tential from the proximal electrode is not observed because of collision. When the proximal site is stimulated after the distal site, only one action potential is recorded until the interval between the stimulations is just longer than the time necessary for the action potential initiated by the distal electrode to propagate via the coupling site to the proximal electrode. At this time interval, the action potential initiated by the distal electrode has propagated just beyond the proximal electrode and can no longer collide with the action potential activated by the proximal electrode. Thus, two action potentials are observed; the direct action potential and the coupled action potential. Since the action potential stimulated by the distal electrode propagates across the coupling site in the direction opposite that stimulated by the proximal electrode, bidirectionality of the coupling is demonstrated.

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7. The collision experiments provide data for the action potential conduction times from one electrode to another. From the distance between the electrodes, the distance from the recording electrode to the coupling site can be computed if the following three assumptions are made: (i) The conduction velocity of the coupled fibers is uniform along their length. (ii) The time required for the action potential to cross the coupling site is negligible compared to the total conduction time. (iii) The distance to the coupling site from the distal stimulating electrode is the same for the two fibers. The ratio of the computed distance to the coupling site divided by the measured distance from the recording electrode to the receptive field of the nociceptor was close to unity (0.98 ± 0.06, $n = 9$); this indicates a high degree of correspondence and thus provides further evidence that the coupling site was at or near the receptor.
8. In this series of experiments, we did not determine the total number of A and C fibers tested. However, in three experiments in the superficial radial nerve of the baboon, we determined that an average of 4.9 ± 0.5 A fibers and 5.3 ± 0.6 C fibers were present in each recorded strand ($n = 30$). Using these numbers, we estimate that in this study of 164 strands we tested 804 A fibers and 869 C fibers.

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Do Frogs Communicate with Seismic Signals?

Abstract. *Male white-lipped frogs exhibit conspicuous behavioral responses to calling conspecific males that are nearby but out of view. Since the calls often are accompanied by strong seismic signals (thumps), and since the male white-lipped frog exhibits the most acute sensitivity to seismic stimuli yet observed in any animal, these animals may use seismic signals as well as auditory signals for intraspecific communication.*

White-lipped frogs (*Leptodactylus albilabris*) inhabit the Luquillo Mountains and adjacent lowlands of Puerto Rico, where males of the species often are found calling from moist ground. We observed that isolated calling males (those stationed far from calling conspecific males) often drastically alter their calling patterns in response to remote, very light footfalls, indicating acute sensitivity to substrate-borne vibrations (seismic stimuli). Therefore, we decided to study the seismic sense of the animal both physiologically and behaviorally. Knowing that the American bullfrog (*Rana catesbeiana*) derives acute seismic sensitivity from its saccule and lagena (*1*), we focused our physiological studies on the white-lipped frog's auditory-vestibular nerve. There we found acute seismic sensitivity, with individual axons exhibiting linear transfer ratios as large as 20,000 spikes per second (axon firing rate) per cm/sec^2 (substrate vibration amplitude) and 70 spikes per second per 10^{-10}m for dorsoventral sinusoidal vibration of the whole animal (*2*). Each seismic axon exhibited band-pass properties and could be characterized in part by a frequency (best vibratory frequency) approximately at the point of the passband's peak transfer ratio. We found these clustered in two groups, one ranging from 20 to 160 Hz, the other ranging from 220 to 300 Hz (Fig. 1a).

Field experiments were carried out during June of 1983 to determine the ability of the male white-lipped frog to detect seismic stimuli in its natural habitat. We placed a vertical geophone and a cardioid microphone approximately 1 m from isolated, calling frogs (*3*). This was done between 1800 and 2130 hours in remote areas of the Luquillo Mountains where there was virtually no automobile traffic or other forms of human activity. A calling, isolated white-lipped frog typically emits 40-msec chirps at a rate of approximately 4 per second. This call probably serves as an advertisement of the male's location (*4*). Using a stereophonic system with headphones to monitor and record simultaneously the microphone and geophone responses, we immediately noticed a transient geophone response (a "thump") at the onset of each chirp. Analysis of the recorded waveforms verified this observation, showing transient vertical (Rayleigh) waves with peak accelerations in the neighborhood of 2 cm/sec^2 (1 m from the frog) concomitant with each chirp (Fig. 1b). Analysis with fast fourier transforms revealed that the power in each thump was distributed over frequencies below 150 Hz, extending down to at least 10 Hz (the low-frequency corner of our geophone response) (*3*), but confined predominantly between 20 and 70 Hz (Fig. 1c). The thump spectrum thus corresponded well to the lower range of seismic sensitivity in the frog's ear (Fig. 1a). Subsequent studies of Rayleigh waves, which are conducted along substrate surfaces, in the same soils in Puerto Rico and a variety of similar soils in California have shown that the waveform and spectrum of the frog thump are typical of vertical geophone responses to impulsive seismic stimuli (for example, taps on the soil surface with a rubber mallet). The frequency range of the airborne acoustical power in the recorded chirps was distinctly higher than that of the substrate-borne thumps, being from about 1.0 to 2.3 kHz (Fig. 1d). The carrier frequency of each chirp began near 1 kHz and then increased in about 15 msec to its final level of approximately 2.3 kHz (Fig. 1e).

Among 11 calling frogs studied (at 11 different sites) five produced thumps and six did not. For 8 of the 11 frogs, we were able to identify the substrate from which they were calling. Four of these were thumpers and were found to be directly on mud; four were nonthumpers and were found to be perched either on grass or on loose, gravelly substrate. Two of these nonthumpers, observed in the act of calling, were found to have their gular pouches suspended above the ground as a result of the dense grass. The typical posture of the calling frog from muddy substrate is prone, with its gular pouch pressed against the substrate. We were unable to produce thumps by playing recorded airborne chirps through a loudspeaker positioned either above the ground or directly against it. Thus the thump appears not to be a consequence of acoustical coupling of the call itself to the ground, but possibly a consequence of the motion of the gular pouch during the explosive onset of the chirp. This conjecture is supported by the consistent concomitancy of the onset of the Rayleigh wave and the rising phase of the

first, large peak in the envelope of the airborne chirp (Fig. 1e).

Acoustical playback experiments provided evidence for communication by airborne calls. At the onset of playback of a series of chirps, chirping isolated frogs typically responded with a pause, followed by one or more chuckles (Fig. 1f). Each chuckle was much longer than a single chirp, typically beginning with frequencies close to 2.3 kHz, then descending in pitch to about 1 kHz. From frogs on muddy substrates, chuckles were accompanied by chains of thumps. As playback of chirps continued, some frogs continued to produce irregular pauses and chuckles, others reverted to periodic chirps. In one case playback of chuckles was presented to an isolated frog already calling in the chuckling mode. The animal responded with much louder, more prolonged and higher-pitched chuckles. On the basis of these observations, we tentatively conclude that chuckles are involved in male-male interactions.

The possibility of involvement of the seismic channel in communication, as well as in detection of danger, was supported by our observations of responses to uncalibrated impulsive seismic stimuli. One such stimulus was light, nearly periodic finger tapping at distances of 1 to 2 m, with amplitude approximately equal to that of the thump (as judged by volume-unit (VU) meter responses on the field tape recorder). Isolated chirping male frogs typically responded to this stimulus with pauses and chuckles, then shifted back to periodic chirping. Stronger impulsive seismic stimuli (light footfalls or gentle taps with a rubber mallet at distances of several meters) typically led to a prolonged pause (up to several minutes), eventually broken by a few chuckles and irregular pauses, followed by reestablishment of periodic chirps.

Thus we have demonstrated that the male white-lipped frog can generate impulsive seismic waveforms concomitant with its putative advertisement calls, that it has a sensory apparatus sufficiently sensitive and appropriately tuned to detect such waveforms, and that the isolated male responds to impulsive seismic stimuli by shifting to a call type that is likely involved in male-male interaction. On the basis of these observations, we propose that the white-lipped frog communicates, in part, through seismic signals.

Rayleigh waves over moist soil surface propagate at velocities in the neighborhood of 100 m/sec, approximately 1/3 the velocity of sound in air. In the presence of rain or strong wind, the Rayleigh-wave and airborne-sound channels are both cluttered with noise. In the absence of such interference, the ambient background in the Rayleigh-wave channel is sufficiently low to allow very large signal-to-noise ratios close to the thumping frog, as exemplified by Fig. 1, b and e. Under such circumstances, thump signals typically merged with the seismic background at distances of approximately 3 to 6 m from the thumping frog. At that point, the peak amplitude (~0.1 cm/sec^2) of the signal still would be sufficient to elicit strong responses from most of the frog's seismic axons. Therefore, the frog presumably could detect thump signals at least to their point of mergence with the background. At our study sites, male white-lipped frogs tended to cluster with nearest neighbors typically 1 to 2 m apart (that is, within distances over which thump signals should remain distinctly above the seismic background).

While there seem to be clear-cut examples of animal communication, there are no wholly adequate definitions of the process of communication itself. The quintessential skeleton of any definition of that process presumably would be "transfer of information from one individual to another," where "information" is defined in analogy to the Shannon sense, namely "reduction of uncertainty concerning the surroundings." Three physical prerequisites for communication, in this sense, would be (i) presence of a channel through which information-bearing signals can be transmitted, (ii) presence in the sender of the wherewithal to generate or encode signals and couple them into the channel, and (iii) presence in the receiver of the wherewithal to extract signal energy from the channel and to detect or decode the signals. Ethologists have fleshed out this skeleton with additional criteria to arrive at working definitions of communication among animals. For example, Lewis and Gower (*5*) have added an evolutionary criterion, namely that "selection has favored both the production and the reception of the signals." Green and Marler (*6*) have included a similar evolutionary criterion (namely, the presence of specialized adaptations for signal production, signal reception, or both) and have added two more, namely that the signal dynamics conveying information be rapid in comparison with the life-cycle dynamics of the animal, and that the signal reflect (internal) states of the sending animal and be capable of altering (internal) states of the receiving animal.

Our observations on the white-lipped frog have demonstrated the presence of all three physical prerequisites for seismic communication in the sense of the previous paragraph. Furthermore, Green

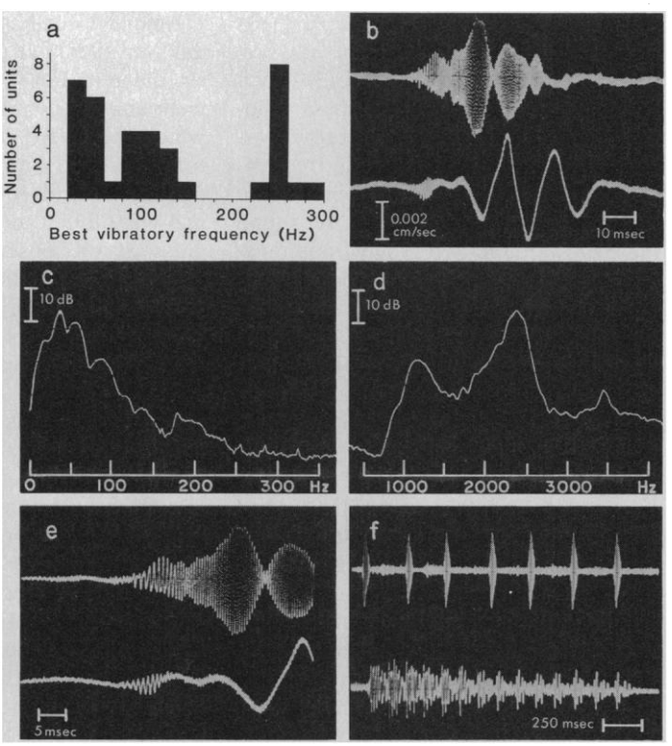

Fig. 1. Vibration sensitivity, vocalizations, and ground-thumping in the white-lipped frog. (a) Distribution of best vibratory frequencies of 37 eighth-nerve afferent fibers. (b) Acoustical chirp recorded from microphone (upper trace) and corresponding seismic thump recorded with vertical geophone 1.0 m from calling frog (lower trace). (c) Thump velocity spectrum taken with Hanning window, 3-Hz bandwidth, over 256 samples. (d) Chirp spectrum taken with Hanning window, 30-Hz bandwidth, over 256 samples. (e) Onset of chirp (upper trace) and thump (lower trace) recorded from microphone and vertical geophone, respectively. (f) Microphone records of a sequence of chirps (upper trace) and a single chuckle (lower trace).

and Marler's final two criteria appear to be met by the white-lipped frog's seismic signals—they are short-lived relative to the animal's life-cycle dynamics, they reflect internal states of the sender, and they are capable of altering internal states of the receiver. With respect to the evolutionary criteria, the frog saccule seems to be a specialized sensor of seismic signals (*1, 2*); one can argue for selective advantage of seismic communication—for example, the difference in velocities between the airborne chirp and the substrate-borne (Rayleigh-wave) thump provides a temporal clue (approximately 7 msec/m) to the distance from the source (as in the lightning-thunder phenomenon), and thus could help male frogs establish and maintain closely spaced territories.

Acute seismic sensitivity has been reported in reptiles, but has not been implicated in intraspecific communication (*7*). Several mammalian species exhibit ground-thumping behavior, but the intraspecific communication channel in such cases has been assumed to be auditory (airborne) rather than vibratory (substrate-borne) (*8*). Ground thumping has also been observed in arthropods, and in some cases may be involved in intraspecific communication (*9*). Direct vibrotactile communication has been convincingly demonstrated in some amphibian species (*10*). As far as we know, however, the evidence we have presented here provides the first strong implication of the use of substrate-borne seismic signals in intraspecific communication in vertebrates.

EDWIN R. LEWIS
Electronics Research Laboratory, University of California, Berkeley 94720
PETER M. NARINS
Department of Biology, University of California, Los Angeles 90024

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Detection of Two Viral Genomes in Single Cells by Double-Label Hybridization in Situ and Color Microradioautography

Abstract. *Double labeling and color microradioautography were used in a new method of hybridization in situ to identify different genes in individual cells. The method is based on the unequal penetration of ^3H and ^{35}S into two layers of nuclear track emulsion separated by a thin barrier film. Hybridization of a ^{35}S-labeled probe specific for one kind of gene results in silver grains over cells in both layers of emulsion; a ^3H-labeled probe for a second gene provides grains only in the first layer of emulsion. Silver grains are converted to magenta-colored grains in the first layer and to cyan-colored grains in the second to facilitate enumeration of grains in each layer. This technique should be widely applicable in analyses of differential gene expression in single cells or in discrete populations of cells.*

With recent advances in hybridization in situ, single copies of viral genomes can be detected in cells or single genes in chromosomes (*1–6*). These sensitivities (in the range of 10^{-18} g of specific nucleotide sequences per cell) have been achieved with improvements in the hybridization methodology itself and the high specific radioactivities of probes labeled with ^3H or ^{125}I precursors (*7*). The recently introduced ^{35}S-labeled precursors (*8*) afford even greater sensitivities on light microscopy, in part because of the increased efficiency of formation of silver grains in radioautographs (about 0.5 grain per disintegration for ^{35}S versus about 0.1 grain per disintegration for ^3H (*9*). The higher energy of ^{35}S responsible

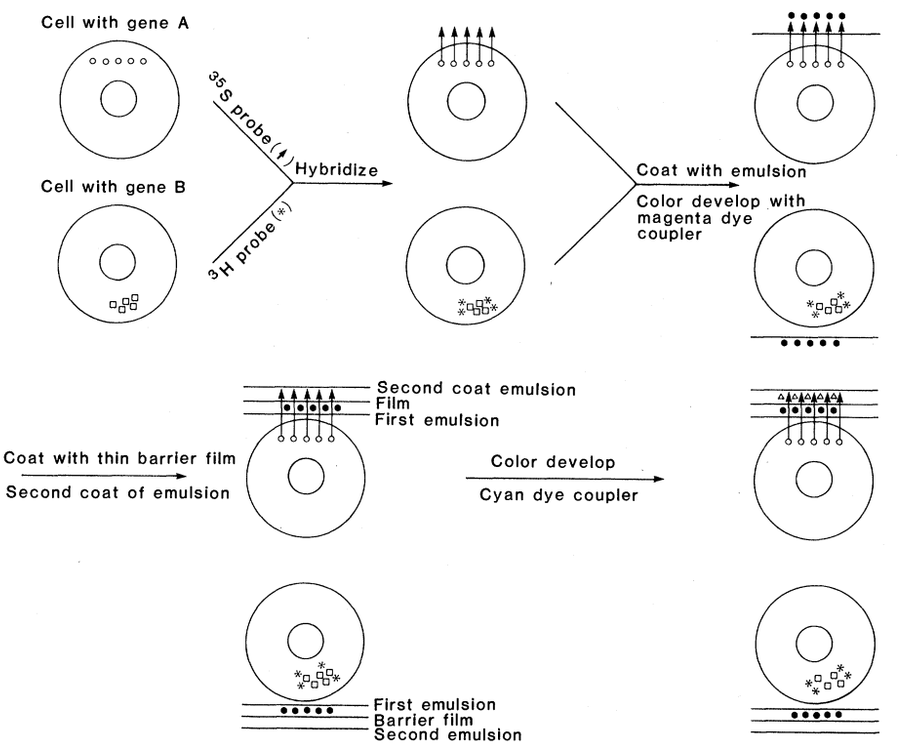

Fig. 1. Principles and major steps of double-label hybridization in situ and color radioautography. Cells with gene A (○) are hybridized to a probe labeled with ^{35}S (↑); cells with gene B (□) are hybridized to a probe labeled with ^3H (*). After slides are coated with nuclear track emulsion, the grains in the first layer are color-developed with a magenta dye coupler (●). A thin barrier film and a second coat of emulsion are applied and color developed with a cyan dye coupler (△).