Two-Round Ca\textsuperscript{2+} transient in papillae by mechanical stimulation induces metamorphosis in the ascidian 
\textit{Ciona intestinalis} type A

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Marine invertebrate larvae are known to begin metamorphosis in response to environmentally derived cues. However, little is known about the relationships between the perception of such cues and internal signalling for metamorphosis. To elucidate the mechanism underlying the initiation of metamorphosis in the ascidian, \textit{Ciona intestinalis} type A (\textit{Ciona robusta}), we artificially induced ascidian metamorphosis and investigated Ca\textsuperscript{2+} dynamics from pre- to post-metamorphosis. Ca\textsuperscript{2+} transients were observed and consisted of two temporally distinct phases with different durations before tail regression which is the early event of metamorphosis. In the first phase, Phase I, the Ca\textsuperscript{2+} transient in the papillae (adhesive organ of the anterior trunk) was coupled with the Ca\textsuperscript{2+} transient in dorsally localized cells and endoderm cells just after mechanical stimulation. The Ca\textsuperscript{2+} transients in Phase I were also observed when applying only short stimulation. In the second phase, Phase II, the Ca\textsuperscript{2+} transient in papillae was observed again and lasted for approximately 5–11 min just after the Ca\textsuperscript{2+} transient in Phase I continued for a few minutes. The impaired papillae by Foxg-knockdown failed to induce the second Ca\textsuperscript{2+} transient in Phase II and tail regression. In Phase II, a wave-like Ca\textsuperscript{2+} propagation was also observed across the entire epidermis. Our results indicate that the papillae sense a mechanical cue and two-round Ca\textsuperscript{2+} transients in papillae transmits the internal metamorphic signals to different tissues, which subsequently induces tail regression. Our study will help elucidate the internal mechanism of metamorphosis in marine invertebrate larvae in response to environmental cues.

1. Background

The larvae of marine invertebrates, including crustacea (barnacles), molluscs, ascidians, echinoderms, and Annelida (polychaetes), eventually settle into the substratum and begin metamorphosis [1–5]. In order to initiate metamorphosis, these larvae require specific cues such as simple contact [6], adhesion via an adhesive organ [7–9], temperature, light, and various chemical cues [10,11]. Therefore, it was thought that a specific organ is responsive to external cues and transduces them to internal organs for the subsequent metamorphosis [12–14]. Ca\textsuperscript{2+} signalling is instrumental in the induction of metamorphosis in metazoans [15–19]. However, little is known about how external environmental cues are translated into internal metamorphic signals.

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Ascidian papillae form a transient sensory adhesive organ that serves to attach the larva to a substrate, thereby ensuring settlement and the onset of metamorphosis into the filter-feeding adult [20]. After the papillae-mediated adhesion to a substrate, ascidian metamorphosis is characterized by tail regression [7,21]. Papillae seem to permit a larva to assess a substrate’s suitability for settlement and metamorphosis. Papillae-mediated larval adhesion is essential for tail regression and tail regression is reportedly abolished in papillae-cut larva [7]. In the larval stage, the ascidian Ciona has three papillae. Foxg is expressed at the papillae under the ERK pathway [22]. Each papilla contains four glutamatergic neurons [8], which are considered sensory neurons [23,24]. Although papillary sensory neurons have been proposed to have both chemosensory and mechanosensory functions [25,26], there is no direct evidence that papillae can perceive external cues.

We have previously reported the Ca\(^{2+}\) dynamics of Ciona embryo from gastrulation to the tailbud stages [27], but Ca\(^{2+}\) dynamics in later developmental stages have not been reported before. Here, we present direct evidence of mechanical cues to the papillae playing a role in initiating Ciona metamorphosis via two-round Ca\(^{2+}\) transients.

2. Material and methods

(a) Samples

Ciona adults were obtained from Maizuru Fisheries Research Station (Kyoto University), Misisaki Marine Biological Station (University of Tokyo) through the National Bioresource Project (NBRP), and Onagawa Field Center (Tohoku University), Japan. Eggs were collected by dissecting gonoducts. Fertilized eggs were incubated at 18°C until observation. As Ciona larvae acquire competence of tail regression after 29.5 h post-fertilization (hpf) [28], we opted to use the stage 29 larvae [29] after 30 hpf.

(b) Preparation of reporter constructs and microinjection and pharmacological treatment

GCaMP6s mRNA was obtained as previously described [27] (see electronic supplementary material).

(c) Fixation of swimming larva and artificial papillae stimulation

The larvae were fixed to a Petri dish coated with poly-D-lysine (PDL) or poly-L-lysine (PLL), referring to practical tips for imaging ascidian embryos [30] (see electronic supplementary material).

(d) Artificial mechanical stimulation of papillae

After establishing the trunk fixation method, we designed a device that consists of a mechanical stimulator, its manipulator, and a holder to apply mechanical stimuli artificially to the papillae of Ciona larva. We called this device an ‘artificial papillae stimulator’ (see electronic supplementary material).

(e) Microscopy

Samples were observed with three different microscopy methods, namely fluorescence microscopy (FM) with a 3CCD camera, confocal laser scanning microscopy (CLSM), and light-sheet microscopy (LSM). For imaging by FM, we followed previous methods [27] (see electronic supplementary material). For LSM, we employed dual inverted selective plane illumination microscopy (disSPIM) with 40x water immersion lens (NA 0.8, Nikon) mounted on objective pieces with fibre-coupled digital micro-mirror scanners (Applied Scientific Instrumentation, USA) for light-sheet generation. Bidirectional stack images measuring 512 x 512 pixels were acquired by an scMOS camera (Zyla 4.2, Andor) at a time interval of 7 s using dispm plugin running on ImageJ Micromanager. Images from two directions were registered and fused using MIPAV (NIH) generate fusion plugin.

3. Results

(a) Artificial induction of tail regression by a new experimental system

At first, we verified that the Ca\(^{2+}\) indicator GCaMP6s can sense Ca\(^{2+}\) dynamics even at later developmental stages (electronic supplementary material, figure S1, stage 36 [29], late body axis rotation period; see electronic supplementary material). To observe Ca\(^{2+}\) dynamics in larva up to the tail regression stage controlling the timing of adhesion, we established a new experimental system for applying artificial mechanical stimulation to the papillae of an individual swimming larva [31]. The larval trunk is immobilized using a PDL-coated glass base to avoid vibration caused by swimming (figure 1a). In the absence of stimulation, 94.6% of immobilized larva retained the tail after 29.5 hpf. At this time, Ciona larvae can undergo tail regression if they receive adhesive stimulation (figure 1b,c). By contrast, when the artificial mechanical stimulus was applied to immobilized larvae (figure 1d), tail regression was induced in 83% of 12 larvae (figure 1e). These results indicate that trunk immobilization prevented tail regression and that our new experimental system can induce tail regression by controlling the timing of mechanical stimulation.

Interestingly, we found that posterior trunk epidermal cells moved towards the posterior at the onset of tail regression (electronic supplementary material, figure S2; Video S1; Video S2; Video S3 from 0:09:41.360), which was the first observable change in the initiation of Ciona metamorphosis earlier than the onset of tail regression. Thus, we defined this backward movement of the posterior trunk epidermis as the timing of the start of tail regression, and we could align the time axis for metamorphosis among individuals, thereby linking each developmental stage.

(b) Two-round Ca\(^{2+}\) transients in papillae were observed prior to tail regression

To examine Ca\(^{2+}\) dynamics during tail regression, we artificially induced tail regression after 29.5 hpf using our new experimental system and observed Ca\(^{2+}\) dynamics from early adhesion up until the late body axis rotation period (up to 52 hpf). Hence, prior to artificial mechanical stimulation, in addition to the known Ca\(^{2+}\) transient in epidermal cells called CTEC [27], spontaneous Ca\(^{2+}\) increases were observed at the posterior sensory vesicle and nerve cord (figure 2a, red arrowheads; Video S3, before adhesion), the timing of which coincided partially with larval swimming behaviour, which suggests the excitation of motor neurons. After mechanical stimulation was applied, Ca\(^{2+}\) transients were observed in specific tissues (figure 2b–g and Video S3). Observed transients were composed of two temporally different Ca\(^{2+}\) transients.
A new experimental system for artificial induction of tail regression. (a) A schematic of a new experimental system for observing Ca²⁺ dynamics during artificial induction of tail regression. GCaMP6s mRNA, which encodes a Ca²⁺ indicator, was injected into Ciona eggs and the injected eggs incubated until observation. PDL-coated (red) dish traps the lateral side of the larval trunk. The papillae can avoid adhesion. Stimuli were applied artificially to the palps using the tip of a glass pole (mechanical stimulator). (b) Stereomicroscopic view of a larva immobilized to a PDL-coated dish at 24.5 hpf. (c) Stereomicroscopic view of the trapped larva that is not subject to stimulation does not undergo tail regression even at 48 hpf. (d) Stereomicroscopic view of a trapped larva subjected to stimulation of the papillae by the mechanical stimulator. The dotted line indicates the larval edge. (e) Stereomicroscopic view of a larva subjected to artificial induction of tail regression by mechanical stimulation. Adhesion by the mechanical stimulator started at time 0. At 20 min, the tail tip started to shrink. The tail retracted up to 100 min. The dotted line indicates the larval tail edge before tail regression. Abbreviations: A, anterior; D, dorsal; P, posterior; V, ventral. Scale bar: 100 μm. (Online version in colour.)

(figure 2h). In the first phase, Phase I, a relatively short-range Ca²⁺ transient was observed immediately after mechanical stimulation. Subsequently the second phase, called Phase II, includes the Ca²⁺ transients observed within 10 min after Phase I.

Phase I was observed immediately after mechanical stimulation and lasted an average of 1.3 min in papillae (figure 2a–e and i; electronic supplementary material, table S1). The Ca²⁺ transient in Phase I showed fluctuation which suggests that Ca²⁺ transient consisted of the accumulation of multiple Ca²⁺ spikes (figure 2i). During Phase I, the Ca²⁺ transient was first observed at both the papillae (figure 2b; orange arrowhead) and the dorsal subregion in the posterior trunk (figure 2b, white arrowhead; electronic supplementary material, Video S3, time point = 0:01:00). Immediately after the Ca²⁺ transient in the dorsal subregion, the propagation to the ventral region was observed to the endodermal subregion (figure 2c,d, blue arrowheads; Video S3, time point = 0:01:06). Interestingly, the Ca²⁺ transient in two areas, the papillae and the endoderm, is temporally different but frequently observed to be coupled (figure 2b–d and i). The Ca²⁺ transient in the papillae reached its maximum intensity an average of 3.6 s earlier than in the endodermal region.

After Phase I, Phase II including the second Ca²⁺ transient in papillae without coupling with the endoderm was observed. The second Ca²⁺ transient reached a peak at 3.2 ± 1.0 min after the maximum peak of the first Ca²⁺ transient (electronic supplementary material, table S1, c). It took 5.3 ± 0.8 min from the peak to stable state (electronic supplementary material, table S1, d). After 8.9 ± 3.3 min lasting of the second Ca²⁺ transient, larvae started tail regression (electronic supplementary material, table S1, f). During Phase II, various tissues, including the epidermis and epidermal sensory neurons, as well as CNS, endoderm, and papillae, exhibited increased Ca²⁺ activity (see electronic supplementary material, Video S3). In particular, wave-like propagations by strong Ca²⁺ increases were observed at whole epidermal cells (n = 6/6, figure 2f; see electronic supplementary material, Video S3, time point 0:03:17–0:06:21) before tail regression. Compared with before tail regression, the Ca²⁺ activity of the extraembryonic region located in the larval tunic, including motile test cells and extracellular ciliated structure, ASNET [32,33], increased.
As two-round Ca\textsuperscript{2+} transients (Phase I and Phase II) at the papillae were observed in all tail-regressed larvae (table 1), Phase I and Phase II appear to be involved in inducing Ciona tail regression.

(c) The Ca\textsuperscript{2+} transient in Phase I responding to the mechanical stimulation

Phase I starts immediately after adhesion (figure 2h). In addition, the anatomical investigation has suggested that ascidian papillae sense mechanical stimulation [8,25,33–36]. These two results suggest that the papillae sense a mechanical stimulus and the Ca\textsuperscript{2+} transient in Phase I is in response to papillary mechanical stimulation. To understand the detailed profile of Phases I and II in tail regression, we first determined the relationship between the mechanical cue and induction of Phase I. Mechanical stimulation of different durations (2, 5, and 13 s) were applied to the larval papillae after 26.5 hpf (figure 3a) and Ca\textsuperscript{2+} responses were recorded. The Ca\textsuperscript{2+} transient in papillae and trunk region was observed in a similar manner to the Ca\textsuperscript{2+} transient in Phase I (electronic supplementary material, table S2) for all stimuli. The Ca\textsuperscript{2+} transient...
Table 1. The number of samples showing Phase I, Phase II and tail regression after different lengths of mechanical stimuli. From the left-hand column, the duration of mechanical stimulation was 0 s, 10 s, and continuous.

| stimulus       | ctrl      | Foxg MO  |
|----------------|-----------|----------|
| 0 s            | Phase I   | 0/8      | 3/3      | 6/6      | 4/13     |
| 10 s           | Phase II  | 0/8      | 0/3      | 6/6      | 0/13     |
| continuous     | tail regression | 0/8 | 0/3 | 6/6 | 3/13 |

papillae was followed by the trunk region (figure 3b; blue arrowheads; electronic supplementary material, table S2) occurring 1.95 s later, as suggested by cross-correlation analysis (figure 3c; electronic supplementary material figure S4; Video S4; electronic supplementary material, table S3). Regardless of the length of mechanical stimulation, the trend of Ca\(^{2+}\) transients (figure 3c) was similar to the trend of Ca\(^{2+}\) transient during Phase I (figure 2i, electronic supplementary material, figure S5).

Based on these results, we concluded that papillae sensed mechanical stimulation and the Ca\(^{2+}\) transient in Phase I is triggered by the mechanical stimulation of the papillae and Ca\(^{2+}\) transient in the posterior trunk region coupled with that of the papillae. Consistent with this conclusion is the observation that swimming larvae did not show Phase I without adhering to the substrate (figure 4a) while all individuals that experienced tail regression experienced Phase I (figure 2b; electronic supplementary material, figure S3A–E).

(d) The Ca\(^{2+}\) transient in Phase II coupled with tail regression

Next, we characterized Phase II, where a relatively long-lasting second Ca\(^{2+}\) transient was observed after Phase I. Because Phase II was just before tail regression (figure 2h; electronic supplementary material, Video S3) and larvae require continuous adhesion for at least 28 min to start tail regression [28], Phase II was assumed to comprise Ca\(^{2+}\) transients that arise during the continuous adhesion required to initiate tail regression. In order to determine the necessity of the second Ca\(^{2+}\) transient in papillae to start tail regression, we verified two requirements. The first requirement is that the second Ca\(^{2+}\) transient happens specifically before tail regression while the second is that the second Ca\(^{2+}\) transient never happens in response to brief stimulation because only continuous adhesion can induce tail regression.

First, we investigated whether the second Ca\(^{2+}\) transient occurs only before tail regression. We compared Ca\(^{2+}\) dynamics between no stimulation and continuous adhesion in larvae after 29.5 hpf (figure 4a,c). The second Ca\(^{2+}\) transient did not occur in the absence of stimulation (figure 4a and table 1, column of 0 s). By contrast, all larvae that underwent metamorphosis displayed the second Ca\(^{2+}\) transient (figure 4c and table 1, column of continuous). Phase II occurred only when tail regression was observed. We therefore propose that the second Ca\(^{2+}\) transient of papillae is essential for tail regression.

Second, to determine if the second Ca\(^{2+}\) transient specifically occurs when the larva is stimulated for a sufficiently long duration to induce tail regression, we applied 10 s of continuous stimulation after 29.5 hpf (figure 4b,c). Following brief stimulation, the Ca\(^{2+}\) transients in Phase I was observed but the Ca\(^{2+}\) transient in Phase II and tail regression did not occur (figure 4b and table 1, column for 10 s stimulus). By contrast, all larvae subject to longer stimulation metamorphosed and showed Ca\(^{2+}\) transient in both Phase I and Phase II (figure 4c and table 1 (n = 6)). Since the second Ca\(^{2+}\) transient was observed only when continuous stimulation was applied, continuous stimulation (12 min average from adhesion to start of tail regression in this study) appears to induce the second Ca\(^{2+}\) transient. Moreover, only larvae that showed the second Ca\(^{2+}\) transient began tail regression (table 1) suggesting that the second Ca\(^{2+}\) transient induces tail regression. These results suggest that Ca\(^{2+}\) transients in Phase II are essential for the start of tail regression.

In addition, to clarify how the papillae differentiation is associated with the induction of Ca\(^{2+}\) transient in Phase I and Phase II and subsequent tail regression, we examined the dynamics of Ca\(^{2+}\) transient and tail regression in Foxg-knockdown larva. It has been reported that Foxg is expressed in larval papillae where it functions to specify the papillae as sensory neurons [22]. During embryogenesis, Foxg expression in neural plate cells is controlled by the mitogen-activated protein kinase (MAPK)/ERK. In Foxg-knockdown larva, short Ca\(^{2+}\) transients were observed at the anterior trunk epidermis under continuous stimulation. However, neither the second Ca\(^{2+}\) transient in Phase II nor tail regression was observed (figure 4d and table 1). This result suggests that Foxg-specified sensory neurons are required for the generation of the second Ca\(^{2+}\) transient and tail regression.

(e) Identification of tissues observed during the first round of Ca\(^{2+}\) transients

Finally, we identified precise anatomical regions where Ca\(^{2+}\) activity increased during Phase I. In Phase I, the Ca\(^{2+}\) transient was first observed at both the papillae (figure 2 and figure 5, orange) and the dorsal subregion in the posterior trunk (figure 2 and figure 5, white arrowhead), before the Ca\(^{2+}\) increase in the dorsal subregion propagated to the ventral endodermal region (figure 2 and figure 5, blue).

Comparing these locations with our phalloidin staining results (figure 5b), the Ca\(^{2+}\) transient in the papillae include the more posterior part called the preoral lobe (figure 5b, orange). The dorsal subregion corresponded to cells located dorsally above the neck region of the central nervous system and the epidermal region (figure 5b, white arrowheads) [23,32,33,37]. The Ca\(^{2+}\) transient in the endodermal region (figure 2) was identified as endoderm surrounding the anterior tip of the notochord (figure 5b, blue). Because this is the first report of a Ca\(^{2+}\) transient being observed in the endoderm at the beginning of metamorphosis, we focused on the region expressing the Ca\(^{2+}\) transient in response to the mechanical cue. To derive more anatomical information about this region, we performed in vivo Ca\(^{2+}\) imaging using LSM. Consistent with our phalloidin staining results, a simultaneous Ca\(^{2+}\) transient was observed in both the papillae and the endodermal cells contacting the anterior part of the notochord.

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This region was identified as the future digestive tract, in agreement with a previous study that identified the same region as the future digestive tract [38]. These results indicate that the papillary Ca$^{2+}$ transient was coupled with that of the dorsally localized cells in the posterior trunk and the primordial digestive tract in Phase I.

4. Discussion

How larvae perceive environmental cues, such as mechanical stimuli, chemical ligands, temperature, and light, before triggering metamorphosis had evaded researchers until now. In this study, we revealed novel insights about the role of Ca$^{2+}$ transients in ascidian to link external cues to inner signals that control metamorphosis through precise timing and targeting of specific tissues.

We have identified a two-round Ca$^{2+}$ transient in the adhesive papillae that respond to mechanical cues to initiate metamorphosis in *Ciona* larvae. The observed Ca$^{2+}$ transients resulting from mechanical stimulation up until tail regression (electronic supplementary material, Video S3) are summarized as follows (figure 6).

Phase I

1. Ca$^{2+}$ transient in papillae in response to mechanical stimulation (figure 3)
2. Ca$^{2+}$ transient in dorsally localized cells after (1) (figure 5)
3. Ca$^{2+}$ transient in dorsal endoderm near the dorsally localized cells (figure 2c)
4. Ca$^{2+}$ propagation from the dorsal endoderm (3) to the ventral endoderm (figure 2d)

Phase II

5. 2nd-round Ca$^{2+}$ transient in the papillae (figure 2h; electronic supplementary material, figure S3)
6. Ca$^{2+}$ increase in cells across the entire epidermis with wave-like propagation (figure 2f; electronic supplementary material, Video S3)
7. Backward movement of posterior trunk epidermis (figure 1; electronic supplementary material, figure S2, Video S1, S2 and S3)
8. Tail regression
Phase I was strongly coupled to mechanosensation (figure 3, figure 4 and table 1) and Phase II was coupled to tail regression (figure 4c and table 1). We considered that the two rounds of Ca^{2+} transients in these phases were essential for Ciona tail regression during metamorphosis. According to a previous study, at least 28 min of adhesion is necessary for tail regression, and individuals more than 29.5 hpf start tail regression after an average adhesion time of 32 min [28]. By contrast, five out of six larvae start tail regression less than 28 min after adhesion in our experiments. The average time between adhesion and tail regression was 12 ± 4.3 min. These different response times may be due to differences in the substrate or in the strength of adhesion.

Is there a causal connection between Phase I and Phase II? We consider there is a temporal threshold of Ca^{2+} in Phase I that is prerequisite for activation of Phase II. From our results, less than 10 s stimulation induced only Phase I whereas an average of 12 min continuous stimulation induced both Phase I and Phase II Ca^{2+} transients. In our experimental system, Phase II is only observed after Phase I occurs (table 1). Therefore, we think Phases I and II are tightly coupled also in the natural condition. However, inhibition of the specification of the papilla sensory neurons by FoxgMO decoupled them (figure 4d). Further studies of papillary sensory neurons will provide a better understanding of the mechanisms that will cause Phase II.

(a) Papillary cells activated by mechanical stimulation
Our results suggest that the papillae include mechanically sensitive sensory cells. There are three types of cells in the papillae, namely four axial columnar cells (ACC), four lateral primary sensory neurons (PNS), and 12 central collocytes (CC). Their nuclei are in posterior papillae processes and tissues that make up the anterior trunk [8]. The mechanism
of metamorphosis signalling from papillae to other cells remains unsolved, but CCs and PNSs have been suggested to have exocytosis function [8]. This suggests that it may release signalling ligands via Ca²⁺-dependent exocytosis.

We assumed that several ACCs, PNS, and CCs react to the mechanical stimulus. Although ACC and CC are not neurons, we need to determine whether they sense mechanical stimuli. Identifying the channels that control Ca²⁺ influx would help elucidate the underlying mechanism for mechanosensing by the papillae.

(b) Transmission of Ca²⁺ signals among different tissues during Phase I and Phase II

Our comprehensive Ca²⁺ imaging revealed the interplay of Ca²⁺ signalling in different tissues at the start of Phase I. The Ca²⁺ activity in the papillae (1) is followed by Ca²⁺ transients in the dorsally localized cells (2) and the dorsal endoderm (3). The Ca²⁺ transient in the dorsal endoderm propagates to the ventral endoderm (4). In Phase II, the Ca²⁺ transients observed in papillae (5) are followed by a second round of Ca²⁺ transient in papillae and an increase in the entire epidermis with wave-like propagation (6). Subsequently, tail regression (7) occurs. (Online version in colour.)
metamorphosis. One of the surprising results in our study is the Ca\(^{2+}\) transient in endodermal cells surrounding the notochord in response to mechanical stimulation (figure 3b). Ca\(^{2+}\) transient has not been observed in the endoderm from gastrulation to the tailbud stage in previous studies [27]. Since this endodermal region differentiates into the digestive tract during metamorphosis, the Ca\(^{2+}\) transient in the endoderm may be related to the promotion of digestive tract differentiation. It is also interesting that dorsally localized cells in the posterior trunk responded during Phase I (figure 2 and figure 5, white arrowhead). The dorsally localized cells are located beneath the epidermis and close to the central nervous system (CNS). Although these cells are unknown, they do not appear to be identical to posterior apical trunk epidermal neuron (pATEN) nor anterior apical trunk epidermal neuron (aATEN) because the cells are more posterior (figure 5b).

During Phase II, wave-like Ca\(^{2+}\) propagations were observed in cells across the entire epidermis (figure 2f, electronic supplementary material, Video S3). This shows the direct evidence of metamorphic signals spread through epithelial conduction of Ca\(^{2+}\). A similar epithelial-conduction model of metamorphic signal propagation has been proposed for the hydrozoan cnidarian Mitrocornella polyidiadium [15]. Our method developed in this study can be applied to other species to test whether the Ca\(^{2+}\) transients that cause metamorphosis are evolutionarily conserved in other marine invertebrates.

Considering the backward movement of the epidermis that occurred following the Ca\(^{2+}\) propagations, these Ca\(^{2+}\) propagations might increase the tension within the entire epidermis to generate the pulling force required for tail regression. The tail epidermis has been proposed to generate a sufficiently strong force to absorb the axial organs into the trunk region [39] during tail regression. It was also confirmed that tail regression is inhibited by using cytochalasin B, indicating that actin fibres play an important role in tail regression [40]. Another hypothesis of the tail epidermis contraction to explain the tail regression during ascidian metamorphosis is based on apoptosis [21,41,42]. Krasovec et al. [41] reported that the tail regression depends on a postero-anterior wave of a caspase-dependent apoptosis coupled with a contraction event. This apoptosis wave might be triggered by the Phase II wave-like Ca\(^{2+}\) propagations in the epidermis.

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