A casein kinase 1 and PAR proteins regulate asymmetry of a PIP(2) synthesis enzyme for asymmetric spindle positioning

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Abstract

Spindle positioning is an essential feature of asymmetric cell division. The conserved PAR proteins together with heterotrimeric G proteins control spindle positioning in animal cells, but how these are linked is not known. In C. elegans, PAR protein activity leads to asymmetric spindle placement through cortical asymmetry of Galpha regulators GPR-1/2. Here, we establish that the casein kinase 1 gamma CSNK-1 and a PIP(2) synthesis enzyme (PPK-1) transduce PAR polarity to asymmetric Galpha regulation. PPK-1 is posteriorly enriched in the one-celled embryo through PAR and CSNK-1 activities. Loss of CSNK-1 causes uniformly high PPK-1 levels, high symmetric cortical levels of GPR-1/2 and LIN-5, and increased spindle pulling forces. In contrast, knockdown of ppk-1 leads to low GPR-1/2 levels and decreased spindle forces. Furthermore, loss of CSNK-1 leads to increased levels of PIP(2). We propose that asymmetric generation of PIP(2) by PPK-1 directs the posterior enrichment of GPR-1/2 and LIN-5, leading to posterior spindle displacement.

Reference


DOI : 10.1016/j.devcel.2008.06.002
PMID : 18694560
Supplemental Data

A Casein Kinase 1 and PAR Proteins Regulate Asymmetry of a PIP₂ Synthesis Enzyme for Asymmetric Spindle Positioning

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Figure S1. GOA-1 and GPA-16 Distribution Is Unaltered in csnk-1(RNAi) and ppk-1(RNAi) Embryos

Wild-type (A, B), csnk-1(RNAi) (C, D) and ppk-1(RNAi) (E, F) embryos stained for GOA-1 (A, C, and E-left panels) and GPA-16 (B, D, and F-left panels). Tubulin is shown in the right panels.
Figure S2. Cortical GPR-1/2 Is Increased in csnk-1(RNAi) and Decreased in ppk-1(RNAi) Gonads

(A–C) Dissected gonads of (A) wild-type (B) csnk-1(RNAi), and (C) ppk-1(RNAi) adult hermaphrodites stained for GPR-1/2. Cortical GPR-1/2 is higher in (B) csnk-1(RNAi) gonad and lower in (C) ppk-1(RNAi) gonad compared to (A) wild-type gonad.

(D) Quantification of cortical and cytoplasmic GPR-1/2 pixel intensity in wild-type, csnk-1(RNAi) and ppk-1(RNAi) gonads (mean ± SEM). For measurement of cortical intensity, the average peak intensity of the three most proximal oocyte cortices is given. n, number of individual cortices. Cytoplasmic number gives the average pixel intensity of the most proximal oocyte. n, number of oocytes analysed.

(E) Western blot of wild-type (lane 1), csnk-1(RNAi) (lane 2) and gpr-1/2(RNAi) (lane 3) embryo extracts, probed with anti-GPR-1/2 (top) or alpha-tubulin loading control (bottom). Quantification was carried out using Gels Analyze tool in Image J. Ratio of GPR-1/2 to tubulin in wild type was normalised to 1. In csnk-1(RNAi) embryos, this ratio is 0.96 indicating no difference in GPR-1/2 levels.
Figure S3. Increased Cortical LIN-5 in csnk-1(RNAi) Embryos
(A, D) Wild-type and (B, E) csnk-1(RNAi) embryos stained for LIN-5 (left panels) and DNA (right panels). (A, B) One-cell embryos during centration. (D, E) One-cell embryos at anaphase. LIN-5 is weakly enriched at the posterior in wild-type anaphase embryos (D). csnk-1(RNAi) embryos show increased cortical LIN-5 (B, E), anterior enrichment of LIN-5 during centration (B) and no asymmetry in anaphase (E). (C, F) Average anterior (light grey) and posterior (dark grey) cortical LIN-5 pixel intensities in specified genotypes of embryos (C) at pronuclear meeting and (F) during meta to telophase (bar represents SEM).
* indicates p value < 0.01 compared to corresponding wild-type.

Figure S4. csnk-1(RNAi) Embryos Have Normal PAR Polarity
Wild-type (A, C) and csnk-1(RNAi) (B, D) embryos stained for PAR-2 (A, B left panels) and PAR-3 (C, D left panels). Tubulin is shown in the right panel for each image.
Figure S5. GPR-1/2 Distribution Depends on CSNK-1 and PAR-3
GPR-1/2 (left panels) and tubulin (right panels) staining in embryos of indicated genotypes.
(A-D) One-cell embryos at telophase.
(E) Average anterior cortical (0-25%; light grey) and posterior cortical (75-100%; dark grey) GPR-1/2 pixel intensities in specified genotypes of embryos at during metaphase to telophase (bar represents SEM).
* indicates p value < 0.01. Overall intensity signifies the average intensities of whole embryonic cortices.