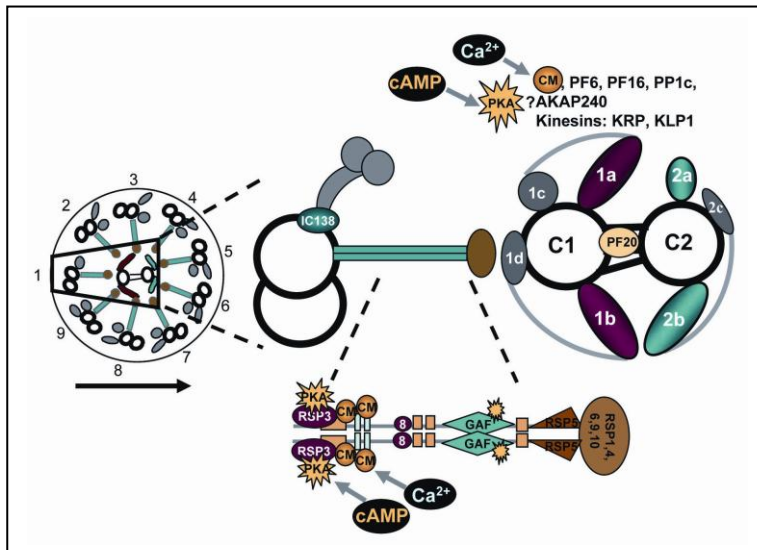




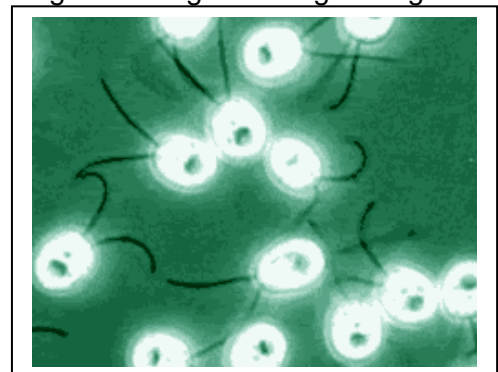
Elizabeth F. Smith
103A Centerra BioLabs
Regulation of Eukaryotic Ciliary/Flagellar Assembly and Motility
<http://www.dartmouth.edu/~esmith>

Cilia and flagella are found on diverse cell types including sperm cells of vertebrates and some invertebrates, unicellular protozoa, and several vertebrate epithelial cell types. In mammals, for example, motile cilia found on cells lining the brain ventricles circulate cerebrospinal fluid; cilia in the respiratory tract sweep debris from the lungs; and oviduct cilia move the fertilized egg to the uterus. In addition, epithelial cilia present early in development are involved in left-right axis determination. Some epithelial cells, such as retinal photoreceptor cells and certain renal epithelial cells, possess immotile cilia which we now know play important sensory roles in cell function. Individuals with motility impaired cilia / flagella or defects in ciliary / flagellar assembly may have any number of serious disorders including hydrocephaly, retinal degeneration, respiratory distress, polycystic kidney disease, and infertility.



Despite the diversity of cell types utilizing these organelles for motile functions, the architecture and the molecules which construct ciliary and flagellar axonemes are highly conserved. The motility of these organelles requires the precise regulation of at least four different dynein motor complexes. This regulation is mediated in part by a signal transduction pathway that includes second messengers such as cAMP and calcium as well as a network of kinases and phosphatases anchored to the axoneme. Our goal is to understand how these signal transduction pathways are integrated

to produce the complex waveforms typical of beating cilia and flagella. Using the biflagellate green alga *Chlamydomonas reinhardtii* as a model system, we have the most complete and advanced array of biochemical, molecular, and genetic techniques with which to study flagellar motility. The high degree of conservation in axonemal structure and composition across species is well illustrated by the finding that the amino acid sequences of virtually all flagellar proteins in *Chlamydomonas* share high sequence identity with proteins predicted from the human genome sequence. In fact, virtually all of the genes in which mutations result in human disease were first identified in *Chlamydomonas*. For a complete list of Smith lab publications, visit our web site.



Current projects include:

1. Identifying calmodulin binding proteins in the axoneme.

For virtually all cell types with cilia and flagella, modulating ciliary and flagellar beating involves changes in intraflagellar free calcium concentration. Using *Chlamydomonas* as a model system, we have identified a role for calmodulin and a calmodulin dependent kinase in a signal transduction pathway that regulates dynein activity. Using a highly specific antibody we

generated against *Chlamydomonas* calmodulin, we have successfully immunoprecipitated at least three different calmodulin containing complexes and have identified the constituent polypeptides of these complexes using mass spectrometry. Current projections include biochemically characterizing these calmodulin binding proteins as well as determining their function using RNAi technology.

2. Modulating motility by post translational modifications.

Substantial evidence indicates that kinases and phosphatases are anchored to the axonemal microtubules and play an important role in regulating motility. We have obtained evidence to indicate that the phosphorylation level of numerous proteins is modulated in response to changes in calcium. Potential projects include determining what kinases are activated in response to changes in calcium. We would also like to identify potential targets of these axonemal kinases assess the function of phosphorylation in modulating motility.

Recent Publications:

- DiPetrillo, C. and E.F. Smith. Pcdp1 (primary ciliary dyskinesia protein 1) and is a calcium-sensitive calmodulin binding protein required for motility. (in preparation for September 2009 submission)
- DiPetrillo, C. and E.F. Smith. (2009) Calcium regulation of axonemal function: Analysis of axonemal calcium binding proteins. *Methods in Cell Biology*. (in press)
- Yang, P. and E.F. Smith. (2008) The flagellar radial spokes. In *The Chlamydomonas Sourcebook. Vol3: Cell Motility and Behavior*. Ed G.B. Witman. Academic Press. pp 207-231
- Dymek, E.E., and E.F. Smith. (2007) A conserved calmodulin and radial spokes associated complex mediates regulation of flagellar dynein activity. *Journal of Cell Biology* 179(3):515-26
- Smith, E.F. (2007) Hydin seek – finding a function in ciliary motility. *Journal of Cell Biology* 176:403-404.
- Dymek, E.E. Tal Kramer, and E.F. Smith. (2006) A kinesin-like calmodulin binding protein localizes to the flagella and microtubule organizing center in *Chlamydomonas*. *Journal of Cell Science* 119:3107-3116
- Wargo, M., E.E. Dymek and E.F. Smith. (2005) PF6 is associated with the flagellar central apparatus as a complex that includes calmodulin. *Journal of Cell Science* 118:4655-4665
- Dymek, E.E. P.A. Lefebvre and E.F. Smith. (2004) PF15 is the *Chlamydomonas* homologue of the katanin p80 subunit and is required for assembly of the flagellar central apparatus. *Eukaryotic Cell* 3(4):870-879.
- Wargo, M., M.A. McPeck, and E.F. Smith. (2004) Analysis of microtubule sliding patterns in *Chlamydomonas* flagellar axonemes reveals dynein activity on specific doublet microtubules *Journal of Cell Science* 117(12):2533-2544.
- DiBella, L.M., E.F. Smith, R. Patel-King, K. Wakabayashi, and S.M. King. (2004) A novel Tctex2-related light chain is required for stability and motor activity of inner dynein arm I1 from the *Chlamydomonas* flagellum. *Journal of Biological Chemistry* 279(20):21666-21676.

For a recent review article describing new found functions for cilia as well as links between cilia and numerous diseases, see:

Marshall WF. 2008 The cell biological basis of ciliary disease. *J Cell Biol.* 180(1):17-21

Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. 2007 *Nat Rev Mol Cell Biol.* 8(11):880-93.

Ainsworth, C. 2007. Tails of the Unexpected. *Nature.* 448(9):638-641

Singla V, Reiter JF. 2006. The primary cilium as the cell's antenna: signaling at a sensory organelle. *Science.* 4;313(5787):629-33.