PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Gloria Venturini

Title of PhD research: Dissecting FMRP domains to restore the synaptic function in Fragile X- Syndrome.

Name of PhD supervisor: Prof. Daniele Di Marino
Research lab name: MasBic-Istituto Superiore di Sanità

Cycle:
[ ] XXXVI
[x] XXXVII

PhD Curriculum:
[ ] Marine biology and ecology
[x] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[x] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):

Fragile X syndrome (FXS) is a genetic disorder causing cognitive and behavioral deficits. It results from CGG triplet expansion in the 5' UTR of the *Fmr1* gene, leading to hypermethylation and absence of fragile X messenger ribonucleoprotein 1 (FMRP).FMRP absence disrupts mRNA translation regulation, leading to protein overexpression in neurons, contributing to FXS and chancing in morphology of dendritic spines.FMRP consists of three domains and their specific functions are unclear.This study aims to identify binding partners and roles of each domain, analyzing their combined impact on protein synthesis and spine morphology. To achieve this HEK293T cells will be transfected with plasmids containing various FMRP constructs (single/multiple domains).Immunoprecipitations (IP) will identify new FMRP protein partners.Results will be validated in Fmr1-KO primary neurons to understand specific FMRP domains' influence on rescuing FXS. This research may offer potential therapy to mitigate the syndrome's pathophysiology.

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

- BACKGROUND

Fragile X-syndrome is one of the most common forms of intellectual disabilities (ID),with cognitive and behavioral deficit. The genetic cause of FXS is a CGG triplet expansion in the 5' UTR of the *Frm1* gene leading to hypermethylation and absence of fragile X messenger ribonucleoprotein 1 (FMRP).

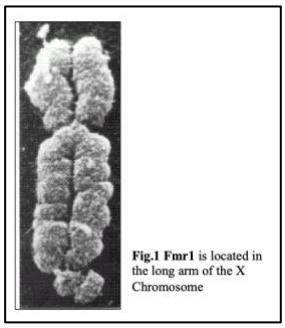
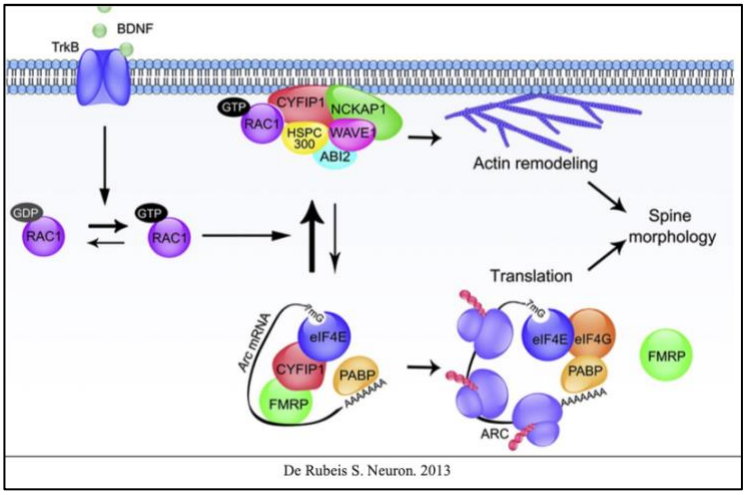


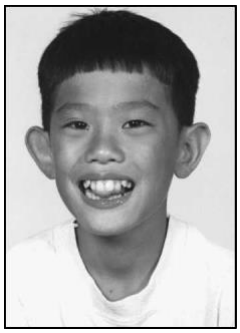
Fig.1 Fmr1 is located in the long arm of the X Chromosome

FMRP is an RNA binding protein that recognizes specific mRNAs which code for proteins crucial for synaptic plasticity and neuronal development. From the mechanistic point of view, FMRP repress translation interacting with Cytoplasmatic Fmrp Interacting Protein1 (CYFIP1) and Eukaryotic Translation Initiation Factor 4E (eIF4E).[1,2] The absence of FMRP results in inadequate regulation of mRNA translation, leading to overexpression of specific proteins in neurons, which therefore contributes to the onset of FXS, in fact postmortem brain analysis in FXS patients showed delayed neuronal spine maturation, characterized by an increase in long, thin dendritic spines.



De Rubeis S. Neuron. 2013

The patients affect by FXS present a typical characteristics of Autistic Spectrum Disorder like:



Physical Characteristics:

- Large body size
- Macroorchidism
- Large ears
- Poor eyesight
- Frontal bossing

Cognitive and Behavioural Characteristics:

- Intellectual disability
- Hyperactivity

- Gastrointestinal problems
- Sleep disturbance
- Motor problems
- Socialization

- SCIENTIFIC AIMS

It's known that, structurally, FMRP is composed by three domains (N-terminal, central, and C-terminal), all involved in RNA binding, the specific function of each domain is not yet fully understood.[4] This study aims to identify binding partners and roles of each domain and their combined effect on protein synthesis and spine morphology.[3,5]

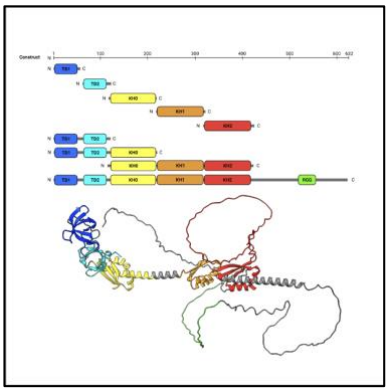


Fig.2 3D structure of FMRP predicted by AlphaFold

	DOMAINS	AA (from-to)	kDa
1	TD1	**53** (1-53)	6.2
2	TD2	**58** (64-121)	6.8
3	KH0	**76** (127-202)	8.8
4	KH1	**68** (216-283)	7.6
5	KH2	**128** (281-408)	14.1
6	TD1-TD2	**121** (1-121)	14.1
7	TD1-TD2-KH0	**202** (1-202)	23.5
8	KH0-KH1-KH2	**282** (127-408)	31.7
9	TD1-TD2-KH0-KH1-KH2	**408** (1-408)	46.3
FL	Full Length	**632**	71.2

Fig.3 Domains of FMRP

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Objective.

The major aim is identifying the binding partners and roles of FMRP's domains, to achieve this, the first objective is using HEK293T cells by transfecting with plasmids containing different FMRP constructs, each of which included single or multiple FMRP domains.

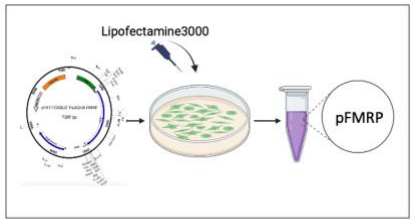


Fig.4 Imagine of Transfection

Methods.

Cells culture (HEK293T)

Transfection

PCR

Cloning

Expected/Obtained Results.

HEK293T cells were transfected with a plasmid containing the full-length (FL) version of the FMRP N-terminal HA-FLAG tags (pFRT-TODestFLAGHAhFMRPiso1) using LIpofectamine3000.

FMRP FL protein was successfully expressed in HEK293T cells.

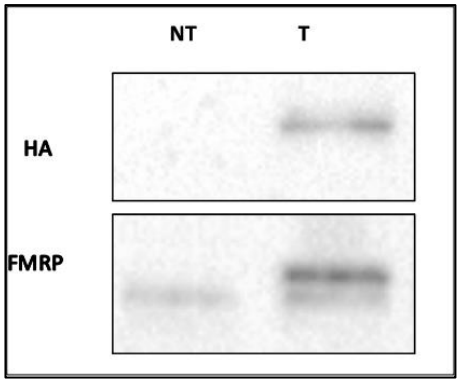
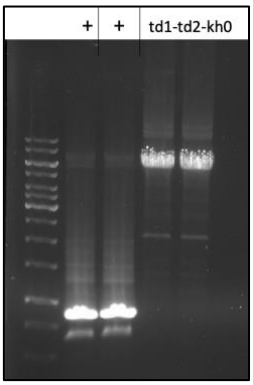
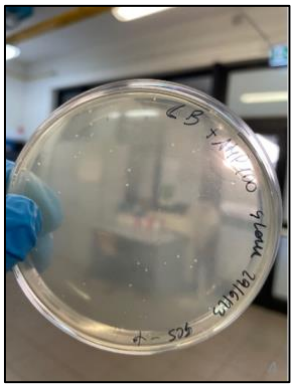


Fig.6 Representative immunoblot indicating the transfection in HEK293T cells. Ab: anti-HA and Ab anti-FMRP

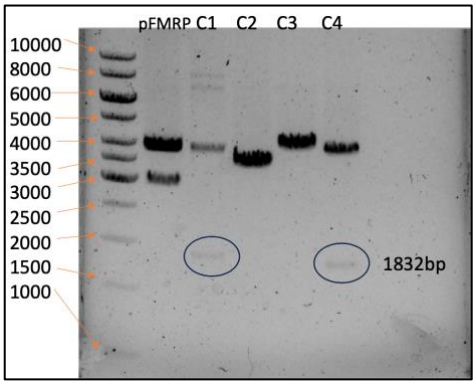
After that, cloning of individual domains began. Here we see the cloning step to obtain TD1TD2KH0 domain and each others.



1°step: PCR



2°step: after extration from agarose gel, transformation to GC5 competent cells



3°step: digestion with restriction enzymes to control the efficiency of transformation and select colonies for sequencing.

WP 2. Objective.

Subsequently, immunoprecipitations (IP) were carried out to identify new protein partners of FMRP.

Methods.

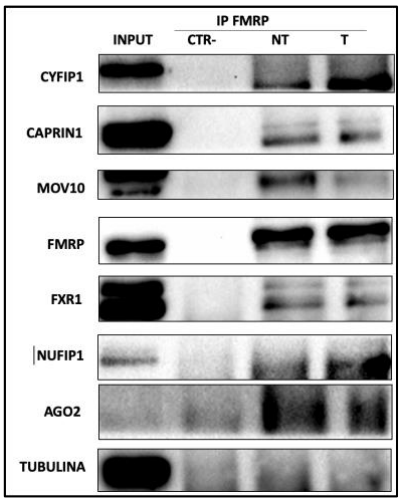
Immunoprecipitation

SDSpage

Western Blot

Expected/Obtained Results.

After the transfection the HEK293T cells non transfected and transfected is used to Immunoprecipitation Assay, to locate a possible interaction with different partner. preliminary results show that the full lengh FMRP it appears to bind to different proteins, in the picture at right we see HEK293T cell lysate were used for immunoprecipitation with antibodies specific to FMRP (shown on the right). CTR- indicates negative controls. The immunoprecipitation (IP) was performed on non transfected (NT) e transfected (T) HEK293T cell lysate with pFMRP and analyzed with anti CYFIP1, anti-CAPRIN1, anti-MOV10, anti-AGO2, anti-FMRP, anti-FXR1, anti-TUBULINA, anti-NUFIP1.



These results will be also validated in Fmr1-KO primary neurons in order to understand which specific FMRP domain or domains may have a more pronounced impact on rescuing the FXS phenotype, with a focus on protein translation and spine morphology. Therefore, the administration of FMRP domains could potentially completely or partially reverse the FXS-associated phenotype. This suggests a potential therapy to mitigate the pathophysiology of the syndrome.

- REFERENCES

[1]Napoli I. et al. *Cell.* 2008

[2]De Rubeis S. et al. *Neuron.* 2013

[3]Myrick L.K et al. *Human Molecular Genetics.* 2015

[4]D'Annessa I. et al. *Progress in biophysics and molecular biology. 2019*

[5]D'Souza M. N. et al. *Molecular Neurobiology*. 2022

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Design of Research (European Projects) – Prof. Paone Nicola

2. Technology Transfer and Innovation – Prof. Iacobucci Donato

3. LaTeX – Prof. Spinozzi Francesco

4.Confocal laser scanning microscopy: CLSM – Prof. AndreaFrontini

5. Theory and application of complex networks –Prof.ssa Maria grazia Ortore (pending)

4. Seminar by Dr. Petrucciani– A shot of science (08.11.2022)

5. Seminar by Dr.ssa Maracci-A shot of science (29.11.2022)

6. Seminar by Dr.ssa Ritacca – A shot of Science (10.05.2023)

7. Seminar by Dr.ssa Flavia Akemi Nitta Fernandez – A shot of science (20/06/2023)

8. Seminar by Dr.ssa, Monica Mattioli Belmonte Cima, PhDweek "In vitro models mimicking human tissues and their cross-talk" (13.06.23)

List of periods spent abroad

List of conferences/workshops attended and of contributions eventually presented

1. **Biophysics@Rome** Conference,April 19th-20th 2023 Roma, Italy. *Poster presentation:* Dissecting Fmrp domains structure to restore the synaptic sunction in Frigile X-Syndrome. G.Venturini, I.Cirilli, A.Roscioni, A.Romagnoli, A.La Teana, D.Di Marino.

2. **3rd DISVA Biochemistry, Molecular Biology and Cellular Physiology Summer Retreat,** 31 August- 4 September 2023, Calcinaia sul lago, Italy.

3. **Third MaSBiC Symposium** - Advances in Protein Science: Exploring Structure, Function, and Beyond, 20th-22th 2023 September 2023, Ancona, Italy. *Poster presentation*: Dissecting Fmrp domains structure to restore the synaptic sunction in Frigile X- Syndrome. G.Venturini, I.Cirilli, A.Roscioni, A.Romagnoli, A.La Teana, D.Di Marino.

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

List of publications on conference proceedings

List of other publications (books, book chapters, patents)

14.10.2023

Student signature

Supervisor signature