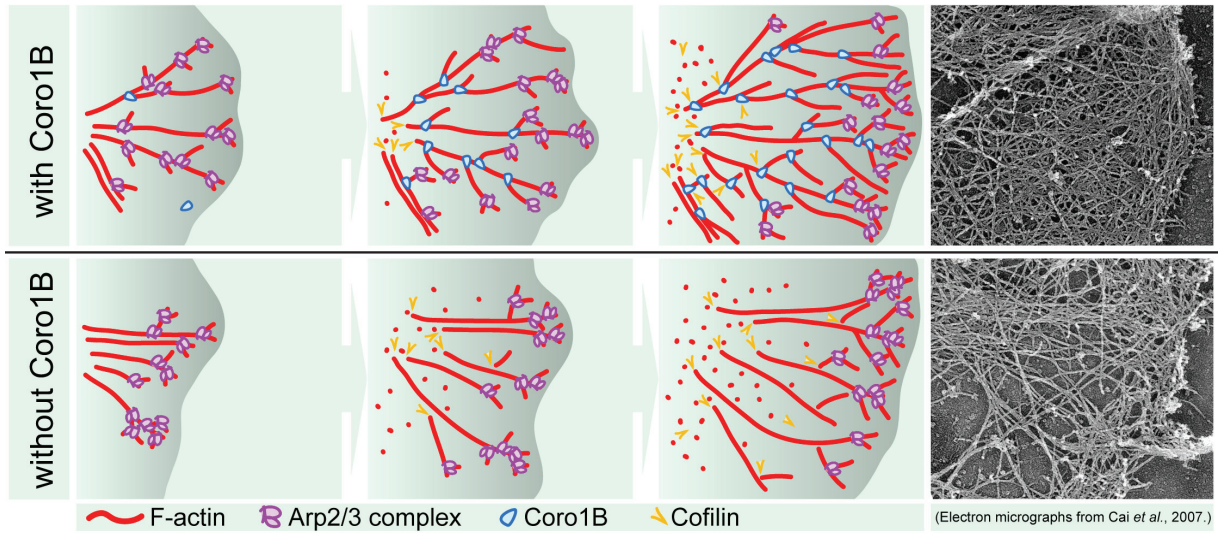
Coronin 1B, conductor of the actin dynamics orchestra

A symphony of cell movements orchestrate embryonic development, wound healing, and immune responses. Dysregulated cell movement contributes to human disease, including metastasic cancer and autoimmune disorders. Dynamic cytoskeletal remodeling, especially remodeling of actin filaments, drives cell movement. Actin filaments assemble from actin monomers and filaments break down at a later stage, recycling monomers. Arp2/3 complex and Cofilin are two key regulators of actin dynamics in moving cells. Since the discovery of these two pivotal elements, it is generally agreed that Arp2/3 complex makes actin filaments in a branched fashion to form highly ordered actin networks at the leading edge; while Cofilin breaks down the actin filaments to recycle monomers at the rear. However, how these two processes of assembly and disassembly are



Coro1B remodels branched actin network to create flexibility. Arp2/3 complex makes actin filaments in a branched fashion to form highly ordered actin network at the leading edge. Coro1B replaces Arp2/3 complex at branched junctions. The resulting Arp2/3-free Coro1B containing actin branches have altered geometry, and providing flexibility for further remodeling.

coordinated is not known. Who conducts the orchestra of actin dynamics, to coordinate these separate players?

I discovered that an actin binding protein named Coronin 1B (Coro1B) remodels branched actin filaments and tunes highly ordered networks into flexible networks. I began my research studying how Coro1B interacts with actin filaments (F-actin) (*1*). When over-expressed in *Drosophila* S2 cells, Coro1B induced formation of actin filament bundles. By probing the Coro1B-actin interface, I identified mutations on surface-exposed residues that revealed a conserved Arginine residue (Arginine-30) that was likely to be crucial for actin filament bundling by Coro1B. Using biochemical experiments, I confirmed Arginine-30 was part of Coro1B's actin-binding site. Further, I demonstrated that Coro1B preferentially binds ATP-F-actin rather than to ADP-F-actin. Actin filaments are assembled at the leading edge from ATP-loaded actin monomers; filaments gradually age as they flow rearward, during which ATP is hydrolyzed. The preferential binding of Coro1B to ATP-F-actin is consistent with its unique localization at the leading edge of migrating cells where ATP-F-actin is abundant.

Coro1B is localized with Arp2/3 complex at the leading edge (*2*). While Arp2/3 complex is making actin filaments at the leading edge, what is Coro1B doing? I found that Coro1B inhibits the Arp2/3 complex, in part, by remodeling the actin filaments at branched junctions. Coro1B remodels branches by replacing Arp2/3 complex at the junctions where filaments intersect. This remodeling alters the branch angle, and as a result, most of Coro1B-containing junctions are oriented at larger angles than Arp2/3-containing ones. Consistent

with these findings, Coro1B and Arp2/3 complex have distinct dynamics at the leading edge of moving cells, with Arp2/3 complex always leading and Coro1B following. I propose that the essential step for remodeling the highly ordered networks occurs when Coro1B replaces Arp2/3 complex at branched junctions (*3*).

Although Coro1B plays an important role in orchestrating network remodeling, it is not the only protein that can modulate branched actin filaments formed by Arp2/3 complex. Cortactin, another F-actin binding protein, stabilizes filament branches *in vitro* (*4*). Cortactin is also enriched at the leading edge, where Coro1B and the Arp2/3 complex are located. Not surprisingly, I found that Coro1B and Cortactin compete with each other at branched junctions: Cortactin prevents Coro1B from remodeling the actin filaments, and *vice versa*. In living cells, depleting Coro1B maintains Arp2/3 complex in the actin networks longer than in cells with normal amounts of Coro1B. Conversely, depleting Cortactin shortens the period that Arp2/3 complex stays in the networks. These findings indicate that Coro1B antagonizes Cortactin and thus remodels branched filaments formed by Arp2/3 complex (*3*), creating flexible actin networks.

Although flexible networks are optimal for cell movement, networks with too much flexibility can be problematic, leading, for example, to metastasis of cancer cells. The network remodeling activity of Coro1B must be precisely controlled in order to fine tune the flexibility of the actin networks. Since Coro1B was initially discovered as a substrate of protein kinase C (*5*), I asked how Coro1B's activity is regulated. The interaction between Coro1B and the Arp2/3

complex is regulated by phosphorylation at Serine-2 of Coro1B and that the phosphorylated form of Coro1B is the inactive form (*2*).

However, a factor that regulates the assembly of actin filaments is not sufficient to coordinate actin dynamics, which involves both filament assembly and disassembly. Coro1B also orchestrates actin filament breakdown by influencing Cofilin activity. This unexpected role for Coro1B became clear during my search for the activating phosphatase for Coro1B. Using a panel of phosphatase inhibitors, I narrowed down the candidate to a member of the Serine and Threonine phosphatase family, Slingshot, which dephosphorylates Cofilin protein on Serine-3 (*6*). A specific isoform, Slingshot 1L, dephosphorylates Coro1B on Serine-2 (*7*). Surprisingly, Coro1B directs the cellular localization of Slingshot 1L, thus regulating its ability to activate Cofilin, the protein that breaks down actin filaments.

Through its ability to remodel Arp2/3-dependent actin networks and filaments turnover, Coro1B is the “conductor” of the orchestra of actin dynamics: on one side, Coro1B regulates the assembly of actin filaments by remodeling the branches formed by Arp2/3 complex; on the other side, Coro1B regulates filament breakdown by Cofilin. This role for Coro1B in coordinating actin dynamics extends our understanding of how actin networks are regulated to drive cell movement, and ultimately may provide new therapeutic avenues to treat human disease.

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