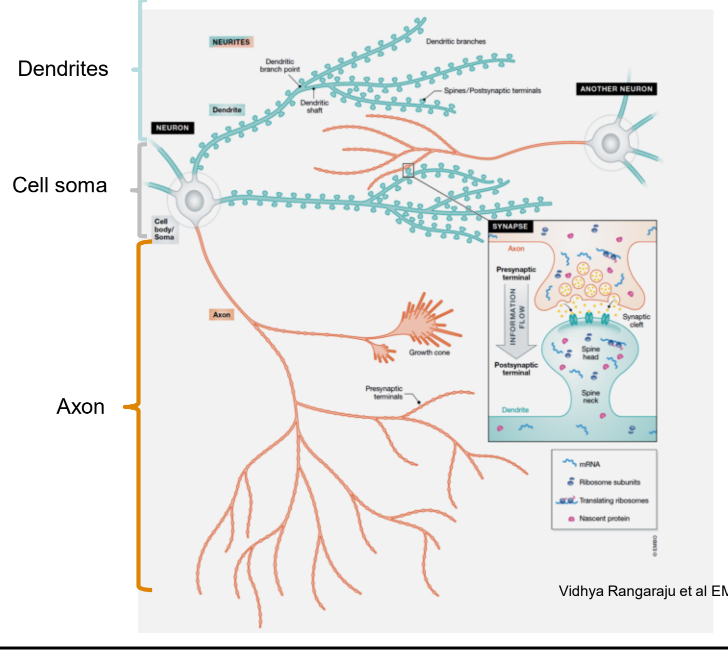
Lesson 13

Regulation of local translation in axons and dendrites

Neuronal local translation compartments



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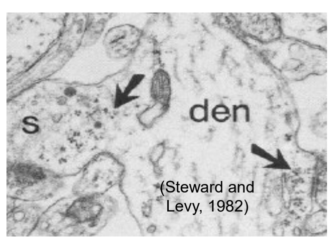
Is there a synthetic machinery for the local protein synthesis in dendrites and axons?

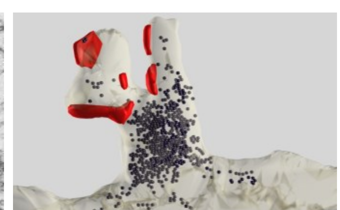
Protein synthesis machinery in dendrites

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

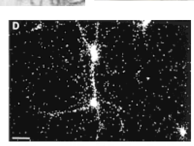
Oswald Steward



(Steward and Levy, 1982)

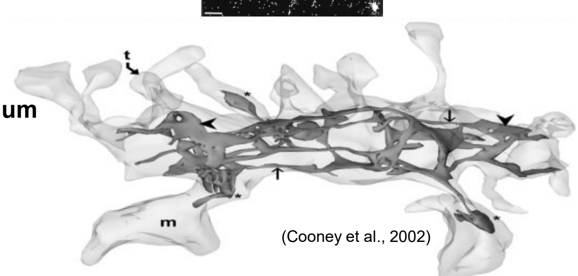

K. Harris

• **tRNAs, aminoacil-tRNA sinthetase, translation factors**
(Tiedge and Brosius, 1996)



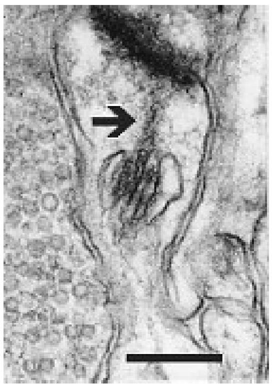
(Tiedge and Brosius, 1996)

•**Endoplasmatic Reticulum**
Spacek, 1985
Pierce et al., 2000;
Cooney et al., 2002;
Aridor et al., 2004

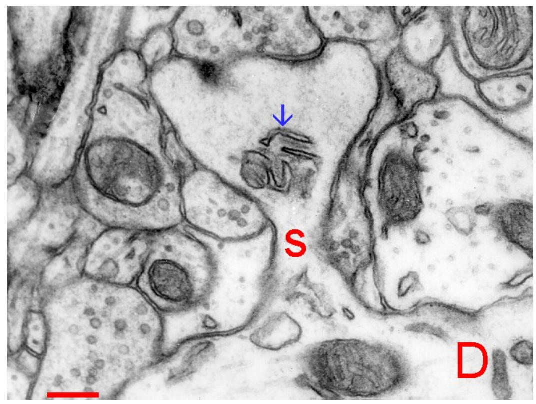


(Cooney et al., 2002)

The spine apparatus

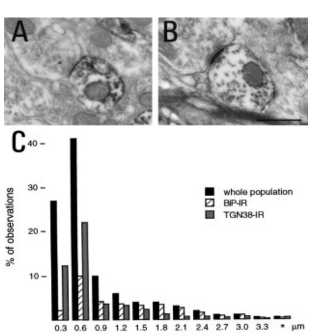


J. Spacek, 1985

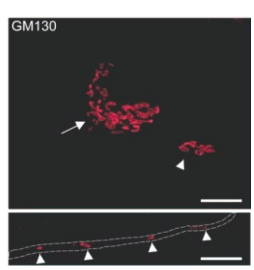


Machinery for protein syntesis: the Golgi apparatus

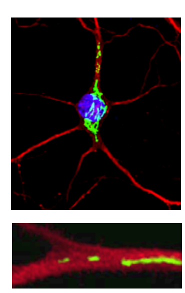
- *trans* **Golgi Network in dendrites up to 0,6 µm Ø(Gardiol et al., 1999)**
- **Golgi outposts even in distal dendrites (Horton and Ehlers, 2003)**
- **Central Golgi cystermae along apical dendrites (Horton et al., 2005)**
- **Demostration of glycosilation in dendrites (Torre e Steward, 1996)**



(Gardiol et al., 1999)

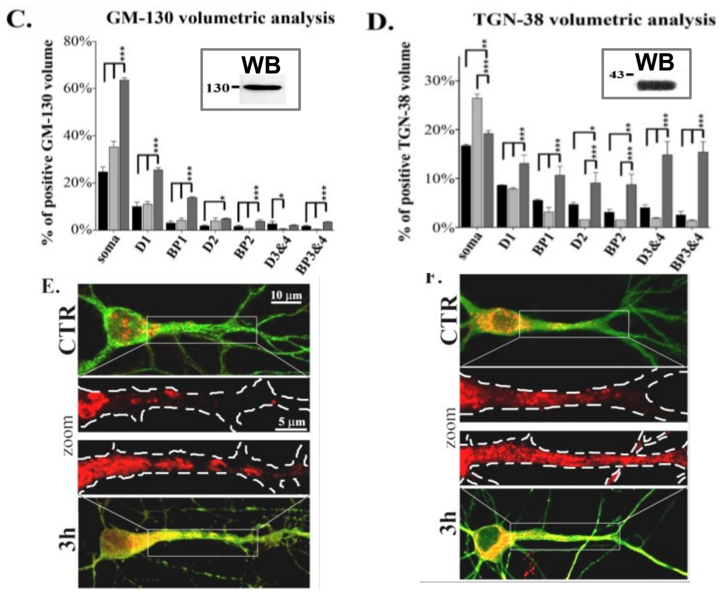


(Horton and Ehlers, 2003)

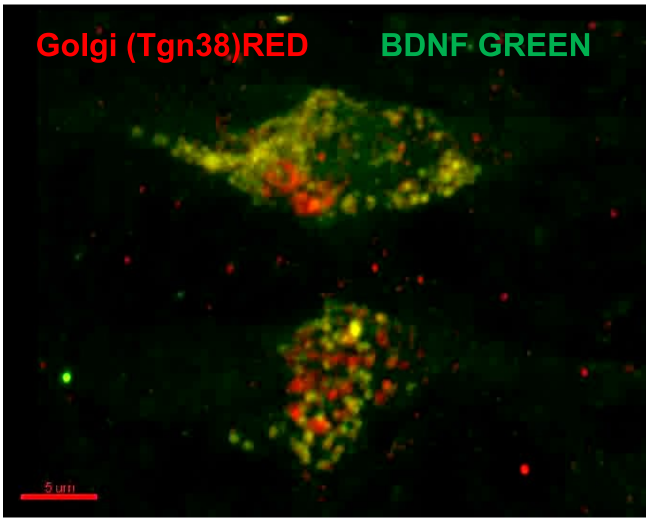


(Horton et al., 2005)

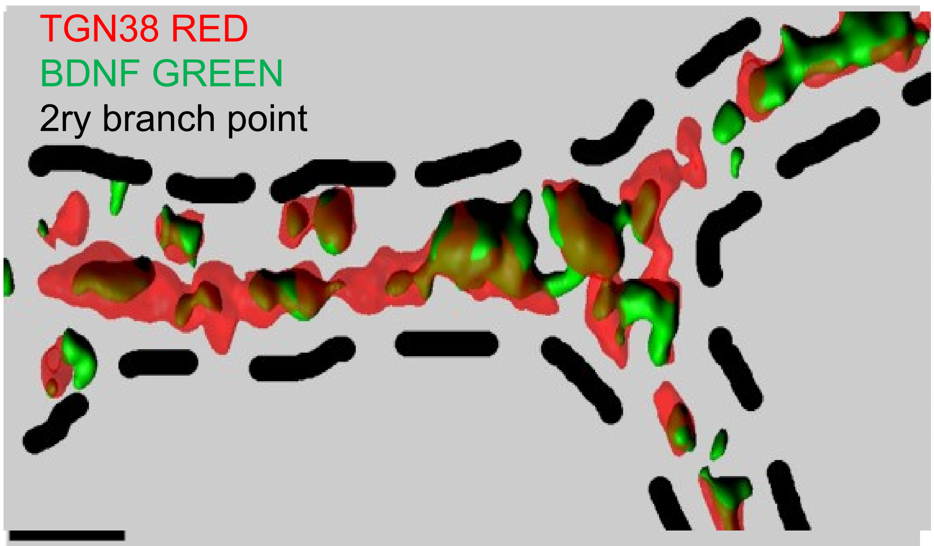
Golgi elements localization after electrical stimulation in vitro



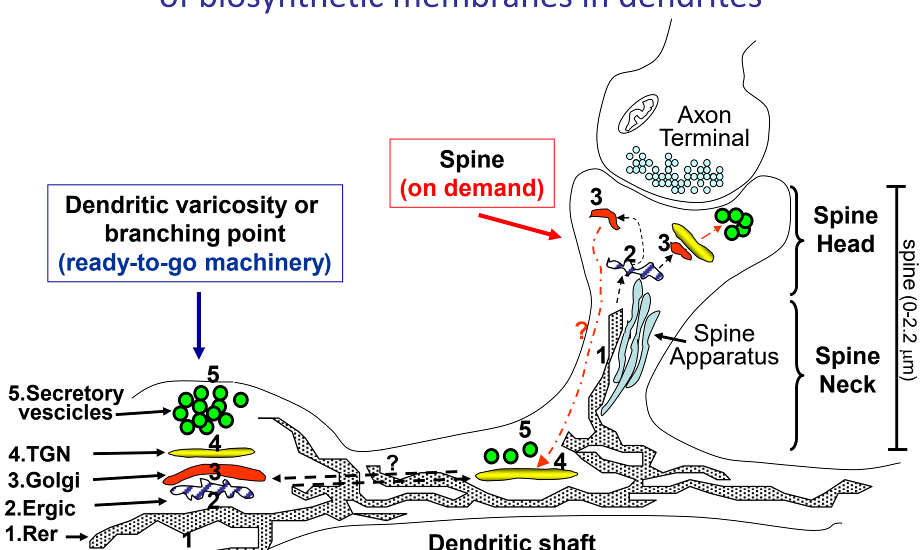
BDNF & Golgi elements co-localization after electrical stimulation in vitro



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

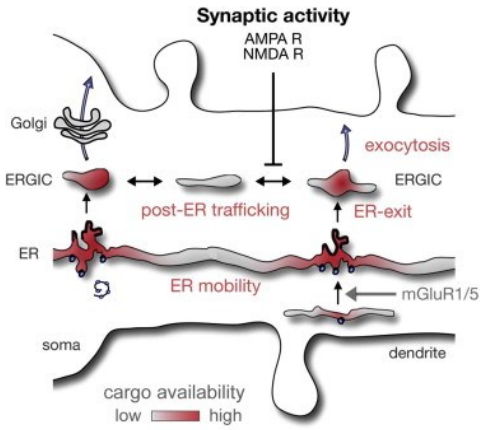


Model of the distribution of biosynthetic membranes in dendrites



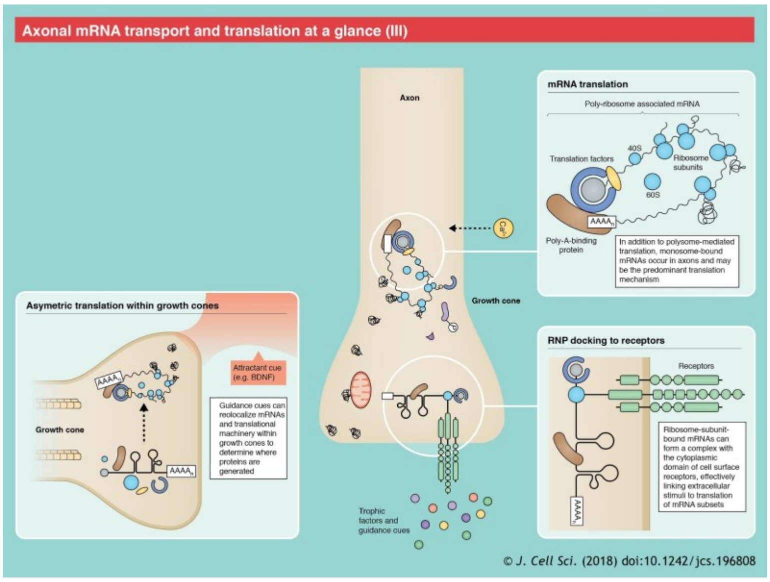
Tongiorgi 2008, Neuroscience Research

Stationary and mobile compartments of the secretory pathway in dendrites

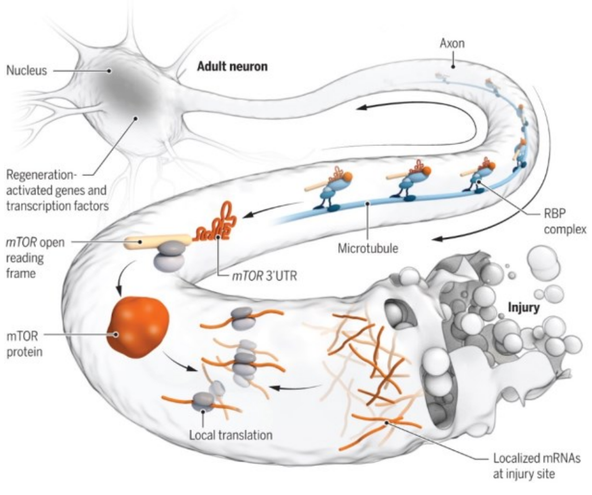


(red = high stability)
(grey = high mobility)

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

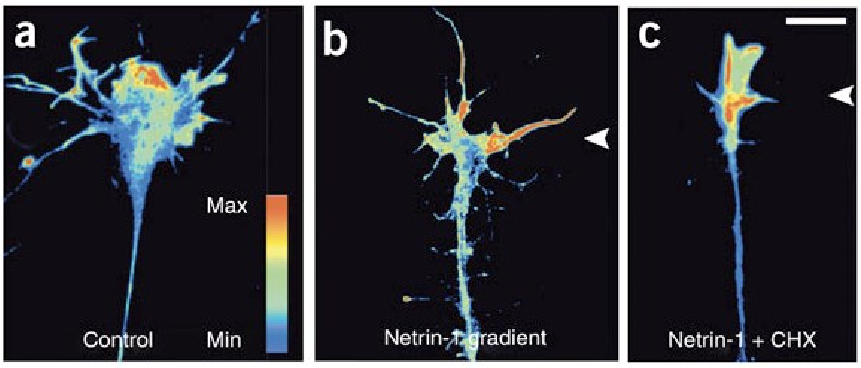


Evidence of mRNA and ribosomes in axons following an injury



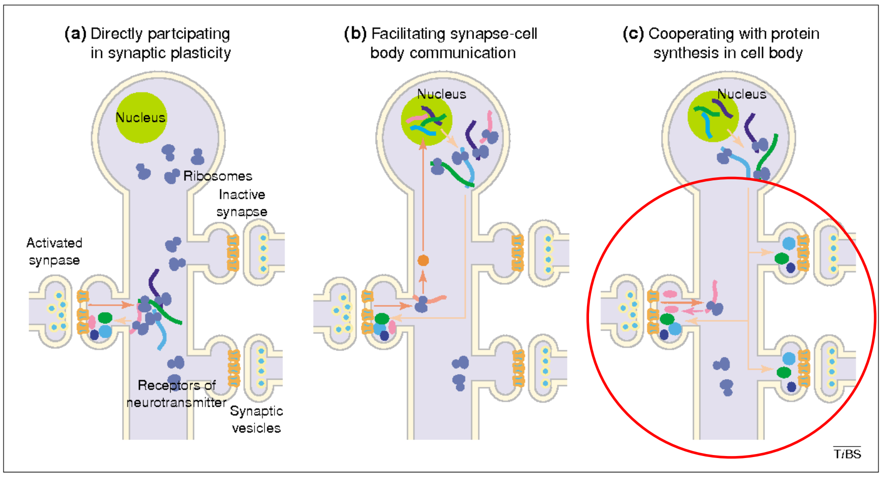
Science 23 Mar 2018
Antonella Riccio

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

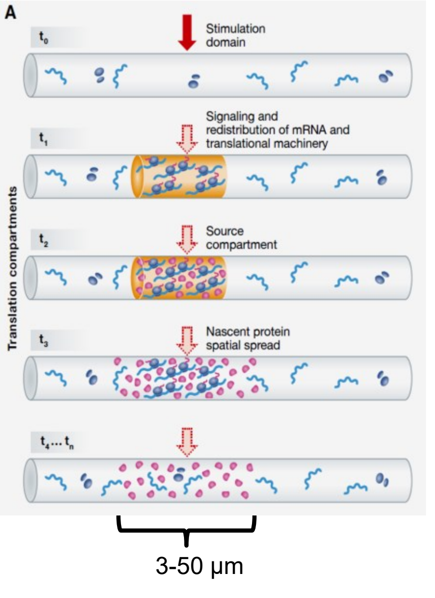


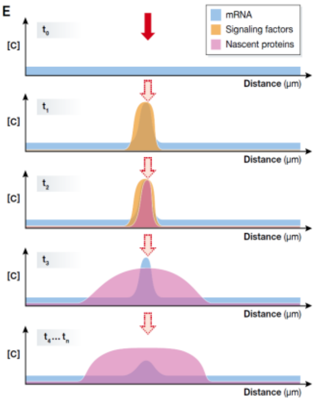
Leung et al. (2006) Nat Neuroscience

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



How local is local translation of dendritic and axonal mRNAs?





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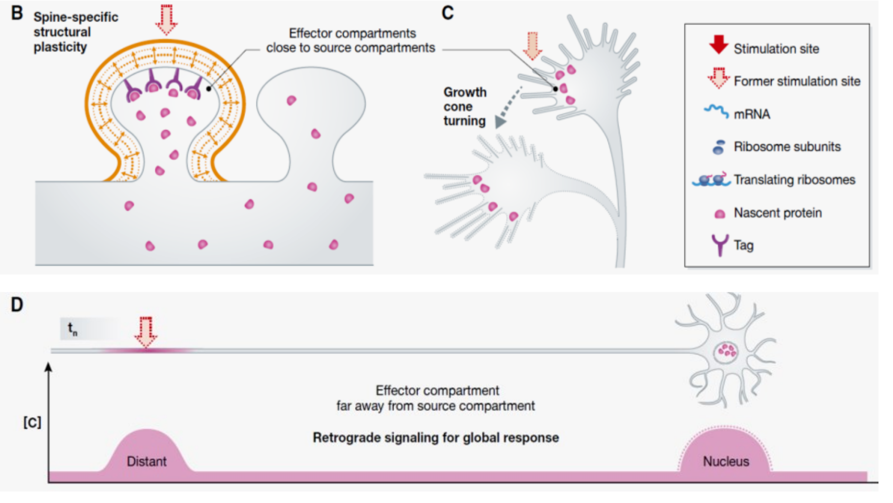
Relatioship between Source and Effector compartments



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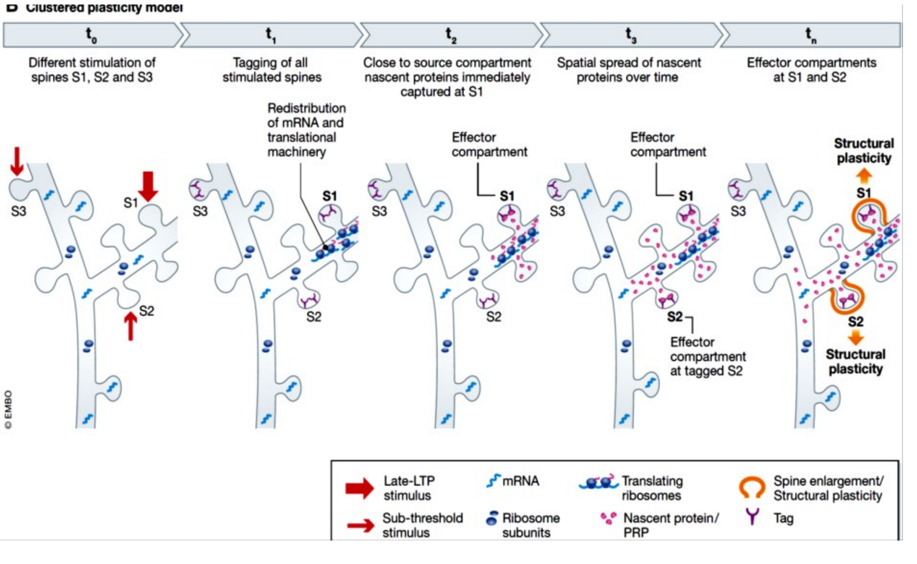
Models of local translation support to plasticity at synapses



Brief recapitulation of the different phases of Translation in Eukaryotes

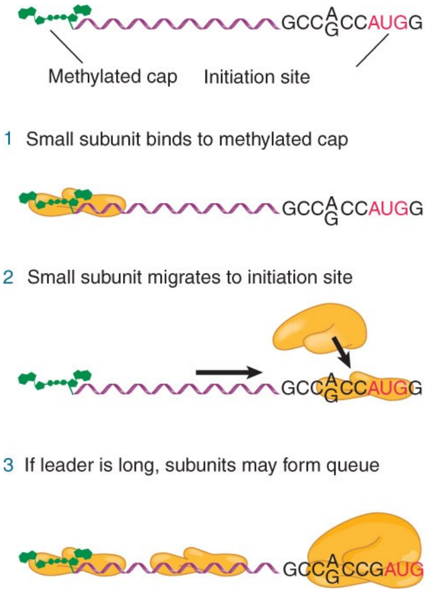
Translation is divided into two stages: initiation and elongation.

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

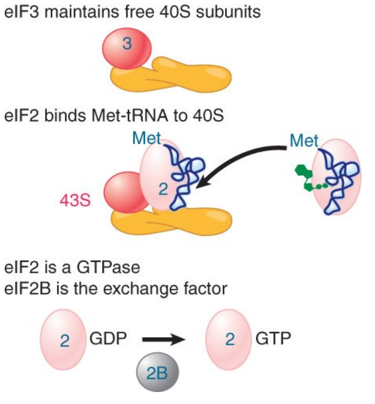
Elongation then continues to assemble amino acids to form the protein.

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.



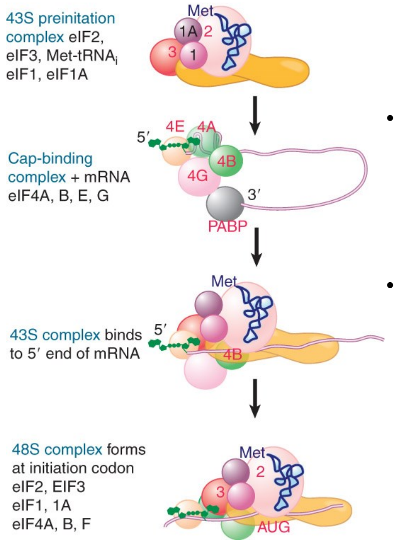
Eukaryotes Use a Complex of Many Initiation Factors



- 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

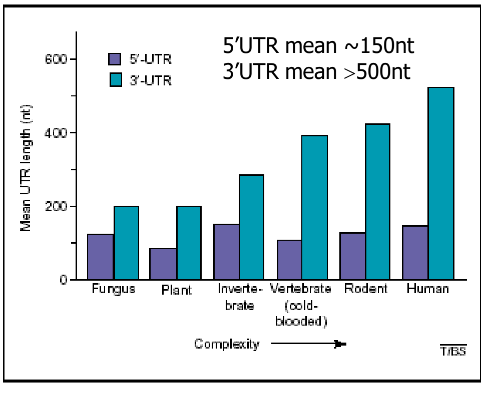


- Initiation factors are required for all stages of initiation, including binding the initiator tRNA, 40S subunit attachment to mRNA, movement along the mRNA, and joining of the 60S subunit.
- Eukaryotic initiator tRNA is a Met-tRNA that is different from the Met-tRNA used in elongation, but the methionine is not formylated.

Control mechanisms of local protein synthesis in dendrites

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

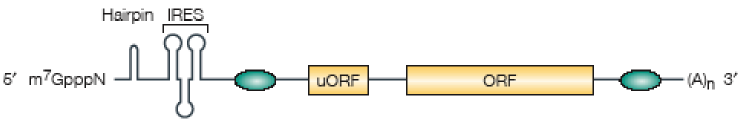
The control of translation occurs both at 5′ and 3′UTR



- **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-independant 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

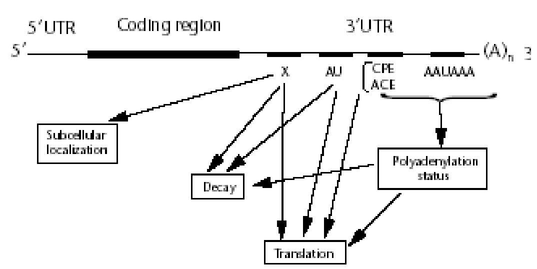


Fig. 1 Multiple functions for mRNA 3' UTRs. Typical *cis*-acting regulatory determinants in the 3' UTR of mRNA. CPE, cytoplasmic polyadenylation element; ACE, adenylation control element.

•AAUAAA exanucleotide important for polyadenilation

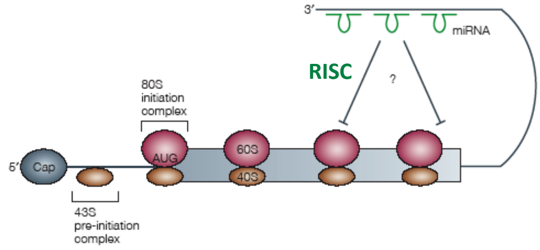
•cytoplasmic polyadenilation elements (CPEs) UUUUAU

•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)

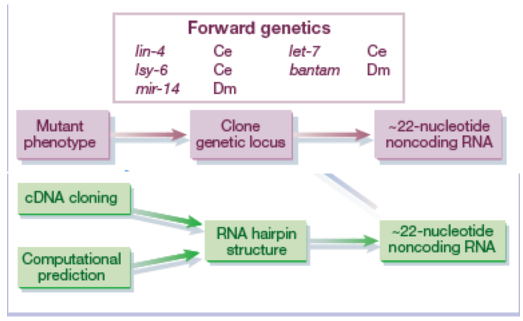
Eukaryotic miRNAs regulate translation, generally, via 3'UTR

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 (hld)

Ambros (2004) Nature 431:351



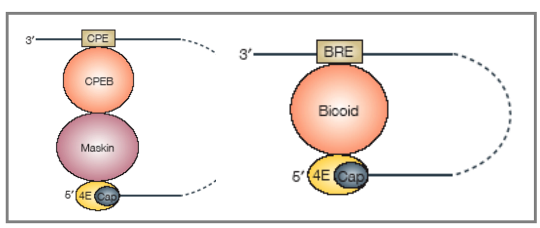
Target of miRNA? (20% of mRNA…)
(complementarity, stability, structure…)
How miRNA inhibits translation?
Identify regulatory networks…



Three 5' mechanisms of regulation of the initiation complex assembly

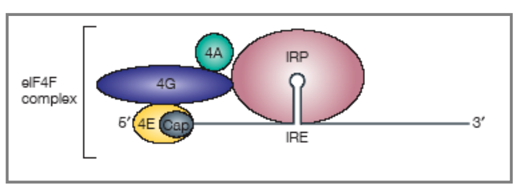
Sequestration of eIF-4E

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



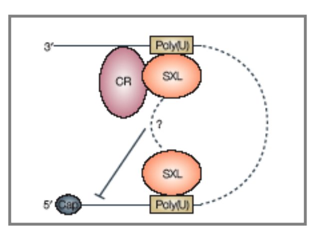
Steric blockage

(iron metabolism)



Cap-independent inhibition
Interference with ribosome scanning

(SXL prevents X-chromosome dosage compensation in drosophila)

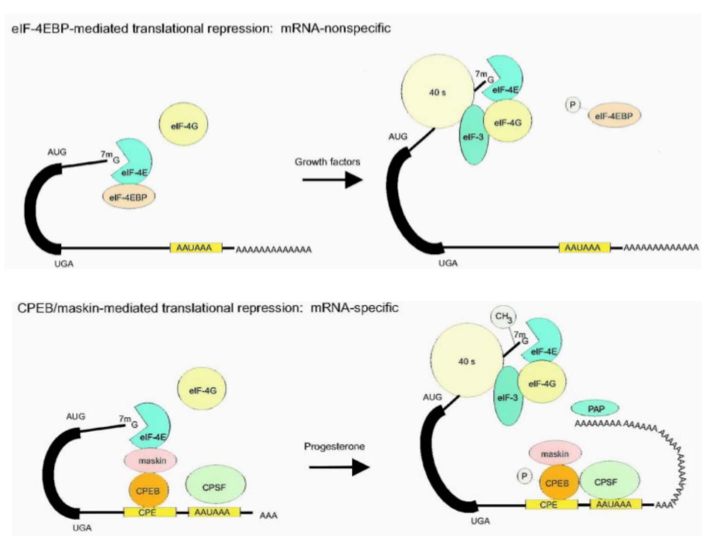


Gebauer & Hentze (2004) Nature Rev Moll Cell Biol. 5:827

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

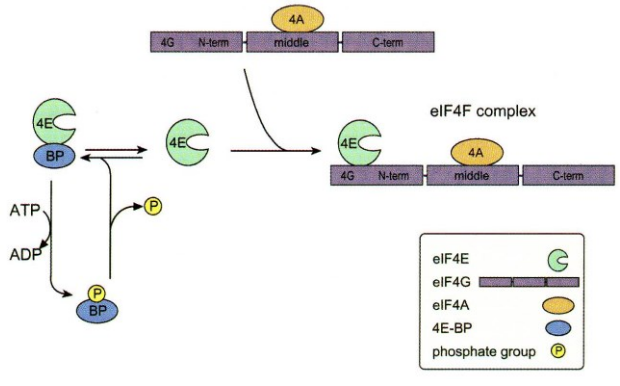


Phosphorylation of 4EBP Allows eIF4E Association with eIF4G

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

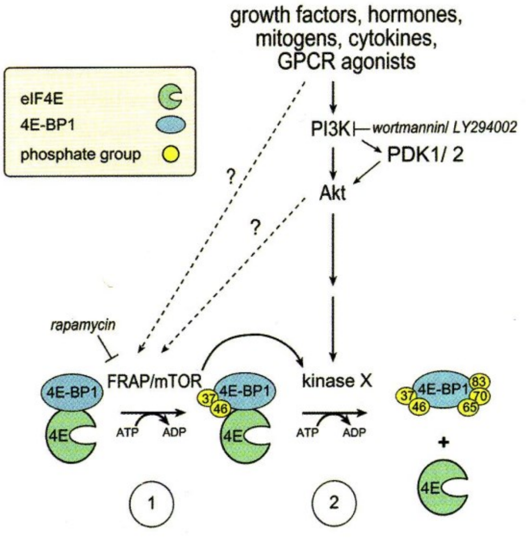
Why does this help?

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



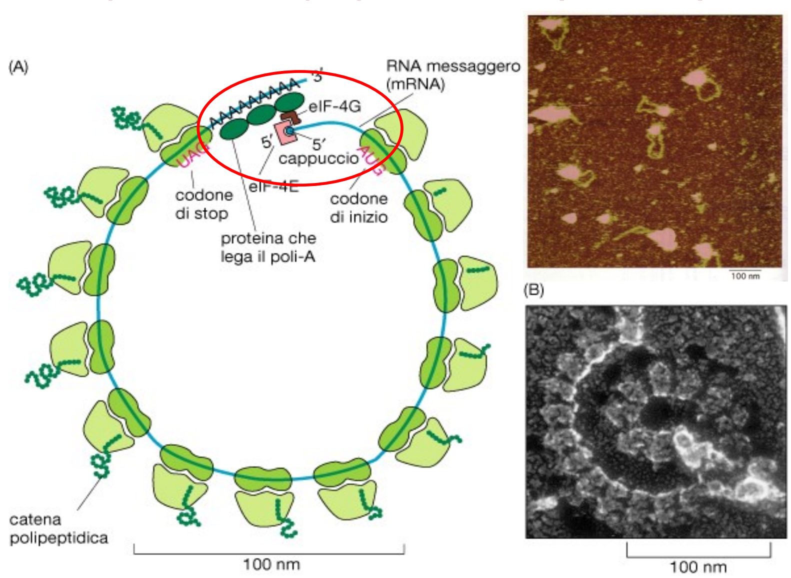
Regulation of the initiation complex assembly

1. Phosphorylation of the eIF4E binding proteins, the 4E-BPs.
2. Binding of polyadenylate binding protein (PABP) to eIF4G.

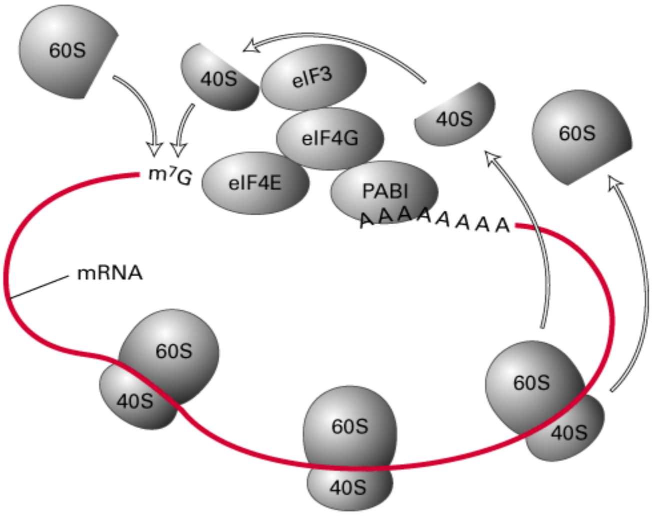
   Why?

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

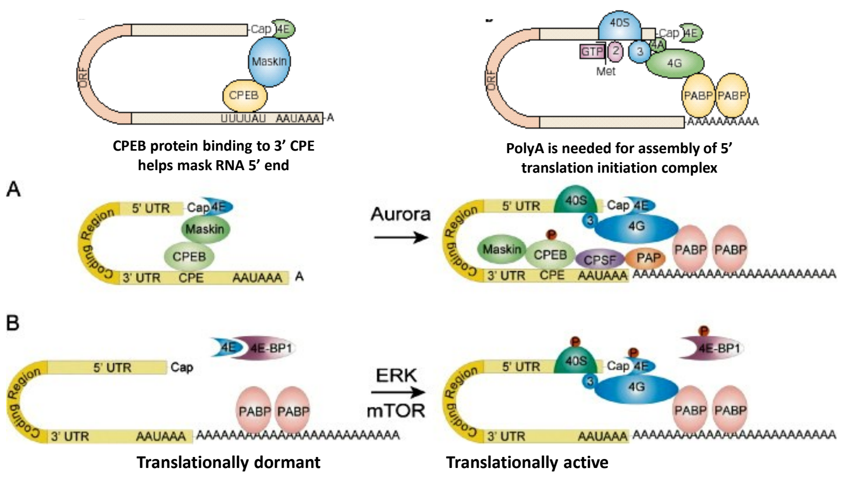
Protein synthesis on polyribosomes (rosettes)



Polyadenylation and Circularization of mRNA Through Binding of PABP to eIF4G



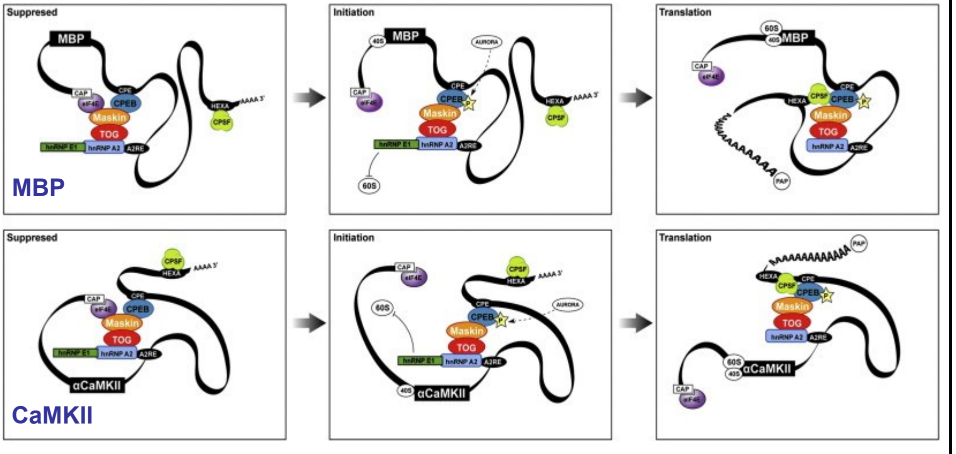
Regulation of the initiation complex assembly: Sequestration of eIF-4E coupled to 3′UTR regulation



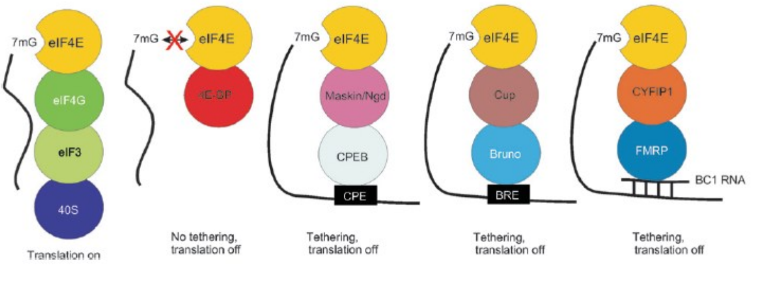
CPEB phosphorylation by Aurora allows for recruitment of polyA polyermerase (PAP)

Polyadenylation of dormant RNA allows assembly of 5' translation initiation complex

Similar models of translational regulation for MAP2 and CaMKII



Similar sequence-specific control of translation



Regulation of the initiation complex assembly

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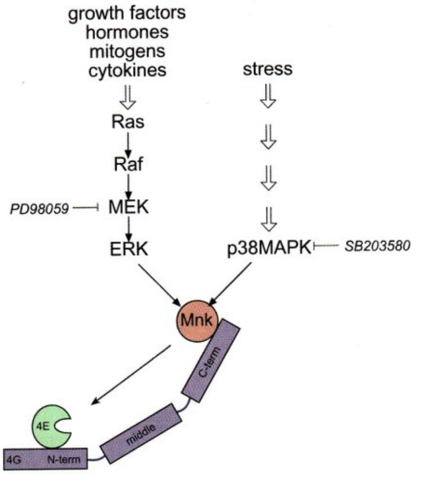
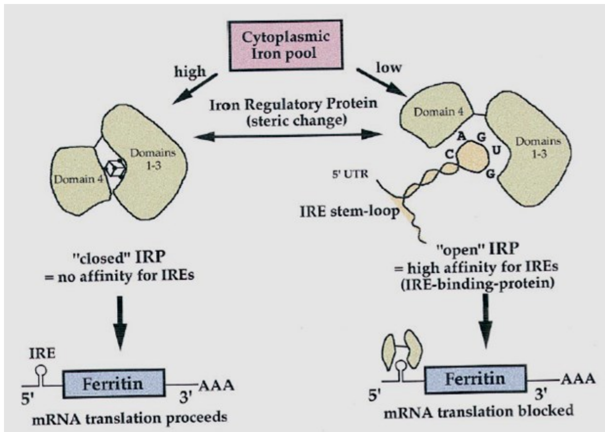
MAPK-Dependent Phosphorylation of eIF4E Is Mediated by the eIF4G Associated Kinase Mnk



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.

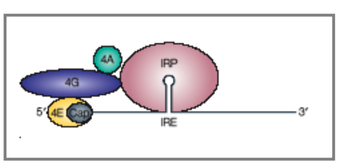


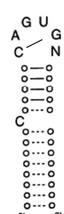
Hereditary hyperferritenemia-Cataract Syndrome (HHCS) is associated with at least 4 mutations on ferritin's IRE
(Girelli et al, Blood (1997), 5: 2084-88)

Other examples of Steric blockage of translation

One regulatory protein targets functionally related mRNAs
(Keene 2003 Mol Cell 12: 1347)

Iron regulatory protein



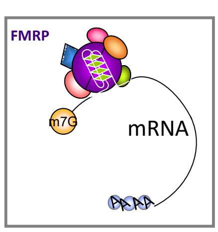


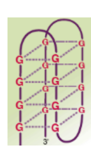
IRP and iron metabolism

Ferritin
Transferrin receptor
e-ALAS
Mitochondrial aconitase…

Dandekar & Hentze (1995 TIG 11:45)

Fragile X-syndrome Protein
(associated in large mRNPs)





FMRP and neuronal function
(actin cytoskeleton remodeling…)

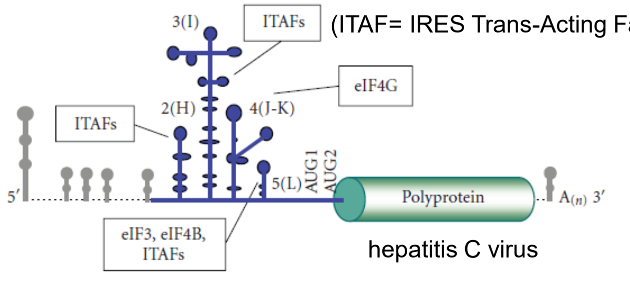
FMR1*
PP2Ac (Rac1 pathway)**
MAP1b
FGF-2*?…**

***Schaeffer et al. 2001 EMBO J 20:4803**
****Castets et al. 2005 Hum Mol Gen. 14:835**
*****Bonnal 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



(ITAF= IRES Trans-Acting Factors)

hepatitis C virus

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
αCaMKII	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
Growth factors	
Fibroblast growth factor 2 (FGF2)	Vagner et al. 1995a
Platelet-derived growth factor B (PDGF/c-*sis*)	Bernstein et al. 1997
Vascular endothelial growth factor (VEGF)	Akiri et al. 1998; Stein et al. 1998; Huez et al. 1998
Cyr61	Johannes et al. 1999
Transcription factors	
Antennapedia	Oh et al. 1992; Ye et al. 1997
Ultrabithorax	Ye et al. 1997
MYT2	Kim et al. 1998
NF-κB repressing factor NRF	Oumard et al. 2000
AML1/RUNX1	Pozner et al. 2000
Gtx homeodomain protein	Chappell et al. 2000a
Oncogenes	
c-myc	Nabru et al. 1997; Stoneley et al. 1998
Pim-1	Johannes et al. 1999
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
Translation factors	
Eukaryotic initiation factor 4G (eIF4G)[a]	Gan and Rhoads 1996
Eukaryotic initiation factor 4GI (eIF4GI)[a]	Johannes and Sarnow 1998
Death-associated protein 5 (DAP5)	Henis-Korenblit et al. 2000
Activators of apoptosis	
Apoptotic protease activating factor Apaf-1	Coldwell et al. 2000
Dendritically localized proteins	
Activity-regulated cytoskeletal protein (ARC)	Pinkstaff et al. 2001
α subunit of calcium calmodulin-dependent kinase II dendrin	Pinkstaff et al. 2001
Microtubule-associated protein 2 (MAP2) neurogranin (RC3)	Pinkstaff et al. 2001
Others	
Immunoglobulin heavy chain binding protein (BiP)	Macejak and Sarnow 1991
La autoantigen	Carter and Sarnow 2000
β subunit of mitochondrial H^+-ATP synthase	Izquierdo et al. 2000
Ornithine decarboxylase	Pyronnet et al. 2000

5′UTR control of translation: Translation of mRNAs containing 5′TOP sequences

(terminal oligo-pyridine tract = 4-14 pyridine)

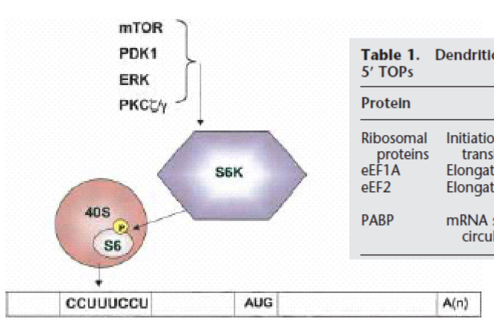


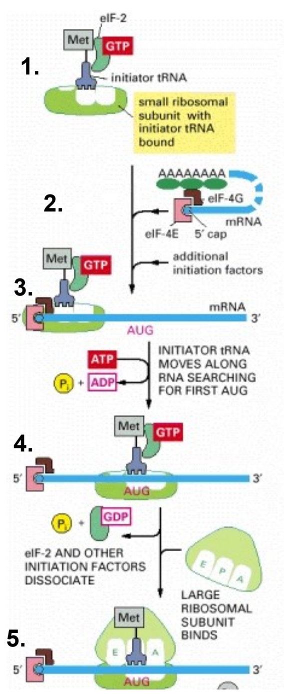
Figure 3 S6-directed translation. S6 kinase (S6K1) is activated by multiple kinases, including PDK-1, ERK, PKCζ/γ, and mTOR. Activated S6K1 phosphorylates ribosomal protein S6, whose phosphorylation is correlated with the selective translation of mRNAs that contain 5′TOPs in their 5′UTR.

Table 1. Dendritic Proteins Encoded by mRNAs Containing 5′ TOPs

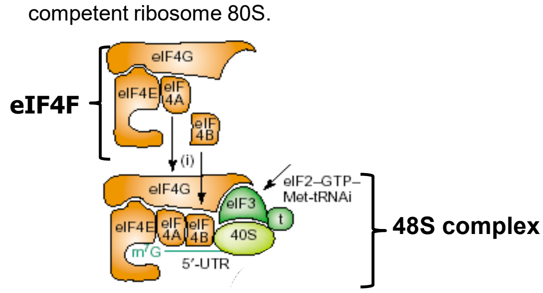
Protein	Function	Reference(s)
Ribosomal proteins	Initiation, scanning, translocation	Steward and Levy 1982; Asaki et al. 2003[a]
eEF1A	Elongation of peptide chain	Inamura et al. 2003
eEF2	Elongation of peptide chain	Asaki et al. 2003; Inamura et al. 2003
PABP	mRNA stability, circularization of mRNA	Mudashetty et al. 2002

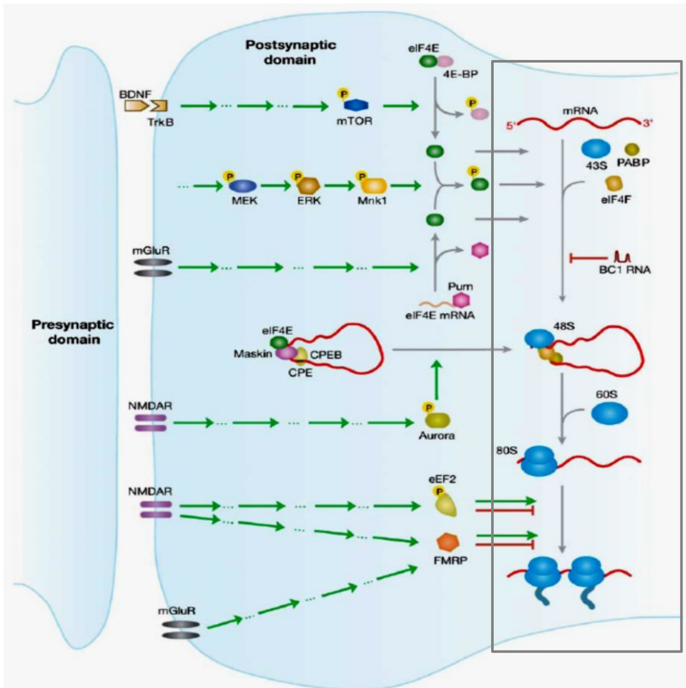
- **What intracellular signals regulate translation in dendrites ?**

The initiation process can be divided in 5 events



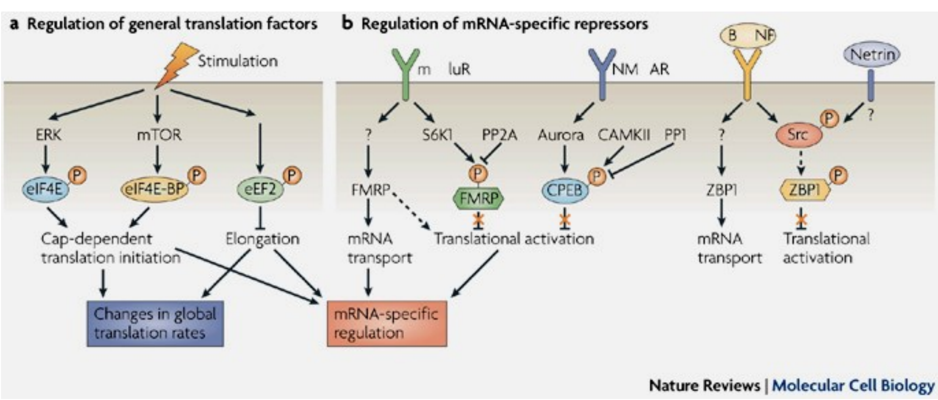
1. Met-tRNA and eIF2 bind to the small ribosomal subunit
2. eIF4F (eukaryotic initiation factor) is recruited at the level of the 5'cap (m^7GpppNcap)
3. The 43S pre-initiation complex binds to eIF4F forming the 48S complex
4. The 48S complex scans the 5'UTR region to reach the AUG and stop
5. When the AUG triplet is reconized by Met-tRNA, there is hydrolisis and dissociation of the initiation factors and the 60S subunit is recruited to form the translation-competent ribosome 80S.



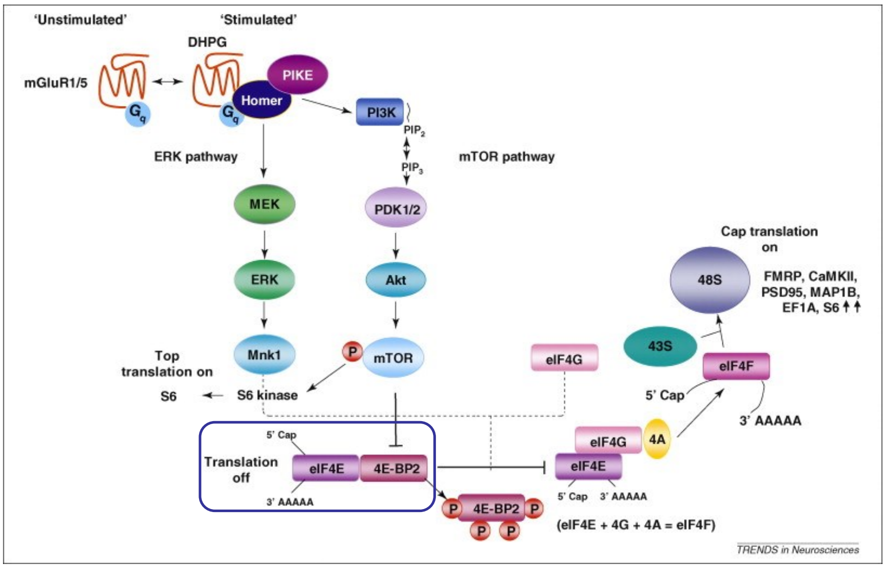


Signalling cascades that influence translation of neuronal mRNAs at 5′ or 3′ UTRS

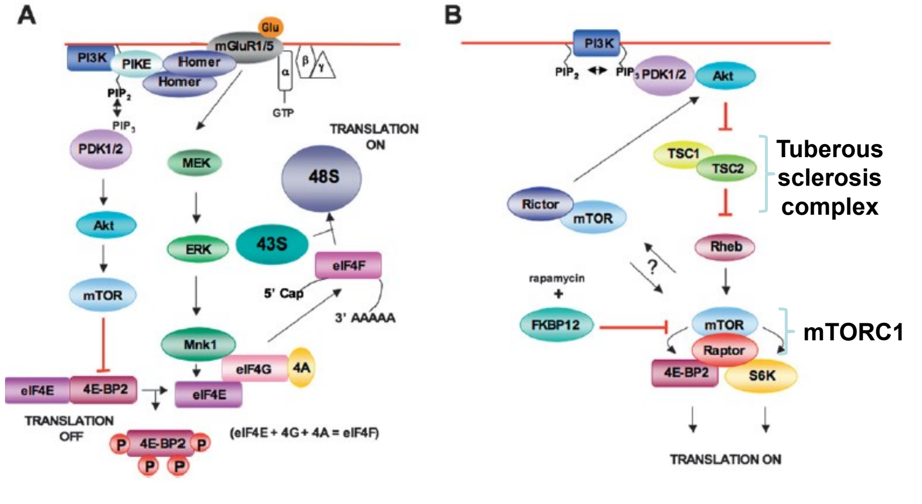
5'UTR **3'UTR**



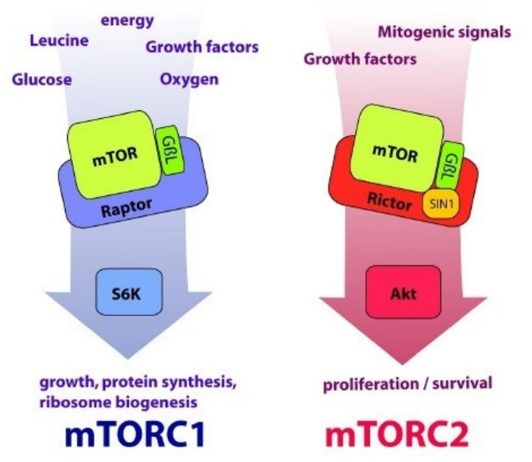
mTOR =mammalian Target of Rapamycin



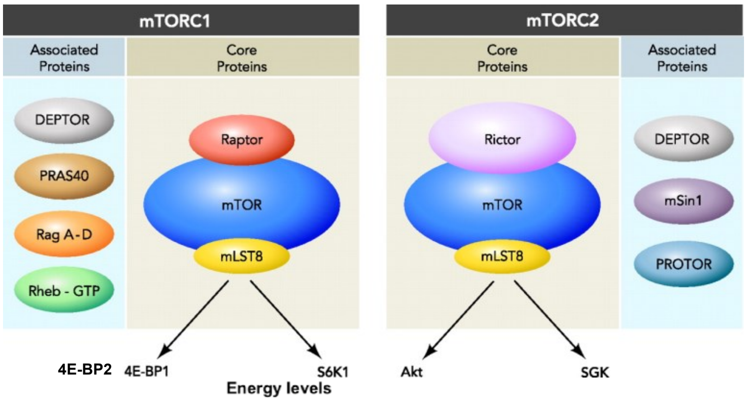
mTOR =mammalian Target of Rapamycin

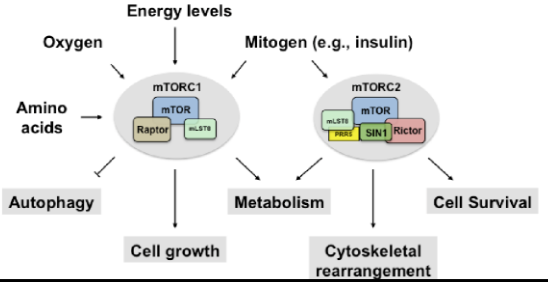


mTOR functions in two distinct complexes and pathways

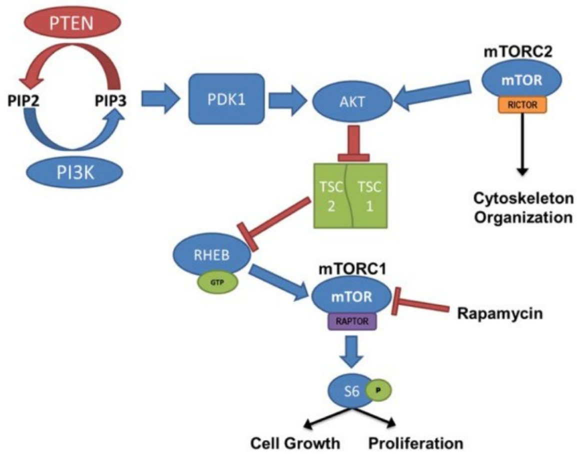


mTOR forms two complexes with different targets





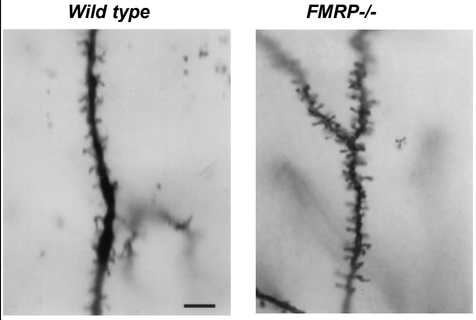
PI3K/PTEN–mTORC pathway

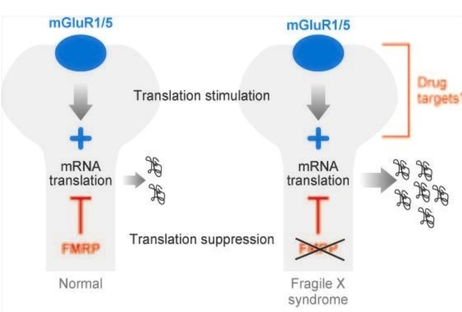


mTOR is activated by signaling through the PI3K–Akt pathway, regulating cell growth and proliferation. Phosphatase and tensin homolog (PTEN), TSC1, and TSC2 act as negative regulators of the mTOR pathway, and removal leads to hyperactivation of mTOR. mTOR complex 1 (mTORC1) signaling requires activation of the adaptor protein raptor, and mTOR complex 2 (mTORC2), which is largely insensitive to acute rapamycin treatment, requires activation of the rictor protein. Although regulation of mTORC2 is less clearly defined, it has been linked to cytoskeleton organization.

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

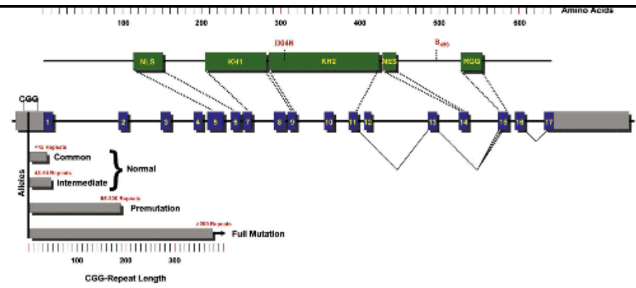
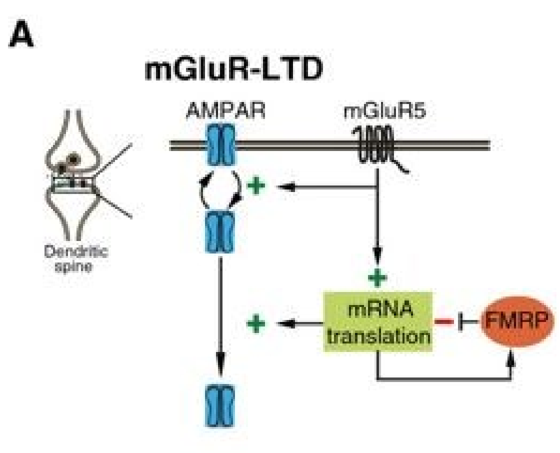


Table 1. Validated FMRP Target mRNAs

[redacted]					
App		+	+	Co-IP	Westmark and Malter, 2007
Arc	+		+	Co-IP	Steward and Worley, 2001; Waung et al., 2008; Park et al., 2008
CamKIIα	+		+	Co-IP	Bramham and Wells, 2007; Muddashetty et al., 2007; Hou et al., 2006; Zalfa et al., 2003
eEF1A	+		+	Co-IP; in vitro	Huang et al., 2005; Sung et al., 2003
Fmr1	+	+	+	in vitro	Weiler et al., 1997; Antar et al., 2004; Schaeffer et al., 2001
GluR1/2	+		+	Co-IP	Muddashetty et al., 2007
Map1b	+	+	+	Co-IP; in vitro; biophysical	Brown et al., 2001; Darnell et al., 2001; Antar et al., 2005; Davidkova and Carroll, 2007; Hou et al., 2006; Menon et al., 2008
Psd95	+	+	+	Co-IP; in vitro; CLIP	Todd et al., 2003; Zalfa et al., 2007; Muddashetty et al., 2007
Sapap3/4	+		+	Co-IP	Brown et al., 2001; Kindler et al., 2004; Narayanan et al., 2007; Dictenberg et al., 2008
Sema3F		+		Co-IP; biophysical	Darnell et al., 2001; Menon and Mihailescu, 2007
Rgs5	+		+	APRA; in vitro	Miyashiro et al., 2003; Dictenberg et al., 2008
Gaba-Aδ	+		+	APRA; in vitro	Miyashiro et al., 2003; Dictenberg 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 hyperferritenemia-cataract syndrome (HHCS)** (Girelli et al, 1997 , Blood, 5: 2084-88)
- **Charcot-Marie-Tooth disease**
- **myelome (c-myc)**

3'UTR

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