Acvr2a is conserved in humans. In addition, while zebrafish embryos depleted for maternal and zygotic Dicer manage to establish an axis and even form somites, mouse embryos mutant for *Dicer* fail to establish a body plan, which demonstrates a greater and earlier reliance on miRNA-mediated regulation in mammals (Bernstein et al., 2003; Tang et al., 2007). As well as being necessary for the patterning of the early embryo, the Nodal signaling pathway has been implicated in the maintenance of stem cells' pluripotency and in tumorigenesis. These two studies open exciting perspectives for the study of these phenomena.

REFERENCES

Bernstein, E., Kim, S.Y., Carmell, M.A., Murchison, E.P., Alcorn, H., Li, M.Z., Mills, A.A., Elledge, S.J., Anderson, K.V., and Hannon, G.J. (2003). Nat. Genet. *35*, 215–217.

Bushati, N., and Cohen, S.M. (2007). Annu. Rev. Cell Dev. Biol., in press. Published online May 21, 2007. 10.1146/annurev.cellbio.23.090506.123406.

Choi, W.Y., Giraldez, A.J., and Schier, A.F. (2007). Science, in press. Published online August 30, 2007. 10.1126/science.1147535.

Giraldez, A.J., Cinalli, R.M., Glasner, M.E., Enright, A.J., Thomson, J.M., Baskerville, S., Hammond, S.M., Bartel, D.P., and Schier, A.F. (2005). Science *308*, 833–838.

Giraldez, A.J., Mishima, Y., Rihel, J., Grocock, R.J., Van Gongen, S., Inoue, K., Enright, A.J., and Schier, A.F. (2006). Science *312*, 75–79.

Martello, G., Zacchigna1, L., Inui, M., Montagner, M., Adorno1, M., Mamidi, A., Morsut, L., Soligo, S., Uyen Tran, U., Dupont, S., et al. (2007). Nature *449*, 183–188.

Shen, M.M. (2007). Development *134*, 1023–1034.

Tang, F., Kaneda, M., O'Carroll, D., Hajkova, P., Barton, S.C., Sun, Y.A., Lee, C., Tarakhovsky, A., Lao, K., and Surani, M.A. (2007). Genes Dev. *21*, 644–648.

Putting the Brakes on Cytokinesis with α-Actinin

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Although signal transduction pathways provide spatiotemporal control of cytokinesis, additional regulation likely occurs through complex cytoskeletal network interactions. In this issue of *Developmental Cell*, Mukhina et al. (2007) show that myosin-II modulates the cortical lifetime of the actin crosslinker α-actinin, which in turn tunes actin filament dynamics, thereby controlling furrow ingression.

Cytokinesis is the process of reshaping one cell into two daughter cells and is driven by actin, actin crosslinkers, and myosin-II. Classically, cytokinesis contractility is thought to occur through the constriction of a sarcomeric-like contractile ring (a pursestring) of actin and myosin-II filaments. However, contractile ring structure varies widely among different organisms, from a highly ordered ring in *S. pombe* to a more disordered actin network in some mammalian cell types (including the normal rat kidney [NRK] cells used in the study by Mukhina et al., [2007]). α-actinin is an actin crosslinker that localizes to the cleavage furrow region in a variety of cells, from yeast to mammals. Yet, until Mukhina et al. (2007), α-actinin's function in cytokinesis in a cell type with a more disorganized contractile network had not been studied. Mukhina et al. (2007) discovered that α-actinin modulates furrow ingression dynamics and actin turnover and that myosin-II activity modulated the lifetime of α-actinin at the equatorial cortex.

To determine how α-actinin contributes to cytokinesis, Mukhina et al. (2007) studied how increasing or decreasing α-actinin expression levels influences the actin cortex and cytokinesis fidelity. When they overexpressed α-actinin, furrow ingression slowed down or reversed, leading to failure of cytokinesis. Under these conditions, the authors observed increased equatorial F-actin levels and slower actin turnover, which may explain the effects on furrow formation. Increased concentration of F-actin at the furrow likely increases viscoelasticity, slowing the removal of cytoskeleton and cytoplasm during furrow ingression. Higher concentrations of α-actinin may also enhance the stability of the polymerized actin network. Indeed, one function of myosin-II at the cleavage furrow is to increase actin turnover, facilitating furrow ingression (Murthy and Wadsworth, 2005).

Conversely, when Mukhina et al. (2007) reduced α-actinin expression by RNAi, ectopic furrows formed and furrow ingression rates increased. Again, the impact of lowering α-actinin levels on cytokinesis shape changes may be through α-actinin's modulation of F-actin, since F-actin levels are reduced by α-actinin RNAi. These results suggest that inhibition of actin turnover by α-actinin may act as a brake to
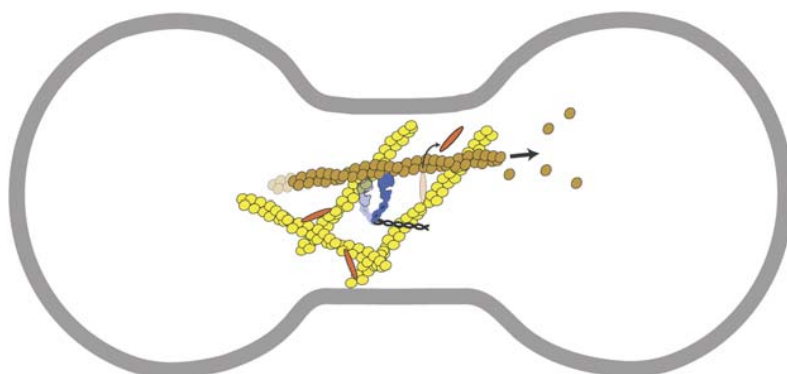
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Figure 1. Dynamic Interplay between Actin, Crosslinkers, and Myosin-II during Furrow Ingression
Cartoon of a dividing cell depicting how myosin-II, going through its working stroke, causes α-actinin to release from the network, allowing the filament to be pulled from the furrow cytoskeleton. Actin filaments, yellow; myosin-II, blue; α-actinin, orange; membrane, gray.

allow cytokinesis to proceed by controlled, stereotypical cell shape changes.

The ability of actin crosslinkers to act as cytokinesis brakes is not unprecedented; dynacortin slows the rate of furrow ingression and contributes to cortical tension and viscoelasticity (Girard et al., 2004; Zhang and Robinson, 2005). It is unclear from Mukhina et al. (2007) whether α-actinin also impacts the viscoelastic properties of the actin cortex. Using glass needles to push against cells attached to the substrate, α-actinin overexpression did not appear to significantly increase the stiffness of the cortex, although differences in cell-substrate adhesion complicate this assay. As overexpression or removal of dynacortin increases or decreases cortical viscoelasticity, respectively (Girard et al., 2004), it is possible that α-actinin does also, especially as the authors show that α-actinin overexpression has a drastic impact on F-actin dynamics and concentration.

Finally, Mukhina et al. (2007) used fluorescence recovery after photobleaching (FRAP) analysis to measure α-actinin lifetimes. They found that α-actinin has a shorter lifetime at the equatorial cortex than at the polar cortex, suggesting that cytoskeletal dynamics are different at these two regions of the dividing cell cortex. They then examined whether myosin-II can influence α-actinin's lifetime, using blebbistatin, an inhibitor of myosin-II

that blocks tight binding of the myosin-II motor to the actin filament. In this analysis, they discovered that myosin-II increases α-actinin recovery rates. In summary, all of the results in Mukhina et al. (2007) suggest an antagonistic interplay between myosin-II and α-actinin: α-actinin provides a braking function, slowing contractility, while myosin-II shortens α-actinin's lifetime in the cleavage furrow network.

How might such an antagonistic interplay between myosin-II and α-actinin work to promote and control cytokinesis contractility? Early in vitro work found that the extent of crosslinking impacts myosin-II contraction of actin networks. In the absence of any crosslinkers, myosin-II does not contract the network (Janson et al., 1991). However, increasing the crosslinker: myosin-II ratio above a threshold also inhibits network contraction by promoting isometric rather than isotonic tension (Janson et al., 1992). Thus, some crosslinker binding is necessary to allow productive myosin-II force generation, but crosslinker release is required to allow contraction to proceed.

Another way α-actinin could contribute to contractility is by increasing myosin-II's duty ratio (the ratio of bound motor heads to the total number of available motor heads). α-actinin can increase myosin-II's Mg^{2+}ATPase activity (Condeelis et al., 1984), which would enhance the proportion of actin-bound myosin-II heads. Myosin-II's duty ratio is also increased by

mechanical load, which locks the motor heads onto the actin filament by decreasing the ADP-off-rate. Therefore, α-actinin enrichment at the equator may increase the duty ratio of myosin-II by stimulating the myosin-II ATPase activity and/or by creating a crosslinked network that generates enough resistance (load) to slow ADP release. Either or both mechanisms would increase the tension at the furrow.

In contrast, myosin-II appears to modulate α-actinin binding, suggesting another way by which cytokinesis contractility may be regulated. The enhanced α-actinin dynamics at the equatorial cortex, as observed by Mukhina et al. (2007), may be due to strain induced by equatorially enriched myosin-II. By pulling on F-actin that is crosslinked by α-actinin, myosin-II may lead to an increased α-actinin off-rate, perhaps due to slip-bond behavior of α-actinin. Similar behaviors have been observed in vitro; for example, myosin-II can disperse filaments from fimbrin-crosslinked actin networks (Prassler et al., 1997). In the furrow, myosin-II may increase actin removal by increasing crosslinker off-rates, allowing filaments to be transported away from the furrow, or allowing access to the actin severing protein, cofilin, which is needed to maintain constant cleavage furrow actin levels as the furrow ingresses (Figure 1).

Although different tissue environments and cell compositions may result in different cellular mechanics, it is likely that the universally conserved cytokinesis machinery in eukaryea, consisting of actin, actin crosslinkers, and myosin-II, interacts in a similar manner to generate contractile force. Mukhina et al. (2007) propose that mammalian cleavage furrows contract by remodeling of the actin cortex rather than by constricting a sarcomeric contractile ring. Remodeling may occur through interplay of cytoskeletal components, wherein myosin-II alters crosslinker binding to F-actin, allowing the actin filaments to slide through the network or to be turned over. Additionally, the interdependent modulation of cytoskeletal dynamics may allow for feedback control of cell shape during mechanical perturbation (Effler et al., 2006). This self-regulation of the cytoskeletal

components of the contracting network may make cytokinesis highly adaptable to the differing mechanical environments that cells might encounter.

REFERENCES

Condeelis, J.S., Vahey, M., Carboni, J.M., Demey, J., and Ogihara, S. (1984). J. Cell Biol. *99*, 119–126.

Effler, J.C., Kee, S., Berk, J.M., Tran, M.N., Iglesias, P.A., and Robinson, D.N. (2006). Curr. Biol. *16*, 1962–1967.

Girard, K.D., Chaney, C., Delannoy, M., Kuo, S.C., and Robinson, D.N. (2004). EMBO J. *23*, 1536–1546.

Janson, L.W., Kolega, J., and Taylor, D.L. (1991). J. Cell Biol. *114*, 1005–1015.

Janson, L.W., Sellers, J.R., and Taylor, D.L. (1992). Cell Motil. Cytoskeleton *22*, 274–280.

Murthy, K., and Wadsworth, P. (2005). Curr. Biol. *15*, 724–731.

Mukhina, S., Wang, Y., and Murata-Hori, M. (2007). Dev. Cell *13*, this issue, 554–565.

Prassler, J., Stocker, S., Marriott, G., Heidecker, M., Kellermann, J., and Gerisch, G. (1997). Mol. Biol. Cell *8*, 83–95.

Zhang, W.W., and Robinson, D.N. (2005). Proc. Natl. Acad. Sci. USA *102*, 7186–7191.

G'rab'bing the Microenvironment for Invasion

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Transformation and metastasis require cell invasion. In this issue of *Developmental Cell*, Caswell et al. describe a unique mechanism regulating tumor cell invasion. The authors report that an interaction between β1 integrin and Rab25, a GTPase that has been linked to tumor aggressiveness and metastasis, regulates the recycling of α5β1 integrin to the leading edge of cell pseudopodia.

In order to invade, tumor cells must negotiate a dense 3D stromal matrix that consists of interstitial collagens (I and III), various proteoglycans, and provisional matrices such as fibronectin and tenascin. The cancer cell achieves this goal by either exploiting preexisting migratory channels within the stroma or actively remodeling the matrix to create a new migratory path. Cell-directed matrix remodeling involves cycles of matrix receptor-dependent adhesion and de-adhesion, dynamic remodeling of the plasma membrane and cytoskeleton, and spatially directed proteolysis—processes that are functionally linked to RhoGTPases, MMPs, and integrins (Page-McCaw et al., 2007). The RabGTPase-dependent endocytic/exocytic cycle of integrins is increasingly being recognized as a key regulator of the appropriate targeting of integrins and the dynamic remodeling of integrin-containing adhesion sites. In this issue of *Developmental Cell*, Caswell et al.

(2007) now demonstrate that the GTPase Rab25 promotes a mode of cell migration within a 3D matrix that is characterized by the extension of long pseudopodia and the rapid recycling of β1 integrins at these membrane protrusions. They show how Rab25 specifically promotes the localization of a spatially restricted pool of β1 integrin to the plasma membrane at the pseudopodial tips, and permits the retention of this pool of cycling β1 integrin at the cell front. Rab25-dependent localized integrin treadmilling at the invading pseudopod then promotes the persistent and directed migration of the cell. These findings indicate that Rab25 could contribute to tumor progression by directing the localization of integrin recycling vesicles, thereby enhancing the ability of tumor cells to directionally invade the extracellular matrix (ECM).

Although tumor cells can invade in the absence of MMP activity, MMPs are essential for matrix remodeling

(Page-McCaw et al., 2007). Indeed, a simulation model that explains a given cell's ability to deform mechanically and digest the matrix during invasion into a 3D matrix demonstrated a bimodal dependence of cell speed and persistence on matrix pore size that implies a stringent requirement for MMP activity (Zaman et al., 2007). Localized subcellular activity of the membrane-anchored matrix metalloproteinase MT1-MMP (also known as MMP14) in particular has been implicated in branching morphogenesis of the mammary gland, in neocapillary structure sprouting during angiogenesis, and in the localized invasion of tumor cells (Page-McCaw et al., 2007). However, the mechanisms underlying the spatiotemporal regulation of MT1-MMP during physiological invasion have not been defined. Recently, Rab8 GTPase was shown to regulate MT1-MMP redistribution to invasive structures, suggesting exocytic vesicle trafficking could be important for