Characterization of a Laminin-like Protein in the Kinetodesmal Fibers of Tetrahymena thermophila

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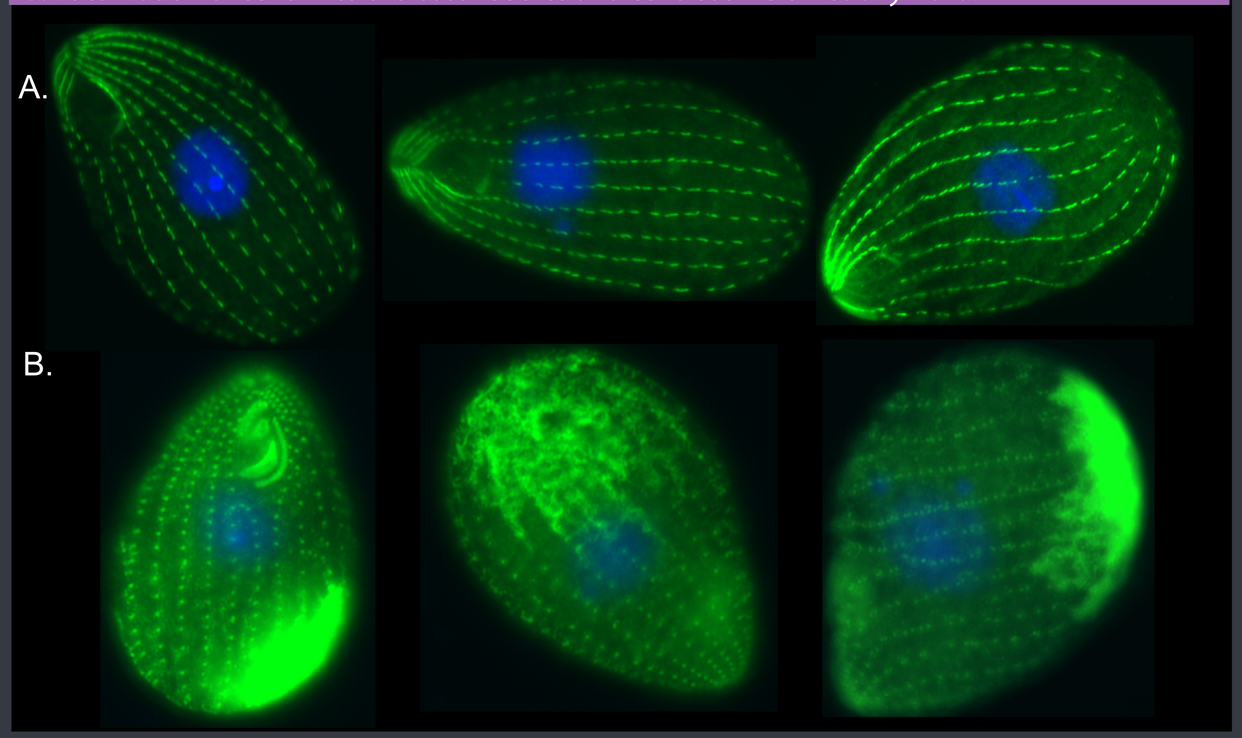
Figure 1. A. Localization of a laminin-like protein to the kinetodesmal fibers of *Tetrahymena*.
B. Localization of centrin to the basal bodies and centrosome of *Tetrahymena*.



A laminin-like protein localizes to kinetodesmal fibers in *Tetrahymena thermophila*. This protein colocalizes with centrin in immunofluorescence as well as CO-IP.

Abstract

Tetrahymena thermophila are free-living ciliated organisms belonging to Kingdom Protista. These organisms possess large numbers of cilia that are used for feeding as well as locomotion. The cilia are attached to microtubule organizing centers (MTOC) called basal bodies, which are rich in centrin, a calcium binding protein present in MTOCs. Because of the mechanical stress that ciliary beating puts on the plasma membrane, these cells have kinetodesmal fibers, composed of bundled coiled-coil proteins which grow from the proximal end of the basal bodies and stabilize the plasma membrane.

Laminin is a cross-linking protein found in the extracellular matrix of animals. Using an antibody against laminin-β1, we found that the antibody localized to kinetodesmal fibers which overlapped the basal bodies stained by the anti-centrin antibody. To determine whether these proteins were in the same complex, we did co-immunoprecipitation using both our anti-centrin and anti-laminin antibodies. Both antibodies precipitated centrin (a doublet at 20 kD) as well as a number of presumed laminin-like proteins between 37 and 50 kD. We also found a number of laminin homologues in the *Tetrahymena* Genome Database. Further studies will be needed to determine which of these proteins is present in the kinetodesmal fiber.

Figure 2. Colocalization of a laminin-like protein with centrin in *Tetrahymena*.
Red: laminin, green: centrin blue: DAPI, yellow (far left) merged image showing colocalization

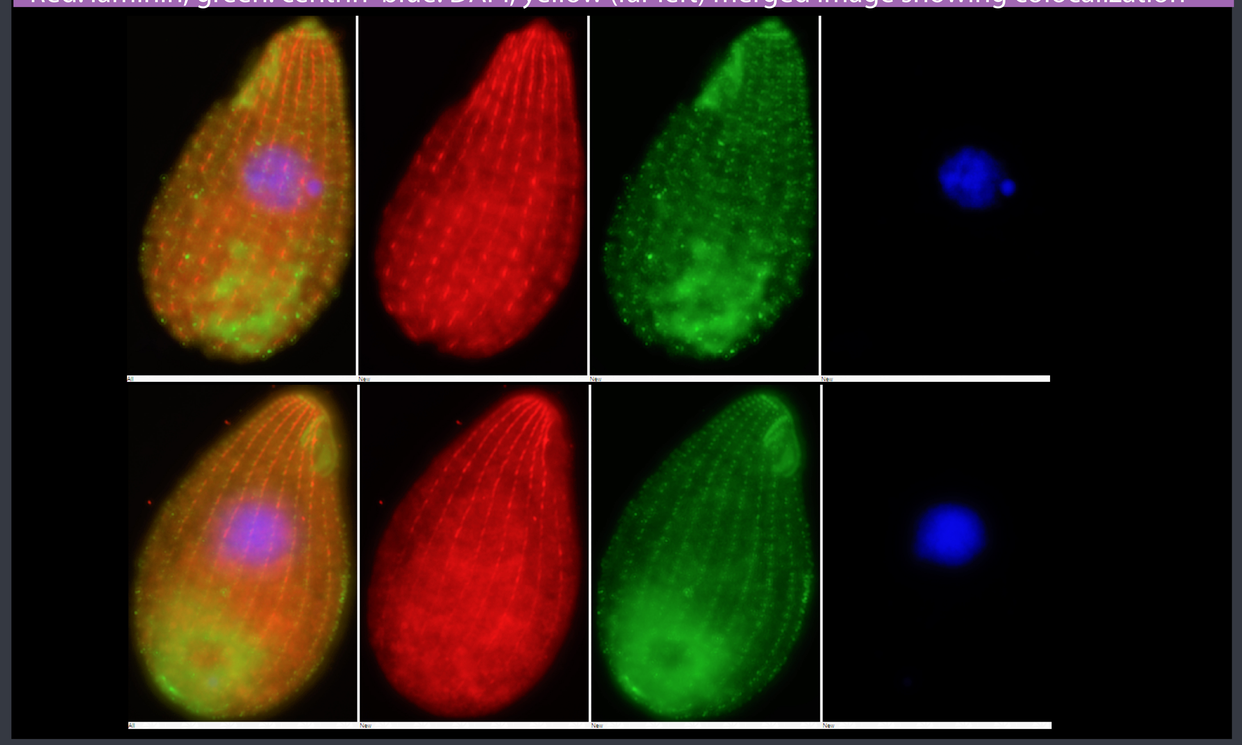
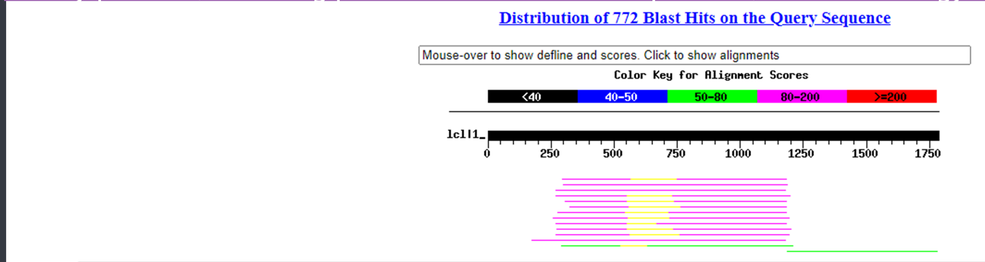


Figure 3. BLAST alignment of laminin (β1) with the predicted *Tetrahymena* proteome shows many proteins, including putative transmembrane proteins, show homology with LAMβ1.



Sequences producing significant alignments:		Score (bits)	E Value
TTHERM_00694440	zinc finger lsd1 subclass family protein	102	4e-21
TTHERM_01416240	GCC2 and GCC3 family protein	93	3e-18
TTHERM_00129550	giardia variant specific surface protein	92	4e-18
TTHERM_00459360	EGF-like domain protein	91	7e-18
TTHERM_00479090	zinc finger lsd1 subclass family protein	91	1e-17
TTHERM_01006510	Guanine nucleotide-binding protein subunit bet...	90	2e-17
TTHERM_00459370	EGF-like domain protein	90	2e-17
TTHERM_00318530	TAP65 LA domain protein	89	4e-17
TTHERM_000096780	transmembrane protein putative	88	6e-17
TTHERM_00037650	transmembrane protein putative	88	6e-17
TTHERM_01419330	zinc finger lsd1 subclass family protein	88	6e-17
TTHERM_00478070	zinc finger lsd1 subclass family protein	88	6e-17
TTHERM_000478080	zinc finger lsd1 subclass family protein	88	8e-17
TTHERM_00579250	transmembrane protein putative	87	1e-16
TTHERM_01178660	hypothetical protein	87	1e-16
TTHERM_01485660	REJ domain protein	87	1e-16

Figure 3A. Results of BLAST alignment of laminin (β1) with the proteins listed in the *Tetrahymena* genome database (ciliate.org). Colored lines (top) indicate regions of alignment between the two protein sequences. High bit scores and low E values indicate a high degree of homology between the proteins being compared.

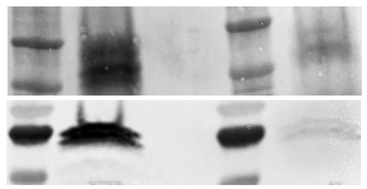


Figure 3B. Western blot shows immunoprecipate of *Tetrahymena* whole cell extract with anti-centrin antibody (lane 2) and anti-laminin antibody (lane 5). Both antibodies pulled down centrin (20 kDa, bottom row, lanes 2 and 5) as well as laminin-like proteins between 37 and 50 kDa (top row, lanes 2 and 5).

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