8. Gibbons, I.R., and Rowe, A.J. (1965). Dynein: A protein with adenosine triphosphatase activity from cilia. Science *149*, 424–426.
9. Kruger, K., Grabowski, P.J., Zaug, A.J., Sands, J., Gottschling, D.E., and Cech, T.R. (1982). Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. Cell *31*, 147–157.
10. Honts, J.E., and Williams, N.E. (2003). Novel cytoskeletal proteins in the cortex of Tetrahymena. J. Eukaryot. Microbiol. *50*, 9–14.
11. Mochizuki, K., Fine, N.A., Fujisawa, T., and Gorovsky, M.A. (2002). Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in Tetrahymena. Cell *110*, 689–699.
12. Taverna, S.D., Coyne, R.S., and Allis, C.D. (2002). Methylation of histone h3 at lysine 9 targets programmed DNA elimination in Tetrahymena. Cell *110*, 701–711.
13. Nowacki, M., Vijayan, V., Zhou, Y., Schotanus, K., Doak, T.G., and Landweber, L.F. (2008). RNA-mediated epigenetic programming of a genome-rearrangement pathway. Nature *451*, 153–158.
14. Aury, J.M., Jaillon, O., Duret, L., Noel, B., Jubin, C., Porcel, B.M., Segurens, B., Daubin, V., Anthouard, V., Aiach, N., *et al*. (2006). Global trends of whole-genome duplications revealed by the ciliate Paramecium tetraurelia. Nature *444*, 171–178.
15. Eisen, J.A., Coyne, R.S., Wu, M., Wu, D., Thiagarajan, M., Wortman, J.R., Badger, J.H., Ren, Q., Amedeo, P., Jones, K.M., *et al*. (2006). Macronuclear genome sequence of the ciliate Tetrahymena thermophila, a model eukaryote. PLoS Biol. *4*, e286.
16. Zufall, R.A., McGrath, C.L., Muse, S.V., and Katz, L.A. (2006). Genome architecture drives protein evolution in ciliates. Mol. Biol. Evol. *23*, 1681–1687.
17. Janke, C., Rogowski, K., Wloga, D., Regnard, C., Kajava, A.V., Strub, J.M., Temurak, N., van Dijk, J., Boucher, D., van Dorsselaer, A., *et al*. (2005). Tubulin polyglutamylase enzymes are members of the TTL domain protein family. Science *308*, 1758–1762.
18. Wilkes, D.E., Watson, H.E., Mitchell, D.R., and Asai, D.J. (2008). Twenty-five dyneins in Tetrahymena: A re-examination of the multidynein hypothesis. Cell Motil. Cytoskeleton *65*, 342–351.
19. Malone, C.D., Falkowska, K.A., Li, A.Y., Galanti, S.E., Kanuru, R.C., Lamont, E.G., Mazzarella, K.C., Micev, A.J., Osman, M.M., Piotrowski, N.K., *et al*. (2008). Nucleus-specific importin alphas and nucleoporins regulate protein import and nuclear division in the bi-nucleate *Tetrahymena thermophila*. Eukaryot. Cell *7*, 1487–1499.
20. Wloga, D., Camba, A., Rogowski, K., Manning, G., Jerka-Dziadosz, M., and Gaertig, J. (2006). Members of the NIMA-related kinase family promote disassembly of cilia by multiple mechanisms. Mol. Biol. Cell *17*, 2799–2810.

Biology Department, Washington University, St. Louis, Missouri 63130, USA.
E-mail: dchalker@biology2.wustl.edu

DOI: 10.1016/j.cub.2008.07.080

Cell Biology: Watching the First Steps of Podosome Formation

Podosomes and invadopodia are actin-rich structures that have come under intense scrutiny over the past several years due to their critical roles in cell migration and invasion. Examination of the initial stages of podosome formation has revealed an important role for the phosphoinositide PI(3,4)P_2 in anchoring the scaffold protein Tks5 to the plasma membrane.

Marc Symons

Podosomes are plasma membrane protrusions that play diverse roles in cell adhesion and migration. These specialized structures are found at the ventral side of a wide range of cells, including osteoclasts, macrophages and endothelial cells [1]. Invasive cancer cells display structures that are similar to podosomes, called invadopodia, that represent the major sites of matrix degradation in these cells [2,3]. The importance of podosomes and invadopodia in many physiological functions has made these structures of burgeoning interest to cell biologists active in fields as diverse as immunology and cancer research.

The regulation of podosome structure and function is exceedingly complex. We now know an impressive array of molecular players that are essential for podosome formation [1,4]. A key mediator is the tyrosine kinase c-Src, which is both necessary and sufficient for podosome formation [1,4,5], and several other critical components of podosomes/invadopodia are Src substrates. Central among these are Tks5, a scaffold protein that binds members of the ADAM family of membrane-spanning proteases [6,7], the Wiskott-Aldrich Syndrome proteins WASp and N-WASp, which stimulate Arp2/3-mediated actin nucleation [8], and cortactin, a protein that stabilizes Arp2/3-mediated actin filament branches [9]. Notably, Tks5, (N-)WASp and a host of other podosome-enriched proteins bind to and are controlled by phosphoinositides, which serve to anchor proteins to various membrane compartments, suggesting that phosphoinositides play an important role in podosome regulation.

Although many critical components of podosomes have been identified, the sequence of molecular events that lead to podosome formation is still largely unknown [1]. A recent study by Oikawa *et al.* [10] provides a new paradigm for dissecting the initial stages of Src-mediated podosome formation and highlights the role of phosphoinositides in this process [10].

The authors examined the subcellular localization of different species of phosphoinositides using fluorescent versions of specific phosphoinositide-binding pleckstrin homology (PH) domains [11]. They showed that phosphoinositide-3, 4-bisphosphate (PI(3,4)P_2) is highly enriched in podosomes that are induced by constitutive activation of Src. PI(3,4,5)P_3 is also found in podosomes, although it localizes to lamellipodia and intracellular vesicles as well. Importantly, overexpression of the PI(3,4)P_2-binding PH domain of Tapp1 suppresses podosome formation, presumably by sequestering the lipid. In line with this observation, overexpression of the PH domain of Akt, which binds to both PI(3,4)P_2 and PI(3,4,5)P_3 has a more marked inhibitory effect on podosome formation. Moreover, both PI 3-kinase, the kinase that produces PI(3,4,5)P_3 using PI(4,5)P_2 as a substrate, and synaptojanin 2, a phosphatase that hydrolyzes PI(3,4,5)P_3 to produce PI(3,4)P_2, are essential for the formation of podosomes and invadopodia [10,12]. Together, these findings strongly indicate critical roles for both PI(3,4,5)P_3 and PI(3,4)P_2 in podosome formation. A candidate binding partner of PI(3,4)P_2 is Tks5, which uses its PX domain to bind to this phosphoinositide [6]. Of note, PI(4,5)P_2 was not detected in podosomes, suggesting that its conversion to PI(3,4,5)P_3 is very efficient.

To follow the first steps of Src-stimulated podosome formation,

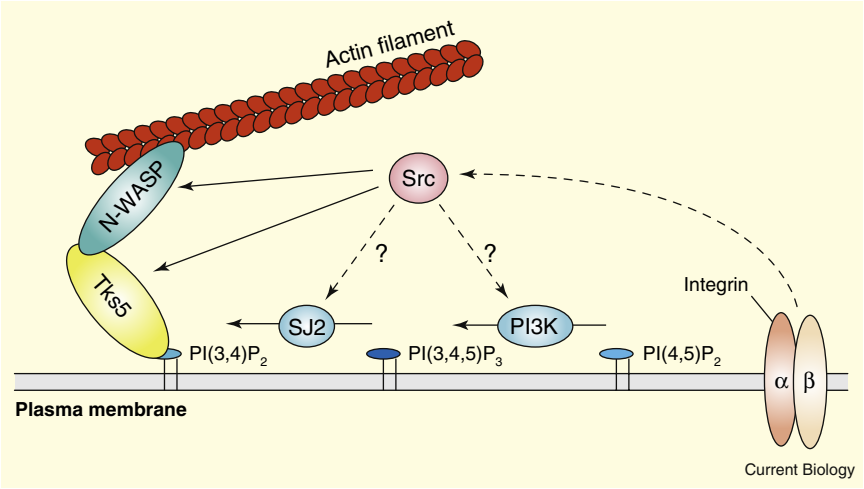

Figure 1. Src kinase is a central regulator of initial events involved in podosome/invadopodia formation.

Src is activated by integrin ligation, and both Tks5 and N-WASP are Src substrates. Tks5 localizes to podosomes/invadopodia by binding to PI(3,4)P_2. Whether and how the phosphoinositide-metabolizing enzymes PI 3-kinase (PI3K) and synaptojanin 2 (SJ2) are regulated by Src remains to be elucidated.

Oikawa *et al.* [10] used live-cell fluorescence microscopy of fibroblasts that stably express a constitutively active version of Src. They started by inhibiting Src kinase activity using the small-molecule inhibitor PP2 and subsequently washed out the inhibitor. Remarkably, PI(3,4)P_2 accumulation could be detected within 5 minutes of Src reactivation and was followed by the slower recruitment of Tks5 and the adaptor protein Grb2. The authors also used incubation and washout of the monomeric actin-binding drug latrunculin B to examine the kinetics of actin polymerization and showed that actin filaments accumulate with similar kinetics to those of Tks5. Furthermore, mass spectrometric analysis and immunoprecipitation studies showed that all five of the Tks5 Src homology 3 (SH3) domains interact with N-WASp. These observations strongly suggest that Tks5 acts as a scaffold for the recruitment of N-WASp, thereby promoting actin polymerization.

Together these findings support a model in which Src either activates or otherwise orchestrates the activities of PI 3-kinase and synaptojanin 2, leading to the accumulation of PI(3,4)P_2 and recruitment of Tks5 (Figure 1). Phosphorylation by Src likely also directly contributes to the activation of the Tks5 scaffold. Once activated and in place, Tks5 can take on its role as a central organizer of podosome actin dynamics and perhaps also of vesicular trafficking, as Oikawa *et al.* [10] also provide evidence that Tks5 binds to dynamin, an important regulator of membrane dynamics that also has been shown to be essential for podosome formation.

A limitation of the otherwise very powerful approach used by Oikawa *et al.* [10] is that it relies on the overexpression of a constitutively active form of Src, obscuring the signaling mechanisms that control Src activation during podosome formation. Thus, it will be important, albeit challenging, to extend these studies to a more physiological setting. It is generally accepted, however, that the signals that initiate podosome formation predominantly derive from the extracellular matrix and are mediated by integrins [1,4], although the adhesive properties of invadopodia are less well documented than those of podosomes [13]. Moreover, Src family kinases play critical roles in integrin signaling in a large number of different cell systems [14], strongly suggesting that Src also mediates integrin-initiated signaling during podosome formation (Figure 1).

Two additional papers published recently in *Current Biology* [15,16] suggest an intriguing new link between integrins and podosomes/invadopodia and provide compelling evidence that podosomes and invadopodia also are major sites through which the cell senses mechanical forces [17]. Collin *et al.* [15] demonstrated that podosomes in Src-transformed fibroblasts can exert tractions with a magnitude that is comparable to that generated underneath focal adhesions, while Alexander *et al.* [16] observed a striking increase in the matrix-degrading activity of invadopodia in breast carcinoma cells by increasing the rigidity of the extracellular matrix. As previously observed in osteoclasts and macrophages, inhibition of myosin contractility led to podosome disassembly in both the fibroblasts and breast carcinoma cells, in line with the notion that mechanotransduction is mediated by integrin–actomyosin interactions. Interestingly, in the breast carcinoma cells, modulating contractility affected the mature, matrix-degrading, but not the immature, inactive invadopodia, strongly suggesting that matrix rigidity facilitates the maturation of invadopodia rather than their initiation [16].

Our increasing appreciation of the roles of invadopodia in tumor cell invasion and metastasis makes these structures very attractive targets for cancer therapy [13]. In osteoclasts, podosomes are intimately involved in the formation of a sealing ring that establishes an isolated compartment where bone is degraded [18,19]. Therefore, targeting this structure may be beneficial in the treatment of osteoporosis. Thus, the recent identification of new molecular components of podosomes and invadopodia and the elucidation of their roles suggests novel therapeutic strategies.

References

1. Gimona, M., Buccione, R., Courtneidge, S.A., and Linder, S. (2008). Assembly and biological role of podosomes and invadopodia. Curr. Opin. Cell Biol. *20*, 235–241.
2. Weaver, A.M. (2008). Invadopodia. Curr. Biol. *18*, R362–R364.
3. Wolf, K., and Friedl, P. (2008). Mapping proteolytic cancer cell-extracellular matrix interfaces. Clin. Exp. Metastasis, in press.
4. Linder, S., and Aepfelbacher, M. (2003). Podosomes: adhesion hot-spots of invasive cells. Trends Cell Biol. *13*, 376–385.
5. Destaing, O., Sanjay, A., Itzstein, C., Horne, W.C., Toomre, D., De Camilli, P., and Baron, R. (2008). The tyrosine kinase activity of c-Src regulates actin dynamics and organization of podosomes in osteoclasts. Mol. Biol. Cell *19*, 394–404.
6. Abram, C.L., Seals, D.F., Pass, I., Salinsky, D., Maurer, L., Roth, T.M., and Courtneidge, S.A. (2003). The adaptor protein fish associates with members of the ADAMs family and localizes to podosomes of Src-transformed cells. J. Biol. Chem. *278*, 16844–16851.

7. Seals, D.F., Azucena, E.F., Jr., Pass, I., Tesfay, L., Gordon, R., Woodrow, M., Resau, J.H., and Courtneidge, S.A. (2005). The adaptor protein Tks5/Fish is required for podosome formation and function, and for the protease-driven invasion of cancer cells. Cancer Cell *7*, 155–165.
8. Yamaguchi, H., Lorenz, M., Kempiak, S., Sarmiento, C., Coniglio, S., Symons, M., Segall, J., Eddy, R., Miki, H., Takenawa, T., *et al*. (2005). Molecular mechanisms of invadopodium formation: the role of the N-WASP-Arp2/3 complex pathway and cofilin. J. Cell Biol. *168*, 441–452.
9. Weaver, A.M. (2008). Cortactin in tumor invasiveness. Cancer Lett. *265*, 157–166.
10. Oikawa, T., Itoh, T., and Takenawa, T. (2008). Sequential signals toward podosome formation in NIH-src cells. J. Cell Biol. *182*, 157–169.
11. Furutani, M., Tsujita, K., Itoh, T., Ijuin, T., and Takenawa, T. (2006). Application of phosphoinositide-binding domains for the detection and quantification of specific phosphoinositides. Anal. Biochem. *355*, 8–18.
12. Chuang, Y.Y., Tran, N.L., Rusk, N., Nakada, M., Berens, M.E., and Symons, M. (2004). Role of Synaptojanin 2 in glioma cell migration and invasion. Cancer Res. *64*, 8271–8275.
13. Weaver, A.M. (2006). Invadopodia: specialized cell structures for cancer invasion. Clin. Exp. Metastasis *23*, 97–105.
14. Mitra, S.K., and Schlaepfer, D.D. (2006). Integrin-regulated FAK-Src signaling in normal and cancer cells. Curr. Opin. Cell Biol. *18*, 516–523.
15. Collin, O., Na, S., Chowdhury, F., Hong, M., Shin, M.E., Wang, F., and Wang, N. (2008). Self-organized podosomes are dynamic mechanosensors. Curr. Biol. *18*, 1288–1294.
16. Alexander, N.R., Branch, K.M., Parekh, A., Clark, E.S., Iwueke, I.C., Scott, A., Guelcher, S.A., and Weaver, A.M. (2008). Extracellular matrix rigidity promotes invadopodia activity. Curr. Biol. *18*, 1295–1299.
17. Bershadsky, A.D., Balaban, N.Q., and Geiger, B. (2003). Adhesion-dependent cell mechanosensitivity. Annu. Rev. Cell Dev. Biol. *19*, 677–695.
18. Jurdic, P., Saltel, F., Chabadel, A., and Destaing, O. (2006). Podosome and sealing zone: specificity of the osteoclast model. Eur. J. Cell Biol. *85*, 195–202.
19. Luxenburg, C., Geblinger, D., Klein, E., Anderson, K., Hanein, D., Geiger, B., and Addadi, L. (2007). The architecture of the adhesive apparatus of cultured osteoclasts: from podosome formation to sealing zone assembly. PLoS ONE *2*, e179.

The Feinstein Institute for Medical Research at North Shore-LIJ, 350 Community Drive, Manhasset, New York 11030, USA.
E-mail: msymons@nshs.edu

DOI: 10.1016/j.cub.2008.08.034