Proteolytic Processing

I wish to state unequivocally that our position has never been that proinsulin is cleaved to insulin "before being packaged in secretory granules" as implied by the citation of our work (reference 42) in the article "Pathways of protein secretion in eukaryotes" by Regis Kelly (*1*). Our view on the localization of the conversion process has been that it occurs mainly within newly formed secretory granules after these bud off from the Golgi, but may begin even before packaging is completed in the (trans) Golgi elements. Our early antimycin experiments (*2*) did not exclude the possible involvement of the Golgi. However, the elegant experiments of Orci *et al.* (*3*) with monoclonal antibodies specific for intact proinsulin developed in our laboratory by Madsen (*4*) show that transport to and through the Golgi stack is halted by antimycin and confirm that a major site of processing is the (pro)secretory granule compartment, including clathrin-coated vacuolar structures in the trans-Golgi compartment. These findings are thus compatible with the concept that the sorting functions of the Golgi bring the converting proteases and prohormones into contact during packaging and sustain our often stated view of conversion as a Golgi/granule process (*5*).

It should be pointed out, however, that some degree of processing before sorting of prohormones need not necessarily abrogate a receptor-mediated sorting process in the Golgi, if one indeed exists for primary secretory products such as proinsulin. Unfortunately, we simply do not know yet whether most of the precursor proteins (for example, pro-opiomelanocortin, proglucagon, and so forth) have an organized globular structure that may resist dissociation even after some degree of limited proteolysis. A more current example than the "classical" ribonuclease S-peptide complex might well be the binding of vasopressin and oxytocin during transport to their respective neurophysins with which they are cosynthesized as single chain precursors (*6*). We clearly need much more information on the three-dimensional organization of precursor molecules as well as on the biochemical properties, subcellular localization, and activation of the prohormonal processing proteases.

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Response: I agree entirely with Steiner's statements on proteolytic processing. I also am in complete agreement with his conclusions in his published work and had hoped to describe them fairly and accurately in that section of my review. In trying to understand why my statement was objectionable to him I realize that there are ambiguities in usage in this area that can lead to unnecessary confusion, as in this case. A secretory vesicle matures from a precursor secretory vesicle in the trans-Golgi region of the cell. In general, the mature vesicle is the organelle that fuses with the plasma membrane when the cell is stimulated. No one questions that in the mature vesicle a large fraction of polypeptide hormone precursor is proteolytically processed. Can it be processed before packaging into the mature secretory vesicle? Steiner and I are in no disagreement that the answer is yes. As Steiner points out, the site of conversion of proinsulin has been localized by Orci and his colleagues to an immature secretory granule, with a clathrin coat and an electron dense core (*1*). Such an immature granule might not be well separated from mature granules on isolation. If so, processing would certainly occur in a vesicle fraction, but not necessarily in mature secretory vesicles. Whether or not some processing occurs in mature vesicles, proteolysis can occur in a trans-Golgi region, where sorting is also thought to take place. It is therefore valid to question, as I did, whether sorting precedes processing or vice versa.

Steiner raises an excellent point in stressing that we do not know what effects proteolytic cleavage have on the structure of the peptide hormones precursor. If the cleavage products remain associated after proteolysis, then the logical argument I made that sorting should precede proteolysis loses its force. If processing does precede sorting, cosorting of peptide fragments could be explained if the fragments moved from Golgi to hormone-containing secretory vesicles by passive fluid flow (*2*). A completely passive flow mechanism, however, would seem to be unlikely since not all secreted proteins are packaged into dense-core secretory vesicles. What features of proteins are involved in selective packaging remain to be determined.

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