

aubergine enhances oskar translation in the Drosophila ovary

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SUMMARY

Although translational regulation of maternal mRNA is important for proper development of the Drosophila embryo, few genes involved in this process have been identified. In this report, we describe the role of aubergine in oskar translation. Previously, aubergine has been implicated in dorsoventral patterning, as eggs from aubergine mutant mothers are ventralized and seldom fertilized (Schüpbach, T. and Wieschaus, E. (1991) Genetics 129, 1119-1136). We have isolated two new alleles of aubergine in a novel genetic screen and have shown that aubergine is also required for posterior body patterning, as the small fraction of eggs from aubergine mothers that are fertilized develop into embryos which lack abdominal segmentation. Although aubergine mutations do not appear to affect the stability of either oskar mRNA or protein, the level of oskar protein is significantly reduced in aubergine mutants. Thus, aubergine is required to enhance oskar translation. While aubergine-dependence is conferred upon oskar mRNA by sequences in the oskar 3’ UTR, aubergine may influence oskar translation through an interaction with sequences upstream of the oskar 3’ UTR.

Key words: aubergine, oskar, translation, maternal mRNA, Drosophila

INTRODUCTION

Regulated translation of maternal mRNAs is a notable feature in the early development of many animals (reviewed by Davidson, 1986). Often, maternal mRNAs are stored in an inactive form in immature eggs. Upon fertilization or maturation, the bulk of the maternal mRNA is recruited into polyribosomes and translated. The resulting proteins support early development prior to the onset of zygotic transcription. The existence of related, but more selective processes in which the translation of individual maternal mRNAs are specifically regulated has long been suspected. In recent years, a number of examples of this phenomenon have been described (reviewed by Curtis et al., 1995). These regulated mRNAs commonly encode proteins with key roles in development. Consequently, analysis of the mechanisms underlying the selective translation of particular mRNAs will contribute to our understanding of early development.

Drosophila has proved to be a valuable system for studying the maternal factors that direct early embryonic development. Large-scale genetic screens for maternal-effect mutations that alter embryonic patterning have identified morphogens that direct body patterning, as well as many of the factors that restrict the activities of these morphogens to specific regions of the egg or embryo. Some of the morphogens are provided as maternal mRNAs, which are localized within the oocyte. Therefore it is not surprising that many of the genes responsible for proper embryonic patterning act in mRNA localization (reviewed by St Johnston and Nüsslein-Volhard, 1992). Although the need for regulated translation of these localized mRNAs could be surmised, no genes with obvious roles in translational control were initially identified among maternal patterning mutants. Instead, the importance of translational regulation was first revealed by detailed analysis of the mRNAs encoding these patterning morphogens. Spatial restriction of hunchback (hb) activity in the early embryo is achieved through local repression of hb mRNA translation, and factors involved in this process have been identified (Wharton and Struhl, 1991; Murata and Wharton, 1995). Translational control has since been demonstrated for several localized maternal mRNAs. Translation of the anteriorly localized bicoid (bcd) mRNA is activated upon fertilization by a mechanism involving regulated cytoplasmic polyadenylation (Sallés et al., 1994). In addition, several posteriorly localized messages are also translationally regulated. For example, in the early embryo translation of the cyclin B mRNA is repressed in the pole cells, but not elsewhere (Dalby and Glover, 1993). Conversely, translation of nanos (nos) mRNA is repressed throughout the embryo, but activated at the posterior pole (Gavis and Lehmann, 1994). In the ovary, oskar (osk) mRNA is subject to a variety of translational controls. Prior to localization of osk mRNA at the posterior pole of the oocyte, translation is repressed by bruno, a protein that binds to bruno response elements (BREs) in the osk mRNA 3’ UTR. This repression is crucial for proper development; when osk activity is provided by a transgene in which the BREs have been mutated, osk protein is precociously expressed in the ovary, and the resulting ectopic activation of posterior body patterning is lethal (Kim-Ha et al., 1995). osk mRNA translation is also subject to positive control. In staufen (stau) mutants, osk
protein levels are greatly reduced, even when osk mRNA is relieved of bruno-mediated repression; thus, stau activates osk translation (Kim-Ha et al., 1995). Another form of positive control involves localization of osk mRNA, which is normally essential for the accumulation of osk protein (Kim-Ha et al., 1995; Markussen et al., 1995; Rongo et al., 1995).

These studies have shown that translational regulation does indeed contribute to proper embryonic development in Drosophila and have also identified a few of the trans-acting factors involved in this process. Moreover, it has become clear that individual mRNAs may be under more than one form of translational regulation, as is evident for osk.

Given the prevalence of translational regulation in early Drosophila development, it is puzzling that few genes involved in this process have been recognized despite the existence of extensive collections of maternal patterning mutants. There are at least two likely explanations to this conundrum. First, the maternal roles of genes with essential zygotic functions cannot easily be assessed by standard genetic methods. Although the maternal roles of some of these loci have been tested using weak or conditional alleles or by germ-line clonal analysis (for examples, see Spradling, 1993), the maternal functions of the majority remain unknown and could involve translational regulation. Second, mutations in genes with multiple maternal functions might give rise to phenotypes which obscure a role in translation. This is indeed the case for stau, initially shown to function in osk mRNA localization (Ephrussi et al., 1991; Kim-Ha et al., 1991) and subsequently found to act in osk translation (Kim-Ha et al., 1995). For these reasons, we expect that additional genes involved in the translational regulation of maternal mRNAs have yet to be identified or recognized.

Here we describe one such gene, aubergine (aub). aub was first identified by its role in dorsoventral patterning (Schüpbach and Wieschaus, 1991). In a novel screen for genes involved in anteroposterior body patterning (J. E. W., J. E. C., J. Schlenker and P. M. M., unpublished data), we have isolated additional aub mutants. We have shown that aub is required for posterior body patterning, as embryos from mutant mothers lack abdominal segmentation and fail to form pole cells. A molecular analysis of the aub- phenotype reveals that the levels of osk and gurken (grk) proteins are reduced. We have studied this effect on osk in greater detail and find that aub mutations do not appear to alter the stability of osk mRNA or protein. We conclude that aub enhances osk mRNA translation.

MATERIALS AND METHODS

Transgenic flies

The OBI transgene includes 315 nt of osk 5’ flanking sequences, the osk coding region (modified to introduce six histidine residues immediately before the stop codon; osk-6His), and the 3’ UTR and 3’ flanking sequences of the bicoid (bcd) gene, all cloned into the CaSpeR transformation vector (Pirotta, 1988). The minimal 5’ flanking sequences and poly-histidine insertion were included to limit osk activity, and the bcd 3’ UTR was added to mislocalize the mRNA to the anterior pole of the embryo. We also refer to the chromosome carrying this transgene as OBI (this is a second chromosome and likely carries two copies of the transgene). Flies with the OBI chromosome misexpress osk at the anterior of the oocyte, produce bicaudal embryos, and are dominant maternal-effect lethal. The OBI chromosome was used in the mutant screen from which new aub alleles were recovered (see below) and in the analyses reported here. The P[osk+] and P[oskBRE] transgenes have previously been described by Smith et al. (1992) and Kim-Ha et al. (1995), respectively.

Mutant stocks

Mutants N11 and K86 were isolated in a screen for dominant modifiers of the OBI bicaudal phenotype (J. E. W., J. E. C., J. Schlenker and P. M. M., unpublished data). Segregation analysis and meiotic mapping placed both N11 and K86 between dp and b on the left arm of the second chromosome, and finer mapping of N11 indicated a map position of 33±5. Complementation tests revealed that both N11 and K86 are alleles of aub (Schüpbach and Wieschaus, 1991); these alleles have been designated aubN11 and aubK86, aubK86, aubG62, aubK93, aubHN56, and aubAWE13 were kindly provided by Trudi Schüpbach. Like aubN11 and aubK86, all five of these original aub alleles also act as dominant partial suppressors of the OBI bicaudal phenotype. aubN11, aubK86, aubK93, and aubG62 are significantly stronger suppressors than are the other three aub alleles. There are no deficiencies that remove aub, so we do not know if the existing mutants are null. For the experiments described here, we used the aubN11/aubK86 allelic combination. The osk- flies were osk3/4Df(3R)Ypl103.

RNA analysis

In situ hybridizations to whole-mount ovaries and embryos were performed as described by Tautz and Pfeifle (1989), using antisense RNA probes labeled with digoxigenin, and previously described modifications (Kim-Ha et al., 1991). For northern blot analysis, RNAs were purified as described by Macdonald et al. (1986), and 32P-labelled DNA probes were generated by random-primed synthesis.

Antibodies and protein analysis

Antibodies directed against vasa (vas), nanos (nos), and exuperantia (exu) have been described by Macdonald et al. (1991); Smith et al. (1992). The rabbit anti-osk antiserum was raised against a bacterially expressed osk fusion protein. The osk expression construct (Ephrussi and Lehmann, 1992) was a generous gift from Anne Ephrussi. Antibodies against the 28-kD and 32-kD aub proteins were provided by Robert Cohen and by F. Shira Neuman-Silberberg and Trudi Schüpbach. Antibodies against kelch (kel) and singed (sn) (Cant et al., 1994; Xue and Cooley, 1993) were provided by Lynn Cooley. Robert Boswell provided anti-tudor (tud) antiserum (Bardsey et al., 1993).

Immunohistochemical staining of whole-mount ovaries and embryos was as described by Bartscher et al. (1991). For detection of osk protein, the extracts were sonicated prior to electrophoresis. Electroblotting was performed in a Genie blotter (Idea Scientific Co.) according to the manufacturer’s protocols. For tub westerns, the standard blotting buffer was modified as recommended for high molecular weight proteins (Bardsley et al., 1993). Proteins were detected using the WesternLight Chemiluminescence Detection System (Tropix, Inc.), according to supplier’s instructions.

RESULTS

aub is required for anteroposterior as well as dorsoventral patterning

The characterization of maternal-effect mutants that produce embryos with abdominal defects led to the discovery of the ‘posterior group’ genes, which act in posterior body patterning (reviewed by St Johnston and Nüsslein-Volhard, 1992). Additional maternally acting genes are likely to participate in this process, but they may not have been identified as posterior group
genes because they function at multiple stages of development, with the resulting phenotypes obscuring any posterior patterning defects. An approach to identify such genes was suggested by our finding that the bicaudal phenotype resulting from over- or mis-expression of osk (Smith et al., 1992; Ephrussi and Lehmann, 1992) could be partially suppressed if the mothers were also heterozygous for a mutation in one of the posterior group genes, such as vas or tud. Similarly, Mohler and Wieschaus (1986) found that the BicaudalD phenotype was suppressed in vas− and tud− heterozygotes. Apparently, these levels of these proteins are limiting in the formation of bicaudal embryos. A screen for mutations which, when heterozygous, partially suppress the formation of bicaudal embryos might therefore uncover additional genes involved in posterior body patterning. We performed such a screen, using the OBI transgene to produce a bicaudal phenotype (J. E. W., J. E. C., J. Schlenker and P. M. M., unpublished). The OBI transgene is similar to the osk/bcd 3′ UTR construct described by Ephrussi and Lehmann (1992) and consists of a slightly modified osk coding region fused to the bcd 3′ UTR. As the bcd 3′ UTR contains the bcd mRNA localization signal, the encoded osk protein is misexpressed at the anterior pole of the oocyte and embryo. Among the mutants recovered were two new alleles of aubergine (aub) (see Materials and Methods). aub was previously identified as one of a class of female-sterile mutants in which the eggshell is ventralized to variable extents (Schüpbach and Wieschaus, 1991; Fig. 1B). Eggs laid by these mutants are typically not fertilized and consequently do not secrete cuticles, making it difficult to detect any additional patterning defects. The partial suppression of the bicaudal phenotype in aub− heterozygotes led us to examine the eggs laid by aub− mothers (for simplicity, we refer to embryos by the genotype of their mothers). We find that approximately 2% of aub mutant eggs are fertilized. All of the resulting embryos lack abdominal segments, the classic posterior group mutant phenotype (Fig. 1D). (At least half of these cuticles display additional abnormalities, which presumably reflect substantial defects in both dorsoventral and anteroposterior body patterning.) Thus aub is required for posterior patterning of the embryo. We also find that the fertilized eggs of aub mutant mothers lack pole cells (data not shown); the same is true for most other posterior group mutants (St Johnston and Nüsslein-Volhard, 1992).

In the following sections we document two events in which aub acts in posterior body patterning, and one in which aub acts in dorsoventral body patterning. Considered in most detail is the enhancement of osk translation by aub. Curiously, the effect of aub mutations on osk translation does not appear to be related to the partial suppression of the OBI bicaudal phenotype. We return to this issue at the end of the Results.

**Localization of mRNAs in aub mutant ovaries**

A simple explanation for the action of aub in anteroposterior and dorsoventral patterning is that aub plays a role in a process that these two systems utilize in common. mRNA localization is one such process (Berleth et al., 1988; Ephrussi et al., 1991; Kim-Ha et al., 1991; Wang and Lehmann, 1991; Neuman-Silberberg and Schüpbach, 1993), and we therefore compared the distributions of several localized mRNAs in wild-type and aub mutant ovaries. The distributions of bcd (data not shown) and grk mRNA (Fig. 2B) are wild-type in aub mutants. The early phases of osk mRNA localization, including concentration in the oocyte of early egg chambers and the initial localization to the posterior pole during stages 7-8, are also not affected by aub mutations (data not...
shown). In contrast, *aub* mutants are defective in maintenance of *osk* mRNA localization. Normally, *osk* mRNA remains tightly localized to the posterior cortex of later-staged oocytes and early embryos. In *aub* mutants, *osk* mRNA prematurely diffuses from the posterior of oocytes (Fig. 2D) and can only rarely be detected at the posterior of embryos (data not shown).

**aub affects the accumulation of *osk* and *grk* proteins**

Although the results presented above could suggest that *aub* acts differently in anteroposterior and dorsoventral patterning, it is also possible that the primary role is in a single type of process, and that the requirement for *aub* in *osk* mRNA maintenance is indirect. One possibility is that *aub* mutants are defective in the accumulation of specific proteins. One affected protein would be osk, as osk protein acts to maintain *osk* mRNA localization (Kim-Ha et al., 1991; Webster et al., 1994; Rongo et al., 1995), and another would be a protein required for dorsoventral patterning. To test this idea, we compared the levels of osk protein in wild type and *aub* mutants. Despite normal amounts of *osk* mRNA (Fig. 2E), the levels of osk protein are substantially reduced. At least two protein products of the *osk* gene are detected on western blots: an abundant protein of approximately $55 \times 10^3$ M_r and a rarer protein of about $70 \times 10^3$ M_r (Markussen et al., 1995; Rongo et al., 1995). The amounts of both osk proteins are decreased in *aub* mutants, although the $55 \times 10^3$ M_r protein is affected to a greater degree (Fig. 3E). We also used immunohistochemistry to monitor osk protein. Under normal staining conditions, very little osk protein is detected in *aub* mutant ovaries, and its appearance is slightly delayed relative to wild-type (Fig. 3D). By extending the staining reaction we detect some osk protein in almost all *aub* mutant ovaries, with the protein appearing dispersed over the posterior third of the oocyte, rather than tightly concentrated at the posterior cortex as in wild-type ovaries.

The level of *grk* protein is also greatly reduced in *aub* mutants (Fig. 3B), while the level of *grk* mRNA remains normal (data not shown). This decrease in the amount of grk protein may give rise to the dorsoventral patterning defects of *aub* mutants. Nevertheless, the grk protein present in *aub* mutants is apparently sufficient for establishment of correct anteroposterior polarity, as *aub* mutants do not exhibit general defects in RNA localization or in migration of the oocyte nucleus to the anterior, two processes which rely on *grk* (González-Reyes et al., 1995; Roth et al., 1995). In addition, *aub* is not generally required for the accumulation of proteins during oogenesis, as *aub* mutations do not affect the levels of any of several other ovarian proteins tested, including vas, exu, kel, and sn (Fig. 3F). 

**aub enhances osk translation**

Because *osk* mRNA levels remain normal in *aub* mutants, but osk protein is reduced, *aub* must influence either the synthesis almost all wild-type oocytes. By stage 10, osk protein can be detected in nearly all of both wild-type and *aub* oocytes, although the levels are much lower in the latter. We do not know if initiation of osk translation is delayed in *aub* mutants, or if the lag in protein appearance simply reflects the time required to accumulate detectable amounts. (E,F) Western blot analysis of ovarian proteins. (E) osk protein in wild-type, *osk* and *aub* oocytes. Two bands are missing in the *osk* sample. These bands correspond to the two forms of osk protein described previously; a minor, $70 \times 10^3$ M_r form and a major, $55 \times 10^3$ M_r form (Markussen et al., 1995; Rongo et al., 1995). Both forms of osk protein are reduced in *aub* extracts, although the effect is more substantial on the $55 \times 10^3$ M_r form (several background bands are present in both wild-type and mutant extracts). (F) Western blot analyses of additional ovarian proteins: vas, exu, kel, and sn. All proteins tested appear at similar levels in wild-type and *aub* oocytes.
or stability of osk protein. Evidence that argues strongly against a role for aub in stabilizing osk protein comes from experiments employing the OB1 transgene. osk protein is normally detected at both poles of OB1 transgenic embryos; the anterior protein is from the transgene, while the posterior protein is from the endogenous osk gene (Fig. 4A). In aub−; OB1 embryos, the posterior osk protein is largely missing, however, the anterior osk protein encoded by the OB1 mRNA remains present at a high level (Fig. 4B). A similar effect is found in aub−; OB1 ovaries (Fig. 5). To rule out the possibility that the slight modification of the osk protein encoded by

Fig. 4. aub is not required for ectopic osk protein accumulation at the anterior of OB1 embryos. osk protein was detected in whole-mount embryos by immunostaining. (A) osk protein appears at both poles of OB1 embryos. (B) In aub−; OB1 embryos, the anterior osk protein expressed from the OB1 transgene remains present at a high level, even though very little of the endogenous osk protein can be detected at the posterior pole.

Fig. 5. OB1 translation is enhanced by aub mutations. Detection of osk protein in whole-mount ovaries. (A) Low levels of uniformly distributed osk protein can be detected in the presumptive oocyte of early OB1 egg chambers. (B) Higher levels of osk protein are seen in early aub−; OB1 oocytes. This enhancement is evident in later-staged oocytes as well, when endogenous osk protein can be detected at the posterior, and the osk protein encoded by the OB1 transgene accumulates at the anterior. These effects are both significant and highly reproducible. (C) In addition to wild-type levels of posterior osk protein, a few discrete patches of osk protein can be detected at the anterior of stage 10, OB1 oocytes. (D) While the endogenous osk protein at the posterior appears reduced, significantly more osk protein is detectable along the anterior margin of similarly staged aub−; OB1 oocytes.

Fig. 6. The effects of aub mutations on P[oskBRE] translation. (A-D) Detection of osk protein in whole-mount preparations. (A) No osk protein is detected in early aub−; P[osk+] egg chambers. (B) As in P[oskBRE] ovaries, osk protein is detected in the presumptive oocytes of early aub−; P[oskBRE] egg chambers. (C) Only a small amount of diffuse osk protein is detected at the posterior pole of later-staged, aub−; P[osk+] oocytes. (D) A similar low level of diffuse osk protein is detected in later-staged, aub−; P[oskBRE] oocytes. (E) Western blot analysis of ovarian proteins. Even when an additional osk transgene is present, a clear reduction in the levels of osk protein in aub mutants is seen (compare P[osk+] and aub−; P[osk+] lanes). The reduction in osk protein levels in aub−; P[osk+] and aub−; P[oskBRE] ovaries is similar. Note that the effects of aub mutations on osk protein accumulation are less pronounced when the osk gene dosage is elevated (compare with E). This may be a general feature of translational regulation, as stau, another factor required for osk translation (Kim-Ha et al., 1995), becomes partially dispensable when osk gene dosage is increased (Smith et al., 1992).

Fig. 7. aub is required for the activity, but not translation, of the OB1 transgene. (A,B) Detection of osk protein in whole-mount embryos. osk protein accumulates at both poles of OB1 embryos (A), but in aub−; OB1 embryos can only be detected at high levels in the anterior (B). (C,D) Visualization of nos mRNA in whole-mount embryos by in situ hybridization. nos mRNA is present at the anterior, as well as posterior, pole of OB1 embryos (C), but cannot be detected at either pole of aub−; OB1 embryos (D). (E,F) Ventral cuticular phenotypes. OB1 embryos exhibit a bicaudal cuticular phenotype, as evidenced by the duplication and inversion of the posterior-most denticle bands in place of the normal head and thoracic structures (E). In contrast, aub−; OB1 embryos display a posterior group mutant cuticular phenotype, a lack of most or all abdominal segments (F).
the OBI transgene (see Materials and Methods) affects the accumulation of anterior osk protein in aub mutants, these experiments were repeated using the mobs transgene (Webster et al., 1994). Like OBI, this transgene misexpresses osk protein at the anterior pole of oocytes and embryos. However, mobs encodes a wild-type osk protein. The distributions of osk protein in aub−; mobs ovaries and embryos are indistinguishable from those in aub−; OBI animals (data not shown). Because the proteins encoded by osk and mobs are identical (the genes differ only in their 3′UTRs), aub is unlikely to affect the stability of osk protein. Thus, aub appears to enhance translation of osk mRNA.

Sequences in the osk 3′ UTR confer aub-dependence upon translation of osk mRNA

The differential dependence of osk and OBI mRNAs on aub for translation also provides some insight into which features confer aub-dependence on the osk mRNA. Because the mRNAs differ only in their 3′UTRs, we can conclude that the requirement for aub is conferred by the osk mRNA 3′ UTR.

Further results suggest that aub may interact, directly or indirectly, with sequences outside of the osk 3′ UTR to influence translation. While translation of osk mRNA is reduced in aub mutants, translation of the OBI message is increased. Although only low levels of osk protein are detected in the oocytes of early-staged, OBI egg chambers (Fig. 5A), high levels of osk protein are seen when these transgenic ovariies are additionally mutant for aub (Fig. 5B). Furthermore, the few, small patches of anteriorly localized osk protein that are first detected in OBI egg chambers in stages 7 and 8 (Fig. 5C) are expanded to outline the anterior margin of aub−; OBI egg chambers (Fig. 5D). This effect is both substantial and highly reproducible. It is unlikely that the increased translation of OBI mRNA in aub mutants is mediated through the bcd 3′ UTR, which is present in the OBI message, as the levels of bcd protein (determined by whole-mount immunostaining and Western analysis) are not affected by aub mutations (data not shown). Instead, wild-type aub function appears to modestly inhibit the translation of the hybrid message, and this effect must be mediated by sequences in the osk mRNA 5′ UTR or coding region. We comment further on the disparate effects of aub mutations on osk and OBI translation in the Discussion.

aub is required for osk translation even in the absence of bruno-mediated repression

bruno protein binds sequences in the osk 3′ UTR termed BREs and represses osk translation (Kim-Ha et al., 1995). One potential role for aub, as a positive regulator of osk translation, could be to override this known form of repression. If so, an osk mRNA not repressed by bruno would not require aub for the normal high levels of translation. To test this possibility, we used the P[oskBRE−] transgene, which differs from wild-type osk only by point mutations in the BREs. The P[oskBRE−] and control P[osk+] transgenes were introduced into aub− flies. Comparing the levels of osk protein in aub−; P[oskBRE−] and aub−; P[osk+] ovaries by western analysis, we find no difference (Fig. 6E). Thus the absence of bruno-mediated repression does not eliminate the requirement for aub in osk translation.

Minor differences in the translation of the P[oskBRE−] and P[osk+] transgenes might not be detected by western blot analysis, as this method examines total ovarian protein. Consequently, we used whole-mount immunostaining as an additional measure of translation. osk protein patterns in the later stages of oogenesis are indistinguishable between aub−; P[oskBRE−] and aub−; P[osk+] ovaries, consistent with the results of the Western analysis (Fig. 6C,D). However, the precocious translation of the P[oskBRE−] transgene at early stages persists even in an aub− background, while no osk protein is detected in aub−; P[osk+] egg chambers at similar stages (Fig. 6A,B). Thus the requirement for aub in osk translation does not extend throughout oogenesis, but appears to be limited to the later stages.

A second requirement for aub in posterior body patterning

The results presented above clearly implicate aub in translation of osk. However, they do not fully explain the posterior body patterning defects caused by the absence of aub. First, the reduced levels of osk protein found in aub mutants might not be expected to eliminate abdominal segmentation completely, as osk protein normally appears to be present in substantial excess. Instead, the dispersal of osk protein across much of the posterior third of the oocyte in aub mutants might be expected to cause ectopic posterior body patterning (Webster et al., 1994). Nevertheless, there is little or no abdominal segmentation in embryos from aub− mothers. Second, while the OBI bicaudal phenotype is partially suppressed in aub− heterozygotes, translation of the OBI transgene does not require aub: anterior osk protein accumulates in OBI ovaries (and also in embryos), even when both alleles of aub are mutant.

An explanation for the extreme posterior patterning defects of aub mutants, and the partial suppression of the OBI bicaudal phenotype in aub− heterozygotes, is revealed by further analysis of embryos from OBI or aub−; OBI mothers. In OBI embryos, anterior osk protein ectopically activates nos and results in the production of bicaudal embryos. Although anterior osk protein is also present in aub−; OBI embryos (Fig. 7B), there is no corresponding anterior recruitment of nos mRNA (Fig. 7D) or accumulation of nos protein (data not shown), despite normal levels of nos mRNA (data not shown). Not surprisingly, the resulting embryos lack abdominal segmentation (Fig. 7F). Therefore aub is also required for a second event in posterior body patterning, intermediate between osk mRNA translation and nos mRNA localization. Again, this event might involve translational regulation. Candidate mRNAs for this second regulatory event are encoded by the vas and tud genes, both of which are conventionally portrayed as intermediate between osk and nos in the posterior body patterning hierarchy and are also required for the activity of anteriorly localized osk protein (Ephrussi and Lehmann, 1992). By Western analysis, however, both proteins remain at wild-type levels in aub mutant ovaries (vas protein, Fig. 3F; tud protein, data not shown). Consequently, the second requirement for aub in posterior body patterning remains incompletely defined.

DISCUSSION

Although the regulated translation of maternal mRNAs has long been recognized as a common feature in the development
of many animals, the importance of this process in early *Drosophila* development was not immediately apparent. Among the collections of maternal-effect mutants with specific defects in embryonic body patterning, many affected localization of particular mRNAs, but none appeared to be involved in translational control. Detailed analyses of the localized mRNAs have, nevertheless, revealed that many are regulated at the translational level. Furthermore, in some cases translational regulation has been shown to be essential for proper development. Despite growing evidence of the importance of translational control of maternal mRNAs in *Drosophila*, few genes involved in this process have been identified.

We have shown that *aub*, a gene previously found to act in dorsoventral patterning (Schüpbach and Wieschaus, 1991), also acts in anteroposterior patterning; embryos from *aub* mutant mothers lack abdominal segmentation and fail to form pole cells. The levels of osk protein are reduced in *aub* mutants, yet there is no change in the stability of *osk* mRNA or protein. Therefore, *aub* enhances *osk* translation. The demonstration that a gene involved in translational regulation affects multiple patterning events provides one explanation for the paucity of translational control factors identified by analyses of genes required only for single patterning systems.

**How direct is the role of *aub* in *osk* translation?**

The role played by *aub* in translation of *osk* mRNA could be either direct or indirect. Various observations suggest two possible scenarios for specific indirect roles; neither is fully consistent with our results. The first possibility is that reduced (but still detectable) levels of grk protein are indirectly responsible for the *osk* mRNA localization and translation defects observed in *aub* mutants. *grk*, in addition to its role in dorsoventral patterning, participates in establishing anteroposterior polarity (Gonzáles-Reyes et al., 1995; Roth et al., 1995). Because this polarity is a prerequisite for the posterior localization and translation of *osk* mRNA, *grk* mutants mislocalize *osk* mRNA (Gonzáles-Reyes et al., 1995; Roth et al., 1995) and have no detectable osk protein (Markussen et al., 1995). However, the anteroposterior patterning defects of *aub* and *grk* mutants differ substantially. In *grk* mutants anteroposterior polarity is reprogrammed, such that both poles have anterior character, and many processes are affected (Gonzáles-Reyes et al., 1995; Roth et al., 1995). For example, the oocyte nucleus is no longer redirected to the anterior margin during mid-oogenesis, and several mRNAs are mislocalized. Among these mRNAs is *osk*, which never moves to the posterior pole; instead, it accumulates in a diffuse zone in the center of the oocyte. In contrast, in *aub* mutants anteroposterior polarity is established correctly. Repositioning of the oocyte nucleus is normal, as is the localization of most mRNAs. While *osk* mRNA localization is affected, the resulting pattern is distinct from that observed in *grk* mutants: *osk* mRNA is spread out from the posterior pole. This defect is very similar to that observed in the strongest *osk* mutants, in which maintenance of *osk* mRNA localization is specifically affected (Ephrussi et al., 1991; Kim-Ha et al., 1991). Thus two distinct stages of *osk* mRNA localization appear to be affected in *grk* and *aub* mutants. Because the phenotype of *aub* mutants is qualitatively different from that of weak *grk* mutants, the posterior defects of *aub* mutants are not likely to be a consequence of limiting levels of *grk* protein.

A second way in which *aub* mutants might indirectly reduce *osk* translation is through an effect on *osk* mRNA localization, which in turn would reduce *osk* translation. A number of mutants, including *orb*, *mago nashi*, *cappucino* and *spire*, have been shown to have such an indirect effect on *osk* translation (Kim-Ha et al., 1995). However, all of these mutants are defective in the initial localization of *osk* mRNA to the posterior pole (Ephrussi et al., 1991; Kim-Ha et al., 1991; Christerson and McKearin, 1994; Newmark and Boswell, 1994), while *aub* mutants display *osk* mRNA localization patterns that are suggestive of a defect in maintenance of localization. At present there is no evidence to indicate that *osk* mRNA maintenance, as distinct from initial posterior localization, is essential for translation. In fact, another mutant isolated in our screen is defective in *osk* mRNA maintenance, yet produces significant levels of osk protein (J. E. W., J. E. C., J. Schlenker and P. M. M., unpublished data), suggesting that maintenance per se is not required for translation. Thus, a more likely relationship between the *osk* mRNA localization and translation defects of *aub* mutants is that the primary defect is in translation, which then causes a failure of mRNA maintenance.

A further difficulty with any model in which *aub* acts indirectly through *osk* mRNA localization to enhance *osk* translation is raised by our results with the *OBI* transgene. Although the anterior localization of the *OBI* mRNA is in no way affected by *aub* mutations, *OBI* translation is altered, albeit in a positive way (see below); this effect clearly cannot be attributed to changes in mRNA localization.

Our results argue against these specific cases in which *aub* could indirectly affect *osk* mRNA translation, yet it remains unclear whether osk protein production is affected directly or indirectly by *aub*. Our data are, however, fully consistent with a model in which *aub* directly enhances the translation of a particular class of ovarian mRNAs, including *osk*, *grk*, and at least one other mRNA (see below). Because osk protein acts in *trans* to maintain its own mRNA (Kim-Ha et al., 1991; Webster et al., 1994; Rongo et al., 1995), the reduced and possibly delayed translation of osk protein in *aub* mutants would underlie the defects in *osk* mRNA maintenance. The decrease in the level of grk protein in *aub* mutants readily explains the eggshell defects of *aub* mutants, as grk mutants are themselves ventralized (Schüpbach, 1987). Finally, the specific effect of *aub* mutations on *OBI* translation is also consistent with a direct role for *aub* in translational regulation.

**aub** and translational regulation of *osk* mRNA

Efficient translation of *osk* mRNA requires *aub*. In contrast, translation of the *OBI* message, which includes the *osk* 5′ UTR and coding region but lacks the *osk* 3′ UTR, does not require *aub*. Therefore, some feature of the *osk* 3′ UTR acts negatively to regulate *osk* mRNA translation, and *aub* acts to overcome this negative regulation. In what fashion might the initial 3′ UTR-dependent negative regulation occur? There are several reasonable possibilities. First, the osk mRNA may be folded so that the 3′ UTR interferes with translation. For example, parts of the 3′ UTR could base pair with regions of the 5′ UTR and impede movement by the 43S preinitiation complex. *aub* could act to disrupt the folded structure and promote translation. While it is difficult to rule out such a
model, we find no extensive 5’ UTR-3’ UTR interactions in predicted foldings of the osk mRNA.

A second potential mechanism for negative control involves regulated cytoplasmic polyadenylation (reviewed by Wickens, 1992; Richter, 1991): the osk 3’ UTR may specify a short poly(A) tail, which must be extended in an aub-dependent fashion for efficient translation. Sallés et al. (1994) have shown that osk mRNA poly(A) tail length remains constant between oogenesis and embryogenesis; however, it remains possible that polyadenylation of osk mRNA may be temporally or spatially regulated during oogenesis. A third possibility for 3’ UTR-dependent, negative control of osk mRNA translation involves repression by a trans-acting factor. In this model, aub would act to override the repression. osk mRNA has already been shown to be negatively regulated by one repressor; an ovarian protein, bruno, binds sequences (BREs) in the osk 3’ UTR and prevents translation of osk mRNA prior to posterior localization (Kim-Ha et al., 1995). If the role of aub were to relieve bruno-mediated repression, then an osk mRNA with nonfunctional BREs would no longer require aub for efficient translation. As we have shown here, this is not the case. However, aub may override the action of a different translational repressor.

Dependence on aub for efficient translation is conferred by the osk 3’ UTR, but there is no indication that aub must itself act through the 3’ UTR. In fact, the behavior of the OBI mRNA raises another possibility. Although the OBI mRNA does not require aub for translation, as noted above, we still observe an interaction between aub and OBI; specifically, in aub mutants, the OBI mRNA is translated at higher levels than in wild-type. It is unlikely that this effect is mediated by the bcd 3’ UTR sequences present in the OBI mRNA, as we find no alteration in bcd protein levels in aub mutants (data not shown). Instead, aub apparently influences OBI translation through an interaction, direct or indirect, with the osk 5’ UTR or coding region, which comprise the remainder of the OBI mRNA. How could such an interaction enhance translation of osk mRNA, but reduce translation of OBI mRNA? One possibility is that aub normally acts to relieve repression of osk mRNA translation via a binding interaction (of aub or an aub-dependent component) with the osk mRNA 5’ UTR. By itself, this interaction could modestly inhibit translation by impeding binding or movement of the 43S preinitiation complex. This type of inhibitory effect has been shown for several proteins (Klausner et al., 1993; Stripecke et al., 1994) and would apply to the situation with the OBI mRNA. However, for osk mRNA this slight negative effect would be accompanied by a substantial increase in translation resulting from aub-dependent relief from repression, and the overall effect would be to enhance translation. While quite speculative, this model can account for the observed effects and should soon be testable.

**aub is pleiotropic**

aub mutations suppress a bicaudal phenotype, a property that allowed us to isolate novel aub alleles. However, translation of the OBI transgene, which was used to create the bicaudal phenotype, is not reduced in aub mutants. Exploring this apparent discrepancy, we found that aub acts more than once in posterior body patterning, first in translation of osk mRNA and again at a later stage prior to the posterior localization of nos mRNA. It is the second instance of aub action in posterior body patterning that presumably underlies suppression of the OBI-induced bicaudal phenotype by aub mutants. Given our results with osk and grk, it seems likely that in all cases the requirement for aub involves translational control, and so we expect that the translation of at least one additional mRNA is affected in aub mutants.

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**REFERENCES**


Murata, Y. and Wharton, R. P. (1995). Binding of pumilio to maternal mRNA raises another possibility. Although the interaction between OSK mRNA and bruno, an ovarian protein, bruno, binds sequences (BREs) in the osk 3’ UTR and prevents translation of osk mRNA prior to posterior localization (Kim-Ha et al., 1995). If the role of aub were to relieve bruno-mediated repression, then an osk mRNA with nonfunctional BREs would no longer require aub for efficient translation. As we have shown here, this is not the case. However, aub may override the action of a different translational repressor.

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