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Understanding the Developmental Code of the Spinal Cord

Researchers have discovered some of the basic control signals that govern the organization of the spinal cord in the developing embryo—findings that quite likely will apply to the brain as well. Insights such as these are yielding new information about an underlying code involving homeobox, or *Hox*, regulatory genes that orchestrate spinal cord development.

The researchers, led by Howard Hughes Medical Institute (HHMI) investigator Thomas Jessell at Columbia University, published their findings in the October 30, 2003, issue of the journal *Nature*. Jessell collaborated on the studies with HHMI research associate Jeremy Dasen at Columbia and Jeh-Ping Liu at the University of Virginia School of Medicine.

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— Thomas M. Jessell

The identity and function of the control signals that govern the formation of neuronal columns in the brain and spinal cord are two of the big questions facing developmental neurobiologists. Columns contain bundles of neurons that are grouped according to function. Despite their localized groupings, the influence of columns on connectivity may be felt at quite a distance because the axons extending from neurons innervate other areas of the body.

"In the spinal cord, for example, the grouping of motor neurons into columns predicts whether the motor neurons send axons to innervate limb muscles, or whether they send axons to axial muscles," Jessell explained. "So, columns represent major functional subdivisions of neurons that ensure that all of the peripheral muscles get innervated by a distinct set of motor neurons."

In earlier studies, the researchers found that transcription factors of the homeodomain family govern how motor neurons acquire a general identity that distinguishes them from adjacent sets of interneurons—through a signaling process that operates along the dorsoventral (front-to-back) axis of

the spinal cord.

That earlier work on the dorsoventral determination of motor neurons in the spinal cord also revealed that a concentration gradient of a signaling molecule known as Sonic hedgehog initially determines which homeodomain protein will be switched on in the motor neuron—a key decision that leads to the establishment of a developmental identity for the neuron. Those neurons that “see” an intermediate concentration of the hedgehog signaling molecule switch on homeodomain proteins that specify motor neuron fate—distinguishing them from neurons that are exposed to a lower hedgehog concentration.

The researchers decided to apply what they had learned from their earlier studies to see how, or whether, a similar gradient signaling system controlled the expression of *Hox* genes along the front-to-rear—or anterior to posterior—length of the developing spinal cord. They also sought to find out whether those *Hox* genes, in turn, triggered the developing motor neurons to assume a specific columnar identity.

“A simple way of viewing this problem is that there must be genes arrayed along the anterior-posterior axis of the spinal cord that somehow are relevant to the emergence of motor neuron columnar identity,” said Jessell. “And despite an enormous amount of work over the last ten to fifteen years, we didn't know very much about those genes; in fact, the only set of genes that had shown any sort of positional differences along the anterior/posterior axis of the spinal cord, were *Hox* genes.”

The earlier studies by Jessell and Liu using an *in vitro* culture system suggested how individual members of the group, called *Hox-c* genes, were expressed at different positions along the length of the spinal cord. Dasen's new *in vivo* experiments established that *Hox* gene expression along the length of the developing spinal cord is controlled by a gradient of fibroblast growth factor (FGF).

“Jeremy's studies show that as you change FGF levels, you dramatically alter *Hox* expression, and motor-neuron columnar identity,” said Jessell. Thus, different levels of FGF signaling determined whether a motor neuron would express *Hoxc5*, *Hoxc6*, *Hoxc8* or *Hoxc9* genes, he said “The experiments also showed that this relatively simple *Hox* code of expression is really driving the columnar differentiation of these motor neurons,” said Jessell.

In the process, the experiments also revealed that the *Hoxc* genes, once expressed, acted to repress one another, in a sort of “molecular battle” to specify more precisely which kind of neurons would make up a given column.

“Because the FGF gradient is presumably a bit ambiguous, individual cells responding to intermediate levels of FGF could have a hard job knowing whether they're supposed to be a motor neuron of one columnar type or another,” said Jessell. “But if you have this winner-take-all strategy—that only one of two competing *Hox* genes can be expressed, through this mutual

cross-repression—then even under conditions of ambiguous FGF signaling, you force the neurons to choose whether they're going to be one of these two columnar subtypes.”

The researchers also found that the *Hoxc* genes function to determine columnar identity in both immature neural progenitor cells and in mature motor neurons. “This finding argues very strongly that motor neurons are plastic after they exit the cell division cycle,” said Jessell. “It also illustrates how the developing nervous system doesn't have to get everything right from the word ‘go’—that there is a steady nudging of the embryonic neurons in a particular direction of differentiation,” he said. “It provides an important added versatility in neural development.”

According to Jessell, the new discoveries regarding the regulation of the development of columns in the spinal cord could well yield insights into how organization of the brain occurs. “We believe that other regions of the central nervous system are constructed through a similar set of principles,” said Jessell.

Although these findings are basic, said Jessell, they could spark new understanding of inherited neurodegenerative diseases such as Lou Gehrig's disease. “A major challenge in these diseases is understanding why motor neurons are selectively vulnerable to genetic insults,” said Jessell. “It may well be that there are certain molecular or functional properties of motor neurons that underlie their selective vulnerability. So, one way to understand this vulnerability in degenerative disease is to understand what makes a motor neuron different from all the other neurons in the spinal cord, and what makes subsets of motor neurons different from each other. This is a step toward that understanding,” he said.

Jessell and his colleagues speculate that the *Hox* genes might also play a more specific role in governing the wiring of motor neurons to particular muscles. “If that is true, it's going to be extremely interesting and exciting, because it may go a long way toward explaining the functional specificity of motor behaviors that reside within the spinal cord,” he said.