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Suppression of *nekl*-associated molting defects by induction of L2d

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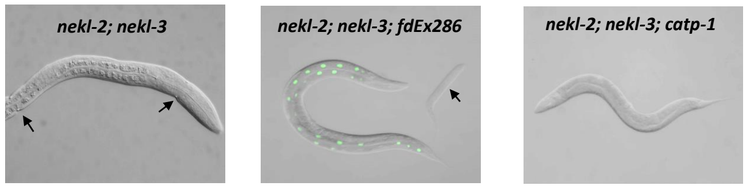
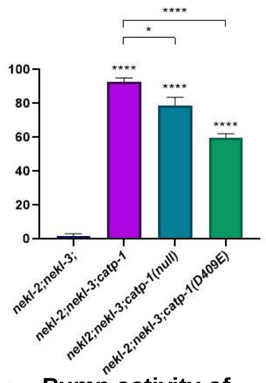
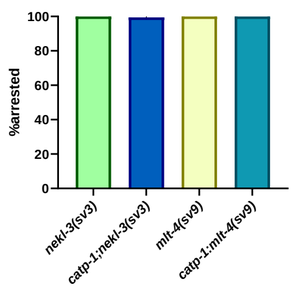
Introduction

C. elegans molting is a complex process that occurs in tight coordination with developmental progression. We have shown that the NIMA-related kinases, NEKL-2 and NEKL-3 (the NEKLs), promote molting through their involvement in the trafficking of epidermal cargos. To better understand the molting process and the functions of NEKLs, we screened for mutations that suppress molting defects in *nekl* mutants. We identified *catp-1* as a suppressor of *nekl*-associated molting defect. CATP-1 is predicted to have ATP-dependent Na+/K+ pump activity. Previous studies indicated loss of *catp-1* results in a lengthened L2 stage. This study addresses the question whether L2d like state induced by loss of *catp-1* is responsible for suppression of *nekl-2/3* molting defect.

catp-1 is a suppressor of *nekl-2;nekl-3*

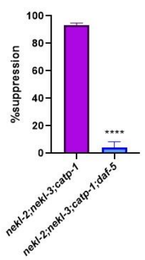




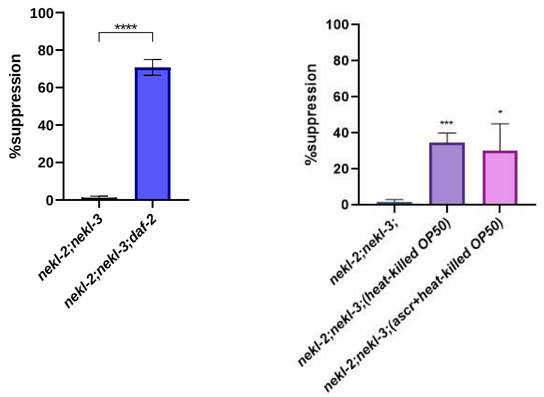


- **Pump activity of CATP-1 is critical as disruption of pump activity (D409E) suppress.**
- ***nekl-3(sv3)* and *mlt-4(sv9)* are hypomorphic alleles.**
- ***catp-1* can only suppress weak loss of function of NEKLs which arrest at L2/L3 molt.**

- **Mutation in *daf-5* which inhibits entry into L2d, strongly reduces suppression.**



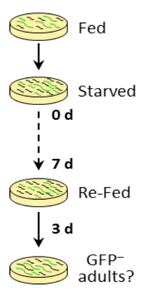
Inducing L2d by *daf-2,* dauer pheromone and growth on heat-killed *E. coli* suppress *nekl-2/3* molting defect

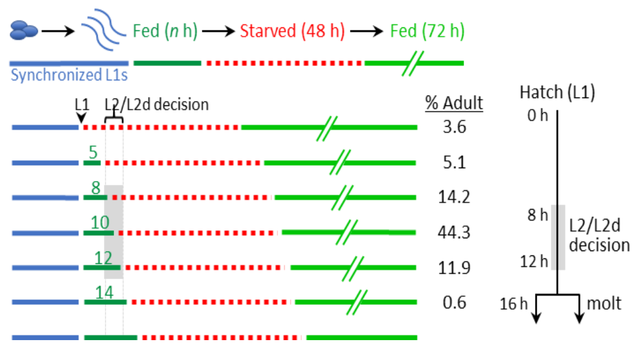


Growth on heat-killed *E. coli* induces only uncommitted L2d, which seems to be enough for suppression of molting defect.

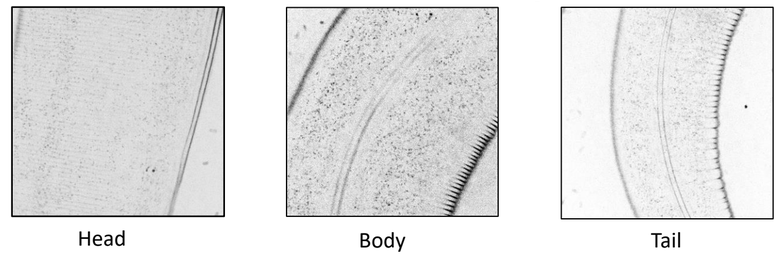
Starvation induced L2d suppress molting defect

		% GFP⁻ adults/total adults			
		Days starved			
	Genotype	0 d	1 d	3 d	7 d
L1/L2 arrest	*nekl-2(fd90)*	1.2 (83)	0 (590)	0 (475)	0.2 (492)
	nekl-2(gk839)	0 (61)	0 (515)	0 (449)	0 (541)
	nekl-3(gk506)	0 (186)	0 (521)	0 (497)	0 (510)
	mlt-3(fd72)	0 (44)	0 (471)	0 (522)	0 (513)
	nekl-2(fd81); nekl-3(gk894345)	0 (207)	13.8 (550) $p < 0.0001$	14.4 (571) $p < 0.0001$	11.1 (515) $p < 0.0001$
L2/L3 arrest	*nekl-2(fd91)*	0 (195)	0 (513)	0 (487)	ND
	nekl-3(sv3)	0 (102)	0 (490)	0 (488)	0 (534)
	mlt-4(sv9)	0 (93)	0 (575)	0 (515)	0 (533)

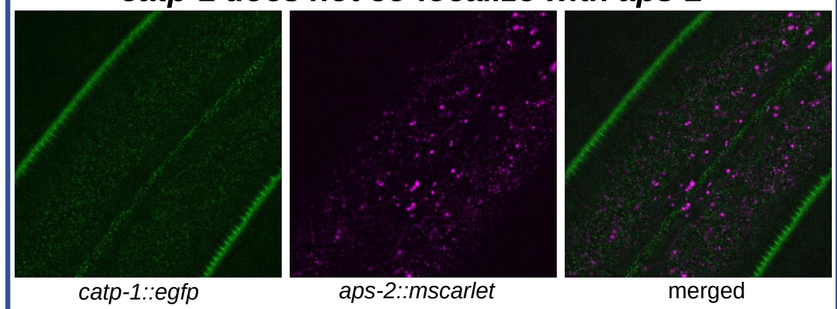




catp-1 is expressed in the epidermis



catp-1 does not co-localize with aps-2



- **Unlike *nekl 2/3*, *catp-1* might not be directly involved with clathrin-mediated endocytosis**

Take-home message

- Loss of *catp-1* induces an L2d-like state and its pump activity is critical for its function.
- L2d is different than normal L2 in terms of sensitivity to loss of *nekl-2/3*.
- Starvation, exposure to dauer pheromone, and growth on heat-killed bacteria, conditions which induce L2d, can suppress *nekl* molting defects.
- Notably, suppression by L2d occurs only with weak or partial loss-of-function (LOF) *nekl* alleles, which typically arrest at the L2/L3 molt, but not stronger LOF alleles, which typically arrest at ~L1/L2.

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