p27^{Kip1} Modulates Axonal Transport by Regulating α-Tubulin Acetyltransferase 1 Stability

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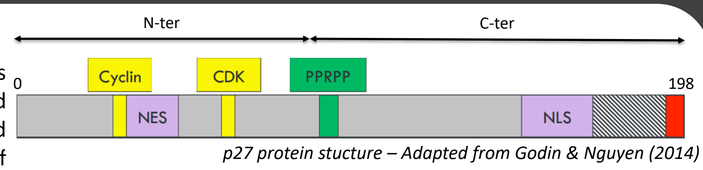
Introduction

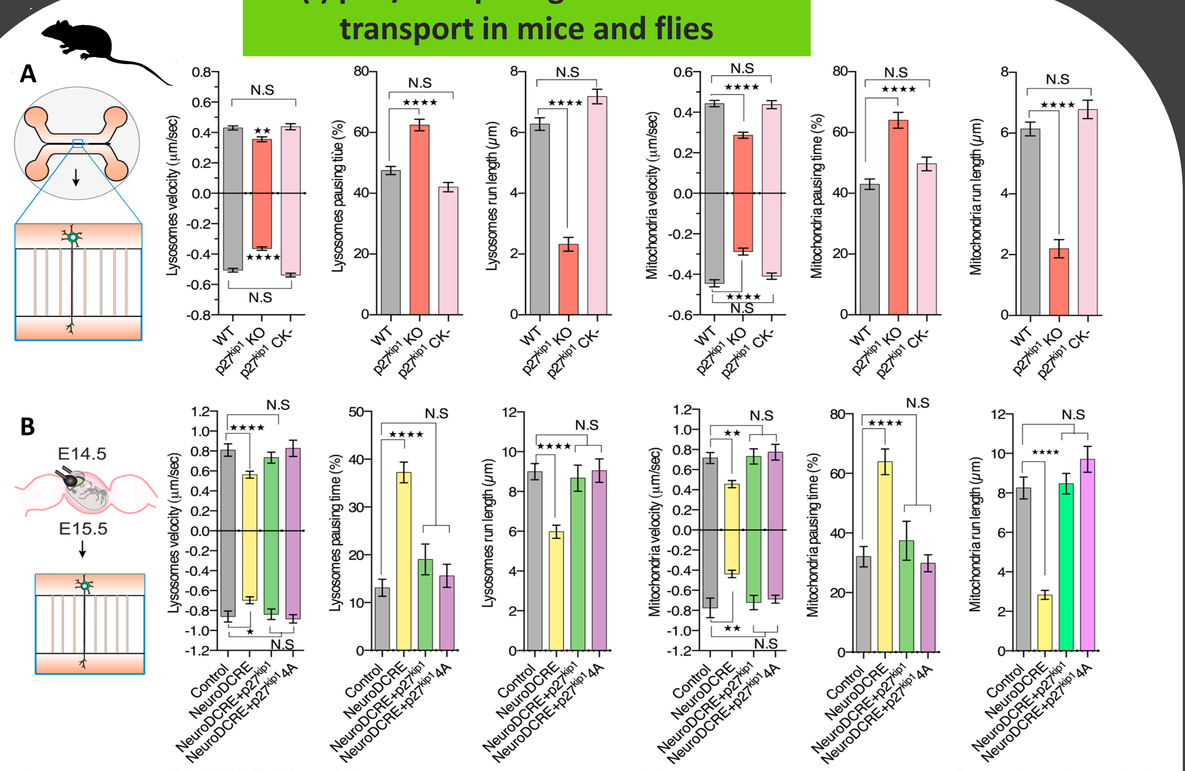
Axonal transport is a dynamic process in which cargoes are transported along microtubule tracts to maintain the homeostasis of neuronal synapses. Anterograde transport replenishes axons and presynaptic sites with protein supply while retrograde transport allows the recycling of proteins and organelles from the axon terminals. Axonal transport is a tightly regulated process with many actors contributing to the spatial and temporal distribution of cargoes. Post-translational modifications of microtubules have been shown to affect transport and notably, impaired acetylation of α-tubulin leads to a reduction in the velocity of organelles and vesicles. This post-translational modification is regulated by the antagonistic activities of the α-tubulin acetyltransferase 1 (ATAT1) and the deacetylase histone deacetylase 6 (HDAC6). Interestingly, p27^{kip1} (p27) has been associated with poorly acetylated microtubules. p27 is a multifunctional protein that was first discovered as a cell cycle regulator but also exhibits versatile roles during cerebral cortex development. p27 binds and stabilizes Neurogenin-2 to regulate neuronal differentiation but it also regulates neuronal migration and neurite branching via signaling pathways converging towards the actin an microtubule cytoskeletons.

Here, we show that the neuronal depletion of p27 in mice or its ortholog, *dacapo*, in *Drosophila Melanogaster* disrupts the axonal transport of organelles *in vitro* and *in vivo* respectively. At the molecular level, p27 binds and stabilizes ATAT1, thereby promoting the acetylation of microtubules. Taken together, our data show that p27 modulates axonal transport by promoting α-tubulin acetylation.

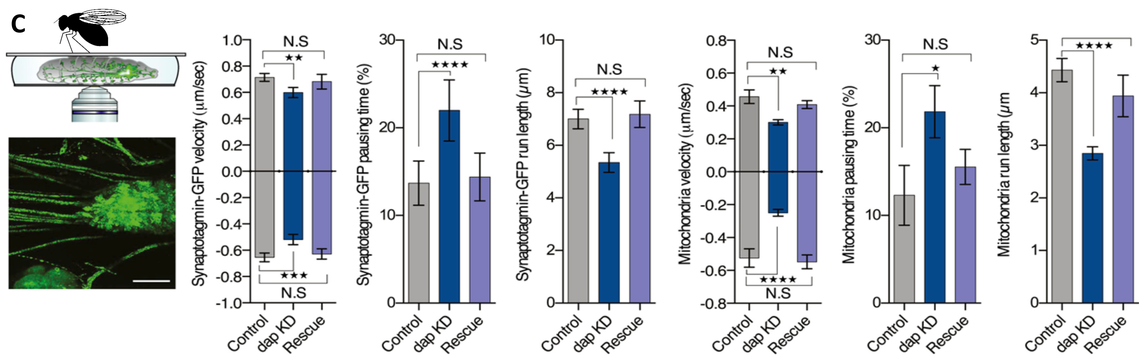


p27 protein stucture – Adapted from Godin & Nguyen (2014)

(I) p27/dacapo regulates axonal transport in mice and flies



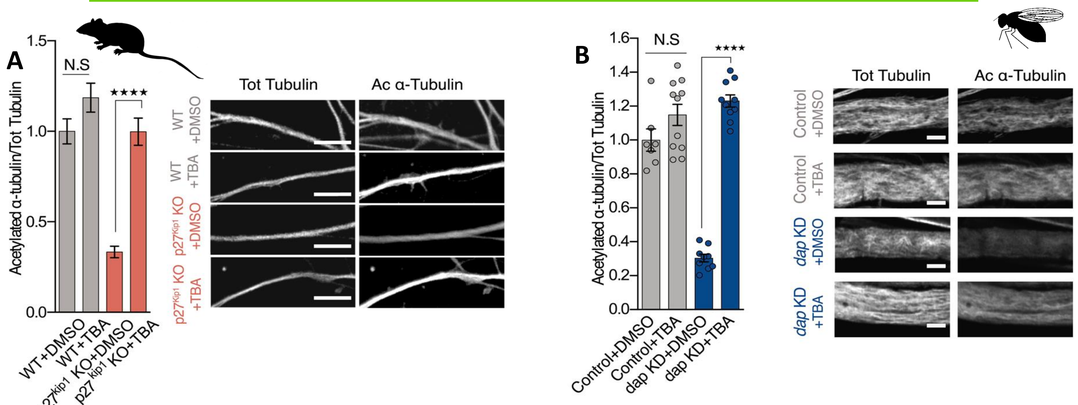
(A) Cortical neurons of E14.5 WT, KO or CK- (no cell-cycle dependent activity) embryos were cultured for 7 days in microfluidic devices and the movement of lysosomes and mitochondria was then recorded using LysoTracker and MitoTracker.
(B) E14.5 lox/lox embryos were electroporated with Cre and p27 expression was subsequently rescued with the full form or a mutant form without microtubule polymerization activity (p27 4A).

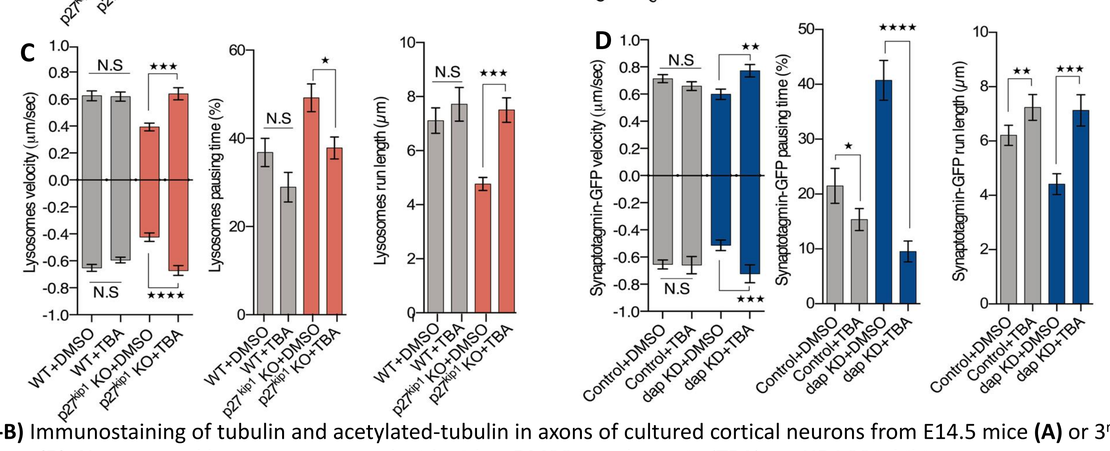


(C) *In-vivo* live imaging of 3rd instar larvae motoneuron axons expressing either Synaptotagmin-GFP or Mito-GFP with a control RNAi , dacapo RNAi (dap KD) or dacapo RNAi with dacapo overexpression (Rescue).

- **p27 KO reduces the transport velocity of mitochondria and lysosomes in the axons of E14.5 DIV7 projection neurons independently from its cell-cycle activity**
- **Reexpressing p27 or a mutant lacking microtubule polymerisation activity fully rescues the transport defects**
- **Knocking-down dacapo in larvae has the same effect with a reduction of mitochondria and synaptic vesicles velocity**

(II) HDAC6 inhibition rescues axonal transport together with tubulin acetylation upon loss of p27 or dacapo

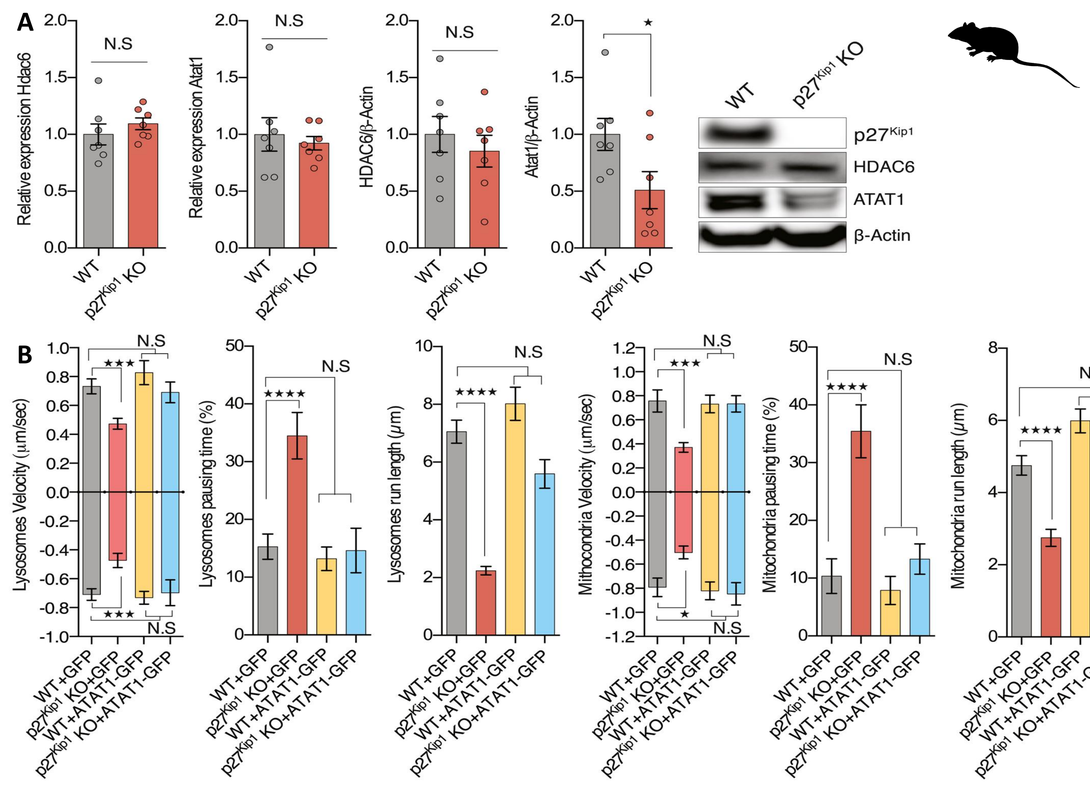




(A-B) Immunostaining of tubulin and acetylated-tubulin in axons of cultured cortical neurons from E14.5 mice **(A)** or 3rd instar larvae **(B)**. Neurons and larvae were treated with either DMSO or tubastatin (TBA), an HDAC6 inhibitor.
(C) Projection neurons from WT or KO E14.5 embryos were treated with DMSO or TBA and imaged using LisoTracker and MitoTracker (not shown).
(D) 3rd instar larvae expressing Synaptotagmin-GFP or Mito-GFP (not shown) were treated with DMSO or TBA and were imaged with videomicroscopy.

- **p27 KO or dacapo depletion results in a reduction of microtubule acetylation levels**
- **HDAC6 inhibition rescues both the microtubule acetylation levels and the axonal transport defects in mouse projection neurons and 3rd instar larvae**

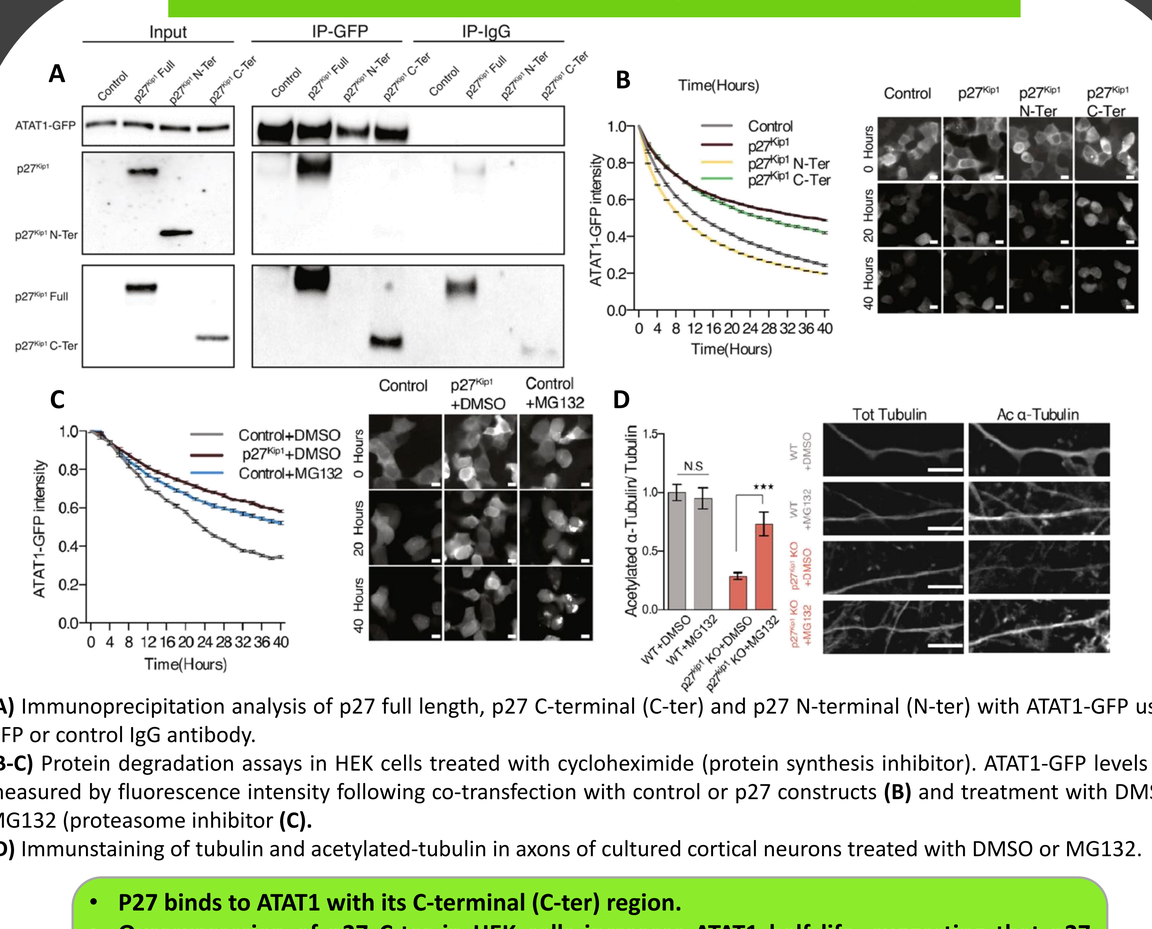
(III) p27 modulates axonal transport by regulating ATAT1 protein levels



(A) ATAT1 and HDAC6 expression at the transcript and protein levels in P0 cortical extracts from WT and p27 KO mice
(B) Time lapse imaging of lysosomes and mitochondria in WT or p27 KO projection neurons electoporated with GFP or a fused ATAT1-GFP protein.

- **ATAT1 protein levels are reduced in p27 KO mice without a change in mRNA levels.**
- **Re-expressing ATAT1 in p27 KO neurons rescues axonal transport defects**

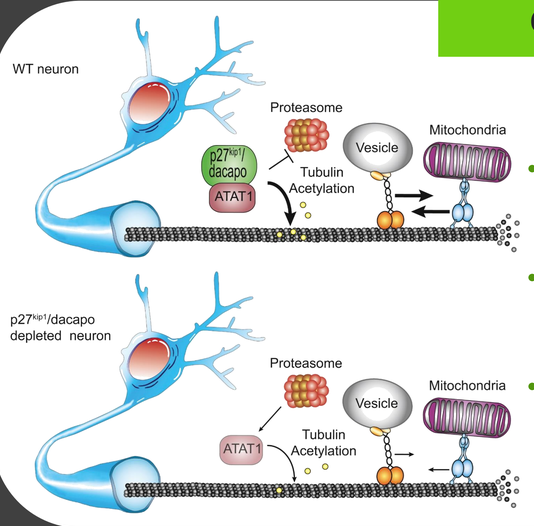
(IV) p27 binds to ATAT1 to regulate its stability



(A) Immunoprecipitation analysis of p27 full length, p27 C-terminal (C-ter) and p27 N-terminal (N-ter) with ATAT1-GFP using a GFP or control IgG antibody.
(B-C) Protein degradation assays in HEK cells treated with cycloheximide (protein synthesis inhibitor). ATAT1-GFP levels were measured by fluorescence intensity following co-transfection with control or p27 constructs **(B)** and treatment with DMSO or MG132 (proteasome inhibitor **(C).**
(D) Immunstaining of tubulin and acetylated-tubulin in axons of cultured cortical neurons treated with DMSO or MG132.

- **P27 binds to ATAT1 with its C-terminal (C-ter) region.**
- **Overexpression of p27 C-ter in HEK cells increases ATAT1 half life, suggesting that p27 regulates ATAT1 levels by stabilizing it**
- **Inhibiting proteasomal degradation is as efficient as p27 overexpression to increase ATAT1 stability and concomitantly rescues microtubule acetylation levels in p27 KO neurons.**

Conclusion



- **p27 binds to ATAT1 and stabilizes it by preventing its degradation by the proteasome.**
- **Upon p27 depletion, ATAT1 is less stable leading to an overall decrease of microtubule acetylation.**
- **Reduced levels of microtubule acetylation result in a slow-down of axonal transport in mice and flies.**

For more details see:
Morelli, G. *et al.* p27 Kip1 Modulates Axonal Transport by Regulating α-Tubulin Acetyltransferase 1 Stability. *Cell Rep.* **23,** 2429–2442 (2018).