The ALS/FTD C9ORF72 hexanucleotide repeat expansion disrupts nucleocytoplasmic transport

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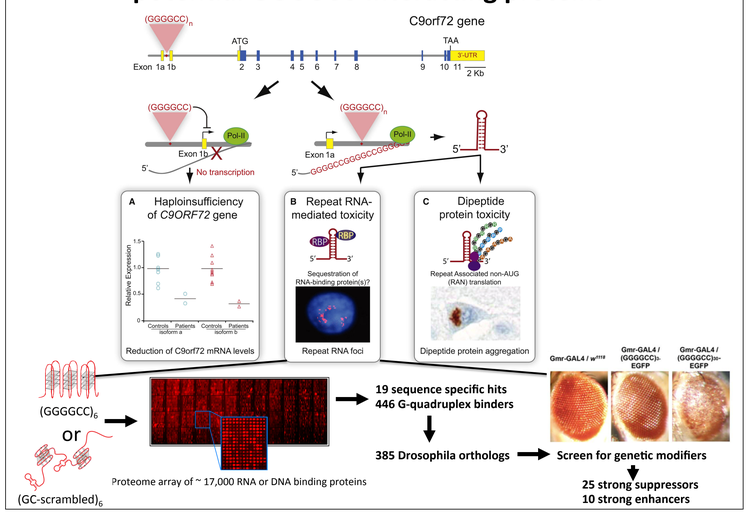
Abstract

Expansion of GGGGCC (G4C2) hexanucelotide repeats in C9ORF72 is the most common genetic cause of ALS/FTD spectrum disorders. Recent evidence suggests that the hexanucleotide expansion likely causes neuronal toxicity via a gain-of-function mechanism and may be mitigated by antisense oligonucleotides (ASOs). Using a recently generated Drosophila model of ALS caused by overexpression of 30 G4C2 repeats (Xu et al, PNAS, 2013), we have identified Ran-GAP1, a key regulator of nuclear transport, as a potent suppressor of G4C2-mediated neurodegeneration. Interestingly, Ran-GAP1 binds to the hexanucleotide repeat sequence of C9ORF72 (Donnelly et al, Neuron, 2013), and accumulates within perinuclear punctae in iPS neurons derived from C9ORF72 ALS patients. Importantly, genetic perturbations that inhibit nuclear export also suppress G4C2-mediated neurodegeneration, whereas genetic inhibition of nuclear import dramatically enhances the phenotype. Furthermore, expression of G4C2 blocks nuclear import of coexpressed GFP containing a nuclear localization signal (NLS:GFP). Consistent with these findings, we find that the RNA-binding protein TBPH (Drosophila TDP-43), which normally cycles between the cytoplasm and nucleus, is mislocalized to and accumulates in the cytoplasm. Thus, we hypothesize that generalized nucleocytoplasmic transport defects might underlie the cytoplasmic accumulation of TDP-43 and contribute to the pathogenesis of ALS/FTD.

A proteomic screen + *Drosophila* genetic screen identifies potential GGGGCC-interacting proteins



RanGAP gain of function rescues photoreceptor degeneration caused by G4C2 repeats

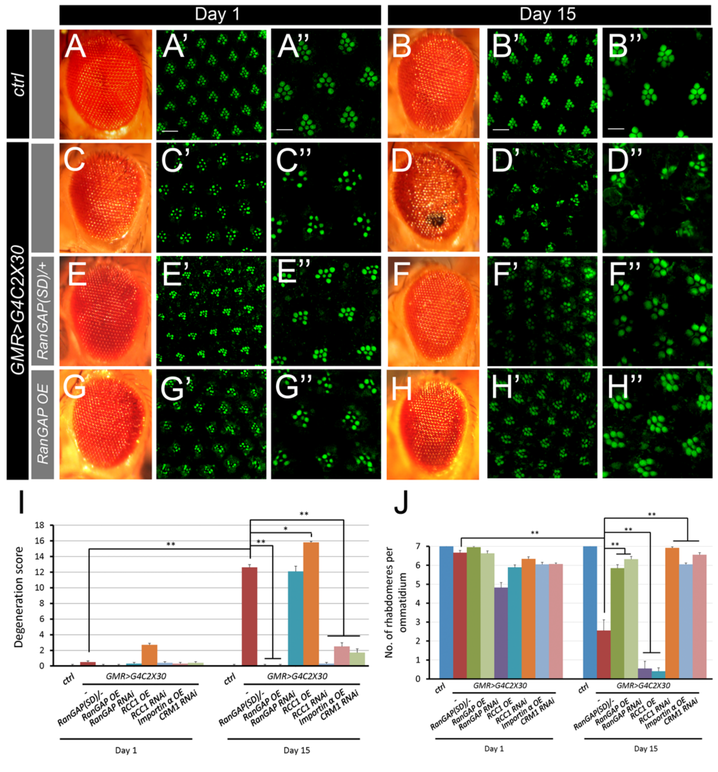


Fig. 2: RanGAP gain of function rescues photoreceptor degeneration caused by G4C2 repeats. Photoreceptor degeneration caused by G4C2-repeat expression worsens with age, compared to the control. Heterozygous mutant of a gain-of-function RanGAP allele (SD) rescues these defects. Green (phalloidin) labels photoreceptor rhabdomeres.

Genetic manipulations in the nucleocytoplasmic transport pathway modify neurodegeneration caused by G4C2 repeats

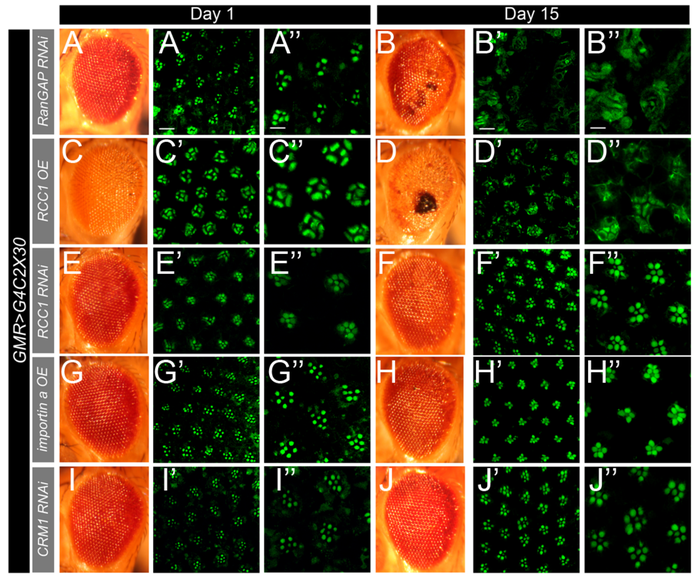


Fig. 3: Nuclear transport machinery genetically interacts with G4C2. Photoreceptors co-expressing G4C2 and different genes/RNAi. RNAi of RanGAP (A and B) or overexpressiong of RCC1 (C and D) enhance the defects, whereas RCC1 (E and F) or CRM1 (I and J) RNAi, or overexpression of importin alpha (G and H) suppress the defects.

G4C2 repeats causes nuclear transport defects

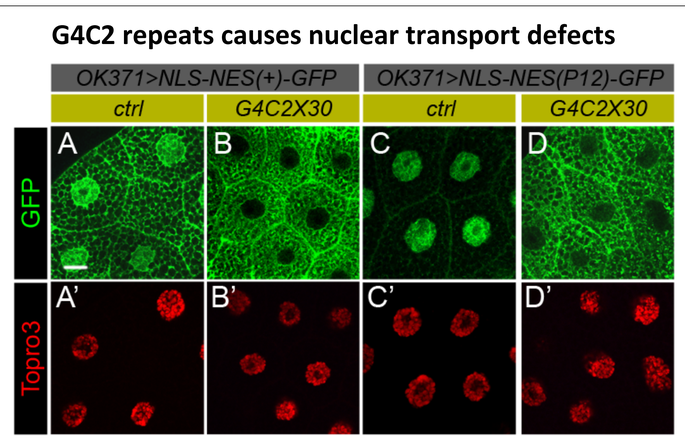


Fig. 4: Nuclear transport defects caused by G4C2 can be rescued by ASO. Expression of NLS-NES-GFP or NLS-NES(P12)-GFP in control (A and C) or G4C2-repeat-expressing (B and D) salivary gland.

NLS-Tomato-NES exhibits reduced nuclear import in C9ORF72 iPS neurons

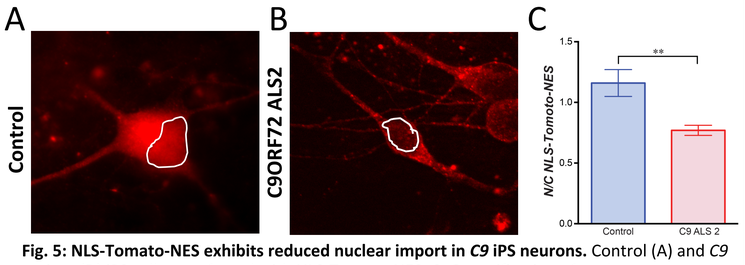


Fig. 5: NLS-Tomato-NES exhibits reduced nuclear import in *C9* iPS neurons. Control (A) and *C9* patient (D) iPS cells transfected with NLS-Tomato-NES. C: Quantification of nuclear versus cytoplasmic Tomato signal.

RanGAP1 is accumulated in the motor cortex of *C9* patients

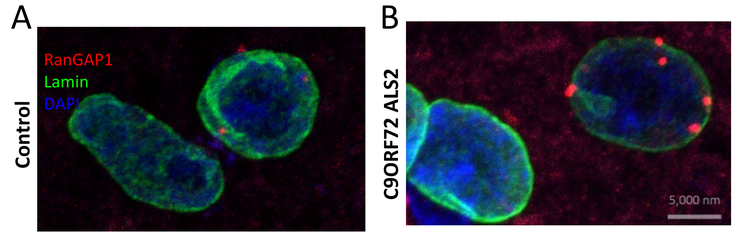


Fig. 6: RanGAP1 is accumulated in the motor cortex of *C9* patients. Immunofluorescent staining of RanGAP1 (human RanGAP), nuclear Lamin (a nuclear envelop marker), and DAPI in control (A) and *C9* patient (B) motor cortex.

RanGAP/RanGAP1 physically interacts with G4C2 repeats mRNA

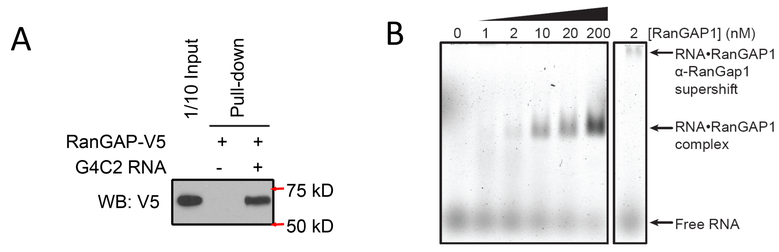


Fig. 7: RanGAP/RanGAP1 physically interacts with G4C2 mRNA. (A) Pull-down of RanGAP-V5 and biotinylated G4C2 RNA using avidin-agarose beads, blotted with a V5 antibody. (B) Left panel: EMSA shows that RNA and RanGAP1 form a complex. Right panel: RNA, RanGAP1, and a RanGAP1 antibody form a super-complex.

KPT-276 rescues transport defects

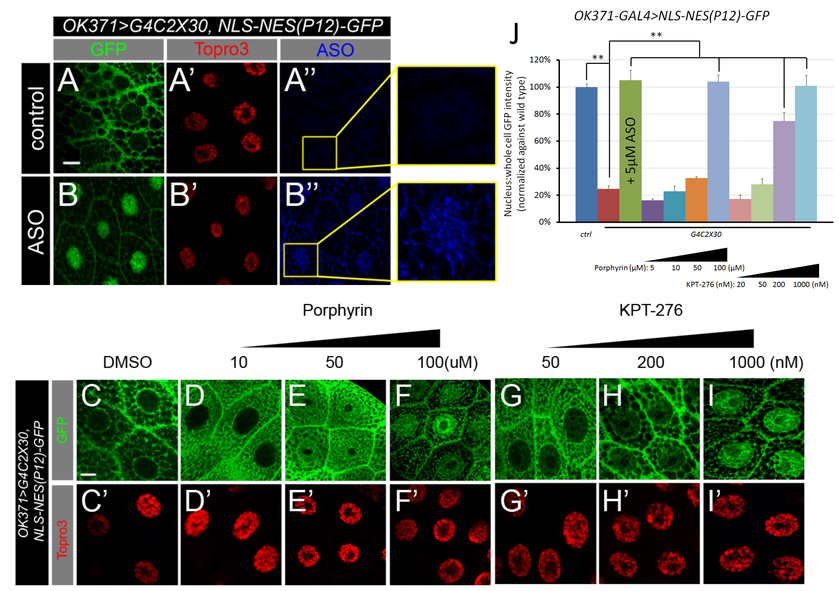


Fig. 9: Pharmacological rescue of nucleocytoplasmic transport defects. Salivary gland of flies expressing G4C2 repeats and NLS-NES(P12)-GFP that are treated with a mock control (A) and 5μM ASO (B). Blue depicts the ASO staining. (C-I) The same flies are treated with a mock control (C) or porphyrin (TMPyP4) or KPT-276 at various concentrations (D-I). Quantification in J.

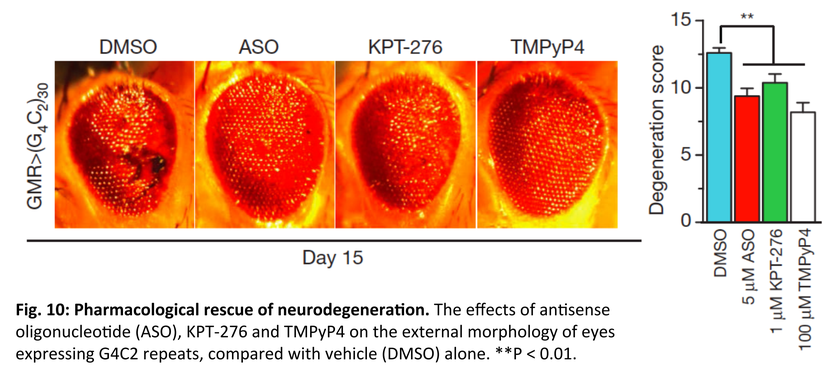
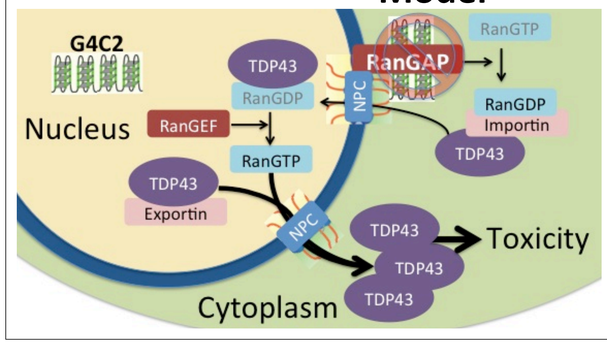


Fig. 10: Pharmacological rescue of neurodegeneration. The effects of antisense oligonucleotide (ASO), KPT-276 and TMPyP4 on the external morphology of eyes expressing G4C2 repeats, compared with vehicle (DMSO) alone. **P < 0.01.

Model



1. G4C2 repeat mRNA interacts with and inhibits RanGAP function to convert RanGTP to RanGDP.
2. Disruption of RanGTP/GDP gradient causes nucleocytoplasmic transport defects and TBPH/TDP43 cytoplasmic mislocalization.
3. These transport defects may be rescued by small molecules.

Reference

1. Xu Z., Poidevin M., Li X., Li Y., Shu L., Nelson D.L., Li H., Hales C.M., ... Jeng P. (2013). Proc. Natl. Acad. Sci. U. S. A 110: 7778-7783.
2. Donnelly C.J., Zhang P.W., Pham J.T., Heusler A.R., Mistry N.A., ... Sattler R*, Rothstein JD*. (2013). Neuron 80: 415-428.
3. Zhang K*, Donnelly CJ*, Haeusler AR, Grima JC, ..., Matunis MJ, Wang J, Sattler R, Lloyd TE*, Rothstein JD*. The C9ORF72 repeat expansion disrupts nucleocytoplasmic transport. Nature 2015: 525(7567): 56-61.