Metody badania RNA w neuronach

Magdalena Dziembowska

Laboratory of Neurobiology
The Nencki Institute of Experimental Biology, Warsaw
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
Polyribosomes localized in dendritic spines

Steward and Levy w 1982
Synaptic spines are "structural units" that undergo plastic changes in response to synaptic 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
Determination whether neuronal activity regulates dendritic/synaptic MMP-9 mRNA translocation, translation and release at the activated synapses.

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 mRNA

FMRP

control

20 min bicuculline

MMP-9 mRNA

FMRP

10 min bicuculline

Number of granules per 50μm of dendrite

0 2 4 6 8 10 12 14 16

ctrl 10 min 20 min

***

ctr 10 min 20 min

***
Sushi belt model

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

© 2011 European Molecular Biology Organization.
MS2 system to stain targeted mRNA in the living cell

MS2 system to stain targeted mRNA in the living cell
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
Synaptoneurosomy w mikroskopie elektronowym
MMP-9 polyadenylation measured by PAT ssay in synaptoneurosomes after glutamate stimulation

A

MMP-9 CDS

STOP

PolyA I

CPE I

CPE II

PolyA II

PolyA III

3' UTR

B

CPE I

R. norvegicus

M. musculus

2416 ACCUUUUGUUUUUUAUGG

2433

2502 ACCUUUUAAUUUUUGUGUG

2519

CPE II

R. norvegicus

M. musculus

2500 CCCUUUAUUAUAUAUGU

2517

2592 CCCUUUAUUAUAUAUGU

2609

C

glutamate

- NS

3' 6' 12' M.

MMP-9

Polyadenylated fraction

Oligoadenylated fraction

D

Intensity (a.u.)

0 100 200 300 400 500 600 700

Poly(A) tail length (nt) MMP-9

E

glutamate

- NS

3' 6' 12' M.

αCaMKII

Polyadenylated fraction

Oligoadenylated fraction

F

Intensity (a.u.)

0 100 200 300 400 500 600 700

Poly(A) tail length (nt) αCaMKII
Polysomal fractionation
Synaptoneurosomes stimulation and lysis

Radioactive RT-PCR:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>unstimulated</th>
<th>stimulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>MMP-9</td>
</tr>
<tr>
<td>3</td>
<td>„spike“</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NFM</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

J. Miłek
Local synthesis of MMP-9 protein with Click-iT chemistry

- Synaptoneurosomes
  - Incubation with methionine analogue (HPG) and stimulation
  - Protein isolation (lysis in 1% SDS)
    - And Click-iT reaction with AHA tagged with biotin
  - Precipitation on streptavidin dynabeads
  - Western blot with anti-MMP-9 antibody

<table>
<thead>
<tr>
<th>C</th>
<th>glut</th>
<th>C</th>
<th>glut</th>
</tr>
</thead>
</table>
| - | -    | + | +    | anisomycin

MMP-9

Arc
Fragile X syndrome is a genetic syndrome which results in a spectrum of characteristic physical, intellectual limitations, emotional and behavioral features which range from severe to mild in manifestation. Some individuals with the fragile X syndrome also meet the diagnostic criteria for autism.

The syndrome is associated with the expansion of a single trinucleotide gene sequence (CGG) on the X chromosome. Mutation at that site is found in 1 out of about every 2000 males and 1 out of about every 259 females.

FMR1 protein was shown to be involved in the process of dendritic spines maturation, regulation of local translation and transport of locally translated mRNAs in neurons.
Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model

T V Bilousova, L Dansie, M Ngo, J Aye, J R Charles, D W Ethell and I M Ethell

MMP-9 mRNA contains G quartets, the consensus sequence for FMRP RGG RNA-binding domain

\[
\text{DW GG N(0-2) DWGG N(0-1) DWGG N(0-1) DWGG N(0-3)}
\]
\[
D \text{ is any nucleotide except C, W is U or A and N is any nucleotide}
\]
\[
ggaggaaaggaggagtgg a 2243-2260 MMP9 3' UTR
\]
Malformations of the dendritic spines may lead to cognitive disorders

One of the characteristic features of FXS neurons is the abnormal dendritic spine morphology (Rudelli 1985). Immature, long and thin spines are also observed in FMR1 KO mice, a model of FXS.
MMP-9?
Synaptoneurosomes fractionation isolated from WT and Fmr1KO

**Hippocampus**

- **Homogenate (H)**
  - HOMOGENIZATION
  - FILTRATION (PVDF 100, 50, 30, 20 μm)
  - CENTRIFUGATION (1000 rpm, 30 min)

- **Filtrate (F)**

- **Synaptoneurosomes (SN)**
  - Synaptoneurosomes fractionation isolated from WT and Fmr1KO

- **Cytosol (C)**

**Western Blot Analysis**

- **PSD-95**
  - WT KO WT KO WT KO WT KO
  - H-homogenate
  - F-supernatant after filtration
  - SN- synaptoneurosomes
  - C-cytoplasm

- **β-DG**
  - WT KO WT KO WT KO WT KO

- **GFAP**
  - WT KO WT KO WT KO WT KO

- **GAPDH**
  - WT KO WT KO WT KO WT KO
Coimmunoprecipitation of FMRP protein with MMP-9 mRNA

1. Precleared extract (Input)
2. Antibody-bound beads
3. Immunoprecipitated complexes
   - 1. Western blot
   - 2. RNA isolation and RT-PCR

Synaptoneurosome extract

anti-FMRP antibody or normal IgG

Dynabeads A
Local translation of MMP-9 is regulated by FMRP

![Diagram of synaptic function and molecular components](image)