1 *Research Article*

2 **Microtubule dynamics is required for rapid coiling of haptonemata in haptophyte**
 3 **algae**

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22

1 **Summary Statement**

2 Microscopy observations and pharmacological experiments revealed that the rapid
3 coiling of a non-motor microtubule-based motile organelle, the haptonema, is explained
4 by conformational changes of microtubules, including twisting and writhing.

5

6 **ABSTRACT**

7 A haptonema is an elongated microtubule-based motile organelle uniquely present in
8 haptophytes. The most notable and rapid movement of a haptonema is “coiling”, which
9 occurs within a few milliseconds following mechanical stimulation in an unknown
10 motor-independent mechanism. Here, we analyzed the coiling process in detail by
11 high-speed filming and showed that haptonema coiling was initiated by left-handed
12 twisting of the haptonema, followed by writhing to form a helix from the distal tip. On
13 recovery from a mechanical stimulus, the helix slowly uncoiled from the proximal
14 region. Electron microscopy showed that the seven microtubules in a haptonema were
15 arranged mostly in parallel but that one of the microtubules often wound around the
16 others in the extended state. The persistence lengths calculated from the curvature of the
17 haptonematal microtubules indicated their unusual flexibility. A microtubule stabilizer,
18 paclitaxel, inhibited coiling and induced right-handed twisting of the haptonema in the
19 absence of Ca^{2+} , suggesting changes in the microtubule surface lattice. Addition of Ca^{2+}
20 caused bend propagation toward the proximal region. These results indicate that
21 switching microtubule conformation with the aid of Ca^{2+} -binding
22 microtubule-associated proteins is responsible for rapid haptonematal coiling.

23

1 INTRODUCTION

2 Haptophytes are the group of microalgae that are widely distributed in oceans.
 3 They show similarities to heterokonts in chloroplast structure and chlorophyll species
 4 but are classified into an independent phylum owing to several cytological properties,
 5 including the lack of mastigonemes on flagella and the presence of extracellular scales
 6 or coccoliths (Christensen et al., 1962; Andersen, 2004). A haptonema is a filiform
 7 organelle uniquely present in haptophytes (Parke et al., 1955). It extends from a position
 8 between the bases of two flagella, reaching up to more than 100 μm in some species
 9 (Gregson et al., 1993a,b). A variety of functions have been demonstrated for haptonema,
 10 including attachment and gliding on a substrate, formation of food aggregates, food
 11 capture and transport, and reception of mechanical stimuli (Manton, 1967; Leadbeater
 12 and Manton, 1971; Kawachi and Inoue, 1991, 1995). When haptophytes receive
 13 mechanical stimuli, they fully coil the haptonema within only a few milliseconds. By
 14 contrast, “uncoiling”, the process to resume the extended state, is much slower than
 15 coiling. The coiling state is thought to be a low-energy form, because the haptonema is
 16 always coiled when it is detached or when haptophytes are dead (Estep and MacIntyre,
 17 1989).

18 Haptonema coiling is inhibited by EGTA depletion of extracellular Ca^{2+} or by
 19 the addition of an inhibitor of Ca^{2+} -induced Ca^{2+} release, indicating that it is triggered
 20 by Ca^{2+} influx followed by efflux from a Ca^{2+} store (Kawachi and Inouye, 1994). Upon
 21 receipt of a mechanical stimulus, haptonematal coiling accompanies a change in
 22 waveform and an increase in beat frequency of flagella, possibly from changes in
 23 intracellular Ca^{2+} concentrations. This results in a quick response to avoid the stimulus
 24 (Gregson et al., 1993b; Kawachi and Inouye, 1994). The mechanism for the cytoskeletal

1 response to Ca^{2+} is not well understood, except that centrin is localized as a small
2 dot-like structure at the distal tip of haptonematal microtubules (Lechtreck, 2004).

3 Ultrastructural observations show that six to seven microtubules pass in
4 parallel through a haptonema. In cross section, they are arranged in a circle of ~100 nm
5 in diameter in the middle region of the haptonema, and in an arc-shape with
6 invagination of endoplasmic reticulum at the basal region. The number of microtubules
7 reduces to three at the distal most region (Manton, 1964, 1967, 1968; Gregson et al.,
8 1993b). It is well known that microtubules of flagellar axonemes have post-translational
9 modifications (Wloga and Gaertig, 2010), but the patterns of modifications of
10 haptonema microtubules are different from those of axonemes (Lechtreck, 2004). The
11 microtubules are surrounded by fenestrated cisternae in the major part of a haptonema
12 beneath the plasma membrane. From thin-section electron microscopy observations, the
13 circular arrangement of microtubules tends to change to a crescent arrangement after
14 coiling. An electron dense structure in the center of the microtubule ring and a structure
15 that links neighboring microtubules are observed (Gregson et al., 1993b). However, no
16 structure that potentially represents motor proteins, such as dyneins or kinesins, has
17 been observed. Thus, the molecular mechanism for rapid coiling of haptonemata is
18 completely unknown.

19 Here we examined the structure of haptonemata and the process of their
20 coiling using a newly identified marine species of the genus *Chrysochromulina*.
21 Although the structures of this haptonema share common properties to those reported
22 for other species (Gregson et al., 1993), we obtained new information regarding
23 microtubule configurations in this haptonema. Furthermore, we found that a
24 microtubule stabilizer, paclitaxel (taxol), inhibits haptonematal coiling, indicating that

microtubule flexibility and dynamics are responsible for the induction of rapid haptonema coiling.

RESULTS

Morphological characterization of *Chrysochromulina* sp. NIES-4122

The genus *Chrysochromulina* (Prymnesiophyceae) is characterized by the development of a relatively long haptonema in the subclass Chrysochromulinaceae (Edvardsen et al., 2011). Here, we used a *Chrysochromulina* species that was collected in Tokyo bay in 2013. The cell strain was established by clonal culture. This species has no calcareous coccoliths but has organic scales with no spine (Fig. S1A-C) and is morphologically classified into the genus *Chrysochromulina*. However, the shapes of the scales are distinct from any known *Chrysochromulina* species. This species possesses a haptonema of up to ~150 μm in length, which is a little longer than that in *C. simplex*, *C. acantha* and *C. hirta* (Fig. S1B; Kawachi and Inouye, 1995; *C. hirta* is changed to *Haptolina hirta* after Edvardsen et al., 2011). When compared with other *Chrysochromulina* species, such as NIES-1333, the haptonema of this species was more resistant to mechanical stimuli that cause detachment from the cell body. Coiling was partially inhibited by depletion of Ca^{2+} in artificial sea water and completely inhibited by chelating intracellular Ca^{2+} (Fig. S1D). This strain has been deposited with the National Institute for Environmental Studies (NIES), Japan, as *Chrysochromulina* sp. NIES-4122.

Observation of haptonematal coiling by high-speed recording

The haptonema of *Chrysochromulina* sp. NIES-4122 occasionally showed coiling during observation under a light microscope. Gentle tapping of the microscope

1 stage induced almost 100% haptonematal coiling (Movie 1). The coiling occurred very
2 rapidly and was complete within 5-10 msec (Movie 2), which is considerably faster than
3 that observed in *C. acantha* (10-20 msec; Leadbeater and Manton, 1971). In contrast,
4 uncoiling was much slower, taking ~480 msec to complete extension (Movie 3).

5 One might expect that the coiling would start from the tip of a haptonema.
6 However, detailed observation of the high-speed images revealed that this is not the
7 case; the distal half of the haptonema first began to bend in gentle helices, followed by
8 sequential coiling from the tip (Fig. 1A). The coil appeared to be left-handed, which
9 was more clearly observed in the process of uncoiling (Fig. 1B). Uncoiling initiated
10 from the proximal region of a haptonema while the distal most part remained curled,
11 which was then gradually unwound during the last step of extension.

12

13 **Microtubule configuration in haptonemata in extended and coiled states**

14 As reported in other species, including *C. chiton* (Manton, 1967), *C. simplex* and *C.*
15 *acantha* (Gregson et al., 1993b), thin-section electron microscopy showed that in the
16 extended haptonema seven microtubules are arranged in a ring, which is peripherally
17 surrounded by cisternae (Fig. 2A-D). This circular arrangement of microtubules was
18 distorted in the coiled haptonema and one of the microtubules was often invaginated
19 towards the center (Fig. 2E-H). We measured the center-to-center distances between
20 adjacent microtubules (Fig. S2A, B). The distances were relatively constant in an
21 extended haptonema among sequential sections (Fig. S2C) but in a coiled haptonema
22 one of the inter-filament distances sometimes became deviated, as if the ring was torn
23 open (Fig. S2D). The deviated microtubule often changed its position relative to the
24 adjacent microtubule (Fig. S2D). This pattern with an interfilament deviation distance

1 of more than 15 nm was observed in 0% and 13% of extended and coiled haptonemata,
2 respectively.

3 In longitudinal sections 70–80 nm thick, we were able to observe a parallel
4 arrangement of three, sometimes four microtubules of up to 1 μ m and 200 nm in
5 extended and coiled haptonemata, respectively (Fig. 2I, J). This indicates that
6 microtubules are arranged in a more or less parallel manner in both extended and coiled
7 stages. To confirm the parallel arrangement of microtubules, we treated a small plate of
8 polymerized Epon resin with poly-lysine. This was then coated with bovine serum
9 albumin. Fixed haptophytes were deposited on the coated resin, fixed and embedded in
10 Epon. This procedure provided a fixed landmark to measure the relative positions of
11 each microtubule between sequential sections (Fig. 3A-C). The position of each
12 microtubule relative to a landmark would change periodically if the haptonematal
13 microtubules were arranged helically. In the case of parallel arrangement, the positions
14 of seven microtubules would shift in parallel in the sequential sections (Fig. 3D). These
15 results indicated that the microtubules are arranged in parallel, at least up to lengths of
16 400 nm and 320 nm for the extended and most of the coiled haptonemata, respectively,
17 (Fig. 3E, F). However, four out of 24 sequential images of the coiled haptonema
18 showed twisted arrangements (Fig. 3G).

19 Next, haptophytes were deposited on a grid, demembrated by NP-40 and
20 observed by negative staining electron microscopy (Fig. 4). The diameters of flagellar
21 axonemes and haptonemata were distinct, so that a long haptonema could be distinctly
22 observed at lower magnifications by negative staining (Fig. 4A). As reported by
23 Gregson et al. (1993b), haptonematal microtubules are bound together after
24 demembration but are partly dissociated in some regions of the extended haptonema.
25 Microtubules of the extended and coiled parts of a haptonema are mostly parallel to

each other without any torsion. However, we often observed a microtubule loosely wound around the other six microtubules (Fig. 4B). This peculiar microtubule winding was not clearly observed in the coiled region where all the microtubules are mostly arranged in parallel. Instead, we observed that microtubules were stacked at two opposite positions in the coil (Fig. 4C). This is compatible with the observations from thin-sectioned sequential images of some microtubules being twisted in the coiled region (Fig. 3).

The diameter of the haptonema coil was $1.2 \mu\text{m} \pm 0.03 \mu\text{m}$ ($N = 83$), as estimated from negative stain images. The microtubules in the coils are largely curved (Fig. 4C), suggesting higher flexibility than microtubules from other sources. To evaluate the flexibility of haptonematal microtubules, we calculated the persistence length, a measure of flexural rigidity of filamentous structures. The persistence lengths for DNA, an actin filament, and a microtubule have been reported to be 50 nm, 13 μm , and $\sim 6 \text{ mm}$, respectively (Taylor and Hagerman, 1990; Gittes et al., 1993; Käs et al., 1994), indicating that microtubules are very rigid structures. However, the persistence lengths of haptonematal microtubules calculated according to the method of Gittes et al. (1993) for the extended and coiled regions of the haptonema were $93.1 \pm 17.7 \mu\text{m}$ ($N = 57$) and $1.7 \mu\text{m} \pm 0.2 \mu\text{m}$ ($N = 78$), respectively (Fig. S3). Although these values were calculated using the negative stain images of microtubules fixed on a carbon membrane and thus do not represent the true values of the persistence length, the results indicate that haptonematal microtubules have high flexibility.

It is likely that binding of haptonema-specific microtubule-binding proteins (MAPs) prevents the curved microtubules from depolymerization. In fact, negative stain images comparing haptonematal microtubules (Fig. 4D, lower) and microtubules polymerized from purified tubulin (Fig. 4D, upper) showed that the haptonematal

1 microtubules are covered with additional proteins. When haptonematal microtubules
 2 were depolymerized, they often appeared to stay bundled (Fig. 4G, left), again
 3 indicating the presence of large amounts of MAPs. We also occasionally observed
 4 filamentous structures with diameters that were much smaller than those of
 5 microtubules. They were observed spreading out from microtubule bundles (Fig. 4E).
 6 Another unique structure we observed was a mass of small particles. These masses were
 7 observed at the distal tip of a haptonema and usually existed as a pair (Fig. 4F),
 8 suggesting a cap structure at the distal end of haptonematal microtubules. Alternatively,
 9 they may simply be masses of tubulins depolymerized from the distal part of
 10 haptonematal microtubules because they are structurally similar to the depolymerized
 11 microtubules (Fig. 4G).

12

13 **Taxol inhibits haptonematal coiling**

14 Ultrastructural observation of the haptonema indicated dynamic structural changes
 15 between coiled and extended states. To examine the possibility that microtubule
 16 dynamics are involved in the coiling mechanism, we used taxol and nocodazole, which
 17 inhibit microtubule depolymerization and polymerization, respectively. We incubated
 18 the haptophytes with each drug and induced coiling by tapping the microscope stage.
 19 Nearly 80% of nocodazole-treated and control haptophytes showed coiling. However,
 20 haptophytes treated with taxol showed significantly inhibited coiling (Fig. 5A). In the
 21 extended state without mechanical stimulation, control haptonemata remained straight
 22 but taxol-treated haptonemata showed bending with low curvatures. The overall length
 23 of taxol-treated haptonema was significantly increased (Fig. 5B), which might indicate
 24 elongation of the microtubules, possibly from the tubulin pool at the distal tip.

Decreased length was observed for nocodazole-treated haptonema but this was not significant.

To quantitatively evaluate the taxol-induced bending, we measured the curvature along the haptonema (Fig. S4). The curvature was almost zero in control and nocodazole-treated haptophytes throughout the haptonema (Fig. 5C, E), but taxol-treated haptonema showed a large peak in the proximal region with the maximum at around 15-20 μm from the base (Fig. 5D, F; Fig. S5). This bending was initially formed in the distal to middle region 5 min after treatment and then propagated toward the proximal region (Fig. S6A).

Changes of taxol-induced haptonema bending by Ca^{2+}

To explore the relationship between the mechanism of coiling and taxol-induced bend formation, we examined the effect of Ca^{2+} on haptonemata after treatment with taxol in Ca^{2+} -free conditions. In contrast to taxol-treated haptonemata in normal sea water with Ca^{2+} (Fig. 5D), the haptonema in Ca^{2+} -free conditions did not show planar bending but rather twisted or helical shapes (Fig. 6A). Careful observation by altering the microscope focus showed that the helix was right-handed. The helix was maintained from 5 min to 60 min but became gentle in pitch to extend toward the tip (Fig. S6B).

To examine the requirement of Ca^{2+} , taxol-treated haptonemata were first incubated in Ca^{2+} -free conditions containing EGTA/BAPTA-AM for 60 min, and then 50 mM CaCl_2 was added. The twist observed 60 min after the incubation in Ca^{2+} -free conditions gradually became planar and shifted toward the base of the haptonema after the addition of CaCl_2 (Fig. 6B). The final waveform became similar to that observed in taxol-containing artificial sea water (Fig. 5D).

1 **DISCUSSION**

2 Our present data demonstrate that the quick coiling of haptonema is driven by the
 3 configurational changes and dynamics of seven singlet microtubules, not through
 4 microtubule-sliding driven by conventional molecular motors. Another eukaryotic
 5 example showing non-motor rapid movement by microtubule structures is the
 6 contraction of heliozoan axopodia (Tilney and Porter, 1965; MacDonald and Kitching,
 7 1967; Suzuki et al., 1980; Hausmann et al., 1983), in which Ca^{2+} -dependent cataclysmic
 8 breakdown of microtubules as well as their depolymerization induce the contraction
 9 (Febvre-Chevalier and Febvre, 1986). However, heliozoan axopodia do not make coiled
 10 structures, and our data showed that the microtubules in coiled haptonemata are not
 11 depolymerized. An example showing a similar movement to haptonematal coiling is
 12 quick contraction of the spasmoneme in the “stalk” of the peritrichous ciliates, such as
 13 *Vorticella* and *Zoothamnium*. The spasmoneme contraction proceeds very quickly to
 14 form a spiral stalk in a Ca^{2+} -dependent but ATP-independent manner. Cytoskeletal
 15 elements of the stalks are not microtubules but helically coiled structures, mainly
 16 constructed of a 20 kDa centrin-related Ca^{2+} -binding protein, called spasmin (Amos et
 17 al., 1975). Thus, haptonematal coiling is a unique type of microtubule-dependent
 18 motility, distinct from any other known eukaryotic motile machinery.

19

20 **Microtubule dynamics is involved in haptonematal coiling**

21 We found that taxol inhibited the rapid coiling of a haptonema. Although this
 22 inhibition may indicate involvement of microtubule depolymerization in the coiling
 23 process, electron microscopy showed no depolymerization of microtubules in the coiled
 24 haptonema. Thus, the inhibition is considered to be caused by another mechanism.
 25 Taxol binds to β -tubulin, resulting in a longitudinal extension of the $\alpha\beta$ -tubulin dimer

spacing, formation of straight protofilaments and stabilization of microtubules (Arnal and Wade, 1995; Caille et al., 2007; Mitra and Sept, 2008). Application of taxol to polymerized microtubules significantly changes their flexural rigidity (Dye et al., 1993; Mickey and Howard, 1994; Venier et al., 1994) and overall radial flexibility (Donhauser et al., 2010). Therefore, the gentle coiling of haptonema in the presence of taxol is likely to be caused by changes in the mechanical property of microtubules.

The flexural rigidity of biological filaments is often evaluated using 'persistence length'. Compared with double-stranded DNA or actin-filaments, whose persistent lengths are reported to be 50 nm (Taylor and Hagerman, 1990) and 10 μm (Gittes et al., 1993; Käs et al., 1994), respectively, microtubules are rigid filaments and their persistence length is large, ranging from 80 μm to 5.2 mm depending on the measurement condition (Gittes et al., 1993; van den Heuvel et al., 2008). Persistence lengths also depend on filament length, and microtubules covalently grafted to a grid showed persistence lengths ranging from 110 to 5,035 μm for filament lengths of 2.6 to 47.5 μm (Pampaloni et al, 2006). We obtained persistence lengths of 93.1 and 1.7 μm in the extended and coiled regions of haptonemata, which are the smallest values among those reported for microtubules. Notably, the microtubules in coiled haptonemata showed unusually curved structures, which cannot be explained by previously-reported persistence length values. Such a high curvature is expected to cause a large distortion of the microtubule surface lattice.

Formation of helical shapes (Fig. 6A) by taxol treatment should be accompanied by distortion of the tubulin lattice. Taxol alters the tubulin lattice and supertwist of microtubules (Arnal and Wade, 1995); therefore, it is possible that the microtubule structures of taxol-treated haptonemata in the absence of Ca^{2+} are distorted and fixed to make the right-handed helical shape. When trapped in this structural state, the changes

in configuration needed for the left-handed coiling of haptonemata may be inhibited. It is likely that haptonematal microtubules need extra structural reinforcement to prevent them from breakage during coiling (Fig. 4D). The microtubule destabilizer, nocodazole, induces no apparent morphological change in haptonemata, such as shortening by depolymerization, indicating the presence of structural reinforcement for microtubule stability. Together, these data indicate that the coiling of haptonematal microtubules is caused by mechanical changes of microtubules with the aid of specific MAPs.

The role of Ca^{2+} in configuration changes of haptonematal microtubules

Haptonemata coiling occurs within 5–10 msec; therefore, haptonematal microtubules are thought to have a structural basis that allows a rapid change in conformation in response to an increase in intracellular Ca^{2+} . Since *in vitro* polymerized microtubules are straight, a mechanical strain must be imposed on microtubules for coiling. This strain is probably induced by MAPs, among which Ca^{2+} -binding proteins seem to play major roles in keeping distinct microtubule configurations in the presence of Ca^{2+} (Fig. 7). Ca^{2+} binding to the haptonematal MAPs alters their conformations, imposing a strain on the microtubule (Fig. 7B). This strain may be relaxed when the microtubule coils (Fig. 7C). The haptonemata of dead cells and detached haptonemata are always coiled (Leadbeater and Manton, 1969; Gregson et al., 1993b), and the intracellular Ca^{2+} of these haptonemata is high. Therefore, the coiled configuration of haptonematal microtubules seems to be stable in the presence of Ca^{2+} -bound MAPs. When Ca^{2+} is released from the MAPs bound to the coiled microtubule, the microtubule conformations change back to the extended form (Fig. 7D). The conformation of MAPs is assumed to be different between Ca^{2+} -bound (Fig. 7B) and Ca^{2+} -free (Fig. 7D) forms.

1 This might result in the different microtubule configurations observed during coiling
2 (gentle helices) and uncoiling (uncurling) (Fig. 1).

3 There is also a possibility that Ca^{2+} binds directly to tubulin to change the
4 microtubule structure. Although *in vitro* microtubules are known to depolymerize in the
5 presence of Ca^{2+} , haptonematal MAPs may prevent the microtubules from disassembly.
6 The electron microscopy images showed haptonema microtubules to be covered with
7 associated proteins (Fig. 4D), which probably include more than one kind of MAP.
8 These MAPs may stabilize the microtubules and prevent them from breakage even in
9 the presence of Ca^{2+} or under mechanical strain.

10 The taxol-induced twist is converted into bending, which propagates from the distal
11 to the proximal region of the haptonema by the addition of Ca^{2+} , indicating that another
12 mechanical state of haptonematal microtubules is induced by Ca^{2+} . The direction of
13 propagation of taxol-induced bending coincides with that of normal haptonematal
14 coiling, supporting the idea that the Ca^{2+} -dependent configuration change is linked to
15 coiling.

16 In this study we observed structures associated with haptonematal microtubules
17 (Fig. 4). In addition to the MAPs that cover the microtubule surface, we saw a
18 filamentous structure that spread out from the bundle of haptonematal microtubules.
19 This filament may be present with haptonematal microtubules to bundle them and might
20 be involved in the Ca^{2+} -dependent configuration change of microtubules for coiling.
21 Another structure we observed was a mass of small particles at the tip of a haptonema,
22 which may play roles both in fixing the distal end of the haptonema and in initiating
23 structural changes of the haptonema for coiling, although it is still possible that these
24 structures are a mass of depolymerized tubulins. Centrin is localized to haptonemata in
25 a spotted pattern, mostly near the distal end (Lehtreck, 2004). However, it is not clear

whether the mass of small particles that we observed (Fig. 4F) is simply comprised by centrin. Identification of the Ca^{2+} -binding proteins will be key to understanding of the molecular mechanism of haptonemal coiling.

Possible models for haptonemal coiling

To explain the mechanism of coiling, we present three models. In the first model, coiling is accounted for by Ca^{2+} -dependent structural changes of MAPs that connect the neighboring microtubules (Fig. 8A). We hypothesize that the configuration of microtubules in the coiled haptonema represent a stable state when Ca^{2+} is bound to the MAPs (Fig. 7; Fig. 8A, right). In the absence of Ca^{2+} , haptonemal microtubules are aligned approximately in parallel (Fig. 8A, left). When Ca^{2+} binds to the MAPs, their structural change imposes a tension between the neighboring microtubules, making the energy state of the haptonema higher, to induce twisting of the microtubules (Fig. 8A, middle), resulting in writhing to make a coil.

The second model (Fig. 8B) is based on our electron microscopy observation that one or two of the microtubules wind around the parallel bundle of the other microtubules (Fig. 4B; Fig. 8B, left). This model assumes that one of the seven haptonemal microtubules has specific Ca^{2+} -binding MAPs that link this microtubule to the other six microtubules. Ca^{2+} -binding to the MAPs may then change the structure of this special microtubule, enforcing the other six microtubules to twist (Fig. 8B, middle). The torsional force would be relaxed when the haptonema is coiled (Fig. 8B, right). This model is somewhat similar to the model that explains coiling of the *Vorticella* stalk, where a thin filament of a contractile protein, spasmin, winds around the elastic rod of the stalk. Once Ca^{2+} binds to spasmin, the thin filament quickly changes the conformation to coil the thick filament (Misra et al, 2010). This model agrees with the

fact that one microtubule deviates from the other six microtubules in its distribution (Fig. 3G; Fig. S2). Therefore, it is reasonable to assume that one of the haptonematal microtubules is associated with specific Ca^{2+} -binding proteins and drives the rapid coiling.

The above two models attribute coiling of a haptonema to structural changes of the MAPs that link two haptonematal microtubules; however, the third model (Fig. S7) assumes association of Ca^{2+} -binding MAPs to each protofilament that constitutes a microtubule. Structural changes of these MAPs would alter the conformation of the protofilament and its interaction with neighboring protofilaments (Fig. 7). These specific MAPs could bind either to one or two of the protofilaments (Fig. S7D), or to all of the protofilaments (Fig. S7G). The former case resembles the model explaining coiling of the bacterial flagellar filament, a helical machinery consisting of 11 protofilaments. They are composed of flagellin, which can adopt two slightly different conformations. The flagellar filament is straight when all the subunits have the same conformation but becomes helical when some of the protofilaments adopt the different structural state. In that way, the flagella can instantly switch from left-handed to right-handed helical structures (Calladine, 1978; Namba and Vonderviszt, 1998). In the case of a haptonema, if some of the MAPs specifically bind to one or two of the protofilaments, as is the case with axonemal doublet microtubules, these protofilaments would change their structure (Fig. S7D), resulting in coiling of the haptonematal microtubules (Fig. S7E). Coiling of the haptonema can be explained if the coiling of individual microtubules is coordinated (Fig. S7F).

Microtubule coiling could also occur when all of the protofilaments change their structure because of conformational change of the MAPs. Changes in the inter-protofilament interactions could twist the protofilaments (Fig. S7G), causing a

torsional force on the microtubule, which may be relaxed when the microtubule coils (Fig. S7H, I). In all of these models, coiling of the haptonema is explained by conformational changes of the protofilaments or microtubules, which are made possible by Ca^{2+} -dependent structural changes of MAPs associated with the protofilaments or microtubules (Fig. 7).

Microtubules are the most rigid cytoskeletal elements but can form flexible arcs with short wavelengths in living cells (Brangwynne et al, 2006). Some MAPs are known to cause conformational changes in protofilaments and overall microtubule structures, as seen in the case of stathmin (Sobel et al, 1989; Brouhard and Rice, 2014). The combination of taxol and MAPs has novel effects on the mechanical properties of microtubules (Hawkins et al., 2013), which is consistent with our present data. Further studies on the structures and Ca^{2+} -dependent configurational changes of haptonematal microtubules and their associated proteins using cryoelectron microscopy and tomography should shed light on the mechanism of rapid coiling induced without microtubule-based motors. Identification of the Ca^{2+} -binding MAPs will greatly clarify the molecular basis for the mechanism.

Materials and methods

Haptophytes

Chrysochromulina sp. was isolated from Tokyo bay in 2013 using micropipette isolation (Anderson and Kawachi, 2005). It was then characterized (Fig. S1) and registered as a culture collection (NIES-4122). *Chrysochromulina* sp. NIES-4122 was maintained in Daigo IMK medium (Nippon Seiyaku Co., Osaka, Japan) at 20°C under a 14:10 h light: dark regime. The haptophyte could be recovered as a pellet after centrifugation but the haptonemata were mostly detached owing to the mechanical

1 stimulus of colliding with the bottom of the centrifuge tube. To increase the number of
 2 cells in a microscopic field, we concentrated haptophytes using a non-toxic density
 3 gradient medium, Percoll. Percoll (GE healthcare) was diluted with two times
 4 concentrated artificial seawater (ASW; 460.3 mM NaCl, 10.11 mM KCl, 9.18 mM
 5 CaCl₂, 35.91 mM MgCl₂, 17.49 mM MgSO₄, 0.1 mM EDTA and 10 mM
 6 HEPES-NaOH, pH 8.2) to make 50% Percoll. Ten milliliters of culture was layered on
 7 100 µl of 50% Percoll in a 15 ml Falcon tube and was centrifuged at 2,800 g for 2 min.
 8 A hundred microliters of concentrated cells were collected from the boundary between
 9 the culture medium and Percoll.

10

11 **Light microscopy observation of haptonematal coiling and uncoiling**

12 Coiling and uncoiling were induced by tapping of the microscopic stage (Kawachi and
 13 Inouye 1994). Haptonemata were observed with a phase contrast microscope (BX51,
 14 Olympus, Tokyo, Japan). To record haptonematal coiling, the microscope was
 15 connected to a high-speed CCD HAS-D3 camera (Ditect, Tokyo, Japan). Haptonematal
 16 bending was analyzed from high-speed camera images using Bohboh software (Bohboh
 17 Soft, Tokyo, Japan). To examine the effects of Ca²⁺, 100 µl of concentrated cells were
 18 mixed with 900 µl of Ca²⁺-free ASW (462.01 mM NaCl, 9.39 mM KCl, 59.08 mM
 19 MgCl₂, 10 mM HEPES, pH 8.0), 10 mM EGTA in Ca²⁺-free ASW, or 10 µM EGTA
 20 and 50 µM BAPTA-AM (Dojindo, 50 mM stock solution in DMSO) in Ca²⁺-free ASW.
 21 Paclitaxel (taxol) and nocodazole were dissolved in dimethyl sulfoxide (DMSO) to 20
 22 mM and added to media to a final concentration of 20 µM.

23

24 **Electron microscopy**

1 Thin-section electron microscopy was performed as described previously (Konno et al,
2 2010) with some modifications. To examine microtubule configurations in sequential
3 images, a flat surface of polymerized Epon resin was treated with 1 mg/ ml poly-lysine
4 for 30 min, followed by coating with 10 mg/ml bovine serum albumin (BSA).
5 Harvested haptophytes were fixed with 2.5% glutaraldehyde and were then deposited
6 onto the poly-lysine coated surface by centrifugation at $200 \times g$ for 10 min. The samples
7 were post-fixed with 1% OsO₄, dehydrated and embedded again according to a general
8 procedure for thin-section electron microscopy. For negative staining, harvested cells
9 were incubated in Ca²⁺-free ASW containing 10 mM EGTA for 10 min. Cells were then
10 gently mounted on a carbon-coated Cu grid and demembrated with 0.5% NP-40 in
11 100 mM HEPES-NaOH (pH 7.2), 2 mM MgSO₄, 5 mM EGTA for 15 sec. The grid was
12 rinsed with the HEPES buffer for 1 min and negatively stained with 1% uranyl acetate.
13 Electron microscopy was carried out using a JEOL 1010 or Tecnai F20 transmission
14 electron microscope. Persistence lengths (L_p) were calculated according to Gittes et al
15 (1993) using the following equation:

$$\langle \cos[\theta(s) - \theta(0)] \rangle = \exp\left(-\frac{s}{2L_p}\right),$$

16 where $\theta(0)$ and $\theta(s)$ are the tangent angles of the filament at the origin (0) and at each
17 point s along the filament. s denotes the arc length. Negative stain images were
18 processed using ImageJ to obtain coordinates for calculation.

19

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Author contributions

K.In. conceived the study; K.A., T.N., K.Is. collected and identified haptophytes; M.N., K.A., K.H., K.S. K.In. designed experiments; M.N., K.A., K.H., K.S., K.In. performed experiments; N.M., K.A., K.S. analyzed data; K.I. wrote the manuscript; all authors agreed to the final version of the manuscript.

Competing interests

The authors declare no competing financial interests.

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Supplementary information

Supplementary figures and movies accompany this paper.

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1 **Figure legends**

2

3 **Fig. 1. High-speed analysis of haptonematal coiling and uncoiling.**

4 (A) Coiling process of a haptonema. High speed images recorded at 1000 fps. Gentle
5 helices that initially formed in the distal half of a haptonema are indicated by white
6 arrowheads. Stacked coils are indicated by black arrowheads. Bar, 20 μ m. (B)
7 Uncoiling process of a haptonema. High speed images recorded at 200 fps. Bar, 20 μ m.
8 The inset represents the trace of the haptonema at 70 msec for clarification of the
9 coiling direction.

10

11 **Fig. 2. Thin-section images of extended and coiled haptonemata.**

12 (A) Extended haptonema at low magnification. Bar, 500 nm. (B-D) High magnification
13 images of an extended haptonema. Bar, 100 nm. (E) Coiled haptonema at low
14 magnification. Bar, 500 nm. (F-H) High magnification images of a coiled haptonema.
15 Dashed yellow circles show a microtubule invaginated into the center. Bar, 100 nm. (I
16 and J) Longitudinal images of extended (I) and coiled (J) haptonemata. Bar, 500 nm.

17

18 **Fig. 3. Arrangement of microtubules along a haptonema.**

19 (A) Extended haptonema adjacent to the poly-lysine-BSA coated Epon surface
20 (landmark). To position each microtubule, a line was drawn from the center of the
21 microtubule bundle at right angles to the surface. Bar, 200 nm. (B) Approximately 80
22 nm sequential sections were made. For example, five sections covered a 400 nm length
23 of a haptonema along the longitudinal axis. (C) Example of sequential images of an
24 extended haptonema. Bar, 200 nm. (D) Definition of the distance from the landmark
25 and the relative position of each microtubule. (E) Typical plot of microtubule positions

1 in five sequential images of an extended haptonema. (F) Typical plot of microtubule
2 positions in five sequential images of a coiled haptonema. (G) Plot showing a helical
3 arrangement of the seven microtubules in a coiled haptonema. Four out of 24 sets of
4 sequential images of the coiled haptonema showed this pattern.

5

6 **Fig. 4. Negative stain images of extended and coiled haptonemata.**

7 (A) Negative stain image of a haptophyte. Because of demembranation, most parts of
8 the cell body are removed. f, flagellum; h, haptonema. A long haptonema is observed on
9 the right side with the tip coiled. Bar, 10 μ m. (B) Three images showing that one,
10 occasionally two microtubules (arrows), wind around the other microtubules in the
11 extended region. Bar, 1 μ m. (C) Coiled region. Microtubules are mostly parallel to each
12 other, but there seems to be a twist at the two opposite regions of the coil (arrowhead).
13 Bar, 500 nm. (D) Microtubules polymerized from purified brain tubulin (upper) and
14 microtubules in a demembranated haptonema (lower). Bar, 50 nm. (E) Image showing
15 filamentous structures (white arrowhead) emanating from the microtubule bundle. Bar,
16 100 nm. (F) Mass of small particles observed at the distal tip of a haptonema. They are
17 frequently observed in a pair. Bar, 200 nm. (G) Depolymerization of microtubules
18 occasionally observed in demembranated haptonemata. The depolymerized
19 microtubules are still bundled probably because of MAPs. The morphology of the small
20 particles in F is similar to depolymerized tubulin dimers. Bar, 200 nm.

21

22 **Fig. 5. Effects of drugs that affect microtubules on haptonematal coiling.**

23 (A) Rates of coiling in the presence of taxol or nocodazole. Error bars show the
24 standard deviation. N=5. (B) Length of haptonemata at 60 min after treatment with each
25 drug. Error bars show the standard deviation. N=5. The asterisk represents that the

difference is significant at $p < 0.01$ (Student's t-test). **(C-E)** Effects of each drug on the curvature along the axis of a haptonema. Corresponding phase-contrast images are shown above each plot. Bar, 10 μm . **(F)** Absolute values of the curvature showing the extent of bending along the haptonema. Error bars show the standard deviation. $N=20$.

Fig. 6. Effects of Ca^{2+} on taxol-induced haptonematal bending.

(A) A phase contrast image of a haptophyte treated with taxol in the absence of Ca^{2+} (CFSW+EGTA+BAPTA-AM) showing a helical configuration of the haptonema (left). The plot to the right shows the curvature along the haptonema. Bar, 10 μm . **(B)** Ca^{2+} -dependent propagation of a taxol-induced haptonematal helix. The helix is also converted to planar bending with the maximum curvature of bend propagating to the proximal region. Trace images of haptophytes are shown as insets.

Fig. 7. Model for configuration changes in haptonematal microtubules driven by Ca^{2+} -binding MAPs.

The three grey tubes represent either the three neighboring microtubules in a haptonema or three neighboring protofilaments in a haptonematal microtubule. In the former case, the microtubules are linked by Ca^{2+} -binding MAPs (pink). When Ca^{2+} is not bound to the MAPs, the microtubules are stable in the straight conformation (A). When Ca^{2+} (yellow) binds, the MAPs change their structures, causing a shearing force between the microtubules (B). The tension is reduced when the microtubule bundle becomes coiled (C). When Ca^{2+} is released from the MAPs, their structure changes to a conformation different from the Ca^{2+} -bound form in B (D). This conformational change induces uncoiling of the microtubules and the haptonema extends. In the case when the grey tubes represent protofilaments, the MAPs are bound to each protofilament. Ca^{2+} -binding to the

MAPs changes the structure of the protofilament and thus the interactions between neighboring protofilaments, which results in a torsional force exerted on individual microtubules (B). The force is relaxed when the microtubule becomes coiled (C). When Ca^{2+} is released, the MAPs take another conformation (D), which changes the inter-protofilament interactions, resulting in uncoiling. In either case, release of Ca^{2+} from the MAPs brings the microtubule conformation back to the extended state.

Fig. 8. Two possible models for the mechanism of haptonematal coiling based on the twisting and writhing of haptonematal microtubules.

(A) Microtubules in the extended haptonema are mostly parallel to each other in the absence of Ca^{2+} (left). Upon Ca^{2+} binding to MAPs that link the neighboring microtubules, the microtubules become twisted, which induces the formation of a gentle helix (middle). The haptonema would then form a coil by writhing, in which the microtubules are again mostly parallel, except that two opposite regions are twisted (right). This state is energetically stable with the aid of MAPs and Ca^{2+} . (B) In the extended state, one microtubule winds around the other six haptonematal microtubules (left), connected with Ca^{2+} -binding MAPs. Binding of Ca^{2+} induces the configuration change of this microtubule to twist the other microtubules, resulting in the formation of a gentle helix (middle). After writhing, the haptonema finally forms a coil (right).

Supplementary Figures

Supplementary Fig. S1. Characterization of morphology and haptonematal coiling in *Chrysochromulina* sp. NIES-4122.

(A) Negative staining image showing extracellular scales without spines. Bar, 100 nm. (B) A differential interference contrast image of a whole haptophyte. Bar, 10 μm . (C)

1 Thin section electron microscopy image of the cell body. Bar, 1 μm . (**D**)
2 Ca^{2+} -dependent haptonematal coiling. Haptophytes were suspended in artificial sea
3 water (ASW), Ca^{2+} -free sea water (CFSW), CFSW with 10 mM EGTA, or CFSW with
4 10 mM EGTA plus 50 μM BAPTA-AM. Error bars represent the standard deviation.
5 $N=5$. * $P<0.01$ (Student's test).

6

7 **Supplementary Fig. S2. Changes in the center-to-center distance between adjacent**
8 **haptonematal microtubules.**

9 The center-to-center distances between adjacent microtubules were measured from six
10 sequential thin-section electron microscopy images. (**A, B**) Sequential thin-section
11 images of an extended (A) and coiled (B) haptonemata. The section numbers and the
12 letters on the microtubules correspond to those in C and D. Bars, 100 nm. (**C, D**)
13 Center-to-center distances between adjacent microtubules were plotted against the
14 sequential section number, showing changes in the distance along the longitudinal axis
15 of the haptonema. The extended haptonema (**C**) shows relatively constant distances
16 among sequential sections but the coiled haptonema sometimes shows deviation for one
17 of the inter-microtubule distances, which changes with the section number (**D**).

18

19 **Supplementary Fig. S3. Measurement of persistence length.**

20 (**A**) Plots along a negative stain image of a haptonema using ImageJ. The inset shows a
21 magnified image of a part of the coiled region. (**B, C**) Distribution of persistence
22 lengths. Green and red bars represent counts of the persistence length of the coiled and
23 extended regions, respectively. The histogram in (B) is plotted with a bin width of 10
24 μm . (C) shows the region shorter than 10 μm , with the bin width of 1 μm .

25

1 **Supplementary Fig. S4. Definition of curvatures.**

2 (A) The curvature of a certain region of a haptonema was defined as the reciprocal of
3 the radius of the inscribed circle (dashed blue or orange line). (B) Plot of curvature
4 against distance from the base of a haptonema. The curvature values are either positive
5 or negative, depending on the bending direction. The absolute value of curvature is used
6 in Fig. 5F and Fig.S5 to show the extent of bending.

7
8 **Supplementary Fig. S5. Comparison of the maximum curvature of bends induced
9 by microtubule-modifying drugs.**

10 Based on the analysis in Fig. 5, the average of absolute curvature values was calculated.
11 Bars represent the standard deviation. N=20.

12

13 **Supplementary Fig. S6. Bend propagation of taxol-treated haptonemata.**

14 The curvature along the haptonema was measured at 5, 30 and 60 min after treatment
15 with taxol. (A) In the presence of Ca^{2+} (ASW); (B) In the absence of Ca^{2+}
16 (CFSW+EGTA+BAPTA-AM). Note that the peak of the maximum curvature (arrows)
17 propagates toward the base of the haptonema with time in the presence of Ca^{2+} and that
18 the gentle helix propagates toward the tip of the haptonema with time in the absence of
19 Ca^{2+} .

20

21 **Supplementary Fig. S7. Basic model for the microtubule coiling mechanism.**

22 Diagrams illustrating the arrangement of tubulin dimers in a microtubule (A, D, G), the
23 state of a microtubule (B, E, H), and the state of a bundle of seven microtubules as in a
24 haptonema (C, F, I). The Ca^{2+} -bound MAPs that are responsible for coiling are
25 associated with either a set of protofilaments (D-F) or to all protofilaments (G-I). When

1 Ca^{2+} is not bound to the MAPs, the microtubules are straight in both cases (A-C). In **D**,
 2 Ca^{2+} -binding to the MAPs changes the structure of the protofilament to which the MAPs
 3 are attached (pink protofilament). If we assume a mechanism similar to that of bacterial
 4 flagella, a slight decrease in the subunit spacing and changes in the interaction between
 5 neighboring protofilaments result in bending and twisting of the microtubule (D), which
 6 lead to coiling of the microtubule (E) and thus coiling of the haptonema (F). In **G**, Ca^{2+}
 7 binding to the MAPs alters the structures of all of the protofilaments and
 8 inter-protofilament interactions. This would twist the protofilaments, imposing a
 9 torsional force on the microtubule, and make the coiled conformation more stable.

10

11 **Movie 1. Real-time movie of haptonematal coiling and uncoiling.**

12 The coiling and uncoiling were induced by tapping the microscopic stage. The movie
 13 was recorded at 100 fps and plays at real time. The process of coiling is too rapid to be
 14 seen but that of uncoiling proceeds more slowly.

15

16 **Movie 2. High-speed movie of haptonematal coiling.**

17 The movie was recorded at 1000 fps and plays at a speed of 0.03×.

18

19 **Movie 3. High-speed movie of haptonematal uncoiling.**

20 The movie was recorded at 200 fps and plays at a speed of 0.15×.

Figure 1

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A

B

Figure 2

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Figure 3

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Figure 4

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Figure 5

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Figure 6

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Figure 7

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Figure 8

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A

Extended

+Ca²⁺
→
twist

→
writhing

Coiled

B

Extended

+Ca²⁺
→
twist

→
writhing

Coiled