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stall-Mediated Extrinsic Control of Ovarian Follicle Formation in Drosophila

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ABSTRACT

Complex patterns of morphogenesis require intricate coordination of multiple, regulatory processes that control cellular identities, shapes, and behaviors, both locally and over vast distances in the developing organism or tissue. Studying Drosophila oogenesis as a model for tissue morphogenesis, we have discovered extraovarian regulation of follicle formation. Clonal analysis and ovary transplantation have demonstrated that long-range control of follicle individualization requires *stall* gene function in cells outside of the ovary. Although tissue nonautonomous regulation has been shown to govern follicle maturation and survival, this is the first report of an extraovarian pathway involved in normal follicle formation.

FOLLICLE formation in *Drosophila melanogaster* takes place in the germarium, which is located at the anterior tip of each ovariole (Figure 1A; for a review, see Spradling 1993). Both germline stem cells (GSC) and somatic stem cells (SSC) reside in the germarium and receive cues from the anteriorly positioned terminal filament cells for their division and maintenance. A germline cyst is initiated when a GSC divides asymmetrically to produce a single cystoblast, while regenerating the GSC. The cystoblast undergoes four rounds of mitosis with incomplete cytokinesis to form a germline cyst, composed of 16 interconnected cystocytes. One of these cystocytes becomes specified as an oocyte, while the remaining cystocytes become nurse cells. As the cyst matures and moves posteriorly through the germarium, somatic cells produced by SSC divisions move to surround each cyst to form a single-layered cuboidal epithelium. The completed follicle consists of a single germline cyst enveloped by the somatic epithelium and, as it exits the germarium, a single column of 6–10 somatic cells separates it from neighboring follicles for continued maturation.

The complex array of cellular events that contribute to ovarian follicle formation is regulated by an equally complex assortment of regulatory mechanisms. Signal transduction cascades that act within the ovary during the earliest stages of follicle formation include the *decapentaplegic* and *fs(1)Yb/piwi/hedgehog* pathways for the maintenance of GSC and SSC fates, as well as for the regulation of mitotic divisions (Xie and Spradling 1998; King and Lin 1999; Cox *et al.* 2000; King *et al.* 2001). Interfollicular communication is mediated by the *Notch/Delta* and *JAK/STAT* pathways (Torres *et al.* 2003), and intrafollicular communication requires *daughterless* (Cummings and Cronmiller 1994) for the specification of cellular identities in the established follicle. Intracellular regulators have been shown to control the establishment and maintenance of the cystoblast fate [*e.g.*, *bag of marbles* (Chen and McKearin 2003) and *benign gonial cell neoplasm* (Lavoie *et al.* 1999)] or to monitor the balance between germline and soma production (*daughterless*; Smith *et al.* 2002). Finally, extraovarian signaling via the insulin pathway regulates germline cyst production in response to the nutritional state of the fly (Drummond-Barbosa and Spradling 2001). We show here that long-range signaling regulates follicle formation as well and that *stall* (*stl*) function is an essential component of this morphogenetic control.

MATERIALS AND METHODS

Drosophila stocks: Flies were maintained on molasses-cornmeal-yeast medium at 25°. Fly stocks used in this study are listed in Table 1.

Genetic analysis of *stl*: Originally isolated as *fs(2)A16* (Bakken 1973), the stl^{a16} allele fails to complement all other known EMS-induced alleles of *stl* (Jones 1999). To aid in the interpretation of mutant phenotypes, we subjected several of the various *stl* mutant chromosomes to recombination to remove unrelated extraneous mutations in linked genes.

Staining and analysis of ovarian tissue: Fixed ovarian tissue was stained with DAPI as described previously (Cummings and Cronmiller 1994). Stained ovaries were visualized in all cases on Zeiss Axiophot/Axioscope microscopes, and images were captured in black and white by either Pixera (germline clones) or Olympus Magnafire (all other experiments) digital cameras. Images were false-colored for GFP, fluorescein, and rhodamine in Adobe Photoshop.

Larval and pupal gonad preparation: Gonads were dissected from either wandering third instar larvae or pharate adult pupae and fixed in 4% paraformaldehyde. Fixed gonads were stained with antibodies against Hts (1B1) and Vasa, as described previously (Smith and Cronmiller 2001).

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TABLE 1

Drosophila stocks used in this study

Stock genotype	Originally obtained from
Oregon-R (wild-type strain)	
cn stl^{PA49} *bw/CyO*	T. Schüpbach
cn stl^{PH57} *bw/CyO*	T. Schüpbach
stl^{WU40} *bw/CyO*	T. Schüpbach
c stl^{AWK26} bw^{D}*/CyO*	T. Schüpbach
w/w; al b pr cn stl^{a16}*/CyO*	R. Nagoshi
w; $P\{w^{+mW.hs}=FRT(w^{hs})\}G13$	Bloomington Stock Center
w^{1118}; $P\{w^{+mW.hs}=FRT(w^{hs})\}G13$ $P\{w^{+mC}=Ubi\text{-}GFP.nls\}2R1$ $P\{Ubi\text{-}GFP.nls\}2R2$	Bloomington Stock Center
$P\{ry^{+t7.2}=hsFLP\}1$, w^{1118}; Adv^{1}*/CyO*	Bloomington Stock Center
$P\{w^{+mW.hs}=FRT(w^{hs})\}G13$ $P\{w^{+mc}=ovo^{D1\text{-}18}\}32X9a$ $P\{w^{+mc}=ovo^{D1\text{-}18}\}32X9b$/ $Dp\{?;2\}bw^{D}$, S^{1}, $wg^{Sp\text{-}1}$ $Ms(2)M^{1}bw^{D}$/*In(2LR)O, Cy* dp^{hvl} *pr* cn^{2}	Bloomington Stock Center
w/w; $P\{w^{+mW.hs}=FRT(w^{hs})\}G13$ stl^{a16}*/CyO*	
w/w; $P\{w^{+mW.hs}=FRT(w^{hs})\}G13$ stl^{PA49} *bw/CyO*	

Mosaic analysis of *stl*: For germline clone analysis, progeny from an *hs-FLP w/+*; *FRT G13* stl^{a16} × *w*; *FRT* ovo^{D1}*/CyO* cross were heat-shocked at 37° for 2 hr on each of 2 consecutive days. Progeny were allowed to mature at 25° until eclosion. At 3–5 days posteclosion, ovaries were dissected, fixed in 4% paraformaldehyde, and stained with DAPI.

To generate marked somatic clones of stl^{PA49} and stl^{a16}, *w/w*; *FRT G13 stl/Cy* virgin females were crossed to *hs-FLP w*; *FRT G13 GFP* males. Progeny were heat-shocked at 37° for 2 hr on each of 2 consecutive days, during first, second, or third instar larval stages. Progeny were raised at 25° and, after eclosion, *w/w*; *FRT G13 stl/FRT G13 GFP* flies were aged 4–7 days before ovary dissection. Tissue was fixed in 4% paraformaldehyde and stained with DAPI.

Ovary transplantation: Transplantation of germarial tissue from donor females to male hosts was performed according to the protocol outlined in Lin and Spradling (1993) with the following modifications: Germaria were dissected in Shields and Sang M3 insect medium (Sigma, St. Louis) and held at room temperature for no more than 20 min before transfer to male host abdomens. Hosts were allowed to recover for 11 days before dissection and staining with DAPI.

RESULTS

The *stl* mutant phenotype: Our analysis of the *stl* mutant phenotype endorsed initial assessments of this gene's essential role during oogenesis (Bakken 1973; Schüpbach and Wieschaus 1991). We found follicle formation in adults to be severely disrupted, even in newly eclosed females. For example, the interfollicular stalks that normally separate adjacent wild-type follicles were completely absent in *stl* ovarioles (Figure 1, B and C). In addition, these mutant ovarioles lacked the somatic epithelial layers that normally envelop individual germline cysts. As a consequence of these defects, each *stl* ovariole appeared essentially as a single irregular somatic epithelium that contained multiple germline cysts. This phenotype resulted from failed follicle individualization, rather than from persistent germline cell division, because mutant ovarioles contained germ cells at varying stages of maturation and because groups of 16 interconnected germ cells that would ordinarily have identified a single germline cyst were still recognizable. Moreover, this disruption of oogenesis in *stl* mutant adults was exacerbated by degeneration of both germline and somatic cells (Figure 1C). These ovary defects were identical not only in homozygotes of all five known alleles of *stl*, but also in all heteroallelic combinations of those alleles (Figure 1D, for example). We were unable to document unambiguously the *stl* hemizygous phenotype, since all of the putative deletion chromosomes that should have uncovered the *stl* locus proved to be structurally complex. Nevertheless, because of the similar nature of the *stl* homozygous and heteroallelic follicular defects and because none of the *stl* alleles has ever been observed to have any dominant effects (data not shown), the ovarian phenotype associated with these alleles most likely represents the loss of *stl* gene function.

Since ovarian follicle formation was completely disrupted already in newly eclosed *stl* mutant females, we examined larval (third instar) and pupal gonads to determine the onset of the *stl* phenotype. On the basis of the organization of germline and somatic cells, larval gonads looked morphologically normal (Figure 2, compare A with B). Pupal gonads, however, lacked complete follicle individualization: Although adjacent germline cysts were mostly separated from each other by clusters/layers of somatic cells, there was no evidence of interfollicular stalks (Figure 2, compare C with D). Thus, wild-type *stl* function must be required at least as early as the pupal stage for normal follicle morphogenesis. To address the question of whether *stl* normally functions in the germline and/or somatic cells of the ovary, we carried out clonal analysis.

Mosaic analysis of *stl*: Clonal analysis of *stl* function revealed unexpected and complex cellular requirements for the gene, failing to detect exclusive requirements for the gene in either the germline or the soma.

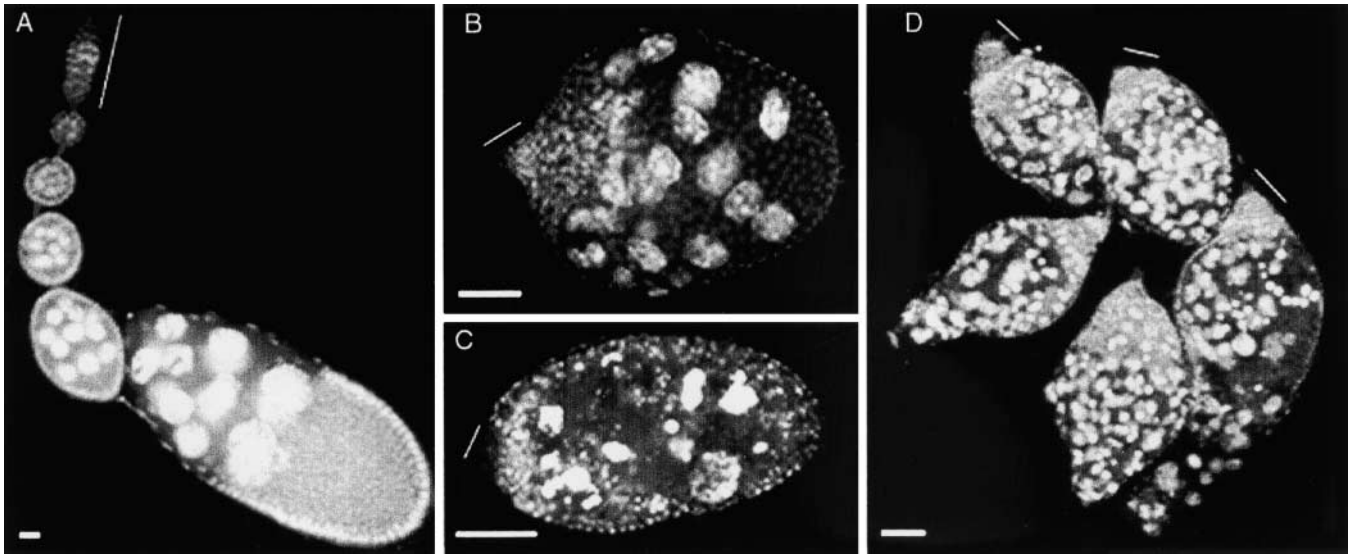

FIGURE 1.—Disruption of follicle morphology in ovaries of *stl* mutant adults. (A) Normal ovarian morphology is illustrated by a wild-type ovariole. (B) In contrast, abnormal follicle formation is already apparent in *stl* homozygous ovarioles that were dissected 1 day posteclosion. Although morphology is disrupted, there is little evidence of cell degradation in these young ovarioles. (C) The defective ovariole structure of *stl* homozygous ovaries shows increasing amounts of cell degeneration with age, as illustrated by this 22-day posteclosion ovariole. Both germline and somatic cells appear to be degrading. (D) The heteroallelic combination of stl^{a16}/stl^{PA49} displays an ovarian phenotype that is identical to that of either homozygote: There is severe mispackaging, as well as degeneration in all five of the ovarioles shown here. All ovaries were stained with the nuclear dye DAPI, to visualize tissue organization. Thin lines indicate the positions of germarium regions in the ovarioles. In this and all subsequent figures, anterior is upward or to the left. Bars, 100 μm.

We first used the dominant female sterile technique to generate stl^{a16} germline clones that were characterized by the absence of the ovo^{D1} phenotype (DFS-FLP/FRT; CHOU and PERRIMON 1996). Since ovo^{D1} homozygous follicles arrest at stage 4, germline clones were recognizable as follicles that were more mature. We induced mitotic recombination during larval stages and recovered ovo^{+} clones in 19% of ovarioles ($n = 279$; Figure 3A). Of 54 stl^{a16} clones, only 7 showed weak *stl*-like follicle formation defects (Figure 3B). Among the remaining 225 stl^{+} (ovo^{D1}) ovarioles, 41 showed similar follicle formation defects, superimposed on the characteristic ovo^{D1} phenotype (Figure 3C). The frequency of defects in ovarioles with mutant germline clones (13%) was not statistically different from that in ovarioles without (18%; Student's *t*-test, $P = 0.67$), suggesting that *stl* function is not required in the germline for normal follicle morphogenesis. To determine whether the follicle defects observed in this experiment resulted from the perturbation of *stl* function in critical somatic cells, unrelated to the *stl* genotype in the germline, we generated *stl* mutant somatic clones that were identifiable by the absence of a GFP marker (LUSCHNIG *et al.* 2000). Marked mutant clones were recovered in 14–84% of ovarioles, depending on the recombination induction protocol used; two independent *stl* alleles produced essentially indistinguishable results. Mutant clones were found in all somatic cell types, including the follicular epithelium, as well as the terminal filament (TF), cap (CAP), and inner sheath (IS) cells of the germarium. Clones restricted to the small population of specialized somatic cells in the germarium (TF, CAP, and IS) were not associated with any increase in the frequency of defective follicles (data not shown); however, we found that *stl* mutant clones in somatic epithelial cells were variably associated with follicular defects. To identify clone features that were most likely to result in follicular defects, we analyzed the *stl* somatic clones in greater detail.

Severe *stl*-like follicle formation defects were most often associated with large *stl* clones in the somatic ovary; however, not every large somatic clone exhibited such severe defects. We classified follicular defects as severe, weak, or absent, while simultaneously categorizing the corresponding ovarioles according to whether they contained large, small, or no somatic clones. Severe morphological defects were found exclusively in ovarioles that contained mutant clones. Further, gross follicular disruptions were most often correlated with large clones that encompassed a majority of the somatic epithelium (Figure 4A), although they occasionally coincided with small mutant patches (Figure 4B). Phenotypic severity, however, was not strictly correlated with somatic clone size, since rare large clones were recovered that exhibited no visible defects (Figure 4C). Similarly, while weak follicular defects were often associated with small clones, they were just as often present in ovarioles that lacked clones altogether (Figure 4D).

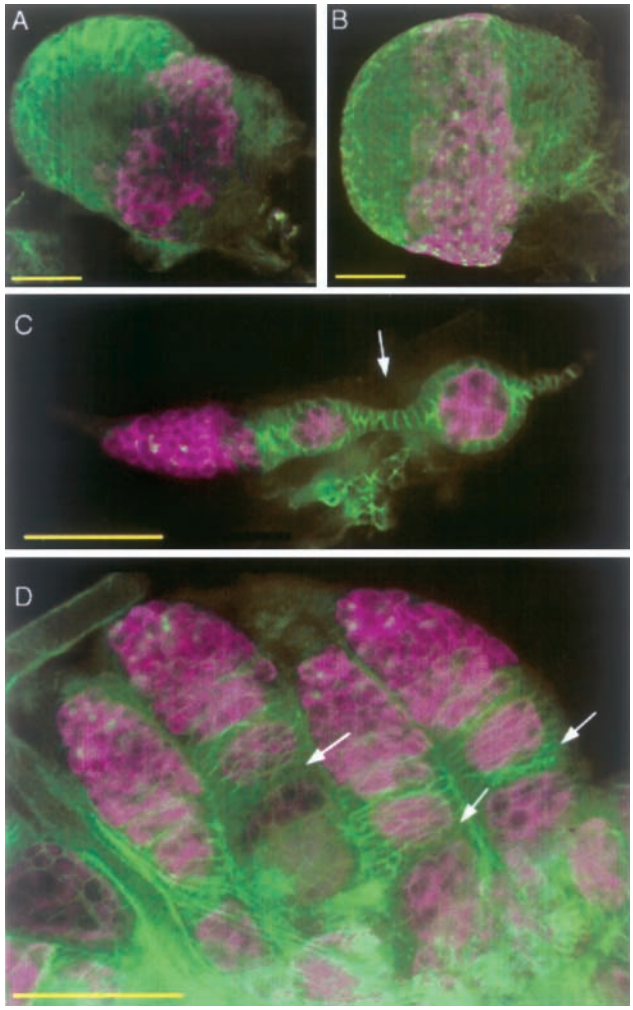

FIGURE 2.—Disruption of follicle morphology in *stl* preadult ovaries. Larval and pupal gonads were stained with anti-Hts and anti-Vasa to mark somatic cells and germline cells, respectively. (A) Morphology of a normal larval gonad. (B) Gonads from *stl* larvae appear morphologically normal. (C) An ovariole from a normal pupal gonad, showing interfollicular somatic stalks between follicles (*e.g.*, arrow). (D) In *stl* pupal gonads, interfollicular stalks do not form (arrows), resulting in a failure in the initial follicle individualization in mutant ovaries. Bars, 50 μm.

Thus, although clone size did influence phenotypic severity, it was not the only determinant; another critical factor of the *stl* phenotype appeared to originate outside the ovary. This interpretation was supported by a quantitative review of the ovarian phenotypes that were produced during our mitotic clonal analysis, even in the absence of ovarian clones. We calculated the percentage of defective ovarioles per female following induction of clones in either wild-type or *stl* genotypic backgrounds. If disruption of *stl* function in extraovarian cells increased the likelihood of follicle formation errors, we would expect to recover clusters of defective ovarioles within individual females, regardless of the presence and/or size of their ovarian clones. The highest per female percentage of defects in the controls was 7.9%; only two females exhibited percentages >5% (Figure 4E). In contrast, among experimental females the percentage of defective ovarioles per female was mostly >5% and reached as high as 30%. In addition to this clustering of ovarian defects, an increased incidence (contingency χ^2, $P < 0.1$) of weak defects in experimental (6.0%) *vs.* control (2.9%) females suggests that weak defects often represent the impact of nonovarian *stl* clones. Overall, these mitotic clone data suggest a model in which *stl* normally contributes to ovarian follicle formation not only in somatic cells of the ovary itself, but also through a previously unidentified regulatory pathway that originates in cells outside of the ovary. Moreover, the extraovarian cells that express *stl*'s function are probably no longer dividing in adults, since we did not recover significant ovarian defects (0.8% of ovarioles) when clones were initiated in adults, whereas ~11% of ovarioles had defects when mutant clones were induced during larval stages. We tested our nonautonomy model by performing ovary transplantations.

Ovarian transplantation analysis: We carried out reciprocal ovary transplantations between genetically wild-type and homozygous *stl* mutant adults and confirmed an extrinsic, *i.e.*, extraovarian, role for *stl* during ovarian follicle formation. Wild-type control transplants confirmed that oogenesis proceeded normally following transfer of germarial tissue to the abdomens of male hosts (Figure 5A; LIN and SPRADLING 1993). Transplantation of wild-type germaria into two different *stl* host genotypes, however, resulted in morphologically abnormal ovarioles that were similar to, but not as severe as, those of homozygous mutant females. In some cases, recovered ovarioles did contain portions of interfollicular somatic epithelium, which has never been observed in *stl* homozygous ovaries (Figure 5B). However, all cases showed one or more typical *stl* ovarian defects, including germline cyst mispackaging, absence of interfollicular stalks, and widespread cellular degeneration (Figure 5, B and C). Reciprocal transplantations, namely *stl* mutant ovary tissue into wild-type hosts, were problematic, since transplanted tissue was never recovered in these experiments. However, the number of transplants performed and the host survival rates were comparable in both transplant operations, suggesting that the *stl* mutant tissue may have been too fragile to survive the stringent transplantation procedure. Although our transplant experiments could not address the sufficiency of extraovarian *stl* function for normal oogenesis, they do directly demonstrate the necessity for such a function.

DISCUSSION

Our results collectively support a model in which *stl* function is essential for proper ovarian follicle formation both in somatic cells in the ovary and in extraovarian somatic cells. Indeed, although hormone regulation

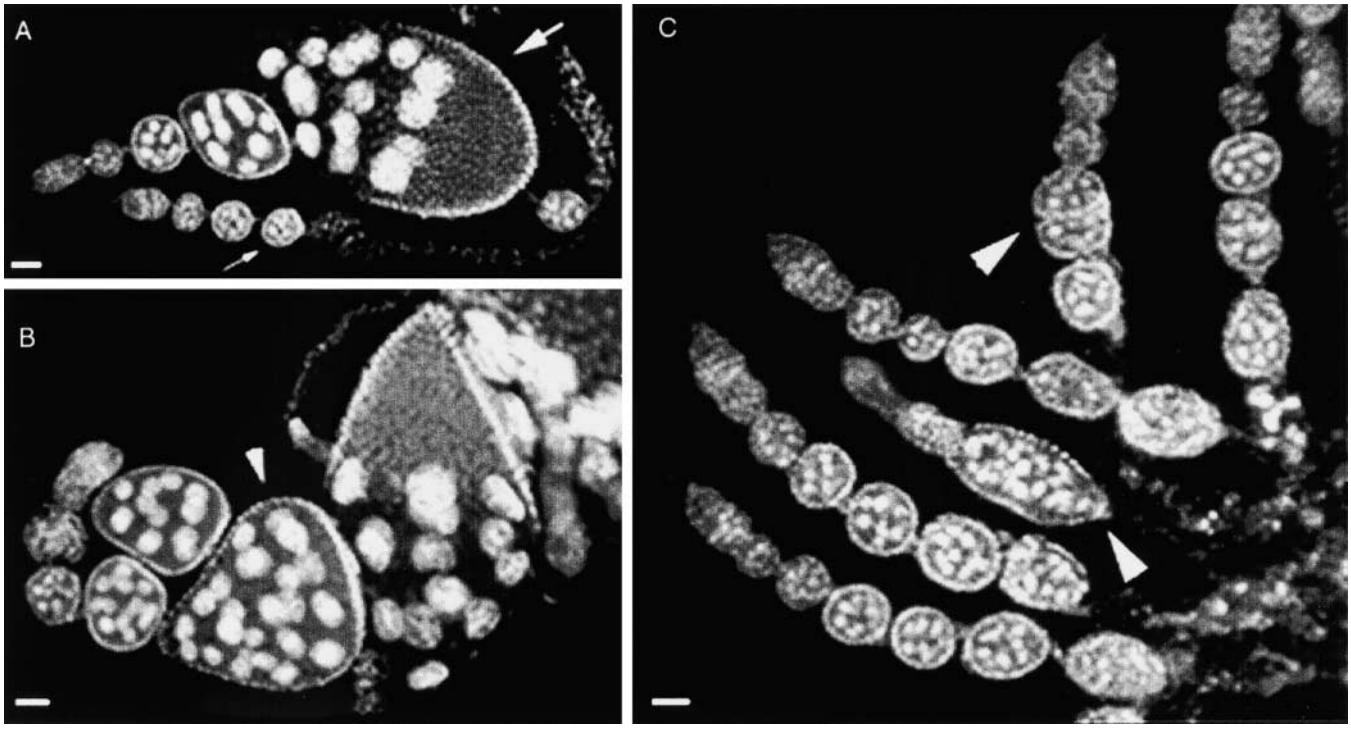

Figure 3.—Clonal analysis of *stl* in the germline. Clones were initiated in first and second instar larvae; germline mosaics were identified by the loss of the ovo^{D1} phenotype. (A) Despite the *stl* clone in the germline, an ovo^{+} stl^{a16} ovariole forms normally maturing, discrete follicles (*e.g.*, large arrow). An ovo^{D1} stl^{+} ovariole in the same ovary (small arrow) shows the absence of maturing follicles that is typical of the ovo^{D1} mutant phenotype. (B) Follicular defects were observed occasionally in ovo^{+} stl^{a16} ovarioles. In this example more than one germline cyst is enveloped by a single somatic epithelium (arrowhead). (C) Similar follicular defects (arrowheads) are shown in ovo^{D1} stl^{+} ovarioles. All ovaries were stained with DAPI to visualize tissue organization. Bars, 100 μm.

has been linked to a germline survival checkpoint in flies (Drummond-Barbosa and Spradling 2001), our discovery of *stl*'s extraovarian contribution to oogenesis provides the first evidence for a tissue nonautonomous pathway that regulates follicle individualization. While our transplantation experiments definitively demonstrate the requirement for *stl* function outside of the ovary, our interpretation that the gene is simultaneously required within the ovary is supported by several observations. First, our inability to recover *stl* tissue from wild-type transplant hosts could indicate an essential *stl* ovarian function, especially if that function were required prior to the patterning of the adult ovariole during pupal gonadogenesis, since *stl* mutants already show follicle defects in pupal ovaries. However, an alternative interpretation could be that the transplanted *stl* tissue simply did not survive our transplantation procedure. More significantly, the phenotypic defects exhibited by the wild-type ovarian tissue grown in *stl* mutant hosts were less severe than those associated with the ovaries of homozygous mutant females. Finally, in our clone generation experiment, weak defects were observed even in the absence of ovarian clones, and severe defects were most often observed when large ovarian clones were present. We propose that weak follicular defects arising from clones outside of the ovary were exacerbated by the simultaneous disruption of *stl* function within the ovarian soma. Thus, we conclude that both the somatic ovarian and the extraovarian roles for *stl* are essential for proper folliculogenesis, but neither is sufficient, indicating that these functions are nonredundant.

Our evidence for *stl*'s extraovarian participation in ovarian follicle formation raises a number of intriguing questions about the nature of such a regulatory process. Since *stl* males appear morphologically normal and yet perturbing *stl* function in males (homozygous *stl*) was enough to disrupt oogenesis in transplanted ovarioles, the *stl*-mediated signaling pathway could be functionally female specific under normal conditions, but inducible in males by transplanted ovarian tissue. Alternatively, it is possible that *stl* contributes to a universal signal that is normally present in both males and females. In this case, at least one of its primary targets would be ovary specific and thereby female specific. Of the five extant alleles of *stl*, four were recovered from an exclusively female sterile mutagenesis screen (Schüpbach and Wieschaus 1991), while the remaining allele was isolated in a nonsaturating sex nonspecific sterility screen (Bakken 1973). It is possible, therefore, that any or all of these alleles of *stl* are female-specific lesions in a gene with broader functions. Regardless of the specific-

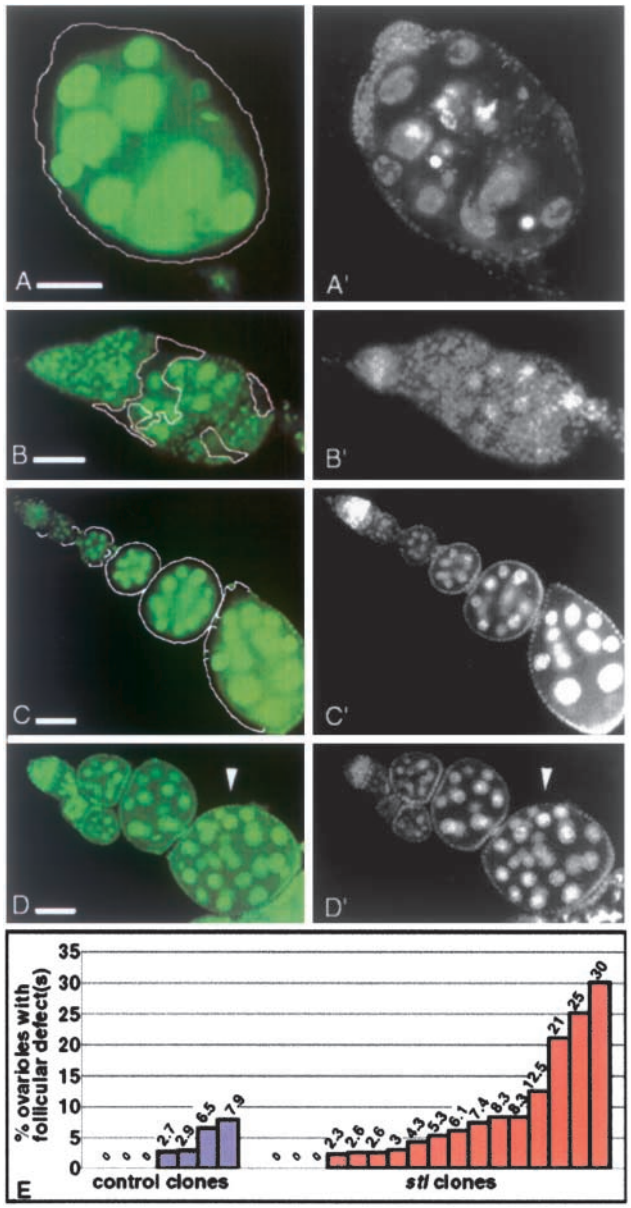

Figure 4.—Variation in phenotypic severity among marked *stl* mutant clones. *stl* mutant clones in an otherwise wild-type background were initiated in first and second instar larvae and identified by the absence of a GFP marker (A, B, C, and D); DAPI staining reveals the structure of these ovarioles (A′, B′, C′, and D′). (A and A′) A large somatic clone that encompasses an entire ovariole (GFP^- stl^{a16} is outlined) exhibits follicle formation defects that are as severe as those observed in homozygous *stl* ovaries. (B and B′) An ovariole with relatively small patches of stl^{a16} mutant soma (outlined) shows severe follicular defects. All other somatic cells, including TF, IS, and CAP, are wild type in this ovariole. (C and C′) In another ovariole, however, follicles are formed properly, in spite of a large area of stl^{a16} soma (outlined). (D and D′) Weak follicular defects (arrowhead) are present in an ovariole that contains no *stl* mutant clones. Bars, 100 μm. (E) Increased incidence of multiple follicular defects among mutant (*FRT GFP/FRT* stl^{A16} or *FRT GFP/FRT* stl^{PA49}) *vs.* wild-type (control, *FRT GFP/FRT* stl^+) females, following clone induction. Each bar represents a single female; the percentage of follicle defects is indicated above each bar. Follicular defects were scored independently of clone frequencies.

ity of the signal's origin, no particular cellular mechanism during follicle formation that requires the long-range signal has yet been discovered; however, it has been shown that *stl* is not required for expression of the polar or interfollicular stalk cell fates in the soma (Tworoger *et al.* 1999). Molecular characterization of *stl*, together with genetic interaction studies, should provide clues to the identities of ovarian targets of the long-range signaling, defined by this gene's function. One intriguing possibility is the transcriptional regulatory gene, *daughterless*, on the basis of its strong dominant mutant interaction with *stl* (Smith and Cronmiller 2001). Finally, since hormones are known to control checkpoints in oogenesis both early (Drummond-Barbosa and Spradling 2001) and late (Soller *et al.* 1999), it is possible that *stl* is directly involved in generating or sending a similar hormonal signal to control follicle formation. Such a signal could originate in the nervous system, given the numerous peptides and hormones that are synthesized in specialized cells in the brain and consistent with the paucity of ovarian defects observed following clone induction in adults.

The discovery of extraovarian control of Drosophila follicle formation prompts the question of whether similar long-range signaling is involved in mammalian folliculogenesis. There are certainly similarities between Drosophila and mammals with respect to follicle maturation and survival, given that those stages of oogenesis in both systems are regulated indirectly via the alteration of ecdysone levels (in response to juvenile hormone) (Jowett and Postlethwait 1980; Soller *et al.* 1999) and progesterone synthesis (in response to follicle stimulating hormone) (Chun *et al.* 1996; Makrigiannakis *et al.* 2000), respectively. Another striking similarity is the importance of intraovarian regulation that mediates extensive cell-cell communication between the oocyte/germline and the surrounding somatic cells in control of cell proliferation or tissue organization; such regulation involves a number of signal transduction pathways, including Notch or EGFR (in Drosophila) (Goode *et al.* 1992; Torres *et al.* 2003) and GDF-9 or bFGF (in mammals) (Fortune 2003). Little is known, however, about the control of mammalian oogenesis prior to follicle activation and growth, and it is the early events, including the migration of granulosa cells to encapsulate individual oocytes, that are most analogous to Drosophila follicle formation, during which somatic cells migrate to surround individual germline cysts. Finally, Drosophila and human follicle formation share the same practical restrictions with respect to *in vitro* culturing. Only Drosophila follicles that are fully formed at the time of explant complete maturation during ovary *in vitro* culturing; this experimental limitation has hindered our progress toward understanding how follicle formation works at the cellular level. Similarly, cultured preantral human follicles exhibit low meiotic competence, uncoordinated granulosa cell and oocyte growth, and failed

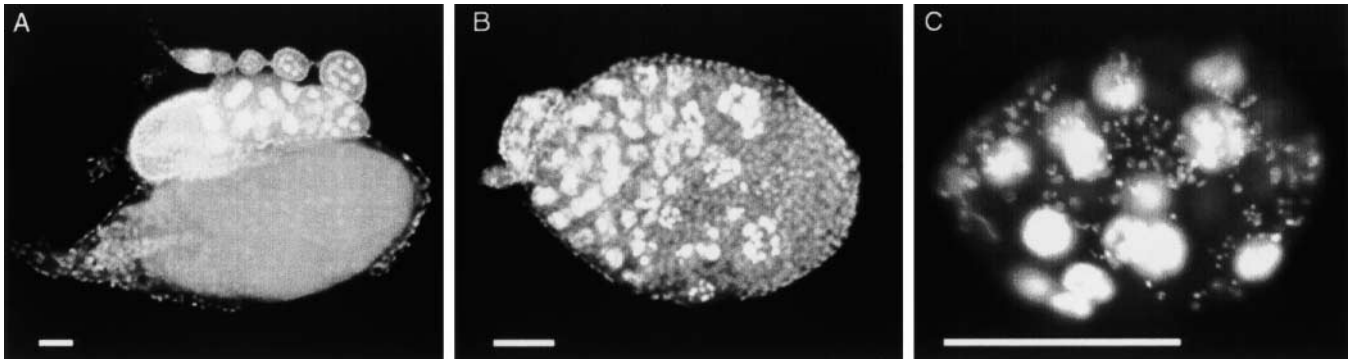

FIGURE 5.—Ovary transplantation. (A) A control ovariole that was recovered following transplantation of a wild-type donor germarium into a wild-type adult male host is morphologically normal. Because the transplanted tissue did not include its associated sheath or muscle layers, the ovariole doubled over as it grew in the host. A total of 220 control transplants were performed with a host survival rate of 10.9%. Wild-type morphogenesis was observed in all eight ovarioles recovered. (B) An ovariole that was recovered following transplantation of a wild-type donor germarium into a stl^{a16}/stl^{a16} adult male host exhibits severe morphological defects. (C) This grossly abnormal follicle that was recovered following transfer of a wild-type germarium into a stl^{PA49}/stl^{PA49} host exhibits substantial somatic degeneration. A total of 362 wild-type to mutant (stl^{a16} or stl^{PA49}) transplants were performed with a host survival rate of 10.5%. Aberrant morphogenesis was observed in all 5 donor ovarioles recovered. All ovarioles were stained with DAPI to visualize tissue morphology. Bars, 100 μm.

maturation (SMITZ and CORTVRINDT 2002); the inability to culture these early stage follicles has eliminated them as a source of oocytes for alternative reproductive strategies. It is an exciting possibility that preindividualized Drosophila follicle stages and preantral human follicles represent comparable premeiotic stages of oogenesis. If they involve comparable extrinsic physiologic controls, the *stall*-mediated signaling function could identify a more universal regulatory mechanism; it could be the missing ingredient that is needed for successful *in vitro* culturing of both tissues. The molecular identification of *stall* will allow this possibility to be explored further.

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