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Correlation between Membrane Potential Responses and Tentacle Movement in the Dinoflagellate *Noctiluca miliaris*

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ABSTRACT—Membrane potential responses and tentacle movement of the marine dinoflagellate *Noctiluca miliaris* were recorded simultaneously and their time relationships were examined. The food-gathering tentacle of *Noctiluca* exhibited slow extension-flexion movements in association with the spontaneously recurring membrane potential responses termed the tentacle regulating potentials (TRPs). The flexion of the tentacle began during the slow depolarization of the TRPs. The rate of the flexion increased after the hyperpolarizing (negative) spike following the slow depolarization. The tentacle then extended slowly during the hyperpolarized level of the TRPs. A TRPs-associated flexion did not occur when the external Ca^{2+} ions were removed. On the contrary, the tentacle showed conspicuous flexion (coiling) when the external Ca^{2+} concentration was raised. In association with the stimulus-evoked action potential, which triggers bioluminescent flash (flash-triggering action potential; FTP), the tentacle coiled quickly. The FTP-associated coiling took place even in the Ca^{2+}-deprived condition. The coupling mechanisms of the TRPs-associated and FTP-associated tentacle movements were compared, and their biological significance was discussed.

Key words: *Noctiluca*, action potential, cell motility, bioelectric control, tentacle

INTRODUCTION

Movement of the food-gathering tentacle in the marine dinoflagellate *Noctiluca miliaris* is under the control of membrane electric events. A slow flexion-extension movement of the tentacle is always associated with spontaneously recurring membrane potential responses termed tentacle regulating potentials (TRPs; Eckert and Sibaoka, 1967; Sibaoka and Eckert, 1967). The TRPs consist of a depolarizing spike followed by a slow depolarization and a subsequent hyperpolarizing spike (Eckert and Sibaoka, 1967, Oami *et al*., 1988). The depolarizing spike is obscure in normal seawater. However, it becomes obvious when the external Ca^{2+} concentration is lowered (Oami *et al*., 1995a, b).

Eckert and Sibaoka (1967) examined the time relationship between TRPs and the tentacle movement by employing cinematography. They concluded that the slow depolarization triggers the flexion of the tentacle. They did not noticed a temporal relationship between the hyperpolarizing spike and the tentacle movement. However, Hisada (1957) had observed that the flexion of the tentacle occurred in close association with the hyperpolarizing spike. Thus, the temporal relationship between TRPs and the tentacle movement remained to be examined.

In addition to the spontaneously recurring TRPs, *Noctiluca* exhibits different kinds of stimulus-evoked membrane potential responses, which trigger a bioluminescent flash (flash-triggering potential, FTP; Eckert, 1965). A quick flexion of the tentacle associated with the FTP was reported by Eckert and Sibaoka (1967). However, neither time relationship nor coupling mechanism between the FTP and the tentacle coiling have been clarified.

Recently, the generation sites and ionic mechanisms of the FTP and TRPs have been determined. The TRPs are generated across the outer membrane facing the extracellular medium (Oami *et al*., 1988). The depolarizing spike is dependent on the Na^+ ions, while the hyperpolarizing spike is dependent on the Cl^- ions (Oami *et al*., 1988; Oami *et al*., 1995a). On the other hand, the FTP is generated across the inner membrane facing the intracellular vacuole and is dependent on the H^+ ions (Eckert and Sibaoka, 1968; Nawata and Sibaoka, 1976; Nawata and Sibaoka, 1979). Interestingly, the contractile mechanism in the tentacle of *Noctiluca* was activated by the H^+ ions, unlike any other contractile elements so far examined (Oami and Naitoh, 1989). ATP was not necessary for the H^+-dependent flexion of the tentacle. The flash mechanism in the cytoplasm was also activated by H^+ ions (Nawata and Sibaoka, 1979).

To understand the bioelectric mechanisms controlling

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the tentacle movement, it is necessary to examine the time relationship between membrane potential responses and the tentacle movement. The correlation between these two events was, therefore, examined. The results clearly showed that the TRPs-associated flexion of the tentacle occurred during the slow depolarization and was suddenly accelerated by the hyperpolarizing spike. The TRPs-associated flexion is assumed to be produced by an increase in the cytoplasmic H^+ concentration mediated by the influx of the Ca^{2+} ions from the extracellular medium into the cytoplasm. On the other hand, a quick flexion of the tentacle was accompanied by the FTP. The FTP-associated flexion of the tentacle was produced by H^+ ions directly introduced from the vacuole into the cytoplasm of the tentacle by the H^+-dependent FTP.

MATERIALS AND METHODS

Specimens of *Noctiluca miliaris* were cultured in artificial seawater (ASW; in mM=500 NaCl, 10 KCl, 30 $MgSO_4$, 20 $MgCl_2$, 10 $CaCl_2$, 5 Tris-HCl buffer; pH 8.0) as previously described (Oami *et al*., 1988). $CaCl_2$ was removed from the ASW to make a Ca^{2+}-deficient ASW. The residual Ca^{2+} concentration in Ca^{2+}-deficient ASW was 0.01 mM.

The membrane potential responses were measured through a glass capillary microelectrode filled with 3 M KCl inserted into the flotation vacuole of the specimen. In some experiments, a stimulating current was injected into the specimen to evoke the hyperpolarizing spike of the TRPs through another microelectrode inserted into the specimen as described elsewhere (Oami *et al*., 1988). The flash-triggering potential was evoked asynchronously by injecting the inward current through a holding suction pipette (internal diameter of tip; about 50 μm, Eckert, 1965).

The membrane potential responses and the tentacle movement were recorded simultaneously on videotapes (Sony, SL-

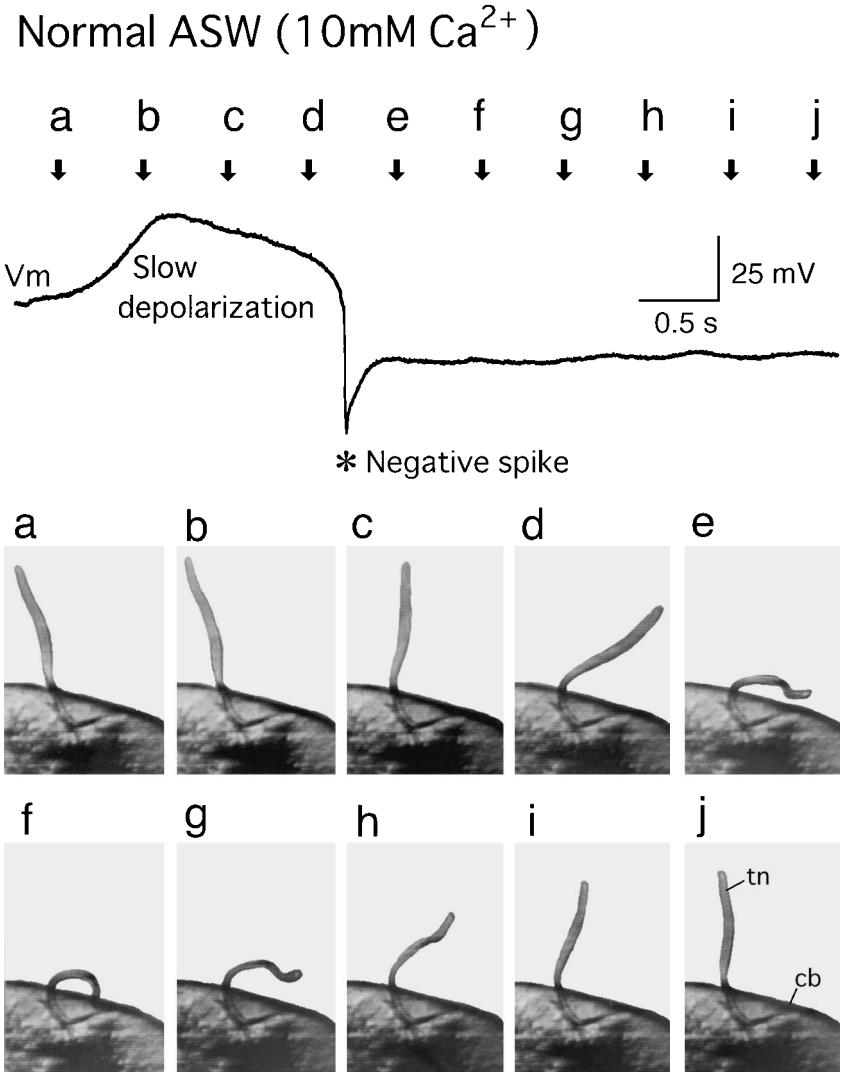

Fig. 1. Simultaneous recordings of the tentacle movement and the tentacle regulating potentials (TRPs) of a specimen of *Noctiluca miliaris* in normal artificial seawater (ASW). During the spontaneous TRPs (trace labeled by Vm), the tentacle was photographed every 0.5 sec and is shown in the lower panels. The arrows above the Vm trace indicate the time when the photograph of the tentacle was taken. **a–j** on the arrows correspond to the **a–j** on the lower panels, respectively. **tn**: tentacle, **cb**: cell body. The background of each photograph was removed to provide a clear view of the the tentacle.

HF3000). The images of the tentacle were video-recorded through a 10× objective. Membrane potential responses were first converted into a train of pulses by using a voltage-frequency (V–F) converter in which the amplitude of the membrane potential was encoded as the frequency of the pulses. The input potential range of the V–F converter was 0–10 V, and the dynamic range of the output was 3–10 KHz. The membrane potential was amplified in advance to obtain the adequate potential range for the performance of the V–F converter. The converted voltage was then recorded on the sound track of the videotape together with the pictures showing the movement of the tentacle. Photographs of the tentacle were taken every 0.5 sec from replayed pictures on a television screen. At the same time, membrane potential responses obtained through a frequency-voltage (F–V) converter were written on a pen recorder. The signal once converted into train of pulses through V–F converter then reconverted into amplitude of the potential by F–V converter delayed about 1 msec with respect to the original input voltage.

For the measurements of the degree of flexion of the tentacle, temporal changes in the position of the tentacle tip were recorded every 0.1 sec. The distance of the tentacle tip from its most extended position along with its trail was then measured, and the degree of the flexion was expressed as a value relative to the most flexed position.

All the experiments were conducted at room temperature ranging from 18°C to 23°C.

RESULTS

A specimen of *Noctiluca miliaris* arrested at the tip of a suction pipette showed spontaneous membrane potential responses that are known as tentacle regulating potentials (TRPs; Eckert and Sibaoka, 1967). The waveform of the TRPs varied with time and among the specimens examined and the movement of the tentacle changed according to the changes in the wave form of the TRPs. In the present study, experiments were performed mainly when the specimen exhibited the basic TRPs described by Eckert and Sibaoka (1967). The basic TRPs consisted of a depolarizing spike followed by a slow depolarization and a subsequent hyperpolarizing spike (Oami *et al.*, 1988). The depolarizing spike is obscure in normal sea water.

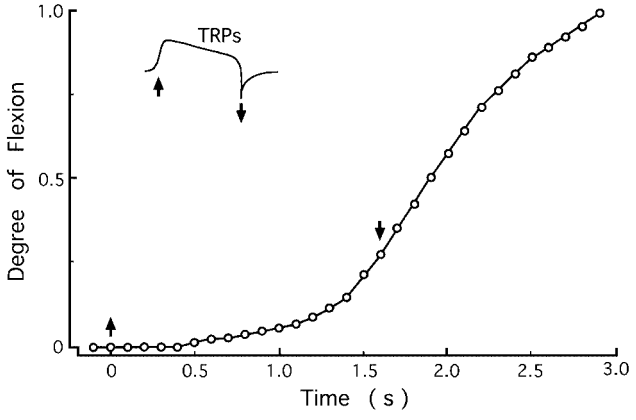

Fig. 2. Measurements of the degree of flexion of the tentacle associated with the TRPs. The upward arrow indicates the onset of the slow depolarization in the TRPs, while the downward arrow shows the hyperpolarizing spike (see inset). The onset of the slow depolarization was taken as time 0.

Fig. 1 shows simultaneous recordings of the basic TRPs and the tentacle movement. During the hyperpolarized level before the slow depolarization, the tentacle was kept extended (Fig. 1a). A slow flexion of the tentacle began when the specimen exhibited the slow depolarization (Fig. 1c) and continued during the slow depolarization (Fig. 1d). The degree of the flexion was accelerated after the hyperpolarizing spike (Fig. 1e) and the flexion became maximal after the quick flexion (Fig. 1f). The tentacle extended slowly during the hyperpolarized level after the hyperpolarizing spike (Fig. 1g–j).

To examine the correlation of the tentacle movement and the TRPs more precisely, I measured the degree of the flexion associated with the TRPs (Fig. 2). In the figure, the onset of the slow depolarization of the TRPs was taken as time 0 and is indicated by an upward arrow, while the peak of the negative spike is indicated by a downward arrow (see also inset). The tentacle began to flex within 1 sec after the onset of the slow depolarization. The degree of the flexion increased more or less linearly during the slow depolarization. The rate of increase of the flexion was accelerated when the membrane potential began to shift toward the

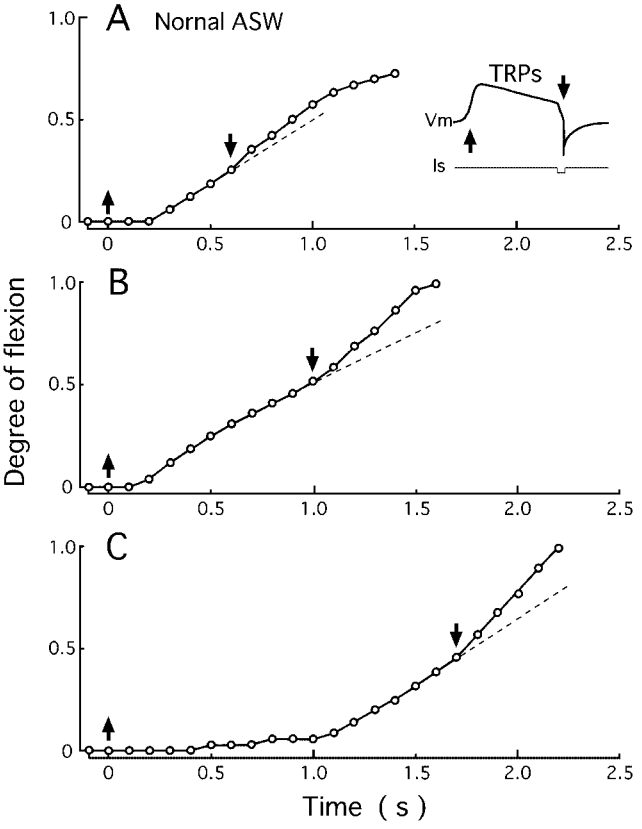

Fig. 3. Three series of measurements of the degree of flexion of the tentacle associated with the TRPs. In each series, the hyperpolarizing spike of the TRPs was evoked by injecting the current at various time from the onset of the slow depolarization (0.6 sec in A, 1.0 sec in B, 1.7 sec in C). For comparison of the rate of increase in the tentacle flexion before and after the hyperpolarizing spike, the plots just before the hyperpolarizing spike was extrapolated as broken lines. Other explanations are the same as in Fig. 2.

hyperpolarizing direction about 0.2 sec prior to the hyperpolarizing spike. This flexion was further accelerated in association with the hyperpolarizing spike. This accelerated flexion persisted several hundred milliseconds after the negative spike, and the tentacle then showed maximum flexion.

To clarify the effects of the hyperpolarizing spike on the tentacle movement more precisely, I evoked the hyperpolarizing spike at various timings during the slow depolarization. The hyperpolarizing spike was evoked by an injection of the inward current into the cell during the slow depolarization (Oami *et al.*, 1988). Fig. 3 shows three series of measurements of the tentacle flexion associated with the TRPs. In each series, the negative spike was evoked at the various time from the onset of the slow depolarization. As shown in the figure, the rate of increase in the degree of flexion was accelerated suddenly after the current-evoked hyperpolarizing (negative) spike irrespective of the timing from the onset of the slow depolarization. The increase in the flexion of the tentacle terminated 500 to 1500 ms after the hyperpolarizing spike.

I next examined the effects of external Ca^{2+} concentration on the TRPs-associated tentacle movement. Fig. 4 shows the representative results. When the external Ca^{2+} was deprived, a conspicuous depolarizing (positive) spike appeared at the beginning of the slow depolarization of the TRPs (Oami *et al.*, 1988). The tentacle stopped its movement at its most extended position even though spontaneous TRPs persisted (Fig. 4). When the external Ca^{2+} concentration was lowered to 1 mM, the flexion of the tentacle was eliminated at its distal half-region. In this condition, the flexion took place subsequently after the hyperpolarizing spike (data not shown).

When the external Ca^{2+} concentration was raised to 40 mM, the waveform of the TRPs was similar to that recorded in the normal ASW. However, the tentacle showed strong coiling even before the slow depolarization was initiated. The degree of the coiling increased during the slow depolarization and became maximal after the hyperpolarizing spike (Fig. 5).

In the next series of experiments, I examined the tentacle movement associated with the flash-triggering action potential (FTP; Eckert, 1965). Simultaneous recordings of tentacle movement and the FTP are shown in Fig. 6. At the time indicated by an asterisk, the FTP was evoked asynchronously by passing the inward current through a holding pipette (Eckert, 1965). A quick coiling of the tentacle began suddenly after the FTP. Within 500 msec after the FTP, the tentacle became tightly coiled.

Fig. 7 shows the tentacle movement associated with the FTP recorded in the Ca^{2+} deficient condition. As described previously, the tentacle did not show flexion in association with the TRPs in this condition. However, a quick flexion took place in association with the FTP, although the degree of the flexion was smaller than that in

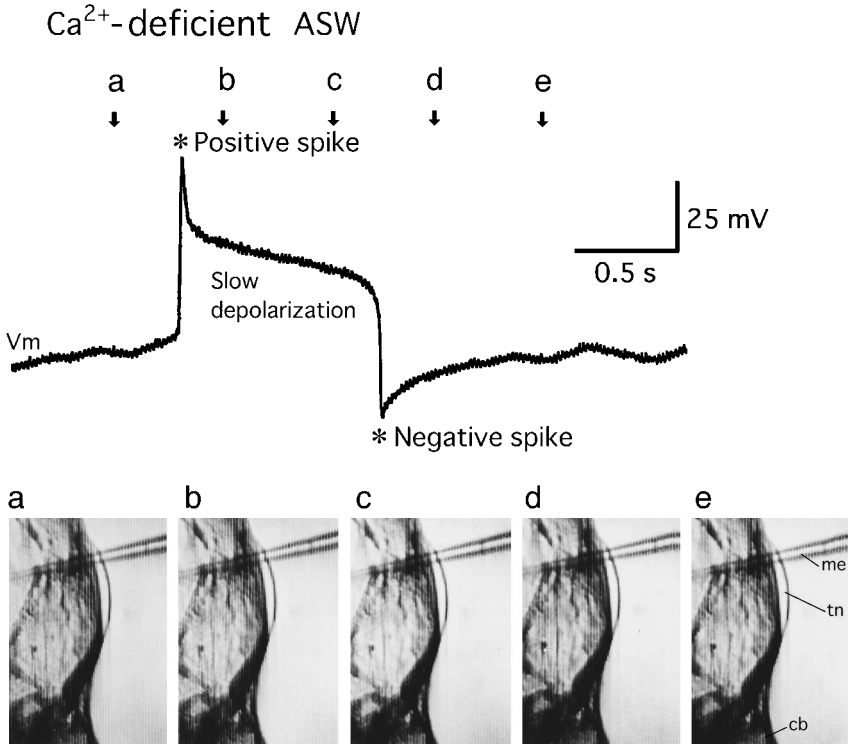

Fig. 4. Simultaneous recordings of the tentacle movement (a–e in the lower panels) and the TRPs (Vm) in the Ca^{2+}-deficient condition. Note that, in this condition, the conspicuous depolarizing spike (positive spike) appeared in addition to the hyperpolarizing spike (negative spike). **tn**: tentacle ; **cb** cell body; **me**: microelectrode. Other explanations are the same as in Fig. 1. In each photograph, the tentacle was kept extended along the surface of the cell body.

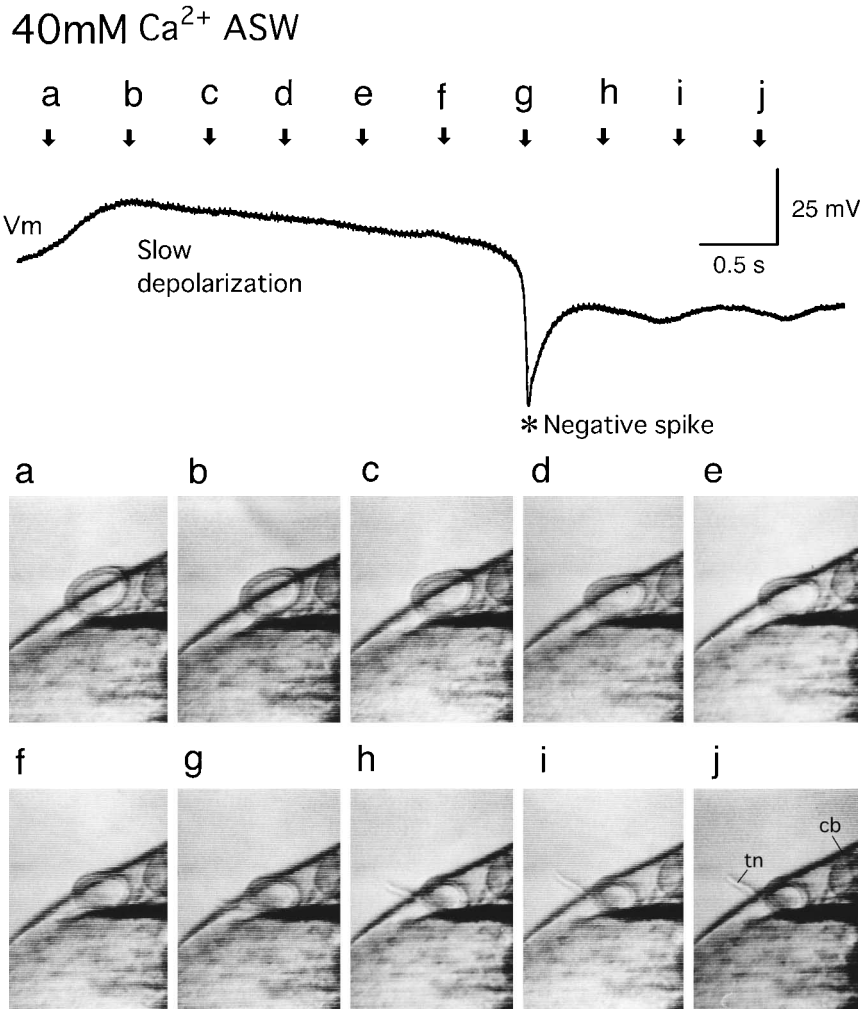

Fig. 5. Simultaneous recordings of the tentacle movement (a-j in the lower panels) and the TRPs (Vm) in the Ca^{2+}-rich (40 mM) condition. Other explanations are the same as in Fig. 1. In the photographs, the tentacle exhibited coiled appearance. The larger diameter of the coil (*e.g.* Fig. 4a) indicates more or less relaxed and the smaller diameter (*e.g.* Fig. 4j) indicates contracted states of the coiled tentacle.

the normal Ca^{2+} concentration.

DISCUSSION

Temporal relationship between TRPs and the tentacle movement

The present experiments clearly demonstrated that the flexion of the tentacle of the specimen in *Noctiluca miliaris* began during the slow depolarization of the tentacle regulating potentials (TRPs; Figs. 1 and 2) and was accelerated suddenly after the hyperpolarizing spike (Fig. 3). Eckert and Sibaoka (1967) pointed out that the tentacle flexion took place in association with the slow depolarization. However, they overlooked the quick flexion associated with the hyperpolarizing spike. In their experiments, *Noctiluca* exhibited a rather long slow depolarization, during which the degree of the flexion reached its maximum. Therefore, enhancement of the flexion by the hyperpolarizing spike was difficult to observe. The acceleration of the flexion associated with the hyperpolarizing spike, as shown in Fig. 3, corresponds to Hisada's observation (Hisada, 1957) that the hyperpolarizing spike in the TRPs is correlated with the tentacle flexion.

In Fig. 2, the increase in the rate of tentacle flexion began slightly prior to the hyperpolarizing spike. The timing of the acceleration well coincided with the hyperpolarizing phase between the slow depolarization and the hyperpolarizing spike. Therefore, the increase in the rate of the flexion was produced by the hyperpolarization following the slow depolarization. When the specimen produced hyperpolarizing spike, the increase in the flexion was conspicuous because the hyperpolarization was quick and its amplitude was large.

Coupling mechanism between tentacle movement and the TRPs

Eckert and Sibaoka (1967) reported that the external Ca^{2+} ions are necessary for TRPs-associated flexion. This was confirmed in the present study. The tentacle stop move-

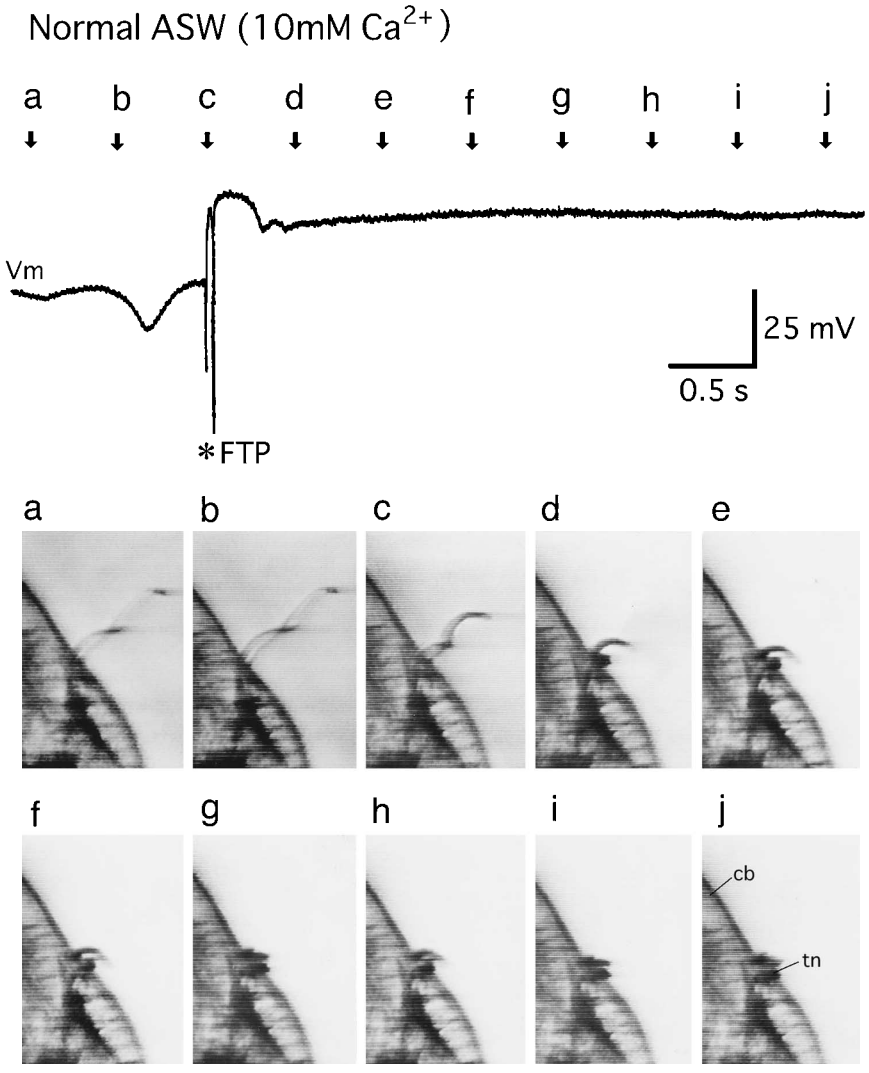

Fig. 6. Simultaneous recordings of the tentacle movement and the flash-triggering action potential (FTP) of the specimen of *Noctiluca miliaris* in normal ASW. The electrically evoked FTP is indicated by an asterisk. **a–j** on the Vm trace correspond to **a–j** in the lower panels, respectively.

ment at its most extended position when the external Ca^{2+} ions were deprived (Fig. 4). Contrary to the Ca^{2+}-deficient condition, the tentacle showed conspicuous coiling when the external Ca^{2+} concentration was raised (Fig. 5). These facts indicate that Ca^{2+} ions introduced from external medium into the cytoplasm of the tentacle are necessary for the TRPs-associated flexion of the tentacle. Because the contractile mechanism in the tentacle is activated directly by H^+ ions (Oami and Naitoh, 1989), the Ca^{2+} ions introduced into the cytoplasm are not assumed to be the activators of the contractile elements in the tentacle. Therefore, Ca^{2+} ions most probably act as mediators between TRPs and increase in the cytoplasmic H^+ concentration. Since the flexion of the tentacle began during the slow depolarization, it is probable that the depolarization-sensitive Ca^{2+} channels are present in the membrane facing the external medium (outer membrane) of *Noctiluca*. When the Ca^{2+} channels are activated by the slow depolarization of the TRPs, Ca^{2+} ions flow from the external medium into the cytoplasm. The increase in the cytoplasmic Ca^{2+} concentration somehow mediates the H^+-dependent contraction of the tentacle. The driving force for the Ca^{2+} ions is transiently increased by the hyperpolarizing spike. This causes the increase in the Ca^{2+} influx through the activated Ca^{2+} channels. The increase in the cytoplasmic Ca^{2+} concentration most probably produces the acceleration of the tentacle flexion associated with the hyperpolarizing spike.

Temporal relationship between FTP and the tentacle movement

The tentacle showed quick and conspicuous flexion when the specimen exhibited the flash triggering potential (Fig. 6). In contrast to the TRPs-associated flexion, the FTP-associated flexion took place even in the absence of external Ca^{2+} ions (Fig. 7). The FTP is generated across the inner membrane facing the intracellular flotation vacuole and

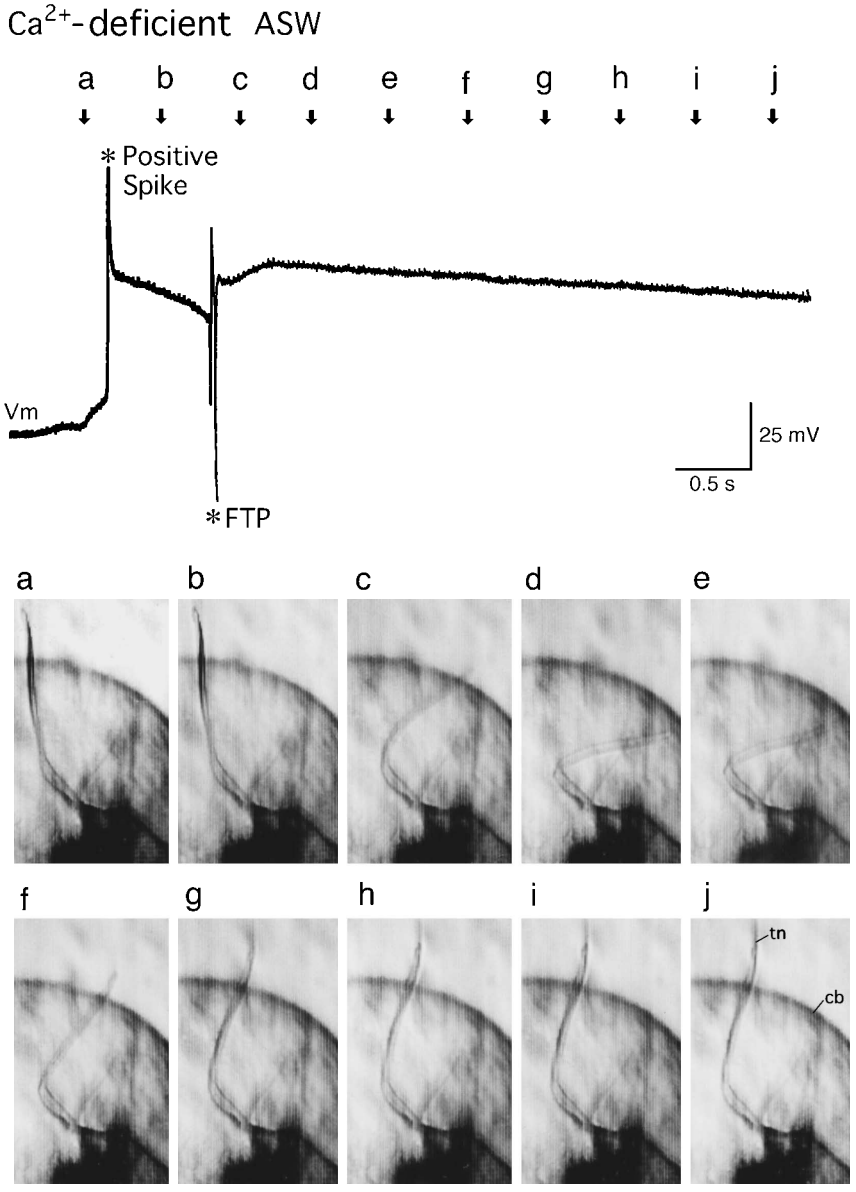

Fig. 7. Simultaneous recordings of the tentacle movement and the flash triggering action potential in the Ca^{2+}-deficient ASW. Other explanations are the same as in Fig. 6.

is dependent on H^+ ions (Eckert and Sibaoka, 1968; Nawata and Sibaoka, 1979). The flash mechanism in the cytoplasm is activated by the H^+ ions introduced into the cytoplasm associated with the FTP. As described earlier, activation of the contractile mechanism in the tentacle also depends on the cytoplasmic H^+ ions (Oami and Naitoh, 1989). Therefore, the increase in the H^+ concentration due to FTP directly activates the contractile mechanism in the tentacle as well as the flash mechanism. Since the FTP takes place across the inner membrane facing the intracellular floatation vacuole, the FTP-associated flexion took place even when the external Ca^{2+} ions were removed (Fig. 7).

Biological significance of the bioelectric regulations of tentacle movements in *Noctiluca*

The present experiments show that the flexion of the tentacle is controlled by two distinct kinds of membrane potential responses, the TRPs and the FTP. The TRPs-associated slow extension-flexion movement is responsible for gathering and endocytotic ingestion of food (Hisada, 1957; Eckert and Sibaoka, 1967; Oami *et al*., 1988; Nawata and Sibaoka, 1986; Nawata and Sibaoka, 1987). On the other hand, the time required to complete the FTP-associated coiling was very rapid (about 500 ms; Fig. 6). This is several times faster than the time required for the TRPs-associated flexion (2–3 sec; Figs 1–3). The FTP-associated coiling seems to be too fast for food gathering. However, the tightly coiled tentacle is certainly secure from damage

caused by the mechanical agitation that triggers FTP in nature. The H^+-dependent contractile mechanism of the tentacle is precisely controlled by the TRPs when *Noctiluca* exhibits feeding activity. However, when *Noctiluca* faces an emergency (e.g., jostling by a big surf), the H^+ ions carried by the FTP directly activate the H^+-dependent contractile mechanism to produce quick and strong flexion of the tentacle.

Noctiluca exhibits prominent differentiation of the membrane function within a single cell to control different kinds of effecter activities, the tentacle movement and the bioluminescent flash (Oami *et al*., 1989; Oami *et al*., 1990). Interestingly, the present study revealed that the tentacle movement is controlled by two distinct kinds of membrane potential responses, the TRPs and the FTP. Therefore, it is an example of the regulation of motile activity by multiple bioelectric activities within a single cell.

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