Characterization of Erinacine-A

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Lucky RMP4

Shimizu et al. found the significance of chemical signaling in axon regeneration in response to injury. This process mainly occurred in peripheral nervous system. In *Caenorhabditis elegans*, the axon regeneration occurred through EGL-30 Gqα-JNK MAP Kinase cascade which is an intrinsic regulator of axon regeneration. The objective was to investigate the process through which this cascade actually spark of. They explained this cascade that genes involving SRG-36 and SRG-37, act as upstream G-Protein coupled receptors (GPCRs). It activate the EGL-30 for dauer inducing ascaroside (ascr # 5) which is the pheromone secreted by *C.elegans*.

In the first step, on receiving the signal, GPCRs activate the EGL-30 Gqα which triggered EGL-8 PLCβ. It converts phosphatidylinositol bisphosphate [PI (4,5) P2] to DAG. DAG stimulated TPA-1 PKC, which activated the JNK pathway, which promotes axon regeneration mostly in young adults. KGB-1 JNK was inactivated by the MAPK phosphatase VHP-1.

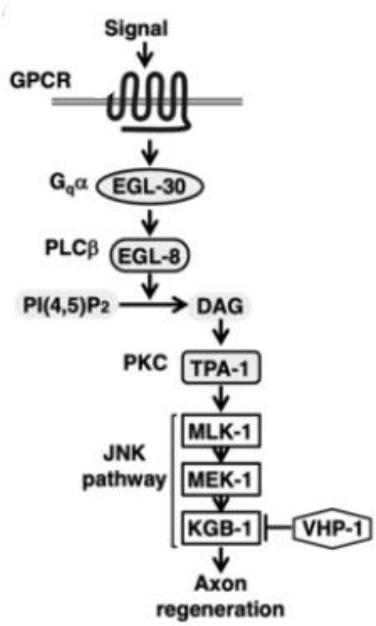

Fig 1. EGL-30 pathway regulation axon regeneration

The next step was to determine which ascaroside controls axon regrowth. Ascarosides divided into two categories in *C. elegans*: ω -ascarosides and (ω-1)-ascarosides were two types of ascarosides. They were produced through two β-oxidation routes, each involving different ACOX enzymes. Percentage of axons that started regenerating 24 hours following laser surgery in young adults. The number of axons inspected displayed here

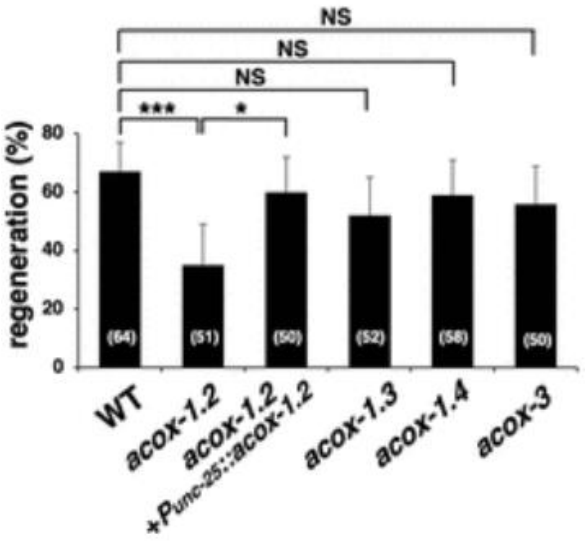

Fig 2. ACOX 1.2 required for axon regeneration

They found out that ACOX 1.2 were causing the mutated reduced axon regeneration while other ACOX causing mutated and disrupted induced axon regeneration.

They investigated the effect of synthetic ascr#5 on axon regeneration since ACOX-1.2 influences the formation of an ascaroside with a short side chain. In diagram (Fig 3) shows that animals entering the dauer stage (A). The percentage of dauers showed in B. Motor neurons in animals after 24h surgery showed in C. They first gave ascr#5 to adult-stage ACOX-1.2 mutants and then measured the frequency of axon regrowth. When injected from an embryo, ascr#5 showed to be adequate to promote dauer formation in ACOX-1.2(gk386052) mutant larvae, and it dramatically corrected the axon regeneration deficit in ACOX-1.2(gk386052) mutants when introduced at the young adult stage. Ascr#5 involved in axon regeneration, based on these findings. D represents the percentage of axon regeneration.

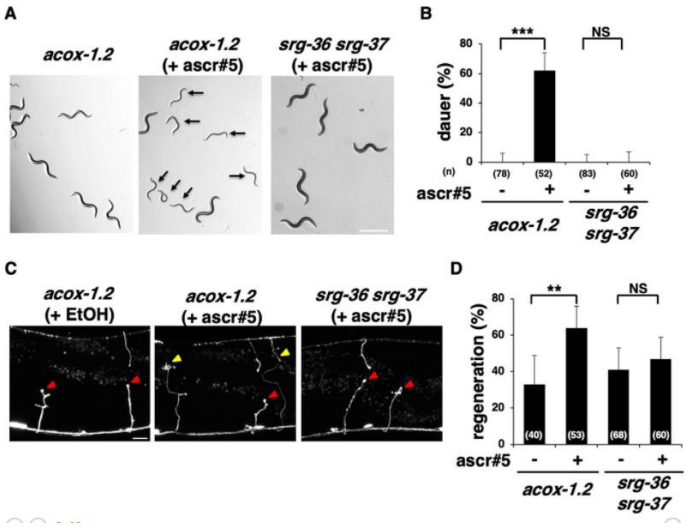

Fig 3. Effect of Ascr#5 on axon regeneration

Motor neurons (magenta) have ventral cell bodies and dorsally extending axonal commissures. Touch neurons (grey) have long axons that run parallel to the long body axis and practically perpendicular to the axons of D-type motor neurons. Percentages of D-type motor axons that started regenerating 24 hours following laser surgery in young adults. The touch neuron of acox-1.2 mutants expressing the acox-1.2 gene is indicated in blue.

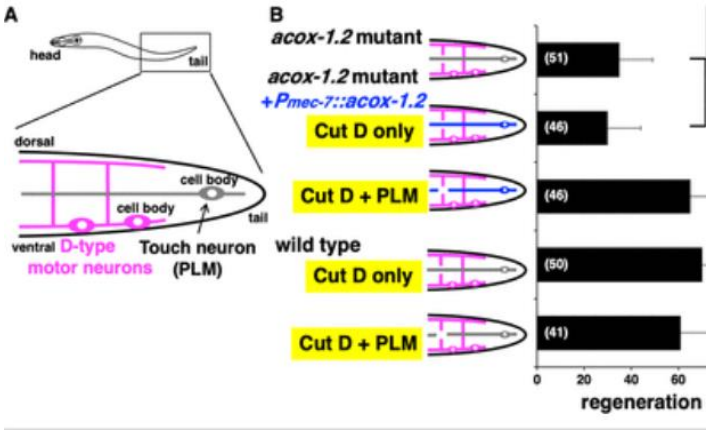

Fig4. Effect of ACOX 1.2 on regeneration of motor axon

The srg-36 and srg-37 genes are members of the nematode-specific GPCR family that encode ascr#5 receptors. As a result, we investigated whether these GPCRs are involved in axon regeneration. The kyIR95 allele deletes both the srg-36 and srg-37 genes because they are close in the genome. We discovered that the frequency of axon regeneration was reduced in srg-36 and srg-37 at the young adult stage (kyIR95). SRG-36 and SRG-37 work together to support dauer development in response to ascr#5.

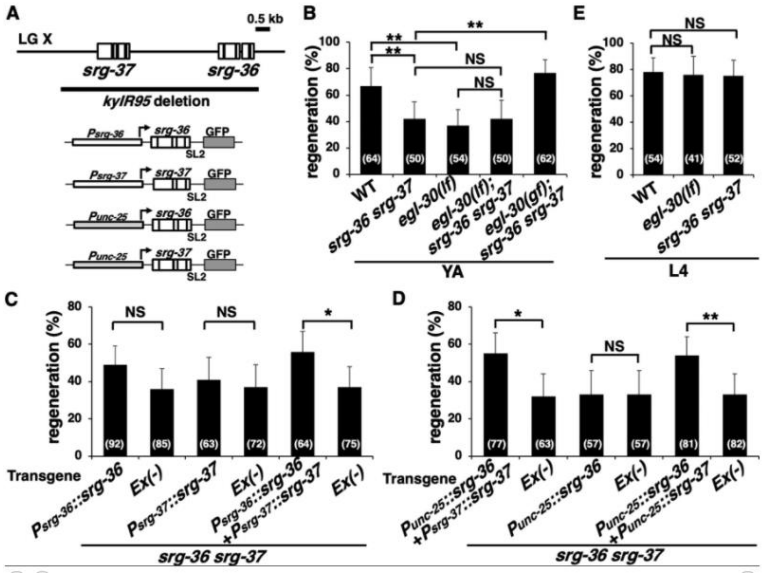

Fig 5. SRG-36 and SRG-37 are involved in axon regeneration

Table 1. Raw data for genotypes tested by axotomy.

Strain	Genotype (juIs76 background)	Stage	Animals, n	Axons, n	Regenerations, n (% of total)	p value	Compared with
KU501	wild type	YA	24	68	42 (62%)		
KU1549	*srx-16(tm7585)*	YA	19	53	32 (60%)	0.8493	KU501
KU501	wild type	YA	24	64	43 (67%)		
KU1550	*acox-1.1(ok2257)*	YA	32	53	22 (42%)	0.0086	KU501
KU1551	acox-1.1(ok2257); Ex[Pacox-1.1::acox-1.1]	YA	23	52	38 (73%)	0.0015	KU1550
KU456	egl-30(lf)	YA	18	50	20 (40%)		
KU1571	acox-1.1(ok2257); egl-30(lf)	YA	23	56	27 (48%)	0.5645	KU1550
KU457	egl-30(gf)	YA	13	30	21 (70%)		
KU1572	acox-1.1(ok2257); egl-30(gf)	YA	33	50	34 (68%)	0.0099	KU1550

Shimizu et al. discovered that ascaroside signaling modulates neural functions in *C.elegans* and the lack of ascaroside synthesis inhibits axon regrowth in particular. Furthermore, the EGL-30 Gqα signaling pathway regulates axon regeneration by detecting ascaroside via GPCRs. By GPCRs NPR-19/NPR-32, AEA activate the Goα protein GOA-1, which antagonizes EGL-30 and prevents axon regrowth. The balance of stimulatory (ascr#5) and inhibitory (AEA) chemical signals transduced via G protein signaling pathways determines axon regeneration in *C.elegans.*

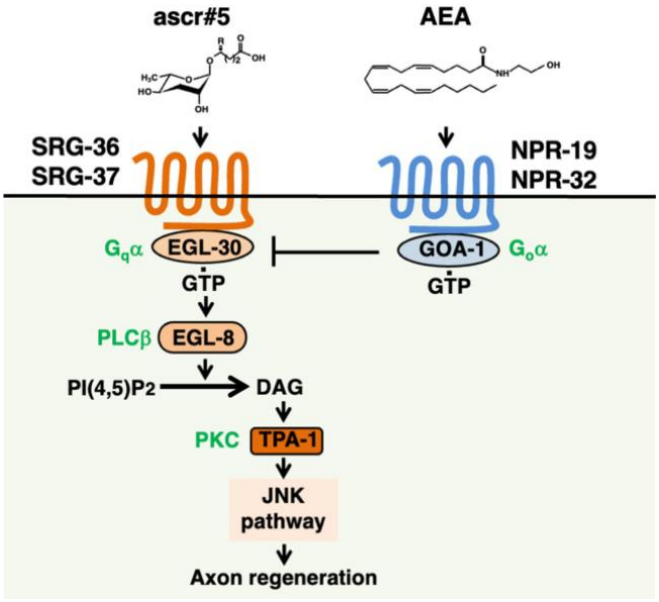

Fig 6. A schematic model for regulating axon regeneration by chemical signaling pathways

The methods used in the article have some similarities and differences from our lab module, primarily by using microbial diagnostic techniques to compare and contrast culture-based microbial identifications vs. molecular-based identifications. Microbial diagnostic techniques used in same way as in our lab but we don't use axotomy in our lab. Like ACOX, we did not use it in our lab module, we used ACOX-1.2, and it used to find molecular-based differentiation and identifications in all of it. Shimizu et al. used expression pattern data graphing and recorded every point like in our lab module with Dr. Griffin.

As you can see that in these methods, materials obtained from different sources which are integrated. In biochemistry and genetics, the GPCRs used in different process through which datasets could be easily integrated. We used a genetic method to inactivate the mTOR kinase, which allowed us to regenerate axons. If mTOR is regulated by melanopsin via the classic GPCR signaling pathway.

Literature Cited

Shimizu, T., Sugiura, K., Sakai, Y., Dar, A. R., Butcher, R. A., Matsumoto, K., & Hisamoto, N. (2022). Chemical signaling regulates axon regeneration via the GPCR–Gqα pathway in Caenorhabditis elegans. *Journal of Neuroscience*, *42*(5), 720-730.