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TITLE: “Exaggerated Cap-Dependent Translation as a Mechanism for Corticostriatal Dysfunction in Fragile X Syndrome Model Mice”

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14. ABSTRACT

Our two laboratories – Klann and Bagni - are committed to understanding the detailed molecular abnormalities associated with developmental disabilities and how these result in synaptic dysfunction and aberrant behavior. Our overall hypothesis is that repetitive and perseverative behaviors exhibited by FXS patients that can be recapitulated in the FXS model mice are caused by affected cortico-striatal synapses. To test this hypothesis, we propose two specific aims: **1) To determine cortico-striatal synaptic composition, function and plasticity in FXS model mice; 2) To determine whether altered cortico-striatal synaptic plasticity and repetitive/perseverative behaviors displayed by FXS model mice are reversed by novel cap-dependent translation inhibitors.** Our specific tasks are centered on a proteomic study of FXS striatal synapses by using a transgenic mouse model that allows to capture "native" synapses. Purified synapse will be analyzed by mass spectrometry and the data will be validated using biochemical and cellular methods. The comparison of the synaptic proteome between the wild type and the FXS mice during development will identify which complexes are affected in FXS and possibly in other synaptopathies. These data will complement the electrophysiological and behavioral studies performed by the coordinator.

15. SUBJECT TERMS

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Introduction

Our two laboratories – Klann and Bagni - are committed to understanding the detailed molecular abnormalities associated with developmental disabilities and how these result in synaptic dysfunction and aberrant behavior. Our overall hypothesis is that repetitive and perseverative behaviors exhibited by FXS patients that can be recapitulated in the FXS model mice are caused by affected cortico-striatal synapses. To test this hypothesis, we propose two specific aims: **1) To determine cortico-striatal synaptic composition, function and plasticity in FXS model mice; 2) To determine whether altered cortico-striatal synaptic plasticity and repetitive/perseverative behaviors displayed by FXS model mice are reversed by novel cap-dependent translation inhibitors.** Our specific tasks are centered on a proteomic study of FXS striatal synapses by using a transgenic mouse model that allows to capture "native" synapses. Purified synapse will be analyzed by mass spectrometry and the data will be validated using biochemical and cellular methods. The comparison of the synaptic proteome between the wild type and the FXS mice during development will identify which complexes are affected in FXS and possibly in other synaptopathies. These data will complement the electrophysiological and behavioral studies performed by the coordinator.

Key Words

Fragile X syndrome (FXS), synaptic proteome, synaptic structure, postsynaptic density, PSD-95, cortex, hippocampus, striatum.

Accomplishments

The accomplishments during year 2 are described, task by task, in the next section. A summary is also given at the end of the following section.

Major goals of the project (year 2)

Task 1-Subtask 2

The colony has been transferred to the new institute in Lausanne, and has been expanded to yield sufficient animals for validation and phosphoproteomic experiments. Since the phosphoproteomic, once performed and analysed, will also need validation, the breeding will continue to the very end of the project to collect the number of animals which are required for experiments with statistical power.

Task 1-Subtask 3

This task according to the revised SoW, has to be completed by month 30, a deadline which we will be able to meet. Remarkably, while the original project proposed to look at the NR2b subunit of the receptor, our analysis of the mass spectrometry data (subtask 1-4) shows that the NR1 subunit may also change in a small but significant way. We have therefore set up the Western blot analysis of both subunit and will complete it in the near future.

Task 1-Subtask 4

The post-synpatic densities (PSDs) are purified by "tandem-affinity purification" (TAP) from mice that have the PSD-95 protein modified with the respective TAP tag. As stated in the revised SoW, the purification of the first set of PSDs has been performed as well as the mass spectrometry. In particular, following the tissue collection we set up the conditions to isolate the protein complexes for mass spectrometry (MS) analysis (Year 1). For all three brain regions, we used conditions previously established for the hippocampal extracts (Fernández et al., 2009). We performed 54 purifications: three brain areas (cortex, striatum and hippocampus), at 2 developmental stages (P30 and P150), from 2 genotypes (PSD-$95^{TAP/TAP}$ x $Fmr1^{+/y}$, $95^{TAP/TAP}$ x $Fmr1^{-/y}$ mice and WT) and in triplicates. The mass spec has been performed by the Facility at Gent (VIB proteomic core, https://corefacilities.vib.be/pec). A bioinformatics is currently finalizing the analysis. Our preliminary data show several important changes, especially in proteins related to the cytoskeleton. An example is shown in Figure 1. We are currently validating these results as described under subtask 1-11.

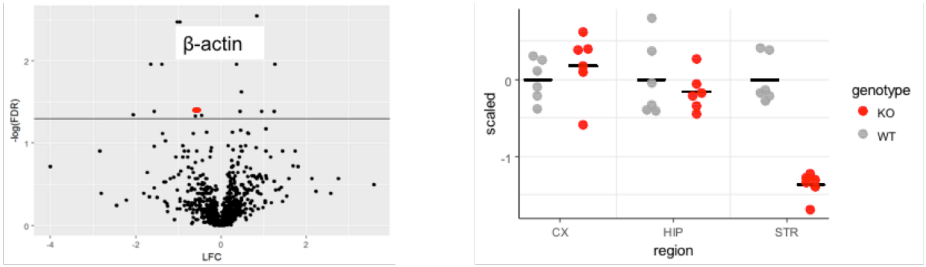
Furthermore, we are currently analysing the preliminary phosphoproteomic analysis that we received as a by-product of the first mass-spectrometry measurements. As described in the updated SoW, we will also perform a dedicated phospho-proteomic analysis, to have a better dataset. The mice needed for this dataset are breeding; we will perform the purification of the post-synaptic density and the mass spectrometry in the first half of the last year.

Task 1-Subtask 7

The post-synaptic analyses have been isolated and are currently being analysed by mass spectrometry. To be completed by month 30 as envisioned in the updated SoW.

Task 1-Subtask 11

This task was envisioned for the third year of the project. Because of the interesting outcome of subtask 1/4, we have already started the validation of this dataset, generating new, independent purifications of the post-synaptic densities.



Others: Ank2, Map4, Rps19, Rps20, Rpl14, Hmgb1

N = 3

Figure 1. Left panel, Vulcano plot of all proteins detected in the PSD. As significance of P<0.05 (1.4 log units) is indicated as black line. Among the dysregulated proteins (above the black line) we found beta Actin (red dot). Right panel, beta actin expression in the post-synaptic densities is altered in Fragile X mice in a brain-region-specific manner. Shown are the log2 values of the mass spectrometry quantification of Actb in post-synaptic densities purified from cortex, hippocampus, and striatum of Fmr1 KO mice or their WT littermates. For the technical control we obtained the respective values of a purification performed with mice which do not express the TAP-tagged PSD-95 protein; these values thus represent the background levels.

Task 2-Subtask 2:

This subtask was envisioned to be completed in the last year of the project.

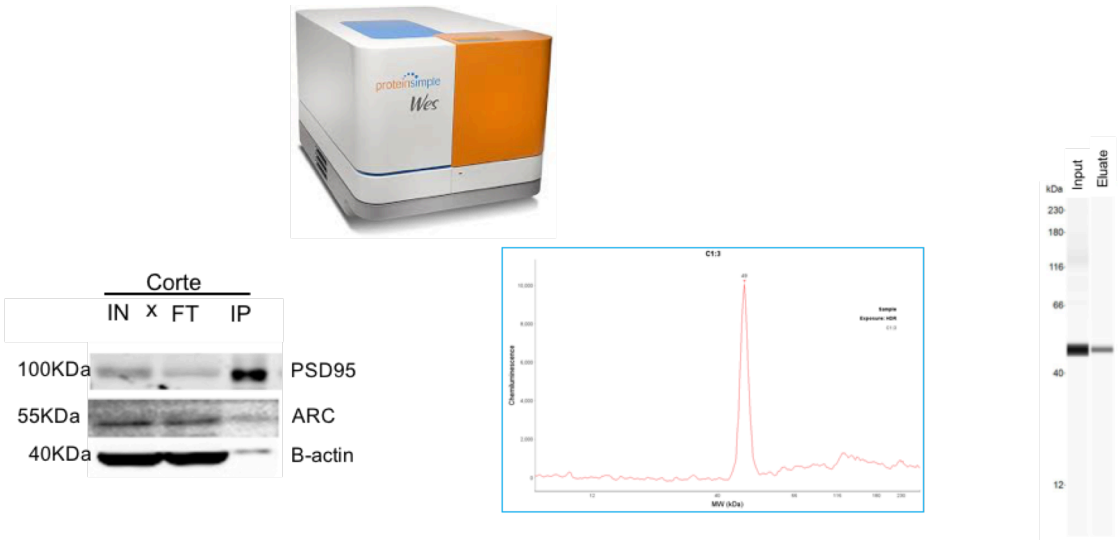


Figure 2. Detection of low protein amount using the WES system. Top, image of the WES system which is used for quantitative validation of the mass spec data. Lower left, regular Western blot shows that low but detectable amount of bactin are present in the TAP- purified PSD. Lower middle, bactin in the TAP-PSD is detected by WES and displayed as an electropherogram (see main peak). Lower right, bactin is displayed as a single band in a lane, similarly to what we would see in a traditional Western blot. This system provides a reliable quantification of very small amounts.

Summary of accomplishments

PSD purifications have been performed and subjected to mass spectrometry

- Mass spectrometry of 54 purifications. Several interesting differences between WT and Fmr1 knock-out mice are now being validated.
- A preliminary dataset for the PSD phosphoproteome has also been obtained and it is now being evaluated bioinformatically.

In addition, the 'TAP' purification of native post-synaptic densities has revealed how the protein Arc (Activity Regulated Cytoskeletal Protein) is integrated into these complexes, shedding a light on its contribution to neurodevelopmental disorders (E. Fernández et al., 2017: Cell Reports 21, 679–691). This grant has been acknowledged for its support of the 'TAP' purification studies.

Impact

Our laboratories are committed to understanding the detailed molecular abnormalities associated with developmental disabilities and how these result in synaptic dysfunction and aberrant behavior. Thus, military families with members afflicted with these disorders will benefit from these studies. In the short term, our studies will provide information whether the composition and the fine regulation (phosphorylation) of the synapses are different in FXS and non-affected mouse models. In the long term, our studies will provide information for the design and use of novel compounds to therapeutically target pathways affected in FXS and other developmental disabilities such as ASD.

Changes/Problems

During the second year of the project, our laboratory moved to the University of Lausanne/Switzerland, which create some delayes in the time line necessary. These changes were agreed in a revised version of the "Statement of Work" (SoW) document. We would like to point out that the revised SoW forsees the fulfilment of all initial goals. As outlined above, we are well on track to reach these goals by the end of the last year.

Products

There has been one publication based on this work so far (Fernandez et al. 2017). Funding from the CDMRP has been acknowledged in this publication.

In addition, a paper from the Klann and Bagni labs has been published in *Science Signaling* on November 7, 2017 in which we demonstrated that 4EGI-1 can reverse a number of hippocampal phenotypes displayed by FXS model mice (Santini et al. (2017) *Sci. Signal.* 10: eaan0665). Funding from the CDMRP has been acknowledged in this publication.

Furthermore, data generated in this work (year 2) in term of methodology we used and the impact on the understanding of FXS synapses have been presented to a few meetings and research Institutions.

Publication:

Fernández E., Collins, M.O., Frank, R.A.W., Zhu, F., Kopanitsa, M.V., Nithianantharajah, J., Lemprière, S.A., Fricker, D., Elsegood, K.A., McLaughlin, C.L., Croning, M.D.R., Mclean, C., Armstrong, J.D., Hill, W.D., Deary, I.J., **Cencelli, G., Bagni, C.,** Fromer, M., Purcell, S.M., Pocklington, A.J., Choudhary, J.S., Komiyama, N.H., and Grant, S.G.N. (2017). "Arc Requires PSD95 for Assembly into Postsynaptic Complexes Involved with Neural Dysfunction and Intelligence." Cell Reports 21: 679-691.

Oral presentation to meetings and research Institutions

1) Cajal School “Advanced Techniques for Synpase Biology”. Bordeaux, France. 2017
2) Telethon Convention Symposium, Riva del Garda, Italy. 2017
3) Max Planck Florida Institute, Jupiter, USA. 2017
4) Cardiff University, Wales, UK. 2017
5) Albert Einstein College of Medicine, NYC, USA. 2017

Participants and other collaborating organizations

Name: **Claudia Bagni**
Project role: Principal investigator
Person Months worked: 2 calendar months
Contribution to the project: Design and supervise experiments and interpret data

Name: **Esperanza Fernández**
Project role: postdoctoral fellow
Person Months worked: Esperanza lead the project during its first year and then left the laboratory, because she could not join the move to Switzerland.
Contribution to the project: Design and perform experiments and analyze the data.

Name: **Giulia Cencelli**
Project role: visiting graduate student.
Person Months worked: 3 months during the first year of the project.
Contribution to the project: Help with the 'TAP' purification.

Name: **Valentina Mercaldo**
Project role: postdoctoral fellow
Person Months worked: 7 calendar months at 50% effort.
Contribution to the project: Valentina and Denise have taken over the project from Esperanza. They are responsible for design and realization of the experiment, as well as analysis of the data.

Name: **Denise Gastaldo**
Project role: PhD student
Person Months worked: 6 calendar months.
Contribution to the project: Valentina and Denise have taken over the project from Esperanza. They are responsible for design and realization of the experiment, as well as analysis of the data.

Name: **Nuria Domínguez**
Project role: PhD student
Person Months worked: 7 calendar months at 60% effort.
Contribution to the project: Help the project with breeding and genotyping mice; preparing brain slices and brain extracts for the experiments.

Name: **Joanna Vigue**
Project role: Technician
Person Months worked: 8 calendar months at 20% effort (paid by Institutional Funding)
Contribution to the project: Help the project with breeding and genotyping mice