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Movement of microtubules by single kinesin molecules

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Kinesin is a motor protein that uses energy derived from ATP hydrolysis to move organelles along microtubules. Using a new technique for measuring the movement produced *in vitro* by individual kinesin molecules, it is shown that a single kinesin molecule can move a microtubule for several micrometres. New information about the mechanism of force generation by kinesin is presented.

MYOSIN, dynein and kinesin are motor proteins which share the ability to convert chemical energy derived from the hydrolysis of ATP into mechanical work. This chemomechanical transduction process involves a cyclic reaction of a motor molecule with a cytoskeletal polymer; an actin filament in the case of myosin and a microtubule in the instances of dynein and kinesin. After binding to the filament, the motor protein is thought to undergo a conformational change, the power stroke, that produces an increment of movement. The protein then releases the filament before rebinding at another site along the filament and thus initiating another cycle. Changes in the affinity of the motor protein for the polymer and the timing of the power stroke are believed to be controlled by transitions between the intermediate states in the ATPase cycle[1,2].

To elucidate the molecular events underlying chemomechanical transduction, one must determine the force produced by an individual molecule, the distance through which a filament is displaced on ATP hydrolysis and the transition rates between successive mechanical and biochemical states. Several factors conspire, however, to obscure these molecular details. For example, it is difficult to extrapolate results to the molecular level when dealing with muscle fibres that possess as many as 10^9 myosin molecules, or with cilia that contain in excess of 10^4 dynein molecules. There are also many different cytoplasmic proteins that modify or regulate the actions of the motor proteins themselves. Motility assays that involve either coating a small bead and observing its movement along a filament[3,4], or coating a surface with a purified motor protein and observing the translocation of a complementary purified filament[5,6], have circumvented some of these difficulties. Although it is now possible to perform measurements of force[7] and displacement[4] with such *in vitro* assays, interpretation of the results at the molecular level is precarious owing to uncertainties about the numbers and possible interactions of the motor molecules involved.

We report here the development of an *in vitro* assay in which purified bovine brain kinesin is adsorbed at low density to the glass surface of an experimental chamber and the ATP-dependent movement of individual microtubules is observed by dark-field microscopy. The stoichiometry of the reaction indicates that a microtubule can be translocated for several micrometres by one kinesin molecule, a tetramer[8,9] comprising two light chains (relative molecular mass 62,000 (62K)) and two identical heavy (120 K) chains that terminate in globular, ATP- and microtubule-binding heads[10-14]. By measuring the speed of movement of microtubules driven by known numbers of kinesin molecules, we can delimit several properties of kinesin's cyclic reaction with a microtubule, including the time of attachment between a kinesin molecule and a microtubule, the force against which one kinesin molecule can work and the magnitude of the displacement produced on hydrolysis of one ATP molecule.

Microtubules move at low kinesin density

To determine the number of kinesin molecules required to move a microtubule, we decreased the density of kinesin adsorbed to successive glass surfaces by exposing them to solutions containing kinesin at progressively lower concentrations. In this way we reduced the number of motor molecules that could potentially interact with the microtubules suspended in a solution that was subsequently introduced into the chamber. If only a single kinesin molecule were required, movement would be expected at very low kinesin densities. To the contrary, movement occurred only when the kinesin density exceeded a relatively high threshold value (Fig. 1); kinesin adsorbed directly onto the glass surface of the chamber, from a solution containing a concentration of 100 nM, resulted in the attachment of numerous microtubules which then moved along the glass surface, but microtubules failed to adhere to a surface treated with kinesin at a concentration of 50 nM. Like kinesin[15], both myosin[16] and dynein[17] must exceed a minimum density before supporting filament movement *in vitro.* Because virtually all of the kinesin molecules in such solutions are adsorbed onto a glass surface within the incubation time of 120 s, this threshold for movement corresponds to a kinesin density of 2,000–4,000 μm^{-2} (see Fig. 1 legend for the calculation).

The density threshold could be interpreted to indicate that the movement of a microtubule is a highly cooperative event that requires several kinesin molecules. An alternative explanation is that, when kinesin molecules adsorb to glass, most denature on the surface and are unable to produce movement. Only when the surface is nearly covered might the last few kinesin molecules adsorb in a configuration that can sustain

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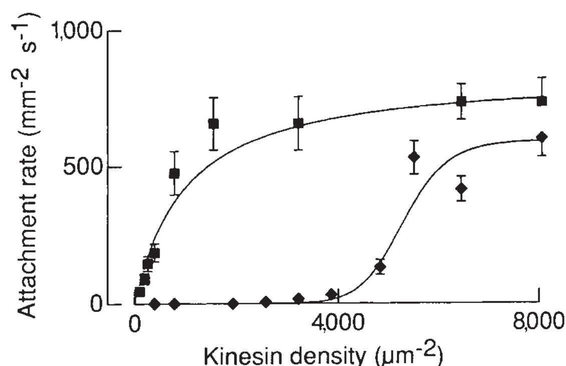
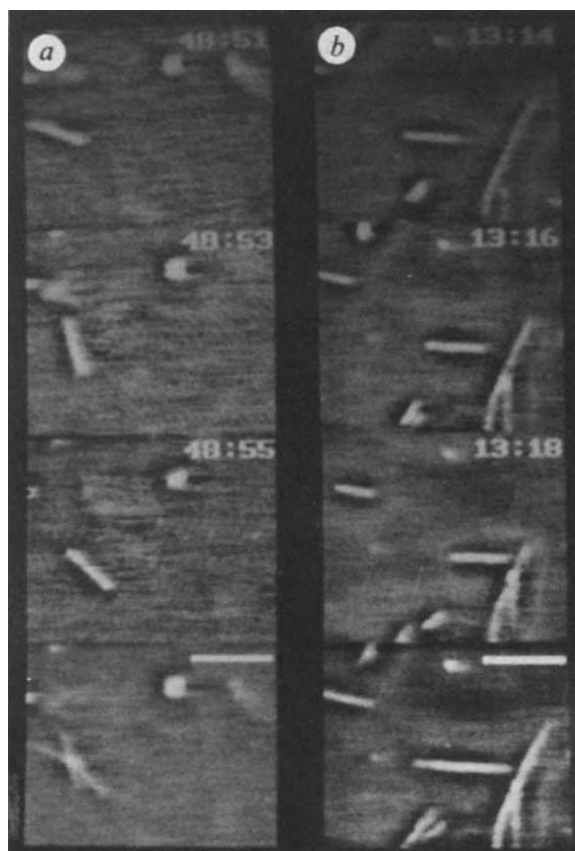


FIG. 1 The rate of attachment of microtubules to a kinesin-coated surface. When kinesin is adsorbed directly onto a glass surface (◆), a threshold density is necessary for microtubules to attach and move. On the other hand, if the glass surface is first pretreated with tubulin and cytochrome *c*, a threshold is no longer apparent and attachment and movement occur at much lower densities (■).

METHODS. The kinesin was prepared at 70–90% purity from bovine brain[23] and its concentration, estimated by gel densitometry with a bovine-serum-albumin standard[23], refers to the tetramer composed of equimolar heavy and light subunits[8,9]. Tubulin of concentration 50 μM, prepared from bovine brain[29], was polymerized in the presence of 4 mM $MgCl_2$, 1 mM GTP, and 10% dimethyl sulphoxide. In 100 μM ATP, the ATP turnover rate per kinesin molecule increased from $0.06\ s^{-1}$ to $1.8\ s^{-1}$ on addition of 7 μM polymerized, dimeric tubulin. This microtubule-stimulated ATPase activity is comparable to that reported by others[22,23]. Experimental solutions were introduced into a chamber, of dimensions 18 mm × 4.5 mm × ~110 μm, composed of a coverslip and a microscope slide (Gold Seal, Clay Adams) separated by greased slivers of coverslip glass. For one curve (◆), the chamber was first filled for 120 s with standard buffer solution (80 mM PIPES, 1 mM EGTA, 2 mM $MgCl_2$, adjusted to pH 6.85 with KOH) into which kinesin had been diluted. Control experiments showed that kinesin adsorbs to the surface of the chamber with a half life of 20–30s, which is roughly the time necessary for a kinesin molecule to diffuse through the depth of the chamber. Before being infused into the chamber, microtubules were diluted ~100-fold to a final concentration of 0.5 μM into the standard buffer solution augmented with 5 μM taxol and 1 mM ATP. The continuous curve is a plot of the equation $(600\ mm^{-2}\ s^{-1})\rho^{12}/(1+\rho^{12})$, in which ρ is the kinesin density (see below) divided by 5,300 μm^{-2}. For the other curve (■), as well as for the experiments shown in the following figures, the chamber was first filled for 120 s with the undiluted tubulin solution to pretreat the surface, then filled for 120 s with the kinesin-containing standard buffer solution to which 4 μM cytochrome *c* (Sigma) had been added. The solution into which the microtubules had been diluted was also augmented with 4 μM cytochrome *c* prior to infusion into the chamber. The continuous curve is a plot of the Langmuir isotherm $(850\ mm^{-2}\ s^{-1})\rho/(1+\rho)$, in which ρ is the kinesin density divided by 1,000 μm^{-2}. The density of kinesin on the glass surface was calculated from the concentration of kinesin, the chamber's volume (~9 μl), and the area of the glass surface (160 mm^2) with the assumption that all the kinesin adsorbed. We confirmed that at least 80% of the kinesin adsorbs within 120 s by introducing the kinesin-depleted solution from one experimental chamber into a second pretreated chamber and measuring the rate of microtubule attachment to the glass surface. The geometry of the chamber minimized the gradient of protein adsorbed to the glass surface when solutions were introduced from one side of the chamber: the chamber's ~100 μm depth permitted the infusion of solutions within ~1 s, a time much shorter than the time required for diffusion to the surface. By measuring the microtubule attachment rate across the chamber we confirmed that over the central 2 mm of the chamber, the region from which the reported data were derived, the density variation was less than 15%. The slide surface of the chamber was viewed in an inverted microscope (IM-35, Zeiss) with darkfield optics (condensor lens, numerical aperture 1.2–1.33, Olympus; 50× objective lens, numerical aperture 0.5–0.85, Nikon) with illumination by visible light from a 100 W mercury-arc lamp (HBO 100W/2, Osram). Images of microtubule movements were projected through a 20× ocular onto a silicon-intensified-target camera (C2400-8, Hamamatsu). The attachment of microtubules was measured by sequentially observing several fields of view (~25 μm × ~35 μm) for times ranging from one to several minutes and counting the number of microtubules that attached to and began to move across the surface. The attachment rate was this number divided by the observation time and the viewing area. Experiments were performed at room temperature (20–23 °C).



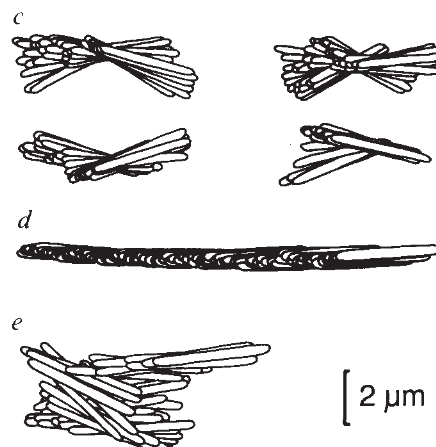


FIG. 2 Photographed video images (*a* and *b*) and tracings of images (*c*, *d* and *e*) of microtubules moving across surfaces coated with kinesin. The three upper panels of (*a*) or (*b*) depict the positions of microtubules at 2 s intervals; the lowest panel shows a superposition of the three images. The tracings present successive video images at 133 ms intervals. In each of the examples in *a* and *c*, for which the kinesin density was 24 μm^{-2}, the microtubule pivoted about a nodal point where a kinesin molecule was presumably located. In three of the traced instances, the microtubule moved until its trailing end nearly reached this nodal point before leaving the surface; the fourth dissociated precociously. The ATP concentrations ranged from 30 μM to 1 mM. At a kinesin density of 2,400 μm^{-2} (*b* and *d*) and an ATP concentration of 100 μM, microtubules moved smoothly and with negligible torsion. A detached microtubule undergoing Brownian motion near the glass surface (*e*) produced an erratic pattern of movement quite distinct from that in the other panels.

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movement. This would explain why the critical density corresponds to an almost complete surface coating by the 80 nm long, 5–15 nm diameter kinesin molecules[11,13,18] and why long microtubules, which would be expected to interact with a larger number of kinesin molecules, require the same critical density as short microtubules.

To test this hypothesis, we attempted to prevent denaturation of kinesin by pretreating the glass surface with other proteins before adsorbing kinesin. Pretreatment with haemoglobin (15 μM), dimeric tubulin (10 μM), or cytochrome *c* (4 μM) enabled microtubules to move at densities less than one-third of those required with untreated glass. After the most successful pretreatment, exposing the surface to tubulin (50 μM) followed by cytochrome *c* (4 μM) and kinesin, the movement of microtubules occurred at kinesin densities as low as $\sim 2\ \mu m^{-2}$. The threshold behaviour was no longer observed and the number of moving microtubules decreased gradually as the kinesin density was reduced (Fig. 1).

The behaviour of individual microtubules moving at low kinesin density on a pretreated glass microscope slide suggested that few motor molecules were participating. Each moving microtubule rotated erratically about a roughly vertical axis through a fixed point on the surface (Fig. 2*a*, *c*), presumably as the result of thermal forces, or of torques produced when a kinesin molecule bound to different protofilaments. When its trailing end reached this nodal point, the microtubule dissociated from the surface and diffused back into solution. By contrast, microtubules exhibited negligible rotation when moving across a surface coated with kinesin at a high density, presumably because the microtubules were restrained by numerous kinesin molecules attached along their lengths (Fig. 2*b*, *d*). Both patterns of movement were distinct from the brownian motion of free microtubules (Fig. 2*e*).

One kinesin molecule moves a microtubule

At low kinesin densities, microtubules thus appear to be attached at only one point, where we infer that at least one kinesin molecule is situated. To determine how many molecules lie at such a point and thus the stoichiometry of the reaction between kinesin molecules and microtubules, we measured the rate at which microtubules attached to and moved across the surface as a function of kinesin density. When the surface was treated with tubulin and cytochrome *c*, the microtubule attachment rate was directly proportional to the kinesin density (Fig. 3*a*); the slope of unity in the double-logarithmic plot is consistent with the hypothesis that the movement reaction requires only one kinesin molecule. Had motion required the cooperation of two or more kinesin molecules, the association rate would have fallen more abruptly as the kinesin density was reduced.

The rate at which microtubules left the surface, the detachment rate, also accorded with the hypothesis that only a single kinesin molecule is required for movement. It can be shown that, if moving a microtubule required several kinesin molecules situated along its length, then the probability of a microtubule moving a distance greater than its length would increase abruptly from zero to unity as the kinesin density was raised above the critical value. The probability instead increased gradually, as expected if one kinesin molecule suffices (Fig. 3*b*). At kinesin densities less than $\sim 20\ \mu m^{-2}$, each microtubule had a low probability of moving more than its length, presumably because there was only a slight chance that it encountered another kinesin molecule before detaching; at these densities we believe that movement was due to single kinesin molecules. From the data of Fig. 3*b*, we estimate that at a density of $60\ \mu m^{-2}$, the average distance between kinesin molecules along the path of a microtubule is equal to the mean length of the microtubules ($2.5 \pm 1.4\ \mu m$, mean ± s.d., $n = 233$). If only half of the kinesin molecules are correctly oriented with respect to the microtubule's axis, then the distance over which a kinesin molecule can reach and react with a microtubule may be estimated at

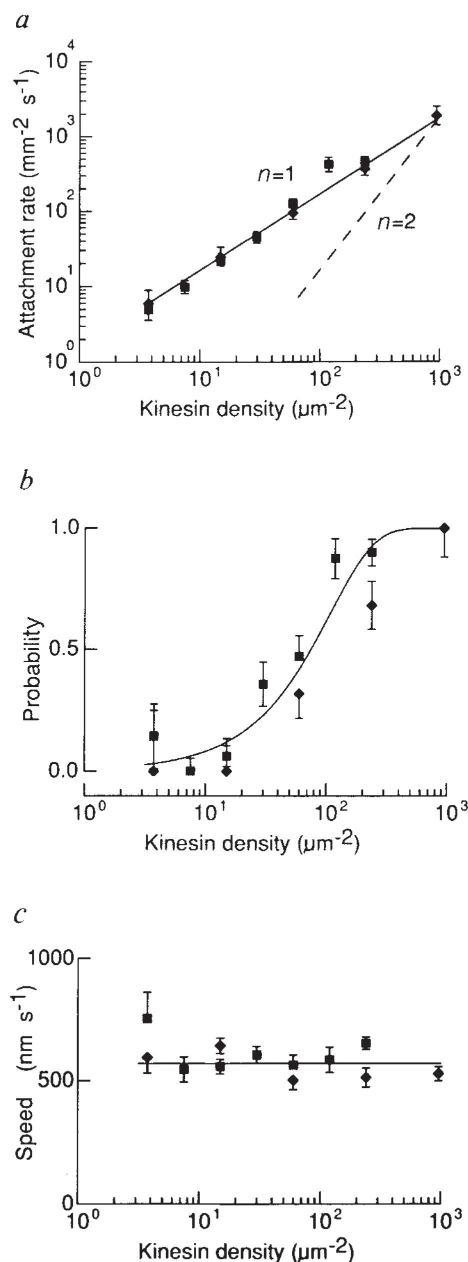

FIG. 3 *a*, Dependence of the microtubule attachment rate upon the kinesin density on a glass surface. The solid line, with a slope of one in this doubly logarithmic plot, is the relation expected if one kinesin molecule sufficed to move a microtubule; for comparison, the dashed line, with a slope of two, represents the result expected if attachment and movement required the cooperation of two kinesin molecules. *b*, The relation between the probability that a microtubule moves a distance greater than its length and the logarithm of kinesin density. The solid line is a plot of the equation $1-\rho\, e^{-\rho}/(1-e^{-\rho})$, in which ρ is the kinesin density divided by $60\ \mu m^{-2}$; this equation corresponds to the prediction of a model in which a single kinesin molecule suffices to move a microtubule (J.H., in preparation). *c*, Dependence of the speed of microtubule movement upon kinesin density. Speeds of microtubule motion were measured from drawings by hand on acetate-sheet overlays of taped video images acquired at 33 ms intervals. At low kinesin density, the speed was determined as the rate at which the microtubule's trailing end approached the fixed (nodal) point at which the kinesin molecule was located. The glass surface was pretreated with tubulin and cytochrome *c* as described in the legend to Fig. 1. The data in *a*, *b* and *c* correspond to observations from a total of 233 individual microtubules in two different experiments (distinguished by symbols). The ATP concentration was 1 mM. Before being infused into the chamber, the diluted microtubules were thrice passed through a 30-gauge needle to shear them into filaments of length $2.2 \pm 1.4\ \mu m$ (mean ± standard deviation, $n = 233$). The error bars correspond to standard errors of the means.

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13 nm. This distance, which is compatible with the dimensions of kinesin molecules and microtubules, is a lower limit because the calculation assumes that all the kinesin molecules on the surface are functional.

The greatest microtubule-moving activity in the low-density assay co-fractionated with the 9*S* peak of kinesin[8,9] on a sucrose-density gradient containing 4 μM cytochrome *c* (data not shown). Individual kinesin molecules can therefore elicit movement. This finding in conjunction with the several lines of evidence described above—movement at low kinesin density, attachment of a microtubule at one point on the substrate and the attachment and detachment rates—indicates that a single kinesin molecule is sufficient to move a microtubule. One possible reservation is that kinesin might preferentially aggregate at the glass surface; the binding of one kinesin to the surface might then nucleate the binding of others. But the formation of such complexes is an unlikely prerequisite for movement because, at low kinesin concentrations, aggregation would necessarily be slow and incomplete. For example, if kinesin's hypothetical association occurred with a rate constant of $10^7\ M^{-1}s^{-1}$, a value approaching the diffusion limit and greater than the on-rate for tubulin polymerization[19], then at the lowest kinesin concentration used in Fig. 3 (100 pM), less than 5% of the bound kinesin would occur in aggregates. Because no deviation from linearity is seen in Fig. 3, we conclude that the functional motor consists of a single kinesin tetramer.

Determinants of microtubule movement

Measurements of microtubule motion at high and low kinesin densities show how the speed of movement is influenced by the number of motor molecules interacting with the microtubule. At an almost saturating ATP concentration (1 mM), the speed of microtubule movement was independent of the kinesin density (Fig. 3*c*); we infer than one kinesin molecule can move a microtubule as quickly as can several molecules. At kinesin densities $<20\ \mu m^{-2}$, at which microtubules are being moved by single kinesin molecules, the speed was independent of the microtubule length, which ranged from 0.7 to 7 μm.

As the ATP concentration was reduced, the speed of movement declined and became dependent on the number of participating motors. For low kinesin densities, at which we expect only one kinesin molecule to be involved in a microtubule's movement, the speed followed the Michaelis-Menten equation[20]; at low ATP concentrations, the Hill coefficient was one (Fig. 4). This result is anticipated if just one kinesin molecule hydrolyses a single ATP to produce an incremental microtubule movement. On the other hand, when the kinesin density was raised to levels at which we expected several molecules to contribute to a microtubule's movement, we obtained a seemingly paradoxical result: the more kinesin molecules that participated, the slower was the motion (Fig. 4). Although at high ATP concentrations the maximal speed was independent of kinesin density, at lower ATP concentrations the speed depended on kinesin density, so that several motors moved a microtubule more slowly than did a single motor. This effect became more pronounced as the ATP concentration was reduced; the Hill coefficient exceeded unity. Control experiments showed that the decrease in speed with decreasing ATP concentration was not caused by depletion of ATP by kinesin's hydrolytic activity (see Fig. 4 legend). Instead, the simplest explanation for this behaviour is that kinesin heads in rigor, those bound to the microtubule but lacking ATP, impeded the movement produced by active heads interacting with the same microtubule. Such an explanation has been considered to account for the noncompetitive inhibition by adenyl-5′-yl imidodiphosphate (AMPPNP) and vanadate of the speed at which sea-urchin-egg kinesin moves microtubules[15]. Because movement persisted at low ATP concentrations, however, this impedance must have been incomplete, perhaps because the bound kinesin molecules were quite compliant or because the rigor bonds could be broken by active heads.

Implications for the reaction cycle

The speed of microtubule motion produced by a single kinesin molecule places limits on the force and displacement generated during each cycle of ATP hydrolysis. Because one motor molecule can move a microtubule as quickly as can several, the force exerted by a single kinesin molecule must significantly exceed the viscous drag from the surrounding fluid. The drag force acting on a microtubule moving across the surface at a speed of $600\ nm\ s^{-1}$ is about 2-3 fN per micrometre of microtubule length[21]. A single kinesin molecule must produce a force in excess of 60 fN to account for the measured lack of dependence of speed on microtubule length. The force thought to be produced by a molecule of muscle myosin[1,7] (500 fN) is thus of the same order of magnitude as what we consider a reasonable lower limit for the force produced by a kinesin molecule.

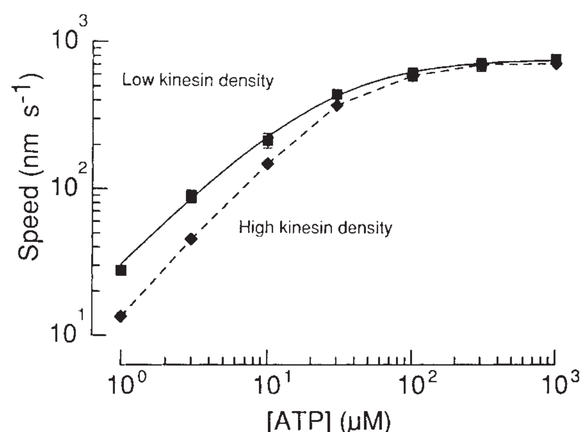

FIG. 4 Dependence of the speed of microtubule movement upon ATP concentration. The kinesin densities were $24\ \mu m^{-2}$ (■) and $\sim 2400\ \mu m^{-2}$ (◆). METHODS. The experimental protocol was identical to that of Fig. 1*b* except that the nucleotide concentration of the microtubule-containing buffer solution was reduced to $\ll 1\ \mu M$ by a double application of the following procedure: microtubules were diluted 100-fold into nucleotide-free buffer solution, centrifuged for 300 s at $10^6\ m\ s^{-2}$, and the pellet was then resuspended in nucleotide-free buffer. In control experiments in this nominally nucleotide-free solution, microtubules bound to the kinesin-coated surface but movement was undetectable at a resolution limit of $0.5\ nm\ s^{-1}$, or one-twentieth of the speed measured in the presence of 1 μM ATP. ATP was added to the nucleotide-free buffer at the stated concentrations. All speed measurements were made from observations of 6–21 individual microtubules; standard errors of the means are indicated by error bars except when smaller than the symbols. To rule out the possibility that the lower speeds measured at high kinesin density and low ATP concentration were caused by the hydrolysis of ATP by kinesin, the speed was measured over a period of 300 s beginning about 30 s after adding the microtubules to the chamber. Only at the lowest ATP concentration (1 μM) was there a significant slowing of the microtubules over time: the fractional decrease in speed over time was $0.0011 \pm 0.0004\ s^{-1}$ (mean ± standard error, $n = 10$) and led to an error of less than 10%. Maintenance of an ATP concentration of 1 μM with an ATP-regeneration system (2 μM creatine phosphokinase and 2 mM phosphocreatine) had no effect on the measured speed. At low kinesin density the probability that a microtubule moved a distance greater than its own length was 0.22 ± 0.06 (mean ± standard error, $n = 50$), confirming that movement was mainly due to single kinesin molecules (see Fig. 3*b*). At high kinesin density it is estimated that 10–40 kinesin molecules were moving each microtubule. From Fig. 3*b*, a density of $\sim 60\ \mu m^{-2}$ corresponded to an average of one kinesin molecule per microtubule; we accordingly expect about 40 molecules per microtubule at a density of $2400\ \mu m^{-2}$. By the model discussed in the legend to Fig. 3, a lower limit of about 10 kinesin molecules per microtubule follows from the observation that even the shorter microtubules ($<2\ \mu m$ long) had only a small probability of dissociating (<0.1) before they had moved ten times their own length.

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It is remarkable that the speed of microtubule movement at high ATP concentrations does not depend on the number of participating kinesin molecules (Fig. 3*c*). Consistent with this observation, the speed at high kinesin density and over a wide range of ATP concentrations is essentially independent of microtubule length, and hence of the number of kinesin molecules involved. Because microtubule movement can be achieved by a single kinesin molecule, knowledge of the ATPase activity of kinesin in our assay would allow determination of the step size, or the displacement per cycle of ATP hydrolysis. If the microtubule-activated ATPase were identical to that in solution in the presence of high ATP and microtubule concentrations[22,23], $V_{max} = 20-30\ s^{-1}$, then a maximal speed of 500-750 nm s^{-1} would imply a step size of 15-30 nm. This tentative value constitutes a big conformational change that exceeds the 4-nm estimate made from video recordings[4].

An important corollary of our conclusion that one kinesin molecule can move a microtubule is that a single molecule can also confine a microtubule at the glass surface. During movement at low kinesin density and high ATP concentration, the microtubule is strongly associated with the surface; in the experiment of Fig. 3, for example, there was a 70% probability that a microtubule's trailing end reached the nodal point at which the motor was located. It may be calculated from our results that one kinesin molecule can maintain its grip on a microtubule over 5 ± 2 μm, which is equivalent to a period of 9 ± 3 s or to some 200-1,000 cycles of ATP hydrolysis. It is possible that kinesin's two globular heads work hand-over-hand, so that one is always bound and prevents the microtubule from diffusing away. Alternatively, the two heads may work independently, as is suggested by experiments using single-headed myosin[7,24,25] and dynein[17,26]. If this is so, the time in the reaction cycle during which the kinesin heads are detached from the microtubule must be so brief, probably less than 1 ms, that the microtubule is unlikely to diffuse out of reach of the kinesin molecule. In either case, the ability of one kinesin molecule to hold and move a microtubule may represent a functional adaptation; during organelle transport, in which it is thought that only a few kinesin molecules produce movement[27], detachment would be disadvantageous because diffusion of the organelle away from a microtubule would greatly reduce the speed of transport.

Conclusion

In this study, we describe an assay for measurement of the motile properties of individual motor molecules. Experimental systems of this sort provide information that studies of populations of molecules cannot; it should be possible, for example, to detect heterogeneity in populations of derivatized or enzymatically cleaved motor molecules. In conjunction with high-resolution systems for measuring force and displacement, such assays should also allow analysis of the force-generating step, an event that has been difficult to resolve using larger populations of motor proteins. Like patch-clamp recording from ion channels[28], the study of movement produced by single motor molecules provides an assay sensitive enough to monitor the activity of an individual protein molecule. □

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LETTERS TO NATURE

A millisecond pulsar in a 32-minute binary orbit

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We report the discovery of a millisecond pulsar in the globular cluster 47 Tucanae (NGC104). It has a spin period of 4.479 ms and is a member of a binary system with an orbital period of 32 min and an eccentricity of 0.32. The mass function, $2.9 \times 10^{-8} M_{\odot}$, is the smallest value for any known binary system. We find that the observations are consistent with a neutron star of mass 1.4 $M_{\odot}$ and a white-dwarf companion of mass 0.8 $M_{\odot}$. The millisecond spin period combined with the very short orbital period offers, for the first time, the possibility of observing spin–orbit coupling effects predicted by general relativity theory.

We began a search for millisecond pulsars in globular clusters using the Parkes radio telescope in November 1987. We observed at frequencies of 430 and 610 MHz using dual-polarization receivers with system-noise equivalents ≈120 Jy. The receiver pass bands were resolved into 32×33 kHz or 24×250 kHz channels per polarization. In each case signals from the two polarizations were summed after detection, filtered and sampled every 250 μs with only the signal polarity being recorded (one-bit quantization). At each telescope position we took samples continuously for 10^4 s and recorded them as a single data set. We combined the outputs from the filter channels after correcting for the relative time delays that are due to interstellar dispersion. We repeated this for a range of dispersion measures and searched for periodicities at each dispersion using power spectrum analysis followed by superposed epoch analysis at selected periods. The search was sensitive to periods in the range 0.5-40 ms for dispersion measures up to 200 cm^{-3} pc.

We discovered[1] two millisecond pulsars in the direction of 47 Tuc (right ascension (1950) = $00^h21^m54^s$, declination (1950) = $-72° 21' 00''$) the first cluster observed. Here we give details of the short-period binary pulsar PSR0021-72A; details of the other

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