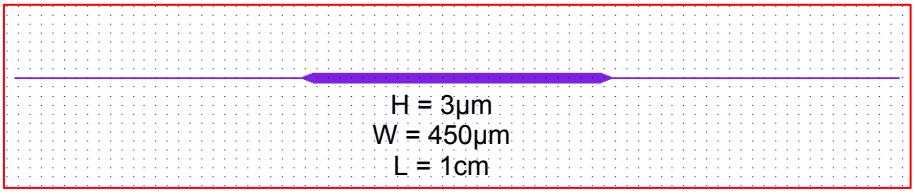

Figure 1: Microfluidic chip layout

Only cells dividing twice were evaluated. The fluorescence intensity corresponds to the amount of expressed protein at a certain point in time because of the full replacement of the original proteins by the fusion constructs. For the GcrA protein, which is homogeneously distributed over the whole cell, a varying fluorescence intensity is observed over the cell cycle.

For the DivK protein, which is responsible for flagellar synthesis, chemotaxis and septum formation, additionally the localization on one pole region is visible in brighter fluorescence intensity especially directly before cell division (Figure 2).

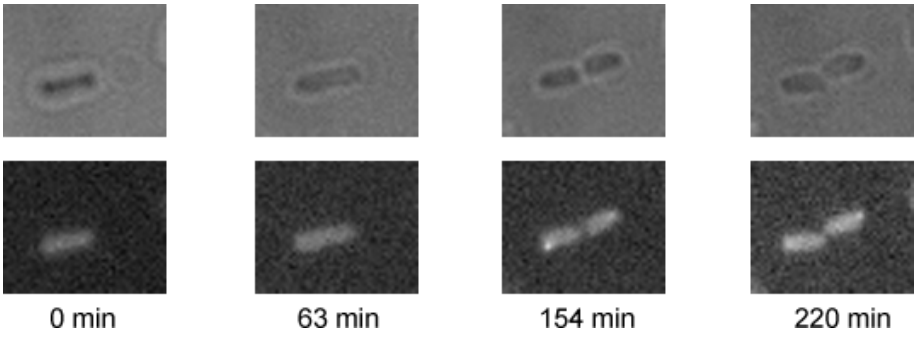

Figure 2: DivK oscillation with localisation at one pole (Rm2011 DivK-EGFP): bright field and fluorescence images over time.

In Figure 3 the mean fluorescence intensity of the GcrA as well as the max/mean intensity of the DivK, indicating the amount of protein asymmetrically localized on one pole, were plotted over time for all observed cells, resulting in the protein dynamics of a hypothetical cell.

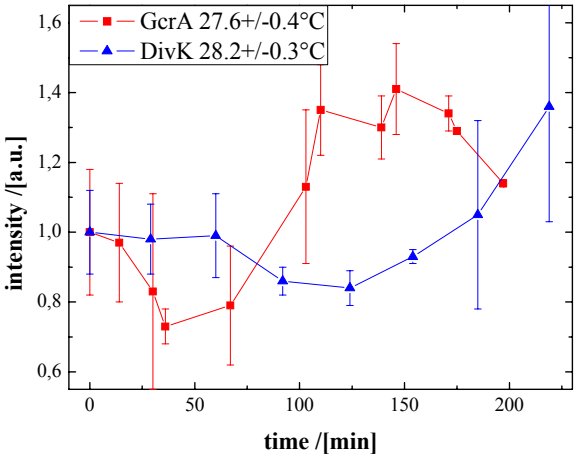

Figure 3: For the GcrA the mean fluorescence intensity and for the DivK the maximal intensity divided by the mean value (max/mean) integrated over the cell are presented for all cells showing at least two cell divisions.

CONCLUSIONS

We collected TLFI data for monitoring the dynamics of essential proteins involved in the control of the bacterial cell cycle in individual cells. The observed division time of about 200 min is close to the reported doubling time of 3 h in liquid cultures during the exponential growth phase [3] and demonstrates the exclusion of any harmful influence. These results are in good agreement with previously theoretical predicted data for *C. crescentus* [4], which was based on the investigation by conventional biochemical, genetic and cell biological assays [5]. Moreover, our straightforward method represents a competitive alternative in comparison to traditional techniques to determine expression patterns (e.g. cell synchronization and RT-PCR or PAGE and immuno- or mass spectrometric detection).

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