UNRAVELING MECHANO-STRESS RESPONSIVE SIGNALING NETWORKS IN BUDDING YEAST VIA MICROFLUIDIC DEVICES

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ABSTRACT

We present a microfluidic platform for in-situ monitoring of single cell mechanotransduction. Despite its fundamental importance, progress on mechano-sensing signaling study has been slow mainly due to technical limitations to specifically activate these pathways and monitor the intracellular dynamics. We applied mechanical stretch to budding yeast cell membrane by 1) pressing and releasing yeast cells using soft elastic polymer (PDMS) and 2) hypo-osmotic pressure. By utilizing these microfluidic approaches, we monitor in-situ single cell mechanotransduction. (e.g. intracellular response the rapid (less than minute) translocation of Crz1 into the nucleus, the Ca^{2+} associated transcription factor). We expect the established platform coupled to single cell analysis will allow us to quantitatively test existing biological models and to reveal new insights into this fundamental but poorly explored mechanotranduction.

KEYWORDS

Microfluidics, Mechano-stress, Signaling pathway

INTRODUCTION

Cells living in natural condition are exposed to many stresses. When these stresses occur, cells sense their extra-and intracellular environment and respond by activating their complex intracellular signaling networks to continuously adapt to the changing situation. Among the stress conditions, mechano-stress has long been recognized as an important physiological response. Indeed, mechano-sensitive feedback mechanisms modulate cellular functions as diverse as migration, proliferation, differentiation and apoptosis, and are crucial for organ development and homeostasis [1]. However, despite its fundamental importance, progress on mechano-sensing signaling has been slow mainly due to technical limitations to specifically activate these pathways.

Here, we utilize two distinct microfluidic platforms to focus on intracellular response upon mechanical stress acting on the cell wall. Despite their different approach to stimulate cells, similar out coming results suggest that the device can be recognized as an alternative tool to clarify certain signaling pathway system.

EXPERIMENT

In yeast, cells are thought to translate physical stimuli (e.g. cell shrinkage/expansion and external force) into various paths of intra-cellular biochemical signals. Here, we demonstrate the result such as changes in intracellular concentration of calcium and translocation of fluorescently tagged proteins.

Hypotonic stress with media switch

To trigger the signaling pathway system in interest, firstly we approached with microfluidic chamber to deform cell membrane by hypoosmotic stress via instantly switching the concentration of media from 1M sorbitol to 0M sorbitol with relevant nutrients. Chamber has 3-5 μm heights, relatively low as yeast can be initially trapped. The trapped cells are not washed away during the medium switching.

While the expansion of cell membrane according to the hypo-osmotic condition by media switch, stretch-activated ion channel flows in calcium, which works as a second messenger and activates the phosphatase calcineurin. This in turn dephosphorylates the transcription factor Crz1 and thereby promotes its rapid translocation into the nucleus (Figure 1A) [2]. Indeed, in microfluidic chamber, cells are rapidly expanded upon hypotonic stress and express a Ca^{2+}-sensitive FRET-sensor [3]. This expression is confirming that the rapid and transient increase of

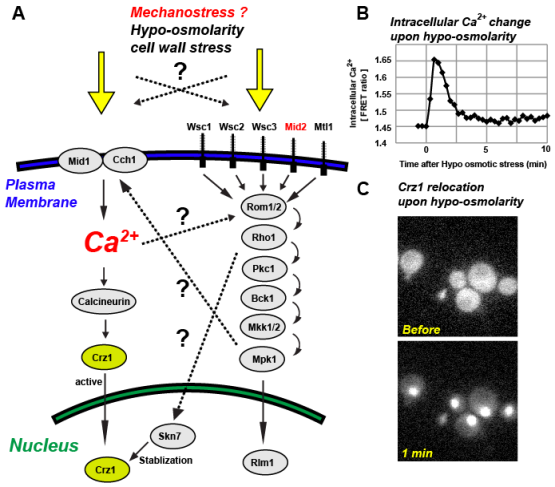

Figure 1: (A) Schematic illustration of the yeast signaling networks relevant for sensing mechano-stress. Arrows indicate activation. Dashed arrows with question marks depict cross-talk by unknown mechanisms. (B) Change of intracellular calcium (Ca2+) concentration upon hypo-osmolality (from 1M to 0M). The concentration was quantified using a FRET -based reporter (C) Nuclear translocation of Crz1-GFP before and 1 minute after applying hypo-osmolality stress conditions was visualized by GFP-microscopy in a microfluidic device.

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calcium inside the cytoplasm (Figure 1B). At the same time, GFP-tagged Crz1 is recognized to be accumulated inside nuclei suggesting the signaling pathway is activated (Figure 1C).

Cell wall deformation occurs with squeezing chip

The approach of hypotonic stress to cells can simply trigger the pathway; however, cells can adapt the change of osmorality and quickly return to their original size. As another platform, we developed innovative microfluidic device that are able to mechanically deform cell membrane in a controlled and reproducible manner. Cells are delivered underneath of PDMS micro-patch array. The PDMS micro-patch expands / recovers by on/off of air pressure (Figure 2A and B). The magnitude and duration of cell membrane stress are controlled by digital air compressor connected with solenoid valve.

We compared the cells geometries in 3D before and after the mechano squeezing and confirmed they expand in horizontal and contract in vertical (Figure 3). The magnitude of the applied pressure controls the degree of cell membrane stretch (the section area in Figure 3), raising the possibility to use such devices for controlled mechano-sensing experiments.

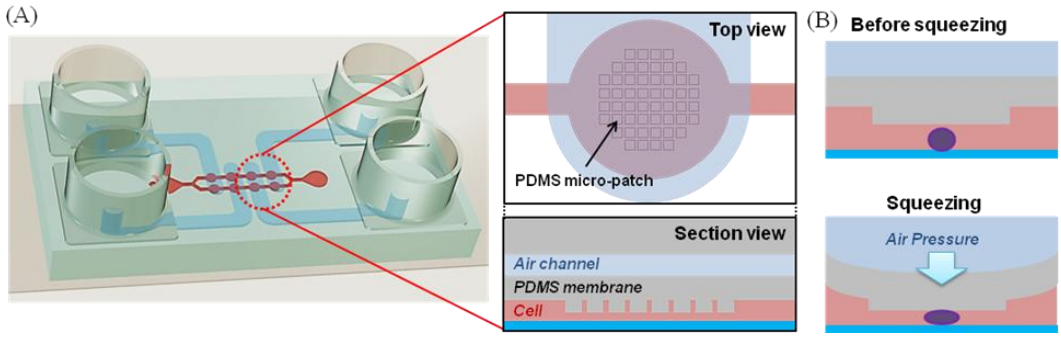

Figure 2: Schematic of the device to deform cell membrane. (a) Double layered microfluidic device with micro-patch on a PDMS membrane. (Patch size generally about 100 μm X 100 μm). (b) Operation of the device; cells are squeezed while air pressure is loaded to the air channel.

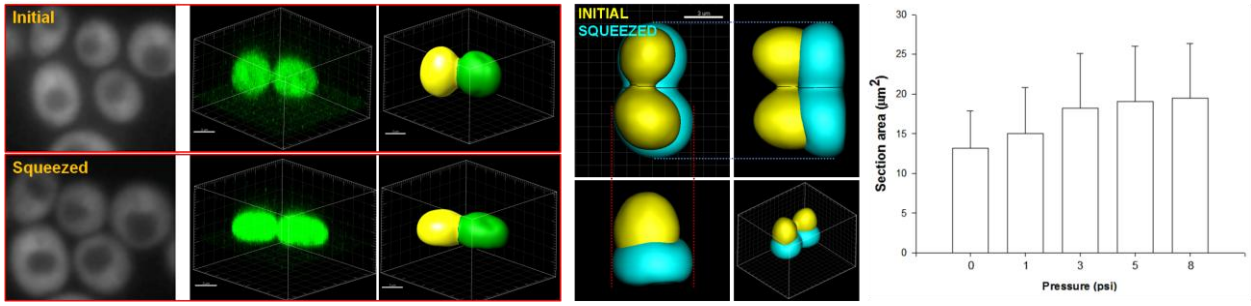

Figure 3: Cell deformation is compared with initial state and when squeezed according to the device.

RESULTS AND DISCUSSION

We observe the rapid relocation of GFP-tagged Crz1 in yeast cell under hypotonic stress, and mechanical expansion by PDMS squeezing chip. Before the stimulation, Crz1 is distributed evenly inside the cytosol. When the stress occurs and triggers the proper sensors, the Crz1 rapidly relocates to the nucleus which can be detected with fluorescent microscope. The dynamics are quantified normalized intensity in nucleus (Figure 4). Hypotonic stress made Crz1 relocation within one minute as well as mechanical expansion by PDMS does. After their highest peak, the one with hypotonic stress gradually return to their initial state during 10 minutes. At the same time, while cells are continuously squeezed during 7 minutes, it took half fold of time for Crz1 to disperse compared to hypotonic stress. Also, right after the release of the squeezing chip, another small peak was instantly observed. This indicates calcium signaling may trigger during recovery process but the molecular mechanism needs to be identified..

Also, we are able to active a general stress response

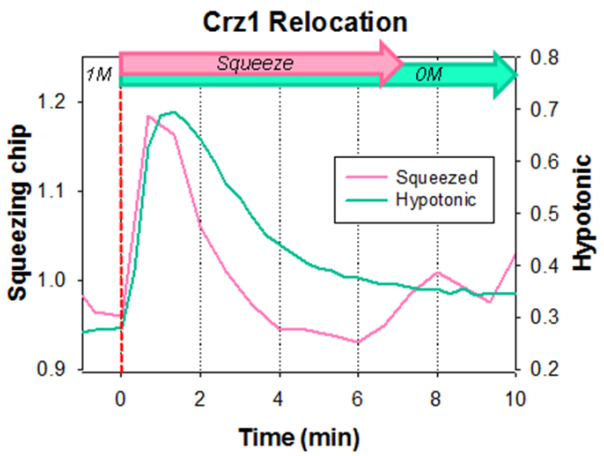

Figure 4: Crz1 relocation to nucleus upon mechano-stress: hypotonic stress and squeezed by the device; the results from two different method show similar tendency of relocation behavior.

factor Msn2 which refers to the nuclear accumulation. Importantly, when the squeezing chip was released, the accumulated Msn2 was dispersed to cytoplasm. We found mechano-stress may activate the general stress response factor Msn2 by a mechanism that involves, at least in part, the stress sensor; Mid2 (Figure 5A). GFP-tagged Msn2 were observed with and without the stress sensor Mid2. While the wild type yeast showed twice-stronger maximum accumulation of Msn2., compared to the cells without Mid2 gene. In the case of single cell, unlike stress given with the hypo-osmotic condition, the peak was observed several times while it was forced to be squeezed. This interesting result can be explained for now that the cell continuously activates their complex co-related pathways to make themselves into initial shape which they cannot (Figure 5 B).

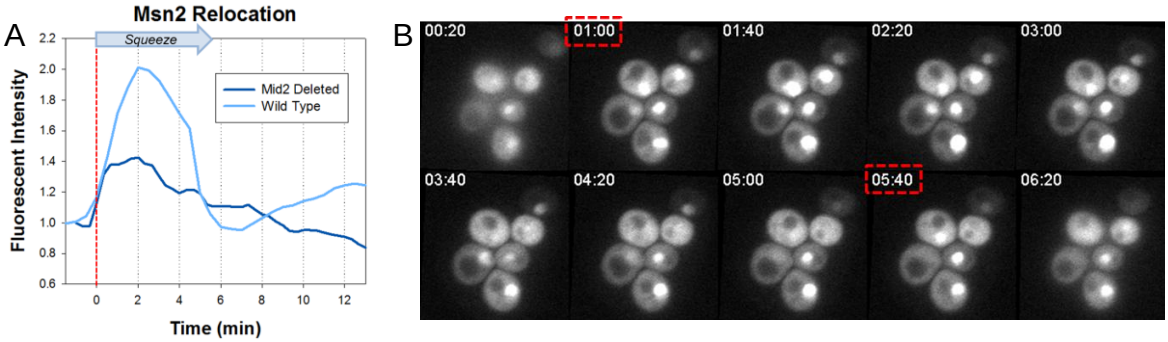

Figure 5: (a) Msn2 relocation to nuclei; wild type yeast is two times higher than the one without mid2 gene. (b) Image of Msn2 relocation in time-course.

CONCLUSION

Taken together, we demonstrate microfluidic-based quantitative assay platform for unraveling mechano-stress responsive signaling networks. Establishing an experimental platform based on microfluidic devices coupled to single cell analysis will allow us to quantitatively test existing biological models, and provide the experimental basis to start mathematical modeling of such dynamic processes for systems biology and to reveal new insights into this fundamental but poorly explored signaling pathway.

Table 1. Comparison of two approaches

	Hypotonic stress	**Squeezing chip**
Trigger Source	Chemical difference	Physical difference
Mechanical Stress	Transiently induced	Controllable stress duration
Common Pro	Able to monitor a dynamic response by live cell imaging	
Common Con	Difficulty in harvest of cells after stress for further biochemical assays	

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