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SHORT COMMUNICATION

Contributions of aminoacyl-tRNA synthetase-interacting multifunctional protein-3 to mammalian translation initiation

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Abstract Aminoacyl-tRNA synthetase-interacting multifunctional protein-3 (AIMP3) stabilizes and protects mammalian methionyl-tRNA synthetase (MRS) and eukaryotic initiation factor 2 subunit gamma (eIF2γ), factors involved in the formation and the delivery of Met-$tRNA_i^{Met}$ respectively, through the binding interactions. Due to the protections that MRS and eIF2γ are provided from the interactions with AIMP3, cellular levels of MRS and eIF2γ may be able to be maintained high enough for their canonical and/or non-canonical functions.

Keywords Aminoacyl-tRNA synthetase-interacting multifunctional protein-3 (AIMP3) · Methionyl-tRNA synthetase (MRS) · Eukaryotic initiation factor 2 subunit gamma (eIF2γ) · Protein–protein interaction · Translation initiation

Introduction

Aminoacyl-tRNA synthetases (ARSs) catalyze aminoacylation reaction, which connects specific amino acids to the tRNAs bearing corresponding anticodon sequences (Lee et al. 2004; Park et al. 2005). This process, which is also called as "tRNA charging", requires a formation of aminoacyladenylate intermediate prior to the attachment to the 3′ terminus of tRNA and forms aminoacyl-tRNA (Fig. 1a) (Lee et al. 2004; Park et al. 2005).

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In eukaryotic cells, multi-tRNA synthetase complex (MSC) is formed by the protein–protein interactions among 9 ARSs and 3 non-enzymatic scaffold proteins, aminoacyl-tRNA synthetase-interacting multifunctional proteins (AIMP); AIMP1 (also known as p43), AIMP2 (p38), and AIMP3 (p18). Each scaffold protein interacts with specific ARSs, thus comprise three subdomains within the large ARS complex (Lee et al. 2004; Park et al. 2005, 2010). From the three non-enzymatic factors, AIMP3, which is also known as a tumor suppressor p18, interacts with methionyl-tRNA synthetase (MRS) (Rho et al. 1999; Park et al. 2005; Han et al. 2006). The amino-terminal domain of human MRS has a high homology with the glutathione *S*-transferase (GST) and plays critical roles in the interaction with the GST-like domain of AIMP3 (He et al. 2009). AIMP3 is also known for interacting with ATM, which is upstream kinase of p53, and the interface of AIMP3 in this interaction was proposed to be distinct from the interface in the binding interaction with MRS (Park et al. 2005; Kim et al. 2008).

Among the ARSs, MRS may be particularly interesting since it is involved in translational initiation by producing Met-$tRNA_i^{Met}$, which is required for the formation of multi-protein complex with other translation initiation factors (Stolboushkina and Garber 2011), and it may have roles in oncogenesis (Marshall et al. 2008). While MRS produces Met-$tRNA_i^{Met}$, eukaryotic initiation factor 2 (eIF2), which is composed of α, β and γ subunits, delivers Met-$tRNA_i^{Met}$ to the 40S ribosome in dependent to GTP (Stolboushkina and Garber 2011; Sonenberg and Hinnebusch 2009). Among the eIF2 subunits, eIF2γ is known to be extremely unstable (Suragani et al. 2006).

Here we present possible mechanisms how AIMP3 contributes to mammalian translation initiation. AIMP3 protects MRS, thus may secure the production of Met-$tRNA_i^{Met}$.

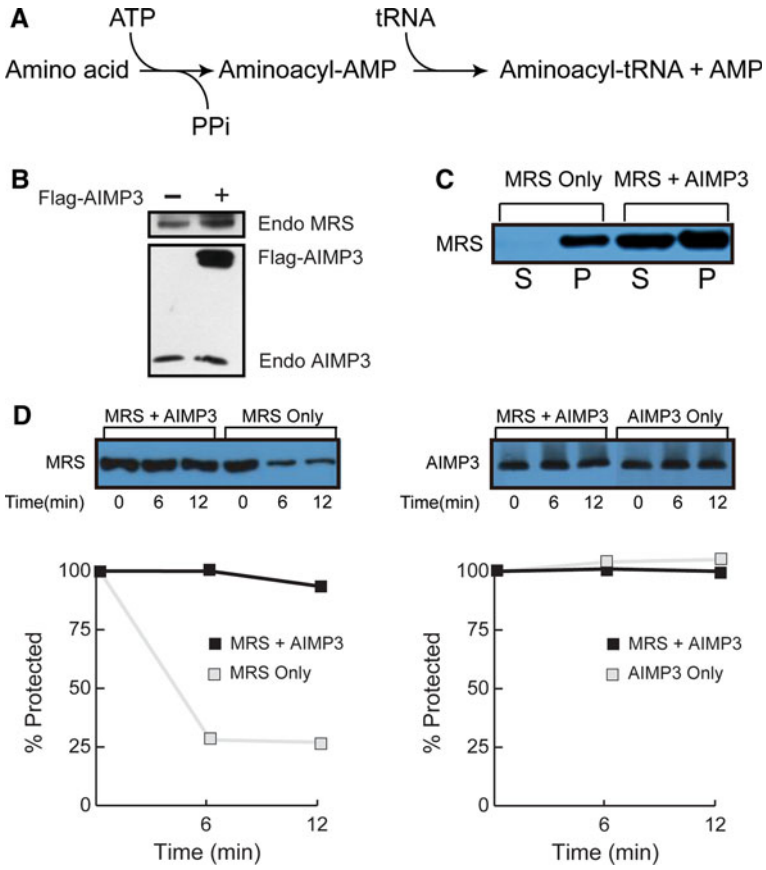

Fig. 1 AIMP3 stabilizes and protects MRS. **a** Diagram of the aminoacylation reaction (tRNA charging). "tRNA charging" is a two-step reaction which are catalyzed by tRNA synthetase. **b** Over-expression of exogenous AIMP3 increases the expression level of endogenous MRS. **c** AIMP3 increases the solubility of MRS. The supernatant (indicated as "S") and pellet (indicated as "P") fractions were separated by SDS-PAGE and the existence of MRS was detected by immunoblotting. **d** MRS is extremely sensitive to the proteolysis of elastase. MRS is protected by AIMP3 from proteolytic digestion of elastase (*left panel*). AIMP3 was not affected by elastase (*right panel*)

At the same time, AIMP3 also protects eIF2γ, thus may contribute to the delivery of Met-tRNA$_i^{Met}$ to the 40S ribosome. As a consequence of protections of MRS and eIF2γ by MRS-AIMP3 and eIF2γ-AIMP3 interactions, the cellular levels of MRS and eIF2γ may be maintained high enough to be involved in canonical and/or non-canonical cellular functions.

Materials and methods

Transfection and determination of endogenous expression level of MRS and eIF2γ

Human embryonic kidney 293 cells or A549 cells were cultured in DMEM medium supplemented with 10 % fetal bovine serum (FBS) and 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5 % CO_2 incubator. To determine the effect of AIMP3 over-expression on the expression level of endogenous MRS or eIF2γ, HEK293 cells or A549 cells were transfected with pFLAG-VN173-AIMP3 by Turbofect transfection reagent (Fermantas). Cells were lysed with 50 mM Tris–HCl (pH 7.4), 0.5 % Triton X-100, 5 mM EDTA, 10 % glycerol, 150 mM NaCl containing protease and phosphate inhibitor. The cell lysates were separated by SDS-PAGE and detected by immunoblotting with anti-MRS, anti-eIF2γ and anti-AIMP3 antibodies.

Expressions of MRS, eIF2γ and AIMP3

Human AIMP3 and MRS genes were cloned into the His-tag multiple cloning sites (MCS) of pETDuet or pACYCDuet (Novagen) to produce His-tagged AIMP3 and MRS. The MRS, eIF2α, eIF2β and eIF2γ genes were cloned into the S-tag MCSs of pETDuet or pACYCDuet to produce MRS and eIF2γ without His-tag. The single or the pairs of each protein were expressed as per the manufacturer's manual (Qiagen). Co-expressions of pairs of proteins were confirmed by immunoblotting.

MRS and eIF2γ digestion by elastase

Methionyl-tRNA synthetase and eIF2γ (15 μg) were digested with 0.6 U of elastase (Sigma) for indicated time at 37 °C in the presence or absence of AIMP3. AIMP3 was

also digested in the presence or absence of MRS or eIF2γ for the control experiments. Digested samples were separated by SDS/PAGE and detected by Western blot analysis.

Results and discussion

AIMP3 stabilizes and protects MRS

High yield preparation of human MRS has been critically required for the functional studies of MRS. However, it has been difficult and challenging to get high expression level of MRS in bacterial system. In an effort to get high yield preparation of human MRS for the functional study, we tried to express MRS under various conditions (temperatures, time, etc.) or to construct fusion proteins with various tagging protein domains. The problem has been relieved by the attachment of large size protein domain, maltose-binding protein (MBP), to make a MBP–MRS fusion protein (Kwon et al. 2011). In regard to the strong association between MRS and AIMP3, we naturally raised a possibility that AIMP3 may affect the expression of MRS. In addition, the earlier observation of decreased level of MRS upon knock-down of AIMP3 (Han et al. 2006), in turn, may implicate the possibility that AIMP3 may stimulate the intracellular expression level of MRS. To test this hypothesis, we tried over-expression of exogenous AIMP3 (tagged with Flag) and check the expression level of the endogenous MRS by Western blot analysis. As seen in Fig. 1b, the expression level of the endogenous MRS was significantly stimulated by the over-expression of exogenous Flag-AIMP3. Previously, it was reported that the expression level of AIMP3 does not affect the transcription level of MRS (Han et al. 2006). Thus, we hypothesized that the stability of MRS may be increased by the consequence of MRS–AIMP3 interaction and the expression level of MRS looks like to be up-regulated. To test this idea, we tried over-expression of MRS in the presence or absence of AIMP3 to investigate whether AIMP3 affect the solubility of over-expressed MRS. We constructed dual expression plasmid over-expressing both MRS and AIMP3. For comparison, we also prepared single expression vector only for MRS. The co-expression of MRS with AIMP3 resulted in dramatically increased solubility of MRS and significant amount of MRS was found in the soluble supernatant fraction (Fig. 1c). Since MRS and AIMP3 are closely related to perform their functional roles in the cell, the stability of MRS may be significantly improved through the binding interaction with AIMP3.

We also investigated whether AIMP3 protects MRS from the proteolytic attack of a protease. In the AIMP3–MRS complex, the regions of MRS, susceptible for the proteolysis, may be covered by AIMP3 and protected from the proteolytic attacks of proteases. To test this possibility, we designed experiment using elastase digestion. Compared to AIMP3, MRS was highly sensitive to the proteolysis by protease (Fig. 1d). When AIMP3 was added to form a MRS–AIMP3 complex, MRS was clearly protected

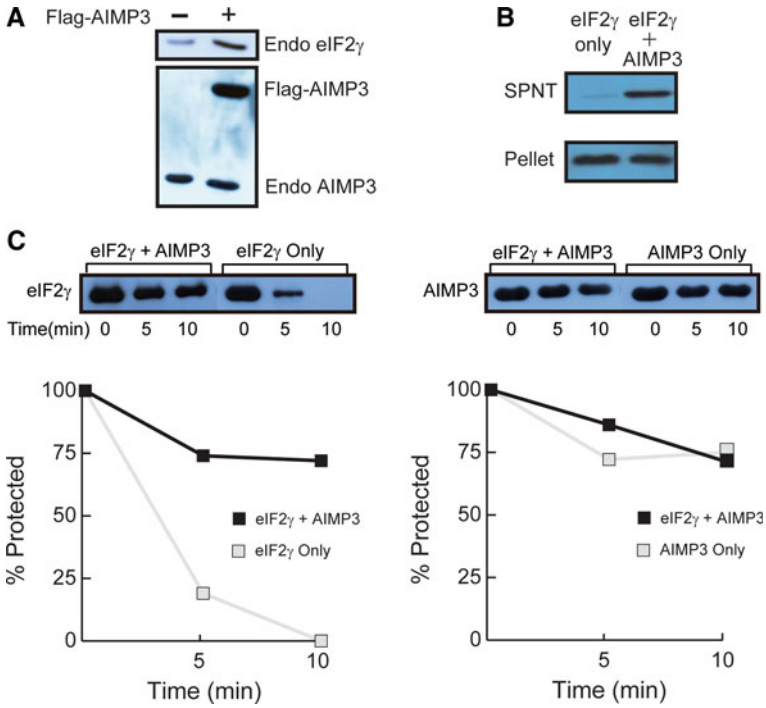

Fig. 2 AIMP3 stabilizes and protects eIF2γ. **a** Over-expression of exogenous AIMP3 increases the expression level of endogenous eIF2γ. **b** AIMP3 increases the solubility of eIF2γ. The supernatant (indicated as "S") and pellet (indicated as "P") fractions were separated by SDS-PAGE and the existence of eIF2γ was detected by immunoblotting. **c** eIF2γ is protected by AIMP3 from proteolytic digestion of elastase (*left panel*). AIMP3 was not affected by elastase (*right panel*)

by the existence of AIMP3 against elastase digestion (Fig. 1d) and this may show that AIMP3 provide MRS protection against the proteolytic digestion of protease.

AIMP3 stabilizes and protects eIF2γ

Methionyl-tRNA synthetase catalyzes the formation of Met-tRNA$_i^{Met}$, which is a critical component of ternary complex with eIF2 components (α, β, γ subunits) and GTP for the translation initiation. Among the three subunits of eIF2, eIF2γ grasps particular interest since it binds with Met-tRNA$_i^{Met}$ and GTP, which are the two critical components of the translation initiation ternary complex. Strong binding interaction between MRS and AIMP3 may implicate the possible involvement of AIMP3 in translation initiation as a scaffold protein connecting MRS and eIF2γ (Kang et al. 2012). In addition, it was reported that the GTP-binding domain of eIF2γ and the GST-like domain of AIMP3 are the interfaces of the eIF2γ–AIMP3 interaction (Kang et al. 2012). To test whether cellular level of eIF2γ is affected by the level of AIMP3 through the eIF2γ–AIMP3 interaction, we checked the effect of AIMP3 over-expression on the cellular level of eIF2γ. As seen in the case of MRS, cellular level of the endogenous eIF2γ was significantly stimulated by the over-expression of exogenous AIMP3 (Fig. 2a).

This result allowed us to proceed further investigations regarding functional roles of AIMP3 in the AIMP3–eIF2γ complex. eIF2γ has been known to be unstable and get into insoluble fraction upon over-expression in bacterial system for the preparation (Suragani et al. 2006). Based on the binding interactions of eIF2γ with AIMP3, we tested the possibility whether stability of eIF2γ can be positively affected by the co-expression with AIMP3. As seen in Fig. 2b, solubility of eIF2γ was significantly increased when it was co-expressed with AIMP3 as MRS was. Then we also tested whether AIMP3 protects eIF2γ from proteolytic attack from elastase. eIF2γ was clearly protected from the proteolysis of elastase (Fig. 2c).

Conclusion

Here, we propose that AIMP3 may contribute to mammalian translation initiation by securing factors involved in the formation of Met-tRNA$_i^{Met}$ and the delivery of it to ribosome. Owing to the stabilization and protection provided by AIMP3, the cellular level of MRS and eIF2γ may be able to be maintained high enough for the functional roles of MRS and eIF2γ, formation and delivery of Met-tRNA$_i^{Met}$, respectively. The two characteristics of MRS and eIF2γ, the instability and the sensitivity to the proteolysis, which seem to be the week points of those factors, may allow AIMP3 a chance to contribute to mammalian translation initiation through the maintenance of levels of both proteins.

These results may have significant implications for understanding the functional roles of protein–protein interactions among the factors involved in the translation initiation and possibly of other protein–protein interactions in the multi-enzyme complexes.

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