

Communication

Receptor-Mediated Endocytosis in Plants is Energetically Possible¹

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ABSTRACT

A detailed examination of the arguments of Cram (1980 *New Phytol* 84: 1–17) against the uptake of major nutrients in plants by endocytosis shows that the arguments do not exclude the possibility of receptor-mediated endocytosis.

Receptor-mediated endocytosis² may be involved in various important physiological processes in plants. Membrane recycling (15, 24) may be accomplished by endocytosis. We have hypothesized that some phytoalexin elicitors operate by crosslinking membrane receptors and entering the cell by endocytosis (25). Certain toxins enter animal cells by receptor-mediated endocytosis (19); it would be surprising if no plant pathogen exploits this mode of penetration. Viruses are known to enter animal cells by endocytosis (6), but the situation in plant cells is controversial (18). In a review, Takebe (32) states that “results of electron microscopic observations of inoculated protoplasts are largely consistent with the hypothesis that virus particles enter protoplasts via an endocytic process,” while Watts *et al.* (36) maintain that endocytosis “does not appear to play a significant part in inoculation.”

A variety of substances enter animal cells by receptor-mediated endocytosis (1, 20, 26): hormones such as insulin and gonadotropin-releasing hormone, nutrients such as low-density lipoprotein (a cholesterol carrier) and transferrin (an iron carrier), and viruses. In a common form of the mechanism, ligands bind to receptors in the plasma membrane and cross-link them. The cross-linked receptors then collect in coated pits, which are internalized as coated vesicles. The receptors are decomposed or recycled, and the ligands decomposed or delivered to internal sites.

The existence of coated vesicles has been demonstrated in yeast (17) and in higher plants (for example, Ref. 4). Endocytosis has been observed in protoplasts of various higher plants (7, 9, 33, 34) and in spheroplasts of yeast (11); these cells are held at zero turgor pressure. Endocytosis has also been observed in intact, turgid cells: in various plant cells by the use of heavy metal salts (for example, Refs. 8, 37), and in yeast cells by the use of lucifer yellow (23), α -amylase, and fluorescein-dextran (12, 13).

Cram (2) argued that endocytosis cannot be the principal means of transporting major nutrients into plant cells. We shall demonstrate that Cram's results do not rule out the possibility of receptor-mediated endocytosis in plant cells. The vesicles involved in endocytosis are small, with a large surface-to-volume ratio, so that a large area of membrane can be internalized with a small turnover of volume. Thus, endocytosis is an inefficient means of uptake of nutrients from bulk solution, as Cram points out, but very effective means of uptake of any species that binds to the cell surface.

One of Cram's arguments involves the pV work needed to form a vesicle against the turgor pressure p of the plant cell. Cram finds that the cell does not produce enough power to do the pV work required for endocytosis at the observed rate of nutrient uptake.

We evaluate the pV work required to internalize the plasma membrane. Consider a vesicle of radius r , area $a = 4\pi r^2$, and volume $v = 4\pi r^3/3$, and a spherical cell of radius R , area A , volume V , and density ρ . The work to form one vesicle is then pv ; the number of vesicles needed to internalize the entire plasma membrane is A/a ; and the work required to internalize the entire plasma membrane is pvA/a . If the entire plasma membrane is internalized in a time τ , then the power needed per cell is $pvA/a\tau$, and the power per unit mass is $(pvA/a\tau)(1/\rho V) = (p/\rho\tau)(r/R)$. Observations of coated vesicles of plants by electron microscopy give a diameter of approximately 100 nm or less (4). If we assume a cell diameter of 50 μm , a density of 1 g/cm^3 , and a turgor pressure of 6 bar (0.6 MPa), then the power per unit mass to internalize the entire plasma membrane in 10 min is 2 $\mu\text{W}/\text{g}$, much less than the range of respiratory rates cited by Cram of 270 to 2000 $\mu\text{W}/\text{g}$ (fresh weight).

If a secretory cell maintains a constant size, the membrane flow to the plasma membrane by secretory vesicles must be balanced by a return flow from the plasma membrane to the cell interior. We calculate the power required for endocytotic return flow, again assuming spherical cells and a turgor pressure p of 6 bar. We also assume that the endocytotic vesicles are the same size as the secretory vesicles observed. For secretion of slime by root cap cells of maize, Morr  and Mollenhauer (16) report $r = 0.25 \mu\text{m}$, $A = 3100 \mu\text{m}^2$, and $\tau = 3.75 \text{ h}$, giving a power requirement of 1.2 $\mu\text{W}/\text{g}$. For secretion of slime by *Mimulus* gland hairs, Schnepf and Busch (27) found $a = 0.8 \mu\text{m}^2$, $A = 4000 \mu\text{m}^2$, and $\tau = 32 \text{ min}$, so that the power required is 4.4 $\mu\text{W}/\text{g}$. From the data of Kristen and Lockhausen (10) for secretion by ovary glands of *Aptenia*, we obtain 18.3 $\mu\text{W}/\text{g}$. For an extreme case, the water gland of *Monarda*, $A = 1000 \mu\text{m}^2$, and $\tau = 0.5 \text{ min}$ (5). If the vesicle radius r is 0.45 μm , the power required is 1000 $\mu\text{W}/\text{g}$, still within the range of respiratory rates cited by Cram (2). So the power requirements are not excessive if the

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² Following Morr  *et al.* (15), we define endocytosis as the uptake of extracellular material by invagination of the plasma membrane. Endocytosis is therefore equivalent to pinocytosis as defined by Wheeler and Hanchey (37) and by Cram (2).

Table I. Observed Turnover Rates and Times for Plasma Membrane

System	Rate	Turnover Time	Reference
	$\mu\text{m}^2/\text{min}$	min	
Model	785	10	Assumed
Root cap slime	14–26	40–70	5
Slime (<i>Drosophyllum</i>)	26	0.4	5
Water glands (<i>Monarda</i>)	2000	0.5	5
Moss protonema tip cells (<i>Funaria</i>)	170	Not applicable	22, 28
Pollen tubes			
(<i>Lilium</i>)	300	Not applicable	^a
(<i>Tradescantia</i>)	350–600	Not applicable	^b
Plant cells			
Growing		20–40	4
Not growing		60–115	4
Animal cells	18.3–198	7–600	35

^a From observed growth rate (15). ^b From observed accumulation rate and diameter of vesicles (21). Cytochalasin D inhibits production as well as transport of vesicles (29), so the value given is a lower limit.

membrane is recycled by endocytosis of vesicles the same size as the secretory vesicles. The power required is proportional to the radius of the endocytotic vesicle, so the requirement is lower if membrane recycling takes place by means of 100-nm coated vesicles, and still lower if membrane recycling is by micelles or individual molecules, as suggested by Schnepf and Busch (27). Staehelin and Chapman (30) recently proposed that both endocytosis and internalization of individual lipid molecules are involved in membrane recycling.

Some values of exocytosis rates of plant cells, endocytosis rates of animal cells, and turnover times for plasma membrane are given in Table I. (Turnover times are not applicable to growing tip cells.) Note that the biochemical turnover times for degradation and synthesis of lipids are much greater, about 10 to 100 h (14).

The power requirement for pV work is usually a small fraction of the power available from respiration; it is also negligible compared to the power required by a mucilage cell or a root cap cell to synthesize carbohydrates at the rate they are excreted.

If the secretion rate of the polysaccharides is \dot{V} (volume/time) and the turgor pressure is p , then the power P_w to do the pV work for the return flow of membrane is simply $p\dot{V}$. The power required for the synthesis of the carbohydrate is $P_{\text{syn}} = \dot{V}cg$, where c is the concentration of polysaccharide, and g is the heat of formation of the polysaccharide from CO_2 and H_2O . So the ratio $P_w/P_{\text{syn}} = p/cg$. If $p = 6$ bar, $c = 10$ mM glucose, and $g = 2.87$ MJ/mol glucose, the ratio is 0.02. Even at this low a concentration of carbohydrate, the cost of transporting the carbohydrate is negligible compared to the cost of producing the carbohydrate by photosynthesis.

If the cell size remains constant, the flow of plasma membrane into the cell must be balanced by a flow from the interior to the plasma membrane. In a secretory cell, if the return flow is by means of endocytotic vesicles formed at the site to which exocytotic vesicles are delivered, the endocytotic vesicles will pick up some of the material just secreted. One way of avoiding this problem is to form the endocytotic vesicles elsewhere in the plasma membrane. Another possibility is to use smaller vesicles (or micelles) for endocytosis than for exocytosis; this will reduce the volume flow into the cell by the ratio of the radii of endocytotic to exocytotic vesicles.

Cram also argues that if a plant cell takes up nutrient solution at the observed rate, it cannot get rid of water fast enough. Again, this argument does not rule out receptor-mediated endocytosis. If the entire plasma membrane is internalized in a time τ , the

total water flow is $(v/\tau)(A/a)$, so that the water flux is $J = v/a\tau$. But the flux is related to the pressure gradient Δp by $J = L_p\Delta p$, where L_p is the hydraulic conductivity. So the pressure increase due to endocytosis is $\Delta p = v/a\tau L_p = r/3\tau L_p$. If we assume $r = 50$ nm as before, we obtain a water flux of 3×10^{-11} $\text{m}^3 \text{m}^{-2} \text{s}^{-1}$ for one complete turnover of the plasma membrane in 10 min. If the hydraulic conductivity L_p of the membrane is the highest value assumed by Cram, 10^{-8} $\text{m s}^{-1} \text{bar}^{-1}$, the increase in turgor pressure is only 0.003 bar. For Cram's lowest value, 10^{-11} $\text{m s}^{-1} \text{bar}^{-1}$, the increase is 3.0 bar, clearly high. The accuracy of the lowest value of L_p has been questioned (3). The range of values of L_p from pressure probe measurements (31) is 10^{-9} to 10^{-7} $\text{m s}^{-1} \text{bar}^{-1}$; with these values Δp would be at most 0.03 bar.

Again, if the cell is not expanding, there has to be a flow of lipid back to the plasma membrane. If the flow is by way of exocytotic vesicles, there will be a corresponding flow of water out of the cell, compensating partially or entirely for the endocytotic water intake.

Note that on account of the surface-to-volume ratio of the vesicles, the turnover of cell volume is very slow even if the turnover of cell surface is fast. If the total water flow is $(v/\tau)(A/a)$, the turnover time τ_v for the cell volume is $\tau_v = (V/v)(a/A)\tau = (R/r)\tau$. For the dimensions assumed, and a turnover time $\tau = 10$ min for the plasma membrane, $\tau_v = 5000$ min = 80 h.

Cram states that "it clearly remains possible that a low rate of pinocytosis might occur in plants, providing a mechanism, possibly specific, for the uptake of macromolecules that could not be taken up by any other means." As we have shown in detail, receptor-mediated endocytosis is such a mechanism.

Note Added in Proof. The results in yeast with fluorescein-dextran (12, 13) are artifactual, according to RA Preston, RF Murphy, EW Jones 1987 *J Cell Biol* 105: 1981–1987.

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