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

Justen Andrews, David Garcia-Estefania, Isabelle Delon, Jining Lü, Maryvonne Mével-Ninio, Anne Spierer, François Payre, Daniel Pauli and Brian Oliver.

**INTRODUCTION**

Transcription regulation is a linchpin in the gene networks controlling development. In flies, nematodes, mice and humans, ovo+ genes encode putative C2H2 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*+ (*still*+) 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* 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 (ovoD) strongly suggest 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 ovoD 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 ovoD show strongly down-regulated ovo and otu promoter activity (Oliver et al., 1994; Mével-Ninio et al., 1996; Andrews 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 ovoD

The ovoD 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 ovoamk and ovoamv 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 ovoD, ovoamk and ovoamv 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 ovoD
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, ovoD 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 ovoD 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
applying 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 trpl-901 leu2-3,112 ura3-52 his3-200 gal4
YAC80A 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 w67c1 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 2 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
ovoD (encoding OVO-A) have opposite transcriptional effects on the
otto promoter (Lü et al., 1998). However, as with any

---

**Table 1. Alleles and transgenes used**

<table>
<thead>
<tr>
<th>Allele (no. of transgenic lines)</th>
<th>Notes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ovoD11v2</td>
<td>Germline amorph. Insertion of gypsy transposon at ~ +900 bp in ovoD1½</td>
</tr>
<tr>
<td>ovoD12v3</td>
<td>Germline amorph. Insertion of HMS Beagle ~4.0-4.6 kb in ovoD1‡</td>
</tr>
<tr>
<td>ovoD13</td>
<td>Germline and shavenbaby amorph‡</td>
</tr>
<tr>
<td>P[owoD1½+ ] (4)</td>
<td>Wild-type allele: Genomic ovo DNA 0 to ~10.5 kb, in pCaSpeR2§</td>
</tr>
<tr>
<td>P[owoUAS.B] (4)</td>
<td>Encodes only OVO-B. Genomic ovo DNA 980 bp to ~10.5 kb from povoD1½, in UASP (Rørth, 1998)</td>
</tr>
<tr>
<td>P[owoD1(¢)] (2)</td>
<td>Encodes only OVO-B. povoD1½ deleted for 1246-1857 bp. Sequence at insertion = 5’-GGCGCGCCTAGCTA-3’</td>
</tr>
<tr>
<td>P[owoD1(¢)] (1)</td>
<td>Encodes only OVO-B. povoD1½ with T to A substitution at 464 bp</td>
</tr>
<tr>
<td>P[owoD1(¢)] (13)</td>
<td>Encodes only OVO-B. povoD1½ with A to G substitution at 463 bp, and G to C substitution at 465 bp, in pW6</td>
</tr>
<tr>
<td>P[owoD1½+ ] (2)</td>
<td>Encodes only OVO-B. povoD1½ deleted for 345-493 bp, in pCaSpeR2 §</td>
</tr>
<tr>
<td>P[owoUAS.B] (6)</td>
<td>Encodes only OVO-B. povoD1 with DNA 1140-38664755-54175513~6500 bp, in pUAST</td>
</tr>
<tr>
<td>ovoD1½</td>
<td>Encodes OVO-A and OVO-A-like. Genomic ovo DNA 0 to ~7.2 kb in pW6</td>
</tr>
<tr>
<td>ovoD1½</td>
<td>Encodes OVO-A and OVO-A-like. G to A substitution at 1572 bp‡</td>
</tr>
<tr>
<td>ovoD1½</td>
<td>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§</td>
</tr>
<tr>
<td>P[owoUAS.A] (3)</td>
<td>Encodes OVO-A, povoUAS.B with insertion of 5’-AAAATGGGACCCTCGGGGATCC-3’ at 1082 bp (Initiation codon underlined)</td>
</tr>
<tr>
<td>P[owoUAS.D] (5)</td>
<td>Encodes OVO-A-like, povoUAS.B, with A to T substitution at 1282 bp</td>
</tr>
<tr>
<td>P[owoE] (3)</td>
<td>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</td>
</tr>
<tr>
<td>P[owoD1.EN] (2)</td>
<td>Encodes OVO-A:EN and OVO-A-like:EN. Genomic ovoD1 DNA 0 to ~7.0 kb, with 916 bp fragment encoding EN repression domain inserted at 5408 bp, in pCaSpeR4</td>
</tr>
<tr>
<td>P[owoUAS.B] (4)</td>
<td>Encodes OVO-A:EN, povoUAS.B with 916 bp fragment encoding EN repression domain inserted at genomic 5408 bp</td>
</tr>
<tr>
<td>P[owoUAS.D] (4)</td>
<td>Encodes OVO-A-like:EN, povoUAS.B with 916 bp fragment encoding EN repression domain inserted at 5408 bp, in pUAST</td>
</tr>
<tr>
<td>P[gal47D] (1)</td>
<td>700 bp nanos promoter fused to GAL4:VP16 coding region**</td>
</tr>
<tr>
<td>GALAP22C2</td>
<td>Enhancer trap expressing GALA ubiquitously‡</td>
</tr>
<tr>
<td>GALAP22</td>
<td>Enhancer trap expressing GALA in embryonic ectoderm‡</td>
</tr>
<tr>
<td>P[otto::lacZ+ ] (2)</td>
<td>Genomic oto DNA ~423+118 bp (convention of Comer et al., 1992) in pCaSpeR-AUG-βgal</td>
</tr>
<tr>
<td>P[otto::lacZ+ ] (1)</td>
<td>Genomic oto DNA 0 to 1082 bp. Linker at insertion site = 5’-AAATGGGACCCTCGGGGATCC-3’ (initiation codon underlined), in pCaSpeR-βgal</td>
</tr>
<tr>
<td>P[otto::lacZ+ ] (9)</td>
<td>povo::lacZ+ with deletion of 345-493 bp</td>
</tr>
<tr>
<td>P[otto::lacZ+ ] (3)</td>
<td>povo::lacZ+ with deletion of 831-978 bp</td>
</tr>
</tbody>
</table>

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

Table 2. Yeast one-hybrid plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGB</td>
<td>Empty pGBDU-C yeast vector encodes S. cerevisiae GAL4 DNA-binding domain*</td>
</tr>
<tr>
<td>pGB-OVO-B</td>
<td>Composite ovo cDNA in pGBDU-C. Encodes OVO 378-495/549-940/958-1400, and GAL4 DNA-binding domain</td>
</tr>
<tr>
<td>pGB-OVO-A</td>
<td>Composite ovo cDNA in pGBDU-C. Encodes OVO 13-940/549-958-14000 and GAL4 DNA-binding domain. Linker (5’-CCGGTCGGGGTCCC-3’) at 5’ of cDNA encodes 5 unrelated residues</td>
</tr>
<tr>
<td>pGB-R2</td>
<td>Truncated pGB-OVO-B, encodes OVO 378-495/549-940-1156</td>
</tr>
<tr>
<td>pGB-R4</td>
<td>Truncated pGB-OVO-B, encodes OVO 378-495/549-875</td>
</tr>
<tr>
<td>pGB-R3</td>
<td>Truncated pGB-OVO-B, encodes OVO 875-940/958-1400</td>
</tr>
<tr>
<td>pGB-ZF</td>
<td>Truncated pGB-OVO-B, encodes OVO 1222-1400</td>
</tr>
<tr>
<td>pGB-R7</td>
<td>Truncated pGB-OVO-B, encodes OVO 378-495/549-632</td>
</tr>
<tr>
<td>pGB-R8</td>
<td>Truncated pGB-OVO-B, encodes OVO 574-875</td>
</tr>
<tr>
<td>pGB-AN1</td>
<td>Truncated pGB-OVO-A, encodes OVO 13-377</td>
</tr>
<tr>
<td>pGB-AN2</td>
<td>Truncated pGB-OVO-A, encodes OVO 251-377</td>
</tr>
<tr>
<td>pGB-R1L</td>
<td>Truncated pGB-OVO-B, encodes OVO 251-495/549/940/958-1400</td>
</tr>
<tr>
<td>pGB-R1</td>
<td>Truncated pGB-OVO-B, encodes OVO 251-495/549/958-1400</td>
</tr>
<tr>
<td>pGB-GA</td>
<td>Encodes S. cerevisiae GAL4 activation domain, 767-881</td>
</tr>
<tr>
<td>pGB-AN1-GA</td>
<td>pGB-AN1 insert fused in frame to the GAL4 activation domain from pGB-GA</td>
</tr>
<tr>
<td>pGB-AN2-GA</td>
<td>pGB-AN2 insert fused in frame to the GAL4 activation domain from pGB-GA</td>
</tr>
</tbody>
</table>

*James et al., 1996.

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).
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 expression is crucial.
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-A-specific reporter (ovo::lacZ^en). 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^en 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^ap). 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^+...
Since four dominant-negative female-sterile \( \text{ovo}^\Delta \) 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 \( \text{ovo}^\Delta \) and expressing OVO-A mRNAs from an exogenous promoter (\( \text{ovo}^{\text{GAL4A}} \)) driven by \( \text{GAL4}^{\text{pos}} \) 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, \( \text{ovo}^{\Delta 1} \) (Fig. 4E) or \( \text{ovo}^{\Delta D} \) (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 overexpression is responsible for the \( \text{ovo}^\Delta \) phenotypes. The \( \text{ovo}^{\text{GAL4A}} \) 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 \( \text{ovo}^{\Delta 1} \) 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 \( \text{ovo}^\Delta \) 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 \( \text{ovo}^\Delta \) is required in the anterior of segments for denticles belt formation (Fig. 5E,F; Payre et al., 1999). We found that ectopic expression of OVO-B cDNA in the embryo (\( \text{ovo}^{\text{GAL4A82}} \)) driven by \( \text{GAL4}^{\text{22C2}} \) induced extra denticles formation in the normally naked region of each segment (Fig. 5G). Because the production of denticles is a wild-type function of somatic \( \text{ovo}^\Delta \), the production of ectopic denticles is another indication that OVO-B has positive function, and may suggest that OVO-B-like isoforms provide \( \text{ovo}^\Delta \) function in the soma. In contrast, expression of OVO-A in the epidermis of \( \text{ovo}^\Delta \) embryos (\( \text{ovo}^{\text{GAL4A4}} \)) resulted in normal cuticle (Fig. 5H), mimicking the phenotype of individuals homozygous for \( \text{ovo}^\Delta \), or \( \text{shavenbaby} \), alleles affecting somatic \( \text{ovo} \) 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 normal cuticle. 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-
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 the female germline. Previous data show negative regulation of ovo-A (Lü et al., 1998) and over-all ovo expression data. We therefore examined females showing high levels of ovo reporter activity (Fig. 6B), while females heterozygous for ovo-A, without an OVO-B encoding transgene, expressed low levels of ovo 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 (ovoAmk, ovoBap), 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 ovoD1, ovoD2 or ovoD3 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 ovoD alleles. Misexpression of OVO-A results in severely disrupted oogenesis (including absence of germ cells in the case of ovoD4) that complicates the interpretation of expression data. We therefore examined females showing weaker dominant-negative phenotypes (e.g. ovoD2 or ovoD3 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 ovoD2 (Fig. 7D-F) or ovoD3 (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 ovoAmk or ovoBap 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 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 ovoD1 alleles (ovoD2, ovoD3, or ovoD4) 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 (ovoΔa2, ovoamk or ovoΔap) 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 Gaureen 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 ovoD 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-
OVO-B and OVO-A function in the female germline

The function of ovo in, and for, female germline development appears to be relatively straightforward (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.
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 expression is expressed 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 competing 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-B. Likewise, the maternal-effect sterility phenotype could be due to absence of OVO-A in the germline, excessive OVO-B, or both.

We thank E. Bellefroid, C. Desplan, R. Lehman, C. Thummel, P. North, and E. Wimmer for plasmids and fly strains used in this study; J. Fahrni, J. Faustinio, C. Ardourel and V. Boulais for invaluable technical help; A. Vincent for support; and A. Vincent, A. Kimmel, A. P. Mahowald, D. Klein, J. Dean and L. Sanchez for helpful discussion and comments.

REFERENCES


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

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.


