Review

PUF proteins: repression, activation and mRNA localization

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The eukaryotic family of RNA-binding proteins termed PUF (Pumilio and FBF) is known for its roles in cell division, differentiation and development. The best-characterized function of PUFs is as posttranscriptional repressors. Recent studies have indicated that PUFs can also activate gene expression. Moreover, it is becoming clear that PUFs facilitate mRNA localization for spatial control of expression. Here, we review the emerging concept of PUF proteins as versatile posttranscriptional regulators. We discuss how the functions of PUFs as repressors and mRNA targeting factors could be integrated by focusing on Puf3 and Puf6 from yeast and propose a model for how the roles of Puf3 in mRNA targeting to the mitochondria and mRNA repression might promote cotranslational import into mitochondria and mitochondrial biogenesis.

Introduction

The PUF family of RNA-binding proteins are posttranscriptional regulators present throughout the eukaryotic domain. PUFs bind to specific recognition sequences in the 3′ untranslated regions (3′ UTRs) of mRNAs to control the stability and translation of transcripts [1,2]. All PUFs contain a PUM-HD-type RNA-binding domain [3,4], reviewed in [1,2], which folds into an arc-like shape and can contact both RNA and protein partners [5,6] (Box 1).

The number of genes encoding PUF family members varies hugely between organisms (Box 2). Moreover, PUFs bind to a large number of mRNA targets and their cellular functions are diverse (Box 2 and Table 1). The roles of PUFs include differentiation and development [7–12], germline functions [13–19], neuronal function and memory [20–25], the cell cycle [26,27] and mitochondrial biogenesis [28–30]; reviewed in [1,2] (Table 1). Wickens and colleagues proposed that the ancestral role of these posttranscriptional regulators is the maintenance of the mitotic potential of stem cells, with other functions acquired later [1]. Recently, a conserved set of PUF mRNA targets with functions in stem cell control among *Drosophila*, *Caenorhabditis elegans* and humans was identified [31].

Studies of the PUF family of RNA-binding proteins have been driven by the fact that these regulators are prototypes for understanding posttranscriptional regulation exerted via the 3′ UTRs of mRNAs. The canonical role of PUFs is as posttranscriptional repressors (reviewed in [1,2]). In addition to this well-established role, new findings in several organisms indicate two further functions for PUFs. Recent evidence has suggested that they can contribute to the activation of mRNA expression [32–35]. Furthermore, new reports have demonstrated that PUFs contribute to the targeting of mRNAs to specific subcellular locations to provide for spatial control of expression [24,29,30,36–39].

Here, we review the molecular mechanisms of PUF protein action, focusing on recent advances in understanding the molecular details of mRNA repression by PUFs and new evidence of the function of PUFs in the activation of mRNA expression and mRNA targeting. We discuss more specifically how the roles of PUFs in mRNA localization could be integrated with their roles in translational repression with two examples from budding yeast for which most is known, Puf6 and Puf3.

PUFs as repressors of mRNA expression

The mechanism of mRNA repression by PUF proteins was first described by the Wickens laboratory [40]. Yeast Puf5 was shown to bind directly and specifically to the Pop2 subunit of the Ccr4-Pop2-NOT mRNA deadenylase complex, thereby recruiting the deadenylase to mRNAs [40] (Figure 1). This major cytoplasmic exonuclease shortens mRNA poly(A) tails [41], influencing both mRNA stability and translation [42–44] (Box 3).

In the closed-loop model, the 5′ and 3′ ends of an mRNA are in close proximity (Figure 1 and Box 3), thereby allowing a 3′ UTR-binding regulator, such as Puf5, to effectively act on the 5′ mRNA end as well. In fact, the decapping activator and translational repressor Dhh1 and the decapping enzyme Dcp1 interact with the Ccr4-Pop2-NOT complex [45,46] and are also recruited to mRNAs by the Pop2–Puf5 interaction [40] (Figure 1). Dhh1 and Dcp1 further cause mRNA repression by affecting the hydrolysis of the 5′ cap (decapping), as well as functioning as translational repressors [47]. The recruitment of factors affecting the 5′ cap could explain PUF-dependent repression observed in the absence of deadenylation [40,48,49].

The generality of the Ccr4-Pop2-NOT recruitment mechanism is supported by the fact that yeast PUFs Puf4 and Puf3 [40,49–51], *Drosophila* Pumilio (Pum) [18], the *C. elegans* FBF and human Pum1 [33,40] also interact with the Ccr4-Pop2-NOT complex. A recent report that *C. elegans* FBF can function to repress translation in the heterologous yeast system also provides evidence for a universal mode of PUF-dependent mRNA repression [52].

PUF-dependent repression mechanisms additional to the recruitment of the Ccr4-Pop2-NOT deadenylase have also been proposed. They include the inhibition of translation initiation factors and their interaction with the 5′

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Box 1. RNA binding by PUF proteins

The canonical PUM-HD RNA-binding domain contains eight alpha helical repeats of a motif of ≈36 amino acids and folds into an arc-like shape [5,6]. The PUM-HD domain interacts with RNA via the concave surface of the arc, whereas protein partners bind to the outside region [5,6]. As such, the PUM-HD domain is both necessary and sufficient for the function of PUFs [40,84]. In a typical PUF–RNA interaction, each of the eight PUM repeats binds to a single nucleotide in a consensus binding motif located in the 3′ UTR of the target mRNA [6,85]. For all defined PUF–mRNA interactions, this motif contains a conserved UGUR (where R is a purine) at the 5′ end and variable 3′ sequences that determine the specificity of PUF–cognate mRNA interactions [1]. Recent studies have illuminated how PUFs bind specifically and exclusively to their target mRNAs, although the amino acids making RNA contacts are highly conserved. On one hand, the unambiguous binding of Puf3 to specific mRNA targets in *S. cerevisiae* is determined by the presence of a distinctive binding pocket in repeat 8, which binds to a cytosine two bases 5′ of the conserved UGUA sequence [86]. On the other hand, changes to the curvature of the arc in yeast Puf4 and the *C. elegans* FBF and flipping of the bases in the cognate RNA sequence determine binding to a longer consensus motif than the conventional 8 nt motif [87,88]. For recent reviews on structural aspects of PUF–RNA interactions see [89,90].

Box 2. The distribution and functions of PUF proteins

Including noncanonical PUFs such as yeast Puf6, *Drosophila* has two PUFs, vertebrates three, yeast six, *C. elegans* 12 and *Arabidopsis* 26 [1,91]. In yeast, the PUF family members bind to distinct subsets of mRNAs [59] and display different phenotypes when inactivated, arguing that each PUF has unique aspects to its function [27,28,30]. However, functional overlap also exists, as suggested by the phenotypic analysis of *puf* mutants and the sharing of mRNA targets between PUFs [49,59,74]. Similarly, in *C. elegans* there is redundancy but also unique functions of the different PUF family members [3,15,16,92–94]; reviewed in [89]. For example, FBF-1 and FBF-2 are redundant [3,15,16]. However, FBF is non-redundant with PUF-8 in the germline [94]. The human PUFs PUM1 and PUM2 bind to overlapping but also specific mRNA targets, again suggesting both common and unique functions [72]. Recent revelations that PUFs bind to groups of functionally related transcripts have suggested that they are central coordinators of gene networks for the coregulated control of expression at the posttranscriptional level [31,59,70–72].

For all PUFs analyzed to date, whole-transcriptome analysis has revealed a large number of putative partner transcripts. In comparison, only a moderate number of mRNAs have been validated as targets by direct assays (Table 1). In some cases, such as for yeast Puf3, it is likely that the majority of the putative mRNA partners are relevant targets *in vivo* [29,59]. In other cases, this is less likely [2,26,74]. For example, only two out of 20 putative targets of yeast PUFs showed differences in half-lives in *puf* mutants in one study [74] and seven out of 15 in another study [26]. Future experiments will need to validate the mRNA targets identified by whole-transcriptome approaches as well as test how PUFs regulate their expression and/or localization.

mRNA cap, changes to the ribonucleoprotein structure and effects on translation elongation and termination.

For example, *Drosophila* Pum can recruit to mRNAs the translational inhibitor d4EHP, via its cofactor Brain tumor (Brat) [11]. d4EHP inhibits translation by competing with the translation initiation factor eIF4E for binding to the cap [53]. In *Xenopus*, Pum2 binds to the cap structure, thereby inhibiting the binding of eIF4E [54]. The noncanonical yeast PUF Puf6 represses the translation of the *ASH1* mRNA by interacting with the translation initiation factor eIF5B/Fun12 and inhibiting its function [37].

Changes to the ribonucleoprotein structure have been suggested for yeast Puf3. In addition to binding to the

Table 1. mRNA targets of model Puf proteins.

Organism	Puf protein	Target transcript[a]	Mode of regulation[b]	Biological process	Ref.	Number of putative targets (whole transcriptome analysis)	Ref.
S. cerevisiae	Puf1	*HXK1*	R	Hexokinase; metabolism	[74]	40	[59]
		TIF1	R	eIF4A; translation initiation factor	[74]		
	Puf2	None	–	–		146	[59]
	Puf3	*COX17*	R, L	Cytochrome *c* oxidase activity; mitochondrial function	[29,60,61]	220	[59]
		COX23	R, L	Cytochrome *c* oxidase activity; mitochondrial function	[29]		
		BSC1	L	Mitochondrial AAA ATPase	[29]		
	Puf4	*HO*	R	Endonuclease; mating type switching	[49]	205	[59]
		Nucleolar proteins[c]	R	Ribosome biogenesis factors; ribosomal subunits	[59,62,75]		
	Puf5	*HO*	R	Endonuclease; mating type switching	[40,49,50,52,76]	224	[59]
		TEC1	R	Transcription factor; pseudohyphal growth	[12]	69	[26]
		STE7	R	MAP-kinase kinase; pseudohyphal growth	[12]	(25/69 found to bind to 3′ UTRs)	
		CIN8[d]	R	Kinesin motor; mitotic spindle assembly; chromosome segregation	[26,52]		
		LPD1[d]	R	Metabolism; lipoamide dehydrogenase	[26]		
		LRG1	R	GTPase activity; Pkc1-mediated signaling pathway; cell wall integrity	[26,77]		
		DHH1[d]	R	RNA helicase; decapping	[26,52]		
		RAX2[d]	R	Bud-site selection, cell cycle	[26,52]		
		ASE1[d]	R	Spindle elongation	[26]		
		UTR1[d]	R	ATP NADH kinase; iron homeostasis	[26]		
		SWD3[d]	R	Chromatin structure; COMPASS complex	[26]		
		PEX17	L	Peroxisome biogenesis	[38,59]		
	Puf6	*ASH1*	R	Transcriptional repressor; mating type switching	[36,37]	N/A	

Table 1 (*Continued*)

Organism	Puf protein	Target transcript[a]	Mode of regulation[b]	Biological process	Ref.	Number of putative targets (whole transcriptome analysis)	Ref.
Drosophila	Pum	*hunchback*	R	Transcription factor; posterior patterning	[7,9]	1090	[70]
		Bicoid	R	Transcription factor; anterior patterning	[10]		
		cyclin B	R	Cyclin; germline function	[14,18]		
		eIF4E	R	Translation initiation factor; neuronal function	[21]		
		para	R	Voltage-gated sodium channel; neuronal function	[22,25]		
C. elegans	FBF	*fbf-1*	R	PUF protein	[16]	1350	[31]
		fbf-2	R	PUF protein	[16]		
		fog-1	R	RNA-binding protein; germline; spermatogenesis	[78]		
		fem-3	R	Stimulates the ubiquitin ligase activity of CBC/FEM1; germline; spermatogenesis	[3,79]		
		gld-1	R, A	RNA-binding protein; germline; entry into meiosis	[15,33,79]		
		gld-3S	R	GLD-2/GLD-3 poly(A) polymerase; germline; entry into meiosis; spermatogenesis	[80]		
		lip-1	R	MAP kinase phosphatase; germline	[81]		
		mpk-1	R	MAP kinase; germline	[19]		
		egl-4	A	Kinase; olfactory adaptation	[34]		
Human	Pum1	*Cyclin B1*[d]	R	Cyclin; cell cycle	[71]	726	[71]
		Cyclin E2[d]	?	Cyclin; cell cycle	[71]	1766	[72]
		Cks2[d]	R	Cyclin-dependent kinase; cell cycle	[71]		
		PCNA[d]	R	Cell cycle	[71]		
		SLBP[d]	R	Histone mRNA-binding protein; mRNA expression; cell cycle	[71]		
		INTS2[d]	?	Integrator complex; transcription	[72]		
		DCUN1D3[d]	?	Cullin neddylation; cell cycle	[72]		
		Dll1[d]	?	Differentiation; Notch signaling pathway	[72]		
		SDAD1[d]	?	Export of ribosomal subunits to the cytoplasm	[72]		
		VEGF-A[d]	?	Vascular endothelial growth factor A	[72]		
		MET[d]	?	Hepatocyte growth factor receptor	[72]		
	Pum2	*SCH1*	R	Voltage-gated sodium channel; neuronal function	[39]	751	[72]
		eIF4E	R	Translation initiation factor; neuronal function	[39]	61	[89]
		DUSP6	R	Phosphatase	[72,82]		
		CEP3	?	Cdc42 effector	[83]		
		ERK2	R	MAP kinase; human embryonic stem cells	[19]		
		p38α	R	MAP kinase; human embryonic stem cells	[19]		
		INTS2[d]	?	Integrator complex; transcription	[72]		
		DCUN1D3[d]	?	Cullin neddylation; cell cycle	[72]		
		Dll1[d]	?	Differentiation; Notch signaling pathway	[72]		
		VEGF-A[d]	?	Vascular endothelial growth factor A	[72]		
		SDAD1[d]	?	Export of ribosomal subunits to the cytoplasm	[72,73]		

[a]As determined by direct assays and/or expression analysis in *puf* mutants
[b]R – repression, L – localization, A – activation
[c]A large number of transcripts encoding nucleolar proteins have been found to coimmunoprecipitate with Puf4 and are upregulated in *puf4Δ* mutants [59,62,75]
[d]Confirmed by direct assay and/or expression analysis following identification in whole transcriptome analysis

Ccr4-Pop2-NOT mRNA deadenylase, Puf3 can affect deadenylation driven by the alternative deadenylase Pan2 [51]. An interaction between Pan2 and Puf3 could not be detected, suggesting that Puf3 affects the structure of the mRNA and the poly(A) tail-binding protein Pab1, thereby exposing the poly(A) tail to deadenylation [51].

Finally, even the prototypical Pop2-dependent PUF Puf5 could use alternative repression mechanisms. The extent of repression by Puf5 *in vitro* is influenced by the distance of the 3′ UTR from the termination codon and a novel "AUU" sequence in the 3′ UTR of the Puf5 target mRNA *HO* [52]. Puf5 might be recruiting a partner protein via the AUU element, the two repressors forming a complex that inhibits translation elongation and/or termination [52].

In conclusion, PUFs are versatile repressors. The recruitment of the Ccr4-Pop2-NOT deadenylase and consequent mRNA deadenylation is a conserved and prevalent mechanism of repression by PUF proteins; however, as described above, other mechanisms are in play as well. The mechanisms of PUF-dependent repression are likely to differ depending on the structure and regulatory elements within the mRNA target and the presence of cofactors (for example, *Drosophila* Pum operates with cofactors such as Nos and Brat, whereas yeast PUFs do not) [1]. It is also likely that PUFs can employ multiple mechanisms for the repression of their mRNA targets. The Pum-dependent repression of *hunchback* mRNA in the *Drosophila* embryo includes both deadenylation [8] and the recruitment of the translation initiation inhibitor d4EHP [11]. Yeast Puf5 is also likely to repress *HO* mRNA by multiple mechanisms [40,52].

Adding to the versatility of PUFs, several recent reports have provided evidence that, in addition to their roles as

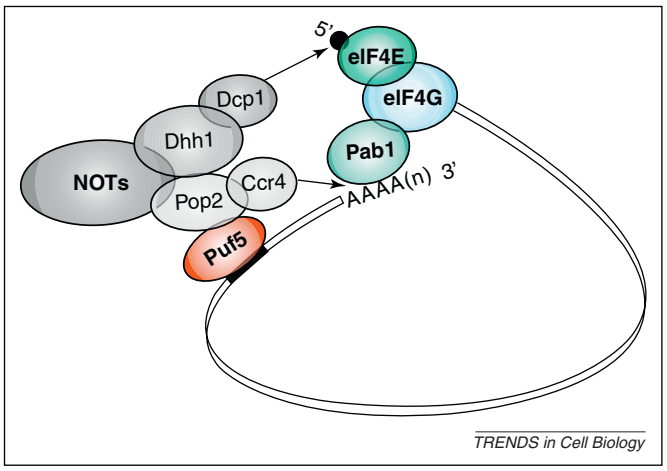

Figure 1. PUF proteins repress mRNAs by recruiting the Ccr4-Pop2-NOT deadenylase complex. PUF proteins, such as yeast Puf5, bind to the recognition sequences in the 3′ UTR of their target mRNAs and recruit the Ccr4-Pop2-NOT mRNA deadenylase via their interaction with the Pop2 subunit [40]. In addition to the deadenylase, the repressive complex recruited by Puf5 includes the decapping factors Dhh1 and Dcp1, which are associated with Ccr4-Pop2-NOT [40,45,46]. Dhh1 and Dcp1 act on the cap to activate decapping and inhibit translation. By recruiting factors affecting both the mRNA poly(A) tail and the 5′ cap, Puf5 can effectively cause deadenylation and translation repression [40]. Whether Dhh1 and Dcp1 homologs are recruited by PUFs in other organisms remains to be determined.

repressors, PUFs could act as activators of mRNA expression [32–35]. The possible mechanisms of PUF-dependent activation are discussed next.

PUFs as activators of mRNA expression

An activator function for PUFs is a new concept, but one that is gaining evidence in several organisms and with various PUF mRNA targets [32–35]. Evidence suggests that the effects of PUFs on mRNA activation are likely to be direct: PUFs bind to the mRNAs that they activate, the levels of these transcripts are upregulated in *puf* mutants and PUF-dependent regulation depends on PUF-binding sites in the 3′ UTRs of these mRNAs [32–35].

The mechanisms of PUF-dependent activation are poorly defined, but several possibilities have been proposed (Figure 2). In *C. elegans*, FBF regulates the activation of *gld-1* in the germline [33]. A possible mechanism is linked to cytoplasmic polyadenylation, extension of the mRNA poly(A) tail by cytoplasmic poly(A) polymerase, which represents a key mechanism for translational activation during development [55]. FBF interacts with the *gld-1* mRNA as well as with the cytoplasmic polyadenylase GLD-2/GLD-3, indicating it might recruit GLD-2/GLD-3 to its target transcript [33] (Figure 2a). FBF also stimulates the activity of GLD-2/GLD-3 *in vitro* [33]. This stimulation depends on the FBF-binding site in the substrate RNA, indicating a direct effect [33]. Therefore, FBF could act in a dual mode: recruiting the polyadenylase to its substrate and stimulating its activity there.

C. elegans FBF has also been proposed to activate the translation of another target mRNA, *egl-4* [34]. The translational activation of *egl-4* by FBF in olfactory sensory neurons is important for adaptation to odors [34]. Here again, FBF binds to the 3′ UTR of its *egl-4* target, and both FBF and the FBF-binding site are required for regulation, suggesting a direct effect [34]. The mechanism of FBF-dependent activation of *egl-4* translation is unclear, but because the expression controlled by the *egl-4* 3′ UTR requires microRNA-binding sites in addition to FBF-binding sites, it has been proposed that PUFs and microRNAs might cooperate in achieving translational activation [34].

A further example of translational activation by PUFs comes from *Xenopus*. In *Xenopus* oocytes, the Pum-binding element (PBE) contributes to translational activation mediated by the cytoplasmic polyadenylation element-binding (CPEB) protein and its cognate cytoplasmic polyadenylation element (CPE) [32]. The requirement for the PBE for translational activation is more stringent when a noncanonical CPE is used compared with a canonical one, and abolished if a dimer of the CPEB is present

Box 3. Effects of mRNA poly(A) tail length on mRNA stability and translational efficiency

Poly(A) tail shortening is the first step of the major mRNA decay pathway, in which the shortening of the tail is followed by the hydrolysis of the 5′ mRNA cap and 5′ to 3′ exonucleolytic degradation of the mRNA (reviewed in [44]). The length of the poly(A) tail also influences the efficiency of translation [42,43,69]. The mRNA poly(A) tail is coated by the Pab1 protein, with Pab1 interacting with the translation initiation factor eIF4G, which in turn binds to the mRNA 5′ cap binding initiation factor eIF4E (reviewed in [69]). This enables the formation of a closed-loop mRNA structure, in which the 5′ and 3′ ends of the mRNA are in close proximity, which is important for translation initiation [42,43,95,96]; reviewed in [69]. The longer the poly(A) tail, the more efficient translation [42], with at least two molecules of Pab1 being required for the stimulated translation that stems from stable closed-loop formation [43].

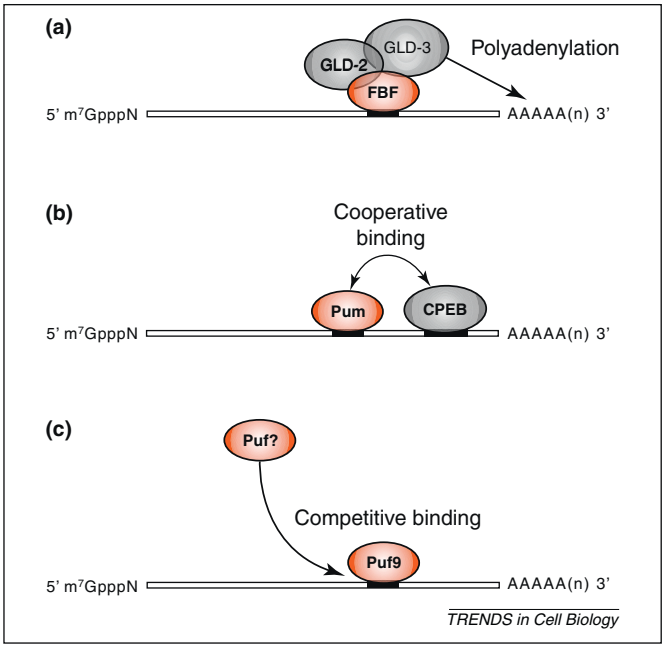

Figure 2. Possible mechanisms of mRNA activation by PUF proteins. **(a)** *C. elegans* FBF interacts with and stimulates the activity of the poly(A) polymerase GLD-2/GLD-3, possibly affecting mRNA polyadenylation and translational activation [33]. **(b)** In *Xenopus cyclin B* mRNA, Pum-binding sites cooperate with CPEB-binding sites to achieve translational activation. The proposed mechanism is the cooperative binding of Pum with CPEB, stabilizing CPEB on the transcript [32]. **(c)** In the parasite *T. brucei*, Puf9 stabilizes its interacting mRNAs, possibly by inhibiting the binding of a repressor. The identity of the repressor is not known, but the fact that mutating the Puf9-binding site resulted in stabilization suggests that the repressor could be another PUF protein, competing for binding with Puf9 [35].

[32]. This argues that the positive effect of Pum could be because of cooperative binding, resulting in the stabilization of CPEB binding to the transcript [32] (Figure 2b). Mutating the PBE did not affect polyadenylation, even though translational activation was compromised, indicating that Pum-dependent stabilization of CPEB could affect a function additional to polyadenylation [32].

The final example of PUF-dependent mRNA activation is from the kinetoplastid parasite *Trypanosoma brucei*. In *T. brucei*, Puf9 stabilizes mRNA substrates during the S-phase of the cell cycle [35]. The depletion of *PUF9* diminishes the abundance of Puf9-interacting mRNAs, indicating that Puf9 stabilizes it targets, but mutating the presumed Puf9-binding motif results in mRNA stabilization [35]. The most straightforward explanation is that Puf9 is competing with a repressor for binding to the 3′ UTR of targeted mRNAs [35] (Figure 2c).

Whether all PUFs can be activators and repressors, or whether this is restricted to some PUFs only, remains an open question. It is also unclear whether there is a "universal mode" of PUF-dependent activation, similar to a general mode of repression by PUFs via the recruitment of the Ccr4-Pop2-NOT mRNA deadenylase. Furthermore, for the PUFs that can both activate and repress (for example the *C. elegans* FBF), an unanswered question is whether they will repress some mRNAs and activate others or whether these functions are integrated to regulate both positively and negatively the same transcript. Based on both the repression and activation of *gld-1* expression by FBF, it has been proposed that PUFs might represent a "molecular switch" that is regulated by the physiological context requiring the repression or activation of their targets [33]. The molecular triggers of the switch from repression to activation could be conformational changes induced by the binding of partner proteins (for example, the binding of the Ccr4-Pop2-NOT complex could result in changes that affect the subsequent binding of the poly(A) polymerase complex), the binding of additional regulators to the mRNAs or posttranslational modifications of the PUFs. There is no experimental evidence that PUFs can act as molecular switches or how this could be achieved; however, a precedent for posttranslational modifications of a 3′ UTR regulator serving as a trigger for a switch from repression to activation has been described for CPEB [56]. In *Xenopus* oocytes, the CPEB complex on *cyclin B1* mRNA contains both a repressive activity, the deadenylase PARN, and an activating factor, the poly(A) polymerase GLD-2 [56]. Initially, *cyclin B1* mRNA is repressed because the activity of PARN is more robust than that of GLD-2. Upon oocyte maturation, CPEB is phosphorylated by Aurora kinase, causing the dissociation of PARN from the complex [56] and consequently enabling translational activation of *cyclin B1* [56]. Analogous mechanisms might be acting on PUFs to enable them to switch from repressors to activators.

mRNA localization by PUF proteins

Another layer of versatility is added to the function of the PUFs by recent evidence that they work as mRNA targeting factors, thereby contributing to spatial control of expression. Most examples come from yeast. Puf3 localizes mRNAs to the mitochondria [28–30], Puf6 contributes to the asymmetric localization of *ASH1* during transport to the yeast bud [36] and Puf5 influences the localization of *PEX14* mRNA with peroxisomes [38]. Additionally, in olfactory neurons in *C. elegans*, FBF could activate the translation of *egl-4* mRNA near the cell body and sensory cilia [34], and mammalian Pum2 might be involved in localized mRNA translation in neurons [24,39]. To discuss how PUFs might work to promote mRNA localization and the spatial control of expression, we will focus on several recent studies in yeast, investigating the activity of Puf6 and Puf3.

Puf6 regulates the localization and translation of the *ASH1* mRNA [36,37] (Figure 3). *ASH1* encodes a transcriptional repressor expressed only in daughter cells, but not in mother cells of yeast. This is achieved via the asymmetric localization and expression of the encoding mRNA [36,37]; reviewed in [57]. Puf6 binds to an element in the *ASH1* 3′ UTR and inhibits its translation during transport to the bud tip by interacting with and inhibiting the function of the translation initiation factor eIF5B/Fun12 [36,37]. The asymmetric localization of *ASH1* mRNA depends on translational inhibition. Asymmetric localization is perturbed in *puf6* mutants and this can be rescued by the insertion of a

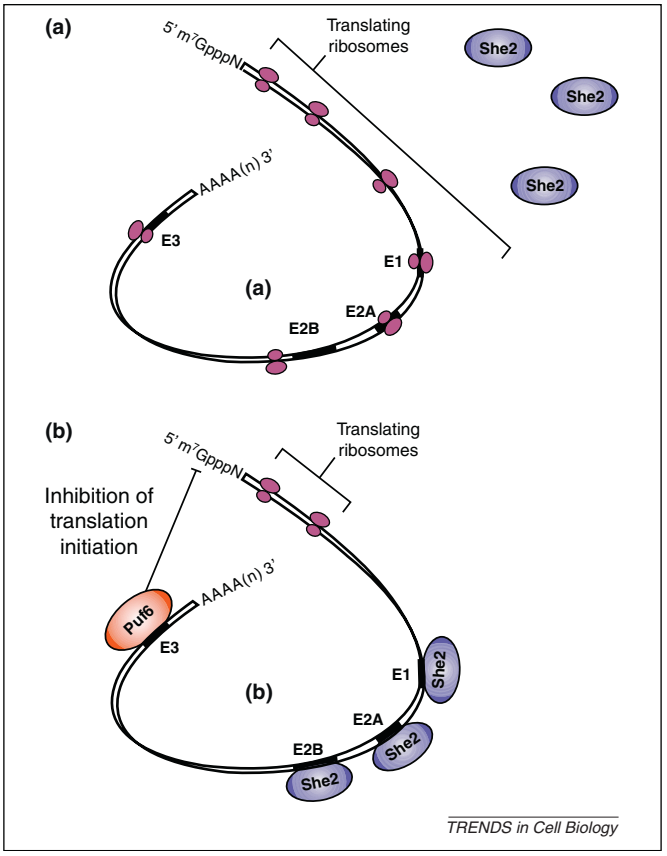

Figure 3. Model of mRNA localization and translational repression by Puf6. **(a)** In the absence of repression by Puf6, translating ribosomes on the *ASH1* mRNA compete with the binding of the locasome component She2 to the localization elements within the *ASH1* ORF. **(b)** Puf6 binding to the *ASH1* 3′ UTR causes the repression of *ASH1* translation due to inhibition of the translation initiation factor eIF5B/Fun12 [36,37]. Translational repression by Puf6 could act to limit competition between translating ribosomes and the locasome component She2 for binding to the E1, E2A and E2B elements in the *ASH1* ORF, thereby promoting mRNA localization [58]. For simplicity, the binding of She2 to the E3 element is not depicted.

stem–loop in the 5′ UTR that slows translation [36]. Three of the four localization elements in the *ASH1* transcript are in the open reading frame (ORF) and bind She2, a subunit of the "locasome complex" required for the localization of mRNAs to the yeast bud [57,58]. It has been proposed that slowing translation contributes to She2 binding to the localization elements in the ORF by diminishing the competition between translating ribosomes and She2 for binding [58] (Figure 3). Once the *ASH1* mRNA has reached its destination at the bud tip, Puf6 is phosphorylated by the CK2 kinase, which lifts *ASH1* repression probably by affecting the binding of Puf6 to the mRNA [37]. To our knowledge, this is the only example of a defined molecular mechanism of control of PUF-dependent gene expression by posttranslational modifications.

Puf3 interacts with mRNAs encoding proteins destined to the mitochondria [59] and contributes to their mitochondrial localization [29,30]. There are four lines of experimental evidence for a direct role for Puf3 in mitochondrial mRNA targeting: (i) the coimmunoprecipitation of mitochondria-localized mRNAs with Puf3 [29,59]; (ii) the mislocalization of mitochondria-localized mRNAs in a *puf3* mutant [29,30]; (iii) that specific Puf3-binding sites in the 3′ UTR of these mRNAs are essential for the mitochondrial localization of the mRNA [29]; and (iv) the binding of Puf3 to mitochondria via its interaction with the ERMES (ER-Mitochondria Encounter Structure) subunit Mdm12 [28].

In addition to contributing to their localization, Puf3 causes the deadenylation and repression of its mRNA targets [28,51,60–62]. It is presently not understood whether or how the roles of Puf3 in mRNA localization and repression are functionally related. It is also unclear what the significance of Puf3 function is for mitochondrial biogenesis.

The localization of mRNAs to mitochondria is thought to facilitate mitochondrial biogenesis by aiding the cotranslational import of proteins [63–67]. Importing proteins cotranslationally is energetically favorable because it negates the need to keep fully translated proteins from folding prematurely. Premature folding prevents protein import across mitochondrial membranes [64].

Mitochondrial proteins bind to the translocase in the outer mitochondrial membrane (TOM complex) via sequences in the amino terminus, meaning that the initial segment of polypeptide leaving the ribosome can bind the TOM receptor subunit Tom20 in the mitochondrial outer membrane. This initiates cotranslational import and also tethers the translating mRNAs, contributing to their association with mitochondria [30,68] (Figure 4). The direct interactions of the mRNA with Puf3 at the mitochondrial surface further contribute to mitochondrial localization [29,30] and should therefore have a positive impact on cotranslational import and mitochondrial biogenesis. By contrast, because of its repressive function [28,51,60–62], Puf3 is expected to inhibit mitochondrial biogenesis.

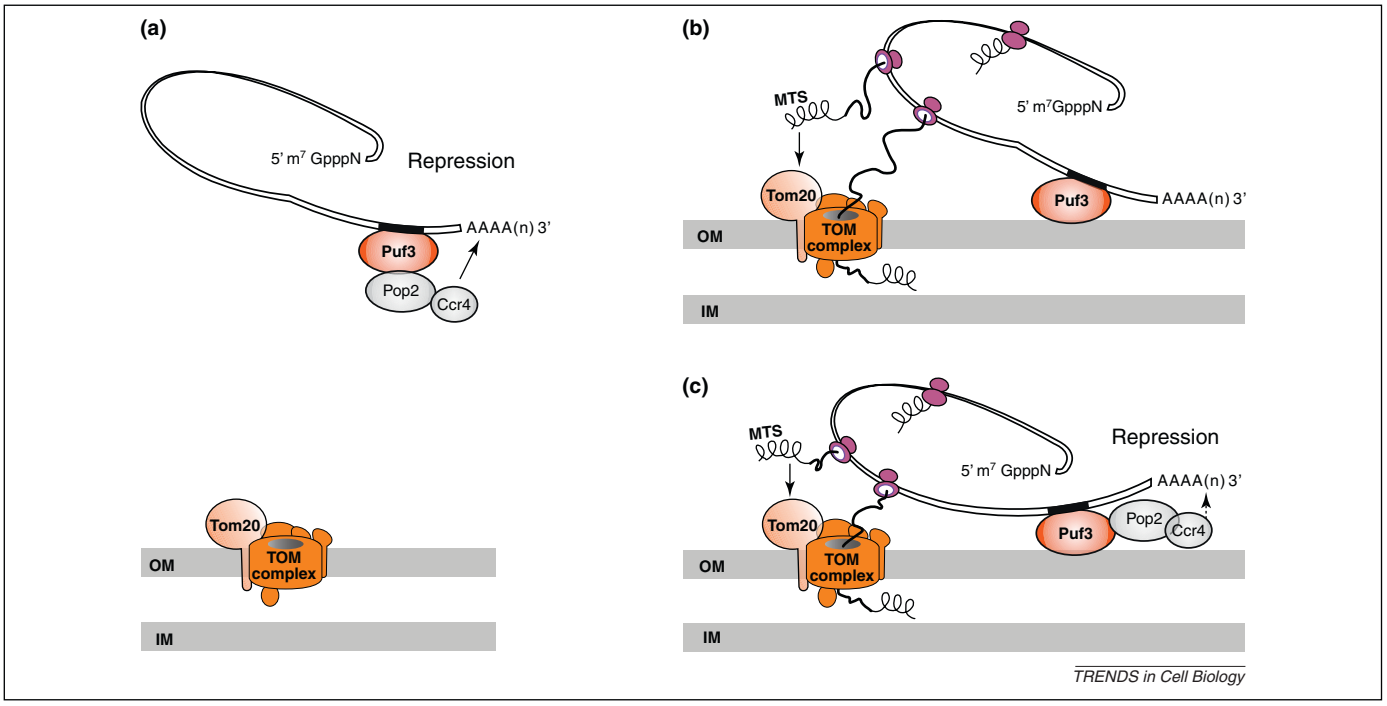

Figure 4. Models of Puf3 function in mRNA repression, the localization of transcripts to the mitochondria and mitochondrial biogenesis. **(a)** Non-mitochondrial Puf3 represses the expression of its interacting mRNAs. It does so by promoting deadenylation via the recruitment of the Ccr4-Pop2-NOT mRNA deadenylase and possibly other mechanisms affecting the structure of the ribonucleoprotein complex [51,60–62]. For simplicity, the NOT subunits of the Ccr4-Pop2-NOT complex are not depicted and instead only Ccr4 and Pop2, which are essential for deadenylation, are shown. **(b)** At the mitochondria, the repressive function of Puf3 is inhibited. Here, the interaction of Puf3 with its partner mRNAs serves to stabilize the localization of the transcripts at the mitochondria, such that mitochondrial targeting sequences (MTS) emerging from ribosomes bind efficiently to Tom20 and are imported cotranslationally via the TOM complex. **(c)** In an alternative scenario, at the mitochondria Puf3 not only contributes to the localization of its interacting mRNAs, but also represses their translation at this location. Puf3-dependent deadenylation could be modulated at the mitochondria to slow, but not fully inhibit, translation. For cotranslational import to proceed with optimal efficiency, the processes of translation and import into the mitochondria have to be balanced. Slowing translation in a Puf3-dependent manner could aid cotranslational protein import into the mitochondria by affording time for the TOM complex to interact with and translocate the proteins before a fully translated protein domain starts to fold.

In support of a repressive role, Puf3 levels decline upon the induction of respiratory metabolism (which promotes mitochondrial biogenesis) [28], and Puf3 no longer destabilizes reporter transcripts in such conditions [62].

These potentially conflicting effects have also been observed in phenotypic studies. *PUF3* overexpression leads to impaired respiratory growth [29], but so does the deletion of *PUF3* [30,59]. Although a *puf3Δ* single mutant is only modestly compromised for growth on a non-fermentable carbon source like glycerol [59], Puf3 becomes essential for respiratory growth on glycerol in a strain lacking the mitochondrial import receptor Tom20 [30]. This synthetic genetic interaction between *TOM20* and *PUF3* is a strong indication that Puf3 has a positive role in mitochondrial biogenesis, probably because of its mRNA localization function. In summary, these data suggest that Puf3 might regulate a dynamic balance among mRNA translation, targeting and protein import that is crucial for mitochondrial function.

One could imagine several possibilities for how the mRNA localization and mRNA repression functions of Puf3 might be controlled. It is possible that these two roles are distinct (Figure 4a and b). As suggested by only partial colocalization of GFP-Puf3 with DAPI-stained mitochondrial DNA and Mdm12 [28], there are likely to be two pools of Puf3 in the cytoplasm: a mitochondrial and a non-mitochondrial pool. The non-mitochondrial pool of Puf3 could act as a repressor, causing deadenylation and inhibiting the expression of mRNAs encoding mitochondrial proteins (Figure 4a), thereby perhaps contributing to the repression of mitochondrial biogenesis during nonrespiratory growth [28]. By contrast, the mitochondrial pool of Puf3, by definition, contributes to mRNA localization to mitochondria, thereby aiding cotranslational import. In this scenario, the mRNA deadenylation and repression function of Puf3 is inhibited at the mitochondrial surface, and Puf3 only functions as an mRNA-tethering factor (Figure 4b). Changes to the Puf3–mRNA complexes owing to the interactions of Puf3 with mitochondrial proteins, such as Mdm12 [28], as well as the interactions of the translating mRNA with the Tom20 receptor and the mitochondrial import machinery during cotranslational import could act to override the repressive function of Puf3.

Alternatively, it is possible that Puf3 not only assists mRNA tethering at the mitochondrial surface but also represses translation at this location (Figure 4c). Puf3-dependent shortening of the poly(A) tail on its mRNA targets at the mitochondria could serve to slow their translation by limiting translation initiation [42–44,69] (Box 3). Although seemingly counterintuitive, this could help cotranslational import. If cotranslational import is to proceed at a maximum rate, the rate of import has to be balanced with the rate of translation to prevent premature translation and folding of the proteins [64]. Therefore, Puf3-dependent slowing of translation could allow time for cotranslational import to proceed with maximum efficiency once the translating mRNAs have docked onto the TOM complex (Figure 4c).

This model implies that, at the mitochondria, Puf3-dependent deadenylation would be controlled in such a way that the poly(A) tail remains long enough to enable translation and compete with decay. Again, it can be envisaged that, at the mitochondria, changes occur to the complex of Puf3 with mRNAs and other partners (such as the Ccr4-Pop2-NOT mRNA deadenylase), modulating the rate of deadenylation to slow, but not fully inhibit, translation.

Experiments comparing the half-lives of the mitochondria-associated mRNA pool versus the non-mitochondrial transcripts in the presence or absence of Puf3, as well as the identification of Puf3 partner proteins and potential posttranslational modifications depending on mitochondrial localization, could help distinguish the best model of Puf3 function in mitochondrial biogenesis.

Concluding remarks

Recent evidence has indicated that PUFs are versatile mRNA regulators, acting as repressors, activators and mRNA targeting factors. Given the impact of these regulators on processes such as differentiation, development and stem cell maintenance, it is exciting to envisage that PUFs might employ multiple mechanisms to regulate their mRNA targets and contribute to their cellular functions. It remains to be determined how the repressive and activating functions of PUFs are integrated and mechanistically enacted. It is also still unclear how widely PUFs contribute to mRNA localization and how the targeting role is integrated with mRNA repression and activation.

The challenge now is to understand how PUFs regulate the multitude of their putative mRNA targets [31,59,70–73]: do they activate these targets, repress them or both, and how does PUF-dependent regulation change in response to extracellular and intracellular signals? Moreover, addressing the subcellular localization of PUF mRNA targets and how that localization depends on PUFs should establish the generality of the mRNA targeting function of PUFs and enlighten us to the function of PUFs in coordinating gene expression in space and time.

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