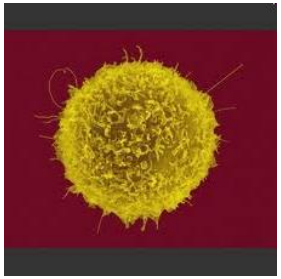
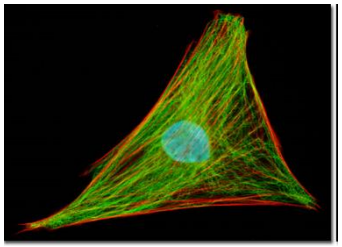
Metody badania RNA w neuronach

Magdalena Dziembowska

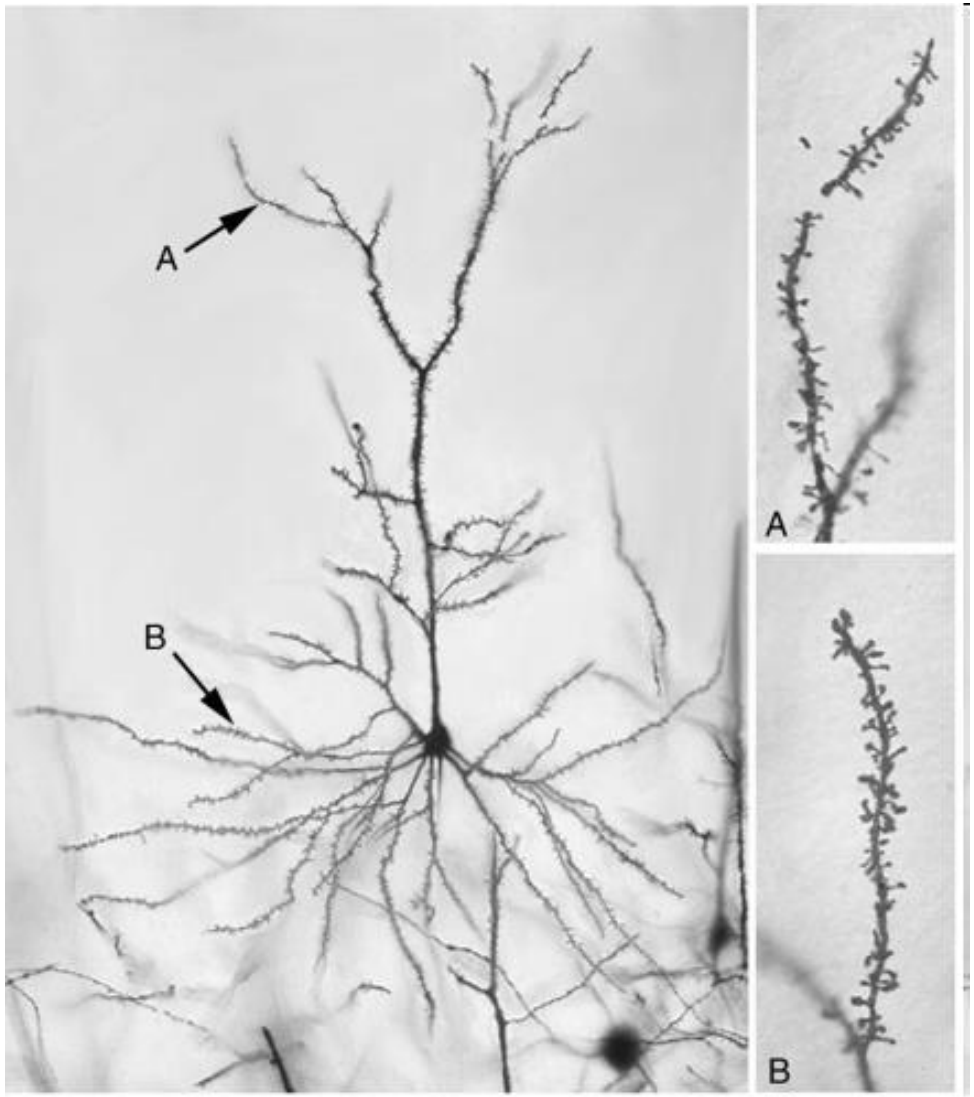
LABORATORIUM
MOLEKULARNYCH PODSTAW
PLASTYCZNOŚCI
SYNAPTYCZNEJ



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**

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

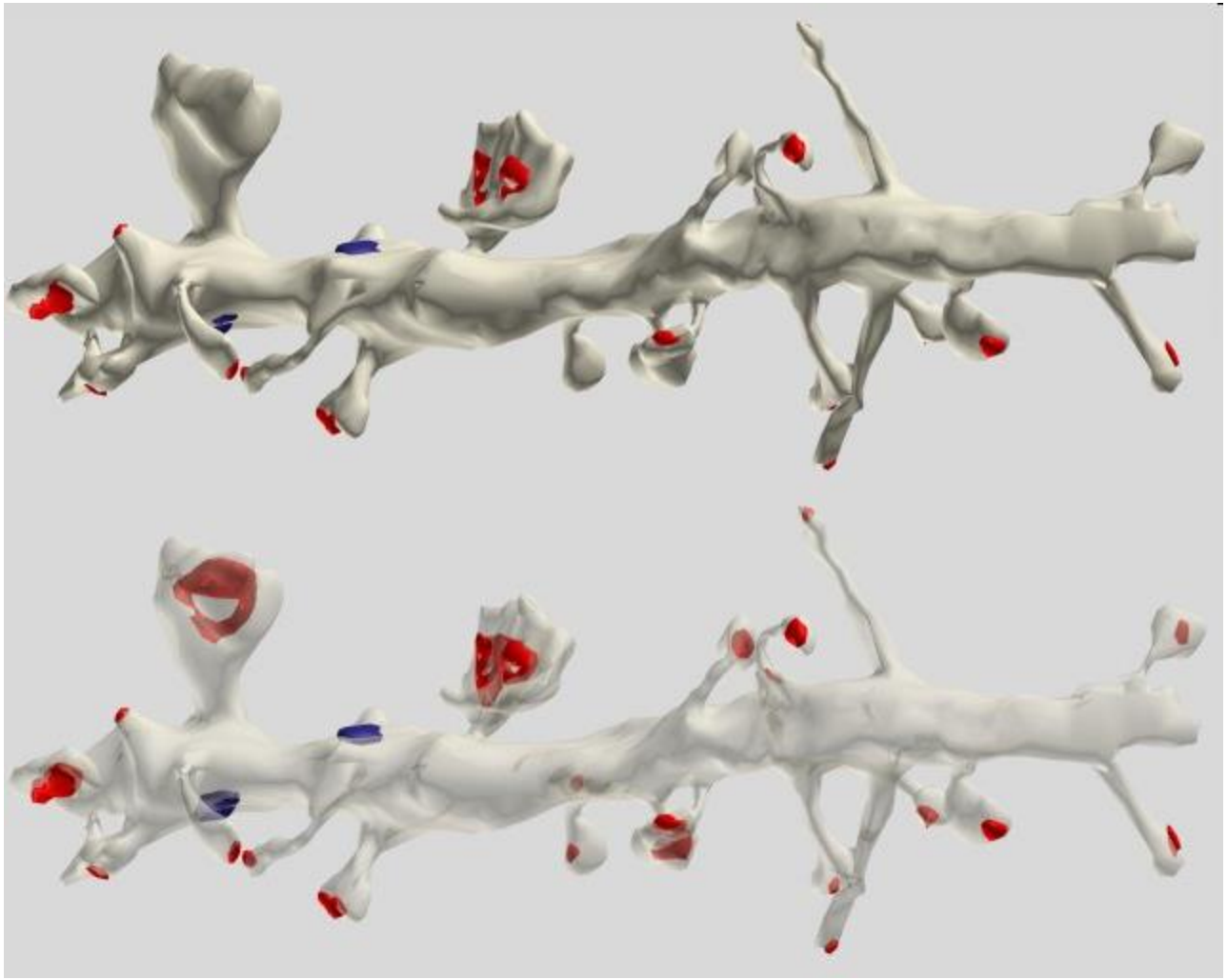
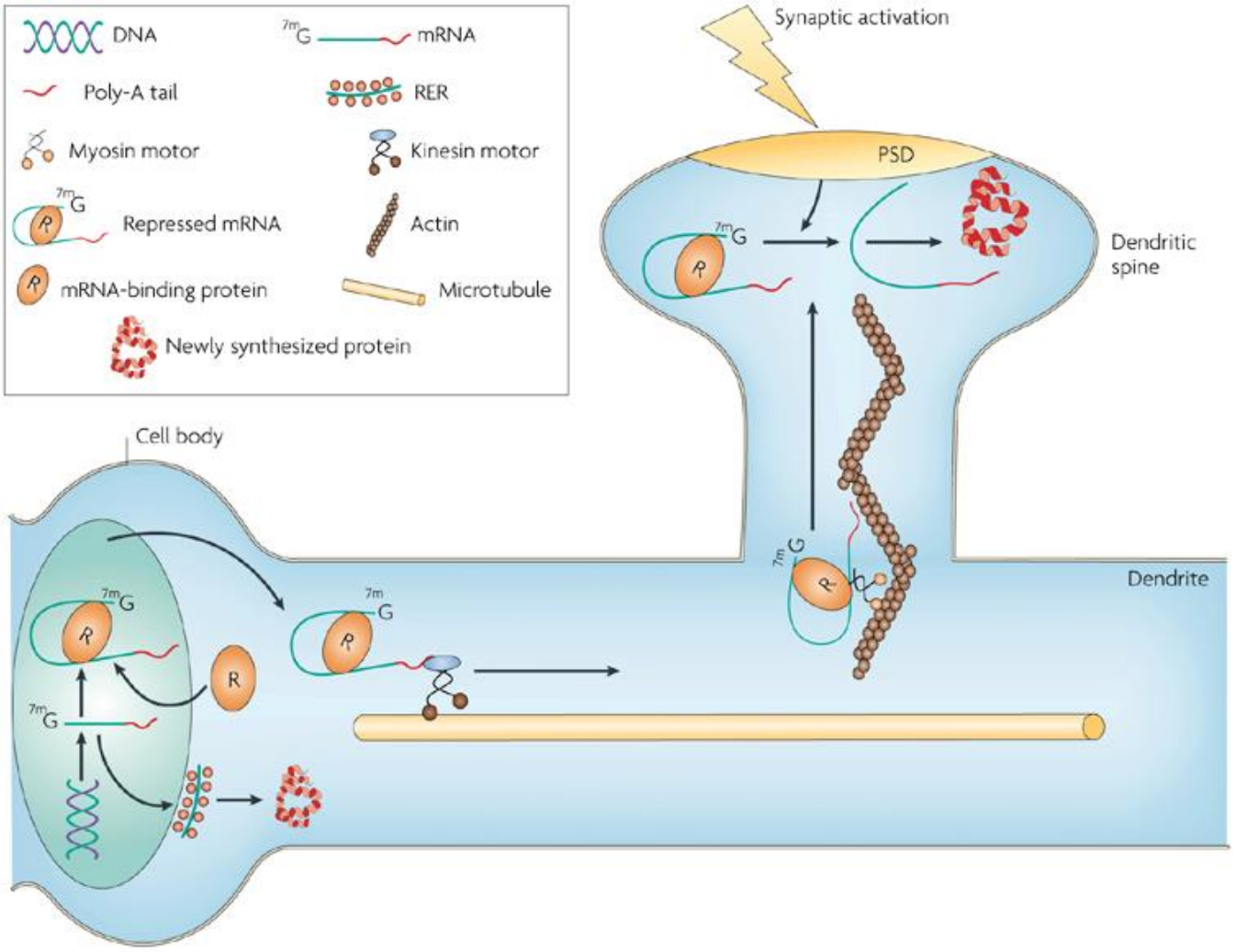
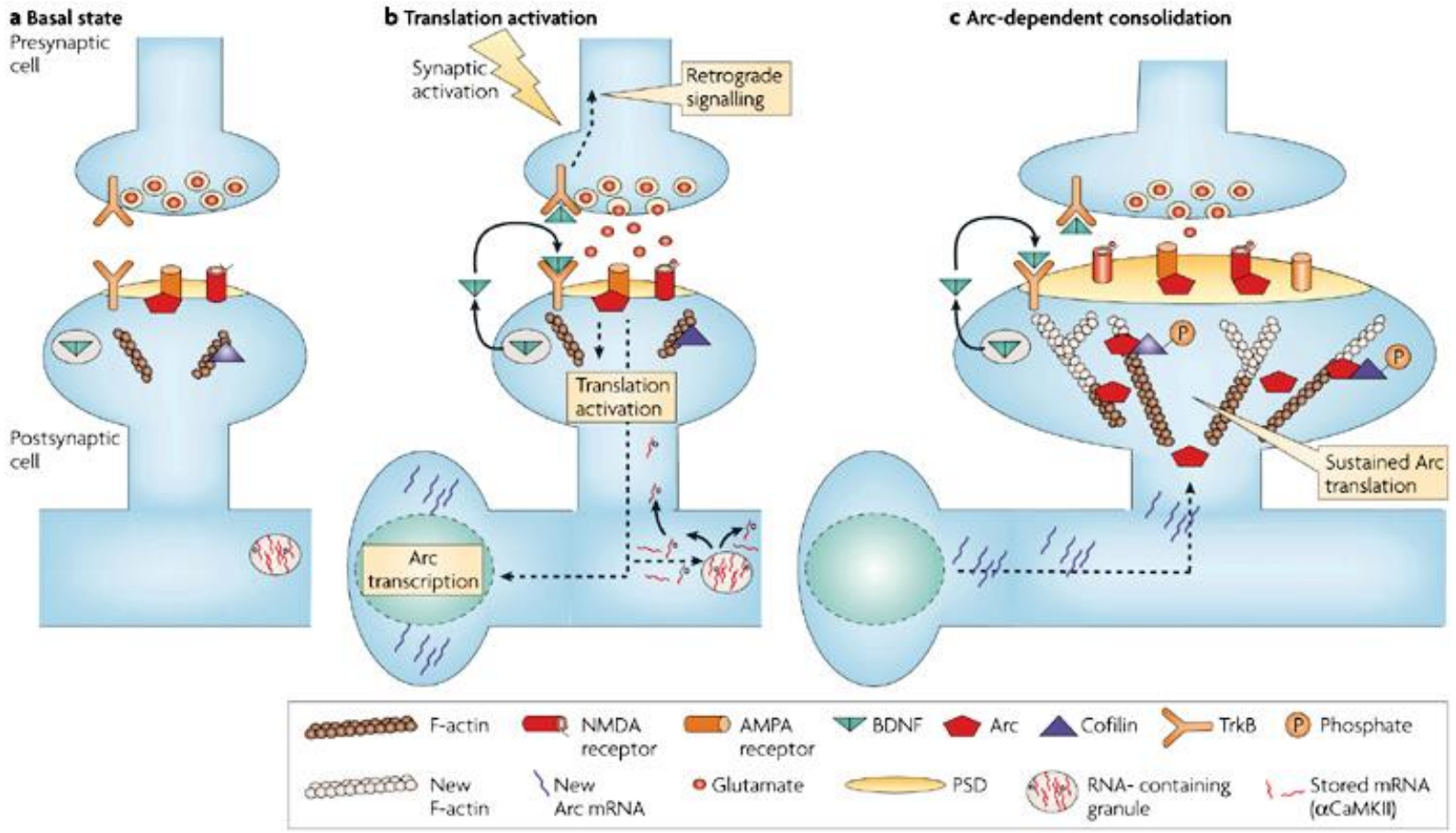


Fig. 1: A segment of pyramidal cell dendrite from stratum radiatum (CA1) with thin, stubby, and mushroom-shaped spines. Spine synapses colored in red, stem (or shaft) synapses colored in blue. The dendrite was made transparent in the lower image to enable visualization of all synapses. *Photo by Josef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

Local mRNA translation in dendritic spines

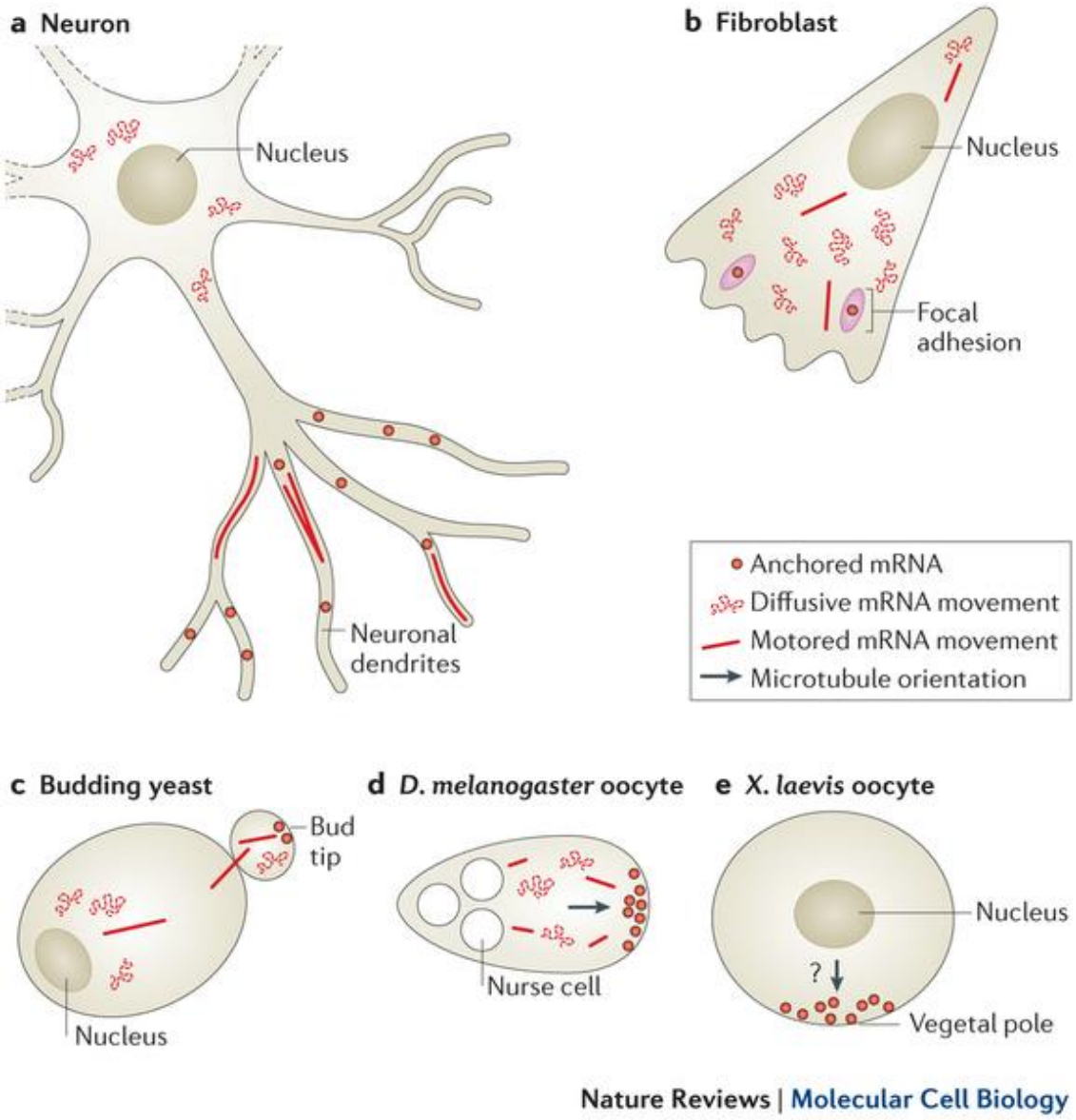


A model of Arc-dependent LTP consolidation in the dentate gyrus



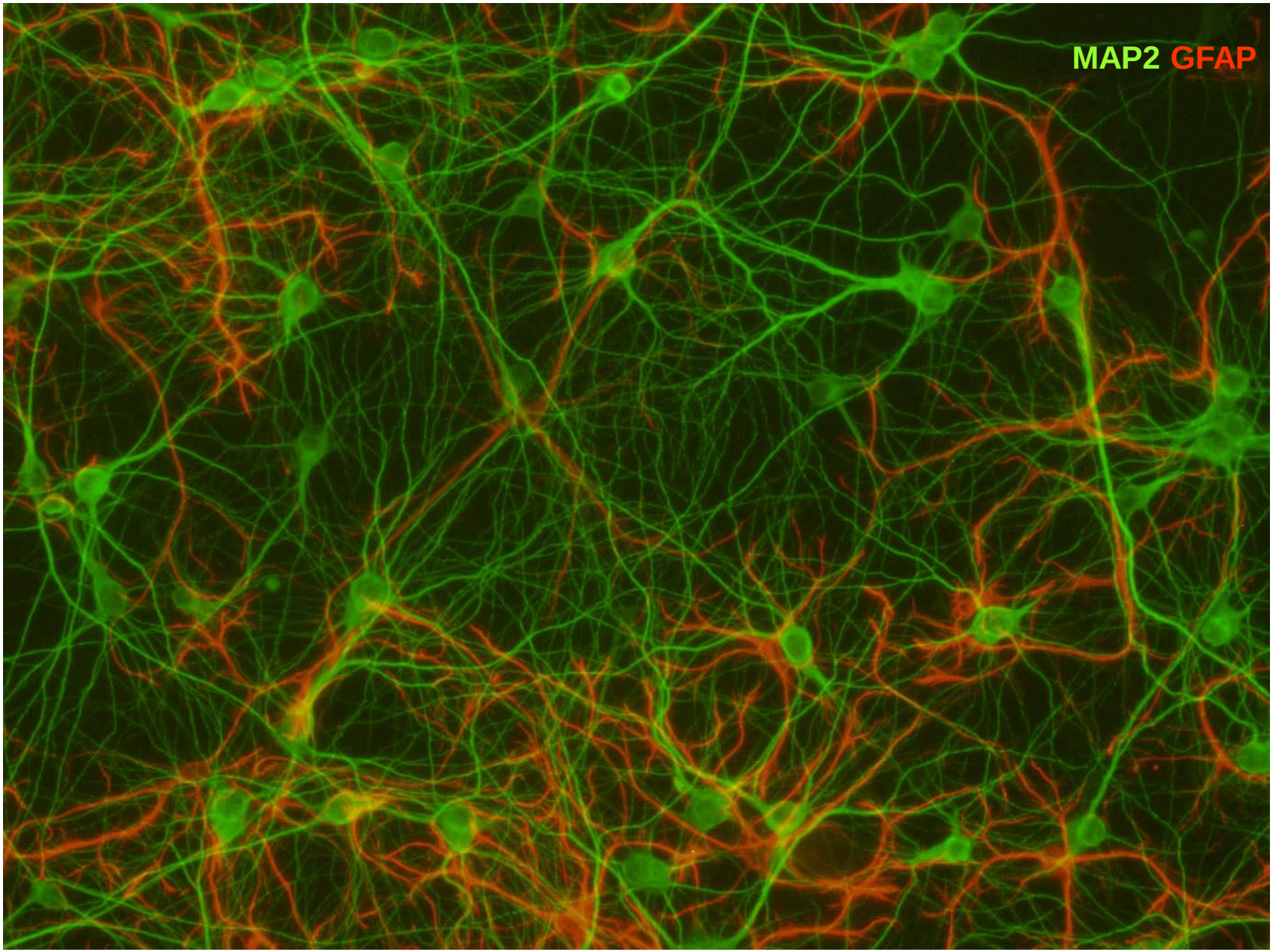
Nature Reviews | Neuroscience

Różna lokalizacja mRNA w zależności od typu komórek

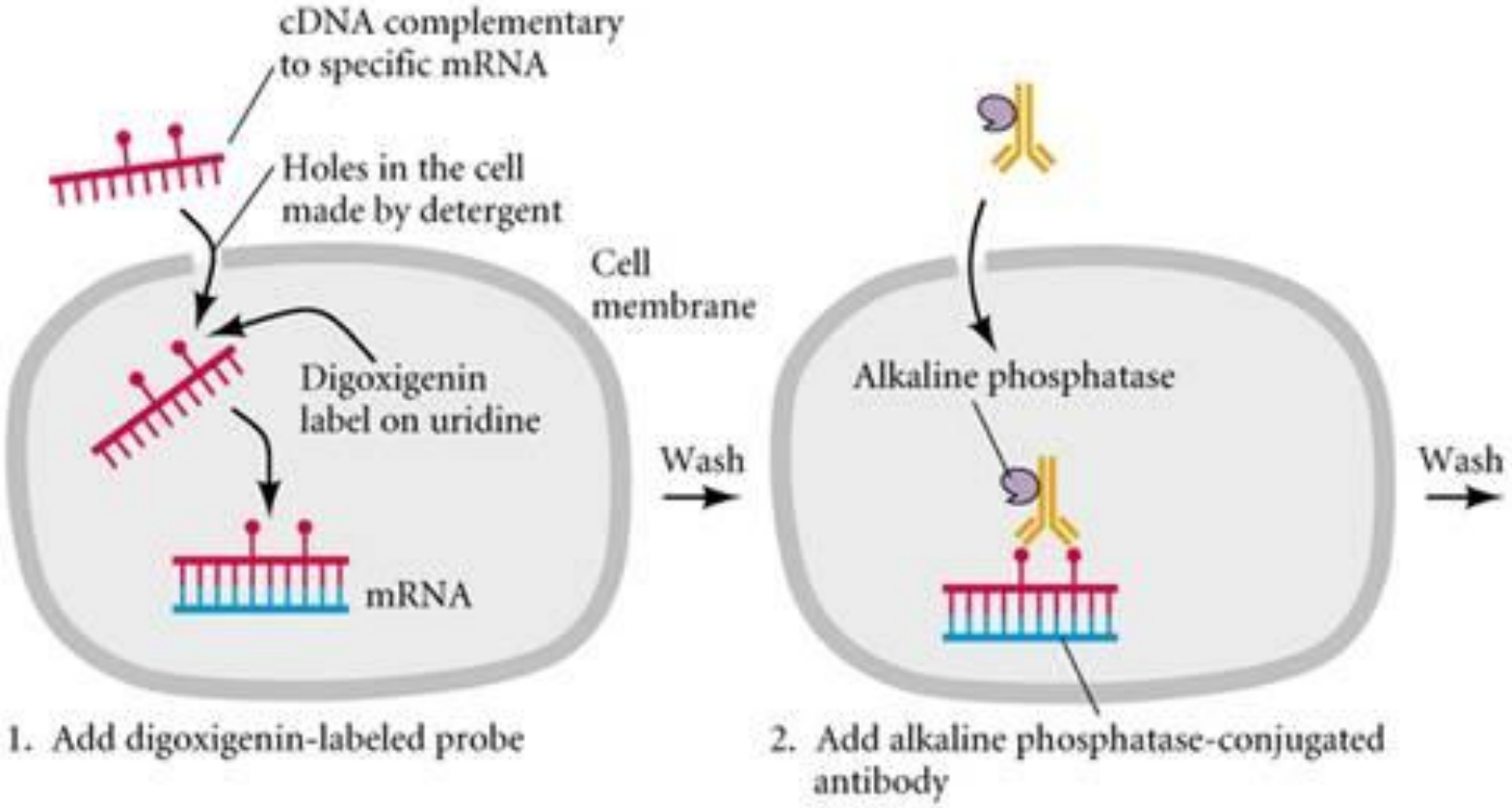


Nature Reviews | Molecular Cell Biology

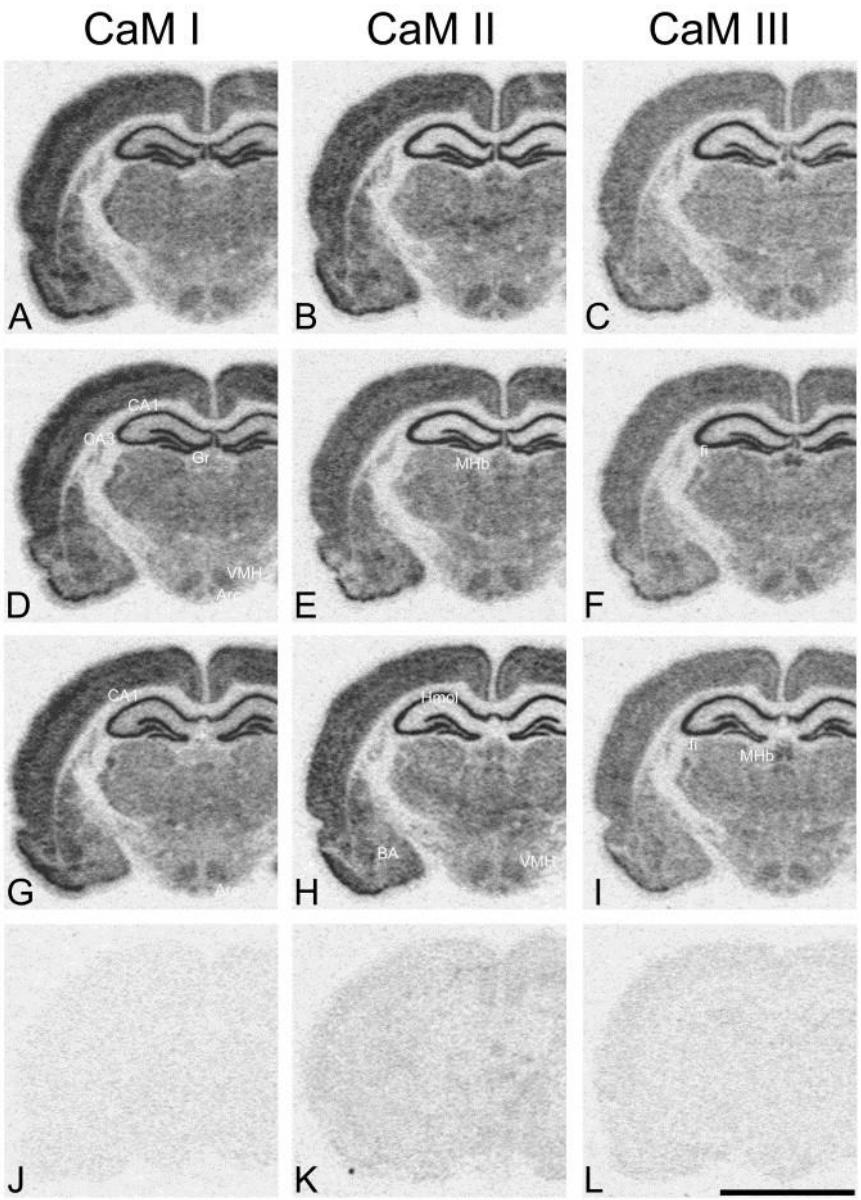
Metody wizualizacji mRNA w neuronach



In situ hybrydyzacja z sondą RNA wyznakowaną digoksygeniną



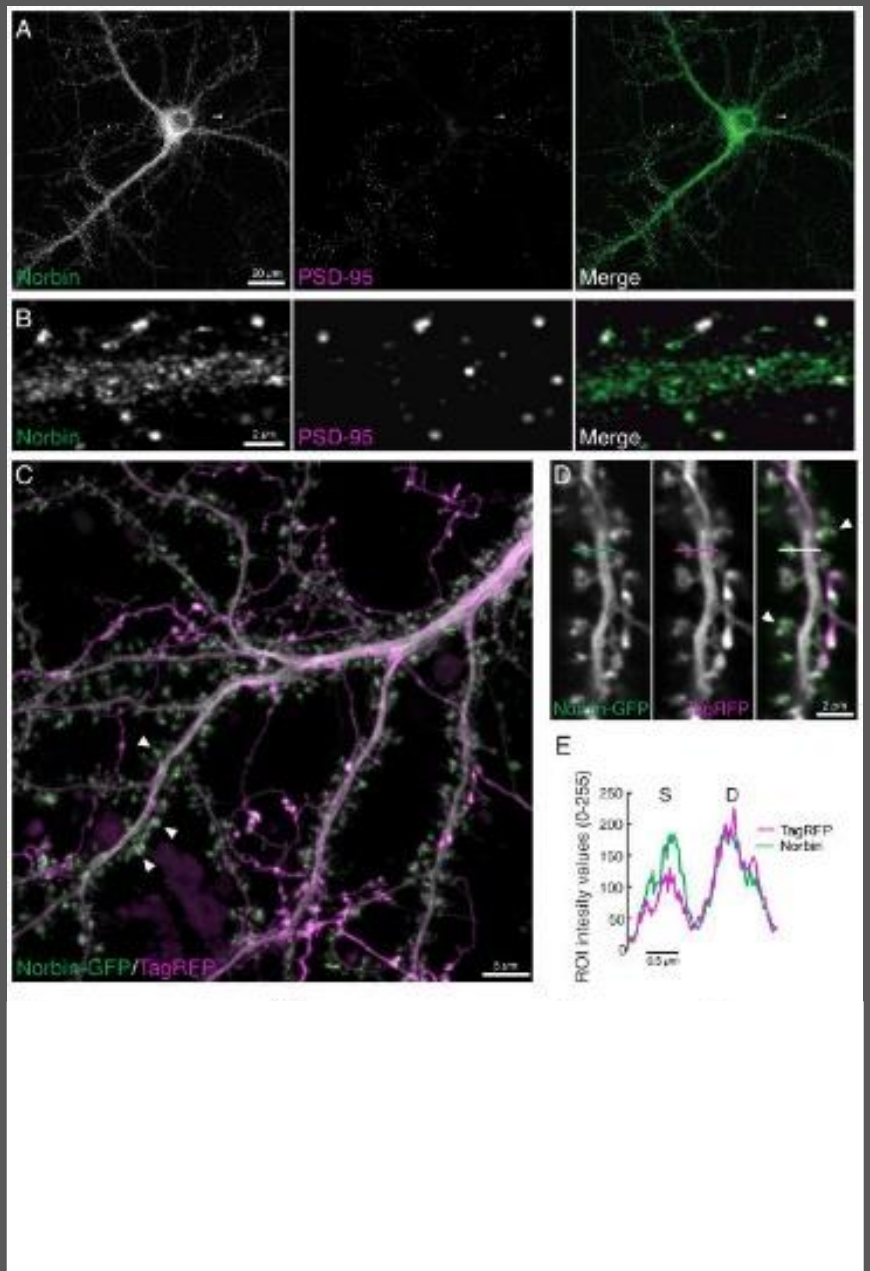
in situ hybrydyzacja z sondą RNA wyznakowaną radioaktywną siarką

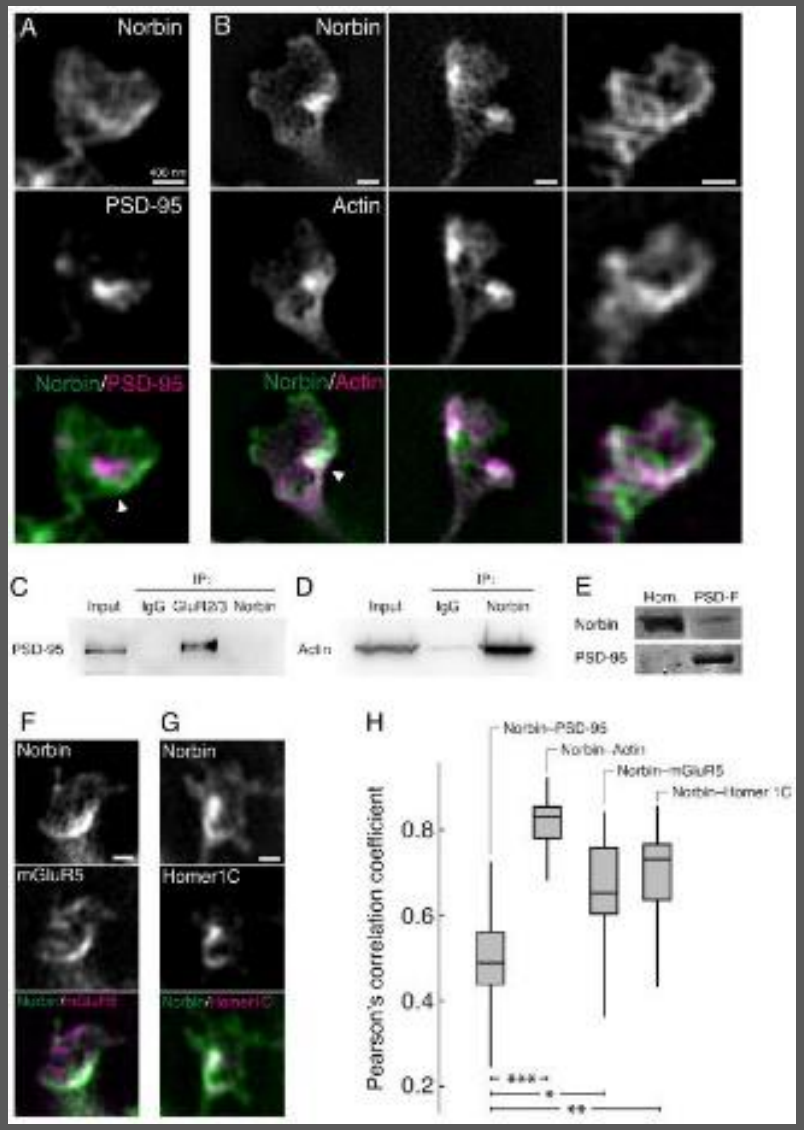


Vizi S. i wsp.,
Brain Research Protocols 2001

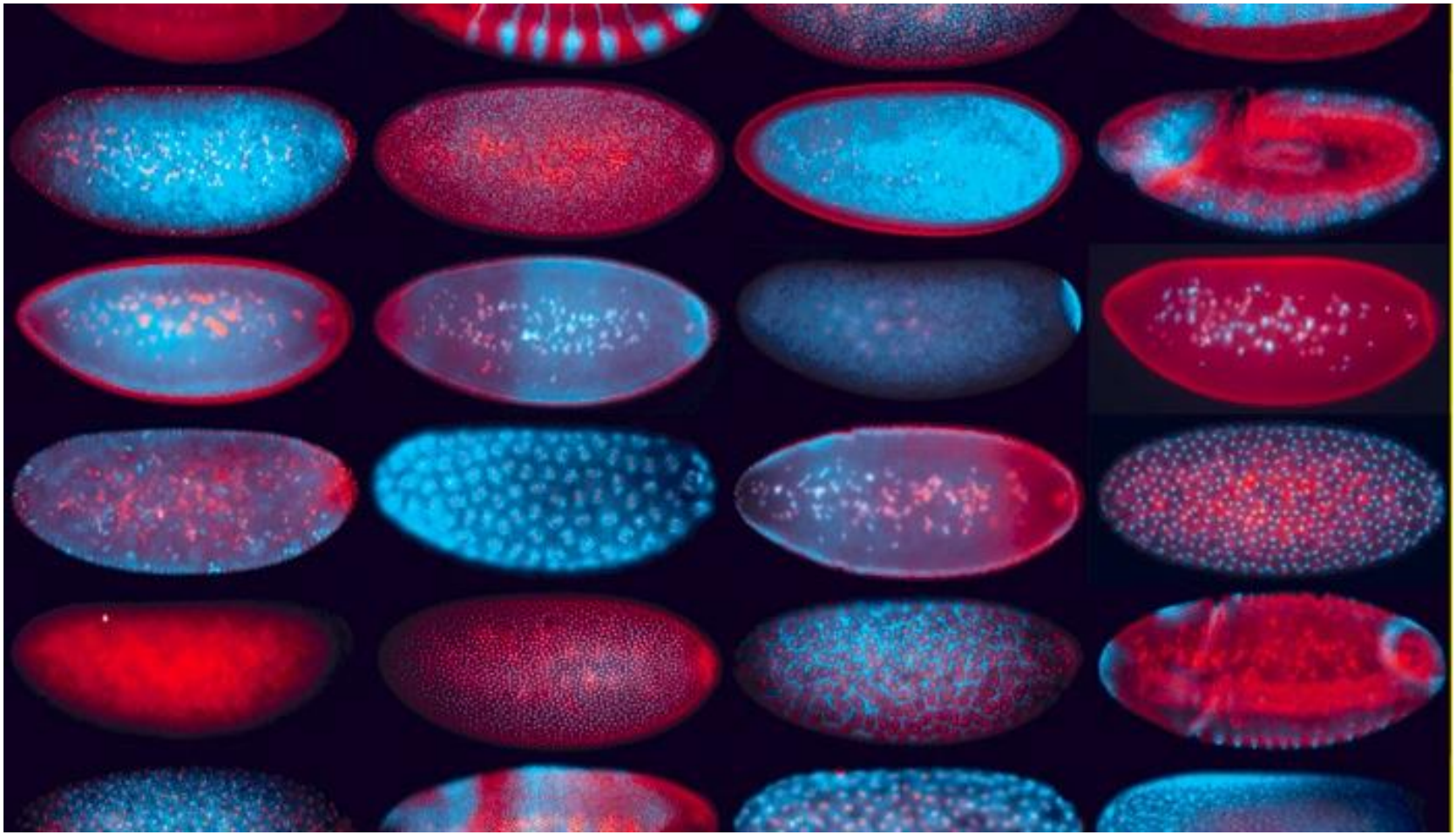
in situ hybrydyzacja w mikroskopie wysokorozdzielczym

(poniżej 200 nm)



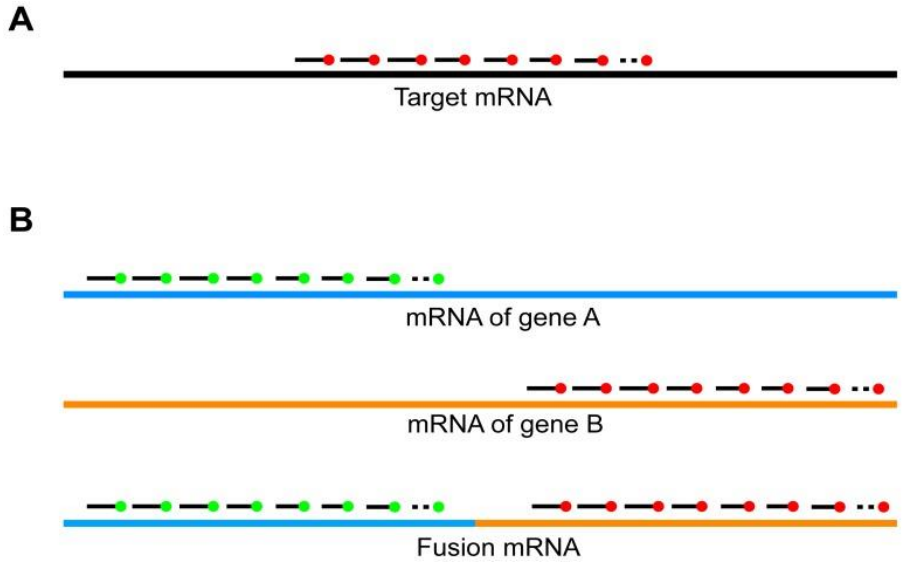


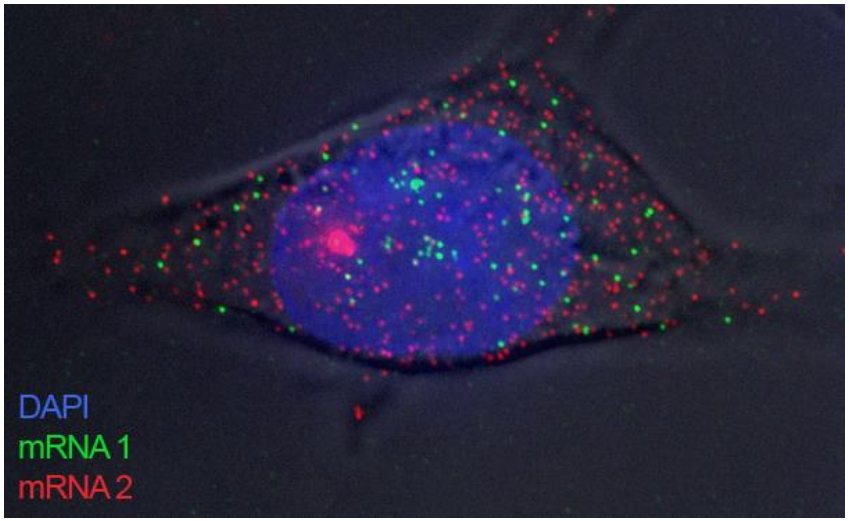
High-resolution fluorescent in situ hybridization procedure to comprehensively evaluate mRNA localization dynamics during early *Drosophila* embryogenesis.



Lecuyer et al. 2007

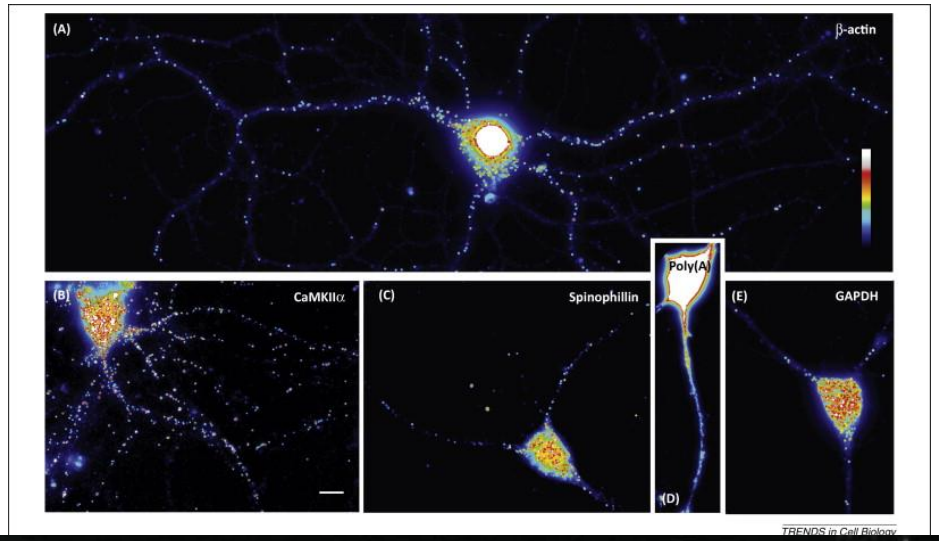
„Single molecule FISH” to metoda in situ hybrydyzacji pozwalająca na obrazowanie pojedynczej cząsteczki mRNA w komórce dzięki wykorzystaniu wielu fluorescencyjnie wyznakowanych sond zaprojektowanych do rozpoznawania sekwencji w obrębie tej samej cząsteczki mRNA

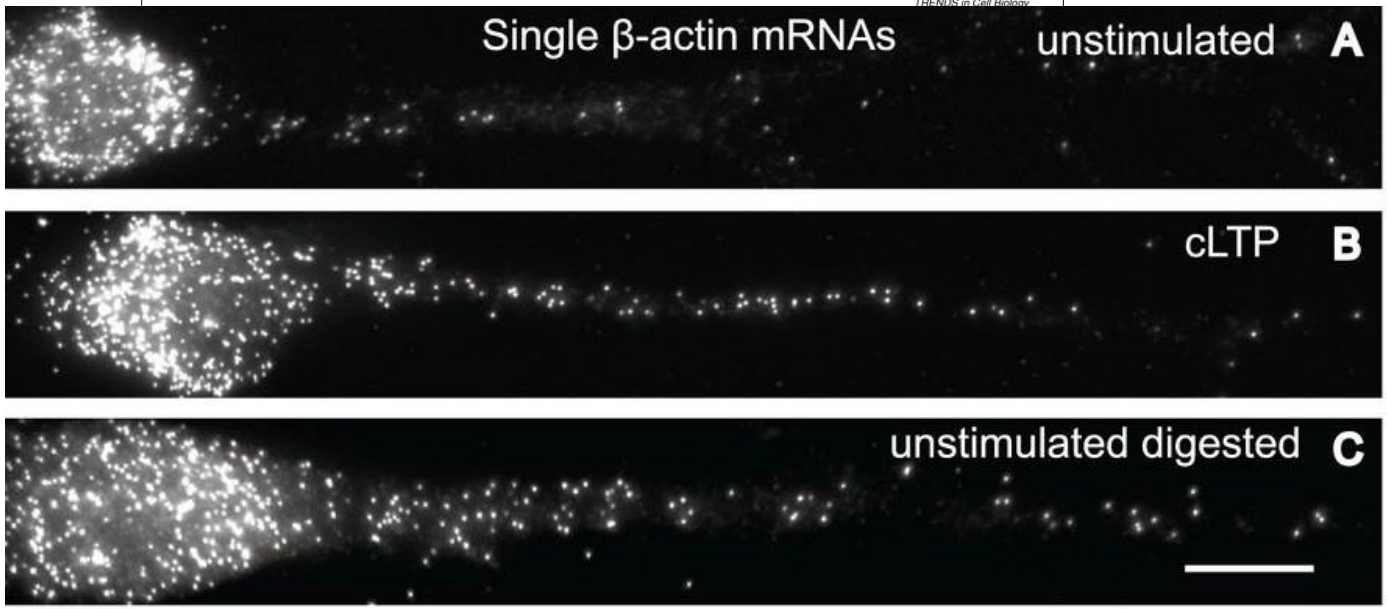




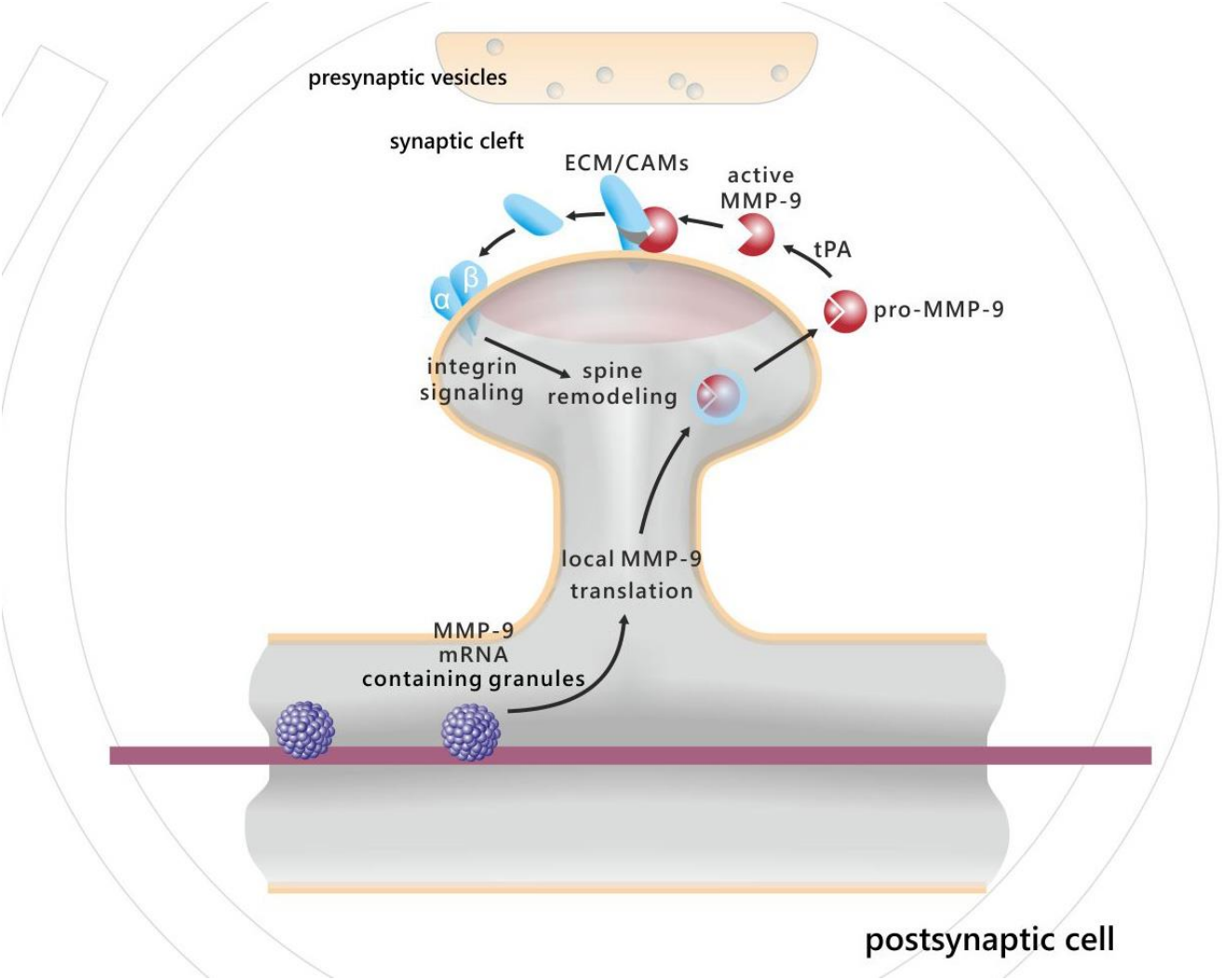
Przy użyciu tej metody można np. jednocześnie wykrywać dwa różne mRNA w komórce lub mRNA powstałe w wyniku fuzji 2 transkryptów (translokacji genomowych) jak np. BCR-ABL

Obrazowanie mRNA w dendrytach komórek nerwowych z wykorzystaniem smFISH





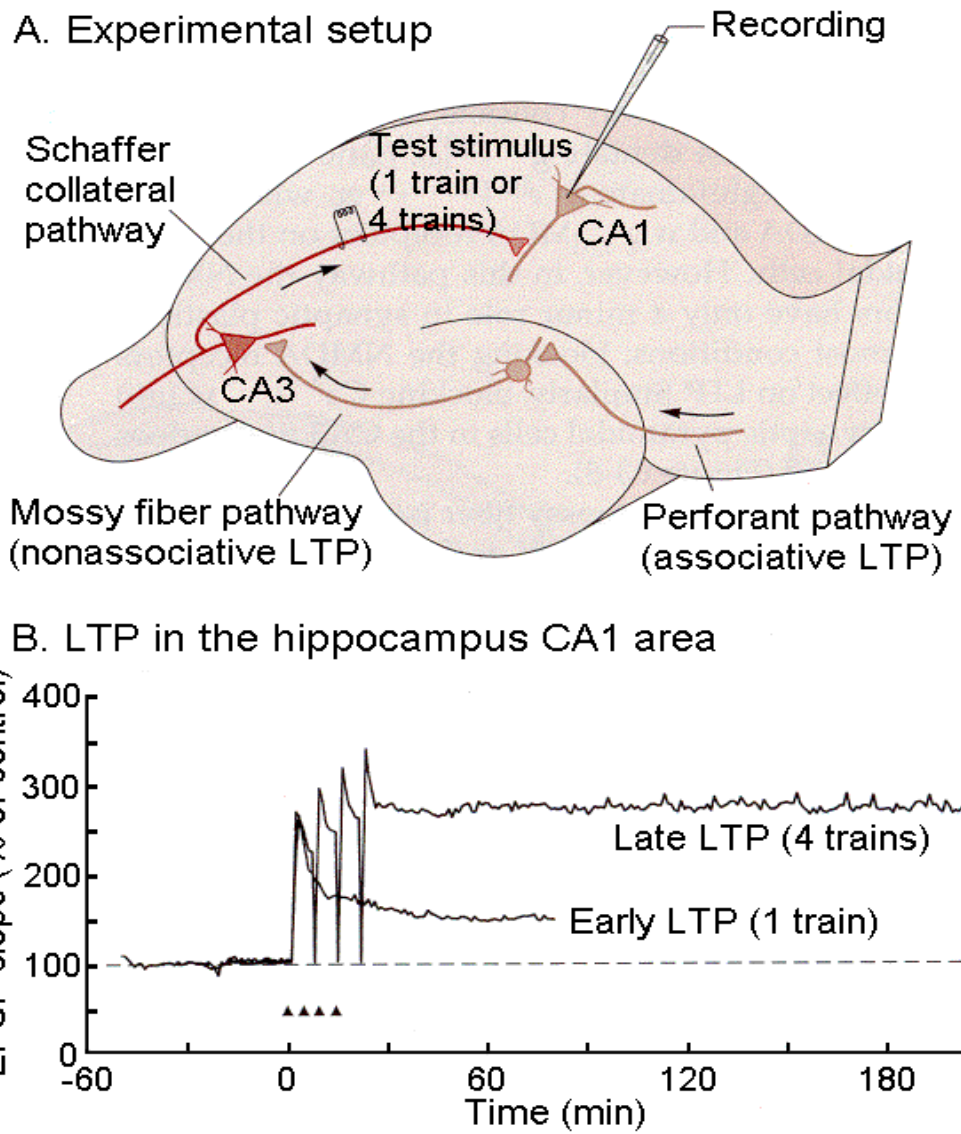
Activity-dependent local translation of MMP-9



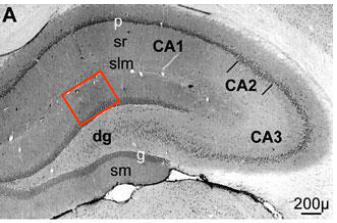
Dziembowska et al., J Neuroscience 2012

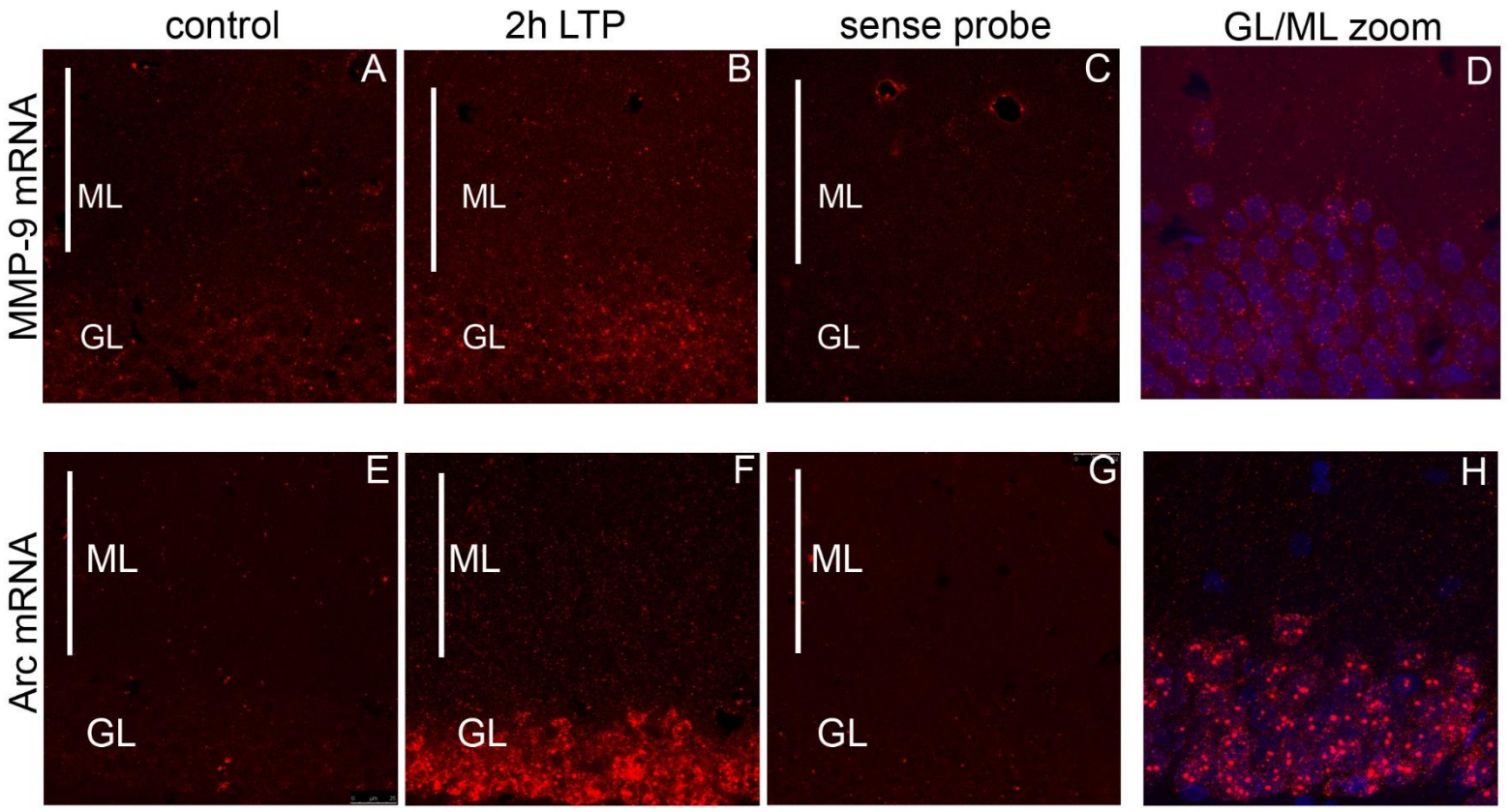
Dziembowska and Wlodarczyk, Int J Biochem Cell Biol **,** *2012*

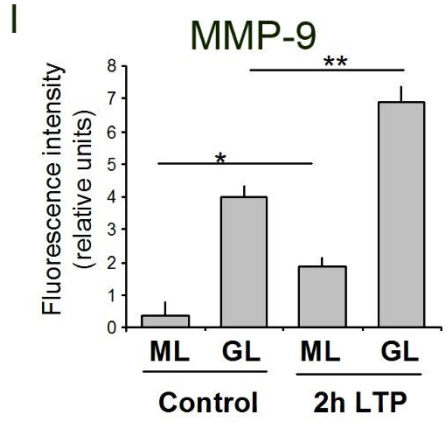
Medial perforant path LTP - a well established model of synaptic plasticity

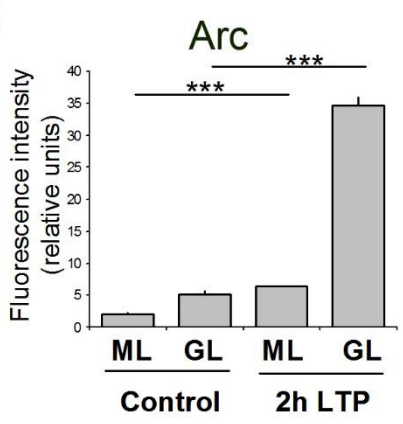


In situ hybridization shows increse in MMP-9 expression in granular layer and molecular layer of dentate gyrus 2h after medial perforant path LTP

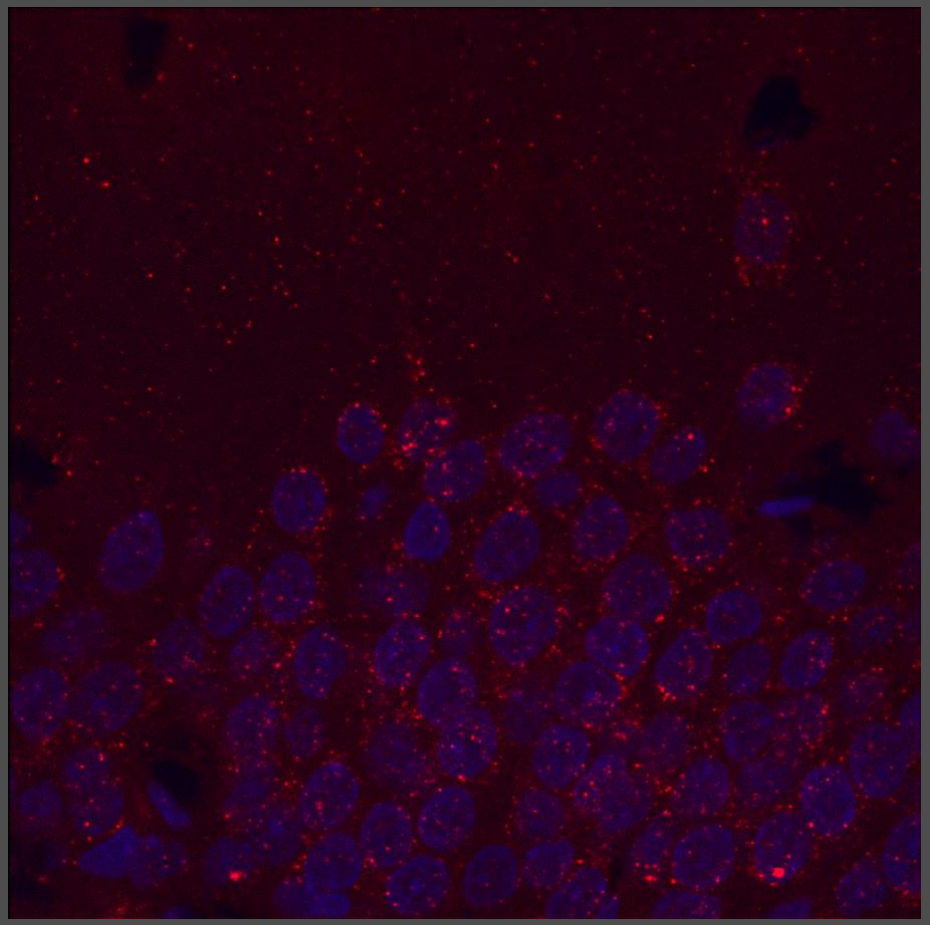




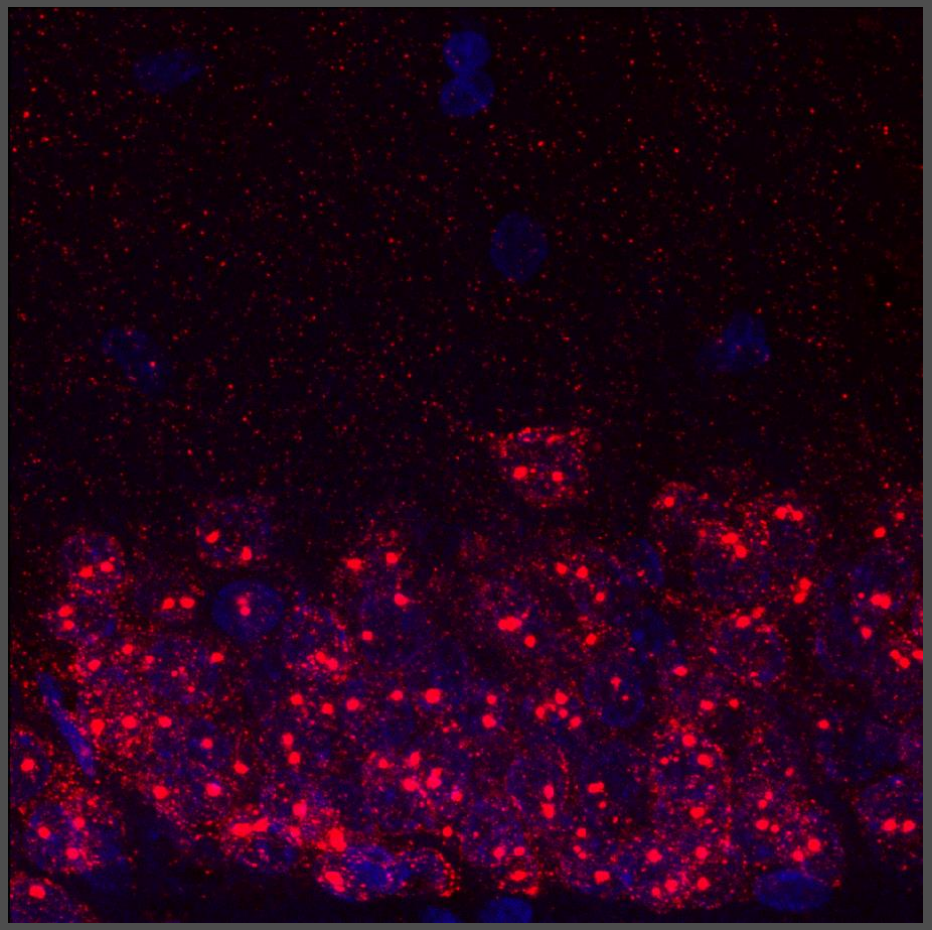




MMP-9 in situ hybridization



Arc in situ hybridization



Sushi belt model

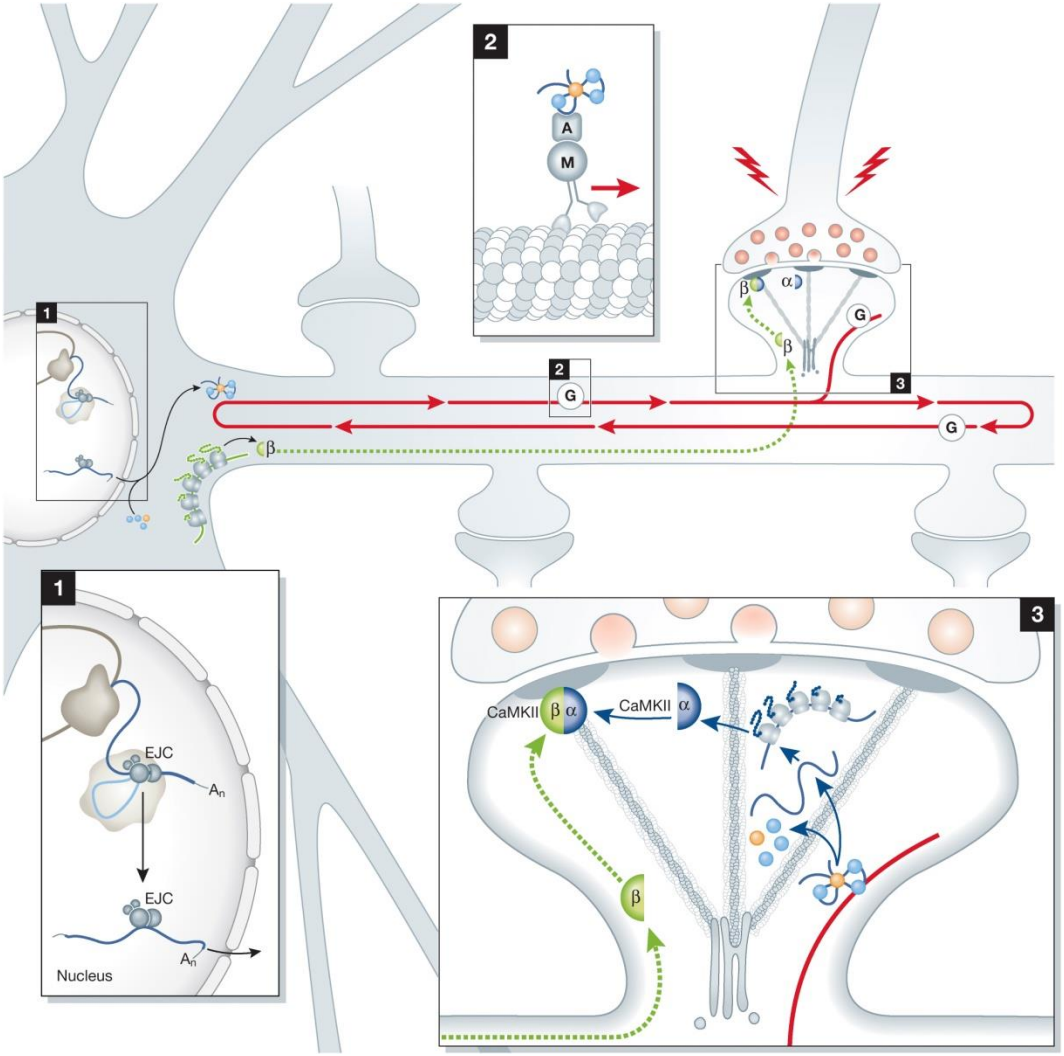
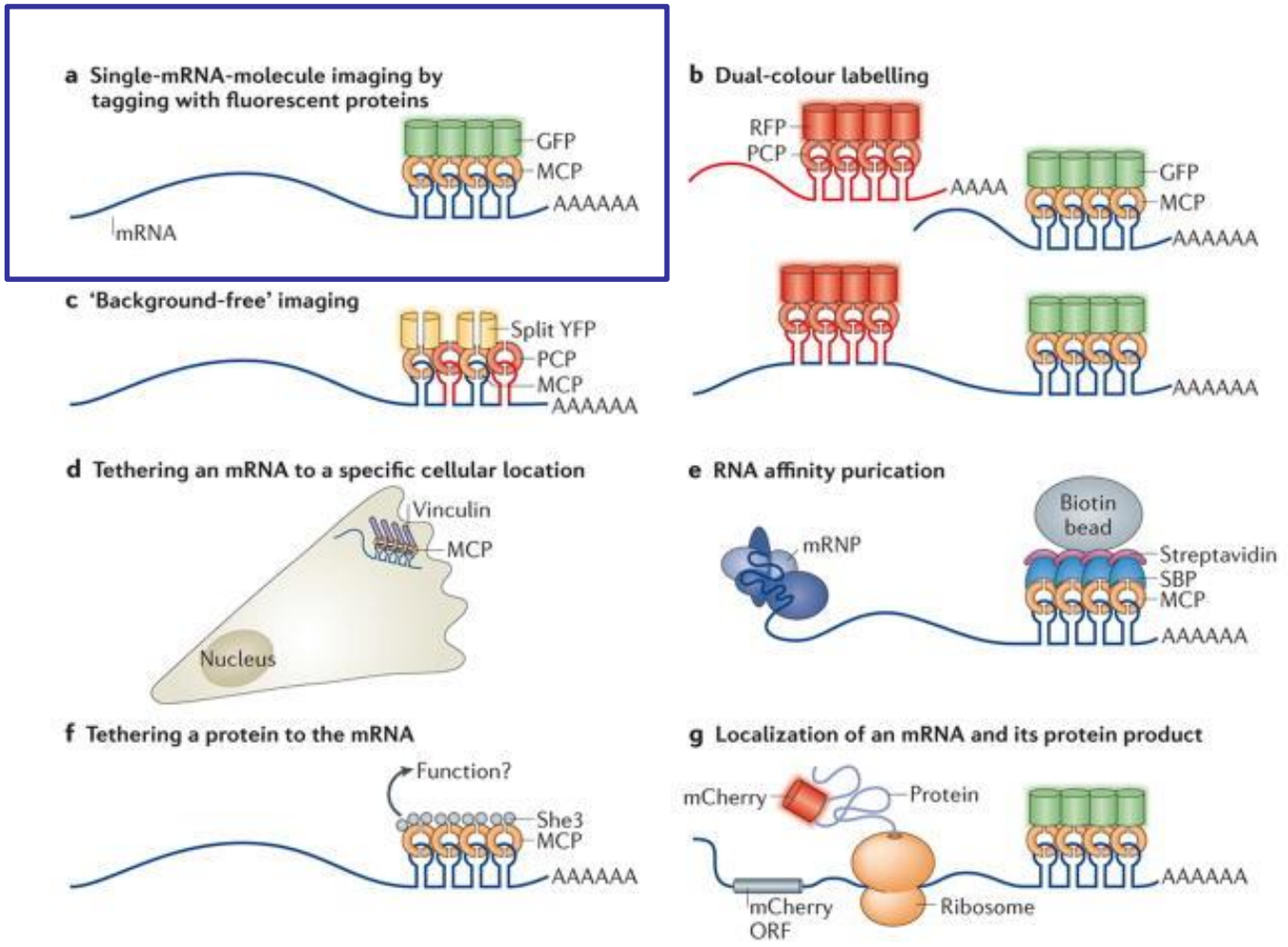


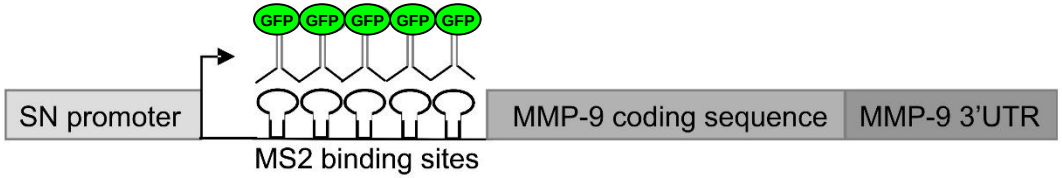
Figure 2 from Michael Doyle and Michael A Kiebler
The EMBO Journal online publication
doi:10.1038/emboj.2011.278

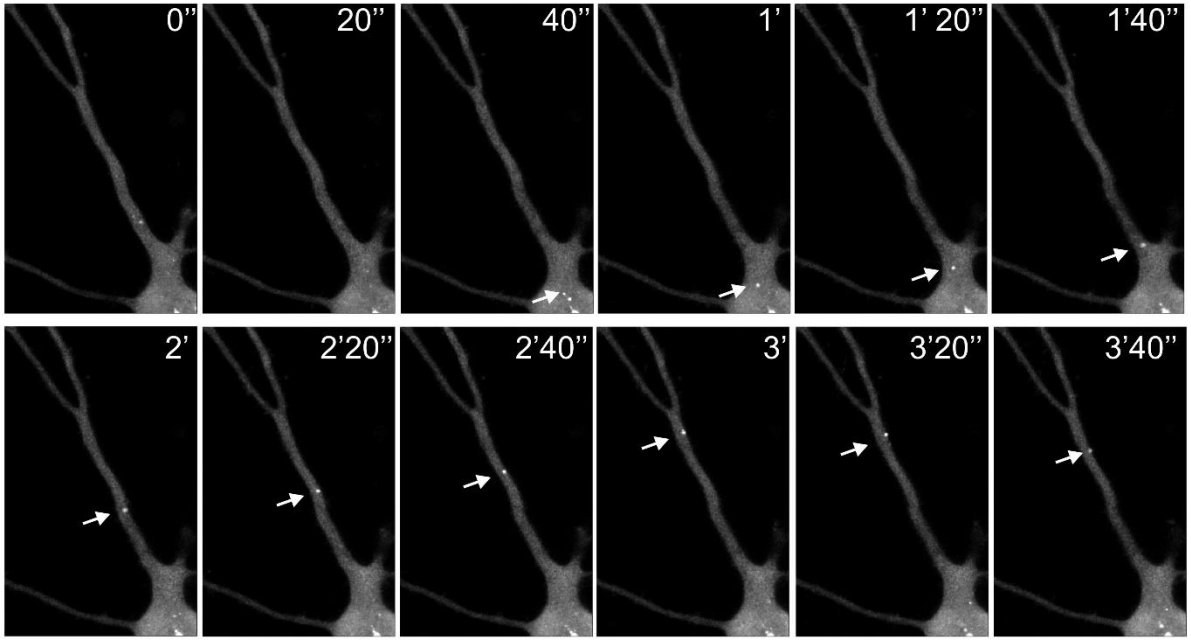
Traditional and novel uses of MS2-like systems to investigate mRNA biology



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

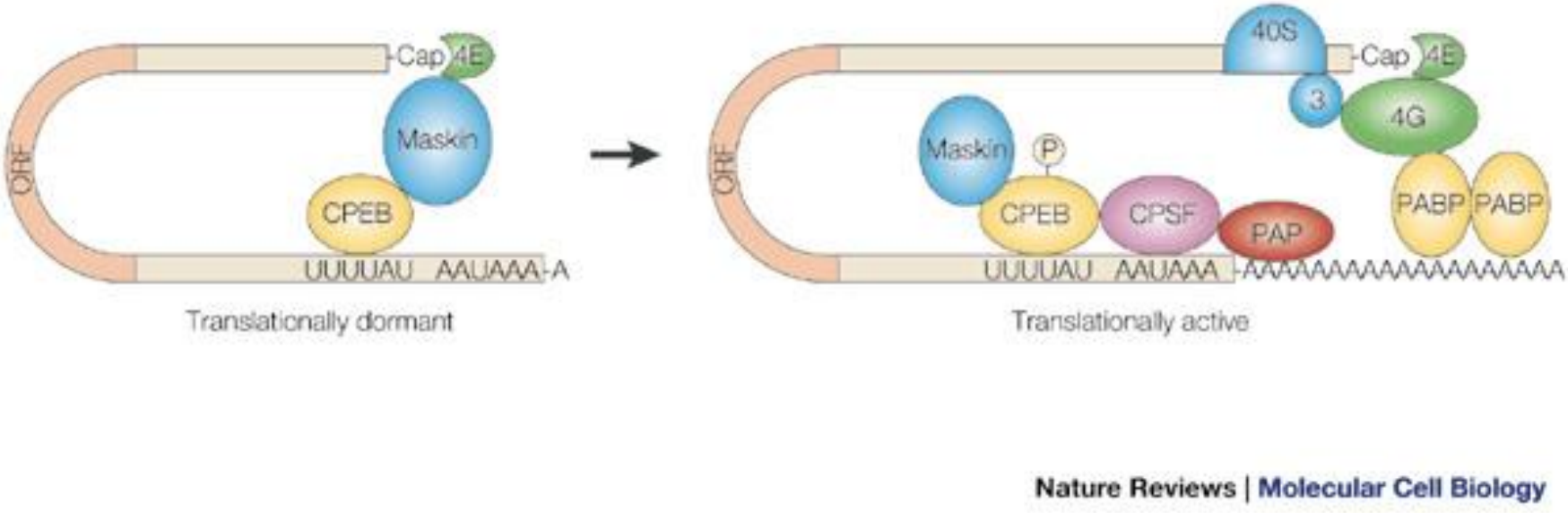
MS2 system to stain targeted mRNA in the living cell





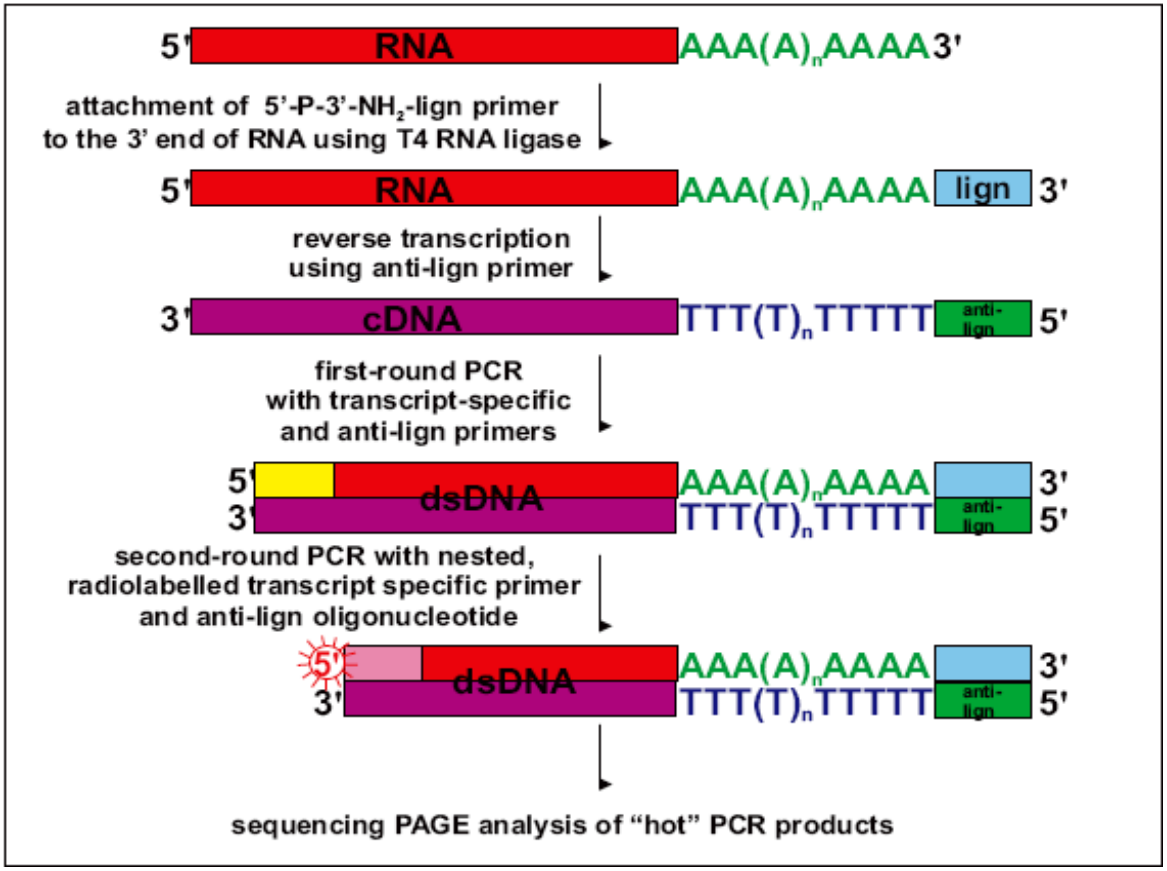
Dziembowska et al., J Neurosci. 2012

Cytoplasmic polyadenylation promotes translation

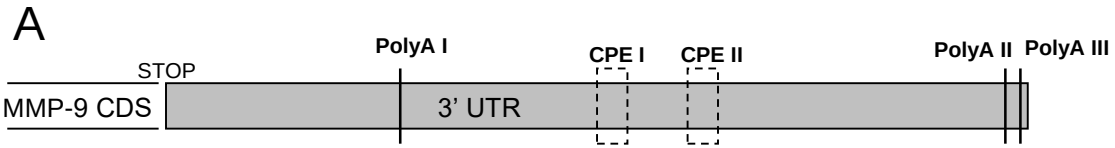


Mendez, R. & Richter, J. D. Translational control by CPEB: a means to the end. *Nature Reviews Molecular Cell Biology* 2, 521–529 (2001)

PAT assay

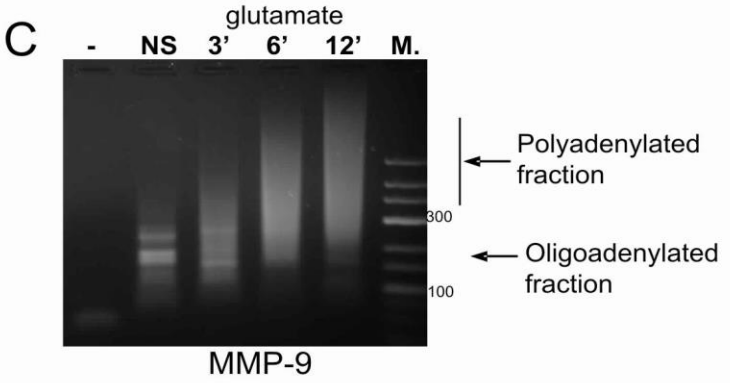

5' RNA AAA(A)nAAAA 3'
attachment of 5'-P-3'-NH2-lign primer
to the 3' end of RNA using T4 RNA ligase
5' RNA AAA(A)nAAAA lign 3'
reverse transcription
using anti-lign primer
3' cDNA TTT(T)nTTTTT anti-lign 5'
first-round PCR
with transcript-specific
and anti-lign primers
5'
dsDNA
AAA(A)nAAAA 3'
3'
TTT(T)nTTTTT 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

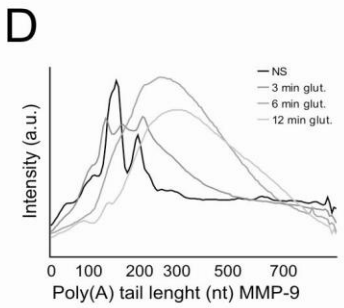
MMP-9 polyadenylation measured by PAT ssay in synaptoneurosomes after glutamate stimulation

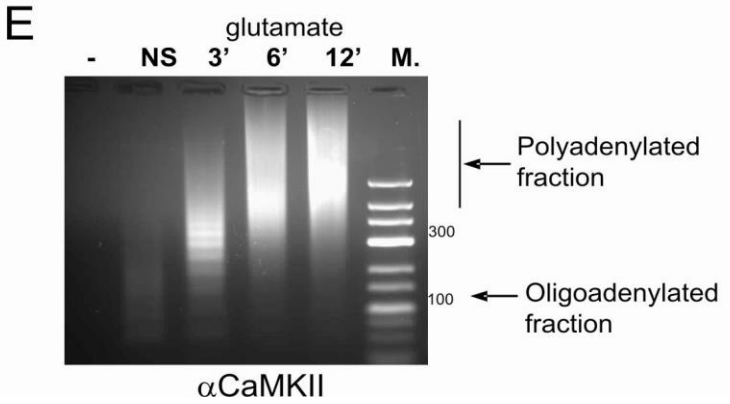


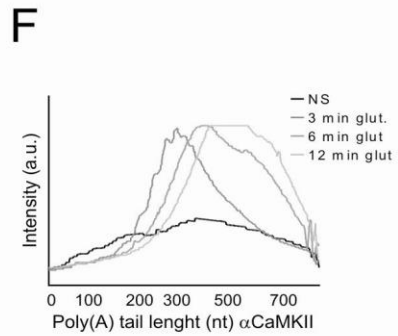
B

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

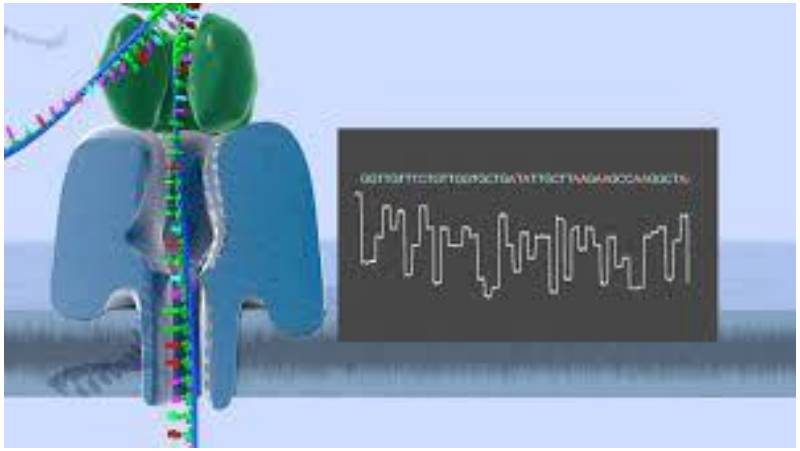


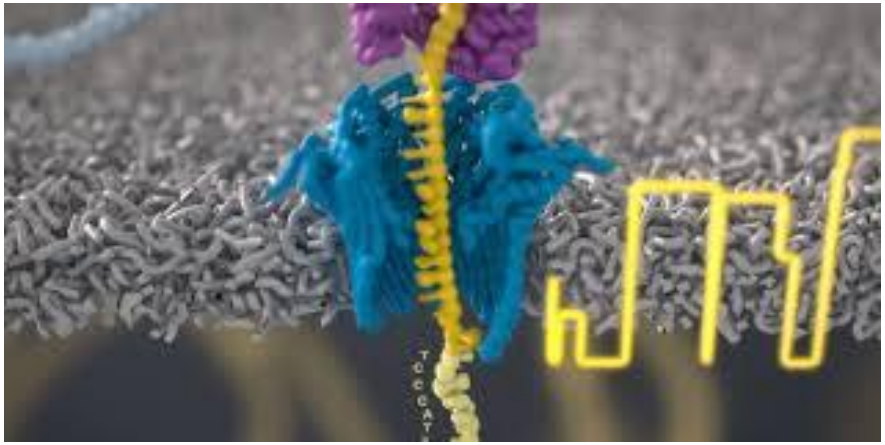






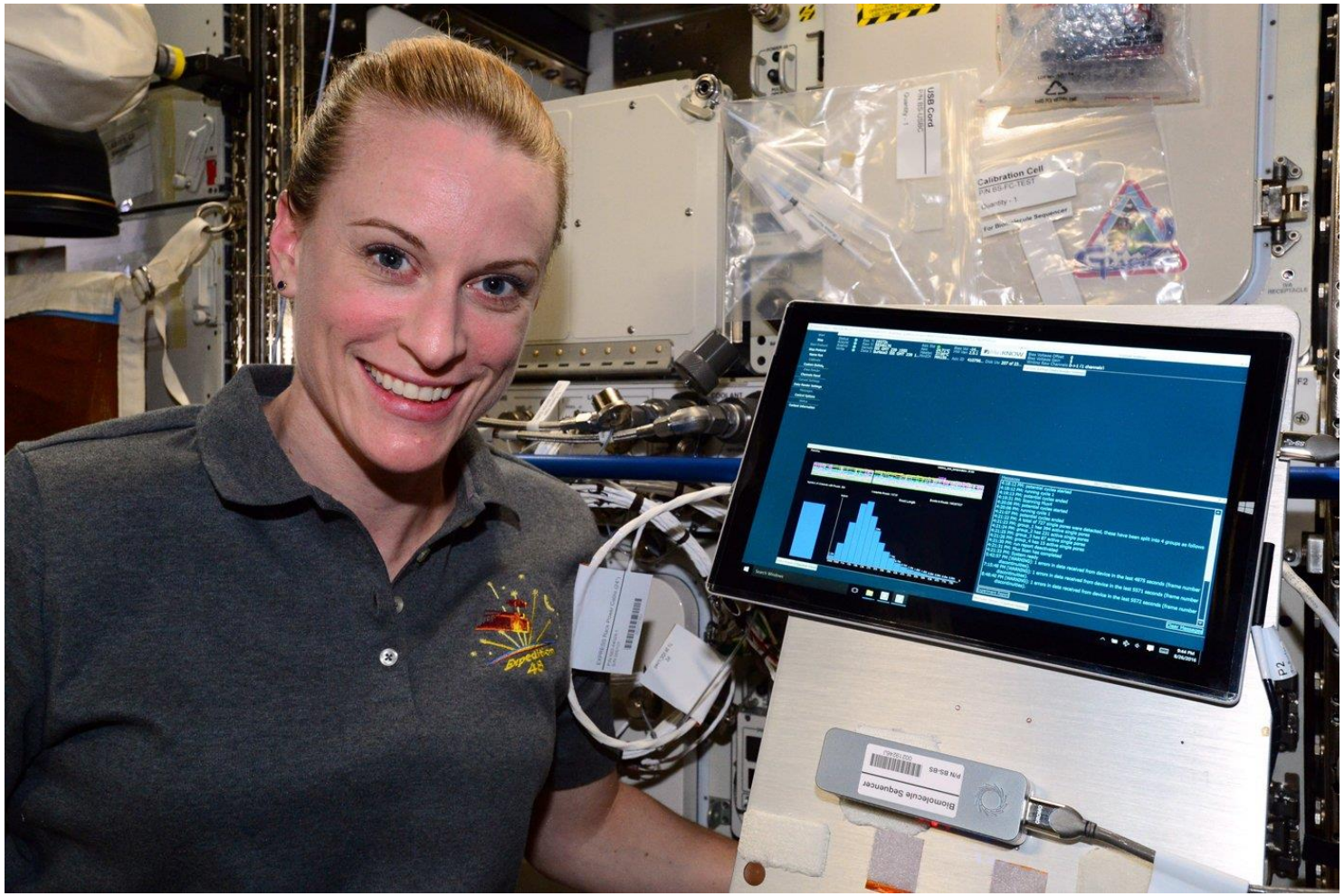
Nanopore Technology and Its Applications in Gene Sequencing





Lin B et al., Biosensors 2021

MinION (Oxford Nanopore) na Międzynarodowej Stacji Kosmicznej



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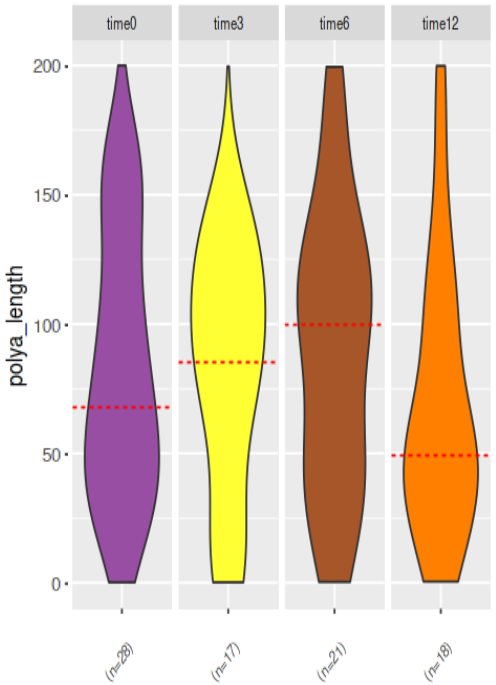
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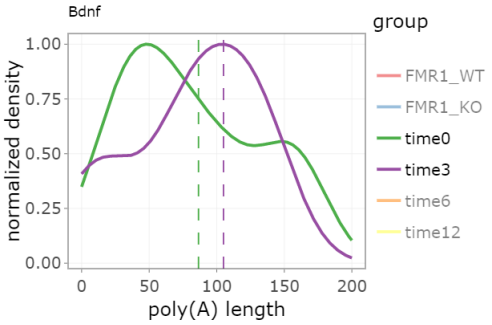
(n=17)
(n=21)
(n=18)


Bdnf
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0.50
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group
FMR1_WT
FMR1_KO
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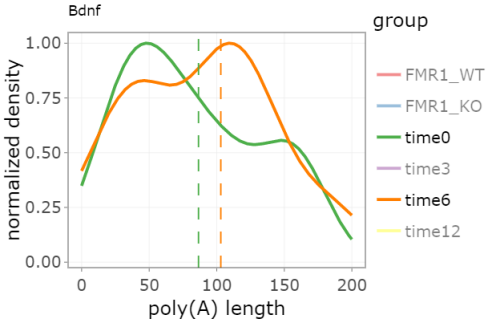
time12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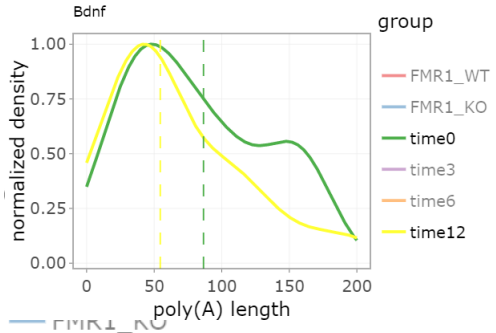
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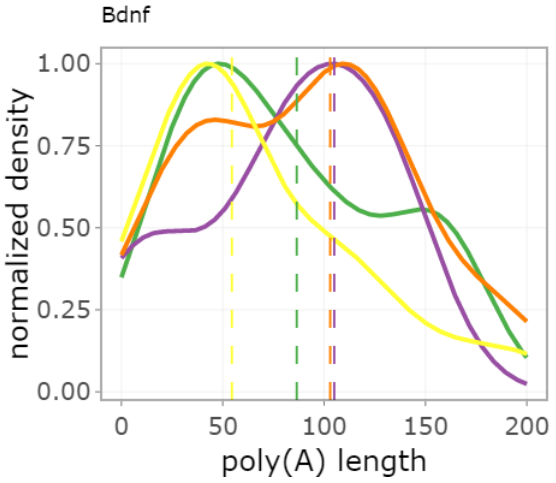
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time0
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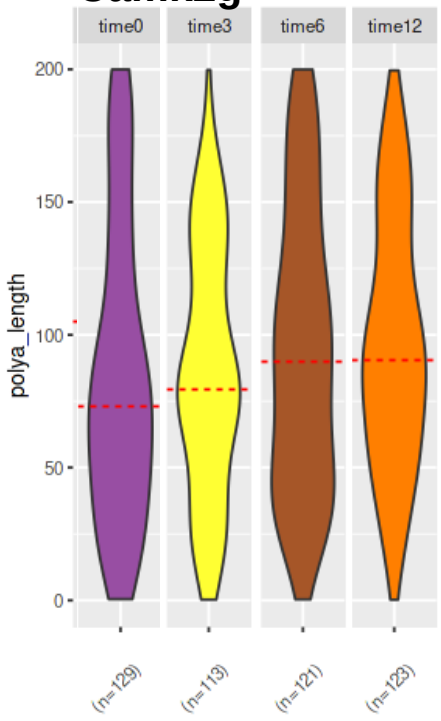
Camk2g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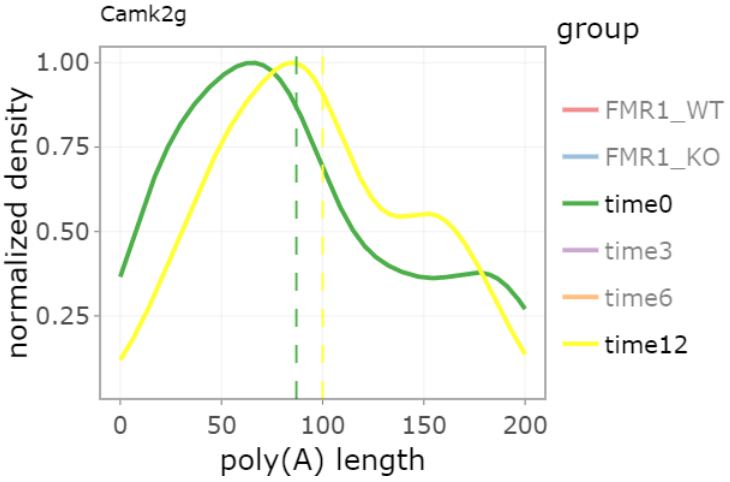
Camk2g
normalized density
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0.50
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poly(A) length
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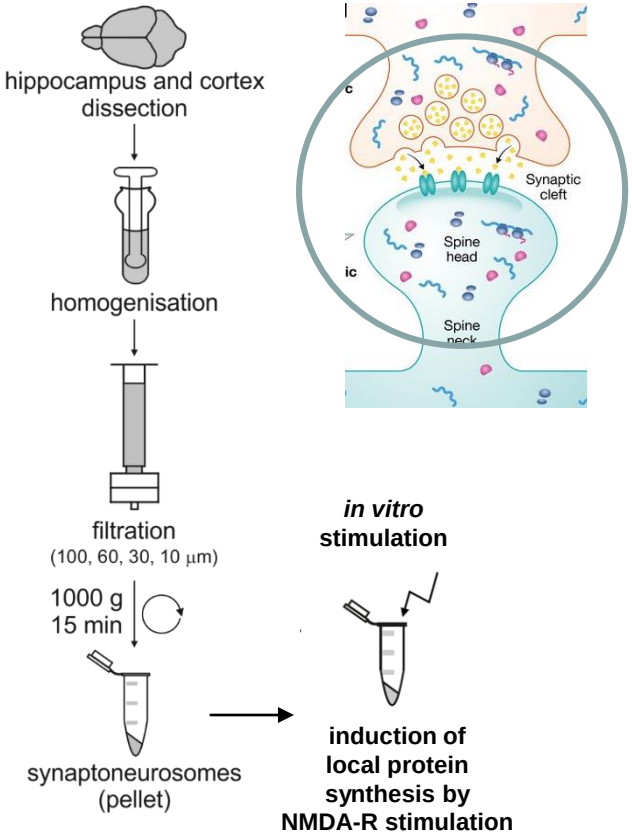
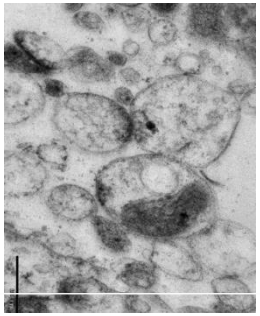
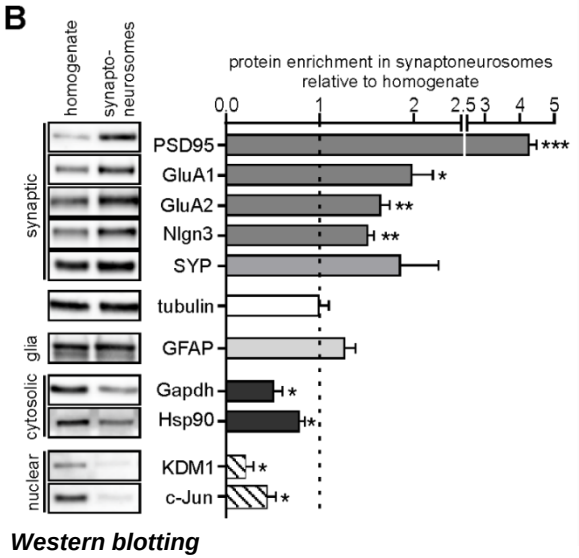
Synaptoneurosomy, model do badań procesów biochemicznych zachodzących w symapsie



Electron microscopy; A. Janusz





Western blotting

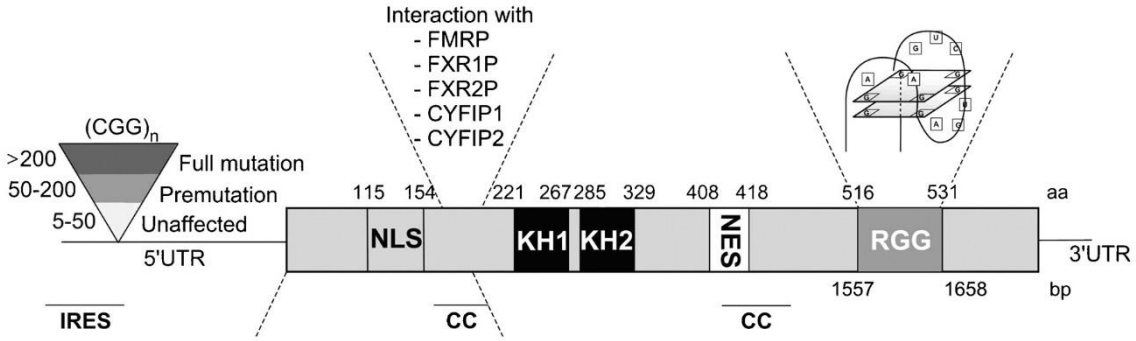
Badanie oddziaływania białko-RNA (na przykładzie FMRP-mRNA MMP-9)

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.

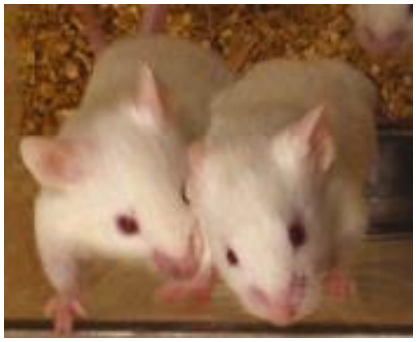
Występuje u 1:4000 mężczyzn oraz 1:8000 kobiet i odpowiada za 5% zdiagnozowanych przypadków autyzmu.

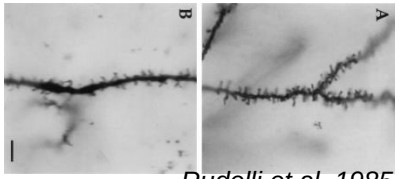
Zespół łamliwego chromosomu X jest spowodowany wyciszeniem genu Fmr1 i wskutek tego, brakiem białka łamliwego chromosomu X (FMRP).



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

***Myszy Fmr1* KO**

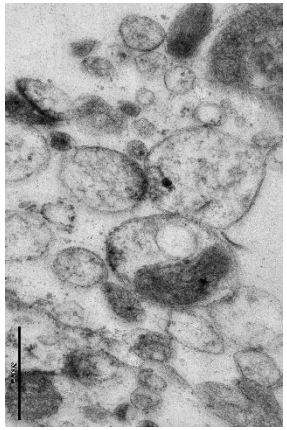


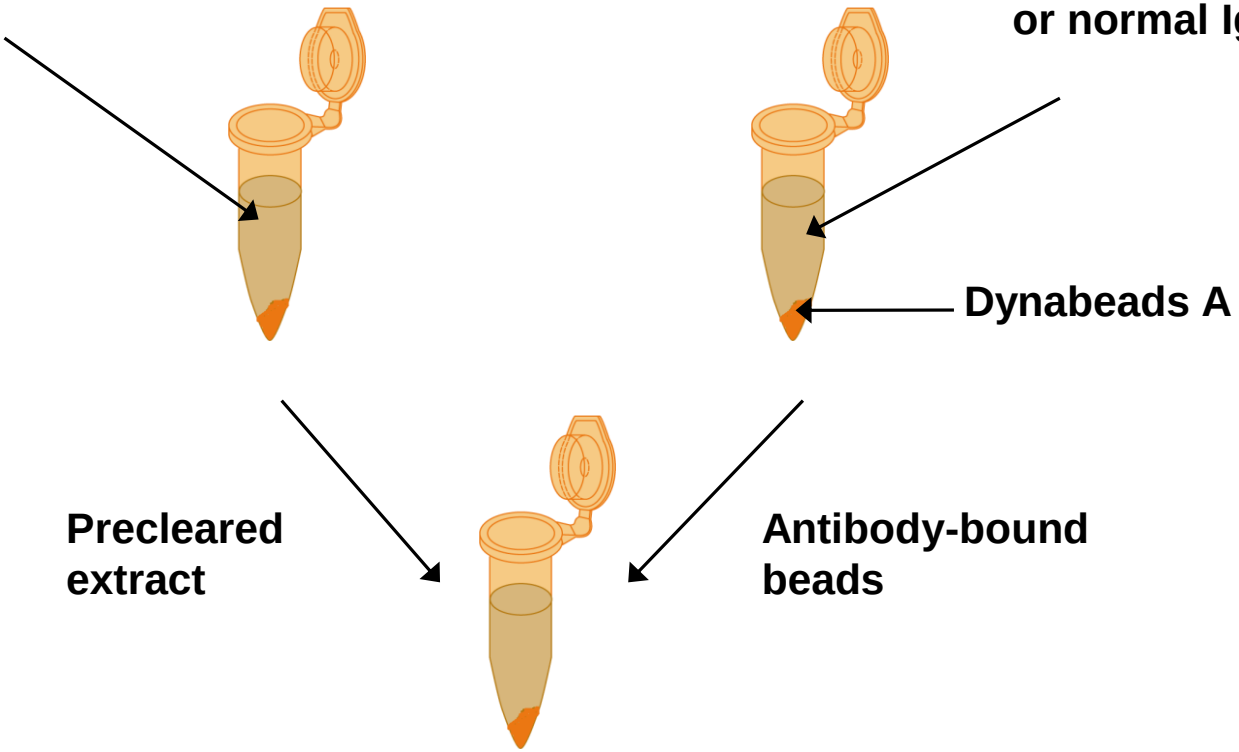


Rudelli et al.,1985

Koimmunoprecipitacja białka FMRP z mRNA MMP-9

Synaptoneurosome extract

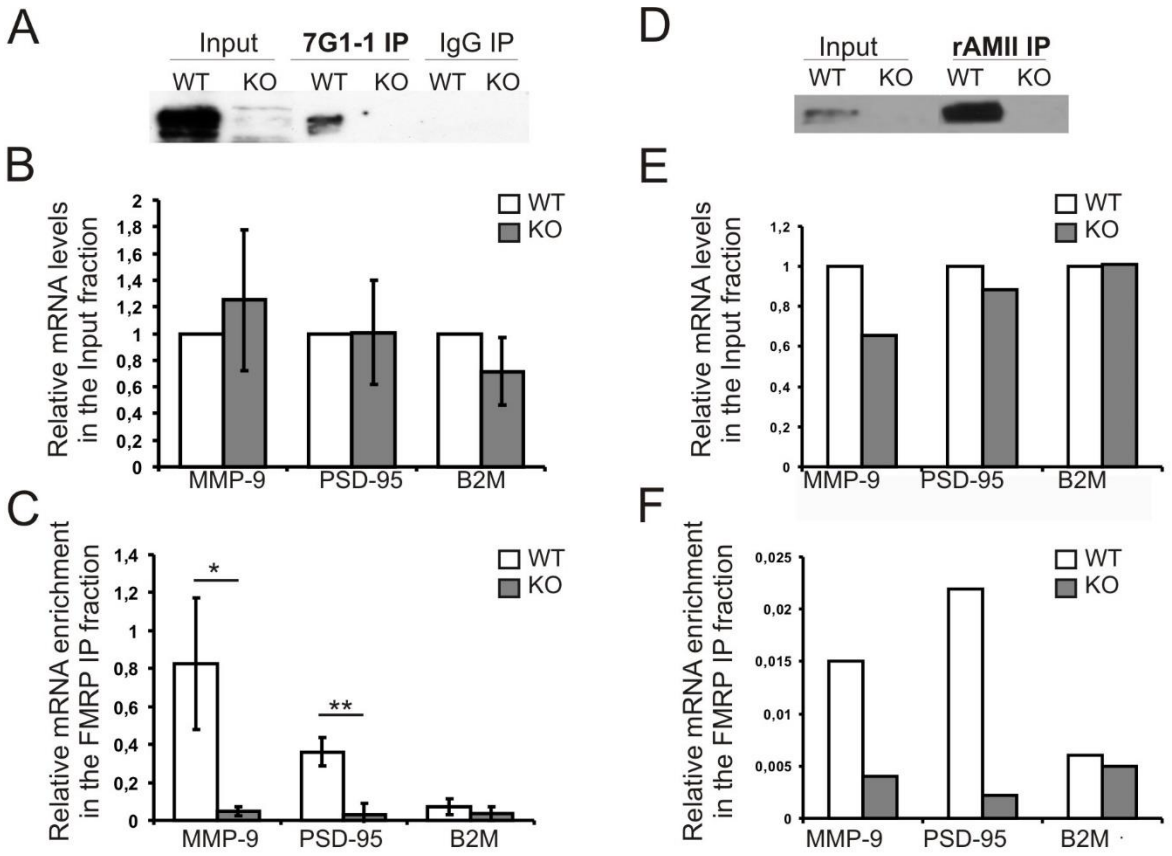




Immunoprecypitated complexes

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

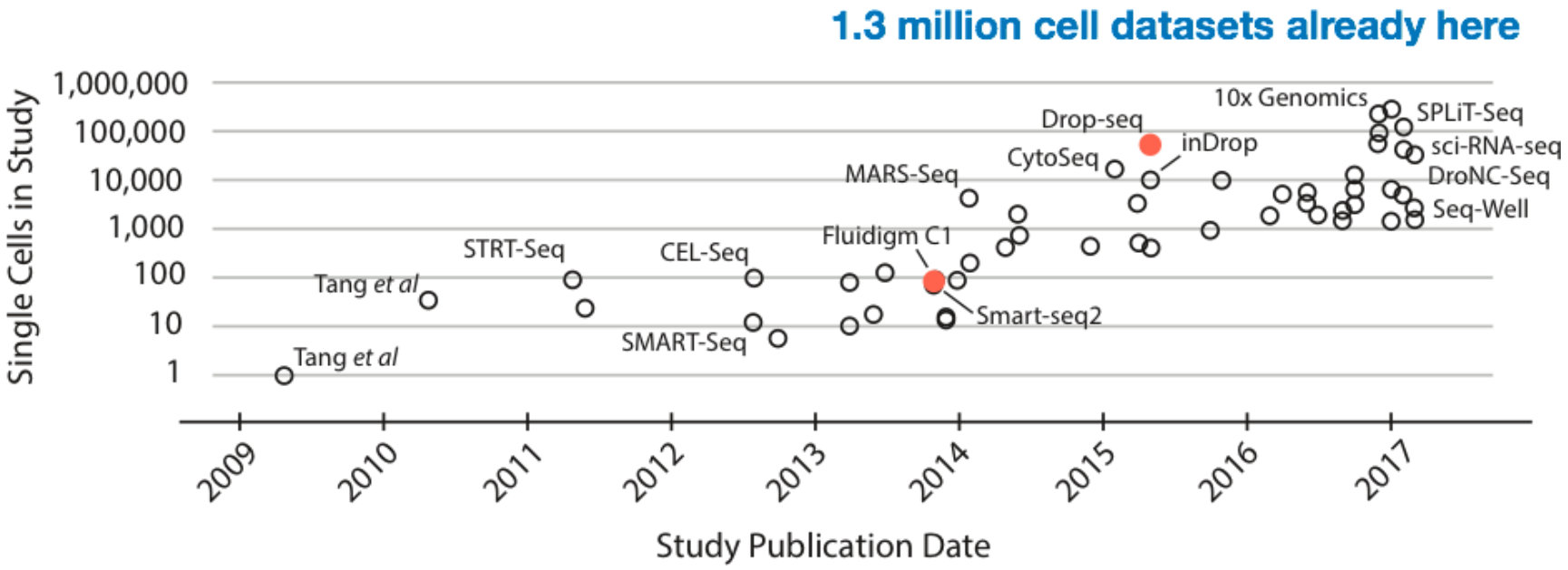
Koimmunoprecipitacja białka FMRP z mRNA MMP-9

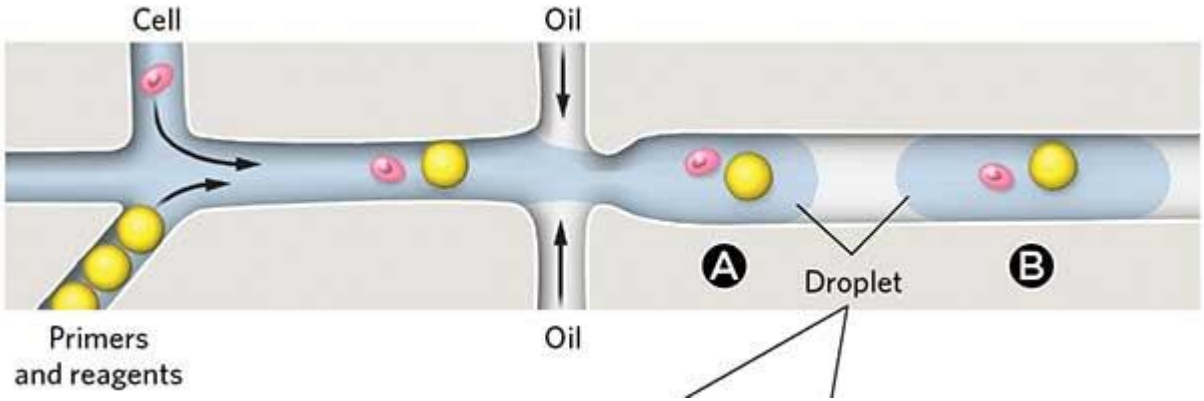


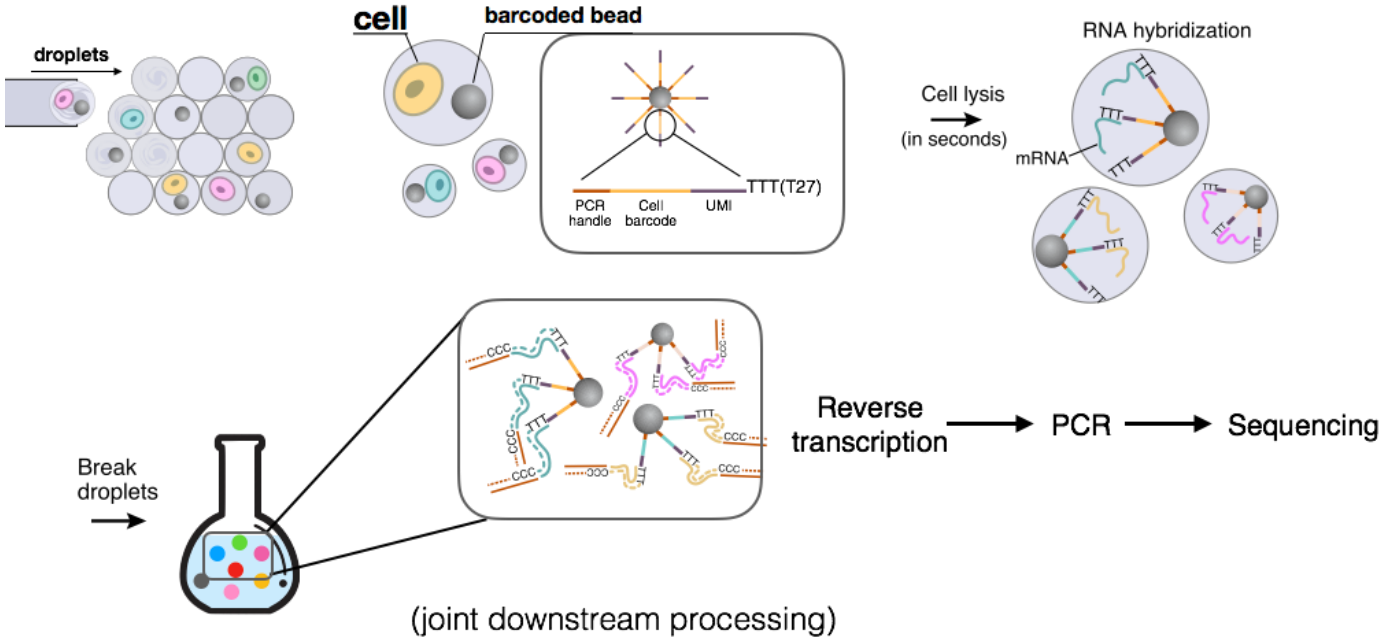
Janusz et al., J Neuroscience 2013

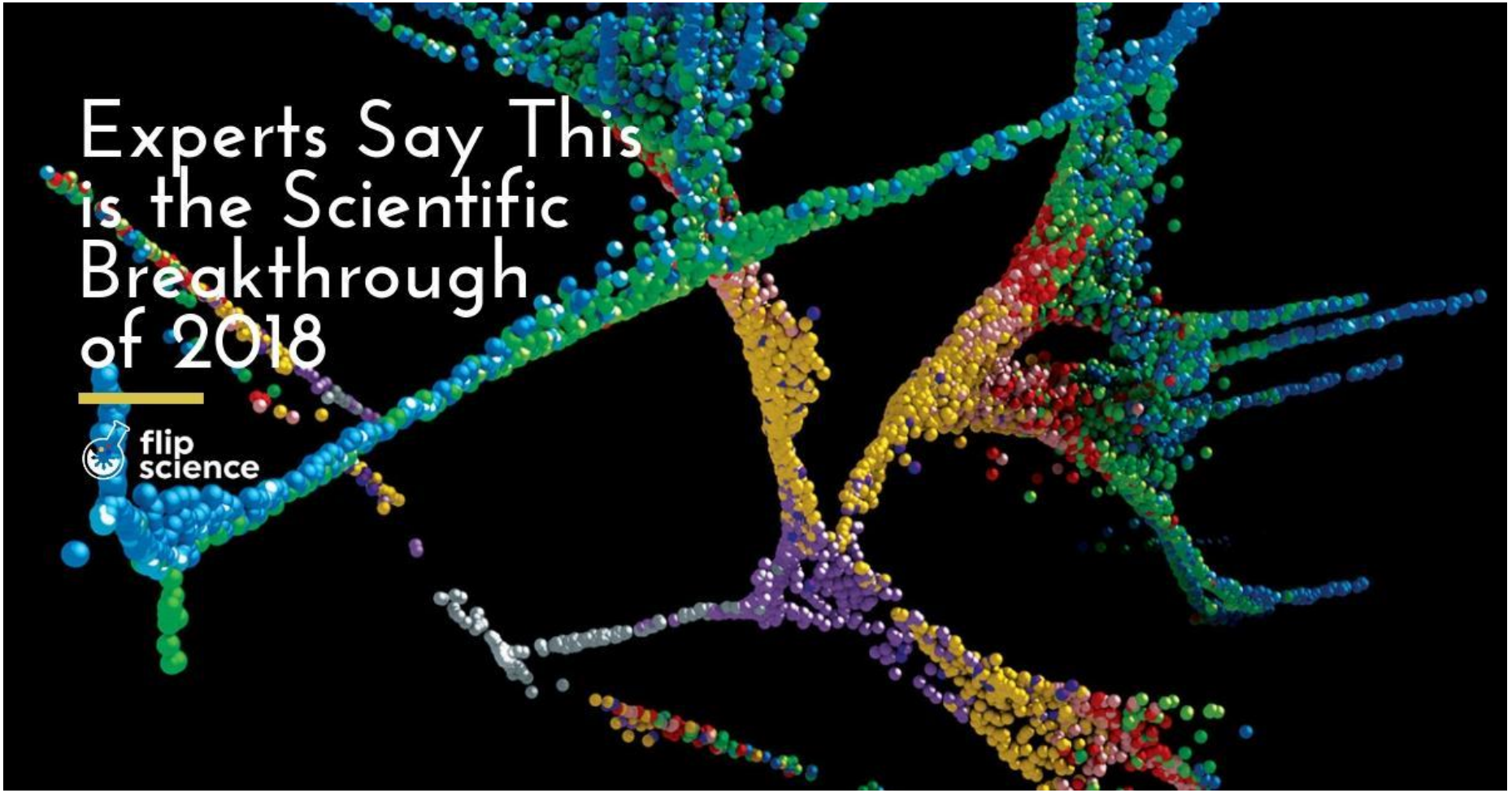
Rozwój nowych technologii – sekwencjonowanie mRNA z pojedynczej komórki

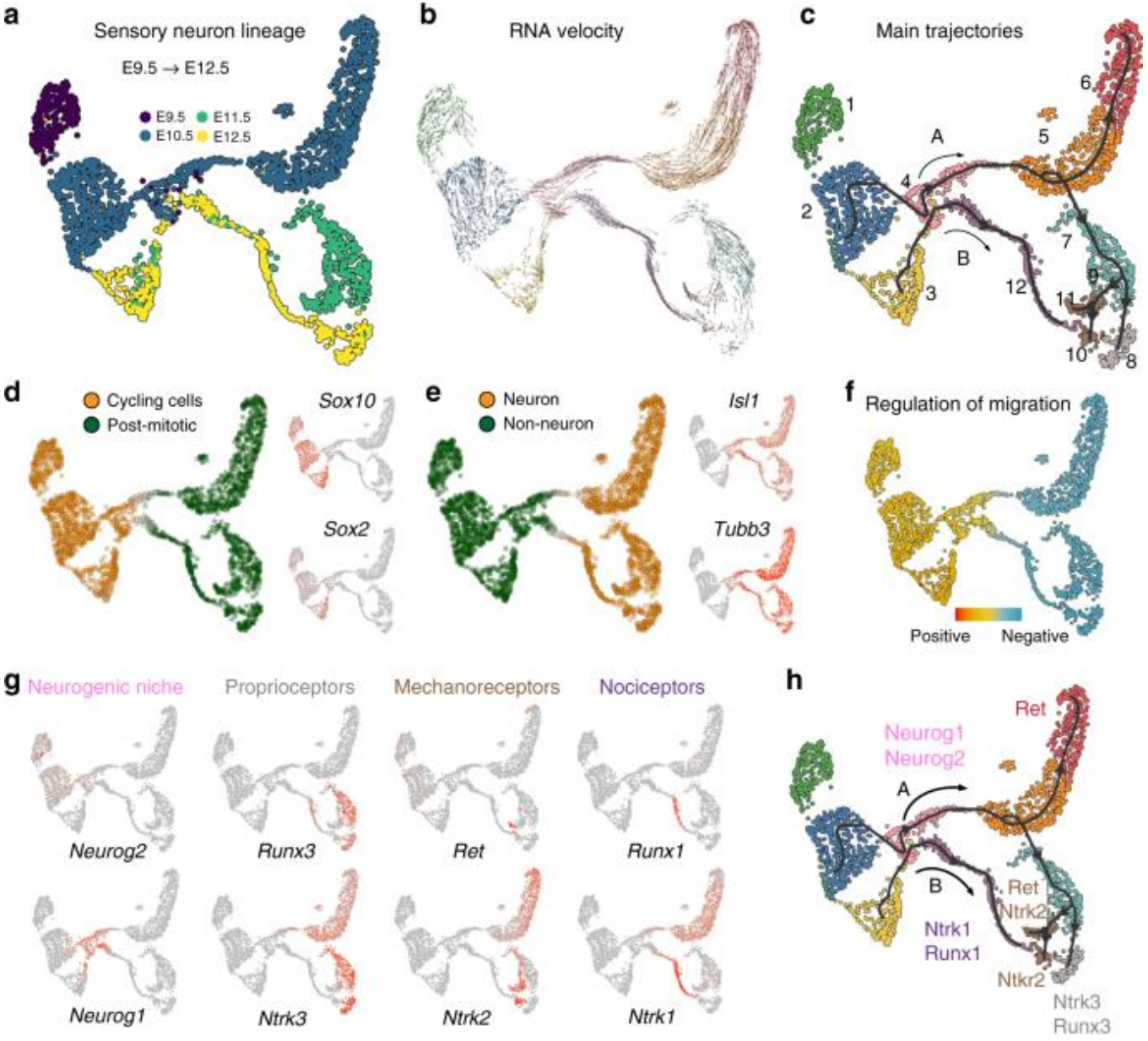
Rozwój technologii sekwencjonowania




Cell
Oil
A
Droplet
B
Primers
and reagents
Oil


droplets
cell
barcoded bead
TTT(T27)
PCR handle
Cell barcode
UMI
RNA hybridization
Cell lysis
(in seconds)
mRNA
Break droplets
Reverse transcription
PCR
Sequencing
(joint downstream processing)


Experts Say This
is the Scientific
Breakthrough
of 2018
flip
science



Faure L et al., Nature Communication 2020

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Single cell RNA sequencing identifies early diversity of sensory neurons forming via bi-potential intermediates

Louis Faure[1], Yiqiao Wang[2], Maria Eleni Kastriti[1], Paula Fontanet[2], Kylie K. Y. Cheung[2], Charles Petitpré[2], Haohao Wu[2], Lynn Linyu Sun[2], Karen Runge[3], Laura Croci[4], Mark A. Landy[5], Helen C. Lai[5], Gian Giacomo Consalez[4], Antoine de Chevigny[3], François Lallemend[2,6], Igor Adameyko[1,7] & Saida Hadjab[2]✉