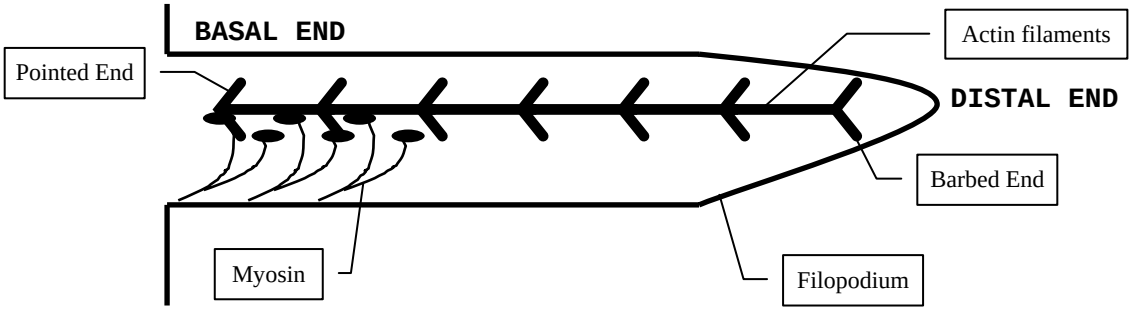
Section.B.

1. According to myosin ATPase cycle, adding ATP will make the myosin detach from the actin filament; while in ATP depleted environment, myosin will tightly attach on the actin filament. Adding ATP will prevent G-actin polymerization. In **step 1**, I will add ATP to keep the myosin in the supernatant (detaching from the tissue endogenous actin filament). In **step 2**, I will use ATP free system. Let the myosin attach to the F-actin and be pelleted. In **step 3**, I will add ATP into the solution, detach the myosin from the actin filament, and pellet the F-actin.

2.



Conventional myosin reels on the actin filament: if the F-actin is fixed, myosin moves to the barbed end of the filament; if the myosin is attached, F-actin moves towards its pointed-end direction. The above diagram shows my hypothesis about the filopodium dynamics. Filopodia contain arrays of uniformly polarized (barbed end distal) actin filaments just as reported by Lewis & Bridgman [1]. Actin quick polymerization at the barbed end stimulates the extension of filopodium. Some kind of conventional myosin located at the basal end attaches to some structure of the filopodium, and its activity will cause F-actin's retrograde flow. As myosins mainly locate near the basal end, it is possible to make all the F-actin flow out of the filopodium. According to surface tension and membrane integrity, if there is no other bundle or solid architecture, the filopodium will retract to disappear following F-actin's retrograding. So, there is a balance: actin polymerization causes filopodium protrusion; myosin reeling causes filopodium retraction. Filopodium dynamics is based on these two activities, both of which need ATP hydrolysis. Sometime, the concentration of ATP in distal end is higher than basal end, so actin polymerization contributes more to filopodium's movement, and filopodium protrudes. Sometime, the concentration of ATP in basal end is higher than distal end, so myosin motor activity contributes more to filopodium's movement, and filopodium retracts. I am sure that there are many other pathways also take part in the regulation of filopodium dynamics, and the truth can not be such simple as I mentioned above. If you add a drug to specifically block the myosin in a rigor binding state, the retrograde F-actin flow will be inhibited. In this situation, the filopodium can only extend, but can not retract or contract. If these filopodium is at the rear of a moving cell, you will see threadlike tails remain following the trace of cell movement. More drug treatment, more tails and longer tails you will find.

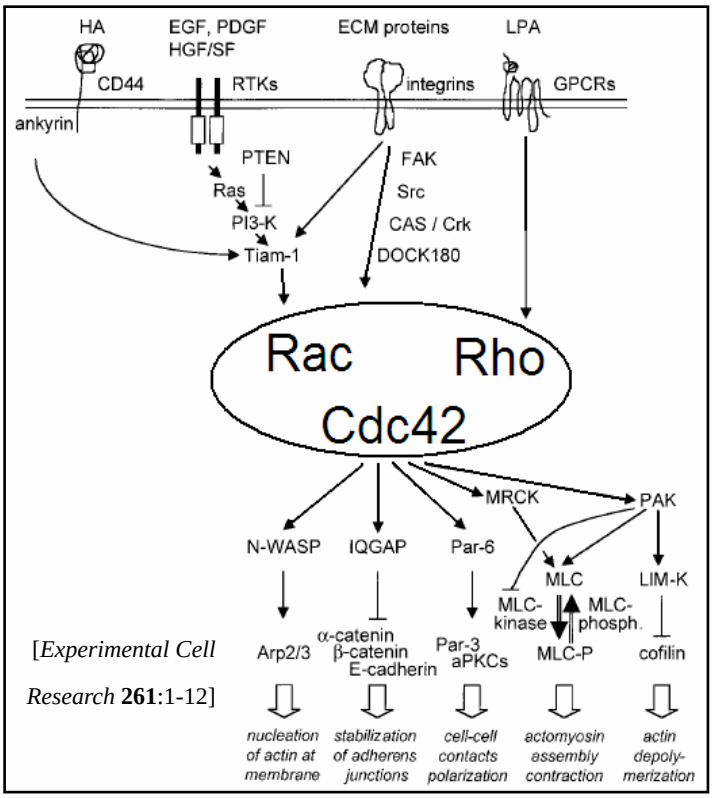
[1] Lewis AK, Bridgman PC. *J Cell Biol* **119**:1219-1243 (1992).

Section.C.

1. The Rho family member Cdc42 can signal through a number of cellular pathways fundamentally in cell regulation. Here, I will focus on Cdc42's function in regulating cell adhesion and actin cytoskeleton activity.

Compared to negative Cdc42 mutation disrupting lamellipodia/filopodia formation, expression of constitutively active Cdc42 will cause the protrusion of many long filopodia at the cell periphery that form adhesive contacts with the substratum [2], produce more than enough membrane ruffling, and may increase cell's invasion ability.

Extracellular stimuli produce active Cdc42 proteins, which bind to the GTPase Binding Domains (GBD) of auto-inhibited WASp/Scar proteins (inhibited by interaction of the GBD with the C terminus) and activate them (PIP_2 also takes part in this activation). Active WASp/Scar proteins cooperate with Arp2/3 complex and actin filaments (depending on the VCA domains of WASp/Scar proteins) to stimulate formation of new barbed ends linking nucleation and branching. Actin nucleation and polymerization regulate the organization of the actin cytoskeleton.



Cdc42 proteins help barbed ends to escape capping [3], which help elongation of actin filaments and increase the formation of filopodia. Cdc42 activate PAK, which activate LIM kinase to inhibit cofilin activity and decrease severing/depolymerizing to actin filaments. Integrin-mediated adhesion activates Cdc42, promoting protrusion and regulating cytoskeleton. Cdc42 proteins down regulate alpha-catenin/beta-catenin/E-cadherin (through IQGAP) and stabilize adhesions. Cdc42 also takes part in regulation of fascin to affect cell motility.

The diagram here shows how Cdc42 is activated and how Cdc42 affects cell by various pathways. Though Cdc42 is primarily involved in the formation of actin based protrusive structures, Cdc42 also influence microtubule dynamics [4]. Cdc42 may also regulate transcription of certain genes to affect cytoskeleton.

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2 *MBoC4*: 948.
3 Huang M, Yang C, *et al*. *Curr Biol* **9**:979-982 (1999).
4 Daub H, Gevaert K, *et al*. *J Biol Chem* **276**:1677-1680 (2001).

2. Fascin was discovered in the 1970s as a 55kD actin-binding protein from cytoplasmic extracts of sea urchin oocytes or coelomocytes.

Actin based structures	Fascins function
cortical structures (filopodia, spikes, lamellipodial ribs, oocyte microvilli, dendrites of dendritic cells)	roles in ***cell-matrix adhesion***, ***cell interactions*** and ***cell migration***
cytoplasmic actin bundles	role in ***cell architecture***

The table summarizes location and cellular function of fascins. *In vitro* and *in vivo* experiments show that fascin assembles very tightly packed and unipolar ordered parallel F-actin bundles [5]. One role of fascin-actin bundles in cells is to impart rigidity to dynamic cortical protrusions; such as in oocytes, fascin rapidly co-distributes with F-actin into microvilli extended upon fertilization. Fascin localizes in ruffles at the leading edges of motile cells, which is required in cell locomotion. ECM components induce or stabilize bundling of actin by fascin. Adhesion to matrix correlates with downregulatation of E-cadherin, which depends on the formation of fascin microspikes. Long continuous actin bundles in *Drosophila* bristles are constructed by overlapping short filaments, which is secured by fascin cross-bridge [6]. Melanoma cell migration is mediated through the actin-bundling protein fascin [7].

Fascin distribution is regulated by **phosphorylation at Ser39**. Phosphorylation at Ser39 will inhibit fascin actin-binding activity. Small GTPases, **Cdc42 and Rac**, regulate formation of fascin spikes and influence cell adhesion. **Caldesmon/tropomyosin** may influence fascin and dissociate fascin-actin bundles. **Drebrin** may compete with fascin and contributes to the dynamics of filopodial extension, adhesion or retraction. As the human fascin gene promoter region contains multiple TCF and NF-kB consensus binding sites, fascin may be regulated by **c-erb-2/HER-2 signaling** [8].

Overexpression of fascin may disorganize adhesions and decrease cell-cell attachment activity, as it prevents the rearrangement of occluding and other structural components into functional tight junctions. The mechanism is believed to involve an interaction between fascin and beta-catenin that could affect the function of beta-catenin in cadherin- and occluding-dependent adhesion complex [9]. And the change of cell adhesion property may increase the ability of cell invasion. Overexpression of fascin will inhibit normal cell migration, as excessive fascin-actin interaction and actin bundles generate rigidity cell architecture overmuch. Fascin overexpression will compete with other actin cross-link proteins, and change the actin network in cell, which may change the shape of cell, affect the cell cytoskeleton, distress the normal activities in cell, and even influence the viability.

Human HIV-related lymphoid hyperplasia evolves in loss of fascin positive follicular and interdigitating dendritic cells. Human B cell lymphoma evolves in decreased fascin-positive dendritic cells in germinal centres. I think that fascin knockout mouse will be sterile even lethal, as fascin is very important in fertilization and fascin-actin bundles are very important in cell structure and function.

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5 Yamashiro-Matsumura S, Matsumura F, *et al*. *J Biol Chem* **260**:5087-5097 (1985).
6 Guild GM, Connolly PS. *J Cell Biol* **162**:1069-1077 (2003).
7 Shonukan O, Bagayogo I. *Oncogene* **22**:3616-3623 (2003).
8 Grothey A, Hashizume R, *et al*. *Oncogene* **19**:4864-4875 (2000).
9 Nina K, Vasileia S, *et al*. *BioEssays* **24**:350-361 (2002).

3. In a word, sequestering actin monomer will inhibit the polymerization of F-actin, and accelerate the depolymerization of pre-existing actin filaments. The concentration of sequestering protein is very important here: very low concentration treatment may only interfere with the actin cytoskeleton; appropriate concentration of newfilin will block specific actin based cellular motility; even higher density may kill the cell.

A) Cell migration is dead.

According to dendritic nucleation/array treadmilling model, protrusion of leading edge is based on the polymerization of actin filaments from G-actin. Retraction/contraction is based on the depolymerization of actin filaments. Sequestering actin monomers inhibits protrusion, the cell will round up and the migration will be blocked.

B) Cytokinesis is blocked/influenced.

Cytokinesis of animal cells involves the formation of the contractile ring along the equatorial plane, which is based on recruitment of preexisting actin filaments into the cleavage furrow. Reorganization depends on depolymerization and polymerization of actin filaments, which will be blocked by sequestering actin monomers. As plant cells build new cell wall outward to the cortex in cytokinesis, it may be only slightly influenced by overexpressing newfilin.

C) Phagocytosis is blocked.

Phagocytosis requires the participation of a dynamic and interactive actin cytoskeleton that acts to deform cellular membranes. A 'polymeric contractile scaffold' between biological membranes and actin filaments is needed to take charge of cytoskeletal rearrangements. Sequestering actin monomer blocks actin based cytoskeletal rearrangements and blocks phagocytosis.

D) Muscle contraction is influenced.

Dynamic changes in the actin cytoskeleton contribute to the processes of contractile activation and mechanical adaptation in smooth muscle. Though sequestering actin monomers don’t affect the sliding between actin thin filaments and myosin thick filaments, reorganizing the actin cytoskeleton is required by smooth muscle cells for acutely adjust the functional properties, which is dramatically influenced by depleting actin monomers.