Università di Torino

MBC

Molecular Biotechnology Center

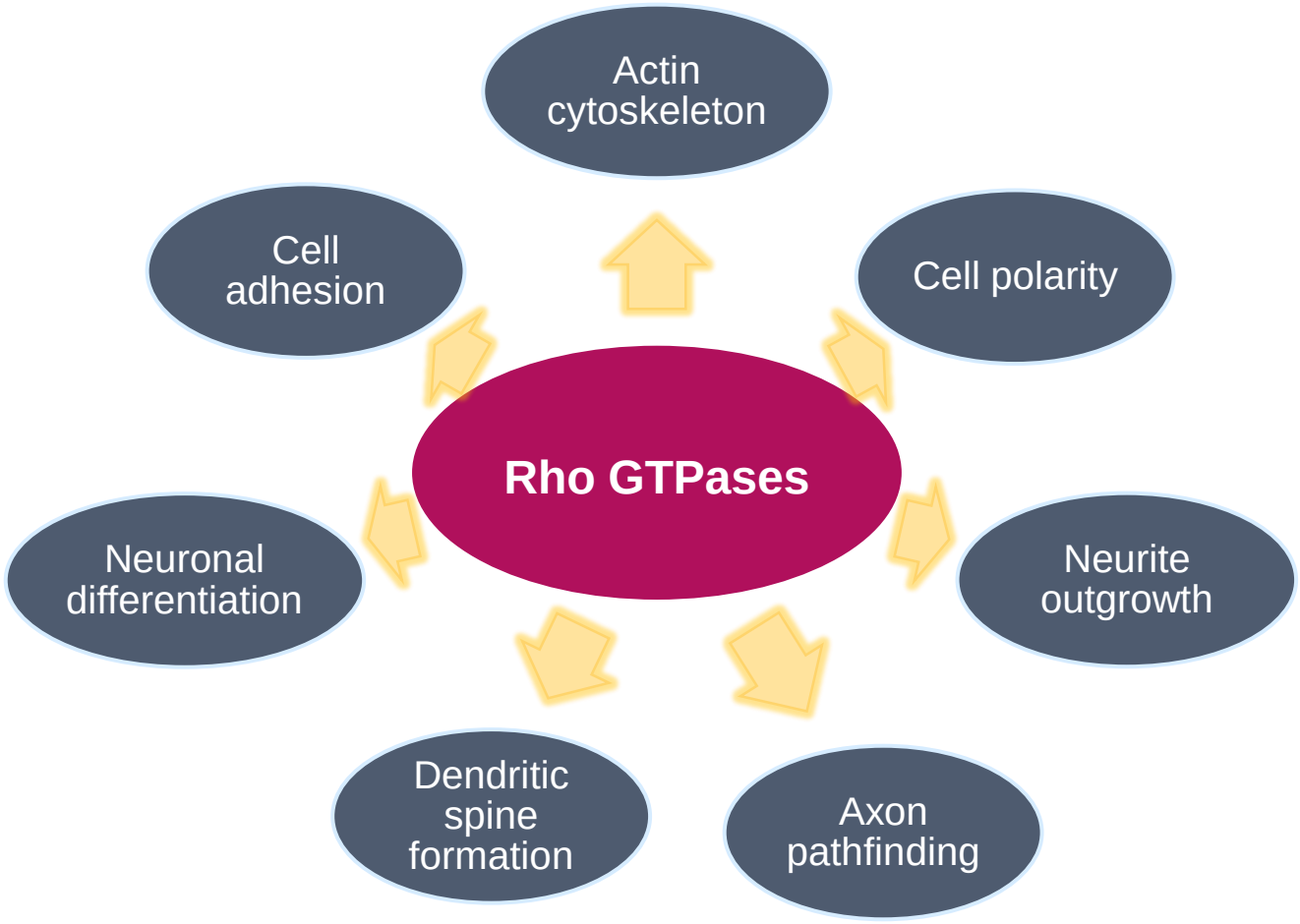
Rho GTPases and their regulators in the control of actin cytoskeleton

Valentina Zamboni

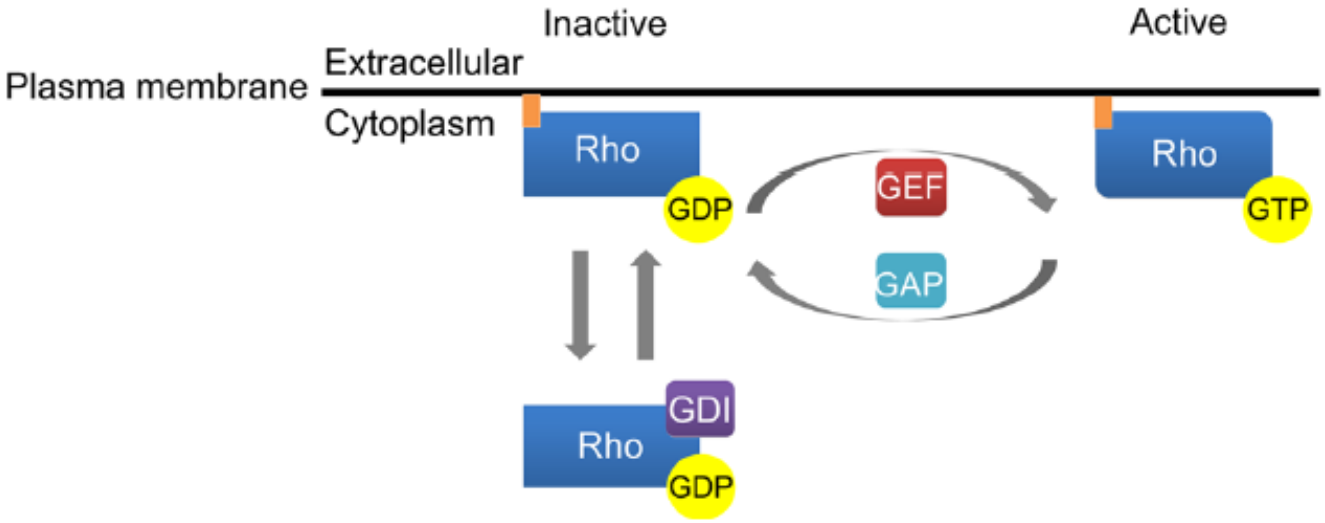
PhD student in Molecular Medicine XXXI cycle

24th May 2017

Small Rho-GTPases



Small Rho-GTPases regulation



GEFs: guanine nucleotide exchange factors
GAPs: GTPase activating proteins
GDIs: guanine dissociation inhibitors

Stankiewicz and Linseman (2014), *Frontiers in Cellular Neuroscience*.

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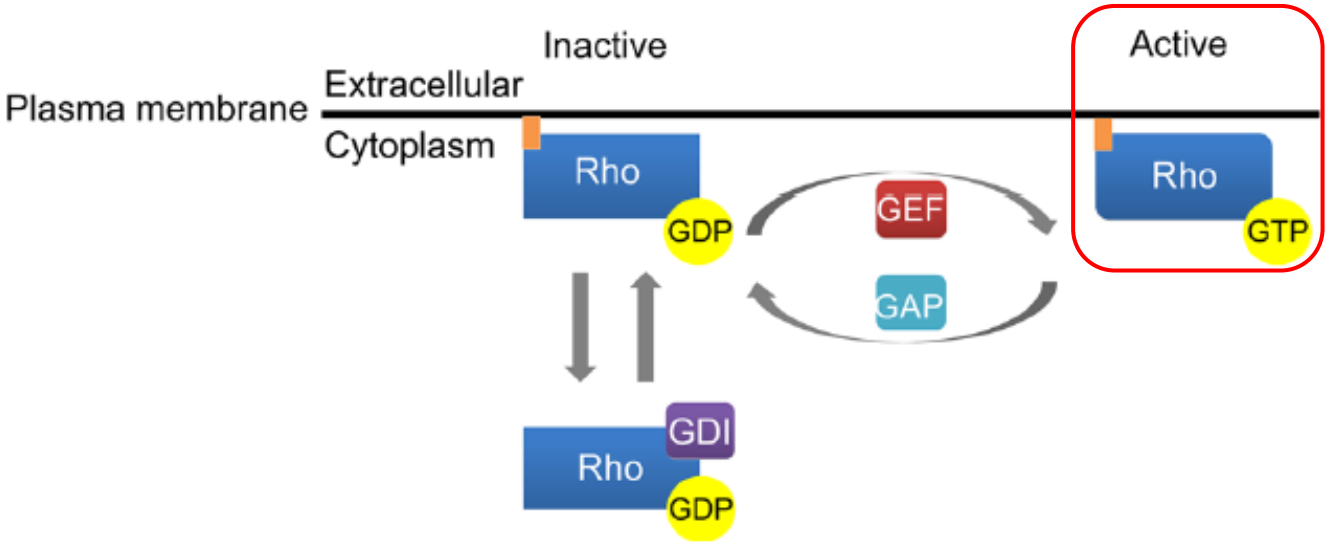

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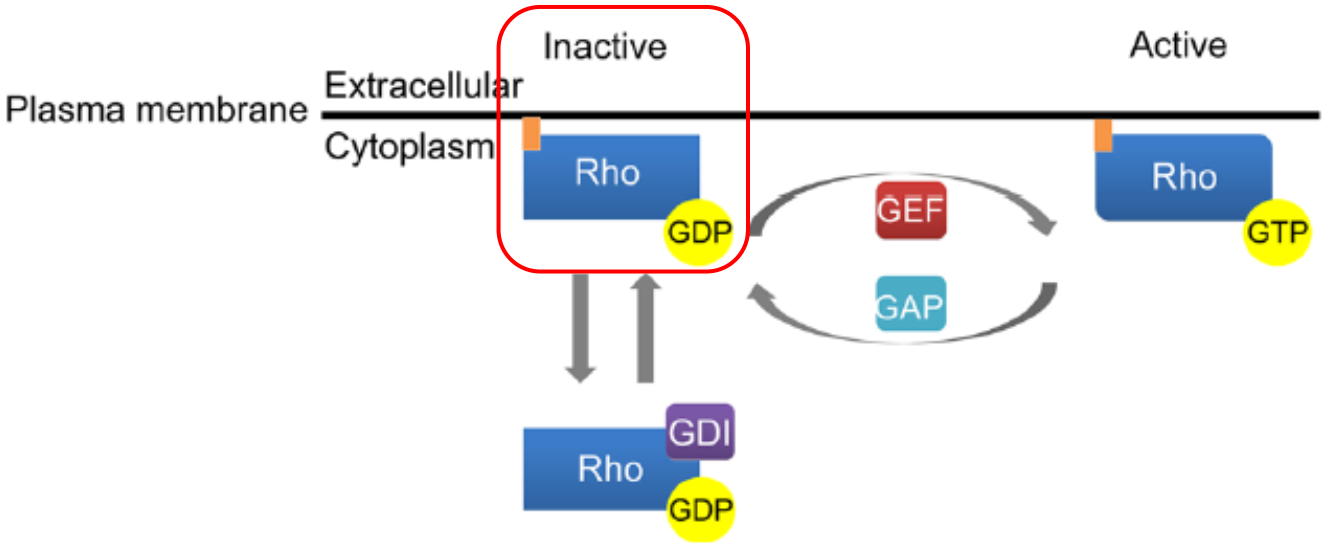

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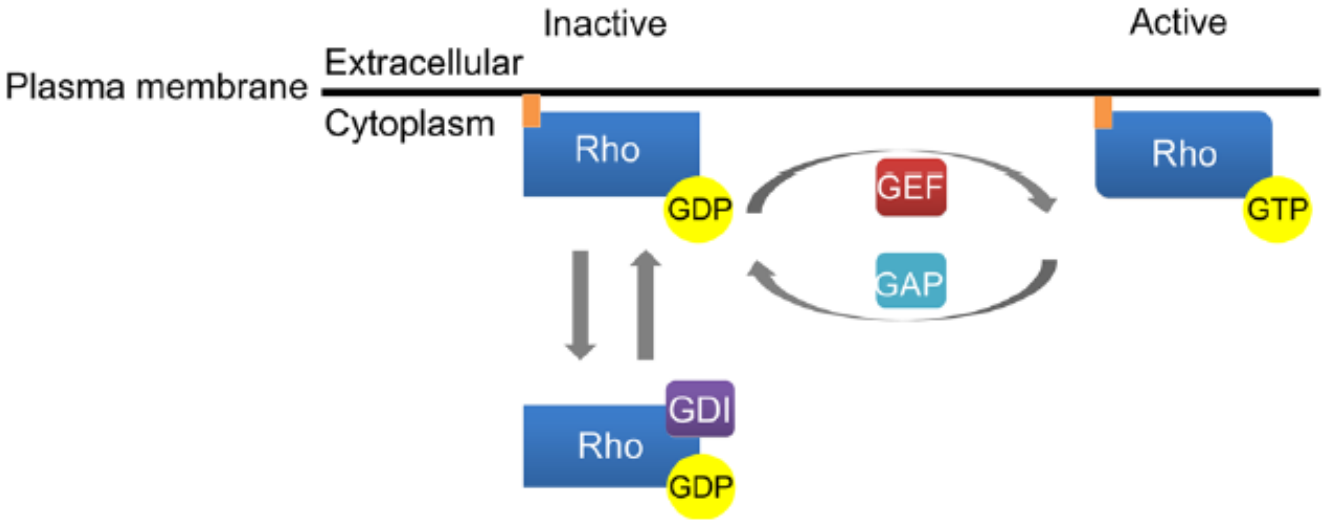

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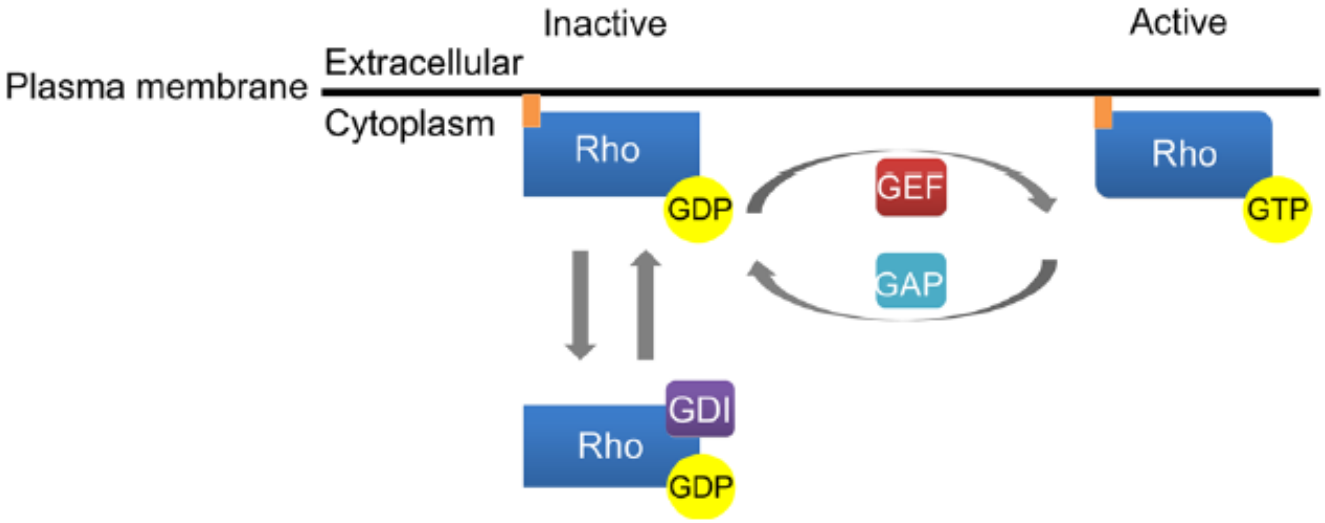

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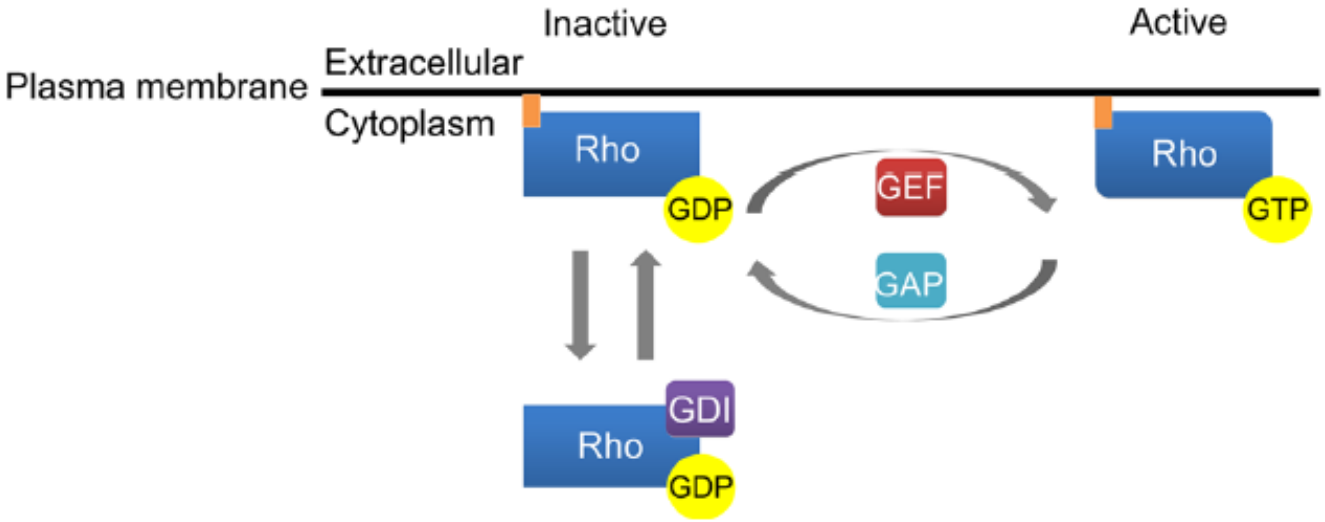

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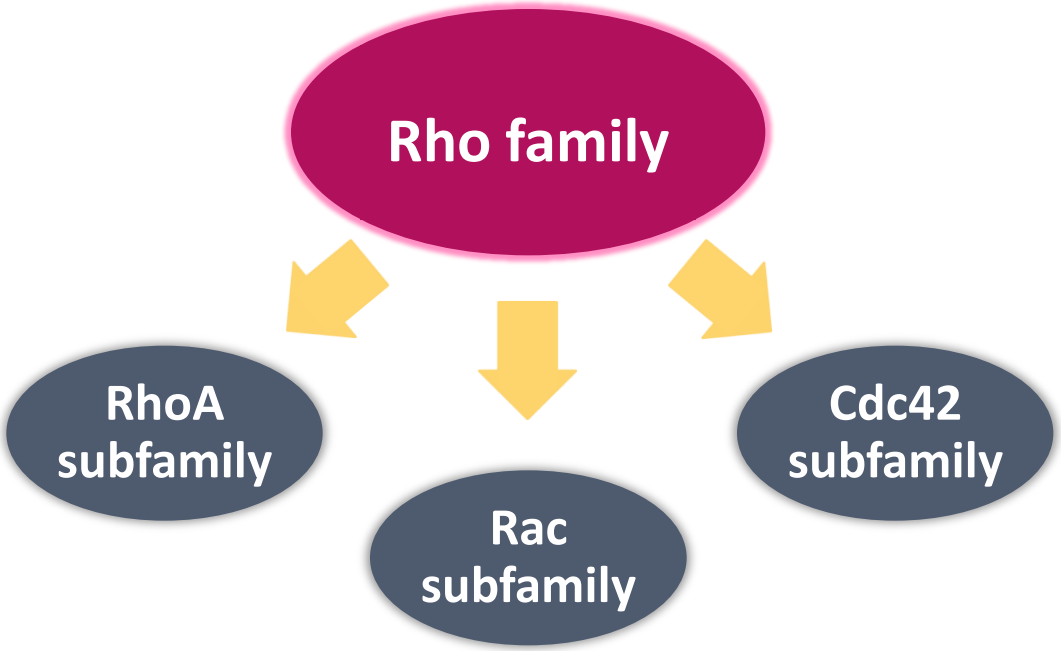
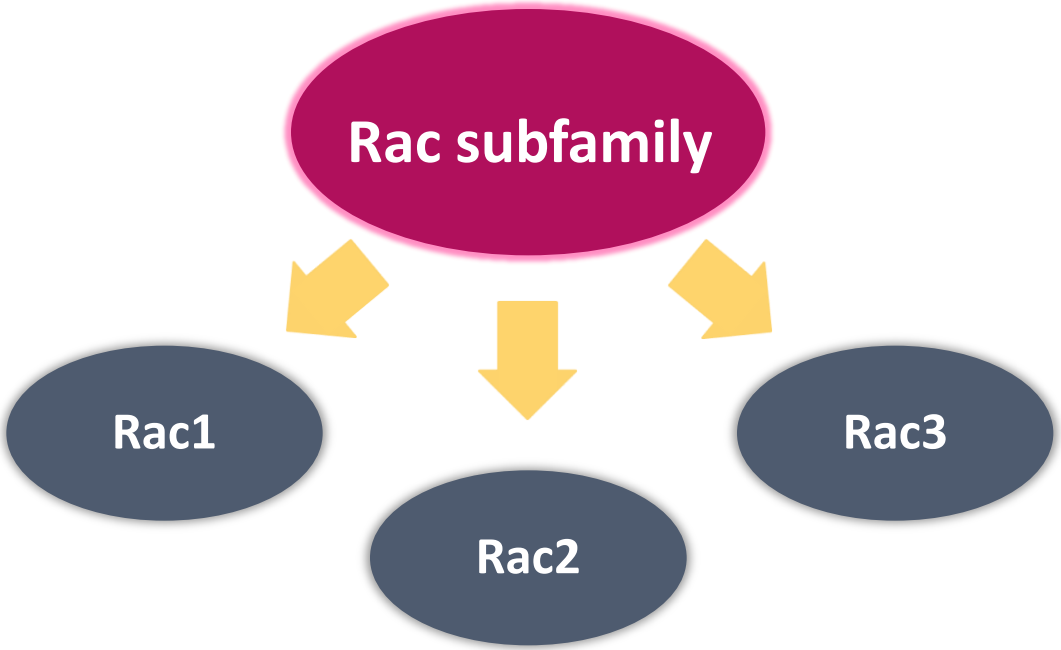
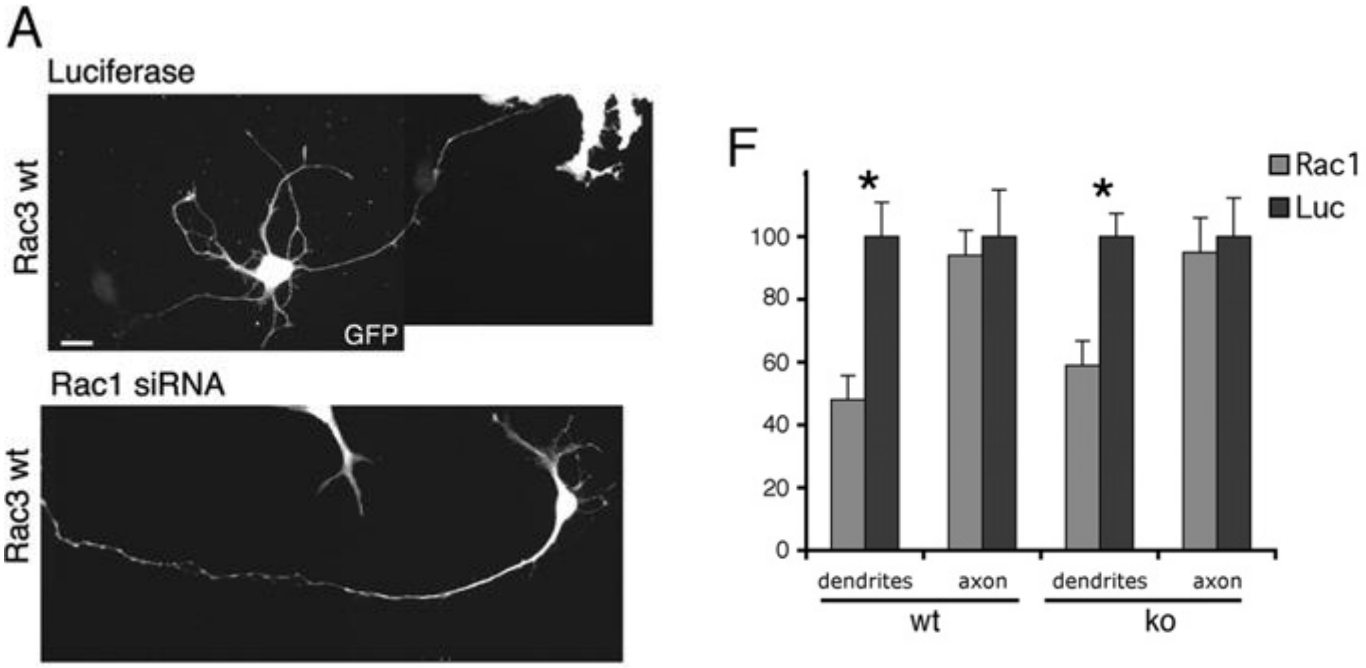
The Rho family of GTPases



The Rac family of GTPases

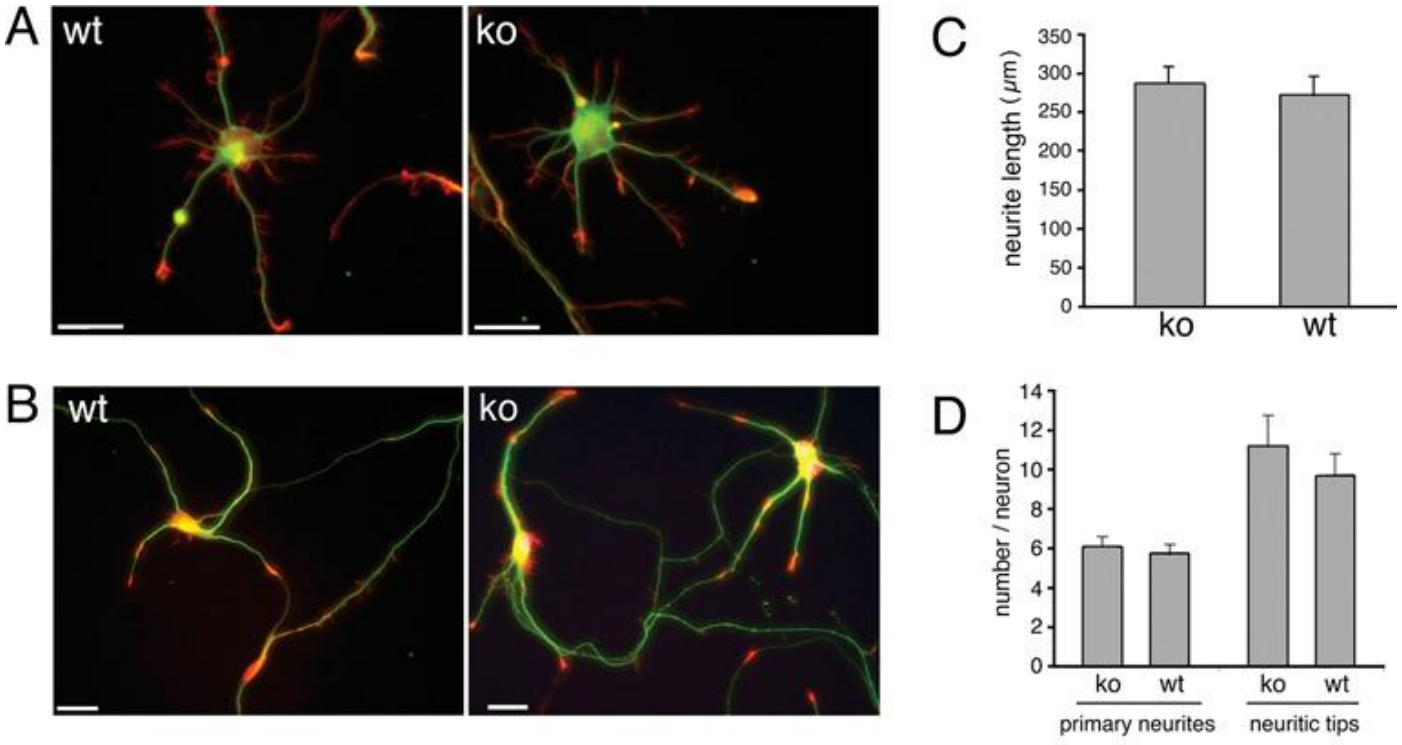


Rac1 knockdown induced a strong reduction in the dendritic tree in hippocampal cultures



Gualdoni et al. (2007), *Bio. Cell*.

Hippocampal neurons isolated from Rac3 KO mice developed normally in culture



Gualdoni et al. (2007), *Bio. Cell*.

Given the high similarity between Rac1 and Rac3, it is possible that these GTPases have redundant functions during development, and that Rac1 could at least partially compensate Rac3 depletion.

Rho GTPases in CNS diseases

Neuroscience and Biobehavioral Reviews 46 (2014) 285–301

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review

Aberrant Rho GTPases signaling and cognitive dysfunction: *In vivo* evidence for a compelling molecular relationship

Bianca De Filippis[a,*], Emilia Romano[a,b], Giovanni Laviola[a]

[a] Sect. Behavioural Neuroscience, Department of Cell Biology & Neuroscience, Istituto Superiore di Sanità, Roma, Italy
[b] Bambino Gesù, Children Hospital, IRCCS, Roma, Italy

EXPERIMENTAL CELL RESEARCH 319 (2013) 2368–2374

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/yexcr

Review Article

Rho GTPase signaling at the synapse: Implications for intellectual disability

Wei Ba[a], Jori van der Raadt[a], Nael Nadif Kasri[a,b,]*

Rho GTPases, Dendritic Structure, and Mental Retardation

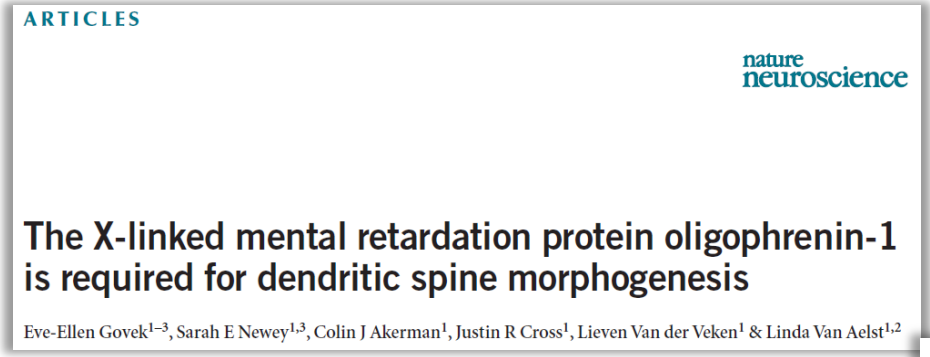
Sarah E. Newey, Vanisree Velamoor, Eve-Ellen Govek, Linda Van Aelst

Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, New York 11724

Received 18 October 2004; accepted 22 November 2004

Rho GTPases in Intellectual Disability and Mental Retardation (ID-MR)

ID-MR affects ~2%–3% of children and young adults. It is characterized by reduced cognitive function, defined by an intelligence quotient lower than 70, together with associated functional deficits in adaptive behavior.

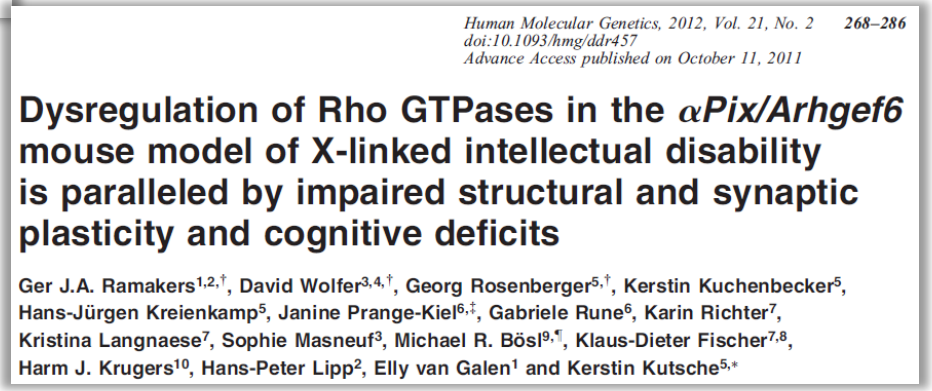


ARTICLES

nature neuroscience

The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis

Eve-Ellen Govek[1–3], Sarah E Newey[1,3], Colin J Akerman[1], Justin R Cross[1], Lieven Van der Veken[1] & Linda Van Aelst[1,2]



Human Molecular Genetics, 2012, Vol. 21, No. 2 **268–286**
doi:10.1093/hmg/ddr457
Advance Access published on October 11, 2011

Dysregulation of Rho GTPases in the *αPix/Arhgef6* mouse model of X-linked intellectual disability is paralleled by impaired structural and synaptic plasticity and cognitive deficits

Ger J.A. Ramakers[1,2,†], David Wolfer[3,4,†], Georg Rosenberger[5,†], Kerstin Kuchenbecker[5], Hans-Jürgen Kreienkamp[5], Janine Prange-Kiel[6,‡], Gabriele Rune[6], Karin Richter[7], Kristina Langnaese[7], Sophie Masneuf[3], Michael R. Bösl[9,¶], Klaus-Dieter Fischer[7,8], Harm J. Krugers[10], Hans-Peter Lipp[2], Elly van Galen[1] and Kerstin Kutsche[5,*]

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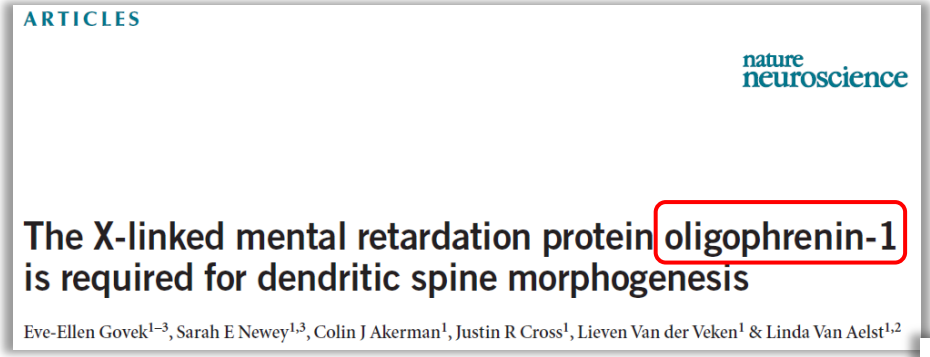

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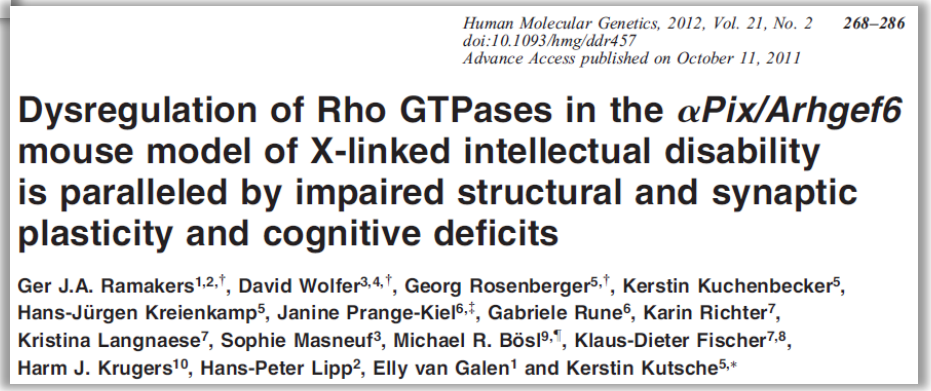

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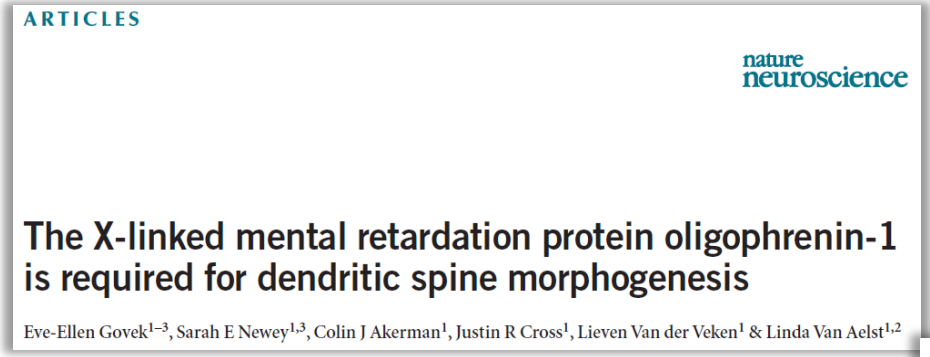

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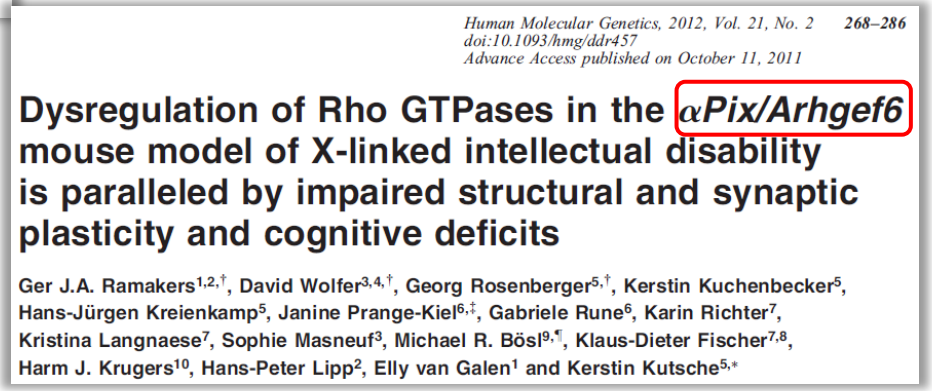

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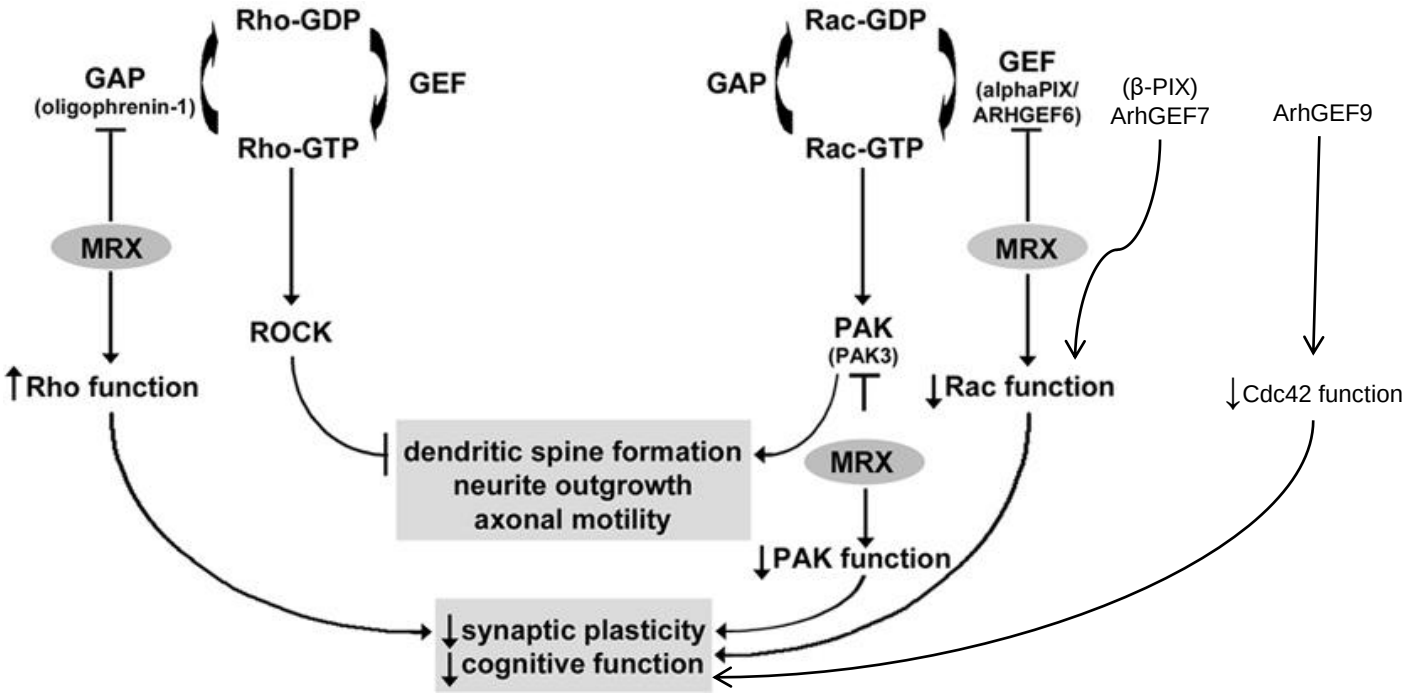
Rho GTPase signaling molecules mutated in X-linked mental retardation (MRX)



Modified from: Linseman et al. (2008), *Frontiers in Bioscience*.

GEF/GAP/GTPase signalling network combinations are numerous and complex

Multiple GTPases (with antagonistic functions) can be activated in response to the same guidance cue.
There are over 70 GEFs and 80 GAPs described in mammals. Many of them regulate several different Rho-family GTPases, and a particular GTPase might be regulated by numerous GEFs and GAPs that are all residing within the same cell.

How can this complex network of interactions be functionally explained?

Rho-family GTPase spatial localization and activation

Spatial compartmentalization of Rhofamily GTPase regulators might allow the same GTPase to be regulated by distinct GEFs or GAPs in different locations.

Key concepts

- Rho GTPases themselves, but also their GEFs and GAPs, are essential regulators of neuronal development.

- Activation of the Rho GTPases under normal conditions depends upon the presence of spatially and temporally regulated Rho GTPase regulators, and it is the fine balance between these regulators that determines the Rho GTPase activity.

- Mutations in several proteins involved in Rho GTPase signaling are causative in some forms of mental retardation.

GEF and GAP proteins

Gene	Behavioral alterations		Synaptic plasticity	Neuronal morphology
	Cognition	Other domains		
Rac1/Cdc42 GAPs				
SRGAP3 (MEGAP or WRP)	↓ Y-maze (spontaneous alternation) ↔ Morris-water maze ↔ Novel object recognition test ↔ Fear conditioning	↓ Open field ↓ Social interaction ↓ Light/dark test ↓ Plus maze ↓ PPI spontaneous tics (SHIRPA-protocol) ↔ Conditioned taste aversion		Alterations of spine length (apical and basal dendrites)
SRGAP3 (MEGAP or WRP)	↓ Long-term memory in novel object recognition test ↓ Morris-water maze (in retest and reversal) ↓ Passive avoidance ↔ Y-maze spontaneous alternation	↔ Sensitivity to foot shock ↔ Rotarod ↔ Anxiety-like responses		↓ Mushroom-shaped spine
BCR and ABR	Mild deficits in: Morris-water maze Novel object recognition test	↔ Open field ↔ Plus maze ↔ Rotarod test	↓ Maintenance of LTP ↔ PTP ↔ LTD	↓ Dendritic spine density (Slight) ↑ number of spines
RhoA GAPs				
OPHN1	↓ Morris-water maze	↓ Open field ↔ Light/dark test ↔ Elevated-Zero-Maze ↓ Social interaction	↓ PPF ↔ LTP ↔ LTD	↓ Synapse density ↑ Dendritic protrusion Immaturity of dendritic spines
OPHN1			↓ LTD	
p190RhoAGAP regulat				
ARG	↓Novel-object-recognition test		↓ PPF ↔ LTP ↔ LTD	↓ Dendrite arbors ↓ Synapse density
ABL	↔ Novel-object-recognition test		↓ PPF ↔ LTP ↔ LTD	↓ Total length and branchpoint number of basal dendrites
Integrin α3	↓ Novel-object-recognition test	↓ Body weight on pnd 42		↓ Dendrite arbors ↓ Synapse density
Rac1/Cdc42 GEFs				
KALRN	↓ Morris-water-maze ↓ Y-maze (spontaneous alternation) ↔ Y-maze (reference memory)	↓ PPI ↓ Sociability ↓ Social approach ↑ Open field (hyperactivity)		↓ Spine density
KALRN	↓ Contextual fear conditioning ↓ Cued fear conditioning	Hyperactivity	Modest ↓ in the maintenance of LTP ↔ LTD ↓ PPF	↔ Hippocampal spines ↓ Cortical spine density
KALRN	↔ Novel object recognition task ↑ Passive avoidance	↓ Anxiety in Elevated zero maze test ↓ Locomotor activity		
KALRN	↔ Radial arm maze task ↔ Novel object recognition test ↓ Contextual fear conditioning ↓ Passive avoidance	↓ Anxiety in Elevated zero maze test	↓ LTP ↓ PFP	↓ Hippocampal spine density ↔ Dendritic length
ARGEF6	Perseveration in Morris-water maze ↓ Complex positional learning	↑ Reactivity to novel environmental stimuli Disinhibition in object exploration	↓ LTP ↑ LTD	↓ Spine synapses ↔ Density of dendrites ↑ Dendrite length ↑ Branch points ↑ Spine densities Mild alteration in dendritic morphology

De Filippis et al. (2014), *Neuroscience and Biobehavioral Reviews*.

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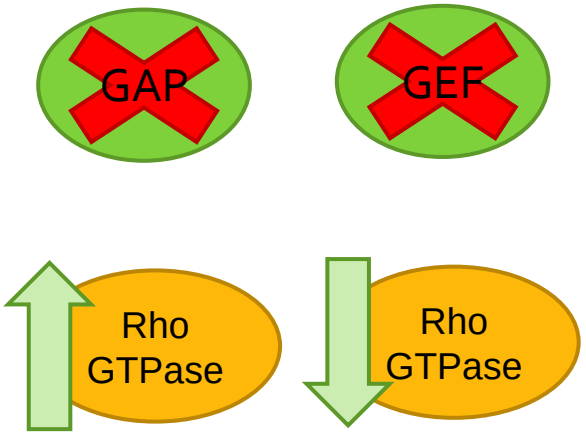


De Filippis et al. (2014), *Neuroscience and Biobehavioral Reviews*.

Tiam1, a Rac1-specific GEF, promotes axon formation

The Journal of Neuroscience, April 1, 2001, 21(7):2361–2372

Evidence for the Involvement of Tiam1 in Axon Formation

Patricia Kunda,[1] Gabriela Paglini,[1] Santiago Quiroga,[2] Kenneth Kosik,[3] and Alfredo Cáceres[1]

[1]Instituto Mercedes y Martín Ferreyra (INIMEC-CONICET), 5000 Cordoba, Argentina, [2]Departamento Química Biologica (CIQUIBIC-CONICET), Universidad Nacional Córdoba, 5000 Córdoba, Argentina, and [3]Department of Neurology (Neuroscience), Harvard Medical School and Center for Neurological Diseases, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115

The EMBO Journal (2004) 23, 1075–1088 | © 2004 European Molecular Biology Organization | All Rights Reserved 0261-4189/04
www.embojournal.org

THE EMBO JOURNAL

Tiam1 mediates neurite outgrowth induced by ephrin-B1 and EphA2

Masamitsu Tanaka[1,2], Riuko Ohashi[1,3], Ritsuko Nakamura[1], Kazuya Shinmura[1], Takaharu Kamo[1], Ryuichi Sakai[2] and Haruhiko Sugimura[1,*]

[1]First Department of Pathology, Hamamatsu University School of Medicine, Handayama, Hamamatsu, Japan and [2]Growth Factor Division, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo, Japan

↑ Overexpression of Tiam1 promotes the formation of several long, thin axon-like processes.

↓ Suppression of Tiam1 prevents axon formation.

Why are there so many GAPs?

Name	Specificity *In vitro*	*In vivo*
p50RhoGAP	**Cdc42**, Rac1, RhoA	RhoA
BPGAP1	**Cdc42**, RhoA	RhoA
Bcr	Cdc42, **Rac1**, Rac2	Rac1
Abr	Cdc42, Rac1,Rac2	ND
mCdGAP	Cdc42, Rac1	Cdc42, Rac1
TCGAP	**Cdc42**, **Rac1**, RhoA	No activity
GRIT	**Cdc42**, Rac1, **RhoA**	**Cdc42**, Rac1, **RhoA**
hCdGAP	Cdc42, Rac1	Cdc42, Rac1
d-CdGAPr	ND	ND
ARAP1	Cdc42, Rac1, **RhoA**	RhoA
ARAP2	ND	ND
ARAP3	Cdc42, Rac1, **RhoA**	RhoA
ARAP3	RhoA	RhoA
srGAP1	ND	Cdc42, RhoA
srGAP2	ND	ND
srGAP3	**Rac1**, Cdc42	ND
p115	ND	RhoA
p85-α	None	None
p85-β	ND	ND
RIP1	**Cdc42**, Rac1	ND
RalBP1	**Cdc42**, Rac1	ND
RLIP76	**Cdc42**, Rac1	ND
DLC-1	**RhoA**, Cdc42	ND
DLC-2	**RhoA**, Cdc42	RhoA
p122RhoGAP	RhoA	RhoA
RhoGAP80C	ND	Rho1, Rac1, Rac2
α1-Chimaerin	Rac1	Rac 1
β1-Chimaerin	Rac1	ND
RICH-1	Cdc42, Rac1	Cdc42, Rac1
RICH-2	Cdc42, Rac2	ND
Nadrin	Cdc42, Rac1, RhoA	ND
3BP-1	Cdc42, Rac1	Rac1
Oligophrenin-1	Cdc42, Rac1, RhoA	RhoA, Cdc42
Graf	Cdc42, RhoA	**RhoA**
Graf-2	Cdc42, RhoA	ND
PSGAP	**RhoA**, Cdc42	RhoA, **Cdc42**
GMIP	RhoA	RhoA
PARG1	Cdc42, Rac1, **RhoA**	RhoA
ArhGAP9	**Cdc42**, **Rac1**, RhoA	ND
ArhGAP12	ND	ND
Arhgap15	Rac1	Rac1
CAMGAP1	Cdc42, Rac1	ND

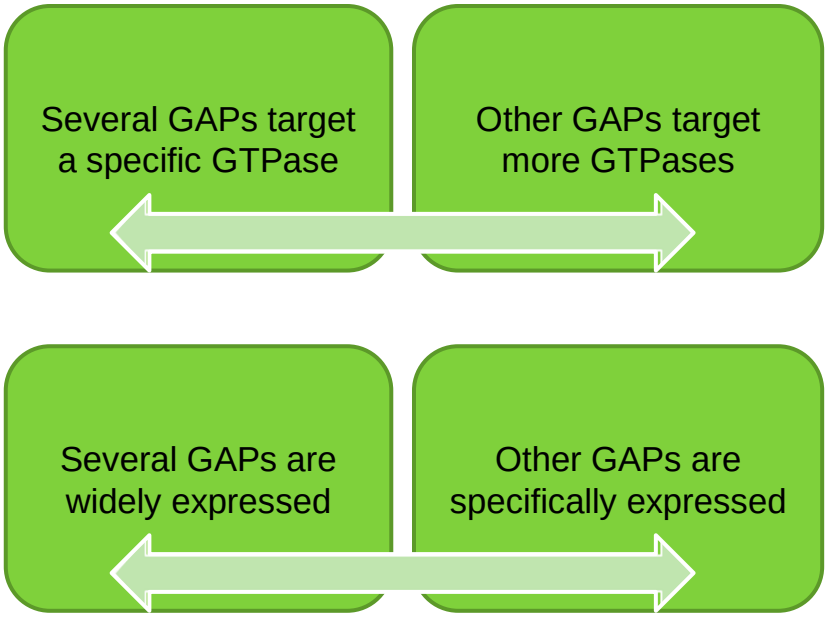
Name	Specificity *In vitro*	*In vivo*
Myosin-IXb	RhoA	ND
Myr5	Cdc42, **RhoA**, Rac1	RhoA
Myr7	RhoA	RhoA
ArhGAP10	**Cdc42**, RhoA, Rac1	ND
XrGAP	ND	ND
CeGAP	Rac1, Cdc42, RhoA	ND
MgcRacGAP	Rac1, Cdc42, RhoA	Cdc42, RhoA
RnGAP	Rac1, Cdc42	Rac1, Cdc42
DRacGAP	ND	Rac1, Cdc42
CYK-4	**Rac1**, **Cdc42**, RhoA	ND
RARhoGAP	RhoA	RhoA
tGAP1	No activity	ND
FilGAP	Cdc42, Rac1	Cdc42, **Rac1**
p73RhoGAP	ND	RhoA
p68RacGAP	Rac1	Rac1
ArhGAP6	RhoA	RhoA
OCRL-1	Rac1	Rac1
Vilse	**Rac1**, Cdc42	Rac1, Cdc42
SYD-1	None	ND
p190-A	Rac1, Cdc42, **RhoA**	RhoA
p190-B	Rac1, Cdc42, **RhoA**	ND
p190	ND	RhoA
Sac7p	Rho1p	Rho1p
Bag7p	Rho1p	Rho1p
Rga1p	Cdc42	Cdc42p
Rga2p	Cdc42	Cdc42p
Bem3p	Cdc42p	Cdc42p
Lrg1p	Cdc42p, Rho2p	ND
Bem2p	Rho1p	Rho1p
Rgd1p	Rho3p, Rho4p	Rho3p
Rgd2p	Cdc42p, Rho5p	ND
DdRacGAP1	DdRac1A, DdRacC	ND

Tcherkezian and Lamarche-Vane (2007), *Bio. Cell.*

Why are there so many GAPs?

The over-abundance of GAPs indicates that:

- each GAP may play a specialized role;
- each GAP activity may be precisely regulated, spatially and temporally.



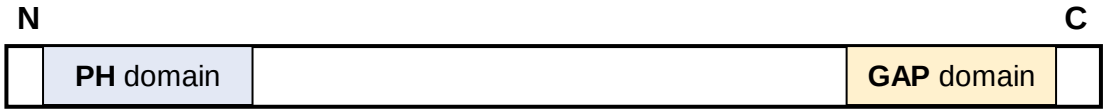
Why are there so many GAPs?

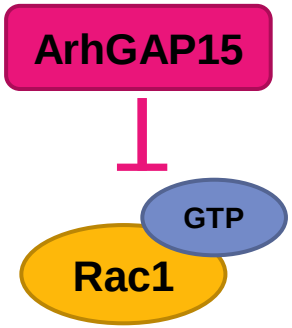
Name	Specificity *In vitro*	*In vivo*
p50RhoGAP	**Cdc42**, Rac1, RhoA	RhoA
BPGAP1	**Cdc42**, RhoA	RhoA
Bcr	Cdc42, **Rac1**, Rac2	Rac1
Abr	Cdc42, Rac1,Rac2	ND
mCdGAP	Cdc42, Rac1	Cdc42, Rac1
TCGAP	**Cdc42**, **Rac1**, RhoA	No activity
GRIT	**Cdc42**, Rac1, **RhoA**	**Cdc42**, Rac1, **RhoA**
hCdGAP	Cdc42, Rac1	Cdc42, Rac1
d-CdGAPr	ND	ND
ARAP1	Cdc42, Rac1, **RhoA**	RhoA
ARAP2	ND	ND
ARAP3	Cdc42, Rac1, **RhoA**	RhoA
ARAP3	RhoA	RhoA
srGAP1	ND	Cdc42, RhoA
srGAP2	ND	ND
srGAP3	**Rac1**, Cdc42	ND
p115	ND	RhoA
p85-α	None	None
p85-β	ND	ND
RIP1	**Cdc42**, Rac1	ND
RalBP1	**Cdc42**, Rac1	ND
RLIP76	**Cdc42**, Rac1	ND
DLC-1	**RhoA**, Cdc42	ND
DLC-2	**RhoA**, Cdc42	RhoA
p122RhoGAP	RhoA	RhoA
RhoGAP80C	ND	Rho1, Rac1, Rac2
α1-Chimaerin	Rac1	Rac 1
β1-Chimaerin	Rac1	ND
RICH-1	Cdc42, Rac1	Cdc42, Rac1
RICH-2	Cdc42, Rac2	ND
Nadrin	Cdc42, Rac1, RhoA	ND
3BP-1	Cdc42, Rac1	Rac1
Oligophrenin-1	Cdc42, Rac1, RhoA	RhoA, Cdc42
Graf	Cdc42, RhoA	**RhoA**
Graf-2	Cdc42, RhoA	ND
PSGAP	**RhoA**, Cdc42	RhoA, **Cdc42**
GMIP	RhoA	RhoA
PARG1	Cdc42, Rac1, **RhoA**	RhoA
ArhGAP9	**Cdc42**, **Rac1**, RhoA	ND
Arhgap15	Rac1	Rac1
CAMGAP1	Cdc42, Rac1	ND

Name	Specificity *In vitro*	*In vivo*
Myosin-IXb	RhoA	ND
Myr5	Cdc42, **RhoA**, Rac1	RhoA
Myr7	RhoA	RhoA
ArhGAP10	**Cdc42**, RhoA, Rac1	ND
XrGAP	ND	ND
CeGAP	Rac1, Cdc42, RhoA	ND
MgcRacGAP	Rac1, Cdc42, RhoA	Cdc42, RhoA
RnGAP	Rac1, Cdc42	Rac1, Cdc42
DRacGAP	ND	Rac1, Cdc42
CYK-4	**Rac1**, **Cdc42**, RhoA	ND
RARhoGAP	RhoA	RhoA
tGAP1	No activity	ND
FilGAP	Cdc42, Rac1	Cdc42, **Rac1**
p73RhoGAP	ND	RhoA
p68RacGAP	Rac1	Rac1
ArhGAP6	RhoA	RhoA
OCRL-1	Rac1	Rac1
Vilse	**Rac1**, Cdc42	Rac1, Cdc42
SYD-1	None	ND
p190-A	Rac1, Cdc42, **RhoA**	RhoA
p190-B	Rac1, Cdc42, **RhoA**	ND
p190	ND	RhoA
Sac7p	Rho1p	Rho1p
Bag7p	Rho1p	Rho1p
Rga1p	Cdc42	Cdc42p
Rga2p	Cdc42	Cdc42p
Bem3p	Cdc42p	Cdc42p
Lrg1p	Cdc42p, Rho2p	ND
Bem2p	Rho1p	Rho1p
Rgd1p	Rho3p, Rho4p	Rho3p
Rgd2p	Cdc42p, Rho5p	ND
DdRacGAP1	DdRac1A, DdRacC	ND

Tcherkezian and Lamarche-Vane (2007), *Bio. Cell.*

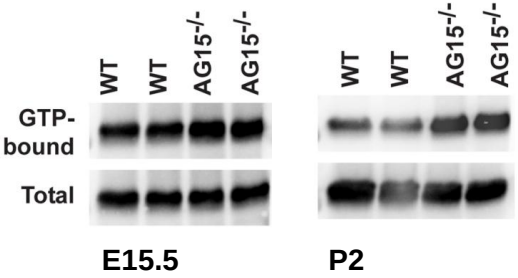
ArhGAP15, a Rac-specific GAP

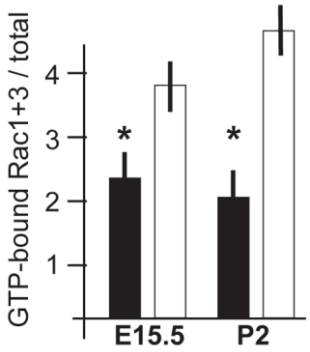


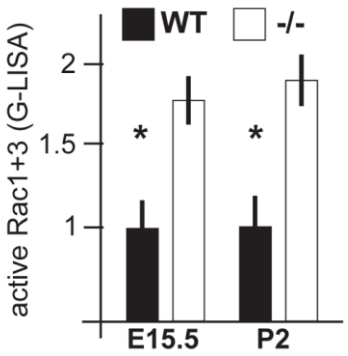


GTP-bound Rac1: active state

GDP-bound Rac1: inactive state

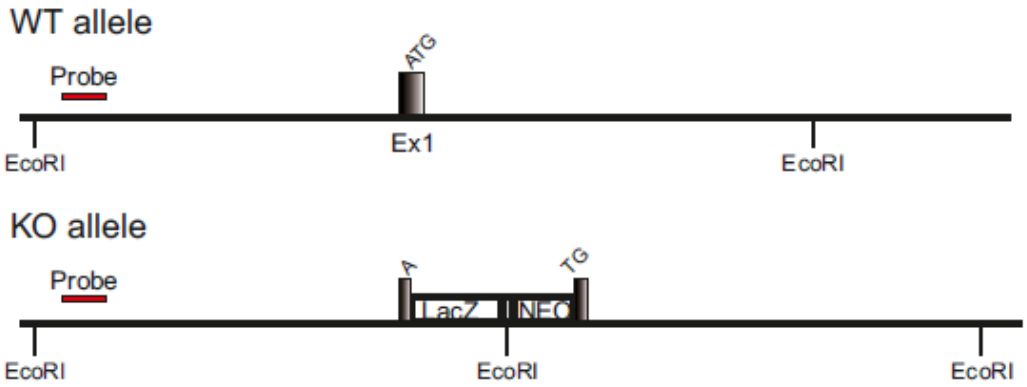






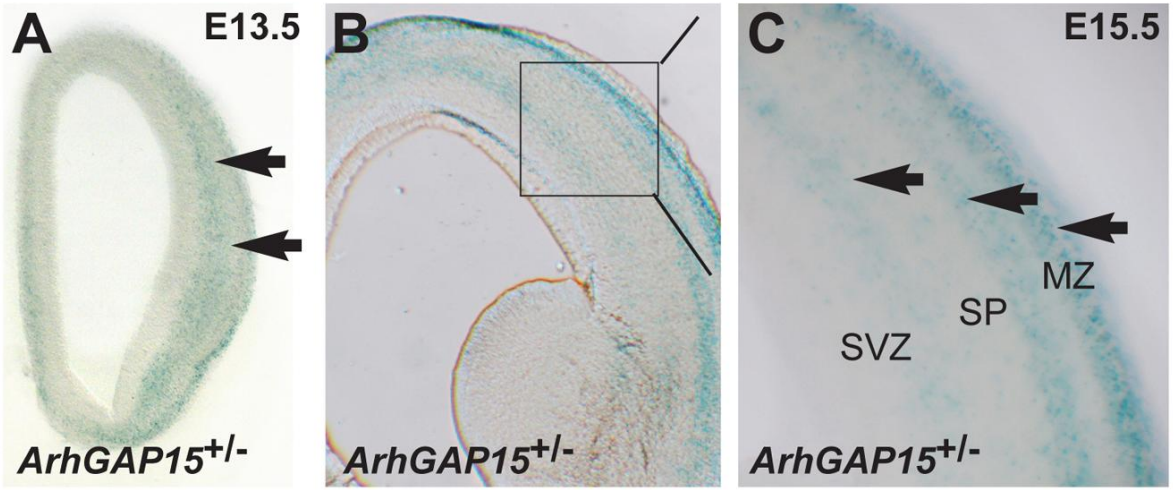
Zamboni et al. (2016), *Scientific Reports*.

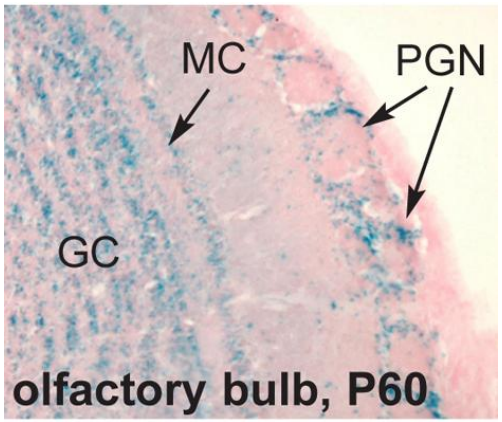
ArhGAP15 KO mouse

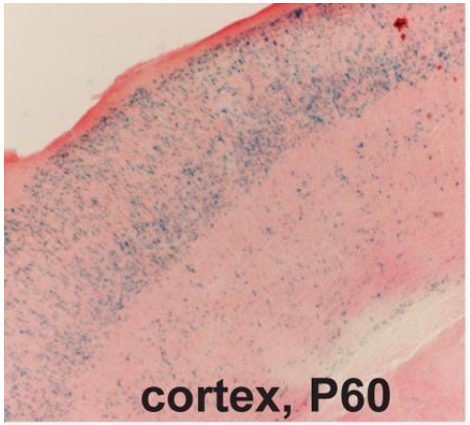


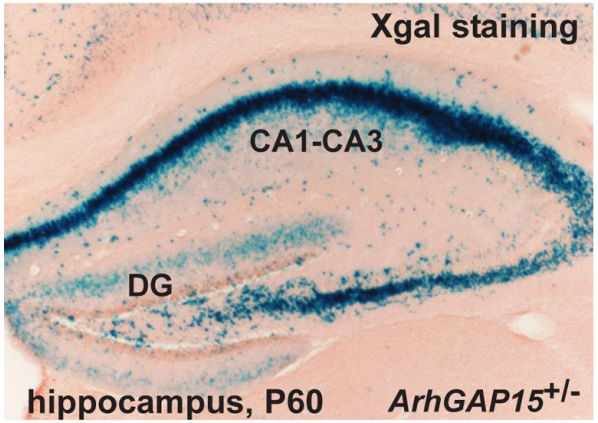
Modified from: Costa et al. (2011), *Blood*.

ArhGAP15 expression in the brain

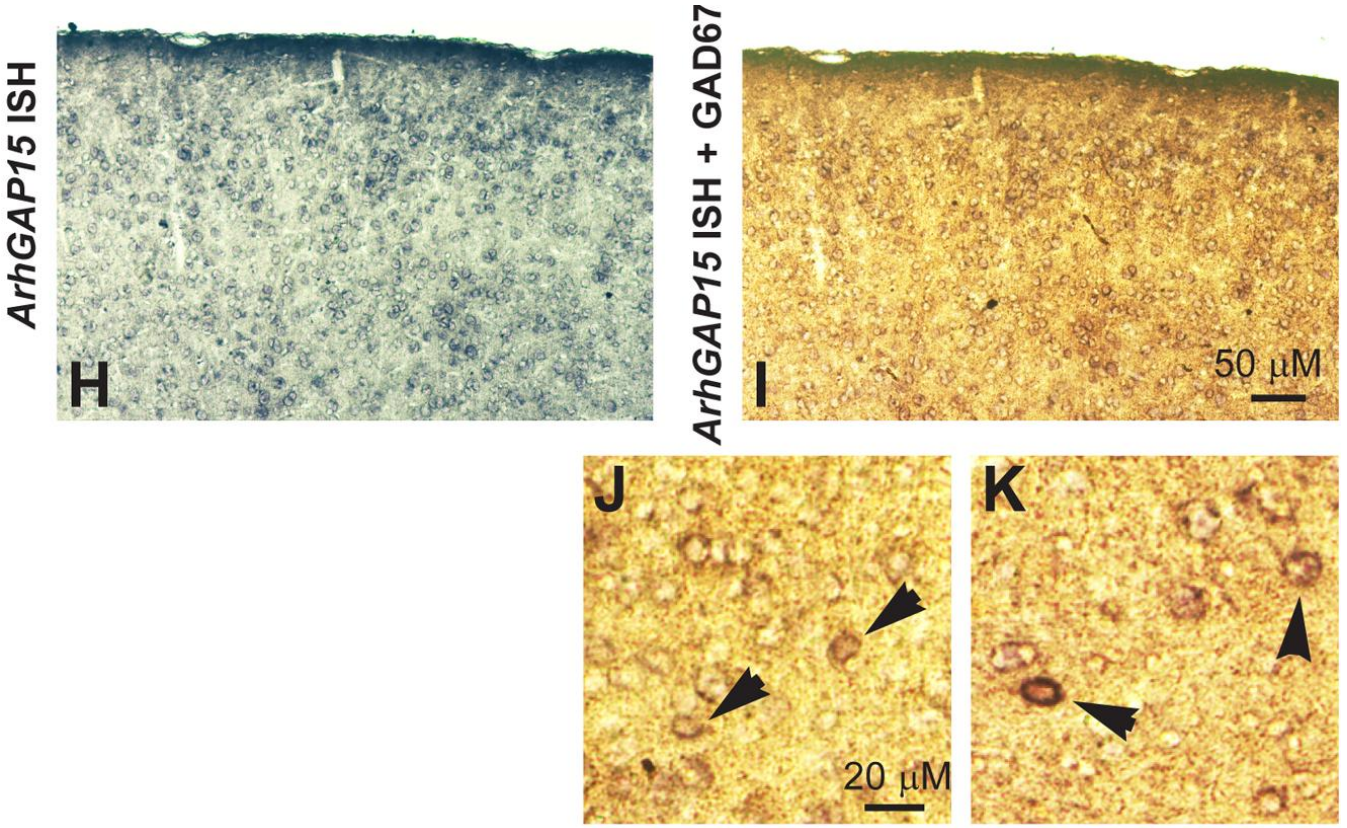




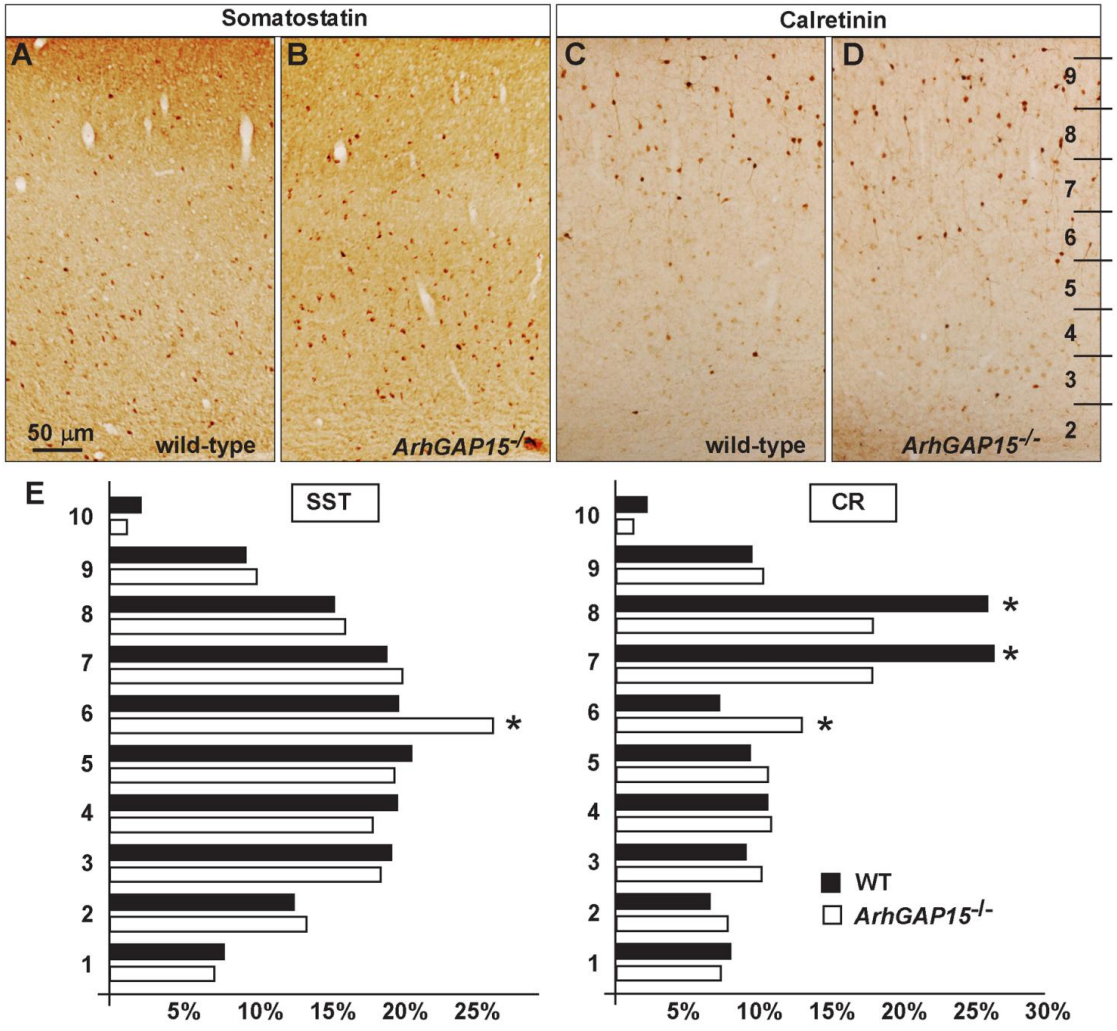




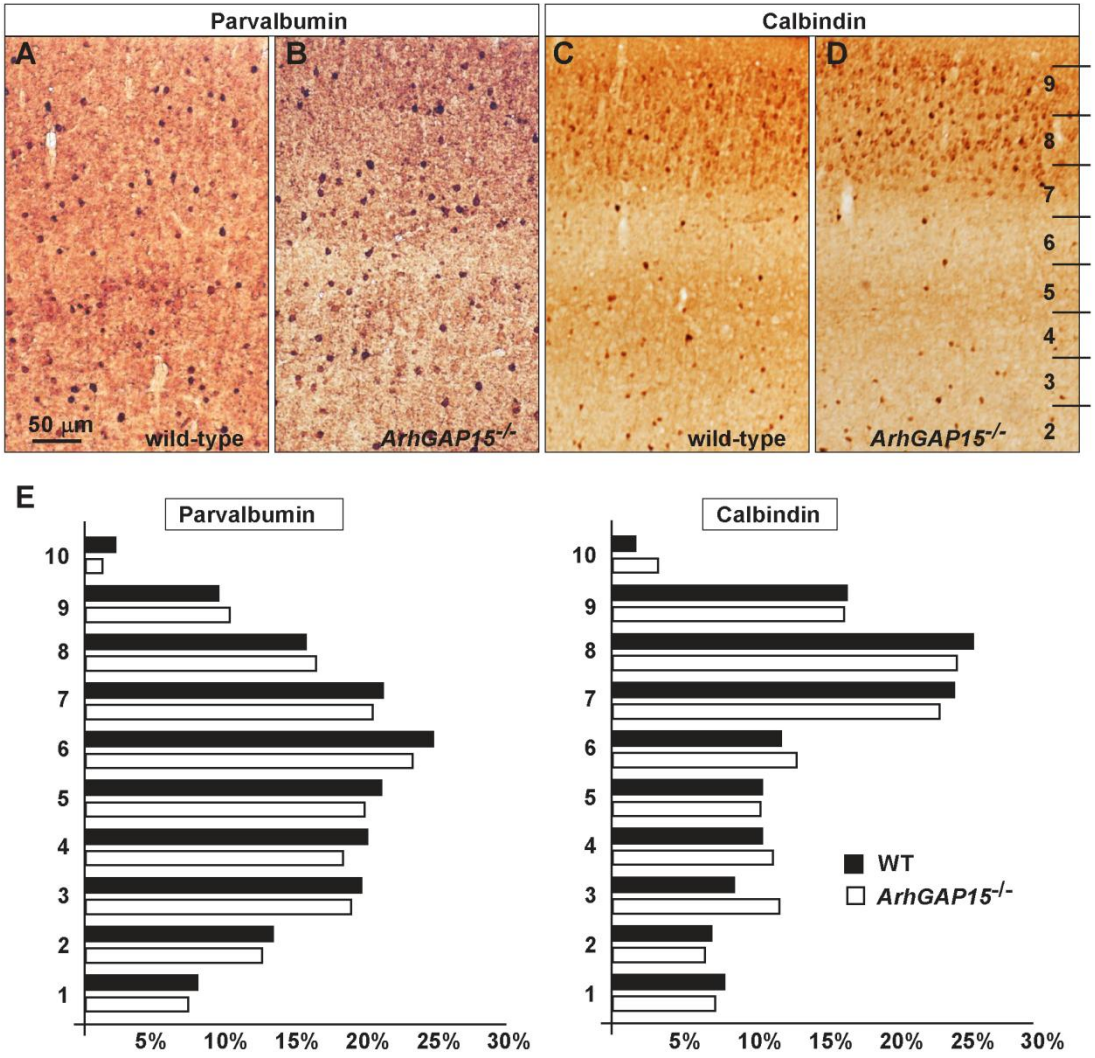
ArhGAP15 is expressed in interneurons



Stratification of adult cortical interneurons is altered in the absence of *ArhGAP15*

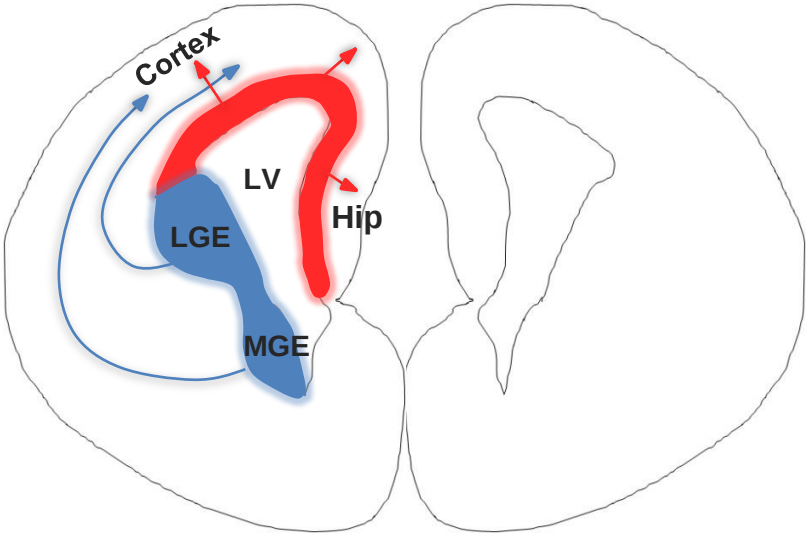


Stratification of adult cortical interneurons is altered in the absence of *ArhGAP15*



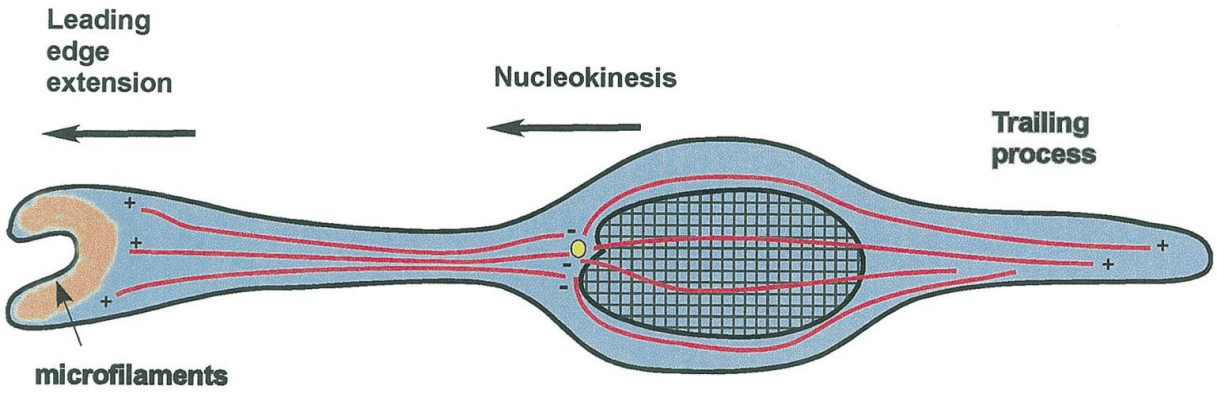
Does the loss of *ArhGAP15* affect neuronal migration?

Neuronal migration during brain development



Glutamatergic excitatory neurons: radial migration
GABA+ interneurons: tangential migration

Neuronal migration is a key feature of nervous system development

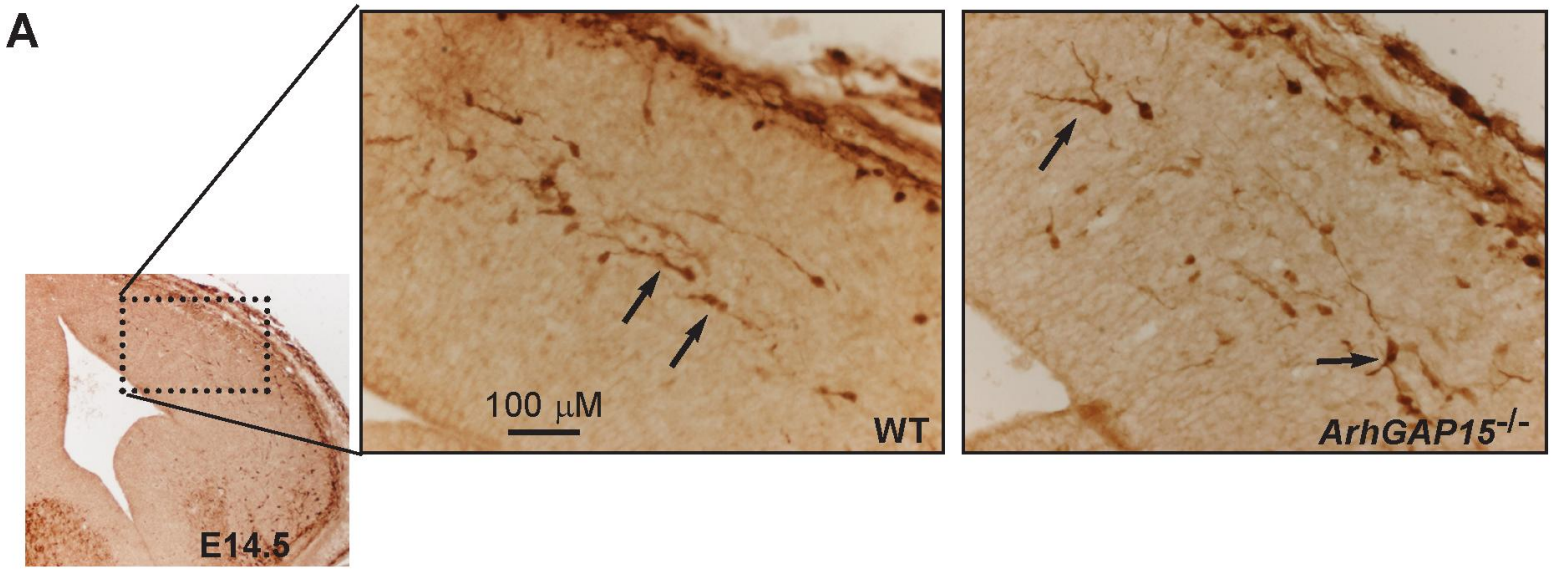


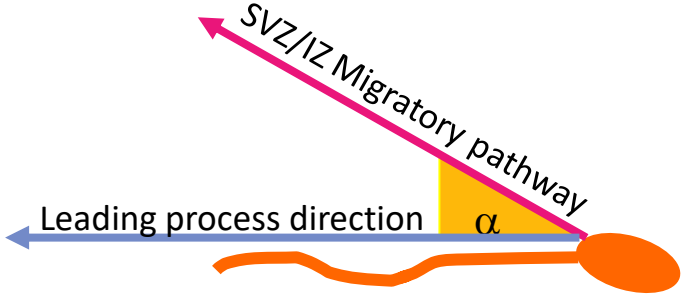
Neuronal migration occurs in three stages:

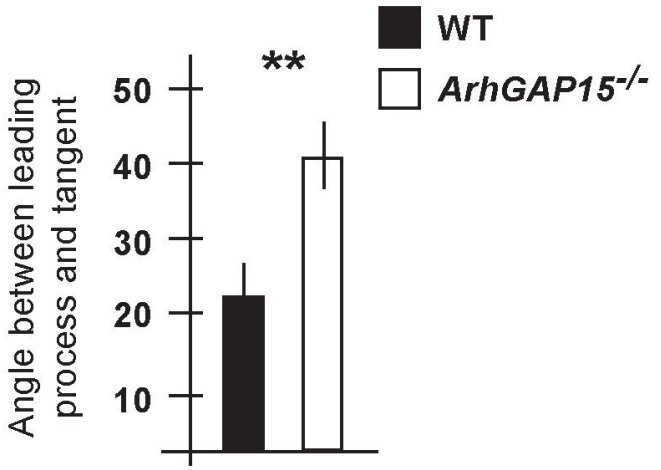
1. Leading Edge Extension
2. Nuclear Translocation (Nucleokinesis)
3. Retraction of Trailing Process

C. Lambert de Rouvroitl and A.M. Gofffinet (2002), Mechanisms of development.

The loss of *ArhGAP15* affects the directionality during tangential migration of interneurons, *in vivo*

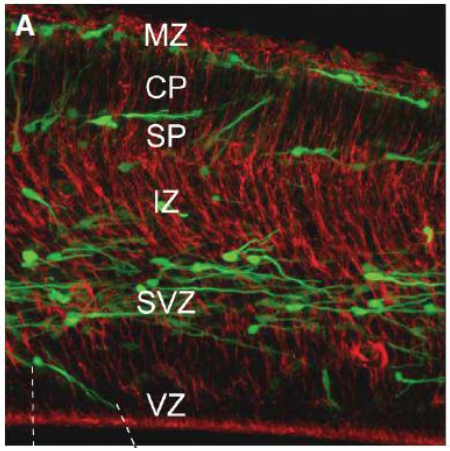


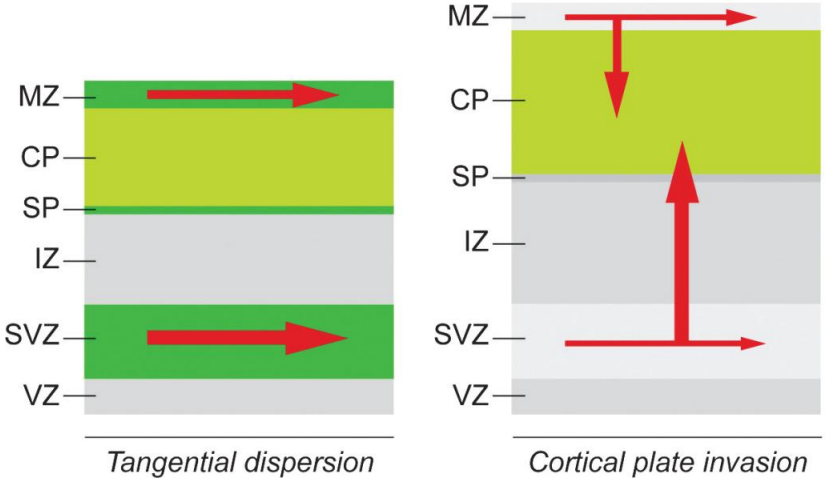




The hyperactivation of Rac1 alters the control of cell directionality during tangential migration.

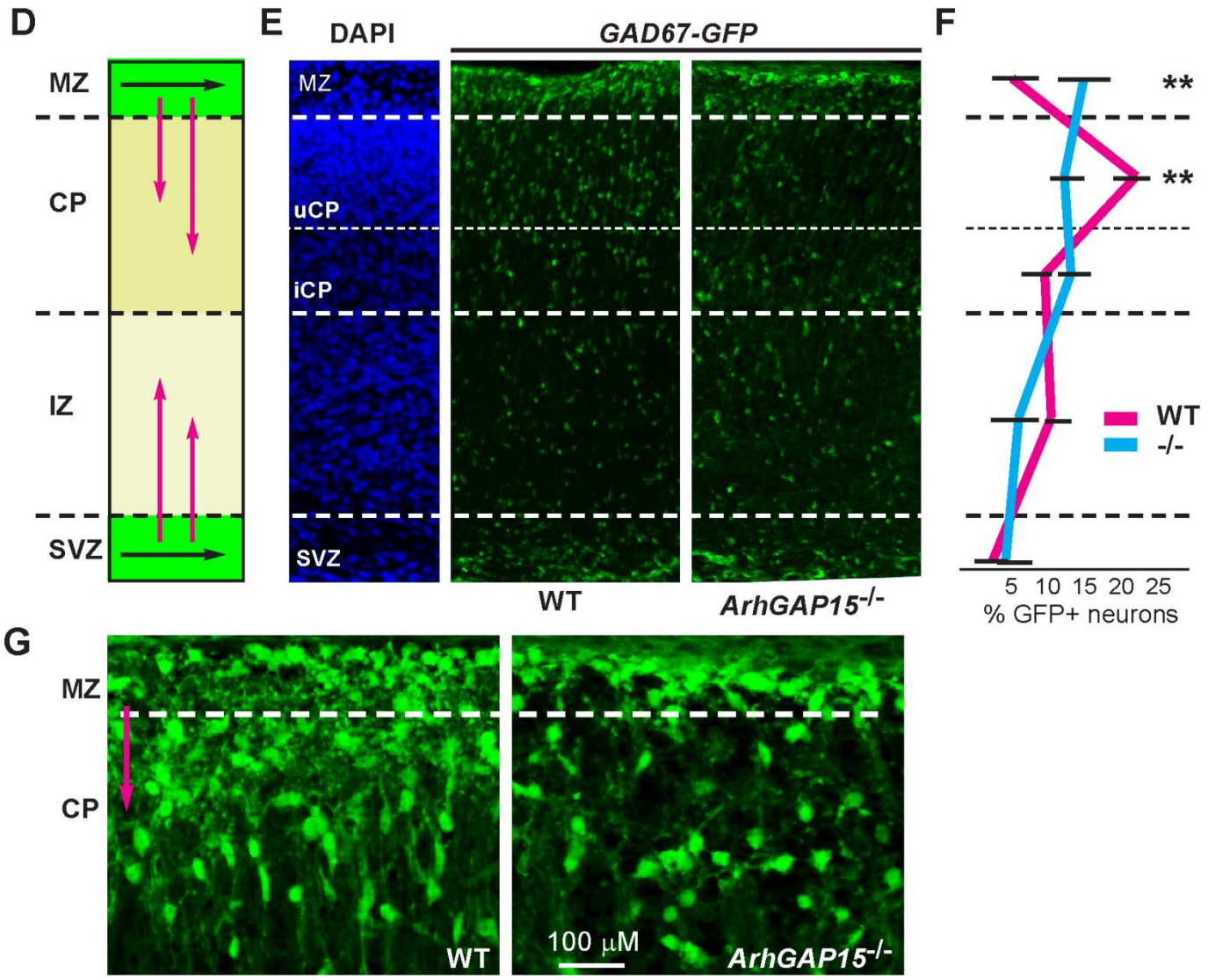
Migratory streams and intracortical dispersion of interneurons



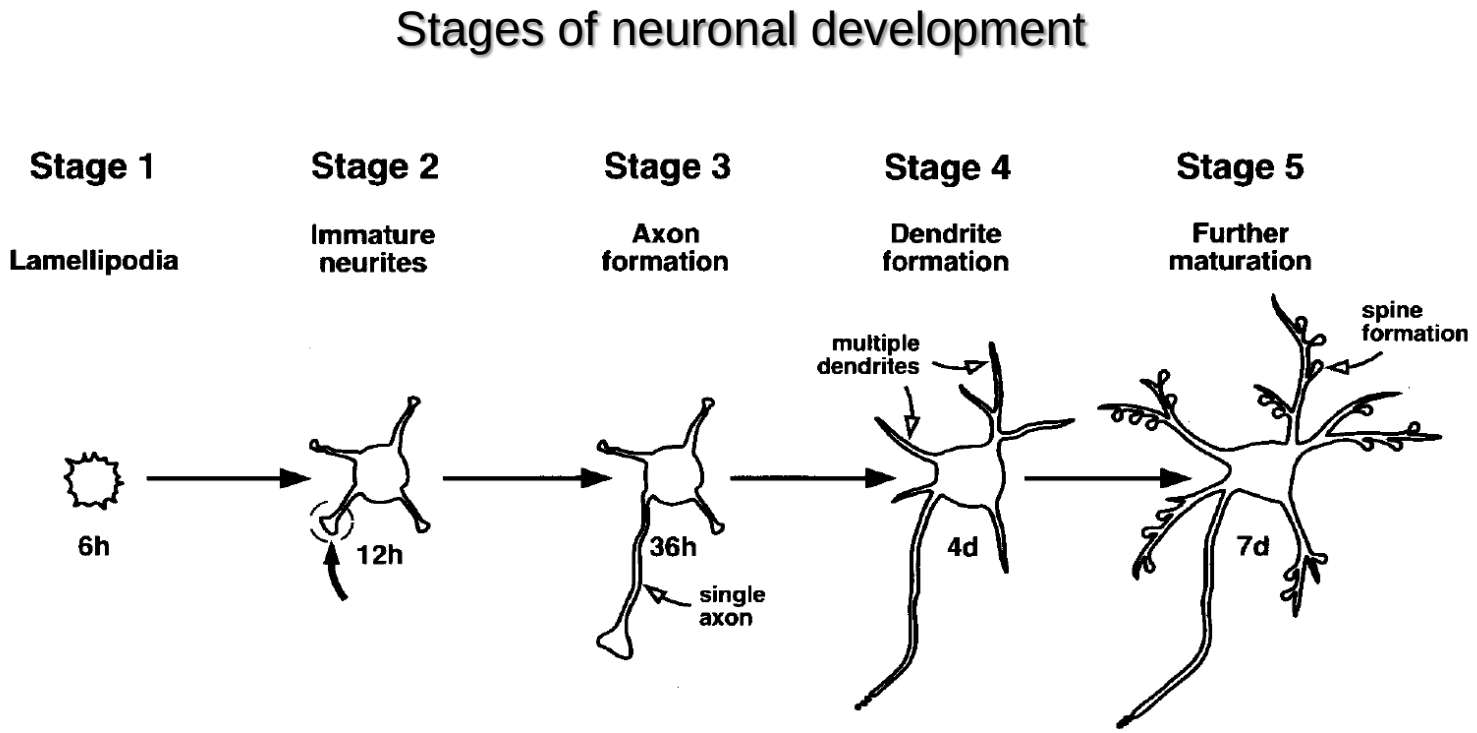


Oscar Marin (2013), *European Journal of Neuroscience*.

The loss of *ArhGAP15* affects the directionality during migration of interneurons

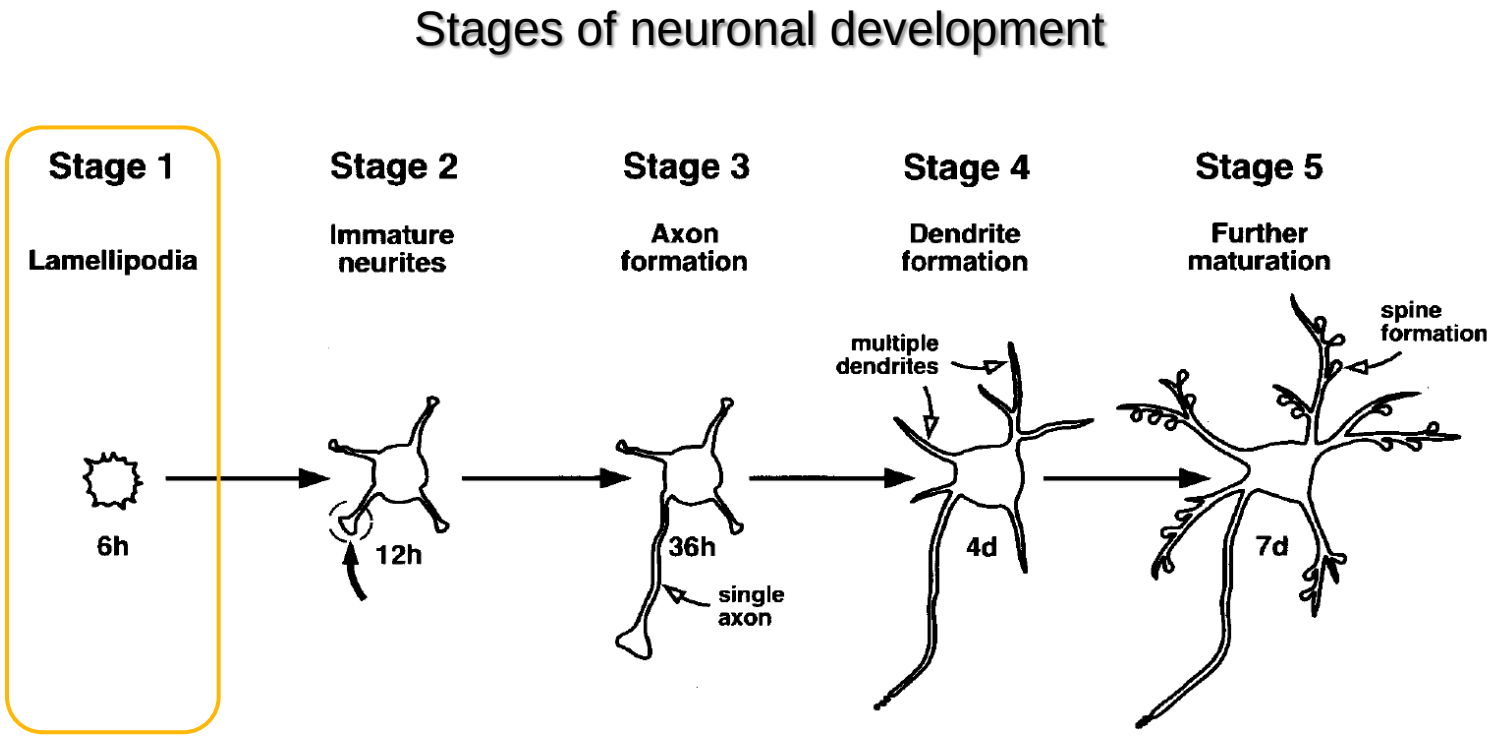


How does a neuron develop the unique and intricate architecture?



Govek et al. (2005), *Genes & Development*.

How does a neuron develop the unique and intricate architecture?



Govek et al. (2005), *Genes & Development*.

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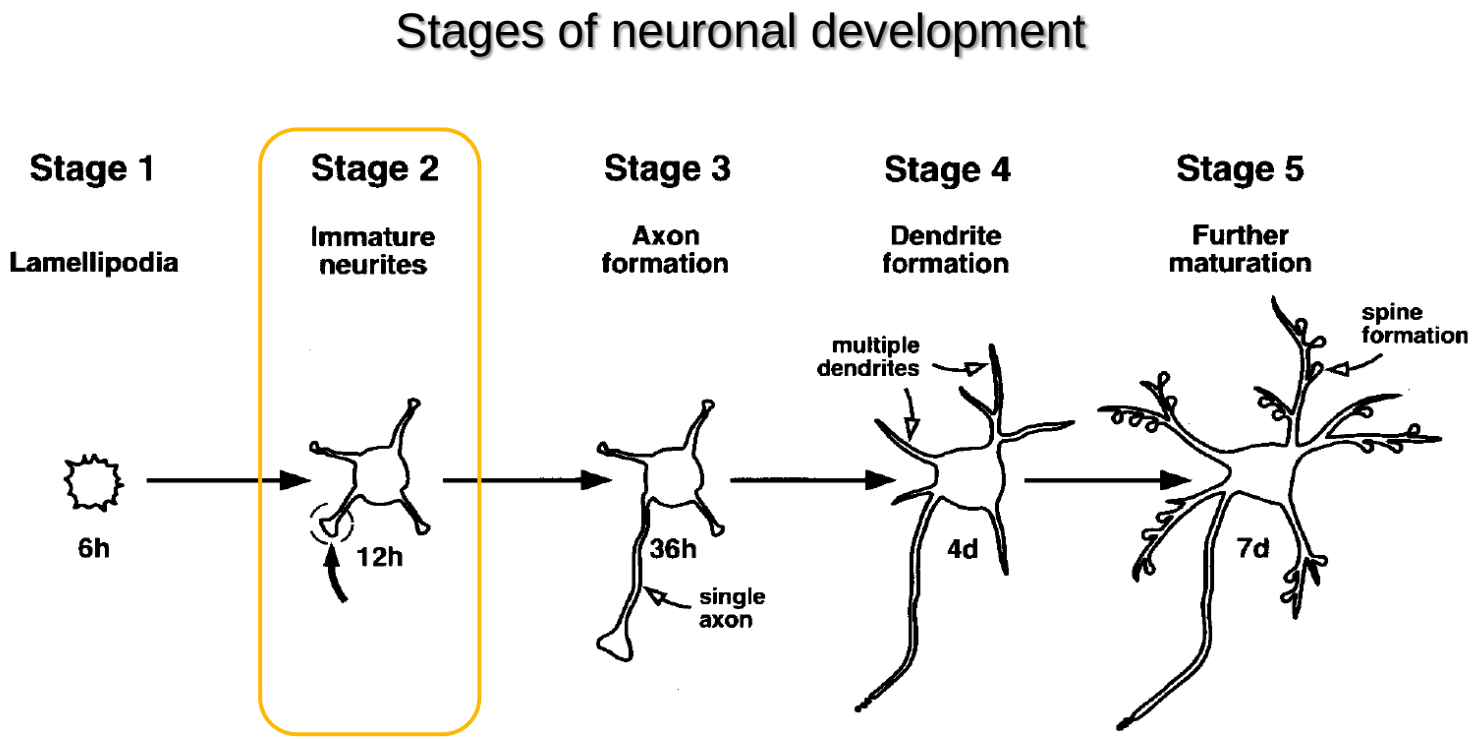

Govek et al. (2005), *Genes & Development*.

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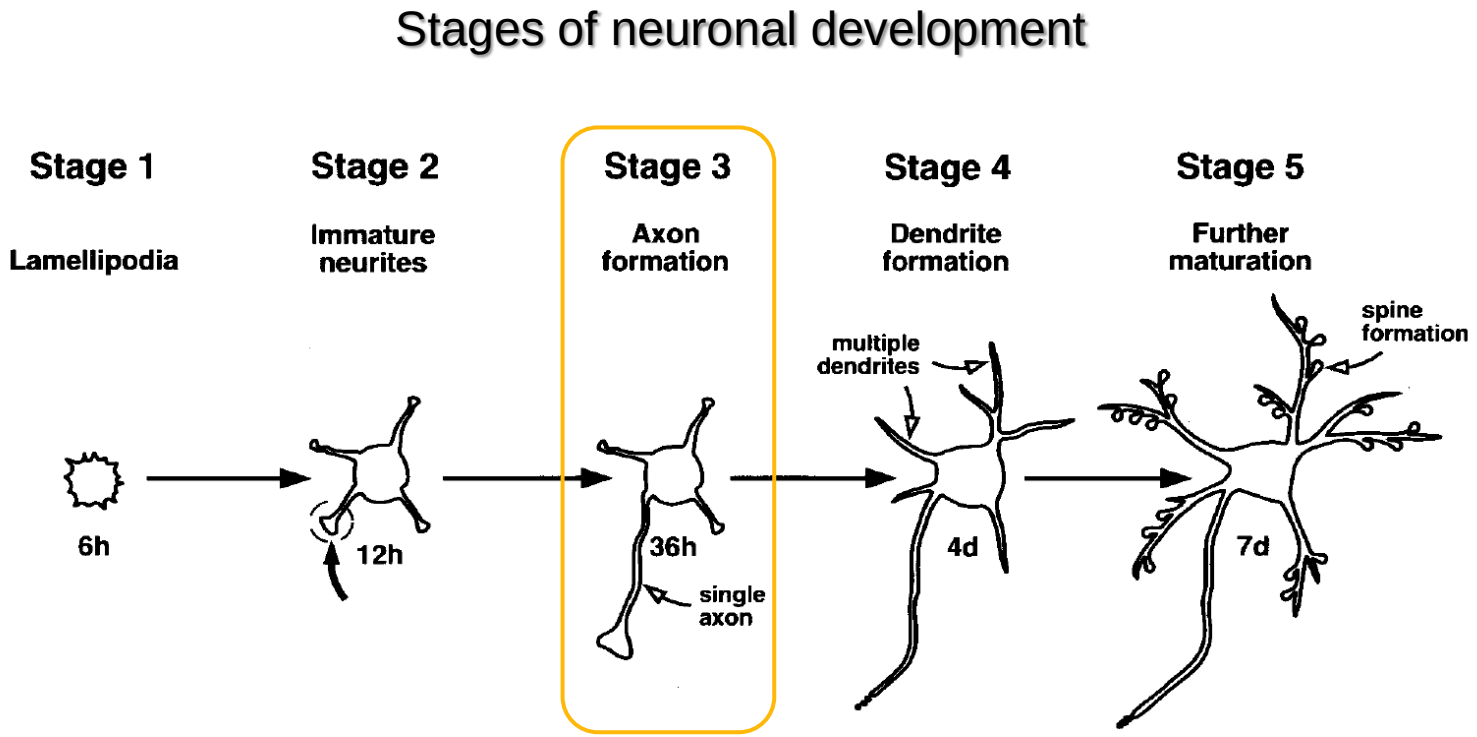

Govek et al. (2005), *Genes & Development*.

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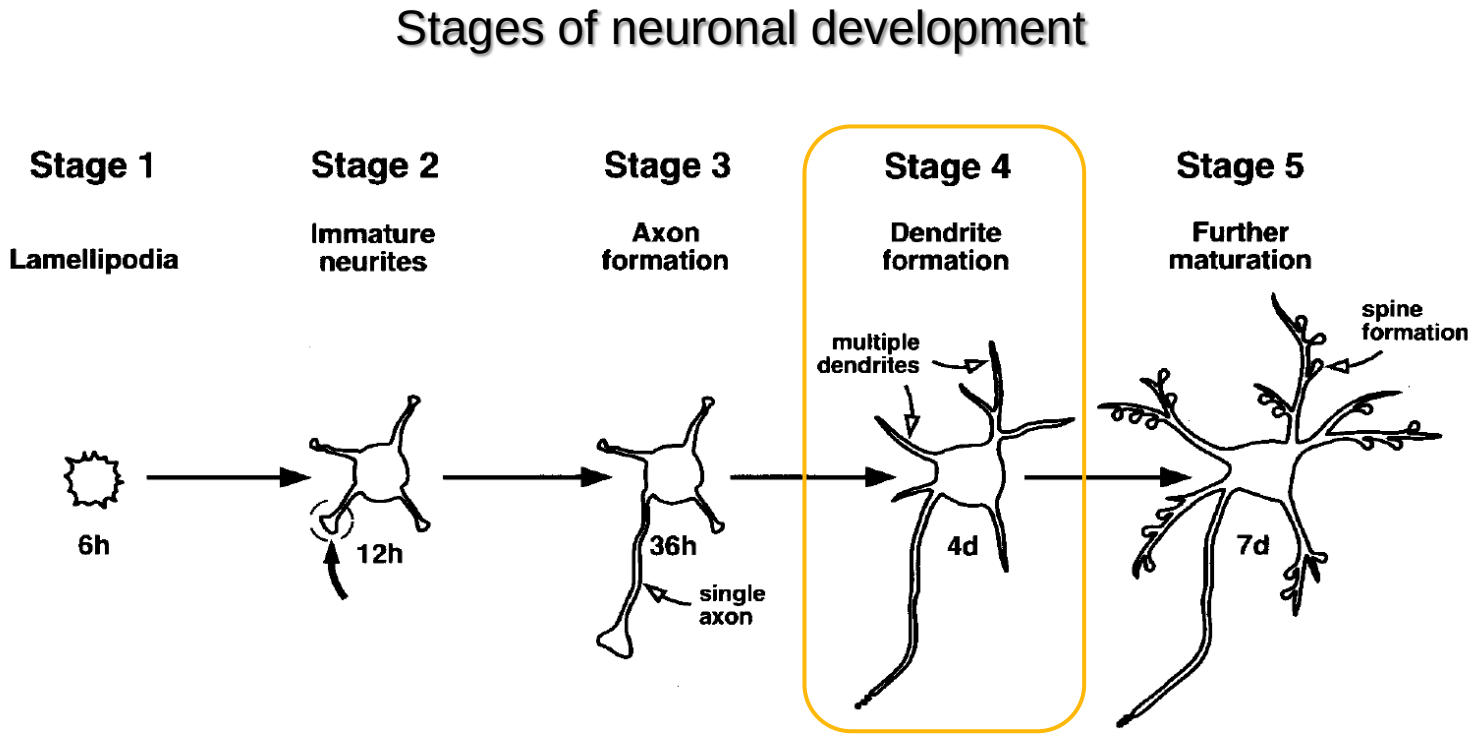
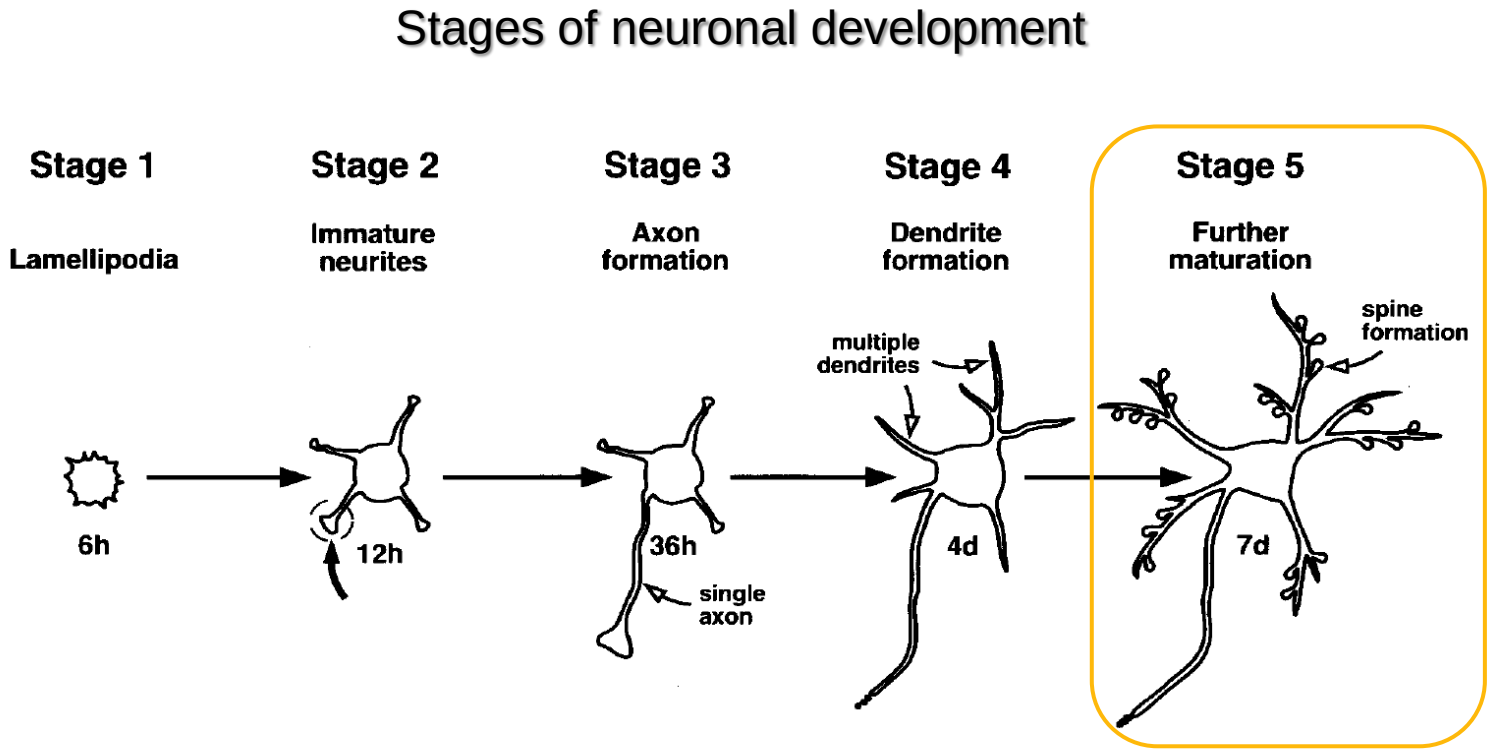


Govek et al. (2005), *Genes & Development*.

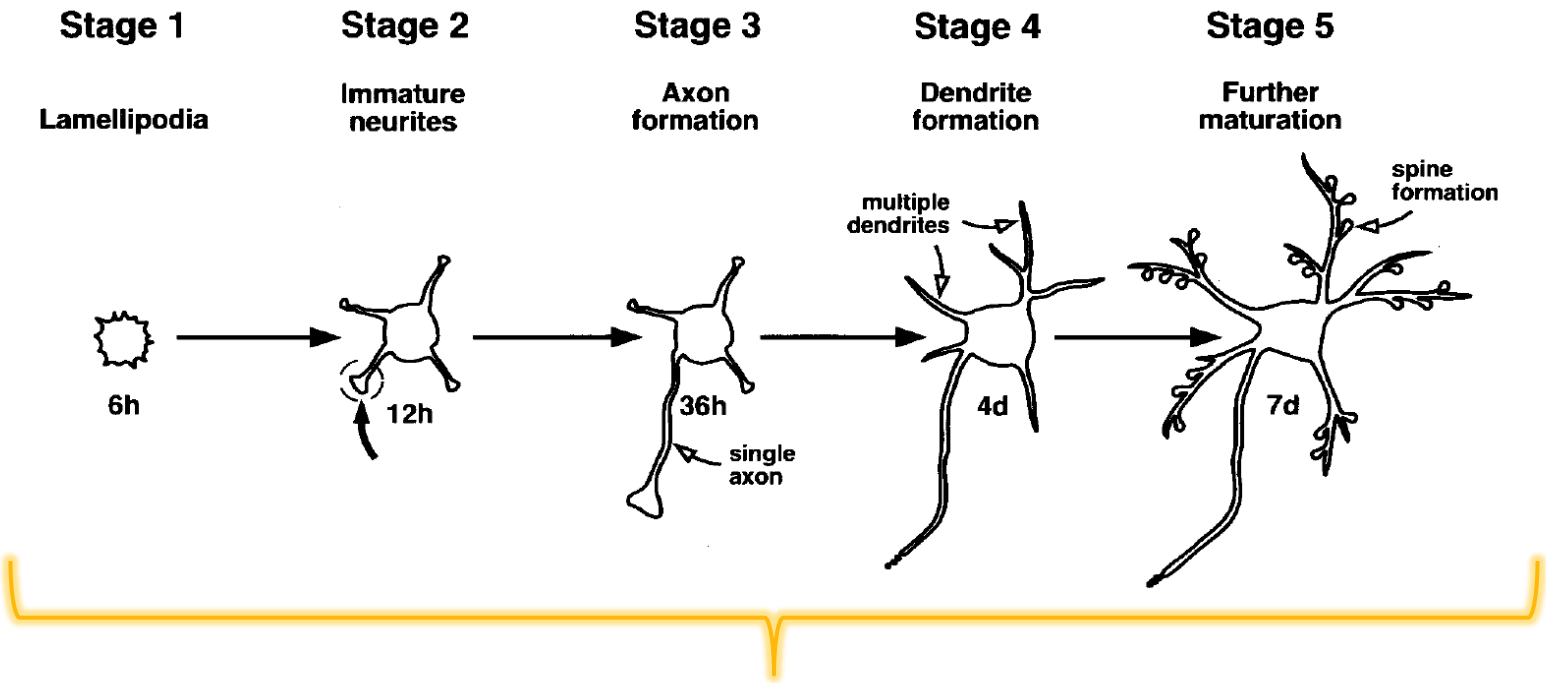
How does a neuron develop the unique and intricate architecture?



Govek et al. (2005), *Genes & Development*.

How does a neuron develop the unique and intricate architecture?

Stages of neuronal development



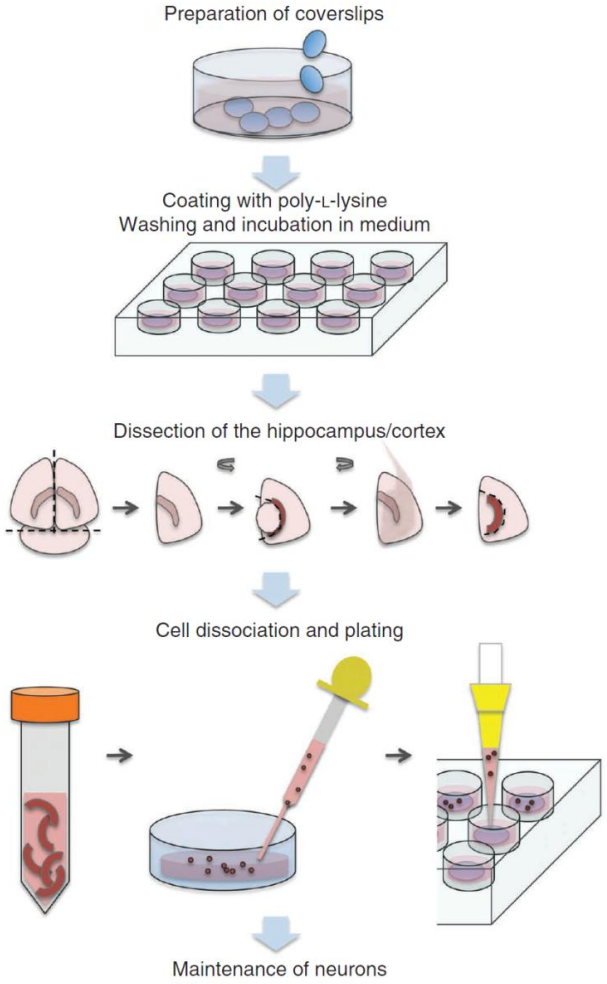
Changes in the actin cytoskeleton

Govek et al. (2005), *Genes & Development*.

Are migration defects caused by alteration in neuronal morphology and neuritogenesis during development?

Primary neuronal cultures

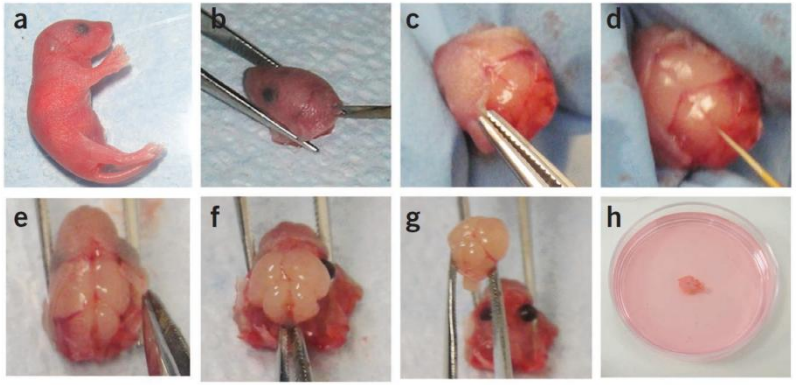
Primary neuronal cultures are powerful model systems used to study neuronal morphology and differentiation, synaptic function and neurotransmitter release.

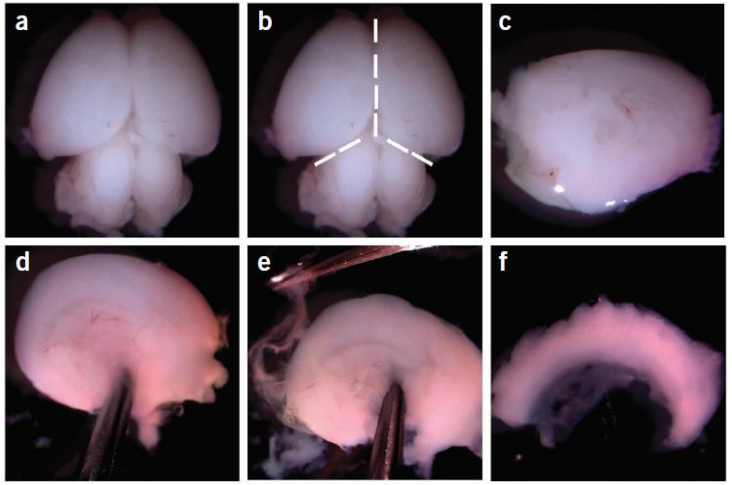


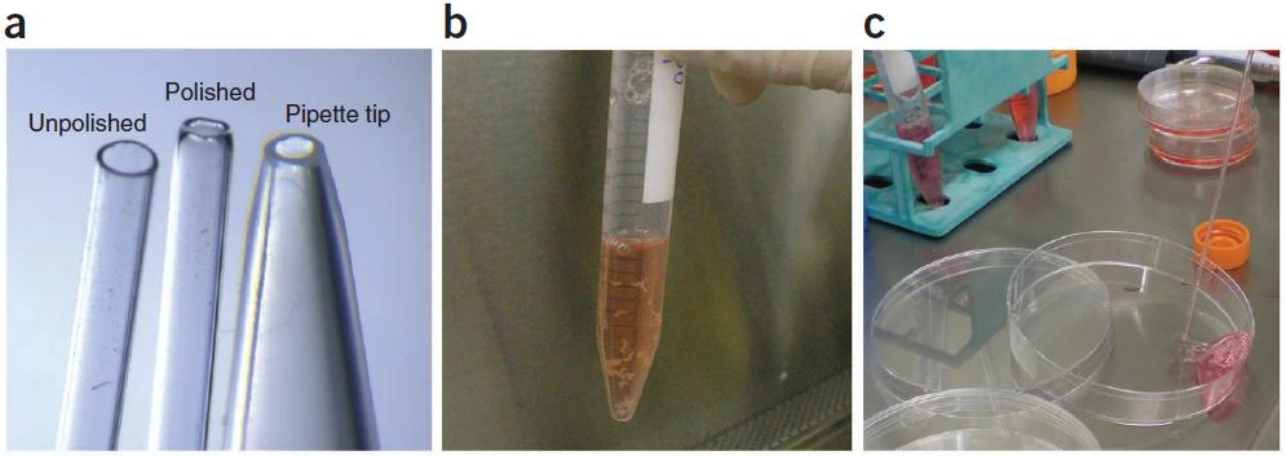
Gerard M J Beaudoin III et al. (2012), *Nature Protocols*.

Primary neuronal cultures

Dissection → dissociation → plating and maintenance

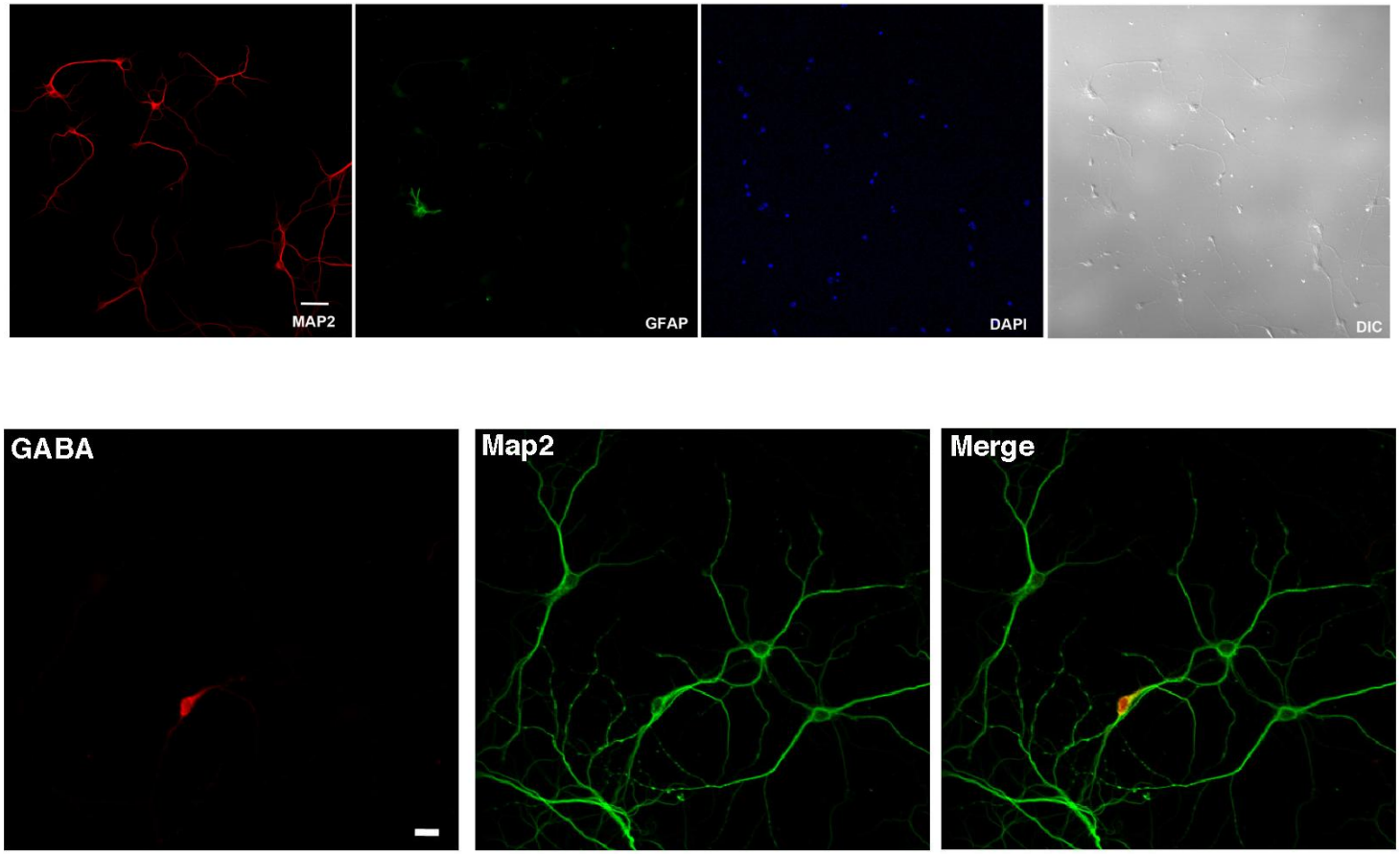






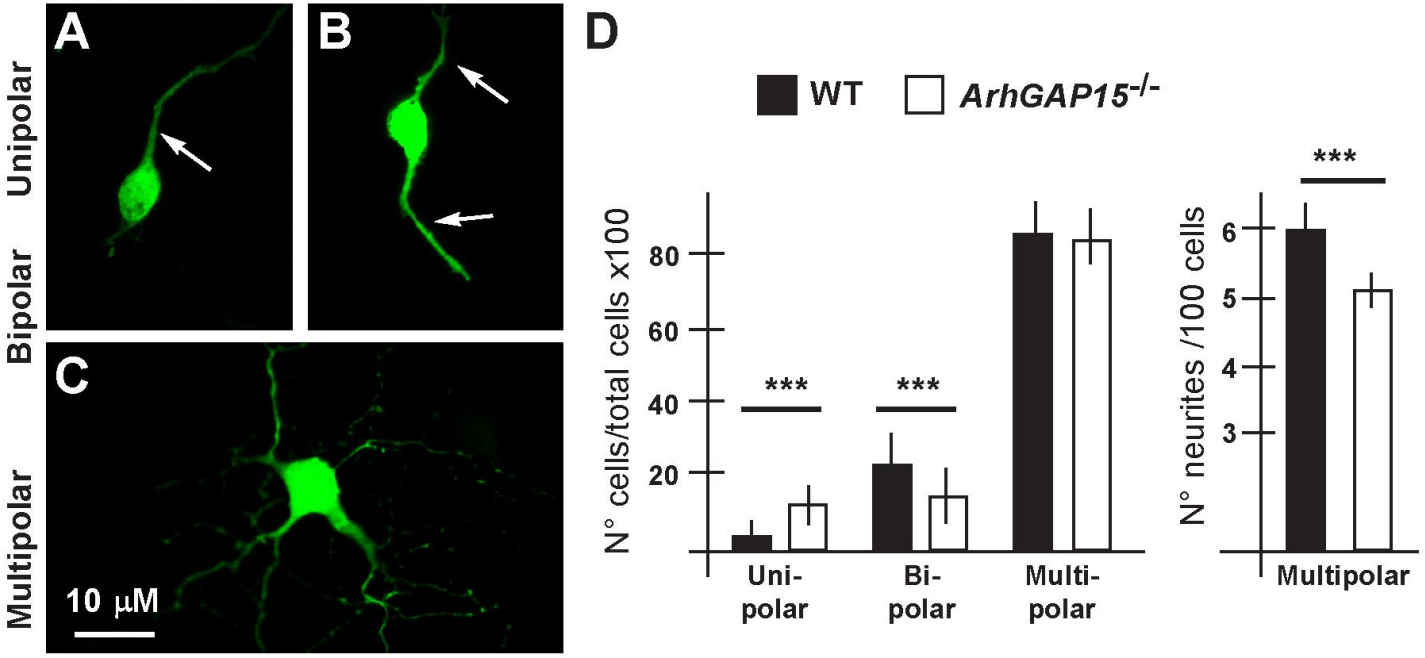
Gerard M J Beaudoin III et al. (2012), *Nature Protocols*.

Primary neuronal cultures are well characterized

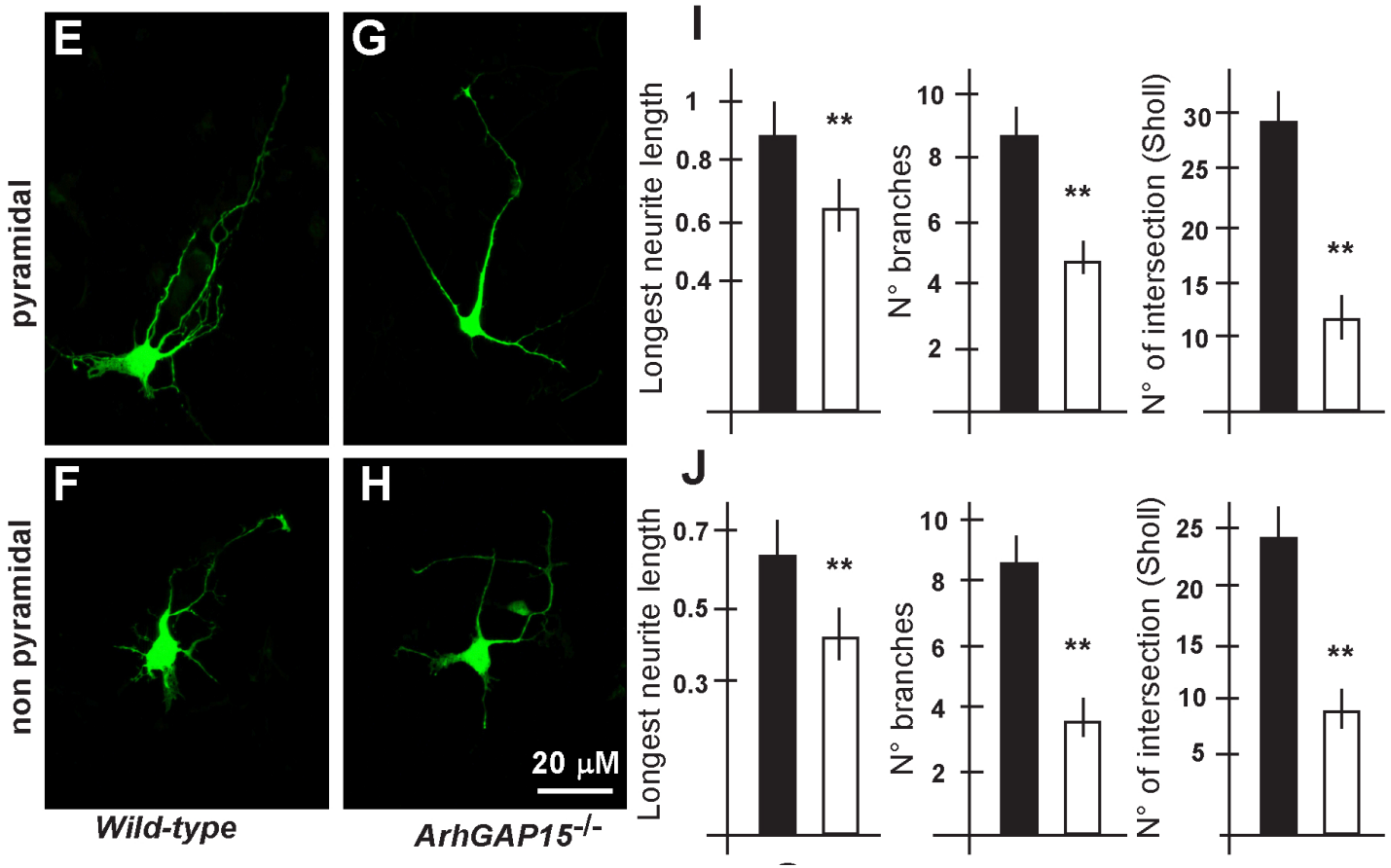


Gerard M J Beaudoin III et al. (2012), Nature Protocols.

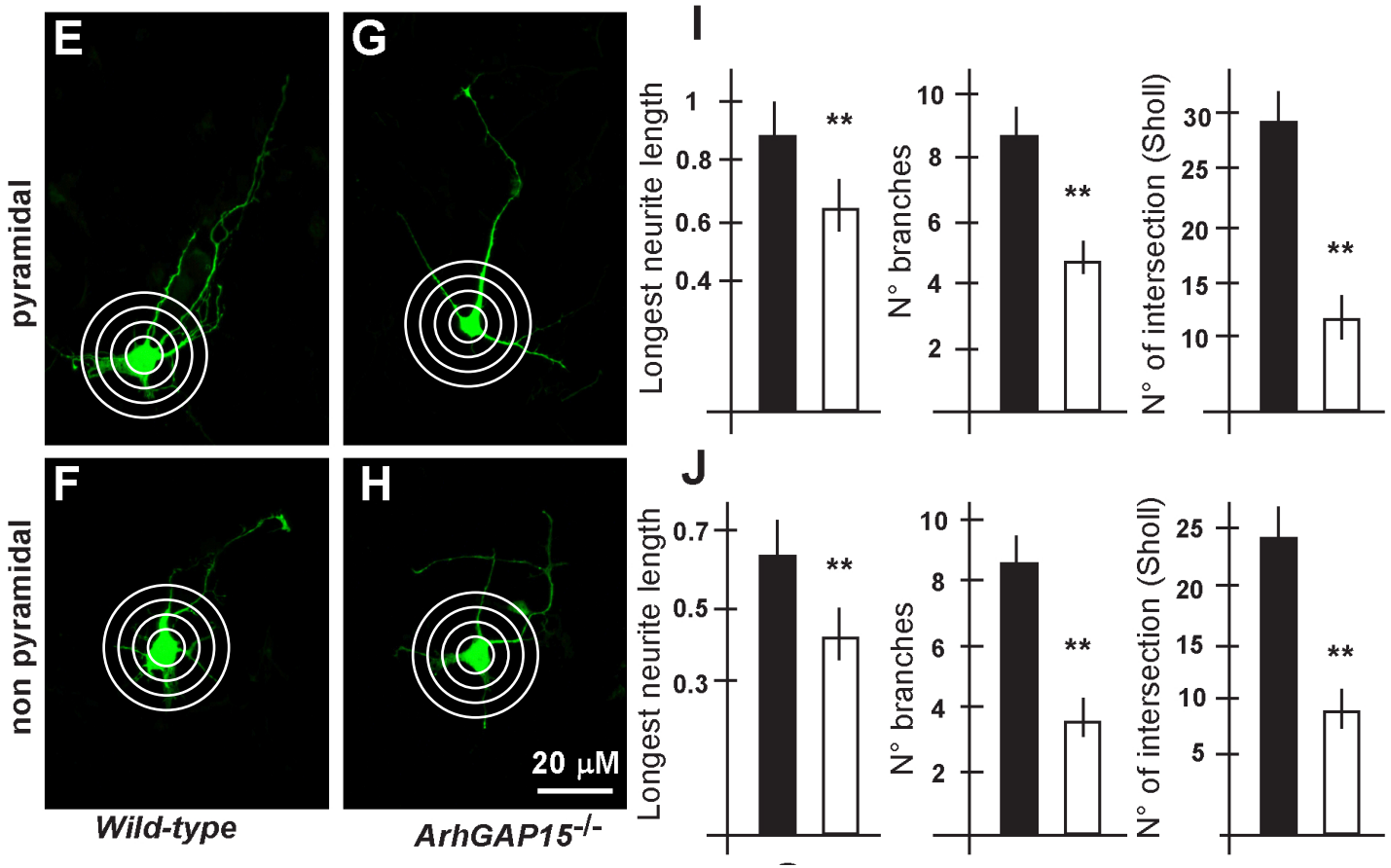
ArhGAP15 is required by immature cortical neurons to achieve a more elaborated morphology



Reduced efficiency of neurite elongation and branching of cortical neurons in the absence of *ArhGAP15*



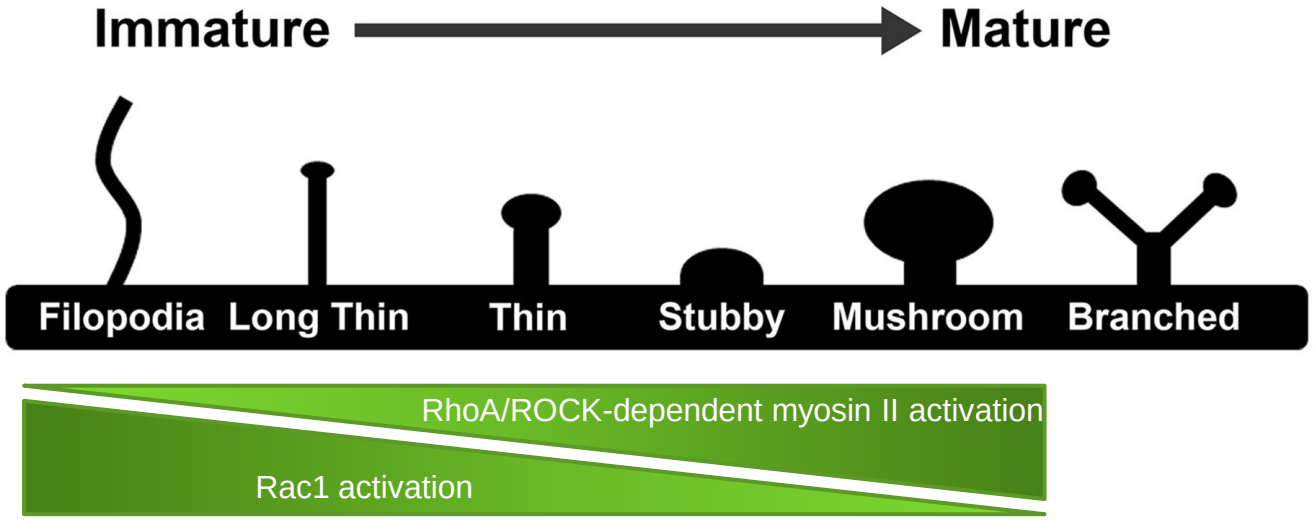
Reduced efficiency of neurite elongation and branching of cortical neurons in the absence of *ArhGAP15*



Do the loss of ArhGAP15 affect spinogenesis?

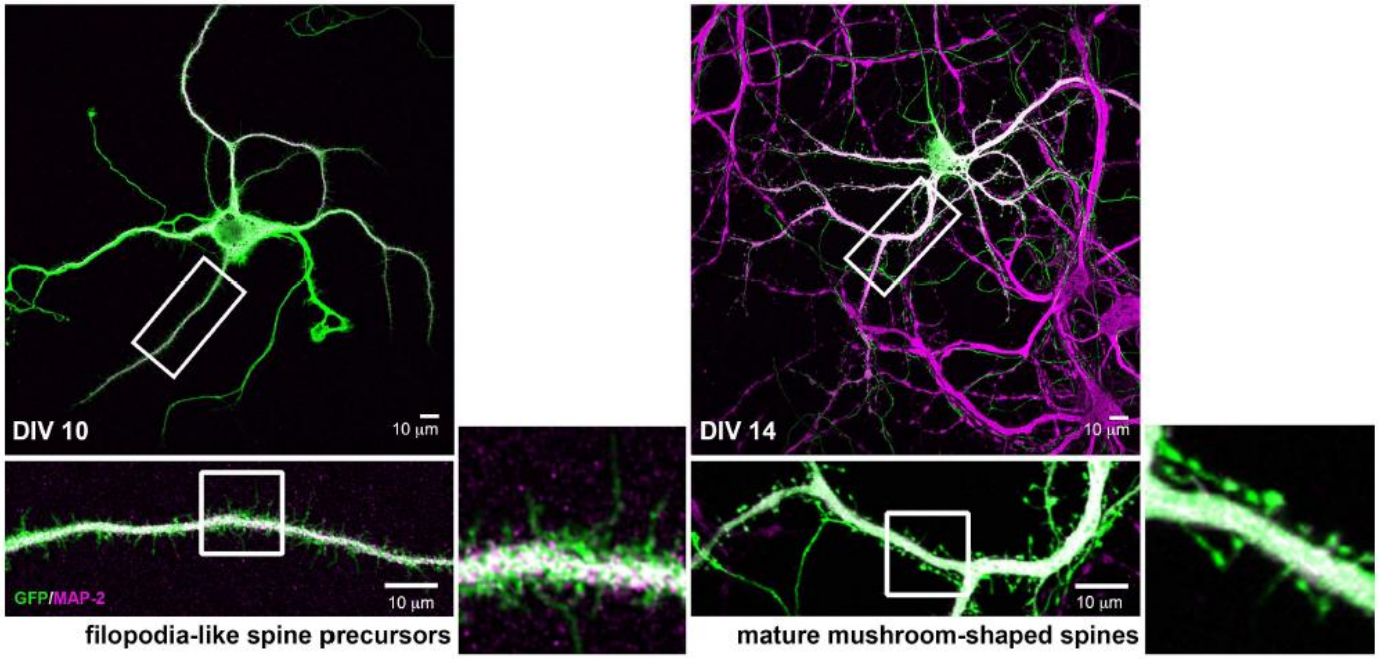
RhoGTPase regulators orchestrate distinct stages of synaptic development

Rac1 promotes the formation of filopodia-like spine precursors that subsequently mature through RhoA/ROCK-dependent myosin II activation into polarized mushroom-shape spines.



Risher et al. (2014), *Cell BiologyNeuroscience*

How RhoGTPase regulators function throughout synaptic development?



Martin-Vilchez et al. (2017), *PLOS ONE*.

How RhoGTPase regulators function throughout synaptic development?

	RhoGTPase Target	Proteome ID	Known Synaptic Function(s)	Neuronal Disease Association	Chromosome Location	Autism-Associated Copy Number Variants*
GEFs						
FRABIN (FDG4)	Cdc42 [53]	[28]	• Unknown	Mutated in Charcot-Marie Tooth [37,38]	12p11.21 (32,655,040–32,798,983)	• Deletion in Autism with Scoliosis Case [54] • 6 Duplications [55] • 4 Duplications and 1 Deletion [56] • 1 Reported Duplication in each publication [57,58] • 1 Deletion [54]
ARHGEF9 (COLLYBISTIN)	Cdc42 [59]	[31]	• Promotes inhibitory synapse formation through gephyrin clustering [26,27]	X-linked Mental Retardation [60,61]	Xq11.1 (62,854,848–63,005,426)	• 2 Duplications and 1 Deletion [56]
ARHGEF7 (β-PIX)	Rac [62]	[28,31]	• Promotes synaptic vesicle recruitment [63] • Increases synaptic Rac activity, resulting in increased dendritic protrusions [5,23]	Mutations in β-PIX isoform (on X Chromosome) result in non-syndromic mental retardation [64]	13q34 (111,767,624–111,958,081)	• 24 Deletions and 1 Duplication [65] • 16 Deletions and 16 Duplications [56] • 1 Deletion and 1 Duplication [55] • 1 Reported Deletion in each publication [57,58,66] • 1 Duplication and 1 Unspecified CNV Reported [67] • 1 Duplication [19]
VAV2	Rac (can also regulate Cdc42 and RhoA *in vitro*) [68]	[31]	• Promotes dendritic development [69,70] • Activated in Response to BDNF and increases spine head size [71]	None Reported	9q34.1 (136,627,016–136,857,726)	• 12 Duplications [56] • 1 Reported Duplication in each publication [72,73] • 1 Deletion [74] • 1 Deletion [19]

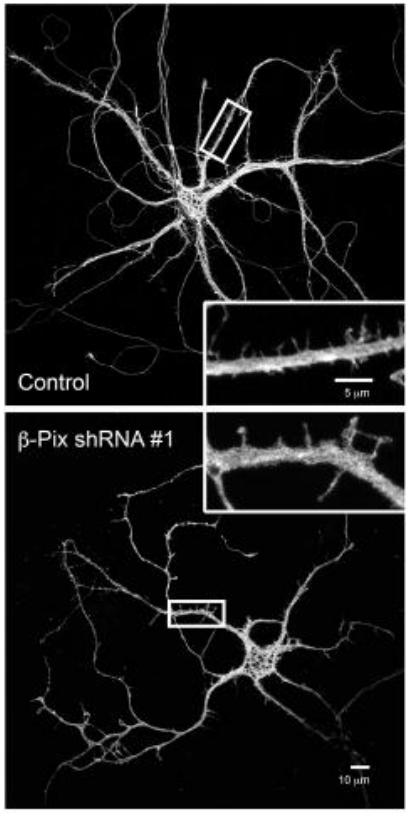
Martin-Vilchez et al. (2017), *PLOS ONE*.

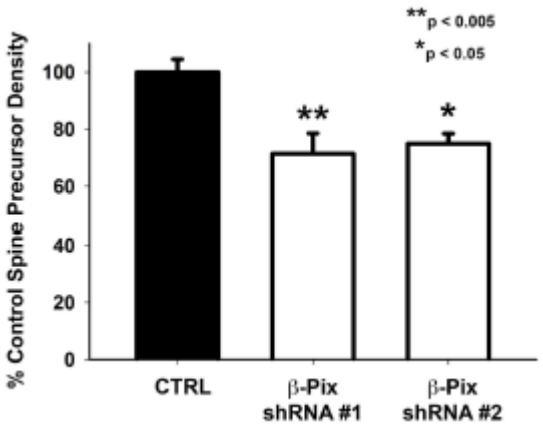
How RhoGTPase regulators function throughout synaptic development?

	RhoGTPase Target	Proteome ID	Known Synaptic Function(s)	Neuronal Disease Association	Chromosome Location	Autism-Associated Copy Number Variants *
GAPs						
ARHGAP23	• unknown	[32]	• Unknown	None Reported	17q12 (36,584,719–36,668,627)	• 1 Reported Duplication in each publication [56,75] • 1 Duplication and 1 Deletion [65]
OLIGOPHRENIN-1	RhoA	N/A	• Regulates activity-dependent strengthening of excitatory synapses through interaction with Homer [76] • Regulates spine length and maturation [15,18]	X-linked Mental Retardation [16]	Xq12 (67262186–67653299	• 36 Duplications and 11 Deletions [56] • 3 Duplications [77] • 1 Reported Duplication in each publication [78–80] • 1 Mosaic Duplication [74] • 1 Reported Deletion in each publication [57,81]

Martin-Vilchez et al. (2017), *PLOS ONE*.

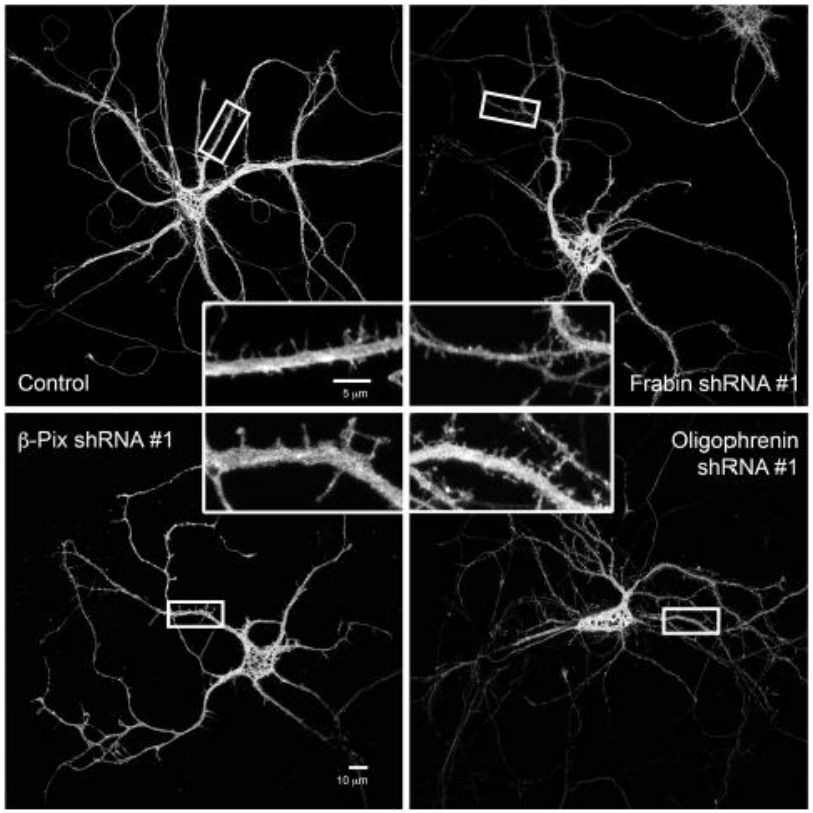
The Rac1 GEF, β-PIX, drives spine precursor formation

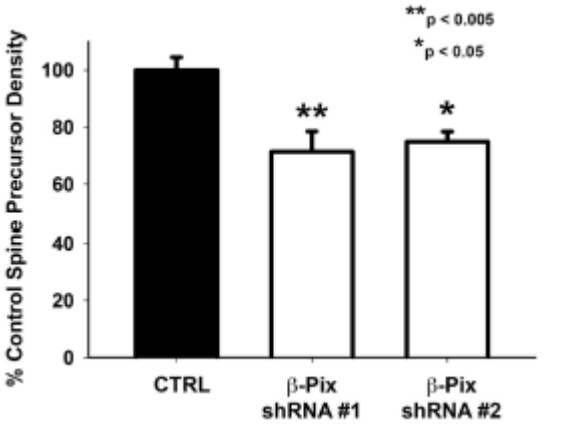


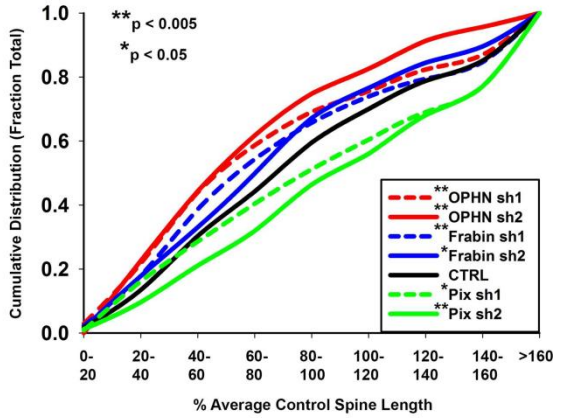


Martin-Vilchez et al. (2017), *PLOS ONE*.

Balanced RhoA and Cdc42 activities regulate spine precursor elongation



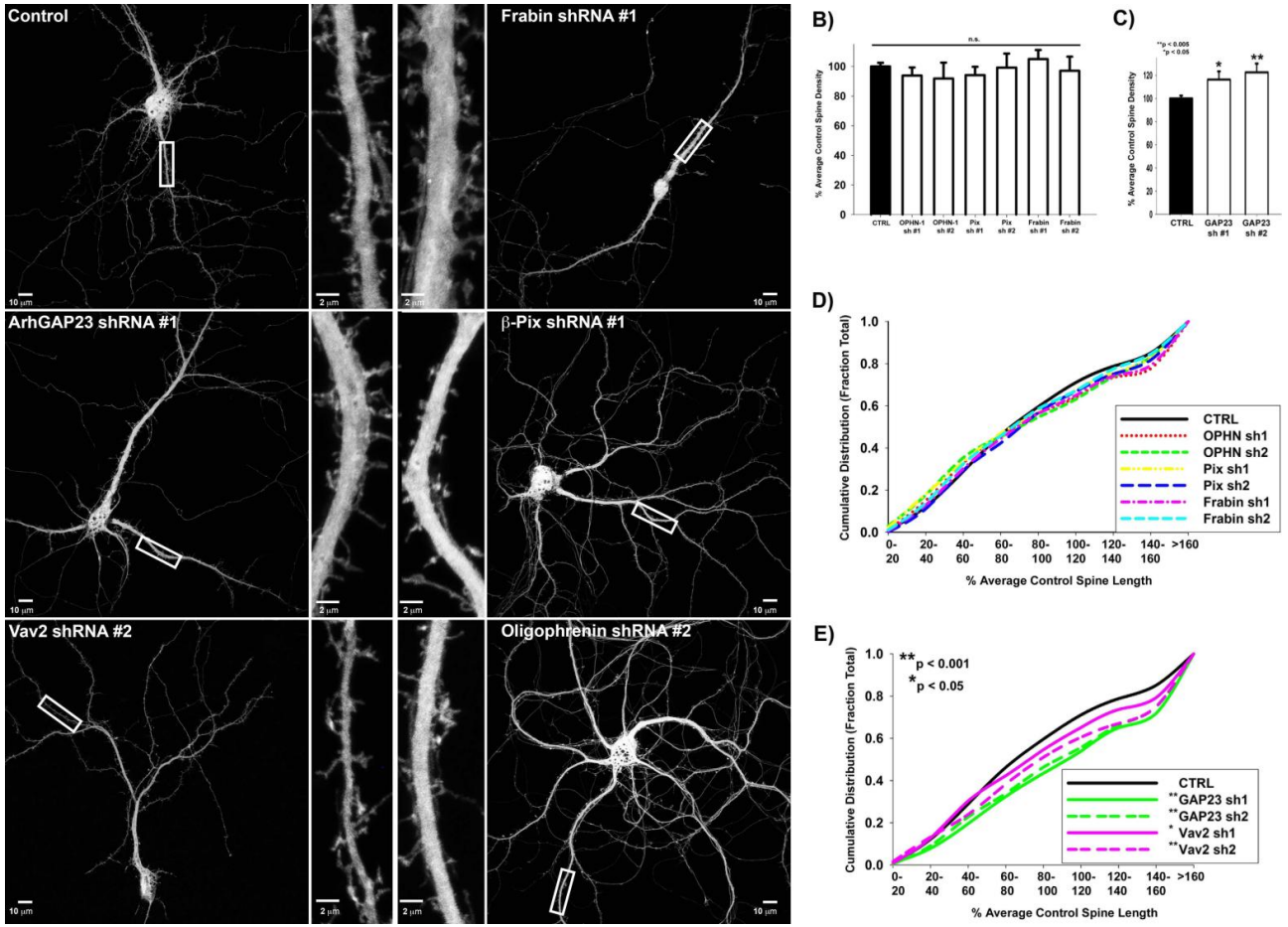




Martin-Vilchez et al. (2017), *PLOS ONE*.

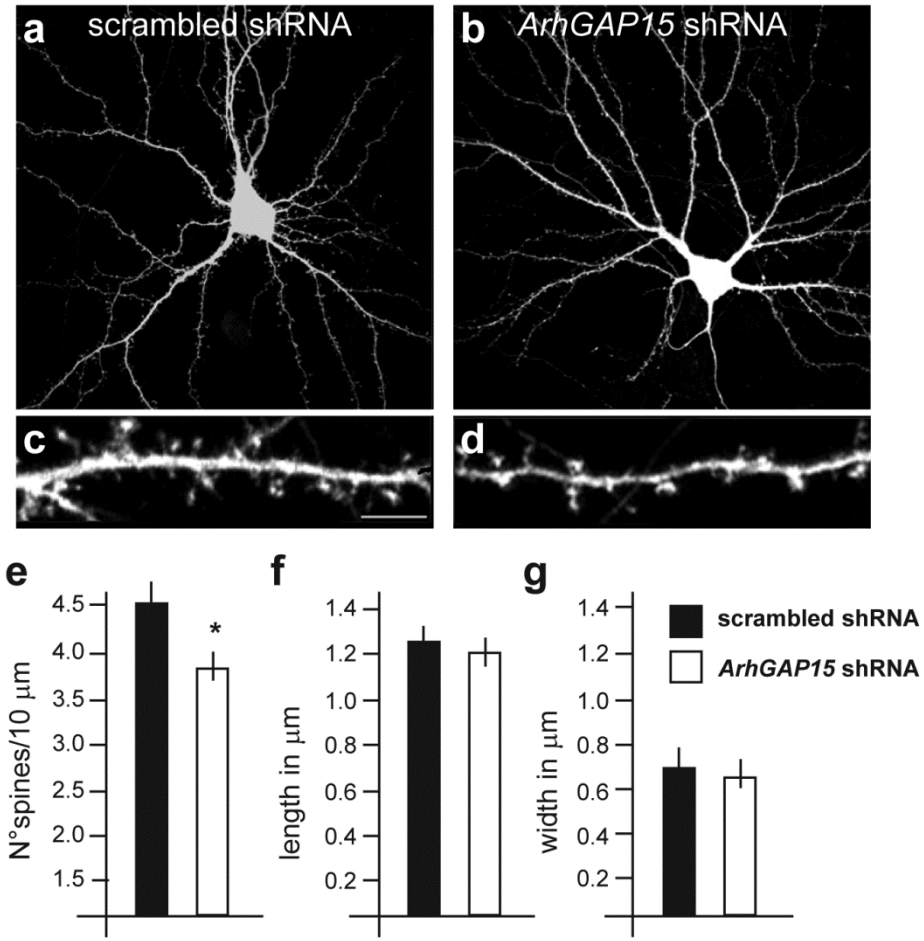
ArhGAP23 promotes spine maturation

While actin polymerization drives spine precursor formation, RhoA/ROCK-mediated myosin II activation is necessary for spine maturation into a polarized mushroom-shape.



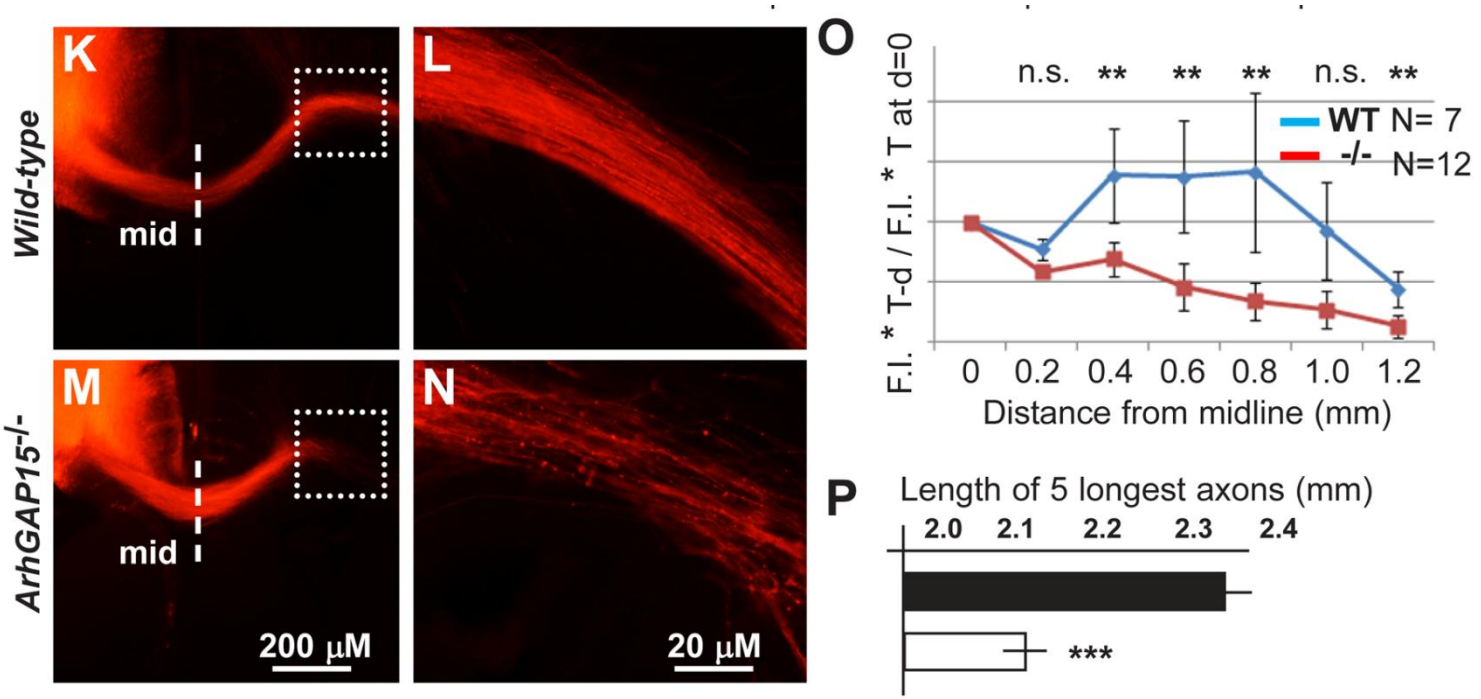
Martin-Vilchez et al. (2017), *PLOS ONE*.

Reduced spine density upon downmodulation of *ArhGAP15*



Zamboni et al. (2016), *Scientific Reports*.

Cortical callosal axons are retarded in *ArhGAP15*$^{-/-}$ neonatal brain



Are reduced neuritogenesis and axonogenesis linked to altered actin dynamic at the growth cone?

Actin remodeling in neurons

Cell migration

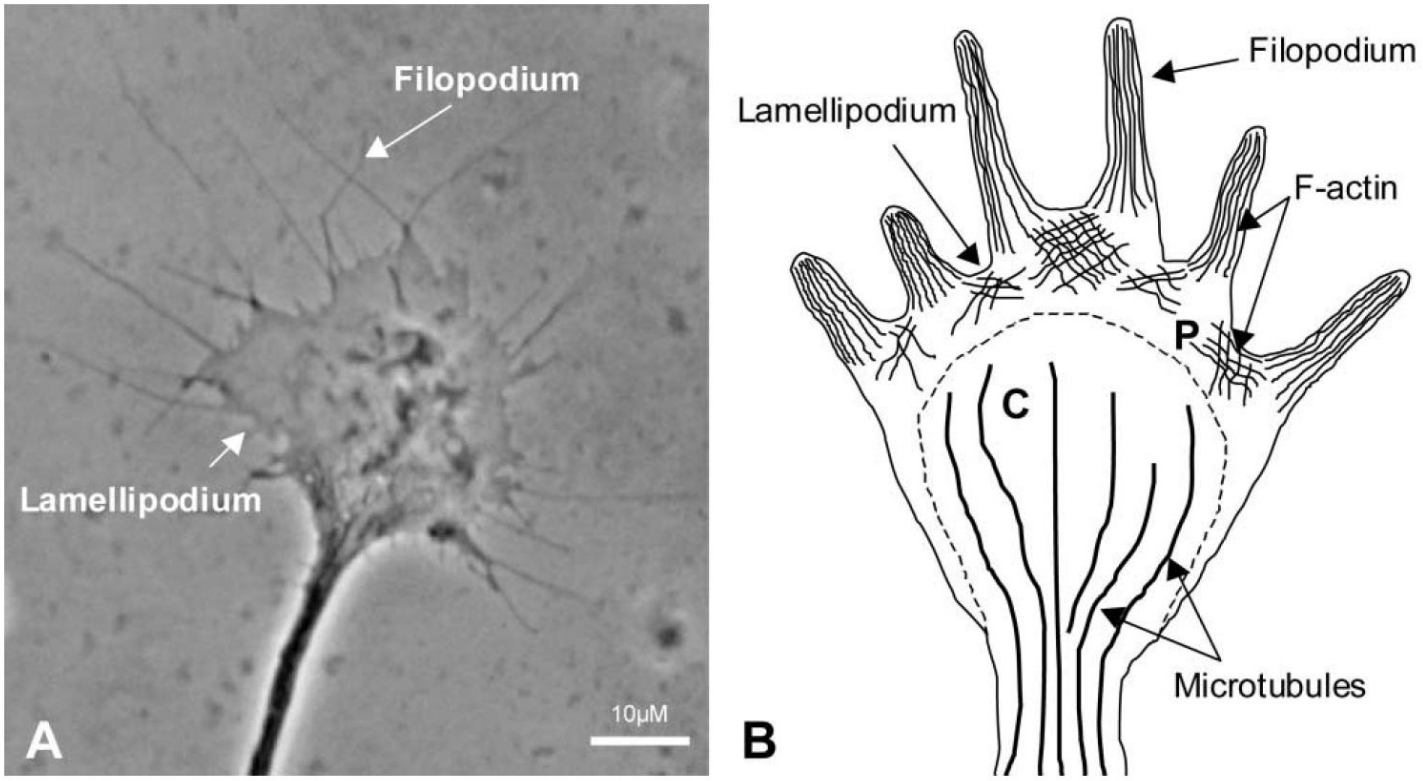
Leading edge

Neurite elongation

Growth cone

The neuronal growth cone: the structure

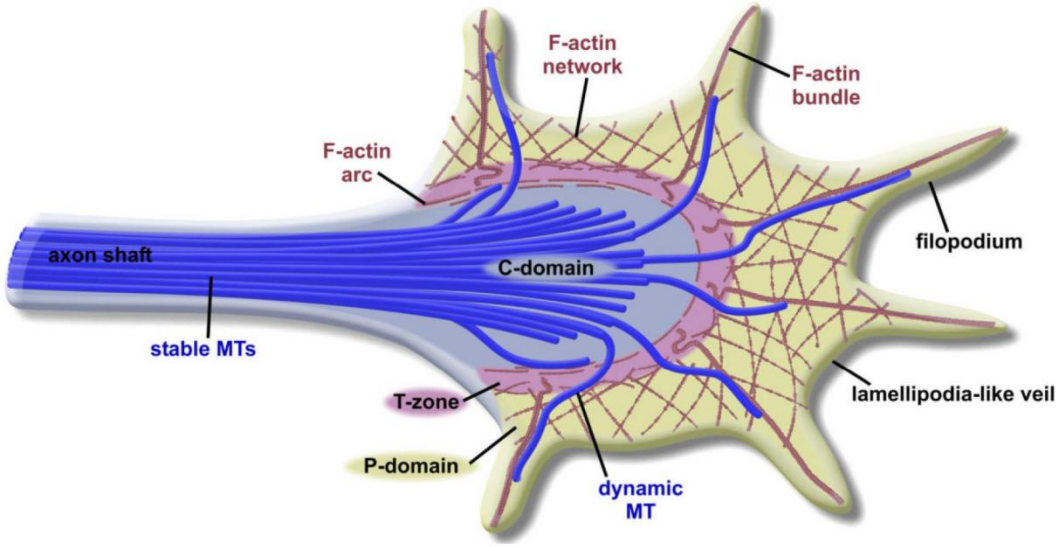
The **filopodia** contain bundles of actin filaments (F-actin).
The **lamellipodia** are flat regions of dense actin meshwork.



Ravine A. Gungabissoon and James R. Bamburg (2003), *The Journal of Histochemistry & Cytochemistry*.

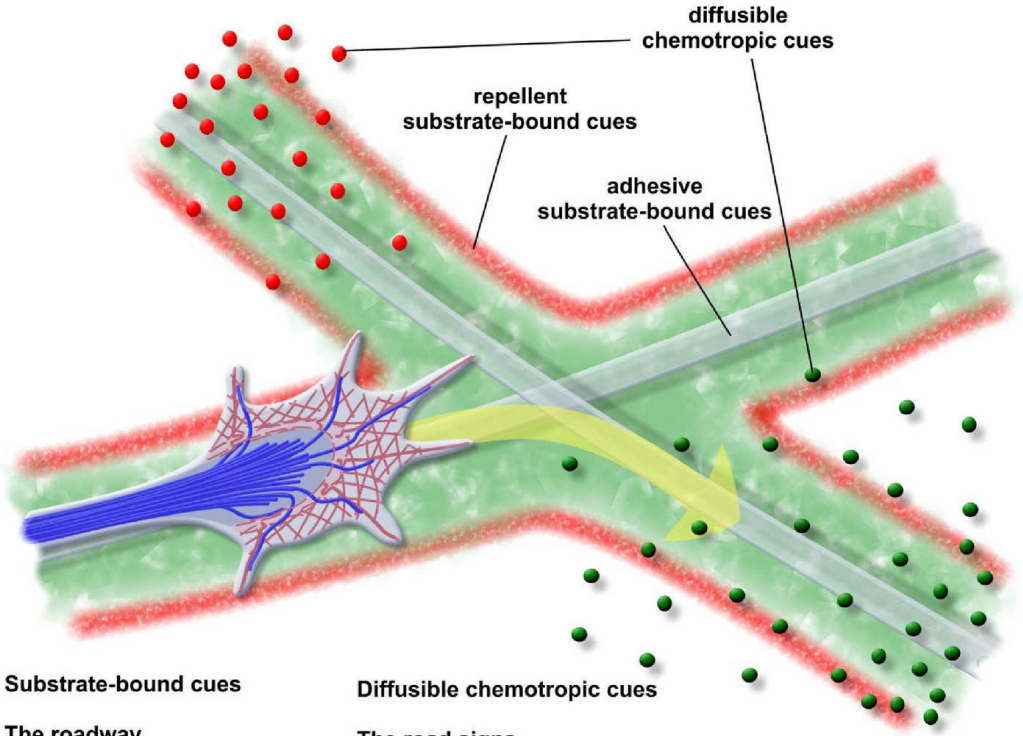
The neuronal growth cone: the structure

The **peripheral domain** is the region surrounding the outer edge of the growth cone, and it is composed of an actin-based cytoskeleton.
The **central domain** is located in the center of the growth cone, and it is composed of a microtubule-based cytoskeleton.
The **transitional domain** is located in the thin band between the central and peripheral domains.



Lowery and Van Vactor (2009*), Nat Rev Mol Cell Biol*.

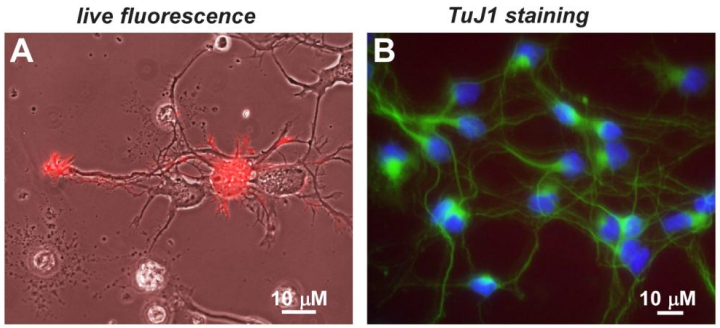
Directions for the trip



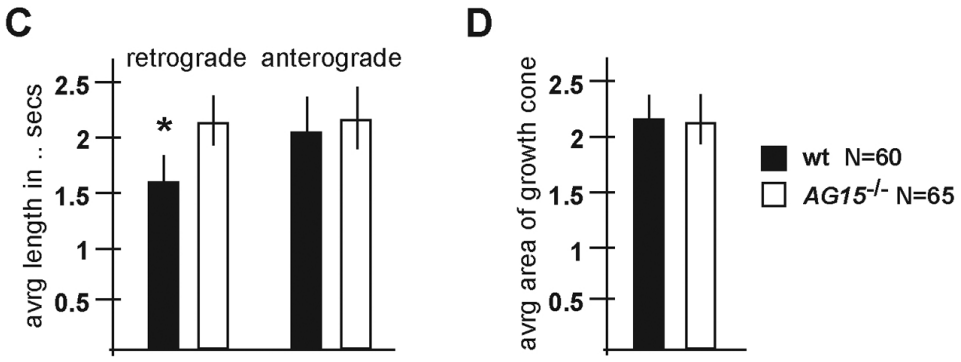
Lowery and Van Vactor (2009*), Nat Rev Mol Cell Biol*.

Analyses of actin cytoskeleton dynamics

Live imaging of the actin cytoskeleton is crucial for the study of many biological processes.

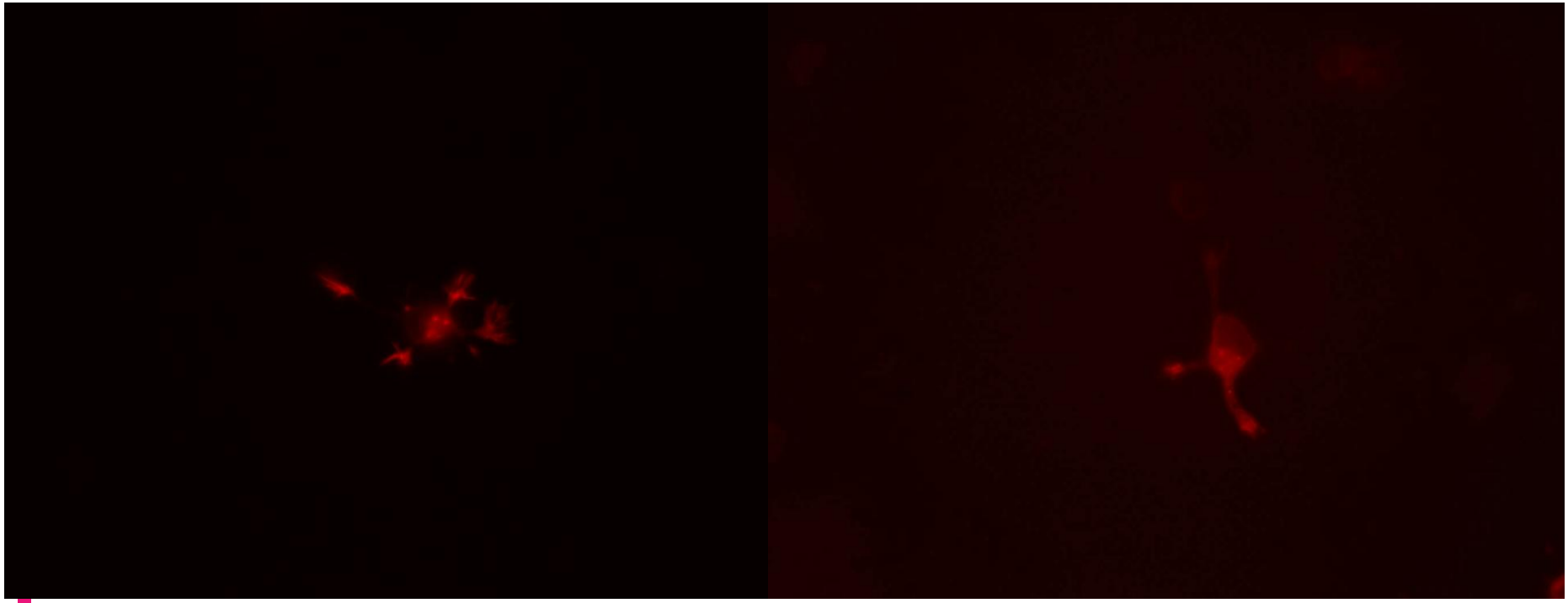


Retrograde actin flow is increased in the absence of *ArhGAP15*



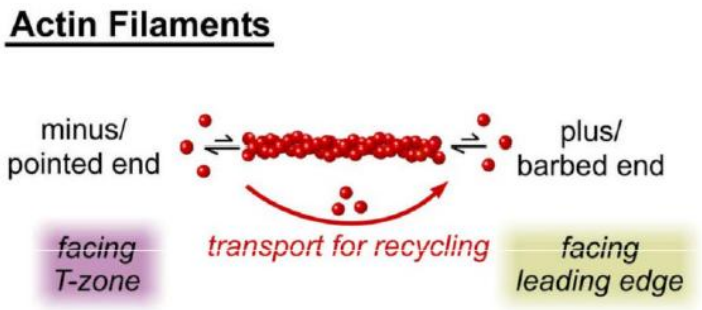
Retrograde actin flow is increased in the absence of *ArhGAP15*

Wild-type **ArhGAP15 KO**



Cytoskeletal dynamics

Growth cone motility and protrusion of the leading edge membrane depend on the dynamic properties of actin.
Actin filaments are polarized polymers composed of actin monomers and their formation, stability and destruction are carefully regulated at every stage.
Changes in equilibria of polymerization dynamics depend on whether ATP or ADP is associated with actin. ATP-actin is usually added to the **`plus' (or barbed) end**.
ATP hydrolyzes to form ADP-actin, and ADP-actin disassembled at the **`minus' (or pointed) end**.

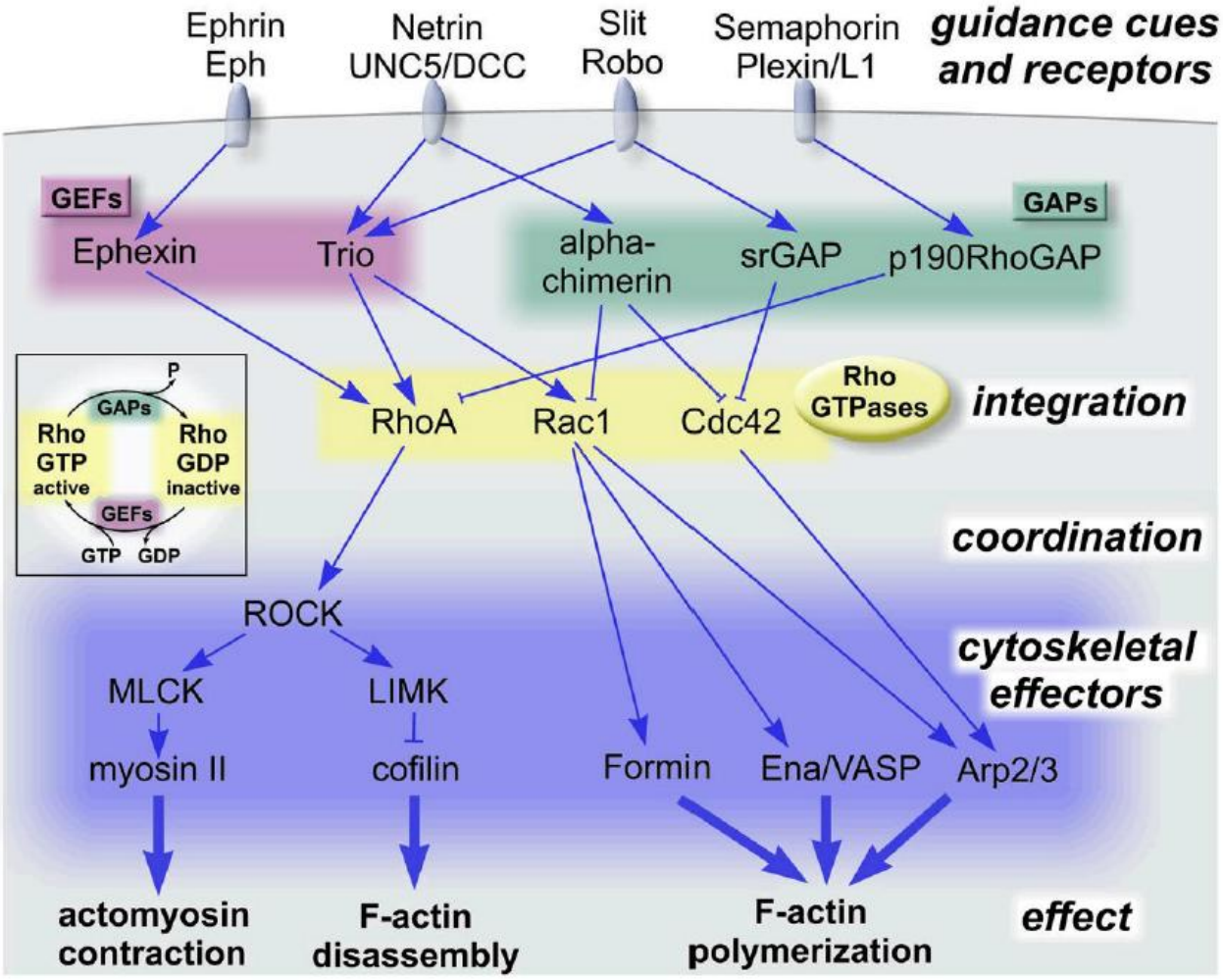


Types of Actin-binding proteins

- monomer binding proteins that either promote growth by adding to barbed end *(profilin)* or inhibit growth by sequestering *(beta-thymosin)*
- F-actin capping proteins that block growth *(neuromodulin)* or block disassembly *(Ena/VASP)*
- F-actin severing proteins *(ADF/cofilin)*
- F-actin stabilization proteins *(tropomyosin)*

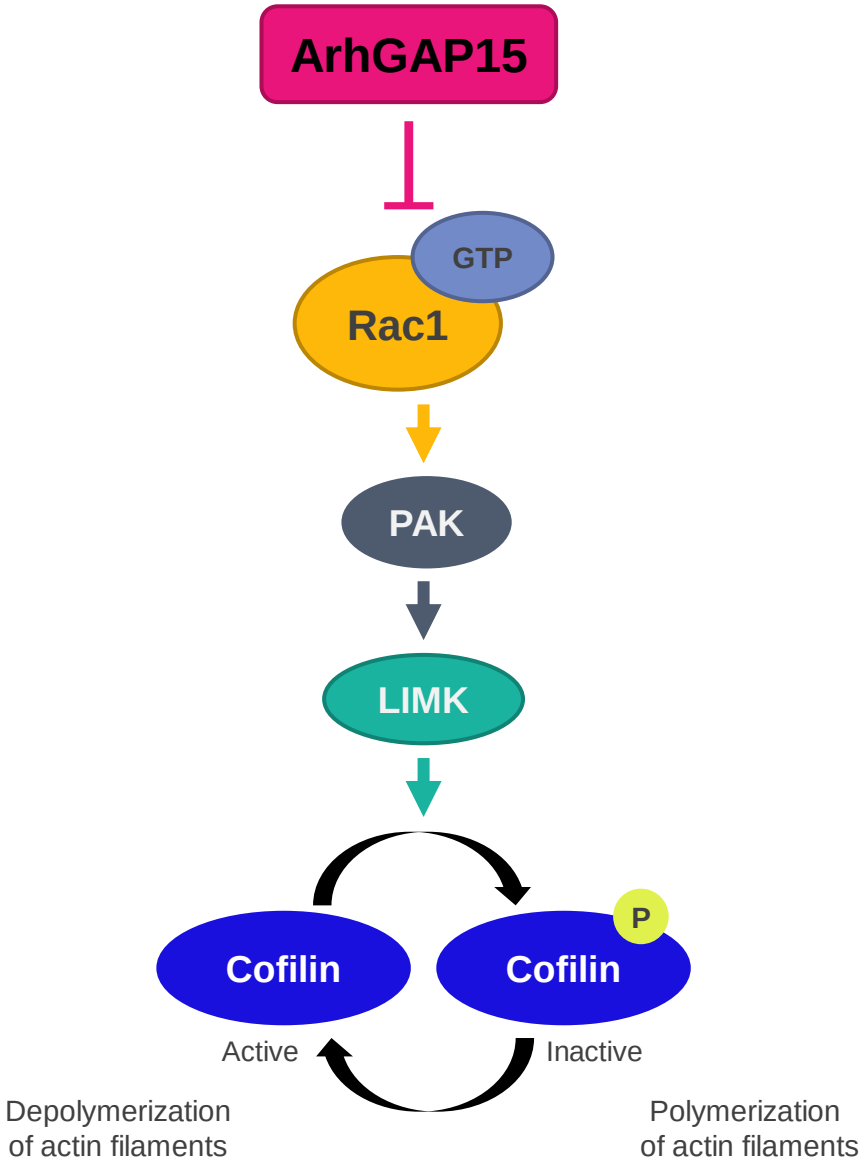
Lowery and Van Vactor (2009*), Nat Rev Mol Cell Biol*.

The growth cone as a `navigator'

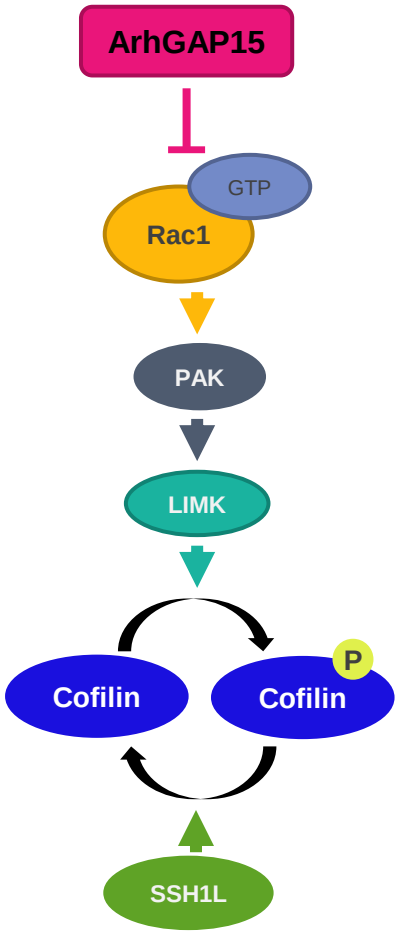


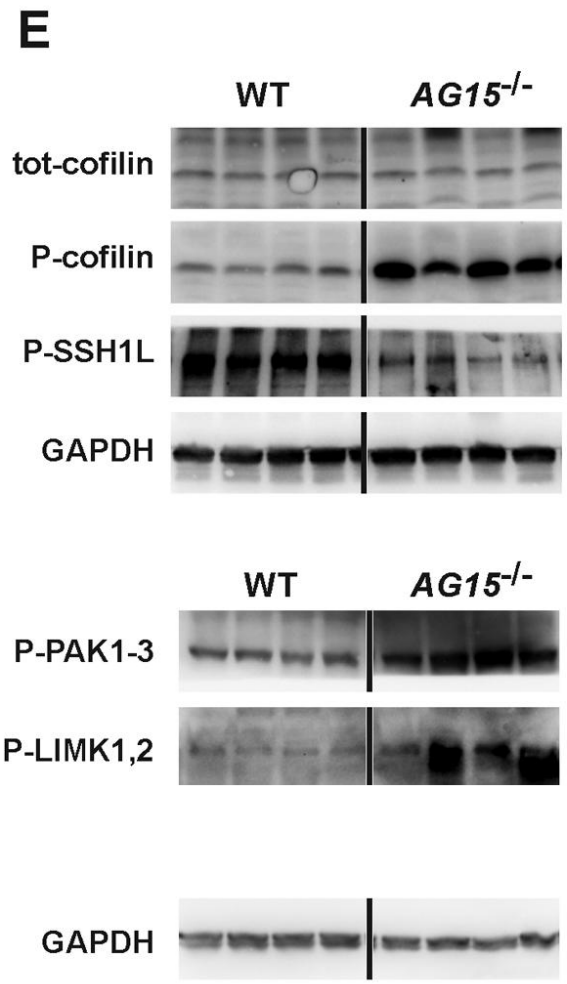
Lowery and Van Vactor (2009*), Nat Rev Mol Cell Biol*.

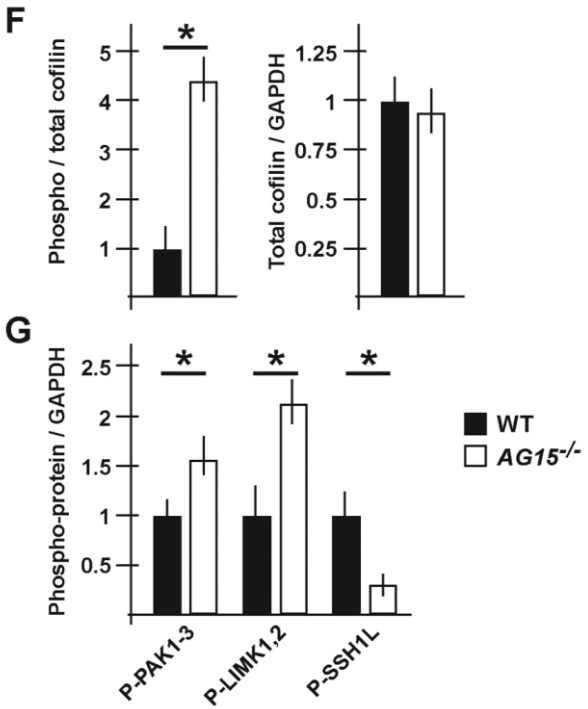
Regulation of actin dynamics signaling pathway



Loss of *ArhGAP15* results in increased cofilin phosphorylation via the PAK-LIMK pathway







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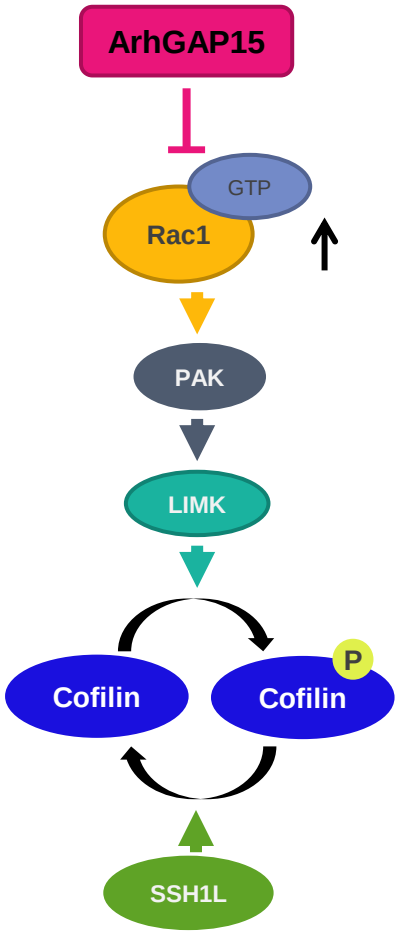

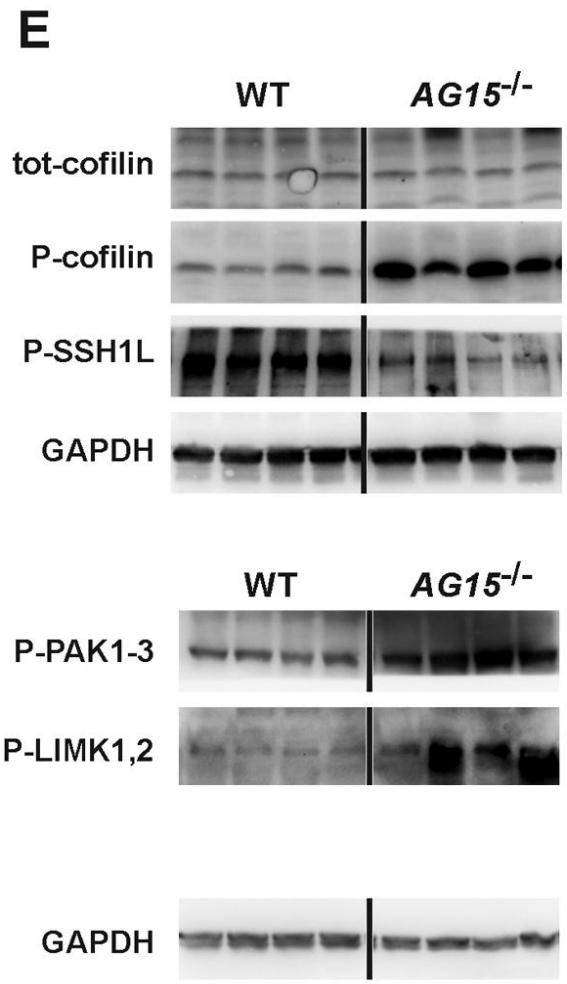

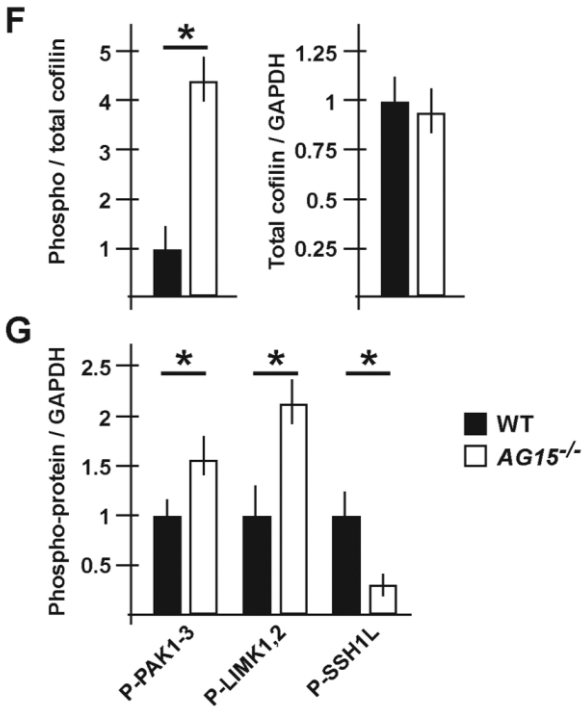

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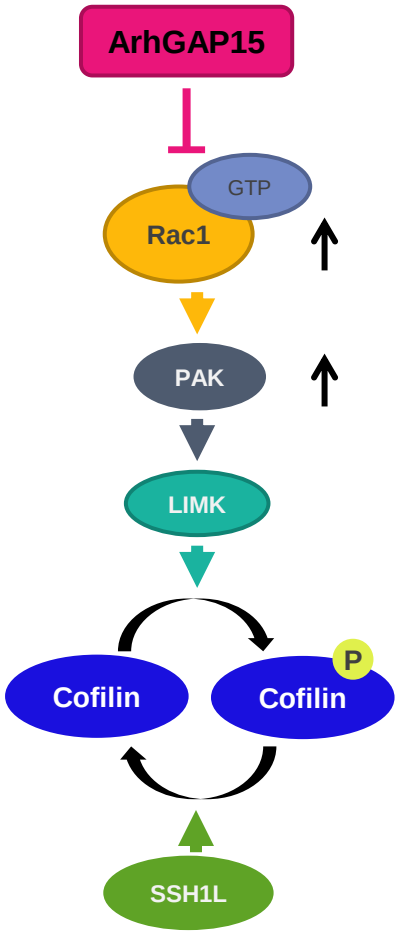

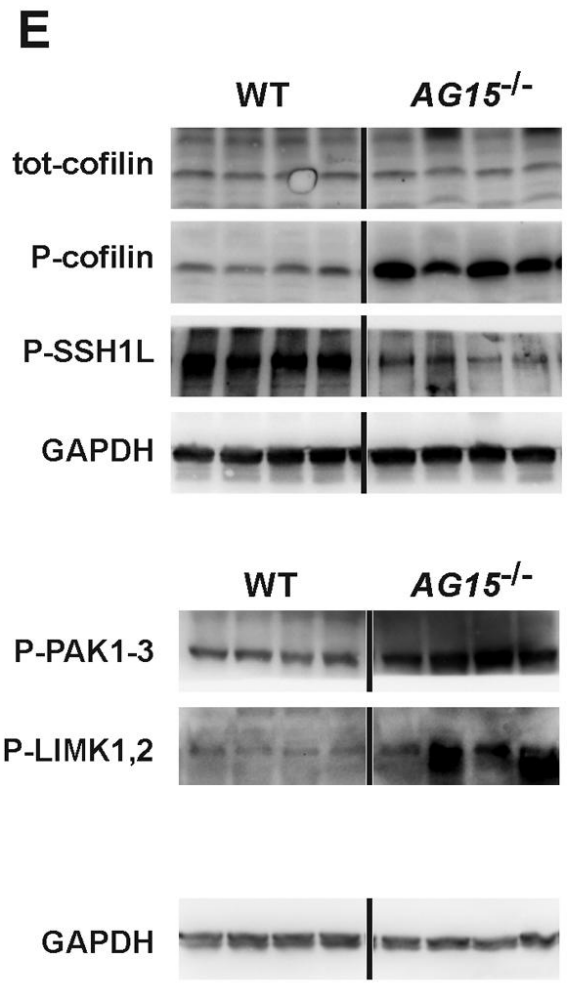

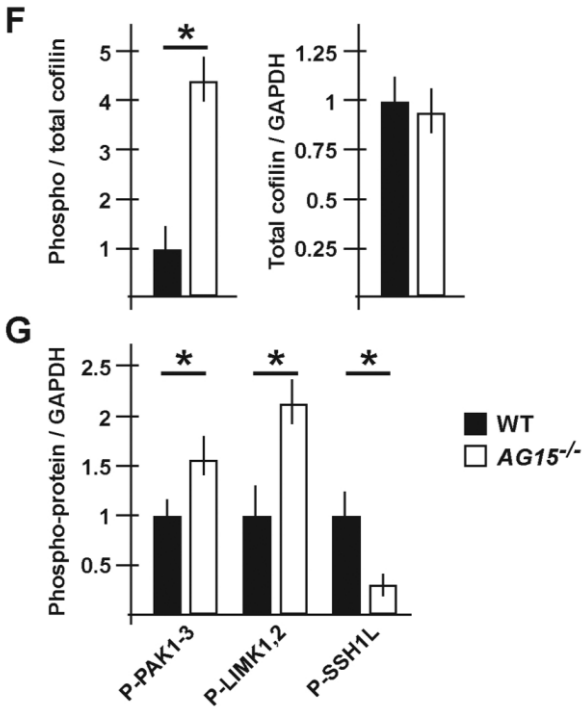

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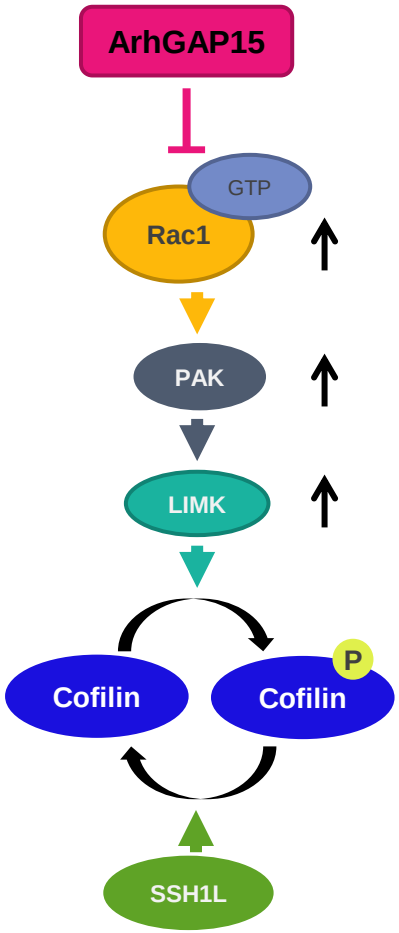

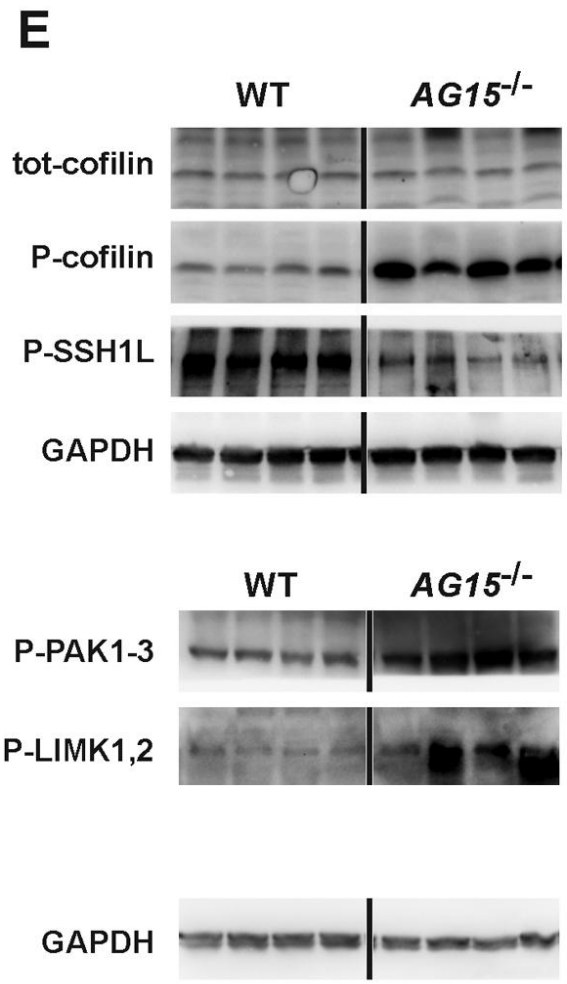

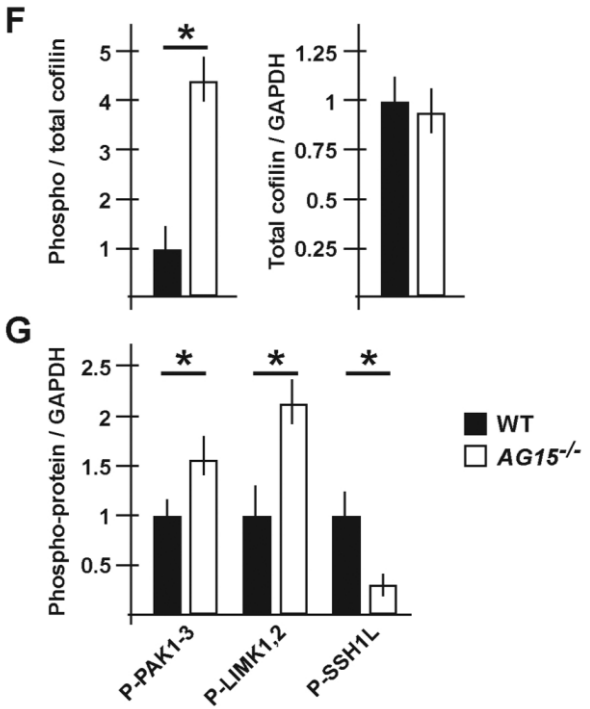

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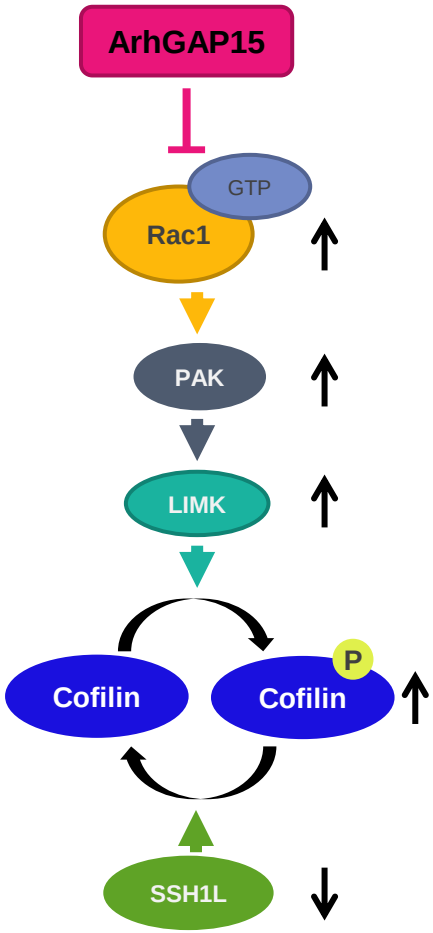

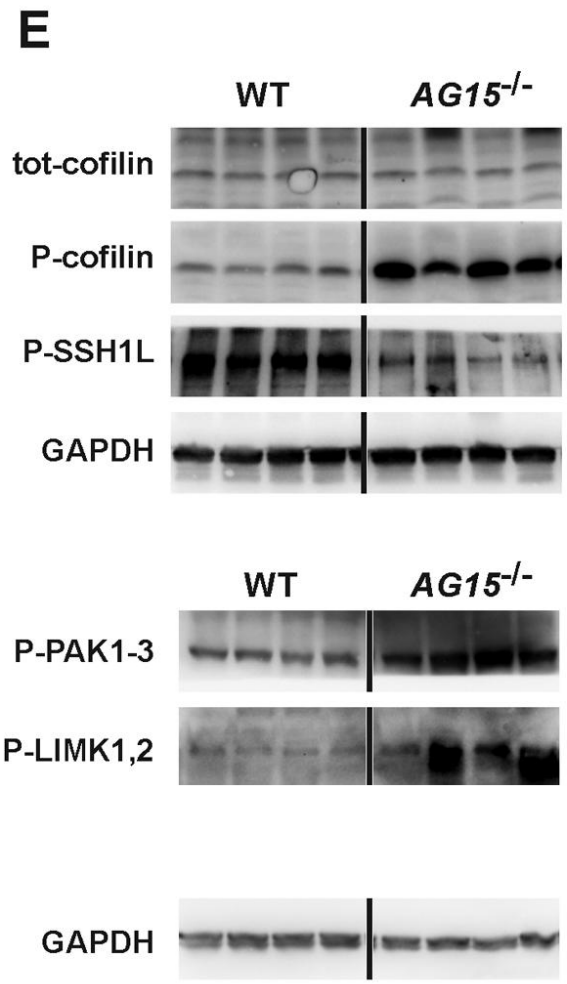

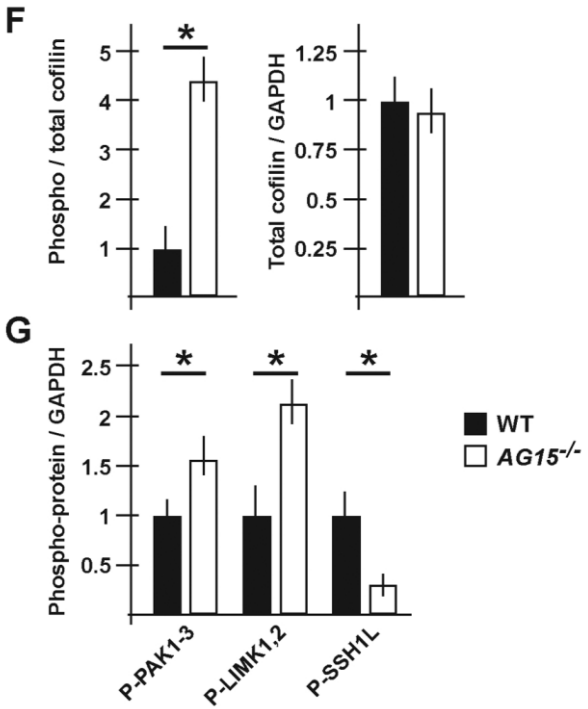

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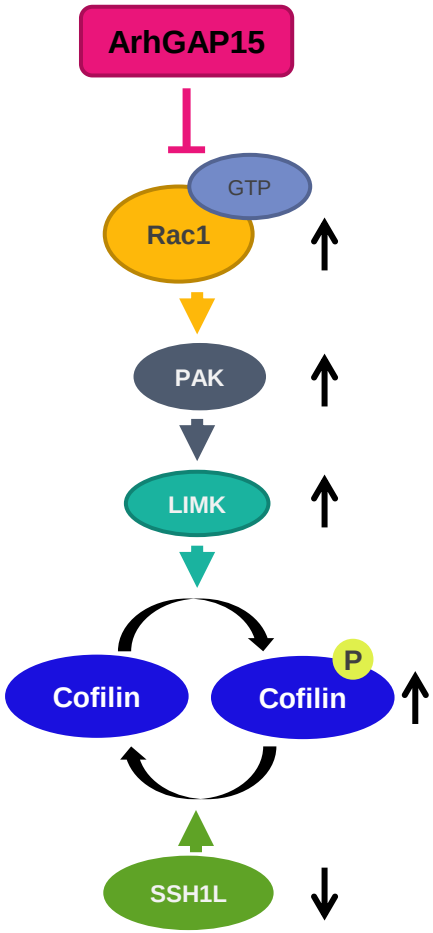
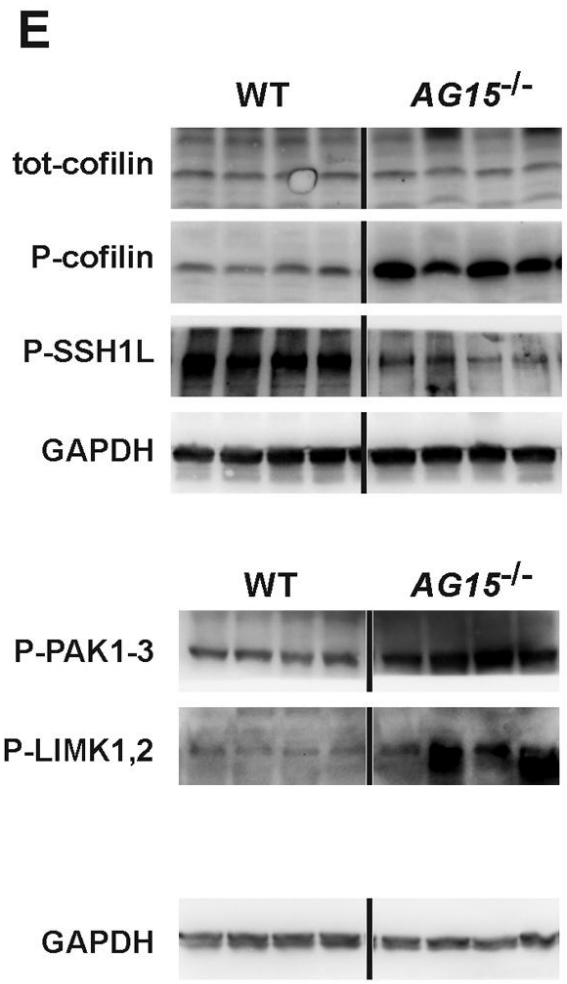
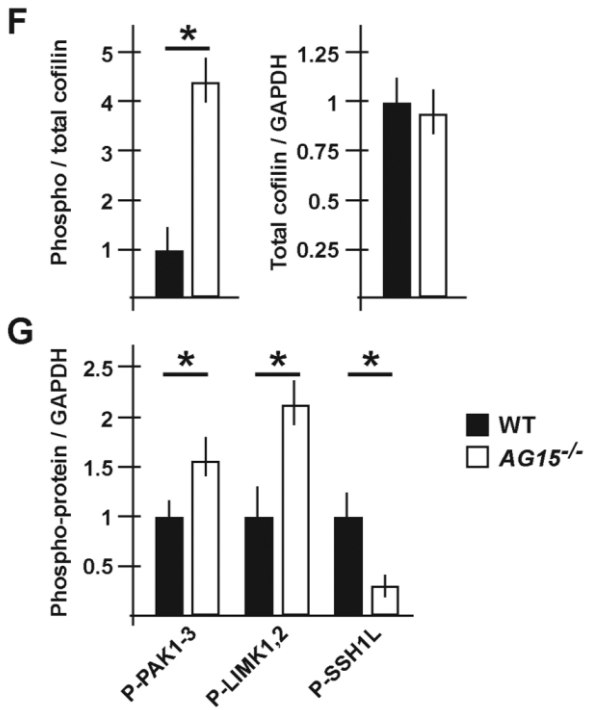




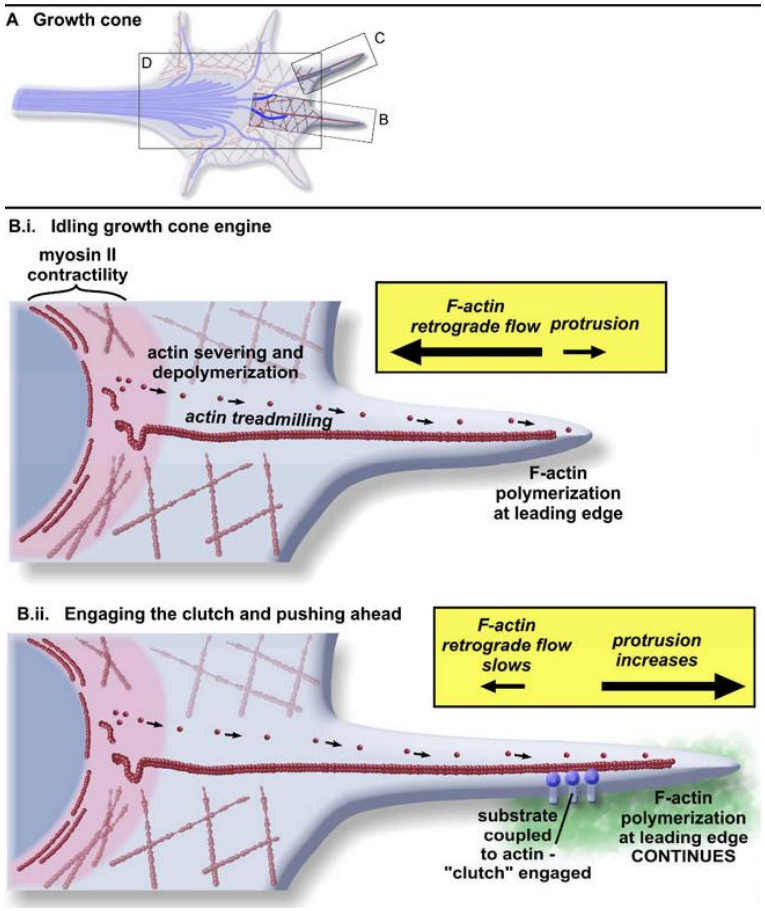


Increased cofilin phosphorylation is consistent with reduced actin dynamics; this could validate the reduced efficiency of neuritogenesis and branching, observed in the absence of *ArhGAP15*.

How does the growth cone utilize the actin engine to move forward?

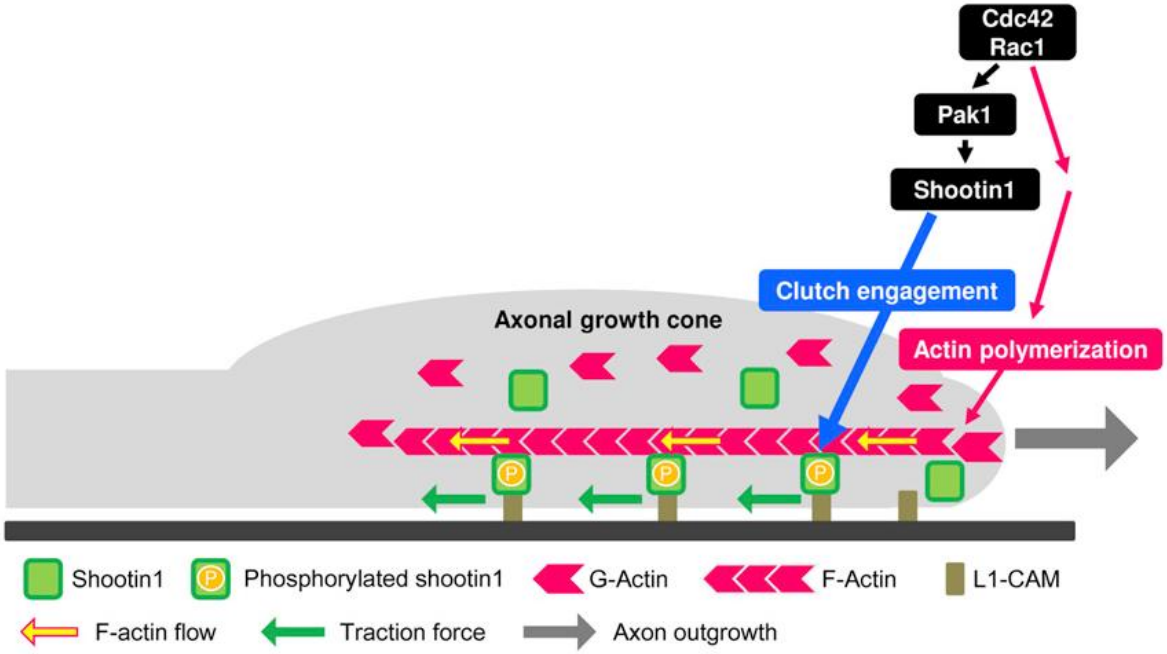
The growth cone `vehicle'

F-actin treadmilling: F-actin polymerization at leading edge, F-actin severing at transition (T)-zone, and recycling of these subunits back to leading edge.
F-actin retrograde flow: F-actin moving backwards towards T-zone, driven both by contractility of the motor protein myosin II, and the `push' from F-actin polymerization in the P-domain.



Lowery and Van Vactor (2009*), Nat Rev Mol Cell Biol*.

Linkage between actin filament retrograde flow and cell adhesion molecules

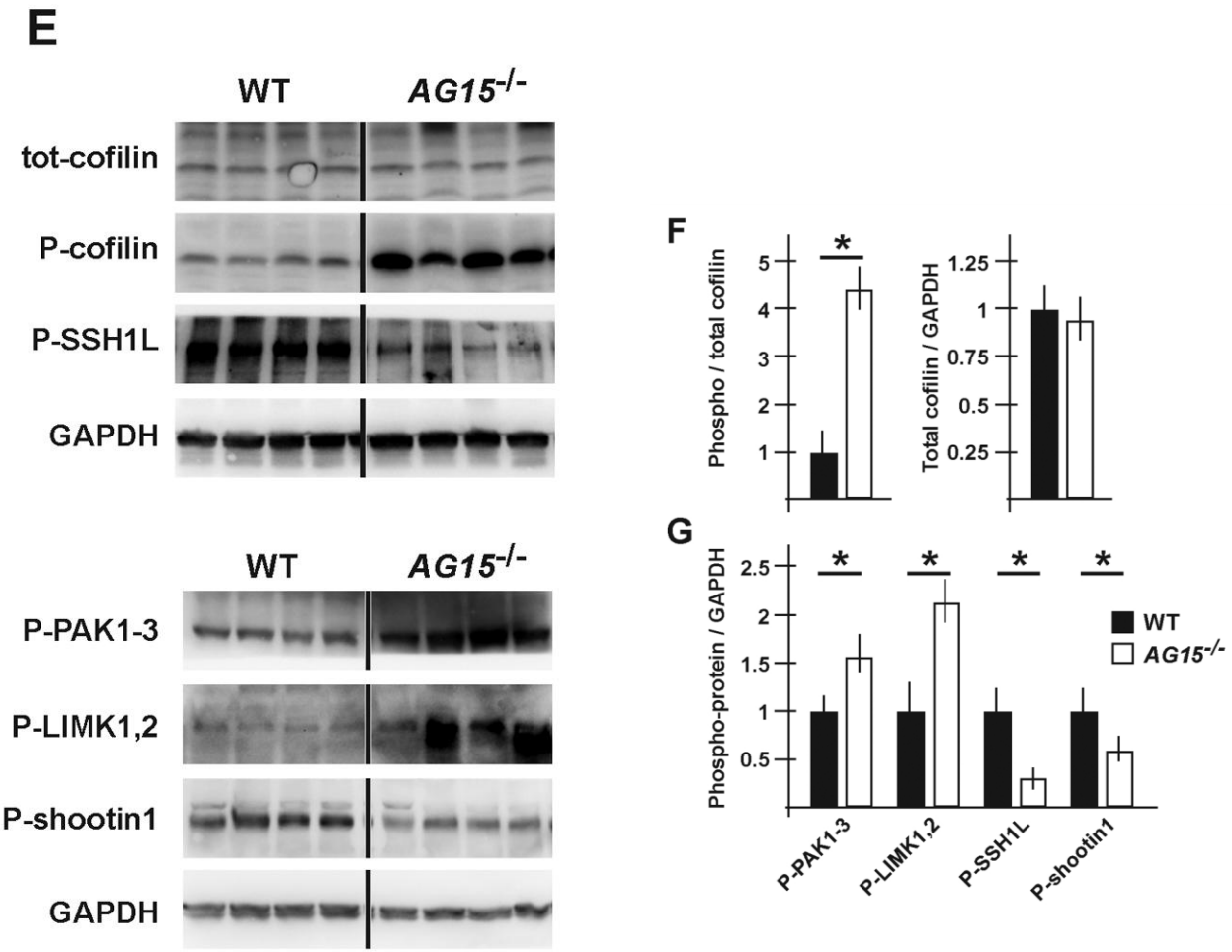


Modified from: Toriyama et al., *Current Biology* (2013).

Shootin1 functions as a linker molecule that couples F-actin retrograde flow and the substrate at neuronal growth cones to promote axon outgrowth:

- shootin1 phosphorylation enhances the interaction between shootin1 and F-actin retrograde flow, promoting filopodium extension and axon outgrowth.

Loss of *ArhGAP15* results in uncoupling between the actin cytoskeleton and cell adhesion molecules



In our model shootin1 does not act as a linker molecule between retrograde actin flow and adhesion system.

Conclusions

- *ArhGAP15* is expressed in three distinct tangential streams, reminiscent of the tangential migration routes of the immature interneurons.

- In the absence of *ArhGAP15*:
  - reduced efficiency of neuritogenesis and branching;
  - increased retrograde actin flow;
  - impaired interneuronal tangential migration;
  - pyramidal cortical neurons are hyperexcitable;
  - *ArhGAP15*KO mice show spontaneous subclinical epileptic spikes.

- Hyperactivation of Rac1-downstream pathway:
  - increased levels of phospho-PAK1/2/3;
  - increased levels of phospho-LIMK1/2;
  - increased levels of phospho-cofilin;
  - reduced levels of phospho-slingshot.

- Uncoupling between the actin cytoskeleton and cell adhesion molecules:
  - decreased levels of phospho-shootin1.

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