Biography of Constance L. Cepko

Constance (Connie) Cepko, a developmental biologist and head of Harvard Medical School's Biological and Biomedical Sciences graduate program, has made extraordinary contributions toward understanding development of the vertebrate CNS. A Howard Hughes Medical Institute Investigator, Cepko has based most of her studies on the retina, a particularly accessible part of the CNS and an ideal model for other neural tissues. Through her pioneering use of genomics tools, such as serial analysis of gene expression (SAGE) and microarrays (1–3), Cepko and her colleagues have developed a comprehensive library of gene expression in mouse retinal development (ref. 3 and S. Blackshaw, S. Harpavat, J. Trimarchi, L. Cai, H. Huang, W. Kuo, K. Lee, R. Fraioli, S.-H. Cho, R. Yung, E. Asch, W. Wong, and C. Cepko, unpublished data). Her research team has traced the complicated migration routes of cells in several areas of the developing CNS of rodents and chicks by using lineage studies (4, 5). Cepko's laboratory also has identified several environmental factors that help determine the fate of undifferentiated retinal cells (6). Her studies have not only contributed basic knowledge to the study of neural development but also have paved the way toward understanding and eventually treating retinal diseases such as macular degeneration and retinitus pigmentosa.

Cepko's work has earned her honors ranging from induction in 1999 to the American Academy of Arts and Sciences to receiving a Leading Women Award in 2003, presented by the Patriots' Trail Girl Scout Council in Boston. Cepko was one of 17 women elected to the National Academy of Sciences in 2002. Her Inaugural Article, titled "Electroporation and RNA interference in the rodent retina *in vivo* and *in vitro*," is featured in this issue of PNAS (7).

An Early Mentor

Cepko's career at the bench began at a seventh grade science fair in her hometown of Laurel, Maryland. Her project on yeast growth, based on an idea from a children's classroom science magazine, won first prize in her school's microbiology section. John Palmer, a judge at the science fair and then a scientist at the Department of the Interior's Forest Disease Laboratory in Beltsville, Maryland, was so impressed by Cepko's project that he invited her to work with him. Cepko quickly accepted.

Constance L. Cepko, photograph taken in 2003.

For several years, Cepko performed experiments over the weekends in Palmer's laboratory, a white clapboard building set deep in the woods. Each experiment focused on yeast nutritional requirements and was geared toward the next science project at upcoming fairs. Palmer tutored Cepko in basic scientific techniques and methods, helping her choose and construct her experiments. "[Palmer] was unbelievable; he would come in on Saturdays and teach this junior high kid science," said Cepko. "He is the reason I'm here."

Although Palmer left the laboratory for a faculty position at the University of Wisconsin when Cepko was still in high school, his mentorship inspired her to continue studying science. She attended the University of Maryland for her undergraduate degree, majoring in microbiology and biochemistry. Cepko's classes sparked her interest in viruses, and when she was ready to apply to graduate school, she chose two schools with strong virology programs: Massachusetts Institute of Technology (MIT), in Cambridge, and the University of Wisconsin, in Madison. Her choice was made after visiting both schools.

"I visited the University of Wisconsin in February, and it was too cold. One of the students told me that his tears would freeze in his eyes when he rode his bike. Since I commute by bike, that was too much for me," said Cepko.

Cepko received her doctoral degree from MIT in 1982 under the mentorship of virologist Phil Sharp. Her thesis, titled "Interaction of the adenovirus 100K and hexon proteins: Analysis using monoclonal antibodies and temperature-sensitive mutants," investigated formation of the adenovirus outer coat (8). Adenovirus causes respiratory infections in humans, with some varieties responsible for the common cold. However, researchers discovered in the late 1970s that if part of the adenovirus genome became integrated into cells, it could induce tumor formation. Cepko initially was interested in adenovirus because of its oncogenic properties but eventually became more interested in a set of structural proteins that are not involved in causing cancer. Her thesis (8) determined that one of these proteins, 100K, helps assemble other proteins that form the adenovirus capsid.

Cepko stayed at MIT for the first 2 years of her postdoctoral studies and moved to the school's newly constructed Whitehead Institute for her last year. Her time was spent constructing some of the first retroviral vectors with her mentor, Richard Mulligan (9). Now a common tool for genetic study, retroviral vectors are used to insert foreign genes into a cell's genome. Part of Cepko and Mulligan's research involved creating vectors that could coexpress a gene of interest and a selectable marker at the same time, thus allowing cells infected with the vector to be recognized easily. This type of vector would later prove invaluable in Cepko's analyses of genes in the retina.

A Fascinating Piece of Tissue

As Cepko finished up her postdoctoral studies in 1985, she pondered her next step with retroviral research: she could either continue to make more and better retroviral vectors, or she could apply these powerful tools to a biological problem. Cepko decided to employ retroviral vectors to study development of the CNS, which was relatively understudied compared to virology. Knowing little then about neurobiology, she read extensively about the nervous system's anatomy and physiology and sat in on an introductory neurobiology course taught by Harvard Medical School professors David Potter and Ed Furshpan.

Eventually, Cepko chose to focus on the retina. The retina has neural networks that develop and operate much like those in the brain, spinal cord, and other CNS tissues, and its size and loca-

This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 16.

www.pnas.org/cgi/doi/10.1073/pnas.0307955100

tion make it accessible for experiments *in vivo* or *in vitro*. These qualities make it an ideal model for CNS study.

"I liked the retina, especially coming from a nonneurobiology perspective. The brain's complex anatomy and physiology are a bit overwhelming, and I didn't understand much of it. But the retina seemed like it was very approachable, and it offered some valuable technical advantages in terms of access for gene delivery or other kinds of molecules," said Cepko. "I thought then, as I do now, that it is a very fascinating piece of tissue."

Immediately after her postdoctoral studies, Cepko accepted a professorship at Harvard Medical School and opened a laboratory to study neural development. Some of the laboratory's initial experiments involved lineage studies of cells during retinal development, a sort of "who begets who" analysis of cell division and migration. To track specific cells of interest, the researchers infected retinal cells with a retroviral marker similar to those Cepko created during her postdoctoral studies. In a 1987 study published in PNAS (10), Cepko and her colleagues Jack Price and David Turner inserted a gene into developing rat retinas that expressed the bacterial protein β-galactosidase, which caused the original cells to change color. Thus, Cepko's team was able to visually follow the trail of marked cells as they descended from completely undifferentiated retinal precursor cells to photoreceptors, neurons, and glia, nonneuronal cells that support neurons (11).

The sibling cells in these areas migrated over long and complex routes, making assignment of sibling relationships so difficult that Cepko and her collaborators needed to create a novel method of tracing sibling relationships. The new method also used retroviral vectors but involved analysis of the viral genome from individual cells taken from the brain (4, 12). This method provided some of the first evidence that sibling cells not only can migrate over long distances along circuitous routes but also can populate different functional domains of the brain (4, 5). The researchers later applied their technique with similar success in the mouse and rat cerebral cortex (4, 13) and several areas of the chick brain (5, 14).

Over the past decade, Cepko's laboratory has gradually moved from studying development at the cellular level to examining it at the genetic level. In 2001, Cepko's team published an article identifying nearly all of the known genes responsible for developing photoreceptors (3). By using SAGE, the researchers extracted RNA from mouse retinal cells in various phases of development. After converting the RNA to several thousand "tags," or short pieces of cDNA, the researchers were able to search databases of mouse and human genes and match the tags to corresponding genes. Cepko and colleagues identified ≈300 photoreceptor genes, five times the number previously known. Of the new genes discovered, 241 have homologs in humans. Isolating these genes narrows the search for the genetic roots of retinal diseases such as macular degeneration and retinitus pigmentosa and could eventually lead to ways to replace dead or damaged cells.

Although many of the genes associated with vision are known, the details of how these genes function are still poorly understood. Cepko and her laboratory address this issue in her Inaugural Article (7) by using electroporation, a technique that involves shocking a cell to create holes in its membrane, thus allowing the cell to take up foreign DNA or RNA. Electroporation is commonly used to insert foreign genes into individual cells in tissue culture or some embryonic tissues (15–17) but had not previously been used in the retina. Here, Cepko's colleague Takahiko Matsuda optimized this technique for the retina, and the two researchers demonstrated electroporation's utility in several applications (7). For example, Cepko and Matsuda inserted RNA interference (RNAi), a small section of RNA that temporarily reduced transcription of particular genes, into photoreceptor cells in newborn mice. As the mice matured, their retinal phenotypes were similar to knockout mice missing the corresponding genes. Cepko and Matsuda also were able to coelectroporate up to three plasmids that gave cell-type-specific transcription in three different kinds of cells.

Keeping a Low Profile

Cepko acknowledges that her laboratory is best known for these large genomics and lineage studies. However, she says, "the lower profile research is perhaps more significant over the long term." Although more incremental and less publicized, Cepko's laboratory has gathered a considerable amount of information about how the fate of retinal cells is determined both by genetic makeup and through chemical cues in the cellular environment (18). "It's a huge area; people are trying to figure out what is the signal from the environment, how does the cell receive the signal, and how does the cell interpret that signal. It's a daily, minute-by-minute thing the cell is doing," she said.

Like many other researchers, Cepko believes that a combination of genomic and environmental studies will some day give researchers a complete picture of retinal development and function, knowledge of enormous significance for preventing blindness. "If you can figure out how to slow down cell death or stop that process even by some small percentage, you can actually preserve people's vision," said Cepko. "I don't know how far we'll get on that, but we'd love to have something to contribute in this area."

Christen Brownlee, *Science Writer*

1. Sharon, D., Blackshaw, S., Cepko, C. L. & Dryja, T. P. (2000) *Proc. Natl. Acad. Sci. USA*. **99,** 315–320.
2. Livesey, F., Furukawa, T., Steffen, M. A., Church, G. M. & Cepko, C. L. (2000) *Curr. Biol.* **10,** 301–310.
3. Blackshaw, S., Fraioli, R. E., Furukawa, T. & Cepko, C. L. (2001) *Cell* **107,** 579–589.
4. Walsh, C. & Cepko, C. L. (1992) *Science* **255,** 434–440.
5. Golden, J. A. & Cepko, C. L. (1996) *Development (Cambridge, U.K.)* **122,** 65–78.
6. Altshuler, D., Lo Turco, J., Rush, J. & Cepko, C. L. (1993) *Development (Cambridge, U.K.)* **119,** 1317–1328.
7. Matsuda, T. & Cepko, C. L. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 16–22.
8. Cepko, C. L. (1982) Ph.D. thesis (Massachusetts Institute of Technology, Cambridge).
9. Cepko, C. L., Roberts, B. E. & Mulligan, R. C. (1984) *Cell* **37,** 1053–1062.
10. Price, J., Turner, D. & Cepko, C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 156–160.
11. Turner, D. L. & Cepko, C. L. (1987) *Nature* **32,** 131–136.
12. Lin, J. C. & Cepko, C. L. (1999) *Dev. Biol.* **211,** 177–197.
13. Golden, J., Fields-Berry, S. C. & Cepko, C. L. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5704–5708.
14. Austin, C. P. & Cepko, C. L. (1990) *Development (Cambridge, U.K.)* **110,** 713–732.
15. Saito, T. & Nakatsuji, N. (2001) *Dev. Biol.* **240,** 237–246.
16. Tabata, H. & Nakajima, K. (2001) *Neuroscience* **103,** 865–872.
17. Itasaki, N., Bel-Vialar, S. & Krumlauf, R. (1999) *Nat. Cell Biol.* **1,** E203–E207.
18. Belliveau, M. J. & Cepko, C. L. (1999) *Development (Cambridge, U.K.)* **126,** 555–566.