



Published in final edited form as:

Int J Biochem Cell Biol. 2018 June ; 99: 197–202. doi:10.1016/j.biocel.2018.04.015.

Aminoacyl tRNA synthetase complex interacting multifunctional protein 1 simultaneously binds Glutamyl-Prolyl-tRNA Synthetase and Scaffold Protein Aminoacyl tRNA synthetase complex interacting multifunctional protein 3 of the Multi-tRNA Synthetase Complex

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Abstract

Higher eukaryotes have developed extensive compartmentalization of amino acid (aa) - tRNA coupling through the formation of a multi-synthetase complex (MSC) that is composed of eight aa-tRNA synthetases (ARS) and three scaffold proteins: aminoacyl tRNA synthetase complex interacting multifunctional proteins (AIMP1, 2 and 3). Lower eukaryotes have a much smaller complex while yeast MSC consists of only two ARS (MetRS and GluRS) and one ARS cofactor 1 protein, Arc1p (1), the homolog of the mammalian AIMP1. Arc1p is reported to form a tripartite complex with GluRS and MetRS through association of the N-terminus GST-like domains (GST-L) of the three proteins (2). Mammalian AIMP1 has no GST-L domain corresponding to Arc1p N-terminus. Instead, AIMP3, another scaffold protein of 18 kDa composed entirely of a GST-L domain, interacts with Methionyl-tRNA synthetase (MARS)(3) and Glutamyl-Prolyl-tRNA Synthetase (EPRS)(4). Here we report two new interactions between MSC members: AIMP1 binds to EPRS and AIMP1 binds to AIMP3. Interestingly, the interaction between AIMP1 and AIMP3 complex makes it the functional equivalent of a single Arc1p polypeptide in yeast. This interaction is not mapped to AIMP1 N-terminal coiled-coil domain, but rather requires an intact tertiary structure of the entire protein. Since AIMP1 also interacts with AIMP2, all three proteins appear to compose a core docking structure for the eight ARS in the MSC complex.

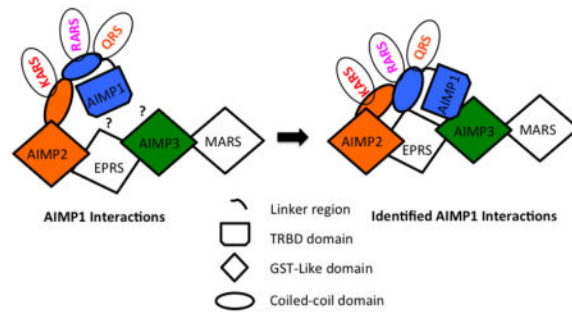
Graphical Abstract

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Conflict of interest statement: The authors have declared that no conflict of interest exists

Author Contributions: MAS designed experiments, interpreted data, and wrote/revised the manuscript, DDL designed experiments, data acquisition and analysis, revised the manuscript, and SB performed *in vitro* experiments, assisted in data acquisition, and Biacore X SPR experiments. All authors approved the manuscript.

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Keywords

aminoacyl tRNA synthetase; RNA-protein interaction; transfer RNA (tRNA)

INTRODUCTION

Protein translation efficiency depends on many aspects, including how quickly transfer RNAs can be recharged and localize toward ribosomes undergoing elongation of peptides. Depending on cellular demands and the organism, the supply and assembly of aminoacyl-tRNA synthetases into a multi-synthetase complex (MSC) varies. For example, in yeast, MSC is simplified containing a scaffold protein, Arc1p, methionyl-tRNA synthetase (MetRS) and glutamyl tRNA synthetase (GluRS). Compared to yeast, mammalian cells feature additional proteins within the MSC complex: 3 scaffold proteins named aminoacyl tRNA Synthetase Complex Interacting Multifunctional Protein 1–3 interacting with 9 aminoacyl-tRNA synthetases. The hypothesized ability of aminoacyl tRNA synthetase family members to adjust aminoacyl-tRNA coupling to the rate of cellular metabolic needs may explain their “moonlighting” functions (5).

In yeast, Arc1p (mammalian homolog, AIMP1) tightly associates with two cytosolic enzymes, MetRS and GluRS. The simplicity of the single Arc1p scaffold with the two enzymes has distinct growth advantages. For example, genetic disruption of Arc1p reduced MetRS activity while slowing growth, therefore imparting a survival advantage in a range of temperatures, between 15 and 23 degrees Celsius (1). In fact, it appears that the two enzymes within the Arc1p complex might not be a random choice. Without enzymes to metabolize specifically glutamine, yeast utilizes glutamate as their substrate. Yeast with methionine restriction paired with elevated glutamic acid was reported to contribute to its longevity, independent of glucose. Importantly, aside from glutamic acid, availability of any other non-essential amino acid had no such effect (6).

Arc1p is made up of three domains: (1) an N-terminus GST-L domain; (2) unstructured; (3) tRNA binding domain (TRBD). The first domain is sufficient for the MetRS and GluRS binding followed by an undefined unstructured middle region and finally the TRBD which binds to tRNA (7). In yeast, the primary function for the Arc1P-GluRS-MetRS complex is the synchronization of nuclear transcription and mitochondrial translation of ATP synthetase genes (8). In contrast to yeast, mammalian cells contain a splitting of the GST-L domains from the TRBD domain. Within mammalian MSC, AIMP2 and AIMP3, two of the 3

scaffold proteins mentioned above, both contain GST-L binding domains. However, AIMP1, the third scaffold protein, has no GST-like domain. Instead, it contains approximately a 70 amino acid (a.a.) long coiled-coil domain, followed by an unstructured region and TRBD domains (9). The AIMP1 coiled-coil domain is further subdivided into two regions; an N terminal part that interacts with the coiled-coil domain of AIMP2 and the distal part that interacts with RARS. The remainder of AIMP1, (also known as p43), retains 54% identity to the Arc1p protein (by NCBI-BLAST).

Interestingly, at least 4 of the ARS enzymes within this complex and all 3 scaffold proteins have secondary, moonlighting functions (5). AIMP1 is one such protein, as it is reported to undergo cleavage, and the C-terminal fragment (AIMP1: a.a. 146-312), was identified as extracellular Endothelial Monocyte-Activating Peptide II (EMAP II) – an anti-angiogenic protein and positive mediator of macrophage migration (10–12) (13). Dissociation of one MSC protein may affect the release of its interacting partners as well; therefore, the physiological effect of the MSC should be considered. Our studies determined that full-length AIMP1 binds to AIMP3. Furthermore, the N-terminus of AIMP1 is also a binding partner with the GST-L domain of EPRS (Glutamyl-Prolyl-tRNA Synthetase). Binding of AIMP1 to AIMP3, or its known binding partner AIMP2, could participate in EPRS binding. Our studies support AIMP1, while linking the other two scaffold proteins, as the only scaffold protein to bind all four components of the GST-tetrameric complex suggesting that AIMP1, the only TRBD protein, may function as a docking site for tRNA and GST-L domain proteins of the MSC complex.

RESULTS

AIMP1 binds to the GST-like domain of the ARS protein EPRS (Glutamyl-Prolyl-tRNA Synthetase)

To assess interactions of the full length AIMP1 protein, an AIMP1-EGFP fusion construct was transiently expressed in HEK293 cell line and pulled down with anti-EGFP antibodies. Samples resolved on an SDS-PAGE gel followed by silver staining, revealed co-immunoprecipitation (co-IP) of a prominent 175 kDa band, with additional less pronounced smaller size proteins and no clear resolving proteins smaller than 35 kDa (Fig. 1A). MALDI-TOF mass-spectroscopy identified the unique peptides of the 175 kDa protein as EPRS (Table 1). Cells co-transfected with AIMP1 full length (a.a. 1-312) and truncated (t) EPRS (a.a. 1-970t) confirmed binding of AIMP1 to EPRS using co-IP (Fig. 1B). Deletion constructs of AIMP1 identified the N-terminus of AIMP1 binds to and is necessary to bind to EPRS 1-907t as the AIMP1 C-Terminus (a.a. 146-312) deletion construct did not immunoprecipitate with EPRS 1-970t (Fig. 1B).

EPRS consists primarily of four domains: (1) GST-L (a.a. 51-162); (2) Glu-ARS (a.a. 425-502); (3) 3 consecutive WHEP elements (a.a. 749-810, 822-888, and 900-966); (4) and Pro-ARS (a.a. 1023-1296). To identify the region of EPRS that AIMP1 interacts with, deletion mutants of EPRS were co-transfected with full length AIMP1 (a.a. 1-312). Deletion of the EPRS C-terminal domains Glu-ARS, WHEP, and Pro-ARS had no effect on AIMP1:EPRS binding, suggesting that AIMP1 binds to the N-terminus of EPRS (a.a. 1-200). (Fig 1D). Further deletion analysis identified the GST-L domain of EPRS was

sufficient for co-IP with endogenous AIMP1 (Fig. 1E) confirming an independent report on GST-L domains within the MSC as one of the two main protein-protein interaction domains (4).

Binding of full-length AIMP1 to AIMP2 participates in AIMP1-EPRS binding

Based on the previously documented multitude of interactions within the MSC (14), EPRS is likely to be recruited to AIMP1 indirectly, through binding of other components of the MSC complex. The other common protein-protein interaction module in MSC is the coiled-coil domain, such as N-terminal 1 to 70 a.a. region of AIMP1. As GST-L domains exhibit homotypical interactions, AIMP2 was identified as a possible facilitator for AIMP1-EPRS interaction as AIMP2 contains both a coiled-coil and GST-L domain, while AIMP3 is composed entirely of a GST-L domain making it less likely to mediate this reaction.

The EPRS GST-L domain forms complexes with AIMP3 and AIMP2 (4). Previous studies suggest that AIMP2 can reportedly bridge EPRS and AIMP1 through an interaction with the coiled-coil domain of AIMP1 (15). As the amount of endogenous AIMP2 seems to be the limiting factor for this association, we were able to bind AIMP1:EPRS co-IP by co-expressing HA-tagged AIMP2 (Fig. 2A). In addition, previous studies have shown that EPRS binds to AIMP2 and AIMP3 (4). To differentiate whether the EPRS pull-down by AIMP1 was mediated solely by coiled-coil domain interactions, we tested GST-L pull-down using full-length AIMP1 or the AIMP1 N-terminal coiled-coil domain alone. Full-length and AIMP1 N-terminal coiled-coil domain of AIMP1 protein pulls down endogenous EPRS. (Fig. 2B).

The full-length of AIMP1 binds AIMP3

AIMP1-AIMP2 interaction maps to the 1-70 a.a. coiled-coil region (15). We tested whether the AIMP3 scaffold protein could be a contributing factor by interacting with AIMP1 outside of its coiled-coil domain. First, we tested co-IP of AIMP3 with full length of AIMP1 (a.a. 1-312) or AIMP1's coiled-coil domain (a.a. 1-70) alone. Endogenous AIMP3 and EPRS immunoprecipitated with full-length AIMP1 protein and AIMP1's coiled-coil domain (Fig. 2B). However, unlike AIMP2, AIMP3 is found abundantly in cells and it seems to be stable even when it is not in complex within MSC (4). In order to test the AIMP1-AIMP3 interaction while excluding the contribution of other proteins, we performed an *in vitro* test using recombinant proteins (Fig. 2C) to determine direct AIMP1 to AIMP3 binding. AIMP3 was found to interact with full-length AIMP1 (Fig. 2D). Previously, AIMP1 has been reported to bind arginyl-tRNA synthetase (RARS) and AIMP2 through the N-terminal coiled-coil motif (15,16). When the first 70 a.a. of AIMP1 were tested for interaction with AIMP3, it was evident that the coiled-coil region is not sufficient to bind AIMP3 (Fig. 2D), although previous studies found that the first 70 a.a. of AIMP1 is sufficient to maintain robust RARS binding (16). These findings suggest that rather the entirety of AIMP1 with an intact intramolecular N- to C-terminus bond (9) was required for efficient AIMP3 binding. We hypothesize that the interaction site for AIMP3 is formed due to this intramolecular interaction within AIMP1. Lastly surface plasmon resonance (SPR) was utilized as an independent evaluation of AIMP1-AIMP3 interaction (Fig. 2E, F). Histidine-tagged AIMP1 and AIMP3 were purified from bacterial lysates by metal-chelating chromatography. As

AIMP1 protein is more than twice the molecular weight than AIMP3, it was immobilized on the chip and AIMP3 was used as ligand. As shown in Fig. 2F at both a low and high concentration, AIMP3 interaction exhibits reversible binding to AIMP1 with an SPR Equilibrium Binding Constant (K_D) of $25.3 \pm 1.13 \times 10^{-13}$ nM.

DISCUSSION

Translation is an energy-costly process with two high-energy phosphate bond used in each of the three steps - aminoacyl-tRNA coupling, trans-peptidase activity and ribosomal translocation. Adaptation for more efficient and potentially co-regulated aa-tRNA coupling by ARS enzymes was necessary for mammalian eukaryotes compared to simpler Archaea. AIMP1/Arc1p is the only scaffold protein shared by eukaryotes, and there are no scaffolds in minimal prokaryotic and archaeic complexes (14). Instead of a GST-L domain found in Arc1p, the AIMP1 N-terminus contains a coiled-coil motif. All previously reported interactions for AIMP1 were mapped to this coiled-coil region (9). In addition, we previously reported intra-molecular bond within the AIMP1 protein (9). Here we report the observation of AIMP1 binding to AIMP3 in addition to independently reported AIMP1 and AIMP2 interaction (15).

Importantly, AIMP1 lacks a GST-L domain. However interaction of MARS, EPRS and a protein scaffold through their GST-L domain appears to be highly conserved. AIMP3 may function as a duplication of the EPRS N-terminal GST-L domain, rather than being derived from Arc1p (based on sequence similarity). Furthermore, as the Arc1p-GluRS-MetRS complex in yeast synchronizes nuclear transcription and mitochondrial translation of ATP synthetase genes (8), AIMP1 binding of EPRS, MARS, and the GST-L scaffold proteins AIMP2 and AIMP3 could facilitate a similar function in mammalian cells. This points to a tantalizing possibility for the emergence of MARS-scaffold-EPRS complex twice in the evolution of eukaryotes. Leading to the proposition that binding of AIMP1 to AIMP3 is the structural mammalian analogue to Arc1p, so that two polypeptides have the same function as the single polypeptide in yeast. This observation leads us to believe that methionine/glutamic acid ratio that reported to affect yeast longevity (6), might have similar significance in mammalian cells.

MATERIALS AND METHODS

cDNA constructs, cell culture and immuno-precipitation

HEK293 cells (ATCC) were cultured in DMEM supplemented with 10% FBS (Hyclone), 1x GlutaMax and penicillin and streptomycin. Transfections using TransIT-293 reagent (Mirrus). Immuno-precipitations (IP) with anti-eGFP llama single chain antibodies beads were performed as per manufacturer's protocol (GFP-nAbTM Agarose, Allele). Equal protein amounts were utilized for western blotting of whole cell lysate and equal protein amounts were utilized for IP experiments. For IP and Western Blotting (WB) antibodies: anti- c-myc, anti-EGFP and C-terminal EPRS antibodies (Santa Cruz, sc-40, sc-9996 and sc-393505 respectively), AIMP1 antibody (sc-376019), N-terminal specific anti-EPRS and anti-HA-epitope (Bethyl, A303-957 and A190-108).

AIMP1-EGFP and AIMP1-EGFP a.a. 1-70 expression constructs were described previously (9). Sequence of the primers and restriction sites are listed in Supplemental Table 1S. AIMP1: a.a. 71-312 deletion mutant was cloned in-frame to pEGFP-N3 vector (Clontech). cDNA of AIMP3 ORF (PCR on MGC clone, Dharmacon, MHS6278-202839379), was cloned into pET-28a sites. Constructs for bacterial expression of GST-AIMP1 and GST-AIMP1 a.a. 1-70 fusions were described previously (9).

Myc-tagged deletion mutants of EPRS (EPRS a.a.1-200; EPRS a.a.1-70; EPRS a.a. 71-200 and EPRS a.a. 31-200) were cloned into pCMV-MYC-N vector (Clontech) of the PCR products with MGC clone 4997672 (Dharmacon MHS1010-202697793) as template. Myc-tagged EPRS a.a. 1-163 was made by excising KpnI-NotI fragment from EPRS a.a. 1-200 construct. EPRS a.a. 1-410 and EPRS a.a. 1-657 were made by removing HindIII and BamHI fragments, respectively, from MGC clone 4997672, which by itself corresponds to EPRS a.a. 1-970.

AIMP2 constructs for mammalian and bacterial expression were produced by PCR on AIMP2 cDNA (Dharmacon MHS6278-202755556) and cloned into pCMV-HA-N (Clontech) at XhoI and NotI restriction sites, and re-cloned into pGEX4-3 using SalI and NotI for the fragment's excision.

In vitro binding assay

GST- and His-tagged proteins were produced as described as previously described (9). GST fusion proteins bound to Glutathione Agarose beads equilibrated and His-tagged AIMP3 eluted with 300mM imidazole in PBS and dialyzed. Agarose beads with bound recombinant GST- fusion proteins were incubated with His-tagged AIMP3 in PBS, 0.01% NP-40 at 4°C. Bound proteins were eluted in 1X Sample Buffer. The samples were resolved on 4–12% gradient gel (Invitrogen) and probed with anti AIMP3 antibodies (Santa Cruz Biotechnology sc-376019)

Mass-spectroscopy

Mass spectrometry was performed after in-gel tryptic digest using Matrix Assisted Laser Desorption Ionization-time of flight (MALDI-TOF) mass spectroscopy analysis at the University of Notre Dame Mass-spectroscopy and Proteomic facility.

Surface Plasmon Resonance

GST-AIMP1 protein immobilized on MC5 chip (manufacturer's guidelines, GE Life Sciences). Amine coupling by covalently binding amine-containing substances to the sensor chip using 60 µL 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) with 60 µL of N-hydroxysuccinimide (NHS) and 90µL of the mixture was injected into the biacore machine. The AIMP1 protein was diluted (10mM sodium acetate buffer, pH 5.0, concentration 200 µg/mL) with 90 µL of 200 µg/mL AIMP1 injected into the machine. 1.0M ethanolamine-HCl, pH 8.5 was injected for chip deactivation. The AIMP3 protein was diluted to obtain a 200 µg/mL concentration (high concentration solution). Dilution of the concentrated AIMP3 solution ten fold gave the low concentration of AIMP3 (20 µg/mL). Low concentration of AIMP3 was injected followed by regeneration solution 50 mM NaOH.

A second injection of high concentration of AIMP3 was injected followed by regeneration solution. Using BIAevaluation software, the resonance Unit (RU) was quantified and the point of saturation on the curve was noted to obtain a saturation profile. Using the KaleidaGraph software and the plotted protein concentration, the RU responses were determined and appeared on the graph as the apparent K_d of the interaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Nikolay Malinin for his technical support and contribution to the manuscript, Haiming Xu for many of the AIMP1 constructs, and Kathryn Del Vecchio for her assistance with the SPR analysis. This publication was made possible in part by 5R01HL114977 (MAS) from NIH and the Lilly Endowment, Inc. Physician Scientist Initiative (MAS).

Abbreviations

EMAP II	Endothelial-Monocyte Activating Polypeptide II
AIMP	<u>a</u> minoacyl tRNA synthetase complex <u>i</u> nteracting <u>m</u> ultifunctional protein
aa	amino acids
MSC	multi-synthetase complex
ARS	aa-tRNA synthetases
Arc1p	<u>A</u> RS <u>c</u> ofactor <u>1</u> protein
MARS	Methionyl-tRNA synthetase
EPRS	Glutamyl-Prolyl-tRNA Synthetase
RARS	Arginyl-tRNA Synthetase
GST-L	GST-like
TRBD	tRNA (TR) binding domain
MALDI-TOF	Matrix Assisted Laser Desorption Ionization-time of flight

References

1. Simos G, Segref A, Fasiolo F, Hellmuth K, Shevchenko A, Mann M, Hurt EC. The yeast protein Arc1p binds to tRNA and functions as a cofactor for the methionyl- and glutamyl-tRNA synthetases. *EMBO J.* 1996; 15:5437–5448. [PubMed: 8895587]
2. Koehler C, Round A, Simader H, Suck D, Svergun D. Quaternary structure of the yeast Arc1p-aminoacyl-tRNA synthetase complex in solution and its compaction upon binding of tRNAs. *Nucleic Acids Res.* 2013; 41:667–676. [PubMed: 23161686]
3. Quevillon S, Robinson JC, Berthonneau E, Siatecka M, Mirande M. Macromolecular assemblage of aminoacyl-tRNA synthetases: identification of protein-protein interactions and characterization of a core protein. *J Mol Biol.* 1999; 285:183–195. [PubMed: 9878398]

4. Cho HY, Maeng SJ, Cho HJ, Choi YS, Chung JM, Lee S, Kim HK, Kim JH, Eom CY, Kim YG, Guo M, Jung HS, Kang BS, Kim S. Assembly of Multi-tRNA Synthetase Complex via Heterotetrameric Glutathione Transferase-homology Domains. *J Biol Chem.* 2015; 290:29313–29328. [PubMed: 26472928]
5. Guo M, Schimmel P. Essential nontranslational functions of tRNA synthetases. *Nat Chem Biol.* 2013; 9:145–153. [PubMed: 23416400]
6. Wu Z, Song L, Liu SQ, Huang D. Independent and additive effects of glutamic acid and methionine on yeast longevity. *PLoS One.* 2013; 8:e79319. [PubMed: 24244480]
7. Simader H, Hothorn M, Kohler C, Basquin J, Simos G, Suck D. Structural basis of yeast aminoacyl-tRNA synthetase complex formation revealed by crystal structures of two binary sub-complexes. *Nucleic Acids Res.* 2006; 34:3968–3979. [PubMed: 16914447]
8. Frechin M, Enkler L, Tetaud E, Laporte D, Senger B, Blancard C, Hammann P, Bader G, Claudermunster S, Steinmetz LM, Martin RP, di Rago JP, Becker HD. Expression of nuclear and mitochondrial genes encoding ATP synthase is synchronized by disassembly of a multisynthetase complex. *Mol Cell.* 2014; 56:763–776. [PubMed: 25453761]
9. Xu H, Malinin NL, Awasthi N, Schwarz RE, Schwarz MA. The N terminus of pro-endothelial monocyte-activating polypeptide II (EMAP II) regulates its binding with the C terminus, arginyl-tRNA synthetase, and neurofilament light protein. *J Biol Chem.* 2015; 290:9753–9766. [PubMed: 25724651]
10. Kao J, Houck K, Fan Y, Haehnel I, Libutti SK, Kayton ML, Grikscheit T, Chabot J, Nowygrod R, Greenberg S, et al. Characterization of a novel tumor-derived cytokine. Endothelial- monocyte activating polypeptide II. *J Biol Chem.* 1994; 269:25106–25119. [PubMed: 7929199]
11. Lee DD, Lal CV, Persad EA, Lowe CW, Schwarz AM, Awasthi N, Schwarz RE, Schwarz MA. Endothelial Monocyte-Activating Polypeptide II Mediates Macrophage Migration in the Development of Hyperoxia-Induced Lung Disease of Prematurity. *Am J Respir Cell Mol Biol.* 2016; 55:602–612. [PubMed: 27254784]
12. Schwarz MA, Kandel J, Brett G, Li J, Hayward J, Schwarz RE, Chappay O, Wautier J, Chabot J, Lo Gerfo P, Stern D. Endothelial-Monocyte Activating Polypeptide II, A Novel Antitumor Cytokine that Suppresses Primary and Metastatic Tumor Growth, and Induces Apoptosis in Growing Endothelial Cells. *Journal of Experimental Medicine.* 1999; 190:341–353. [PubMed: 10430623]
13. Park SG, Choi EC, Kim S. Aminoacyl-tRNA synthetase-interacting multifunctional proteins (AIMPs): a triad for cellular homeostasis. *IUBMB Life.* 2010; 62:296–302. [PubMed: 20306515]
14. Havrylenko S, Mirande M. Aminoacyl-tRNA synthetase complexes in evolution. *International journal of molecular sciences.* 2015; 16:6571–6594. [PubMed: 25807264]
15. Ahn HC, Kim S, Lee BJ. Solution structure and p43 binding of the p38 leucine zipper motif: coiled-coil interactions mediate the association between p38 and p43. *FEBS Lett.* 2003; 542:119–124. [PubMed: 12729910]
16. Armstrong L, Biancheri R, Shyr C, Rossi A, Sinclair G, Ross CJ, Tarailo-Graovac M, Wasserman WW, van Karnebeek CD. AIMP1 deficiency presents as a cortical neurodegenerative disease with infantile onset. *Neurogenetics.* 2014; 15:157–159. [PubMed: 24958424]

Highlights

- AIMP1 binds the N-terminal GST-like domain of the ARS protein EPRS
- AIMP1 binds the GST-like domain of the MSC scaffold protein AIMP3
- Binding of full-length AIMP1 to AIMP2 enhances AIMP1-EPRS binding

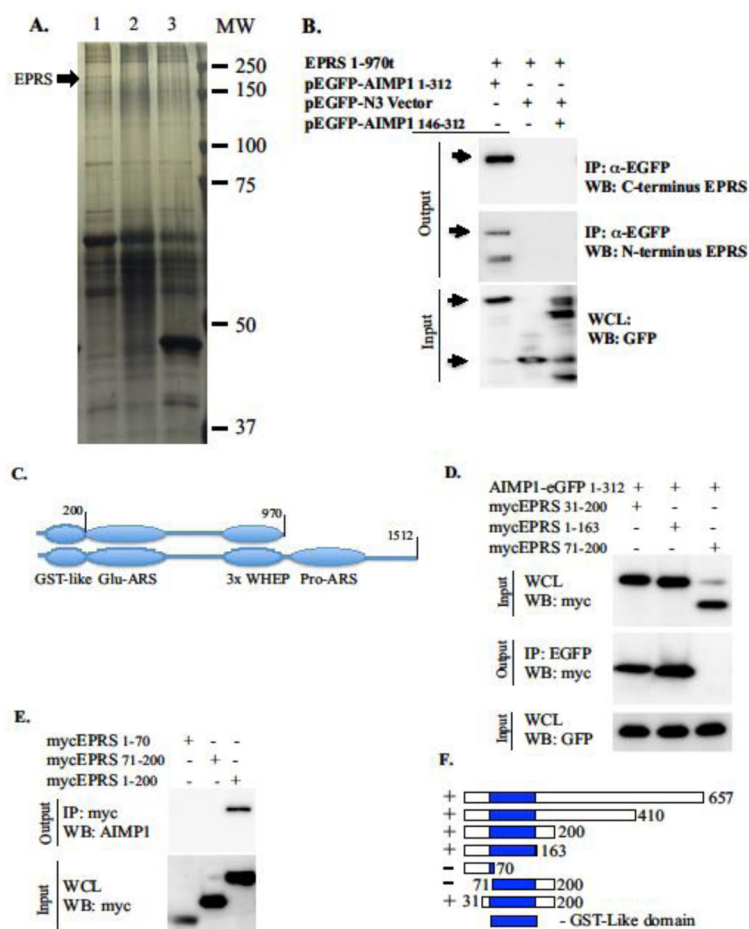


FIGURE 1. AIMP1 a.a. 1-312 co-precipitates with the MSC complex protein EPRS whose GST-like domain is sufficient for AIMP1 interaction

HEK293 cells transfected (A) with (1) AIMP1- EGFP fusion (2) pEGFP-N3 vector (3) AIMP1- EGFP a.a. 146-312. Silver staining of protein immuno-precipitated with anti-EGFP llama antibodies identified a unique p175 band (lane 1, arrow) identified as EPRS (11 unique peptides, MALDI-TOF) (endogenous AIMP1 IP sent for MS confirmed EPRS co-precipitation data not shown, n=3). HEK293 cells transfected with EPRS1-970 truncated (t) aa protein (B) and EGFP fusions as indicated: AIMP1 a.a. 1-312, pEGFP-N3 vector, and AIMP1- EGFP a.a. 146-312 were examined for binding to EPRS. Input analysis determined equal transfection (whole cell lysates:WCL, anti-GFP antibody). IP for αEGFP probed for C-terminus EPRS or N-terminus EPRS antibodies (B, Output Arrows) determined that full length AIMP1 (a.a.1-312) co-precipitated with EPRS while the truncated C-terminus of AIMP1 (a.a. 146-312) and empty N3 Vector did not. Schematic representation of EPRS domain architecture: GST-like domain, Glu-ARS, WHEP domain, and a Pro-ARS domain (C). Deletion analysis of EPRS and AIMP1 using myc tagged EPRS deletion mutants with AIMP1- EGFP a.a. 312 EGFP fusion protein (all samples). Co-IP with EGFP and WB myc and input analysis confirmed expression of deletion construct and fusion protein expression (D). Pull down of myc-EPRS deletion mutants with myc IP and endogenous AIMP1 WB endogenous (E). Summary of the EPRS deletion mutants co-IP with AIMP1- EGFP: “+”

designating interaction with AIMP1, “–” indicating no interaction. WHEP repeat domains are near the C-terminal residue 657 (F).

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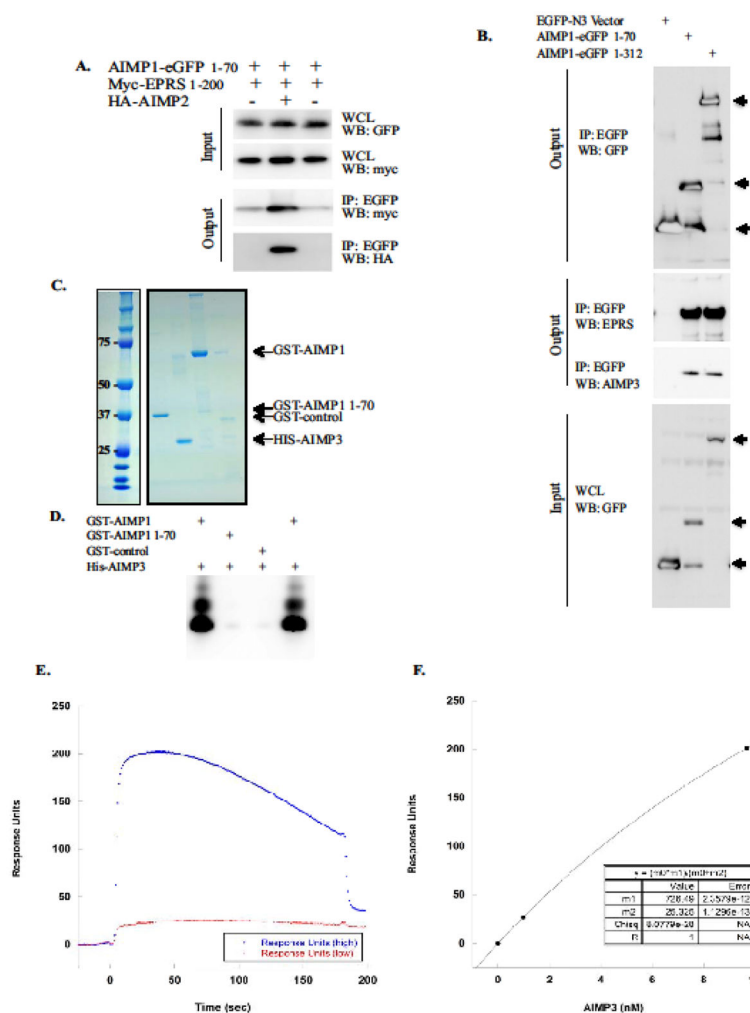


FIGURE 2. AIMP2 associates with AIMP1 binding to EPRS, while full length AIMP1 a.a. 1-312 binds AIMP3

AIMP1-EGFP a.a. 1-70 fusion protein transiently expressed in HEK293 cell line with myc-EPRS a.a. 1-200 and HA-AIMP2. Validation of transfection efficiency determined by input analysis with WB of EGFP and myc antibodies (A). Co-immunoprecipitation with EGFP and WB analysis of myc and HA (Output). Immuno-precipitation of overexpressed EGFP-N3 vector, AIMP1-EGFP a.a. 1-70 or AIMP1-EGFP a.a. 1-312 was probed for endogenous EPRS and AIMP3 (B). Input and output analysis of GFP confirmed expression. In vitro AIMP1 and AIMP3 interaction used purified recombinant His-tagged AIMP3, GST-control, GST-tagged AIMP1 and GST-tagged AIMP1 a.a. 1-70 purified from bacterial lysates (C). AIMP3 was eluted from nickel beads, while GST fusions left bound to glutathione beads used for pull down. WB analysis was performed using an AIMP3 antibody (D). Surface plasmon resonance using histidine-tagged AIMP1 and AIMP3 purified from bacterial lysates. AIMP1 was immobilized on the chip and AIMP3 was used as a ligand at low concentration (10 fold dilute, 20 $\mu\text{g/mL}$, Blue line: E) and high concentration (Red line: E, 200 $\mu\text{g/mL}$) ($n=5$, representative) with reversible binding demonstrated and an SPR Equilibrium Binding Constant of $25.325 \pm 0.13 \times 10^{-13}$ SD K_d (nM) determined using

equilibrium constants where injection of AIMP3 where each point represents a saturation point on the sensogram. K_d values were determined by fitting 5 points with a nonlinear least squares analysis of the binding isotherm ($R_{eq} = R_{max} / (1 + K_d/C)$). E, is a representative sensogram from the data showing AIMP1/AIMP3 binding responses over time. Equilibrium binding curves of AIMP1/AIMP3 at given concentrations and K_d values determined from an average of 3 separate experiments \pm SD (F).

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Table 1

MALDI-TOF Mass-Spectroscopy of excised 175 kDa band from AIMP1 Immunoprecipitation (Fig. 1A).

Sequence	# PSMs	Protein Group Accessions
SLYDEVAAQGEVVR	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
DQVDIAVQELLQLK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
MFEIVFEDPK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
FAGGDYTTTIEAFISASGR	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
EENLADWYSQVITK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
WFGFLEAQAFQSVGTK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
mFEIVFEDPK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
QFAYQnSWGLTTR	3	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
LNLNNTVLSK	2	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
VAVQGDVVR	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
EVIPVNVPEAQEEEmK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]
AYVDDTPAEQMK	1	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=4 - [SYEP_HUMAN]