Synthesizing the Opioid Peptides

The opioid peptides are synthesized as parts of large precursor molecules that may be split to yield different products in different cells

The biosynthesis of the opioid peptides illustrates what seems to be a general trend in neurobiology. Like many other peptides that act in the nervous system, the opioids, which are best known for their analgesic and euphoric effects, are not synthesized individually. Instead, a single gene encodes a large inactive polypeptide that contains within its structure the sequences of several small active molecules that are subsequently split from the precursor. Often the individual active peptides perform different functions.

Such an arrangement confers a great deal of subtlety and flexibility on the workings of the nervous system. It may help to coordinate the separate actions that combine to produce complex behaviors and responses, as has also been suggested for the egg-laying hormone system of the marine snail *Aplysia* (*Science*, 14 May 1982, p. 720). Moreover, if the sites where the precursor is split vary from tissue to tissue, it will be possible to generate different combinations of peptides from a single gene product.

Both of these possibilities have proved to be true for the opioid precursor called pro-opiomelanocortin (POMC). Researchers have also shown that synthesis of the POMC peptides may be regulated both at the level of gene transcription into messenger RNA (mRNA), the first step of protein synthesis, and at later stages during the processing of the POMC polypeptide to the final products.

Since the discovery in 1975 of Met-enkephalin and Leu-enkephalin, the list of opioid peptides has grown to include at least eight or nine members, all of which have either the Met-enkephalin or the Leu-enkephalin sequence as their amino terminals. It takes three distinct genes, including the POMC gene, to code for the polypeptide precursors of these peptides.

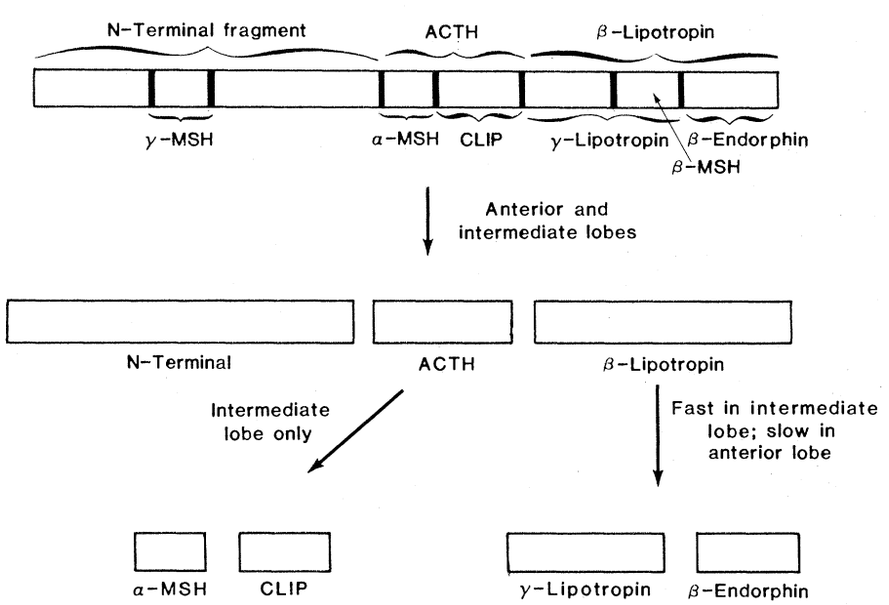
POMC was the first of the precursors to be identified. The gene was cloned and sequenced in 1979 by Shosaku Numa of Kyoto (Japan) University and his colleagues.

The complete POMC molecule contains the sequences of no less than seven active peptides, including the opioid β-endorphin, adrenocorticotropic hormone (ACTH), and α-, β-, and γ-melanocyte–stimulating hormones (α-, β-, and γ-MSH's). The individual peptides in the precursor are bounded on both ends by pairs of basic amino acid residues, usually containing one lysine and one arginine residue. These pairs, which are the sites recognized and cut by the protein-splitting enzymes that release the active opioids, are common features of precursor polypeptides.

The POMC polypeptide is made in the anterior and intermediate lobes of the pituitary gland, in the hypothalamus and other areas of the brain, and in several peripheral tissues including the placenta, gastrointestinal tract, and lungs. Early work by Edward Herbert and James Roberts of the University of Oregon, and by Betty Eipper and Richard Mains of the University of Colorado showed that POMC is processed differently in the anterior pituitary of mice and rats than in the intermediate pituitary. (Humans lack a distinguishable intermediate lobe.) In the anterior lobe, the initial product is cleaved to produce ACTH, a hormone that stimulates glucocorticoid production by the cortex of the adrenal gland, and β-lipotropin, the function of which is poorly understood. Some of the β-lipotropin is further split, thus releasing β-endorphin.

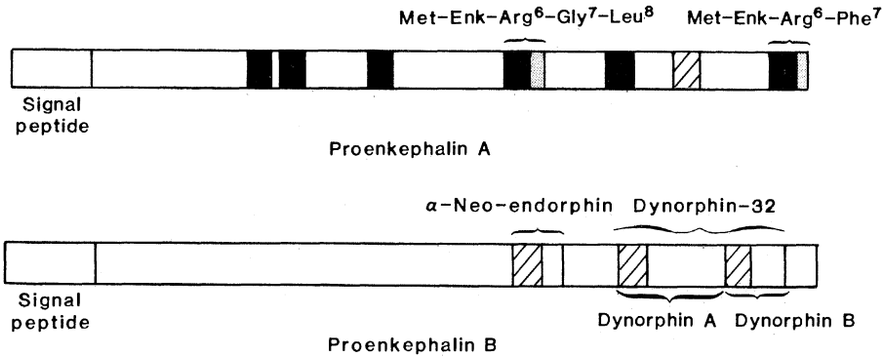
The intermediate lobe does not produce either ACTH or β-lipotropin as final products. These molecules are formed just as they are in the anterior lobe, but all the ACTH is cleaved to produce α-MSH and corticotropin-like intermediate lobe peptide (CLIP). And the β-lipotropin is cut into β-endorphin and γ-lipotropin.

This type of differential processing might be explained if the POMC molecules produced by the two tissues were not the same, with the structural variations dictating how the polypeptides are to be split. This seems unlikely, however, as investigators have generally found only one active POMC gene per haploid genome. Herbert and Michael Uhler, who is now at Stanford University, found two copies of the gene in the mouse, but one turned out to be a pseudogene—that is, it does not yield an



POMC processing in the pituitary

The complete POMC molecule is diagrammed at the top of the figure. The dark vertical bars represent the pairs of basic amino acids that bound the active peptides and serve as the targets for the enzymes that split the precursor. The early processing follows the same path in both lobes of the pituitary, but additional cleavages in the intermediate lobe produce smaller final products.



Proenkephalin structures

Proenkephalin A is the precursor of the enkephalins and proenkephalin B is the precursor of the dynorphins. The dark bars represent Met-enkephalin fragments and the cross-hatched bars are Leu-enkephalin segments.

active product. Roberts, who has moved to Columbia University, says, "You have a system expressing a single gene in the genome but the protein product is processed differently to give different products."

The two tissues may have alternative sets of enzymes for processing the POMC molecule, Herbert and Roberts suggest. Peptides that are destined to be secreted, as these are, make their way in an orderly fashion from the site of synthesis, the ribosomes attached to the membranes of the endoplasmic reticulum, to the outside of the cell. Processing enzymes may work at any of the steps along the way. POMC is split to produce smaller fragments in the intermediate lobe of the pituitary than in the anterior lobe, probably because the intermediate lobe cells have enzymes available at the later stages of transit that are not possessed by the anterior lobe cells.

Another possibility is that the RNA transcripts of the POMC gene may be spliced to give different mRNA's in the anterior and intermediate lobes. This gene, like most of the genes of higher organisms, contains noncoding regions called introns, which are transcribed into RNA but must be spliced out of the final mRNA. At present, there is no information about whether the two lobes have different splicing enzymes.

Although the processing of POMC itself or of its mRNA determines which peptides will finally be produced in the anterior and intermediate lobes of the pituitary, transcription of the POMC gene into mRNA is also regulated—and regulated differently—in the two tissues. Release of ACTH is part of the animal's response to stress. It is triggered by corticotropin-releasing factor (CRF), a hypothalamic hormone that is secreted into small blood vessels leading to the anterior pituitary. The ACTH in turn stimulates secretion of glucocorticoids by the adrenal gland; these increase glucose metabolism, thereby providing the energy needed to cope with a stressful situation. In a classic feedback situation, the adrenal hormones act on the anterior pituitary to shut off ACTH production and release.

The glucocorticoids shut off the production of ACTH by inhibiting transcription of the POMC gene, Herbert and Roberts find. If the animal is deprived of adrenal hormones by removing the gland, the POMC mRNA content of the anterior pituitary increases dramatically after a lag of several hours. By 20 days after the adrenalectomy, it is 20 to 30 times the normal concentration in the anterior lobe.

The concentration of the POMC-derived peptides in the anterior pituitary at first decreases after the adrenal glands are removed, but then begins to increase after the concentration of the mRNA increases. "Some of these effects are due to the stress of the operation," Herbert explains, "but most are due to the loss of the glucocorticoids. If you inject glucocorticoids, the mRNA level comes back down to normal. The hormone inhibits synthesis of the mRNA."

Cloned, radioactively labeled genes can be used as probes to detect the corresponding mRNA's in individual cells, even if these constitute only a small fraction of a diverse population. Using this method, Roberts showed that the increase in POMC mRNA in the anterior pituitary was caused both by greater messenger production per cell and by an increase in the number of cells transcribing the POMC gene.

Part of the glucocorticoid effect might be mediated indirectly by CRF. The hypothalamus releases more of this agent in response to adrenalectomy and less when glucocorticoids are given. Roberts says, "That is a possibility, that CRF contributes. But you do know that you can get the glucocorticoid effect in cultured cells," without the hypothalamic hormones.

POMC gene transcription increases only by a factor of 2 to 3 in the cultured cells, not the 20- to 30-fold increase seen in the pituitary. Some of this difference may be due to the influence of CRF in the whole animal, Roberts says, possibly because it is the agent that causes the increase in the number of cells producing POMC.

According to Herbert and Roberts, adrenalectomy has no effect on the transcription of the POMC gene by cells of the intermediate pituitary lobe, and glucocorticoid administration only a slight effect. But activation of dopaminergic neurons, which originate in the hypothalamus and innervate the intermediate pituitary, inhibits synthesis of POMC mRNA by this lobe. "Two days after treatment with a dopamine agonist [mimic], transcription into the mRNA has decreased and the concentration of POMC has decreased," Roberts explains. Conversely, treatment with a compound that blocks dopamine action causes an increase in the mRNA concentration.

Studying expression of the POMC gene in brain is much more difficult than studying it in the pituitary. However, work by Dorothy Krieger of the Mt. Sinai Medical School and others indicates that POMC processing in the hypothalamus is somewhat different from that in both the anterior and intermediate lobes of the pituitary. Krieger and Jeffrey McKelvy of the State University of New York at Stony Brook have evidence that the processing may take different routes in various cells in the hypothalamus.

Very little is known about the expression of the other two genes coding for opioid polypeptides, largely because these were first cloned within the past year or so. Only then did the complete amino acid sequences of these polypeptides become available.

The Numa and Herbert groups and that of Sidney Udenfriend of the Roche Institute of Molecular Biology in Nutley, New Jersey, cloned the gene coding for the polypeptide now called proenkephalin A. And the Numa group cloned the third gene, which codes for the polypeptide designated proenkephalin B.

The polypeptide sequences, which were deduced from the sequences of the cloned genes, confirmed what was already suspected, namely that proenkephalin A, not POMC, is the precursor of Met- and Leu-enkephalin and also some larger enkephalin-containing pep-

tides. According to Udenfriend, some of these larger peptides are more active than the enkephalins themselves. Proenkephalin B contains the sequences of α-neo-endorphin, dynorphins A and B, and Leu-enkephalin.

The structures of these polypeptides show the potential for differential processing. Although the work is much less advanced than that on POMC, the Udenfriend group has evidence suggesting that proenkephalin A may undergo differential processing. At Stanford University, Jack Barchas and his colleagues have been studying the brain distributions of the peptides contained within the proenkephalin B molecule and have found indications that the manner in which this molecule is split may vary with its cellular location.

Whether the three genes have the same evolutionary ancestor is unclear. The overall organization of the three is similar. They resemble each other in size and in the positions of their introns. And all the polypeptides contain six cysteine residues which are clustered near the amino terminals. Evidence of repeated nucleotide sequences within the individual genes suggest that each arose by the duplication of a short primordial gene segment.

They may not all have arisen from the same segment, however. Except for the short sequences coding for the enkephalin moieties found in all the active opioid peptides, the nucleotide sequences of the genes are dissimilar. Roberts suggests, "It almost looks like convergent evolution, but these genes appear to be extremely old." Opioid peptides have even been found in single-celled organisms such as *Tetrahymena*. Their lengthy evolutionary history could have allowed many changes in nucleotide sequence to accumulate.

However the genes evolved, the result is that a large number of different but related neuropeptides can be made with a great deal of economy and the flexibility to allow the composition of the final mixture of products to vary in response to dissimilar circumstances.

—JEAN L. MARX

Additional Readings

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Are the Ocean's Deserts Blooming?

According to the standard method the central oceans are impoverished, but new measurements show them to be many times more productive

Are the vast central regions of the ocean biological deserts? They are, according to measurements using carbon-14, the standard method of the past 30 years for measuring the primary production of the oceans. Although they cover almost half the area of the world ocean, these least productive or oligotrophic waters have been thought to account for only about 20 percent of the organic matter created through marine photosynthesis, or perhaps 10 percent of all primary production. But many biological oceanographers have grown leery of the carbon-14 method as various potential methodological problems have arisen, ranging from the inadvertent removal of some photosynthetic organisms to mass poisoning with trace metals.

Investigations of the carbon-14 method itself have not yet revealed the magnitude of the error in oligotrophic waters, but some physical oceanographers are claiming that their methods reveal such areas to be many times more productive than indicated by the carbon-14 method. A major difference between the two approaches is the scale of an experiment. Biological oceanographers pour a few hundred milliliters of seawater into a glass bottle, add carbon-14–labeled carbonate, and wait a few hours while the phytoplankton, floating microscopic plants, convert the carbonate into organic matter. Then, the experimenter filters the water and measures the decay of carbon-14 incorporated in the trapped particulate matter. Physical oceanographers, on the other hand, measure the effects of photosynthesis on tens or thousands of cubic kilometers of seawater over months, years, or decades.

In one such large-scale experiment, Eric Shulenberger of the Natural History Museum in San Diego and Joseph Reid of the Scripps Institution of Oceanography used a bottle, of sorts, that forms every summer over hundreds of kilometers of the central North Pacific. The sun-warmed water within 20 meters or so of the surface forms a stable layer or cap over the cooler water below, bottling up the oxygen produced there by photosynthesis. The bottle lasts several months, but fails to contain oxygen produced within the warm surface layer. That gas steadily leaks to the atmosphere.

Ignoring this leakage and other losses that would increase their measured productivity, Shulenberger and Reid calculated the rate of primary production that could account for the trapped oxygen. At oligotrophic sites north of Hawaii that were surveyed in 1975, the productivity implied by the trapped oxygen was two to seven times higher than carbon-14 values determined at the same sites. Because of the conservative assumptions, the gap between the results of the two methods may be even greater.

> The productivity measured by Jenkins is only double the accepted rate for such waters, but his measurement neglects most of the organic matter photosynthetically produced in the central ocean.

Recently, William Jenkins of the Woods Hole Oceanographic Institution experimented with a longer lasting bottle that stretches from one side of the subtropical North Atlantic to the other. In-