Autism-Misregulated eIF4G Microexons Control Synaptic Translation and Higher Order Cognitive Functions

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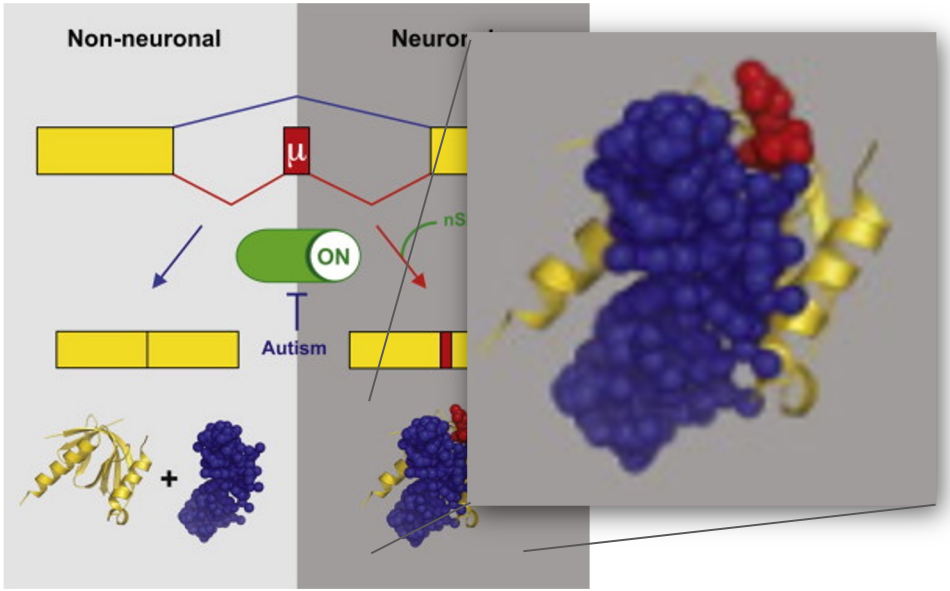
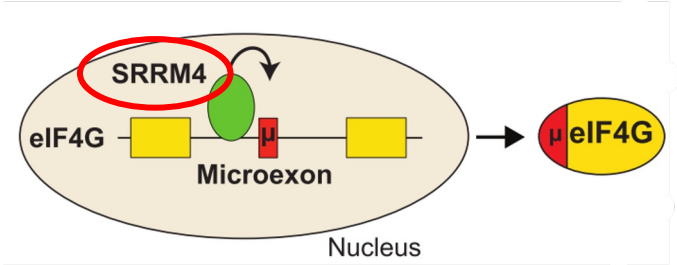
Presented by: Louis Wu, Bilin Nong, Yiqi Zhang (Group 12)

Introduction

Misregulated alternative splicing of eIF4G microexon leads to ASD





Inclusion of microexon

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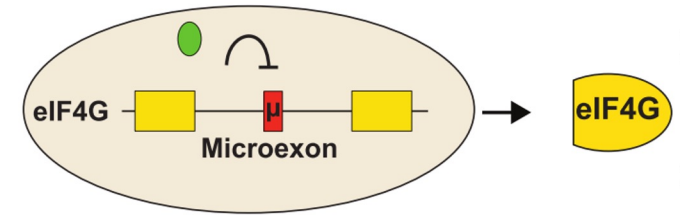
Normal neuronal activity



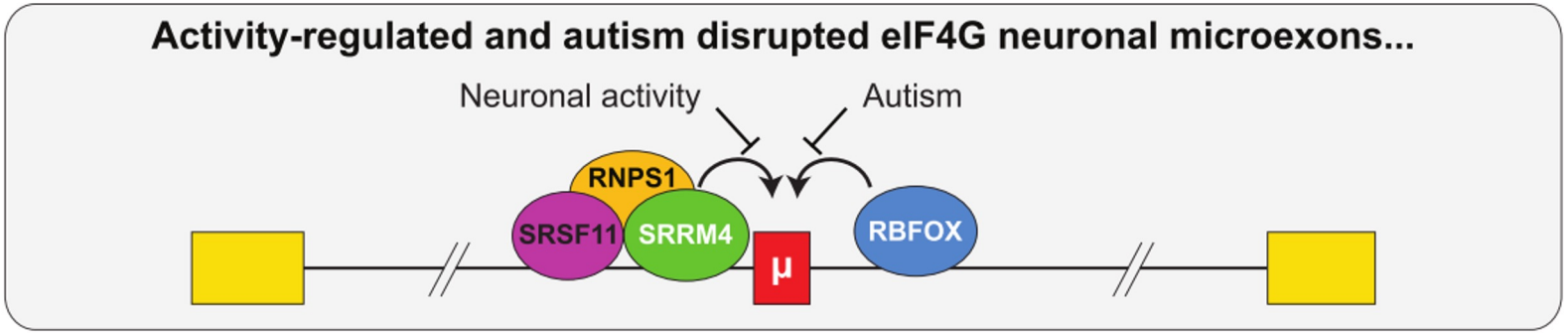
Exclusion of microexon

↓

ASD features

Introduction

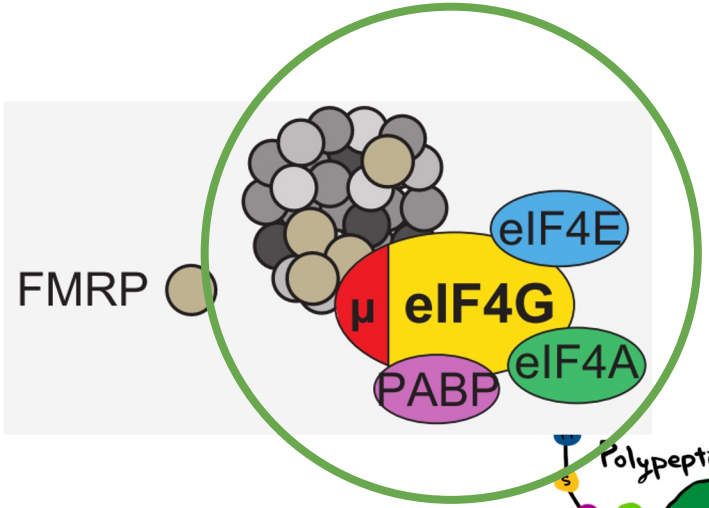
What regulates the alternative splicing of eIF4G microexon?

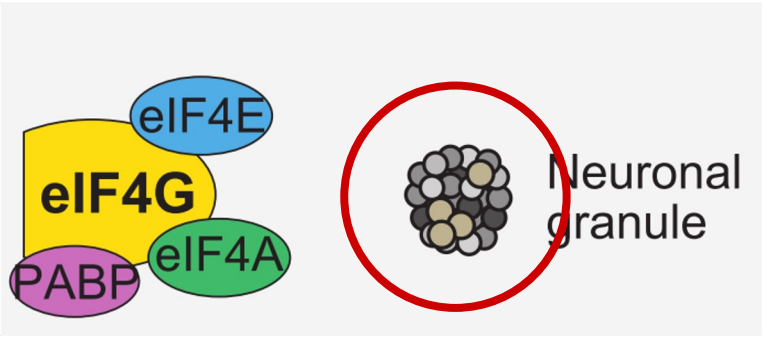


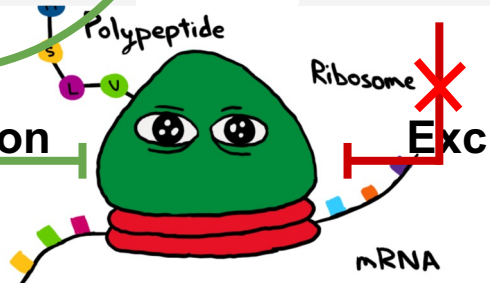
Introduction

Condensate **includes** complex

Condensate **excludes** complex



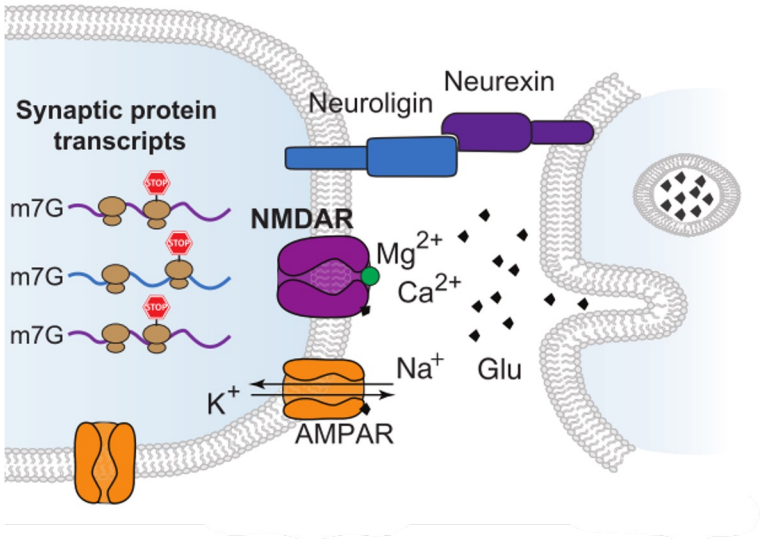




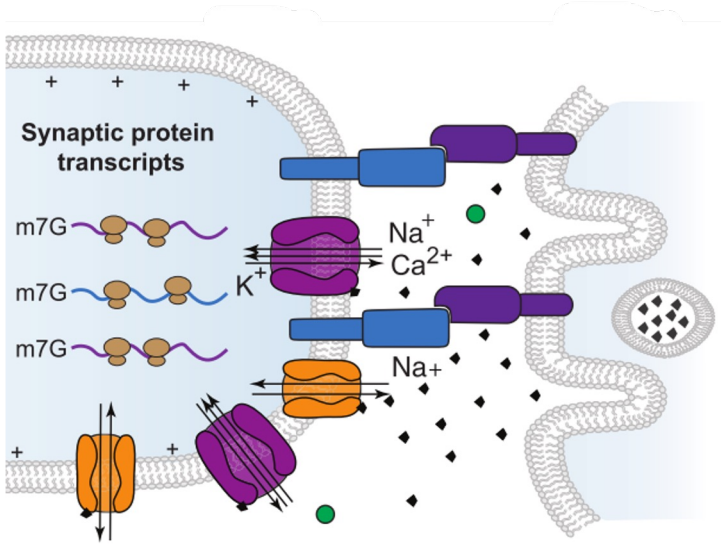
Inclusion of microexon

Exclusion of microexon

Introduction

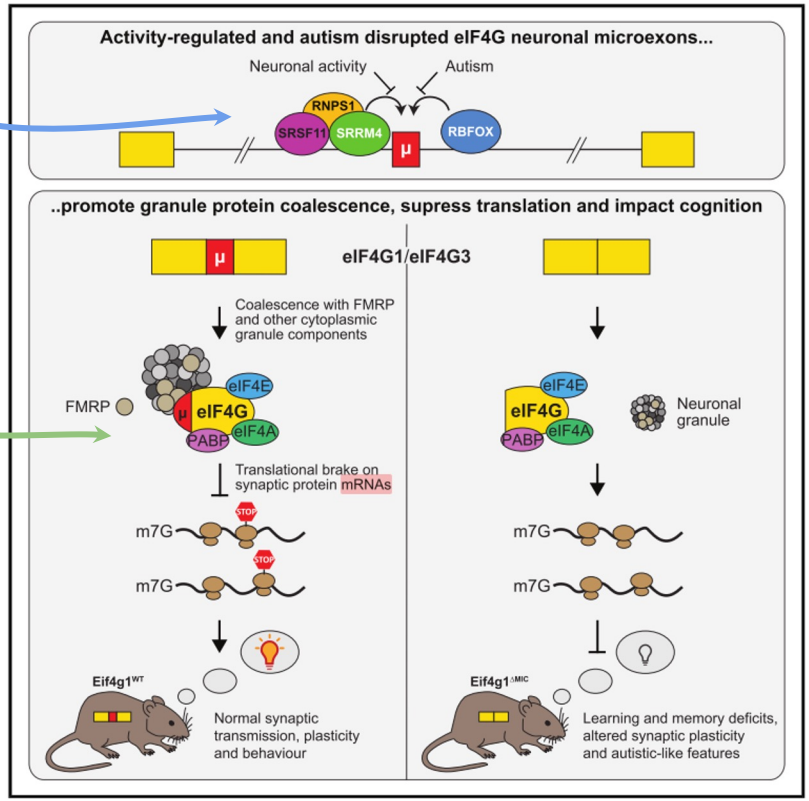


Inclusion of microexon



Exclusion of microexon

Is regulated alternative splicing of microexons associated with the misregulation of neuronal translation which leads to ASD phenotypes?

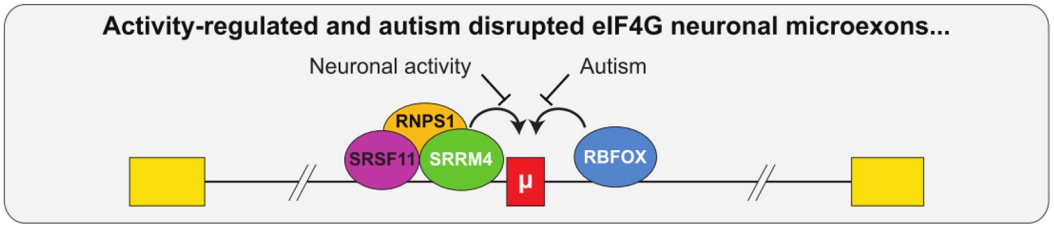


Regulated Alternative Splicing

Is regulated alternative splicing of microexons associated with the misregulation of neuronal translation that leads to ASD phenotypes?

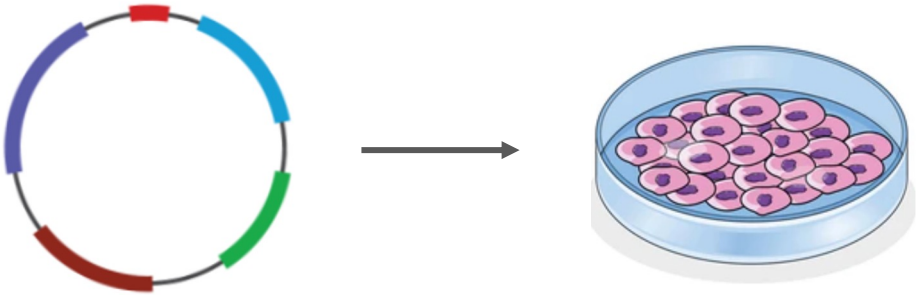
Regulation via:

- Proteins
- Neuronal activity

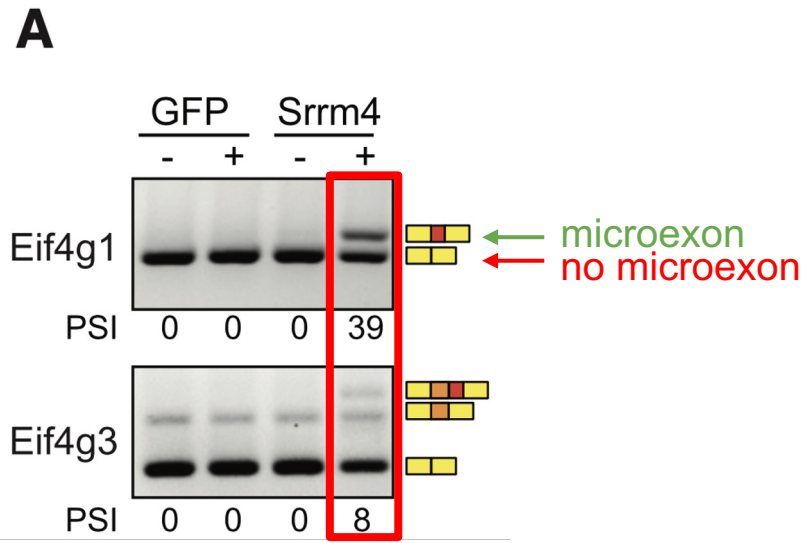


Protein Regulation of Alternative Splicing

What proteins regulate neuronal alternative splicing of EIF4G microexons?



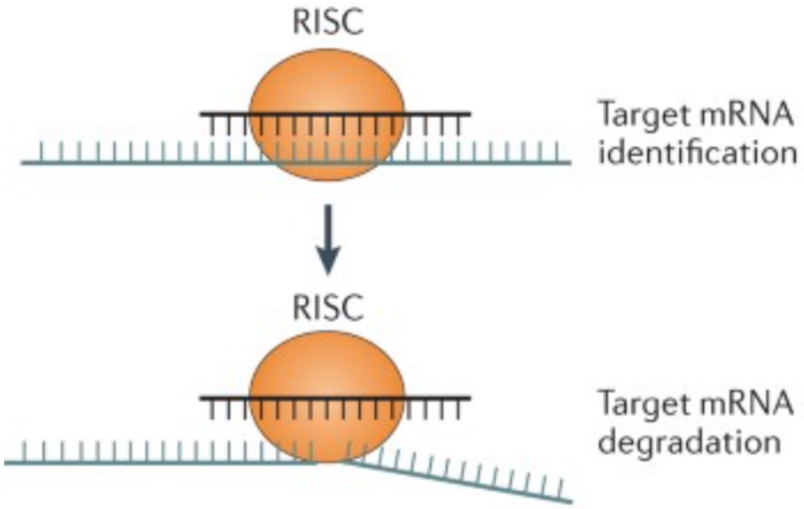
Srrm4 plasmid transfection into mESC



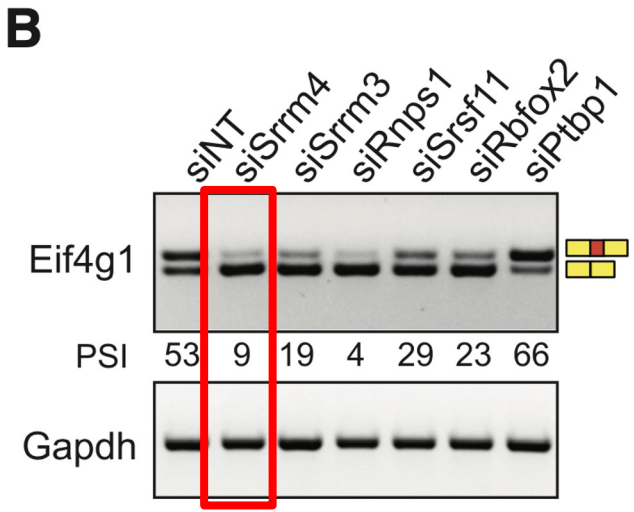
Upregulation of Srrm4

Protein Regulation of Alternative Splicing

What proteins regulate neuronal alternative splicing of EIF4G microexons?



siRNA degradation of Srrm4 mRNA

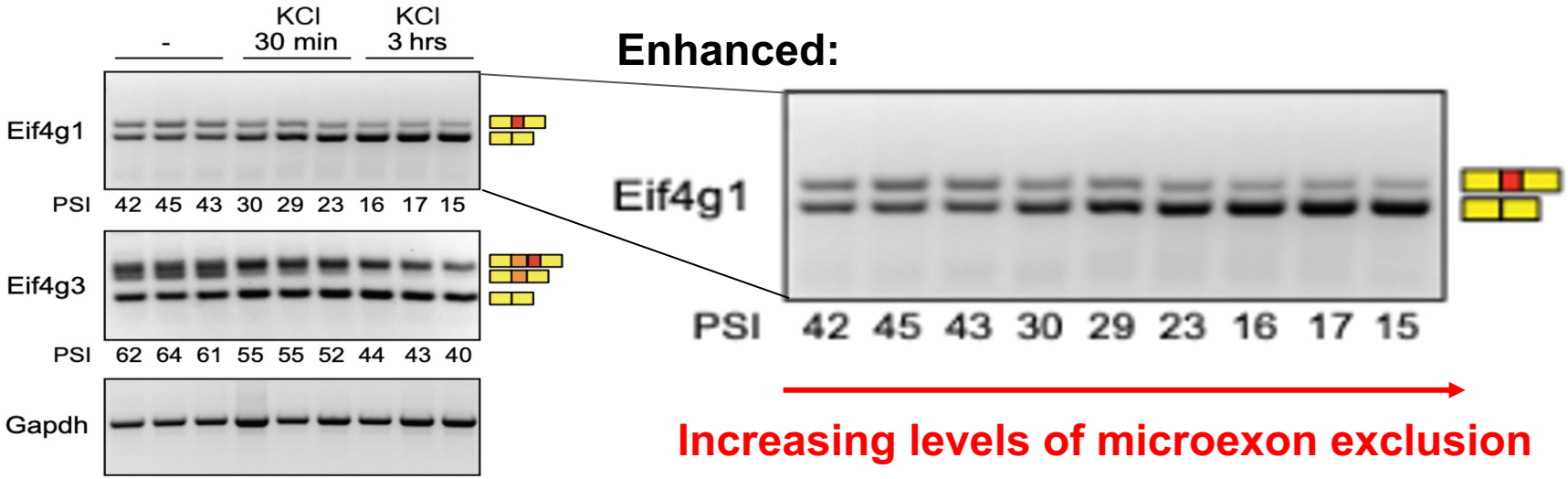


Downregulation of Srrm4

Neuronal activity regulation of alternative splicing

Does neuronal activity regulate alternative splicing of EIF4G microexons?

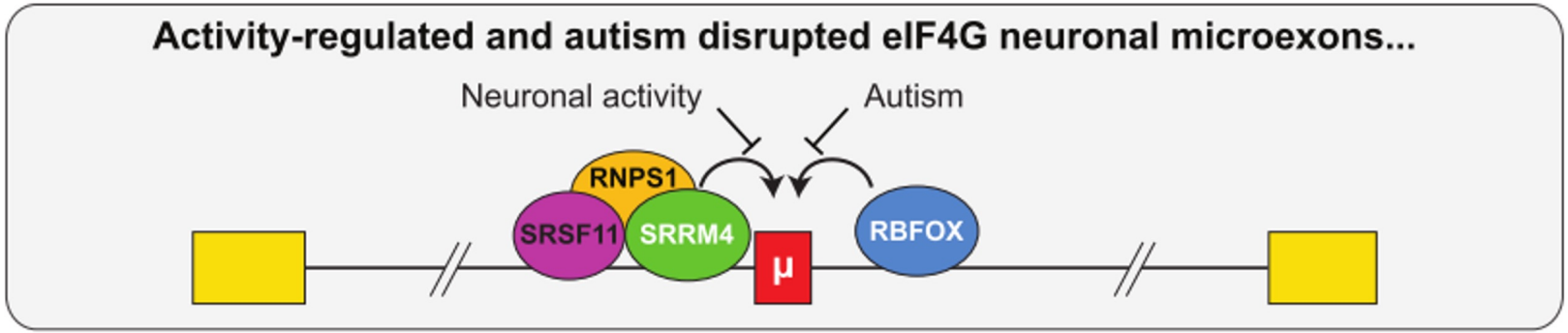
KCl depolarizes cell membrane to emulate neuronal activity



Results

Through regulation of alternative splicing:

- SRRM4 **promotes** the **inclusion** of eIF4G microexon.
- Neuronal activities **inhibit** the **inclusion** of the microexon

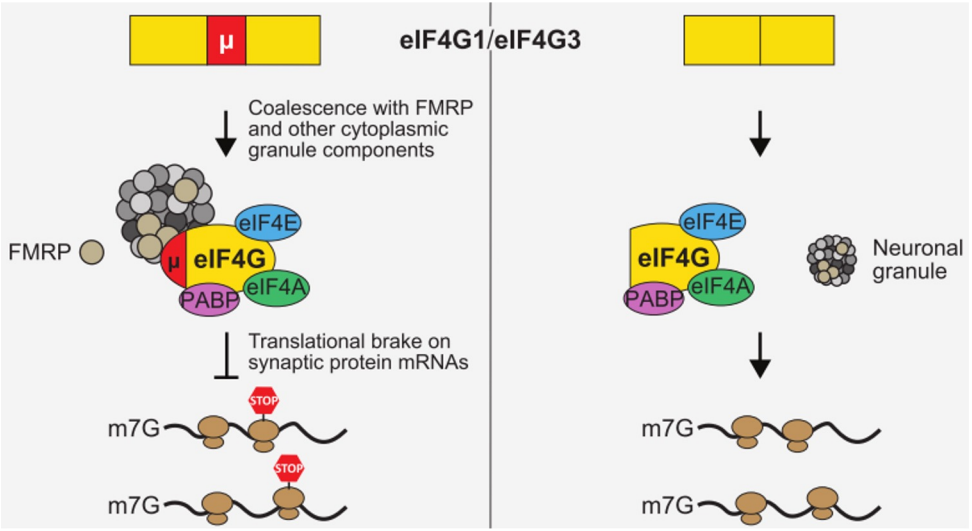


Misregulation of Neuronal Translation

Is regulated alternative splicing of microexons associated with the misregulation of neuronal translation which leads to ASD phenotypes?

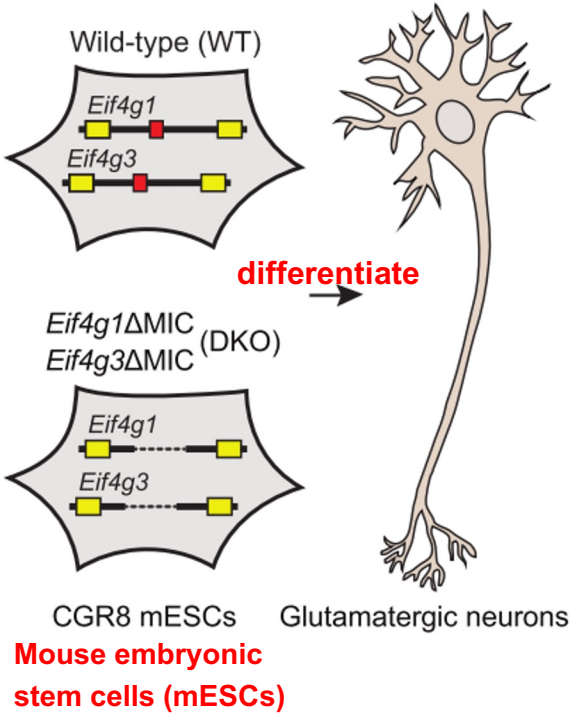
How we can measure translation:

1. The amount of **proteins**
2. The formation of **RNA granules**

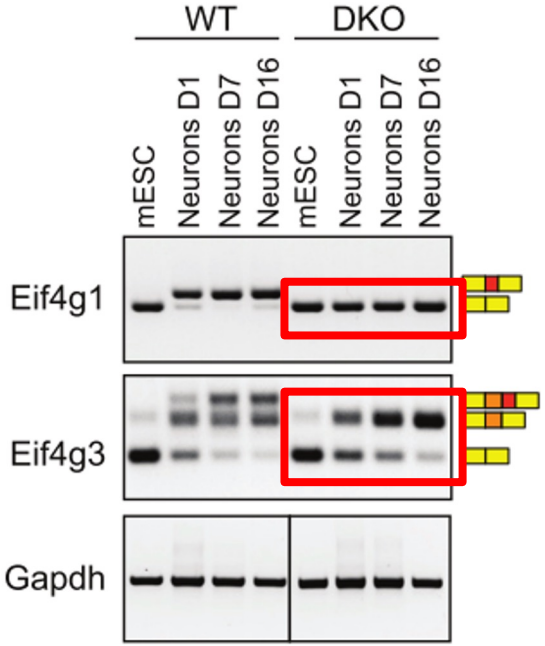


Do EIF4G microexons regulate neuronal translation?

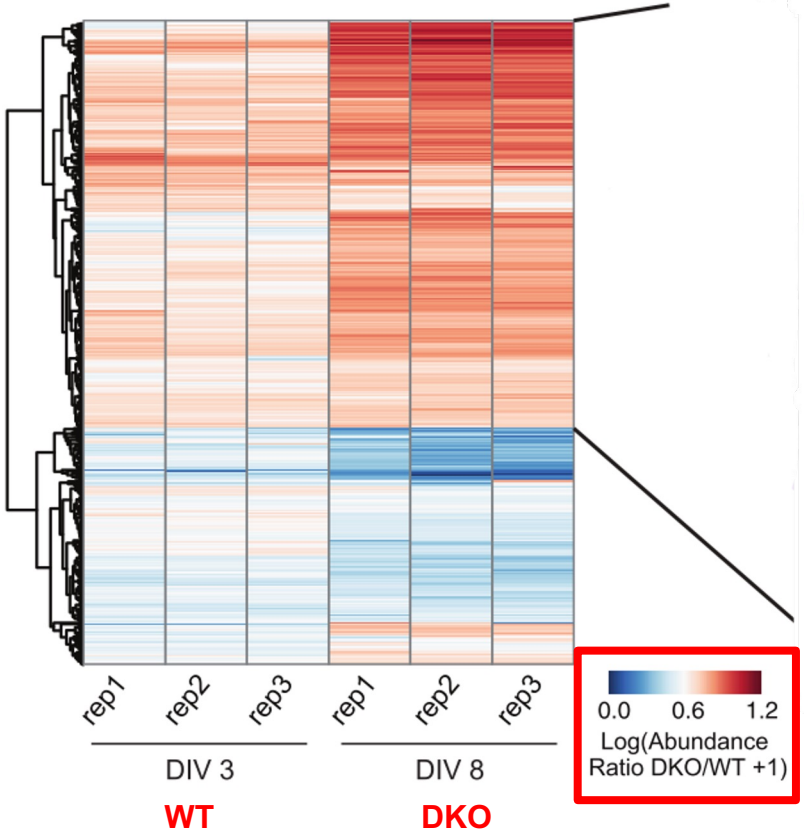
CRISPR-Cas9 DKO of Eif4g microexon

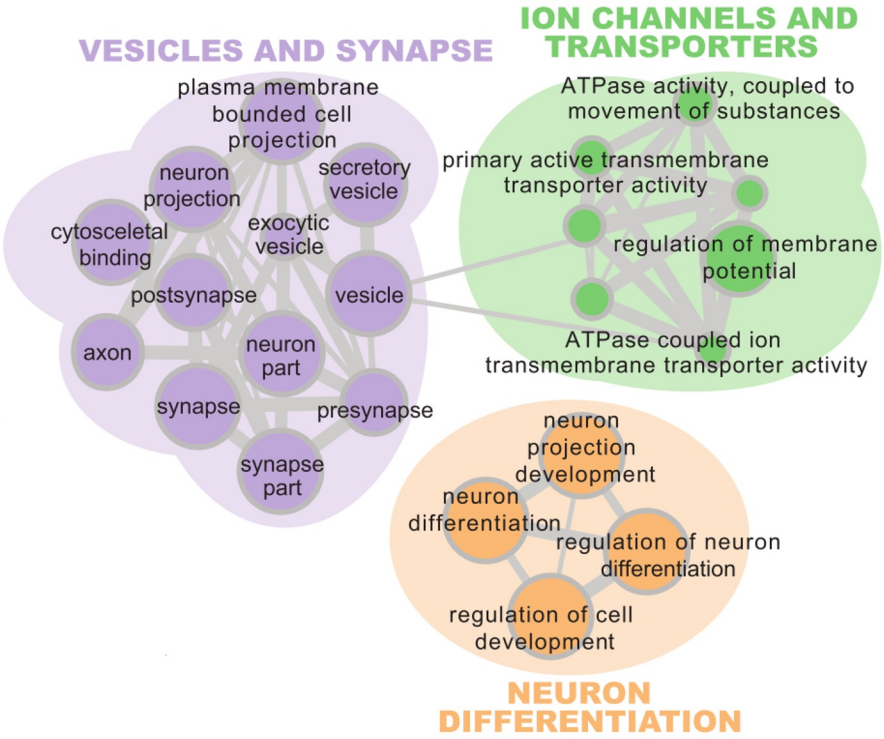


RT-PCR monitoring of neurons



Quantitative mass spectrometry of proteins



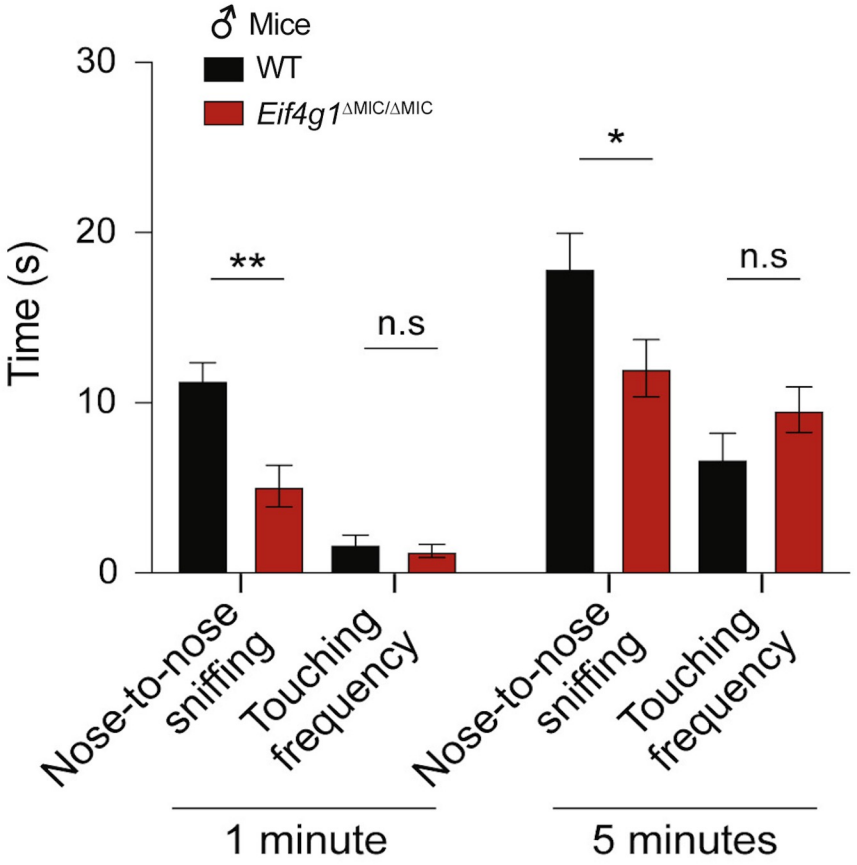


Neurodevelopmental and Behavioural Phenotypes

Does regulated alternative splicing of microexons lead to ASD phenotypes?

- Tests for **sociability**
  - Reciprocal interaction test
- Tests for **learning & memory ability**
  - Contextual fear-conditioning test

Reciprocal Interaction Test

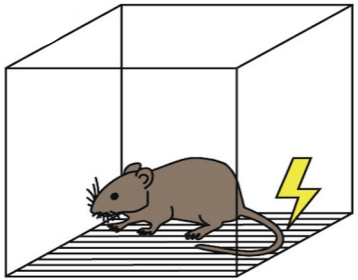


Eif4g1 homozygous-deletion mice **interact significantly less** than WT mice

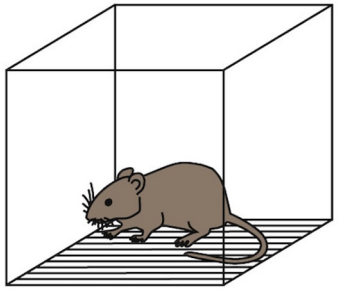
→ **Abnormal Sociability**

Contextual Fear-Conditioning Test

Day 1: Training

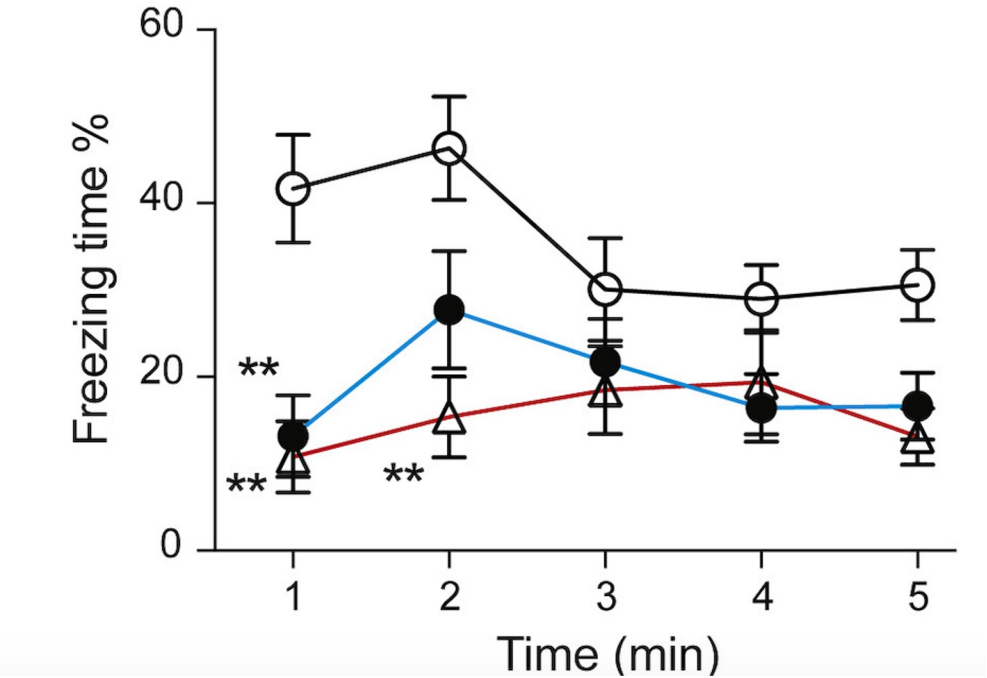


Day 2: Contextual Fear Conditioning



- Conditioning requires **hippocampal-dependent memory**
- Context ~ electric shock ~ fear response (freezing time)

Contextual Fear-conditioning Test



♂ *Eif4g1*

+/+

ΔMIC/+

ΔMIC/ΔMIC

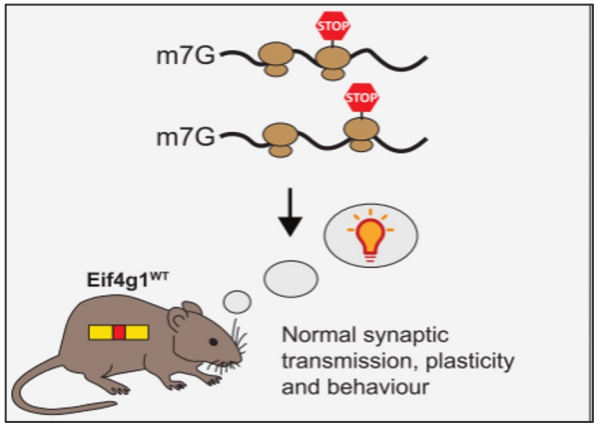
Eif4g1 homozygous-deleted Mice show **less freezing time (fear response)** than WT mice

→ **Impaired episodic memory**

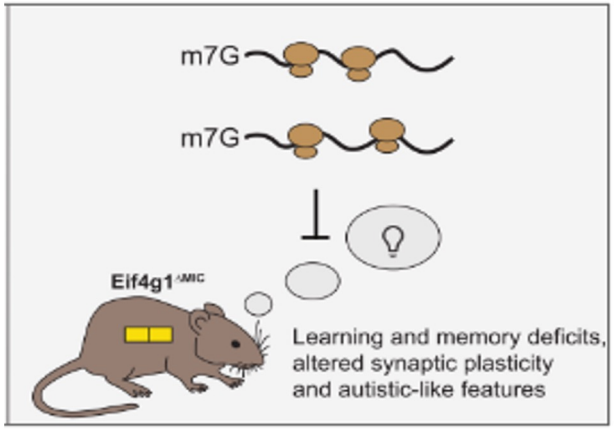
Results

eIF4G microexon homozygous-deletion mice exhibit **ASD phenotypes**:

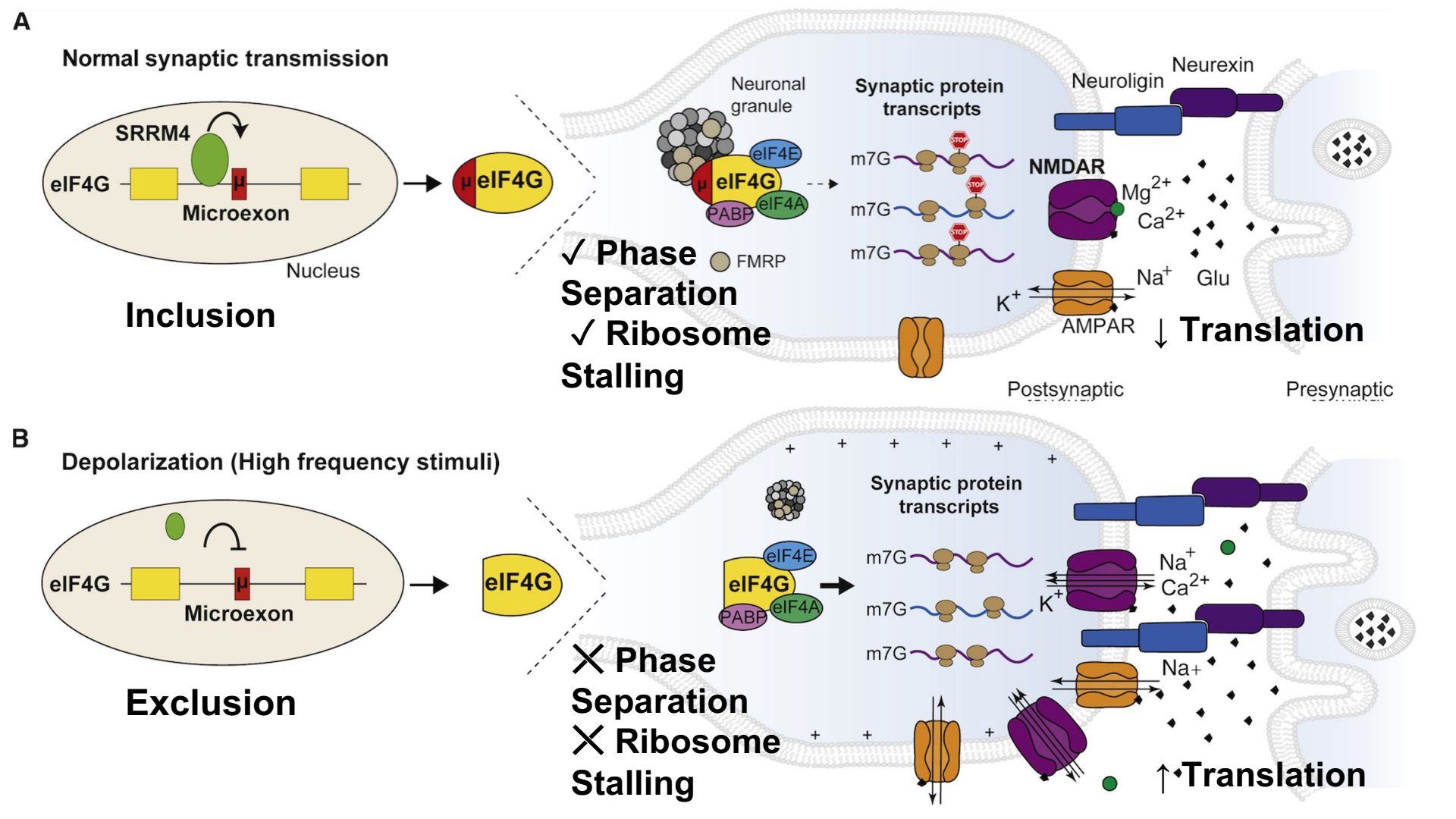
1. **Social behaviour abnormalities**
2. **Impaired hippocampal memory**



Included microexon



Excluded microexon


A
Normal synaptic transmission
SRRM4
eIF4G
μ
Microexon
Nucleus
Inclusion
μ eIF4G
Neuronal granule
eIF4E
eIF4G
PABP
eIF4A
FMRP
Synaptic protein transcripts
m7G
STOP
✓ Phase Separation
✓ Ribosome Stalling
Neuroligin
Neurexin
NMDAR
Mg2+
Ca2+
Na+
Glu
K+
AMPAR
↓ Translation
Postsynaptic
Presynaptic
B
Depolarization (High frequency stimuli)
eIF4G
μ
Microexon
Exclusion
eIF4G
Synaptic protein transcripts
eIF4E
eIF4G
PABP
eIF4A
m7G
✕ Phase Separation
✕ Ribosome Stalling
Na+
Ca2+
K+
↑ Translation

Discussion

- **Potential treatment methods** for neurological disorders related to altered splicing & translational control.

ILLUSTRATION BY KATIE CAREY

Questions?