New and Notable

Heart of the Beat (the Flagellar Beat, that Is)

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The mechanism that makes a eukaryotic flagellum or cilium beat is one of cell biology's most enduring puzzles. As long ago as 1835, Dr. W. Sharpey (1) noted that the beating of cilia is a confirmed biological phenomenon that warrants further study. Today, we know that the motor protein dynein powers the bending of cilia and flagella. Dynein does this by creating traction between the outer doublets of the microtubule scaffolding called the axoneme that is at the heart of all cilia and flagella. The article by Dr. Charles Brokaw (2) in this issue of *Biophysical Journal* provides new insight into the mechanism of the interaction that generates the flagellar and ciliary beat. The study provides pivotal information on the nature of the dynein tubulin interaction, and is useful to discriminate between the different proposals regarding the mechanism of ciliary and flagellar beating.

One of the more elegant experiments investigating the interaction of dynein and the doublet microtubules in the flagellum was conducted by Aoyama and Kamiya (3). They showed that individual doublets of ''frayed'' flagellar axonemes of the green algae *Chlamydomonas reinhardtii* occasionally will reassociate with each other and establish a kind of beat-like cycle. The doublets adhere to each other, bend, and subsequently separate from each other, from the base to the tip, and then repeat the sequence. This outer-doublet microtubule pair is the simplest flagellar fragment that still shows the ability to construct beat-like oscillations. Therefore, it is likely that it is telling us something important about the oscillation mechanism underlying the beating of the whole flagellar structure. Dr. Brokaw uses images from published studies of the Kamiya group and applies the best biophysical modeling tools to simulate these classic experiments and elucidate the physics of the two-doublet system.

Currently, there are three rather different views of how flagella and cilia convert the action of the dynein motors into a rhythmic beat. The first view is that a complex of regulatory proteins residing on the central pair and radial spokes actively sends signals to the dynein motors through a dynein regulatory complex located on the surface of the outer doublets. It is hypothesized that a regulatory enzymatic cascade originates at the central pair-spoke interaction sites and acts to turn the dyneins on and off in the proper order to produce beating (4). The second view maintains that the dynein molecules themselves are tuned oscillators that can be entrained to synchronize their cross-bridge cycles into waves of activity under the appropriate loading conditions present in the intact flagellum (5). The third view contends that the dyneins are engaged and disengaged by changes in the interdoublet spacing as the axonemal structure is distorted by stress (6). This last view is called the ''geometric clutch'' hypothesis.

The simulations conducted by Dr. Brokaw indicate that the oscillations of two doublets isolated from the intact flagellum can be explained satisfactorily by a mechanism whereby the dyneins detach from their binding sites when the transverse stress between the doublets exceeds the capacity of the dyneins to hold on, as illustrated in Fig. 1. This supports the contention that dynein action can be regulated by the transverse force (t-force) that develops between adjacent doublets. If this mechanism of oscillation also functions in the intact axoneme, it would lend support to the geometric clutch interpretation of the flagellar beat. This does not rule out the possibility that the other major hypotheses could also help explain the generation of the beat, but it does lend credibility to the hypothesis that t-force between the doublets can modulate the activity of dynein. It also tells us that the flagellum possesses a means of generating repetitive cycles of oscillation that is independent of the influence of the central apparatus. The switching mechanism in the two-doublet experiments appears to be more dependent on the t-force acting on the dyneins than on the cargo load the dyneins are carrying.

The authors of the original experimental report (3) dismissed the geometric clutch interpretation of their results as inconsistent with their observations. This was based on the fact that the doublets are often seen to separate when there is no initial curvature, and curvature is necessary for the development of transverse stress. Dr. Brokaw specifically addresses this concern in his analysis and shows that the buckling instability of the member of the doublet pair that is under compression creates a local curvature, as illustrated in Fig. 1, and that the resultant t-force acting on the dynein increases rapidly due to this dynamic instability.

The computational approach used by Dr. Brokaw provides new and valuable quantization of the dynein-tubulin interaction and has the potential to be of value in developing an accurate model of flagellar and ciliary functioning. By fitting the modeled simulations to the experimental data, the analysis gives us a first estimate of the adhesion force with which real dynein motors hold on to the microtubule to which they are bound. It also provides a first look at the kinetics of disassociation and reassociation of the motors with their binding sites. These quantitative parameters of adhesion, disassociation, and reassociation of dynein with tubulin are elements of the geometric clutch model,

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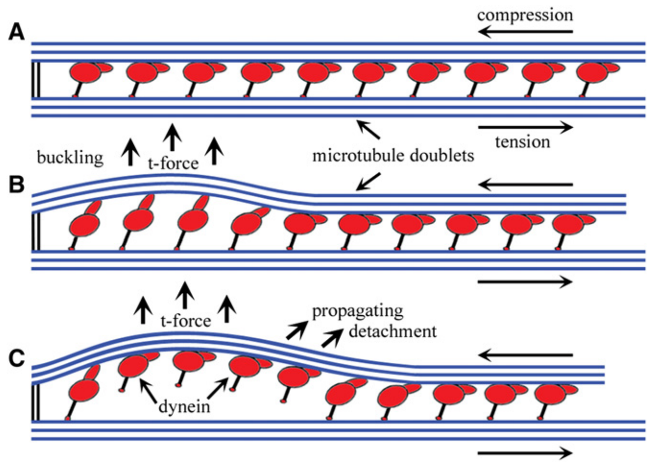

FIGURE 1 Proposed mechanism of dynein dissociation in the two-doublet experiment. (*A*) Two adjacent doublets associate by dynein attachment. Dynein motor activity results in compression of the upper doublet and tension on the lower doublet. (*B*) The doublet under compression undergoes buckling, which creates a curvature and t-force. (*C*) The t-force increases and overcomes the adhesion of the dyneins to produce a spreading wave of detachment.

but to date they have not been amenable to experimental study or to quantization. Dr. Brokaw's approach allows useful information to be mined from the two-doublet studies. Ultimately, this will permit a much better understanding of the kinetics of dynein tubulin interaction and the mechanism of flagellar beating.

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