Faculty of Science

Study programme: Molecular Biology and the Biochemistry of Organisms

Alexandra Popova

Human non-canonical translation initiation factors EIF4E2 and EIF4E3
Lidské nekanonické translační iniciační faktory EIF4E2 a EIF4E3

Type of thesis: Bachelor´s thesis

Supervisor: RNDr. Martin Pospíšek PhD.

Prague, 2025

Acknowledgments:

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU and by the Project OP JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy, co-funded by the European Union.

I would also like to thank my family for supporting me for every step of the way, and my advisor RNDr. Martin Pospíšek PhD. for guiding me while writing this thesis.

Declaration:

I declare that I have prepared this thesis independently and that I have listed all the information sources and literature used. Neither this thesis nor any substantial part of it has been submitted to obtain another or the same academic degree

Prague, 29.04.2025

Signature

Alexandra Popova

Abstract

The family of eukaryotic translation initiation factors 4E (EIF4E) comprises of a group of proteins that recognise the 7-methylguanosine cap at the 5’ mRNA end to initiate translation. In humans, there are three isoforms, EIF4E1, EIF4E2 and EIF4E3. EIF4E1 participates in global translation and is considered canonical. EIF4E2 and EIF4E3 are non-canonical translation initiation factors. This thesis summarizes current knowledge on EIF4E2 and EIF4E3. EIF4E2 and EIF4E3 structures differ from EIF4E1, with some key amino acid residues being replaced. This reduces their binding affinity to the m7G cap 40-200 times in comparison to EIF4E1. Therefore, availability of active EIF4E1 needs to be decreased for EIF4E2 and EIF4E3-mediated translation initiation. EIF4E2 is necessary for both translation repression and translation initiation. Besides others, EIF4E2 has a role in cognitive function and development by repressing specific mRNAs. It also can promote tumorigenesis by initiating translation in hypoxia. EIF4E3 seems to have many different functions but its specific role is not known. Its absence in the body can impact development and feeding in humans. EIF4E3 facilitates translation in stress conditions when EIF4E1 isn’t available. It mostly plays a tumour-suppressive role in cancers, preferentially translating a different set of proteins than EIF4E1 does.

Key words: Translation, EIF4E2, EIF4E3, Cancer, Translation Initiation factor, Mammals

Abstrakt

Rodina eukaryotických translačních iniciačních faktorů 4E (EIF4E) zahrnuje skupinu proteinů, které rozpoznávají 7-methylguanosinovou čepičku na 5' konci mRNA a iniciují translaci. U člověka existují tři izoformy: EIF4E1, EIF4E2 a EIF4E3. EIF4E1 se podílí na globální translaci a je považován za kanonický. EIF4E2 a EIF4E3 jsou nekanonické translační iniciační faktory. Tato práce shrnuje současné poznatky o EIF4E2 a EIF4E3. Struktury EIF4E2 a EIF4E3 se liší od struktury EIF4E1, přičemž některé klíčové aminokyselinové zbytky jsou nahrazeny. To snižuje jejich vazebnou afinitu k čepičce m7G 40x - 200x ve srovnání s EIF4E1. Proto musí být dostupnost aktivního EIF4E1 snížena pro iniciaci translace zprostředkovanou EIF4E2 a EIF4E3. EIF4E2 je nezbytná jak pro represi translace, tak pro její iniciaci. Kromě jiného má EIF4E2 roli v kognitivních funkcích a vývoji tím, že potlačuje specifické mRNAs. Může také podporovat nádorové bujení tím, že iniciuje translaci při hypoxii. Zdá se, že EIF4E3 má mnoho různých funkcí, ale jeho specifická úloha není známa. Jeho absence v těle může mít vliv na vývoj a výživu u lidí. EIF4E3 usnadňuje translaci ve stresových podmínkách, kdy není k dispozici EIF4E1. V nádorových onemocněních hraje většinou úlohu potlačující vznik nádorů a přednostně překládá jinou sadu proteinů než EIF4E1.

Klíčová slova: Translace, EIF4E2, EIF4E3, Rakovina, Translační iniciační faktor, Savci

List of abbreviations

4E-BP	4E-binding protein
4E-BP1	4E-binding protein 1
4E-BP2	4E-binding protein 2
4E-BP3	4E-binding protein 3
4EHP	4E homologous protein
4E-SE	4E-sensitive elements
4E-T	4E-transporter
AF	Atrial fibrillation
AI	Androgen-independent
ANG	Angiogenin
ASD	Autism spectrum disorder
CaP	Prostate cancer
CCR4-NOT	Carbon catabolite repression-negative on TATA-less deadenylase complex
CITE	Cap-independent translation enhancers
COS	Chronic oxidative stress
ECM	Extracellular matrix
EDF1	Endothelial differentiation factor 1
EIF1	Eukaryotic translation initiation factor 1
EIF1A	Eukaryotic translation initiation factor 1A
EIF2	Eukaryotic translation initiation factor 2
EIF3	Eukaryotic translation initiation factor 3
EIF4A	Eukaryotic translation initiation factor 4A
EIF4E	Eukaryotic translation initiation factor 4E Family
EIF4E1	Eukaryotic translation initiation factor 4E1
EIF4E2	Eukaryotic translation initiation factor 4E2
EIF4E3	Eukaryotic translation initiation factor 4E3
EIF4F	Eukaryotic translation initiation complex 4F
$EIF4F^{H}$	Hypoxic eukaryotic translation initiation complex 4F
$EIF4F^{S}$	Stress eukaryotic translation initiation complex 4F
EIF4G1	Eukaryotic translation initiation factor 4G1
EIF4G3	Eukaryotic translation initiation factor 4G

EIF5 Eukaryotic translation initiation factor 5

EMT Epithelial-mesenchymal transition

ERK1/2 Extracellular signal-regulated kinases 1/2

GIGYF Grb10-interacting GYF

GRHL3 Grainy-head like transcription factor 3

GSK3β Glycogen synthase kinase 3β

HHARI Human homolog of *Drosophila* Ariadne

HIF Hypoxia-inducible factor

HTM Human trabecular meshwork

IFN Type 1 interferons

IOP Intraocular pressure

IRES Internal ribosomal entry sites

ISG15 Interferon-stimulated gene 15

m7G 7-methylguanosine

miR microRNA

miRISC microRNA-induced silencing complex

MNK1/2 MAP kinase interacting kinase 1/2mTOR

mTOR Mammalian target of rapamycin

mTORC1 Mammalian target of rapamycin complex 1

mTORC2 Mammalian target of rapamycin complex 2

PABP Poly-adenylate binding proteins

P-bodies Processing bodies

PCOS Polycystic ovary syndrome

PIC Translation pre-initiation complex

RBM4 RNA-binding motif protein 4

rHRE RNA hypoxia response element

SKCM Skin cutaneous melanoma

TME Tumour microenvironment

TNRC6A Trinucleotide repeat-containing adaptor 6A

TRS Threonyl-tRNA synthetase

TTP Tristetraprolin

UTR Untranslated region

VEGF Vascular endothelial growth factor

WM Working memory

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Introduction

Eukaryotic mRNAs have 7-methylguanosine (m7G) caps on their 5‘ ends that are essential for mRNA stability, export from the nucleus to cytoplasm and translation initiation (Keith and Fraenkel-Conrat 1975, Muthukrishnan, Both et al. 1975, Dostie, Ferraiuolo et al. 2000). Eukaryotic translation initiation factor 4E (EIF4E) recognises these caps and binds EIF4G and EIF4A to form the EIF4F complex (Gross, Moerke et al. 2003, Marintchev, Edmonds et al. 2009). The EIF4F complex can then recruit the translation pre-initiation complex (PIC) which kickstarts translation initiation (Gross, Moerke et al. 2003).

The EIF4E protein family consists of three isoforms in humans. EIF4E1 is the canonical isoform and participates in global translation. EIF4E2 and EIF4E3 are non-canonical isoforms and do not participate in global translation. (Joshi, Lee et al. 2005)

EIF4E2 can both repress and initiate translation. EIF4E2 can form many alternative complexes, that when bound to the m7G cap of mRNA, can impact whether translation occurs or not. (Fu, Olsen et al. 2016, Jeong, Park et al. 2019) Notably, EIF4E2 can also mediate mRNA translation in hypoxia, thus substituting EIF4E1 (Uniacke, Holterman et al. 2012).

The specific function of EIF4E3 remains unknown, but its impact can be felt across many processes (Landon, Muniandy et al. 2014, Huang, Fan et al. 2023, Xu, Zhao et al. 2023). EIF4E3 can create alternative EIF4F complexes capable of initiating translation in stress conditions, thus also substituting EIF4E1 (Osborne, Volpon et al. 2013). EIF4E3 preferentially promotes translation of different mRNAs than the canonical EIF4E1 protein. (Landon, Muniandy et al. 2014)

EIF4E2 and EIF4E3 both play important roles in tumours, albeit in different ways. EIF4E2 supports the tumour microenvironment (TME) due to its ability to initiate translation in hypoxia (Uniacke, Holterman et al. 2012). Ability to growth in hypoxia is one of the key features of cells in solid tumours and their metastases (For review on hypoxia and tumours refer to (Li, Zhao et al. 2021)). EIF4E3, on the other hand, plays more of a tumour-suppressive role (Xu, Zhao et al. 2023). It is important to note that EIF4E2 and EIF4E3 functions varies among different kinds of cancer.

The objective of this thesis is to summarize different functions EIF4E2 and EIF4E3 have in the body and how they can initiate translation in a non-canonical manner. More specifically, this thesis aims to contribute to understanding the role of EIF4E2 and EIF4E3 in human pathologies including cancerogenesis.

Translation Initiation

Translation initiation is a key regulatory step in eukaryotic translation (Harnett, Ambrozkiewicz et al. 2022). It is highly controlled, consisting of eukaryotic translation initiation factors that interact together to lead to the recruitment of the 40S small ribosomal subunit to the mRNA. Subsequently, the 60S large ribosomal subunit attaches to form the complete 80S protein synthesis machinery. (For review on translation initiation refer to (Querido, Díaz-López et al. 2024)) The mRNA is not just a chain of ribonucleotides. It is modified on both ends. The 5' end is linked to a m7G cap with a 5'-5' triphosphate linkage. (Keith and Fraenkel-Conrat 1975, Muthukrishnan, Both et al. 1975) The 3' end is polyadenylated (Edmonds and Abrams 1960, Lim and Canellakis 1970). These modifications allow translation initiation factors to bind to mRNA, recruit the ribosomal subunits and initiate translation (Muthukrishnan, Both et al. 1975, Wells, Hillner et al. 1998).

Translation initiation starts with the formation of the 43S PIC. The 40S small ribosomal subunit binds to eIF1 (Passmore, Schmeing et al. 2007), eIF1A (Fekete, Applefield et al. 2005), eIF3 , eIF5 and a ternary complex consisting of eIF2, guanosine 5'-triphosphate and methionyl initiator transfer RNA (Asano, Clayton et al. 2000, Hashem, des Georges et al. 2013). The EIF4F complex binds to the mRNA and recruits the 43S PIC complex to the mRNA to form the 48S initiation complex (Gross, Moerke et al. 2003, Brito Querido, Sokabe et al. 2020). This complex scans the nucleotides in a search of the AUG start codon and binds to it (Kozak 1978). Finally, the 60S large ribosomal subunit is recruited to form the 80S ribosomal complex capable of elongation (Unbehaun, Borukhov et al. 2004).

The rate-defining step in translation initiation is the formation of the EIF4F complex. The EIF4F is hetero-trimeric complex that consists of EIF4E, a cap-binding protein, EIF4A, a DEAD-box helicase and EIF4G, a scaffolding protein. (Gross, Moerke et al. 2003, Marintchev, Edmonds et al. 2009) EIF4E is present in the lowest concentrations out of all translation initiation factors in the cell (Duncan, Milburn et al. 1987), hence it being part of the rate limiting step.

The EIF4E in the EIF4F complex binds to the 5' m7G cap of mRNA (Sonenberg and Hinnebusch 2009) while EIF4G binds to polyadenylate binding proteins (PABPs) present on the 3'-end poly(A) tail of mRNA. This results in the topological circularization of mRNA (Wells, Hillner et al. 1998, Amrani, Ghosh et al. 2008). Through the subsequent interaction of EIF4G with EIF3 (Korneeva, Lamphear et al. 2000), the 43S pre-initiation complex is recruited to mRNA, forming the 48S initiation complex (Gross, Moerke et al. 2003). This highlights the key role EIF4E plays in cap-dependent translation initiation.

EIF4E1

As mentioned above, EIF4E is the cap-binding protein present in the EIF4F translation initiation complex. EIF4E exists in several different isoforms and their variants that share some common

features. They are small proteins with a size of around 25 kDa. On a structural level, they have a disordered N-terminus and a conserved globular domain with a cupped hand shape. Their structure consists of a curved anti-parallel β-sheet that is supported by three long α-helices on the dorsal surface. (Marcotrigiano, Gingras et al. 1997)

Some species including the baker's yeast *Saccharomyces cerevisiae* possess only a single EIF4E isoform (Altmann, Handschin et al. 1987) while other species have an extremely multiplied number of EIF4E-coding genes. As an example of the latter can serve the fruit fly in which eight different isoforms of EIF4E have been found (Hernández, Altmann et al. 2006). In humans, three different isoforms are present, EIF4E1, EIF4E2 and EIF4E3 (Joshi, Lee et al. 2005). EIF4E1 is considered to be the canonical isoform, with EIF4E2 and EIF4E3 unable to replace EIF4E1 functionally in *S. cerevisiae* missing the *Eif4e1* gene (Joshi, Cameron et al. 2004).

EIF4E1 participates in global or "standard" translation. Its gene is located on chromosome 4 in humans and alternative splicing can give rise to its different variants (Mrvova, Frydryskova et al. 2018). EIF4E1 is crucial, its complete loss causes death before the sixth embryonic day in *Eif4e* KO -/- mice (Truitt, Conn et al. 2015). Embryonic mice still need translation and protein-synthesis to occur despite the lack of EIF4E1. EIF4E1b, an isoform of EIF4E1, initiates translation instead. EIF4E1b expression is exclusive to oocytes, ovaries and early embryos (Evsikov, Graber et al. 2006). In mice, EIF4E1b selectively binds to certain maternal mRNAs, promoting their translation and regulating development (Yang, Xin et al. 2023). EIF4E1b can bind to the cap, but cannot bind to EIF4G, and therefore would not be able to form a canonical EIF4F translation initiation complex. In zebrafish, EIF4E1b was found to repress translation instead. EIF4E1b localises to processing bodies (P-bodies) and binds to mRNA with shorter poly A tails, repressing them translationally. Overall, EIF4E1b overexpression leads to a reduction in protein abundance in zebrafish cells. (Lorenzo-Orts, Strobl et al. 2024)

On a subcellular level, EIF4E1 is found in the cytoplasm, where it plays a significant role in binding to the m7G cap of mRNA to initiate translation. In the cytoplasm, EIF4E1 can form part of P-bodies where mRNA decay machinery is found (Bashkirov, Scherthan et al. 1997). EIF4E1 can also be found in stress granules which contain parts of active translation apparatus. (Fujimura, Sasaki et al. 2012). Stress granules are specifically formed as a protection mechanism where the cell promotes mRNA turnover and repression of translation. Upon heat shock or arsenic stress, EIF4E1 localises both to P-bodies and stress granules (Frydryskova, Masek et al. 2016).

However, in some cell lines, up to 70% of EIF4E1 is found in the nucleus, (Lejbkowicz, Goyer et al. 1992). In the nucleus, EIF4E1 plays a role in the processing of mRNA, playing a significant role in steady-state capping of mRNA (Culjkovic-Kraljacic, Skrabanek et al. 2020). EIF4E1 associates with the

spliceosome in the nucleus, where it can both elevate and impair splicing levels, in an alternative manner (Ghram, Morris et al. 2023). The transportation of EIF4E1 from the cytoplasm to the nucleus is mediated by 4E-T, a nucleocytoplasmic shuttling protein (Dostie, Ferraiuolo et al. 2000).

RNA-seq was used to analyse the transcriptomes of 27 different human tissues. *EIF4E1* was expressed in all 27 tissues analysed, with expression being highest in the thyroid and testis tissues and lowest in the pancreas tissues. (Fagerberg, Hallström et al. 2014) This indicates how important EIF4E1 is for global translation all over the body.

A 2.2A resolution X-ray structure of murine EIF4E1 with cap analogue, m7GDP, shows exactly how EIF4E1 recognises the 5' mRNA cap. EIF4E1 recognises the cap analogue through π–π stacking interactions between Trp56 and Trp102 residues. However, that isn't the only interaction that supports cap binding. Three hydrogen bonds interact with the side chain carboxylate functional group of Glu103 and the NH peptide backbone of Trp102. Van der Waals contact is formed with the N-(7)-methyl group of the cap analogue and Trp166. Finally, the diphosphate chain of the cap analogue is responsible for salt bridges and hydrogen bonds with Trp102 NH sidechain group, Trp166 indole rings and Arg112, Lys162 and Arg157 side chains. (Marcotrigiano, Gingras et al. 1997)

EIF4E1 has a binding site on the dorsal surface that allows it to form complexes with both EIF4G and 4E-BP through the BXXYDRXFLΦ sequence with B representing a conserved basic residue, X representing a variable amino acid and Φ representing a conserved hydrophobic residue (Imataka, Gradi et al. 1998). EIF4G and 4E-BP compete for this binding spot, with 4E-BP acting as a regulator of the availability of EIF4E1 (Marcotrigiano, Gingras et al. 1999). EIF4G-binding is crucial because it significantly increases affinity of EIF4E1 to the mRNA cap (Haghighat and Sonenberg 1997). This motif also allows EIF4E1 to bind to 4E-T, ensuring the transport of EIF4E1 from the cytoplasm to the nucleus (Dostie, Ferraiuolo et al. 2000).

4E-BP regulates the availability of EIF4E1 (Gingras, Raught et al. 2001) with the help of the activity of the kinase mammalian target of rapamycin (mTOR) (Beretta, Gingras et al. 1996). When hyper-phosphorylated by mTOR, 4E-BP loses the ability to bind to EIF4E1, and EIF4G binds instead, leading to the subsequent formation of the EIF4F complex that initiates translation (Gingras, Raught et al. 2001). The phosphorylation sites of 4E-BPs are Thr37, Thr46, Ser65, Thr70, Ser83 and Ser112 (Fadden, Haystead et al. 1997). 4E-BPs are initially phosphorylated on the Thr37 and Thr46, which then lead to the subsequent phosphorylation of the other sites. (Gingras, Raught et al. 2001)

mTOR is a Ser/Thr kinase that can respond to both extracellular and intracellular signals such as the presence of amino acids or energy ratio. This kinase forms complexes known as mammalian target of rapamycin complexes 1/2 (mTORC1/2). 4E-BP is a substrate of mTORC1 that consists of the

mTOR kinase and regulatory proteins. (Beretta, Gingras et al. 1996) During the amino acid starvation or when the ATP/AMP ratio is low, mTOR kinase no longer phosphorylates 4E-BP.As a result, EIF4E1 is sequestered. This prevents EIF4G from binding to EIF4E1, thus inhibiting translation initiation. (Tokunaga, Yoshino et al. 2004)

Activity of EIF4E1 can further be regulated through its phosphorylation at Ser209 by MAP kinase-interacting kinases 1/2 (MNK1/2). Phosphorylation at Ser209 decreases its affinity to 4E-BP, allowing it to bind to the cap and initiate translation. The suggested mechanism through how this works is electrostatic repulsion that occurs between the negatively charged 4E-BP and negatively charged phosphate groups of phosphorylated EIF4E1. Cap-binding is also enhanced by a salt bridge formed between Lys159 and phosphorylated Ser209. This salt bridge has thus a stabilising effect of the mRNA in the cap-binding slot. (Batool, Majeed et al. 2020). Phosphorylation at the Ser209 residue is the determining factor for whether EIF4E1 is sequestered or not as experiments showed that EIF4E1 can bind to both phospho-deficient and phospho-mimicked 4E-BP (Showkat, Beigh et al. 2014).

Phosphorylation at the EIF4E1 Ser209 site is also crucial for many key cellular processes. For example, exporting a specific subset of mRNA is made possible due to phosphorylation of EIF4E1 (Phillips and Blaydes 2008). It fulfils an important function in neurons as well. *EIF4E*^{Ser209Ala} phospho-mutant knock-in mice (*4Eki*) (Gkogkas, Khoutorsky et al. 2014) displayed a depression-like phenotype (Amorim, Kedia et al. 2018). Furthermore, phosphorylated EIF4E1 upregulates *Period1* and *Period2*, genes producing proteins necessary for resetting the circadian clock (Cao, Gkogkas et al. 2015). It is important to note that Ser209 phosphorylation of EIF4E1 has been linked to cellular transformation and formation of malignant cells (Batool, Majeed et al. 2020).

EIF4E1 is a proto-oncogenic protein that can promote tumorigenesis (Lazaris-Karatzas, Montine et al. 1990) and rescue cells from apoptosis when overexpressed (Polunovsky, Rosenwald et al. 1996). If upregulated in cancer cells, its cytoplasmic role promotes translation of many oncogenic proteins. These proteins, known as EIF4E-sensitive, are specifically selected due to the presence of the increased complexity of the 5'UTR region. (Koromilas, Lazaris-Karatzas et al. 1992) This leads to the upregulation of proteins such as vascular endothelial growth factor (VEGF), a protein necessary for promoting vasculature growth, BCL-2 which inhibits cell death, collagenase MMP-9 which promotes metastasis and c-MYC, a transcription factor. As a result, dramatic phenotypic changes are observed and cancer cells are formed. (For review of the eIF4E1 role in cancer refer to (Montanaro and Pandolfi 2004))

The nuclear function of EIF4E1 contributes to its oncogneic potential. EIF4E1 exploits its ability to preferentially export certain oncogenic mRNAs from the nucleus to the cytoplasm. As a result, these

oncogenic mRNAs are translated more. This is known as EIF4E-mediated export (Topisirovic, Kentsis et al. 2005). EIF4E1 facilitates export of the selected mRNAs by binding to their 4E-sensitive element (4E-SE), a 100 nucleotides long conserved sequence at their 3' UTR. The mRNAs containing 4E-SE include but are not limited to proteins that drive the cell cycle forward and have proliferative properties such as cyclin D1, cyclin E1 and c-MYC, and proteins that play a role in apoptosis and DNA repair. (Culjkovic, Topisirovic et al. 2006) c-MYC, arguably the most important of the aforementioned proteins, is a proto-oncogenic transcription factor that besides other functions, increases EIF4E expression, driving thus tumorigenesis forward exponentially (Rosenwald, Rhoads et al. 1993).

EIF4E2

EIF4E2, also known as 4EHP, is a non-canonical cap-binding translation initiation factor coded by a gene located on the long arm of chromosome 2 in humans (Joshi, Cameron et al. 2004). Unlike EIF4E1 that participates in global translation, EIF4E2 has functions in both translation repression and translation initiation in transcript-specific circumstances.

The globular region of EIF4E proteins is shaped like a cupped hand and EIF4E2 is not an exception (Rosettani, Knapp et al. 2007). However, there are some notable differences between EIF4E2 and EIF4E1. EIF4E2 has shorter N and C terminal domains (Kubacka, Kamenska et al. 2013). Similarly to EIF4E1, the dorsal side is responsible for interaction with binding partners while the ventral side binds to 5' m7G cap of mRNA. (For review refer to (Christie and Igreja 2023)) Analysis of EIF4E2 on an amino acid level shows that EIF4E2 is 30% identical to EIF4E1 and has a similarity of 60%(Rom, Kim et al. 1998).

Tissue localisation does not differ significantly in EIF4E2 when compared to EIF4E1. EIF4E2 is expressed ubiquitously in all tissues. Mouse EIF4E2-mRNA was found in the highest levels in testis, with high expression also in kidney and liver. On the other hand, heart and lung tissues display markedly lower levels of EIF4E2-mRNA. In direct comparison, EIF4E2 is expressed at lower levels than EIF4E1. (Joshi, Cameron et al. 2004).

To underline the significance of EIF4E2, *Eif4e2* KO mice were phenotypically analysed. These mice were not viable, with all of them dying at postnatal day 0. Analysing their embryo revealed that these mice had smaller brains and unexpanded lungs. All mice were cyanotic at birth, due to lack of sufficient oxygenation. (Morita, Ler et al. 2012). Therefore, we can assume that EIF4E2 is essential for all mammals.

EIF4E2 was firstly reported to only be found exclusively in the cytoplasm (Rom, Kim et al. 1998) however later studies showed that it can also be found in the nucleus. The precise way how EIF4E2 shuttles between the cytoplasm and nucleus is unknown, however EIF4E2 is considered to be a 4E-T

independent nucleocytoplasmic shuttling protein. EIF4E2 binds to 4E-T similarly to EIF4E1, but its nuclear localization doesn't depend on 4E-T. Tests on EIF4E2 shuttling were conducted on cells where 4E-T was depleted and the cells were treated with Leptomycin B, an inhibitor of the CRM1 receptor. (Kubacka, Kamenska et al. 2013) The CRM1 receptor binds CRM1 protein which is a crucial nuclear export factor (Stade, Ford et al. 1997). Despite all that, EIF4E2 still shuttled to the nucleus while EIF4E1 in absence of 4E-T could not shuttle and stayed in the cytoplasm.(Kubacka, Kamenska et al. 2013). Despite EIF4E2 not using 4E-T for shuttling, it can still bind to 4E-T to form a complex that is a part of microRNA-mediated gene silencing machinery (Chapat, Jafarnejad et al. 2017).

EIF4E2, like EIF4E1, can localise to P-bodies and stress granules. EIF4E2 localises to both P-bodies and stress granules upon heat shock. Contrary to this, EIF4E2 does not localise to stress granules and appears only in P-bodies in the arsenite-stressed cells. This suggests that EIF4E2 participates in different cellular pathways and has a different role in different stress conditions.(Frydryskova, Masek et al. 2016)

Glycogen levels in the cell can play a role in the expression of EIF4E2. Mice that are not able to store glycogen effectively have their EIF4E2 gene upregulated by around 1.57-fold. The opposite applies with downregulation of the EIF4E2 gene expression by 2.08-fold in mice that accumulate glycogen. (Parker, Pederson et al. 2006) This suggests an inverse relationship between glycogen levels and expression of EIF4E2 and points to the possible role of EIF4E2 in dealing with lack of energy in cells.

The role of EIF4E2 in stress conditions is further emphasised by the upregulation of the *Eif4e2* gene in the microgravity stress conditions. This was discovered through an experiment with human fibroblast that were cultured in the outer space. (Liu and Wang 2008)

EIF4E2 binds cap with lower affinity then EIF4E1

EIF4E2 can also interact with the m7G cap of mRNA, with a 40-200-time lower binding affinity than EIF4E1 (Rom, Kim et al. 1998, Joshi, Cameron et al. 2004, Rosettani, Knapp et al. 2007, Zuberek, Kubacka et al. 2007). This means EIF4E2 cannot compete with EIF4E1 in cap-binding and EIF4E1 needs to be sequestered, inactivated or not present to allow EIF4E2 to initiate translation.

The differences between EIF4E1 and EIF4E2 can explain the differences in affinity. First of all, out of the eight tryptophan residues in EIF4E1, only six of them are conserved in EIF4E2 with Trp43 and Trp56 being replaced by tyrosine residues. (Joshi, Cameron et al. 2004). The replaced Trp56 is crucial in the aforementioned π–π stacking interactions of EIF4E1 cap-binding (Marcotrigiano, Gingras et al. 1997). However, when conducting experiments on a mutant EIF4E1 with a Trp56-Tyr

replacement, the amino acid switch seems to enhance binding by around 1,5 times (Zuberek, Kubacka et al. 2007).

The tryptophan to tyrosine replacement therefore does not seem to explain the lower cap-binding affinity of EIF4E2. Other structural differences such as where the tryptophan or tyrosine residues are located play a significant role in explaining lower EIF4E2 binding affinity to the mRNA 7mG cap. The Trp56 residue in EIF4E1 is located between two β-sheets in the loop S1-S2 (Tomoo, Shen et al. 2003) while the same region in EIF4E2 has an additional five amino acids not present in EIF4E1. As a result, the longer loop region does not create a perfect stacking interaction between the m7G moiety and the tyrosine residue (Zuberek, Kubacka et al. 2007).

This all is anchored by hydrogen bonds with Glu125 side chain and Trp124 main chain, similarly to the hydrogen bonds found in EIF4E1 cap binding. However, despite the similarity of the binding sites, many interactions can take place in different ways between EIF4E1 and EIF4E2. In EIF4E2, the α-phosphate group of m^7GTP cap binds through a hydrogen bond with His110 side chain while the β-phosphate of m^7GTP cap is involved through an interaction with the Arg174 guanidium group. In EIF4E1, these amino acid residues and interactions are different. The His110 residue is replaced by glycine and the Arg174 equivalent residue interacts with the α-phosphate instead. Additionally, the Lys162 residue interacts with the β-phosphate of m^7GTP cap. This interaction has no direct equivalent in EIF4E2 as the Lys162 residue is replaced with an isoleucine that is incapable of interacting with the phosphate group. (Rosettani, Knapp et al. 2007) Figure 1 shows the interactions between EIF4E2 and the cap.

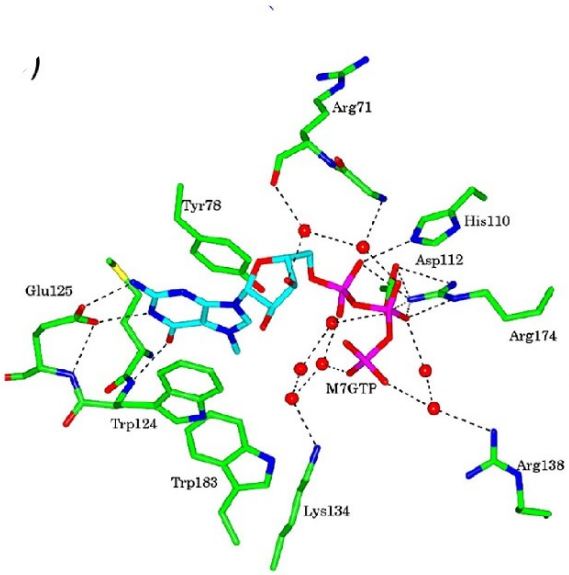

Figure 1- EIF4E2 cap-binding, taken from (Rosettani, Knapp et al. 2007)

ISGylation of EIF4E2 enhances cap-binding

Interferon-stimulated gene 15 (ISG15) is classified as an ubiquitin-like protein (Haas, Ahrens et al. 1987). When the innate immune response is activated, various cytokines play different roles in modulating the immune response. Increased levels of the type 1 interferons (IFN) are specifically significant in such conditions. (For review on IFN refer to (García-Sastre and Biron 2006)). Increased IFN production leads to the increased expression of the set of interferon-stimulated genes including ISG15, allowing it to conjugate with proteins. This helps the cell to rapidly change the proteome and respond to pathogens or stresses directly.

ISG15 can conjugate to EIF4E2 using human homolog of *Drosophila* Ariadne (HHARI), the UbcH8-interacting E3 ligase, (Tan, Ardley et al. 2003) through two separate target sites, Lys134 and Lys222. This increases cap-binding affinity of EIF4E2. Since ISG15 on its own cannot bind to the cap, it is thought that ISGylating EIF4E2 changes the conformation of the translation initiation factor in such a way that allows it to bind with a higher affinity to the cap. (Okumura, Zou et al. 2007)

Another effect ISGylation has on EIF4E2 is enhancing translation-inhibition compared to non-ISGylated EIF4E2 (Okumura, Zou et al. 2007). This way, translation and protein production can be modulated in response to a breach of the immune system or stress conditions.

In genotoxic stress conditions where DNA is damaged, large quantities of HHARI interacts with EIF4E2 initiating translational arrest, and thus protecting the cell (von Stechow, Typas et al. 2015).

Despite EIF4E1 and EIF4E2 being 30% identical and 60% similar on an amino acid level, EIF4E1 cannot be modified by ISG15 (Okumura, Zou et al. 2007).

EIF4E2 has multiple binding partners

EIF4E2 has different binding partners. These binding partners contain a conserved binding motif that EIF4E2 recognises and binds to. It has the sequence $YXYX_4L\Phi$ with X representing any amino acid and Φ representing a hydrophobic residue (Cho, Poulin et al. 2005, Jeong, Park et al. 2019). Different binding partners can modulate the function of EIF4E2 and allow it to participate in different cellular processes. Binding partners include Grb10-interacting GYF 2 (GIGYF2) (Morita, Ler et al. 2012), 4E-T (Kubacka, Kamenska et al. 2013), Threonyl-tRNA synthetase (TRS) (Jeong, Park et al. 2019), Prep1 (Villaescusa, Buratti et al. 2009) and to a weaker extent, 4E-BP. Three different isoforms of 4E-BP exist, 4E-BP1, 4E-BP2 and 4E-BP3. Specifically, EIF4E2 prefer to bind 4E-BP2 and 4E-BP3 rather than 4E-BP1. Similarly, EIF4E2 cannot bind to EIF4G1, binding to EIF4G3 instead. (Joshi, Cameron et al. 2004)

EIF4E2 participates in many key cellular processes

Translational repression

EIF4E2 can participate in translation repression using various mechanisms and can fulfil different roles inside the cell as well as different roles in different tissues and different species.

1. MicroRNA-induced silencing complex (miRISC)effector machinery

The miRISC effector machinery works by topologically circularizing mRNA, similar to how EIF4G binds PABP on the 3' end tail of mRNA in canonical translation. The 4E-T creates a bridge between EIF4E2 and CCR4-NOT complex. The CCR4-NOT complex binds to the miRNA that is responsible for the translational repression effect. It is important to note that this is only made possible because EIF4E2 bound to 4E-T has four times higher affinity to the cap than the standalone EIF4E2. Despite this, the increase in affinity is still not enough to compete with EIF4E1 cap-binding and it is thought that in vivo, the entire miRISC effector machinery may have some additional interactions that stimulate affinity to the cap. Another explanation could be that due to 4E-T having a higher binding affinity to EIF4E2 than to EIF4E1, it is able to concentrate more EIF4E2 around the cap, allowing EIF4E2 to successfully compete with EIF4E1. (Chapat, Jafarnejad et al. 2017). This mechanism can be seen in Figure 2.

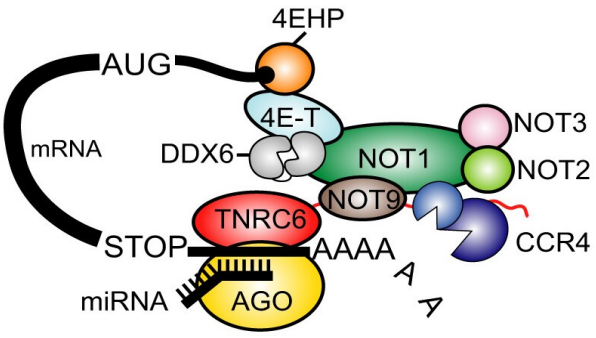

Figure 2- Repression through miRISC, taken from (Christie and Igreja 2023)

Dusp6 is a gene that is repressed by miRISC effector machinery (Jafarnejad, Chapat et al. 2018). *Dusp6* codes for the protein dual specifity phosphatase 6 (Dusp6) (Muda, Boschert et al. 1996). This protein is a phosphatase that acts on extracellular signal-regulated kinases 1/2 (ERK1/2) (Muda, Theodosiou et al. 1996). When *Dusp6* is repressed, ERK1/2 remains phosphorylated. This increases cell proliferation and decreases apoptosis in cells. In *EIF4E2* KO cells, Dusp6 levels increase, which inactivates the ERK1/2 pathway. This reduces cell proliferation. (Jafarnejad, Chapat et al. 2018)

2. Repression through GIGYF2 complex

GIGYF2 is a major binding partner for EIF4E2 that binds to the EIF4E2 dorsal surface through the conserved EIF4E2-binding motif present closer to the N-terminal (Morita, Ler et al. 2012). As an adaptor protein, GIGYF2 is able to bind to different proteins, which can give the EIF4E2-GIGYF2 complex multiple different roles in the cell. These proteins are ZFN598 (Morita, Ler et al. 2012), DDX6 (Peter, Ruscica et al. 2019) and trinucleotide repeat-containing adaptor 6A (TNRC6A) (Sobti, Mead et al. 2023).

To strengthen its interaction with EIF4E2, GIGYF2 proteins have auxiliary sequences in the C terminal domain that specifically interact with EIF4E2 regions not present in EIF4E1. This makes the GIGYF2-EIF4E2 interaction more specific and more stable. (Peter, Weber et al. 2017)

GIGYF2 and *EIF4E2* expression is mutually balanced. In *EIF4E2* KO mice, *GIGYF2* expression decreases . The same effect is seen vice versa. When GIGYF2 is depleted, EIF4E2 levels decrease as well. (Morita, Ler et al. 2012)

When it comes to translational repression, two important GIGYF2 binding motifs play a role in forming the final translation repression appartatus. One is the Me31B/DDX6-binding motif resposible for binding RNA-dependent ATPase DDX6 (Peter, Ruscica et al. 2019). The other sequence is the GYF motif responsible for binding to the PPGΦ (where Φ represents a hydrophobic residue) motifs found in zinc finger protein ZNF598 (Morita, Ler et al. 2012) or ZFP36/Tristetraprolin (TTP) (Fu, Olsen et al. 2016).

The complex with TTP and ZFN598 represses and degrades cytokine mRNAs, which play an important role in controlling the innate immune system and restriction of the inflammatory response (Tollenaere, Tiedje et al. 2019). The ZFN598 complex also plays an additional role in mouse embryonic development, specifically in neurogenesis and lung development. (Morita, Ler et al. 2012)

TNRC6A can also bind to GIGYF2 through the PPGΦ binding motif. This complex with EIF4E2 is responsible for repressing translation of certain miRNA targets. (Sobti, Mead et al. 2023)

The GIGYF2-EIF4E2 complex also plays a role in the ribosome quality control. The ribosome quality control is a rescue pathway in eukaryotes which is activated when two ribosome collide at a stall site, forming a binding site for E3 ligase ZFN598. This enzyme conjugates ubiquitin to the RPS3A, RPS10 and RPS20 proteins of the small ribosomal subunit. (Garzia, Jafarnejad et al. 2017) A small protein, endothelial differentiation related factor 1 (EDF1), binds to 40S small ribosomal subunit and makes up part of the overall ribosome quality control machinery. EDF1 stabilises GIGYF2 (Sinha, Ordureau et al. 2020) while GIGYF2 and EIF4E2 blocks translation initation (Hickey, Dickson et al. 2020) or triggers cotranslational degradation of mRNA (Weber, Chung et al. 2020). This has the effect of reducing ribosome loading and ensuring the quality of proteins produced.

GIGYF2 mutations which impair binding to EIF4E2 can lead to autism spectrum disorder (ASD)-like behavior in mice. The impaired GIGYF2-EIF4E2 complex is unable to repress the translation of specific mRNAs in excitatory neurons, allowing increased levels of hippocampal mGluR-LTD and an observed diminished sociability in mice. Synaptic plasticity dysfunction, a key characteristic of ASD, was also observed. (Wiebe, Meng et al. 2020)

3. Repression of *Hoxb4* in mice to regulate oogenesis

Cytoplasmic Prep1 forms a complex with EIF4E2 through its conserved binding motif in the cytoplasm of oocytes. This complex binds to the 3'UTR of *Hoxb4* mRNA, repressing its translation. This repression is necessary in the development of oocytes, as the presence of Hoxb4 protein in developing oocytes leads to increased formation of cysts and growth failure of oocytes (Villaescusa, Buratti et al. 2009). This mechanism is shown in Figure 3.

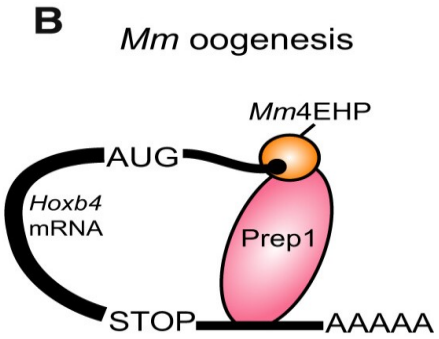

Figure 3- Repression of Hoxb4, taken from (Christie and Igreja 2023)

4. EIF4E2-mediated repression plays an important role in cognition

EIF4E2 in excitatory and inhibitory neurones is necessary for the spatial working memory (WM) to be established in mice. WM is a short-term memory that is necessary in decision-making. Tests were performed on EIF4E2-cKO^{exc} mice which have EIF4E2 knocked out of their excitatory neurones. They were tested on their ability to navigate T-mazes. Mice were first placed in conditional training for 10 minutes, where they were allowed to explore these T-shaped mazes with one of the arms blocked. After an hour, the mouse re-entered the maze where it was allowed to explore all of the maze unhindered. The mice's ability to navigate the T-maze was quantified and showed that EIF4E2-cKO^{exc} mice have their WM diminished. This test was then repeated on EIF4E2-cKO^{inh} mice. These mice have EIF4E2 knocked out in their inhibitory neurones. Their WM was severely impacted too. This is because EIF4E2 is necessary for the repression of certain proteins that control the mTORC1 pathway. This loss of repression in EIF4E2-cKO^{exc} and EIF4E2-cKO^{inh} mice can attenuate the mTORC1 pathway, which in turn diminishes the WM. (Wiebe, Huang et al. 2023)

Translation initiation

In specific cases and while binding to certain proteins, EIF4E2 has been found to initiate translation. It does not do so on a global scale, but more so under specific conditions and for specific transcripts.

1. TRS-mediated translation initiation

Translation is initiated by the formation of a complex that corresponds to the EIF4F complex in canonical translation. This complex consists of EIF4E2, TRS and EIF4A. TRS binds to PABP, similarly to EIF4G, to circularize mRNA. Furthermore, it also links to EIF3, initiating translation in a similar mechanism to canonical translation. (Jeong, Park et al. 2019)

The EIF4E2 and TRS interaction is highly specific with TRS being the only aminoacyl-tRNA synthetase EIF4E2 can bind to. Similarly, TRS cannot bind to EIF4E1 and EIF4E3 (Jeong, Park et al. 2019).

The very specific interaction between the two proteins is due to the UNE-T region present in TRS that contains the conserved binding motif necessary for interacting with EIF4E2. In fact, the TRS-EIF4E2 complex is very similar to the complexes EIF4E2 forms with other interaction partners. The TRS region is found in all eukaryotes, but more specifically, different immunoassays have showed that the UNE-T region is only found in vertebrates. This means the EIF4E2-UNE-T interaction is exclusive to vertebrates. (Jeong, Park et al. 2019)

TRS-mediated translation plays a crucial role in translation initiation for mRNAs required for vascular development such as VEGF and angiogenin (ANG). In cell lines where TRS or EIF4E2 was suppressed, lower levels of VEGF and ANG were subsequently observed (Jeong, Park et al. 2019).

TRS-mediated translation initiation can be seen in Figure 4.

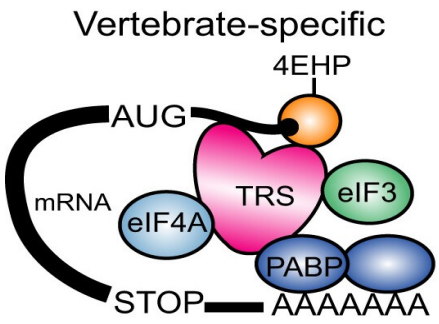

Figure 4- Translation initiation with EIF4E2 and TRS, taken from (Christie and Igreja 2023)

2. Translation in hypoxic conditions

Because of a family of transcription factors known as hypoxia inducible factors (HIF), cells are able to modulate key cellular processes in response to lower oxygen concentrations (Semenza and Wang 1992). These transcription factors are heterodimeric, consisting of an alpha and a beta subunit (Wang and Semenza 1995). Three alpha isoforms have been discovered to date, HIF1-α, HIF2-α and HIF3-α, and their expression is oxygen-regulated. On the other hand, the beta subunit, HIF1-β is constitutively expressed (Wang, Jiang et al. 1995).

In hypoxic conditions, the cell arrests many high-energy processes, including EIF4E1-dependent global translation. In chronic low oxygen conditions, mTORC1 no longer phosphorylates 4E-BPs, leading to its activation. Hypophosphorylated 4E-BP sequesters EIF4E1, preventing it from binding to the mRNA m7G cap. This disrupts global translation (Arsham, Howell et al. 2003). However, the cell needs to maintain a basal level of translation and synthesise proteins necessary to respond to hypoxia. It does this through both cap-independent translation and cap-dependent translation. In cap-independent translation, the cell uses highly structured sequences known as cellular internal ribosomal entry sites (IRES) (Young, Wang et al. 2008) or cap-independent translation enhancers (CITEs) (Terenin, Andreev et al. 2013). The cell can also exploit a special form of cap-dependent translation where an alternate $EIF4F^{H}$ complex is formed (Ho, Wang et al. 2016).

HIF2-α, in its standalone form, can regulate translation by forming a non-canonical $EIF4F^{H}$ complex that can initiate translation in hypoxic conditions. HIF2-α cannot interact with RNA directly, and interacts with RNA hypoxia response elements (rHRE) through another protein, RNA binding motif protein 4 (RBM4). RBM4 binds to rHRE, independent of the oxygen concentration, and in hypoxic conditions, RBM4 binds HIF2-α through its amino terminal region. The HIF2-α and RBM4 complex recruits EIF4E2. (Uniacke, Holterman et al. 2012) Together with EIF4G3 and EIF4A, the complex $EIF4F^{H}$ is formed, which initiates translation (Ho, Wang et al. 2016). This complex is shown clearly in Figure 5.

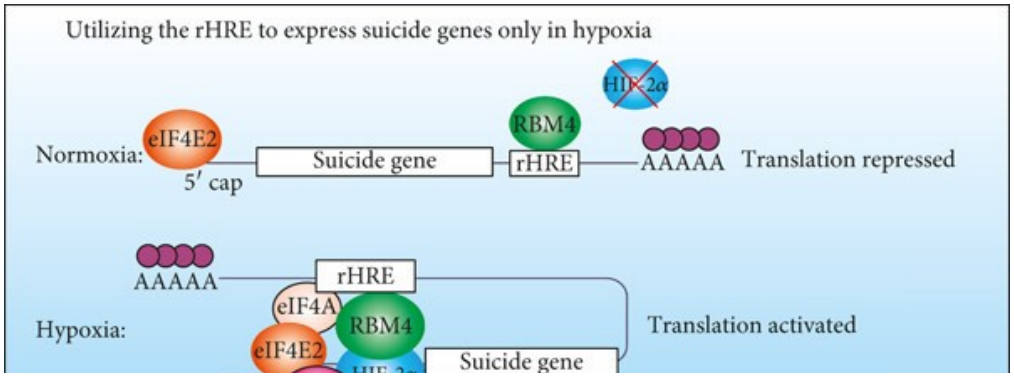

Figure 5- EIF4E2-mediated translation in hypoxia, taken from (Melanson, Timpano et al. 2017)

EIF4E2 participates in other cellular processes as well. EIF4E2 forms a complex with the protein Glycogen synthase kinase-3β (GSK3β) which is a Ser/Thr kinase that can stimulate or repress senescence of the cell under different conditions. When in complex with EIF4E2, it maintains phosphorylation of p53. When p53 is dephosphorylated, senescence in cells can be increased by repressing transcription. Under hypoxia, GSK3β Cys317 is S-nitrosyled. This inhibits the EIF4E2- GSK3β pathway, which has the effect of inhibiting phosphorylation of p53. This has the effect of increasing senenscence levels in hypoxia. (Sun, Yang et al. 2022)

Due to the hypoxic tumour microenvironment (TME) (Franko and Sutherland 1979), EIF4E2 can promote tumorigenesis by allowing translation initiaiton to occur in an alternative manner with the help of HIF2-α. Depletion of EIF4E2 slows down the spread of cancer cells and formation of tumours (Uniacke, Perera et al. 2014).

Variants of EIF4E2

EIF4E2 itself has been found to have variants of its own as well. One of them is of course, EIF4E2_A, the standard full-length form of the protein that is used in all the studies. Other non-standard versions are EIF4E2_C and EIF4E2_CRA_a. EIF4E2_C is a protein that has a shorter C-terminal region due to exon 7a being replaced with exon 7c. EIF4E2_CRA_a retains the same C-terminal region as EIF4E2_C while also having a different N terminus (Frydryskova, Masek et al. 2016). Furthermore, some variants utilize different exons than the ones found in EIF4E2_A. EIF4E2_B has alternate exons 7b and 8. The EIF4E2_D variant has exon 7a replaced with exon 7c and is missing exon 3.These non-standard variants of EIF4E2 vary in length and have not been studied in detail yet. (Mrvova, Frydryskova et al. 2018)

EIF4E3

EIF4E3, the least studied non-canonical isoform in the EIF4E family, is coded by a gene on chromosome 3 in humans (GenBank Overview). It is 25-30% identical and 45-55% similar to EIF4E1 and EIF4E2. (Joshi, Cameron et al. 2004) Five EIF4E3 transcripts code for two EIF4E3 isoforms, known as EIF4E3_A , the full form of the protein, and EIF4E3_B, the truncated form of the protein missing the N-terminal region (Frydryskova, Masek et al. 2016).

Some EIF4E3 transcripts share transcription start sites with the exon present on the GPR27 anti-sense strand (Chopra, Yiv et al. 2020). GPR27, is a G-protein that positively regulates the production of insulin (Ku, Pappalardo et al. 2012). This could suggest GPR27 and EIF4E3 somehow being transcriptionally linked.

The specific function of EIF4E3 remains unknown, but it has been found to play a role in both tumorigenesis and tumour suppression in different cancers (Deng, Lin et al. 2022, Xu, Zhao et al. 2023). Its ability to bind EIF4G indicates its ability to participate in translation (Joshi, Cameron et al. 2004).

EIF4E3 is not an essential protein, with *Eif4e3 -/-* KO mice being viable, however other features seem to be significantly affected by the lack of EIF4E3. KO mice display a lack of fluidity in movement, resulting in an abnormal gait. Furthermore, KO mice have a significantly lower body weight than their WT mice counterparts. (IPMC:www.mousephenotype.org (Groza, Gomez et al. 2022))

In stress conditions induced by Torin1, mitochondrial respiration was significantly lowered in N2a *Eif4e3* KO cell lines. This reduction was at around 3.4 fold in comparison to WT which only had mitochondrial respiration lowered by 2-fold. This resulted in lower ATP production in the KO cells, in comparison to the WT cells. (Weiss, Allen et al. 2021)

EIF4E3 is not expressed as ubiquitously in all tissues like EIF4E1 and EIF4E2 are, with highest levels of EIF4E3-mRNA found in muscle tissue. Lower levels of EIF4E3 are found in the lung and spleen, with possibly even lower levels of mRNA in other tissues in which it was not detected. Corresponding translated protein was also not detected, suggesting that outside of special circumstances, EIF4E3 levels in the cells are extremely low. (Joshi, Cameron et al. 2004)

Another source indicates EIF4E3 is detected on an mRNA level in all tissues, but it doesn't always translate to form an EIF4E3 protein product, with protein levels being markedly absent from the eye, the skin and soft connective tissue. Furthermore, despite its presence in most tissues, EIF4E3 was often notably absent from specific tissue and organs. These include but are not limited to the cerebellum, hippocampus, vagina and ovaries. (Human Protein Atlas proteinatlas.org (Uhlen, Fagerberg et al. 2015)

On a subcellular level, EIF4E3 is present in the cytoplasm, indicating its function in translation. It also localises to the nucleus, where its function remains unknown. Specifically, in the cytoplasm, it localises to stress granules and P-bodies, with the two EIF4E3 isoforms localising differently under different kinds of stress. Under heat shock, EIF4E3_A localised to stress granules and was missing from P-bodies. On the other hand, EIF4E3_B was missing from both. Under arsenic stress, similar results were found with EIF4E3_A again localising to stress granules only and EIF4E3_B not being found in either stress granules or P-bodies. (Frydryskova, Masek et al. 2016)

Structurally, EIF4E3 does not differ from other EIF4E members in some aspects, maintaining central beta sheets and three alpha helices on the dorsal surface. Where it differs is at the disordered N-terminus, with EIF4E3 having an additional alpha helix, that is not present in EIF4E1. Furthermore,

EIF4E3 has a disordered C terminus, and does not contain the residues needed to form the eighth beta strand present in other EIF4E family members. (Osborne, Volpon et al. 2013)

EIF4E3 binds the cap with lower affinity than EIF4E1, in an abnormal way

Unlike other EIF4E isoforms that characteristically have two conserved aromatic residues critical for recognition of the cap, EIF4E3 subverts expectations. In place of the conserved Trp56 residue found in EIF4E1, EIF4E3 has a non-aromatic Cys52, that together with Trp98 is capable of recognising the cap, albeit binding to it in a manner different than an aromatic cap sandwich. This difference, however, does not seem to be the sole reason for the 10-40 lower binding affinity to the cap in comparison to EIF4E1. When engineering an EIF4E3 Cys52Trp mutant, binding affinity was reduced by three to five fold. (Osborne, Volpon et al. 2013)

Trp98 forms aromatic stacking interactions when binding to the m7G cap, similar to its equivalent Trp102 in EIF4E1. On the other hand, Cys52 forms an alpha helix in the S1-S2 loop and together with other residues present in the S1-S2 loop, all interact with the mRNA m7G cap, to create the most extensive network of bonds out of all the EIF4E family members. Specifically, these residues are Ala47 and Ala49, which form both sidechain and backbone contacts with the purine ring. Meanwhile, Ser43 side chains and Leu44 and Pro45 backbones interact with the sugar of the cap. The disordered C terminus also plays a role in cap-binding. The cluster of these extensive hydrophobic and charged interactions are not present in EIF4E1 or EIF4E2 cap-binding. (Osborne, Volpon et al. 2013) The interactions between EIF4E3 and the cap can be seen in Figure 6.

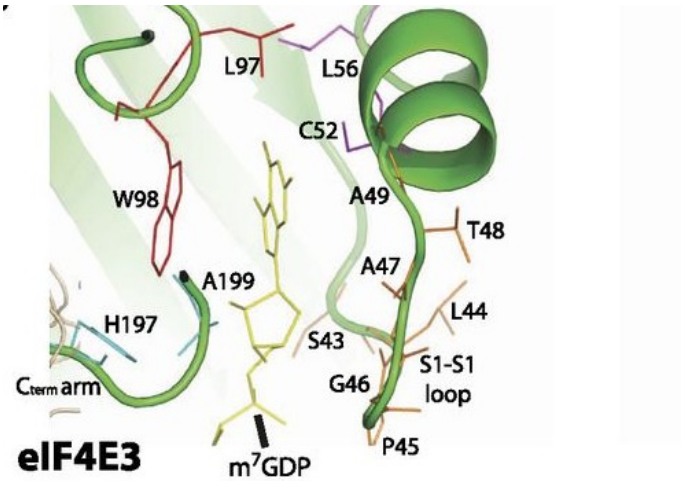

Figure 6- EIF4E3 cap-binding, taken from (Osborne, Volpon et al. 2013)

EIF4E3 interaction with proteins

EIF4E3 is capable of binding to EIF4G1 and EIF4G3 (Frydryskova, Masek et al. 2016), indicating its ability to initiate translation and form active EIF4F complexes. However, EIF4E3 is not regulated by 4E-BPs, with no interaction or any kind of binding between them found (Joshi, Cameron et al. 2004). This is important because if EIF4E3 was regulated by 4E-BPs, it would be sequestered alongside EIF4E1. However, because 4E-BPs cannot bind EIF4E3, when EIF4E1 is sequestered, EIF4E3 can form a non-canonical active $EIF4F^S$ complex capable of initiating translation. (Weiss, Allen et al. 2021)

EIF4E3 expression regulated by EIF4E1 availability and MNK1/2

As previously mentioned, EIF4E1 availability can be regulated not just by 4E-BPs, but also by MNKs, phosphorylating the Ser209 residue, rendering it available for cap-binding (Batool, Majeed et al. 2020). An experiment conducted on Diffuse large B-cell lymphoma (DLBCL) showed that MNKs regulate both EIF4E1 and EIF4E3, albeit in different ways. This was proven through the inhibition of MNK1/2, which expectedly led to the decrease of phosphorylation of Ser209 in EIF4E1, reducing its cap-binding potential. However, it also led to the upregulation of EIF4E3 (Landon, Muniandy et al. 2014), suggesting that EIF4E3 might function as replacement for EIF4E1-mediated translation, to maintain protein translation, or drive the translation of a different subset of mRNAs. (Weiss, Allen et al. 2021)

Specifically, unphosphorylated EIF4E1 is the factor that drives the upregulation of EIF4E3. DLBCL cell lines that contained uninhibited functional MNK1/2 and “phospho-null” EIF4E1 that cannot be phosphorylated had higher EIF4E3 expression levels. (Landon, Muniandy et al. 2014)

EIF4E3 impact in cells modulated by microRNAs and lncRNAs

EIF4E3 expression can be regulated by various microRNAs. This allows EIF4E3 to have a varied response in different tissues.

Starting with an experiment conducted on human trabecular meshwork (HTM) cells, micro RNA-1298 (miR-1298) was found to negatively regulate EIF4E3 expression by binding to a sequence on the 3’ UTR region of the EIF4E3 mRNA. Considering that miR-1298 is downregulated in chronic oxidative stress (COS), this indicates that EIF4E3 is partially responsible for the pathogenesis of glaucoma. (Ruibin, Zheng et al. 2018) Glaucoma is a neurodegenerative disease, characterised by a gradual loss of retinal ganglion cells that can eventually lead to blindness (For review on glaucoma refer to (Weinreb, Aung et al. 2014)) One of its leading causes is an increase in intraocular pressure (IOP) (Kapetanakis, Chan et al. 2016). An increase in IOP can occur due to extracellular matrix (ECM)

being accumulated in trabecular meshwork (Villarreal, Oh et al. 2011) which occurs due to the induction of apoptosis by COS in trabecular meshwork cells (Alvarado, Murphy et al. 1984).

Another experiment was conducted on the MKN45 and HGC27 cell lines that were overexpressing miR-592. MiR-592 binds to the *EIF4E3* mRNA, thus decreasing its levels. miR-592 can be sequestered by circVDAC3, a circular mRNA. This sequestration has the effect of upregulating EIF4E3, which is crucial in the suppression of gastric cancer. This is further proven by the fact that in gastric cancer tissues, *EIF4E3* is expressed in significantly lower levels, indicating a relationship between the circVDAC3-miR-592 axis, EIF4E3 and the progression or suppression of gastric cancer. (Yang, Xiao et al. 2024)

miR-148b-3p was also found to have an impact on EIF4E3 in liver cancer cells, with cells that contain a high level of miR-148b-3p having low levels of EIF4E3. This might indicate EIF4E3's tumour-suppressing properties. MiR-148b-3p is regulated by LINC01554, a long non-coding RNA. The effect of LINC01554 is the downregulation of miR-148b-3p and thus inhibition of viability and migration of cell and promoting of apoptosis. This, of course, has the effect of ensuring less transformation of healthy cells to malignant cells. LINC01554 upregulates a set of proteins, including EIF4E3, that are responsible for tumour-suppressive actions. (Ren, Wang et al. 2024)

In medulloblastoma, pediatric brain cancer, miR-584-5p acts as a tumour suppressor by targeting the *EIF4E3* and *HDAC1* genes. *HDAC1* codes for the histone deacetylase complex. Proteins produced by these two genes have tumour-promoting functions in medulloblastoma. MiR-584-5p lowers the expression of the *Eif4e3* and *Hdac1* genes. This leads to the reduction of the viability and migration of the medulloblastoma cells. The miR-584-5p–eIF4E3/HDAC1 axis regulates cell cycle as cells treated with miR-584-5p mimic had their cell cycle arrested at G2/M. This seems to imply that EIF4E3 might play a role in the progression of the cell cycle. The role of the miR-584-5p–eIF4E3/HDAC1 axis goes even further. Cells depleted of EIF4E3 and HDAC1 have collapsed or multipolar spindles. These cells also could not repair DNA damage through non-homologous end joining (NHEJ) or homologous recombination (HR). (Abdelfattah, Rajamanickam et al. 2018)

Atrial fibrillation (AF), or abnormal heart rhythm, can lead to many serious secondary problems such as strokes, clots and even heart failure. In people suffering from AF-related stroke, EIF4E3 is upregulated in the left and right atrium of the heart. Furthermore, *hsa-miR-198* and *hsa_circ_0018657* are upregulated in the left and right atrium of the heart. This is important because EIF4E3 is hsa-miR-198's target, which is a target of hsa_circ_0018657, indicating that this regulatory pathway plays a role in the development of an AF-related stroke. (Huang, Fan et al. 2023)

MiR-101 is upregulated in hypoxic conditions and can reduce EIF4E3 levels by binding to 3'UTR region of its mRNA. This reduction in EIF4E3 activity leads to the upregulation of EIF4E1, which activates the HIF-1 signalling pathway. VEGF-A is produced as a result, causing proliferation and migration of endothelial cells. (Pang, Ye et al. 2020) These cells need blood vessels to supply them with nutrition, which is where miR-101 plays another role in promoting angiogenesis by binding to the protein Cullin 3 under hypoxic conditions (Kim, Lee et al. 2014). This can indicate, that EIF4E3 in fact, represses angiogenesis in normal non-hypoxic conditions (Pang, Ye et al. 2020).

EIF4E3 expression is reduced by high expression levels of *MiR-21* and *MiR-206*. These miRNAs are linked to the onset of the atrophy of muscles seven days after denervation. This implies that EIF4E3, might play a role in the building of muscles, when the muscle is properly innervated. (Soares, Cagnin et al. 2014)

EIF4E3 has various functions within the cell

<u>EIF4E3 translates in stress conditions that affect EIF4E1 and EIF4E2 availability</u>

When EIF4E1 is sequestered by 4E-BPs, EIF4E3 can form alternative EIF4F complexes that can impact the translatome. These complexes are known as $EIF4F^{S}$. $EIF4F^{S}$ can further be divided into two groups, depending on which EIF4G participates in the complex. With EIF4G1, EIF4E3 forms the $EIF4F^{S1}$ complex and with EIF4G3, EIF4E3 forms $EIF4F^{S3}$ complex (Frydryskova, Masek et al. 2016) Sometimes, EIF4F complexes containing EIF4E3 are also referred to as EIF4F-3 (Landon, Muniandy et al. 2014). From here on after, I will refer to those complexes as $EIF4F^{S}$.

EIF4E3 preferentially translates transcripts that have a shorter 5' terminal length, leading to their upregulation. On the other hand, EIF4E3 represses the translation of transcripts that have longer 5' terminal lengths, with these genes being significantly downregulated as a result. (Weiss, Allen et al. 2021) This is necessary to maintain basal levels of cell function, with most short 5' terminal length transcripts belonging to those of housekeeping genes (Elfakess, Sinvani et al. 2011).

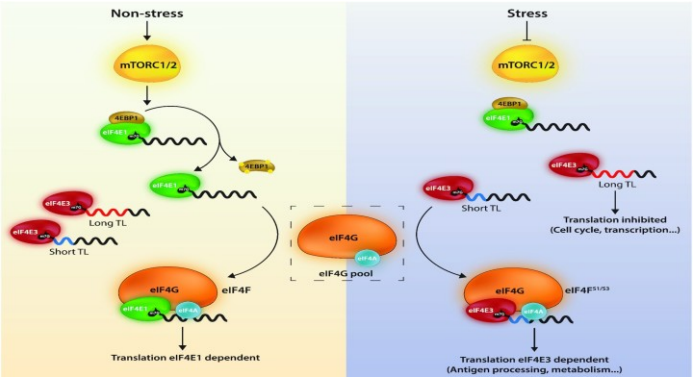

Figure 2- EIF4E3 replaces EIF4E1 in stress conditions, taken from(Weiss, Allen et al. 2021)

EIF4E3 can also impact the translatome slightly, translating proteins in higher or lower quantities than in EIF4E1-mediated translation. This impacts microRNA maturation, with EIF4E3 reducing the expression of DICER1, an Rnase III enzyme whose substrate is dsRNA or hairpin RNA (Macrae, Zhou et al. 2006). Other protein expressions that are affected by EIF4E3 are ADAR, which is responsible for RNA stability, and other transcription factors like n-MYC and TWIST1 (Landon, Muniandy et al. 2014).

EIF4E3 functions within tumours

As mentioned earlier, microRNAs can regulate EIF4E3 in different ways to either promote or suppress tumorigenesis. EIF4E3 also plays a role in different types of cancers directly, without the influence of microRNAs.

Tumour-suppressing

In head and neck squamous cell carcinoma, *EIF4E3* expression decreases, indicating its tumour-suppressing abilities. This seems to be due to higher infiltration of immune cells, namely M1 macrophages and CD8+ T cells when EIF4E3 is expressed more. These cells show higher expression of many immunomodulatory targets, which leads to higher sensitivity to immunotherapy. These cells also express more of *CCL4, CCL5, CXCL9, CXCL10* genes. The presence of these chemokines strengthens the immune system against tumours, explaining EIF4E3's tumour-suppressing abilities. (Xu, Zhao et al. 2023)

Oral squamous cell carcinoma can be caused by chromosomal aberrations, one of them being the deletion of the 3p region of chromosome 3. This region contains *EIF4E3*, indicating that its loss can lead to oncogenic transformation of the cells in the oral cavity. (Cha, Kim et al. 2011)

In breast cancer, *EIF4E3* is considered to be a favourable breast cancer gene (BRCA) prognostic factor, with its expression being markedly decreased in breast cancer cell lines in comparison to healthy breast cell lines (Huang, Mo et al. 2023).

EIF4E3 was also found to have tumour-suppressing properties in hepatocellular carcinoma, with it being significantly downregulated in tumour cells in comparison to normal cells (Chen, Yao et al. 2022).

In skin cutaneous melanoma (SKCM), high expression of *EIF4E3* is linked to better survival, indicating once more, EIF4E3's tumour-suppressing properties. It is thought that it fights tumours by activating immune responses. (Zhang, Miao et al. 2022)

In bladder cancer, the regulated co-expression of *EIF4E3* and *Grainyhead-like transcription factor 3 (GRHL3)* has been found to correlate with the prolonged survival of patients. Furthermore, characteristic GRHL3 binding motifs were found in EIF4E3 in intron 5, suggesting some formation of a complex (Lammert, Pannhausen et al. 2024). The GRHL3 binding motifs consist of two adjacent Grainyhead consensus sequence repeats and two tandem core CNNG motifs separated by 5 bases (Werth, Walentin et al. 2010, Chung, Tan et al. 2016).

In colorectal cancer, *EIF4E3* expression is lowered. This can be used as a valuable prognostic marker in patients suffering from colorectal cancer (Huang, Zhu et al. 2022).

Tumour-progression

Contradictorily, in melanoma, increased *EIF4E3* expression can be used as a main prognostic marker in advancement of cancer . It has been suggested that EIF4E3 promotes melanoma through the modulation of some immune responses and maturation of CD8+ T cells but the exact mechanism of how it does so remains unknown (Deng, Lin et al. 2022). Similarly, in renal cell carcinoma, EIF4E3 is also upregulated, indicating its ability to promote tumorigenesis (Human Protein Atlas proteinatlas.org (Uhlen, Fagerberg et al. 2015).

Prostate cancer (CaP) controlled by androgens can transform into the deadly androgen-independent (AI) prostate cancer (CaP). This transformation is mediated by the CD168 signalling pathway (Ying and Lin 2004). CD168 interacts with hyaluronan (HA), activating PI3K-IP3 signalling in AI CaP. CD168's downstream signalling genes which include EIF4E3 were found to be overexpressed in AI CaP, preferentially promoting AI CaP over androgen-dependent CaP. (Lin, Chang et al. 2007)

Possible neuronal and developmental role

A child with a deletion of 785kb in the 3p14.1p13 region in chromosome 3 was found to have speech delay, blepharophimosis, contractures and hypertonia. This chromosomal deletion spans many genes, *FOXP1, GPR27, PROK1* and *EIF4E3* (Pariani, Spencer et al. 2009). This could suggest that EIF4E3 plays a role in certain neuronal processes, however when compared to other cases with similar deletions (Sichong, Bui et al. 1981, Hertz, Coerdt et al. 1988, Crispino, Cardoso et al. 1995), these phenotypic changes seem more likely to be due to the deletion of *FOXP1* (Pariani, Spencer et al. 2009). Despite that, the impact of EIF4E3 cannot be excluded.

Four patients were reported to have a microdeletion in 3p14.1p13 region, deleting the *EIF4E3* gene, the first three exons of *FOXP1* and its intronic enhancer, hs1149. These four patients displayed feeding difficulties, gross psychomotor delay and multiple contractures. Many of these patients displayed severe developmental delays and facial abnormalties as well. This could be attributed to lack

of EIF4E3, as *FOXP1* KO mice did not seem to display a similar phenotype (Thevenon, Monnier et al. 2014).

Role in immunity

As mentioned above, EIF4E3 can impact the immune system by upregulating certain chemokines. These chemokines promote an immune response against tumour cells in head and neck squamous cell carcinoma (Xu, Zhao et al. 2023).

Another way, EIF4E3 plays a role in the immune system through differentiation where increased *EIF4E3* expression is correlated with increased monocyte differentiation (Xu, Zhao et al. 2023).

One of the ways EIF4E3 suppresses the formation of tumours is through the activation of the immune system and the increased infiltration of different immune cells (Zhang, Miao et al. 2022, Xu, Zhao et al. 2023).

In polycystic ovary syndrome (PCOS), *EIF4E3* is expressed in higher levels (Zhao, Liu et al. 2024). This correlates with the fact that PCOS patients have higher infiltration of immune cells like natural killer cells and leukocytes and higher levels of IL-1β and IL-18 in follicular fluids (He, Mao et al. 2020, Liu, Liu et al. 2021).

Role in the cardiovascular system

As mentioned above, EIF4E3 has been shown to inhibit angiogenesis in non-hypoxic conditions (Pang, Ye et al. 2020). Furthermore, when co-expressed with other genes, it promotes the development of AF-related strokes This shows that EIF4E3 does have a role in the cardiovascular system. The role of EIF4E3 is further emphasised in AF-related strokes. When MiR-198 binds to the 3'UTR region of the EIF4E3 mRNA, the chances of an AF-related stroke developing increases. (Huang, Fan et al. 2023)

EIF4E2 and EIF4E3 role in tumours

EIF4E2 and EIF4E3 have been proven to play an important role in tumours, whether by promoting their formation or suppressing them. This chapter will summarize how exactly do EIF4E2 and EIF4E3 impact different tumours and cancers.

EIF4E2 translation in hypoxia drives tumorigenesis

Tumours proliferate further than their vasculature does, often resulting in core regions, where oxygen is in lower concentrations than in normoxic cells (Soares, Cagnin et al. 2014). This can impact translation initiation and the resulting translatome, as cancer cells observe a marked decrease in global

translation rates. This allows the malignant cell to save energy and resources. (Liu, Cash et al. 2006, Uniacke, Holterman et al. 2012). In this condition, the tumour cells may switch from EIF4E1-mediated global translation, to an EIF4E2-mediated hypoxic translation, supplementing the tumours with key proteins needed for proliferation and migration. Hypoxia is a significant inhibitor of mTORC1, causing hypophosphorylated 4E-BPs to sequester EIF4E1, preventing it from binding the mRNA cap. (Tinton and Buc-Calderon 1999) This allows EIF4E2 to step in, and preferentially translate mRNAs with rHRE, thus promoting tumour growth (Uniacke, Holterman et al. 2012). I already explained how EIF4F^{H}, the hypoxic translation initiation complex, assembles and functions in Chapter titled "EIF4E2".

EIF4E2 upregulates a set of genes that have the ability to modify the extracellular matrix (ECM) in hypoxic conditions. This drives the epithelial-mesenchymal transition (EMT), which promotes metastasis and the spread of cancer cells throughout the body. In colorectal cancer, through the EIF4F^{H} complex, EIF4E2 promotes translation of *PLOD2* and *P4HA1* mRNAs. PLOD2 and P4H are enzymes responsible for the post-translational modifications of collagen, necessary to for the correct alignment and stiffening of collagen. P4HA1 is the alpha subunit of P4H. (Li, Hung et al. 2025)

EIF4E2 isn't the only translation initiation factor from the EIF4E family capable of tumorigenesis. EIF4E1 overexpression and phosphorylation on its Ser209 residue have been found in many different kinds of tumours (Lazaris-Karatzas, Montine et al. 1990). This is because EIF4E1 is necessary for the translation of mRNAs with highly complex 5' UTR regions (Koromilas, Lazaris-Karatzas et al. 1992). These mRNAs belong to proliferative proteins like c-MYC, VEGF and cyclin D1 (Montanaro and Pandolfi 2004).

The importance of EIF4E1 in tumours is further highlighted in lung adenocarcinoma. METTL16 is an enzyme that transfers a methyl group to N^6 position of the adenosine residues in RNA (m6A Writer). This protein is overexpressed in lung adenocarcinoma and plays a crucial role in the transformation of cells into malignant cells. METTL16 directly interacts with EIF4E2, sequestering it, preventing it from competing with EIF4E1 for mRNA cap binding. This facilitates EIF4E1-mediated translation initiation and the subsequent translation of proliferative proteins. (Wang, Zhang et al. 2023)

Generally, however, EIF4E2 is found to be overexpressed in most tumours, with some tumours even being confirmed to have a non-canonical EIF4F^{H} complex necessary for transformation and growth. Table 1 highlights the effect EIF4E2 has in different kinds of tumours.

Table 1:

Cancer	EIF4E2	Reference
Uveal melanoma	Increased expression	1
Lung adenocarcinoma	EIF4E2 sequestered, EIF4E1 upregulated	2
Lung squamous cell carcinoma	Increased expression	3,4
Liver hepatocellular carcinoma	Increased expression	3,4
Renal cell carcinoma	EIF4E2-mediated translation initiation activated	5
Acute myeloid leukemia	Increased expression	6
Colorectal cancer	EIF4E2-mediated translation initiation activated	5, 7
Glioblastoma	EIF4E2-mediated translation initiation activated	5,8
Colon adenocarcinoma	Increased expression	3,4
Breast cancer	EIF4E2-mediated translation initiation activated	8

Table 1, Legend

No role in tumours

Increased expression, possible tumour-progressive properties

Confirmed tumour-progressive properties

References: [1](Yang, Gu et al. 2021), [2](Wang, Zhang et al. 2023), [3] www.proteinatlas.org, *[4](Uhlen, Zhang et al. 2017), [5](Uniacke, Perera et al. 2014),[6](Zhao, Yang et al. 2024), [7](Li, Hung et al. 2025), [8](Kelly, Varga et al. 2018)*

EIF4E3, unknown molecular mechanism in tumours

As explained in the previous chapter, EIF4E3 can play a role in both the progression and the suppression of tumours (Deng, Lin et al. 2022, Xu, Zhao et al. 2023). The specific molecular mechanism through which it impacts tumours is unknown, but many different hypotheses attempt to explain how it could work. EIF4E3 translation impacts the translatome, allowing different proteins to be produced

(Landon, Muniandy et al. 2014). It can also activate the immune response, increasing infiltration of immune cells (Xu, Zhao et al. 2023).

EIF4E3 initiates translation by forming alternative EIF4F^{S} complexes when EIF4E1 is sequestered by 4E-BPs. This can explain how EIF4E3 suppresses tumours, considering the aforementioned role of EIF4E1 in promoting tumours (Osborne, Volpon et al. 2013).

Table 2 summarises briefly the effect EIF4E3 has on different kinds of tumours. For a more detailed explanation on exactly how EIF4E3 works in these tumours, please refer to the chapter titled "EIF4E3".

Table 2:

Cancer	EIF4E3	Reference
Medullablastoma	Increases viability and migration of cancer cells	1
Head and neck squamous cell carcinoma	Decreased expressiom	2
Oral squamous cell carcinoma	Deletion of gene	3
Breast cancer	Decreased expression	4
Skin cutaneous melanoma	Decreased expression	5
Melanoma	Increased expression	6
Bladder cancer	Decreased expression	7
Renal cell carcinoma	Increased expression	8,9
Androgen-independent prostate cancer	Increased expression	10
Hepatocellular carcinoma	Decreased expression	11
Colorectal cancer	Decreased expression	12
Diffuse Large B cell lymphoma	Does not participate in translation Decreased expression	13

Table 2, Legend

- *Increased expression, possible tumour-progressive properties*
- *Confirmed tumour-progressive properties*
- *Decreased expression, possible tumour-suppressive properties*
- *Confirmed tumour-suppressive properties*

References: [1](Abdelfattah, Rajamanickam et al. 2018), [2](Xu, Zhao et al. 2023), [3](Cha, Kim et al. 2011), [4](Huang, Mo et al. 2023), [5](Zhang, Miao et al. 2022), [6](Deng, Lin et al. 2022), [7](Lammert, Pannhausen et al. 2024), [8]www.proteinatlas.org, *[9](Uhlen, Zhang et al. 2017),[10](Lin, Chang et al. 2007), [11](Chen, Yao et al. 2022), [12](Huang, Zhu et al. 2022), [13](Landon, Muniandy et al. 2014)*

Discussion

EIF4E2, despite its many similarities to EIF4E1, has many unique differentiating features that allows it to play a versatile role in the cell. EIF4E2 binds the m7G cap with lower affinity than EIF4E1 (Rom, Kim et al. 1998). EIF4E2 also binds different initiation factors, binding EIF4G3 instead of EIF4G1. EIF4E2 also preferentially binds 4E-BP2 and 4E-BP3 while EIF4E1 binds 4E-BP1. (Joshi, Cameron et al. 2004)

In the presence of IFN, EIF4E2 can be ISGylated on the target sites Lys134 and Lys222. This ISGylation increases EIF4E2 cap-binding affinity, enabling it to perhaps better compete with EIF4E1 during an interferon response. EIF4E1 cannot be ISGylated, due to its lack of lysine residues in the equivalent positions. (Okumura, Zou et al. 2007) In the alignment between EIF4E1 and EIF4E2 published by Frydryskova et al. (Frydryskova, Masek et al. 2016), EIF4E1 contains Arg132 and Thr225 at the positions occupied by lysines in EIF4E2. This would indicate that in an interferon response, the cell switches from EIF4E1-mediated global translation initation to a more transcript specific EIF4E2-mediated translation initiation. Further studies would be necessary to discover how EIF4E2 ISGylation impacts the cellular translatome and thus the cellular and whole body response to the increased levels of IFN which would for example occur in the course of a viral infection.

EIF4E3, on the other hand, is even less studied than EIF4E2. Its specific function remains largely unknown. It localises to both the nucleus and cytoplasm, just like EIF4E1 does (Joshi, Cameron et al. 2004). In the cytoplasm, EIF4E3 participates in translation initiation, similar to EIF4E1 (Osborne, Volpon et al. 2013). However, the nuclear role of EIF4E3 remains unknown. EIF4E1 in the nucleus aids in the processing, steady-state capping of mRNA (Culjkovic-Kraljacic, Skrabanek et al. 2020), splicing (Ghram, Morris et al. 2023) and even export of mRNA (Topisirovic, Kentsis et al. 2005). Due to the similarities shared between EIF4E1 and EIF4E3, we can assume EIF4E3 plays a similar role in the nucleus, preferentially exporting mRNAs coding for the stress-related proteins.

EIF4E1 and EIF4E3 differ greatly in their binding to 4E-BPs. EIF4E1 activity is controlled by the phosphorylation state of 4E-BPs. These 4E-BPs are regulated by the mTOR pathway. (Beretta, Gingras et al. 1996) EIF4E3 does not bind to 4E-BPs, and is therefore not regulated by them (Joshi, Cameron et al. 2004). This suggests that EIF4E3 escapes control by the mTOR pathway and may be regulated by different proteins or not regulated at all. This could indicate that EIF4E3 might maintain some basal

level of translation that keeps the cell alive when EIF4E1-mediated translation or other kinds of translation are not running. For example, EIF4E1 might be made inactive in stress conditions, and in such situations EIF4E3 might be crucial in rescuing the cell and allowing it to survive through difficult conditions.

EIF4E2 and EIF4E3 both play important roles in tumours, whether it is through supporting or fighting their progress. They can be used as valuable prognostic markers in different cancers (Chen, Yao et al. 2022, Deng, Lin et al. 2022, Huang, Zhu et al. 2022, Huang, Mo et al. 2023). EIF4E2-mediated translation in hypoxic conditions has already been proven to promote tumours (Uniacke, Holterman et al. 2012), but the presence of the alternate $EIF4F^{H}$ complex in different cancers should be studied more and its possible usage as a target in cancer treatment should also be explored. EIF4E3 impacts many different kinds of cancers,mostly in a tumour-suppressive manner. This could tie back to the fact that EIF4E3 initiaties translation when EIF4E1 is inactive (Osborne, Volpon et al. 2013). EIF4E1 is known to preferentially translate oncogenic proteins (Koromilas, Lazaris-Karatzas et al. 1992), so the switch to EIF4E3-mediated translation could explain its tumour-suppressive function. EIF4E3 should be studied to understand exactly in what conditions does the cell switch to EIF4E3-mediated translation. This could help us better understand its exact role in cancer.

Both EIF4E2 and EIF4E3 play an important role in the brain and cognitive functions. In EIF4E2, its ability to repress mRNA expression plays a crucial role in regulating the short-term memory (Wiebe, Huang et al. 2023) and social behaviour (Wiebe, Meng et al. 2020) of mice. This could not be the only role EIF4E2 fulfills in the brain. Its localisation to stress granules (Frydryskova, Masek et al. 2016) could play an important role in human brain pathologies, as the formation of stress granules in neuronal cells can form protein aggregates which can cause the onset of many neurodegenerative diseases (For review on stress granules and neurodegenerative diseases refer to (Advani and Ivanov 2020)).

EIF4E3 seems to play an important role in the neuronal tissue as well. *EIF4E3* KO mice have an abnormal walking gait (mousephenotype.org) and patients with microdeletion syndromes that impact the *EIF4E3* gene suffer from psychomotor delays and feeding difficulties (Thevenon, Monnier et al. 2014). It could be worth exploring what impact exactly does EIF4E3 have in the brain and whether it can be used as an avenue for the treatment of some neurodegenerative diseases.

Conclusion

EIF4E2 and EIF4E3, despite not being canonical translation initation factors, still play an important role in the human body. They have a wide range of functions that can impact many processes in the body. Their involvement in tumour cells and some brain functions highlights a possible avenue for exploring novel treatment options.

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