SHORT REPORT
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G Protein Regulator 1 (GPR-1) Localizes to Cortical Sites of Artificial Mechanical Indentation in *Caenorhabditis elegans* Zygotes

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Cytokinesis and spindle positioning require the cortical force regulator G Protein Regulator 1/2 (GPR-1/2). GPR-1/2 is thought to localize to sites of cortical force generation. Does GPR-1/2 also act as a sensor for mechanical stimulation? I mechanically stimulated the cortex by indenting it with a glass needle and observed the cortical localization of a YFP::GPR-1 transgene. I found that cortical YFP::GPR-1 accumulated at the site of mechanical indentation. This phenomenon occurred on most of the cortical areas except the site of prospective cytokinesis furrow formation. This result suggests that GPR-1/2 can sense mechanical properties of the cortex, which may be important for GPR-1/2 function regulating spindle positioning and cytokinesis. © 2012 Wiley Periodicals, Inc

Key Words: cytokinesis, mechanosensation, GPR-1/2, *C. elegans*

Introduction

During cell division, the mitotic spindle and the cell cortex provide cues to each other to properly divide the cell: the cell cortex determines the position of the mitotic spindle and the mitotic spindle positions the cytokinesis cleavage furrow [Grill et al., 2001; Rappaport, 2005]. Spindle positioning and cytokinesis require force to divide the cell. Force-generating biochemical pathways can also act as force sensors. An example for a molecule that is a force generator as well as a force sensor is the motor protein myosin [Luo et al., 2012].

The *C. elegans* zygote is an established system to study cell division [Schneider and Bowerman,2003; Cowan and Hyman, 2004; Begasse and Hyman, 2011; Oegema and Hyman, 2006]. Before division, cell polarity is established by the sperm-derived centrosome. The position of the centrosome determines the posterior of the embryo [Goldstein and Hird, 1996]. The maternal pronucleus and the paternal pronucleus meet in the posterior of the embryo to form the pronuclear centrosomal complex (PCC). The PCC then moves to the center of the cell. The axis of the PCC is determined by the two centrosomes. The PCC rotates to align on the long axis of the embryo. After nuclear envelope breakdown, a mitotic spindle is formed. During anaphase, the mitotic spindle elongates and is displaced toward the posterior of the cell. The cytokinesis furrow then bisects the spindle between the two asters and divides the cell into two daughter cells of unequal size and content [Schneider and Bowerman, 2003; Cowan and Hyman, 2004; Begasse and Hyman, 2011; Oegema and Hyman, 2006].

Microtubule asters play an important role in positioning the mitotic spindle and in cytokinesis furrow positioning. The cortex exerts pulling forces acting on microtubule asters to center the PCC, rotate it, elongate the spindle, and displace it to the posterior during anaphase [Grill et al., 2001; Couwenbergs et al., 2007; Goulding et al., 2007]. Microtubule asters also provide the first of two signals that specify the position of the cytokinesis cleavage furrow [Bringmann et al., 2005, 2007]. G Protein Regulator (GPR) was identified first as a regulator of spindle positioning [Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003], and second as a regulator of cytokinesis [Bringmann et al., 2007]. GPR consists of two almost identical proteins that function redundantly and are collectively called GPR-1/2. In the absence of GPR-1/2, centration of the mitotic spindle is slow, spindle displacement and microtubule-dependent cortical forces are low, and aster-positioned cytokinesis is absent [Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003; Pecreaux et al.,

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2006; Bringmann et al., 2007]. Overexpression of GPR-1 causes increased microtubule-based pulling forces preventing the formation of a normal spindle [Redemann et al., 2011]. GPR-1/2 localizes to centrosomes and to the cell cortex. Cortical GPR-1/2 is increased in the posterior during anaphase spindle displacement and is decreased at the site of cytokinesis furrow formation [Colombo et al., 2003; Gotta et al., 2003; Bringmann et al., 2007; Park and Rose, 2008]. These observations indicate that GPR-1/2 controls cortical force generation and that sites of cortical GPR-1/2 accumulations are sites of force generation. Here, I investigated the possibility that GPR-1/2 not only acts as a force generator, but also as a force sensor. I mechanically stimulated the cell cortex with a glass needle and found an accumulation of YFP::GPR-1 at the site of stimulation. This mechanosensitive localization was quickly reversible and occurred along most of the cortex except a small equatorial region of generally low local YFP::GPR—the site at which the cytokinesis furrow later ingressed. The results suggested that localization of GPR-1 is mechanosensitive, suggesting a role of mechanosensation in GPR-1/2 function.

Results and Discussion

GPR-1 Localizes Dynamically to the Cell Cortex as Two Circumpolar Bands

The localization of GPR-1/2 has been studied with immunofluorescence and yellow fluorescent protein (YFP)-tagged transgenes [Gotta et al., 2003; Tsou et al., 2003; Bringmann et al., 2007; Park and Rose, 2008; Redemann et al., 2011]. I first wanted to reexamine the dynamics of cortical YFP::GPR-1 localization during the first cell division of *C. elegans*. Using a spinning disc microscope, I filmed zygotes expressing a functional YFP::GPR-1 transgene during the first cell division and quantified cortical YFP::GPR-1 [Redemann et al., 2011]. I dissected zygotes from transgenic adults and sandwiched them between an agarose hydrogel pad and a glass coverslip. Every 2 min, I took first a differential interference contrast (DIC) image and then a fluorescence image. During pronuclear migration (prophase), polarity was established in the posterior of the embryo and the anterior (maternal) pronucleus migrated toward the posterior (paternal) pronucleus. The anterior cortex appeared ruffled during this time, whereas the posterior cortex appeared smooth. Between anterior and posterior cortex, there was a transient furrow called pseudocleavage furrow [Schneider and Bowerman, 2003; Cowan and Hyman, 2004; Begasse and Hyman, 2011; Oegema and Hyman, 2006]. YFP::GPR-1 localized unevenly to the entire anterior ruffled cortex. On the posterior cortex, YFP::GPR-1 localized as a circumpolar band and was locally reduced at the pole (the site of polarity establishment). A small local reduction was also seen at the posterior half of the pseudocleavage furrow (Fig. 1A). During centration (prophase to prometaphase), the PCC migrated toward the center of the cell and then rotated onto the long axis of the embryo. Before the PCC was fully centered, there was a strong anterior circumpolar YFP::GPR-1 band and relatively little YFP::GPR-1 at the anterior pole and the posterior cortex (Fig. 1B). Once centration was completed (prometaphase to metaphase), the anterior circumpolar GPR-1 band was still present and YFP::GPR-1 increased on the posterior cortex to also form a circumpolar band of approximately similar YFP::GPR-1 intensity (Fig. 1C). During early anaphase, the spindle was displaced toward the posterior and still two circumpolar GPR-1 bands were visible (Fig. 1D). However, the size of the posterior band was now increased compared with the anterior band. Between the two bands, a clear local minimum was visible. At the site of this local minimum, the cytokinesis furrow ingressed later during anaphase (Fig. 1E). I conclude that cortical GPR-1 localized dynamically to the cortex. The localization was consistent with the view that cortical GPR-1 increases microtubule dependent forces to position the spindle: cortical YFP::GPR-1 was higher in the anterior during centration, and higher in the posterior during anaphase spindle displacement. Although the relative amounts of cortical YFP::GPR-1 changed during cell division, there were five areas along the cortex with consistent GPR-1 localization: There were local minima at the anterior pole and the posterior pole. There were local maxima on the anterior and posterior lateral cortex that can be described as two circumpolar bands, and there was a local minimum between these two bands at the central lateral region. These results are consistent with and partially overlapping with previously reported localization studies [Gotta et al., 2003; Tsou et al., 2003; Bringmann et al., 2007; Park and Rose, 2008; Redemann et al., 2011].

GPR-1 Localizes to the Site of Mechanical Stimulation on Most of the Cortex except the Site of Prospective Cytokinesis Furrow Formation

I wanted to test whether localization of YFP::GPR-1 responds to mechanical or geometrical stimulation. I mechanically perturbed different areas of the cortex by pressing a glass needle into it and observed and quantified cortical YFP::GPR-1. To stimulate the cortex, I first dissected zygotes expressing YFP::GPR-1 from adult *C. elegans*, transferred them with a glass pipette, and glued them onto a glass coverslip in an open drop of embryo buffer (EB). I observed the zygotes using DIC microscopy to identify the cell cycle stage and mechanically stimulated the zygotes between prometaphase and early anaphase. For mechanical stimulation, I moved a horizontal glass needle relative to the zygote using a micromanipulator and automated stage and pressed the needle into the zygote. This

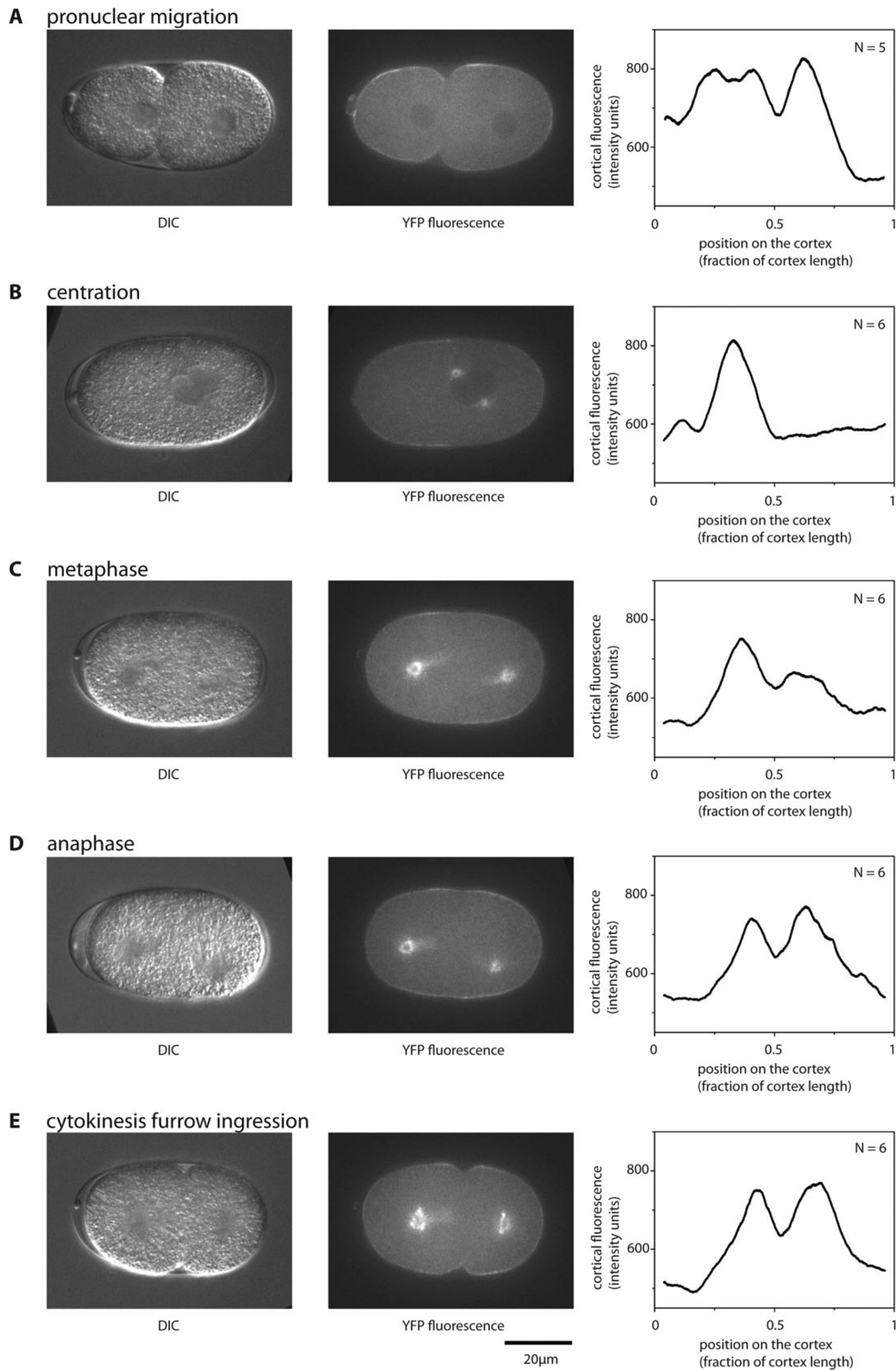

Fig. 1. YFP::GPR-1 localizes dynamically to sites of force generation. (**A**) Pronuclear migration. (**B**) Centration, the PCC migrates to the anterior and YFP::GPR-1 is enriched in the anterior. (**C**) Metaphase. The spindle is centered and aligned onto the anterior-posterior axis and YFP::GPR-1 localizes to two circumpolar bands. (**D**) Early anaphase, the spindle is displaced toward the posterior and the posterior circumpolar band is increased. (**E**) Cytokinesis furrow ingression, YFP::GPR-1 is increased in the posterior circumpolar band and is decreased at the site of asymmetric cytokinesis furrow formation.

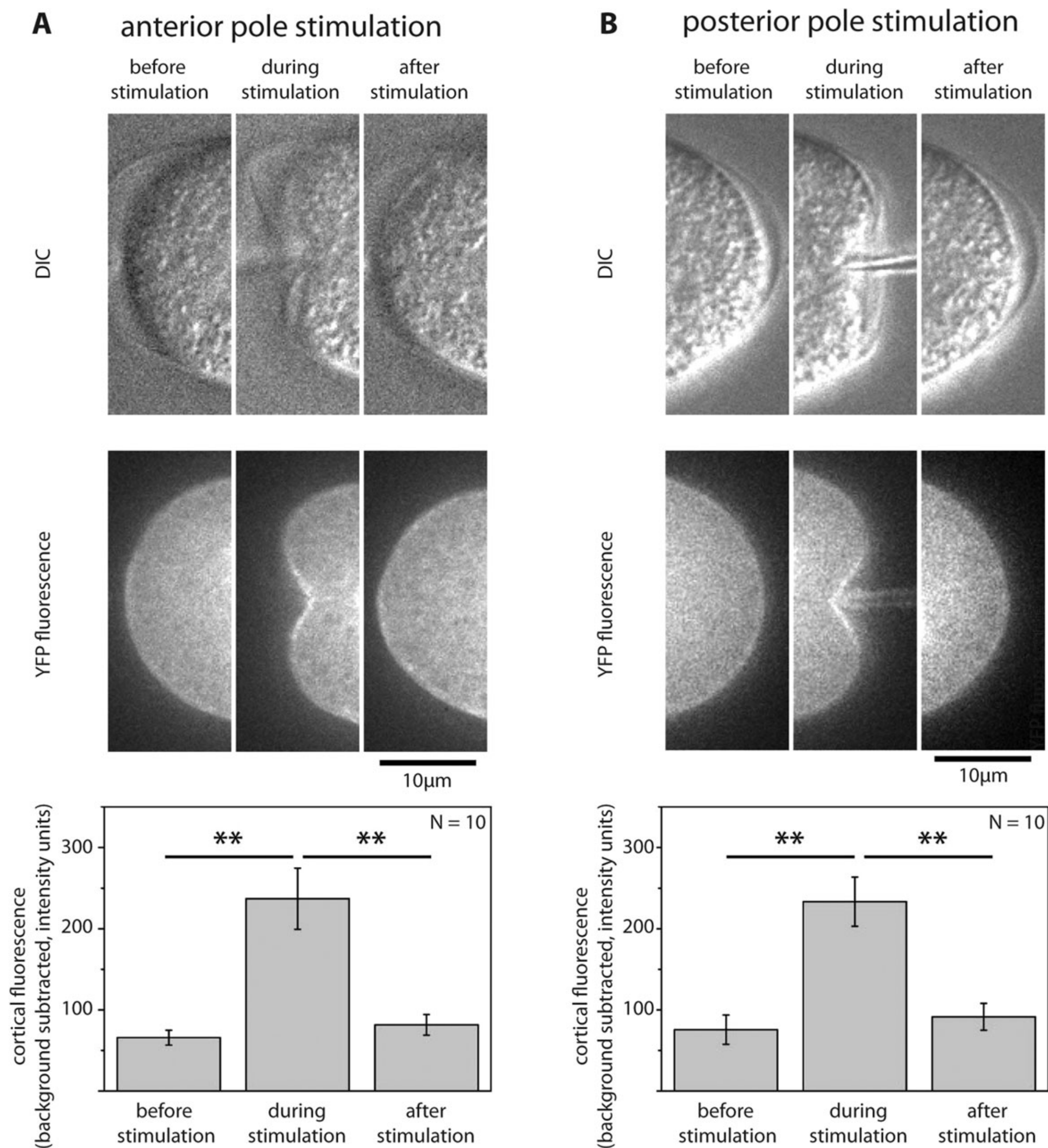

Fig. 2. Artificial mechanical indentation at the poles caused a massive YFP::GPR-1 accumulation. (**A**) Indentation at the anterior pole. (**B**) Indentation at the posterior pole.

caused an indentation of the soft eggshell surrounding the zygote as well as the underlying cortex but did not penetrate either of them. I pressed the needle so that the cortex was indented about 5 microns deep. Then I pulled the needle off the embryo, which reversed the indentation. After the experiment, the embryos did not show any signs of damage, they did not leak, and appeared to develop normally. I filmed zygotes using spinning-disc microscopy to observe YFP::GPR-1 before, during, and after the stimulation. I probed the five cortical areas of YFP::GPR-1 localization as defined above: anterior and posterior pole, anterior and posterior lateral region, and central lateral region. Pressing the needle into the anterior or posterior polar cortex were YFP::GPR-1 is normally minimal, caused an approximately threefold increase in cortical YFP::GPR-1 in the indented area ($P < 0.01$, Fig. 2). Pressing the needle into the anterior or posterior lateral cortex where YFP::GPR-1 is normally high, caused a 1.7-fold increase in cortical YFP::GPR-1 in the indented area ($P < 0.05$, Figs. 3a and 3c). Pressing the needle into the equatorial cortex where YFP::GPR-1 is minimal, caused no significant increase of cortical YFP::GPR-1 in the indented area ($p = 0.3$, Fig.3b). Removing the needle reversed stimulation-induced YFP::GPR-1 accumulation in less than 20 s. The results show that cortical YFP::GPR-1 reversibly accumulated at the site of mechanical stimulation caused by a glass needle. The relative effect was strongest at the poles of the zygote were

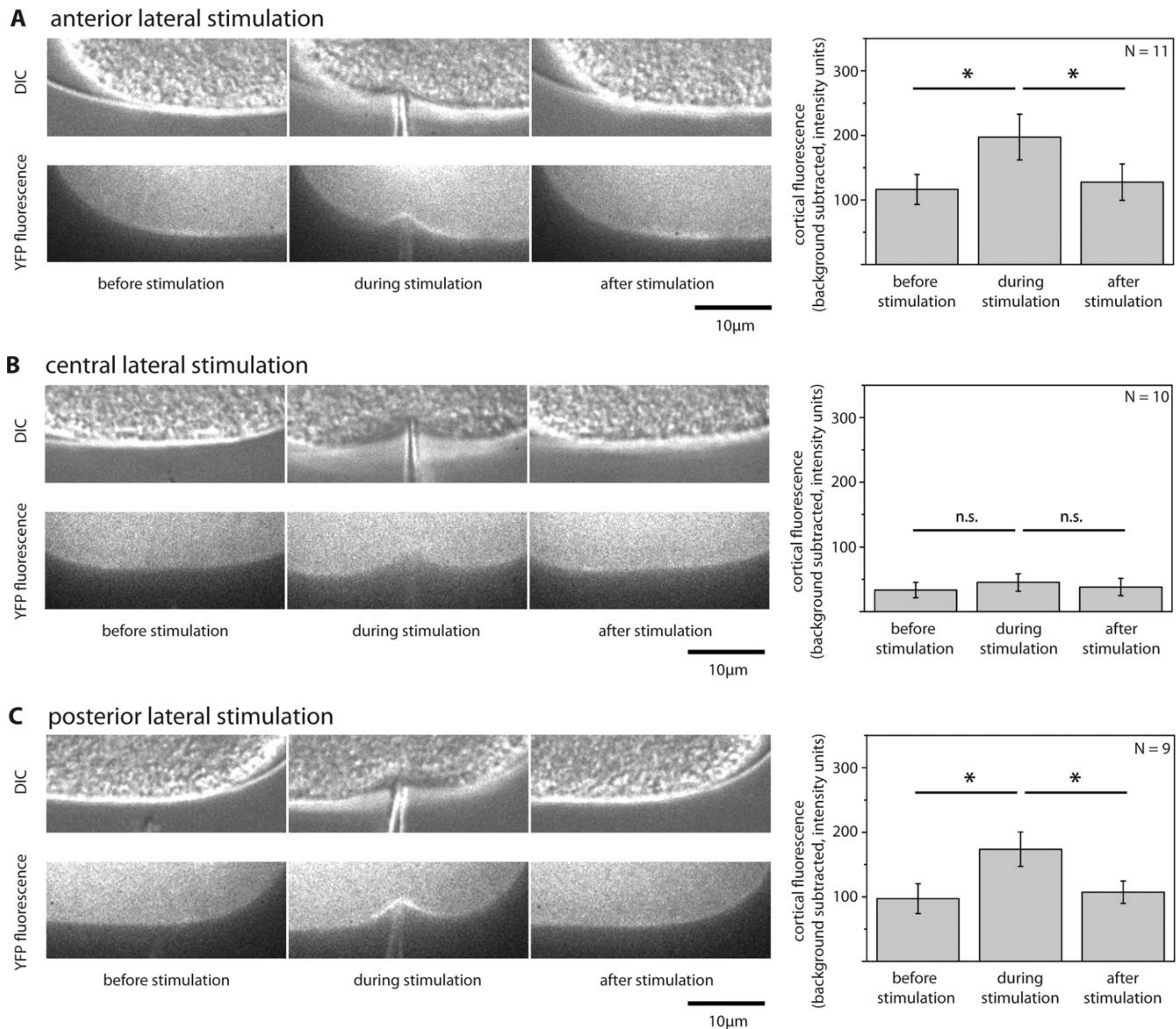

Fig. 3. Artificial mechanical indentation at the anterior lateral and the posterior lateral cortex but not a the central lateral cortex caused significant YFP::GPR-1 accumulation. (A) Indentation at the anterior lateral cortex. (**B**) Indentation at the central lateral cortex. (**C**) Indentation at the posterior lateral cortex.

YFP::GPR-1 is normally low. YFP::GPR-1 accumulation was also observed at the lateral cortex where GPR-1 normally has a local maximum. However, cortical YFP::GPR-1 accumulation could not be observed at the relatively small equatorial region where cortical YFP::GPR-1 has a local minimum and where the cytokinesis furrow later ingressed.

Discussion

To my knowledge, these experiments provide the first experimental evidence that cortical GPR-1 localization responds to artificial mechanical stimulation.

Is mechanosensitive accumulation a general feature of all or many plasma membrane associated proteins? I also looked at one additional plasma membrane marker, a Pleckstrin homology domain fused to GFP [Audhya et al., 2005], which did not show mechanosensitive accumulation (Supporting Information, Fig. S1). Thus, although mechanosensitive accumulation does not seem to be a property of all plasma membrane associated proteins, additional experiments are required to reveal whether other plasma membrane associated proteins also have mechanosensitive properties.

I have used a YFP-tagged version of GPR-1 that has been shown to be functional because its localization is identical to immunostainings performed by other labs, and because YFP::GPR-1 rescues the phenotype caused by GPR-1/2 RNAi [Redemann et al., 2011]. Nevertheless, it would be interesting to confirm mechanosensitive localization of GPR-1/2 by immunostaining the endogenous GPR-1/2 during mechanical stimulation.

Why was the effect strongest at the poles? First, the cortical YFP::GPR-1 baseline was low at the poles potentially

allowing a stronger relative increase. Second, it was easier to indent the polar cortex so that the indentation was deeper and the average stimulation was thus probably stronger at the poles making a direct comparison of stimulation of polar and lateral cortical regions difficult. Why does cortical GPR-1 not accumulate at the small central lateral cortex, the future site of furrow ingression? The small central lateral YFP::GPR-1 minimum is most prominently seen during early anaphase, when it is displaced slightly posterior exactly at the site of prospective cytokinesis furrow ingression. The DEP domain protein LET-99, which is also required for cytokinesis, has been shown to localize to this area. LET-99 has been shown to inhibit accumulation of YFP::GPR-1 at the site of cytokinesis furrow formation [Bringmann et al., 2007]. Thus, a possible explanation would be that LET-99 prevents accumulation of GPR-1 in this area during artificial mechanical stimulation. What does GPR-1 sense? I have stimulated the cell cortex by indenting it with a glass needle and found an accumulation of cortical YFP::GPR-1. However, this stimulation changes many parameters of the cell such as cortical curvature, cortex-microtubule interface (angle at which microtubules are touching the cortex), cortical tension, and other parameters. What is the parameter that is sensed by GPR-1? Additional mechanical manipulation experiments need to be performed to find out. Is the endogenous localization of GPR-1 influenced by mechanical or geometrical cues, and what is the role of the stimulation-sensitivity of GPR-1? We still know too little about the properties of the cortex during spindle positioning and cytokinesis and too little about the localization of GPR-1/2 to answer this question. Although my results suggested that GPR-1 localization could be influenced by artificial mechanical stimulation, this observation opened up many new questions regarding the role of mechanical cues and GPR-1 to cortex function such as spindle positioning and cytokinesis.

Materials and Methods

Culturing *C. elegans*

C. elegans embryos were cultured as described at 15°C [Brenner, 1974]. The strains TH252, *unc-119(ed3)III; ddIs34[pie-1::gpr-1(synthetic, CAI 0.6, artificial introns)::yfp; unc-119(+)]* [Redemann et al., 2011] and OD58, *unc-119*(*ed3*) *III; ltIs38 [pAA1*; *pie-1/GFP::PH*(*PLC1delta1*); unc-119 (+)] [Audhya et al., 2005] were used.

Fluorescence Microscopy

All fluorescence images were taken with a Nikon TiE inverted microscope (Japan) and an "Andor Revolution" spinning disc system equipped with a 488 nm Laser and an iXon EMCCD camera at a magnification of 1000× (Belfast, UK).

Mechanical Stimulation

Coverslips were first coated with Cell-Tak (BD Bioscience, Franklin Lakes, NJ, USA) using the "spreading" method described in the manufacturers protocol. Briefly, a 1 μl drop of Cell-Tak solution was placed onto the coverslip and was allowed to dry. Using a hydrophobic pen, a circle with a diameter of about 1–1.5 cm was drawn around the Cell-Tak area so that the Cell-Tak was in the center of the circle. Coverslips were then washed with first pure water and then with pure ethanol. After drying, coated coverslips were used within the next 6 h and not stored longer. A borosilicate needle was pulled using a needlepuller (Sutter Novato, CA, USA). The needle was placed into a custom build needle holder that kept the needle almost horizontally compared with the focal plane (about 5% tilt). The needle holder was placed into a micromanipulator (Eppendorf, Patchman, Hamburg, Germany). The tip of the needle was broken off to make the needle blunt by moving the needle against a glass capillary using the micromanipulator. Zygotes were taken from gravid hermaphrodites. Adult hermaphrodites were placed in a drop of EB (10 mM Tris-Cl, pH 8.5) placed on an uncoated coverslip. Hermaphrodites were cut open using steel needles, and early zygotes were selected using a fine glass mouth pipette. I transferred zygotes onto the coverslips coated with Cell-Tak and covered with EB. I placed the embryo onto the microscope and observed it at 60× magnification to identify polarity and orientation. I rotated the coverslips containing the embryos first so that the long axis of the embryo was orthogonal to the axis of the needle and then pressed the glass needle down onto the embryo for a few seconds at about the middle of embryo length. This pressing step tightly fixed the embryo to the glass coverslip. I then rotated the embryo so that the cortical area of interest faced the tip of the needle. I then changed to 1000× magnification and moved the needle and the embryo so that the needle was just a few microns away from the site of stimulation. I then took a fluorescence and then a DIC image to document the YFP::GPR-1 localization and cell cycle stage before stimulation. I moved the needle into the zygote by moving it with a micromanipulator and also moved the embryo with an automated stage (Prior Proscan, Rockland, MA, USA). I then took again a DIC and a fluorescence image, moved the needle away from the embryo, and took a DIC and fluorescence image again. All experiments were performed at 18°C.

Intensity Measurements for GPR-1

For measuring cortical YFP::GPR-1 intensity along the cortex, a line was drawn manually along the cortex from the anterior to the posterior pole. Intensity along this line was averaged for a line width of 5 pixels and was quantified with iQ software (Andor, Belfast, UK). For measuring cortical YFP::GPR-1 intensity at the site of stimulation, a

line was drawn across the cortex at the site of stimulation and the intensity was quantified with iQ. The maximum YFP::GPR-1 intensity was determined and the background was subtracted. Data were statistically processed using Origin software. The statistical test used was Wilcoxon paired sample.

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