

Supplemental Materials

Molecular Biology of the Cell

Zhuravlev et al.

Supplemental Information: Zhuravlev *et al.* 2017

Title

CYK-4 regulates Rac, but not Rho, during cytokinesis

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CYK-4 and Rac in cytokinesis

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Supplementary Figure Legends

Figure S1: *cyk-4(ts)* mutant embryos take longer to form a double membrane and fail in cytokinesis at the 2-cell stage via a *Rac*-dependent mechanism

A) Time-lapse montage of the division plane region over time during cytokinesis in control and *cyk-4(ts)* mutant 1-cell embryos with and without *Rac(RNAi)*. The plasma membrane is labeled with GFP::PH. B) Analysis of the time from anaphase onset to double membrane formation in embryos from the genotypes shown. All of the controls but only a few *cyk-4(ts)* mutant embryos have formed a double membrane at 150 sec post-AO due to the slower rate of contractile ring constriction in *cyk-4(ts)* mutants (e.g. Figure 1A and C). Error bars represent the SD; p-values were obtained by an unpaired, two-tailed t-test (Table S3). C) Schematic of the 2-cell *C. elegans* embryo indicating the anterior AB and posterior P1 cells; and bar graph showing cytokinesis completion/failure rates of AB and P1 cells in *cyk-4(ts)* mutant 2-cell embryos with or without *Rac(G60R)* at restrictive temperature. The number of embryos per genotype is indicated on each individual bar; p-values were obtained by both Fisher's and Barnard's exact tests (Table S3). AO=anaphase onset. n.s.= $p>0.05$; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$; ****= $p<0.0001$. Scale bars: yellow=60 sec, white=10 μm .

Figure S1: Zhuravlev et al. (2017)

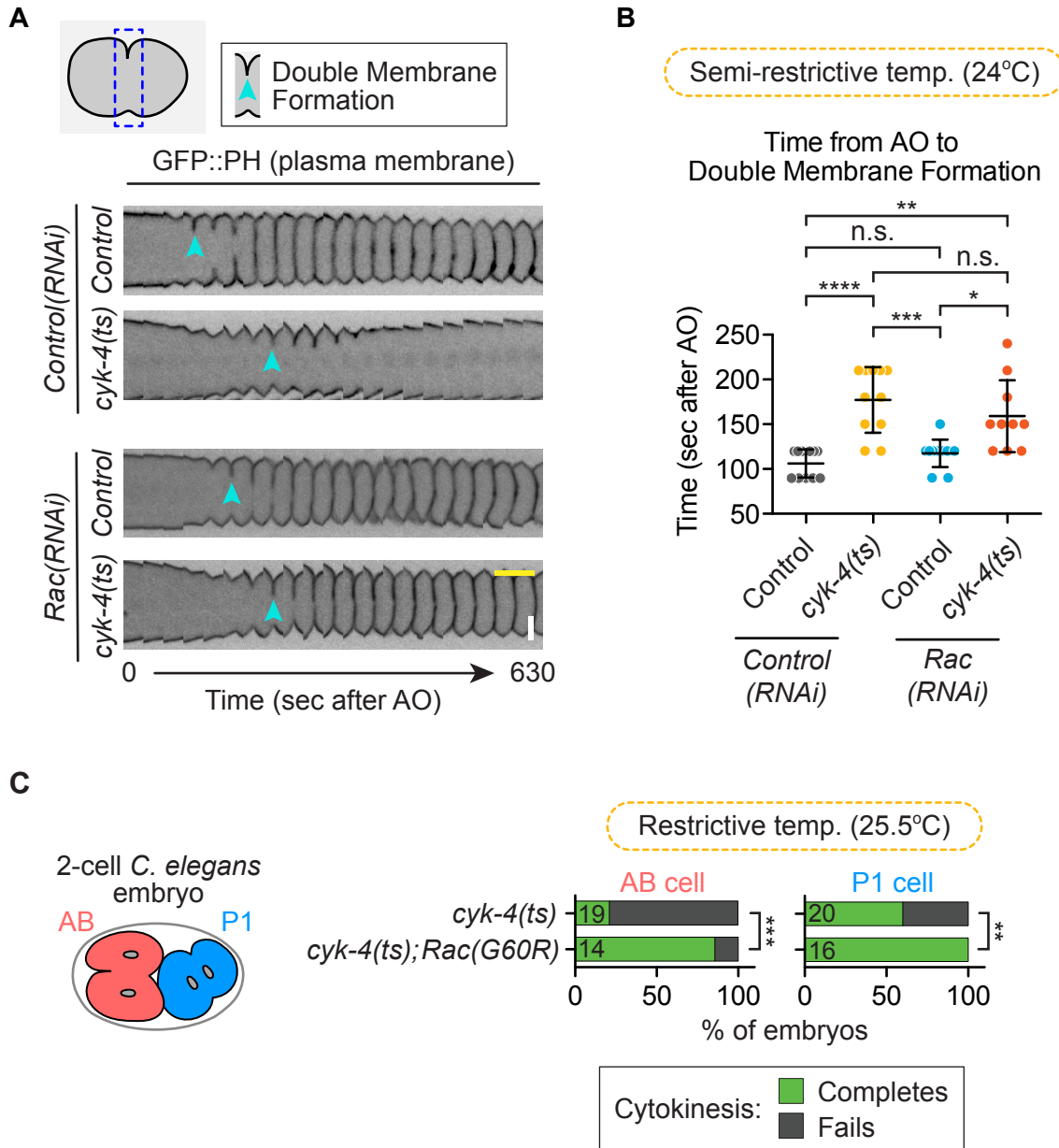
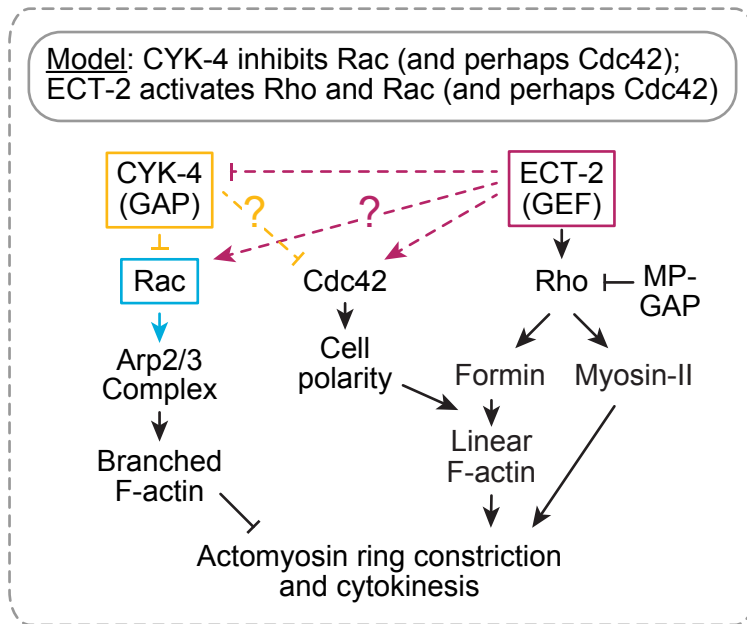


Figure S2: Genetic model for Rho family GTPase signaling during cytokinesis

Revised genetic model for the molecular regulation of Rho family small GTPases during cytokinesis. Note: We cannot rule out a role for ECT-2 in regulating Rac and/or CYK-4 activity. We also cannot rule out a role for ECT-2 and/or CYK-4 in regulating Cdc42 activity.

Figure S2: Zhuravlev et al. (2017)



Supplementary Table Legends

Table S1: *Strain names and genotypes*

List of all strain names and genotypes used in this study.

Table S2: *Feeding RNAi constructs*

Plasmid names, oligos for RNAi feeding constructs, and DNA templates with the Ahringer clone number and/or cloning vector information used in this study.

Table S3: *Statistical analysis.*

Matrix of all statistical analysis, p-values, and experimental numbers used throughout this study.

Table S1: Zhuravlev et al. (2017)

Strain Names and Genotypes

Strain	Genotype
N2	<i>wild-type (ancestral)</i>
OD95	<i>unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ltIs37[pAA64; pie-1::mCherry::his-58 unc-119(+)]IV</i>
JCC176	<i>unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ced-10(n3246)IV</i>
OD239	<i>cyk-4(or749ts) unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ltIs37[pAA64; pie-1::mCherry::his-58 unc-119(+)]IV</i>
JCC146	<i>cyk-1(or596ts) unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ltIs37[pAA64; pie-1::mCherry::his-58 unc-119(+)]IV</i>
JCC177	<i>cyk-1(or596ts) unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ced-10(n3246)IV</i>
JCC192	<i>nmy-2(ne3409ts) dpy-5**; unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ltIs37[pAA64; pie-1::mCherry::his-58 unc-119(+)]IV</i>
JCC203	<i>nmy-2(ne3409ts), dpy-5**; unc-119(ed3)* ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ced-10(n3246)IV</i>
JCC925	<i>ect-2(ax751ts)II; unc-119(ed3)*, ltIs38 [pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III</i>
JH2754	<i>ect-2(ax751ts)II</i>
MG592	<i>ect-2(ax751ts)II; ced-10(n1993)IV</i>
MT5013	<i>ced-10(n1993)IV</i>
JCC719	<i>mgSi3[cb-unc-119(+)] eGFP:utrophin]II; unc-119(ed3)*III; ltIs37[pAA64; pie-1::mCherry::his-58 unc-119(+)]IV</i>
JCC765	<i>mgSi3[cb-unc-119(+)] eGFP:utrophin]II; unc-119(ed3)* cyk-4(or749ts)III; ltIs37[pAA64; pie-1::mCherry::his-58 unc-119(+)]IV</i>
JCC541	<i>unc-119(ed3)*III; ltIs37 [pAA64; pie-1::mCherry::his-58; unc-119(+)]IV; zuls45[nmy-2::nmy-2::eGFP unc-119(+)]V</i>
OD235	<i>unc-119(ed3)* cyk-4(or749ts)III; zuls45[nmy-2::nmy-2::eGFP unc-119(+)]V.</i>
JCC178	<i>unc-119(ed3)* cyk-4(or749ts) ltIs38[pAA1; pie-1::eGFP::PH(PLC1delta1) unc-119(+)]III; ced-10(n3246)IV</i>
BA17	<i>fem-1(hc17)IV</i>
OD227	<i>cyk-4(or749ts)III</i>
EU1303	<i>cyk-4(or570ts)III</i>

*The *unc-119(ed3)* mutation was present in the parental strains but has not been directly sequenced in these strains to determine if the *unc-119* gene is mutated.

**The exact *dpy-5* allele is unknown but presumed to be *e61*, as published in (Carvalho et al. 2009. *Cell*. 137:936-37).

Table S2: Zhuravlev et al (2017)

Feeding RNAi constructs

Plasmid	Gene(s)	Oligo 1 (5'-3')	Oligo 2 (5'-3')	Template	Cloning vector
pJC90	<i>ced-10</i> (C09G12.8)	GCGCG <u>AAGCTT</u> TCAAATGTGTCGTCGTTGGT	GCGCG <u>AAGCTT</u> GATCGCCTCATCGAAAATTG	N2 cDNA	L4440 (empty vector)
pJC53	<i>cdc-42</i> (R07G3.1)	Acquired from Ahringer library (Clone # II-5P13)			