Research Group Details

Lab Name	Garcia lab
Group Leader	Susana M. D. A. Garcia
lab www-address	http://www.biocenter.helsinki.fi/bi/
Contact email address	garcia@molbio.mgh.harvard.edu

Short Introduction to the lab

The Garcia laboratory is interested in understanding the mechanisms associated with cellular toxicity caused by the expression of transcripts bearing nucleotide repeats.

Short-tandem nucleotide repeats are widely present in the human genome. Expansions within these repeat regions result in a number of degenerative disorders, including Myotonic Dystrophies, Fragile X Syndrome and Amyotrophic Lateral Sclerosis, amongst others. RNAs bearing expanded nucleotide repeats in non-coding regions disrupt essential molecular processes and lead to severe cellular degeneration. The molecular disruptions caused by these toxic RNAs are poorly understood, as well as the mechanisms that underlie the cellular degeneration leading to the different human disorders. One example is provided by myotonic dystrophy 1 (DM1), an autosomal, dominant, neuromuscular degenerative disease that results from CTG expansions in the 3' untranslated region (UTR) of the DMPK protein-kinase gene.
Our laboratory's research goal is to understand how repeat-bearing RNAs hijack normal cellular function to execute toxicity. In particular, we want to identify the cellular components and pathways responsible for the regulation of RNA toxicity.

The Garcia laboratory is particularly interested in the surveillance role played by the nonsense mediated mRNA decay (NMD) pathway. NMD was recently identified as a suppressor of Myotonic Dystrophy 1 toxicity, however how NMD recognizes toxic RNAs and the mechanism of RNA toxicity suppression is not understood. NMD is a conserved pathway present in all eukaryotes and is implicated in a variety of biological processes. Understanding the mechanisms of RNA target recognition, identifying the NMD-interacting pathways in transcript degradation, as well as the cellular localization of these essential processes is crucial.

Project Outline

Title

Degradation of toxic RNAs in the cell – mechanisms and impact on degenerative disorders

Short introduction to the research problem

The goal of this research project is to dissect the mechanism of nonsense mediated mRNA decay (NMD) recognition of toxic RNAs bearing expanded GC-rich repeat sequences.

NMD suppresses repeat RNA toxicity through degradation of transcripts bearing expanded CUG repeats. Transcript recognition by NMD is dependent on the GC nucleotide content of the 3'UTR. Still, there is a limited understanding of the mechanism of NMD substrate selection in general, and nothing

is known of the mechanism of toxic RNA transcripts recognition. Which pathways are involved in toxic RNA recognition and recruitment by NMD, which components are involved in transcript degradation, and where do these events occurs in the cell? In addition, what are these pathways contributions for RNA-dependent neurodegenerative disorders?

Statement of experimental aims and methods

Aims:
Aim 1: Identify the genes and pathways involved in the recognition of GC-rich toxic RNAs for degradation in the cell. Distinguish amongst the identified genes, which correspond to NMD-dependent and to NMD-independent RNA degradation pathways.

Aim 2: Dissect the mechanisms of transcript recognition by the NMD-dependent RNA-degradation pathways identified. What factors are involved in toxic RNA recognition? How does cross talk occur between the 'new' identified factors and NMD? Do these new genes/pathways also function as modulators of RNA toxicity associated to myotonic dystrophy? What additional biological roles do these factors play, in conjunction with NMD, in a multicellular organism?

Aim 3: Analyze how do the genes and pathways identified regulate expanded repeat-associated disorders, such as Myotonic dystrophy. Test whether these genes are specific to RNA-dependent diseases or do they play a role in the regulation of broader repeat-dependent disorders.

Methods:
A C. elegans genome RNAi screen identified a preliminary set of candidate genes involved in the degradation of transcripts bearing a 3'UTR GC-rich sequence. This set of genes will form the basis for the identification of new genes/pathways that interact with NMD in the degradation of toxic repeat RNAs in the cell.
A combination of C. elegans and mammalian cell culture will be used as tools in addressing mechanisms of cellular degradation of toxic RNAs, with particular emphasis on the NMD pathway. Genetics, including RNAi screening, the generation of mutants and reporter C. elegans strains will be a regularly used set of tools. In addition, high-resolution imaging and complex animal behavior analysis, together with biochemical approaches will be used to address the aims indicated above.

Project time table

Predicted project duration: 4 years
Time table:
Year 1:Analyze genes with reporter strains and perform epistasis analyzes to establish genes/pathways dependence on the NMD pathway. Characterize the involvement of the genes of interest in the modulation of expanded CUG toxicity. Start to dissect the mechanism of interaction between NMD and the pathways identified.
Year 2: Insight into biological significance of the pathways identified; establish mechanism of RNA degradation, including biological significance for the organism. Start to write paper. Address conservation of mechanism in a mammalian system.
Year 3: Submit paper for publication. Dissect additional regulatory pathways associated with the genes being studied. Test conservation of these mechanisms for repeat-associated disorders in general. Proceed with follow up studies.
Year 4: Write paper. Address the main points of the follow up studies in a mammalian system. Submit paper.

Collaborators or external secondments relevant to the project

This project will be executed in collaboration with the Tabach laboratory at the Hebrew University, Israel. The collaborators at the Hebrew University will contribute with data analysis, integration with other screens and databases, as well as through phylogenetic analysis, clustering methods, etc. The potential to travel to the Hebrew University for a short period of time for work is a possibility.

Max 5 references relevant to the project

- - Identification of genes in toxicity pathways of trinucleotide-repeat RNA in C. elegans.
- Garcia SM, Tabach Y, Lourenço GF, Armakola M, Ruvkun G.
- Nat Struct Mol Biol. 2014 Aug;21(8):712-20.
-
- - Identification of small RNA pathway genes using patterns of phylogenetic conservation and divergence.
- Tabach Y, Billi AC, Hayes GD, Newman MA, Zuk O, Gabel H, Kamath R, Yacoby K, Chapman B, Garcia SM, Borowsky M, Kim JK, Ruvkun G.
- Nature. 2013 Jan 31;493(7434):694-8.
-
- - Neuronal signaling modulates protein homeostasis in Caenorhabditis elegans post-synaptic muscle cells.
- Garcia SM, Casanueva MO, Silva MC, Amaral MD, Morimoto RI.
- Genes Dev. 2007 Nov 15;21(22):3006-16.