An E-cadherin-mediated hitchhiking mechanism for C. elegans germ cell internalization during gastrulation

Daisuke Chihara and Jeremy Nance

SUMMARY
Gastrulation movements place endodermal precursors, mesodermal precursors and primordial germ cells (PGCs) into the interior of the embryo. Somatic cell gastrulation movements are regulated by transcription factors that also control cell fate, coupling cell identity and position. By contrast, PGCs in many species are transcriptionally quiescent, suggesting that they might use alternative gastrulation strategies. Here, we show that C. elegans PGCs internalize by attaching to internal endodermal cells, which undergo morphogenetic movements that pull the PGCs into the embryo. We show that PGCs enrich HMR-1/E-cadherin at their surfaces to stick to endoderm. HMR-1 expression in PGCs is necessary and sufficient to ensure internalization, suggesting that HMR-1 can promote PGC-endoderm adhesion through a mechanism other than homotypic trans interactions between the two cell groups. Finally, we demonstrate that the hmr-1 3’ untranslated region promotes increased HMR-1 translation in PGCs. Our findings reveal that quiescent PGCs employ a post-transcriptionally regulated hitchhiking mechanism to internalize during gastrulation, and demonstrate a morphogenetic role for the conserved association of PGCs with the endoderm.

KEY WORDS: Gastrulation, Primordial germ cell (PGC), Endoderm, Cadherin, 3’ UTR, Adhesion

INTRODUCTION
Gastrulation is a crucial morphogenetic event when cells that will give rise to internal tissues and organs move to interior regions of the embryo. Although both somatic cells (endodermal and mesodermal precursors) and primordial germ cells (PGCs) internalize during gastrulation, most studies of gastrulation have focused on somatic cells because they comprise the vast majority of internalizing cells. Somatic cells and PGCs are set apart during the initial stages of embryogenesis and follow distinct developmental trajectories. Soon after somatic cells are born, they activate specific transcription factors that begin to restrict their developmental potential. Cells that acquire endodermal or mesodermal fates then move into the interior of the embryo during gastrulation. Cell fate specification and gastrulation are often coordinated by the same transcription factors, which regulate both cell-identity genes and genes that control gastrulation movements. For example, the C. elegans endodermal precursor E is specified through the combined action of GATA transcription factors END-1 and END-3 (Maduro, 2006). The daughters of E (Ea and Ep) initiate gastrulation when they constrict their apical surfaces and ingress into the interior of the embryo (Nance and Priess, 2002; Lee and Goldstein, 2003). Disrupting function of the end genes prevents the E lineage from producing endoderm and also blocks Ea and Ep ingress (Nance and Priess, 2002; Lee et al., 2006; Owraghi et al., 2010). An analogous coupling between cell fate specification and gastrulation is well documented in vertebrate mesendodermal cells (for example, by Nodal signaling) and fly specification and gastrulation is well documented in vertebrate embryos (Leptin, 2005; Heisenberg et al., 2010). An analogous coupling between cell fate specification and gastrulation is well documented in vertebrate embryos (Leptin, 2005; Heisenberg et al., 2010).

Finally, we demonstrate that the conserved association of PGCs with the endoderm.

© 2012. Published by The Company of Biologists Ltd

RESEARCH ARTICLE

DEVELOPMENT

Accepted 3 May 2012

1Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY 10016, USA. 2Department of Cell Biology, NYU School of Medicine, New York, NY 10016, USA.

*Author for correspondence (jeremy.nance@med.nyu.edu)
end-1P::GFP both Z2 and Z3 sank into the embryo. Ingression times were normalized to cells in somatic lineages were chosen for analysis [E, Ea; MSw, MSppp; unc-119(ed3) (Invitrogen), the pCFJ150 destination vector (Frokjaer-Jensen et al., 2008) from plasmid pJN254 (Nance et al., 2003) was cloned into the (a gift from Andy Fire, Stanford School of Medicine, CA, USA).

Material and methods

Strains
All hmr-1 mutants were hmr-1(zu389), which contains a nonsense mutation prior to sequences encoding the transmembrane domain (Broadbent and Pettitt, 2002). All unc-119 mutants were unc-119(ed3). The following strains were used (all FT strains were created in this study): N2 (wild type); CX2993, sac-7(by46); kyd4 (Zallen et al., 1999); FT233, hmr-1; xnsEx42 [hmr-1(+); dpy-17::RFP, end-1P::GFP]; FT598, xnsEx11 [mex-5P::GFP; PHPLC::nos-2::UTR]; xns91 [end-1P::mCherry-PHPLC]; FT669, xnsEx12; xnsEx11; xns99 [end-1P::mCherry-PHPLC]; FT741, xns6 [mex-5P::HMR-1-GFP::hmr-1::UTR]; unc-119(ed3); FT774, xns17 [mex-5P::GFP-PHPLC::hmr-1::UTR]; zub244 [nmy-2P::PGL-RFP]; unc-119; FT776, hmr-1; xnsEx42; xns6; FT834, xns13 [mex-5P::GFP-PHPLC::tbb-2::UTR]; zub244; unc-119; FT850, xns18 [mex-5P::HMR-1-GFP-ZF1::hmr-1::UTR]; unc-119; FT853, zub240 [pie-1P::sec-GFP]; zub244; lts14 [pie-1P::mCherry-PHPLC]; FT295, qts12 [pal-1P::YFP]; xns91; FT1040, hmr-1; xns375 [hmr-1P::HMR-1-ZF1-GFP::hmr-1::UTR]; and MS1248, end-1(ok538) end-3(ok1448); trEx568 [end-1(+)], end-3(+), sur-5::RFP] (Owragh et al., 2010).

Transgene construction
mex-5P::HMR-1-GFP::hmr-1::UTR, mex-5P::HMR-1-GFP-ZF1::hmr-1::UTR, mex-5P::GFP-PHPLC::nos-2::UTR, mex-5P::GFP-PHPLC::hmr-1::UTR and mex-5P::GFP-PHPLC::tbb-2::UTR were created using Multisite Gateway (Invitrogen), the pCFJ150 destination vector (Frokjaer-Jensen et al., 2008) and the following entry clones: 5', pJA252 (mex-5P) and pJA254 (mex-5P::GFP) (Zeiser et al., 2011); middle, pJN527 (mex-5P::GFP-PHPLC); pDC21 (mex-5P::GFP-ZF1) and pDC08 (PHPLC::3', pDC10 (nos-2::UTR), pCM1.36 (tbb-2::UTR)) (Merritt et al., 2008) and pNS22 (hmr-1::UTR). Gateway entry clones were constructed as follows. pNS27 (HMR-1-GFP::hmr-1::UTR); pNS27 (HMR-1-GFP::hmr-1::UTR); and pNS22 (hmr-1::UTR). Gateway entry clones were constructed as follows. pNS27 (HMR-1-GFP::hmr-1::UTR); pNS27 (HMR-1-GFP::hmr-1::UTR); and pNS22 (hmr-1::UTR).

Non-Gateway plasmids were constructed as follows. hmr-1P::HMR-1-GFP-ZF1 was cloned from hmr-1P::HMR-1-GFP::unc-54::UTR (Achilleos et al., 2010) digested with XbaI to remove gef and gfp and the unc-54 3' UTR, gfp, gfp and the hmr-1P::HMR-1-GFP-ZF1 were inserted using Gibson end-joining (Gibson et al., 2009). hmr-1P::HMR-1-GFP-ZF1 was created by cloning the end-1 promoter from hmr-1P::GFP (Nance et al., 2003) into mCherry plasmid pGC326 (a gift from E. J. Hubbard, Skirball Institute, New York, USA) digested with HindIII and Apel, then inserting PCR-amplified rat PLC1 domain (Nance et al., 2003) was cloned into the pDONR221 (Invitrogen) and gfp was inserted into a BsiWI site engineered before the stop codon. pDC21 (HMR-1-GFP-ZF1) and pDC08 (PHPLC::3', pDC10 (nos-2::UTR), pCM1.36 (tbb-2::UTR)) (Merritt et al., 2008) and pNS22 (hmr-1::UTR).

Results

Gastrulation
Gastrulation movements of PGCs and surrounding cells during gastrulation To determine whether PGC gastrulation movements are active or inactive, we asked if the corpse of a laser-irradiated PGC could be observed. Laser irradiation was performed on a Zeiss AxioImager using a 100× 1.3 NA objective and MicroPoint laser with Coumarin dye cell. Embryos were mounted on 4% agarose and the targeted nucleus was pulse-irradiated until refractile debris appeared. Founder cells were laser-irradiated in each targeted lineage, except for the Cx3 lineage where Cap and Cpp were targeted. Irradiated embryos were analyzed only if irradiated cell(s) ceased dividing and cell divisions were normal.

Fluorescence microscopy and analysis of cell adhesion and movements
Fluorescence time-lapse movies of embryos expressing endoderm and PGC surface markers (strain FT696) were acquired using a Leica SP5 confocal microscope, 488 nm and 594 nm lasers, and 63× 1.3 NA water-immersion objective. Embryos were suspended from the coverslip in water. Dorsal shift was measured by calculating the distance from the dorsal-most endodermal or Z2 cell surface to the eggshell. To analyze cell contacts and separations in wild-type embryos, strain FT583 was used. Separations between PGcCs and endoderm were examined using strain FT598 (wild type) and strain FT696 (hmr-1).
laser-irradiated each of these blastomeres individually to prevent movements of the D, MS, Cxp or E blastomere descendants, we considered the possibility that movements of the D, MS or Cxp descendants could push the PGCs into the embryo, or that the internal E descendants could pull the PGCs into the embryo. To determine whether PGC ingression requires morphogenetic movements of the D, MS, Cxp or E blastomere descendants, we laser-irradiated each of these blastomeres individually to prevent their internalization, then captured 4D DIC movies of gastrulation. In each experiment, we analyzed the ingression time of PGCs as well as of mesodermal cells within the D and MS lineages, and endodermal cells within the E lineage. We captured movies up to the 210-minute stage, when all of the analyzed cells in unirradiated embryos had completed their ingression (Fig. 1C,D). PGCs that remained on the surface at the 210-minute stage were scored as failing to ingress.

Whereas irradiating the D, MS or Cxp blastomeres did not affect PGC ingression (Fig. 2A,D; supplementary material Movie S3), irradiating the E blastomere prevented the PGCs from ingressing in most embryos (Fig. 2B,D; supplementary material Movie S4). Importantly, irradiating the E blastomere did not disrupt gastrulation broadly, as mesodermal cells ingressed normally after E irradiation (supplementary material Fig. S1). To prevent endodermal cell ingression using a less invasive approach, we analyzed end-1 end-3 double mutant embryos, in which the E cell acquires a mesectodermal fate (Owraghi et al., 2010) and the transformed E daughters remain on the surface or ingress at a later stage (supplementary material Fig. S1C). Similar to embryos with a laser-irradiated E blastomere, most PGCs failed to ingress in end-1 end-3 double mutant embryos (Fig. 2C,D), whereas mesodermal cells ingressed normally (supplementary material Fig. S1). Taken together, these experiments indicate that endodermal cells, which are positioned in the interior of the embryo adjacent to the PGCs, are required specifically for PGC ingression.

**Dorsal movements of endodermal cells are required for PGC ingression**

Given that ingression of the endodermal cells Ea and Ep is complete 1 hour before PGC ingression begins (Fig. 1D), Ea and Ep do not pull the PGCs with them as they ingress. Therefore, we considered the possibility that the internal descendants of Ea and Ep might undergo a subsequent morphogenetic movement that

---

**PGC ingestion requires endodermal cells**

To determine whether PGC ingestion requires morphogenetic movements of the D, MS, Cxp or E blastomere descendants, we laser-irradiated each of these blastomeres individually to prevent
endodermal cells. In comparison with control embryos, the distance
in many cases remained on the surface immediately dorsal to the
division. The position of the irradiated C corpse was variable, but
movements, we laser-irradiated the C blastomere to block its
thinning of the dorsal cell layer could block endodermal cell dorsal
(Fig. 3G,H, compare arrows). To test whether preventing the
plane of the embryo, reducing the thickness of the layer over time
dorsal green cells). The C descendants divided within the surface
derived predominantly from the C blastomere, covered the
cells. During gastrulation, a single superficial layer of dorsal cells,
cell dorsal movements might require a redistribution of more dorsal
(Nance and Priess, 2002). Therefore, we reasoned that endodermal
movement of endodermal cells without killing or transforming the
for PGC ingression, we sought a means to prevent the dorsal
surfaces (Fig. 3D,E; Fig. 4E). These observations suggest that
partially enveloped the internalized PGCs by wrapping around their
moved dorsally a similar distance (Fig. 3F). Endodermal cells also
ventral surface into the interior, the internal endodermal cells
out of six embryos] (Fig. 3A-C). As PGCs ingressed from the
ventral surface into the interior, the internal endodermal cells
associated continuously throughout the period of gastrulation [six
out of six embryos] (Fig. 3A-C). As PGCs ingressed from the
ventral surface into the interior, the internal endodermal cells
moved dorsally a similar distance (Fig. 3F). Endodermal cells also
partially enveloped the internalized PGCs by wrapping around their
surfaces (Fig. 3D,E; Fig. 4E). These observations suggest that
dorsal movements of internal endodermal cells might pull the
attached PGCs into the interior of the embryo.

To test whether endodermal cell dorsal movements are required
for PGC ingestion, we sought a means to prevent the dorsal
movement of endodermal cells without killing or transforming the
cells. Because embryonic cells are tightly packed and surrounded
by a confining eggshell, significant movements of one cell group
must be accompanied by compensatory movements of another
(Nance and Priess, 2002). Therefore, we reasoned that endodermal
cell dorsal movements might require a redistribution of more dorsal
cells. During gastrulation, a single superficial layer of dorsal cells,
derived predominantly from the C blastomere, covered the
endodermal cells and separated them from the eggshell (Fig. 3G,H,
dorsal green cells). The C descendants divided within the surface
plane of the embryo, reducing the thickness of the layer over time
(Fig. 3G,H, compare arrows). To test whether preventing the
thinning of the dorsal cell layer could block endodermal cell dorsal
movements, we laser-irradiated the C blastomere to block its
division. The position of the irradiated C corpse was variable, but
in many cases remained on the surface immediately dorsal to the
endodermal cells. In comparison with control embryos, the distance
that PGCs ingressed and that endodermal cells shifted dorsally was
greatly diminished following C laser irradiation (Fig. 3I; 
supplementary material Fig. S2), and in four out of 10 embryos,
PGCs remained on the ventral surface. These observations are
consistent with the hypothesis that dorsal movements of
endodermal cells, rather than signals provided by these cells, are
required for PGC ingestion.

In order to be pulled into the embryo by endodermal cells, PGCs
would need to adhere tightly to endodermal cell surfaces yet be
able to break contacts with adjacent mesodermal cells. To examine
contacts that the PGCs make with endodermal cells and
mesodermal cells, we created a strain expressing transgenes that
separately mark all cell surfaces (membrane-localized mCherry),
tercellular separations (secreted GFP) and PGCs (PGL-1-RFP,
‘P-granules’). During gastrulation, we failed to detect secreted GFP
between PGCs and endodermal cells, indicating that the surfaces
of PGCs and endodermal cells were juxtaposed (supplementary
material Fig. S3). By contrast, pockets of secreted GFP
accumulated between PGCs and mesodermal cells, as well as
between endodermal cells and mesodermal cells (supplementary
material Fig. S3). We conclude that PGCs and endodermal cells
make more contiguous contacts with each other than either group
of cells makes with its mesodermal neighbors.

**HMR-1/E-cadherin mediates PGC-endoderm
adhesion**

We wondered how PGCs adhere to the dorsally shifting
endodermal cells during gastrulation. SAX-7/L1CAM and HMR-
1/E-cadherin are known to mediate adhesion between *C. elegans*
early embryonic cells; removing SAX-7 and HMR-1 together, but
not individually, causes blastomeres to become more rounded and
prevents endodermal cells from ingressing (Grana et al., 2010). In
early embryos, both SAX-7 and HMR-1 are found at sites of cell-
cell contact (Costa et al., 1998; Chen et al., 2001; Grana et al.,
2010). We examined SAX-7 and HMR-1 localization during PGC
gastrulation to determine whether either protein is found at contacts
between the PGCs and endodermal cells. As in early embryos,
SAX-7 localized uniformly to all sites of cell-cell contact
(supplementary material Fig. S4A). However, HMR-1 was
markedly enriched at the surfaces of the PGCs relative to all other
cells (Fig. 4A,B). HMR-1 enrichment was first evident in P1 and
persisted in Z2 and Z3 throughout gastrulation stages, suggesting
that HMR-1 may have a specific function in the PGCs.

sax-7 null mutants are viable and fertile, and PGCs ingressed
normally in sax-7 mutant embryos (supplementary material Fig.
S4B). Embryoszygotically mutated for hmr-1 arrest at late stages of
embryogenesis and gastrulation phenotypes have not been reported,
although mutant embryos still contain maternal HMR-1 protein
(Costa et al., 1998; Grana et al., 2010). We were able to reduce but
not to eliminate both maternal and zygotic HMR-1 using RNAi
(supplementary material Fig. S4C,D), and a small number of hmr-
l(RNAi) embryos displayed PGC ingestion defects (Fig. 4C). To
deplete HMR-1 levels further, we rescued hmr-1 mutants with an
extrachromosomal array (xtEx42, hereafter hmr-1Ex) that robustly
expressed zygotic HMR-1 (supplementary material Fig. S5A) but
expressed maternal HMR-1 only at very low levels (due to germ-
line transgene silencing; supplementary material Fig. S4E). In 11
out of 29 hmr-1; hmr-1Ex embryos, PGCs failed to ingress (Fig.
4C,D). By treating hmr-1; hmr-1Ex embryos with hmr-1 RNAi, we
depleted HMR-1 below the level of detection by immunostaining
(supplementary material Fig. S4F), and PGC ingestion was
blocked (13 of 14 embryos) (Fig. 4C). Endodermal and

**Fig. 2. Requirement of interacting cells for PGC ingestion.**

(A-C) Embryos (~50 μm in length) are oriented anterior towards the left and
are shown from the ventral perspective. PGC ingestion following
D irradiation (A), E irradiation (B) and in end-1 end-3 mutant embryos
(C). PGCs, cyan; E, yellow; D, orange. The position of internalized PGCs
is indicated with dashed cyan outlines, while PGCs that failed to ingress
are shaded in cyan. The corpse of the irradiated cell remaining on the
surface is indicated with hatched fill. (D) Ingression of PGCs in laser-
irradiated and mutant embryos (wild-type, n=10; D irradiation, n=12;
MS irradiation, n=11; Cxp irradiation, n=6; E irradiation, n=21; end-1
end-3, n=8). Asterisks indicate a significant difference in whether PGCs
ingressed relative to unirradiated controls (Fisher’s exact test, **P<0.01,
***P<0.001).
Mesodermal cells ingressed normally in embryos lacking detectable HMR-1 (supplementary material Fig. S4G-I), although we observed later defects in closure of the gastrulation cleft, which normally occurs well after PGC ingression is complete (Fig. 4D, dashed area) (Grana et al., 2010). Therefore, failed PGC ingression in hmr-1 mutants is not caused by a general defect in cell adhesion or gastrulation, suggesting a specific role for HMR-1 in PGC internalization.

To determine whether HMR-1 is required for adhesion between PGCs and endodermal cells, we depleted HMR-1 in embryos expressing endoderm and PGC cell surface markers. We examined embryos just after the stage when PGC ingression is normally complete and determined whether PGCs and endodermal cells were in contact or were visibly separated. In contrast to control embryos, in which PGCs were always partially wrapped by endodermal cells (Fig. 4E), we detected separations between PGCs and endoderm with increasing frequency as HMR-1 levels were reduced (Fig. 4G). When examined at higher resolution by acquiring confocal z-stacks, at least one of the PGCs in hmr-1 embryos was partially or fully detached from endodermal cells and remained on the surface (Fig. 4F, 10 of 11 embryos), and the contact interface between PGCs and endodermal cells was significantly reduced (Fig. 4H, seven out of 11 hmr-1 embryos had a PGC-endoderm interface falling below the 95% confidence interval of the interface in wild-type embryos). Reducing HMR-1 levels did not cause PGCs to dissociate from one another, although in a small number of embryos the PGC-PGC contact interface was reduced (data not shown). We conclude that HMR-1 is needed for PGCs to adhere to endodermal cells during gastrulation.

HMR-1 is required in PGCs but not somatic cells

We used transgenes to determine whether HMR-1 is required in PGCs, somatic cells or both cell types. HMR-1 in the embryo arises from two sources – a maternally inherited pool that is present in both somatic cells and PGCs, and a zygotically expressed pool that is probably found only in somatic cells (because of transcriptional inhibition in PGCs) (Costa et al., 1998; Achilleos et al., 2010). Therefore, we first asked whether maternal HMR-1 is essential for PGC ingression, as this is the likely source of HMR-1 protein in PGCs. To test the requirement for maternal HMR-1, we allowed hmr-1; hmr-1Ex estradiol to self-fertilize and compared PGC ingression in embryos that inherited the hmr-1Ex extrachromosomal array with those that did not. Both classes of embryos contained very low levels of maternal HMR-1 derived from the partially silenced hmr-1Ex transgene (supplementary material Fig. S5A). Embryos showed equivalent defects in PGC ingression irrespective of whether they inherited hmr-1Ex, suggesting that maternal HMR-1 is required for PGC ingression (Fig. 5A, compare 2nd and 3rd columns). To determine whether supplying maternal HMR-1 is sufficient for PGC ingression, we created a transgene expressing HMR-1-GFP from the maternal mex-5 promoter and the hmr-1 3' UTR (mex-5P::HMR-1-GFP::hmr-1UTR), and crossed it into hmr-1 mutant embryos. HMR-1-GFP was present in all cells, but, like endogenous HMR-1, was markedly enriched in PGCs (Fig. 6A) and PGC ingression was rescued completely (Fig. 5A, compare 2nd and 4th columns). We conclude that maternal HMR-1 is necessary and sufficient for PGC ingression. Additionally, the...
How does HMR-1 become enriched in PGCs? Laser irradiation of PGCs but not in somatic cells (see Discussion).

Finding that expressing hmr-1 zygotically in somatic cells (from hmr-1<sup>139</sup>) was insufficient to rescue PGC internalization suggests that HMR-1 is required in PGCs but not in somatic cells, but arguments against a requirement solely in somatic cells.

To determine whether PGC ingestion requires that HMR-1 be present in PGCs and somatic cells, or just in PGCs, we created a transgene that would allow us to supply maternal HMR-1 specifically to PGCs. Maternally expressed proteins tagged with the zinc finger 1 (ZF1) domain from PIE-1 are degraded rapidly in somatic cells of early embryos, but are protected in germ-line precursor cells and PGCs (Reese et al., 2000; Nance et al., 2003; Anderson et al., 2008; Wehman et al., 2011). We tested whether the ZF1 tag could be used to restrict maternal HMR-1 to PGCs by inserting the tag into mex-5<sup>P::HMR-1-GFP::hmr-1UTR</sup> reporters.

**HMR-1 enrichment in PGCs occurs through 3’ UTR-mediated translational control**

How does HMR-1 become enriched in PGCs? Laser irradiation of the E cell did not prevent HMR-1-GFP enrichment in the PGCs (n=7), indicating that endodermal cells do not induce HMR-1 upregulation and suggesting that HMR-1 enrichment might be an autonomous property of the PGCs. We considered it unlikely that
HMR-1 enrichment resulted from zygotic hmr-1 transcription, given that PGCs are thought to be transcriptionally inactive, and because we observed that HMR-1-GFP driven by the heterologous mex-5 promoter also became enriched in PGCs (Fig. 6A). To confirm that mex-5::HMR-1-GFP::hmr-1 UTR does not contain a cryptic promoter that drives zygotic expression in PGCs, we introduced the transgene at fertilization by crossing wild-type hermaphrodites with mex-5::HMR-1-GFP::hmr-1 UTR males. None of the outcross embryos expressed HMR-1-GFP in PGCs (0/54 embryos), in contrast to embryos produced from transgenic mothers (57/66 embryos). In addition, although laser irradiation of the E nucleus prevented zygotic expression of a pes-10::::GFP reporter (data not shown), we were unable to prevent enrichment of HMR-1-GFP in P4 by laser-irradiating the P4 nucleus (seven out of seven embryos), potentially explaining why the irradiated cells were internalized (Fig. 1E,F). We conclude that HMR-1 enrichment in PGCs arises from maternal hmr-1 mRNA or protein rather than from zygotic transcription of hmr-1 in PGCs.

To determine whether hmr-1 mRNA is preferentially inherited or stabilized in PGCs, we performed in situ hybridization. A hmr-1 antisense probe labeled all early embryonic cells uniformly and was not enriched in P4 (107/107 embryos) (supplementary material (Subramaniam and Seydoux, 1999), showed a visible enrichment of probe staining in P4 (33/45 embryos) (supplementary material). This finding suggests that HMR-1 enrichment is mediated by the hmr-1 3' UTR, which is the only hmr-1 regulatory or coding element remaining in the transgene. Indeed, replacing the hmr-1 3' UTR with that of the housekeeping gene tbb-2 (β-tubulin) (mex-5::GFP-PHPLC::tbb-2 UTR) caused GFP-PHPLC to be expressed uniformly in both soma and germ line (Fig. 6C). Together, these experiments indicate that PGC enrichment of HMR-1 protein occurs post-transcriptionally, and that the hmr-1 3' UTR is sufficient to mediate PGC-enriched expression.

To test whether the hmr-1 3’ UTR affects HMR-1 translation in PGCs, we performed fluorescence recovery after photobleaching (FRAP) experiments on embryos expressing mex-5::GFP-PHPLC::hmr-1 UTR. Just after the birth of P4, somatic cells and P4 expressed equivalent levels of GFP-PHPLC (Fig. 6D,G,I). We photobleached whole embryos at this stage to quench most GFP fluorescence (Fig. 6E), then assayed expression levels again after the birth of Z2 and Z3 to measure nascent translation of the maternally supplied transgene mRNA. New GFP expression occurred in both somatic cells and PGCs, but was significantly higher in PGCs (Fig. 6F,G,I). By contrast, in control experiments performed using the mex-5::GFP-PHPLC::tbb-2 UTR transgene, both cell types showed an equivalent recovery following photobleaching (Fig. 6H,I). We conclude that the hmr-1 3’ UTR mediates HMR-1 enrichment in the PGCs by promoting increased translation of the hmr-1 mRNA in PGCs relative to somatic cells.

**DISCUSSION**

Our findings indicate that PGCs ingest using a hitchhiking mechanism that is enabled by post-transcriptionally regulated adhesion. We have shown that endodermal cells are required for PGCs to internalize and contact the PGCs directly throughout gastrulation. As the PGCs ingest, endodermal cells shift dorsally a similar distance, and blocking the dorsal shift prevents PGC ingestion. Therefore, after endodermal cells internalize during gastrulation, they undergo a second morphogenetic movement that pulls the attached PGCs into the embryo (Fig. 1). PGCs express high levels of the cell-adhesion protein HMR-1/E-cadherin, which we have shown is upregulated post-transcriptionally in PGCs and functions to promote their robust adhesion to endoderm and subsequent internalization (Fig. 7). Thus, although the PGCs move into the embryo using a hitchhiking mechanism, they play an active
role in their internalization by raising the levels of HMR-1/E-cadherin – an adhesion protein that enables their morphogenetic movements.

E-cadherin regulation has been shown to be crucial for gastrulation in many species, although in these instances E-cadherin is downregulated in internalizing cells. For example, murine cells inhibit E-cadherin expression as they migrate through the primitive streak (Ciruna and Rossant, 2001; Zohn et al., 2006), and sea urchin primary mesenchyme cells rapidly internalize surface E-cadherin as they ingress into the blastocoel (Miller and McClay, 1997). E-cadherin downregulation is needed for these ingressing cells to delaminate from epithelial sheets as they migrate to form a new germ layer. The findings we present here demonstrate that E-cadherin upregulation can also promote ingression, by facilitating adhesion to other cells whose movements provide forces for internalization.

Although we found that the most severe depletion of HMR-1 resulted in an almost complete failure in PGC ingression, we detected a smaller percentage of embryos that showed clear PGC-endoderm separation (compare Fig. 4C,G). This discrepancy probably results from two factors. First, we scored PGC ingression as successful only if both PGCs dropped from the surface of the embryo; therefore, one PGC could still remain associated with endoderm whereas the other detached. Second, separations between the two cell groups are difficult to detect unless they are viewed from a lateral perspective – an orientation embryos adopt infrequently when standard mounting methods are used. Indeed, when we examined only those embryos in a lateral orientation at high resolution by acquiring confocal z-stacks, at least one of the two PGCs were partially or fully detached from endoderm in 10 of 25 embryos, and the remaining contact interface with endoderm was significantly reduced (Fig. 4H).

E-cadherin mediates adhesion largely through homotypic interactions with cadherins on the adjacent cell (Borghini and Nelson, 2009; Harris and Tepass, 2010). HMR-1 is expressed in both PGCs and endoderm (see Fig. 4), and trans-interactions between HMR-1 on each of these cell groups could contribute to their adhesion. How, though, can we reconcile our finding that PGC ingression can occur when HMR-1 is detectable only in PGCs? Although we cannot exclude the possibility that trace levels of somatic HMR-1 below our limit of detection are sufficient to promote PGC ingression, we envision two models that could explain a PGC-specific role for HMR-1. One possibility is that HMR-1 interacts with a heterotypic ligand, such as SAX-7, that is present on the surfaces of endodermal cells. Heterotypic trans interactions between classic cadherins and other adhesion proteins have been described in a few cell types and shown to promote adhesion. For example, direct interactions between E-cadherin on epithelial cells and integrins on lymphocyte cell surfaces mediate adhesion between the two cell types (Cepok et al., 1994; Higgins et al., 1998). A second model that can explain our findings is that HMR-1 mediates robust PGC-endoderm adhesion by promoting changes in PGC cell surface tension, which in turn induces endodermal cells to spread over and ‘hug’ the PGCs. Experiments with cultured cells and tissues have shown that overexpression of cadherins within a cell group increases the surface tension of the tissue (Foty and Steinberg, 2005). Tissues with different surface tensions placed adjacent one another behave like immiscible liquids, wherein the tissue with lower surface tension wraps around that with higher surface tension, irrespective of the type of adhesion protein that each tissue expresses (Foty et al., 1996; Foty and Steinberg, 2005; Lecuit and Lenne, 2007). High levels of HMR-1 expression in PGCs could increase their surface tension, promoting a spreading behavior by the adjacent endodermal cells and resulting in a tight association between the two cell groups. Ubiquitously expressed adhesion proteins such as SAX-7 could facilitate adhesion and spreading between the two cell groups. In support of this model, we observed that endodermal cells not only adhere to PGCs, but also wrap dramatically around the PGC surfaces (see, for example, Fig. 3E, Fig. 4E).

Why do PGCs preferentially adhere to endodermal cells rather than the ring of mesodermal cells that surround them on the embryo surface? Our ability to rescue PGC internalization by providing HMR-1 solely to PGCs indicates that differences in HMR-1 levels between mesodermal and endodermal cells cannot explain the preferential association of PGCs and endoderm. Rather, we propose that preferential association of PGCs with endodermal cells may reflect differences in the relative stability of adhesive interactions that endodermal cells and mesodermal cells make with their neighbors. Mesodermal cells are highly mobile as they ingress from the ventral surface and in many cases migrate extensively within the embryo (Schnabel et al., 1997; Viveiros et al., 2011). Therefore, mesodermal cells behave like mesenchymal cells and must be able to make and break new contacts rapidly; by contrast, endodermal cells remain as a unified cell group (Schnabel et al., 1997). Our analysis of cell separations supports this view, as we detected separations between mesodermal cells and their neighbors, but not between the two PGCs, between endodermal cells, or at the PGC-endoderm interface (supplementary material Fig. S3).

Our findings reveal for the first time the different molecular strategies that C. elegans PGCs and somatic cells use to internalize during gastrulation. Somatic cells rely on lineage-specific cell fate transcription factors to trigger their ingression movements (Nance and Priess, 2002; Lee et al., 2006; Harrell and Goldstein, 2011). Endodermal cell ingressions, and probably those of mesodermal cells, occur when myosin accumulates at the apical surface and promotes apical constriction (Nance and Priess, 2002; Lee and Goldstein, 2003; Nance et al., 2003). Laser-irradiation experiments show that somatic cell ingression movements are largely autonomous, as killing one group of ingressing cells does not affect the ingestion of another (Nance and Priess, 2002). In contrast to somatic cells, we have shown that PGC ingression is regulated post-transcriptionally by 3′ UTR-mediated HMR-1 upregulation and occurs through a hitchhiking mechanism that is dependent on...
endodermal cell movements. Our data support a role for the hmr-1 3’ UTR in regulating HMR-1 translation, rather than stability or localization of the hmr-1 mRNA, as hmr-1 mRNA appears uniformly distributed in all embryonic blastomeres. 3’ UTR-mediated post-transcriptional regulation is a conserved mechanism that germ cells in both invertebrates and vertebrates use to control levels of proteins important for their development and differentiation (Knaut et al., 2002; Kataoka et al., 2006; Merritt et al., 2008; Rangan et al., 2009; Suzuki et al., 2010). The data we present here show that PGCs also use 3’ UTR control to regulate the level of proteins needed for morphogenesis. Given that PGCs are transcriptionally quiescent in early embryos of many animals, we anticipate that UTR regulation could be a conserved mechanism that PGCs use to control proteins important for gastrulation.

The association between embryonic endoderm and primordial germ cells is observed in a wide variety of species, including worms, flies, sea urchins, frogs and mice (Santos and Lehmann, 2004; Juliano et al., 2006), but the significance of this association is not known. Our analysis of PGC expression demonstrates a role for endoderm-PGC association in helping to ensure that PGCs are properly internalized during gastrulation. Other species may use similar strategies. For example, in some other nematodes, PGCs are born at a distance from the endoderm and are repositioned to lie adjacent the endoderm before gastrulation commences (Wiegener and Schierenberg, 1998; Lahl et al., 2009). Drosophila PGCs require E-cadherin to adhere to endodermal cells as both cell groups are internalized during gastrulation by extension of the germ band (DeGennaro et al., 2011). Sea urchin PGCs might also rely on association with endoderm to become internalized, as they are found at the tip of the invaginating archenteron (primitive gut) during gastrulation (Juliano et al., 2006; Yajima and Wessel, 2011). PGCs in frogs also associate with the presumptive endoderm as it involutes during gastrulation (Whitington and Dixon, 1975; Nishiumi et al., 2005). Thus, the ancient association between PGCs and endoderm may reflect a morphogenetic role for endoderm in helping place PGCs in the proper position within the embryo.

Acknowledgements
We thank Julie Ahringer, Oliver Hobert, Jane Hubbard, Craig Hunter, Morris Maduro, Jonathan Pettitt, Jim Priess, Geraldine Seydoux, Kuppuswamy Subramaniam, Ann Wehman and Eva Zeiser for their generous gifts of strains and reagents; and Lionel Christiaen, Jane Hubbard, Thomas Hurd, Holger Knaut, Ruth Lehmann and Nance lab members for comments on the manuscript. Some strains and antibodies were provided by the NIH-supported CGC and DSHB.

Funding
This work was funded by grants to J.N. from the National Institutes of Health [R01GM078341, R21HD058953], NYSTEM [C024301] and the Irma T. Hirschl Charitable Trust. Deposited in PMC for release after 12 months.

Competition interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at http://dev.biologists.org/lookup/suppl?doi=10.1242/dev.079863-JDC1

References


