How to apply FLUCS in single cells and living embryos

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Method Article

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Abstract

This protocol is associated to the publication "Non-invasive perturbations of intracellular flow reveal physical principles of cell organization" by Mittasch et al., published in Nature Cell Biology. In the following, we describe the procedure for maintenance and preparation of C. elegans zygotes for imaging and application of FLUCS. Preparation of S. cerevisiae cells for microscopy and intracellular flow perturbations. S. cerevisiae cells were grown into logarithmic stage. Subsequently the S. cerevisiae cells were either grown for 2 hours or alternatively energy depleted through addition of 2-Desoxy-D-Glucose \(inhibition of glycolysis) and antimycin A \(inhibition of mitochondrial ATP production) for 2 h prior to flow induction. This treatment causes a more than 95% reduction in cellular ATP 2. Potentially this protocol is suitable to energy deplete all S. cerevisiae strains and closely related yeast species. A specific marker such as µNS-GFP1is needed to observe subsequent flow perturbations by microscopy.

Introduction

In the following, we describe the procedure for maintenance and preparation of C. elegans zygotes for imaging and application of FLUCS.

Reagents

\[A] C. elegans M9 buffer: 22 mM KH2PO4, 42 mM Na2HPO4, 86 mM NaCl, 1 mM MgSO4 15 -25 µm diameter beads \(Polybead, Polysciences, Germany) standard transparent nail polish We used the following transgenic C. elegans strains: SWG007 \(lifeact::mKate2; GFP::NMY-2, genotype: nmy-2\(cp8 \[myo-2::GFP unc-119+]) I; gesIs001 \[Pmex-5::Lifeact::mKate::nmy-2UTR, unc-119+]), TH120 \(PAR-2::GFP, PAR-6::mCherry, genotype: unc-119\(ed3) III; ddIs25\[pie-1::GFP::par-2\[RNAi res. SacI/MluI])b + unc-119]; ddIs26\[mCherry::T26E3.3 \(par-6) + unc-119\(+)]), TH306 \(NMY-2::GFP, PAR-2::mCherry, genotype: unc-119\(ed3) III; zuIs45\[nmy-2::NMY-2::GFP + unc-119\(+)] V; ddIs31\[pie-1::mCherry::par-2 + unc-119\(+)], LP675 \(PAR-2::mNeonGreen, PAR-6::mKate2, genotype: par-6\(cp60\[par-6::mKate2::3xMyc + LoxP unc-119\(+) LoxP]) I; par-2\(cp329\[mNG^PAR-2]) III \[B] Yeast AntimycinA \(Sigma-Aldrich - A8674), 2-Desoxy-D-Glucose \(ROTH – CN96.3), Synthetic defined \(SD)-medium \()/synthetic complete \(SC)-medium \(Formedium), glucose, concanavalin A \(SIGMA-ALDRICH Chemie GmbH - C7275-250MG) Cell line: W303 ADE+ pAG415GPD-EGFP-µNS1 \[W303 MATα can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 pAG415GPD-EGFP-µNS]

Equipment

FLUCS setup

Procedure

\[A] C. elegans 1. Transgenic C. elegans strains were maintained at 18◦C and imaged at room temperature. Prior to imaging, C. elegans was transferred to 24◦C for 12-16 h. 2. To obtain embryos, adult worms were dissected in 6 µl of M9 buffer mixed with diluted spacer of typically 15 µm diameter beads, on a standard 18 X 18 µm^2 cover slip. 3. This cover slip was successively placed on a high thermal conductive sapphire cover slide \(SMS-7521, UQG Optics, UK), sealed by standard transparent nail polish and placed on the microscope for imaging. 4. To reproducibly induce flows in embryos using FLUCS, we focused into the center of the embryo. This was performed by focusing on the surface of the coverslip and on the surface of the sapphire slide and then setting the z-position of microscope to exactly half the distance i.e. half the chamber height. 5. To induce a specific flow field i.e. a line profile or a circular flow, we used a custom-written LabVIEW software that superimposes the scan path of the infrared laser with the high-resolution image of the camera. A specific line profile is drawn manually onto camera image, and the laser parameters are set. Typical laser parameters were: scan frequency of 2 kHz, a step size of the laser beam in the focal plane of about 1 µm, and a minimum scan length of about 30 µm. 6. While flow induction, we globally cooled the entire flow chamber using Peltier elements typically -3 K to around 19˚C. 7. While flow induction, we simultaneously imaged the embryo in an alternating fashion from bright-field to fluorescent i.e. GFP or mCherry. 8. Flow profiles and cortical velocities were analysed in a post-processing step \(see methods of manuscript) \[B] Yeast 1. Inoculate approximately 1/4 of a yeast colony into 50 ml SC-medium + 2 % glucose \(if no plasmid based selection is used) or SD-medium \(with the dropout that is needed for plasmid-selection) 2. Grow yeast cells at 25°C, 180 rpm shaking for ~18 h \(till an optical density at 600 nm of 0.2-0.4 is reached) Energy depletion: 3. 2 X washing: centrifuge at 1940 G for 4 min and resuspend in 50 ml SC-medium without glucose 4. Finally: centrifuge at 1940 G for 4 min and resuspend in 50 ml SC-medium without glucose + 20 mM 2-Desoxy-D-Glucose + 10 mM antimycin A 5. Incubate yeast cells at 25°C, 180 rpm shaking for 2 h 6. Centrifuge at 1940 G for 4 min and resuspend in 1.5 ml SC-medium without glucose + 20 mM 2-Desoxy-D-Glucose + 10 mM antimycin A Attachment of yeast cells to cover glass for microscopy: 7. Preparation of Concanavalin A solution: Mix 2.2ml 1x PBS, 125µl 0.2M K2HPO4 pH=6, 2.5µl 1M CaCl2 , 5µl 10% NaN3, 250µl 5mg/ml Concanavalin A 8. Add 1 µl of Concanavalin A solution to a cover glass and spread with a pipette tip. Wait till dry 9. Add 2.0 µl of yeast cells on a standard 18 X 18 µm^2 cover slip, leading to thin chamber of about 5-7 µm, reducing unnecessary heating. 10. This cover slip was successively placed on a high thermal conductive sapphire cover slide \(SMS-7521, UQG Optics, UK) and placed on the microscope for imaging. 11. To reproducibly induce oscillatory flows in yeast cell using FLUCS, we focused onto the fluorescently-labelled µNS particles. 12. To induce oscillatory flows, we used following laser parameters: scan frequency of 2 kHz, a step size of the laser beam in the focal plane of about 1 µm, and a scan length of 30 µm. 13. To obtain a given oscillation frequency the laser is scanned N times in one-direction and then N times in the other direction. To obtain an oscillation frequency of 0.5 Hz, N=2000 repetitions are required, for 6.25 N=160, and for 50 Hz N=20. 14. While flow induction, we simultaneously imaged the µNS particle trajectory fluorescently in the GFP channel, with 8 frames per full oscillation. 15. µNS particle trajectories were analysed as described in the methods section of the manuscript.

Timing

Building a FLUCS setup might require 1 month with calibration, for this please contact us and we can give you a full list of parts that you need to order. Mounting an embryo and performing FLUCS is quick, typically this doesn't take longer than 30 s.

Troubleshooting

First of all make sure that your FLUCS setup operates after assembly. For this immerse fluorescent tracer beads in a viscous solution such as glycerol and see if you can induce hydrodynamic flows. If this works reliably you might want to induce flows in embryos. If you see visible modifications of the tissue during FLUCS application, you might denature the specimen. A potential reason for this might be that it is getting to hot, which is often due to a to thick chamber. Therefore, make sure that your chamber is not thicker then typically 20 µm.

Anticipated Results

By using FLUCS you might be able to perform intracellular flow experiments. This will allow you to move proteins in the cytoplasm, or in the cortex. Moreover, you will be able to measure material properties of the cytoplasm locally and dynamically

References

1. Munder, M. C. et al. A pH-driven transition of the cytoplasm from a fluid- to a solid- like state promotes entry into dormancy. eLife Sciences 5, e09347 \(2016). 2. Serrano, R. Energy requirements for maltose transport in yeast. Eur. J. Biochem. 80, 97–102 \(1977).