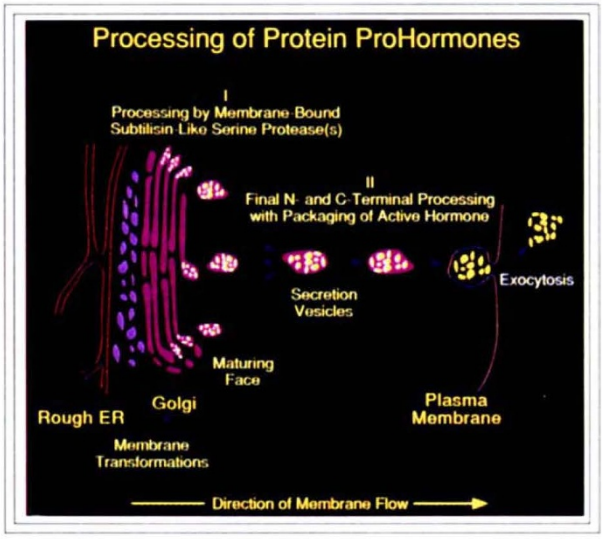
MAMMALIAN SUBTILISINS

NEW KEY TO PROTEIN PROCESSING?

CAMBRIDGE, Mass.—Three years ago Genentech's (So. San Francisco, CA) Cori Gorman had a novel solution to an identity crisis. In the 20 plus years since the proinsulin precursor was found to convert to active insulin by "dibasic site" cleavage, scientists could not find the enzyme responsible. As the list of proteins that underwent the enzymatic knife grew to those in viruses, the ubiquity of this cellular mechanism made the dilemma even more frustrating. Commercially, the problem is critical: many biomedically important "mature" proteins exist initially in the "pro" form. Understanding the enzyme that processes proteins means cost-effective production.



Gorman studied consensus sequences of her employer's insulin-like recombinant relaxin—a cervix-dilating hormone that induces labor—and thought a subtilisin-like serine protease might be responsible. "Everyone threw up their hands," she says. "They all told me, `Don't you know those are only found in procaryotes?'"

But Gorman had her revenge on the naysayers at a recent Serono Symposium in Norwell, MA, entitled "Cell Biology and Biotechnology: Novel Approaches to Increased Cellular Productivity." Data from her group,[1,2] and other researchers, strongly implicate a new enzyme family as responsible for prohormone cleaving, mammalian subtilisin-like enzymes.

Three flavors

So far, the newly identified mammalian subtilisins come in three flavors. PACE (Paired basic Amino acid residue Cleaving Enzyme) is apparently found in almost all mammalian tissue, suggesting activity in constitutive protein processing. PC1 is localized in the anterior and posterior lobes of the pituitary, while PC2 restricts itself to the intermediate lobe of that gland. The gland-associated enzymes hint that they normally process proteins following a regulated secretory route.

Gorman decided to test PACE's ability to process neurotrophic growth factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3). In a transient expression system using human kidney (HK) 293 cells, Gorman found that PACE converts the NGF precursor efficiently, but falters with NT-3 and languishes in its attempts to process BDNF. Noting intermediate protein sizes during NGF processing, Gorman correlated three upstream dibasic cleavage sites that suggested PACE cuts progressively, whittling the precursor down to its active form. Supporting this hypothesis was the observation that NT-3, with one upstream site, processed better than BDNF, with none. To test this idea, Gorman put in upstream dibasic sites in BDNF and saw efficient processing. "The take-home message," says Gorman, "is that changing the substrate can enhance protein processing dramatically."

Switching gears to look at PC1 and PC2, Gorman took on a formidable challenge: produce recombinant relaxin in mammalian cells that don't have a regulated secretory pathway. Present industry standards dictate expressing the cDNA coding for mature relaxin in *Escherichia coli* then lysing the bacteria and purifying and refolding the protein, a series of time-consuming and costly processes.

In the same HK 293 co-transfection system, Gorman showed that PC1 could successfully process relaxin. Here the message was clear: "Adding the right mammalian subtilisin-like enzyme," says Gorman, "can successfully produce properly folded protein."

Despite these exciting results, Gorman cautions that they are preliminary: the mammalian subtilisin family is new and must withstand more scientific scrutiny before they are mechanistically understood and completely accepted by the research community.

Regulation of PACE, PC1, and PC2 activity is still a major mystery. The enzymes may themselves be zymogens, requiring cleavage by other proteases for activation, much like their substrates. Tracking down this series of activating cleavages appears at first like staring into a hall of mirrors. But an appealing hypothesis suggests that PACE may cleave itself, as well as PC1 and PC2.

Fine-tuning cell systems that normally don't have a regulated secretory pathway presents another challenge. "We've seen secretion of PC1 and PC2 in 293 cells, despite the fact the cell line doesn't have secretory vessels," says Gorman. "This secretion may make an enzyme concentration problem in attempting high levels of expression in certain proproteins." But, so far at least, introducing this mechanism doesn't seem to harm the cells.

Cost effective?

Gorman staunchly defends mammalian-cell protein production as the future method of choice. Genentech routinely does several-thousand liter runs of mammalian cells, with multiple harvests of protein over antibody columns. With relaxin—at the bench level—Gorman claims 70 percent yields of properly folded hormone. With *E. coli*, on the other hand, "you wind up doing all the work for the bacteria," she says. Quality control presents a nightmare in *E. coli* systems: out of a typical 30 percent relaxin yield, as little as 5 percent is biologically active. "For neurotrophic factors especially," Gorman says, "you can make grams of them, but they aren't correctly folded. So where are you?" She sees the cost of large-scale mammalian cell culture coming down with more experience.

The explosion of interest in this area should soon deliver up a judgment on mammalian-cell protein production. "Sometimes you are afraid to go to the library," says Gorman, "for fear that someone has published what you are working on."

—Stephen M. Edgington

References

1. Smeekens, S., and D. Steiner. 1990. *J. Biol. Chem.* **265:**2997-3000.
2. Seidah, N., Gaspar, L., Mion, P., Marcinkiewicz, M., Mbikay, M., and M. Creitein. 1990. *DNA and Cell Biology* **9**:415-424.