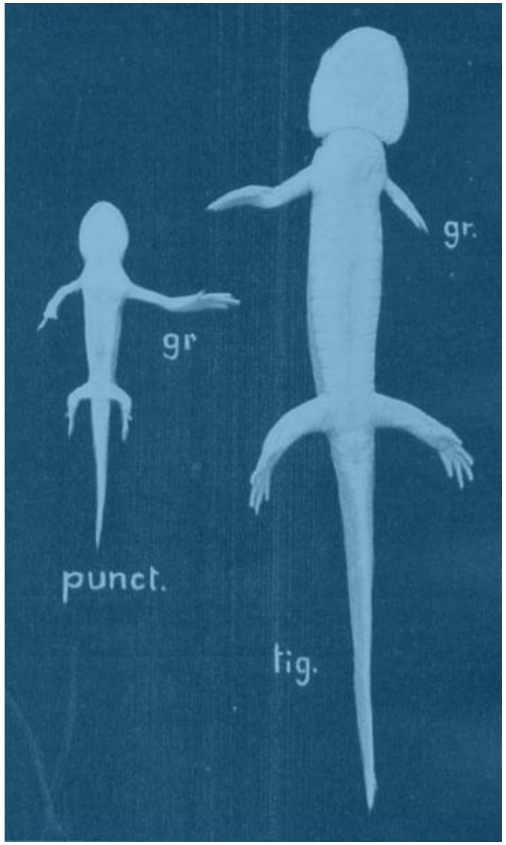
Organism and Tissue Growth



Course 3: Organ intrinsic control of growth

Thomas Lecuit

chaire: Dynamiques du vivant

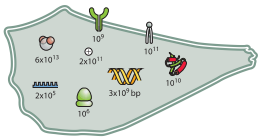
COLLÈGE DE FRANCE
1530

• Motor, Constraints and Regulation of Growth

Energy supply

carbohydrates
amino-acids
ions etc

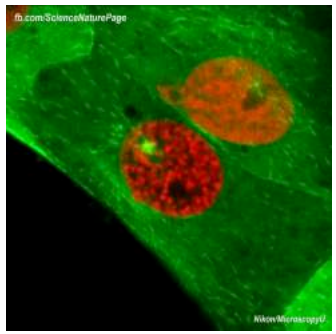
Metabolism



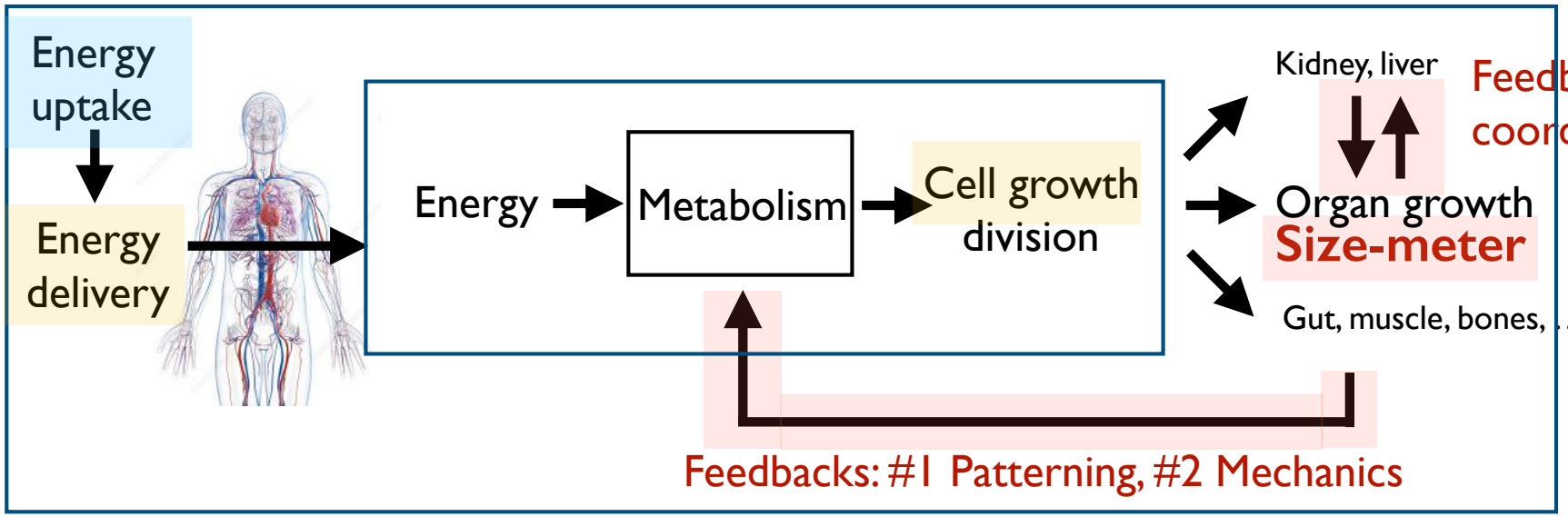
Synthesis:
ATP, amino-acids, proteins, lipids, carbohydrates, etc

H_2O flux

Cell growth, division



kidney cell



• Organismal Growth: Metabolism and Size

—Life is a self-sustaining (heritable) organisation of matter brought out of equilibrium locally and persistently

—The organisation, growth, and maintenance at all levels of organisation, molecules, organelles, cells, organs and whole organisms requires constant energy conversion

—Evolution is constrained by energy demands, delivery and conversion across scales

Metabolic rates (Power)

• Summary — Organism Scaling Laws

1. Size of animals and plants varies over many orders of magnitude including within a given taxon or families
2. Animals and plants show characteristic allometric relationships: relative growth and self-similarity
3. Allometry reflects internal and external constraints in organisation, namely:

   — Mechanical constraints (elastic similarity)
   — Energy delivery to all cells in the organism

4. The West-Brown-Enquist model provides a quantitative framework that explains the ubiquity of ¼ exponents in allometry, in particular Kleiber's law

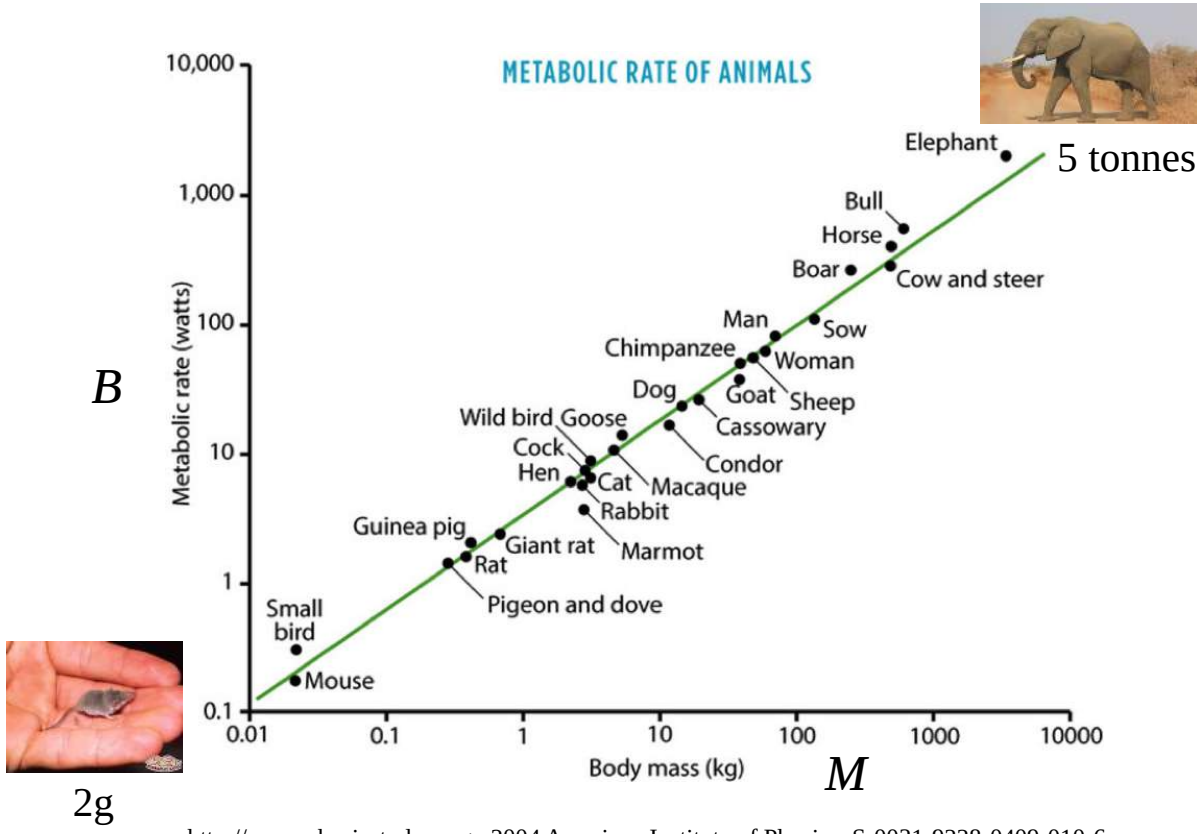
   —Key feature: Hierarchical self-similar branching network with invariant terminal units and minimisation of energy dissipation

5. The WBE model yields a universal ontogenetic bounded growth curve
6. The WBE model redefines a universal biological clock adjusted for mass (internal constraint) and temperature (external constraint), where the clock ticks slower as size increases.

7. There are obvious limits to this model and some features are incorrect (eg. planarian) but it provides a compelling 0th order model to explain organismal growth and size.

• Organismal Growth: Metabolism and Size

$$B = B_0 M_b^{3/4} \qquad t \propto M_b^{1/4} e^{E/kT}$$



5 tonnes

2g

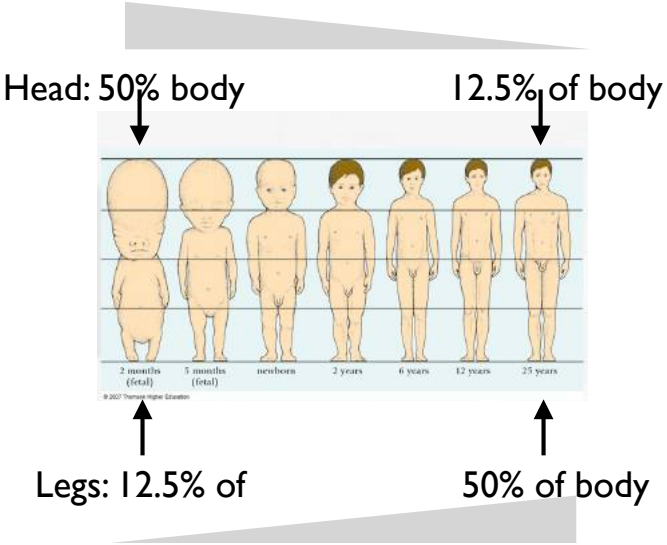
http://www.physicstoday.org . 2004 American Institute of Physics, S-0031-9228-0409-010-6

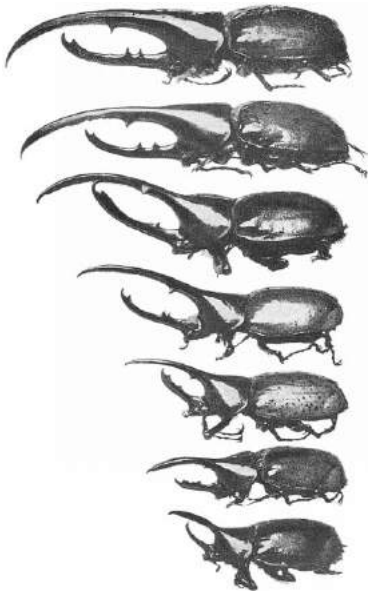
• Allometry - Law of *relative* growth

—Questions:

- what dictates the relative partitioning of organismal growth among organs, limbs etc?
- How are proportions monitored and controlled?

- How is growth symmetry ensured?
  And when it is asymmetric, how is this controlled?

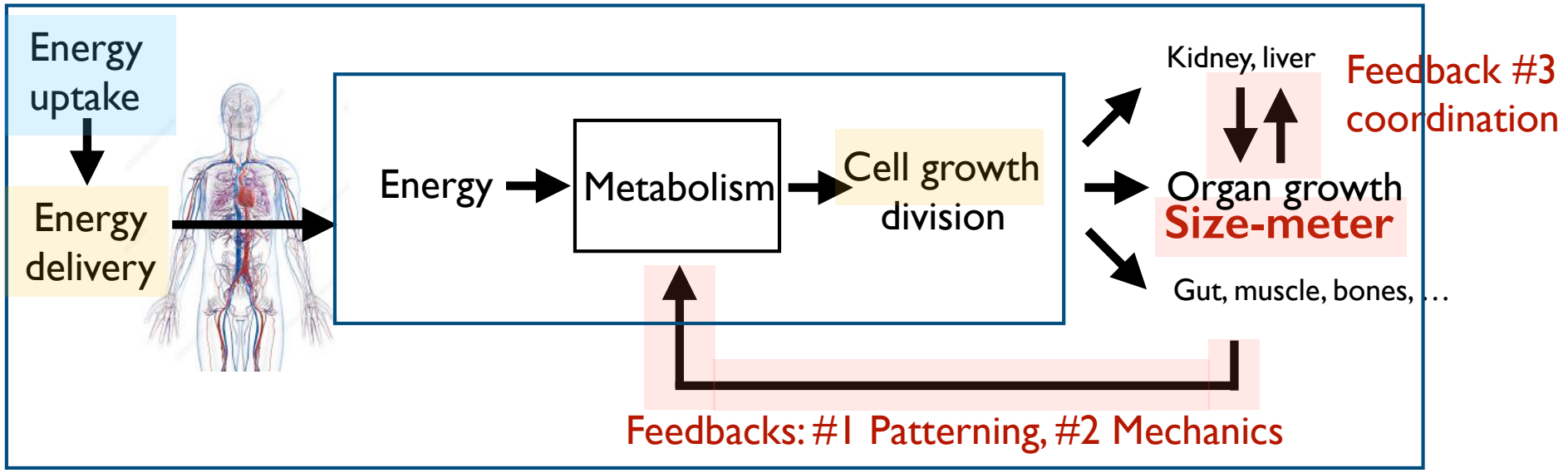




C. Champy (1924)

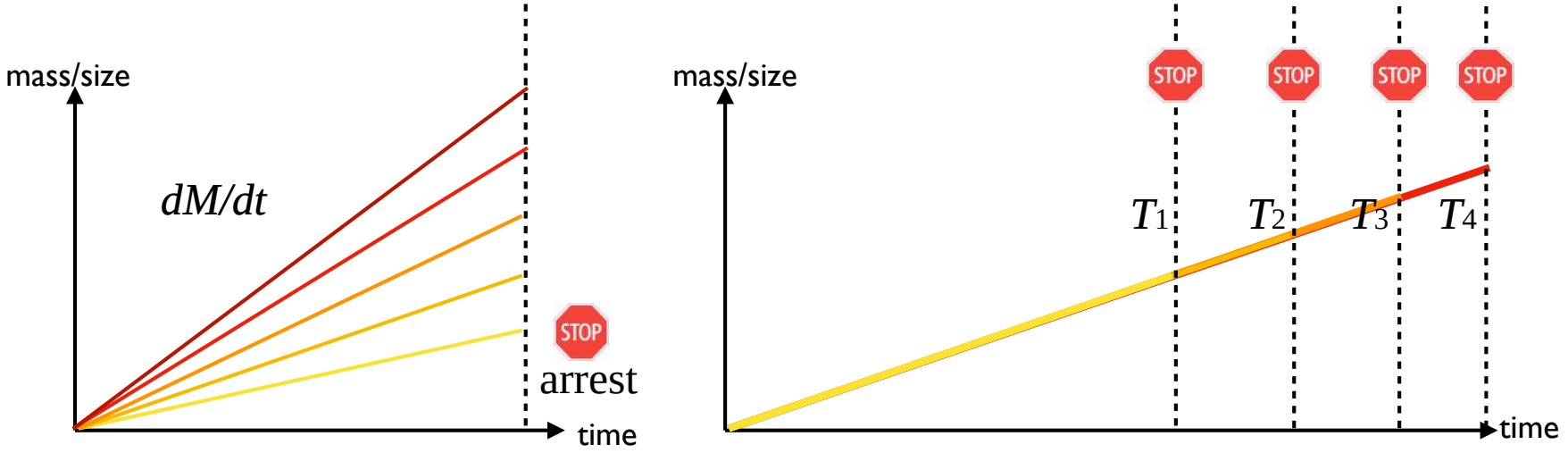
fiddler crabs

- Motor, Constraints and Regulation of Growth



• Growth Rate, Duration/Arrest

— Organ and organism size may be regulated by:
Growth duration and/or growth rate.
— Need to regulate growth arrest (determinate growth).

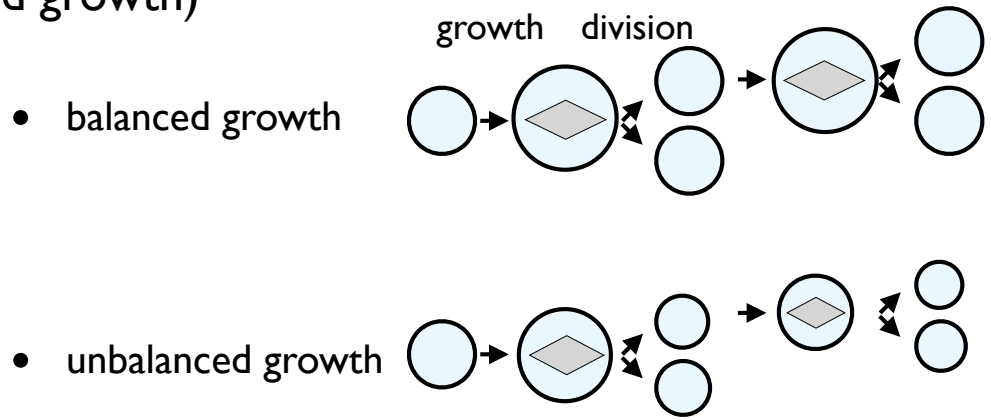


— Question: What is measured? Intrinsic « ruler » of growth
Cell intrinsic process, organ specific, organism specific?
Interactions/coordination at each scale (ie. between cells) and across scales (feedbacks?)

- In search of Sensor(s) and Coordinator(s)

• Cellular versus Organ scale models of growth arrest

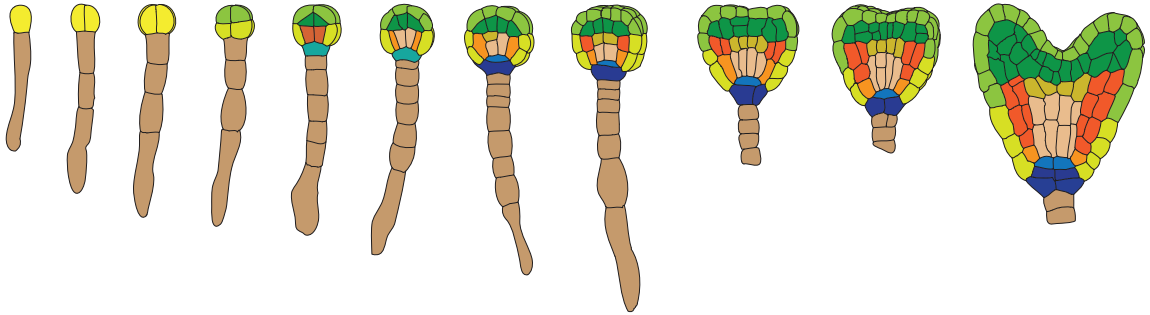
— In plants and animals tissue growth is driven by both cell division (increase in cell number) and cell growth (increase in cell size).
—The two processes are coordinated (balanced growth) but can be independently regulated (unbalanced growth)



— Question: What controls growth arrest?
Hypothesis: Cell-scale model: counting cell size, cell number, cell divisions etc.

If so the pattern of cell division should affect organ shape and size.

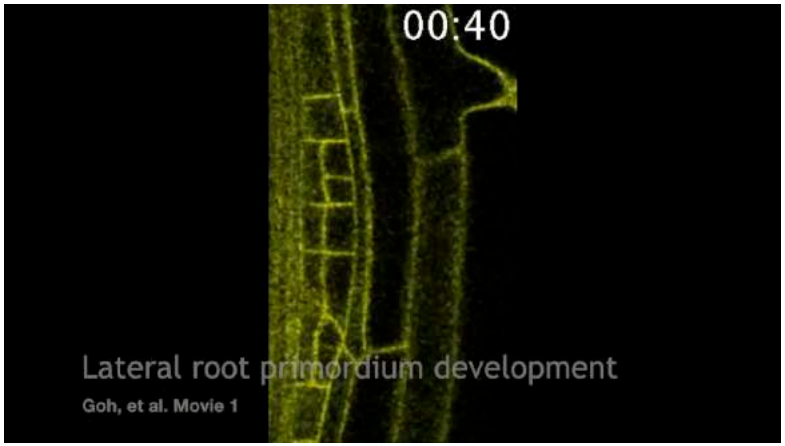
• Coordination of Cell Division and Cell Growth in plants



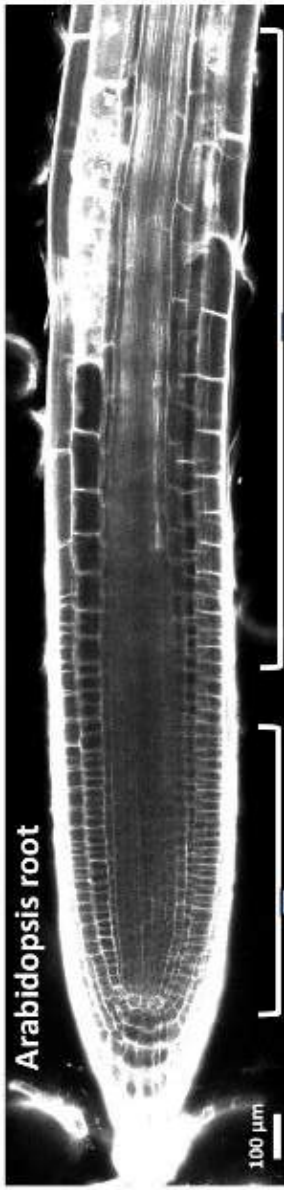
Arabidopsis embryogenesis

Galetti R., Verger S., Hamant O.& Ingram GC. *Development.* 143:3249. 2016

Arabidopsis lateral root growth



Goh T. et al, and Guyomarc'h S. *Development.* 143:3363. 2016



Cell Growth

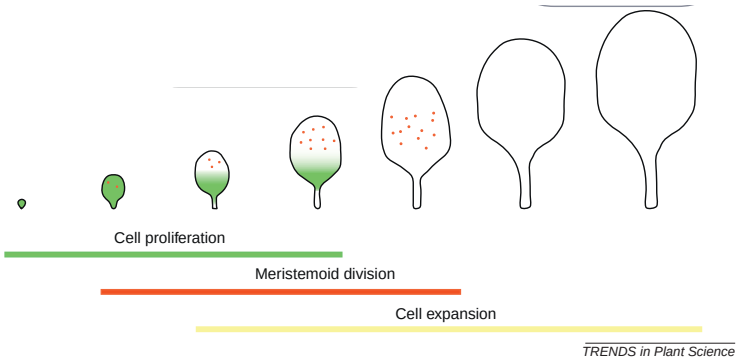
Cell Division

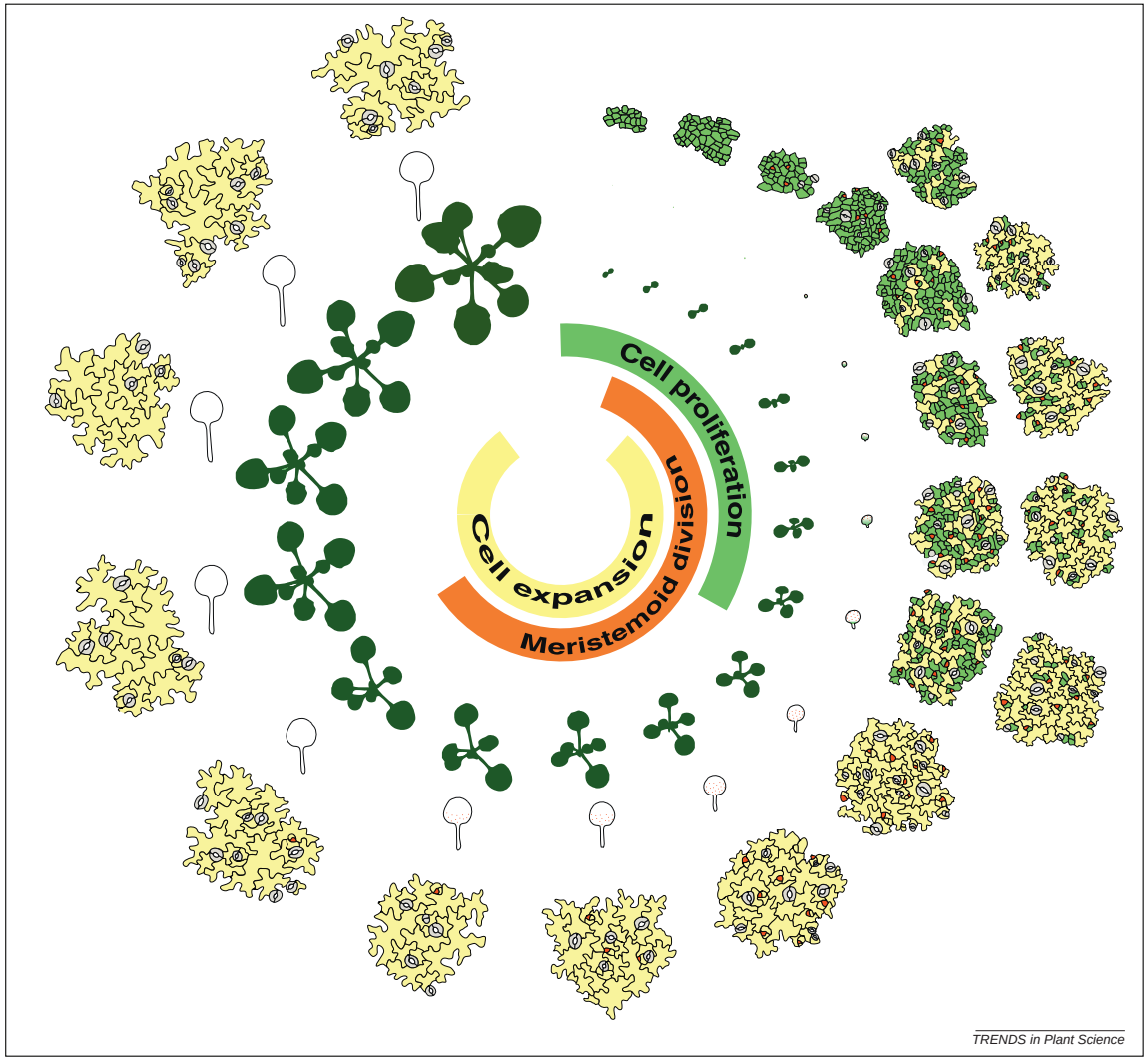
Primary root growth

Takatani et al *Scientific Reports* (2017). DOI: 10.1038/s41598-017-08453-5

• Coordination of Cell Division and Cell Growth in plants

- Transition from mitotic cycles (proliferation) to cell growth (expansion) is developmentally controlled





N. Gonzalez et al. and D. Inze *Trends in Plant Science* 17:332

• Growth compensation in plants

— Growth can occur without cell division in plants: (indeterminate growth)

- γ-ray induced block of cell division affects overall growth but does not block growth completely (« gamma-plantelets »)
- Dry mass synthesis (proteins) so growth cannot be solely attributed to water flux
- Cells become larger: 260 vs 28µm in mesophyll
- Differential growth of roots and shoots

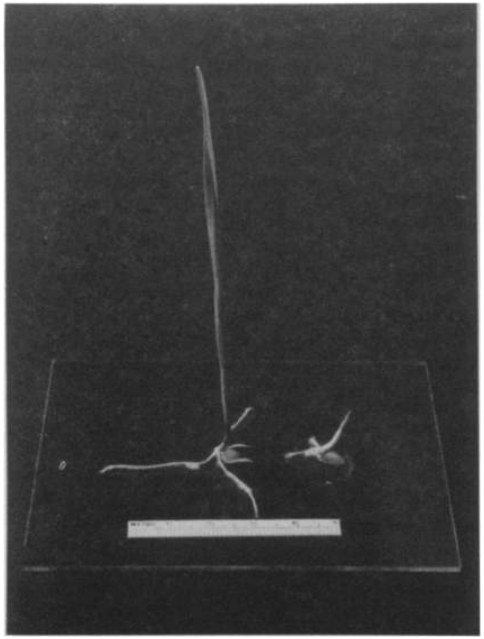


Fig. 1. Nine-day-old wheat grown from irradiated grain. Left: 9-day-old unirradiated control seedling. Right: 9-day-old seedling growing without cell division from irradiated (800 kr) grain.

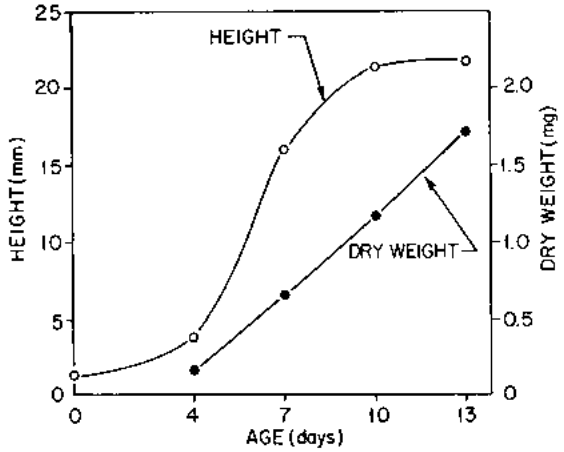


Fig. 2. Length and dry weights of first foliage leaf of gamma-plantlets. Length and dry weight measurements were taken from 4 independent groups of 38 plants for each day (4, 7, 10, 13). The tallest 7 leaves (minus coleoptiles) were measured and then dried for weight determinations.

TABLE 1. *Effects of metabolic and growth inhibitors on the germination of gamma-plantlets*

Treatment	Germination	Length of first leaf (12 days)
Water controls	87%	10.8 mm
10^{-3} M 2,4-dinitrophenol	0%	
10^{-2} M sodium azide	0%	
0.2 M nicotine	0%	

OxPhos inhibitor
Cytochrome oxydase inhibitor in Gram- bact

TABLE 2. *Protein content and dry weight of gamma-plantlets*

Material analyzed		Protein content (µg/plant)	Dry weight (mg/plant)
Unsown embryo	minus scutellum	107	0.50
	with scutellum	330	1.23
9-day-old gamma-plantlets	minus scutellum	489	5.60
	with scutellum	980	7.23
8-day-old unirradiated seedlings	minus scutellum	480	5.33
	with scutellum	708	6.39

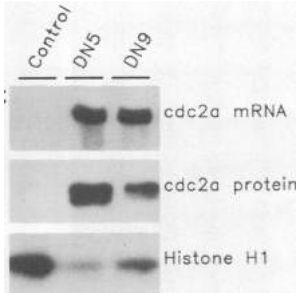
Haber AH., Carrier W. and Foard D, 1961. Metabolic studies of g-irradiated wheat growing without cell division. *Am J Bot* 48: 431-438

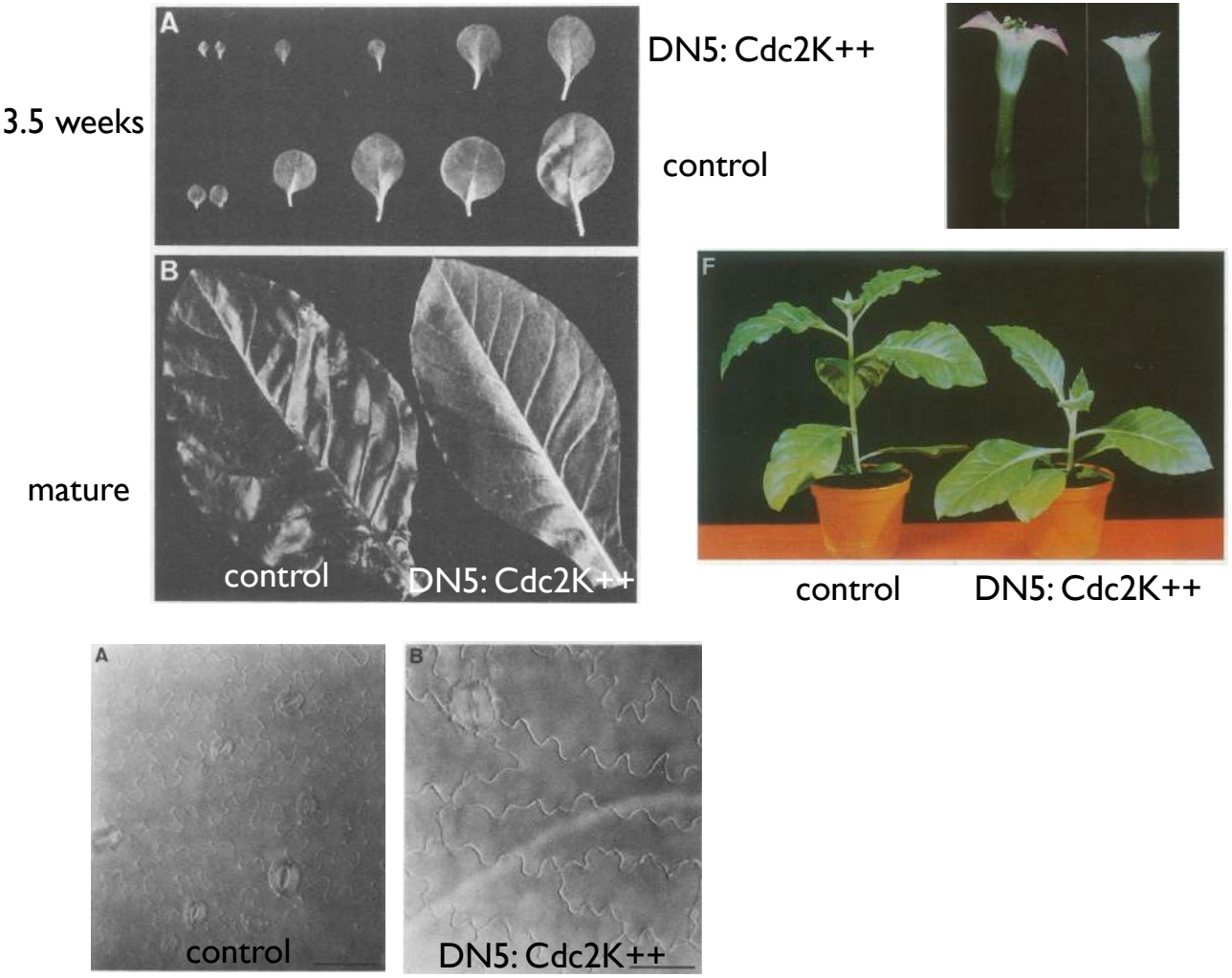
Foard D, Haber AH. 1961. Anatomic studies of g-irradiated wheat growing without cell division. *Am J Bot* 48: 438– 446.

• Growth compensation in plants

— Growth can occur without cell division in plants:
(lateral organs with determinate growth)

- Overexpression of dominant negative mutant of Cdc2/CDK1 Kinase reduces cell cycle in *Tobacco*
- Plant morphology is preserved (though overall growth is slightly reduced)
- Cells are larger than in controls





A. Hemerly et al . *EMBOJ.* 14:3925-3936 (1995)

• Growth compensation in plants

— Growth can occur without cell division in plants: (lateral organs with determinate growth)

- Transition from mitotic cycles to endocycle is developmentally controlled and regulated by the Cdk1/Cdc2 inhibitor KRP2
- This reduces cell number and correlatively increases cell size about 4X.

(via endoreplication: see Course #1, 12 Nov)

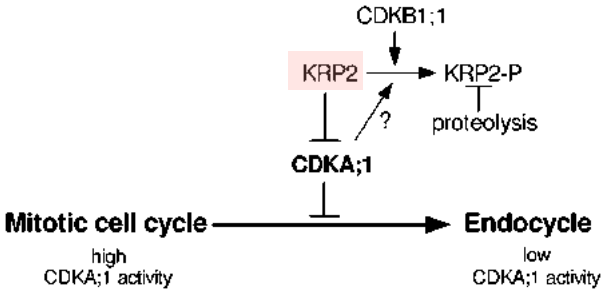
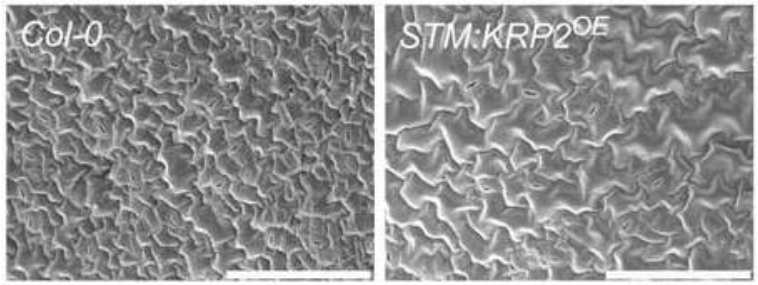
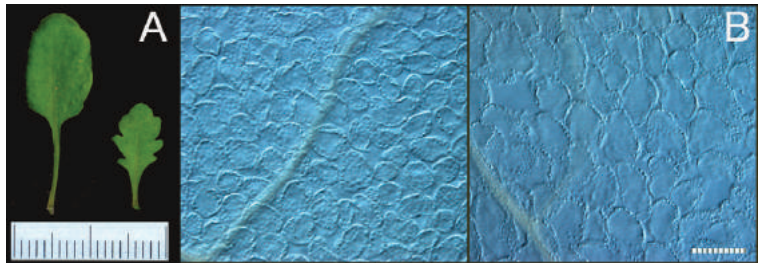


Table 1. Abaxial Epidermis Cell Size and Cell Number in Leaves of Wild-Type and *KRP2*^{OE} Plants

Line	Leaf Size (mm^2)	Abaxial Epidermal Cells	
		Estimated Number	Size (μm^2)
Col-0	15.03 ± 1.02	13,532 ± 875	1,160 ± 33
CaMV 35S:KRP2 S1	7.68 ± 0.37	1,895 ± 205	4,330 ± 494
CaMV 35S:KRP2 S2	8.98 ± 0.23	1,770 ± 93	5,250 ± 358





doi:10.1371/journal.pbio.0060174.g001

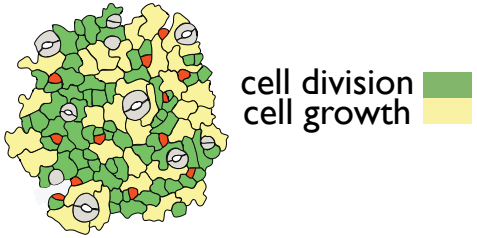
A. Verkest et al . *The Plant Cell* (2005); 17: 1723-1736

• Growth compensation in plants

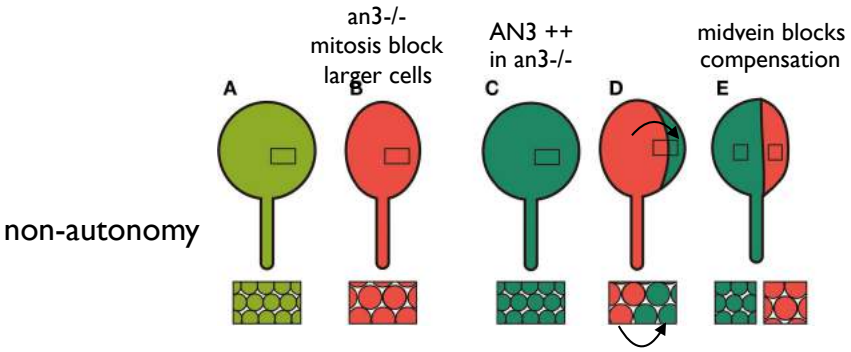
- *Unidirectional* compensation:

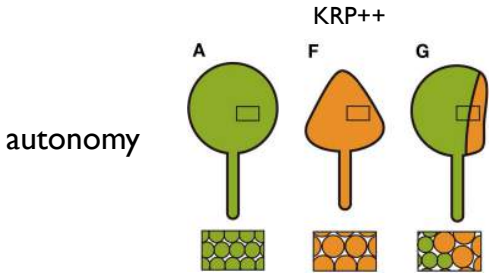
Block of cell division increases compensatory cell growth but, in general, induced growth does not affect cell division

- Existence of a threshold of cell division inhibition to induce compensatory growth: cell division sensing?
- Cell autonomy and non-autonomy of compensatory mechanisms:



ANGUSTIFOLIA3/GIF1 (*AN3/ GIF1*)





- Mechanisms of compensation remain largely unknown.

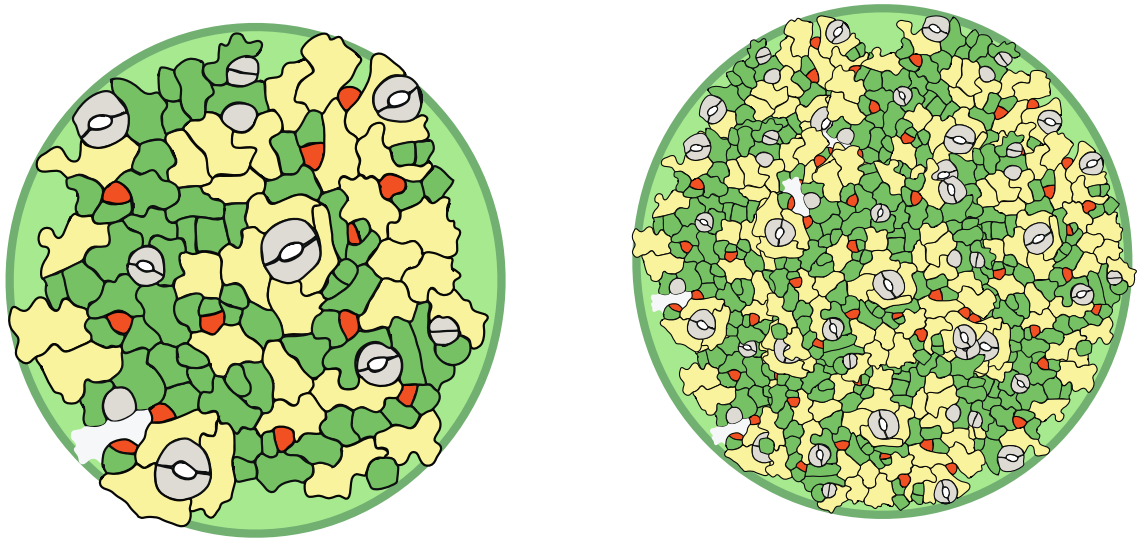
—induced endocycling and polyploidy in some instances but not systematically

G. Horiguchi and H.Tsukaya *Frontiers in Plant Sci.* 2011. doi: 10.3389/fpls.2011.00024

• Determinants of tissue size in *Plants*

— Neither cell number nor cell size per se determine organ size

— Non-autonomous compensation reveals organ (or organism)-scale regulation of tissue size

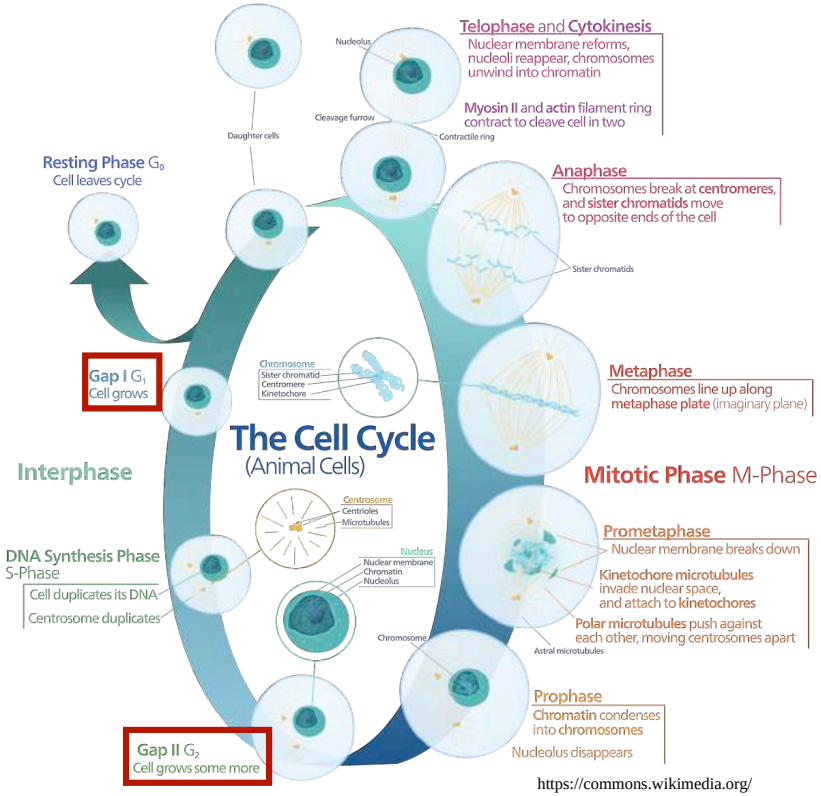


G. Horiguchi and H.Tsukaya *Frontiers in Plant Sci.* 2011. doi: 10.3389/fpls.2011.00024

• Cell division and cell growth in animals

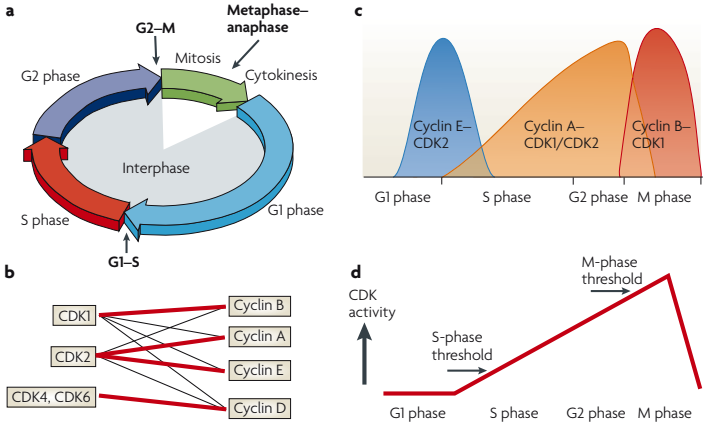
- In general animal cells divide (mitosis) after doubling their volume during the G1, S and G2 phases.
- This is also true in Yeast *S. cerevisiae*.
- As a result cells keep often a nearly constant volume over many cell generations.
- If so, tissue growth is dependent on cell division, and counting cell number could be a proxy for « measuring » tissue size.

- How is cell growth coordinated with cell division?
- How to affect cell size?
  - — DNA content
  - — Cell cycle regulators



https://commons.wikimedia.org/

- Work in Yeast revealed the dominance of cell growth over cell division: proliferation has no impact on cell growth, but the converse is not true.



H. Hochegger; S. Takeda, and T. Hunt (2008) *Nature Reviews MCB* 9:910-916.

• Growth compensation revealed by polyploidy

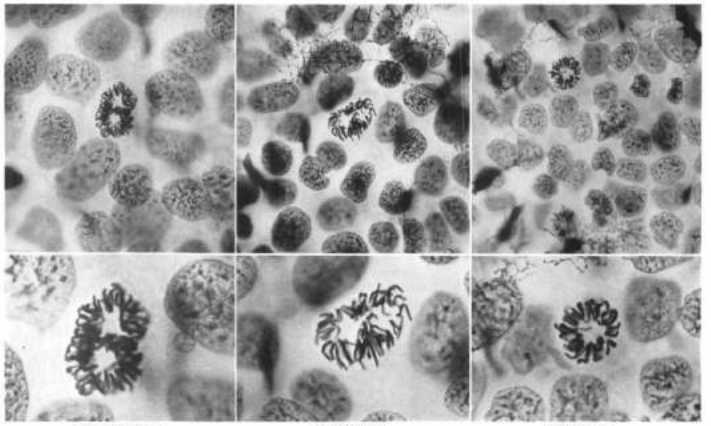
Gerhard Fankhauser (1901–1981)

- Polyploid cells are larger (see course #1 12 Nov)

POLYPLOIDY IN THE SALAMANDER, *EURYCEA BISLINEATA*

GERHARD FANKHAUSER
Department of Biology, Princeton University

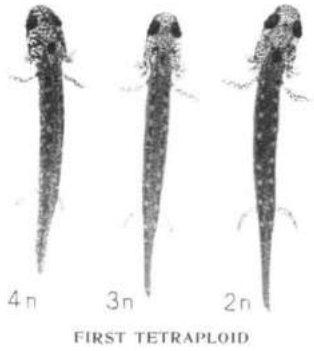
Fankhauser G. 1939. *J Hered* 30: 379–388.



4n 3n 2n

- Polyploid larvae have normal morphology and quasi normal size

5. As in triploid larvae of the newt, *Triturus viridescens*, gigantism of polyploid Eurycea larvae is prevented by a reduction in cell number which compensates the increase in size of the individual cells.



4n 3n 2n

• Growth compensation revealed by polyploidy

MAINTENANCE OF NORMAL STRUCTURE IN HETEROPLOID SALAMANDER LARVAE, THROUGH COMPENSATION OF CHANGES IN CELL SIZE BY ADJUSTMENT OF CELL NUMBER AND CELL SHAPE

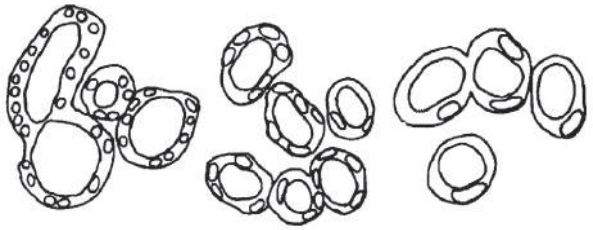
G. FANKHAUSER
Department of Biology, Princeton University, New Jersey

- In spite of large differences in cell size due to polyploidy, cells adapt their morphology to produce normally shaped organ.
- The diameter and thicknesss of epithelial tubes and layers are preserved.

Notophthalmus viridescens

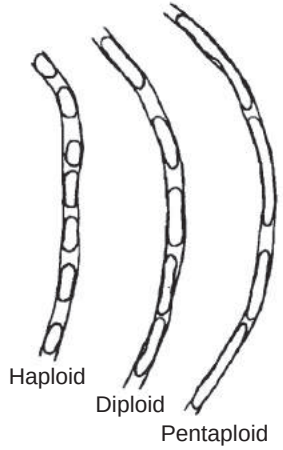
Pentaploid Diploid

Notophthalmus viridescens pronephric tubules



Haploid Diploid Pentaploid

Notophthalmus viridescens lens epithelium



Haploid
Diploid
Pentaploid

Fankhauser G. 1945. *J Exp Zool* 100: 445–455.

see also Review: D. Levy and R. Heald. *Cold Spring Harb Perspect Biol* 2016;8:a019166

• Growth compensation in *Drosophila*

—Comparison of tissue (wing) and cell size in *Drosophila*

- The wing blade area is not proportional to cell surface area
- If cell number and cell area change in proportion then the slope of log transformed data should be greater than 1
- If they varied independently the slope should be closed to 1
- *If there was a complete compensation then the slope would be 0 and partial compensation would have slopes between 0 and 1.*

  — evidence of partial compensation

- Selection for large and small wings gives rise to increased compensation

—selection for large and small wings

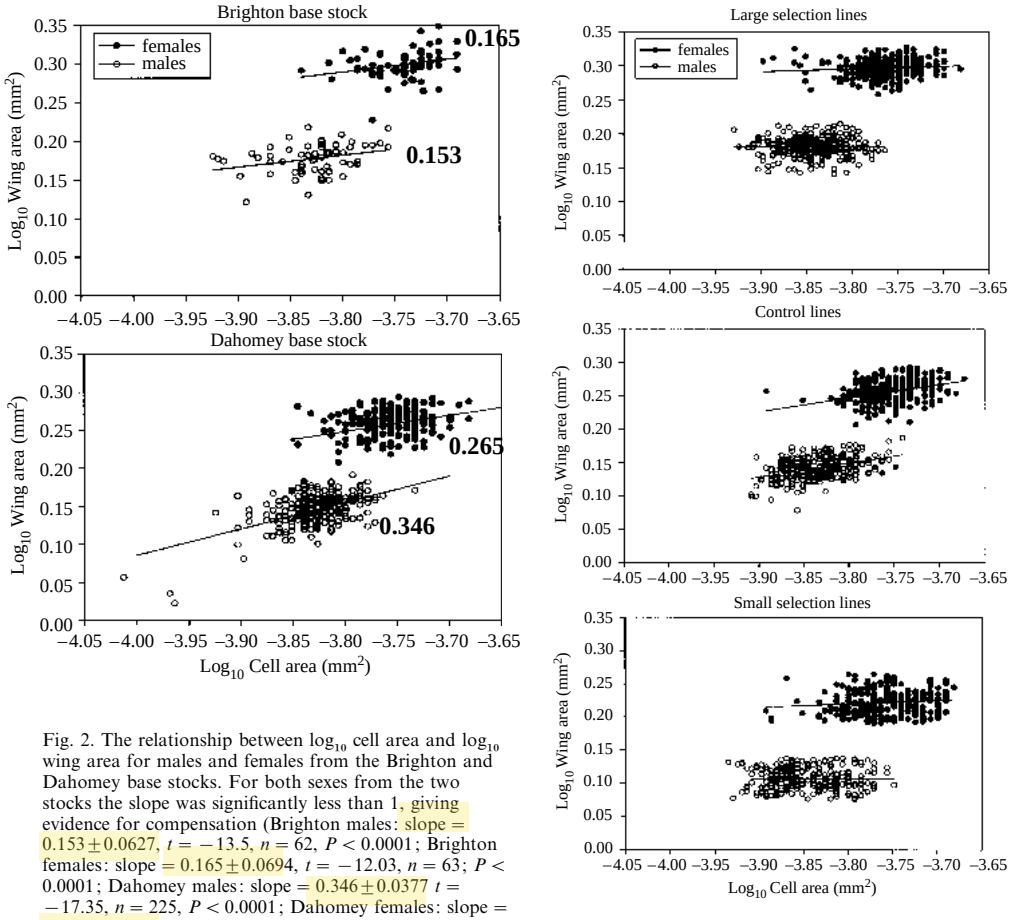
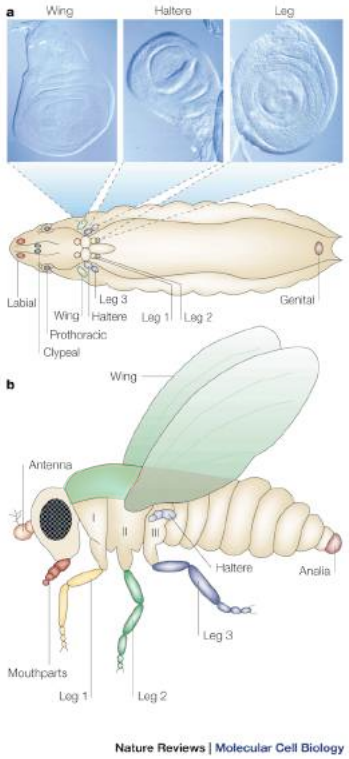


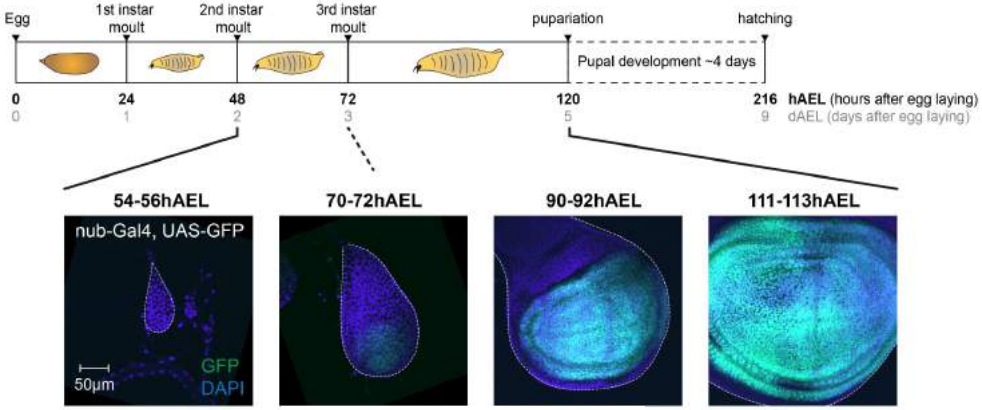
Fig. 2. The relationship between $\log_{10}$ cell area and $\log_{10}$ wing area for males and females from the Brighton and Dahomey base stocks. For both sexes from the two stocks the slope was significantly less than 1, giving evidence for compensation (Brighton males: slope = 0.153 ± 0.0627, $t = -13.5$, $n = 62$, $P < 0.0001$; Brighton females: slope = 0.165 ± 0.0694, $t = -12.03$, $n = 63$; $P < 0.0001$; Dahomey males: slope = 0.346 ± 0.0377 $t = -17.35$, $n = 225$, $P < 0.0001$; Dahomey females: slope = 0.265 ± 0.039, $t = -18.84$, $n = 225$, $P < 0.0001$). The

McCabe J, French V, Partridge L (1997). *Genet Res* 69: 61–68.

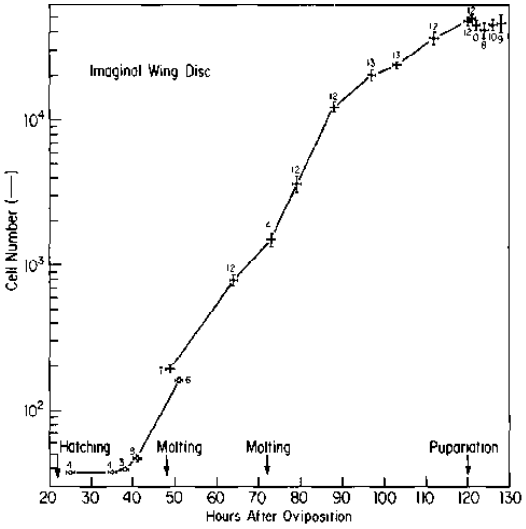
• Growth compensation revealed by cell division regulators

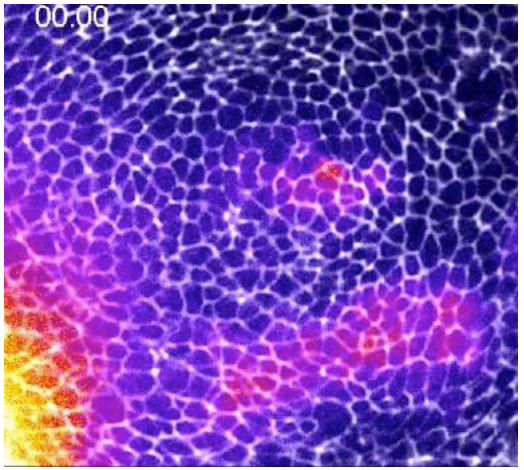
- Larval imaginal discs grow exponentially from 50 to 50.000 cells in 4 days





Stefan Harmansa (IBDM, Marseille)





Stefan Harmansa (IBDM, Marseille)

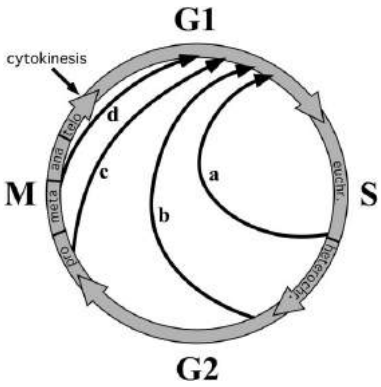
MC. Diaz de la Loza and B. Thompson. *Mechanisms of Development* 144 (2017) 23–32

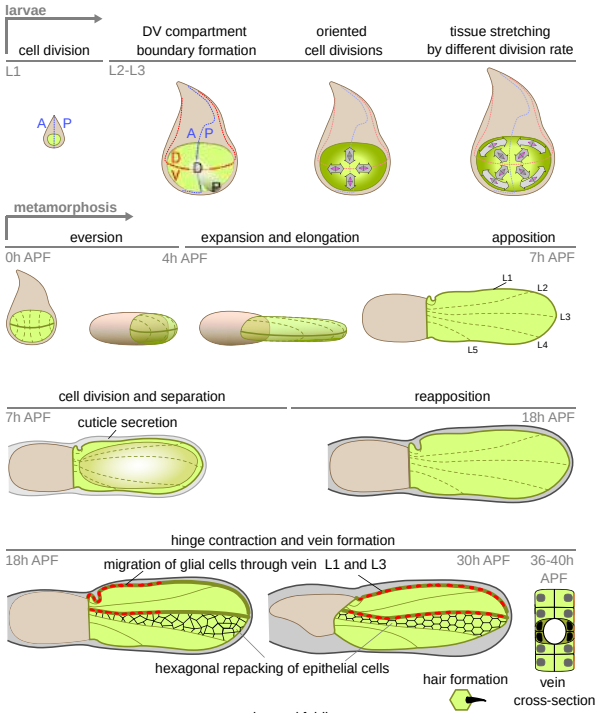
Bryant PJ, Levinson P: Intrinsic growth control in the imaginal primordia of *Drosophila*, and the autonomous action of a lethal mutation causing overgrowth. *Dev Biol* 1985, 107:355-363.

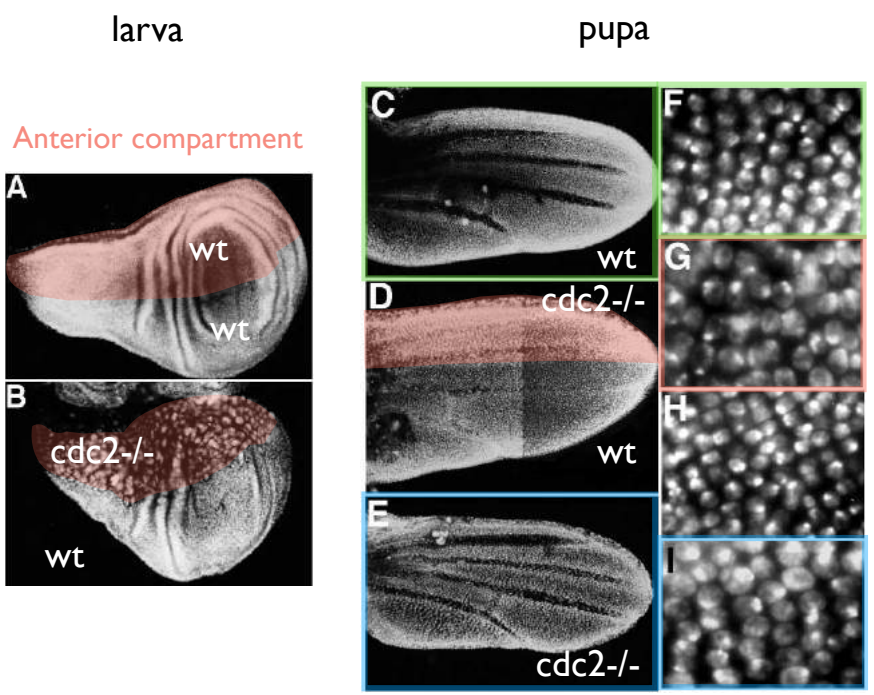
• Growth compensation revealed by cell division regulators

- in Cdc2/CDK1 mutants, cells go through S phase but do not enter into mitosis and instead, endoreplicate and grow larger than controls

- The larval and pupal wing tissues acquire quasi normal dimensions when cells are much larger.
- This rules out cell number and cell size as a sensing mechanism: rather this suggests a global (organ scale) sensing mechanism that measures tissue dimensions (area, volume?)
- Sustained growth is due to endoreplication: reveals impact of DNA content but not of cell growth per se.



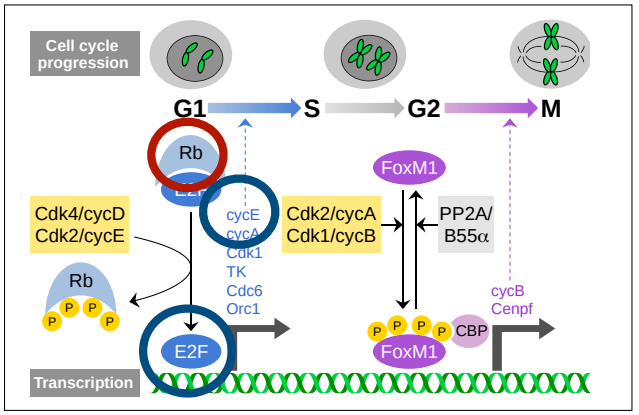




• Growth compensation revealed by cell division regulators

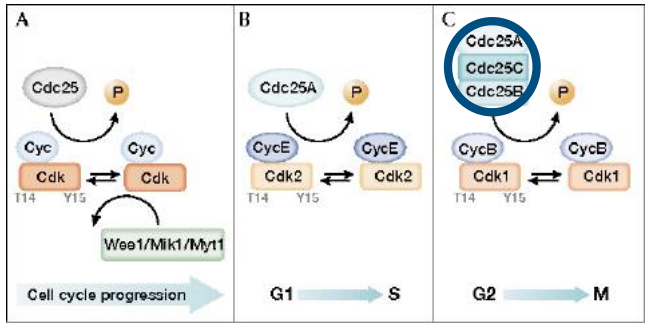
- Impact of cell proliferation on cell growth: E2F, pRB

- E2F promotes G1/S transition by inducing S phase Cyclin and other S phase genes (eg. thymidine kinase, origin of replication proteins etc).
- The retinoblastoma protein Rb inhibits E2F.

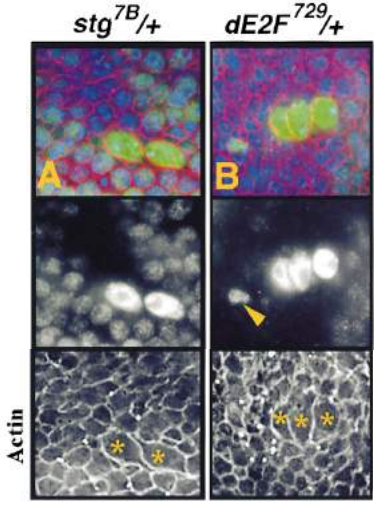


S. Lim and P. Kaldis. *Development* 140, 3079-3093 (2013) doi:10.1242/dev.091744

- E2F induces also G2/M transition via String/Cdc25 expression



- Stg/Cdc25 and E2F mutant cells do not divide and are larger.

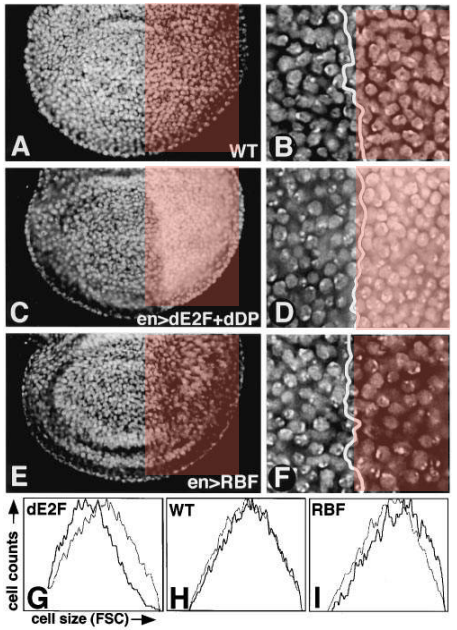


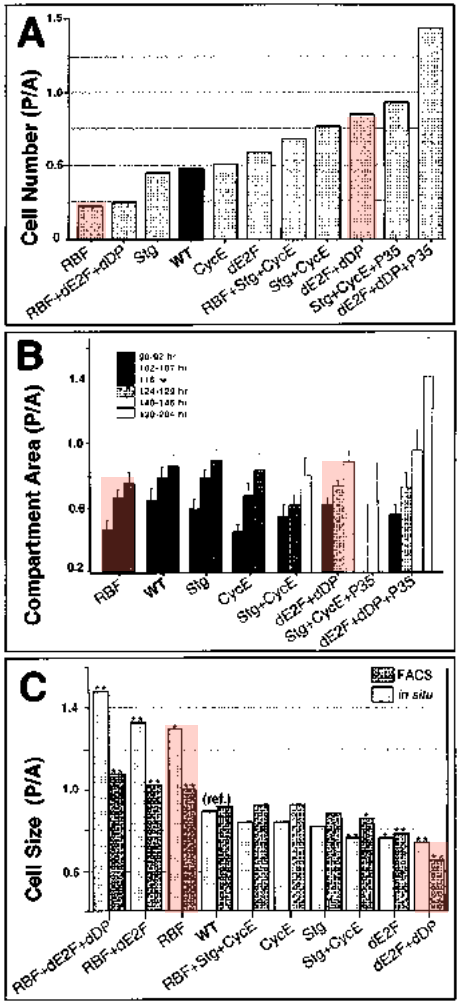
Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA (1998) Coordination of growth and cell division in the Drosophila wing. *Cell* 93: 1183–1193.

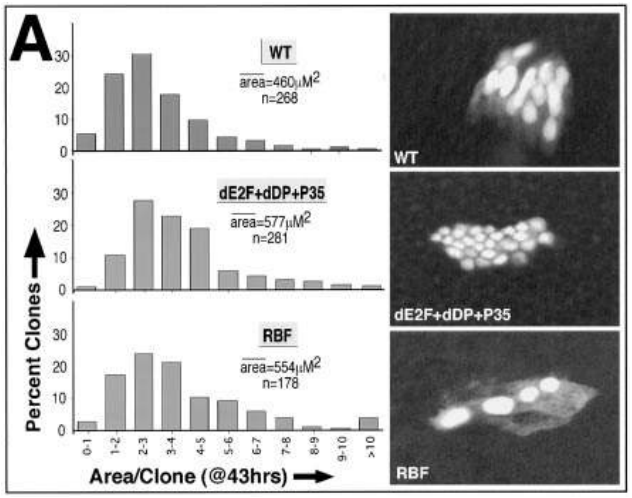
• Growth compensation revealed by cell division regulators

- A gradual increase in cell division rate causes a gradual decrease in cell size: E2F and DP overexpression.
- A decrease in cell division rate causes cell enlargement, but such overgrowth cannot be sustained (without endoreplication): RB over expression

- Conclusions:
  —There is a dominance of cell growth over cell division (cell division does not affect cell growth)
  —Growth is monitored/measured at the tissue scale (wing imaginal disc), but not at the cellular scale.



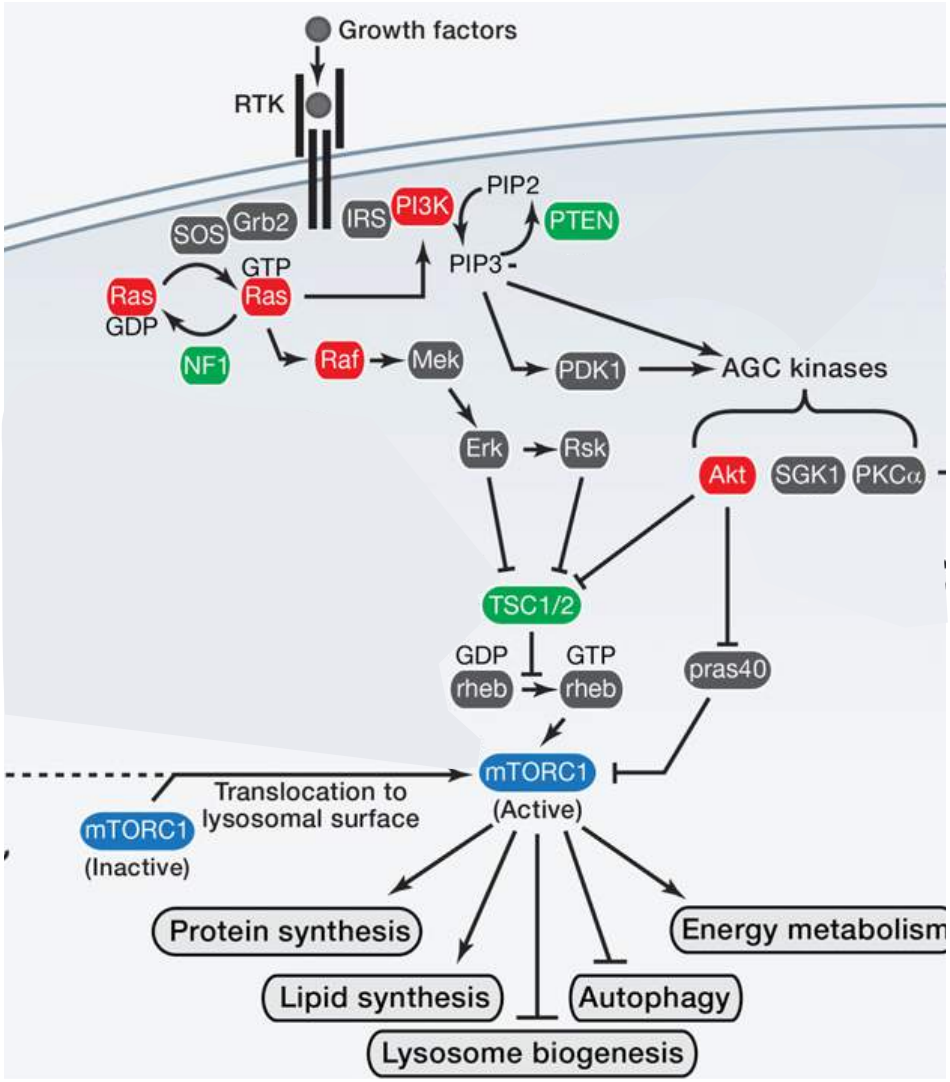




Neufeld TP, de la Cruz AF, Johnston LA, Edgar BA (1998) Coordination of growth and cell division in the Drosophila wing. *Cell* 93: 1183–1193.

• Intrinsic regulators of cell and tissue growth

- Ras, Pi3K and TOR signalling control cellular anabolism and growth



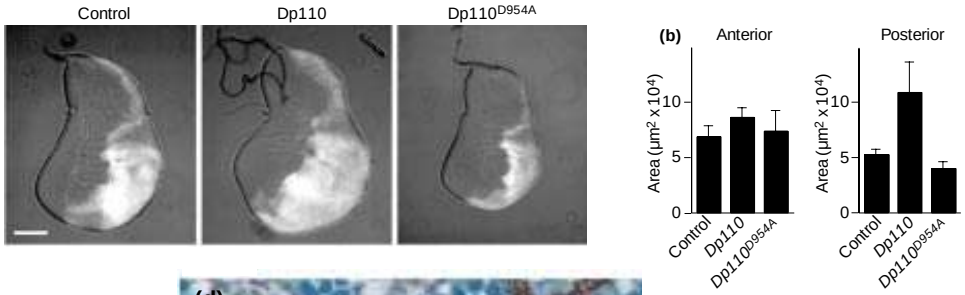
Laplante and Sabatini D. (2012) *Cell* 149:274

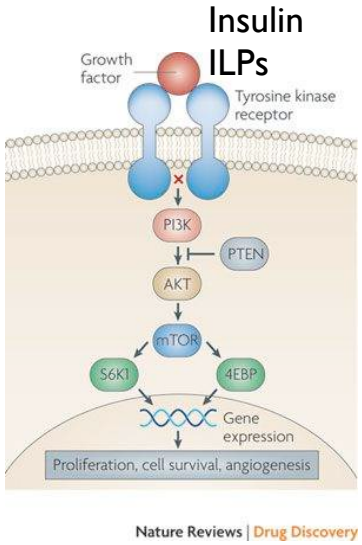
• Intrinsic regulators of cell and tissue growth

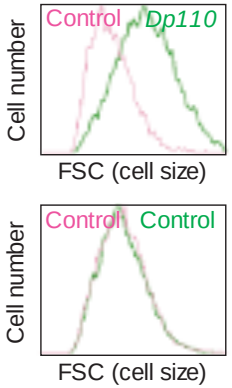
- PI3Kinase controls cell and tissue growth autonomously

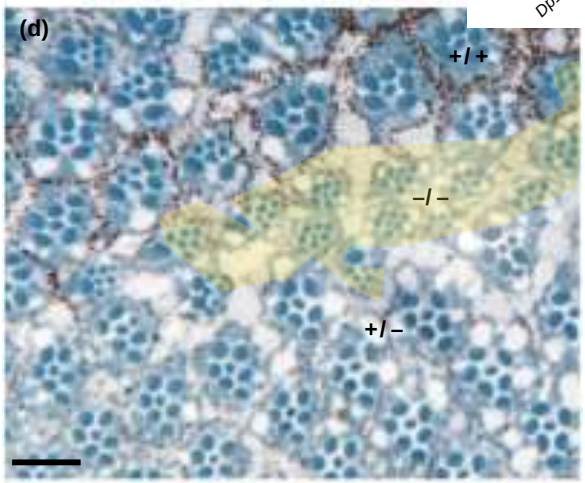
— Overexpression of p110 increases wing size
— Overexpression of a mutant (dominant negative form reduces wing size)
— The same effect is seen in tissue compartments
— Cell autonomous control of growth: unbalanced growth (ie. growth without cell division)

—Consistent with dominance of growth on cell division in Animals as in Yeast.







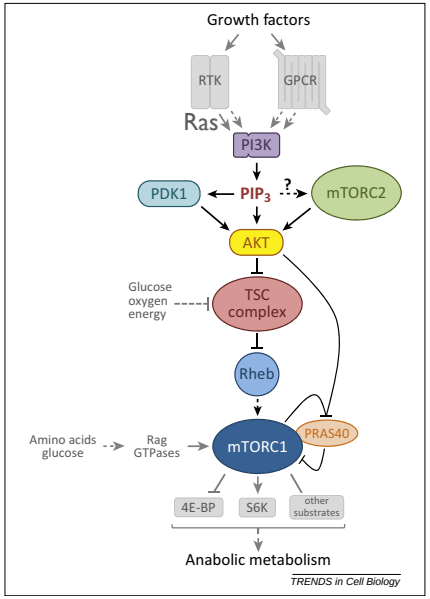


Leevers SJ, Weinkove D, MacDougall LK, Hafen E, Waterfield MD: *EMBO J* 1996, 15:6584-6594.

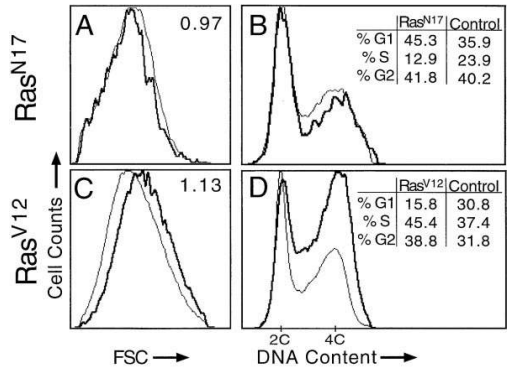
Weinkove, D., Neufeld, T.P., Twardzik, T., Waterfield, M.D., and Leevers, S.J. (1999). *Curr. Biol. 9*, 1019–1029.

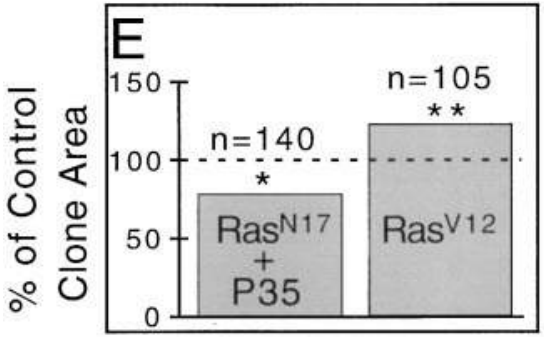
• Intrinsic regulators of cell and tissue growth

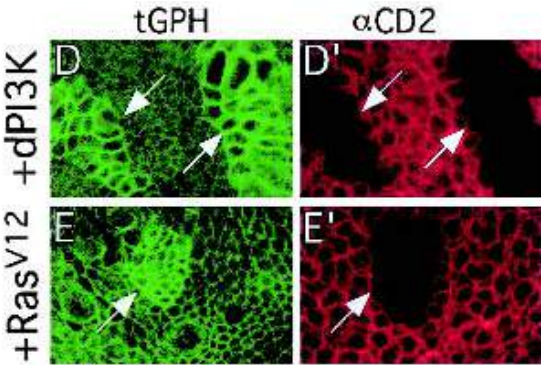
- Ras signalling promotes cell division and cell growth as well as clone size

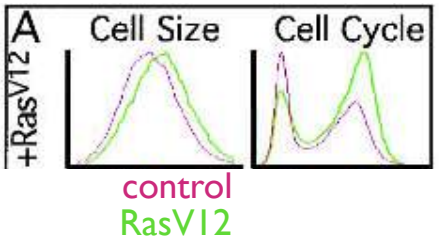


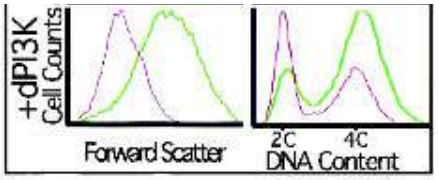
- A constitutively active form of Ras (RasV12) activates Pi3K signalling (Pi3P)
- Ras signals via Pi3K, linking tissue signalling (Receptor Tyrosine Kinase) to cell and tissue growth.











Prober, D.A., and Edgar, B.A. (2000). *Cell* 100, 435–446.
Prober, D.A., and Edgar, B.A. (2002). *Genes Dev. 16*, 2286–2299.

• Determinants of tissue size in *Animals*

— Cell autonomous growth compensation: cells that do not divide grow larger due to the **dominance of growth on cell division**

— Cell intrinsic growth requires the Ras, Pi3K and TOR pathway signalling

— Neither cell number nor cell size per se determine organ size

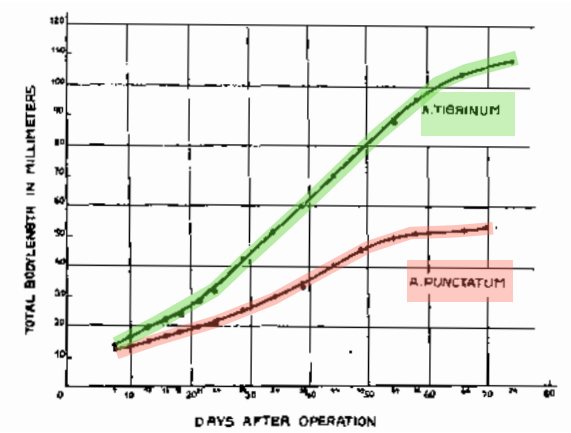
— Growth is measured as a tissue/organ scale quantity (volume, surface etc).

• Organ-specific size measurement

THE GROWTH OF EYES AND LIMBS TRANSPLANTED HETEROPLASTICALLY BETWEEN TWO SPECIES OF AMBLYSTOMA

VICTOR C. TWITTY AND JOSEPH L. SCHWIND
Osborn Zoölogical Laboratory

1931

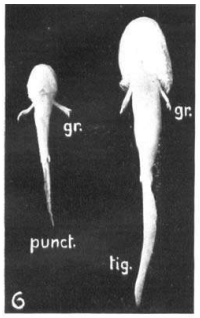


Different growth rates

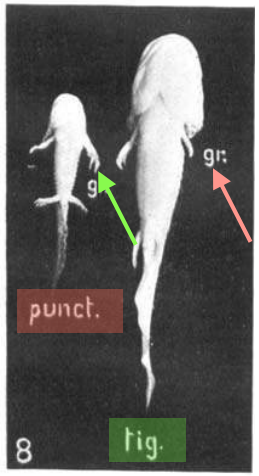
Ambystoma tigrinum

Ambystoma punctatum

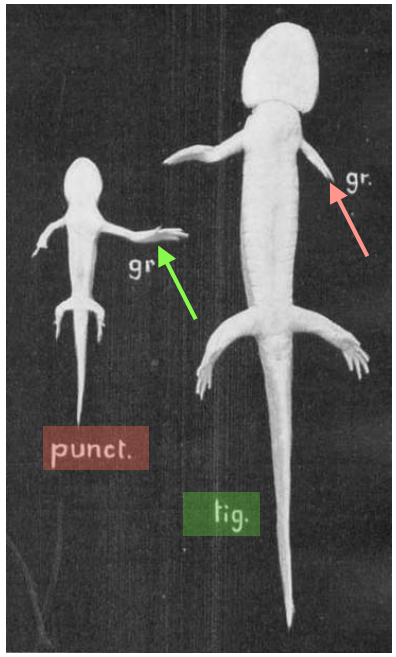
- Heteroplastic graft experiments between two salamander species with different growth rates
- Limbs grow according to graft growth rate.



+21 days
(after graft)



+40 days

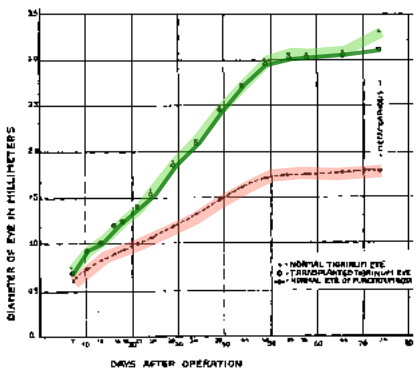


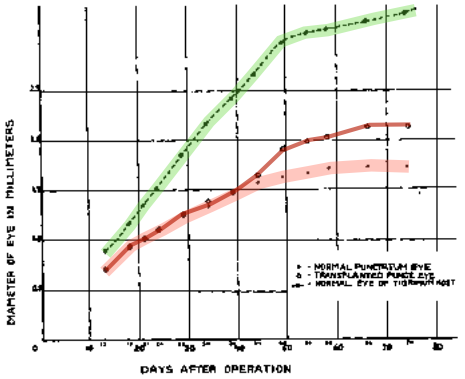
after metamorphosis

V. Twitty and J. Schwind. 1931. *J. Exp. Zool.* 59:61-86. https://doi.org/10.1002/jez.1400590105

• Organ-specific size measurement

—Tissue and cell intrinsic properties

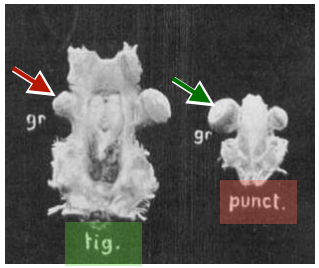




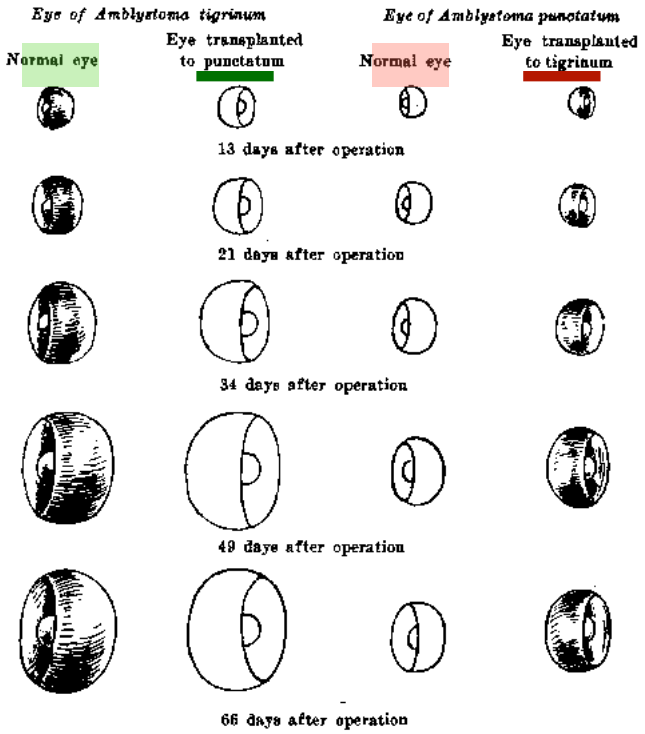
- Heteroplastic graft experiments between two salamander species with different growth rates.
- Eyes grow according to graft growth rate.

- The observations reveal a level of control over organ size which is independent of total organism energy supply:
  —*tigrinum* feeds more than *punctatum* yet in equivalent energy input (feeding) and delivery (WBE model) its limbs scale autonomously and in a species specific manner.

Asymptotic organism mass: $M = (a/b)^4 = (B_0 m_c / B_c)^4$



- Growth arrest signals are not simply monitoring tissue/organ size independent of local cellular/tissue properties (eg. cell metabolic power B_c?, organ « sizemeter »?)
- In other words the arrest signal must originate from local organ size measurement and is dependent on cellular intrinsic (species specific) properties.

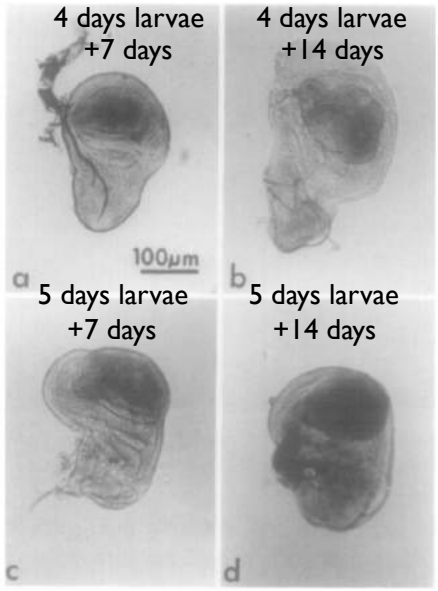


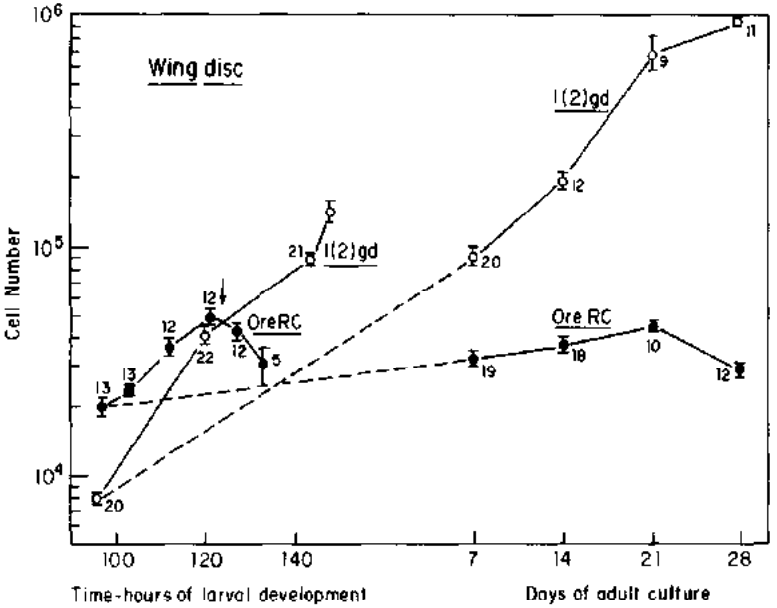
V. Twitty and J. Schwind. 1931. *J. Exp. Zool.* 59:61-86. https://doi.org/10.1002/jez.1400590105

• Organ-specific size measurement

— Organ intrinsic control mechanisms of tissue growth

- Imaginal discs from late larvae transplanted in the growth permissive environment of adult females grow to their final size.
- A stop signal is intrinsic to the tissue and does not depend on the environment.
- in these conditions, *l(2)gl* mutants do not stop.





Garcia-Bellido A: *J Insect Physiol* 1965, 11:1071-1078.

Bryant PJ, Levinson P: Intrinsic growth control in the imaginal primordia of *Drosophila*, and the autonomous action of a lethal mutation causing overgrowth. *Dev Biol* 1985, 107:355-363.

• Organ-specific size measurement

— Cell extrinsic « organic » properties: communication at scale of organism

- Manifestation of regulative growth by extrinsic/environmental factors in *heteroplastic grafts* *where growth stage of graft and host are different*

- The young graft accelerates growth with respect to host which does not grow in experimental conditions

(a phenomenon also called « catch-up growth », see Course #6)

- The old graft retards growth with respect to host
- So growth is regulative until correct proportions (size ratio) between organ of different species within the same chimera are reached

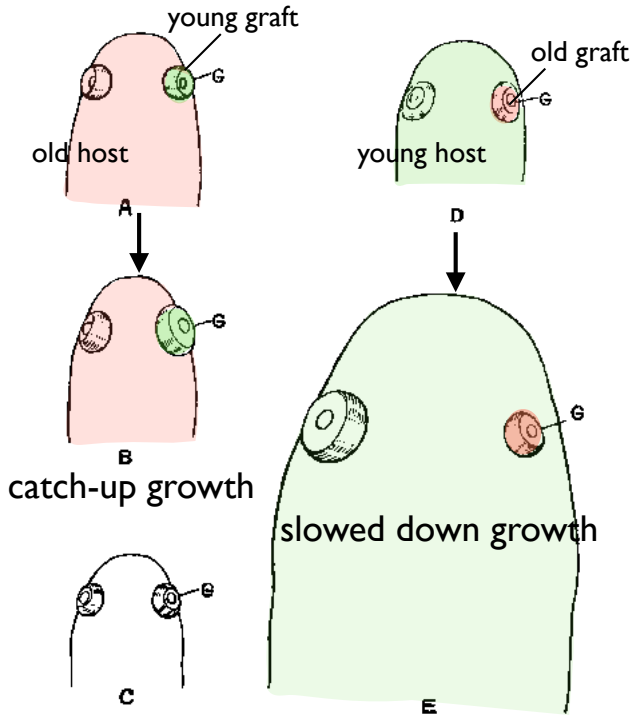
« **From the results of earlier experiments cited and those presented here, one is inclined to conclude that the potential size of the eye is largely determined by intrinsic factors, but that the expression or realization of this potentiality during the growth of the animal depends upon its interaction with a gradually changing organic environment.** »

- Importance of cellular/tissue environment

REGULATION IN THE GROWTH OF TRANSPLANTED EYES

VICTOR C. TWITTY
Osborn Zoölogical Laboratory, Yale University

1930

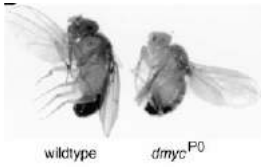


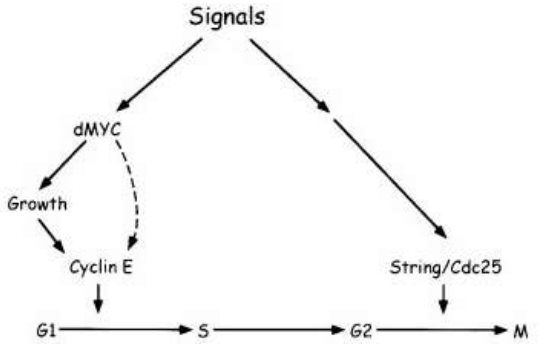
V. Twitty 1930. *J. Exp. Zool.* 55:43-52.

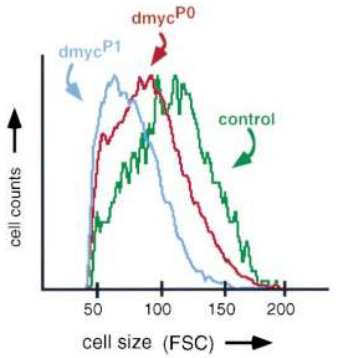
• Cell growth regulator indicates non-autonomous compensation

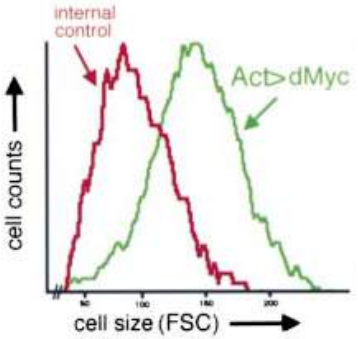
- Myc regulates cellular growth, and size of cellular clones
- Myc does does not induce cell division (independently regulated by Stg/Cdc25).

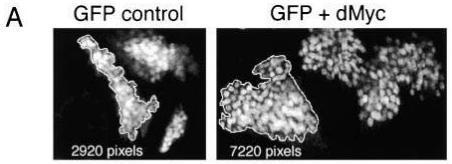
- Myc mutant animals are smaller (smaller cells)
- But over expression of Myc in clones does not produce larger animals… Yet cells are larger and divide normally.
- Points to another level of growth compensation which is non-autonomous (ie. depends on cellular environment) and operates within a tissue: cell competition

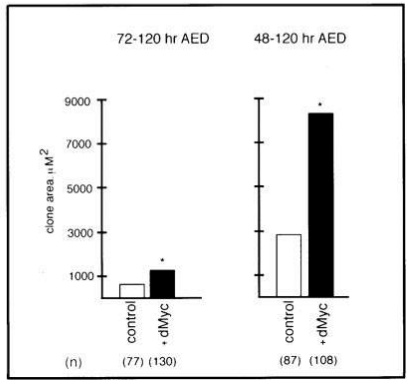








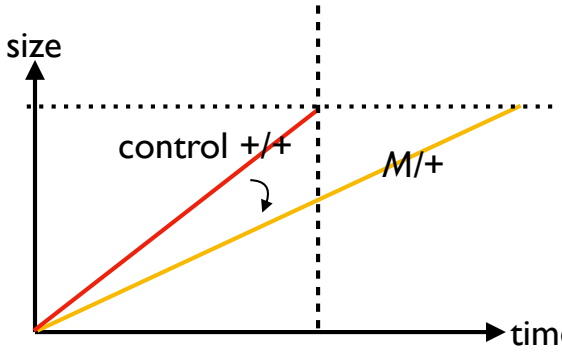




Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P (1999) *Cell* 98: 779–790.

• Non-autonomous growth compensation: cell competition

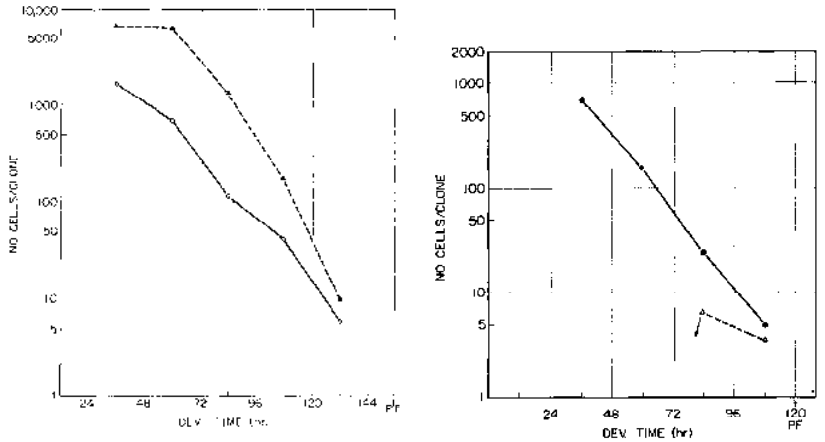
- *Minute* genes encode ribosomal proteins required for protein translation
- *Minute* mutations are recessive lethal mutants that slow down development dominantly
- *Minute* mutant cells divide more slowly
- Cells and adults have a normal size (and shape)
- This indicates that size is not controlled by a determination of time/duration but of size



Minutes: Mutants of Drosophila Autonomously Affecting Cell Division Rate

GINÉS MORATA[1] AND PEDRO RIPOLL
Instituto de Genética y Antropología, Centro de Investigaciones Biológicas, C.S.I.C., Velázquez, 144, Madrid 6, Spain

1975



- Clones of M+/M+ (wildtype) cells have a faster exponential growth that clones of M/+ mutant cells
- Clones of M/+ mutant cells have a slow growth rate and as such are outcompeted.
- *Minute* mutants with more severe growth defects are lost if induced early in development.

- The normal size of wings indicate regulation of growth at the organ scale
- Strong perturbation of growth patterns are corrected to produce normally sized limbs and animals

The normal shape and size of mosaic mesothoraces in which more than one-third of the cells derive from not more than ⅛ of the anlage (Ripoll, 1972) indicates the existence of appreciable regulation in the developing wing disc.

Apparently Minute cells, although viable, have a lower mitotic rate that lowers their ability to compete with normally growing cells.

Such cell competition would therefore explain the complete absence of clones arising in earlier stages.

The phenomenon of cell competition can also account for the smaller final number of Minute cells in mosaic wings of Minute individuals if it is imagined that they are overgrown by more rapidly dividing non-Minute cells.

Morata, G., and P. Ripoll. 1975. *Dev. Biol.* 42:211–221. http://dx.doi.org/10.1016/0012-1606(75)90330-9

Simpson, P. 1979. *Dev. Biol.* 69:182–193. http://dx.doi.org/10 .1016/0012-1606(79)90284-7

• Non-autonomous growth compensation: cell competition

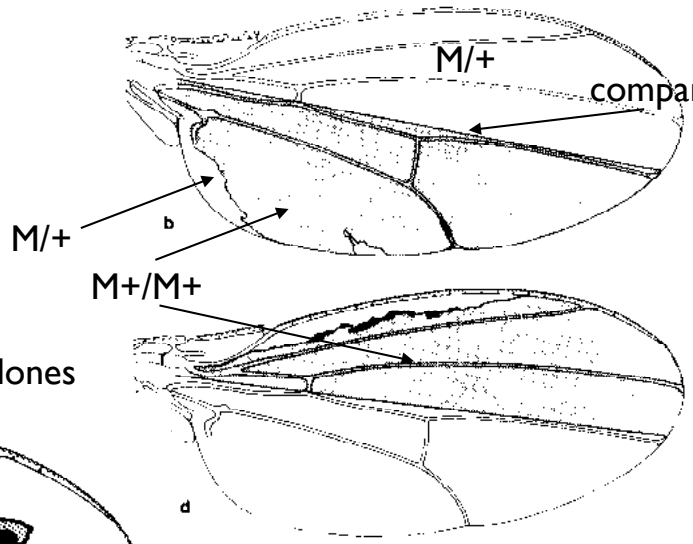
—Cells gauge their respective growth rates and any discrepancy is corrected so as to produce normally sized tissues and organs

Differential Mitotic Rates and Patterns of Growth in Compartments in the *Drosophila* Wing

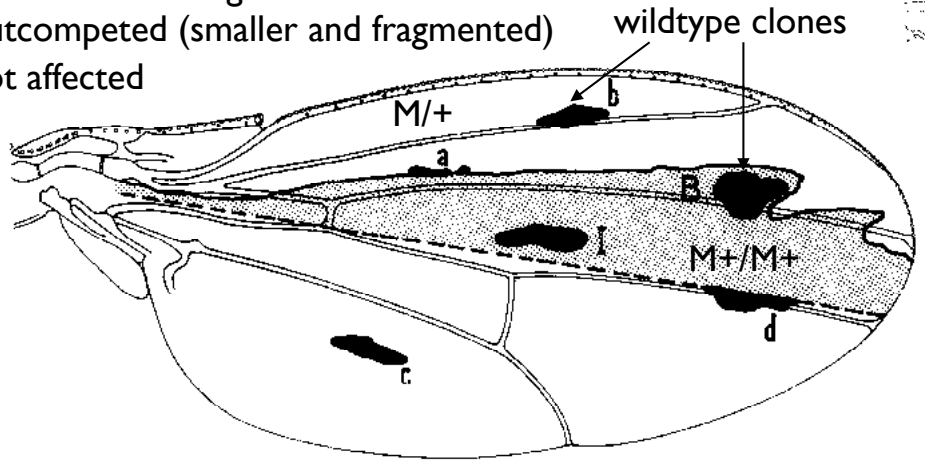
PAT SIMPSON[1] AND GINÈS MORATA[2]

Centre de Génétique Moléculaire, CNRS 91190 Gif-sur-Yvette, France

- Cell competition is higher when mitotic rate differences are stronger
- Cell competition induced by *Minute* mutations operate within « compartments », which work as unite of growth control.
- Cell competition is a result of local interactions between fast (M+/M+) and slow dividing cells (*M/+*)



Sequential induction of clones:
— M+/M+ clone in a *M/+* background
— ***a*** clone is outcompeted (smaller and fragmented)
— ***b*** clone is not affected



Simpson, P., and G. Morata. 1981. *Dev. Biol.* 85:299–308. http://dx.doi.org/10.1016/0012-1606(81)90261-X

• Non-autonomous growth compensation: cell competition

—Cells gauge their respective growth rates and any discrepancy is corrected so as to produce normally sized tissues and organs

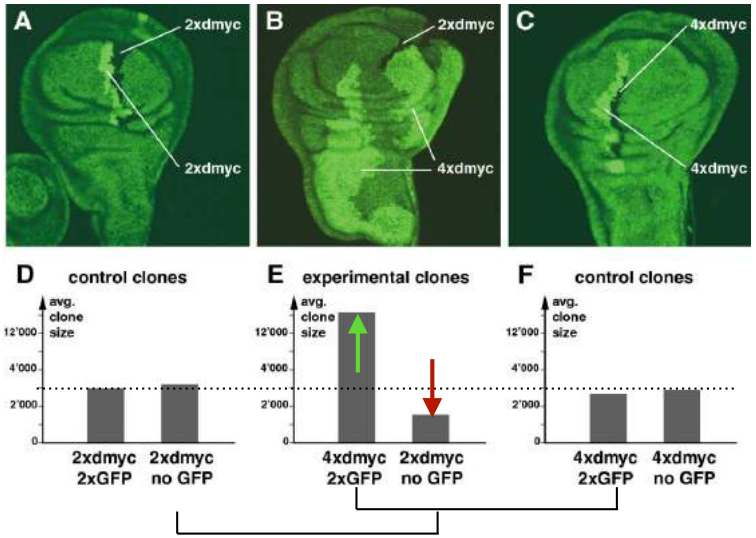
—Cell competition is induced by cells expressing different levels of Myc

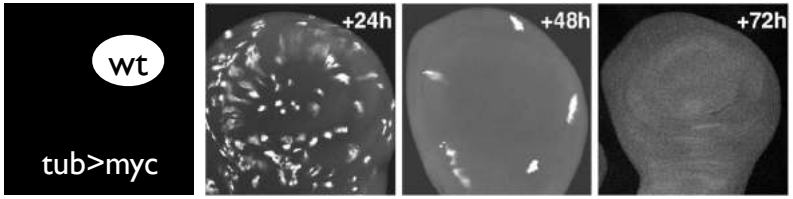
- Myc is a major regulator of cell anabolism
- Changing the levels of Myc expression causes cell competition

- Cells grow and divide as a function of their cellular environment:
  — 4xmyc cells grow more only when juxtaposed to 2xmyc cells
  — 2xmyc clones grow less when juxtaposed to 4xmyc cell
  —control clones surrounded by Myc overexpressing cells are eventually eliminated

- Yet clones of cells with higher levels of Myc do not cause aberrant morphology (size and pattern).

- Myc level is very frequently upregulated in cancers





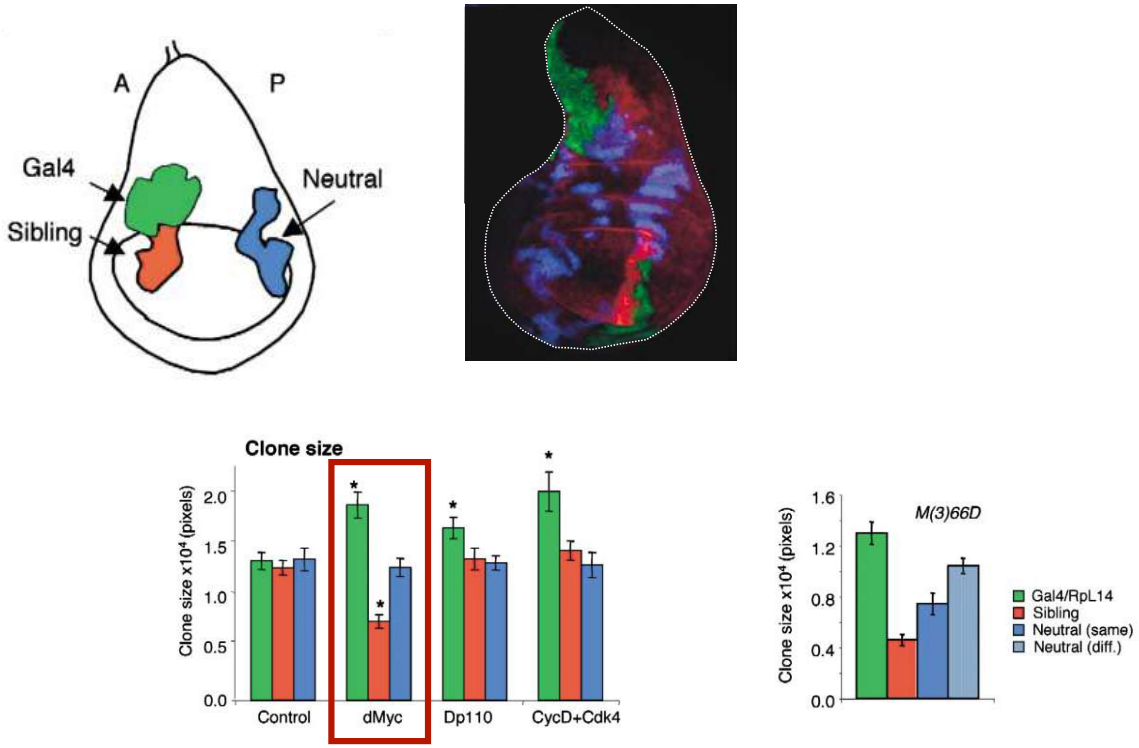
E. Moreno and K. Basler *Cell*, Vol. 117, 117–129, April 2, 2004

• Non-autonomous growth compensation: cell competition

—Cells gauge their respective growth rates and any discrepancy is corrected so as to produce normally sized tissues and organs

- Changing the levels of Myc expression causes cell competition similar to *Minute* mutations
- Cell competition is not a systematic consequence of juxtaposing cell populations with differential growth rates

(Pi3K pathway does not induce cell competition, nor does cell proliferation)

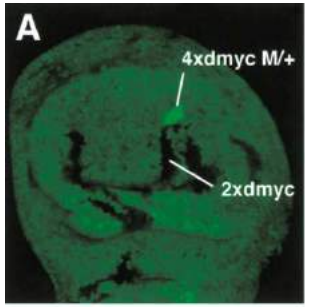


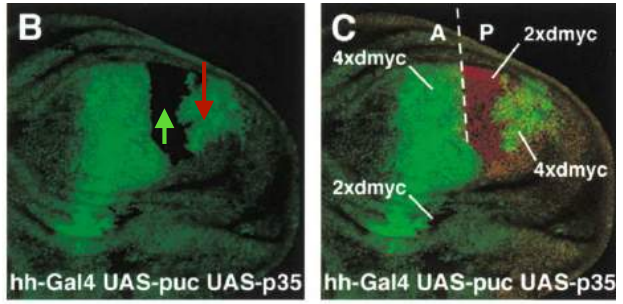
C. de la Cova et al, L.A. Johnston. *Cell*, Vol. 117, 107–116, April 2, 2004

• Non-autonomous growth compensation: cell competition

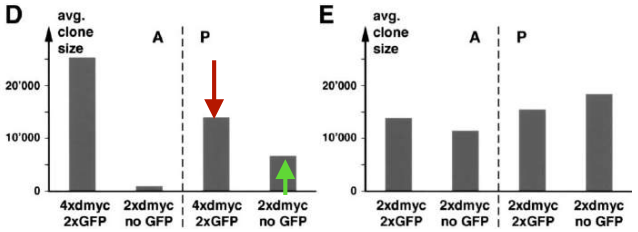
—Cells gauge their respective growth rates and any discrepancy is corrected so as to produce normally sized tissues and organs

—Cell competition is induced by cells expressing different levels of Myc



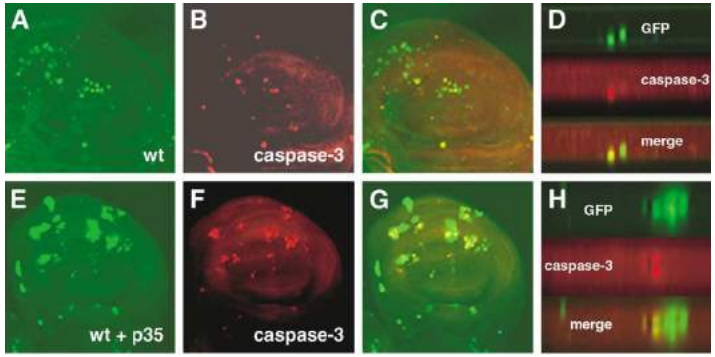


- Myc « super-competitor » cells require translational machinery (ribosomes assembly).



- Compensatory mechanisms: apoptotic cell death is induced in 2xmyc cells adjacent to 4xMyc cells
  p35: caspase inhibitor reduces cell competition

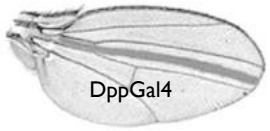
wt clones (GFP+)
in a tub>>Myc background
are outcompeted, unless cell death is suppressed

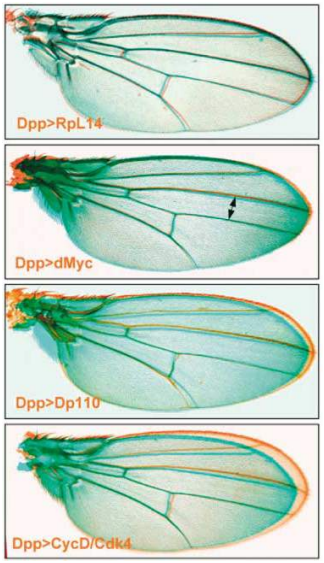


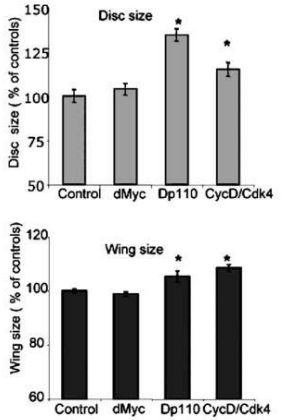
E. Moreno and K. Basler *Cell*, Vol. 117, 117–129, April 2, 2004
C. de la Cova et al, L.A. Johnston. *Cell*, Vol. 117, 107–116, April 2, 2004

• Non-autonomous growth compensation: cell competition

— Myc controls tissue size by inducing cell-competition via cell death







- Cell competition buffers local over-growth induced by Myc or Minute protein overexpression.

—Overexpression of RpL4 (ribosomal protein) and Myc in a stripe in the middle of the wing or in clones does not affect wing size.

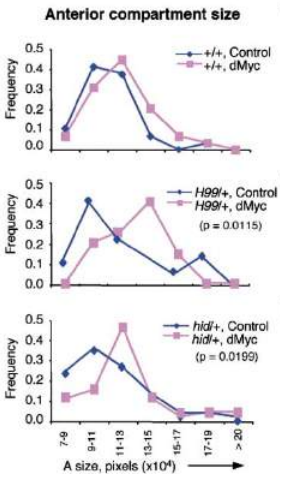
—However, ubiquitous over expression of Myc increases wing size 20%

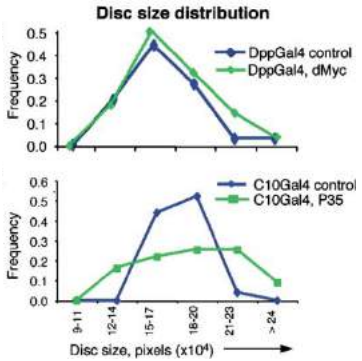
Ubiquitous expression of dMyc

Overall size, fold over control

	Disc (n)	Wing (n)
Control	1.00 (33)	1.00 (21)
Tub>*dmyc, y+*>Gal4 (No HS)	1.23 (15)	1.16 (17)
Tub>Gal4 (+ HS)	1.08 (30)	1.05 (25)

- Pi3K and S phase cyclin/cdk expand wing size due to absence of cell competition





- Reduced cell death (*hid*/+ or *H99*/+) blocks cell competition and induces compartment overgrowth due to Myc clonal over expression
- Blocking cell death does not cause tissue overgrowth (mean) but increases variability in wing size

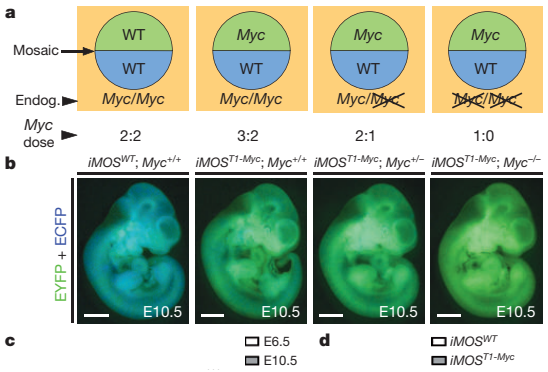
C. de la Cova et al, L.A. Johnston. *Cell*, Vol. 117, 107–116, April 2, 2004

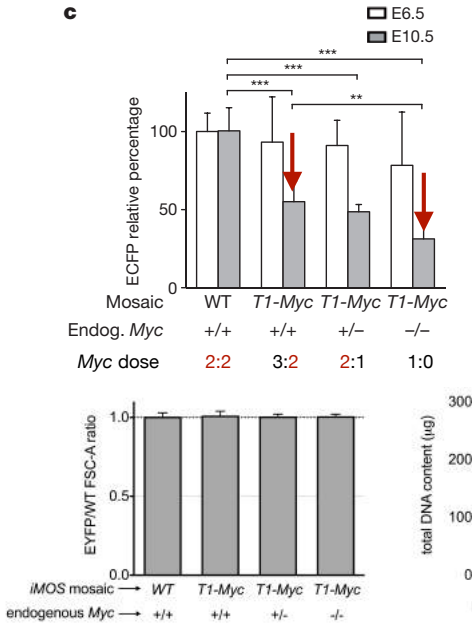
• Non-autonomous growth compensation: cell competition

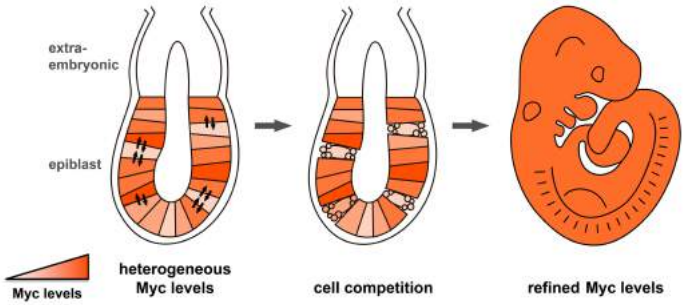
— Myc induced cell-competition in vertebrate embryos

- Cell competition is induced in the mouse epiblast using a genetic mosaic system that changes randomly the levels of Myc between cell populations labeled with a different fluorescent protein (CFP and YFP)
- It is the relative level of Myc in cells that determines their growth rate (not the absolute level)

- Cell size and embryo size is not affected.







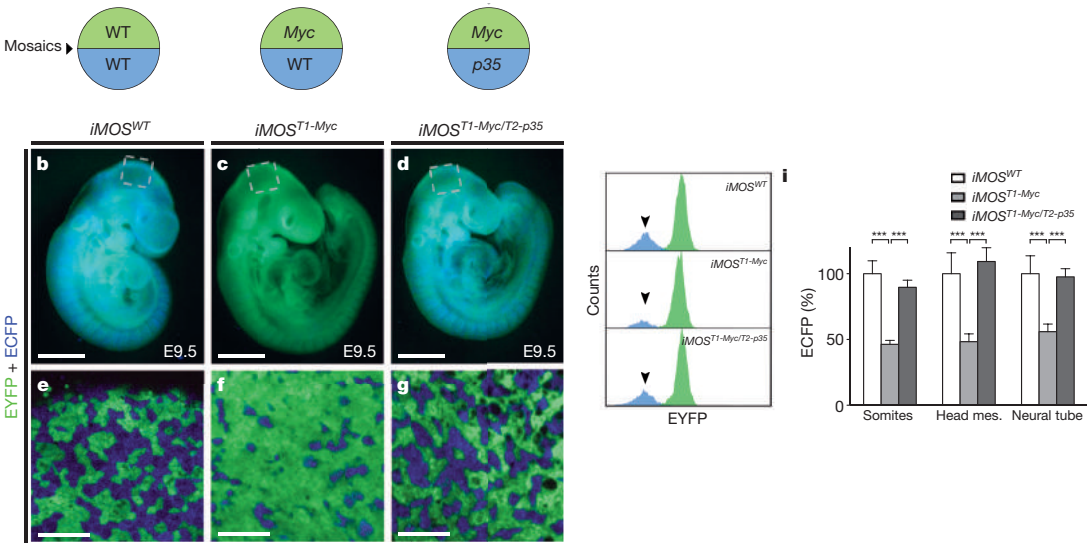
C. Claveria et al. and M. Torres. *Nature.* 2013 ;500(7460):39-44. doi: 10.1038/nature12389.

• Non-autonomous growth compensation: cell competition

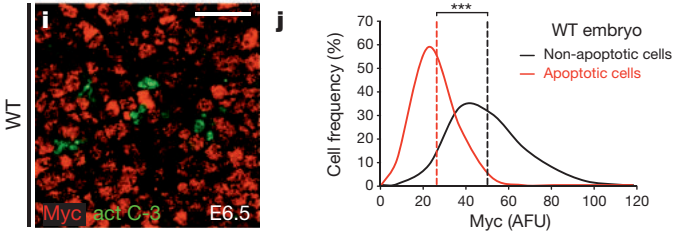
— Myc induced cell-competition in vertebrate embryos

- Cell competition requires cell death in « loser » cells: —blocking cell death (p35 expression) in loser cells rescues cell proportions .

- Endogenous levels of Myc in the epiblast (but not in extra embryonic tissues) is highly heterogeneous.
- Cell death is induced in low Myc expressing cells



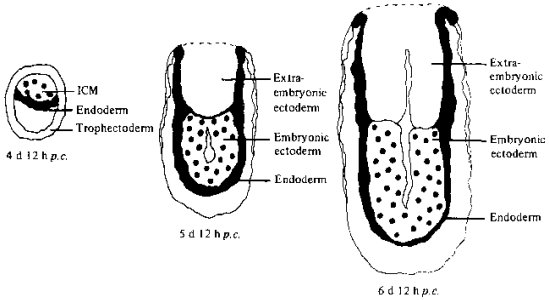
- Blocking apoptosis does not affect embryo pattern (and size a priori)
- This suggests that cell competition more likely operates as a quality control mechanism in « long lived » tissues/organisms .
- Unclear whether it affects embryo/tissue size…?
- Yet, cell competition exists in vertebrates and its implication is organ size control/regeneration and homeostasis should be addressed



C. Claveria et al. and M. Torres. *Nature.* 2013 ;500(7460):39-44. doi: 10.1038/nature12389.

• Growth compensation in the mouse embryo

—Both smaller and larger embryos reveal compensatory growth to attain a normal embryo size



- Mouse embryos treated with MitomycinC are initially much smaller but recover in size and morphology
- This involves upregulation of mitotic activity

Table 1 Cell numbers and mitotic activity in control and MMC-treated $7\frac{1}{2}$-d embryos

		Control	MMC
n		6	5
No. of cells	Ectoderm	7,618 ± 443	978 ± 157
	Mesoderm	3,743 ± 256	374 ± 53
	Endoderm	1,019 ± 57	374 ± 33
M/A index	Ectoderm	3.9 ± 0.2	12.5 ± 1.5
	Mesoderm	1.9 ± 0.2	5.2 ± 0.7
	Endoderm	4.5 ± 0.6	6.1 ± 0.7

Camera lucida drawings were made of transverse sections and tissue volumes computed from planimeter measurements of tissue areas. Cell numbers were then calculated from a knowledge of cell volume[13].

We have shown that the mouse embryo can be reduced to around 10% of its normal size at a time when it is about to begin organogenesis, but is nearly normal again before that phase of development is complete. Much of this regulation, especially morphogenesis, is accomplished within 48 h of the damage being inflicted. In particular, the formation of the brain and head seems to be achieved in about half the time it normally takes.

Snow, M. H. L., Tam, P. P. L. (1979) Nature 279, 555-557

J. Embryol. exp. Morph. Vol. 72, pp. 169–181, 1982
Printed in Great Britain © Company of Biologists Limited 1982

Mechanism of size regulation in mouse embryo aggregates

By N. E. LEWIS, AND J. ROSSANT
From the Department of Biological Sciences, Brock University, St Catharines, Ontario

- Double-sized embryos produced by fusion of two 8-cell stage embryos recover within 24h their size
- This is not associated with cell death or a reduction in the fraction of dividing cells
- It correlates with increased cell cycle length

Days *p.c.*	Number of embryos: Double	Number of embryos: Control	Mean cell number ± S.E.: Double	Mean cell number ± S.E.: Control
4 d, 0 h	7	6	160 ± 15·4	63 ± 14·6
4 d, 8 h	6	4	248 ± 31·4	115 ± 18·8
4 d, 16 h	10	7	309 ± 21·0	160 ± 14·8
5 d, 0 h	7	5	413 ± 43·0	198 ± 14·8
5 d, 8 h	7	5	389 ± 45·6	199 ± 40·8
5 d, 16 h	11	7	749 ± 64·7	324 ± 34·2
6 d, 0 h	4	5	1209 ± 94·2	891 ± 54·8
6 d, 8 h	8	5	1542 ± 62·1	1216 ± 110·8
6 d, 16 h	6	6	2253 ± 313·2*	2086 ± 233·0*

Table 4. *Cell cycle length (hours) of double and control embryos derived from mitotic index values*

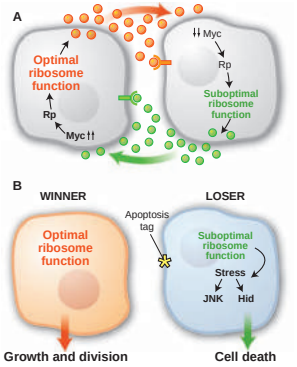
Days *p.c.*	Embryonic ectoderm: Control	Embryonic ectoderm: Double	Extraembryonic ectoderm: Control	Extraembryonic ectoderm: Double	Endoderm: Control	Endoderm: Double
5 d 16 h	5·4	6·9	7·1	8·0	12·5	11·8
6 d 0 h	4·1	5·4	6·7	8·7	8·0	11·1
6 d 8 h	3·7	5·3	4·9	6·9	5·9	9·5
6 d 16 h	4·2	4·3	5·6	5·0	6·6	6·9

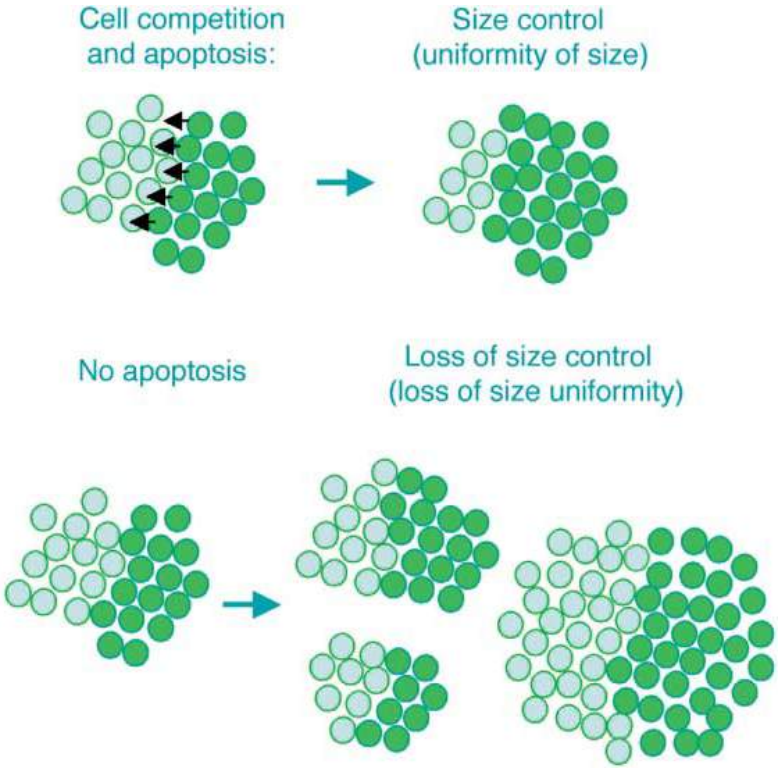
Lewis, N. E., Rossant, J. (1982) *J. Embryol. exp. Morph* 72, 169-181

• Non-autonomous growth compensation: cell competition

— Myc controls tissue size by inducing cell-competition via cell death

— local sensing of growth rate between cells.
— local correction ensure correct tissue size, and tissue fitness/homeostasis



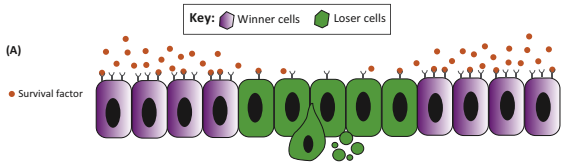


C. de la Cova et al, L.A. Johnston. *Cell*, Vol. 117, 107–116, April 2, 2004

• Non-autonomous growth compensation: cell competition

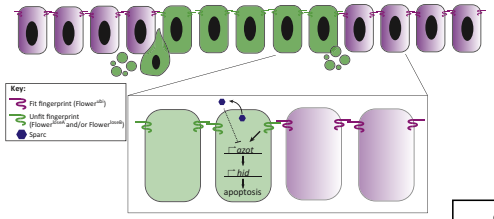
— Mechanisms of cell competition

- competition for limiting extracellular pro-survival factors



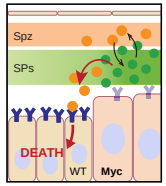
Moreno, E. et al. (2002) *Nature* 416, 755–759

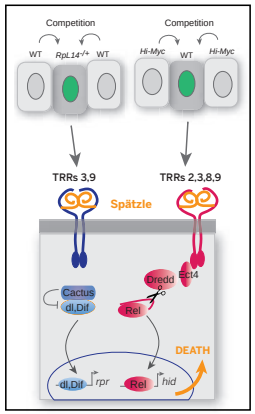
- communication through direct cell–cell contact



Rhiner, C. et al. (2010) Dev. Cell 18, 985–998

- communication through secretory signals

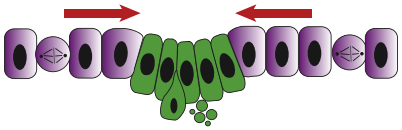




Meyer, S.N., et al, Basler, K., and Johnston, L.A. (2014). *Science 346*, 1258236.

Alpar, L., Bergantinos, C., and Johnston, L.A. (2018). *Dev. Cell 46*, 706–719.e705.

- mechanical stress

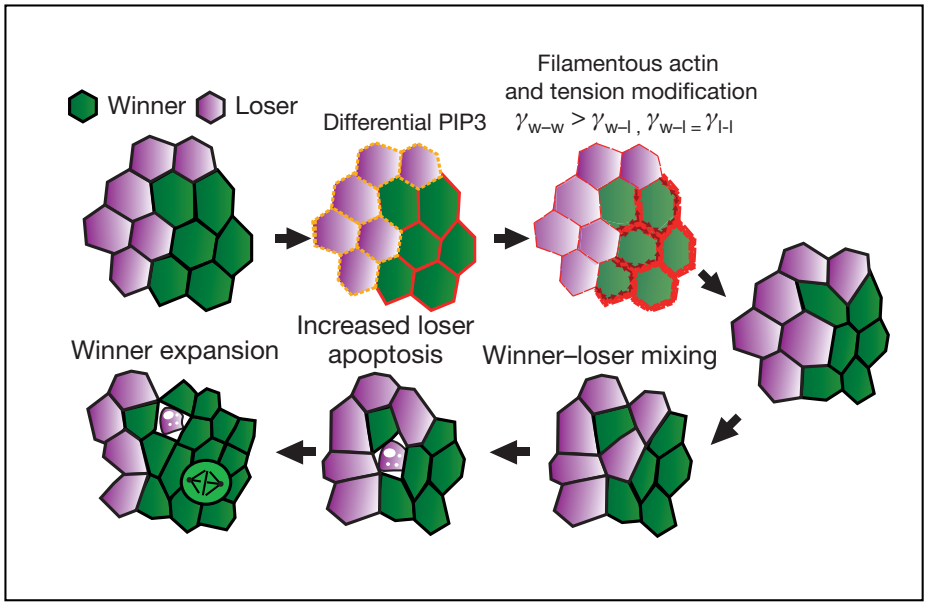


• Non-autonomous growth compensation: cell competition

— Mechanisms of cell competition

- Cell competition (induced by Myc) requires cell mixing by differential tension and cell intercalation
- Tissue crowding and competition for space (mechanically induced) induces cell elimination

(see Course #5, 10 December)



Levayer R, Hauert B, Moreno E.
Nature. 2015 Aug 27;524(7566):476-80. doi: 10.1038/nature14684.

Levayer R, Dupont C, Moreno E.
Curr Biol. 2016 Mar 7;26(5):670-7. doi: 10.1016/j.cub.2015.12.072.

Moreno E, Valon L, Levillayer F, Levayer R.
Curr Biol. 2019 Jan 7;29(1):23-34.e8. doi: 10.1016/j.cub.2018.11.007.

• Conclusion: intrinsic and organ specific growth sensing

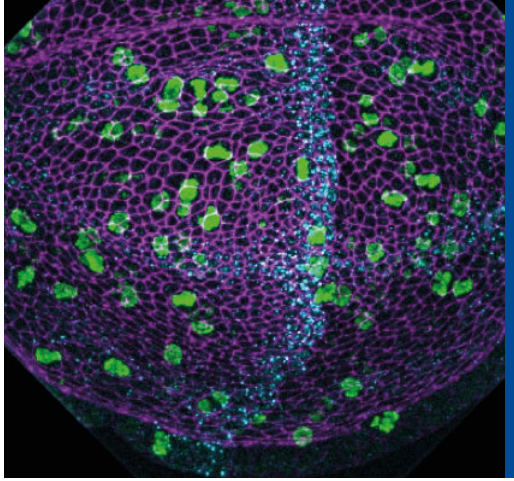
— In plants and animals tissue growth is driven by both cell division (increase in cell number) and cell growth (increase in cell size).
—The two processes are coordinated but can be independently regulated

— Question: What controls growth arrest?
Hypothesis: Cell-scale model: counting cell size, cell number, the number of cell division
If so the pattern of cell division should affect organ shape and size.
But in general it does not

- Cell growth is controlled intrinsically (cellular anabolism: Ras, Pi3K, TOR signalling)
- The pattern of cell division does not affect tissue size.

- Perturbations in cell growth (via ploidy, Cdc2 and endoreplication, *Minute*, *Myc* mutations and cell competition) does not affect tissue size revealing compensatory mechanisms and **local** regulation.

- These compensatory mechanisms are **specific to each organ** (and species)
- Compensation reveals **organ specific tissue size sensing/measurement**



COLLÈGE DE FRANCE
1530

CHAIRE DYNAMIQUES DU VIVANT
Année académique 2019-2020

Thomas LECUIT

Moteurs, contraintes et régulations de la croissance

Cours les mardis de 10h à 11h30
Amphithéâtre Guillaume Budé

Cours :

12 novembre 2019	Introduction : comment la taille biologique est-elle codée ?
19 novembre 2019	Lois d'échelle, allométrie et croissance des organismes
26 novembre 2019	Croissance des organes et contrôle interne
03 décembre 2019	Contrôle interne et patterning
10 décembre 2019	Contrôle interne et mécanique
17 décembre 2019	Coordination et symétrie - Conclusion

Colloque :

Contraintes et plasticité au cours du développement et de l'évolution
(avec Denis Duboule, chaire Évolution des génomes et développement)

Le mardi 30 juin et le mercredi 1er juillet, de 9h à 18h
Amphithéâtre Maurice Halbwachs