How Peptide Hormones Get Ready for Work

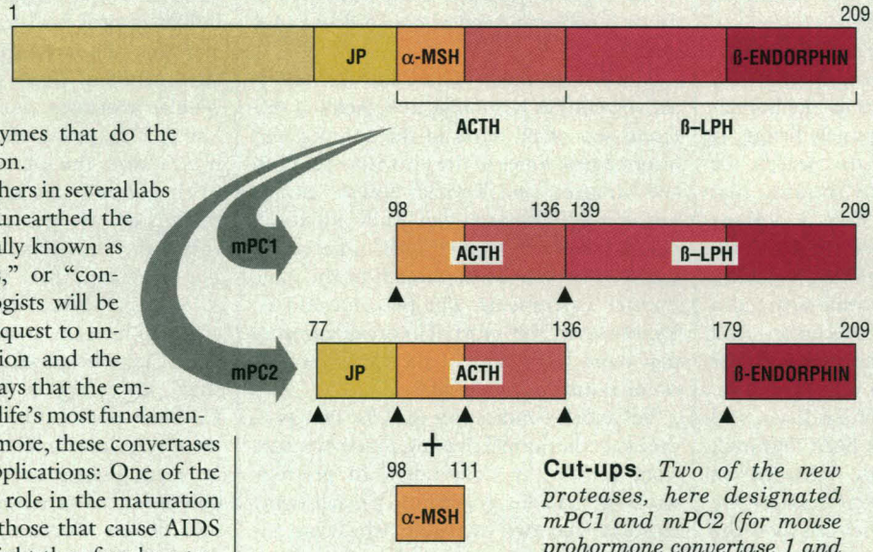
Researchers seem to have found the enzymes that clip peptide hormones and neurotransmitters out of their precursors

FOR MORE THAN 25 YEARS, CELL BIOLOGISTS have been hunting for an elusive—but critical—set of enzymes. The body makes an array of peptides that have a pervasive role in regulating essentially all physiological activities. These include hormones such as insulin, factors that stimulate cell growth, and neurotransmitters that carry signals from nerve cells to their targets. But these peptides are not born ready for action; they are made as part of larger proteins and have to be clipped out before they can go to work. Until the last year or two, however, the main product of the search for the protease enzymes that do the clipping was frustration.

No longer. Researchers in several labs seem to have finally unearthed the elusive enzymes, usually known as "converting enzymes," or "convertases." If so, biologists will be greatly aided in their quest to understand brain function and the developmental pathways that the embryo follows—two of life's most fundamental mysteries. What's more, these convertases could have clinical applications: One of the enzymes might play a role in the maturation of viruses, including those that cause AIDS and influenza, and might therefore be a target for antiviral drugs.

Perhaps influenced by their troubles in the past, the researchers doing the work are still cautious. It's a bit too soon, they say, to be absolutely certain that the recently identified enzymes are in fact the long-sought convertases. As biochemist John Hutton of the University of Cambridge in England notes, during the 25-year search "many candidates have been put forward—and eventually shot down." But now researchers have cloned mammalian genes for three new protein-splitting enzymes of a type not previously thought to occur in mammals and are beginning to acquire evidence indicating that they do function as convertases in living cells. "The whole field is starting to jell," says Gary Thomas of the Vollum Institute at the Oregon Health Sciences University in Portland, who has been hunting convertases for several years.

For most of the past 25 years, researchers tried to isolate the convertases by conventional biochemical methods. And as Hutton indicates, they did not lack for candidates. The problem was that mammalian cells contain many protease enzymes that clip at the same sites as the convertases, which usually attack at pairs of basic amino acids, such as lysine plus arginine or two arginines. So the biochemists had no trouble pulling out enzymes that would cut the large precursor "prohormones" in the right locations in the test tube. They ran aground, however, when they tried to prove that these were bona fide convertases in the cell.



Cut-ups. *Two of the new proteases, here designated mPC1 and mPC2 (for mouse prohormone convertase 1 and 2), cleave POMC, a large prohormone that contains several peptides, including adrenocorticotrophic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), and β-lipotrophin (β-LPH).*

The lead that would eventually end the impasse was uncovered about 7 years ago by Jeremy Thorner and his colleagues at the University of California, Berkeley. While studying a yeast mutant called *kex2*, identified because it fails to make two peptides, one a factor needed for sexual mating by yeast and the other a cell-killing toxin, these researchers noted that the peptides are normally cut out of larger precursor proteins much as mammalian peptide hormones are. The Thorner group then went on to trace the *kex2* mutation to the gene encoding the protease that does the cutting.

That suggested to Thorner—and to the researchers hunting the mammalian convertases as well—that the yeast protease might be a prototype for the mammalian enzymes. Granted, the yeast enzyme resembled a bacterial protease called subtilisin and at the time no subtilisin-like enzyme had been found in mammals. Nevertheless, the specificity of the *kex2* product was right; it clips at dibasic amino acids.

More direct support for the idea that the yeast enzyme might be a prototype for the mammalian convertases was provided by gene transfer experiments done in 1987 by Robert Fuller, then a postdoc in Thorner's lab, working in collaboration with Thomas and his Oregon colleagues. Those experiments showed that the yeast enzyme would correctly cut a mammalian prohormone in mammalian cells. "We felt that meant," Thomas says, "that the [mammalian converting] enzymes must be functionally similar to the *kex2* protein." Presumably their structures would also be similar, so that the *kex2* gene might serve as a probe for pulling out the genes for the mammalian enzymes.

But the researchers hunting the mammalian enzymes had to endure a couple more years of frustration, because, says long-time convertase hunter Nabil Seidah of the Clinical Research Institute of Montreal, the *kex2* gene probe did not pick up any mammalian genes after all. Meanwhile, however, Fuller, Thorner, and their Berkeley colleague Anthony Brake decided to take another tack. They performed a computer search for protein sequences resembling those of the *kex2* product. The result was a surprise: A mammalian *kex2*-related gene had already been found, but no one suspected that it might encode a protease.

The mammalian relative of *kex2* had actually been discovered in 1986 by Anton Roebroek, Wim Van de Van, and their colleagues at the University of Nijmegen in the Netherlands, who weren't interested in convertases at all but were studying the *fes/fps* oncogene. In the course of those investigations, they had identified a new gene located near the beginning of the oncogene. Because the structure of the new gene, which was designated *fur* (for *fes/fps u*p-

stream region), suggested that it encoded a membrane protein, those researchers hypothesized that the protein might be a receptor that could interact with the oncogene product to transmit growth stimulatory signals into cells.

When the Berkeley group found the *kex2* resemblance, however, a whole new possibility opened up: Instead of being a growth factor receptor, furin, as the *fur* gene product is known, might be a mammalian converting enzyme. And furin's not the only candidate convertase. Last year Steven Smeekin and Donald Steiner of the Howard Hughes Medical Institute at the University of Chicago, and Seidah, Michel Chrétien, and their colleagues in Montreal, with the aid of the polymerase chain reaction, independently cloned two additional mammalian *kex2*-related genes.

> ***"The whole field is starting to jell."***
>
> —GARY THOMAS

If the two new genes do encode mammalian convertases—as everyone in the field now thinks—their discovery by the Steiner and Chrétien groups is extremely fitting. It was Steiner who set off the search for convertases in the 1960s by showing that insulin has to be cleaved from a prohormone. Chrétien's early work helped establish that certain pituitary peptides, including a melanocyte stimulating hormone are also synthesized as part of larger precursors. And both researchers managed to stick with the research through thick and thin ever since.

Given how long that trail has been, and how many false scents have been followed, why do the researchers now think they've come up with authentic convertases? One reason, Seidah says, is that they can show the genes are active in the cells where prohormone cleavage is known to take place. They've found, for example, that expression of the two genes discovered by his and Steiner's groups is particularly high in the pituitary gland and certain regions of the brain, such as the hypothalamus, and also in the insulin-secreting cells of the pancreas. That suggests the proteases made by those genes are the ones that clip neuroendocrine peptides out of their precursors, an idea supported by the finding that the enzymes are located in the intracellular secretory granules where the peptides are processed and stored until being released.

These localization results do not prove that these proteases are in fact the convertases. But the availability of the genes has made possible another—much more definitive—experiment. The researchers can now transfer the protease genes, together with the genes encoding prohormones, into cells where the prohormones would not normally be cut. That's one of the biggest differences between the older work with isolated enzymes and the newer work, Seidah points out. Now the activity of the proteases can be studied in living cells, and if they cut the prohormones there it will be strong evidence that they are in fact convertases.

Results from the early transfer experiments are positive. The Montreal group and Thomas, Steiner, and their colleagues have recently completed experiments in which they transferred the genes for their "prohormone convertases" into cells along with the gene encoding proopiomelanocortin (POMC), a prohormone that is made in the pituitary gland and contains within its structure no fewer than seven active peptides, including the endogenous opiate β-endorphin and adrenocorticotrophic hormone.

The processing of POMC into its various active peptides is complicated, yielding different sets of products in the anterior and intermediate lobes of the pituitary. Yet both the Montreal and Thomas-Steiner groups got similar, although not quite identical, results showing that POMC processing could essentially be duplicated in the gene transfer experiments. The two convertases, Steiner says, "form sort of a core of enzymes that reproduce many of the cleavages that occur normally, if not all."

Yet another indication that the new proteases are the normal cleaving enzymes comes from Hutton at Cambridge. In previous work, he and his colleagues biochemically characterized two enzymes, which are located in secretory granules where they cleave several prohormones at lysine-arginine sequences. Now, the researchers have done antibody studies showing that one of the secretory granule enzymes corresponds to one of the new proteases. "That marries the enzymology to the gene clone," Hutton says.

The picture that is emerging from all this is that the two proteases discovered by the Chicago and Montreal groups cleave neuropeptides and hormones, such as insulin, that are stored in the secretory granules of cells and released only in response to appropriate stimuli. Furin, meanwhile, appears to have a somewhat different role. It is synthesized in more types of cells than the other two proteases. Indeed, furin is made in all the cell types studied so far. And localization studies suggest that it works on proteins moving through the Golgi apparatus on their way out of the cell, not in proteins stored in the secretory granules.

Furin has the potential to cut a wide variety of protein precursors, Thomas says. For example, his group has shown that it cleaves the active form of nerve growth factor from its precursor. And both the Van de Van group, which is now at the University of Louvain in Belgium, and another group including Randal Kaufman of Genetics Institute and Philip Barr of Chiron Corp. showed that it also releases active von Willebrand factor, one of the factors needed for normal blood clotting, from its precursor. "The working hypothesis is that furin is responsible for the maturation of a number of seemingly unrelated precursors," Thomas says. What they all have in common is a motif of four amino acids (arginine-X-lysine/arginine-arginine), that marks the site of the cutting.

In addition to nerve growth and von Willebrand factors, the proteins with this motif include the precursors of the AIDS virus envelope protein gp120 and of the influenza virus hemagglutinin protein, both of which are needed to produce infectious virus particles. These precursors are cut by cellular proteases, and now, Thomas says, "20 groups, going on 50" want to find out if furin does the job.

That is only one of many questions that researchers are going to want to answer about the prohormone-cleaving enzymes. For one thing, there may be more than the three found so far. How the various cleaving enzymes interact to produce the large number of peptide products known to require such processing is another question. As Chrétien points out, the patterns of protease activity are likely to be particularly interesting in the brain, because differential processing of prohormones like POMC is one of the ways in which the immense functional diversity of brain neurons can be generated. "The enzymes stand at the crossroads of all the pathways synthesizing neuropeptides," Chrétien notes.

And then there is the possible role of the enzymes in embryonic development, a line of inquiry that will be aided by two recent discoveries. Thomas and his grad student Joel Hayflick have identified a *kex2*-related gene that is active early in the development of the fruit fly. And Ken Peters, a graduate student in Ann Rose's lab at the University of British Columbia in Vancouver has identified one that affects the development of the roundworm *Caenorhabditis elegans*. Both of those organisms are much more amenable to genetic and developmental studies than mammals.

Finally, there are suggestions that the convertases themselves may require processing in order to be activated. If so, that, too, would only seem fitting. ■ JEAN MARX