

Filopodia dynamics and automated image analysis



Richard Butler (Fiji plugin)
& Vasja Urbancic (biology & R scripts)

Neubias Training School TS7
Szeged, 28 January 2018

Checklist: Data, Software, Scripts

Data:

- Full dataset (8GB)
- Minimal dataset (416MB)
- Local server: 'Vasja' (full data), 'Vasja_Minimal' (minimal data)
location: \\HVPC\TS
username: INF\ts2018, password: Training
- ~~Google drive location: TS7_Vasja, TS7_Vasja_Minimal~~

Software

- Fiji (Life-Line June 2014)
- R (3.3.2 or above)

Plugin

- ~~Filopodyan_.jar - new v. for Java8~~ → Filopodyan-1-2-SNAPSHOT.jar (Java8, new Fiji)
- CAD_Bounder.jar - old v. for Java6, more robust; *requires old Fiji* (Lifeline June 2014)

Scripts

- Scripts_FilopodyanR

Workshop structure

Part 1

Introduction

What are filopodia and how have they been studied

Part 2

Intro to Filopodyan workflow (Fiji)

A guided walk through the plugin for segmentation & tracking using a simple demo file

Part 3

Intro to Filopodyan workflow (R)

Phenotype comparison; correlations between properties; filopodium initiation; tip elongation

Part 4

a. **Analysis: Phenotype comparison**

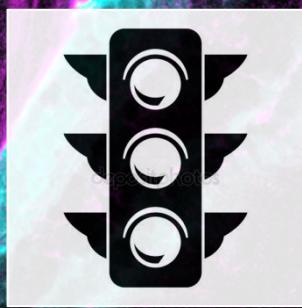
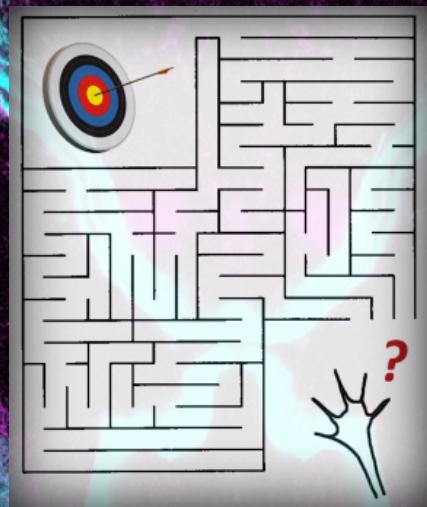
Batch processing (Fiji) and downstream analysis

b. **Analysis: Fluorescence & tip movement**

Tip fitting, direction-corrected tip movement, cross-correlation analysis

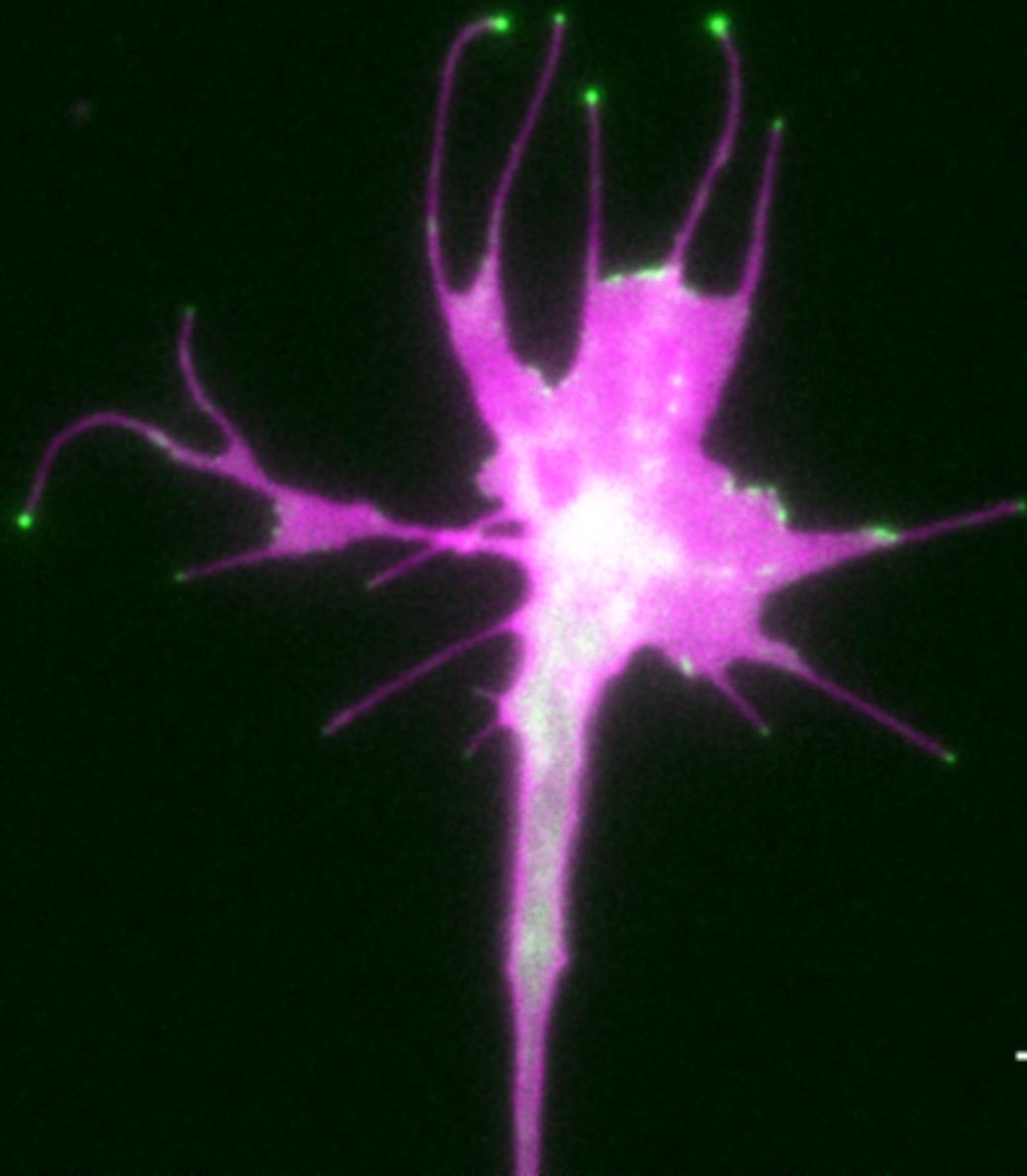
Part 5:

Deconstruction



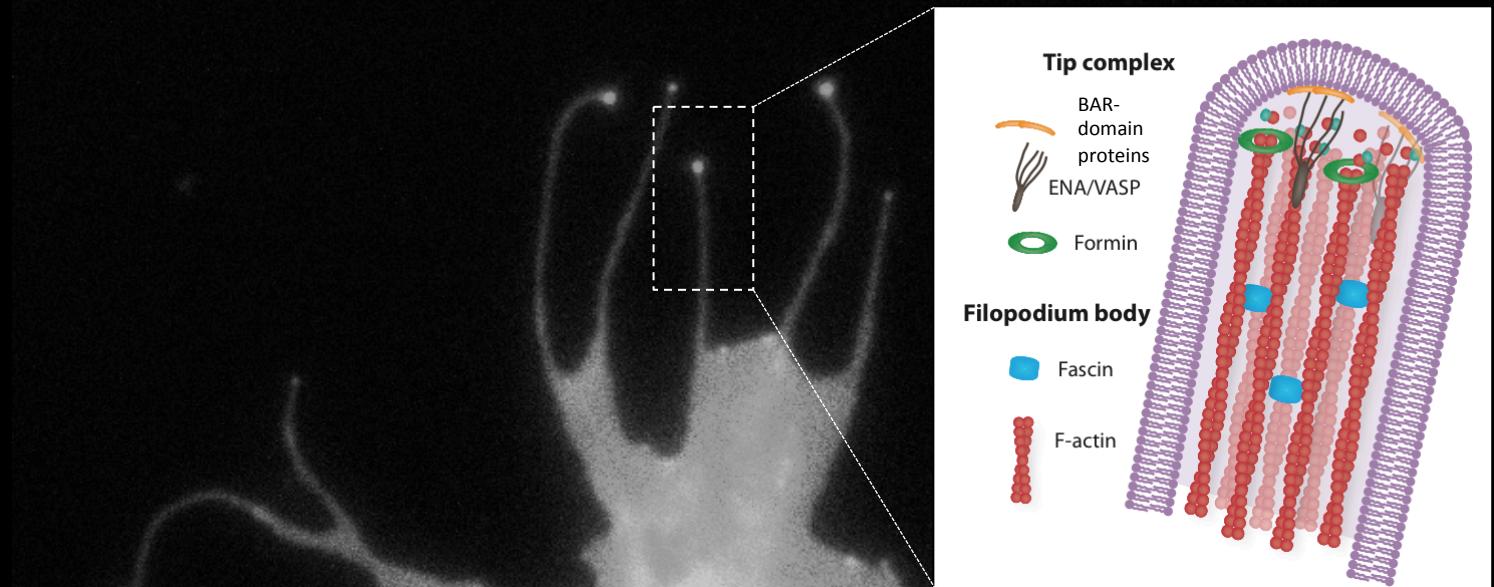
GAP-RFP NeonGreen-ENA

00:00



5 μm

What are filopodia?

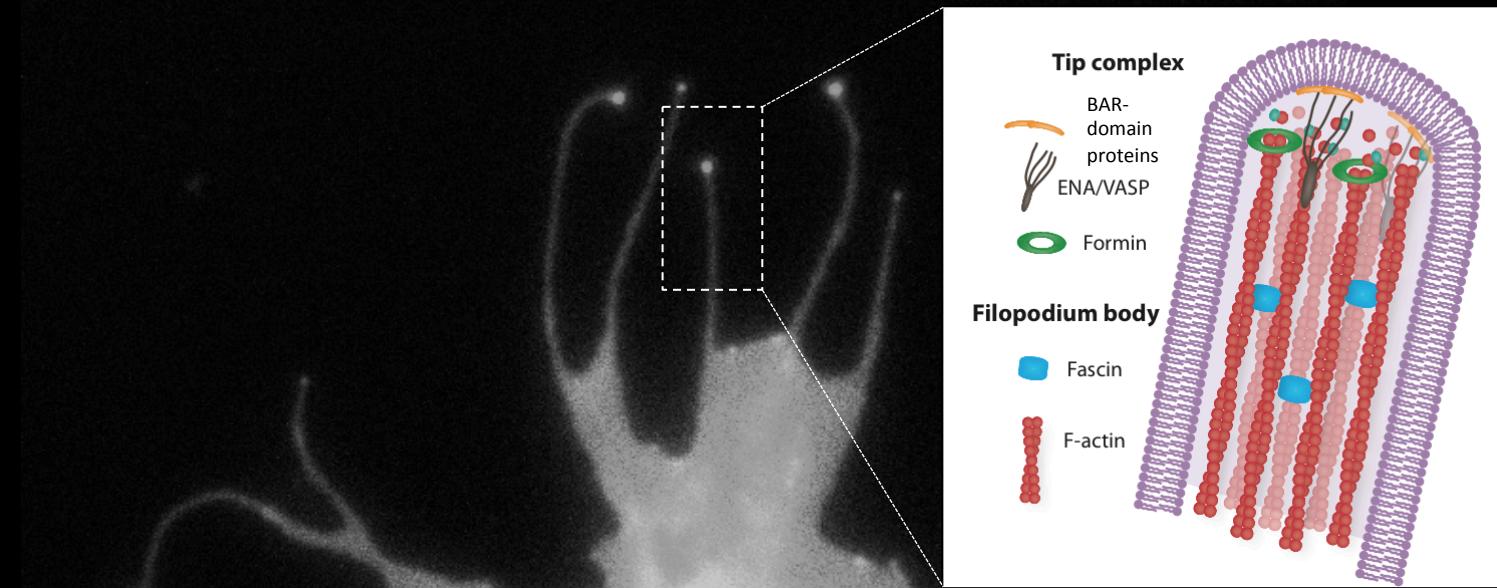


Thin finger-shaped protrusions

Filled with **parallel actin filaments** (and organising proteins e.g. fascin)

Topped with a “**tip complex**” – extension and retraction

Essential roles of filopodia in neurons



Filopodia are vital for:

- **sensing of guidance cues** (Zheng et al. 1996, McConnell et al. 2016)
- **motility and navigation** through developing brain (Chien et al. 1993, Dwivedy et al. 2007)
- **branching and formation of synaptic connections** (Dwivedy et al. 2007, Lohmann et al. 2008)

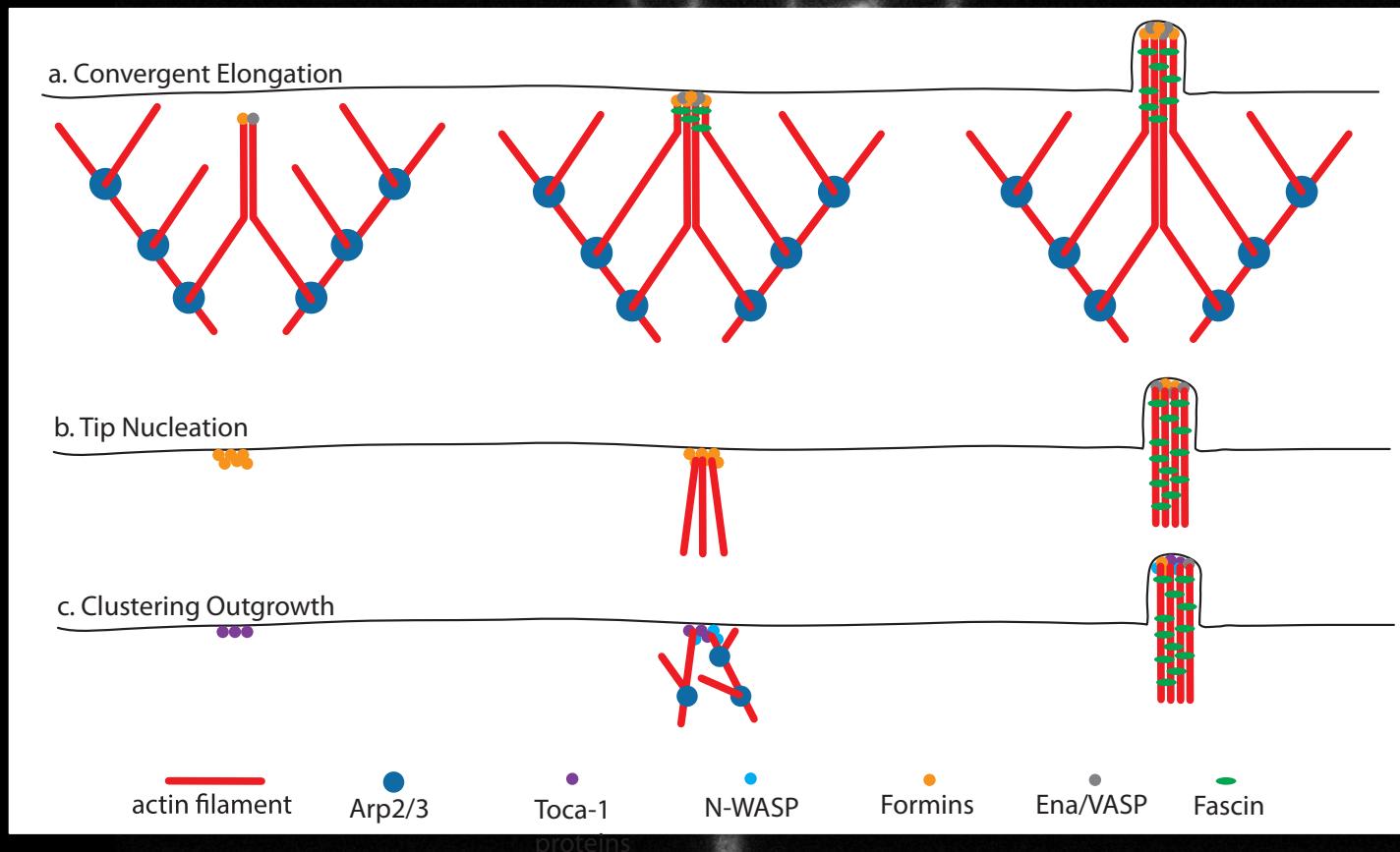
Filopodial proteins:

- **genetic association with neurodevelopmental disorders and intelligence** (e.g. CYFIP-1 in schizophrenia; TOCA-1 in intelligence, Benyamin et al. 2014)

Important remaining questions in filopodia research

How do filopodia form?

Convergent elongation vs tip nucleation vs membrane organisation



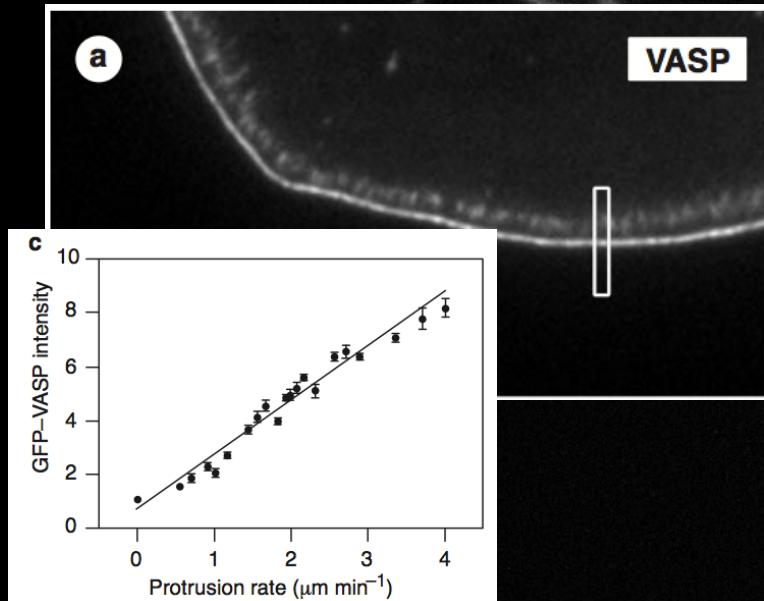
Important questions in filopodia research

How do filopodia form?

Convergent elongation vs tip nucleation vs membrane organisation

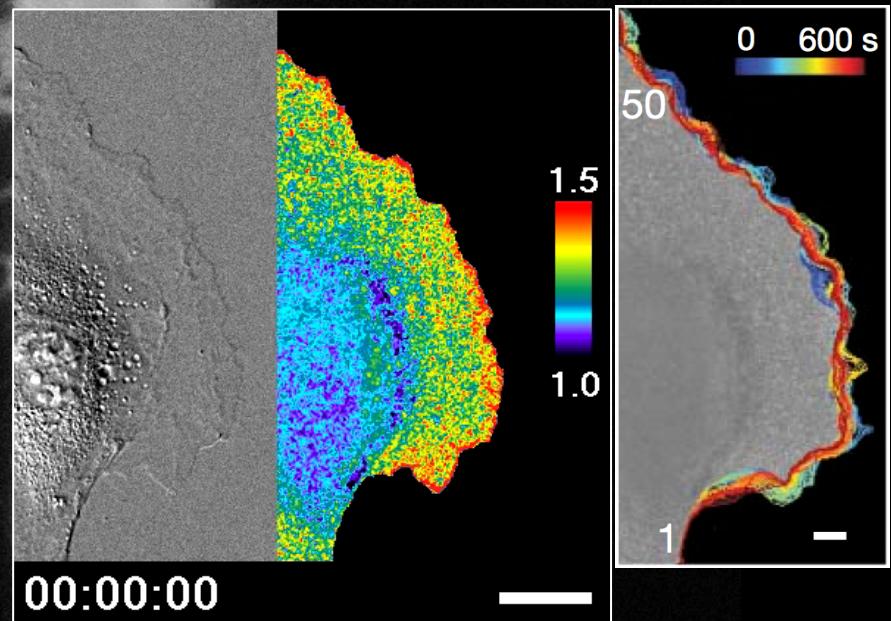
How do filopodia elongate?

More VASP → faster protrusion



Rottner et al. 1999

RhoA activity and speed of protrusion:



Machacek et al. 2009

Important questions in filopodia research

How do filopodia form?

Convergent elongation vs tip nucleation vs membrane organisation

How do filopodia elongate?

e.g. evidence for different molecular types of elongation:

Formin-driven vs ENA/VASP-driven

Similarity vs diversity in molecular mechanisms:

intercellular diversity – filopodia in different cell types?

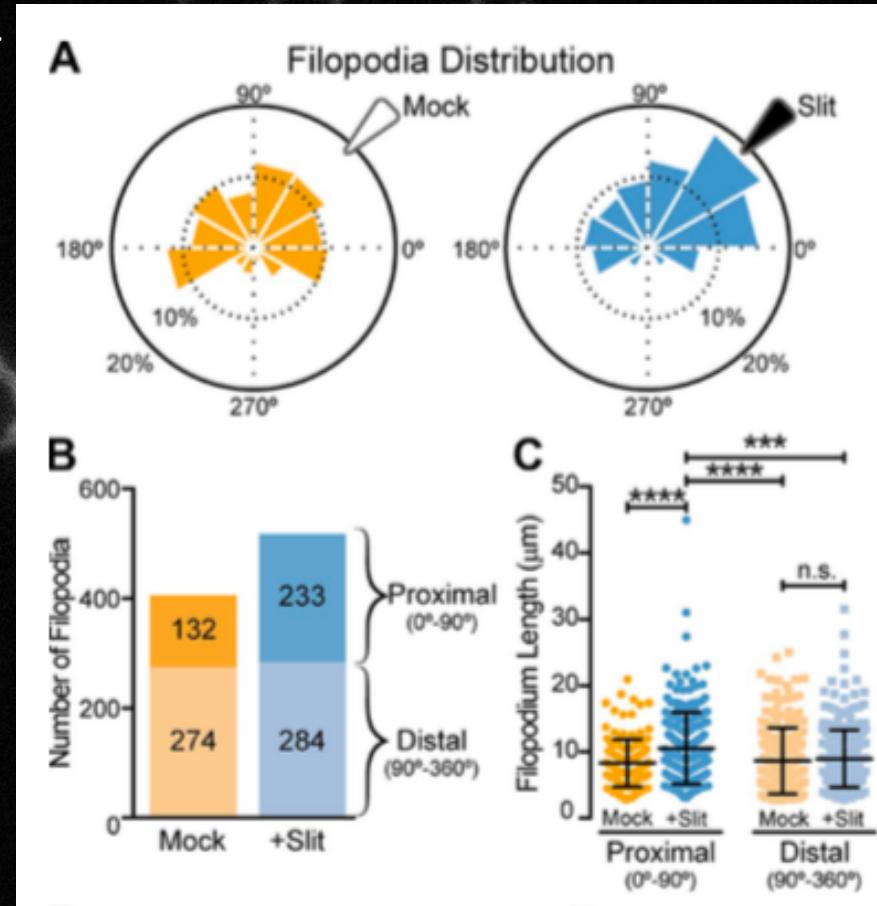
intracellular diversity – different filopodia within the same cell?

How have filopodia previously been analysed? (1)

Manual analysis:

- counting,
- length,
- angle, ...

e.g.



McConnell et al. 2016

How have filopodia been analysed? (2)

Automated analysis tools:

1. FiloDetect [Matlab]

(Nilufar et al. 2013)

2. CellGeo [Matlab]

(Tsygankov et al. 2014)

3. F-dynamics [Matlab]

& DFMA [Fiji]

(Hendrisusdottir & Bergmann 2014)

(Tarnok et al. 2015)

4. ADAPT [Fiji]

(Barry et al. 2015)

5. (Saha et al. 2016) [MATLAB]

6. FiloQuant [Fiji]

(Jacquemet et al. 2017)

... and key advancements:

- thresholding segmentation and erosion-dilation
- measuring number and length
- tracking individual filopodia over time
- dynamics of broad protrusions (lamellipodia)
- specialised for dendritic filopodia
- lateral as well as longitudinal movements
- centre of mass displacements
- versatile suite incl. filopodia identification and tracking, cell migration, peripheral fluorescence & membrane velocity
- axial fluorescence measurements
- suitable in cell sheets & 3D environments
- filopodia density

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Question 1: How are new filopodia formed?

Competing models of filopodium formation:

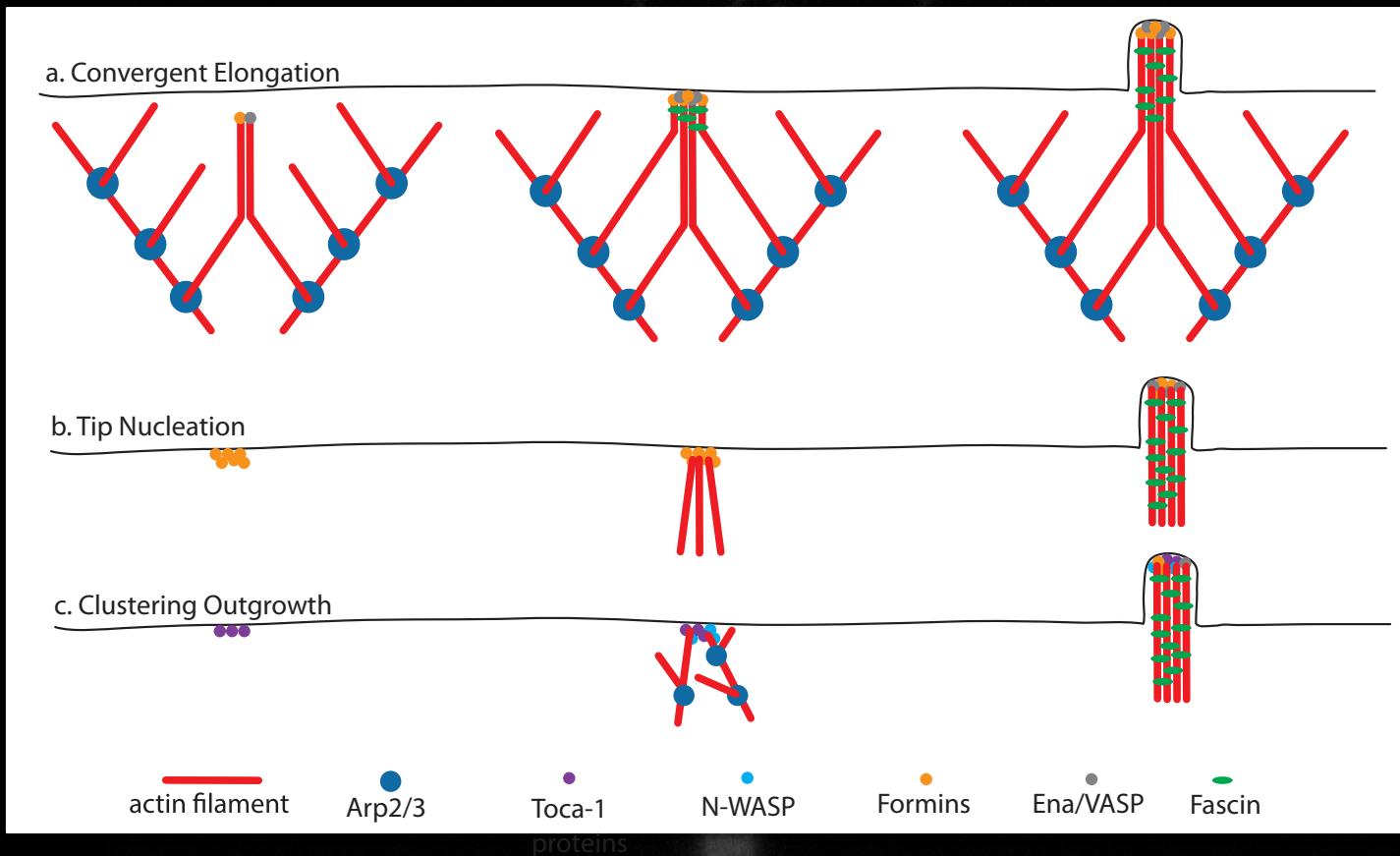
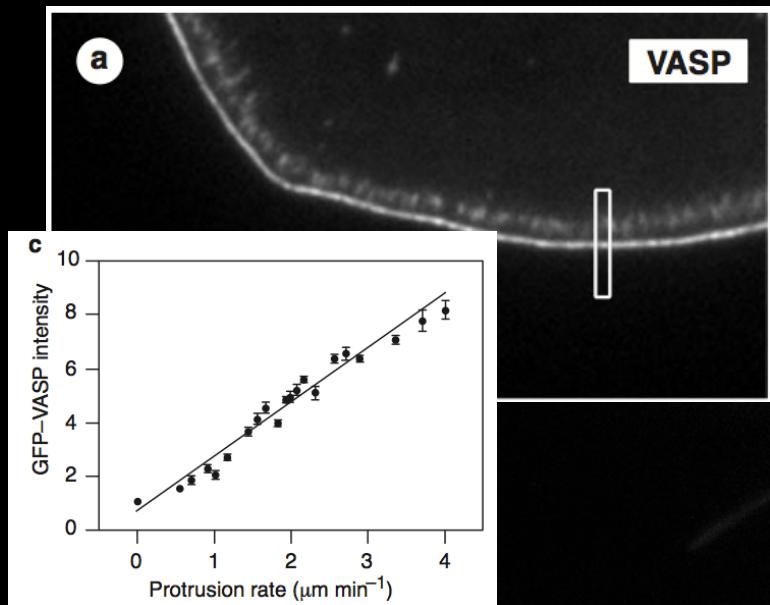


image by
Helen Fox

Question 2: How do filopodia elongate?

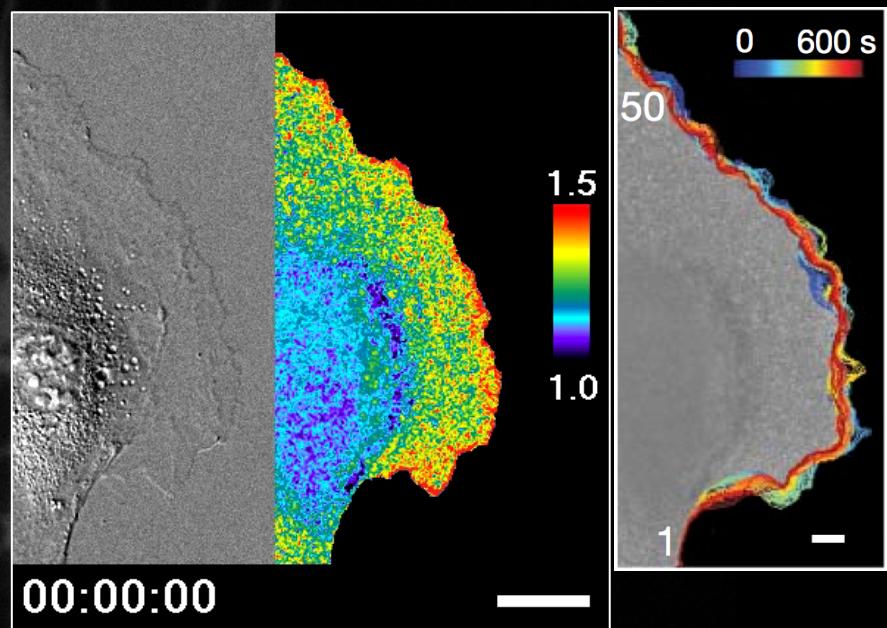
In lamellipodia:

More VASP → faster protrusion



Rottner et al. 1999

RhoA activity and speed of protrusion:



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Do similar effects exist in filopodia?