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Experimental approaches for investigation of aminoacyl tRNA synthetase phosphorylation

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ABSTRACT

Phosphorylation of many aminoacyl tRNA synthetases (AARSs) has been recognized for decades, but the contribution of post-translational modification to their primary role in tRNA charging and decryption of genetic code remains unclear. In contrast, phosphorylation is essential for performance of diverse non-canonical functions of AARSs unrelated to protein synthesis. Phosphorylation of glutamyl-prolyl tRNA synthetase (EPRS) has been investigated extensively in our laboratory for more than a decade, and has served as an archetype for studies of other AARSs. EPRS is a constituent of the IFN-γ-activated inhibitor of translation (GAIT) complex that directs transcript-selective translational control in myeloid cells. Stimulus-dependent phosphorylation of EPRS is essential for its release from the parental multi-aminoacyl tRNA synthetase complex (MSC), for binding to other GAIT complex proteins, and for regulating the binding to target mRNAs. Importantly, phosphorylation is the common driving force for the context- and stimulus-dependent release, and non-canonical activity, of other AARSs residing in the MSC, for example, lysyl tRNA synthetase (KARS). Here, we describe the concepts and experimental methodologies we have used to investigate the influence of phosphorylation on the structure and function of EPRS. We suggest that application of these approaches will help to identify new functional phosphorylation event(s) in other AARSs and elucidate their possible roles in noncanonical activities.

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1. Introduction

The 20 aminoacyl-tRNA synthetases (AARSs) are ubiquitous and evolutionarily conserved enzymes that catalyze the highly specific acylation of amino acids to cognate tRNAs [1]. Thus, AARSs are essential for accurate decoding of the triplet genetic code and for sustenance of all domains of life, including bacteria, archaea, and eukarya [2,3]. However, AARSs also perform critical cellular activities unrelated to their primary function in aminoacylation. These noncanonical activities include regulation of gene transcription, mRNA translational control, angiogenesis, apoptosis, amino acid sensing, and cell signaling, among others [4–6]. The evolutionary emergence of noncanonical activities approximately coincided with the incorporation of a subset of AARSs into a macromolecular complex, namely, the multi-aminoacyl tRNA synthetase complex (MSC) [4,7]. The 1.5 mDa mammalian MSC comprises nine AARS activities in eight polypeptides, EPRS, MARS, DARS, KARS, IARS, LARS, QARS, and RARS, bridged by three scaffolding proteins, AIMP (aminoacyl tRNA synthetase complex interacting multifunctional protein) 1, 2, and 3 [5,8]. EPRS is unique among the mammalian synthetases as a bifunctional enzyme containing two AARSs, EARS and PARS, in a single polypeptide chain [9]. The physiological function of the MSC is not clear. MSC binding to ribosomes might permit "channeling" of charged tRNAs directly to the A-site to improve efficiency of protein synthesis [10–12]. Alternatively, the MSC can serve as a cytoplasmic "depot" for context- and stimulus-dependent release of AARSs for performance of noncanonical activities [13,14]. Phosphorylation of eukaryotic AARS has been recognized for decades, but most studies show little influence on synthetase activity [15]. However, recent studies suggest that post-translational modifications, particularly phosphorylation, are key drivers of the noncanonical activities of AARSs. In the earliest, and perhaps the most extensively studied example, interferon (IFN)-γ-induced phosphorylation of EPRS was shown to induce transcript-selective translation inhibition in in myeloid cells [16,17]. In subsequent studies, phosphorylation of KARS was observed upon immunologic challenge in mast cells to activate a noncanonical transcriptional activity; and by laminin in cancer cells to control cell migration [18,19]. For both, EPRS and KARS, phosphorylation is the signal-dependent event that drives their release from the MSC to permit interaction with other binding partners, intracellular relocalization (for example to the nucleus and plasma membrane in the case of KARS), and ultimately, execution of the AARS-specific noncanonical activity [19–21]. Based on these examples, future studies are likely to reveal additional AARSs, on or off the MSC, which are activated by phosphorylation to perform novel noncanonical functions.

In human myeloid cells, IFN-γ induces phosphorylation of EPRS at Ser^{886} and Ser^{999} in the noncatalytic linker domain that joins the two synthetase cores [20]. The two phosphorylation events co-ordinate multiple activities in the GAIT complex-mediated translational control system. These events include (i) release of EPRS from the MSC, (ii) assembly of the active heterotetrameric GAIT complex by interaction with ribosomal protein L13a, NS1-associated protein-1 (NSAP1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and (iii) repression of translation via direct binding to a bipartite, stem-loop GAIT element in the 3′ untranslated region (UTR) of a family of inflammation-related transcripts, including ceruloplasmin (Cp), vascular endothelial growth factor (VEGF)-A, and several chemokines and their receptors [22–24]. Ser^{886} phosphorylation is required for interaction of EPRS with NSAP1 to form an intermediate "pre-GAIT" complex, whereas Ser^{999} phosphorylation is critical for binding with L13a and GAPDH to form the functional, four-component GAIT complex that represses translation of GAIT element-bearing mRNAs by blocking ribosome binding to eIF4G in the translation-initiation complex [20]. Interestingly, the heterotrimeric GAIT complex in mice lacks NSAP1, and the EPRS linker does not contain the NSAP1-binding phosphorylation site at Ser^{886} attesting to the critical importance of Ser^{999} phosphorylation [25]. Recently, viral infection has been shown to induce phosphorylation of EPRS at Ser^{990} in the linker domain, and subsequent release from the MSC for viral clearance [14].

Identification and elucidation of the signaling pathway that regulates stimulus-dependent phosphorylation, and particularly the upstream and proximal kinases, can provide important clues to the physiological function of the phosphorylation event. Cyclin-dependent kinase-5 (Cdk5), in conjunction with its activator protein p35, is the proximal kinase that phosphorylates Ser^{886} in human EPRS, and is upstream of an unidentified AGC kinase group that mediates EPRS Ser^{999} phosphorylation [26]. This finding was unexpected because Cdk5 has been considered a central nervous system-specific kinase [27]. However, recent studies have shown its induction in lipopolysaccharide-stimulated macrophages [28], and its regulation of PPARγ function in adipocytes [29], implicating the kinase in inflammation and metabolism, respectively. In mast cells and cardiomyocytes, KARS, another integral constituent of MSC, is phosphorylated at Ser^{207} by mitogen-activated protein kinase (MAPK) phosphorylation inducing a conformational alteration that releases it from the MSC [21,30]. Released cytosolic KARS translocates to the nucleus, enhances production of the 2nd messenger, diadenosine tetraphosphate (Ap_4A), which activates micropththalmia-associated transcription factor (MITF) by inhibiting the Hint-1 repressor. Thus, KARS phosphorylation induces positive regulation of MITF target gene transcription. Thr^{52} phosphorylation of KARS by p38MAPK also induces its release from MSC and translocation to the plasma membrane for laminin-dependent cell migration with metastatic implications [19].

Although phosphorylation of several other AARSs displaying noncanonical activities has been reported, little is known about their specific role in controlling these activities [31–34]. We

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