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Remodelling during the development of nematocysts in a siphonophore

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Abstract

Remodelling of a smoothly tapering internal tubule to form the enlarged shaft of microbasic mastigophores of the siphonophore *Rosacea* takes place very late in development. It occurs after deployment of the nematocyst in its final position, and after the pleats of the internal tubule have been completely obliterated in the shaft region by the growth of spine material. The optical and mechanical properties of the internal tubule at the earlier (bedspring) stage give no hint that a shaft will develop. The possible ways in which remodelling might occur inside the capsule and remote from the cytoplasm are discussed.

Introduction

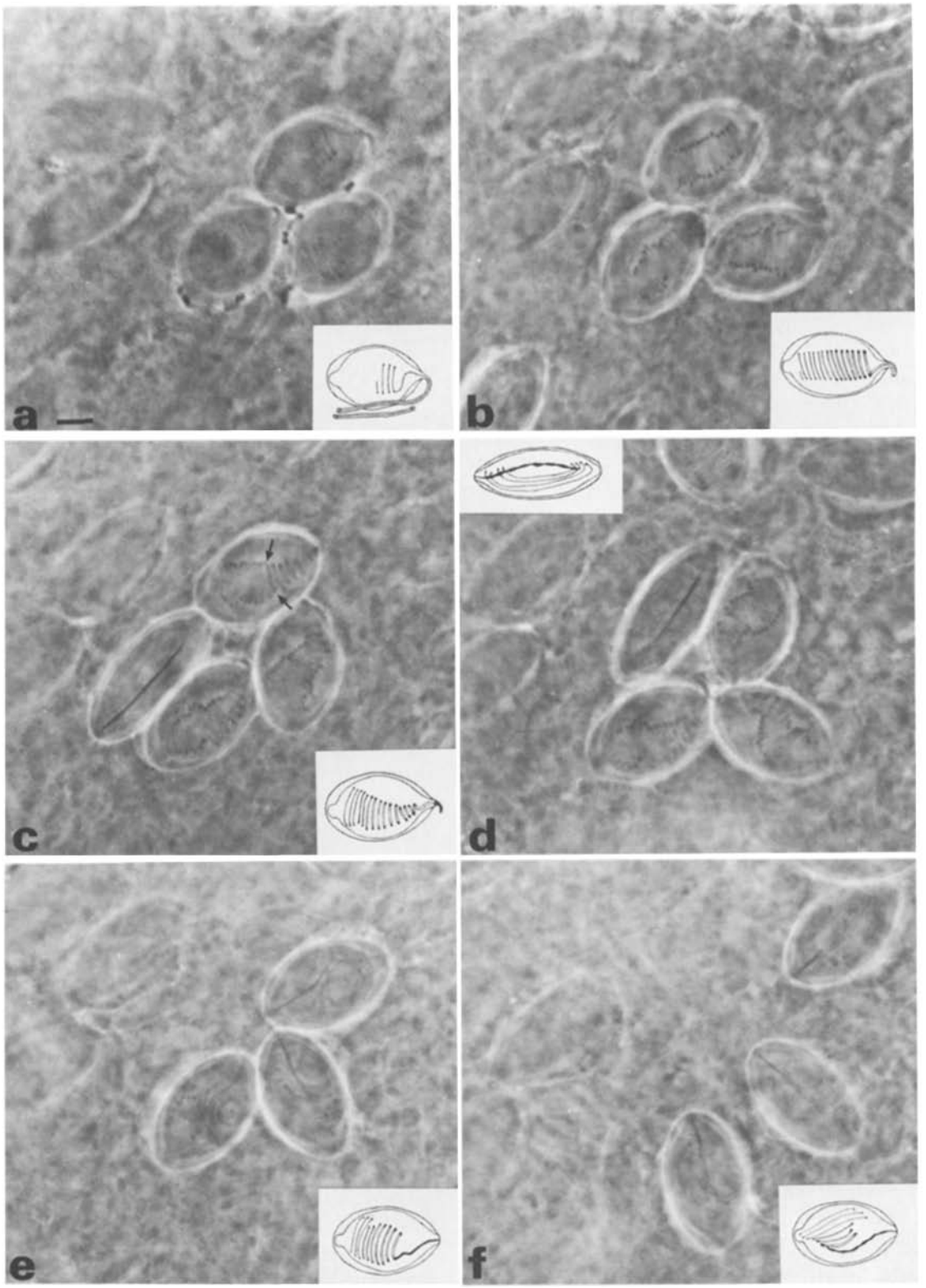
The shaft of the nematocyst tubule is secreted as an enlarged part of the external tubule in developing nematocysts such as the stenoteles of *Hydra* (Holstein, 1981), *Dipurena* (Günzl, 1973) or of *Physophora* (Skaer, 1973). In other cases, the external tubule shows no sign of the shaft that is present on the discharged tubules of, say, microbasic mastigophores (Skaer, 1973). The shaft must form by remodelling after the external tubule has withdrawn into the capsule. Both the remodelling and the growth of spines, therefore, occur remote from the cytoplasm itself. Since this is counterintuitive (for immediately after withdrawal into the capsule, there is only a tiny amount of barb material; Skaer, 1973), the situation was reexamined to see how remodelling and the growth of spines could occur. In this paper I present a time-lapse, developmental sequence which confirms that the developing capsule with an internal, bedspring shaped tubule (i.e., in isodiametric helical coils) is indeed the same capsule that later has a large shaft that extends almost the full length of the capsule, as reported in Skaer (1973). A time-lapse video (European VHS or Pneumatic tape) of this sequence is available on application to the author. The changes and the causes of remodelling are discussed in the light of evidence from these phase-contrast pictures and from electron microscopy.

Material and methods

The gastrozooids of living *Rosacea cymbiformis* (Chiaje) (Siphonophora) were examined as described by Skaer (1973). The sequence was photographed with phase-contrast microscopy at approximately 2.5 min intervals.

Results

The time-lapse sequence shown in this paper covers a period of approximately 2 h, and shows


a
b
c
d
e
f

a cluster of 3 large (microbasic mastigophore) nematocysts as they develop in the living animal. The nematocyst capsules elongate extensively and become slimmer as development proceeds. The external tubule, recognizable as completely black profiles in Fig. 1a, is uniformly tapered. At this stage it is not visibly differentiated into shaft and tubule. Even when the tubule is interiorized into the nematocyst capsule it initially coils, independent of the capsule wall (Fig. 1b), as a uniform bedspring that is isodiametric about a straight axis. Thus its mechanical properties indicate that there is no differentiation into 2 components (shaft and tubule) when it is first inside the capsule. Only later does the tubule differentiate inside the capsule into the basally enlarged shaft and the smaller-diameter internal tubule.

Although, in the bedspring stage (Fig. 1b), the coil oif the internal tubule is isodiametric, the coil diameter at the opercular end soon becomes smaller whereas that at the free end progressively enlarges so that the tubule appears to be wound around a cone (Fig. 1c).

During this particular sequence (Fig. 1c-d), a migrating cnidoblast collides with the cluster of 3, and displaces it. This migrating cnidoblast is of the same type as the cluster of 3 but is at a later developmental stage, with a clearly defined longitudinal shaft region. Later still, at the end of the sequence, when each of the 3 have also developed a visible shaft region, they separately migrate out of the field of view (Fig. 1e-f).

Discussion

This recorded sequence incontrovertibly links the earlier, bedspring stage without a shaft, with the migrating stage in which a shaft region is visible. Despite the visibility by phase-contrast of the shaft region in the migrating stage, electron microscopy at this stage reveals that a true shaft, defined as a region of the tubule with a significantly increased diameter, is not present (Skaer, 1973). The cause of the increase in refractive index, visibility, and straightening of the tubule in this region is the presence of 3 large masses of spine material in the future shaft region but not elsewhere. These masses, though large, do not at this stage extend into the 3 helical pleats of the tubule wall (Skaer, 1973; Fig. 13).

Later still, when the nematocyst arrives at its

Fig. 1. Selections from the video sequence showing the development of a group of 3 microbasic mastigophore nematocysts. Bar = 10 μm.

a: Formation of the internal tubule. 3–4 coils are present in the capsule; the external tubule can be seen as black circular profiles around the outside of the capsule.

b: Formation of the internal tubule almost complete. This is the bedspring stage. The internal tubule tapers smoothly from one end to the other, and the coils are isodiametric. Thus there is no indication of the formation of a shaft.

c: Differentiation of the internal tubule on the basis of thickness and change in the diameter of coiling. The region of tubule near the operculum has become thicker and appears as if coiled around a cone. Arrows mark a sudden change in diameter of the tubule that may delimit the future shaft. A migrating form of the same nematocysts (i.e., at a later stage of differentiation) has collided with the original clump of 3 (at middle left).

d: Coiling of the internal tubule is now about a helical axis. The free end of the tubule i.e., the part away from the operculum becomes less distinct and more laxly coiled. The migrating form (upper left) continues on towards top right, displacing 2 of the original 3 nematocysts. The inset diagram shows the migrating stage, i.e., at a slightly later stage than the inset in Fig. 1f. Note that in the migrating cell the operculum end of the capsule trails at the rear.

e: Further formation of the shaft. The opercular end of the internal tubule is more distinct and straight. The remainder of the tubule is very indistinct and laxly coiled.

f: Beginning of migration of the original group of 3. The cells are moving apart, with the shaft at the opercular end trailing. Optical sectioning effects prevent the full length of the shaft being apparent in this picture. The stage rapidly becomes like the migrating form shown in Fig. 1d.

The entire sequence extends over *ca* 2 h.

Insets (slightly reduced scale) are modified from Skaer (1973), reproduced with permission from The Company of Biologists Ltd.

final destination alongside the cnidoband in the cnidosac, the shaft region is so filled with spine material that the pleats of the tubule disappear. In this respect the shaft region differs from that of the stenoteles of *Hydra*. Despite growth of the 3 large spines, in *Hydra* the shaft nevertheless retains its pleats (Chapman, 1961). These pleats, on discharge, undoubtedly provide the wall material inflated during the ultrarapid discharge so effectively shown by Tardent & Holstein (1982). The shaft in *Rosacea* is not created simply by the accumulation of spine material until the pleats disappear. Although precise measurements of tubule surface area of the sort carried out by Skaer & Picken (1965) cannot be made on this material, it is clear that the shaft region at this stage, though its wall has doubled in thickness relative to the tubule (Skaer, 1973: Figs 13 and 15), has not significantly increased in diameter compared with the smoothly tapering tubule. It is only during maturation in its final position in the cnidosac that the shaft region becomes a true shaft with an enlarged wall area that on discharge inflates to give an increased diameter.

The time-lapse sequence reveals the remarkably sharp demarcation that occurs between the developing shaft region as it straightens, and the rest of the tubule whose coils become more lax. The question is, therefore, not only how is the shaft created, but also, how is the precise extent of the shaft set? One possibility is that the distinction, though initially invisible and not reflected in the mechanical properties, is nevertheless built in chemically from the start, when the external tubule is secreted. A monoclonal antibody might show such a distinction. Alternatively, the distinction might be created physically. The first sign in the sequence of formation of the shaft region occurs at the end where the operculum will develop – where also the fixed but open end of the inverted tubule contacts the cytoplasm. As described above, the tubule, which was an isodiametric coil, changes so that it resembles a coil around a cone, with the point of the cone at the opercular end. This appearance is consistent with some unwinding of the helix along its length, but carried out against a constraint. Although there is a small amount of spine material within the tubule, its accumulation in the shaft region must be largely by entry of new material into the tubule at the opercular end. This new material must be used both for spines and for the increase of wall thickness in the future shaft region. Entry of this material at the opercular end will stiffen, straighten and untwist the tubule, both by tending to reduce the bedspring coiling, and also by inflating somewhat the bases of the three pleats. Unwinding from this fixed end (for example, by insertion of spine material from the cytoplasm) would either cause the rest of the tubule to rotate, or, if this were impeded, would cause parts adjacent to the fixed end to become overwound. The result would be the observed decrease in coil diameter. Some unwinding at the free end of the tubule would give the increase there in coil diameter.

The cause of a general unwinding cannot just be spine material entering from the cytoplasm, for, at this stage there is almost no visible spine material along the majority of the internal tubule: the helical pleats in the wall are not obviously unwound or opened out (Skaer, 1973: Fig. 13). Transfer of a very small amount of spine material to the shaft from the rest of the tubule is also a possibility. Since we do not know what creates the twist as the tubule invaginates – a twist that forms the 3 helical pleats and results in the isodiametric coil – we do not know what allows the coiling to relax. It could be that slow relaxation of the coil is an inevitable consequence of time, and just happens to coincide with entry of spine material at the opercular end.

Although the internal tubule is clearly in a left-handed coil (Fig. 1a,b) the handedness of the pleats on the tubule itself has not been determined in this species. We do not know, therefore, what effect straightening of the tubule coil, such as occurs during shaft formation, will have on the pleats: they may be unaffected or become overwound or unwound (i.e., tending to open out). The manner in which straightening of the coil is achieved, moreover, whether with or without rotation, cannot be discovered from the video, for the tubule has no visible markers to enable one to

detect rotation. The tip of the tubule certainly moves during early stages in the formation of the shaft, but this cannot be distinguished from movement due to unwinding of the coil.

Overwinding the tubule from one end, and unwinding from the other (free) end, might create a kink in the tubule beyond which spine material in significant amounts could not penetrate from the opercular end; but although some visible distinction of the tubule into 2 sections can be made at this stage (small arrows, Fig. 1c), these particular sites are not associated with a sudden local change in the diameter of the coil.

Campbell (1988) suggests that it is mechanical stretching due to growth of the spines that might cause remodelling. This would apply to the enlargement of the shaft. The suggestion could be tested if anisorhizas with only very small spines were to be found that were secreted, as here, with a smoothly tapering external tubule.

As the shaft extends, the capsule extends longitudinally (Skaer, 1973). While the capsule extends, its diameter reduces; while the shaft extends, its diameter increases. In the mature capsule the shaft is almost as long as the capsule. Were the shaft to be longer, it is doubtful if the sharp curvature would allow it to discharge. The functional capsule is a visible indication of the precision of cellular control processes.

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