08

Inner Junction of doublet microtubules in motile and non-motile cilia

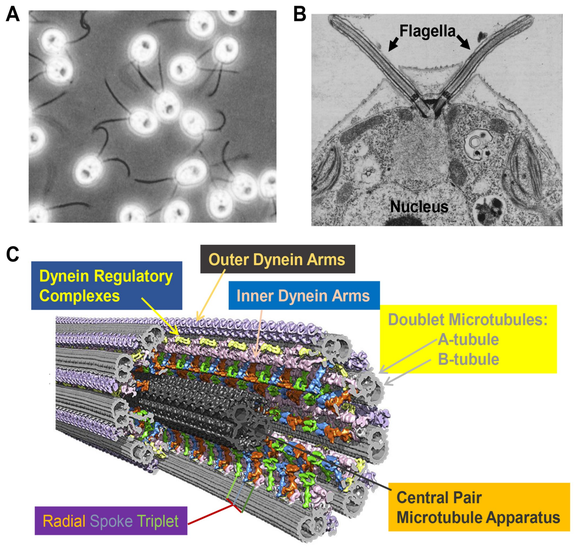
-----Exploring the flagella axoneme of Chlamydomonas reinhardtii as a synthetic template

Hongmin Qin[1], Rene Garcia[1], Timothy D. Phillips[2],

[1] Department of Biology, College of Science *[2] Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences*

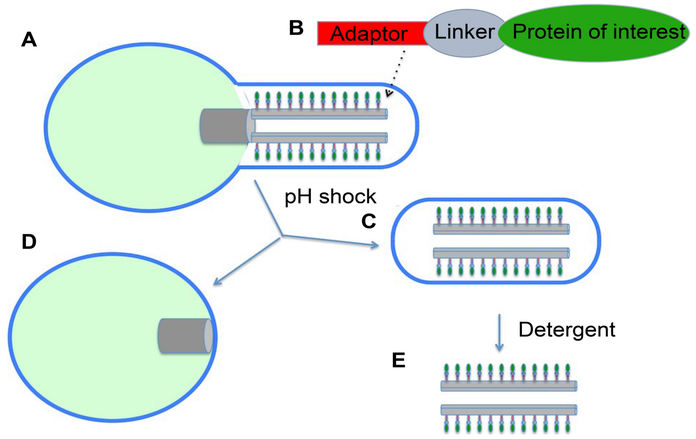
T3: TEXAS A&M TRIADS FOR TRANSFORMATION
A President's Excellence Fund Initiative

1. *Chlamydomonas reinhardtii* is free-living single-cell green algae



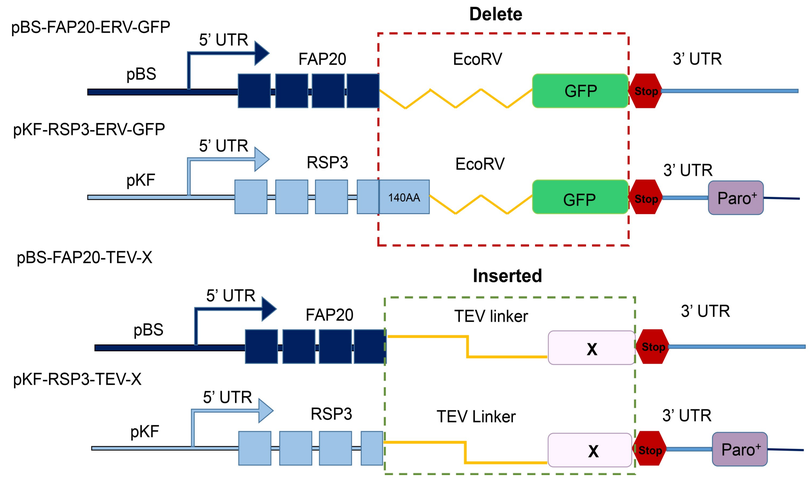
The flagellar axoneme of the green alga Chlamydomonas reinhardtii presents an ideal scaffold for constructing linear protein nanoarrays. The eukaryotic flagellum is a motile structure that consists of a protrusion of the plasma membrane supported by the axoneme, a protein-based assembly consisting of nine doublet microtubules (DMTs) together with several hundred associated proteins involved in building the axoneme and driving flagellar motility. Flagellar proteins are synthesized in the cell body first and then make their way to the flagellum. A complex molecular machinery known as the intraflagellar transport (IFT) system uses a combination of motors and protein chaperones to transport insoluble proteins into the axoneme and incorporate them into the appropriate positions Many different proteins incorporate into the axoneme with fixed spatial repeats, for example, radial spokes and dynein arms binds to the axoneme with an underlying 96 nm periodicity that is generated by molecular rulers aligned to the axonemal lattice . (A) Cells using two flagella (B) to swim. (C) The architecture of substructures within the flagellar axoneme.

2. Harnessing the flagellar axoneme as a biologically self-assembling protein nanoarray.



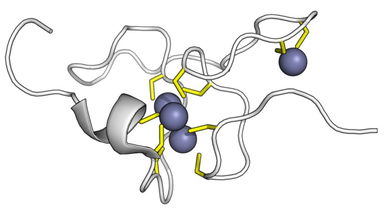
Cell (A) with a protruding flagellum, which consists of an extension of the cell membrane overlaying a protein structural core axoneme. (B) By fusing a protein of interest to one of the axonemal proteins, the axoneme protein can serve as an adaptor to attach many copies of the protein of interest into the axoneme, forming a protein array. The flagellum (C) can be cleanly detached from the cell body by transiently reducing the pH of the media (pH shock), which releases the cell body intact. (D). Treatment of the isolated flagella with detergent leads to removal of membrane, and the axoneme (E) can then be purified by a single centrifugation step. An important feature of this system is that is allows the protein array to be isolated in either a membrane-bound vesicle form (C) or a solvent exposed membrane-less form (E).

3. Constructs for expressing fusion proteins that can be anchored on doublet microtubules.



FAP20 and RSP3 are two doublet microtubule proteins. The constructs of pBS-FAP20-GFP and pKF-RSP3-GFP (Yanagisawa et al., 2014) were modified to express the fusion proteins. The fusion protein was connected to FAP20 or RSP3 with a TEV-linker. The restriction enzyme EcoRV digestion sequence and the GFP gene were deleted in both of the original constructs. In addition, the C-terminal 140 amino acids of RSP3 were deleted as well in the pKF-RSP3-GFP construct. The TEV-linker and the target gene (X), such as βLac, were fused to the C terminus of either FAP20 or RSP3.

4. Engineering strains expressing Metallothionein (MTs) fused to FAP20 and RSP3



MTs are a family of small, highly conserved, cysteine-rich metal-binding proteins that are important for zinc and copper homeostasis, protection against oxidative stress, and buffering against toxic heavy metals.

5. Applications

The cells expressing MT fusion proteins are ready to be used to develop novel biosorbents that will reduce heavy metal exposures from contaminated food and water during an environmental disaster and emergency. These enterosorbents (when administered to humans and animals) will decrease metal bioavailability and toxicity, and can be used to protect vulnerable communities, first responders and cleanup personnel at the site.