Nanos suppresses somatic cell fate in *Drosophila* germ line

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Nanos (Nos) is one of the evolutionarily conserved proteins known to direct germ-line development. In *Drosophila*, maternal Nos protein maintains transcriptional quiescence in the germ-line progenitors or pole cells to repress ectopic expression of somatic genes. Here we show that maternal Nos is required to establish and maintain germ-line identity by preventing apoptosis and somatic cell fate. The pole cells lacking maternal Nos were degraded by apoptosis during mid to late embryogenesis. When apoptosis was suppressed by *Df(3L)H99*, some pole cells lacking Nos adopted somatic cell fates. These pole cells expressed somatic markers ectopically and lost the germ-line marker Vasa. We further found that some Nos-negative pole cells were able to migrate into the gonads, but they failed to develop as functional germ cells during postembryonic stages. We propose a model in which Nos establishes germ-line/soma dichotomy and is also required to maintain germ-line fate.

The mechanism underlying the segregation of germ line from somatic line is a century-old issue in developmental biology. In many animal groups, maternal factors required for germ-line formation are localized in a histologically remarkable region in egg cytoplasm, or germ plasm, and are inherited in the germ-line progenitors (1, 2). In *Drosophila*, the germ-line progenitors known as pole cells are first formed at the posterior pole of the blastoderm embryos. During later embryogenesis, the pole cells pass through midgut epithelium into hemocoel and migrate within the embryos to reach the embryonic gonads, where they differentiate as functional germ line (1–4). Previous works have demonstrated that none of the pole cells becomes incorporated into any somatic tissues and contributes to somatic development during embryogenesis (5, 6). However, it remains elusive how the developmental fate of pole cells is regulated by germ plasm components.

One of the germ plasm components, Nanos (Nos), is evolutionarily conserved and has a widespread role in germ-line development (7–13). Maternal *nos* mRNA is enriched in germ plasm, and its protein product is partitioned into pole cells when they are formed and remains detectable in these cells throughout embryogenesis (14). Nos is required in pole cells for their proper migration into the embryonic gonads (15, 16). Within pole cells, Nos is involved in maintaining transcriptional quiescence (17) and is also required for maintenance of a germ-line-specific chromatin status that correlates with transcriptional inactivity (18). In the absence of maternal Nos activity, ectopic expression of somatic genes is detectable in pole cells (17). These results led us to speculate that pole cells lacking Nos may adopt somatic cell fate.

Here, we show that pole cells are able to adopt both germ-line and somatic cell fates and undergo apoptosis. Nos is required to repress the pathway leading to somatic differentiation and apoptosis. Thus, we conclude that Nos is essential for germ-line/soma dichotomy and for germ-line maintenance.

Materials and Methods

Fly Stocks. The *nos* allele used was nos^{BN}. *Df(3L)H99* was as described in ref. 19. PLHΔ23 is described in ref. 20. Using these lines, we generated nos^{BN} *Df(3L)H99* PLHΔ23/TM3 (nos^{BN}-*H99-PLHΔ23*/TM3). In the experiment to examine the fate of pole cells homozygous for *Df(3L)H99* and *nos*-H99 pole cells in the third-instar larvae, we used TM6 ubi-GFP instead of TM3. TM6 ubi-GFP expresses GFP under the control of the ubiquitin promoter in germ and somatic lines (data not shown).

***In Situ* Hybridization for Midgut Marker Genes.** The dechorionated embryos were fixed in 2 ml of a 1:1 mixture of heptane and fixative I [3.7% formaldehyde in PBS (130 mM NaCl/7 mM Na_2HPO_4/3 mM NaH_2PO_4)]. The fixed embryos were processed for whole-mount *in situ* hybridization as described in ref. 21 with several modifications. Digoxigenin-labeled RNA probes were synthesized from a 706-bp cDNA fragment from *midgut expression 1* (*mex1*), 3,137-bp cDNA of *integrin β neu* (*bInt-n*) (LD09848), 3061-bp cDNA of *dGATAe* (LD08432), and a 589-bp cDNA fragment from the 3′-terminal region of *CG11267*. *mex1*, *bInt-n*, and *dGATAe* were reported to be expressed in the embryonic midgut (refs. 22 and 23 and T. Okumura and R. Murakami, personal communication). *CG11267* was selected to be expressed in the midgut by our screen. These probes were *in situ* hybridized with midgut cells after stage 13. However, neither of these genes were expressed in pole cells within the gonads and hemocoel during normal development (data not shown). To label almost all of the midgut epitherial cells intensely, we used mixed probes (*mex1*/*bInt-n* and *dGATAe*/*CG11267*) for *in situ* hybridization. Hybridization was performed at 60°C in a solution containing 50% formamide, 5× standard saline citrate (SSC) (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7), 0.1% Tween 20, 0.1 mg/ml yeast tRNA, 10 mM DTT, and 10% dextran sulfate. The embryos were washed six times at 60°C in a solution containing 50% formamide, 5× SSC, and 0.1% Tween 20. Signal was detected with a TSA Biotin System (PerkinElmer). The embryos were then stained for β-galactosidase (β-gal) and Vasa (Vas).

Immunostaining. Immunofluorescent staining of embryos was carried out as described in ref. 24. For Vas staining, the dechorionated embryos were fixed in 2 ml of a 1:1 mixture of heptane and fixative II (4% paraformaldehyde in PBS). The fixed embryos were treated with a rat anti-Vas antibody (1:2,000 dilution) and then with a Texas red-conjugated anti-rat IgG (1:50, Cappel).

For triple staining, the *in situ* hybridized embryos were stained for β-gal and Vas. We used a rabbit anti-β-gal antibody (1:400, Chemicon) and a rat anti-Vas antibody (1:2,000). As secondary antibodies, an Alexa 647-conjugated anti-rat IgG (1:1,000, Molecular Probes) and an Alexa 488-conjugated anti-rabbit IgG

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Abbreviations: β-gal, β-galactosidase; Nos, Nanos; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling; Vas, Vasa.

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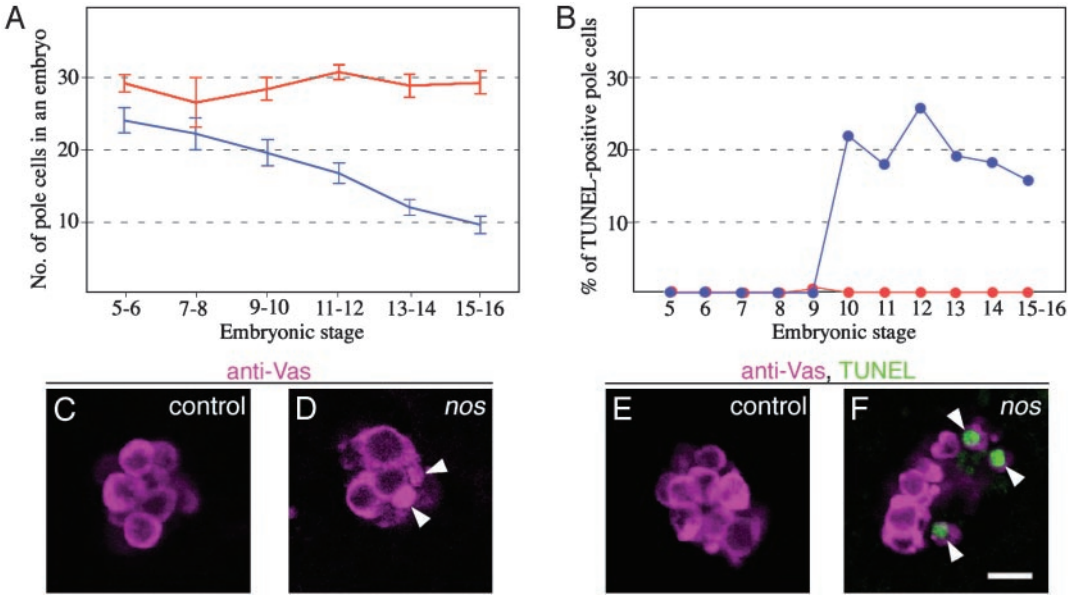

Fig. 1. Removal of Nos activity causes apoptosis of pole cells during embryogenesis. Embryos from $nos^{BN}/TM3$ females (*C* and *E*) (control embryos) and from nos^{BN}/nos^{BN} females (*D* and *F*) (*nos* embryos) were stained with an anti-Vas antibody (*A, C,* and *D*) and double-stained with an anti-Vas antibody and TUNEL labeling (*B, E,* and *F*). (*A* and *B*) The number of pole cells per embryo (*A*) and the percentage of pole cells with TUNEL signal (*B*) in control (red) and *nos* (blue) embryos are plotted against the developmental stage. Embryos (3–30) of each genotype were examined at each stage. (*C–F*) Stage-13 embryos. Vas signal is shown in magenta, and TUNEL signal is shown in green. Arrowheads show pole cells with altered morphology associated with apoptosis. (Scale bar = 10 μm.)

(1:400, Molecular Probes) were used. Stained embryos were then mounted in Vectashield (Vector Laboratories) and observed under a confocal microscope (Leica Microsystems, Tokyo).

For immunostaining larval ovaries and testes, the dissected gonads were fixed in fixative II for 20 min and treated three times with methanol. Fixed gonads were rinsed in PBS containing 1% Triton X-100 for 15 min and treated with rabbit anti-β-gal, rat anti-Vas, and mouse anti-GFP (1:400, Wako Pure Chemicals, Osaka) antibodies. As secondary antibodies, an Alexa 647-conjugated anti-rat IgG (1:500, Molecular Probes), an Alexa 568-conjugated anti-rabbit IgG (1:1,000, Molecular Probes), and an Alexa 488-conjugated anti-mouse IgG (1:500, Molecular Probes) were used. The stained samples were then mounted in Vectashield and observed under a confocal microscope (Leica Microsystems).

Double Staining with Terminal Deoxynucleotidyltransferase-Mediated dUTP Nick End Labeling (TUNEL) and Immunostaining Methods. TUNEL was performed essentially according to the method reported in ref. 19 with several modifications. Embryos were fixed with fixative I for 30 min and devitellinized. Those embryos were washed in PBS containing 0.3% Triton X-100 and in TUNEL buffer [TUNEL dilution buffer (Roche Applied Science) containing 0.3% Triton X-100 and 1.5 mM $CoCl_2$]. The embryos were incubated for 3 h at 37°C in TUNEL buffer containing 1× TUNEL enzyme (Roche Applied Science) and 10 μM dUTP [1:2 mix of Bio-16-dUTP and dUTP (Roche Molecular Biochemicals)] and washed in PBS containing 0.3% Triton X-100 . The embryos were treated with FITC-avidin DN (1:200 dilution, Vector) for 1 h. The embryos were then stained for Vas protein by the method described above.

Pole Cell Transplantation and β-Gal Staining. Pole cell transplantation and β-gal staining were performed as described in ref. 15.

Results and Discussion

We wished to examine the developmental fate of pole cells lacking Nos (*nos* pole cells) as embryogenesis proceeds. However, beginning at stage 9/10 most pole cells were lost in the embryos derived from *nos* homozygous mothers (*nos* embryos) (24) (Fig. 1*A*). Because *nos* pole cells sometimes showed irregular shapes characteristic of apoptotic cells (Fig. 1*D*) and were TUNEL-positive (25) (Fig. 1 *B* and *F*), we concluded that these pole cells were eliminated by apoptosis. To further test this conclusion and potentially to be able to analyze the developmental fate of *nos* pole cells, we used *Df(3L)H99* (H99), a small deletion that uncovers the genomic region including *reaper* (*rpr*) (19), *head involution defective* (*hid*) (26), and *grim* (27), which are all involved in apoptosis. As expected, H99 deficiency suppressed apoptosis of *nos* pole cells. In embryos lacking maternal Nos and zygotic H99 activity (*nos*-H99 embryo), none of the pole cells showed TUNEL signal (Table 1), showing that apoptosis of *nos* pole cells requires activities of genes within the H99 deficiency.

To examine the developmental fate of *nos*-H99 pole cells, we transplanted them into host embryos and examined their developmental fate, because *nos*-H99 embryos were lethal. As shown in Table 2 and Fig. 2 *D–I*, *nos*-H99 pole cells were integrated within somatic tissues, such as midgut epithelium, tracheal

Table 1. H99 deficiency suppresses apoptosis of *nos* pole cells

Embryos examined*	No. of pole cells observed	No. of pole cells with TUNEL signal (%)	Significance†
nos	380	75 (19.7)	
nos-H99	524	0 (0)	$P < 0.0001$

*Stage 13 embryos derived from nos^{BN}/nos^{BN} females mated with WT males (*nos*) and nos^{BN} *Df(3L)H99*/nos^{BN} females mated with *Df(3L)H99*/*TM3* males (*nos*-H99) were double-stained with an anti-Vas antibody and TUNEL labeling. Because the lack of H99 results in repression of apoptosis in somatic tissues (19), the embryos without TUNEL signal in the somatic region, such as gnathal segments and the dorsal folds, were judged to be homozygous for *Df(3L)H99*.

†Significance was calculated by using Fisher's exact probability test.

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Table 2. Developmental fate of transplanted pole cells

Donor embryos*	Transplants	Surviving embryos	No. of embryos with labeled cells: Total	Within gonads (%)	Integrated within somatic tissue (%)
Control	134	86	35	20 (57.1)†	0 (0)
nos	431	218	64	0 (0) ‡	0 (0) ‡
nos-H99	539	361	112	28 (25.0) ‡	20 (17.9)§ ‡

*The embryos derived from nos^{BN}-*H99-PLHΔ23*/TM3 females mated with *Df(3L)H99*/TM3 males were referred to as control embryos. The embryos from nos^{BN}-*H99-PLHΔ23*/nos^{BN} females mated with WT males were referred to as *nos* embryos. All *nos* embryos lack maternal Nos activity but retain H99 activity. The embryos derived from nos^{BN}-*H99-PLHΔ23*/nos^{BN} females mated with *Df(3L)H99*/TM3 males were referred to as *nos*-H99 embryos. All of the embryos obtained from this cross lack maternal Nos activity, and about half of the embryos carrying PLHΔ23 lack zygotic H99 activity. We transplanted pole cells from these embryos to host embryos. Because the transplanted pole cells with PLHΔ23 express β-gal after heat treatment, we can follow their developmental fate after heat treatment and staining for β-gal (15). After transplantation, the hosts were developed until stages 15–17 and were heat-treated and stained with β-gal or an anti-β-gal antibody.

†There is no significant difference in the percentage of pole cells incorporated into the gonads between control and normal embryos (data not shown), although about half of control embryos were homozygous for *Df(3L)H99*. This indicates that pole cells are able to enter the gonads, irrespective of the presence or absence of H99 activity.

‡$P < 0.005$. Significance was calculated by using Fisher's exact probability test.

§The percentages of host embryos with *nos*-H99 pole cells integrated into midgut epithelium, tracheal epithelium, and gastric ceca were 60% (12 of 20), 5% (1 of 20) and 5% (1 of 20), respectively. *nos*-H99 pole cells were also found to be integrated into somatic tissues, such as Malpighian tubules (2 of 20), hindgut (1 of 20), and fat body (1 of 20). These data suggest that pole cells have a tendency to become midgut cells. This phenotype might be explained by contamination of the transplanted cells with the midgut precursors locating just beneath pole cells in the blastodermal embryos. However, that is not the case, because we transplanted pole cells from the embryos at early syncytial blastoderm stage, when somatic nuclei still remain uncellularized.

epithelium, and gastric ceca, whereas neither control nor *nos* pole cells ever were. These "integrated" *nos*-H99 pole cells were morphologically distinct from pole cells that were merely lost in midgut lumen and hemocoel. Pole cells integrated within somatic tissues were morphologically indistinguishable from their neighboring host cells (Fig. 2 *D–I*). For example, pole cells within midgut epithelium were columnar in shape (Fig. 2 *D* and *G*). In contrast, the "lost" *nos*-H99, *nos*, and control pole cells were round in shape and were neither associated with nor embedded within somatic tissues (Fig. 2*B*). Moreover, we found that the integrated pole cells expressed somatic markers ectopically. When *nos*-H99 pole cells were integrated into the midgut epithelium, all of them expressed the midgut marker genes *CG11267* and *dGATAe* ($n = 13$ pole cells examined) and *bInt-n* and *mex1* ($n = 9$) (Fig. 3 *B* and *D*). Conversely, the germ-line marker Vas was lost or significantly reduced in these pole cells (Fig. 3*C*). In contrast, all of the lost pole cells were Vas-positive but failed to express midgut markers (*mex1*/*bInt-n*) ($n = 11$) (Fig. 3 *F–J*). These results clearly show that *nos* pole cells can differentiate as somatic cells when their apoptosis is suppressed.

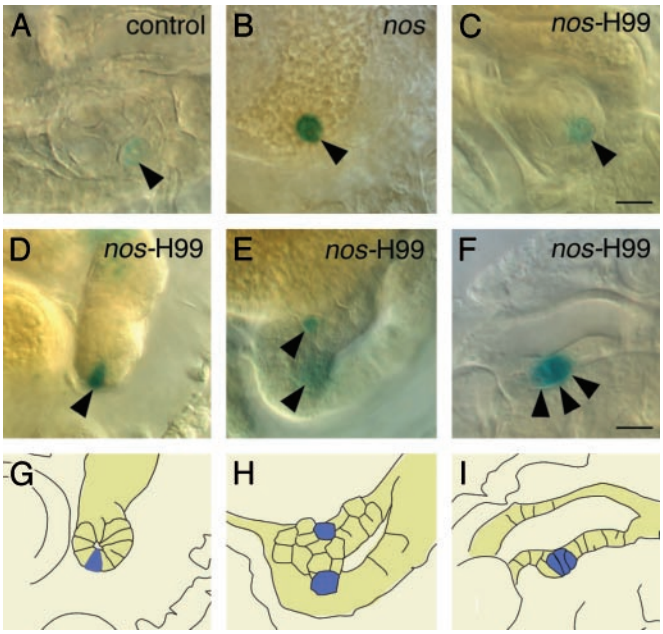

Fig. 2. *nos*-H99 pole cells have the ability to adopt somatic cell fate and to enter the embryonic gonads. Pole cells from *nos* (*B*), *nos*-H99 (*C–F*), and control (*A*) embryos were transplanted into hosts, and the hosts were developed until stages 15–17. The transplanted pole cells were identified as blue cells after heat treatment and staining for β-gal (arrowheads). The transplanted control (*A*) and *nos*-H99 (*C*) pole cells were observed within the gonads, whereas *nos* pole cells remained outside the gonads (*B*). Some *nos*-H99 pole cells were integrated within somatic tissues (*D–F*). (*D*) *nos*-H99 pole cell within midgut epithelium is shown. Note that *nos*-H99 pole cell and the neighboring midgut epithelial cells were columnar in shape at stage 17. *nos*-H99 pole cells within gastric ceca (*E*) and tracheal (*F*) epithelium are also shown. The lower cell indicated by an arrowhead in *E* was out of focus. (*G–I*) Drawings tracing the integrated *nos*-H99 pole cells (blue) and the surrounding somatic cells in *D–F*, respectively. (Scale bar = 10 μm.)

We have shown that transplanted *nos* pole cells never migrate into the host gonads (15). To our surprise, their ability to enter the gonads was restored by suppressing apoptosis. In ≈25% of the host embryos, *nos*-H99 pole cells were incorporated within the gonads (Figs. 2*C* and 3 *K–O* and Table 2). This percentage is less than that observed when control pole cells were transplanted into the hosts, presumably because only half of the *nos*-H99 embryos used as donors are homozygous for H99 (Table 2). Thus, the ability of *nos* pole cells to migrate into the gonads is fully restored by suppressing apoptosis in our transplantation experiments.

nos-H99 pole cells incorporated within the embryonic gonads were indistinguishable from the host pole cells in their morphology and Vas staining (Figs. 2*C* and 3 *K–O*). However, these pole cells did not contribute to egg and sperm production. In the ovaries, the cells derived from transplanted *nos*-H99 pole cells were detectable until at least the end of the third instar, but their size was much smaller than that of normal germ-line cells, and their Vas expression was significantly reduced (Fig. 4 *B* and *C* and Table 3). In the testes of the third-instar larvae, the cells derived from transplanted *nos*-H99 pole cells were no longer detectable (Fig. 4*F* and Table 3). These results indicate that *nos*-H99 pole cells are unable to complete gametogenesis. As a control, we transplanted pole cells from H99-homozygous em-

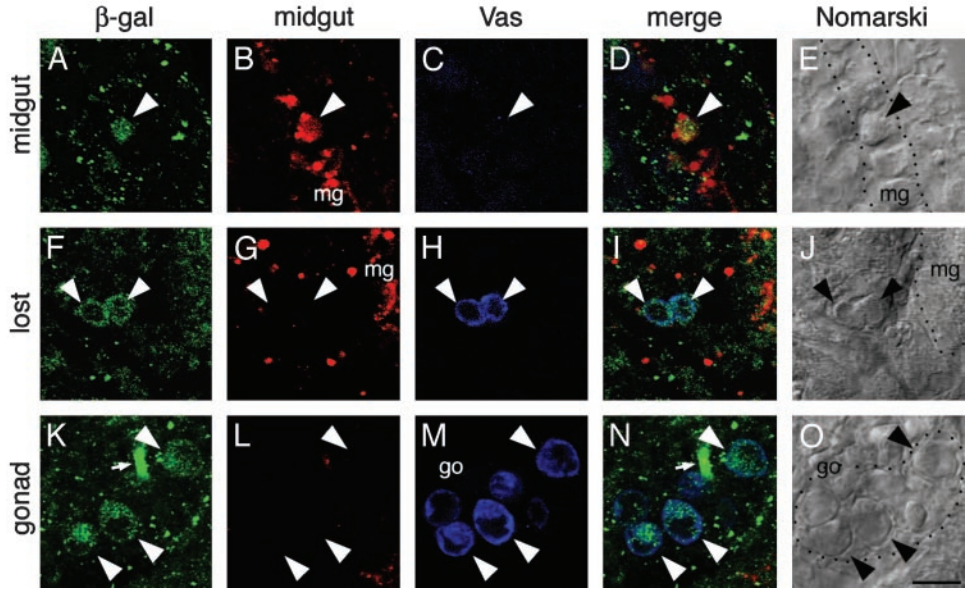

Fig. 3. *nos*-H99 pole cells within midgut epithelium expressed midgut marker genes but lost germ-line marker Vas. We examined expression of midgut marker genes and Vas protein in *nos*-H99 pole cells within midgut epithelium (*A–E*), hemocoel (lost pole cells) (*F–J*), and gonad (*K–O*) of host embryos. The transplanted pole cells were identified as the cells expressing β-gal (marked by arrowheads). The embryos at stages 13–15 were triple-stained with probes for the midgut marker genes (*bInt-n/mex1*; red) (*B, G,* and *L*) and antibodies against β-gal (green) (*A, F,* and *K*) and Vas protein (blue) (*C, H,* and *M*). *nos*-H99 pole cell integrated within midgut epithelium (mg), like the neighboring host cells, was cuboidal at stage 14 (*E*). The integrated pole cell expressed midgut marker genes (*B*) but not Vas protein (*C*). In contrast, *nos*-H99 pole cells, which were found within hemocoel in the vicinity of midgut (*J*), maintained Vas expression (*H*), but never expressed midgut marker genes (*G*). Within the gonad, *nos*-H99 pole cells and the host pole cells were both negative for midgut markers (*L*). Arrows in *K* and *N* indicate nonspecific staining of tracheal lumen. Merged images (*D, I,* and *N*) and photographs taken with a compound microscope equipped with Nomarski optics (*E, J,* and *O*) are shown. Dotted lines indicate midgut epithelia in *E* and *J* and gonad (go) in *O*. (Scale bar = 10 μm.)

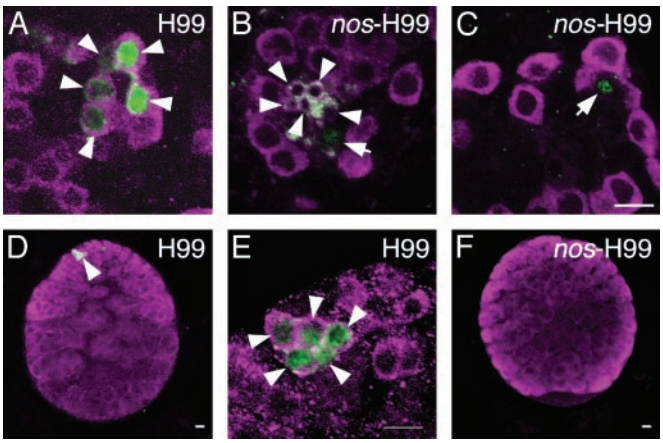

Fig. 4. The developmental fate of *nos*-H99 pole cells incorporated into the gonads. We examined the developmental fate of pole cells homozygous for H99 (*A, D,* and *E*) and *nos*-H99 (*B, C,* and *F*) pole cells in ovaries (*A–C*) and testes (*D–F*) in third-instar larvae. H99-homozygous pole cells were obtained from embryos produced from nos^{BN}-*H99-PLHΔ23/TM6 ubi-GFP* females mated with *Df(3L)H99/TM6 ubi-GFP* males (for details, see *Materials and Methods*). The germ-line cells derived from the transplanted pole cells were identified as the cells expressing β-gal (green, marked by arrowheads in *A, D,* and *E*). Among these cells, GFP-negative cells were judged to be homozygous for H99 (data not shown). *nos*-H99 pole cells were obtained from embryos derived from nos^{BN}-*H99-PLHΔ23*/nos^{BN} females mated with *Df(3L)H99/TM6 ubi-GFP* males. Because pole cells lacking maternal Nos alone are unable to enter the gonads, the β-gal-positive cells within the gonads were judged to be homozygous for H99 (green, marked by arrows and arrowheads in *B* and *C*). Germ-line cells from *nos*-H99 pole cells were much smaller than normal germ-line cells (*B*, arrowheads), and their Vas expression (magenta) was significantly impaired (*B* and *C*, arrows). β-gal signal was frequently seen in the nuclei of the transplanted cells (*A–E*), but some transplanted cells showed β-gal signal in their cytoplasm (*B*, arrowheads). (*E*) A higher-magnification image of β-gal-positive germ-line cells shown in *D*. *nos*-H99 pole cells were no longer discernible in a testis of third-instar larvae (*F*). (Scale bars = 10 μm.)

bryos into hosts. These pole cells were able to migrate into the embryonic gonads (data not shown) and were found normally within the ovaries and testes of the third-instar larvae (Fig. 4 *A*, *D*, and *E*).

Our results imply that pole cells are multipotent, in that they are able to adopt both germ-line and somatic cell fates and undergo apoptosis. Nos is required to repress the pathway leading to somatic differentiation and apoptosis and, thus, to direct germ-line development. Removal of Nos and H99 activities causes pole cells to differentiate into soma. However, some pole cells retain the ability to migrate properly into the gonads. These different behaviors of *nos*-H99 pole cells could be explained by differences in the cellular environment encountered by the pole cells. Alternatively, there may be a heterogeneity among pole cells, one with and one without the competence to adopt somatic cell fate. This idea is supported by the observation that there are Nos-independent transcriptional repression mechanisms in pole cells. Somatic gene expression is derepressed only in a subset of *nos* pole cells (17, 18). Furthermore, a nucleosomal histone modification, methylated lysine 4 on histone H3, that correlates well with open chromatin is accumulated only in a subset of *nos* pole cells (18). We propose that transcriptional derepression is a prerequisite for somatic differentiation of pole cells, although it remains unclear how somatic cell types are specified.

Our data also show that Nos is not essential for pole cell migration because *nos*-H99 pole cells can be normally incorporated within the gonads. It is reasonable to conclude that Nos represses apoptosis in pole cells, allowing their proper migration into the gonads. However, *nos*-H99 pole cells do not complete gametogenesis, suggesting that Nos has another function in later germ-line development (16). It has been reported that zygotic Nos is required in the germ-line cells to prevent their precocious entry into oogenesis during larval development (28). However, in the larvae lacking zygotic Nos, the prematurely formed cysts

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Table 3. The ability of pole cells to become germ-line cells in the gonads of the third-instar larvae

	No. of ovaries observed			No. of testes observed		
Donor embryos*	Total	With cells derived from transplanted cells	With abnormal germ-line cells†	Total	With cells derived from transplanted cells	With abnormal germ-line cells
H99	86	6‡	0 §	86	5‡ §	0
nos-H99	72	11¶	11	78	0¶	0

*We examined the developmental fate of pole cells homozygous for H99 and *nos*-H99 pole cells in the ovaries and testes of the third-instar larvae. H99-homozygous pole cells were obtained from embryos produced from nos^{BN}-*H99-PLHΔ23*/TM6 ubi-GFP females mated with *Df(3L)H99*/TM6 ubi-GFP males. *nos*-H99 pole cells were obtained from embryos derived from nos^{BN}-*H99-PLHΔ23*/nos^{BN} females mated with *Df(3L)H99*/TM6 ubi-GFP males. Pole cells from these donor embryos were transplanted into *yw* host embryos. The hosts were allowed to develop until the third-instar larvae and were heat-treated at 36°C for 2 h followed by incubation at 25°C for 1 h. The gonads dissected from these larvae were triple-stained for β-gal, GFP, and Vas.

†The number of the gonads with abnormal germ-line cells derived from the transplanted pole cells is shown. The abnormal germ-line cells were significantly smaller in size and their Vas expression was reduced compared with the neighboring host germ-line cells.

‡The cells derived from the transplanted pole cells were identified as the cells expressing β-gal. Among these cells, GFP-negative cells were judged to be homozygous for *Df(3L)H99.* The number of gonads with the β-gal-positive and GFP-negative cells are shown.

§$P < 0.05$. Significance was calculated by using Fisher's exact probability test.

¶Because pole cells lacking Nos are unable to enter into the gonads, the β-gal-positive cells within the gonads were judged to be homozygous for H99. The number of the gonads with the β-gal-positive cells are shown.

fail to execute oogenesis and eventually degenerate. It is possible that maternal Nos may also be required in pole cells to repress their premature differentiation. Alternatively, the defect of *nos*-H99 pole cells could result simply from their failure to establish germ-line fate.

In nematode, zebrafish, and mouse embryos, Nos homologues are required for maintenance of the germ-line progenitors (10, 12, 13). These results, and those we have observed in *Drosophila*, indicate that Nos is involved in an evolutionarily conserved mechanism required for germ-line maintenance. Moreover, in *Caenorhabditis elegans* and *Drosophila*, Nos is required to establish germ-line-specific histone modifications that correlate with transcriptionally inactive chromatin (18). We propose that Nos also acts as a part of conserved mechanism repressing somatic gene expression and differentiation to establish germ/soma dichotomy.

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1. Beams, H. W. & Kessel, R. G. (1974) *Int. Rev. Cytol.* **39,** 413–479.
2. Eddy, E. M. (1975) *Int. Rev. Cytol.* **43,** 229–280.
3. Rongo, C. & Lehmann, R. (1996) *Trends. Genet.* **12,** 102–109.
4. Campos-Ortega, J. A. & Hartenstain, V. (1985) *The Embryonic Development of Drosophila melanogaster* (Springer, Heidelberg), pp. 9–84.
5. Underwood, E. M., Caulton, J. H., Allis, C. D. & Mahowald, A. P. (1980) *Dev. Biol.* **77,** 303–314.
6. Technau, G. M. & Campos-Ortega, J. A. (1986) *Roux's Arch. Dev. Biol.* **195,** 489–498.
7. Wang, C. & Lehmann, R. (1991) *Cell* **66,** 637–647.
8. Mosquera, L., Forristall, C., Zhou, Y. & King, M. L. (1993) *Development (Cambridge, U.K.)* **117,** 377–386.
9. Pilon, M. & Weisblat, D. A. (1997) *Development (Cambridge, U.K.)* **124,** 1771–1780.
10. Subramaniam, K. & Seydoux, G. (1999) *Development (Cambridge, U.K.)* **126,** 4861–4871.
11. Mochizuki, K., Sano, H., Kobayashi, S., Nishimiya-Fujisawa, F. C. & Fujisawa, T. (2000) *Dev. Genes Evol.* **210,** 591–602.
12. Köprunner, M., Thisse, C., Thisse, B. & Raz, E. (2001) *Genes Dev.* **15,** 2877–2885.
13. Tsuda, M., Sasaoka, Y., Kiso, M., Abe, K., Haraguchi, S., Kobayashi, S. & Saga, Y. (2003) *Science* **301,** 1239–1241.
14. Wang, C., Dickinson, L. K. & Lehmann, R. (1994) *Dev. Dyn.* **199,** 103–115.
15. Kobayashi, S., Yamada, M., Asaoka, M. & Kitamura, T. (1996) *Nature* **380,** 708–711.
16. Forbes, A. & Lehmann, R. (1998) *Development (Cambridge, U.K.)* **125,** 679–690.
17. Deshpande, G., Calhoun, G., Yanowitz, J. L. & Schedl, P. D. (1999) *Cell* **99,** 271–281.
18. Schaner, C. E., Deshpande, G., Schedl, P. D. & Kelly, W. G. (2003) *Dev. Cell* **5,** 747–757.
19. White, K., Grether, M. E., Abrams, J. M., Young, L., Farrell, K. & Steller, H. (1994) *Science* **264,** 677–683.
20. Kobayashi, S., Kitamura, T., Sasaki, H. & Okada, M. (1993) *Development (Cambridge, U.K.)* **117,** 885–893.
21. Tautz, D. & Pfeifle, C. (1989) *Chromosoma* **98,** 81–85.
22. Robert, A. S., Xie, X., Andres, A. J. & Galewsky, S. (1991) *Dev. Biol.* **143,** 206–211.
23. Yee, G. H. & Hynes, R. O. (1993) *Development (Cambridge, U.K.)* **118,** 845–858.
24. Asaoka-Taguchi, M., Yamada, M., Nakamura, A., Hanyu, K. & Kobayashi, S. (1999) *Nat. Cell Biol.* **1,** 431–437.
25. Gavrieli, Y., Sherman, Y. & Ben-Sasson, S. A. (1992) *J. Cell Biol.* **119,** 493–501.
26. Grether, M. E., Abrams, J. M., Agapite, J., White, K. & Steller, H. (1995) *Genes Dev.* **9,** 1694–1708.
27. Chen, P., Nordstrom, W., Gish, B. & Abrams, J. M. (1996) *Genes Dev.* **10,** 1773–1782.
28. Wang, Z. & Lin, H. (2004) *Science* **303,** 2016–2019.