Lissencephaly-1 controls germline stem cell self-renewal through modulating bone morphogenetic protein signaling and niche adhesion

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In the *Drosophila* ovary, bone morphogenetic protein (BMP) signaling activated by the niche promotes germline stem cell (GSC) self-renewal and proliferation, whereas E-cadherin–mediated cell adhesion anchors GSCs in the niche for their continuous self-renewal. Here we show that *Lissencephaly-1* (*Lis1*) regulates BMP signaling and E-cadherin–mediated adhesion between GSCs and their niche and thereby controls GSC self-renewal. *Lis1* mutant GSCs are lost faster than control GSCs because of differentiation but not because of cell death, indicating that *Lis1* controls GSC self-renewal. The *Lis1* mutant GSCs exhibit reduced BMP signaling activity, and *Lis1* interacts genetically with the BMP pathway components in the regulation of GSC maintenance. Mechanistically, Lis1 binds directly to and stabilizes the SMAD protein Mothers against decapentaplegic (Mad), facilitates its phosphorylation, and thereby regulates BMP signaling. Finally, the *Lis1* mutant GSCs accumulate less E-cadherin in the stem cell–niche junction than do their wild-type counterparts. Germline-specific expression of an activated BMP receptor thick-veins (Tkv) or E-cadherin can partially rescue the loss phenotype of *Lis1* mutant GSCs. Therefore, this study has revealed a role of *Lis1* in the control of *Drosophila* ovarian GSC self-renewal, at least partly by regulating niche signal transduction and niche adhesion. It has been known that *Lis1* controls neural precursor/stem cell proliferation in the developing mammalian brain; this study further suggests that *Lis1*, which is widely expressed in adult mammalian tissues, could regulate adult tissue stem cells through modulating niche signaling and adhesion.

In adult animal tissues, stem cells normally undergo asymmetric division to generate self-renewing stem cells and differentiated cells that replace lost cells. Their behavior is tightly controlled by the concerted actions of extrinsic and intrinsic factors in a variety of systems (1, 2). Interestingly, signals from the niche often function within one cell diameter, repressing expression or functions of differentiation-promoting genes and thereby maintaining stem cell self-renewal (3). To self-renew continuously, stem cells must be anchored to the niche and constantly receive niche signals to maintain their undifferentiated state. Stem cell anchorage to the niche often is achieved through cadherin- or integrin-mediated cell adhesion (2, 4). However, how niche signaling and niche anchorage are coordinately regulated in adult stem cells remains largely unknown.

Drosophila ovarian germline stem cells (GSCs) are an attractive system for studying stem cells and the niche at the molecular and cellular level (5). Two or three GSCs are located at the tip of the germarium and directly contact cap cells and escort stem cells, which constitute a GSC niche (Fig. 1*A*) (6–8). In addition, they contain an intracellular spherical structure known as the "spectrosome," which is rich in cytoskeletal proteins such as Hu li-tai shao (Hts) (Fig.1*B*). (9) Differentiating GSC daughters, cystoblasts, move away from the niche and further divide four times synchronously to form 16-cell cysts identified by branched fusomes. Bone morphogenic protein (BMP) signaling is necessary and sufficient for maintaining GSC self-renewal by directly repressing the expression of differentiation-promoting genes such as *bag of marbles* (*bam*) (7, 10, 11). In addition, E-cadherin–mediated cell adhesion is required to keep GSCs in the niche for continuous self-renewal (12). *bam* transcriptional repression by BMP signaling in the GSC is incomplete, leaving low levels of Bam expression (13). Bam-mediated E-cadherin repression in the GSC controls stem cell competition for niche occupancy, functioning as a quality control mechanism to ensure that differentiated GSCs are displaced rapidly from the niche and then are replaced by functional ones through competition (13).

Lissencephaly-1 (*Lis1*) first was identified as the causative gene for the human disease lissencephaly and later was shown to be involved in the regulation of neural precursor proliferation and neuronal migration in the developing brain (14, 15). Like its counterpart in other nervous systems, *Lis1* is required for controlling asymmetric division of *Drosophila* neuronal precursors, neuroblasts, through regulating spindle orientation and for dendrite formation of differentiated neuronal cells (16, 17). Interestingly, Lis1 also regulates cyst division, oocyte formation, and oocyte nucleus migration in the *Drosophila* ovary (18–20). In this study, we show that it functions as an intrinsic factor to control GSC self-renewal, at least in part through regulating BMP signal transduction and E-cadherin–mediated cell adhesion.

Results and Discussion

Lis1 Is Required for GSC Self-Renewal. To investigate the potential role of the Lis1 gene in GSC maintenance in the *Drosophila* ovary, we used Flippase (FLP)-mediated FLP-recognition target (FRT) mitotic recombination to generate marked *armadillo* (*arm*)-*lacZ*–negative *Lis1* mutant GSCs and examined their maintenance and relative division rates as previously reported (7, 12). The GSCs were identified as the most anterior single germ cells containing an anteriorly anchored spectrosome and directly contacting cap cells; the marked and unmarked GSCs were identified by the absence or presence of *arm-lacZ* expression, respectively (Fig. 1 *A* and *B*). About 75–80% of the *arm-lacZ*–negative marked wild-type GSCs detected during the first week after clone induction (ACI) still remained in the niche 3 wk ACI (Fig. 1 *B–D*). In contrast, only 2% and 17%, respectively, of *arm-lacZ*-negative marked $Lis1^{D}$ and $Lis1^{G10.14}$ ($Lis1^{G}$) mutant GSCs detected 1 wk ACI remained in the niche 3 wk ACI, (Fig. 1 *D–F*). $Lis1^{D}$ and $Lis1^{G}$, encoding truncated proteins, have been shown previously to represent severe or null *Lis1* mutants (18).Thus, most of the germaria had already lost the marked mutant GSCs 3 wk ACI, as

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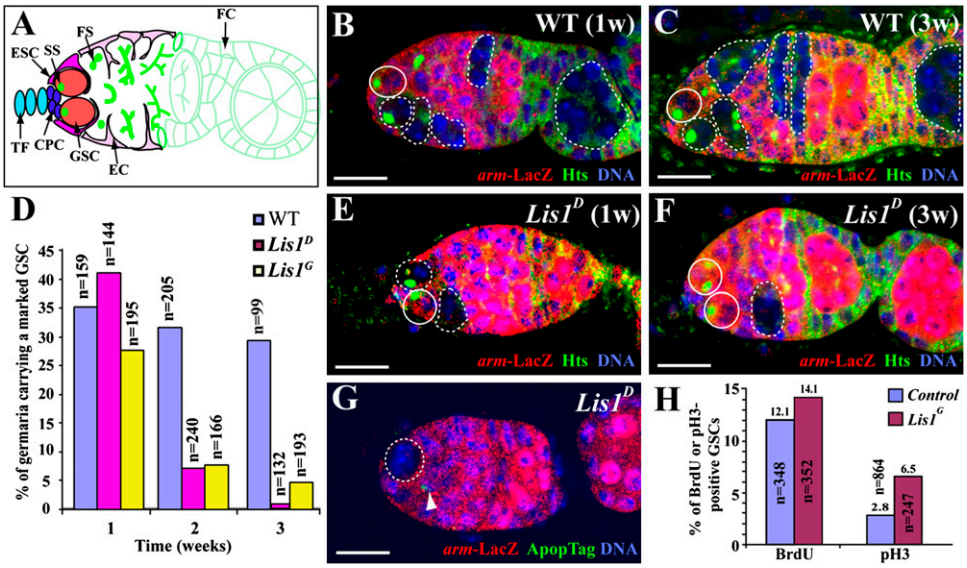

Fig. 1. Requirement of Lis1 in controlling GSC self-renewal. (*A*) Schematic diagram of a wild-type germarium showing different cell types and organelles. CPC, cap cell; EC, escort cell; ESC, escort stem cell; FC, follicle cell. FS, fusome; GSC, germline stem cell; SS, spectrosome; TF, terminal filament. (*B* and *C*) Germaria carrying an unmarked GSC (solid circle) and a 1-wk-old (*B*) or 3-wk-old (*C*) marked wild-type GSC clone in which the marked GSC and progeny are indicated by a broken circle and broken lines, respectively. (*D*) Changes in percentages (*y* axis) of the germaria carrying a marked control and *Lis1* mutant GSC clone with time (*x* axis). *n* represents the number of total germaria examined. (*E*) Germarium carrying an unmarked wild-type GSC (solid circle) and a marked *Lis1* mutant GSC (broken circle), which produces a marked mutant cyst (broken lines). (*F*) Germarium carrying two unmarked wild-type GSCs (solid circles) and a marked *Lis1* mutant cyst (broken line), indicative of a lost marked *Lis1* mutant GSC. (*G*) Germarium carrying an ApopTag-negative marked *Lis1*-mutant GSC (broken circle) with a dying escort cell (arrowhead). (*H*) *Lis1* mutant GSCs may divide slightly faster than control GSCs. There are almost two times more pH3-positive $Lis1^G$ mutant GSCs than control GSCs, but only slightly more $Lis1^G$ mutant GSCs than control GSCs are positive for BrdU. *n* represents the number of total GSCs examined. (Scale bars: 10 μm.)

was evident from the presence of marked differentiated cysts in the germaria or late egg chambers (Fig. 1*F*). To determine further whether apoptosis contributes to the loss of the marked *Lis1* GSCs, we used a TUNEL- based ApopTag labeling assay to detect the dying cells in the ovaries. Although we could detect dying somatic cells in germaria easily, we failed to detect apoptotic marked $Lis1^G$ and $Lis1^D$ mutant GSCs ($n = 156$), further reinforcing the idea that *Lis1* mutant GSCs are lost because of differentiation rather than apoptosis (Fig. 1*G*). Taken together, these results demonstrate that *Lis1* is required for controlling GSC self-renewal but not survival in the *Drosophila* ovary.

During the analysis of the marked *Lis1* mutant clones, we noticed that marked *Lis1* mutant GSCs produced much fewer germline cysts than the marked control GSCs, suggesting that *Lis1* is required for GSC division, cyst survival, or both (compare Fig. 1 *B* and *E*). *Lis1* has been shown previously to regulate M-phase progression and spindle orientation in *Drosophila* and mouse neural stem cells (16, 21, 22). To determine if *Lis1* is required for GSC division, we performed BrdU labeling and phosphorylated histone H3 (pH3) staining on unmarked control GSCs and marked *Lis1* mutant GSCs. Surprisingly, the percentage of the BrdU-positive marked *Lis1* mutant GSCs is similar to or even slightly higher than that of the unmarked control GSCs, and the percentage of the pH3-positive marked *Lis1* mutant GSCs is almost two times higher than that of the control GSCs, suggesting that *Lis1* mutant GSCs may cycle normally or slightly faster than control GSCs (Fig. 1*H* and Fig. S1). Because we did not observe TUNEL-positive *Lis1* mutant cysts in the germaria carrying a *Lis1* mutant GSC, this result suggests that the fewer number of mutant cysts produced by an *Lis1* mutant GSC probably results from cystoblast death and the quick disappearance of the dying cystoblasts. Like *Drosophila* neuroblasts, *Lis1*-mutant GSCs exhibited a misoriented spindle more frequently than control GSCs (Fig. S2). The published studies show that the defect in spindle orientation might cause the slowdown of the M phase of the *Lis1* mutant stem cells in the mouse brain and *Drosophila* testis (22, 23). Our finding that *Lis1* mutant GSCs exhibit misoriented spindles but do not appear to proliferate more slowly than wild-type GSCs suggests that *Lis1* also may be required for regulating the checkpoint control in GSCs.

To investigate if the spindle misorientation is responsible for the loss of *Lis1* mutant GSCs, we generated *arm-lacZ*–marked *hts* mutant GSCs, which exhibit misoriented spindles (24), and determined their maintenance over time. Surprisingly, the marked *hts* mutant GSCs still were well maintained 2 wk ACI, in contrast with the dramatic loss of marked $Lis1^G$ and $Lis1^D$ GSCs 2 wk ACI (Table S1). This finding suggests that spindle misorientation could not be fully responsible for the loss of *Lis1*-mutant GSCs.

Lis1 Promotes BMP Signaling in GSCs and Facilitates Their Maintenance. Because niche-initiated BMP signaling is required for maintaining GSC self-renewal (7, 10), we explored the possibility that Lis1 regulates BMP signal reception in the GSC. In the GSC, BMP signaling up-regulates *Daughters against decapentaplegic* (*Dad*) expression and represses *bam* transcription, effects that can be recapitulated by the reporters *Dad-lacZ* and *bam-GFP*, respectively (10, 11). Therefore we examined the expression of *Dad-lacZ* and *bam-GFP* in the marked *Lis1* mutant GSCs. As expected, in the unmarked control GSCs, *Dad-lacZ* was expressed and *bam-GFP* was repressed (Fig. 2 *A*–*B*′). In contrast, *Dad-lacZ* expression was much lower in the marked $Lis1^D$ GSCs (17/34) and $Lis1^G$ GSCs (15/40) than in the neighboring unmarked control GSCs (Fig. 2 *A* and *A*′). Consistent with lower BMP signaling in the *Lis1* mutant GSCs, *bam-GFP* expression was higher in the marked $Lis1^D$ GSCs (36/74) and $Lis1^G$ GSCs (18/59) than in the neighboring unmarked control GSCs (Fig. 2 *B* and *B*′). In *Drosophila*, BMP signal transduction leads to the production of phosphorylated Mothers against decapentaplegic (pMad), which works with SMAD4/Medea to regulate gene expression (25). In the control, the marked and unmarked wild-type GSCs had similar levels of

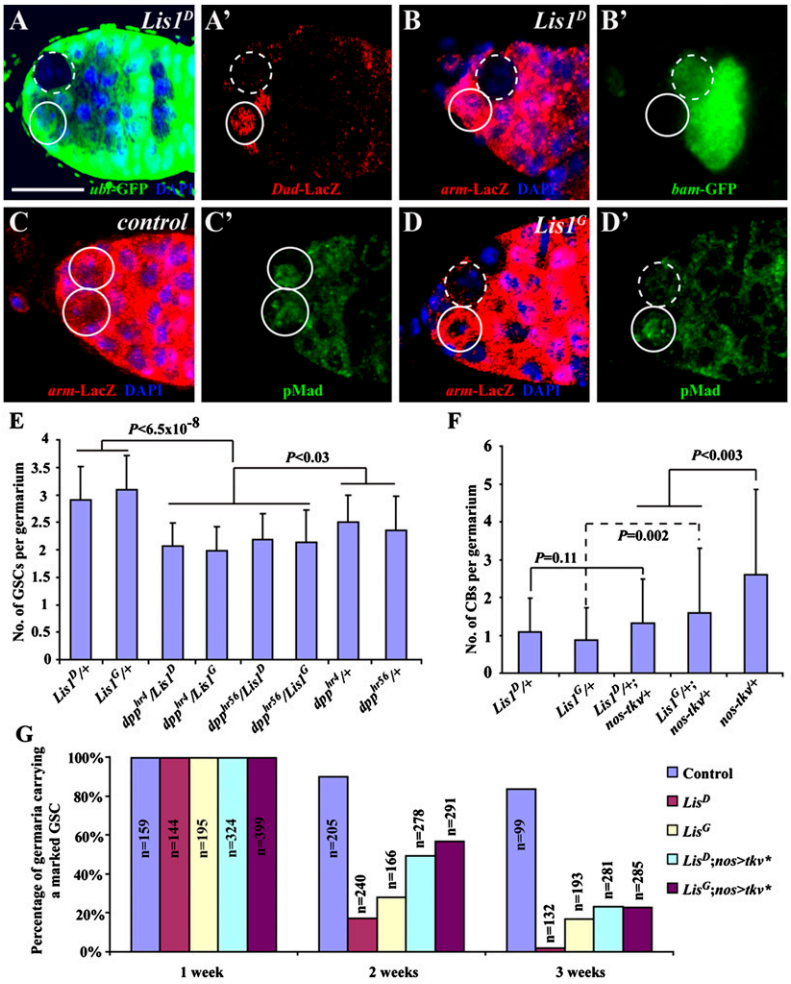

Fig. 2. Requirement of Lis1 in maintaining BMP signaling in GSCs. (*A* and *A′*) A marked *Lis1* mutant GSC (broken circle) downregulating *Dad-lacZ* expression compared with an unmarked wild-type GSC (solid circle) in the same niche. (*B* and *B′*) A marked *Lis1* mutant GSC (broken circle) up-regulating bam-GFP expression compared with an unmarked wild-type GSC (solid circle) in the same niche. (*C* and *C′*) Two unmarked wild-type GSCs (solid circles) with similar levels of nuclear pMad expression. (*D* and *D′*) A marked *Lis1* mutant GSC (broken circle) with lower nuclear pMad expression than its neighboring unmarked wild-type GSC (solid circle). (*E*) Germaria of *Lis1* and *dpp* heterozygous double mutants have significantly fewer GSCs than germaria of *Lis1* or *dpp* heterozygous single mutants. Error bars represent SD. (*F*) Heterozygous mutation in *Lis1* can suppress excess cystoblast phenotype caused by germline-specific Tkv overexpression (*nos-tkv* represents *nos-gal4* driving *UASp-tkv*). (*G*) Germline-specific expression of an activated BMP receptor Tkv can partly slow the loss of the marked *Lis1* mutant GSCs. For all genotypes, the initial percentages of the germaria carrying a marked GSC are normalized to 100%. *nos > tkv**, *nos-gal4 UAS-tkv** (tkv* is a constitutively active form of tkv). *n* represents the number of total germaria examined.

pMad expression (Fig. 2 *C* and *C′*). However, the expression of pMad was down-regulated dramatically in marked $Lis1^D$ or $Lis1^G$ mutant GSCs compared with the neighboring unmarked wild-type GSCs (Fig. 2 *D* and *D'*). Taken together, these results demonstrate that *Lis1* is required in the GSC to maintain BMP signaling activity.

We have shown previously that GSC self-renewal is very sensitive to the dosage of *decapentaplegic* (*dpp*), which encodes a niche BMP (7, 10). To probe further the relationship between BMP signaling and *Lis1* in GSC regulation, we carried out genetic interaction studies on *Lis1* and *dpp* mutants. Interestingly, the germaria of *Lis1* and *dpp* double heterozygotes had significantly fewer GSCs than the germaria of *Lis1* or *dpp* heterozygotes, indicating that *Lis1* and *dpp* interact genetically with each other in GSC regulation (Fig. 2*E*). GSC differentiation also is sensitive to BMP signaling dosage: More BMP signaling favors self-renewal (7, 10). Consistently, when a wild-type *thickveins* (*tkv*), encoding a BMP type I receptor (26, 27), was overexpressed in germline cells using *nos-gal4* and *UASp-tkv*, the germaria contained an average 2.6 ± 2.3 cystoblasts per germarium, in comparison with about one cystoblast per wild-type germarium (28), indicating that boosting BMP signaling promotes GSC proliferation or slows down cystoblast differentiation (Fig. 2*F*). $Lis1^D$ and $Lis1^G$ heterozygous germaria had about one cystoblast, behaving like wild-type germaria (Fig. 2*F*). However, $Lis1^D$ or $Lis1^G$ heterozygous germaria overexpressing *tkv* had significantly fewer cystoblasts than wild-type germaria overexpressing *tkv*, indicating that reduction of *Lis1* function can suppress BMP signaling in the control of GSC proliferation or differentiation (Fig. 2*F*). To test further that the reduced BMP signaling contributes to the loss of *Lis1* mutant GSCs, we used *nos-gal4*–driven *UAS-tkv** (a constitutively active form of *tkv*) to boost BMP signaling in the marked *Lis1*-mutant GSCs. Germline-specific *tkv** expression could slow the loss of the 2-wk-old marked *Lis1* mutant GSCs dramatically, but this rescue effect became less dramatic for the 3-wk-old mutant GSCs, indicating that increasing BMP signaling can, at least partially, slow the loss of the *Lis1* mutant GSCs (Fig. 2*G*). Therefore, all the genetic data show that *Lis1* interacts genetically with the *dpp*/BMP

pathway in controlling the balance between GSC self-renewal and differentiation.

Lis1 Interacts with Mad to Regulate Its Stability and Phosphorylation. Because of the difficulty in obtaining sufficient GSCs, we sought to use S2 cells to carry out biochemistry studies to obtain further mechanistic insights into how *Lis1* regulates BMP signaling. Dpp stimulation led to the nuclear accumulation of pMad in S2 cells in comparison with no detectable pMad in the S2 cells without Dpp, confirming the previous finding that S2 cells are capable of responding to Dpp/BMP (Fig. 3 *A* and *B*) (29). Interestingly, knockdown of *Lis1* expression using dsRNAs also resulted in the reduction of nuclear pMad expression, just as in *Lis1*-mutant GSCs, indicating that *Lis1* regulates BMP signaling in S2 cells (Fig. 3*C*). Two independent dsRNAs could knock down Lis1 protein expression efficiently, as was verified by Western blotting (Fig. 3*D*). In the *Lis1* knockdown S2 cells, both Mad and pMad expression levels were significantly reduced, as compared with controls, indicating that *Lis1* is required for maintaining Mad protein expression and/or phosphorylation (Fig. 3*D* and Fig. S3). Interestingly, pMad levels still remained lower in the *Lis1* knockdown cells in which total Mad levels were brought back nearly to normal by forced expression than in the controls, suggesting that *Lis1* also is involved in the regulation of Mad phosphorylation (Fig. 3*E*). Therefore, *Lis1* is required for maintaining Mad protein expression levels and facilitating Mad phosphorylation.

To investigate how *Lis1* regulates Mad protein expression and phosphorylation, we tested whether Lis1 and Mad proteins could interact physically in S2 cells. Interestingly, Flag-tagged Lis1 and HA-tagged Mad immunoprecipitated each other down in the S2 cells (Fig. 3*F*). Furthermore, Flag-tagged Lis1 could bring down HA-tagged Mad more efficiently in the presence of Tkv than in its absence, indicating that Tkv can enhance the Lis1–Mad interaction (Fig. 3*G*). The interaction between Lis1 and Mad became even stronger in the presence of both Punt and Tkv than in the presence of Tkv alone, indicating that the BMP receptor complex can help strengthen the Lis1–Mad interaction (Fig. 3 *G* and *H* and Fig. S3*C*). In *Drosophila*, Dpp requires the kinase functions of both the type I receptor Tkv and the type II receptor Punt for transducing its signal (26, 27, 30, 31). Interestingly, wild-type Tkv and kinase-dead Tkv^{KR} could equally promote the Lis1–Mad interaction, but the presence of Tkv^{KR} caused the reduction of pMad expression, suggesting that BMP receptors, not BMP signaling per se, facilitate the Lis1–Mad interaction (Fig. 3*H*). A comparison of the amount of Mad and pMad brought down by Lis1 indicated that Lis1 had stronger interaction with the unphosphorylated form of Mad than with pMad, suggesting that Lis1 releases Mad following its phosphorylation (Fig. 3*H*). Taken together, these results suggest two distinct roles of Lis1 in the regulation of BMP signal transduction: stabilizing Mad protein through physical interaction and promoting Mad phosphorylation, possibly by facilitating interaction between Mad and the receptor complex.

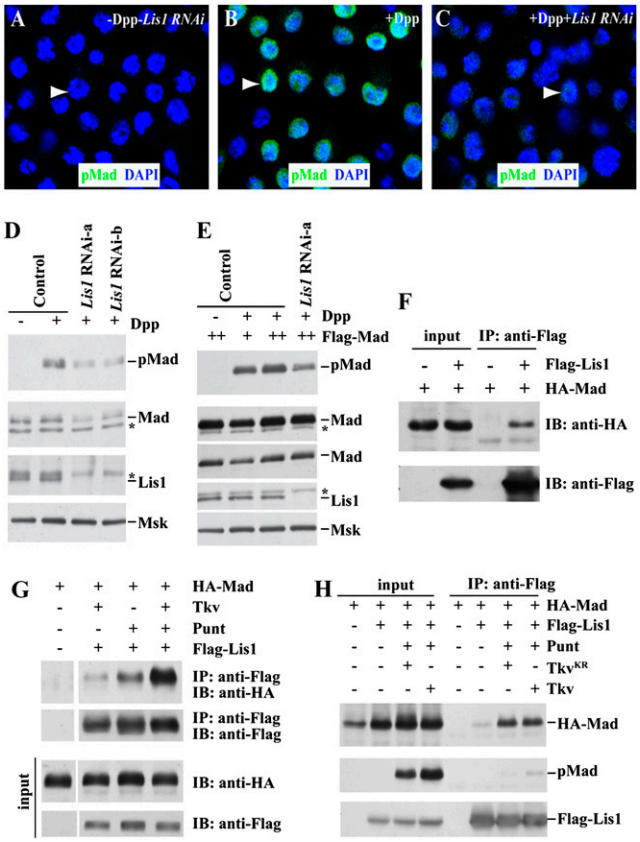

Fig. 3. Regulation of Mad stabilization and phosphorylation by Lis1. (*A–C*), Suppression of Dpp-stimulated nuclear pMad expression by *Lis1* RNAi knockdown Untreated S2 cells have low pMad expression (*A*). Dpp treatment promotes nuclear accumulation of pMad in S2 cells (*B*), which can be suppressed by RNAi-mediated *Lis1* knockdown (*C*). Arrowheads point to nuclei of representative cells. (*D*) Knockdown of *Lis1* expression by two independent dsRNAs causing the reduction of both Mad and pMad protein expression. Moleskin (Msk) a nuclear import factor for Mad, acts as a loading control. (*E*) Knockdown of *Lis1* expression still exhibits the reduction of pMad expression even in the presence of normal Mad levels. The normal Mad level is achieved through using a higher concentration of CuSO4. The two lanes showing Mad protein expression represent two different exposures. Asterisks in *D* and *E* denote nonspecific protein ban. (*F*) Flag-tagged Lis1 can specifically bring down HA-tagged Mad in S2 cells. IB, immunoblot. (*G*) The presence of Tkv or Punt can strengthen the physical interaction between Lis1 and Mad. IP, immunoprecipitation. (*H*) The physical interaction between Lis1 and Mad is enhanced by the presence of Punt and wild-type Tkv and even by kinase-dead Tkv.

Lis1 Maintains Normal E-Cadherin Accumulation in the GSC–Niche Junction. E-cadherin is important for anchoring GSCs in the niche and thus for continuous GSC self-renewal (12). To investigate if *Lis1* also is required to maintain the accumulation of E-cadherin in the stem cell–niche junction, we measured and compared E-cadherin levels in marked *Lis1* mutant GSCs and their wild-type neighboring GSCs by reconstructing 3D images based on thin confocal sections, according to our published experimental procedures (13). Interestingly, the marked $Lis1^D$ and $Lis1^G$ mutant GSCs had significantly less E-cadherin accumulation in the stem cell–niche junction than the unmarked control GSCs in the same niches (Fig. 4 *A–C*). Recently, we showed that a GSC expressing more E-cadherin gradually gains more contact area with the niche than a GSC expressing less E-cadherin and, competing for niche occupancy, gradually pushes the latter out of the niche (13). Indeed, the $Lis1^G$ and $Lis1^D$ mutant GSCs had less contact area than the unmarked control GSCs in the same niches, indicating that E-cadherin–mediated competition might be responsible in part for loss of *Lis1* mutant GSCs (Fig. 4*C′*). Our previous finding that Bam up-regulation in GSCs results in E-cadherin down-regulation raises the interesting possibility that the decrease of E-cadherin expression in *Lis1* mutant GSCs could result from the up-regulation of *bam* shown earlier (28). To investigate the possibility that *Lis1* regulates E-cadherin accumulation independently of *bam*, we examined E-cadherin expression in *Lis1*-mutant follicle cells, in which *bam* is not expressed. *Lis1* mutant follicle cells had less E-cadherin accumulation in the germ cell–follicle interface than neighboring wild-type follicle cells in the same egg chambers, suggesting that *Lis1* can regulate E-cadherin levels independently of *bam* (Fig. 4 *D* and D′). To test further whether the reduced E-cadherin accumulation in the GSC–

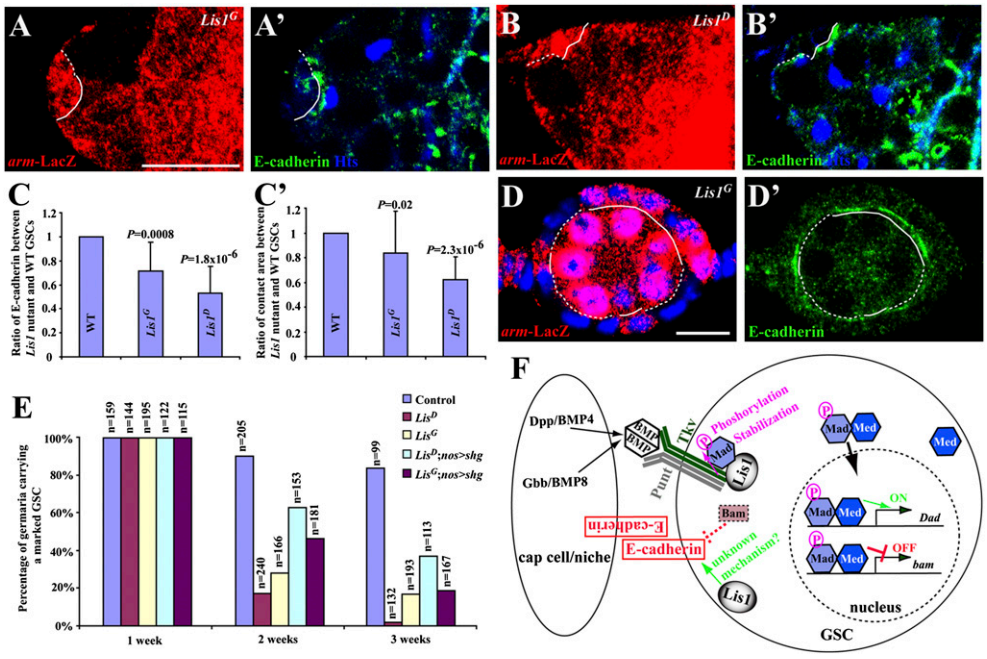

Fig. 4. Regulation of E-cadherin accumulation in the stem cell–niche interface by Lis1. (*A*–*B*′) The interface (broken lines) between a marked *Lis1*^{G} mutant GSC (*A* and *A*′) or a *Lis1*^{D} mutant GSC (*B* and *B*′) and the niche accumulates much less E-cadherin than the interface (solid line) between an unmarked wild-type GSC and the niche. (*C* and *C*′) Marked *Lis1* mutant GSCs have significantly less E-cadherin accumulation in the GSC–niche junction (*C*) and have a significantly smaller contact area with the niche (*C*′) than unmarked control GSCs. (*D* and *D*′) Marked *Lis1* mutant follicle cells (broken lines) have much less E-cadherin in their interface with germ cells than unmarked wild-type follicle cells (solid lines). (Scale bars in *A* and *D*: 10 μm.) (*E*) Germline-specific E-cadherin expression can partly slow the loss of the marked *Lis1* mutant GSCs. For all genotypes, the initial percentages of the germaria carrying a marked GSC are normalized to 100%. *nos* > *shg* = *nos-gal4 UAS-shg*. (*F*) A working model explaining how Lis1 might regulate BMP signaling in the GSC and E-cadherin accumulation in the GSC–niche junction. Gbb, glass-bottom boat; Med, Medea.

niche junction contributes to the loss of *Lis1* mutant GSCs, we used *nos-gal4*–driven *UAS-shotgun* (*shg*, which encodes *Drosophila* E-cadherin) to increase E-cadherin expression in the marked *Lis1*-mutant GSCs. Germline-specific E-cadherin expression could slow the loss of the 2-wk-old marked *Lis1* mutant GSCs dramatically, and the rescue effect also decreased for the 3-wk-old mutant GSCs, indicating that increasing E-cadherin accumulation can, at least in part, slow the loss of *Lis1* mutant GSCs (Fig. 4*E*). Together, these results argue strongly that *Lis1* maintains E-cadherin accumulation in the stem cell–niche junction, contributing to GSC maintenance.

In this study, we show that *Lis1* controls GSC self-renewal by maintaining BMP signaling in GSCs and E-cadherin accumulation in the stem cell–niche interface. To our knowledge, this is the first time that *Lis1* has been shown to regulate signaling and adhesion in any cell type, including stem cells. We also show that *Lis1* regulates BMP signaling by modulating Mad stability and phosphorylation, probably through physical interaction. Based on our findings, we propose that Lis1 can stabilize Mad protein and facilitate its phosphorylation by the BMP receptor complex, likely through direct association, and also can promote E-cadherin accumulation in the stem cell–niche junction, possibly through *bam*-dependent and independent mechanisms (Fig. 4*F*). Because BMP signaling is known to regulate self-renewal of different stem cell types, and E-cadherin is known to regulate stem cell anchorage and cell migration, our findings may provide insight into how *Lis1* controls neural stem cell self-renewal and neuronal migration in mammalian systems. In addition, this study also demonstrates the function of *Lis1* in regulating an adult stem cell type other than neural stem cells or precursors. Because *Lis1* is widely expressed in mammalian adult tissues, this study raises the possibility that *Lis1* also regulates other adult stem cell types in mammals, including humans.

Materials and Methods

Drosophila Stocks and Genetic Clonal Analysis. The information about the different *Drosophila* strains (*bam-GFP*, *Dad-lacZ*, *dpp*^{hr4}, *dpp*^{hr56}, *Lis1*^{D}, *Lis1*$^{G10.14}$, *nos-gal4*, *UASp-tkv*, *UASp-tkv**, *UASp-shg*, *FRT*$_{G13}$, *armadillo-lacZ*, and *ubiquitin-GFP*) is described in FlyBase (http://flybase.org). All of the *Drosophila* crosses and cultures were done at 25 °C. The experimental procedures for using FLP-mediated FRT recombination to generate the marked control and *Lis1* mutant GSCs were described previously (7, 12). For statistical analyses, the student's *t* test was used.

Immunohistochemistry. The following antisera were used: monoclonal mouse anti-Hts antibody 1B1, 1:4 [Developmental Studies Hybridoma Bank (DSHB)]; monoclonal rat anti-E-cadherin DCAD2, 1:6 (DSHB); polyclonal rabbit anti-β-galatosidase antibody, 1:300 (Cappel); monoclonal mouse anti-β-galatosidase antibody, 1:100 (Promega); polyclonal rabbit anti-GFP antibody, 1:200 (Molecular Probes); D-PLP, 1:1,000 (a gift from J. Raff University of Oxford, Oxford), pMad, 1:200 (a gift from P. ten Dijke, Leiden University Medical Center, Leiden, the Netherlands); pH3, 1:500 (Upstate); and Alexa 488- and Alexa 568-conjugated goat anti-mouse, anti-rabbit, and anti-rat, 1:300 (Molecular Probes). The immunostaining protocol and the TUNEL assay using the ApopTag kit from Chemicon have been described previously (7, 12, 32). All micrographs were taken using a Leica TCS SP2 confocal microscope.

Experimental details for RNAi in S2 cells, constructs, immunoprecipitation, and immunostaining of S2 cells are provided in *SI Materials and Methods*.

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