

Formation of the Apical Flaps in Nematocysts of Sea Anemones (Cnidaria: Actiniaria)

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Abstract. Using scanning and transmission electron microscopy, we studied formation of the structure at the apical end of sea anemone nematocysts through which the tubule everts at discharge. In anemones of the genus *Metridium*, we found that each of the three solid triangular apical flaps comprises two layers that are continuous with those of the capsule wall: the electron-lucent inner layer is bound to the electron-dense outer layer. The two-layer structure is obvious in some discharged capsules in which, perhaps due to fixation, the layers part at the flap's periphery. Before the nematocyst discharges, a channel leads from a pore at the tip of the joined flaps into the lumen of the inverted tubule. The thin laminate layer that coats each flap lines the channel. The base of the nematocyst tubule adheres to the capsule wall near the capsule's apical end, and a branch of the tubule underlies part of the laminate layer that coats the flaps. Thus the tubule is not continuous with the capsule wall but structurally separate from it. This helps reconcile differences in understanding of the number of layers constituting the capsule wall, and makes clear that the tubule should be considered part of the capsule contents.

Introduction

Nematocysts, one of three types of cnida, are intracellular capsules produced only by members of the phylum Cnidaria (*e.g.*, jellyfish, sea anemones, corals). Nematocysts are used in capture of prey, defense against predators, and inter- and intra-specific aggression (*e.g.*, Mariscal, 1974a, 1984). Be-

fore discharge, the apical end of a mature nematocyst is covered by either three flaps or a single operculum (summarized by Mariscal, 1984; Watson and Mariscal, 1985). Discharge is effected when the flaps reflex outward (Westfall and Hand, 1962; Westfall, 1965; Salleo *et al.*, 1991) or the operculum opens, allowing the hose-like tubule, which is somewhat to very much longer than the capsule that had contained it, to evert (turn inside-out).

The operculum, a hinged closure that opens much like a cap on a tube of toothpaste and that has been recorded from non-anthozoans, is circular to trilobed in form (Östman, 1982). Its diameter, based on published figures, varies from about 0.8 μm (Rifkin and Endean, 1983: fig. 15) to 1.8 μm (Rifkin and Endean, 1983: fig. 2; Yanagihara *et al.*, 2002: figs. 3F and 6D). Flaps are characteristic of anthozoans; published figures for base-to-tip length of each of the three triangular flaps are 0.5 μm (basitrichous isorhiza in Mariscal, 1974a: fig. 11), 0.6 μm (basitrichous isorhiza in Salleo *et al.*, 1991: fig. 3B), and 1.2 μm (microbasic mastigophore in Godknecht and Tardent, 1988: fig. 3C).

The main objective of this research was to understand the formation of apical flaps, including how flaps relate developmentally and morphologically to the rest of the nematocyst. This led us to consider how the tubule connects to the capsule.

Studies of various types of nematocysts, both operculate and non-operculate, have led many to consider the capsule double-walled (Allman, 1871; Schneider, 1900; Westfall, 1965; Blake *et al.*, 1988; Watson and Wood, 1988; Özbek *et al.*, 2004). Watson and Wood (1988) recommended that the two wall layers Schneider (1900) had termed the sclera and the propria be referred to simply as outer and inner layers, respectively. In addition to what he termed the sklera and propria in nematocysts of two hydrozoans, Holstein (1981)

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found that an electron-dense innermost third layer, which he termed the interna, forms when the external tubule (the state designated “preinverted” by Watson, 1988) invaginates. In their study of microbasic mastigophores of the sea anemone *Anemonia sulcata* (Pennant, 1777), Godknecht and Tardent (1988: p. 85) asserted “The capsule wall is composed of four layers, the interna and the inner, middle and outer layer.” We infer from our results that the capsule wall and the apical flaps of a mastigophore of *Metridium* each consists of two layers and that each layer is continuous between capsule and flaps. In addition, the flap surface that is exposed upon nematocyst discharge is covered by a thin laminate layer.

Weill (1934) considered the tubule to be an extension of the capsule wall, but did not determine with which wall layer the tubule is continuous. Hyman (1940: p. 382) summarized early observations, noting that the tubule is “fastened to the narrower end and is therefore continuous with the capsule wall.” Many, including Godknecht and Tardent (1988), Engel *et al.* (2001), and Özbek *et al.* (2004), did not discuss the connection between the two parts; others, such as Manuel (1988) and Szczepanek *et al.* (2002), explicitly accepted the continuity between the tubule and capsule wall asserted by Weill (1934), and Chapman and Tilney (1959b) even termed the base of a discharged stenotele the “evaginated capsular wall.” Westfall and Hand (1962) and Westfall (1965) demonstrated that the tubule is continuous with the inner layer of the capsule wall. In a review of nematocyst terminology, Watson and Wood (1988: p. 22) left ambiguous the relationship between the tubule and capsule wall, commenting that the tubule is “continuous with, or attached to, the apex of the capsule.” Using a variety of fixatives, we found that the basal part of the tubule adheres to the apical end of the capsule just proximal to the point where the capsule and flaps meet. We infer that this skirt-like portion of the tubule was previously interpreted as the layer of the capsule wall referred to as the “interna.” Being separate from the capsule wall, the tubule should be considered part of the capsule contents.

Materials and Methods

In early transmission electron microscopy (TEM) studies of nematocysts from sea anemone acontia and tentacles, Westfall (1965, for example) used the name *Metridium senile* (Linnaeus, 1761) for these animals. Subsequently, it was recognized that the similar-looking anemone *M. farcimen* (Tilesius, 1809) occurs in the same area (Bucklin, 1987; Fautin *et al.*, 1990; Fautin and Hand, 2000). We cannot be certain which species of *Metridium* was used for the TEM in our research, but the taxonomic uncertainty affects neither our results nor our conclusions. Likewise, although we could identify the type of nematocyst in many micrographs, we were able to identify those in some TEM

figures only as mastigophores (based partly on the fact they were from acontia and normal tentacles). We are confident that this uncertainty does not affect our study of the apical end of the capsule: Westfall (1965) found microbasic amastigophores and microbasic *b*-mastigophores of *Metridium* fundamentally alike, differing only in spination of the tubule.

For TEM, small bits of tissue were fixed in 2% OsO₄ and 1% K₂Cr₂O₇ in 78% seawater at pH 7.2 (Westfall, 1965), in 10% acrolein plus 2% OsO₄, or in 5% glutaraldehyde followed by 2% OsO₄ in cacodylate buffer. All specimens were dehydrated in ethanol and flat-embedded in Epon. Thin sections cut on a Porter-Blum ultramicrotome using a diamond knife were doubly stained in uranyl acetate and lead citrate. Micrographs were taken on an RCA EMU 3G electron microscope at the University of California, Berkeley.

For scanning electron microscopy (SEM), live specimens of *M. senile*, collected from Lynn Harbor, Massachusetts, were anesthetized with menthol. Tentacles and acontia were cut from each individual. Samples were placed in a 1 mol l⁻¹ sodium citrate solution for 15 min to induce the nematocytes to expel the nematocysts. After three washes with distilled water, samples were placed in 1% OsO₄ solution overnight. All specimens were dehydrated in ethanol, then critical-point-dried with CO₂. Each sample was sputter-coated with gold palladium in a Hummer sputter coater and was examined using a LEO 1550 field emission scanning electron microscope at the University of Kansas, Lawrence.

Length of apical flaps was measured on micrographs from the tip of an opened flap to midway along its joint with the capsule.

Results

Nematocyst maturation

The nematocyst develops in a nematocyte with a homogeneous nucleus. A TEM image of a mastigophore nematocyst at an early stage of formation reveals an immature tubule in a moderately electron-dense matrix enclosed by a thin double-walled capsule (Fig. 1). The nematocyst has a closed apex; an apical cap of electron-dense material is continuous with the thin electron-dense outer wall of the capsule that underlies the nematocyst membrane. As the nematocyst matures, the tubule becomes complexly folded and the lucent layer of the capsule wall thickens, although the dense layer remains thin (Fig. 2). A ring of circular fibers in the cytoplasm forms a collar below the apex of the nematocyst.

During maturation, electron-lucent material continuous with that of the capsule infiltrates the electron-dense material at the apex to create three flaps (Fig. 3). The electron-dense material lies outside the thicker electron-lucent material (Figs. 4, 5, 6). The flaps are not differentiated in an

immature nematocyst in which the tubule has invaginated (Fig. 1) but is still thin and straight, and lies within a dense matrix. Images in which the tubule is mature, being folded complexly and lying in clear surroundings (*e.g.*, Fig. 2), show fully formed flaps.

The nematocyst membrane surrounds the entire capsule until it breaks upon discharge of the tubule (Fig. 5).

Flaps

Each flap is solid and is coated by a thin layer that appears laminate in some figures: near the base of the parting flaps in Figure 4, on the surface of reflexed flaps in Figure 5 (arrows), lining the longitudinal channel in Figure 7 (arrows). Because this layer covers the surface of each flap, it is visible where the flaps meet as a tripartite seam (Figs. 3, 6).

The flaps of a mature nematocyst do not occlude all the apical space: an electron-dense channel runs lengthwise through the center of the region where they meet. The section of Figure 7 is in a plane that shows the entire channel; this nematocyst is immature. The section in Figure 2 includes a bit of the channel; this nematocyst is mature. A pore, visible where the tips of the flaps converge (Fig. 6), is the external opening of the channel. The other end of the channel opens into the space of the (inverted) tubule (Fig. 7). The channel is lined by the thin laminate layer coating the flaps. An amorphous dense material, prominent in Figure 5 beneath the laminate layer of the reflexed flaps, is also visible in cross section in Figure 6 along the edges of the seams.

When the apex opens at discharge, the flaps separate where they abut one another, each reflexing against the apical capsule wall (Fig. 5). In the reflexed state, the base-to-tip length of flaps of tentacle amastigophores was 0.65–0.71 μm ($n = 5$) and that of acontia amastigophores was 1.09–1.26 μm ($n = 6$). The lucent portion lies between the dense portion that had constituted the external surface of the flap and the amorphous dense material underlying the laminate surface (Fig. 5). A ridge is visible on the surface of some or all reflexed flaps (*e.g.*, Fig. 8, arrows). In most nematocysts, the electron-dense and electron-lucent portions of the flap remain cohesive after discharge of the tubule (Figs. 5, 8), but in some, the flap splits at its periphery (Figs. 9, 10).

Tubule attachment

The thin internal tubule is anchored apically to the capsule in both immature (Fig. 1) and mature (Fig. 2) capsules. The material of the tubule is electron-dense in our Figures 2 and 5. In Figure 2, this material can clearly be traced along the base of the flaps onto the inner capsule wall. In Figures 1 and 4, in which the tubule is intermediate in electron density, it can also be traced onto the capsule wall. In three

dimensions, this basal portion of the tubule is thus a skirt applied against the inner wall of the capsule at its apical end.

A branch of the tubule wall extends apically to form another short skirt underlying the basal portion of the thin laminate layer covering the flaps; it thus anchors the tubule to the base of the flaps and serves as a hinge when the flaps reflex. Upon discharge, when the flaps part at their seams and reflex against the capsule, this tubule material can be seen extending to the basal portion of each flap (Fig. 5; the everted tubule is not in the plane of the section).

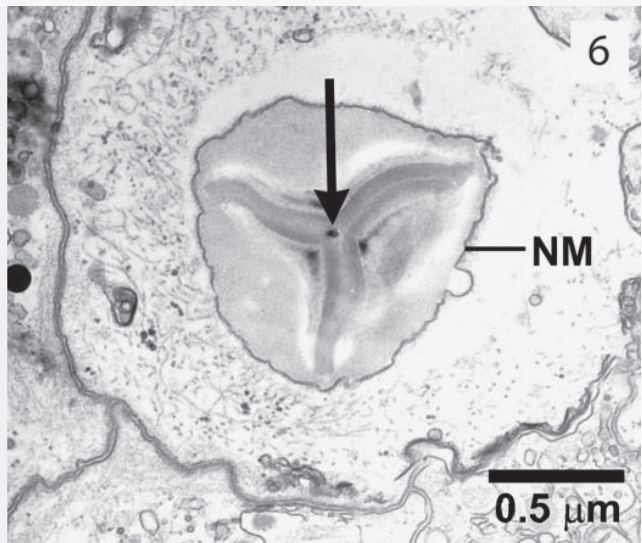
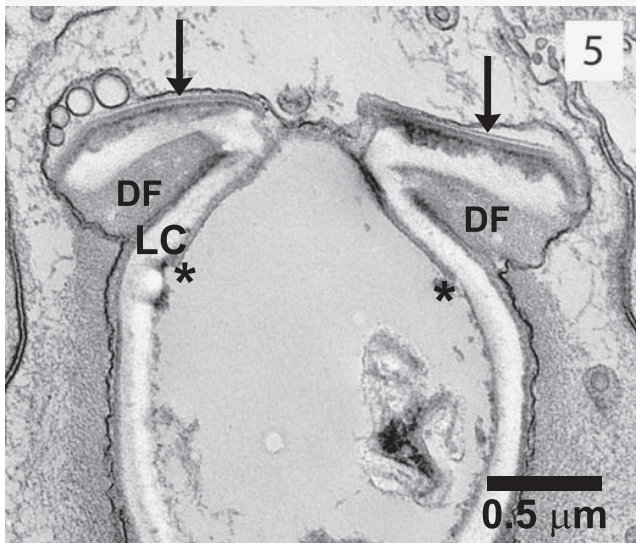
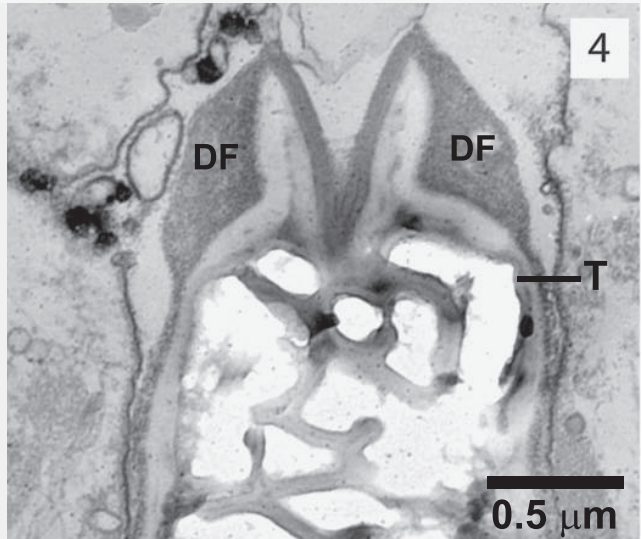
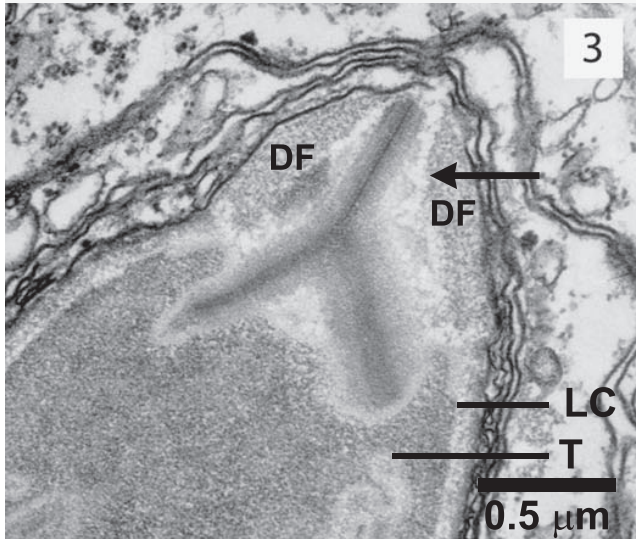
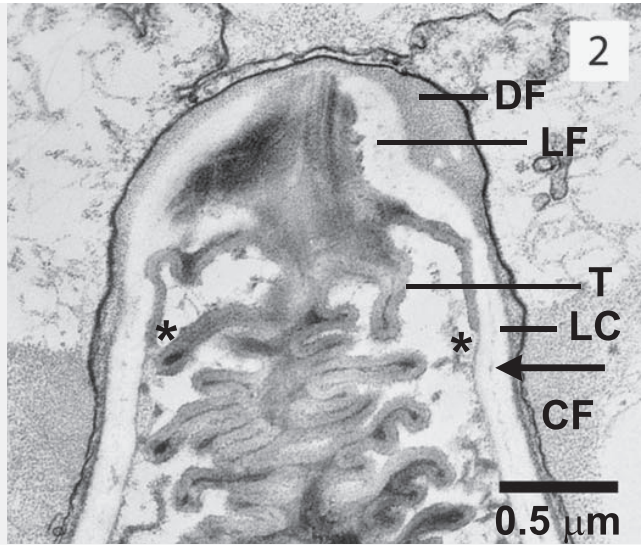
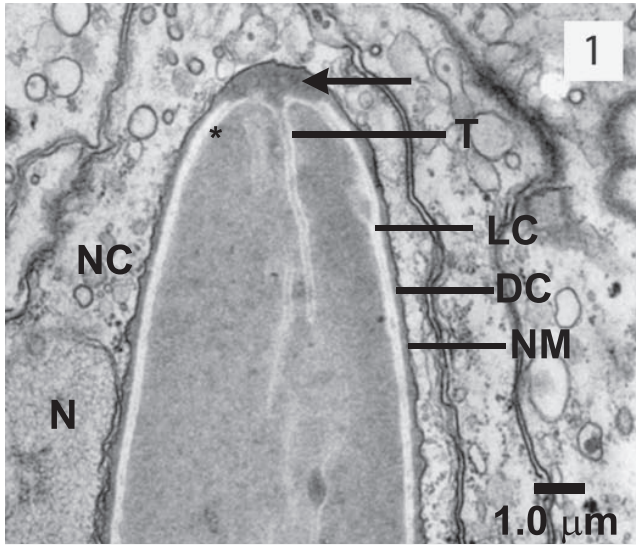
Discussion

Our understanding of the juxtaposition of capsule, tubule, and flaps at the apex of a nematocyst closed by flaps is summarized in Figures 11 and 12. Our findings support the conclusions of Westfall and Hand (1962) that apical flaps are continuous with the capsule wall. However, the tubule is not continuous with the capsule wall; its basal end is anchored in two places at the apical end of the capsule, and it should be considered part of the capsule contents. Our understanding of the structure of the flaps and capsule differs in two respects from that shown in the schematic of Godknecht and Tardent (1988; fig. 4): we regard the interna not as a component of the capsule wall but as the base of the tubule, and we find that a skirt of tubule material underlies the inner part of the thin laminate layer that covers the surface of the reflexed flaps.

Apical flap formation and composition

The flaps must form after inversion of the preinverted tubule, but we cannot determine if the tubule is completely assembled prior to differentiation of the flaps; in our images of a capsule lacking flaps, the tubule is thin and straight, whereas in those of a capsule with fully formed flaps, the tubule is mature. In the mastigophores we studied, the outer component of the flaps differentiates from a mat of electron-dense material that surrounds the capsule between it and the nematocyst membrane, as noted also by Watson and Mariscal (1985); this outer layer is bonded to an inner electron-lucent layer, and both layers are continuous with the corresponding layer of the capsule wall.

Watson and Mariscal (1985) extensively analyzed the radial laminae bordering the seams where three flaps abut one another, and termed the point where the three flaps meet (Fig. 6) a tube. That point is a pore—the two-dimensional terminus of a three-dimensional channel (Fig. 7), seen also in figures 3 and 7 of Blake *et al.* (1988), and traced by Watson and Mariscal (1985) in serial sections. The channel may deliver material into the nematocyst, perhaps effecting discharge (see below). The channel is lined by laminae like those of the seams, as seen also in figure 3 of Blake *et al.* (1988). Watson and Mariscal (1985) noted the similarity between the electron-lucent lining of the channel and the



inner wall of the everted nematocyst tubule, but the two cannot be the same because the inner wall of the everted tubule had been the outer layer of the coiled and folded tubule lying in the capsule. We conclude that the entire surface of each reflexed flap that is exposed upon nematocyst discharge is covered by a thin laminate layer (*e.g.*, Fig. 5, arrows; Watson and Mariscal, 1985: fig. 7) that in an undischarged nematocyst is visible at the edges of the flaps where it borders the seams, and along the channel.

The granular material noted by Watson and Mariscal (1985) where the edges of two flaps meet (not visible in Fig. 6, perhaps because it is so distal) is presumably the “glue” that holds the flaps closed prior to discharge. To understand nematocysts better, the composition of this material should be studied with techniques such as those used to localize minicollagens (Adamczyk *et al.*, 2008; David *et al.*, 2008). (Understanding the nature of the adhesive holding the two layers of the capsule together and that holding tubule to capsule might also benefit from such an approach.)

Base-to-tip length of apical flaps of amastigophores is greater in capsules from acontia than in those from tentacles, probably because in *Metridium*, mastigophores of acontia are larger than those of tentacles (Hand, 1956). Published sizes of apical flaps (given in the Introduction) correspond well with what we found. The ridge from its tip to the base of many flaps visible after the flaps reflex back (Fig. 8) are obvious also in Godknecht and Tardent (1988: fig. 3C), who did not remark on them. We infer that the lack of a ridge on some flaps is only apparent, perhaps because of the shallow plane of focus at such high magnification. The ridge is a consequence of the flap being triangular in cross section, thickest in the central portion and tapering at the edges, as is obvious in, for example, Figure 6. If the flaps were of equal thickness throughout, the space among

them in an undischarged nematocyst would be a cone, not a channel.

The two layers of a reflexed flap that has split at its periphery (*e.g.*, Figs. 9, 10) are of similar thickness. We therefore conclude that this separation, which is presumably a fixation artifact, is between the outer layer (*i.e.*, the dense layer in TEM) and the inner layer (*i.e.*, the lucent layer in TEM), not between the latter and its thin laminate coating. The outer layer folds against the capsule (Fig. 10), from which we infer that it is a more flexible layer than the inner one.

Are apical flaps and opercula homologous?

The fundamental differences between flaps and opercula, which serve the same purpose, have been obscured in part because of terminology: Godknecht and Tardent (1988) and Koch *et al.* (1998) referred to the flaps as “opercular flaps”; Kass-Simon and Scappaticci (2002) called the series of flaps an operculum. The term operculum should be applied only to the discrete cap of a nematocyst; opercula have thus far been found only in non-anthozoan cnidarians.

Westfall (1966a) supported the idea that the operculum develops from the capsule wall, as we have established for flaps. However, Koch *et al.* (1998) found the major component of the operculum to be spinalin, the material that forms nematocyst spines and is imported from the capsule matrix through the inverted tubule wall. Simultaneous development of operculum and spines led Holstein (1981: p. 288) to conclude that the two structures are “physically connected”: we now understand that the connection is, instead, temporal, as neither structure can develop until spinalin is present. The alternating electron-lucent and electron-dense lamellae in TEM cross sections of opercula

Figures 1–6. Transmission electron micrographs of apical end of nematocysts from the sea anemone *Metridium*. CF: circular fibers; DC: dense layer of capsule wall; DF: dense layer of flaps; LC: lucent layer of capsule wall; LF: lucent layer of flaps; N: nucleus; NC: nematocyte; NM: nematocyst membrane; T: tubule; * indicates end of tubule attachment to capsule wall. **Fig. 1.** Longitudinal section of immature nematocyst from base of tentacle (fixed in OsO₄ and dichromate); matrix of fibrous electron-dense material at apex of capsule (arrow) is continuous with dense layer of capsule wall beneath nematocyst membrane. **Fig. 2.** Longitudinal section of mature microbasic *b*-mastigophore from acontium (fixed in OsO₄ and dichromate) with highly folded internal tubule attached apically to inner capsule wall. Electron-dense matrix forms outer layer of flaps. Intermittent dense line (arrow) appears to separate inner and outer portions of electron-lucent part of capsule wall. Cytoplasmic ring of circular fibers forms a collar below apex. **Fig. 3.** Oblique section through developing tripartite apical flaps of maturing nematocyst from tentacle (fixed in glutaraldehyde and OsO₄) reveals intrusion of electron-lucent capsular material (arrow) into dense material of flaps. **Fig. 4.** Separated apical flaps of capsule from acontium (fixed in acrolein and OsO₄); note flat surface of flaps where they have parted, and anchorage of the tubule, which extends along inner wall of capsule to the level of the line leading from “T.” **Fig. 5.** Discharged nematocyst from acontium (fixed in acrolein and OsO₄) shows two of three flaps, each with a flat laminate surface (arrows) beneath ruptured nematocyst membrane. Layer of electron-dense material lies between laminate surface and lucent layer of flaps. Tubule extending from its attachment at * distally to the underside of the laminate surface is particularly visible on right side. **Fig. 6.** Surface section through apex of nematocyst from acontium (fixed in OsO₄ and dichromate) showing central pore (arrow) where the three flaps meet. Radial seams separate the flaps.

(Slautterback, 1961; Westfall, 1966b; Holstein, 1981) are presumably formed by condensed spalinin. Watson and Mariscal (1985) extensively discussed the laminate borders of the radial seams, which we conclude are the edges of the material covering the entire surface of each flap. The laminate portions should be investigated biochemically as a possible homology between flaps and opercula.

Nematocysts closed by opercula differ from those closed by flaps in other ways, too. An operculate nematocyst is triggered to discharge by a cnidocil (Schulze, 1873; Weill, 1934; Chapman and Tilney, 1959a), and a nematocyst with flaps by a flagellum called a kinocilium (Weill, 1934; Pantin, 1942; Westfall, 1965, 1966a, b; Mariscal, 1974a, b; Peteya, 1975; Westfall *et al.*, 1998; it was referred to as a short cnidocil by Hwang *et al.*, 2008). Even in the process of discharge, the two differ. Holstein and Tardent (1984) observed that eversion of the tubule always begins immediately after the operculum opens, but this is not so for flaps: the delay between flap opening and initiation of tubule eversion can be as much as 60 s (Godknecht and Tardent, 1988)—or the tubule may not evert at all (Salleo *et al.*, 1991), as seems to be the case in our Figure 4. Another possible difference involves discharge. If influx of compounds is critical to this process (discussed by Watson and Mariscal, 1985), and if those compounds enter the nematocyst through the channel that leads from the pore where the flaps converge, nematocysts with an operculum must allow the compounds access to the capsule interior in some other way.

Capsule wall composition and tubule attachment

Blake *et al.* (1988: p. 219) summarized many studies in stating “Nematocyst capsular walls are generally characterized as bilayered, being composed of a thin, outer, electron-dense layer overlaying a thicker, electron-lucent region,” an

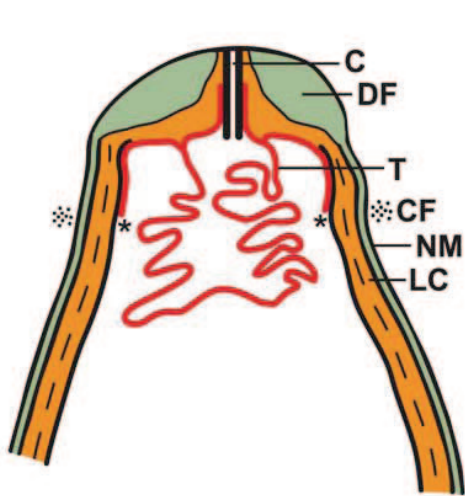
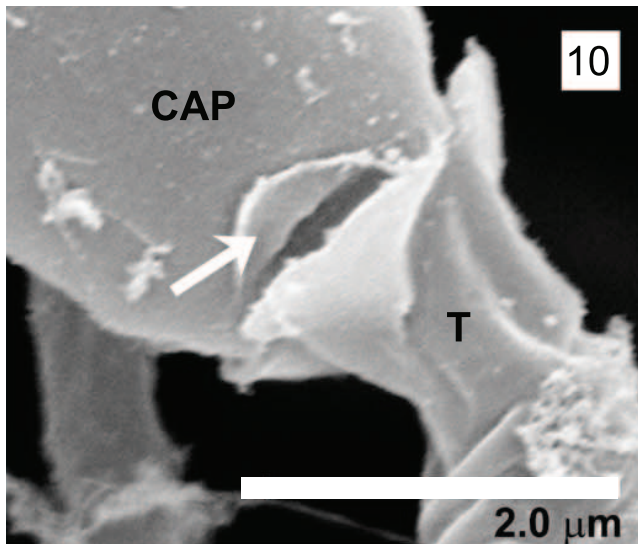
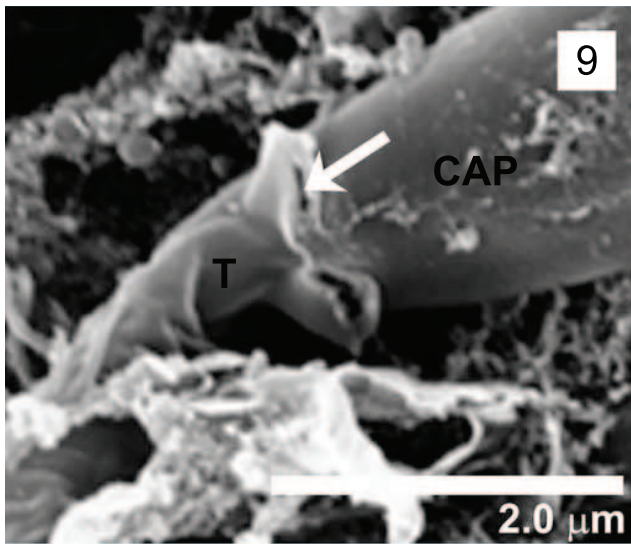
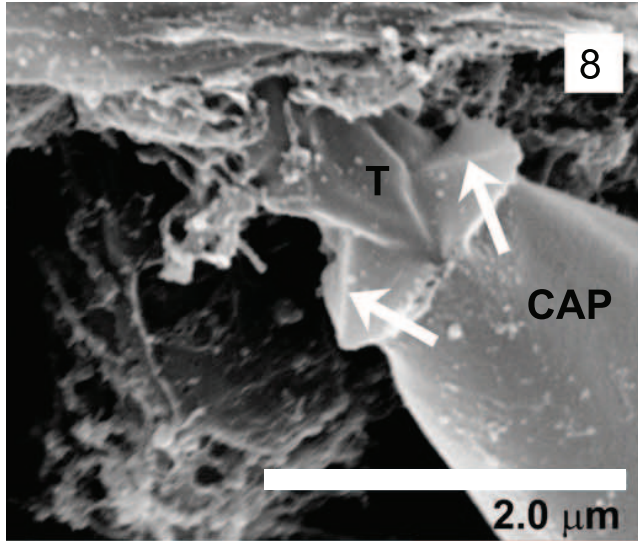
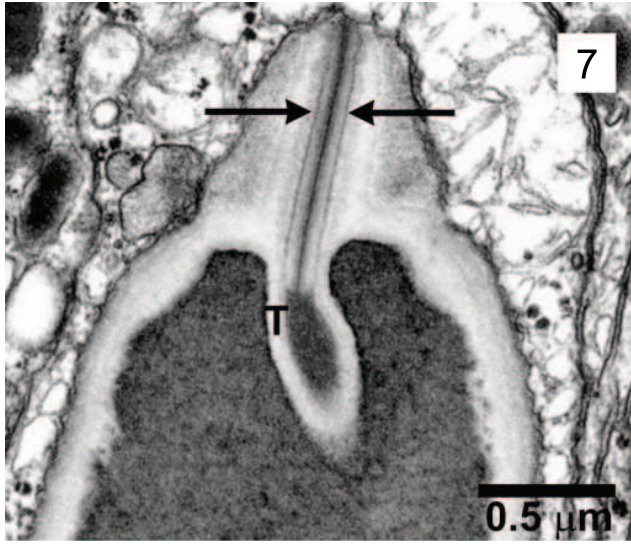
appearance illustrated in our Figures 1, 3, 5, and 7. However, Holstein (1981) considered the wall to have three layers, and Godknecht and Tardent (1988) four.

The inner electron-lucent layer of the capsule appears double in some micrographs (*e.g.*, our Fig. 2; Westfall and Hand, 1962: fig. 3; Godknecht and Tardent, 1988: fig. 2E; Watson, 1988: fig. 13). After examining many micrographs, we conclude there is a single electron-lucent layer, the appearance of two being an artifact. Fixation can affect capsule wall appearance; because of impermeability of the wall, mature nematocysts are difficult to fix for electron microscopy (Westfall, 1965; Watson and Mariscal, 1985), especially using conventional fixatives such as glutaraldehyde and OsO₄. Most images in which the wall appears to have a single electron-lucent layer are from material fixed conventionally. Our images of an apparent double layer are from material fixed with OsO₄ and dichromate or acrolein and OsO₄, the latter also used by Godknecht and Tardent (1988). (Acrolein and OsO₄ may also be responsible for the partial opening of the flaps without tubule eversion: compare our Figure 2 with an acrolein-fixed nematocyst in figure 3B of Godknecht and Tardent, 1988.) Further, thickness of the capsule wall changes with maturity (*e.g.*, Watson and Mariscal, 1984; Watson, 1988).

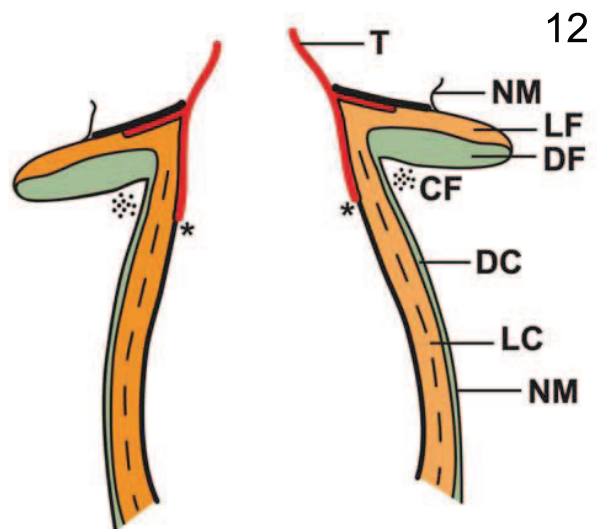
Such an artifact may have been responsible for some of the uncertainty about the number of wall layers. It is also possible that wall structure may differ with nematocyst type, just as the wall of a spirocyst differs from that of a nematocyst (*e.g.*, Mariscal *et al.*, 1976). We consider it unlikely that nematocysts vary greatly in this regard, but knowing that other facets of nematocysts differ, including tubule length and spination, we emphasize that our findings are from mastigophores of a sea anemone.

Holstein (1981: p. 285) considered the capsule wall to consist of three layers: “the outer sclera, the middle propria,

Figure 7–12. **Fig. 7.** Transmission electron micrograph of longitudinal section of immature nematocyst from tentacle (fixed in OsO₄ and dichromate); note channel extending from pore at capsule apex into invaginated tubule (T). Arrows indicate laminate layer that forms wall of channel. **Figures 8–10.** Scanning electron micrographs of apical end of discharged microbasic amastigophores from tentacles of the sea anemone *Metridium*. CAP: nematocyst capsule; T: tubule. **Fig. 8.** Reflexed flaps attached at junction of apex of capsule and base of everted tubule. Note ridges (arrows) extending from pointed tip to broad base of each triangular flap. **Figs. 9, 10.** Discharged nematocysts with split (arrows) between the two layers of a flap. **Figures 11–12.** Diagram of apical end of nematocysts in undischarged (Fig. 11) and discharged (Fig. 12) states. C: channel extending from pore at capsule apex into invaginated tubule; CF: circular fibers; DC: dense layer of capsule wall; DF: dense layer of flaps; LC: lucent layer of capsule wall (which appears double in some preparations—hence dashed line in the middle); LF: lucent layer of flaps; NM: nematocyst membrane; T: tubule; * indicates end of tubule attachment to capsule wall. **Fig. 11.** Undischarged capsule, surrounded by nematocyst membrane, is characterized apically by three flaps formed of two layers, an outer dense layer and an inner layer that forms from ingrowth of lucent layer of the capsule wall. Channel is lined by laminar material; tubule is attached both to capsule wall and beneath the channel. **Fig. 12.** At discharge, apical flaps reflex back, breaking nematocyst membrane, allowing invaginated tubule to evert. Laminar material that had lined the channel is visible on surface of reflexed flaps. The dense layer of flaps, which had been outermost, lies beneath the lucent part and is continuous with dense layer of capsule wall beneath nematocyst membrane. Below reflexed flaps is a circular band of thin fibers that may hold the capsule in place when nematocyst discharges.



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12

and the inner interna.” As we understand his interpretation of his figures 20–22, he considered the interna to be at once the inner layer of the capsule and the outmost layer of the tubule. He thereby posited continuity between the capsule wall and tubule, as have many others (*e.g.*, Weill, 1934; Hyman, 1940; Chapman and Tilney, 1959b; Westfall and Hand, 1962; Westfall, 1965), and as did Mariscal *et al.* (1976) for spirocysts.

However, capsule and tubule have very different functions, so it stands to reason that the materials that compose them differ correspondingly. The capsule is rigid, as a result of disulfide bonds between molecules of minicollagen (Watson and Mariscal, 1984; Engel *et al.*, 2001; David *et al.*, 2008). The tubule, by contrast, is flexible (Watson and Mariscal, 1984; Watson and Mire-Thibodeaux, 1994) so it can be coiled and twisted (Skaer and Picken, 1965) to invert and be packed within the capsule, then evert by uncoiling, untwisting, and expanding upon discharge. Disulfide reducing agents dissolve the capsule but not the tubule (Blanquet and Lenhoff, 1966; Mariscal and Lenhoff, 1969; Mariscal, 1971). Phelan and Blanquet (1985), who found the amino acid composition of capsule and tubule to be extremely similar, took advantage of their minor differences to separate their proteins. The finding of Adamczyk *et al.* (2008) that some minicollagens associate only with the tubule and others only with the capsule wall is further evidence that the tubule cannot be a continuation of the capsule wall.

The tubule begins to be assembled external to the capsule, but before it has become very long, it is invaginated into the capsule, where its assembly is completed (Jickeli, 1883; Nussbaum, 1887; Murbach, 1894; Iwanzoff, 1896; Slautterback and Fawcett, 1959; Slautterback, 1961; Carré, 1972; Skaer, 1973; Holstein, 1981; Watson, 1988). Before the pre-inverted tubule inverts, a clear transition at its junction with the capsule has been observed in both operculate (Skaer, 1973; Holstein, 1981) and non-operculate (Westfall, 1966a) nematocysts. Weill (1934), Hyman (1940), Chapman and Tilney (1959b), and Watson and Wood (1988) all found the connection between the inverted tubule and the capsule wall to be unclear.

Holstein (1981) reported that the interna appears after the external tubule invaginates, but his images do not show its extent. Although the precise details of how the tubule is anchored to the capsule wall may differ between operculate and non-operculate nematocysts, we infer that what Holstein (1981) termed the interna is the electron-dense, thick layer we saw along the inside of the capsule only at the apical end, where it lies against the layers that can be traced completely around the capsule wall (*e.g.*, Figs. 1, 2, 5). Such an apical layer has been observed in undischarged nematocysts by one of us (Westfall and Hand, 1962: fig. 3; Westfall, 1965: fig. 2) and by Blake *et al.* (1988: fig. 7), and in discharged nematocysts by Watson and Mariscal (1984: figs. 12, 15). Despite this evidence, the relationship between

capsule wall and tubule was still unsettled: figure 4 of Godknecht and Tardent (1988: 88) illustrates a mastigophore of *Anemonia sulcata*, the caption of which states that what we interpret as the anchorage of the tubule is “where electron dense interna of the evaginating structures goes over into the less electron dense interna covering inner face of capsule,” and that they considered to be a fourth layer of the capsule wall. In all published figures, this layer extends only to where it does in our figures; we see no evidence that it extends beyond the apical end or changes in electron opacity.

Nematocyst discharge is an extraordinarily rapid, forceful process (Holstein and Tardent, 1984). Were the tubule attached only along the apical end of the capsule, it would be in danger of being peeled from the capsule at discharge. We posit that the tubule material is anchored also under the thin laminate layer that is exposed on the surface of a reflexed flap (Figs. 11, 12). Evidence of such a branch is in micrographs of both undischarged capsules such as figure 18 in Westfall (1965) and discharged ones (Fig. 5, in which the anchorage of the tubule at the apical end of the capsule appears to extend into the base of the reflexed flap). This skirt, which presumably does not extend as far apically between the flaps as within them, may have other functions as well. It might be a hinge for the flaps. It might be important in tubule eversion by exerting radial tension on the basal end of the tubule, thereby facilitating passage of the rest of the tubule through it. The amorphous electron-dense band underlying the laminate surface of each flap (Figs. 2, 5; Westfall and Hand, 1962: fig. 3; Watson and Mariscal, 1985: fig. 6, dbd; Blake *et al.*, 1988: fig. 7) may help anchor the skirt of the tubule. This material does not appear in our conceptual diagrams of nematocysts (Figs. 11, 12).

Likewise, we infer that the circular fibers visible in Figure 2 hold the capsule in the nematocyte after nematocyst discharge. This interpretation is contrary to the conclusion of Westfall (1965) that the fibers function in discharge. They are not part of the “microtubule array in the cytoplasm surrounding the capsule tip” that Watson and Mariscal (1985: p. 207, 208) inferred holds the “capsule at the cell surface until discharge.” Rather, we interpret them as functionally analogous to the fibrous collar described by Westfall (1970) in the neck region of operculate nematocysts in the hydromedusan *Gonionemus vertens*.

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