Supplementary information

Figure legends

Figure S1. Effect of Caf1 D161A, Pan2 D1083A, Tob(1-160), and Tob(110-218) on deadenylation of BGG (1-39)-MS2bs mRNA.
(A) T-REx-HeLa cells were co-transfected with the pFlag-CMV5/TO-BGG (1-39)-MS2bs reporter plasmid, pCMV-5xFlag-EGFP reference plasmid and either pCMV-5xMyc-Caf1 D161A (lanes 6-10), pCMV-5xMyc-Pan2 D1083A (lanes 11-15) or, as a control, pCMV-5xMyc (lanes 1-5). The transcriptional pulse-chase analysis was performed as described in Figure 7B. Steady-state BGG (1-39)-MS2bs mRNA (lane 16) and the deadenylated (A0) mRNA (lane 17) were analyzed as in Figure 7B. 5xFlag-EGFP mRNA served as a transfection/loading control.
(B) T-REx-HeLa cells were co-transfected with the pFlag-CMV5/TO-BGG (1-39)-MS2bs reporter plasmid, pCMV-5xFlag-EGFP reference plasmid and either pCMV-Myc-Tob (1-160) (lanes 6-10) or pCMV-Myc-Tob (110-218) (lanes 11-15) or, as a control, pCMV-Myc (lanes 1-5). The transcriptional pulse-chase analysis was performed as described in Figure 7B. Steady-state BGG (1-39)-MS2bs mRNA (lane 17) and the deadenylated (A0) mRNA (lane 16) were analyzed as in Figure 7B. 5xFlag-EGFP mRNA served as a loading control.
(C) The amount of the BGG(1-39)-MS2bs mRNA at each time point in Supplementary Figure S1A was quantitated, and the level of the mRNA at 0-time point was defined as 100% (left panel). The average length of the poly(A) tails at each time point was calculated from Supplementary Figure S1A (middle) and S1B (right) by the auto peak search analysis in Image Gauge ver. 4.23 (FUJIFILM) software. Error bars represent the standard deviation of three independent experiments.

Figure S2. Downregulating Tob partially represses the CPEB3-accelerated deadenylation of BGG (1-39)-MS2bs mRNA.
(A) T-REx-HeLa cells were co-transfected with the pFlag-CMV5/TO-BGG (1-39)-MS2bs reporter plasmid, pCMV-5xFlag-EGFP reference plasmid either pMS2-HA (lanes 3-7, 13-17) or pMS2-HA-CPEB3 (lanes 8-12, 18-22), and either Tob/Tob2
siRNA (lanes 13-22) or a control luciferase siRNA (lanes 3-12). The transcriptional pulse-chase analysis was performed as described in Figure 7B. mRNA half-lives of 5.8 h, 2.7 h, 6.2 h, and 4.8 h were calculated from lanes 3-7, 8-12, 13-17, and 18-22, respectively. Steady-state BGG (1-39)-MS2bs mRNA (lanes 1 and 23) and the deadenylated (A0) mRNA (lanes 2 and 24) were analyzed as in Figure 6(F). 5xFlag-EGFP mRNA was served as a loading control. Results are representative of three independently performed experiments.

(B) The levels of BGG (1-39)-MS2bs mRNA were quantified by real-time PCR with the level of the mRNA from 0-h time point defined as 100%. Results are the average of three independently performed experiments.

(C) Down-regulation of Tob/Tob2 in T-REx-HeLa cells. Tob mRNA, Tob2 mRNA and 28S rRNA were analyzed by real-time PCR.

(D) T-REx-HeLa cells were transfected with either Tob/Tob2 siRNA (lane 2) or a control luciferase siRNA (lane 1). Total cell lysate was analyzed by Western blotting using anti-Tob or anti-GAPDH. Tob/Tob2 siRNAs reduced the level of Tob protein to 41%.

(E) COS-7 cells were transfected with pHA-CMV5-CPEB3 and either pME-Flag (lanes 1, 4), pME-Flag-Tob (lanes 2, 5), or pME-Flag-Tob2 (lanes 3, 6). The cell extracts were subjected to an immunoprecipitaion assay (IP) using anti-Flag antibody. The immunoprecipitates (lanes 4-6) and inputs (lanes 1-3, 10% of the amount immunoprecipitated) were analyzed by Western blotting (WB) using the indicated antibodies.

(F) The average length of the poly(A) tails at each time point was calculated from lanes 3-7 and 13-17 in Supplementary Figure S2A by the auto peak search analysis in Image Gauge ver. 4.23 (FUJIFILM) software. Error bars represent the standard deviation of three independent experiments.

Figure S3. HA-CPEB3 binds endogenous Tob and Caf1.

HeLa cells (lanes 1-4) or COS-7 cells (lanes 5-8) were transfected with pHA-CMV5 (lanes 1, 3, 5, 7) or pHA-CMV5-CPEB3 (lanes 2, 4, 6, 8). The cell extracts were subjected to an immunoprecipitaion assay (IP) using anti-HA antibody. The immunoprecipitates (lanes 3, 4, 7, 8) and inputs (lanes 1, 2, 5, 6, 10% of the amount immunoprecipitated) were analyzed by Western blotting (WB) using anti-Tob, anti-Caf1, or anti-HA.
Hosoda Fig S3

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(kDa)

47  35  84

1  2  3  4  5  6  7  8

Tob (WB: anti-Tob)
Caf1 (WB: anti-Caf1)
HA-CPEB3 (WB: anti-HA)