Abstract

In addition to the canonical eIF4E cap-binding protein, eukaryotes have evolved sequence–related variants with distinct features, some of which have been shown to negatively regulate translation of particular mRNAs, but they remain poorly characterised. The vertebrate eIF4E proteins have been divided into three classes, with class I representing the canonical cap-binding protein eIF4E1a. eIF4E1a binds eIF4G to initiate translation, and proteins that act as molecular mimics–of eIF4G such as 4E-BPs and 4E-T prevent this interaction by binding eIF4E1a with the same consensus sequence $YX_4L\phi$.

This study focuses on *Xenopus* and human eIF4E1b as well as human eIF4E2 isoforms. eIF4E1b, a class I eIF4E protein, closely related to the canonical eIF4E1a (70% identical in sequence), is highly expressed in mice, *Xenopus* and zebrafish oocytes, while eIF4E2, a class II eIF4E protein, which exhibits only 30% identity to eIF4E1a, is ubiquitously expressed. As components of two different protein complexes, they participate in repressing the cap-dependent translation of specific mRNAs, although they both bind the cap weakly.

Using fluorescence titration, circular dichroism and homology modeling, a detailed study of *Xenopus* and human eIF4E1b interactions with cap analogues was undertaken. The predicted structure of eIF4E1b maintains the α+β fold characteristic for eIF4E proteins and its cap-binding pocket is similarly arranged by critical amino acids: Trp56, Trp102, Glu103, Trp166, Arg112, Arg157, Lys162 and residues of the C-terminal loop. However, it was demonstrated that eIF4E1b exhibits three fold weaker binding to the cap than eIF4E1a, both proteins being highly stimulated by methylation at N^7 position of guanine. Moreover, eIF4E1b proteins are distinguishable from eIF4E1a ones by a set of conserved amino acid substitutions, several of which are located near cap-binding residues. Indeed, eIF4E1b possesses several distinct features, namely enhancement of cap-binding by a benzyl group at N^7 position of guanine, a reduced response to increasing length of the phosphate chain and improved binding to a cap separated by a linker from Sepharose, suggesting differences in the arrangement of the protein core. In agreement, mutagenesis of the amino acids differentiating eIF4E1b from eIF4E1a proteins, reduces cap-binding by eIF4E1a two-fold, demonstrating the role of these additional residues in modulating cap-binding.

The investigation of the human eIF4E2 (4EHP) involved its interaction with a new protein partner called eIF4E –Transporter, 4E-T. It was demonstrated that eIF4E2 binds 4E-T in the yeast two hybrid assay, as well as in pull-down assays and by recruitment to P-bodies in mammalian cells. It was also shown that while both eIF4E1a and eIF4E2 bind 4E-T via the canonical $YX_4L\phi$ sequence, nearby downstream sequences also influence eIF4E:4E-T interactions. Indirect immunofluorescence was used to demonstrate that eIF4E2, normally homogeneously localised in the cytoplasm, does not redistribute

to stress granules in arsenite-treated cells, in contrast to eIF4E1a. Moreover, eIF4E2 shuttles through nuclei in a Crm1-dependent manner, but in an 4E-T–independent manner, unlike eIF4E1a. Altogether we conclude that while both cap-binding proteins interact with 4E-T and can be recruited by 4E-T to P-bodies, eIF4E2 functions are likely to be distinct from those of eIF4E1a, both in the cytoplasm and nucleus, further extending our understanding of mammalian class I and II cap-binding proteins.