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OVO transcription factors function antagonistically in the *Drosophila* female germline

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SUMMARY

OVO controls germline and epidermis differentiation in flies and mice. In the *Drosophila* germline, alternative OVO-B and OVO-A isoforms have a common DNA-binding domain, but different N-termini. We show that these isoforms are transcription factors with opposite regulatory activities. Using yeast one-hybrid assays, we identified a strong activation domain within a common region and a counteracting repression domain within the OVO-A-specific region. In flies, OVO-B positively regulated the *ovarian tumor* promoter, while OVO-A was a negative regulator of the *ovarian tumor* and *ovo* promoters. OVO-B isoforms supplied *ovo*$^+$ function in the female germline and epidermis, while OVO-A isoforms had dominant-negative activity in both tissues. Moreover, elevated expression of OVO-A resulted in maternal-effect lethality while the absence of OVO-A resulted in maternal-effect sterility. Our data indicate that tight regulation of antagonistic OVO-B and OVO-A isoforms is critical for germline formation and differentiation.

Key words: Sex determination, Transcription, Epidermis, Denticle, Maternal-effect sterility, Maternal-effect lethality, Germline, *Drosophila melanogaster*

INTRODUCTION

Transcription regulation is a linchpin in the gene networks controlling development. In flies, nematodes, mice and humans, *ovo*$^+$ genes encode putative C_2H_2 zinc-finger transcription factors (Mével-Ninio et al., 1991; Chidambaram et al., 1997; Dai et al., 1998; Lü et al., 1998; Masu et al., 1998). The *Drosophila* OVO zinc-finger domain has sequence-specific DNA-binding activity (Lü et al., 1998), strongly suggesting that all OVO proteins bind DNA. Furthermore, the sex-specific reproductive defects and poor hair formation seen in flies and mice mutant for *ovo* (Oliver et al., 1987; Dai et al., 1998; Payre et al., 1999), reveal a conservation of OVO function over an enormous evolutionary distance. This suggests a conservation of biochemical activity and genetic function, and makes the study of OVO broadly relevant.

Drosophila ovo$^+$ is required in at least two tissues. In the embryonic ventral epidermis *ovo* (formerly known as *shavenbaby*; Oliver et al., 1987; Payre et al., 1999) promotes the elaboration of denticles, protrusions from single cells resembling hairs, in the anterior half of thoracic and abdominal segments. Expression of *OVO* mRNA is negatively regulated by WINGLESS and positively regulated by EGF signaling pathways in this tissue (Payre et al., 1999). Flies lacking *ovo*$^+$ in the soma show global defects in denticles and other 'hairs' throughout development. In females, *ovo*$^+$ is required for the viability and differentiation of XX germ cells (Oliver et al., 1987, 1990; Oliver and Pauli, 1998). Since OVO contains a zinc-finger domain similar to many known transcription factors, it is likely to regulate target promoters. Indeed, in the germline, *ovo*$^+$, *stand still*$^+$ (*stil*$^+$) and somatic sex determination signals activate *ovarian tumor* (*otu*$^+$) (Pauli et al., 1993; Lü et al., 1998; Hinson and Nagoshi, 1999; Sahut-Barnola and Pauli, 1999). The recent demonstration that OVO directly binds multiple sites near the *otu*$^+$ promoter raises the possibility that this regulation is direct (Fig. 1B; Lü et al., 1998).

The *ovo*$^+$ locus encodes a family of proteins sharing an extensive C-terminal region that contains a zinc-finger DNA-binding domain, but have substantially different N termini (Mével-Ninio et al., 1995). This organization could be important, as transcription factors are modular proteins with separate DNA-binding and effector domains (reviewed by Ptashne and Gann, 1997). Germane to this study are two major classes of germline *OVO* transcripts driven by the *ovo-A* and *ovo-B* promoters (Fig. 1A,B; Garfinkel et al., 1994; Mével-Ninio et al., 1995). Although, *OVO-B* and *OVO-A* transcripts differ only by their short first exons (77 and 126 n.t.) OVO-B has 374 fewer residues than OVO-A. *OVO-B* mRNAs encode only OVO-B isoforms from an AUG initiation codon in exon 2, whereas OVO-*A* transcripts encode longer OVO-A isoforms

from an AUG initiation codon in exon 1A, and potentially encode OVO-B from the downstream AUG.

Four dominant-negative *ovo* alleles (*ovo*^{D}) strongly suggests that inclusion of the OVO-A N-terminal region is functionally significant (Mével-Ninio et al., 1996; Andrews et al., 1998). All four mutations introduce novel in-frame AUG codons upstream of the OVO-B initiation codon. Consequently, *OVO-B* transcripts from *ovo*^{D} alleles encode slightly truncated OVO-A isoforms (we refer to these OVO-A-like isoforms as OVO-A throughout the manuscript). OVO-A functions to down regulate target promoters, as females heterozygous for *ovo*^{D} show strongly down-regulated *ovo* and *otu* promoter activity (Oliver et al., 1994; Mével-Ninio et al., 1996; Andrews et al., 1998; Lü et al., 1998). In this study, the results of multiple assays strongly indicate that OVO-B and OVO-A are transcriptional regulators with opposite activities.

If the differences between wild-type OVO-B and OVO-A biochemical activity are relevant to the fly, then OVO-B and OVO-A would be expected to show non-coordinate expression in the germline. The overlapping nature of the *OVO-B* and *OVO-A* transcripts makes distinguishing between mRNA forms difficult. However, it seems clear that *OVO-A* transcripts are relatively rare, as they are only occasionally seen by primer extension (Garfinkel et al., 1994; Mével-Ninio et al., 1995), nuclease protection, or RT-PCR (unpublished data). *OVO-B* isoforms are easily detected by these methods. The best spatial information comes from the expression of β-gal tagged OVO proteins. Those data showed that overall OVO expression is strong during all of oogenesis, while OVO-A is expressed very weakly and only in nearly mature follicles (Fig. 1C; Mével-Ninio et al., 1996). These data suggest that expression of OVO-B is early while expression of OVO-A is late. Consistent with this differential pattern of expression, we show that OVO-B function is required during oogenesis, while OVO-A is important for subsequent embryonic germline development. Thus, OVO-B and OVO-A are indeed required for different developmental events.

MATERIALS AND METHODS

Cloning

We used standard molecular biology techniques throughout (Sambrook et al., 1989). All *ovo* DNA (Mével-Ninio et al., 1991; accession number X59772) and amino acid sequence positions are by convention (a.a. 1 is the first and 1400 the last residue of the longest predicted OVO-A isoform). We verified constructs by restriction mapping and/or sequencing using fluorescent dye terminators (ABI-PRISM Big Dye Terminator Cycle Sequencing Kit and an ABI-377 sequencer, Perkin-Elmer).

Alleles and transgenes

Because of the largely overlapping nature of the *OVO-A* and *OVO-B* transcription units, it is quite difficult to make mutations that would unambiguously affect only one transcription unit. To circumvent this concern, we made transgenes that disrupt the function of the OVO isoforms in different ways (Table 1). These constructs were then introduced into flies by P-element-mediated transformation.

The *ovo*$^{\Delta a2}$ transgene has a large deletion that removes the region encoding most of the N-terminal portion of the OVO-A, takes the ORF out-of-frame, and introduces stop codons in all three frames. This deletion also removes much of the 5′-UTR of *OVO-B*, but leaves the coding region intact. The *ovo*^{amk} and *ovo*^{amv} transgenes have point mutations converting the first AUG initiation codon of the OVO-A ORF into either lysine or valine codons that are highly unlikely to function as translation initiation codons. The next initiation AUG codon in front of a long ORF in *OVO-A* mRNAs is the first AUG initiation codon in the OVO-B ORF. Thus, the *ovo*$^{\Delta a2}$, *ovo*^{amk} and *ovo*^{amv} transgenes eliminate the OVO-A-specific ORF, should have no effect on *OVO-B* mRNA expression, and should result in expression

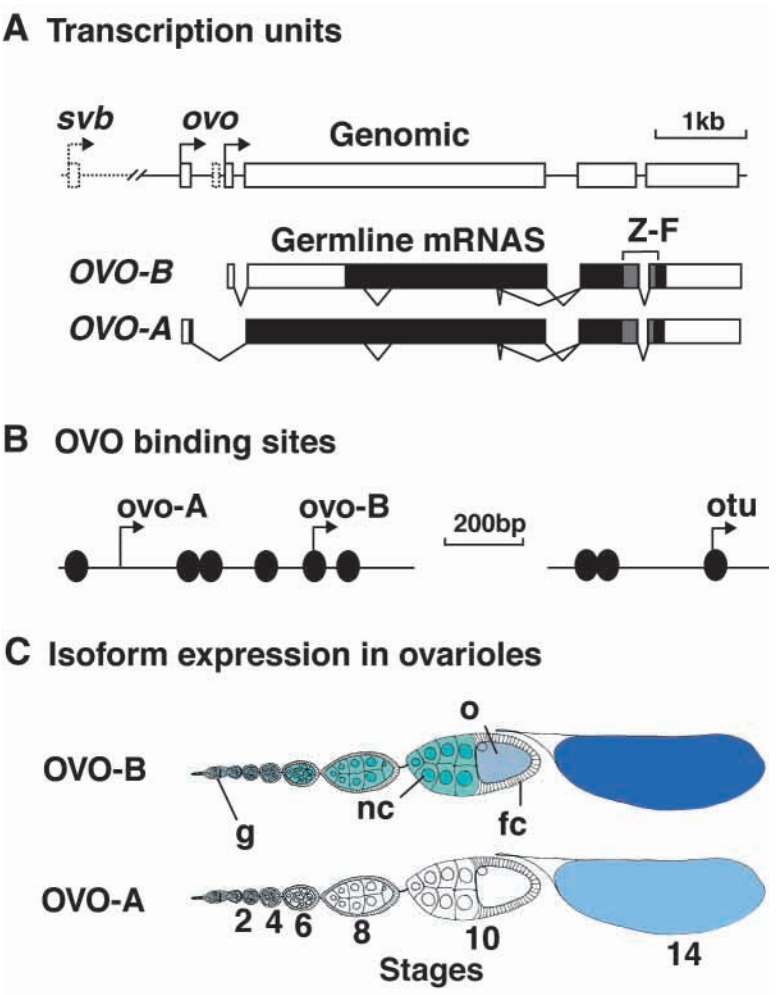

Fig. 1. Structure and function of *ovo*. (A) Organization of the *ovo* locus (top) and germline transcripts (bottom). *ovo* promoters (solid bent arrows), exons (bars), introns (straight lines, top; bent lines, bottom), open reading frames (filled bars) zinc-finger DNA-binding domain (Z-F, grey) are shown. The *svb* promoter, 5′-exon(s), and an *OVO-B* class exon (accession no. AA820712.1, The FlyBase Consortium, 1999) yet to be characterized are dashed. The two first alternative splice sites in the first common exon (exon 2) are newly reported (accession no. AA390588, The FlyBase Consortium, 1999 and unpublished data). (B) The structure of the *ovo* and *otu* promoter regions showing OVO binding sites identified by footprinting (filled circles). (C) Expression pattern of OVO-B and OVO-A isoforms based on translational reporter gene expression. Darker shading denotes stronger expression. The germarium (g), nurse cells (nc), oocyte (o), follicle cells (fc), and follicle stage numbers are indicated.

of OVO-B when and where OVO-A is normally expressed. The $ovo^{\Delta ap}$ transgene has a deletion of the DNA encoding the first exon and the first AUG initiation codon of *OVO-A*. This deletion leaves the *OVO-B* mRNA fully intact, but removes sequences upstream of the *ovo-B* promoter. Thus, $ovo^{\Delta ap}$ knocks out the expression of OVO-A and should supply OVO-B in the correct spatial and temporal pattern.

Several preexisting *ovo* alleles (classical or transgenic) encode OVO-A at the expense of OVO-B. The ovo^{D} alleles produce OVO-A isoforms from *OVO-B* mRNAs, and thus, are expected to result in precocious expression of OVO-A in the germline.

We also used the modular GAL4/UAS system (Brand and Perrimon, 1993; Rørth, 1998) to drive transcription of *OVO-B* or *OVO-A* transcripts in the germline or epidermis. The GAL4 transcriptional activator was expressed in a tissue to activate *OVO* transcription from GAL4 responsive UAS promoters in separate transgenes. We have also engineered several additional constructs to mimic the predicted negative transcriptional activities of OVO-A by appending the active repression domain from *Drosophila* ENGRAILED (EN; cf. John et al., 1995) to OVO isoforms.

Yeast one-hybrid

Yeast were grown at 30±0.5°C. We introduced plasmids into yeast strain PJ69-4A (*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*; James et al., 1996) and cultured transformed yeast on synthetic medium lacking either uracil for plasmid selection, or uracil, histidine and adenine for growth assays. Constructs are detailed in Table 2. We measured β-galactosidase activity in liquid culture lysates using the o-nitrophenyl β-D-galactopyranoside substrate (Clontech Laboratories, Inc., 1999).

Genetics

We used standard *Drosophila* techniques throughout (Ashburner, 1989). See Table 1 and The FlyBase Consortium (1999) for allele descriptions. We cultured flies at 25±0.5°C. We examined ovaries live under phase contrast and Nomarski optics, or stained with DAPI under epifluorescence. We examined larval cuticles with phase-contrast or dark-field optics following Hoyer's/lactic acid treatment. We tested fertility by mating individuals to one or two $y\ w^{67c1}$ flies, or in the case of fully penetrant phenotypes, by mating en masse (approx. 30 individuals). We dissected a minimum of 100 individuals for germline phenotype experiments. We stained for β-galactosidase according to Pauli et al. (1993), except that we pre-incubated tissues for 24 hours at approx. 4°C. Experimental and control tissues were co-processed using aliquots of identical reagents.

RESULTS

OVO-B and OVO-A function antagonistically in yeast one-hybrid assays

We have shown that ovo^{+} (encoding OVO-B and OVO-A) and ovo^{D} (encoding OVO-A) have opposite transcriptional effects on the *otu* promoter (Lü et al., 1998). However, as with any

Table 1. Alleles and transgenes used

Allele (no. of transgenic lines)	Notes*
ovo^{D1rv22}	Germline amorph. Insertion of *gypsy* transposon at ~ +900 bp in ovo^{D1}‡
ovo^{D1rv23}	Germline amorph. Insertion of *HMS Beagle* ~4.0-4.6 kb in ovo^{D1}‡
ovo^{svb-1}	Germline and *shavenbaby* amorph‡
$P\{ovo^{D1r+}\}$ (4)	Wild-type allele. Genomic *ovo* DNA 0 to ~10.5 kb, in pCaSpeR2§
$P\{ovo^{UAS.B}\}$ (4)	Encodes only OVO-B. Genomic *ovo* DNA 980 bp to ~10.5 kb from $povo^{D1r+}$, in UASp (Rørth, 1998)
$P\{ovo^{\Delta a2}\}$ (2)	Encodes only OVO-B. $povo^{D1r+}$ deleted for 1246-1857 bp. Sequence at insertion = 5′-GGCGCGCCTAGCTA-3′
$P\{ovo^{amk}\}$ (1)	Encodes only OVO-B. $povo^{D1r+}$ with T to A substitution at 464 bp
$P\{ovo^{amv}\}$ (13)	Encodes only OVO-B. $povo^{S-7.2}$ (Mével-Ninio et al., 1991), with A to G substitution at 463 bp, and G to C substitution at 465 bp, in pW6
$P\{ovo^{\Delta ap}\}$ (2)	Encodes only OVO-B. $povo^{D1r+}$ deleted for 345-493 bp, in pCaSpeR2
$P\{ovo^{UAS.B2}\}$ (6)	Encodes only OVO-B. *ovo* cDNA = 1140-3866/4755-5417/5513-~6500 bp, in pUAST
ovo^{D1}	Encodes OVO-A and OVO-A-like. A to T substitution at 1282 bp‡
$P\{ovo^{D1-7}\}$ (5)	Encodes OVO-A and OVO-A-like. ovo^{D1} DNA 0 to ~7.2 kb in pW6¶
ovo^{D2}	Encodes OVO-A and OVO-A-like. G to A substitution at 1572 bp‡
ovo^{D3}	Encodes OVO-A and OVO-A-like. A to T substitution at 1336 bp‡
$P\{ovo^{D4}\}$ (2)	Encodes OVO-A and OVO-A-like. *ovo* DNA 0 to ~10.5 kb with insertion at 1142 bp that creates new AUG translation initiation codon, in pCaSpeR2§
$P\{ovo^{UAS.A}\}$ (3)	Encodes OVO-A. $povo^{UAS.B}$ with insertion of 5′-AAAATGGGACCGGTCGGGGATCCC-3′ at 1082 bp (Initiation codon underlined)
$P\{ovo^{UAS.D1}\}$ (5)	Encodes OVO-A-like. $povo^{UAS.B2}$, with A to T substitution at 1282 bp
$P\{ovo^{EN}\}$ (3)	Encodes OVO-A::EN and OVO-B::EN. Genomic *ovo* DNA 0 to ~7.0 kb, with 916 bp fragment encoding ENGRAILED (EN) repression domain inserted at 5408, in pCaSpeR4
$P\{ovo^{D1.EN}\}$ (2)	Encodes OVO-A::EN and OVO-A-like::EN. Genomic ovo^{D1} DNA 0 to ~7.0 kb, with 916 bp fragment encoding EN repression domain inserted at 5408 bp, in pCaSpeR4
$P\{ovo^{UAS.B2.EN}\}$ (4)	Encodes OVO-B::EN. $povo^{UAS.B2}$ with 916 bp fragment encoding EN repression domain inserted at genomic 5408 bp
$P\{ovo^{UAS.D1.EN}\}$ (4)	Encodes OVO-A-like::EN. $povo^{UAS.D1}$ with 916 bp fragment encoding EN repression domain inserted at 5408 bp, in pUAST
$P\{GAL4^{nos}\}$ (1)	700 bp *nanos* promoter fused to GAL4::VP16 coding region**
$GAL4^{e22C2}$	Enhancer trap expressing GAL4 ubiquitously‡
$GAL4^{69B}$	Enhancer trap expressing GAL4 in embryonic ectoderm‡
$P\{otu::lacZ^{wt}\}$ (2)	Genomic *otu* DNA −423-+118 bp (convention of Comer et al., 1992) in pCaSpeR-AUG-βgal
$P\{ovo::lacZ^{1.1}\}$ (1)	Genomic *ovo* DNA 0 to 1082 bp. Linker at insertion site = 5′-AAAATGGGACCGGTCGG-3′ (initiation codon underlined), in pCaSpeR-βgal
$P\{ovo::lacZ^{\Delta ap}\}$ (9)	$povo::lacZ^{1.1}$ with deletion of 345-493 bp
$P\{ovo::lacZ^{\Delta bp}\}$ (3)	$povo::lacZ^{1.1}$ with deletion of 831-978 bp

*This study unless indicated otherwise.
‡The FlyBase consortium, 1999.
§Andrews et al., 1998.
¶Mével-Ninio et al., 1994.
**Van Doren et al., 1998.

Table 2. Yeast one-hybrid plasmids

Plasmid	Notes
pGB	Empty *pGBDU-C* yeast vector encodes *S. cerevisiae* GAL4 DNA-binding domain*
pGB-OVO-B	Composite *ovo* cDNA in pGBDU-C. Encodes OVO 378-495/549-940/958-1400, and GAL4 DNA-binding domain
pGB-OVO-A	Composite *ovo* cDNA in pGBDU-C. Encodes OVO 13-940/958-1400 and GAL4 DNA-binding domain. Linker (5′-CCGGTCGGGGATCCC-3′) at 5′ of cDNA encodes 5 unrelated residues
pGB-R2	Truncated pGB-OVO-B, encodes OVO 378-495/549-940/958-1156
pGB-R4	Truncated pGB-OVO-B, encodes OVO 378-495/549-875
pGB-R3	Truncated pGB-OVO-B, encodes OVO 875-940/958-1400
pGB-ZF	Truncated pGB-OVO-B, encodes OVO 1222-1400
pGB-R7	Truncated pGB-OVO-B, encodes OVO 378-495/549-632
pGB-R8	Truncated pGB-OVO-B, encodes OVO 574-875
pGB-AN1	Truncated pGB-OVO-A, encodes OVO 13-377
pGB-AN2	Truncated pGB-OVO-A, encodes OVO 251-377
pGB-R1L	Truncated pGB-OVO-B, encodes OVO 251-495/549-940/958-1400
pGB-R1	Truncated pGB-OVO-B, encodes OVO 251-495/549-940/958-1156
pGB-GA	Encodes *S. cerevisiae* GAL4 activation domain, 767-881
pGB-AN1-GA	pGB-AN1 insert fused in frame to the GAL4 activation domain from pGB-GA
pGB-AN2-GA	pGB-AN2 insert fused in frame to fthe GAL4 activation domain from pGB-GA

*James et al., 1996.

genetic analysis, this does not necessarily mean that OVO-B and OVO-A proteins have opposite biochemical activities. Given that transcriptional effector domains often function across species (Guarente and Bermingham-McDonogh, 1992), we probed the biochemical activity of OVO-B and OVO-A (Fig. 2A,B) in yeast one-hybrid assays. We made a series of plasmids encoding OVO polypeptides fused to the yeast GAL4 DNA-binding domain (GAL4DB) and transformed these into a yeast strain containing three reporter genes driven by GAL4 upstream activation sequences. To identify and map OVO transcriptional effector domains, we monitored the effect of fusion proteins on reporter gene expression. Growth was assayed on selective media and β-galactosidase activity in lysates.

The control plasmid encoding only GAL4DB did not support growth or appreciable β-galactosidase activity (Fig. 2C). In contrast, a plasmid encoding GAL4DB::OVO-B fusion protein resulted in strong growth and a 51-fold increase in β-galactosidase activity (Fig. 2D). Strikingly, a plasmid encoding GAL4DB::OVO-A fusion protein showed no significant effect in either assay (Fig. 2E). These data indicate that OVO-B has a transcriptional activation domain and are consistent with the presence of an OVO-A-specific domain that blocks activation.

To map the OVO-B activation and the OVO-A repression regions, we created a series of plasmids encoding truncated GAL4DB::OVO fusion proteins (Fig. 2F-O). Plasmids encoding C-terminal OVO fusion proteins, including the zinc-finger domain, were not active in either assay (Fig. 2H,I). In contrast, yeast bearing plasmids encoding fusions with the N-terminal region of OVO-B grew well and showed up to 232-fold induction of β-galactosidase activity (Fig. 2F,G,J,K). These data indicate that there are one or more activation domains in the N-terminal region of OVO-B. Yeast bearing plasmids encoding fusion proteins containing the OVO-A-specific region grew poorly and showed only a 3- to 4-fold activation of β-galactosidase, even though these proteins also include the OVO-B activating region (Fig. 2N,O). Lack of activation function could be due to any of several causes, but these OVO-A constructs do have ‘bait’ activity in two-hybrid experiments (unpublished data), indicating that the fusion proteins are produced and make their way to the nucleus. Therefore, these data suggest that the OVO-A-specific region

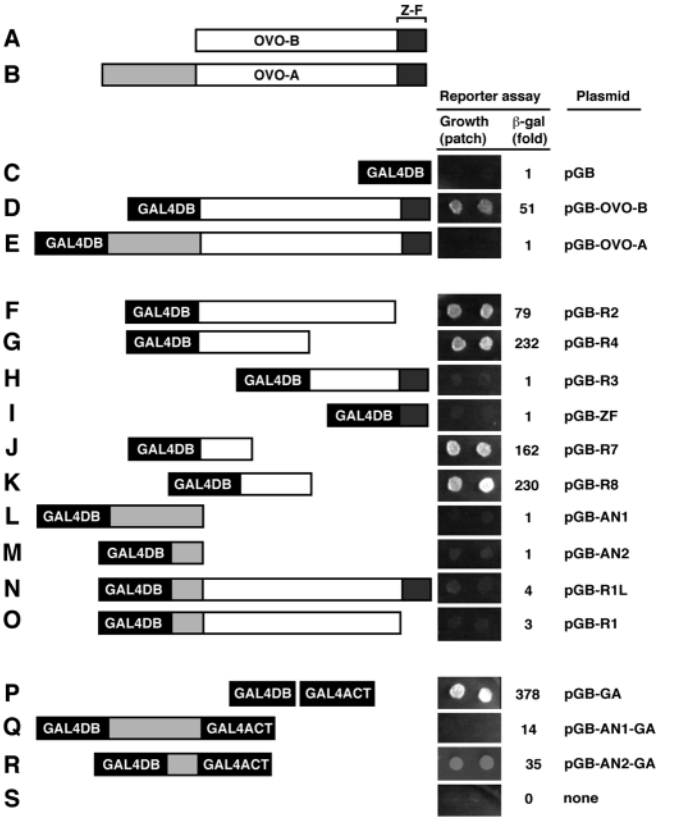

Fig. 2. OVO-B and OVO-A in yeast one-hybrid assays. (A,B) Diagram of OVO-B and OVO-A isoforms showing the zinc-finger (Z-F, dark grey) and OVO-A-specific domains (light grey). (C-R) Yeast one-hybrid assays of protein fusions containing OVO polypeptides (open), and the GAL4 DNA-binding domain (GAL4DB; filled) and activation domain (GAL4ACT; filled). Reporter assays are lawn growth on selective media and β-galactosidase enzyme activity given as fold induction relative to pGB (C).

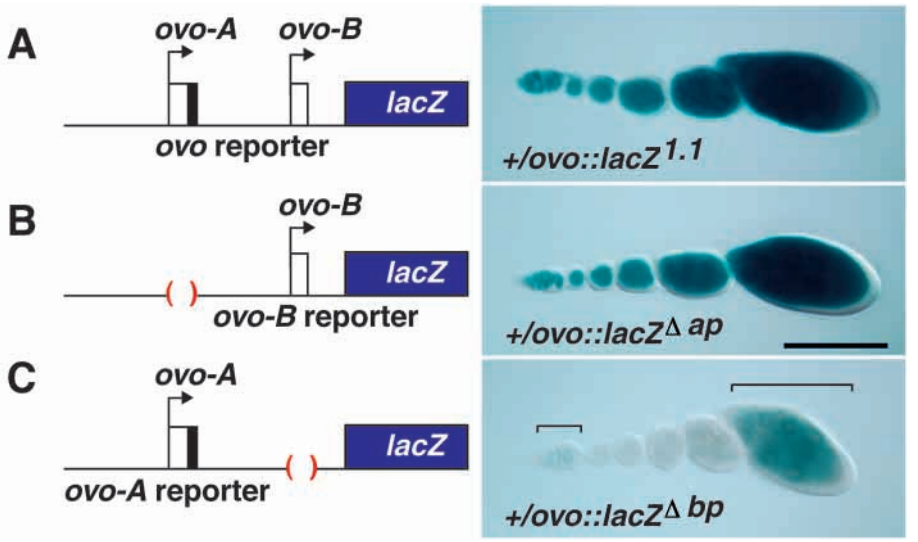

Fig. 3. Expression of *ovo* reporter genes in the female germline. (A-C) Cartoons of the reporter genes show promoters (bent arrows), *OVO* exons (black or white bars), deletions (red brackets) and the lacZ encoding fragment (blue bars). Histochemically stained ovarioles were viewed under DIC optics. (A) Base reporter for both *ovo* promoters. (B) The *ovo-B*-specific reporter. (C) The *ovo-A*-specific reporter. Location of staining is indicated by brackets. Genotypes are: (A) *y* w^{67c1}; +/*P{ovo::lacZ*$^{1.1}$*}*, (B) *y* w^{67c1}; +/*P{ovo::lacZ*$^{\Delta ap}$*}*, (C) *y* w^{67c1}; +/*P{ovo::lacZ*$^{\Delta bp}$*}*. Bar, 500 μm.

contains a *cis*-acting repression domain that counteracts the activation domain found in both OVO-B and OVO-A proteins.

If the OVO-A-specific region contains an active repression domain, one would predict that it could be transferred to a heterologous transcription factor. Indeed, the OVO-A-specific region blocked the activation domain from the GAL4 protein (GAL4ACT). Plasmids encoding GAL4DB::GAL4ACT proteins resulted in very strong growth and a 378-fold increase in β-galactosidase activity (Fig. 2P; cf. Ptashne and Gann, 1997). However, the inclusion of OVO-A-specific sequences resulted in poor growth and only a 14- to 35-fold increase in β-galactosidase activity (Fig. 2Q,R). Thus, the OVO-A-specific domain blocked GAL4ACT function. These data indicate that a repression domain within the OVO-A-specific region is able to counteract at least two different positive effector domains.

The *ovo-B* promoter is strong and acts early, whereas the *ovo-A* promoter is weak and acts late

At the protein level, OVO-B is expressed during early oogenesis, while OVO-A is expressed late, but the activity of the *ovo-A* and *ovo-B* promoters is unknown. Because we will use these promoters to drive expression of specific isoforms in later experiments, understanding the timing and level of

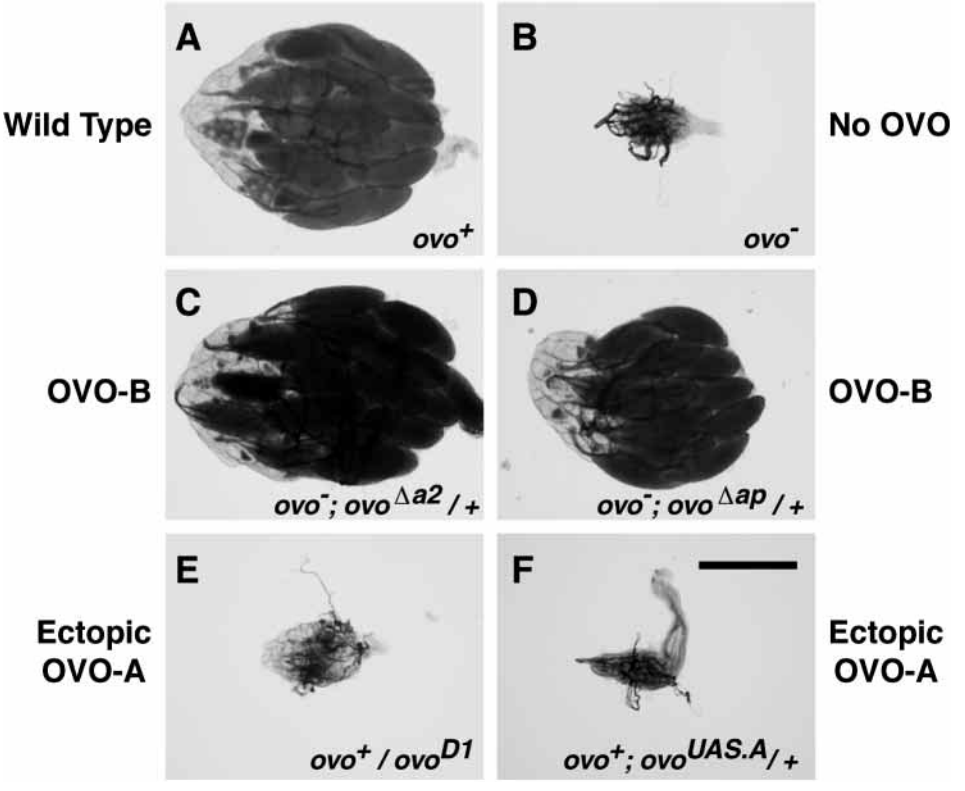

Fig. 4. Isoform function in the germline. Whole-mount ovaries viewed by phase contrast microscopy. Anterior is to the left. (A) Wild type. (B) Homozygous for a strong *ovo* allele. (C,D) Homozygous for the same strong *ovo* allele, but bearing OVO-B encoding transgenes. (E) Heterozygous for an allele encoding OVO-A from an *OVO-B* mRNA. (F) Homozygous for *ovo*$^{+}$ but expressing an *OVO-A* mRNA from an UAS promoter active in the germline. Genotypes are: (A) *y* w^{67c1}, (B) *y* w^{67c1} ovo^{D1rv23}. (C) *y* w^{67c1} ovo^{D1rv23}; *P{ovo*$^{\Delta a2}$*}*/+, (D) *y* w^{67c1} ovo^{D1rv23}; *P{ovo*$^{\Delta ap}$*}*/+, (E) *y* w^{67c1}/ovo^{D1} v^{24}, (F) *w; P{ovo*$^{UAS.A}$*}*/*P{GAL4*^{nos}*}*. Bar, 500 μm.

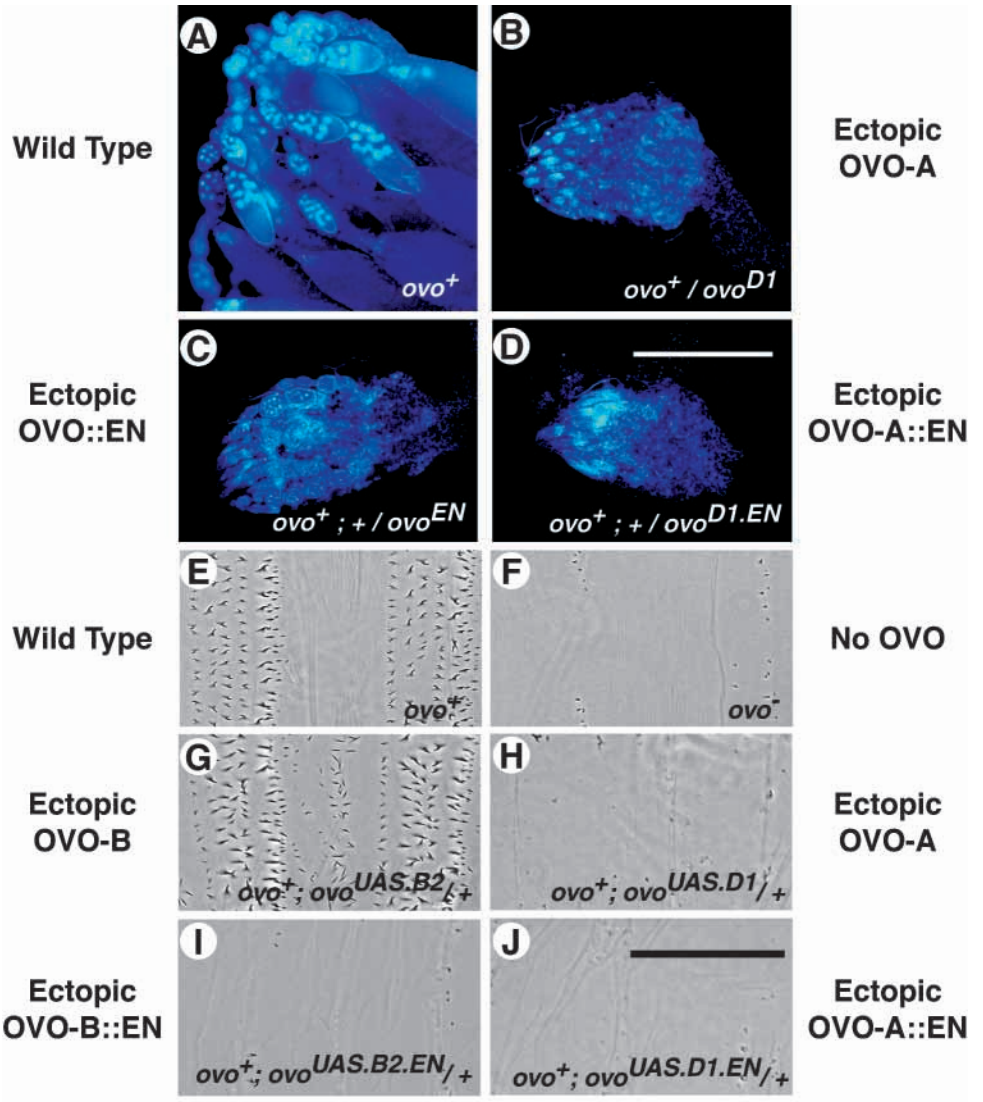

Fig. 5. OVO::EN mimics OVO-A in the germline and soma. (A-D) Single ovaries of the indicated genotype stained with DAPI and viewed under epifluorescence. (E-J) Ventral views of larval abdominal segments A3 and A4 (anterior to the left) viewed by phase contrast illumination. (A) Wild type (half an ovary). (B) Heterozygous for an allele encoding OVO-A from an *OVO-B* mRNA. (C) Wild type for *ovo* with a transgene encoding both OVO-B::EN and OVO-A::EN. (D) Wild type for ovo^{+} with transgene encoding only OVO-A::EN. (E) Wild type. (F) Hemizygous for a strong loss-of-function *ovo* allele for somatic functions. (G) Wild type for *ovo* and expressing ubiquitous OVO-B encoding transcripts from an exogenous UAS driven promoter. (H) Wild type for *ovo* and expressing ubiquitous OVO-A encoding transcripts from an exogenous UAS driven promoter. (I) Wild type for *ovo* and expressing ubiquitous OVO-B::EN encoding transcripts from an exogenous UAS driven promoter. (J) Wild type for *ovo* and expressing ubiquitous OVO-A::EN encoding transcripts from an exogenous UAS driven promoter. Genotypes are: (A,E) +, (B) $+/ovo^{D1}\ v^{24}$, (C) $+/P\{ovo^{EN}\}$, (D) $+/P\{ovo^{D1.EN}\}$, (F) $ovo^{svb\text{-}1}/Y$, (G) $P\{ovo^{UAS.B2}/P\{GAL4^{e22C2}\}$, (H) $P\{ovo^{UAS.D1}/P\{GAL4^{e22C2}\}$, (I) $P\{ovo^{UAS.B2.EN}/P\{GAL4^{e22C2}\}$, (J) $P\{ovo^{UAS.D1.EN}/P\{GAL4^{e22C2}\}$. (A-D) Bars, 500 μm; (E-J) 50 μm.

promoter activity is critical. We constructed, and introduced into flies, *lacZ* reporter genes for the *ovo-A* and *ovo-B* promoters and compared chromogenic activity in females bearing these constructs (Fig. 3). We used these identical upstream regions in later OVO encoding transgenic constructs.

We observed expression of the base reporter (of both *ovo-B* and *ovo-A*) in all the germline cells of the adult female (Fig. 3A; stem cells, dividing gonial cells, and egg chambers). Germarial staining was strong, expression was marginally weaker in early egg chambers (stage 2-4), and was very strong in eggs that had begun accumulating yolk. The pattern of base reporter expression is thus fully consistent with previous reporter and in situ hybridization patterns for bulk *OVO* transcripts (Garfinkel et al., 1994; Mével-Ninio et al., 1995, 1996).

Deleting the *ovo-A* promoter and flanking regions, including DNA encoding the OVO-A AUG in exon 1A (Fig. 3B), resulted in the *ovo-B*-specific reporter ($ovo::lacZ^{\Delta ap}$). Flies bearing this construct expressed β-galactosidase activity at high levels in all the adult female germ cells. The chromogenic pattern and expression strength in $ovo::lacZ^{\Delta ap}$ females was very similar to that seen with the reporter of both *ovo-B* and *ovo-A* promoters, suggesting that the bulk of *OVO* transcripts in the adult ovary are *OVO-B*.

Deleting the *ovo-B* promoter and first exon (Fig. 3C) resulted in the *ovo-A*-specific reporter ($ovo::lacZ^{\Delta bp}$). Females bearing this *ovo-A*-specific reporter showed much weaker staining that was similar in strength and pattern to translational reporters for OVO-A (Mével-Ninio et al., 1996). Very weak chromogenic activity was present in the germline stem cells and dividing cystocytes within the germarium. Expression was even lower in early to middle stages of egg chamber differentiation. Stronger staining occurred only from middle vitellogenic stages through egg maturity, but even in these late stages, *ovo-A* reporter staining was weaker than *ovo-B* reporter staining. These, along with previous data (cf. Fig. 1C), indicate that the *ovo-A* promoter is relatively weak and acts late in relation to *ovo-B*.

OVO isoforms function antagonistically in zygotic female germline development

To investigate OVO isoform function in flies, we generated an extensive series of transgenes encoding either OVO-B or OVO-A. We first asked if transgenes encoding OVO-B provide ovo^{+}

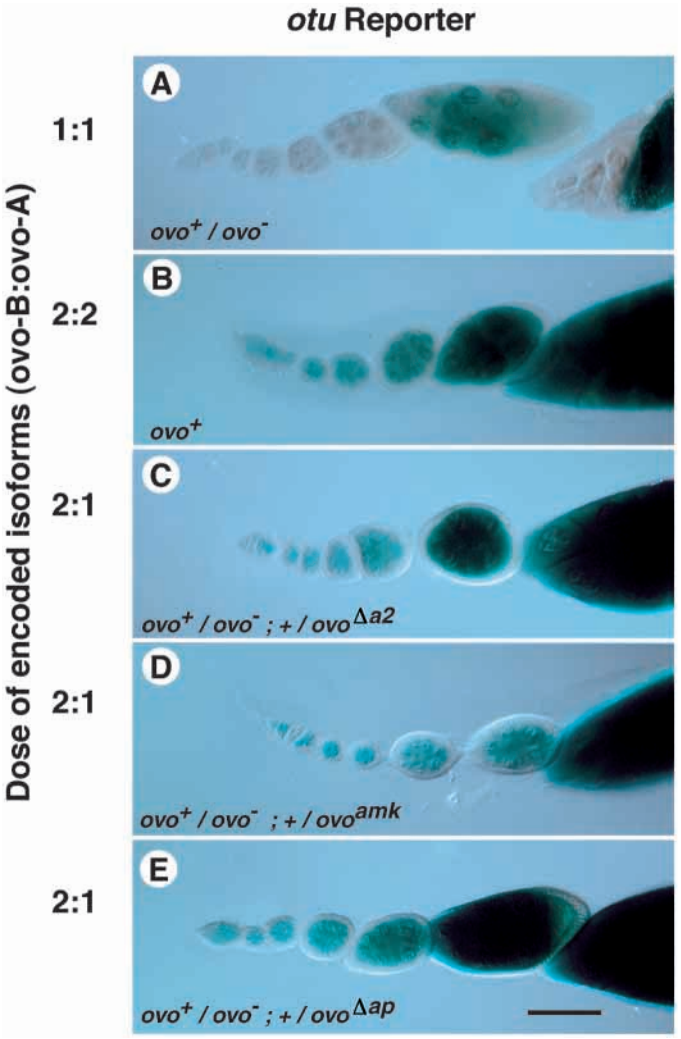

Fig. 6. Effect of transgenes encoding OVO-B on the *otu* promoter. Histochemically stained ovarioles of viewed under DIC optics. All females carried a single copy of the same *otu::lacZ* reporter line. The genetic dose of OVO-B and OVO-A encoding alleles is shown (left). (A) Heterozygous for *ovo*. (B) Wild type. (C-E). Heterozygous for *ovo* with an OVO-B encoding transgene. Genotypes are: (A) w^{1118}/y w^{67c1} ovo^{D1rv23}; $P\{otu::lacZ^{wt}\}/+$, (B) *FM7/ y* w^{67c1}; $P\{otu::lacZ^{wt}\}/+$, (C) w^{1118}/y w^{67c1} ovo^{D1rv23}; $P\{otu::lacZ^{wt}\}/+;+/P\{ovo^{\Delta a2}\}$, (D) w^{1118}/y w^{67c1} ovo^{D1rv23}; $P\{otu::lacZ^{wt}\}/+;+/P\{ovo^{amk}\}$, (E) w^{1118}/y w^{67c1} ovo^{D1rv23}; $P\{otu::lacZ^{wt}\}/+;+/P\{ovo^{\Delta ap}\}$. Bar, 500 μm.

genetic activity in the germline. Wild-type females had fully populated ovaries (Fig. 4A), while those of females homozygous for loss-of-function *ovo* mutations were devoid of germ cells and were thus completely sterile (Fig. 4B). In contrast, *ovo* mutant females carrying a single copy of any of several OVO-B encoding transgenes, $ovo^{\Delta a2}$ (Fig. 4C), $ovo^{\Delta ap}$ (Fig. 4D), ovo^{amv}, or ovo^{amk} (not shown) were fully fertile and had morphologically wild-type ovaries. Importantly, as these four transgenes eliminate OVO-A expression in different ways, phenotypic rescue is not construct-specific. The strong rescue of ovo$^-$ phenotypes by transgenes expressing only OVO-B unambiguously indicates that OVO-B provides zygotic ovo^+ function in the female germline.

Since four dominant-negative female-sterile ovo^D alleles encode OVO-A isoforms at the expense of OVO-B, we have hypothesized that precocious OVO-A expression has negative genetic activity in the female germline. Further support for this comes from females homozygous for ovo^+ and expressing *OVO-A* mRNAs from an exogenous promoter ($ovo^{UAS.A}$ driven by $GAL4^{nos}$ in the germline). These females had small atrophic ovaries exhibiting no discernible germline (Fig. 4F). This phenotype is more extreme than that seen in flies heterozygous for the strong dominant-negative alleles, ovo^{D1} (Fig. 4E) or ovo^{D4} (Andrews et al., 1998), which always show at least limited germline development in the presence of a wild-type allele. These data demonstrate that OVO-A over-expression is responsible for the ovo^D phenotypes. The $ovo^{UAS.A}$ allele is probably stronger, because it is not subject to negative-feedback regulation at the exogenous GAL4/UAS promoter.

The ENGRAILED repression domain mimics the OVO-A-specific domain

If OVO-A is a repressor in flies, then an engineered OVO-B isoform with a different repressor domain should mimic OVO-A. We constructed such an OVO-A mimic from a fusion of OVO-B and the EN repression domain. Wild-type females had well-developed ovaries with egg chambers in various states of completion (Fig. 5A), while females heterozygous for the OVO-A encoding ovo^{D1} allele had small ovaries bearing only early egg chambers (Fig. 5B). Females bearing the OVO::EN encoding alleles showed a strong mutant phenotype (Fig. 5C,D) like that exhibited by females heterozygous for strong ovo^D alleles. Early egg chambers were present, but they degenerated before vitellogenic stages.

We also looked at the epidermal activity of OVO-B, OVO-A, or OVO::EN. Somatic ovo^+ is required in the anterior of segments for denticle belt formation (Fig. 5E,F; Payre et al., 1999). We found that ectopic expression of OVO-B cDNA in the embryo ($ovo^{UAS.B2}$ driven by $GAL4^{e22C2}$) induced extra denticle formation in the normally naked region of each segment (Fig. 5G). Because the production of denticles is a wild-type function of somatic ovo^+, the production of ectopic denticles is another indication that OVO-B has positive function, and may suggest that OVO-B-like isoforms provide ovo^+ function in the soma. In contrast, expression of OVO-A in the epidermis of ovo^+ embryos ($ovo^{UAS.D1}$ driven by $GAL4^{e22C}$) resulted in naked cuticle (Fig. 5H), mimicking the phenotype of individuals homozygous for ovo^-, or *shavenbaby*, alleles affecting somatic *ovo* functions (Fig. 5F). Thus, ectopic OVO-A and OVO-B have opposite genetic activities in the embryonic epidermis; OVO-B providing wild-type activity and OVO-A a counteracting negative activity. Similarly, we found that expression of transgenes encoding the EN repression domain fused to either OVO-B (Fig. 5I), or OVO-A (Fig. 5J) resulted in naked cutilce. These data show that OVO proteins fused to an active repression domain from EN mimic the effect of OVO-A proteins in both the germline and epidermis. By inference, these data suggest that OVO-A acts by repression.

Target promoters activated by OVO-B and repressed by OVO-A

If the transcriptional regulatory activity we found in yeast reflects wild-type OVO isoform activities in flies, then OVO-

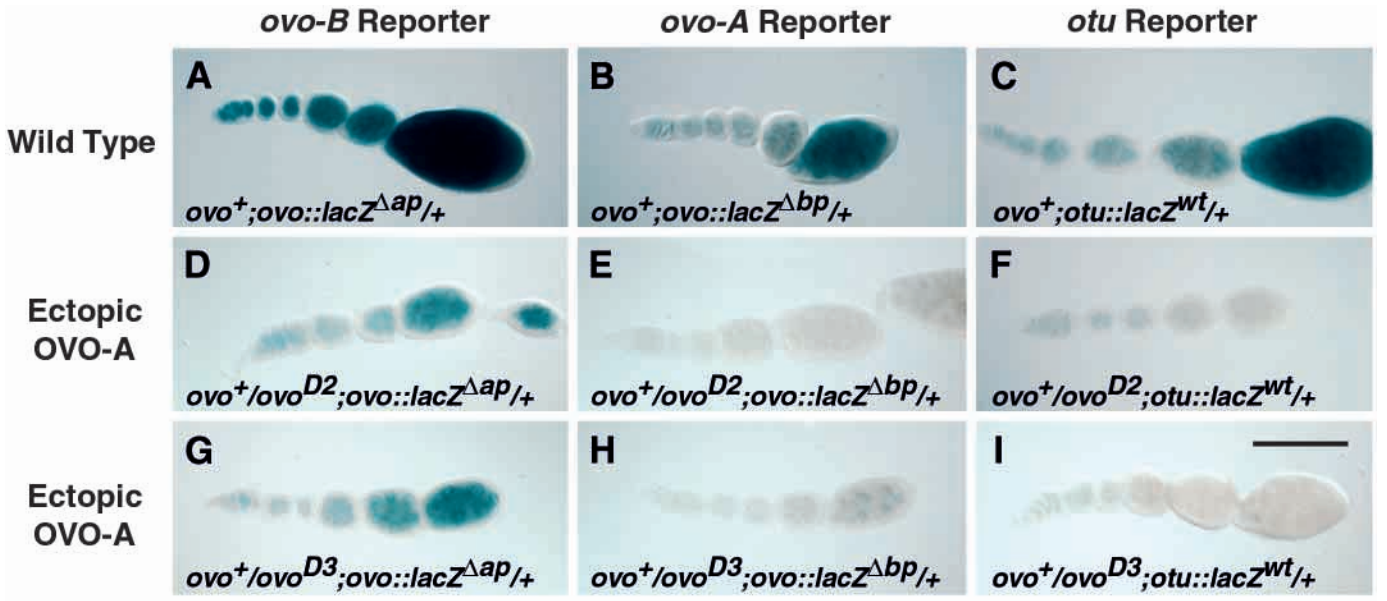

Fig. 7. Effect of OVO-A on *ovo-B*, *ovo-A*, and *otu* reporters. Whole-mount chromogenic staining viewed under DIC optics. Both *ovo* (rows) and reporter (column) genotypes are shown. Reporters are: (A,D,G) *ovo-B*, (B,E,H) *ovo-A*, and (C,F,I) *otu*. Isoforms encoded are: (A-C) both OVO-B and OVO-A, (D-I) wild type plus OVO-A from *OVO-B* mRNAs. Genotypes are: (A) *y* w^{67c1}*; P{ovo::lacZ*$^{\Delta ap}$*}/+*, (B) w^{1118}*/y* w^{67c1}*; P{ovo::lacZ*$^{\Delta bp}$*}/+*, (C) *FM7/* w^{1118}*; P{otu::lacZ*^{wt}*}/+*, (D) *y* w^{67c1}*/ovo*^{D2} v^{24}*; P{ovo::lacZ*$^{\Delta ap}$*}/+*, (E) w^{1118}*/ovo*^{D2} v^{24}*; P{ovo::lacZ*$^{\Delta bp}$*}/+*, (F) w^{1118}*/ ovo*^{D2} v^{24}*; P{otu::lacZ*^{wt}*}/+*, (G) *y* w^{67c1}*/ovo*^{D3} v^{24}*; P{ovo::lacZ*$^{\Delta ap}$*}/+*, (H) w^{1118}*/ovo*^{D3} v^{24}*; P{ovo::lacZ*$^{\Delta bp}$*}/+*, (I) w^{1118}*/ ovo*^{D3} v^{24}*; P{otu::lacZ*^{wt}*}/+*. Bar, 500 μm.

B will activate, and OVO-A will repress *ovo* and *otu* target promoters. We cannot look at the effect of OVO-B absence, as female germline viability requires OVO-B. We therefore examined the effect of adding transgenes encoding only OVO-B in an *ovo*$^{-}$*/ovo*$^{+}$ background. Females homozygous for *ovo*$^{+}$ show high levels of *otu* reporter activity (Fig. 6B), while females heterozygous for *ovo*$^{+}$, without an OVO-B encoding transgene, expressed low levels of *otu* reporter activity (Fig. 6A; Lü et al., 1998). The addition of a single copy of either a wild-type *ovo*$^{+}$ transgene, or any of three transgenes encoding only OVO-B (*ovo*$^{\Delta a2}$, *ovo*^{amk}, *ovo*$^{\Delta ap}$), resulted in wild-type expression of the *otu* reporter (Fig. 6C-E), clearly indicating that transgenes expressing only OVO-B up-regulate the *otu* promoter. Therefore, the positive activity of *ovo*$^{+}$ (encoding OVO-B and OVO-A) in this assay (Fig. 6B) is almost certainly due to the high expression of OVO-B during oogenesis.

Previous data show negative regulation of *otu* (Lü et al., 1998) and over-all *ovo* (*ovo-A* and *ovo-B*; Oliver et al., 1994; Mével-Ninio et al., 1996) reporter activity in females bearing the *ovo*^{D1}, *ovo*^{D2} or *ovo*^{D3} alleles suggesting that OVO-A mediates negative *ovo* autoregulation. Since negative autoregulation could occur via different mechanisms such as, down-regulation of *ovo*, up-regulation of *OVO-A* expression, down-regulation of *OVO-B* expression, or both, we determined the response of these promoters to OVO-A isoforms encoded by *ovo*^{D} alleles. Misexpression of OVO-A results in severely disrupted oogenesis (including absence of germ cells in the case of *ovo*$^{UAS.A}$) that complicates the interpretation of expression data. We therefore examined females showing weaker dominant-negative phenotypes (e.g. *ovo*^{D2} or *ovo*^{D3} heterozygotes). Under long incubation periods *ovo-B* (Fig. 7A), *ovo-A* (Fig. 7B), and *otu* (Fig. 7C) reporters show robust expression in the ovarioles of wild-type females. Under identical conditions, expression of each of the reporters was severely reduced in ovarioles from females heterozygous for either *ovo*^{D2} (Fig. 7D-F) or *ovo*^{D3} (Fig. 7G-I). Thus, OVO-A isoforms significantly repress expression of the *otu*, *ovo-B*, and *ovo-A* promoters in the female germline.

Distinct maternal effects due to OVO-B and OVO-A

The above data showed that OVO-B and OVO-A have opposing activities. However, the excellent fertility of *ovo*$^{-}$ females bearing OVO-B encoding transgenes indicated that there was no obligatory OVO-A function in, and for, the female germline. This raises the question of whether or not OVO-A activities are relevant to the development of the germline. Females with no endogenous *ovo* and heterozygous or homozygous for the OVO-B encoding *ovo*$^{\Delta a2}$ or *ovo*^{amk} transgenes were fertile, however 10-40% of the resulting progeny were sterile. Like wild-type flies (Fig. 8A,B), the fertile siblings usually had normal appearing gonads: ovaries full of developing follicles and coiled testes. In stark contrast, dissection of sterile progeny of females lacking OVO-A expression revealed no evidence of a germline. Ovaries were small and atrophic (Fig. 8C). Similarly, testes were shriveled and uncoiled (Fig. 8D). Interestingly, an occasional individual showed one atrophic gonad and one phenotypically wild-type gonad. There were no overt somatic defects. This maternal-effect sterility, or grandchildless, phenotype could be due to absence of OVO-A, or de-repression of OVO-B. Regardless, these data indicated that maternal OVO-A plays a role in germline development.

We observed a different maternal-effect phenotype when precocious or excessive OVO-A was present. The sterilizing effect of the OVO-A encoding *ovo*^{D} alleles (*ovo*^{D2}, *ovo*^{D3}, or *ovo*^{D4}) can be titrated by the addition of supernumerary copies

of *ovo*$^{+}$. In an intermediate situation, where there was sufficient *ovo*$^{+}$ activity to partially restore viable egg production, but not full fecundity, embryos developed but died before hatching showing defective embryonic patterning (c.f. Andrews et al., 1998). Wild-type embryos develop cuticles replete with easily recognizable pattern features (Fig. 8E). The dead progeny showed gross and variable defects in cuticle differentiation (Fig. 8F) including: holes, especially anteriorly; segment deletions and fusions; failed head involution and deletions of anterior elements of the mouthparts. Significantly, we did not observe defects in the elaboration of denticle belt hairs, so this maternal-effect lethality phenotype is unrelated to the shavenbaby phenotype. Embryos escaping from maternal-effect lethality developed into adult progeny with frequent segmentation defects, but showed normal germline development. It is possible that OVO-A was simply toxic to the embryonic soma, or prevented deposition of critical components during oogenesis, however, the maternal-effect lethality was rescued by OVO-B encoding transgenes (*ovo*$^{\Delta a2}$, *ovo*^{amk} or *ovo*$^{\Delta ap}$) suggesting that a proper maternal ratio of OVO-B to OVO-A is critical, directly or indirectly, for development of the embryonic soma.

DISCUSSION

Alternative promoter selection controls the expression of germline OVO isoforms with identical DNA-binding domains but different N termini. Briefly, our data show that OVO-B acts as a transcriptional activator in yeast, is sufficient for all known *ovo*$^{+}$ functions in the zygotic germline, supplies *ovo*$^{+}$ function in the epidermis, and positively regulates transcription of the downstream *otu* locus. In each case, OVO-A has an opposing activity. OVO-A acts as transcriptional repressor in yeast, is dominant-negative in the germline and epidermis and negatively regulates *ovo-A*, *ovo-B*, and *otu* promoters. OVO isoforms also show different maternal activities.

OVO-B and OVO-A have different transcriptional effector domains

The activation and repression regions in OVO-B and OVO-A show features found in the effector domains of other transcription factors (Fig. 9; reviewed by Gaurente and Bermingham-McDonogh, 1992; Cowell, 1994; Hana-Rose and Hansen, 1996; Ptashne and Gann, 1997). The activation region between residues 378 and 632 contains a glycine-rich region (61%) and an acidic region (pI=3.4). Similarly, the activation region between residues 574 and 875 contains a second acidic region (pI=3.7) and an extensive glutamine/histidine-rich region. The region of overlap between these two fragments (a.a. 574-632) does not contain primary sequence motifs associated with activation domains. The repression region identified in yeast (a.a. 251-377) contains a charged basic region (57% charged residues, pI=11.9) and a serine-rich domain (46 %). Significantly, all the *ovo*^{D} alleles encode mutant proteins from novel initiation codons (Mével-Ninio et al., 1996; Andrews et al., 1998) which include this repression region (Fig. 9B). This is consistent with the presence of conserved activation- and repression-domain binding proteins in yeast and flies.

While absence of activity is not necessarily due to repression, we present data strongly suggesting that OVO-A proteins contain an N-terminal domain that acts as a *cis*-dominant-repressor. We cannot rule out OVO-A action by binding to and titrating out activators, or by competing with activators for DNA binding sites, or even directing the destruction of OVO-A/OVO-

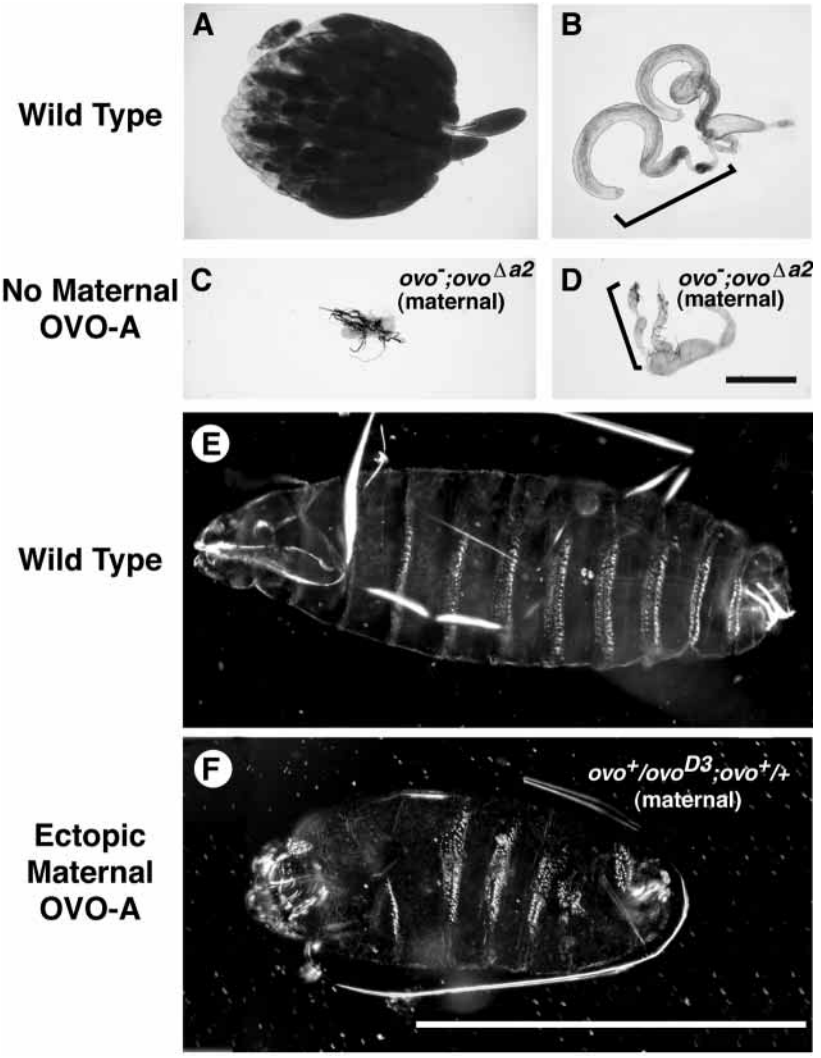

Fig. 8. Maternal effects of *ovo*. (A-D) Maternal-effect sterility. Whole-mounted gonads viewed by phase contrast optics. (E,F) Maternal effect lethality. Larval cuticle preparations viewed under dark-field optics. (A) Wild-type ovary. (B) Wild-type testes. One coiled testis is indicated by the bracket. (C) Atrophic ovary with no evidence of germline development. (D) Atrophic testes. One deflated and uncoiled testis is indicated by the bracket. (E) Wild type. (F) Cuticle of larva from a mother heterozygous for *ovo*$^{+}$ and an allele encoding OVO-A from *OVO-B* mRNA, plus an extra copy of *ovo*$^{+}$. Maternal genotypes are: (A,B,E) *y w*^{67c1}, (C,D) *y w*^{67c1} *ovo*^{D1rv23}*; P{ovo*$^{\Delta a2}$*}*/+, (F) *w*^{1118}*/ ovo*^{D3} *v*^{24}*; P{ovo*$^{D1r+}$*}*/+. Bars (A-D and E,F) 500 μm.

B heterodimers. However, it is more likely that OVO-A represses transcription through inhibitory protein-protein interactions with enhancer or promoter binding proteins (reviewed in Hanna-Rose and Hansen, 1996), or, given the position of OVO binding sites at transcription start sites (Lü et al., 1998), through steric hindrance of initiation complex formation. OVO-A repression activity requires the DNA-binding domain (Andrews et al., 1998) suggesting that repression occurs at target promoters. Additionally, *cis*-dominant OVO-A repression is transferable to the GAL4 protein in yeast and furthermore the EN active repression domain and the OVO-A repression domain have the same effect on OVO-B activity in flies. While, these fusion proteins could have the same negative activity through different mechanisms, the simple hypothesis is that OVO-A is a repressor.

OVO-B and OVO-A function in the female germline

The function of *ovo* in, and for, female germline development appears to be relatively straight forward (Fig. 10A). Our data indicate that OVO-B supplies the essential ovo^+ function in the female germline. This is fully consistent with the expression of *OVO-B* isoforms early in oogenesis where ovo^+ activity is required. Genetic and molecular data indicate that ovo^+ acts upstream of otu^+ (Pauli et al., 1993; Oliver et al., 1994; Lü et al., 1998) and ultimately Sxl^+ (Bopp et al., 1993; Oliver et al., 1993; Pauli et al., 1993). We find that OVO-B positively regulates *otu* transcription in the female germline, suggesting that part of the function of OVO-B is to up-regulate OTU production. There are at least three known positive regulators of *otu* promoter activity: *ovo* (Lü et al., 1998; this study); somatic signals (Hinson and Nagoshi, 1999); and *stil* (Sahut-Barnola and Pauli, 1999). While we do not know how OVO-B activates *otu* in conjunction with these other regulators, STIL is a germline restricted chromatin associated protein that is present at cytological sites of transcription (Sahut-Barnola and Pauli, 1999). Thus, STIL and OVO-B may act directly at the promoter. The somatic sex determination signals that influence *otu* expression are undefined.

Whereas maternal OVO-A is required for the germline of the progeny, it is extremely toxic when produced during early oogenesis. Consequently, there must be a tightly regulated way to produce OVO-A late in oogenesis, and indeed, the major phase of OVO-A protein production appears to be during terminal oogenesis (Mével-Ninio, et al., 1996). Perhaps late OVO-A acts to shut down production from *ovo-B*, *otu* and ultimately *Sxl* at the end of oogenesis (Fig. 10B). This may occur too rapidly to be detected by our reporter genes as we have failed to detect differences in *ovo-B*, *ovo-A*, or *otu* reporter expression between females encoding both OVO-B and OVO-A versus those that encode only OVO-B. Nevertheless, it is quite clear that OVO-A is able to repress target genes that are known to be part of the genetic hierarchy including *ovo*.

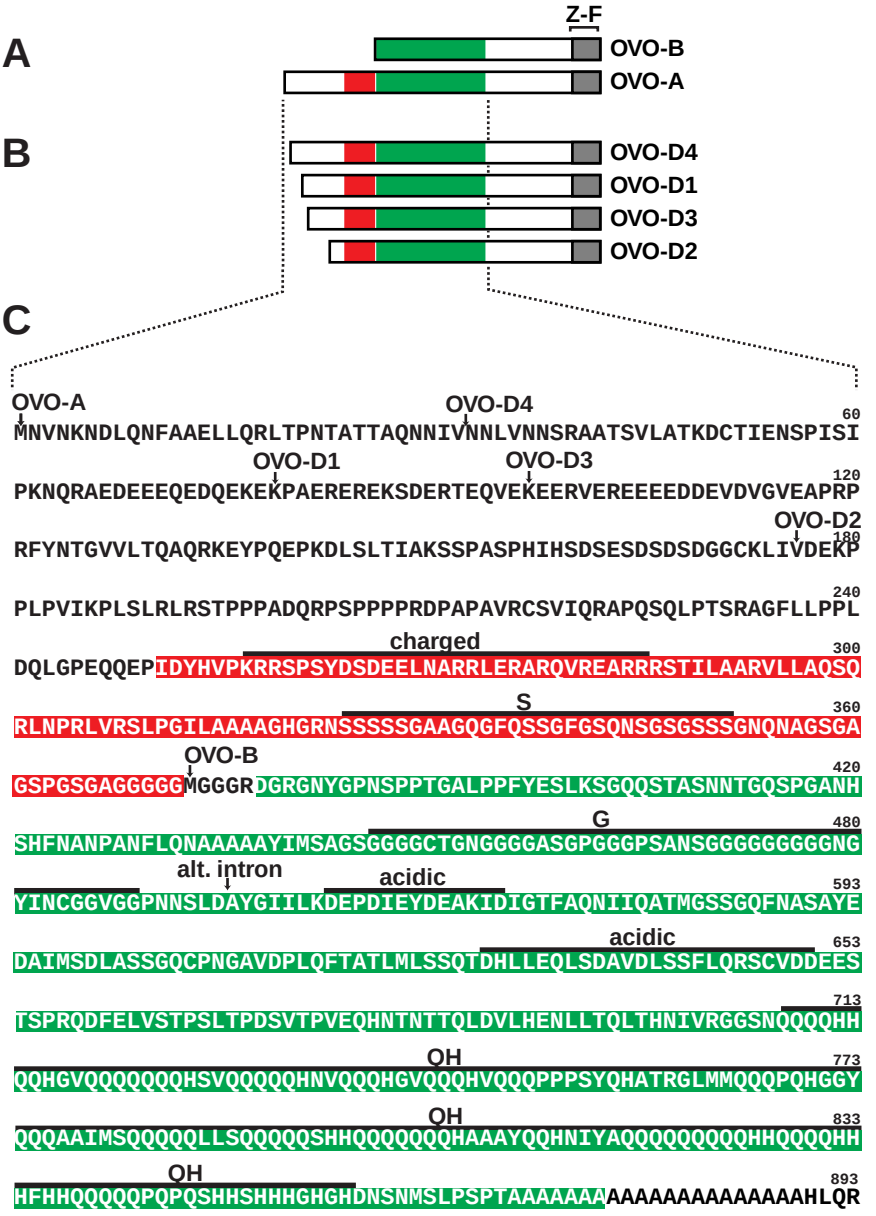

Fig. 9. Effector regions in OVO isoforms. (A) Wild-type OVO-B and OVO-A isoforms. (B) The repressive OVO-A isoforms encoded by ovo^D alleles. (C) Amino acid sequence of the regions mapped in vivo by the wild-type and ovo^D encoded OVO-A isoforms, with the effector domains mapped in yeast in color (red, repression; green, activation). Charged, acidic and low complexity regions (S, G and QH rich regions) are indicated above the sequence. Some OVO isoforms have additional residues at the indicated position due to alternative introns use (alt. intron). Because none of the ovo^D alleles result in a shavenbaby phenotype (unless they are expressed ectopically) the somatic *OVO* mRNAs probably do not include the region bearing the ovo^D point mutations or repression domain. The alternative splice may serve to append somatic specific residues to the activation region. In this case, the repression domain would be restricted to germline isoforms.

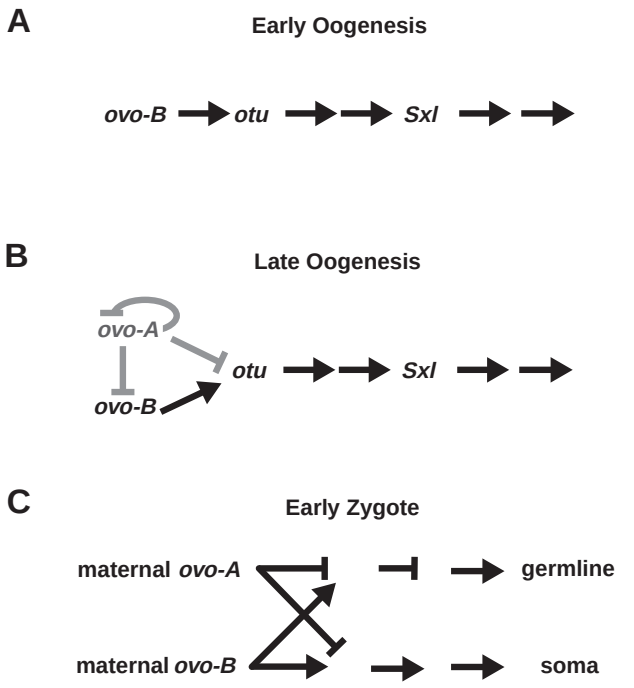

Fig. 10. Model of *ovo* function for oogenesis and early embryogenesis. (A) During most of oogenesis OVO-B is necessary and sufficient for female germline development, while OVO-A has no role. Part of the function of OVO-B is to regulate *OTU* mRNA production, probably directly. OTU regulates *Sxl* expression by an unknown mechanism. (B) Precocious expression of OVO-A clearly indicates that OVO-A down-regulates three target promoters, including both *ovo* promoters and the *otu* promoter. Negative control of *otu* could be direct, or mediated through negative regulation of *ovo-B*, or more likely both. The late expression of endogenous OVO-A may play a similar role. (C) In the maternally charged egg both OVO-B and OVO-A function to direct formation of germline and somatic tissues, perhaps through cross-regulation of target genes. Relatively high OVO-A predominates in the germline to repress transcription of target genes while relatively high OVO-B predominates in the soma to activate transcription of target genes in that tissue. We do not model the onset of transcription of *ovo* in the embryonic soma or germline.

Our data points to two significant roles for maternally deposited OVO-A and OVO-B (Fig. 10C). The maternal-effect-sterility phenotype seen when mothers lack OVO-A, indicates that *ovo* is required maternally for germline formation or maintenance. Parsimony suggests that OVO-A acts as a transcriptional repressor of genes that must be off in the embryonic germline. This is quite interesting in light of the limited transcriptional activity in the early germline (Van Doren et al., 1998), and the finding that premature transcription of at least some broad classes of genes are detrimental to germ cell migration and survival (Kobayashi et al., 1996; Asaoka et al., 1998). Similar transcriptional repression controls germline determination in *C. elegans* (reviewed by Seydoux and Strome, 1999). While we have not directly determined when the germline defect becomes apparent, the occasional observation of unilateral germ cell-less gonads is an argument for a sparse population of primordial germ cells. The few primordial germ cells that successfully migrate to a gonadal primordium can then fully populate an adult gonad (germline development reviewed by Spradling 1993).

The maternal effect on somatic development is also a newly described ovo phenotype. In this case, we suggest a simple model that maternal OVO-B is required to activate target genes required for the soma (FIG. 10C). Unfortunately, we cannot test for a maternal effect of OVO-B directly, as it is required for egg formation. The experimental evidence is that excessive OVO-A proteins causes maternal-effect lethality that can be titrated by supplying additional copies of transgenes encoding OVO-B. We can firmly suggest that maternal OVO is not likely to play a direct role in somatic *ovo* expression required for cuticle development. Our results show that ectopic expression of OVO-A during the time that somatic ovo^+ is expressed results in naked cuticle, and we did not observe reduced denticle belts in embryos that where overstocked with maternal OVO-A product.

A simple model is that OVO-A repression is required for germline development and that OVO-B activation is required for the soma (horizontal lines in Fig. 10C). However, given the presence of both OVO-B and OVO-A in late oogenesis and in early embryos, it is likely that OVO-B and OVO-A compete for similar binding sites during these stages. This might result in cross-regulation of target genes by the opposing activities of OVO-B and OVO-A (diagonal lines in Fig. 10C). Indeed, the maternal-effect lethality phenotype is best explained by interfering cross-regulation by OVO-A. Likewise, the maternal-effect sterility phenotype could be due to absence of OVO-A in the germline, excessive OVO-B, or both.

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REFERENCES

Andrews, J., Levenson, I. and Oliver, B. (1998). New AUG initiation codons in a long 5′ UTR create four dominant negative alleles of the Drosophila C_2H_2 zinc-finger gene *ovo*. *Dev. Genes Evol.* **207**, 482-487.

Asaoka, M., Sano, H., Obara, Y. and Kobayashi, S. (1998). Maternal Nanos regulates zygotic gene expression in germline progenitors of *Drosophila melanogaster*. *Mech. Dev.* **8**, 153-158.

Ashburner, M. (1989). *Drosophila. Volume I A Laboratory Handbook. Volume II A Laboratory Manual.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Bopp, D., Horabin, J. I., Lersch, R. A., Cline, T. W. and Schedl, P. (1993). Expression of the *Sex-lethal* gene is controlled at multiple levels during *Drosophila* oogenesis. *Development* **118**, 797-812.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Chidambaram, A., Allikmets, R., Chandrasekarappa, S., Guru, S. C., Modi, W., Gerrard, B. and Dean, M. (1997). Characterization of a human homolog (OVOL1) of the Drosophila *ovo* gene, which maps to chromosome 11q13. *Mamm. Genome* **8**, 950-951.

Clontech Laboratories, Inc. (1999) Yeast Protocols Handbook, PT3024-1 (PR91200), www.clontech.com.

Comer, A. R., Searles, L. L. and Kalfayan, L. J. (1992). Identification of a genomic DNA fragment containing the *Drosophila melanogaster ovarian tumor* gene (*otu*) and localization of regions governing its expression. *Gene* **118**, 171-179.

Cowell, I. G. (1994). Repression versus activation in the control of gene transcription. *Trends Biochem. Sci.* **19**, 38-42.

Dai, X., Schonbaum, C., Degenstein, L., Bai, W., Mahowald, A. and Fuchs, E. (1998). The *ovo* gene required for cuticle formation and oogenesis in flies is involved in hair formation and spermatogenesis in mice. *Genes Dev.* **12**, 3452-3463.

Garfinkel, M. D., Lohe, A. R. and Mahowald, A. P. (1992). Molecular genetics of the *Drosophila melanogaster ovo* locus, a gene required for sex determination of germline cells. *Genetics* **130**, 791-803.

Garfinkel, M. D., Wang, J., Liang, Y. and Mahowald, A. P. (1994). Multiple products from the *shavenbaby-ovo* gene region of *Drosophila melanogaster*: relationship to genetic complexity. *Mol. Cell. Biol.* **14**, 6809-6818.

Guarente, L. and Bermingham-McDonogh, O. (1992). Conservation and evolution of transcriptional mechanisms in eukaryotes. *Trends Genet.* **8**, 27-32.

Hanna-Rose, W. and Hansen, U. (1996). Active repression mechanisms of eukaryotic transcription repressors. *Trends Genet.* **12**, 229-234.

Hinson, S. and Nagoshi, R. N. (1999). Regulatory and functional interactions between the somatic sex regulatory gene *transformer* and the germline genes *ovo* and *ovarian tumor*. *Development* **126**, 861-871.

James, P., Halladay, J. and Craig, E. A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* **144**, 1425-1436.

John, A., Smith, S. T. and Jaynes, J. B. (1995). Inserting the Ftz homeodomain into Engrailed creates a dominant transcriptional repressor that specifically turns off Ftz target genes in vivo. *Development* **121**, 1801-1813.

Kobayashi, S., Yamada, M., Asaoka, M. and Kitamura, T. (1996). Essential role of the posterior morphogen *nanos* for germline development in Drosophila. *Nature* **380**, 708-711.

Lü, J., Andrews, J., Pauli, D. and Oliver, B. (1998). Drosophila OVO zinc-finger protein regulates *ovo* and *ovarian tumor* target promoters. *Dev. Genes Evol.* **208**, 213-222.

Masu, Y., Ikeda, S., Okuda-Ashitaka, E., Sato, E. and Ito, S. (1998). Expression of murine novel zinc finger proteins highly homologous to Drosophila *ovo* gene product in testis. *FEBS Lett.* **421**, 224-228.

Mével-Ninio, M., Terracol, R. and Kafatos, F. C. (1991). The *ovo* gene of Drosophila encodes a zinc finger protein required for female germ line development. *EMBO J.* **10**, 2259-2266.

Mével-Ninio, M., Guénal, I. and Limbourg-Bouchon, B. (1994). Production of dominant female sterility in *Drosophila melanogaster* by insertion of the ovo^{D1} allele on autosomes: use of transformed strains to generate germline mosaics. *Mech. Dev.* **45**, 155-162.

Mével-Ninio, M., Terracol, R., Salles, C., Vincent, A. and Payre, F. (1995). *ovo*, a Drosophila gene required for ovarian development, is specifically expressed in the germline and shares most of its coding sequences with *shavenbaby*, a gene involved in embryo patterning. *Mech. Dev.* **49**, 83-95.

Mével-Ninio, M., Fouilloux, E., Guénal, I. and Vincent, A. (1996). The three dominant female-sterile mutations of the *Drosophila ovo* gene are point mutations that create new translation-initiator AUG codons. *Development* **122**, 4131-4138.

Oliver, B., Perrimon, N. and Mahowald, A. P. (1987). The *ovo* locus is required for sex-specific germ line maintenance in Drosophila. *Genes Dev.* **1**, 913-923.

Oliver, B., Pauli, D. and Mahowald, A. P. (1990). Genetic evidence that the *ovo* locus is involved in Drosophila germ line sex determination. *Genetics* **125**, 535-550.

Oliver, B., Kim, Y.-J. and Baker, B. S. (1993). *Sex-lethal*, master and slave: a hierarchy of germ-line sex determination in *Drosophila*. *Development* **119**, 897-908.

Oliver, B., Singer, J., Laget, V., Pennetta, G. and Pauli, D. (1994). Function of *Drosophila* ovo^{+} in germ-line sex determination depends on X-chromosome number. *Development* **120**, 3185-3195.

Oliver, B. and Pauli, D. (1998). Suppression of distinct ovo phenotypes in the Drosophila female germline by $maleless^{-}$ and $Sex\text{-}lethal^{M}$. *Dev. Genet.* **23**, 335-346.

Pauli, D., Oliver, B. and Mahowald, A. P. (1993). The role of the *ovarian tumor* locus in *Drosophila melanogaster* germ line sex determination. *Development* **119**, 123-134.

Payre, F., Vincent, A. and Carreno, S. (1999). *ovo/svb* integrates the antagonistic WINGLESS and DER pathways to control epidermis differentiation. *Nature* **400**, 271-275.

Ptashne, M. and Gann, A. (1997). Transcriptional activation by recruitment. *Nature* **386**, 569-577.

Rørth, P. (1998). Gal4 in the Drosophila female germline. *Mech. Dev.* **78**, 113-118.

Sahut-Barnola, I. and Pauli, D. (1999). The *Drosophila*gene *stand still* encodes a germline chromatin-associated protein that controls the transcription of the *ovarian tumor* gene. *Development* **126**, 1917-1926.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Seydoux, G. and Strome, S. (1999). Launching the germline in *Caenorhabditis elegans*: regulation of gene expression in early germ cells. *Development* **126**, 3275-3283.

Spradling, A.C. (1993). Developmental genetics of oogenesis. In *The Development of Drosophila.* (ed. M. Bate and A. Martinez-Arias). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

The FlyBase Consortium (1999). The FlyBase database of the Drosophila Genome Projects and community literature. *Nucl. Acids Res.* **27**, 85-88.

Van Doren, M., Williamson, A. L. and Lehmann, R. (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. *Curr. Biol.* **8**, 243-246.