

# Subcellular Centrin Localization within Distinct Compartments of *Vorticella convallaria*

Katarzyna Konior, Suzanne M. McCutcheon, and <sup>1</sup>Howard E. Buhse, Jr.

Department of Biological Sciences,  
University of Illinois at Chicago, Chicago, Illinois 60607

<sup>1</sup>Corresponding Author

Phone: 312-996-2997; FAX: 312-996-2805; e-mail: spasmin@uic.edu

## ABSTRACT

The stalked ciliated protozoan, *Vorticella convallaria*, uses a mode of contraction that differs from most other eukaryotic motile systems. Because calcium alone induces this contraction, we have undertaken the molecular characterization of the calcium-binding proteins, centrin/spasmins, associated with the contractile organelles in this organism. Many species have evolved centrin/spasmin multi-gene families with the prediction of an increased range of functions among these organisms. To define these functions in *V. convallaria*, we have initiated immunolocalization studies at the light and the ultrastructural level utilizing anti-centrin and anti-stalk antibodies. We have localized these centrin/spasmin antibodies to various contractile cytoskeletal structures within the cell and our transmission electron microscopy localization data indicate a further restriction to compartments within these organelles. The results obtained from this study allowed us to present a model in which we hypothesize the mechanism of contraction and relaxation in this ciliated protozoan and allows us to begin to dissect the function of this multi-gene family.

**Key Words:** Calcium-binding proteins, calcium-induced contraction, contractile organelles, fibrillar mass, membrane-bounded tubules, myoneme, nanofibers, spasmoneme

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## INTRODUCTION

*Vorticella convallaria* is a polymorphic, ciliated protozoan that spends the majority of its life cycle as an actively-feeding, sessile cell attached to a substrate by its contractile stalk (Fig. 1A). In nature, a mechanical stimulation of these cells results in a rapid contraction of the cell body and stalk (Fig. 1B). This contraction is brought about by an integrated, cytoskeletal fiber system (myonemes and spasmoneme) that transverses from the oral opening, through the cell body and terminates at the end of the stalk (Amos 1972). The myonemes are the fiber system within the cell body that are bundled together to form the spasmoneme, the stalk's contractile organelle (Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). Ultra-structural studies showed the myonemes and the spasmoneme

are morphologically identical (Allen 1973; Amos 1972; Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). Additionally, these studies revealed two distinguishable regions within these organelles: a “fibrillar mass” composed of longitudinally oriented 2-5 nm fibers and “membrane-bounded tubules” evenly distributed throughout the fibrillar mass (Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). Interestingly, the membrane-bounded tubules are also filled with longitudinally oriented 2-5 nm fibers (Allen 1973; Amos 1972). The biochemical composition of these fibers and the role they play in the fibrillar mass and within the membrane-bounded tubules are still unknown. Our research is focused on understanding the molecular basis of these fibers and their possible function in this unique contractile system.

During contraction, the stalk coils to 20-30% of its extended length in 2-4 milliseconds and biochemical studies have demonstrated that this contraction is calcium-driven and ATP-independent (Amos 1971; Amos 1972; Hoffman-Berling 1958). Amos showed that a change from  $10^{-8}$  M to  $10^{-6}$  M calcium was sufficient to induce contraction of isolated stalks (Amos 1971). Supporting the importance of calcium in contraction, Carasso and Favard (1966a; 1966b) localized calcium within the membrane-bounded tubules and postulated a calcium storage/release role for this compartment. Biochemically, Amos et al. (1975) determined that the major constituents of the spasmoneme were small, acidic calcium-binding proteins, denoted spasmins. He proposed they formed the 2-5 nm fibers found within this organelle. Maciejewski et al. (1999) isolated a *Vorticella* spasmin cDNA and showed its evolutionary relatedness to another family of small, acidic EF-hand calcium-binding proteins, centrins.

It is now well-established that centrin proteins are present in all eukaryotic cells and involved in a number of processes in the cell, but its molecular mechanism of action is still poorly understood. Centrins are localized to the centrioles/basal bodies where they function in the duplication (Baum et al. 1986; Koblenz et al. 2003; Salisbury et al. 2002; Stemm-Wolf et al. 2005) or the proper positional orientation of this organelle (Ruiz et al. 2005). Additionally, centrins have been associated with a number of other cellular processes: DNA repair via nucleotide excision repair (Araki et al. 2001; Molinier et al. 2004), nuclear mRNA export (Fischer et al. 2004), and signal transduction (Pulvermuller et al. 2002). Finally, centrins are integral components of calcium-triggered contractile fibers (Hart and Salisbury 2000; Levy et al. 1996). In many protists, these fibers systems have been expanded into elaborate contractile cytoskeletons with a concomitant increase in the number of centrin genes. For instance, in *Paramecium*, the contractile cytoskeleton (the infraciliary lattice) is mainly composed of nine centrin subfamilies and two subfamilies of a high molecular weight centrin-binding Sfi1p-like protein (Gogendeau et al. 2007; Gogendeau et al. 2008). The recent identification of Sfi1p and Sfi1p-like proteins suggests that the centrin-associated contractile fibers are composed of two units (Kilmartin 2003; Salisbury 2004). This model proposes a Sfi1p/centrin fiber with Sfi1p constituting the flexible backbone and centrins inducing the calcium-regulated contraction. The conformational change in the centrin protein, upon binding calcium, exerts torsional forces along the Sfi1p-backbone and results in a local twisting, bending, and shortening of the fiber. Recent support for this model has been demonstrated in the contractile infraciliary lattice of *Paramecium* where Sfi1-like proteins and various centrins mediate a calcium-responsive contraction of the cytoskeletal network (Gogendeau et al. 2007). In *Vorticella*, a similar expansion of a centrin gene family has been identified and consists of at least

one spasmin and six centrins (Maciejewski et al. 1999; McCutcheon et al. 2002). However, the exact nature of the 2-5 nm fibers, the function of the multiple centrins/spasmins or the presence of a *V. convallaria*-like Sfi1p are unknown. Additionally, the morphology of *Vorticella*'s cytoskeleton is more complex with membrane-bounded tubules embedded within the fibrillar mass. It has been proposed that the membrane-bounded tubules are calcium storage/release compartments but no explanation was given for the presence of 2-5 nm fibers within the tubules. The various fibers systems within *Vorticella* make it a good organism to address the various roles of centrins/spasmins within the contractile fiber and may yield insights into their mode of action. Therefore, we initiated an ultra-structural study of two *V. convallaria* centrin/spasmin proteins to begin to address these questions. Our results will show that both *V. convallaria* centrin/spasmin localize to the contractile cytoskeleton yet have a differential distribution within these organelles. A novel model incorporating the complex morphology with the current view of centrin/spasmin-based contraction is discussed.

## MATERIALS AND METHODS

### Cell culture

*Vorticella convallaria* cells were grown in a bacteria-containing medium of boiled wheat grass according to the methods of Vacchiano et al. (1991). We obtained large number of cells ("rejuvenated cells") by replacing the culture medium with fresh medium and allowing cell growth for 48 hrs, replacing the medium again and shaking the culture for 24 hrs. All cells used in our experiments were "rejuvenated cells."

### Antibodies

1F5-antibody is a mouse monoclonal antibody produced against isolated *V. convallaria* stalk protein (Vacchiano 1992). N-termVcCentrin4-antibody, a polyclonal rabbit antibody, was raised against a synthetic peptide of the derived N-terminal sequence of VcCentrin4 (McCutcheon et al. 2002). FITC-conjugated goat anti-rabbit and anti-mouse antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Gold conjugated goat anti-rabbit (5 nm) and anti-mouse (15 nm) antibodies were purchased from Ted Pella, Inc. (Redding, CA). Alkaline Phosphatase (AP)-conjugated anti-rabbit antibody was purchased from Promega Co. (Madison, WI) and AP-conjugated anti-mouse antibody was purchased from Jackson ImmunoResearch Laboratories. 1F5-antibody, a mouse monoclonal antibody detects a recombinantly expressed spasmin protein (Vacchiano 1992).

### Electrophoresis and western blot

*V. convallaria* protein preparations from whole cell lysates were isolated using Tri Reagent (Molecular Research Center, Inc. Cincinnati, OH) according to manufacturer's instructions. BioRad Tris-HCl Criterion Precast Gels (18%) were used for SDS-gel electrophoresis and for western analysis. The gels were electrophoresed at a constant voltage of 200 V for 1hr 15 min. Following electrophoresis, the separated proteins were transferred to Immuno-blot PVDF membrane (Bio-Rad Laboratories, Hercules, CA) using the BioRad Criterion Blotter and processed for western analysis using BioRad Immun-Star<sup>TM</sup>AP Substrate according to manufacturer's instructions. The primary antibody dilutions were as follows; 1:2,000 for N-termVcCentrin4-antibody and 1:100 for 1F5-anti-

body and 1:6,500 for anti-rabbit IgG-AP conjugate and 1:5,000 for anti-mouse IgG-AP conjugate.

### **Immunofluorescent studies**

For immunofluorescent studies, rejuvenated cells (see above) were allowed to attach overnight to coverslips. Prior to fixation, the cells were exposed to relaxation buffer (10 mM EGTA, 50 mM Tris-base, 10 mM KPO<sub>4</sub>, 7.5 mM NH<sub>4</sub>Cl, 240 μM KCl, 240 μM MgSO<sub>4</sub>, 0.2% Triton X-100) for two min. They were then fixed in 100% methanol containing 100 μl of 24 mM MgSO<sub>4</sub> and 100 μl of 24 mM KCl per 10 ml at -20°C for 10 min. Following three 5-min washes in modified phosphate buffered saline (PBS) buffer (130 mM NaCl, 2 mM KCl, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, 2 mM KH<sub>2</sub>PO<sub>4</sub>, 10 mM EGTA, 2 mM MgCl<sub>2</sub>, 0.1% Tween 20 at pH 7.4), the attached cells were incubated in the primary antibody (1:1,000 for N-termVcCentrin4-antibody and 1:10 for 1F5-antibody) for one hr. Cells were then washed three times in modified PBS buffer for 10 min and incubated in secondary antibody (1:500 dilution for both antibodies) for 30 min. Following the three 10-min washes in PBS, the cells were mounted in Vectashield mounting medium (Vector Laboratories Inc., Burlingame, CA). The treated coverslips were placed on glass slides and viewed using a ZeissAxioskop microscope equipped with phase and fluorescent objectives.

### **Transmission Electron Microscopy**

*V. convallaria* cells were fixed in 2% paraformaldehyde, 0.1% glutaraldehyde, and 0.1 M cacodylate (pH 7.2), then transferred through a series of acetone dehydrations (30, 50, 75, 90, and two washes in 100% acetone) under vacuum in a PELCO BioWave™ 34700 microwave oven (Ted Pella, Inc., Redding, CA). Each cycle consisted of 40 sec at 100% power followed by one min at 0% power. LR White Resin (London Resin Company Ltd., Reading, Berkshire RG7 4YG England) infiltration consisted of three changes (10 min at 100% power, under vacuum) of acetone to resin: 2:1, 1:1, 1:2. The resin was polymerized at 80°C for 45 min without vacuum. Eighty nm sections were produced using a Reichert Ultracut E microtome (Leica, Deerfield, IL) and collected on 200-mesh nickel grids (Ted Pella, INC., Redding, CA) for immuno-gold processing. The grids were blocked for 45 min in blocking buffer (0.8% BSA, 0.1% IGSS quality gelatin, 5% non-immune serum in PBS, pH 7.4) and washed twice for five min each using wash buffer (0.8% BSA, 0.1% IGSS quality gelatin in PBS, pH 7.4). The grids were then incubated with primary antibodies overnight [N-termVcCentrin4 (1:50, 1:100 dilution of 5 nm gold conjugated anti-rabbit) and 1F5 (1:2.5, 1:75 dilution of 15 nm gold conjugated anti-mouse)]. Four 5-min washes in wash buffer were applied followed by incubation with gold-conjugated secondary antibodies for four hrs. Again, the grids were taken through six-5 min washes in wash buffer and two-5 min washes in PBS (pH 7.4). The grids were then exposed to 2% glutaraldehyde in PBS (pH 7.4) for ten minutes and washed two times in double deionized water for five minutes. The grids were post-stained in 2% aqueous uranyl acetate for five min, followed by one min in lead citrate and viewed on a JOEL 1200E electron microscope. The distribution of labeled antibodies was analyzed by examining printed micrographs.

In order to determine the respective areas occupied by the fibrillar mass and the tubules, *Vorticella* cells were fixed under conditions that are optimal for staining membranes using a protocol by David H. Hall. The protocol is found at the following website:

<http://neuroscience.aecom.yu.edu/labs/halllab/wormem/methods.htm>. Following fixation, eighty nm sections were produced and viewed on a JOEL 1200E electron microscope. Ten micrographs (8 X 11 in) were enlarged 25 times and the percent ratio of membrane-bounded tubules to fibrillar mass within the spasmoneme was determined using an area meter model LI-3100 (LI-COR, Inc., Lincoln, NE). The total surface area of the spasmoneme was measured then the area of the membrane-bounded tubules and fibrillar mass was determined.

## RESULTS AND DISCUSSION

Previously, we identified a multi-gene family of centrin/spasmin (Maciejewski et al. 1999; McCutcheon et al. 2002; Vacchiano 1992) proteins in *V. convallaria*. Because our primary interest is concerned with dissecting the role of centrins/spasmins in cytoskeletal contraction, we focused initially on localization (by immunofluorescence) and distribution (by immuno EM) of two centrin/spasmin proteins using two antibodies, N-termVcCentrin4-antibody and 1F5-antibody. The 1F5-antibody is one of several monoclonal antibodies isolated from a mouse monoclonal antibody screen using enriched stalk proteins as the antigen (Vacchiano, 1992). The 1F5-antibody was characterized and detected a *V. convallaria* calcium-binding protein (Vacchiano, 1992). Subsequently, this antibody was shown to recognize a recombinantly expressed *V. convallaria* spasmin protein (Maciejewski et al., 1999). Western blot analysis, using protein from *Vorticella* whole cell lysates, was performed to confirm that each antibody recognized distinct *Vorticella* centrin/spasmin proteins. The western blot analysis (Fig. 2) indicates that N-termVcCentrin4-antibody detects a single protein with a relative molecular weight of 22.5 kDa. 1F5-antibody recognizes two proteins with relative molecular weights of 23 and 19.6 kDa. Since the 1F5-antibody recognizes two proteins, the epitope is likely to a conserved calcium-binding domain. This data combined with previous results indicates that these antibodies recognize different *V. convallaria* centrin/spasmin proteins.

Since each antibody recognizes a distinct protein or a unique subset of proteins, these antibodies were used in cellular localization studies. At the light microscopy level, two contractile organelles, the myonemes and spasmoneme, are detected by both antibodies (Fig. 3). The myonemal fibers transverse the cell body and as they approach the posterior end (scopular region) of the cell body, they bundle together to form one massive spasmoneme that traverses through the length of the stalk. Although these contractile fibers are localized to different parts of the cell, cell body versus stalk, they are morphologically identical and perform the same function which is rapid contraction of the whole organism (Allen 1973; Amos 1972; Fauré-Fremiet et al. 1956; Sotelo and Trujillo-Cenóz 1959). The immunofluorescent studies revealed that the myonemal fibers of the cell bodies are strongly labeled by both N-termVcCentrin4-antibody and 1F5-antibody (Fig. 3). Using the descriptive myonemal fiber system developed for another peritrich ciliate *Campanella umbellaria* (Shi et al. 2004), our study shows N-termVcCentrin4-antibody labels the *Vorticella* myonemal system composed of longitudinal fibers running from the base of the peristomial disc/lip of the cell body to the junction between the scopular lip and the stalk (Fig. 3). The labeling pattern reveals that the longitudinal myonemes are uniformly distributed around the cell body with a separation of approximately 1.4  $\mu\text{m}$ , and they bifurcate along their length forming interconnecting branches linking individual longitudinal myonemes to one another resulting in a web-like fiber network. The longitudinal myo-

nemes are, in turn, connected with the peristomial myonemes that circumscribe the anterior part of the cell. In the contracted state, when the oral cilia are retracted inside the cell, the circular myonemes are most prominent. When the cell is relaxed, very delicate anastomosing fibers linking the peristomial myonemes are observed. A weak fluorescent signal is detected at the aboral end of the cell body localizing to the ciliary wreath. Additionally, the N-termVcCentrin4-antibody localizes to the spasmoneme but the labeling is only revealed in the parts of the cell where the sheath was dislodged from the cell body. (At this point, we do not know why the labeling of the spasmoneme is only seen when the sheath is removed.)

The overall labeling pattern for the 1F5-antibody is similar to the N-termVcCentrin4-antibody, albeit there are minute differences. First, though both antibodies label the whole length of the spasmoneme, the 1F5-antibody labels the spasmoneme without removing the extracellular sheath as was necessary to localize N-termVcCentrin4-antibody to the spasmoneme. Second, the peristomial ring formed by the myonemes does not fluoresce as strongly as with the N-termVcCentrin4-antibody. With the exception of these two minor variations, we were unable to discern any other differences between the localization of these antibodies at the light microscopic level. At this resolution level, the colocalization of these proteins to the same subcellular compartment was unclear, so we initiated ultrastructural studies to further define the localization of these two antibodies.

The most interesting finding is that our data show a differential localization of these proteins to distinct sub-compartments within the spasmoneme (Fig. 4-7). 1F5-antibody localizes almost exclusively to the fibrillar mass of the spasmoneme, while the N-termVcCentrin4-antibody labels both the fibrillar mass and the membrane-bounded tubules. Quantification of the gold particle distribution showed that 26% of N-termVcCentrin4-antibody grains are located inside the membrane-bounded tubules and 68.5% localize within the fibrillar mass (Fig. 8). For the 1F5-antibody, 93.5% of the gold particles localized to the fibrillar mass and only 6.5% were distributed inside the membrane-bounded tubules (Fig. 8). Localization of centrin/spasmin proteins within the fibrillar mass, the major contractile force of the spasmoneme, is consistent with their association with contractile fibers. However, (even though the membrane-bounded tubules contain a fibrous core) the localization of a centrin protein within the membrane-bounded tubules would not be predicted from previous models of the tubules as simple calcium storage/release compartments (Carasso and Favard 1966a; 1966b). Based on this observation, we hypothesize that besides a calcium storage/release role, the membrane-bounded tubules have a structural function within the spasmoneme and a centrin/spasmin protein plays an important role in this process.

If the membrane-bounded tubules have a structural role, then they should constitute a significant cross-sectional area of the spasmoneme. Results from our morphological investigation, a finding consistent with previous studies, revealed the spasmoneme is composed of a fibrillar mass and membrane-bounded tubules containing 2-5 nm fibers (Fig.7) (Allen 1973; Amos 1972). Our analysis of these subcomponents showed that the membrane-bounded tubules represent approximately 15% and the fibrillar mass 85% of the area of the spasmoneme. Additionally, the membrane-bounded tubules appear continuous through the whole length of the spasmoneme. The even dispersal of the membrane-bounded tubules, the significant cross-sectional area occupied by them, and the

longitudinal extension of these structures throughout the spasmoneme suggest a potential structural function for these substructures in addition to the proposed calcium storage/release function.

We propose a novel, testable model that incorporates the calcium storage/release function of the membrane-bounded tubules and explains the presence of fibers within the fibrillar mass and in the membrane-bounded tubules and the distribution of the centrin/spasmin antibodies. In our model, the membrane-bounded tubules serve two roles, as calcium storage/release sites and as structural support for the spasmoneme. This dual function facilitates both the contraction (calcium release, fiber disassembly) and extension (calcium uptake, fiber reassembly) of the spasmoneme. We propose that the tubular fibrils are formed by self-assembled centrin/spasmin proteins (Wiech et al. 1996; Yang 2006; Zhao et al. 2009) while the fibrillar mass fibers are composed of bundles of Sfi1-like proteins with bound centrin/spasmin proteins analogous to the contractile infraciliary lattice in *Paramecium* (Gogondeau et al. 2007). During stalk contraction, calcium released from the membrane-bounded tubules into the fibrillar mass initiates contraction through cumulative conformational changes of the fibrillar fibers caused by calcium binding to centrin/spasmin which in turn initiates torsional forces along the Sfi1p-backbone resulting in their twisting and shortening as first proposed by Salisbury (Salisbury 2004). Within the membrane-bounded tubules following calcium release, the aggregated centrin/spasmin fibers disassemble and the membrane-bounded tubules lose their rigidity offering minimal resistance during spasmonemal contraction. During stalk relaxation, the calcium concentration within the tubules increases, initiating self-aggregation of centrin/spasmin proteins into a meshwork of fibers that lends structural integrity and assists in the lengthening of the stalk by stiffening of the spasmoneme. The self-assembly of centrins (Yang 2006; Zhao et al. 2009) and aggregation in elevated calcium has been reported (Wiech et al. 1996; Yang 2006). Therefore, the centrin/spasmin proteins with their calcium-binding properties would participate in both the contraction and the relaxation cycles of this unique contractile system. To test our hypothesis, we plan to identify and characterize a *Vorticella*'s Sfi1 protein(s). Our model predicts the Sfi1p will localize exclusively to the fibrillar mass.

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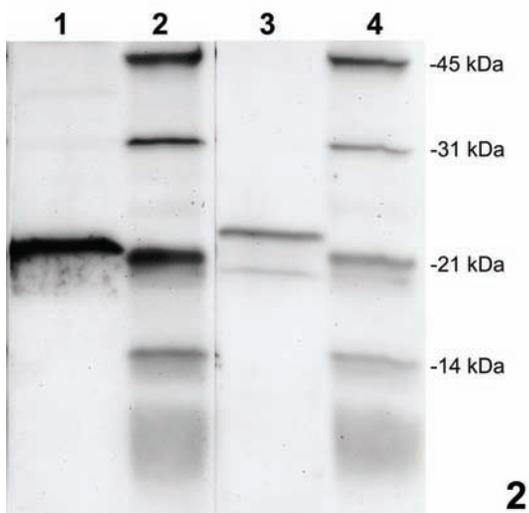
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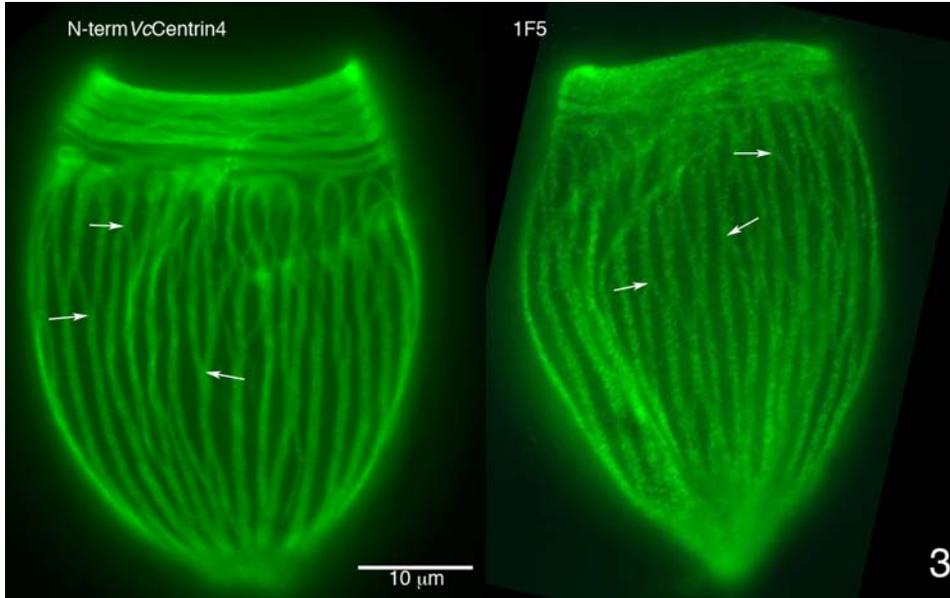
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**Figure 1.** Phase contrast images of *Vorticella convallaria*. A. Phase contrast image of a non-contracted fully extended cell. B. Phase contrast image of a fully-contracted cell.



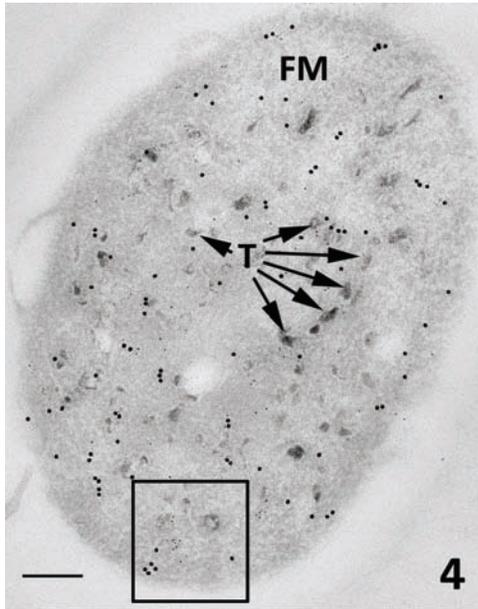
**Figure 2.** Immunodetection of centrin antibodies in *Vorticella convallaria*. Western blot analysis of N-termVcCentrin4 and 1F5 antibodies. Lanes 1 and 3 contain *V. convallaria* whole cell lysates. Lanes 2 and 4 are standard markers whose molecular weights are indicated on the right. Lane 1 was probed with an anti-N-termVcCentrin4-antibody and detected with Alkaline Phosphatase-conjugated anti-rabbit antibody. Lane 3 was probed with an anti-stalk 1F5-antibody and detected with Alkaline Phosphatase-conjugated anti-mouse antibody.



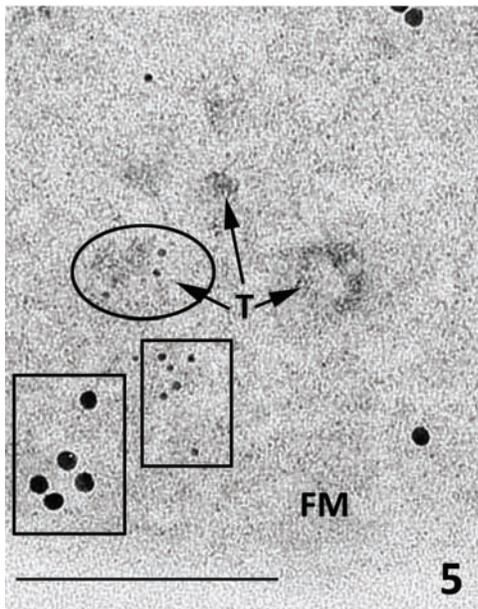
**Figure 3.** Contractile myonemes of *V. convallaria* shown at the light microscopy level. The cells were labeled with an anti-N-termVcCentrin4-antibody or with an anti-stalk 1F5-antibody and detected with FITC-conjugated goat anti-rabbit antibody and FITC-conjugated goat anti-mouse antibody, respectively. For clarity, only the cell bodies are shown. The cells are oriented with the oral apparatus toward the top of the figure. Arrows indicate bifurcations along the longitudinal myonemes forming a web-like fiber network. Scale bar = 10 μm.

**Figures 4-7.** *Vorticella convallaria* spasmoneme ultrastructure.

**Figures 4 & 5.** TEM immuno-localization of N-termVcCentrin4 (1:50, 1:100 dilution of 5 nm gold conjugated anti-rabbit) and 1F5 (1:2.5, 1:75 dilution of 15 nm gold conjugated anti-mouse) antibodies within the spasmoneme. FM denotes fibrillar mass. T designates membrane-bounded tubules. Individual tubules are indicated by arrows. Individual tubules are indicated by arrows.

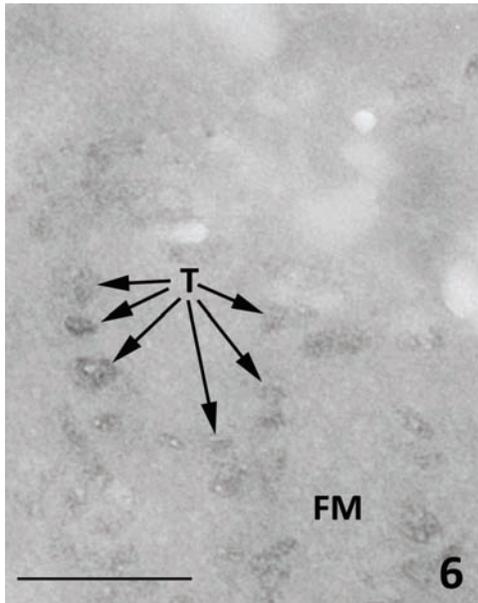


**Fig. 4.** Cross-section of the spasmoneme showing distribution of gold particles for double labeling with N-termVcCentrin4 and 1F5 antibodies. At the ultrastructural level, the spasmoneme consists of two morphologically distinct compartments: the electron dense membrane-bounded tubules and the less dense fibrillar mass. Mag.= 20 K. Scale bar = 200 nm.

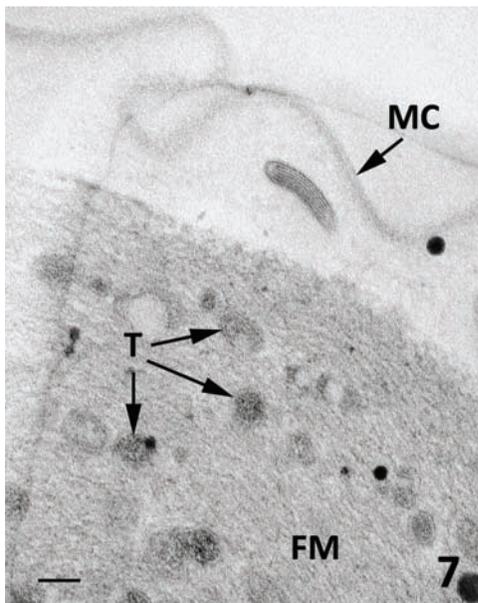


**Fig 5.** Higher magnification of the boxed area in Fig. 4. N-termVcCentrin4 and 1F5 antibodies have distinct localizations within the spasmoneme. Their localization is indicated by ovals (within membrane-bounded tubules) and rectangles (within fibrillar mass). N-termVcCentrin4 antibody (5 nm gold) is found within the membrane-bounded tubules and the fibrillar mass. 1F5 antibody (15 nm gold) is localized almost exclusively to the fibrillar mass (see Fig. 8). Scale bar = 200 nm.

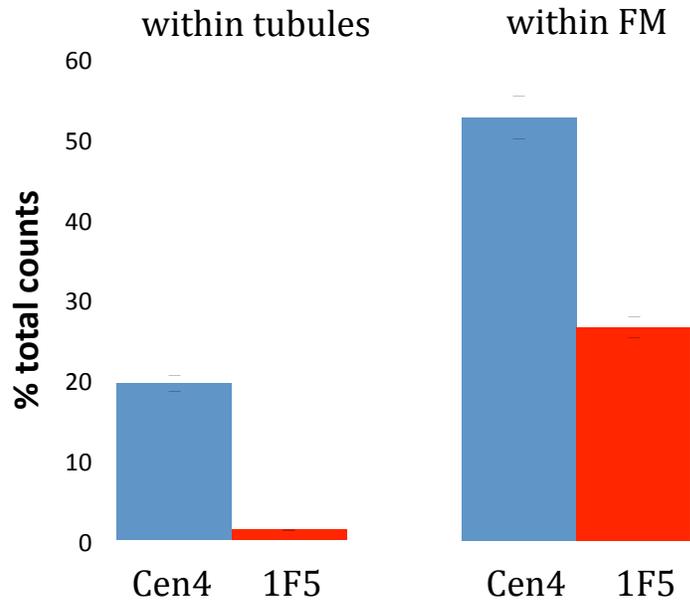
**Figures 4-7, cont'd.** *Vorticella convallaria* spasmoneme ultrastructure.



**Fig 6.** Cross-section of spasmoneme-negative control for double labeling with N-termVcCentrin4 and 1F5 antibodies. Negative control grids were incubated with secondary antibodies only. Mag.= 30 K. Scale bar = 200 nm.



**Fig 7.** Ultrastructural morphology of the spasmoneme. Tangential TEM section showing a portion of the spasmoneme. The tubules are membrane-bounded. The membranes appear as halos around these electron dense structures which stand out from the background of fibrillar mass. FM denotes fibrillar mass. MC indicates the triple membrane complex (plasma membrane plus inner and outer membranes of alveolar sacs), a feature unique to all ciliates. T labels membrane-bounded tubules. Individual membrane-bounded tubules are indicated by arrows. Mag.=60 K. Scale bar =100 nm.



**Figure 8.** Analysis of the distribution of N-termVcCentrin4 antibody and 1F5-antibody within the spasmoneme of *Vorticella convallaria*. The graph depicts results from five separate experiments that include analysis of thirty separate electron micrographs of the Vorticellan spasmoneme. Shown on the left is the distribution on N-termVcCentrin4-antibody (blue) and 1F5-antibody (red) within the tubular compartment. On the right is the distribution of these antibodies within the fibrillar mass (FM) compartment. These data are presented as a percentage of the total number of gold particles that localize to the spasmoneme.