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EFFECTS OF ATP CONCENTRATION ON THE FORCE-VELOCITY RELATION OF SLIDING MICROTUBULES IN SEA URCHIN SPERM FLAGELLA.

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We studied the force-velocity relation of sliding microtubules in demembranated sperm flagella of the sea urchin *Hemicentrotus pulcherrimus* over a range of Mg-ATP concentration from 3.7 to 350 μM by using the method of Kamimura & Takahashi (Nature,293:566,1981) with some improvements. The shape of the force-velocity curve under 'auxotonic' conditions was independent of the concentration of ATP and appeared almost linear or had a reverse curvature to that of the hyperbolic force-velocity curve of muscle. The sliding velocity under loads of <40% of the maximal sliding force (F_{max}) showed a slight decrease with increasing load. Above 0.4 F_{max}, the sliding velocity decreased linearly and steeply, and abruptly decreased to zero near the maximal force. The power calculated as the product of velocity and force passed through a peak at c. 0.7 F_{max}. This shows that the maximal power is attained at a larger relative load than in muscle. The maximal sliding velocity obtained by extrapolation of the force-velocity curves to zero load showed a Michaelis-Menten type dependence on the Mg-ATP concentration, with a Km of 180 μM and V_{max} of c. 18 $\mu m \cdot sec^{-1}$. The maximal force did not significantly change over a Mg-ATP concentration range 3.7 to 350 μM.

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TRANSITION FROM CILIARY TO FLAGELLAR TYPE MOVEMENT OF *CHLAMYDOMONAS* FLAGELLA INDUCED BY ELECTRICAL STIMULATION.

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When a single *Chlamydomonas reinhardtii* cell was stimulated with an electric pulse (0.2-1.2μA, 1ms) delivered through a suction electrode holding the cell body, the flagella, which normally show a ciliary-type beating pattern, began to beat in a flagellar-type pattern. We filmed the flagellar response with a high speed 16mm camera at 400f.p.s. and analysed the flagellar movement during the transition from the ciliary to flagellar type beating. The transition had a well-defined beginning and was complete in 2-3 beat cycles. At the onset of transition, the length of time for the development of principal and reverse bends at the flagellar base sometimes changed: the principal bends took shorter time to grow before they began to propagate while the reverse bends stayed longer at the base. On the other hand, principal bends that were propagating along the flagellum did not change in curvature and sliding velocity at the onset. The sliding velocity in the distal region of the propagating reverse bends decreased whereas that in the proximal region did not change; the curvature of the propagating reverse bends increased.

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FLAGELLAR MOVEMENTS OF CHLAMYDOMONAS DURING PHOTOSTIMULATION.

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To understand behavior of Chlamydomonas during phototaxis, it is essential to know how flagellar beat pattern changes in response to photostimulation. We have developed a system; a single cell is captured and held immobile in a micropipet while its flagellar movements are recorded by high-speed (240 fields/s), high sensitivity video microscopy using stroboscopic flashes passed through a red filter. In the absence of a stimulus beam, the two flagella generally beat synchronously at 41-48 Hz with periods of asynchronous beating that resulted when the frequency of the trans-flagellum (the one farthest from the eyespot) transiently increased within 3 Hz. When cells were stimulated with a dim white light, the beat frequency of the trans-flagellum increased about 30% after 0.1 s. The waveforms didn't appear to change. Cells stimulated by a brighter white light underwent a photophobic response in which the flagella changed from a ciliary to a flagellar beat pattern about 20 ms after stimulation, and returned to the ciliary mode after 0.5 s. In some cells, beating in the flagellar mode was followed by quiescence in which the flagellar were held nearly straight.

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A SEMINAL PLASMA PROTEIN FACTOR INHIBITS THE FLAGELLAR MOVEMENT OF THE DEMEMBRANATED SEA URCHIN SPERMATOZOA.

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The mammalian seminal plasma contains a factor which inhibits the movement of the reactivated mammalian sperm flagella (Lamirande et al, Biol. Reproduct., 28, 788, 1983). The factor was purified from the boar seminal plasma by means of ion exchange column chromatography and ammonium sulfate cut. The SDSPAGE revealed that the component of the purified fraction which inhibited the movement of the reactivated sea urchin sperm flagella as well as that of the boar was an oligomer containing 16, 13 and 12 KD polypeptides. The factor inhibited the flagellar movement completely at approximately 30 μg/ml. The effects of the factor on beat frequency was very small in the lower concentration than that. The inhibition occurred near the critical concentration. When the concentration of the factor was high the flagellar bend was preserved as if it was frozen (looked like the rigor bend). However, the "frozen bend" propagated very slowly at the early stage of the incubation (less than 1 μm/sec) and stopped propagation within a few minutes maintaining the bend. The factor partially inhibited ATPase activity of dynein. It was likely that the factor increased the shear resistance among the outer doublet microtubules to inhibit the movement.