

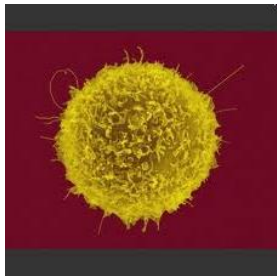


Metody badania RNA w neuronach

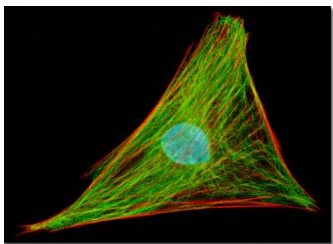
Magdalena Dziembowska



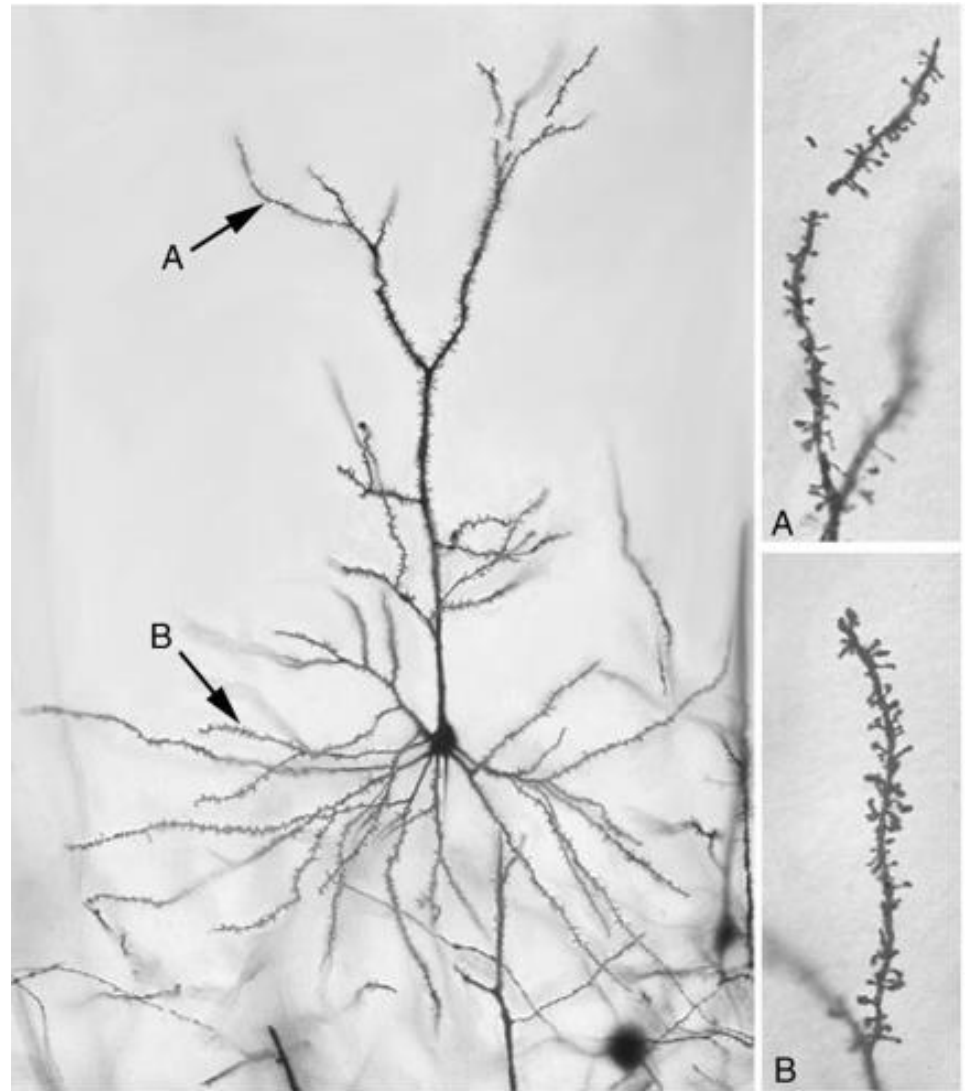
LABORATORIUM
MOLEKULARNYCH PODSTAW
PLASTYCZNOŚCI
SYNAPTYCZNEJ



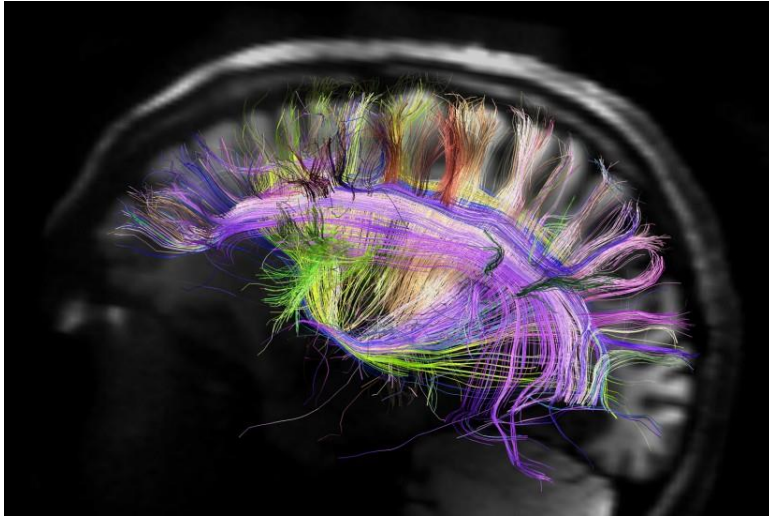
T cell



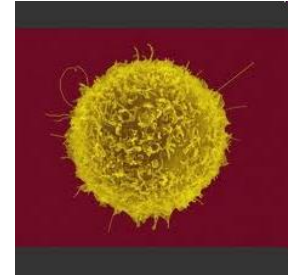
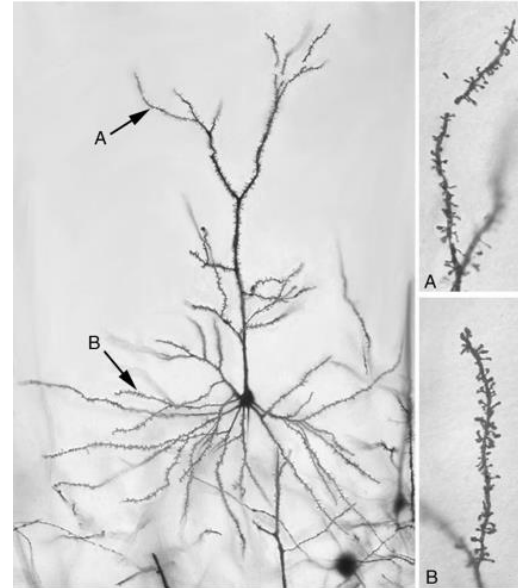
fibroblast



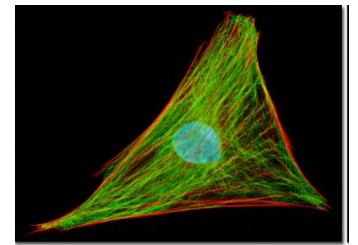
A Golgi-stained pyramidal cell in the parietal cortex of a rat. The high power images at the right show dendritic spines on apical and basilar dendritic branches. Photo by **Grazyna Gorny**



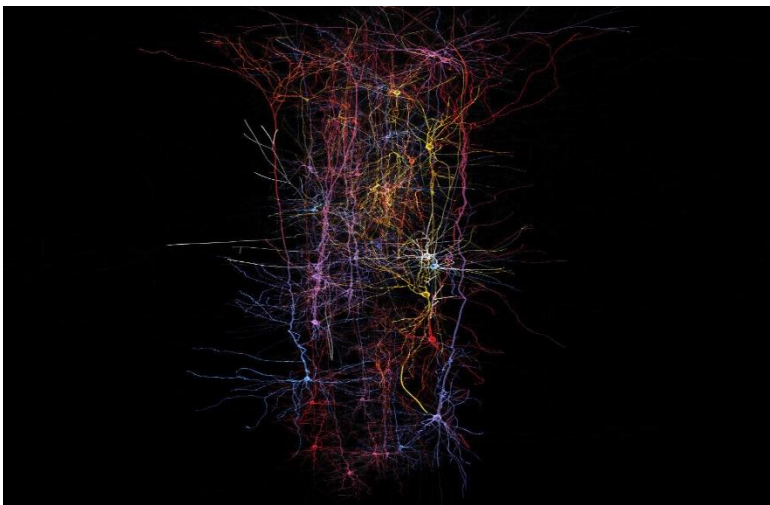
Brain Research Through Advancing Innovative
Neurotechnologies (BRAIN) initiative.



T cell



fibroblast



The Blue Brain Project



Synapsy są zlokalizowane na kolcach dendrytycznych. Kolce dendrytyczne to dynamiczne struktury, które mogą zmieniać kształt w odpowiedzi na pobudzenie.

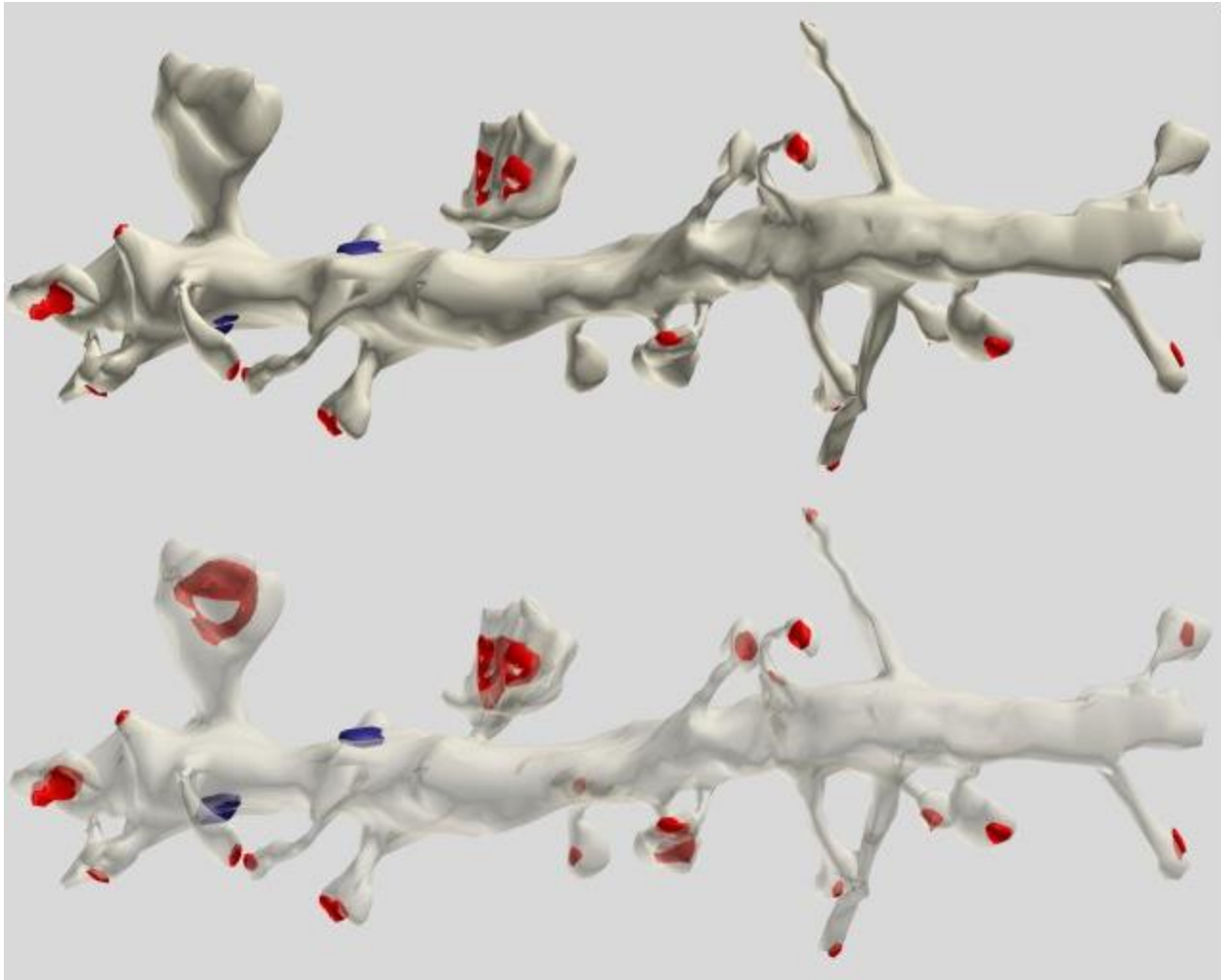
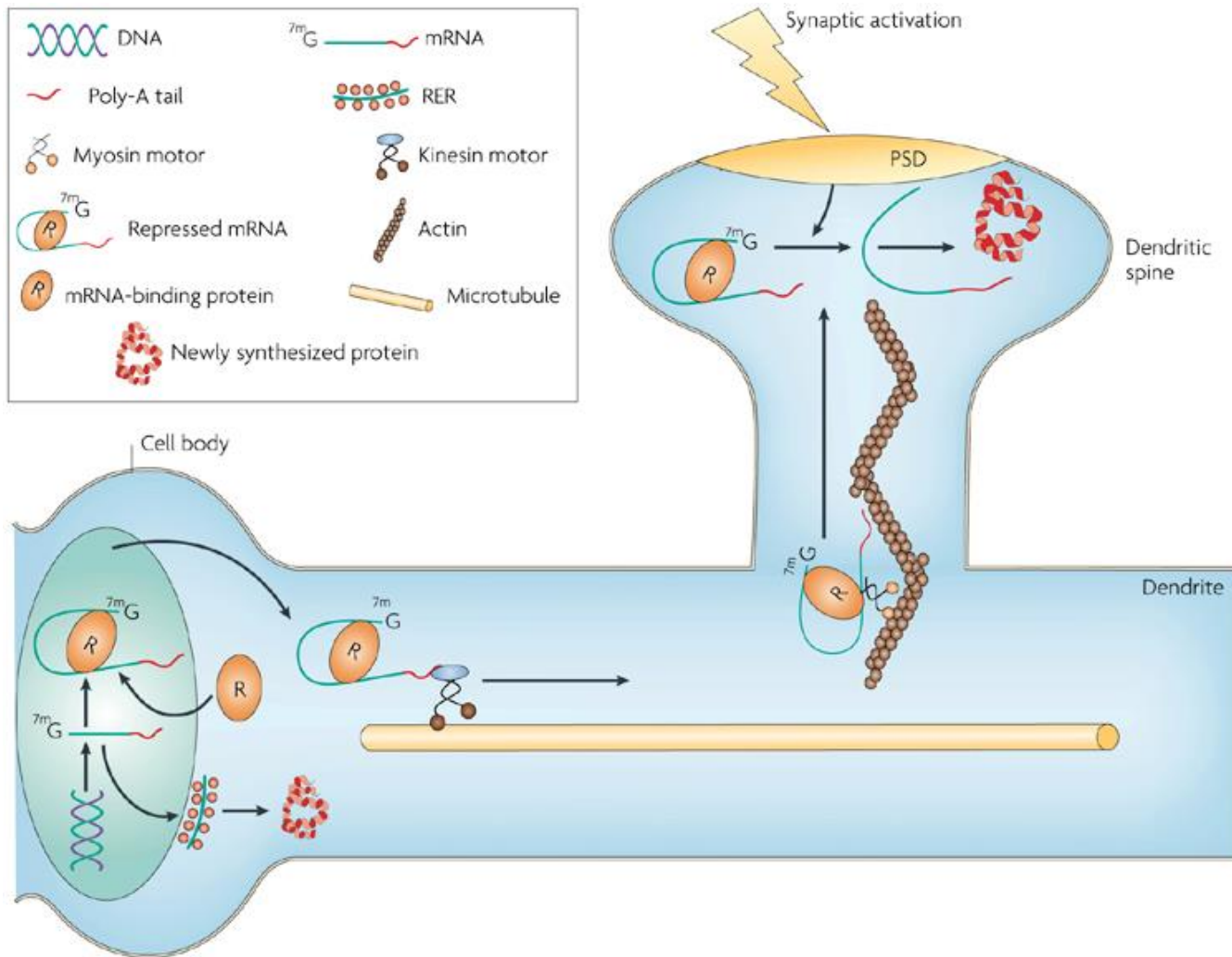
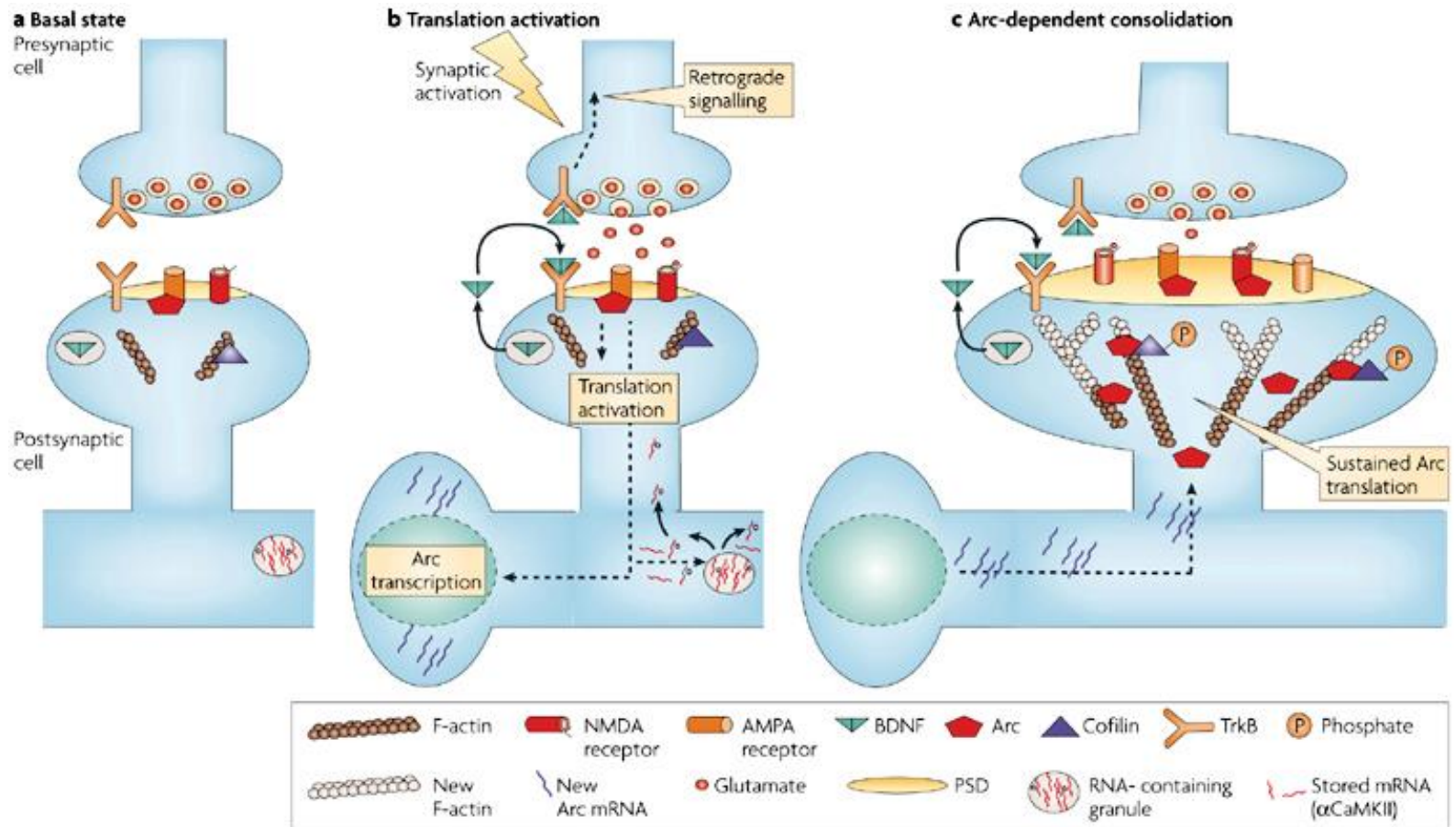


Fig. 1: A segment of pyramidal cell dendrite from stratum radiatum (CA1) with thin, stubby, and mushroom-shaped spines. Spine synapses colored in red, stem (or shaft) synapses colored in blue. The dendrite was made transparent in the lower image to enable visualization of all synapses. *Photo by [Josef Spacek](#).*

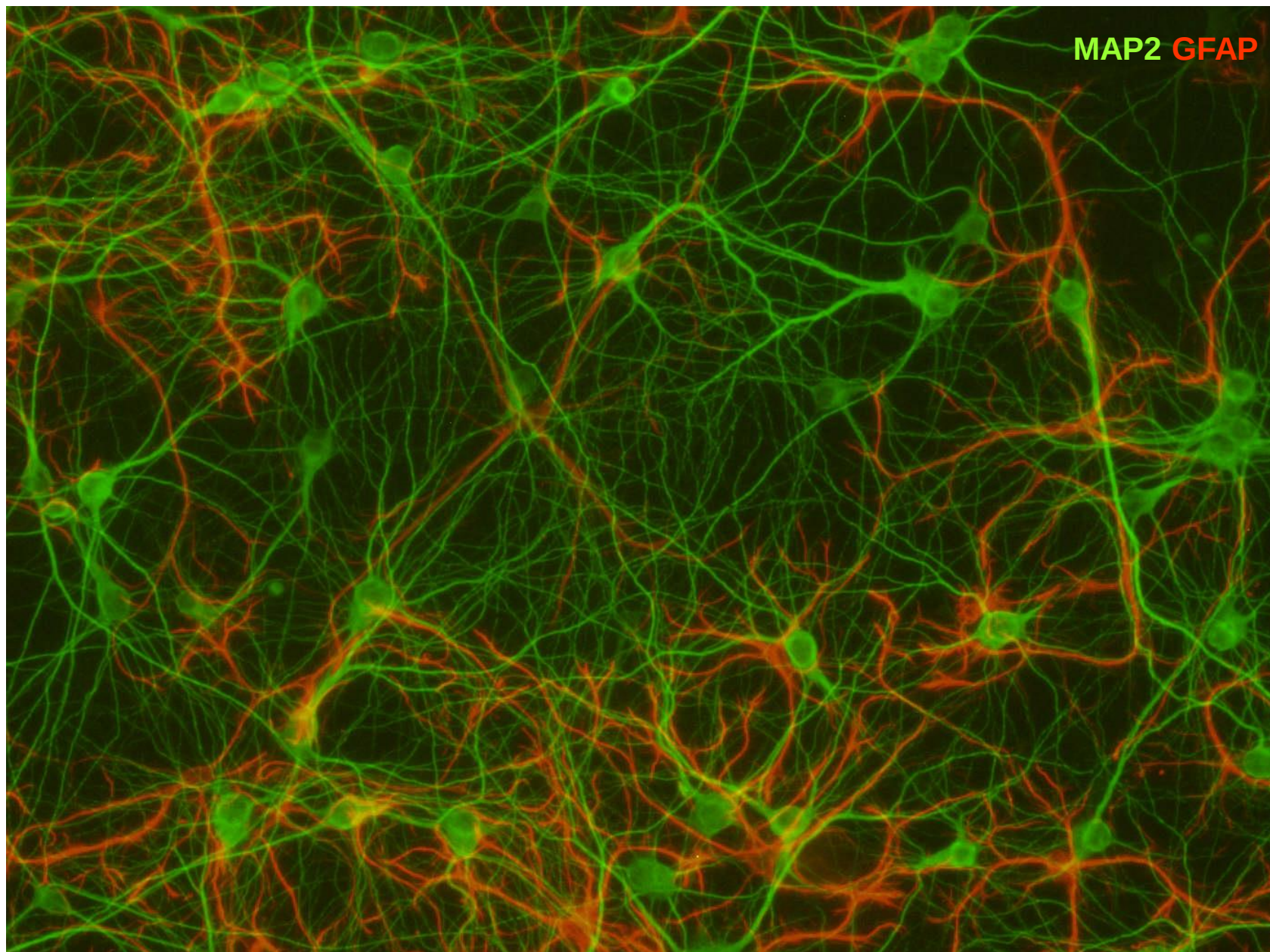
Local mRNA translation in dendritic spines



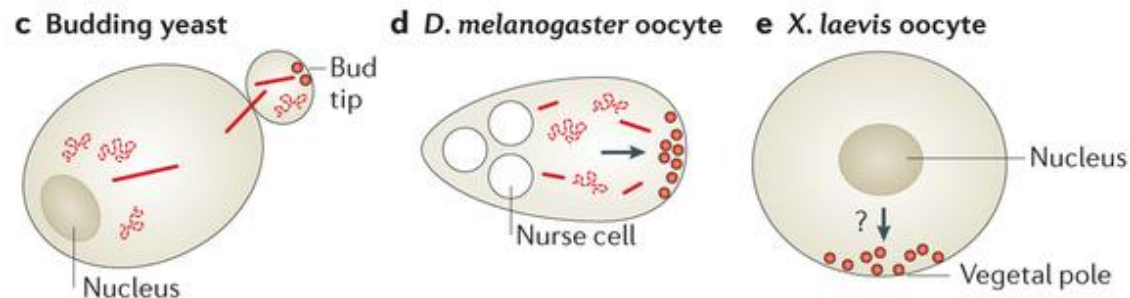
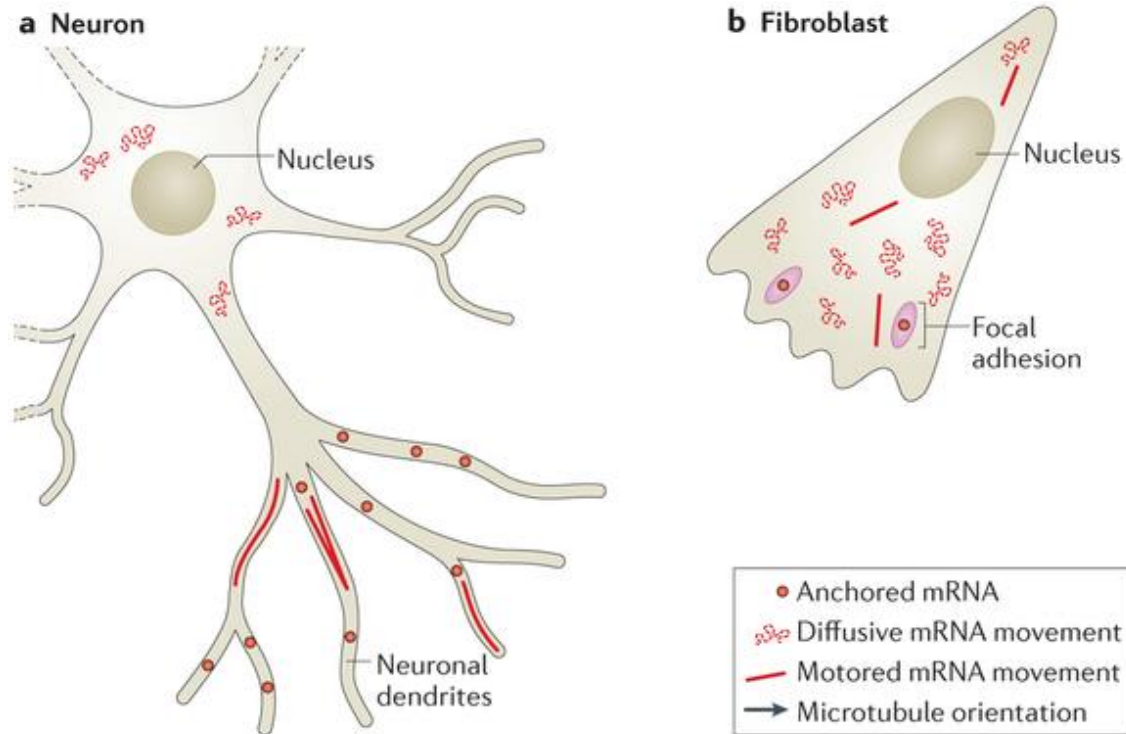
A model of Arc-dependent LTP consolidation in the dentate gyrus



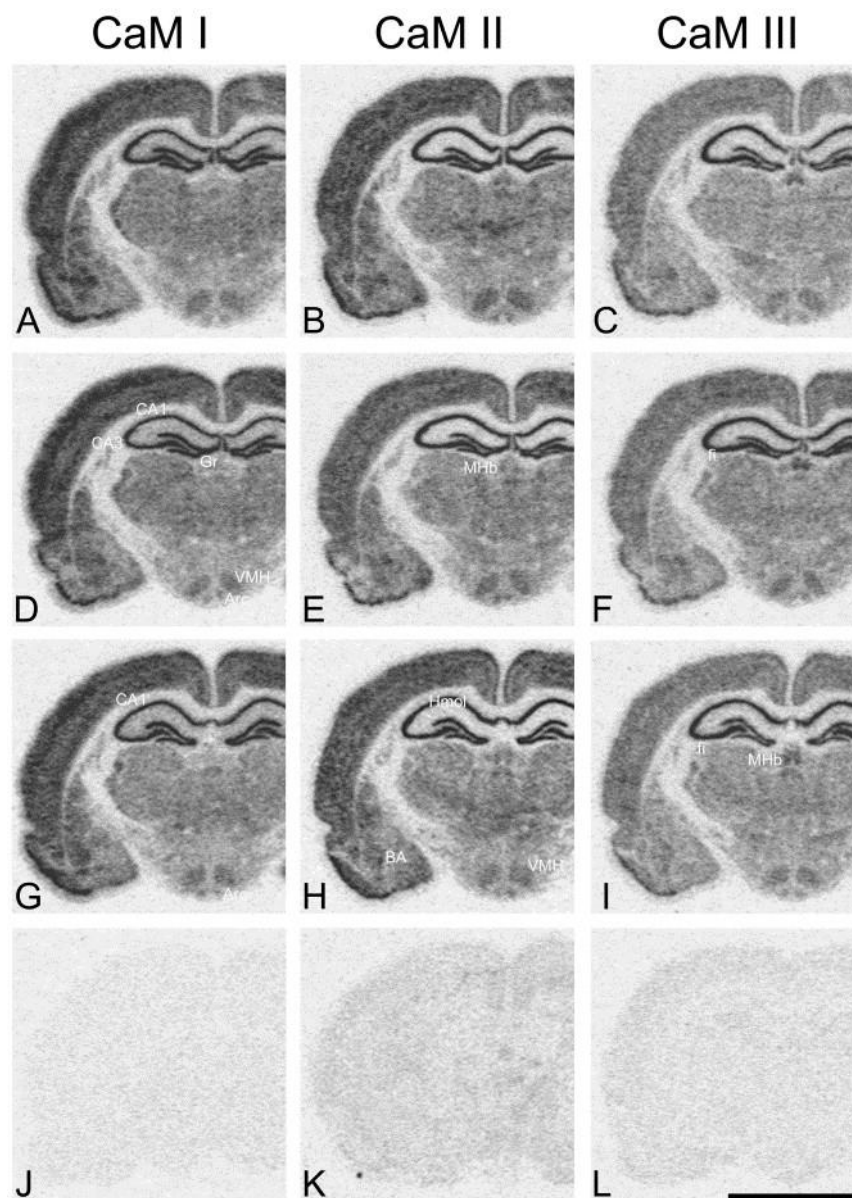
Metody wizualizacji mRNA w neuronach



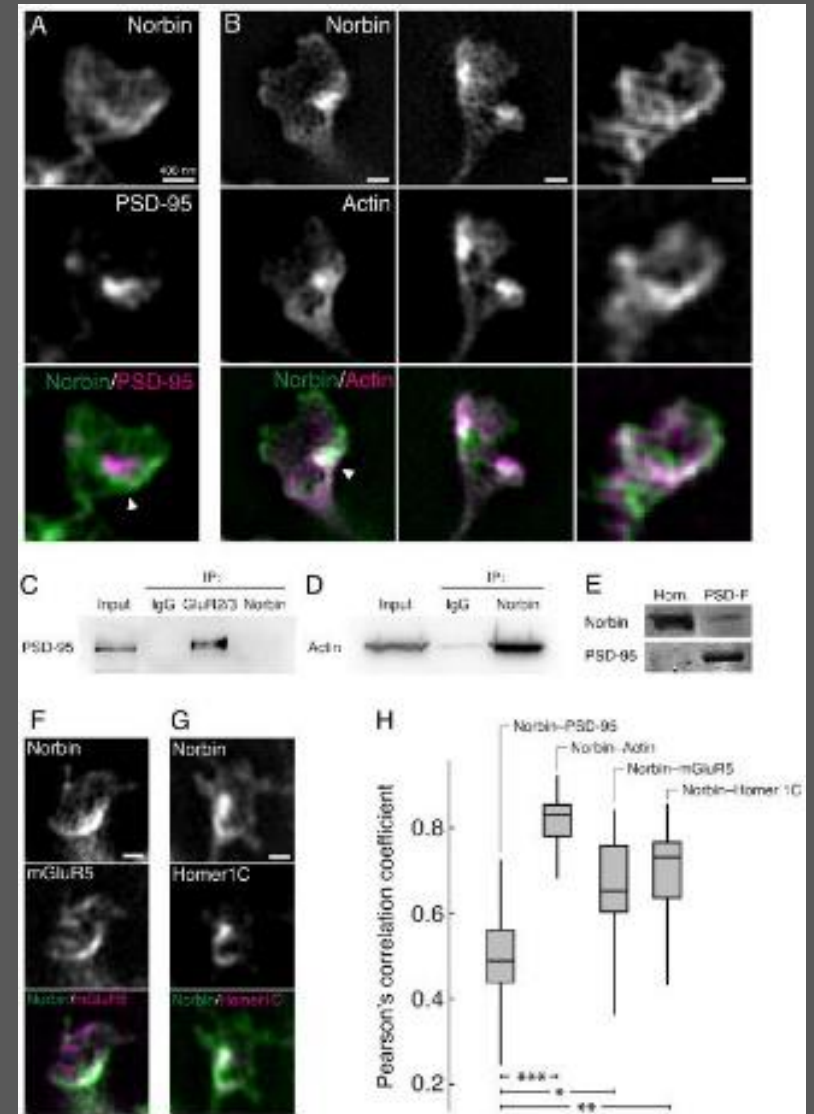
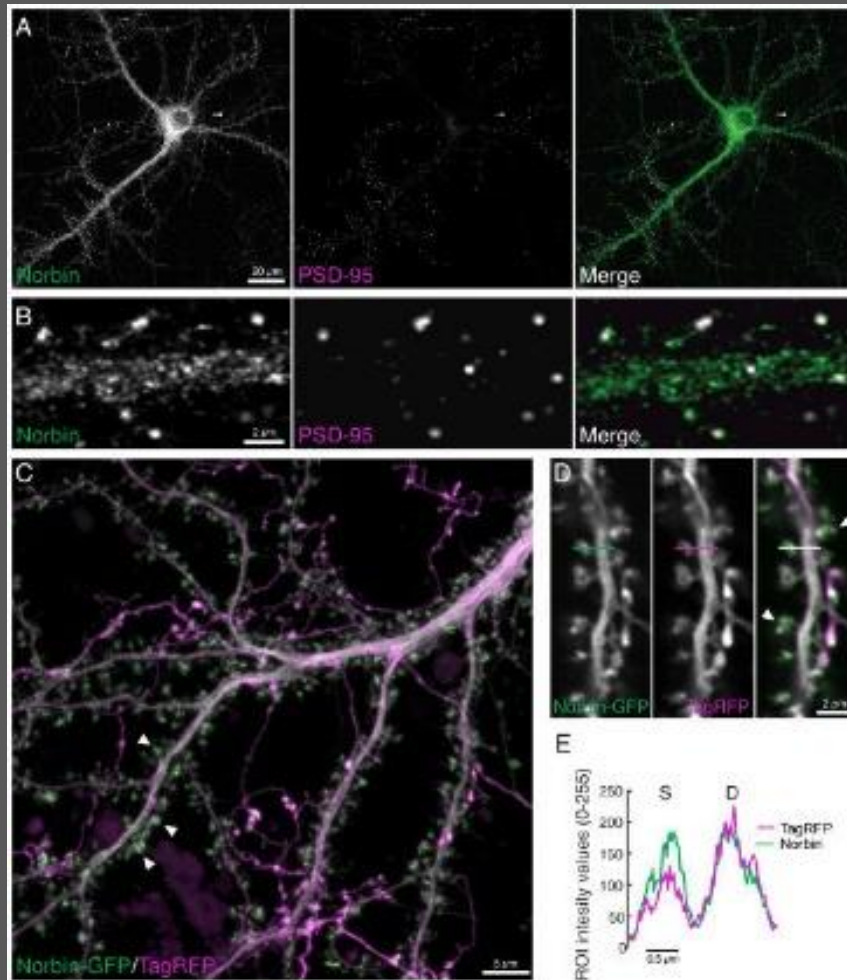
Different types of mRNA movements depend on subcellular location and on cell type



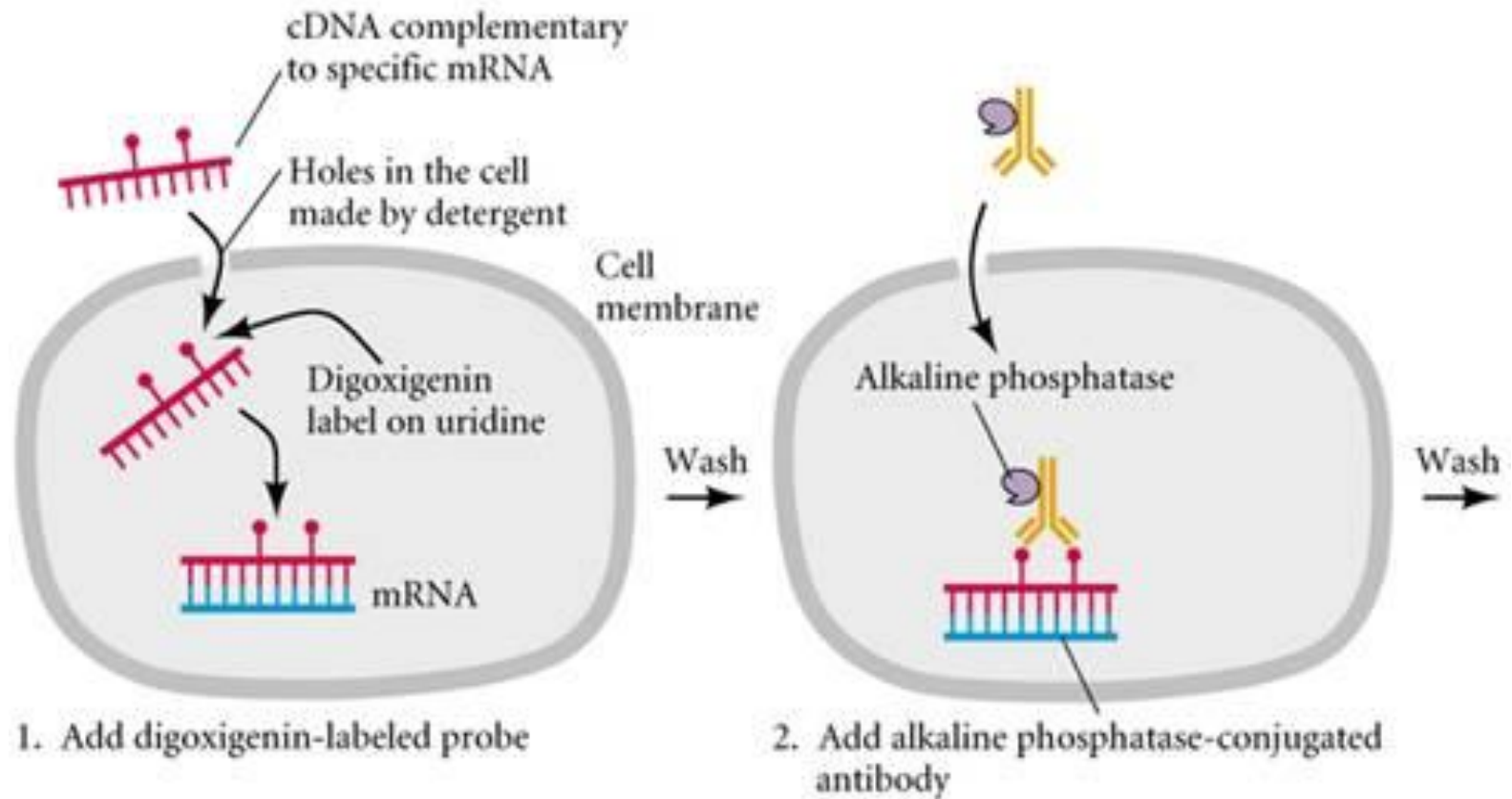
***in situ* hybrydizacja z sondą RNA wyznakowaną radioaktywną siarką**



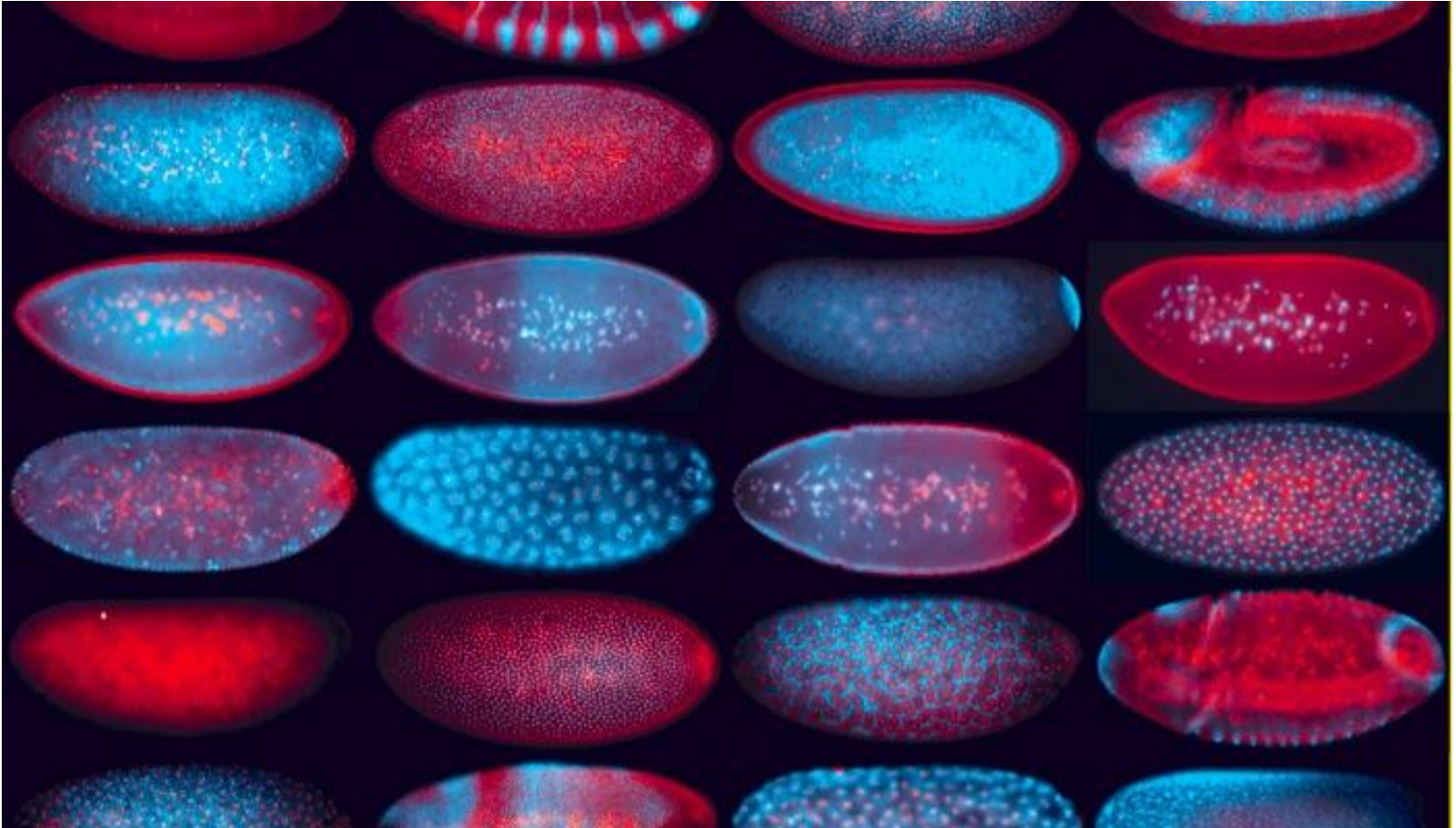
Super resolution microscopy



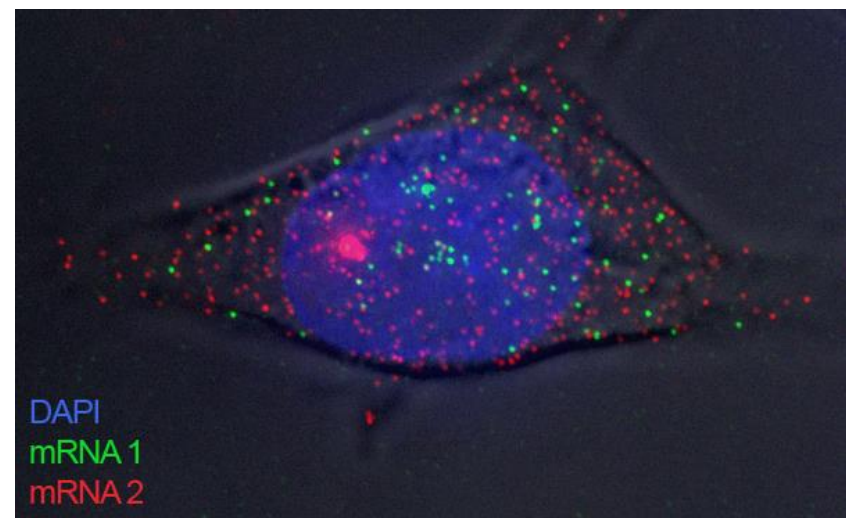
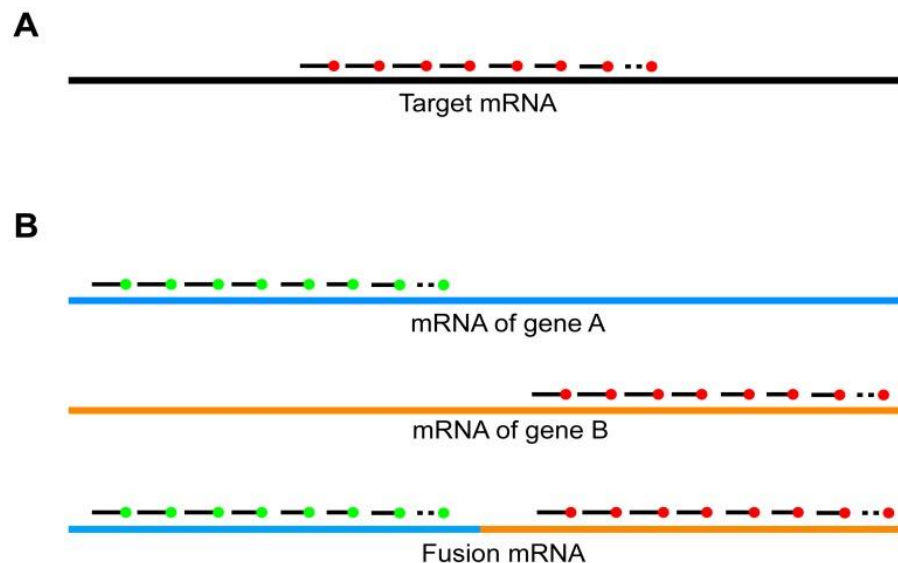
In situ hybridization with a digoxigenin-labeled RNA probe



High-resolution fluorescent in situ hybridization procedure to comprehensively evaluate mRNA localization dynamics during early *Drosophila* embryogenesis.

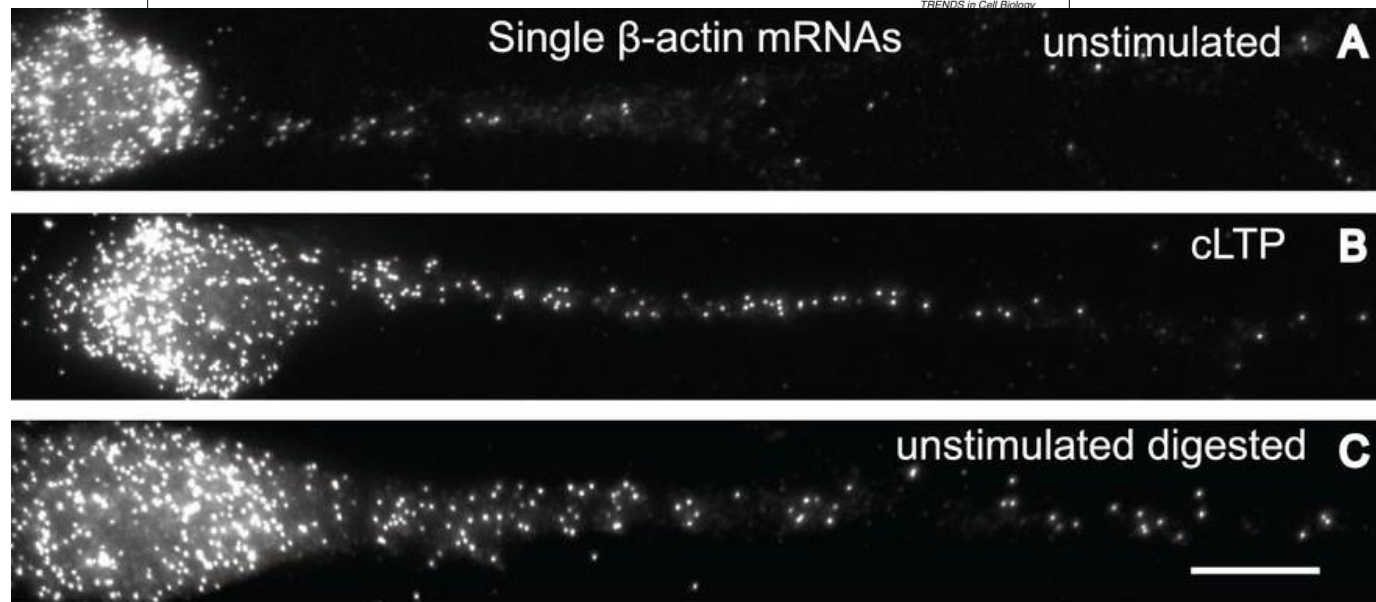
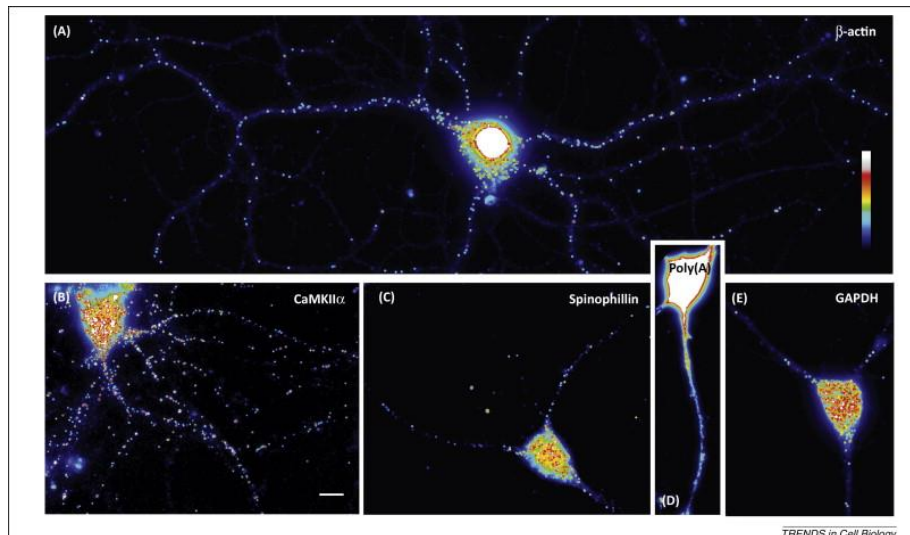


„Single molecule FISH” to metoda in situ hybrydyzacji pozwalająca na obrazowanie pojedynczej cząsteczki mRNA w komórce dzięki wykorzystaniu wielu fluorescencyjnie wyznakowanych sond zaprojektowanych do rozpoznawania sekwencji w obrębie tej samej cząsteczki mRNA

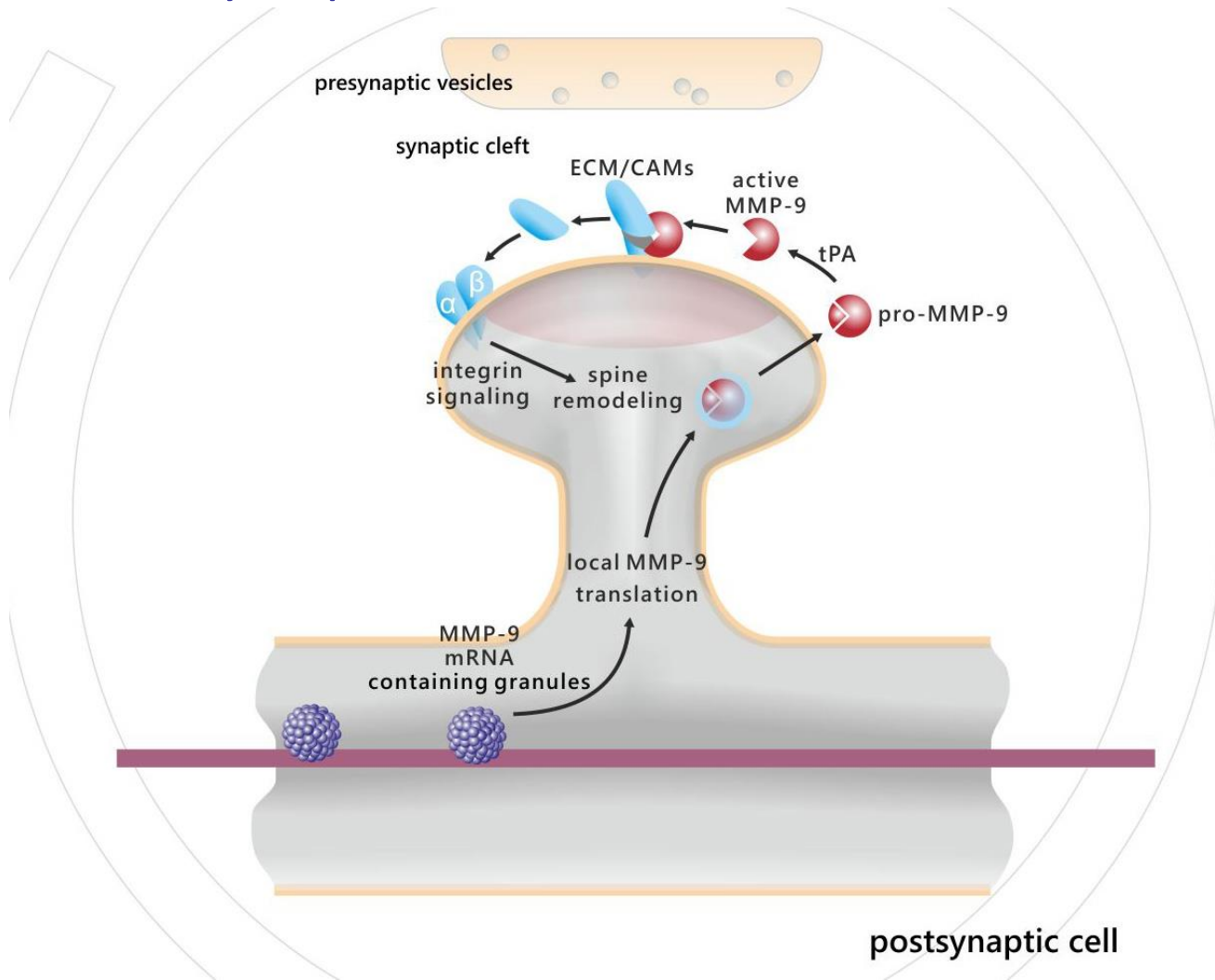


Przy użyciu tej metody można np. jednocześnie wykrywać dwa różne mRNA w komórce lub mRNA powstałe w wyniku fuzji 2 transkryptów (translokacji genomowych) jak np. BCR-ABL

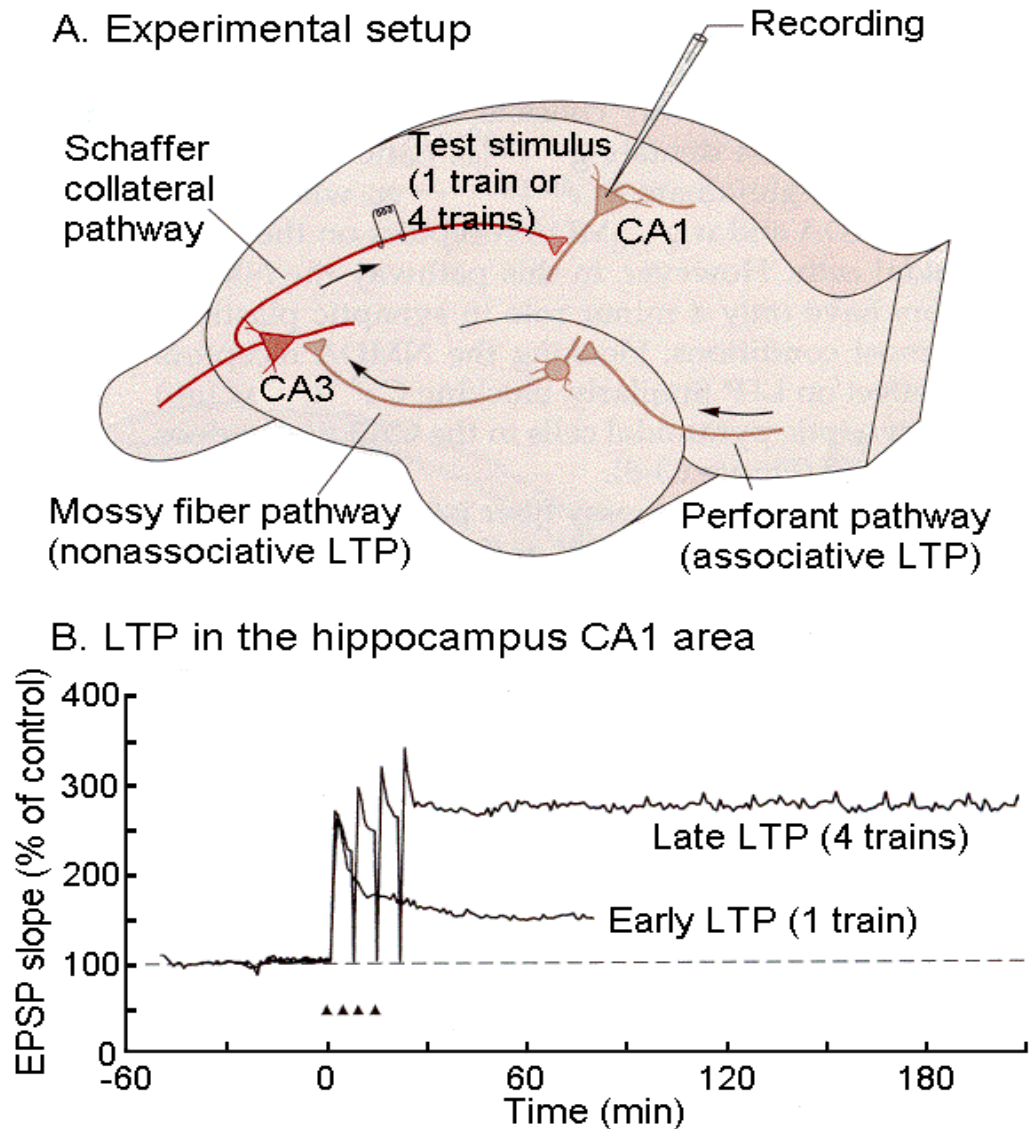
Obrazowanie mRNA w dendrytach komórek nerwowych z wykorzystaniem smFISH



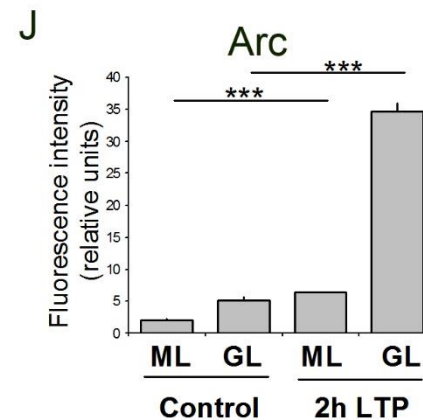
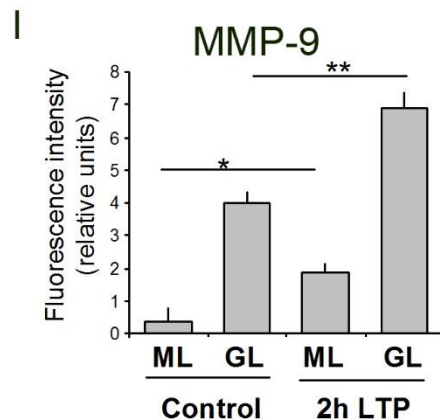
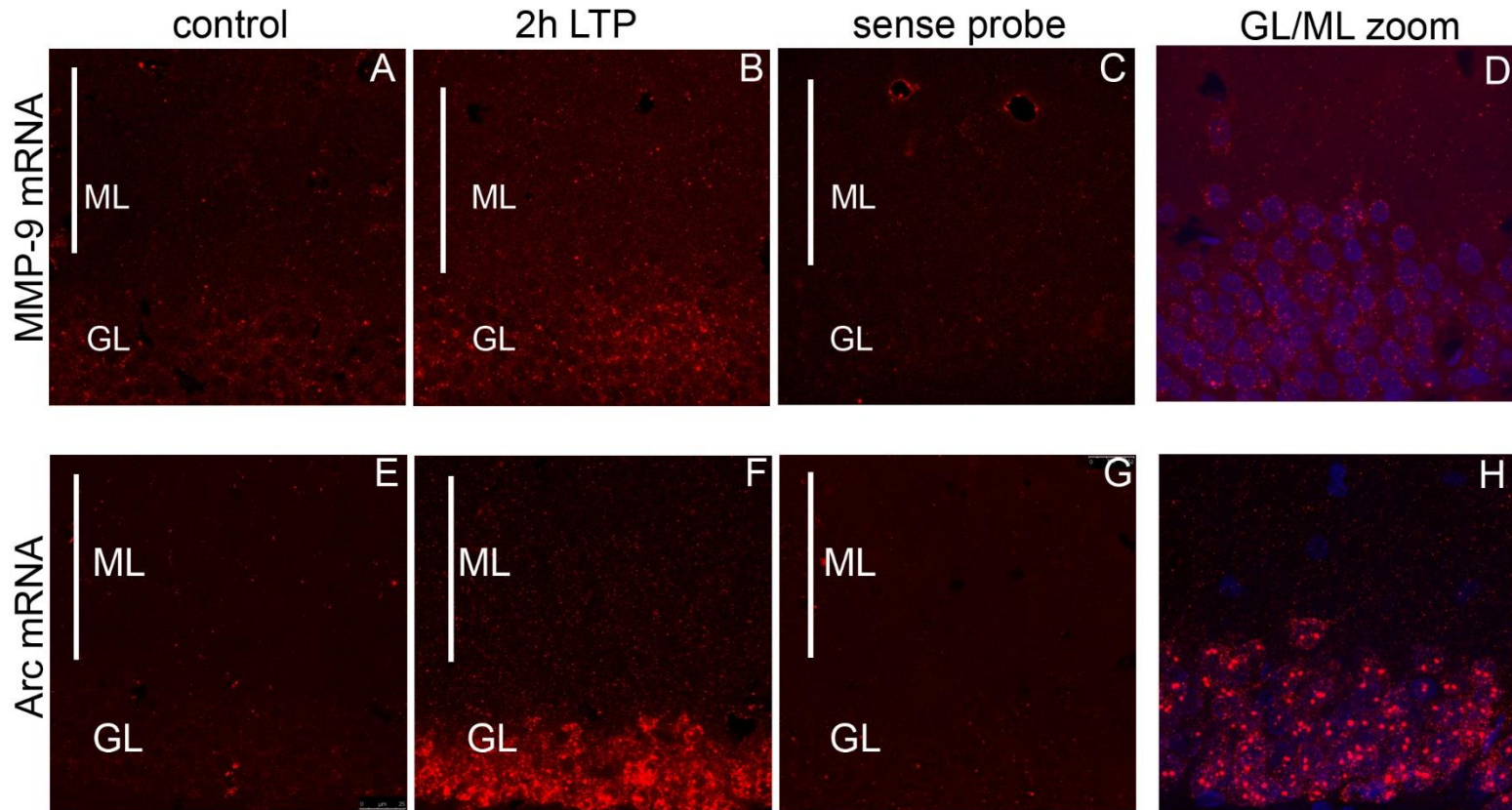
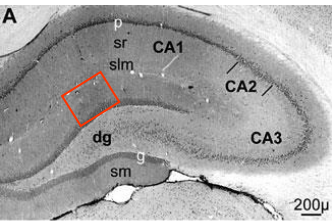
Activity-dependent local translation of MMP-9



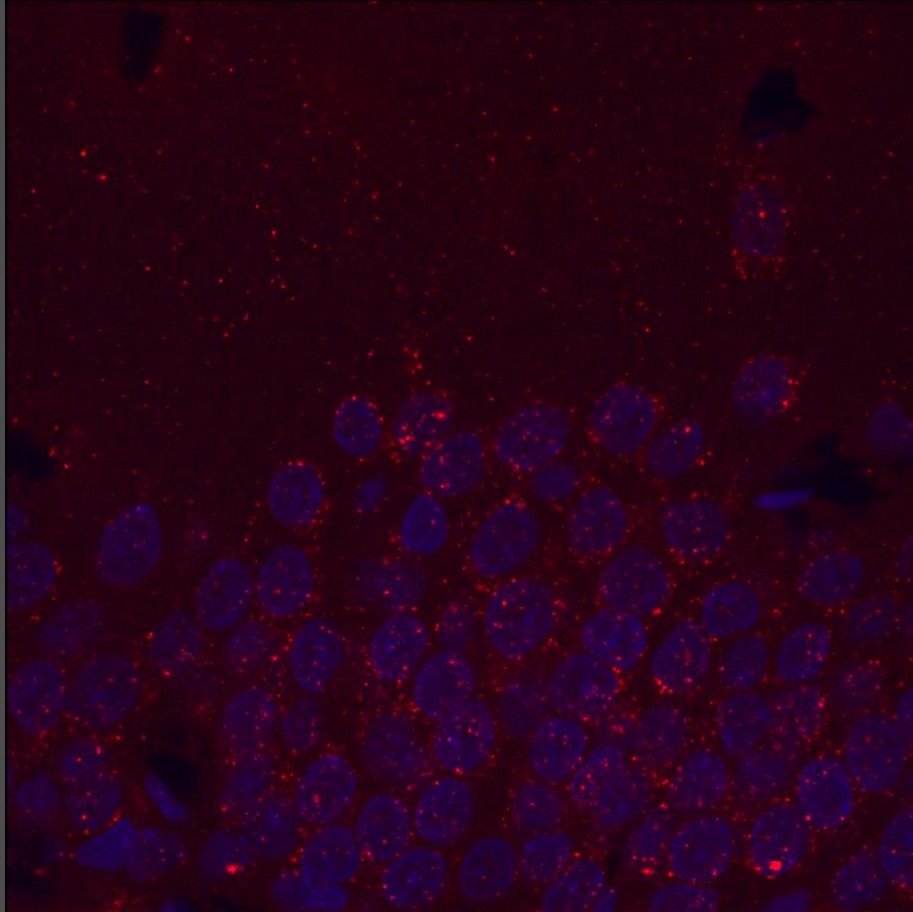
Medial perforant path LTP - a well established model of synaptic plasticity



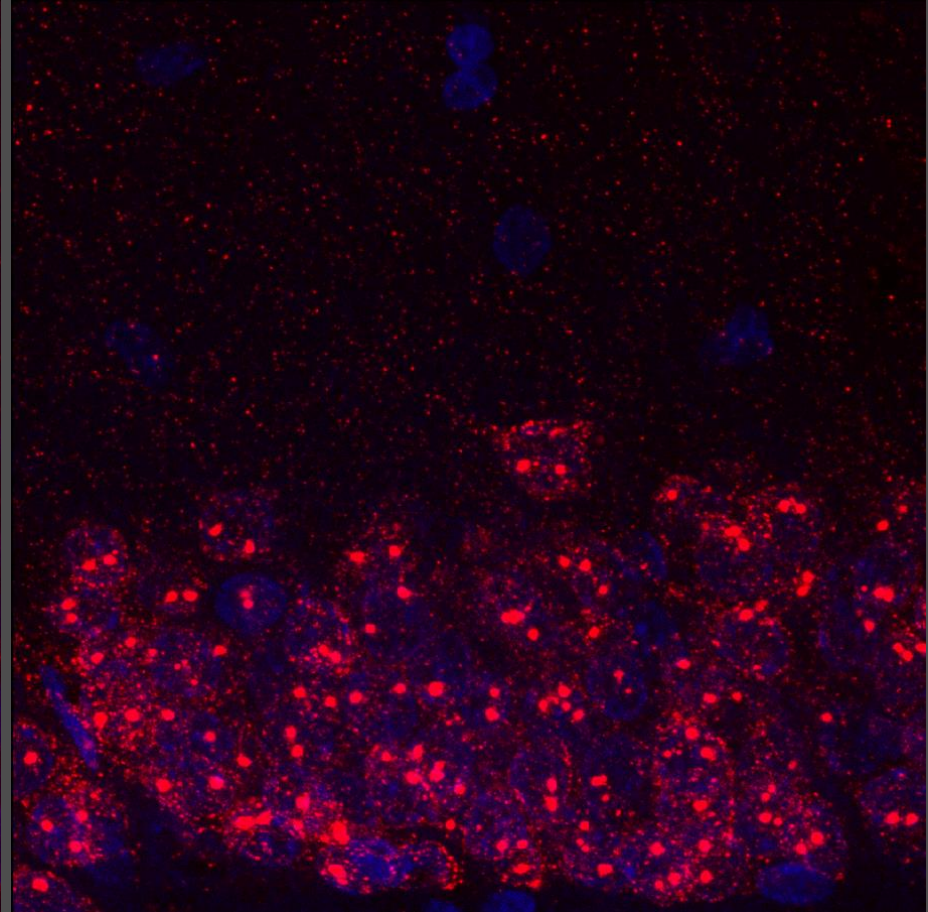
In situ hybridization shows increase in MMP-9 expression in granular layer and molecular layer of dentate gyrus 2h after medial perforant path LTP



MMP-9 in situ hybridization



Arc in situ hybridization



Sushi belt model

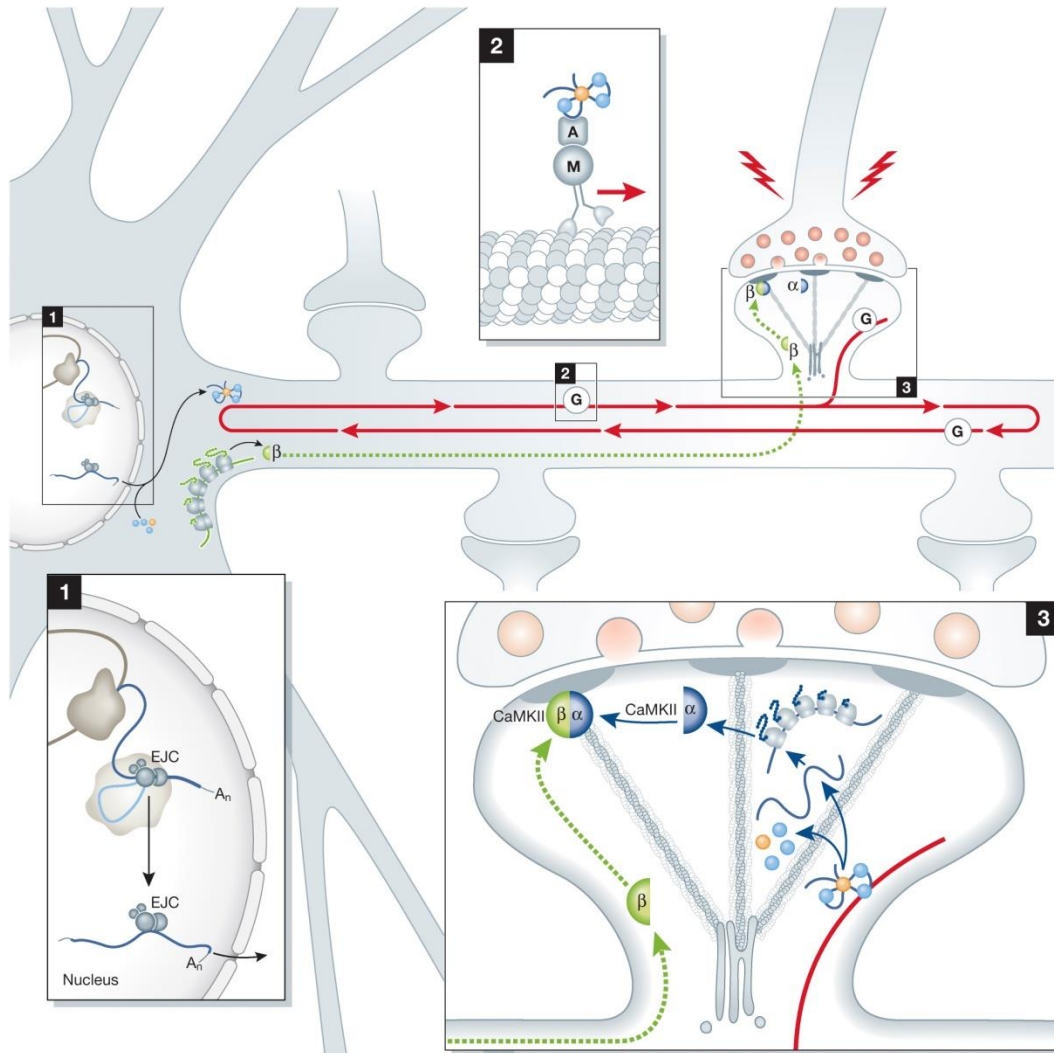
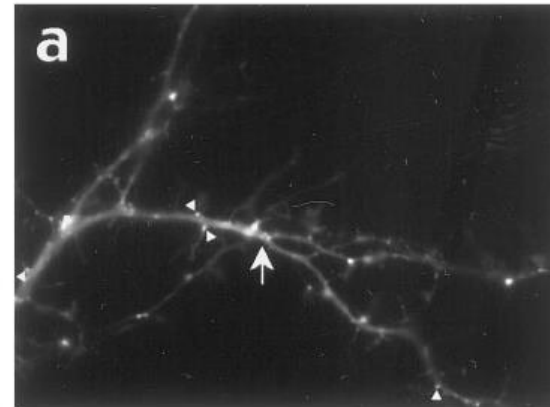
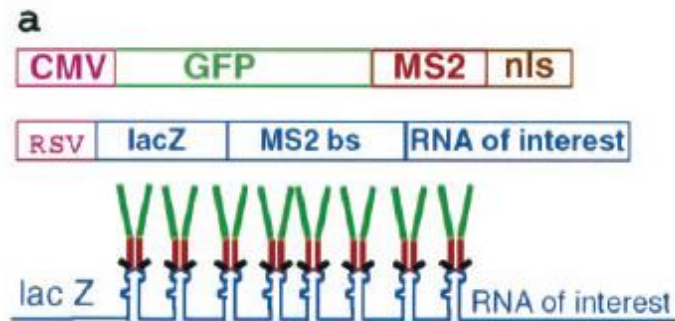


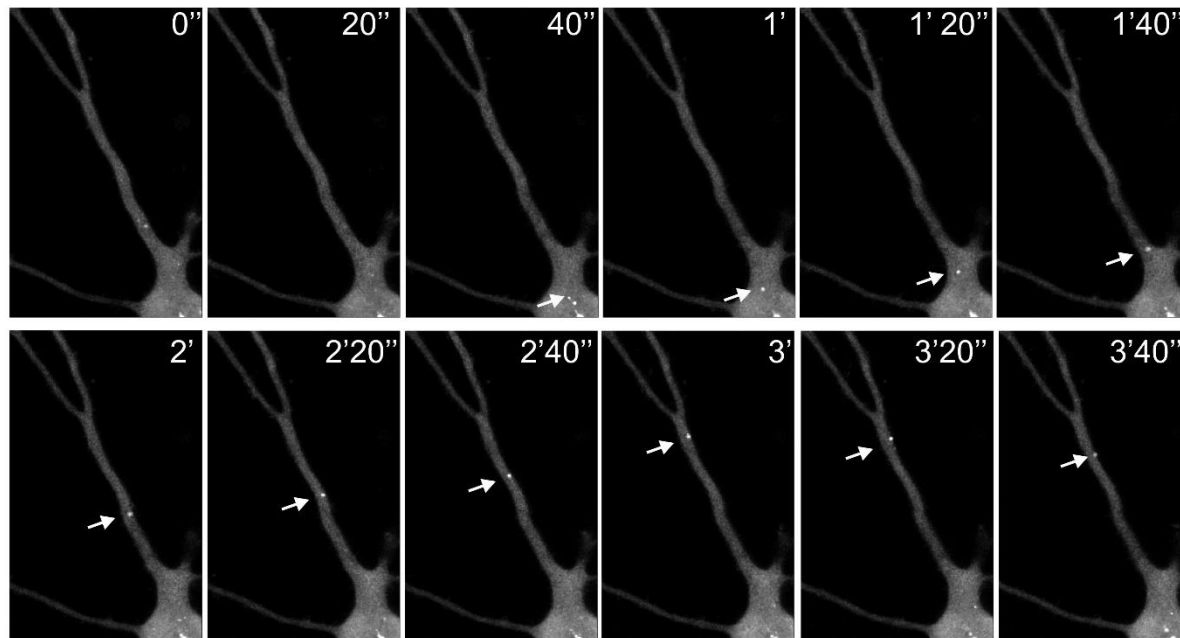
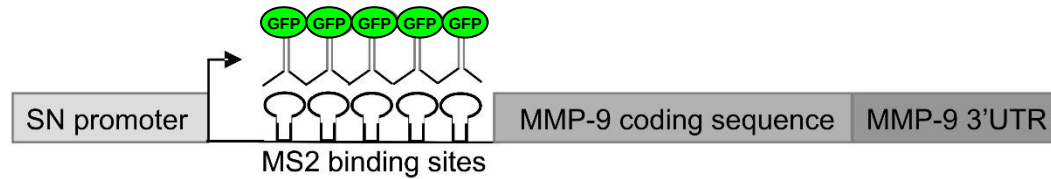
Figure 2 from Michael Doyle and Michael A Kiebler
The EMBO Journal online publication
 doi:10.1038/emboj.2011.278

MS2 system to stain targeted mRNA in the living cell

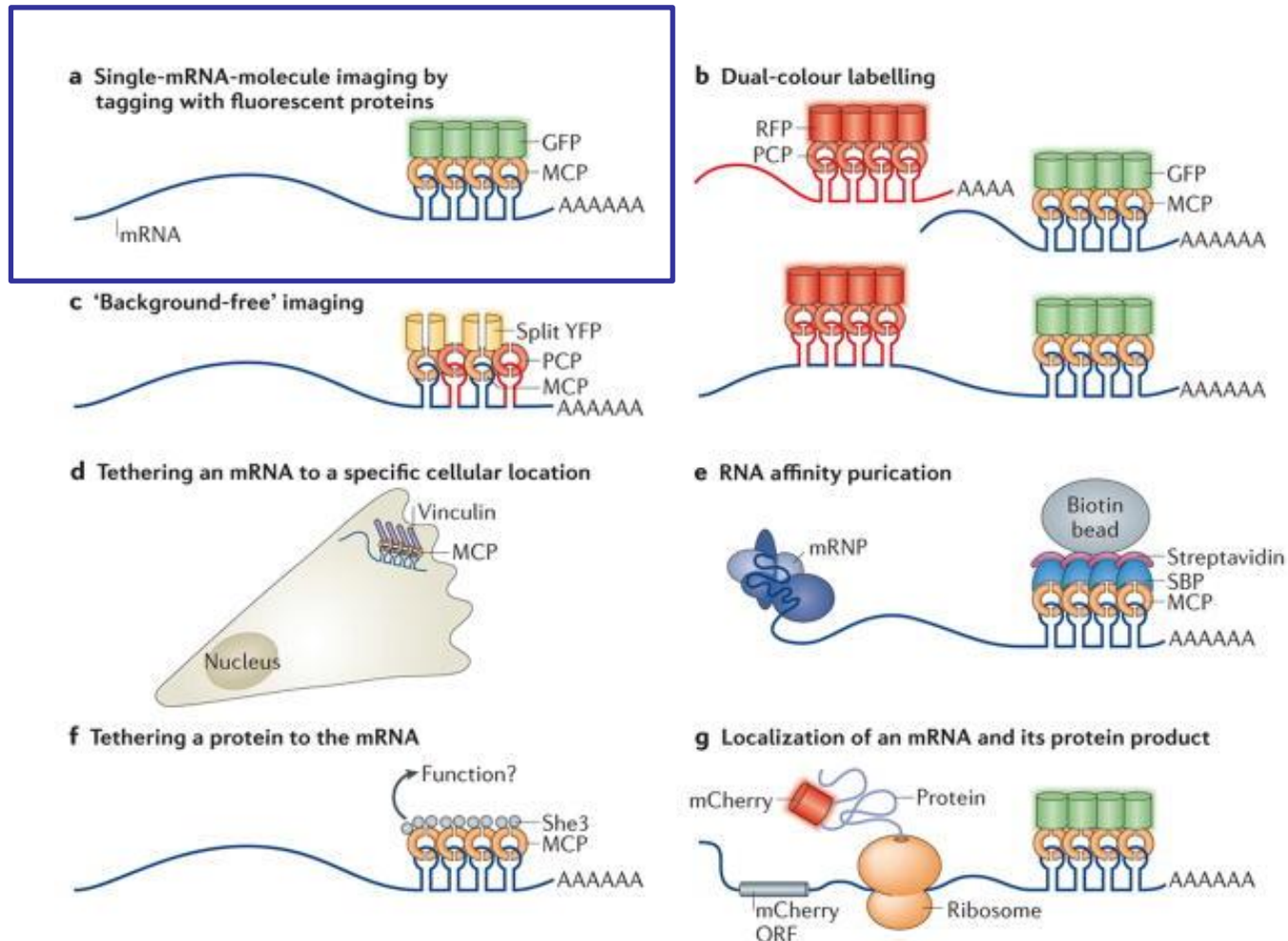


Martha S. Rook, Mei Lu, and Kenneth S. Kosik The Journal of Neuroscience, September 1, 2000, 20(17):6385–6393

MS2 system to stain targeted mRNA in the living cell



Traditional and novel uses of MS2-like systems to investigate mRNA biology

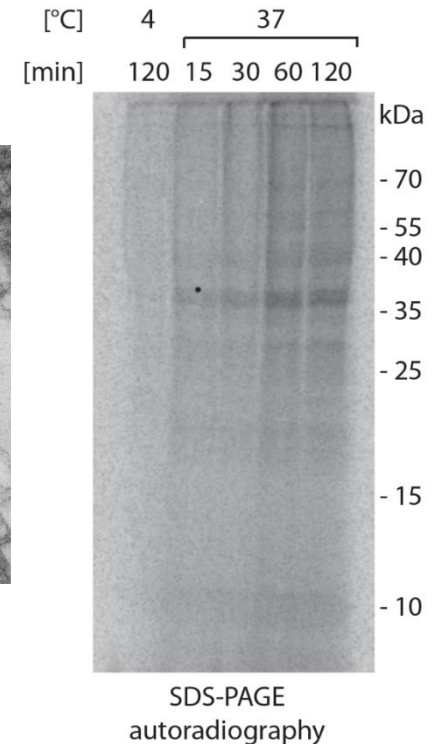
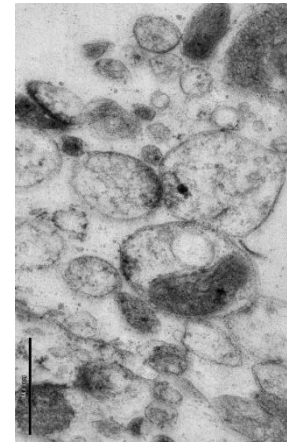
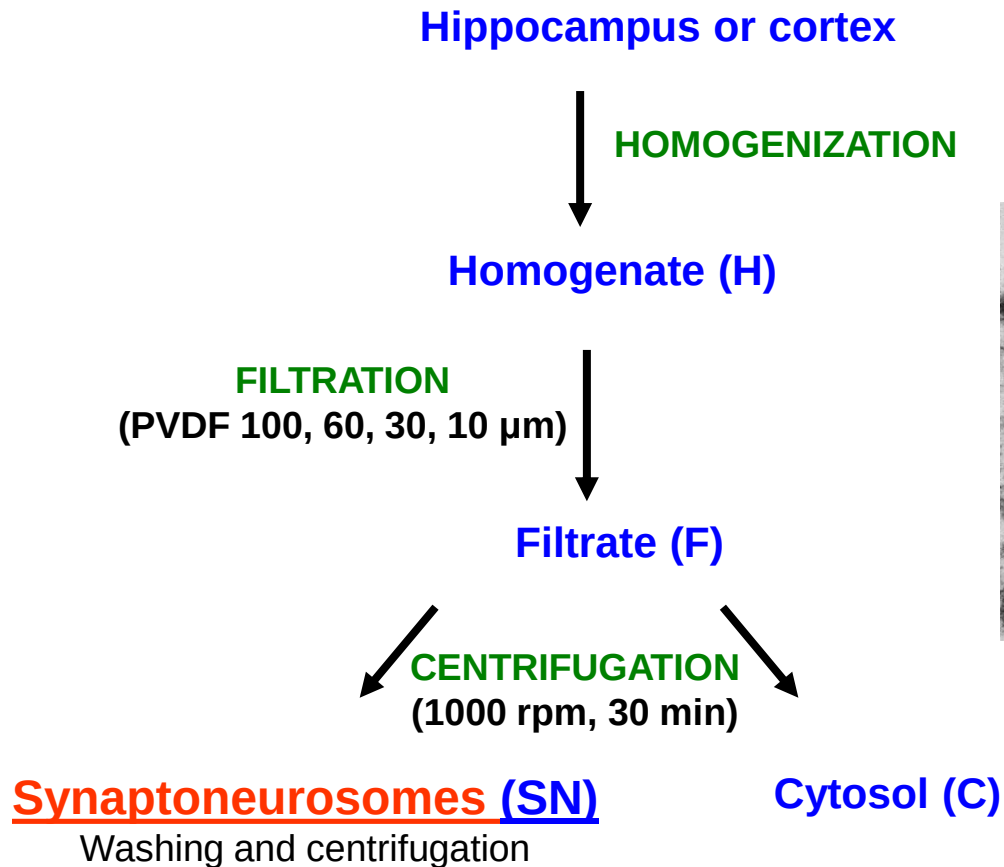


[In the right place at the right time: visualizing and understanding mRNA localization.](#)

Buxbaum AR, Haimovich G, Singer RH.

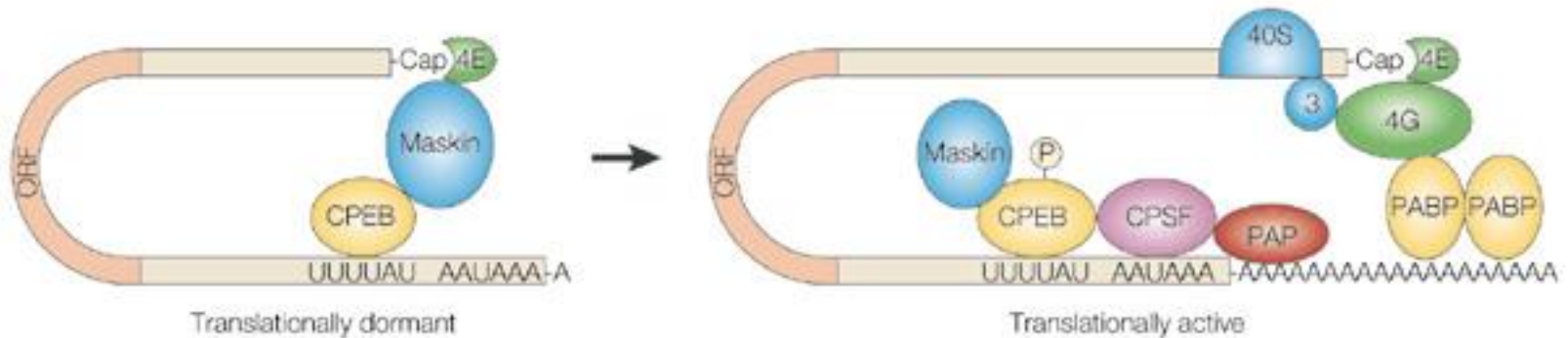
Nat Rev Mol Cell Biol. 2015

MMP-9 protein is enriched in the synaptoneurosomal fraction



1. Polyadenylation of MMP-9 in response to synaptic stimulation
2. Isolation of polyribosomes
3. Direct study of MMP-9 protein synthesis by Click-iT chemistry

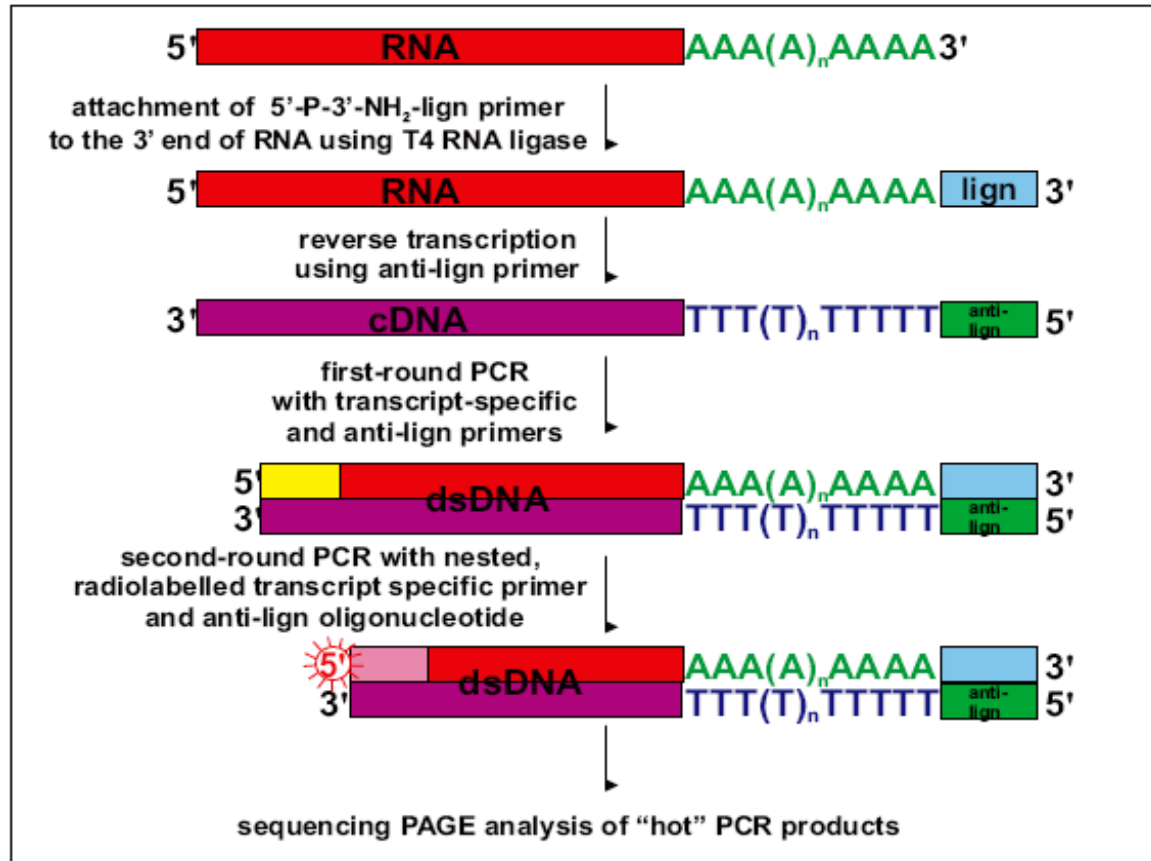
Cytoplasmic polyadenylation promotes translation



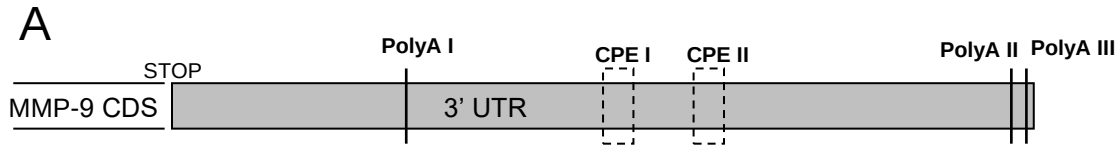
Nature Reviews | Molecular Cell Biology

Mendez, R. & Richter, J. D. Translational control by CPEB: a means to the end. *Nature Reviews Molecular Cell Biology* 2, 521–529 (2001)

PAT assay

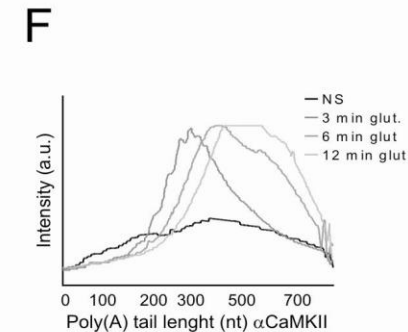
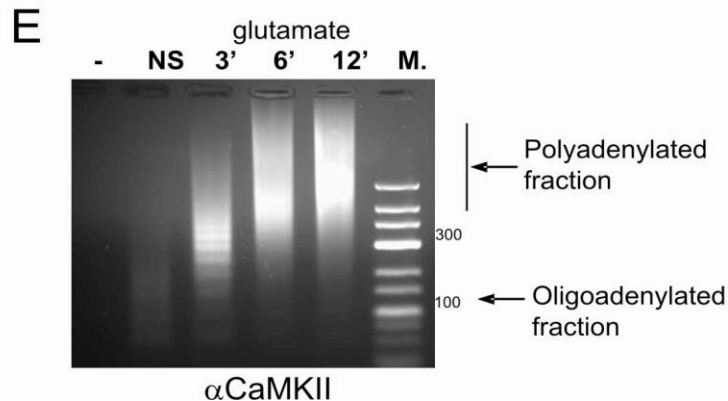
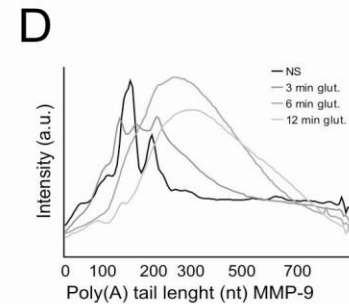
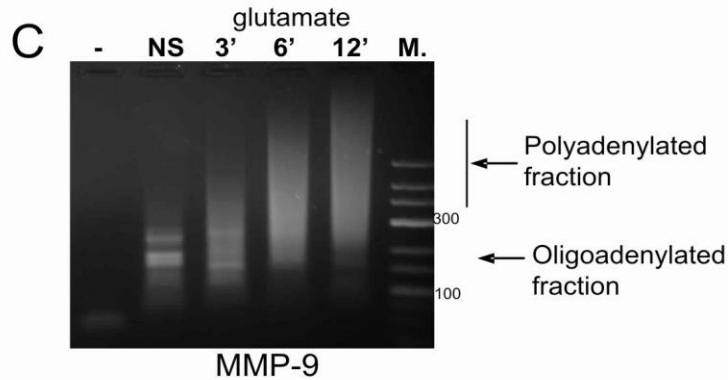


MMP-9 polyadenylation measured by PAT ssay in synaptoneurosomes after glutamate stimulation

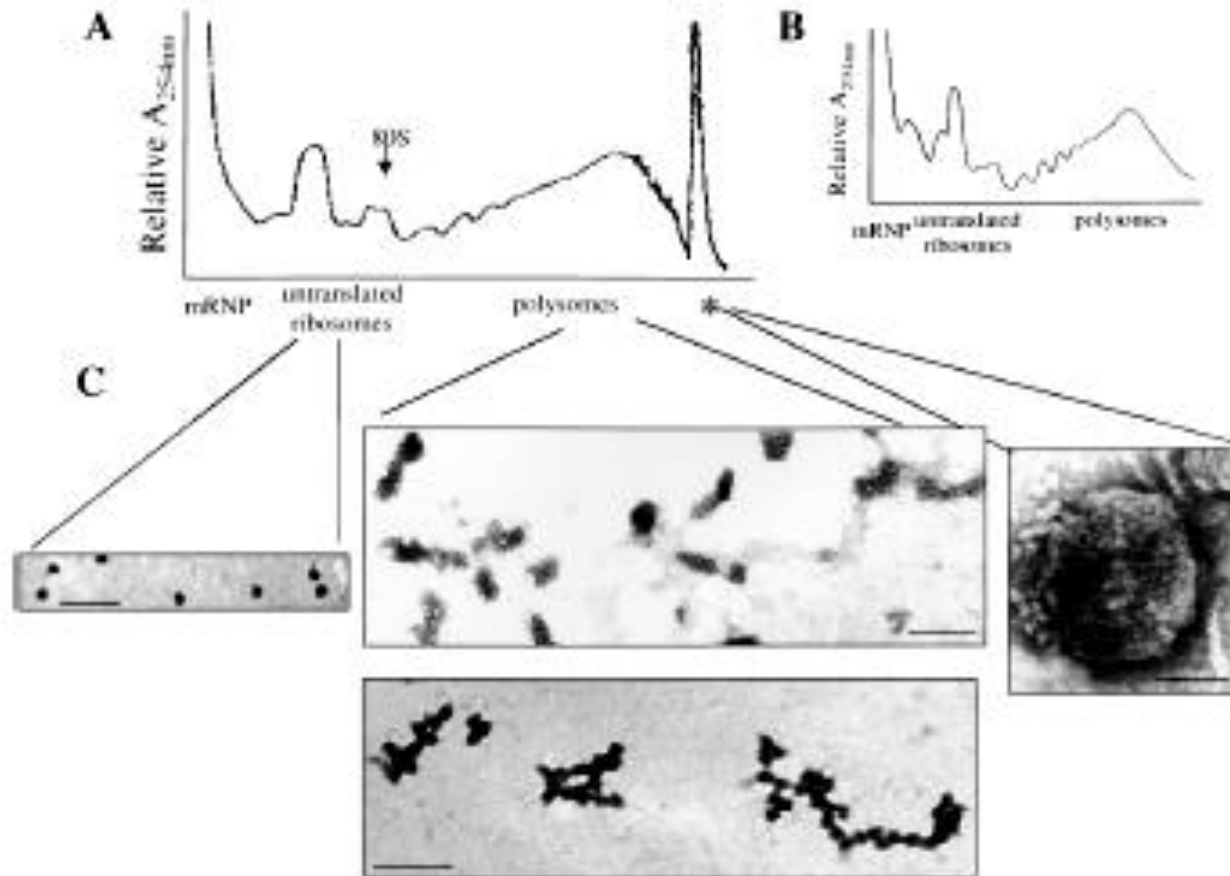


B

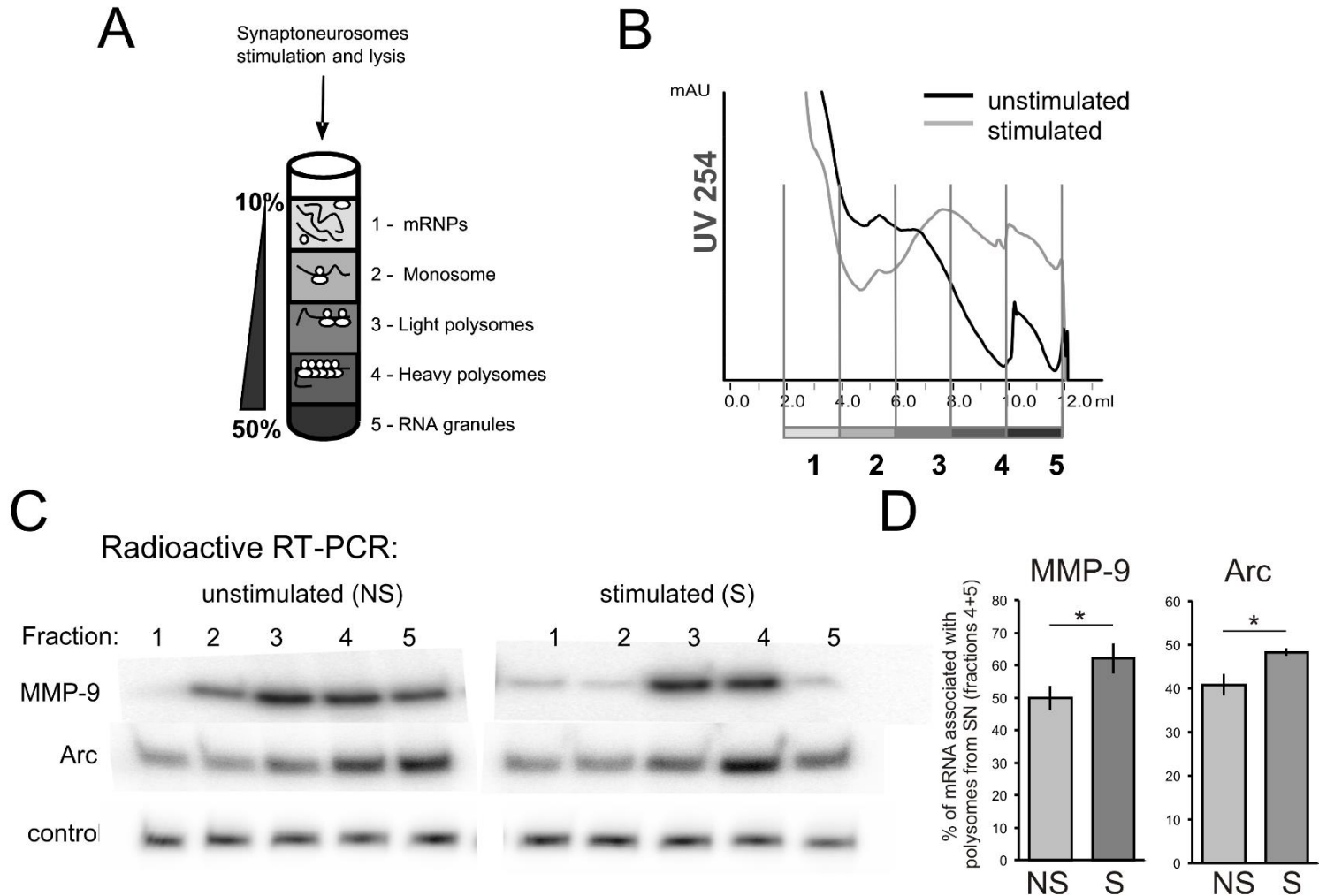
CPE I	R. norvegicus	2416	ACCUUUUGUUUUUAUGGG	2433
	M. musculus	2502	ACCUUUUAUUUUUGUGUG	2519
CPE II	R. norvegicus	2500	CCCUUUUAUUUAUUAUGU	2517
	M. musculus	2592	CCCUUUUAUUUAUUAUGU	2609



Polysomal fractionation



Polyribosome association of MMP-9 mRNA in synaptoneurosomes



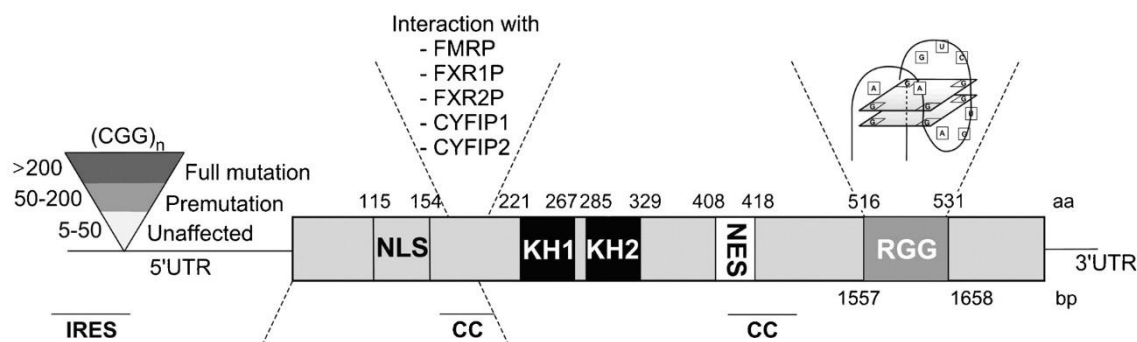
Badanie oddziaływania białko-RNA (na przykładzie FMRP-mRNA MMP-9)

Brak FMRP prowadzi do zespołu łamliwego chromosomu X (Fragile X syndrome, FXS)

to choroba genetyczna skutkująca między innymi opóźnieniem rozwoju umysłowego i zaburzeniami ze spektrum autyzmu.

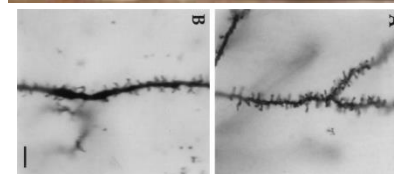
Występuje u 1:4000 mężczyzn oraz 1:8000 kobiet i odpowiada za 5% zdiagnozowanych przypadków autyzmu.

Zespół łamliwego chromosomu X jest spowodowany wyciszeniem genu *Fmr1* i wskutek tego, brakiem białka łamliwego chromosomu X (FMRP).



C D'Hulst, R F Kooy, J Med Genet 2009

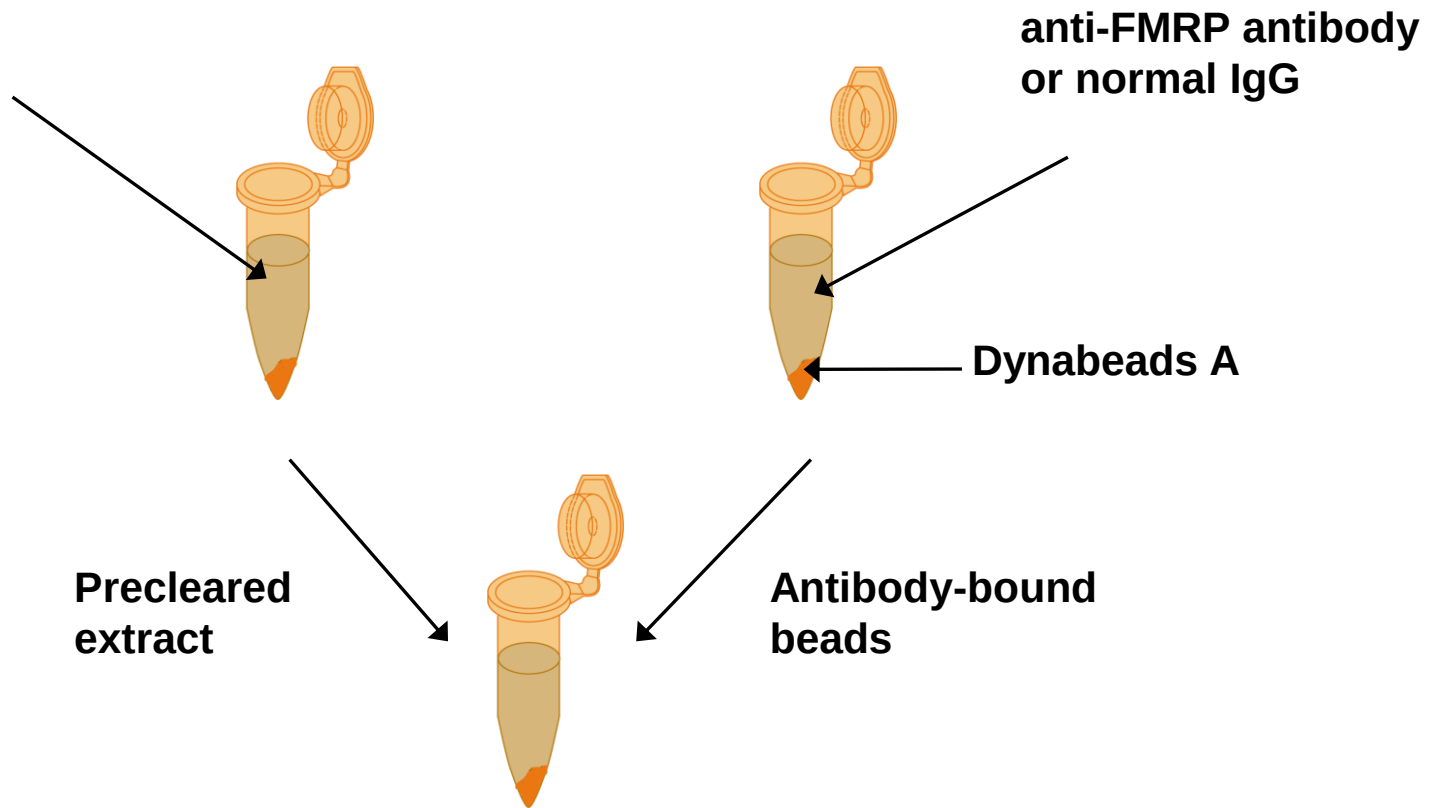
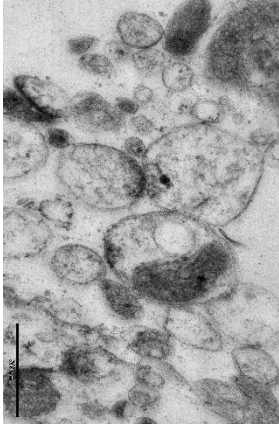
Myszy *Fmr1* KO



Rudelli et al., 1985

Coimmunoprecipitation of FMRP protein with MMP-9 mRNA

Synaptoneurosome
extract



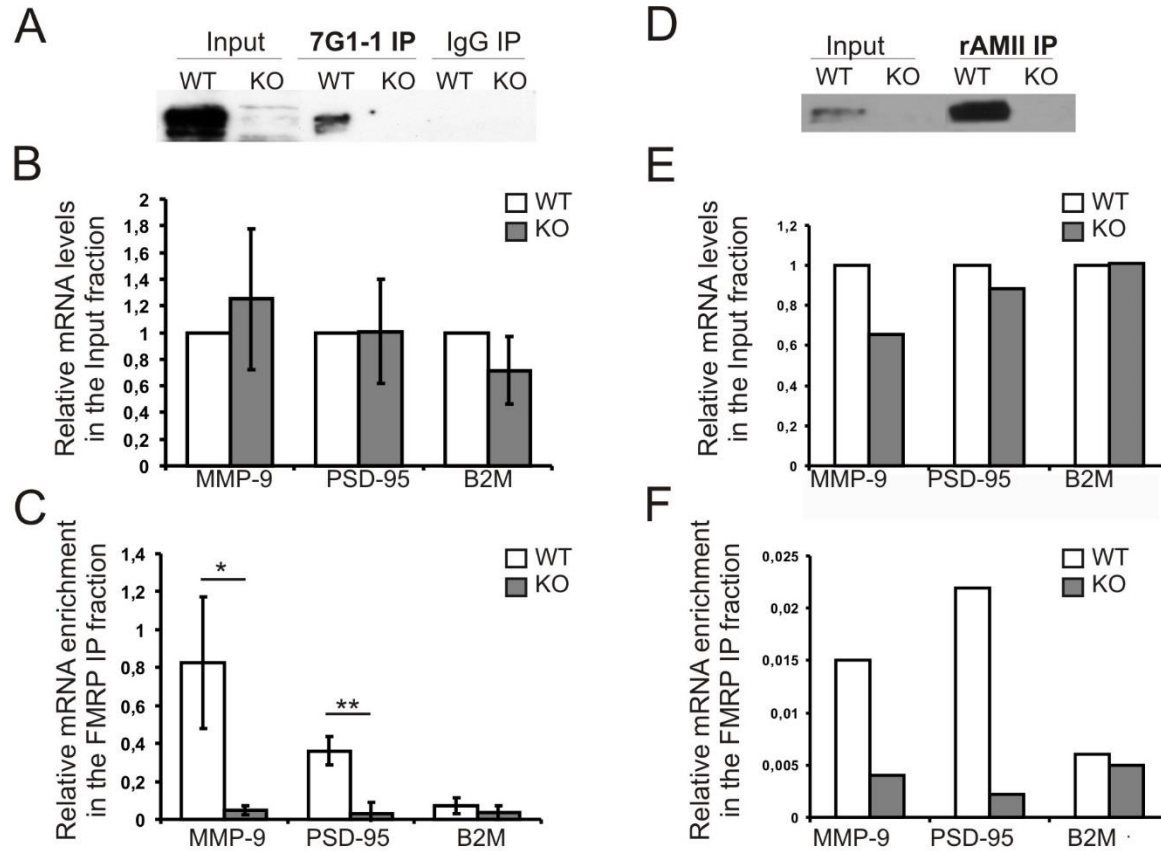
Precleared
extract

Antibody-bound
beads

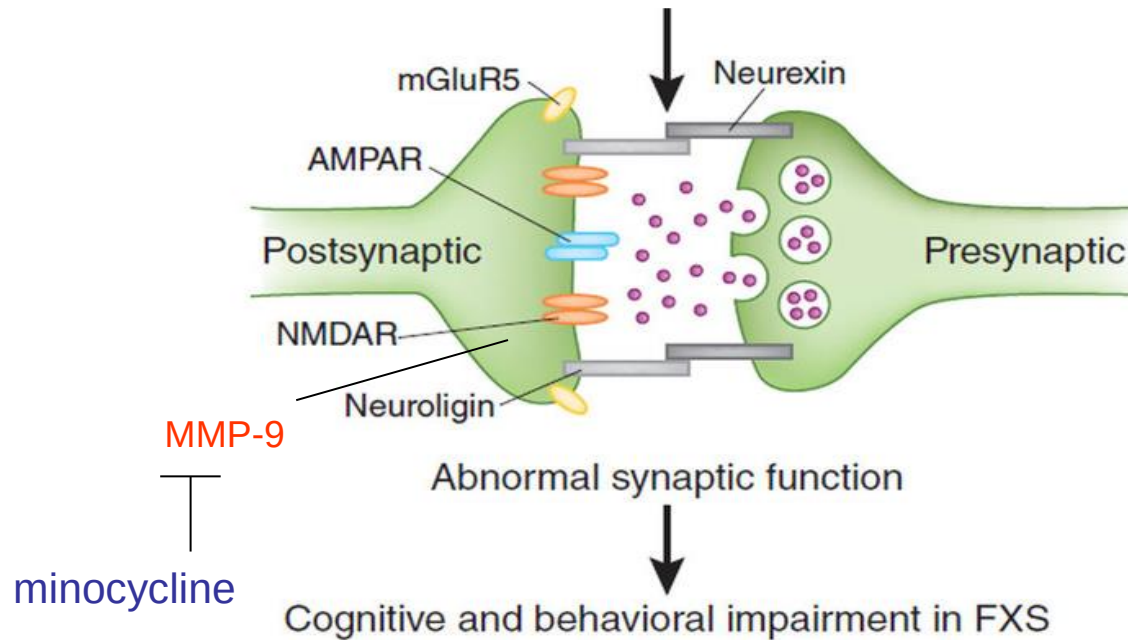
Immunoprecipitated complexes

1. Western blot
2. RNA isolation and RT-PCR

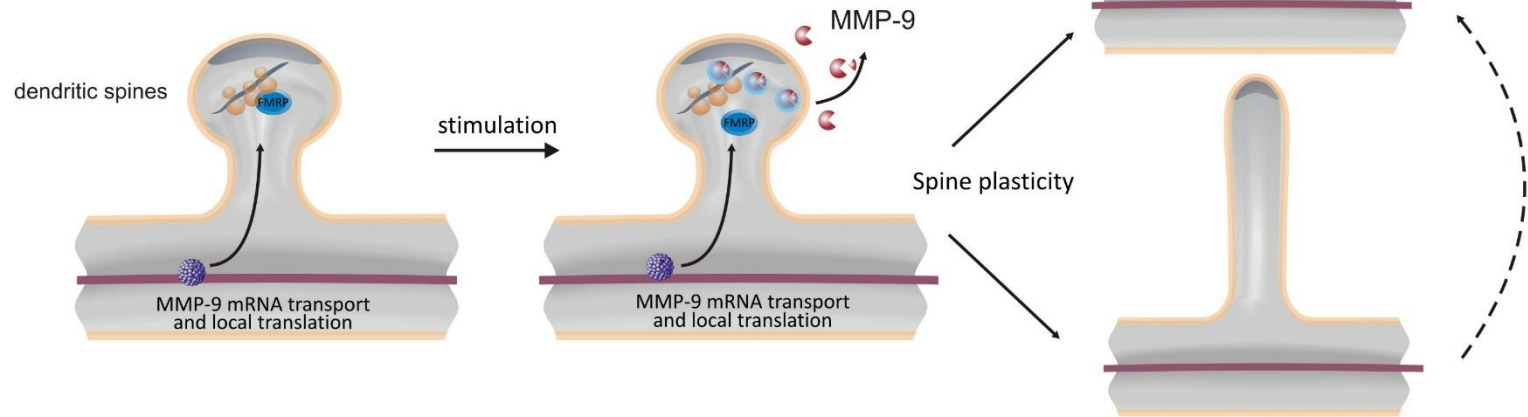
MMP-9 mRNA is precipitated with anti-FMRP antibodies



Local translation of MMP-9 is regulated by FMRP



Healthy



FXS

