RNA toxicity in a zebrafish model for C9orf72
ALS is abrogated by Pur-alpha and p62

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1) Research Question

5' - CTAGGGGCCCCGGGCGGGGCGG
3' - GATCCCCGGGCCCCGGGCCC

Evidence for direct RNA toxicity in an *in vivo* model?

2) Research model

Vertebrate model

DPR toxicity

RNA toxicity

C9orf72 LOF

Motor Neurons

Axons

Lemmens et al, HMG 2007

Background

Evidence for direct RNA toxicity in an *in vivo* model?
1) Is repeat RNA toxic in zebrafish?

**Sense RNA is toxic**

- A: 
  - Graph showing Axonal Length (%) for GFP, 3S, 4S, 10S, 35S, 70S, 90S.
  - Bars indicate toxicity levels.

- B: 
  - Graph showing Affected embryos (%) for GFP, 3S, 4S, 10S, 35S, 70S, 90S.
  - Bars indicate toxicity levels.

**Antisense RNA is toxic**

- C: 
  - Graph showing Axonal Length (%) for GFP, 35AS, 70AS.
  - Bars indicate toxicity levels.

- D: 
  - Graph showing Affected embryos (%) for GFP, 35AS, 70AS.
  - Bars indicate toxicity levels.
2) Are DPRs toxic in zebrafish?

**PR and GR are toxic**

**All (except PA) DPRs are detected**

**Codon optimized DPR constructs**
3) Can repeat RNA toxicity be caused by DPR toxicity?

**GR, PR and GP are not detected in repeat RNA zebrafish**

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

![Graph E](image5.png)

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T. Gendron & L. Petrucelli
3) Can repeat RNA toxicity be caused by DPR toxicity?

Mizielinska et al, Science 2014

**No synergistic toxicity between low doses of DPRs**

**‘RNA only’ constructs are toxic in zebrafish**
4) Can repeat RNA toxicity be modified?

Purα, hnRNPA1 and hnRNPH1 bind repeat RNA

Purα prevents RNA toxicity

No effect of hnRNPA1 and hnRNPH1

Purα does not rescue DPR toxicity
5) p62 in RNA toxicity zebrafish model?

Almeida et al, Acta Neuropathol 2013

Dafinca et al, Stem Cells 2016

p62 is increased in C9orf72 iPSNs

*p62 levels not altered in zebrafish model*

Pur-alpha increases p62 levels

p62 prevents RNA toxicity
6) What is the mechanism of the rescue by Purα?

Deletion of G-rich or PUR2 domain abrogates rescue of Purα
6) What is the mechanism of the rescue by Purα? – PUR2 domain

The **PUR2** domain mediates p62 induction

**Patient derived fibroblasts**
6) What is the mechanism of the rescue by Purα? – G-rich domain

Daigle et al, Acta Neuropathol 2016:
- Pur-alpha regulates SG dynamics
- Pur-alpha localizes to SGs
- G-rich domain is low complexity domain

**The G-rich domain is involved in SGs (?)**
Summary

1) Arginine rich DPRs (PR & GR) are toxic in zebrafish

2) Sense and antisense repeat RNA cause direct RNA toxicity in the zebrafish independent of DPR toxicity

3) Overexpression of Purα prevents direct RNA toxicity
   - PUR2 domain: p62
   - G-rich domain: stress granules
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