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Pheromone Receptors Need "Escorts"

Howard Hughes Medical Institute (HHMI) researchers and their colleagues have discovered that escort molecules are required to usher pheromone receptors to the surface of sensory neurons where they are needed to translate chemical cues.

In an interesting twist, the researchers found that the escort molecules belong to a family of proteins, called the major histocompatibility complex (MHC), which plays an important role in the immune system. The researchers speculate that in addition to being escort molecules, the MHC proteins might actively modulate an animal's response to pheromones. Modulation of pheromone activity might aid in the recognition of other animals.

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for the mechanism of pheromone detection."**

— **Catherine Dulac**

The studies in mice add “a novel and unexpected layer of complexity to the process of pheromone detection,” the researchers wrote in an article published in the March 7, 2003, issue of the journal *Cell*. The article was published online on March 4, 2003. The findings also suggest that, similarly, escort molecules, although of a different kind, may be important in smell and taste receptors.

HHMI investigators Catherine Dulac at Harvard University and Kirsten Fischer Lindahl at the University of Texas Southwestern Medical Center led the research teams that collaborated on the studies.

The pheromone communication system, which is found in a wide range of mammals, involves detection of chemical odorants released by animals. Detection of pheromones takes place in a specialized structure, called the vomeronasal organ (VNO). Although the VNO resides in the nasal cavity, the pheromone sensory system is distinct from the sense of smell, as are the chemical receptors involved. In animals possessing a pheromone sensory system—including mice, dogs, cats and elephants—the system governs a range of genetically preprogrammed mating, social ranking, maternal, and territorial defense behaviors.

According to Dulac, untangling the complexity of the pheromone system has been a daunting task for researchers. “For example, if you compare the

number of receptors, which ranges between two hundred and four hundred, and the number of behaviors they trigger, which ranges up to a dozen, there is a huge discrepancy,” she said. “So, you can either postulate that there are hundreds of behaviors not yet described, or more likely a given behavior involves the activation of multiple receptors.”

To begin sorting out the functions of the multitude of pheromone receptors, Dulac and her colleagues decided to study a subpopulation of sensory neurons in the VNO. The researchers knew they could distinguish neurons that expressed one family of receptors, called V2R, from another family, called V1R, so they used a technique called “subtractive differential screening of single cell cDNA libraries” to compare the genes that are switched on in neurons bearing the two different types of pheromone receptor.

Their comparisons—as well as sequencing of the discovered genes and searches of gene databases—yielded evidence that two families of MHC genes called M1 and M10 were preferentially activated in these neurons, said Dulac. The finding was surprising because MHC proteins commonly function on the surface of immune cells to present foreign proteins to the immune system to trigger destruction of invading pathogens. The M10 proteins found in the VNO were different in structure and obviously in function from other such molecules.

Dulac's and Fischer Lindahl's research teams set out to explore the structure and function of the M10 type of MHC proteins that the genes produced. Their studies revealed that the MHC genes were exclusively expressed in the VNO and in no other tissue. And within the VNO, they were only expressed in V2R-positive VNO neurons. The researchers observed that each type of V2R receptor apparently had a specific type of M10 protein associated with it.

“So, we found that there is a population of neurons in which each neuron expresses only one type of pheromone receptor gene,” said Dulac. “We also were able to show that these individual neurons express only one type of M10 gene. This told us there was some type of logic in that association.”

Additional studies showed that the M10 gene was activated only after birth, which suggested that M10 only functions in pheromone sensing in the adult animal. The researchers showed that the M10 proteins, like the pheromone receptor proteins, were localized to the tips of neurons, called dendrites, where chemical reception takes place.

Their studies showed that the M10 protein, as well as an “accessory” molecule, beta2-microglobulin, that accompanies such M10 proteins, directly interacted with the pheromone receptor molecule. Finally, they found that the M10 protein and its accessory molecule were necessary for the pheromone receptor to reach the surface of the neuron.

The researchers also explored the effects of knocking out the key M10 accessory molecule, beta2-microglobulin, in mice. They found that the beta2-microglobulin-knockout male mice lacked V2R receptors in their

VNOs and also failed to exhibit the normal aggressive behavior toward other males.

According to Dulac, the scientists' findings show that M10 plays a crucial escort role for pheromone receptors, but it might well have a modulatory role. "The fact that the receptor needs M10 to go to the surface, doesn't prove it's the exclusive role of the protein," she said. "We do know that each time researchers have described an association between a particular receptor and another molecule at the cell surface, it has always been the case that the specificity of the original receptor is being modified. So, we have found new molecular players, if you will, in the game of pheromone detection."

Dulac said that the newly discovered MHC molecule involvement could have important implications for understanding the pheromone system. "This association opens all sorts of possibilities for the mechanism of pheromone detection, because we know the animal can modulate its behavior according to the sex of another animal, its genetic background and the elements that make up the identity of an animal."

The discovery of escort molecules in the pheromone system could have implications for understanding the molecular machinery involved in smell and taste, Dulac said. Researchers knew that in cell cultures, olfactory and taste receptors seemed to require additional molecules to reach the surfaces of cells. That observation hints at the need for still-undiscovered escort molecules for those receptors, as well as for the VIR-expressing class of pheromone receptors, she said.