Protection of specific maternal messenger RNAs by the P body protein CGH-1 (Dhh1/RCK) during *Caenorhabditis elegans* oogenesis

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During oogenesis, numerous messenger RNAs (mRNAs) are maintained in a translationally silenced state. In eukaryotic cells, various translation inhibition and mRNA degradation mechanisms congregate in cytoplasmic processing bodies (P bodies). The P body protein Dhh1 inhibits translation and promotes decapping-mediated mRNA decay together with Pat1 in yeast, and has been implicated in mRNA storage in metazoan oocytes. Here, we have investigated in *Caenorhabditis elegans* whether Dhh1 and Pat1 generally function together, and how they influence mRNA sequestration during oogenesis. We show that in somatic tissues, the Dhh1 orthologue (CGH-1) forms Pat1 (*patr-1*)-dependent P bodies that are involved in mRNA decapping. In contrast, during oogenesis, CGH-1 forms *patr-1*–independent mRNA storage bodies. CGH-1 then associates with translational regulators and a specific set of maternal mRNAs, and prevents those mRNAs from being degraded. Our results identify somatic and germ cell CGH-1 functions that are distinguished by the involvement of PATR-1, and reveal that during oogenesis, numerous translationally regulated mRNAs are specifically protected by a CGH-1–dependent mechanism.

Introduction

Accurate temporal and spatial regulation of mRNA translation and degradation is critical for control of gene expression. In a dramatic example of this regulation, developing oocytes produce thousands of different mRNAs that must be maintained in a translationally silent state in order to prevent premature differentiation (Johnstone and Lasko, 2001; Ciosk et al., 2006; Richter, 2007). Many of these mRNAs are provided to the embryo maternally and then destroyed during the transition to zygotic gene expression (Schier, 2007; Stitzel and Seydoux, 2007). Much has been learned about how specific mRNA-binding proteins control translation of individual maternal mRNAs in oocytes and early embryos (Lipshitz and Smibert, 2000; Wickens et al., 2002; Richter, 2007). However, less is understood about how these mRNAs are influenced by mechanisms that affect mRNA translation or turnover on a broader scale.

Various mechanisms that repress translation or degrade mRNAs are concentrated in cytoplasmic structures known as processing bodies (P bodies; also referred to as GW or Dcp bodies; Anderson and Kedersha, 2006; Eulalio et al., 2007a; Parker and Sheth, 2007). These mechanisms include decapping- and nonsense-mediated mRNA degradation, as well as translational silencing and mRNA degradation directed by small RNAs. P bodies are defined by a set of "core" proteins that are involved in decapping-dependent decay, a process in which the protective 5′ cap is removed from a deadenylated mRNA, thereby exposing it to 5′-to-3′ degradation (Parker and Sheth, 2007). These core P body proteins, as described in yeast, include the decapping enzyme subunits Dcp1 and Dcp2 and a set of accessory proteins that form a decapping complex on mRNAs.

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Abbreviations used in this paper: IP, immunoprecipitate; miRNA, microRNA; P body, processing body; PABP, poly-A–binding protein; RIP-Chip, RNA IP and microarray analysis; RNP, RNA–protein; RT-qPCR, quantitative RT-PCR; SAGE, serial analysis of gene expression; UTR, untranslated region; WT, wild type.

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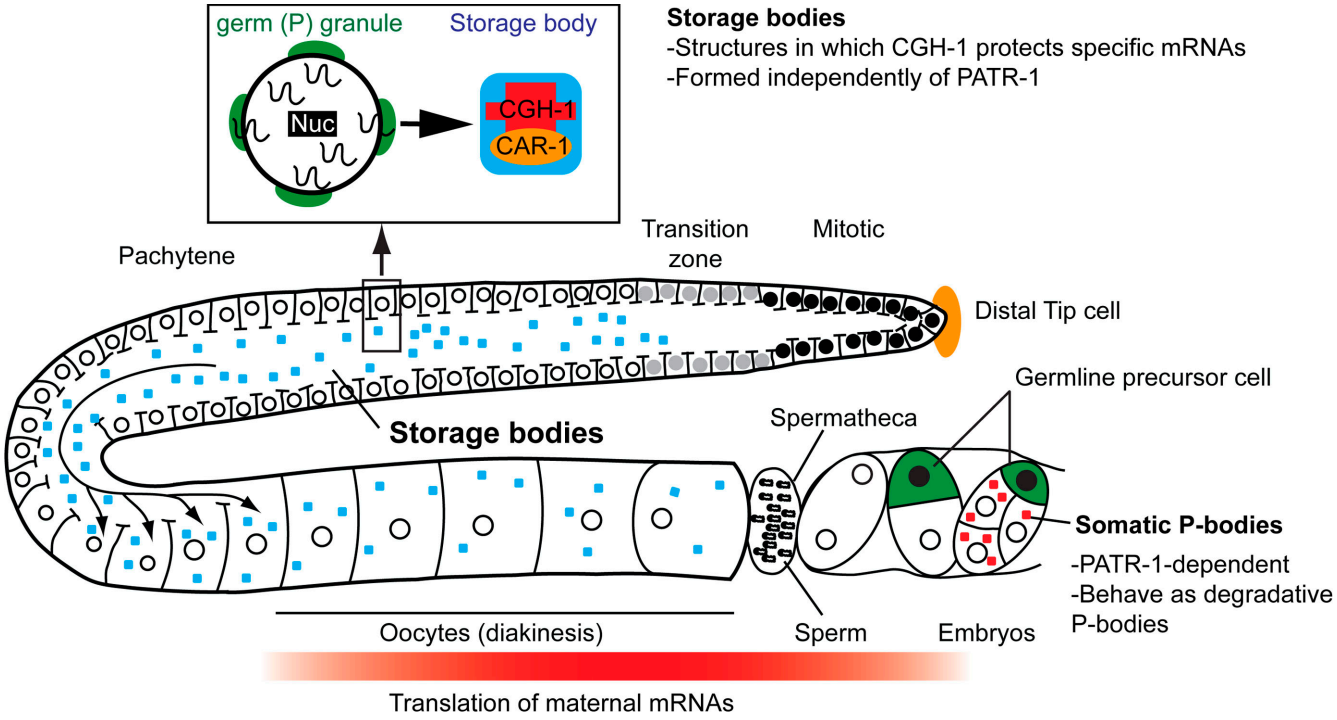

Figure 1. **A *C. elegans* hermaphrodite gonad, illustrating models discussed in the text.** Germ cells develop in an "assembly line" fashion from stem cells that are regulated by the somatic distal tip cell (Hubbard and Greenstein, 2005). Each of two gonad arms produces sperm during the fourth larval stage, then oocytes during adulthood. Germ cells initially share a common cytoplasm, which flows as shown by arrows (Wolke et al., 2007). Transcription increases sharply upon progression through the transition zone and entry into meiosis I. As new mRNAs move into the syncytial gonad core, some appear to pass through germ (P) granules (inset, green), which are located just outside of most nuclear pores (Pitt et al., 2000; Schisa et al., 2001). CGH-1 associates with and protects particular translationally regulated maternal mRNAs in the context of storage bodies, which may be assembled on germ (P) granules (inset). Oocytes enlarge and become cellularized in the proximal region, and fertilization occurs in the spermatheca. Transcription ceases as oocytes enter diakinesis (Walker et al., 2007). Further development depends upon translationally regulated maternal gene products until embryonic transcription begins at the four-cell stage (Seydoux and Dunn, 1997). CGH-1 then associates with PATR-1 in *patr-1*–dependent somatic P bodies (red dots).

Among these decapping accessory proteins are the Pat1 protein and the DEAD box RNA helicase Dhh1, which inhibit translation competitively and promote decapping complex assembly and P body formation (Coller and Parker, 2005; Pilkington and Parker, 2007). If decapping is prevented, this mechanism can maintain mRNAs in a translationally arrested state, providing a model for how silenced mRNAs might be sequestered in potentially any cell type. Evidence from human and *Drosophila melanogaster* cell lines indicates that the Dhh1 orthologue is important for microRNA (miRNA)-induced translational silencing (Chu and Rana, 2006; Eulalio et al., 2007c), which suggests that this inhibitory mechanism might be involved in posttranscriptional regulation in many contexts.

In developing oocytes, early embryos, and neurons, the Dhh1 orthologue and other core P body proteins are found in RNA–protein (RNP) granules that are proposed to correspond to mRNA storage particles (Navarro et al., 2001; Coller and Parker, 2004; Eulalio et al., 2007a; Parker and Sheth, 2007). During *Caenorhabditis elegans* oogenesis (Fig. 1), Dhh1 (CGH-1) colocalizes with the P body protein CAR-1 in RNP particles that accumulate in the syncytial gonad cytoplasm in parallel to newly synthesized mRNAs (Navarro et al., 2001; Boag et al., 2005; Lall et al., 2005; Jud et al., 2008). In *D. melanogaster*, translational silencing of some oocyte mRNAs depends upon the Dhh1 orthologue Me31B (Nakamura et al., 2001). In these and other metazoans, the Dhh1 helicase is found in a germ line RNP complex that includes CAR-1 and Y-box proteins (Table I; Wilhelm et al., 2000; Nakamura et al., 2001; Minshall and Standart, 2004; Weston and Sommerville, 2006). In early biochemical studies of *Xenopus laevis* oocytes, it was proposed that this set of proteins "masks" mRNAs to prevent their translation (Richter and Smith, 1984; Weston and Sommerville, 2006). It is still unknown whether the Dhh1 helicase associates with metazoan maternal mRNAs broadly or specifically, and how in general it influences their regulation in vivo. It has also not been determined whether the putative oocyte mRNA storage particles that are defined by this helicase correspond simply to a form of P body in which mRNA degradation has been blocked, or a different type of structure.

In *C. elegans*, maternally provided CGH-1 and CAR-1 perdure in the embryo. They then form particles that begin to disappear around the four-cell stage in somatic precursors but persist until around the 200-cell stage in the germ lineage (Navarro et al., 2001; Audhya et al., 2005; Boag et al., 2005). In the embryonic germ line, CGH-1 and CAR-1 colocalize with the germ plasm, an RNP structure that maintains germ cell identity in many species (Strome and Lehmann, 2007). *C. elegans* germ plasm particles are called P granules, but we will refer to them as germ (P) granules to avoid confusion. The embryonic distribution of CGH-1 is remarkably similar to that of a large

Table I. **Biochemically-demonstrated interactions with CGH-1 orthologues**

Protein type	*C. elegans* (adult hermaphrodite[o])	*D. melanogaster* (adult ovary)	*X. laevis* (oocytes)	*S. cerevisiae*	*H. sapiens* (HeLa cells)
DEAD box RNA helicase	CGH-1	Me31B	Xp54	Dhh1p	RCK
Sm-like domain	CAR-1[b,c]	Tral[b,g]	RAP55[h,i]	?	?
CPEB family	?	?	CPEB[h,i]	–	?
Y-box	CEY-2, 3, 4[b]	Yps[d,e,f,g]	FRGY2[h,i]	–	?
KH domain	ATX-2[a]	?	?	?	ataxin-2[l]
Poly A–binding	PAB-1[a]	Pabp[g]	PABP[h]	Pbp1p[j]	?
eIF4E	?	eIF4E[f,g]	?	?	eIF4E[m]
eIF4E-binding	?	Cup[f,g]	eIF4E-T[h]	?	?
Decapping enzymes	?	?	?	Dcp1p[j] Dcp2p[j]	hDcp1[m] hDcp2[m,n]
Enhancer of decapping/ translational repressor	PATR-1[a]	?	P100[h]	Pat1p[j]	?
Enhancer of decapping	?	?	?	Edc3p[j]	Edc3[n]
Decapping factors	?	?	?	Lsm1-7p[j,k]	?

The proteins shown have each been demonstrated to interact physically with CGH-1 orthologues. Interactions that have been identified only by yeast two-hybrid are not included. Some proteins that colocalize to P bodies, such as the CAR-1 orthologues Scd6 (yeast) and RAP55 (human), are also not included, as they have not been biochemically identified to interact with the corresponding CGH-1 orthologue. CPEB, cytoplasmic polyadenylation element–binding protein; ?, unambiguous orthologue exists, but no information is available. –, no clear orthologue described.

[a]This paper.

[b]Boag et al., 2005.

[c]Audhya et al., 2005.

[d]Nakamura et al., 2001.

[e]Wilhelm et al., 2000.

[f]Nakamura et al., 2004.

[g]Wilhelm et al., 2005.

[h]Tanaka et al., 2006.

[i]Weston and Sommerville, 2006.

[j]Krogan et al., 2006.

[k]Coller et al., 2001.

[l]Nonhoff et al., 2007.

[m]Chu and Rana, 2006.

[n]Fenger-Gron et al., 2005.

[o]Protein extracts were prepared from whole adult hermaphrodites in which sperm production had ceased and oocytes were being produced.

group of maternal mRNAs (class II mRNAs; Seydoux and Fire, 1994), which is consistent with the idea that CGH-1 might associate with maternal mRNAs.

Here, we have used genetics and biochemistry to investigate how core P body mechanisms contribute to oogenesis and maternal mRNA storage in *C. elegans*. We show that in somatic tissues, CGH-1 forms Pat1 (*patr-1*)-dependent P bodies that are associated with decapping-dependent mRNA decay. In contrast, in developing oocytes, *patr-1* is not required for formation of CGH-1 RNP particles, which we refer to as storage bodies. During oogenesis, CGH-1 associates primarily with a set of translation regulators, and a large but specific set of translationally regulated maternal mRNAs. Surprisingly, CGH-1 is then required to protect these particular mRNAs from degradation. Our findings identify three distinct structures that contain CGH-1 (germ [P] granules, storage bodies, and somatic P bodies), and, for the first time, demonstrate tissue-specific and Pat1-independent functions for this core P body helicase. They also reveal that many maternal mRNAs are regulated not only by interactions with individual RNA-binding proteins that control their translation, but also by a CGH-1–dependent protective mechanism.

Results

Somatic but not germ line CGH-1 particles correspond to decapping-associated P bodies

We first investigated whether the CGH-1 particles that are present during oogenesis are assembled by the same mechanism as "typical" P bodies, and whether such structures are present in *C. elegans* tissues. P bodies are dynamic structures that accumulate in proportion to their steady-state supply of mRNA (Parker and Sheth, 2007). If Pat1 or Dhh1 (CGH-1) is lacking, entry of mRNA into the decapping pathway is inefficient and P bodies are extremely small (Sheth and Parker, 2003; Eulalio et al., 2007b). In contrast, P bodies accumulate mRNA and become large if mRNA decapping is prevented. This paradigm is now well established in yeast and mammalian cells. As CGH-1 is a core P body component (Parker and Sheth, 2007), it is possible to detect *C. elegans* P bodies that are involved in decapping-dependent decay by identifying CGH-1 particles that are smaller when PATR-1 (Pat1) is lacking, and larger when decapping-dependent degradation is blocked.

We performed these analyses using RNAi against *patr-1* and decapping-associated genes, and by examining previously uncharacterized predicted null mutations in *patr-1* and *dcap-2*, the *C. elegans* orthologue of *Dcp2* (decapping enzyme catalytic subunit; Fig. S1, A–C; and Table S1, available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1). *dcap-2*(*tm2470*) homozygotes produce fertile offspring, though in reduced numbers, which makes it possible to examine the consequences of an absence of mRNA decapping in animals of any stage. In contrast, *patr-1*(*tm2402*) homozygotes produce offspring that arrest development during late embryonic or early larval stages.

To examine how lack of *dcap-2* or *patr-1* affects the distribution of CGH-1 during oogenesis, we used antibody staining to visualize endogenous CGH-1 in gonads. In wild-type (WT) hermaphrodites, in the mitotic precursors at the distal gonad end, CGH-1 and CAR-1 are apparent on germ (P) granules around the nuclear periphery (Fig. 1; Navarro et al., 2001; Boag et al., 2005). As germ cells enter meiosis, CGH-1 and CAR-1 together form particles that accumulate within the syncytial gonad core and lack characteristic germ (P) granule proteins (Figs. 1 and S2 A, available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1; Navarro et al., 2001; Boag et al., 2005). Strikingly, this distribution of CGH-1 and CAR-1 was not detectably altered by lack of either PATR-1 or DCAP-2 (Fig. S2 A), which suggests that these structures are formed differently from "standard" P bodies. We will refer to the CGH-1/CAR-1 particles that are seen in the gonad core as storage bodies because of this distinction, and because we have found that CGH-1 protects many oocyte mRNAs from degradation (Fig. 1; see subsequent paragraphs).

Our failure to identify PATR-1–dependent CGH-1 particles in the germ line made it important to investigate whether such structures might be present in *C. elegans* somatic tissues. In the embryo, CGH-1 staining overlaps with CAR-1, DCAP-2, and its cofactor DCAP-1, as predicted for P bodies (unpublished data; Audhya et al., 2005; Boag et al., 2005; Squirrell et al., 2006). In somatic precursors, CGH-1 particles become increasingly less prominent after the four-cell stage in the WT but were far more prevalent at later stages after RNAi of *dcap-1* and *dcap-2* (*dcap-1/-2[RNAi]*; Fig. 2). This was also true in *dcap-2 (tm2470)* embryos and after RNAi of the decapping-associated 5′-to-3′ exonuclease XRN-1 (unpublished data), which indicates that the decapping pathway is required for the normal disappearance of CGH-1 particles from the soma. In striking contrast, after the four-cell stage, CGH-1 foci were far less prevalent in *patr-1(RNAi)* and *patr-1(tm2402)* embryos than in WT so that by the 100-cell stage, *patr-1(RNAi)* embryos contained 12-fold fewer CGH-1 foci (Fig. 2, B–D). In somatic embryonic cells, CAR-1 antibody staining was affected similarly by *dcap-1/-2* and *patr-1*RNAi (unpublished data), as was a rescuing transgenic fusion protein in which CGH-1 is linked to GFP (Fig. 2 E). During postembryonic stages in WT animals, antibody staining detects CGH-1 and CAR-1 only in germ cells (Navarro et al., 2001; Boag et al., 2005). In contrast, in *dcap-2(tm2470)* larvae and adults, CGH-1 particles were readily apparent in many somatic tissues (Figs. 3 A and S1 F). Western blotting indicated that total CGH-1 levels were not dramatically affected in either *patr-1*(*tm2402*) or *dcap-2*(*tm2470*) adults (Fig. 3 C), which suggests that these postembryonic CGH-1 foci may be formed mainly through aggregation, not new CGH-1 synthesis. We conclude that these somatic CGH-1 foci, which we will refer to as somatic P bodies, may represent sites of decapping-mediated mRNA turnover.

Association of PATR-1 with somatic P bodies

We next investigated whether PATR-1 is present in these various types of CGH-1 particles (somatic P bodies, germ [P] granules, and storage bodies). Western blotting with an antiserum against a PATR-1 N-terminal epitope (PATR-1[N]) revealed that PATR-1 is expressed primarily in somatic tissues (Figs. 3 D and Fig. S1 D), and we did not detect PATR-1(N) immunostaining in dissected gonads (not depicted). In the embryo, PATR-1(N) staining was diffuse and barely detectible through the two-cell stage. Robust PATR-1(N) staining then appeared specifically in somatic P bodies at the four-cell stage (Fig. 3 B and not depicted), just before these structures became *patr-1* dependent (Fig. 2 B). Subsequently, PATR-1(N) staining appeared in the somatic daughter of the germ line precursor shortly after each successive division and persisted in somatic cells until late embryonic stages (Fig. 3 B and not depicted). In *dcap-2(tm2470)* mutants but not the WT, PATR-1(N) staining that colocalized with CGH-1 was readily detectable during larval and adult stages (Figs. 3 A and S1 F). It is possible that in some cells, PATR-1 levels might be too low for detection, or the PATR-1(N) epitope might not be accessible. However, our finding that PATR-1(N) staining is detectable in CGH-1 foci only in somatic cells suggests that it represents a specific marker of degradative somatic P bodies.

Interactions between CGH-1 and translational regulators during oogenesis

Having observed that germ line CGH-1 particles seem to be formed differently from these somatic P bodies, we examined the germ line CGH-1 RNP complex further. In WT adults, the bulk of CGH-1 protein is found in germ cells (Boag et al., 2005). When we previously immunoprecipitated endogenous CGH-1 from adult extracts, we detected interactions between CGH-1 and CAR-1, Y-box proteins, and two prominent proteins of ∼75 and ∼70 kD (Boag et al., 2005). Through mass spectroscopy, we have now identified those last two proteins as ATX-2 (ataxin 2) and PAB-1 (poly-A–binding protein [PABP]), respectively. ATX-2 and PAB-1 are RNA-binding proteins that are involved in translational regulation, and PABP protects mRNAs from deadenylation and degradation (Mangus et al., 2003; Ciosk et al., 2004). These two proteins interact with CGH-1 orthologues and each other in multiple species, but neither is typically found in P bodies (Table I; Mangus et al., 2003; Ciosk et al., 2004; Ralser et al., 2005; Wilhelm et al., 2005; Nonhoff et al., 2007; Anderson and Kedersha, 2008). We could detect PATR-1 in CGH-1 immunoprecipitates (IPs) by Western blotting (Fig. 3 E), which is consistent with evidence that this helicase interacts with Pat1 in other species (Ho et al., 2002; Coller and Parker, 2005; Krogan et al., 2006; Eulalio et al., 2007b). Neither actin nor the P body

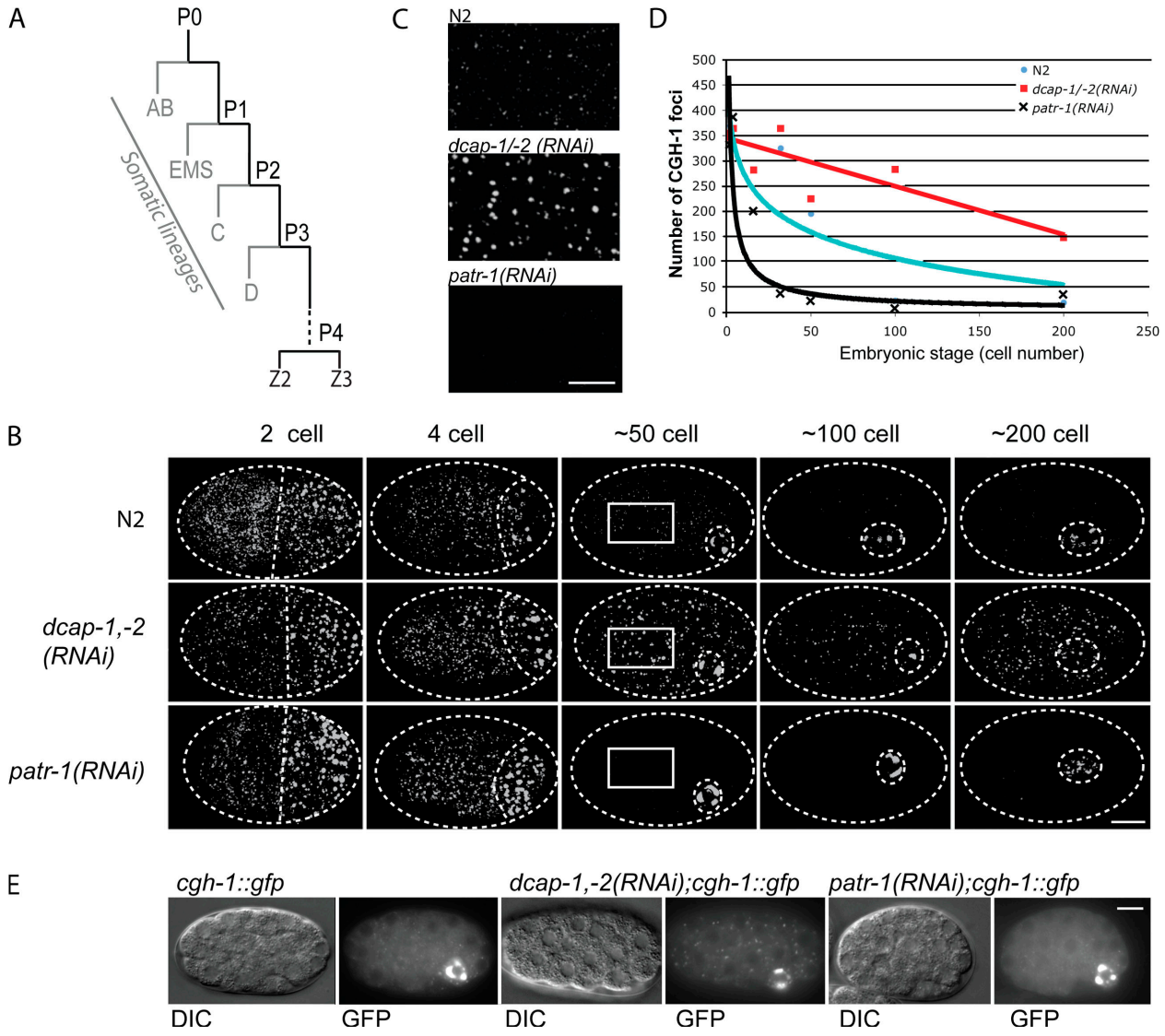

Figure 2. **Somatic embryonic CGH-1 foci have characteristics of decapping-associated P bodies.** (A) Embryonic founder cells. Four successive asymmetrical divisions of the germ cell precursor each produce a somatic and germ cell daughter. The founding germ line cell P4 gives rise to the germ cell precursors Z2 and Z3 before the 200-cell stage. (B) Effects of *dcap-1,-2* and *patr-1* RNAi on embryonic CGH-1 foci. Embryos were immunostained for CGH-1 and then analyzed by confocal microscopy. Z series projections of staining throughout the embryo are shown. In germ cell precursors (posteriorly and to the right, outlined by broken lines), most CGH-1 particles colocalize with germ (P) granules (Navarro et al., 2001). At the two- and four-cell stages, CGH-1 foci are distributed similarly in N2 (WT), *dcap-1/-2(RNAi)*, and *patr-1(RNAi)* embryos. At later stages, in somatic cells, CGH-1 foci are much more prominent in *dcap-1/-2(RNAi)* embryos than in N2, and are almost absent in *patr-1(RNAi)* embryos. In each experimental set, >50 embryos were examined at each stage shown. (C) Detailed images of somatic P bodies in 50-cell embryos, corresponding to the boxes in B. (D) Quantitation of somatic embryonic P bodies after *dcap-1,-2* or *patr-1* RNAi. CGH-1 foci were quantitated in confocal z series projections, with foci of fewer than six pixels and germ (P) granules excluded. Data points each correspond to two-to-seven embryos. (E) Effects of *dcap-1,-2* and *patr-1* RNAi on embryonic CGH-1::GFP foci. Single plane differential interference contrast (DIC) and fluorescent images are shown of representative ~30-cell embryos from a rescuing CGH-1::GFP transgenic strain. CGH-1::GFP foci in germ (P) granules were unaffected by these RNAi treatments, but somatic foci were more prominent in decapping-defective embryos than WT, and rarely visible in *patr-1(RNAi)* embryos. In each set, >40 embryos were examined at this and other early embryonic stages. Bars: (B) 10 µm; (C) 5 µm; (E), 10 µm.

protein LSM-2 were similarly detectable in these CGH-1 IPs (unpublished data), which indicates that the interaction between CGH-1 and PATR-1 is specific. However, our CGH-1 IP/mass spectrometry analyses did not identify PATR-1, decapping enzyme subunits, or other core P body components among the prominent protein species that interact with CGH-1 in adults (unpublished data; Boag et al., 2005). We conclude that during oogenesis, CGH-1 interacts primarily with a conserved set of translational regulators, not the decapping apparatus (Table I).

Association of CGH-1 with specific maternal mRNAs during oogenesis

Interactions between CGH-1 and these proteins were abolished by RNase treatment, which suggests that mRNA might be present in this germ line CGH-1 complex (Boag et al., 2005). To test this idea, we performed four experiments in which we used RNA IP and microarray analysis (RIP-Chip) to detect mRNAs that coIP with CGH-1 (Fig. 4 A). We performed these IPs using extracts that were prepared from intact 1-d-old adults in a way

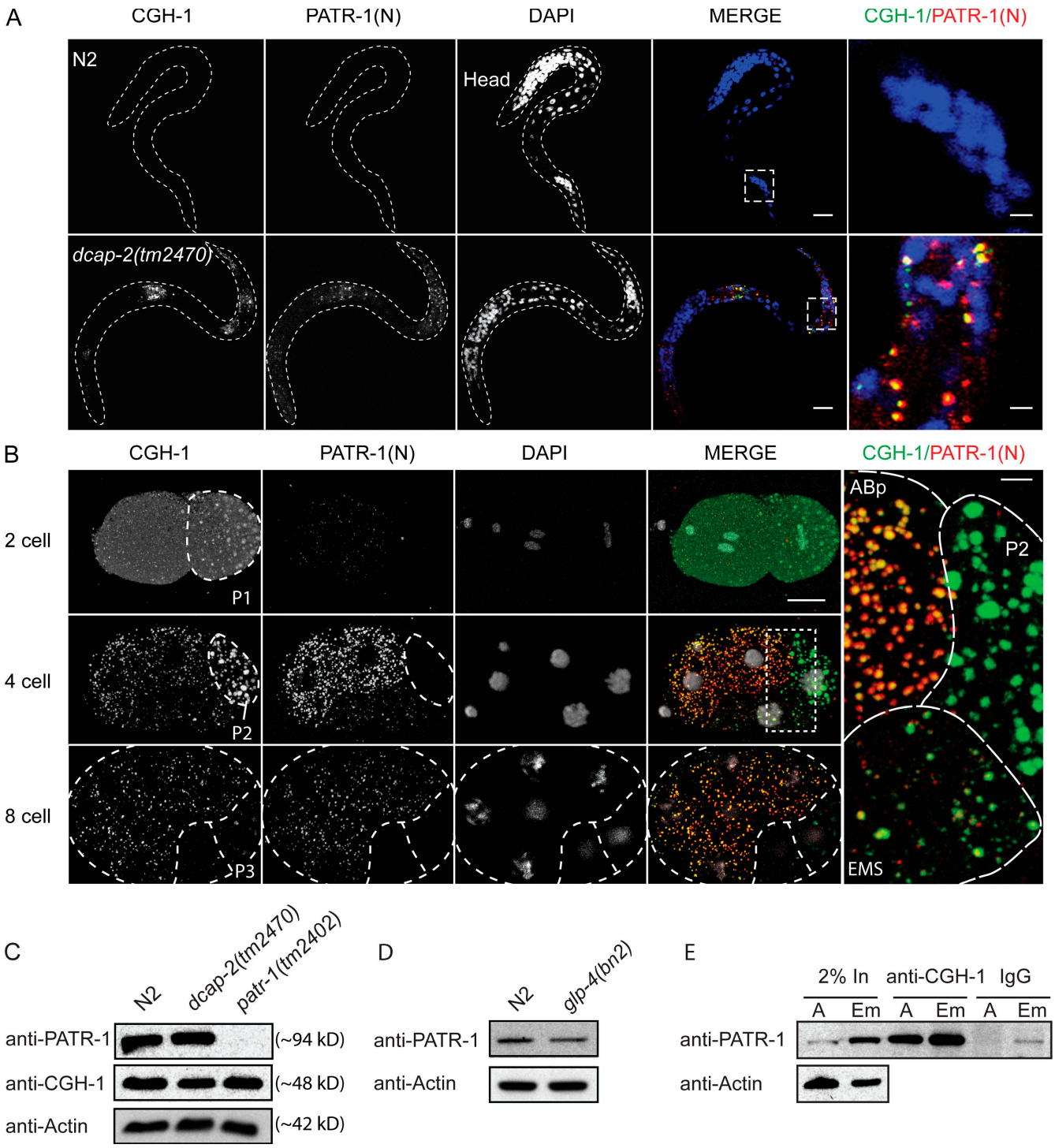

Figure 3. **Association of CGH-1 and PATR-1 in somatic P bodies.** (A) Somatic P bodies in *dcap-2(tm2470)* larvae. Single-plane confocal images reveal colocalizing CGH-1 (green) and PATR-1(N)-staining (red) particles in many somatic cells in *dcap-2(tm2470)* but not N2 (WT) L1-stage larvae (outlined by broken lines). Regions outlined by dashed boxes are enlarged on the far right. $n > 100$ for each set. Bars: 10 µm; (far right) 2 µm. (B) PATR-1(N) staining marks somatic P bodies in the embryo. WT embryos were analyzed by antibody staining and confocal microscopy (z series projections are shown). Germ cell precursors are outlined by broken lines, and in eight-cell embryos, the somatic sister of P3 (C blastomere) is similarly delineated. Robust PATR-1(N) staining is first detected in four-cell embryos, in which it colocalizes with CGH-1 in somatic blastomeres. After each successive division of the germ cell precursor PATR-1(N), staining is initially low, then increases. These low levels are apparent in the EMS blastomere (detail of the boxed area shown on the right), and also in the C blastomere (sister of P3) at the eight-cell stage. More than 50 embryos in each set were examined in each of at least two experiments. Bars: 10 µm; (far right) 2 µm. (C) CGH-1 and PATR-1 levels in N2, *dcap-2(tm2470)*, and *patr-1(2402)* adult hermaphrodites. PATR-1 was detected with anti–PATR-1(N). (D) PATR-1 expression in N2 and *glp-4(bn2)* adults, which essentially lack germ cells when grown at the restrictive temperature of 25°C. Densitometry indicated that *glp-4(bn2)* adult PATR-1 levels were ~68% of WT. (E) PATR-1 coIPs with CGH-1. CGH-1 IPs from 2.5 mg of adult (A) and embryo (Em) protein extracts were Western blotted for PATR-1.

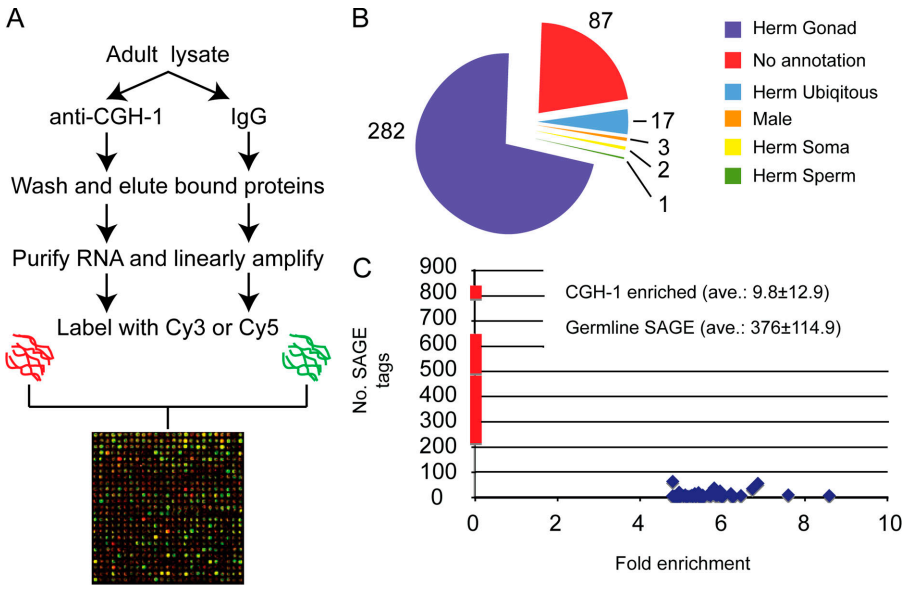

Figure 4. **CGH-1 associates with specific maternal mRNAs.** (A) Identification of CGH-1–associated mRNAs by RIP-Chip. CGH-1 was immunoprecipitated from extracts from 1-d-old adult hermaphrodites, then mRNA was extracted from material that was eluted by the immunogenic CGH-1 peptide, a protocol that isolates the CGH-1 complex (Boag et al., 2005). The control was rabbit IgG. After linear amplification, samples were labeled with Cy3 or Cy5 and hybridized to microarrays. Fold enrichment was averaged from four RIP-Chip experiments. (B) Expression profiles of CGH-1–enriched mRNAs. The vast majority of annotated CGH-1–enriched mRNAs are expressed primarily in adult hermaphrodite gonads, which produce only oocytes (Reinke et al., 2000, 2004). (C) CoIP with CGH-1 does not correlate with abundance. The relative enrichment in CGH-1 IPs and the number of nonambiguous SAGE tags (normalized to 100,000) are plotted for the 50 mRNAs that were most enriched in CGH-1 IPs (blue) and the 50 most abundant mRNAs (red) from a dissected gonad SAGE library (SW040; http://tock.bcgsc.bc.ca/cgi-bin/sage140).

that excluded embryos so that they were enriched for CGH-1 that is expressed in adult germ cells. We then identified mRNAs that were on average at least threefold overrepresented in CGH-1 IPs relative to IgG controls because we expected that this arbitrarily defined level would identify specific interactions. Surprisingly, only 392 mRNAs met this criterion (Fig. 4 B, and Tables II and S2, available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1).

If CGH-1 associates with *C. elegans* mRNA nonspecifically, our IPs should have isolated a random distribution of the most abundant somatic and germ line mRNAs because they were performed from whole animal extracts. Significantly, however, nearly all of the mRNAs that were enriched in our CGH-1 IPs are known to be expressed primarily in germ cells and to be provided to embryos as maternally supplied mRNA. Of the 305 CGH-1–associated mRNAs for which expression patterns have been characterized (Reinke et al., 2004), 92% (282) are expressed mainly in the hermaphrodite gonad and not during spermatogenesis, which indicates that they are produced during oogenesis. In addition, most CGH-1–associated mRNAs (85%) have been classified as maternal mRNAs (present in embryos before the onset of zygotic transcription), and 52% have expression profiles consistent with class II maternal mRNAs (Baugh et al., 2003). These CGH-1–associated mRNAs correspond to a significant but restricted fraction of the ~6,000 genes that are expressed in the hermaphrodite gonad (Baugh et al., 2003; Reinke et al., 2004). Taken together, our findings suggest that CGH-1 may associate with a limited set of maternal mRNAs.

To test this idea further, we examined the relative abundance of CGH-1–associated mRNAs by interrogating *C. elegans* serial analysis of gene expression (SAGE) databases (McKay et al., 2003; Khattra et al., 2007). The number of SAGE tags recovered for each mRNA is indicative of its relative abundance. If CGH-1 simply associates randomly with mRNAs that are present in developing germ cells, the most abundant germ line mRNAs should have been overrepresented in our CGH-1 IPs. This was not the case, however. None of the 50 most abundant gonad mRNAs were enriched at least threefold in our CGH-1 IPs (unpublished data). In addition, although the 50 most highly enriched CGH-1–associated mRNAs were represented by a mean of 9.8 nonambiguous SAGE tags (per 100,000) in a gonad mRNA library, the mean number of SAGE tags for the 50 most abundant gonad mRNAs was >38-fold higher (Fig. 4 C). This indicates that our CGH-1 IPs did not isolate the most abundant germ line mRNAs. A similar difference was seen when these CGH-1–associated mRNAs were compared with the 50 most abundant of all adult *C. elegans* mRNAs (Fig. S3 A, available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1). We conclude that during oogenesis, CGH-1 associates specifically with a discrete subset of germ line mRNAs, nearly all of which are expressed during oogenesis and provided to the embryo maternally.

The proteins encoded by these CGH-1–associated mRNAs are involved in various processes, including RNA binding, ubiquitylation, and gene regulation (Fig. S3 B). These functional categories are also highly represented among mRNAs that are present in mammalian oocytes (Evsikov et al., 2006; Kocabas et al., 2006). Many CGH-1–associated mRNAs are involved in embryonic fate specification or cell cycle control (i.e., *nos-2*, *pos-1*, *cdk-1*, and *cdk-5*; Tables II and S2). In addition, many of these mRNAs are known to be translationally regulated during oogenesis, in that their protein products are not detected until late oogenesis or early embryonic stages (i.e., *nos-2*, *pos-1*, and *pie-1*; Tenenhaus et al., 1998; Subramaniam and Seydoux, 1999; Tabara et al., 1999). Together, the data suggest that CGH-1 associates with a particular subset of maternal mRNAs, many of which are subject to posttranscriptional regulation during oogenesis.

This interaction between CGH-1 and maternal mRNAs predicts that in oocytes, mRNA should be present in storage bodies along with CGH-1. To test this idea, we performed CGH-1 antibody staining simultaneously with in situ hybridization with a probe for SL1, a trans-spliced leader sequence that is found on

Table II. **mRNAs that are most highly enriched in CGH-1 IPs**

ORF	Gene	CGH-1/IgG ratio	Baugh expression[a]	Reinke germ line expression[b]	Protein domain/KOG classification[c]
F39B2.11		8.6	SMD		Translocase of outer mitochondrial membrane complex [KOG3028]
ZK1127.1	*nos-2*	7.6	SMD	Oogenesis-enriched	RNA-binding, germ line development
F52E1.1	*pos-1*	6.9	SMD	Oogenesis-enriched	Zn finger, posterior embryonic fate specification
C09G9.6	*oma-1*	6.7	SMD	Oogenesis-enriched	Zn finger, oocyte maturation
T20D3.8		6.5	SMD	Oogenesis-enriched	Phosphatidylinositol biosynthesis [KOG3059]
CD4.7		6.3	MD	Oogenesis-enriched	Zn-finger protein [KOG3362]
T21C9.13		6.2	SMD	Oogenesis-enriched	Unknown
T24D1.3		6.0	SMD	Oogenesis-enriched	Unknown
K10B2.3	*clec-88*	6.0	SMD	Oogenesis-enriched	C-type lectin [KOG4297]
F08F3.6		5.9	SM	Oogenesis-enriched	Unknown, F-box containing
C30B5.2		5.9	MD		Leptin receptor gene-related protein [KOG2174]
F42A9.8		5.9	SMD	Oogenesis-enriched	Unknown
Y32F6A.1		5.8	ME	Oogenesis-enriched	Unknown
Y65B4BL.5		5.8	ME	Oogenesis-enriched	Long-chain acyl-CoA synthetases (AMP-forming) [KOG1256]
C48B4.7		5.8	SMD	Oogenesis-enriched	Unknown
C17E4.5	*pab-3*	5.8	SMD	Oogenesis-enriched	Poly-A–binding protein [KOG4209]
F55A11.8		5.7	SMD	Oogenesis-enriched	Unknown
T19B10.7	*ima-1*	5.7	SMD	Oogenesis-enriched	Karyopherin (importin) α [KOG0166]
T21C9.4		5.5	ME		Enhancer of rudimentary (cell cycle) [KOG1766]
C30F12.4		5.5	SMD	Oogenesis-enriched	Unknown

The CGH-1/IgG ratio indicates fold enrichment.

[a]Microarray expression data from Baugh et al. (2001). MD, maternal degradation class (genes that are the subset of maternal genes that decrease without first increasing in abundance; ME, maternal-embryonic class (mRNAs with similar expression levels in pre- and postzygotic embryos); SMD, strictly maternal degradation class (genes are the subset of maternal degradation genes that are not also classified as embryonic).

[b]Microarray expression data from (Reinke et al. (2004). Intrinsic, similar expression in gonads that produce either oocytes or sperm; Oogenesis-enriched, mRNAs that were more abundant during oogenesis than spermatogenesis.

[c]Eukaryotic clusters of orthologous groups of proteins database classification. A complete list of CGH-1 associated mRNA is available in Table S2 (available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1).

~60% of *C. elegans* mRNAs (Zorio et al., 1994). When *C. elegans* oogenesis is arrested, mRNA accumulates in large cytoplasmic foci, greatly enhancing its detection (Schisa et al., 2001). We therefore performed our experiments in the feminized strain *fog-2(q71)*, which ovulates infrequently because it does not produce sperm (Schedl and Kimble, 1988). In *fog-2(q71)* oocytes, CGH-1 formed large particles that colocalized with the SL1 signal, which was also detected in smaller particles that lacked CGH-1 staining (Fig. 5, A–C). In *fog-2(q71)*, this CGH-1 staining has been reported to colocalize with large CAR-1 foci, only some of which overlap with germ (P) granules (Jud et al., 2008). Taken together, this suggests that some but not all SL1-containing mRNA colocalizes with CGH-1 and CAR-1 in storage bodies and germ (P) granules.

Mislocalization and specific destabilization of germ line mRNAs in the absence of CGH-1

Our results raise the important question of how CGH-1 influences the localization, stability, or translational regulation of maternal mRNAs during oogenesis. Null *cgh-1(ok492)* and *cgh-1(RNAi)* hermaphrodites are sterile but produce oocytes in which gross abnormalities in gene expression have not been detected (Navarro et al., 2001; Boag et al., 2005). This makes it very unlikely that the lack of CGH-1 results simply in a widespread release of germ line translational suppression, which would lead to somatic differentiation in the gonad (Ciosk et al., 2006). CGH-1 is required for appropriate localization of CAR-1, however. If CGH-1 is absent, CAR-1 forms aberrant sheetlike structures within the syncytial gonad core (Fig. S2 B; Audhya et al., 2005; Boag et al., 2005). This appears to involve a defect in storage body formation because germ (P) granules remain largely intact during oogenesis in *cgh-1* null mutants (Fig. S2 B; Boag et al., 2005). We examined how lack of CGH-1 affects the distribution of mRNAs during oogenesis by hybridizing with the SL1 probe, which in WT animals detects small foci throughout the syncytial core (Fig. 5 D). Remarkably, in *cgh-1(ok492)* animals, some SL1-containing mRNA was similarly found in small puncta, but a significant proportion of the SL1 signal colocalized with the large CAR-1 sheets (Fig. 5, F–I). We conclude that during oogenesis, CGH-1 is required for correct localization of a substantial proportion of SL1-containing mRNAs.

We next investigated whether a lack of CGH-1 differentially affects the localization or stability of representative mRNAs that our RIP-Chip experiments identified as being associated with CGH-1. For controls, we examined germ line enriched mRNAs (Reinke et al., 2004; Reinke et al., 2000) that were indicated by SAGE databases to be of comparable abundance but had not been detected as CGH-1–associated in our IPs. We first performed in situ hybridization of dissected gonads

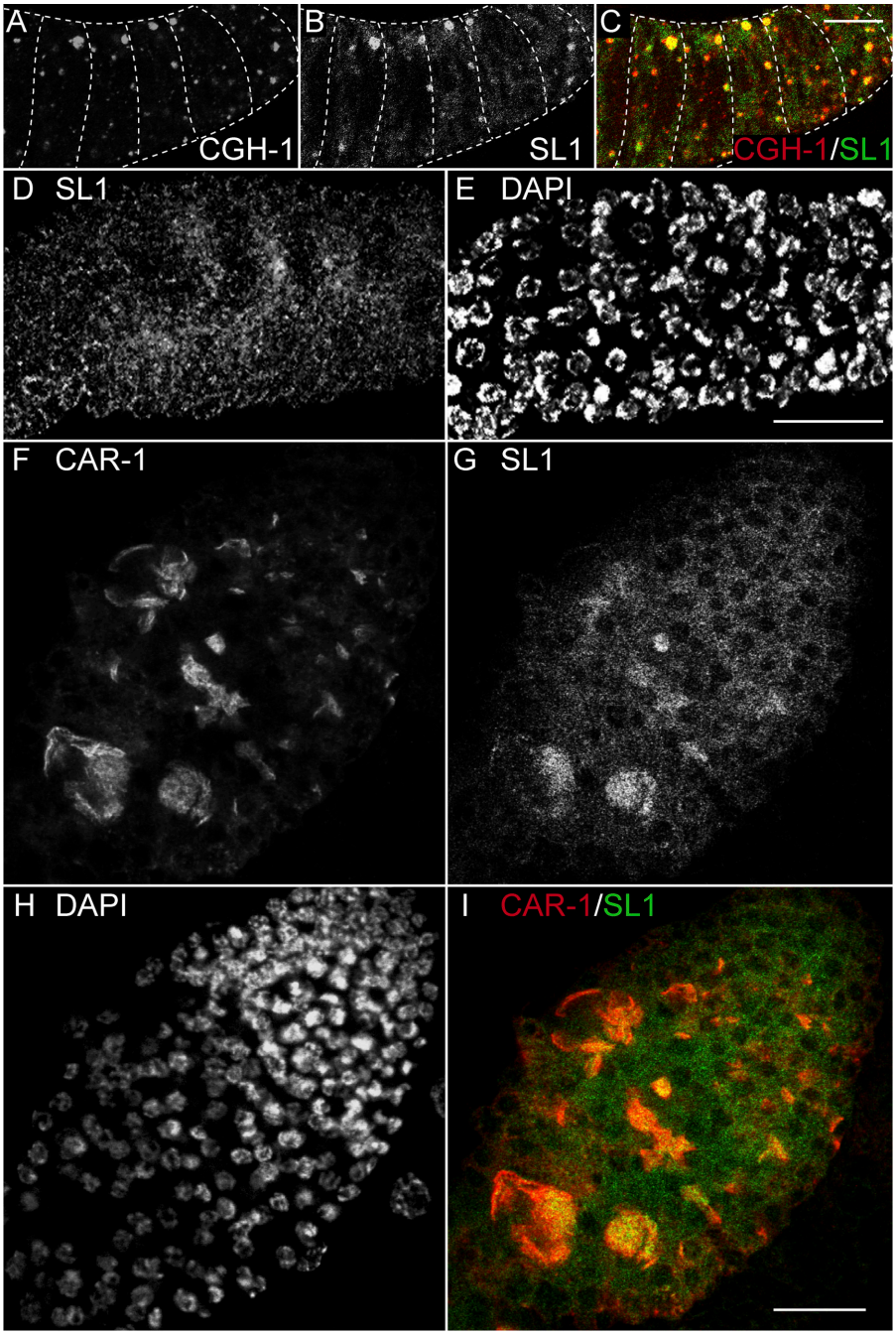

Figure 5. **CGH-1 affects mRNA localization during oogenesis.** (A–C) CGH-1 and SL1 leader sequence localization in proximal oocytes in *fog-2(q71)* females. Extruded gonads from 1-d-old adults were stained for CGH-1 and analyzed by in situ hybridization to an Alexa 488–linked SL1 antisense probe. SL1-containing mRNA accumulates in large foci that colocalize with CGH-1 and in separate small puncta. In parallel, an SL1 sense probe showed little or no diffuse signal (not depicted). Oocytes are outlined by broken lines. $n > 60$. (D and E) SL1 localization in WT hermaphrodite gonads analyzed by in situ hybridization. (F–I) Abnormal mRNA localization during oogenesis in *cgh-1(ok492)*. Extruded hermaphrodite gonads were analyzed by in situ hybridization for SL1 (green) and staining with anti–CAR-1 (red) and DAPI. The transition zone and pachytene region of a typical gonad are shown, with distal toward the top right. CAR-1 and some SL1-containing mRNAs colocalize in sheetlike structures. The bulbous shape of this gonad is characteristic of many animals that lack CGH-1 (Navarro et al., 2001). Confocal z series projections are shown. For D–I, $n > 60$ for WT and *cgh-1(ok492)*. Bars, 10 µm.

(Lee and Schedl, 2006), a technique that reveals approximate mRNA levels during the different stages of oogenesis. Across multiple experiments, the levels of all six CGH-1–associated mRNAs that we tested were dramatically and consistently reduced throughout the gonad in young *cgh-1(ok492)* adults (Figs. 6 A and S3 C). In contrast, the expression patterns of six out of seven control mRNAs that we examined were indistinguishable between *cgh-1(ok492)* and WT (Figs. 6 A and S3 D). We next examined the relative abundance of these mRNAs in *cgh-1(ok492)* and WT animals using quantitative RT-PCR (RT-qPCR). The results of these experiments were strikingly similar to our in situ hybridization findings (Fig. 6 B). Additional experiments were performed using RNA extracted from dissected gonads of WT and *cgh-1(ok492)* adult hermaphrodites, and similar results were obtained (unpublished data). Within a population of *cgh-1(ok492)* animals, each of the CGH-1–associated mRNAs that we tested was present at dramatically lower levels, but a reduction was seen for only one of the control mRNAs (*gld-1*; Fig. 6 B).

Our findings suggest that CGH-1–associated mRNAs become differentially destabilized if CGH-1 is absent. Alternatively, it might be possible that *cgh-1(ok492)* animals have lower levels of these maternal mRNAs because they do not ovulate and therefore produce fewer oocytes, or if they have a germ cell differentiation block. *cgh-1(ok492)* and *cgh-1(RNAi)* animals switch from sperm to oocyte production at the appropriate stage, however, and

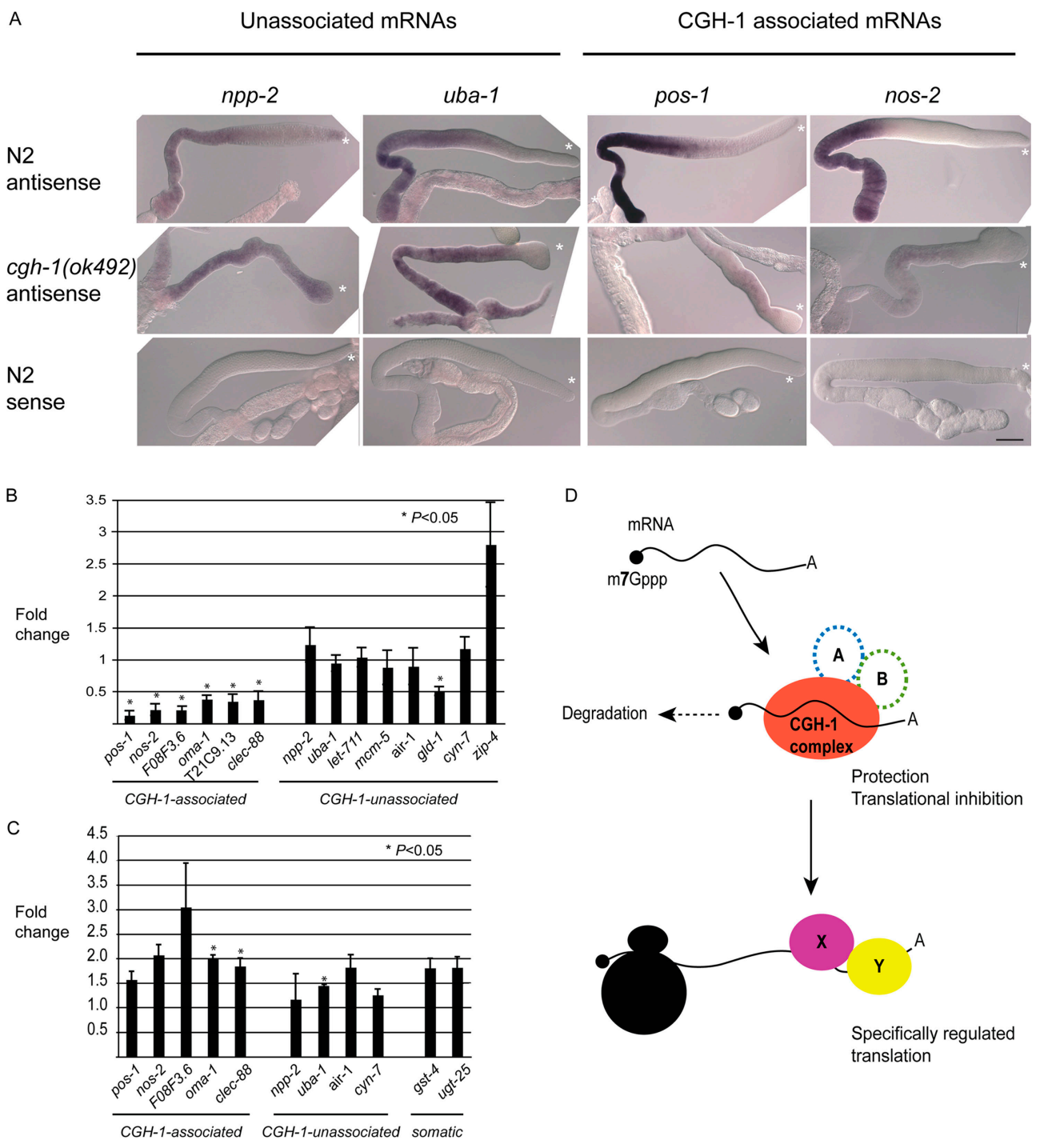

Figure 6. **CGH-1 binds and stabilizes particular mRNAs during oogenesis.** (A) Analysis of mRNA abundance and distribution by in situ hybridization. Probes were hybridized to dissected gonads from 1-d-old N2 and *cgh-1(ok492)* animals. For each probe and genotype tested, at least 20–25 gonads were examined in each of two or more experiments. Six of seven mRNAs that were not associated with CGH-1 did not differ detectably between N2 and *cgh-1* mutant animals (Fig. S3, C and D, available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1). In contrast, all six CGH-1–associated mRNAs analyzed were present at markedly lower levels in *cgh-1(ok492)* gonads. Distal is shown to the right (asterisks). Each in situ hybridization experiment gave consistent results among gonads examined, for which representative images are shown. Bar, 50 µm. (B) Relative abundance of CGH-1–associated and unassociated mRNAs in *cgh-1(ok492)* adults compared with WT, assayed by RT-qPCR. Each mRNA value was normalized to actin (*act-1*), and WT levels were converted to 1. The mean of three biological experiments is graphed. (C) Relative abundance of CGH-1–associated and unassociated mRNAs in *fog-2(q71)* adults compared with WT. Data are from two independent experiments. Error bars indicate SEM. (D) A model for germ line CGH-1 functions. Many maternal mRNAs rely on the CGH-1 complex to protect them from degradation and possibly for translational silencing. Some CGH-1–associated mRNAs are known to be translationally regulated through binding of sequence-specific proteins to their 3′ UTRs (see text). Such proteins might be present on individual mRNAs in conjunction with the CGH-1 complex or could assume control of these mRNAs after they are released by this complex.

as adults, produce only oocytes (Navarro, et al., 2001; Boag, et al., 2005). Furthermore, *cgh-1* mutants express typical oocyte-specific genes. For example, three of the CGH-1–unassociated mRNAs that we tested (*gld-1*, *air-1*, and *zip-4*) are expressed primarily during oogenesis and not in larvae (Reinke et al., 2004), and only one of these (*gld-1*) is present at modestly lower levels in *cgh-1(ok492)* hermaphrodites (Figs. 6 B and S3 D). Finally, mRNAs that are associated with (and dependent upon) CGH-1 are present at normal or increased levels in *fog(q71)* females, which also ovulate infrequently (Fig. 6 C). We conclude that during oogenesis, CGH-1 is needed to protect the specific group of mRNAs with which it normally associates but not most other mRNAs, in striking contrast to its previously described role in promoting mRNA decay in P bodies (Coller and Parker, 2004; Eulalio et al., 2007a; Parker and Sheth, 2007).

Discussion

Studies in yeast, cultured cells, and in vitro have shown that Dhh1(CGH-1) and Pat1 act in concert to recruit mRNAs to P bodies, inhibit their translation, and promote decapping (see Introduction). We now find that in *C. elegans*, the oogenesis functions of CGH-1 are distinguishable genetically from its role in "typical" P bodies. In somatic tissues, CGH-1 is found in *patr-1*–dependent decapping-associated P bodies, but during oogenesis, CGH-1 forms storage bodies independently of *patr-1*, interacts primarily with a set of translational regulators, and protects a specific group of maternal mRNAs from degradation.

PATR-1–dependent somatic CGH-1 particles have properties of decapping-associated P bodies

When we investigated whether germ line or embryonic CGH-1 particles have characteristic properties of P bodies (Parker and Sheth, 2007), we found that this was the case only in somatic cells. The CGH-1 particles that are present in somatic cells after the four-cell stage (somatic P bodies) were stabilized if decapping-associated factors were lacking, and were essentially undetectable in the absence of PATR-1 (Fig. 2, B–E). PATR-1(N) staining was seen almost exclusively in somatic P bodies, and in *dcap-2(tm2470)* animals, somatic P bodies that contained both PATR-1 and CGH-1 were prominent into adulthood (Figs. 3 A and S1 F). We conclude that PATR-1 dependence and PATR-1(N) staining are specific characteristics of somatic P bodies, and that in metazoan, Pat1 may represent a functional marker of degradative P bodies.

The somatic P bodies we identified are likely to correspond to previously described embryonic particles containing DCAP-2 and the miRNA-associated proteins ALG-1 (argonaute) and AIN-1 (GW182; Ding et al., 2005). Cell culture experiments suggest that mammalian CGH-1 (RCK) is required for gene silencing by miRNAs (Chu and Rana, 2006), but *cgh-1(ok492)* homozygotes do not show either the devastating germ line defects caused by lack of the nuclease dicer (*dcr-1*) or the heterochronic phenotypes associated with some miRNAs (Pasquinelli et al., 2000; Knight and Bass, 2001). Although this suggests that CGH-1 is not generally essential for miRNA function, it will be important to investigate whether CGH-1 might have redundant or more specific functions in miRNA-directed silencing.

Somatic CGH-1 particles became *patr-1* dependent approximately as decay of class II maternal mRNAs begins (see Introduction), which suggests that class II mRNAs might be degraded through decapping. However, class II mRNAs that we have analyzed by in situ hybridization and RT-qPCR (*pos-1* and *nos-2*) were not detectably stabilized in *dcap-2(tm2470)* embryos (unpublished data). Perhaps the decapping pathway degrades only some class II mRNAs or acts redundantly with other mechanisms. Consistent with the latter idea, *dcap-2(tm2470)* mutants develop to adulthood and are fertile (Table S1), which suggests that the decapping pathway is unlikely to be the most important mRNA decay mechanism in *C. elegans*.

CGH-1 binds and stabilizes specific maternal mRNAs

In contrast to somatic P bodies, the CGH-1–containing storage bodies found in germ cells were not detectably influenced by lack of *dcap-2* or *patr-1*, and they lacked PATR-1(N) staining (Fig. S2 and not depicted). In addition, neither the decapping proteins nor PATR-1 were among the most prominent CGH-1 interactors in *C. elegans*, in which the bulk of CGH-1 expression derives from developing oocytes (Table I; Boag, et al., 2005). During oogenesis, CGH-1 therefore appears to act in a manner that is distinct from its P body functions. Accordingly, we identified two prominent CGH-1 interactors to be ATX-2 and PAB-1, which in mammalian cells are not found not in P bodies but are characteristic of stress granules, structures in which some mRNAs are transiently sequestered during stress (Anderson and Kedersha, 2008). We speculate that CGH-1 and the translational regulators with which it forms a germ line RNP (Table I; Boag, et al., 2005) may assemble on an mRNA to form the functional unit of the storage body, much as the decapping complex is the "building block" of the P body (Parker and Sheth, 2007). Although germ line storage bodies are distinct from P bodies, it should be remembered that core P body mechanisms have important functions during oogenesis: *dcap-2* and *patr-1* mutants show minor germ line abnormalities (Fig. S1 C and Table S1), and in *D. melanogaster*, *Dcp1* is required for localization of some maternal mRNAs (Lin et al., 2006).

We investigated the specificity with which CGH-1 acts on mRNA during oogenesis by performing a systems analysis of its interactions with mRNA. In RIP-Chip experiments, we obtained the unexpected finding that CGH-1 interacts specifically with a particular set of oocyte and maternal mRNAs (Fig. 4 and Table S2). Our stringent criteria for CGH-1 association might have underestimated the number of mRNAs that interact specifically with CGH-1 in vivo, but we established that these CGH-1–associated mRNAs are functionally linked to CGH-1, as we obtained the surprising finding that they were differentially destabilized in *cgh-1(ok492)* animals (Fig. 6 and Fig. S3, C and D). Our findings indicate that CGH-1, and by extension the conserved CGH-1 germ line complex, defines a large but discrete maternal mRNA storage pathway.

What is the main function of the CGH-1 complex in regulating these mRNAs? In *cgh-1(ok492)* animals, we did not detect premature translation of the CGH-1–associated mRNA *pos-1* by antibody staining (not depicted), although the destabilization of this mRNA (Fig. 6, A and B) might have masked any translational derepression. It is intriguing that CGH-1 protects particular maternal mRNAs from degradation, in striking contrast to its more familiar role in promoting decapping. In the protozoan *Plasmodium berghei*, the CGH-1 orthologue (DOZI) is also required for female fertility and stabilization of certain mRNAs that are translationally silenced in female gametocytes, the female gamete precursor (Mair et al., 2006). Individual translationally suppressed mRNAs coIP from gametocytes along with DOZI, and without DOZI, many mRNAs that are normally translationally silenced in the gametocyte are degraded. This striking similarity to our findings suggests that a protective function for CGH-1 in reproduction might be conserved. One possibility is that the degradation that occurs in the absence of CGH-1 is a "default" fate that eliminates errant mRNAs that have escaped from being appropriately localized or translationally silenced (Fig. 6 D).

It is an intriguing question how CGH-1 interacts with and protects particular mRNAs because DEAD box helicases generally bind mRNA nonspecifically (Rocak and Linder, 2004). This specificity might be provided by other CGH-1 complex components (Table I), by transient interactions with other factors, or by proteins that interact specifically with particular subgroups of CGH-1–associated mRNAs (Fig. 6 D). For example, in mammalian cells, the decapping machinery can be targeted to mRNAs by sequence-specific regulators (Franks and Lykke-Andersen, 2007). One possibility is that the CGH-1 complex is formed on mRNAs at germ (P) granules, at which some CGH-1 is found throughout oogenesis (Figs. 1 and S2 A). Some maternal mRNAs seem to pass through germ (P) granules as they emerge from the nuclear pores, and the mRNAs that are most robustly detected in germ (P) granules by in situ hybridization (*mex-1*, *pos-1*, and *nos-2)* are prominent in CGH-1 IPs (Tables II and Table S2; Pitt et al., 2000; Schisa et al., 2001). The RNA-dependent association of PAB-1 with CGH-1 we have described here (see Results) suggests a plausible if speculative model for how the CGH-1 complex could protect these mRNAs. CGH-1 might allow these mRNAs to interact stably with PAB-1, binding of which inhibits deadenylation and degradation (Mangus et al., 2003).

Considerable progress has been made in various systems toward understanding how translation of individual maternal mRNAs is controlled through binding of regulatory proteins to their 3′ untranslated regions (UTRs; Lipshitz and Smibert, 2000; Wickens et al., 2002; Richter, 2007). In contrast, it has remained largely unknown to what extent CGH-1 or other putative "masking" proteins (Weston and Sommerville, 2006) might influence regulation of these same mRNAs. Many of the mRNAs that we determined are bound and protected by CGH-1 are known to be translationally regulated during oogenesis (see Results). For example, one of the most prominent CGH-1–associated and –stabilized mRNAs is *nos-2* (*nanos* orthologue; Fig. 6, A and B; and Table II), the translation of which is controlled through interactions between specific regulators and UTR sequences (Jadhav et al., 2008). CGH-1 thus defines a broader level of regulation that may act on many maternal mRNAs for which translation is controlled by sequence-specific UTR-associated mechanisms, raising the interesting question of how the CGH-1 complex might interact functionally with those mechanisms (Fig. 6 D).

Developmental regulation of CGH-1 and PATR-1 functions

Our results in *C. elegans* have shown that CGH-1 functions differently in the oogenic germ line and soma, and have distinguished three types of CGH-containing particles (germ [P] granules, storage bodies, and somatic P bodies; Fig. 1). In this simple metazoan, therefore, CGH-1 functions are tissue-specific and restricted with respect to its mRNA targets, at least during oogenesis. We conclude that CGH-1, PATR-1, and possibly other core P body mechanisms may have surprisingly complex functions during development. It will be important to elucidate how these mechanisms are targeted to individual mRNAs, and how CGH-1 and PATR-1 functions are regulated in different cellular and developmental contexts.

Materials and methods

Strains

WT (N2 Bristol) and mutant strains were maintained as in Brenner (1974). Deletion mutants were generated by the National BioResource Project for the Nematode, balanced, and maintained as the strains *patr-1(tm2402)II/* mln1[dpy-10(e128) mls14] and *dcap-2(tm2470)IV/* nT1[qIs51] (Table S1). Each was outcrossed at least seven times to N2. Additional strains used were: *glp-4(bn2)I*, *unc-119*(ed3)*III*, and *cgh-1(ok492)III*, *fog-2(q71)V*.

RNAi

Young adult hermaphrodites were injected with double-stranded RNA (~1 µg/µl total concentration) and allowed to lay eggs for 8–10 h before being transferred to new plates. F1 adult hermaphrodites were dissected and used for either gonad or embryo staining.

RIP-Chip

The CGH-1 RNP was immunoprecipitated from adult extracts as described previously (Boag et al., 2005) with the following modifications. Salmon sperm DNA–coated protein A beads (Millipore) were pretreated with 2 mg tRNA before addition to the protein lysate. After elution with CGH-1 peptide, RNA was purified from the eluted RNP mixture by extraction with Tri Reagent (Sigma-Aldrich) and subjected to one round of linear amplification using either the SuperScript System (Invitrogen) or as described previously (Baugh et al., 2001). Four independent coIPs and linear amplifications were conducted, with microarray analysis performed as described previously (Reinke et al., 2004). We averaged the relative abundance of each gene across four independent CGH-1 versus IgG microarray experiments, and mRNAs with more than a threefold fold increase in CGH-1 IPs were defined as CGH-1–associated. SAGE data were obtained from the Genome BC *C. elegans* Gene Expression Consortium (http://elegans.bcgsc.bc.ca/).

Antibody production and microscopy

Rat polyclonal antibodies were raised against the 19 N-terminal amino acids of PATR-1 (Cocalico Biologicals, Inc.), and affinity purified (Sulfolink; Pierce). Staining of *patr-1(RNAi)* or *tm2402* gonads showed only a low level of diffuse background signal (Fig. S1 E). Gonad staining was performed as described previously (Navarro et al., 2001). For PATR-1(N) staining, embryos were fixed in methanol (−20°C) for 10 min and placed in cold acetone for a further 10 min, then washed, blocked, and stained similarly to gonads. Antibodies to POS-1 and GLH-2 were provided by Y. Kohara (National Institute of Genetics, Shizuoka, Japan) and K. Bennett (University of Missouri, Columbia, MO), and staining was carried out as described previously (Tabara et al., 1999). Secondary antibodies were species-specific Alexa A488 and A555 (Invitrogen), and samples were mounted in Vectashield mounting medium (Vector Laboratories). Confocal image acquisitions were performed at room temperature using a microscope

(LSM 510 META) equipped with an oil immersion Plan-Apochromat 1.4 NA 63× objective (all from Carl Zeiss, Inc.). Z series projections were interrogated for CGH-1 particle size and number using MetaMorph software (MDS Analytical Technologies). Live imaging of CGH-1::GFP–expressing embryos was conducted at room temperature using an Axioplan 2 MOT microscope equipped with an oil immersion Plan-Apochromat 1.4 NA 63x objective and controlled by AxioVision software, and images were acquired with a camera (AxioCam; all from Carl Zeiss, Inc.). Processing of all images was performed using Photoshop CS2 and Illustrator CS2 (both from Adobe).

Identification of additional CGH-1 complex proteins

ATX-2 and PAB-1 were identified here as corresponding to species that were previously detected in CGH-1 IPs (Boag et al., 2005). Briefly, CGH-1 and associated proteins were eluted from the protein A beads, separated on a 10% polyacrylamide gel, and stained. Individual bands were excised from the gel, digested with trypsin, and subjected to tandem mass spectrometry analysis. Proteins were identified by searching the National Center for Biotechnology Information Inr database using the Mascot program. ATX-2 and PAB-1 were identified by the presence of five and nine distinct high-confidence peptides that cover 6% and ~17% of the predicted proteins, respectively.

Immunoprecipitation and Western analysis

IP of endogenous CGH-1 was conducted as described previously (Boag et al., 2005). For Western analysis of PATR-1 expression in N2 or *patr-1(tm2402)*, adult hermaphrodite worms or dissected N2 gonads were frozen and thawed in the presence of 1× reducing sample buffer and denatured at 95°C for 6 min. Samples were run on 10% SDS-PAGE gels and transferred to nitrocellulose membranes using standard methods. Proteins were detected with species-specific HRP-conjugated secondary antibodies. The relative abundance of PATR-1 in *glp-4(bn2)* compared with WT was calculated by densitometry using ImageJ (National Institutes of Health). Actin was used as a reference standard.

In situ hybridization

Digoxigenin-labeled sense and antisense probes were produced by asymmetrical PCR from cDNA templates (~1 kb), and dissected gonads were fixed in 3% paraformaldehyde, 0.25% glutaraldehyde, and 0.1 M K2HPO4, pH7.2, and processed as described previously (Lee and Schedl, 2006). This protocol provided consistent results across >50 individual gonads examined for each mRNA and genotype. Fluorescence RNA in situ hybridizations were performed essentially as described previously (Schisa et al., 2001) using Alexa 488–conjugated sense and antisense SL1 probes. This probe specifically detects SL1-containing spliced mRNAs in the cytoplasm (Seydoux and Fire, 1994).

RT-qPCR analysis

RNA was extracted from 1-d-old adults using Tri Reagent and cDNA prepared from total RNA using SuperScript II (Invitrogen). Primer sequences were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). RT-qPCR was performed using SYBR green PCR master mix (Applied Biosystems) and the ABI Prism 7700 Sequence Detection system (Applied Biosystems). Samples were run in duplicate, and relative abundance was determined using the ΔΔCt method (Livak and Schmittgen, 2001) and *act-1* (actin) as the internal standard. For analysis of mRNA levels in *cgh-1(ok492)* and WT animals, three independent biological experiments were conducted for each mRNA. Additional experiments were performed using RNA extracted from dissected gonads of WT and *cgh-1(ok492)* adult hermaphrodites, and similar results were obtained. For comparison of mRNA levels in *fog-2(q71)* and WT animals, two independent experiments were conducted.

Production and analysis of transgenic *CGH-1::GFP* strains

The *CGH-1::GFP* translational fusion construct pCGH-1::GFP-unc119 was constructed by cloning genomic fragments of 3,658 bp (predicted *cgh-1* promoter and coding region) and 1,128 bp (the region 3′ to the *cgh-1* ORF) into the *NotI* and *SacII* sites, respectively, of the *unc-119* rescue plasmid pBS-unc119-GFP. Transgenic animals were produced by biolistic transformation (Praitis et al., 2001). Animals were grown on nematode growth medium plates until the adult stage, then bleached to obtain embryos that were subsequently cultured for 3 d in S-basal medium containing HT115(DE3) bacteria. Gold beads that were coated with pCGH-1::GFP-unc119 were bombarded into these young adults using the PDS-1000/He with Hepta adaptor system (Bio-Rad Laboratories). Animals were allowed to recover for 1 h, transferred to NGM plates seeded with HT115(DE3) bacteria, grown at 25°C for 3 wk, and then screened for WT animals (*unc-119* rescued). By genetic crossing, we obtained two strains in which the *CGH-1::GFP* transgene rescued *cgh-1(ok492)* animals to fertility. One of these strains (*ldIs100*[$P_{cgh\text{-}1}$*::cgh-1::GFP::3′UTR*$_{cgh\text{-}1}$*; unc-119(+)*]) was analyzed in Fig. 2 E. Single focal plane images of transgenic embryos were acquired using a Axioskop-2 microscope (Carl Zeiss, Inc.).

Online supplemental material

Fig. S1 diagrams the *dcap-2(tm2470)* and *patr-1(tm2402)* deletions, and shows how these mutations affect the organization of the gonad. A comparison of PATR-1 levels in WT, *patr-1(tm2402)*, and dissected gonads is also presented. Staining for endogenous CGH-1 and PATR-1 in *patr-1(tm2402)* and *dcap-2(tm2470)* is shown, demonstrating the specificity of the PATR-1(N) antisera and the persistence of CGH-1 and PATR-1 foci in somatic tissues of *dcap-2(tm2470)* adults. Fig. S2 shows representative images of CGH-1 and CAR-1 localization in N2, *dcap-2(tm2470)*, and *patr-1(tm2402)* gonads, along with CAR-1 and GLH-2 staining in the null *cgh-1(ok492)* mutant. Fig. S3 provides supporting evidence that CGH-1 associates with and stabilizes a specific subset of maternal mRNAs. Table S1 compares offspring production in the WT (N2) and the predicted null mutants *dcap-2 (tm2470)* and *patr-1(tm2402)*. Table S2 lists the CGH-1–associated mRNAs. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200801183/DC1.

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Note added in proof. The Evans laboratory has shown that during *C. elegans* oogenesis, CGH-1 colocalizes in particles with the maternal mRNA *glp-1* and is required to maintain its stability under conditions in which oogenesis is arrested (Noble, S.L., B.L. Allen, L.K. Goh, K. Nordick, and T.C. Evans. 2008. *J. Cell Biol.* 182:559–572).

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