Examining Mechanisms of Nuclear Lamina Disruption in Neurodegeneration

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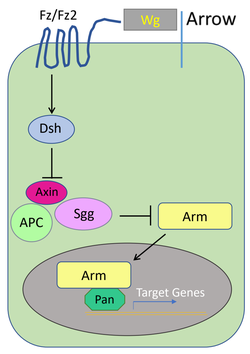
Abstract

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease which typically leads to death within 2-5 years after diagnosis. The most common genetic cause of ALS is a hexanucleotide repeat expansion within the C9orf72 gene. Recent work has implicated an upregulation in Wnt signaling (*Drosophila* Wingless, Wg) in ALS pathogenesis, both in ALS patient tissue and SOD1 ALS model systems. However, the role of this pathway in C9-ALS, and the mechanism of upregulation and its role in disease pathogenesis in other ALS subtypes, have yet to be characterized. We aim to examine the role of this pathway using a fly model of C9-ALS which expresses the repeat expansion under control of the Gal4/UAS system. We found that Wg is upregulated in our C9-ALS model, indicating its potential relevance to pathogenesis in this model. Disruption of the nuclear pore complex and of nucleocytoplasmic transport have been increasingly implicated in neurodegenerative disease pathogenesis, including in ALS, Alzheimer's disease, and Huntington's disease. Interestingly, upregulation of Wg signaling using the Gal-4/UAS system leads to altered expression of Nup214 and Nup98 as well as defects in nucleocytoplasmic transport of a shuttle GFP construct which mimics phenotypes seen in C9-ALS models. We further aim to examine the molecular mechanism of Wg upregulation as well as potential downstream pathogenic cascades. These studies have the potential to uncover the role of Wg signaling in C9-ALS, which may suggest novel therapeutic targets.

Background

- ALS is a devastating neurodegenerative disease leading to death within 2-5 years post onset.
- A GGGGCC hexanucleotide repeat expansion in C9orf72 accounts for up to 50% of fALS and 10% of sALS.
  - Most individuals have between 2-23 repeats, pathogenic expansions can reach up to 1000s.
- Wnt signaling is upregulated in other subtypes of ALS, and this may be protective or detrimental.
- Nuclear pore complex (NPC) and nucleocytoplasmic transport (NCT) defects are common to many neurodegenerative diseases.



NCT Disruption Commonly Occurs in C9-ALS Models and Drug Induced Restoration of NCT Ameliorates Toxicity

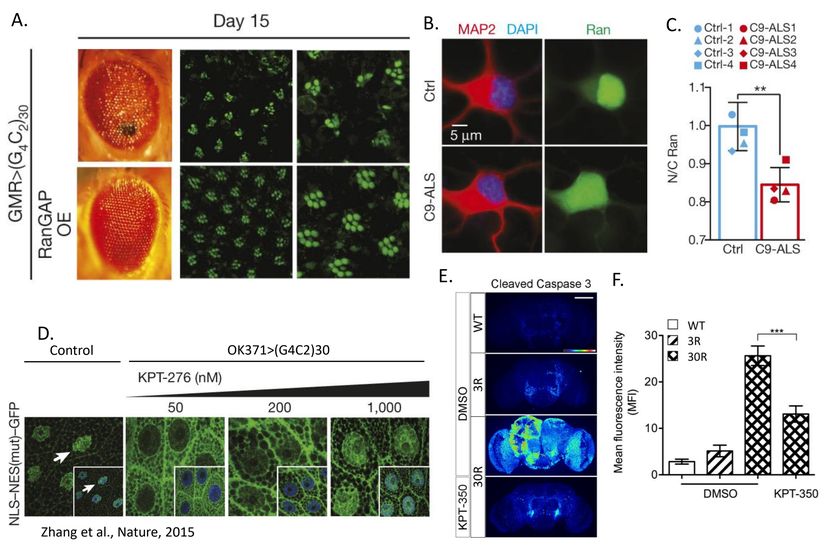


Figure 1: A) A screen for modifiers of (G4C2)30-mediated degeneration in the fly eye uncovered strong modifiers in the NCT pathway. B-C) The Ran gradient is disrupted in C9 iPSC cells. D) Shuttle NLS-NES(mut)-GFP construct is mislocalized to the cytosol in (G4C2)30 fly salivary glands, this mislocalization is rescued with the Selective Inhibitor of Nuclear Export (SINE) KPT-276. E-F) Expression of (G4C2)30 in adult fly neurons is rescued by the SINE KPT-350.

Wg Protein is Upregulated in C9-ALS Fly Neurons at Multiple Timepoints

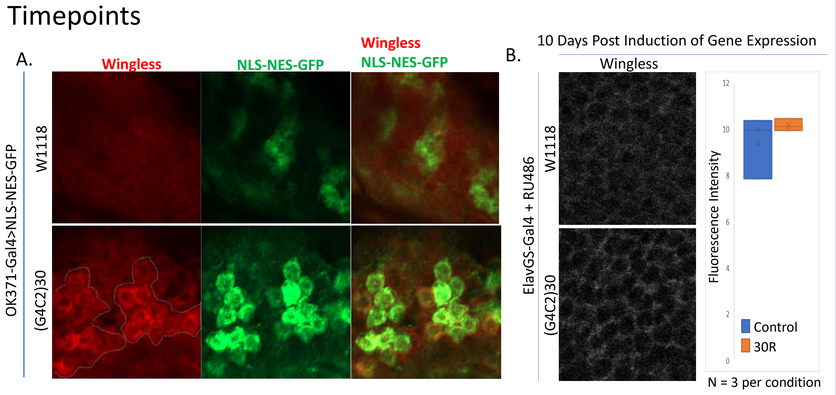


Figure 2: A) Expression of (G4C2)30 in larval VNC using OK371-Gal4 causes upregulation of Wg in construct expressing cells, as identified by GFP marker, in comparison to non-expressing cells. B) RU486-inducible expression of (G4C2)30 in all neurons via ElavGS-Gal4 in adults shows slight upregulation of Wg protein after 10 days of drug feeding. RU486 fed in food at a concentration of 200uM, data shown is from female flies.

References

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Upregulation of Wg Signaling Mimics C9-ALS induced Lamina Disruptions

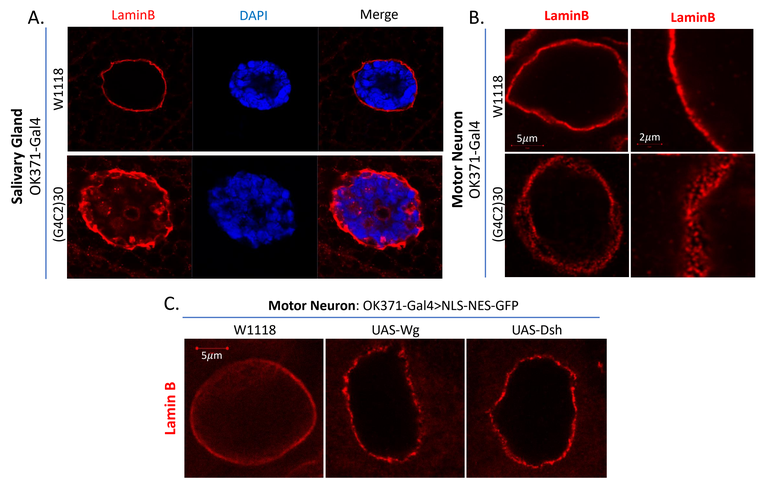

Figure 3: A) Expression of (G4C2)30 in Drosophila salivary gland cells via OK371-Gal4 leads to disruptions of the nuclear lamina as seen using anti-Lamin B staining, along with accumulations of Lamin B within the nucleus. B) Similar defects are seen in motor neurons when expansion microscopy is used to examine the lamina at high magnification. C) Upregulation of Wg signaling leads to nuclear lamina defects mimicking those caused by C9-ALS constructs in motor neurons.

Upregulation of Wg Signaling Causes Nuclear Lamina Defects but Downregulation Does Not

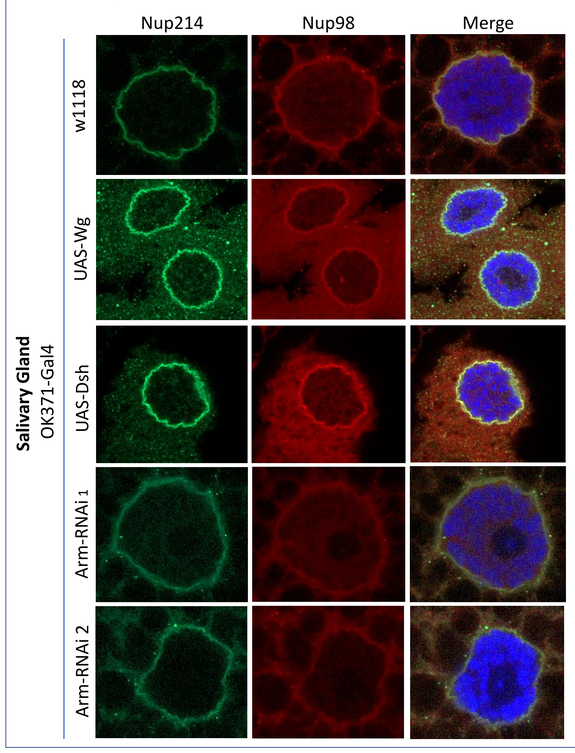

Figure 4: Upregulation of Wg signaling via overexpression of Wg ligand or Dsh leads to defects in both Nup214 and Nup98, two nucleoporins which have been previously shown to have defects in C9-ALS models. However, knockdown of Arm does not cause changes in Nup214 or Nup98. OK371-Gal4 used to drive expression in larval salivary glands.

Upregulation of Wg Signaling Mimics C9-ALS Mediated Functional Defects in Nucleocytoplasmic Transport

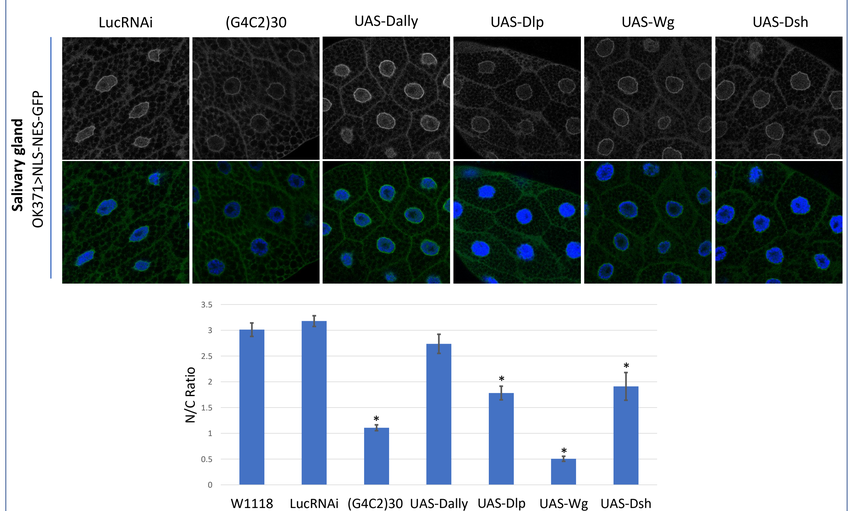

Figure 5: Upregulation of Wg signaling leads to defects in nucleocytoplasmic transport as shown by NLS-NES-GFP shuttle construct. OK371-Gal4 used to overexpress components of Wg pathway in larval salivary glands. Ratio of nuclear to cytoplasmic GFP measured and reported. * = P<0.05, n = 8-12 for all conditions except UAS-Wg where n = 4.

Acknowledgements:

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Manipulation of Wg Signaling Modifies C9-ALS Defects in Eye Degeneration, Eclosion, and Climbing Ability

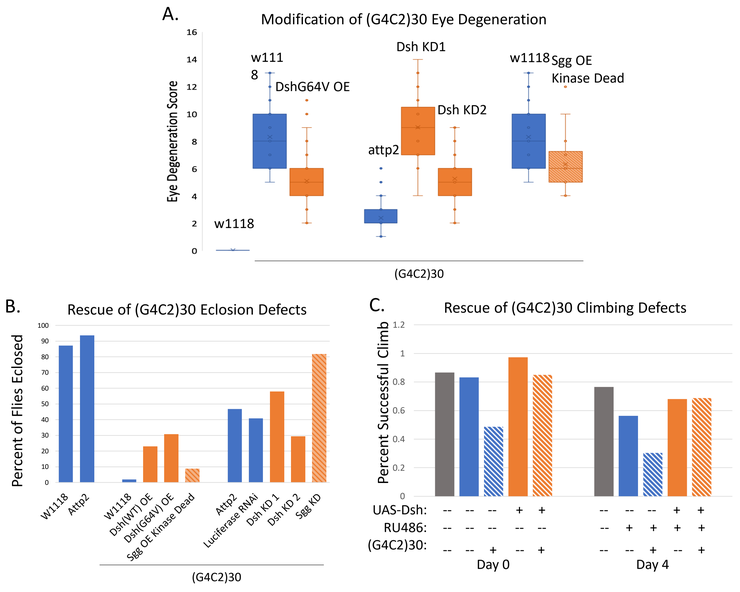

Figure 6: A) Co-expression of UAS overexpression and RNAi knockdown constructs manipulating Wg signaling with (G4C2)30 in eyes using GMR-Gal4 modifies eye degeneration scores. B) Co-expression of Wg manipulation constructs in motor neurons using OK371-Gal4 modifies eclosion rates. C) RU486 drug inducible overexpression of UAS-Dsh in all neurons in adult female flies via ElavGS-Gal4 modifies climbing ability.

Adult-Inducible Upregulation of Wg Signaling Causes Nuclear Pore Defects

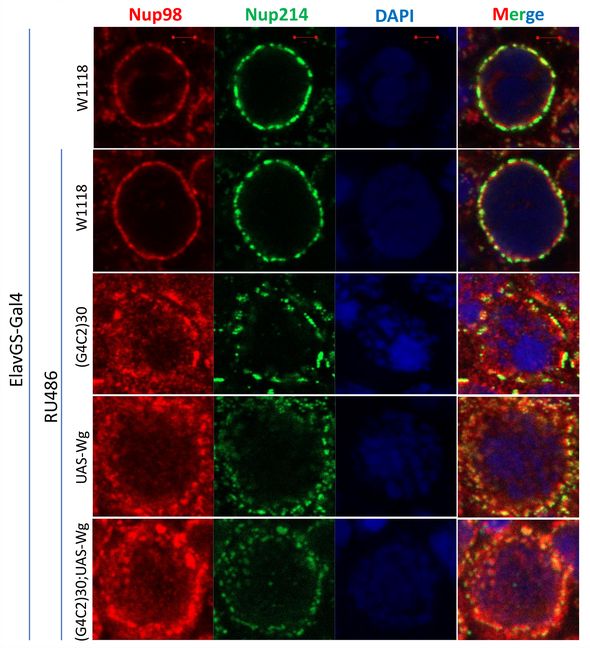

Figure 7: Upregulation of Wg signaling via ElavGS-Gal4 in adult flies leads to defects in both Nup214 and Nup98 in neurons, mimicking defects seen in our C9 model under the same conditions. Nups visualized via expansion microscopy and antibody labeling. RU486 fed in food at a concentration of 200uM. Data shown is from female flies 10 days post drug feeding.

Adult Drosophila Neurons Retain the Ability to Express Cell Cycle Markers, but This is Not Seen in C9-ALS Model

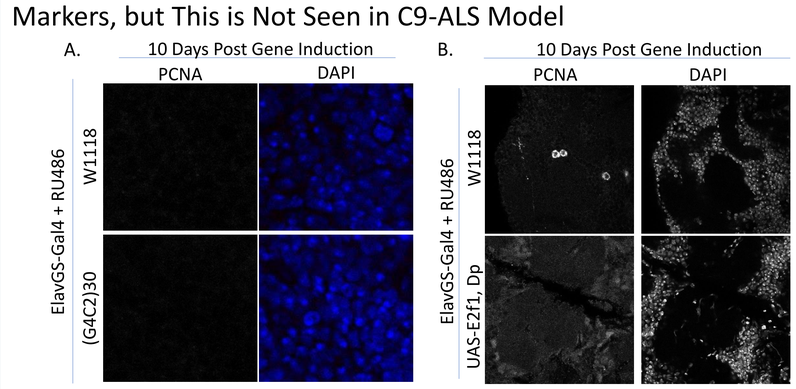

Figure 8: A) Inducible expression of (G4C2)30 does not cause neurons to re-express PCNA, a marker of late G1 to G2 phase. B) Inducible expression of S-phase transcription factors E2f1 and Dp leads to expression of PCNA. Expression driven by ElavGS-Gal4, RU486 fed in food at 200uM. N = 5 per condition. Data from female flies.

Conclusion

- Wg protein is upregulated at multiple timepoints in our C9-ALS fly model.
- Upregulation of Wg signaling can disrupt both the nuclear lamina and components of the nuclear pore complex leading to functional defects, mimicking C9-ALS models.
  - Upregulation of Wg rescues behavioral phenotypes in our C9-ALS model.
- It is unlikely that Wg upregulation is mediating these defects through disruptions in the cell cycle as may have been expected due to Myc and Cyclin D1 targets of Wg signaling.
- Given NCT disruptions seen in ALS, whether upregulation of Wg protein leads to an upregulation in functional signaling needs to be assessed.