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Phylogenetic comparison of *oskar* mRNA localization signals

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ABSTRACT

As a way to spatially control the expression of genes within cells, RNA localization is being recognized as an important process by which proteins are restricted to specific subcellular domains, which occurs in more diverse types of tissue than previously considered. Although many localized RNAs have been identified, information on *cis*-acting elements of localization is still limited. As transcripts of *oskar* (*osk*) are known to localize to the posterior pole of oocytes, we computationally analyzed a conserved sequence among eight *Drosophila* species and tested its role as a localization element. Dimerization of *osk* mRNA did not occur when the motif was deleted, but this did not affect assembly of *osk* mRNA-containing ribonucleoprotein (RNP) complexes. Without the motif, however, large RNP complex particles accumulated in nurse cells, and only a small fraction of these RNP complexes was transported into oocytes and properly localized to the posterior pole. Therefore, this motif may be required for the early transport of *osk* mRNA into oocytes. Also, as dimerization of *osk* mRNA does not seem to be a prerequisite for the assembly of RNP complexes, a dimerization-independent mechanism may also serve to localize *osk* mRNA to the posterior pole.

1. Introduction

The establishment of polarity is important for many developmental processes and the maintenance of organismal structures. Disruption of polarity not only affects normal development but is also associated with many human diseases such as gastrointestinal disorders, renal failure, and metastatic cancer [1]. To establish polarity, many molecules are localized at specific sites, with mRNA localization being one of the major occurrences of polarization [2]. Since the discovery of localized mRNAs in various cells including oocytes, neurons, and fibroblasts [3–6], the *Drosophila* system has contributed substantially to our understanding of the role of and mechanisms for mRNA localization. In addition, a recent report on the isolation of large amounts of localized mRNAs that were previously known to be localized as proteins confirmed the importance of mRNA localization in many cellular processes [7,8]. Analysis of localization elements often provides valuable information on the mechanisms required by organisms to assure successful development. For example, analysis of a localization signal not only revealed its abundance in the 3′ untranslated region (UTR) but also the importance of translational control activity associated with the localization process [9–13]. Also, the use of reporter constructs to analyze *cis*-regulatory elements has revealed the coupling of splicing to cytoplasmic localization as well as novel localization mechanisms such as dimerization and hitchhiking [14,15].

Although studies on *trans*-acting localization elements have made substantial progress [16–19], relatively little information is available on *cis*-acting localization elements [20,21]. As many localized mRNAs have been found, progress in this area should accelerate. Identification of *cis*-acting localization elements has been a tedious task, however, as it involves the construction of several recombinant DNAs containing serial deletions and the generation of transgenic flies. Recently, whole genome sequences of 12 *Drosophila* species were reported [22]. This information can be useful for identifying conserved elements that are essential for animal survival. Many localized mRNAs have been characterized in *Drosophila* oocytes, with *oskar* (*osk*) mRNA being one major RNA required for the establishment of posterior polarity and germline during *Drosophila* embryonic development [23,24]. Therefore, we used *osk* mRNA to search for a conserved *cis*-localization element. We found a motif that was conserved across eight sequenced *Drosophila* species and experimentally confirmed its role in *osk* mRNA transport. Also, we found that dimerization of *osk* mRNA does not seem to be required to initiate assembly of the *osk* mRNA-transporting ribonucleoprotein (RNP) complex.

2. Materials and methods

2.1. Fly stocks

Drosophila ananassae (TSC#14024-0371.13), *Drosophila pseudoobscura* (TSC#14011-0121.94), *Drosophila sechellia*

Abbreviations: osk, *oskar*; UTR, untranslated region; Orb, oo 18 RNA-binding protein; RNP, ribonucleoprotein.

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(TSC#14021-0248.25), *Drosophila simulans* (TSC#14021-0251.194), *Drosophila virilis* (TSC#15010-1051.87), *Drosophila willistoni* (TSC#14030-0811.24), and *Drosophila yakuba* (TSC#14021-0261.01) were purchased from the *Drosophila* Species Stock Center (University of California, San Diego, USA).

2.2. Plasmid construction

For the analysis of a candidate localization element, reporter plasmids consisting of *osk* gene promoter and start codons and a green fluorescent protein (GFP) coding region replacing most of the *osk* coding region and 3′ UTR were constructed. A DNA fragment containing the 479-nucleotide *osk* promoter region, 15-nucleotide *osk* 5′ UTR, and 183-nucleotide N-terminal coding region was fused to the 730-nucleotide BamHI-NotI digested DNA fragment from pEGFP-N2 (GenBank Accession # U57608). The wild-type 3′ UTR or 61-nucleotide-deleted *osk* DNA fragment was fused to this construct.

A 61-nucleotide deletion in the 3′ UTR was generated using the QuickChange Site-directed mutagenesis kit (Agilent Technologies Company, USA) following the manufacturer's instructions. A template plasmid containing the wild-type *osk* 3′ UTR was denatured and annealed with two oligonucleotides containing the 61-nucleo tide region deletion (5′ATGCTGCATTTTGGCCGTAAATTATTGATGTG CTCAAGCAAA3′ and 5′TGCTTGAGCACATCAATAATTTACGGCCAAAAT GCAGCATGG3′). Pfu Turbo DNA polymerase was used for new DNA strand synthesis, and the non-mutated parental DNA template was digested with DpnI. The circular, nicked, newly synthesized double-stranded DNA was transfected into DH5α-competent cells. Construction of the mutated plasmid was confirmed by polymerase chain reaction (PCR) and sequencing analysis (data not shown).

2.3. RNA in situ hybridization

Digoxigenin (DIG)-labeled antisense probes were prepared as described by Kim-Ha et al. [23]. To detect the hybridized signal, alkaline phosphatase-mediated conversion of BCIP/NBT substrate or peroxidase-tyramide-mediated amplification was performed. Tyramide signal amplification was performed following the manufacturer's instructions (Invitrogen Ltd., UK) with some modifications. Specifically, for penetration of the probe, ovaries were treated with 3 μg/ml proteinase K for 13 min at room temperature followed by 30 min incubation on ice. Probe hybridization was performed at 55 °C.

2.4. RNA–protein double-labeling

For RNA–protein double-labeling, ovaries were treated as described for RNA *in situ* hybridization with the following modification. Instead of proteinase K treatment to enhance probe accessibility, ovaries were treated with cold 80% acetone for 10 min at −20 °C as described by Pare et al. [25] and then washed with 1× phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBT). After post-fixation in 4% paraformaldehyde for 20 min, ovaries were washed with PBT. After performing RNA probe hybridization and washes as described above, the primary antibody against oo 18 RNA-binding (Orb) protein (6H4, DSHB, University of Iowa, USA) was added to the biotin-labeled anti-DIG antibody (Jackson ImmunoResearch, USA) at a 1:10 ratio. After primary antibody incubation and washes, ovaries were incubated with Alexa Flour 488-conjugated goat anti-mouse antibody to detect Orb and horseradish peroxidase-conjugated streptavidin (Invitrogen Ltd., UK). Ovaries were washed and labeled with tyramide solution to detect hybridized RNAs.

3. Results

3.1. Localization of osk mRNA is conserved throughout the Drosophila genus

The 12 *Drosophila* species with sequenced genomes span a wide range of global distribution and vary considerably in morphology and behavior [26]. The phylogenetic distance between *Drosophila melanogaster* and the other 11 *Drosophila* species shows a gradient of evolutionary distances ranging from 1 million years to more than 30 million years [22,26]. As a range of divergence would be useful for comparing conserved regulatory motifs, we investigated whether the *osk* gene is conserved across these *Drosophila* genomes. Orthologous sequences to the *osk* gene were found in all 12 species. These sequences shared more than 70% of amino acids in the protein-coding region (data not shown). As *osk* is localized to the posterior pole of the oocyte during oogenesis, and this process is critical for posterior body patterning and germ cell formation, we selected seven *Drosophila* species in addition to *D. melanogaster* to include at least one of each phylogenic lineage and examined the localization of *osk* ortholog transcripts. *D. simulans*, *D. sechellia,* and *D. yakuba* belong to the melanogaster group of subgenus Sophophora. *D. pseudoobscura* and *D. willistoni* also belong to subgenus Sophophora, but one is in the obscura group and the other is in the willistoni group. *D. virilis* is most distantly related to *D. melanogaster* and belongs to subgenus Drosophila. In *D. melanogaster*, *osk* mRNA is synthesized in nurse cells and transported to the oocyte during early oogenesis (stages 1–6). During mid-oogenesis (stages 7–9), transportation from nurse cells to the oocyte continues, and transported *osk* mRNA transiently accumulates at the anterior margin of the oocyte but eventually becomes localized at the posterior pole (Fig. 1) [23,24]. As oogenesis progresses (stages 9–10), accumulation of *osk* mRNA at the posterior pole becomes apparent, and anteriorly localized *osk* mRNA disappears. A similar localization pattern of *osk* orthologous mRNAs was observed in all seven *Drosophila* species tested (Fig. 1). As the posterior localization of *osk* orthologs was detected even in very distantly related species, this conserved pattern indicates that *osk* mRNA localization is an essential step for early embryonic development throughout *Drosophila* species.

3.2. Identification of highly conserved sequences in the 3′ UTR of osk mRNA

As the localization pattern of *osk* mRNA and its orthologs is very similar, localization elements might be conserved across the eight tested *Drosophila* species. To identify conserved *cis*-acting regulatory elements, we compared sequences of *osk* and its orthologous genes among the eight species. As the presence of a *cis*-localization element of *osk* mRNA in the 3′ UTR was reported in *D. melanogaster*, we specifically compared sequences in 1 kilobase (kb) regions downstream from the termination codons of *osk* orthologs. Two regions were found to contain highly conserved patches. The upstream conserved motif was located within a sequence spanning 61 nucleotides (Fig. 2). The downstream conserved motif was present near the polyadenylation site (data not shown). As the replacement of this downstream region with a heterologous tubulin polyadenylation signal has been reported previously [27], we did not further analyze this region. Instead, the 61-nucleotide region was further analyzed for the presence of a localization signal.

3.3. The conserved sequence is required for the initial transport of osk mRNA from nurse cells to the oocyte

To test the significance of the conserved sequence in the localization process, we constructed a reporter gene containing the *osk* 3′

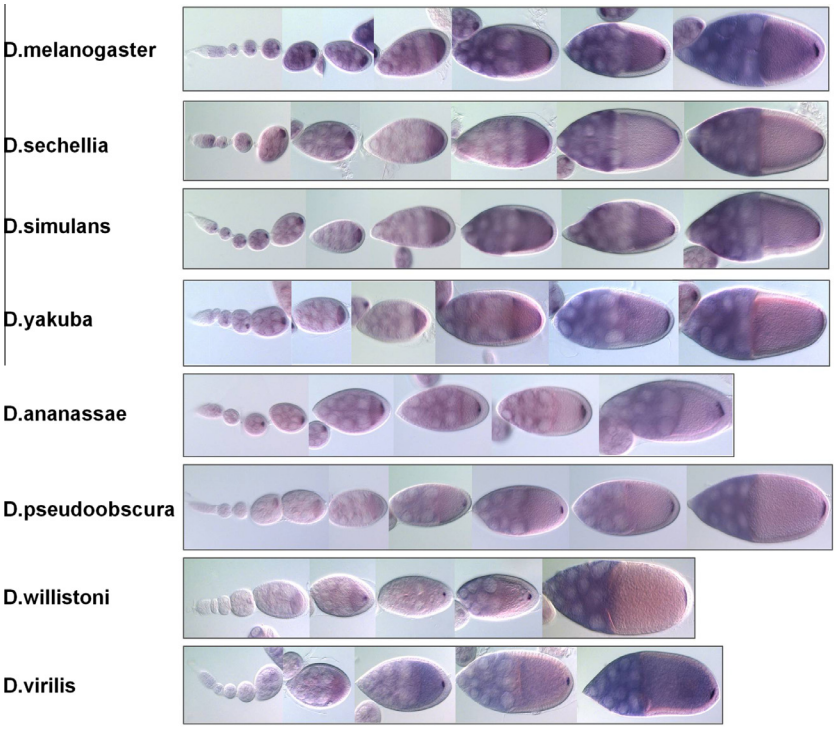

Fig. 1. *Osk* mRNA localization in various *Drosophila* species. Localization patterns of *osk* and its homologous transcripts from eight *Drosophila* species were examined using *in situ* hybridization. Localized RNAs appear as dark purple deposits. Progressively older egg chambers are displayed from left to right. In each egg chamber, the oocyte is located posteriorly (right side) relative to 15 anteriorly located nurse cells. *D. simulans*, *D. sechellia*, and *D. yakuba* are very closely related to *D. melanogaster*. By contrast, *D. virilis* is distantly related to *D. melanogaster*. The *osk* probe created from *D. melanogaster* was applied to the egg chambers of all species except *D. virilis*, for which a probe created from the orthologous gene of this species was used. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Dsim    629TGAAAATGCA-CTTGCTTTACTTGGAAAATTCGCTTGCACAAAATCAACGCCGCGGCTGATTT691
Dsec    630TGAAAATGCA-CTTGCTTCACTTGGAAAATTCGCTTGCACAAAATCAACGCCGCGGCTGATTT692
Dmel    630TGAAAATGCA-CT-GCTTTACTTGGAAAATTCGCTTGCACAAAATCAACGCCGCGGCTGATTT692
Dyak    618TGAAAATGCA-CTTGCTTTACTTGGAAAATTCGCTTGCACAGAATCAACGCCGCGGCTGATTT680
Dpse    662TGAAAATGCA-CTTGCTTCACTTTAAGCCTTCGCTTGCACAGAATCAACGCCGCGGCTGATTT724
Dana    617TGAAAATGCA-TTTGCTTCCCTTGAAGCACTCGCTTGCACAAAATCAACGCCGCGGCTGATTT679
Dvir    560TGAAAATGCA-TTTGCTTCACTT-ATAGCCTGGCTTGCACAAAATCAATACCGCGGTTGATTT622
Dwil    556TGAAAATGCAATTCGCCTCACTTCACATTCAAGCTTGGGGAAAATCAACGCCGCGGCTGATTT617
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Fig. 2. Computational analysis of conserved sequences and motifs in the 3′ UTR of *osk* homologs. Comparison of 3′ UTR sequences from eight *Drosophila* species was performed using the ClustalW program. The first nucleotide sequence after the stop codon was numbered as one for each species. Among the 1-kb 3′ UTR sequences, two patches of conserved sequences were identified. For the first region, conserved identical sequences are indicated by red asterisks. The second region was a conserved sequence motif previously found to be required for polyadenylation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

UTR with deletion of the 61-nucleotide conserved sequence (GFP-$osk^{\Delta 61}$). The transcript of the control construct, consisting of the *osk* promoter, reporter GFP gene, and wild-type *osk* 3′ UTR (GFP-osk^{wt}), showed a localization pattern identical to that of wild-type endogenous *osk* mRNA (Fig. 3A and B). By contrast, the GFP-$osk^{\Delta 61}$ transcript introduced a defect into the initial localization step, greatly reducing the transport of the transcript from nurse cells to the oocyte. Furthermore, retention of large particles containing the GFP-$osk^{\Delta 61}$ mRNA in nurse cell cytoplasm was apparent (Fig. 3A). Wild-type endogenous *osk* mRNA was also detected as particles in nurse cells. However, they were much smaller and detected at a much lower frequency (Fig. 3B). During later stages of oogenesis (stages 9–10), a relatively small amount of GFP-$osk^{\Delta 61}$ mRNA compared with GFP-osk^{wt} mRNA was localized at the posterior pole of oocytes (Fig. 3A), indicating proper function of the posterior localization process that directs movement of the GFP-$osk^{\Delta 61}$ transcript into the oocyte. In other words, the posterior localization process (i.e., later steps of the localization process) was not defective for the GFP-$osk^{\Delta 61}$ transcript. As the amount of large particles in nurse cells decreased compared with that detected in earlier stages, the RNP particles that were not transported into the oocyte appeared to disassemble or degrade between stage 8 and 9. To confirm that the smaller amount of *osk* transcript accumulation was not due to lower level of expression of the transgenic transcript, we examined expression levels of transgenic transcripts using both end-point and real-time reverse transcription PCR analysis and found that transgenic lines showed similar transcript expression levels (data not shown).

3.4. GFP-$osk^{\Delta 61}$ transgenic transcripts form a transport-defective RNP complex

As the posterior localization of *osk* mRNA is known to be Orb protein-dependent [28,29], the distribution of Orb protein was

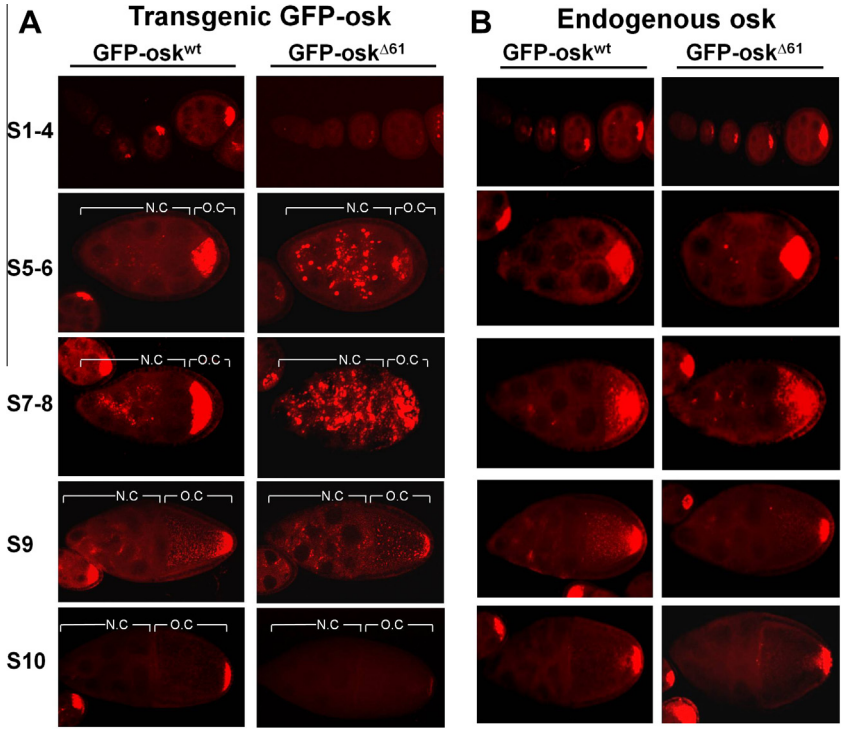

Fig. 3. Mutation in the conserved motif sequence leads to impaired transcript localization. (A) Transgenic fly strains expressing modified forms of the *osk* gene were tested for localization defects. Transcripts are shown as red fluorescent signals. Transcripts of the transgene harboring wild-type 3′ UTR (GFP-osk^{wt}) showed a localization pattern similar to that of wild-type endogenous *osk* transcripts. Complete deletion of the conserved motif (GFP-$osk^{\Delta 61}$) resulted in severe defects in early transport of *osk* transcripts into the oocyte (stages 1–4). Retention of transgenic transcripts as large particles in nurse cells was apparent for conserved motif-deleted transcripts (GFP-$osk^{\Delta 61}$) during stages 5–8. These large particles disappeared by stage 9, perhaps via dissociation or degradation, as the overall transcript signal decreased in stage 9 nurse cells. Conserved motif-deleted transcripts that entered the oocyte became localized to the posterior pole. Positions of the nurse cell (N.C.) and oocyte (O.C.) are indicated. (B) Localization pattern of endogenous *osk* transcripts was not affected in egg chambers harboring either GFP-osk^{wt} or GFP-$osk^{\Delta 61}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

examined in egg chambers containing wild-type or mutated transgenic transcripts. Transport of Orb protein into the oocyte was observed, and the localization pattern of Orb within the oocyte was similar in egg chambers containing wild-type or mutated transgenic transcripts. However, retention of Orb-containing large particles in nurse cells of mutant egg chambers was apparent during mid-oogenesis stages (Fig. 4A). As transgenic transcript-containing large particles were observed in GFP-$osk^{\Delta 61}$ transgenic egg chambers, we examined whether Orb and localization-detective GFP-$osk^{\Delta 61}$ transgenic transcripts are retained in the same particle. RNA–protein double-labeling showed that GFP-$osk^{\Delta 61}$ transcripts co-localized with Orb proteins within the large particles retained in nurse cells (Fig. 4B). Thus, these large particles observed in nurse cells appear to be defective RNP particles that are less efficient for early transport processes.

4. Discussion

mRNA localization has long been considered a special event that occurs in polarized cells and involves only a limited number of mRNAs. Recently, however, a large number of genes with localized protein products were found to produce mRNAs that become localized to specific tissue regions. Localization of diverse mRNAs to specific tissue sites might be controlled by shared or discrete RNA motifs. To understand the mechanism of RNA localization, we sought to identify RNA localization elements. By conducting a phylogenetic comparison of orthologous genes from diverse Drosophila species, we verified the usefulness of this method in identifying conserved regulatory motifs for RNA localization. Furthermore, by analyzing mRNAs deficient in localization elements, we gained insight into the mechanism of mRNA localization.

For *osk* mRNA localization, it has been suggested that a fusion construct with the coding region replaced by a reporter gene cannot localize by itself without the help of endogenous *osk* mRNA. Rather, the localization of intron-less lacZ-*osk*-3′ UTR may be explained by its hitchhiking onto endogenous *osk* mRNA localization complexes, forming a higher-order *osk* messenger RNP complex [14]. However, this dimerization and hitchhiking step is not necessary for either nuclear export or the early phase of *osk* mRNA transport from nurse cells into the oocyte [14]. Based on these findings, we examined whether dimerization occurs between endogenous and transgenic *osk* mRNA. As GFP-$osk^{\Delta 61}$ transgenic mRNA was retained in nurse cells as large particles, endogenous *osk* mRNA may also be retained in these particles if dimerization occurs. However, retention of endogenous *osk* mRNA as large particles was not observed (Fig. 3B). As the transport of mRNAs from nurse cells to the oocyte is not dependent on dimerization [14], our deleted motif might be required for interactions with other molecules that are critical for transport of mRNA from nurse cells into the oocyte. Most GFP-$osk^{\Delta 61}$ transcripts could not enter the oocyte, but after their successful entry, they properly localized to the posterior pole of the oocyte (Fig. 3A). As dimerization and assembly of an RNP complex would occur before entry into oocytes, dimerization of *osk* mRNA does not appear to be the first step in messenger RNP complex assembly. Also, the ability of the GFP-$osk^{\Delta 61}$ transcript, which did not dimerize with endogenous *osk* mRNA, to localize at the posterior pole upon entry into the oocyte implies the existence of additional localization mechanisms independent of dimerization.

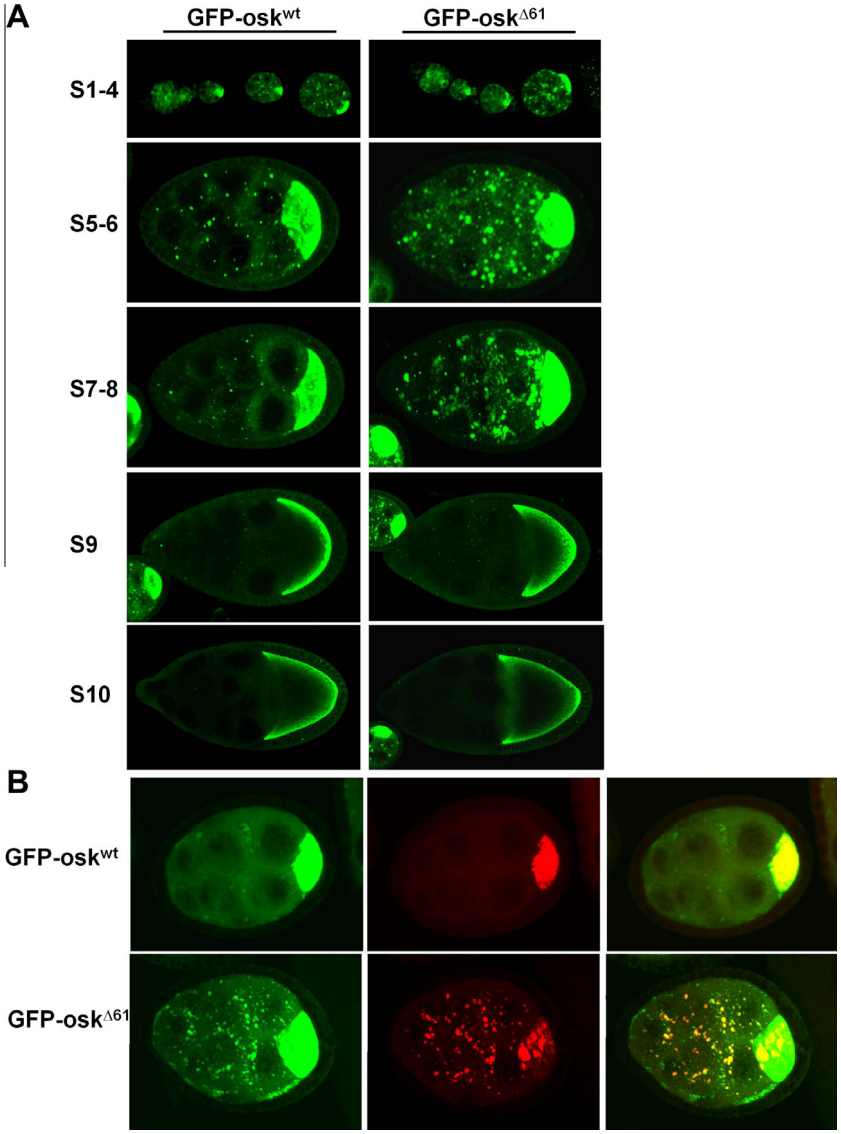

Fig. 4. Localization-defective mRNAs form RNP complexes with Orb protein. Localization of Orb protein was examined in both GFP-*osk*^{wt} and GFP-*osk*$^{\Delta 61}$ egg chambers. (A) Orb-containing particles (green) were observed during stages 5–8, but these particles were rarely seen during stage 9. Larger particles were detected in GFP-*osk*$^{\Delta 61}$ than in GFP-*osk*^{wt} egg chambers. (B) Egg chambers underwent RNA–protein double-labeling. Co-localization (yellow, right) of Orb protein (left, green) with GFP-*osk*$^{\Delta 61}$ transcripts (middle, red) was observed in both nurse cells and the oocyte. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Acknowledgments

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References

[1] J.P. Thiery, Epithelial–mesenchymal transitions in development and pathologies, Curr. Opin. Cell Biol. 15 (2003) 740–746.
[2] T.G. Du, M. Schmid, R.P. Jansen, Why cells move messages: the biological functions of mRNA localization, Semin. Cell Dev. Biol. 18 (2007) 171–177.
[3] W.R. Jeffery, C.R. Tomlinson, R.D. Brodeur, Localization of actin messenger RNA during early ascidian development, Dev. Biol. 99 (1983) 408–417.
[4] R. Lehmann, C. Nusslein-Volhard, Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*, Cell 47 (1986) 141–152.
[5] J.N. Wilcox, J.L. Robers, B.M. Chronwall, J.F. Bishop, T. O'Donohue, Localization of proopiomelanocortin mRNA in functional subsets of neurons defined by their axonal projections, J. Neurosci. Res. 16 (1986) 89–96.
[6] D.A. Melton, Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus oocytes*, Nature 328 (1987) 80–82.
[7] E. Lecuyer, H. Yoshida, N. Parthasarathy, et al., Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function, Cell 131 (2007) 174–187.
[8] S. Kwon, Single-molecule fluorescence in situ hybridization: quantitative imaging of single RNA molecules, BMB Rep. 46 (2013) 65–72.
[9] J. Kim-Ha, K. Kerr, P.M. Macdonald, Translational regulation of *oskar* mRNA by Bruno, an ovarian RNA-binding protein, is essential, Cell 81 (1995) 403–412.
[10] S.E. Bergsten, E.R. Gavis, Role for mRNA localization in translational activation but not spatial restriction of nanos RNA, Development 126 (1999) 659–669.
[11] B. Reveal, N. Yan, M.J. Snee, et al., BREs mediate both repression and activation of *oskar* mRNA translation and act in trans, Dev. Cell 18 (2010) 496–502.
[12] Y. Kato, A. Nakamura, Roles of cytoplasmic RNP granules in intracellular RNA localization and translational control in the *Drosophila* oocyte, Dev. Growth Differ. 54 (2012) 19–31.
[13] P. Lasko, MRNA localization and translational control in *Drosophila* oogenesis, Cold Spring Harb. Perspect. Biol. 4 (2012) a012294.
[14] O. Hachet, A. Ephrussi, Splicing of *oskar* RNA in the nucleus is coupled to its cytoplasmic localization, Nature 428 (2004) 959–963.
[15] H. Jambor, C. Brunel, A. Ephrussi, Dimerization of *oskar* 3′UTR promotes hitchhiking for RNA localization in the *Drosophila* oocyte, RNA 17 (2011) 2049–2057.

[16] M. Claussen, B. Suter, BicD-dependent localization process: from *Drosophila* development to human cell biology, Ann. Anat. 187 (2005) 539–553.
[17] D. Nashchekin, S. St Johnston, Egalitarian recruitment of localized mRNAs, Genes Dev. 23 (2009) 1475–1480.
[18] D. Dubin-Bar, A. Bitan, A. Bakhrat, et al., *Drosophila* javelin-like encodes a novel microtubule-associated protein and is required for mRNA localization during oogenesis, Development 138 (2011) 4661–4671.
[19] C.W. Chang, D. Nashchekin, L. Wheatley, et al., Anterior-posterior axis specification in *Drosophila oocytes*: identification of novel bicoid and oskar mRNA localization factors, Genetics 188 (2011) 883–896.
[20] P.M. Macdonald, K. Kerr, J.L. Smith, A. Leask, RNA regulatory element BLE1 directs the early steps of bicoid mRNA localization, Development 118 (1993) 1233–1243.
[21] V. Van De Bor, E. Hartswood, C. Jones, et al., Gurken and the I factor retrotransposon RNAs share common localization signals and machinery, Dev. Cell 9 (2005) 51–62.
[22] Drosophila 12 Genomes Consortium, Evolution of genes and genomes on the *Drosophila* phylogeny, Nature 450 (2007) 203–218.
[23] J. Kim-Ha, J.L. Smith, P.M. Macdonald, Oskar mRNA is localized to the posterior pole of the *Drosophila* oocyte, Cell 66 (1991) 23–35.
[24] A. Ephrussi, L.K. Dickinson, R. Lehmann, Oskar organizes the germ plasm and directs localization of the posterior determinant nanos, Cell 66 (1991) 37–50.
[25] A. Pare, D. Lemons, D. Kosman, et al., Visualization of individual Scr mRNAs during *Drosophila* embryogenesis yields evidence for transcriptional bursting, Curr. Biol. 19 (2009) 2037–2042.
[26] T.A. Markow, P.M. O'Grady, Drosophila biology in the genomic age, Genetics 177 (2007) 1269–1276.
[27] J. Kim-Ha, P.J. Webster, J.L. Smith, P.M. Macdonald, Multiple RNA regulatory elements mediate distinct steps in localization of oskar mRNA, Development 119 (1993) 169–178.
[28] L.B. Christerson, D.M. McKearin, Orb is required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis, Genes Dev. 8 (1994) 614–628.
[29] J.S. Chang, L. Tan, P. Schedl, The *Drosophila* CPEB homolog, orb, is required for oskar protein expression in oocytes, Dev. Biol. 215 (1999) 91–106.