Molecular Physiology

# Worm sheds light on mammalian cold sensation

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Temperature sensing is vital for living things. Noxious coldness could evoke severe tissue damage or even life-threatening issue in animals. Therefore, to cope with cold temperature, organisms have evolved specific physiological and behavioural responses, which rely on the function of the cold-sensing receptor [1].

Understanding about the molecular identity of the cold-sensing receptors remains unsatisfactory. In 2007, two independent research groups had reported that one member of the TRP family, the TRPM8, serves as a cool sensor in mice with an activation threshold of 26℃ [4],[5]. In addition, some other TRP channels have been reported to be involved in cold sensation in other species like TRPA1, but their role in thermal sensation appear to be complicated. Mammalian TRPA1 was firstly cloned as a receptor for noxious cold with a cold activation threshold at around 17℃ in vitro. However, behavioural study found that TRPA1 might function as a polymodal sensor affecting the sensation of mice to heat rather than cold. So far, the molecular identities of cold receptors still remain elusive. For instance, human could absolutely discriminate 26 degree from degrees that lower than 26 and TRPM8 mutant mice retain noxious cold evoked behaviours, suggesting that there are some novel cold receptors remain to be discovered [4],[5].

To fill the blank in such area, Shawn Xu and his lab members used *C.elegans*, a model animal widely used in sensory biology [7], to study the molecular identity of the cold receptor. Their previous result showed that the gut of the worm could robustly response to cold [3]. To screen for novel cold sensing receptors, they conducted random mutagenesis and performed functional imaging with a RT-PCR thermal cycler. After screening for more than 30,000 strains, they obtained a mutant worm with strong deficit in cold sensing. Whole-genome sequencing maps the mutation to the locus of *glr-3*, which encodes a kainite-type glutamate receptor. They looked into the expression profile of *glr-3* and found that it is expressed in the intestine and a sensory neuron ASER. Specifically reintroducing *glr-3* in ASER rescued the cold-sensing deficit in *glr-3* null mutant. Following results suggest that ASER is a cold-sensitive neuron and the GLR-3 has dual independent functions as the cold sensor and the glutamate receptor.

To further verify that GLR-3 is a cold sensor, they ectopically expressed the GLR-3 in

body wall muscle cells, conferring the cold-insensitive cells with cold sensitivity. Moreover, transfection of GLR-3 in mammalian CHO, COS-7 and Hela cell lines came out with the same result.

Next, they wanted to know whether the cold sensitivity of GLR-3 is evolutionally conserved. GluK2 is the ortholog of GLR-3 in mice and is also a kainate-type glutamate receptor. Expression of GluK2 in *glr-3* mutant worm rescued the calcium response in ASER neuron and the cold-avoidance behaviour.

Further characterization of GLR-3/GluK2 demonstrated that the activation thresholds of them are around 18 ℃ and their cold sensitivities are independent of its channel activity. By utilizing several G protein inhibitors, they found that GLR-3/GluK2 rely on Gi/o protein signalling and function as metabotropic receptors to transmit cold sensation.

Next, they tested whether the GluK2 is expressed in the DRGs in mice. They adopted RNAscope assay, and detected GluK2 mRNA in the DRG neurons. Knocking down GluK2 expression did not affect the response of DRGs to cool temperatures, while their responses to noxious cold temperatures are severely disrupted.

Moreover, in their recent study, they generated the GluK2 KO mice. The phenotype of the KO mice recapitulates what they have discovered in C.elegans. GluK2 KO mice retain their ability to sense mechanical, heat and even cool stimuli, while their cold sensation is severely defective [6].

To summarize, they identifies a novel cold receptor by conducting screening in *C.elegans*. Compared with previous attempts in screening new cold sensor by backward screening with specific target genes [1], their excellent work also reinforces the powerfulness and feasibility of unbiased forward screening with the tiny and elegant model organism.

# References

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