An intimate look at LET-23 EGFR trafficking in the vulval cells of live *C. elegans* larvae

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Precise cell fate specification is essential for organ formation. A simple view is that one or several signal sending cells emit a ligand to a group of signal receiving cells that express the corresponding receptor, which transduces the signal through intracellular enzyme pathways. All these events must be spatio-temporally regulated to achieve the proper strength, duration and output of the signaling pathways. In particular, the production and secretion of the ligand has to be coordinated with the expression and accessibility of the receptor in the signal receiving cells. Furthermore, removal of the ligand or receptor is key to achieve proper signal termination and prevent excess cell differentiation and proliferation. Improper regulation of any of these events may cause developmental defects and human disease. *C. elegans* is an excellent model to systematically identify genes that control the localization and activity of the Epidermal Growth Factor Receptor (EGFR) homolog LET-23. To identify regulators of LET-23 trafficking, Haag et al. observed LET-23 localization in the vulva precursor cells (VPCs) of RNAi treated larvae by live fluorescent microscopy. In this comment, we provide an overview of the newly identified regulators of LET-23 trafficking and discuss the role of the Ezrin/Radixin/Moesin homolog ERM-1 as a temporal regulator of EGFR signaling.

The Hermaphrodite Vulva As A Model to Study EGFR Signaling

The hermaphrodite vulva is necessary for egg-laying and copulation. The formation of the polarized vulval precursor cells

(VPC) occurs during the L1 and L2 stages by specification of six VPCs out of 11 Pn.p cells in the ventral epidermis.¹ From the L2 stage on, the gonadal anchor cell (AC) secretes the EGF ortholog LIN-3. The underlying VPCs express, initially at equal levels, the EGFR homolog LET-23.² The VPC that is nearest to the AC, P6.p, receives most of the inductive LIN-3 signal, up-regulates LET-23 expression and adopts a 1° cell fate through activation of the conserved LET-60 RAS/MPK-1 MAPK pathway.¹ P6.p produces several DELTA ligands, which induce the lateral LIN-12 NOTCH pathway in the neighboring VPCs P5.p and P7.p to specify the secondary (2°) cell fate.^{3,4} NOTCH signaling in the 2° cells down-regulates LET-23 expression and inhibits RAS/MAPK signaling.⁵⁻⁷ The distal VPCs, which receive little inductive EGF and lateral DELTA signals, adopt the tertiary (3°) un-induced cell fate. Thus, in a wild-type hermaphrodite the six VPCs adopt an invariant 3°-3°-2°-1°-2°-3° pattern of vulval cell fates (Fig. 1A,B).¹ The acquisition of these cell fates at the beginning of the L3 stage determines the following cell division pattern and morphogenetic events that ultimately lead to the formation of a vulval tube, which connect the uterus to the exterior.⁸

Mutations that perturb EGFR/RAS/MAPK or NOTCH signaling result in easily detectable phenotypes such as Egg-Laying-defective (Egl), Protruding Vulva (Pvl), abnormal Eversion of the vulva (Evl) and in severe cases a Vulvaless (Vul) phenotype. On the other hand, mutations that hyper-activate the EGFR or NOTCH pathway cause a MultiVulva (Muv) phenotype. Muv animals usually have a functional vulva and additional

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Abbreviations: EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; VPCs, vulval precursor cells; AC, anchor cell; VUL: vulva-less; MUV, multi-vulva; PVL, protruding vulva; syn-Muv, synthetic multivulva genes; ERM, ezrin, radixin, and moesin; FERM, 4.1 protein, ezrin, radixin, and moesin; PDZ, post synaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1); FRAP, fluorescence recovery after photobleaching.

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Figure 1. LET-23 expression and localization in the vulval epithelium. (A) Schematic drawing of an L2 larva with the location of the VPCs (P3.p through P8.p) and AC. (B) LET-23 EGFR and NOTCH signaling network controlling 1° and 2° vulval fate specification. P6.p adopts the 1° and P5.p & P7.p the 2° fate. The remaining distal VPCs (not shown) adopt the non-vulval 3° fate. (C) Basolateral and apical LET-23::GFP expression (green) in the 1° P6.px cell after cell fate specification. Low levels of LET-23::GFP are observed in the 2° P5.p and P7.p descendants due to LIN-12 NOTCH mediated lateral inhibition. The AC is labeled with an mCherry::plc δ PH reporter (magenta)³⁴. (D-I) Examples of phenotypes identified in the LET-23 localization screen¹⁶. (H) Apical enrichment of LET-23::GFP due to an increased dose of LIN-3, which causes receptor endocytosis from the basolateral membrane. (I) LET-23::GFP expression in adjacent VPCs due to AC duplication. (Figure is adapted from¹⁶).

ventral protrusions formed from vulval tissue. Genetic screens, especially in sensitized genetic backgrounds, have been extremely successful in identifying new components of the 2 signaling pathways as well as genes required for VPC polarity, proliferation and vulval morphogenesis.^{8,9}

A Conserved Protein Complex Required For LET-23 Localization and Signaling

The first forward screens done by Sydney Brenner and his collaborators^{10,11} identified three genes, *lin-2*, *lin-7* and *lin-10*, that encode components of a conserved protein complex required for

basolateral LET-23 localization.¹²⁻¹⁴ In wild-type larvae, LET-23 EGFR is equally localized at the basolateral and apical plasma membrane of the VPCs (Fig. 1B-D). While apical receptor localization appears to be the default state, the retention of LET-23 at the basolateral membrane depends on the interaction of LET-23 with the tripartite LIN-2/LIN-7/LIN-10 complex (Fig. 1B).² This interaction involves the direct binding of the PDZ domain of LIN-7 to a PDZ binding motif at the C-terminus of LET-23.¹³ Mutations in any of these three genes or a C-terminal truncation of LET-23 result in a loss of basolateral retention and an increased apical accumulation of LET-23. Since LIN-3 EGF is secreted by the AC

located on the basal side of the VPCs, apical mis-localization prevents efficient receptor activation.² After ligand binding, LET-23 is endocytosed and either recycled back to the basolateral plasma membrane, transcytosed to the apical compartment or degraded in lysosomes. All these steps are regulated in order to fine-tune receptor activity and inhibit EGFR/RAS/MAPK signaling in the 2° VPCs. For example, the RAB-5 repressor EPS-8 is recruited into the LIN-2/LIN-7/LIN-10 complex in 1° cells, while NOTCH-mediated lateral inhibition down-regulates *eps-8* expression in 2° cells.⁷ This mechanism results in the basolateral retention of LET-23 in 1° cells and an increased rate of LET-23 endocytosis in 2° cells.

Furthermore, the RAB-7 GTPase regulates LET-23 trafficking to the lysosome targeting it for degradation. However, is not known if RAB-7 is differentially expressed in 1° versus 2° VPCs.¹⁵

An *in vivo* Screen For Additional LET-23 Regulators

While the classical forward genetic screens resulted in the isolation of the first three receptor localization genes, further screens failed to identify additional regulators of LET-23 trafficking. To discover new components that regulate LET-23 localization and expression, a transgenic line expressing a functional LET-23::GFP translational reporter that recapitulates the LET-23 pattern previously observed by antibody staining was used by Haag *et al.* (Fig. 1C,D).^{2,16} This reporter allows the direct observation of LET-23 localization in different tissues and opened the door to a quantitative analysis of LET-23 trafficking and dynamics, for example by performing fluorescence recovery after photobleaching (FRAP) measurements in different subcellular compartments. One insight gained by this type of analysis is that the LET-23 localization observed at a given time point is an indicator of the amount and source of the LIN-3 EGF signal a cell has previously received. For example, increasing the dose of LIN-3 produced by the AC causes an increase in the apical and decrease in the basolateral pool of LET-23, while an ectopic source of LIN-3 results in persistent LET-23 expression in multiple adjacent VPCs (Fig. 1H,I).

Finally, the LET-23::GFP reporter was used for a first *in vivo* screen to directly identify regulators of LET-23 localization in the vulval cells.¹⁶ An RNAi screen of 705 genes annotated to cause an aberrant protruding vulval phenotype (Pvl) identified 81 genes regulating various aspects of receptor dynamics and expression.¹⁶ A broad spectrum of receptor mis-localization phenotypes, such as enrichment in the apical compartment, punctate or diffuse cytoplasmic localization, or persistent expression in 2° cells was observed using high resolution fluorescent microscopy (Fig. 1D-G). As expected, a subset of the

newly identified regulators of LET-23 localization also controls receptor activity, which manifests in an altered strength of the RAS/MAPK signal generated in the VPCs of RNAi treated larvae.

Newly Identified Regulators of LET-23 Localization and Signaling

This section gives an overview of the various regulators of LET-23 trafficking identified by Haag *et al.*¹⁶ according to the different mis-localization phenotypes. The most frequent phenotypes observed were apical enrichment (24 genes) and persistent 2° expression (31 genes) (Fig. 1G,I).¹⁶ 2 caveats are that these 2 mis-localization phenotypes may be more easily scored and that these they may preferentially cause a Pvl phenotype, for which the candidates were already pre-selected. Interestingly, many of the genes that exhibit an apical enrichment phenotype encode for proteins with predicted housekeeping functions. For example, C30C11.1 or T13H5.5 both encode mitochondrial ribosomal proteins and *paf1-1* (C55A6.9) an RNA polymerase I and II associated factor. While apical LET-23 localization of LET-23 appears to be the default route, while basolateral receptor localization likely depends on many global factors that maintain cellular integrity. This view also explains why no apical localization signal has been found, neither in *C. elegans* LET-23 nor in the vertebrate EGFRs.

Some of the genes exhibiting an apical mis-localization phenotype may not be involved in basolateral retention of LET-23, but rather act upstream by controlling LIN-3 production. Since a higher dose of LIN-3 increases the rate of the receptor endocytosis from the basolateral compartment, this leads to a gradual accumulation of LET-23 in the apical compartment (Fig. 1H). This probably explains the apical mis-localization phenotype observed after *met-2* RNAi. The class B *synMuv* gene *met-2* encodes a histone methyltransferase that represses in combination with *met-1* the transcription of *lin-3* in the surrounding hypodermis.¹⁷ Elevated LIN-3 expression from the hypodermis results in

enhanced endocytosis and apical accumulation of LET-23 in all VPCs.

Some of the “usual suspects” know to be involved in VPC fate specification are found in the category of persistent 2° expression. Examples are *lin-31*, which encodes a *forkhead* transcription factor that inhibits 1° fate specification in a complex with LIN-1 ETS¹⁸ or *fbf-2*, which encodes a translational regulator that is required for proper 2° fate execution in P5.p and P7.p together with *fbf-1*.¹⁹ Inactivation of these genes may cause a 2° to 1° fate transformation and hence prevent the downregulation of LET-23 in the ectopic 1° cells (Fig. 1G).

Another interesting case is *sys-1*, which encodes a distant member of the β -catenin family.²⁰ SYS-1 controls the asymmetric cell divisions of the somatic gonad precursor cells that generate, among others, the distal tip and ACs. In *sys-1* mutants, an extra AC is produced instead of a distal tip cell, leading to LET-23 expression in multiple adjacent VPCs that have received an inductive signal from the second, ectopic AC (Fig. 1I). A similar scenario may explain the expanded LET-23 expression pattern observed after *cdc-42*, *par-2* or *par-3* knock-down. The small GTPase CDC-42 and the PDZ protein PAR-3 are components of the anterior PAR complex, while the C3HC4-type RING-finger protein PAR-2 is a component of the posterior PAR complex required for cell polarity in the early embryo.²¹ Inactivation of *cdc-42* or *par-3* causes partitioning of the gonad into 2 separate sections, each one containing one AC due to a loss of LIN-12 NOTCH signaling between the 2 AC precursors.²² In addition, CDC-42 and the PAR proteins may act cell-autonomously to maintain overall VPC polarity, which could indirectly affect LET-23 endocytosis and down-regulation in 2° cells.

RNAi against *ego-2* caused a diffuse accumulation of LET-23 in the cytoplasm of all VPCs, suggesting a defect in intracellular transport (Fig. 1F). *ego-2* encodes a BRO1 domain protein related to mammalian PTPN23, which regulates the transport of ubiquitinated EGFR through the ESCRT III complex to the intraluminal vesicles of multivesicular bodies.²³ Another study found that *ego-2* regulates GLP-1 NOTCH signaling during germ

cell development and embryogenesis, as well as LIN-12 NOTCH signaling during somatic gonad development.²⁴ Thus, *ego-2* might simultaneously regulate LET-23 and LIN-12 through control of their endocytic transport in the VPCs and thereby coordinate the 1° versus 2° fate decision during vulva development.

ERM-1 Is a Temporal Regulator of LET-23 Signaling

One of the genes that exhibits a similar apical mis-localization phenotype as *lin-2*, *lin-7* or *lin-10* mutants is *erm-1*. RNAi against *erm-1* or an *erm-1* null mutant caused strong apical enrichment of LET-23 (Fig. 2A,B).¹⁶ *erm-1* encodes a member of the Ezrin, Radixin and Moesin

(ERM) family and was previously shown to be required for apical lumen formation in the intestine.^{25,26} ERM proteins contain an N-terminal FERM domain and a C-terminal actin-binding domain.^{27,28} They switch from a closed inactive conformation localized in the cytoplasm to an open active conformation at the plasma membrane.²⁹ In the open conformation, the FERM domain interacts with plasma membrane proteins either directly or indirectly through adaptor proteins,²⁷ while binding of the actin cytoskeleton to the C-terminus regulates the activity of the complex.²⁸

FRAP and LIN-3 dosage experiments indicated that ERM-1 inhibits LET-23 mobility and endocytosis at the basolateral plasma membrane.¹⁶ Furthermore, ERM-1 binds to LET-23 through its N-terminal

FERM domain, and this interaction is independent of the tri-partite LIN-2/LIN-7/LIN-10 complex (Fig. 2C). Surprisingly, genetic epistasis experiments with an *erm-1(0)* mutant showed that ERM-1 negatively regulates LET-23 signaling unlike mutations in the tri-partite complex, which result in reduced receptor activity due to the apical receptor mis-localization.¹²⁻¹⁴ One explanation for this observation is that in *erm-1(0)* mutants more LET-23 molecules can be recruited into the tri-partite complex where they can bind LIN-3 and activate the RAS/MAPK pathway (Fig. 2C).

What may be the physiological function of the inhibitory effect ERM-1 exerts on LET-23? One idea is that ERM-1 is part of a “buffering” mechanism that prevents the instant activation of the entire basolateral pool of LET-23 once the AC begins to secrete LIN-3 at the mid L2 stage. According to this model, ERM-1 sequesters LET-23 in an inactive compartment at the basolateral membrane by linking LET-23 to the cortical actin cytoskeleton (Fig. 2C). This mechanism permits the VPCs and their descendants to maintain a fraction of LET-23 in the basolateral compartment over a longer time period and thus generate a continuous RAS/MAPK signal in the 1° cells. Consistent with this model, the expression of the RAS/MAPK target gene *egl-17* showed a gradual decrease in the 1° vulval cells of *erm-1(0)* mutants rather than the continuous increase seen in wild-type animals.¹⁶ Continuous RAS/MAPK signaling after vulval induction may be necessary for the execution of the 1° cell fate during the subsequent phase of vulval morphogenesis.³⁰

The “receptor buffer model” raises several interesting questions: How is LET-23 recruited into and released from the ERM-1 complex? Do downstream components of the LET-23/RAS/MAPK pathway regulate the ERM-1-LET-23 interaction in a feedback loop? The phosphorylation status of ERM-1 likely plays a crucial role in the switch from the closed to the open conformation. It is probably the open conformation of ERM-1 that is recruited to the basolateral plasma membrane where ERM-1 interacts with LET-23. Further insights into the regulation of ERM-1 may be gained by identifying the kinase(s) that

Figure 2. ERM-1 inhibits LET-23 at the basolateral plasma membrane. (A) Nomarski image and (A') LET-23::GFP expression in a heterozygous *erm-1(tm677)/+* and (B, B') a homozygous *erm-1(tm677)* larva at the Pn.px stage. Arrows indicate the basal and arrowheads the apical membrane domains. (C) “Receptor buffer” model for the regulation of LET-23 by ERM-1. The LIN-2/LIN-7/LIN-10 complex promotes receptor activation, while the ERM-1 complex prevents LET-23 signaling from basolateral membrane by sequestering LET-23 in an actin-rich membrane compartment. LET-23 might cycle through both complexes through regulation of ERM-1 activity. The dashed arrows depict a putative feedback from the RAS/MAPK pathway regulating ERM-1. (Figure is adapted from¹⁶).

regulates the switch from the closed to the open conformation. Moreover, mammalian, Ezrin is required for RAS activation at the plasma membrane through the regulation of Son-of-Sevenless (SOS), suggesting yet another role for ERM-1 in the regulation of RAS signaling.^{31,32}

C. elegans ERM-1 is not the first example of an ERM protein regulating EGFR signaling: Mammalian Nf2/Merlin, another more distant member of the ERM family, inhibits EGFR signaling in cultured epithelial cells.³³ However, Nf2/Merlin acts differently. Its active state is the closed rather than the open conformation and Nf2/Merlin inhibits EGFR at apical cortex upon cell-cell contact formation by binding to E-cadherin and the actin network near the adherens junctions.³³ This diversification in ERM function raises the possibility that multiple ERM proteins regulate receptor signaling in different subcellular compartments of polarized cells.

In summary, an unbiased screen using direct microscopic observation of the vulval epithelium has identified a variety of regulators of LET-23 localization and expression. These genes act in diverse processes such as ligand production, cell fate determination, cell polarization and intracellular trafficking.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Sternberg PW. Vulval development. Wormbook 2005; 1-28; PMID:18050418
- Kaech SM, Whitfield CW, Kim SK. The LIN-2/LIN-7/LIN-10 complex mediates basolateral membrane localization of the *C. elegans* EGF receptor LET-23 in vulval epithelial cells. Cell 1998; 94:761-71; PMID:9753323; [http://dx.doi.org/10.1016/S0092-8674\(00\)81735-3](http://dx.doi.org/10.1016/S0092-8674(00)81735-3)
- Chen N, Greenwald I. The lateral signal for LIN-12/Notch in *C. elegans* vulval development comprises redundant secreted and transmembrane DSL proteins. Dev Cell 2004; 6:183-92; PMID:14960273; [http://dx.doi.org/10.1016/S1534-5807\(04\)00021-8](http://dx.doi.org/10.1016/S1534-5807(04)00021-8)
- Simske JS, Kim SK. Sequential signalling during Caenorhabditis elegans vulval induction. Nature 1995; 375:142-6; PMID:7753169; <http://dx.doi.org/10.1038/375142a0>
- Bersert T, Hoier EF, Battu G, Canevascini S, Hajnal A. Notch inhibition of RAS signaling through MAP kinase phosphatase LIP-1 during *C. elegans* vulval development. Science 2001; 291:5; <http://dx.doi.org/10.1126/science.1055642>
- Yoo AS, Bais C, Greenwald I. Crosstalk between the egfr and lin-12/notch pathways in *C. elegans* vulval development. Science 2004; 303:5; <http://dx.doi.org/10.1126/science.1091639>
- Stetak A, Hoier EF, Croce A, Cassata G, Di Fiore PP, Hajnal A. Cell fate-specific regulation of EGF receptor trafficking during Caenorhabditis elegans vulval development. EMBO J 2006; 25:11; <http://dx.doi.org/10.1038/sj.emboj.7601137>
- Schindler AJ, Sherwood DR. Morphogenesis of the caenorhabditis elegans vulva. Wiley Interdiscip Rev Dev Biol 2013; 2:75-95; PMID:23418408; <http://dx.doi.org/10.1002/wdev.87>
- Wang Z, Sherwood DR. Dissection of genetic pathways in *C. elegans*. Methods Cell Biol 2011; 106:113-57; PMID:22118276; <http://dx.doi.org/10.1016/B978-0-12-544172-8.00005-0>
- Brenner S. The genetics of Caenorhabditis elegans. Genetics 1974; 77:24.
- Horvitz HR, Sulston JE. Isolation and genetic characterization of cell-lineage mutants of the nematode Caenorhabditis elegans. Genetics 1980; 96:435-54; PMID:7262539
- Hoskins R, Hajnal AF, Harp SA, Kim SK. The *C. elegans* vulval induction gene lin-2 encodes a member of the MAGUK family of cell junction proteins. Development 1996; 122:97-111; PMID:8565857
- Simske JS, Kaech SM, Harp SA, Kim SK. LET-23 receptor localization by the cell junction protein LIN-7 during *C. elegans* vulval induction. Cell 1996; 85:195-204; PMID:8612272; [http://dx.doi.org/10.1016/S0092-8674\(00\)81096-X](http://dx.doi.org/10.1016/S0092-8674(00)81096-X)
- Whitfield CW, Bénard C, Barnes T, Hekimi S, Kim SK. Basolateral localization of the Caenorhabditis elegans epidermal growth factor receptor in epithelial cells by the PDZ protein LIN-10. Mol Biol Cell 1999; 10:2087-100; PMID:10359617; <http://dx.doi.org/10.1091/mbc.10.6.2087>
- Skorobogata O, Rocheleau CE. RAB-7 Antagonizes LET-23 EGFR signaling during vulva development in caenorhabditis elegans. PLoS ONE 2012; 7:e36489; PMID:22558469; <http://dx.doi.org/10.1371/journal.pone.0036489>
- Haag A, Gutierrez P, Bühler A, Walser M, Yang Q, Langouët M, Kradolfer D, Fröhli E, Herrmann CJ, Hajnal A, et al. An in vivo EGF receptor localization screen in *C. elegans* identifies the Ezrin homolog ERM-1 as a temporal regulator of signaling. PLoS Genet 2014; 10(5):e1004341
- Andersen EC, Horvitz HR. Two *C. elegans* histone methyltransferases repress lin-3 EGF transcription to inhibit vulval development. Development 2007; 134:2991-9; PMID:17634190; <http://dx.doi.org/10.1242/dev.009373>
- Tan PB, Lackner MR, Kim SK. MAP kinase signaling specificity mediated by the LIN-1 Ets/LIN-31 WH transcription factor complex during *C. elegans* vulval induction. Cell 1998; 93:569-80; PMID:9604932; [http://dx.doi.org/10.1016/S0092-8674\(00\)81186-1](http://dx.doi.org/10.1016/S0092-8674(00)81186-1)
- Walser CB, Battu G, Hoier EF, Hajnal A. Distinct roles of the Pumilio and FBF translational repressors during *C. elegans* vulval development. Development 2006; 133:3461-71; PMID:16908630; <http://dx.doi.org/10.1242/dev.02496>
- Miskowski J, Li Y, Kimble J. The sys-1 gene and sexual dimorphism during gonadogenesis in Caenorhabditis elegans. Dev Biol 2001; 230:61-73; PMID:11161562; <http://dx.doi.org/10.1006/dbio.2000.9998>
- Boyd L, Guo S, Levitan D, Stinchcomb DT, Kempthues KJ. PAR-2 is asymmetrically distributed and promotes association of P granules and PAR-1 with the cortex in *C. elegans* embryos. Development 1996; 122:3075-84; PMID:8898221
- Welchman DP, Mathies LD, Ahringer J. Similar requirements for CDC-42 and the PAR-3/PAR-6/PKC-3 complex in diverse cell types. Dev Biol 2007; 305:347-57; PMID:17383625; <http://dx.doi.org/10.1016/j.ydbio.2007.02.022>
- Ali N, Zhang L, Taylor S, Mironov A, Urbé S, Woodman P. Recruitment of UBYPY and ESCRT exchange drive HD-PTP-dependent sorting of EGFR to the MVB. Curr Biol 2013; 23:453-61; PMID:23477725; <http://dx.doi.org/10.1016/j.cub.2013.02.033>
- Liu Y, Maine EM. The Bro1-domain protein, EGO-2, promotes Notch signaling in Caenorhabditis elegans. Genetics 2007; 176:2265-77; PMID:17603118; <http://dx.doi.org/10.1534/genetics.107.071225>
- Van Firden D, Johnson K, Segbert C, Bossinger O. The *C. elegans* ezrin-radixin-moesin protein ERM-1 is necessary for apical junction remodelling and tubulogenesis in the intestine. Dev Biol 2004; 272:262-76; PMID:15242805; <http://dx.doi.org/10.1016/j.ydbio.2004.05.012>
- Göbel V, Barrett PL, Hall DH, Fleming JT. Lumen morphogenesis in *C. elegans* requires the membrane-cytoskeleton linker erm-1. Dev Cell 2004; 6:865-73; PMID:15177034; <http://dx.doi.org/10.1016/j.devcel.2004.05.018>
- Hughes SC, Fehon RG. Understanding ERM proteins—the awesome power of genetics finally brought to bear. Curr Opin Cell Biol 2007; 19:6; <http://dx.doi.org/10.1016/j.ceb.2006.12.004>
- Turunen O, Wahlström T, Vaheri A. Ezrin has a COOH-terminal actin-binding site that is conserved in the ezrin protein family. J Cell Biol 1994; 126:1445-53; PMID:8089177; <http://dx.doi.org/10.1083/jcb.126.6.1445>
- Nakamura F, Amieva MR, Furdumayr H. Phosphorylation of threonine 558 in the carboxyl-terminal actin-binding domain of moesin by thrombin activation of human platelets. J Biol Chem 1995; 270:31377-85; PMID:8537411; <http://dx.doi.org/10.1074/jbc.270.52.31377>
- Pellegrino MW, Hajnal A. The transcription factor VAB-23 links vulval cell fate specification and morphogenesis. Worm 2012; 1(3):170-175.
- Sperka T, Geißler KJ, Merkel U, Scholl I, Rubio I, Herrlich P, Morrison HL. Activation of Ras Requires the ERM-Dependent Link of Actin to the Plasma Membrane. PLoS ONE 2011; 6:e27511; PMID:22132106
- Geißler KJ, Jung MJ, Riecken LB, Sperka T, Cui Y, Schacke S, Merkel U, Markwart R, Rubio I, Than ME, et al. Regulation of Son of sevenless by the membrane-actin linker protein ezrin. Proc Natl Acad Sci USA 2013; 110:20587-92; <http://dx.doi.org/10.1073/pnas.1222078110>
- Curto M, Cole BK, Lallemand D, Liu C-H, McClatchey AI. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. J Cell Biol 2007; 177:893-903; PMID:17548515; <http://dx.doi.org/10.1083/jcb.200703010>
- Ziel JW, Hagedorn EJ, Audhya A, Sherwood DR. UNC-6 (netrin) orients the invasive membrane of the anchor cell in *C. elegans*. Nat Cell Biol 2009; 11:183-9; PMID:19098902; <http://dx.doi.org/10.1038/ncb1825>