CAR-1 and Trailer hitch: driving mRNP granule function at the ER?

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The targeting of messenger RNAs (mRNAs) to specific subcellular sites for local translation plays an important role in diverse cellular and developmental processes in eukaryotes, including axis formation, cell fate determination, spindle pole regulation, cell motility, and neuronal synaptic plasticity. Recently, a new conserved class of Lsm proteins, the Scd6 family, has been implicated in controlling mRNA function. Depletion or mutation of members of the Scd6 family, *Caenorhabditis elegans* CAR-1 and *Drosophila melanogaster trailer hitch*, lead to a variety of developmental phenotypes, which in some cases can be linked to alterations in the endoplasmic reticulum (ER). Scd6/Lsm proteins are RNA binding proteins and are found in RNP complexes associated with translational control of mRNAs, and these complexes can colocalize with the ER. These findings raise the possibility that localization and translational regulation of mRNAs at the ER plays a role in controlling the organization of this organelle.

Translation of mRNAs at specific locations within the cell is important for a variety of processes

Localized translation of mRNAs is important for numerous biological processes in eukaryotes, with a particularly significant role in the control of early development (for reviews see Gray and Wickens, 1998; Palacios and St. Johnston, 2001). For example, the anterior–posterior and dorsal–ventral axes in *Drosophila melanogaster* are defined by the proper localization and translation of *bicoid*, *oskar*, and *gurken* maternal mRNAs to three different positions within the oocyte (for review see Ephrussi and St. Johnston, 2004). Similarly, in *Caenorhabditis elegans*, the establishment of the germ cell lineage is dependent on the partitioning of maternal mRNAs in P-granules into the posterior cell during the first embryonic cell division (Strome and Wood, 1982; Kawasaki et al., 1998). Localized translation

also plays a role in somatic cells. For example, the spindle pole is regulated by the translation of a cyclin mRNA localized to this structure (Groisman et al., 2000). Similarly, the control of local translation in neurons influences growth cone guidance and synaptic plasticity (for review see Martin, 2004).

There are many processes involved in localized translation of mRNAs. mRNAs must accumulate at specific subcellular sites either by active transport or by diffusion and then entrapment. The mRNA as it is being transported, as well as the corresponding unlocalized mRNA, must be translationally repressed. Finally, the translation of the localized mRNA must be activated. These processes are illustrated by the regulation of β-actin mRNA by zipcode binding protein 1 (ZBP1), which binds specific sequences in the 3′ untranslated region (UTR) of β-actin mRNA and is required for its localization to sites of actin polymerization at the cell periphery (Farina et al., 2003). ZBP1 binds β-actin mRNA in the nucleus and represses its translation while the mRNA is transported. Once β-actin mRNA reaches the cell periphery, ZBP1 is phosphorylated by Src, which reduces binding of ZBP1 to the mRNA, thus allowing for translation (Huttelmaier et al., 2005). Translation repression complexes associated with mRNAs, including those that are localized, are often found in large aggregates referred to as RNP particles or granules. The aggregation of RNP complexes may facilitate the transport of specific mRNAs to discrete regions of the cell and possibly help to establish or maintain the repressed state.

Recent results have identified a conserved set of proteins that are involved in both translation repression and packaging of repressed mRNAs into RNA granules in both somatic cells and early development. A variety of eukaryotes, including yeast, *C. elegans*, and mammals, concentrate at least some untranslated mRNAs in cytoplasmic RNP granules referred to as processing bodies (P-bodies) but also called GW- or Dcp-bodies (Bashkirov et al., 1997; Ingelfinger et al., 2002; Lykke-Andersen, 2002; van Dijk et al., 2002; Sheth and Parker, 2003; Lall et al., 2005). P-bodies can be thought of as a purgatory for mRNA because transcripts within these complexes can either return to translation or be subject to degradation by mRNA decapping and 5′ exonuclease digestion (Cougot et al., 2004; Teixeira et al., 2005; Brengues et al., 2005). P-bodies may be involved in a wide variety of translation repression events. For example, proteins within P-bodies are required for general translation control in yeast (Coller and Parker, 2005), and translation repression by microRNAs appears to involve the accumulation of

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Abbreviations used in this paper: P-body, processing body; UTR, untranslated region; ZBP1, zipcode binding protein 1.

target mRNAs in P-bodies (Jakymiw et al., 2005; Liu et al., 2005; Pillai et al., 2005). The dual role of P-bodies in translation repression and mRNA degradation leads to these complexes containing both translation repressor proteins and enzymes for mRNA degradation, such as the decapping enzyme.

The same proteins that function in translation control and P-body formation in somatic cells appear to underlie the function of maternal mRNA storage granules. For example, the yeast Dhh1p and its homologue in mammalian cells RCK/p54, which are members of the DEAD-box family of RNA helicases, function both in translation repression and P-body formation in yeast and mammals (Andrei et al., 2005; Coller and Parker, 2005). Importantly, homologues of Dhh1p are required for proper translation control of maternal mRNAs and are found in maternal storage granules in a variety of organisms. For example, the *D. melanogaster* homologue Me31b is found within maternal mRNA granules and is required for the proper translational control of the *oskar* mRNA (Nakamura et al., 2001). The *C. elegans* homologue CGH-1 colocalizes with P-granules, the maternal mRNP granules that are localized to the germline in *C. elegans* (Navarro and Blackwell, 2005). Xp54, the *Xenopus laevis* homologue, is present in stored RNP granules (Ladomery et al., 1997), and tethering Xp54 to specific mRNAs represses their expression (Minshall and Standart, 2004). P-bodies and maternal mRNA storage granules share other components, as the decapping enzyme can also be found in P-granules in *C. elegans* (Lall et al., 2005; Squirrell et al., 2006). Similarly, the homologue of yeast Pat1p, a key component of yeast P-bodies, is a component of translationally repressed maternal mRNPs in *X. laevis* oocytes (Murray et al., 1991; Rother et al., 1992; Coller and Parker, 2005). These results suggest that maternal mRNA storage granules and P-bodies are both structurally and functionally related, although one anticipates that maternal mRNA storage granules will have additional mechanisms to allow spatial and temporal control of mRNAs in a more precise manner.

The Scd6 family is a conserved class of Lsm proteins associated with translationally repressed RNA complexes

In a current set of papers, members of the Scd6 family of proteins have been identified as a new conserved component of the translation repression complex found in P-bodies and maternal mRNA storage granules. This protein family was first identified through computational analyses as a new class of Lsm proteins (Albrecht and Lengauer, 2004; Anantharaman and Aravind, 2004). The family is named after the *Saccharomyces cerevisiae* member Sdc6p (Anantharaman and Aravind, 2004) and contains several subclasses of closely related proteins (Albrecht and Lengauer, 2004). Each Scd6 family member contains two RNA-associated motifs: the Lsm, or like-Sm domain, at the NH$_2$ terminus, and clusters of RGG motifs, which would be predicted to form an RGG box RNA binding domain. Like-Sm domains are ancient protein domains that are found in eubacteria, archaea, and eukaryotes (for reviews see Khusial et al., 2005; Wilusz and Wilusz, 2005). Like-Sm domains interact with each other to form six- or seven-membered ring structures that can bind RNA. Both the Lsm region and a protein fragment containing

the RGG region of the *C. elegans* Scd6 orthologue CAR-1 bind poly(U) in vitro, verifying that this class of Lsm proteins is capable of directly binding RNA (Audhya et al., 2005). Scd6 family members also contain the newly recognized FDF motif of unknown function, although it is shared with the Edc3 family of Lsm proteins (Albrecht and Lengauer, 2004; Anantharaman and Aravind, 2004), which are also components of P-bodies in yeast and mammals (Kshirsagar and Parker, 2004; Fenger-Gron et al., 2005).

Scd6 orthologues have been found to associate with RNP particles containing translationally repressed mRNAs in numerous organisms. In *D. melanogaster* oocytes, Trailer hitch colocalizes with RNP particles containing Me31b that have previously been shown to contain translationally repressed mRNAs (Boag et al., 2005; Wilhelm et al., 2005). In developing oocytes and embryos in *C. elegans*, CAR-1 localizes to two types of RNP granules, both of which contain CGH-1, P-granules, and smaller cytoplasmic foci, which may be analogous to P-bodies in yeast and humans (Audhya et al., 2005; Boag et al., 2005; Squirrell et al., 2006). Scd6p localizes with Dhh1p in P-bodies in yeast (Johnson, N., personal communication). In addition, the mammalian homologue Rap55 localizes to P-bodies in mouse and human cells (Yang et al., 2006). The localization of Scd6 proteins with these RNP particles suggests that they function in the control of mRNA translation and/or degradation. This idea is supported by the observation that Scd6 proteins physically associate with other proteins involved in translation repression. Trailer hitch and CAR-1 affinity purify with Me31b or CGH-1, respectively, as well as with other proteins associated with translation repression (Audhya et al., 2005; Boag et al., 2005; Wilhelm et al., 2005). Some of these interactions are dependent on RNA, suggesting that the proteins may be linked together on the same RNA molecules. In other cases, the proteins may directly interact with each other because their copurification is resistant to RNase treatment. The observations that mRNAs copurify with Trailer hitch RNP complexes (Wilhelm et al., 2005) and that CAR-1 can bind RNA (Audhya et al., 2005) suggest that Scd6 proteins may bind specific mRNAs and control their translation (see last section).

Scd6 proteins are functionally and physically associated with the ER

An unanticipated connection is that these Lsm proteins appear to have functional and physical links with the ER. This connection was first suggested by the observation that overexpression of the *S. cerevisiae* homologue Scd6p suppresses a deficiency in clathrin (Nelson and Lemmon, 1993), suggesting that Scd6p could affect the flux of proteins to or from the membrane. Subsequently, the *D. melanogaster* homologue *trailer hitch* was found to be required for the efficient secretion of Gurken, a member of the TGF-α family, and Yolkless, the vitellogenin receptor, in oocytes (Wilhelm et al., 2005). Both Gurken and Yolkless proteins accumulated in large foci within the oocyte, although some of the protein was still secreted, suggesting that *trailer hitch* has a general role in the secretory process. An important step in secretion is the exit of proteins from the ER to the Golgi. The COPII complex is required for ER-to-Golgi

trafficking and is located at discrete sites in the ER associated with ER exit (for reviews see Mancias and Goldberg, 2005; Tang et al., 2005). In *trailer hitch* mutants, a component of the COPII complex, Sar1, is mislocalized from small discrete foci to abnormally large patches in nurse cells and the oocyte (Wilhelm et al., 2005). The mislocalization of Sar1 protein indicates that *trailer hitch* is required for normal ER exit site distribution and morphology. The defect in ER exit sites would lead to defects in the secretion of proteins like Gurken and Yolkless.

The *C. elegans* Scd6 orthologue CAR-1 has also been found to be required for ER dynamics in embryos (Squirrell et al., 2006). Normally, the organization of the ER undergoes changes in conjunction with the cell cycle (Poteryaev et al., 2005). In *C. elegans* embryos, the ER is in a dispersed state during interphase. The ER changes to a more ordered, reticulated state in mitosis. During mitosis, the ER also associates strongly with the mitotic spindle, both at the poles and in the area between the poles, referred to as the midzone. As the cleavage furrow forms, the reticulated state rapidly disassembles back to the dispersed state. In embryos that have been depleted of CAR-1 by RNAi treatment, the ER is found in large patches and thick strands both at interphase and during mitosis (Squirrell et al., 2006). During mitosis, the association of the ER with the spindle, particularly with the midzone region, is reduced compared with wild-type. Thus, depletion of CAR-1 significantly disrupts ER organization. Whether the defects in ER organization in *C. elegans* are due to a disruption of ER exit site function or the abnormal distribution and morphology of ER exit sites in *D. melanogaster* are due to a more global disruption of ER organization remains to be determined. However, it is clear that this family of Lsm proteins is required directly or indirectly for normal ER organization.

CAR-1 and Trailer hitch may affect ER organization directly, given that both proteins can localize at or near to the ER (Wilhelm et al., 2005; Squirrell et al., 2006). In *C. elegans* embryos, the majority of small RNP granules containing CAR-1 overlap with ER structures in interphase. During mitosis, CAR-1 concentrates on the spindle region, including the midzone, as mitosis progresses, suggesting that it may associate with the ER at the spindle; however, this needs to be confirmed by colocalization studies. In *D. melanogaster*, Trailer hitch colocalizes with the ER in nurse cells and oocytes (Wilhelm et al., 2005). Although the colocalization in oocytes is difficult to interpret because the oocyte is so densely packed with ER, it is likely to be relevant, given that there is a defect in exit from the ER in both cell types. The physical connection between RNP granules containing CAR-1 and Trailer hitch and the ER has led to the suggestion that these proteins regulate mRNAs that are associated with the ER (Wilhelm et al., 2005; Squirrell et al., 2006).

Depletion or mutation of CAR-1 or *trailer hitch* results in a diverse set of phenotypes, some of which may be a consequence of the disruption of ER organization. Mutations in *trailer hitch* cause defects in dorsal–ventral patterning in *D. melanogaster*, so that mutant eggs lack or have reduced dorsal appendages (Wilhelm et al., 2005). The ventralization of *trailer hitch* mutant eggs is likely due to the lack of secretion of Gurken, as it is a major determinant of dorsal fate.

A major phenotype of CAR-1 in *C. elegans* may also result from the disruption of the ER. Depletion or mutation of CAR-1 causes dramatic failure in cytokinesis in early *C. elegans* embryos (Audhya et al., 2005; Boag et al., 2005; Squirrell et al., 2006). When CAR-1 is defective, the cleavage furrow begins to form normally during anaphase, but then it regresses and membrane fails to accumulate at the cleavage furrow. In wild-type embryos during anaphase, interzonal or midzone microtubule bundles form between the two separating masses of chromosomes associated with the spindles. These interzonal microtubule bundles are thought to signal the proper positioning of the cleavage furrow (Bringmann and Hyman, 2005) Interzonal microtubule bundles are completely absent in CAR-1–depleted embryos (Audhya et al., 2005; Squirrell et al., 2006). Squirrell et al. (2006) propose that the lack of microtubules in the midzone is a result of the reduced accumulation of ER with the spindle in CAR-1 RNAi embryos. The close association of the ER with the spindle could allow the ER to stabilize the microtubules by regulating the local calcium concentration. In addition, the normal distribution and function of exit points in the spindle-associated ER may be necessary for the elaboration of new membrane and membrane proteins required for completion of cytokinesis. Therefore, one simple model is that the failure of cytokinesis results from the disruption of the ER organization caused by the lack of functional CAR-1. Alternatively, because CAR-1 associates with the spindle and defects in midzone spindle formation can affect ER organization at the midzone (Squirrell et al., 2006), it is formally possible that CAR-1 affects spindle function, which then leads to the defect in ER organization at the spindle; however, this model does not explain why depletion of CAR-1 has a global effect on ER organization.

CAR-1 defects in *C. elegans* also give rise to other phenotypes. CAR-1 got its name from its effects on cytokinesis and apoptosis as well at its association with RNA. In *C. elegans*, about half of all oocytes undergo apoptosis just before individual oocytes form from a syncytium in the gonad, apparently to provide cytoplasmic components to the oocytes that do form (Gumienny et al., 1999). Depletion of CAR-1 increases physiological germ cell apoptosis and impairs oogenesis (Boag et al., 2005). It is not clear whether these alterations in physiological apoptosis and oogenesis are related to defects in ER function. However, it is interesting that depletion of CAR-1 causes extracellular cytoplasmic "spheres" to accumulate that contain high levels of the secreted yolk receptor protein, RME-2 (Boag et al., 2005). It is tempting to speculate that these "spheres" are produced by some perturbation in the secretory process. Alternatively, CAR-1 may regulate a variety of mRNAs that affect cellular processes other than ER function.

The study of Scd6/Lsm proteins has illuminated new aspects of RNP granules

The investigation of the Scd6 protein family has also revealed some interesting features of mRNA granules in eukaryotic cells. First, several observations now suggest that there may be different classes of P-bodies or P-body–like granules within a single cell. A clear example of this idea is the existence of both P-bodies

and P-granules in developing oocytes and early embryos of *C. elegans* (Audhya et al., 2005; Boag et al., 2005; Lall et al., 2005; Navarro and Blackwell, 2005; Squirrell et al., 2006). Interestingly, these particles share many of the same components but are clearly distinct in that P-granules contain unique proteins and segregate preferentially to the germline. In addition, not all P-bodies in *C. elegans* embryos, as detected by an antibody to the decapping enzyme, are compositionally the same because some lack the CAR-1 protein (Squirrell et al., 2006). Consistent with multiple classes of P-bodies in both *D. melanogaster* egg chambers (Wilhelm et al., 2005) and in mammalian cells in culture (Kedersha et al., 2005), some P-bodies are motile within the cell, whereas others are more stationary, perhaps because they are associated with the ER. A second interesting observation is that association of CAR-1 and CGH-1 with P-bodies in *C. elegans* is temporally regulated during early development. P-bodies containing the decapping protein are numerous in single-cell embryos during pronuclear migration; however, at this time there are very few CAR-1 foci (Squirrell et al., 2006). Localization of CAR-1 with P-bodies increases dramatically by the four-cell stage of development. In contrast, CGH-1 localization to P-bodies decreases after several embryonic divisions (Navarro and Blackwell, 2005), whereas the decapping enzyme persists in P-bodies throughout embryonic development (Lall et al., 2005). These observations suggest that there may be multiple classes of P-bodies and that the components of P-bodies will be spatially and temporally regulated, presumably with how mRNA function is being controlled. The possibility that P-bodies differ compositionally and functionally suggests that they may have significant specificity in controlling mRNA function.

What is the function of the Scd6 family of Lsm proteins?

An unresolved issue is the precise function of these Scd6 proteins. Three lines of arguments lead to the hypothesis that the Scd6 family of Lsm proteins plays some role in the regulation of mRNA translation and/or degradation. First, CAR-1, Trailer hitch, Rap55, and Scd6p can all be found in mRNA granules containing untranslated mRNAs and coimmunopurify with other proteins involved in translational control. Second, depletion of the DEAD box RNA helicase, CGH-1, causes defects in cytokinesis and apoptosis similar to those seen with CAR-1 depletion, supporting the idea that CAR-1's function is related to mRNA (Audhya et al., 2005; Boag et al., 2005). Third, these proteins can bind RNA and coimmunoprecipitate mRNAs. In this view, phenotypes of strains lacking these proteins would be due to misregulation of specific mRNAs. For example, Scd6 proteins could regulate particular mRNAs that control the spatial organization of the ER. Localized translation would allow the concentrated expression of the encoded proteins, which would then influence the formation of functional subdomains of the ER. Consistent with this idea, the mRNAs for Sar1 and Sec13, components of the COPII complex, which is associated with ER exit sites, are present in immunoprecipitates of Trailer hitch (Wilhelm et al., 2005). Concentrated localized expression of Sar1 mRNA on the ER would be an effective mechanism for controlling the size and distribution of ER exit sites, as recruitment of Sar1 is the first step in assembly of COPII complexes on the ER membrane. The idea that localized mRNA translation may be involved in determining ER organization is novel and raises the possibility that this is an important mechanism for controlling ER dynamics through the cell cycle and during development.

It should be pointed out that there is no direct evidence that members of this family of Lsm proteins act as translational repressors. Because targeted translation of mRNAs involves the reactivation of the repressed mRNA, CAR-1 and Trailer hitch could act as translational activators or as both activators and repressors. This latter possibility is suggested by the fact that cytoplasmic polyadenylation element binding protein, an mRNA binding protein involved in local control of translation in a variety of settings, functions first to repress the translation of mRNAs and later to activate them (for review see Mendez and Richter, 2001). Finally, one has to consider the possibility that these proteins may work independently of translational control. This is suggested by the observation that the *X. laevis* Scd6 orthologue Rap55 is associated with RNP particles that are thought to control mitotic spindle assembly by a mechanism proposed to be independent of new translation (Blower et al., 2005). This finding suggests that RNA or proteins within the particle may serve a direct structural or regulatory role in spindle assembly. Thus, Scd6 proteins could theoretically influence RNP function without modulating translation. Future work identifying the mRNAs bound by this class of Lsm proteins and how their function is affected by these proteins will be important for understanding the specific role of this protein family and for determining the roles that control of mRNA localization, translation, and decay play in eukaryotic cells.

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