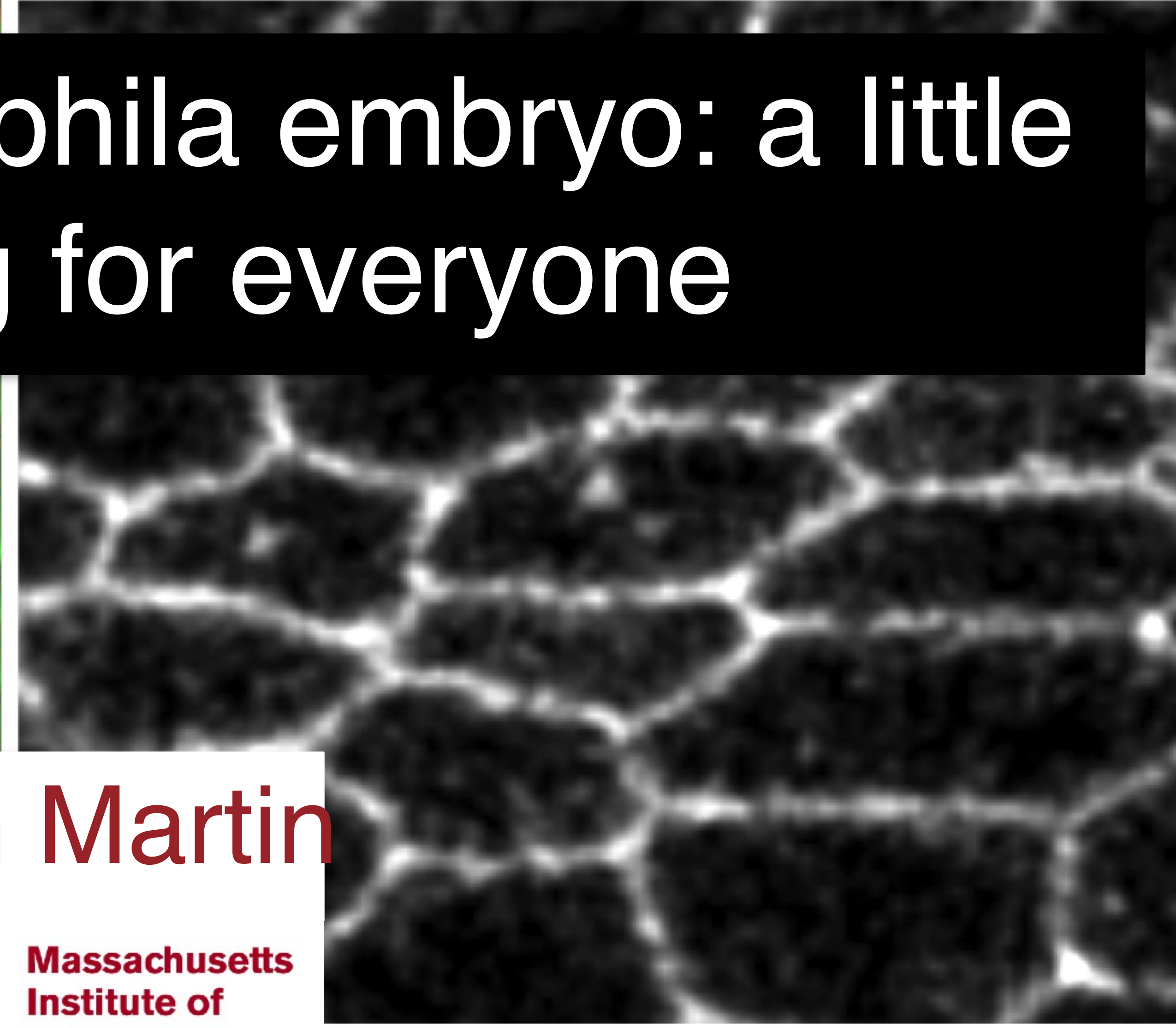
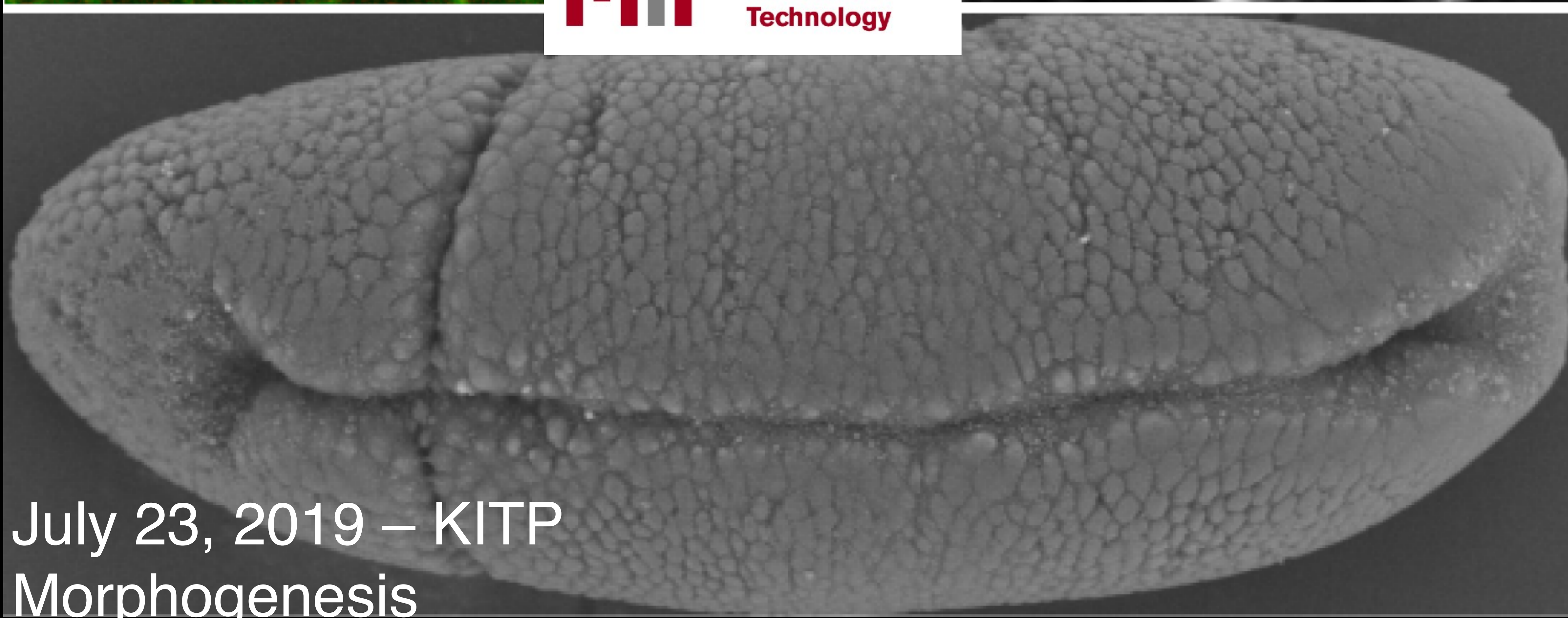
The early Drosophila embryo: a little something for everyone



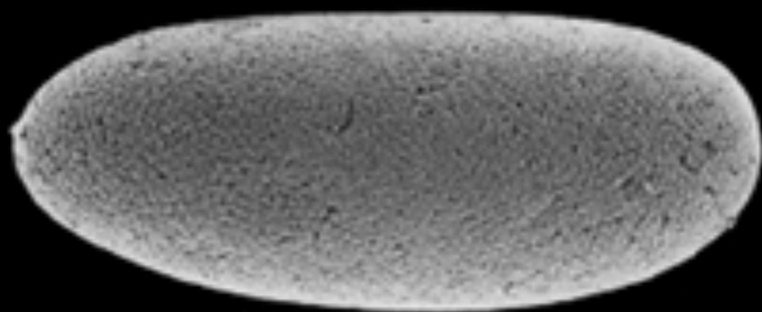
Adam Martin

Massachusetts Institute of Technology



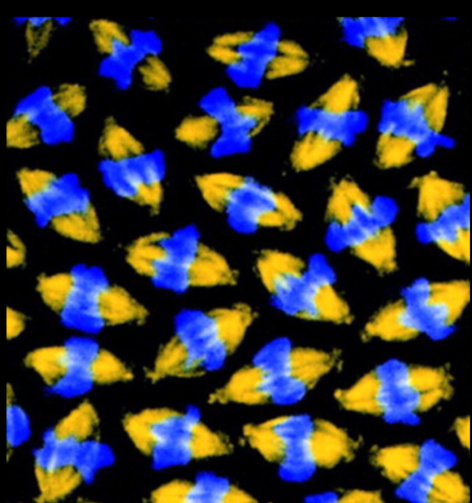
July 23, 2019 – KITP Morphogenesis

Drosophila embryo: Everything a Biophysicist could want (in a couple hrs)



Time (4 hours)

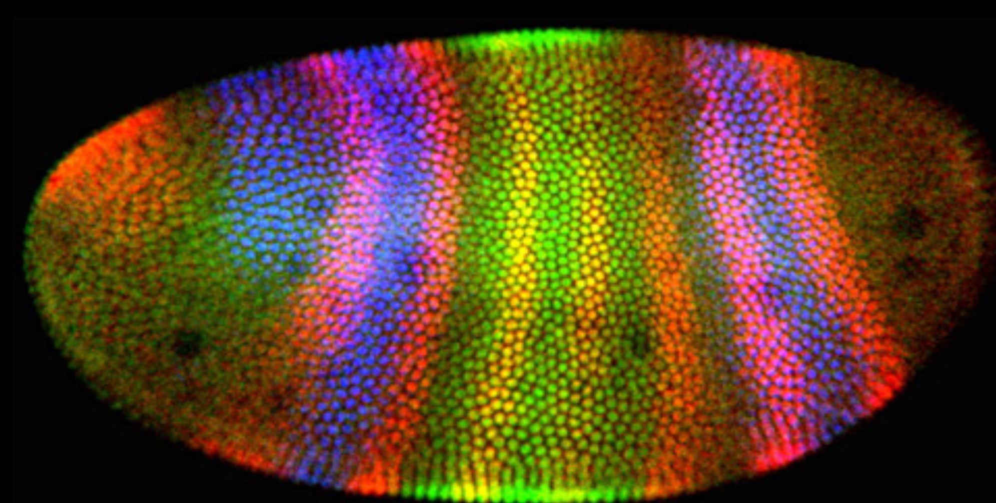
1) Nuclear division



Microtubules

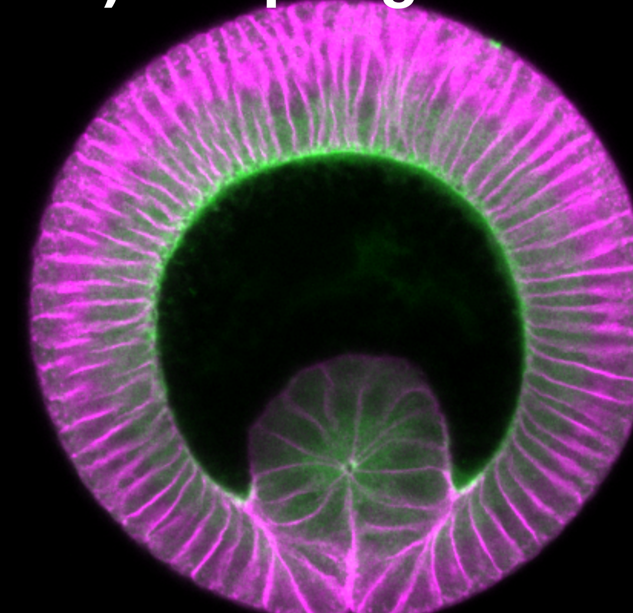
DNA

2) Patterned gene expression
3) Cellularization



Different transcription factors

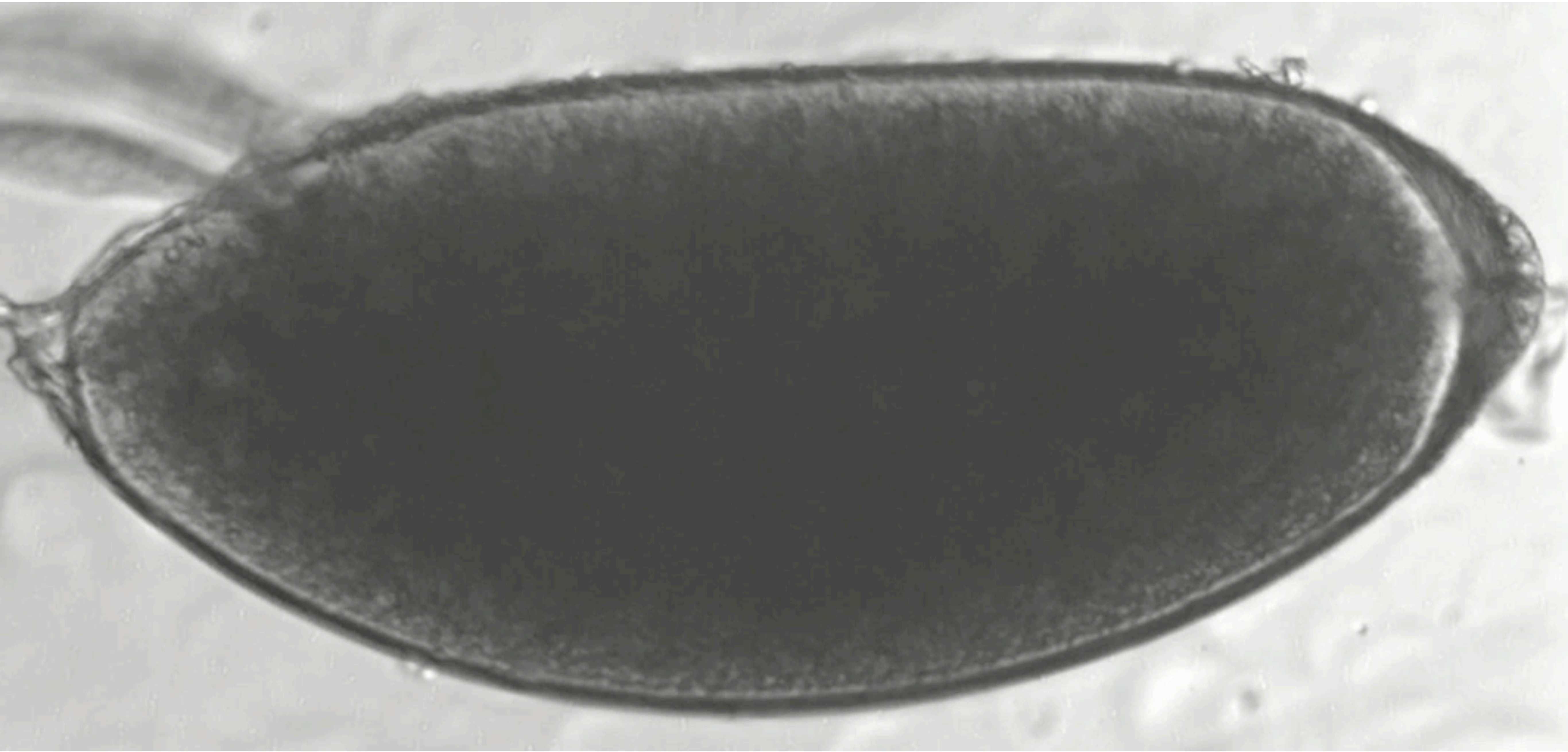
4) Morphogenesis



Plasma Membrane

Myosin motor

Development involves global tissue movements



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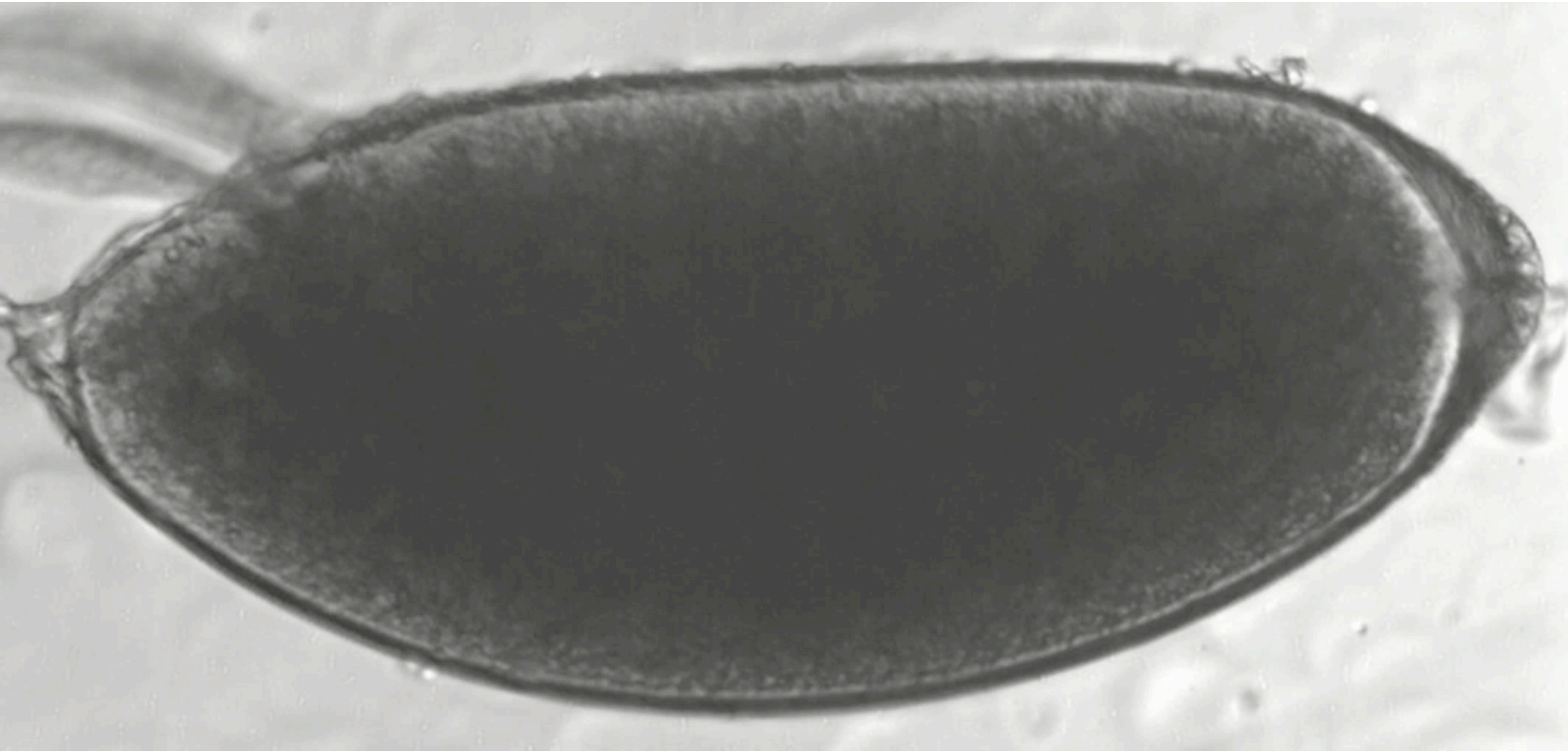

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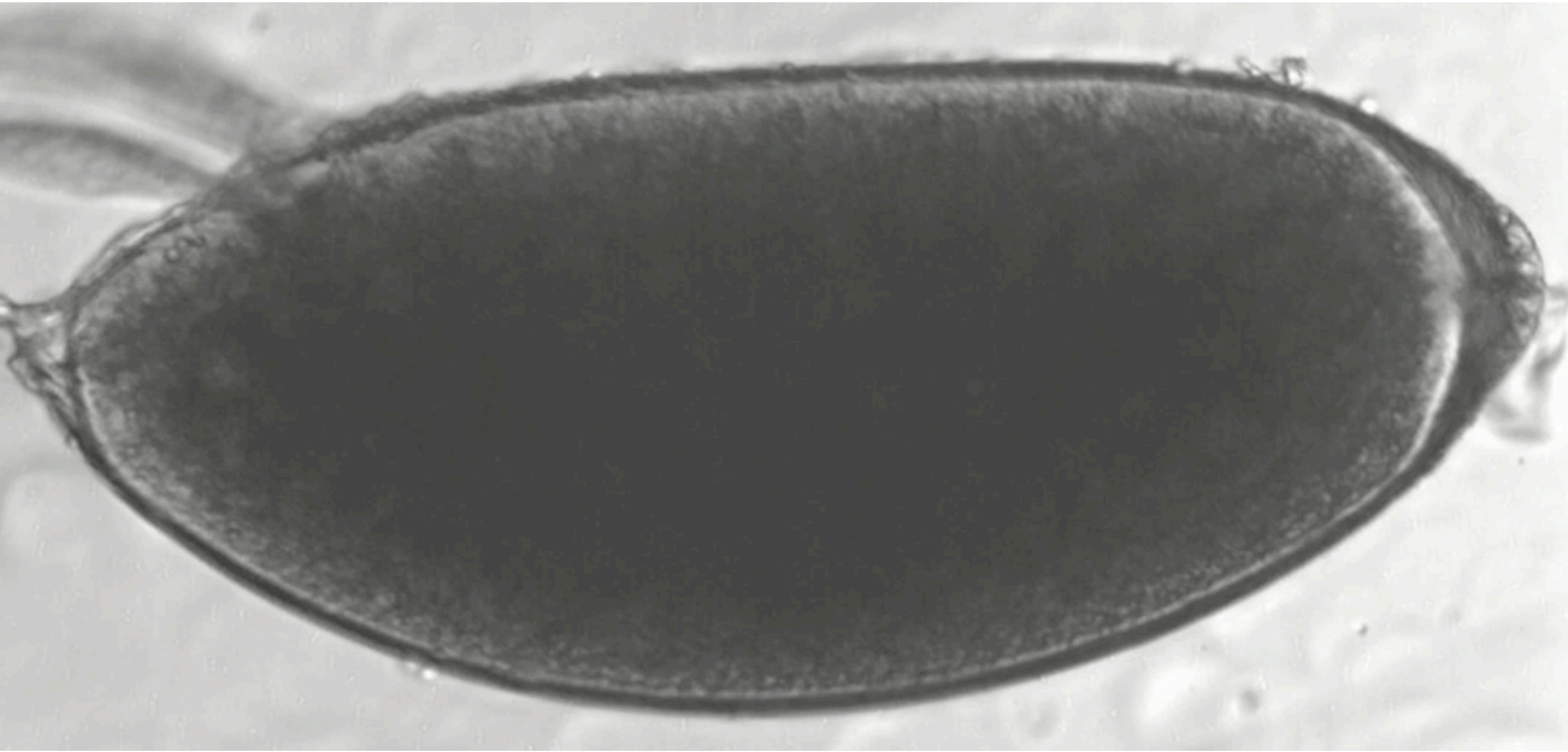
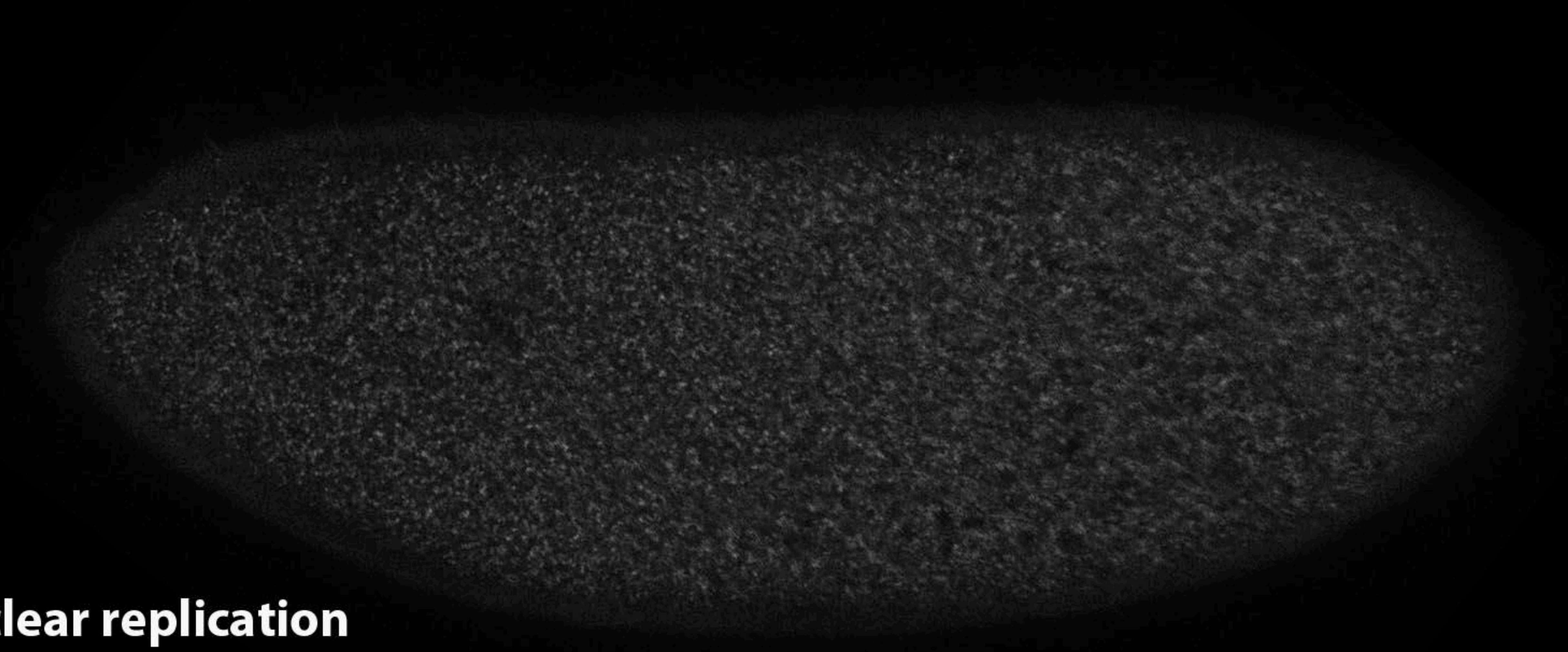


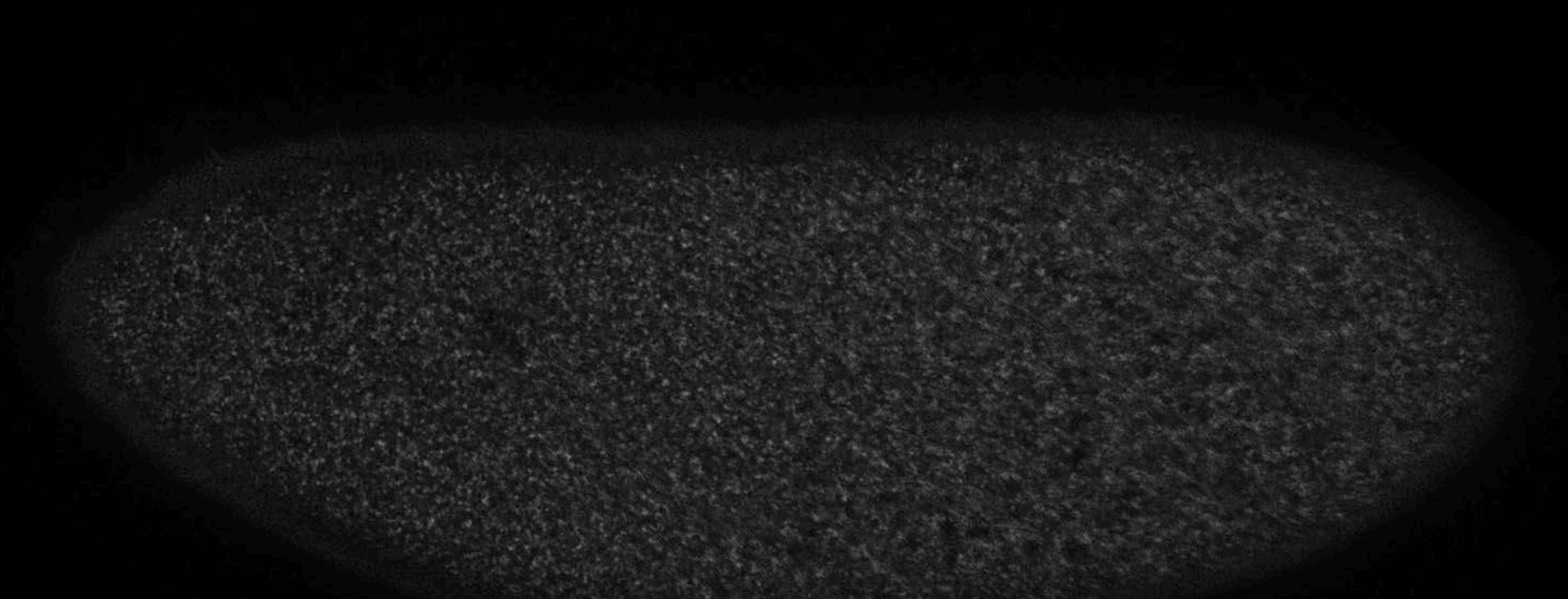
Cell cycle transitions during early development



nuclear replication
(cell cycle ~nine minutes)

Eric Wieschaus

Cell cycle transitions during early development



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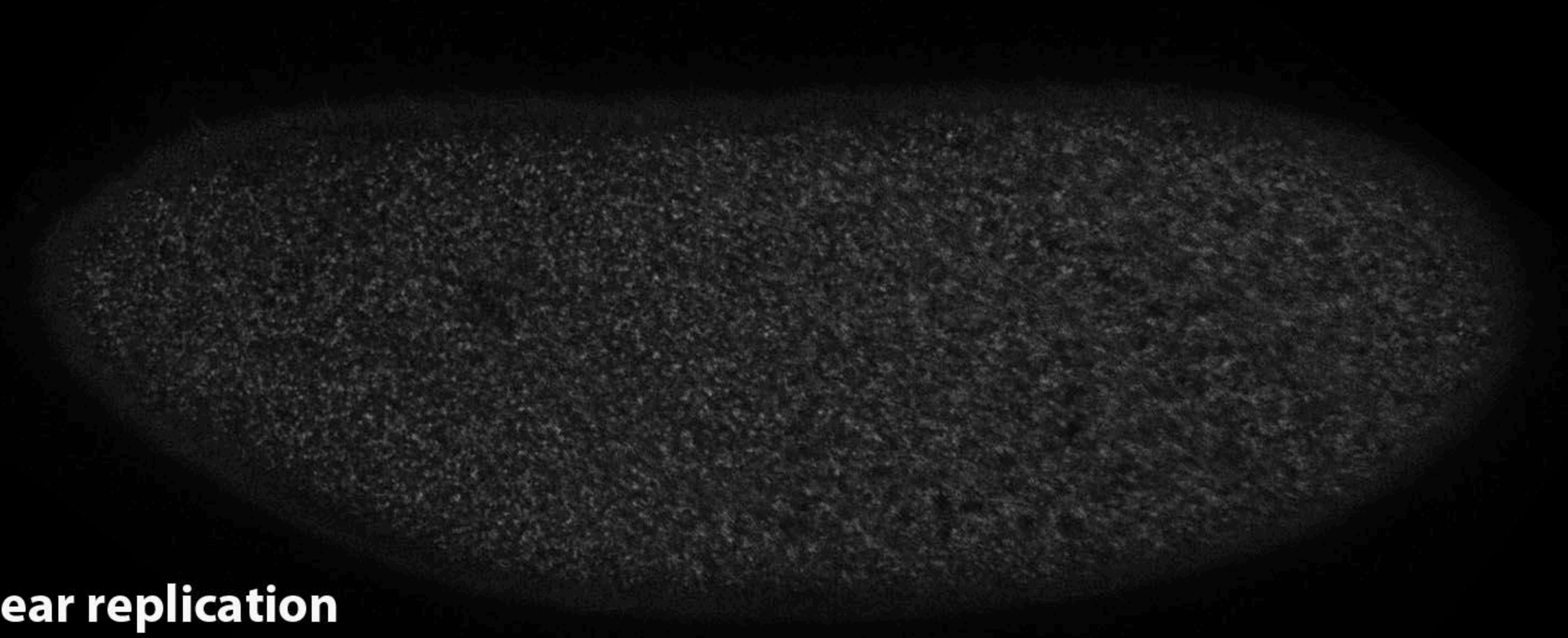
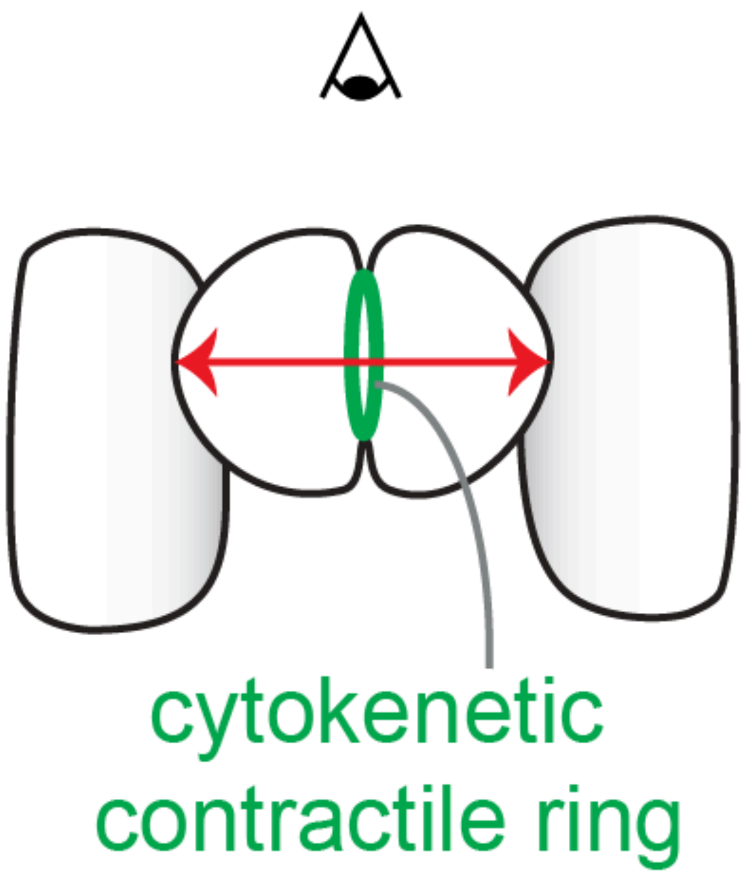
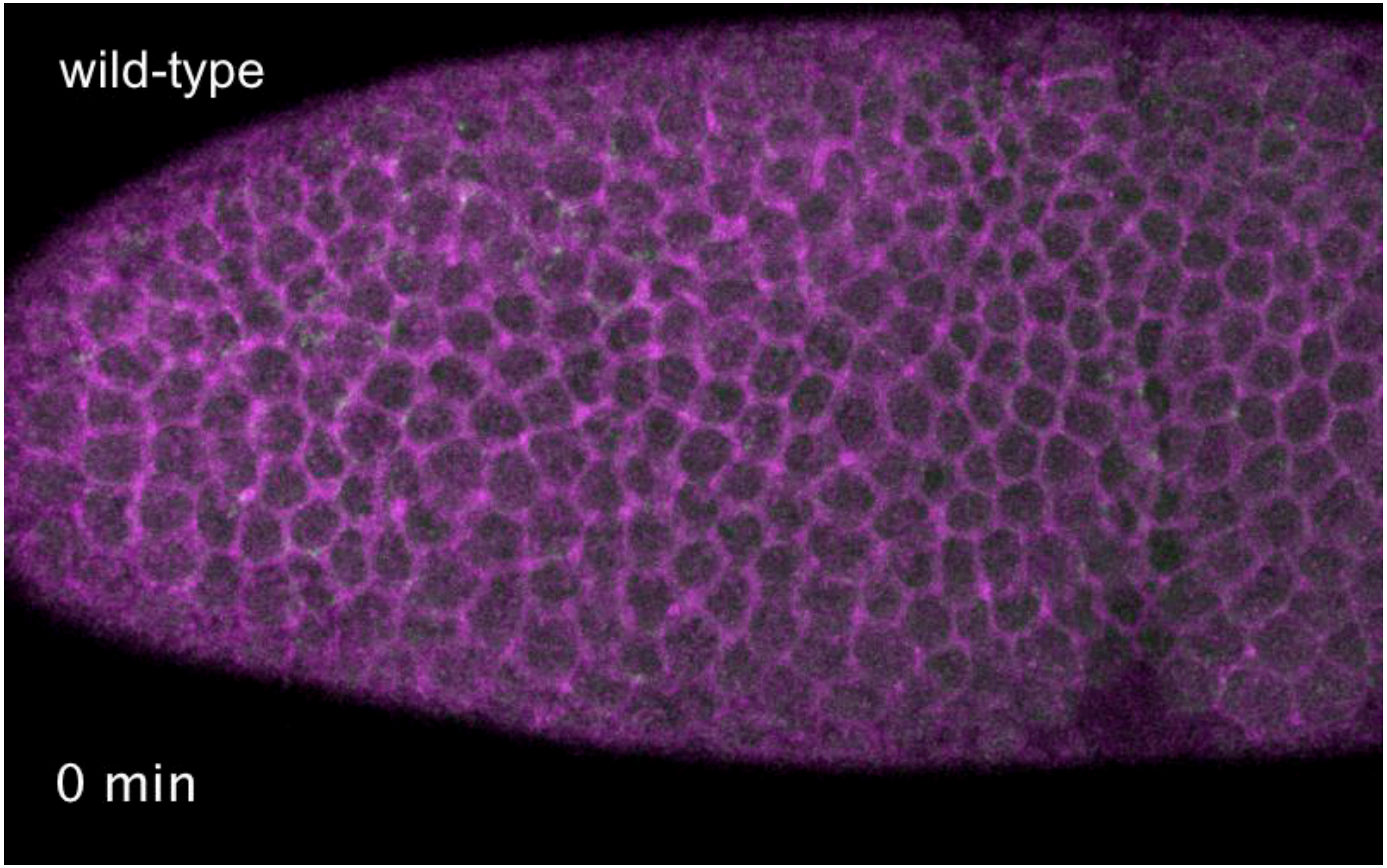


nuclear replication
(cell cycle ~nine minutes)

Eric Wieschaus

After cells form, cell division occurs in mitotic domains





Chanet et al., 2017

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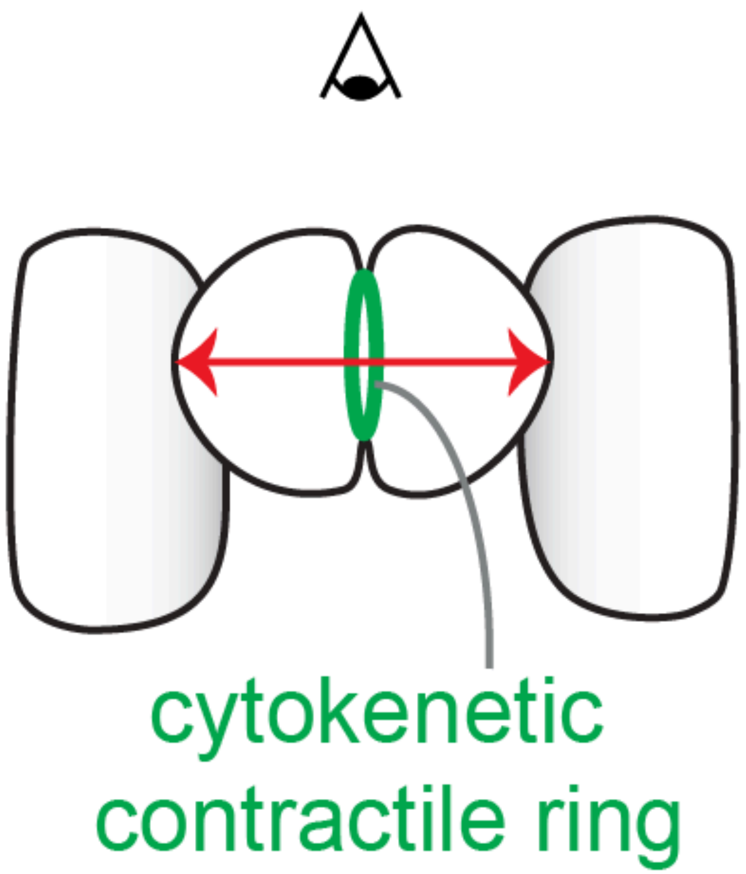

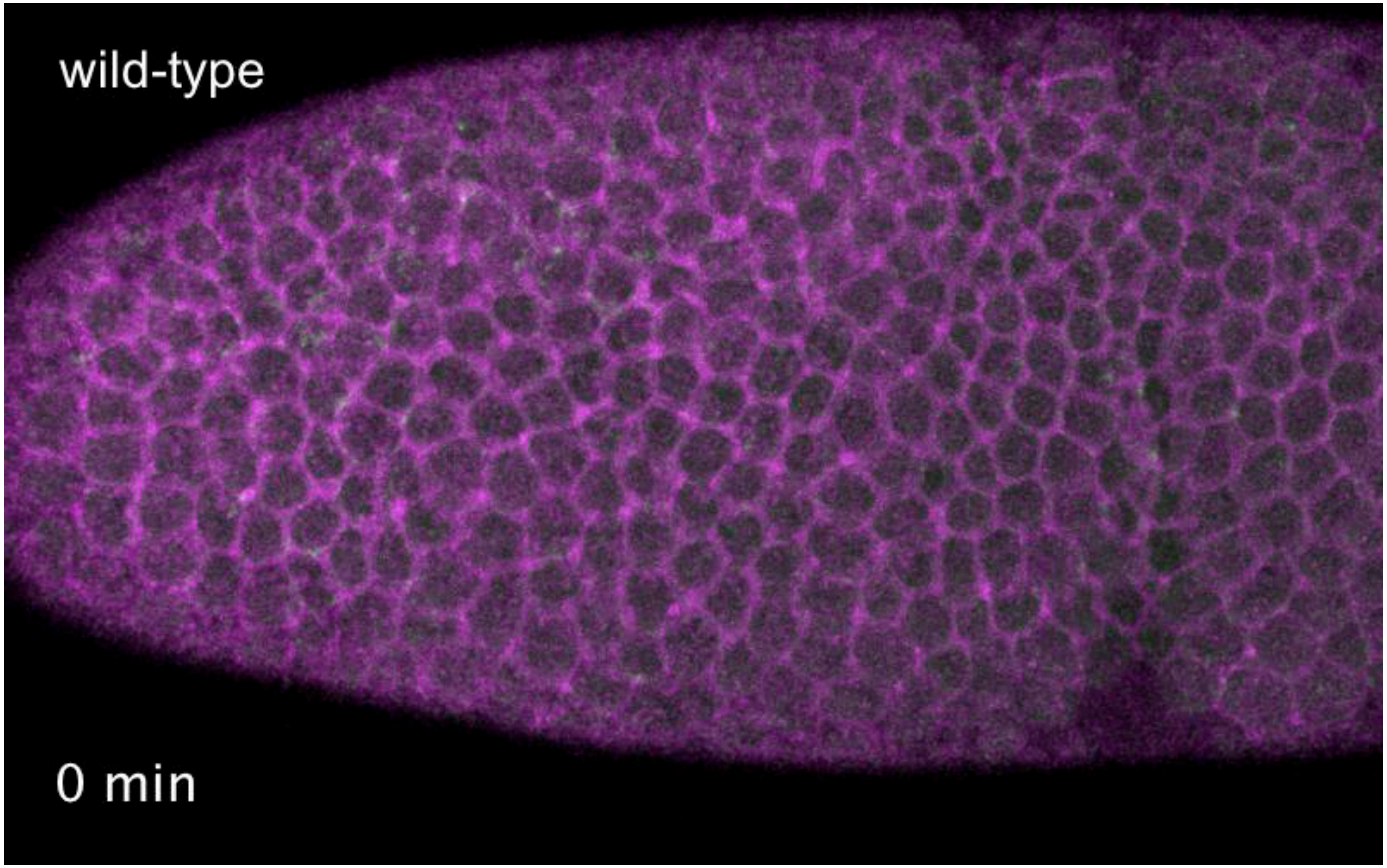

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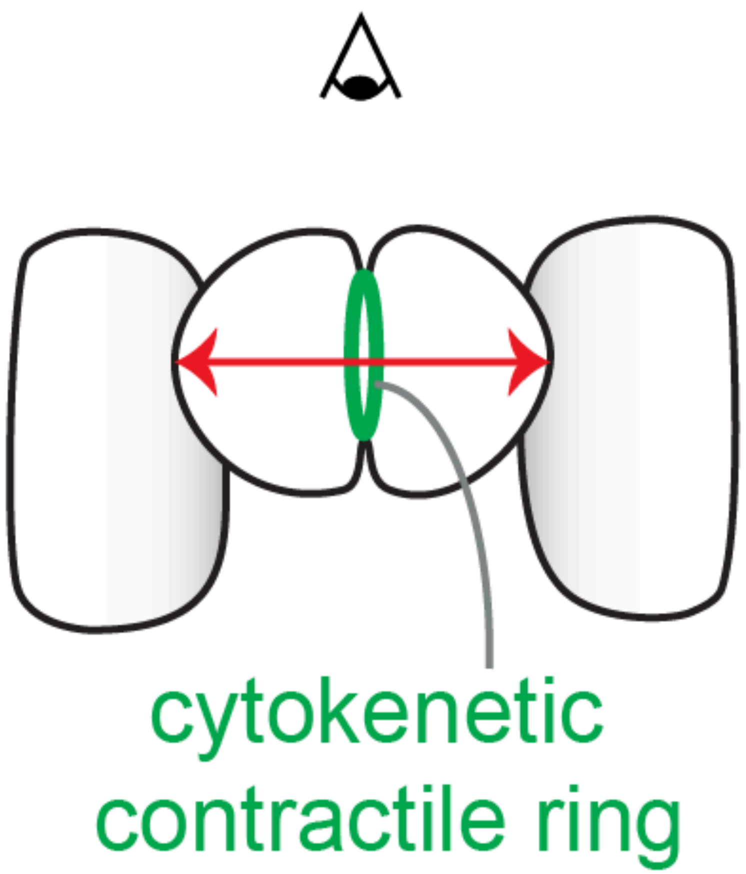
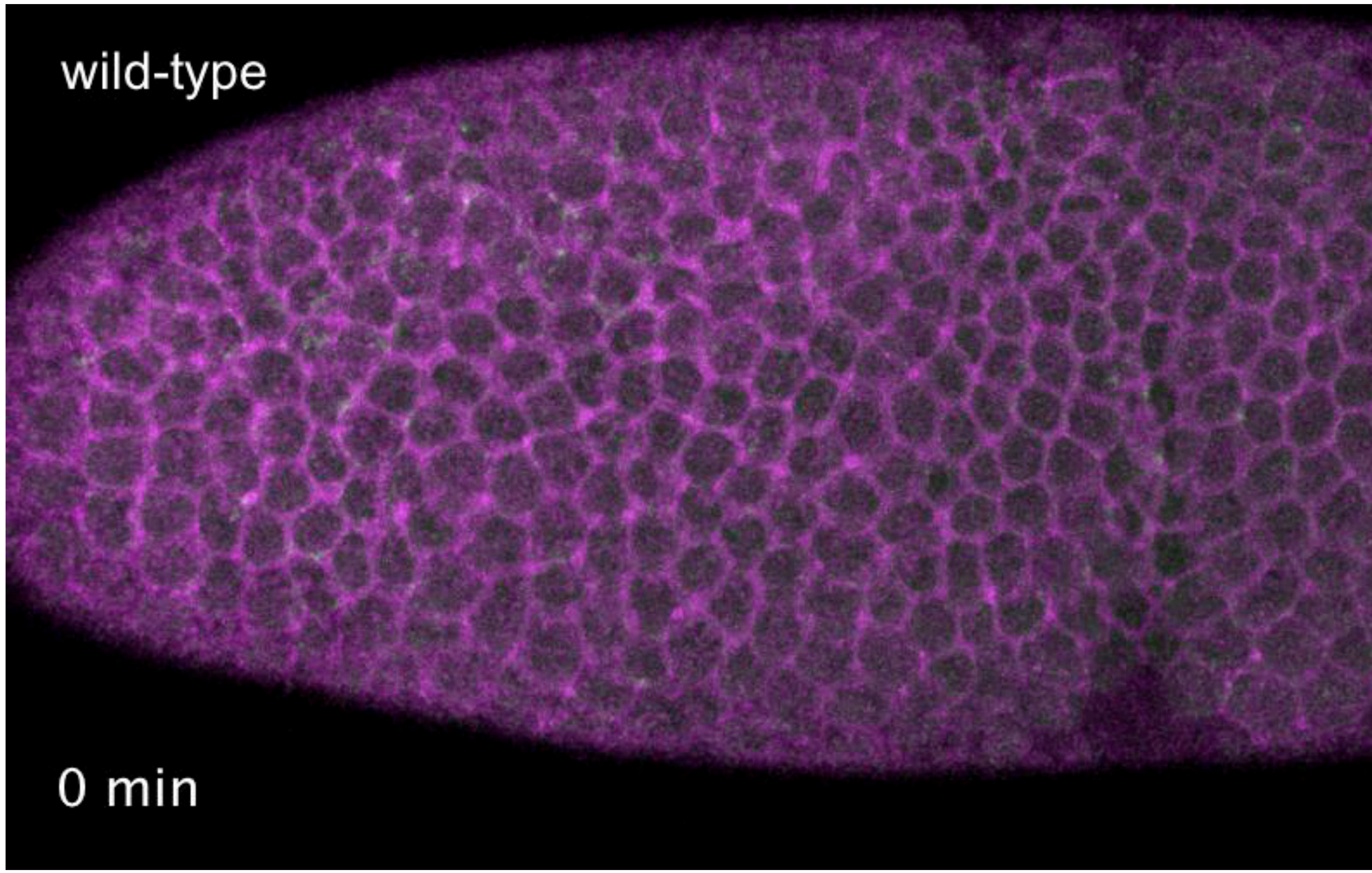




Chanet et al., 2017

Making heads or tails of development

Embryo

Making heads or tails of development

Embryo

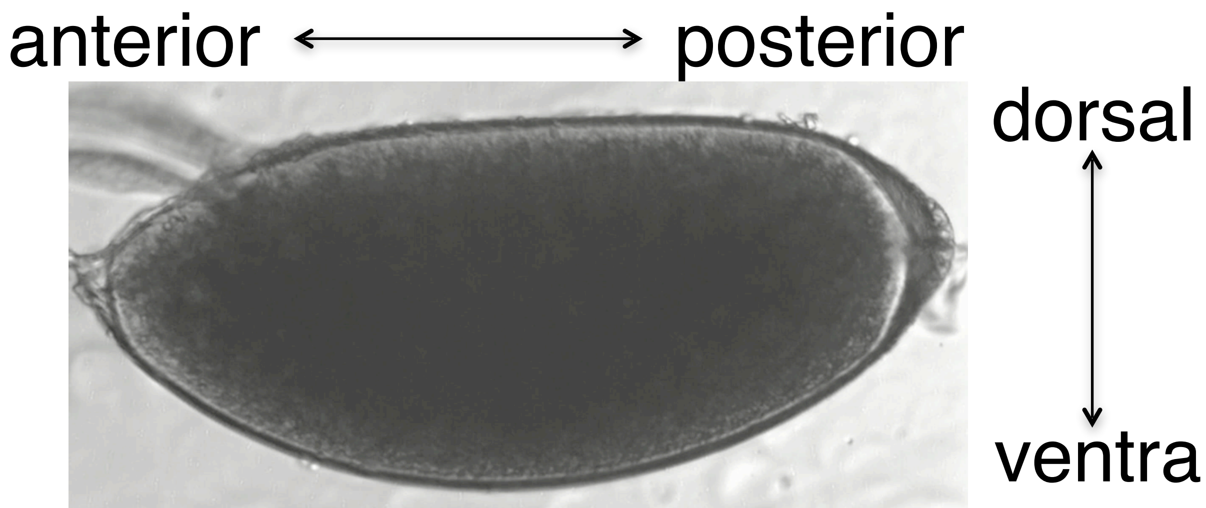
Making heads or tails of development

anterior ⟷ posterior

Embryo

Making heads or tails of development

Embryo



Making heads or tails of development

anterior ⟷ posterior

Embryo

Head and thorax

Abdominal segments

Larva

Making heads or tails of development

anterior ↔ posterior

dorsal

ventral

Embryo

Head and thorax

Abdominal segments

Larva

Adult

Making heads or tails of development

anterior ↔ posterior

dorsal ↕ ventral

Embryo

Head and thorax

Abdominal segments

Larva

Adult

Maternal effect gene

↓

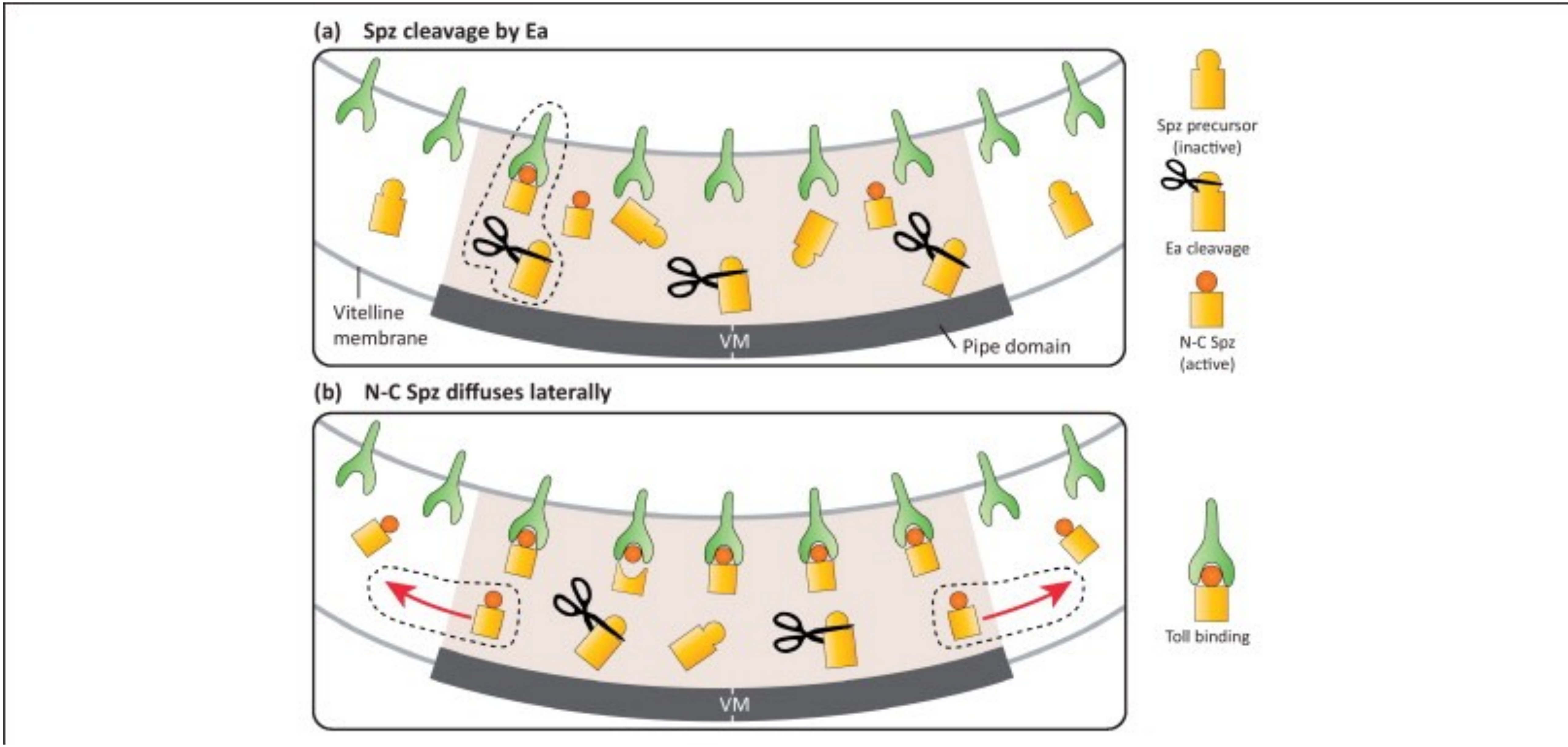
Zygotic genes

↙ ↘

Cell fate

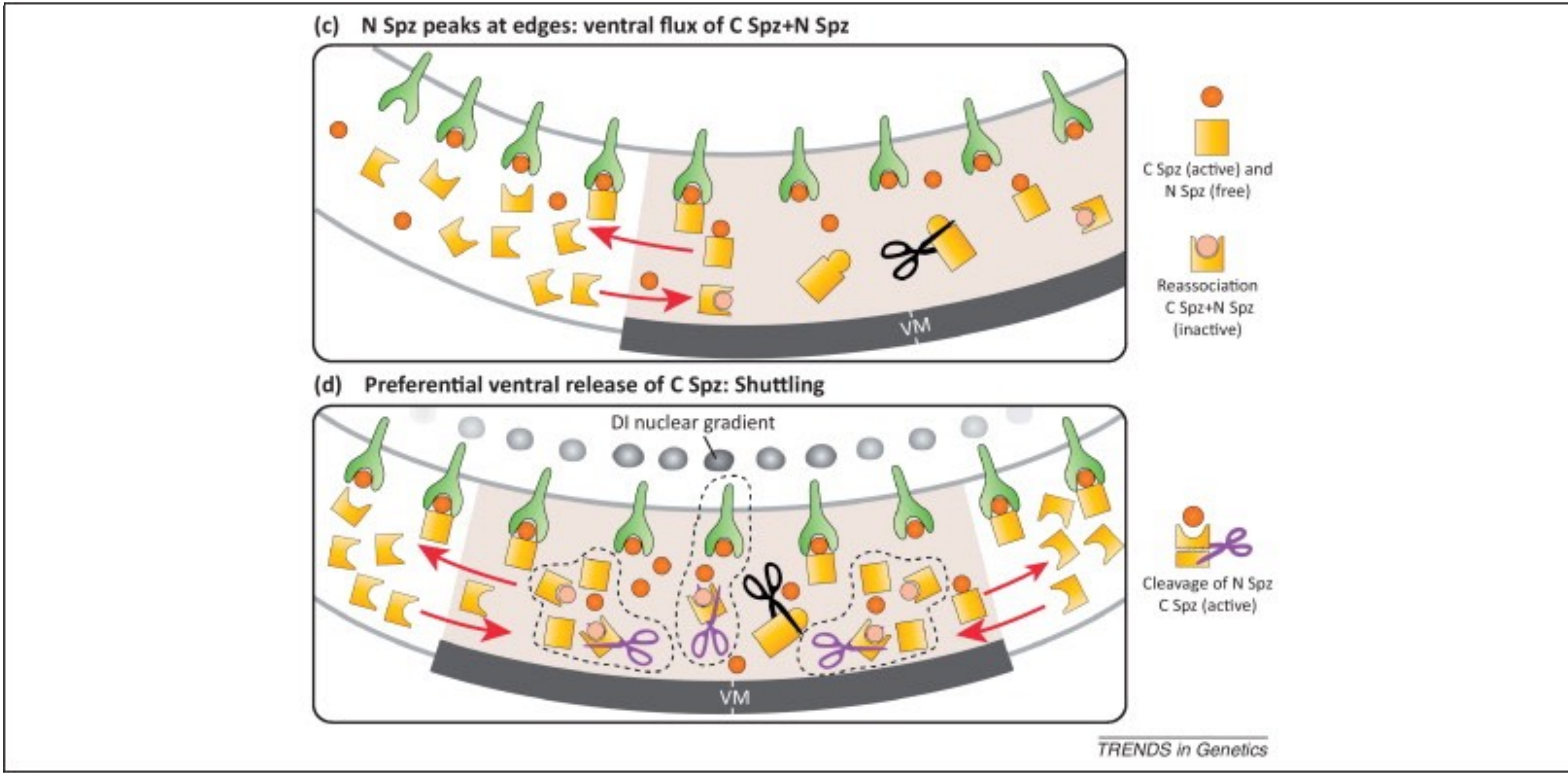
Forces/Form

Morphogen signaling leads to gradient in nuclear Dorsal



Shilo et al., 2013

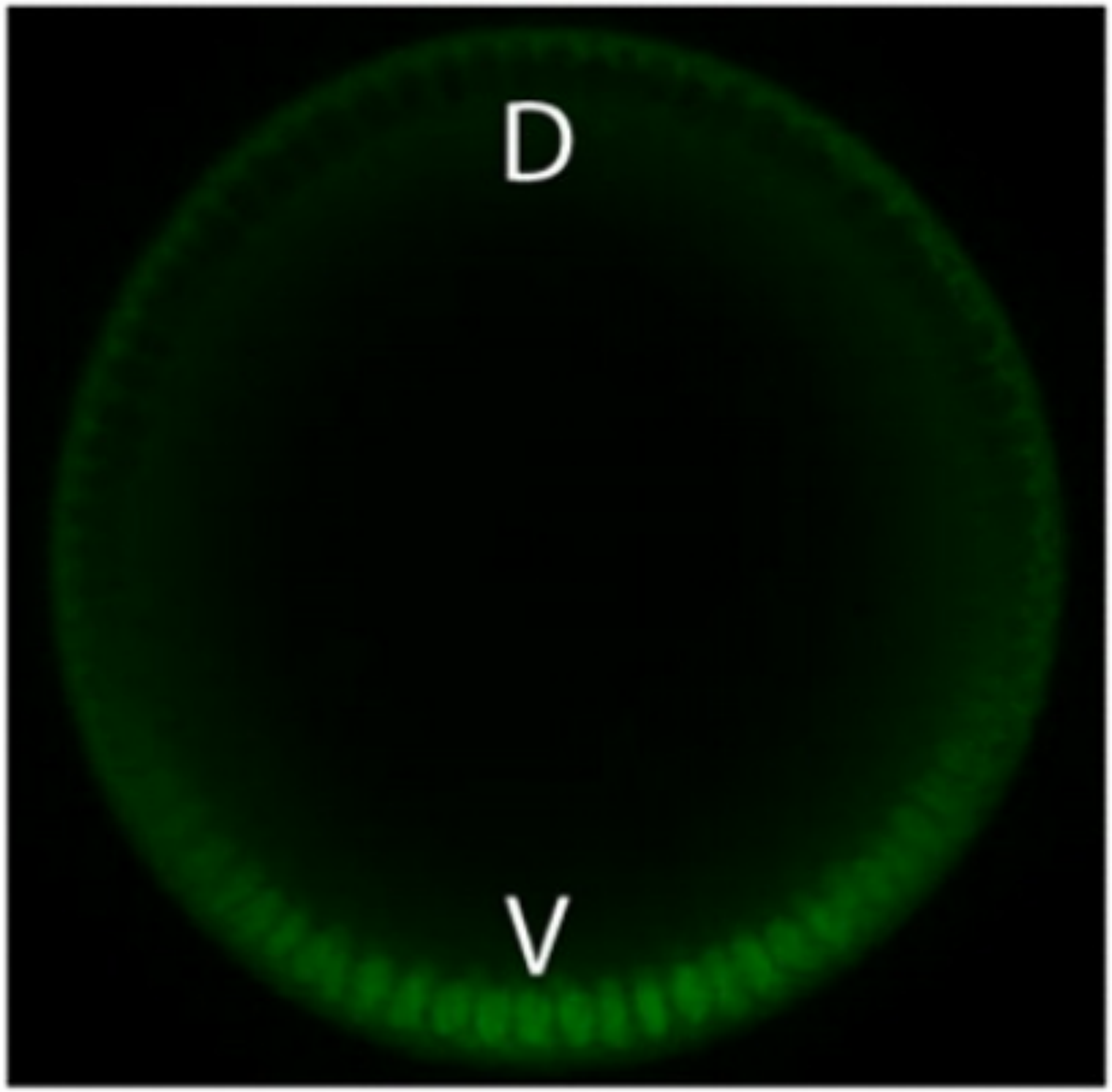
Morphogen signaling leads to gradient in nuclear Dorsal



Shilo et al., 2013

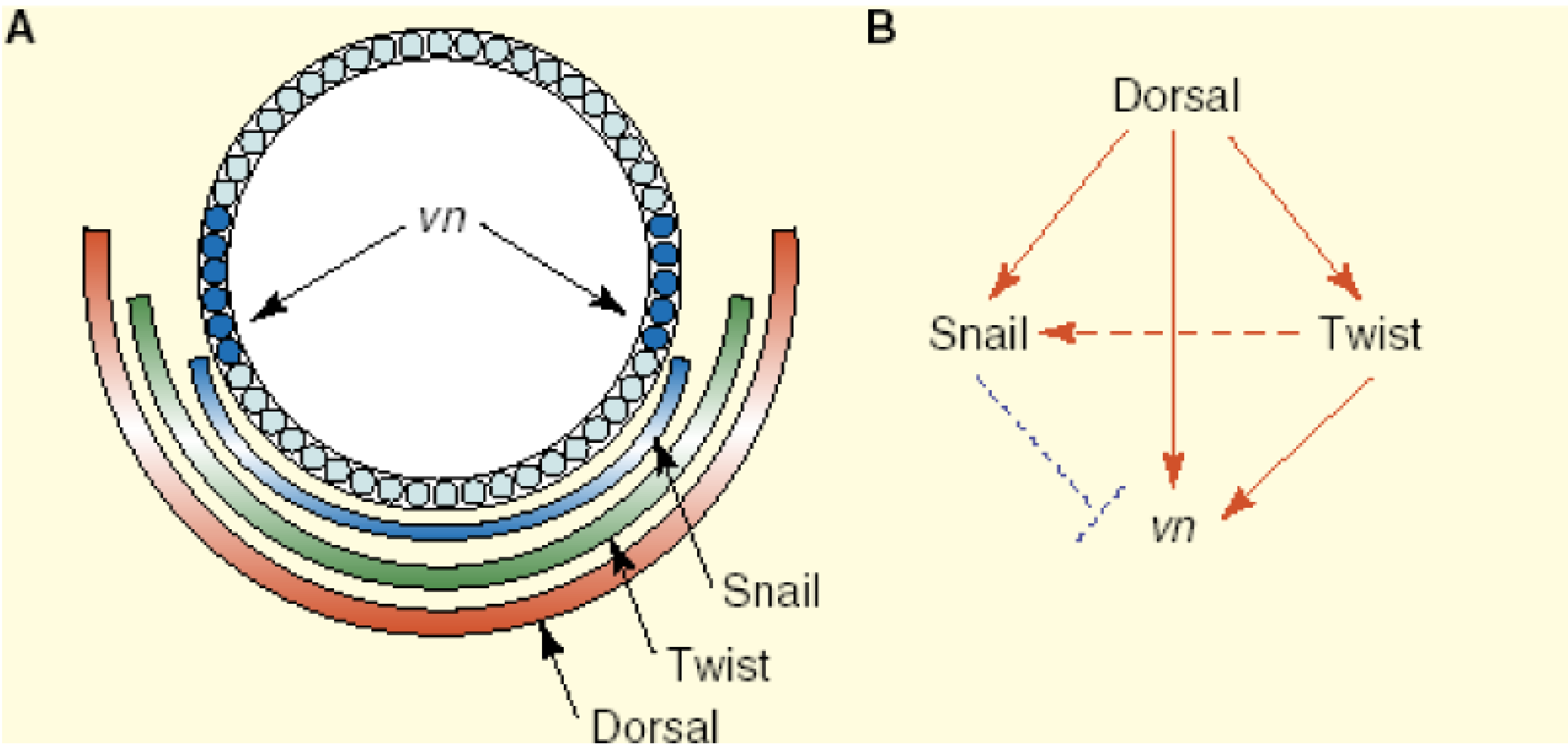
The Dorsal protein defines the ventral side of the embryo

Dorsal protein (NF-kB transcription factor)



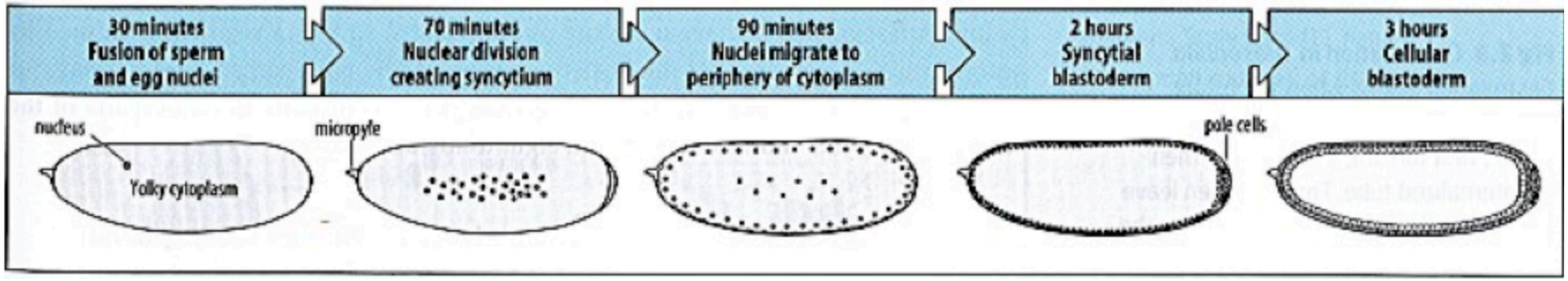
Kanodia et al., 2009

Dorsal concentration and cross-regulation establish domains of gene expression



M. Levine's lab

Cellularization: 1 to 6,000 cells in 1 hour



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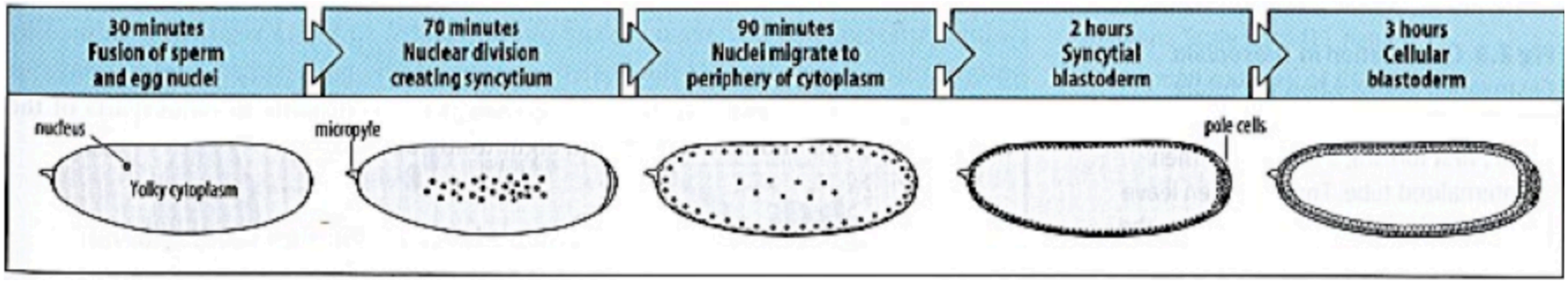

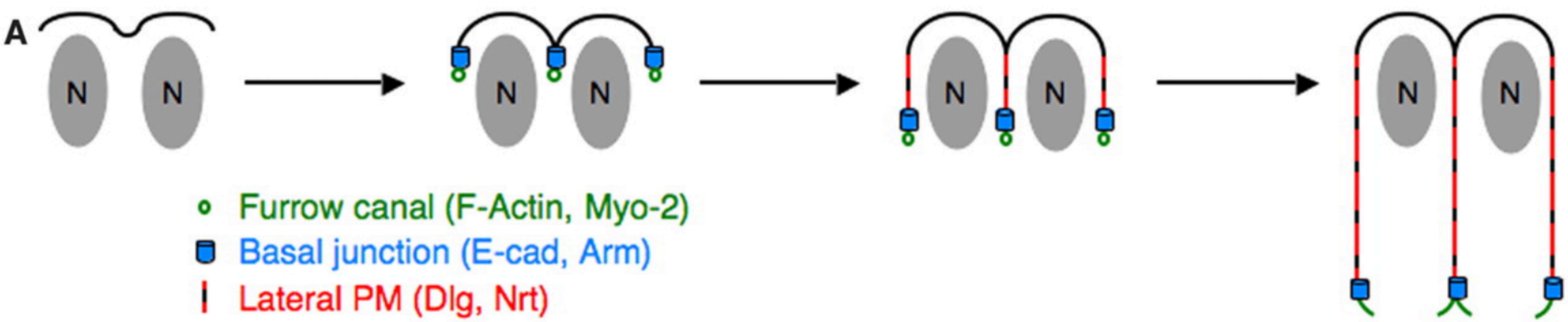

Sokac & Wieschaus, 2008

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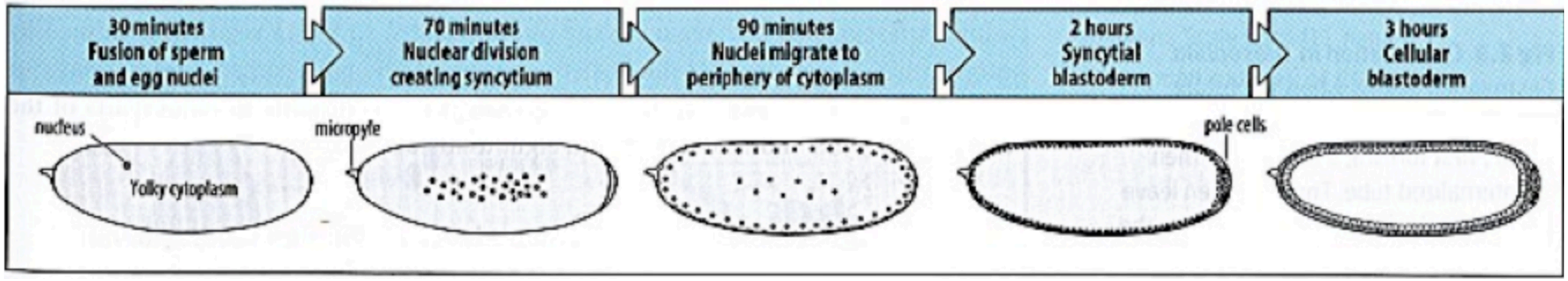
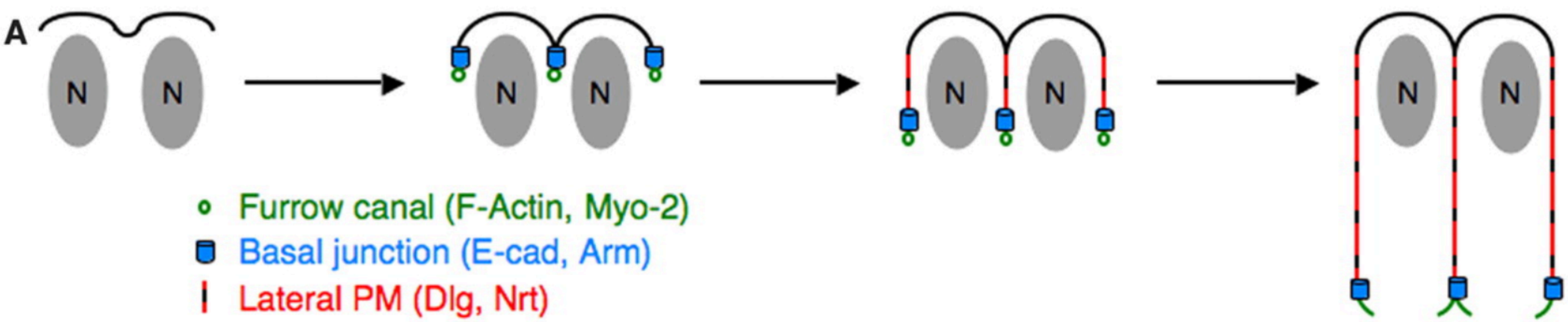
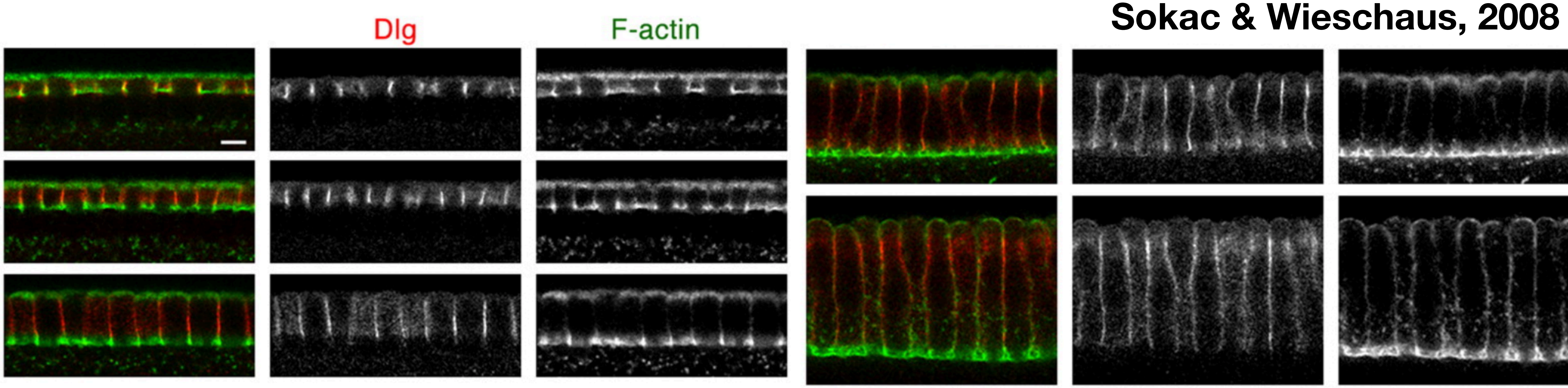
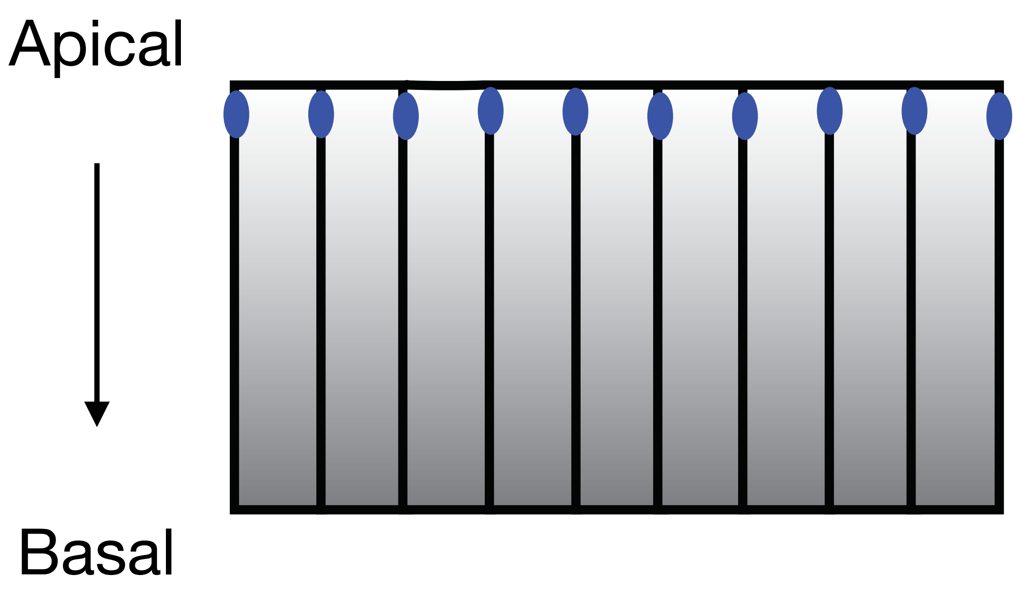




Sokac & Wieschaus, 2008



Cells that form are epithelial in nature



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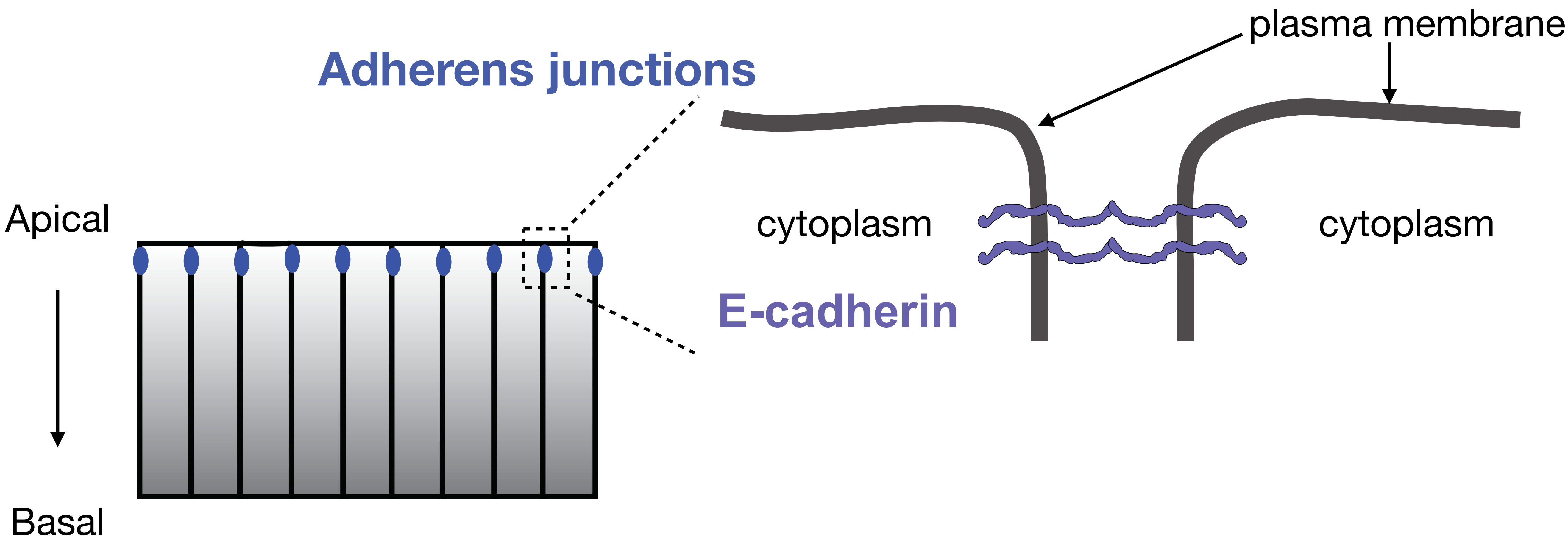
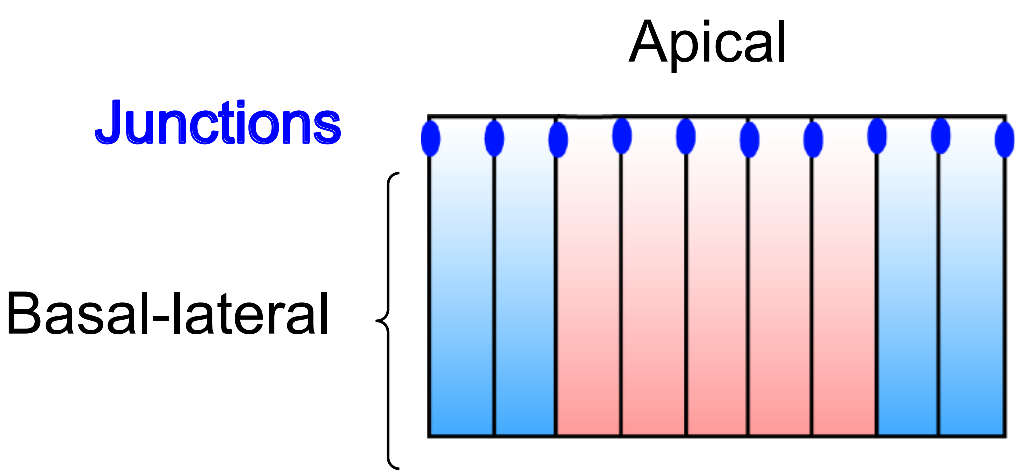


Epithelial cells undergo various shape changes and rearrangements to sculpt tissues



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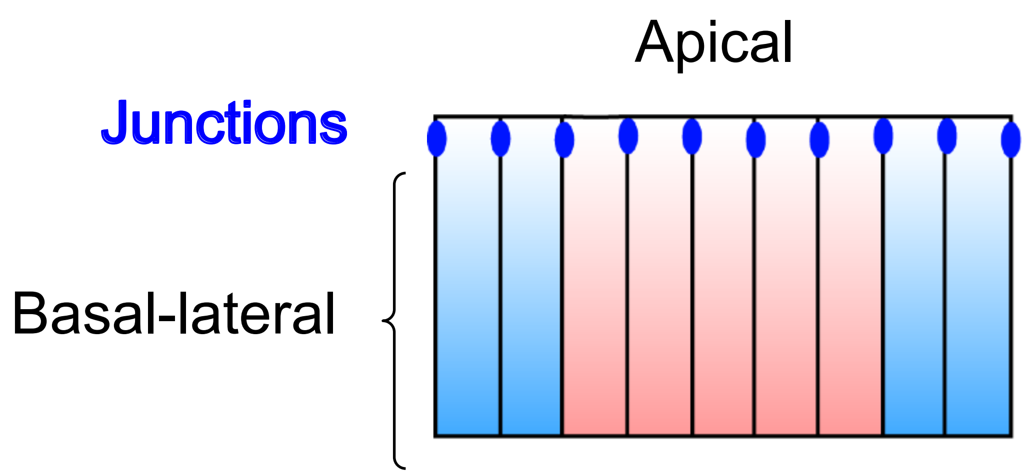
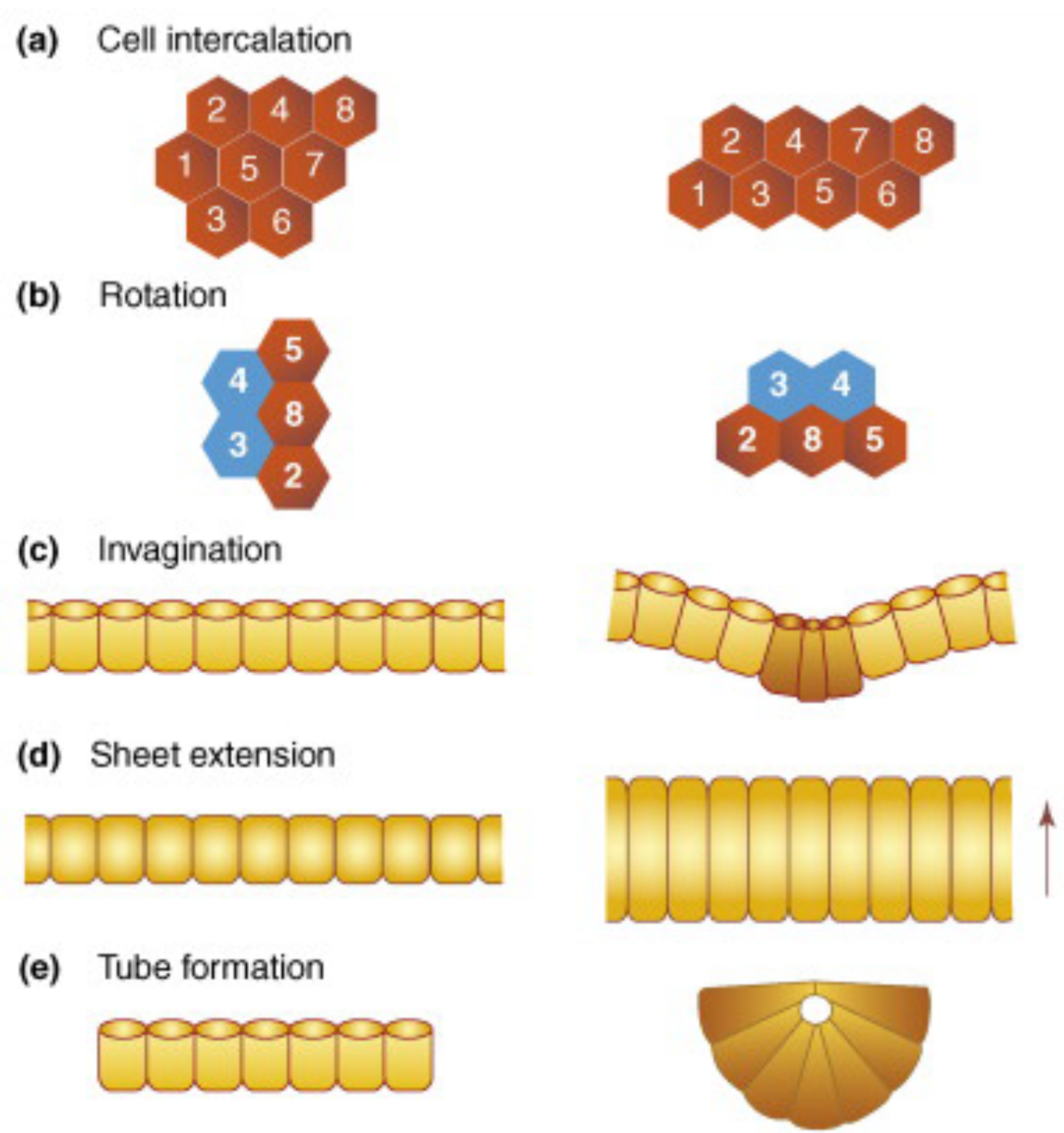
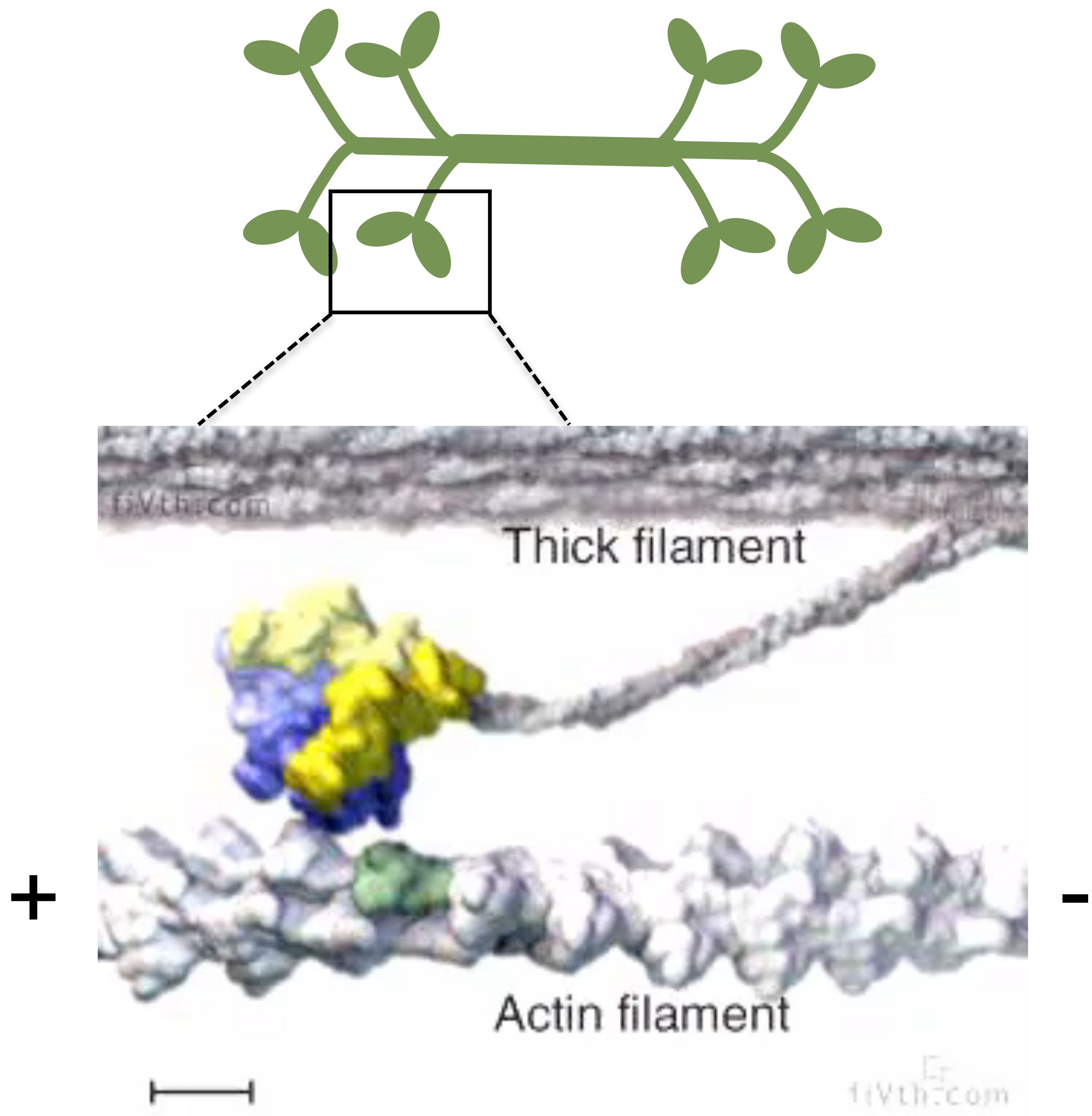




Quintin . . . Labouesse.
Trends Genet. 2008.

Myosin 2 (myosin) is thought to generate force to drive morphogenesis



Vale & Milligan, *Science* (2000).

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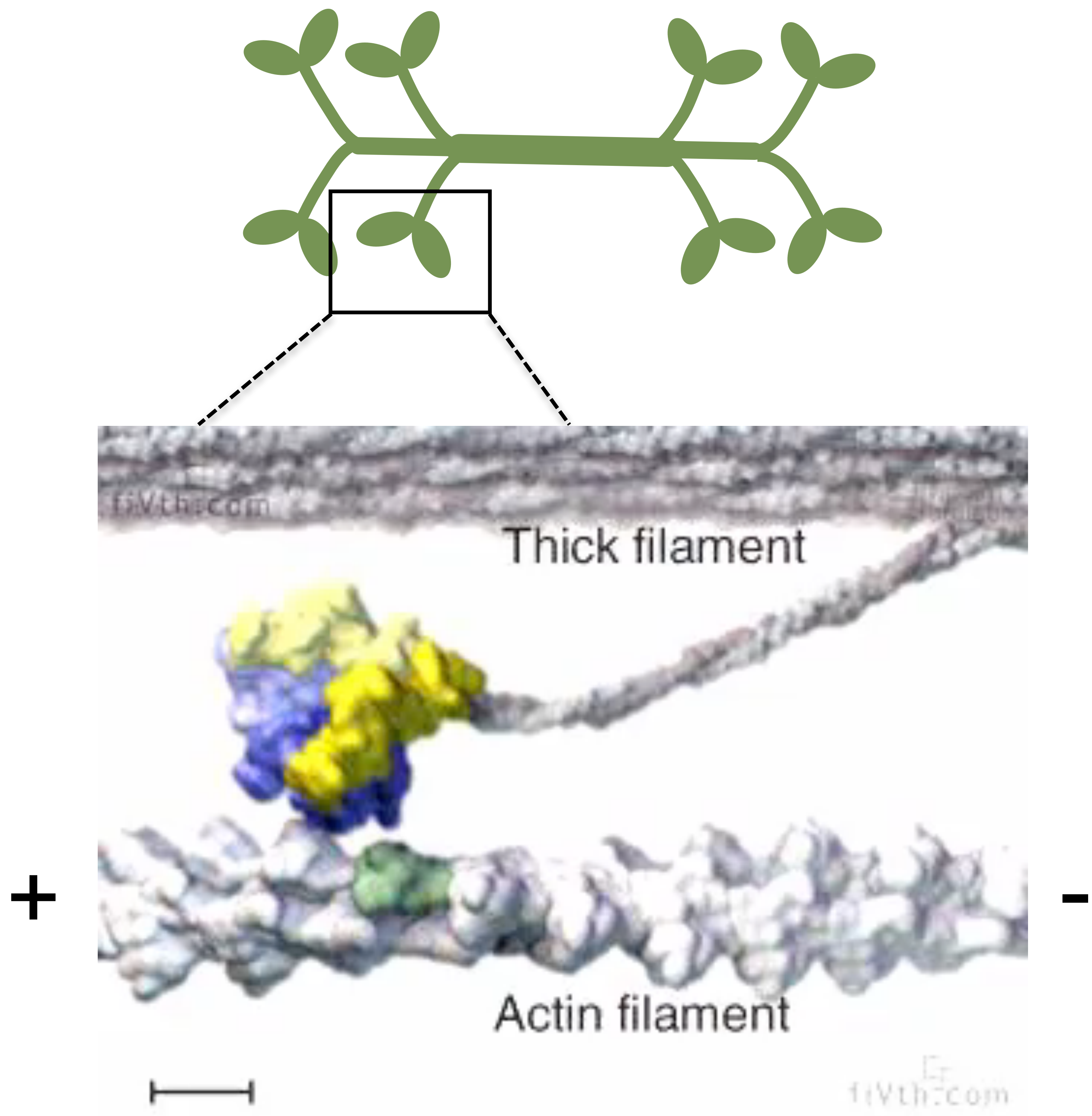

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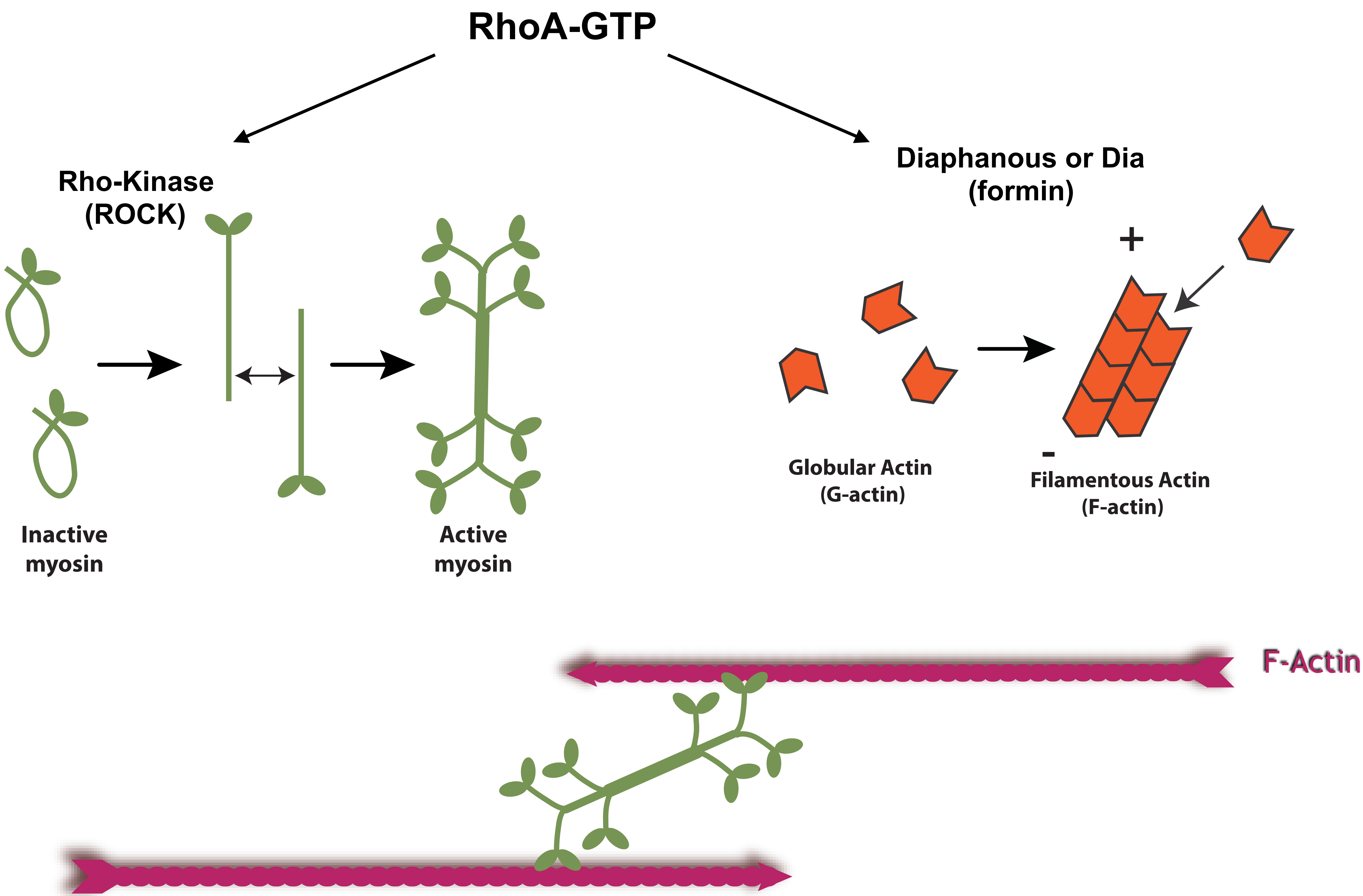
RhoA promotes actomyosin contraction



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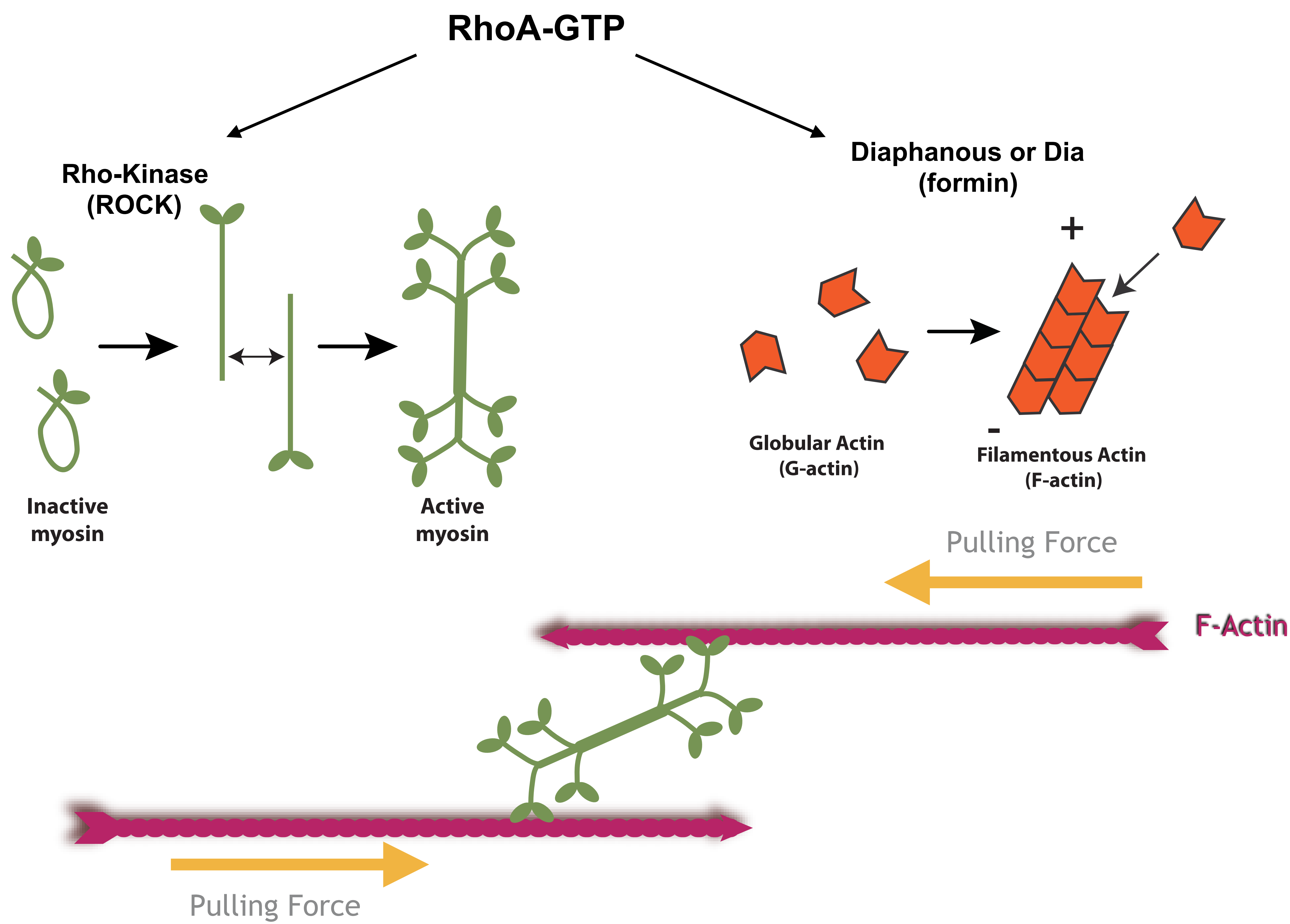

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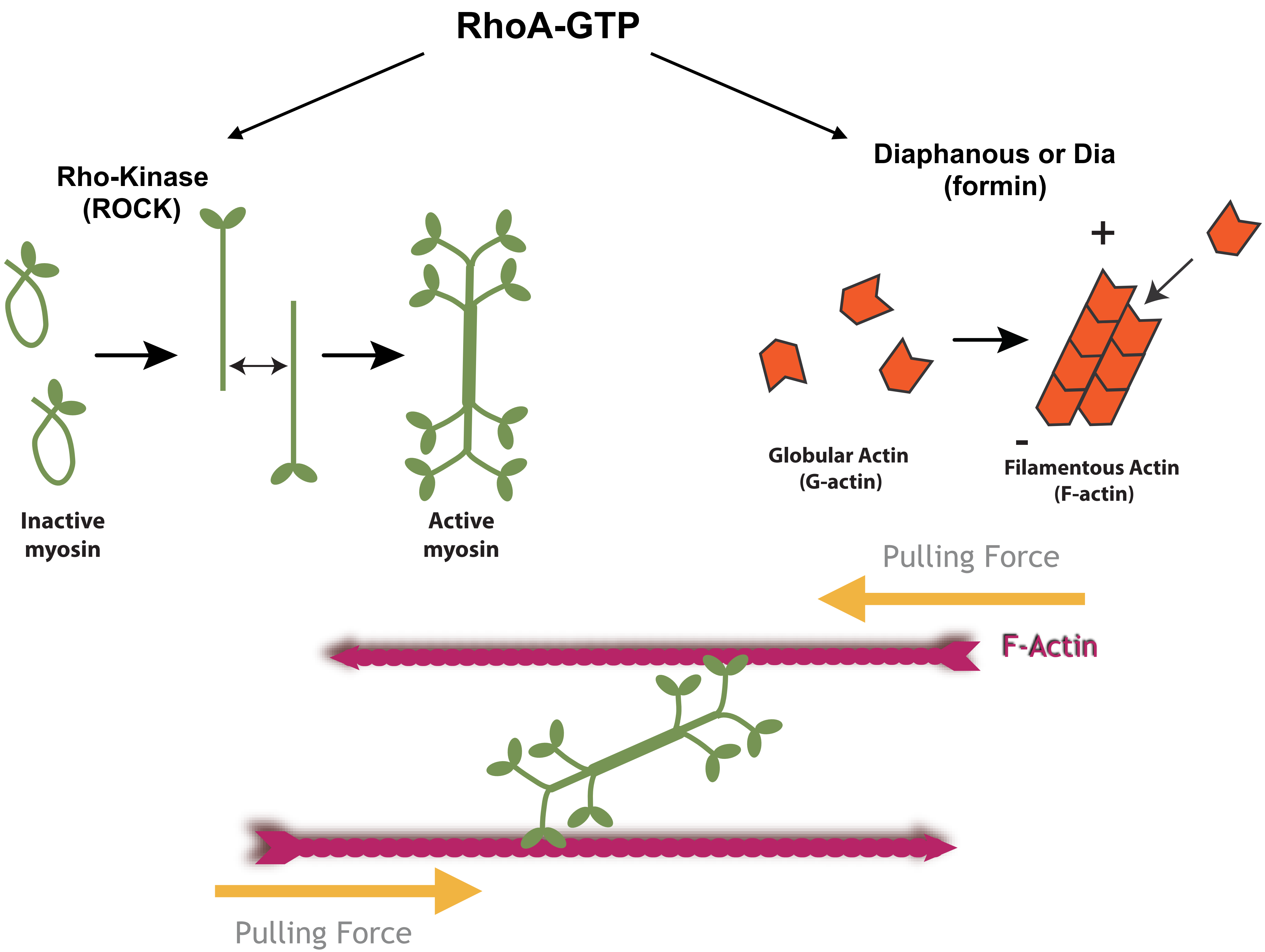

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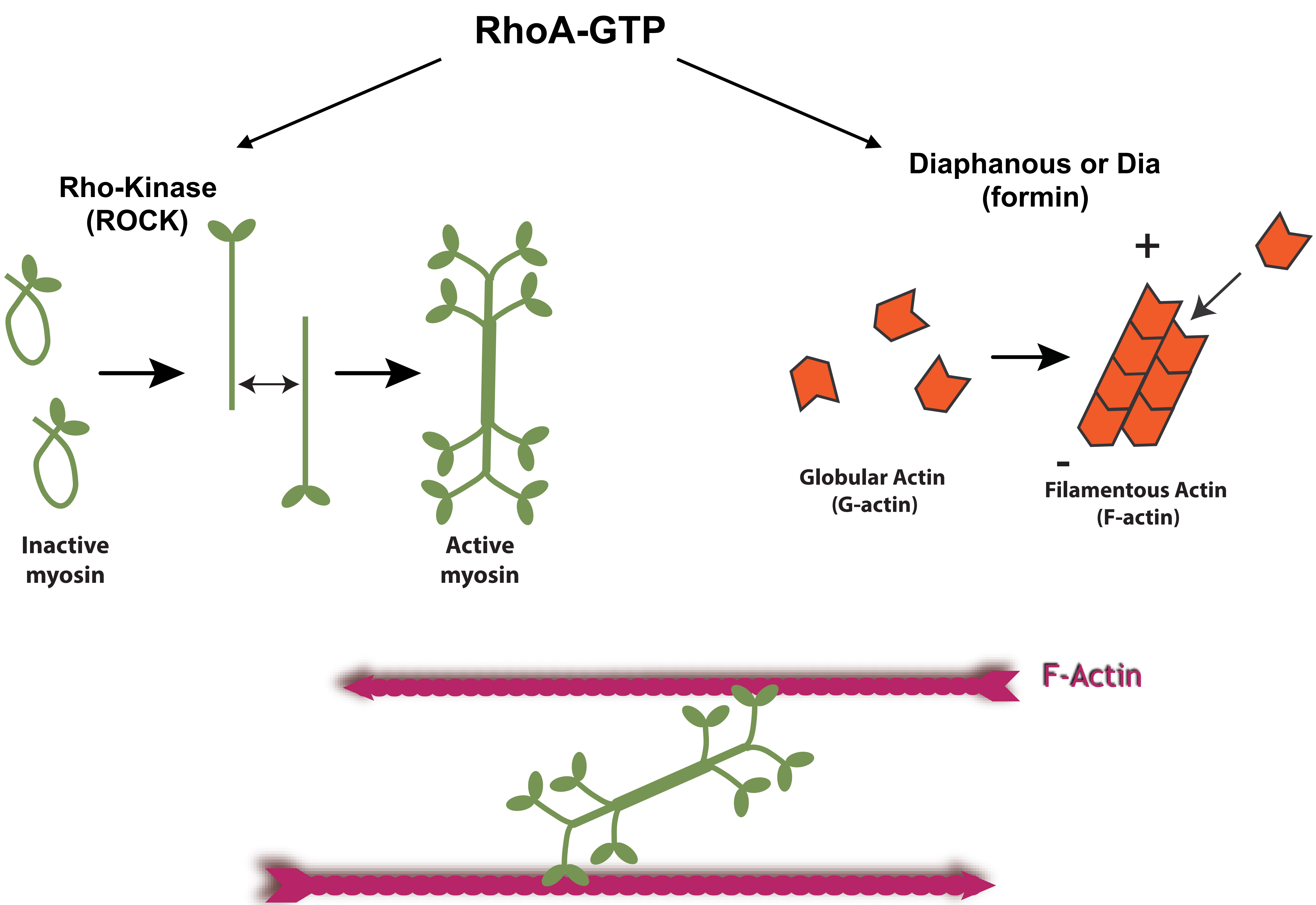
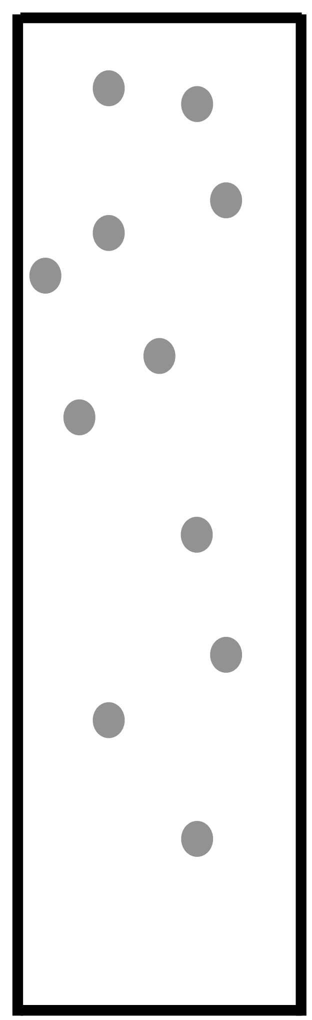


Simple model for constriction

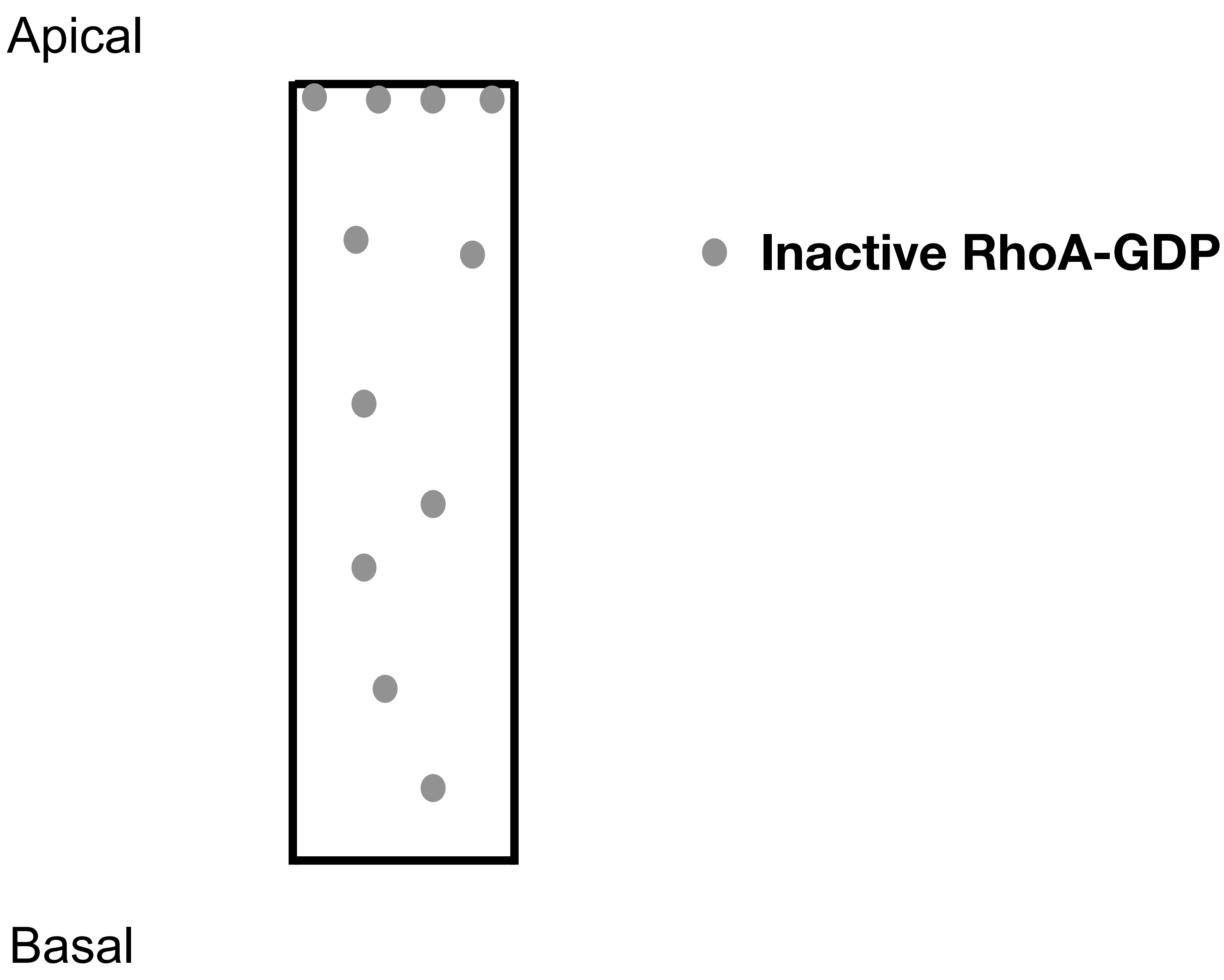
Apical



Inactive RhoA-GDP

Basal

Simple model for constriction



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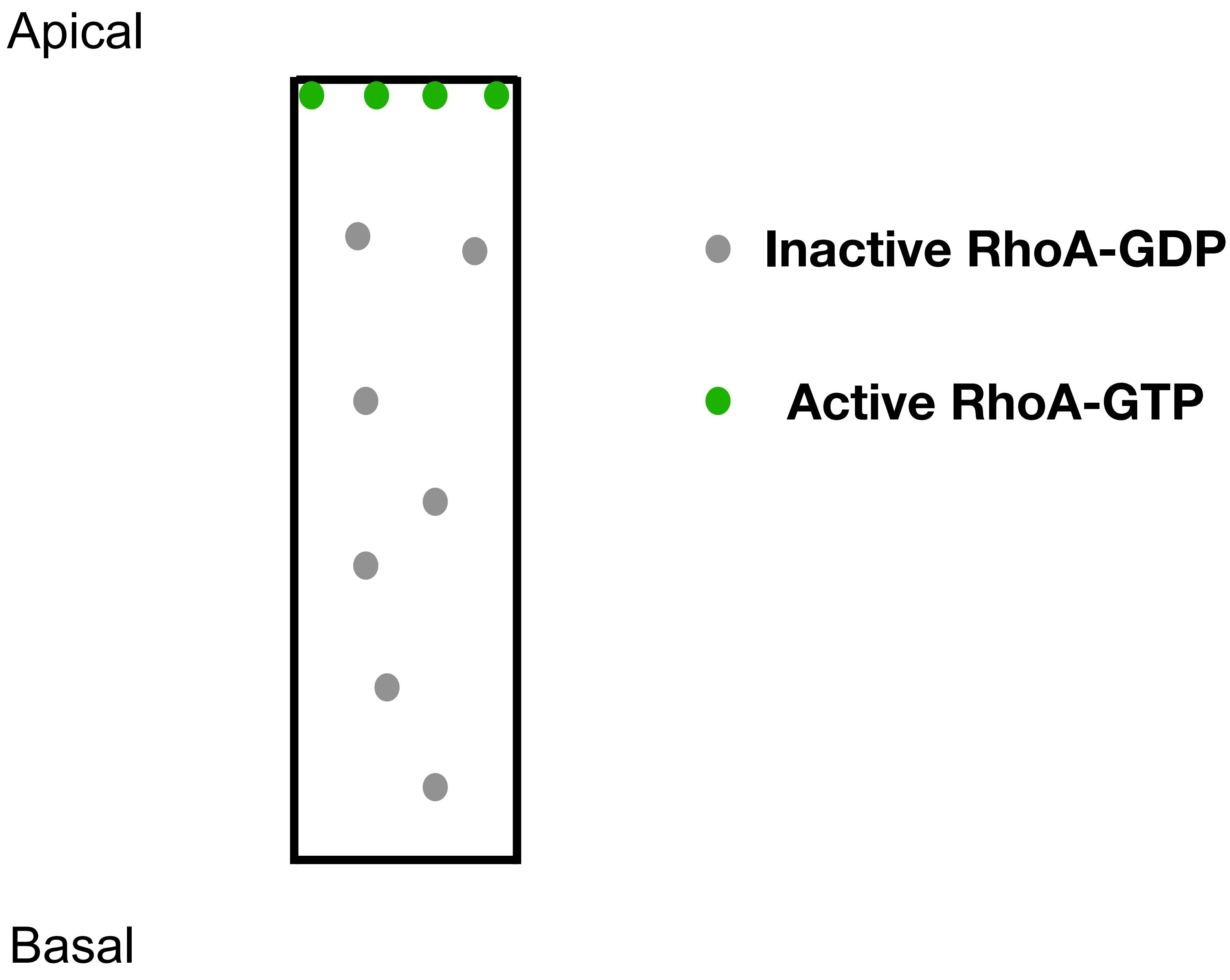

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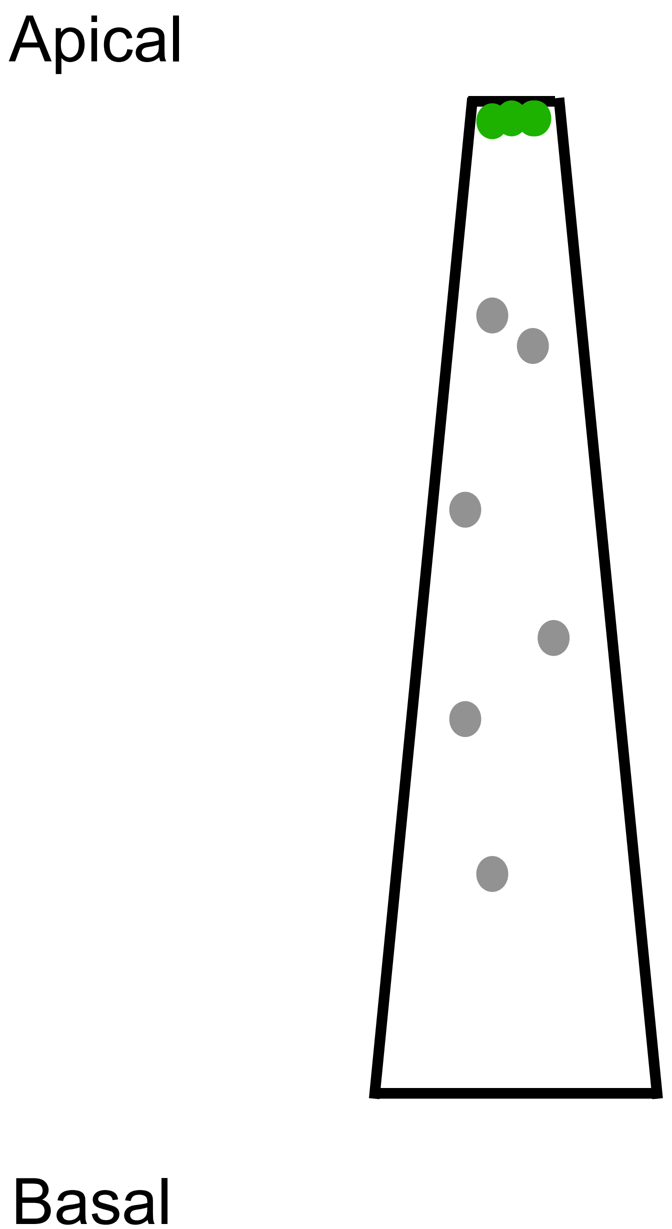

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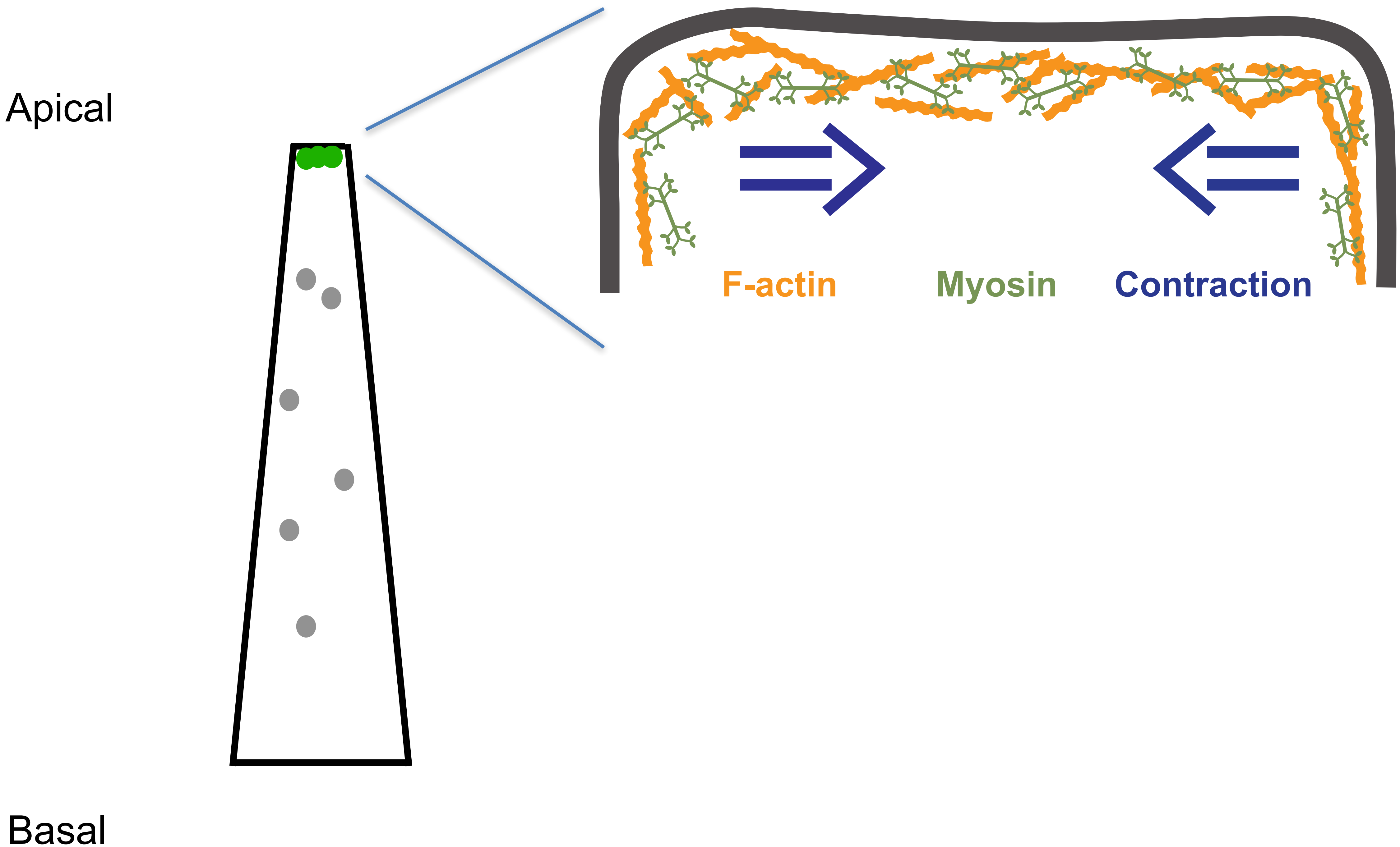
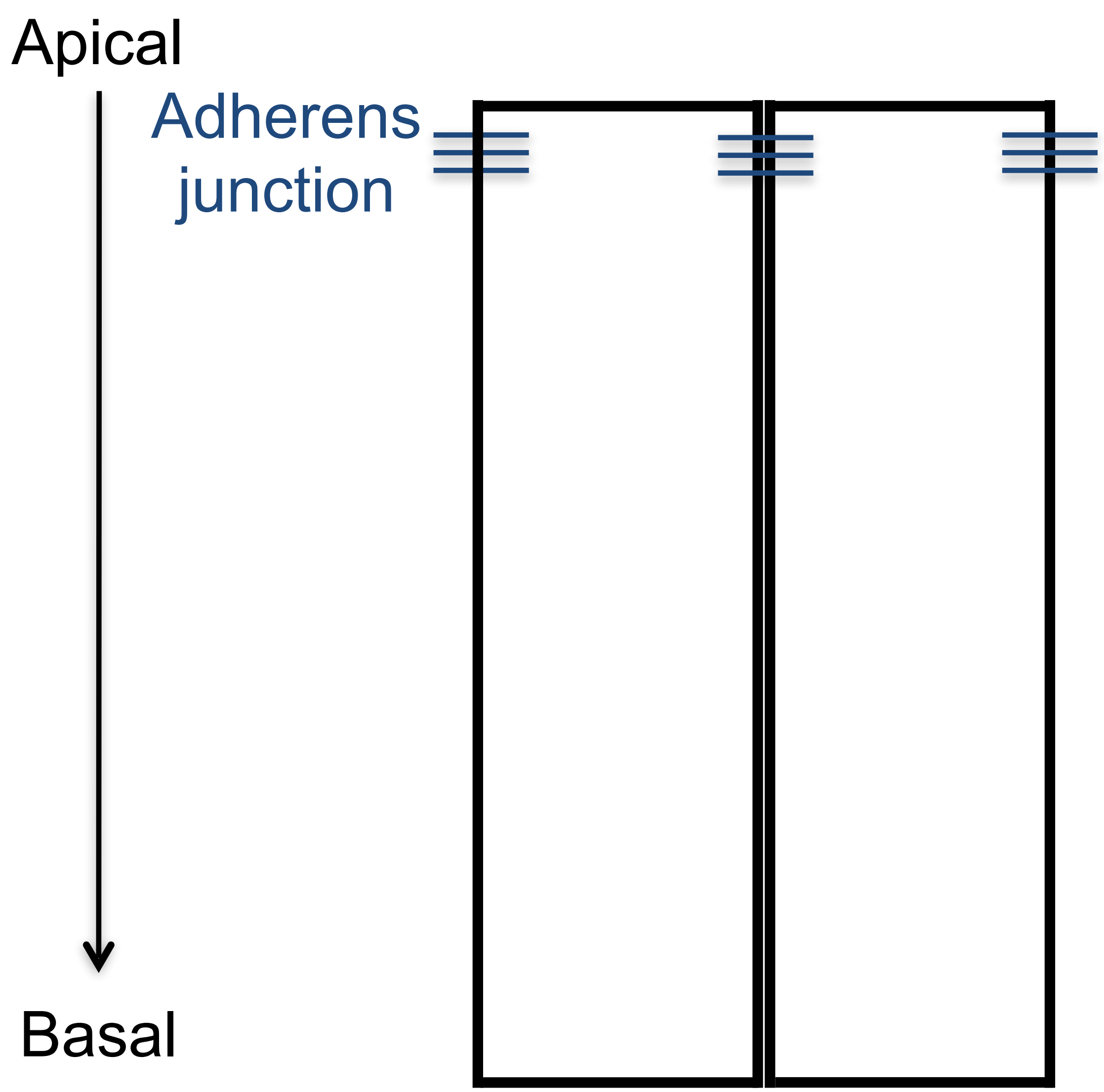


Adherens junctions serve as anchor points for contraction to pull



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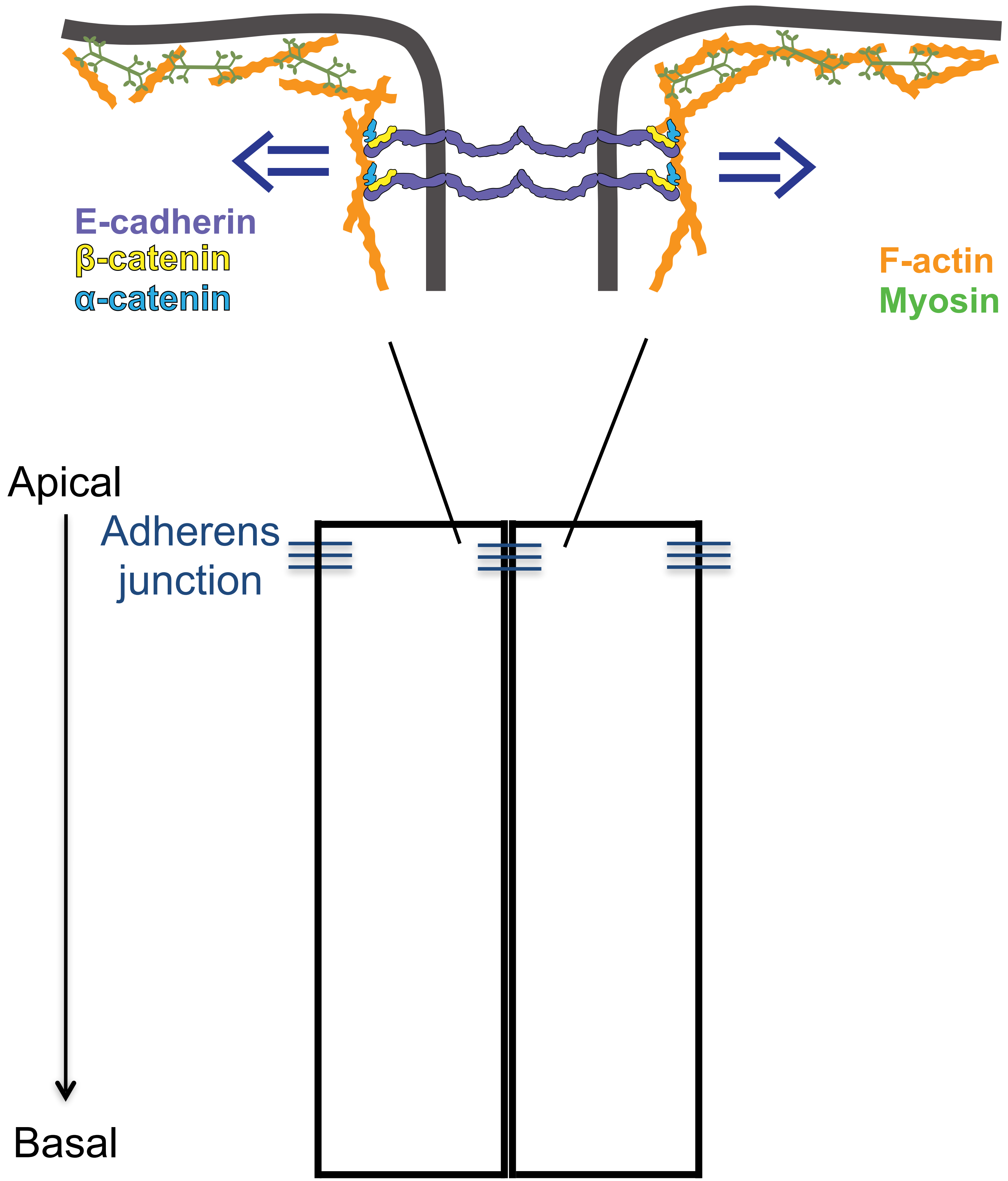
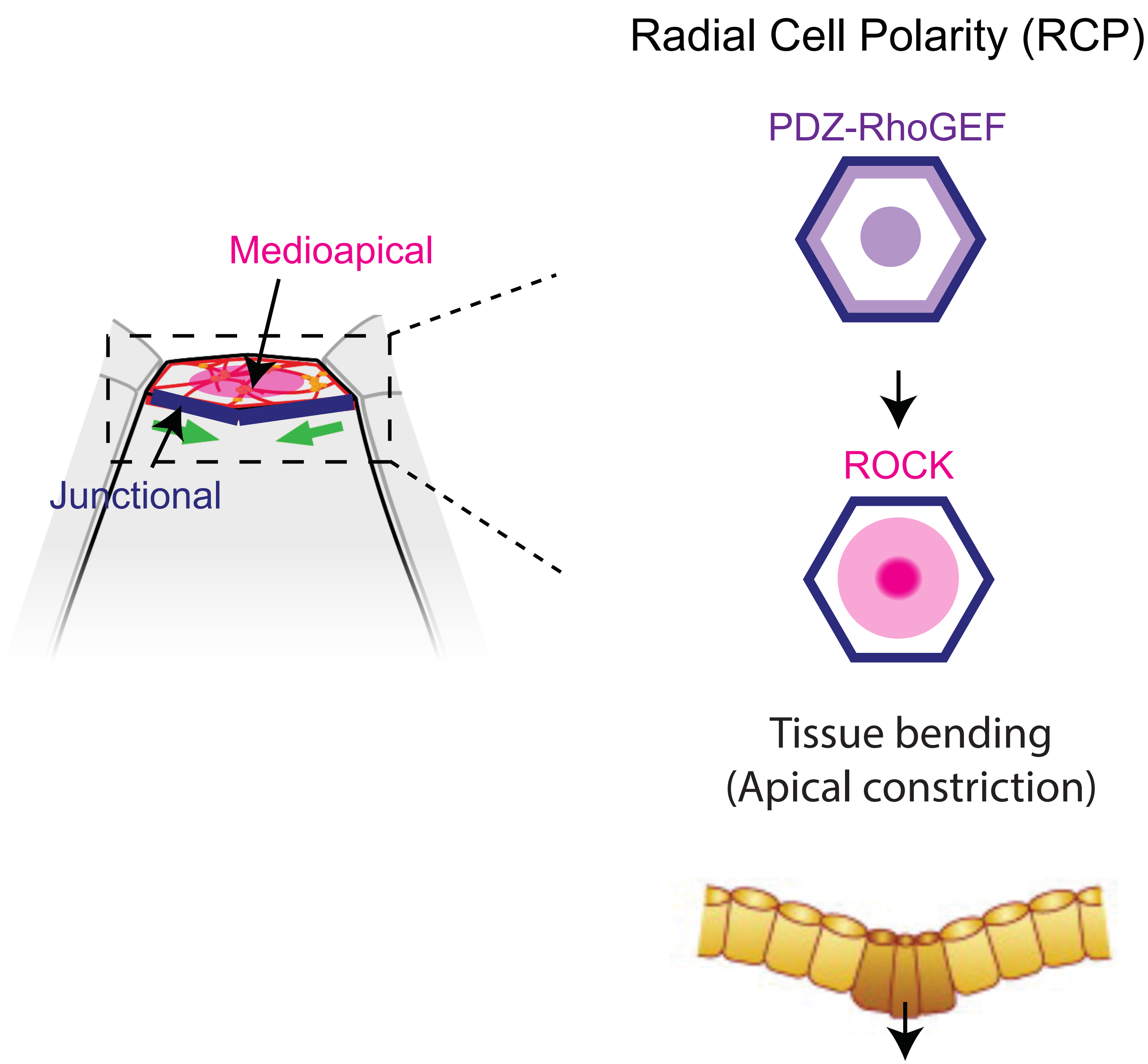


Distinct morphogenetic programs arise from different patterns of cell contractility



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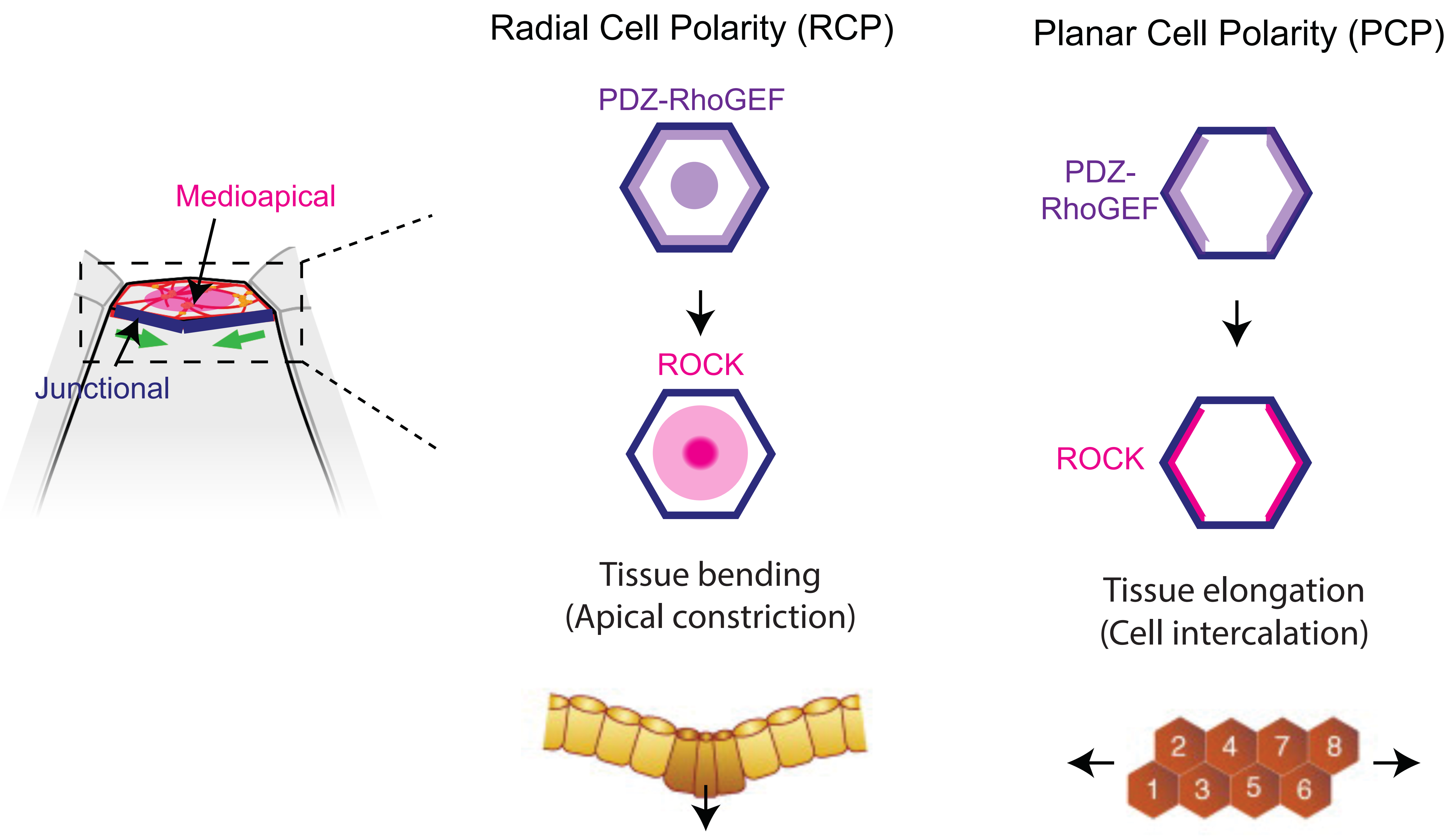
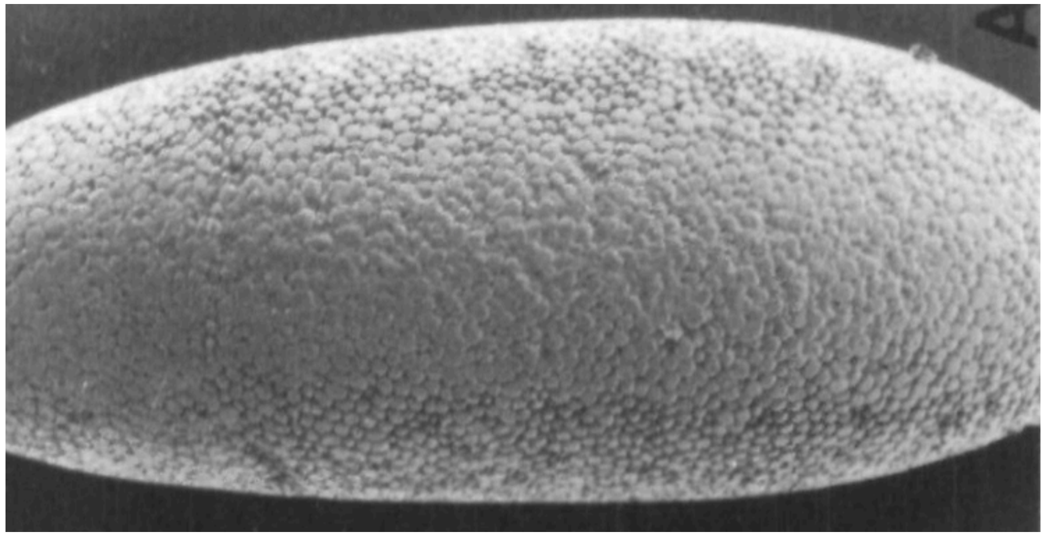


In Drosophila, ventral cells fold inward / invaginate



Sweeton, D., et al. *Development* (1991)

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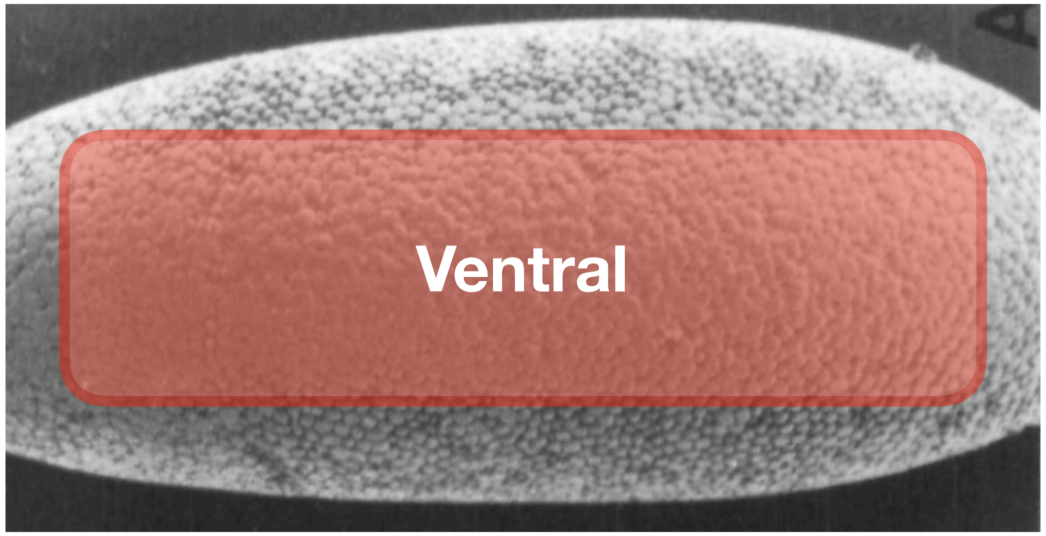

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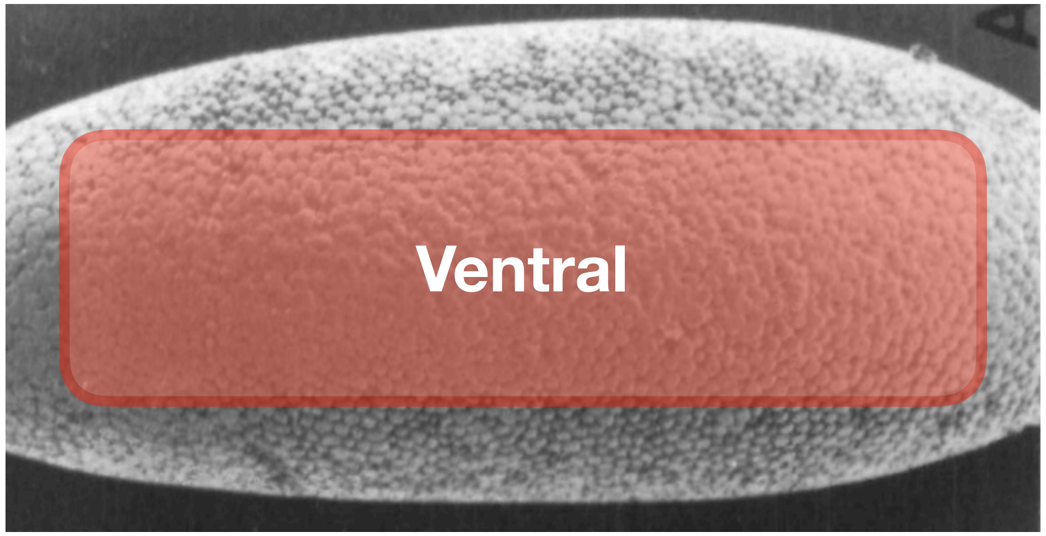

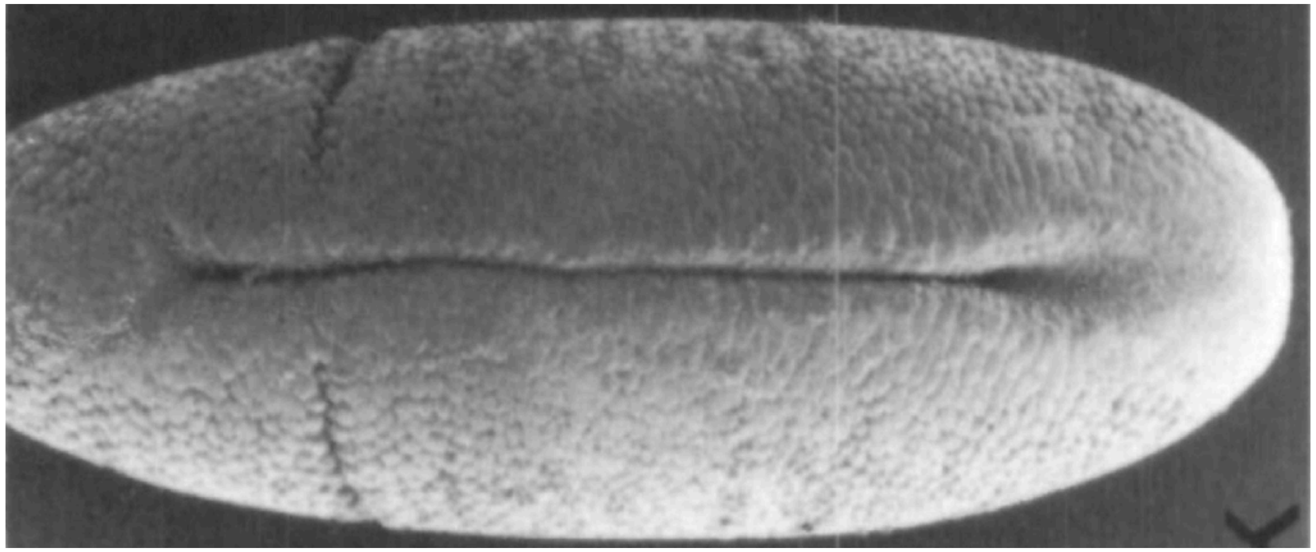

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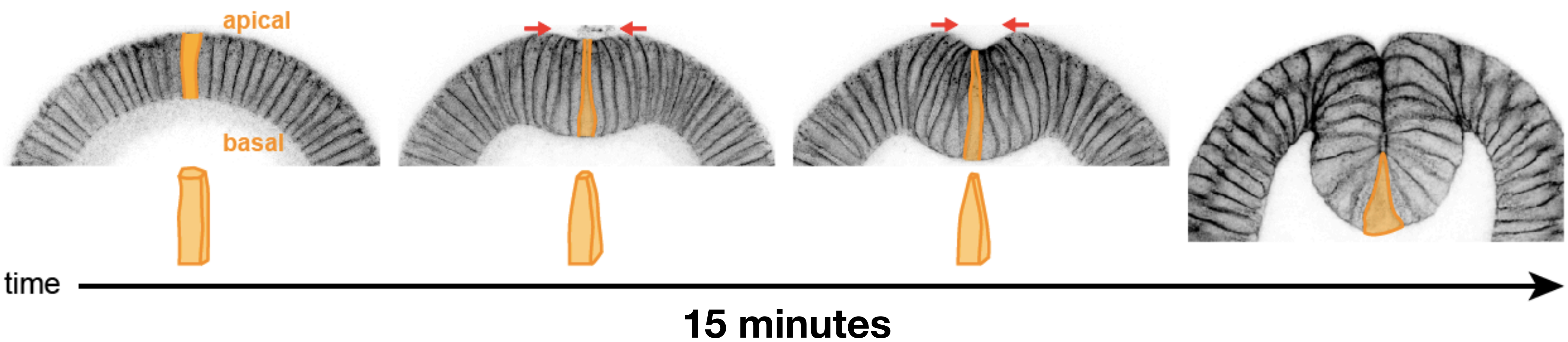
Apical constriction is associated with tissue folding



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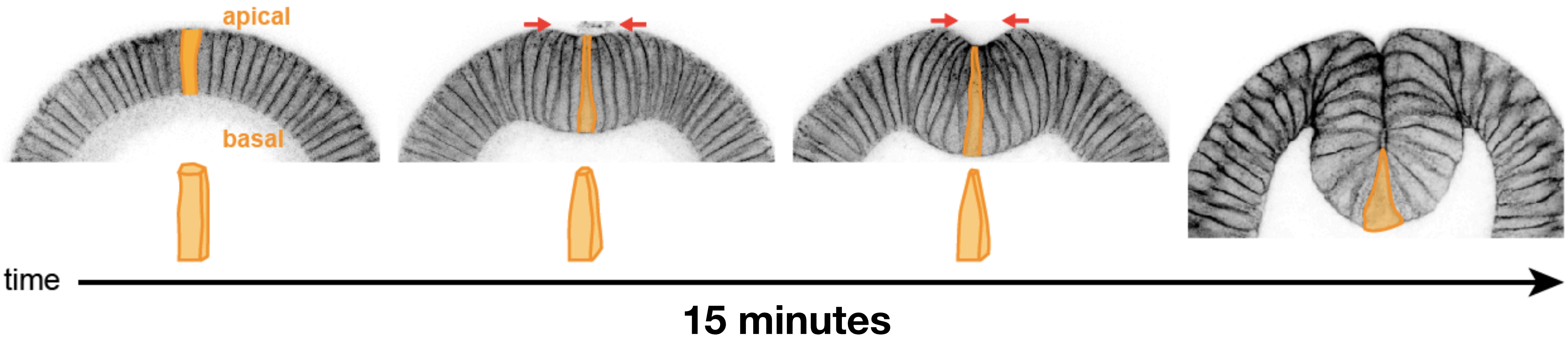
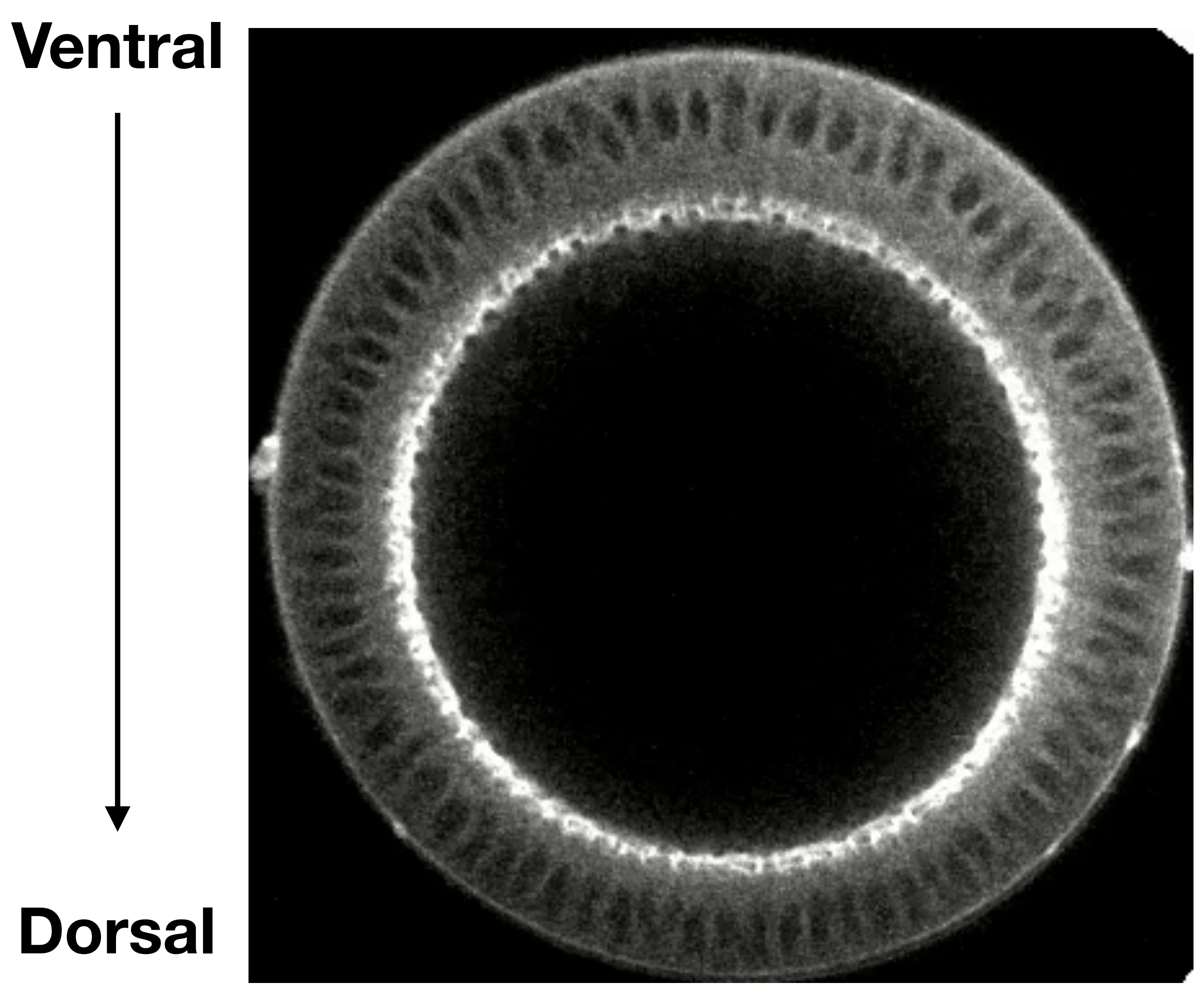
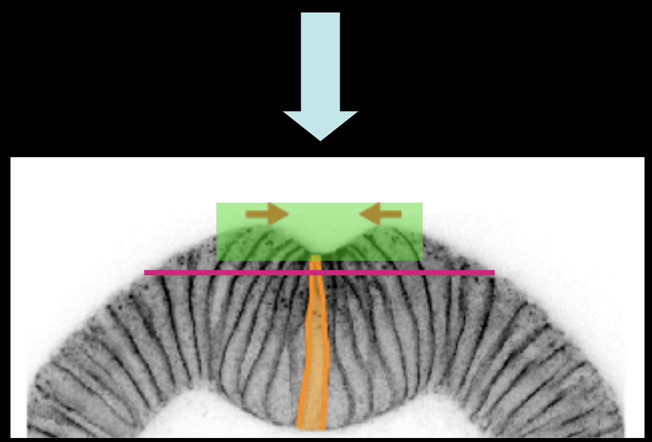




Brodland et al., *PNAS* (2000).

Myosin 2 (myosin) accumulates apically during folding



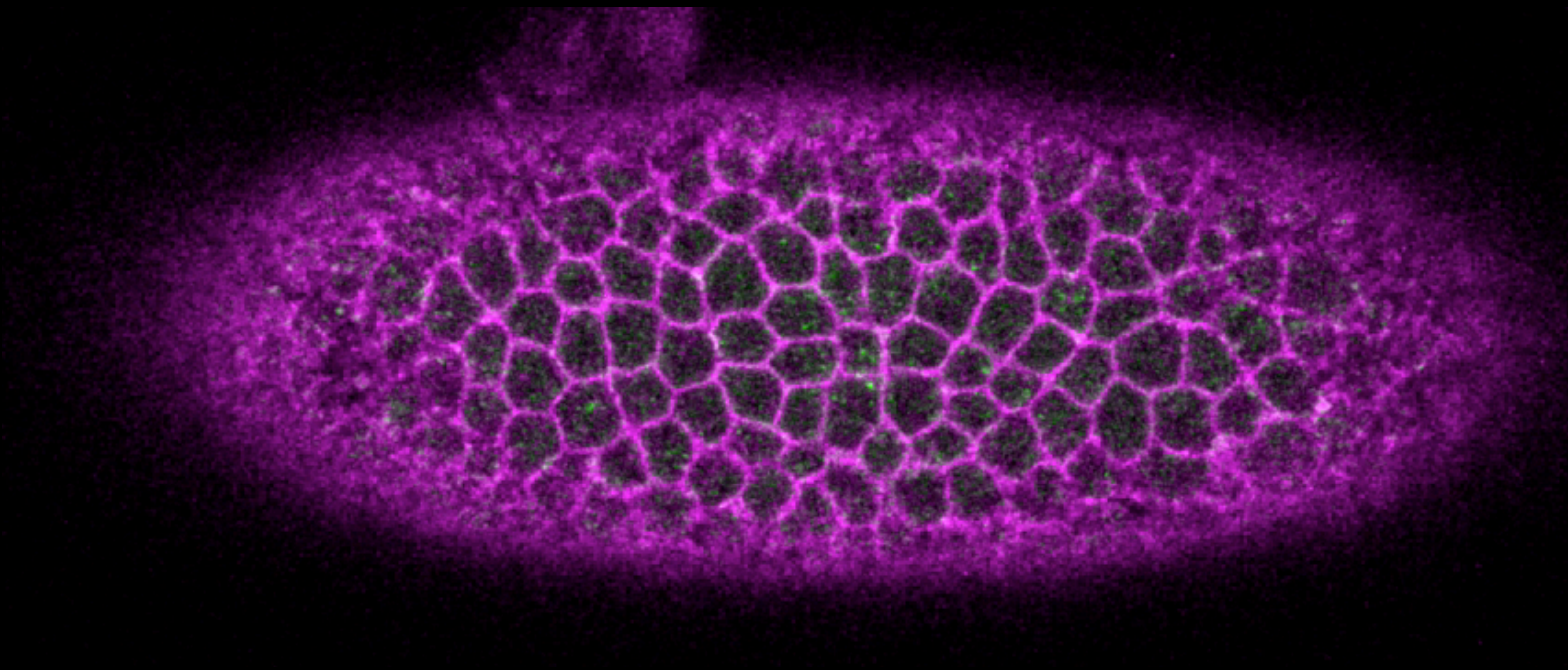
Myosin

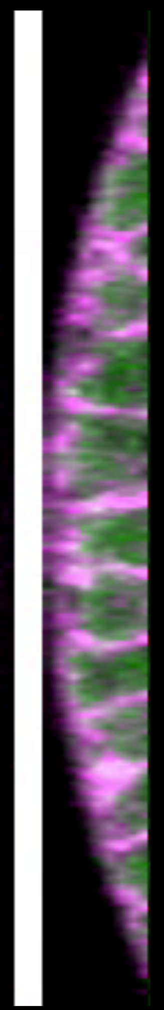
Membrane

Claudia Vasquez

Cross-Section

Apical → Basal





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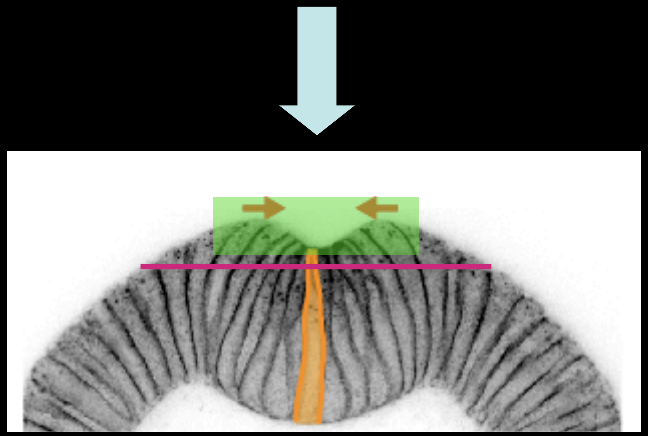
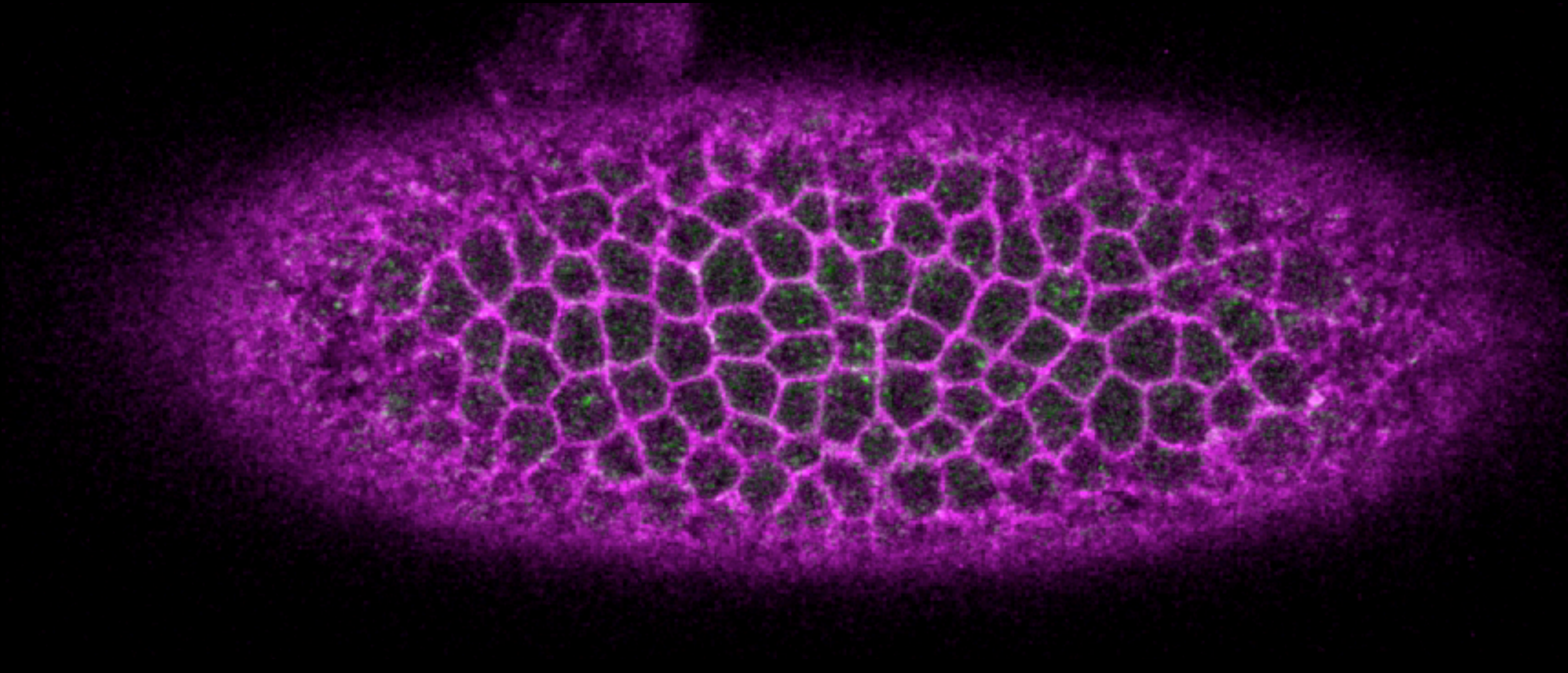


Myosin

Membrane

Claudia Vasquez



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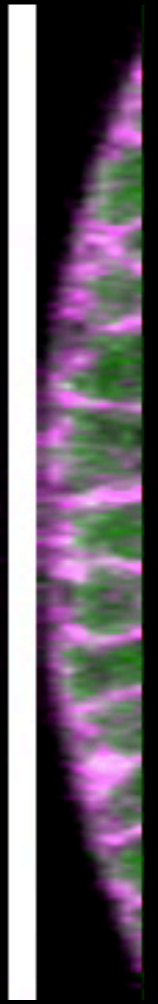

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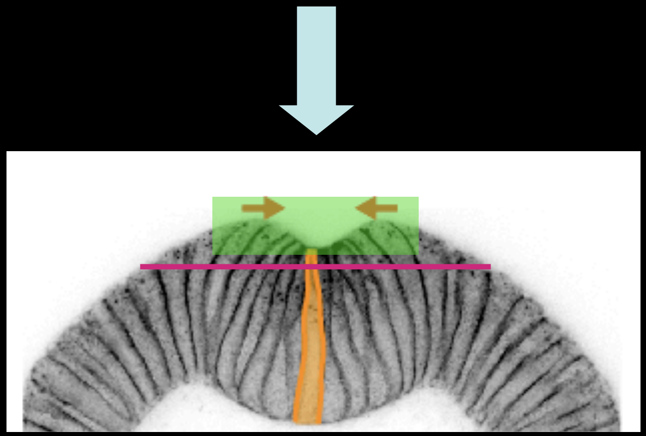
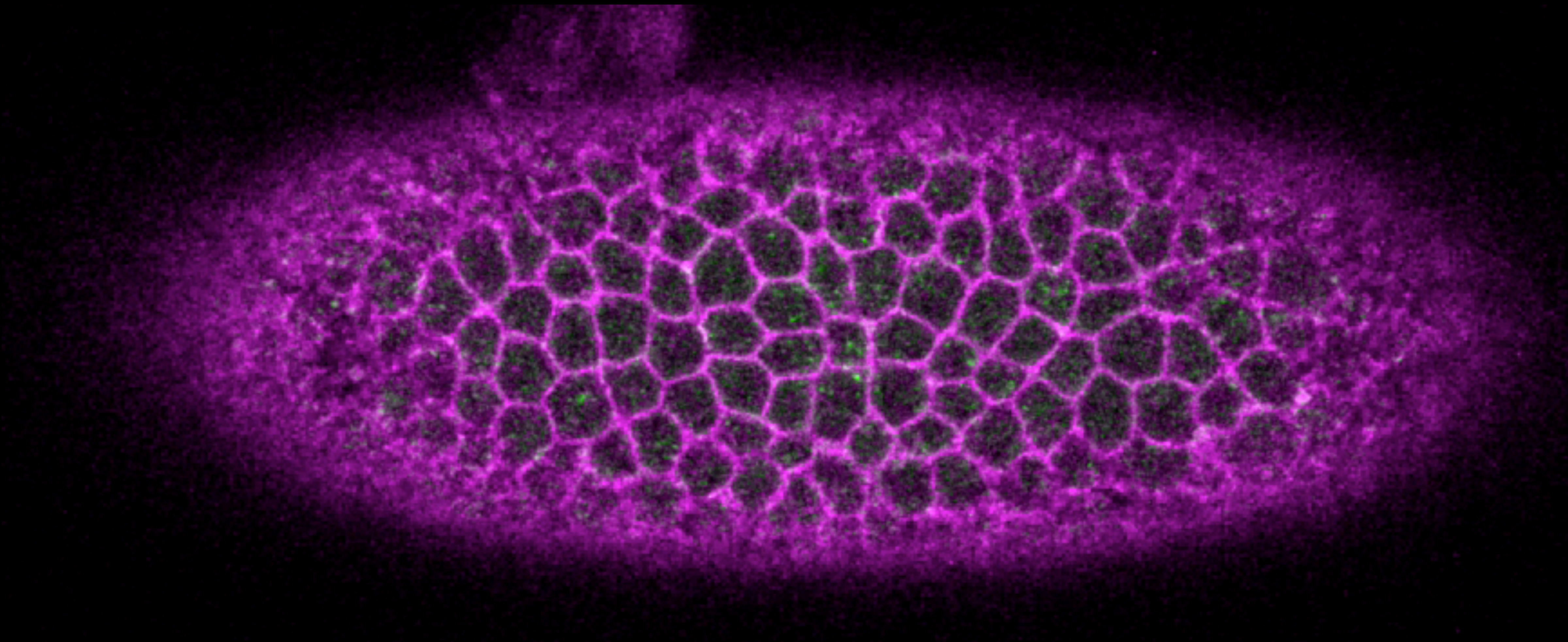


Myosin

Membrane

Claudia Vasquez



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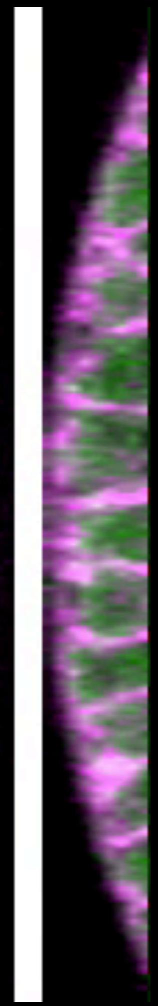

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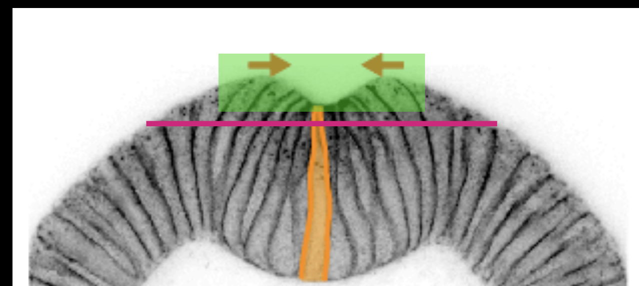
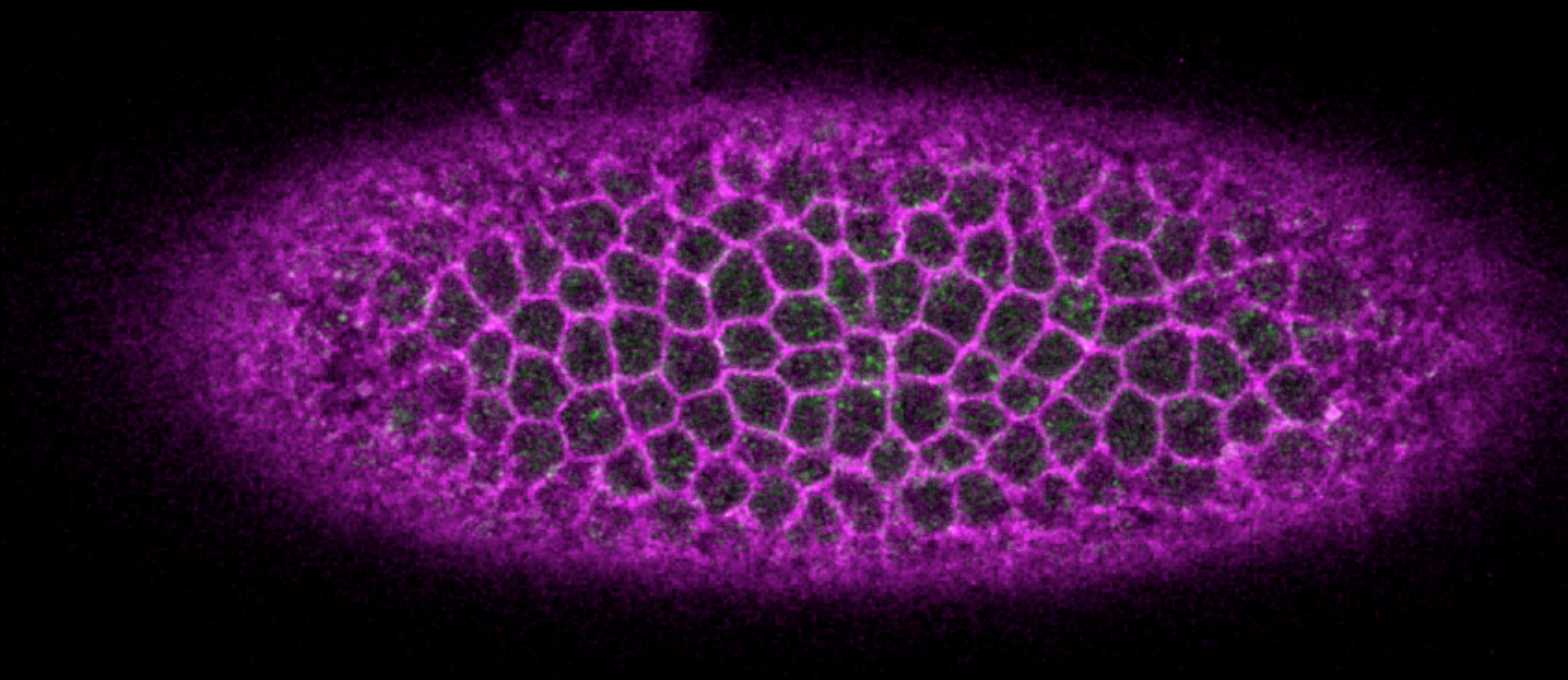


Myosin → Cytoskeleton /Regulator

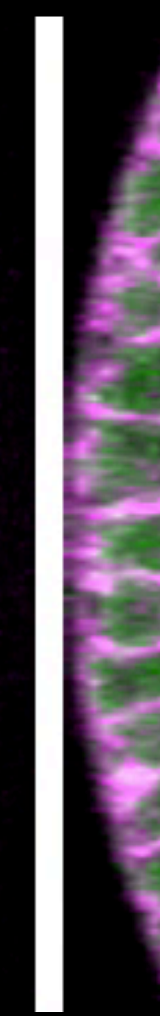
Membrane → Cell outline

Claudia Vasquez



Cross-Section

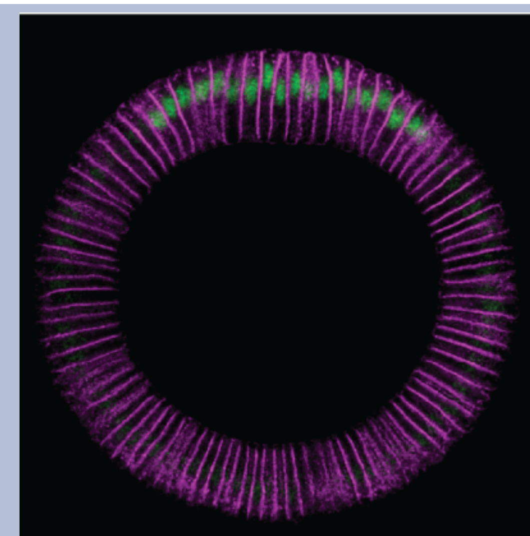
Apical →Basal



Ventral transcription factor promotes contractility

Regulation of gene expression

Snail and Twist Transcription factors



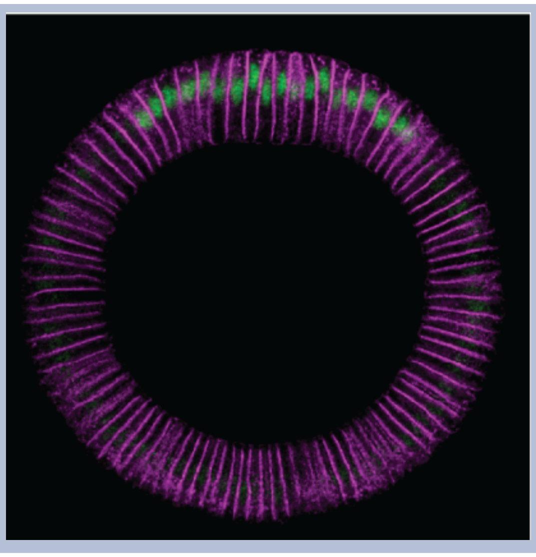
Ventral

Dorsal

Ventral transcription factor promotes contractility

Regulation of gene expression

Snail and Twist Transcription factors



Ventral

Dorsal

Leptin and Grunewald, 1990; Leptin, 1991, Costa et al., 1994.
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Ventral transcription factor promotes contractility

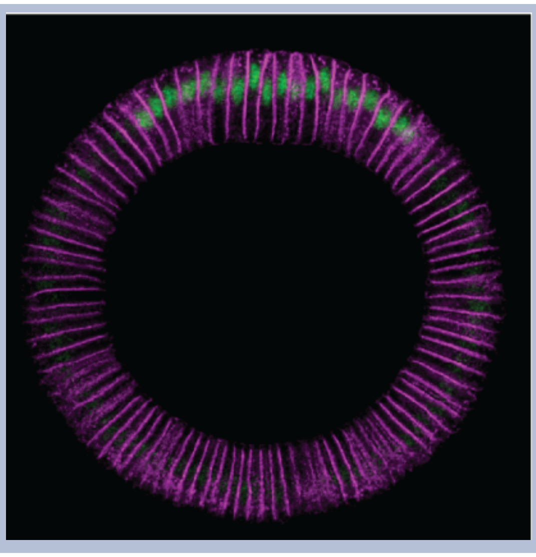
Regulation of gene expression

Snail and Twist Transcription factors

Ligand **T48**

GPCR

RhoGEF2



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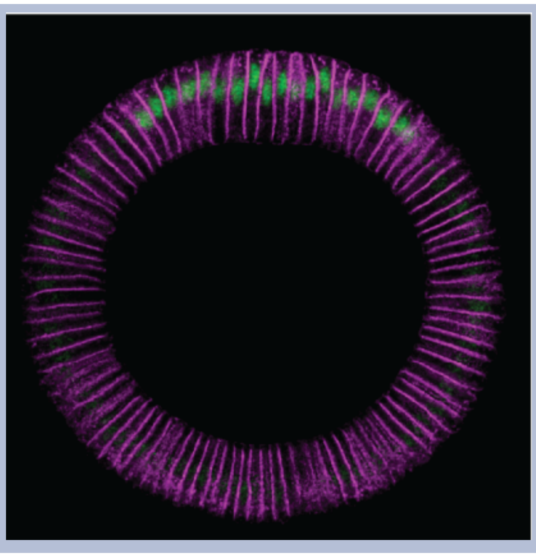

Ventral

Dorsal

Signal transduction (post-transcriptional)

GPCR

RhoGEF2

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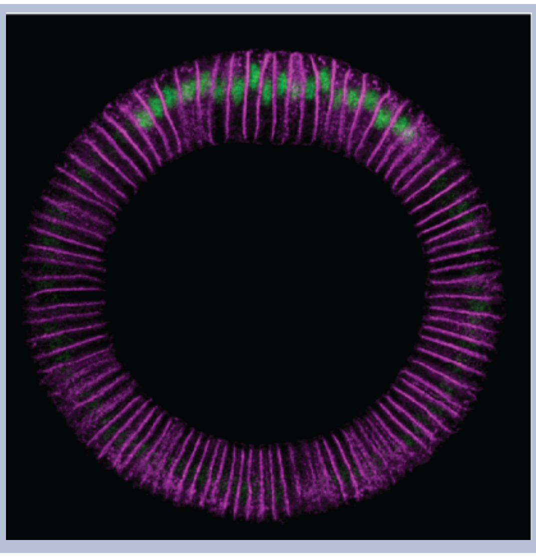

Ventral

Dorsal

Signal transduction (post-transcriptional)

GPCR

RhoGEF2

RhoA GDP

RhoA GTP

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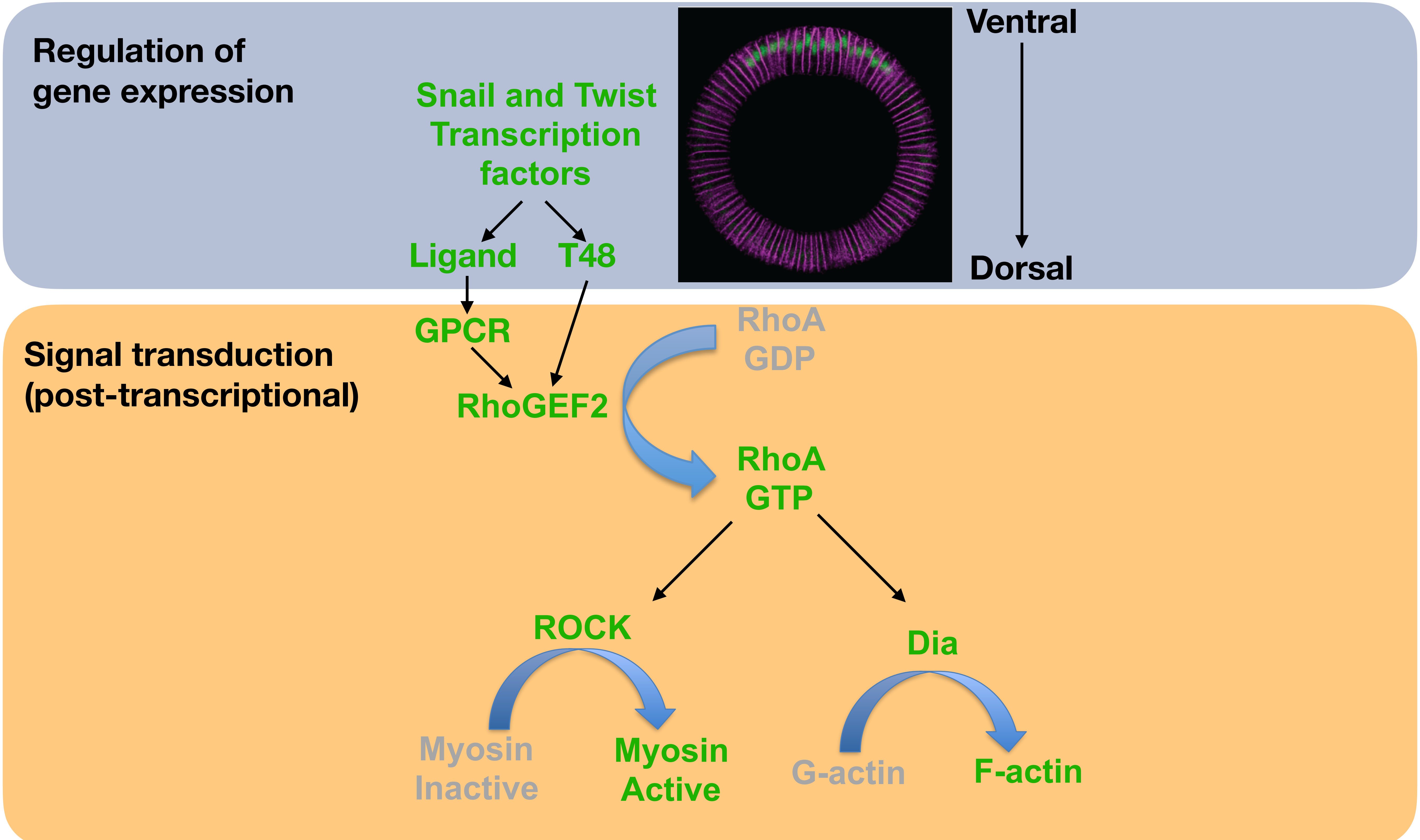

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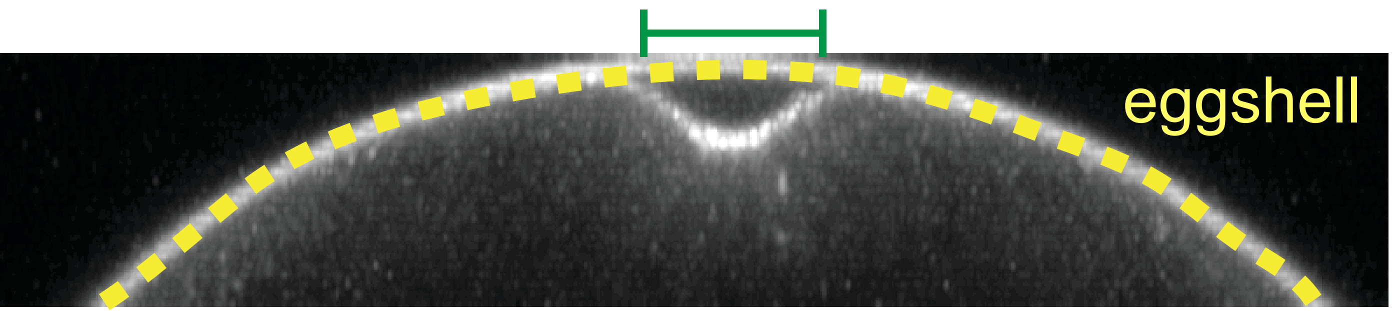
ROCK is polarized within the apical domain

Wild-Type



GFP::Rho-Kinase

Frank Mason, Ph.D.

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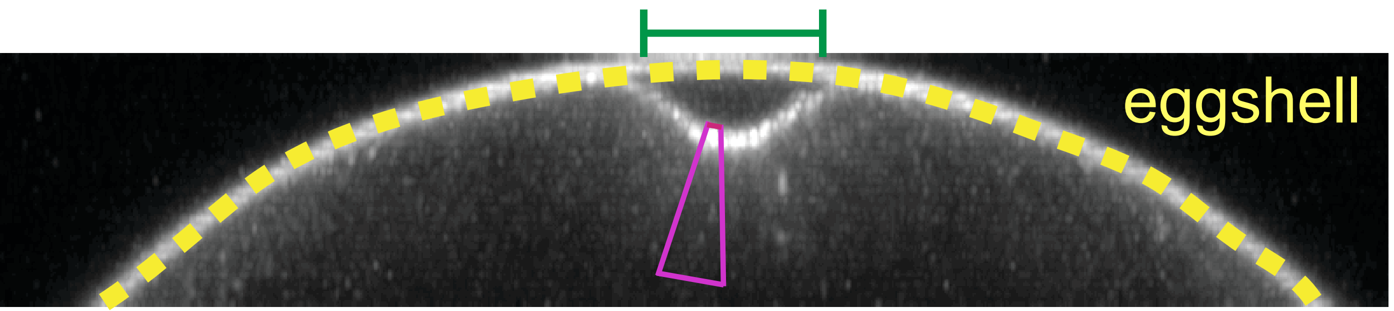
Wild-Type



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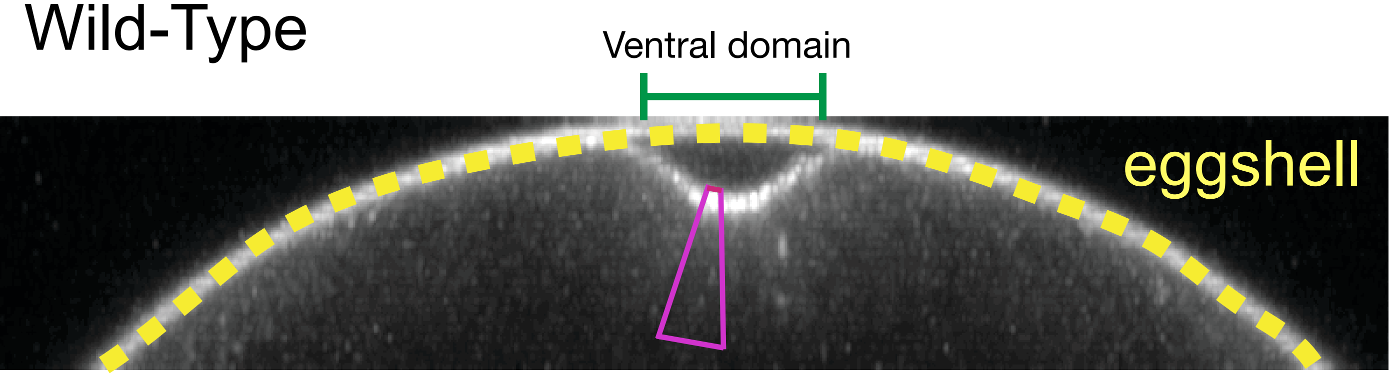
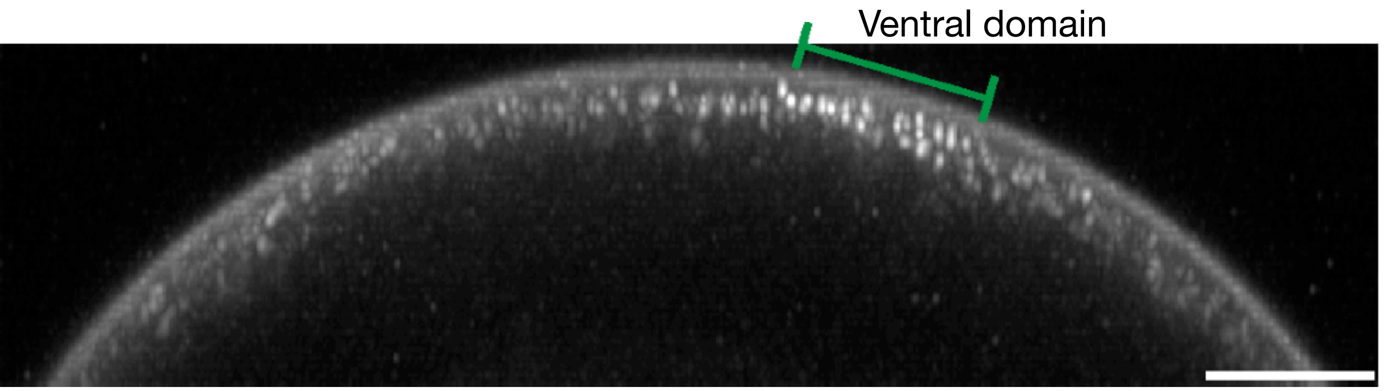


GFP::Rho-Kinase

twist mutant



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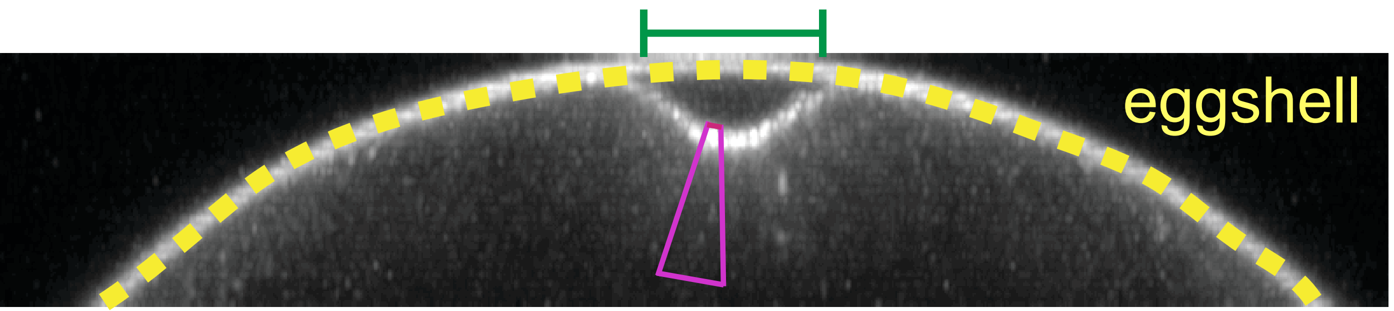

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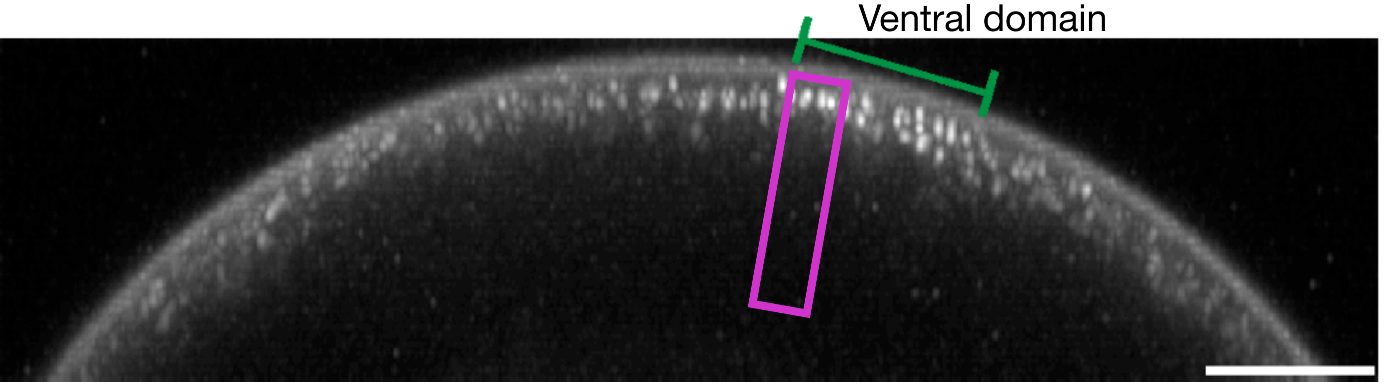

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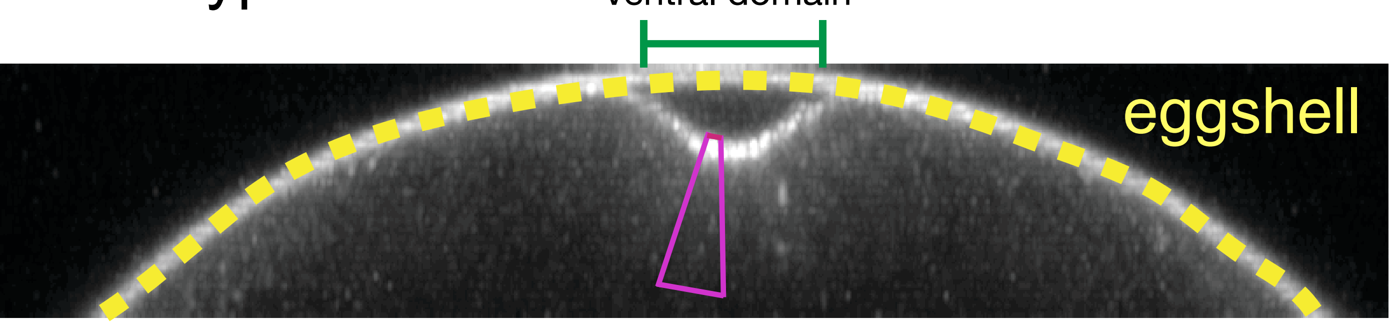
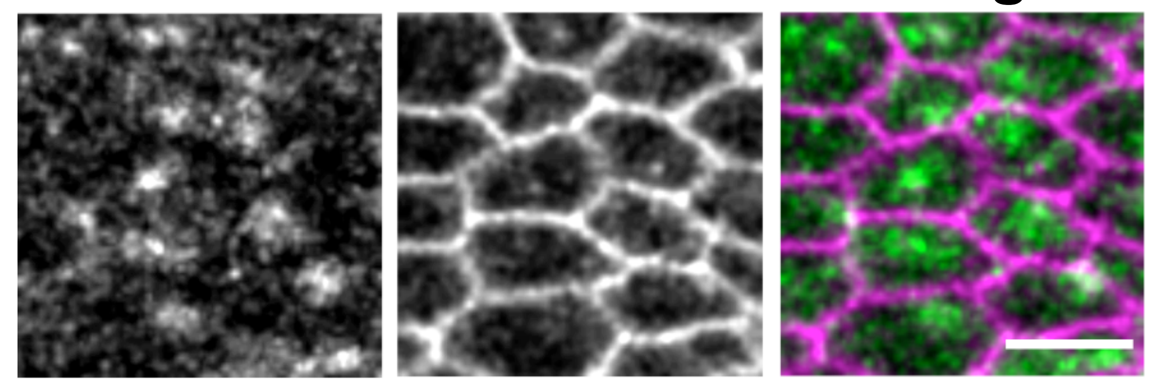
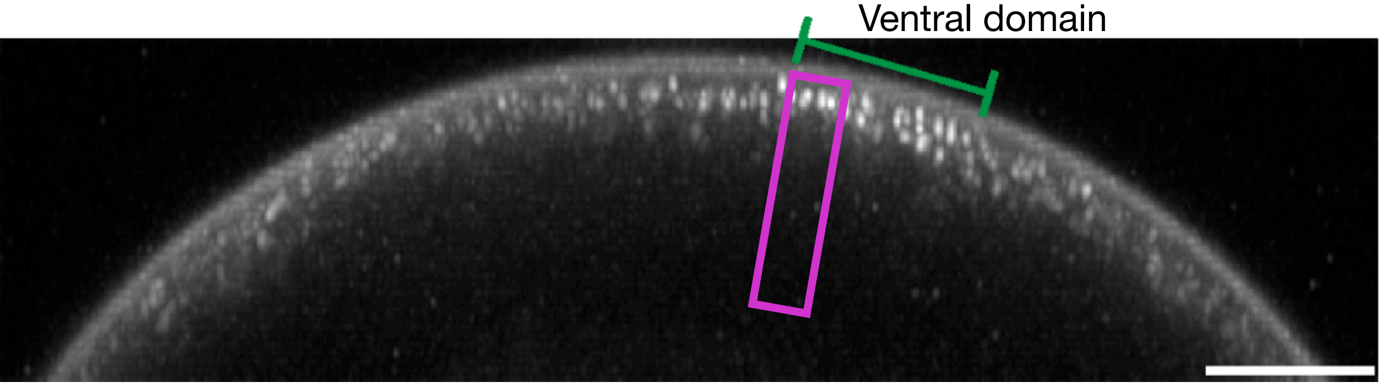


GFP::Rho-Kinase



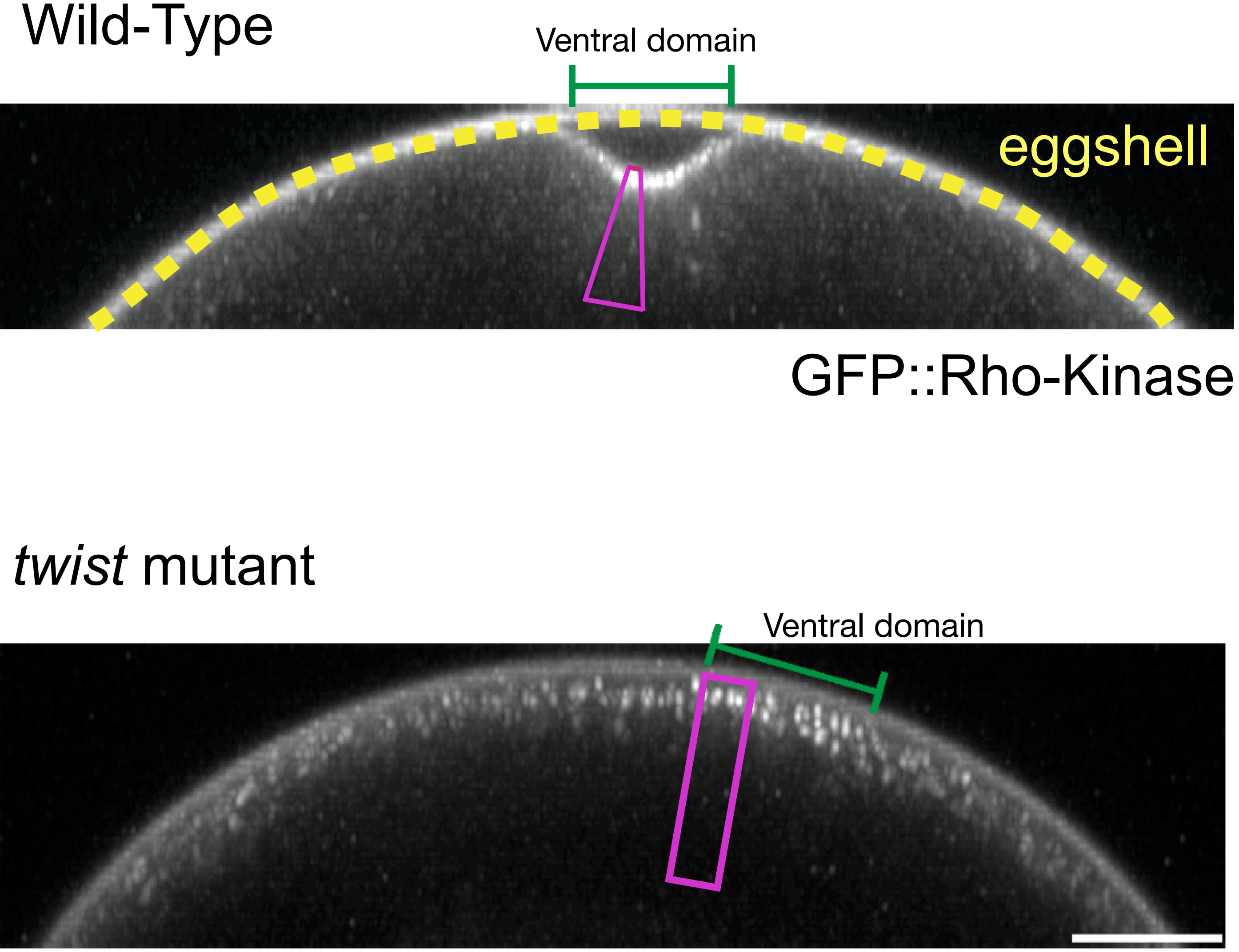
twist mutant

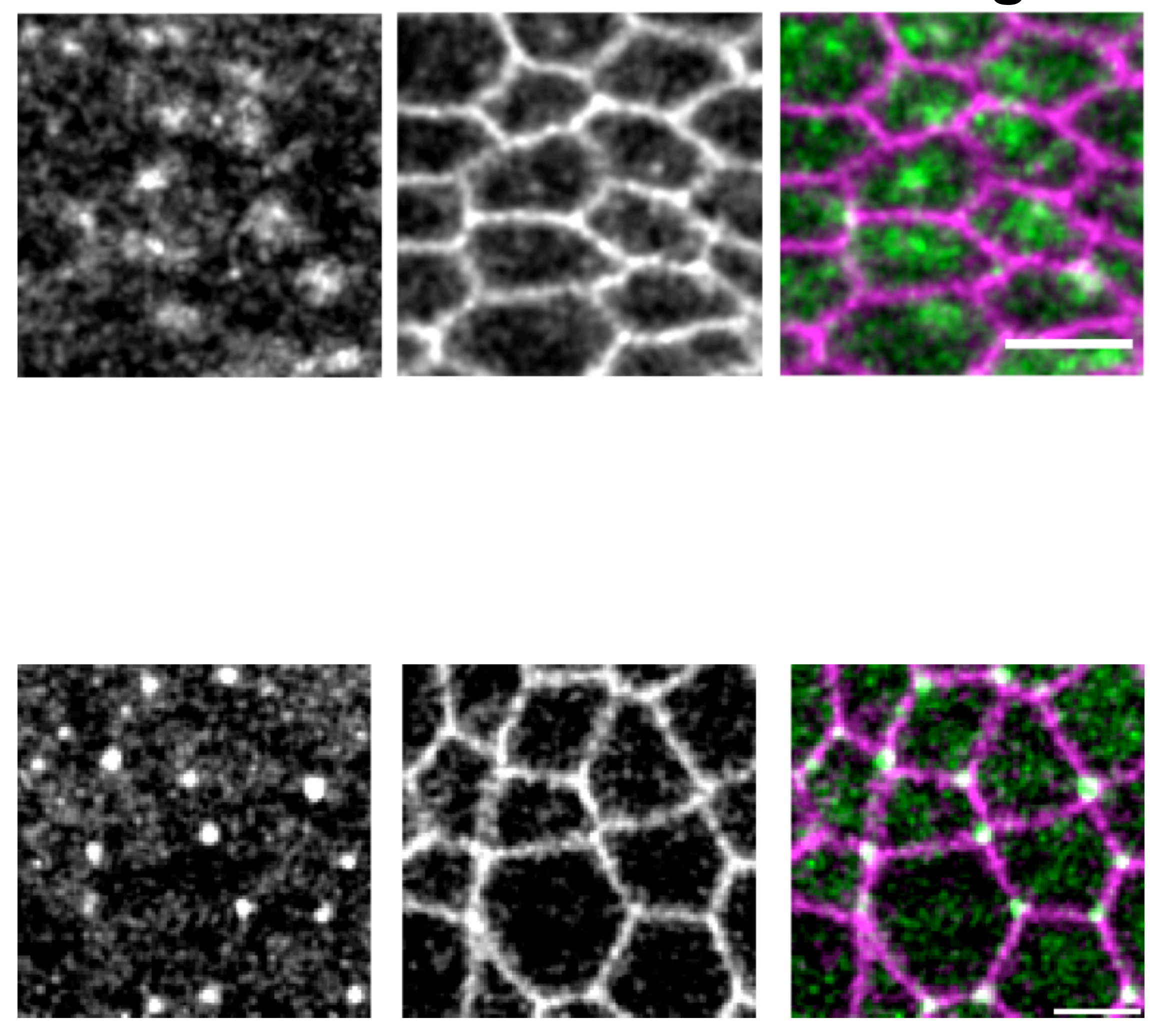


GFP::Rho-Kinase

Frank Mason, Ph.D.

ROCK is polarized within the apical domain





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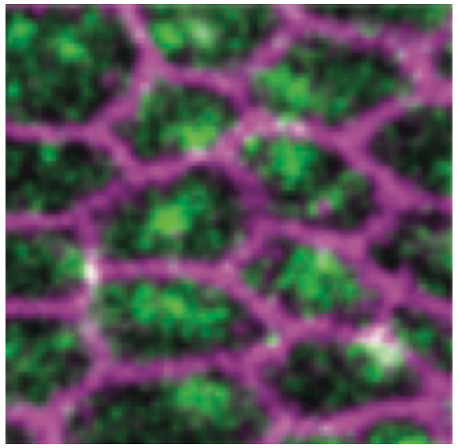
RhoA signaling is polarized within apical domain

GFP::ROCK

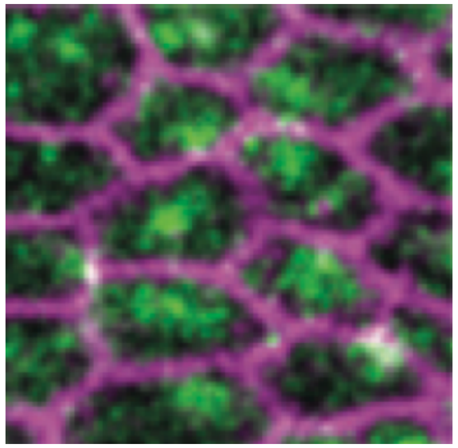
Surface view



RhoA signaling is polarized within apical domain

GFP::ROCK

Surface view

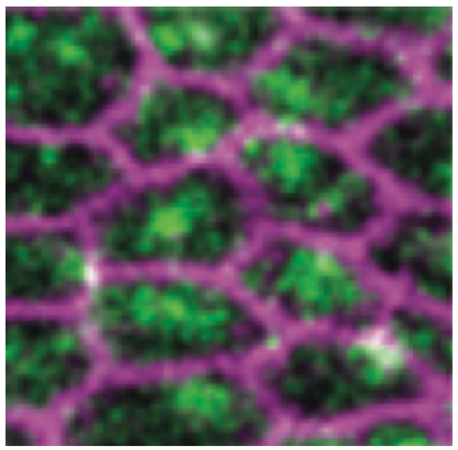


Depends on RhoA activity
Active RhoA is also medioapically polarized

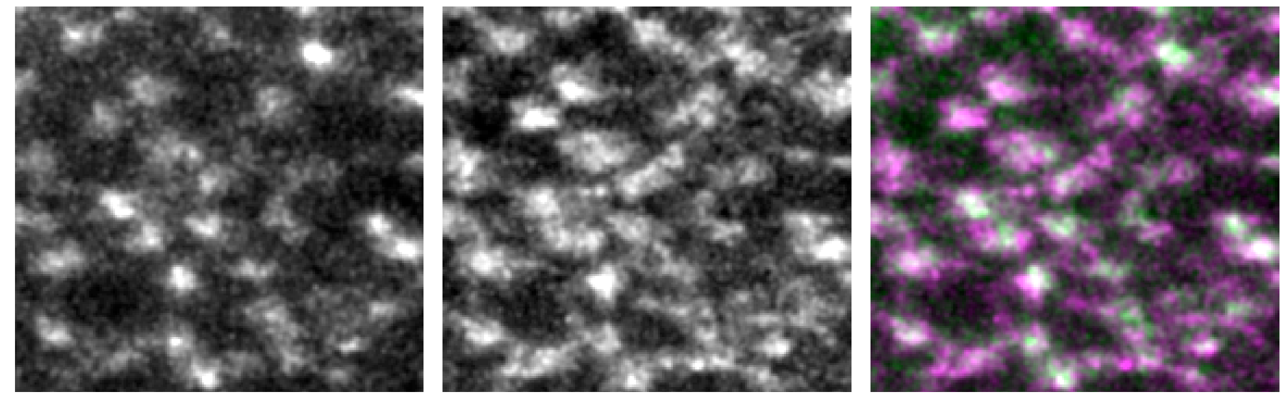
RhoA signaling is polarized within apical domain

GFP::ROCK

Surface view

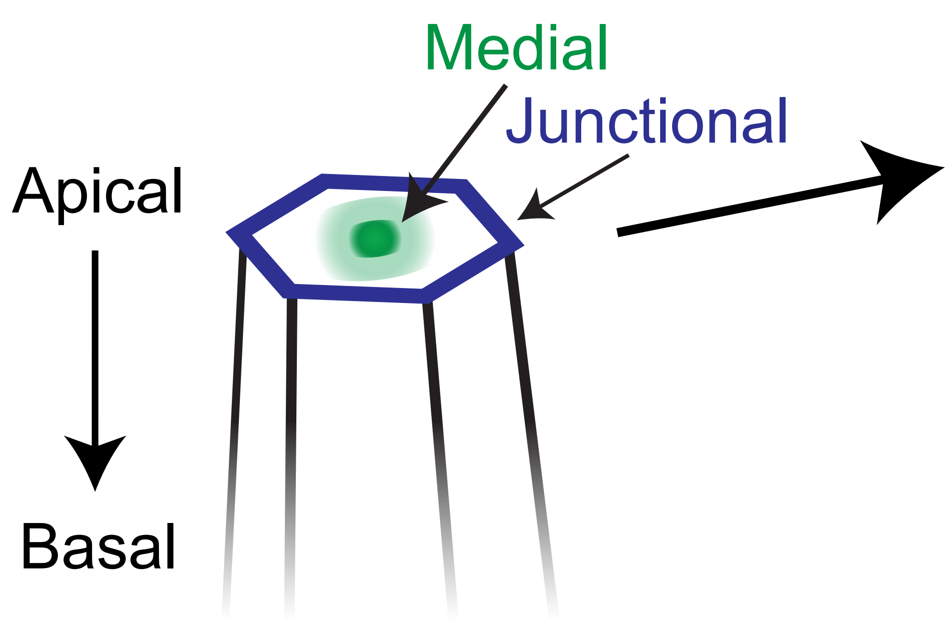


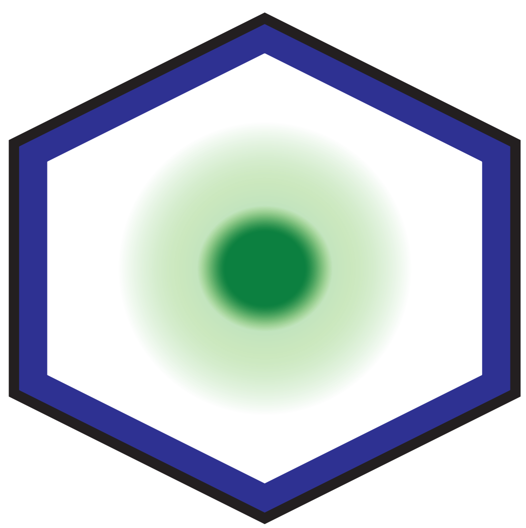
GFP::ROCK Myosin Merge



Depends on RhoA activity
Active RhoA is also medioapically polarized

Constricting cell apex has a spatial pattern of ROCK/myosin



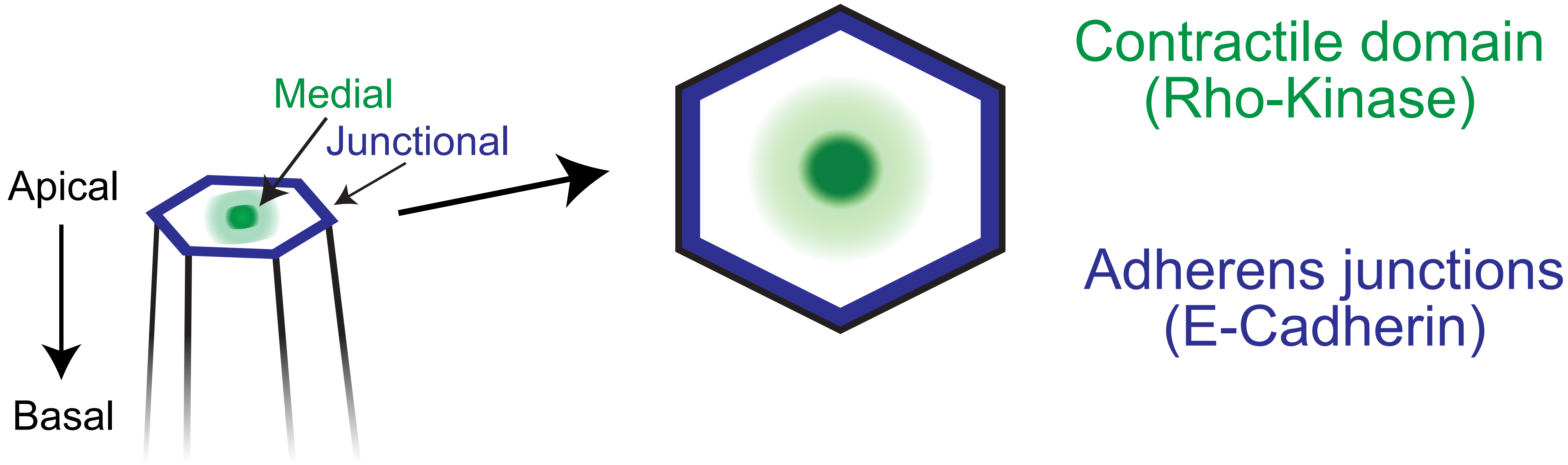


Contractile domain
(Rho-Kinase)

Adherens junctions
(E-Cadherin)

Mason FM, Tworoger M, Martin AC. *Nat Cell Biol.* (2013).
Mason et al., *J. Cell Biol.* (2016).

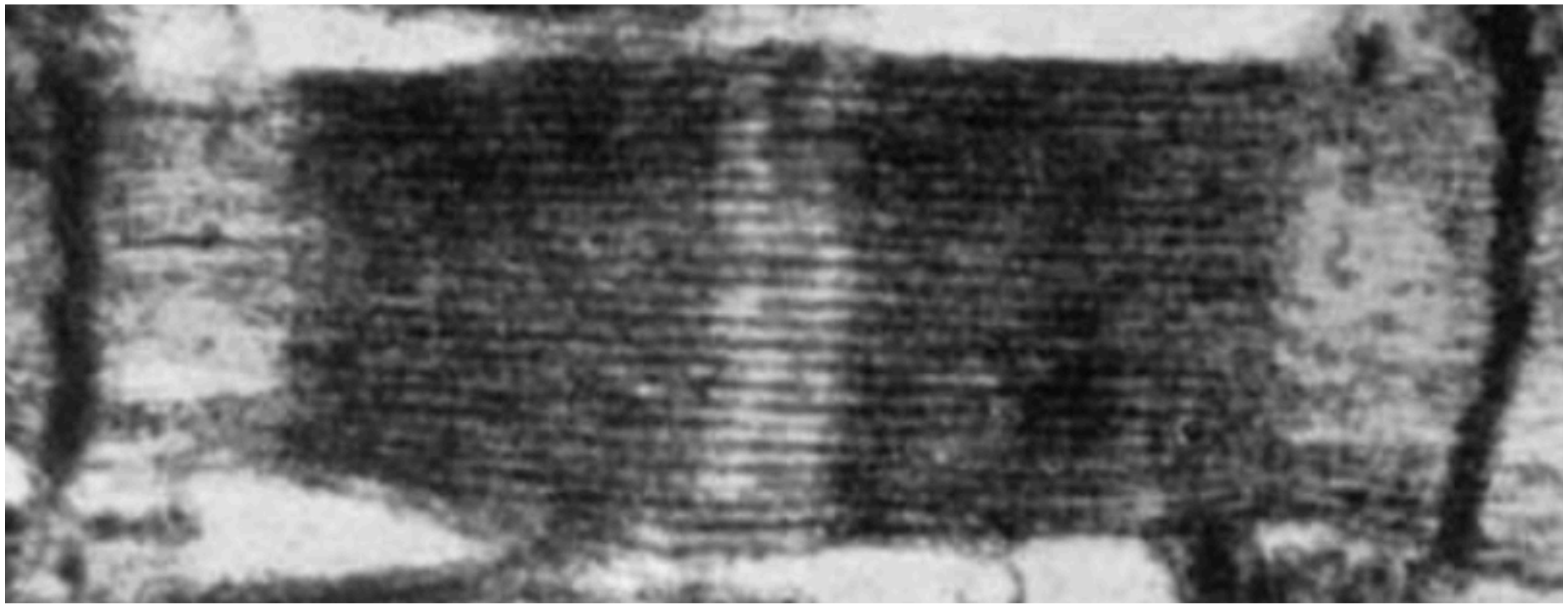
<u>Constricting cell apex has a spatial pattern of ROCK/myosin</u>



Why is ROCK/myosin in the middle?

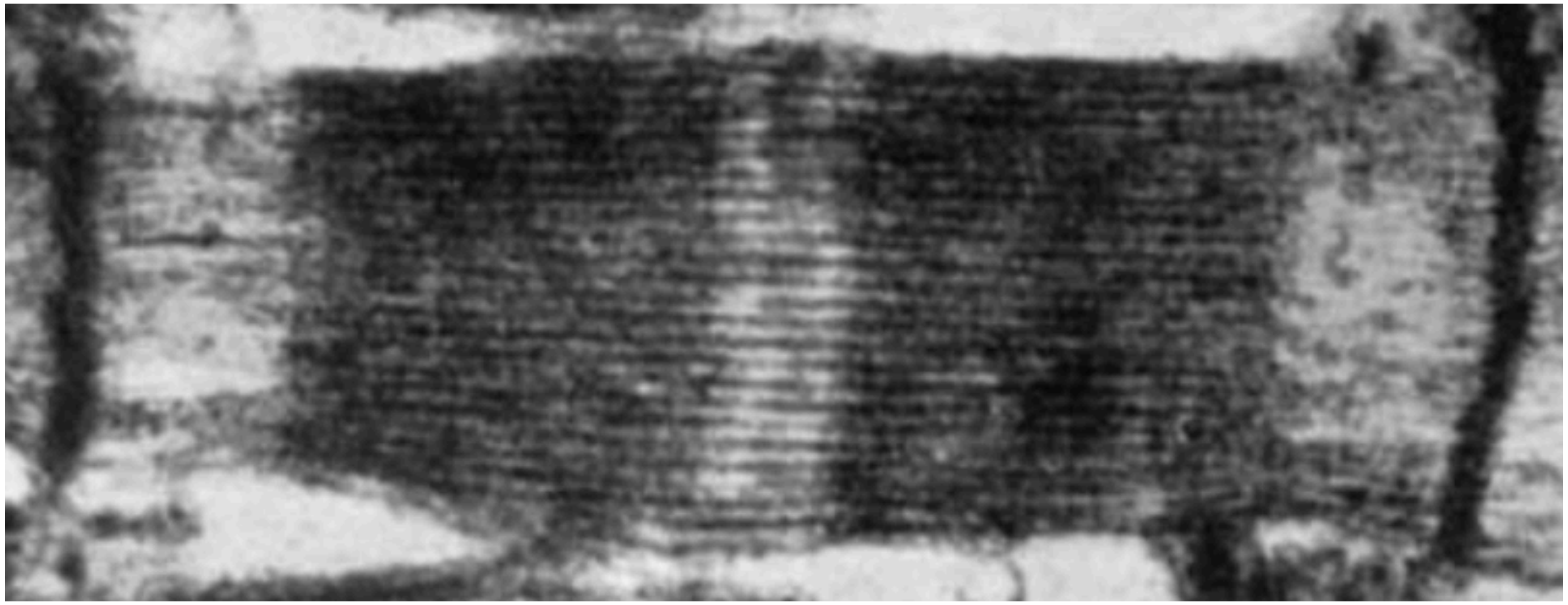
Mason FM, Tworoger M, Martin AC. *Nat Cell Biol.* (2013).
Mason et al., *J. Cell Biol.* (2016).

Sarcomere is the contractile unit of a muscle - myosin in the middle

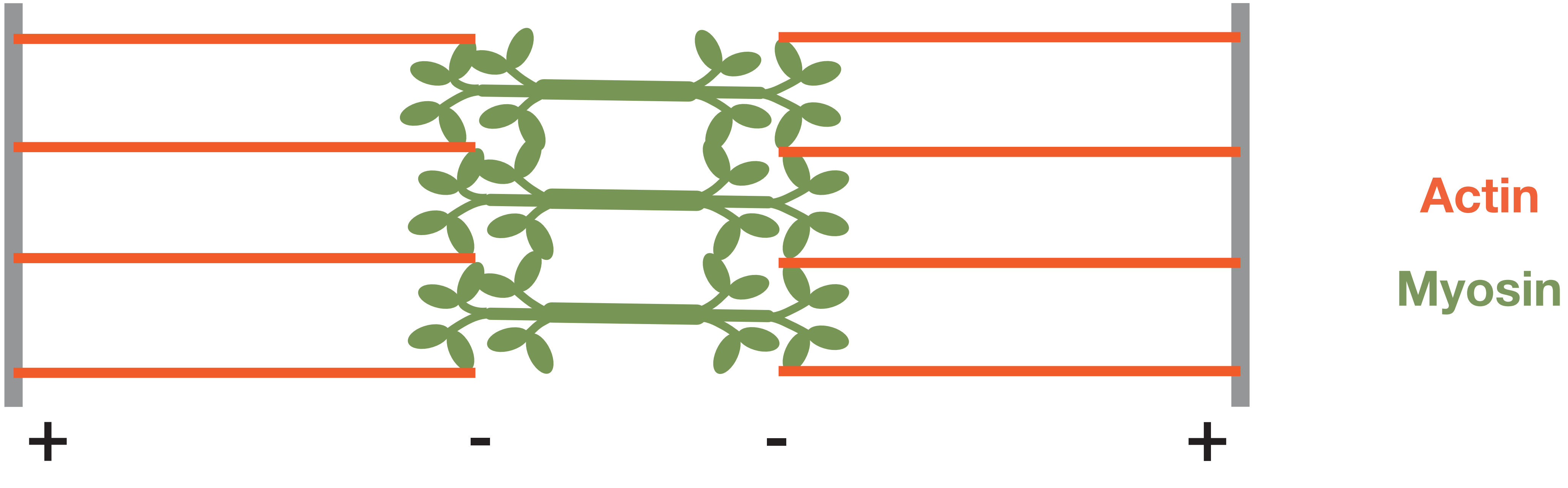


Hanson and Huxley, *Nature* (1953).

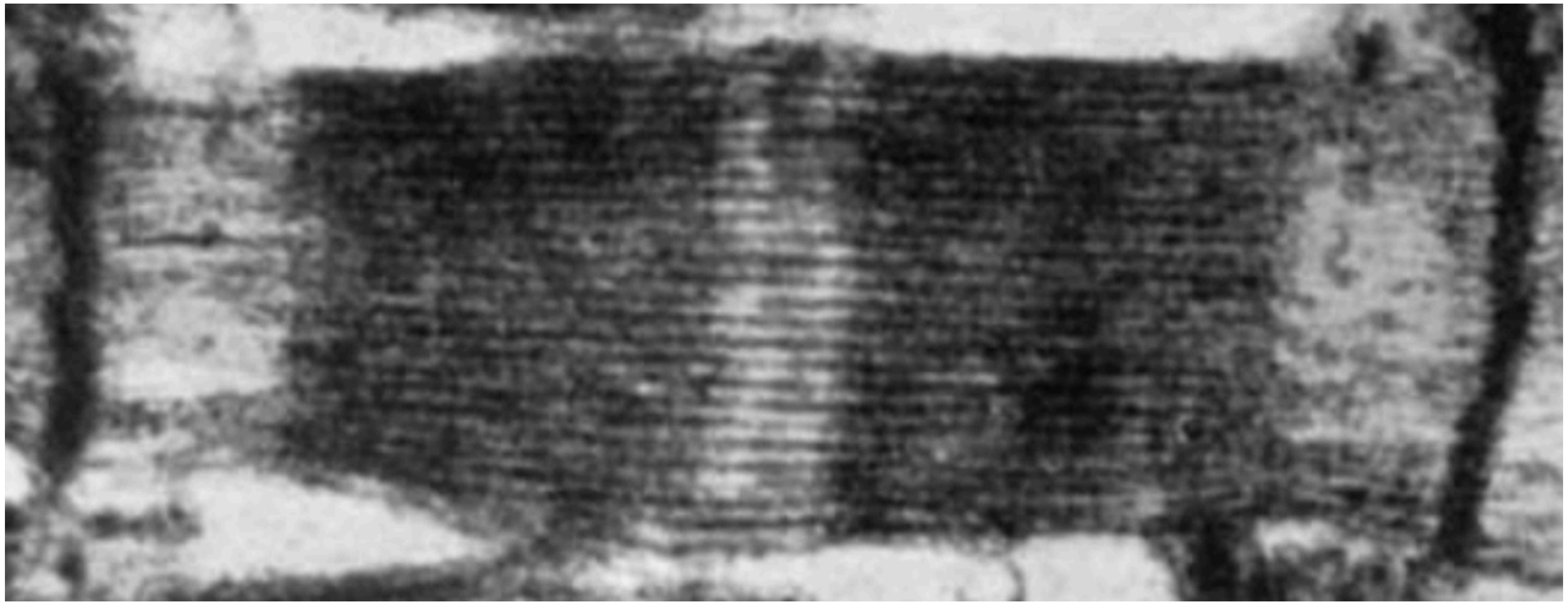
Sarcomere is the contractile unit of a muscle - myosin in the middle



Hanson and Huxley, *Nature* (1953).



Sarcomere is the contractile unit of a muscle - myosin in the middle



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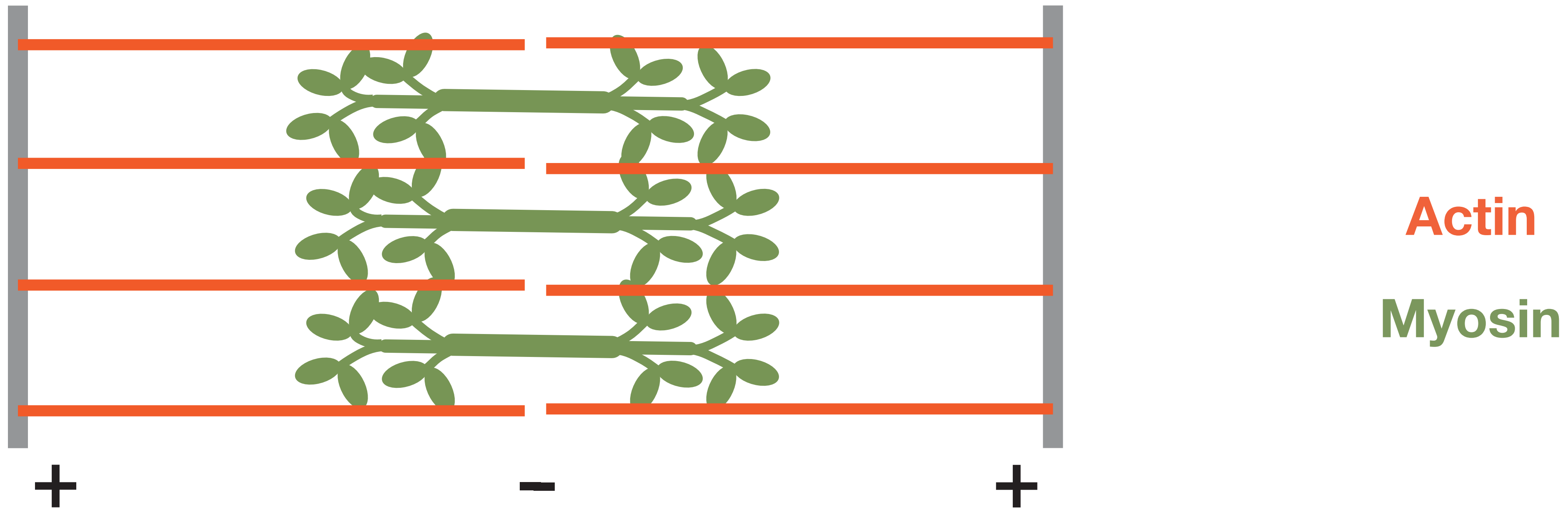
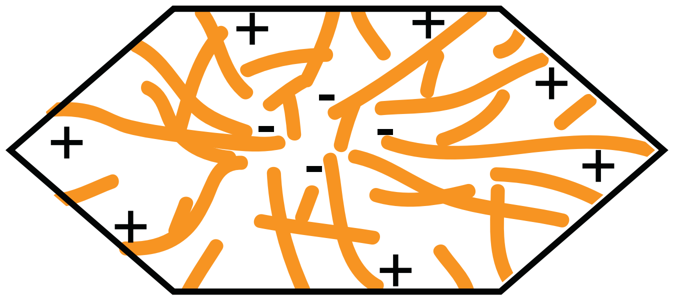


Apical cortex polarity resembles that of sarcomere

Polarized actin cortex



Jonathan Coravos, Ph.D.

Coravos and Martin, *Developmental Cell* (2016).

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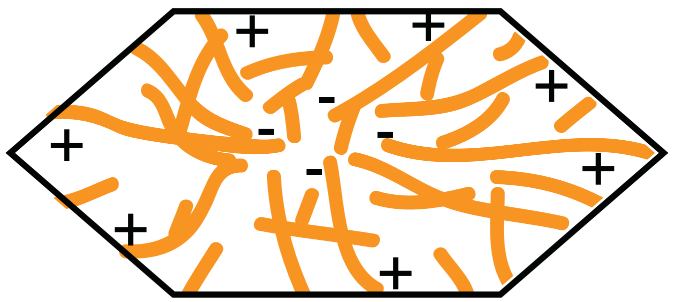
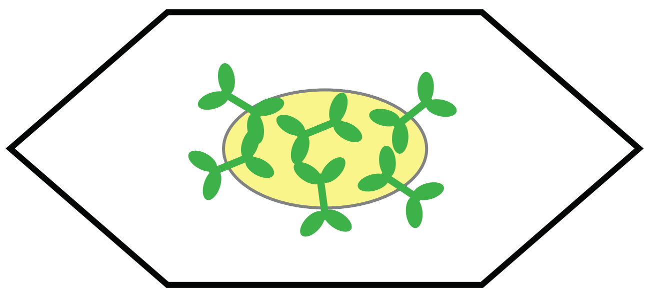


Jonathan Coravos, Ph.D.

Central ROCK and myosin II



Frank Mason, Ph.D.

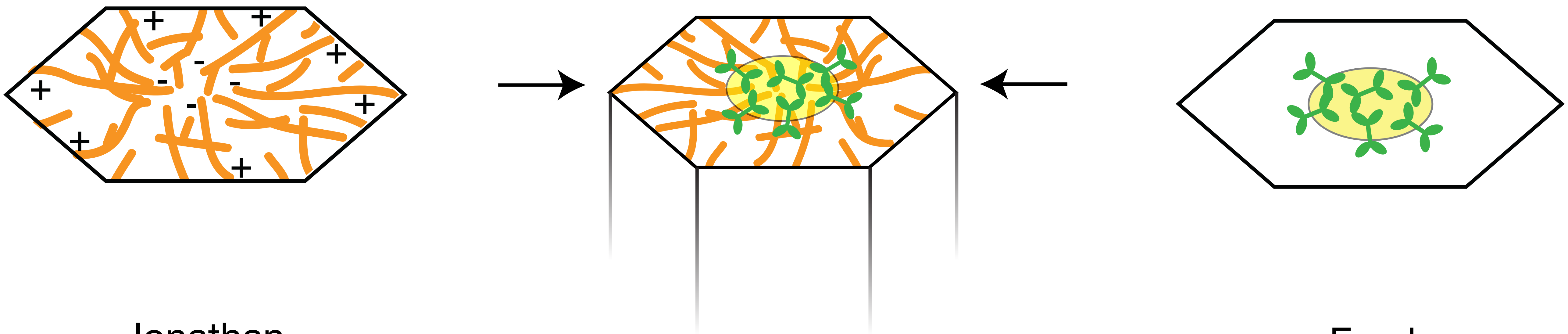
Coravos and Martin, *Developmental Cell* (2016).

Apical cortex polarity resembles that of sarcomere

Polarized actin cortex

"Radial Sarcomere"

Central ROCK and myosin II



Jonathan Coravos, Ph.D.

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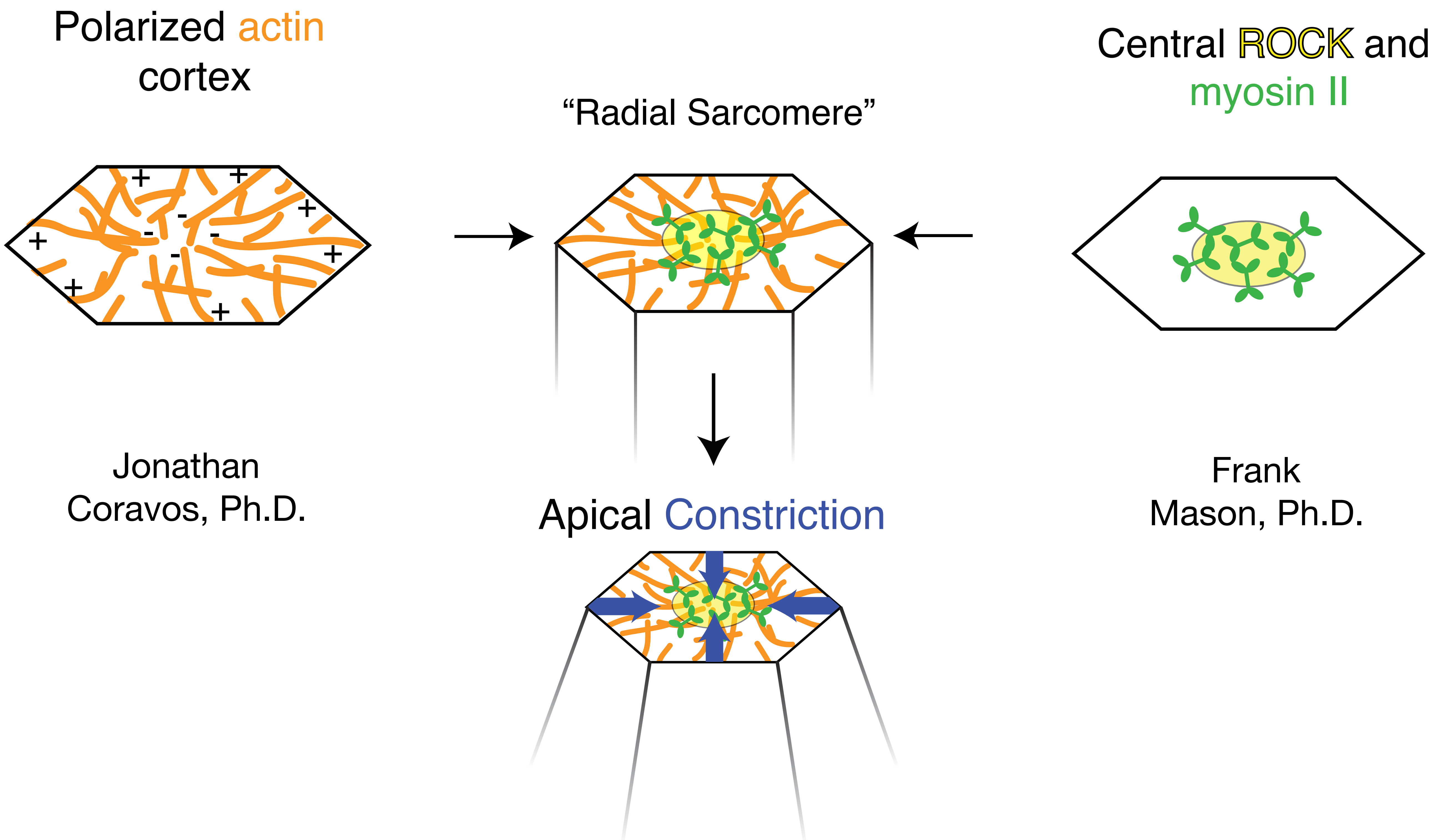

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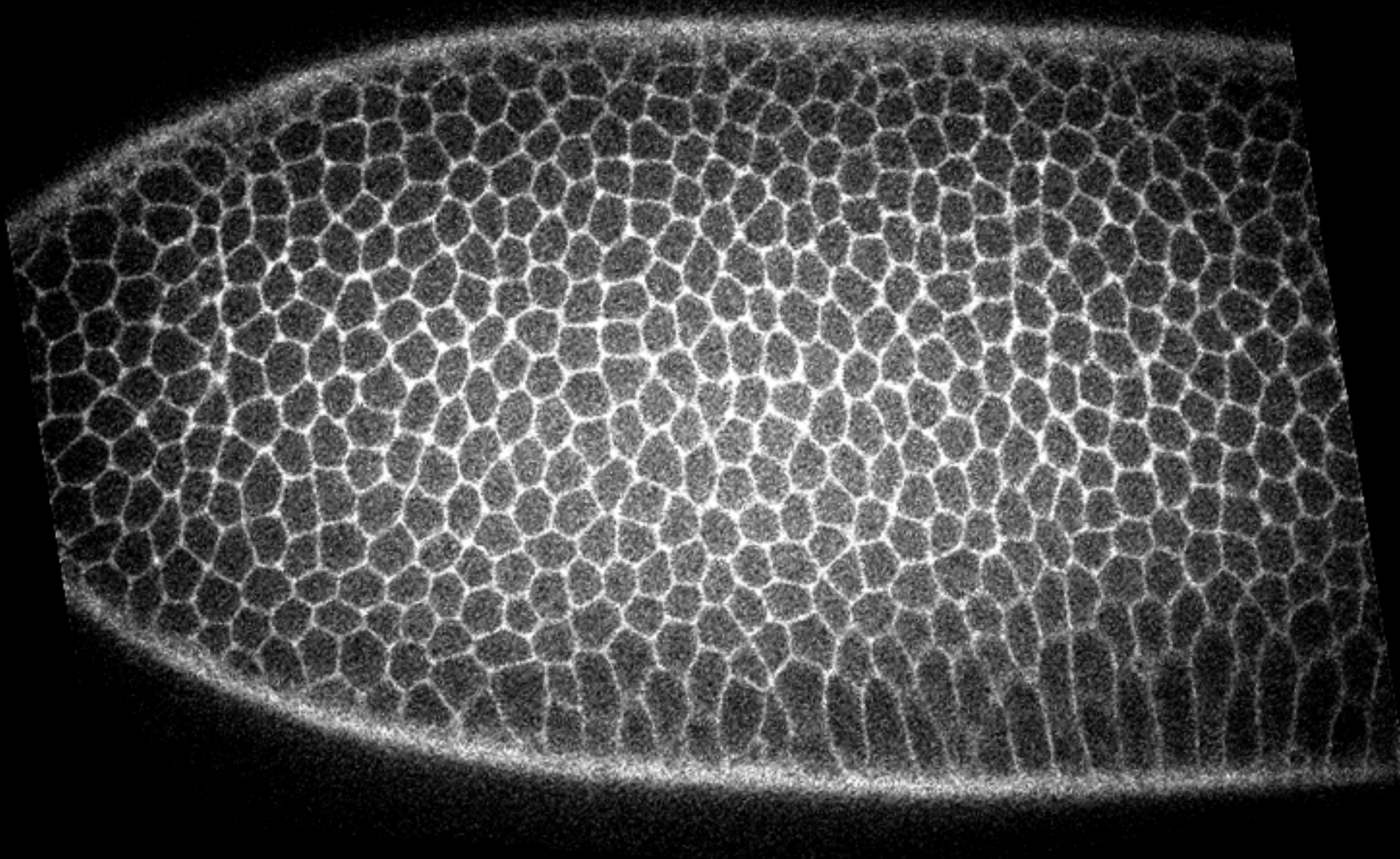
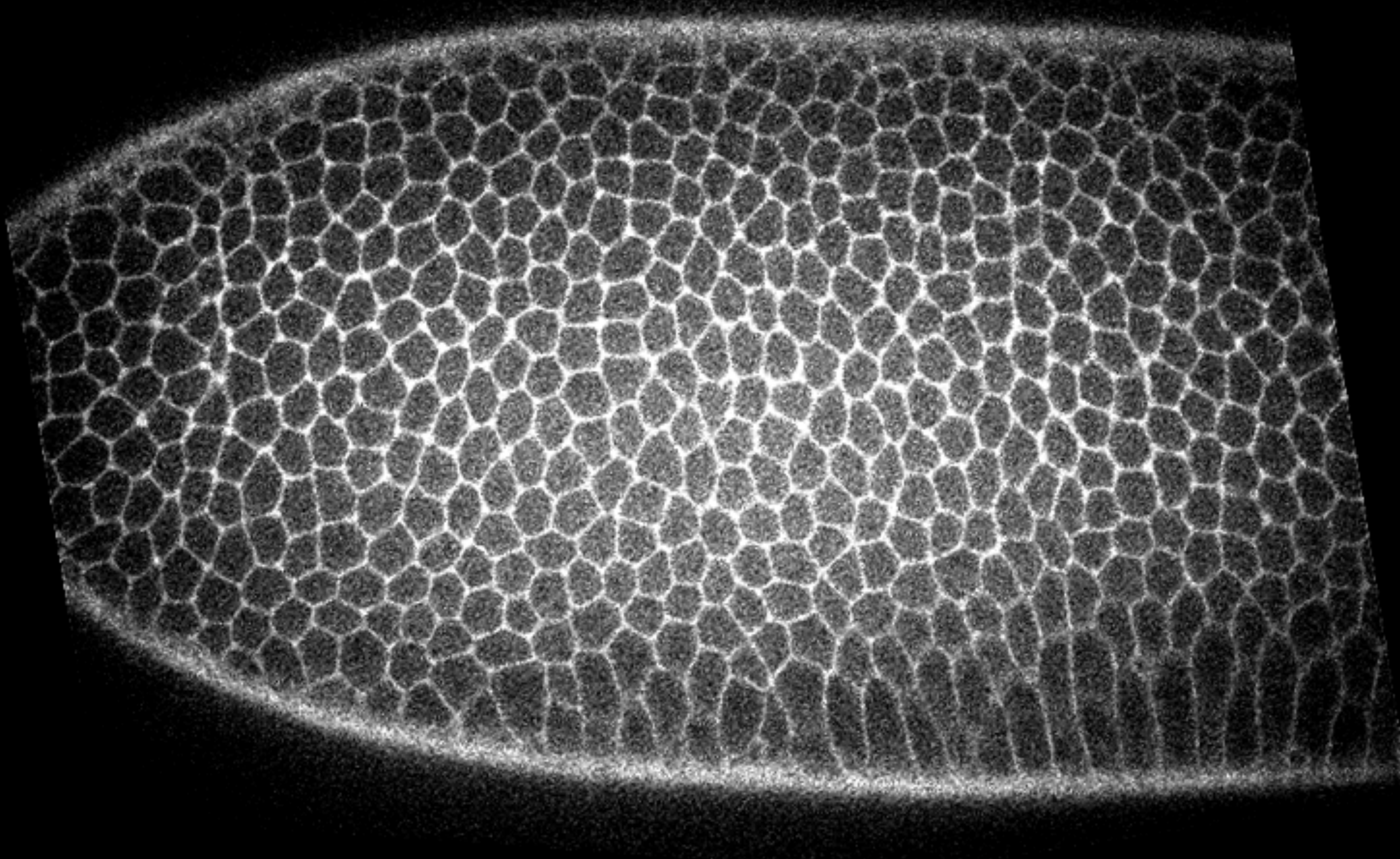
Tissue extension in Drosophila



Zallen lab

Tissue extension in Drosophila



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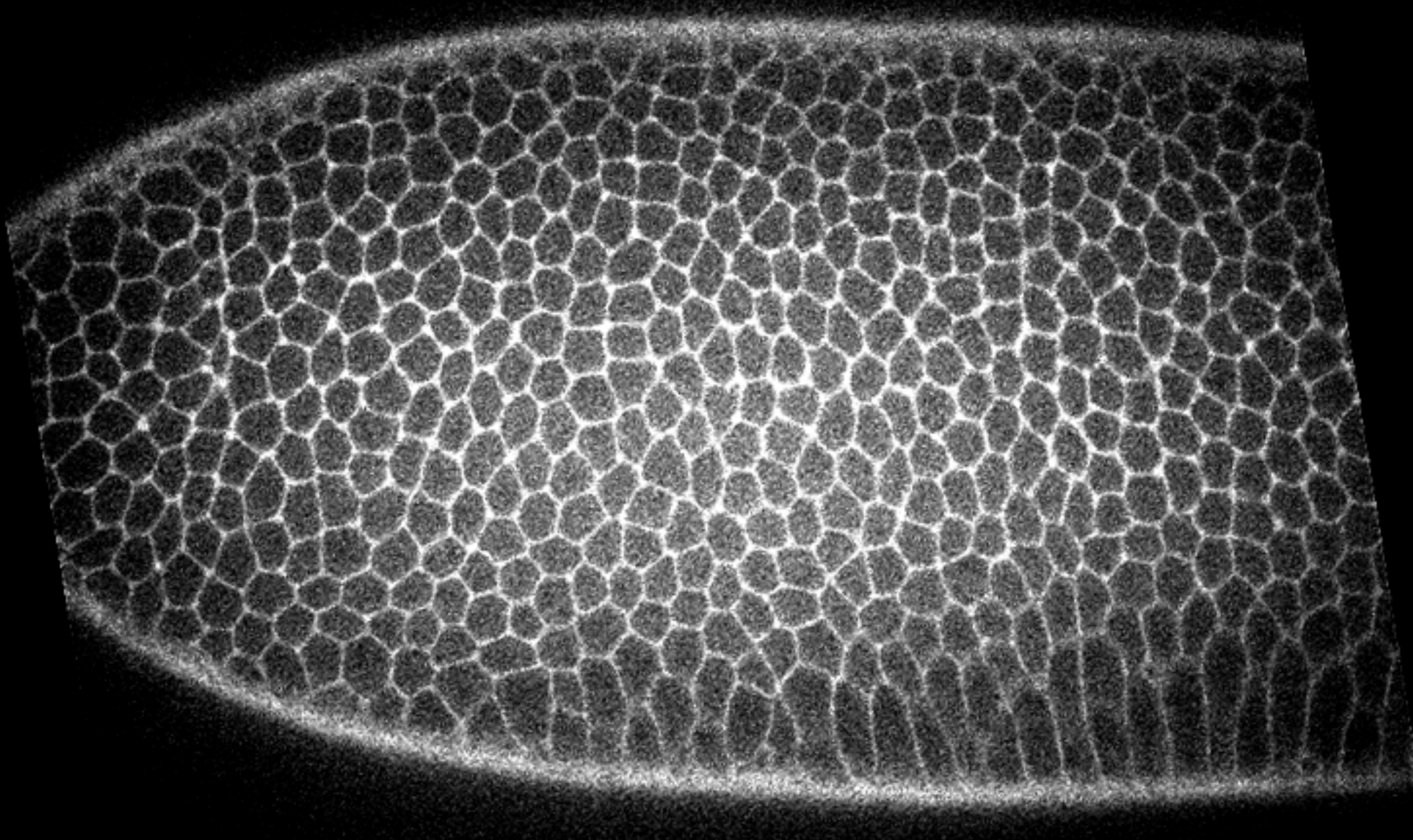
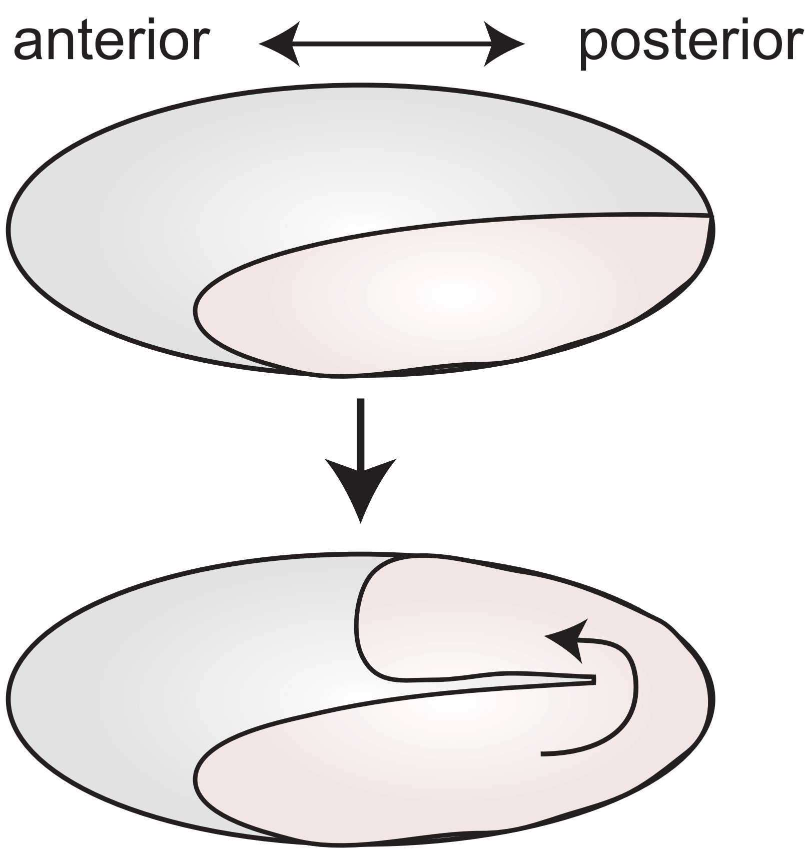


Zallen lab

Ordered exchange of intercellular contacts can drive extension

1



Mason & Martin, 2011

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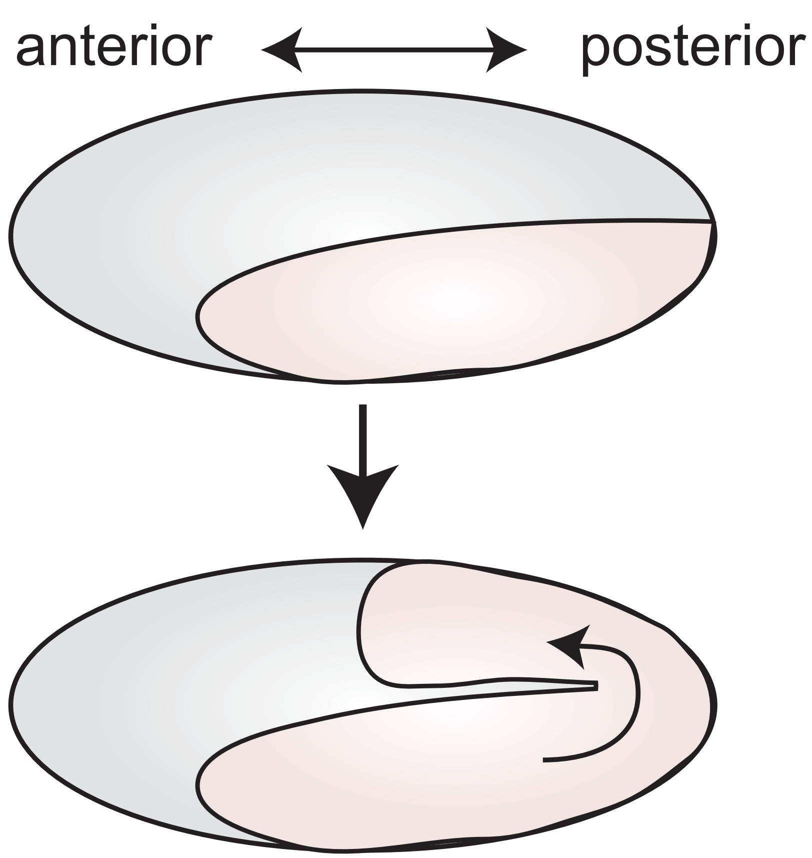
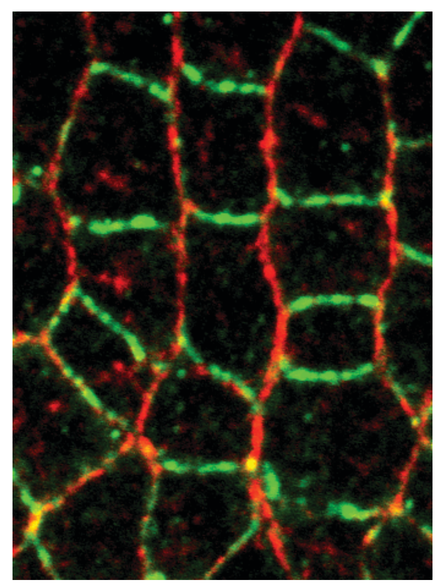
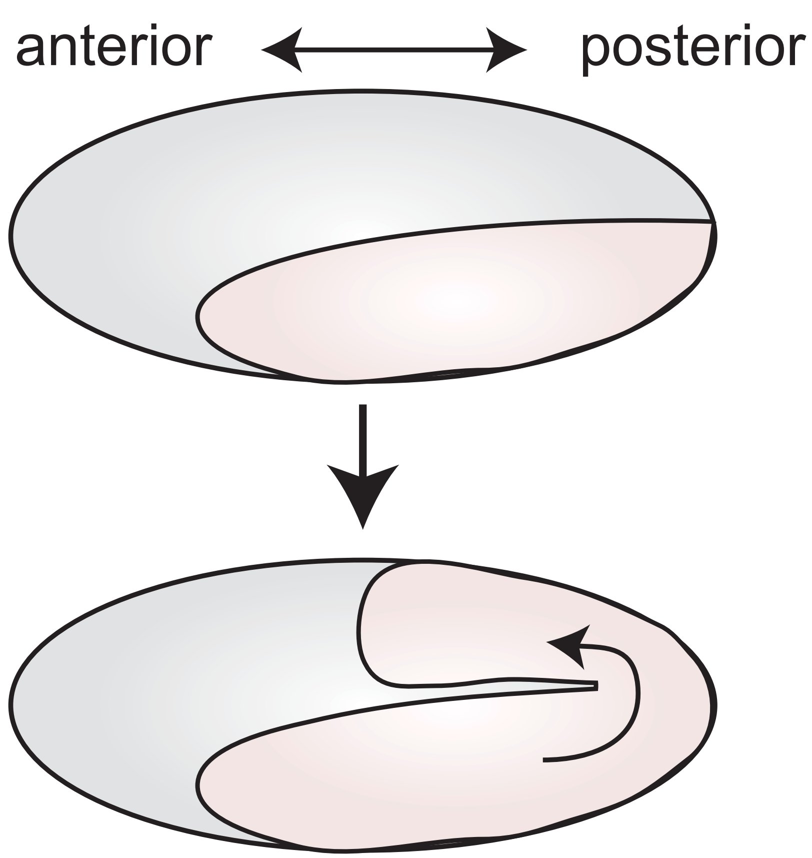


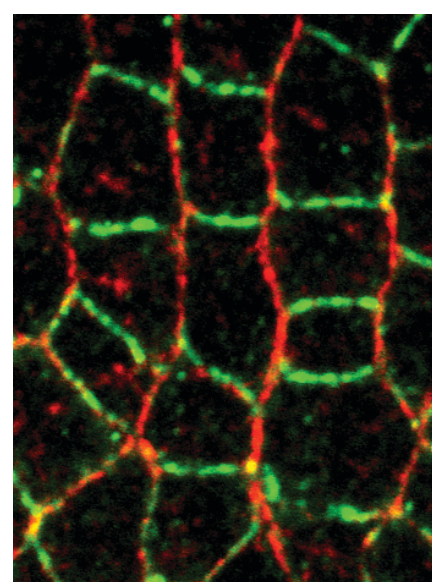


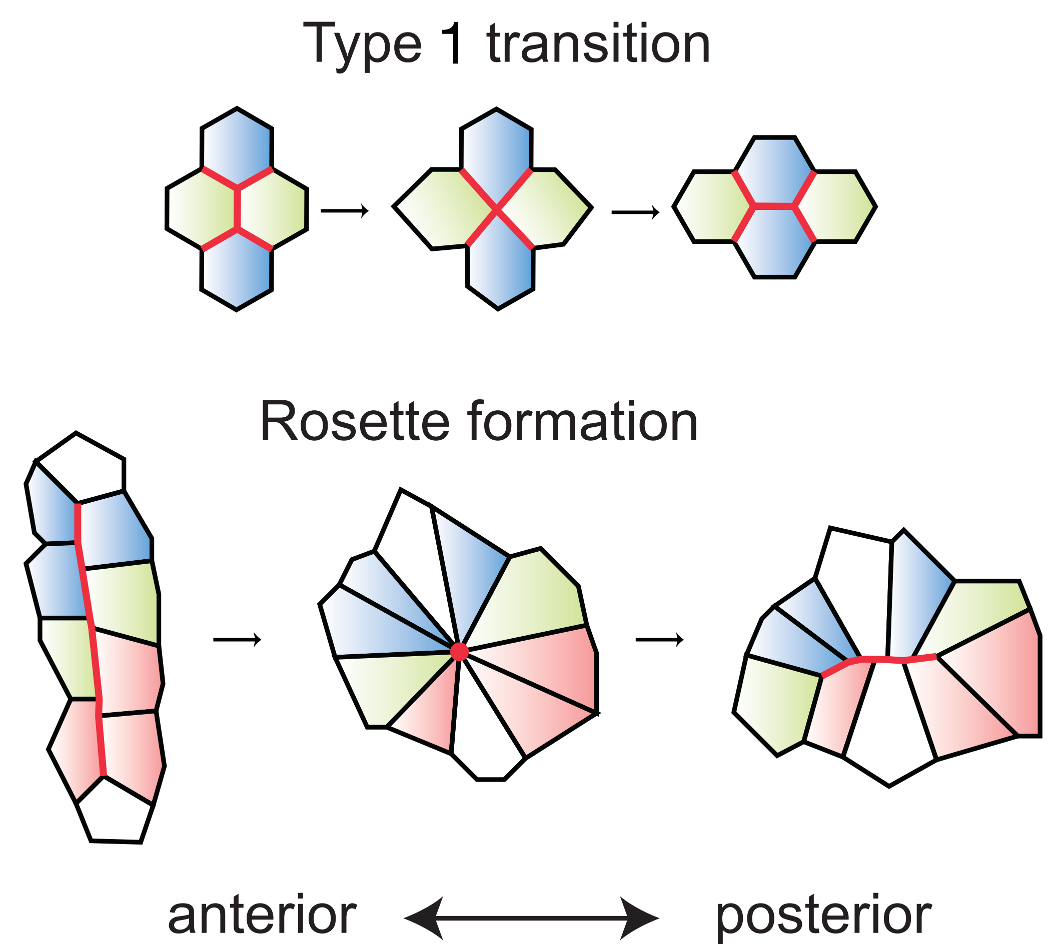
Myo-II
Baz/Par-3

Mason & Martin, 2011

Ordered exchange of intercellular contacts can drive extension

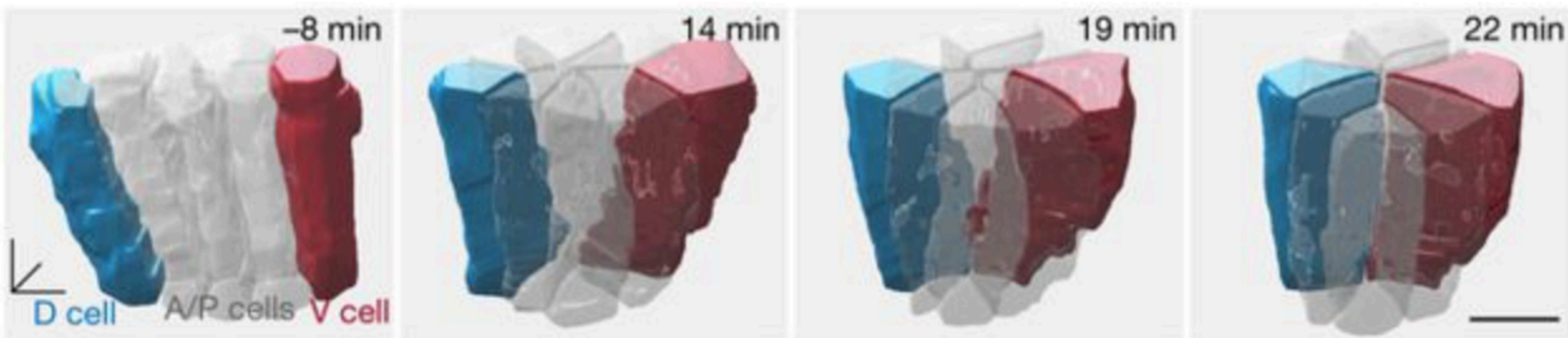






Mason & Martin, 2011

Cell protrusion is also important for convergent extension



Sun et al., 2017

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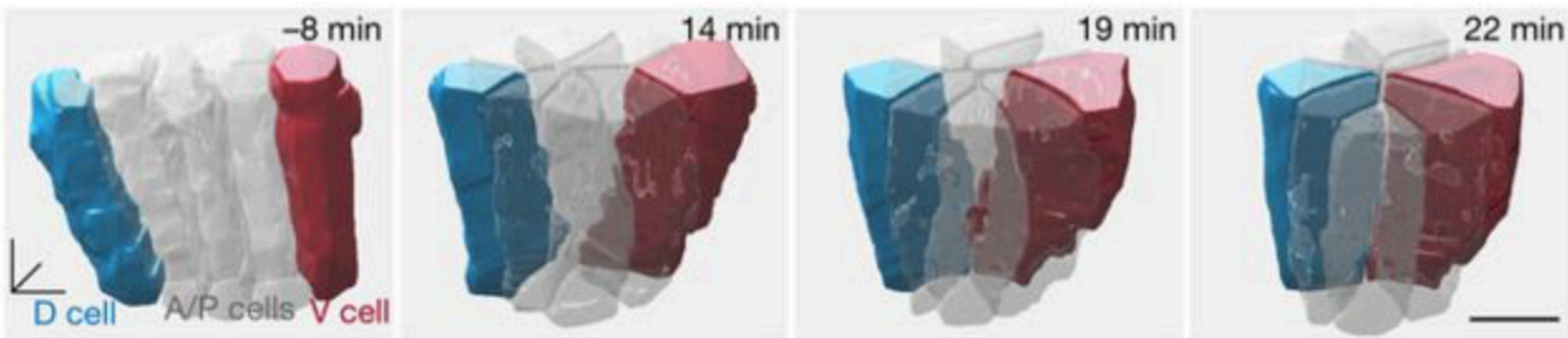

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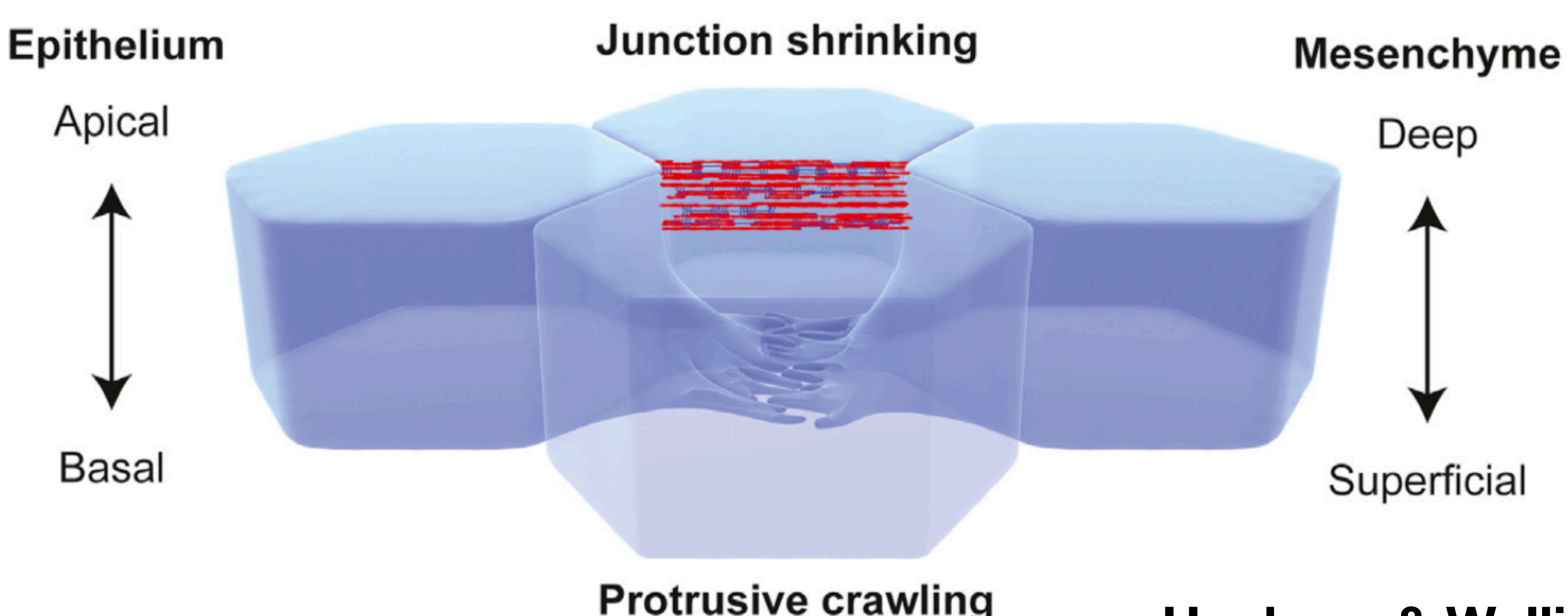

Huebner & Wallingford, 2018

How do we know whether myosin functions as a ‘motor’?

ATPase Activity of Myosin Correlated with Speed of Muscle Shortening

MICHAEL BÁRÁNY

1967

From the Institute for Muscle Disease, Inc., New York

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1967

Claudia Vasquez

ATPase rate scales with muscle contraction velocity

TABLE II

RELATIONSHIP BETWEEN CONTRACTION TIME AND ATPASE ACTIVITY OF MYOSIN IN MUSCLES OF CAT AND SLOTH

Muscle	Contraction time*	ATPase activity‡ in the presence of: Actin	Ca^{++}+0.05 M KCl	Ca^{++}+0.5 M KCl	EDTA	ATP sensitivity‡
	msec	*μmoles P_i/mg/min*	*μmole/P_i/mg/min*	*μmole P_i/mg/min*	*μmole P_i/mg/min*	%
Cat extensor digitorum longus....	19–19.5	1.46	0.67	0.45	0.61	131
Sloth extensor digitorum longus..	122–135	0.26	0.18	0.12	0.17	100
Cat gastrocnemius medialis.......	22.5–27	1.41	0.68	0.39	0.58	128
Sloth gastrocnemius medialis.....	109	0.25	0.20	0.12	0.19	122

* 37–38°C and 34–35°C for muscles of cat and sloth, respectively. References for contraction times: cat extensor digitorum longus, Gordon and Phillips (13, 14); cat gastrocnemius, Wills (15) and Buller et al. (11); sloth muscles, Goffart et al. (12).

‡ 37.5°C and 34.5°C for myosin of muscles of cat and sloth, respectively.

ATPase rate scales with muscle contraction velocity

BLE II

N CONTRACTION TIME AND
IN MUSCLES OF CAT AND SLOTH

	n	ATPase activity‡ in the presence of				
		Actin	Ca^{++}+0.05 M KCl	Ca^{++}+0.5 M KCl	EDTA	ATP sensitivity‡
		μmoles P_i/ mg/min	*μmole/P_i/ mg/min*	*μmole P_i/ mg/min*	*μmole P_i/ mg/min*	%
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ATPase rate scales with muscle contraction velocity

BLE II

CONTRACTION TIME AND

IN MUSCLES OF CAT AND SLOTH

ATPase activity‡ in the presence of

	n	Actin	Ca^{++}+0.05	Ca^{++}+0.5		ATP
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ATPase rate scales with muscle contraction velocity

BLE II

N CONTRACTION TIME AND

IN MUSCLES OF CAT AND SLOTH

	n	ATPase activity‡ in the presence of				
		Actin	Ca^{++}+0.05 M KCl	Ca^{++}+0.5 M KCl	EDTA	ATP sensitivity‡
		μmoles P_i/ mg/min				
Cat extensor digitorum longus....	19–19.5	1.46				
Sloth extensor digitorum longus..	122–135	0.26				
Cat gastrocnemius medialis.......	22.5–27	1.41				
Sloth gastrocnemius medialis.....	109	0.25				

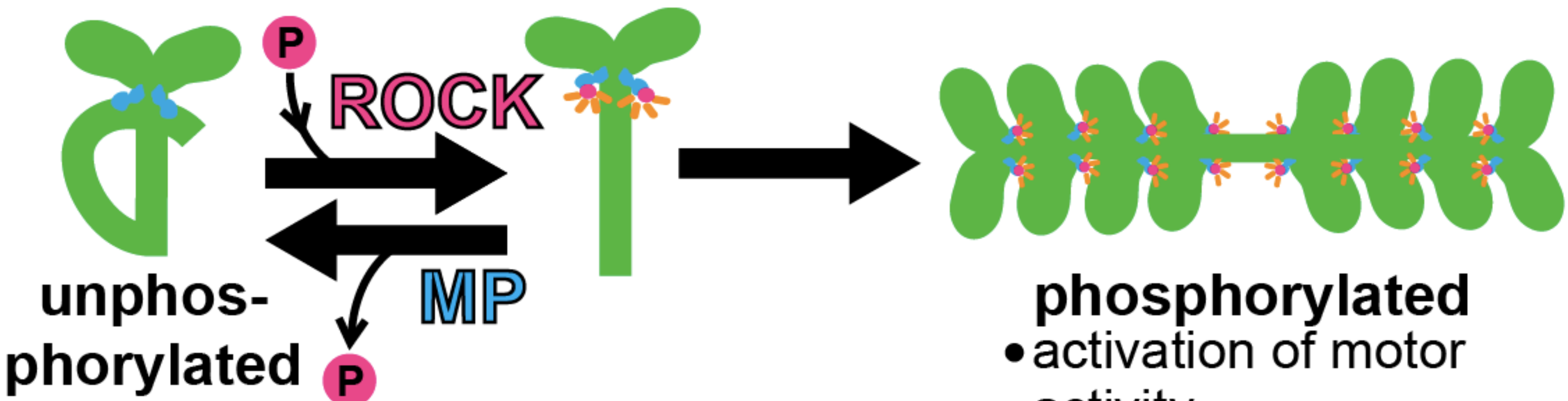
* 37–38°C and 34–35°C for muscles of cat and sloth, resp

times: cat extensor digitorum longus, Gordon and Phillip

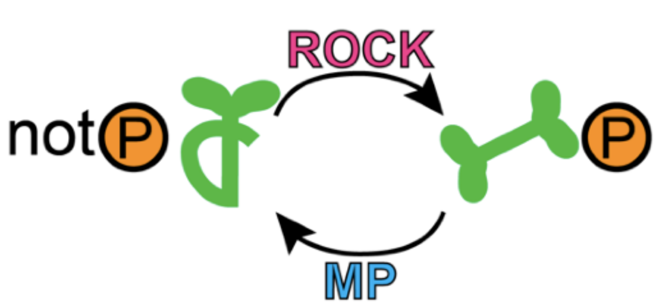
(15) and Buller et al. (11); sloth muscles, Goffart et al. (12).

‡ 37.5°C and 34.5°C for myosin of muscles of cat and sloth, r

Myosin activity and assembly is regulated by phosphorylation

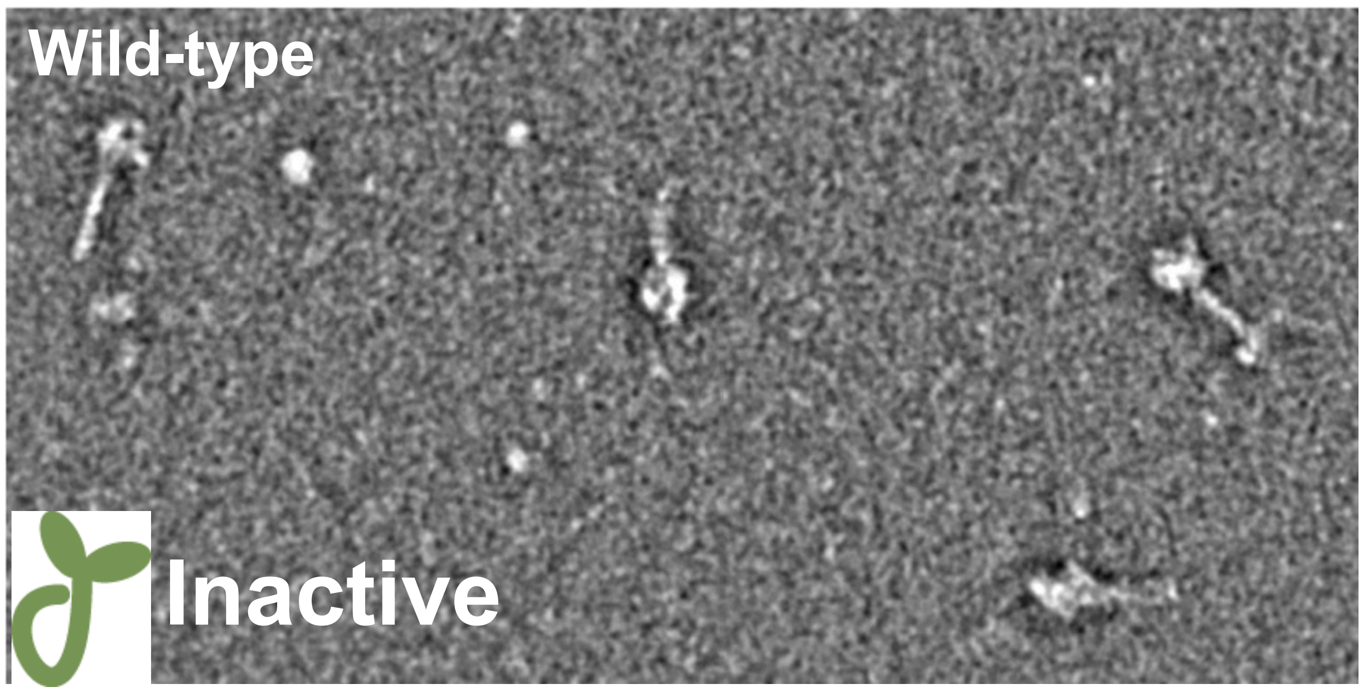


- activation of motor activity
- assembly into bipolar mini-filaments



Drosophila myosin 2 is regulated by phosphorylation similar to mammalian myosin 2

- Phosphorylation



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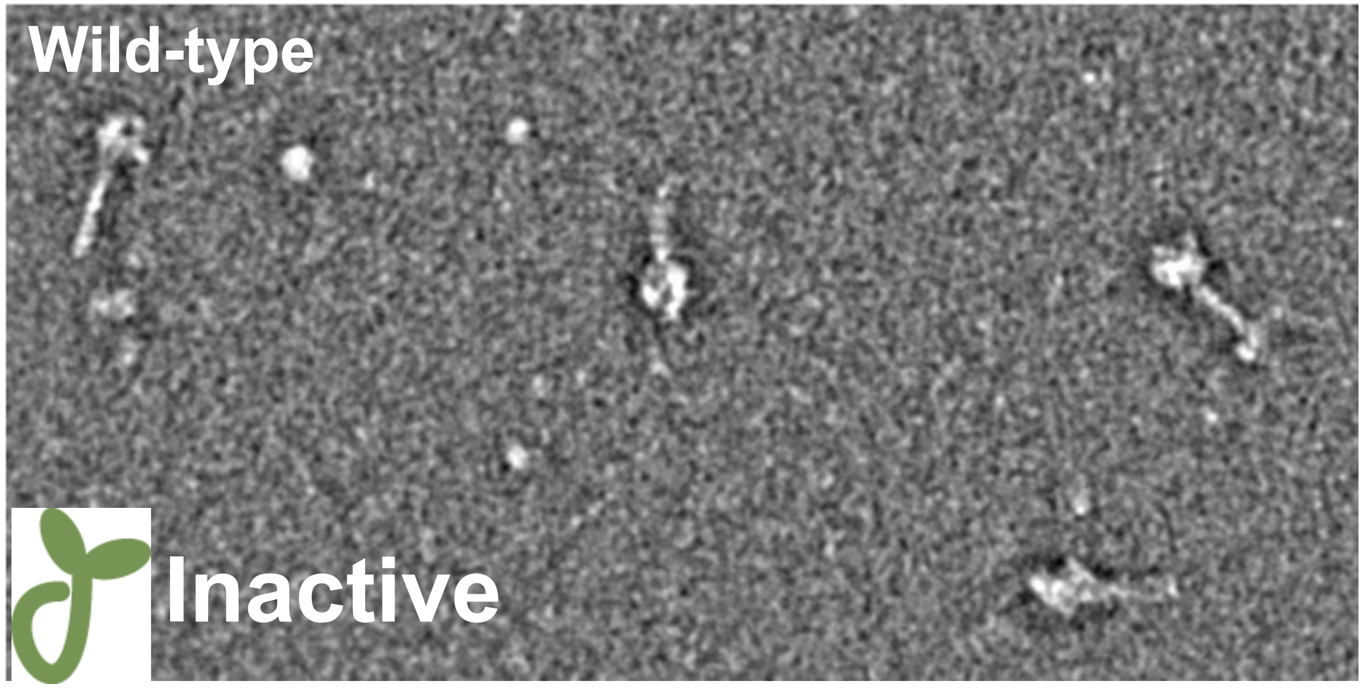
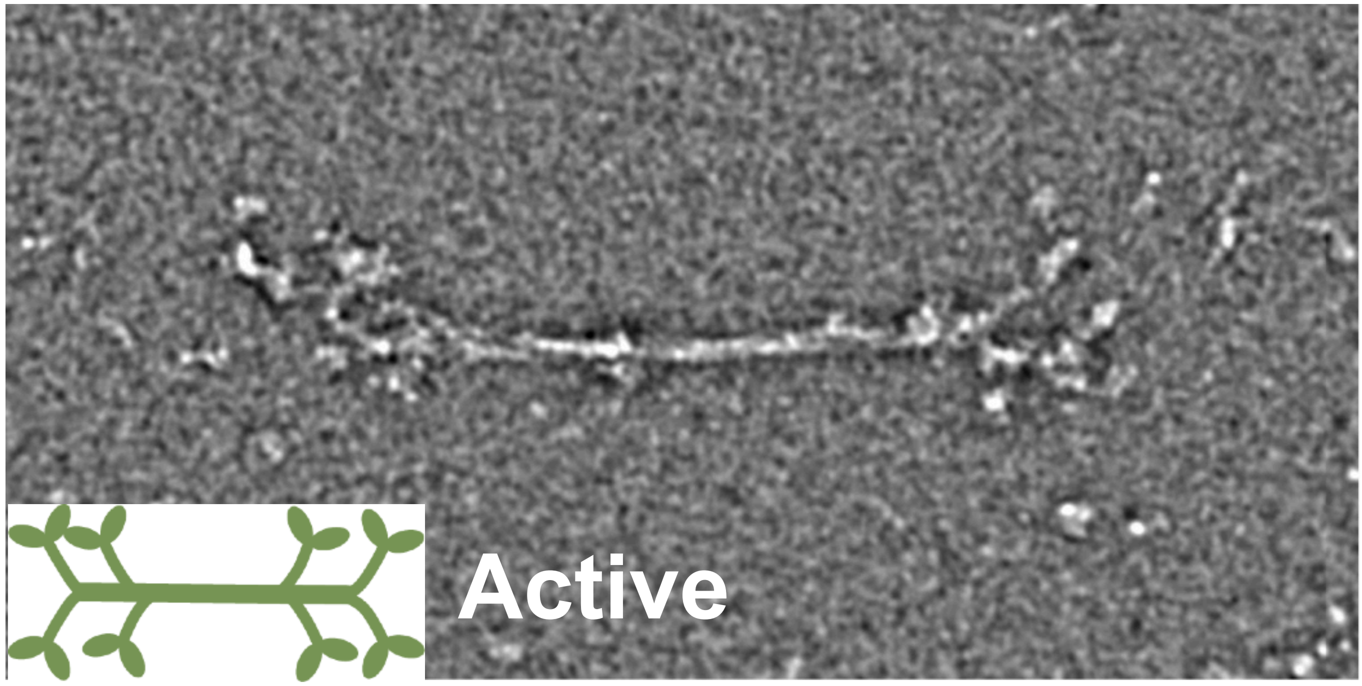
Drosophila myosin 2 is regulated by phosphorylation similar to mammalian myosin 2

- Phosphorylation



+ Phosphorylation



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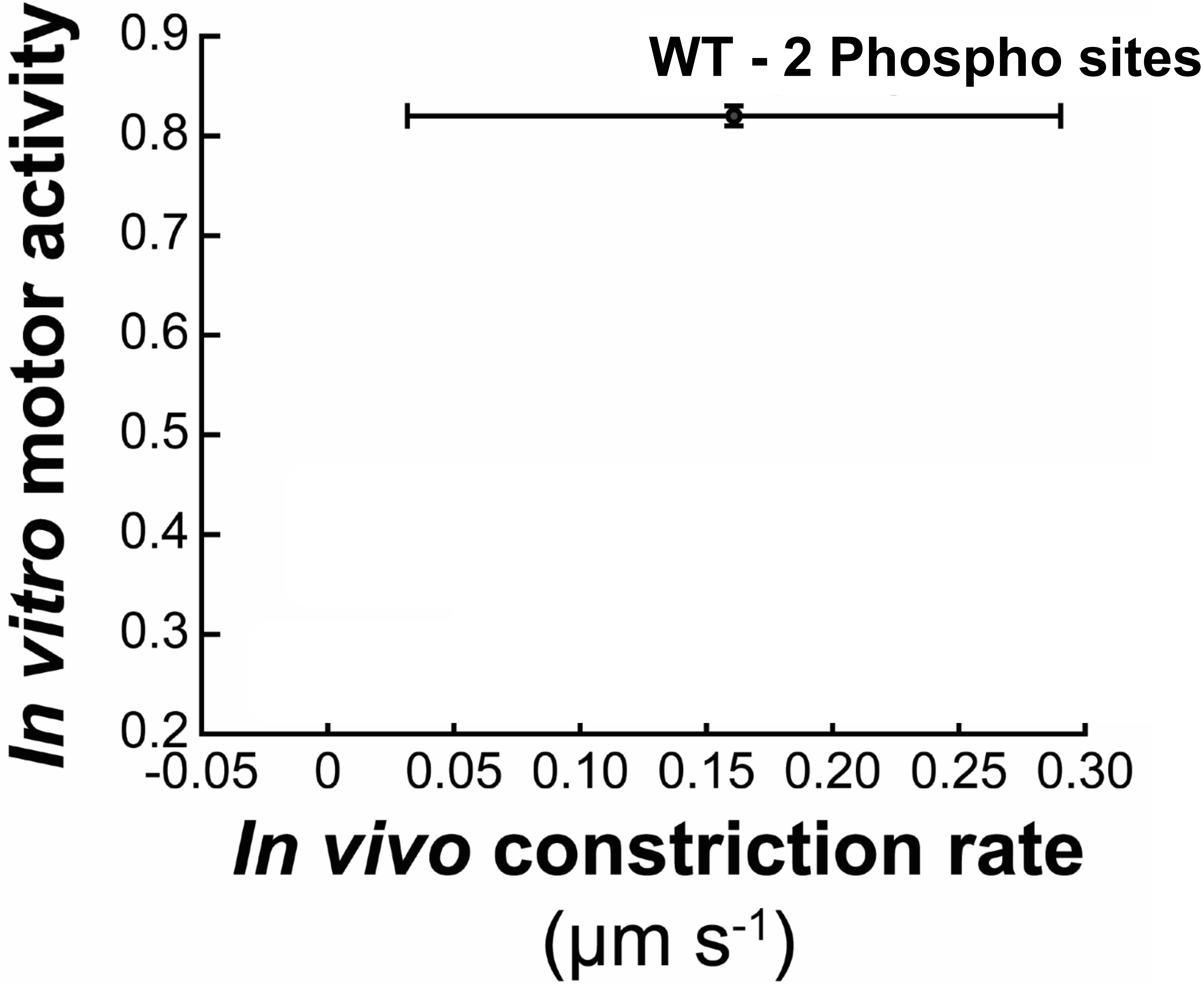
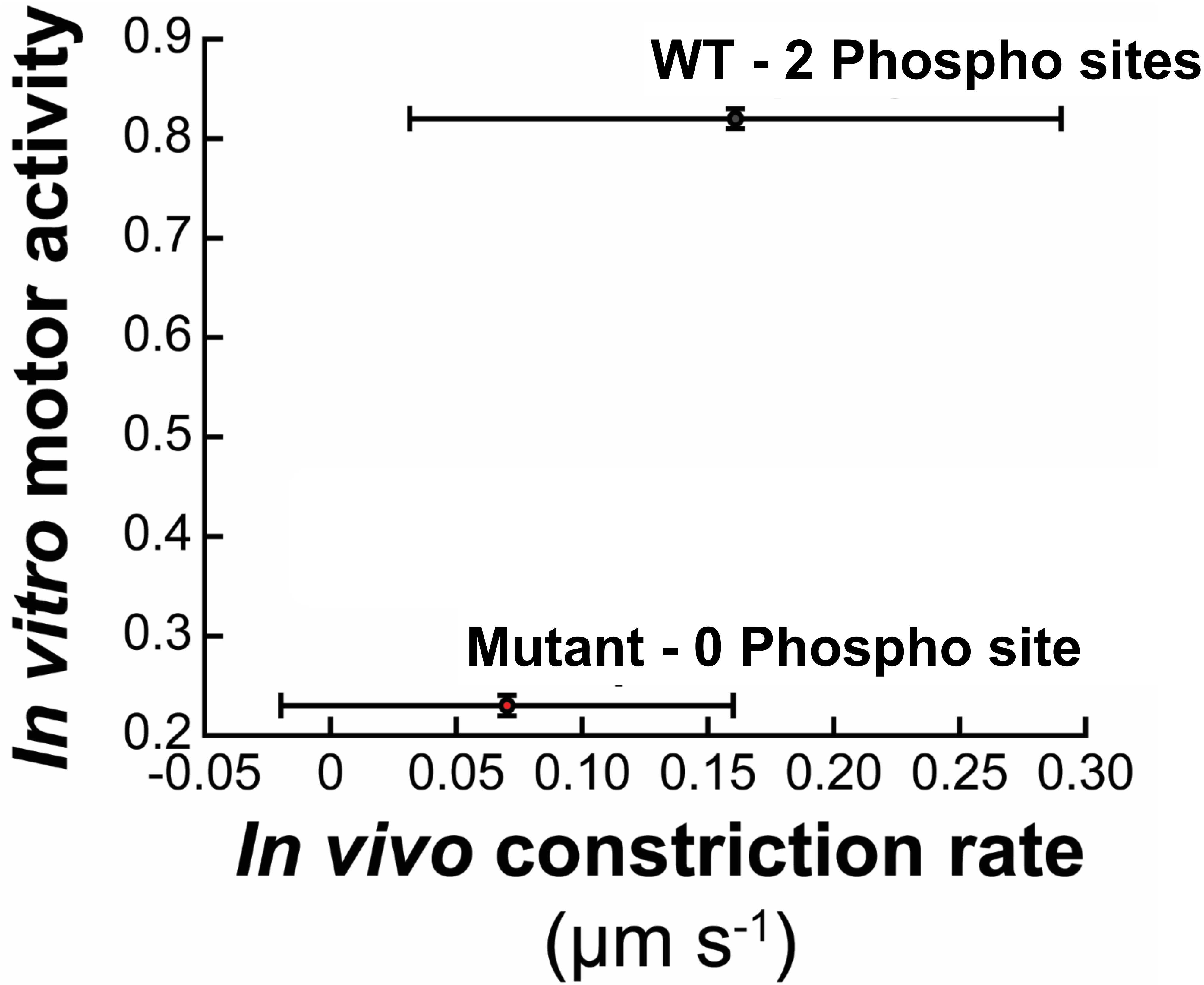
James Sellers

Myosin motor activity scales with contraction rate



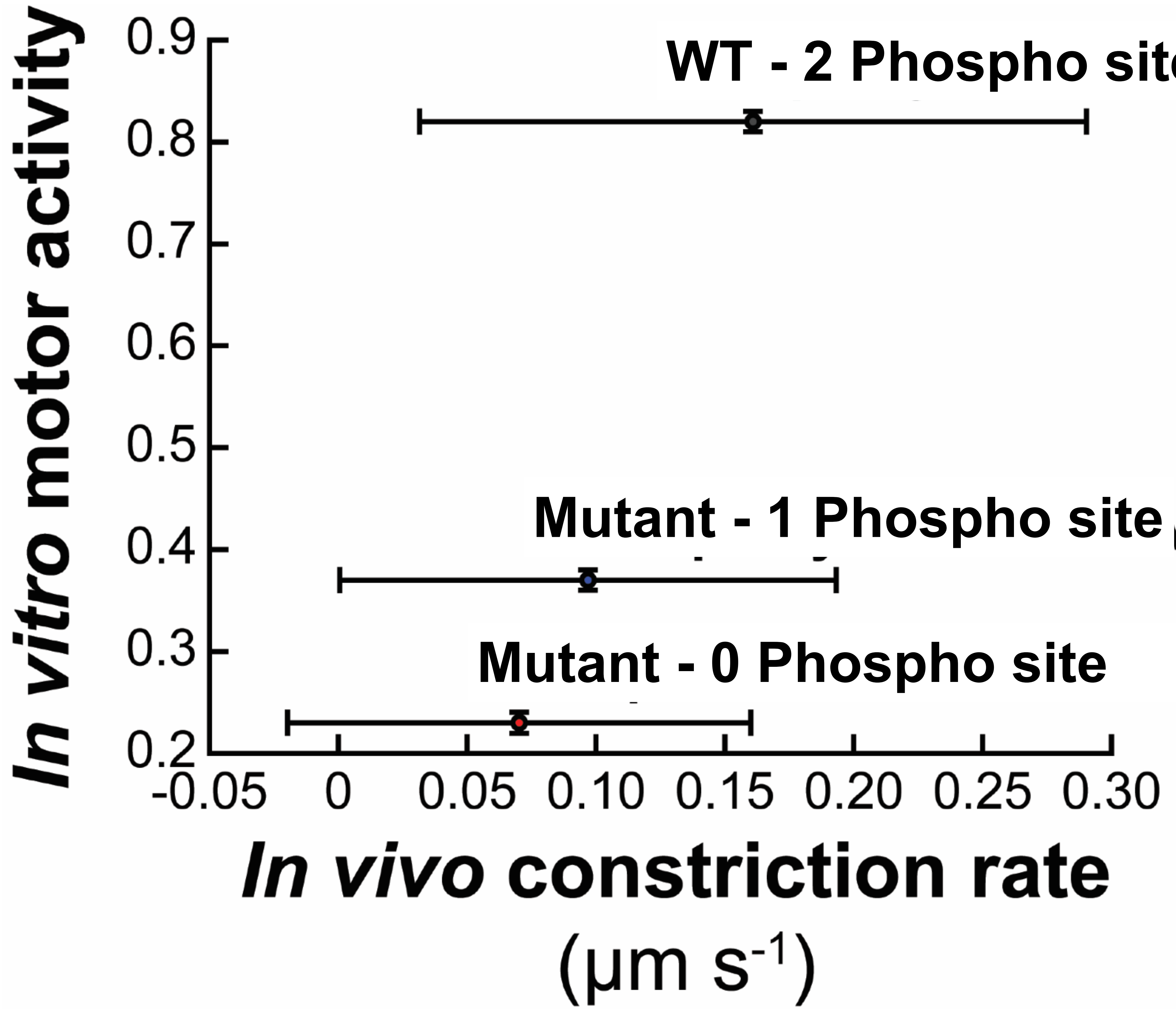
Vasquez, et al., *eLife* (2016).

Myosin motor activity scales with contraction rate



Vasquez, et al., *eLife* (2016).

Myosin motor activity scales with contraction rate



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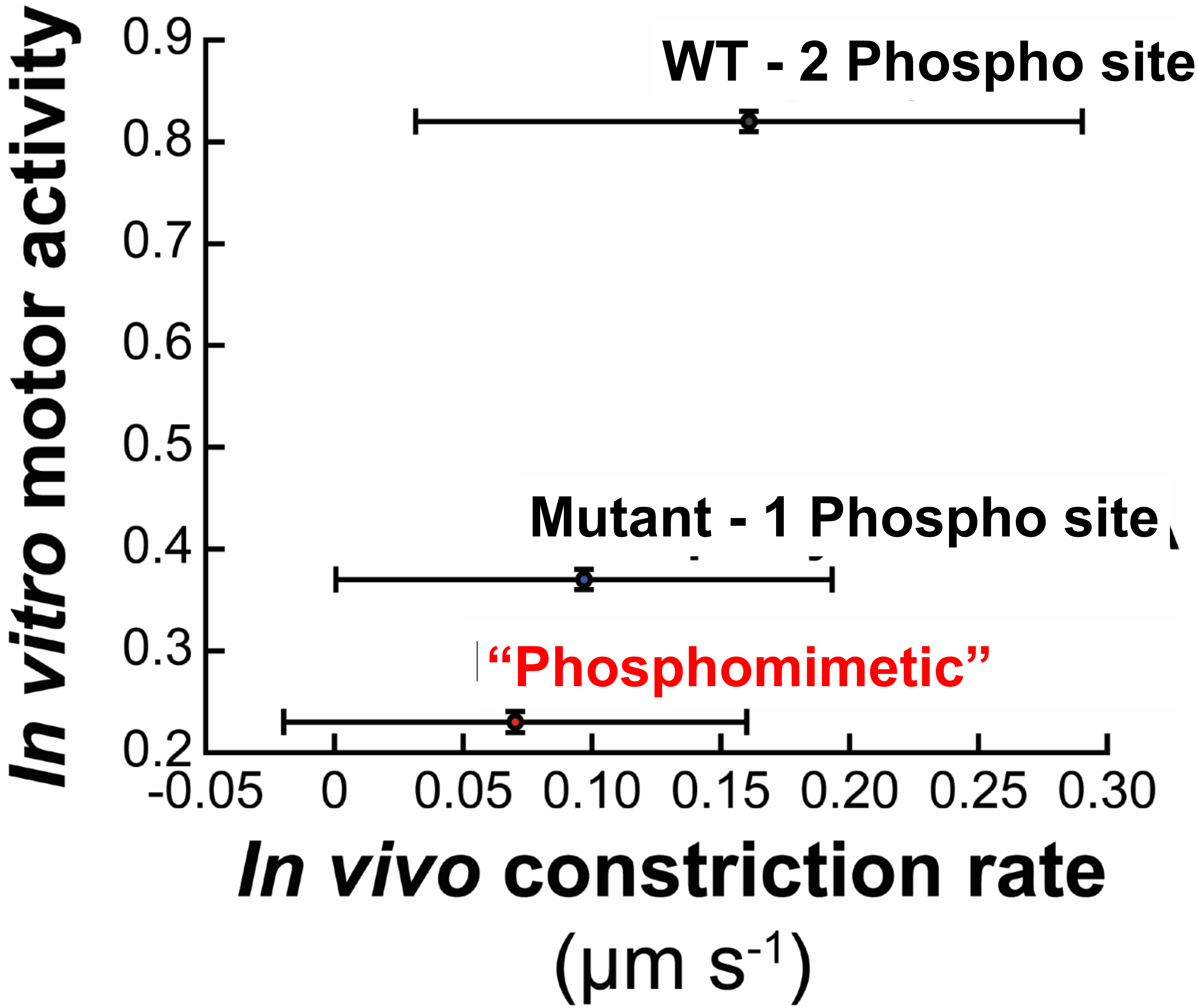
Myosin motor activity scales with contraction rate



Vasquez, et al., *eLife* (2016).