Cell-non-autonomous signaling from AWC olfactory neurons regulates immune response in *Caenorhabditis elegans*

Preview for "Foster *et al*. (2020) Innate Immunity in the *C. elegans* Intestine Is Programmed by a Neuronal Regulator of AWC Olfactory Neuron Development. Cell Reports, "

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Understanding how environmental cues regulate animal survival and identifying the key molecular players that modulate animal chemosensation have been challenging biological questions. In this paper, the authors utilize the powerful model system *Caenorhabditis elegans (C. elegans)* to investigate the neuro-immune crosstalk and characterize the underlying mechanism that links chemosensation with intestinal immune control. Although chemosensation is highly divergent between mammals and *C. elegans*, some regulatory and behavioral mechanisms appear to be more conserved.

The anatomy and developmental lineage of every *C. elegans* neuron have been fully mapped. Out of the total 302 neurons, a group of 32 ciliated cells named chemosensory neurons, evaluates the environment and modulates worm physiological response and behaviors (Ward. et al 1975). These behaviors include differentiation of nutritious bacteria from pathogens, chemotaxis, changes in overall motility, and entry/exit cycle from the alternative dauer developmental stage (Culotti and Russell, 1978; Lewis and Hodgkin, 1977, Hu et al., 2007). Among the *C. elegans* chemosensory neurons, the olfactory system is presumed to be crucial in detecting volatile odors, and consists of three pairs of olfactory neurons including AWA, AWB and AWC. Unlike the other olfactory neurons, AWC neurons are bilaterally asymmetric (Wes and Bargmann, 2001). Each animal has one AWC^{ON} and one AWC^{OFF}, stochastically established during late embryogenesis via the signaling pathway mediated by the gap junction network and the calcium-activated MAP kinase cascade, and their neuro-identities are maintained during adulthood (Chuang et al., 2007). Both neurons have similar morphology, but distinct functions and olfactory receptor gene expression. AWC^{ON} senses the odor 2-butanone by the expression of the G- protein -coupled receptor (GPCR) gene, *str-2,* resulting in either attractive or repulsive behavior, while AWC^{OFF} responds to 2,3-pentanedione through the GPCR *srsx-3* resulting in attractive behavior. In a screen looking for AWC symmetry regulators, *olrn-1(ky626)* mutation was isolated as it results in two AWC^{OFF} neurons and no AWC^{ON} neurons. In brief, OLRN-1 activation inhibits the kinase pathway consisting of UNC-43 (CaMKII)/NSY-1/SEK-1 cascade in AWC^{ON} cell-autonomously. Previously, AWC bilateral asymmetry mechanism was shown to be crucial in defining two neuron identities with distinct olfactory specificity, which results in attractive or repulsive behaviors. However, recent evidences pointed out how the downstream circuits of those two neurons are involved in regulating metabolic pathways in peripheral tissues as well, including proteostasis (Finger at al, 2019) and lipid metabolism (Mutlu et. al, 2020). The role of AWC or any olfactory neurons in modulating innate immune activity was unknown, until this study by Foster *et al.* that found an interesting link between OLRN-1 and the intestinal immune response pathways.

C. elegans lives in the soil with a diversity of pathogens that can overcome innate immune response and infect different tissues resulting eventually in the worm death. Although worms lack specialized immune cells and adaptive immunity, they still provide a robust platform to investigate aspects of bacterial pathogenesis. Pathways associated in anti-microbial defense include mitogen-activated protein kinase (MAPK) signaling cascades which consist of p38 MAPK signaling, c-Jun N-terminal kinase (JNK) signaling and ERK signaling (Nicholas, H.R., and Hodgkin, J, 2004, Mizuno et al., 2004). In *C. elegans*, *pmk-1* encodes a MAPK, *sek-1* encodes a MAPK kinase (MAPKK), and *nsy-1* encodes a MAPK kinase kinase (MAPKKK), and these three proteins constitute the core of p38 MAPK signaling pathway, required for the control of both pathogen and stress responses (Kim et al., 2002; Kim et al., 2004, Troemel et al., 2006). Follow-up works have demonstrated the implication of this pathway is host response upon bacterial, fungal or viral infection in the intestine and epidermis. SKN-1/Nrf and ATF-7/bZIP usually act as downstream targets for PMK-1 in the regulation of different biological events (van der Hoeven R et. al 2011, Shivers RP et. al, 2010). Overall, p38/PMK-1 MAPK cascade and its downstream transcriptional machinery mediated by SKN-1 and ATF-7 are the master regulators of innate immunity in *C. elegans*.

The mechanisms by which pathogenic bacteria modulate innate immunity in distinct aspects of physiology and behavior are the study goals of many research labs, and more interest is raising on how the nervous system influences these mechanisms. Neuronal-expressed genes proposed to be involved in innate immunity include *ins-7,* that inhibit intestinal DAF-16 after being released from dense core vesicles (DVCs) of several sensory neurons, and *npr-1* which encodes a GPCR. Mutations in both genes are associated with an induced pathogen susceptibility. On the other hand, mutations in *octr-1* gene, encoding for the octopamine receptor, negatively regulate the non-canonical UPR by conferring an increased resistance to infection (Styer et al., 2008; Reddy et al., 2009, Sun et al., 2011). In addition, the expression of *tph-1,* encoding the enzyme necessary for serotonin biosynthesis in ADF neurons, is induced upon pathogenic infections (Anderson et al., 2013, Zhang et al., 2005). All of those examples show that the field is starting to scratch the surface of the neuro-immune crosstalk, pointing out that more investigations are further required to clarify the specific mechanisms.

In this paper, Foster *et al.* first conducted a forward genetic screen in *C. elegans* to identify recessive mutations that lead to constitutive activation of the innate immunity reporter, *Pirg-4::GFP,* which is under the control of p38/ PMK-1 MAPK pathway. They found two different alleles in *olrn-1* gene: *ums9* which is a nonsense mutation, and *ums11* which is a mutation in a splice acceptor site, that activate *Pirg-4::GFP* reporter. They performed most of the further experiments with the *ums9* allele, demonstrating that both innate immune activation markers, *irg-4* and *irg-5,* are transcriptionally upregulated in *olrn-1* mutants. Furthermore, these mutants exhibit increased resistance to pathogenic bacteria, *P. aeruginosa,* and expressing *olrn-1* under the control of its endogenous promoter can rescue these phenotypes. Interestingly, although OLRN-1 is an important protein involved in olfactory neuron development, the increased resistance to *P. aeruginosa* infection in *olrn-1* mutants is not due to enhanced avoidance of *P. aeruginosa* as these mutant animals occupy the bacterial lawn as much as their wild-type controls. The authors demonstrated less accumulation of *P. aeruginosa* in *olrn-1* mutants' intestine despite unchanged pharyngeal pumping, suggesting enhanced bacterial clearance from the gut and thus, increased resistance to pathogenic bacteria. Those mutants also showed a slower development rate and smaller brood size suggesting that in wild-type worms *olrn-1* functions to prevent the deleterious effects of unnecessary immune activation on development and reproduction.

The authors then performed RNA-sequencing in *olrn-1* mutants to dissect the transcriptional changes underlying the enhanced resistance to *P. aeruginosa* infection. They identified more than 500 genes that are upregulated in *olrn-1* mutants when compared to wild-type, and about one third of them overlapped with genes regulated by the p38/PMK-1 MAPK pathway. Accordingly, *olrn-1* mutants have increased levels of active phosphorylated PMK-1 and inactivation of the pathway components, such as *pmk-1* and *tir-1*, suppresses the transcriptional increase in innate immune response markers *irg-4* and *irg-5*, as well as the pathogen-resistance phenotype conferred by *olrn-1* mutants. To sum up, p38/PMK-1 MAPK pathway seems to orchestrate the immunity response downstream of *olrn-1*.

Since *olrn-1* is not expressed in the intestinal epithelium, the authors then set out to find where it functions to regulate intestinal immune response. They found that expressing *olrn-1* under chemosensory neurons specific promoter *odr-3*, suppresses the increase in immune response genes, the developmental defect and pathogen resistance phenotypes observed in *olrn-1* mutants. Additionally, they showed that overexpressing *pmk-1* using intestinal promoter is sufficient to restore pathogen resistance and immune response activation in *olrn-1; pmk-1* double mutants. Thus, *olrn-1* acts in the *C. elegans* chemosensory neurons to signal p38/PMK-1 MAPK pathway in the intestine to regulate immune response and pathogen resistance in a cell-non-autonomous manner.

The mechanisms underlying the immune response to pathogen infections can be variable and dependent on the worms's susceptibility. *C. elegans* is an attractive model for identifying endogenous regulators of known described pathways or novel modulators. More evidence suggests the involvement of the nervous system in regulating the immune system to maintain a systemic homeostasis as previously mentioned. However, the detailed mechanisms of these modulations remain largely unsolved. In this paper, Foster *et al.* uncovered a new role for the gene *olrn-1* that functions cell-non-autonomously in AWC neurons to suppress the p38/PMK-1 MAPK pathway in the intestine. This discovery introduces a new role for OLRN-1 previously reported to be crucial on AWC asymmetry specification. Although this pathway is particularly intriguing, different questions remain still unsolved.

First of all, the authors tested only one pathogen, but it is interesting to know whether this mechanism is solely due to *P. aeruginosa* or it is shared by other bacterial pathogens such as *Staphylococcus aureus* or viral and fungal infections. Second, although *olrn-1* mutants do not exhibit enhanced avoidance of *P. aeruginosa*, this still may not rule out that olfactory receptor activities in AWC neurons are required for innate immune activity. An intriguing experiment could be to expose the worms to solely the smell of *P. aeruginosa* and observe any differences in innate immune reporters. If there are changes, it would be interesting to understand the identity and the modality, by which specific volatile molecules from *P. aeruginosa*, that are sensed by AWC neurons, result in the immune response activation in the intestine. Indeed, the receptor(s) on AWC neurons responding to a specific class of chemical cues mediating this downstream response are unknown. Third, the cell-non-autonomous signaling could be mediated by neurotransmitters, neuropeptides, and/or neurohormones. It will be fascinating to test their involvement in future investigations, which will discover the downstream mediators of neuron-intestine communication in innate immune response. Knowing the identity of the neuropeptides or the neurotransmitters may lead to chemical screens aiming on delineating exogenous antagonistic or agonistic drugs that can be used as neuronal interventions to control pathogen infections. Finally, not much is known about the OLRN-1 protein structure and whether it employs similar mechanisms to activate the same MAPK cascade in AWC

neuron and the intestine. Those future studies will help uncover the upstream regulators of p38 MAPK PMK-1 pathway and open new avenues on understanding the effects on sensory neuron activity during immune response induction.

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