Figure S4. The alternatively spliced intron is required for feedback regulation of Rbp1 and Rbp1-like. (A) Intron-containing transgenes. Diagram shows the structures of Rbp1 and Rbp1-like genomic constructs driven by the Actin5C promoter. Arrows indicate locations of PCR primers; the forward primer targets Actin5C sequences to distinguish transgene products from those of the endogenous genes or of the stably integrated cDNA constructs used for overexpression of the RBP1-RS and RBP1L-RS proteins under control of the metallothionein promoter. Gel photographs show RT-PCR analysis of the transgene RNAs in SL2 cells overexpressing RBP1-RS or RBP1L-RS. The ALT and RS isoforms were amplified with separate primers for 27 cycles (Rbp1) or 25 cycles (Rbp1-like). Although relative expression of the ALT isoforms from the transgenes is higher than for the endogenous gene (compare with Fig. 4), feedback regulation of the ALT/RS ratio is maintained. This is seen clearly for RS isoform levels in the photographs. The effects on ALT isoform levels seem small because the constructs (which overexpress the proteins and thus already trigger feedback) invert the ALT/RS ratio relative to the endogenous RNAs. However, even the more subtle increase in ALT forms upon overexpression of RBP1-RS is statistically significant, as shown by the quantitation relative to the lacZ cotransfection standard (graph at the right; P values are for differences from control; N=3 in each case). (B) Transgenes without introns. Diagram shows the structures of Rbp1 and Rbp1-like cDNA constructs corresponding to the RS isoforms, driven by the Actin5C promoter. Arrows indicate locations of PCR primers as in part A. Gel photographs show the analysis of the transgene RNAs as in part A (only RS isoforms can be produced in this case). No significant differences from the control were observed.

Methods: Rbp1 and Rbp1-like genomic DNAs were amplified using Platinum Taq-HIFI (Invitrogen, CA). Forward primers spanned the start codon and had an Xba I extension; reverse primers spanned the stop codon (primer sequences are shown in Online Supplementary Information Table II). The PCR products were digested with Xba I and cloned between the Xba I and Hpa I sites of pPAc-PL (gift of Carl Thummel). SL2 cells stably transfected with cDNA expression constructs under control of the metallothionein promoter (see text) were induced for 24 hours with 300 micromolar cupric sulfate before transient transfection with the reporters using Invitrogen’s calcium chloride protocol (Drosophila Expression System technical manual, Version H). A lacZ construct in plasmid pPAc-PL was co-transfected as a standard. Transfection was followed after 16 hr by a wash with Schneider medium and replating with cupric sulfate for 3 hr before RNA extraction. Expression of the reporter genes was analyzed by RT-PCR using primers in the Act5C 5’ UTR (F1pPAc) or 3’ UTR (B1pPAc) with an opposing primer targetting within the reporter gene as indicated in the figures (sequences shown in Online Supplementary Information Table I). The forward primer within the lacZ construct was LacZF1.
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Actin5C sequences to distinguish transgene products from those of the endogenous genes or of the stably
integrated cDNA constructs used for overexpression of the RBP1-RS and RBP1L-RS proteins under
control of the metallothionein promoter. Gel photographs show RT-PCR analysis of the transgene
RNAs in SL2 cells overexpressing RBP1-RS or RBP1L-RS. The ALT and RS isoforms were amplified
with separate primers for 27 cycles (Rbp1) or 25 cycles (Rbp1-like). Although relative expression of the
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