Methods to study RNA in neurons

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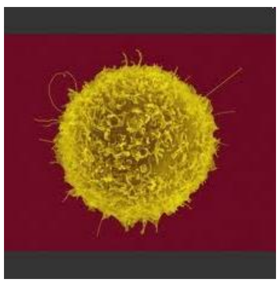
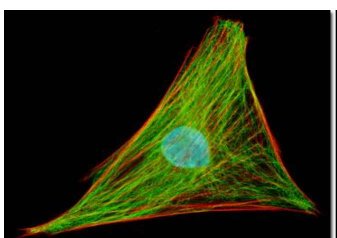
LABORATORIUM MOLEKULARNYCH PODSTAW PLASTYCZNOŚCI SYNAPTYCZNEJ

UNIWERSYTET WARSZAWSKI

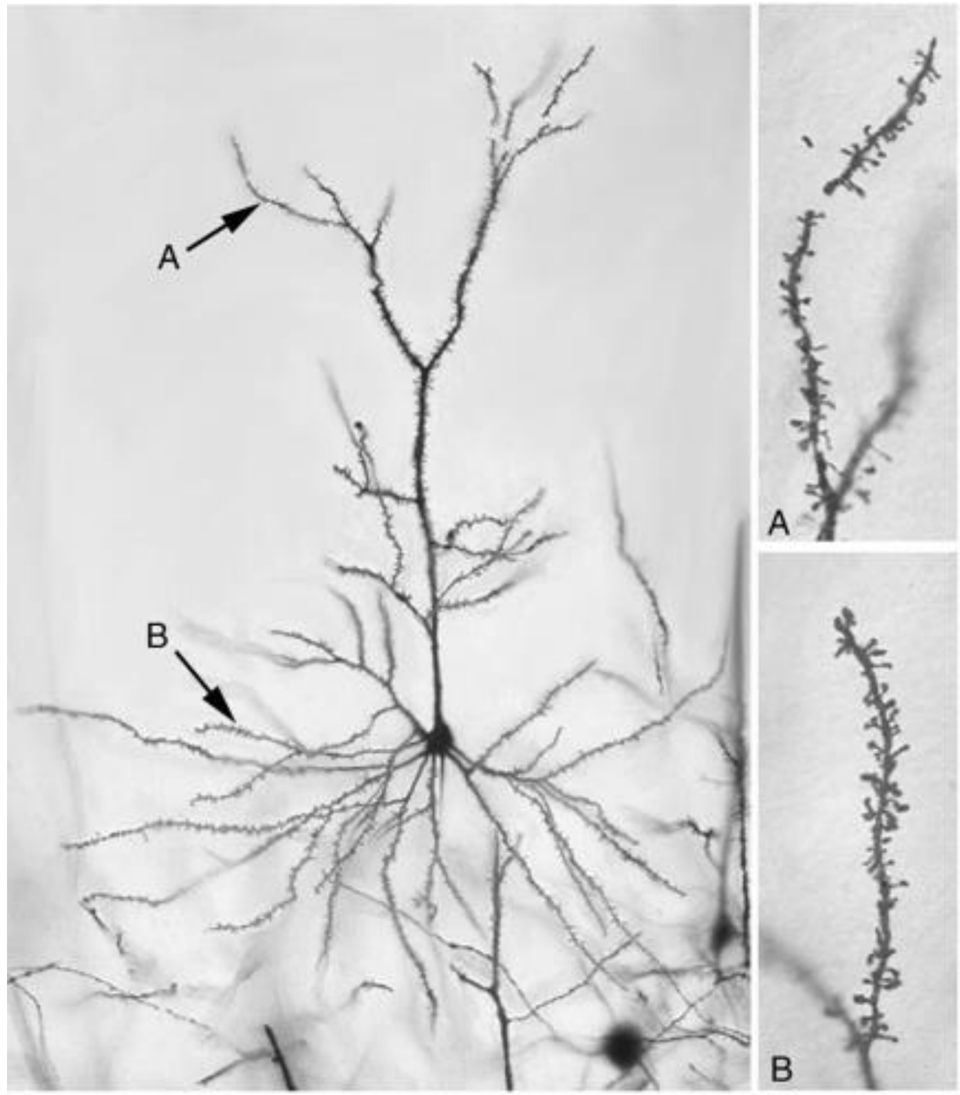
CeNT CENTRUM NOWYCH TECHNOLOGII



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**

Synapses are located on dendritic spines. Dendritic spines are dynamic structures that can change shape in response to stimulation.

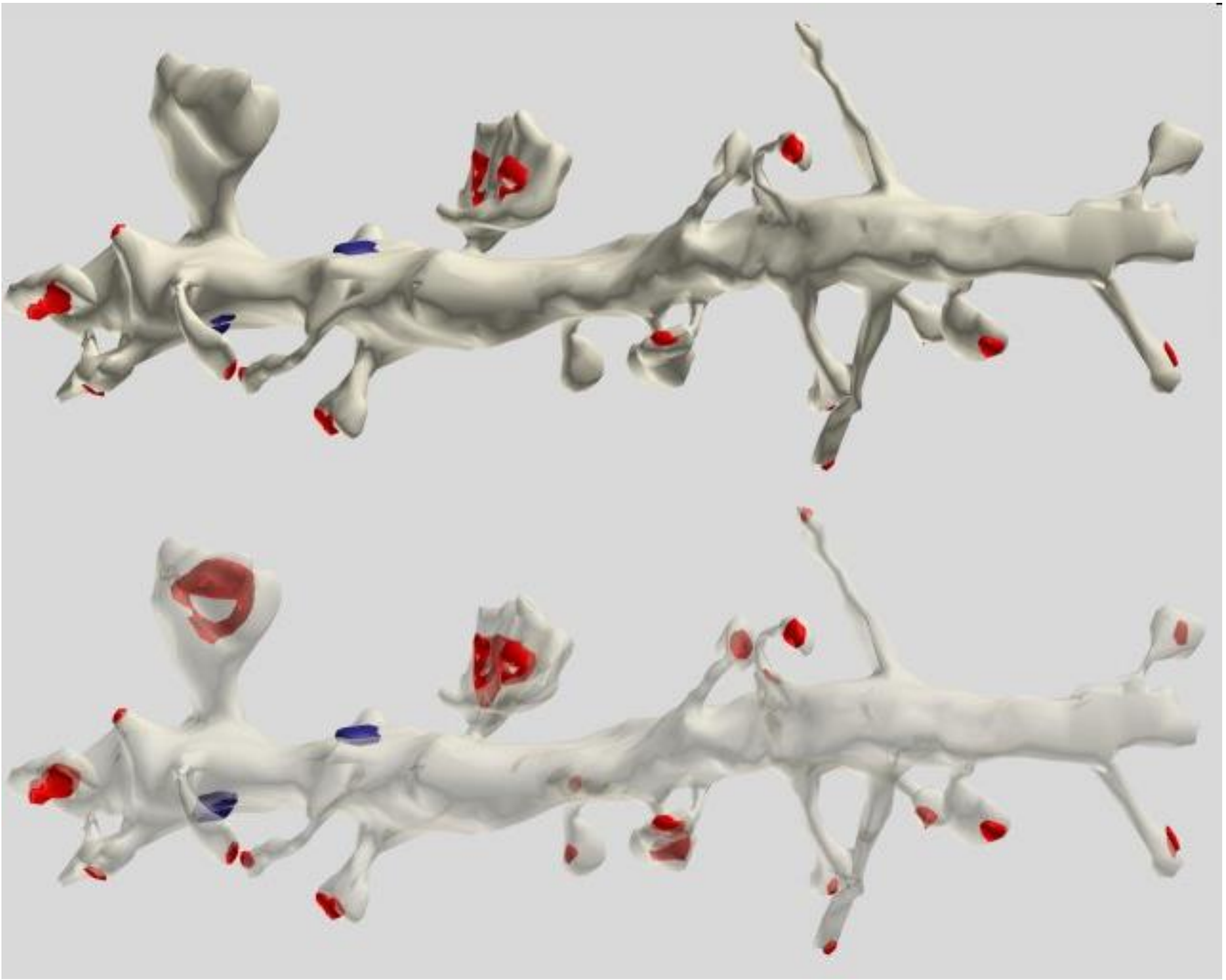
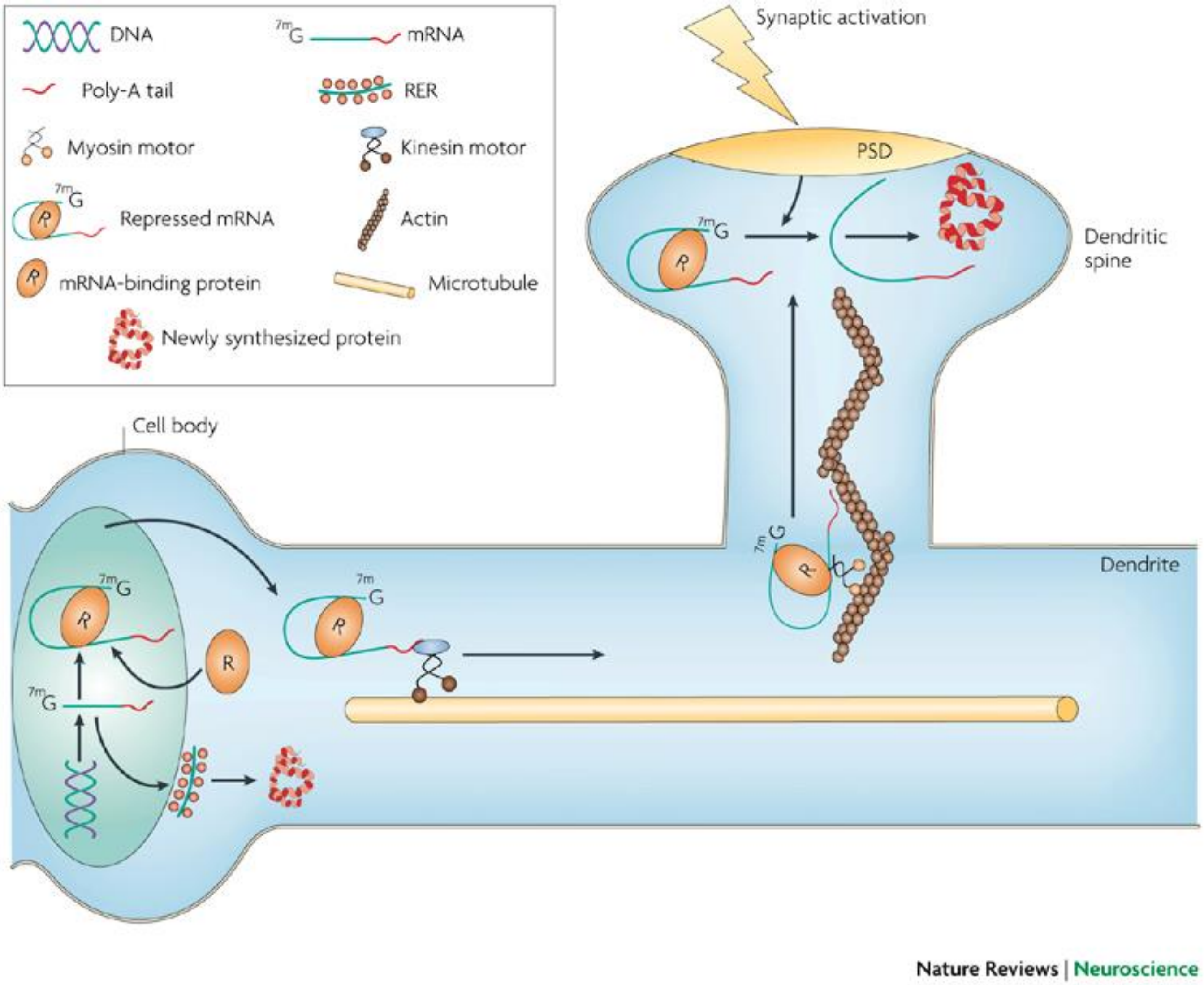
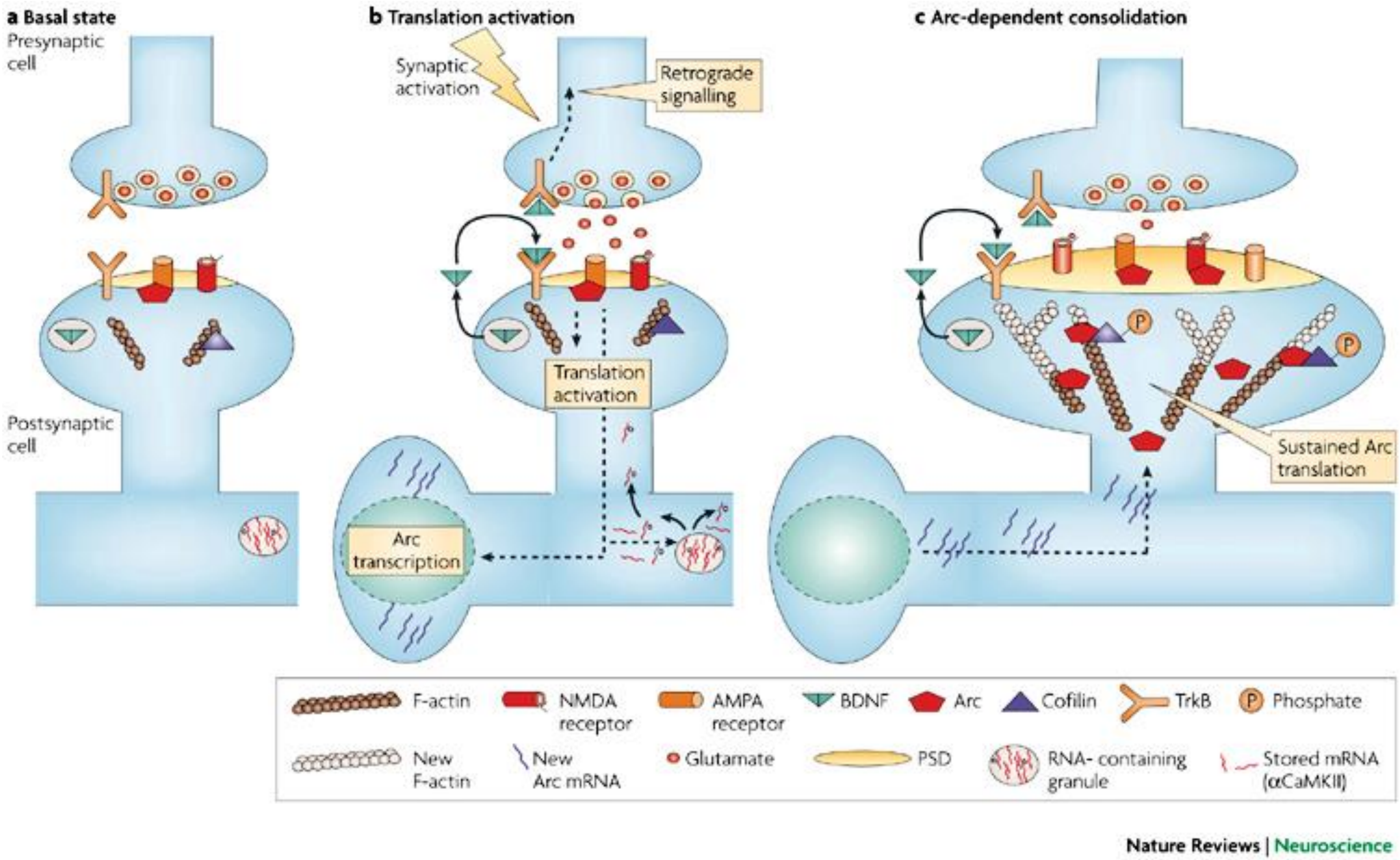


Fig. 1: A segment of pyramidal cell dendrite from stratum radiatum (CA1) with thin, stubby, and mushroom-shaped spines. Spine synapses colored in red, stem (or shaft) synapses colored in blue. The dendrite was made transparent in the lower image to enable visualization of all synapses. *Photo by* Josef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

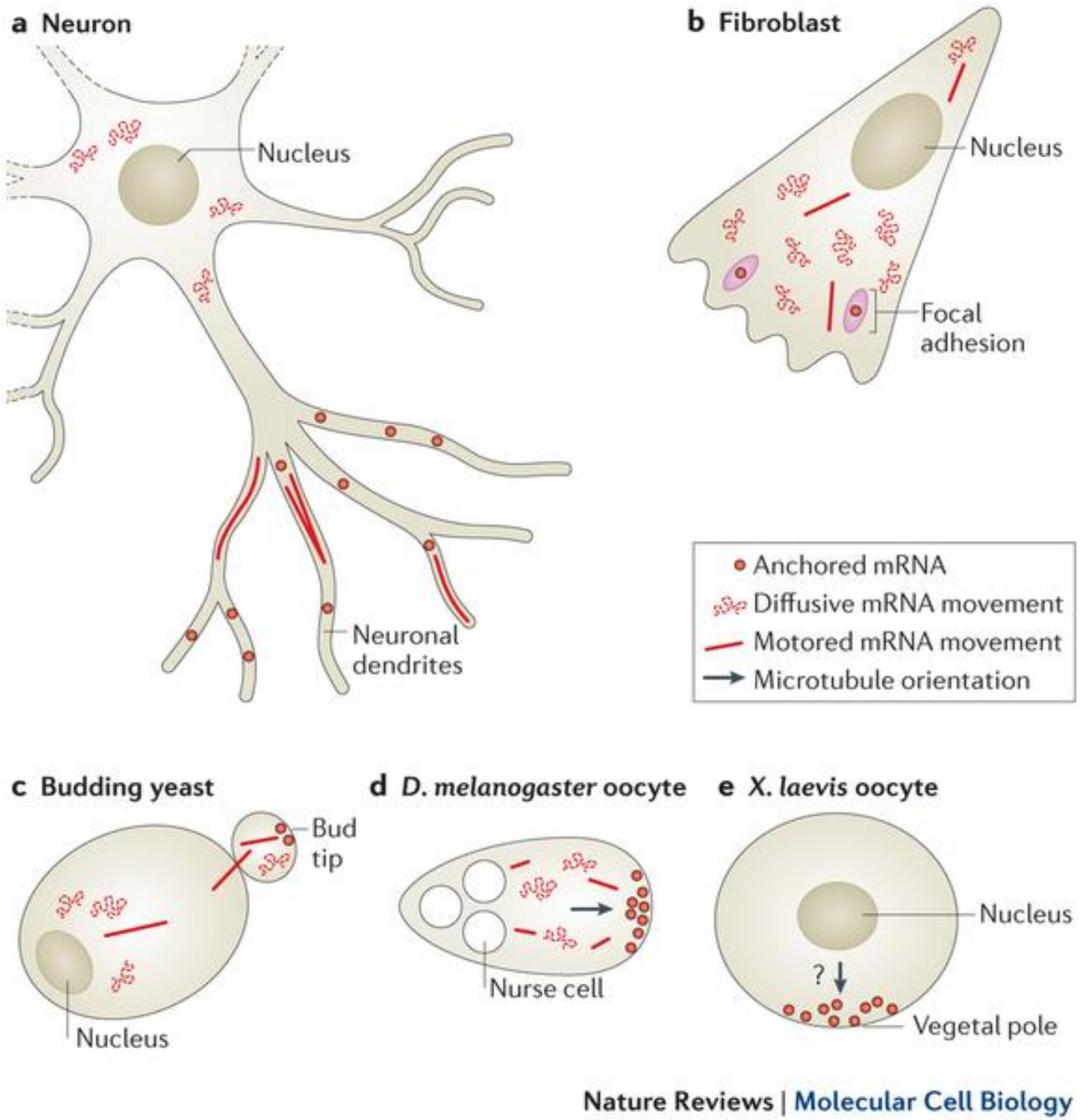
Local mRNA translation in dendritic spines



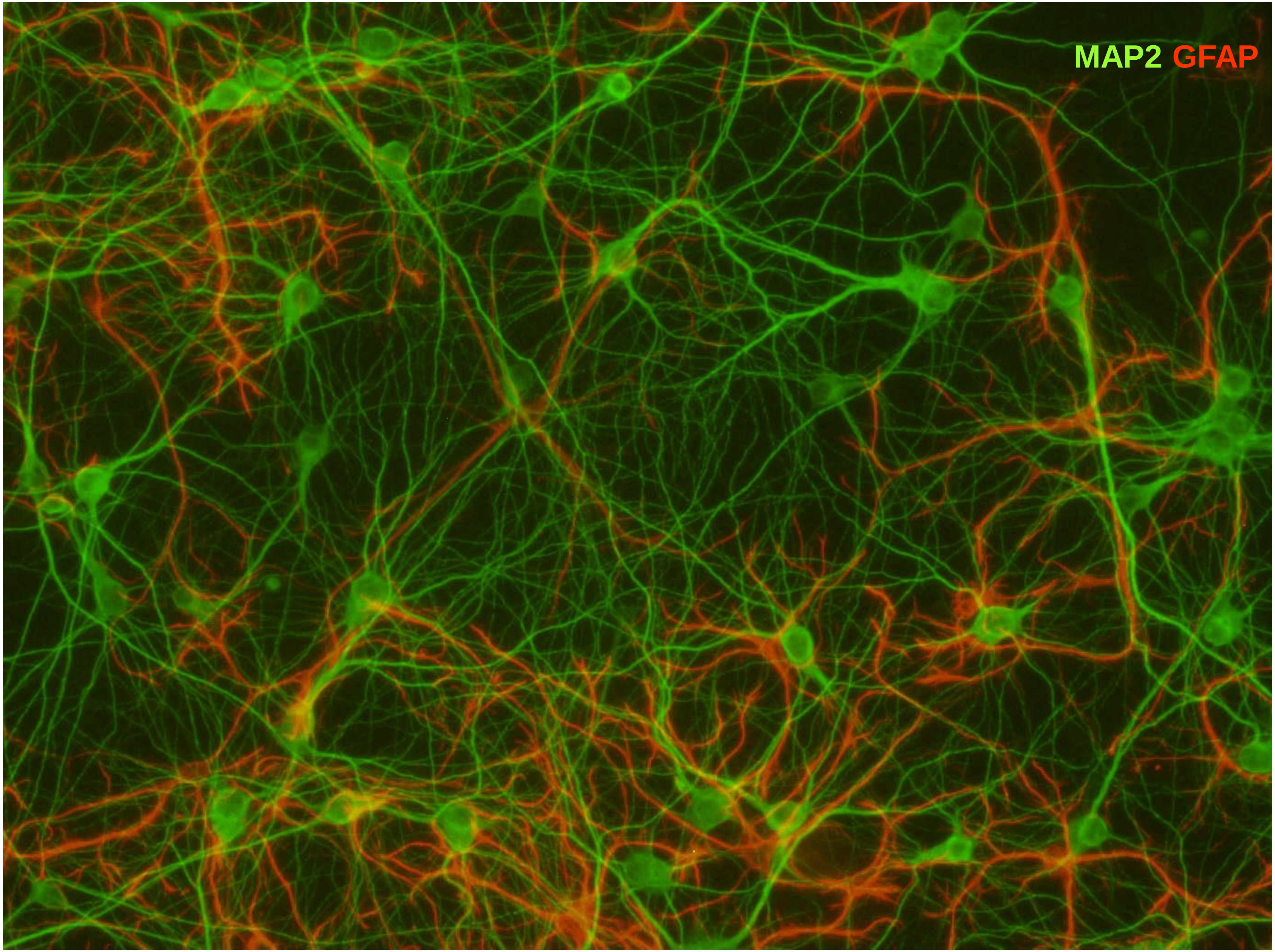
A model of Arc-dependent LTP consolidation in the dentate gyrus



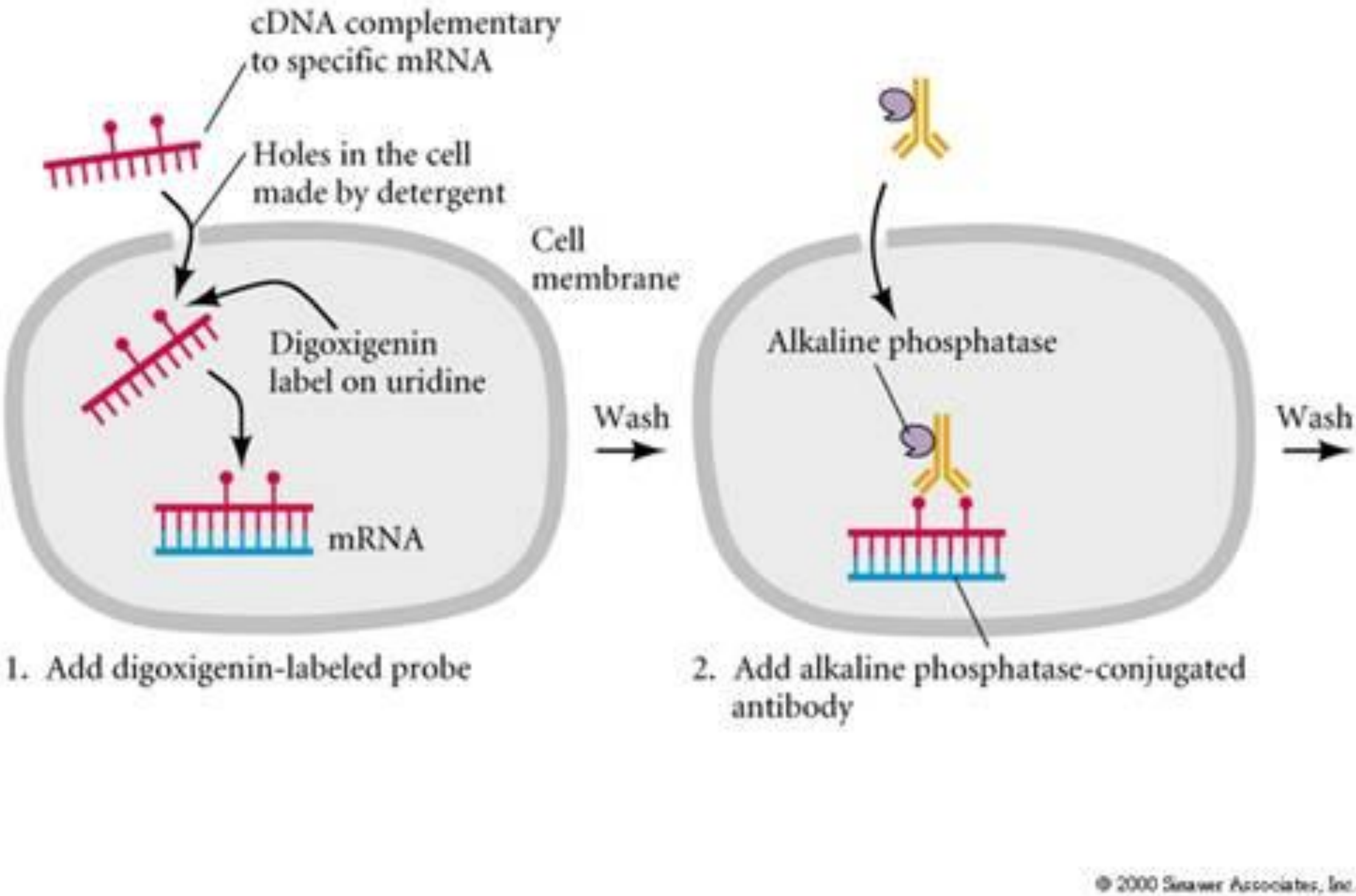
Differential mRNA localization depending on cel types



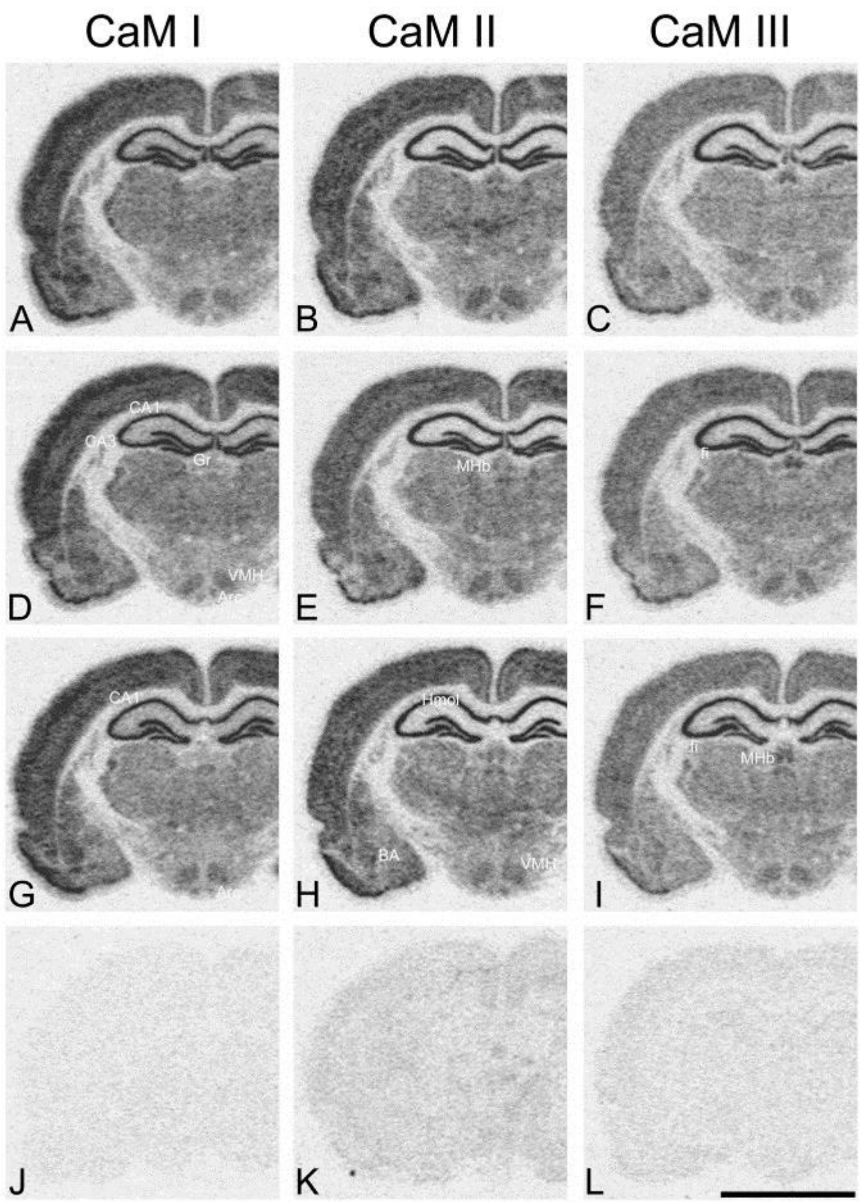
Methods of mRNA visualization in neurons



In situ hybridization with RNA probe labelled with Digoxigenine

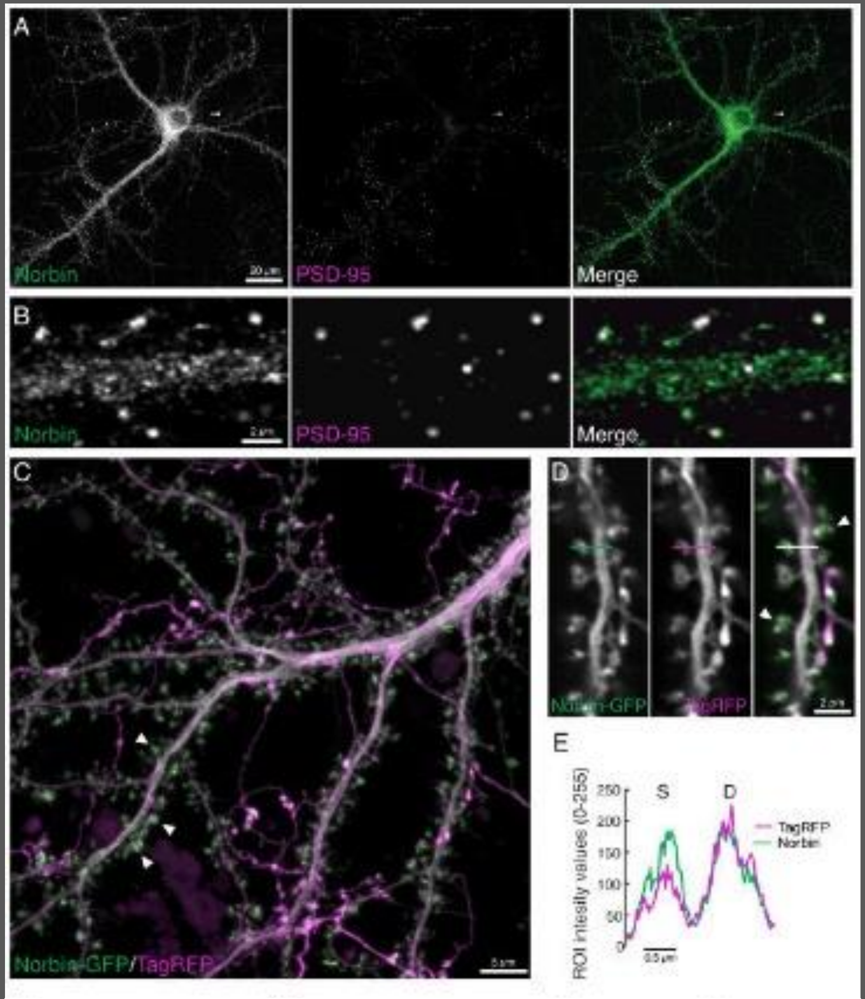


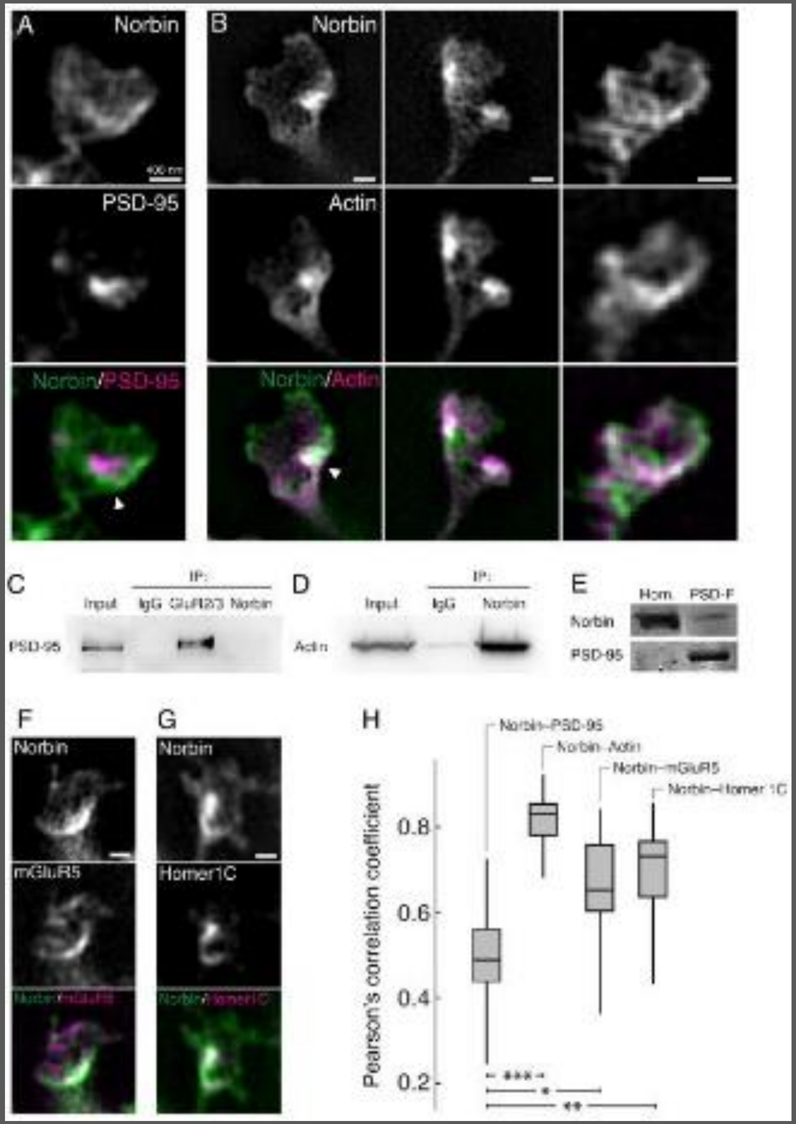
in situ hybridization with RNA probe labelled with radioactive Sulphure



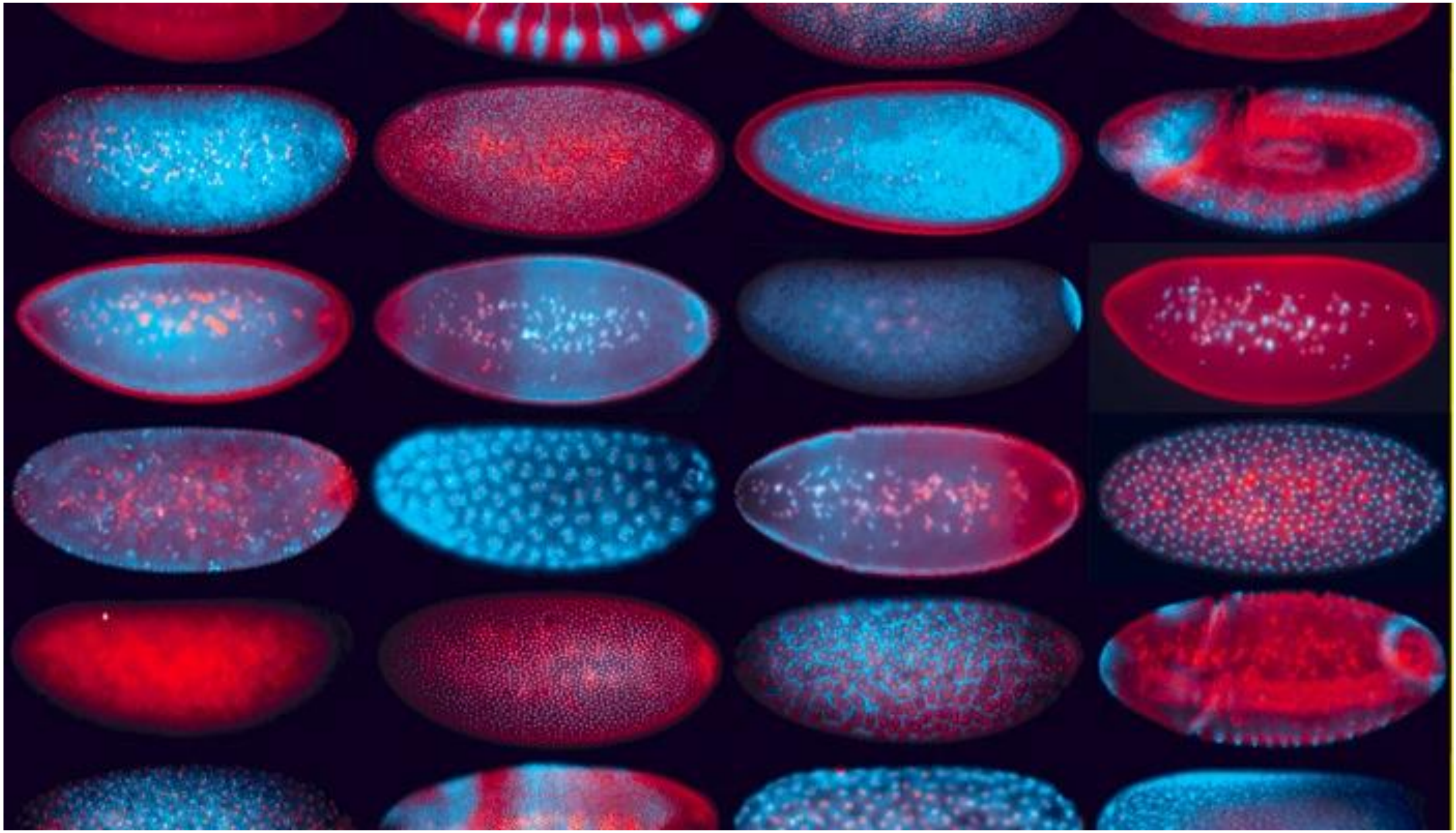
Vizi S. i wsp.,
Brain Research Protocols 2001

in situ hybridization in high resolution microscopy
(below 200 nm)



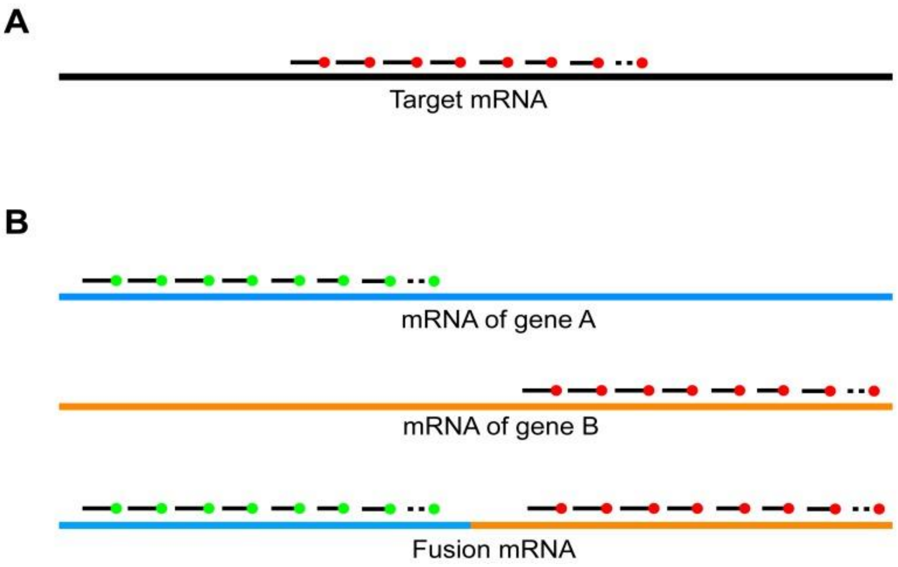


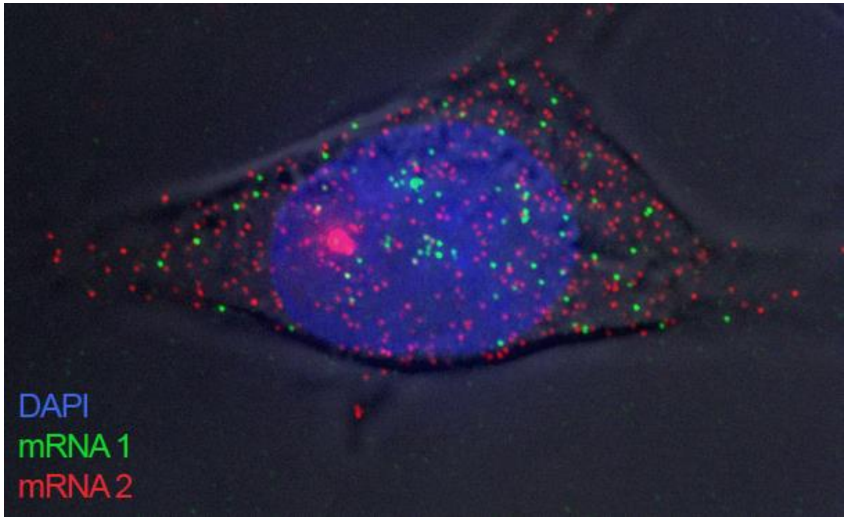
High-resolution fluorescent in situ hybridization procedure to comprehensively evaluate mRNA localization dynamics during early *Drosophila* embryogenesis.



Lecuyer et al. 2007

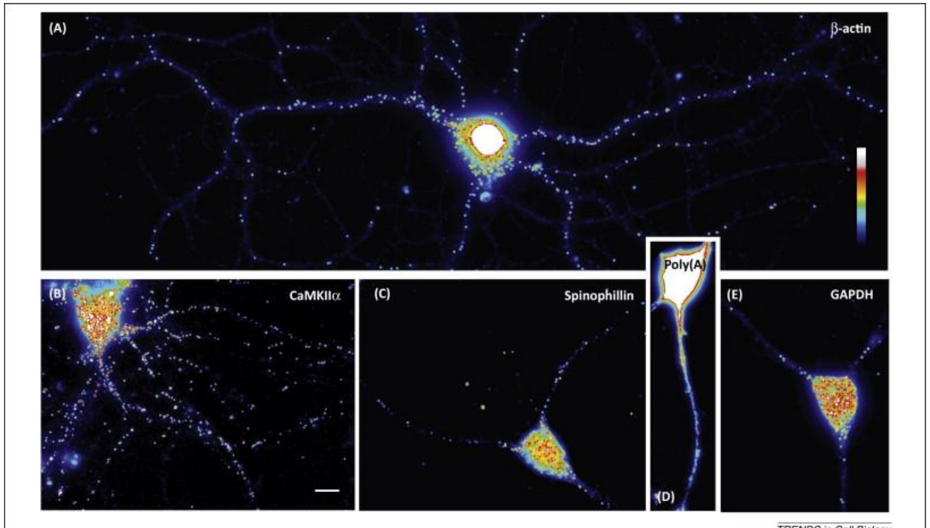
„Single molecule FISH” is an in situ hybridization method that allows imaging of a single mRNA molecule in a cell by using multiple fluorescently labeled probes designed to recognize sequences within the same mRNA molecule

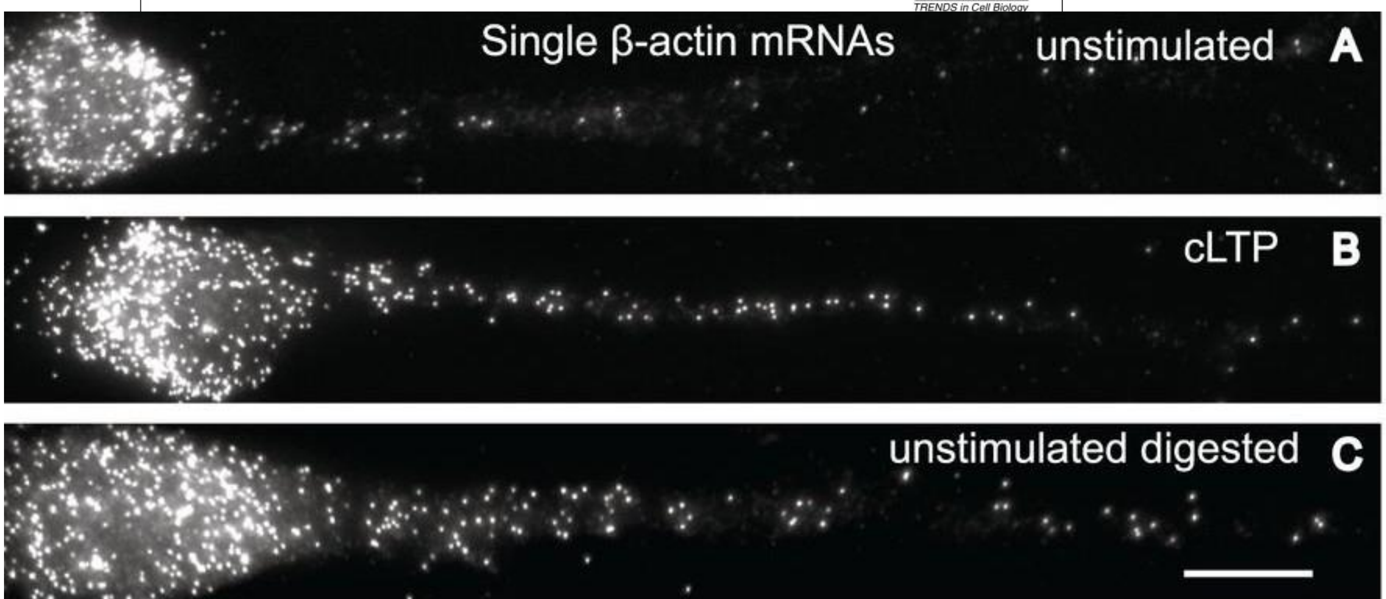




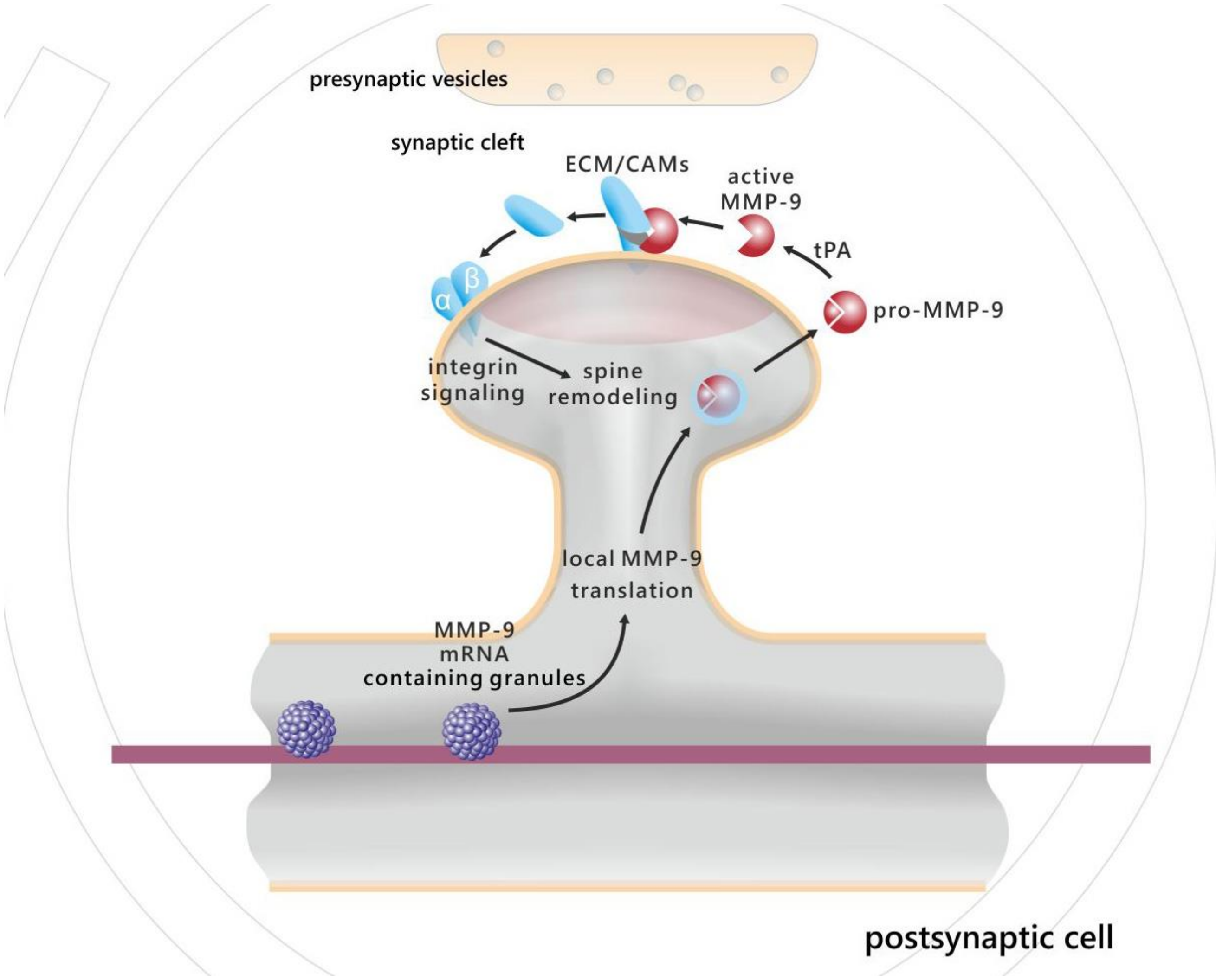
Using this method, it is possible, for example, to simultaneously detect two different mRNAs in a cell or mRNAs resulting from the fusion of 2 transcripts (genomic translocations) such as BCR-ABL

Imaging mRNA in nerve cell dendrites using smFISH





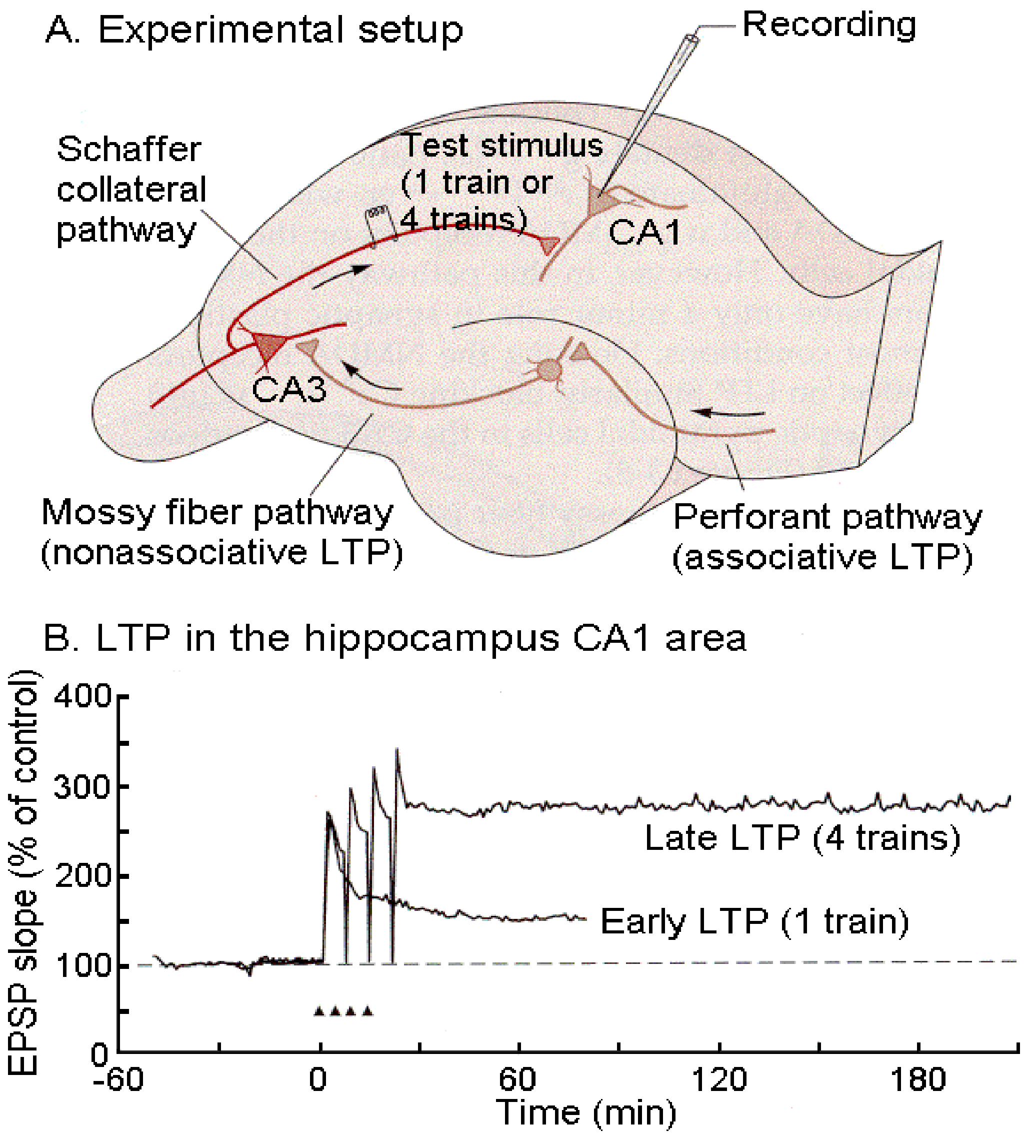
Activity-dependent local translation of MMP-9



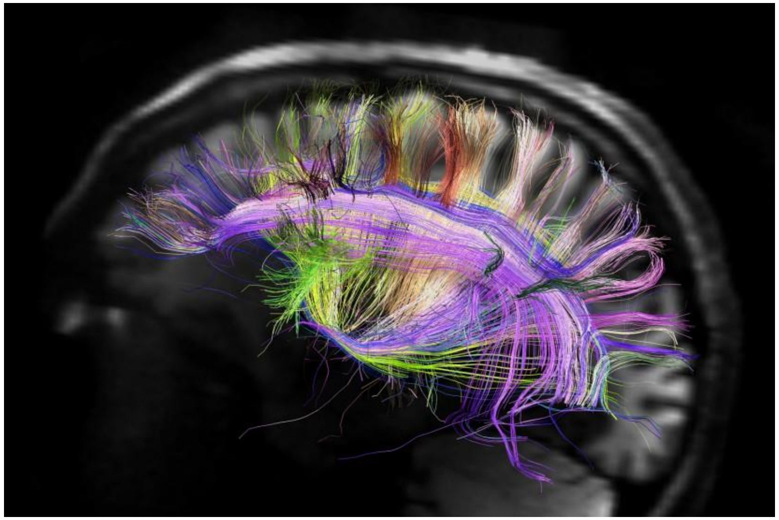
Dziembowska et al., J Neuroscience 2012

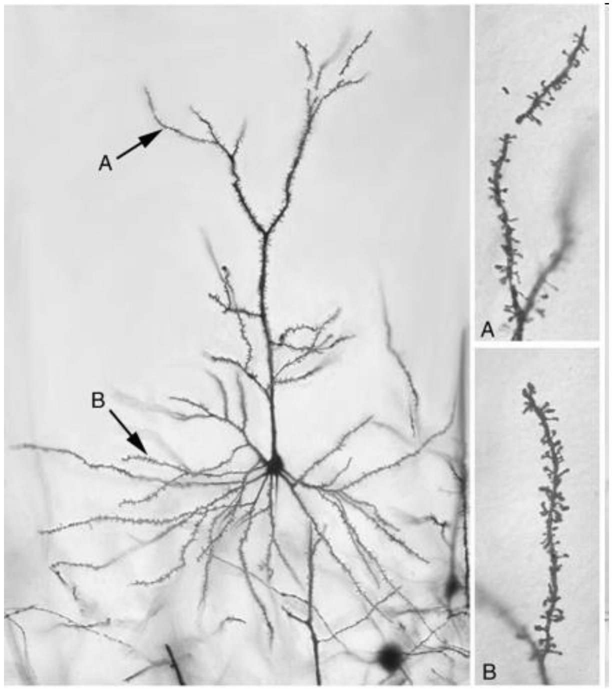
Dziembowska and Wlodarczyk, Int J Biochem Cell Biol **,** *2012*

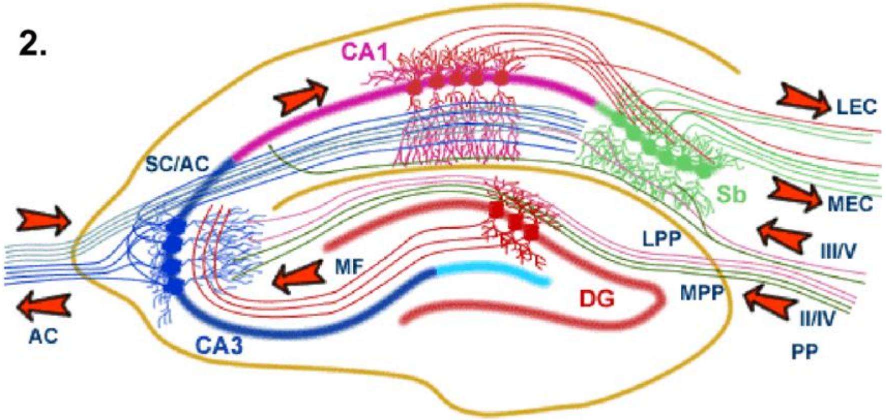
Medial perforant path LTP - a well established model of synaptic plasticity



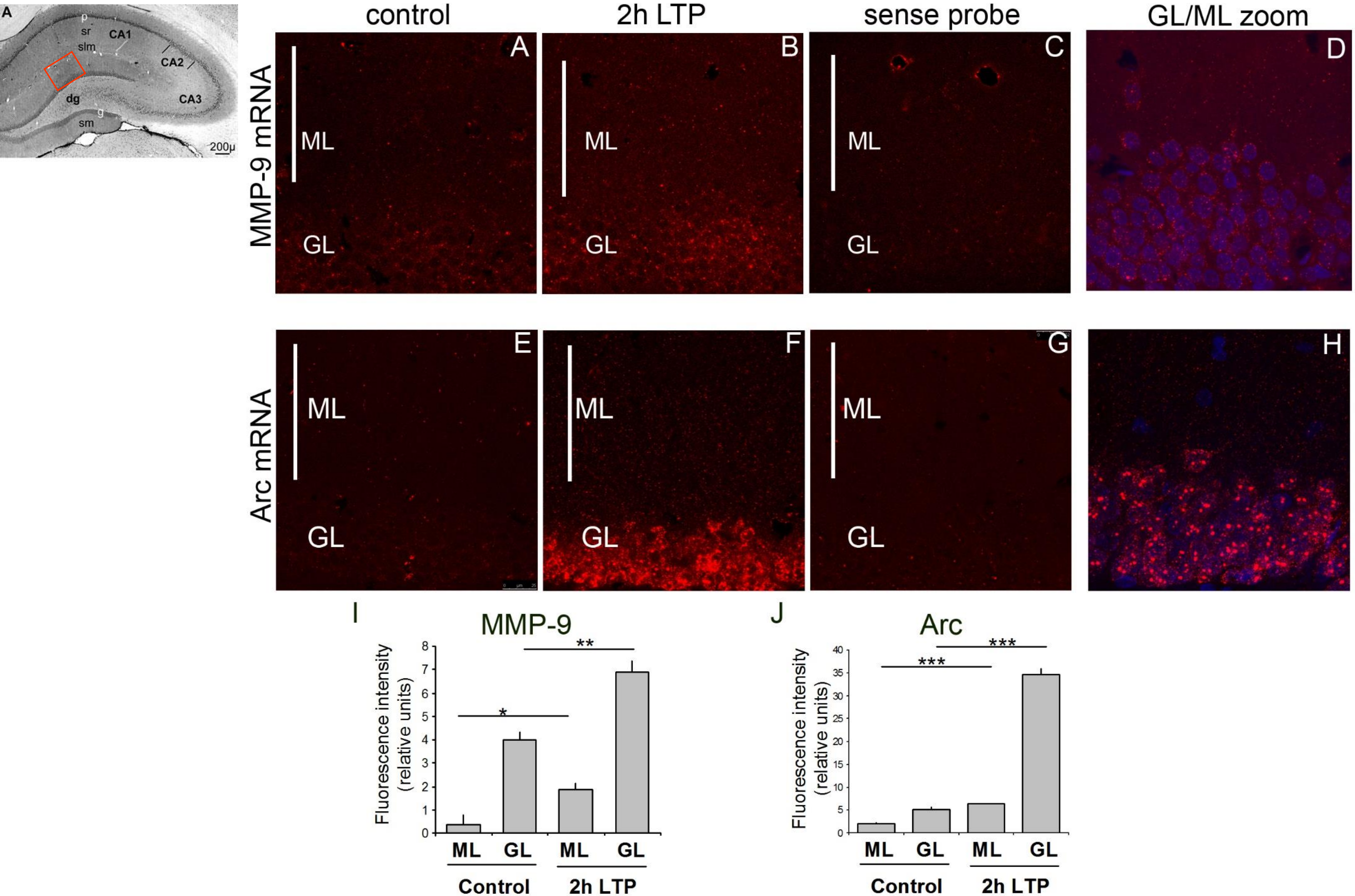
Brain circuits



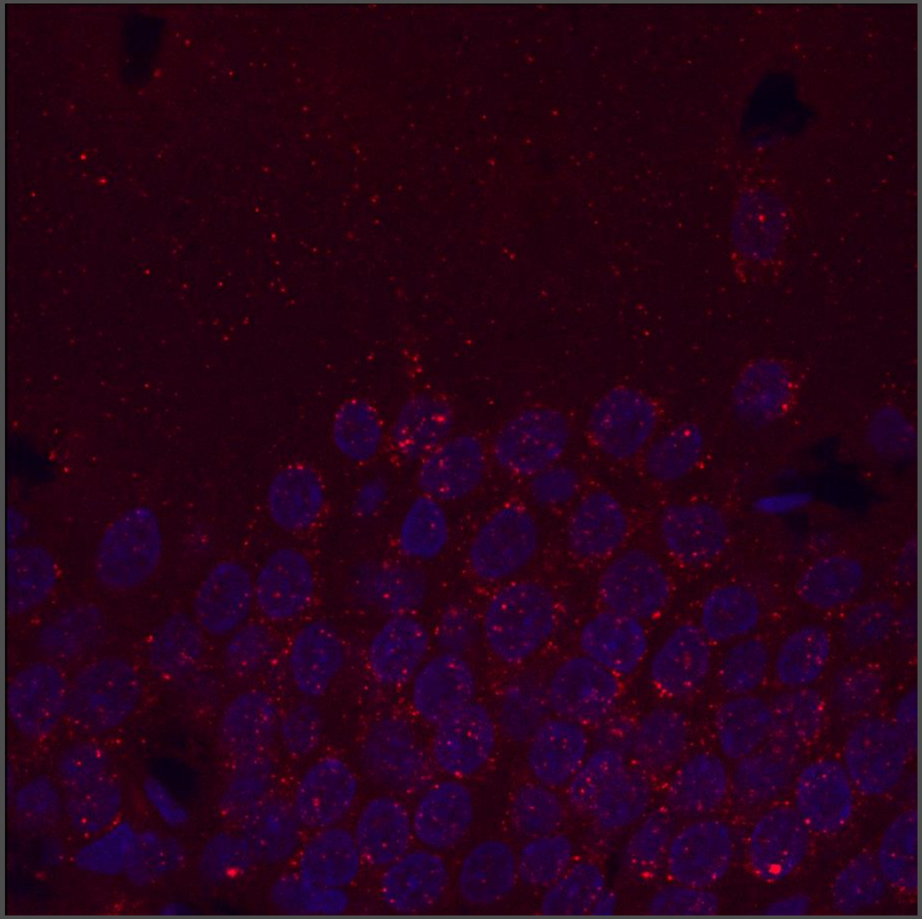




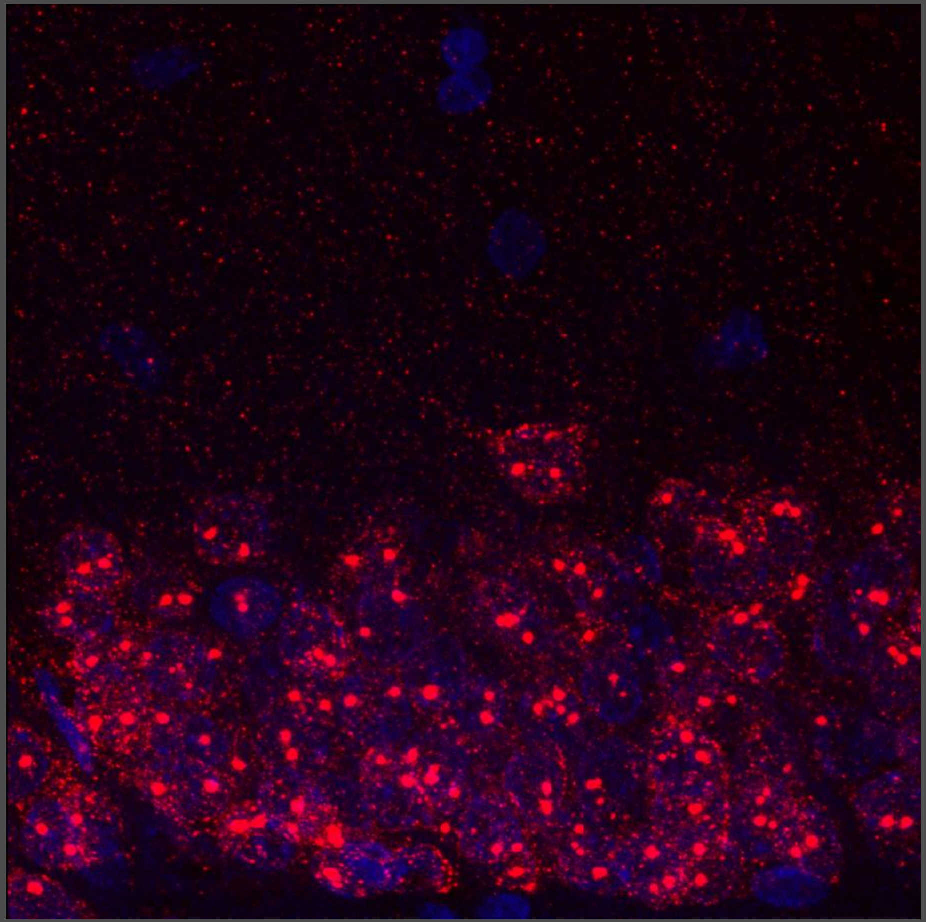
In situ hybridization shows increse in MMP-9 expression in granular layer and molecular layer of dentate gyrus 2h after medial perforant path LTP



MMP-9 in situ hybridization



Arc in situ hybridization



Sushi belt model

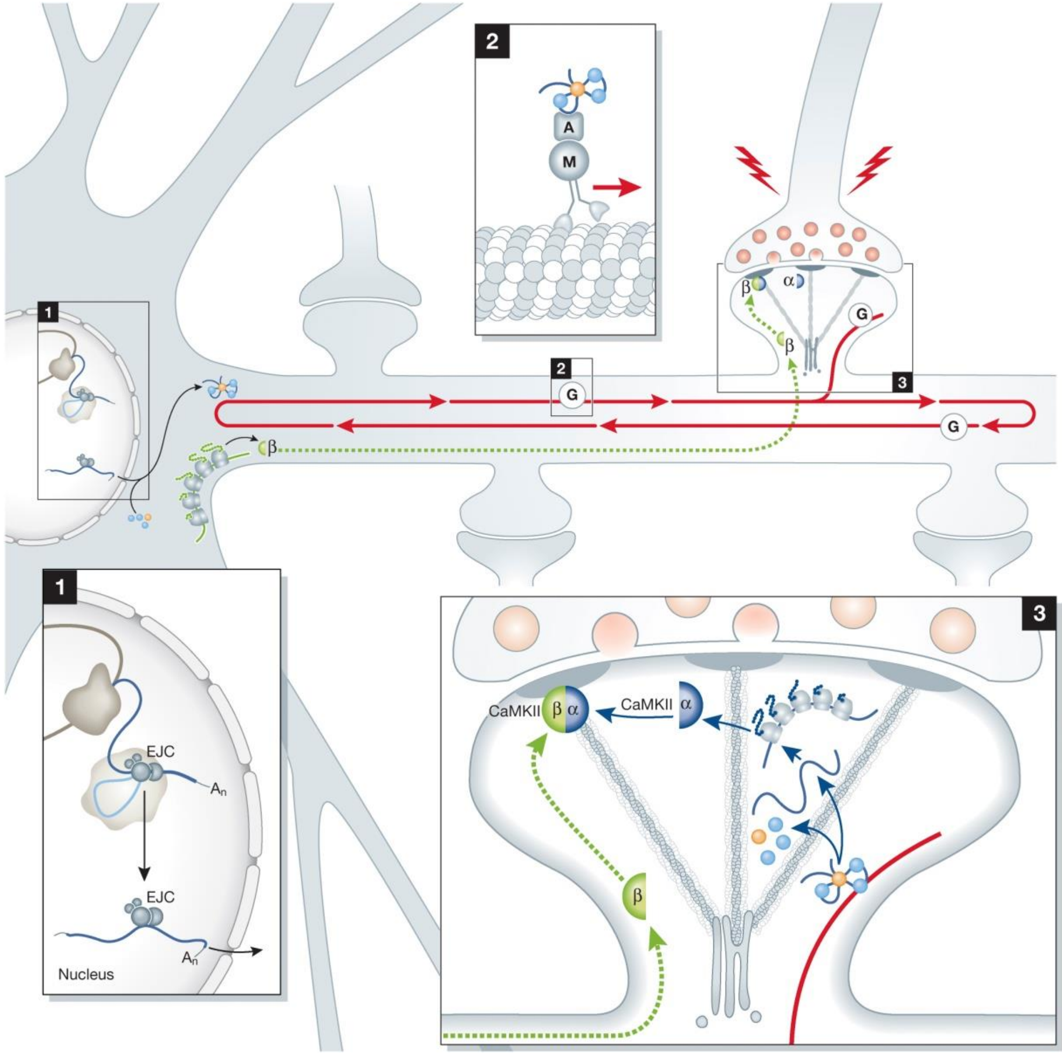
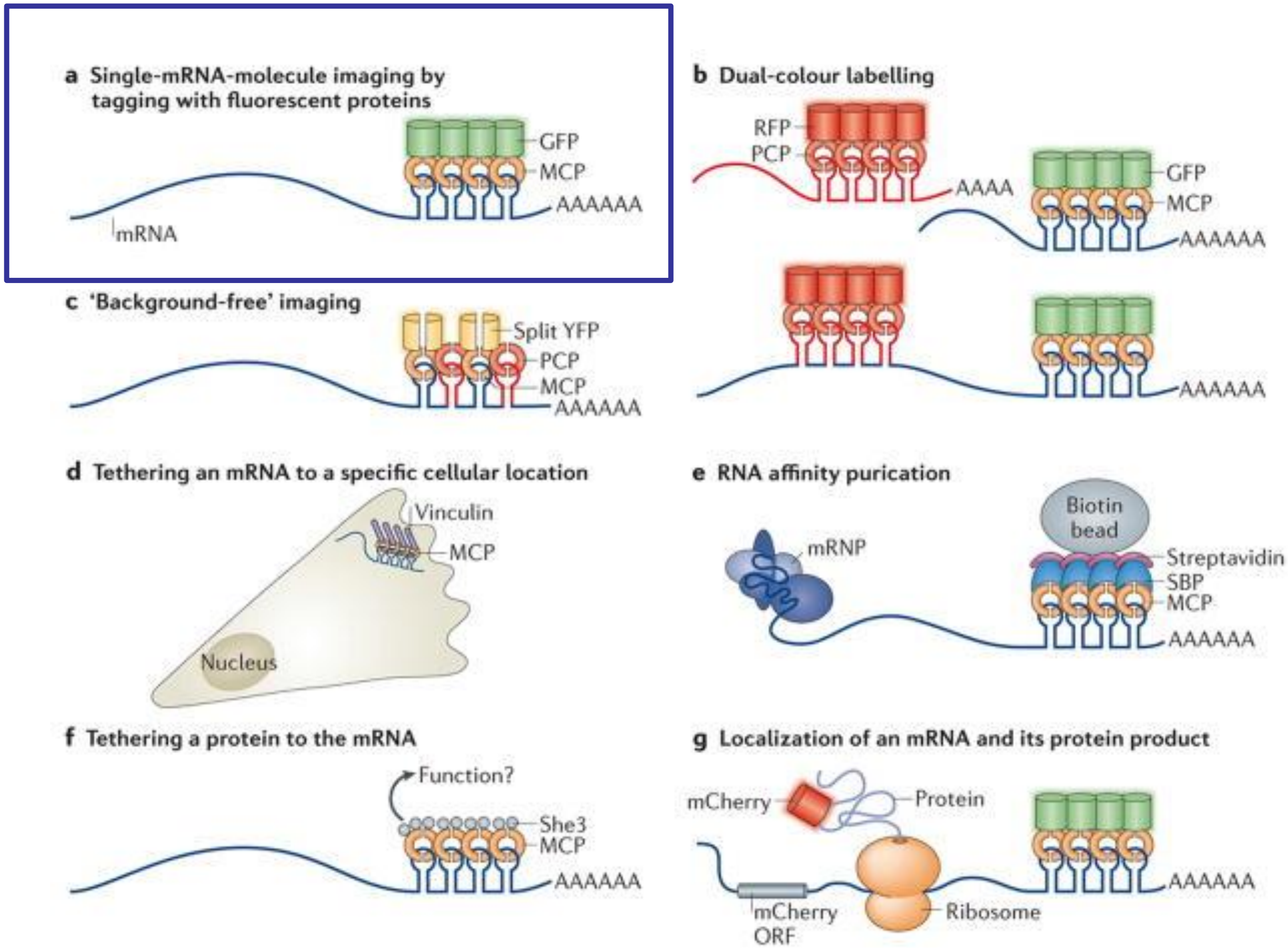


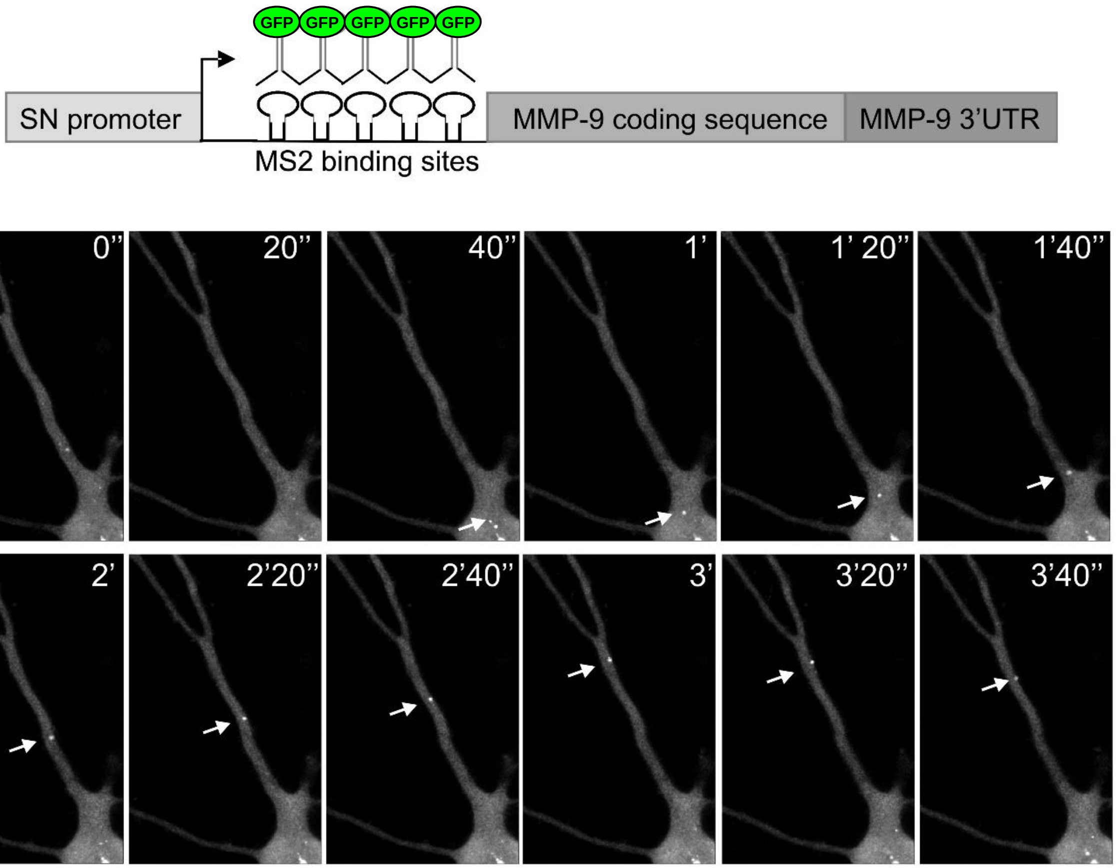
Figure 2 from Michael Doyle and Michael A Kiebler
The EMBO Journal online publication
doi:10.1038/emboj.2011.278

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



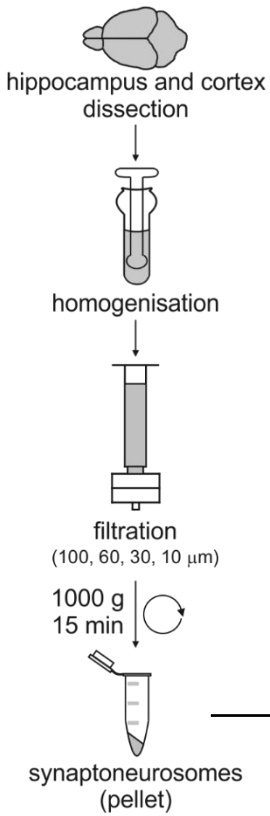
[In the right place at the right time: visualizing and understanding mRNA localization.]
Buxbaum AR, Haimovich G, Singer RH.
Nat Rev Mol Cell Biol. 2015

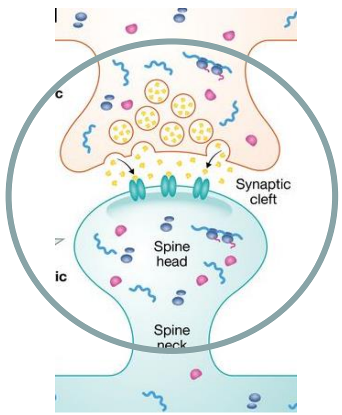
MS2 system to stain targeted mRNA in the living cell



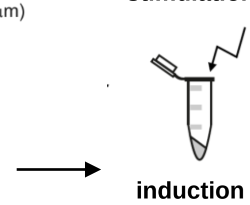
Dziembowska et al., J Neurosci. 2012

Synaptoneurosomes, a model for studying biochemical processes occurring in the synapses



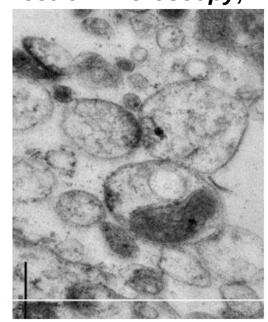


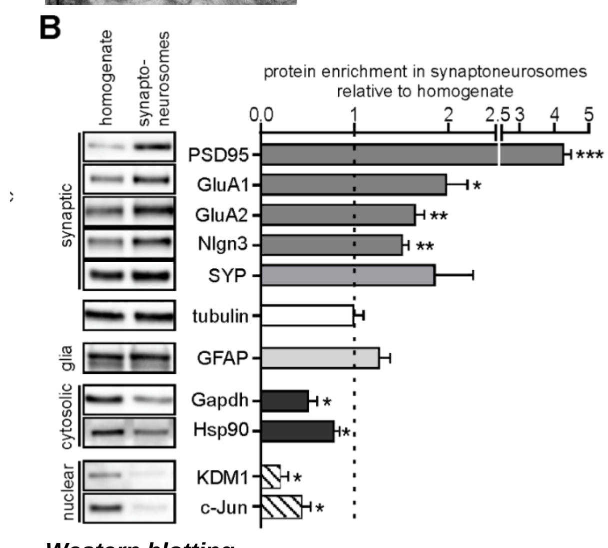
***in vitro* stimulation**



induction of local protein synthesis by NMDA-R stimulation

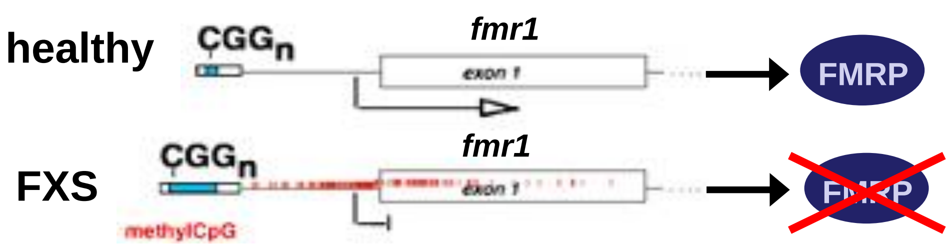
Electron microscopy; A. Janusz

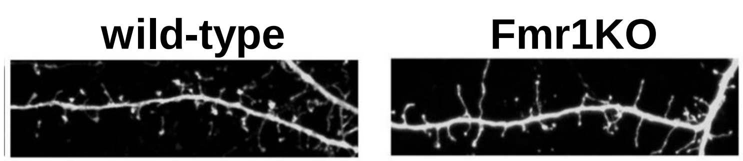




Western blotting

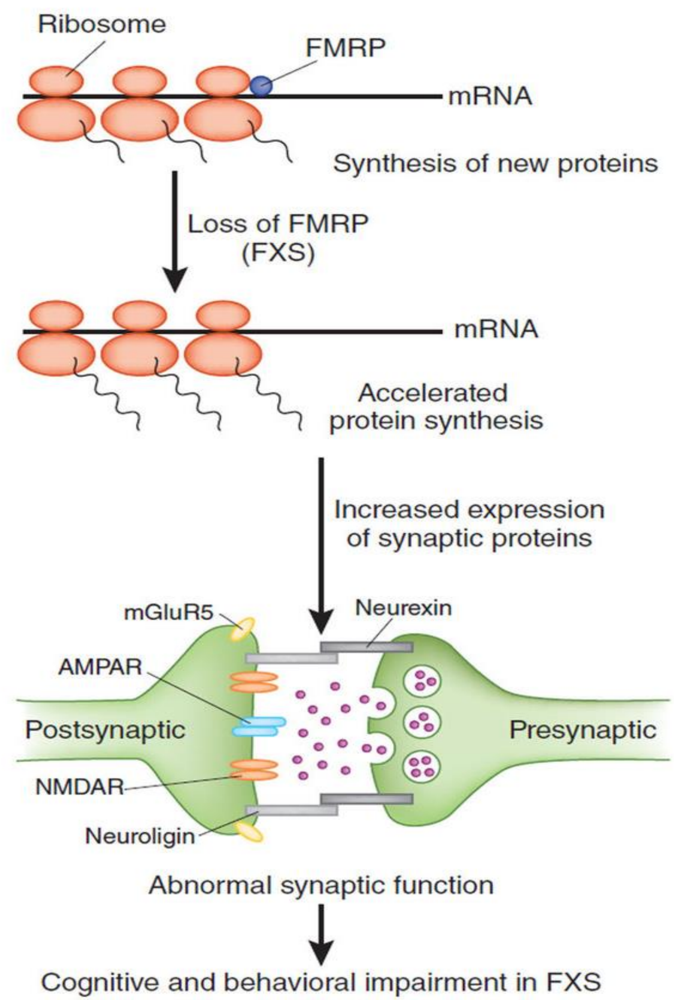
Fragile X syndrome is the most common form of inherited intellectual disability with behaviors characteristic of autism spectrum disorder (ASD).
Syndromic autism
Fragile X syndrome is caused by transcriptional silencing of the Fmr1 gene and consequent loss of expression of the FMRP protein.





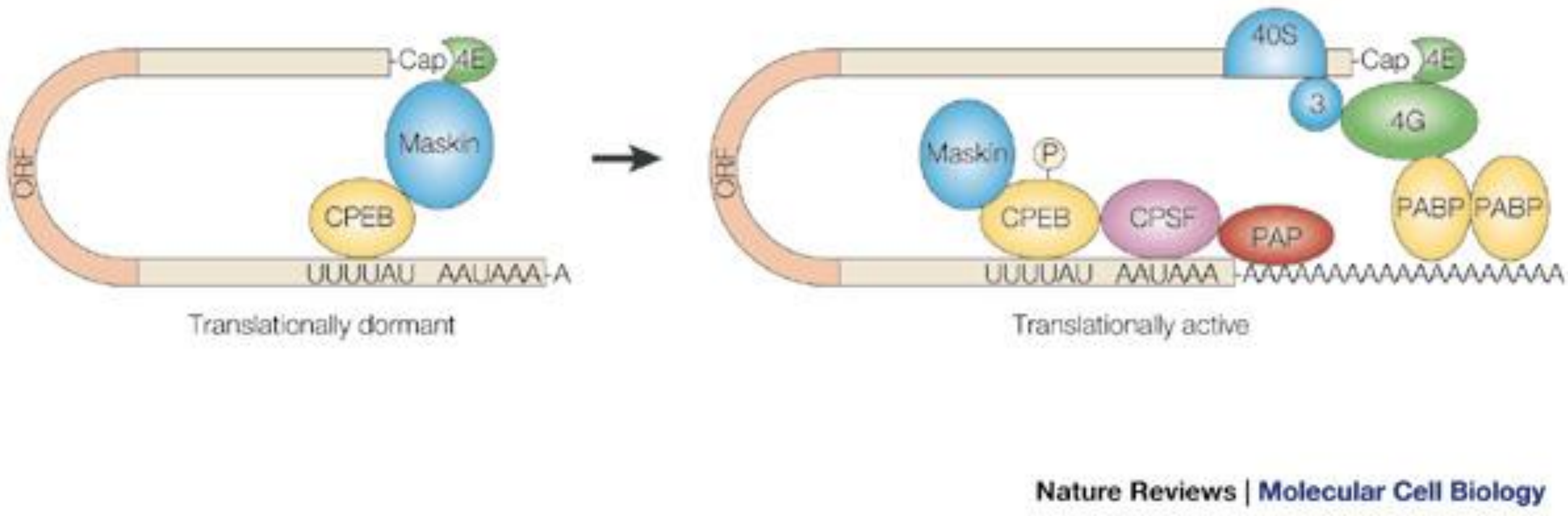
Jasinska et al., Mol. Neurobiol. 2015

***Fmr1* KO mice**



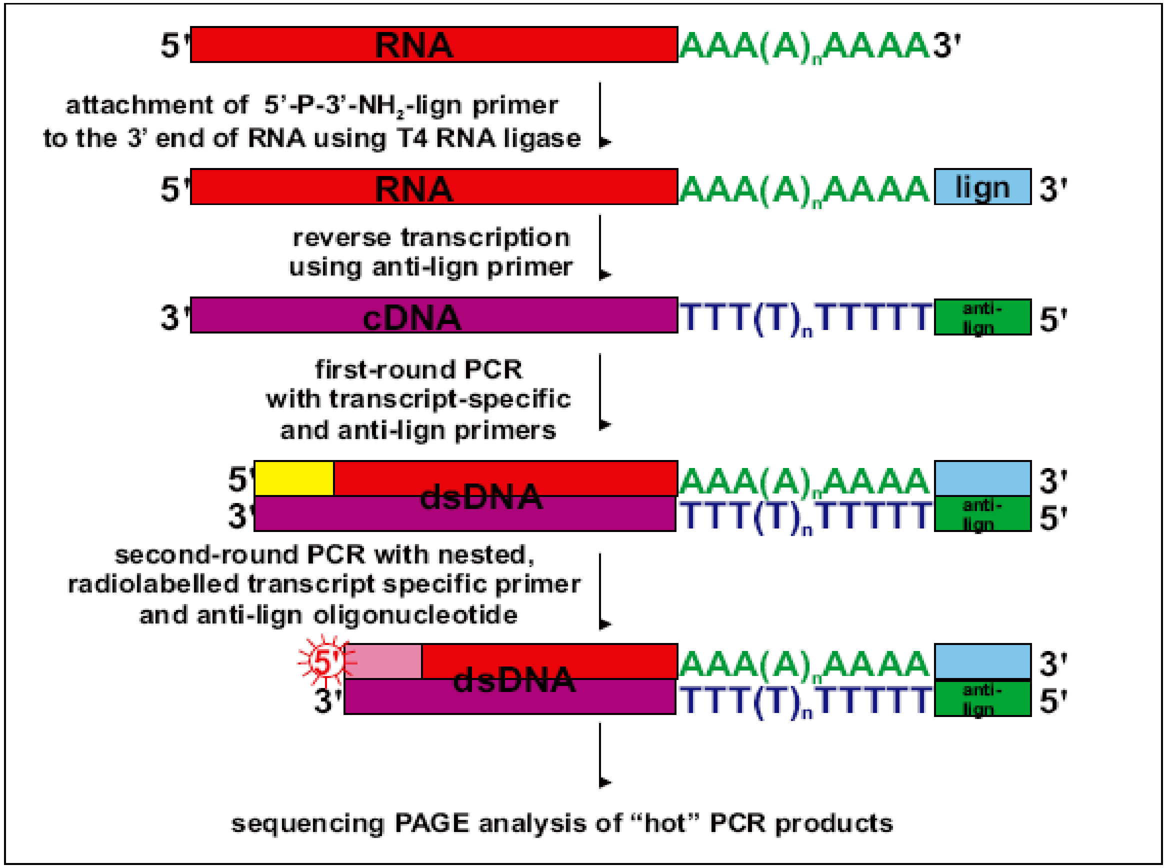
Nature Medicine (2011)

Cytoplasmic polyadenylation promotes translation



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

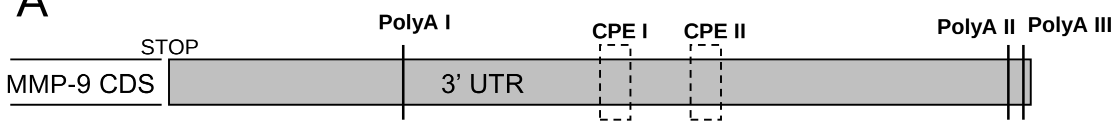
PAT assay



5' RNA AAA(A)nAAAA 3'
attachment of 5'-P-3'-NH2-lign primer
to the 3' end of RNA using T4 RNA ligase
5' RNA AAA(A)nAAAA lign 3'
reverse transcription
using anti-lign primer
3' cDNA TTT(T)nTTTTT anti-lign 5'
first-round PCR
with transcript-specific
and anti-lign primers
5' dsDNA AAA(A)nAAAA 3'
3' TTT(T)nTTTTT anti-lign 5'
second-round PCR with nested,
radiolabelled transcript specific primer
and anti-lign oligonucleotide
5' dsDNA AAA(A)nAAAA 3'
3' TTT(T)nTTTTT anti-lign 5'
sequencing PAGE analysis of "hot" PCR products

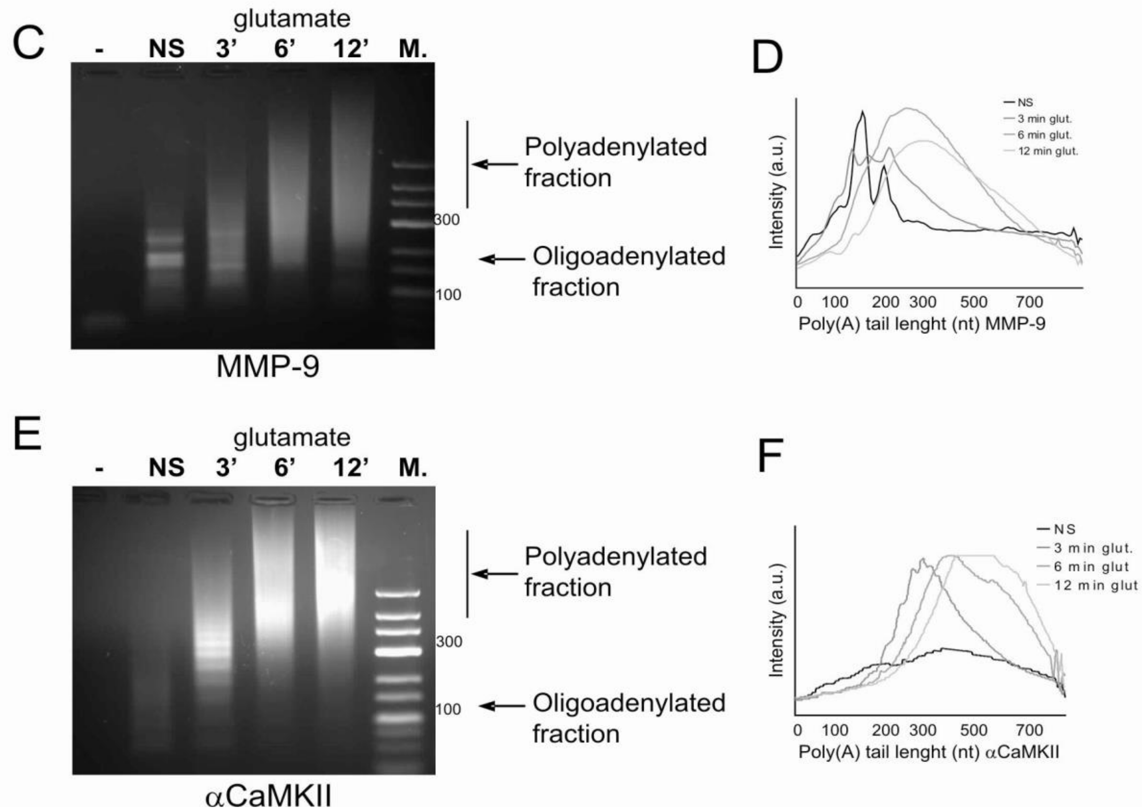
MMP-9 polyadenylation measured by PAT ssay in synaptoneurosomes after glutamate stimulation

A

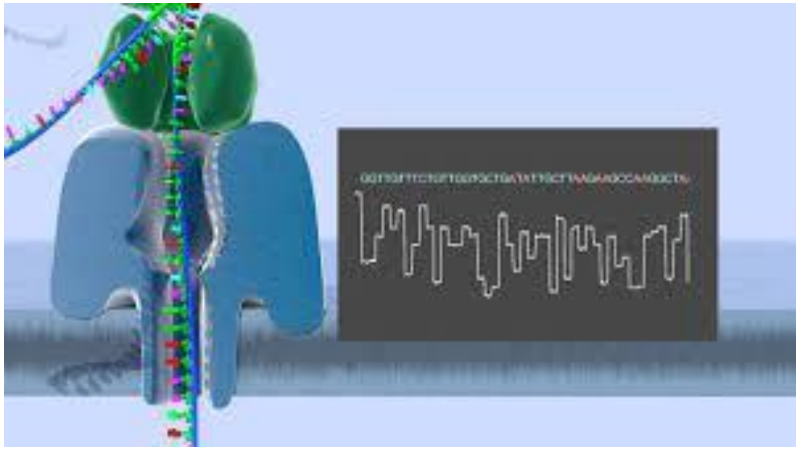


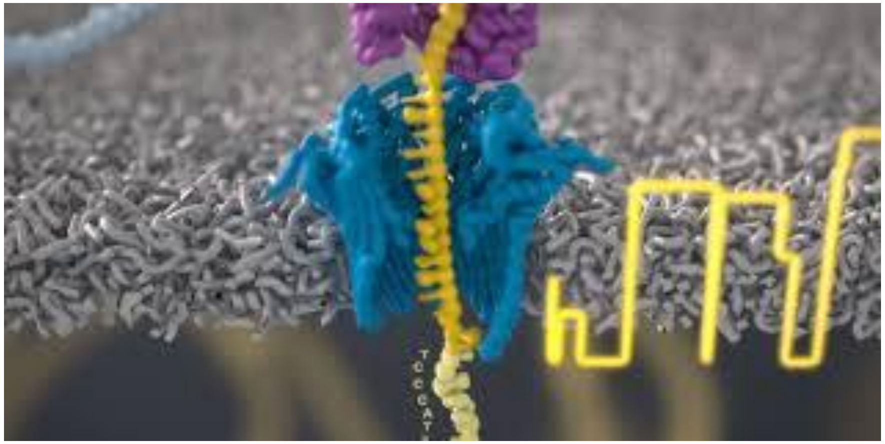
B

CPE I	R. norvegicus	2416	ACCUUUUG**UUUUUAU**GGG	2433
	M. musculus	2502	ACC**UUUUAU**UUUUGUGUG	2519
CPE II	R. norvegicus	2500	CCC**UUUUAU**UUAUUAUGU	2517
	M. musculus	2592	CCC**UUUUAU**UUAUUAUGU	2609



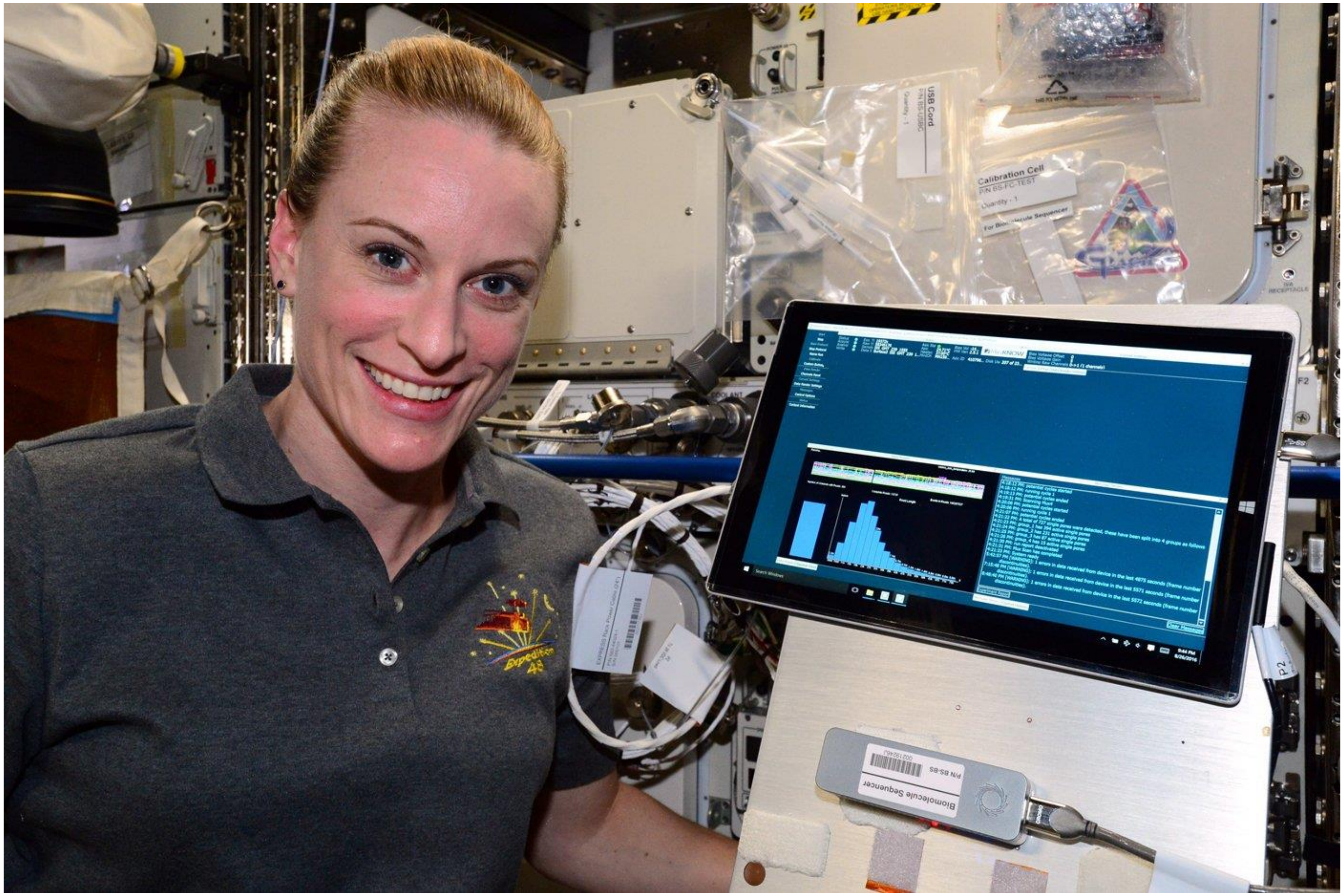
Nanopore Technology and Its Applications in Gene Sequencing

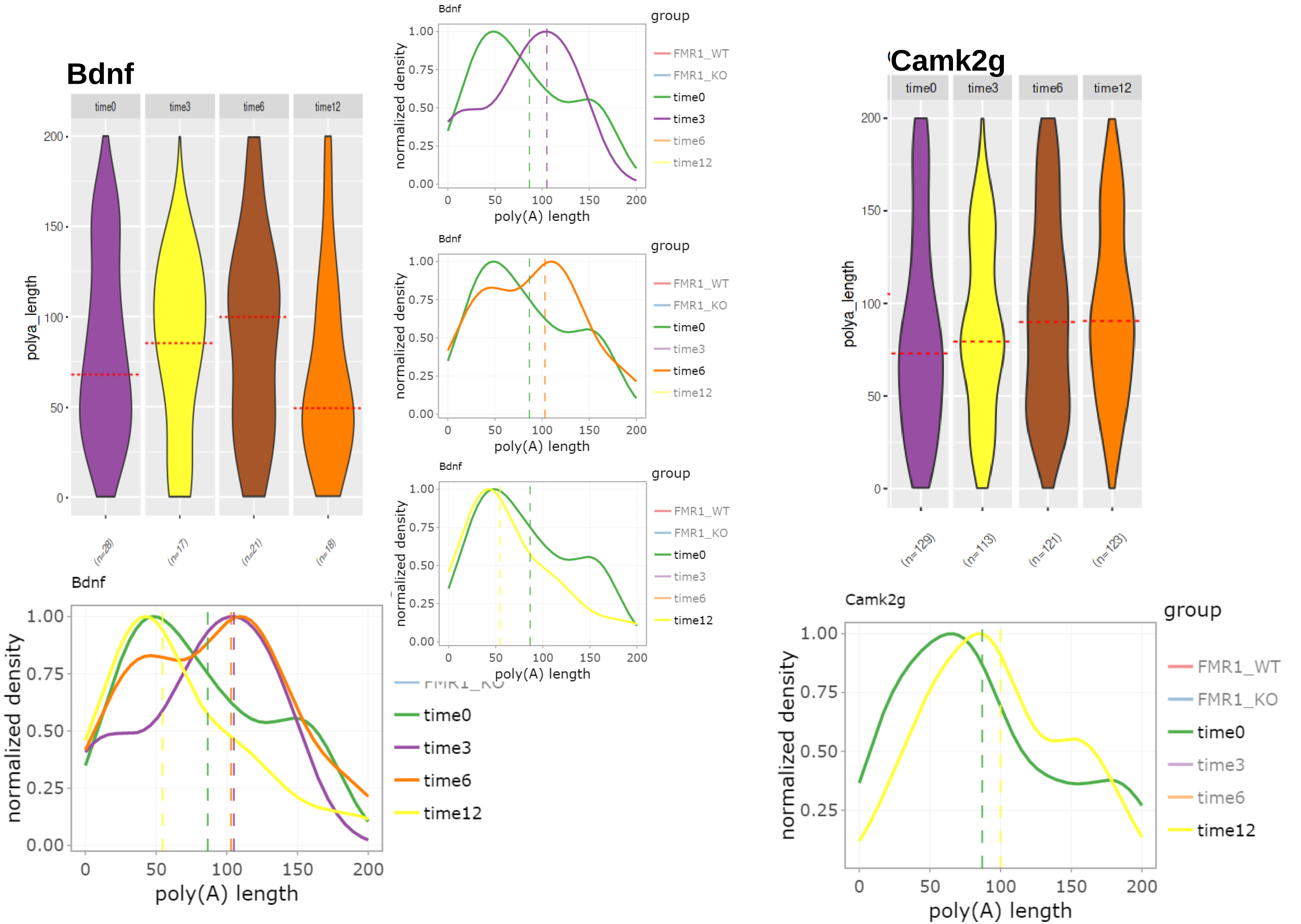




Lin B et al., Biosensors 2021

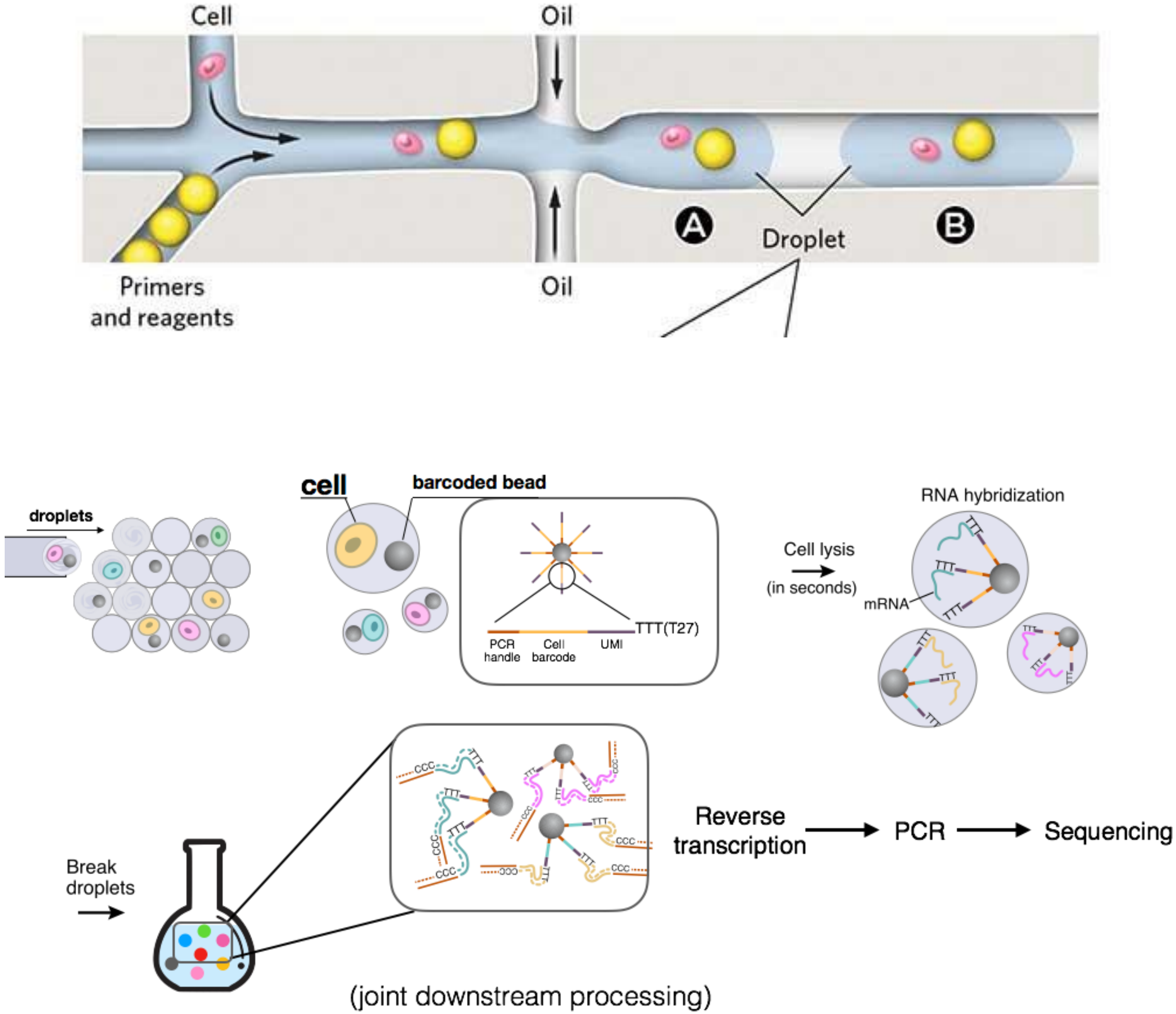
MinION (Oxford Nanopore) on the International Space Station



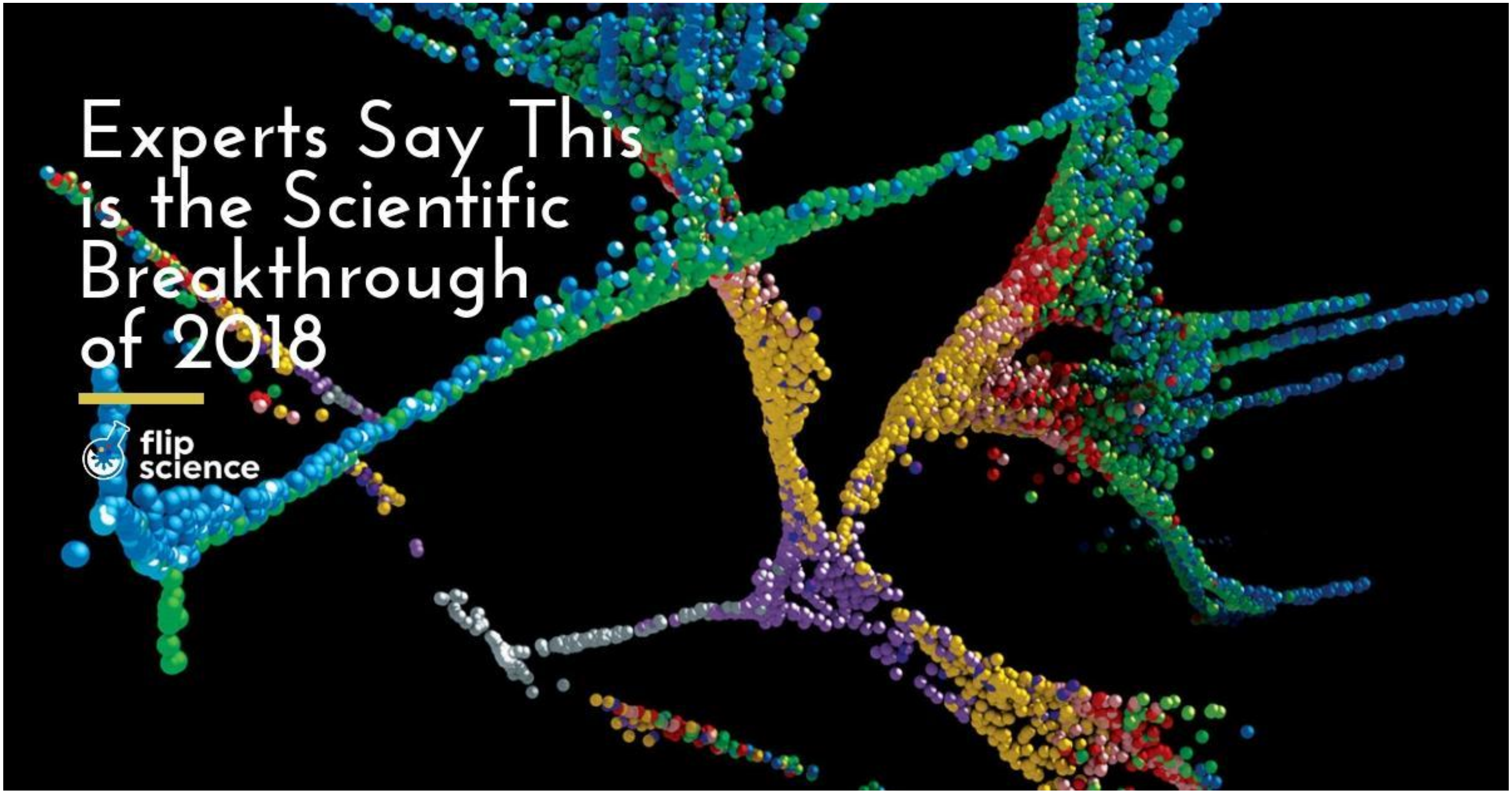


Bdnf
time0
time3
time6
time12
polya_length
(n=28)
(n=17)
(n=21)
(n=18)
Bdnf
normalized density
poly(A) length
group
FMR1_WT
FMR1_KO
time0
time3
time6
time12
Camk2g
(n=129)
(n=113)
(n=121)
(n=123)

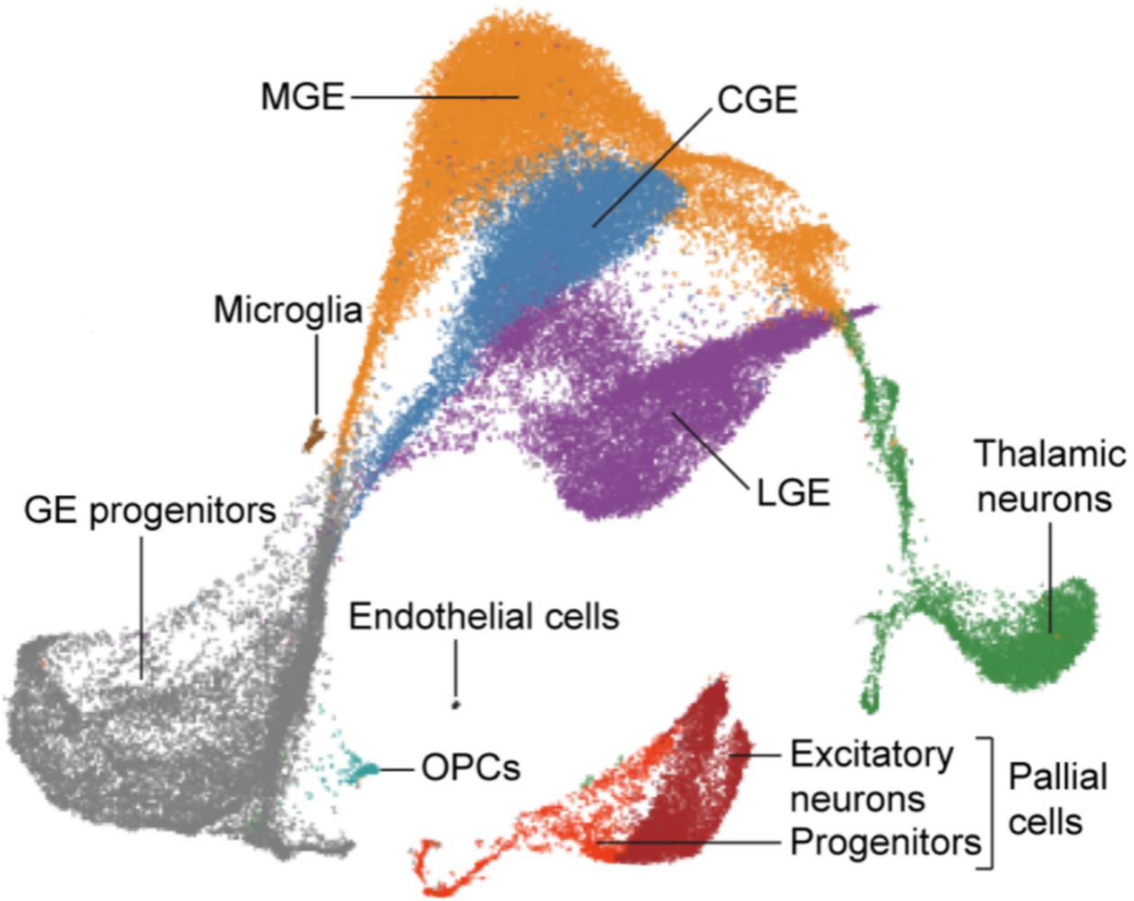
Rozwój nowych technologii – sekwencjonowanie mRNA z pojedynczej komórki



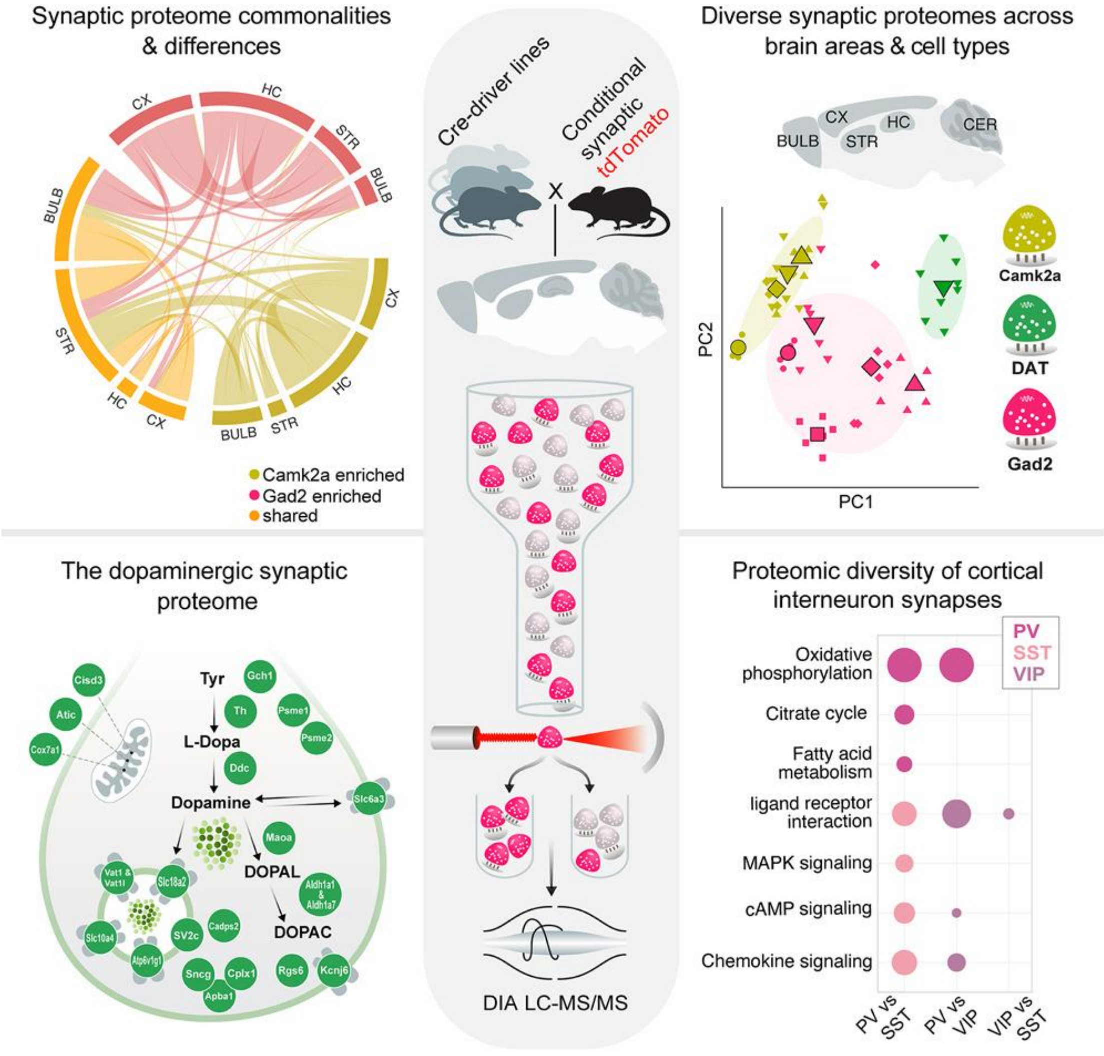
Cell
Oil
Primers
and reagents
Oil
A
Droplet
B
droplets
cell
barcoded bead
TTT(T27)
PCR
handle
Cell
barcode
UMI
Cell lysis
(in seconds)
RNA hybridization
mRNA
Break
droplets
Reverse
transcription
PCR
Sequencing
(joint downstream processing)


Experts Say This is the Scientific Breakthrough of 2018
flip science

Gene expression clusters



The authors annotated these cells manually, using the expression of some known marker genes, as shown in this figure from the paper



Marc van Oostrum, Thomas M. Blok, Stefano L. Giandomenico, ..., Nicole Fu¨ rst, Julian D. Langer, Erin M. Schuman

Cell 2023 *The proteomic landscape of synaptic diversity across brain regions and cell types*