of avoiding the wrong competitive encounters as much as precipitating the right ones.

Competitive interactions between colonies on a coral reef often occur over relatively short distances at zones of contact (*16*). Thus, whether two colonies will meet and interact will depend largely on patterns of recruitment and normal, asexual growth. The erect and planar growth form of *Millepora* and many gorgonians makes sustained contact and interaction relatively unlikely. *Millepora*'s ability to actively detect and pursue a predictably inferior competitor from some distance has some interesting implications for the mechanics of interactions on coral reefs. In effect, this interaction begins not when contact is made (and observed) but when the target gorgonian is detected. This means that, as an interactive unit, the effective boundaries of a potential target gorgonian extend beyond its physical margins to the distance over which it can be detected. Since detection is dependent on directional water flow, the initial settlement patterns of potentially interacting colonies will profoundly affect future events.

The basic ecological difference between mobile and sessile organisms is the ability to actively change locations and thereby influence specific encounters and interactions. For *Millepora*, and perhaps other sessile marine taxa which use directed growth rather than motion; this apparently fundamental difference is more one of method and time scale than one of effect.

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6. The use of "attack" branch is meant to convey the functional and morphological distinction between specialized branches that serve to encounter and encrust another organism and normal, noninteractive branches within *Millepora* colonies, regardless of colony shape; it in no way implies intent.
7. Seventy-five interactions were examined in an area of approximately 200 m² at Gorgo City, 3 miles west of Discovery Bay, Jamaica. Surge axis is north-south. Frequency of positions of attacking *Millepora* relative to target gorgonians (north, 40; south, 31; east, 1; west, 3) were significantly different as judged by the χ^2 goodness-of-fit test. Down-current attacks did not differ significantly between north and south.
8. *Millepora*-target pairs were clamped facing the current on parallel P.V.C. pipes separated by 15 cm; pairs were separated by 60 cm. The experiment ran for 10 months between 1 October 1977 and 1 August 1978 at the site in (*7*). Horizontal growth in *Millepora* colonies constituted a positive response. Loss and replacement of some *Millepora* colonies created unequal final sample sizes; all were paired longer than the minimum response time (1.5 months).
9. Interestingly, *Millepora* attacked the scleraxonian *Briareum asbestinum* whose internal skeleton of loosely compacted spicules is unsuitable for encrustation.
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Endocytotic Sucrose Uptake in *Amoeba proteus* Induced with the Calcium Ionophore A23187

Abstract. *The calcium ionophore A2187 brings about an influx of calcium and uptake of sucrose by endocytosis in* Amoeba proteus. *The amount of endocytotic sucrose uptake elicited by the ionophore depends upon the external calcium ion concentration. Calcium ion movements may serve to couple the surface phase of endocytosis with cytoplasmic uptake of the endocytotic inducer.*

Endocytosis is a basic cellular process by which extracellular solute is taken up by cell surface membrane infolding and vesiculation. Uptake of solute in this manner, distinct from carrier-mediated transport processes, is a physiological mechanism responsible for the uptake of large, normally impermeant, solute molecules. There is increasing evidence that Ca^{2+} plays an important role in endocytosis, as it apparently does in exocytosis (*1*). In *Amoeba proteus*, a model system used to investigate endocytosis (pinocytosis), maximum endocytotic activity requires an optimal concentration of Ca^{2+} in the external medium, on the order of $10^{-4}M$. In addition, the inducer of endocytosis in the amoeba displaces a dose-dependent amount of calcium from the cell surface (*2*). In this study with amoebas, addition of the calcium ionophore A23187 to the external medium resulted in an influx of Ca^{2+} and the endocytotic uptake of sucrose from the external medium—further evidence that Ca^{2+} plays a pivotal role in the mechanism of endocytosis.

In *Amoeba proteus*, endocytosis is normally brought about by a variety of positively charged solute molecules that bind to the surface of the cell (*3*). This is followed by cessation of locomotion, the development of numerous surface projections, and the invagination of channels leading from the tips of these projections into the cytoplasm. Finally, the bases of the channels are pinched off into vesicles containing surface membrane with adsorbed inducer and a portion of the bulk medium (*4*). How the initial interaction of the endocytotic inducer with the surface is coupled with its eventual uptake by endocytosis is not known, but it has been established that the onset of endocytosis in the amoeba is accompanied by physiological and structural changes in the cell membrane. These include an increase in membrane conductance and a reversible increase in the thickness of the membrane lipid lamella (*5*). Associated with these changes in the cell membrane is a possible movement of the calcium ion, which may serve to couple the surface phase of endocytosis with subsequent cytoplasmic events (*1*). This possibility was investigated by use of the calcium ionophore A23187 (*6*).

This ionophore is an antibiotic that acts as a carrier for Ca^{2+}. Structurally, A23187 is a relatively complex carboxylate compound with a molecular weight of 523. The ionophore forms a complex with Ca^{2+} only in its deprotonated anionic form, with two A23187 anions taking up a pseudocyclic conformation around one calcium ion. In this configuration the

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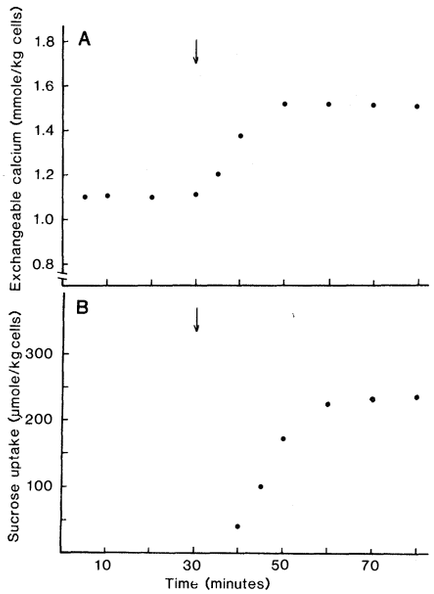

Fig. 1. (A) Ionophore-stimulated ^{45}Ca exchange in *Amoeba proteus* in control medium (0.0 m*M* Ca^{2+}). The ^{45}Ca (1 μCi/ml) was added to a suspension of amoebas, and the exchange was observed over time. After a 30-minute equilibration period, A23187 (50 μg/ml) was added to the cell suspension (arrow). (B) Ionophore-induced sucrose uptake by endocytosis in *Amoeba proteus*. A suspension of amoebas was equilibrated in control medium (0.1 m*M* Ca^{2+}) with 1 m*M* sucrose and [^{3}H]sucrose (2 μCi/ml). After 30 minutes, A23187 (50 μg/ml) was added to the cell suspension (arrow). Sucrose uptake was corrected for extracellular space (24 percent) determined during the 30-minute equilibration period.

polar groups of the ionophore surround the calcium ion, while the exterior of the complex is hydrophobic and consequently lipid soluble (*7*). The ionophore-Ca^{2+} complex can then migrate from the bulk phase, across the cell membrane, releasing Ca^{2+} into the cell interior. Calcium ionophores are then a means of introducing Ca^{2+} into a cell and have been used extensively to investigate the role of Ca^{2+} movements in a variety of biological systems (*8*).

Addition of A23187 (50 μg/ml) to a suspension of amoebas that have been equilibrated with ^{45}Ca for 30 minutes elicits an increase in the amount of calcium associated with the cell (Fig. 1A) (*9*). Under normal circumstances, calcium exchange in *Amoeba proteus* is initially very rapid (1.1 mmole per kilogram of cells in less than 1 minute when the external Ca^{2+} concentration is 0.1 m*M*), with no significant further exchange over the next several hours; thus the labeled Ca^{2+} appears to be exchanging with a fraction of the cell surface calcium (*10*). When A23187 is added to the cell suspension, however, the amount of calcium associated with the cell increases by approximately 0.4 mmole per kilogram of cells. In addition to eliciting an apparent Ca^{2+} influx, A23187 stimulates the uptake of labeled sucrose from the external medium (Fig. 1B).

Under control conditions, the surface of *Amoeba proteus* is impermeable to sucrose, and sucrose itself is not an inducer of endocytosis. Sucrose is used as an extracellular marker to measure the intensity of bulk-phase endocytosis in the amoeba (*11*). When A23187 is added to a suspension of cells, in the presence of 1 m*M* sucrose, the cells begin to take up sucrose after an initial lag of 5 to 10 minutes. About 30 minutes after the addition of A23187, sucrose uptake levels off at approximately 240 μM, corrected for an extracellular space of 24 percent. Calcium exchange and sucrose uptake are both increased as the A23187 concentration in the external medium is increased, maximum sucrose uptake occurring when the external A2187 level is 50 μg/ml. The amount of endocytotic sucrose uptake in the presence of A23187 in the amoeba is a function of the external Ca^{2+} concentration (Fig. 2). In Ca^{2+}-free medium, sucrose uptake induced by A23187 (50 μg/ml) is minimal, but as the external Ca^{2+} concentration is increased to 0.1 to 1.0 m*M*, the uptake of sucrose by endocytosis is maximally stimulated. Further increases in the external Ca^{2+} concentration do not elicit increased sucrose uptake.

The results of this study suggest that movement of Ca^{2+} across the cell surface of the amoeba may act to couple the initial binding of the endocytotic inducer to the cell surface with subsequent uptake into the cytoplasm. Induction of endocytosis in the amoeba begins as the inducer binds to the cell surface, displacing a fraction of cell surface calcium (*2*). Displacement of surface calcium may in turn increase the permeability of the plasmalemma and allow an increase in solute movements across the cell surface (*12*), including an influx of Ca^{2+}. If cytoplasmic Ca^{2+} activity is maintained at very low levels, even a small influx of Ca^{2+} would be sufficient to increase Ca^{2+} activity, at least transiently, in localized areas of the cytoplasm beneath the cell surface. Increases in cytoplasmic Ca^{2+} activity may then stimulate or participate in the contractile response of filaments associated with the surface of the amoeba (*13*), with resulting surface invagination, channel formation, and vesiculation. In this particular instance, the ionophore A23187 does not elicit a Ca^{2+}-dependent endocytotic uptake of sucrose by binding to the cell surface and displacing surface calcium, as do the usual

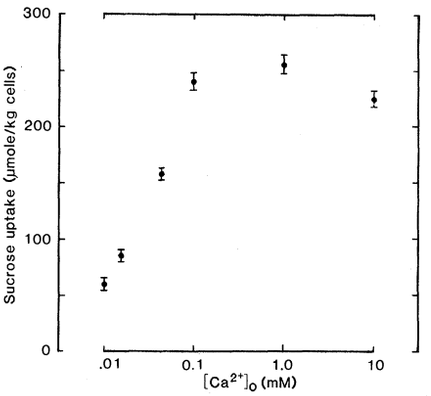

Fig. 2. Ionophore-induced sucrose uptake in *Amoeba proteus* as a function of the external Ca^{2+} concentration. Cell portions were equilibrated in control medium with 1 m*M* sucrose and [^{3}H]sucrose (2 μCi/ml) and varying Ca^{2+} concentrations, with A23187 (50 μg/ml) for 30 minutes. Each point represents the mean of eight determinations, and the vertical bar represents ± 1 standard error of the mean.

cationic inducers of endocytosis, but acts by short-circuiting the usual intermediate sequences of events in the endocytotic cycle. This in turn elevates cytoplasmic Ca^{2+} activity, which directly stimulates the endocytotic uptake of the external medium and labeled sucrose.

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