In vivo imaging of Ras protein's activity in olfactory neurons

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Method Article

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Abstract

Signal transduction pathways play essential roles to adapt environmental changes and composed of various signaling proteins. Therefore, observation of activity of a specific protein in vivo is important for understanding the signal transduction pathways. However temporal dynamics of signaling proteins in living animals are not well known. Here we tried to monitor the activities of Ras protein which is one of the key regulators for the Ras-MAPK signal transduction pathway. It was previously reported that the Ras-MAPK pathway functions in olfactory neurons in Caenorhabditis elegans. Thus, we observed Ras activity in olfactory neuron in living animals using Raichu-Ras, a FRET-based sensor for Ras activity. We succeeded in detecting quick changes of Ras activity in response to odor stimuli.

Introduction

Signal transduction cascades play essential roles for various organisms to adapt environmental changes and consist of various signaling molecules. Therefore, to analyze mechanisms of signal transduction pathways, it is important to observe dynamics of signaling molecules _in vivo_. For example, intracellular ion level has been observed using _in vivo_ imaging analyses such as calcium imaging[1]. However, signal transduction pathways are also composed of various signaling proteins. Signaling proteins alter their status in response to continuous environmental stimuli. Even though observation of specific protein activity _in vivo_ is crucial for understanding the signal transduction pathways, temporal dynamics of signaling proteins in living animals are largely unknown. To answer this question, we tried to monitor the activities of Ras protein which is one of the key regulators for cell growth, differentiation, olfactory reception and other biological processes[2-4]. Thus, we observed Ras activity in olfactory neuron in a living nematode worm _Caenorhabditis elegans_[5]. We used Raichu-Ras, a FRET-based sensor for Ras activity6. Raichu-Ras was expressed in AWC olfactory neurons in _C. elegans_ and we monitored Ras protein activity in vivo after stimulation of isoamyl alcohol which is an odorant sensed by the AWC neurons. Here we describe a detailed protocol for imaging of specific protein's activity in intact animals. _In vivo_ imaging of protein activity may be more difficult than calcium imaging since its dynamics of activity change is weaker than that of intracellular ion level in many cases. Therefore it is important to restrain animal's movement by gluing the animal on the agarose pad to minimize noise of fluorescent changes from the animal motion.

Reagents

Imaging buffer 80 mM NaCl 20 mM D-glucose 10 mM Hepes 5 mM KCl 5 mM $MgCl_2$ 1 mM $CaCl_2$ Agarose pad 4% Bacto agar \(diluted in imaging buffer) Odorant Isoamyl alcohol The Odorant was diluted in ethanol and imaging buffer \(odorant : ethanol : imaging buffer = 1 : 5 : 4).

Equipment

Cover glass Glass capillary tube Glue 35 mm laboratory dish Pipetman \(P-20) Pipette tips Fluorescence microscope Digital camera device Imaging software

Procedure

1. Pick up individual adult animals expressing Raichu-Ras probe to a bacteria-free NGM plate to remove bacteria. And then, pick up the animal and put them on 4% agarose pad made on a cover glass. 2. Immobilize the animal on the agarose pad by glue which is delivered through a drawn glass capillary tube. The tip of the animal's head is always kept free from glue. 3. Place the cover glass with glued animal in the 35 mm laboratory dish and cover with 4 ml imaging buffer solution. 4. Capture the images of cell body of the olfactory neuron expressing the probe by a microscope equipped with 40× or 63× objective lens and a digital camera device. During imaging analysis, room temperature is kept at 20-23 ^{o}C. 5. During collection of the images, add 20 µl odorants solution to the buffer covering animals using pipetman as the odorant stimuli. The final concentration of the odorants are 5×10^{-4}, 1×10^{-3}, 1.5×10^{-3} after the first, second and third stimulation, respectively. 6. After the imaging experiment, exclude crosstalk between the YFP and CFP emission spectra using a linear unmixing method to distinguish weak signals representing Ras activity from noise \(optional). 7. Calculate fluorescent intensity using the imaging software.

Anticipated Results

Ras imaging clearly showed its activity changes were occurred after an odorant stimulus in olfactory neurons in living animals \(Fig. 1). After application of odorants, the changes in YFP and CFP fluorescence intensities opposed each other and ratio changes were also observed \(Fig. 1b, c). Ratio changes of Raichu-Ras occurred within few seconds after odor addition. After reaching the peak, the ratio decreased to the basal level within 2 – 3 sec even if the odorant was still present. Therefore, it revealed that responses of Ras to odorants were quick and transient in the olfactory neurons. This protocol enabled to measure such temporal properties of single protein activity _in vivo_.

References

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Figures

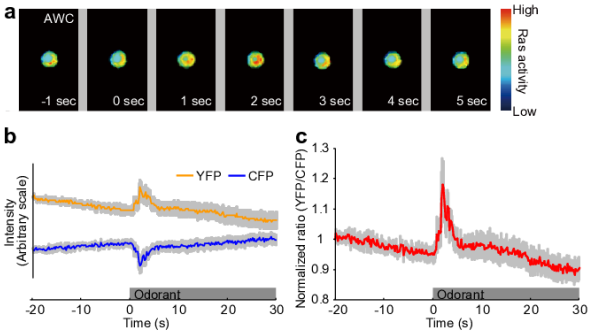

Figure 1

In vivo imaging of Ras activity (a) Images of cell body of an AWC olfactory neuron expressing Raichu-Ras. Colours indicate values of YFP/CFP intensity ratio, with a high ratio (red) corresponding to high Ras activity. (b, c) Temporal profiles of YFP and CFP fluorescence and the mean intensity ratio (YFP/CFP) of

Raichu-Ras in AWC neurons after application of odorant. The shaded region around the plotted data represents SEM.