Control of clathrin-mediated endocytosis by NIMA family kinases

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Abstract

NEKL kinases are highly conserved members of the NIMA-kinase family, which have been predominantly implicated in cell division and ciliogenesis. We previously reported, that NEKL-2/NEK8/9 and NEKL-3/NEK6/7 are required within the *C. elegans* epidermis, and that their knockdown causes molting defects that results in larval lethality. In addition, we discovered a conserved set of ankyrin repeat proteins, MLT-2/ANKS6, MLT-3/ANKS3, and MLT-4/INVS, that partner with the NEKLs to regulate their intracellular localization.

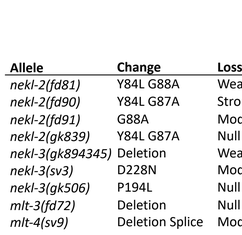
To better understand the mechanistic functions of NEKL-MLTs we obtained genetic suppressors of molting defects in *nekl-2*; *nekl-3* double mutants. Strikingly, our screen identified several mutations that directly affect the activity of AP2, a conserved heterotetrametric adaptor complex required for clathrin mediated endocytosis. Knockdown of individual AP2 subunits, or an allosteric activator of AP2, FCHO-1, strongly suppress molting defects in *nekl* mutants. Conversely, mutations that increase the levels of active AP2 strongly enhance *nekl* molting defects. In addition, inhibition of *nekls* suppress morphological defects associated with reduced AP2 function, attesting to the tight functional connection between the NEKLs and AP2.

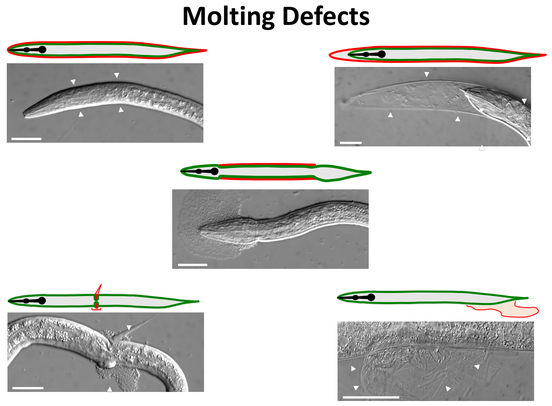
To determine the functions of NEKLs in endocytosis, while avoiding potential confounding effects caused by *nekl*-mutant larval arrest, we used auxin inducible degradation methods to deplete NEKLs specifically at the adult stage. Loss of NEKLs led to greatly elevated levels of clathrin at the epidermal apical membrane and to a profound reduction in apical clathrin mobility. Adult NEKL depletion also adversely affected uptake of LRP-1, an epidermal cargo critical for molting. These results are consistent with depletion of NEKLs leading to a defect in the ability of vesicles to release clathrin after scission and suggest that NEKLs promote clathrin uncoating. Notably, both defects were largely alleviated by a reduction in AP2 or FCHO-1 activities. These findings suggest that reduced AP2 activity may facilitate clathrin uncoating and indicate that reduced AP2 activity suppresses *nekl* molting defects through the restoration of normal trafficking.

Lastly, we show that human NEK6 and NEK7, orthologs of NEKL-3, can rescue both molting and trafficking defects in NEKL-3 depleted strains, suggesting that the control of intracellular trafficking is an evolutionarily conserved function of NEK family kinases.

Molting Defects

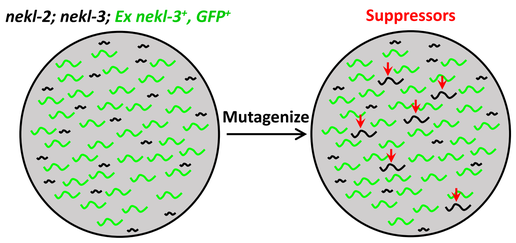
Larval Molting



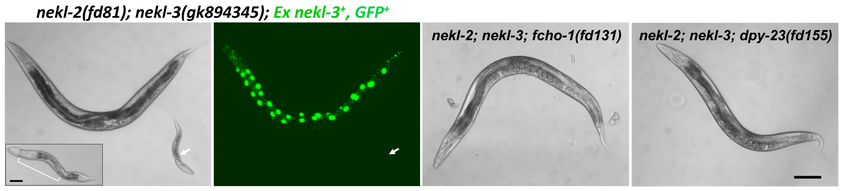


Suppressor screen

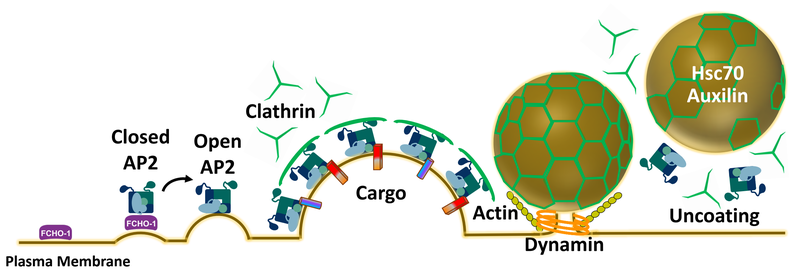
Allele	Change	Loss of function
nekl-2(fd81)	Y84L G88A	Weak
nekl-2(fd90)	Y84L G87A	Strong
nekl-2(fd91)	G88A	Moderate
nekl-2(gk839)	Y84L G87A	Null
nekl-3(gk894345)	Deletion	Weak
nekl-3(sv3)	D228N	Moderate
nekl-3(gk506)	P194L	Null
mlt-3(fd72)	Deletion	Null
mlt-4(sv9)	Deletion Splice	Moderate



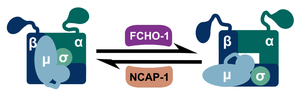
nekl-2(fd81); nekl-3(gk894345); Ex nekl-3+, GFP+



Several suppressors connected to clathrin mediated endocytosis

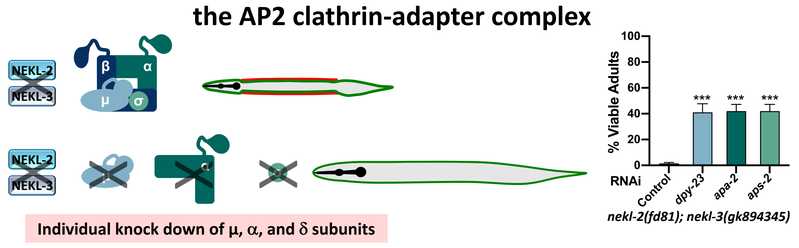


AP2 exist at least in two conformations

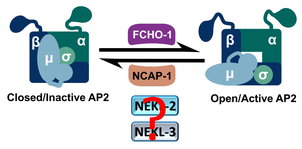


AP2 heterotetramer consists of two independently partially functional hemi complexes

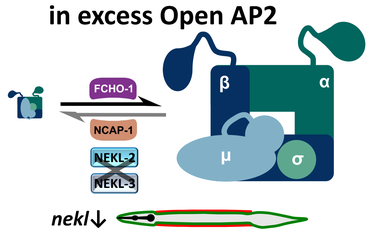
nekl molting defects are suppressed by decreased function of the AP2 clathrin-adapter complex



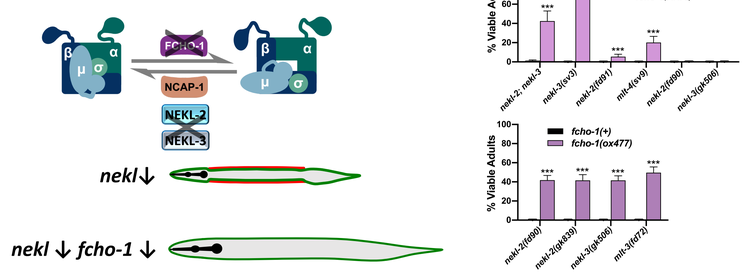
Model that NEKLs may promote closed AP2



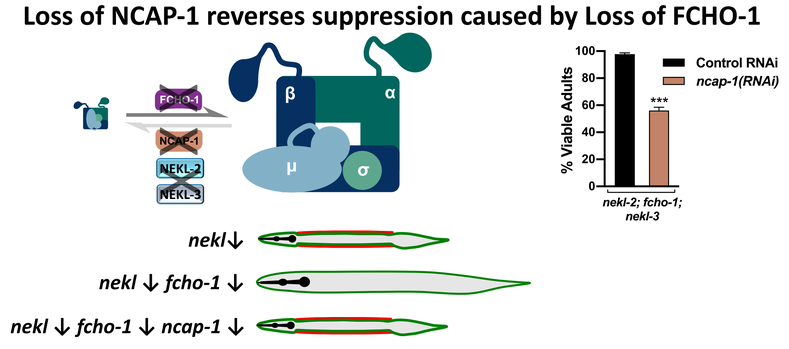
Loss of NEKLs function may result in excess Open AP2



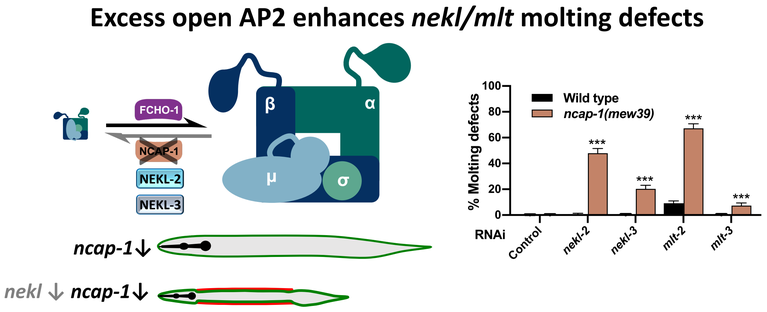
Loss of FCHO-1 suppresses *nekl* molting defects

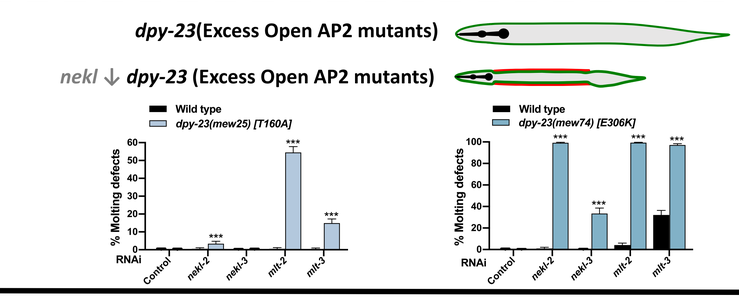


Loss of NCAP-1 reverses suppression caused by Loss of FCHO-1

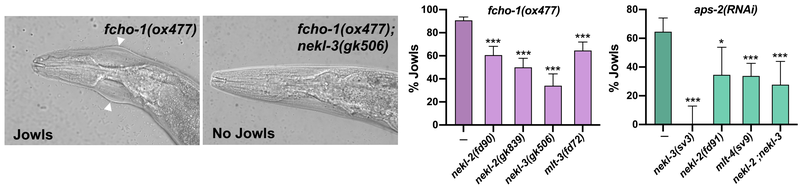


Excess open AP2 enhances *nekl/mlt* molting defects

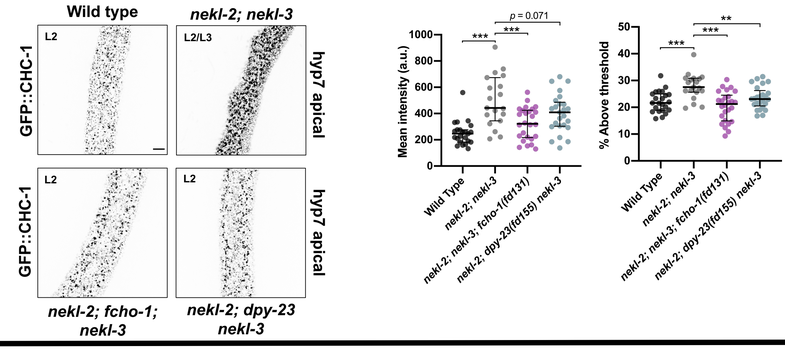




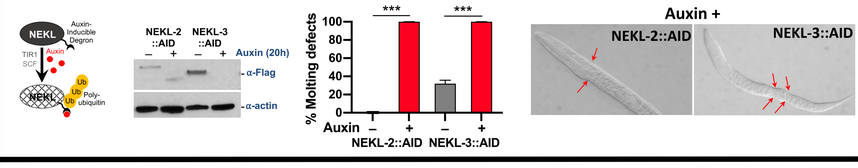
Jowls phenotype suppressed by *nekl-mlt* mutants



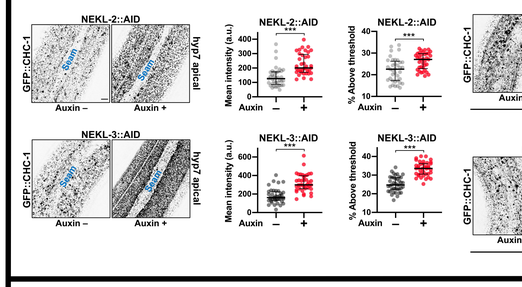
Loss of NEKL changes epidermal clathrin localization



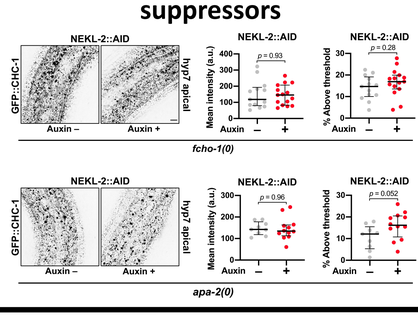
Inducible repression of NEKLs



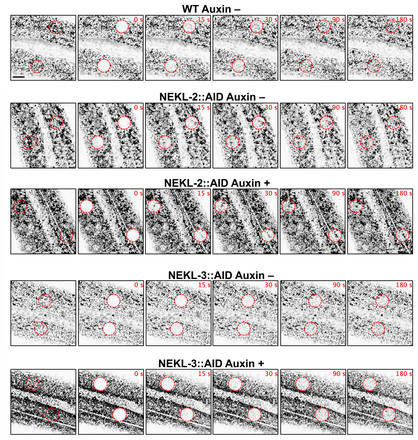
Acute Depletion of NEKLs in adults alters clathrin localization

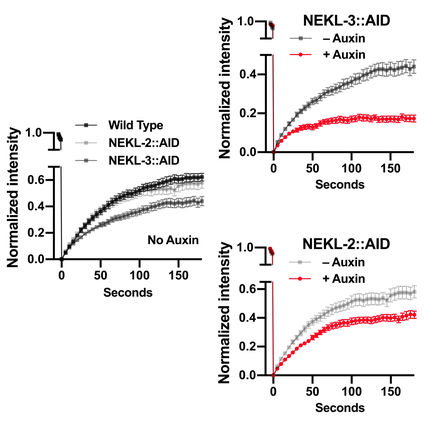


Clathrin defects are corrected by suppressors

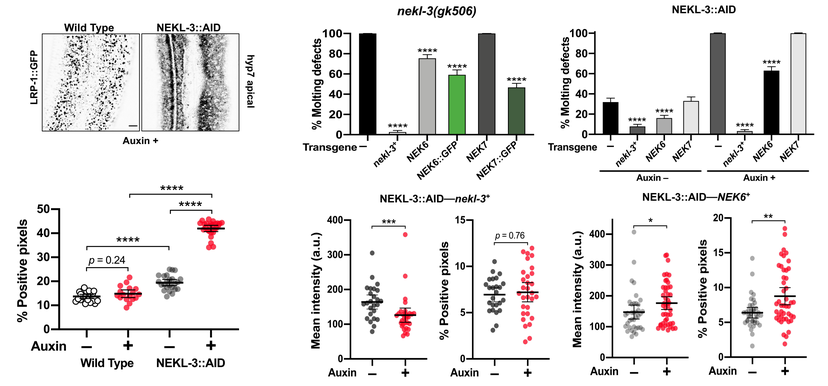


Acute loss of NEKLs reduces clathrin puncta mobility in the epidermis





Cargo trafficking is disrupted in NEKL-depleted adults



The human orthologs rescue molting and trafficking defects

Funding

This project is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). This work was supported by NIH grants GM066868 (to DSF), P20 GM103432 (Wyoming INBRE).