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The Crystal Structure of the C-Terminal DAP5/p97 Domain Sheds Light on the Molecular Basis for Its Processing by Caspase Cleavage

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DAP5/p97 (*d*eath-*a*ssociated *p*rotein 5) is a member of the eukaryotic translation initiation factor 4G family. It functions as a scaffold protein promoting cap-independent translation of proteins. During apoptosis, DAP5/p97 is cleaved by caspases at position 792, yielding an 86-kDa C-terminal truncated isoform (DAP5/p86) that promotes translation of several mRNAs mediated by an internal ribosome entry site. In this study, we report the crystal structure of the C-terminal region of DAP5/p97 extending between amino acids 730 and 897. This structure consists of four HEAT-Repeats and is homologous to the C-terminal domain of eIF4GI, eIF5, and eIF2Bε. Unlike the other proteins, DAP5/p97 lacks electron density in the loop connecting α3 and α4, which harbors the caspase cleavage site. Moreover, we observe fewer interactions between these two helices. Thus, previous mapping of this site by mutation analysis is confirmed here by the resolved structure of the DAP5/p97 C-terminus. In addition, we identified the position of two conserved aromatic and acidic boxes in the structure of the DAP5/p97 C-terminus. The acidic residues in the two aromatic and acidic boxes form a continuous negatively charged patch, which is suggested to make specific interactions with other proteins such as eIF2β. The caspase cleavage of DAP5/p97 removes the subdomain carrying acidic residues in the AA-box motif, which may result in exposure of a hydrophobic surface. These intriguing structural differences between the two DAP5 isoforms suggest that they have different interaction partners and, subsequently, different functions.

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Keywords: protein translation; DAP5; caspase; AA boxes; HEAT-Repeat

Introduction

Initiation of translation in eukaryotes is a highly regulated process. The majority of initiation events in the cell occur through a cap-dependent mechanism. This mode of initiation involves the assembly of the preinitiation complex at the mRNA 5′Cap m^7GpppX structure. The preinitiation complex consists of several initiation factors including, among others, the ternary complex of eukaryotic translation initiation factor (eIF) 4E, eIF4A, and eIF4G. eIF4E is a protein that specifically recognizes the cap structure,[1,2] eIF4A is an ATP-dependent RNA helicase that serves to unwind secondary structures within the 5′ untranslated region (UTR) of the mRNA,[2,3] and eIF4G is a scaffold protein that bridges eIF4E and eIF4A. In addition, eIF4G recruits eIF3, which in turn interacts with the small ribosomal subunit. eIF4G also binds the poly(A)-binding protein, allowing circularization of the mRNA and as well as the Mnk1 protein kinase that phosphorylates eIF4E in these complexes.[4,5]

An alternative mechanism of translation initiation involves the recruitment of the ribosome to the

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Abbreviations used: eIF, eukaryotic translation initiation factor; UTR, untranslated region; IRES, internal ribosome entry site; AA box, aromatic and acidic box; HR, HEAT-Repeat.

mRNA at a position close to or directly at the initiator codon. This mode of initiation requires the presence of an internal ribosome entry site (IRES) element within the mRNA's 5′UTR, which, by interacting with the translation machinery, enables ribosome binding independent of the 5′Cap structure. The cap-dependent mechanism of initiation is frequently blocked by various cellular stresses. A characteristic of IRES-mediated translation initiation is its ability to proceed under conditions in which cap-dependent translation is inhibited.[6–8]

DAP5/p97 (*d*eath-*a*ssociated *p*rotein 5), a member of the eIF4G protein family,[9,10] has been implicated in mediating cap-independent translation. The homology of DAP5/p97 to eIF4G is largely confined to the central segment, which corresponds to the eIF4A and eIF3 binding regions.[10–13] The N-terminal part of eIF4G that contains the binding sites for eIF4E and poly(A)-binding protein is completely missing from DAP5/p97.[10,11,14–16] Consistent with sequence data, it was shown that DAP5/p97 indeed binds eIF3 and eIF4A, while it fails to bind eIF4E necessary for cap-dependent translation.[11,14,16–19] The C-terminal parts of eIF4G and DAP5/p97 contain two aromatic and acidic boxes (AA boxes) (aromatic/aliphatic and acidic residues; also known as eIF5C or W2 domain).[20–23] Notably, both eIF4G and DAP5/p97 bind to Mnk1 using the AA-box motif. In contrast, DAP5/p97, but not eIF4G, binds to eIF2β through the AA-box motif.[24] eIF2β is a subunit of the adaptor protein eIF2, which brings the initiator Met-tRNA to the preinitiation complex. The differential binding of eIF2β suggests that functional differences between DAP5/p97 and eIF4G may also reside in their C-terminal regions, in addition to the abovementioned differences in the N-terminal region.

DAP5/p97 was identified and cloned simultaneously by four independent groups.[11,16,25,26] In our laboratory, DAP5/p97 was isolated through a genetic screen aimed at identifying novel prodeath genes essential for interferon-γ-induced cell death.[16] While this protein was being studied as part of the cell death process, it was revealed that, under apoptotic conditions, DAP5/p97 undergoes proteolytic cleavage, giving rise to a 86-kDa fragment (DAP5/p86). By using a panel of inhibitors for various cellular proteases, the cleavage of DAP5/p97 was attributed to caspases. Examination of the amino acid sequence of DAP5/p97 revealed the existence of two potential caspase cleavage sites of the motif DXXD (DETD^{792} and DHVD^{825}), which are capable of yielding a cleavage product that is close to the expected size. DAP5/p97 mutants carrying a single substitution of the aspartic residue with alanine (DXXD → DXXA) in both potential sites were constructed, and the ability of each mutation to abolish the cleavage site upon death stimulus was examined. This work revealed that DAP5/p97 was converted into DAP5/p86 by a single caspase cleavage event at DETD^{792}, whereas the second potential DHVD^{825} site was silent. The cleavage at D792 generated an isoform of the protein lacking the last 115 C-terminal amino acids.[18]

Subsequently, it was demonstrated, either by ectopically expressing DAP5/p86 in cells[27–29] or by adding the recombinant isoform to cell-free systems,[27] that the cleaved isoform is more active than the full-length protein in promoting the IRES-driven translation of some mRNAs coding for apoptotic-related genes. In addition, it was shown that the 5′UTR of the DAP5/p97 mRNA itself fulfils the set of criteria defining an IRES element, and that DAP5/p97 is capable of supporting translation from its own IRES in dying cells.[18] These DAP5/p86-mediated activities are considered to be part of the cellular response during apoptotic cell death manifested when cap-dependent translation is compromised.[9] In recent years, DAP5/p97 protein has also been found to display positive effects on translation, promoting the IRES-driven translation of target mRNAs and enhancing general uncapped mRNA translation in reconstituted cell-free systems.[30–32] In addition, it has been demonstrated that endogenous DAP5/p97 cosediments with polysomes in sucrose gradients, suggesting that it is actively involved in translation even at steady-state growth conditions.[24,33] Recent work, based on knocking down DAP5/p97 in unstressed HeLa cells, established that the full-length protein promotes the cap-independent translation of mRNAs encoding two prosurvival proteins, Bcl-2 and CDK1. The depletion of DAP5/p97 from cells and the resulting decline in the expression of Bcl-2 and CDK1 caused caspase-dependent cell death that was most prominent in the mitotic phase of the cell cycle.[34] Although both DAP5 isoforms display important roles in protein translation, the detailed functional differences between the two have not been established yet. This further stresses the importance of crystallizing the C-terminal domain of DAP5/p97 harboring the caspase cleavage site. Moreover, comparison with the existing structure of the close family member eIF4G should highlight the unique functions of DAP5/p97 in light of its rising importance in the regulation of translation during cell death/survival processes.

In this study, we report the crystal structure of the C-terminal region of DAP5/p97 extending between amino acids 730 and 897 (designated as DAP5-CTD). This structure belongs to the subclass of proteins containing HEAT-Repeat (HR) domains that include eIF4GI, eIF5, and eIF2Bε, all of which contain AA-box motifs.[22,23,35] The first AA box of DAP5-CTD consists of helices α6 and α7, whereas the second motif begins at the C-terminal half of α8 and extends until residue 900. The AA boxes, which are rich in acidic residues, form a continuous negatively charged patch that was shown to interact with the positively charged segments of Mnk1 and eIF2β. The structure of DAP5-CTD is similar to those of eIF4GI, eIF5, and eIF2Bε, with the exception of the loop connecting α3 to α4, which lacks electron density in DAP5-CTD. The sequence of this loop is considerably longer in DAP5-CTD and includes the established caspase cleavage site at position D792. Following cleavage at this site, the