Published in final edited form as:

Science. 2005 October 21; 310(5747): 486–489.

Movement of Eukaryotic mRNAs Between Polysomes and Cytoplasmic Processing Bodies

Muriel Brengues^{*}, Daniela Teixeira^{*}, and Roy Parker[†]

Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, University of Arizona, Tucson, AZ 85721–0106, USA

Abstract

Eukaryotic cells contain nontranslating messenger RNA concentrated in P-bodies, which are sites where the mRNA can be decapped and degraded. We present evidence that mRNA molecules within yeast P-bodies can also return to translation. First, inhibiting delivery of new mRNAs to P-bodies leads to their disassembly independent of mRNA decay. Second, P-bodies decline in a translation initiation–dependent manner during stress recovery. Third, reporter mRNAs concentrate in P-bodies when translation initiation is blocked and resume translation and exit P-bodies when translation is restored. Fourth, stationary phase yeast have large P-bodies containing mRNAs that reenter translation when growth resumes. The reciprocal movement of mRNAs between polysomes and P-bodies is likely to be important in the control of mRNA translation and degradation. Moreover, the presence of related proteins in P-bodies and maternal mRNA storage granules suggests this mechanism is widely adapted for mRNA storage.

A key aspect of the regulation of eukaryotic gene expression is the control of mRNA translation and degradation, which often occurs by decapping, followed by 5' to 3' decay (1). Translation and mRNA degradation via decapping are tightly linked. To decap, an mRNA exits translation and assembles into a translationally repressed messenger ribonucleoprotein (mRNP) lacking translation initiation factors and containing the decapping enzyme and several accessory proteins (1,2). These translationally repressed mRNPs accumulate within P-bodies (also referred to as GW or Dcp bodies) (3–8), where decapping can occur (9,10). The formation of a P-body mRNP is also important for control of translational repression (2,11,12). An unresolved issue is whether P-bodies can store mRNAs and later release them to reenter translation.

To determine whether mRNAs in P-bodies were committed to decapping, we examined P-bodies in *dcp1Δ* cells where decapping is blocked (1) after 10 min of cycloheximide treatment, which prevents mRNAs from exiting translation and entering P-bodies (7,9,10,13). We directly observed P-body proteins by using green fluorescent protein (GFP)–tagged versions of the components Dcp2p and Dhh1p, whose presence in P-bodies is dependent on RNA, thus also providing an indirect manner of observing P-body mRNAs (7). We observed that P-bodies in *dcp1Δ* (Fig. 1A) and *xrn1Δ* cells (fig. S1) declined after cycloheximide treatment, suggesting that mRNPs can exit P-bodies in the absence of decapping and 5' to 3' degradation.

[†] To whom correspondence should be addressed. E-mail: rrparker@u.arizona.edu.

^{*} These authors contributed equally to this work.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1115791/DC1

Materials and Methods

Figs. S1 to S3

Table S1

References

If mRNAs within P-bodies are committed to degradation, then the reduction in P-bodies seen in the *dcp1Δ* strain could be due to 3' to 5' degradation. Thus, we examined P-bodies in *dcp1* mutant strains lacking Ski2p, which is required for 3' to 5' degradation of mRNAs. Because blocks to decapping and 3' to 5' degradation of mRNA are lethal in combination (14), we inhibited decapping in the *ski2Δ* strain by using the temperature-sensitive allele *dcp1-2*. After a shift to 37°C and because of inhibition of mRNA decapping, P-bodies increased in size and number in the *dcp1-2* and *dcp1-2 ski2Δ* strains but still declined after cycloheximide addition (Fig. 1B). Moreover, proteins required for 3' to 5' degradation of mRNAs are not concentrated in P-bodies (fig. S2). These results suggest that mRNAs exit P-bodies independent of mRNA degradation, possibly to return to translation.

To determine whether P-body mRNAs could enter translation, we examined translation regulation by the presence of glucose (15). We assessed P-bodies by following the subcellular distribution of Dhh1p and Dcp2p as well as the subcellular distribution of two reporter mRNAs (PGK1-U1A and MFA2P-U1A), which are visualized by the binding of a U1A-GFP fusion protein to their 3' untranslated region (UTR) (16). Glucose deprivation leads to a rapid loss of polysomes (Fig. 2F) and an increased accumulation of Dcp2p, Dhh1p, and PGK1-U1A and MFA2P-U1A mRNAs in P-bodies (Fig. 2, G to J). Readdition of glucose leads to rapid restoration of polysomes (Fig. 2K) and loss of Dcp2p, Dhh1p, and PGK1-U1A and MFA2P-U1A mRNAs from P-bodies (Fig. 2, L to O). The MFA2P-U1A mRNA accumulated in P-bodies before glucose deprivation and after glucose restoration (Fig. 2, E and O), consistent with the poorer translation of the MFA2 mRNA (~24% untranslated) compared with PGK1 mRNA (~2% untranslated). These observations demonstrate that restoration of translation after glucose deprivation leads to disassembly of P-bodies, either by decapping and degradation of the mRNA within P-bodies or through return of the mRNAs within P-bodies to polysomes.

To evaluate whether decapping was required for the decline of P-bodies after glucose restoration, we examined the response to glucose deprivation and restoration in the *dcp1-2* strain. Similar to wild-type cells at 37°C (Fig. 3A), glucose deprivation in *dcp1-2* cells led to Dhh1p, Dcp2p, PGK1-U1A, and MFA2P-U1A mRNAs accumulating in P-bodies (Fig. 3B, images g to j) and a decline in polysomes (Fig. 3B, image f). Readdition of glucose to the *dcp1-2* strain led to reformation of polysomes (Fig. 3B, image k) and a decline in P-bodies (Fig. 3B, images l to o). The disassembly of P-bodies that occurs upon restoration of glucose is therefore not dependent on decapping and degradation of mRNA within P-bodies. However, because P-bodies do not completely return to basal amounts in the *dcp1-2* strain after glucose restoration, some mRNAs may be targeted for decay under these conditions.

To determine whether translation initiation was required for the disassembly of P-bodies after glucose restoration, we examined the response to glucose deprivation and restoration in a strain carrying a conditional temperature-sensitive allele of a subunit of eukaryotic initiation factor 3 (eIF3), *prt1-63*. Similar to wild-type cells (Fig. 3A), glucose deprivation in *prt1-63* cells led to an accumulation of Dhh1p, Dcp2p, and PGK1-U1A and MFA2P-U1A reporter mRNAs in P-bodies (Fig. 3C, images g to j) and a decline in polysomes (Fig. 3C, image f). Re-addition of glucose led to a limited reformation of polysomes (Fig. 3C, image k), and P-bodies largely persisted (Fig. 3C, images l to o). The small decrease in P-bodies seen under these conditions is likely due to the partial restoration of polysomes in the *prt1-63* strain at 37°C and to some mRNAs being degraded by decapping, consistent with the persistence of small P-bodies in the *dcp1-2* strain after glucose restoration. This indicates that the decline of P-bodies that occurs upon restoration of glucose requires translation initiation, presumably to allow the dynamic movement of mRNAs between polysomes and P-bodies to shift to the translating state. This result argues that mRNAs within P-bodies are reentering translation.

To demonstrate that P-body mRNAs were reentering translation, we examined the translation status of specific mRNAs on sucrose gradients before and during glucose deprivation and after glucose restoration. The MFA2P-U1A mRNA was primarily associated with polysomes during log growth (~76% polysome-associated), shifted to non-translating fractions of the gradient during glucose deprivation (~36% polysome-associated) where P-body components sediment (2,17,18), and shifted back into the polysome region of the gradient during glucose restoration (~72% polysome-associated) (Fig. 4A). These results are consistent with the subcellular location of the MFA2P-U1A mRNA, whose concentration in P-bodies is increased during glucose deprivation and then decreased by glucose restoration (Fig. 2). This result argues that mRNAs can return to translation from a P-body state. Similar shifts were observed with endogenous mRNAs, including RPL41A, PGK1, and CYH2 transcripts (Fig. 4B, lower images).

In principle, the transcripts associated with polysomes during glucose restoration could be products of new transcription and not those previously localized to P-bodies. To verify that the appearance of mRNAs in the translating pool does not require new transcription, we performed similar experiments by using a tetracycline repression construct (Tet-Off promoter) with the MFA2pG and the MFA2P-U1A reporters, thereby allowing repression of transcription at the time of glucose deprivation. We observed a similar movement of these mRNAs from the polysome pool to the nontranslating pool and back to translation in response to glucose availability (Fig. 4B), demonstrating that the appearance of MFA2 transcripts in the translating pool after glucose readdition is caused by the restored translation of preexisting transcripts.

The ability of mRNAs to return to translation from P-bodies suggests that P-bodies can serve as sites of mRNA storage. Because P-bodies increase in size with cell density (7), we hypothesized that P-bodies might serve as site of mRNA storage during stationary phase in yeast, where cells enter a G0-like state (19). In stationary phase yeast, we observed that polysomes are reduced and P-bodies are enlarged, as judged by either proteins (7) or the MFA2P-U1A mRNA (Fig. 5).

Several observations indicate that mRNAs within P-bodies at stationary phase enter translation when nutrients are provided. First, in the presence of fresh media, polysomes are restored and P-bodies decrease within 30 min (Fig. 5). Second, when we inhibited the transcription of the Tet-Off MFA2pG reporter mRNA at the time of nutrient readdition, we observed that these transcripts moved from being predominantly untranslated in stationary phase cells (~22% polysome-associated) to the polysome pool upon media readdition (~73% polysome-associated) (Fig. 5). This demonstrates that during stationary phase P-bodies store mRNAs, which can later enter translation.

Previous work has demonstrated that mRNAs can move from polysomes to P-bodies when translation is repressed (2,7). Our results indicate that mRNAs can also exit P-bodies to reenter translation, suggesting a movement of yeast mRNAs from polysomes to P-bodies, where they can either be degraded or return to polysomes. Because most yeast mRNAs undergo decapping and therefore associate with P-body components (1), and P-body proteins are required for the general repression of translation (2), we anticipate that most yeast mRNAs can move between polysomes and P-bodies. Moreover, because P-bodies decline in *dcp1Δ* cells after cycloheximide addition (Fig. 1A), there is likely to be a continual flux of mRNAs in and out of P-bodies. Mammalian P-bodies are similar to yeast P-bodies, suggesting that the movement of mRNAs from P-bodies to polysomes is likely to be a fundamental property of eukaryotic cells. Lastly, ribosomal components and translational factors are not concentrated in yeast P-bodies, even under stress (7) (fig. S3), indicating that the movement of mRNAs into P-bodies requires the loss of ribosomes and translation initiation factors.

Movement of mRNAs in and out of P-bodies can function to store mRNAs, one example being yeast in stationary phase, where RNAs enter translation once growth is resumed. Because maternal mRNA storage granules and P-bodies contain related proteins (1), we hypothesize that many forms of mRNA storage will share a conserved mechanism. This work also adds evidence that translating and nontranslating pools of mRNAs are spatially segregated in the cytoplasm between polysomes, stress granules, and P-bodies (20,21). The presence of multiple discrete biochemical and cytological states for cytoplasmic mRNAs strongly implies that mechanisms that control the movement of mRNAs between these states will be important in the regulation of mRNA translation and degradation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References and Notes

1. Collier J, Parker R. *Annu Rev Biochem* 2004;73:861. [PubMed: 15189161]
2. Collier J, Parker R. *Cell*. in press
3. Bashkurov VI, Scherthan H, Solinger JA, Buerstedde JM, Heyer WD. *J Cell Biol* 1997;136:761. [PubMed: 9049243]
4. Ingelfinger D, Arndt-Jovin DJ, Luhrmann R, Achsel T. *RNA* 2002;8:1489. [PubMed: 12515382]
5. Lykke-Andersen J. *Mol Cell Biol* 2002;22:8114. [PubMed: 12417715]
6. van Dijk E, et al. *EMBO J* 2002;21:6915. [PubMed: 12486012]
7. Teixeira D, Sheth U, Valencia-Sanchez MA, Bregues M, Parker R. *RNA* 2005;11:371. [PubMed: 15703442]
8. Eystathioty T, et al. *Mol Biol Cell* 2002;13:1338. [PubMed: 11950943]
9. Sheth U, Parker R. *Science* 2003;300:805. [PubMed: 12730603]
10. Cougot N, Babajko S, Seraphin SB. *J Cell Biol* 2004;165:31. [PubMed: 15067023]
11. Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. *Nat Cell Biol* 2005;7:719. [PubMed: 15937477]
12. Sen GL, Blau HM. *Nat Cell Biol* 2005;7:633. [PubMed: 15908945]
13. Andrei MA, et al. *RNA* 2005;11:717. [PubMed: 15840819]
14. Anderson JS, Parker RP. *EMBO J* 1998;17:1497. [PubMed: 9482746]
15. Ashe MP, De Long SK, Sachs AB. *Mol Biol Cell* 2000;11:833. [PubMed: 10712503]
16. Brodsky AS, Silver PA. *RNA* 2000;6:1737. [PubMed: 11142374]
17. Bonnerot C, Boeck R, Lapeyre B. *Mol Cell Biol* 2000;20:5939. [PubMed: 10913177]
18. Hilleren P, Parker R. *RNA* 2001;7:753. [PubMed: 11350039]
19. Gray JV, et al. *Microbiol Mol Biol Rev* 2004;68:187. [PubMed: 15187181]
20. Anderson P, Kedersha N. *J Cell Sci* 2002;115:3227. [PubMed: 12140254]
21. Nover L, Scharf KD, Neumann D. *Mol Cell Biol* 1983;3:1648. [PubMed: 6633535]
22. We thank the Parker lab for discussions, especially Kristian Baker and Carolyn Decker for critical reading of the manuscript. Supported by Howard Hughes Medical Institute and NIH (GM45443). D.T. was supported by Fundacao para a Ciencia e Tecnologia (SFRH/BD/2739/2000), Portugal.

Fig. 1. mRNAs exit P-bodies in the absence of decapping. **(A)** Wild-type (WT) and *dcp1Δ* cells grown in yeast extract, peptone, and glucose (YPGlu) at 30°C (control) were treated with cycloheximide (CYH, 100 μg/ml) for 10 min. **(B)** Cells grown in YPGlu at 23°C were shifted to 37°C for 30 min followed by CYH treatment for 10 min.

Fig. 2. Disassembly of P-bodies after translation restoration. WT cells expressing a GFP-tagged version of Dcp2p (**B**, **G**, and **L**), Dhh1p (**C**, **H**, and **M**), or co-expressing U1A-GFP with PGK1-U1A (**D**, **I**, and **N**) or MFA2P-U1A (**E**, **J**, and **O**), were grown in glucose containing medium at 30°C (Glu), shifted for 10 min without glucose (10 min -Glu), followed by readdition of glucose (5 min +Glu). Polysome profiles under each condition are shown (**A**, **F**, and **K**).

Fig. 3.

Translation initiation, but not decapping, is required for mRNAs to exit P-bodies. Cells were grown in glucose-containing medium at 23°C (Glu), shifted to 37°C for 10 min without glucose (10 min -Glu), followed by readdition of glucose (10 min +Glu). Polysome profiles under each condition are shown (a, f, and k). (A) WT, (B) *dcp1-2*, and (C) *prt1-63* cells expressing a GFP-tagged version of Dcp2p (b, g, and i), Dhh1p (c, h, and m), or co-expressing the plasmids U1A-GFP and PGK1-U1A (d, i, and n), or U1A-GFP and MFA2P-U1A (e, j, and o) are shown.

Fig. 4.

Movement of mRNAs between a translating and a nontranslating pool. (A) WT cells co-expressing U1A-GFP with MFA2P-U1A or (B) expressing a plasmid containing MFA2P-U1A or MFA2pG under the control of a tetracycline promoter (Tet-Off MFA2P-U1A and Tet-Off MFA2pG) were grown at 30°C in synthetic complete (SC) medium plus glucose (Glu), shifted for 10 min to (A) SC with no glucose or (B) SC with no glucose containing doxycycline (10 min -Glu), followed by readdition of glucose (5 min +Glu). (A) Polysome profiles of the collected sucrose gradients are shown. Fraction 1 corresponds to the top of the gradient. (A and B) Northern blots for the indicated mRNA are shown.

Fig. 5. mRNA present in P-bodies in stationary phase can return to translation. Cells expressing Tet-Off MFA2pG or co-expressing U1A-GFP with MFA2P-U1A were grown until stationary phase (SP). Then glucose, with or without doxycycline, was added to the culture (30 min +Glu). Polysome profiles and Northern blotting of cells expressing the plasmid Tet-Off MFA2pG are shown (**top**). Cells co-expressing U1A-GFP and MFA2P-U1A are visualized (**bottom**). In stationary phase, ~80% of the cells show large foci; after 30 min +Glu, ~20% of the cells have foci, and the remaining foci are considerably diminished.