Acquisition of Lubrol Insolubility, a Common Step for Growth Hormone and Prolactin in the Secretory Pathway of Neuroendocrine Cells

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Rat prolactin in the dense cores of secretory granules of the pituitary gland is a Lubrol-insoluble aggregate. In GH₄C₁ cells, newly synthesized rat prolactin and growth hormone were soluble, but after 30 min about 40% converted to a Lubrol-insoluble form. Transport from the endoplasmic reticulum is necessary for conversion to Lubrol insolubility, since incubating cells with brefeldin A or at 15 °C reduced formation of insoluble rat β-S-prolactin. Formation of Lubrol-insoluble aggregates has protein and cell specificity; newly synthesized human growth hormone expressed in AtT20 cells underwent a 40% conversion to Lubrol insolubility with time, but albumin did not, and human growth hormone expressed in COS cells underwent less than 10% conversion to Lubrol insolubility. del32–46 growth hormone, a naturally occurring form of growth hormone, and P89L growth hormone underwent conversion, although they were secreted more slowly, indicating that there is some tolerance in structural requirements for aggregation. An intracellular compartment with an acidic pH is not necessary for conversion to Lubrol insolubility, because incubation with chloroquine or bafilomycin slowed, but did not prevent, the conversion. GH₄C₁ cells treated with estradiol, insulin, and epidermal growth factor accumulate more secretory granules and store more prolactin, but not more growth hormone, than untreated cells; Lubrol-insoluble aggregates of prolactin and growth hormone formed to the same extent in hormone-treated or untreated GH₄C₁ cells, but prolactin was retained longer in hormone-treated cells. These findings indicate that aggregation alone is not sufficient to cause retention of secretory granule proteins, and there is an additional selective process.

Secretory proteins are synthesized on polysomes attached to the endoplasmic reticulum and transported through its membranes into its lumen, where the proteins fold. Vesicular or tubular structures transport folded proteins to the cis region of the Golgi complex, and the proteins process through the stacks of the Golgi complex to the trans side, after which vesicles deliver secretory proteins to the cell surface. Neuroendocrine cells, exocrine cells, peptidergic neurons, mast cells, and some other secretory cells have a specialized pathway to the cell surface in which certain secreted proteins are stored in concentrated forms in vesicles called secretory granules, so that the proteins are rapidly available in large amounts when needed. Concentration of protein hormones in granules is extensive; prolactin, for example, is 200 times more concentrated in dense cores of secretory granules than in the lumen of the endoplasmic reticulum (1).

A possible mechanism for concentrating proteins is self-association or aggregation; the formation of insoluble aggregates of prolactin in secretory granules is evidence for such a mechanism. Giannattasio et al. (2) isolated intact prolactin-containing secretory granules from rat pituitary glands and then removed the granule membranes with detergent. When the nonionic detergent Lubrol was used, the granule membranes dissolved, but the prolactin contents remained insoluble, and the dense cores of the granules retained their morphology (2). Dense cores of secretory granules with no surrounding membrane appear occasionally in the pericapillary space of the pituitary gland, indicating that the cores dissolve relatively slowly after exocytosis in animals (3, 4). Aggregates of prolactin resembling what are found in secretory core granules are detectable by electron microscopy in the lumen of the trans-Golgi cisternae, so the process begins before the granules form (5). Prolactin in solution has a tendency to aggregate at mildly acidic pH (6, 7); the tendency to aggregate in solution at acidic pH is a property shared by many proteins that are stored in granules (8–13). Such aggregation in solution has led to one of the simplest models for concentrating secretory granule proteins, which is that secretory proteins are soluble in the secretory pathway until they reach the trans-Golgi region, where they aggregate, facilitated by the decrease in pH that occurs there. In this model, aggregation in the trans-Golgi cisternae may play a role in sorting proteins as well as concentrating secretory granule proteins (14, 15), since small vesicles carry off soluble proteins, leaving behind aggregated proteins, too large to remove, to form dense cores of secretory granules.

Prolactin and growth hormone have several properties that make them useful for understanding the processes that lead to storage in secretory granules. They are small (22 kDa), monomeric proteins that are stored in granules and neither glycosylated nor proteolytically processed beyond the cleavage of the signal sequence necessary for transport into the endoplasmic reticulum. They are structurally similar proteins (16). Despite these similarities, their storage is regulated differently in a pituitary cell line. GH₄C₁ cells are rat pituitary tumor cells that make prolactin, growth hormone, chromogranin B, and secretogranin II. These cells accumulate secretory granules with dense cores of prolactin and store prolactin when cultured with insulin, estradiol, and epidermal growth factor (17). The patterns of prolactin release from GH₄C₁ cells cultured in these
conditions and prolactin release from primary cultures of lactotrophs are similar when release is stimulated by several different mechanisms (18), indicating that the regulated pathway of secretory granules in these cells behaves as it does in normal lactotrophs. When GH4C1 cells are cultured in the absence of estradiol, insulin, and epidermal growth factor, however, there are few dense core secretory granules in the cells, and prolactin storage is reduced (17). Treatment does not increase storage of other proteins in secretory granules in these cells, including growth hormone, despite its similarity to prolactin (17, 19–21). We have investigated whether hormone treatment increases the ability of cells to store prolactin by regulating their ability to aggregate prolactin preferentially.

MATERIALS AND METHODS

**Pulse-Chase Experiments**—GH4C1 cells, 1.5 × 10^5 cells/60-mm plate, were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-10 nutrient mixture plus 15% gelding serum with or without hormone treatment (1 nM estradiol, 300 nM insulin, and 5 nM epidermal growth factor), as indicated, and used 4 days after plating. For incorporation of 35S-amino acids, cells were incubated with 200 μCi of Express 35S-Protein Labeling Mix (PerkinElmer Life Sciences) in cytosine- and methionine-free Dulbecco's modified Eagle's medium with 10 mM MES, 10 mM HEPES, 5% gelding serum (Cryoprepare, Inc.), and 5% nonessential amino acids. The constructions were sequenced by the W. M. Keck Foundation Biotechnology Resource Laboratory and determined to be correct. 

**Antisera**—Antisera to human growth hormone was from the NIDDK National Hormone and Pituitary Program and A. F. Parlow and goat anti-rabbit antiserum conjugated to Texas red (Molecular Probes) as a second antibody.

**RESULTS**

The properties of newly synthesized proteins were assessed immediately after a 10-min incubation with 35S-amino acids and also during a subsequent incubation with unlabeled amino acids to follow changes as proteins progressed through the secretory pathway. At the end of a 10-min pulse with 35S-amino acids, 35S-prolactin was isolated in the culture. In GH4C1 cells treated with hormones, over 90% of newly synthesized 35S-prolactin was soluble when cell membranes were disrupted with Lubrol. At longer times after synthesis, some of the intracellular 35S-prolactin became insoluble in Lubrol (Fig. 1), a property of prolactin aggregates in the dense cores of secretory granules (2). Over 50% of the total 35S-prolactin (intracellular plus extracellular) in the culture, C, 35S-prolactin that was insoluble in Lubrol following the 10-min pulse as a percentage of total 35S-prolactin in the culture. Data in B and C represent the mean of two or more experiments, and the bars represent the ranges or the S.E. When no bars are shown, they fall within the symbols. Squares, prolactin in cultures with no drugs in the chase medium; circles, prolactin in cultures with 30 μM chloroquine in the chase medium.

**Fig. 1. Solubility and secretion of rat prolactin (PRL) in GH4C1 cells treated with estradiol, insulin, and epidermal growth factor.** A, incorporation of 35S-amino acids into rat prolactin after a 10-min pulse or after a 10-min pulse followed by a 30-min chase period. S, supernatant of a 50,000 × g centrifugation at 4 °C of cells lysed in 1.5% Lubrol. P, pellet from the same centrifugation. M, medium from the same sample. B, 35S-prolactin secreted into the medium following the 10-min pulse, as a percentage of the total 35S-prolactin (intracellular plus extracellular) in the culture. C, 35S-prolactin that was insoluble in Lubrol following the 10-min pulse as a percentage of total 35S-prolactin in the culture. Data in B and C represent the mean of two or more experiments, and the bars represent the ranges or the S.E. When no bars are shown, they fall within the symbols. Squares, prolactin in cultures with no drugs in the chase medium; circles, prolactin in cultures with 30 μM chloroquine in the chase medium.
cholate or SDS (Table I) than when they were disrupted by Lubrol. Purified prolactin remains at the top of a sucrose gradient in 0.3 M sucrose, but aggregates of prolactin in the dense cores of secretory granules sediment through 1.2 M sucrose, analyzed by velocity sedimentation over a sucrose cushion (2). This characteristic is shared by much of the Lubrol-insoluble form of 35S-prolactin in GH4C1 cells. The proportion of 35S-prolactin in GH4C1 cells that had the size and density to sediment through 1.2 M sucrose increased with time after synthesis (Table II). In most experiments, we added detergent directly to cells to obtain rapid and complete recovery of 35S-prolactin, but in one experiment, we lysed the cells with a ball homogenizer and removed the nuclei with a low speed spin before adding Lubrol. The results were similar to when cells were lysed directly; the proportion of intracellular 35S-prolactin that was Lubrol-insoluble after a 30-min chase period was 53 ± 2.5%, and 38 ± 1.6% sedimented through 1.2 M sucrose, indicating that the presence of nuclear components did not contribute to the sedimentation properties.

Although dense cores of prolactin secretory granules dissolve slowly enough so that they are detected outside cells of the pituitary gland after exocytosis (3, 4), the cores ultimately dissolve. Lubrol-insoluble prolactin in GH4C1 cells remains insoluble for over 1 h after the membranes are disrupted when incubated at 4 °C but becomes soluble rapidly when incubated at 37 °C, behavior consistent with the properties of prolactin in vivo (Table III).

GH4C1 cells cultured in the absence of estradiol, insulin, and epidermal growth factor contain few secretory granules and store little prolactin relative to what they make (17). Newly synthesized 35S-prolactin, however, showed the same conversion to a Lubrol-insoluble form in GH4C1 cells treated with hormones (Fig. 2). Thirty minutes after the incubation with 35S-amino acids, over 50% of the total 35S-prolactin was Lubrol-insoluble. At this time, little 35S-prolactin had been secreted, but over 70% was secreted by 2 h after the pulse (Fig. 2). The intracellular Lubrol-insoluble form decreased as 35S-prolactin was secreted from the cells, indicating that this form, and not just soluble prolactin, was secreted. After 2 h, more 35S-prolactin in GH4C1 cells that had not been treated with hormones (Table IV). Over 90% of the Lubrol-insoluble form of 35S-prolactin 30 min after synthesis sedimented through a 1.2 M sucrose solution (not shown), indicating that the insoluble form of prolactin has the same properties in GH4C1 cells that do not store prolactin as in cells that do.

Growth hormone is structurally similar to prolactin and is also stored in a concentrated form in secretory granules in the pituitary gland (16). GH4C1 cells produce both prolactin and growth hormone, but they do not store growth hormone well, and its storage is not increased by the hormone treatment that increases prolactin storage (17). In GH4C1 cells, intracellular rat 35S-growth hormone behaved as prolactin did; it also became Lubrol-insoluble within 30 min after the pulse period, and the amount of the insoluble form declined subsequently as growth hormone was secreted from the cells (Fig. 2). GH4C1 cells secreted most 35S-growth hormone within 2 h after its synthesis, whether cells were treated with hormones or not (Fig. 2 and Table IV).

The conversion to an insoluble form appears to require transport along the secretory pathway. After the pulse of 35S-amino acids, either treating GH4C1 cells with brefeldin A, which disrupts transport from the endoplasmic reticulum (26), or incubating them at 15 °C, which prevents transport from the endoplasmic reticulum (27), greatly reduced formation of the

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**Table I**

| Detergent       | Insoluble |
|-----------------|-----------|
| 1.5% Lubrol     | 59 ± 2    |
| 1.5% deoxycholate | 4.6 ± 0.6 |
| 0.1% SDS        | 9.9 ± 1.0 |

**Table II**

| Time of chase | Insoluble % | Sedimenting rapidly % | Fraction of insoluble that sediments rapidly % |
|---------------|-------------|-----------------------|-----------------------------------------------|
| 15            | 19 ± 2.1    | 12 ± 0.7              | 0.6                                           |
| 30            | 45 ± 1.3    | 36 ± 3.3              | 0.8                                           |
| 60            | 53 ± 2.8    | 48 ± 0.2              | 0.9                                           |

**Table III**

| Temperature °C | Insoluble % |
|---------------|-------------|
| 4             | 52 ± 3.1    |
| 22            | 16 ± 0.8    |
| 37            | 0.9 ± 0     |

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![Fig. 2](image_url)
Lubrol-insoluble form of rat prolactin in GH_4C_1 cells (Fig. 3). Incubating GH_4C_1 cells at 20 °C, which prevents transport from the trans-Golgi cisternae (28), reduced formation of the Lubrol-insoluble form of rat prolactin but not as much as the incubations at 15 °C.

The conversion did not absolutely require an acidic compartment in the secretory pathway. The addition of 30 μM chloroquine at concentrations that neutralize acidic compartments in neuroendocrine cells (29, 30) slowed the conversion of 35S-prolactin to the insoluble form in GH_4C_1 cells but did not prevent the conversion (Fig. 1C). Concentrations of 10 μM but not 1 μM chloroquine exerted similar effects, and the conversion of rat 35S-growth hormone was affected in a similar manner to 35S-prolactin in GH_4C_1 cells (not shown). Bafilomycin A1 inhibits vacuolar proton ATPases and therefore also neutralizes acidic compartments in cells (31) and had a similar effect to chloroquine (Fig. 4). Bafilomycin A1 (1.6 μM) slowed secretion but did not prevent conversion to the insoluble form. We confirmed that acidic compartments in the cells are neutralized by immunostaining for DAMP, which accumulates in acidic compartments (32). Treatment with chloroquine prevented the accumulation of DAMP in the cells (Fig. 5).

The conversion of growth hormone to a Lubrol-insoluble form also occurs in another neuroendocrine cell line, AtT20, a mouse pituitary cell line that produces proopiomelanocortin and stores ACTH in secretory granules. Human 35S-growth hormone, expressed in transiently transfected AtT20 cells, behaved as rat growth hormone in GH_4C_1 cells; it was soluble initially and became about 40% Lubrol-insoluble after 30 min (Fig. 6). Serum albumin is secreted directly from the liver without storage and shows the same behavior when expressed in AtT20 cells (33). 35S-Albumin, expressed in transiently transfected AtT20 cells, was mostly soluble after synthesis and showed no change in solubility with increasing time (Fig. 6).

The conversion of growth hormone to a Lubrol-insoluble form was less pronounced in COS cells than it was in neuroendocrine cells. Less than 10% of the total 35S-growth hormone became insoluble in transiently transfected COS cells (Fig. 7).

The ability to measure Lubrol insolubility in transfected cells allowed us to investigate how rigorous the structural requirements are for the process. Prolactin and growth hormone from almost all species, except a few fish, have a conserved proline in the second α-helix, proline 89 in human growth hormone. This residue is not involved in direct binding to growth hormone receptors but may be conserved because of its structural effect that causes a kink in the second helix so this helix bends toward the other α-helices (34). Substitution of a leucine for this proline (P89L GH) is likely to disrupt the bend; such a form of growth hormone has found with growth hormone deficiency (35). Growth hormone has a long amino acid loop that connects helix 1 to helix 2. A naturally occurring form of human growth hormone that results from alternate splicing of the GH1 gene gene has amino acids 32–46 deleted (del32–46 GH) (36). del32–46 GH is capable of folding almost normally (37), but the molecule has less flexibility in the connecting loop and lacks a small α-helix normally found in residues 38–47 (34). Both P89L GH and del32–46 GH acquire Lubrol insolubility when expressed in AtT20 cells to almost the same extent as wild-type hormone (Fig. 8). Both mutant proteins are secreted slightly more slowly.

### Table IV

**Secretion of newly synthesized prolactin and growth hormone from GH_4C_1 cells**

| Growth hormone | Prolactin |
|----------------|-----------|
| Untreated      | 77 ± 3    | 66 ± 5    |
| Treated        | 72 ± 6    | 36 ± 3    |

*Note: The percentage of intracellular prolactin.

![Figure 3](image3.png)

**Fig. 3.** Effect of agents that affect transport in the secretory pathway on conversion of rat prolactin to a Lubrol-insoluble form in GH_4C_1 cells treated with estradiol, insulin, and epidermal growth factor. Cells were incubated for 10 min with 35S-amino acids, followed by a 30-min chase period in the presence or absence of 5 μg/ml brefeldin A (A) or a 30-min chase period at 37, 20, or 15 °C (B); 35S-prolactin in the pellet after the chase period is presented as a percentage of intracellular prolactin.

![Figure 4](image4.png)

**Fig. 4.** Effect of bafilomycin A on the solubility and secretion of rat prolactin (PRL) in GH_4C_1 cells treated with estradiol, insulin, and epidermal growth factor. Cells were incubated for 10 min with 35S-amino acids, followed by a 30-min chase period. 35S-prolactin in the medium as a percentage of the total in the cultures. Details are as described in the legend to Fig. 1. Circles, prolactin in cultures with no drugs in chase medium. Squares, prolactin in cultures with 1.6 μM bafilomycin A in the chase medium.

![Figure 5](image5.png)

**Fig. 5.** Accumulation of DAMP in the presence or absence of chloroquine in GH_4C_1 cells treated with insulin, estrogen, and epidermal growth factor. Cells were incubated with 150 μM DAMP in the absence (A) or presence (B) of 30 μM chloroquine, and the accumulation of DAMP was assessed by immunofluorescence.
than wild-type GH (Fig. 8). The distribution of these mutants in transfected AtT20 cells is the same as that of wild-type, with heaviest staining in a region near the nucleus, consistent with accumulation in the Golgi complex, and in punctate staining near the plasma membrane, consistent with accumulation in secretory granules (Fig. 9). The difference that exists between wild-type growth hormone and the two mutants is therefore primarily a quantitative difference in the rate at which acquisition of Lubrol insolubility and secretion occur rather than a qualitative difference.

**DISCUSSION**

Rat prolactin in the dense cores of secretory granules is Lubrol-insoluble (2), and rat prolactin in GH4C1 cells is soluble immediately after synthesis and acquires Lubrol insolubility with time. The Lubrol-insoluble form in GH4C1 cells is most likely to be the same form of prolactin found in dense cores of secretory granules for several reasons. The Lubrol-insoluble form in GH4C1 cells has detergent sensitivity and the ability to sediment through sucrose that is similar to prolactin aggregates isolated from secretory granules. The Lubrol-insoluble form in GH4C1 cells becomes soluble at 37 °C, consistent with what happens in vivo. The ability to become soluble at 37 °C indicates that the proteins have not denatured to form an irreversible aggregate of unfolded or misfolded proteins, such as occurs with amyloid formation. Lubrol has been shown to disrupt secretory granule membranes (2). The Lubrol-insoluble forms of prolactin and growth hormone are unlikely to be caused by the failure to disrupt membranes of other compartments in the secretory pathway because albumin does not become insoluble during its transport through the secretory pathway in AtT20 cells, but human growth hormone does. The insoluble forms of secretory proteins are unlikely to be caused by binding to large cellular components not disrupted by
Lubrol, because prolactin and growth hormone are soluble immediately after synthesis and because very little growth hormone becomes insoluble when growth hormone is expressed in COS cells, so the ability to form Lubrol-insoluble aggregates is a cell-specific property. Therefore, the acquisition of Lubrol insolubility provides a measure to follow the aggregation that occurs when prolactin and growth hormone are concentrated into secretory granules in neuroendocrine cells.

Aggregation of prolactin does not occur to any great extent in the endoplasmic reticulum. Immediately after a 10-min pulse of \(^{35}\)S-amino acids, most \(^{35}\)S-prolactin is in the endoplasmic reticulum (1), and most prolactin is soluble. Newly synthesized prolactin moves to the Golgi complex during a 15-min chase period (1), and insoluble \(^{35}\)S-prolactin is formed by that time. If protein transport from the endoplasmic reticulum is prevented either by adding brefeldin A or by incubating cells at 15 °C, formation of the insoluble form is greatly reduced.

Lubrol-insoluble forms of prolactin and growth hormone are detected by 15 min after the initial pulse of \(^{35}\)S-amino acids. In the experiments reported, we measured insolubility after Lubrol treatment using velocity centrifugation at 50,000 \(\times g\) for 60 min to be comparable with conditions that were used for the investigations of the solubility of dense cores of prolactin from secretory granules (2). The same amount of \(^{35}\)S-prolactin after a 10-min chase period, however, was in the pellet after centrifugation at 5,000 \(\times g\) for 10 min in 0.32 M sucrose as with the more extensive centrifugation (not shown). The insoluble aggregates therefore all appear to be large when formed or very soon after formation. The process to make the insoluble form may occur relatively rapidly, or there may be a gradual formation of a Lubrol-soluble aggregate that then rapidly converts to a Lubrol-insoluble form. In contrast, the acquisition by \(^{35}\)S-prolactin of the ability to sediment through 1.2 M sucrose as the cores from secretory granules containing prolactin do is a process that occurs more gradually and increases from 15 to 60 min, consistent with the time it takes for granules to mature (1).

A somewhat unexpected result was that chloroquine and bafilomycin A1 did not substantially prevent the conversion of prolactin and growth hormone to a Lubrol-insoluble form. It has been a reasonable assumption that an acidic environment is important for aggregation of proteins that are stored in secretory granules for two reasons. One is that the environment of the trans-Golgi lumen in neuroendocrine cells becomes acidic (38), and it is in this area that hormone aggregates are detected by electron microscopy (5). The second is that most proteins that are stored in secretory granules aggregate in mildly acidic pH conditions (6–13). Acidic pH, however, is not the only factor that facilitates self-association of secretory granule proteins in solution; prolactin, for example, has been shown to aggregate at neutral pH, especially in the presence of cations (6, 39, 40). An acidic pH is necessary in neuroendocrine cells for the proteolytic processing of proopiomelanocortin and somatostatin (29, 30), and it may be more important for processing than for concentration of secretory granule proteins. An acidic pH may facilitate aggregation of larger cargo proteins for secretory granules, since prolactin aggregation in the presence of chloroquine was slower than without it, although part of the effect may be caused by slowing of transport. An acidic pH, however, does not appear necessary for aggregation of prolactin or growth hormone in the secretory pathway of GH \(_4\)C \(_1\) cells; therefore, additional factors must be responsible for hormone aggregation after cells are transported form the endoplasmic reticulum. Such an interpretation is consistent with the lack of aggregation of human growth hormone in COS cells, although aggregation occurs in AtT20 cells. The failure to form a Lubrol-insoluble form in COS cells is unlikely to be caused by lack of acidification of the trans-Golgi lumen, since this region is acidic in all cells that have been examined (32, 33, 41, 42).

Investigations of the structures required for aggregation in cells is difficult because mutations that affect structures markedly are likely to affect folding and the ability of the protein to leave the endoplasmic reticulum. The two mutants P89L GH and del32–46 GH are transported through the secretory pathway and behave in a fashion that is similar to wild-type growth hormone; the lag in aggregation and secretion of these two mutants is most likely caused by an increase in the time that it takes to fold the mutant molecules. Both have mutations that affect structures in the growth hormone molecule on the side of the molecule not involved in binding to the receptors (34). The ability of the mutants to aggregate indicates that there is at least some leeway in the structures required for assembly into aggregates.

Hormone storage in GH \(_4\)C \(_1\) cells has been assessed previously by measuring the ratio of intracellular to secreted hormone and the cells found to store little growth hormone with or without hormone treatment, to store more prolactin with treatment, and to store secretogranins well with or without treatment (17, 21). All three proteins are in a regulated pathway in GH \(_4\)C \(_1\) cells (19, 21, 43, 44), so the lack of good storage does not reflect secretion via a constitutive pathway. The results reported here with pulse-chase analysis in GH \(_4\)C \(_1\) cells are consistent with the previous results and indicate that newly synthesized prolactin is retained longer in cells with hormone treatment. The ability of the cells to retain hormone, however, did not reflect the ability to aggregate hormone, since the GH \(_4\)C \(_1\) cells formed growth hormone and prolactin aggregates to an equal extent in hormone-treated and untreated cells.

These results indicate that the simplest model for hormone storage is not sufficient to explain what occurs in GH \(_4\)C \(_1\) cells, since some process other than aggregation must cause preferential retention of prolactin in hormone-treated cells. Aggregation has been proposed to be responsible for sorting, because soluble proteins are not retained (14), but these results indicate that it is retention of the aggregate that is regulated. Since this retention occurs differentially for prolactin and growth hormone, it may be considered a separate sorting step from aggregation.

Preferential retention of aggregates may occur by preferential retention of secretory granules. Secretory granule assembly involves correct localization of transmembrane proteins as well as the luminal contents, and the combination of transmembrane proteins that assemble and direct the fate of the granules may depend on the luminal contents. Evidence for such a possibility is found in bag cells of Aplysia. In the trans-Golgi lumen of these cells, egg-laying hormone precursor is cleaved, and two kinds of granules form that contain either aggregates of the C-terminal fragment or aggregates of the N-terminal fragment of egg-laying hormone precursor. The two granules are transported to different areas of the bag cell (45, 46). An alternative possibility is that sorting occurs before aggregation; sorting to parts of the cell more capable of making granules that are retained after they are made is been suggested for why superior cervical ganglion neurons store neuropeptide Y better than proopiomelanocortin (47). The finding that growth hormone and prolactin do not always colocalize in the same part of the secretory pathway in a single GH \(_4\)C \(_1\) cell is consistent with this possibility (48).

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