FAK and talin: Who is taking whom to the integrin engagement party?

Bryan Serrels and Margaret C. Frame

Edinburgh Cancer Research UK Centre, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH2 4RX, Scotland, UK

In this issue, Lawson et al. provide new insight into the relationship between FAK and talin during assembly of integrin adhesions on fibronectin. They show that FAK is upstream of talin, and that talin is not required for FAK recruitment or for integrin activation at nascent adhesions. However, FAK-talin binding is required for adhesion turnover and cell motility. The findings question the view that talin is always upstream of focal adhesion protein recruitment to clustered integrin sites.

Integrins are transmembrane extracellular matrix receptors that not only mediate physical attachment between a cell and its environment, but also permit transduction of signals to control shape, motility, and the cell cycle in response to environmental cues. Integrin adhesions exist in multiple forms in cells, for example focal complexes, focal adhesions, and fibrillar adhesions, and many of the major components of these have now been identified. The assembly of multiprotein complexes initiated by integrin engagement and clustering is critical to their function, and thus controls motility and proliferation. Some mechanisms of integrin activation—its linkage to the actin cytoskeleton and cell motility—have been elucidated, and our knowledge of how cell structure, actin/adhesion dynamics, and cell motility are coordinated with control of proliferation and survival is increasing. Yet we still lack detail on the sequence of events that lead to assembly of the adhesion protein complexes. Two key players are the band 4.1, ezrin, radixin, moesin homology (FERM) domain–containing proteins talin (of which there are two isoforms, talin 1 and talin 2) and focal adhesion kinase (FAK). Talin and FAK can form a complex and both interact with integrins. Several recent studies have begun to elucidate the precise details of how these are recruited to nascent adhesions, and function there to regulate spreading, motility, and signaling. Now, in this issue, Lawson et al. provide evidence that FAK is required for recruitment of talin (rather than the other way around) when mouse embryo fibroblasts (MEFs) are in the process of assembling new adhesions (often termed "nascent" or "early spreading" adhesions), ~15 min after they are plated onto fibronectin. In particular, talin recruitment to nascent adhesions is visibly impaired in FAK-deficient fibroblasts, or FAK knockdown SKOV3 carcinoma cells. Moreover, siRNA-mediated knockdown of talin 1 in human umbilical vein endothelial cells (in which talin 1 is believed to be the predominant talin isoform), has no effect on FAK localization to nascent adhesions. The authors rightly conclude that in the cells they study, FAK is required for recruitment of talin to nascent adhesions. This appears to contradict perceptions in the field that talin lies upstream of FAK recruitment to focal adhesions sites (for example, see Chen et al., 1995; Zhang et al., 2008; Wang et al., 2011).

Notwithstanding the differences in cells and experimental strategies, the apparent differences can be reconciled, at least in part, by considering the time at which adhesion structures were examined. For example, in Wang et al. (2011), adhesions were generally visualized at least 48 h after plating. Under these conditions, small hairpin RNA (shRNA)-mediated depletion of talin in mammary epithelial cells (MECs; which express only the talin 1 isoform) led to impaired recruitment of vinculin, paxillin, integrin-linked kinase, and FAK at mature focal adhesions. When Lawson et al. (2012) examined adhesions at 60 min after plating on fibronectin, they also found that FAK depletion did not prevent talin localization to mature adhesions that already had integral actin linkages at this later time point. The requirement for talin in studies described by Zhang et al. (2008) used genetic knockout of talin 1 with additional siRNA-mediated knockdown of talin 2. This resulted in loss of vinculin- and paxillin-containing foci at edge protrusions in fully spread talin-deficient cells; in their experiments, initial spreading was unaffected (Zhang et al., 2008). Taking all of these observations together, the most likely explanation is that FAK is responsible for talin recruitment to nascent or spreading, but not mature, adhesions; conversely, talin is required for FAK (and other major adhesion components) to localize properly at mature adhesions. Thus, it may be that talin and FAK are required for efficient recruitment of each other under different circumstances, which include the maturation states of adhesion structures (see model in Fig. 1).

It is also intriguing that FAK-deficient MEFs, in which talin is not visible at nascent adhesions, display active β1 integrin. Indeed, β1 integrin is also activated when talin is depleted from MECs (Wang et al., 2011). This is surprising because talin

Correspondence to Margaret C. Frame: m.frame@ed.ac.uk

© 2012 Serrels and Frame This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).

The Rockefeller University Press
J. Cell Biol. Vol. 196 No. 2 185–187
www.jcb.org/cgi/doi/10.1083/jcb.201112128

THE JOURNAL OF CELL BIOLOGY

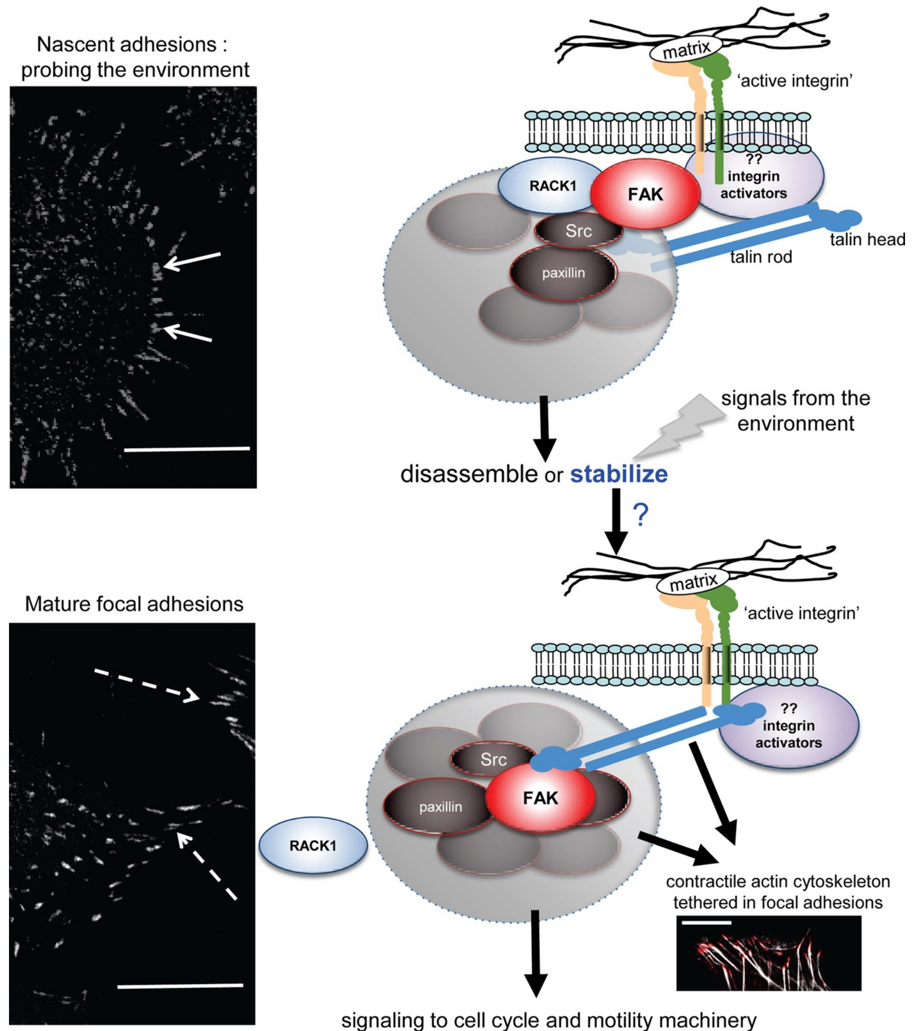

Figure 1. **FAK and talin at nascent and mature focal adhesions.** A model is shown depicting key differences at nascent "environment probing" adhesions (top) and more mature "classical" focal adhesions (bottom), as highlighted by the recent work of Lawson et al. (2012) and other studies referred to in this comment. Key "positioning" of FAK, talin, RACK1, Src, and paxillin and other unknown putative integrin activators are depicted. The confocal images display FAK-stained nascent adhesions (white; solid arrows) in MEFs at 15 min after plating on fibronectin (top) and more mature focal adhesions (white; broken arrows) at 24 h after plating (bottom left image). The bottom right image shows actin stress fibers (depicted in white after phalloidin staining) that are tethered into FAK-containing mature adhesions (shown in red after antibody staining) in fully spread cells. Images were obtained using confocal microscopy. The blue question mark reflects the lack of information concerning whether nascent adhesions stabilize and convert into more mature focal adhesions. Bars, 20 µm.

is known to be a potent integrin activator (Tadokoro et al., 2003; Calderwood, 2004), and the talin head domain was sufficient to restore integrin activation in talin-depleted MEFs in the study by Zhang et al. (2008). Other putative integrin-activating proteins, such as the kindlins (Montanez et al., 2008; Moser et al., 2008), or as yet unidentified activators, might perform this function in some situations (for example when talin is depleted). However, the formal possibility that sufficient residual talin remains after siRNA or shRNA-mediated talin knockdown to permit some integrin activation cannot be ruled out. Although talin does not appear to be necessary for β1 integrin activation during the earliest stages of cell spreading (Lawson et al., 2012), or in spread MECs (Wang et al., 2011), talin may still be needed for full, or sustained, activation of clustered β1 integrin at nascent and/or mature focal adhesions.

Lawson et al. (2012) also took the elegant approach of examining effects of a talin binding–impaired mutant of FAK, namely FAK-E1015A. When expressed in FAK-deficient MEFs, this mutant did not support talin's association with nascent adhesions. FAK-E1015A did not inhibit integrin-stimulated FAK phosphorylation (on Y397), which shows that FAK binding to talin is not required for its activation. This contradicts the lack of FAK-Y397 phosphorylation in talin-depleted cells described in (Zhang et al., 2008), and this difference remains unexplained. Taken at face value though, the data might imply that talin, but not stable talin–FAK association, is required for proper FAK activation in MEFs. Unlike wild-type FAK (FAK-WT), the talin binding–impaired FAK-E1015A protein was unable to rescue the enhanced number of large, more static, focal adhesions displayed by FAK-deficient MEFs, as described previously (Webb et al., 2004; Tomar and Schlaepfer, 2009). Consistent with the reduced dynamic turnover of focal adhesions, cells expressing FAK-E1015A displayed reduced direction-dependent migration and wound repair. Moreover, when compared with FAK-WT, FAK-E1015A could not restore talin cleavage as judged by head domain generation. This is perhaps a consequence of impaired FAK-mediated recruitment of the talin protease calpain (Carragher et al., 2003), which is required for dynamic adhesion turnover and cell motility (Franco et al., 2004).

It is also intriguing that the residues identified on talin required to bind FAK may overlap with those required to bind β1-integrin (Bouaouina et al., 2008), raising the possibility that talin/FAK and talin/β1 complexes are mutually exclusive. Therefore, in some situations FAK may act as a scaffold for talin until it is cleaved by calpain, after which the talin head

domain generated may preferentially bind to, and activate, more integrins. If this occurs, it could enhance intracellular signaling stemming from active integrins, but would also likely lead to disruption of talin-mediated links between integrins and the actin cytoskeleton that are mediated by the talin rod domain (Critchley and Gingras, 2008; Zhang et al., 2008). This could be one way in which signaling is linked to enhanced focal adhesion turnover. There is clearly much more to discover about the complex inter-related roles of FAK and talin in assembly and dynamic turnover of integrin adhesions.

The new work from Lawson et al. (2012) highlights important specific and general issues for future investigation. For example, how is FAK recruited to nascent adhesions? Additional work is also needed to address the nature and relationship between assembly of early spreading/nascent adhesion complexes and the processes involved in assembly of more mature focal adhesion structures. Do nascent adhesions, or a subset of these, mature into more stable focal adhesions in a manner that permits cells to adopt a directional, or polarized, spread phenotype (see model in Fig. 1)? They clearly share many of the same core adhesion components, yet there are also reported differences, including the presence of the scaffold protein RACK1. This was first described at so-called spreading initiation centers (SICs), but was absent in mature focal adhesions (de Hoog et al., 2004). RACK1 was also located at nascent protrusions in squamous carcinoma cells derived from mouse skin tumors (Serrels et al., 2010), and was also found to be absent from mature adhesions in that study. Although the relationship between SICs and nascent adhesions is not well established, RACK1 may represent an example of an adhesion protein that is differentially located at early spreading/nascent and mature adhesion complexes. There will be others, and their identification will provide potential value in marking the types of developing adhesion complexes, particularly at nascent versus more mature or stable adhesion structures. Where this would have particular value is in trying to determine whether each type of adhesion complexes function in vivo, and if so how, when, and where they operate to control cellular function in living animals.

The evidence that FAK and talin display differential requirements for their incorporation into complexes at nascent and mature focal adhesions implies that the precise assembly and disassembly sequences at such adhesions may well be different. There are probably also redundant mechanisms and compensatory adaptation present when we experimentally deplete or mutate key components, which may make absolute conclusions difficult. Together with contextual considerations, for example, cell-type specificity and matrix-associated differences, it may not be possible to discern a single universal model of the precise sequence of events by which nascent or mature adhesions assemble and disassemble. The work from Lawson et al. (2012) is important because, when considered with previously published studies, it highlights the complexity of integrin adhesion dynamics and implies that there is not a single unified way in which integrin adhesion complexes assemble as cells first make "probing" contacts with extracellular matrix in their environment. We can conclude that talin recruitment is not always the first vital step.

Submitted: 22 December 2011
Accepted: 3 January 2012

References

Bouaouina, M., Y. Lad, and D.A. Calderwood. 2008. The N-terminal domains of talin cooperate with the phosphotyrosine binding-like domain to activate beta1 and beta3 integrins. *J. Biol. Chem.* 283:6118–6125. http://dx.doi.org/10.1074/jbc.M709527200

Calderwood, D.A. 2004. Talin controls integrin activation. *Biochem. Soc. Trans.* 32:434–437. http://dx.doi.org/10.1042/BST0320434

Carragher, N.O., M.A. Westhoff, V.J. Fincham, M.D. Schaller, and M.C. Frame. 2003. A novel role for FAK as a protease-targeting adaptor protein: regulation by p42 ERK and Src. *Curr. Biol.* 13:1442–1450. http://dx.doi.org/10.1016/S0960-9822(03)00544-X

Chen, H.C., P.A. Appeddu, J.T. Parsons, J.D. Hildebrand, M.D. Schaller, and J.L. Guan. 1995. Interaction of focal adhesion kinase with cytoskeletal protein talin. *J. Biol. Chem.* 270:16995–16999. http://dx.doi.org/10.1074/jbc.270.28.16995

Critchley, D.R., and A.R. Gingras. 2008. Talin at a glance. *J. Cell Sci.* 121:1345–1347. http://dx.doi.org/10.1242/jcs.018085

de Hoog, C.L., L.J. Foster, and M. Mann. 2004. RNA and RNA binding proteins participate in early stages of cell spreading through spreading initiation centers. *Cell*. 117:649–662. http://dx.doi.org/10.1016/S0092-8674(04)00456-8

Franco, S.J., M.A. Rodgers, B.J. Perrin, J. Han, D.A. Bennin, D.R. Critchley, and A. Huttenlocher. 2004. Calpain-mediated proteolysis of talin regulates adhesion dynamics. *Nat. Cell Biol.* 6:977–983. http://dx.doi.org/10.1038/ncb1175

Lawson, C., S.-T. Lim, S. Uryu, X.L. Chen, D.A. Calderwood, and D.D. Schlaepfer. 2012. FAK promotes recruitment of talin to nascent adhesions to control cell motility. *J. Cell Biol.* 196:223–232.

Montanez, E., S. Ussar, M. Schifferer, M. Bösl, R. Zent, M. Moser, and R. Fässler. 2008. Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev.* 22:1325–1330. http://dx.doi.org/10.1101/gad.469408

Moser, M., B. Nieswandt, S. Ussar, M. Pozgajova, and R. Fässler. 2008. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat. Med.* 14:325–330. http://dx.doi.org/10.1038/nm1722

Serrels, B., E. Sandilands, A. Serrels, G. Baillie, M.D. Houslay, V.G. Brunton, M. Canel, L.M. Machesky, K.I. Anderson, and M.C. Frame. 2010. A complex between FAK, RACK1, and PDE4D5 controls spreading initiation and cancer cell polarity. *Curr. Biol.* 20:1086–1092. http://dx.doi.org/10.1016/j.cub.2010.04.042

Tadokoro, S., S.J. Shattil, K. Eto, V. Tai, R.C. Liddington, J.M. de Pereda, M.H. Ginsberg, and D.A. Calderwood. 2003. Talin binding to integrin beta tails: a final common step in integrin activation. *Science*. 302:103–106. http://dx.doi.org/10.1126/science.1086652

Tomar, A., and D.D. Schlaepfer. 2009. Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility. *Curr. Opin. Cell Biol.* 21:676–683. http://dx.doi.org/10.1016/j.ceb.2009.05.006

Wang, P., C. Ballestrem, and C.H. Streuli. 2011. The C terminus of talin links integrins to cell cycle progression. *J. Cell Biol.* 195:499–513. http://dx.doi.org/10.1083/jcb.201104128

Webb, D.J., K. Donais, L.A. Whitmore, S.M. Thomas, C.E. Turner, J.T. Parsons, and A.F. Horwitz. 2004. FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nat. Cell Biol.* 6:154–161. http://dx.doi.org/10.1038/ncb1094

Zhang, X., G. Jiang, Y. Cai, S.J. Monkley, D.R. Critchley, and M.P. Sheetz. 2008. Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat. Cell Biol.* 10:1062–1068. http://dx.doi.org/10.1038/ncb1765