The 1986 Nobel Prize for Physiology or Medicine

A developmental biologist and a biochemist are honored for discovering nerve growth factor and epidermal growth factor

THE 1986 Nobel Prize for Physiology or Medicine has been awarded to Rita Levi-Montalcini and Stanley Cohen for their discoveries of factors that control cell growth and development. Levi-Montalcini was cited for her contributions to the identification of nerve growth factor (NGF), a protein that is necessary for the growth, development, and maintenance of nerve cells in the peripheral nervous system and apparently also in the brain. Cohen, who collaborated with Levi-Montalcini in some of the early work on NGF, later went on to identify a second distinct growth regulatory protein, which is called epidermal growth factor (EGF).

According to the Nobel Committee of the Karolinska Institute in Stockholm, Sweden, the discoveries have "opened new fields of widespread importance to basic science. As a direct consequence we may increase our understanding of many disease states such as developmental malformations, degenerative changes in senile dementia, delayed wound healing, and tumor diseases."

Although NGF and EGF were the first growth factors to be isolated, numerous additional growth factors, which act on many cell types, have since been identified. Growth factor research has especially burgeoned within the past few years as new techniques, among them the methods of recombinant DNA technology, have facilitated the isolation of the factors and their genes. But the new laureates began their investigations many years before the advent of such technologies.

In the 1940's, Levi-Montalcini was working in her native Italy—during World War II in a laboratory that she had set up in her home when she was barred from university research because of her Jewish heritage. After the war, however, she took up a position at the University of Turin. Levi-Montalcini's early interests included the influences of peripheral tissues on the growth and maintenance of nerve cells. Viktor Hamburger of Washington University in St. Louis was doing related research and, in 1947, invited Levi-Montalcini to St. Louis, thus beginning a collaboration that was to lead to the identification of NGF.

The development of the nervous system, with its myriad precise neuronal connections both within the brain and from the brain to the peripheral tissues, is a formidable task. Early on, the collaboration between Hamburger and Levi-Montalcini led to a greater appreciation of the importance of nerve cell death during the development of connections between embryonic neurons and peripheral tissues. Moreover, the work established that the tissues exert a positive influence on the maintenance of differentiated neurons, presumably by producing "trophic factors."

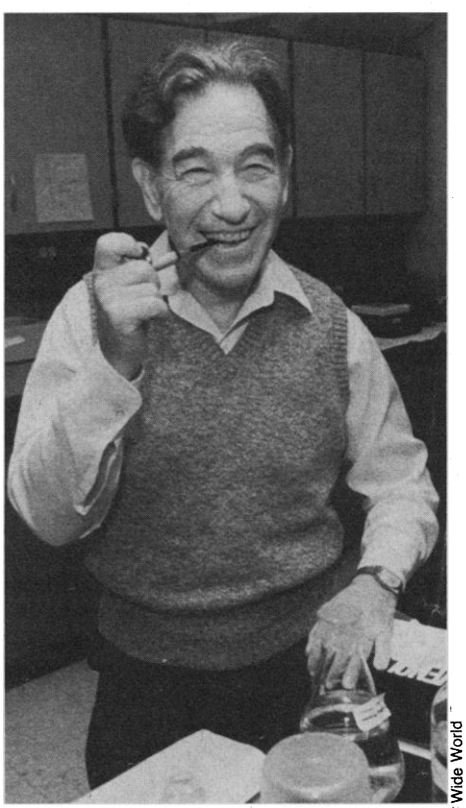
Hamburger postulated that other rapidly growing tissues might also produce factors with trophic effects on nerve cells. One of his students, Elmer Bueker, pursued this suggestion and observed that a mouse tumor, when transplanted into chick embryos, stimulates nerve cell growth. Levi-Montalcini and Hamburger confirmed Bueker's result and went on to show that the stimulation was produced by a molecule—namely NGF—that is secreted by the tumor.

Levi-Montalcini also devised a very sensitive assay for NGF that was to pave the way for the isolation of the molecule. The nerve cells that had been shown at that time to respond to NGF were either sensory neurons, which convey information about sensory stimuli from nerve endings to the brain, or sympathetic neurons, which help to regulate involuntary functions such as the beating of the heart and blood flow. For the assay, Levi-Montalcini incubated either sensory or sympathetic ganglia in laboratory dishes with a source of NGF. When exposed to very small quantities of the growth factor, the ganglion cells rapidly grow projections called neurites, which form halos around the ganglia.

By 1954, the work on NGF had taken a more biochemical direction as efforts to isolate the growth factor molecule got under way. Hamburger withdrew from the research at that time, and Cohen, who had trained as a biochemist and was then also at Washington University, began his collaboration with Levi-Montalcini. Levi-Montalcini and Cohen were able to isolate a nerve-growth stimulating material from the mouse tumor. However, this preparation contained both protein and nucleic acid. In an effort to determine which of these had the NGF activity, Cohen treated the material with snake venom, which contains enzymes that break down nucleic acids. Much to his surprise the venom had a potent NGF activity of its own.

Cohen hypothesized that mammalian salivary glands, which are analogous to the venom glands of snakes, might also prove to be a rich source of the factor. That turned out to be the case, at least for the salivary gland of the male mouse. Cohen was then able to purify NGF from snake venom and mouse salivary glands and make antibodies to the material, which had turned out to be a protein.

In those pre–recombinant DNA days, determination of protein sequences was a tedious affair and many years were to elapse before the complete sequence of NGF was determined. Not until 1970, did Ralph Bradshaw, Ruth Angeletti, and William Frazier of Washington University School of Medicine accomplish this feat. The complete NGF molecule consists of two identical protein chains, each of which has a molecule weight of about 13,250.



Wide World

Stanley Cohen: *helped to isolate nerve and epidermal growth factors.*

Wide World

Rita Levi-Montalcini's *persistence in pursuing nerve growth factor culminates in the Nobel Prize.*

Once the NGF molecule was definitively in hand, research on its biological effects blossomed in several laboratories. Hamburger, for one, took up the work again and Levi-Montalcini has maintained her interest over the years. She had already shown in the 1950's that Cohen's antibody to NGF prevents the development of sympathetic neurons when it is injected into newborn mice and rats. NGF is needed both for the development and maintenance of sympathetic and certain sensory neurons. The molecule, which is made by the neuronal target tissues, binds to specific receptors on the nerves and is then transported up the cell to the nucleus in the cell body, where it exerts its effects.

Until recently, the effects of NGF had been thought to be limited to the peripheral nervous system. However, within the past year or two, a growing body of evidence suggests that it also acts in the brain, although there is a major difference between its peripheral and central effects. In the periphery, NGF acts on sympathetic neurons that use catecholamine neurotransmitters such as norepinephrine and dopamine and sensory nerve cells that make certain neuroactive peptides. But in the brain, NGF acts on neurons that use the neurotransmitter acetylcholine. The brain tracts that respond to NGF include those that degenerate in Alzheimer's and Huntington's diseases. This raises the possibility that failure to produce or respond to NGF might contribute to the development of these serious neurological disorders, although this has not yet been proven.

The expectation is that there will prove to be several factors that stimulate nerve cell growth, thereby helping to coordinate the very complex set of events needed to establish and maintain a complete nervous system. But so far NGF is the only such factor that has been characterized.

Cohen's discovery of EGF was a direct outgrowth of his research on NGF, although the EGF work was largely accomplished after he moved to Vanderbilt University School of Medicine in 1959. Cohen had noted a peculiar effect when the NGF-containing extracts of salivary glands were injected into newborn mice. The eyelids of the animals opened sooner than they usually do and their teeth also grew in ahead of schedule. These effects did not occur with pure NGF, thereby indicating that some additional factor in the extracts must be the cause.

This factor also proved to have growth-stimulatory effects. The eyes opened early, Cohen found, because the skin cells of the lids were stimulated to grow and to differentiate. By 1962, Cohen had isolated the causative factor, which he had called EGF. Cohen and his colleagues determined the amino acid sequence of the protein in the early 1970's. The molecule contains 53 amino acids.

One of the biggest mysteries concerning EGF and the other growth factors that have since been identified concerns how the stimulatory signals transmitted by these agents are conveyed from the cell membrane to the nucleus. According to Cohen, EGF must bind to a receptor on the surface of sensitive cells to exert its effects. It resembles NGF in this regard. The receptor-EGF complex is then brought into the cell, although it is not clear that this has anything to do with signal transmission to the nucleus.

Another consequence of EGF binding, according to Cohen and his Vanderbilt colleagues, is the activation of an unusual enzymatic activity of the receptor. The receptor is a tyrosine kinase enzyme, which means that it attaches phosphate groups to the amino acid tyrosine in proteins. A number of enzymes that have important regulatory roles in the cell are also kinases, but most of them attach phosphates not to tyrosine, but to serine. The supposition is that the addition of phosphate to tyrosine in the target protein or proteins of the EGF receptor participates in the signal transmission. Cohen and others are very interested in identifying the substrates for the receptor. They have some clues, but as things now stand the ways in which growth signals are transmitted to the nucleus remain mysterious.

The problem is important, not just for understanding normal growth control, but also because disturbances in cell growth and differentiation can lead to the development of cancer. One of the major recent findings in cancer research is the link between between the normal machinery of growth regulation—including that involving EGF—and "oncogenes," which can cause cells to acquire cancerous characteristics. Oncogenes are derived from normal cellular genes that have somehow been altered so that they make abnormal products or make their products in an uncontrolled fashion. They occur in certain cancer-causing viruses of animals and have been found in activated form in human tumors.

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Early in 1984, Michael Waterfield of the Imperial Cancer Research Fund, Joseph Schlessinger of the Weizmann Institute of Science in Rehovot, Israel, and Axel Ullrich of Genentech, Inc., in South San Francisco, and their colleagues discovered that the *erbB* oncogene is derived from the gene for the EGF receptor. Their results indicate that the *erbB* gene encodes a truncated version of the EGF receptor that may be locked in the "on" position, thus imparting a continuous growth signal to the cells containing it. *ErbB* is not the only oncogene that has been linked to growth factors. For example, the *sis* oncogene is derived from a gene for platelet-derived growth factor. A better understanding of how growth factors work may thus eventually lead to better cancer therapies.

Meanwhile, with EGF becoming available in larger quantities as a result of gene cloning, the factor may find application in growing skin cells for treating burn victims. In addition, EGF inhibits acid secretion by the stomach. It is identical to the human hormone urogastrone, Cohen notes, and may have a potential application in ulcer treatment.

Before the isolation and characterization of NGF and EGF by Levi-Montalcini and Cohen, the idea of growth factors was somewhat suspect. "This had the advantage that people left you alone and you weren't competing with the world," Cohen says. "The disadvantage was that you had to convince people that what you were working with was real." It is safe to say that people are now convinced. ■ JEAN L. MARX