photodiode to get lower capacitance than in a two-segment photodiode. We use $(A - B)/(A + B)$ as our deflection signal, where A and B are the signals from the two segments of the photodiode. This signal is independent of laser intensity fluctuations, which are otherwise a significant source of noise.
12. M. Mate helped us understand and estimate this force. His AFM measurements of this force are consistent with the formula given by Israelachvili (*13*, p. 221): $F = 4\pi R\delta_s$ where R is the tip radius ($\approx$0.1 μm) and δ_s is the surface tension of the liquid, 7×10^{-2} N/m for water. Thus $F \approx 10^{-7}$ N.
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Cnidocyte Mechanoreceptors Are Tuned to the Movements of Swimming Prey by Chemoreceptors

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Cnidocytes, the stinging cells of cnidarians, discharge nematocysts in response to physical contact accompanied by the stimulation of specific chemoreceptors. Cnidocytes in fishing tentacles of a sea anemone are now found to discharge nematocysts preferentially into targets vibrating at 30, 55, and 65 to 75 hertz. Moreover, in the presence of submicromolar concentrations of known chemosensitizers, such as N-acetylated sugars and mucin, these optima shift to 5, 15, 30, and 40 hertz, frequencies that correspond to the movements of swimming prey. Hence, chemoreceptors for these substances tune cnidocyte mechanoreceptors to frequencies that match the movements of the prey.

Cnidarians, such as hydra, jellyfish, corals, and sea anemones, rely primarily on chemical and mechanical perturbations in the environment to detect prey and predators because, in most cnidarians, vision is absent and photic detection is only crudely developed (*1*). Chemoreceptors are important in regulating prey capture (*2, 3*) and movement of the captured prey to the mouth (*4*). Cnidocytes (cnida-containing cells) discharge nematocysts and other cnidae [intracellular capsules containing eversible tubules (*5*)] into or onto the prey to capture it. This discharge involves a series of cellular (*6*) and mechanical processes that culminate in the rapid eversion of the tubule (*7*). Some everting tubules inject toxins into the prey, whereas others adhere to the surface of the prey or entangle its appendages (*8*).

Substances derived from prey are detected by at least two classes of chemoreceptors (*2*) that predispose cnidocytes to discharge cnidae in response to physical contact (*6*). One class of chemoreceptors is specific for free and conjugated N-acetylated sugars, and the other exhibits broad specificity for low molecular weight amino compounds (*2*). Receptors for N-acetylated sugars occur exclusively on supporting cells (*9*), cells that surround cnidocytes (Fig. 1). With one exception (*10*), the functional characteristics of specific mechanoreceptors in cnidarians have not been described. We now characterize the mechanoreceptors involved in triggering the discharge of nematocysts in the fishing tentacles of a sea anemone.

Monoclonal sea anemones (*Haliplanella luciae*) were reared under defined conditions (*11*) and fed a diet of brine shrimp nauplii (*Artemia salina*) (*12*). Experiments were performed approximately 72 hours after feeding. To test cnidocyte responsiveness, we touched the fishing tentacles with vibrating probes, each of which consisted of a 2-cm segment of monofilament, nylon fishing line having one end coated with a thin layer of gelatin (*11*). Microbasic p-mastigophore nematocysts discharged into the gelatin coating were counted (*11*). Vibrating probes have a flat frequency response from 5 to 100 Hz for displacements at the probe tip ranging from 35 to 700 μm (*13*). A probe

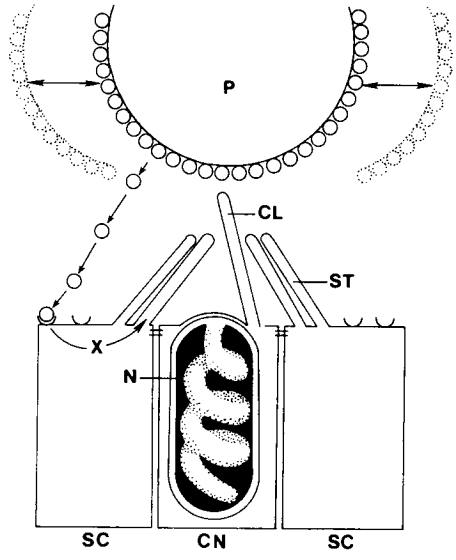

Fig. 1. Diagram of a cnidocyte–supporting cell complex. The cnidocyte (CN) contains a nematocyst (N). Supporting cells (SC) have receptors (curved lines at the top of each SC box) that detect N-acetylated sugars (O) (*9*) on or from prey (P). Receptor-ligand complexes initiate intracellular processes (**X**), which lengthen the stereocilia (ST) (*14*) and tune the cnidocyte mechanoreceptor to frequencies that match movements (double-headed arrows) produced by swimming prey. The mechanoreceptor consists of a single cilium (CL) originating from the cnidocyte and several stereocilia originating from the supporting cells (*20*).

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displacement of 140 μm elicited the discharge of the greatest number of nematocysts (*14*) and was used throughout these experiments.

Cnidocytes discharge nematocysts to bovine submaxillary mucin and to N-acetylated sugars in a dose-dependent manner (*2, 3, 11*), provided that physical contact accompanies exposure to the sensitizer (*6*). Dose responses to these and other chemical sensitizers, measured with static (nonvibrating) probes, show increased discharge of nematocysts at lower concentrations, maximum discharge at higher concentrations, and apparent desensitization with decreased discharge of nematocysts at still higher concentrations (*2, 3, 11*). In the present experiments, a seawater control and doses of mucin in seawater corresponding to distinct regions of the dose-response curve were used: (i) EC_{50}, the effective concentration of mucin that produces a half-maximum response; (ii) EC_{100}, the effective concentration that produces a maximum response; and (iii) DC_{100}, the minimum concentration that causes complete desensitization (a response comparable to that of seawater controls).

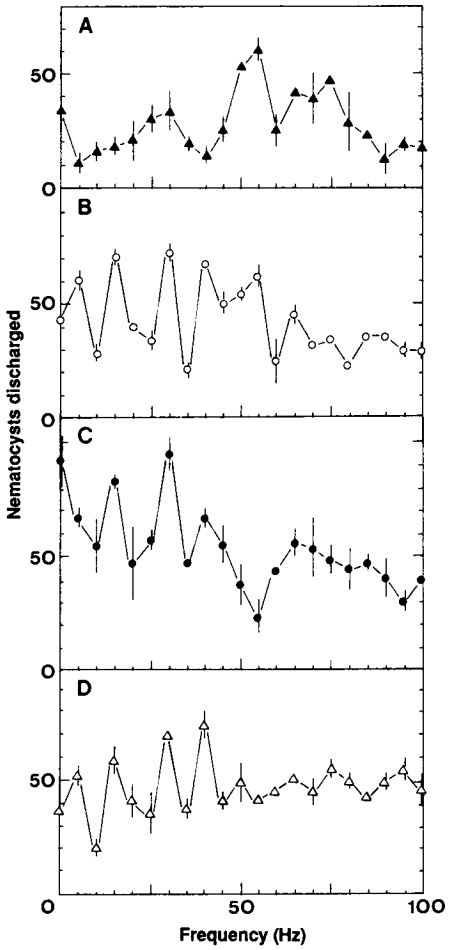

Fig. 2. Frequency responses of cnidocytes. Fishing tentacles were touched with gelatin-coated probes vibrating at a specified frequency 10 min after the sea anemones were exposed to seawater alone (**A**) (▲) or to seawater containing mucin at (**B**) the EC_{50} dose ($4 \times 10^{-8} M$; ○), (**C**) the EC_{100} dose ($4 \times 10^{-7} M$; ●), or (**D**) the DC_{100} dose ($1 \times 10^{-5} M$; △). After contacting the tentacles, each probe was fixed in 2.5% glutaraldehyde in seawater, then prepared as a wet mount. Microbasic p-mastigophore nematocysts discharged into the gelatin coating were counted within a single, microscopic field (450 μm in diameter) (*11*). On a given day, four probes were used for each frequency tested, one for each of four anemones. Each point represents the mean of two daily means (± SEM).

In seawater without mucin, cnidocytes discharged nematocysts maximally into probes vibrating at 55 Hz, and to a lesser degree at 65 to 75 Hz and 30 Hz (Fig. 2A). At each dose of mucin tested, cnidocytes discharged nematocysts maximally into probes vibrating at 5, 15, 30, and 40 Hz (Fig. 2, B, C, and D). Differential frequency-response curves obtained by subtracting data for seawater controls from data for cnidocytes exposed to mucin confirmed that mucin increased the discharge of nematocysts at these frequencies (Fig. 3).

The maximal response of cnidocytes to contact with probes vibrating at 55 Hz in seawater without mucin (Fig. 2A) persisted at the EC_{50} dose of mucin (Fig. 2B) but was lost at the higher doses (Figs. 2, C and D, and 3). At the EC_{100} dose, a modest increase in nematocyst discharge occurred between maxima (at 10, 25, and 35 Hz; Fig. 3). At the DC_{100} dose, cnidocytes readily discharged nematocysts into probes vibrating at 5, 15, 30, and 40 Hz but not into static probes (0 Hz) (Fig. 2D). Hence, the desensitization of cnidocytes observed at relatively high concentrations of sensitizer with static targets (*2, 3, 11*) does not occur with vibrating targets.

Because cnidocytes in fishing tentacles discharge nematocysts primarily to capture prey, we analyzed the movements produced by swimming brine shrimp, the prey of our laboratory-reared anemones. Movements were recorded from a hearing-aid microphone placed at the smaller opening of a specimen chamber containing a single specimen in seawater (*15*). The movements of swimming brine shrimp nauplii and adults are rhythmic, with each cycle consisting of several low-frequency components of varying amplitudes (Fig. 4).

Power spectra of movements produced by single, swimming brine shrimp showed several maxima. Close, overlapping maxima appearing as doublet frequencies are considered here as a single frequency of an intermediate value. Power spectra of movements produced by a swimming brine shrimp nauplius had maxima at 2, 7, 12, 19, 30, 38, and 60 Hz (Fig. 5A), of which 60 Hz is attributed to background electrical noise (Fig. 5B).

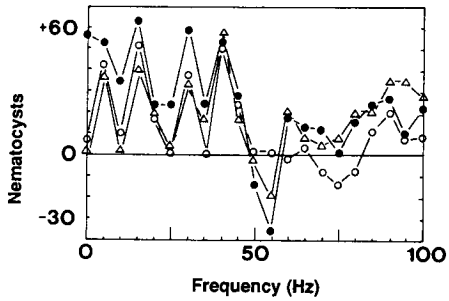

Fig. 3. Differential frequency responses of cnidocytes. Curves were obtained by subtracting data for seawater controls from data for mucin-treated anemones at the EC_{50} dose (○), the EC_{100} dose (●), and the DC_{100} dose (△).

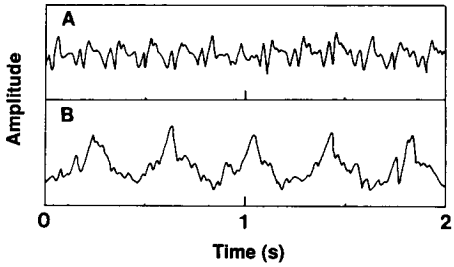

Fig. 4. Recordings of water-borne disturbances produced by swimming prey. Amplified output is shown from a microphone placed at one end of a disposable pipette tip containing (**A**) a brine shrimp nauplius in 25 μl of seawater and (**B**) an adult brine shrimp in 0.2 ml of seawater.

Power spectra of movements produced by an adult brine shrimp showed maxima at 2, 12, 19, 30, and 38 Hz (Fig. 5C). Chemosensitized cnidocytes discharged nematocysts maximally into targets vibrating at about 5, 15, 30, and 40 Hz (Fig. 3), frequencies that approximate those produced by swimming prey (Fig. 5, arrows) and other small crustaceans (*16*), whereas non-chemosensitized cnidocytes discharged nematocysts maximally at predominantly higher frequencies (30, 55, 65, and 75 Hz; Fig. 2A).

Cnidocyte mechanoreceptors (*17*) are similar in morphology to the mechanoreceptors of hair cells in the acousticolateralis systems of vertebrates. In each case, several stereocilia surround or cluster near a single kinocilium (*17, 18*). Stereocilia of hair cells are considered to be structurally static because they contain paracrystalline arrays of cross-linked, filamentous actin (*18*) and functionally static because they exhibit fixed frequency responses (*19*). In contrast, the stereocilia in cnidocyte mechanoreceptors of *H. luciae* increase in length by as much as 70% immediately upon exposure to mucin (*14*). Moreover, mucin-treated cnidocytes discharge nematocysts maximally into targets that are vibrating at lower frequencies (Fig. 3). These observations are consistent with reports that frequency responsiveness

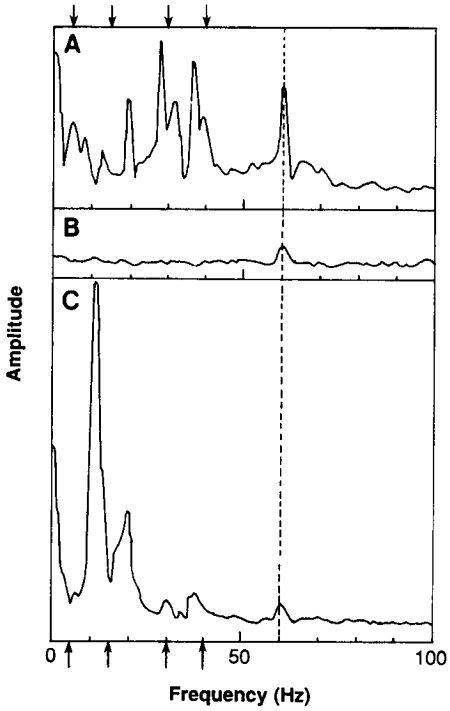

Fig. 5. Tracings of power spectra of swimming prey. Power spectra (plots of the distribution of the intensity of acoustic signals as a function of frequency) of digitized recordings (1.25-s interval) of (**A**) a brine shrimp nauplius in 25 μl of seawater, (**B**) 25 μl of seawater alone, and (**C**) an adult brine shrimp in 0.2 ml of seawater. Arrows mark the optimal frequencies for stimulating the discharge of nematocysts in mucin-treated cnidocytes (Fig. 3). Electrical noise was noted at 60 Hz (dashed line).

in certain free-standing hair cells is correlated with the length of the stereocilia. Hair cells having longer stereocilia respond to lower frequencies (*19*).

In sea anemones, the cilium of each cnidocyte mechanoreceptor originates from the cnidocyte, whereas the stereocilia and the receptors for N-acetylated sugars are located on supporting cells (*9*). Supporting cell chemoreceptors for N-acetylated sugars tune mechanoreceptors involved in discharging nematocysts, possibly by inducing a change in the length of the stereocilia (Fig. 1). Thus, cnidocyte–supporting cell complexes select prey by discriminating among potential prey according to a combination of the chemical and physical cues they present. Our findings raise the possibility that, in other cnidarians as well, preferred prey are selected on the basis of an interplay between chemical and mechanical sensing systems such that chemical substances derived from the prey tune cnidocyte mechanoreceptors to frequencies that match those produced by the moving prey.

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Major Enhancement of the Affinity of an Enzyme for a Transition-State Analog by a Single Hydroxyl Group

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The compound 1,6-dihydropurine ribonucleoside, prepared by reduction of nebularine in the presence of ultraviolet light, is bound by adenosine deaminase approximately 10^8-fold less tightly than 6-hydroxy-1,6-dihydropurine ribonucleoside, a nearly ideal transition-state analog. This difference in affinities, which is associated with the presence of a single hydroxyl group in the second compound, suggests the degree to which one or a few hydrogen bonds may stabilize the transition state in an enzyme reaction of this type.

THE CATALYTIC FUNCTION OF AN ENzyme depends on its ability to discriminate between the substrate in the ground state and the altered substrate in the transition state, binding the latter species more tightly and diminishing the difference in free energy that limits the rate of reaction. These two forms of the substrate tend to be broadly similar in structure, so that discrimination is probably based on a few differences in the immediate neighborhood of the bonds that are formed and broken during the reaction. It is shown that the presence of a single hydroxyl group appears to contribute −9.8 kcal/mol to the free energy of binding of a transition-state analog by adenosine deaminase.

Adenosine deaminase catalyzes the hydrolytic removal of ammonia and other leaving groups from 6-substituted purine ribonucleosides. Hydrolysis cannot occur when hydrogen is present at the 6-position, so that nebularine [purine ribonucleoside (**I** in Scheme 1, where R is ribose] is bound by adenosine deaminase as a competitive inhibitor, exhibiting an enzyme affinity comparable with that of the substrate adenosine (*1*). Until recently, nebularine had appeared to be a typical substrate analog, but ^{13}C nuclear magnetic resonance (NMR) and ultraviolet spectra strongly suggest that nebularine is actually bound in a form that is sp^3-hybridized at the 6-position (*2*). The bound form is probably the rare species [1 part in 10^7 in dilute solution (*3*)] that is covalently hydrated at the 1,6-positions (**II**) (*4*). The apparent affinity of this species for the enzyme (apparent inhibition constant $K_i = 3.0 \times 10^{-13} M$) exceeds the affinities of substrates,

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