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Proteomic analysis identifies endoribouclease EhL-PSP and EhRRP41 exosome protein as novel interactors of EhCAF1 deadenylase☆

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ABSTRACT

In higher eukaryotic cells mRNA degradation initiates by poly(A) tail shortening catalyzed by deadenylases CAF1 and CCR4. In spite of the key role of mRNA turnover in gene expression regulation, the underlying mechanisms remain poorly understood in parasites. Here, we aimed to study the function of EhCAF1 and identify associated proteins in *Entamoeba histolytica*. By biochemical assays, we evidenced that EhCAF1 has both RNA binding and deadenylase activities in vitro. EhCAF1 was located in cytoplasmic P-bodies that increased in number and size after cellular stress induced by DNA damage, heat shock, and nitric oxide. Using pull-down assays and ESI-MS/MS mass spectrometry, we identified 15 potential EhCAF1-interacting proteins, including the endoribonuclease EhL-PSP. Remarkably, EhCAF1 colocalized with EhL-PSP in cytoplasmic P-bodies in trophozoites. Bioinformatic analysis of EhL-PSP network proteins predicts a potential interaction with EhRRP41 exosome protein. Consistently, we evidenced that EhL-PSP colocalizes and physically interacts with EhRRP41. Strikingly, EhRRP41 did not coimmunoprecipitate EhCAF1, suggesting the existence of two EhL-PSP-containing complexes. In conclusion, our results showed novel interactions between mRNA degradation proteins and evidenced for the first time that EhCAF1 is a functional deadenylase that interacts with EhL-PSP endoribonuclease in P-bodies, while EhL-PSP interacts with EhRRP41 exosome protein in this early-branched eukaryote.

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Biological significance

This study provides evidences for the functional deadenylase activity of EhCAF1 and shows a link between different mRNA degradation proteins in *E. histolytica*. By proteomic tools and pull down assays, we evidenced that EhCAF1 interacts with the putative endoribonuclease EhL-PSP, which in turn interacts with exosome EhRRP41 protein. Our data suggest for the first time the presence of two complexes, one containing the endoribonuclease EhL-PSP and the deadenylase EhCAF1 in P-bodies; and another containing the endoribonuclease EhL-PSP and the exosome EhRRP41 exoribonuclease. Overall, these results provide novel data that may help to understand mRNA decay mechanisms in this parasite.

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1. Introduction

Polyadenylated RNA turnover is a critical determinant in eukaryotic gene expression. It results from both synthesis and degradation rates, which are continuously adjusted to cell physiological requirements. In cytoplasm, mRNA degradation is initiated by poly(A) tail shortening by deadenylases, followed by 5′-end cap removal by DCP1 and DCP2 enzymes [1]. Then, mRNA is submitted to 5′-3′ exonucleolytic digestion by XRN1. Alternatively, deadenylated mRNA can be degraded from 3′ end by exosome [2]. Alterations of any regulatory elements participating in these reactions can modify both mRNA stability and deadenylation rate, indicating that poly(A) tail shortening is a rate-limiting step in mRNA decay [3,4].

Deadenylation is performed by multiple poly(A) ribonucleases. CCR4 and POP2 proteins are the main deadenylases in *Saccharomyces cerevisiae* [5,6]. Higher eukaryotic cells have one homologue of yeast CCR4, whereas three POP2 homologues (CAF1, CAF1-like and CALIF) have been identified in human [7–9]. PAN2/PAN3 and PARN are additional poly(A) ribonucleases that participate in specific deadenylation events [10,11]. Interestingly, CAF1 and CCR4 have been involved in other cellular processes. In yeast, their association with five NOT proteins, CAF40 and CAF130 polypeptides, forms the CCR4–NOT complex, which interacts with TBP and TFIID transcription factors, establishing a functional link between mRNA synthesis and degradation [12]. CAF1 also participates in cell cycle regulation interacting with BTG1/2 and ANA/BTG3 anti-proliferative proteins [13,14]. In addition, deadenylase activity of CCR4–NOT complex contributes to DNA damage response in yeast through interactions with DUN1, MRC1, RAD9, and RAD17 checkpoint factors [15]. CAF1 also regulates mRNA poly(A) tail length of Crt1 transcription factor, which controls several DNA damage-induced genes [16]. In addition, PUF proteins that bind 3′ untranslated region (UTR) triggering mRNA decay or translational repression, physically bind POP2 deadenylase in yeast and stimulate the recruitment of CCR4 and two other enzymes involved in mRNA regulation, namely Dcp1p and Dhh1p [17,18].

After deadenylation, the exosome complex of 3′ → 5′ exoribonucleases catalyzes the complete 3′ to 5′ degradation of deadenylated transcripts in cytoplasm. The eukaryotic core exosome is a conserved nine-subunit protein complex. In yeast and human, Rrp44 and Dis3 proteins constitutively associate with the exosome and provide the sole source of processive 3′ to 5′ exoribonuclease activity [19,20]. In addition to its role in RNA decay, the exosome also participates in the processing of 3′ extended precursor molecules to mature stable RNAs, such as small nucleolar and small nuclear RNAs (snoRNAs, snRNAs) and ribosomal RNAs (rRNAs) [21,22]. Exosome activities are regulated through association with other protein complexes, such as SKI-complex factors [23], as well as TRAMP, a polymerase complex that primes structured RNA for degradation [24,25].

There is increasing data evidencing that mRNA degradation occurs in specialized cytoplasmic foci known as mRNA processing bodies (P-bodies) that are enriched in RNA substrates and mRNA turnover proteins. Although the complete protein inventory of P-bodies has not been defined, most proteins involved in the 5′-3′ mRNA-decay pathway, including decapping enzymes, 5′-3′ exonucleases and poly(A) ribonucleases, have been described as components of P-bodies, while the absence of proteins that function in the 3′-5′ decay is remarkable [26,27]. To date, the exosome and SKI-complex factors have not been detected in P-bodies [28].

As in other protozoan parasites, posttranscriptional regulation of gene expression and mRNA degradation mechanisms are poorly understood in *Entamoeba histolytica*, the protozoan parasite responsible for human amoebiasis that affects 50 million people worldwide [29]. Recently, we have reported that mRNA decay machineries, including mainly decapping and deadenylation activities are generally well conserved in *E. histolytica* [30]. We also evidenced that several proteins involved in mRNA degradation, namely the EhXRN2 exoribonuclease, the EhDCP2 decapping enzyme, the EhCAF1 deadenylase and the EhAGO2-2 protein involved in RNA interference, are enriched in cytoplasmic P-body like structures in trophozoites [30,31]. Because of the relevance of poly(A) tail removal in mRNA decay, it is of prime interest to characterize the enzymes involved in deadenylation as they are likely to be target of regulators affecting mRNA stability. Here, we report the functional characterization of a deadenylase in *E. histolytica* and provide experimental evidences for potential EhCAF1 partners, which may help to understand mRNA decay mechanisms in this early-branched protozoan parasite.

2. Material and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations of the Guide for the Use of Laboratory