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A Developing Nerve's Target May Give Final Stamp on Identity

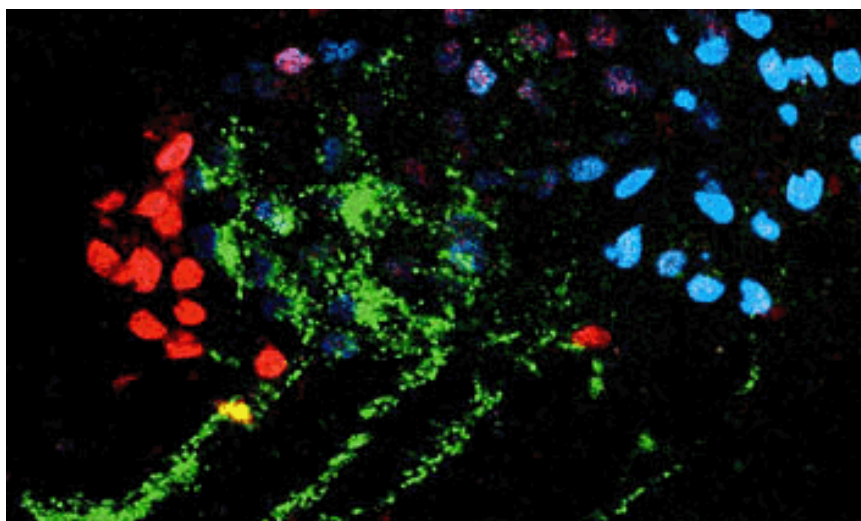


Image Title: The image shows expression of the ETS protein PEA3 (red) and the LIM homeodomain protein Isl1 (blue) in hindlimb level motor neurons. External femorotibialis motor neurons have been labeled with HRP (green). The HRP-labeled motor neurons express neither PEA3 or Isl1. - Ira Schieren and Thomas Jessell/HHMI at Columbia University

An elaborately orchestrated genetic program transforms generic neural cells in vertebrate embryos into motor neurons that control specific muscular movements. Now, researchers have found that one source of the final defining signal in this developmental process comes from cells in the developing limb, the target region of the motor neurons.

Thomas M. Jessell and David J. Anderson, HHMI investigators at Columbia University and California Institute of Technology, and their colleagues have found that the motor and sensory neurons that constitute a nerve circuit express the same genes, transcription factors of the ETS family. *ETS* genes define clusters of developing motor neurons called motor pools that connect with specific embryonic muscles and receive inputs from specific sets of sensory neurons.

One surprise finding of this work is that sensory neurons, the return wires that relay back to the central nervous system information about what muscles are doing, contain the same ETS proteins as their functionally related target motor neurons. This result was unexpected, says Jessell, because sensory neurons develop essentially independently from motor neurons-yet, ultimately, ETS proteins match within a completed circuit.

"One way to achieve that match might be to provide a coordinating signal from the limb," explains Jessell. In other words, the target itself might play a role in constructing the proper connections within the spinal cord.

Indeed, Jessell, Anderson and their colleagues demonstrated that removing limb buds-the small region of tissue that eventually becomes wings and legs-from chick embryos before *ETS* expression begins will yield motor and sensory neurons that lack their final *ETS* identities. As soon as ETS proteins appear, however, neither motor nor sensory neurons need their target to continue developing properly.

The results of these studies, which were performed together with the laboratories of Cynthia Lance-Jones at the University of Pittsburgh School of Medicine and Tetsuichiro Saito at Japan's National Institute of Genetics, are reported in the October 30, 1998, issue of the journal *Cell*.

The article in *Cell* is the latest in a series of experiments in Jessell's laboratory that elucidate the molecular mechanisms of nervous system development. While the developmental chronology of the nervous system's biological structures is largely known, the chemical processes that construct them cell by cell-as fast as 250,000 cells per minute-are only now coming to light.

"The number of different neuronal cell types is thought to exceed 100, far more than any other organ of the body," says Jessell. Their differentiation proceeds in five basic steps.

First, a set of undifferentiated cells in the embryo's outermost layer is tapped to become neural progenitor cells. These generic nerve cells next separate into broad populations of immature neurons and supporting structures called glial cells. Third, the immature neurons migrate to their final position. From there they send axons, the leading edge of a developing neuron, through the growing tissue, to seek their proper muscle target on a limb. Finally, specific motor and sensory neurons set up the connections, or synapses, that form a complete circuit between muscle and nerve center.

The sequence begins in humans at about the third week of gestation. Deep within the early embryo, a thin sheet of cells curls around itself to form a structure called the neural tube. Ultimately, the neural tube will form the scaffold for the brain and spinal cord.

The chemical messenger that determines which cells will become motor neurons comes from a rod of cells beneath the neural tube called the notochord. This messenger is the sonic hedgehog protein-named for the

bristly appearance of fruit flies that lack the related *hedgehog* gene.

Remarkably, hedgehog's effects depend on how much of the protein reaches a particular set of cells. Two parallel bands of cells at an intermediate distance from the notochord receive a dose of hedgehog, which programs these cells to become motor neurons. Jessell's group reported previously that hedgehog's primary function within these cells is to activate a gene called MNR2.

Thus, a cell's position within the embryo largely determines its mature fate. However, developmental biologists have realized that neural tube cells at the same location can differentiate further. "It turns out that the timing of when a motor neuron is born is another critical determinant in making further distinctions," Jessell says.

Jessell and his associate Shanthini Sockanathan discovered that early-born motor neurons secrete retinoic acid, an active metabolite of vitamin A. Late-born motor neurons must migrate through these neurons to arrive at their final position. In the August 21, 1998, issue of *Cell*, Jessell and Sockanathan reported that retinoic acid directs the late-born motor neurons along a different pathway of differentiation. The two populations become the wiring for what are called ventral and dorsal limb muscles, such as those on the top and bottom of the thigh.

The new work may provide the final step of functional fine-tuning. "The nervous system has to coordinate the release and contraction of a variety of muscles just to take a simple step. We've known that," says Jessell, "but nobody has provided any molecular correlation to that."

So what is the chemical signal that appears to emanate from the neurons' muscle target? That, Jessell says, is what the next step of his research is designed to answer.