Translation-dependent mRNA localization to *Caenorhabditis elegans* adherens junctions

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Summary statement

An smFISH-based survey identified a subset of mRNAs coding for junctional components that localize at or in proximity of the adherens junction through a translation-dependent mechanism.

Abstract

mRNA localization is an evolutionarily widespread phenomenon that can facilitate subcellular protein targeting. Extensive work has focused on mRNA targeting through “zip-codes” within untranslated regions (UTRs), while much less is known about translation-dependent cues. Here, we examine mRNA localization in *Caenorhabditis elegans* embryonic epithelia. From an smFISH-based survey, we identified mRNAs associated with the cell membrane or cortex, and with apical junctions in a stage- and cell type-specific manner. Mutational analyses for one of
these transcripts, *dlg-1/disks large*, revealed that it relied on a translation-dependent process and did not require its 5’ or 3’UTRs. We suggest a model in which *dlg-1* transcripts are co-translationally localized with the nascent protein: first the translating complex goes to the cell membrane using sequences located at the carboxy-terminus/3’ end, and then apically using amino-terminal/5’ sequences. These studies identify a translation-based process for mRNA localization within developing epithelia and determine the necessary cis-acting sequences for *dlg-1* mRNA targeting.

**Introduction**

mRNA localization is an efficient means to place the associated translation products in the appropriate subcellular location (Ephrussi et al., 1991; Lecuyer et al., 2007; Takizawa & Herskowitz, 1997). Large-scale studies in diverse organisms have revealed that many mRNAs are enriched at specific subcellular loci (Jambor et al., 2015; Lecuyer et al., 2007). This mechanism is essential to establish embryonic patterning (Frigerio et al., 1986; Rebagliati et al., 1985), distribute determinants asymmetrically in precursor cells (Broadus et al., 1998; Li et al., 1997), and segregate functionally distinct compartments in differentiated and polarized cells like neurons or epithelial cells (Ryder & Lerit, 2018). For example, a global analysis of localized mRNAs in murine intestinal epithelia found that 30% of highly expressed transcripts were polarized, and that their localization coincided with highly abundant regions in ribosomes (Moor, 2017). The frequent close apposition of mRNAs and their translated proteins indicates that one function of mRNA localization is to enrich proteins at their final destinations through localized translation (Das et al., 2021; Ryder & Lerit, 2018). However, other functions exist, such as targeted protein degradation (Chouaib et al., 2020), translational repression (Kourtidis et al., 2017), and RNA stabilization or storage (Standart & Weil, 2018).

Cells use a variety of mechanisms to position mRNAs within cells. UTRs often harbor localization elements (“zip-codes”) that dictate where an mRNA should be delivered (Katz et al., 2012; Kislauskis et al., 1994; Nagaoka et al., 2012). Correct splicing and the presence of exon junction complexes (EJC) can also play a role in mRNA enrichment to certain subcellular localizations (Hachet & Ephrussi, 2004; Kwon et al., 2021). On the other hand, mRNAs coding for transmembrane or secreted proteins can be localized through a translation-dependent mechanism. For example, the localization factor Signal Recognition Particle binds the nascent signal peptide of endoplasmic reticulum (ER)-bound proteins, arrests cytoplasmic translation,
and docks at the ER. Translation is resumed after docking, and a transmembrane machinery allows the translocation of the fully synthetized proteins into the ER (Weis et al., 2013). More recently, studies with translation inhibitors puromycin and cycloheximide have implicated nascent peptides for mRNA localization to other membranous organelles or non-P body foci, but the exact mechanisms for delivery are unknown for most genes (Chouaib et al., 2020).

In *C. elegans*, mRNA localization has been studied mainly in the context of post-transcriptional gene silencing in membraneless organelles, specifically germline P granules, somatic P-bodies, and stress granules, where transcripts are stabilized (Scheckel et al., 2012), protected from degradation or small RNA-mediated gene silencing (Gallo et al., 2008; Ouyang et al., 2019; Shukla et al., 2020), or repressed translationally (Voronina, 2013). In these instances, mRNAs are commonly post-transcriptionally regulated and localized through their 3'UTRs (Parker et al., 2020; Wright et al., 2011). 3'UTR-dependent mRNA localization also occurs in axons of adult neurons (Yan et al., 2009), where mRNA localization is paired with local translation. However, not all localized mRNAs rely on their 3'UTRs. A recent study on the early *C. elegans* embryo demonstrated the dispensability of 3'UTR to localize at least two mRNAs (*erm-1* and *imb-2* (Parker et al., 2020)). However, the mechanisms that localize these mRNAs are currently unknown.

In this study, we focused on mRNA localization during development of *C. elegans* embryonic epithelia. *C. elegans* embryogenesis is highly stereotyped, giving rise to an invariant number and positioning of epithelial cells. Epithelial morphogenesis starts from the embryonic stage possessing eight endodermal cells (8E stage) (Sulston et al., 1983) when cell junctions, commonly referred to as the *C. elegans* Apical Junction (CeAJ) (McMahon et al., 2001), begin to form. CeAJs are fully established during the so-called bean and comma stages (names attributed to the early elongation stages based on the shape of the embryo (Sulston et al., 1983)). *C. elegans* possesses a single type of apical junction that comprises two adjacent adhesion systems, AS-I and AS-II (Bossinger et al., 2015). AS-I is composed of a Cadherin-Catenin Complex (CCC), constituted by HMR-1/E-Cadherin, HMP-1/α-Catenin, HMP-2/β-Catenin, and JAC-1/p120-Catenin, which links to intermediate filaments of the cytoskeleton and F-actin (Costa et al., 1998; Pettitt et al., 2003). Additional cytoskeletal organizers (e.g., SMA-1) contribute to the correct architecture of AS-I (McKeown et al., 1998). In AS-II, a DLG-1/Discs Large and AJM-1 Complex (DAC) provides a link between the proposed adhesion molecule of the AS-II, called SAX-7/L1CAM (Chen & Zhou, 2010), and cytoskeletal-associated components SMA-1/βH-Spectrin, ERM-1/Ezrin/Radixin/Moesin, and actin filaments (Bernadskaya et al., 2015).
2011; Gobel et al., 2004; McKeown et al., 1998; Van Furden et al., 2004). A series of evolutionarily conserved ancillary proteins (actin filaments, claudins, spectrins, PAR proteins, etc.) help form and maintain the CeAJs (Armenti & Nance, 2012).

To investigate the existence of mRNA localization during embryonic development, we conducted a single molecule fluorescence in situ hybridization (smFISH)-based survey on the C. elegans embryo and tested the localization of mRNAs coding for factors belonging to AS-I and AS-II, as well as for proteins involved in CeAJ formation and maintenance. We identified transcripts enriched at the CeAJ in a stage- and cell type-specific manner. Genetic and imaging analyses of transgenic lines for one of the identified localized mRNAs, dlg-1/discs large, mapped domains required for localization. Our data demonstrate that the dlg-1 UTRs are dispensable, whereas translation in cis is required for localization, therefore providing an example of a translation-dependent mechanism for mRNA delivery in C. elegans.

Results

mRNAs coding for the main components of the cell adhesion system II are enriched at the CeAJ

We began our analysis of mRNA localization by surveying twenty-five transcripts that code for the major factors involved in cell polarity and CeAJ formation, as well as some cytoskeletal components (Fig. 1A and Table S1) in epithelial cells during morphogenesis. The protein products of the tested mRNAs are localized differentially along the cell membrane/cortex and cytoplasm (Fig. 1A and Table S1). We identified epithelial cells and the CeAJ using a CRISPR-engineered DLG-1::GFP fusion (Heppert et al., 2018). Our survey revealed mRNAs with varying degrees of localization within epithelia, which we divided into three classes: CeAJ/membrane localized, perinuclearly localized, and unlocalized (Fig. 1B-G, S1, and S2 and Table S1). Five of these transcripts were enriched at specific loci at or near the cell membrane: laterally and at the CeAJ for dlg-1 (Fig. 1C for endogenous/GFP CRISPR-tagged dlg-1::gfp mRNA and S1A for endogenous/non-tagged dlg-1 mRNA), solely at the CeAJ for ajm-1 and erm-1 (Fig. 1D,E), apically and at the CeAJ for sma-1 (Fig. 1F), and apically for vab-10a (Fig. 1G). The degree of enrichment varied for these transcripts with some dramatically (ajm-1, dlg-1) and others only mildly enriched (erm-1). Interestingly, all these transcripts but vab-10a encode the main cytoplasmic components of the AS-II.
Beyond AS-II-coding and *vab-10a* transcripts belonging to the CeAJ/membrane localized mRNA class, our survey detected four mRNAs (*hmr-1, sax-7, eat-20, and let-805*) that showed a few instances of perinuclear localization (Fig. S1B). HMR-1/E-cadherin and SAX-7/L1CAM constitute the transmembrane components (putative for SAX-7) of the CCC and the DAC, respectively (Chen & Zhou, 2010; Costa et al., 1998). EAT-20, a Crumbs-like factor involved in apicobasal polarity, and LET-805/fibronectin 1 are transmembrane proteins as well (Armenti and Nance, 2012; Shibata et al., 1999). Bioinformatic analyses of their sequences confirmed the presence of signal peptides in all the four proteins (Methods). Therefore, the perinuclear localization of their transcripts likely reflects classical ER-associated translation (Hermesh & Jansen, 2013). The rest of our tested mRNAs did not possess any evident subcellular localization at any of the analyzed embryonic stages/tissues and were not further investigated (Fig. S2 and Table S1). Taken together, our smFISH survey revealed nine localized mRNAs, five at the cell membrane and four perinuclear. These data indicate that mRNA membrane localization is a feature of the AS-II cell adhesion system, except for the putative transmembrane protein-coding *sax-7* and *actin* mRNAs.

**dlg-1 and ajm-1 mRNA enrichment at the apical junction varies in a stage- and cell type-specific manner**

Close examination of the smFISH data showed that mRNA localization varied in a stage- and cell type-specific manner, including transcripts encoding components of the same complex. Specifically, DLG-1 and AJM-1 form a complex (Bossinger et al., 2001) yet differed in the spatiotemporal localization of their mRNAs during epithelial morphogenesis (Fig. 2A,B). *dlg-1* and *ajm-1* start to be expressed at the 4E embryonic stage (Von Stetina et al., 2017). Although epidermal CeAJ (eCeAJ) do not exist yet at the 4E stage (Fig. 2A, upper-most panels), we detected some *dlg-1* mRNA localized near the cell membrane, marked by the basolateral factor LET-413 (Fig. S3A). During eCeAJ maturation and formation (8E, 16E, and bean stages), *dlg-1* and *ajm-1* mRNAs showed a peak in enrichment at or next to the membrane (Fig. 2A,B; Table S2), although *dlg-1* mRNA was more enriched than *ajm-1* (81 versus 55% at the 16E stage (negative control, *jac-1*: 30%), and 77 versus 57% at the bean stage (negative control, *jac-1*: 26%)). When eCeAJ were fully established (comma and 1.5-fold stages), forming the typical continuous and circumferential belt-like structure at the apical side of the cell membrane, both *dlg-1* and *ajm-1* showed a decrease in enrichment, although *dlg-1* was consistently more
enriched than \textit{ajm-1} (54 versus 41\% at the comma stage (negative control, \textit{jac-1}: 24\%), and 58 versus 42\% at the 1.5-fold stage (negative control, \textit{jac-1}: 26\%); Fig. 2A,B; Table S2).

Analyses of transverse sections of lateral membranes of epidermal (seam) cells at the bean stage demonstrated that \textit{dlg-1} mRNA did not only co-localize with the CeAJ but was also present laterally (Fig. S3B). The lateral localization of \textit{dlg-1} mRNA diminished at later stages of development (comma stage) in favor of a more consistent co-localization with the fully mature CeAJ (Fig. S3C).

Morphogenesis of the digestive track showed a different pattern for \textit{dlg-1} and \textit{ajm-1} mRNA junctional localization (Fig. 2C,D). Visually, during foregut or pharyngeal CeAJ (pCeAJ) maturation at the 16E stage, and after full formation at the bean stage, \textit{dlg-1} and \textit{ajm-1} mRNA were only mildly colocalizing with CeAJ markers (Fig. 2C). Only at the comma stage, when pCeAJ were fully established, a higher degree of localized mRNA could be observed, especially for \textit{ajm-1} mRNA (61\% for \textit{dlg-1} and 68\% for \textit{ajm-1} (negative control, \textit{jac-1}: 29\%); Fig. 2C,D; Table S2). At later stages of pharyngeal morphogenesis (1.5-fold stage), as observed for the epidermis, mRNA enrichment at the pCeAJ decreased gradually (47\% for \textit{dlg-1} and 58\% for \textit{ajm-1} (negative control, \textit{magi-1}: 20\%); Fig. 2C,D; Table S2). These data demonstrate enrichment at the CeAJ for two of our identified localized mRNAs at distinct stages and cell types of embryogenesis.

Localization of \textit{dlg-1} mRNA at the CeAJ does not depend on its UTRs.

mRNA localization commonly involves recognition of zip-codes located within UTRs (Chaudhuri et al., 2020; Jambhekar & Derisi, 2007). To test whether the localization of one of the identified localized mRNAs, \textit{dlg-1}, relied on zip-codes, we generated extrachromosomal transgenic lines carrying a \textit{dlg-1} gene whose sequence (exons and introns) was fused to an in-frame GFP and to endogenous or exogenous UTRs (Fig. 3A). We used UTRs from mRNAs that do not localize near cell membranes, namely \textit{sax-7} and \textit{unc-54} (Fig. S4A,B). One construct (“3'UTR” reporter) substituted the endogenous \textit{dlg-1} 3'UTR with an \textit{unc-54} 3'UTR (Lockwood et al., 2008; McMahon et al., 2001), and a second construct exchanged both the endogenous 5' and 3'UTRs (“5'-3'UTRs” reporter) by additionally substituting the endogenous \textit{dlg-1} 5'UTR with a \textit{sax-7} 5'UTR to the 3'UTR reporter construct (Fig. 3A). The transgenic constructs were expressed in a wild-type background, and smFISH experiments were conducted with probes against the GFP RNA sequence to assess specifically the localization of the transgenic \textit{dlg-1::gfp} mRNAs (cr.dlg-
1::gfp and tg.dlg-1::gfp). The mRNA localization patterns of the two UTR reporters were compared to the localization of dlg-1::gfp transcripts from the CRISPR line (“wild-type”, Fig. 3A; Heppert et al., 2018). Both reporter strains were enriched at the CeAJ and resembled the wild-type cr.dlg-1::gfp (means: wild-type = 60%; 3’UTR = 71%; 5’-3’UTR = 74%; Fig. 3B,C; Table S2). A slight increase in mRNA localization for the two reporter strains may reflect their different transgenic nature (extra-chromosomal) compared to the wild-type reference (CRISPR). These results indicate that the UTR sequences of dlg-1 mRNA are not required for localization.

Localization of dlg-1 mRNA at the CeAJ is translation-dependent.

Co-translational mechanisms for mRNA delivery have been described for mRNA encoding transmembrane and secreted proteins (Nyathi et al., 2013). Recent studies have suggested that co-translational mRNA localization can also exist for transcripts encoding proteins in other subcellular locations (Chouaib et al., 2020; Li et al., 2021; et al., 2018; Safieddine et al., 2021; Sepulveda et al., 2018). To determine whether dlg-1 mRNA localization occurs co-translationally, we designed a transgene (tg) to interfere with normal translation by deleting two nucleotides (TG) within the start codon of an otherwise wild-type sequence that contained both exons and introns (Fig. 4A,B). Ribosomes scanning transgene mRNA from the 5’ end would encounter two new AUG start codons that are each out-of-frame compared to the wild-type (Fig. 4B). The first in-frame AUG after the deletion is located at position 47 (Fig. 4A,B and S5A; Table S3). We generated transgenic lines in a smg-2 mutant strain (Hodgkin et al., 1989) to avoid mRNA degradation by nonsense-mediated decay (NMD), which recognizes and destroys mRNAs with precocious translation termination (Huang and Wilkinson, 2012; Mango, 2001). As a control, we verified by smFISH that wild-type tg.dlg-1 mRNA was localized normally in smg-2 mutant embryos, demonstrating that NMD does not interfere with targeting dlg-1 transcripts to the CeAJ (Figure 4C,D,F).

Next, we examined our non-translatable dlg-1::gfp mRNA (“ΔATG”) in smg-2 mutant embryos using smFISH paired with fluorescence analysis for DLG-1::GFP protein, to tract the degree of in-frame translation. The carboxy-terminal position of the GFP moiety ensures that mRNAs that initiate in-frame translation anywhere within the DLG-1 coding sequence are GFP-positive. AJM-1 antibody staining was used to identify the CeAJ (Francis and Waterston, 1991; Mohler et al., 1998). The embryos of one of our ΔATG transgenic lines (line1) lacked any detectable DLG-1::GFP protein and displayed a dramatic decrease of mRNA at the CeAJ.
compared to controls (means: full-length; wild-type = 72%; full-length; smg-2 = 70%; ΔATG; smg-2 = 18%; Fig. 4E,F; Table S2). We conclude that translation is required for mRNA localization. Embryos from our second ΔATG transgenic line (line2) displayed a little GFP protein (Fig. S5B,C). We speculate that truncated DLG-1 protein may be generated by one or more of the ten alternative in-frame AUGs that can be found within the dlg-1 mRNA (Fig. 4A and S5A; Table S3). For the second line, we observed some mRNA localized at the CeAJ, and it was always in proximity to DLG-1::GFP protein (Fig. S5B). These data suggest that dlg-1 mRNA localization depends on its ongoing translation (e.g., line 1; Fig. 4E,F), and that even low amounts of translation are sufficient for mRNA delivery to its final location (e.g., line 2; Fig. S5B).

As a second test for the involvement of translation in dlg-1 mRNA localization, we inhibited total translation in a drug-free manner. Drugs like cycloheximide or puromycin, commonly used to block translation, do not penetrate the worm embryo eggshell easily. Instead, we exposed the *C. elegans* embryos to heat. One of the early responses to heat stress is a block of translation caused by “ribosome drop-off” (Spriggs et al., 2010), leading to a global decrease in polysome occupancy (Arnold et al., 2014). Embryos from our *dlg-1::gfp* CRISPR line grown at 20°C for generations were subjected to a 1-hour heat-shock at 34°C or 37°C on plates and immediately processed for smFISH experiments (Fig. S6A-C). In both conditions, we observed a significant loss in mRNA localization at the CeAJ (means: 20°C = 65%; 34°C = 27%; 37°C = 19%; Fig. S6D and Table S2). These results show the loss of mRNA localization upon heat-shock. In both the ATG deletion strains and the heat-shock conditions, endogenous DLG-1 was present at the CeAJ (Figure S6 and data not shown). Therefore, we conclude that translation of dlg-1 mRNA in cis is required for enrichment at the CeAJ.

The carboxy-terminus is necessary and key for mRNA localization to the membrane.

Given the requirement for translation to localize dlg-1 mRNA, we considered mRNA targeting in the context of the DLG-1 protein that would be produced. DLG-1 is a complex protein, with different domains that establish protein localization and function, as diagrammed in Figure 5 (Fig. 5A (Firestein & Rongo, 2001)). To define critical regions for mRNA localization, we deleted these domains using existing (Firestein & Rongo, 2001; Lockwood et al., 2008) and newly generated transgenic lines (Methods) (Table S4). Immuno-staining for endogenous AJM-1 provided a spatial reference for the CeAJ that was unaffected in any of our transgenic strains,
which also expressed endogenous, wild-type DLG-1. smFISH with GFP probes were specific for transgenic *dlg-1::GFP* ("tg.dlg-1"), and GFP fluorescence from transgenic DLG-1 ("tg.DLG-1") provided a readout for the localization of the transgenic protein. We began by analyzing images of top views of epidermal seam cells to determine association to lateral membranes. We focused on the bean stage, when wild-type *dlg-1* RNA is highly localized (Fig. 5B-F and Fig. 2). Fluorescent images and quantitation of our full-length (FL) control revealed lateral enrichment of *tg.dlg-1FL* mRNA (Fig. 5B; mean = 74%; Table S2). Such lateral and CeAJ enrichment of *tg.dlg-1FL* mRNA resembled what was observed with the CRISPR line, indicating that transgenes reflected appropriate regulation (Fig. S3B).

First, we examined the amino-terminal domains. The L27 protein domain is involved in DLG-1 multimerization as well as interactions with the DAC component, AJM-1 (Firestein & Rongo, 2001; Lockwood et al., 2008). Removal of the sole L27 domain (ΔL27; Fig. 5C) did not significantly impair the lateral enrichment of *tg.dlg-1ΔL27* mRNA compared to the full-length (Fig. 5C; mean = 69%; Table S2). These data suggest that the L27 domain sequences make minor contributions to the accumulation of *dlg-1* mRNA to the lateral membranes. Given the interactions of the L27 domain with junctional proteins, this effect may reflect detachment of mutant DLG-1 protein and mRNA from junctions.

A larger amino-terminal truncation removed the PDZ domains as well as the L27 domain, but left the SH3, Hook, and GuK domains intact (SH3-cHk-GuK; Fig. 5D). *tg.dlg-1SH3-cHk-GuK* mRNA was also enriched laterally to a degree that was similar to the ΔL27 line (Fig. 5D; mean = 66%; Table S2). These data suggest that the PDZ domains are not required for lateral mRNA enrichment. We speculate that the carboxy-terminal sequences are largely sufficient to direct *dlg-1* mRNA to lateral membranes.

We examined a construct lacking the Hook and GuK domains (ΔcHk-GuK; Fig. 5E) and observed a significant decrease in mRNA lateral localization compared to both the full-length and the SH3-cHk-GuK construct (Fig. 5E; mean = 54%; Table S2), but higher than those of an unlocalized mRNA (Fig. S7A,B; mean = 41%; Table S2). These data suggest that Hook and GuK domains are key for *dlg-1* mRNA localization to the lateral membrane, but that additional, amino-terminal sequences contribute somewhat. As the Hook and GuK domains have no known role in protein localization (Lockwood et al., 2008), these data reveal a new role for these sequences in localization,
This hypothesis was confirmed when we examined another carboxy-terminal truncation where the SH3 and third PDZ domain were removed in addition to the carboxy-terminus (L27-PDZ1/2; Fig. 5F). \textit{tg.dlg-1}^{L27-PDZ1/2} mRNA lateral localization was highly impaired and reached the levels of an unlocalized mRNA (Fig. 5F and S7B; mean = 43%; Table S2). Together with the previous data, these observations indicate that the Hook and GuK domains are essential for \textit{dlg-1} mRNA localization and account for the large majority of lateral membrane localization of \textit{dlg-1} mRNA, in conjunction with the SH3 domain.

\textit{The amino-terminus is involved in mRNA enrichment at the junction once the mRNA has reached the membrane.}

We examined the apicobasal and junctional distribution of \textit{dlg-1} mRNAs, by analyzing frontal plane views, paired to apicobasal and apical intensity profile analyses (Fig. 6). Fluorescent images of our full-length control showed lateral and CeAJ enrichment of \textit{tg.dlg-1}^{FL} mRNA and CeAJ localization for the corresponding \textit{tg.DLG-1}^{FL} protein (Fig. 6D). These data were confirmed by intensity profile analyses where mRNA peaks (green) largely overlapped with protein peaks (magenta), and these were located at the CeAJ (pink vertical lines) in both apicobasal and apical profiles (Fig. 6D',D''). A few minor mRNA peaks were observed in the apicobasal intensity profile between the CeAJ peaks, indicating a few cytoplasmic mRNAs that might represent mRNA in transit from the nucleus to their final location (Fig. 6D'). Other RNAs were observed close to the CeAJ peaks in the apical intensity profile (Fig. 6D''). These data indicate the bulk of \textit{dlg-1} mRNA is associated with the CeAJ and lateral surfaces.

Next, we analyzed the same transgenic lines examined in Figure 5, starting from the amino-terminus. Deletion of the L27 domain (involved in DLG-1 multimerization and AJM-1 interaction (Firestein & Rongo, 2001; Lockwood et al., 2008)) showed \textit{tg.DLG-1}^{ΔL27} protein localized more broadly along the whole membrane compared to the full-length mRNA, with only partial enrichment at the CeAJ (Fig. 6E). Similar to its encoded protein, \textit{tg.dlg-1}^{ΔL27} mRNA distribution was scattered along the whole membrane (Fig. 6E). Intensity profile analyses confirmed the broader membrane distribution of \textit{tg.DLG-1}^{ΔL27} and the presence of \textit{tg.dlg-1}^{ΔL27} mRNA along the lateral (and partially apical and basal) membranes. Some \textit{tg.dlg-1} mRNA was enriched at the CeAJ, but less than the full-length, and some was cytoplasmic mRNA, more than the full-length (Fig. 6E',E''). These data suggest that the L27 domain contributes to the accumulation of \textit{dlg-1} mRNA and protein at the CeAJ.
The larger amino-terminal truncation, leaving the sole SH3, Hook, and GuK domains intact, showed a broader distribution of \(\text{tlg.DLG-1}\) at the lateral membrane without any enrichment at the CeAJ. \(\text{tg.dlg-1}\) mRNA was also distributed along the membrane, especially laterally (Fig. 6F,F'). This broader distribution along the whole membrane could not be addressed with the sole top views (Fig. 5). The apicobasal view suggests that the mRNA not seen laterally in Fig. 5 may reflect localization at apical or basal membranes. These data show that the carboxy-terminal sequences are sufficient to direct \(\text{dlg-1}\) RNA and protein to the membrane (as also seen in Fig. 5D), but that the amino-terminal L27 and PDZ domains are important for targeting mRNA and protein to the CeAJ (Fig. 6F,F'').

The correlation between mRNA and protein localization for the constructs described above suggests that either protein dictates mRNA localization or translation occurs at defined locations. This correlation was lost for constructs lacking the Hook and GuK domain (Fig. 6G,G',G''). Deletion of the Hook and GuK domains did not affect \(\text{tlg.DLG-1}\) protein localization but did impair CeAJ enrichment of mRNA (Fig. 6G,G',G''). The mRNA was detected at all membrane surfaces: lateral, apical, and basal (Fig. 6G). Other mRNA was detected adjacent to the membrane, but not overlapping, and in the cytoplasm (Fig. 6G,G'). Similar to Fig. 6F, this apicobasal view suggests that mRNA that was not located laterally in top views may also reflect its location at the apical and basal membranes. These data suggest that sequences within the Hook and GuK domains help target \(\text{dlg-1}\) mRNA to the membrane. DLG-1 protein localized at the junction in this mutant strain may reflect localized translation for the subset of \(\text{dlg-1}\) mRNAs at lateral and junctional surfaces or post-translational movement of protein to the junction.

Further removal of sequences from the carboxy-terminus impaired \(\text{tlg.DLG-1}\) protein and \(\text{tg.dlg-1}\) mRNA localization. The construct containing the L27 and the first two PDZ domains produced transgenic protein and mRNA present all over the membrane and in the cytoplasm (Fig. 6H). In intensity profile analysis, mRNA and protein peaks showed minimal overlap, confirming the broader and more randomized distribution observed in the fluorescent images (Fig. 6H',H''). These data suggest that \(\text{dlg-1}\) mRNA and protein can accumulate in ectopic locations without degradation, at least for the amino-terminal half of the protein.

In conclusion, the structure-function analyses revealed that the Hook and GuK domains were required to localize \(\text{dlg-1}\) mRNA and, together with the SH3 domain, sufficient for both protein and mRNA localization to lateral membranes. In addition, PDZ domains together with the L27 were necessary, but not sufficient, to bring DLG-1 and \(\text{dlg-1}\) to the CeAJ (Fig. 5,6).
Discussion

This study has made three contributions towards understanding RNA localization in *C. elegans* embryos. First, the cytoplasmic mRNAs within adhesion system II of the CeAJ are localized within epithelial cells. Second, localization of *dlg-1* mRNA depends on translation in *cis*, and not UTR zip-codes. Third, specific regions within *dlg-1/DLG-1* carboxy-terminus dictate localization at the membrane and, separately, to the CeAJ.

We conducted an smFISH-based survey, which identified endogenous mRNAs that are localized at or near the cell membrane (five out of twenty-five tested mRNAs). Localized mRNA in *C. elegans* has been observed for maternally provided mRNAs in early embryos (Parker et al., 2020) and for RNAs in synapses of adult neurons (Yan et al., 2009). Very recently, a new study addressed membrane-associated localization of mRNA through a PP7/PCP-tagging approach in later embryonic stages and found five mRNA (*erm-1, pgp-1, magu-2, let-413*, and *ajm-1*) to be enriched at these loci (Li et al., 2021). Previous studies of *Drosophila* embryogenesis have shown that many mRNAs are localized subcellularly, including mRNAs coding for cytoskeletal and junctional components (Jambor et al., 2015; Lecuyer et al., 2007; Ryder & Lerit, 2018). Among them, β-actin, *E-cadherin*, and zo-1 mRNAs are localized at the cell cortex of epithelial cells (Ryder & Lerit, 2018). We did not observe orthologues of these mRNAs being cortically localized, suggesting species or cell-type specific differences. Nevertheless, we identified other localized mRNAs, four of which (*dlg-1, ajm-1, erm-1*, and *sma-1*) code for factors that are functionally linked within the AS-II. The transmembrane protein SAX-7 is also supposedly localized in the AS-II, but its mRNA was not membrane associated. Instead, we observed perinuclear sax-7 mRNA, suggesting SAX-7 is localized via the ER.

mRNAs coded by orthologues of *dlg-1* also show a defined subcellular localization in other species in polarized cells such as embryonic cells or neurons. For example, *Drosophila dlg1* mRNA associates transiently to membranes during embryogenesis (mitotic cycle 14): laterally at stage 5 (cellularization) when membranes start to form around nuclei, all around the cell membrane at stages 6 and 7 (cellularization and gastrulation), and it becomes unlocalized at later stages (Lecuyer et al., 2007). In zebrafish, neuronal *dlg1* mRNA localizes stably in myelin sheets of fully differentiated oligodendrocytes (Yergert et al., 2021). These data suggest that *dlg1* mRNA localization may have pivotal roles in development. While *dlg-1* is the most broadly studied, one other mRNA uncovered in our survey, *erm-1*, has been shown to localize in another context. Specifically, *erm-1* mRNA is targeted to the cell periphery in the early *C.
C. elegans embryo (Parker et al., 2020), providing an additional example of membrane enriched mRNA in different developmental contexts.

Localization of **dlg-1** mRNA depended on its translation rather than its UTRs. Substitution of both the 5' and 3'UTRs with UTRs of unlocalized mRNAs did not disrupt association of **dlg-1** mRNA with the CeAJ. This result mirrors orthologues of **dlg-1** in other species, which also do not require their UTRs for subcellular localization (Yergert et al., 2021). Thus, **dlg1** mRNAs are frequently localized within cells, but the mechanism of UTR-independent targeting of this transcripts is unknown for any species. We found that **dlg-1** mRNA required its coding sequences and translation in cis. We analyzed two *C. elegans* lines expressing a full-length **dlg-1::GFP** with the normal ATG deleted (ΔATG). One of these produced no protein and had no mRNA localization, demonstrating the importance of translation. This line demonstrated that DLG-1 protein supplied in trans was not sufficient for targeting to the CeAJ, as all our transgenic lines were analyzed in embryos expressing wild-type, endogenous DLG-1. The second line fortuitously produced a little protein in some cells. In these expressing cells, both protein and mRNA were occasionally localized, suggesting that even a little translation was sufficient to target **dlg-1** mRNA to the membrane and CeAJ.

Besides the ATG mutation, the remainder of the **dlg-1ΔATG** gene was wild-type, including the intron-exon sequences. This wild-type configuration reveals that sequences and complexes associated with the EJC are not sufficient to localize **dlg-1** mRNA. Thus, **dlg-1** likely differs from mRNAs like **oskar** in *Drosophila*, where splicing generates a localization element and EJC binding site, which together target **oskar** mRNA within oocytes (Ghosh et al., 2012).

Our structure function analyses highlighted two pathways for mRNA localization: one dependent on the carboxy-terminus (SH3, Hook, and GuK domains), and a second dependent on the amino-terminus (L27-PDZ domains). The first pathway relied on protein sequences that are known to target and maintain DLG-1 protein to the membrane (SH3) and on the Hook and GuK domain with unknown function. A minimal sequence containing these three domains accounted for the vast majority of localized **dlg-1** mRNA to the membranes (mostly lateral). Complementarily, loss of SH3, Hook, and GuK domains (together with the third PDZ) fully impaired **dlg-1** mRNA localization. We note that the location of the Hook and GuK domains at the carboxy-terminus demonstrates that **dlg-1** mRNA is not delivered analogously to ER targeting, where translation is arrested after translation of amino-terminal signal sequences and resumes when mRNAs are docked at the ER. However, a modified co-translational pathway is a possibility. For example, after the SH3 domain is translated, the translating complex, including
the mRNA, could be delivered to the lateral membranes even in the absence of a full round of translation. This model fits with the known requirement of the SH3 domain for lateral distribution of DLG-1 protein (Lockwood et al., 2008), but does not yet explain the role of the Hook and GuK.

Loss of Hook and GuK sequences showed normal protein localization even in a mutant background. Since a small proportion of this mutant mRNA was found at or near lateral membranes, it is possible that only this cohort was translated. This model would explain the lack of mutant DLG-1 protein observed near mis-localized mRNA. Alternatively, mutant DLG-1 could be translated ubiquitously in epidermal cells and then transported rapidly to the CeAJ. We note that previous studies have shown that the Hook and GuK domains are essential for viability, but the reason is unknown (Lockwood et al., 2008). One intriguing possibility is that the lack of mRNA localization affects DLG-1 levels or turnover. Alternatively, these domains have other physiological roles that have not yet been discovered.

Once at the lateral membrane, a second step may deliver mRNA and protein to the CeAJ. For example, DLG-1 associates with itself and with AJM-1 via the L27 domain, and the PDZ domains are involved in DLG-1 junctional association (Lockwood et al., 2008). Thus, these amino-terminal sequences may either target DLG-1/dlg-1 from lateral surfaces to the CeAJ or retain DLG-1 at the junction.

In summary, our findings suggest a two-step model for targeting a translating dlg-1 mRNA to the CeAJ: first, the carboxy-terminal part of the DLG-1 protein brings the translating complex to the membranes, then the amino-terminus pushes it apically to the CeAJ. None of these domain combinations was sufficient to target dlg-1 mRNA to the junction, suggesting these processes synergize to place dlg-1 mRNA at the CeAJ during embryonic epithelium formation.

Materials and methods

Nematode culture

All animal strains were maintained as previously described (Brenner, 1974) at 20°C. Transgenic lines containing extrachromosomal arrays were grown at 15°C to reduce transgene overexpression. Some lines containing extrachromosomal arrays presented instances of mosaicism and differential expression levels among cells due to their extrachromosomal nature. Therefore,
we focused on cells with consistent patterns of expression. For a full list of alleles and transgenic lines, see Table S4.

**Heat-shock experiments**

Heat-shock experiments (duplicates) were performed on ML2615: the agar of each plate, with a high amount of laid embryos, was split into three new plates. Each new plate was placed for one hour at the three different temperatures for the experiment (20°C, 34°C, and 37°C). Embryos were then collected and processed following the smFISH protocol.

**Generation of transgenic lines**

*dlg-1* deletion constructs ΔATG (SM2664 and SM2663) and ΔL27-PDZs (SM2641) were generated by overlap extension PCR using pML902 as a template. Oligos used to generate ΔATG: PCR1_Fw agaggatccagctccacactaac; ΔATG_PCR1_Rv tgactcgtggatgctccttcttcgg; ΔATG_PCR2_Fw aagaggatccagctccacactaac; ΔATG_PCR2_Rv cgtacggccgactagtaggaac. Oligos used to generate ΔL27-PDZs: PCR1_Fw agaggatccagctccacactaac; ΔL27-PDZs_PCR1_Rv cgtacggccgactagtaggaac; ΔL27-PDZs_PCR2_Fw cgtacggccgactagtaggaac. For *dlg-1* 5'UTR replacement construct (“5'-3'UTRs”, SM2646), sax-7 5'UTR fragments were synthesized as ultramer duplex oligos ordered from IDT (sequence: aatttaatttttcagcttcagttcctccaaattttagaagacttcaggtcagttcctccacgagaagcttcaggtcagttcctccacgaca). Oligos used to generate 5'-3'UTRs: PCR1_Fw agaggatccagctccacactaac; 5'-3'UTRs_PCR1_Rv_a cgttgcagctttctctcctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctctc
smFISH, immunostaining, and microscopy

smFISH experiments were all done in triplicates but those for the heat-shock experiments (Fig. S6; duplicates). smFISH was adapted from (Tsanov et al., 2016). Custom Stellaris smFISH probes labeled with Quasar 570 dye were designed against par-3, par-6, hmp-1, and erm-1 mRNAs using the Stellaris FISH Probe Designer (Biosearch Technologies, Petaluma, CA). Probes against the other mRNAs were designed following the smiFISH approach as previously described (Tsanov et al., 2016). Each open reading frame was run through the Oligostan script in RStudio and 12 - 24 IDT primary smFISH probes were ordered for each mRNA (100 µM in IDTE, pH 8.0; IDT). All probes were designed against the endogenous mRNA sequences but dlg-1, pkc-3, hmp-2, spc-1, let-805, and vab-10a, whose mRNA were detected with gfp probes in their corresponding transgenic lines (Table S4). Exception to this are Fig. S1A and S3A where we used probes against the endogenous dlg-1 mRNA. For a full list of primary probe sequences, see Table S5. Secondary probes (FLAP-Y) with a 5’-acrydite modification and a 3’-Atto565 or a 3’-Atto637 labels were ordered from IDT. An equimolar amount of each set of primary probes was pooled in a 1.5 ml Eppendorf tube and diluted 5 times with IDTE (pH 8.0) to reach a final concentration of 0.833 µM per probe. An in vitro pre-hybridization reaction was set up as follow: 4 µl of primary probe-set pool, 1 µl of secondary FLAP-Y probe, 2 µl of 10x NEBuffer 3 (cat#B7003, New England Biolabs), and 13 µl of water were incubated in a thermocycler (cat#EP950040025, Eppendorf) at 85°C for 3 minutes, 65°C for 3 minutes, and 25°C for 5 minutes. Pre-hybridized FLAP-Y smFISH probes could be placed at 4°C for storage. One 6 cm plate with gravid adults and laid embryos with little bacteria lawn left were washed with 1 ml of water. Adults and larvae were discarded. An additional 1 ml of water was added to the plate. Laid embryos were gently scrubbed off with a gloved finger and transferred to a 1.5 ml Eppendorf tube. A gentle “short” 6-second spin was applied to the Eppendorf tube to pellet the embryos to minimize stress. Extra liquid was removed and embryos were allowed to rest for 10 minutes. Embryos were transferred on poly-L-lysine-coated (cat#P8920, Sigma) slides (cat#ER-303B-CE24, Thermo Scientific) and allowed to settle. Excess water was removed and 50 µl fix (1% PFA in PBS with 0.05% Triton) was added and incubated for 15 minutes. After removing the fixative solution and adding a coverslip, slides were quickly transferred on a metal plate on dry ice and stored at -80°C over-night. After a freeze-crack, slides were immediately transferred in a Coplin jar with ice-cold methanol for 5 minutes. Subsequent washes with PBS (5 minutes), PBS with 0.5% Tween-20 (10 minutes, and 20 minutes), and again PBS (5 minutes) were applied to the slides. 100 µl of hybridization solution (dextran sulfate (10% W/V) in 1 part of Formamide, 1 of 20x SSC, and 8 of water) were applied to the sample area and slides were
then transferred in a humidity chamber and incubated for 1 hour at 37°C. After removal of the hybridization solution, 50 µl of new hybridization solution containing 1 µl of pre-hybridized FLAP-Y smFISH probes or 0.5 µl of Stellaris probes were applied to the sample area and slides were incubated again in a humidity chamber at 37°C for 4 hours in the dark. After incubation, hybridization solution was wicked off, and samples were washed twice with wash buffer (1 part of formamide, 1 of 20x SSC, and 8 of water). Slides with 100 µl of wash buffer were incubated for 1 hour at 37°C in a humidity chamber in the dark. The wash buffer was finally wicked off and samples were washed twice with wash buffer and mounted with 12 µl VECTASHIELD® Antifade Mounting Medium with DAPI (H-1200, Vector Laboratories). For AJM-1 antibody staining (MH27, DSHB, 1:100; Francis and Waterston, 1991) coupled to smFISH was needed, primary antibodies were added to the hybridization solution during the 4-hour incubation, and secondary antibodies (Alexafluor 546 goat anti-mouse: A-11030, Invitrogen, 1:250) to the wash buffer in the last 1-hour incubation. A widefield microscope FEI “MORE” with total internal reflection fluorescence (TIRF) and a Hamamatsu ORCA flash 4.0 cooled sCMOS camera and a Live Acquisition 2.5 software was used for capturing images. Pictures were deconvolved with the Huygens software and then processed in OMERO (https://www.openmicroscopy.org/omero/) or ImageJ (https://imagej.net/). Figures were prepared in Adobe Illustrator (https://www.adobe.com/).

Image analysis and quantitation

ImageJ (https://imagej.net/) was used for post processing (Z-stack/channel extrapolation and transversal projections (as in Fig. S3B',C')) and GFP intensities quantification (as in Fig. S5B). FISH-quant v.3 (Mueller et al., 2013) was used for image analysis and quantitation. The mRNA counts for each seam cell from top views in maximum intensity projections (Fig. S8A) were obtained by drawing outlines: (a) along the cell borders marked by DLG-1::GFP or AJM-1 (Fig. S8B,C) for “total” mRNA (Fig. S8D); (b) parallel to the cell border for “cytoplasmic + nuclear” mRNA (Fig. S8E,F); (c) around the DNA marked by DAPI staining (Fig. S8G) for “nuclear” mRNA (Fig. S8H). We implemented the FISHquant script (see Data availability) to allow us to define units (single mRNAs) to estimate the amount of mRNA per identified dot. Units were identified by drawing outlines around five of the lightest mRNA dots and averaging their intensities (“Amplitude” in FISHquant; Fig. S8I). The intensities of each identified dot was divided by the intensity of our unit. The difference between “total” and “cytoplasmic + nuclear” mRNA provided the value for “membrane” mRNA (enriched at or in the proximity of the lateral
In all the analyses but the one for figure 5, the amount of localized mRNA has been calculated as “membrane” mRNA divided by “total” mRNA. In figure 5, due to the high transcriptional signal in some of the transgenic lines, the amount of localized mRNA has been calculated as “membrane” mRNA divided by “total” minus “nuclear” mRNA. All the lines, including positive (“full-length”; Fig. 5B) and negative (jac-1; Fig. 5 and S7) controls, have been undergone the same type of analysis. This type of calculation was made to avoid underestimation of localized mRNA in the lines with a high transcriptional signal compared to the others. It needs to be taken into account that this type of calculation determines an overestimation of localized mRNA in all the lines analyzed (e.g., Fig. S7B). The mRNA counts for each pharynx from transversal views in maximum intensity projections (Fig. 2D) were obtained by drawing an “apical” outline along the apical side of all the pharyngeal cells marked by cr.DLG-1::GFP, and a “total” outline boxing the whole body of the pharyngeal cells taken into account with the “apical” outline. The amount of apically localized mRNA for pharynxes has been calculated as “apical” mRNA divided by “total” mRNA. Statistical analyses were performed in the software R (R Core Team, 2021; https://www.R-project.org/) and dot plots with box plots were generated with the ggplot2 package (Wickham, 2016; https://ggplot2.tidyverse.org). Each graph possesses a thick black line within the box that represents the median, two hinges for the first and third quartiles, two whiskers that define the upper and lower limits, and outlying dots are represented individually beyond the whiskers. A red dots represent the mean. A t-test was employed to test the statistical differences between the conditions analyzed.

**Intensity profile analyses**

Apicobasal and apical intensity profile analyses were performed extracting the data for signal intensities from the Multi Plot tool from ROI Manager of ImageJ (https://imagej.net/). Regions of interests (ROIs) were chosen as it has been schematically described in Fig. 6A,B. Signal intensities were acquired for *dlg-1::gfp* mRNA (channel 1), AJM-1 (channel 2), DLG-1::GFP (channel 3), and DNA (channel 4). To avoid the quantification of nuclear mRNA signal, not relevant for our analyses, we removed from the *dlg-1::gfp* channel the signal overlapping with the DNA staining (Fig. S9).
Protein sequence analysis

To identify putative signal peptide sequences in amino acid sequences of selected proteins, we took advantage of the SignalIP-5.0 Server (Center of Biological Sequence Analysis – CBS). Nucleotide or amino acid sequences analyses were performed with Expasy (Swiss Institute of Bioinformatic – SIB) or Clustal Omega (European Molecular Biology Laboratory-European Bioinformatic Institute – EMBL-EBI).

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Competing interests

The authors declare that they have no conflict of interest.

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Data availability

The modified FISH-quant script has been deposited on Zenodo (https://zenodo.org/record/5544017#.YVrzBtpBxaQ) with the DOI number 10.5281/zenodo.5544017.
References


Figure 1. Five mRNAs coding for DAC components, basolateral polarity factors, and a fibrous organelle-bound protein are enriched at the cell membrane. A. Simplified color-coded schematics of a *C. elegans* epithelial cell, highlighting the classes of factors involved in apicobasal polarity and maintenance of epithelial morphology. Black line: apical polarity factors.

B. Fluorescent micrographs of a *C. elegans* embryo at the comma stage (upper panels) and zoom-ins (lower panels) showing smFISH signal of an unlocalized mRNAs (*jac-1* – green), fluorescent signal of the endogenous CRISPR-engineered GFP-tagged DLG-1 protein (cr.DLG-1::GFP, magenta), and merges. To the left of the images, bars color-coded as in (A) to indicate the sub-class of the factor the mRNA codes for. C-G. Fluorescent micrographs of entire *C. elegans* embryos (left panels) and zoom-ins (right panels) showing smFISH signal of localized mRNAs (*dlg-1::gfp* in epidermal cells of a bean stage (C), *ajm-1* in pharyngeal cells of a late comma stage (D), *erm-1* in epidermal cells of a bean stage (E), *sma-1* in epidermal and pharyngeal cells of a late comma stage (F), and *vab-10a::gfp* in epidermal cells of a comma stage (G) – green), fluorescent signal of CeAJ markers (cr.DLG-1::GFP or endogenous AJM-1, magenta), and merges. Specific embryonic stages were selected for each transcript based on the highest degree of mRNA localization they exhibit. To the left of each image, bars color-coded as in (A) to indicate the sub-class of factors the mRNAs code for. White squares: portion of the embryo shown in the zoom-ins. Scale bar (entire embryos): 10 µm. Scale bars (zoom-ins): 5 µm.
Figure 2. *dlg-1* and *ajm-1* mRNA localization changes dynamically during epithelial morphogenesis. **A.** Left: names and schematics of the analyzed embryonic stages (4E: no junctions; 8E: nascent junctions; 16E: junction maturation; bean: junction formation; comma-1.5-fold: established junctions). Red squares: portion of the embryo shown on the right. Right panels: fluorescent micrographs of epidermal and seam cells of *C. elegans* embryos showing smFISH signal of two localized mRNAs, *dlg-1* (cr.dlg-1::gfp, green) and *ajm-1* (cyan), fluorescent signal of the CRISPR-engineered GFP-tagged DLG-1 protein (cr.DLG-1::GFP, magenta), and merges. Scale bars: 5 µm. **B.** Dot plot with box plot: each dot represents the percentage of laterally localized versus total cellular *jac-1* (unlocalized control, grey), *dlg-1::gfp* (green), and *ajm-1* (cyan) mRNAs (Y axis) in each seam cell analyzed at the stated embryonic
stages (X axis: 16E (n = 25), bean (n = 25), comma (n = 25), and 1.5-fold (n = 25)). Data derived from 5 different embryos for each stage. Here and in the next dot blots with box plots: a thick black line represents the median; the two hinges, first and third quartiles; the two whiskers define the upper and lower limits; outlying dots are represented individually. Red dots represent the mean. Significance of statistical analyses (t-test, two tails): n. s. = not significant; ** = < 0.01; *** = < 0.001. C. Same as in (A) but for pharyngeal cells. D. Dot plot with box plot: each dot represents the percentage of apically localized versus total cellular magi-1 (unlocalized control, grey), dlg-1::gfp (green), and ajm-1 (cyan) mRNAs (Y axis) in each pharynx analyzed at the stated embryonic stages (X axis: comma (n = 5 for magi-1 and n = 15 for dlg-1::gfp and ajm-1) and 1.5-fold (n = 5 for magi-1 and n = 15 for dlg-1::gfp and ajm-1). Significance of statistical analyses (t-test, two tails): * = < 0.05; ** = < 0.01; *** = < 0.001.
Figure 3. *dlg-1* endogenous 5' and 3'UTR are not required for its localization. A. Schematic representations of the three analyzed transgenes carrying a GFP-tagged *dlg-1* (comprising exons (“ex”) and introns (“int”) combined with endogenous or exogenous UTRs that are not competent to localize their own mRNAs. “wild-type”: CRISPR-engineered line with endogenous *dlg-1* 5' and 3'UTRs (black). “3’UTR”: multicopy extrachromosomal transgenic line with *dlg-1* 5'UTR (black) and *unc-54* 3'UTR (grey). “5'-3'UTRs”: multicopy extrachromosomal transgenic line with *sax-7* 5'UTR (white) and *unc-54* 3'UTR (grey). The schematics are not in scale with the actual size of the corresponding sequences. UTR lengths: *dlg-1* 5'UTR: 61 nucleotides; *sax-7* 5'UTR: 63 nucleotides; *dlg-1* 3'UTR: 815 nucleotides; *unc-54* 3'UTR: 280 nucleotides. B. Fluorescent micrographs of a lateral portion of seam and a ventral epithelial cells at the comma stage of *C. elegans* embryos showing smFISH signal of CRISPR or extrachromosomal transgenic *dlg-1* mRNAs (cr.*dlg-1::gfp* and tg.*dlg-1::gfp*, respectively, green), fluorescent signal of CRISPR or extrachromosomal transgenic GFP-tagged DLG-1 protein (cr.DLG-1::GFP and tg.DLG-1::GFP, magenta), and merges. Scale bars: 5 µm. C. Dot plot with box plot: each dot represents the percentage of laterally localized versus total cellular *dlg-1::gfp* mRNA in “wild-type” (black; mean = 60.13; standard deviation (StDev) = 15.70), “3’UTR” (grey; mean = 70.95; StDev = 13.16), and “5’-3’UTRs” (white; mean = 73.85; StDev = 16.18) (Y axis) in each seam cell analyzed at comma stages (n = 25 for each transgenic line). Data derived from 5 different embryos. Significance of statistical analyses (*t*-test, one tail): n. s. = not significant; ** = < 0.01.
Figure 4. *dlg-1* mRNA localization depends on its translation. A. Upper part: schematic representation of transgenic *dlg-1::gfp* mRNA and domain-coding portions. Magenta asterisks: the 11 possible in-frame AUG along the coding sequence. Red circle: the first AUG whose corresponding TG nucleotides were elicited from the transgenic “ΔATG” sequence. Scale bar: 150 nucleotides (nt). UTRs and GFP are not in scale. Lower part: zoom-in the L27-coding sequence. Magenta asterisks: the first 2 AUG belonging to the main frame (“Frame_1”). Blue asterisks: the first two AUG out-of-frame (“Frame_2”). Scale bar: 25 nucleotides (nt). B. Nucleotide sequence (green) and its corresponding amino acid translation for the first (magenta) and the second (blue) frames. The amino acid sequence highlighted in blue represents full coding sequences (from a methionine to a stop codon) that are out of frame (“Frame_2”).
amino acid sequence highlighted in magenta represents the first alternative in frame sequences in the transgenic “ΔATG”. In red: the two nucleotides elicited from the transgenic “ΔATG” sequence and the corresponding amino acid that cannot be translated in the two frames. **C-E.** Fluorescent micrographs of multicopy extrachromosomal transgenic lines of a lateral portion of seam and epidermal cells at a comma stage of *C. elegans* embryos. smFISH signal of wild-type (“full-length”, (C-D)) and altered ATG (“ΔATG”, line1 (E)) *tg.dlg-1::gfp* mRNAs (green), immunofluorescent signal of the endogenous AJM-1 protein (red), fluorescent signal of the corresponding *tg.DLG-1::GFP* protein (magenta), and merges. Corresponding genotypes are at the bottom: the “full-length” transgene in (C) is expressed in a wild-type background (“wild-type b.g.”); “full-length” and “ΔATG” transgenes in (D) and (E) are expressed in a null mutant background for an NMD component (“smg-2 b.g.”). Arrowheads indicate examples of localized mRNA. Scale bars: 5 µm. **F.** Dot plot with box plot: each dot represents the percentage of laterally localized versus total cellular *dlg-1::gfp* in “full-length; wild-type” (black; mean = 71.74; StDev = 16.88), “full-length; *smg-2*” (grey; mean = 69.61; StDev = 15.64), and “ΔATG; *smg-2*” (white; mean = 17.50; StDev = 22.80) mRNAs (Y axis) in each seam cell analyzed at comma stages (n = 25). Data derived from 5 different embryos. Significance of statistical analyses (*t*-test, two tails for “full-length; wild-type” versus “full-length; *smg-2*”, one tail for “full-length; *smg-2*” versus “ΔATG; *smg-2*”): n. s. = not significant; *** = < 0.001.
Figure 5. Specific domain-coding sequences of *dlg-1* mRNA are required for its normal lateral localization. **A.** Schematic representation of the full-length transgenic DLG-1::GFP protein, highlighting domains and their known functions (Firestein et al., 2001). Blue: L27 domain. Shades of grey: the three PDZ domains. Yellow: SH3 domain. Orange: the conserved stretch of the Hook domain (cHk). Red: GuK domain. Green: GFP, C-terminally tagged. Scale bar: 50 amino acids (aa). **B-F.** Left side of the panels: models showing schematic representations of wild-type (“full-length” (B)) and truncated versions of DLG-1 (ΔL27 (C), SH3-cHk-GuK (D), ΔcHk-GuK (E), and L27-PDZ1/2 (F)). Domains that are present in the transgene are depicted in magenta, deleted ones in light pink surrounded by black dashed lines. In green: mRNAs undergoing translation (with ribosomes in grey) whose size represent a rough estimation of their abundance, quantified at the very right of each panel. Small black parallel lines on the left of the cartoons represent the lateral cell membrane (CeAJ included). Middle part of the panels: top views of fluorescent micrographs (maximum intensity projection of seven Z-stacks) of a lateral portion of seam and epidermal cells at the bean stage of *C. elegans* embryos showing smFISH signal of transgenic *tg.dlg-1::gfp* mRNAs (green), immuno-fluorescent signal of the endogenous AJM-1 protein (red), fluorescent signal of the transgenic GFP-tagged DLG-1 protein.
protein coded by the corresponding transgene (magenta), and merges. The last merge images show mRNA with DNA (orange) to mark the nuclei. Arrowheads: examples of laterally localized mRNAs. Arrows: examples of fluorescent mRNA signal of overexpressed transgenes in the nucleus. Scale bars: 5 µm. Right side of the panels: horizontal dot plot with box plot: each dot represents the percentage of laterally localized versus cellular *tg.dlg-1::gfp* in the different lines analyzed (schematics of the domain structure as in (A) are on the left of each box plot).: “full-length” (blue; n = 25; mean = 74.22; StDev = 15.09), “ΔL27” (black; n = 25; mean = 69.21; StDev = 12.33), “SH3-cHk-GuK” (dark grey; n = 25; mean = 66.41; StDev = 17.71), “ΔcHk-GuK” (light grey; n = 25; mean = 54.22; StDev = 13.81), “L27-PDZ1/2” (white; n = 25; mean = 43.40; StDev = 20.19) mRNAs (Y axis) in each seam cell analyzed at bean stages. Data derived from five different embryos for each line (six for SH3-cHk-GuK). A vertical green dashed line represents the baseline of localization (40.98%) for an unlocalized mRNA, *jac-1*, determined with the same method used for the transgenic lines (see Fig. S7 and Table S2 for details). Significance of statistical analyses (*t*-test, one tail): n. s. = not significant; * = < 0.05; ** = < 0.01; *** = < 0.001.
Figure 6. Specific domain-coding sequences of *dlg-1* mRNA are required for its normal apicobasal localization. A. Schematic representation of the full-length transgenic DLG-1 protein as in Fig. 5A. GFP sequence not in scale. B. Schematic representation of a seam cell in 3D (grey cube). Magenta apical belt: CeAJ. A black rectangle shows a frontal plane view in the middle of the cell used to analyze the images in the rest of the figure. Light grey represents the cytoplasm, a dark grey filled circle the nucleus, and green filled circles mRNAs. C. Top: simplified schematics of the frontal view of a seam cell (B). Green circles: transgenic *dlg-1* mRNA. Magenta rectangles: transgenic DLG-1 protein. Highlighted in yellow (top left) and in blue (top right) the regions of the cell used for apicobasal and apical intensity profile analyses, respectively. Orange asterisks: mRNAs in the nuclei that have not been considered in the intensity profile analyses, as representing transcription sites. Blue asterisk: example of a cytoplasmic mRNA that would be considered in the apicobasal analysis, but not in the apical. Below: projections of mRNA and protein (and nucleus in the left side) present in the schematics above (same color-code). Bottom: exemplified intensity profile graphs based on the projections.
above, where peaks show the positions of transgenic *dlg-1* mRNA (green line) and transgenic DLG-1 protein (magenta). X axis: width of the cell; Y axis: fluorescent intensity. The grey box in the left graph represents the projection of the nucleus whose intensities have been removed from the analysis. **D–H.** Frontal plane views of fluorescent micrographs of three adjacent seam cells at the bean stage of *C. elegans* embryos showing smFISH signal of transgenic *tg.dlg-1-gfp* mRNAs (full-length (D), ΔL27 (E), SH3-chK-GuK (F), ΔChK-GuK (G), and L27-PDZ1/2 (H) (green)), immuno-fluorescent signal of the endogenous AJM-1 protein (red), fluorescent signal of the corresponding transgenic GFP-tagged DLG-1 protein (magenta), and merges. Arrowheads: CeAJ localization. Shaded grey shapes cover the nuclear regions to avoid focusing on transcriptional or general nuclear mRNA signals not relevant for the study. Orange asterisks: unspecific signal staining the eggshell. Scale bars: 5 µm. Right side: simplified schematics based on the fluorescent images on the right. Frontal view of a seam cell (rectangle) modelling transgenic mRNA and protein localizations. Green circles: transgenic *dlg-1* mRNA. Shades of magenta: varying degrees of transgenic DLG-1 protein along the membrane (borders) and in the cytoplasm (middle part). **D’–H’.** Intensity profile graphs of three contiguous cells (apicobasal profile (highlighted in yellow) explained in (C) (left side) for the corresponding fluorescent images on the left (D–H). X axis: cell width (µm); Y axis: measured fluorescent intensities. Green lines: transgenic *dlg-1* mRNA intensities. Magenta lines: transgenic DLG-1 protein intensities. Pink vertical lines: location of the CeAJ, identified by peak values for the intensity profile of AJM-1 fluorescent signal (not shown). Light grey panels: nuclei locations that have been evicted from the mRNA channel to avoid quantification of transcriptional signal, corresponding to the localization of the shaded grey circles in the fluorescent images. **D”–H”.** Intensity profile graphs of the sole apical part of the same cells analyzed on the left (apical profile (highlighted in blue) explained in (C – right side)). Axes and color-codes as in (D’–H’).
**Fig. S1.** Endogenous *dlg-1* is enriched at the CeAJ; transmembrane protein-coding mRNAs show instances of perinuclear localization. **A.** Fluorescent micrographs of the entire *C. elegans* embryos (upper panels) and zoom-ins (lower panels) showing smFISH signal of endogenous *dlg-1* mRNAs (green) in epidermal and seam cells of a bean stage, fluorescent signal of the transgenic GFP-tagged HMR-1 protein (HMR-1::GFP, magenta), and merges. Endogenous/non-tagged *dlg-1* mRNA shows CeAJ/membrane localization like its endogenous/GFP CRISPR-tagged version. Scale bar (upper panels): 10 µm. Scale bars (lower panels): 5 µm. **B.** Fluorescent micrographs of portions and entire *C. elegans* embryos showing instances of perinuclearly localized mRNAs coding for transmembrane proteins (HMR-1, SAX-7, EAT-20, and LET-805::GFP). smFISH signal of localized mRNAs hmr-1, sax-7, eat-20, and let-805::gfp (green), fluorescent signal of the CRISPR-engineered DLG-1::GFP (cr.DLG-1::GFP) or AJM-1 (magenta), and merges. To the left of each image, bars color-coded as in Fig. 1A to indicate to which sub-class of factors the mRNAs code for. Arrowheads: examples of cells with perinuclearly localized mRNAs. Scale bars: 5 µm.
Fig. S2. Fifteen mRNAs coding for CeAJ, cell polarity, and related components do not show any evident subcellular enrichment. A-O. Fluorescent micrographs of entire C. elegans embryos at the bean/comma stages focusing on the epidermis for the stated mRNAs in each image (green). Magenta: CeAJ marker (cr.DLG-1::GFP or AJM-1 for GFP-tagged lines). White asterisks mark unspecific signal from eggshells. Scale bars: 10 μm. A’-O’. Same as in A-O but for the forming digestive track (pharynx and intestine).
Fig. S3. *dlg-1* mRNA is enriched at the cell membrane prior to CeAJ maturation. A. Fluorescent micrographs of developing epithelial cells of a portion of a *C. elegans* embryo at the 4E stage (schematics on the left) showing smFISH signal of the endogenous *dlg-1* mRNA (green), fluorescent signal of the transgenic GFP-tagged LET-413 protein (LET-413::GFP, magenta), and merges. Scale bars: 10 μm. B-C. Top views of fluorescent micrographs of a seam cell at the embryonic bean and comma stages (schematics on the left). Images show smFISH signal of the *dlg-1::gfp* mRNA (cr.*dlg-1::gfp*, green), fluorescent signal of the CRISPR-engineered GFP-tagged DLG-1 protein (cr.DLG-1::GFP, magenta), and merges. Red asterisks mark the site from which transverse projections shown below start from. Scale bar: 5 μm. Below: corresponding transverse sections on a flat plane of the sole rolled out membrane of the seam cell shown above. Red asterisks mark the horizontal location of CeAJ and the starting site shown above. Scale bar: 5 μm.
Fig. S4. The UTRs enrolled in transgenic lines derive from unlocalized mRNAs. A-B. Maximum intensity projections of 5 (A) and 3 (B) Z-stacks of fluorescent micrographs of portions (red box in the cartoon) of *C. elegans* embryos at the 16E (A) and 1.5-fold (B) stages showing smFISH signal of sax-7 (A) and *unc-54* (B) mRNAs (green), fluorescent signal of the endogenous GFP-tagged DLG-1 protein (cr.DLG-1::GFP, magenta), DNA (cyan), and merges. Arrowheads: examples of cells with perinuclearly localized sax-7 mRNA. Such a localization depends on the signal peptide located in the CDS of sax-7 and not on its UTRs. Scale bars: 10 µm.
Fig. S5. *dlg-1* mRNA coding sequence possesses putative alternative start codons that may allow its partial translation. A. Transgenic GFP-tagged (green “GFP” at the carboxy-terminus) DLG-1 protein sequence. Highlighted in magenta are methionine amino acids corresponding to the green asterisks show in Fig. 4A. B. Dot plot with box plot: each dot represents the GFP intensity of pharynxes of adult worms carrying a “full-length” (n = 9; mean = 24.6 x 10^3; StDev = 9.4 x 10^3) or a “ΔATG line2” transgene (n = 15; mean = 2.0 x 10^3; StDev = 0.1 x 10^3). GFP intensities from animals of the “ΔATG line2” not carrying a transgene (recognizable through the “rolling” phenotype from the co-marker rol-6) were used as a negative control (“n. c.”) (n = 3; mean = 0.4 x 10^3; StDev = 0.1 x 10^3). Red dots represent the mean. Significance of statistical analyses (t-test, one tail): *** = < 0.001. C. Fluorescent micrographs of a lateral portion of seam and epithelial cells at the late bean stage of a *C. elegans* embryo showing an example of a “ΔATG” transgene expressed in a null mutant background for an NMD component (“smg-2 b.g.”, bottom), that is able to express its coded protein partially (“ΔATG line2”). As in Fig. 4C-E: smFISH signal of altered ATG (“ΔATG”) *tg.dlg-1::gfp* mRNAs (green), immuno-fluorescent signal of the endogenous AJM-1 protein (red), fluorescent signal of the corresponding ΔATG *tg.DLG-1::GFP* protein (magenta), and merges. Arrowheads: examples of laterally localized mRNAs. Scale bar: 5 μm.
Fig. S6. 
dlg-1 mRNA localization is lost upon heat stress. A-C. Fluorescent micrographs of seam cells of C. elegans embryos at the late bean stage that have been grown at 20°C (A) or subsequently subjected to a 1-hour heat-shock at 34°C (B) or 37°C (C) on plates. The images show smFISH signal of cr.dlg-1::gfp mRNA (green), fluorescent signal of the corresponding cr.DLG-1::GFP (magenta), and merges. Scale bars: 5 µm. D. Dot plot with box plot: each dot represents the percentage of laterally localized versus total cellular cr.dlg-1::gfp mRNA in each seam cell analyzed for embryos grown at 20°C (black; mean = 65.36; StDev = 19.78) or subsequently subjected to 34°C (grey; mean = 27.35; StDev = 14.85) or 37°C (white; mean = 18.70; StDev = 18.55) heat-shock (n = 25 for all conditions). Data derived from 5 different embryos. Significance of statistical analyses (t-test, one tail): * = < 0.05; *** = < 0.001.
Fig. S7. The baseline percentage of lateral localization to call an mRNA unlocalized varies if the nuclear signal is taken under consideration or not. A. Fluorescent micrographs of the five posterior seam cells of a C. elegans embryo at the bean stage. The images show smFISH signal of jac-1 mRNA (green), fluorescent signal of cr.DLG-1::GFP (magenta), DNA staining (orange), and merge. Scale bar: 5 μm. B. Dot plot with box plot: each dot represents the percentage of laterally localized versus “total” (“nuclear signal: yes”; black; mean = 25.87; StDev = 13.36) or versus “total” minus “nuclear” (“nuclear signal: no”; white; mean = 40.98; StDev = 20.05) jac-1 mRNA in each seam cell at the bean stage analyzed (n = 14) (Table S2; Materials and methods). Data derived from 3 different embryos. Significance of statistical analyses (t-test, one tail): *** = < 0.001.
Fig. S8. Quantitation through FISH-quant provides total, cytoplasmic, and nuclear mRNA counts. **A.** Fluorescent micrograph (maximum intensity projection) of the same embryo shown in Fig. S7A showing smFISH signal of jac-1 mRNA (red) from the FISH-quant platform. **B.** Same embryo as in (A) with cr.DLG-1::GFP signal (grey) to identify seam cells. Dashed square: portion of the embryo zoomed-in in (C). **C.** Zoom-in showing the five seam cells that will be undergoing the analysis through FISH-quant. **D.** Example of an outline (green) drawn along the border of a seam cell marked by cr.DLG-1::GFP signal. This outline provides the counts for the “total” cellular mRNA. **E.** Example of an outline (green) drawn parallel (± 500 nm) to the outer border (blue) of the same seam cell. This outline provides the counts for the “cytoplasmic + nuclear” mRNA. **F.** Outlines of the five seam cells that will be undergoing the analysis through FISH-quant. **G.** Same as (F), but showing the DNA signal (grey) instead of the cr.DLG-1::GFP signal. **H.** Same as in (G) showing the outlined DNA signals (small polygons in blue and green) to mark the “nuclear” mRNA. Dashed square: portion of the embryo zoomed-in in (I). **I.** Zoom-in on two of the five seam cells that will be undergoing the analysis through FISH-quant and showing the outlined mRNA dots chosen as units (small ellipses in blue and green).
Fig. S9. Removal of smFISH signal overlapping with DNA in apicobasal and apical profile analyses. Fluorescent micrographs of a seam cell at the bean stage seen in frontal view (DE27 line). In the first three panels, from left to right: smFISH signal of *tg.dlg-1ΔL27* mRNA (green), DNA staining (orange), and merge. White continuous lines: periphery of the cell. Red dashed lines: periphery of the DNA staining. On the very right: smFISH signal of *tg.dlg-1ΔL27* mRNA, (same as on the very left) with the mRNA signal overlapping with the DNA staining being removed for profile analyses shown in Fig. 6 (Methods). Scale bar: 2.5 μm.
Table S1. 

**List of factors enrolled in the smFISH survey.** Names of the factors and their orthologues whose mRNAs were tested in our smFISH-based survey for localized mRNAs highlighted in the same color-code as in Fig. 1A to address to which group of localized protein they belong to (Factor); to what (if any) AS they belong to (Adhesion system); subcellular localization of the stated factor (Protein localization); WormBase identification number (WormBaseID); gene sequence name (Sequence name); mRNA localization observed in this study (Observed mRNA localization); target of smFISH probes (smFISH probes; for full list of probe sequences, see Table S4); strain that has been tested in our survey for the corresponding mRNA/factor (Strain tested); reference for the corresponding factor (Reference).

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Table S2. 

**Raw data for image quantitation.** Raw data (mRNA counts), percentages of localization, means, and standard deviations, for all the quantification analyses in this study.

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Table S3. 

**List of methionine amino acid within the DLG-1 sequence.** Amino acid positions and domain location of the 11 methionine amino acids found in the DLG-1 sequence, corresponding to the green asterisks in Fig. 4A.

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Table S4. 

**List of C. elegans strains.** Detailed list of names, genotypes, and references of C. elegans strains used in this work.

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Table S5. 

**List of smFISH probes.** Sequences of the smFISH primary probes used in this study.

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Supplemental references


