FEBS Letters 452 (1999) 96–99 FEBS 21991

Minireview

Cytoskeleton cross-talk during cell motility

J. Victor Small*, Irina Kaverina, Olga Krylyshkina, Klemens Rottner

Department of Cell Biology, Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, A-5020 Salzburg, Austria

Received 30 March 1999

Abstract Cell crawling entails the co-ordinated creation and turnover of substrate contact sites that interface with the actin cytoskeleton. The initiation and maturation of contact sites involves signalling via the Rho family of small G proteins, whereas their turnover is under the additional influence of the microtubule cytoskeleton. By exerting relaxing effects on substrate contact assemblies in a site- and dose-specific manner, microtubules can promote both protrusion at the front and retraction at the rear, and thereby control cell polarity.

Key words: Actin; Microtubule; Signalling; Rho

1. Cytoskeleton-contact interplay

Of the three polymers that make up the cytoskeleton, it is the filaments of the actin cytoskeleton that underlie the cell membrane and that contribute directly to the maintenance of cell shape and form. Intermediate filaments are dispensable for cell shape determination since in their absence cells can differentiate normally [1], whereas microtubules have an important, but indirect influence on cell shape, a topic to which we will return below.

Much of what we now know about the mechanisms controlling the organisation of the actin cytoskeleton comes from studies of fibroblastic cells in culture. Two general aspects may first be highlighted: (1) the dynamic nature of the actin cytoskeleton; and (2) the variety of assemblies of actin filaments that can be generated in vivo. Among these actin assemblies (reviewed in [2]), the most ubiquitous are the prominent bundles of actin filaments that make up the 'stress fibres' and the 'lamellipodia' and 'filopodia' that form at the cell periphery. The organisation of each of these assemblies is stabilised by specific sets of actin-associated proteins, conferring on them different functions. Stress fibres are contractile and serve to develop strong substrate anchorage, whereas lamellipodia and filopodia are required for spreading and motility. Central players in signalling the formation of these actin filament arrays are the members of the Rho family of small GTPases: Rho, Rac and Cdc42; Rho activity is required for stress fibre formation and Rac and Cdc42 for lamellipodia and filopodia, respectively [3].

To what extent each of these actin filament assemblies is expressed in a cell is largely determined by the cell type and the nature of the substrate on which it is induced to spread, illustrating a feedback between Rho family regulation and the response to extracellular matrix [4,5]. It is now well established that the attachment of a cell to a substrate does not occur uniformly over its ventral surface, but at specific, focalised sites of adhesion. At these sites, transmembrane matrix receptors (integrins) link matrix ligands on the outside of the cell with the actin cytoskeleton on the inside [6]. Notably, it is the dynamic interplay between the formation and turnover of contact sites and the simultaneous generation and re-organisation of the actin cytoskeleton that determines the form and motile behaviour of a cell. Significant in this connection is the existence of different classes of contact sites associated with the different actin assemblies, most readily detected by labelling for established contact components such as vinculin or paxillin (Fig. 1). Fine, linear contacts are associated with a sub-population of filopodia; small, punctate contacts can be found associated with lamellipodia; and prominent linear or chevron-shaped contacts (focal adhesions) mark the termini of stress fibre bundles (e.g. [7]).

Earlier lines of evidence suggested that some of the primary contacts formed beneath filopodia and lamellipodia serve as precursors of focal adhesions [7,8]. The results of more recent studies have substantiated these claims by showing that focal adhesions cannot develop independently, but only from such precursor contacts [9]. In other words, the generation of lamellipodia and filopodia via Rac and Cdc42 provides the structural environment for contact initiation.

2. Contacts and contractility

Chrzanowska-Wodnicka and Burridge [10] have demonstrated that the development and maintenance of focal adhesions, signalled by Rho, requires myosin-II-dependent contractility. Contractility is mediated via the phosphorylation of the myosin regulatory light chain and inhibitors of myosin light chain kinase were shown to cause the dissolution of both stress fibre bundles and focal adhesions. Parallel studies (reviewed in [11]) have pinpointed Rho kinase (p160 ROCK) as the Rho effector responsible for inducing focal adhesions. In this scheme, Rho kinase phosphorylates and inhibits myosin light chain phosphatase, thereby facilitating myosin phosphorylation by the myosin light chain kinase. A key role for Rho kinase in this process has been confirmed by transfecting cells with dominant-negative Rho kinase constructs [12] and by using a newly developed and specific inhibitor of this enzyme [13].

So where do precursor contacts fit into this scheme? Nobes and Hall [14] have described a contact type distinct from focal adhesions that is formed at the cell periphery when Rac is up-regulated and Rho down-regulated. We have now studied the dynamics of these Rac-induced 'focal complexes', labelled in living cells with a fluorescent analogue of the contact protein

*Corresponding author. Fax: (43) (7662) 763961-40.
E-mail: jvsmall@imb.oeaw.ac.at

PII: S0014-5793(99)00530-X

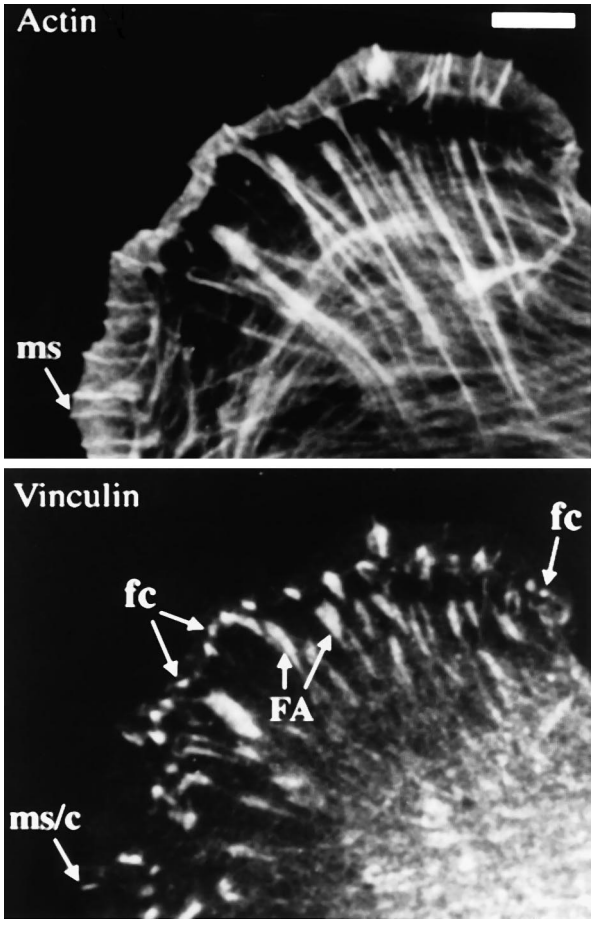

Fig. 1. Contact types in a migrating chick heart fibroblast. Cell was fixed and double-labelled for vinculin and actin. Precursor focal complexes in the lamellipodium include focal complexes (fc) and a microspike-associated contact (ms/c). FA indicates focal adhesions. Bar, 5 μm.

vinculin [9]. It could first be shown that focal complexes can serve as precursors of focal adhesions. Second, like focal adhesions, focal complexes showed the same requirement for myosin contractility for their maintenance, in that they were dissociated by myosin light chain kinase inhibitors. However, whereas Rho kinase inhibition causes the dissolution of focal adhesions and stress fibre bundles [13], it had no effect on focal complexes. Indeed, a block of Rho kinase activity was found to stimulate membrane ruffling and focal complex initiation and turnover, associated with advances of the cell periphery characteristic of protrusive phases in cell motility.

Precursor contacts formed in ruffling lamellipodia thus depend for their existence on myosin contractility, but this is stimulated by a route downstream of Rac that does not involve Rho kinase. Other observations (Kaverina and Rottner, unpublished) indicate that precursor contacts formed beneath filopodia are also Rho kinase-independent. Significantly, it could also be shown that as far as contact dynamics are concerned, Rac and Rho antagonise each others' pathways. Hence, the down-regulation of Rho caused an up-regulation of membrane ruffling and the down-regulation of Rac, an increase in the size of focal adhesions [9]. We will return to this phenomenon in Section 4.

3. Microtubule modulation

We have already seen that the formation and development of adhesion sites is under the control of the Rho family of small G-proteins. Other recent findings [15,16] reveal that microtubules have an equally profound influence on substrate contact turnover, an influence that now explains the dependence of directional locomotion on an intact microtubule cytoskeleton [17].

In living, migrating fibroblasts in which both microtubules and substrate contacts were labelled with fluorescent probes, it was found that microtubule ends specifically targeted vinculin-containing contact sites ([15] and Fig. 2). Via the use of microtubule inhibitors it could also be demonstrated that contact sites nucleate microtubule growth and stabilise microtubule assembly, suggestive of a functional basis of the targeting interaction. It was speculated that microtubules deliver (or sequester) regulatory components to modulate contact development [15,18]. While such components remain to be identified, further work has indicated that microtubules potentiate the dissociation and turnover of substrate contacts. This was indicated by the observation that peripheral focal adhesions that were multiply targeted by microtubules were commonly dissociated from the substrate, in conjunction with the inward retraction of the cell edge [16]. Likewise, by monitoring the contact dynamics in spreading cells that contained either an intact or disassembled microtubule cytoskeleton, it was evident that microtubules were required for the centrifugal turnover of contact sites, necessary for the efficient advance of the spreading cell edge.

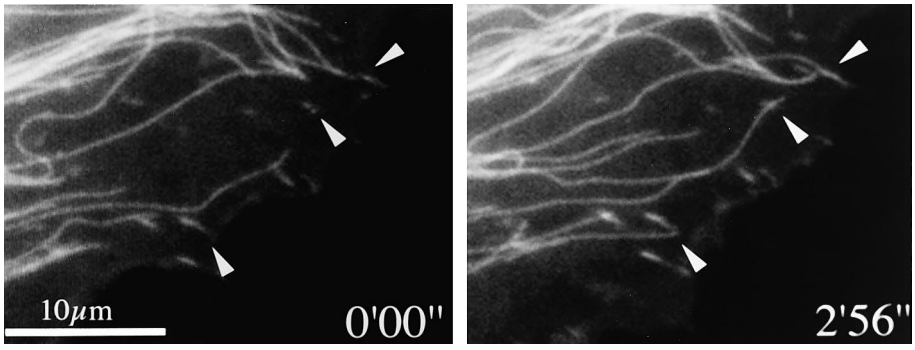

Fig. 2. Targeting of contact sites by microtubules in a living cell. Two video frames are shown of a cell co-injected with vinculin and tubulin, each conjugated with a rhodamine fluorophore. Arrowheads indicate the contacts targeted by growing microtubules in this sequence. Times indicated on the frames are in minutes and seconds.

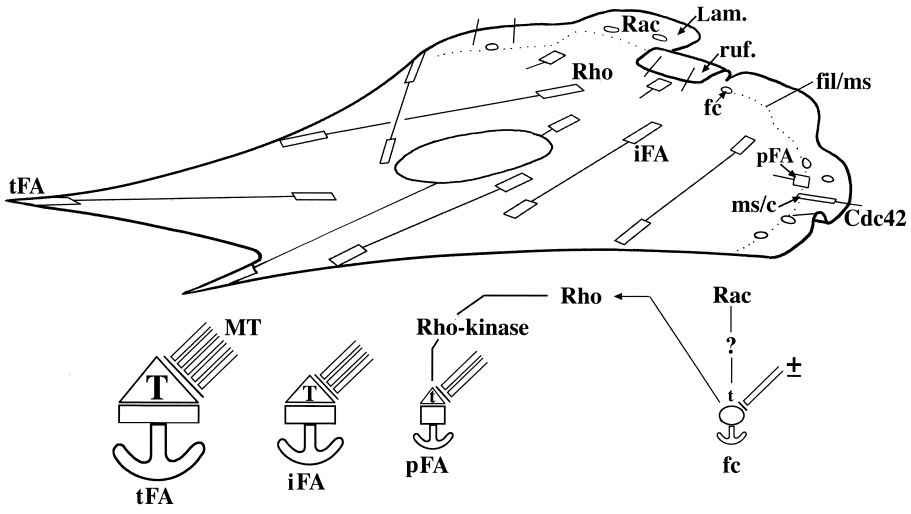

Fig. 3. Schematic illustration of substrate contact dynamics in a moving fibroblast. Upper section: Substrate contacts are initiated in the protruding and ruffling lamellipodium (Lam, ruf). Two classes of primary contacts are depicted: punctate focal complexes (fc) and linear contacts associated with some microspike bundles (ms/c). Their formation is associated with the activation of Rac and Cdc42, respectively. Each type of primary contact can develop into a precursor of a focal adhesion (pFA), involving a transition from Rac (Cdc42) to Rho signalling. Further abbreviations: iFA, intermediate focal adhesion in the body of the cell; tFA, focal adhesion at a trailing cell edge. Lower section: Rho signalling and microtubule-mediated modulation of adhesion. Four types of contact sites with different strengths of anchorage to the substrate (anchors) are depicted and correspond to those in the upper diagram. All sites rely on contractility for their maintenance, indicated by different levels of myosin II-dependent tension (T and t). For precursor (pFA) and mature focal adhesions (iFA, tFA), myosin activation depends on Rho kinase. The contractility of Rac-induced focal complexes is Rho kinase-independent. Microtubules (MT) interface with contact sites and modulate their turnover by locally inhibiting contractility. The relaxing dose is controlled by the total number and frequency of microtubule targeting events. Focal complexes may or may not be targeted by microtubules. Adapted from [16].

When fibroblasts are treated with microtubule antagonists, their stress fibre bundles enlarge [19] and they develop increased tension with their substrate [20]. In starved cells, microtubule depolymerisation leads to the activation of Rho and the induction of focal adhesions [21,22], a process that, as we have mentioned, is also dependent on contractility. On this basis, Bershadsky et al. [21] have suggested that microtubules exert a general inhibition on contractility. Our own findings are not only consistent with this suggestion, but provide evidence for the localised modulation of contact dynamics, both of focal complexes and of focal contacts, by microtubule-mediated relaxation of contact assemblies.

Most illustrative of the relaxing effect of microtubules was the finding that contractility inhibitors, applied locally at a cell edge bearing focal adhesions, caused the same mode of retraction as observed with microtubule targeting. Significantly, the local application of an inhibitor was accompanied by the rapid depolymerisation of microtubules from the contacts at the application site. We may recall that focal adhesions can stabilise microtubules that target them [15] and here the reciprocal effect occurs; contact destabilisation induces microtubule disassembly. Along the same lines, enhanced Rho activity has been found to stabilise microtubules [23], a result that we would attribute to the prior capture of microtubules at focal adhesions. Taken together, these data illustrate a mutual feedback between contact sites and microtubules that influences the stabilities of both of them.

In contradiction to the present scheme, Waterman-Storer and Salmon [24] have suggested that the growth of microtubules towards the cell front activates Rac and the formation of lamellipodia, whereas microtubule depolymerisation towards the rear activates Rho and the formation of focal adhesions, required for retraction. Our own observations are inconsistent with this idea: rather, we suggest that the activation of ruffling after release from nocodazole, on which the idea is based [24], is an indirect result of the down-regulation of Rho, which leads to the stimulation of Rac [9].

To conclude this section, it is proposed that microtubule targeting relies on a tension-sensing mechanism whereby microtubules deliver relaxing signals in a precise way to contact sites destined for diminution or dissociation.

4. Collaborate and move

On the basis of the foregoing, we can speculate on how the pathways involving the actin and microtubule cytoskeletons collaborate in the regulation of cell polarity and locomotion (Fig. 3).

When fibroblasts are injected with constitutively active Rho, Rac or Cdc42 the effect on the actin system is a global one [14]. For example, the peripheral activation of membrane ruffles and filopodia effected by Rac and Cdc42 occurs around most of the cell periphery. The single activation of these small G-proteins is thus insufficient to signal the polarisation of cell form. For this microtubules are required. As we have seen, contact formation and turnover is under the influence of both the small G-proteins and microtubules. They must therefore collaborate in regulating the pattern and reorganisation of substrate contact assemblies.

The possibility must be entertained that microtubules do not provide new regulatory partners, but simply intervene in the Rho family pathways by providing or sequestering downstream effectors. In any case their final effect is to exert a relaxation response. Since both focal complexes and focal adhesions rely on contractility, they can each fall under the influence of relaxation signals transmitted by microtubules. In

this connection, the targeting activity of microtubules provides a convenient way of controlling the dose of relaxing agents. Focal complexes that must be turned over at the cell front to promote protrusion would require smaller doses of relaxation than focal adhesions at the trailing edges of a migrating cell that must be released from the substrate.

The antagonism between the Rac and Rho pathways, as has been noted also with other cells [5,25], would serve to amplify the polarisation reaction. Thus the up-regulation of Rac at the protruding and ruffling front of the cell would tend to suppress the formation of focal adhesions. It remains to be shown if microtubule signalling takes advantage of these antagonistic effects.

These recent studies have exposed the contact machinery as the interface between the actin and microtubule cytoskeletons. By regulating contact dynamics, microtubules can exert a control on both cell shape and locomotion. Many questions, of course, remain to be answered, not least the mechanism by which microtubules are guided to contact sites and the nature and means of transport of their signalling cargo.

Acknowledgements: The authors thank Ms M. Schmittner for technical and photographic assistance and Ms E. Eppacher for manuscript processing. Part of this work was supported by a grant from the Austrian Science Research Foundation.

References

[1] Fuchs, E. and Cleveland, D.W. (1998) Science 23, 514–519.
[2] Small, J.V., Rottner, K., Kaverina, I. and Anderson, K. (1998) Biochim. Biophys. Acta 1404, 271–281.
[3] Hall, A. (1998) Science 279, 509–514.
[4] Hotchin, N.A. and Hall, A. (1995) J. Cell Biol. 131, 1857–1865.
[5] Van Leeuwen, F.N., Kain, H.E.T., Van der Kammern, R.A., Michiels, F., Kranenburg, O.W. and Collard, J.G. (1997) J. Cell Biol. 139, 797–807.
[6] Yamada, K.M. and Geiger, B. (1998) Curr. Opin. Cell Biol. 9, 76–85.
[7] Rinnerthaler, G., Geiger, B. and Small, J.V. (1988) J. Cell Biol. 106, 747–760.
[8] Izzard, C.S. and Lochner, L.R. (1980) J. Cell Sci. 42, 81–116.
[9] Rottner, K., Hall, A. and Small, J.V. (1999) submitted for publication.
[10] Chrzanowska-Wodnicka, M. and Burridge, K. (1996) J. Cell Biol. 133, 1403–1415.
[11] Narumiya, S., Ishizaki, T. and Watanabe, N. (1997) FEBS Lett. 410, 68–72.
[12] Ishizaki, T., Naito, M., Fujisawa, K., Maekawa, M., Watanabe, N., Saito, Y. and Narumiya, S. (1997) FEBS Lett. 10, 118–124.
[13] Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, H., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M. and Narumiya, S. (1997) Nature 389, 990–994.
[14] Nobes, C.D. and Hall, A. (1995) Cell 81, 53–62.
[15] Kaverina, I., Rottner, J. and Small, J.V. (1998) J. Cell Biol. 142, 181–190.
[16] Kaverina, I., Krylyshkina, O. and Small, J.V. (1999) in preparation.
[17] Vasiliev, J.M. and Gelfand, I.M. (1976) in: Cell Motility, pp. 279–304, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[18] Small, J.V., Rottner, K. and Kaverina, I. (1999) Curr. Opin. Cell Biol. 11, 54–60.
[19] Lloyd, C.W., Smith, C.G., Woods, A. and Rees, D.A. (1977) Exp. Cell Res. 110, 427–437.
[20] Danowski, B.A. (1989) J. Cell Sci. 93, 255–266.
[21] Bershadsky, A., Chausovsky, A., Becker, E., Lyubimova, A. and Geiger, B. (1996) Curr. Biol. 6, 1279–1289.
[22] Enomoto, T. (1996) Cell Struct. Funct. 21, 317–326.
[23] Cook, T.A., Nagasaki, T. and Gundersen, G.G. (1998) J. Cell Biol. 141, 175–185.
[24] Waterman-Storer, C.M. and Salmon, E.D. (1999) Curr. Opin. Cell Biol. 11, 61–67.
[25] Kozma, R., Sarner, S., Ahmed, S. and Lim, L. (1997) Mol. Cell. Biol. 17, 1201–1211.