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The double life of the ribosome: when its protein folding activity supports prion propagation

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Abstract

It is no longer necessary to demonstrate that ribosome is the central machinery of protein synthesis. But it is less known that it is also key player of the protein folding process through another conserved function: the protein folding activity of the ribosome (PFAR). This ribozyme activity, discovered more than two decades ago, depends upon the domain V of the large rRNA

within the large subunit of the ribosome. Surprisingly, we discovered that anti-prion compounds are also potent PFAR inhibitors, highlighting an unexpected link between PFAR and prion propagation.

In this review, we discuss the ancestral origin of PFAR in the light of the ancient RNA world hypothesis. We also consider how this ribosomal activity fits into the landscape of cellular protein chaperones involved in the appearance and propagation of prions and other amyloids in mammals. Finally, we examine how drugs targeting the protein folding activity of the ribosome could be active against mammalian prion and other protein aggregation-based diseases, making PFAR a promising therapeutic target for various human protein misfolding diseases.

Keywords

prion, ribosome, PFAR, chaperon, protein folding, RNA

Present in every cell and mitochondria, ribosomes are composed of two subunits, which themselves are made up of ribosomal RNA (rRNA) and proteins. Broadly, the small subunit initiates the translational process and ensures the correct decoding of genetic information, while the large subunit catalyzes the peptidyl transferase activity that covalently links amino acids together. While the central role of the ribosome in protein synthesis is well appreciated, little is known about the second ribozyme activity of the ribosome: its protein folding activity (PFAR).

Since its discovery, the existence of PFAR has been controversial and difficult to gain acceptance within the scientific community. Despite PFAR being first identified in 1994 by the group of C. Das Gupta ¹ and this activity being corroborated by several other teams ², PFAR is often overlooked, as illustrated by its absence from reviews on non-coding RNAs. However, once getting over the initial surprise of the existence of such an activity, it seems self-evident that the biological entity that has evolved to synthesize proteins must also aid them achieve a proper three-dimensional functional state prior to their release. In addition, this is fully consistent with the estimation that, apart from spontaneously folded proteins, only a fraction of the total cellular proteins can be folded by classical protein chaperones ³.

Initially, PFAR was described *in vitro* for bacterial ribosomes and was quickly shown to be a conserved function of any ribosome, whether from bacteria, eubacteria, eukaryotes or even mitochondria ². The conservation of the protein folding capacity of the ribosome among species is not a surprise when considering the high rate of conservation of rRNA in the ribosome core throughout evolution ⁴. As one may expect, PFAR is a versatile process that has been demonstrated to be able to refold any protein challenged so far *in vitro* ² and *in vivo* ⁵⁻⁸. This folding activity appears to be inherent to the conserved RNA domain that also harbors the

peptidyl transferase activity: domain V of the rRNA of the large subunit of the ribosome (23S for bacteria, 25S for yeast and 28S for mammals). However, the nucleotides of domain V involved in its protein folding activity are different from those involved in its peptidyl transferase activity^{9, 10} (**Figure 1**).

The protein folding mediated by domain V is a two-step post-translational process: the neo-synthesized polypeptide is firstly folded by the central loop of domain V (RNA1) and remains associated to it until the intervention of a second part of domain V (RNA2) which is responsible for the release of the folded protein¹¹. The nucleotides involved in PFAR are localized at the interface of the ribosome's small and large subunits⁹. Ribosomal subunits dissociate in the presence of unfolded polypeptides, making them more accessible to PFAR-involved nucleotides and thus enhancing their folding ability². This is in good agreement with the fact that protein synthesis and protein folding are synchronized: as long as the peptidyl transferase activity continues, the protein folding activity of the ribosome is silenced and only becomes operational when translation is completed¹².

In the midst of identifying new anti-prion compounds, we identified anti-prion drugs such as 6-aminophenanthridine (6AP,¹³), guanabenz (GA,¹⁴) and imiquimod (IQ,¹⁵) which are active *in vivo* against yeast and mammalian prions and that were amazingly identified as PFAR inhibitors.

The discovery that 6AP and GA anti-prion drugs are also anti-PFAR drugs led us naturally to suggest that PFAR may be linked to prion propagation. Although the primary role of protein chaperones is to prevent protein misfolding and aggregation, involvement of protein chaperones like PFAR in the propagation of prion conformation is self-evident, as amyloid-forming proteins exist in several conformations and replication corresponds to propagation of differentially folded

states ¹⁶. This hypothesis has strong support as Hsp104p, a yeast heat shock protein, has been shown for a long time to be involved in yeast prion propagation, together with Hsp70 and Hsp40. Similarly to Hsp104p, PFAR misregulation leads to a defect in the propagation of the yeast prion $[PSI^+]$ ⁶ (**Figure 2**). The anti-PFAR drugs we identified were the first of that kind: all the previous anti-PFAR drugs described so far are antibiotics that also bind to domain V and inhibit its peptidyl transferase activity ^{5, 7}. Of note, the fact that some antibiotics specifically target PFAR is a further indication of the central function of this ribozyme activity.

In this review, we consider the ancestral origin of PFAR and the relationship of this emerging ribosomal activity with the global protein folding capacity of the cell. We also discuss PFAR involvement in the propagation of prions and other amyloids in mammals.

PFAR activity: another relic of the ancient RNA world into modern ribosomes?

In current living organisms, the newly synthesized polypeptides undergo several maturation mechanisms in order to be correctly processed and translocated to achieve their functional folded state. These maturation processes are often linked to protein synthesis. They depend on translational speed and on interactions of the nascent polypeptide chain with the peptide exit tunnel and with the nascent chain-interacting protein factors, which are located on and around the ribosomal tunnel exit area. Beyond, a network of other cooperating chaperones acting post-translationally in a coordinated ballet are also involved during the latter stages of the protein folding process ¹⁷. However, this system brings the endless chicken-and-egg paradox back into play: to get mature and functional proteins, the cell needs... mature and functional chaperoning factors! To address this paradox, one has to consider the evolution of the protein folding process and how the ancient foundations may have developed. In the ancient RNA world hypothesis,

earlier forms of life may rely on the dual function of RNA that simultaneously catalyzes enzymatic reactions and store genetic information ¹⁸. This suggests that RNA could be the main actor that carried out the chemical reactions allowing proteins to gradually emerge and become more complex due to the development of more and more sophisticated protein folding capabilities. Many molecular fossils of the ancient RNA world are still present and sometimes still active in modern organisms. Candidates must be catalytic, ubiquitous, and/or central to some aspect of metabolism ¹⁹. Accordingly, one of the most significant piece of evidence to support that the first peptide bonding machines emerged in an RNA world is the fact that the modern ribosome is a ribozyme, or in other words that only RNA performs its key role in peptide bond formation ²⁰. The peptidyl-transferase center (PTC) of the modern ribosome is a universal and highly conserved RNA-only structure located in the domain V of 23S rRNA in bacteria or its equivalent parts in eukaryotic ribosome. It forms a symmetrical pocket in the heart of the large ribosomal subunit and probably originated from the dimerization of two stem–elbow–stem motifs ²¹. The PTC is considered to be the first proto-ribosome dating from the ancient RNA world since it was certainly capable of triggering catalytic reactions by itself. Strikingly, as supported by the binding sites of its protein substrates or inhibitors, PFAR activity is clustered within the PTC binding pocket, surrounding the core where the catalytic reaction takes place ^{9, 22} (**Figure 1**). Therefore, PFAR encompasses most criteria for making it a perfect vestige of the ancient RNA world as it is: *i*) part of another ribozyme, the PTC, *ii*) as ancient as the PTC, *iii*) supported by RNA only, *iv*) conserved from bacteria to eukaryotes and *v*) capable of playing a key role in modern protein synthesis. In this scenario, the first PTCs (proto-PTCs) were formed by the dimerization of short helical structures that created the first random but fragile peptides

aleatory using amino acids. In a process that might be assimilated as “molecular Darwinism”²³, these proto-PTCs would then have evolved into a stable PTC, able to better favor protection, stability and activity of the peptides they synthesized. While one can assume that the earliest peptides were short, single domain and rapidly selected for robust chaperone-independent folding, the emergence of a primitive PFAR certainly conferred a selective advantage to the peptides. In turn, and resulting from the more and more efficient peptides and proteins being produced, the proto-PTC slowly co-evolved into a proto-ribosome and finally into the modern ribosome we recognize today, likely by progressively integrating multiple small ribonucleoprotein complexes into a much more complex machinery²⁴. The traces of these ancient times remain active RNAs such as domain V harboring PTC and PFAR, still embedded in contemporary ribosomes, and may continue to play a role as part of the global protein folding capability of the cell.

Which place PFAR could take in the array of protein chaperones?

Prion propagation in yeast has been shown to be intimately linked to the expression and activity of a plethora of protein chaperones. We recently showed that PFAR and Hsp104p partially compensate each other for $[PSI^+]$ propagation in yeast (**Figure 2**). We indeed observed that PFAR up-modulation can compensate for the partial loss of Hsp104p activity and that PFAR partial down-modulation is also compensated by Hsp104p increase⁶. These results clearly indicate that PFAR is linked to the cellular array of protein chaperones, at least through Hsp104p. To this respect, PFAR participates to the very complex network required for cell proteostasis, *i.e.* the global protein quality control and the maintenance of proteome homeostasis. Of note, polynucleotides, and more particularly RNAs, have been shown to exhibit potent

chaperone activity *in vitro*²⁵. Proteostasis involves hundreds of proteins constituting a highly regulated and integrated network.

One of the main challenges of the cellular response to stress is to synthesize, under adverse conditions, correctly folded proteins essential for survival. Since the PTC and PFAR are intimately linked, PFAR activity directly depends upon the synthesis of proteins, thus allowing the cell to establish a proper equilibrium between protein production and folding activities. In this context, the link between the PTC and PFAR can be seen as a strategy to avoid the synthesis of a polypeptide in the absence of efficient cellular folding activities. Remarkably, in situations of cellular stress, translation is strongly reduced, which consequently increases the population of non-translating ribosomes becoming available for protein folding. As a consequence, the corresponding misfolded proteins emerging from the deficient ribosome will be more prone to proteolysis. Thus, PFAR could also participate to proteostasis as a potential proofreading process of the PTC activity.

While Hsp104, and now PFAR, are key players in dictating the appearance and propagation of naturally occurring amyloid-based yeast prions, members of the cytosolic Hsp70 chaperone machinery, and associated co-chaperones, are important modulators of prion propagation, and in some instances *de novo* prion formation^{16, 26}. The cytosolic Hsp70-Ssa [Stress Seventy Sub-Family A] family members are well characterised as playing an integral role in modulating the propagation of a variety of yeast prions^{27, 28} and the ribosome-associated Hsp70-Ssb [Stress Seventy Sub-Family B] family members are anti-prion in their action since they repress the spontaneous appearance of [*PSI*⁺]²⁹. Of note, contrary to Hsp104p and Hsp70 which expression is induced by stress, PFAR is a ready-to-use protein folding source immediately available in case

of heatshock to supplement Hsp104 or to compensate for its absence, similarly to mammals where there is no *HSP104* homolog.

Where could PFAR “sit” amongst these other cellular factors and how may they interact to influence prion propagation, or other protein folding events *in vivo*? One possibility is that some prion phenotypes consecutive to alterations in PFAR activity could result from indirect effects through alteration of chaperone systems such as the cytosolic Hsp70 machinery. However, such phenotypes are not due to indirect effects on Hsp70 abundance ⁶ and it is unlikely that alterations in PFAR activity could significantly disturb Hsp70 specific functions, unless through indirect effect on Hsp70 co-chaperone activities. PFAR could possibly work in conjunction or in tandem with Hsp70s or other chaperones and the association of Hsp70-Ssb and the interaction with RAC [Ribosome Associated Complex] in prion formation and propagation ³⁰ provides the basis for such interactions to evolve. Chaperones often function as multi-complex machines and it is conceivable that Hsp70 or co-chaperones could be involved into the delivery or processing of substrates acted upon by PFAR, or that PFAR could process substrates acted on by downstream chaperone machinery. The construction and characterisation of yeast cells modified to alter PFAR activity now provides the basis for a thorough genetic assessment of interaction between PFAR and an array of protein chaperone pathways. The involvement of PFAR in protein homeostasis could extend well beyond prion propagation and should it be the case, the integration with other chaperone networks is even more likely.

PFAR: mammalian prion propagation and beyond?

How could drugs targeting the protein folding activity of the ribosome, which mainly lies in the cytoplasm, be active against mammalian prion PrP^{Sc}, which is believed to be modified mostly at

the cell surface or on endocytic compartments? Addressing this question emphasizes our lack of precise knowledge regarding the cellular compartments where prion conversion and subsequent accumulation occur. This is partly due to the fact that direct and dynamic visualization in cells remains a highly challenging issue, notably because tagging the cellular form of the prion protein (PrP^C) most often prevents its conversion into the disease-specific isoform PrP^{Sc}. In many differentiated cell types, a major proportion of PrP^C is detected in lipid rafts at the cell surface, being anchored by a GPI moiety (for review ³¹). PrP^C expression at the cell surface is required for conversion into misfolded PrP^{Sc} ^{32, 33} and this process may occur rapidly ³². Several reports also indicate that a substantial fraction of PrP^C cycles constitutively between the plasma membrane and endocytic compartments and that conversion can also occur in several intracellular compartments all along the endocytic pathway ³⁴⁻⁻³⁶. A minor proportion of PrP^C can be found in the cytosol or in contact with the cytosol, due to retrotranslocation from the endoplasmic reticulum (or escape before translocation) or transmembrane anchoring, respectively (for review ³⁷). In physiological conditions, these forms are rapidly cleared by quality control processes (ERAD (Endoplasmic-reticulum-associated protein degradation), aggresomes). However, in the prion disease state, or with mutated PrP mimicking familial forms of prion diseases, the levels of cytosolic PrP are increased ^{37, 38}. The exact contribution of these variants to prion pathology remains unknown. PrP^C is also involved in many cellular processes and signaling pathways. These processes include response to oxidative stress, ER stress, apoptosis, proliferation and differentiation (reviewed in ³⁹⁻⁻⁴¹). Certain stress can even trigger unusual localization of PrP^C into the nucleus ⁴². Late stages of prion pathogenesis involve over-activation of the PERK (protein kinase RNA-like endoplasmic reticulum kinase) pathway of the

unfolded protein response in experimental mouse models⁴³. Such pathways and the deregulation of the PrP-dependent physiological pathways, or a yet to discover PFAR-dependent proteostatic pathway in mammals⁴⁴, provide plenty of scope for direct or indirect interactions with PFAR to occur.

PFAR involvement goes most probably further than prion-based diseases such as Creutzfeldt-Jacob disease. There is indeed growing evidence that amyloid-based diseases like OPMD (Oculopharyngeal muscular dystrophy), Alzheimer's, Parkinson's and Huntington's diseases share key biophysical and biochemical characteristics with prionopathies: they involve accumulation of aggregates of misfolded host-encoded proteins through prion-like processes of seeded polymerisation⁴⁵⁻⁴⁷. As metazoa have no Hsp104p ortholog, the protein folding activity of domain V of the ribosome might thus correspond to the Hsp104-like activity responsible for amyloid handling that is absent in these organisms. This original hypothesis makes sense in light of our recent findings that some anti-prion compounds with anti-PFAR activity, like 6AP, GA and IQ, are also active in models of other protein aggregation-based diseases like Huntington's disease, Parkinson's disease (unpublished data) and OPMD⁴⁸. Our data thus indicate that PFAR involvement in amyloid handling may be shared by several human protein misfolding-based diseases, hence making PFAR a potential therapeutic target for human protein misfolding diseases. Despite the central role of PFAR in the proteostasis of the cell, it is still a promising therapeutic target to treat this class of diseases, either as a sole target or as part of a collaborative strategy targeting more than one cellular chaperone system. Indeed, its mild inhibition, together with other therapeutics, may help overcome the progression of these invariably fatal pathologies.

Conflict of interest

The authors declare no competing financial interests in relation to the work described.

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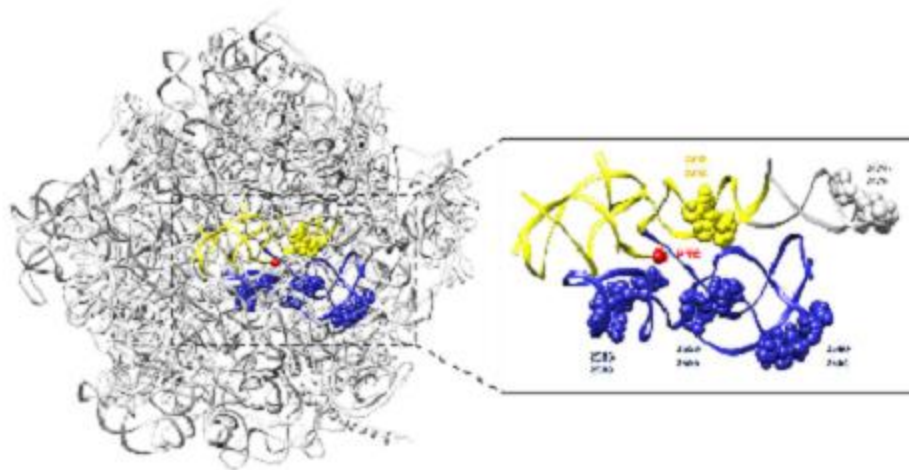


Figure 1. The sites of major importance for PFAR activity are included within the PTC region. The ribosomal large subunit is shown in light grey (Protein Data Bank code 4V6F). The yellow and blue helices indicate the two helices part of the symmetrical region forming the PTC cavity. The nucleotides involved in PFAR activity are numbered and represented as spheres (*Thermus thermophilus* numbering). The core of the PTC is indicated by a red sphere (Adapted from ⁹). Yeast nucleotide U2892 ⁶ corresponds to position 2492.

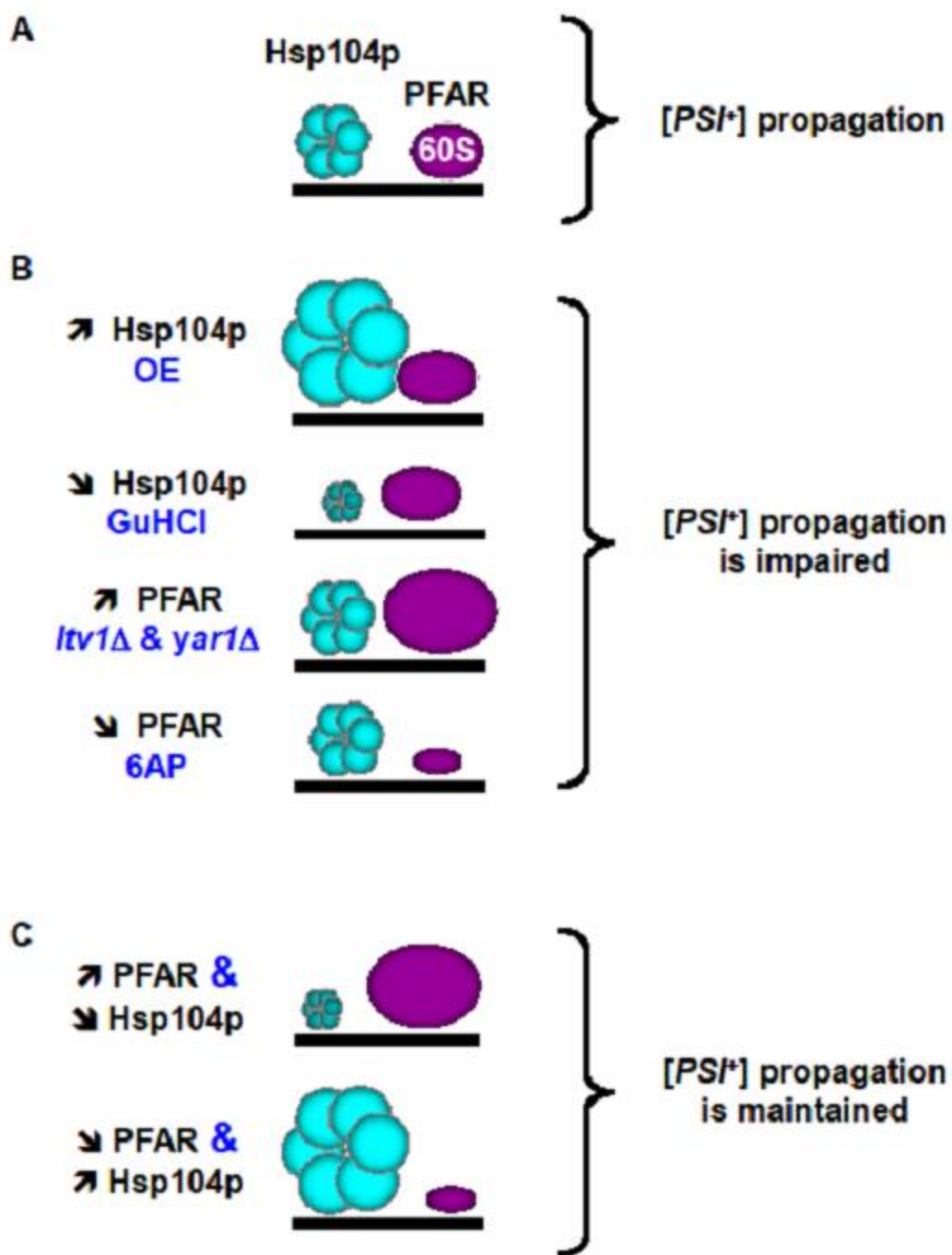


Figure 2. Interplay between protein folding activities of Hsp104p and ribosome in modulating [PSI⁺] propagation (adapted from ⁶). **A** - Hsp104p and PFAR both participate in [PSI⁺] propagation. **B** - [PSI⁺] propagation is affected by enrichment or inhibition of Hsp104p

and PFAR. C - Hsp104p and PFAR interplay results in the compensation of the increase of one by the reduction of the other, and *vice versa*. OE, overexpression. GuHCl, Hsp104p inhibitor guanidine hydrochloride. *ltv1* Δ and *yar1* Δ , PFAR-enriched yeast strains deleted for *LTV1* or *YAR1* genes ⁶.

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