Biochemical and Biophysical Research Communications 455 (2014) 323–331

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Arsenite inhibits mRNA deadenylation through proteolytic degradation of Tob and Pan3

Ryota Yamagishi, Nao Hosoda, Shin-ichi Hoshino *

Department of Biological Chemistry, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya 467-8603, Japan

ARTICLE INFO

Article history:
Received 28 October 2014
Available online 15 November 2014

Keywords:
Deadenylation
Tob
Pan3
Deadenylase
PAM2
HRI

ABSTRACT

The poly(A) tail of mRNAs plays pivotal roles in the posttranscriptional control of gene expression at both translation and mRNA stability. Recent findings demonstrate that the poly(A) tail is globally stabilized by some stresses. However, the mechanism underlying this phenomenon has not been elucidated. Here, we show that arsenite-induced oxidative stress inhibits deadenylation of mRNA primarily through downregulation of Tob and Pan3, both of which mediate the recruitment of deadenylases to mRNA. Arsenite selectively induces the proteolytic degradation of Tob and Pan3, and siRNA-mediated knockdown of Tob and Pan3 recapitulates stabilization of the mRNA poly(A) tail observed during arsenite stress. Although arsenite also inhibits translation by activating the eIF2α kinase HRI, arsenite-induced mRNA stabilization can be observed under HRI-depleted conditions. These results highlight the essential role of Tob and Pan3 in the stress-induced global stabilization of mRNA.

1. Introduction

Eukaryotic cells have evolved various stress response systems to preserve the accurate regulation of gene expression. Of those systems, stabilization of the mRNA poly(A) tail and the inhibition of translation were reported to be important phenomena in mRNA metabolism under stress conditions. They have been shown to induce the reprogramming of translation and mRNA decay to regulate selective gene expression for adaptation and responses to stresses.

Under non-stress conditions, the 3′ poly(A) tail of mRNA binds to the 5′ cap together with the cytoplasmic poly(A) binding protein, PABPC1, which specifically interacts with the poly(A) tail, and circularizes mRNA to activate translation by recruiting the terminating ribosome to the next round of translation [1–3]. On the other hand, the poly(A) tail also regulates the stability of mRNA [4], and poly(A) tail shortening, which is termed deadenylation, is the rate-limiting step in mRNA decay [5]. Thus, the poly(A) tail plays a crucial role in the post-transcriptional control of gene expression. A previous study showed that the poly(A) tail is stabilized under stress conditions [6–8]. However, the molecular mechanism and target of stress that induces stabilization of the mRNA poly(A) tail remain unclear. Deadenylation is generally mediated by two major deadenylase complexes, Pan2–Pan3 and Caf1–Ccr4 [9]. The Pan2–Pan3 complex consists of catalytic subunit Pan2 and regulatory subunit Pan3 [10], and both the Caf1 and Ccr4 subunits exhibit the catalytic activity of deadenylase [11–13]. We previously reported that the termination of translation triggers mRNA deadenylation [14], and proposed a mechanism of mRNA deadenylation in which the termination complex eRF1–eRF3 is released from PABPC1 after the termination of translation and the two deadenylase complexes are then recruited to PABPC1 to degrade the poly(A) tail of mRNA [15,16]. We also demonstrated that the anti-proliferative protein Tob is involved in deadenylation. In this mechanism, Pan3 and Tob mediate the recruitment of deadenylase complexes to PABPC1 by directly binding to PABPC1 via their PAM2 motifs. Furthermore, eRF3 has a PAM2 motif, and regulates the initiation of deadenylation by competing with Pan3 or Tob for the binding of PABPC1 [15,17]. Thus, PAM2 motif-containing proteins as well as deadenylases play pivotal roles in mRNA deadenylation. Therefore, it is conceivable that any of the factors involved in deadenylation may be targeted by stresses to inhibit deadenylation. We herein show that arsenite stress induces degradation of the two PAM2-containing proteins, Tob and Pan3, and downregulation of Tob and Pan3 is the primary cause responsible for the stabilization of the mRNA poly(A) tail.

Abbreviations: HRI, heme-regulated inhibitor kinase; PAM2, PABP-interacting motif 2.

* Corresponding author. Fax: +81 52 836 3427.
E-mail address: hoshino@phar.nagoya-cu.ac.jp (S.-i. Hoshino).

http://dx.doi.org/10.1016/j.bbrc.2014.11.015
0006-291X/

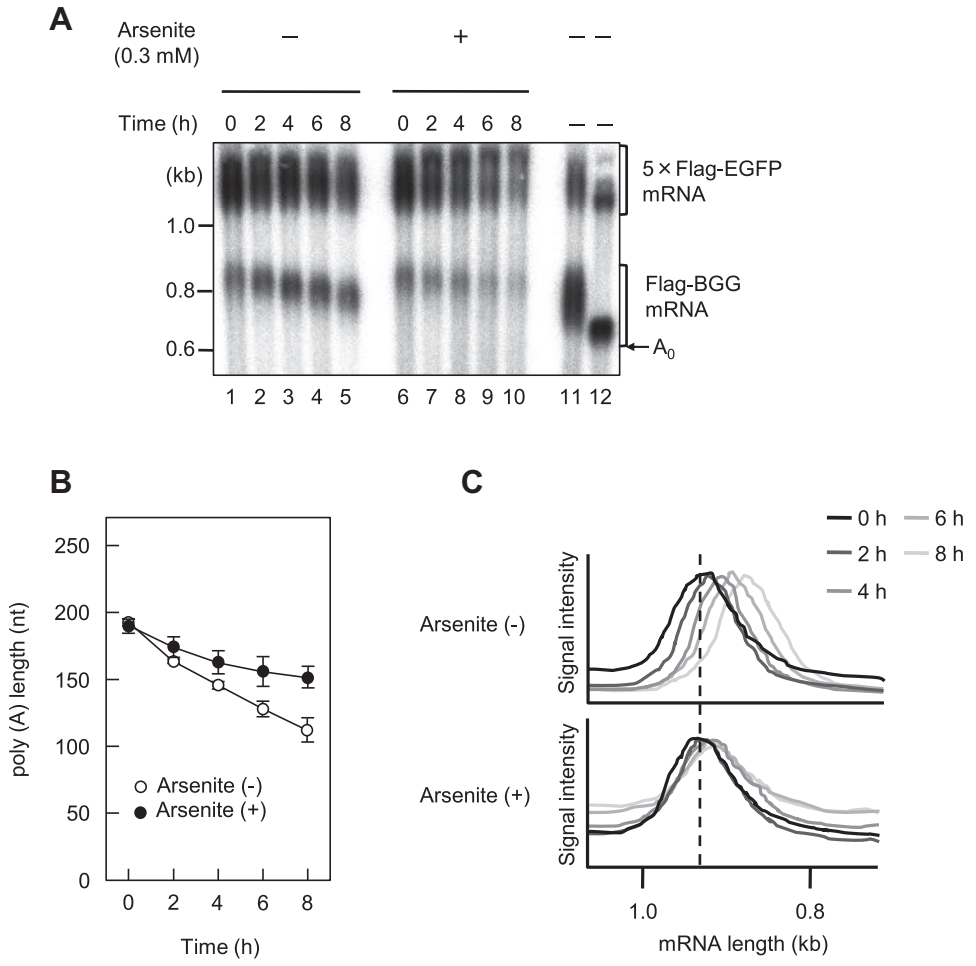

Fig. 1. Arsenite-induced oxidative stress inhibits mRNA deadenylation. (A) T-REx-HeLa cells were co-transfected with a pFlag-CMV5/TO-BGG reporter plasmid and pCMV-5 × Flag-EGFP reference plasmid. One day later, β-globin mRNA was induced by tetracycline for 2 h, and the cells were harvested at a specified time after the shutoff of β-globin mRNA transcription and addition of arsenite (lanes 6–10). To mark the fully deadenylated (A0) β-globin mRNA, β-globin mRNA that was induced to be expressed by the treatment with tetracycline for 24 h (lane 11) was digested with RNase H in the presence of oligo(dT) (lane 12). β-Globin mRNA was detected by Northern blot analysis. (B) The average length of the poly(A) tails at each time point was measured. Error bars represent the standard deviation of three independent experiments. (C) The distribution of β-globin mRNA was visualized by quantifying the signal intensity from (A). (D–F) As in (A–C), except that cells were co-transfected with a pFlag-CMV5/TO-BGG-cMyc 3′ UTR reporter plasmid and pCMV-5 × Flag-GST-CAT reference plasmid.

2. Materials and methods

2.1. Cell culture and DNA/RNA transfection

T-REx HeLa cells were cultured in Dulbecco's modified Eagle's medium (Nissui) supplemented with 5% fetal bovine serum. DNA/RNA transfection was performed using Lipofectamine 2000 (Invitrogen) or Lipofectamine RNAi Max (Invitrogen) as described previously [18,19].

2.2. siRNA

The sequence of siRNAs for luciferase and Tob were described previously [20]. The sequence of other siRNAs were the following: Tob2 siRNA (5′ r(CUCUCUGUCUAUGCAUUCA)d(TT) 3′), Pan3 siRNA (5′ r(GUCUCACAGAUUCCUAUUU)d(TT) 3′), HRI siRNA (5′ r(CUUAAUGAGGUCUGCUAAA)d(TT) 3′).

2.3. Antibodies

The antibodies used in this study were anti-eIF2α (FL-315, SANTA CRUZ) and anti-eIF2α-P (phospho-Ser52, assay designs). Anti-Pan3, and anti-Pan2 antibodies were raised against Pan3 (1-149 a.a.) and Pan2 (1-357 a.a.), respectively. Anti-Tob [20], anti-GAPDH [19], anti-GSPT [21], and anti-PABPC1 [22] were described previously. The anti-Caf1 antibody was a kind gift from Dr. Ann-Bin Shyu [23].

2.4. RNA analysis

The transcriptional pulse-chase analysis, total RNA isolation and Northern blot analysis were performed as described previously [18,20].

3. Results

3.1. Arsenite-induced oxidative stress inhibits mRNA deadenylation

Environmental stress globally inhibits mRNA deadenylation [6–8]. However, the underlying mechanism has not been elucidated. To examine the effects of stress on mRNA deadenylation in more detail, we first confirmed the inhibition of mRNA deadenylation during arsenite-induced oxidative stress using a tetracycline

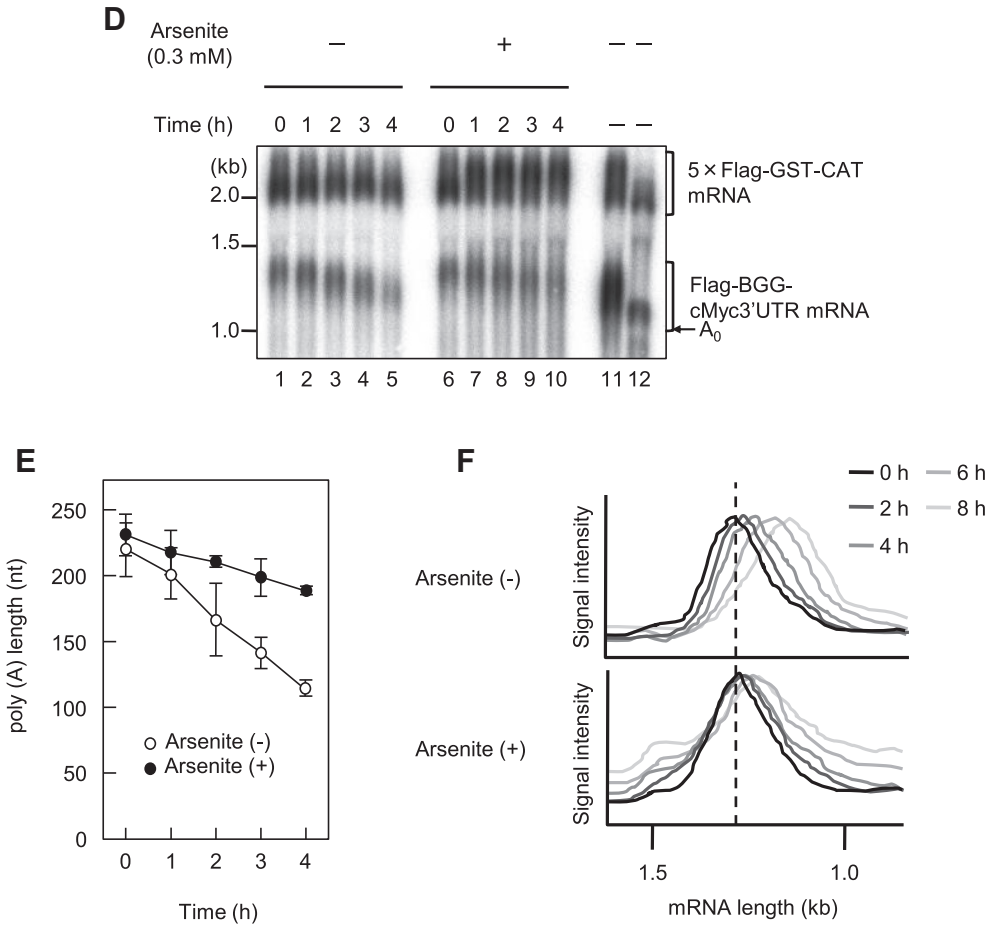

Fig. 1 (*continued*)

regulatory transcriptional pulse-chase analysis [15]. We monitored the kinetics of two reporter mRNAs. One is a stable β-globin reporter with β-globin 3′ UTR (hereafter referred to as β-globin mRNA) [15], and the other is an unstable β-globin reporter with c-myc 3′ UTR (c-myc mRNA) [18]. T-REx HeLa cells were co-transfected with a plasmid expressing either β-globin or c-myc reporter mRNAs and a reference plasmid expressing either 5 × Flag-EGFP or 5 × Flag-GST-CAT mRNAs as a transfection/loading control. After transcription was induced by tetracycline for 2 h, arsenite was added to the cells, and the kinetics of newly synthesized mRNA was analyzed. As shown in Fig. 1A–C, the deadenylation rate of β-globin mRNA was significantly decreased by arsenite, while that of c-myc mRNA was also decreased during arsenite stress (Fig. 1D–F). These results indicate that the deadenylation of both stable β-globin and unstable c-myc reporter mRNAs are inhibited during arsenite-induced oxidative stress.

3.2. Tob and Pan3 are selectively degraded during arsenite-induced oxidative stress

We previously reported the mechanism of mRNA deadenylation, in which the translation termination factor eRF3 and two major cytoplasmic deadenylase complexes, Pan2–Pan3 and Caf1–Ccr4–Tob, play central roles through their binding to PABPC1 [15]. Thus, we hypothesized that any of these factors may be targeted by arsenite-induced oxidative stress to inhibit deadenylation. To examine this, we analyzed the expression levels of Tob, Pan3, PABPC1, eRF3, Caf1, and Pan2 proteins during arsenite stress by Western blotting (Fig. 2A). Among these proteins, the expression level of the Tob protein was rapidly decreased by the exposure to arsenite. The expression level of the Pan3 protein was also decreased, although with slower kinetics than that of Tob (Fig. 2A lanes 5–7). Thus, Tob and Pan3 were selectively degraded during arsenite stress. We attributed the observed decrease in protein levels to proteolysis, because a previous study reported that the Tob protein was degraded by proteasome during ultraviolet (UV)-induced stress [24]. We pretreated T-REx HeLa cells with the proteasome inhibitor MG132 before the addition of arsenite and examined the effects of MG132 on the arsenite-induced degradation of Tob and Pan3 (Fig. 2B, lanes 12–16). As shown in Fig. 2B, MG132 repressed arsenite-induced Tob degradation. On the other hand, MG132 did not significantly affect the expression level of Pan3 (Fig. 2B, lanes 12–16). Taken together, these results indicate that arsenite induces the proteasomal degradation of Tob and other proteases are involved in the arsenite-induced degradation of Pan3.

3.3. siRNA-mediated knockdown of Tob and Pan3 can recapitulates stabilization of the mRNA poly(A) tail observed during arsenite stress

The above results led us to hypothesize that arsenite may inhibit deadenylation through degradation of Tob and Pan3. To test this, we utilized an siRNA-mediated knockdown strategy. First, we investigated the deadenylation kinetics of the stable β-globin mRNA. T-REx HeLa cells were co-transfected with a plasmid expressing β-globin reporter mRNA, a reference plasmid expressing

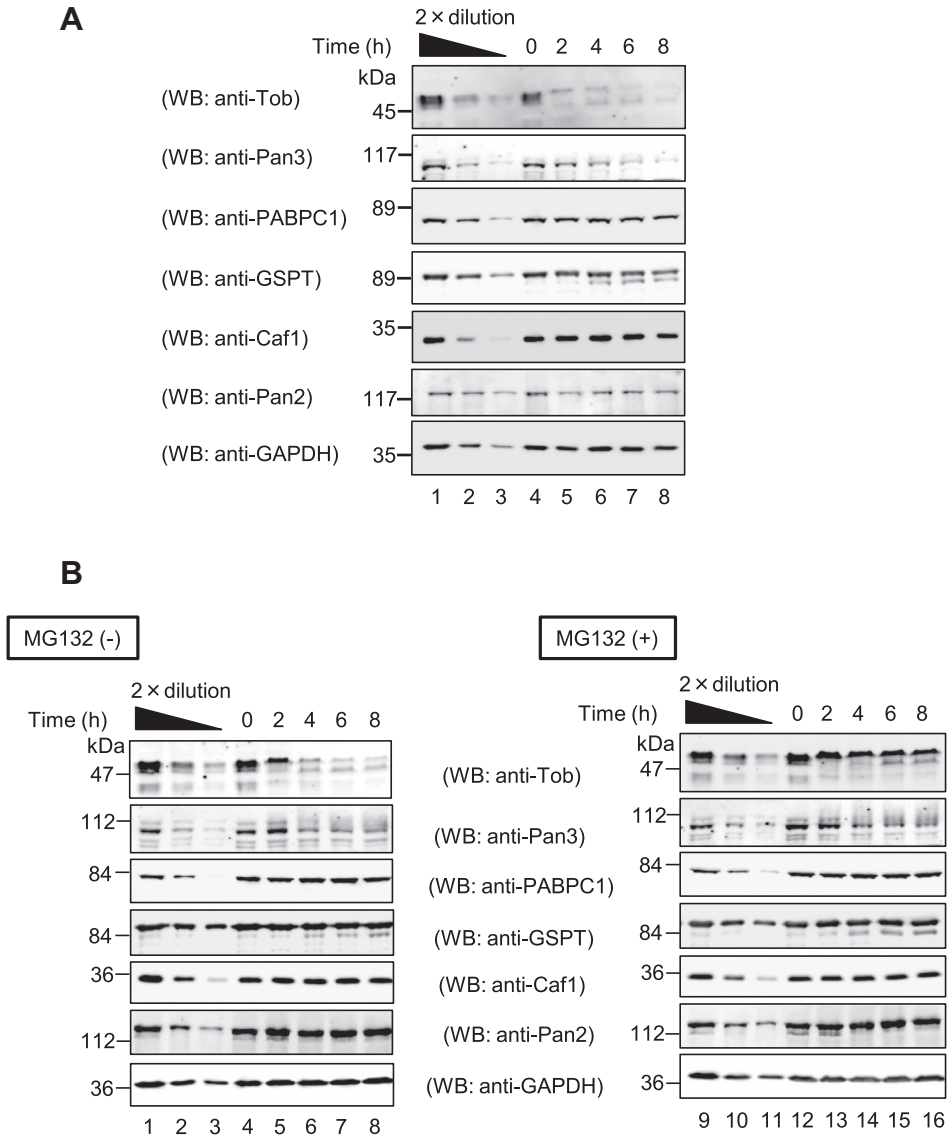

Fig. 2. Tob and Pan3 are selectively degraded during arsenite-induced oxidative stress. (A) T-REx-HeLa cells were harvested at a specified time after the addition of arsenite, and proteins were analyzed by Western blotting with the indicated antibodies. The three leftmost lanes, which analyzed two-fold dilutions of cellular protein, show that the conditions used for Western blotting were semi-quantitative. (B) As in (A), except that MG132 was added to cells 30 min prior to the addition of arsenite.

5 × Flag-EGFP mRNA as a transfection/loading control, and either Tob1/Tob2 siRNA, Pan3 siRNA, or control siRNA, and deadenylation kinetics were monitored as above. The knockdown of Tob inhibited the deadenylation of β-globin mRNA, the kinetics of which were similar to those during arsenite stress (Fig. 3A–C). On the other hand, the knockdown of Pan3 specifically inhibited the early phase of deadenylation (Fig. 3B), the kinetics of which were slightly different from those during arsenite stress. The expression of Tob and Pan3 was confirmed by Western blotting with the indicated antibodies (Fig. 3D). These results suggest that the stress-induced inhibition of β-globin mRNA deadenylation is mainly caused by the degradation of Tob, and only partially by that of Pan3.

Next, we investigated the deadenylation kinetics of unstable c-myc mRNA. To identify the deadenylases responsible for c-myc mRNA deadenylation, the effects of the overexpression of nuclease-deficient Caf1 (Caf1 D161A) and Pan2 (Pan2 D1083A) on c-myc mRNA deadenylation were analyzed. T-REx HeLa cells were co-transfected with a plasmid expressing c-myc mRNA, a reference plasmid expressing 5 × Flag-GST-CAT, and either pCMV-5 × Myc-Pan2 D1083A, pCMV-5 × Myc-Caf1 D161A or, as a control, pCMV-5 × Myc, and deadenylation kinetics were analyzed. The overexpression of Caf1 D161A almost completely repressed the rate of deadenylation of c-myc mRNA (Supplementary Figure lanes 11–15), whereas Pan2 D1083A had no significant effect on the rate of deadenylation (Supplementary Figure lanes 6–10). These results demonstrate that the Caf1–Ccr4–Tob complex, but not the Pan2–Pan3 complex, is responsible for c-myc mRNA deadenylation. Therefore, we hypothesized that arsenite inhibits the deadenylation of c-myc mRNA through degradation of Tob and, thus analyzed the effects of the knockdown of Tob on c-myc mRNA deadenylation. T-REx HeLa cells were co-transfected with a plasmid expressing c-myc mRNA, a reference plasmid expressing 5 × Flag-GST-CAT, and either Tob1/Tob2 siRNA or control siRNA, and deadenylation was monitored as above. As shown in Fig. 3D–F, the knockdown of Tob repressed c-myc mRNA deadenylation, the kinetics of which were similar to those during arsenite stress. These results suggest that the stress-induced inhibition of c-myc mRNA deadenylation is caused by the degradation of Tob. Thus,

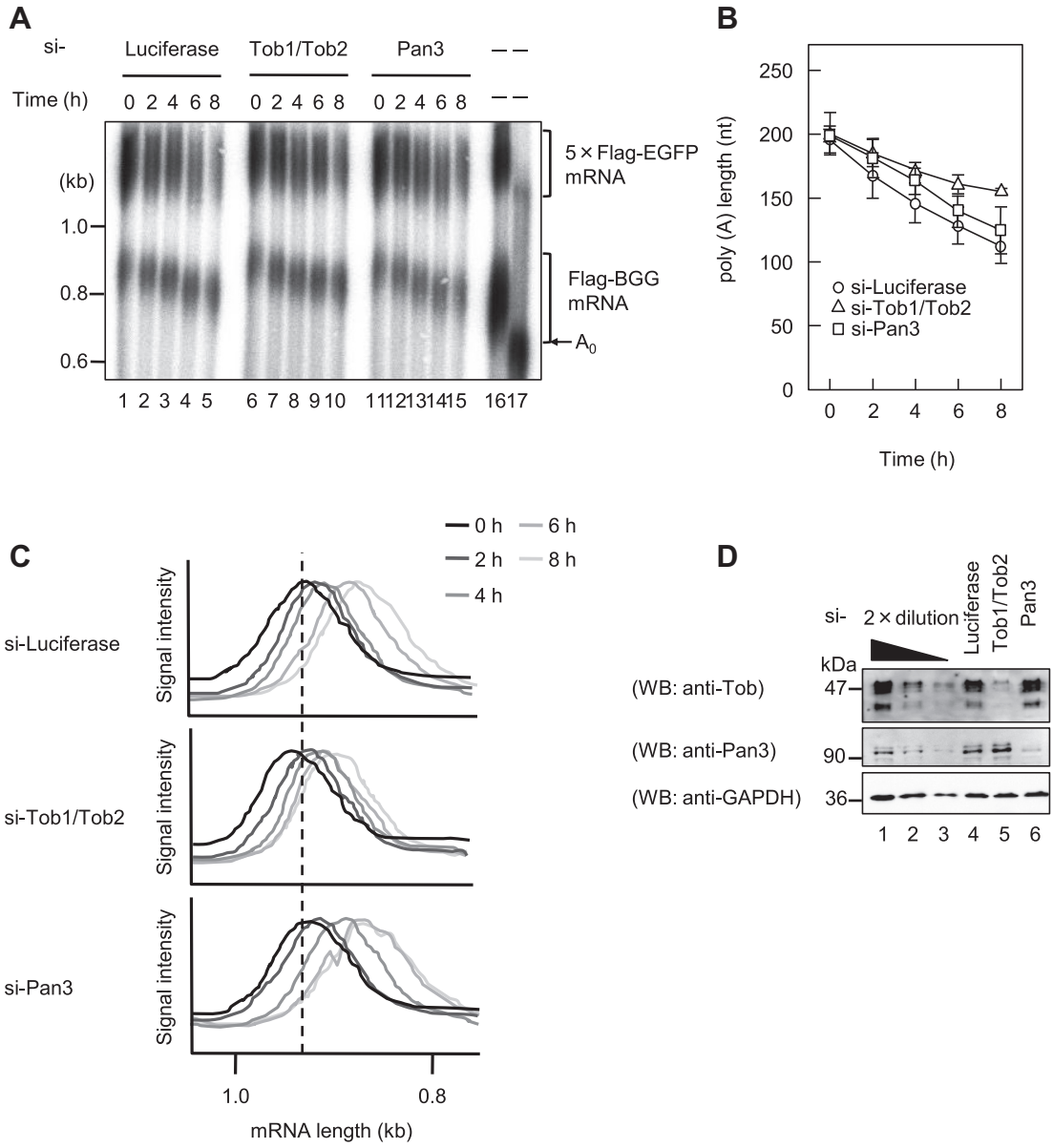

Fig. 3. siRNA-mediated downregulation of either Tob1/Tob2 or Pan3 recapitulates the inhibition of mRNA deadenylation during arsenite-induced oxidative stress. (A) T-REx HeLa cells were co-transfected with a pFlag-CMV5/TO-BGG reporter plasmid, pCMV-5 × Flag-EGFP reference plasmid, and either Luciferase siRNA (lanes 1–5), Tob1/Tob2 siRNAs (lanes 6–10), or Pan3 siRNA (lanes 11–15). One day later, β-globin mRNA was induced by tetracycline for 2 h. Cells were harvested at the specified time after the shutoff of β-globin mRNA transcription. Fully deadenylated mRNA (A0) was marked as in Fig. 1A. (B) The average length of the poly(A) tails at each time point was measured. (C) The distribution of β-globin mRNA was visualized by quantifying the signal intensity from (A). (D) Proteins were analyzed by Western blotting with the indicated antibodies. The three leftmost lanes, which analyzed two-fold dilutions of cellular protein, show that the conditions used for Western blotting were semi-quantitative. (E–H) As in (A–D), except that cells were co-transfected with a pFlag-CMV5/TO-BGG-cMyc 3′ UTR reporter plasmid, pCMV-5 × Flag-GST-CAT reference plasmid, and either Luciferase siRNA ((E) lanes 1–5) or Tob1/Tob2 siRNAs ((E) lanes 6–10).

we conclude that arsenite induces the degradation of Tob and Pan3 to inhibit mRNA deadenylation.

3.4. Arsenite-induced inhibition of mRNA deadenylation is not dependent on HRI-mediated inhibition of translation

Arsenite not only induces stabilization of the mRNA poly(A) tail, but also inhibits protein synthesis by phosphorylating the initiation factor eIF2α, which disrupts the eIF2–GTP–$tRNA^{Met}$ ternary complex [25]. This suggests that the inhibition of mRNA deadenylation by arsenite might be dependent on the inhibition of translation during arsenite stress. A previous study demonstrated that the arsenite-induced phosphorylation of eIF2α and inhibition of protein synthesis requires heme-regulated inhibitor kinase (HRI) [26]. Thus, we performed the knockdown of HRI and analyzed the deadenylation kinetics of reporter mRNA during arsenite stress. T-REx HeLa cells were co-transfected with a plasmid expressing β-globin mRNA or c-myc mRNA, a reference plasmid expressing 5 × Flag-EGFP mRNA or 5 × Flag-GST-CAT mRNA as a transfection/loading control, and either HRI siRNA or control siRNA, and deadenylation kinetics were monitored as in Fig. 1. Arsenite induced the phosphorylation of eIF2α, which was completely inhibited by the knockdown of HRI. Under this HRI knockdown condition, the arsenite-induced inhibition of mRNA deadenylation

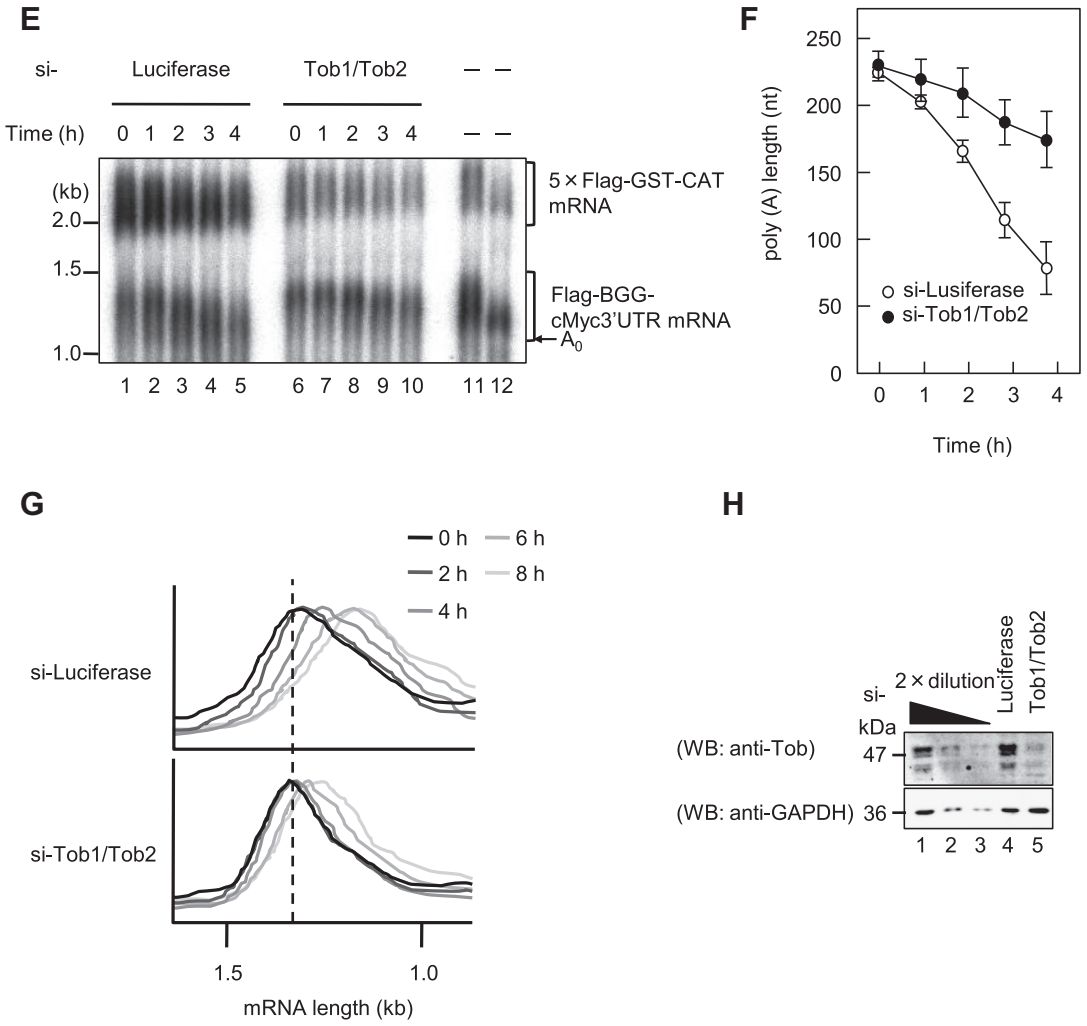

Fig. 3 (*continued*)

was still observed for both β-globin (Fig. 4B, compare lanes 11–15 and 16–20, and C) and c-myc reporter mRNAs (Fig. 4D, compare lanes 11–15 and 16–20, and E). These results indicate that arsenite inhibits mRNA deadenylation irrespective of the HRI-mediated phosphorylation of eIF2α.

4. Discussion

In yeast and mammals, cellular stress generally induces stabilization of the mRNA poly(A) tail. However, the mechanism underlying this phenomenon has not been elucidated. Stress-induced stabilization of the mRNA poly(A) tail is recognized as a global phenomenon regardless of the mRNA species [6–8]. This prompted us to speculate that the factors involved in general mRNA deadenylation may be targeted by stress. We previously proposed a mechanism of mRNA deadenylation, in which the termination factor eRF3 dissociates from the poly(A)-binding protein PABPC1 after the termination of translation, and in turn, the deadenylase complexes, Pan2–Pan3 and Tob–Caf1–Ccr4, associate with PABPC1 to degrade the poly(A) tail [15,16]. Thus, we analyzed the expression levels of Tob, Pan3, PABPC1, eRF3, Caf1, and Pan2 proteins during arsenite stress and found that the levels of Tob and Pan3 proteins are selectively decreased during arsenite stress (Fig. 2A). The arsenite-induced degradation of Tob, but not Pan3, appears to be mediated by the proteasome. The siRNA-mediated knockdown of Tob and Pan3 can recapitulate stabilization of the mRNA poly(A) tail observed during arsenite stress (Fig. 3). Although arsenite also induces the inhibition of translation by activating the eIF2α kinase HRI, the arsenite-induced inhibition of deadenylation can be observed under HRI-depleted conditions (Fig. 4). From these results, we conclude that the stress-induced stabilization of the mRNA poly(A) tail is caused by the degradation of Tob and Pan3. Since the two PAM2-containing proteins Tob and Pan3 mediate the recruitment of Caf1–Ccr4 and Pan2 deadenylases to PABPC1-bound mRNA, it is conceivable that the proteolytic degradation of Tob and Pan3 leads to the dissociation of Caf1–Ccr4 and Pan2 deadenylases from mRNA and, thus, inhibits deadenylation.

We previously showed that, in addition to the role of Tob in the general deadenylation and decay of mRNA [15,16], Tob also functions in the transcript-specific regulation of deadenylation through binding to sequence-specific RNA-binding proteins, CPEB and CPEB3. Tob recruits Caf1 to CPEB and CPEB3 to accelerate deadenylation and decay of their specific targets, including c-myc and AMPA receptor (GluR2) mRNAs [18,20]. The transcript-specific regulation by Tob–Caf1–Ccr4 via binding to CPEBs appears to be dominant over the general regulation of deadenylation via PABPC1 [20]. In this study, we examined c-myc mRNA as a representative example of short-lived mRNAs and found that c-myc mRNA is also stabilized during arsenite stress (Fig. 1D). The siRNA-mediated knockdown of Tob can recapitulate stabilization (Fig. 3). Thus, even in the case of unstable c-myc mRNA, stress-induced stabilization of the poly(A) tail is also explained by the degradation of Tob.

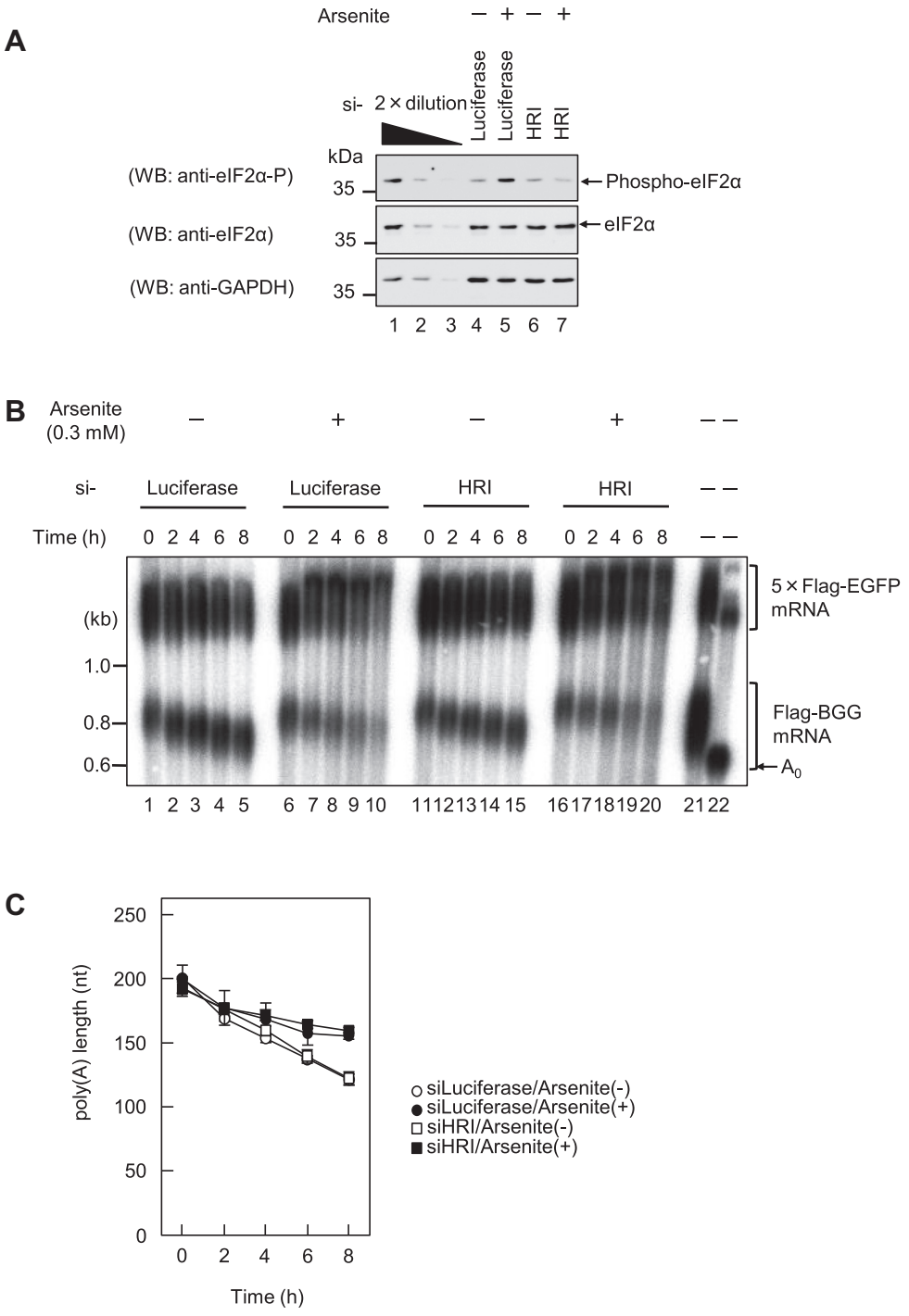

Fig. 4. Inhibition of mRNA deadenylation during arsenite-induced oxidative stress occurs independently of the HRI-mediated phosphorylation of eIF2α. (A) T-REx HeLa cells were transfected with Luciferase siRNA or HRI siRNA. Cells were harvested after exposure to 0.3 mM arsenite for 2 h. eIF2α and its phosphorylation were analyzed by Western blotting with the indicated antibodies. (B) T-REx HeLa cells were co-transfected with a pFlag-CMV5/TO-BGG reporter plasmid, pCMV-5 × Flag-EGFP reference plasmid, and either Luciferase siRNA (lanes 1–10) or HRI siRNA (lanes 11–20). A pulse-chase analysis was performed as in Fig. 1A. (C) The average length of the poly(A) tail was measured. (D) and (E) As in (B) and (C), except that cells were co-transfected with a pFlag-CMV5/TO-BGG-cMyc 3′ UTR reporter plasmid and either Luciferase siRNA ((D) lanes 1–10) or HRI siRNA ((D) lanes 11–20).

We here showed that Pan3 as well as Tob is selectively down-regulated during arsenite stress. However, the degradation of Pan3 is slower than that of Tob (Fig. 2A) and does not appear to be mediated by proteasome (Fig. 2B). Furthermore the knockdown of Pan3 leads to modest stabilization of the mRNA poly(A) tail over that of Tob (Fig. 3B). Consistent with previous findings in which Tob–Caf1–Ccr4 was identified as the major mRNA deadenylase, stress-induced stabilization of the mRNA poly(A) tail appears to be mainly due to the degradation of Tob.

Conflict of interest

The authors declare that they have no conflict of interest.

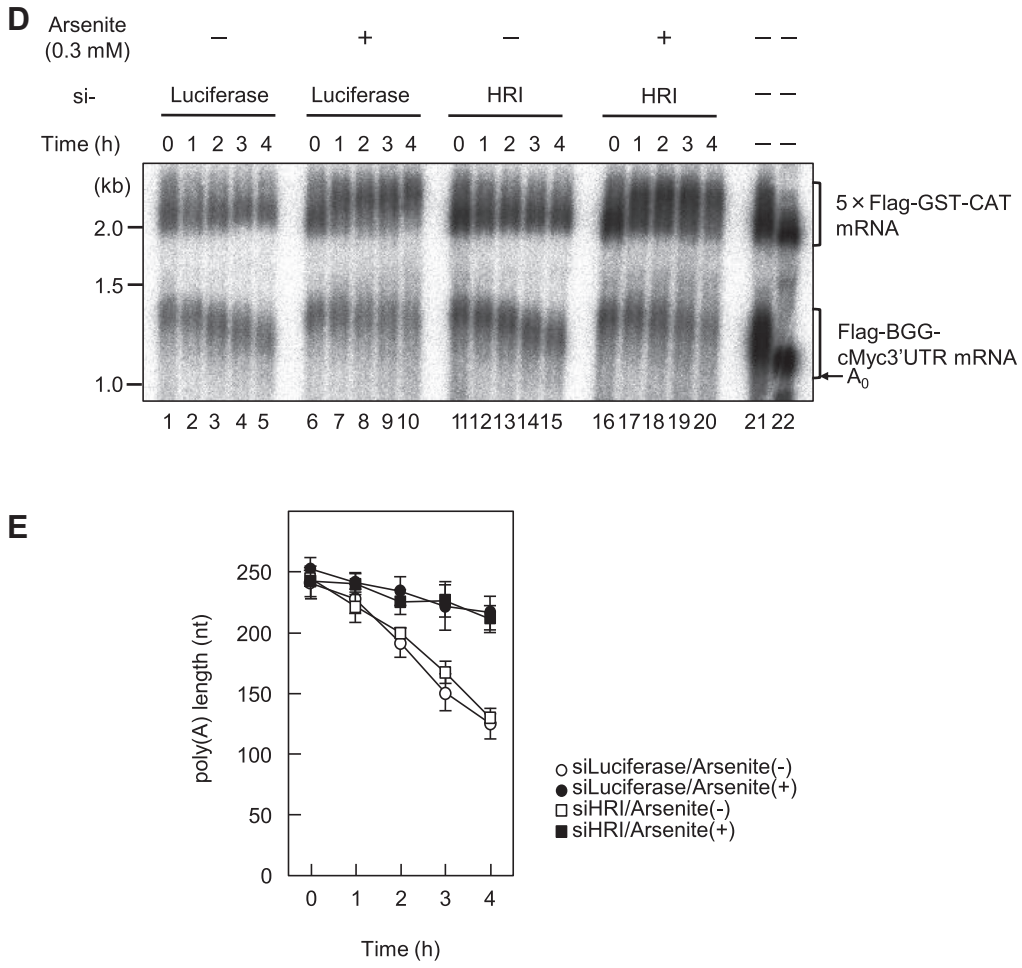

Fig. 4 (*continued*)

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas "RNA regulation" (No. 20112005) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in-Aid for Scientific Research (B) (No. 25291004) from Japan Society for the Promotion of Science, and a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan (to S.H.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.11.015.

References

[1] S.E. Wells, P.E. Hillner, R.D. Vale, A.B. Sachs, Circularization of mRNA by eukaryotic translation initiation factors, Mol. Cell 2 (1998) 135–140.
[2] D.R. Gallie, A tale of two termini: a functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation, Gene 216 (1998) 1–11.
[3] N. Uchida, S. Hoshino, H. Imataka, N. Sonenberg, T. Katada, A novel role of the mammalian GSPT/eRF3 associating with poly(A)-binding protein in Cap/Poly(A)-dependent translation, J. Biol. Chem. 277 (2002) 50286–50292.
[4] J. Coller, R. Parker, Eukaryotic mRNA decapping, Annu. Rev. Biochem. 73 (2004) 861–890.
[5] A.B. Shyu, J.G. Belasco, M.E. Greenberg, Two distinct destabilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay, Genes Dev. 5 (1991) 221–231.
[6] G. Gowrishankar, R. Winzen, F. Bollig, B. Ghebremedhin, N. Redich, B. Ritter, K. Resch, M. Kracht, H. Holtmann, Inhibition of mRNA deadenylation and degradation by ultraviolet light, Biol. Chem. 386 (2005) 1287–1293.
[7] G. Gowrishankar, R. Winzen, O. Dittrich-Breiholz, N. Redich, M. Kracht, H. Holtmann, Inhibition of mRNA deadenylation and degradation by different types of cell stress, Biol. Chem. 387 (2006) 323–327.
[8] V. Hilgers, D. Teixeira, R. Parker, Translation-independent inhibition of mRNA deadenylation during stress in *Saccharomyces cerevisiae*, RNA 12 (2006) 1835–1845.
[9] A. Yamashita, T.C. Chang, Y. Yamashita, W. Zhu, Z. Zhong, C.Y. Chen, A.B. Shyu, Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover, Nat. Struct. Mol. Biol. 12 (2005) 1054–1063.
[10] N. Uchida, S. Hoshino, T. Katada, Identification of a human cytoplasmic poly(A) nuclease complex stimulated by poly(A)-binding protein, J. Biol. Chem. 279 (2004) 1383–1391.
[11] J. Chen, Y.C. Chiang, C.L. Denis, CCR4, a 3′–5′ poly(A) RNA and ssDNA exonuclease, is the catalytic component of the cytoplasmic deadenylase, EMBO J. 21 (2002) 1414–1426.
[12] P. Viswanathan, T. Ohn, Y.C. Chiang, J. Chen, C.L. Denis, Mouse CAF1 can function as a processive deadenylase/3′–5′-exonuclease in vitro but in yeast the deadenylase function of CAF1 is not required for mRNA poly(A) removal, J. Biol. Chem. 279 (2004) 23988–23995.
[13] C. Bianchin, F. Mauxion, S. Sentis, B. Seraphin, L. Corbo, Conservation of the deadenylase activity of proteins of the Caf1 family in human, RNA 11 (2005) 487–494.
[14] N. Hosoda, T. Kobayashi, N. Uchida, Y. Funakoshi, Y. Kikuchi, S. Hoshino, T. Katada, Translation termination factor eRF3 mediates mRNA decay through the regulation of deadenylation, J. Biol. Chem. 278 (2003) 38287–38291.
[15] Y. Funakoshi, Y. Doi, N. Hosoda, N. Uchida, M. Osawa, I. Shimada, M. Tsujimoto, T. Suzuki, T. Katada, S. Hoshino, Mechanism of mRNA deadenylation: evidence for a molecular interplay between translation termination factor eRF3 and mRNA deadenylases, Genes Dev. 21 (2007) 3135–3148.
[16] S. Hoshino, Mechanism of the initiation of mRNA decay: role of eRF3 family G proteins, Wiley Interdiscip. Rev. RNA 3 (2012) 743–757.
[17] L. Ruan, M. Osawa, N. Hosoda, S. Imai, A. Machiyama, T. Katada, S. Hoshino, I. Shimada, Quantitative characterization of Tob interactions provides the

thermodynamic basis for translation termination-coupled deadenylase regulation, J. Biol. Chem. 285 (2010) 27624–27631.
[18] K. Ogami, N. Hosoda, Y. Funakoshi, S. Hoshino, Antiproliferative protein Tob directly regulates c-myc proto-oncogene expression through cytoplasmic polyadenylation element-binding protein CPEB, Oncogene 33 (2014) 55–64.
[19] S. Saito, N. Hosoda, S. Hoshino, The Hbs1–Dom34 protein complex functions in non-stop mRNA decay in mammalian cells, J. Biol. Chem. 288 (2013) 17832–17843.
[20] N. Hosoda, Y. Funakoshi, M. Hirasawa, R. Yamagishi, Y. Asano, R. Miyagawa, K. Ogami, M. Tsujimoto, S. Hoshino, Anti-proliferative protein Tob negatively regulates CPEB3 target by recruiting Caf1 deadenylase, EMBO J. 30 (2011) 1311–1323.
[21] Y. Hashimoto, N. Hosoda, P. Datta, E.S. Alnemri, S. Hoshino, Translation termination factor eRF3 is targeted for caspase-mediated proteolytic cleavage and degradation during DNA damage-induced apoptosis, Apoptosis 17 (2012) 1287–1299.
[22] M. Osawa, N. Hosoda, T. Nakanishi, N. Uchida, T. Kimura, S. Imai, A. Machiyama, T. Katada, S. Hoshino, I. Shimada, Biological role of the two overlapping poly(A)-binding protein interacting motifs 2 (PAM2) of eukaryotic releasing factor eRF3 in mRNA decay, RNA 18 (2012) 1957–1967.
[23] N. Ezzeddine, T.C. Chang, W. Zhu, A. Yamashita, C.Y. Chen, Z. Zhong, Y. Yamashita, D. Zheng, A.B. Shyu, Human TOB, an antiproliferative transcription factor, is a poly(A)-binding protein-dependent positive regulator of cytoplasmic mRNA deadenylation, Mol. Cell. Biol. 27 (2007) 7791–7801.
[24] T. Suzuki, J. Tsuzuku, K. Kawakami, T. Miyasaka, T. Yamamoto, Proteasome-mediated degradation of Tob is pivotal for triggering UV-induced apoptosis, Oncogene 28 (2009) 401–411.
[25] N. Kedersha, S. Chen, N. Gilks, W. Li, I.J. Miller, J. Stahl, P. Anderson, Evidence that ternary complex (eIF2–GTP–tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules, Mol. Biol. Cell 13 (2002) 195–210.
[26] E. McEwen, N. Kedersha, B. Song, D. Scheuner, N. Gilks, A. Han, J.J. Chen, P. Anderson, R.J. Kaufman, Heme-regulated inhibitor kinase-mediated phosphorylation of eukaryotic translation initiation factor 2 inhibits translation, induces stress granule formation, and mediates survival upon arsenite exposure, J. Biol. Chem. 280 (2005) 16925–16933.