Tensile stress stimulates microtubule outgrowth in living cells

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Summary

Cell motility is driven by the sum of asymmetric traction forces exerted on the substrate through adhesion foci that interface with the actin cytoskeleton. Establishment of this asymmetry involves microtubules, which exert a destabilising effect on adhesion foci via targeting events. Here, we demonstrate the existence of a mechano-sensing mechanism that signals microtubule polymerisation and guidance of the microtubules towards adhesion sites under increased stress. Stress was applied either by manipulating the body of cells moving on glass with a microneedle or by stretching a flexible substrate that cells were migrating on. We propose a model for this mechano-sensing phenomenon whereby microtubule polymerisation is stimulated and guided through the interaction of a microtubule tip complex with actin filaments under tension.

Movies available on-line

Key words: Microtubules, Actin cytoskeleton, Adhesion, Tension, Mechanosensor

Introduction

Abercrombie formulated: a cell's relationship to the substratum is the key to cell crawling, and it is the changing adhesion of a cell to the substratum that moves a cell along (Abercrombie, 1978). Movement itself also requires traction, and the need for contractility to provide traction for cell movement was clear to the earliest investigators (Weiss, 1959). We now know that traction is exerted on the substrate via specialised adhesion sites that interface with the actin cytoskeleton. At least two classes of adhesion sites can be distinguished: focal adhesions, which interface with actin stress fibre bundles, and smaller, focal complexes, which are associated with actin filament networks and filopodia (reviewed in Burridge and Chrzanowska-Wodnicka, 1996; Small et al., 1999a). Focal complexes can serve as precursors of focal adhesions through a transition effected by changes in the balance of activities of members of the Rho GTPase family (Nobes and Hall, 1995; Rottner et al., 1999a). And both focal complexes and focal adhesions require contractility in the actin cytoskeleton for their formation and maintenance (Chrzanowska-Wodnicka and Burridge, 1996; Rottner et al., 1999a). Contractility, in turn, probably plays an important part in inside-out signalling events (Shyy and Chien, 1997), possibly through force-induced conformational changes in molecules of the extracellular matrix (Zhong et al., 1998), integrins (Vinogradova et al., 2000; Takagi et al., 2001) and cytoskeleton-associated components (Yamada and Geiger, 1997).

Cell locomotion not only involves the mutual and dynamic reorganisation of the actin cytoskeleton and its associated adhesion sites but also mechanisms that confer polarity on these structural changes. Only then can traction forces in the actin cytoskeleton be converted into net movement (e.g. Beningo et al., 2001). Earlier findings attributed this polarising function to microtubules (Vasiliev and Gelfand, 1976), and more recent studies have revealed how they may achieve this role (Kaverina et al., 1998; Kaverina et al., 1999; Kaverina et al., 2000). It has been shown in fibroblasts that microtubules specifically target substrate adhesion sites and that these targeting events are followed by the turnover of adhesion sites or their dislocation from the substrate (Kaverina et al., 1998; Kaverina et al., 1999). Given the dependence of adhesion site maintenance on contractility, it was speculated that microtubules destabilise adhesions by delivering signals that antagonise the contractility pathway. This contention was supported by the demonstration that dissociation of adhesion sites at the cell edge could be mimicked by the local application of inhibitors of actomyosin contractility (Kaverina et al., 2000).

The question addressed in the present study is what is the mechanism by which microtubules are guided to adhesion sites. We formerly showed that the local application of contractility inhibitors to a cell edge produced a rapid and local depolymerisation of microtubules towards the cell centre. In the present work, we have used alternative approaches to modulate radial stress at the cell periphery. By this means, we demonstrate that microtubules specifically invade regions where stress is locally increased. These findings highlight a stress-dependent feedback mechanism that presumably plays an important role in the selection of adhesion sites for targeted modulation via microtubules.

Materials and Methods

Cells and expression constructs

Cells of the mouse melanoma line B16F1 (ATCC) were maintained

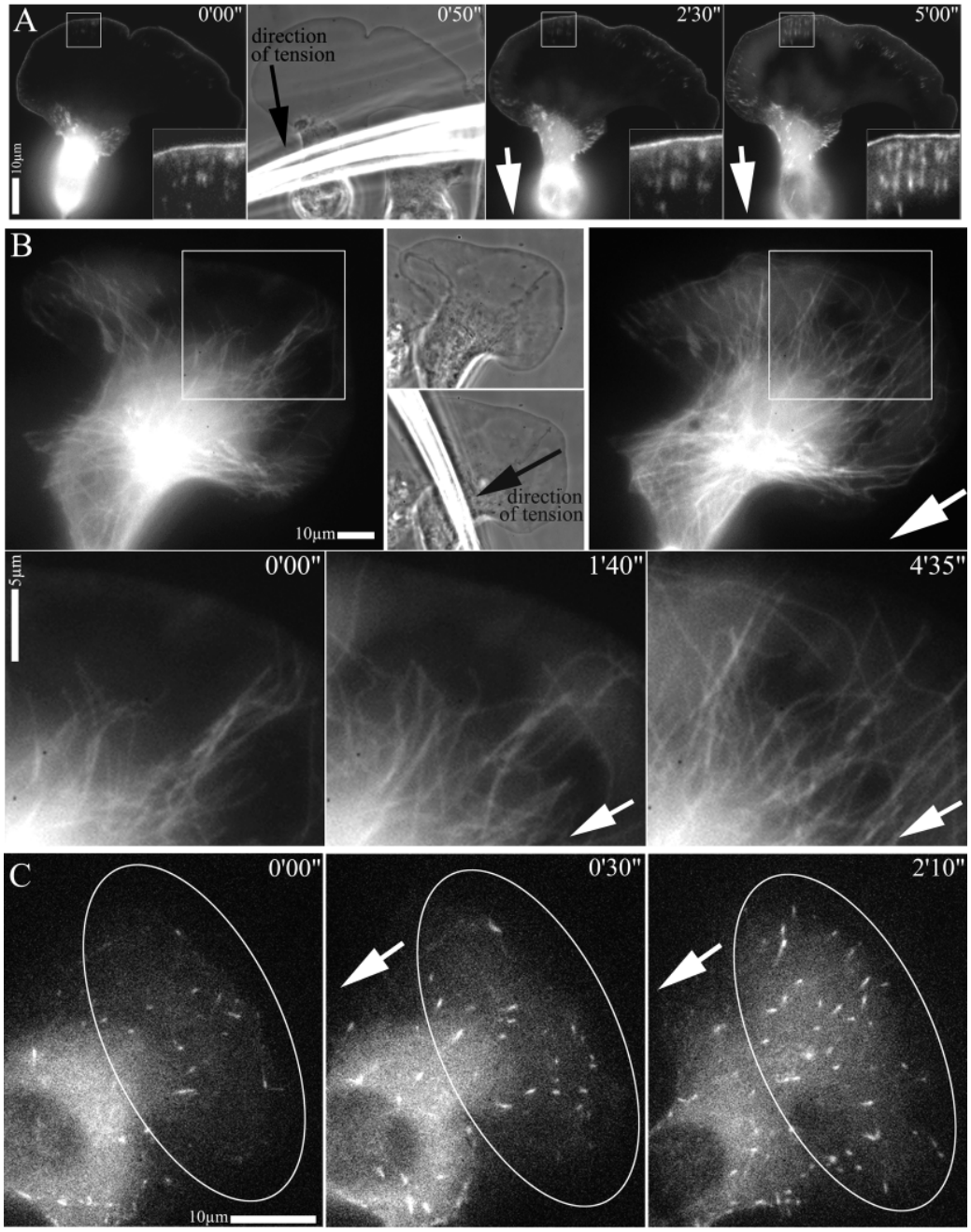

Fig. 1. Cell body displacement stimulates growth of peripheral adhesions in B16 melanoma cells and the polymerisation of microtubules towards the cell edge. (A) Video frames show a motile cell, expressing GFP-VASP, whose cell body was displaced by a microneedle in the direction indicated in the phase contrast image. Panel 0′00″ in this and subsequent figures corresponds to the video frame before tension application. Boxed insets in the fluorescence images show enlargement of peripheral adhesion sites in the region diametrically opposite the cell body. The continued protrusion of the cell edge is indicated by the persistence of the line of GFP-VASP at the tip of the lamellipodium. An example of one from five cells is shown. Times are in minutes and seconds. Bar, 10 µm. (B) The conditions used were the same as in A for a B16 melanoma cell expressing GFP-tubulin. Arrows in the phase contrast and fluorescent images of the video sequence indicate the direction of stress application. Insets show invasion of microtubules into lamella region in the line of applied stress. An example of one from seven cells is shown. (C) The conditions used were the same as in A for a B16 melanoma cell expressing GFP-CLIP-170. Arrows indicate the direction of stress application. Note the increase in number of polymerising microtubules in peripheral lamella (ellipse), which are marked by GFP-CLIP-170 at their tips. An example from one from 15 cells is shown.

in DMEM with 10% FCS at 37°C in 5% CO_2. They were transfected with 6 µl Superfect lipofection agent (Qiagen) and 1 µg DNA per 30 mm dish in 5% serum overnight and plated onto 25 µg/ml laminin-coated coverslips or flexible substrates (see below). Cells were used for experiments 4-8 hours after plating on glass and 12-16 hours after plating on flexible substrates.

Goldfish fin fibroblasts (line CAR, ATCC) were maintained in basal Eagle medium with Hanks' BSS and non-essential amino acids and with 15% FBS at 25°C. They were transfected transiently as described previously (Kaverina et al., 1999). The stable clone Tub3 was produced by selection with 1 mg/ml G418.

Primary keratocytes from scales of black molly fish or Alpine trout were prepared and maintained as described previously (Anderson and Cross, 2000).

The following vectors, kindly provided by J. Wehland and co-workers (Braunschweig, Germany), were used for expression of EGFP-fused proteins: (1) mouse beta 3 tubulin in a pEGFP-C2 vector; (2) human zyxin in a pEGFP-N1 vector (Rottner et al., 2001); and (3) human VASP in a pEGFP-C2 vector (Rottner et al., 1999).

Mouse h1 calponin in pEGFP-C1 (Danninger and Gimona, 2000) was kindly provided by M. Gimona (Salzburg, Austria). CLIP-170 GFP (Hoogenraad et al., 2000) was a generous gift of A. Akhmanova (Rotterdam).

The B16F1 cell line stably expressing EGFP-beta-actin (Ballestrem et al., 1998) was kindly provided by C. Ballestrem (Geneva).

Microinjection and cell manipulation

Injections were performed with sterile Femtotips (Eppendorf, Hamburg) held in a Leitz Micromanipulator with a pressure supply from an Eppendorf Microinjector 5242. Cells were injected with a continuous outflow mode from the needle under a constant pressure

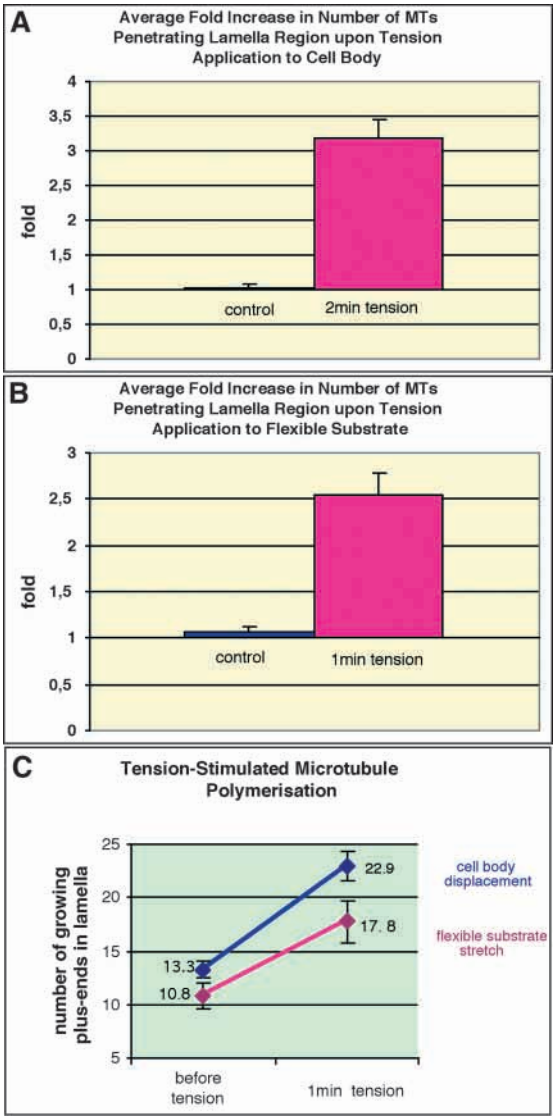

Fig. 2. (A,B) Quantification of the increase in the number of microtubules extending into anterior lamella regions of B16 melanoma cells in response to increased stress imposed by cell body manipulation (A, 22 cells) and stretching of a flexible growth substrate (B, 19 cells). (C) Quantification of the increase of CLIP-170-associated polymerising microtubule tips in lamella regions of B16 melanoma cells in response to increased stress imposed by cell body manipulation (blue, 15 cells) and stretching of a flexible growth substrate (magenta, four cells).

of between 20 and 40 hPa. For local application of drugs, performed with the same system, a constant pressure of 50-100 hPa was used.

Cell manipulations were performed using the same micromanipulator with flamed, curved microinjection needles.

Tetramethyl rhodamine (5-TAMRA; Molecular Probes, USA) conjugated vinculin from turkey gizzard was kindly provided by K. Rottner and M. Gimona. Cy3-conjugated tubulin was kindly provided by J. Peloquin and G. Borisy (Chicago, USA).

For local application through a microneedle, drugs were dissolved in microinjection buffer (2 mM Tris-Acetate pH 7.0, 50 mM KCl). The inhibitor of myosin light chain kinase, ML-7 (Alexis Corporation, Switzerland) was used at a concentration of 300 μM, the actomyosin inhibitor 2,3-butanedione 2-monoxime (BDM) at a concentration 250 mM and H7 (Sigma) at a concentration of 1 mM.

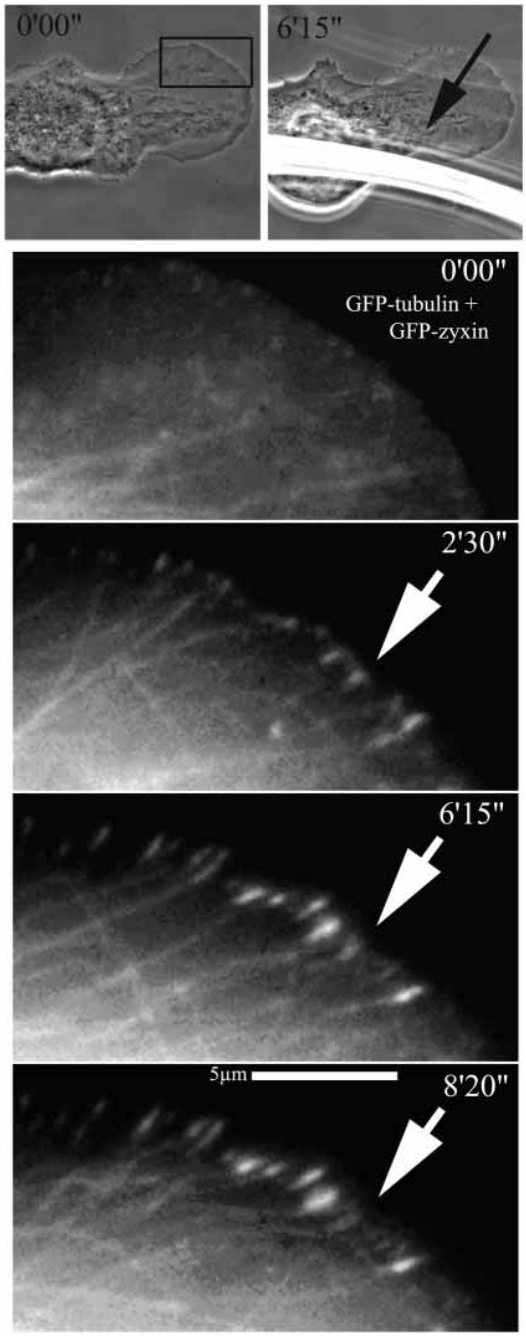

Fig. 3. Microtubules induced to polymerise by increased stress target the adhesion sites that simultaneously enlarge at the cell periphery. The figure shows a B16 melanoma cell that was transfected with GFP-zyxin and GFP-tubulin and subjected to cell body displacement in the direction indicated by the arrows. Upper phase images show the cell just before (left) and 6 minutes 15 seconds (6′15″) after tension application (right) with the microneedle. The area boxed in the left phase image corresponds to the region shown in fluorescence in the lower video frames. An example of one of 15 cells is shown.

Polyacrylamide substrates

Flexible substrates composed of 5% acrylamide and 0.08% Bis-acrylamide were prepared as previously described (Wang and Pelham, 1998; Beningo et al., 2001). 100 μg/mL poly-D-lysine in PBS was covalently coated to the substrates overnight at 4°C following a

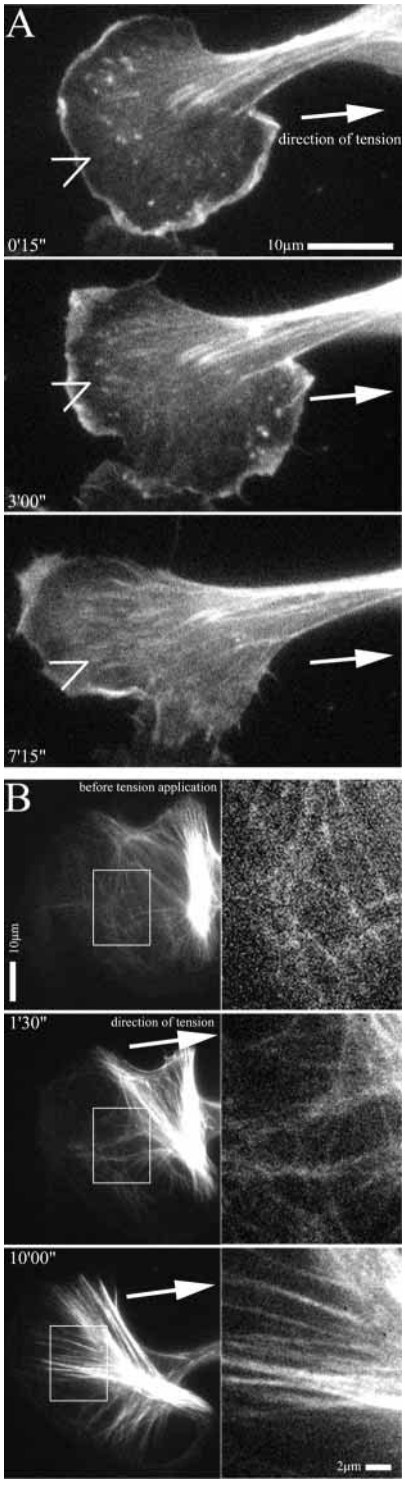

Fig. 4. Cell body displacement stimulates formation of radial bundles of actin filaments that terminate at the cell periphery. Video sequences show a B16 melanoma cell expressing either GFP-actin (A, example from nine cells) or GFP-calponin h1 (B, example from five cells.) that was subjected to cell body manipulation at time 0. The chevrons in (A) indicate regions of bundle formation. Boxed regions in (B) are shown at higher magnification in the right hand panels.

previously published procedure (Wang and Pelham, 1998). After rinsing with PBS, the substrate was coated with 25 μg/mL Laminin (dialyzed in PBS 3 hours on ice) for 1 hour at room temperature just prior to plating.

Video microscopy and image analysis

Cells were injected and observed in an open chamber at room temperature for CAR cells and keratocytes and in a heated chamber (Warner Instruments, Reading, UK) at 37°C for B16 cells on an inverted microscope (Axiovert 135TV; Zeiss, Austria) equipped for epifluorescence and phase contrast microscopy. Injections were performed at an objective magnification of 40× (NA 1.3 Plan Neofluar), and video microscopy was performed with a 100×/NA 1.4 Plan-Apochromat with or without 1.6 optovar intermediate magnification. Tungsten lamps (100 W) were used for both transmitted and epi-illumination. Data were acquired with a back-illuminated, cooled CCD camera from Princeton Research Instruments driven by IPLabs software (both from Visitron Systems, Germany) and stored as 16-bit digital images. The microscope was additionally equipped with shutters (Optilas GmbH, Germany) to allow separate recordings of video sequences in phase contrast and fluorescence channels and with a filter wheel for two fluorescent channels. Times between frames were 10 to 25 seconds.

For quantitative analysis of microtubule penetration in lamella, a line was drawn perpendicular to the direction of tension or, for controls, to the direction of protrusion, such that between 5-15 microtubules crossed the line at time ‘0’. In consecutive video frames the line was fixed at a constant distance from the cell front. Depending on the cell measured, the distance ranged from 4 to 10 μm. The number of microtubules crossing the line was then counted after 1 minute for flexible substrate experiments and after two minutes for cell manipulations.

Fluorescence recovery after photobleaching

Fluorescence recovery after photobleaching (FRAP) was performed in a LSM 5 Pascal confocal microscope (Ziess) using cells expressing GFP-tubulin. A line was bleached across lamella regions and the time-lapse video of the microtubule pattern recorded. ML-7 was applied locally as described above.

Results

Stress application via cell body displacement in melanoma cells

When plated on laminin, B16F1 melanoma cells commonly express broad, protruding lamella regions that contain relatively small numbers of focal adhesions. The adhesion pattern, as revealed in cells transfected with GFP-VASP or GFP-zyxin, comprises a set of focal complexes that turnover rapidly at the base of the protruding lamellipodium (Rottner et al., 1999b) and variable numbers of larger and longer-lived focal adhesion sites in regions behind the lamellipodium (Fig. 1A). The application of force to the cell body with a microneedle in a direction opposing movement induced an enlargement of these peripheral adhesions in the direction of stress application (Fig. 1A). In this situation, protrusion of the cell front was not arrested but continued during the period of adhesion site enlargement, as confirmed by the persistence of GFP-VASP at the lamellipodium tip (Fig. 1A) (Rottner et al., 1999b). These results showing stress-induced enlargement of adhesion foci in motile melanoma cells confirm and extend previous findings using fibroblasts (Riveline et al., 2001).

Lamella regions of B16F1 melanoma cells situated behind rapidly protruding lamellipodia characteristically show few microtubules that extend close to the cell front (see also Ballestrem et al., 2000). However, when stress was applied via a microneedle to the cell body of B16 cells that had been transfected with GFP-tubulin, microtubules were seen to extend towards the base of the protruding lamellipodium (Fig. 1B). Measurements of 20 cells for a period of 2 minutes showed that there was an approximately three-fold increase in

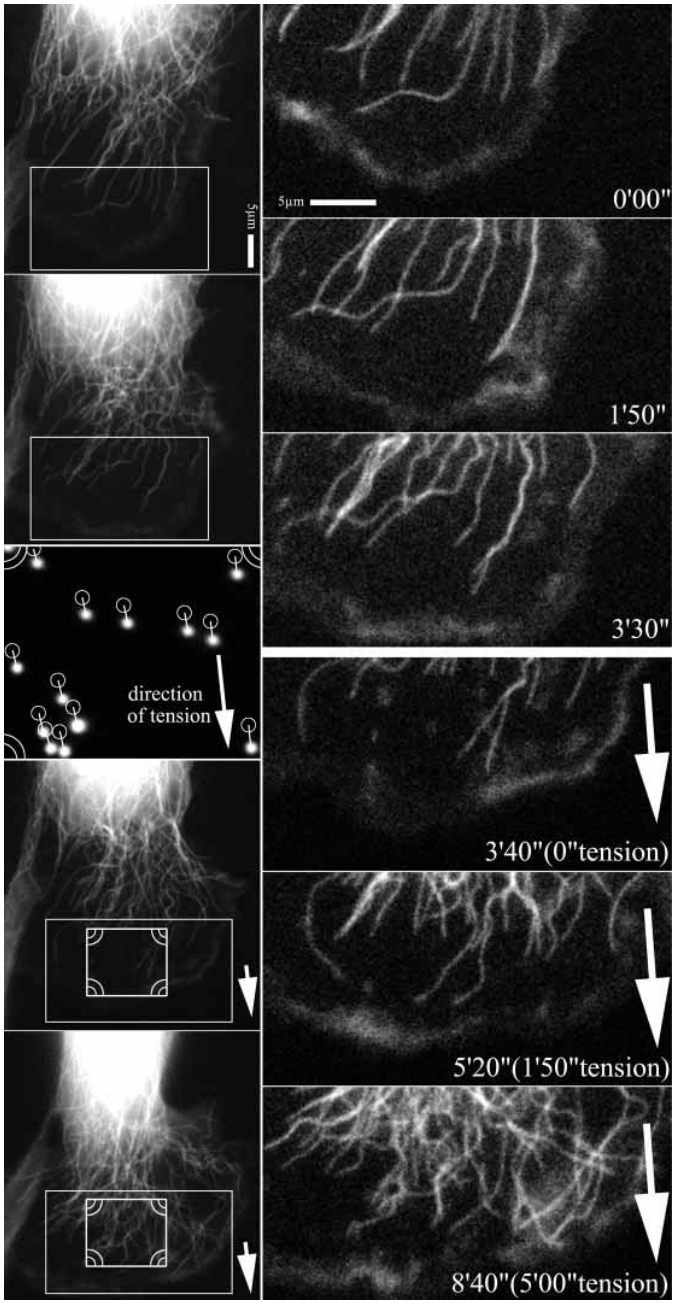

Fig. 5. Tension applied via stretching of the growth substrate beyond the cell front induces microtubule growth at the cell periphery. The figure shows a B16 melanoma cell that was transfected with GFP-tubulin and plated onto a flexible, polyacrylamide substrate impregnated with rhodamine-tagged fluorescent beads. The upper left and right panels (boxed insets enlarged) show video frames of the cell before tension application. The lower frames show the corresponding regions after tension application [at 3 minutes 30 second (3′40″)] by a needle applied to the substrate around 20 μm beyond the cell edge. The direction and magnitude of tension is indicated by the shift of fluorescent beads (middle panel, left), which corresponds to the smaller boxes regions in the lower, left panels. One example from 19 cells is shown.

the number of microtubules that penetrated a given region of a lamella following the application of stress (Fig. 2A).

In GFP-CLIP-170-transfected cells, the number of fluorescent microtubule plus ends in peripheral lamella regions increased dramatically upon stress application (Fig. 1C, Fig. 2C). Since CLIP-170 binds only to polymerising microtubule tips, these findings show that the stress-induced invasion of microtubules is caused by the stimulation of microtubule polymerisation and not by the transport of pre-existing microtubules towards the cell front by other means. By doubly transfecting cells with GFP-tubulin and GFP-zyxin, we could further show that the microtubules that polymerised to the cell front targeted the adhesion sites that were independently amplified at the cell periphery by the increased tension (Fig. 3).

It is notable that the same regions behind rapidly protruding lamellipodia in B16 cells that are depleted of microtubules also lack well defined bundles of actin filaments (Ballestrem et al., 1998). Instead, there commonly exists a loose network of actin filaments that extends from the base of the lamellipodium into the perinuclear region, where bundles become more evident (Rottner et al., 1999b; Small et al., 1999b). In view of the observed growth of both adhesion sites and microtubules in response to applied stress, it was important to establish the accompanying changes in the actin cytoskeleton under the same conditions. For this purpose, we used cells transfected either with GFP-actin (Fig. 4A) or GFP-calponin. The actin-binding protein calponin is a particularly useful probe as it binds preferentially to bundles of actin filaments and not to actin meshworks in lamellipodia or to looser actin filament arrays in cultured cells (Gimona and Mital, 1998). As shown in Fig. 4B, mechanical stress induced a rapid and dramatic appearance of actin bundles extending from the cell centre to the periphery, consistent with the parallel amplification of peripheral focal adhesions that occurs under the same conditions (Figs 1 and 3). The stress exerted in lamellae by cell body displacement was estimated on the basis of manipulations of cells spread on flexible substrates (see below) to be of the order of 5×10^5 dynes/cm^2. This result revealed the continuity of actin filaments in the cytoplasmic network with peripheral anchorage sites as well as the competence of these filaments to form bundles under stress.

Stress application to melanoma cells via a flexible substrate

A second method of applying mechanical stress to adherent cells is to use flexible substrates for growth and to distort the substrate locally with a micropipette (Lo et al., 2000). In this case, the distortion can be monitored by the incorporation of marker beads into the substrate. Accordingly, B16 cells transfected with GFP-tubulin were plated onto flexible polyacrylamide substrates, and the substrate was stretched to induce localised stress at the cell periphery (Fig. 5). The stress exerted on the cell was estimated to be of the order of 10^6 dynes/cm^2 on the basis of the displacement of the substrate and previous simulations (Beningo et al., 2001). As with cell body manipulation, the application of radial stress caused a dramatic induction of microtubule growth, so that after 1 minute of tension application there was an average 2.5-fold increase in the number of microtubules in anterior lamella zones (Fig. 2B).

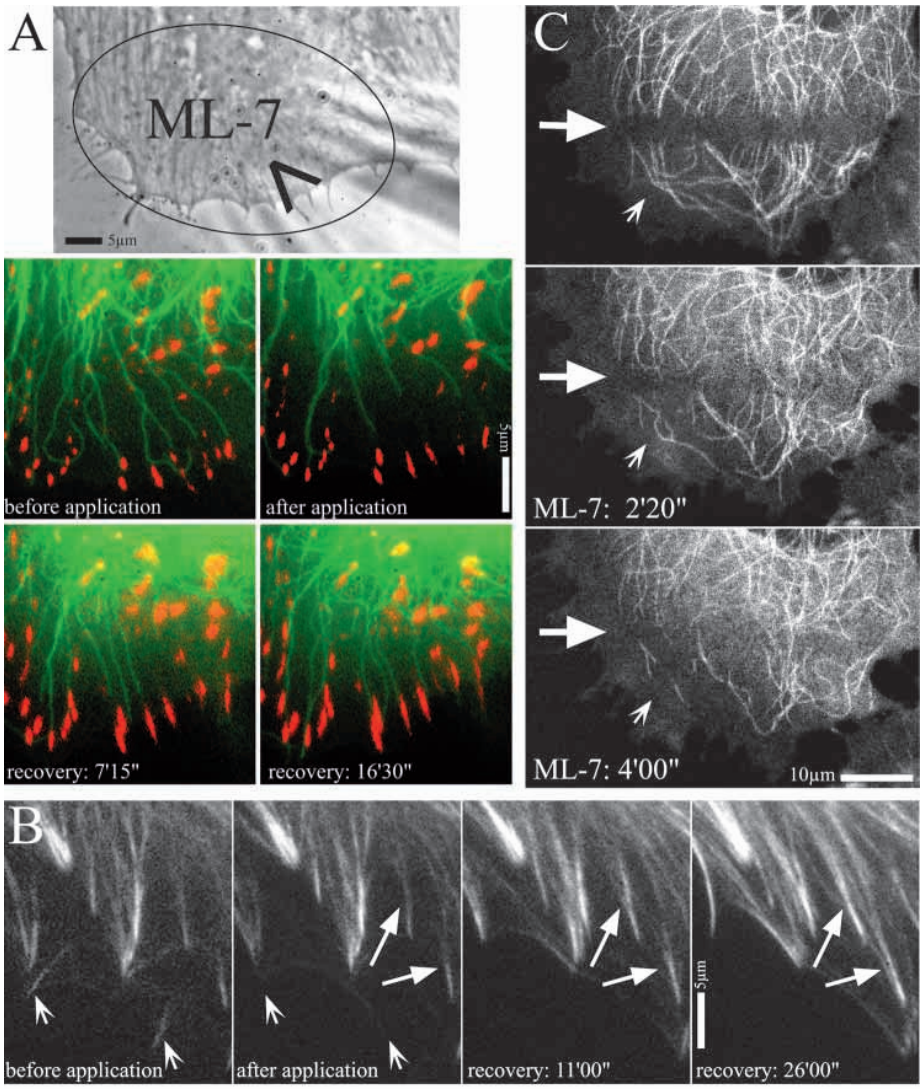

Fig. 6. Recovery from brief, local inhibition of contractility by ML-7 is associated with the repolymerisation of microtubules to peripheral adhesions and enhanced actin bundle formation. (A) A fish fibroblast expressing GFP-tubulin that was injected with TAMRA vinculin. The phase contrast image (left) indicates the region of application of ML-7 (ellipse) via a micropipette (chevron). The period of application was 3 minutes. Fluorescent images show video frames of the region of application at the time points indicated. One example from seven cells is shown. (B) A fish fibroblast was treated as in A but was transfected with GFP-calponin to highlight actin bundles. Arrowheads show peripheral bundles disassembling during application. Arrows indicate stress fibres enhancing during recovery. One example from eight cells is shown. (C) The retraction of microtubules from the cell edge on ML-7 treatment is caused by depolymerisation and not by bulk withdrawal of microtubules. A fish fibroblast expressing GFP-tubulin was photobleached in a narrow region across the base of lamella (arrow) and then exposed to local ML-7 application. Arrowheads indicate depolymerising microtubules in subsequent frames. One example from five cells is shown.

Using GFP-CLIP-170-transfected cells we could show that this effect was again caused by the enhancement of microtubule polymerisation, as indicated by a dramatic increase in the number of fluorescent microtubule plus ends in stressed regions (Fig. 2C).

Recovery from inhibition of contractility in fibroblasts

An increase in stress in the actin cytoskeleton is also stimulated during the recovery of cells from an inhibition of actomyosin contractility. As shown previously, the inhibition of myosin-dependent contractility in fibroblasts causes the dissolution of focal adhesions and the relaxation of substrate traction (Chrzanowska-Wodnicka and Burridge, 1996). When applied locally to a cell edge, myosin inhibitors not only induce the diminution and release of substrate adhesions but also the rapid shrinkage of microtubules (Kaverina et al., 1999). In this study, we monitored the short-term behaviour of microtubules and stress fibres in fish fibroblasts during transient, local treatment with one of three inhibitors (BDM, ML-7 or H-7). Within 2-3 minutes of inhibitor treatment, microtubules withdrew from the cell edge (Fig. 6C). Using the fluorescence recovery after photobleaching (FRAP) technique in combination with inhibitor application, we could confirm that the withdrawal of microtubules was caused by depolymerisation and not by the passive retraction of the microtubule network (Fig. 6C). Following removal of the inhibitor and the recovery of tension in the actin cytoskeleton, microtubules repolymerised and targeted the peripheral adhesion sites (Fig. 6A). Essentially the same result was obtained with all three inhibitors.

The result of using the same experimental protocol with cells transfected with GFP-calponin is presented in Fig. 6B. Typically, the withdrawal of the cell edge was accompanied by the partial dispersion of small peripheral actin bundles, labelled with GFP-calponin. The restoration of contractility following recovery from the inhibitor was marked by bundle growth (Fig. 6B), which is indicative of an increase in the stress level above that before inhibitor treatment. Experiments with GFP-actin-transfected cells gave essentially the same result (data not shown).

Cell body restraint in epidermal keratocytes

In the rapidly migrating fish keratocyte, microtubules are concentrated around the cell body and are not required for polarised locomotion (Euteneuer and Schliwa, 1984). These cells also lack typical focal adhesions and stress fibres but exhibit small punctate adhesion complexes beneath lamellipodia (Anderson and Cross, 2000). Restraint of the keratocyte cell body with a microneedle caused a dramatic

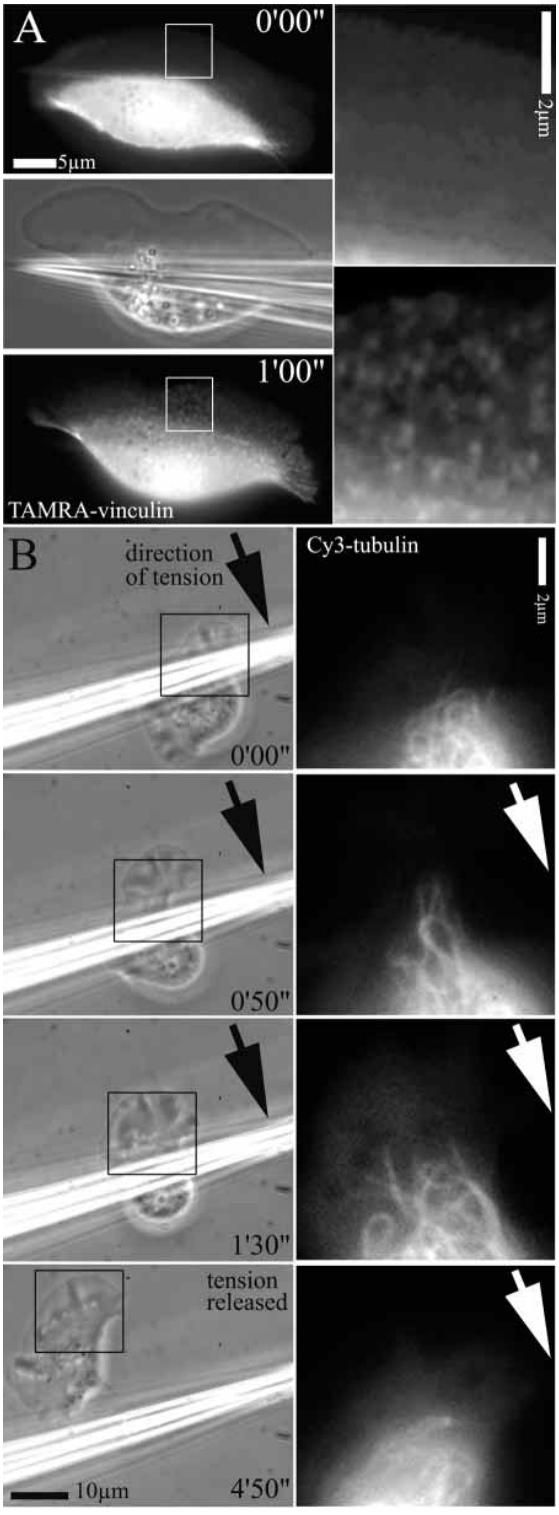

Fig. 7. Mechanical restraint of the keratocyte cell body stimulates growth of adhesion sites and penetration of microtubules into the lamellipodium. A trout keratocyte cell body, injected with rhodamine vinculin, was arrested at time 0 by a micropipette (phase image). The fluorescence images and insets (of boxed regions) are shown at the initiation of cell body arrest (0′0″) and 1 minute later. Note the incorporation of vinculin into multiple, new focal complexes in response to stress (at 1′00″). One example of five cells is shown. (B) The same protocol was used as in A for a black molly keratocyte injected with Cy-3 tubulin. The cell body was released 70 seconds before the last frame (4′50″). The right panels show, in the fluorescence channel, the regions boxed in the phase contrast images (left). An example from 15 cells is shown.

enhancement in the size of adhesion sites in the lamellipodium (Fig. 7A), which is indicative of an increase in stress in the actin network. By microinjecting fluorescent tubulin into keratocytes we were able to monitor changes in microtubule dynamics in response to the same mechanical manipulations. As shown in Fig. 7B, restraint of the cell body leads to the invasion of microtubules into the lamellipodium, which extended in the opposite direction from the applied stress.

Discussion

Riveline et al. recently showed that an increase in stress imposed between the cell body and the periphery of a cell by the application of a microneedle caused the growth of focal adhesions (Riveline et al., 2001). These findings are consistent with the original idea of Vasiliev (Vasiliev, 1985), which were later confirmed experimentally (Chrzanowska and Burridge, 1996), that focal adhesion enlargement can be explained by the alignment of actin filaments from a primary attachment point through contractile interactions with myosin. The results of Riveline et al. demonstrate that tension applied from the outside, without the activation of myosin, is likewise sufficient to drive adhesion site assembly (Riveline et al., 2001). Using similar strategies for locally modulating stress, we now demonstrate that an increase in tension at peripheral substrate attachments signals the outgrowth of microtubules towards these sites in various cell types. In keratocytes, microtubules do not normally invade lamellipodia and are not required for cell guidance. Nevertheless, enhanced stress induces microtubule outgrowth in lamellipodia, indicating the general nature of the phenomenon. Notably, the association of actin filaments in bundles, unlike tension, is apparently not sufficient for microtubule guidance, as microtubules depolymerise from ML7-relaxed zones before bundles disassemble; they also polymerise into lamellae upon stress application prior to the appearance of bundles.

Local tension increases were also induced at the periphery of *Aplysia* growth cones by the application and restraint of beads coated with attachment molecule ligands, and, in line with our findings, microtubules grew towards the restrained beads (Suter et al., 1998a). Taken together with an earlier demonstration (Kaverina et al., 1999) (this study) that localised relaxation of tension causes the depolymerisation of microtubules away from the cell periphery, our results highlight the existence of a tension-sensing mechanism for microtubule growth. The phenomenon we describe should not be confused with the stabilisation of microtubules at adhesion foci (Kaverina et al., 1998) or with the long-term microtubule stabilisation that depends on RhoA and its effector mDia (Cook et al., 1998; Palazzo et al., 2001). Interestingly, the application of tension to chromosomes was recently shown to amplify the number of kinetochore microtubules, again linking tension with microtubule growth or stability (King and Nicklas, 2000). Two primary processes must be invoked to explain the effects we have described: a tension-linked stimulation of microtubule polymerisation and the guidance of microtubules to peripheral sites.

Various possibilities exist for establishing linkages between actin and microtubule networks (reviewed in Gavin, 1996; Goode et al., 2000; Waterman-Storer et al., 2000) that may be relevant for the guidance of microtubules to substrate adhesions.

One possibility is suggested by the demonstration that kinesin and unconventional myosins can cooperate in the transport of vesicles along microtubules and actin filaments (Rodionov et al., 1998). However, the involvement of heterodimeric myosin-kinesin motor complexes (Huang et al., 1999; Beningo et al., 2000) in the guidance of microtubules along actin filaments to adhesion sites (Goode et al., 2000) is unlikely since a block in kinesin activity has no effect on the ability of microtubules to target substrate adhesions (Krylyshkina et al., 2002).

As elaborated in detail by Goode et al., the interaction of microtubules with actin filaments represents a common feature of morphogenetic events in a variety of cell types (Goode et al., 2000). Interestingly, actin cables are needed for maintaining yeast spindle orientation (Palmer et al., 1992; Theesfeld et al., 1999), and this process requires myosin V (Yin et al., 2000), which can bind to the microtubule-associated protein Kar 9. Thus, the sliding of a myosin along actin filaments may serve to direct microtubules toward the cortex (see also Suter et al., 1998b). Myosin V was also found by Waterman-Storer et al. to colocalise with dynamic, co-linear arrays of actin filaments and microtubules observed in *Xenopus* extracts (Waterman-Storer et al., 2000). A possible mode of linkage for an unconventional myosin to microtubules has also been suggested by the interaction of a class VI myosin with a CLIP-170 homologue in *Drosophila* (Lantz and Miller, 1998), but so far an association of myosin VI with microtubules has not been observed (Lantz and Miller, 1998; Buss et al., 1998). In this connection, CLIP-170 (Perez et al., 1999) and other proteins at the tips of growing microtubules (reviewed in Schroer, 2001; Tirnauer and Bierer, 2000; Schuyler and Pellman, 2001) including APC (Mimori-Kiyosue et al., 2000a), EB1 (Mimori-Kiyosue et al., 2000b), members of the dynactin complex (Vaughan et al., 1999) and the CLASP family (Akhamanova et al., 2001) are strategically located where microtubule dynamics as well as the guidance of microtubule growth are most probably controlled.

With regard to the link between microtubule guidance and stress, the results with B16 cells transfected with the abundant smooth muscle isoform of calponin (h1) are particularly interesting. As shown by Gimona and Mital, calponin binds primarily to stress fibre bundles and not to actin meshworks or loose actin filament arrays (Gimona and Mital, 1998). The dramatic appearance of calponin-positive bundles in the lamella regions of B16 cells after tension application suggests that tension induces conformational changes in actin filaments, or proteins associated with them, that facilitate calponin binding. It has already been shown that actin-binding proteins can induce changes in the twist of the long pitch helices of actin filaments (Bamberg et al., 1999; Galkin et al., 2001): by the same token the application of torsion to a ‘relaxed’ filament should induce structural changes allowing interaction with other binding partners. We suggest that conformational changes in actin induced by stretch play a primary role in signalling for microtubule polymerisation and guidance. One plausible scenario includes the linkage of an unconventional myosin to a component of the microtubule tip complex, whereby this myosin is only competent to bind to actin filaments that are under tension. This interaction could induce further conformational changes in the microtubule tip complex itself that promote microtubule polymerisation. Whether or not such a scenario pertains to microtubules and whether other regulatory factors are involved that bind to microtubules (Best et al., 1996; Ren et al., 1998; Glaven et al., 1999) or that influence microtubule assembly (Carazo-Salas et al., 1999) in response to stress-induced signal transduction at adhesion sites remains to be elucidated. Whatever the mechanism, we appear to have revealed a tension-based feedback loop that plays a role in the promotion of substrate adhesion disassembly via microtubules.

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