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Supplemental materials and methods

RNA protein cross-linking immunoprecipitation and RT-PCR

Ovaries were hand dissected from 50 flies, and protein was extracted using a pipette pestle in 100 µl of extraction buffer (25 mM Hepes, pH 6.8, 50 mM KCl, 1 mM $MgCl_2$, 1 mM DTT, 125 mM sucrose, and proteinase inhibitor cocktail [Roche]). The protein extract was then cleared by centrifugation at 12,000 rpm for 5 min, and the supernatant was transferred to a new tube before the addition of 37% formaldehyde to a final volume of 1% (vol/vol). The solution was then incubated for 10 min with slow mixing at RT. The cross-linking reaction was quenched by the addition of glycine, pH 7.0, to a final concentration of 0.25 M. The extract was then incubated with 50 µg of anti-IMP immobilized on protein A–Sepharose and mixed for 2 h at 4°C. The Sepharose was gently spun down (1 min at 2,500 rpm) and washed five times with RIPA buffer (50 mM Tris, pH 7.5, 1% NP-40, 1% sodium decholate, 0.1% SDS, 1 mM EDTA, and 1 M NaCl). 100 µl of extraction buffer was added and heated at 70°C for 45 min to reverse the RNA protein cross-linking. The released proteins and RNAs were removed and placed in a new tube. 300 µl Trizol (Invitrogen) and 80 µl of chloroform were added, mixed, incubated at RT for 10 min, and then spun at 14,000 rpm for 10 min. The aqueous phase and isopropanol ppt were collected with glycoblue (Ambion) by spinning at 14,000 rpm for 10 min and then DNase treated with DNA-free (Ambion) and resuspended in 20 µl RNase-free water. 1 µl of the extracted RNA was taken for RT-PCR using Superscript II (Invitrogen) with Oligo(dt) and for PCR using primers specific for target RNAs. The following primers were used in this study: *tubulin 67c*, forward: GGCAGCCTGAAGACCAAG GAG, and reverse: CACTGCTCTGCGATCTTCTGC; *oskar*, forward: TCCCTGGAGAACATACCACGAG, and reverse: GCATTTACGCTGGCTTGCTG; *bicoid*, forward: CAAGAATGACGAAAGTCCGAGTCTG, and reverse: TCGTGCATTGATATTGGTTCGATTC.