A Kinesin-like Motor Inhibits Microtubule Dynamic Instability

Henrik Bringmann,1,* Georgios Skiniotis,2† Annina Spilker,1‡ Stefanie Kandels-Lewis,1 Isabelle Vernos,1 Thomas Surrey1§

The motility of molecular motors and the dynamic instability of microtubules are key dynamic processes for mitotic spindle assembly and function. We report here that one of the mitotic kinesins that localizes to chromosomes, Xklp1 from Xenopus laevis, could inhibit microtubule growth and shrinkage. This effect appeared to be mediated by a structural change in the microtubule lattice. We also found that Xklp1 could act as a fast, nonprocessive, plus end–directed molecular motor. The integration of the two properties, motility and inhibition of microtubule dynamics, in one molecule emphasizes the versatile properties of kinesin family members.

The activities of a variety of molecular motors are required to spatially organize microtubules and chromosomes in the mitotic spindle (1, 2). Some motors contribute to spindle pole focusing (3, 4), others stabilize antiparallel microtubule overlaps in the spindle midzone (5), and yet others are involved in chromosome-microtubule interactions (6–8). How these different functions depend on the characteristics of the motors’ kinetic behavior is unclear. Local and temporal modulation of microtubule dynamics by stabilizing and destabilizing factors is also necessary for spindle assembly and function (9–14).

Little is known about the interaction between microtubules and chromosome arms that are covered with chromokinesins. One of these kinesins is Xenopus laevis kinesin-like protein 1 (Xklp1), a member of the Kif4 subfamily. Xklp1 is important for chromosome-microtubule interactions and for spindle pole extension (4, 7). Its C-terminal part

References and Notes
16. Materials and methods are available as supporting material on Science Online.
22. K. Murase et al., unpublished data.
28. We thank K. Hinata for advice and providing plant materials, H. Etoto for advice and help, Y. Okamoto for γ irradiation, A. Miyawaki for providing GFP vector, A. Kato for providing tobacco BY-2 cells, T. Tani and H. Shimosato for discussions, and T. Ueda and H. Sugita for technical assistance. This work was partly supported by Grants-in-Aid for Scientific Research [number (no.) 14360066 to S.T. and no. 11238205 and no. 15208013 to A.J.] from the Ministry of Education, Sports, Science, and Technology (MEXT) of Japan, by grant no. RFTF000102 (to S.T.) from the Japan Society for the Promotion of Science, and by the MEXT Grant-in-Aid for the 21st Century Centers of Excellence (COE) Program to Nara Institute of Science and Technology.

Supporting Online Material
www.sciencemag.org/cgi/content/full/303/5663/1516/DC1
Materials and Methods
Fig. S1
References
targets the molecule to chromosomes (15),
but the molecular mechanism of the interaction
of the N-terminal motor domain with microtubules is not understood. To gain
insight into the basic characteristics of this kinesin-like protein, we examined the kinetic
properties of the putative motor domain of Xklp1 and its influence on microtubule dy-
namics in vitro.

A purified fusion protein consisting of the
predicted N-terminal motor domain and part
of the coiled coil of Xklp1 and a C-terminal glutathione S-transferase (GST) tag
(Xklp1506H) was tested for motility in
reactions. Microtubule gliding was observed, and the velocity distribution peaked around 1.4 μm/s in the presence of 1
mM adenosine triphosphate (ATP) (Fig. 1A).
Xklp1 was a plus end–directed motor (Fig. 1
B), like the homologous mouse Kif4 (19). In
contrast to Kif4, however, Xklp1 acted as a
fast molecular motor.

To characterize the processivity of Xklp1,
a construct with a C-terminal His 6-tag
(Xklp1506H) was used. Xklp1506H was in its
dimeric, native state (4, 16) (Fig. S1). The
chemical processivity, i.e., the number of
ATP molecules hydrolyzed per productive
encounter of the motor with a microtubule
(20), was determined by steady state and
pre–steady state kinetic measurements (Fig.
1, D and E) (16). A fast steady-state adeno-
sine triphosphatase (ATPase) turnover rate
kcat of 62 ± 17 s⁻¹ and a Michaelis-Menten
constant Kcat/pre of 58 ± 29 μM (Fig. 1D)
were obtained. The Kcat/pre was two orders of
magnitude higher than that of other motile
kinesins (20–22) and indicated very weak
microtubule binding of the motor in the pres-
ence of ATP. The ratio of kcat/Kcat/pre of
about 1.1 μM⁻¹s⁻¹ is in the order of magni-
tude expected for a diffusion-limited reaction
of a kinesin (23) and does not suggest a
processive mechanism of ATP hydrolysis
(24). The nonprocessive nature of Xklp1 ac-
activity was confirmed by measuring the bimo-
olecular rate of productive encounters kni of
0.28 ± 0.01 μM⁻¹s⁻¹ under pre–steady state
conditions (16, 20, 25) (Fig. 1E). Because
this value is quite low for a kinesin (21, 22)
and comparable to the apparent bimolecular rate kcat/Kcat/pre, it can be concluded that
Xklp1506H is nonprocessive or at most weak-
ly processive (20).

Although Xklp1506H showed adenine nucleotide–dependent binding to paclitaxel-
stabilized microtubules typical for kinesins
(Fig. 1C), its fast motility and its very weak
binding to microtubules when ATP was present
are more reminiscent of myosin II than of other
kinesins (26, 27). Xklp1 probably acts as a
"rower" (28) and may only generate efficient
movement in large assemblies, e.g., on chromo-
somes (similar to myosin II, which assemblies
into large arrays in myosin fibers).

We then tested whether Xklp1 affected
microtubule dynamics. Xklp1506H was added to
pregrown microtubule asters nucleated from
centrosomes, and the dynamics of indi-
vidual microtubules in the presence of 30 μM
tubulin, 1 mM GTP, and 1 mM ATP were
observed by video-enhanced differential inter-
fERENCE contrast (DIC) microscopy (12,
16). Under these conditions, most micro-
tubules were growing. The speed of microtu-
bule polymerization was slowed down with
increasing motor concentration and was com-
pletely inhibited above a concentration of 10
μM of Xklp1506H motor domains, resulting
even in static microtubules (Fig. 2A; fig. S2).
Washing out the motor restored microtubule
polymerization, showing the reversibility of
this effect. Experiments in which the motor
was not washed out indicated a limited sta-
Bility of static microtubules at higher motor
concentrations on a slower time scale.
Whereas static microtubules were still
present after 20 min in the presence of 10 μM
Xklp1506H, half of the microtubules had dis-
appeared after 5 min with 25 μM motor.
Next, we examined the effect of Xklp1 on
shrinkling microtubules. Microtubules
were first grown from centrosomes with
Xklp1506H concentrations that were only
weakly growth-inhibiting. Keeping the motor
concentration constant, tubulin and GTP
were then washed out to induce microtubule
depolymerization. Xklp1506H slowed down
microtubule depolymerization with increas-
ing motor concentration (Fig. 2B; fig. S2). It
completely inhibited microtubule shrinking
at motor concentrations around 1 μM of
Xklp1506H motor domains. Because essen-
tially no free tubulin was present during
depolymerization, Xklp1’s effect must have been
due to an interaction with the microtu-
bule, not with free tubulin. Microtubules
grown from axonemes showed that inhibition
of microtubule dynamics was not restricted to
microtubule plus ends. Thus, Xklp1 was able to
fully inhibit both microtubule polymeriza-
B asion as well as depolymerization.

To learn more about the mechanism of
Xklp1-mediated inhibition of microtubule
dynamics, its nucleotide dependence was ex-
amined. Asters were nucleated from centros-
somes in the presence of Xklp1506H and dif-
dent adenine nucleotides. They were fixed
at defined time points before they had

[Figure 2: Xklp1 is an inhibitor of microtubule dynamics: (A and B) Dynamic microtubule
asters nucleated from centrosomes were visual-
alized by DIC microscopy and the length of
individual microtubules was plotted versus
time. (A) Concentration-dep
endent and reversible
hibition of microtubule growth. Four
experiments with different
Xklp1506H concentrations
are shown (top to bottom).
In each experi-
test, asters grew first in the absence
of motor (left column),
then motor was added
(middle column) and re-
moved again (right col-
num). Tubulin concen-
tration was always 30
μM. (B) Inhibition of mi-
crotubule shrinkage in
the absence of tubulin.
Six experiments are
shown (top to bottom).
After growth of asters
either in the absence
of motor or in the presence
of motor at low concentrations as indicated (left column), tubulin was removed, keeping the
motor concentration constant (right column).
The requirement of higher motor concentrations for complete
inhibition of microtubule dynamics on growing microtubules in the presence of GTP-tubulin can be
explained, assuming that free tubulin competes for Xklp1 binding (Fig. 3A). (C and D) Microtubule
asters nucleated from centrosomes and visualized by fluorescence microscopy. (C) Inhibition of microtubule
growth by dimeric Xklp1506H and monomeric Xklp1354H in the presence of different adenine
nucleotides. Control is without motor. (D) Inhibition of dilution-induced microtubule depolymerization
by the two dimeric constructs Xklp1506H and Xklp1507H/GST and by monomeric Xklp1354H in the
presence of ATP. Control is without motor. Bar, 10 μm.

1520
5 MARCH 2004 VOL 303 SCIENCE www.sciencemag.org
reached their steady-state size and were observed after fixation by fluorescence microscopy (16) (Fig. 2C). The inhibition of aster growth by Xklp1506H was stronger in the presence of the nonhydrolyzable ATP analog β,γ-imidoadenosine 5'-triphosphate (AMP-PNP) (or in the absence of adenine nucleotide) than in the presence of ATP (or ADP).

Thus, the motor-mediated inhibition of microtubule dynamics did not require the hydrolysis of ATP but did correlate with the strength of Xklp1506H binding to microtubules (Fig. 1C). Using Xklp1507GST and a monomeric construct, Xklp1506H (16) (fig. S3), as controls, we showed that neither the presence of the His-tag nor dimerization of the motor were necessary for inhibition of microtubule dynamics (Fig. 2, C and D).

Similar to KIn1 kinesins (13, 29), Xklp1506H also bound to free tubulin (Fig. 3A) and possessed ATPase activity stimulated by free tubulin (Fig. 3B) (16). However, in contrast to KIn1 kinesins (13, 29), the steady-state affinity of Xklp1506H for microtubules at saturating ATP concentrations was still higher than for free tubulin (compare Fig. 1D and Fig. 3B), and immunolocalization showed no specific binding to microtubule ends (Fig. 3C). Thus, Xklp1’s inhibitory effect on microtubule dynamics was probably not due to a direct prevention of loss or gain of tubulin dimers at end structures but rather caused by a change in microtubule structure induced by the presence of bound motor.

To test this hypothesis, we visualized microtubules by cryo-electron microscopy (cryo-EM) after incubation with Xklp1506H and AMP-PNP (16) under conditions in which Xklp1 induced the majority of the microtubules to be static with rare events of microtubule destabilization (as demonstrated by light microscopy). Cryo-EM revealed microtubules that frequently had defects along the entire lattice, occasionally even showing break points (Fig. 4B) and ends that disintegrated into frayed structures (Fig. 4C). Defects were not observed in control experiments without motor or with dimeric conventional kinesin that fully decorated microtubules (Fig. 4A).

The effect of Xklp1506H on the structure of paclitaxel-stabilized microtubules was milder, but also striking. At overstoichiometric ratios of motor, microtubules were unraveled from their predominant supertwisted forms, as indicated by a wider lattice with protofilaments running in parallel. This unwinding, which propagated from the microtubule ends for several hundreds of nanometers, led very often to entirely open and collapsed microtubule walls (Fig. 4E). Partial decoration of Xklp1 was mostly visible along unperturbed microtubule segments (fig. S4A). Similar results were obtained with monomeric Xklp1506H (fig. S4B). Despite the extensive microtubule unraveling, the presence of paclitaxel appeared to inhibit other visible structural defects induced by Xklp1 in nonstabilized microtubules. The extensive unwinding of microtubules was not observed in the absence of Xklp1 or in controls containing constructs of dimeric conventional kinesin (Fig. 4D).
This effect of Xklp1 on the entire microtubule lattice is clearly different from that of destabilizing Kin1 kinesins that produce microtubule ends from which protofilaments peel off. The structural changes in the microtubule lattice induced by Xklp1 suggest an allosteric mechanism for the inhibition of microtubule dynamic instability (Fig. 3D): Xklp1 bound to the microtubules appears to induce global structural changes in the microtubule lattice that influence the microtubule ends, causing inhibition of microtubule dynamics and, eventually, on a slower time scale, microtubule destabilization.

Bound to chromosome arms in cells (7), Xklp1 might contribute to a local decrease of microtubule dynamics. This suggests an extension of the “search and capture” model, which proposes the capture and stabilization of dynamic microtubules exclusively by kinesin-1 and not by the overall chromosome surface (30, 31). Alternatively, Xklp1 could serve to destabilize microtubules close to chromosomes on a slow time scale. In addition, the motor activity of Xklp1 generates plus end-directed movement that is fast in comparison to the velocity of microtubule polymerization and flux in the spindle, which can explain its proposed role in the extension of spindle poles (4). The N-terminal part of Xklp1 containing the motor domain is sufficient for its two activities, motility and inhibition of microtubule dynamic instability.

The motor domain of Xklp1 shares characteristics with two very different classes of kinesin-like proteins: (i) those with a clear transport function like conventional kinesin that undergo ATP-dependent conformational changes upon binding to the microtubule lattice, and (ii) the microtubule-depolymerizing kinesins that cannot translocate along the microtubule lattice in a directed manner, but catalyze ATP hydrolysis when bound to free tubulin. Xklp1 could thus be an evolutionary link between these two classes of kinesins that couple ATP hydrolysis either with motility along the microtubule lattice or with microtubule depolymerization.

References and Notes
15. I. Vernos, unpublished data.
16. Materials and methods are available as supporting material on Science Online.
33. We thank M. Utz for technical assistance, N. Mücke (DKFZ, Heidelberg) for help with the analytical ultracentrifugation, C. Tsivanisaris (Max Planck Institute for Medical Research, Heidelberg) for help with the stopped-flow measurements, A. Hoenger and T. Wendt for help with the cryo-EM, R. Cross (Marie Curie Research Institute, Oxford) for pET17G6ST, A. Popov and F. Nédélec for centrosomes, and I. Arnal (CNRS, Rennes) for axonemes. Supported by DFG grant SU 175/4-1. H.B. was supported by the German National Merit Foundation.

Supporting Online Material
www.sciencemag.org/cgi/content/full/303/5663/1519/DC1
Materials and Methods
Figs. S1 to S4
References
17 December 2003; accepted 16 January 2004

A Toll-like Receptor That Prevents Infection by Uropathogenic Bacteria

Dekai Zhang, Guolong Zhang,* Matthew S. Hayden, Matthew B. Greenblatt, Crystal Bussey, Richard A. Flavell, Sankar Ghosh†

Toll-like receptors (TLRs) recognize molecular patterns displayed by microorganisms, and their subsequent activation leads to the transcription of appropriate host-defense genes. Here we report the cloning and characterization of a member of the mammalian TLR family, TLR11, that displays a distinct pattern of expression in macrophages and liver, kidney, and bladder epithelial cells. Cells expressing TLR11 fail to respond to known TLR ligands but instead respond specifically to uropathogenic bacteria. Mice lacking TLR11 are highly susceptible to infection of the kidneys by uropathogenic bacteria, indicating a potentially important role for TLR11 in preventing infection of internal organs of the urogenital system.

TLRs represent a family of transmembrane proteins characterized by multiple copies of leucine-rich repeats in the extracellular domain and a cytoplasmic Toll/IL-1 (interleukin-1) receptor homology domain (TIR) (1, 2). Currently 10 TLRs have been reported in mammalian species, and these appear to recognize distinct pathogen-associated molecular patterns (PAMPs). Of these known TLRs, TLR2, TLR3, TLR4, TLR5, and TLR9 have been extensively characterized (1, 2). TLR1, TLR6, TLR7, and TLR8 have not yet been shown to independently impart signals after recognition of specific microbial products. Heterodimerization between certain TLRs also helps to increase the diversity of PAMPs that can be recognized (3, 4).

The completion of the human and mouse genome sequence has provided the opportunity to determine whether the mammalian TLR family extends beyond the 10 known members. To identify as yet uncharacterized TLRs, we used the sequence of the TIR domain of TLR4 to search National Center for Biotechnology Information (NCBI) databases. The sequence encoding TLR11 was first detected as an expressed-sequence tag (EST) from a mouse liver EST database. Sequential searches using this EST ultimately led to the recovery of the mouse genomic sequence encoding TLR11. The GENSCAN program (5) was used to predict the putative open reading frame (ORF), and hypothetical translation predicted a 907 amino acid protein with the hallmarks of known Toll receptors, including a leucine-rich domain, a transmem-