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Simultaneous measurement of sensor-protein dynamics and motility of a single cell by on-chip microcultivation system

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Abstract

Measurement of the correlation between sensor-protein expression, motility and environmental change is important for understanding the adaptation process of cells during their change of generation. We have developed a novel assay exploiting the on-chip cultivation system, which enabled us to observe the change of the localization of expressed sensor-protein and the motility for generations. Localization of the aspartate sensitive sensor protein at two poles in Escherichia coli decreased quickly after the aspartate was added into the cultivation medium. However, it took more than three generations for recovering the localization after the removal of aspartate from the medium. Moreover, the tumbling frequency was strongly related to the localization of the sensor protein in a cell. The results indicate that the change of the spatial localization of sensor protein, which was inherited for more than three generations, may contribute to cells, motility as the inheritable information.

Finding

Escherichia coli cells are able to respond to change in environmental chemo-effector concentrations through the reversal of their flagellar motors [1,2]. Attractants (such as aspartate and serine) promote counterclockwise rotation of the flagella, resulting in smooth swimming, whereas repellents (such as phenol and Ni) promote clockwise rotation, resulting in tumbling. These responses are mediated by membrane-bound, methyl-accepting chemoreceptor proteins (MCPs). Immunoelectron microscopy revealed that MCP-CheW-CheA complexes are clustered *in vivo*, predominantly at the cell poles [3], and merely weaker lateral clusters were observed [4,5]. It has been expected that the polar-localization changes according to environmental conditions, whereas there is no evidence showing the dynamics of localization-change have been reported. Conventional group-based experiments do not allow measuring the process of MCPs clustering and its change in consecutive generations in individual cells, which is essential to estimate the change occurring during the alternation of generations. In order to understand epigenetic processes such as adaptation and selection, both the protein-dynamics and the cell-dynamics of particular single cells should be observed continuously for generations.

Previously, we have developed the on-chip culture system exploiting the microfabrication technique and optical trapping [6-8]. The system enabled us to keep the condition around the cells constant by exchanging the fresh medium continuously and by controlling the cell number with optical trapping. Individual cells swimming in microchambers were observed with a spatial resolution of 0.2 μm by phase-contrast/fluorescence microscopy.

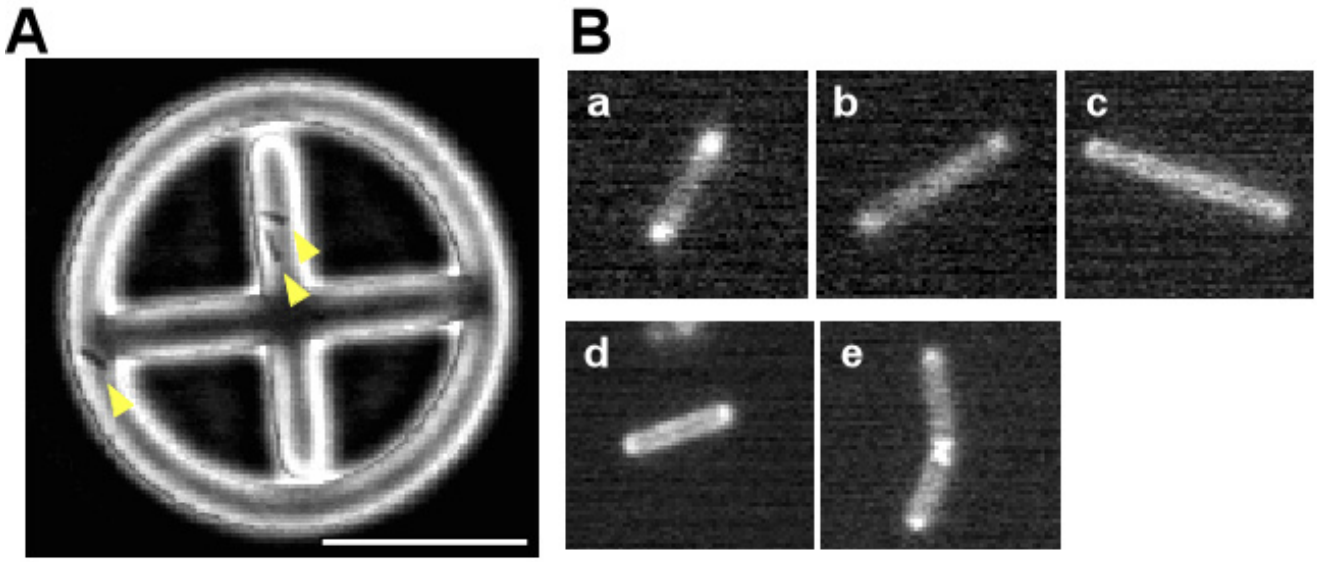

Figure 1
(A) Optical micrograph of the microchamber. Yellow arrows point to an isolated *E. coli* in the microchamber. The bar indicates a length of 20 μm. (B) The magnified micrographs show sensor-protein dynamics of isolated single *E. coli* at times of (a) 0 min, (b) 180 min, (c) 225 min, (d) 315 min, and (e) 435 min after the inoculation.

To measure the localization-dynamics of expressed proteins and the motility of single cells simultaneously, we used the on-chip microcultivation system and assayed intracellular proteins tagged with green fluorescent protein (GFP). In this paper, we report the time course of the motility and the localization of the aspartate receptor protein (Tar) for several cell generations. We also report cellular responses caused by the addition and removal of the aspartate during cultivation.

Figure 1A shows the top-view of the wheel-shaped-microchamber used in this experiment. First, a single bacterium was placed in a microchamber and was isolated in the wheel region to swim along the track by sealing of semi-permiable membrane lid onto the microchamber. Then, the bacterium running around the circle structure was observed continuously by measuring the tumbling frequency and the protein-localization dynamics. When the cell divided into two daughter cells, one of these two daughter cells was picked up by optical tweezers, transported to the axle area and was held in this region with optical tweezers continuously to stop it growing. The microchambers were etched into a 0.2-mm-thick biotin-coated glass slide, and were covered with a semi-permeable avidin-coated cellulose membrane lid (pore size: molecular weight ~25,000) to separate the microchambers from the medium-buffer-exchange region. The cultivation buffer in the microchamber was continuously refreshed by diffusion through the membrane (refresh rate – approximately one minute) to maintain the homogeneous medium environment. A bacterium was gave chemical stimulation by changing contents of medium supplied from the medium-buffer-exchange region.

We measured tumbling frequency and Tar-localization ratio of isolated *E. coli* in M9 minimal medium containing non-attractor amino acid (Asn, Arg, Cys, Ile, Gln, Thr, Thy, Trp, Val, His, Phe, Pro, Met, Lys, and Leu) at 1 mM and 0.2% glucose at 30°C (Fig. 1B and Fig. 2). To visualize Tar protein in living *E. coli* cells, Tar was tagged by green fluorescence protein in AW539 strain [9]. The Tar-localization ratio is estimated by measuring the ratio of the cytoplasm fluorescence intensity and the polar fluorescence intensities.

When the cultivation started, the Tar-localization ratio (red square) was 2.5 and the tumbling-frequency (blue circle) was 0.5 (s-1) (Fig. 1B-a and Fig. 2a). After the 2nd cell division was occurred, minimal medium containing 1 mM aspartate was given to the 3rd generation of cell (135 min after microcultivation). After adding the attractant, tumbling-frequency was decreased immediately compared to the previous generation. Localization of the aspartate sensitive sensor protein at two poles in *Escherichia coli* also decreased quickly by half 45 min following medium change (Fig. 1B-b and Fig. 2b). Finally,

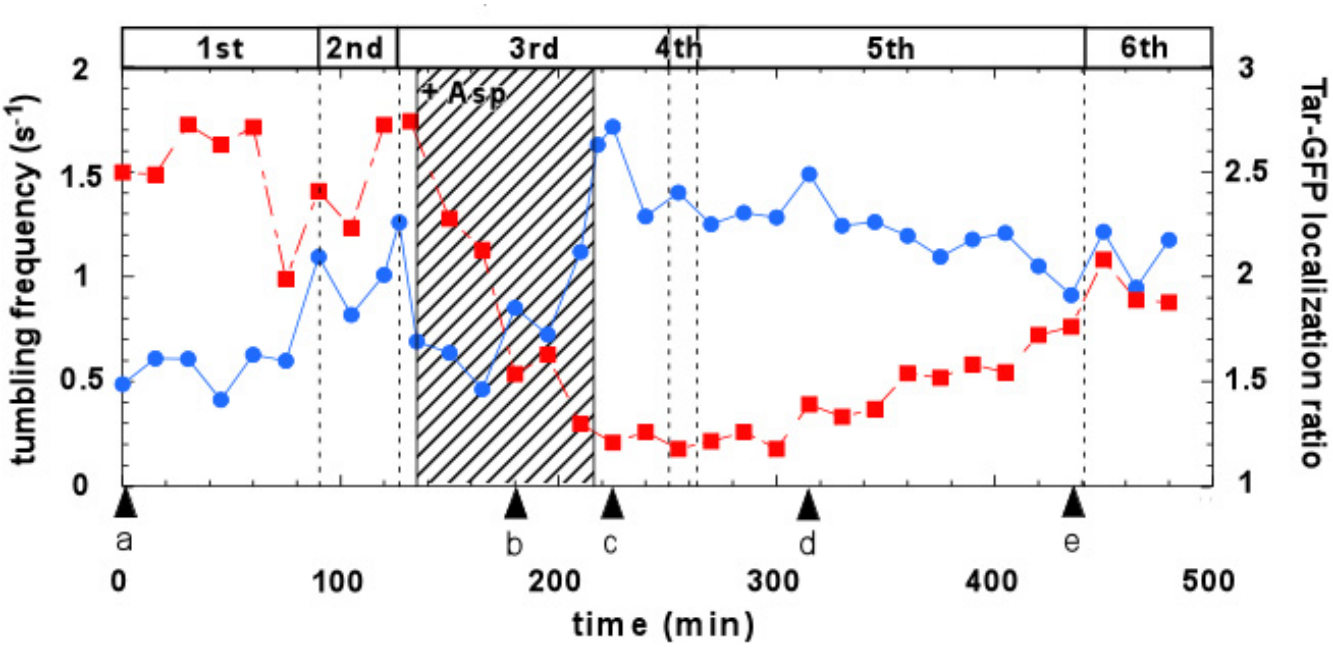

Figure 2
Time course of tumbling frequency (blue circles) and Tar-localization dynamics (red squares) with chemical stimulation. Hatched pattern shows the period when bacterium was stimulated by aspartate. Dashed lines show bacterial division points.

after 80 min of stimulation by aspartate, localized Tar was diffused completely. Then, the aspartate was removed from the cultivation medium and cells were cultivated further to measure the recovery of Tar-locarization dynamics (Fig. 1B-c and Fig. 2c). After the first medium exchange, it took more than three generations to recover original pattern of Tar localization (Fig. 1B-d,1B-f, and Fig. 2d,2f). However, the frequency of tumbling remained higher than the former generations. This may indicate that the formation of Tar-localization requires more time than its diffusion. Such an asymmetric reversibility of protein localization may contribute to the inheritance of the cells' phenomenon caused by environmental change. It also suggests a possibility that change of Tar localization can be inherited by descendant cells and this can affect their motility and therefore their phenotype.

In conclusion, we reported the following two topics:

• A novel assay for observing the protein-localization dynamics and the motility for generations was developed.

• The decreasing and recovery of the Tar-localization in a living bacterium was monitored under environmental change.

This assay can potentially be used for measuring cell-dynamics and inheritance of those information from generation to generation with focusing each individual cell caused by the environmental change.

Authors' contributions

II carried out the microchamber design, cell preparation, single cell observation, image analysis and drafted the manuscript. DS and IK prepared Tar-GFP vector and discussed this study. KY conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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