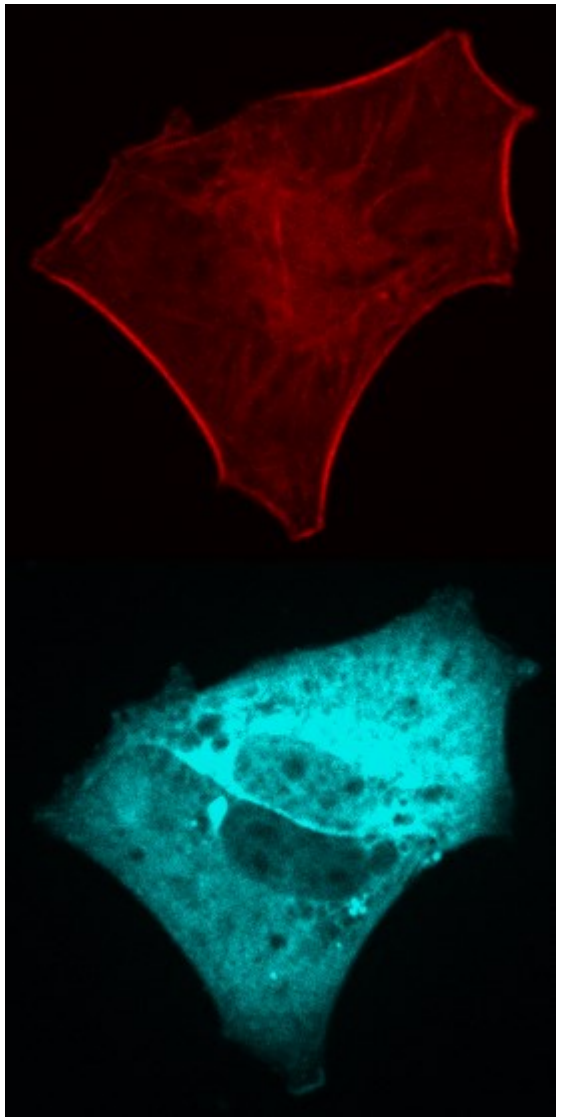
Role of MRTF as a mediator of mechanotransduction in the muscle cell

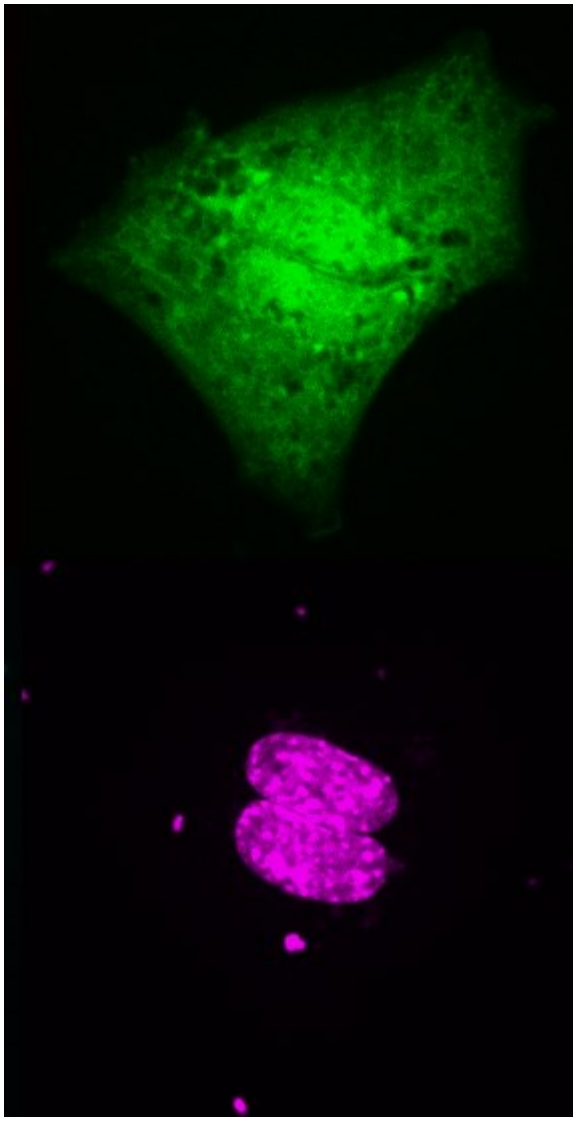


Lorraine Montel

PhD student 3rd year

Complex Systems and Materials
University Paris Diderot
Sylvie Hénon

Collaboration :
Genetics, Development and Physiology of Skeletal muscle
Institut Cochin
Athanassia Sotiropoulos



Mechanotransduction

Mechanical Signal → Biological Response

Mechanotransduction in the muscles

Mechanical Overload

Exercise
Compensation

↓

HYPERTROPHY

Right-handed javelin thrower with bigger arm due to exercise

No Mechanical Load

Immobilization
Denervation
Low gravity environment

↓

ATROPHY

Mechanotransduction

Mechanical Signal → Biological Response

How does that work ?

Mechanical Overload

Exercise
Compensation

↓

HYPERTROPHY

No Mechanical Load

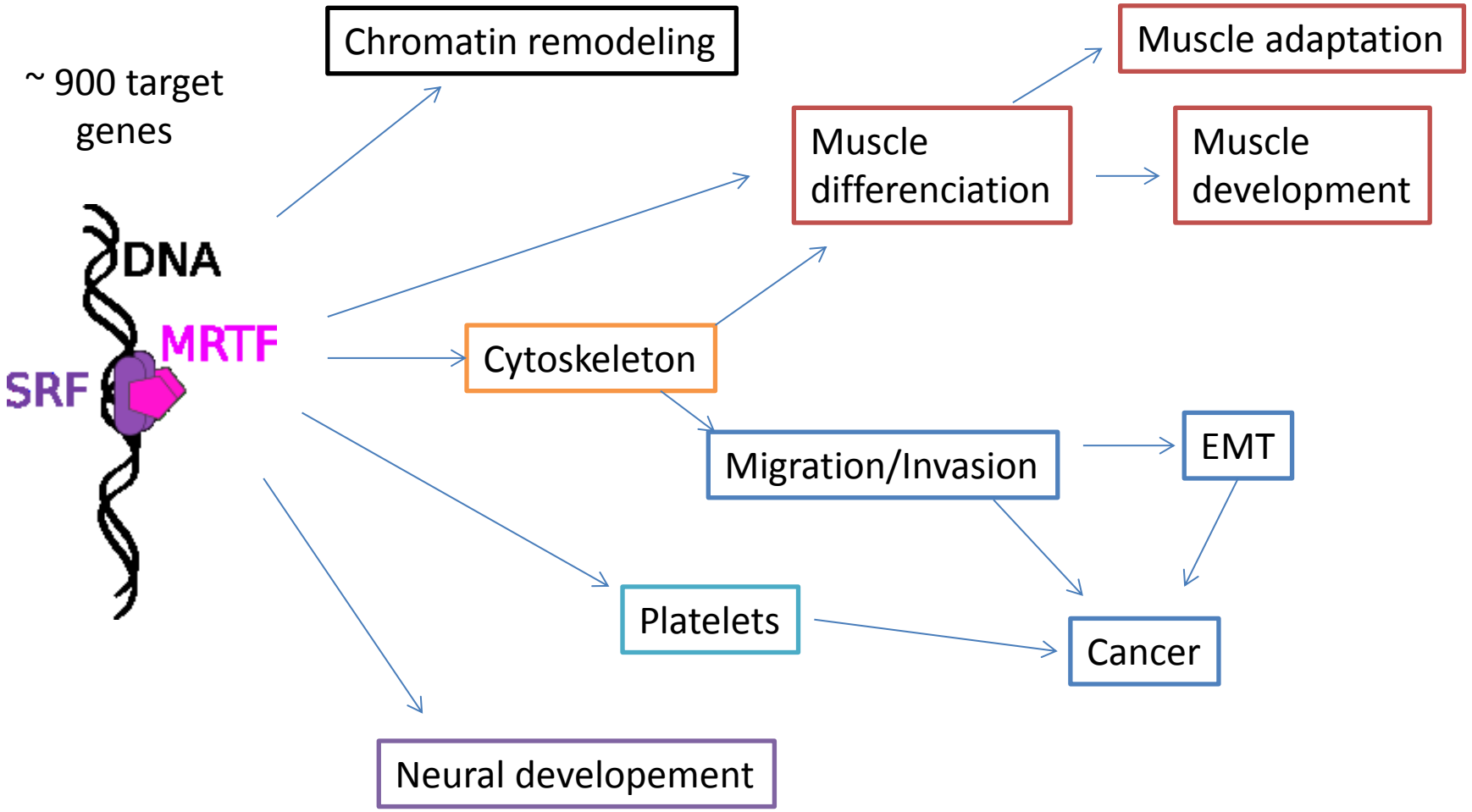
Immobilization
Denervation
Low gravity environment

↓

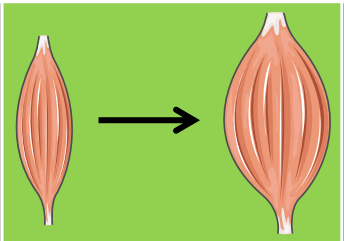
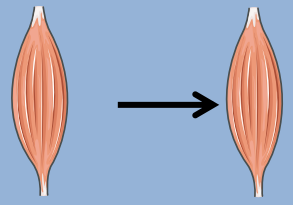
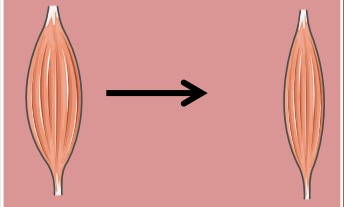
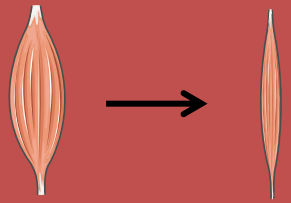
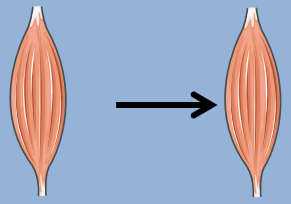
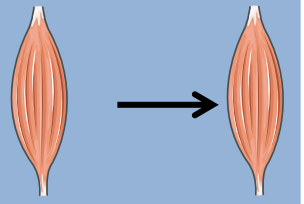
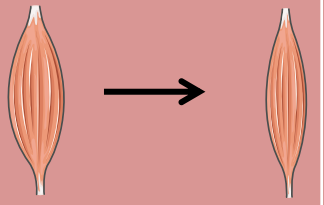
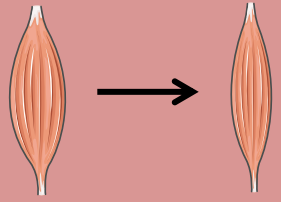
ATROPHY

Right-handed javelin thrower
with bigger arm due to exercise

Serum Response Factor

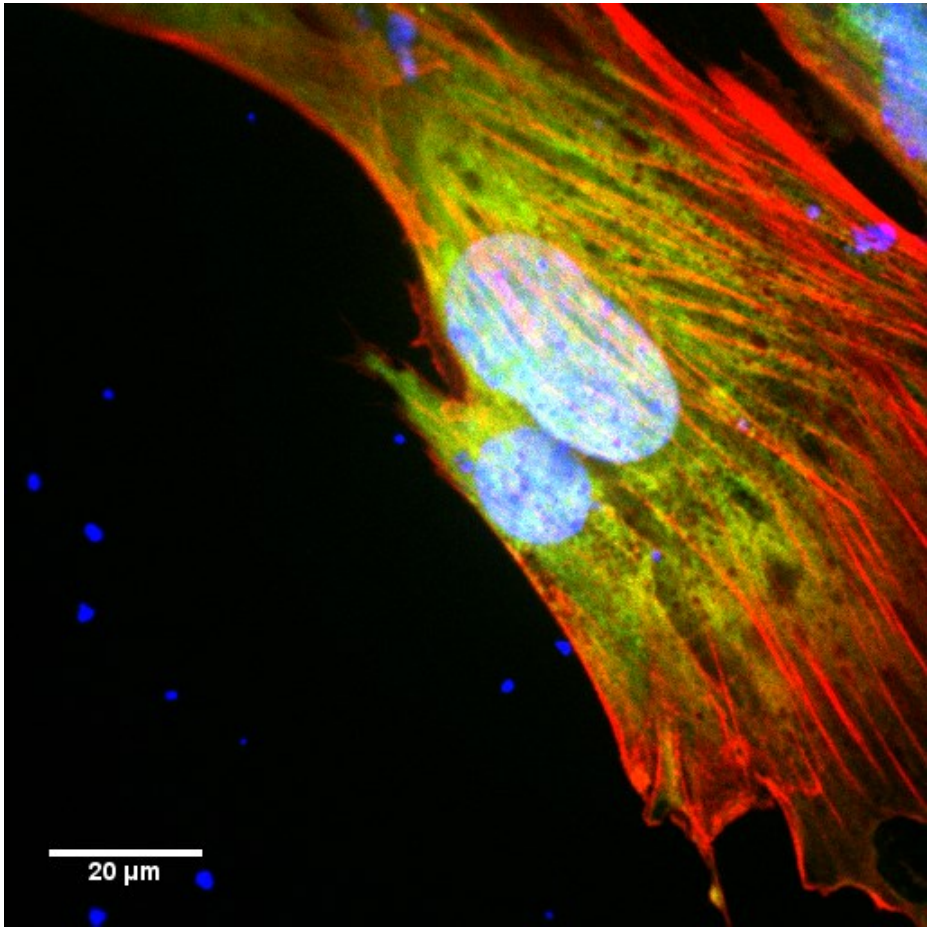


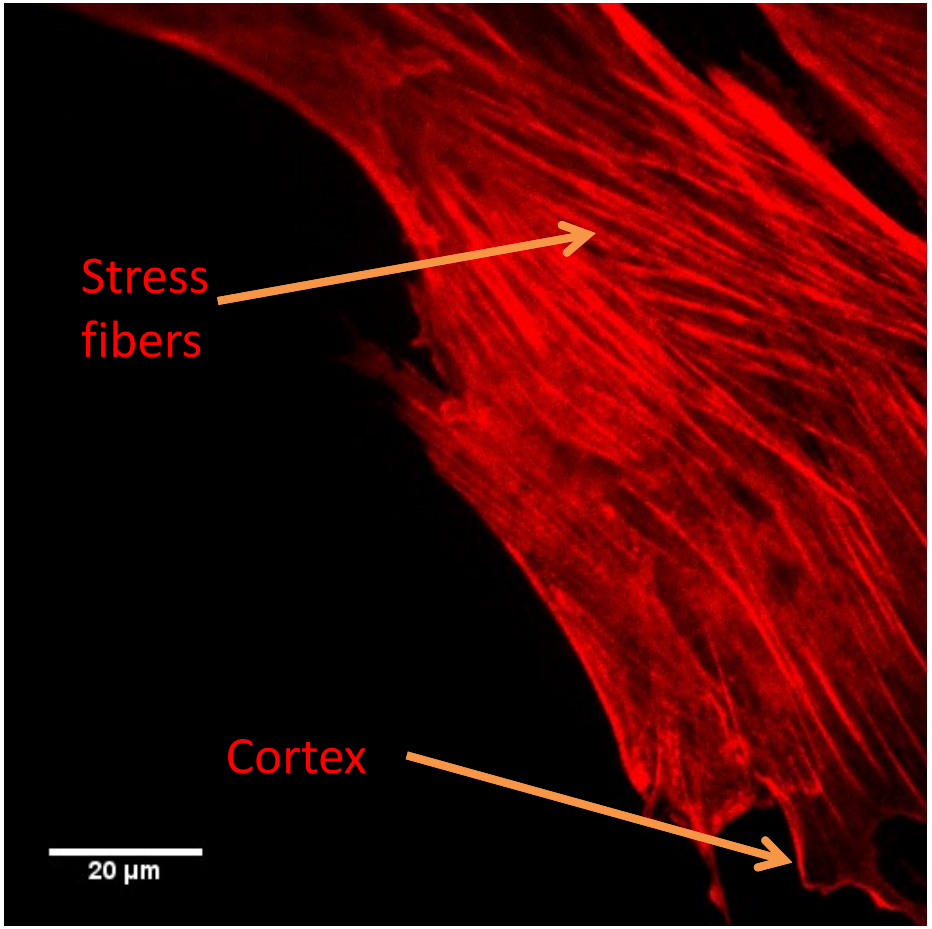
Srf is necessary for mechanically-induced hypertrophy/atrophy

	WT	SRF loss of function	Active SRF	Nuclear MRTF
Overload				
Denervation				
Caloric Restriction				

Work from the Sotiropoulos group from Insitut Cochin

The actin cytoskeleton





Red : Filaments of actin

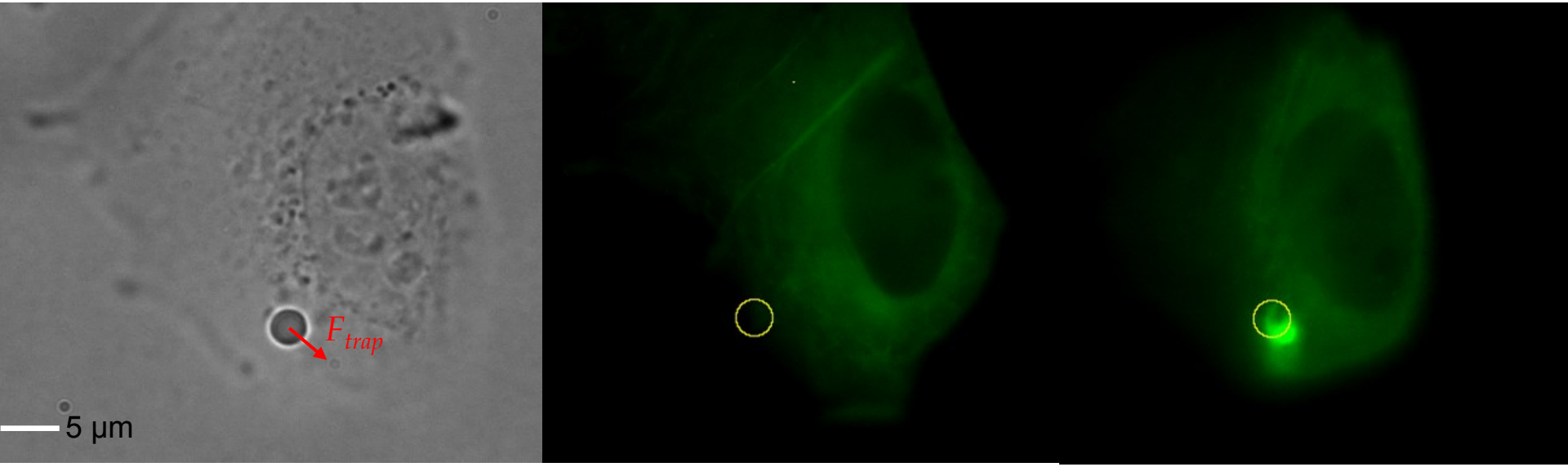
Green : Globular actin

Blue : DNA

Mechanical cues induce modification of the actin cytoskeleton

C2C12 mouse myoblast

GFP Actin

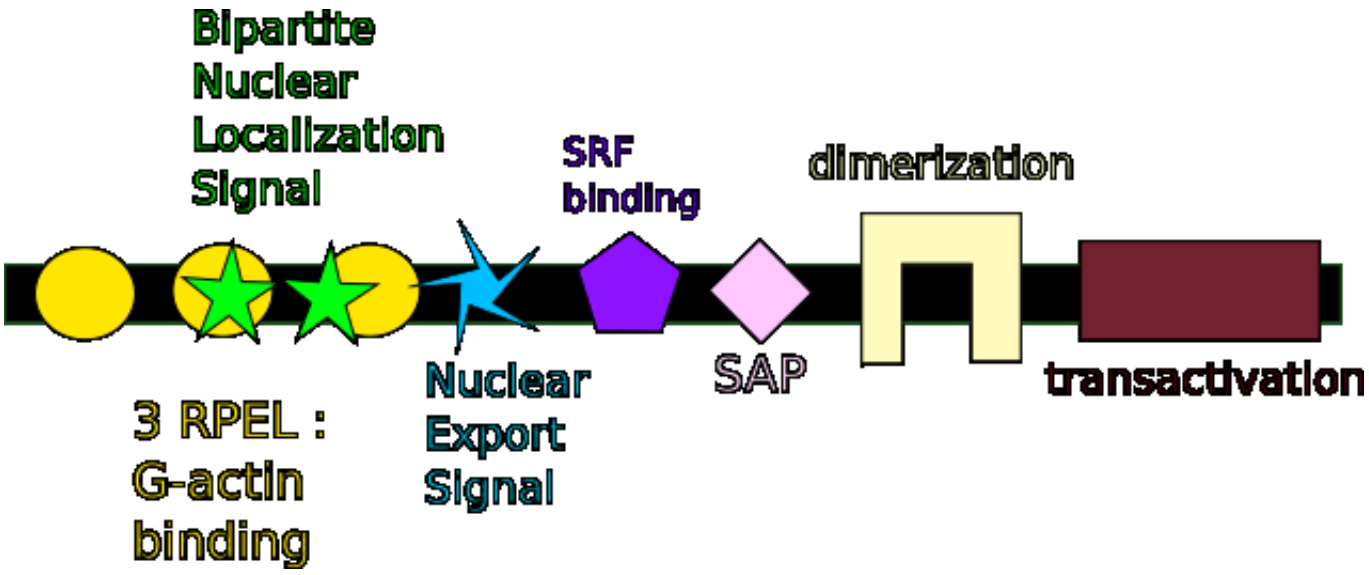


Before

After 60 minutes

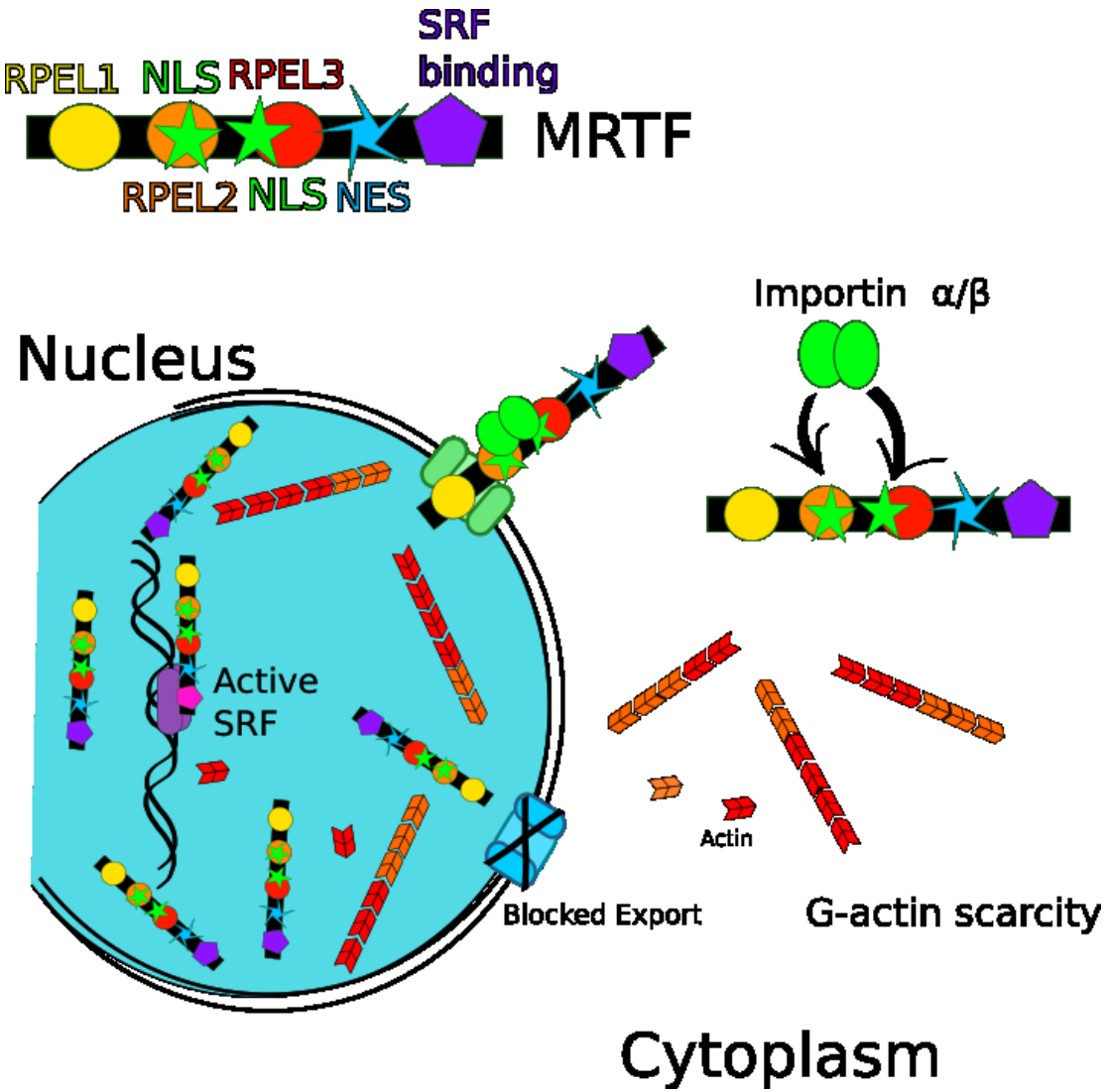
Icard-Arcizet D. *et al* , **Sylvie Hénon team**

Myocardin-Related Transcription Factor : a G-actin sensor

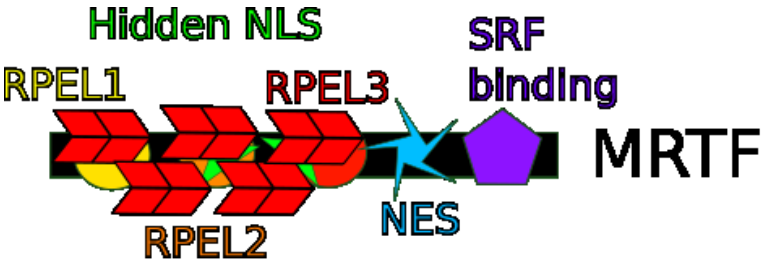


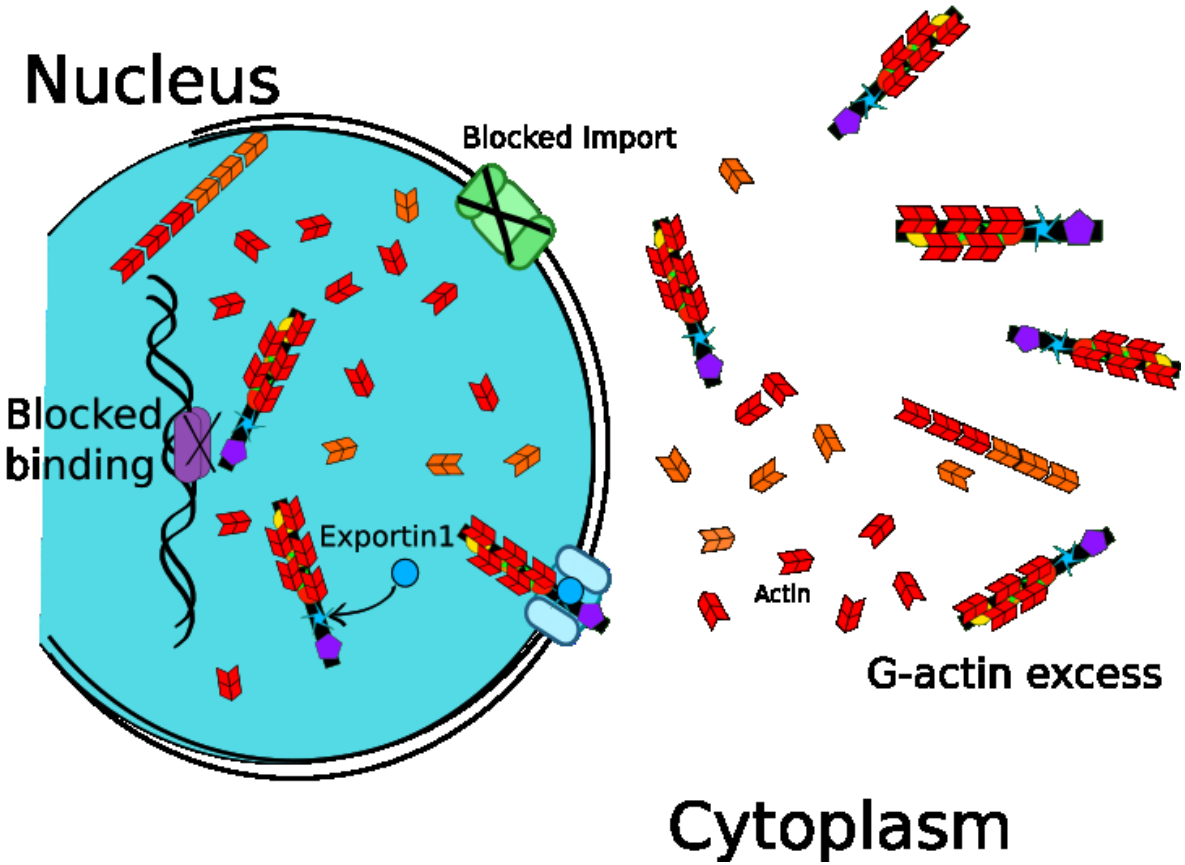
NLS included in G-actin binding zone, linking actin binding and MRTF localization

Nuclear MRTF in G-actin scarcity



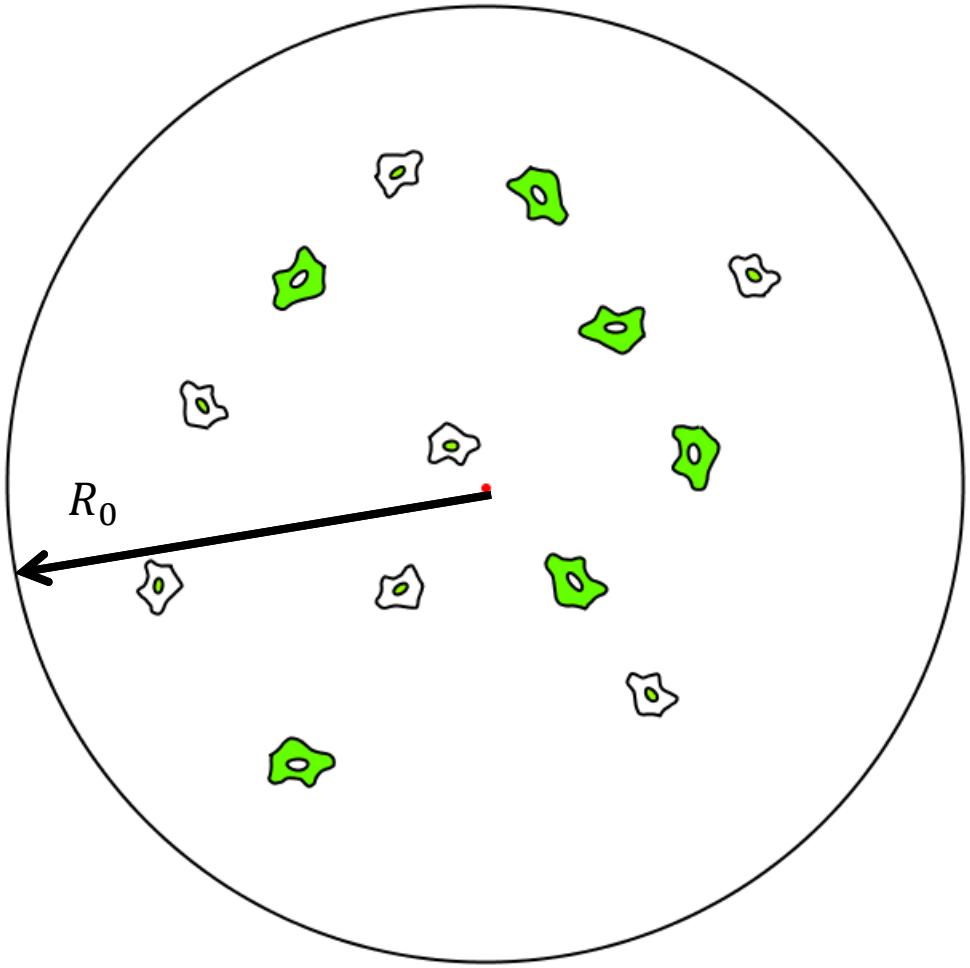
Cytoplasmic MRTF in G-actin Excess





Experimental Set-up:

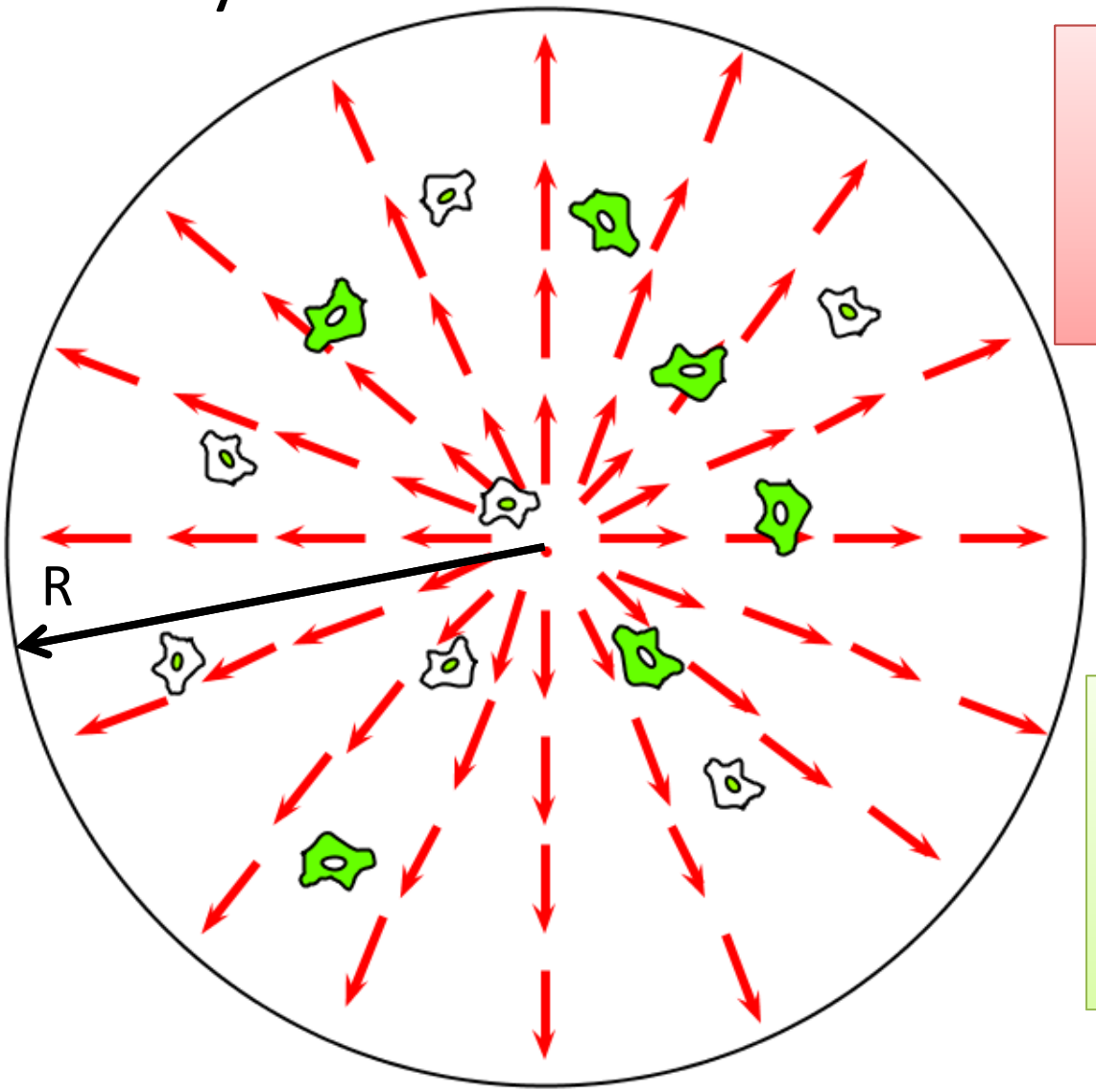
Myoblasts on stretched PDMS substrate



- Stretchable PDMS
- C2C12 Myoblasts
- MRTF-A GFP localization read-out

Experimental Set-up:

Myoblasts on stretched PDMS substrate



Uniform radial constant strain

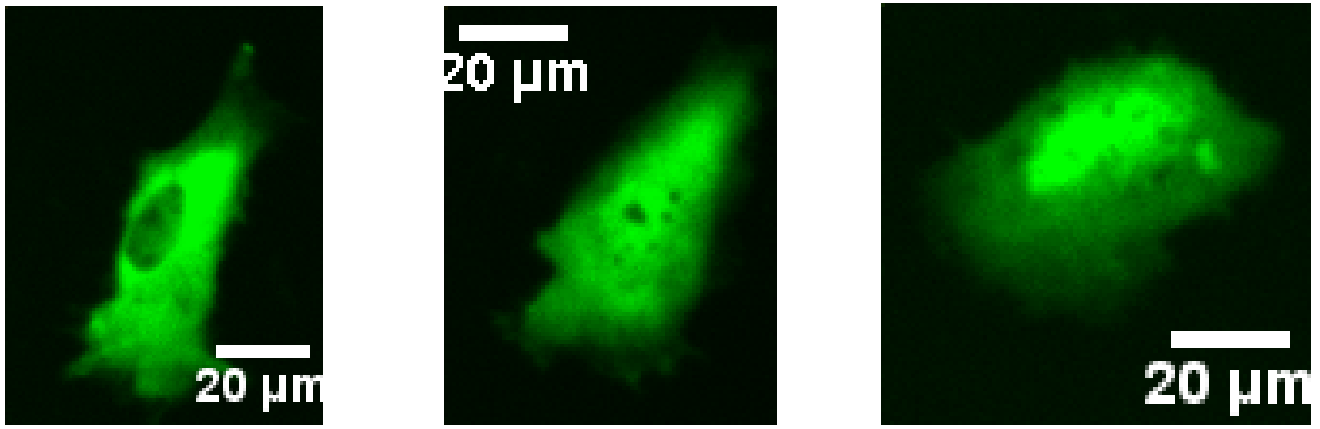
Strain rate : $\frac{R - R_0}{R_0}$

MRTF-A GFP localization read-out

Localization of MRTF-A

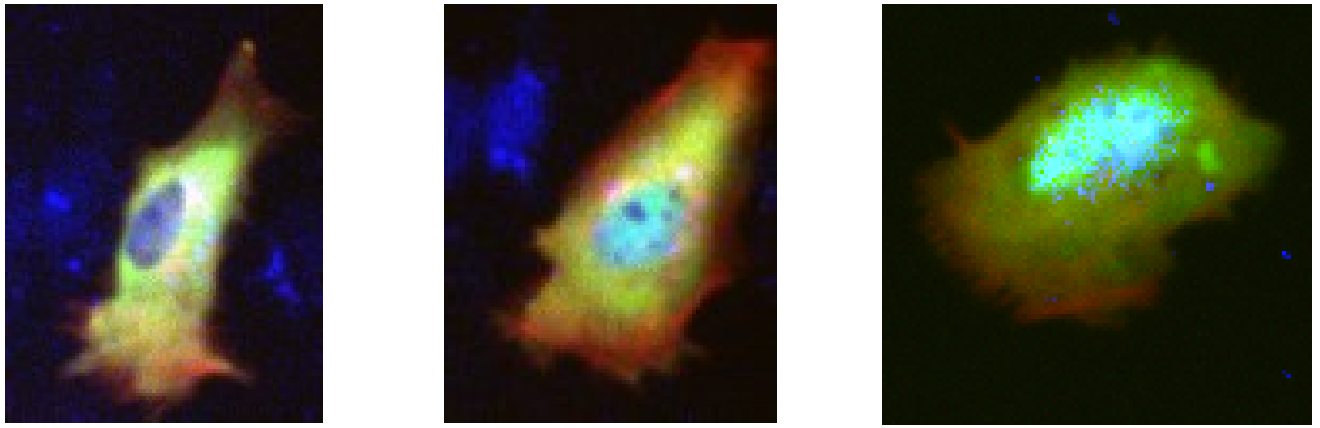
Cytoplasmic Homogeneous Nuclear

MRTF-A GFP

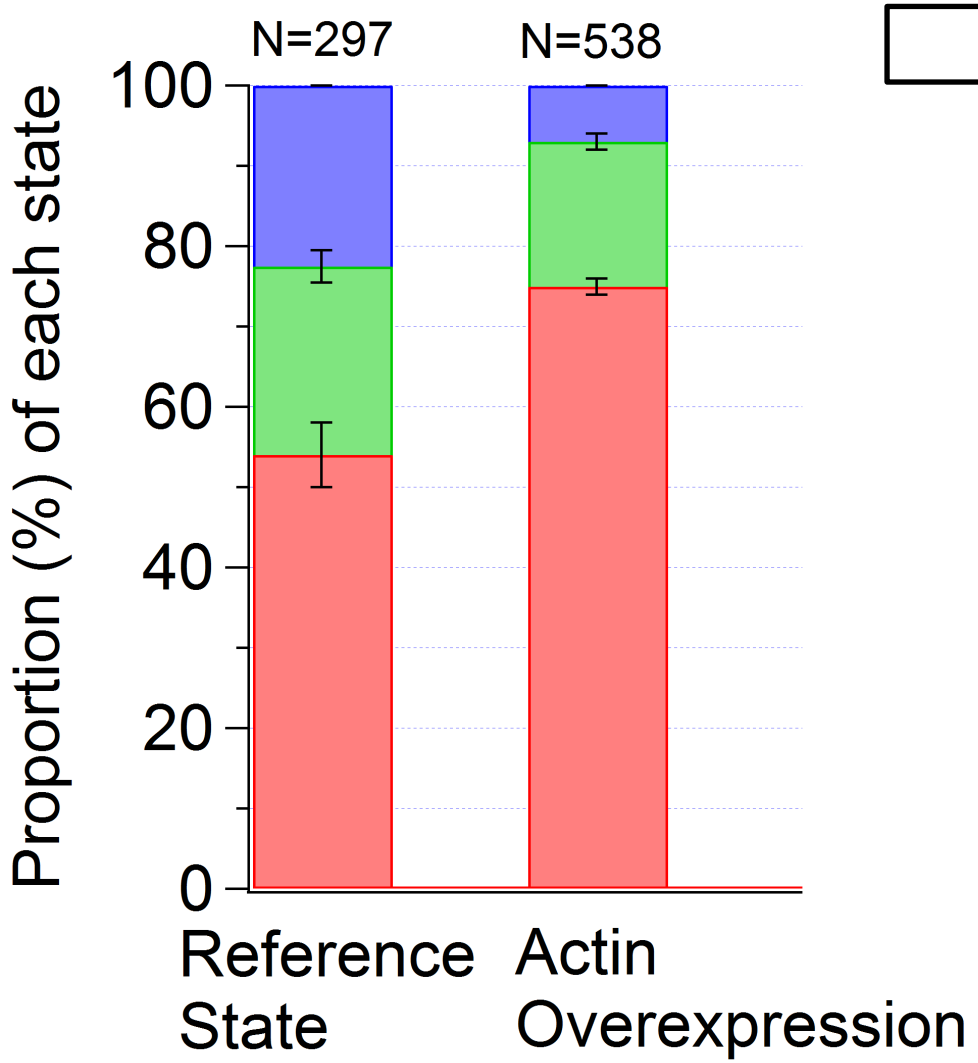


Actin mCherry

DAPI (Nucleus)



Influence of Actin Overexpression on MRTF-A Localization



MRTF-A Localization

- Nuclear
- Homogeneous
- Cytoplasmic

Actin Overexpression

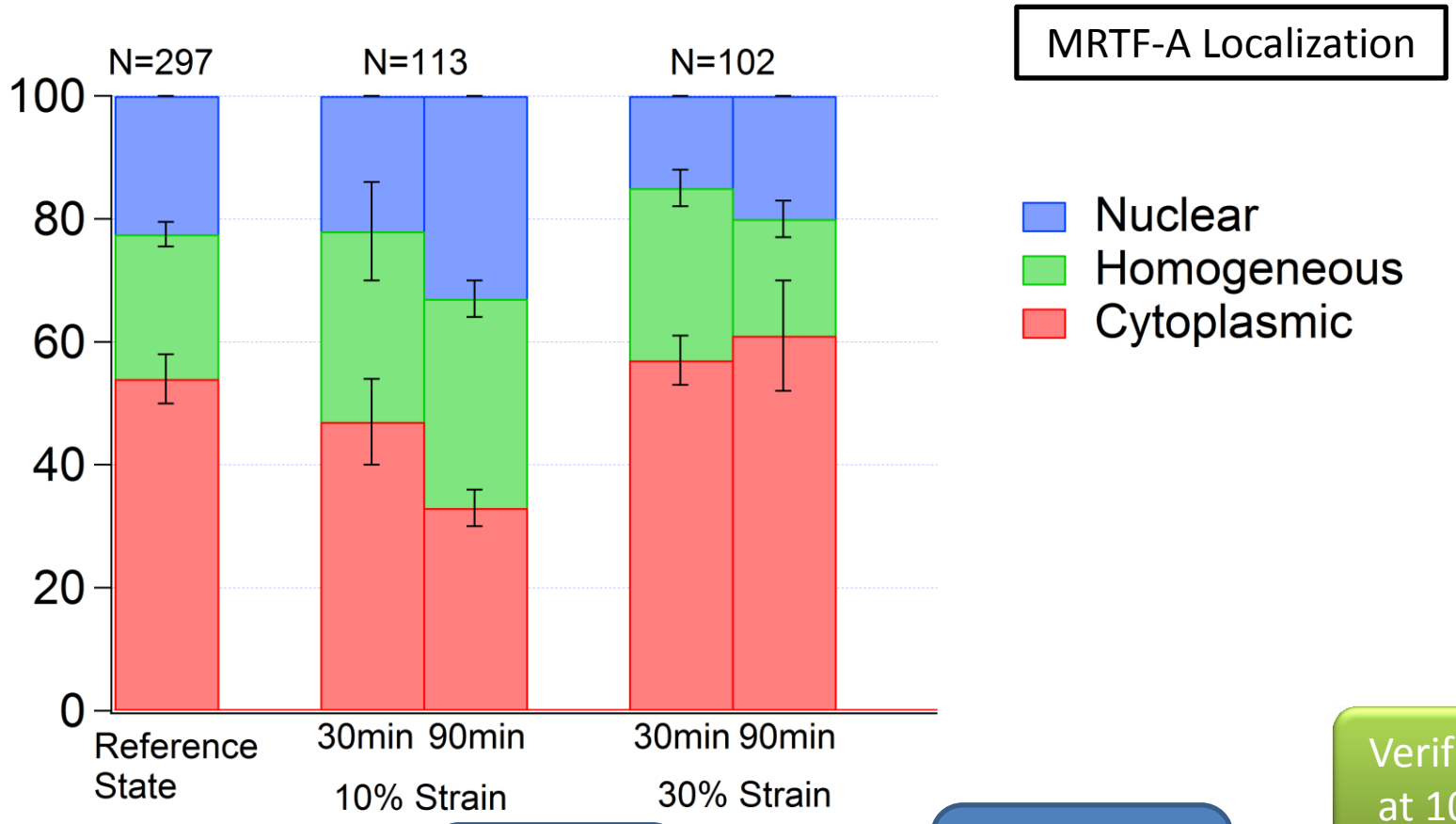
↓

G-Actin Excess

↓

Actin-MRTF-A Complex in the Cytoplasm

Influence of Stretching on MRTF-A localization

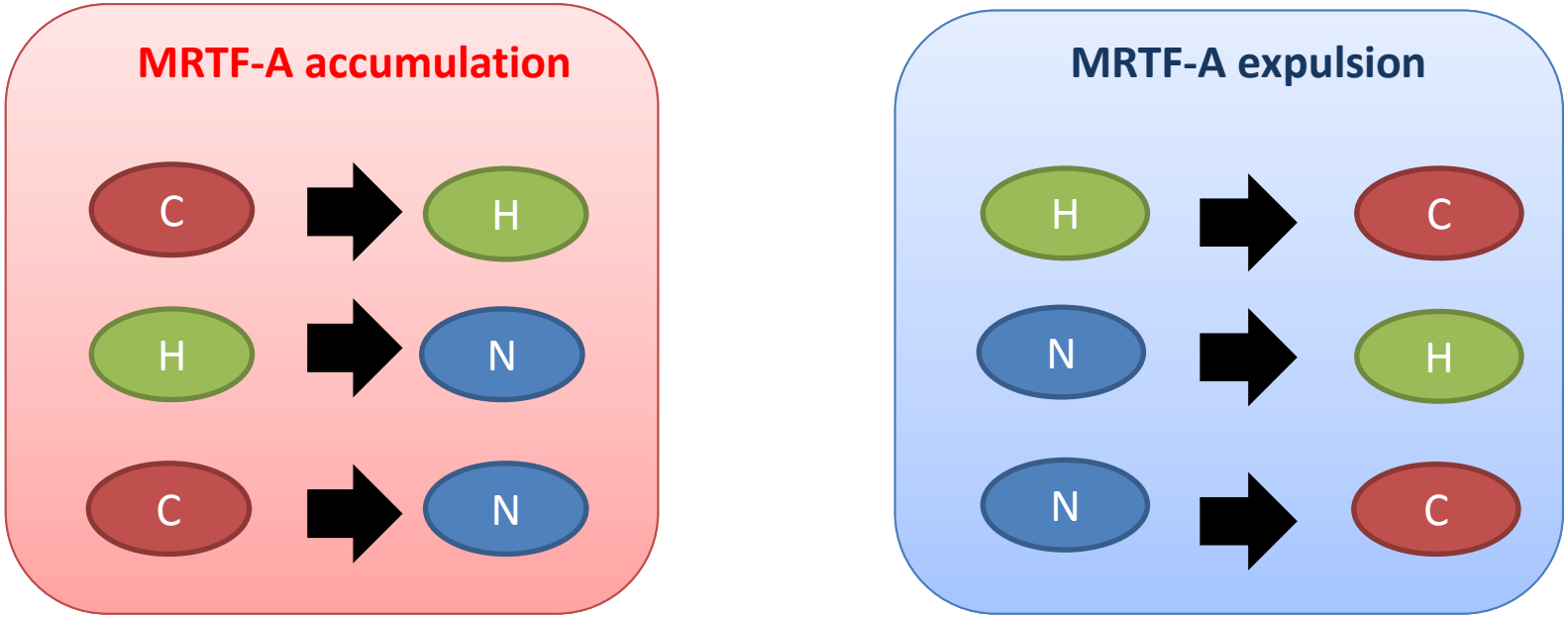


Mechanical Cues → Actin Assembly → MRTF-A Entering the Nucleus

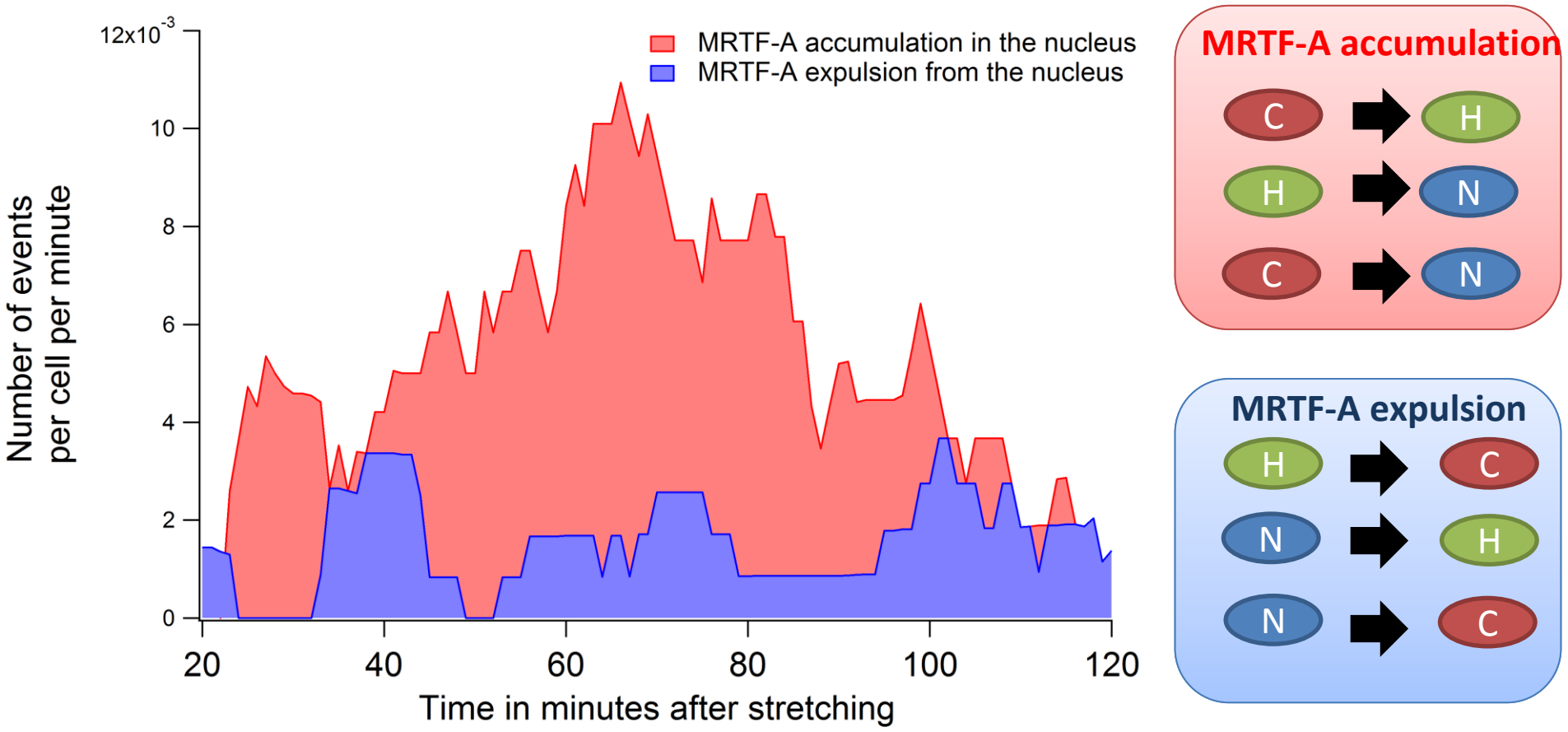
Verified at 10%

? at 30%

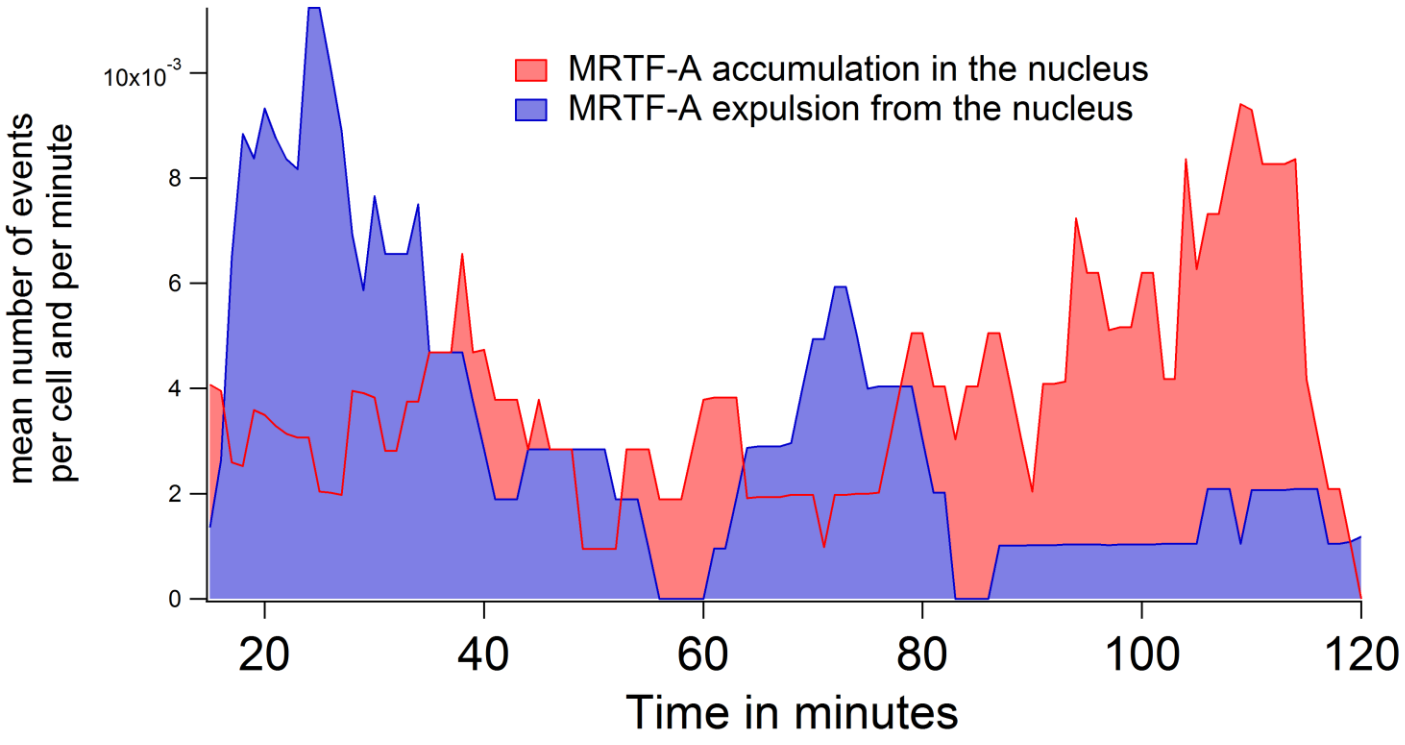
Classification of events



10% Strain Experiments



30% Strain Experiments



MRTF-A accumulation :

C → H

H → N

C → N

MRTF-A expulsion :

H → C

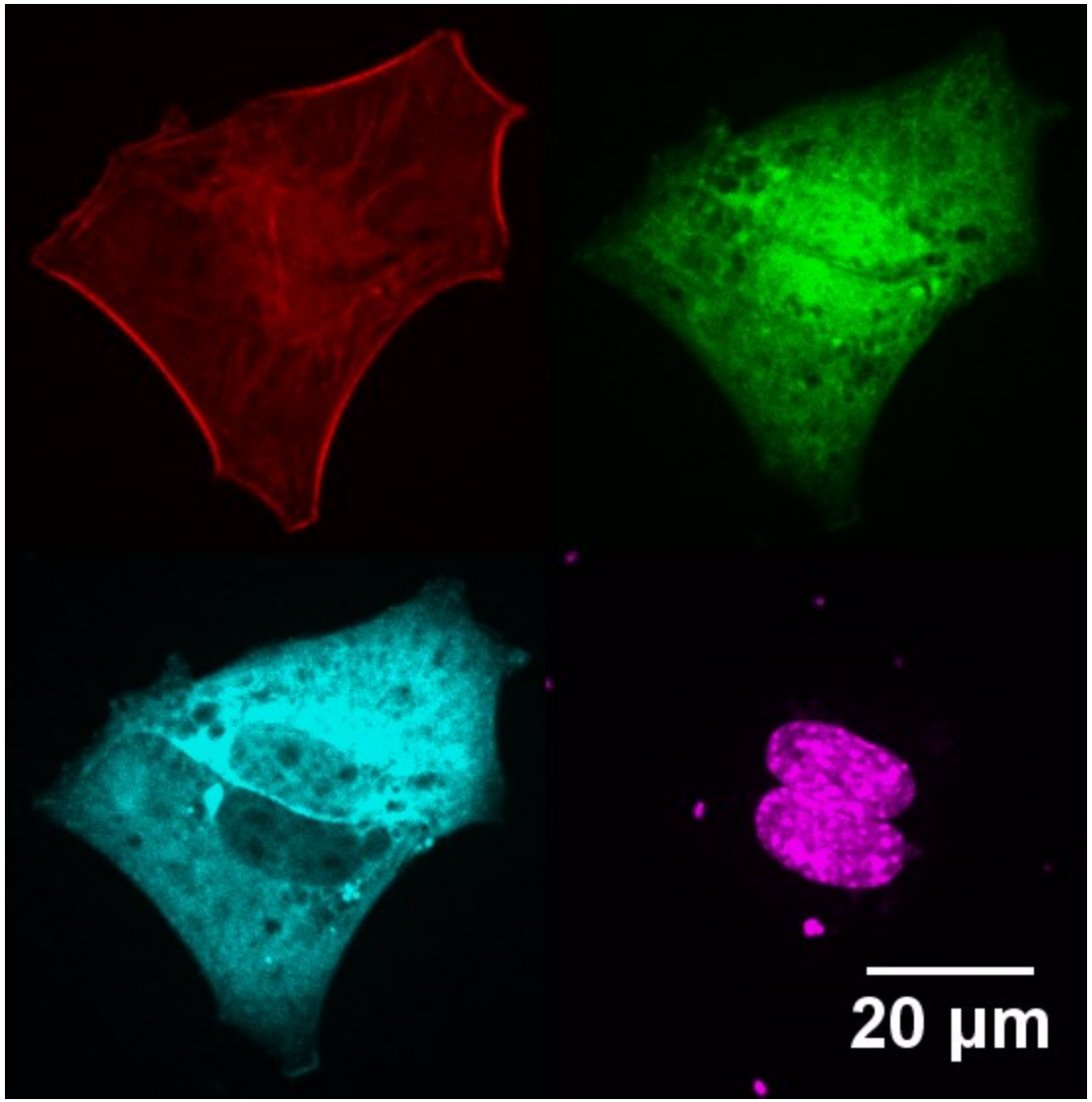
N → H

N → C

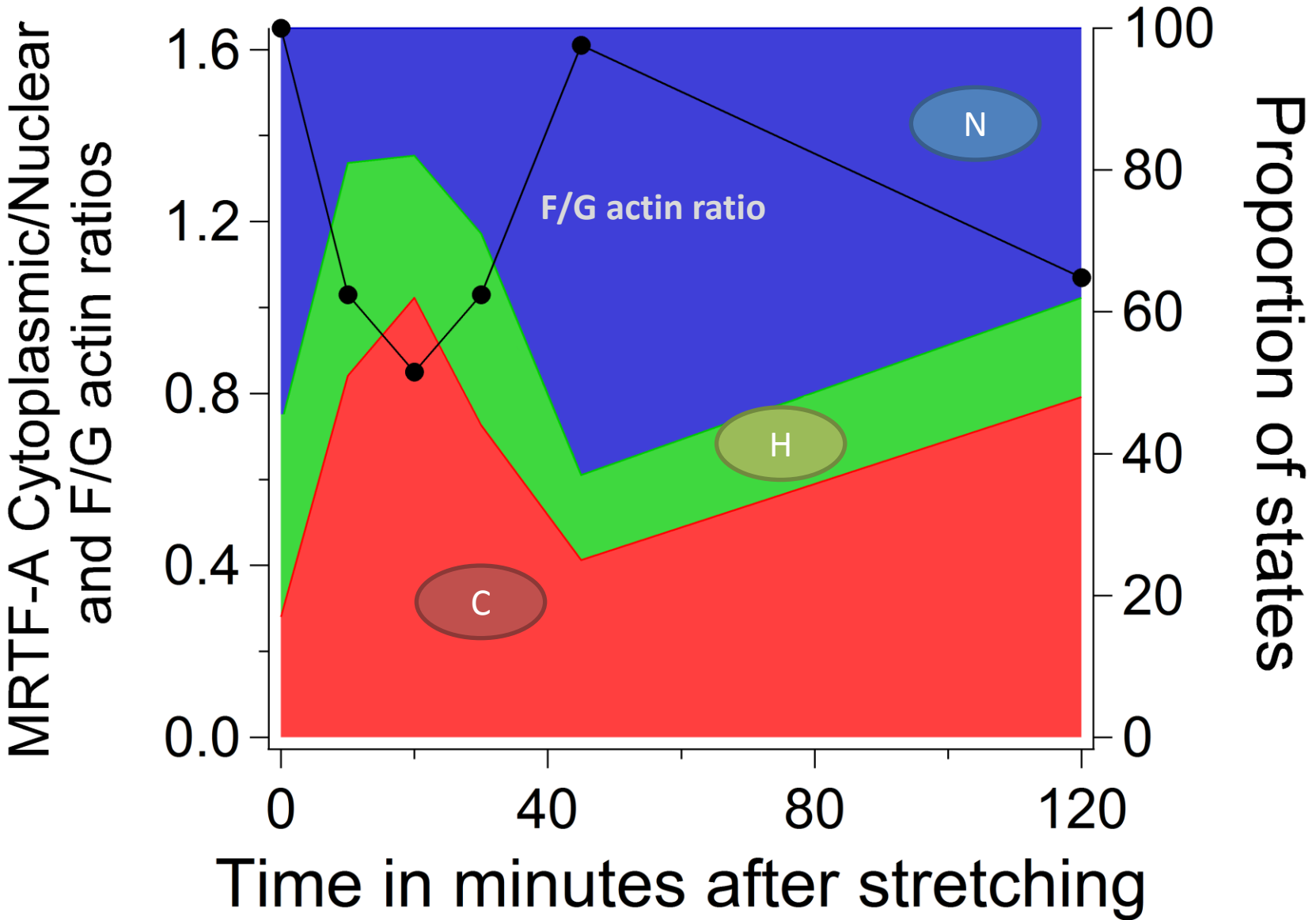
Expulsions at 30 min : Brutal destruction of actin filaments ?

F/G ratio on fixed cells

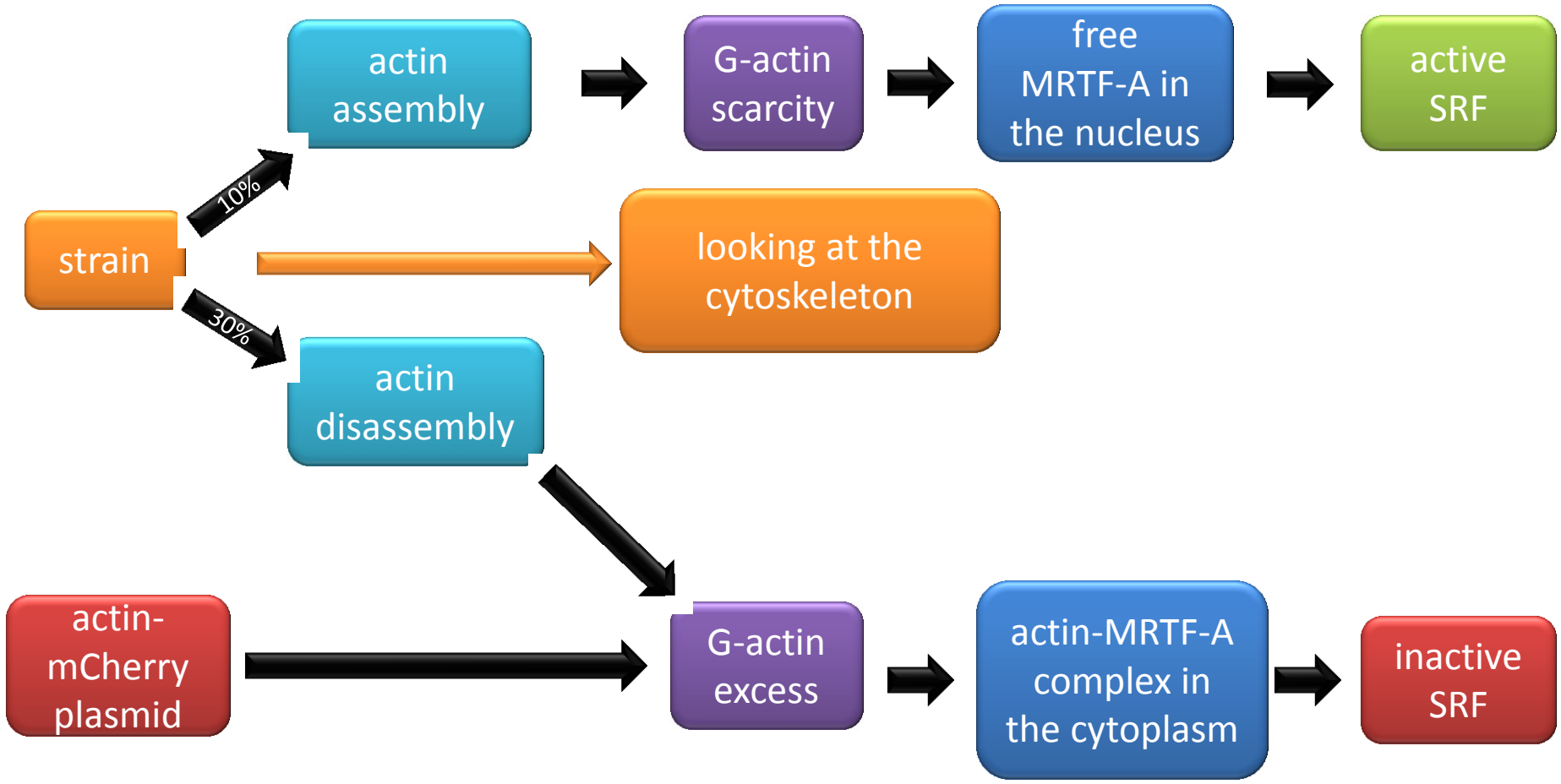
- Red : F-actin (Phalloidin)
- Green : G-actin (DNase I)
- Cyan : MRTF-A GFP
- Magenta : Nucleus (DAPI)



30% Strain Experiments on fixed cells

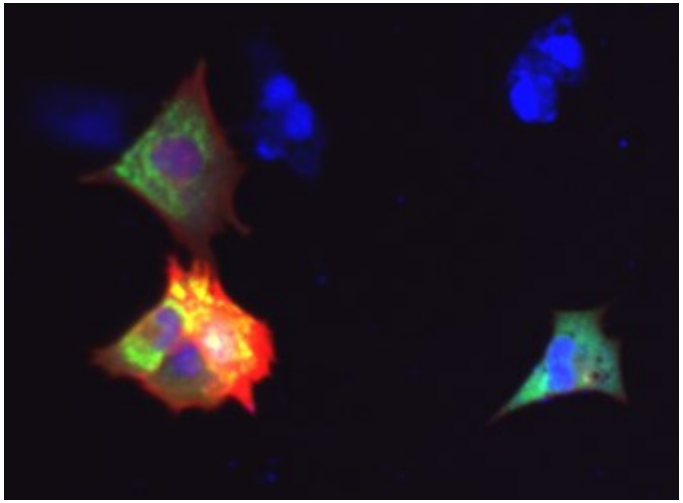


Summary


actin assembly
G-actin scarcity
free MRTF-A in the nucleus
active SRF
10%
strain
looking at the cytoskeleton
30%
actin disassembly
actin-mCherry plasmid
G-actin excess
actin-MRTF-A complex in the cytoplasm
inactive SRF

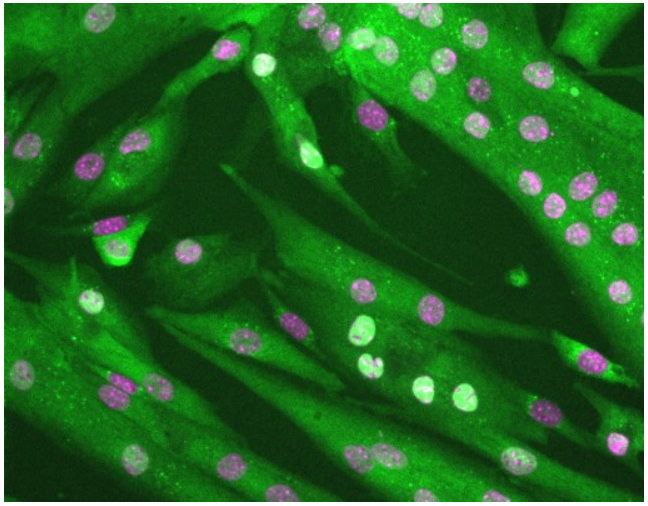
Perspective

Live visualization of the cytoskeleton (SiRactin, F-tractin)



+

AAV-infected MRTF-A GFP primary myoblasts and differenciated myotubes



What is the pathway from mechanics to actin polymerization ?

Which target genes are activated ?

Acknowledgements

Complex Systems and Materials
University Paris Diderot
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A. Pincini
A. Richert
L. Réa

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