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Overactive EGF signaling suppresses a *C. elegans pnc-1* egg-laying phenotype independent of known signaling mediators.

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Abstract

Nicotinamide recycling is critical to the development and function of *Caenorhabditis elegans*. Excess nicotinamide in a *pnc-1* nicotinamidase mutant causes the necrosis of uv1 and OLQ cells and a highly penetrant egg laying defect. An EGF receptor (*let-23*) gain-of-function mutation suppresses the Egl phenotype in *pnc-1* animals. However, gain-of-function mutations in either of the known downstream mediators, *let-60/ Ras* or *itr-1*, are not sufficient. Phosphatidylcholine synthesis is neither required nor sufficient, in contrast to its role in the *let-23gf* rescue of uv1 necrosis. The mechanism behind the *let-23gf* suppression of the *pnc-1* Egl phenotype is unknown.

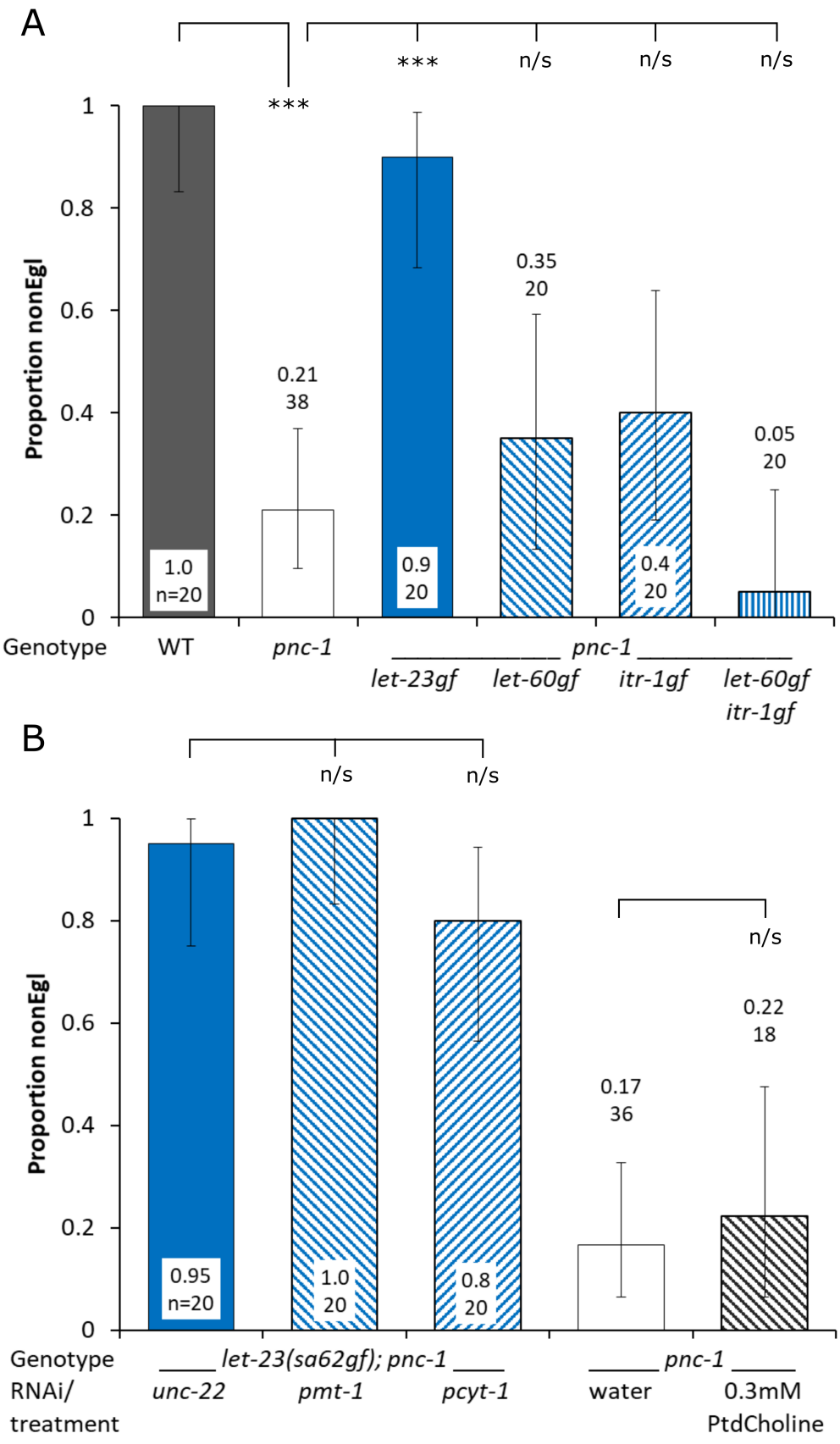
A
***
***
n/s
n/s
n/s
Proportion nonEgl
1
0.8
0.6
0.4
0.2
0
1.0
n=20
0.21
38
0.9
20
0.35
20
0.4
20
0.05
20
Genotype
WT
pnc-1
pnc-1
let-23gf
let-60gf
itr-1gf
let-60gf
itr-1gf
B
n/s
n/s
n/s
Proportion nonEgl
1
0.8
0.6
0.4
0.2
0
0.95
n=20
1.0
20
0.8
20
0.17
36
0.22
18
Genotype
let-23(sa62gf); pnc-1
pnc-1
RNAi/
treatment
unc-22
pmt-1
pcyt-1
water
0.3mM
PtdCholine

Figure 1 : A *let-23(sa62)gf* mutation in *C. elegans* strongly suppresses a *pnc-1(pk9605)* egg-laying (Egl) phenotype, where adult *pnc-1(pk9605)* hermaphrodites form “bags of worms” by four days post L4>adult molt. A) A gain-of-function mutation in *let-23* is sufficient to rescue the *pnc-1* Egl phenotype, but gain-of-function mutations in downstream genes *let-60* and/ or *itr-1* are not. B) Phosphatidylcholine synthesis is neither required nor sufficient for the *let-23(ga62)gf* rescue of the *pnc-1* Egl phenotype. WT = wild-type *C. elegans* N2 Bristol strain. Error bars are 95% confidence intervals, with proportion and sample size in the data labels. Proportions were analysed by pairwise.prop.test in R with Holm p value adjustment; *** and n/s represent p<0.0001 and non-significant, respectively.

Description

NAD^+ is an electron carrier and a co-substrate for NAD^+-dependent enzymes such as poly(ADP-ribose) polymerases (Bouchard *et al.* 2003; Sauve 2008). The byproduct of these enzymatic reactions, nicotinamide (NAM), must be salvaged to maintain a readily available NAD^+ pool. In *Caenorhabditis elegans* the nicotinamidase PNC-1 acts both cell autonomously and non-cell autonomously to convert NAM into nicotinic acid (NA), an NAD^+ precursor in this organism (Huang and Hanna-Rose 2006; Vrablik *et al.* 2009; Crook *et al.* 2014). Loss of PNC-1 function affects NAD^+ pathway metabolites in two ways. It results in an increase in NAM, causing necrosis of OLQ and uv1 cells, and an egg-laying phenotype due to reduced muscle function. It also reduces NAD^+ levels, resulting in gonad developmental delay and a male mating defect (Huang and Hanna-Rose 2006; Vrablik *et al.* 2009; Vrablik *et al.* 2011; Upadhyay *et al.* 2016).

LET-23 is the sole *C. elegans* Epidermal Growth Factor (EGF) receptor and is involved in a range of biological and developmental processes, including vulval development and specification of the uv1 cells (Chang *et al.* 1999; Moghal and Sternberg 2003). A gain-of-function mutation in the extracellular domain, *let-23(sa62)gf*, results in precocious activation of LET-23 independent of its EGF ligand LIN-3 (Katz *et al.* 1996). Overactivation of LET-23 rescues the uv1 cell necrosis phenotype of *pnc-1* loss-of-function mutants, and this rescue requires phosphatidylcholine synthesis (Huang and Hanna-Rose 2006; Crook *et al.* 2016; Crook and Hanna-Rose 2020). We noted that the egg-laying phenotype of *pnc-1* was also ameliorated by overactivation of LET-23 and decided to investigate the mechanism.

To study the role of EGF signaling in the prevention of the egg-laying phenotype we placed individual L4 hermaphrodites on Nematode Growth Medium (NGM) agar plates spotted with *Escherichia coli* OP50. Individual animals were observed after two, three and four days at 20C and scored as non-Egg laying defective (nonEgl) adults or “bags of worms” (Egl), where larvae hatch in the uterus due to a failure to lay eggs. Proportion nonEgl was calculated as the number nonEgl adults/ total number of individuals at day four. All nonEgl adults had laid eggs by day 4. We found that the *pnc-1(pk9605)* loss-of-function allele reduced the proportion of nonEgl adults to 0.21 and that the *let-23(sa62)* gain-of-function (gf) allele in a *pnc-1(pk9605)* background restored that to 0.9 (Fig. 1a). However, gain-of-function mutations in *let-60* or *itr-1*, which mediate signal transduction downstream of *let-23* (Clandinin *et al.* 1998; Chang *et al.* 1999), had no effect on the *pnc-1* egg-laying phenotype (Fig. 1a).

Phosphatidylcholine synthesis is required for *let-23(sa62)gf* mediated rescue of uv1 necrosis and exogenous phosphatidylcholine alone is partially sufficient for uv1 survival (Crook *et al.* 2016). PMT-1 is part of the Sequential Methylation Pathway (SMP) that synthesizes phosphocholine (Brendza *et al.* 2007), and PCYT-1 turns phosphocholine from the Sequential Methylation and Kennedy pathways into CDP-choline, the precursor of phosphatidylcholine (Kennedy and Weiss 1956). To test if phosphatidylcholine synthesis was required for the *let-23(sa62)gf*-mediated rescue of the *pnc-1* egg-laying phenotype we knocked down *pmt-1* or *pcyt-1* by RNAi. *unc-22* (control), *pmt-1* and *pcyt-1* RNAi bacterial cultures were spotted onto NGM plates containing 50 $\mu g.ml^{-1}$ ampicillin and 1 mM IPTG, then individual L4 hermaphrodites were added to each plate and scored as above. We found that neither *pmt-1* nor *pcyt-1* were required for rescue in a *let-23(sa62)gf; pnc-1(pk9605)* background (Fig. 1b). *pmt-1* or *pcyt-1* RNAi did however reduce uv1 cell survival in nonEgl adults in experiments run concurrently with this project (Crook *et al.* 2016), suggesting that RNAi knockdown of the target genes was effective. Next, we wanted to see if phosphatidylcholine alone was sufficient for rescue, as it ameliorates the uv1 necrosis phenotype (Crook *et al.* 2016). We supplemented *pnc-1* animals with 0.3 mM phosphatidylcholine but found no effect on the *pnc-1* egg-laying phenotype (Fig. 1b).

We have shown that overactivation of the *C. elegans let-23* EGF receptor robustly rescues the *pnc-1* egg-laying phenotype, but that gain-of-function mutations in the known downstream signaling mediators *let-60/ Ras* and *itr-1* are not sufficient. Phosphatidylcholine synthesis is not required for the *let-23(sa62)gf* rescue of the egg-laying phenotype and phosphatidylcholine supplementation of *pnc-1* had no significant effect at the sample sizes used, in contrast to the role of phosphatidylcholine in *let-23(sa62)gf* rescue of uv1 necrosis. We have clearly demonstrated another role for *let-23* outside that of growth and development. However, the mechanism by which overactive LET-23 rescues egg-laying in *pnc-1* animals is not

clear. LET-23 may act *via* an as yet unknown pathway that restores uterine or vulval muscle function by either reducing the production of nicotinamide in those tissues or promoting some other compensatory mechanism.

Reagents

Strains:

N2 Bristol

BL5715 *inIs179 (ida-1::gfp)* II

HV560 *inIs179 (ida-1::gfp)* II*; pnc-1(pk9605)* IV

HV639 *inIs179 (ida-1::gfp)* II*; pnc-1(pk9605) let-60(n1046gf) itr-1(sy290gf) unc-24(e138)* IV

HV662 *inIs179 (ida-1::gfp)* II*; pnc-1(pk9605) let-60(n1046gf)* IV

HV663 *inIs179 (ida-1::gfp)* II*; pnc-1(pk9605) itr-1(sy290gf) unc-24(e138)* IV

HV776 *let-23(sa62gf) inIs179(ida-1p::gfp)* II*; pnc-1(pk9605)* IV

The strains used in this study are available from the authors upon request.

We used the following clones from the Ahringer RNAi library: *pmt-1* ZK622.3 II-4G04, *pcyt-1* F08C6.2 X-3N20, *unc-22* ZK617.1 IV-6K06.

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References

Bouchard VJ, Rouleau M, Poirier GG. 2003. PARP-1, a determinant of cell survival in response to DNA damage. Exp Hematol 31: 446-54. PMID: 12829019.

Brendza KM, Haakenson W, Cahoon RE, Hicks LM, Palavalli LH, Chiapelli BJ, McLaird M, McCarter JP, Williams DJ, Hresko MC, Jez JM. 2007. Phosphoethanolamine N-methyltransferase (PMT-1) catalyses the first reaction of a new pathway for phosphocholine biosynthesis in *Caenorhabditis elegans*. Biochem J 404: 439-48. PMID: 17313371.

Chang C, Newman AP, Sternberg PW. 1999. Reciprocal EGF signaling back to the uterus from the induced *C. elegans* vulva coordinates morphogenesis of epithelia. Curr Biol 9: 237-46. PMID: 10074449.

Clandinin TR, DeModena JA, Sternberg PW. 1998. Inositol trisphosphate mediates a RAS-independent response to LET-23 receptor tyrosine kinase activation in *C. elegans*. Cell 92: 523-33. PMID: 9491893.

Crook M, Hanna-Rose W. 2020. Overactive EGF signaling promotes uv1 cell survival via increased phosphatidylcholine levels and suppression of SBP-1. MicroPubl Biol. 10.17912/micropub.biology.000266. PMID: 32666045.

Crook M, Upadhyay A, Ido LJ, Hanna-Rose W. 2016. Epidermal Growth Factor Receptor Cell Survival Signaling Requires Phosphatidylcholine Biosynthesis. G3 (Bethesda) 6: 3533-3540. PMID: 27605519.

Crook M, McReynolds MR, Wang W, Hanna-Rose W. 2014. An NAD(+) biosynthetic pathway enzyme functions cell non-autonomously in *C. elegans* development. Dev Dyn 243: 965-76. PMID: 24753121.

Huang L, Hanna-Rose W. 2006. EGF signaling overcomes a uterine cell death associated with temporal mis-coordination of organogenesis within the *C. elegans* egg-laying apparatus. Dev Biol 300: 599-611. PMID: 16963018.

Katz WS, Lesa GM, Yannoukakos D, Clandinin TR, Schlessinger J, Sternberg PW. 1996. A point mutation in the extracellular domain activates LET-23, the *Caenorhabditis elegans* epidermal growth factor receptor homolog. Mol. Cell. Biol. 16**:** 529-537. PMID: 8552080.

Kennedy EP, Weiss SB. 1956. The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 222: 193-214. PMID: 13366993.

Moghal N, Sternberg PW. 2003. The epidermal growth factor system in *Caenorhabditis elegans*. Exp Cell Res 284: 150-9. PMID: 12648474.

Sauve AA. 2008. NAD+ and vitamin B3: from metabolism to therapies. J Pharmacol Exp Ther 324: 883-93. PMID: 18165311.

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Upadhyay A, Pisupati A, Jegla T, Crook M, Mickolajczyk KJ, Shorey M, Rohan LE, Billings KA, Rolls MM, Hancock WO, Hanna-Rose W. 2016. Nicotinamide is an endogenous agonist for a *C. elegans* TRPV OSM-9 and OCR-4 channel. Nat Commun 7: 13135. PMID: 27731314.

Vrablik TL, Wang W, Upadhyay A, Hanna-Rose W. 2011. Muscle type-specific responses to NAD+ salvage biosynthesis promote muscle function in *Caenorhabditis elegans*. Dev Biol 349: 387-94. PMID: 21092737.

Vrablik TL, Huang L, Lange SE, Hanna-Rose W. 2009. Nicotinamidase modulation of NAD+ biosynthesis and nicotinamide levels separately affect reproductive development and cell survival in *C. elegans*. Development 136: 3637-46. PMID: 19820182.

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