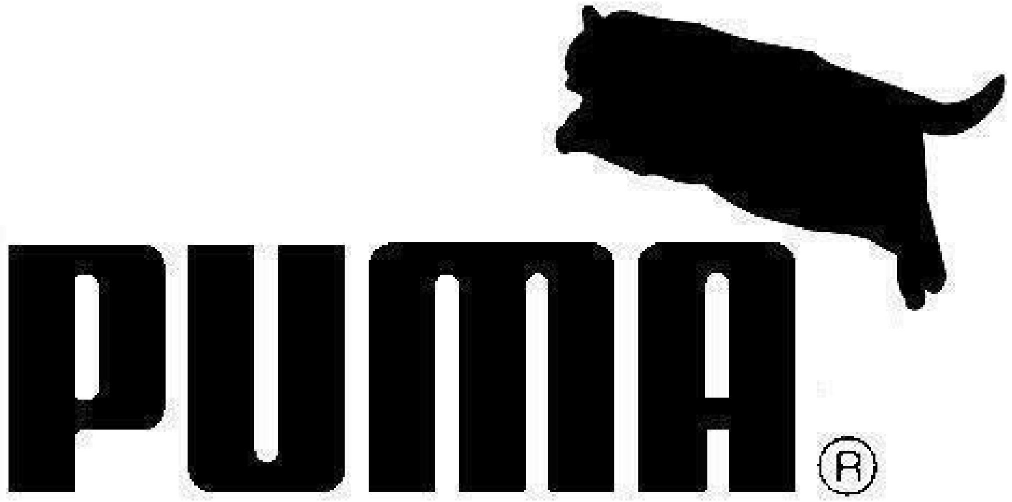
Vývojová biologie

Regulation of cell and organ size in development and examples of their necessity



Tomáš Bárta
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Contents

- Introduction
- Embryo size
- Signaling pathways in individual/organ size regulation
- Aspects of regulation Growth rate/duration
- Examples – Drosophila, Human
- Regulation of organ size

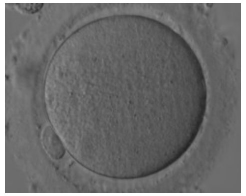
Size control - food for thought

- The Basic Question of Developmental Biology
- We still don't know the clear answer

Suncus etruscus

vs

Balaenoptera musculus



The same plan of the body (propositions must be preserved), the size of tissues and organs largely differs

?

Size control - food for thought

- Size is the most basic phenotype
- It affects many aspects of animal biology: anatomy, physiology, behavior...
- Therefore, control of body/organ size is a key developmental process that ensures that an animal grows to a size that is typical of its species.

- Improper regulation leads to dwarfism, gigantism, and/or hypo- or hyperplasia in organs.

Size control - food for thought

Cell number regulation

Cell size control

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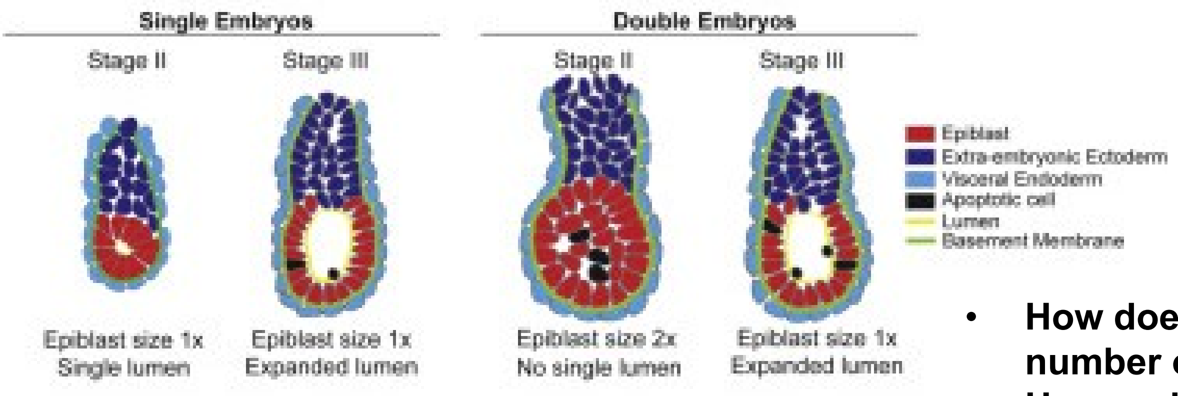
How is the size of embryo regulated?

If we aggregate two embryos together - a normal individual is formed



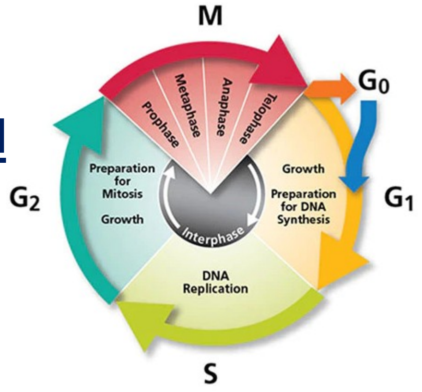
- **How does an embryo know that it should have the right number of cells?**
- **How and when does it adjust the number of cells?**

„Size regulation appeared to be brought about by alteration in cell cycle length. There was no obvious increase in cell death in the double embryos nor an increase in the non-dividing cell population.„

Lewis and Rossant 1982

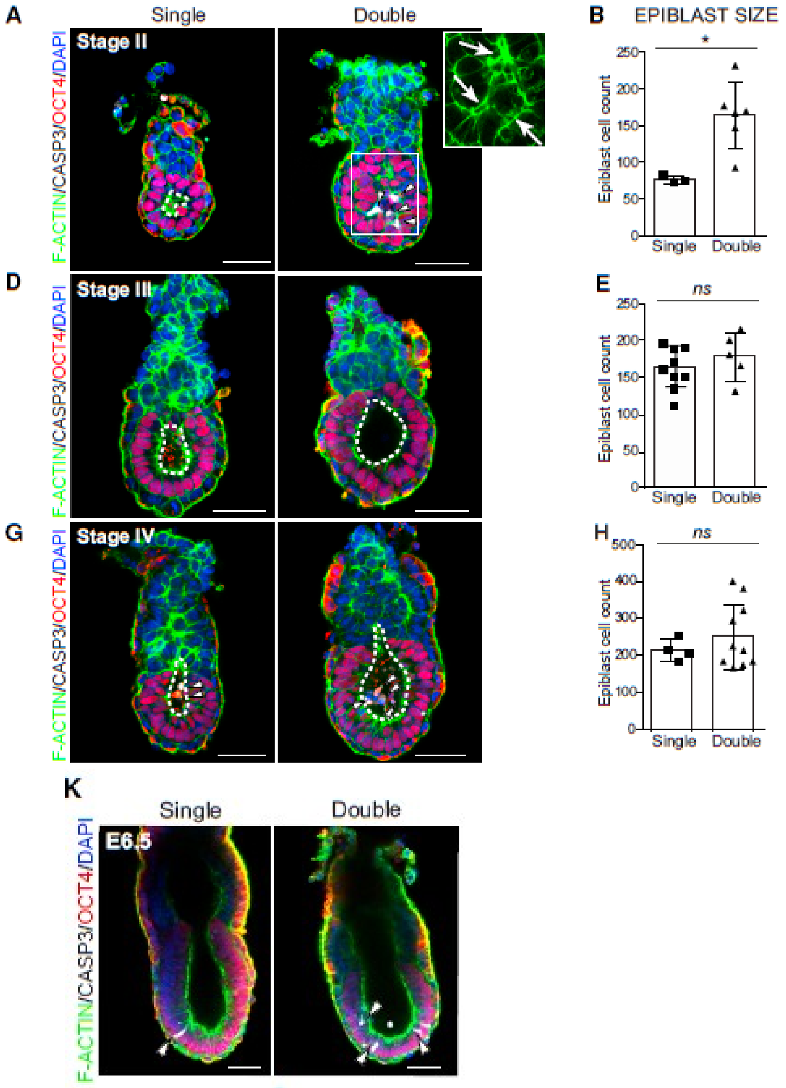
By lengthening the cell cycle, the number of cells is adjusted

This happens after implantation, but before gastrulation

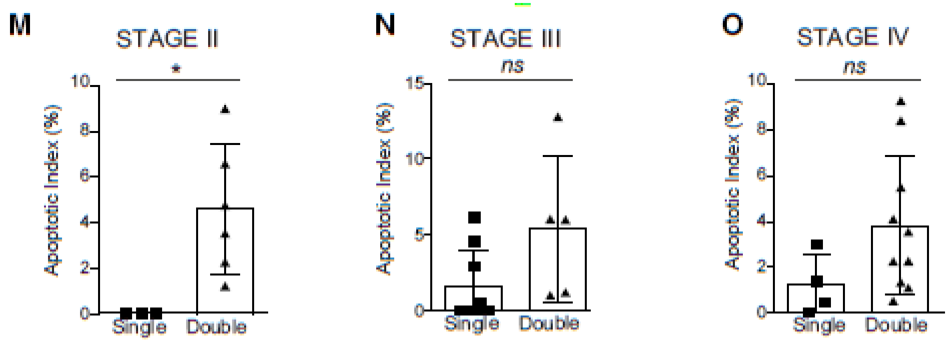


If there are too many cells: How is embryo size regulated?

If we aggregate two embryos together - a normal individual is formed



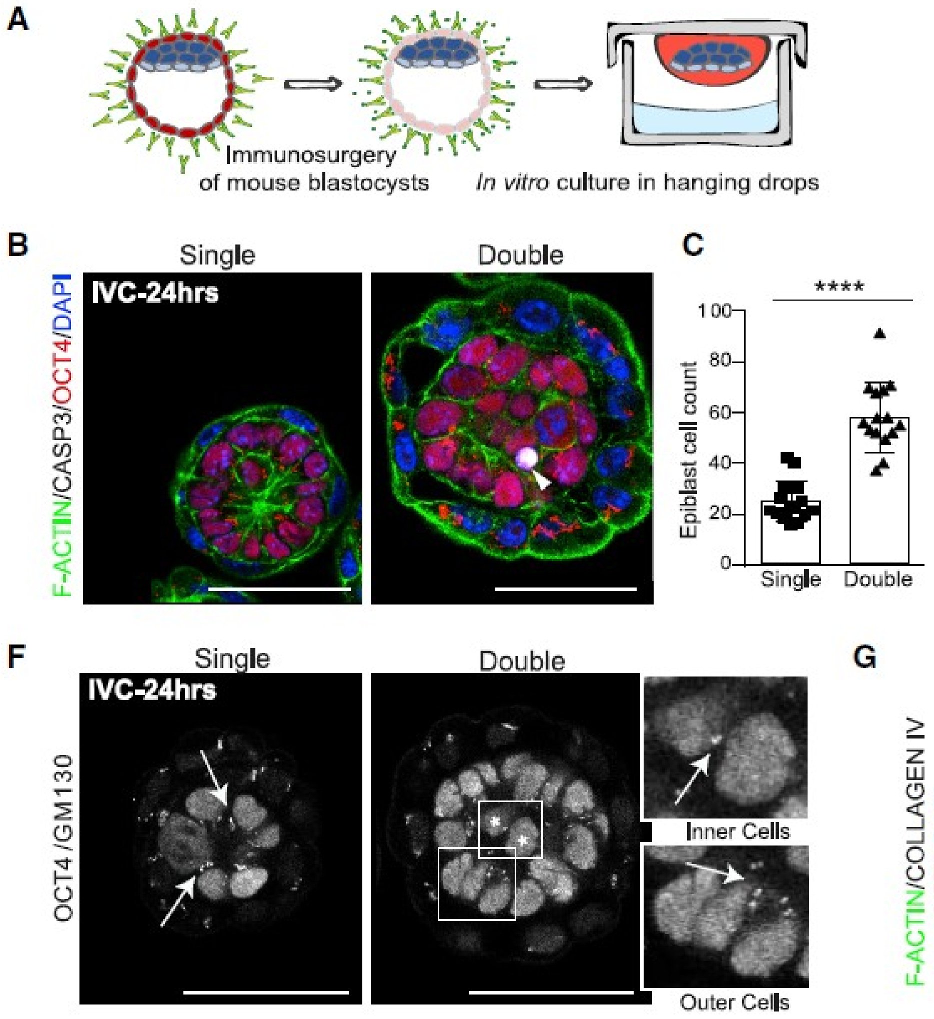
In a double embryo, apoptosis is induced only by epiblast cells (no others) that are not in contact with the basement membrane and only in the early embryonal stage.



Orietti, 2020

If there are too many cells: How is embryo size regulated?

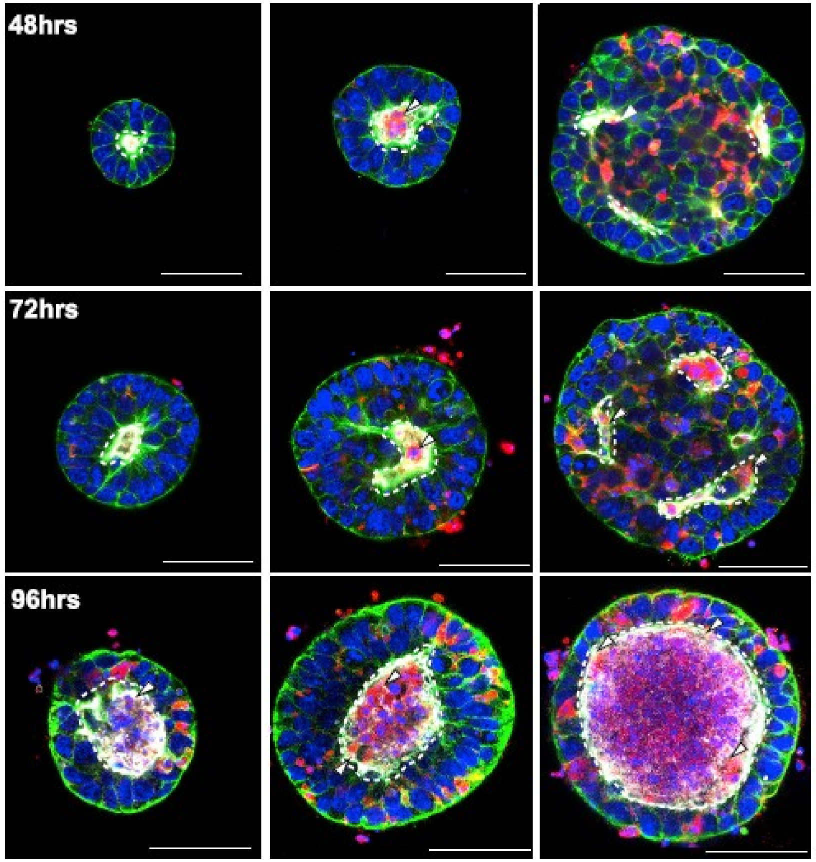
Demonstrated on an isolated epiblast in vitro - without trophectoderm contribution



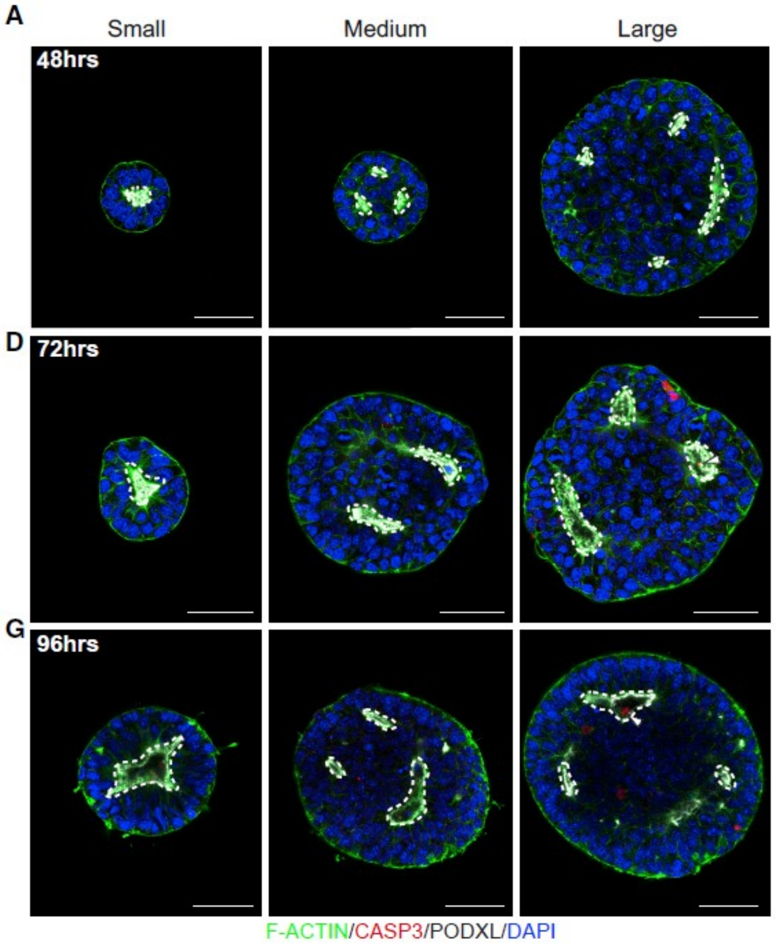
Orietti, 2020

If there are too many cells: How is embryo size regulated?

Demonstrated using na Embryonic Stem Cells



Inhibition of apoptosis

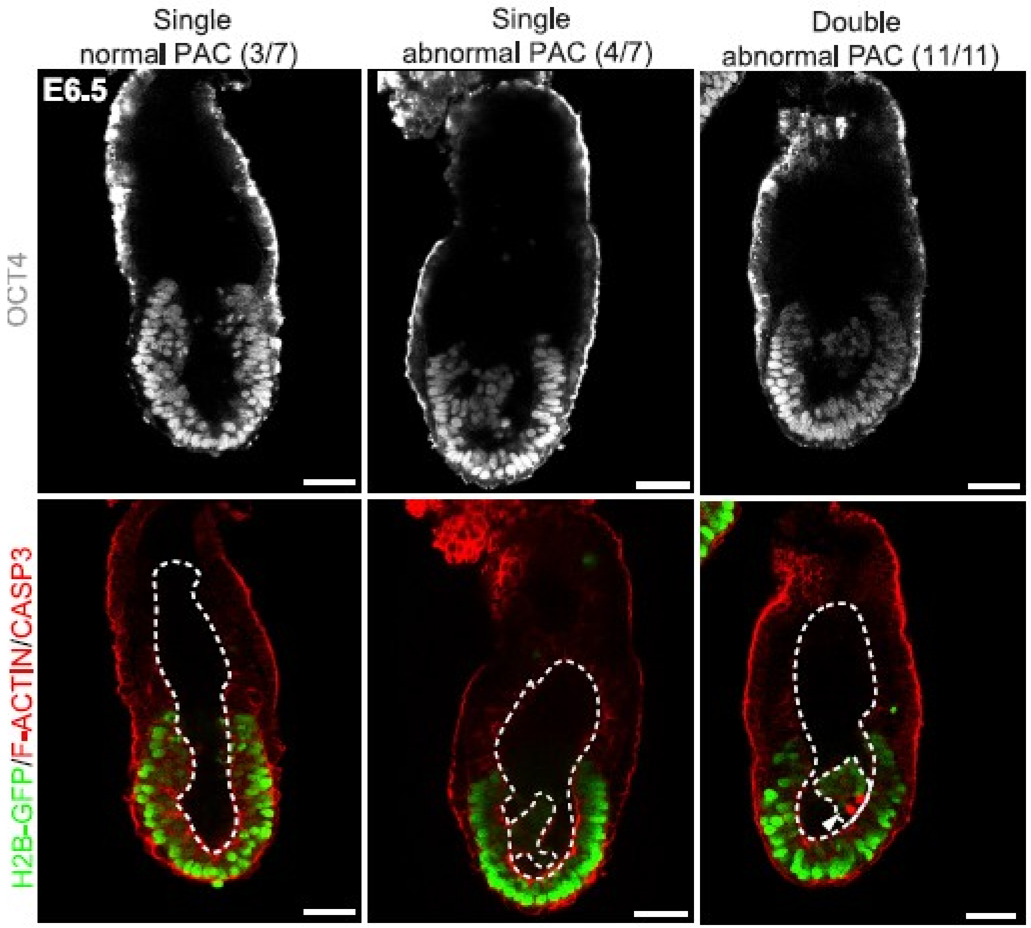


Inhibition of apoptosis did not lead to the formation of an epithelium.

Orietti, 2020

If there are too many cells: How is embryo size regulated?

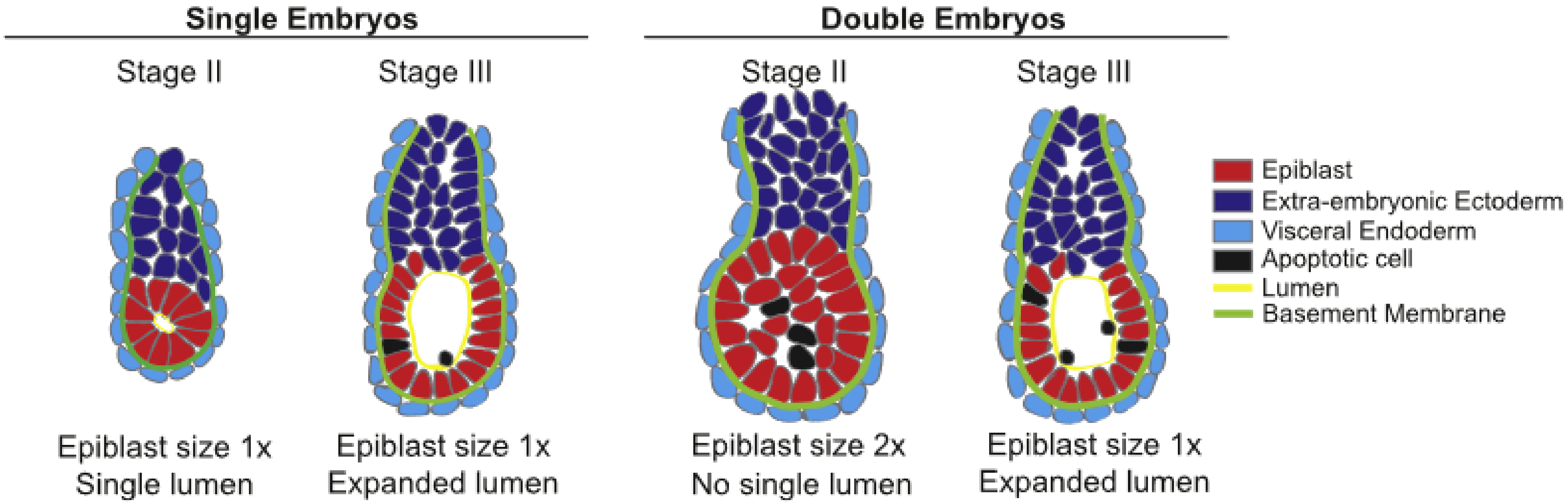
Apoptosis, however, does not regulate the size of the embryo!



Embryos with inhibited apoptosis were able to adjust their size

Orietti, 2020

If there are too many cells: How is embryo size regulated?



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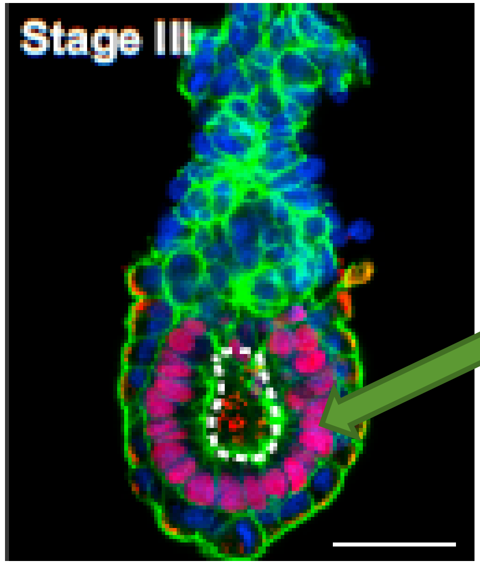
If there are too many cells: How is embryo size regulated?

Summary:

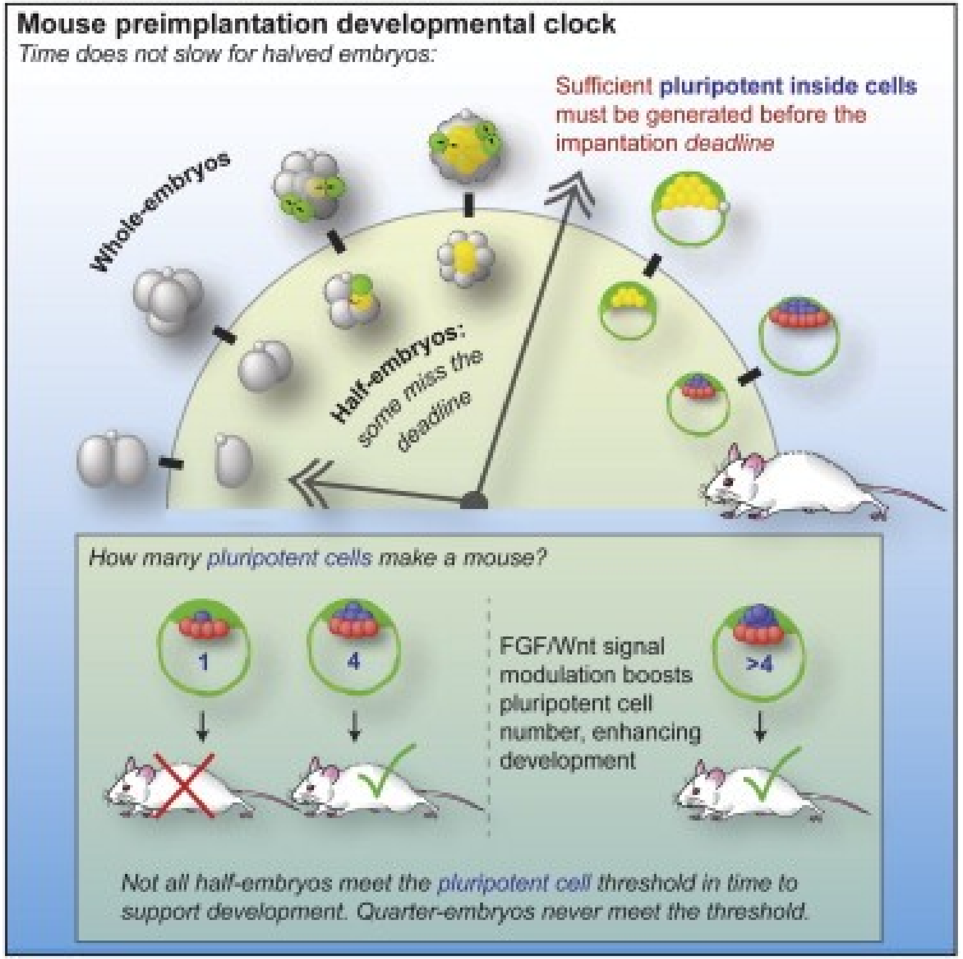
- The embryo compensates for the increased number of cells by lengthening the cell cycle, not by apoptosis.
- However, apoptosis is necessary for the proper formation of the epiblast epithelium.
- Two different mechanisms: apoptosis x slowing of proliferation

Slowing the cell cycle

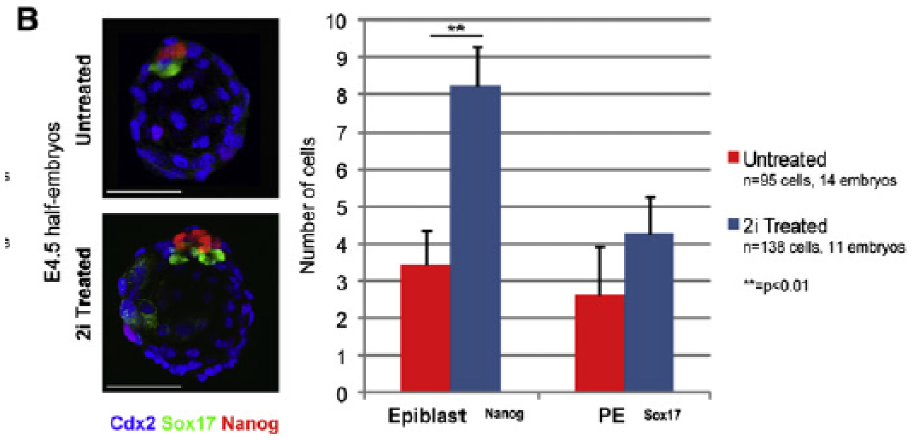


Apoptosis is necessary to arrange the epiblast epithelium in "double" embryos

If there are <u>too few</u> cells: How is embryo size regulated?



► Half-embryos have the same timing of development as normal embryos, but their developmental potential is reduced.
► In order to maintain development, the presence of at least 4 pluripotent cells is required.
► Fgf/Wnt modulation leads to correction of the development of half embryo



So, is there a checkpoint in development where the embryo "checks" its size?

Morris, 2012

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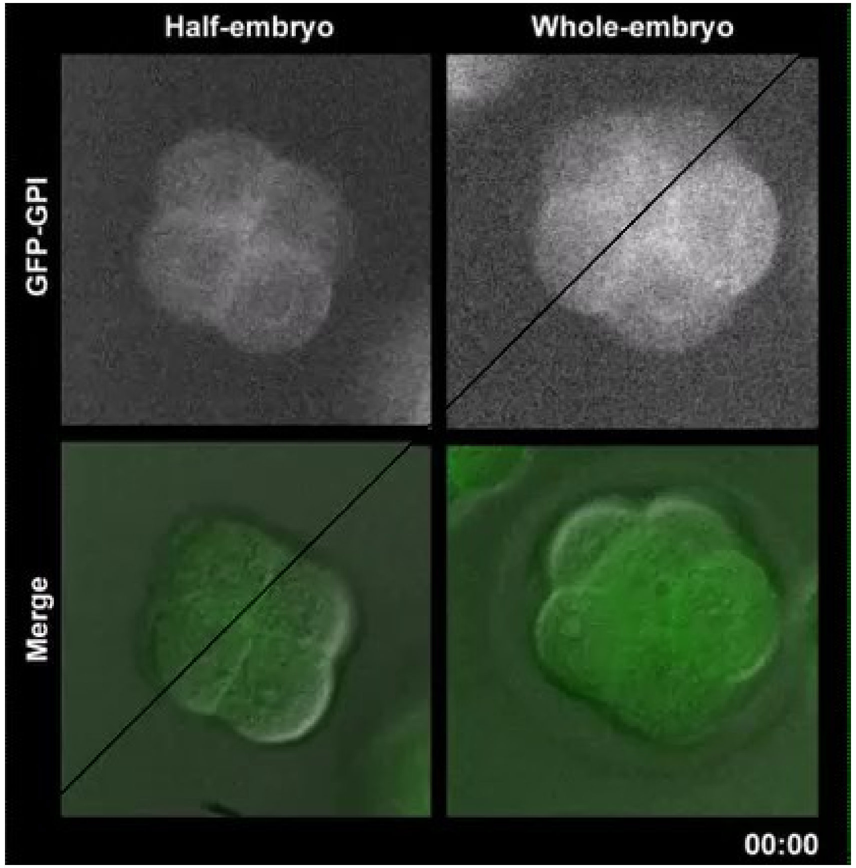
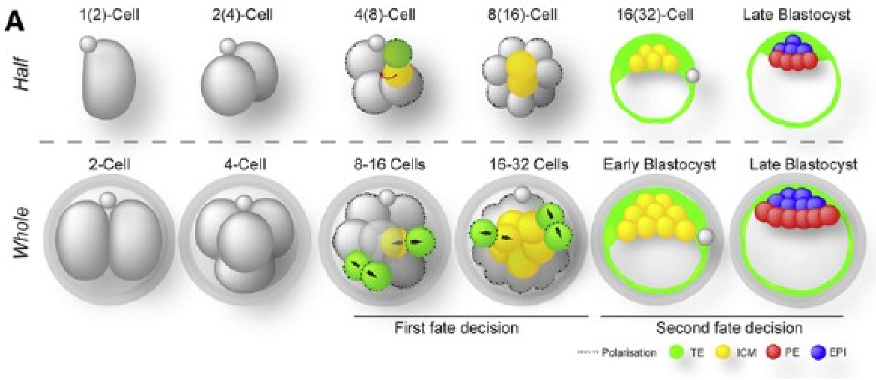
If there are too few cells: How is embryo size regulated?





Morris, 2012

Vývojová biologie

If there are <u>too few</u> cells: How is embryo size regulated?

Summary:

- Half embryos complete their development (not all)
- No 1/4 embryo completes its development - it lacks cells.
- Can be compensated by modulation of FGF, Wnt signaling.

What is involved in size control?

Include:

- Genes
- Signal pathways
- Hormones
- Together they provide a properly proportioned and reasonably sized body

Insulin/IGF-, RAS/RAF/MAPK-, TOR, Hippo, and JNK pathways

- **Crosstalk between them**
- **Growth rate**
- **Length of growth**
- **Size (organ tissues)**
- **Growth coordination**

Signaling pathways that are involved in size control

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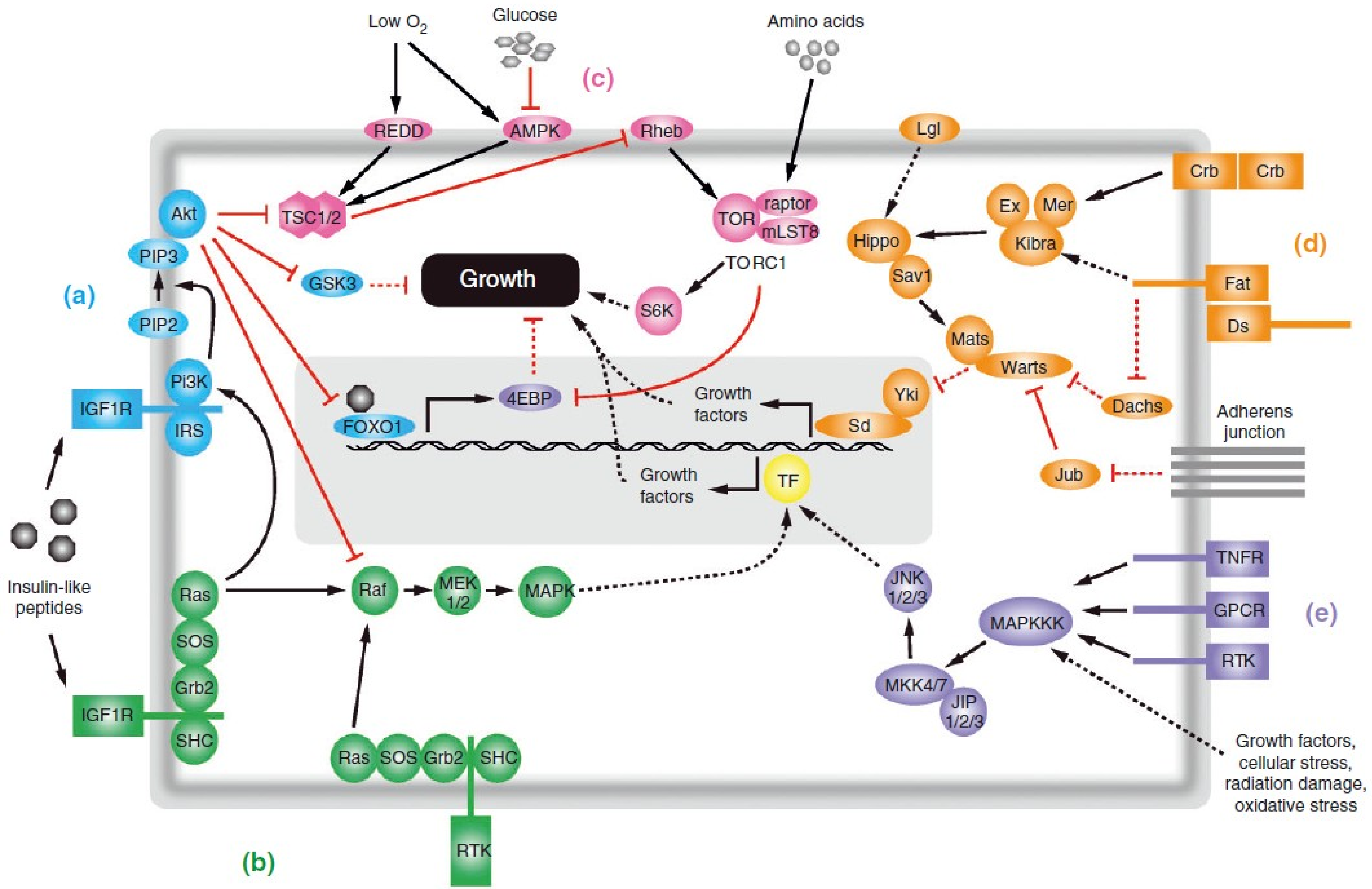
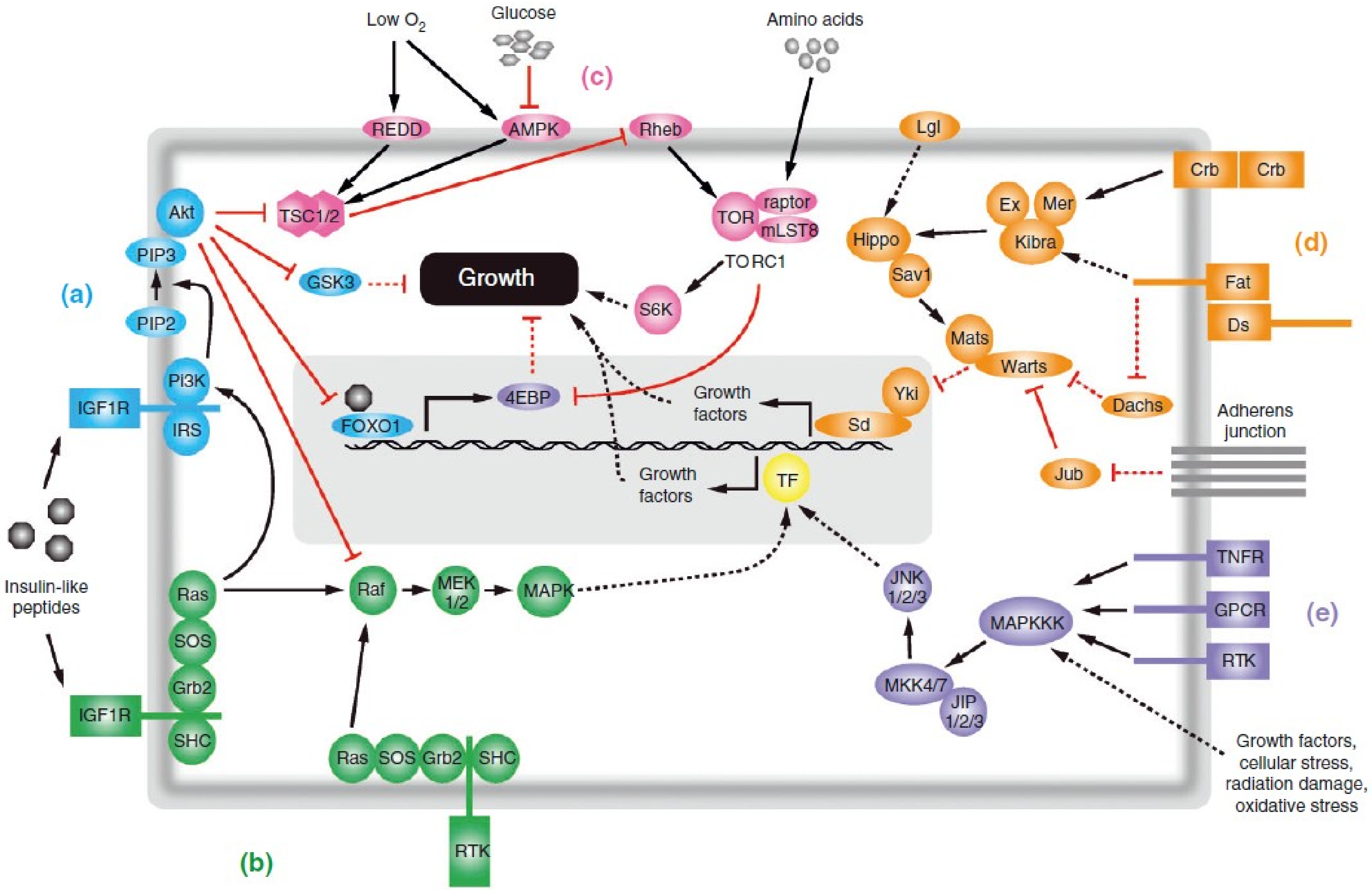


Size – Signalling pathways – Insulin/IGF1

Insulin/Insulin-like growth factor (IGF) Signaling (IIS) pathway
Amount of IGF depending on nutrition/nutrients



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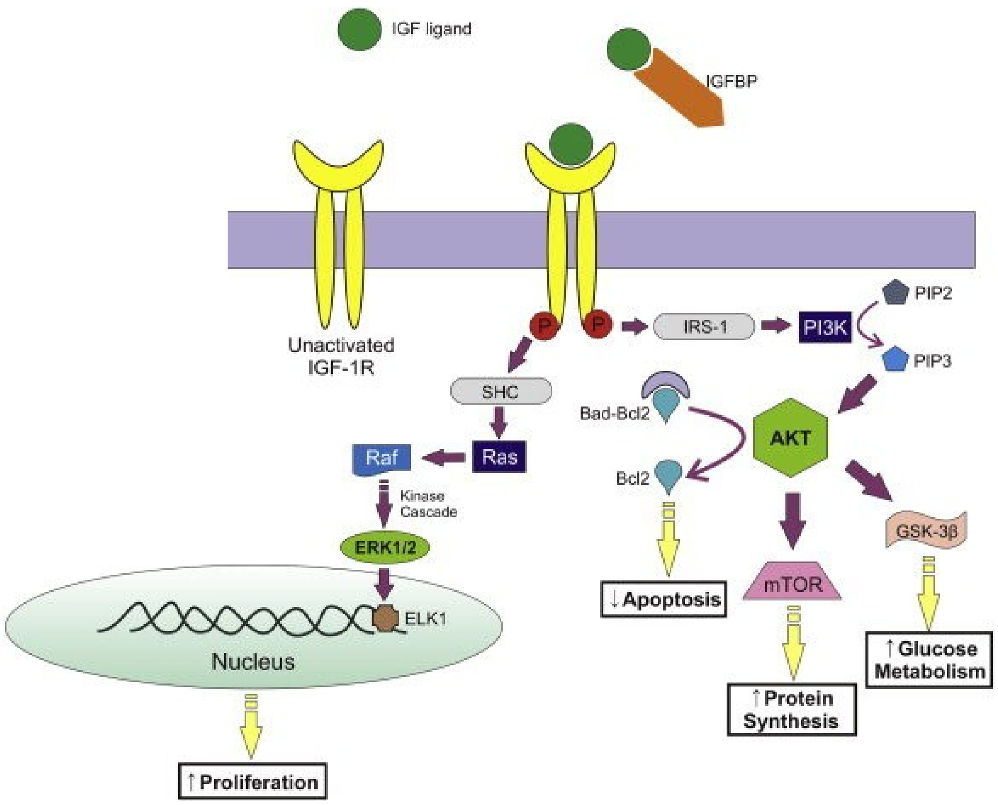
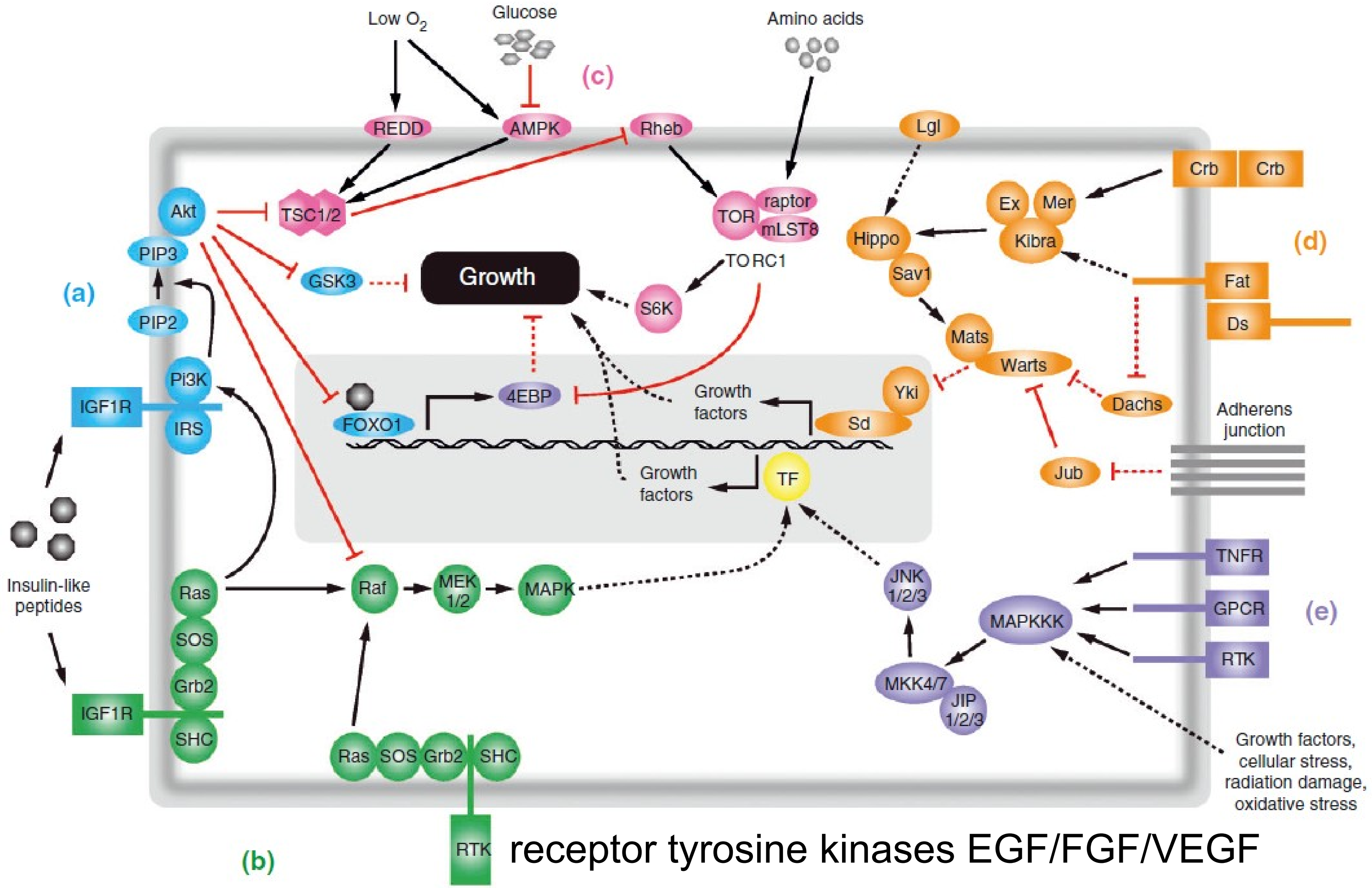
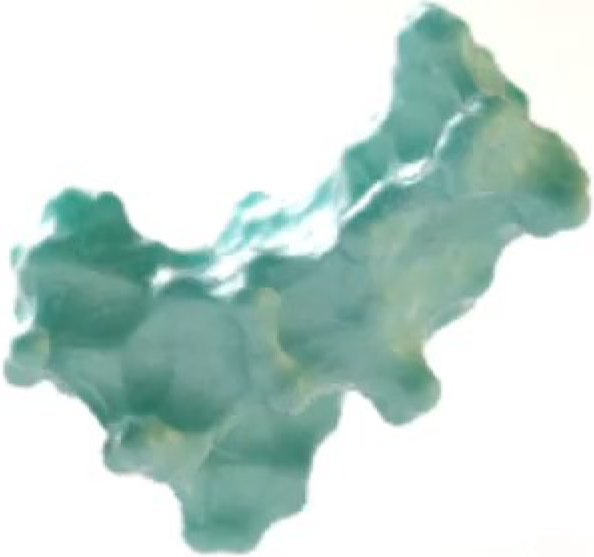


Size - signalling pathways - Ras/Raf/MAPK

IGF activated – see previous slides



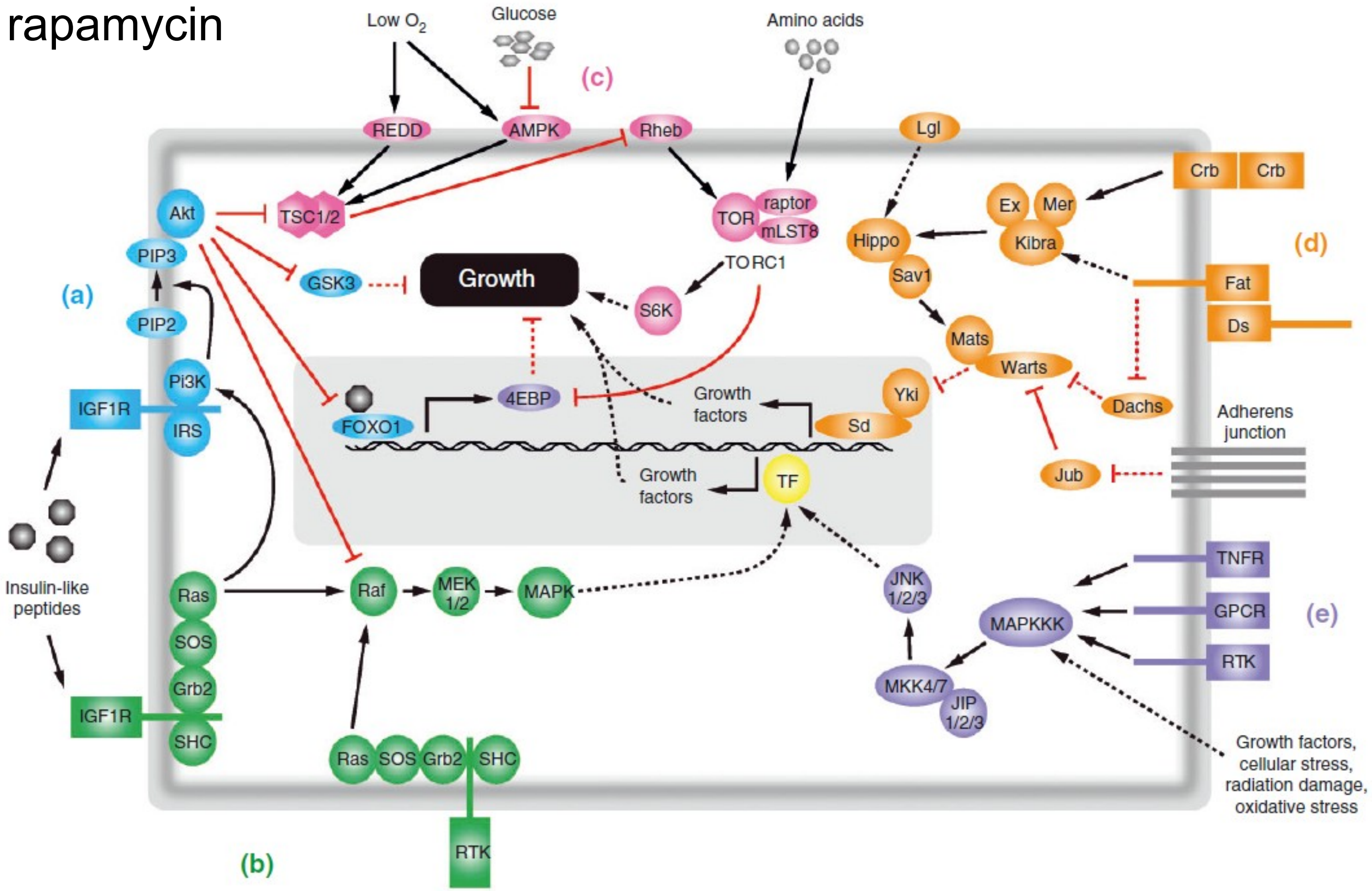
receptor tyrosine kinases EGF/FGF/VEGF



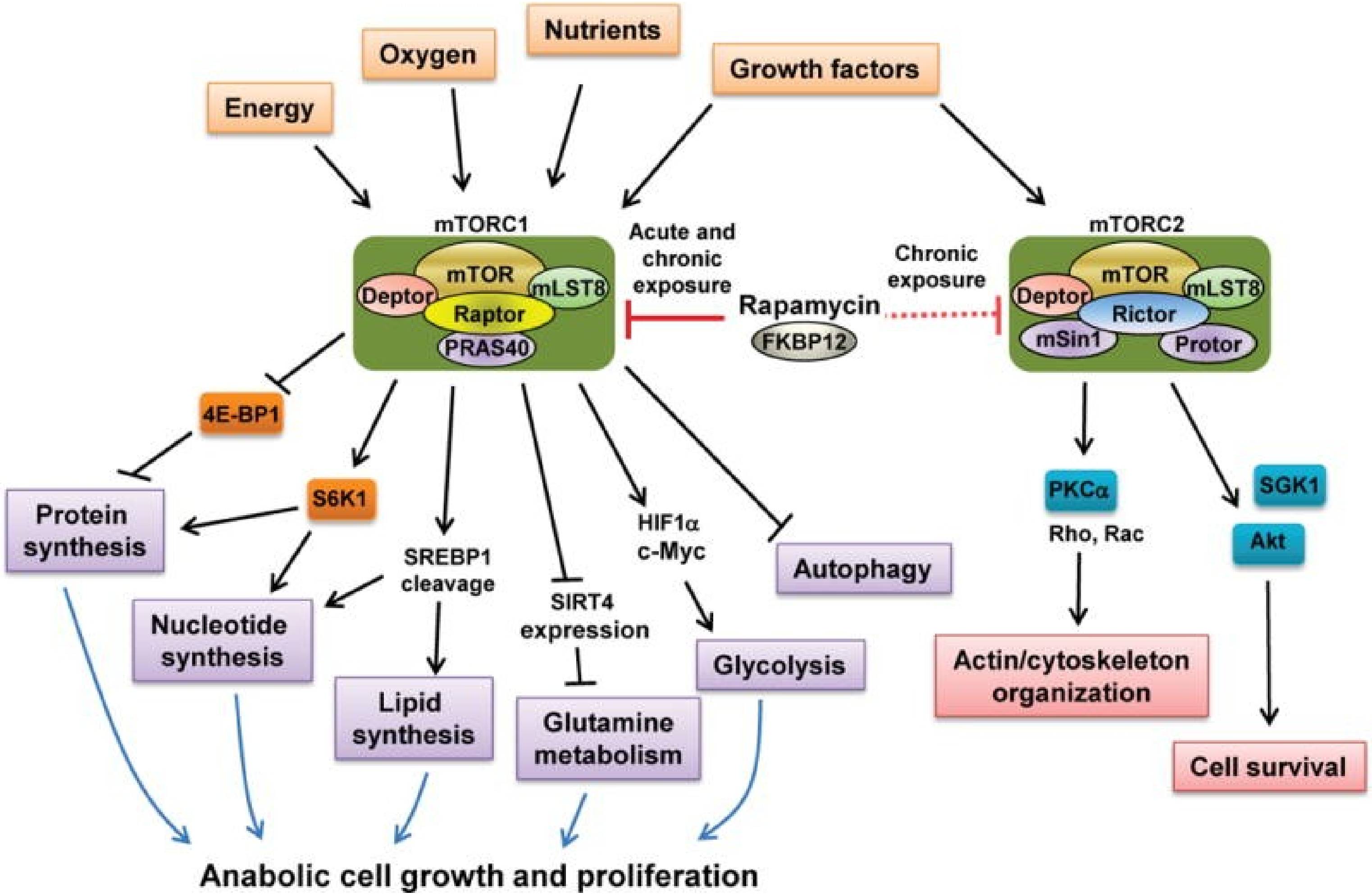
Size - signalling pathways - TOR

- Similar to IGF, it also regulates growth based on the presence of nutrients, energy, oxygen
- Conserved from yeast to man
- IGF and TOR are the major players involved in the transmission of information on the presence of nutrients.

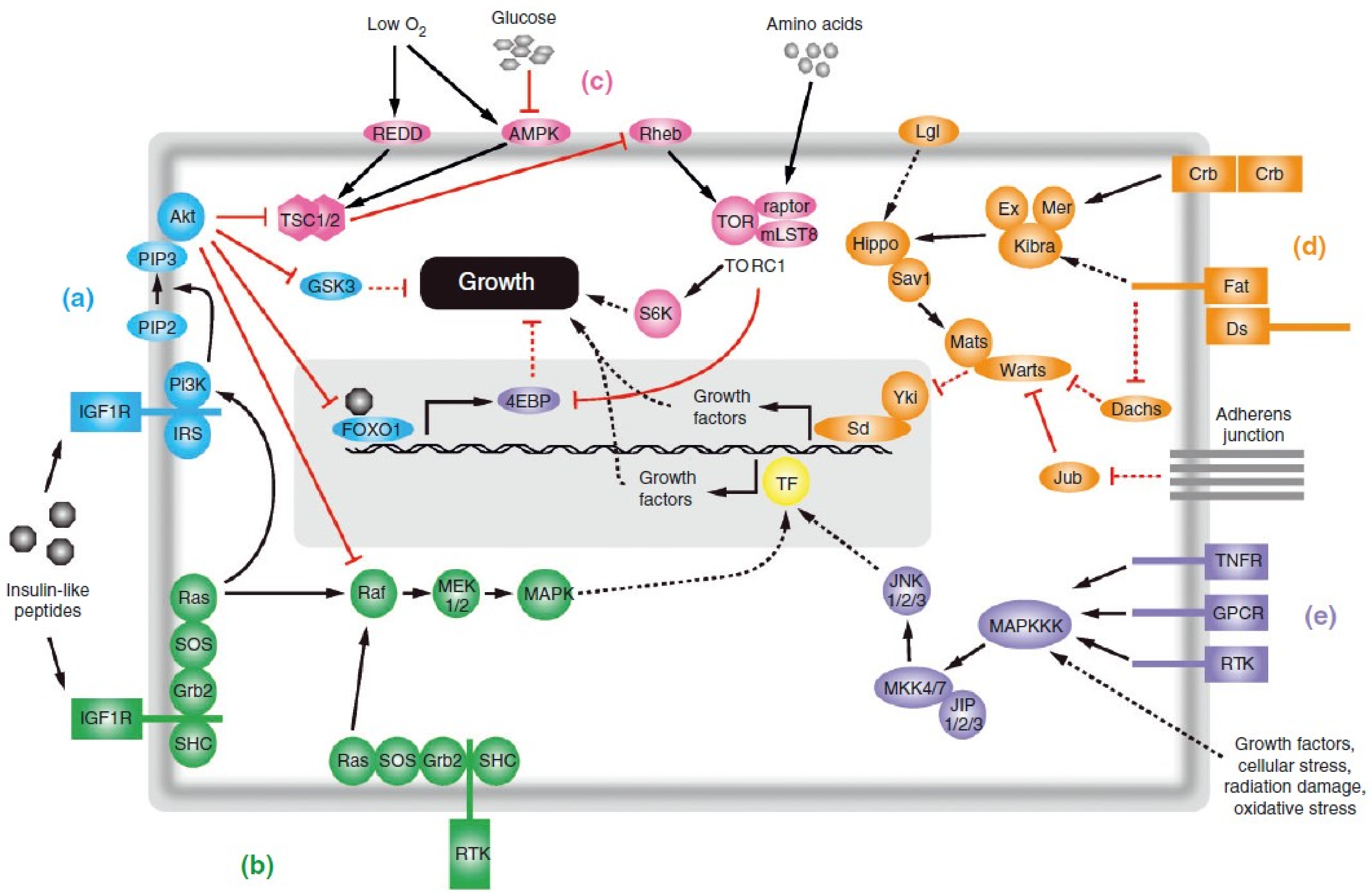
TOR = target of rapamycin



Size - signalling pathways - TOR



Size - signalling pathways - Hippo



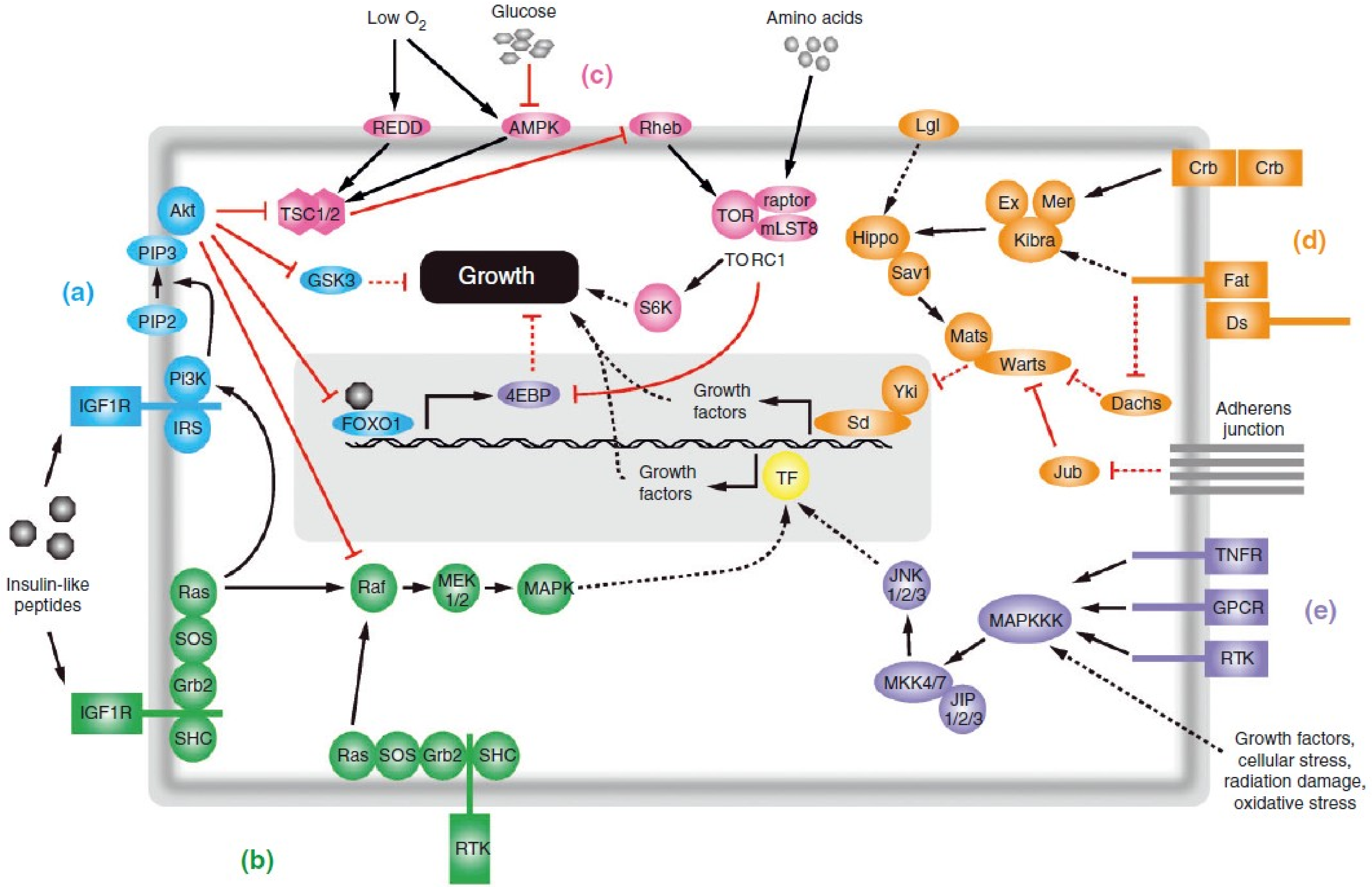
Drosophila v. Mammals
Hippo = Mst1/2
Salvador = Sav1
Warts = Lats1/2
Mats = Mob1A/B

YAPing Hippo Forecasts a New Target for Lung Cancer Prevention and Treatment

Duojia Pan
Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD

Size – signalling pathways - JNK

- Stress pathway, regulates cell death, tissue regeneration, wound healing



Size - signalling pathways - Other

- **Signal pathways that control pattering:**

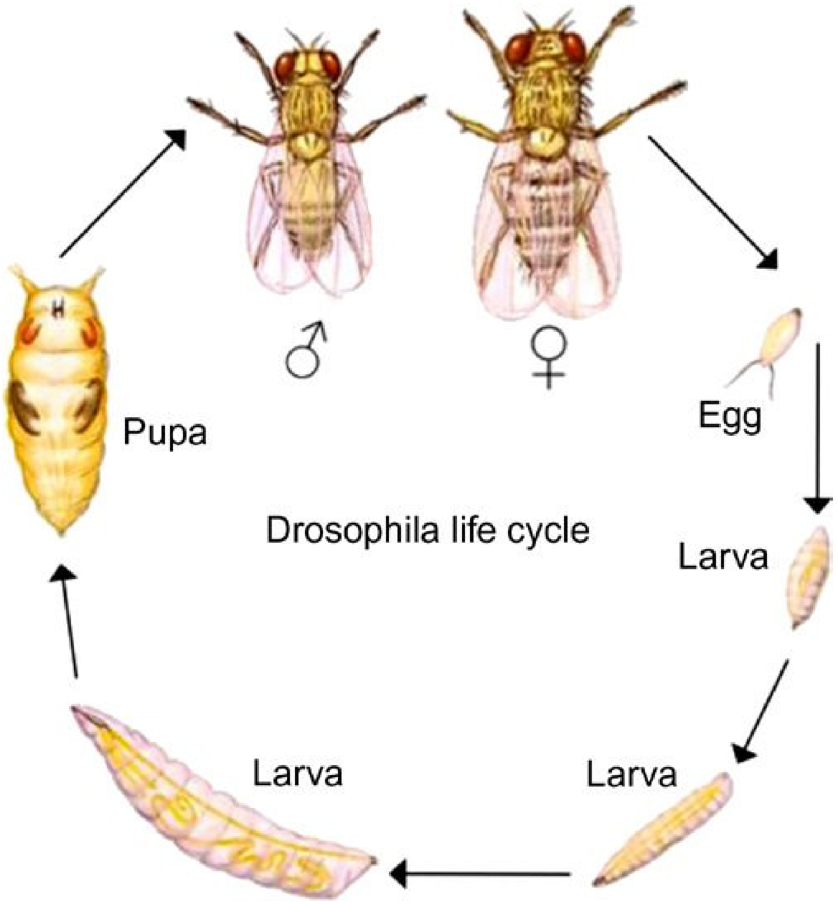
- They control the shape and arrangement of the organ - changes in cell growth and proliferation
- Hh, Wnt, TGF-β

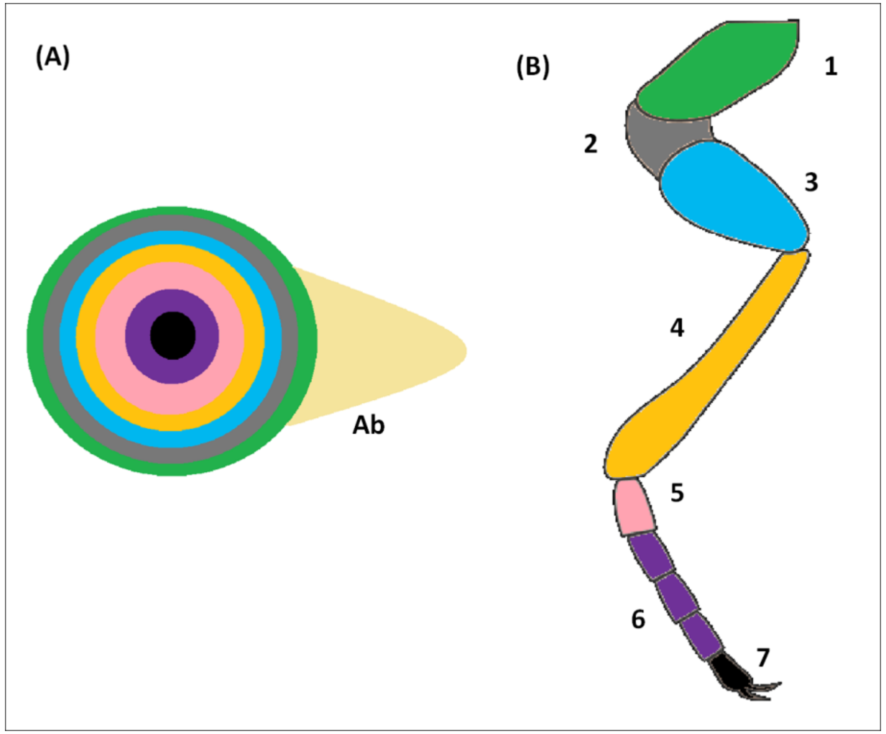
Size – Signalling pathways – Conclusion and Questions

- Know the basic signal pathways that regulate size.

Aspects of Size Control – Drosophila

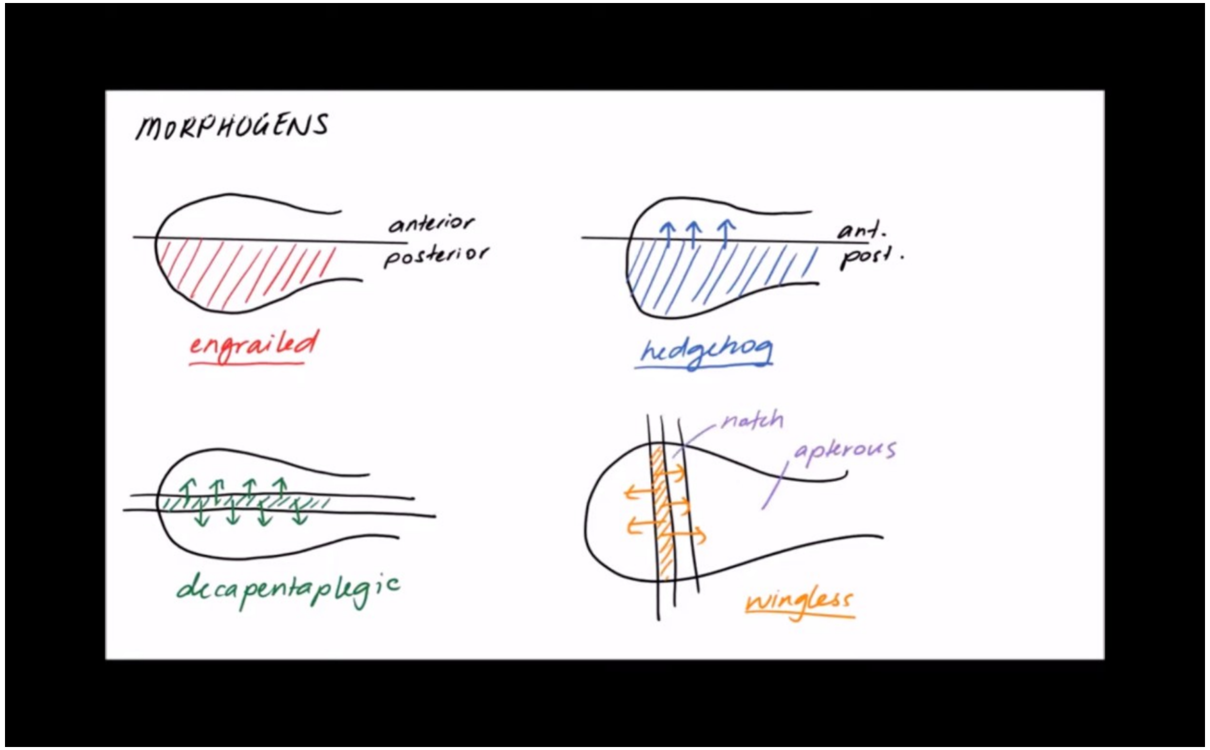
Imaginal discs – model system
The size of the disc determines the size of the organ

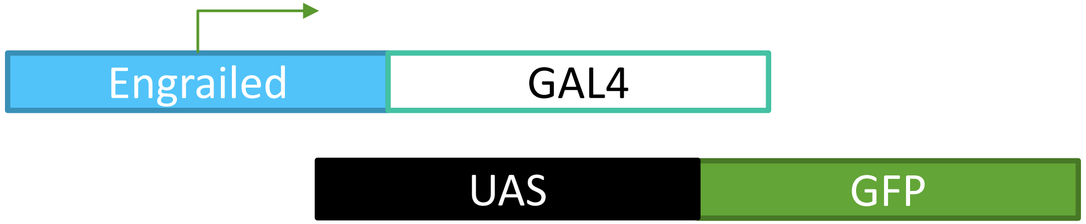




Aspects of Size Control – Drosophila

GAL4/UAS system: a geneticist's Swiss army knife





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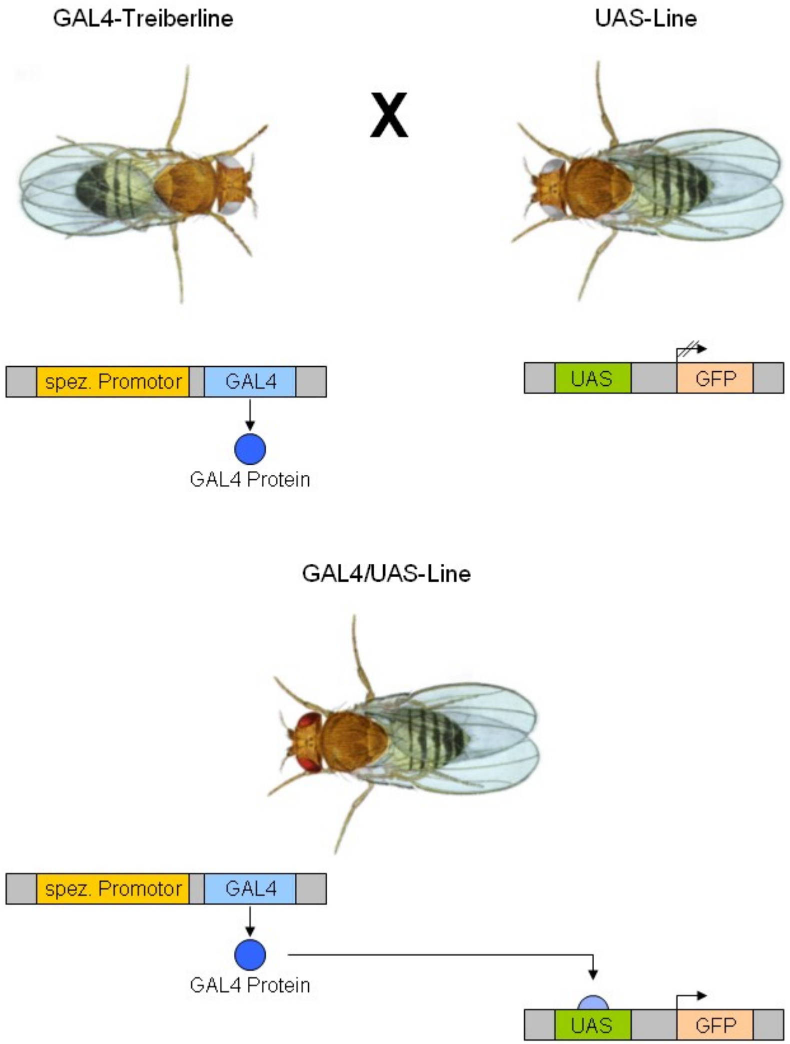
Aspects of Size Control – Drosophila

GAL4/UAS system: a geneticist's Swiss army knife



WingJ

Development of the *Drosophila* wing

Aspects of Size Control

- The above-mentioned (in intro) signaling pathways regulate growth and proliferation, but this does not explain the very nature of size control.
- **The size of the tissue/organ/individual determines the growth rate and the duration of growth.**

Growth rate

Controlled by signaling pathways that regulate cell proliferation and growth

Growth duration

It is regulated by systemic hormonal signals that coordinate the cessation of growth throughout the body, as well as organ-autonomic processes that ensure that organs stop growing once they reach their final size.

Aspects of Size Control

Growth rate

Controlled by signaling pathways that regulate cell proliferation and growth.

Aspects of Size Control – Growth Rate

- Regulated by cell growth and proliferation => more larger cells/time = larger organ/individual
- Growth and proliferation are regulated by different pathways.

Cell growth:
75% of the cell is made up of water, ions and small molecules
18% is made up of proteins => growth depends on protein synthesis.

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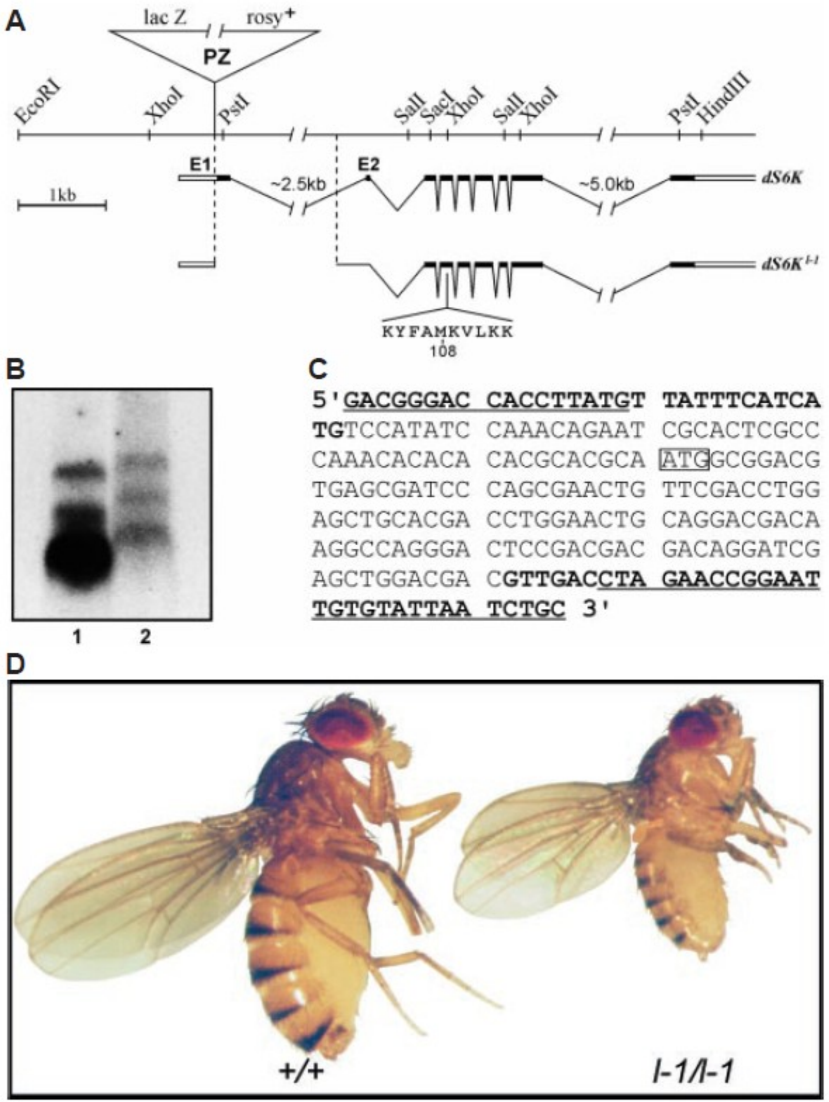
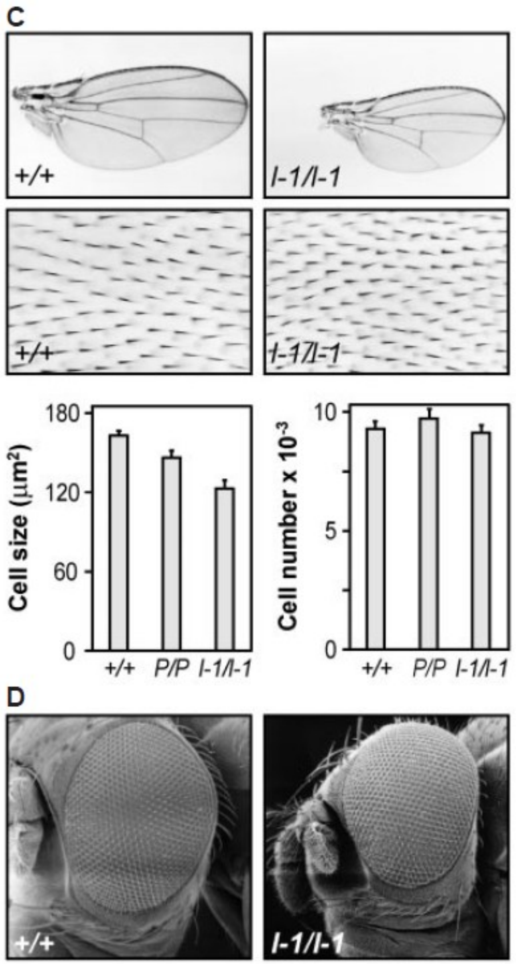
Aspects of Size Control – Growth Rate

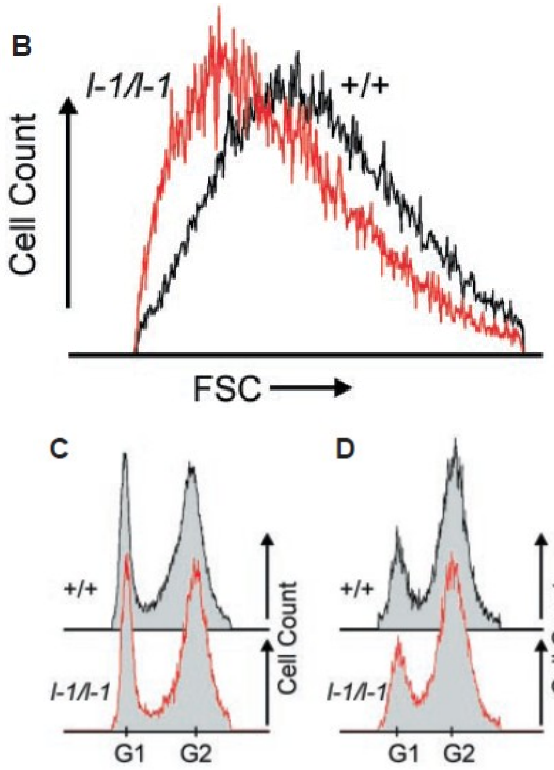
- S6K (S6 kinase) is a ribosomal 40S protein => controls ribosomal protein synthesis
- S6K deficiency (l-1/l-1) – delayed development, smaller individual <u>but the same number of cells</u>

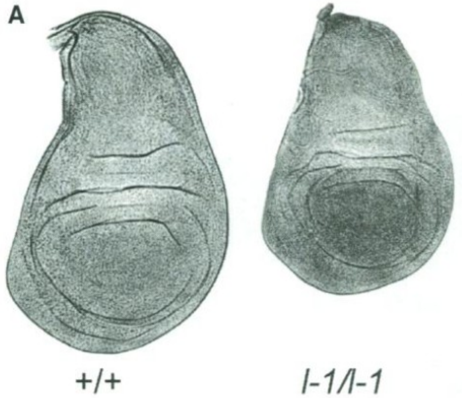


Aspects of Size Control – Growth Rate

S6K is a ribosomal 40S protein => controls protein synthesis
S6K deficiency (l-1/l-1) – delayed development, smaller individual, smaller organs, but the same number of cells



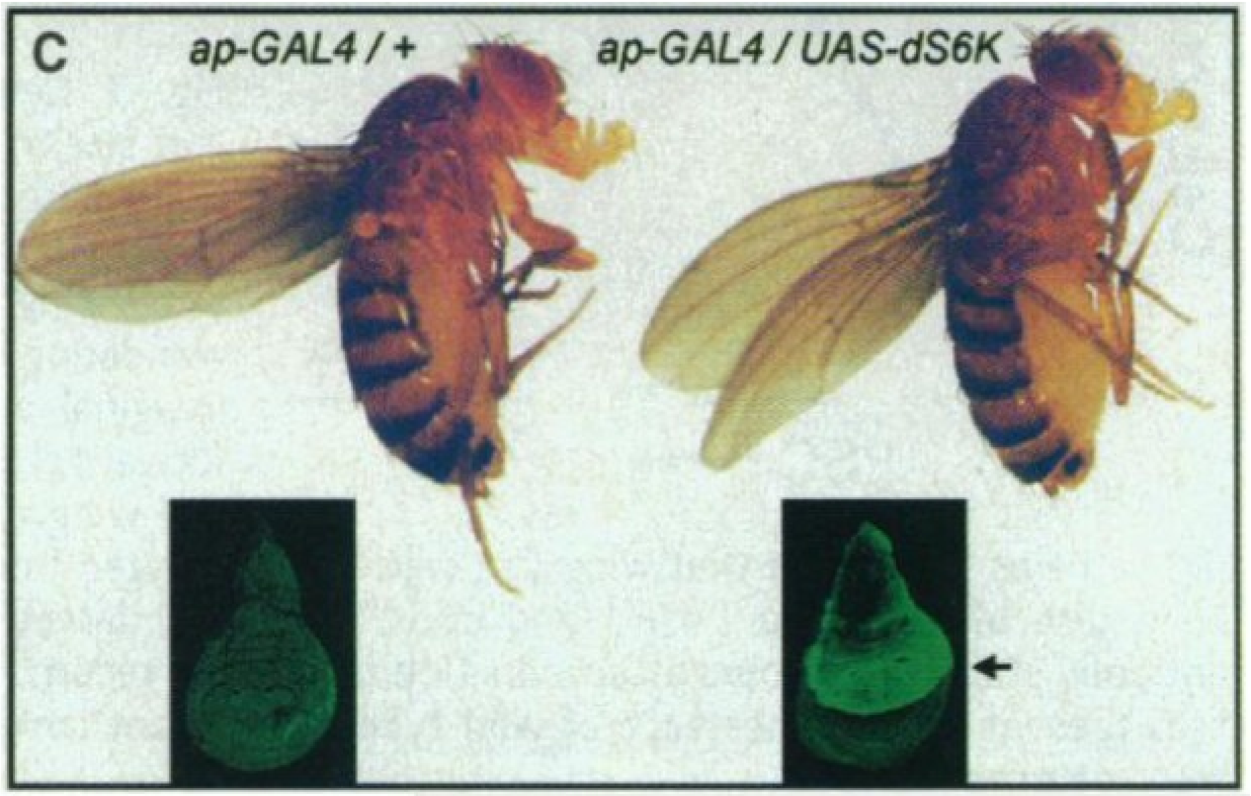




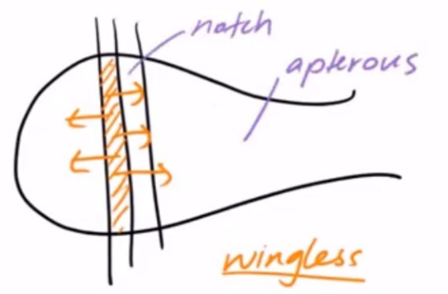
Montagne et al., 1999

Aspects of Size Control – Growth Rate

S6K is a ribosomal 40S protein => controls ribosomal protein synthesis
dS6K (overexpression) – larger cells



Montagne et al., 1999



Aspects of size control - Insulin/Insulin-like growth factor (IGF)

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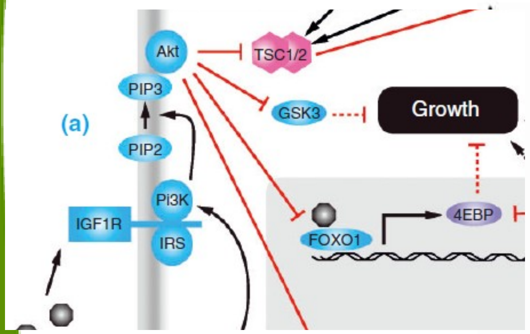
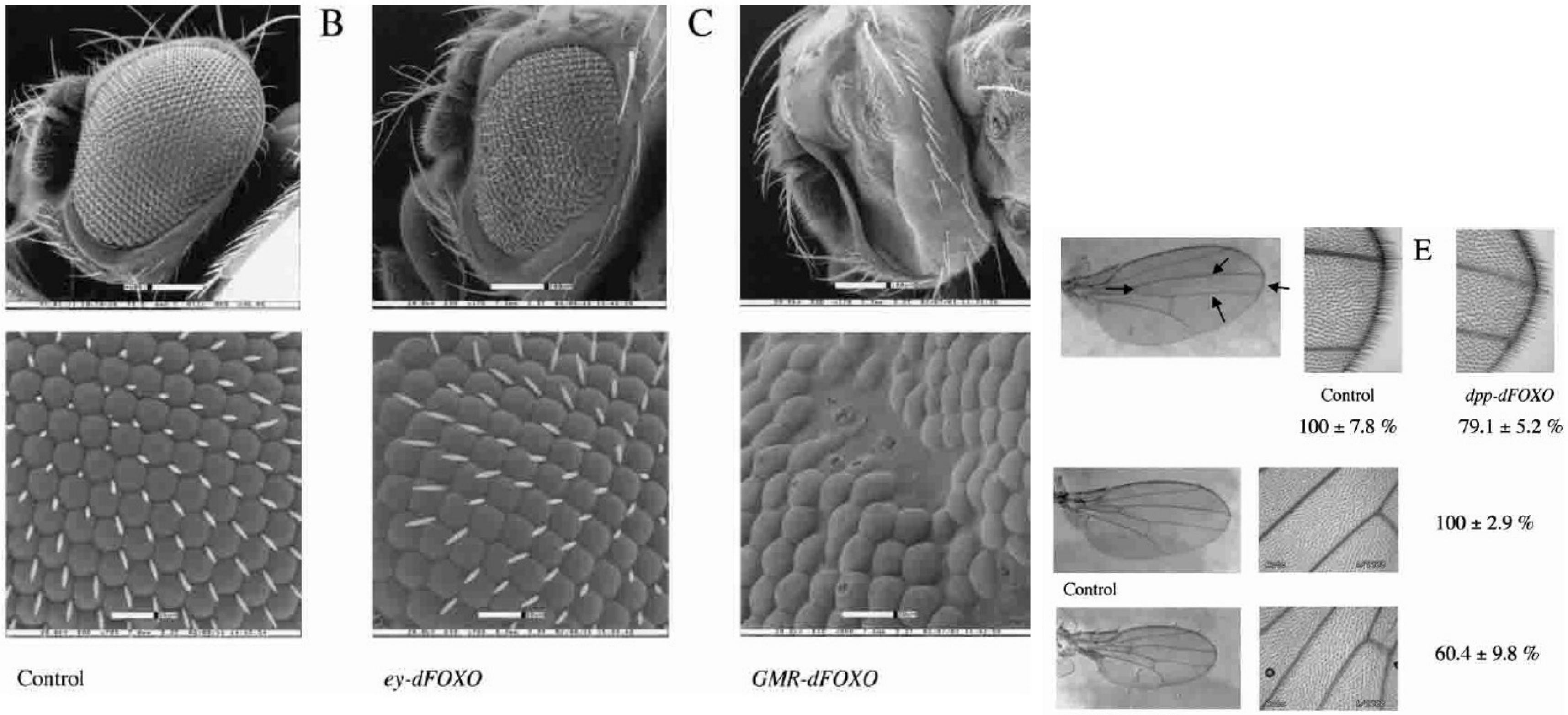
- FOXO regulates organ size <u>by controlling the number of cells</u>
- Higher FOXO expression, fewer cells

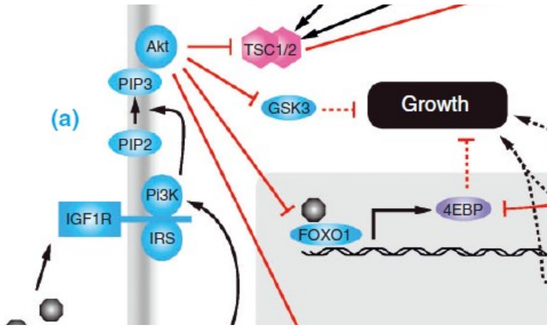


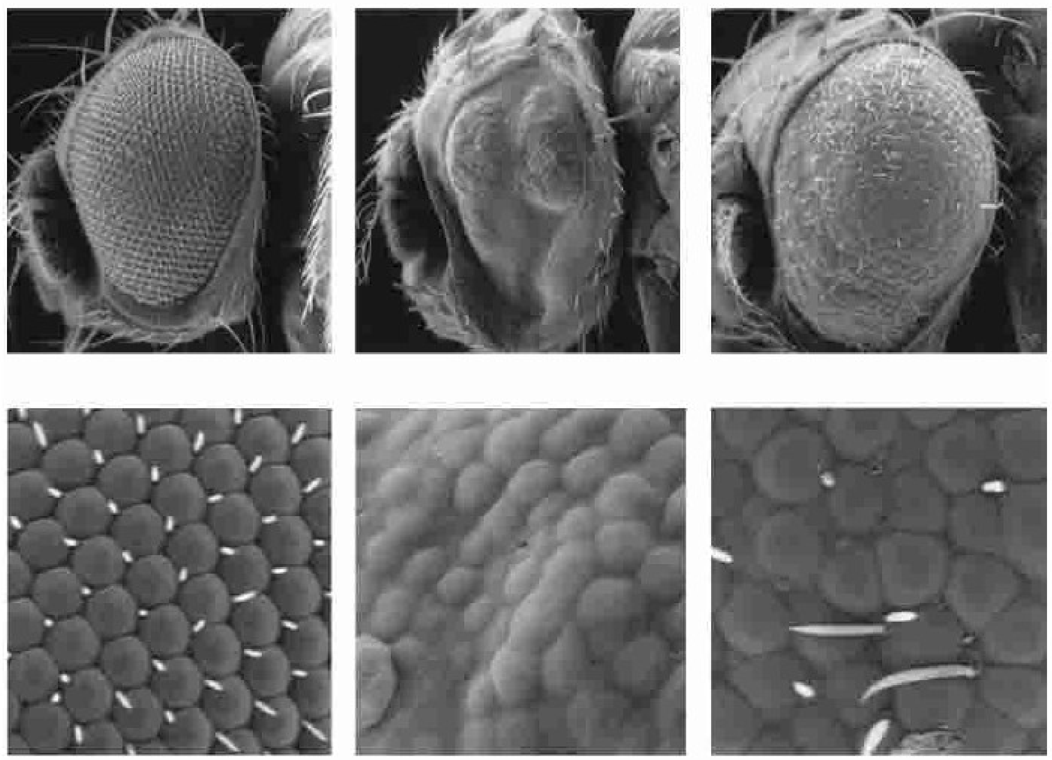


Puig et al, 2003

Aspects of size control - Insulin/Insulin-like growth factor (IGF)

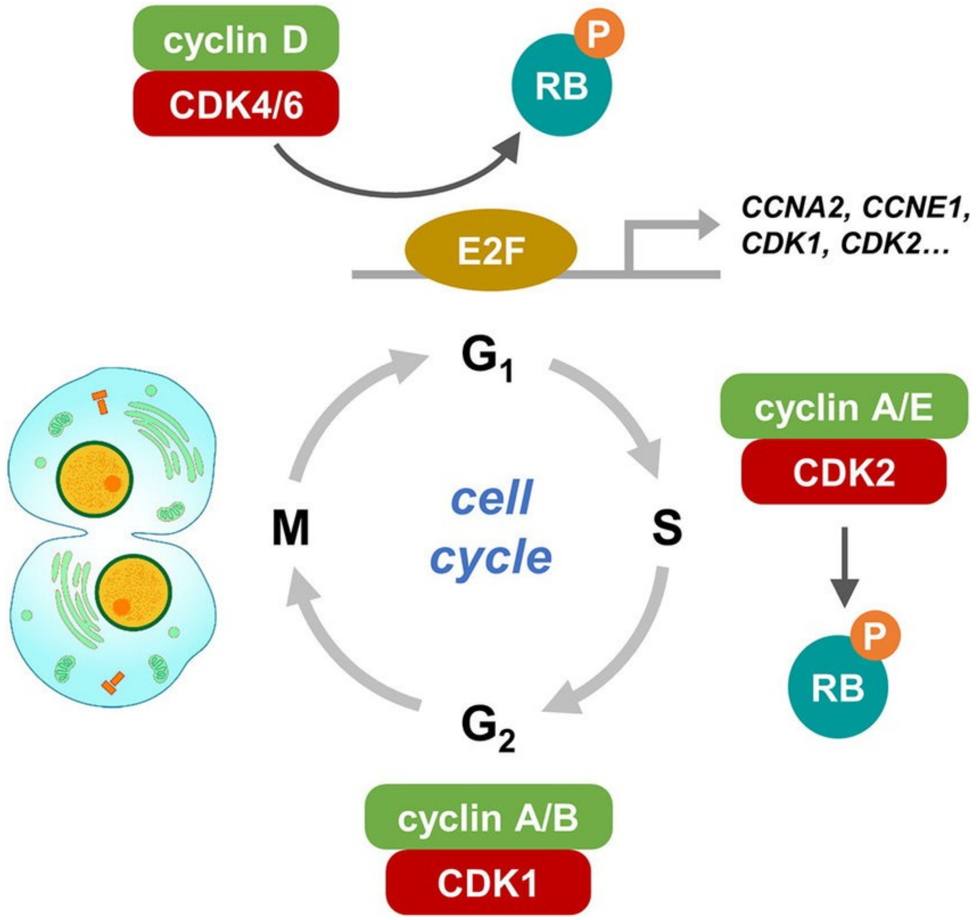
- FOXO regulates organ size by controlling the number of cells
- Higher FOXO expression, fewer cells
- Rescue phenotype through overexpression of dAkt (growth inhibitor inhibition)





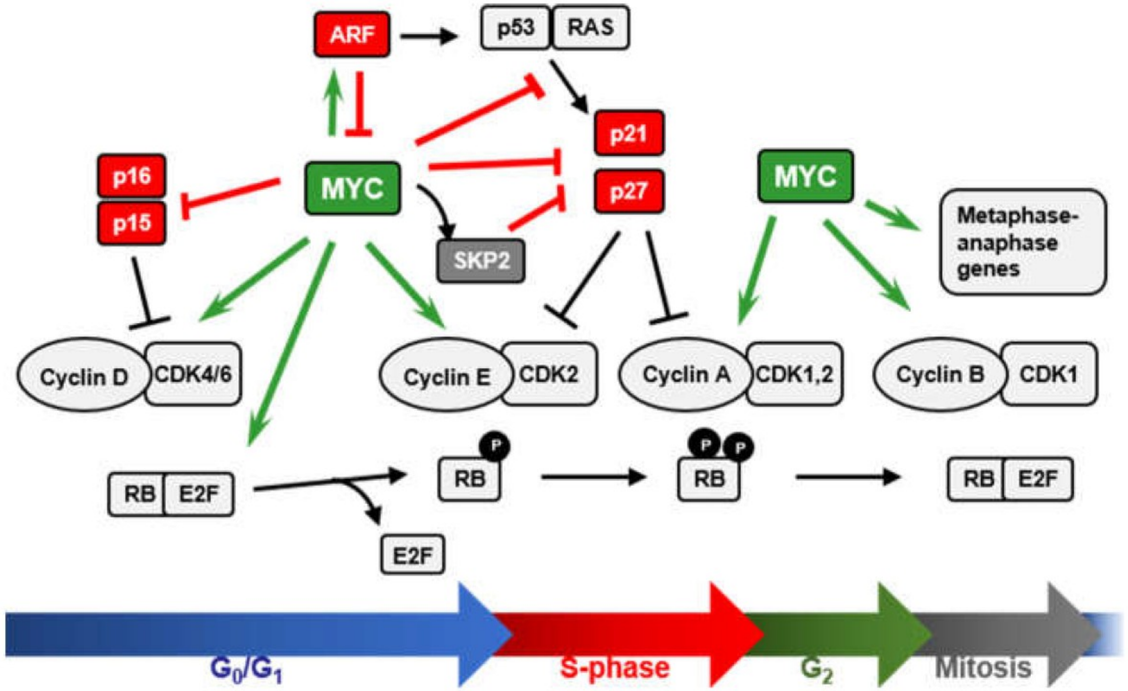
Puig et al, 2003

Aspects of size control – Growth rate – cell cycle



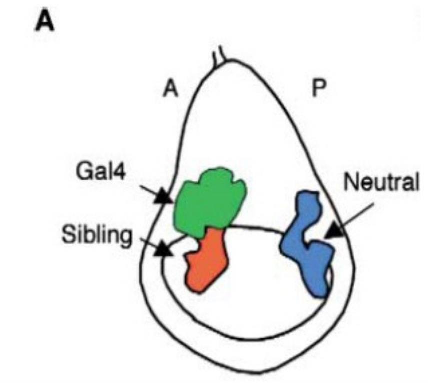
Vývojová biologie

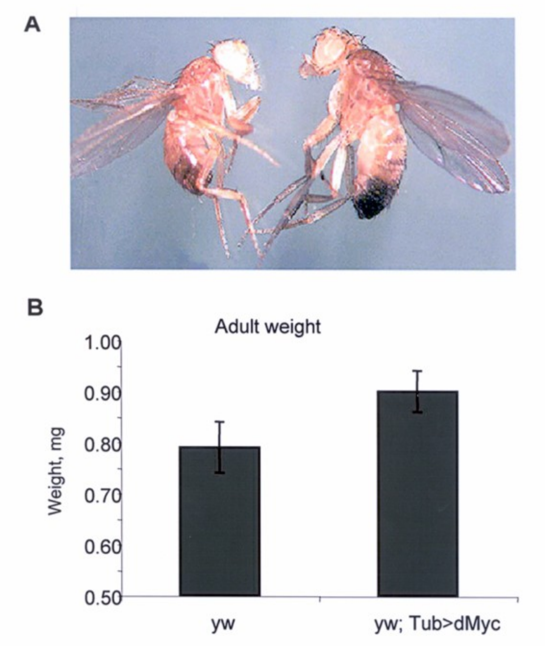
Aspects of size control - Growth rate - cell cycle - MYC



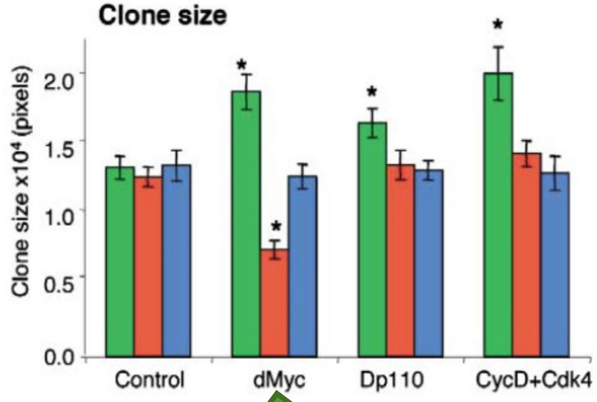
- Oncoprotein
- Induces the expression of many growth factors
- It positively regulates the biogenesis of ribosomes and thus also protein synthesis
-

Aspects of size control - Growth rate - cell cycle - MYC





Myc overexpression



Cells in close contact with the dMyc clone have a growth disadvantage.

Cell, Vol. 117, 107–116, April 2, 2004, Copyright ©2004 by Cell Press

Drosophila Myc Regulates Organ Size by Inducing Cell Competition

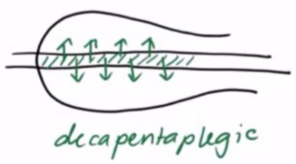
Claire de la Cova,[1] Mauricio Abril,[1]
Paola Bellosta,[2] Peter Gallant,[2]
and Laura A. Johnston[1,*]

In *Drosophil*
marily in ima
to adult app

Is a difference in growth rates between populations of cells sufficient to induce competition?

Claire de la Cova, Cell, 2004

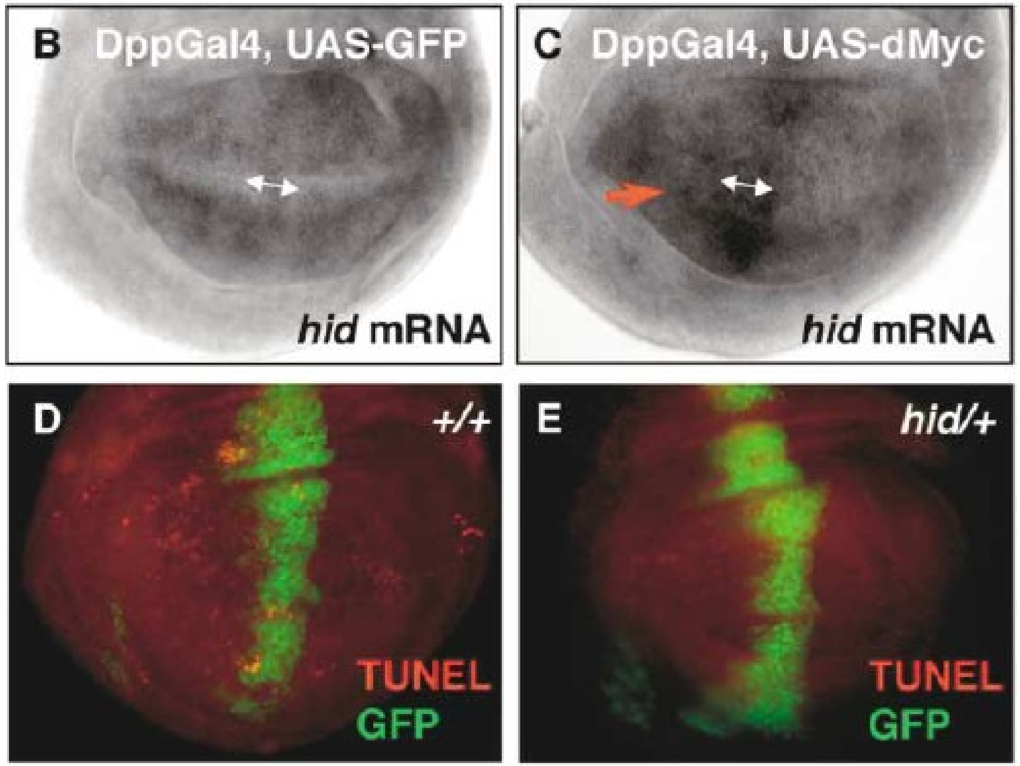
Aspects of size control - Growth rate - cell cycle - MYC

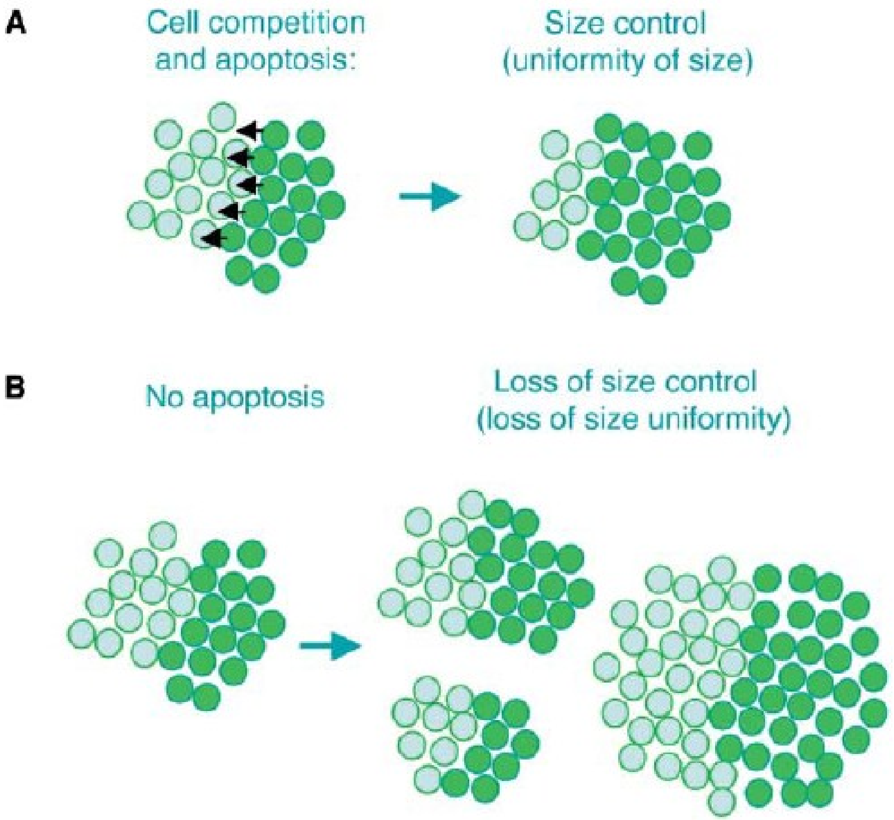


Myc overexpression

A Tunel⁺ cells (fold over control)

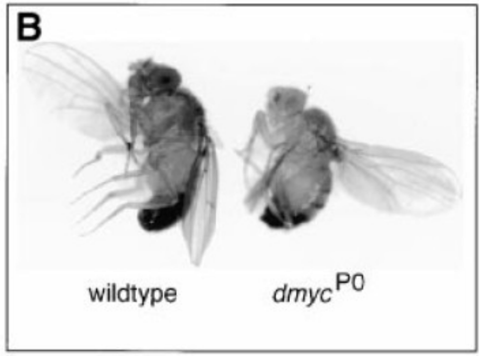
	Control	dMyc	Dp110	CycD/Cdk4	
In clone	1.0	8.3 *	4.2	4.4	
Outside clone	1.0	3.5 *	0.3	0.8	* $p<0.005$

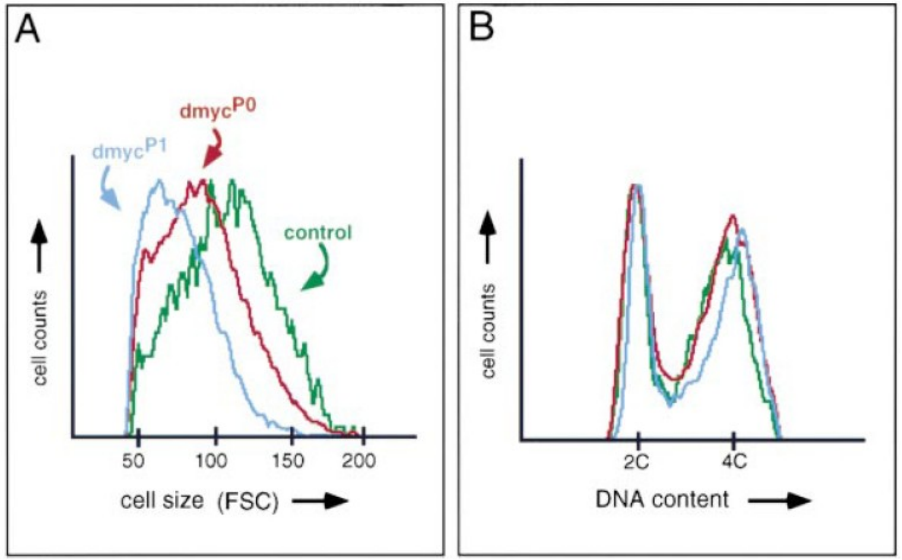


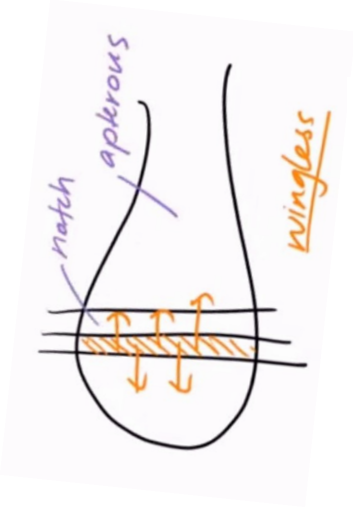


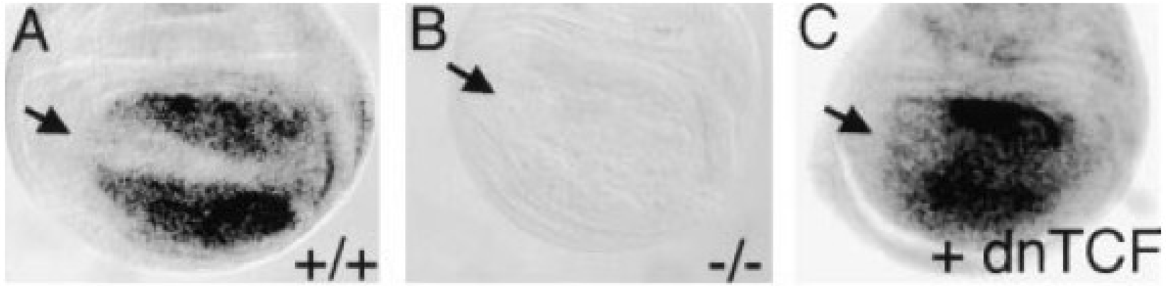
Aspects of size control - Growth rate - cell cycle - MYC

Myc deficiency









Johnston et al., 1999

Wnt controls the size

Aspects of size control - Growth rate - cell cycle - MYC

Summary of MYC:

Myc overexpression = more larger cells, faster proliferation, larger individual, loss of size control

Myc deficiency = smaller cells, smaller individual

The size of the wing is controlled by Wnt (Wg)

Aspects of Size Control – Growth Duration

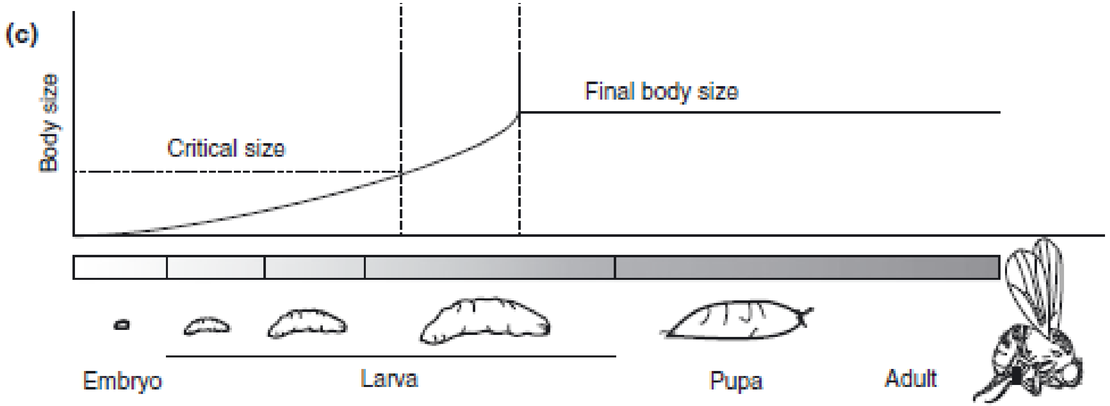
<u>Growth duration</u>

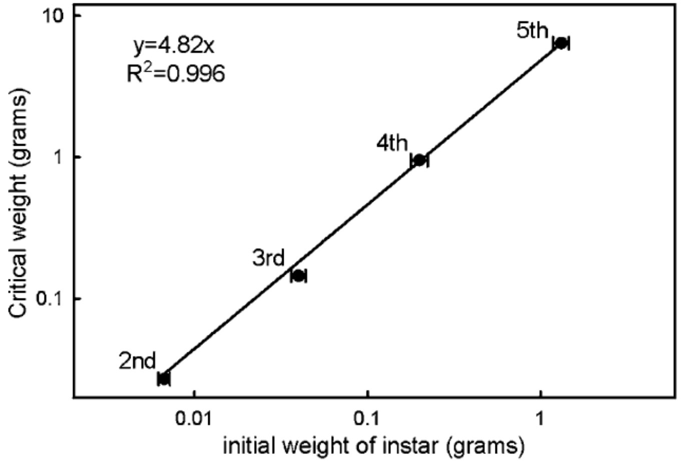
It is regulated by systemic hormonal signals that coordinate the cessation of growth throughout the body, as well as organ-autonomic processes that ensure that organs stop growing once they reach their final size.

Aspects of Size Control – Growth Duration

- Regulation of the growth rate and proliferation is not enough to fully control growth.
- Differences between individuals (and also between species) are due to differences in cell size and number of cells -> also regulated by growth duration
- Many signaling pathways and molecular mechanisms that regulate growth rates are also involved in regulating growth duration.

- Drosophila – 3 larval stages
- The size of the individual is determined by: rigid exoskeleton – there is no further growth, the size of the larva at the stage when it stops receiving food, before the stage of pupa (larval wandering).
- However, the decision to pupate is at a much earlier stage (the beginning of the 3rd instar) and is associated with a certain size (critical size)





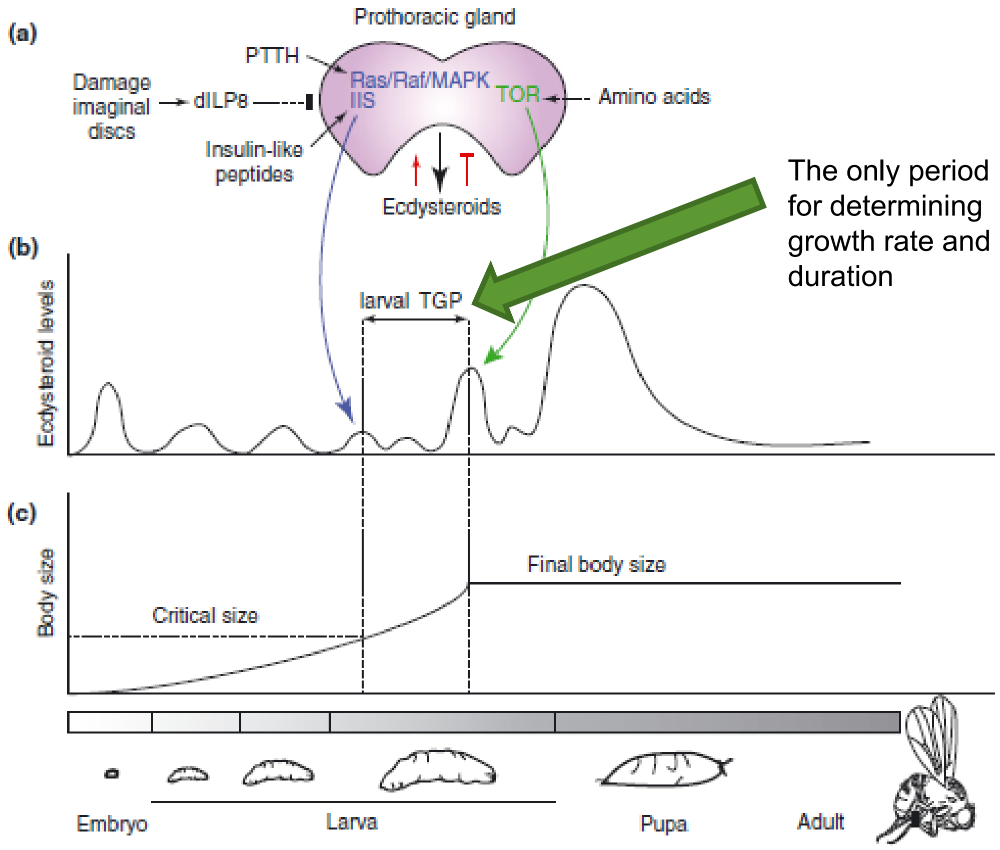
Aspects of Size Control – Growth Duration - IGF

Vývojová biologie

- However, the decision to pupate is at a much earlier stage (the beginning of the 3rd instar) and is associated with the acquisition of a certain size (critical size), which is accompanied by the synthesis of the hormone ecdysone
- Ecdyson hormone is synthesized in increasing pulses, and each pulse is associated with a specific event in development (metamorphosis).

- <u>Critical size is absent in humans.</u>

TGP = Terminal Growth Period

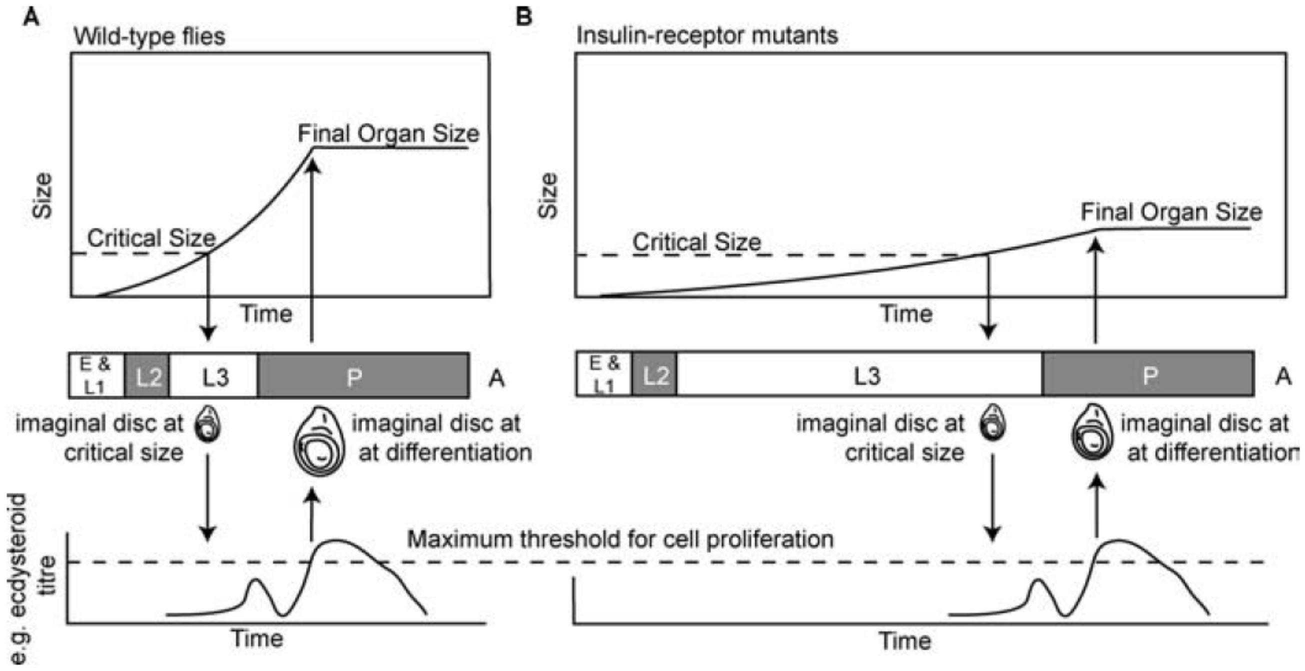


The only period for determining growth rate and duration

Aspects of Size Control – Growth Duration - IGF

IGF regulates growth rate during TGP

When ecdysteroid levels rise above a maximum threshold, the discs cease cell proliferation and undergo differentiation, fixing their final size

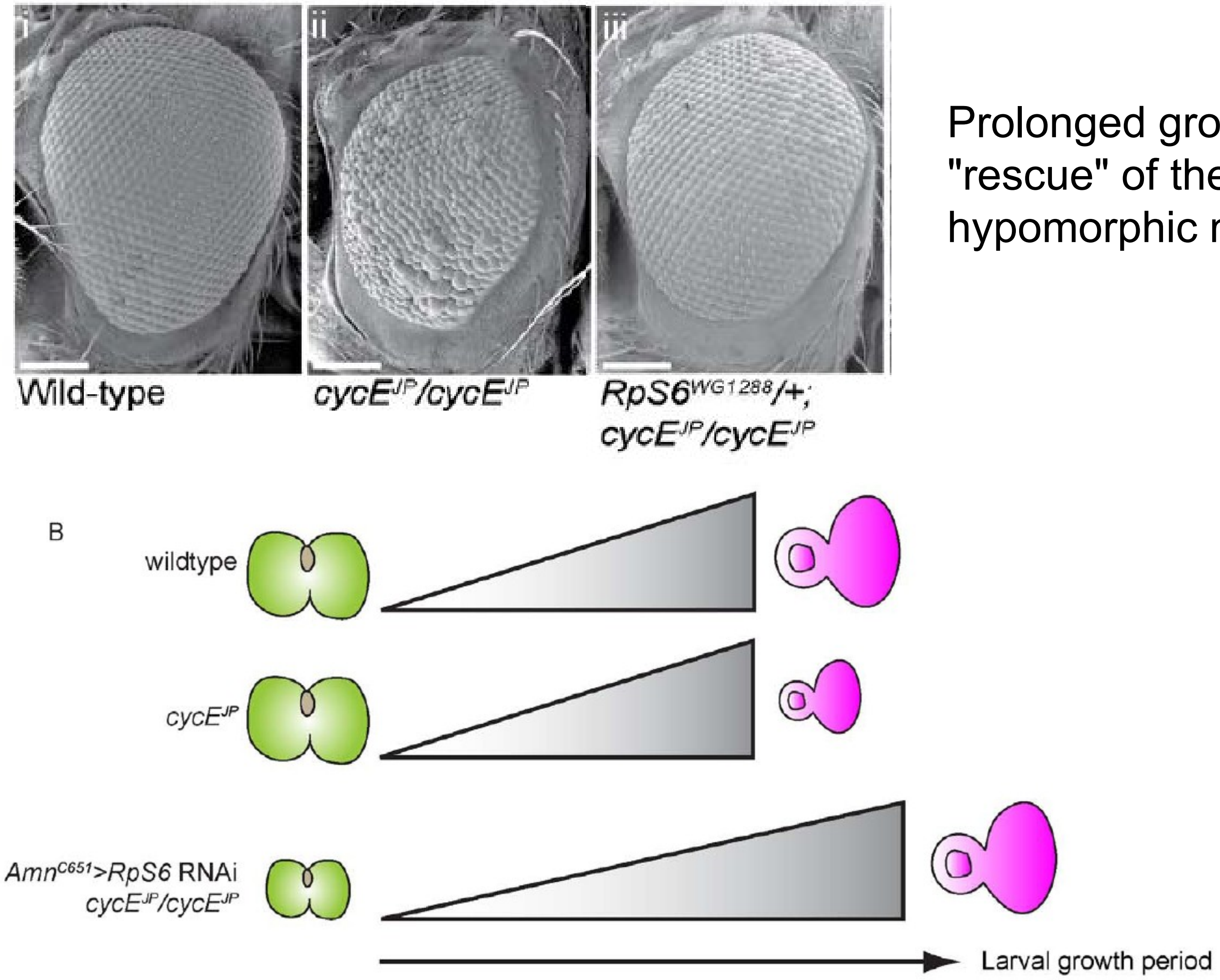


Because the rate of cell proliferation is slowed, the imaginal discs are smaller when they begin to differentiate, reducing final organ size.

Shingleton et al., 2005

Vývojová biologie

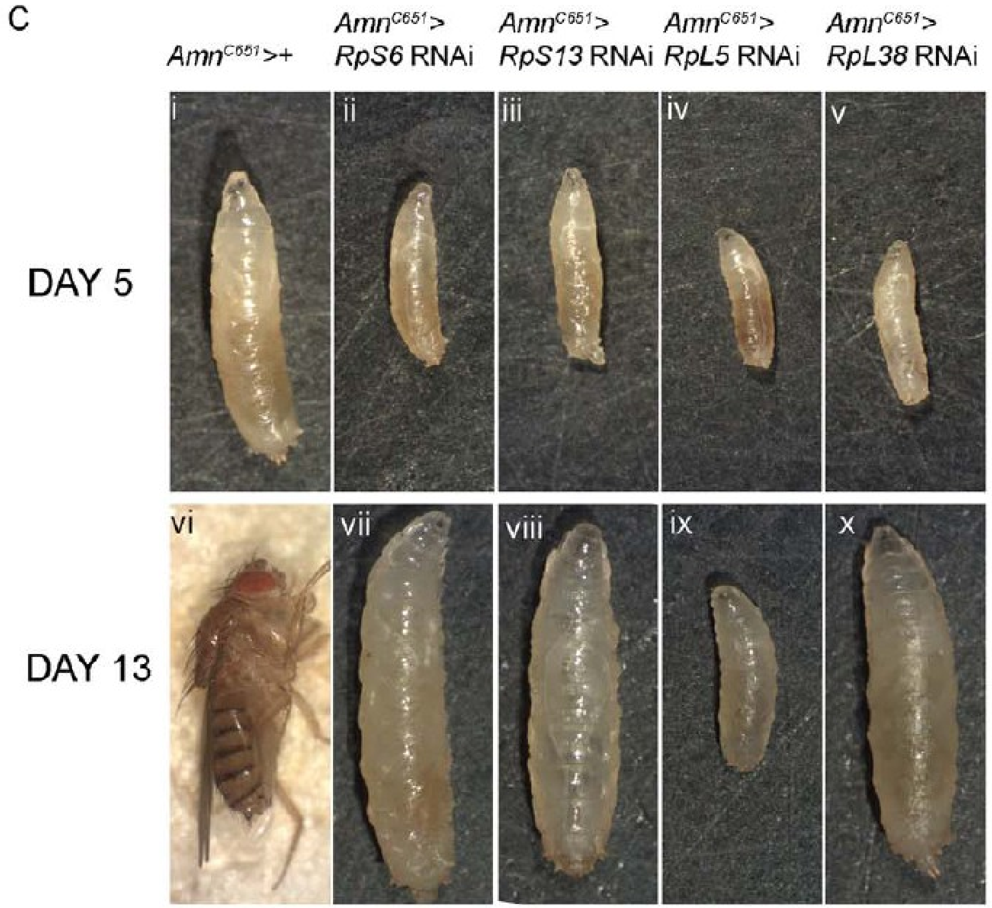
Aspects of Size Control – Growth Duration

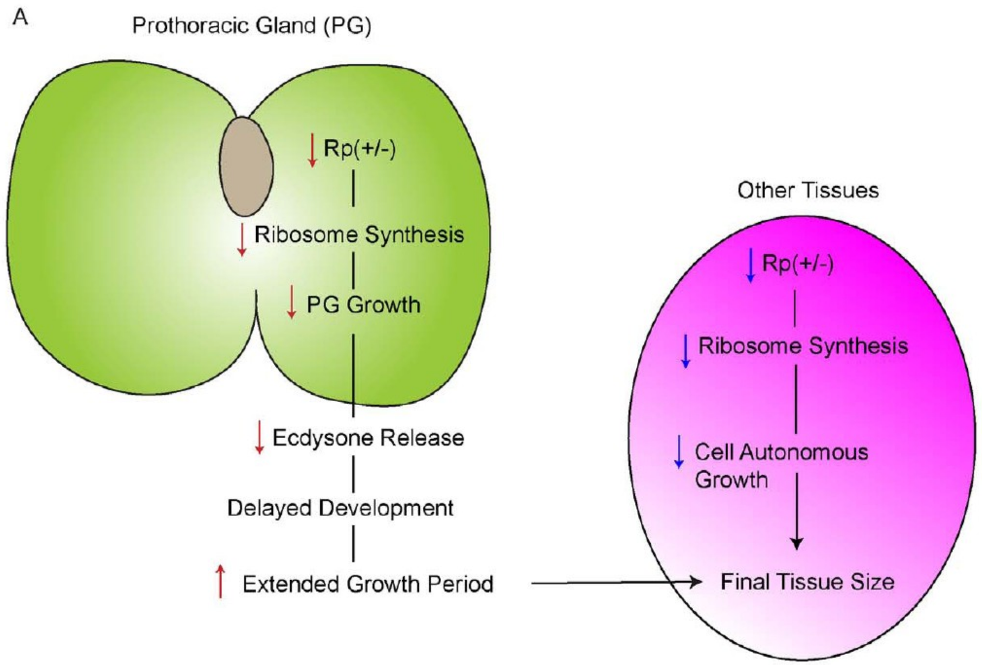


Prolonged growth time leads to the "rescue" of the phenotype of the cycE hypomorphic mutation

Lin et al., 2011

Aspects of size control – Growth duration – protein synthesis





Lin et al., 2011

Aspects of Size Control – Growth Duration

The exact mechanism by which the larvae monitor their critical size is not clear...

however…

PNAS

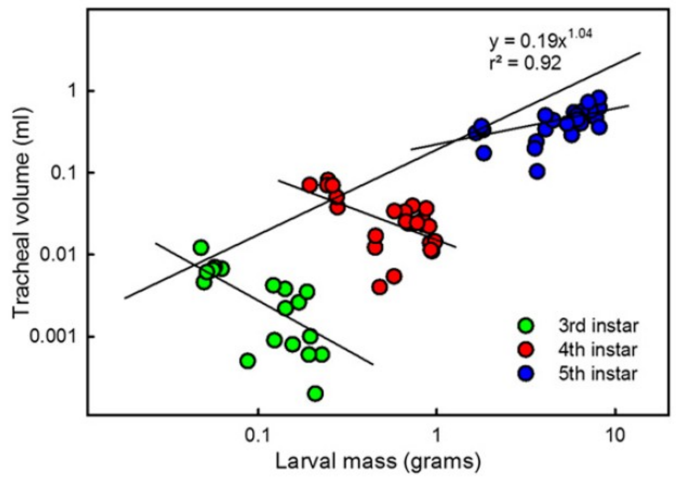
Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis

Viviane Callier and H. Frederik Nijhout[1]

Department of Biology, Duke University, Durham, NC 27708

Edited* by Mary Jane West-Eberhard, Smithsonian Tropical Research Institute, Ciudad Universitaria, Costa Rica, and approved July 28, 2011 (received for review April 27, 2011)

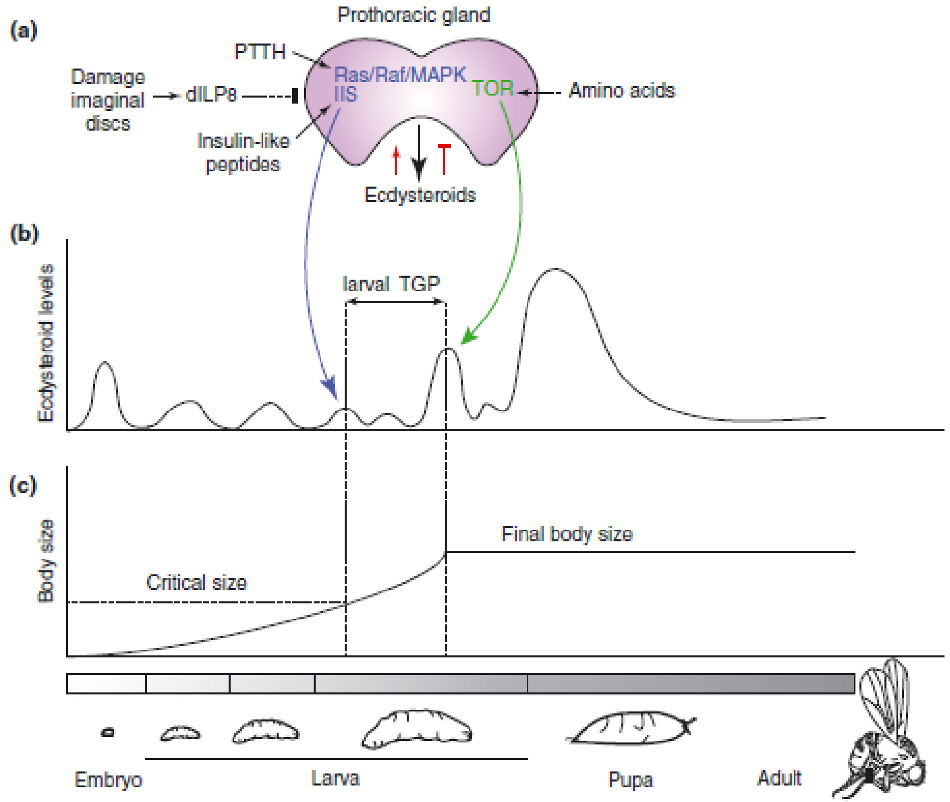
Here we show that this size-sensing mechanism depends on the limited ability of a fixed tracheal system to sustain the oxygen supply to a growing individual. As body mass increases, the demand for oxygen also increases, but the fixed tracheal system does not allow a corresponding increase in oxygen supply. We show that interinstar molting has the same size-related oxygendependent mechanism of regulation as metamorphosis. We show that low oxygen tension induces molting at smaller body size, consistent with the hypothesis that under normal growth conditions, body size is regulated by a mechanism that senses oxygen limitation.



Aspects of Size Control – Growth Duration

The exact mechanism by which the larvae monitor their critical size is not clear, but signaling pathways regulating the synthesis of Ecdysone and thus responding to critical size are known

IIS, TOR, RAS/RAF/MAPK



Aspects of Size Control - Growth Duration - Human

What about human?

Aspects of Size Control - Growth Duration - Human

What about human?

- Growth arrest is associated with the end of puberty -> timing puberty is an important factor in size regulation
- Hormonal changes in puberty are known, but the mechanisms that control when these hormonal changes are initiated are much less understood.

„For example, children displaying precocious puberty are typically tall for their age because of an advanced adolescent growth spurt, but generally become shorter adults because they enter maturation and, therefore, adulthood earlier. Conversely, delayed puberty commonly leads to individuals with high stature."

- Higher BMI, earlier puberty
- <u>This suggests that the timing of puberty and growth stop in humans, as well as in Drosophila, are regulated by the state (amount) of nutrition and body size of adolescents.</u>

Aspects of Size Control - Growth Duration - Human

Vývojová biologie

***LIN28B* the first genetic determinant regulating the timing of human pubertal growth**

nature genetics

Genetic variation in *LIN28B* is associated with the timing of puberty

Ken K Ong[1–3], Cathy E Elks[1,2], Shengxu Li[1,2], Jing Hua Zhao[1,2], Jian'an Luan[1,2], Lars B Andersen[4], Sheila A Bingham[5,6], Soren Brage[1,2], George Davey Smith[7], Ulf Ekelund[1,2,8], Christopher J Gillson[1,2], Beate Glaser[7], Jean Golding[9], Rebecca Hardy[10], Kay-Tee Khaw[11], Diana Kuh[10], Robert Luben[11], Michele Marcus[12–14], Michael A McGeehin[12], Andrew R Ness[15], Kate Northstone[16], Susan M Ring[16], Carol Rubin[12], Matthew A Sims[1,2], Kijoung Song[17], David P Strachan[18], Peter Vollenweider[19], Gerard Waeber[19], Dawn M Waterworth[17], Andrew Wong[10], Panagiotis Deloukas[20], Inês Barroso[20], Vincent Mooser[17], Ruth J Loos[1,2] & Nicholas J Wareham[1,2]

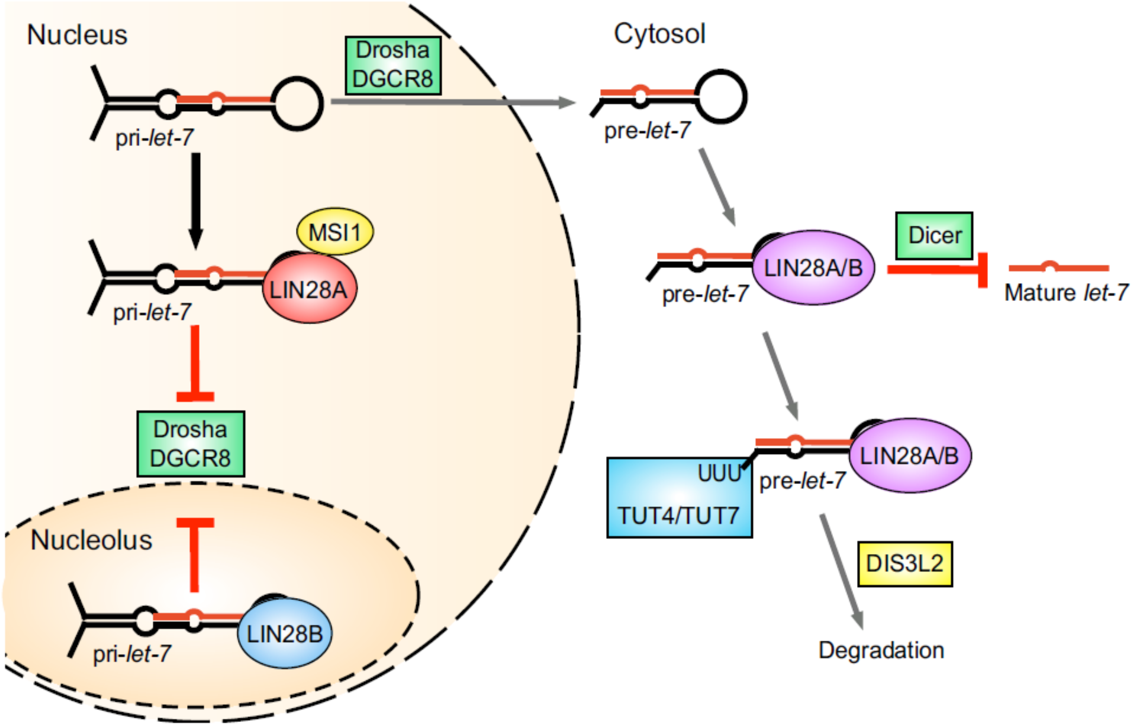
study for age at menarche in 4,714 women

independent replication studies in 16,373 women

„This allele was also associated with earlier breast development in girls; earlier voice breaking and more advanced pubic hair development in boys; **a faster tempo of height growth in girls and boys; and shorter adult height in women and men in keeping with earlier growth cessation.“**

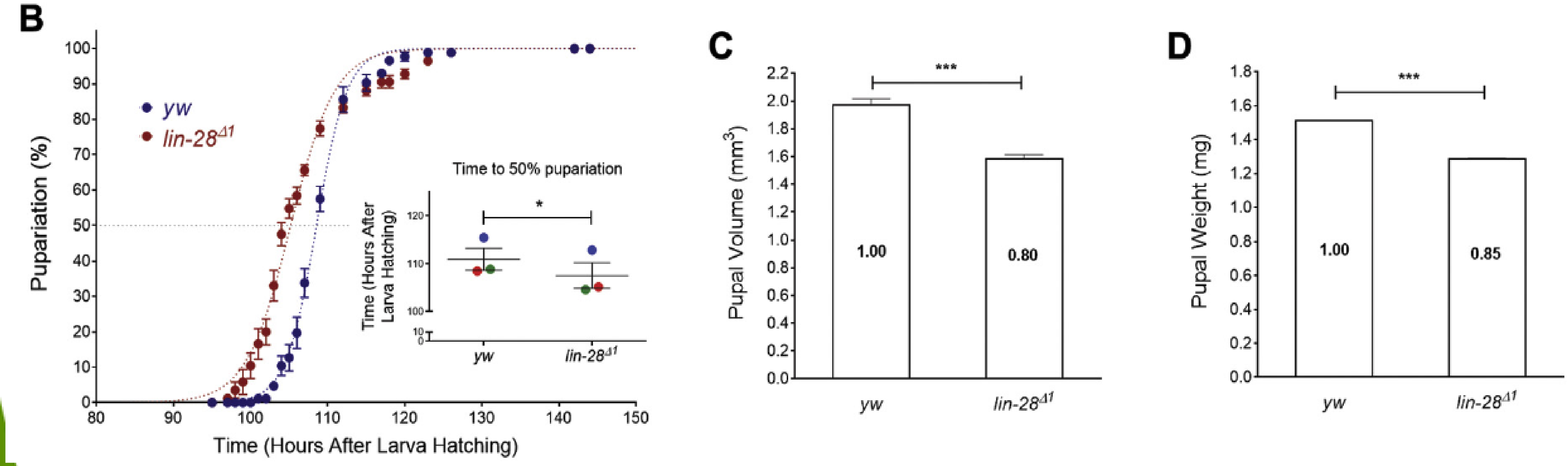
Aspects of Size Control – Growth Duration – LIN28

- Important in microRNA processing (inhibits Let-7).
- Controls growth and metabolis



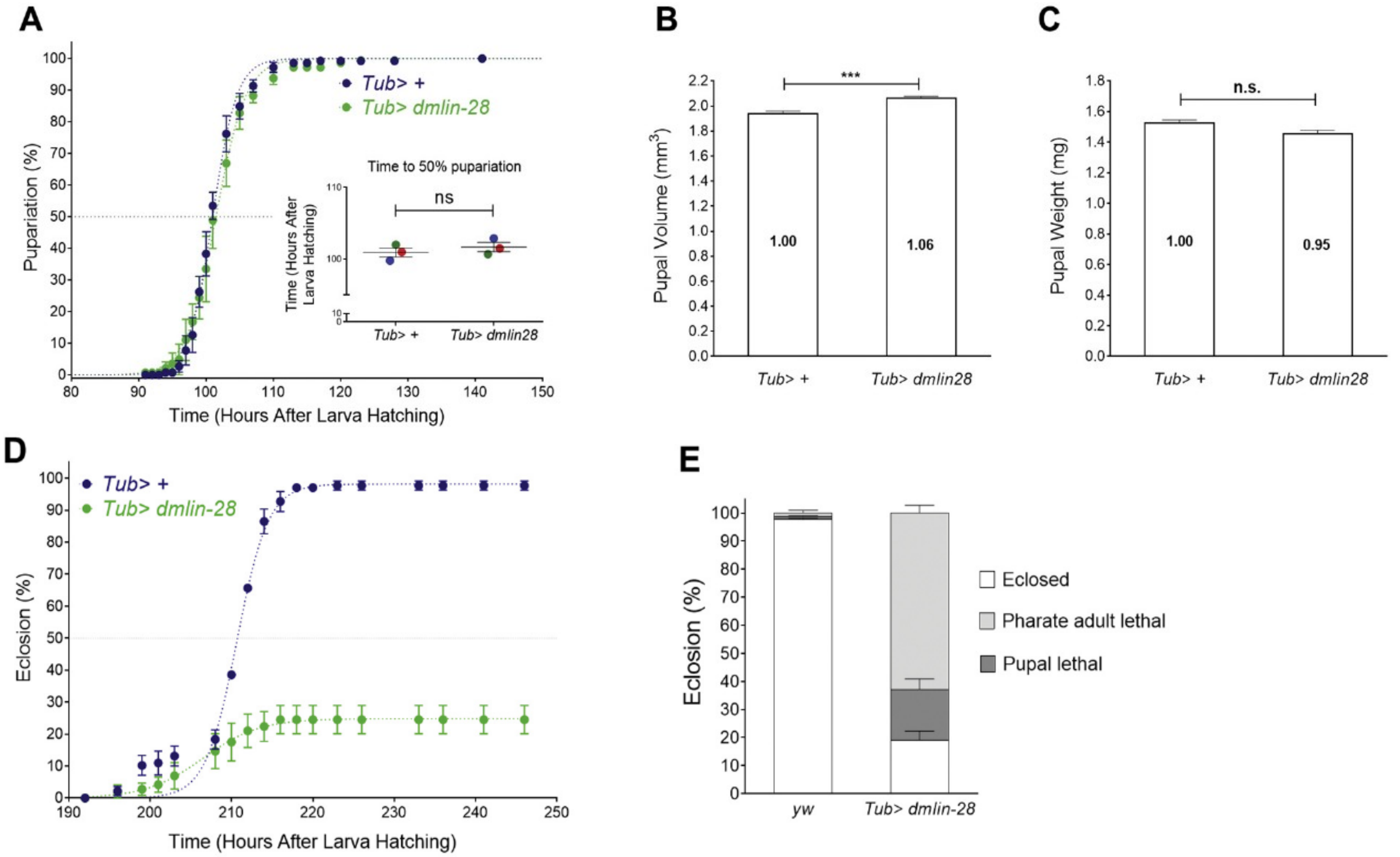
Aspects of Size Control – Growth Duration – LIN28

- Back to the flies
- Lin-28 deficiency leads to earlier pupation, smaller and lighter individuals



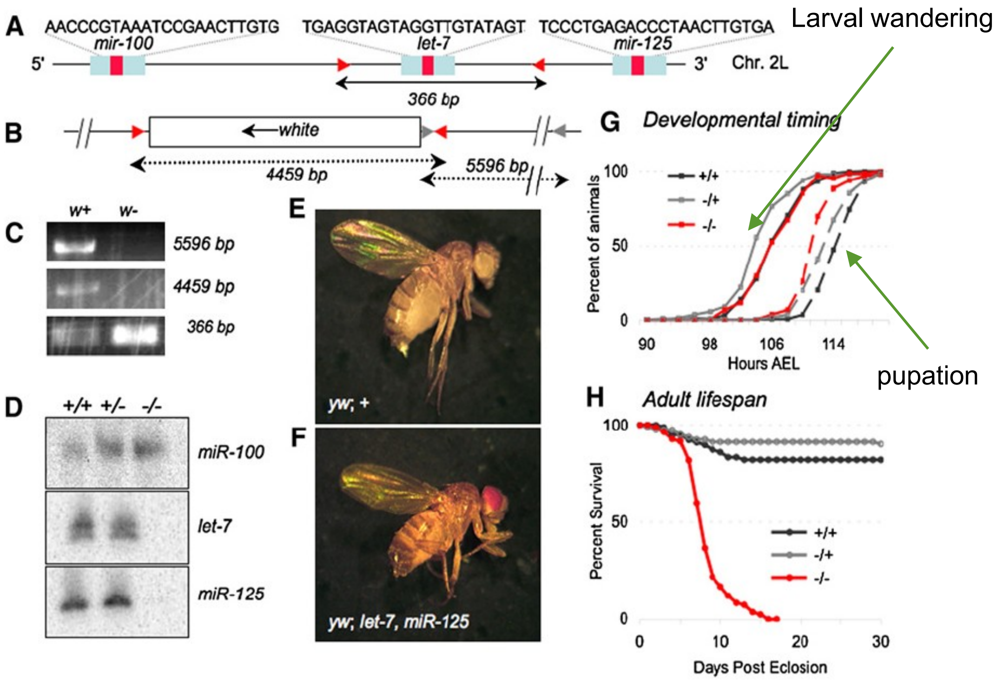
Aspects of Size Control – Growth Duration – LIN28

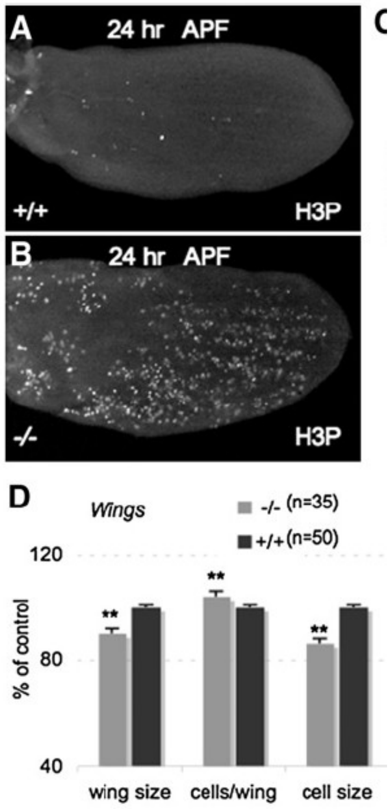
- Back to the flies
- Overexpression of lin-28 leads to larger individuals and problems during hatching (problems during metamorphosis)



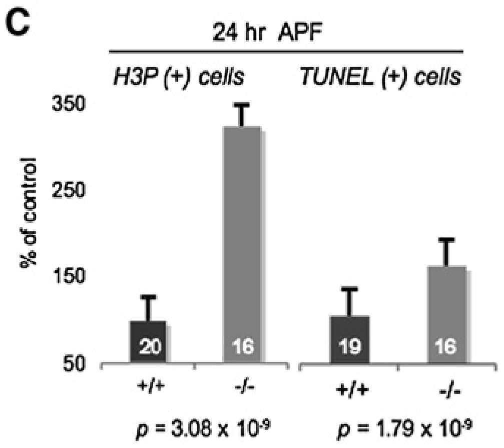
Aspects of Size Control – Growth Duration – LIN28

Let-7 deficiency





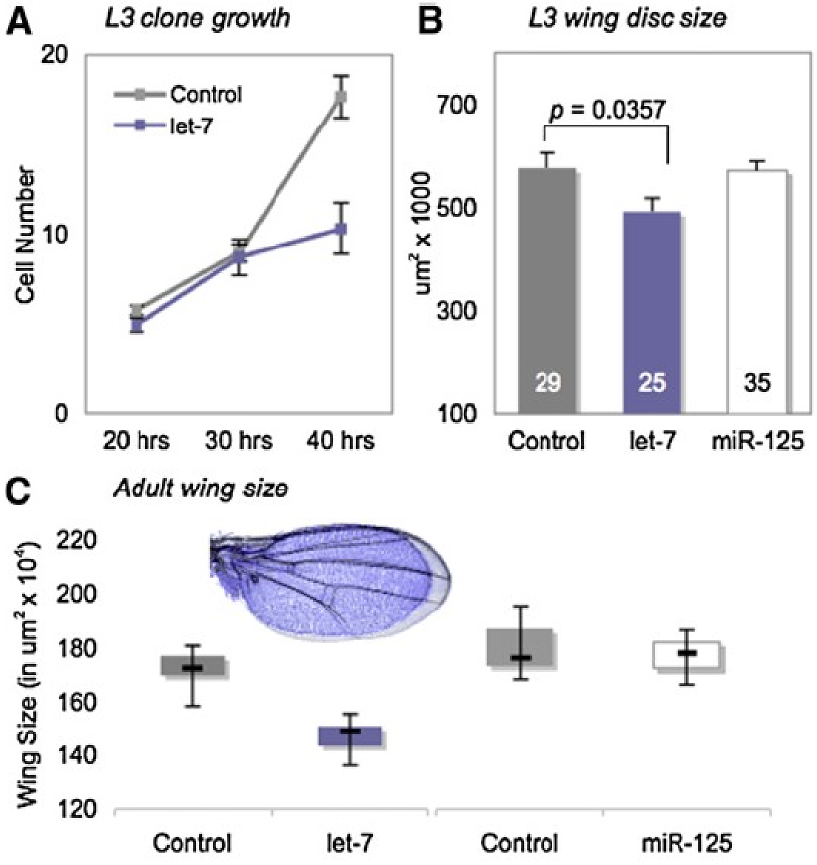
Cells are not able to "get out" of the cell cycle.



Caygill et al., 2008

Aspects of Size Control - Growth Duration - LIN28B

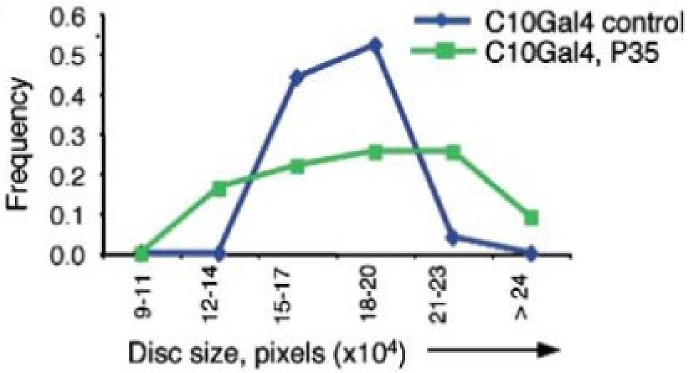
Let-7 overexpression leads to cessation of development in L1, L2 and high lethality => overexpression of miRNA in vestigial (vg) – wing-specific

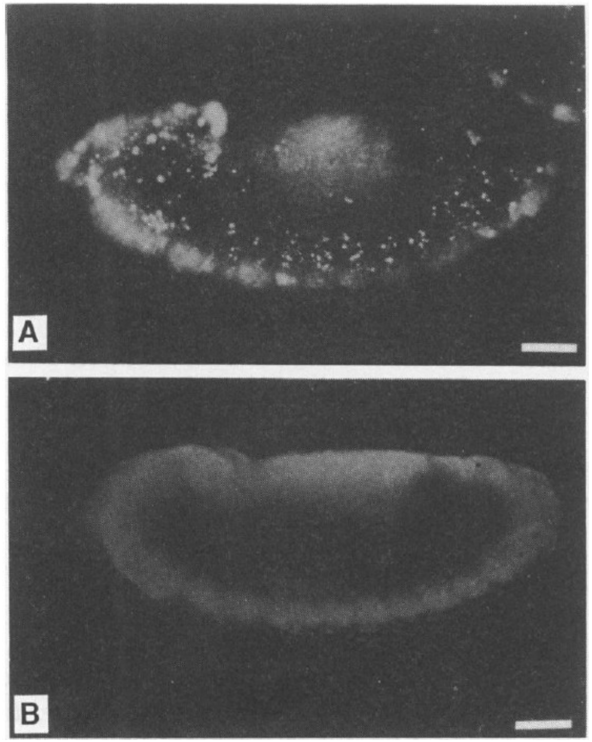


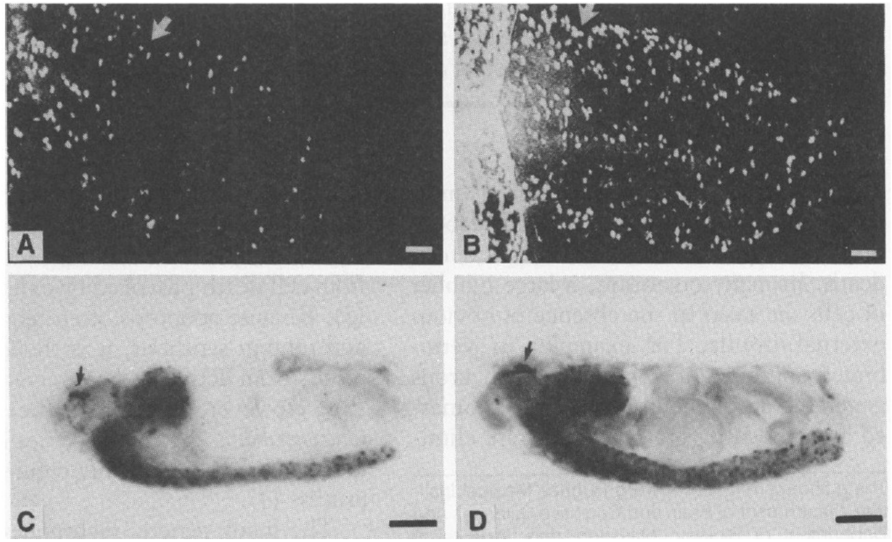
Caygill et al., 2008

Negative growth controls

- Apoptosis







White et al., 1994

Aspects of size control - Size of organs

The organs "know" what size they should be. And they also "know" when to stop growing => autonomy in terms of organ size. The concept was introduced back in the 70s.

Silber, 1976

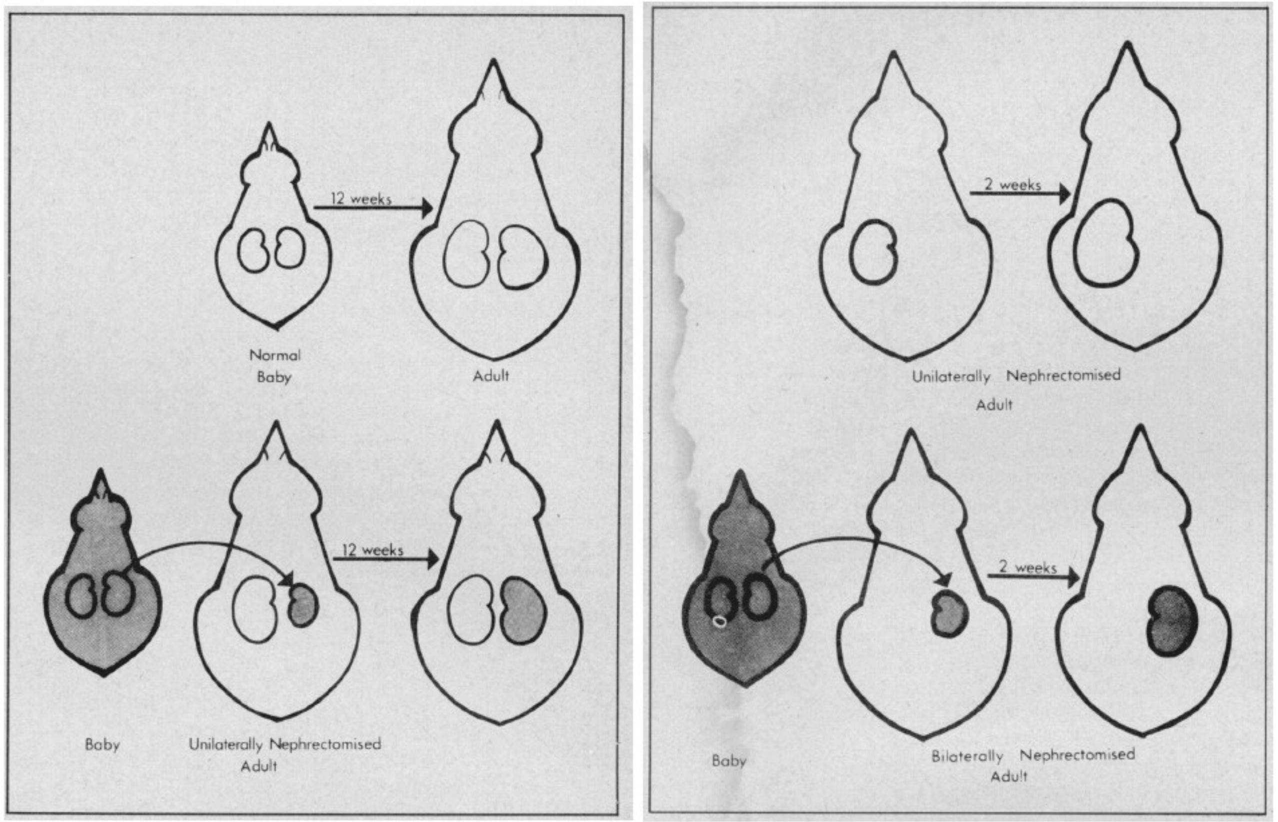


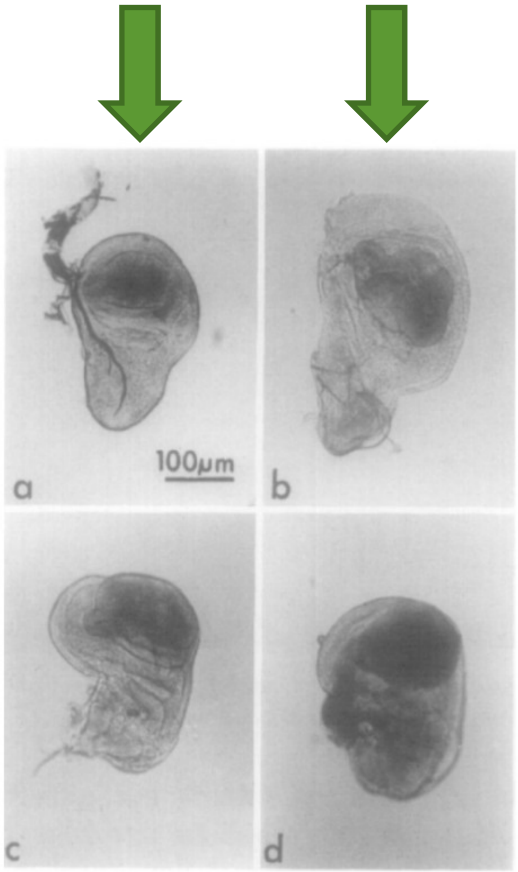
Table 1.—Growth and Hypertrophy of Baby Kidneys in Babies vs Adults

	Rat Age, Weeks	Kidney Age, Weeks	Kidney Size, cm*
Baby rat unilaterally nephrectomized at 4 weeks	4	4	1.27 ± 0.10
	7	7	1.80 ± 0.10
	12	12	2.05 ± 0.13
Baby kidney at 4 weeks transplanted into bilaterally nephrectomized adult rat	12	4	1.30 ± 0.15
	15	7	1.88 ± 0.12
	20	12	2.10 ± 0.14

Aspects of size control - Size of organs

The same goes for the fruit fly.

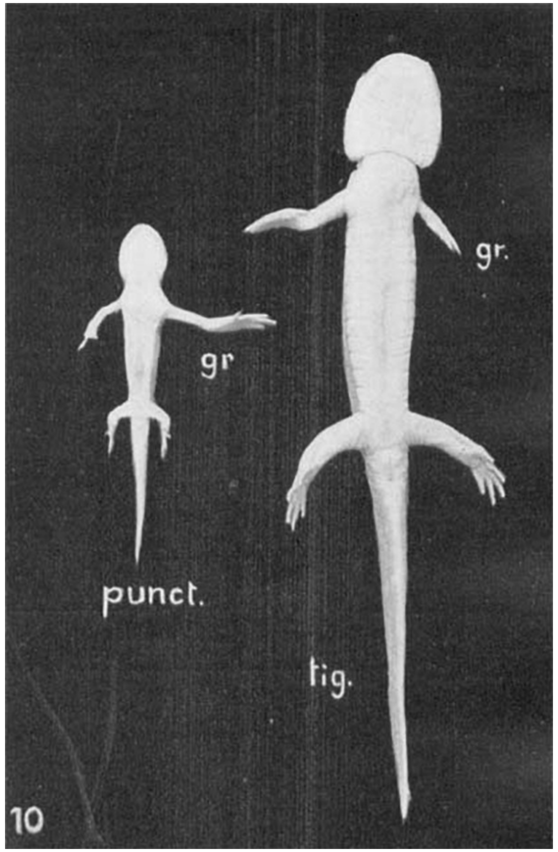
Imaginary wing disc   The same disc, cultured in vivo in an adult



Bryant, Levinson, 1985

Aspects of size control - Size of organs

- Mammals have a similar body plan, but organ sizes vary dramatically
- The size is not random, but the result of thorough regulation
- The size of the organ must also be adapted to physiological needs (when a kidney is removed, the other one hypertrophies)
- Coordination of proliferation and cell death is crucial for the correct size of the organ.



Size was determined by integration of a limb-intrinsic “potential,” which was greater in tigrinum,
and a systemic “regulator” more active in punctatum

- Each organ has autonomous size control (but is also partially subject to system control)
- Growth duration/timing seems to be the key

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Aspects of size control - Size of organs

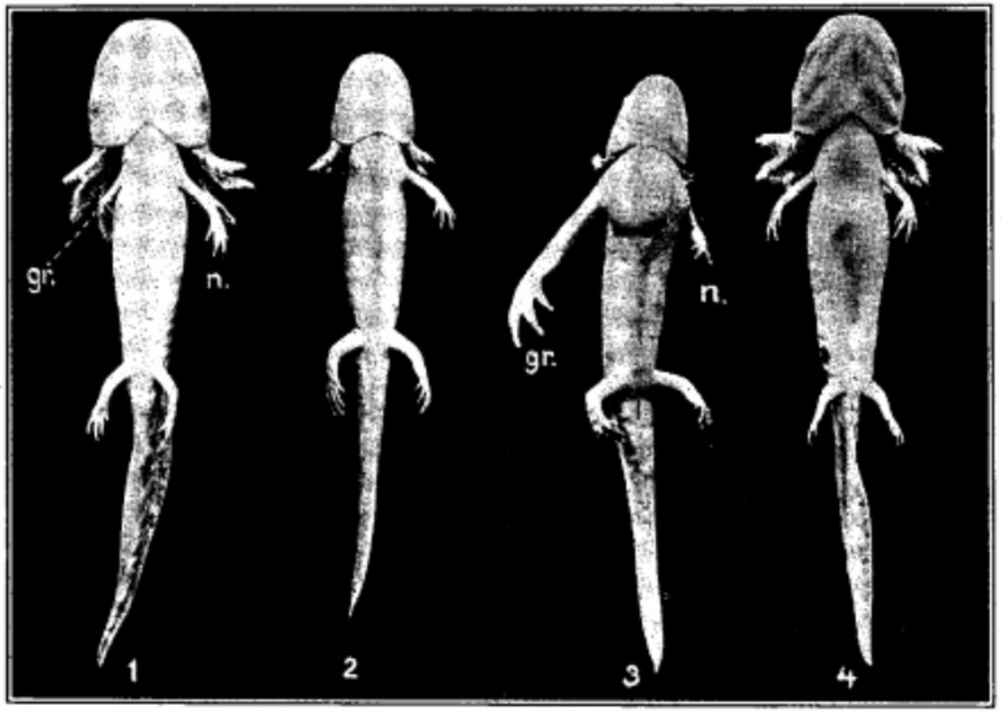


Fig. 1.—Larva of Amblystoma tigrinum with a punctatum fore limb (*gr*) on the right side; *n*, normal limb. Exp. NE.10; specimen preserved 76 days after operation.

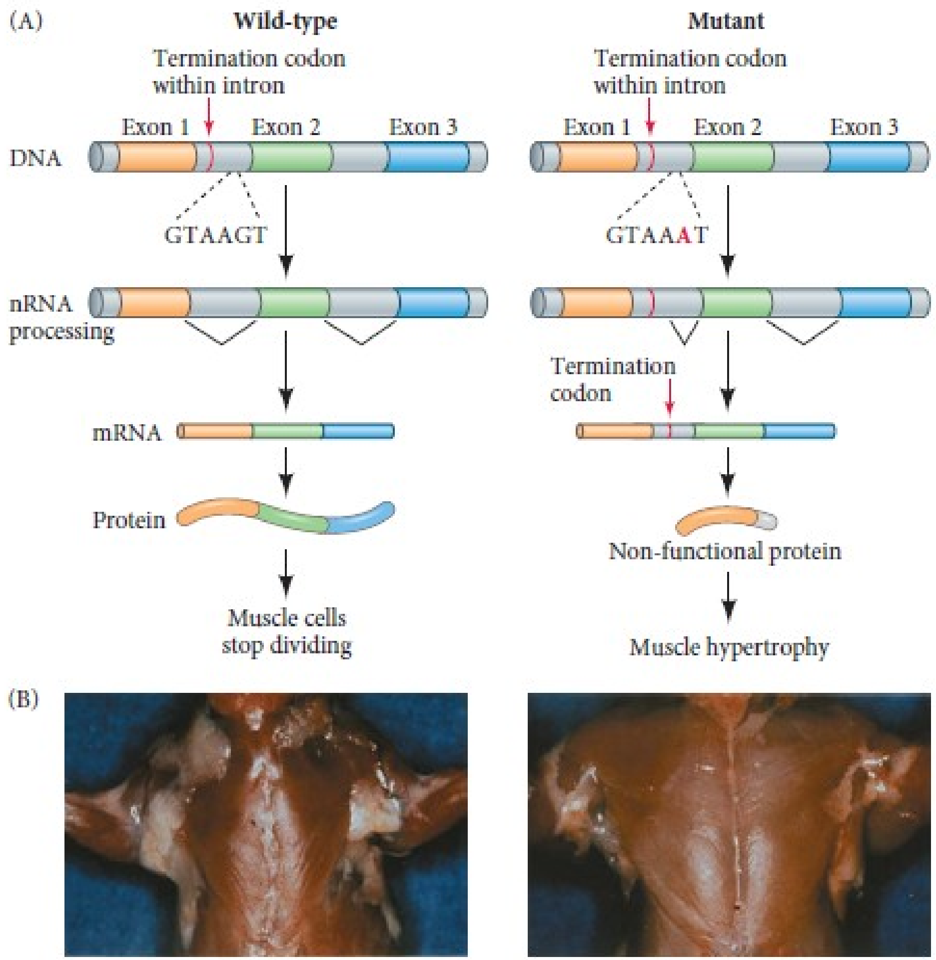
Fig. 2.—Larva of A. punctatum, the donor of the limb shown in fig. 1. Regeneration of the lost limb has not occurred. Specimen preserved 76 days after operation.

Fig. 3.—Larva of A. punctatum, showing a gigantic, but otherwise normal, tigrinum limb (*gr*), grown to this size after having been grafted in the limb bud stage; *n*, normal limb. Exp. NE.13, specimen preserved 72 days after operation.

Fig. 4.—Normal control larva of A. tigrinum of the same age.

Aspects of size control - Size of organs

- Each organ has an autonomous size control
- Link to the first lecture



Aspects of size control - Size of organs

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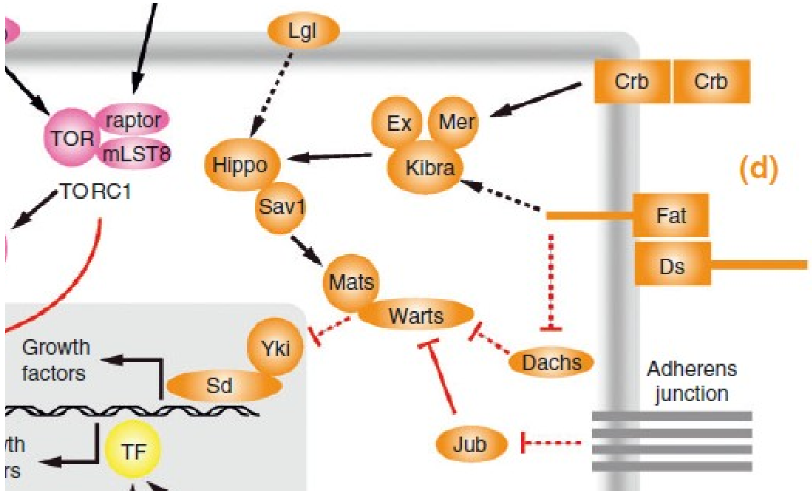
Coordination of proliferation and cell death is crucial for the correct size of the organ.

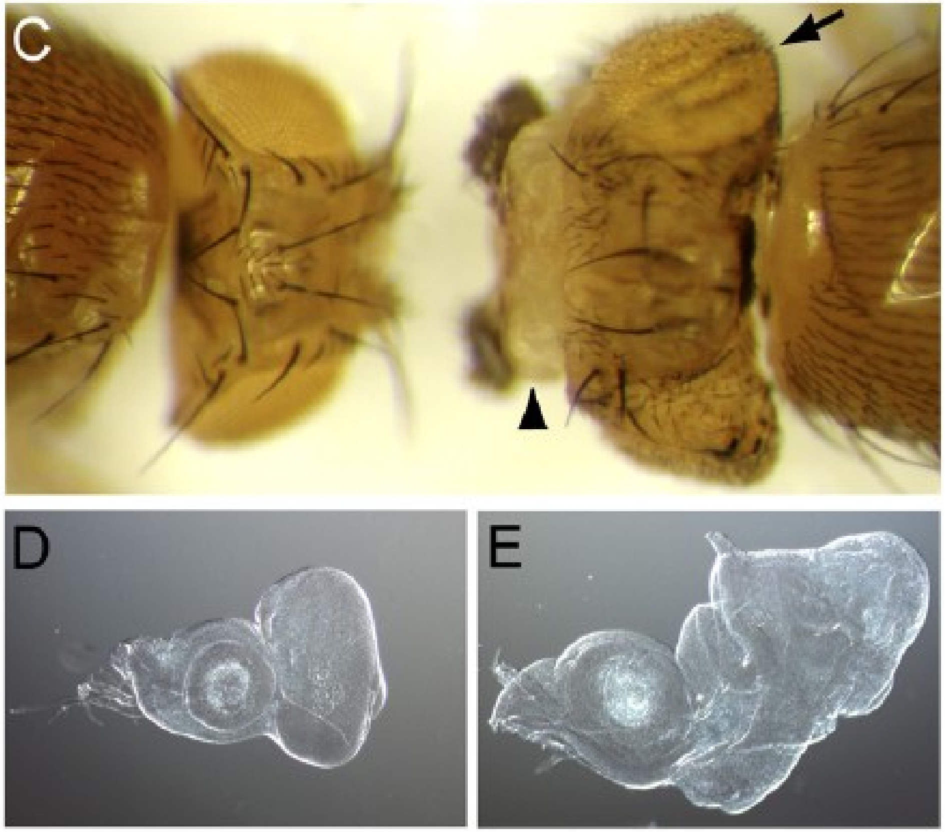
Hippo

Aspects of size control - Organ size - Hippo



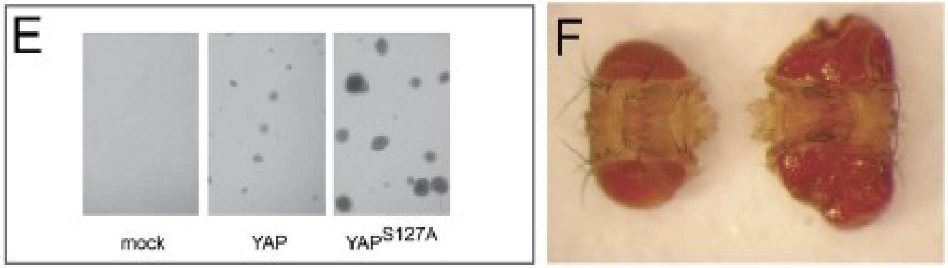
Dong et al., 2007

Yki^{S168A}

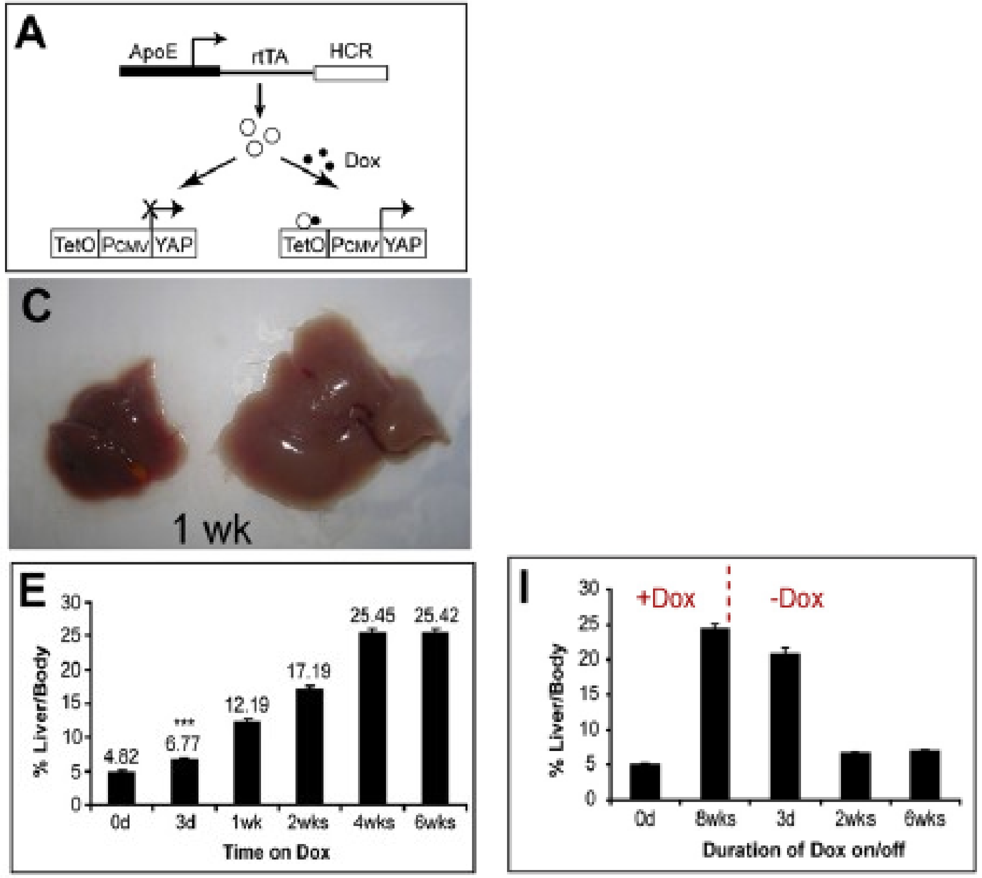


Aspects of size control - Organ size - Hippo

And in mammals?

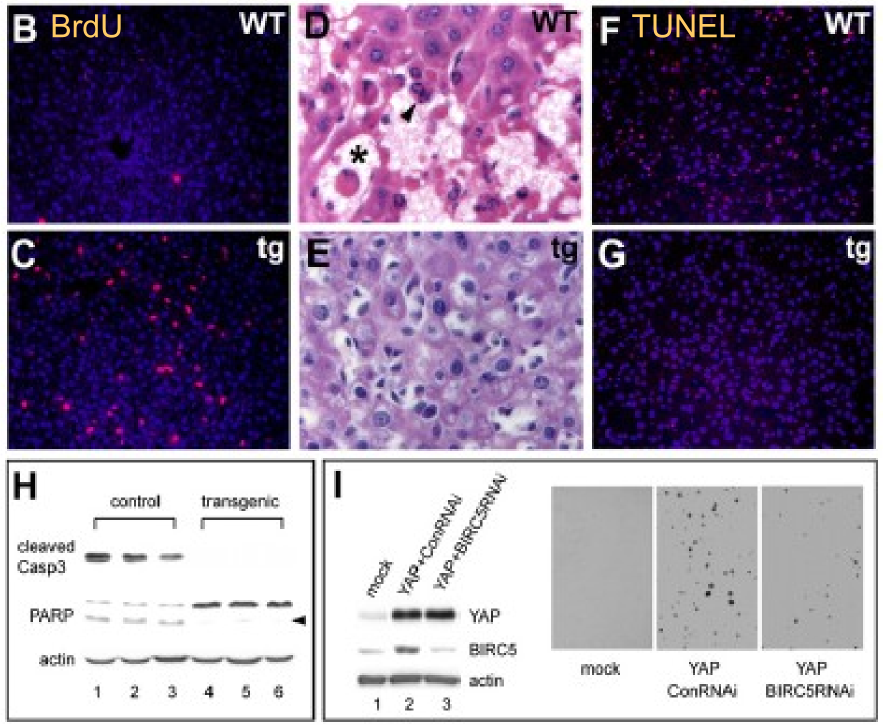


Dong et al., 2007



Aspects of size control - Organ size - Hippo

Hippo and cell death

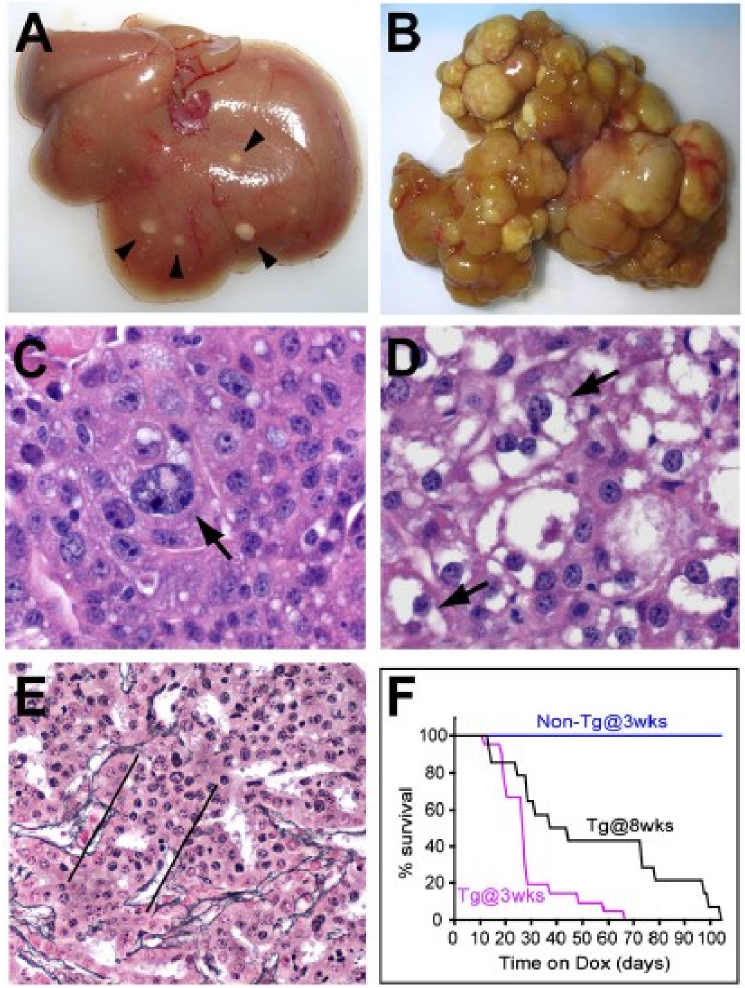


YAP Overexpression

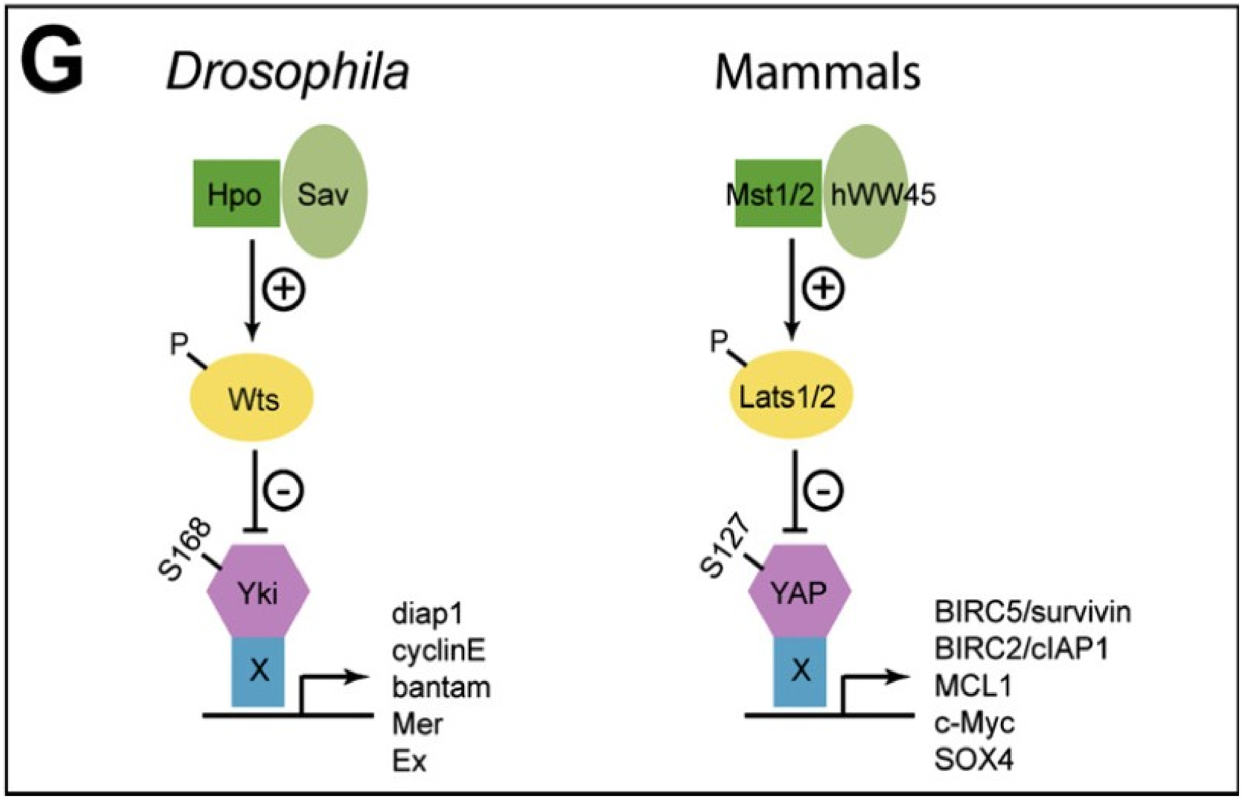
Vývojová biologie

Aspects of size control - Organ size - Hippo

BONUS: Hippo and cancer



Aspects of sizing control — Organ size — Hippo — Conclusion



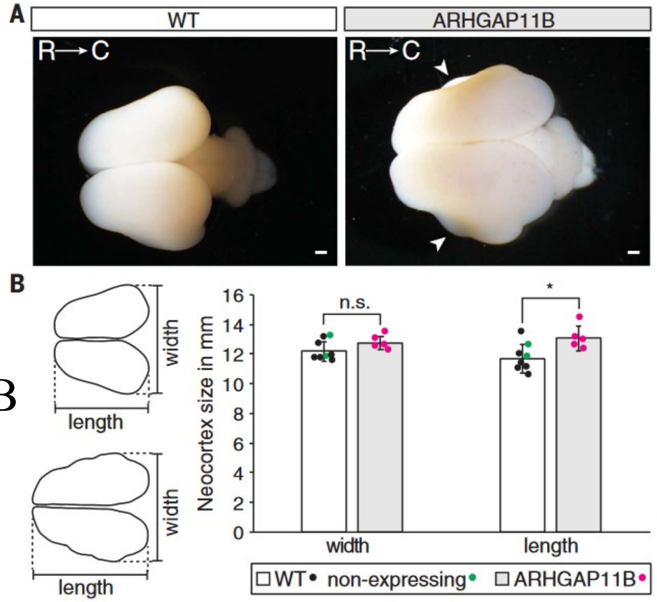
BONUS:Back to the lecture on size control

NEURODEVELOPMENT

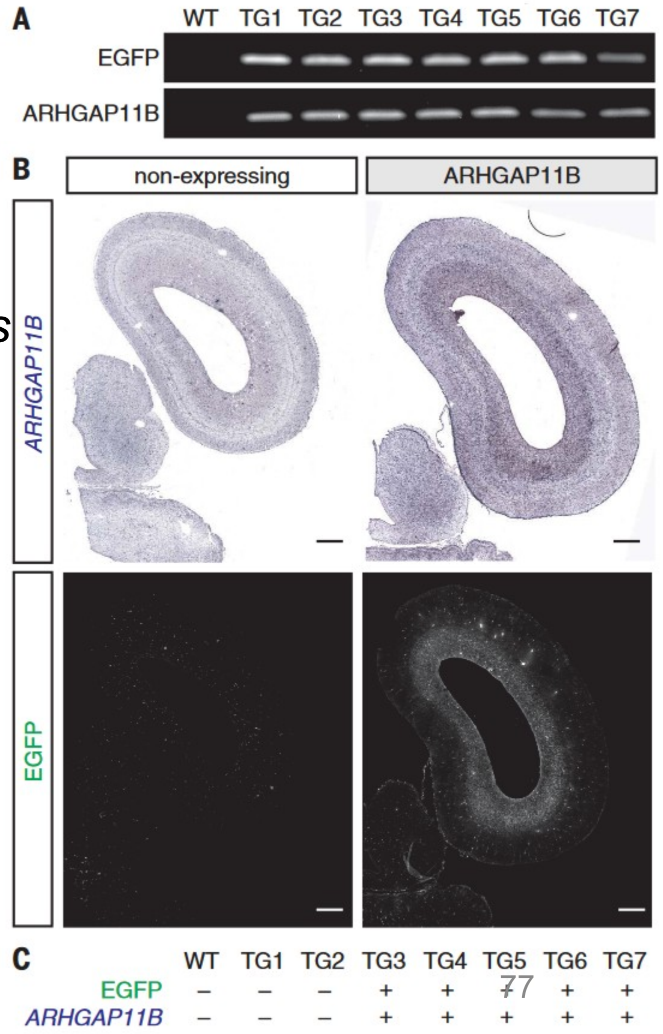
Human-specific *ARHGAP11B* increases size and folding of primate neocortex in the fetal marmoset

Michael Heide[1]*, Christiane Haffner[1], Ayako Murayama[2,3], Yoko Kurotaki[4], Haruka Shinohara[4], Hideyuki Okano[2,3], Erika Sasaki[4], Wieland B. Huttner[1]*

- *ARHGAP11B gene is present is only in humans*
- *It was formed about 5 million years ago by partial duplication of the ARHGAP11A gene (Rho-GAP in the nucleus)*
- *However, ARHGAP11B has lost this activity due to the point mutation and is localized in the mitochondria, where it participates in glutamine metabolism*
- *Increased number of radial glia, neural*
- *precursors of the neocortex*
- *Ethics?*
- *Did this gene really separate human*
- *from primates?*



"The cells amplified upon ARHGAP11B expression in fetal marmoset neocortex exhibited a marker signature consistent with the identity of basal radial glia."



Conclusion and questions

- Control of growth/size: Growth rate vs Growth duration + examples
- Organ growth/size control (Hippo)
- Know that there is also negative regulation.

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Rate of growth X Duration of growth

Cell count regulation X Cell size control

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Thank you for your attention