Cell & Developmental Biology

Pentatricopeptide Repeat Domain-Containing Proteins in Mammals and Budding Yeast

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The pentatricopeptide repeat (PPR) family is composed of three degenerated domains that span between 31 and 36 amino acids [1]. PPR motifs occur in tandem arrays of two to 26 units per protein [2]. Each motif is predicted to comprise two anti-parallel α-helices that contain several projecting amino acidic side groups; therefore the arrays would form a superhelix with a binding surface that is suitable to interact with selected bases [3] and, through basic amino acids, with phosphate groups of RNA molecules [4]. The minimal requirement to bind RNA sequences with high affinity [2] and specificity [5,6] is thought to be a pair of tandem repeats [4]. PPR-containing proteins mainly participate in post-transcriptional events, such as RNA editing [7], translation [8], stability [5], processing [9], and splicing [10]. Nuclear genomes of land plants encode between 400 and 600 members of the PPR family [11], whose products are predicted to be mostly localized in chloroplasts and mitochondria [1]. To date, the TPRpred algorithm [12] has detected seven mammalian PPR proteins [13], albeit with different probabilities (indicated as percentage between parenthesis immediately after the abbreviated name): leucine-rich pentatricopeptide repeat cassette protein (LRPPRC; 100%), mitochondrial DNA-directed (MtRPOL; 2%), RNA polymerase (mitochondrial protein of the small subunit 27 (MRPS27; 93%), mitochondrial RNase P protein 3 (MRPP3; 10%), pentatricopeptide repeat-containing protein 1 (PTCD1; 100%), pentatricopeptide repeat-containing protein 2 (PTCD2; 97%), and pentatricopeptide repeat-containing protein 3 (PTCD3; 100%). These proteins are predicted to contain between two and 20 PPR domains. However, only a few of these motifs have been experimentally proven to be involved in the activity of the corresponding protein. The nuclear-encoded LRPPRC has 20 PPR motifs, arrayed in four clusters of six, two, four, and seven, respectively. The last motif stands alone. Even though 19 N-terminal PPR domains are required for full RNA binding activity *in vitro*, the replacement by β-galactosidase of the last 120 C-terminal amino acids (1272–1392), which deletes 15 amino acids of the 19th motif and the entire 20th motif, causes embryonic lethality in mice [6]. LRPPRC localizes in the mitochondria matrix and forms an RNA-dependent complex with the stem-loop interacting RNA binding protein (SLIRP) that stabilizes mRNAs encoded by the mitochondrial DNA H strand [14]. A single alanine to valine mutation (A354V) [15] located outside the PPR motifs, restricts the import of LRPPRC protein into mitochondria [16]. This replacement causes a severe form of Leigh syndrome, characterized by cytochrome *c* oxidase deficiency that leads to metabolic and/ or neurological fatal episodes [15,17]. The structure of a single-subunit RNA polymerase transcribes the human mitochondrial genome [18] has been solved [19]. It possesses two PPR motifs in tandem, between amino acids 263-296 and 297-330, spatially separated from the active site. The PPR domains are indirectly important for enzymatic activity: when deleted, the polymerase cannot initiate transcription [19]. MRPS27 has been annotated as a member of the mitoribosome small subunit [20] and is predicted to possess six PPR motifs. MRPS27 associates to the Era G-protein-like1 (ERAL1), a RNA chaperone that contributes to the assembly of the mitoribosome small subunit by protecting the ribosomal RNA (rRNA) from degradation [21]. PTCD1 is a mitochondrial matrix protein [22] with nine predicted PPR domains. PTCD1 binds to leucyl-tRNAs and reduces their levels, thus negatively affecting mitochondrial translation

in osteosarcoma cell lines [22]. PTCD2 has five predicted PPR domains and is required to process the NADH dehydrogenase subunit 5 (*ND5*)-cytochrome b (*COB*) pre-mRNA into the corresponding mature transcripts. Lack of PTCD2 causes a dramatic decrease of complex III activity, especially in the heart [23]. PTCD3 has 15 predicted PPR motifs and specifically binds the rRNA of the mitoribosome small subunit, but it is not required for rRNA stability [24]. PTCD3 is now considered a constitutive member of the mitochondrial ribosome [25], even though it has also been found in the mitochondrial transcriptional complex along with transcripts, RNA polymerase, and a putative RNA helicase [26].

In budding yeast (*Saccharomyces cerevisiae*), TPRpred predicts that five proteins have a probability of 90% or higher of containing PPR motifs: Pet309p, Ccm1p, Aep3p, Rpm2p, and Rmd9p. These proteins have between five and thirteen PPR domains. The best characterized is Pet309p, which contains thirteen PPR motifs. Ten of them are arrayed in one cluster between amino acids 312 and 632. At least seven of these motifs are required to translate *COX1* mRNA in mitochondria, but not to stabilize it [8]. Mutations on the fourth PPR motif, neutralizing the positive charges of lysine-424 and arginine-427, results in an inactive protein, as assessed by poor growth on non-fermentable substrates, a measure of mitochondrial functionality in budding yeast. Another yeast protein, Ccm1p was first reported to be essential for the removal of the fourth intron of the mitochondrially-encoded *COB* and *COX1* pre-mRNA, in order to yield the mature transcripts [10], which are required for translation. Ccm1p has six pentatricopeptide repeats (PPR) motifs according to TRPpred. Two of them, located between amino acids 319 and 353, and amino acids 356 and 390 have the most canonical sequences, with P-values of 1.8×10^{-06} and 4.1×10^{-13}, respectively. Both domains are required for activity [10]. Further studies have proven that Ccm1p is also required to maintain the ribosomal RNA of the small subunit (15S rRNA) in *S. cerevisiae* intronless mitochondria [27] and intron-containing mitochondria strains [28]. When the three lysines of the $PPR^{356-390}$ motif are replaced by alanines, a significant decrease in intron-removal activity along is observed [28]. Importantly, the mutation does not affect the activity of other maturases or the levels of 15S rRNA. This observation suggests that both activities share the same motif but involve different sets of amino acids. Aep3p has six predicted PPR domains. It localizes on the matrix side of mitochondrial inner membrane and participates in the processing and/ or stabilization of the mitochondrially-encoded bicistronic *ATP8/AT68* mRNA [29]. Aep3p

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also facilitates the initiation of protein synthesis in mitochondria with unformylated methionine by physically interacting with translation initiator factor 2 [30]. Interestingly a natural Y305N mutation, located outside of the PPR motifs, does not abolish the activity as long as formylmethionine is available. Rmd9p, with five predicted PPR domains, also localizes on the matrix side of mitochondrial inner membrane [31]. This protein is loosely associated with monosomes, albeit partially [31] and participates in the processing and/ or stability of several mitochondrial transcripts [32]. A missense mutation V363I, outside the PPR motifs, does not inactivate the protein but creates a poorly respiring phenotype in the *rsm28Δ*, *rmd9-V363I* double mutant [31]. Rpm2p is the protein component of mitochondrial RNAse P, the RNA-protein complex that processes tRNAs [33]. It also facilitates the translation of several mitochondrially-encoded *COX1*, *COX2*, and *COX3* mRNAs. This activity appears to involve the first and part of the second PPR motifs, since their deletion abolishes this function completely [34]. Taking together, these observations undoubtedly highlight the importance of PPR-containing proteins in mitochondrial RNA metabolism. However direct evidence implicating these motifs is fundamentally lacking and evidently more experimentation is required to understand the mechanisms of PPR motif-RNA interaction in mammalian and yeast mitochondria.

Funding

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