

# CORONIN 1B COORDINATES ACTIN DYNAMICS IN LAMELLIPODIA

by  
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# Abstract

## LIANG CAI: CORONIN 1B COORDINATES ACTIN DYNAMICS IN LAMELLIPODIA.

(Under the direction of James Bear.)

Cell migration is critical for a variety of physiological processes. Coronins are a conserved family of actin binding proteins that affect cell migration. My research focuses on the molecular mechanism through which Coronin 1B coordinates actin dynamics in lamellipodia.

We report that Coronin 1B co-localizes with the Arp2/3 complex in lamellipodia, and co-immunoprecipitates with this complex. This interaction is regulated by PKC phosphorylation on Ser2. Further, we show that Coronin 1B interacts with not only the Arp2/3 complex but also the Slingshot 1L (SSH1L) phosphatase, two regulators of actin filament formation and turnover. Coronin 1B inhibits filament nucleation by Arp2/3 complex and this inhibition is attenuated by the Ser2 phosphorylation, a site targeted by SSH1L. Coronin 1B also directs SSH1L to lamellipodia where SSH1L likely regulates Cofilin activity via dephosphorylation. Accordingly, depleting Coronin 1B increases phospho-Cofilin levels, and alters lamellipodial dynamics and actin architecture. Thus, Coronin 1B coordinates actin assembly by Arp2/3 complex and actin disassembly by Cofilin for effective lamellipodial protrusion.

Analysis of Coronin function has been hampered by the lack of a clear understanding of how Coronin interacts with F-actin. We identify a surface-exposed conserved residue, Arg30, which is critical for Coronin 1B binding to F-actin. We demonstrate that Coronin 1B binds with high affinity to ATP/ADP-Pi F-actin, and the R30D

mutant lacking F-actin binding loses the ability to exert Coronin 1B function.

Using various biochemical assays, we show that Coronin 1B disassembles Arp2/3-containing actin branches by inducing Arp2/3 dissociation from the side of filaments, which is potently antagonized by Cortactin. Coronin 1B localizes to actin branches in a mutually exclusive manner with the Arp2/3 complex, and live-cell imaging reveals a sequential accumulation of these proteins during actin network assembly. Interestingly, depletion of Coronin 1B synchronizes the dynamics of Arp2/3 complex with the actin network. Together, we conclude that Coronin 1B replaces the Arp2/3 complex at actin branches, promotes branched actin network remodeling, and coordinates actin dynamics in lamellipodia.

*This dissertation is dedicated to the following people:*

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# List of Abbreviations

<b>Abp</b>	actin-binding protein
<b>ADF</b>	actin depolymerizing factor
<b>Arp</b>	actin-related protein
<b>ANOVA</b>	analysis of variance
<b>ANCOVA</b>	analysis of covariance
<b>BSA</b>	bovine serum albumin
<b>Coro1B</b>	Coronin 1B
<b>CI</b>	confidence interval
<b>CTTN</b>	Cortactin
<b>EGF</b>	epidermal growth factor
<b>FACS</b>	fluorescence activated cell sorting
<b>FBS</b>	fetal bovine serum
<b>GST</b>	glutathione S-transferase
<b>hr</b>	hour
<b>HOAc</b>	acetic acid
<b>IB</b>	immunoblotting
<b>IF</b>	immunofluorescence
<b>LIMK</b>	LIM kinase
<b>IP</b>	immunoprecipitation
<b>MBP</b>	maltose-binding protein
<b>MEF</b>	mouse embryonic fibroblast
<b>min</b>	minute
<b>N-WASP</b>	neural WiskottAldrich syndrome protein
<b>NGF</b>	nerve growth factor

<b>NPF</b>	nucleation promoting factor
<b>PAGE</b>	polyacrylamide gel electrophoresis
<b>PBS</b>	phosphate-buffered saline
<b>PCR</b>	polymerase chain reaction
<b>PDGF</b>	platelet-derived growth factor
<b>PKC</b>	protein kinase C
<b>PLC</b>	phospholipase C
<b>PMA</b>	phorbol-12-myristate-13-acetate
<b>SAS</b>	Spectrin F-actin seeds
<b>SCAR</b>	suppressor of cAMP receptor
<b>SD</b>	standard deviation
<b>SEM</b>	standard error of the mean
<b>Ser</b>	Serine
<b>SSH</b>	Slingshot
<b>STS</b>	staurosporine
<b>TEM</b>	transmission electron microscopy
<b>TIRF</b>	total internal reflection
<b>TIRFM</b>	total internal reflection microscopy
<b>VCA</b>	verprolin-central-acidic domain of N-WASP
<b>WASP</b>	WiskottAldrich syndrome protein
<b>WAVE</b>	WASP family verprolin homologous
<b>WH2</b>	WASP homology 2
<b>WT</b>	wild type