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Cnidocyst structure and the biomechanics of discharge

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ABSTRACT

The cnidocyst is the defining organelle of the cnidarians, used for capture of prey and defense. It consists of a cylindrical capsule, which releases a long tubule upon triggering. Cnidocysts develop inside a giant post-Golgi vesicle by a sequential accumulation of proteins from the Golgi apparatus. Traditionally three types of cnidocysts are distinguished: nematocysts, spirocysts, and ptychocysts. Here we focus on nematocysts, the prototypic cnidocyst and by far most diverse group of cnidocysts in this phylum. The mature nematocyst capsule comprises a collagenous polymer with remarkable biophysical properties, able to withstand an osmotic pressure of 150 bar. Release of the capsule and discharge is probably initiated by classical exocytosis. High-speed studies revealed the kinetics of discharge to be as short as 700 ns, generating an acceleration of 5,400,000 × *g* and a pressure of 7.7 GPa at the site of impact of the spines onto the prey. Thus nematocysts comprise a powerful molecular spring mechanism releasing energy stored in the wall polymer in the nanosecond time range. During the last few years, genomic, biochemical and structural studies have helped to unravel the molecular composition of the nematocyst supra-structure. Here we summarize these findings and present an integrative view of mechanical and molecular aspects that have shaped the nematocyst during evolution.

1. Nematocysts – structure and function

Cnidarians represent an ancient group of animals (>500 Myr old), which developed one of the first nervous systems in metazoan evolution. The characteristic cnidarian cell type is the stinging cell (cnidocyte, nematocyte). Cnidocysts are unique chemo- and mechanoreceptive neuronal cells that release a highly ordered secretion product upon stimulation, the nematocyst (Tardent and Holstein, 1982; Holstein and Tardent, 1984; Nüchter et al., 2006), the spirocyst or the ptychocysts. Spirocysts are used to mechanically immobilize prey in most anthozoans, and ptychocysts are used by cerianthid anemones to construct the tube in which they live. It is unclear at present whether they represent a different type of cnidocyst or whether they are just a highly specialized nematocyst type (David et al., 2008). Nematocysts are of varying morphology and they are either used for prey capture or for defensive and locomotory functions (Hidaka, 1993; Tardent, 1995; Kass Simon and Scappaticci, 2002). The nematocyst is product of a giant post-Golgi vesicle (Slautterback and Fawcett, 1959; Slautterback, 1963; Carre, 1972, 1974; Carre and Carre, 1973; Skaer, 1973; Engel et al., 2001; Holstein, 1981). It consists of several different protein species that assemble into a large capsular structure with a long spiny tubule inside. The matrix is built up by poly-γ-glutamate, which binds a 2 M concentration of cations, thereby creating a high osmotic intra-capsular pressure of more than 150 bar (Weber, 1989, 1990, 1991; Weber et al., 1990; Gerke et al., 1991; Klug and Weber, 1991). In mature nematocytes, which are mounted in the epithelial cell layer, the nematocyst vesicle is docked at the apical side of the cell. Mechanical stimulation of the sensory receptor of the nematocyte, the cnidocil, elicits an action potential, which in turn triggers Ca^{2+}-dependent discharge (Santoro and

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Salleo, 1991; Gitter, 1994; Gitter et al., 1994; Watson and Hessinger, 1994). Within less than 3 ms the tubule evaginates and injects toxins into the prey (Tardent and Holstein, 1982; Holstein and Tardent, 1984; Nüchter et al., 2006).

On the molecular level, the nematocyst is designed to withstand this extreme mechanical stress by combining high resistance and flexibility. The major constituents of the capsule wall are mini-collagens, a family of unusually short collagens (Kurz et al., 1991; Engel et al., 2001, 2002; Ozbek et al., 2002a; Pokidysheva et al., 2004). They comprise a central collagen triple helix with 12–16 Gly-X-Y repeats flanked by polyproline stretches and terminal cysteine-rich domains (CRDs). A second capsule protein termed NOWA serves as a positional cue in the assembly process (Engel et al., 2002). The organization of the wall and tube structures from these proteins is mainly directed by a membrane-associated assembly and disulfide reshuffling process, resulting in a three-dimensional polymer stabilized by disulfide bonds (Engel et al., 2001). Investigation of the wall surface by atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM) revealed dense globular structures (Holstein et al., 1994) that represent disulfide-dependent hetero-assembly products of mini-collagens and NOWA protein. They form a continuous supra-structure of the nematocyst wall (Ozbek et al., 2002b, 2004; Pokidysheva et al., 2004), which might exhibit a preferential assembly mode leading to fiber-like structures (Holstein et al., 1994).

1.1. Mechanics of nematocyst discharge

Ready-to-discharge nematocysts are docked at the nematocyte's apical pole through their vesicular membrane (Fig. 1). Each nematocyst vesicle is encircled by a tight basket of microtubules and intermediate filaments forming a solid anchoring for the organelle in the cell (Wood and Novak, 1982; Novak and Wood, 1983; Hausmann and Holstein, 1985; Engel et al., 2002). During the entire discharge process the organelle is efficiently anchored by the cytoskeleton and does not alter its position inside the cell.

All nematocysts have a solid, elastic wall and a lid structure, which closes the capsule before discharge. The lid can be shaped as a solid operculum in hydrozoans or it can consist of three opercular flaps in anthozoans (Godknecht and Tardent, 1988). In the discharged form, all capsules have a pear- or banana-shaped capsular body with an extended twisted tubule that normally carries three rows of spines. The tubule morphology of capsules differs considerably among cnidarian species, but the capsules of the highest structural complexity are found in hydrozoans. Here, the stenoteles represent one of the most sophisticated capsule types. The stenotele tubule is enlarged at its basal region, which is called the shaft. The distal shaft carries the stylet apparatus, which contains three huge stylets and three arrays of thinner spines. This stylet apparatus forms a dart when the tubule evaginates, which punches a whole into the prey's cuticle through which the long tubule enters, releasing hemolytic and neurotoxic substances (Tardent, 1995). The dart-like stylet apparatus can penetrate even thick crustacean cuticles (Tardent, 1995).

It is generally accepted today that the high osmotic pressure that is built up at the end of capsule morphogenesis (Szczepanek et al., 2002) in conjunction with the elastically stretched capsular wall is the main driving force of discharge. At the end of morphogenesis the capsule is maximally expanded and during discharge the volume gets suddenly reduced again by about 50% (Tardent and Holstein, 1982; Holstein and Tardent, 1984). The high osmotic pressure is generated by poly-γ-glutamate and 2.80 ± 0.20 μmol of anions found per milligram of isolated and purified dry nematocysts (Weber et al., 1987a,b; Weber, 1989;). They are responsible for the generation and regulation of an internal osmotic pressure that amounts up to 150 bar (Weber, 1989). The anions are organized as linear homopolymers of L-glutamic acids, which are linked by γ-carboxyl-alpha-amino amide bonds. Notably, poly-γ-glutamic acids are known to occur in some selected bacteria, but have not been reported for any eukaryotes. They are the predominant components of all nematocysts in other cnidarian species and thus might represent a class of compounds which is characteristic for a whole phylum of the animal kingdom (Weber, 1989).

It was recently questioned whether capsules are under an initial osmotic pressure (Berking and Herrmann, 2006). Based on the acidic capsular matrix, it was speculated that counter ions may dissociate from the carboxyl groups of the polymeric matrix after triggering. The resulting electrostatic repulsion should then create a pressure (and volume) increase inside the matrix. The proposed mechanism can certainly not explain the dramatic volume changes during discharge (see above), which indicates that a high osmotic pressure inside the capsule is the basic principle of all nematocysts. However, it might explain how the discharge process is initiated. Our high-speed analyses have revealed that in about 20% of all capsules discharge is accompanied by an initial volume increase. This is important, since vesicle swelling is a well-known phenomenon in most exocytotic systems. In nematocysts and in many eukaryotic exocytotic systems (e.g. mast cells), the matrix of the secretory organelle contains a polymeric matrix, which, because of the high density of fixed negative charges, acts like a cationic ion exchanger (Fernandez et al., 1991; Parpura and Fernandez, 1996). After fusion pore formation, an influx of counter ions from the extracellular medium (Na^+ or K^+) is accompanied by a large influx of water causing the matrix to swell osmotically (Fernandez et al., 1991). This basic mechanism can be still found in some nematocysts of the anthozoan *Corynactis*, where capsules swell by >50% immediately prior to discharge and then return to exactly their original dimensions after discharge (Robson, 1972). Nematocysts in *Hydra* are more sophisticated in that they are already under a high initial osmotic pressure prior to discharge (Holstein and Tardent, 1984; Weber, 1989, 1990; Holstein et al., 1994; Szczepanek et al., 2002).

We presume that in the initial phase of discharge the volume of the capsule must swell over a critical threshold value in order to discharge. Alternatively, proteins localized in the operculum might change their supra-molecular architecture upon triggering and in this manner facilitate discharge. Also, we found that the molecular arrangement within the operculum apparently changes during the

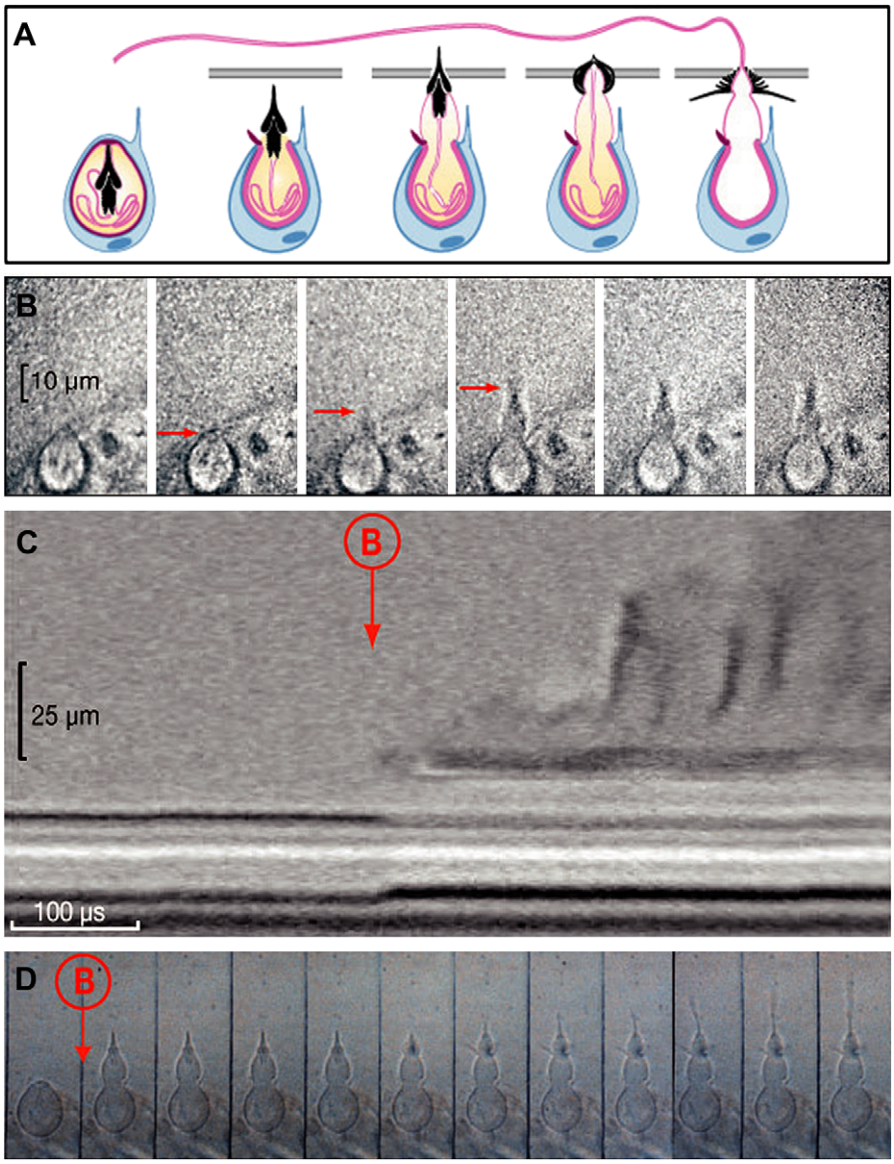

Fig. 1. Morphodynamics of nematocyst discharge. Discharge of a stenotele is depicted schematically (A), recorded with a Hamamatsu C4187 high-speed camera in the framing mode at 1,430,000 frames per second (B), in the streak mode at 3 µs resolution (C), and with a rotary prism camera at 40,000 frames per second (D). Each nematocyte (blue in A) harbors one cyst (pink in A) with stylets that punch a hole into prey. The rapid phase of discharge was only resolved by using the framing mode of the Hamamatsu C4187 camera (B) and is indicated is red in (C) and (D). (A–C) from Nüchter et al. (2006) and (D) from Holstein and Tardent (1984).

discharge process, indicating a more loose arrangement of structural proteins induced by the discharge process (Özbek and Holstein, unpublished). Therefore, we propose that the molecular assembly of the operculum enables it to react to the ionic changes preceding discharge by a molecular rearrangement facilitating capsule wall disintegration at this position. Thus, the release of the increased osmotic pressure would be directed to the preformed opening preventing a random rupture of the wall structure.

1.2. Discharge kinetics

The kinetics of discharge of stenoteles was investigated first by Reisinger (1937) using the rotary mirror high-speed technique. He found that the duration of the entire discharge is in the range of about 3 ms. Holstein and Tardent (1984) analyzed discharge by using a rotary mirror high-speed camera at a rate of 40,000 frames per second(fps). Nüchter et al. (2006) reinvestigated the process, using an ultra-fast electronic framing streak camera (CCD and image intensifier) at 1,600,000 fps. This type of camera can run at several frames per second, although the record lengths are limited to 8 or 16 images in the framing mode. According to the data of Holstein and Tardent (1984) and Nüchter et al. (2006) discharge can be subdivided into 4 phases. Phase A is the initial lag phase, i.e. the interval between membrane depolarization and the flipping open of the operculum. In phase B the capule's cover is opened

and the stylet-bearing portion of the tubule is ejected. Phase C is an intermediate phase during which discharge was arrested and in phase D the long terminal part of the tubule evaginates. Each of these phases will now be described in more detail.

1.2.1. Phase A, the initial lag phase

Nematocyte discharge can be induced by applying a short step depolarization (80 mV) to small pieces of tentacles from *Hydra* polyps (Nüchter et al., 2006). Intracellular recordings using current and voltage clamp methods revealed previously that depolarizing receptor potentials and receptor currents are also generated in stenotele nematocytes of capitate tentacles of the marine hydroid polyp *Stauridiosarsia producta* in response to mechanical stimulation of the cnidocil apparatus (Brinkmann et al., 1996; Gitter and Thurm, 1996). Thus, membrane depolarization mimics an action potential that is normally elicited when a prey mechanically deflects the sensory receptor of the cell, the cnidocil.

Holstein and Tardent (1984) on the basis of limited data reported delay times of about 1 ms. Gitter and Thurm (1996) analyzed the length of the lag phase more quantitatively by using video microscopy with triggered flash illumination. They found a minimal delay of 330–450 μs and 230–350 μs for depolarizing and large hyperpolarizing stimuli, respectively. By using the streak mode of the electronic camera, Nüchter et al. (2006) generated a large number (>500) of streak records of discharge that allowed a precise determination of all discharge phases. The streak mode displays a single continuous "smear" picture of a given process within a chosen time range, so that both temporal and positional information is recorded simultaneously. According to these measurements, the lag phase was of 650 ± 134 μs in normal culture medium. By varying the extracellular Ca^{2+} it was found that the lag phase was strongly Ca^{2+}-dependent. Lowering the Ca^{2+} concentration in the medium significantly postponed or even inhibited discharge, and increasing the Ca^{2+} concentration shortened the delay. At Ca^{2+} concentrations of
6–8 mM, delay could become as short as 180 μs, which is in the delay range found for fast synaptic vesicles (Schneggenburger et al., 2002). Therefore, it is likely that extracellular calcium entering the nematocyte leads to fusion pore formation during nematocyst exocytosis. We presume that saturation of Ca^{2+}-binding sites in the nematocyte represents the rate limiting step leading to fusion pore formation and discharge. Although voltage-gated Ca^{2+} channels as well as Ca^{2+}-binding proteins of the exocytotic machinery (Sudhof, 1995; Augustine, 2001) are present in databases of the *Nematostella* and *Hydra* genome databases, it is unclear so far how Ca^{2+} actually influences the discharge kinetics.

1.2.2. Phase B, the rapid phase of discharge

In phase B the nematocyst vesicle fuses with the nematocyte membrane, and the capsule's discharge process starts. The most obvious event is that the lid is opened and the stylet-bearing portion of the tubule is ejected. Streak recordings using the electronic framing streak camera revealed that ejection is always exceedingly fast. At a temporal resolution of 3 ms, ejection of the stylet-bearing portion of the tube always corresponds to an abrupt, step-like dislocation of the upper capsular trace without any intermediate stage (Fig. 2), which suggests that the critical phase of discharge is accomplished in less than 3 μs.

The ultra-fast phase of the discharge process was resolved in the camera's framing mode of the electronic camera at a framing rate of 1,430,000 fps. At this speed intermediate stages of the ejection phase of the stylets were recorded, although some recordings failed to catch intermediate stages, suggesting that the discharge process was even faster. Nüchter et al. (2006) calculated on a conservative basis that stylet ejection could be as short as 700 ns. Based on these

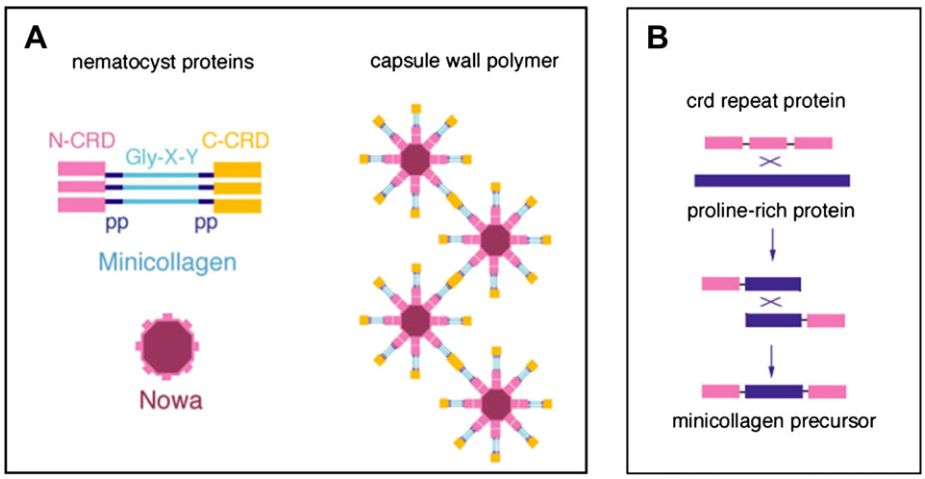

Fig. 2. Molecular structure of the nematocyst wall. (A) The major structural proteins of nematocysts are mini-collagens and NOWA. Mini-collagens are comprised of a central Gly-X-Y triple helical domain with flanking polyproline (pp) rich domains and N- and C-terminal cysteine-rich domains (CRD). NOWA proteins contain multiple CRDs that interact with the C-CRD of mini-collagens to form the capsule polymer. (B) Model to explain the molecular evolution of mini-collagens. It is proposed that mini-collagens arose by recombination events from toxin-derived proteins with multiple CRD repeats and polyproline-rich proteins (see text for details).

calculations, an average velocity (v) of the stylet tip of 18.6 m/s is generated. By assuming constant acceleration (a) they calculated that 5,413,000 $\times$ g is required to produce this average velocity (v) from a standing start. This high acceleration can explain how a small mass can generate sufficient force at the site of impact. The stylets are composed of a keratin-like protein and have an extremely narrow tip. Based on the accelerated mass and the stylets tip (80 nm^2), a pressure (p) of 7.7 GPa was estimated when the stylet tip hits the prey. This force is in the range of technical bullets and explains how a 5 μm thick solid cuticle of a crustacean prey can be effectively perforated by a cnidarian nematocyst with a minimum of mass. This seems to be the fastest known process generated in animal systems. The acceleration of limbs in mantis shrimps (more than 10,000 $\times$ g) were long considered to produce the highest acceleration in the animal kingdom (Patek et al., 2004).

It should be pointed out that in the majority (80%) of all records discharge occurred without any visible changes in the matrix prior to stylet ejection (Nüchter et al., 2006). This indicates that the cyst is in its maximally expanded state and only a minimal increase in pressure is sufficient to elicit discharge. Prior to the flipping open of the operculum further structural changes can be observed inside the capsule. The capsule's shape changed abruptly when the stylets were ejected. This is clearly visible as a step-like displacement of the lower capsular trace towards a smaller capsule diameter. This change was considered to represent the beginning of the contraction process of the capsular wall.

1.2.3. Phase C, the temporary arrest phase

Phase C is a relatively long arrest phase during which the stylets can evaginate from the opening they have created mechanically in order to release the rest of the tubule into the into the prey's body. After this arrest phase the long terminal part of the tubule evaginates. How this arrest phase is terminated is unclear so far. It was proposed that in addition to the poly-γ-glutamate in the capsule matrix, poly-γ-glutamate is also found within the lumen of the tubule shaft region in stenoteles. This poly-γ-glutamate could be required to drive the second phase of the discharge process in stenotele nematocysts (Szczepanek et al., 2002). An alternative explanation is that after the sudden release of the stylet apparatus the intra-capsular pressure must rise again to reach a sufficient threshold value required for further discharge. In line with this argument streak records revealed a distinct volume dependency of the length of the arrest phase C, which increased linearly from 450 μs in small-sized capsules (500 μm^3) to 550 μs in medium-sized capsules (4000 μm^3) (see Nüchter et al., 2006, Fig. S4 for details).

1.2.4. Phase D, the toxin release phase

In phase D, which is the slowest of all, the long terminal part of the tubule is evaginated. During this phase, the long tubule (500 μm) evaginates with a velocity of 0.27 $\pm$ 0.21 m/s (Nüchter et al., 2006). The streak images reveal discrete periodicities along the trace of the evaginating tubule. These pulsations with a 50 μs phase probably reflect the rotary movements of the evaginating tubule, which is twisted around its axis in the undischarged capsule (Tardent and Holstein, 1982). An alternative explanation would be that they reflect step-like contractions of the mini-collagen polymer.

1.3. Supra-molecular structure and self-assembly of the nematocyst

The capsule proteome comprises a total number of more than 200 proteins, the majority of which are provided by structural proteins like the mini-collagen family (Kurz et al., 1991) (Balasubraminian et al., in preparation). Mini-collagens are an integral part of the wall and tubule structures where they form a disulfide-linked polymer. An additional component of this supra-structure is NOWA, a large glycoprotein, which forms irregular globular aggregates that are able to interlink with mini-collagens. Spinalin, a keratin-like glycine- and histidine-rich protein, is involved in the formation of spine and operculum structures. All capsule proteins are secreted into the growing nematocyst vesicle during morphogenesis and undergo spontaneous self-assembly. During the final step of maturation, polymer formation is initiated, which renders most of the capsule proteins insoluble. Reducing agents, which break disulfide connections, can readily solubilize most of the capsule proteins.

1.3.1. Mini-collagens

The *Hydra* genome project has yielded 17 different members of the mini-collagen protein family that vary in length and domain composition (David et al., 2008). Mini-collagens are trimeric molecules that are expressed as soluble precursors, which during nematocyst maturation polymerize by a switch in the disulfide linkage from intra- to intermolecular connections (Engel et al., 2001; Ozbek et al., 2002a,b). They are composed of a central collagen triple helix flanked by polyproline stretches and short cysteine-rich domains (CRDs), which are involved in the formation of the cysteine-linked ultra-stable capsule wall structure (Ozbek et al., 2004). The canonical CRD, which is found in at least one mini-collagen in all cnidarians analyzed so far, has a highly conserved pattern of 6 cysteines within a sequence of 18 amino acids (CXXXCXXXCXXXCXXXCC). The NMR structure of the C-terminal mini-collagen-1 CRD of *Hydra* revealed a tightly packed globular fold with a 1–4, 2–6 and 3–5 cysteine pattern and a central *cis* proline turn between cysteine 3 and cysteine 4 (Pokidysheva et al., 2004). The overall left-handed fold comprises an N-terminal α-helix, followed by three different turns. Surprisingly, the N-terminal mini-collagen-1 CRD, although having an identical cysteine pattern, showed a fundamentally different structure and disulfide bonding (1–5,2–4,3–6) with an overall right-handed fold (Milbradt et al., 2005). Thus, the mini-collagen CRDs clearly serve as a rare example for the divergent evolution of protein folds in closely related domains. Recently, Meier et al. have demonstrated by NMR analysis of mutated N- and C-terminal CRDs that these can interconvert by a single point mutation (Meier et al., 2007a,b). The ability of single CRD sequences to populate two different folds has given strong indications that complex

features, including protein tertiary structures, can develop by smooth evolutionary transitions.

Domain duplications and minute sequence alterations involving the CRDs have apparently yielded structurally different isoforms within this gene family with different functions in nematocyst architecture. This is demonstrated by the finding that mini-collagen-15, which shows an altered C-terminal CRD sequence with the first two cysteines of the canonical pattern missing, resulting in a different structure, is exclusively incorporated into the tubule (Adamczyk et al., 2008), whereas mini-collagen-1 is a capsule wall component. It has therefore been suggested that the evolution of mini-collagens is closely correlated with the evolution of capsule types in cnidarians. This is emphasized by the fact that sequence variations are less pronounced in the more primordial species *Nematostella vectensis*, which contains only 5 mini-collagen genes and a reduced nematocyst repertoire (David et al., 2008).

1.3.2. NOWA

NOWA is a 90 kDa glycoprotein which contains an N-terminal SCP domain, a central C-type lectin domain and a CRD octarepeat at its C-terminus probably acting as a mini-collagen linker motif (Engel et al., 2002). NOWA was originally characterized as an outer wall protein, which forms globular aggregates on the capsule surface. We have shown that recombinantly expressed NOWA as well as its cysteine-rich octarepeat domain (CROD) spontaneously form disulfide-linked globular aggregates that resemble the globular building units of the nematocyst wall (Ozbek et al., 2004a). These NOWA globules are extremely heterogeneous in size ranging from 15 to 45 nm in diameter and form several densely packed layers along the wall profile. Ultrastructural analysis of the wall architecture revealed that the individual globules are interconnected via rod-like protrusions that probably represent mini-collagen. The structural elucidation of several NOWA CRDs demonstrated that despite sequence insertions of varying length into the tightly knit disulfide-rich domains, all NOWA CRDs form an identical, prototypical structure. This fold is similar to the N-terminal mini-collagen-1 CRD structure, strongly suggesting a common precursor of the CRD domain.

NOWA appears early during nematocyst morphogenesis and forms a thin layer at the inside of the nematocyst vesicle membrane (Engel et al., 2002). Mini-collagens, which are expressed at later stages, gradually attach to this layer in a pre-assembly process. Meier et al. could demonstrate that the shared CRD motif between mini-collagens and NOWA spontaneously undergoes intermolecular disulfide formation under Golgi conditions indicating a covalent assembly between NOWA aggregates and mini-collagens at an early stage of capsule morphogenesis, which is later followed by polymerization between the C-terminal mini-collagen CRDs. Mature capsules are composed of a tight NOWA–mini-collagen polymer, which is the basis for the highly stress-resistant capsule wall structure.

1.3.3. Spinalin

The spines on the tubule surface of nematocysts are presumed to have high mechanical strength. Stylets, the large spines of stenoteles, are needed to puncture the prey organism cuticle during capsule discharge, while spines in desmonemes and isorhizas appear to function as barbs allowing binding to prey or to the substrate. Spinalin is a 24 kDa protein that is a constituent of spines and opercula of nematocysts in *Hydra* (Koch et al., 1998; Hellstern et al., 2006). Immunocytochemical analysis of developing nematocysts revealed that spinalin first appears in aggregated protein bodies in the matrix but then is transferred through the tubule wall at the end of morphogenesis to form spines on the external surface of the inverted tubule and to form the operculum. A resistance to DTT treatment is characteristic of spinalin, indicating that it is part of highly condensed structures. On the sequence level it is related to fibrous glycine-rich proteins like loricrins and cytokeratins. Biochemical data have shown that spinalin is an obligate dimer in *Hydra* cell lysates. Recombinantly expressed protein shows a strong dimerization tendency as a prerequisite to aggregate formation.

2. Evolutionary considerations

2.1. Molecular evolution

A prominent open question concerns the unsolved evolutionary origin of the nematocyst. Both genetic and proteomic approaches have been initiated to answer this question. Hwang et al. presented the first transcriptome analysis of nematocytes, which yielded roughly 50 nematocyst-specific genes (Hwang et al., 2007). These include structural proteins that are essential for assembling the capsule, toxin proteins and genes encoding the sensory apparatus (cnidocil) of the nematocyte. Interestingly, the vast majority of these genes did not have an orthologue in other species, indicating a unique molecular composition of the nematocyst. An ongoing proteomic analysis of nematocysts in *Hydra* has revealed more than 200 capsule proteins (Balasubramanian et al., in preparation). The overall picture provided by this study is that many nematocyst proteins are a product of extensive domain shuffling, a process that obviously started off with a limited repertoire of structural motifs. This is particularly emphasized in proteins of the mini-collagen family, which are composed from a small "toolkit" of domains that probably existed as independent proteins, e.g. the multiple CRD tandem repeat of NOWA. In addition, there is a noticeable adaptation of domain motifs from toxin groups by structural capsule proteins. Again mini-collagens might serve as a prominent example. The structurallywell-characterized canonical mini-collagen CRDs show tightly knit folds dominated by three cysteine links, a feature shared by several cnidarian toxins like those acting on voltage-gated channels (Standker et al., 2006; Messerli and Greenberg, 2006). Although short cysteine-rich proteins are extremely diverse in structure and function (Cheek et al., 2006), the finding that mini-collagens and toxins in cnidarians share common propeptide sequences (Anderluh et al., 2000), demonstrated to assist in CRD folding for mini-collagen-1 (Milbradt et al., 2005), is additionally suggestive of a common origin. A possible scenario might have been a recombination event combining CRDs and polyproline stretches in

a first step and the generation of a mini-collagen precursor protein with a central polyproline sequence in a second, equal recombination step (Fig. 2B). In the ongoing proteomic analysis a capsule protein was indeed found, which comprises N- and C-terminal CRDs with a central polyproline stretch but no collagen sequence (Balasubramanian et al., in preparation). It might be speculated therefore that the short central collagen domain has evolved from proline-rich sequences. Proline-rich proteins are common components of plant cell walls, where they are integrated into the wall matrix (Showalter, 1993). In addition, small proline-rich proteins have been shown to function as defensins in plants (Pearce et al., 2007) or antibacterial peptides in insects (Li et al., 2006). Similar proteins have not yet been identified in cnidarians, but it is suggestive that hydroxyproline-rich sequences with structural or defensive functions have been precursors to mini-collagens in cnidarians.

A second example is represented by the putative structure of spinalin. To elicit structural features of spinalin sequence threading approaches were performed, which revealed similarities of the putative 3D structure to the toxin–agglutinin fold. Structural investigations of wheat germ agglutinin and several snake venom toxins revealed domains folded into a series of coiled short loops linked together by 4 invariant disulfide bridges. The lack of secondary-structure elements is compensated in these domains by the strict network of disulfide links. In the case of spinalin, the N-terminal subdomain shows a pattern of several GYGG repeating motifs, which may provide the driving force for dimer formation, as stable dimers are retained after reduction with dithiothreitol (Hellstern et al., 2006). This hypothesis is supported by investigations of the C-type lectin rhodocetin. Both heterodimeric subdomains in rhodocetin are formed by a conserved pattern of disulfide bridges stabilizing several loops. However, the interdomain interface is not stabilized via cystine formation, but by van der Waals contacts between several β-branched side chains.

2.2. Extrusive organelles in protists – a possible origin of nematocysts?

Extrusive organelles sharing morphological and functional characteristics of cnidarian nematocysts are widespread among various groups of protists (Hausmann, 1978). They are generally membrane-bound, cytoplasmatic structures produced by the Golgi apparatus. Some of these extrusive organelles are structurally clearly *not* related to nematocysts since they exhibit completely different discharge mechanisms. The mucocysts of flagellates and ciliates are large para-crystalline secretory bodies that slowly expand upon discharge and the spindle trichocysts of ciliates and flagellates rapidly discharge by a sudden unfolding of a preexisting three-dimensional network of protein filaments (Messer and Ben-Shaul, 1971; Hausmann, 1978; Satir et al., 1988; Kung et al., 2000; Plattner and Kissmehl, 2003). However, there are also "extrusomes" that share distinct similarities with the nematocyst discharge mechanism. The rhabdocysts in ciliates exhibit tune-like structures that become everted upon discharge (Hausmann, 1978; Raikov, 1992). In particular, toxicysts of ciliates (*Didinium*) and flagellates (*Colponema*) and nematocysts of flagellates (*Polykrikos*) and karyorelictid ciliates (*Remanella*) exhibit a highly similar mode of explosive extrusion during which the extrusome vesicle fuses with the plasma membrane and an internal tubule is everted by which toxins are secreted (Rieder, 1968; Hausmann, 1978; Westfall et al., 1983; Lee and Kugrens, 1992; Raikov, 1992; Hoppenrath and Leander, 2006; Hoppenrath and Leander, 2007). The extrusion process is believed to be driven by a high internal pressure of the extrusome. Prey organisms might be other ciliates or bacteria. Although molecular evidence for a possible protist ancestry of nematocysts is largely lacking, Hwang et al. indicated that two calcium-binding proteins identified in their screen for nematocyte-specific genes had significant matches with genes from protists (Hwang et al., 2007). It will therefore be intriguing to screen protist genomes for nematocyst-specific genes to answer the question of which molecular components have possibly been transferred from protists to Cnidaria (Shostak, 1993). The fact that several of these protists with extrusive organelles were colonial organisms suggests that nematocyst-like organelles were a basic and ancestral feature of the first metazoans.

Conflict of interest

None declared.

References

Adamczyk, P., et al., 2008. Minicollagen-15, a novel minicollagen isolated from *Hydra*, forms tubule structures in nematocysts. J. Mol. Biol. 376, 1008–1020.
Anderluh, G., et al., 2000. A common motif in proparts of Cnidarian toxins and nematocyst collagens and its putative role. Biochim. Biophys. Acta 1476, 372–376.
Augustine, G.J., 2001. How does calcium trigger neurotransmitter release? Curr. Opin. Neurobiol. 11, 320–326.
Berking, S., Herrmann, K., 2006. Formation and discharge of nematocysts is controlled by a proton gradient across the cyst membrane. Helgol. Mar. Res. 60, 180–188.
Brinkmann, M., et al., 1996. Mechanoelectric transduction in nematocytes of a hydropolyp (Corynidae). J. Comp. Physiol. 178, 125–138.
Carre, C., Carre, D., 1973. Etude du cnidome et de la cnidogenese chez *Apolemia uvaria* (Lesueur, 1811) (siphonophore physonecte). Exp. Cell Res. 81, 237–249.
Carre, D., 1972. Étude du developpement des cnidocystes dans le gastrozoide de *Muggiaea kochi* (Will, 1844) (siphonophore calycophore). C.R. Hebd. Sianc. Acad. Set., Paris 275, 1263–1266.
Carre, D., 1974. Formation, migration et maturation des nematoblastes et des nematocystes chez les siphonophores. l – Mise en evidence et formation des clones de nematocystes. Annales Embryol. 7, 205–218.
Cheek, S., et al., 2006. Structural classification of small, disulfide-rich protein domains. J. Mol. Biol. 359, 215–237.
David, C.N., et al., 2008. Evolution of complex structures: minicollagens shape the cnidarian nematocyst. Trends Genet. 24, 431–438.
Engel, U., et al., 2002. NOWA, a novel protein with minicollagen Cys-rich domains involved in nematocyst formation in. Hydra. J. Cell Sci., 3923–3934.
Engel, U., et al., 2001. A switch in disulfide linkage during minicollagen assembly in *Hydra* nematocysts. EMBO J. 20, 3063–3073.
Fernandez, J.M., et al., 1991. Reversible condensation of mast cell secretory products in vitro. Biophys. J. 59, 1022–1027.
Gerke, I., et al., 1991. The spatial distribution of cations in nematocytes of Hydra vulgaris. Hydrobiologia 216/217, 661–669.
Gitter, A.H., 1994. Time interval between electrical stimulus and discharge of in situ stenoteles of *Hydra*. Naturwissenschaften 81, 365–366.
Gitter, A.H., et al., 1994. Calcium- and voltage-dependence of nematocyst discharge in *Hydra vulgaris*. J. Comp. Physiol. A. 175, 115–122.

Gitter, A.H., Thurm, U., 1996. Rapid exocytosis of stenotele nematocysts in *Hydra vulgaris*. J. Comp. Physiol. A. 178, 117–124.
Godknecht, A., Tardent, P., 1988. Discharge and mode of action of the tentacular nematocysts of *Anemonia sulcata* (Anthozoa: Cnidaria). Mar. Biol.. (Berlin) 100, 83–92.
Hausmann, K., 1978. Extrusive organelles in protists. Int. Rev. Cytol. 52, 197–276.
Hausmann, K., Holstein, T., 1985. Bilateral symmetry in the cnidocil–nematocyst complex of the freshwater medusa *Craspedacusta sowerbii* Lankester (Hydrozoa, Limnomedusae). J. Ultrastruct. Res. 90, 89–104.
Hellstern, S., et al., 2006. Structure/function analysis of spinalin, a spine protein of *Hydra* nematocysts. FEBS J. 273, 3230–3237.
Hidaka, M., 1993. Mechanism of nematocyst discharge and its cellular control. Adv. Comp. Environ. Physiol., 45–76.
Holstein, T., 1981. The morphogenesis of nematocytes in *Hydra* and *Forskalia*: an ultrastructural study. J. Ultrastruct. Res. 75, 276–290.
Holstein, T., Tardent, P., 1984. An ultrahigh-speed analysis of exocytosis: nematocyst discharge. Science 223, 830–833.
Holstein, T.W., et al., 1994. Fibrous mini-collagens in *Hydra* nematocysts. Science 265, 402–404.
Hoppenrath, M., Leander, B.S., 2006. Dinoflagellate, Euglenid, or Cercomonad? The ultrastructure and molecular phylogenetic position of *Protaspis grandis* n. sp. J. Eukaryot. Microbiol. 53, 327–342.
Hoppenrath, M., Leander, B.S., 2007. Morphology and phylogeny of the pseudocolonial dinoflagellates *Polykrikos lebourae* and *Polykrikos herdmanae* n. sp. Protist 158, 209–227.
Hwang, J.S., et al., 2007. The evolutionary emergence of cell type-specific genes inferred from the gene expression analysis of *Hydra*. Proc. Natl. Acad. Sci. USA 104, 14735–14740.
Kass Simon, G., Scappaticci, A.A., 2002. The behavioral and developmental physiology of nematocysts. Can. J. Zool. 80, 1772–1794.
Klug, M., Weber, J., 1991. An extract from *Hydra vulgaris* (Cnidaria) nematocysts increases cytoplasmic Ca^{2+} levels in fibroblasts. Toxicon 29, 129–133.
Koch, A.W., et al., 1998. Spinalin, a new glycine- and histidine-rich protein in spines of *Hydra* nematocysts. J. Cell. Sci. 111, 1545–1554.
Kung, C., et al., 2000. Recent advances in the molecular genetics of *Paramecium*. J. Eukaryot. Microbiol. 47, 11–14.
Kurz, E.M., et al., 1991. Mini-collagens in *Hydra* nematocytes. J. Cell Biol. 115, 1159–1169.
Lee, R.E., Kugrens, P., 1992. Relationship between the flagellates and the ciliates. Microbiol. Rev. 56, 529–542.
Li, W.F., et al., 2006. Apidaecin-type peptides: biodiversity, structure–function relationships and mode of action. Peptides 27, 2350–2359.
Meier, S., et al., 2007a. Sequence-structure and structure–function analysis in cysteine-rich domains forming the ultrastable nematocyst wall. J. Mol. Biol. 368, 718–728.
Meier, S., et al., 2007b. Continuous molecular evolution of protein-domain structures by single amino acid changes. Curr. Biol. 17, 173–178.
Messer, G., Ben-Shaul, Y., 1971. Fine structure of trichocyst fibrils of the dinoflagellate *Peridinum westii*. J. Ultrastruct. Res. 37, 94–104.
Messerli, S.M., Greenberg, R.M., 2006. Cnidarian toxins acting on voltage-gated ion channels. Mar. Drugs 4, 70–81.
Milbradt, A.G., et al., 2005. The two cysteine-rich head domains of minicollagen from *Hydra* nematocysts differ in their cystine framework and overall fold despite an identical cysteine sequence pattern. J. Mol. Biol. 354, 591–600.
Novak, P.L., Wood, R.L., 1983. Development of the nematocyte junctional complex in *Hydra* tentacles in relation to cellular recognition and positioning. J. Ultrastruct. Res. 83 111–1121.
Nüchter, T., et al., 2006. Nanosecond-scale kinetics of nematocyst discharge. Curr. Biol. 16, R316–R318.
Ozbek, S., et al., 2002a. A switch in disulfide linkage during minicollagen assembly in *Hydra* nematocysts or how to assemble a 150-bar-resistant structure. J. Struct. Biol. 137, 11–14.
Ozbek, S., et al., 2002b. Structure/function relationships in the minicollagen of *Hydra* nematocysts. J. Biol. Chem. 277, 49200–49204.
Ozbek, S., et al., 2004. The glycoprotein NOWA and minicollagens are part of a disulfide-linked polymer that forms the cnidarian nematocyst wall. J. Biol. Chem. 279, 52016–52023.
Parpura, V., Fernandez, J.M., 1996. Atomic force microscopy study of the secretory granule lumen. Biophys. J. 71, 2356–2366.
Patek, S.N., et al., 2004. Biomechanics: deadly strike mechanism of a mantis shrimp. Nature 428, 819–820.
Pearce, G., et al., 2007. Three hydroxyproline-rich glycopeptides derived from a single petunia polyprotein precursor activate defensin I, a pathogen defense response gene. J. Biol. Chem. 282, 17777–17784.
Plattner, H., Kissmehl, R., 2003. Molecular aspects of membrane trafficking in *Paramecium*. Int. Rev. Cytol. 232, 185–216.
Pokidysheva, E., et al., 2004. The structure of the Cys-rich terminal domain of *Hydra* minicollagen, which is involved in disulfide networks of the nematocyst wall. J. Biol. Chem. 279, 30395–30401.
Raikov, I.B., 1992. Unusual extrusive organelles in karyorelictid ciliates: an argument for the ancient origin of this group. Biosystems 28, 195–201.
Reisinger, E., 1937. Der Entladungsvorgang der Nesselkapseln. Verh. Deutsch. zool. Ges. Leipzig 39, 311–315.
Rieder, N., 1968. Electron microscope studies on the resting structure of the toxicysts of *Didinium nasutum*. Z. Naturforsch. B. 23, 569.
Robson, E.A., 1972. The discharge of nematocysts in relation to properties of the capsule. Publ. Seto Mar. Biol. Lab, 653–673.
Santoro, G., Salleo, A., 1991. The discharge of in situ nematocysts of the acontia of *Aiptasia mutabilis* is a Ca^{2+}-induced response. J. Exp. Biol. 156, 173–185.
Satir, B.H., et al., 1988. Aspects of signal transduction in stimulus exocytosis-coupling in *Paramecium*. J. Cell. Biochem. 36, 429–443.
Schneggenburger, R., et al., 2002. Vesicle pools and short-term synaptic depression: lessons from a large synapse. Trends Neurosci. 25, 206–212.
Shostak, S., 1993. A symbiogenetic theory for the origins of cnidocysts in Cnidaria. Biosystems 29, 49–58.
Showalter, A.M., 1993. Structure and function of plant cell wall proteins. Plant Cell 5, 9–23.
Skaer, R.J., 1973. The secretion and development of nematocysts in a siphonophore. J. Cell Sci. 13, 371–393.
Slautterback, D.B., 1963. Cytoplasmic microtubules. I. Hydra. J. Cell Biol. 18, 367–388.
Slautterback, D.B., Fawcett, D.W., 1959. The development of the cnidoblasts of *Hydra*; an electron microscope study of cell differentiation. J. Biophys. Biochem. Cytol. 25, 441–452.
Standker, L., et al., 2006. A new toxin from the sea anemone *Condylactis gigantea* with effect on sodium channel inactivation. Toxicon 48, 211–220.
Sudhof, T.C., 1995. The synaptic vesicle cycle: a cascade of protein–protein interactions. Nature 375, 645–653.
Szczepanek, S., et al., 2002. Poly-gamma-glutamate synthesis during formation of nematocyst capsules in *Hydra*. J. Cell Sci. 115, 745–751.
Tardent, P., 1995. The cnidarian cnidocyte, a high-tech cellular weaponry. Bioessays 17, 351–362.
Tardent, P., Holstein, T., 1982. Morphology and morphodynamics of the stenotele nematocyst of *Hydra attenuata* Pall. (Hydrozoa, Cnidaria). Cell Tissue Res. 224, 269–290.
Watson, G.M., Hessinger, D.A., 1994. Evidence for calcium channels involved in regulating nematocyst discharge. Comp. Biochem. Physiol. Comp. Physiol. 107, 473–481.
Weber, J., 1989. Nematocysts (stinging capsules of Cnidaria) as Donnan-potential-dominated osmotic systems. Eur. J. Biochem. 184, 465–476.
Weber, J., 1990. Poly(gamma-glutamic acid)s are the major constituents of nematocysts in *Hydra* (Hydrozoa, Cnidaria). J. Biol. Chem. 265, 9664–9669.
Weber, J., 1991. A novel kind of polyanions as principal components of cnidarian nematocysts. Comp. Biochem. Physiol. A 98, 285–291.
Weber, J., et al., 1987a. Detection of high concentration of Mg and Ca in the nematocysts of various cnidarians. Experientia (Basel) 43, 1022–1025.
Weber, J., et al., 1987b. Some physical and chemical properties of purified nematocysts of *Hydra attenuata* Pall. (Hydrozoa, Cnidaria). Comp. Biochem. Physiol. B Comp. Biochem. 88, 855–862.
Weber, J., et al., 1990. The nematocyst extract of *Hydra attenuata* causes single channel events in lipid bilayers. Toxicon 28, 403–409.
Westfall, J.A., et al., 1983. Ultrastructure of the dinoflagellate *Polykrikos*. I. Development of the nematocyst–taeniocyst complex and morphology of the site for extrusion. J. Cell Sci. 63, 245–261.
Wood, R.L., Novak, P.L., 1982. The anchoring of nematocysts and nematocytes in the tentacles of *Hydra*. J. Ultrastruct. Res. 81, 104–116.