Serotonin-Mediated Endocytosis of apCAM: An Early Step of Learning-Related Synaptic Growth in *Aplysia*

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The long-term facilitation of synaptic efficacy that is induced by serotonin in dissociated cell cultures of sensory and motor neurons of *Aplysia* is accompanied by the growth of new synaptic connections. This growth is associated with a down-regulation in the sensory neuron of *Aplysia* cell adhesion molecules (apCAMs). To examine the mechanisms of this down-regulation, thin-section electron microscopy was combined with immunolabeling by gold-conjugated monoclonal antibodies specific to apCAM. Within 1 hour, serotonin led to a 50% decrease in the density of gold-labeled complexes at the surface membrane of the sensory neuron. This down-regulation was achieved by a heterologous, protein synthesis–dependent activation of the endosomal pathway, which leads to internalization and apparent degradation of apCAM. The internalization is particularly prominent at sites where the processes of the sensory neurons contact one another and may act there to destabilize process-to-process contacts that normally inhibit growth. In turn, the endocytic activation may lead to a redistribution of membrane components to sites where new synapses form.

A feature of the neuronal changes accompanying long-term memory storage is the formation of new synaptic connections (*1*). Despite the association of morphological changes with different forms of learning, little is known about the subcellular and molecular mechanisms that underlie this synaptic structural plasticity. To study these mechanisms, we have exploited the cellular specificity of a simple behavioral system—the gill-withdrawal reflex of *Aplysia*. Long-term memory for sensitization of this reflex is associated with a growth of new synaptic connections between the sensory and motor neurons (*2*). The duration of this structural change parallels the behavioral retention of the memory (*3*). Similar changes can be reconstituted in dissociated cell cocultures of sensory neurons and identified motor neurons by the repeated presentation of serotonin (5-HT), a facilitating neurotransmitter released by sensitizing stimuli in the intact animal (*4*).

The growth produced by 5-HT is associated with the down-regulation at the cell surface of the sensory neurons of a group of immunoglobulin (Ig)-related cell adhesion molecules, apCAMs (*5*). The transient modulation by 5-HT of the apCAMs may represent an early step in initiating learning-related growth of synaptic connections. Indeed, blockade of apCAMs with a monoclonal antibody (MAb) causes defasciculation, a process that appears to precede synapse formation (*6*). We have therefore investigated the mechanisms by which 5-HT modulates apCAM.

We combined serial, thin-section electron microscopy with immunolabeling using a gold-conjugated MAb specific to apCAM and followed the fate of the apCAMs at the surface membrane of the sensory neuron (*7*). The down-regulation of apCAMs on the cell surface was accomplished by an increase in internalization of the cell adhesion molecules that was dependent on protein synthesis. Within 1 hour of its application, 5-HT led to a 50% decrease in the density of gold-labeled complexes at the surface membrane of the sensory neuron (Fig. 1A). Whereas there were 9.4 ± 0.7 (SEM) complexes per micrometer of surface membrane length in 5-HT–treated cells, there were 19.5 ± 1.4 complexes per micrometer in control cells ($n = 7$; $P < 0.001$, two-tailed t test). A similar decrease in surface labeling in response to 5-HT was seen when we used monovalent Fab′ fragments of the MAb. Accompanying the decrease in the surface labeling was a sevenfold increase in the percentage of gold within the cell (5-HT: 29.4 ± 2%; control: 4.1 ± 0.3%; $n = 7$; $P < 0.001$) (Fig. 1B). This effect required new protein synthesis (*8*); both the down-regulation at the surface and the internalization of apCAM were blocked by the protein synthesis inhibitor anisomycin (Fig. 1). Thus, there were 15.9 ± 3.4 complexes per micrometer of surface membrane length in cells treated with 5-HT in the presence of 10 μM anisomycin ($n = 3$; $P < 0.05$). Similarly, only 4.9 ± 1.3% of the gold complexes were internalized when anisomycin was applied to the cells during 5-HT incubation ($n = 3$; $P < 0.01$).

About 50% of the immunogold-labeled apCAM at the cell surface was internalized after exposure to 5-HT treatment. This reduction is substantially greater than can be detected with immunofluorescence (*5*). The magnitude and time course of this effect are comparable to the ligand-induced internalization of other receptors, such as the β_2-adrenergic and epidermal growth factor (EGF) receptors (*9*, *10*), as well as the phorbol ester–

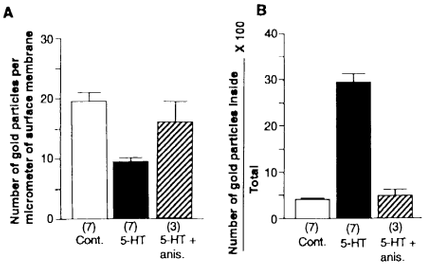

Fig. 1. Serotonin-induced down-regulation of apCAM at the sensory neuron membrane. (**A**) Surface density of apCAM. The number of gold-conjugated MAb complexes was determined as in (*7*). (**B**) ApCAM density inside the cell determined as in (*7*). Anisomycin was 10 μM. Each bar represents the mean ± SEM.

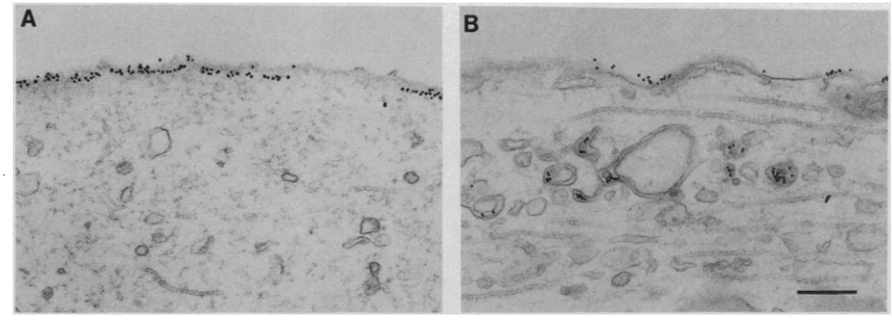

Fig. 2. Structural mechanisms underlying down-regulation at the surface membrane: endocytic activation and internalization of apCAM. (**A**) Distribution of apCAM under control conditions. (**B**) Distribution of apCAM after treatment with 5-HT. Scale, 0.25 μm.

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induced internalization of the receptors to transferrin, insulin, and EGF (*11*). The finding that the down-regulation of apCAM was incomplete may be due to heterogeneity in internalization by different isoforms of apCAM; some isoforms are transmembrane in disposition, whereas others have a phosphoinositide linkage to the membrane. Partial down-regulation is also characteristic of the IgG Fc receptors (*12*) and the receptors for nerve growth factor (NGF) (*13*).

Prior to exposure to 5-HT, the surface membrane of the sensory neuron had a uniform, linear appearance (Fig. 2A). Gold complexes decorated most of the plasma membrane and little, if any, gold was found inside the cell where the cytoplasm contained cytoskeletal elements and membrane-bound vesicular profiles. After a 1-hour exposure to 5-HT, there was a redistribution of apCAM, resulting in a decrease in the number of gold complexes at the surface membrane and a concomitant increase in their internalization into an assortment of endosomal subcompartments (*14*) (Fig. 2B). The surface membrane appeared to be thrown up into a series of undulating contours, and in addition there was an activation of the endosomal pathway, as evidenced by recruitment of additional elements of the cytoskeleton and a variety of polymorphic vesicular and tubular endosomal profiles. In *Aplysia*, these endosomal compartments often appear to be part of an extensive reticular network, similar to that of mammalian cells in culture (*15*).

These changes were specific to the sensory neurons and did not occur in a postsynaptic target of the sensory neurons, the motor neuron L7, in isolation or in cocultures. This lack of internalization of apCAM occurred despite the fact that motor neurons have receptors and respond to 5-HT with a slight hyperpolarization. By contrast, the internalization occurred equally well in the sensory neurons that were isolated as it did in sensory neurons that were cocultured with motor neurons.

To determine if the 5-HT–induced internalization was selective to the apCAMs, we examined the effects of 5-HT on the internalization of other receptors known to undergo receptor-mediated endocytosis. Because we could not label insulin or transferrin receptors on the surface of sensory neurons in *Aplysia* in vivo, we used a more general marker for endocytosis, wheat-germ agglutinin (WGA) (*16*). A 1-hour exposure of cultures to 5-HT led to a 26% decrease in the density of WGA on the surface membrane (5-HT: 18.7 ± 0.6 complexes per micrometer; control: 25.2 ± 2 complexes per micrometer, $n = 3$; $P < 0.05$) and a doubling in the percentage of WGA inside the cell (5-HT: 61 ± 3%; control: 31 ± 4%; $n = 3$; $P < 0.01$). Thus, in addition to stimulating the internalization of apCAM, 5-HT can also induce the internalization of other surface membrane glycoproteins.

The relatively high resting internalization rate of WGA in control cells suggested that the lectin might be stimulating uptake of itself and raised the possibility that the twofold increase in internalization observed after exposure to 5-HT might be artificially low. To address this issue, we repeated the WGA experiments, this time exposing the cells to 5-HT at room temperature in the absence of any lectin and then to WGA at 4°C. Because little label was found inside the cells under these conditions where endocytosis was inhibited (only those surface antigens were labeled that had not undergone internalization during the 5-HT exposure), we used for comparison the surface density of WGA in control and 5-HT–treated cultures. There was a 26% decrease in the density of WGA-labeled complexes at the surface membrane of sensory neurons in cultures that had been incubated at room temperature with WGA and a 29% decrease in surface density in those experiments where the cells were labeled at 4°C after a 1-hour exposure to 5-HT (5-HT: 12.6 ± 0.2 complexes per micrometer; control: 17.7 ± 1 complexes per micrometer; $n = 3$; $P < 0.01$). These results indicate that WGA did not stimulate uptake of itself and suggests the difference in the rate of internalization between experimental and control cultures is an accurate reflection of the effects of 5-HT on the turnover of surface membrane proteins. We have also conducted experiments in which we labeled sensory neurons only after exposure to 5-HT, using gold-conjugated MAbs to apCAM. In a preliminary experiment, exposure of sensory neurons to 5-HT in the absence of MAbs, followed by labeling of the cells with MAbs at 4°C, resulted in a decrease of apCAM on the surface membrane. These findings suggest that the down-regulation of apCAMs is triggered by 5-HT and not by cross-linking

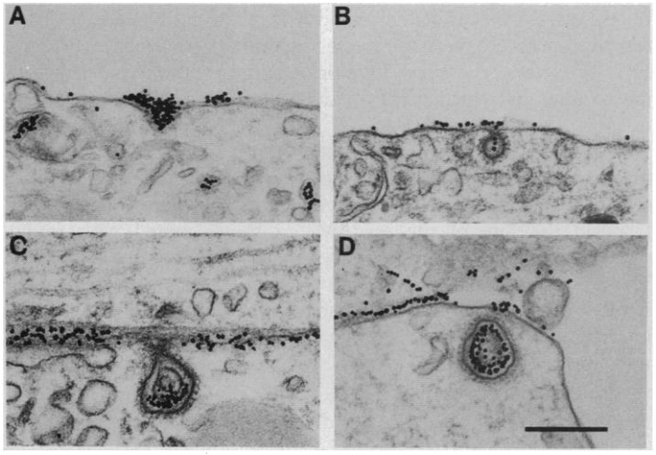

Fig. 3. Internalization of apCAM by means of coated pits. (**A**) The early stages of 5-HT–induced coated pit formation. (**B**) The morphology of coated pits at unapposed membranes. (**C**) The morphology of coated pits at apposed membranes. (**D**) Large coated vesicles containing high concentrations of gold particles. Scale, 0.25 μm.

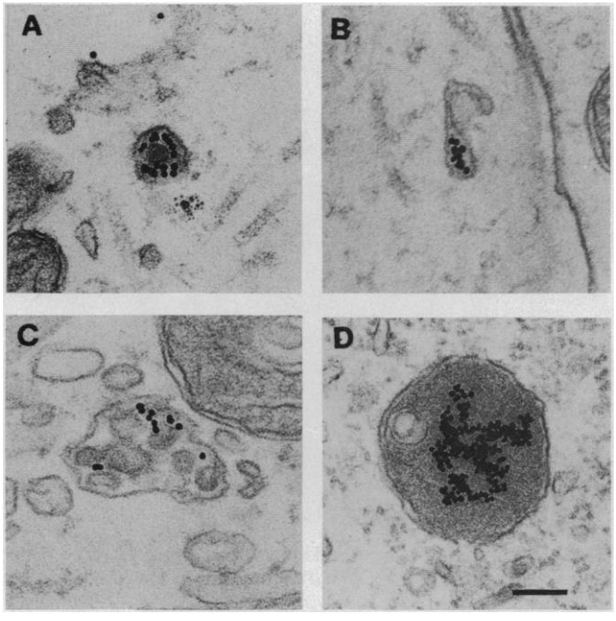

Fig. 4. Intracellular fate of down-regulated apCAM: Internalization and degradation via the endosomal pathway. (**A**) Early smooth-surfaced endosomes. (**B**) Uncoupling (CURL) vesicles or sorting endosomes. (**C**) Multivesicular bodies. (**D**) Mature lysosomes. Scale for (A to C), 0.1 μm; (D) 0.15 μm.

of apCAMs through a multivalent probe.

How is the internalization of apCAM accomplished? To address this question, we next examined the intracellular fate of the apCAM-MAb complex in isolated sensory neurons. We found that the internalization of apCAM was initiated at specialized depressions of the surface membrane of the sensory neuron (Fig. 3). These parts of the plasma membrane appear coated because of an electron-dense, bristle-like layer along the cytoplasmic leaflet. Numerous proteins have been isolated from these membranes in other neuronal and non-neuronal systems, and the best characterized is clathrin. Indeed, concomitant with the down-regulation of the apCAMs, there was an increase in the expression of the light chain of clathrin (*17*). During the earliest stages of the formation of coated pits in response to 5-HT, there was already a prominent accumulation of gold particles, suggesting that as with other forms of receptor-mediated endocytosis these specialized depressions function as molecular sieves that concentrate certain surface proteins and exclude others (Fig. 3A).

Receptors that are constitutively internalized, like the low-density lipoprotein receptor (*18*), the transferrin receptor (*19*), and the asialoglycoprotein receptor (*20*), are concentrated in coated pits even in the absence of ligand. However, in the absence of 5-HT, apCAM does not accumulate in coated pits and is internalized at a very low rate, while WGA is internalized rapidly, suggesting that apCAM is not constitutively internalized. Comparison of the internalization of WGA and apCAM in the absence of 5-HT (31% and 4%, respectively) supports the notion that apCAM is not constitutively internalized. Indeed, apCAM lacks a typical internalization sequence (*21*), and mutated Fc receptors (also members of the Ig superfamily) that lack the cytoplasmic domain, or in which the internalization sequence has been disrupted, are also internalized slowly (*12*).

The mechanisms for internalization of apCAM at regions of contact between the axonal fascicles of the sensory neurons seem to differ in several ways from the mechanisms at naked, unapposed segments of the axon. First, at unapposed sites, the morphology of coated pits and coated vesicles was simple and consisted of the conventional cup-like, coated invagination of the surface membrane (Fig. 3B). By contrast, at apposed sites the coated pits were consistently larger and more complex (Fig. 3C). At sites of membrane apposition, the coated pits were 50% wider (172 ± 6 nm versus 114 ± 7 nm; $n = 10$; $P < 0.001$), extended 63% deeper into the cytoplasm (196 ± 15 nm versus 120 ± 9 nm; $n = 10$; $P < 0.001$), and contained 4.5 times more internalized gold particles (32 ± 6 particles versus 7 ± 2 particles; $n = 10$; $P < 0.001$) than did the more conventional coated pits at unapposed segments of the sensory neuron plasma membrane.

Second, at sites of membrane apposition the coated pits had a more complex internal morphology, often displaying one or more smooth membrane-bound vesicular profiles within the principal invagination. Occasionally, the membranes of these small, interior vesicles appeared continuous with the surface membrane of the apposed neurite. These observations are consistent with the pinching off and internalization of small segments of the plasma membrane of the opposing neurite. Indeed, after the formation of coated pits at sites of membrane apposition, these complex invaginations separated from the surface membrane to form coated vesicles, which still contained internal profiles and a heavy concentration of gold particles consistent with the internalization of two apposed membrane surfaces (Fig. 3D). This cell-cell interaction could serve as a potential signaling mechanism (*22*).

Third, at sites of membrane apposition the 5-HT–induced internalization of apCAM is particularly prominent. This removal of cell adhesion molecules from patches of apparent adhesion may be an important molecular step leading to defasciculation of the axonal processes of the sensory neurons. Moreover, these sites of prominent internalization are often characterized by intense endocytic activity. The clustering of heavily labeled endosomal vesicles near or directly above these sites might reflect an earlier, synchronous burst of endocytic activity at the surface membrane.

We next examined the pathway of internalization activated by 5-HT and found it to be similar to that of receptor-mediated endocytosis. The labeled apCAMs entered into a prelysosomal-endosomal pathway for apparent degradative processing (*23*). Thus, we found the internalized apCAMs in a series of membrane-bound subcompartments, which, based on their similarity in morphology to different classes of endosomes described in other systems, appear to include early smooth-surfaced endosomes (Fig. 4A), more complex structures resembling uncoupling (CURL) vesicles or sorting endosomes (Fig. 4B), as well as a variety of putative late endosomes including multivesicular bodies (Fig. 4C). The final step in this degradative pathway occurs in the cell body, where the internalized gold is heavily concentrated in mature lysosomes (Fig. 4D).

The low pH in the CURL is thought to cause receptors to dissociate from their ligands (*24*). Free receptors targeted for recycling congregate in the thin, tubular membrane section of the CURL. This is thought to break off to form a separate elongated vesicle that recycles the receptor back to the plasma membrane. By contrast, ligand or a ligand-receptor complex that is targeted for degradation is segregated into the swollen portion of the CURL, which ultimately either fuses with multivesicular

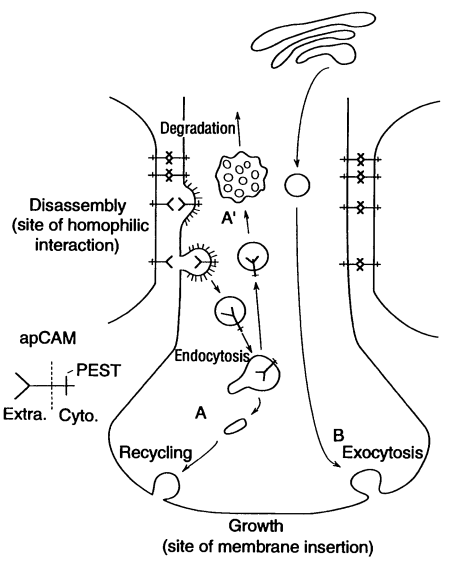

Fig. 5. Internalization, disassembly, and learning-related growth. The onset of synaptic growth is triggered by the facilitating neurotransmitter 5-HT. Serotonin leads to a down-regulation of NCAM-related apCAMs. Part of this down-regulation occurs at the presynaptic membrane, where disassembly is achieved by a transient internalization involving the endosomal pathway. Internalization seems to be particularly active at sites of apposition (depicted here as stable, nongrowing regions where one neurite abuts and adheres to another) and is characterized there by intense endocytic activity. These focal endocytic bursts begin at coated pits and proceed through a series of endosomal precursors, including uncoupling (CURL) vesicles. Here, the internalized plasma membrane can follow one of two pathways. In pathway A, internalized endocytic membrane components are retrieved from sites of apparent adhesion in the tubular extension of the CURL and reinserted at the surface at sites of new synapse formation. In pathway A', the apCAM molecules are targeted for degradation and become segregated within the swollen, vesicular portion of the CURL, which ultimately fuses with or matures into late endosomal compartments such as multivesicular bodies. Additional membrane inserted into the terminal for growth may also come by means of recruitment of new transport vesicles from the trans-Golgi network and the insertion of membrane by exocytosis (pathway B). The cytoplasmic domain of apCAM contains a prominent PEST sequence, which may be proteolytically cleaved and lead to enhanced turnover of newly synthesized protein (*5*), as well as contribute to the rapid internalization that accounts for the altered expression of apCAM at the surface membrane.

bodies or matures into late endosomes and lysosomes. We have found a differential targeting of the internalized apCAM with respect to these two functionally distinct CURL compartments in control and 5-HT–treated sensory neurons. In control preparations, 37 ± 3% (n = 6) of the gold-labeled complexes were in the swollen portion of the CURL and 63% were present in the thin, tubular neck. This distribution is reversed after exposure to 5-HT: 90 ± 3% (n = 7; $P < 0.001$) of the gold particles were within the vesicular, degradative compartment, and only 10% of the label was in the membranous, recycling extension (*25*).

Thus, our results indicate that a neurotransmitter important for learning can stimulate receptor-mediated endocytosis and lead, by this means, to a rapid internalization of an NCAM-related cell adhesion molecule. Moreover, unlike classical receptor-mediated endocytosis, in which internalization is triggered by the binding of ligand to its homologous receptor, the endocytosis of apCAM is triggered by the binding of ligand to a heterologous receptor, the 5-HT receptor, and consequent internalization of apCAM. A similar pathway for internalization can therefore be activated in two distinctly different receptor pathways, through heterologous as well as through homologous receptors.

A possible clue to the heterologous mechanisms for internalization of apCAM is suggested by the presence of a PEST sequence in the cytoplasmic region of the transmembrane form of apCAM (*5*). This sequence is also found in other cell adhesion molecules, NCAM and fasciclin II, and is postulated to be involved in protein degradation. Proteolytic cleavage of apCAM (*26*) induced by 5-HT might alter its conformation or its interaction with cytoskeletal elements, so that apCAM could now be localized to coated pits and subsequently internalized.

Both the down-regulation of apCAM at the level of expression and the internalization of apCAM require new protein synthesis. The requirement for protein synthesis during these initial, endocytic stages of long-term facilitation correlates with the dependence of the later growth changes on the synthesis of new protein and mRNA (*27*). In the case of internalization of apCAMs, this requirement for protein synthesis may reflect the induction of a protein, perhaps a protease, necessary for internalization or the requirement of a protein that turns over rapidly.

The ability of 5-HT to modify the structure of the surface and internal membrane systems of sensory neurons in *Aplysia* by initiating a rapid and protein synthesis–dependent sequence of steps is similar to the changes induced by growth factors such as EGF and NGF in non-neuronal (*28*) and PC12 cells (*29*). This finding suggests that with repeated or prolonged exposure, neurotransmitters such as 5-HT can act like growth factors and cause a persistent alteration in the architecture of the neuron.

Our data further suggest that the 5-HT–induced internalization of apCAM and the consequent membrane remodeling may represent the first morphological steps in the structural program for long-term facilitation. According to this view (Fig. 5), learning-related synapse formation is preceded by and perhaps requires endocytic activation, which can then serve a double function. First, endocytic activation can disassemble adherent fascicles of the sensory neurons (defasciculation). The punctate and discontinuous distribution of apCAM on the cell surface suggests that the adhesive interactions mediated by apCAM occur in patches. The removal of these clusters of apCAM may destabilize adhesive contacts between axonal processes and facilitate defasciculation. Second, massive endocytic activation can lead to a redistribution of membrane components that favors synapse formation. The assembly of membrane components required for initial synaptic growth may involve insertion, by means of targeted exocytosis, of endocytic membrane retrieved from sites of adhesion and recycled to sites of new synapse formation. Synapse formation may require, in addition, the recruitment of new transport vesicles from the trans-Golgi network.

The notion that endocytic activation may provide some of the initial membrane components required for learning-related synaptic growth is consistent with the idea that membrane proteins may come from an endosomal precursor (*30*). This idea is also supported by studies of neurite elongation in culture, which suggest that membrane flow and addition depends on the endocytic-exocytic cycle (*31*). Thus, aspects of the initial steps in learning-related synapse formation may eventually be understood in the context of a heterologous and targeted form of receptor-mediated endocytosis.

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7. See Glanzman and co-workers (*4*) for methodological details on cell culturing. Each sensory culture contained multiple cells; cocultures contained a single sensory neuron and the gill motor neuron L7. Cells were allowed to grow in culture for 5 days before each experiment. MAb 4E8 was purified from ascites fluid by affinity chromatography with protein A, conjugated with 10-nm colloidal gold (Amersham), and incubated for 1.5 hours at room temperature with the cultured cells. After rinsing to remove unbound MAb, 5-HT (Sigma, creatinine sulfate) (2 to 5 μM) was added for 1 hour at room temperature. Cells were fixed in place on poly-L-lysine–coated Aclar 33c coverslips by slow perfusion with a trialdehyde solution containing 1% paraformaldehyde, 1% acrolein, 2.5% glutaraldehyde, and 2.5% dimethylsulfoxide with 10% sucrose plus $CaCl_2$ (0.05%) in 0.1 M cacodylate buffer (pH 7.4) [D. L. Glanzman *et al.*, *Neuron* **3**, 441 (1989)]. After 1 hour at room temperature, this fixative was replaced with 2% paraformaldehyde and 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 16 to 20 hours at 4°C. The cultures were treated with 2% O_SO_4 in 0.1 M cacodylate buffer (pH 7.4) for 1 hour at room temperature. After the cultures were embedded in Epon 812, serial thin sections (50 to 100 sections per block) were cut parallel to the substrate surface, stained with lead and uranyl acetate, and photographed with a Philips 301 electron microscope. A total of 52 cultures was used. Random regions of immunolabeled thin sections were sampled by taking micrographs at regularly spaced intervals. Enlargements of the micrographs (80,000×) were quantitatively analyzed with a blind procedure. To quantitate the gold-labeled complexes, the micrographs were mounted on a Bioquant II digitizing tablet (R & M Biometrics, Inc., Nashville, TN) interfaced with an Apple IIe microcomputer, and the linear extent of surface membrane was obtained by digitized tracing. The total number of gold particles along the surface membrane was counted for each micrograph. An internalization index was computed by dividing the total number of gold particles inside the cell by the total number of gold particles for each micrograph. Data from each culture dish constituted one score or ratio.
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14. For control and experimental groups, each micrograph contained an average of 16 μm of sensory neuron surface membrane. The total number of gold particles in each micrograph from 5-HT–treated cells was 208 ± 15 (SEM) and, for control cells, 313 ± 27. Of the 208 gold particles per micrograph in 5-HT–treated cells, 147 ± 9 were on the surface membrane and 61 ± 7 were in intracellular compartments. For the 313 gold particles per micrograph in control cells, 300 ± 26 were on the surface and 13 ± 2 were internalized. Therefore, of the 50% reduction of gold complexes induced by 5-HT at the surface (147 of 300), internalization can account for approximately half or 20% (61 of 300). The remainder may be due to other factors, for example, release directly from the surface or from inside the cell. Our sampling field did not include cell bodies, which subsequent analysis has indicated contain numerous lysosomes that are heavily labeled with gold after exposure to 5-HT. Thus, the actual figure for internalization is likely to be much higher.
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Colloid Formation During Waste Form Reaction: Implications for Nuclear Waste Disposal

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Insoluble plutonium- and americium-bearing colloidal particles formed during simulated weathering of a high-level nuclear waste glass. Nearly 100 percent of the total plutonium and americium in test ground water was concentrated in these submicrometer particles. These results indicate that models of actinide mobility and repository integrity, which assume complete solubility of actinides in ground water, underestimate the potential for radionuclide release into the environment. A colloid-trapping mechanism may be necessary for a waste repository to meet long-term performance specifications.

Recent emphasis in the U.S. high-level nuclear waste disposal program has focused on determination of the suitability of the potential site at Yucca Mountain, Nevada (*1*). The current reference design (*2*) calls for spent nuclear fuel from commercial reactors and for high-level waste glass to be contained in an engineered barrier system (EBS) that is surrounded by the natural host rock. Ideally, this multiple barrier system (waste form, EBS, rock) limits radionuclide release from the waste material and retard radionuclide migration due to chemical and physical interactions with the rock.

Evaluation of the total system performance has been based on the premise that the release of actinides [for example, Pu and Am (*3*)] from the waste form will be controlled by the solubility of individual elements in ground water and that subsequent radionuclide transport will be determined by factors such as water flow and by radionuclide retardation processes such as sorption (*4–7*). Under this scenario, the waste form itself can react relatively rapidly, yet the transport of radionuclides will be limited by geohydrologic factors.

In this report we demonstrate limitations of the solubility-controlled assumption by examining the release of Np, Pu, and Am from waste glass under simulated storage conditions. Although it is known that radionuclides may form colloids (*8*) that could affect transport properties (*9*, *10*), it has generally been assumed that colloids result from hydrolysis of dissolved species in solution (radiocolloids) or from adsorption of dissolved radionuclides onto suspended mineral particles in the ground water (pseudocolloids) (*5–7*). Both of these processes of colloid formation occur independently of waste form and EBS, and they may be considered secondary processes. However, because glass is a metastable solid, it may transform into a more stable phase assemblage under repository storage conditions (*11*, *12*). This assemblage may be a new source of colloidal material, termed primary colloids, and forms by a process that is dependent on the waste form. The radionuclide content, the sources, and the mechanism of formation of these colloids must be accurately known to assess transport of radionuclides from the EBS to the accessible environment.

Glass may be exposed to a variety of

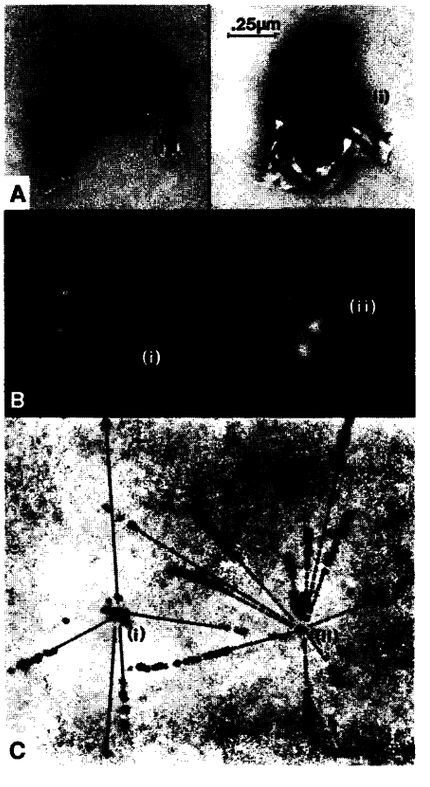

Fig. 1. (**A**) Bright-field electron micrographs of two colloid (residue) particles [labeled (i) and (ii)]. (**B**) Corresponding backscattered electron images. Bright areas indicate high concentrations of brockite (ideally Ca-Th phosphate). (A) and (B) demonstrate that particle (i) contains mostly clay with minor brockite, whereas particle (ii) contains predominantly brockite. (**C**) The α-tracks in nuclear emulsion (with extrapolated trajectories) superimposed on a bright-field image of particles (i) and (ii). The smaller particle, (ii), contains more (Pu- and Am-bearing) brockite and therefore produces more tracks.

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