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

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