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REVIEW ARTICLE

Intracellular degradation of newly synthesized secretory proteins

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Introduction

The synthesis of secretory proteins differs in a fundamental way from the synthesis of most other proteins. Although the mechanism for translating the information contained in mRNA into a sequence of amino acids is universal, proteins destined to be exported from a cell are quickly segregated into a membrane-bound compartment, the rough endoplasmic reticulum. This sequestration begins while the message is being read, that is, while the protein is being elaborated. The rough endoplasmic reticulum is the first of a series of compartments, collectively termed the secretory pathway, through which the protein is transported to the extracellular space. The other major elements of the pathway are the Golgi complex and secretory vesicles (Palade, 1975).

Secretory cells are often divided into two groups, regulated and non-regulated. In regulated cells, proteins are concentrated and stored in vesicles and the rates of secretion can be varied in response to extracellular signals; hormone-producing cells fall in this category. In contrast, secretion rates in non-regulated cells, such as plasma cells, fibroblasts, and liver cells, are maintained at relatively constant levels. This classification is not absolute. Certain types of regulated cells, for example, cells which synthesize parathyroid hormone and prolactin, actually contain two pools of secretory protein; material in one pool is secreted continuously in a manner similar to non-regulated cells, while protein in the other pool is stored and secreted in a fashion typical of regulated cells. It is not known whether a single cell can contain both kinds of pools or the different pools correspond to subcategories of a general cell type.

Topologically, the secretory pathway is separated from both the cell interior and the extracellular space. Within this protected environment polypeptide chains destined for export are subjected to an extensive series of modifications. For example, most secretory proteins are first synthesized as higher molecular weight precursors designated by the prefixes 'pre' and 'pro'. Transformation from the 'prepro' form to the 'pro' form involves cleavage of a peptide at the *N*-terminus soon after it enters the lumen of the rough endoplasmic reticulum (Blobel, 1982). In many cases the 'pro' form is converted to final form in the Golgi complex. Other examples of post-translational modifications are alteration of amino acid side chains, addition and removal of carbohydrates, and formation of covalent bonds between and within polypeptides. These processes occur in the rough endoplasmic reticulum and Golgi complex.

Protein flux through the secretory pathway is not conservative; some of the molecules which enter the rough endoplasmic reticulum do not emerge in the extracellular space, but are broken down along the way. Indeed, in some cells more than half of the protein is destroyed within minutes of being synthesized. Thus, the list of post-translational modifications of secretory proteins must include degradation. The purposes of this review are as follows.

(1) Review the evidence that many different kinds of secretory proteins can be degraded rather than secreted.

(2) From consideration of degradation of specific proteins, attempt to discern common elements and thereby infer general properties of the degradation mechanisms.

(3) Propose a series of questions and speculations which might stimulate further investigation.

The scope of this review is limited to degradation that occurs before proteins are discharged to the extracellular space; specifically excluded from consideration is degradation of secretory proteins which have been secreted and then recaptured or otherwise taken up by a cell.

Methodology

Two basic strategies are used to measure degradation of secretory proteins. One method is based on release from peptide linkage of a marker amino acid or peptide. (Clearly, use of this technique is limited by the availability of appropriate markers.) It has been employed to study degradation of collagen, liver proteins and parathyroid hormone; although it is conceptually very simple, technical problems may make it difficult to use routinely (Bienkowski &

Abbreviations used: Ig, immunoglobulin; PTH, parathyroid hormone.

Engels, 1981). In the other method, the protein of interest is labelled metabolically by incubating cells or tissue with a radioactive amino acid for a specific period, then removing the radioactive precursor and incubating the cells under 'chase' conditions. Degradation is assessed by measuring the decrease in labelled protein during the chase period. (Techniques for assaying the protein often involve use of precipitating antibodies.) This method has two potential difficulties: (1) it may not be suitable for detecting low levels of degradation because it depends on measuring differences accurately; (2) ability to measure degradation may be very sensitive to the length of the labelling period and the sampling schedule during the chase period.

Degradation of secretory proteins

Collagen

Collagen is a major structural protein in mammals. It is a long, thin molecule composed of three subunits called α chains, which twist about each other to form a triple helix. The α chains are synthesized as higher molecular weight precursors, termed pro α chains, which associate in the rough endoplasmic reticulum to form procollagen which, in turn, is transported through the secretory pathway and processed to collagen outside the cell. Collagen has an unusually high content of proline which is a major determinant of the secondary structure of the α chains. Many prolyl residues are hydroxylated after they are incorporated into peptide linkage, and these hydroxyprolyl residues have a critical role in stabilizing the triple helical structure of the collagen molecule. Formation of hydroxyproline takes place in the rough endoplasmic reticulum; the reaction is mediated by the enzyme prolyl hydroxylase and requires Fe^{2+} and ascorbate as cofactors.

There have been more reports on intracellular degradation of newly synthesized collagen than of any other secretory protein (for review, see Rennard *et al.*, 1982). Collagen degradation is studied by incubating cultured cells or tissues with [^{14}C]proline and measuring the release of hydroxy[^{14}C]proline in either free form or low molecular weight peptides (Bienkowski & Engels, 1981). Since hydroxyproline is formed by enzymic hydroxylation of prolyl residues already incorporated into peptide linkage, and since hydroxyproline is found almost exclusively in collagen, release of this imino acid is evidence that either collagen or a collagen precursor has been degraded. When stationary phase cultures of human foetal lung fibroblasts are incubated under conditions which permit maximum hydroxylation of prolyl residues, approx. 15% of the hydroxy[^{14}C]-proline is recovered in a low molecular weight fraction. This is termed the basal level of degradation for these cells. Various lines of evidence suggest that the breakdown process occurs intracellularly: (1) free hydroxyproline appears in the cell layer before collagen can be detected in the culture medium; and (2) labelled collagen or procollagen added to cultures is recovered intact (Bienkowski *et al.*, 1978*a*,*b*; Berg *et al.*, 1980). It can be concluded, therefore, that the molecular species degraded is a collagen precursor, either procollagen or pro α chains. Collagen degradation has been observed in many kinds of connective tissue cells and the levels range from less than 10% to greater than 40%. While it is likely that these differences reflect both real variations among cell types as well as differences in techniques, it should be emphasized that degradation has been observed in every system tested. However, of the five genetically distinct types of collagen (I, II, III, IV and V) it has only been shown that three types are degraded intracellularly (I, II and IV) (Duchene *et al.*, 1981; Palotie, 1983); the question of whether the others (III and V) are also broken down intracellularly must await studies using cells or tissues which synthesize only those types.

Krieg *et al.* (1980) measured degradation in normal and virally transformed human lung fibroblasts; although transformation decreased collagen synthesis by a factor of 20 (which corresponded to the decrease in collagen mRNA), the percentage degradation was not changed significantly. This finding suggests that degradation and synthesis are regulated independently.

Degradation in human fibroblasts increases significantly when culture conditions are manipulated so that the conformation of newly synthesized collagen molecules is abnormal. Structural defects can be introduced by incubating cells with proline analogues which are incorporated into peptide linkage but which prevent correct folding of the triple helix (Bienkowski *et al.*, 1978*a*; Berg *et al.*, 1980). Defects in molecular structure can be introduced at the post-translational level by inhibiting formation of hydroxyproline. For example, collagen synthesized by fibroblasts in exponential phase of growth is underhydroxylated because the level of prolyl hydroxylase is relatively low (Berg *et al.*, 1980; Steinmann *et al.*, 1981); underhydroxylation also results when cells are incubated in the absence of ascorbate (Steinmann *et al.*, 1981). Interestingly, however, degradation is not complete in any of these instances and this suggests that some molecules with abnormal conformation escape being broken down.

In a very important series of experiments, Berg *et al.* (1980) showed that the increase in degradation observed in cultures which synthesized abnormal collagen was blocked when either inhibitors of lysosomal proteinases [chloroquine, NH_4Cl, TLCK (7-amino-1-chloro-3-L-tosylamidoheptan-2-

one) or leupeptin] or a microtubule disruptor (colchicine) was added to the incubation medium. These agents had no effect on the basal level of degradation. To study the fate of abnormal collagen, Berg and colleagues used cells in exponential phase, or stationary cultures incubated with the proline analogue *cis*-hydroxyproline. Recent work in this laboratory has shown that the high level of degradation observed in ascorbate-deprived cultures is also reduced to the basal level when NH_4Cl is present (R. Bienkowski, unpublished work). While experiments involving inhibitors of various cellular functions must always be interpreted with caution, these experiments strongly suggest that degradation of abnormal collagen is caused by lysosomal proteinases.

Fig. 1 presents a scheme which accounts for much of the available experimental evidence. The principal features of this model are as follows.

(1) Intracellular degradation has two components, basal and enhanced.

(2) The basal component operates continuously (even in systems which synthesize normal, fully hydroxylated collagen).

(3) Basal degradation occurs at a point in the secretory pathway before the site of colchicine block. Since this block is thought to be located beyond the Golgi complex, the model predicts that proteinases capable of digesting procollagen or pro α chains are to be found in either the rough endoplasmic reticulum or the Golgi complex.

(4) Basal degradation does not involve lysosomes, since it is not suppressed by inhibitors of lysosomal proteinases.

(5) The enhanced component of degradation, in contrast with the basal component, does involve lysosomes but does not operate continuously; rather, it is 'activated' when abnormal collagen is synthesized. The model predicts the existence of a mechanism which assesses structural integrity of molecules and sorts them accordingly. The model further predicts that molecules with abnormal conformation are transported to a lysosomal compartment where they are degraded.

It must be emphasized that many elements of this scheme are frankly speculative. The model is useful to the extent that it suggests further experiments and makes predictions about their outcomes. However, it clearly has limitations because it does not address several important questions:

(1) Are the molecules degraded by the basal component randomly selected, or do they differ in some way from the molecules that are not degraded?

(2) Why are not all abnormal molecules degraded? (There may be a threshold of deviation from normal conformation that must be reached before a molecule is recognized as defective; alternatively, the recognition mechanism may not operate with absolute efficiency.)

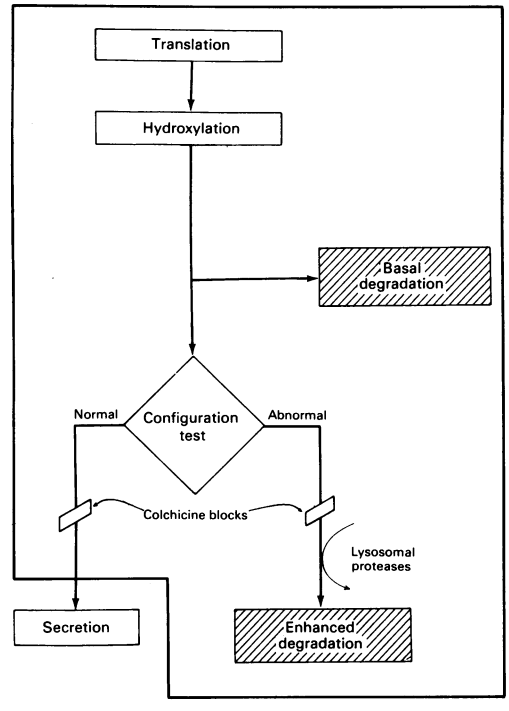

Fig. 1. *Intracellular degradation of newly synthesized collagen*
Measurement of collagen degradation is based on release of hydroxyproline from peptide linkage. This model accounts for the experimental evidence and predicts that degradation has at least two components, basal and enhanced. The pathway leading to enhanced degradation is activated when newly synthesized molecules have abnormal configurations; this component involves lysosomal proteinases. The decision diamond symbolizes a set of (hypothetical) recognition, sorting and transport mechanisms. The basal component is located, both spatially and temporally, beyond the site of hydroxylation of prolyl residues and before the recognition mechanisms. It is not clear whether molecules enter this pathway in a selective or random fashion.

(3) The model does not explain why degradation is elevated in circumstances not associated with synthesis of abnormal collagen, for example, when the intracellular level of cyclic AMP is increased (Baum *et al.*, 1980).

As already noted, intracellular degradation has been characterized more extensively for collagen than for any other secretory protein. To the extent that collagen is an 'ordinary' secretory protein,

insights gained from further studies of its rapid turnover can be expected to have wide applicability.

Immunoglobulin M heavy chains (IgMμ)

Immunologic response is effected in part by the various classes of immunoglobulins produced by B lymphocytes. The kinds and amounts of immunoglobulins made by a given cell depend on its state of differentiation. Undifferentiated B cells produce IgM which is found only on the cell membrane. In contrast, differentiated B cells secrete IgM as well as incorporate it into the membrane. IgM is a high molecular weight glycoprotein that consists of two light chains, designated κ, and two heavy chains, designated μ. The secreted and membrane forms of the heavy chains, μ_s and μ_m, differ slightly in primary structure, and μ_m chains are more extensively glycosylated than are μ_s chains. Various groups have studied intracellular degradation of μ_s chains in normal B cells and in cell lines derived from them. The basic procedure is to incubate the cells with a radioactive amino acid for a relatively short time and then assay the amount of μ_s at various times during the chase period; the net loss in labelled μ_s is taken as a measure of intracellular degradation.

Sidman (1981) showed that resting (undifferentiated) B lymphocytes from mice synthesize μ_s chains but do not secrete IgM; he interpreted this result as evidence for intracellular degradation of the μ_s chains. Dulis *et al.* (1982) reported a similar effect in a line of B lymphoma cells (Daudi) which are phenotypically similar to non-secreting (resting) B cells; they found that Daudi cells synthesized μ_s chains and then degraded them rapidly (half-life about 80 min). The site of degradation was not determined; however, Dulis *et al.* (1982) observed that the μ_s chains were not resistant to digestion with the glycosidase endo H, and they speculated that segregation of molecules destined to be degraded occurred at some point in the secretory pathway before the site of terminal glycosylation (which is thought to be located in the *trans* region of the Golgi complex). The work of Sidman (1981) and Dulis *et al.* (1982) is strong evidence that intracellular degradation has a role in regulating phenotypic expression of resting B cells.

Sidman *et al.* (1981) also studied production of μ_s chains in normal and mutant hybridoma cell lines derived from activated (secreting) lymphocytes. In the normal line μ_s chains were secreted and not degraded. However, in two mutant lines the chains were degraded very rapidly (with half-lives of 10 min and 80 min); these molecules were grossly abnormal in that significant portions of the polypeptide as well as carbohydrate units were missing. The investigators further showed that μ_s chains synthesized by normal cells were degraded almost completely when glycosylation was blocked by tunicamycin. An obvious interpretation of this result is that the nonglycosylated molecules were recognized as 'abnormal'. (Interestingly, tunicamycin had little effect on the rate of degradation in one of the two mutant lines, and it actually slowed turnover of abnormal μ_s in the other line.) Sidman *et al.* (1981) further showed that mixed hybrids of normal and mutant cells synthesized both normal and abnormal μ_s chains, but only normal molecules were secreted. This experiment suggests the existence of mechanisms which can recognize the difference between normal and abnormal molecules and selectively degrade the latter.

Casein

Casein, the principal milk protein, is synthesized by epithelial cells of the lactating mammary gland. Razooki Hasan *et al.* (1982) found that 50–90% of the casein synthesized by cultured mammary glands of pregnant rabbits was degraded intracellularly. The organs were incubated for 2 h with either [^{3}H]-leucine or [^{3}H]proline and then washed thoroughly to remove the label. Incubation was continued for up to 28 h and the amount of labelled casein was measured at various times. Incorporation of label into casein continued during the first 1 h of chase and during the next 2 h the amount of labelled casein decreased to approx. 40% of the peak value. However, the decrease was inhibited completely when chloroquine was included in the chase medium, suggesting that lysosomal proteinases were responsible for the rapid disappearance of the protein. Only casein synthesized during the chase period was degraded; when cycloheximide was included in the chase medium incorporation of label was inhibited and the amount of casein present at the end of the labelling period remained unchanged for the rest of the incubation. Less than 10% of the radioactive casein synthesized by the explants was detected in the medium. Since synthesis of casein is extremely sensitive to hormonal factors and the intracellular level of calcium, it is possible that the culture system did not support optimal phenotypic expression of the lactating gland. Nevertheless, these experiments are important because they demonstrate that degradation can function at the post-translational level to regulate production of a specialized secretory protein. Furthermore, this work indicates that lysosomes are involved in the degradation process.

Certain technical aspects of this work illustrate the difficulties involved in measuring degradation. The ability to detect breakdown of the newly synthesized casein depended largely on continued incorporation of label during the chase period. (This suggests the existence of a pool of amino acids which does not exchange rapidly with the culture medium.) A previous study from the same laboratory of casein production in explanted mammary tissue found no

evidence for degradation (Wilde *et al.*, 1980). Those experiments used a 1 h labelling period and casein was assayed after several hours of chase; because of that sampling schedule, the continued synthesis and subsequent breakdown of casein during the early phase of the chase were missed completely.

Liver proteins

The liver synthesizes and secretes many kinds of proteins. Glaumann *et al.* (1982) considered the question whether any of these are degraded rather than secreted. Rats were injected with [^{3}H]leucine and killed 4 h later. Microsomes (endoplasmic reticulum) and Golgi complexes were isolated from the livers and incubated for 2 h, during which time degradation of labelled protein was monitored by measuring the appearance of acid-soluble radioactivity; approx. three times as much radioactivity was released from microsomes as from the Golgi fraction. When secretion of protein was inhibited by administering vinblastine to the animals 0.5 h after the label, the amount of radioactivity released from the Golgi fraction increased threefold, but there was no change in the amount released from the microsomes. Further studies showed that the pH optimum of proteolysis in the Golgi fraction was in the acidic range (pH 4–5). Ultrastructural studies revealed little contamination with lysosomes and measurement of lysosomal marker enzymes suggested the same conclusion. However, in a separate morphological study, Marzella & Glaumann (1980) showed that when secretion of liver proteins was inhibited by vinblastine, secretory vesicles either became sequestered within lysosomes or fused with them.

This work provides evidence for proteolysis in two components of the secretory pathway, the endoplasmic reticulum and the Golgi complex. Since degradation was observed in organelles isolated from control livers it is possible that at least some fraction of the proteins synthesized by the liver is broken down continuously. Interestingly, lysosomes were involved in degradation only when secretion was inhibited. These features are incorporated into the model for degradation of liver proteins shown in Fig. 2. Note that vinblastine is depicted as having two effects. Secretory vesicles that are already formed are 'side-tracked' and become associated with lysosomes. In addition, secretory material 'backs up' and eventually is degraded within the Golgi complex.

The experiments described by Glaumann *et al.* (1982) considered only 'liver proteins' in general, and it would be extremely useful to determine the extent to which specific proteins such as albumin are broken down.

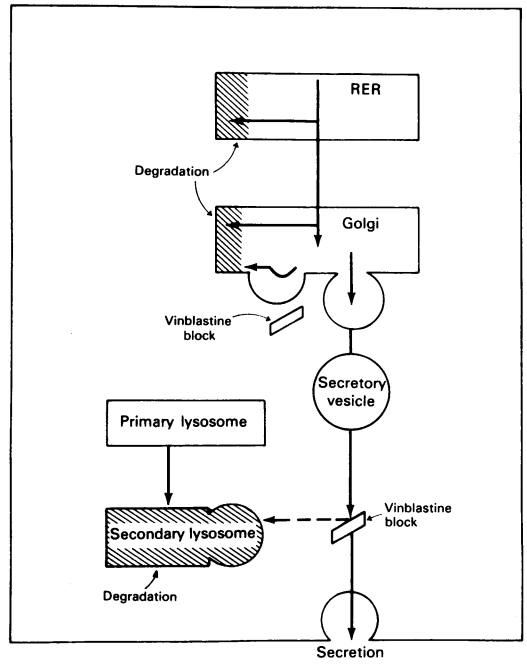

Fig. 2. *Intracellular degradation of newly synthesized liver proteins*

This model, based on the work of Glaumann *et al.* (1982) and Marzella & Glaumann (1980), shows that degradation occurs in the rough endoplasmic reticulum (RER) and Golgi complex (indicated by the shaded areas); the data are not sufficient to decide whether molecules enter these catabolic pathways in either a selective or a random fashion. Inhibiting secretion has two effects: (1) secretory vesicles associate with primary lysosomes to form secondary lysosomes in which secretory protein is degraded; and (2) secretory protein in the Golgi complex 'backs up' and is degraded in that organelle.

Parathyroid hormone

PTH is a low molecular weight protein which has a critical role in regulating the level of calcium in blood. Studies conducted *in vivo* and *in vitro* have shown that there is a strong inverse relationship between PTH production and calcium ion concentration. There are two intracellular pools of PTH; one pool secretes hormone rapidly while the other stores it. Secretion from the second pool can be stimulated by agents such as dibutyryl cyclic AMP or β-agonists (Morrisey & Cohn, 1979*a*).

Several groups have demonstrated that a significant amount of PTH is degraded intracellularly. Morrisey & Cohn (1979*b*) used a pulse–chase technique to follow the fate of newly synthesized PTH in cultured rat parathyroid glands. The tissue was incubated for 10 min with [^{35}S]methionine, then transferred to chase medium and samples were taken

for analysis at frequent intervals (10–20 min). Experiments were carried out at low and high calcium concentrations. At the low concentration the amount of PTH (actually proPTH) was greatest approx. 10 min after the chase was initiated and then it began to decline. After 50 min of chase only 30% of the original (labelled) protein remained. Since proPTH was converted stoichiometrically to PTH, it was concluded that PTH was the molecular species that was degraded. Furthermore, since proPTH is processed to PTH in the Golgi complex it was also concluded that degradation occurred in newly formed secretory granules. (It can be argued, however, that the data do not exclude the possibility that degradation took place in the region of the Golgi complex where secretory vesicles are formed.). When the experiments were carried out at high calcium concentration, an additional component of degradation was observed which accounted for approx. 10% of the original protein; this second degradation process occurred after 60 min of chase, which is much longer than the time for newly synthesized PTH to pass through the Golgi complex.

Hanley *et al.* (1978) detected fragments of PTH released from bovine parathyroid glands cultured in a continuous perifusion system. The peptides were released from both the *N*- and *C*-termini of the molecule. Although the investigators did not calculate the relative amounts of peptides and intact hormone, a conservative estimate based on their data suggests that more than half the newly synthesized PTH molecules were degraded. Hanley *et al.* (1978) argued that production of the fragments occurred intracellularly rather than after PTH was secreted because: (1) the perifusion medium was sampled very soon after it was in contact with the gland; (2) intact PTH, when passed through the system, was not degraded; and (3) production of fragments was directly related to the concentration of calcium ions.

Oldham *et al.* (1971) studied the dynamics of PTH production in organ cultures of pig parathyroid tissue by a morphological technique. In one series of experiments tissue was incubated first in low-calcium medium for 2.5 h and then in high-calcium medium for 2.75 h. Electron micrographs of the tissue showed an increase in the number of secretory vesicles within the cell as well as many secretory granules within autophagic vesicles.

These reports clearly demonstrate that intracellular degradation, operating at the post-translational level, can regulate PTH production in response to extracellular signals such as calcium concentration. However, the most inpressive finding to emerge from this work is that, under normal culture conditions, more hormone is degraded than secreted. Fig. 3 presents a model which accounts for the available data on intracellular degradation of PTH. It suggests that most of the breakdown occurs in the distal region (*trans*) of the Golgi apparatus and that this process generates the relatively large peptides observed by Hanley *et al.* (1978); the manner in which these peptides are released from the cell is not known. The relatively small increase in degradation that is observed when calcium concentration is increased probably takes place in lysosomes. This prediction could be tested by performing an incubation in the presence of lysosomotropic agents.

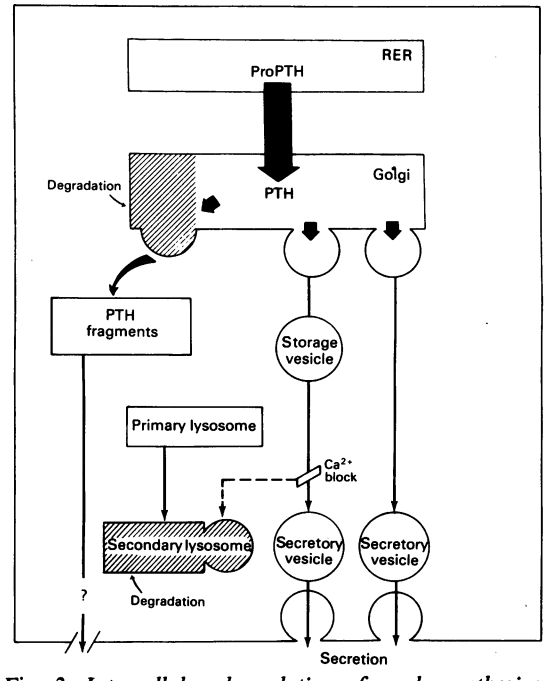

Fig. 3. *Intracellular degradation of newly synthesized parathyroid hormone*

This model suggests that degradation occurring in the Golgi complex (indicated by shading) generates PTH fragments from the *N*- and *C*-termini of the parent molecule. The question mark indicates that the manner in which these peptides are transported out of the cell is not known; neither is it known whether the remaining portion of the PTH molecule is further degraded in the Golgi complex. The model also suggests that increasing the calcium level has the effect of blocking release of PTH from storage vesicles; these vesicles then associate with primary lysosomes to form secondary lysosomes in which PTH is degraded. Abbreviation used: RER, rough endoplasmic reticulum.

Prolactin

Prolactin is synthesized by specialized cells of the anterior pituitary. There are at least two intracellular pools of prolactin, and they differ in the rates at which the hormone is secreted from them. One

pool has a short turnover time, whereas the other appears to store prolactin for relatively long periods. The latter pool is more responsive to agents that are known to stimulate secretion of prolactin (Dannies, 1982).

Intracellular degradation of prolactin has been studied by several groups. Smith & Farquhar (1966) conducted an electron microscopic study of the fate of newly synthesized prolactin in pituitaries of nursing rats; when the stimulus for secretion was blocked (by removing the suckling rats), secretory vesicles containing prolactin accumulated intracellularly for up to 12h and were eventually degraded by lysosomes. Shenai & Wallis (1979) observed degradation of prolactin in organ cultures of female rat pituitaries. In one set of experiments they followed the time course of incorporation of [^{3}H]leucine into prolactin for 8h. The rate of production decreased abruptly (by approx. 75%) after 40min, suggesting that the protein was degraded soon after it was synthesized. In a second set of experiments pituitaries were incubated with label for 4h and then levels of prelabelled prolactin were measured at various times during a chase period; 33% of the prelabelled hormone was lost after 3h. Particular attention should be paid to the labelling schedules used in these two experiments. Because of the complicated kinetics of prolactin production (some is stored and some is secreted rapidly), the substrates for degradation may not be the same in both experiments. The site(s) of degradation is(are) not clear; while the authors did not show electron micrographs, they stated that the organelles of the secretory pathway appeared normal at the ultrastructural level (presumably they did not observe fusion of secretory vesicles with lysosomes).

Dannies & Rudnick (1980) studied degradation of prolactin in primary cultures of male rat pituitary cells. They found no evidence for breakdown under normal conditions; however, when secretion was inhibited by a 3 day exposure to bromoergocriptine, a dopamine agonist, 40% of the prolactin synthesized during a 2h pulse disappeared during a subsequent 9h chase period. Since the chase medium contained cycloheximide (to block incorporation of label into protein) protein synthesis was apparently not necessary for degradation to occur.

Maurer (1980) also studied prolactin degradation in cultures of female rat pituitary cells. The cells were incubated with [^{3}H]leucine for 30min and the labelling period was followed by a chase period of 24h. Only a small, variable amount of prolactin was lost in control cultures, but more than 50% of the hormone was degraded in cells treated with bromoergocriptine. Maurer (1980) also conducted a series of experiments with cycloheximide and concluded that protein synthesis was necessary to initiate, but not maintain, prolactin degradation induced by bromoergocriptine. In a further set of experiments Maurer (1980) found that chloroquine partially inhibited degradation, which indicated that the breakdown process was mediated by lysosomal enzymes.

In attempting to synthesize the work of the various groups into a coherent picture, it appears that the reports of Smith & Farquhar (1966), Dannies & Rudnick (1980) and Maurer (1980) are in basic agreement, i.e., degradation of prolactin is induced when secretion is inhibited, and at least part of this breakdown is due to lysosomal proteinases. There is no consensus, however, regarding the level of degradation under 'control' culture conditions. Whereas Dannies & Rudnick (1980) and Maurer (1980) found little or no degradation in primary cultures of dispersed cells, Shenai & Wallis (1979) observed very high levels of degradation in organ cultures of pituitary glands. This lack of agreement may be due to basic differences in technique; alternatively, different degradation mechanisms may be operating in the two kinds of culture systems.

Insulin

Halban & Wollheim (1980) reported that newly synthesized insulin was degraded intracellularly in cultured pancreatic islets from rats. Cells were incubated with [^{3}H]leucine for a relatively long time (3 days) and then the amount of labelled insulin in the cells was measured; parallel cultures were incubated under chase conditions for an additional 24h, after which time the amounts of labelled insulin in the medium and in the cells were determined. Glucose concentration during the labelling period was 8.3mM, but during the chase period cultures were incubated at either low (2.8mM), high (16.7mM) or control (8.3mM) levels. When the chase medium contained 8.3mM-glucose approx. 41% of the prelabelled insulin was lost from the cells, but only 16% appeared in the medium. Since a control experiment demonstrated that intact insulin added to the cultures was not degraded, it was concluded that 25% of the prelabelled hormone present in the cells at the end of the labelling period was degraded during the subsequent 24h chase. When cells were incubated in low-glucose medium only 4% of the insulin was detected in the medium and 37% was degraded by the cells. However, when the medium contained a high glucose concentration almost half of insulin was released and none was degraded. This report offers clear evidence that net production of insulin by pancreatic islet cells can be modulated post-translationally by an intracellular degradation mechanism. The authors speculated that lysosomes are involved in the breakdown process, but definitive information on this point is lacking, and experiments conducted with lysosomotropic agents would be illuminating.

Discussion

Fig. 4 presents a generalized model for intracellular degradation of newly synthesized secretory proteins. Basically, it is a representation of the secretory pathway in which the various possible sites of degradation have been indicated. Just as the details of the secretory process vary among different types of cells, so too, the mechanisms of degradation may depend on the nature of a particular cell and the protein(s) it produces. Nevertheless, several general features emerge from the analyses of individual cases presented in the previous section.

(1) *Degradation is common.* Among the secretory proteins which can be degraded are three hormones, a structural protein, a milk protein, liver proteins and a major component of the immune system. It is not known whether all secretory proteins can be degraded but it seems reasonable, at least, to hypothesize that intracellular degradation is a universal post-translational process.

(2) *Abnormal proteins are degraded more extensively than normal proteins.* Abnormalities in primary structure due to translation errors (incorporation of amino acid analogues into collagen) or to defects in gene structure (IgM heavy chains) correlate with increased intracellular degradation. [In this regard it should be mentioned that it has been known for several years that abnormal cellular proteins are degraded more rapidly than normal proteins (Goldberg & Dice, 1974; Goldberg & St. John, 1976).] As already noted, these results strongly

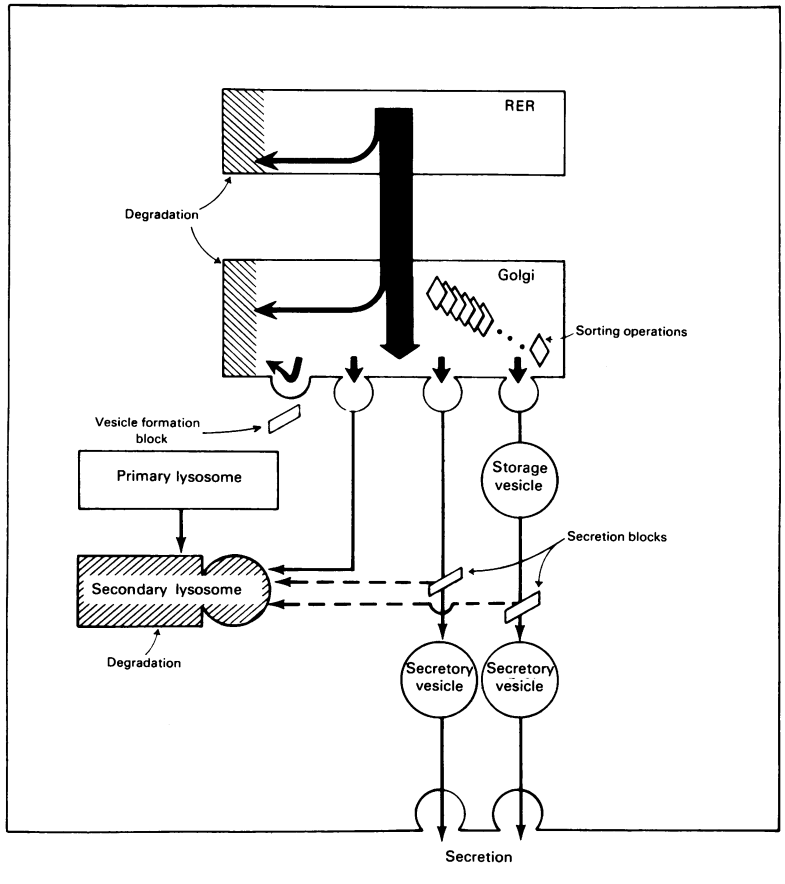

Fig. 4. *Generalized model for intracellular degradation of newly synthesized secretory proteins*
This scheme combines several of the characteristics of models constructed to describe degradation of specific proteins (compare Figs. 1, 2 and 3). The main features are: (1) degradation sites in the rough endoplasmic reticulum (RER) and Golgi complex (indicated by shaded areas); (2) mechanisms in the Golgi complex which recognize and segregate molecules and route them to different destinations; (3) response to inhibiting secretion includes accumulation and increased degradation of secretory protein in the Golgi complex, and formation of secondary lysosomes by fusion of vesicles with primary lysosomes and subsequent degradation of secretory protein.

suggest the existence of recognition and sorting mechanisms. The Golgi complex is a candidate for the location of these mechanisms because this organelle is known to perform various other 'traffic control' operations (Farquhar & Palade, 1981). A very important set of questions is what are the signals that mark a molecule as normal or abnormal, and how is this information processed?

(3) *Degradation increases when secretion is inhibited.* This effect has been observed for liver proteins, prolactin, insulin and PTH. Interestingly, Berg *et al.* (1980) found no increase in collagen degradation in human fibroblasts when the cells were treated with colchicine. Various explanations for this behaviour are possible: for example, the incubation time (6h) used by Berg *et al.* (1980) may not have been long enough to observe an effect of colchicine; alternatively, collagen may be an exception to a rule which holds for most other secretory proteins.

(4) *One component of degradation involves lysosomes.* Direct evidence for this assertion comes from morphological studies which show that secretory vesicles in liver cells and prolactin-producing cells can, under certain circumstances (which usually involve inhibition of secretion), fuse with primary lysosomes. Biochemical studies provide indirect evidence: lysosomotropic agents such as chloroquine and NH_4^+ completely block degradation of casein and prolactin and the increase in degradation observed when fibroblasts synthesize abnormal collagen.

(5) *Intracellular degradation has a non-lysosomal component.* Again, the evidence is both direct and indirect. Studies with subcellular fractions of liver show that the rough endoplasmic reticulum and Golgi complex release acid-soluble radioactivity at constant rates, suggesting that newly synthesized secretory protein can be degraded within these organelles. The indirect evidence is that a significant amount of collagen degradation is not inhibited by lysosomotropic agents. The studies with liver proteins suggest that proteolytic enzymes may be present in the secretory pathway of other types of cells. Indeed, Sidman *et al.* (1981) and Morrisey & Cohn (1979*b*) considered such a possibility in their discussions of mechanisms to explain degradation of IgM heavy chains and PTH.

It is interesting to note that Quinn & Judah (1978) detected cathepsin B in the Golgi fraction of rat livers and they presented evidence that it mediates the conversion of proalbumin to albumin. In addition, these investigators suggested that the same enzyme might have a similar function in processing proinsulin and proPTH. Cathepsin B has a pH optimum in the acidic range and can cleave a variety of peptide bonds, and it is usually thought to be confined to lysosomes. However, various investigators (Smith & van Frank, 1975; Novikoff, 1976) have noted that the so-called lysosomal proteinases can be detected in non-lysosomal compartments within the cell. If such relatively non-specific proteinases are present and active within the secretory pathway, then several questions arise regarding how, or whether, they are controlled so that they do not attack all newly synthesized secretory proteins. (This point is discussed further in section 9 below.)

(6) *Degradation is a mechanism for regulating production of secretory protein at the post-translational level.* This assertion is based on experiments with insulin, prolactin and PTH which show that, in response to appropriate physiological signals, net production can be lowered by increasing the rate of degradation. Evidently, degradation is a means of maintaining a cell in a state of readiness for maximal production of a secretory protein such as a hormone, and what is not needed within a relatively short time is broken down. Of course, production can also be controlled by varying the amount of mRNA available for translation, but this might require much longer periods of time.

(7) *Degradation is a mechanism for regulating phenotypic expression.* This is actually a stronger version of the previous statement. The only protein for which it holds rigorously is IgM μ_s which is synthesized but not secreted by resting B lymphocytes. It is not clear whether the almost complete degradation of casein synthesized by cultured mammary glands falls into this category, or whether it is more appropriately explained as a post-translational control of protein production.

(8) *Degradation occurs on several time scales.* Just as degradation takes place in different locations in the cell, so too can it occur in different time frames. Measured in units of the time necessary to synthesize a protein and transport it to the Golgi complex, breakdown of liver proteins, PTH and collagen is very rapid. In contrast, degradation of insulin, prolactin and some PTH occurs after relatively long times. It is interesting to speculate that rapid degradation occurs in the rough endoplasmic reticulum and Golgi complex, whereas slow degradation occurs in lysosomes.

(9) *Degradation can be extensive.* Consider the relative amounts of newly synthesized protein which can be degraded, rather than secreted, under normal culture conditions; collagen, 15%; insulin, 25%; PTH, 70%; casein, 90%; IgM heavy chains (resting B cells), 100%. An obvious question is what purpose is served by such apparent waste? Some writers have referred to this situation as a 'futile cycle', but this may be more an expression of our lack of understanding than an accurate assessment of a cell's metabolic economy. One can speculate that the breakdown phenomenon is related to regulation. As discussed above (section 6), controlling production

by varying the rate of degradation, in addition to modulating transcription and translation, may afford the cell a finer degree of regulation and enable it to respond appropriately to changes in conditions which occur on several time scales. The high basal levels of turnover may represent the metabolic price of having such a degradation mechanism in place.

Another possibility is that basal degradation is a consequence of having proteinases, which are necessary for processing precursor forms of proteins, in the secretory pathway. In addition to cleaving propeptides, these enzymes may have considerable activity towards other regions of the molecules. Metabolically, it may be 'cheaper' to synthesize more protein than to regulate the specificity of the enzymes.

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