

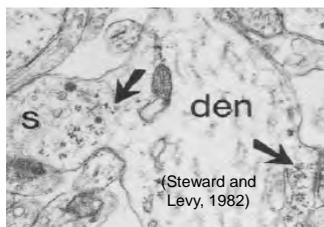
Is there a synthetic machinery for the local protein synthesis in dendrites and axons?

Protein synthesis machinery in dendrites

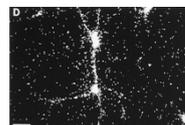
- **Ribosomes**
Steward and Levy, 1982;
Tiedge and Brosius, 1996



Oswald Steward

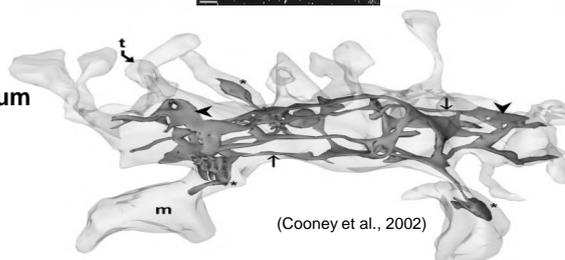


- **tRNAs, aminoacyl-tRNA synthetase, translation factors**
(Tiedge and Brosius, 1996)



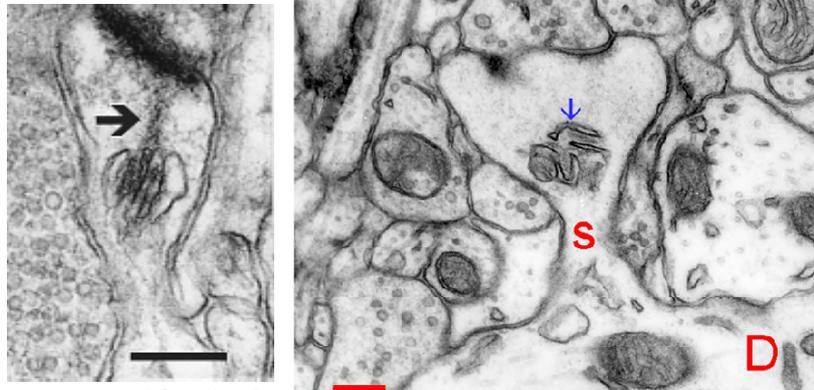
(Tiedge and Brosius, 1996)

- **Endoplasmic Reticulum**
Spacek, 1985
Pierce et al., 2000;
Cooney et al., 2002;
Aridor et al., 2004



(Cooney et al., 2002)

The spine apparatus

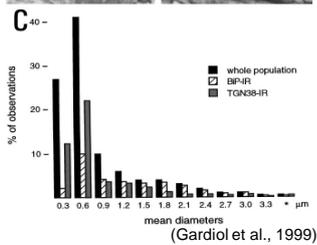
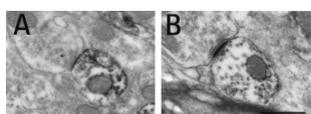


J. Spacek, 1985

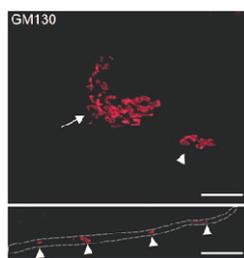


Machinery for protein synthesis: the Golgi apparatus

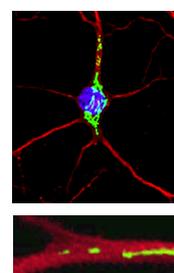
- *trans* Golgi Network in dendrites up to 0,6 μm \emptyset (Gardioli et al., 1999)
- Golgi outposts even in distal dendrites (Horton and Ehlers, 2003)
- Central Golgi cisternae along apical dendrites (Horton et al., 2005)
- Demonstration of glycosilation in dendrites (Torre e Steward, 1996)



(Gardioli et al., 1999)

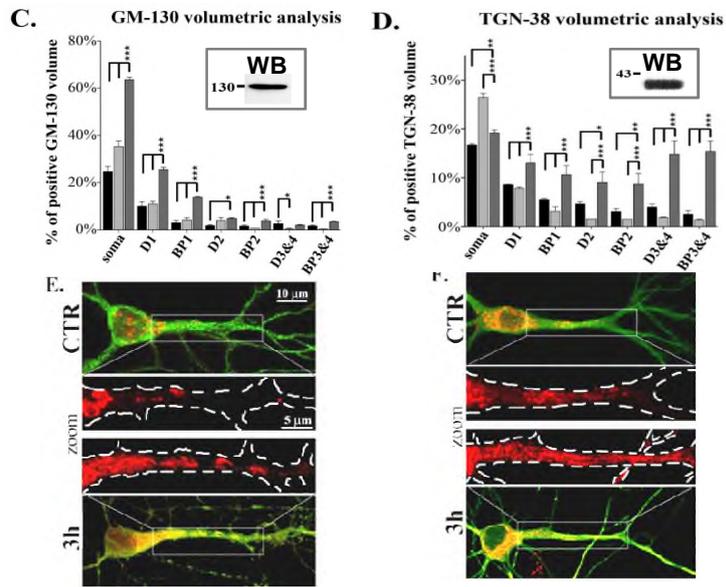


(Horton and Ehlers, 2003)

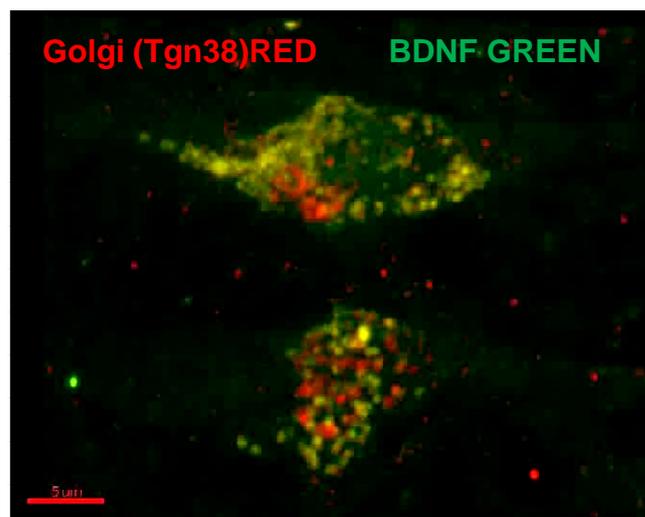


(Horton et al., 2005)

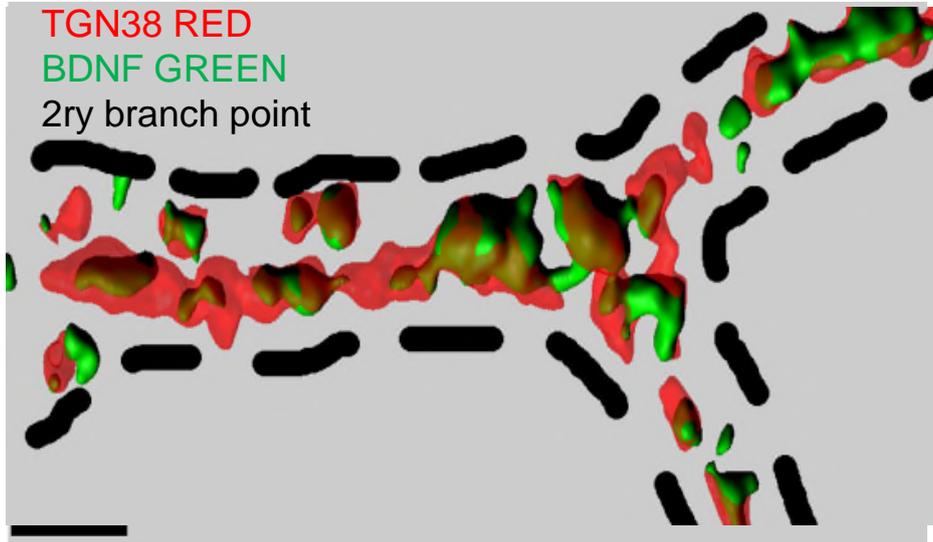
Golgi elements localization after electrical stimulation in vitro



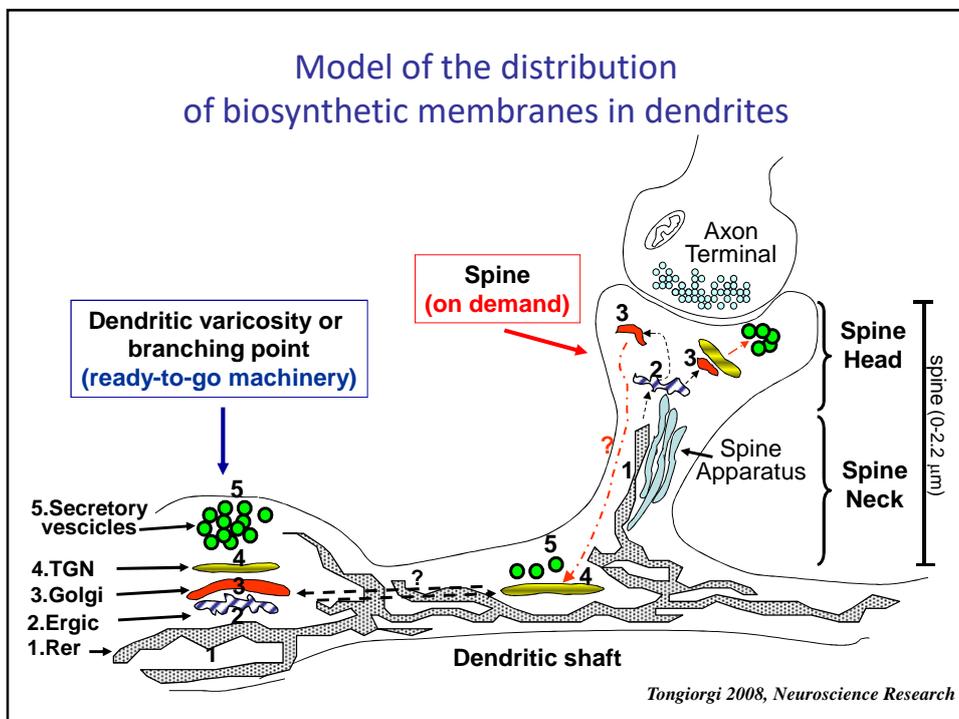
BDNF & Golgi elements co-localization after electrical stimulation in vitro



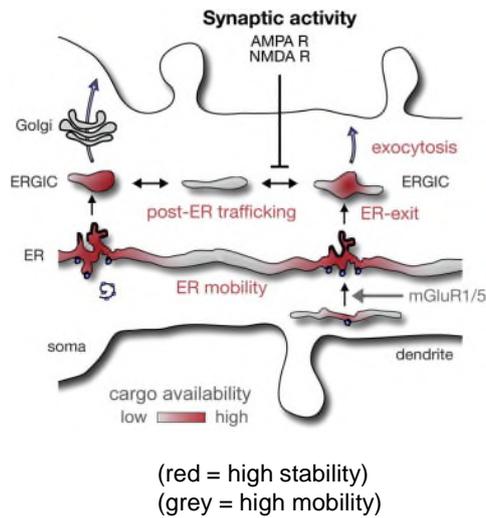
BDNF & Golgi elements co-localization after electrical stimulation in vitro



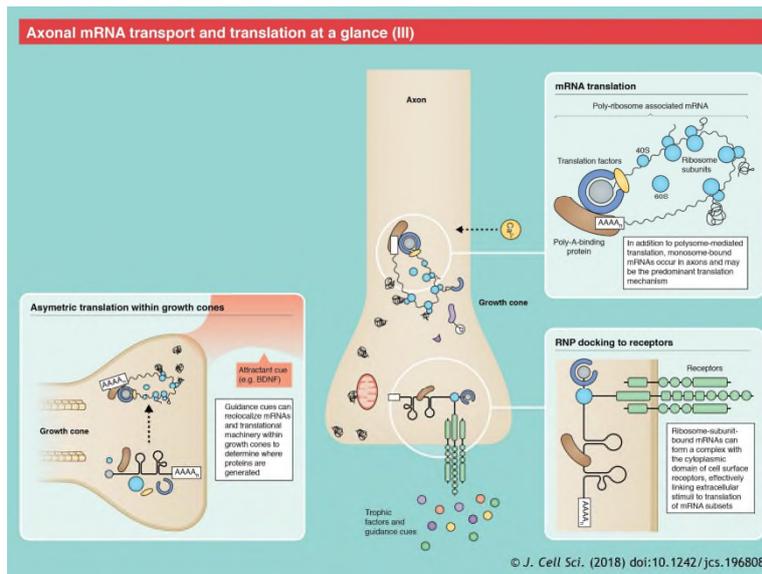
Model of the distribution of biosynthetic membranes in dendrites



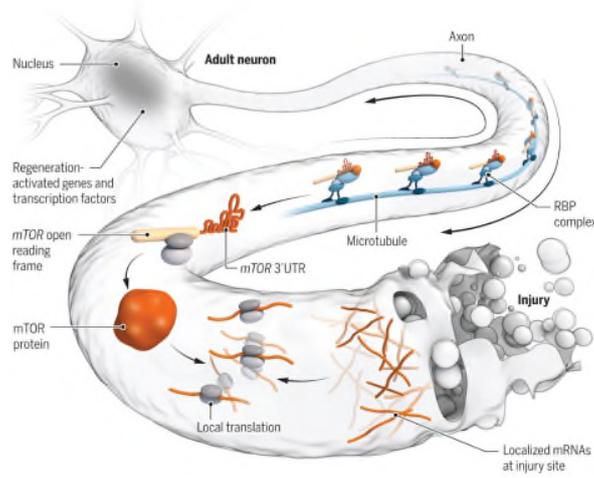
Stationary and mobile compartments of the secretory pathway in dendrites



No evidence for the presence of compartments of the secretory pathway in axons

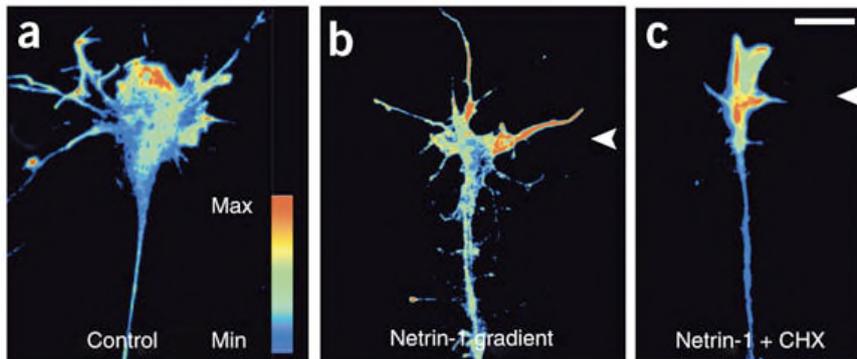


Evidence of mRNA and ribosomes in axons following an injury



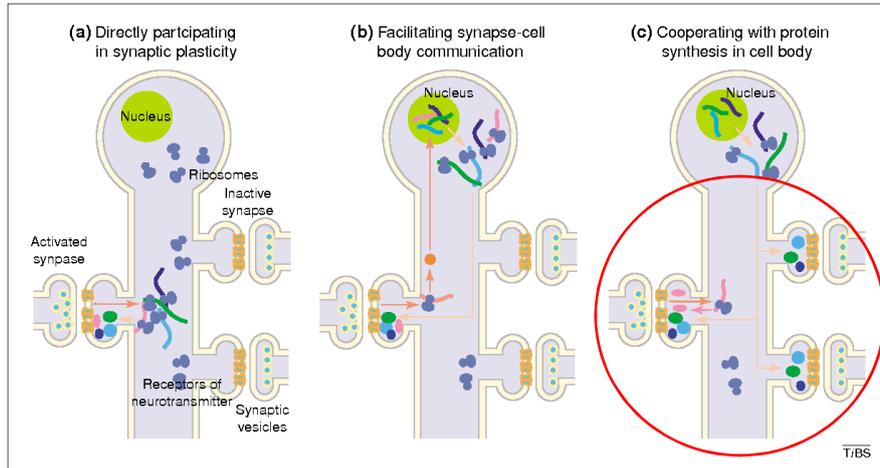
Science 23 Mar 2018
Antonella Riccio

Relocalization of beta-actin mRNA within growth cones: evidence of mRNA, ribosomes and translation factors

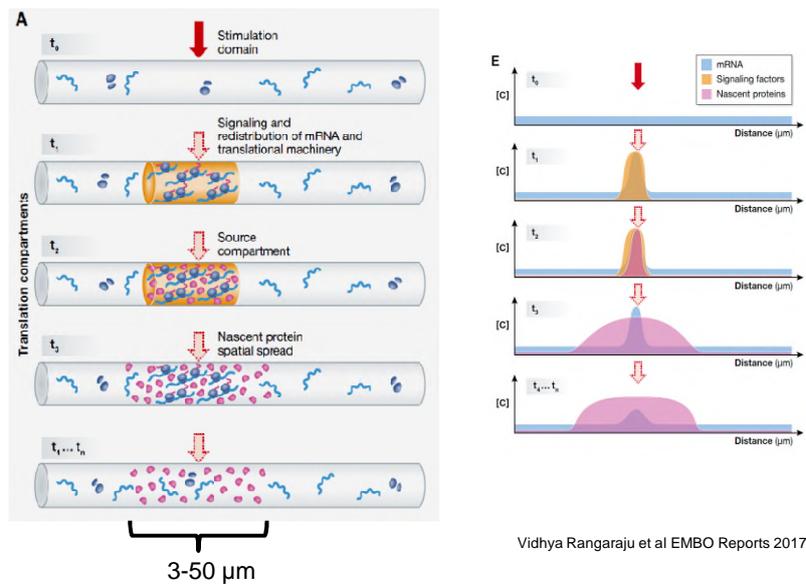


Leung et al. (2006) Nat Neuroscience

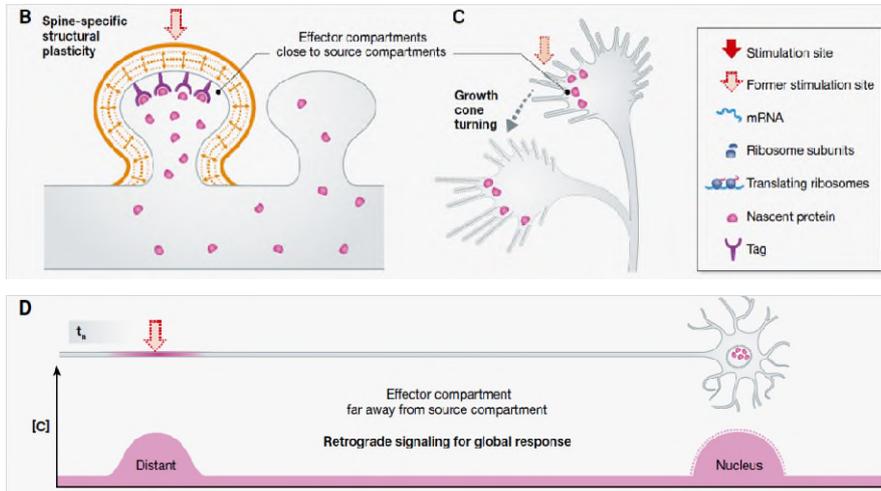
Local translation of dendritic mRNAs supports plasticity at (individual?) synapses



How local is local translation of dendritic and axonal mRNAs?

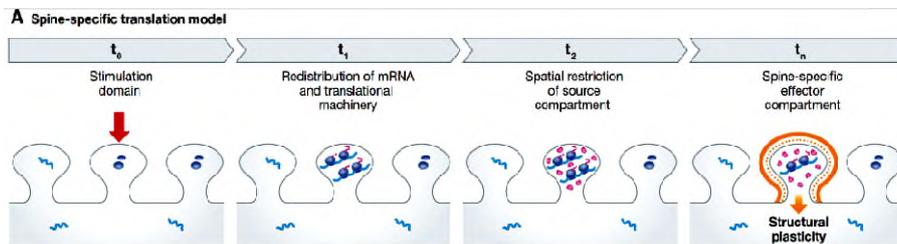


Relationship between Source and Effector compartments

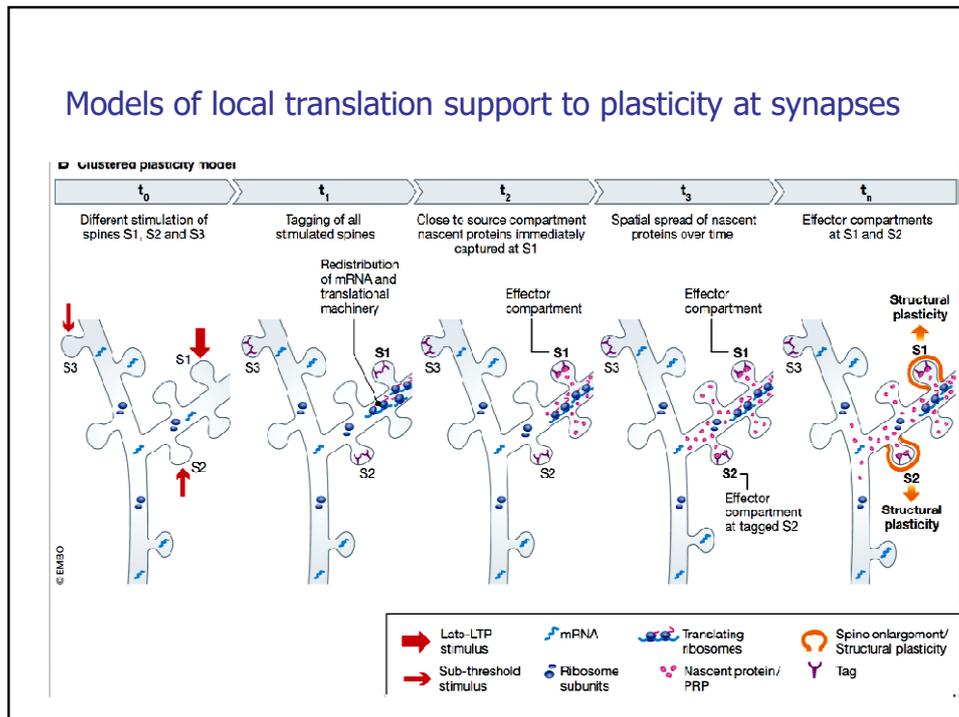


Vidhya Rangaraju et al EMBO Reports 2017

Models of local translation support to plasticity at synapses



Models of local translation support to plasticity at synapses



Brief recapitulation of the different phases of Translation in Eukaryotes

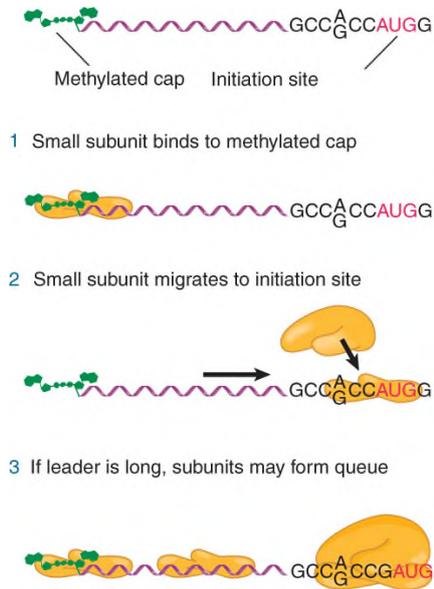
Translation is divided into two stages: initiation and elongation.

Initiation brings the mRNA to the ribosome and uses a large number of initiation factors to assemble the ribosome and begin translation.

Elongation then continues to assemble amino acids to form the protein.

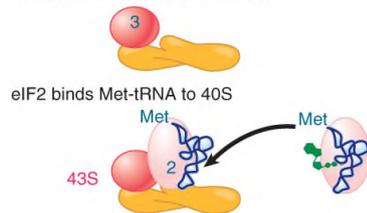
Small Subunits Scan for Initiation Sites on Eukaryotic mRNA

- Eukaryotic 40S ribosomal subunits bind to the 5' end of mRNA and scan the mRNA until they reach an initiation site.
- A eukaryotic initiation site consists of a ten-nucleotide sequence that includes an AUG codon.
- 60S ribosomal subunits join the complex at the initiation site.

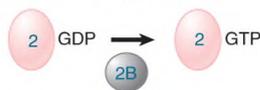


Eukaryotes Use a Complex of Many Initiation Factors

eIF3 maintains free 40S subunits



eIF2 is a GTPase
eIF2B is the exchange factor

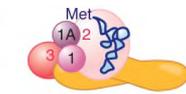


- eIF2 binds the initiator Met-tRNA_i and GTP, forming a ternary complex that binds to the 40S subunit before it associates with mRNA.
- A cap-binding complex binds to the 5' end of mRNA prior to association of the mRNA with the 40S subunit.

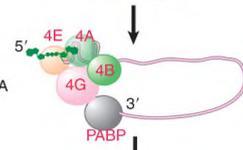
FIGURE 21: 43S complex = 40S subunit + factors +tRNA

Eukaryotes Use a Complex of Several Initiation Factors

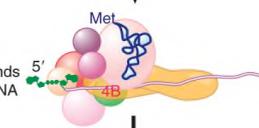
43S preinitiation complex eIF2, eIF3, Met-tRNA_i, eIF1, eIF1A



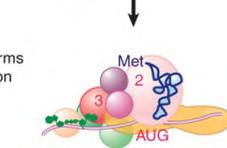
Cap-binding complex + mRNA eIF4A, B, E, G



43S complex binds to 5' end of mRNA



48S complex forms at initiation codon eIF2, EIF3 eIF1, 1A eIF4A, B, F



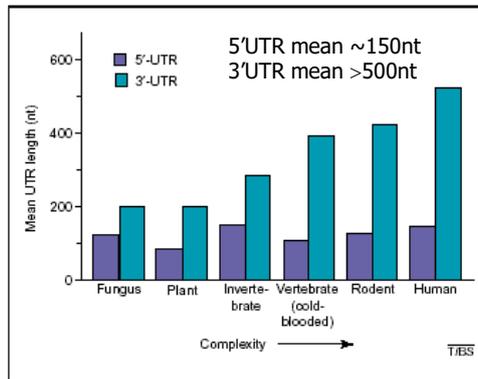
- Initiation factors are required for all stages of initiation, including binding the initiator tRNA, 40S subunit attachment to mRNA, movement along the mRNA, and joining of the 60S subunit.

- Eukaryotic initiator tRNA is a Met-tRNA that is different from the Met-tRNA used in elongation, as the methionine is not formylated.

Control mechanisms of local protein synthesis in dendrites

- 5' UTR (promoters & IRES)
- 3'UTR (rosettes formation)

The control of translation occurs both at 5' and 3'UTR



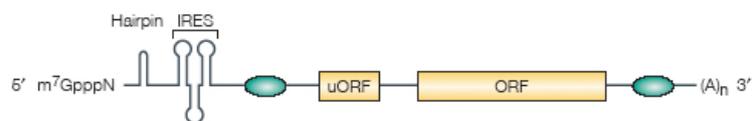
- Less rigid than 5'UTR

- Generally longer *

- Interacts with 5'UTR

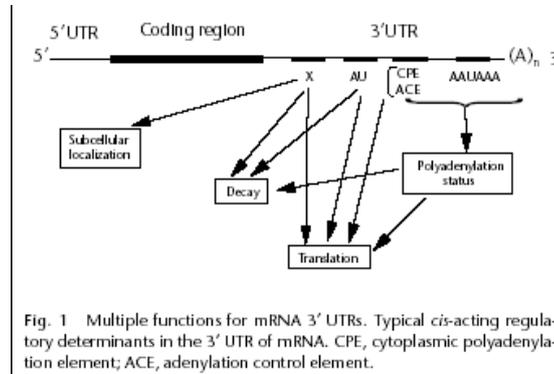
* = The 3'UTR has become longer during evolution correlating with organisms complexity while the 5'UTR length has remained constant

5'UTR elements that influence translation of mRNA



- cap structure and the polyA tails : canonical motifs
- Secondary structures close to the 5' end block translation initiation
- IRES: ribosome entry site mediates cap-independent translation, shunt mechanism...
- short ORF reduced translation of the main ORF
-  - binding sites for trans-acting regulatory factors (protein, miRNA...)

3'UTR sequences are important for mRNA translation



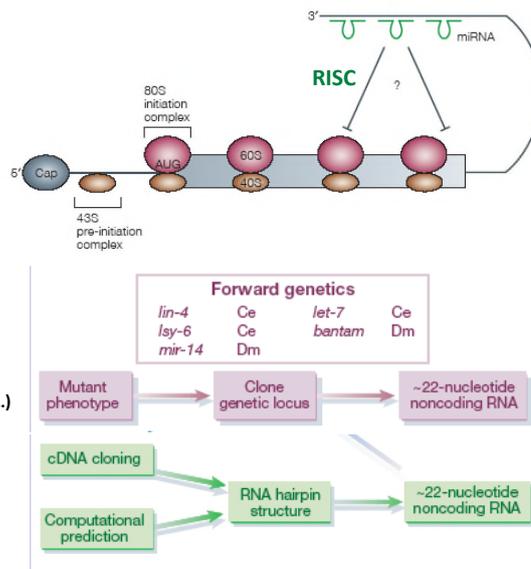
- AAUAAA exanucleotide important for polyadenilation
- cytoplasmic polyadenilation elements (CPEs) UUUUUAU
- AU-rich elements (AREs) confer rapid turn-over to mRNAs but are stabilized by binding proteins of the ELAV family (*embryonic lethal abnormal vision*) BUT may also be bound by other proteins to regulate translation (e.g. FXR1 + Ago2. Vasudevan & Steitz , Cell 2007)

Eukaryotic miRNAs regulate translation, generally, via 3'UTR

- C. elegans lin-4*
Developmental timing (lin-14, lin-28)
- C. elegans let-7*
Developmental timing (lin-14, lin-28)
- C. elegans lsy-6*
Neuronal cell fate (cog-1)
- D. melanogaster bantam*
Cell death (hid)

Ambros (2004) Nature 431:351

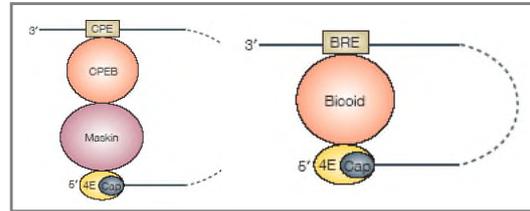
Target of miRNA? (20% of mRNA...)
(complementarity, stability, structure...)
How miRNA inhibits translation?
Identify regulatory networks...



Three 5' mechanisms of regulation of the initiation complex assembly

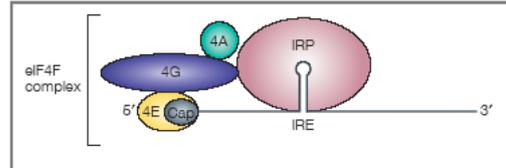
Sequestration of eIF-4E

(CPEB-Maskin : early development in xenopus
 Bicoid: anteroposterior axis in drosophila)



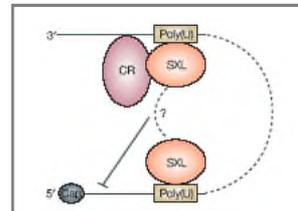
Steric blockage

(iron metabolism)



Cap-independent inhibition Interference with ribosome scanning

(SXL prevents X-chromosome dosage compensation in drosophila)

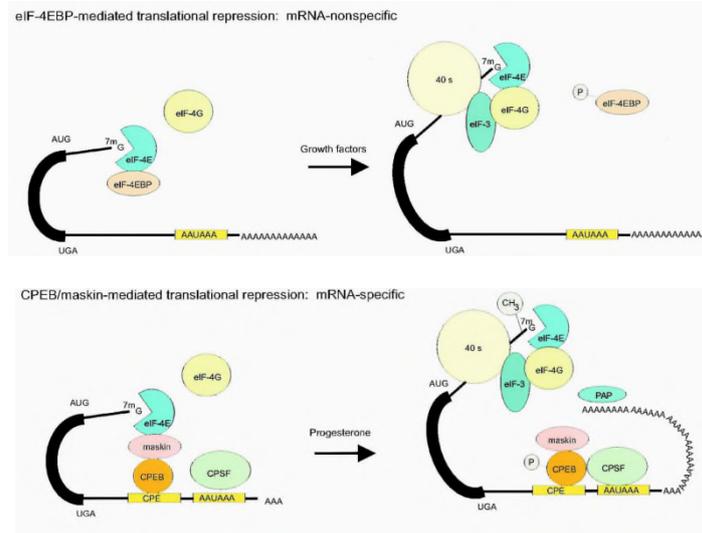


Gebauer & Hentze (2004) Nature Rev Moll Cell Biol. 5:827

Regulation of the initiation complex assembly: Sequestration of eIF-4E

1. Phosphorylation of the eIF4E binding proteins, the 4E-BPs.
2. Binding of polyadenylate binding protein (PABP) to eIF4G.
3. Phosphorylation of eIF4E allows it to detach from the cap and recycle.

Sequence specific vs. non-specific 4EBP-mediated translational repression

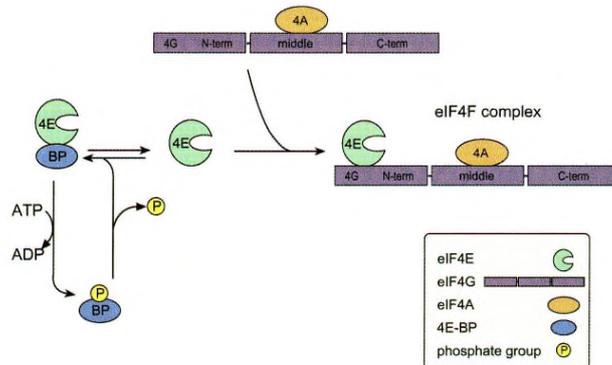


Phosphorylation of 4EBP Allows eIF4E Association with eIF4G

1. Phosphorylation of the eIF4E binding proteins, the 4E-BPs.

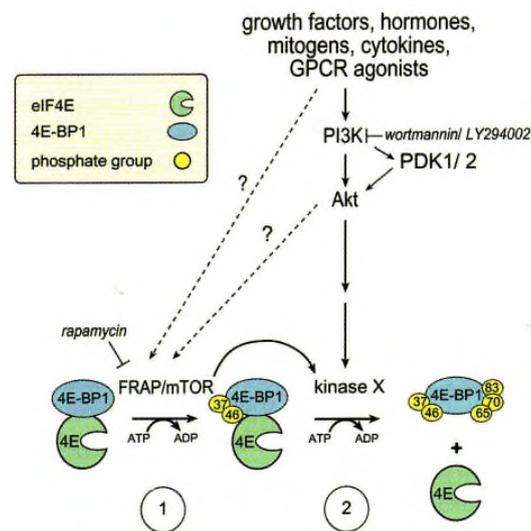
Why does this help?

Because the 4E-BPs inhibit eIF4E's function, and their phosphorylation liberates eIF4E from inhibition.



Sonenberg et al. eds Translational Control of Gene Expression (2000) p. 247

Phosphorylation of 4EBP by mTOR and Upstream Pathway



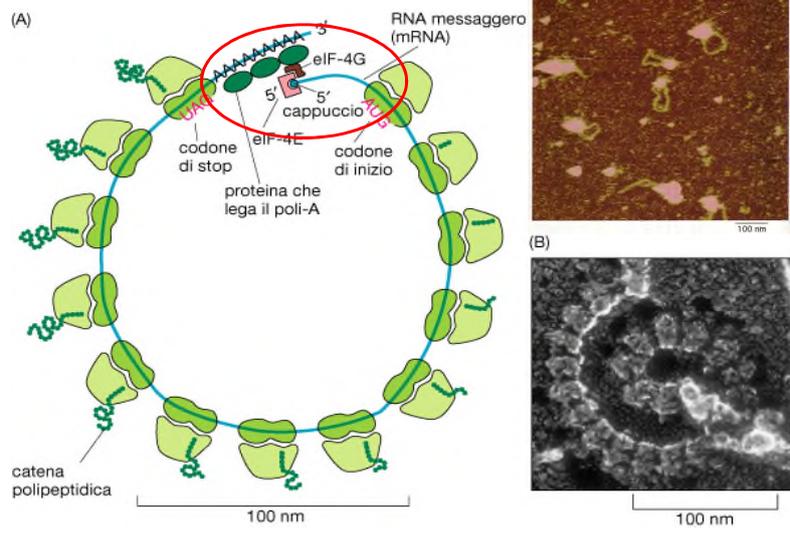
Regulation of the initiation complex assembly

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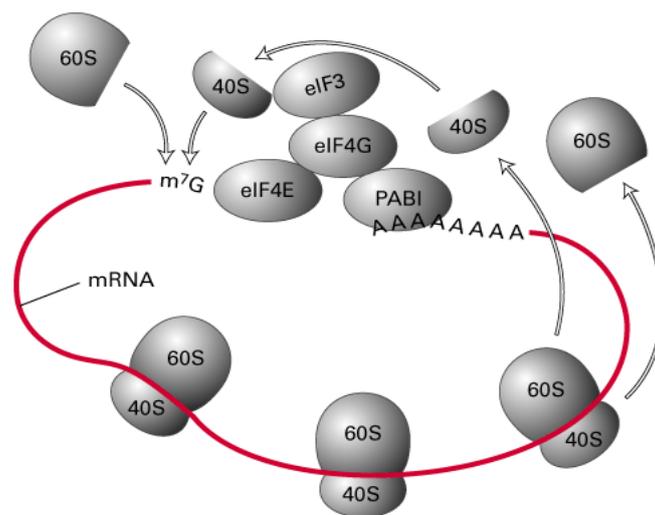
Why?

Because this circularizes the polysome, and allows ribosomal subunits to start new ribosomes.

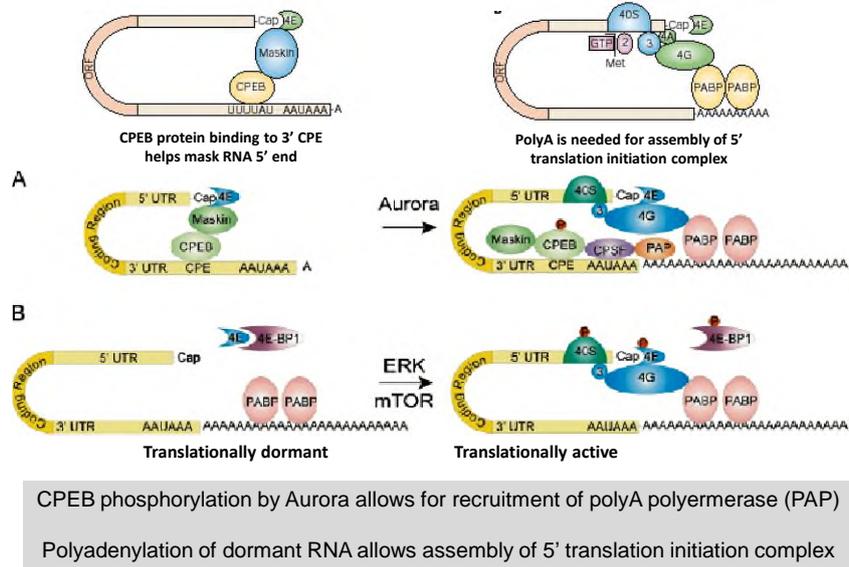
Protein synthesis on polyribosomes (rosettes)



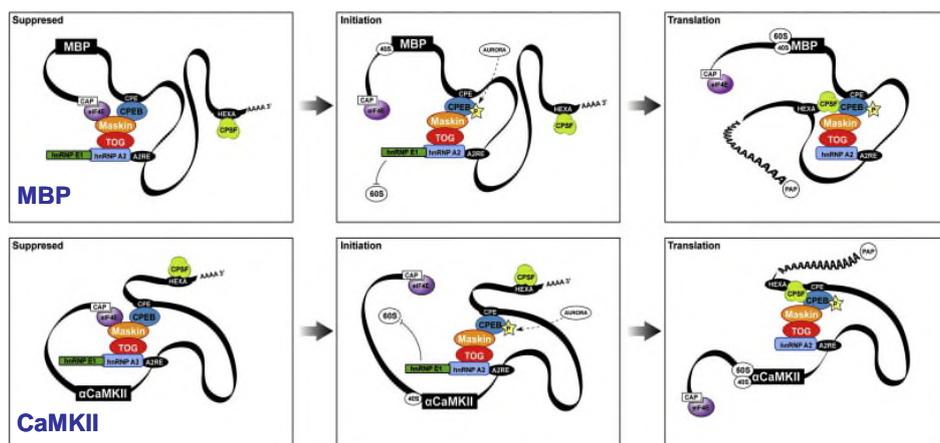
Polyadenylation and Circularization of mRNA Through Binding of PABP to eIF4G



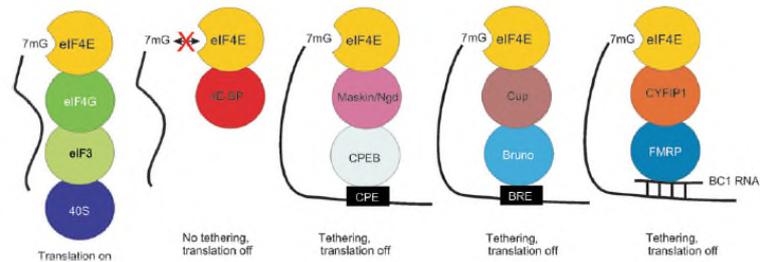
Regulation of the initiation complex assembly: Sequestration of eIF-4E coupled to 3'UTR regulation



Similar models of translational regulation for MAP2 and CaMKII



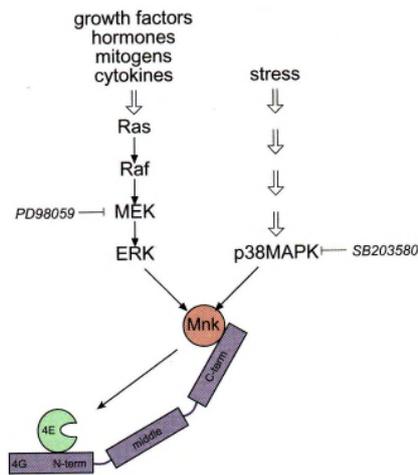
Similar sequence-specific control of translation



Regulation of the initiation complex assembly

1. Phosphorylation of the eIF4E binding proteins, the 4E-BPs.
2. Binding of polyadenylate binding protein (PABP) to eIF4G.
3. Phosphorylation of eIF4E allows it to detach from the cap and recycle.

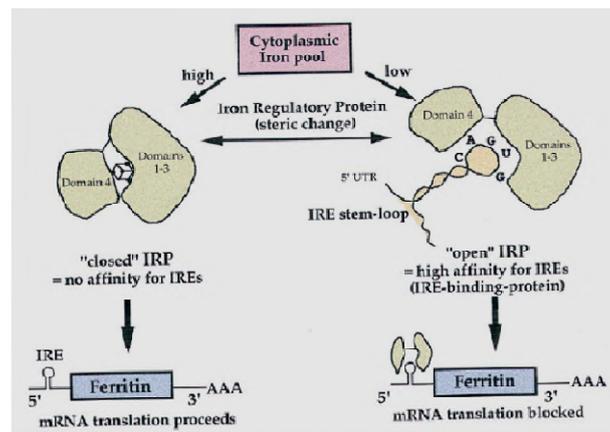
MAPK-Dependent Phosphorylation of eIF4E Is Mediated by the eIF4G Associated Kinase Mnk



Sonenberg et al. eds Translational Control of Gene Expression (2000) p. 270

Regulation of the initiation complex assembly: Steric blockage of translation (5'control)

A typical example is the regulation at the 5'UTR IRE (iron responsive element) of ferritin mRNA.



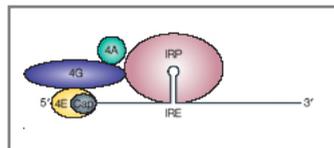
Hereditary hyperferritinemia-Cataract Syndrome (HHCS) is associated with at least 4 mutations on ferritin's IRE (Girelli et al, Blood (1997), 5: 2084-88)

Other examples of Steric blockage of translation

One regulatory protein targets functionally related mRNAs

(Keene 2003 Mol Cell 12: 1347)

Iron regulatory protein

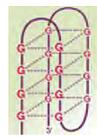
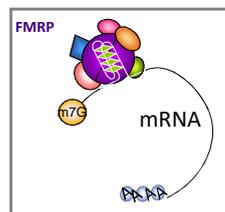


IRP and iron metabolism

Ferritin
Transferrin receptor
e-ALAS
Mitochondrial aconitase...

Dandekar & Hentze (1995 TIG 11:45)

Fragile X-syndrome Protein (associated in large mRNPs)



G quartet

FMRP and neuronal function (actin cytoskeleton remodeling...)

FMR1*
PP2Ac (Rac1 pathway)**
MAP1b
FGF-2***?...

*Schaeffer et al. 2001 EMBO J 20:4803

**Castets et al. 2005 Hum Mol Gen. 14:835

***Bonnalet et al. 2003 JBC 278:39330

Darnell et al. 2001 Cell 107:489; 2005 Genes Dev

Alternative 5'UTR control of translation: the IRES mechanism

- **IRES (internal ribosome entry site)**
– A eukaryotic messenger RNA sequence that allows a ribosome to initiate polypeptide translation without migrating from the 5' end.

IRES-Directed Translation in dendrites

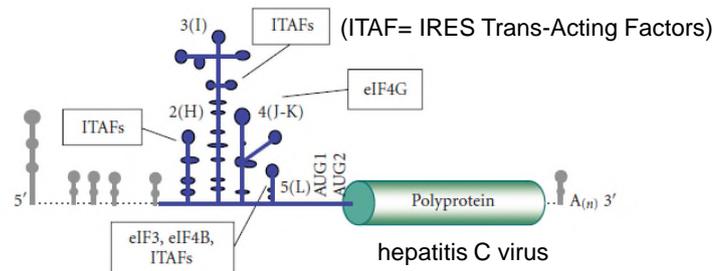


Table 2. Dendritic mRNAs Containing IRESs

mRNA	Protein Function	Reference(s)
Activity-regulated cytoskeletal protein (Arc)	Actin-binding synaptic junctional protein	Pinkstaff et al. 2001
α CaMKII	Multifunctional kinase, calcium signaling	" "
Dendrin	Unknown	" "
Microtubule-associated protein 2 (MAP2)	Microtubule assembly and scaffolding protein	" "
Neurogranin (RC3)	Calcium/calmodulin-binding protein	" "

BDNF & TrkB

Genes having IRES-Directed Translation

Table 3. Internal ribosome entry sites in cellular mRNAs

Gene product	Reference	Translation factors	
Growth factors		Eukaryotic initiation factor 4G (eIF4G) ^a	Gan and Rhoads 1996
Fibroblast growth factor 2 (FGF2)	Vagner et al. 1995a	Eukaryotic initiation factor 4G1 (eIF4G1) ^a	Johannes and Sarnow 1998
Platelet-derived growth factor B (PDGF/ <i>c-sis</i>)	Bernstein et al. 1997	Death-associated protein 5 (DAP5)	Henis-Korenblit et al. 2000
Vascular endothelial growth factor (VEGF)	Akiri et al. 1998; Stein et al. 1998; Huez et al. 1998	Activators of apoptosis	
Cyr61	Johannes et al. 1999	Apoptotic protease activating factor Apaf-1	Coldwell et al. 2000
Transcription factors		Dendritically localized proteins	
Antennapedia	Oh et al. 1992; Ye et al. 1997	Activity-regulated cytoskeletal protein (ARC)	Pinkstaff et al. 2001
Ultrabithorax	Ye et al. 1997	α subunit of calcium calmodulin-dependent kinase II dendrin	Pinkstaff et al. 2001
MYT2	Kim et al. 1998	Microtubule-associated protein 2 (MAP2)	Pinkstaff et al. 2001
NF- κ B repressing factor NRF	Oumard et al. 2000	neurogranin (RC3)	
AML1/RUNX1	Pozner et al. 2000	Others	
Gtx homeodomain protein	Chappell et al. 2000a	Immunoglobulin heavy chain binding protein (BiP)	Macejak and Sarnow 1991
Oncogenes		La autoantigen	Carter and Sarnow 2000
c-myc	Nabru et al. 1997; Stoneley et al. 1998	β subunit of mitochondrial H ⁺ -ATP synthase	Izquierdo et al. 2000
Pim-1	Johannes et al. 1999	Ornithine decarboxylase	Pyronnet et al. 2000
Protein kinase p58 ^{PITSLRE}	Cornelis et al. 2000		
Transporters/receptors			
Cationic amino acid transporter Cat-1	Fernandez et al. 2001		
Nuclear form of Notch 2	Lauring and Overbaugh 2000		

5'UTR control of translation: Translation of mRNAs containing 5'TOP sequences (terminal oligo-pyridine tract = 4-14 pyridine)

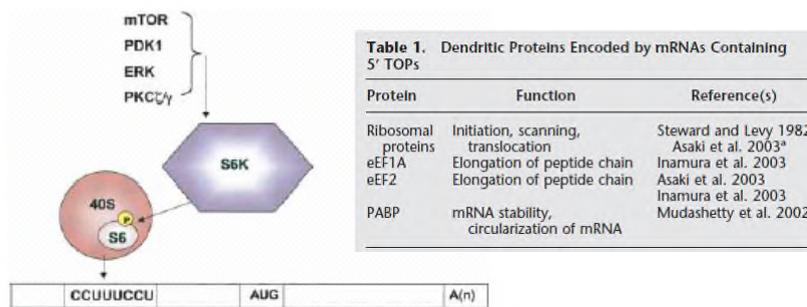
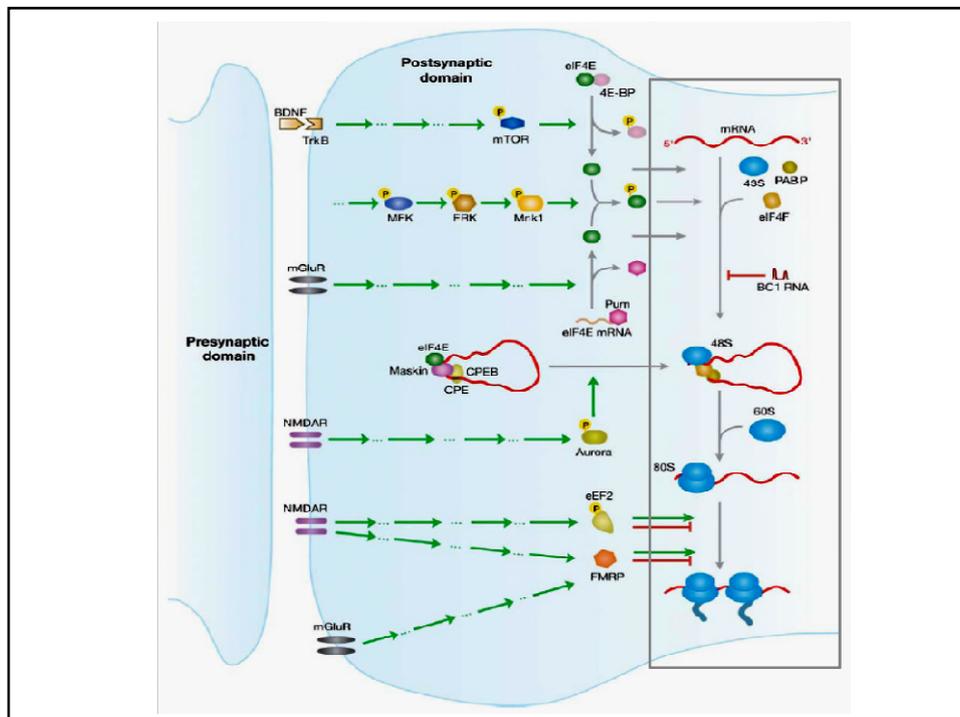
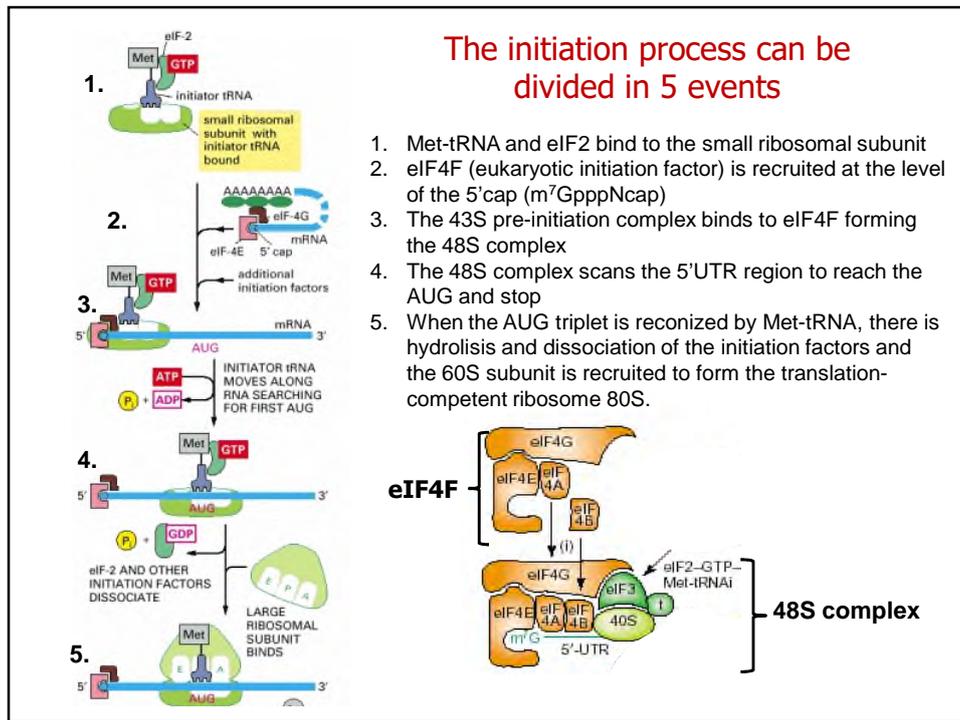
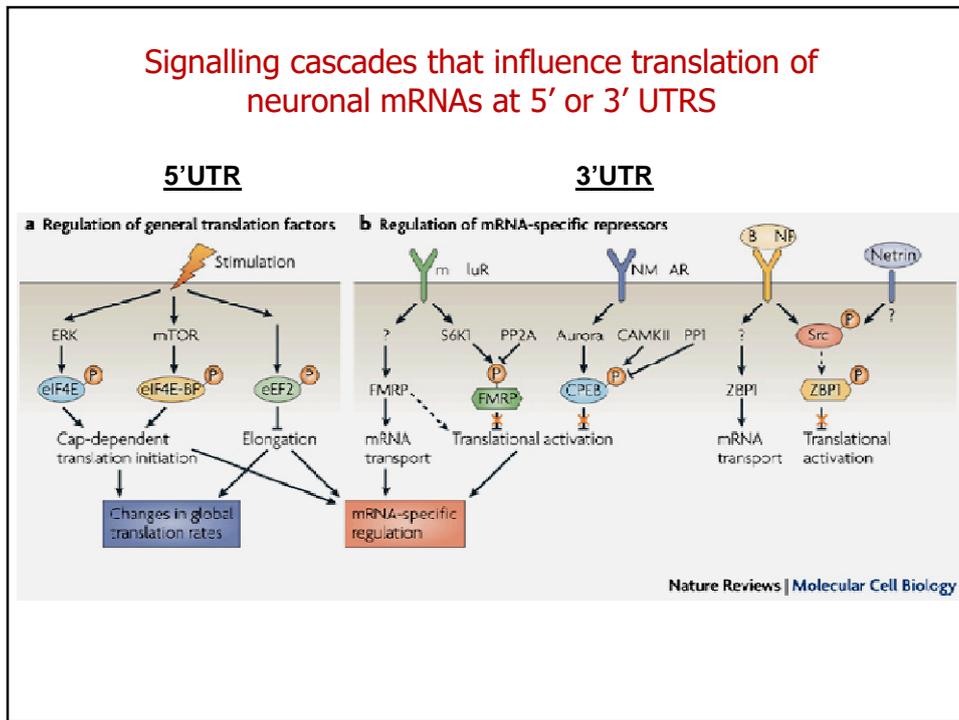


Figure 3 S6-directed translation. S6 kinase (S6K1) is activated by multiple kinases, including PDK-1, ERK, PKC ζ/γ , and mTOR. Activated S6K1 phosphorylates ribosomal protein S6, whose phosphorylation is correlated with the selective translation of mRNAs that contain 5'TOPs in their 5'UTR.

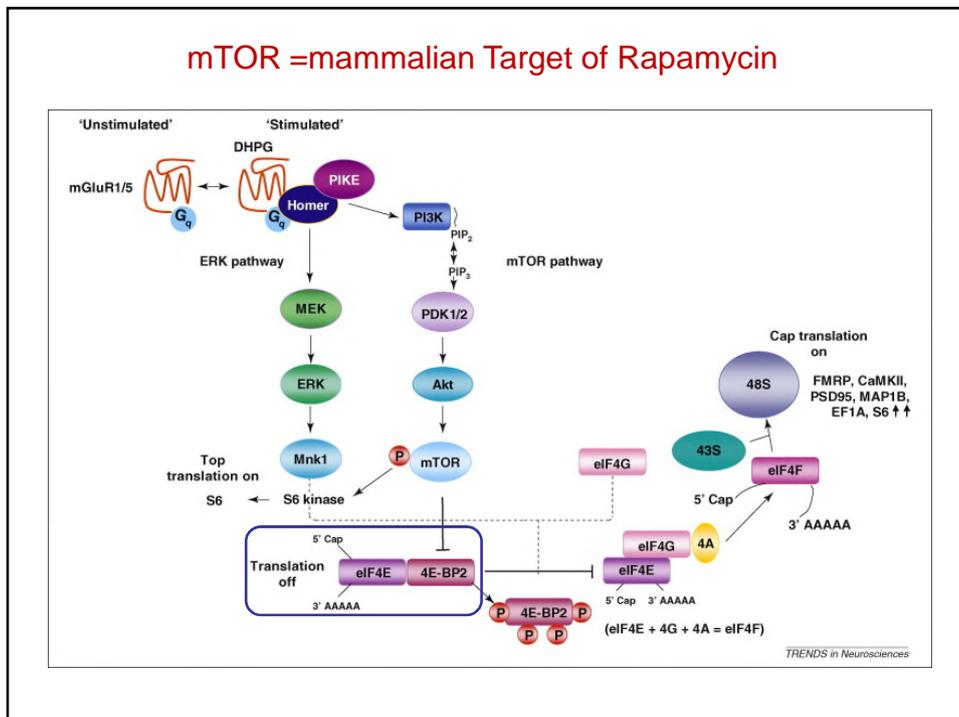
- **What intracellular signals regulate translation in dendrites ?**

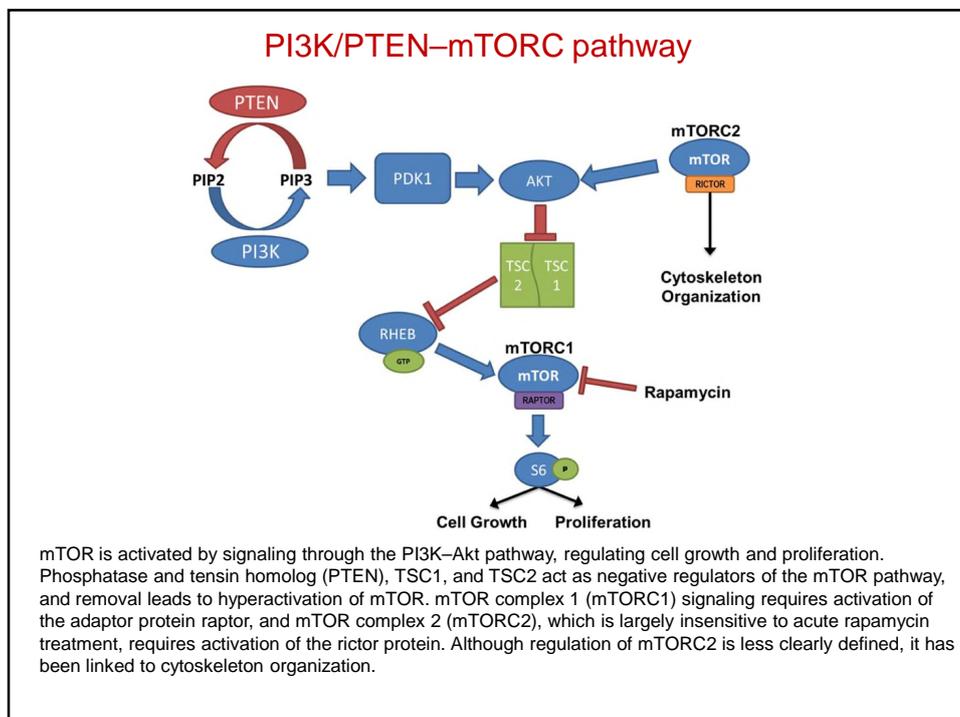
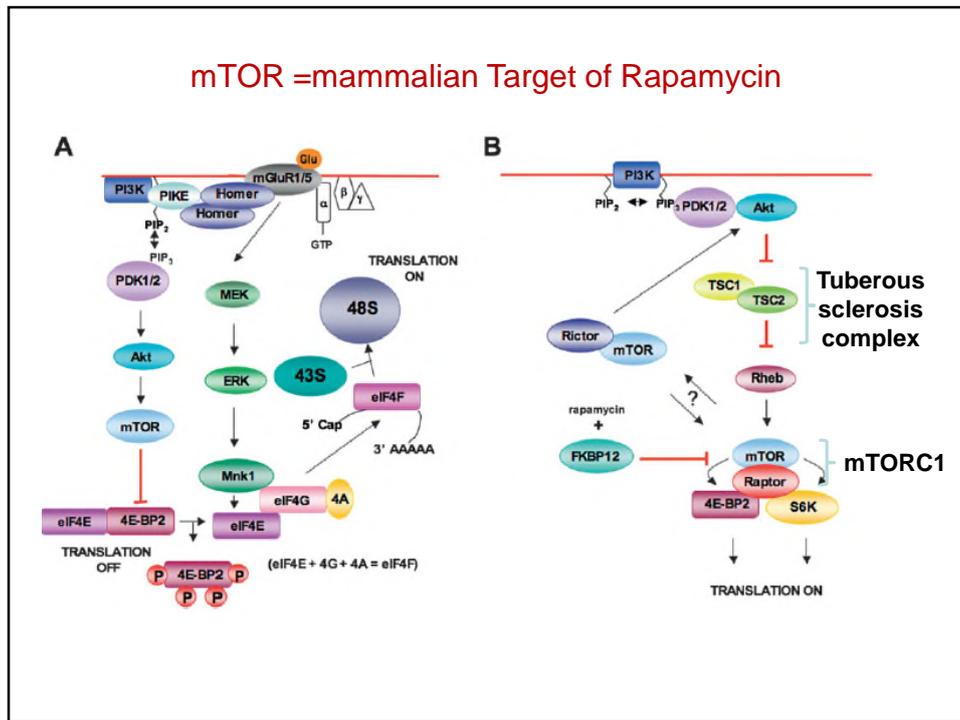


Signalling cascades that influence translation of neuronal mRNAs at 5' or 3' UTRS

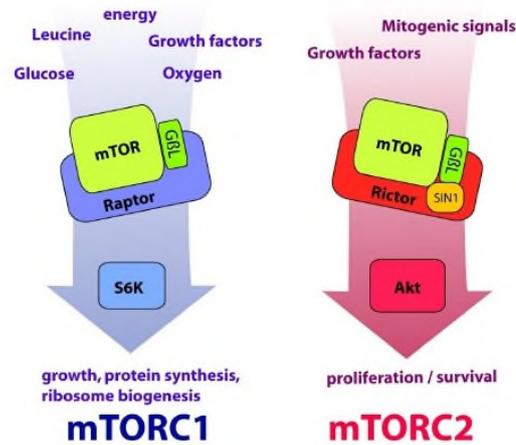


mTOR =mammalian Target of Rapamycin

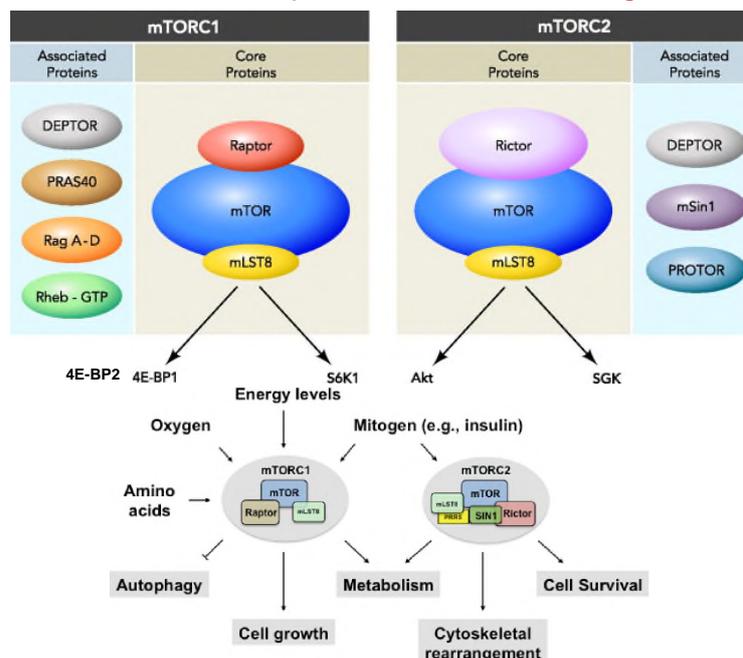




mTOR functions in two distinct complexes and pathways

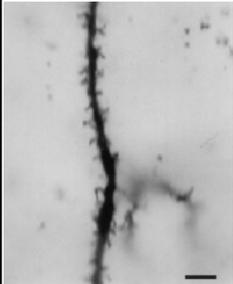


mTOR forms two complexes with different targets

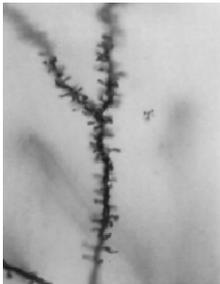


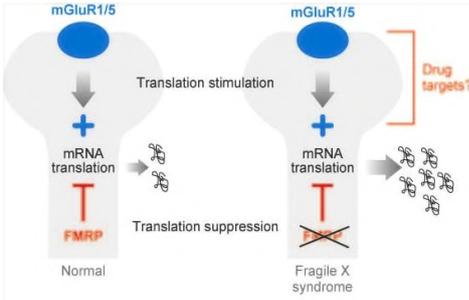
The Fragile X Mental Retardation Protein (FMRP) in dendritic RNA transport/translation

Wild type



FMRP^{-/-}





Comery et al. (1997) PNAS
Penagarikano et al. (2009) Ann Rev Genomics & Hum Gen

FMRP & RNA binding

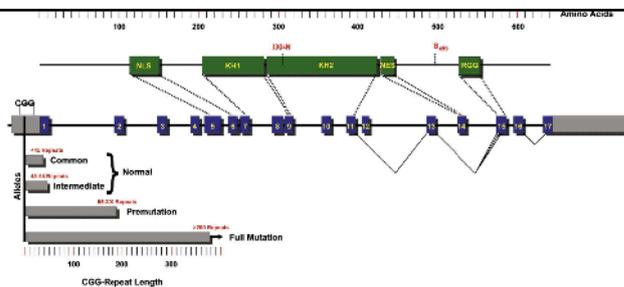
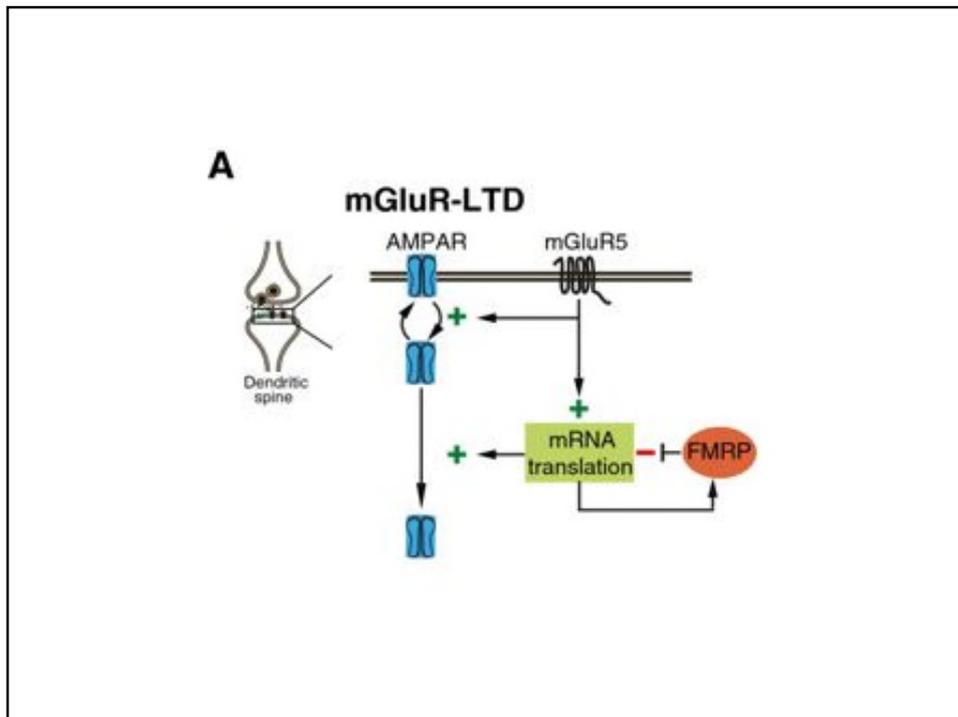


Table 1. Validated FMRP Target mRNAs

mRNA	Dendritic Localization	G-Quartet-like Structure	mGluR Stimulated	Method	References
<i>Atp</i>		+	+	Co-IP	Westmark and Maiter, 2007
<i>Arc</i>	+		+	Co-IP	Steward and Worley, 2001; Wuang et al., 2008; Park et al., 2008
<i>Camk1α</i>	+		+	Co-IP	Bramham and Wells, 2007; Muddasheyy et al., 2007; Hou et al., 2006; Zaifa et al., 2003
<i>eEF1A</i>	+		+	Co-IP; in vitro	Huang et al., 2005; Sung et al., 2003
<i>Fmr1</i>	+	+	+	in vitro	Weiler et al., 1997; Antar et al., 2004; Schaeffer et al., 2001
<i>GluR1/2</i>	+		+	Co-IP	Muddasheyy et al., 2007
<i>Map7b</i>	+	+	+	Co-IP; in vitro; biophysical	Brown et al., 2001; Damell et al., 2001; Antar et al., 2005; Davidkova and Carroll, 2007; Hou et al., 2006; Menon et al., 2008
<i>Psd95</i>	+	+	+	Co-IP; in vitro; CLIP	Todd et al., 2003; Zaifa et al., 2007; Muddasheyy et al., 2007
<i>Sapap3/4</i>	+		+	Co-IP	Brown et al., 2001; Kindler et al., 2004; Narayanan et al., 2007; Dichtenberg et al., 2006
<i>Sern3F</i>		+		Co-IP; biophysical	Damell et al., 2001; Menon and Mihalescu, 2007
<i>Rgs5</i>	+		+	APRA; in vitro	Miyashiro et al., 2003; Dichtenberg et al., 2008
<i>Gaba-Aδ</i>	+		+	APRA; in vitro	Miyashiro et al., 2003; Dichtenberg et al., 2008

Bassell & Warren (2008) Neuron



Pathogenesis at 5' and 3'UTRs

Mutations at 5'UTR or 3'UTR affects the mechanism of translation and cause human diseases (**in red** those affecting the nervous system)

5'UTR

- Hereditary hyperferritinemia-cataract syndrome (HHCS) (Girelli et al, 1997, Blood, 5: 2084-88)
- **Charcot-Marie-Tooth disease**
- myeloma (c-myc)

3'UTR

- **Myotonic Dystrophy (MD)**
- **Neuroblastoma**
- Muscular congenital Dystrophy "Fukuyama-type" (FCMD)
- **alfa-thalassemia**
- Human acute myelogenous leukemia (AML)