Cadherin-mediated cell interaction regulates germ cell determination in mice

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Summary

The germ cell lineage segregates from the somatic cell lineages in early embryos. Germ cell determination in mice is not regulated by maternally inherited germplasm, but is initiated within the embryo during gastrulation. However, the mechanisms of germ cell specification in mice remain unknown. We located precursors to primordial germ cells (PGCs) within early embryos, and show here that cell-cell interaction among these precursors is required for germ cell specification. We found that the expression of a calcium-dependent cell adhesion molecule, E-cadherin, is restricted to the proximal region of extra-embryonic mesoderm that contains PGC precursors, and that blocking the functions of E-cadherin with an antibody inhibits PGC formation in vitro. These results showed that E-cadherin-mediated cell-cell interaction among cells containing PGC precursors is essential to directing such cells to the germ cell fate.

Movies available online

Key words: Primordial germ cell, E-cadherin, Cell interaction, Cell fate

Introduction

The germ cell lineage in mouse embryos is segregated from the pluripotent cell population of the proximal epiblast after the onset of gastrulation (Wylie, 1999; McLaren, 1999; Zhao and Garbers, 2002; Lawson and Hege, 1994; Tam and Zhou, 1996). However, the mechanisms of germ cell specification in mice remain unknown. Although maternal germ cell determinants are critical for germ cell formation in organisms such as *Caenorhabditis*, *Drosophila* and *Xenopus*, they do not seem to be involved in mouse germ cell allocation. Cell lineage analyses have revealed that a portion of proximal epiblast cells in pre- and early streak mouse embryos are common precursors of primordial germ cells (PGCs) and extra-embryonic mesoderm (Lawson and Hage, 1994) and that final cell fate is not yet determined at this time. In addition, after transplantation into the proximal region of the epiblast, distal epiblast cells that normally become ectoderm cells follow the fate of the proximal epiblast and give rise to PGCs as well as extra-embryonic mesoderm (Tam and Zhou, 1996). This also indicates that proximal location in the epiblast is important in becoming committed to PGCs.

We established a primary culture system that can mimic PGC differentiation from the epiblast, and have shown that the extra-embryonic ectoderm induces the conditions required for PGC formation within the adjacent proximal epiblast (Yoshimizu et al., 2001). In addition, studies of knockout mice have revealed that BMP4/8b signaling is critical for this induction event (Lawson et al., 1999; Ying et al., 2000), and recombinant BMPs can induce PGCs from epiblasts in culture (Hayashi et al., 2002). Signals from BMP might induce precursors of PGCs and of extra-embryonic mesoderm, but may not be involved in actual PGC determination. Thus, a second local signal at the posterior end of the embryo might regulate allocation to the PGC lineage (Lawson et al., 1999; Fujiwara et al., 2001). The nature of this presumptive second signal remains obscure. A gene known as *fragilis/mil-1* that is potentially expressed in PGC precursors (Saitou et al., 2002; Tanaka and Matsui, 2002) encodes a transmembrane protein that belongs to the interferon induced transmembrane protein family, and it might be involved in cell-cell contact as well as cell growth control (Deblandre et al., 1995). As yet there is no evidence that this gene functions in PGC formation.

Cadherins are responsible for cell adhesion through calcium-dependent homophilic interactions and have various functions including cell differentiation (Takeichi, 1991; Gumbiner, 1996; Laprise et al., 2002). For example, N-cadherin-dependent cell interactions in a group of muscle progenitors plays a crucial role in promoting differentiation into MyoD-expressing muscle in *Xenopus* embryos (Holt et al., 1994). Although high levels of E-cadherin are expressed in the epiblast but not in the mesoderm, we show here that E-cadherin is expressed in the proximal extra-embryonic mesoderm that contains the PGC precursors and that cell-cell interaction mediated by E-cadherin is crucial for these precursors to be allocated to the germ cell lineage.

Materials and methods

Mouse strains and staging of embryos

Embryos for explant culture and whole-mount immunofluorescence staining were obtained from female mice of the outbred strain ICR that were mated with male BDF1 (C57/B6j × DBA2) mice or with Oct4 (GOF18-ΔPE)-GFP heterozygous male mice (Yoshimizu et al., 1999) maintained in the C57Bl/6 background. Embryos were staged before the experiments as described previously (Downs and Davies, 1993): E6.5, E6.75, E7.0, E7.25 and E7.5 embryos corresponding to early streak, mid-streak, mid to late streak, early allantoic bud and late allantoic bud stages, respectively.

Histological staining

Whole embryos and cultured explants were histologically stained as described previously (Ciruna and Rossant, 2001). Primary antibodies were applied at the following concentrations: 10 μg/ml for rat anti-E-cadherin (ECCD-2) (Takara Shuzo), 3.0 μg/ml for rat anti-Flk1 (AVAS12) (Kataoka et al., 1997) and at a 1:1000 dilution for rabbit anti-PGC7 (Sato et al., 2002). The appropriate species-specific fluorophore-labeled secondary antibodies (Molecular Probes) were applied at a 1:200 dilution. Nuclei were stained with TOTO-3 iodide (Molecular Probes) at a 1:500 dilution in the presence of 100 μg/ml of RNase A. Staining was examined using a confocal microscope (Leica). Specimens were stained with alkaline phosphatase (ALP) after immunostaining as described (Donovan et al., 1986).

Dissection of embryos and explant culture

Embryos were dissected out from decidua and then Reichert's membrane and visceral endoderm were mechanically removed using fine forceps and a tungsten needle to isolate epiblasts or extra-embryonic mesoderm (Hogan et al., 1994). The proximal quarter of epiblasts was dissected at E6.5 and E6.75 as proximal epiblasts. To isolate extra-embryonic mesoderm at E6.75 and E7.0, boundaries of extra-embryonic mesoderm, extra-embryonic ectoderm and epiblast were cut with a tungsten needle. We also isolated the base of allantoic buds as extra-embryonic mesoderm at E7.25 and E7.5. The fragments of extra-embryonic mesoderm were dissociated into single cells by gentle pipetting in 0.025% trypsin/0.75 mM EDTA. Extra-embryonic mesoderm explants were co-cultured with 200 μg/ml of ECCD-1, a blocking antibody for E-cadherin, or with the same concentration of ECCD-2 as a control. Explants were cultured on a feeder layer of STO fibroblast cells as described previously (Yoshimizu et al., 2001).

Time-lapse analysis

Time-lapse images were analyzed using a Leica confocal microscope equipped with a cell culture chamber. Images were captured every hour for 40 hours.

Results

PGC precursors are located within the extra-embryonic mesoderm

To determine the mechanisms of germ cell determination in mice, we initially attempted to locate PGC precursors in early streak embryos by explant culture. At E6.5, explants of proximal epiblast gave rise to PGCs expressing alkaline phosphatase (ALP) and a more specific marker, PGC7/stella (Sato et al., 2002; Saitou et al., 2002), whereas E6.75 explants did not (Table 1 and Fig. 1A-D). These findings suggested that PGC precursors migrated out of the proximal epiblast between E6.5 and E6.75. Because ALP-positive (Ginsberg et al., 1990) and PGC7/stella-positive (Sato et al., 2002; Saitou et al., 2002) PGCs appear first in the extra-embryonic mesoderm at E7.25, and *fragilis/mil-1* (Saitou et al., 2002; Tanaka and Matsui, 2002), which seems to be expressed in PGC precursors, is expressed in the earlier (E6.75) extra-embryonic mesoderm, we cultured fragments of isolated extra-embryonic mesoderm from E6.75 embryos under the same conditions. All of these explants gave rise to PGCs, indicating that PGC precursors are located within the extra-embryonic mesoderm at E6.75 (Table 1, Fig. 1E).

Cell-cell interaction among the precursors is necessary for PGC allocation

We reported that Oct4 (GOF18-deltaPE)-GFP transgenic embryos specifically expressed GFP in PGCs after E8.0 (Yoshimizu et al., 1999). Although GFP is also weakly expressed in somatic tissues before this stage, the E6.75-E7.25 extra-embryonic mesoderm expressed more GFP than anywhere else in the embryo, suggesting that cell populations containing PGC precursors can be distinguished by intense GFP signals (Fig. 2). Explants of E6.75 extra-embryonic mesoderm of the transgenic embryos did indeed express high levels of GFP, and ALP-positive, PGC7/stella-positive PGCs migrated from a cluster of intensely GFP-positive cells after culture (Fig. 3), further indicating that intense GFP expression in the extra-embryonic mesoderm marks a cell population that includes forming PGCs. The intensely GFP-expressing cells formed clusters within the explants before the PGCs started migration (Fig. 3B-D and Supplemental data: http://dev.biologists.org/supplemental). In addition, the cell clusters were formed at 9 hours in culture, but cells were not PGC7 positive until 12 hours in culture (Fig. 7; data not shown), suggesting that cell contact is important in PGC formation before commitment. To test the requirement for cell-cell

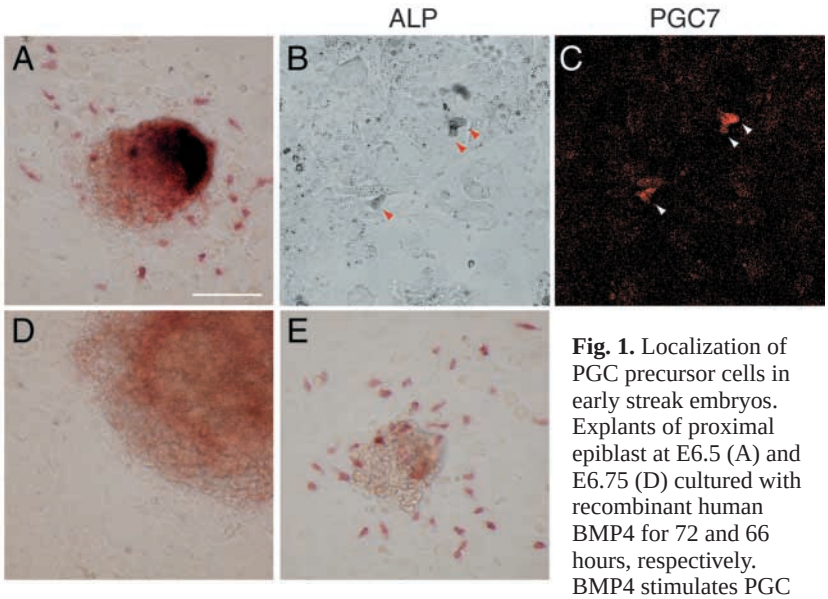

Fig. 1. Localization of PGC precursor cells in early streak embryos. Explants of proximal epiblast at E6.5 (A) and E6.75 (D) cultured with recombinant human BMP4 for 72 and 66 hours, respectively. BMP4 stimulates PGC formation from E6.5 proximal epiblasts (unpublished results). ALP- and PGC7/stella-positive PGCs developed from E6.5 explants (B,C, arrowheads), but no such cells emerged from E6.75 explants (D). The same group of cells is shown in B and C. E6.75 extra-embryonic mesoderm generated ALP-positive PGCs (E). Scale bar: 160 μm (A,D,E) and 100 μm (B,C).

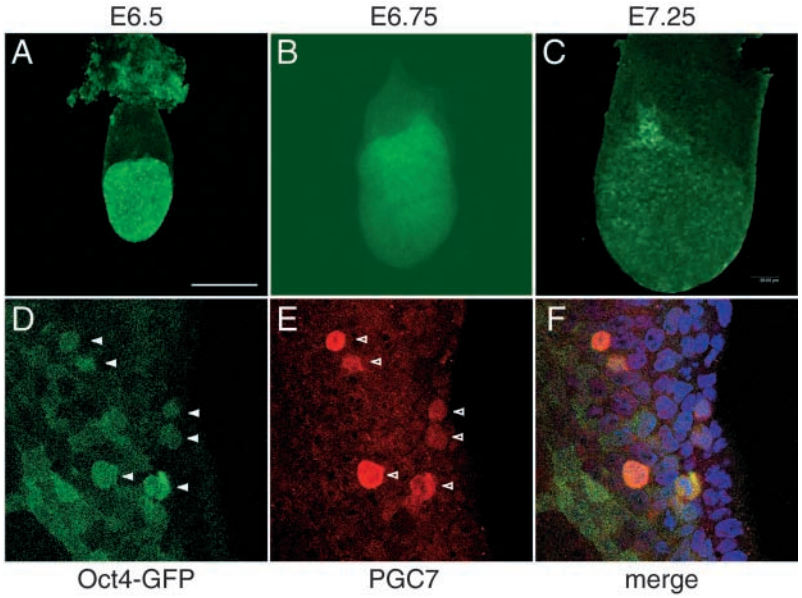

Fig. 2. Expression of Oct4 (GOF18-deltaPE)-GFP transgenes in gastrulating embryos. Fluorescence images of embryos at (A) E6.5 (early streak stage), (B) E6.75 (mid-streak stage), (C) E7.25 (early allantoic bud stage). (D,E,F) Extra-embryonic mesoderm of Oct4-GFP embryos at mid-allantoic bud stage showing expression of Oct4-GFP (D arrowheads) and stained with anti-PGC7 antibody (E). (F) Merged image. The cells that express intense Oct4-GFP (D, filled arrowheads) in base of allantoic bud also stained with anti-PGC7 antibody (E, open arrowheads). Scale bar: 200 μm (A-C); 25 μm (D-F).

interaction, we cultured extra-embryonic mesoderm of E6.75 and E7.5 embryos after dissociating the cells by trypsinization. No PGCs were evident in the dissociated E6.75 explants (Table 2). The frequency of PGC formation gradually increased from E6.75 to E7.5, when 100% of the dissociated explants gave rise to PGCs (Table 2, Fig. 4), suggesting an early requirement for cell contact that is relieved by E7.5. Trypan Blue staining showed that over 80% of both E6.75 and E7.25 cells survived after trypsinization. In addition, the total number of cells after culture, determined by culturing embryos ubiquitously expressing GFP, was 86-90% of the number of plated cells, and no difference was found between E6.75 and E7.25 cells. Thus, the rates of cell survival at E6.75 and E7.25 in vitro were identical. Together, these results suggest that close cell-cell interaction around E6.75 is critical for PGC differentiation from extra-embryonic mesoderm.

E-cadherin expressed in the precursors is critical for PGC allocation

We examined which molecules mediate PGC formation that is cell-interaction dependent. While searching for candidates among cell adhesion proteins, we found that the expression profile of E-cadherin was unique (Saitou et al., 2002). E-cadherin is a cell adhesion molecule that is involved in many aspects of development, including organogenesis and cell differentiation (Takeichi, 1991; Gumbiner, 1996; Laprise et al., 2002). Although E-cadherin expression in early streak embryos is high in the epiblast but undetectable in the mesoderm (Damjanov et al., 1986), we found intense expression at E6.75 in a proximal region of the extra-embryonic mesoderm adjacent to the epiblast (Fig. 5B), and expression was localized to the sites of cell contact. E-cadherin expression was not, however detected in the more distal part of the extra-embryonic mesoderm, which was developing into the allantois (Fig. 5B). By E7.0, cells that might be PGC precursors and that expressed more intense GFP, formed a cluster in the most distal region of the extra-embryonic mesoderm expressing E-cadherin (Fig. 2F). This suggested that E-cadherin is involved in clustering PGC precursors. PGC7/stella was not expressed in these cells,

Table 1. Localization of the forming PGCs in early streak embryos

		E6.5			E6.75		
Type of explants	BMP4 (500 ng/ml)	Explant with PGCs/ total explants	(%)	Range of PGC numbers	Explant with PGCs/ total explants	(%)	Range of PGC numbers
Epiblast+exee	–	9/9	100	4-72	3/3	100	6-56
Exem	–	–	–	–	7/7	100	4-24
Pro.epi.	–	2/13	15	3-6	0/5	0	0
Pro.epi.	+	4/4	100	10-40	1/6	17	9

Exee, extra-embryonic ectoderm; Exem, extra-embryonic mesoderm; Pro.epi., proximal epiblast

Table 2. Requirement of cell-cell contact for PGC formation in extraembryonic mesoderm

	E6.75			E7.0			E7.25			E7.5		
Type of explants	Explant with PGCs/total explants	(%)	Range of PGC numbers	Explant with PGCs/total explants	(%)	Range of PGC numbers	Explant with PGCs/total explants	(%)	Range of PGC numbers	Explant with PGCs/total explants	(%)	Range of PGC numbers
Intact	5/5	100	4-24	5/5	100	17-46	5/5	100	2-37	–	–	–
Dissociated	0/12	0	0	2/12	17	3-4	9/12	75	2-21	10/10	100	1-18

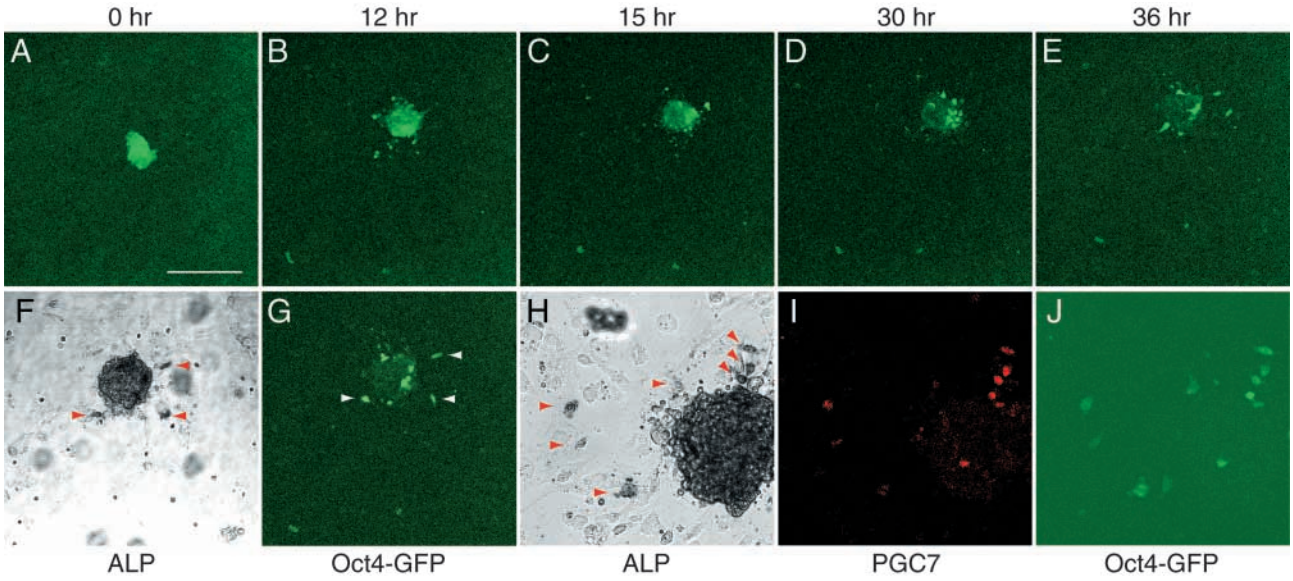

Fig. 3. PGCs from Oct4-positive cell cluster in isolated extra-embryonic mesoderm at E6.75. (A-G) Time-lapse analysis of Oct4-GFP extra-embryonic mesoderm. (A-E,G) Images of cells expressing Oct4-GFP in extra-embryonic mesoderm on an STO feeder layer after (A) 0, (B) 12, (C) 15, (D) 30, (E) 36 and (G) 40 hours in culture. (F) ALP expression of explant in G. Arrowheads indicate ALP- and GFP-positive migrating PGCs. (H,I,J) Co-expression of PGC markers, (H) ALP, (I) PGC7 and (J) Oct4-GFP in PGCs (arrowheads) that developed from cultured E6.75 extra-embryonic mesoderm. Scale bar: 200 μm (A-G) and 100 μm (H-J).

indicating that PGCs had not yet differentiated from precursors at E7.0 (Fig. 5E-H, data not shown). Similar expression profiles persisted at E7.25, when PGC7/stella-positive PGCs were obvious among cells expressing E-cadherin (Fig. 5I-L). Therefore, E-cadherin expression appears to define the cell population containing nascent PGCs within the extra-embryonic mesoderm.

To determine the role of E-cadherin, we tested the effect of an E-cadherin-blocking antibody on PGC formation in culture (Yoshida-Noro et al., 1984). We cultured fragments of extra-embryonic mesoderm from E6.75 embryos with or without the blocking antibody, ECCD-1. In the absence of antibody (Fig. 6A-D) or in the presence of the control antibody, ECCD-2 (Fig. 6I-L), a cluster of PGCs expressing Oct4-GFP and PGC7/stella formed within explants after 15 hours in culture, but not with ECCD-1 (Fig. 6E-H). Although cells often expressed very high levels of GFP in cultured explants in the presence of ECCD-1, they were PGC7 negative (Fig. 6E-G). These cells may be PGC precursors that failed to commit to a PGC fate.

A cluster of PGCs that specifically expressed E-cadherin after culture (Fig. 6M-P) resembled PGCs in the extra-embryonic mesoderm at E7.25 in vivo (Fig. 5I-L). In addition, a cluster of PGC precursors expressing GFP that were negative for PGC7/stella formed as early as 9-10 hours in culture, and the cluster also specifically expressed E-cadherin (Fig. 7). This suggests that E-cadherin plays an essential role in clustering PGC precursors before they become committed to PGCs, and that PGC specification is achieved by E-cadherin-mediated cell-cell interaction among the precursors, but not between the precursors and their neighboring somatic cells.

In contrast to its effect on PGC formation, ECCD-1 did not prevent the expression of Flk1, a marker of extra-embryonic mesoderm and allantois (Kataoka et al., 1997) (Fig. 8). One report has indicated that Flk1 is not expressed in E6.75 extra-embryonic mesoderm (Yamaguchi et al., 1993), and we confirmed that Flk1 expression was undetectable in the explant before culture (data not shown), indicating that differentiation of extra-embryonic mesoderm progressed in culture in the presence of ECCD-1. These results indicate that blocking E-cadherin function inhibits PGC formation, but does not affect the differentiation of extra-embryonic mesoderm cells. After 48-60 hours in culture, ECCD-1-dependent PGC formation was still inhibited in E6.75 explants, while E7.25 explants were not affected (Table 3, Fig. 9), consistent with the conclusion from the cell dissociation

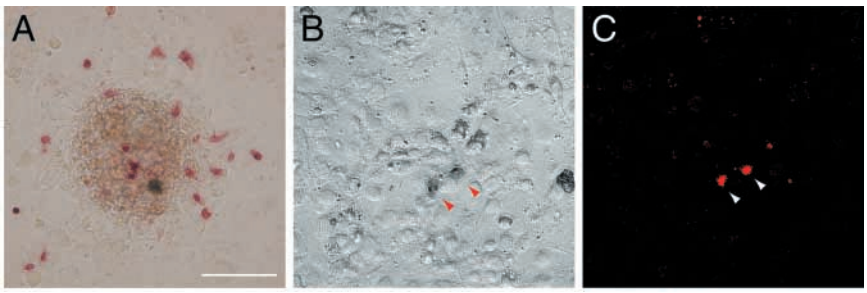

Fig. 4. PGCs from E7.25 extra-embryonic mesoderm. Intact extra-embryonic mesoderm fragment (A) and cells dissociated from a fragment (B,C) were cultured for 48 hours, and then stained for ALP activity (A,B) or for PGC7 (C). ALP-positive cells developed from the intact fragment (A) and from dissociated cells (B, arrowheads). ALP-positive cells in B also stained with the anti-PGC7 antibody (C, arrowheads). Scale bars, 160 μm (A) and 100 μm (B,C) μm.

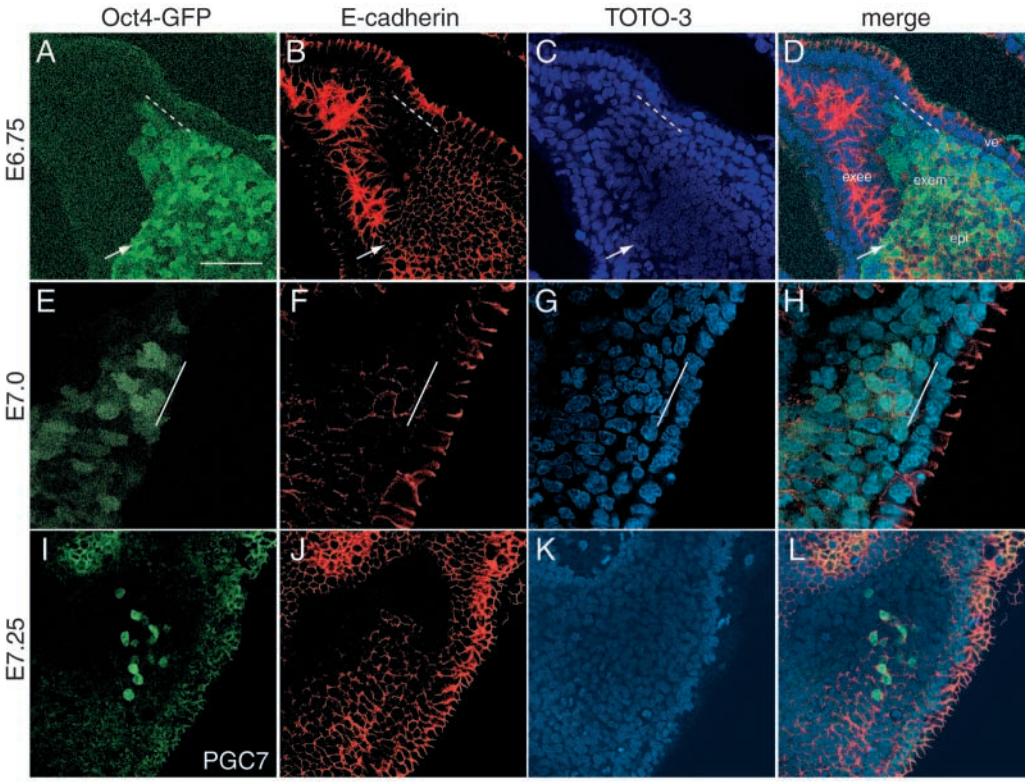

Fig. 5. E-cadherin expression in clustered PGC precursors at base of nascent allantois. Lateral view of embryos at (A-D) mid-streak (E6.75), (E-H) mid- to late streak (E7.0), and (I-L) early allantoic bud (E7.25) stage with anterior to left. (A-D) Oct4-GFP (A) and E-cadherin (B) expression was slightly and obviously lower in the distal portion of extra-embryonic mesoderm (dotted line). Arrows indicate boundary of epiblast and extra-embryonic mesoderm; exee, extra-embryonic ectoderm; exem, extra-embryonic mesoderm; epi, epiblast; ve, visceral endoderm. (E-H) Cells expressing intense GFP (E, solid line) form a cluster at the apical tip of the E-cadherin-expressing region (F) of extra-embryonic mesoderm. (I-L) Committed PGCs expressing PGC7 (I) dispersed in the most distal region in E-cadherin-expressing extra-embryonic mesoderm (J). (C,G,K) TOTO-3 staining of nuclei and (D,H,L) merged images. Scale bar: 50 μm (A-D,I-L) and 25 μm (E-H).

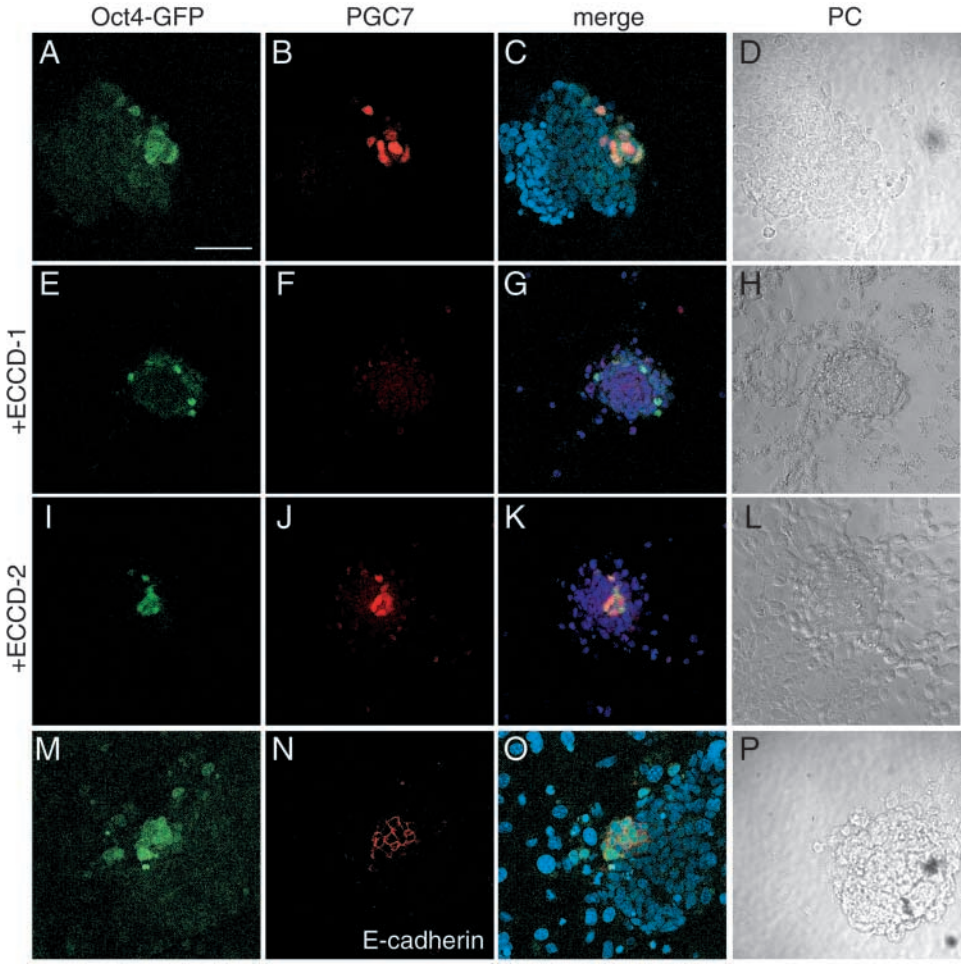

Fig. 6. Antibody blocking of E-cadherin impairs PGC formation in culture. Explants of Oct4-GFP extra-embryonic mesoderm at E6.75 were cultured for 15 hours with no antibody (A-D,M-P), with ECCD-1 that blocks E-cadherin (E-H), or with ECCD-2, a control antibody with no blocking function (I-L). Cultured explants were stained with anti-PGC7 antibody (B,F,J) or with anti-E-cadherin, ECCD-2 (N). (A,E,I,M) Oct4-GFP expression; (C,G,K,O) merged images; (D,H,L,P) phase contrast images. With ECCD-1, scattered Oct4-GFP-positive cells are evident, but they are PGC7 negative (E-G). Oct4-GFP positive clump of cells express E-cadherin, while the remaining explant is E-cadherin-negative in control culture (M-P). Scale bar: 50 μm (A-D,M-P); 80 μm (E,L).

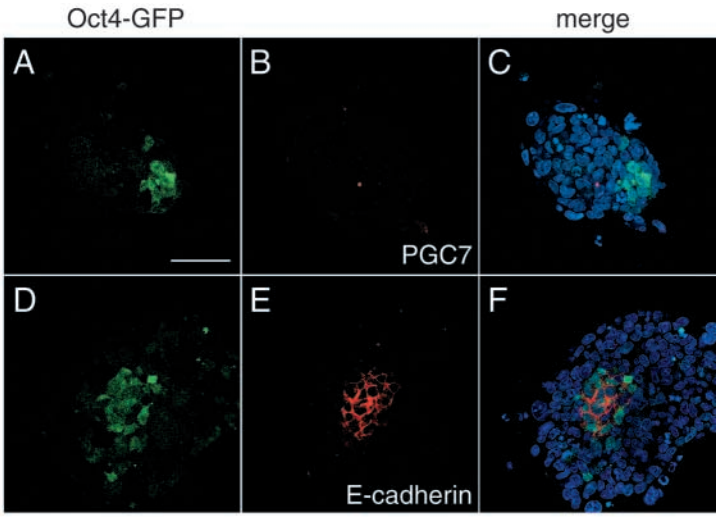

Fig. 7. Specific expression of E-cadherin within the cluster of PGC precursors. Explants of Oct4-GFP extra-embryonic mesoderm at E6.75 were cultured for 9-10 hours, and stained with anti-PGC7 antibody (B) or with anti-E-cadherin antibody, ECCD-2 (E). Oct4-GFP expression (A,D). (C,F) Merged images with TOTO-3 stained nuclei. Cluster of GFP-expressing PGC precursors that are PGC7/stella negative exclusively maintained E-cadherin expression. Scale bar: 50 μm.

study that cell contact was no longer required by E7.25 (Table 2).

Discussion

Cell lineage analyses of pre- and early streak epiblast cells have indicated that PGCs and extra-embryonic mesoderm such as allantois originate from common precursors located within the proximal epiblast (Lawson and Hage, 1994). The expression profiles of *fragilis/mil-1* (Saitou et al., 2002; Tanaka and Matsui, 2002) and Oct4-GFP (Yoshimizu et al., 1999) as well as our culture experiments (Table 1) suggest that these precursor cells migrate to and remain in a region at the posterior of embryos by E6.75. Our present results indicate that E-cadherin-mediated interactions among such common precursor cells regulate their fates and are necessary for their commitment to the germ cell lineage (Fig. 10).

We also examined the involvement of other cell surface molecules in PGC specification. We focused on the Notch family proteins because they regulate cell-type specification and differentiation in many different species via interaction with their ligands, the Delta family of proteins that are

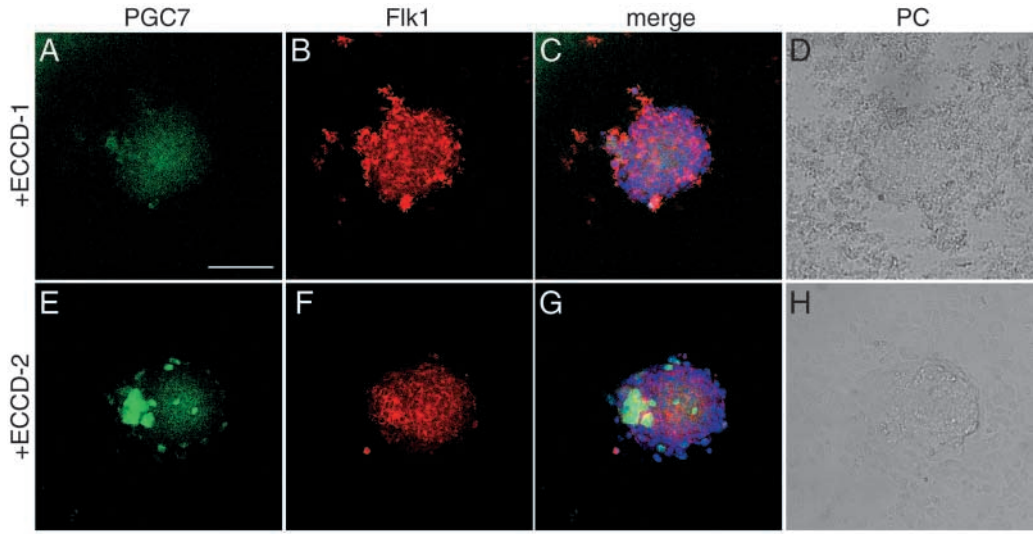

Fig. 8. Extra-embryonic mesoderm develops normally in cultured explants with E-cadherin-blocking antibody. Explants of extra-embryonic mesoderm were cultured for 15 hours with ECCD-1 (A-D), or with ECCD-2 (E-H). Cultured explants were stained with anti-PGC7 (A,E) and anti-Flk1 antibodies to show differentiation of extra-embryonic mesoderm (B, F). (C,G) Merged and (D,H) phase contrast images. Scale bar: 100 μm.

Table 3. Requirement for E-cadherin in PGC formation in extraembryonic mesoderm

	E6.75			E7.25		
Medium	Explant with PGCs/total explants	(%)	Range of PGC numbers	Explant with PGCs/total explants	(%)	Range of PGC numbers
+ECCD-1	0/7	0	0	4/4	100	7-23
+ECCD-2	4/5	80	4-18	4/5	80	4-18
Buffer	5/5	100	8-25	2/2	100	18-30

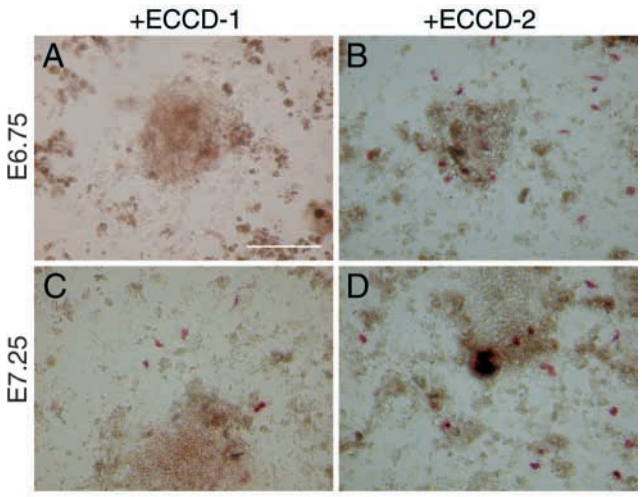

Fig. 9. Inhibition of migrating PGC emergence by E-cadherin-blocking antibody in prolonged culture. Extra-embryonic mesoderm explants at E6.75 (A,B) and E7.25 (C,D) were cultured with ECCD-1 (A,C) or ECCD-2 (B,D) for 48-60 hours. Emergence of PGCs was detected by ALP staining. Scale bar: 200 μm.

expressed in neighboring cells (Artavanis-Tsakonas et al., 1999). Four Notch genes (*Notch1-4*) have been identified in mice and we found by whole-mount in situ hybridization that only *Notch1* and *Notch2* were uniformly expressed in epiblasts at E6.5 (data not shown). However, not all of the Notch genes were expressed in extra-embryonic mesoderm at E7.0 (Williams et al., 1995) (data not shown). In addition, we examined abnormalities of PGC development in homozygous *Notch1* and *Notch2* mutant embryos by whole mount ALP staining, but the numbers and localization of PGCs in the homozygous embryos did not significantly differ from those of the heterozygous and wild type littermates at E8.5. Taken together, we concluded that Notch signaling does not function in PGC development. However, the possibility of redundancy in Notch1 and Notch2 functions cannot be ruled out, as in the roles they play in left-right asymmetry determination (Krebs et al., 2003).

Several mechanisms by which E-cadherin regulates PGC determination are possible. The simplest model is that E-cadherin itself transmits instructive signals for PGC determination. For example, homophilic interactions of E-cadherin transmit intracellular signals by sequestering β-catenin from lymphoid enhancer factor/T cell factor (LEF/TCF) (Hecht and Kemler, 2000) transcription factors (Orsulic et al., 1999). E-cadherin might also facilitate the clustering of signaling molecules with growth factor receptors that are specifically expressed within the cluster. For instance, VE-cadherin in endothelial cells forms a complex consisting of β-catenin, PI 3-kinase and VEGF receptor 2, by which VEGF-A activates Akt (Carmeliet et al., 1999). E-cadherin also stimulates the MAPK pathway through ligand-independent activation of EGFR in epithelial cells (Pece et al., 2000). Because PI 3-kinase is also involved in E-cadherin-dependent activation of MAPK (Pece et al., 1999; Laprise et al., 2002), we examined its involvement in PGC formation by adding the potent PI 3-kinase inhibitors, LY294002 and wortmanin to cultured E6.75 extra-embryonic mesoderm. However, PGC formation was not significantly affected (data not shown). This indicates that E-cadherin-mediated signals are transmitted to PGC precursors via different signaling pathways. *Fragilis/mil-1* (Saitou et al., 2002; Tanaka and Matsui, 2002) is likely to be associated with E-cadherin in signal transmission. *Fragilis/mil-1* is specifically expressed in a cell population in the extra-embryonic mesoderm and in newly formed PGCs, and encodes a transmembrane protein. A role in cell-cell interaction thus seems likely for *fragilis/mil-1*, although clarification of its functions await further study.

Alternatively, E-cadherin might play more permissive roles in transmitting signals for PGC determination. E-cadherin may simply permit close enough contact for other independent instructive signals such as juxtacrine signals or signals via other adhesion molecules including Fragilis/Mil-1. In this regard, E-cadherin could also function as an anchor that settles precursor cells within niches for PGC differentiation. Cell adhesion mediated by DE-cadherin is required in the *Drosophila* ovary to anchor germline stem cells in niches for their renewal (Song et al., 2002). In mouse extra-embryonic mesoderm, E-cadherin expression is restricted to the proximal region that is adjacent to the epiblast, which also expresses E-cadherin, and is not expressed in the distal portion of extra-embryonic mesoderm or in the allantois (Fig. 5B,F,J). This spatial distribution of E-cadherin prompts the notion that homophilic E-cadherin interactions prevent precursors from moving to the distal part of the extra-embryonic mesoderm, where the allantois differentiates (Downs and Bertler, 2000). The continuous expression of E-cadherin in the proximal extra-embryonic mesoderm might thus protect PGC precursors from allantois differentiation (Fig. 10).

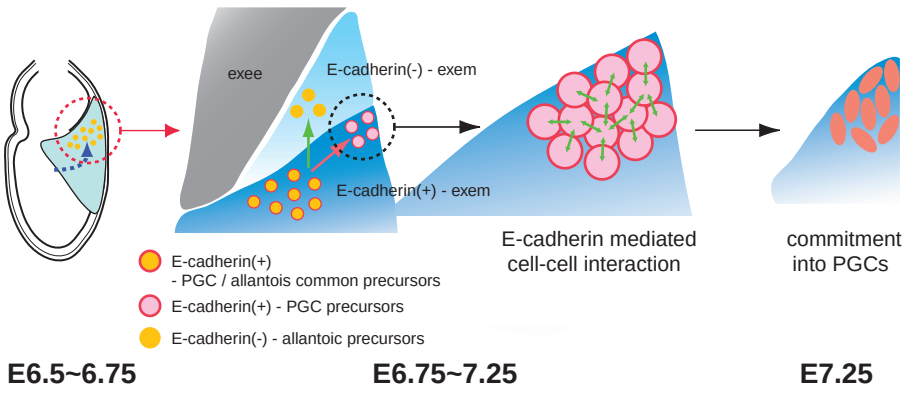

Fig. 10. Possible mechanism of PGC determination by E-cadherin-mediated cell interaction. PGC/allantois common precursors locate within the proximal region of extra-embryonic mesoderm. Cells losing E-cadherin expression move to the distal region of the extra-embryonic mesoderm and differentiate into allantois. In contrast, some cells continuously expressing E-cadherin form clusters and E-cadherin-mediated cell signaling directs them to germ cell fate. exee, extra-embryonic ectoderm.

The present study provides evidence for the importance of E-cadherin-mediated cell interaction in germ cell determination. Signaling molecules that depend on E-cadherin function should be identified. The identification of such molecules will further increase understanding of the process of germ cell determination and help to identify novel germ cell determinants.

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