

# Evidence for Multiple Somatic Pools of Individual Axonally Transported Proteins

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**ABSTRACT** The idea that individual axonally transported proteins can exist in several kinetically distinct pools within the cell body was studied using the presumptive neurosecretory low molecular weight (LMW) proteins of *Aplysia* neurons L11 and R15. Pulse-chase experiments revealed that the loss of labeled LMW proteins from the soma by axonal transport does not follow single exponential kinetics as it should if they are being removed from single pools. Rather, decay of label occurs in at least two phases having half-lives of ~1 and 40 h. The LMW proteins are homogeneous by sequential SDS gel electrophoresis and isoelectric focusing, indicating that individual protein species exhibit multiphasic decay kinetics. Two types of evidence imply that the bulk of cellular LMW protein turns over at the slower rate: the LMW pool does not reach constant specific activity at the rapid rate during continuous exposure to labeled precursor, and long-term blockade of axonal transport does not produce an appreciable accumulation of these species in the cell body. These results suggest that some of the newly synthesized LMW protein enters a small somatic pool from which it is rapidly subjected to axonal transport, while the remainder enters a larger pool where it can mix with previously synthesized protein before transport. A cellular mechanism that would yield this behavior is suggested.

An abundance of neurosecretory granules in the cell soma or in other regions distant from the nerve terminals is a common characteristic of neurosecretory cells (16). This has been presumed to reflect the storage of secretory material in a reservoir to be called upon during periods of intense secretion. Theoretically, this storage process could take one of two forms: once synthesized, each molecule of secretory material could enter a single large somatic pool from which it would be withdrawn at random for commitment to axonal transport; or, alternatively, once synthesized, each molecule would either be committed to transport or, depending on demand, shunted into a storage pool for later withdrawal. These alternatives are distinguishable by the fact that the latter would predict the preferential transport of newly synthesized molecules.

Kinetic studies using the presumptive neurosecretory proteins synthesized by individual characterized neurons of *Aplysia* offer the precision required to distinguish between these alternatives. In this mollusk, certain neuronal types devote 30–50% of their total amino acid incorporation to a group of low molecular weight (LMW) proteins. Members of this LMW group share a number of characteristics in addition to molecular weight and rapidity of labeling: in each cell, they comprise a precursor-product sequence whereby an initial precursor of

~12,000 daltons is progressively reduced to lower molecular weight products (1, 2, 5, 15, 23); the products are subjected to rapid axonal transport (9); and several lines of indirect evidence suggest that they are eventually secreted (3, 8, 13). Moreover, quantitative data imply that axonal transport is the predominant mechanism by which label decays from these proteins (5, 7). This last feature is crucial for the present study because, while the question of kinetically distinguishable pools is most apparent when considering neurosecretory cells, it may be asked equally well with respect to axonally transported proteins in general.

Several groups have studied the turnover of these proteins in the cell body by use of pulse-chase paradigms (1, 5, 11, 15), and the results have been consistent. During the first few hours of the chase period, label is lost from somatic low molecular weight protein with a half-life of 2–4 h. However, the data in these reports also indicate that the decay rate slows down at later times in the chase period, and that significant radioactivity remains in low molecular weight protein in the soma for as long as 20 h after exposure to labeled precursor has been terminated. Because a model in which LMW protein is withdrawn at random from a single somatic pool and committed to axonal transport would predict that the loss of label in such

experiments would follow a single exponential time-course, other causes for this behavior must be sought.

Several hypothetical mechanisms could explain the data: loss of radioactivity from LMW protein might occur by multiple mechanisms having different kinetics, such as proteolysis and transport. The persistence of label might reflect the existence of comigrating proteins that do not belong to the rapidly synthesized and transported LMW group. The decreasing rate of decay late in these experiments might be an artifact resulting from degeneration of the preparation. Finally, the observed multiple decay rates might be attributable to the existence of multiple somatic pools of LMW protein having different turnover rates. Each of these possible explanations for the data is testable, and such tests form the basis of this report.

## MATERIALS AND METHODS

*Aplysia californica* mollusks weighing 100–300 g were obtained from Pacific Bio-Marine Laboratories (Venice, Calif.) and kept at 15°C in Instant Ocean (Aquarium Systems, Wickliffe, Ohio) for no more than 2 wk before use. For pulse-chase studies, abdominal ganglia were removed from the animals, kept at room temperature for 2 h in artificial seawater, then transferred to 1 ml of artificial seawater containing 1% glucose and 100  $\mu$ Ci/ml L-[4,5- $^3$ H]leucine (100 Ci/mM, Amersham Corp., Arlington Heights, Ill.). Labeling was allowed to proceed for 2 h, after which the ganglia were rinsed briefly in artificial seawater and transferred to 10 ml of chase medium, consisting of artificial seawater supplemented with vitamins, amino acids, glucose and 1 mM unlabeled leucine. The composition of these media and the efficacy of the chase procedure have been described previously (3, 5).

At varying times after the beginning of the chase, ganglia were withdrawn and the somata of cells L11 and R15 (10) were dissected. After homogenization in the appropriate sample buffer, individual cells were subjected to electrophoresis on 6  $\times$  75-mm polyacrylamide gels (10%T, 5%C, where T = total acrylamide concentration and C = percent of T that is bisacrylamide). Two electrophoretic systems were used: "SDS gels" contained 0.1% SDS, as described previously (5); "acid-urea gels" were made up in 0.9 M acetic acid and 10 M urea (14). Internal standards of dansylated myoglobin and adrenocorticotrophic hormone were included on SDS gels. Cytochrome *c* was used as a marker in the acid-urea system. Acid-urea gels were kept overnight at 4°C in 15% TCA before being sliced into 1-mm segments for liquid scintillation counting in the fluid of Ward et al. (24). SDS gels were sliced and counted immediately, as free leucine does not enter these gels.

In some cases, labeled peaks from cell extracts separated on SDS gels were eluted and subjected to isoelectric focusing. The SDS gels were kept overnight at room temperature in 40% methanol/10% acetic acid to remove SDS, then sliced longitudinally into two parts. Peaks of interest were identified by liquid scintillation counting of 1-mm slices of one part, and corresponding slices of the other part were then lyophilized from distilled water and eluted with 1% Triton X-100 + 6 M urea for 48 h at room temperature. The eluates were subjected to isoelectric focusing on 5  $\times$  75-mm cylindrical gels containing 1% Triton X-100 and 6 M urea. Bio-Lyte (Bio-Rad Labs, Richmond, Calif.) ampholytes were used, and the gels were prepared according to Bio-Rad technical bulletin no. 1030, except that polymerization was initiated with 0.04% ammonium persulfate and the gels were prerun for 2 h. Isofocusing was performed overnight at a constant voltage of 200 V and a coolant temperature of 10°C. The pH along the gel was measured with a surface electrode before slicing for scintillation counting.

To estimate total LMW content, ganglia were kept for 4 h in artificial seawater at room temperature in the presence or absence of 0.1 mg/ml vinblastine sulfate (Sigma Chemical Co., St. Louis, Mo.). The somata of cells L11 and R15 were then isolated, two cells of each type were combined, and the extracts were subjected to electrophoresis on SDS gels (5%T, 5%C) cast in 100- $\mu$ l capillary tubes (5). The gels were stained overnight in 0.05% Coomassie Brilliant Blue (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) in 40% methanol/10% acetic acid. After 5–6 h of destaining in the methanol-acetic acid solution, the gels were scanned at 580 nm. The areas representing low and high molecular weight (HMW) proteins were determined from densitometer tracings by planimetry.

As in previous kinetic studies on these species, the data are expressed as the ratio of radioactivity or staining density in the low molecular weight proteins to that in the sum of higher molecular weight species. This effectively normalizes for variations in cell size and in total label incorporated by individual cells. For extracts separated on SDS gels, it corresponds to the ratio between proteins smaller than 18,000 daltons and larger species; for acid-urea gels, the ratio is between those proteins migrating at a rate of at least 0.75 relative to cytochrome

*c* and more slowly migrating species. In either gel system, LMW proteins form discrete peaks containing >80% of the total radioactivity in the low molecular weight or high-migration-rate region. The turnover rates of LMW proteins calculated by this method are not absolute but relative to that of total cellular protein. However, the half-life of total cellular protein is too long to be estimated in experiments of the duration used here (23; my unpublished data; Table I in reference 11), and should therefore provide a stable baseline in these experiments.

## RESULTS

Data from pulse-chase experiments in which extracts of cells L11 and R15 were electrophoresed on SDS gels are presented in Fig. 1. Label clearly does not decay from the low molecular weight region of these gels by a single exponential process, a finding that is consistent with previous results (5, 15). This loss of label can be resolved into at least two exponential phases in each cell, although intermediate phases could be present as well. The half-life of the rapid phase is  $\sim$ 1 h in both R15 and L11 at 15°C, whereas the half-life of the slow phase in both cells can be calculated to be  $\sim$ 40 h at this temperature. Extrapolation of the decay curves to zero time indicates that, of the total label in low molecular weight protein at the end of the 2-h labeling period,  $\sim$ 20% decays at the slow rate in L11 and 36% at the slow rate in R15. All of these calculations are necessarily imprecise because of the very slow rate of the slow phase and the variability of the original data. However, the results clearly indicate that there are at least two phases of decay and that these differ in rate by at least an order of magnitude.

Because the LMW proteins in each of these cells comprise a proteolytic processing sequence (1, 2, 5, 15, 23), biphasic decay curves could be interpreted as reflecting the rapid loss of leucine in proteolytic fragments, followed by slower commitment of the product proteins to axonal transport. However, this cannot be the case, because the products, as well as the total LMW group, display multiphasic decay kinetics (5, 15), and

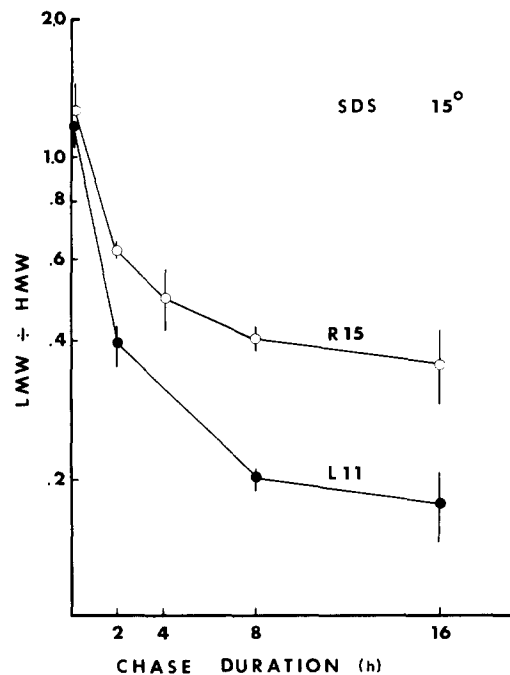


FIGURE 1 Decay of label from somatic LMW proteins of L11 and R15 after a 2-h exposure to tritiated leucine at 15°C with the LMW proteins being identified on SDS gels. Points are the mean  $\pm$  SEM of three to four replications and represent the ratio of radioactivity in proteins smaller than 18,000 daltons to that in higher molecular weight material.

data from both L11 (6) and R15 (23) indicate that the full leucine content of the precursor is conserved in the product. Furthermore, treatments that block axonal transport prevent 80–100% of the loss of label from the LMW proteins of these cells (5, 7), indicating that both the rapid and slow phases of decay involve commitment to axonal transport.

Another possible explanation for this behavior would be that the low molecular weight region of these SDS gels contains at least two groups of proteins: the rapid-turnover LMW group and contaminants that turn over more slowly. This possibility was tested in two ways. First, pulse-chase studies were repeated on cells L11 and R15, with the LMW proteins being identified by electrophoresis on acid-urea gels, the rationale being that because migration on such gels is a function of both molecular charge and molecular weight (19), whereas SDS gels separate proteins by the latter parameter only, it would be unlikely that the same contaminating proteins would comigrate with the LMW proteins on both gel systems. The resolving power of the acid-urea gel system is sufficient to separate the 12,000-dalton LMW precursors of L11 and R15, even though they comigrate on SDS gels and differ by a single net charge at this pH (4). In addition, it has been shown that the 12,000-dalton LMW protein of R15 isolated on these gels contains only quantitatively minor contaminants when eluted and rerun on SDS gels (14).

In L11 cells, tritiated leucine is initially incorporated into an LMW protein that migrates as a discrete peak at a rate of  $0.85 \pm 0.02$  relative to cytochrome *c* (Fig. 2; see also reference 4). This material disappears during the chase period, coincident with the rise of a peak migrating at a rate of  $0.93 \pm 0.02$  relative to the marker. The time-course of this shift in migration rate suggests that it may reflect the 12,000- to 9,000-dalton transition observed on SDS gels (5). Similar peaks and a similar shift in migration rate occur in cell R15, and in this case, the change

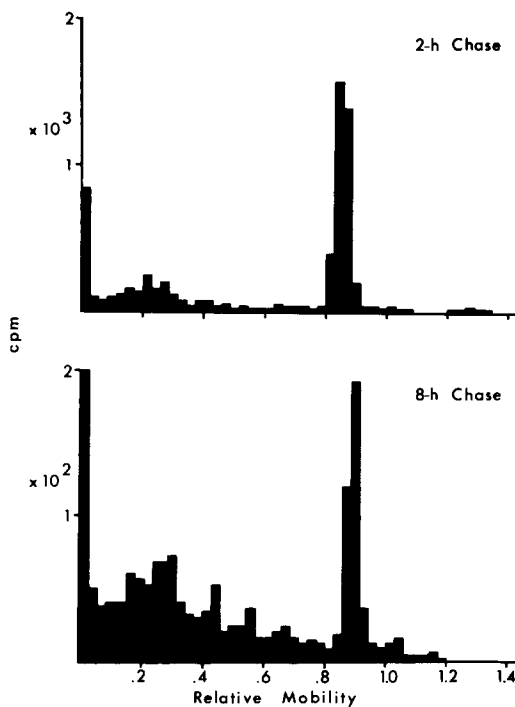


FIGURE 2 LMW protein of cell L11 identified on acid-urea gels. Upper: extract of a cell exposed to tritiated leucine for 2 h, then chased for 2 h. Lower: extract from a cell labeled for 2 h, then chased for 8 h. Abscissa represents mobility relative to cytochrome *c* on both graphs.

in migration rate has been shown to be attributable to a reduction in molecular weight during processing (15). In both cells, label persists for at least 16 h into the chase period as a discrete peak in the same position as the product protein observed at earlier times. As shown in Fig. 3, the radioactivity in these peaks, relative to that in the more slowly migrating species, decays by a multiphasic process, just as it does from the LMW proteins identified by SDS gel electrophoresis. That both separation methods yield multiphasic decay curves suggests that such decay does not result from contaminants, but that the LMW group proteins decay by at least two kinetically distinct processes.

A more direct test for contaminants was performed by eluting LMW peaks from SDS gels and subjecting them to isoelectric focusing. Data from L11 are shown in Fig. 4. The LMW group in this cell consists of two major species: a 12,000-dalton precursor that is cleaved to a 9,000-dalton product, the conversion being 50% complete by ~3 h at 15°C (5). When eluted and rerun on isofocusing gels, these species yield major peaks at pI 8.2–8.6 and 4.8–5.4, respectively. Each also yields a more neutral peak, but this never exceeds 18% of the radioactivity in the major species and thus cannot contribute significantly to the observed decay curves. Moreover, the isoelectric focusing profiles of the 9,000-dalton species from L11 cells extracted for electrophoresis immediately after an 8-h pulse of label, with or without vinblastine, are identical to each other and to that obtained after an additional 16-h chase period in the presence of vinblastine. Thus, differential turnover of multiple components of peaks observed on SDS gels cannot account for the multiphasic decay of label from these peaks. Data obtained from cell R15 are entirely analogous to those from L11 and will be reported elsewhere.

Another explanation for the multiphasic decay of label from these proteins in pulse-chase experiments would be that the

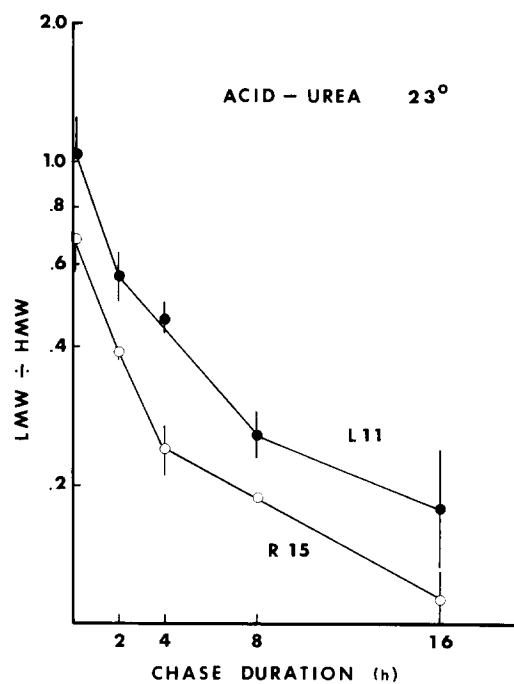


FIGURE 3 Decay of label from somatic LMW proteins of L11 and R15 after a 2-h exposure to tritiated leucine at room temperature with the LMW proteins being identified on acid-urea gels. Points are the mean  $\pm$  SEM of three to four replications and represent the radioactivity in proteins migrating more rapidly than 0.75 relative to cytochrome *c* divided by that in the more slowly migrating species.

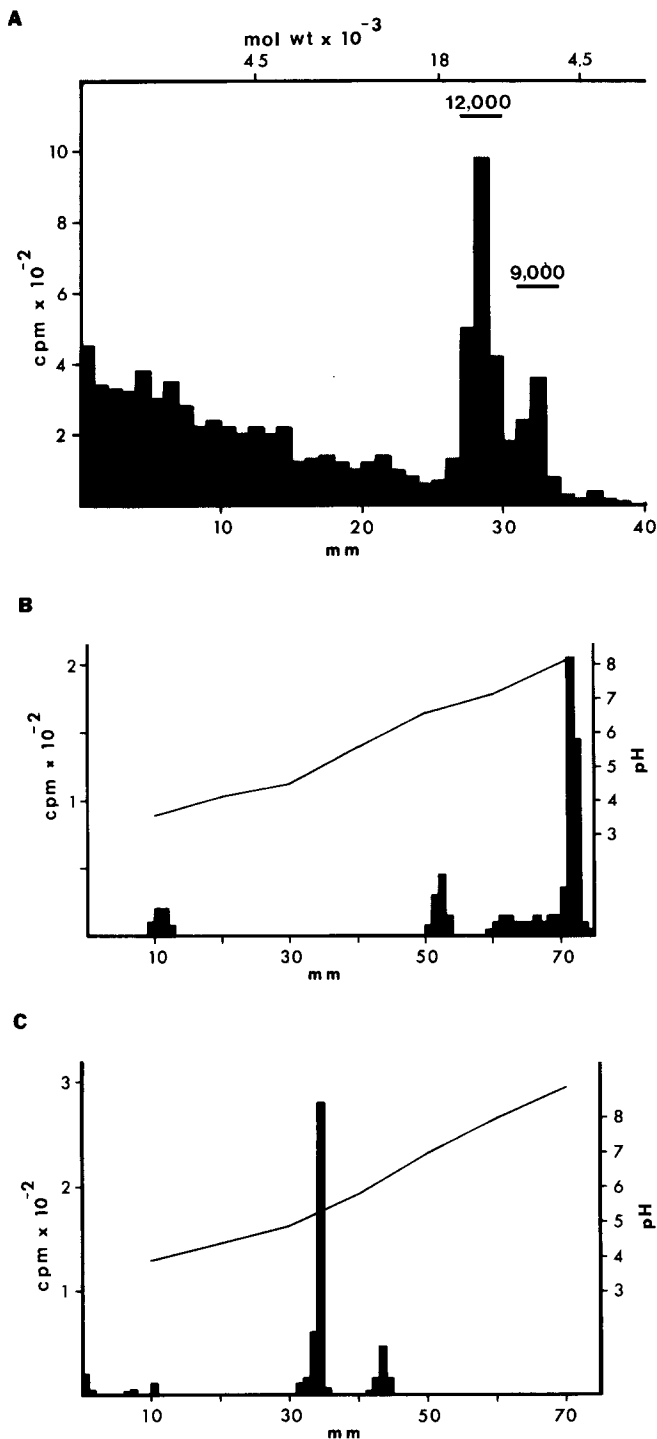


FIGURE 4 Sequential SDS gel electrophoresis and isoelectric focusing of the LMW proteins of cell L11. (A) Proteins synthesized by L11 during an 8-h exposure to [ $^3\text{H}$ ]leucine, separated by SDS gel electrophoresis. Three cells were combined for this assay. (B) Isoelectric focusing profile of protein eluted from region marked 12,000 in A. (C) Isoelectric focusing profile of protein eluted from 9,000 region in A.

rapid phase represents the actual rate of turnover of these species, whereas the slower phase is an experimental artifact resulting from, for example, reutilization of protein-bound precursor during the chase period or degeneration of the preparation during long periods *in vitro*. This idea can be tested by

noting the relative labeling of the LMW group at short and long intervals during continuous exposure to radioactive precursor. If the system is in a steady state, such that the rate of LMW synthesis is equal to the rate of LMW removal from the soma, then the accumulation of label in these proteins represents a rise in specific activity, and the LMW pool should become labeled to constant specific activity with a rate constant given by the half-life of decay. Early in the labeling period, the label in LMW protein relative to that in higher molecular weight species should approximate the relative rates of synthesis of the two groups. However, this ratio should decrease with time as the LMW pool reaches constant specific activity while the higher molecular weight proteins, with longer half-lives, continue to increase in specific activity. If the notion that all LMW protein turns over at the rapid rate is valid, then its pool would have a half-life of  $\sim 1$  h, and should become 94% saturated with label after 4 h of continuous exposure to precursor. Therefore, this hypothesis would predict that the ratio of label in LMW vs. higher molecular weight protein would be substantially lower after 18 h of exposure to tritiated leucine than after 2 h. As shown in Table I, this is not the case, even though the cells obviously continued to incorporate label into protein during this period.

This result indicates that the total LMW pool does not turn over as rapidly as the rapid phase of loss of label in pulse-chase experiments would suggest. Moreover, that incorporation of labeled precursor into low molecular weight protein is maintained at a rate characteristic of LMW synthesis for prolonged periods also argues against the contention that other proteins with a slower turnover comigrate with the LMW group on SDS gels.

It is possible to determine whether the rapid or slow rate of decay more closely approximates the turnover rate of total somatic LMW protein in another way. It was shown previously that loss of LMW protein can be selectively blocked by abolishing axonal transport (7). Because such treatment does not result in increased degradation of these proteins and does not influence their rate of synthesis (7), abolition of axonal transport in these cells should cause total LMW protein to accumulate in the soma at the rate at which it is normally transported. If all LMW protein turns over at the rapid rate, this accumulation should amount to a doubling approximately every hour. To test this, ganglia were incubated for 4 h in the presence of 0.1 mg/ml vinblastine. SDS extracts of control and vinblastine-treated L11 and R15 somata were electrophoresed on microgels and stained with Coomassie Brilliant Blue. Densitometer tracings of the stained gels were divided into high and low molecular weight regions as indicated in Fig. 5, and the ratio of the stain in the low molecular weight region to that in higher molecular weight proteins was calculated. As shown in Table II, transport blockade caused no more than a 10% increase in the cells' relative content of LMW protein by this measure, whereas turnover at the rapid rate would have resulted in a 16-fold increase. It would therefore appear that the bulk of LMW protein has a somatic half-life much longer than 4 h.

This conclusion is valid only to the extent that the ratio of stain density in the two gel regions reflects the relative protein content of these regions. The small quantity of protein obtainable from individual cells required the use of the most sensitive available stain, but Coomassie staining is not always strictly proportional to protein concentration (12). Therefore, it was necessary to determine whether a change in the ratio of LMW

TABLE I  
Lack of Saturation of LMW Pool

Label duration h	L11		R15	
	LMW/HMW	cpm/cell	LMW/HMW	cpm/cell
2	1.08 ± .03 (6)	2,813 ± 845 (6)	1.17 ± .06 (6)	2,028 ± 345 (6)
18	1.12 ± .20 (6)	21,164 ± 7,164 (6)	0.98 ± .08 (5)	31,103 ± 14,933 (5)

LMW/HMW, ratio of radioactivity in species smaller than 18,000 daltons to that in higher molecular weight proteins on SDS gels (10%T, 5%C). Cells were taken from ganglia incubated at 15°C for the times indicated in 100 µCi/ml tritiated leucine. Entries are given as mean ± SEM (number of experiments, n).

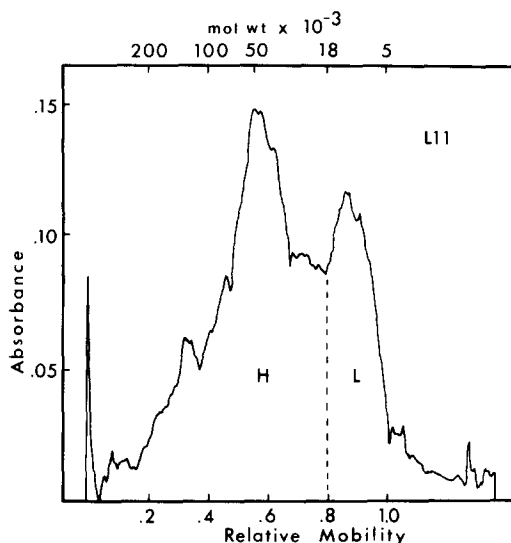


FIGURE 5 Densitometer scan of a Coomassie-stained SDS microgel on which two L11 cells were electrophoresed. H and L designate the gel regions used for calculating data in Tables II and III. Relative mobility refers to ACTH, run on parallel gels.

TABLE II  
LMW/HMW Ratio by Coomassie Staining

	L11	R15
Control	0.34 ± .05 (6)	0.42 ± .03 (5)
VLB	0.36 ± .05 (6)	0.46 ± .05 (6)

Entries are the mean ± SEM (n) of the ratio of absorbance in the low molecular weight region to that in the high molecular weight region of SDS gels (5%T, 5%C). Two cells were combined on each gel. VLB cells were from ganglia incubated for 4 h in the presence of 0.1 mg/ml vinblastine sulfate.

to higher molecular weight protein content would have been reflected in an equivalent change in staining density.

This amounts to determining whether or not the relationship between stain density and protein concentration remains constant in each gel region over the range of protein concentrations employed in these experiments. To test this, several cells of each type were combined and extracted for SDS electrophoresis, and aliquots containing the equivalent of two, three, or four cells were run in parallel. As might be expected, the total staining density of the gels varied from run to run, but when the comparison was made between gels electrophoresed and stained together, total staining was proportional to protein loading: the absorbance of gels containing the equivalent of four L11 cells was  $2.0 \pm 0.3$  (3) times that of gels containing two cells, whereas this ratio was  $1.7 \pm 0.3$  (3) for R15 cells (the numbers in parentheses represent the number of experiments). Furthermore, as shown in Table III, the ratio of stain in the

TABLE III  
Coomassie Staining: Invariance with Protein Loading

No. of cells	L11	R15
2	0.37 ± .02 (3)	0.41 ± .07 (3)
3	0.32 ± .07 (3)	0.45 ± .07 (3)
4	0.44 ± .09 (3)	0.51 ± .13 (3)

Entries are the mean ± SEM (n) of the ratio of absorbance in the LMW region to that in the HMW region of SDS gels (5%T, 5%C).

low and high molecular weight regions did not vary significantly within this range of total protein concentrations. These data indicate that, in this system, over the range of concentrations employed, Coomassie staining is proportional to protein concentration, and that this holds equally for both the high and low molecular weight groups.

## DISCUSSION

These results confirm earlier data indicating that the loss of labeled LMW proteins from the somata of these cells does not follow the single exponential time-course that would be expected if they were being removed from a single pool by a single mechanism. Several hypotheses that could conceivably account for this behavior can be ruled out: loss of label might result from both proteolysis and axonal transport, but blockade of axonal transport is sufficient to abolish the loss of label from these species, indicating that they turn over by a single process. The persistence of labeled low molecular weight protein late in the chase period might reflect the presence of slow-turnover proteins not belonging to the rapidly synthesized and transported LMW group, but both direct and indirect tests for such contaminants proved negative. Finally, one might attribute the slow phase of decay to various sources of experimental artifact, but direct and indirect tests imply that the bulk of the LMW protein actually turns over at this rate. On the other hand, the hypothesis that multiphasic decay reflects the existence of multiple, kinetically distinct somatic pools of LMW protein is consistent with all of the foregoing, and as outlined below, explains the apparent discrepancy between the rate of synthesis and rate of turnover of these proteins.

According to these results, any pool model for the LMW proteins is constrained by three factors: (a) radioactivity is lost from pulse-labeled somatic LMW protein with at least two rate constants of decay, (b) the specific activity of somatic LMW protein rises at a constant rate for at least 18 h, and (c) the turnover rate of total LMW protein is much slower than that predicted by the rapid rate of loss of label from newly synthesized material. The first constraint implies that there exist at least two somatic LMW pools, one of which turns over more rapidly than the other. The distinction between these pools could lie in the velocity at which molecules enter and leave the

pool, or in pool size, as turnover is a function of both factors. The second and third constraints can be used to judge which of these factors is responsible for the two-pool behavior of this system. A simple two-pool model is illustrated in Fig. 6a. If it is assumed that pools A and B are roughly the same size, but that  $V_a > V_b$ , then pool A will turn over more rapidly than pool B. During continuous exposure to labeled precursor, pool A will rapidly saturate with a time constant of  $V_a/A$ . Thereafter, LMW protein will continue to be labeled, but at a slower rate, given by  $V_b/B$ . This is clearly not consistent with the data in Table I. On the other hand, pool A could have a greater turnover rate than pool B if  $V_a = V_b$ , but  $A < B$ . In this case, during continuous exposure to labeled precursor a negligible percentage of the total pool (i.e., pool A) would saturate with a rapid rate constant ( $V_a/A$ ), whereas the majority (pool B) would saturate at a slower rate ( $V_b/B$ ). This is in accord with the data in Table I. Furthermore, the rate of accumulation of pool material when decay is blocked would not be large in relation to total pool size, being approximately  $2V_b/B$ . This is consistent with the data in Table II. Thus the data are most consistent with there being two or more somatic LMW pools that differ in turnover rate by virtue of the relative quantities of material they contain, rather than by any great differences in the velocity at which material enters and leaves them. This latter consideration makes it unlikely that the rapid and slow decay phases represent commitment to rapid and slow axonal transport. It is important to note that although the model of Fig. 6a is the simplest, a number of models, including that illustrated in Fig. 6b, are equally plausible. The key feature is that the pools must differ in size rather than in the velocity at which material enters and exits.

The results described here are therefore best explained by assuming that *Aplysia* neurons contain at least two somatic pools of LMW protein. Some of the newly synthesized LMW material can enter a small pool from which it is rapidly subjected to axonal transport, while the rest enters a larger storage pool or pools where it can mix with previously synthesized material before transport. It follows from this model that, in pulse-labeling studies, newly synthesized molecules are subjected to transport at a higher specific activity than the total being committed to transport. This has two consequences. First, the turnover rate of total LMW protein can be overestimated by pulse-chase data. For example, Loh and Gainer (15) calculated a 3.6-h somatic half-life for LMW protein in R15 at 15°C. The soma of this cell is packed with 1,000–2,000 Å dense-cored granules (10) that are thought to contain the LMW protein (3), and the turnover of such a large quantity of protein so rapidly does not seem reasonable. The present data suggest, rather, that this quantity of protein turns over with a half-life on the order of days.

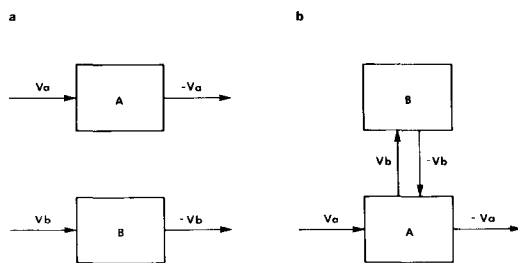


FIGURE 6 LMW pool models.  $V_a$  and  $V_b$  represent the velocities of input or outflow of pools A and B. Further explanation can be found in the text.

A second consequence of the existence of kinetically distinct somatic pools of individual axonally transported proteins is that observations limited to radioactive proteins in nerve trunks may not accurately reflect the types of protein actually being transported. This would be the case in those situations where experimental manipulation alters the relative rates at which the low- and high-specific-activity pools are committed to transport, as well as situations where the transported species are also undergoing time-dependent modifications during transport. The latter situation applies to the preparation used here, as it has been shown that the processing sequence of the LMW proteins that begins in the soma continues in the axon, where they are progressively reduced to a molecular weight of 1,000–2,000 daltons (9). The conversion process is slow in relation to the rate of transport, and labeled intermediate species larger than 3,000 daltons persist in the axon at least 20 h after a 3-h pulse to the cell bodies. However, this is not an accurate reflection of the relative quantity of intermediates and products actually undergoing transport, because the only proteins smaller than 12,000 daltons that can be observed in Coomassie-stained gels from nerve trunks are the 1,000 to 2,000-dalton products (9). This also indicates that newly synthesized LMW proteins appear to be a minor fraction of the total LMW protein committed to transport; that is, the large, low-specific-activity pool actually contributes most of the axonally transported LMW protein.

An interesting parallel can be drawn between the events described here and the disposition of secretory material in nonneural secretory cells. In the parotid (22) and parathyroid (17, 18) glands, as well as in the endocrine (21) and exocrine (20) pancreas, it has been shown that secretory proteins exist in both a rapidly labeled pool and a more slowly labeled pool and that stimulation can evoke preferential release from one pool or the other. Although it has not been shown unequivocally that both pools coexist within individual cells in these systems, it would appear that differential compartmentalization of proteins that are subject to translocation may be a common cellular process.

In the absence of further data, the cellular mechanisms responsible for the compartmentalization process in *Aplysia* neurons must remain the subject of speculation, but one hypothetical mechanism recommends itself by virtue of its simplicity. It seems reasonable to suppose that at some stage in its synthetic sequence, the LMW material passes through the Golgi apparatus into granules that become free to diffuse away into the cytoplasm. Commitment to transport might then occur when a granule reaches a discrete site where it is loaded onto the transport mechanism. If it is further presumed that sites of granule formation exist at various points in the soma, but that the site of loading for transport is spatially restricted, then the products of formation sites located near the transport site will be rapidly committed to transport, whereas those granules formed farther away will mix with previously synthesized material before being transported. Such a process would generate the data observed in this study without invoking any cellular mechanisms other than a discrete transport loading site and multiple sites of granule formation. However, it remains possible that the nerve cell may possess unique cellular mechanisms for dividing newly synthesized molecules between transport and storage pools, and for regulating the apportionment of secretory material between these pools according to demand. The question of which of these alternatives best explains the present data is worthy of further study.

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## REFERENCES

1. Aswad, D. W. 1977. Heterogeneity and processing of low molecular weight protein in cell R15 of *Aplysia californica*. *J. Neurochem.* 28:1137-1140.
2. Aswad, D. W. 1978. Biosynthesis and processing of presumed neurosecretory proteins in single identified neurons of *Aplysia californica*. *J. Neurobiol.* 9:267-284.
3. Berry, R. W. 1975. Functional correlates of low molecular weight peptide synthesis in *Aplysia* neurons. *Brain Res.* 86:323-333.
4. Berry, R. W. 1976. A comparison of the 12,000 dalton proteins synthesized by *Aplysia* neurons L11 and R15. *Brain Res.* 115:457-466.
5. Berry, R. W. 1976. Processing of low molecular weight proteins by identified neurons of *Aplysia*. *J. Neurochem.* 26:229-231.
6. Berry, R. W. 1977. Function and metabolism of neuronal proteins: comparisons among the identified neurons of *Aplysia*. In *Biochemistry of Characterised Neurons*. N. N. Osborne, editor. Pergamon, Oxford, England. 283-308.
7. Berry, R. W. 1978. Synthesis of axonally transported proteins following inhibition of transport. *Brain Res.* 159:427-430.
8. Berry, R. W. 1979. Secretion of neural peptides from the nervous system of *Aplysia californica*. *J. Neurobiol.* 10:499-508.
9. Berry, R. W., and A. W. Schwartz. 1977. Axonal transport and axonal processing of low molecular weight proteins from the abdominal ganglion of *Aplysia*. *Brain Res.* 129:75-90.
10. Frazier, W. T., E. R. Kandel, I. Kupfermann, R. Waziri, and R. E. Coggeshall. 1967. Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *J. Neurophysiol. (Bethesda)*. 30:1288-1351.
11. Gainer, H., and J. L. Barker. 1975. Selective modulation and turnover of proteins in identified neurons of *Aplysia*. *Comp. Biochem. Physiol.* 51B:221-227.
12. Gorovsky, M. A., K. Carlson, and J. L. Rosenbaum. 1970. Simple method for quantitative densitometry of polyacrylamide gels using fast green. *Anal. Biochem.* 35:359-370.
13. Kupfermann, I., and K. R. Weiss. 1976. Water regulation by a presumptive hormone contained in identified neurosecretory cell R15 of *Aplysia*. *J. Gen. Physiol.* 67:113-123.
14. Loh, Y. P., and H. Gainer. 1975. Low molecular weight specific proteins in identified molluscan neurons. I. Synthesis and storage. *Brain Res.* 92:181-192.
15. Loh, Y. P., and H. Gainer. 1975. Low molecular weight specific proteins in identified molluscan neurons. II. Processing, turnover, and transport. *Brain Res.* 92:193-206.
16. Mason, C. A., and H. A. Bern. 1977. Cellular biology of the neurosecretory neuron. *Handb. Physiol.* 1:651-690.
17. Morrissey, J. J., and D. V. Cohn. 1979. Regulation of secretion of parathormone and secretory protein-I from separate intracellular pools by calcium, dibutyryl cyclic AMP, and (1)-isoproterenol. *J. Cell Biol.* 82:93-102.
18. Morrissey, J. J., and D. V. Cohn. 1979. Secretion and degradation of parathormone as a function of intracellular maturation of hormone pools. Modulation by calcium and dibutyryl cyclic AMP. *J. Cell Biol.* 83:521-528.
19. Rodbard, D., and A. Chrambach. 1971. Estimation of molecular radius, free mobility, and valence using polyacrylamide gel electrophoresis. *Anal. Biochem.* 40:95-134.
20. Rothman, S. S., and L. D. Isenman. 1974. Secretion of digestive enzyme derived from two parallel intracellular pools. *Am. J. Physiol.* 226:1082-1087.
21. Sando, H., and G. M. Grodski. 1973. Dynamic synthesis and release of insulin and proinsulin from perfused islets. *Diabetes.* 22:354-360.
22. Sharoni, Y., S. Eimerl, and M. Schramm. 1976. Secretion of old versus new exportable protein in rat parotid slices. *J. Cell Biol.* 71:107-122.
23. Strumwasser, F., and D. L. Wilson. 1976. Patterns of proteins synthesized in the R15 neuron of *Aplysia*: temporal studies and evidence for processing. *J. Gen. Physiol.* 67:691-702.
24. Ward, S., D. L. Wilson, and J. J. Gilliam. 1970. Methods for fractionation and scintillation counting of radioisotope labeled polyacrylamide gels. *Anal. Biochem.* 38:90-97.