C9orf72 repeat expansions cause neurodegeneration in *Drosophila* through arginine-rich proteins

Mizielinska. S. *et al.,*
Science, 2014

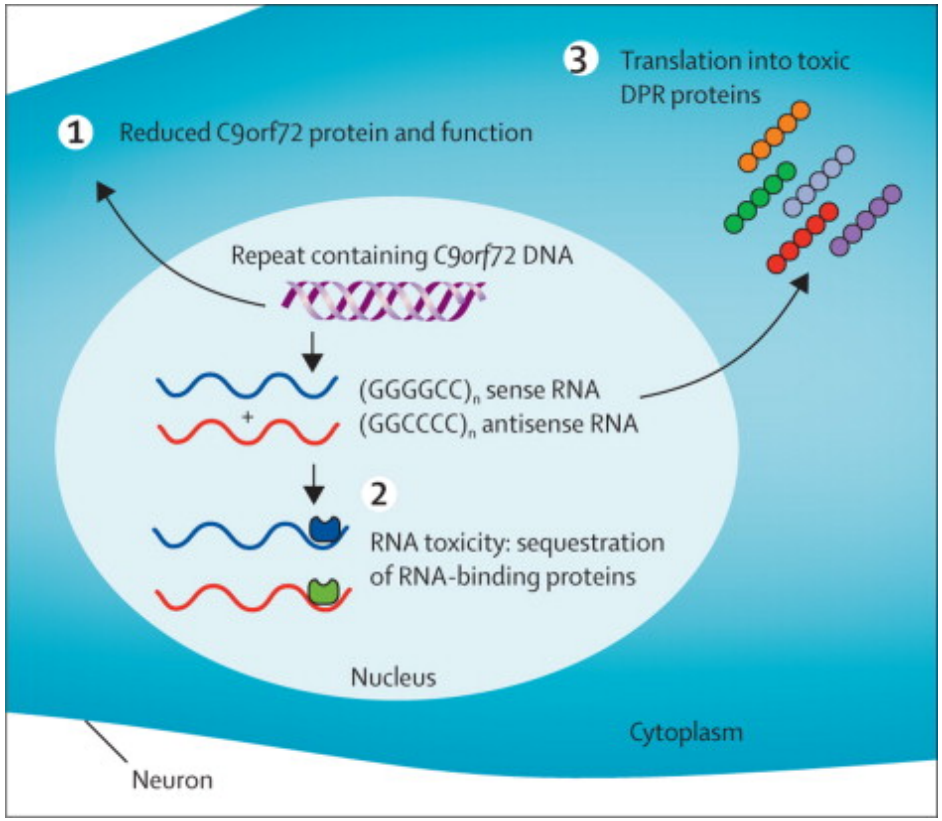
Anand David
26-05-2015

Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD)

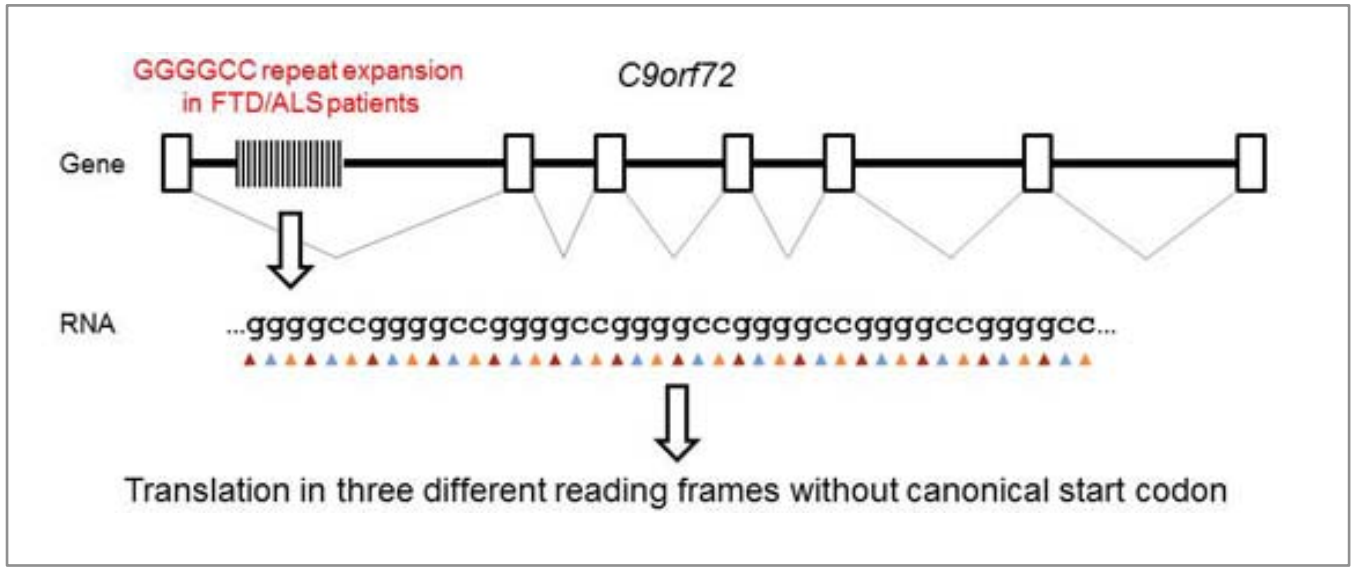
- Mutations in non-coding (intronic) region of chromosome 9 open reading frame 72 (***C9orf72***) gene
- Mutational Mechanism: massive expansion of a hexanucleotide repeat GGGGCC (**G_4C_2**)
- C9FTD/ALS patients carry 400-4400 G_4C_2 repeats

Possible mechanisms by which repeat expansion cause neurodegenerative diseases

Rohrer J.D. ***et al.***, ***Lancet Neurol, 2015***



Repeat-associated non-ATG (RAN) translation



Mori K. ***et al.***, ***Science, 2013***

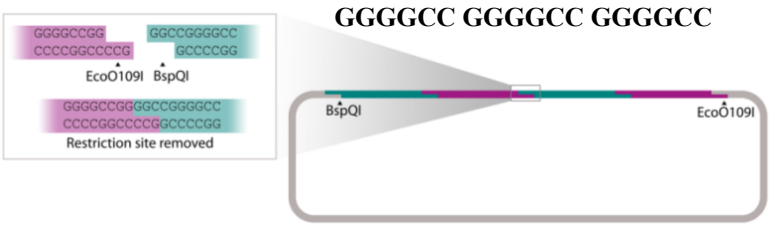
Dipeptide repeat (DPR) protein:

- glycine-alanine **(GA)**
- glycine-proline **(GP)**
- proline-alanine **(PA)**
- glycine-arginine **(GR)**
- proline-arginine **(PR)**

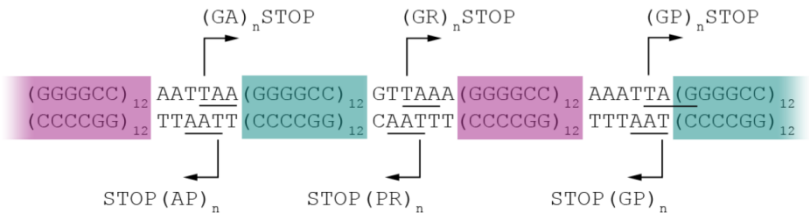
Aim : deciphering contribution of repeat RNA and DPR proteins to *c9orf72*-mediated neurodegeneration

G_4C_2 expanded repeat construct design

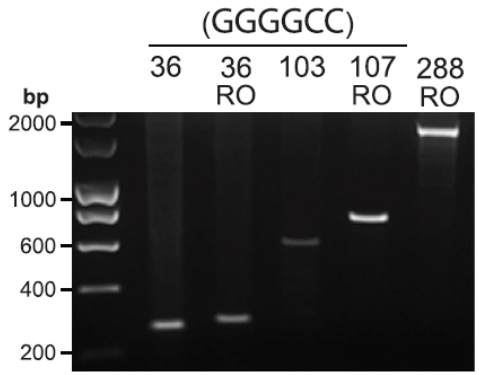
Pure repeat construct:
RNA translated to DPR protein



Repeat RNA-only (RO) / interrupted construct:
RNA expressed but not the dipeptide translation products

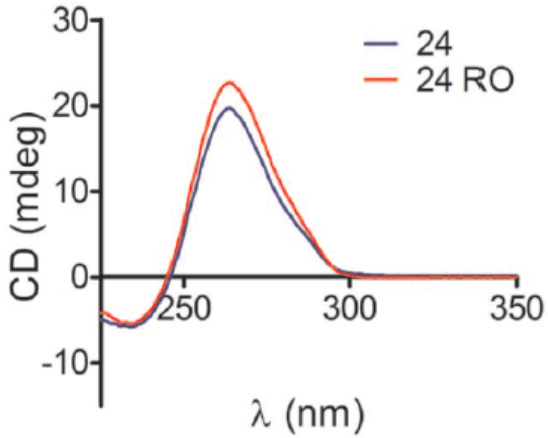


Different lengths of onstructed **pure** and RNA-only (**RO**) repeats



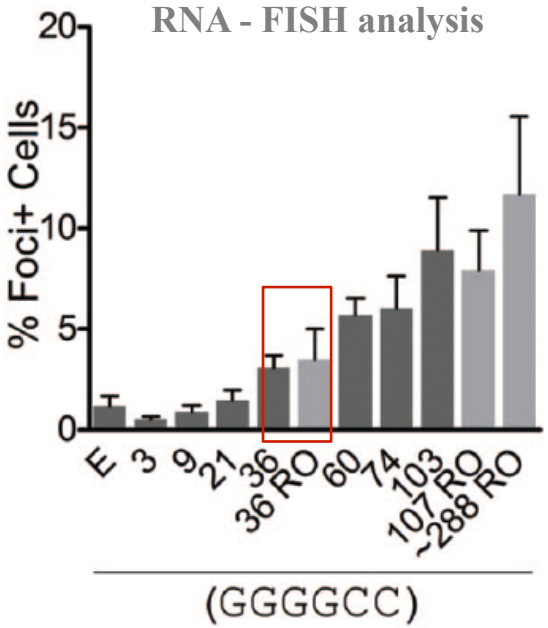
Does the interruption affect the structure and characteristic of G_4C_2 repeat RNA ?

Circular dichroism test



Human neuroblastoma (SH-SY5Y) cell line

RNA - FISH analysis

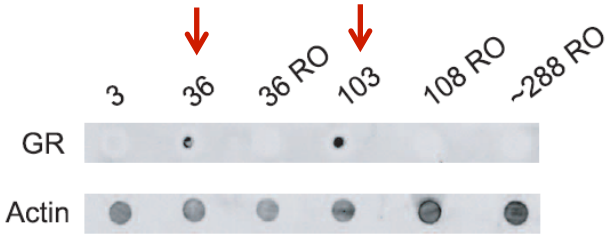


- **Interuptions didnot affect the tertiary structure of G_4C_2 repeat RNA**
- **Formation of RNA foci was lenght dependent for both pure and RO repeats**

Is the toxicity conferred by DPR protein or by repeat RNA ?

Drosophila **expressing pure or RO repeats**

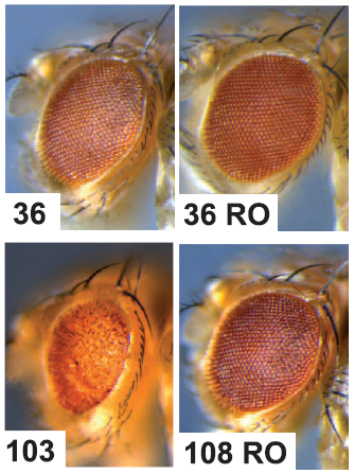
UAS promoter



Dot blot assay

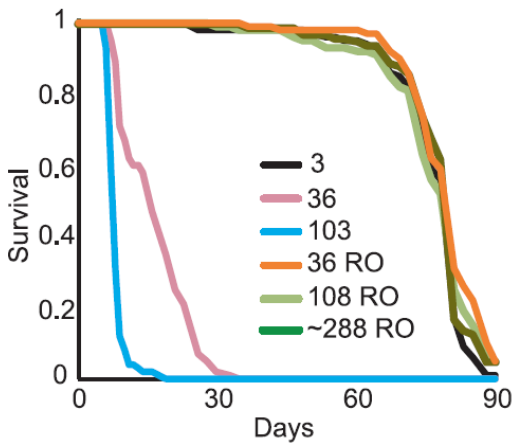
Immunoblotting with an antibody to poly-(GR)

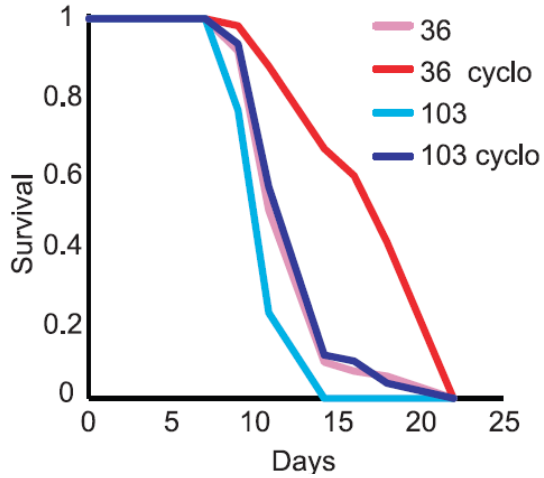
GMR-Gal4 driver



- **Pure repeats generated poly-(GR) proteins but not the RO repeats**
- **Pure repeats were lethal, whereas RO repeats had no effect**

Lifespan assay in flies expressing repeats in adult neuron with Elav-GeneSwitch driver





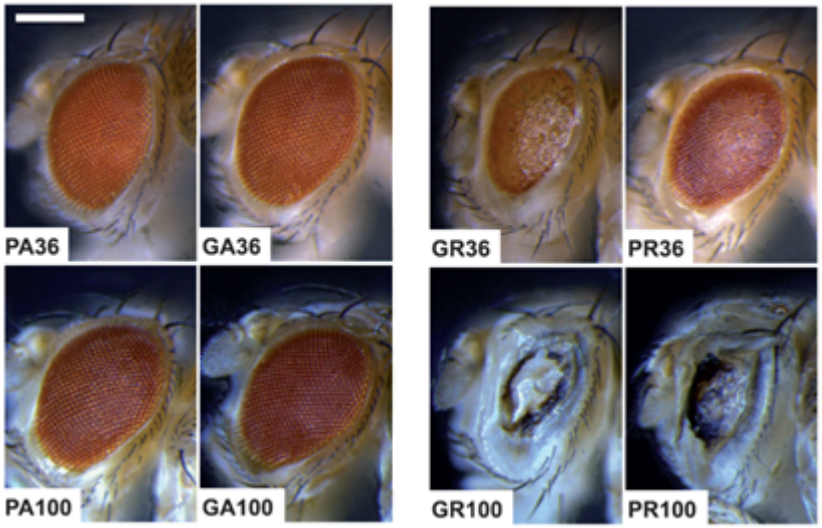
Translation blocked by cycloheximide

- **Neurotoxicity of pure repeats was attributable to DPR protein production**

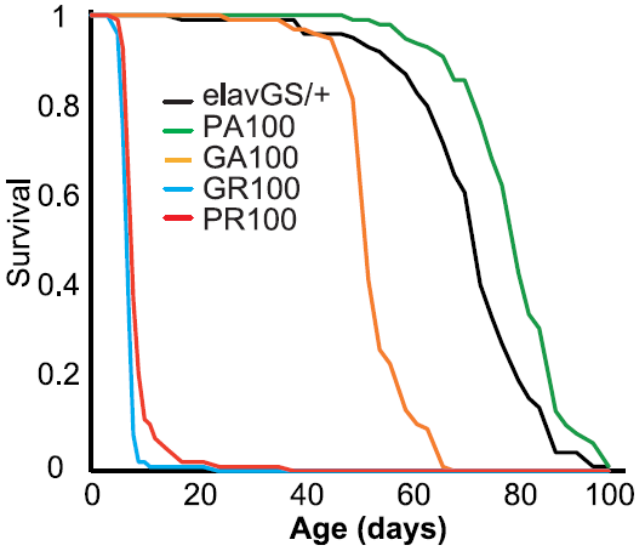
Which of the five DPR proteins translated from *c9orf72* repeat drives neurodegeneration ?

Protein-only constructs for individual DPR protein: different codons to make dipeptides of interest

Expression in *Drosophila* eye



Expression in adult nervous system



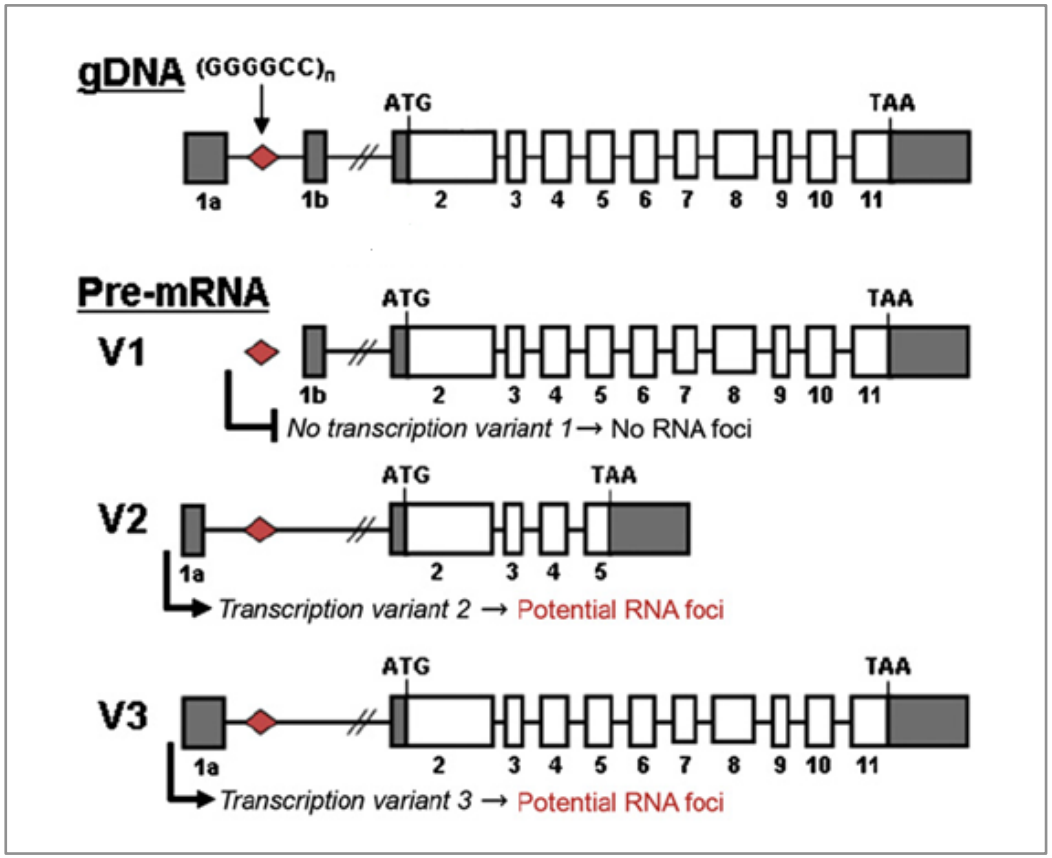
- **Longer GR and PR sequence lead to increased eye degeneration and lethality**
- **Highly basic arginine-containing DPR proteins drove repeat toxicity**

Take home message

- G_4C_2 repeats can cause toxicity through the production of aberrant translation products and not from the RNA alone
- GR and PR (GA to lesser extent) drives neurodegenration caused by *C9orf72* mutations in ALS and FTD, although all five DPR proteins form inclusions in affected brain regions
- RNA foci is observed in brain of C9FTD/ALS patients, thus modeling RNA repeats closer to pathological range can reveal the contribution of RNA toxicity
- Identifying suppressors of dipeptide toxicity can be poteintial therapeutic stratergy

Thank you for your attention !

C9orf72 transcripts preduced by alternative pre-mRNA splicing



Hernandez. M. D. ***et al.*****,** ***Neuron, 2011***