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What controls the size of an animal?

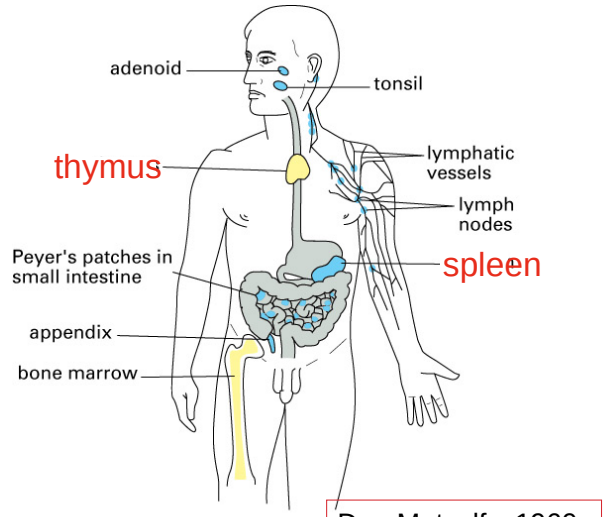
Animal and organ size depends on

1. Total cell mass:
   —cell size
   —cell number
2. Extracellular materials

For mammals, cell numbers matter most

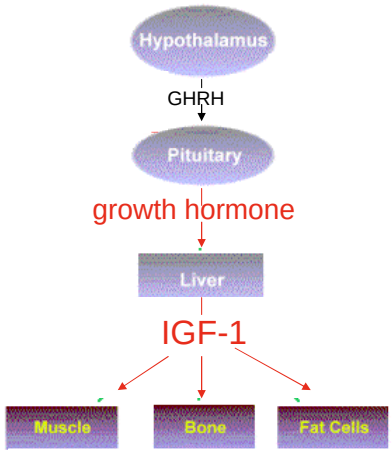
Cell number depends on
—cell division
—cell death

Local versus systemic controls on animal and organ size



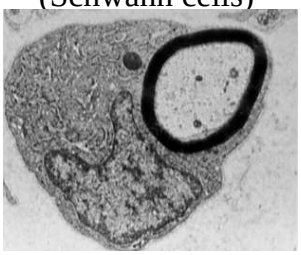
Don Metcalf—1960s

The growth hormone/IGF-1 pathway is the best understood systemic growth control system



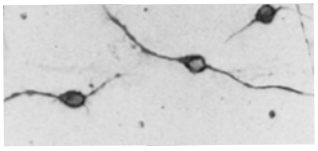
The myelinating glial cells and their precursors

Cell size control
(Schwann cells)

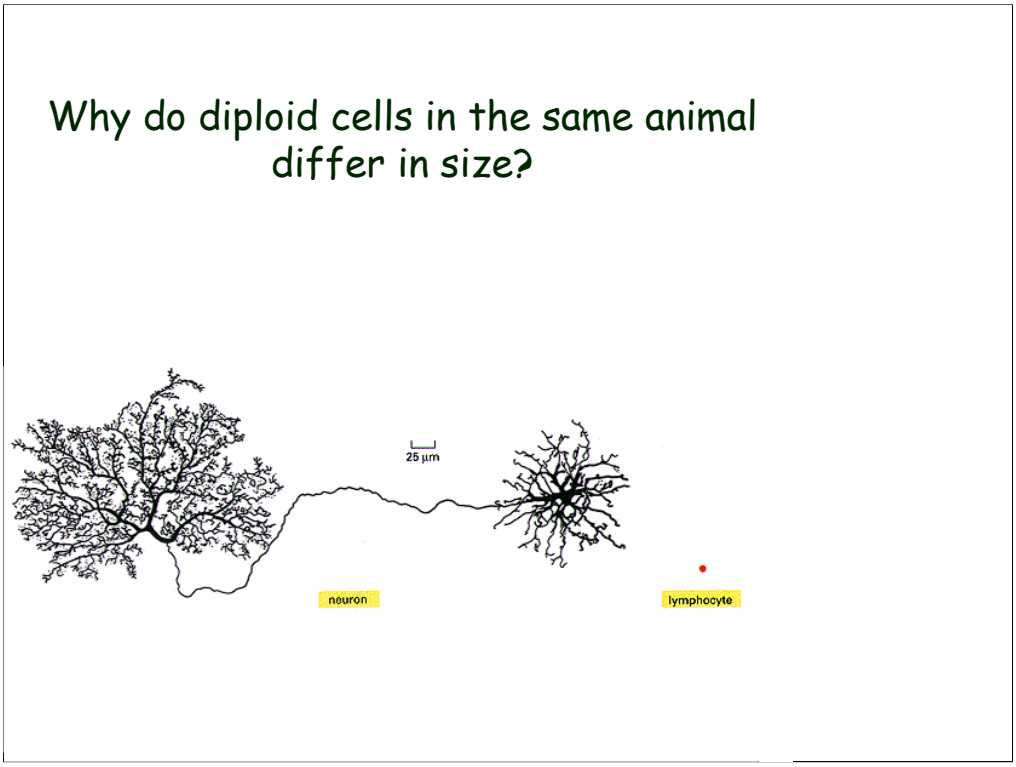


Cell number control
(oligodendrocytes)

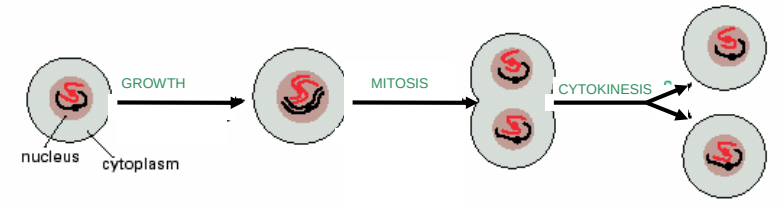
QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.



Cell size control


Why do diploid cells in the same animal differ in size?
25 µm
neuron
lymphocyte

How does a proliferating cell co-ordinate its growth with division?



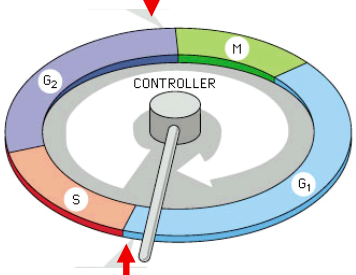
Cell growth is required for any organism to grow

Terminology problem

1. cell growth
2. growth factor

Cell-size checkpoints

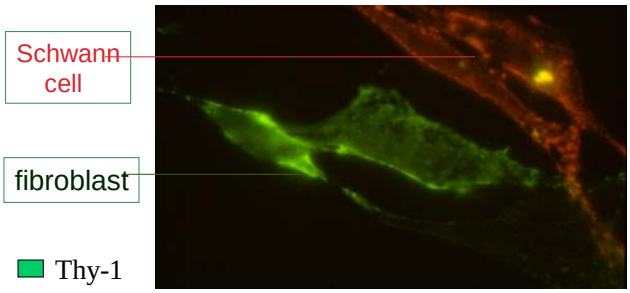
G_2 cell-size checkpoint



G_1 cell-size checkpoint

Ian Conlon

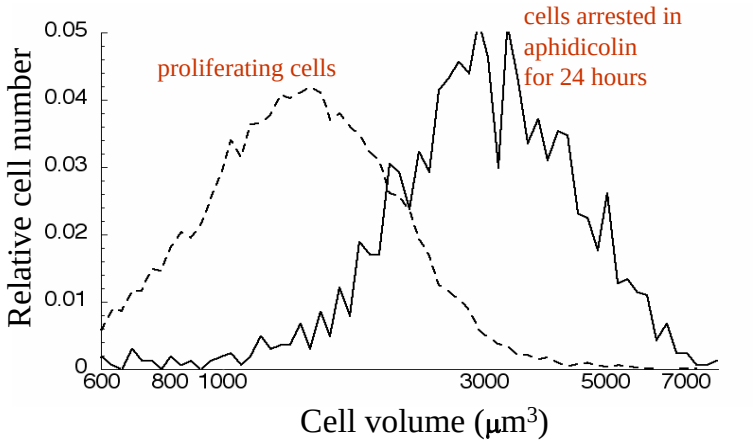
Schwann cells and fibroblasts can be distinguished in cultures of newborn rat sciatic nerve cells

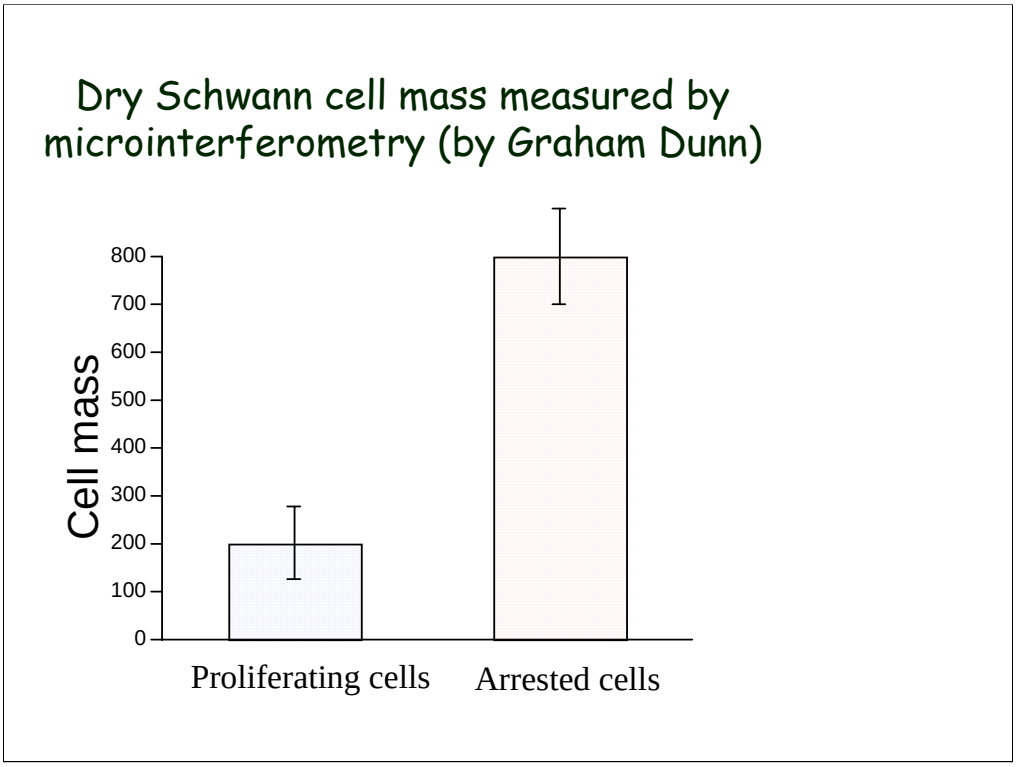



Jeremy Brockes


Kay Fields

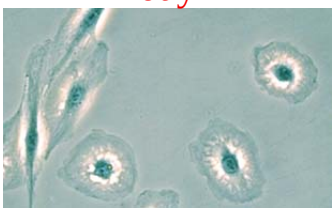
Growth of normal and aphidicolin-arrested Schwann cells in FCS, assessed in a Coulter counter



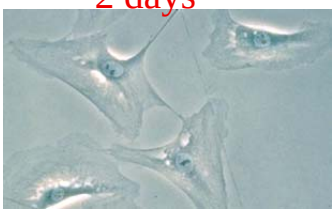

Dry Schwann cell mass measured by microinterferometry (by Graham Dunn)
Cell mass
800
700
600
500
400
300
200
100
0
Proliferating cells
Arrested cells

Progressive growth of aphidicolin-arrested Schwann cells in 3% FCS

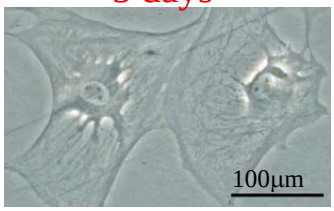
1 day

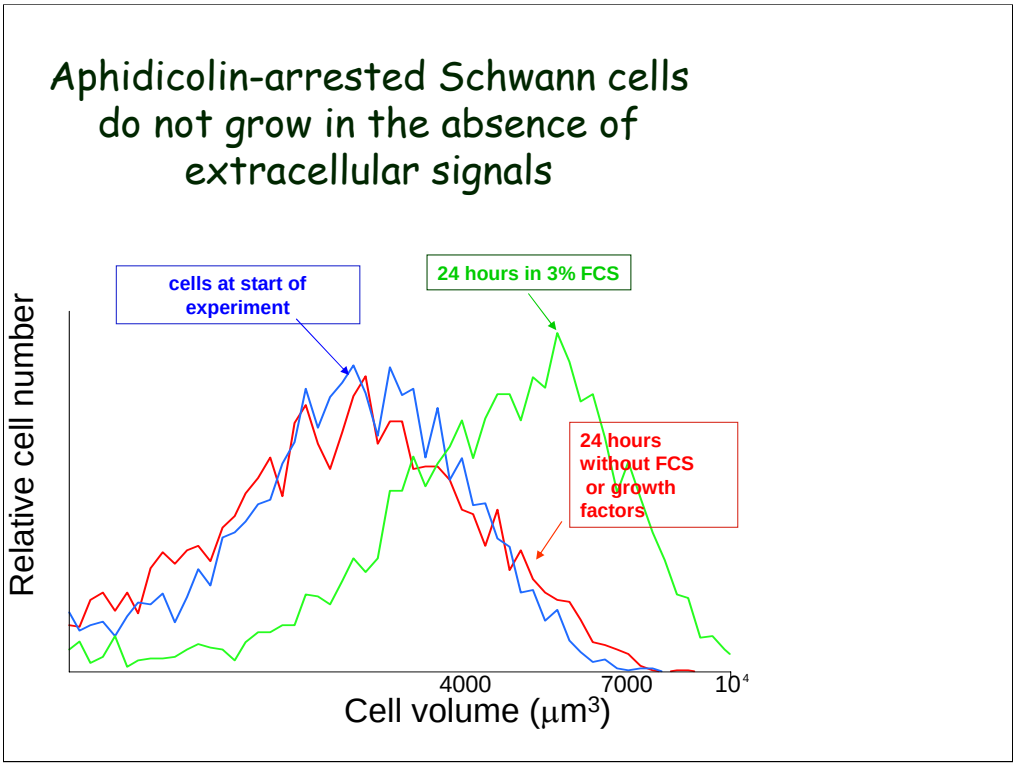


2 days

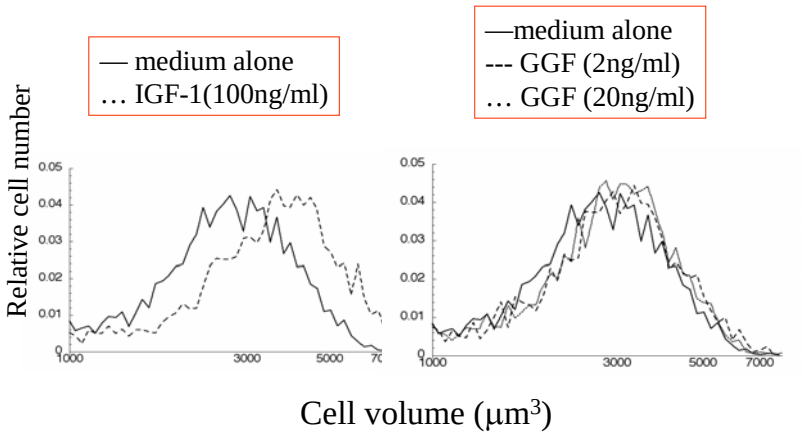


5 days

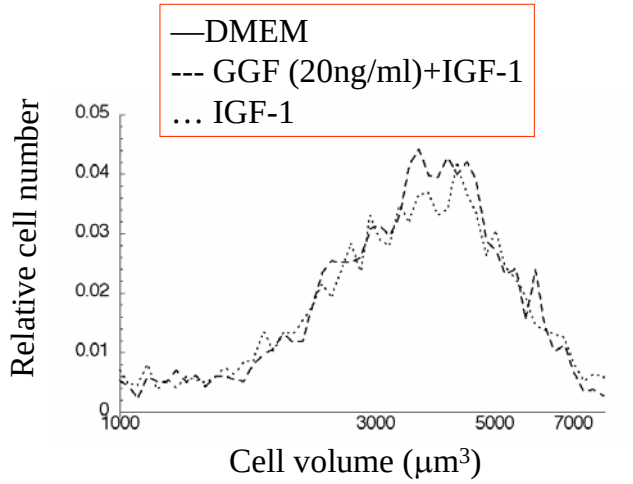



Aphidicolin-arrested Schwann cells do not grow in the absence of extracellular signals
cells at start of experiment
24 hours in 3% FCS
24 hours without FCS or growth factors
Relative cell number
4000
7000
10⁴
Cell volume (μm³)

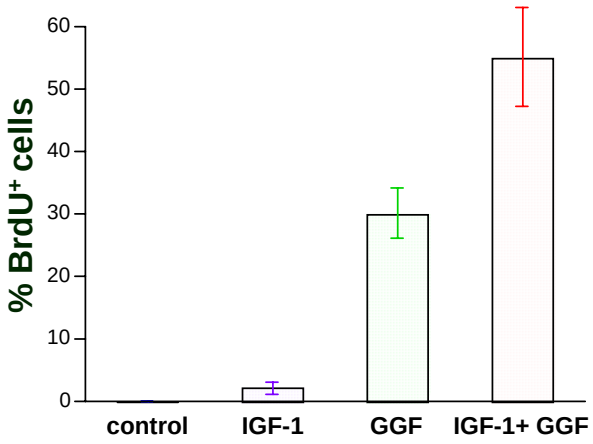
IGF-1 but not GGF stimulates Schwann cell growth



GGF does not increase the cell growth stimulated by IGF



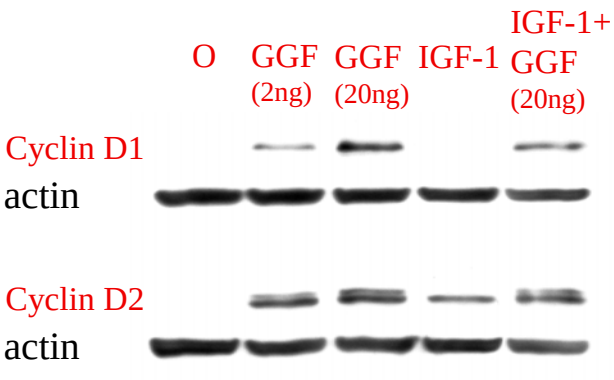
GGF stimulates entry into S phase, and IGF-1 synergizes with GGF



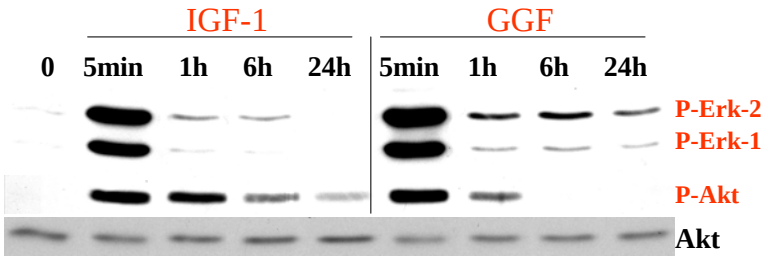
Others have reported similar findings with other cells and mitogens

1. Zetterberg: 3T3 cells (IGF-1 and EGF)
2. Delue et al. : dog thyrocytes (IGF-1 and TSH)

Western blot showing that GGF stimulates the production of cyclin D1 and D2 proteins within 4 hr; IGF-1 does not help



IGF-1 induces a sustained activation of Akt, whereas GGF induces a sustained activation of Erks

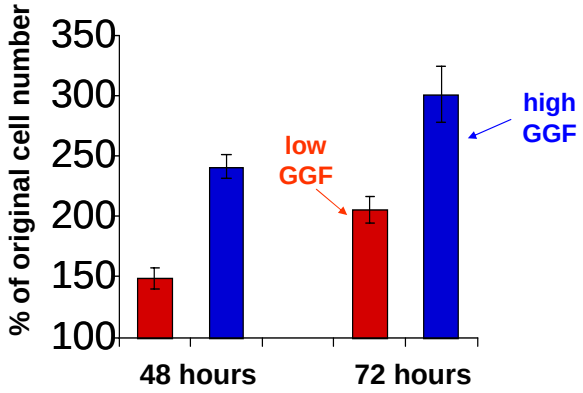


What happens to proliferating Schwann cells if IGF-1 is held constant and GGF is varied?

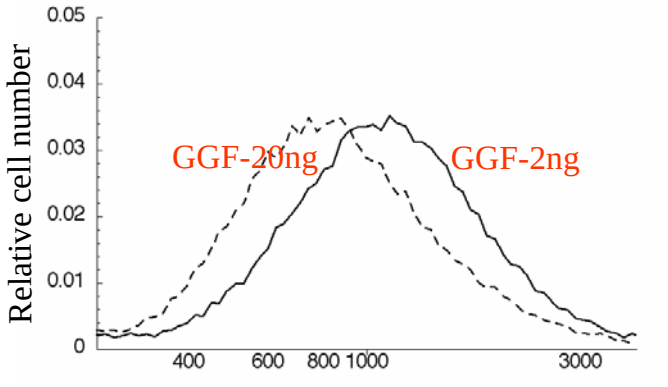
IGF-1=100 ng/ml

GGF= 2ng/ml or 20ng/ml

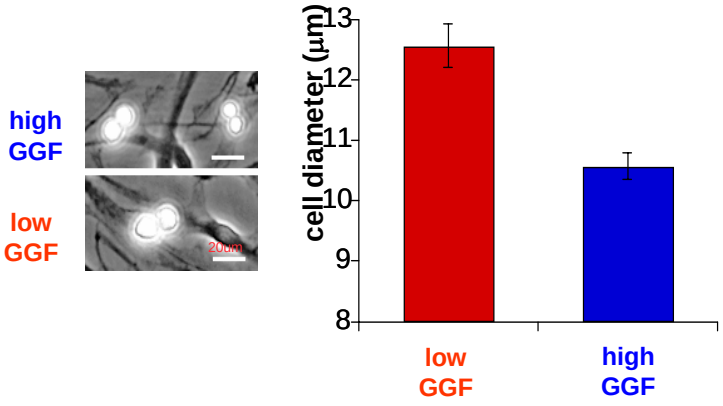
In constant IGF-1, high GGF stimulates faster cell proliferation than does low GGF

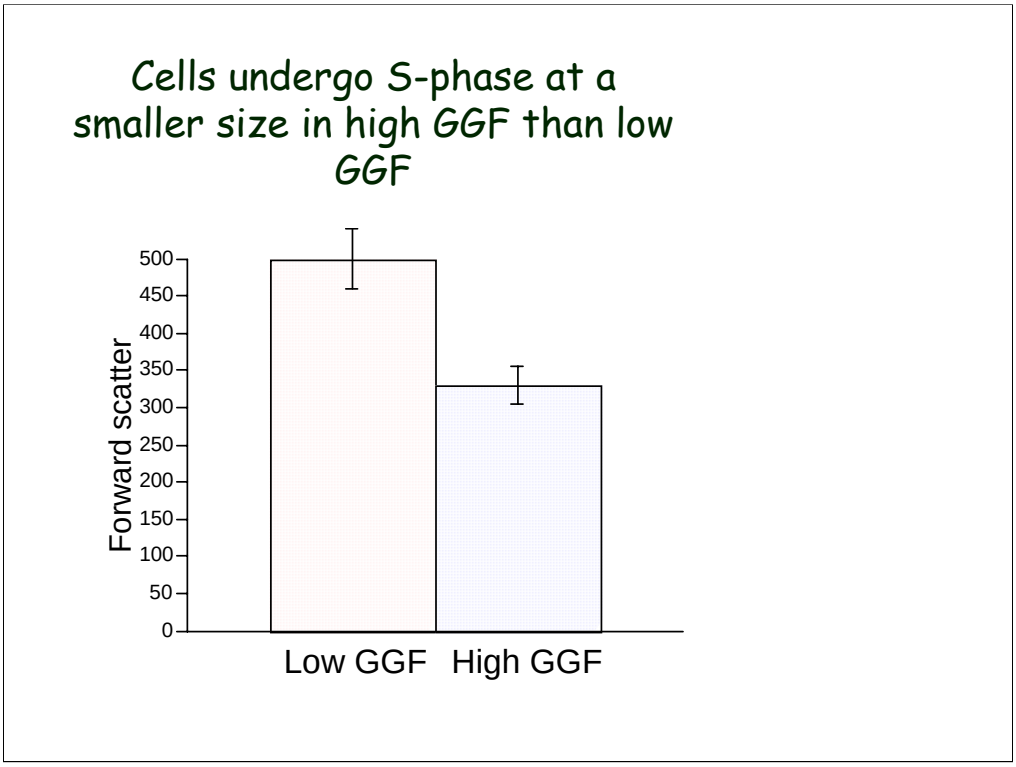


Cells proliferating in high GGF are smaller on average than cells proliferating in low GGF



Cells undergo mitosis at a smaller size in high GGF than low GGF




Cells undergo S-phase at a smaller size in high GGF than low GGF
Forward scatter
500
450
400
350
300
250
200
150
100
50
0
Low GGF
High GGF

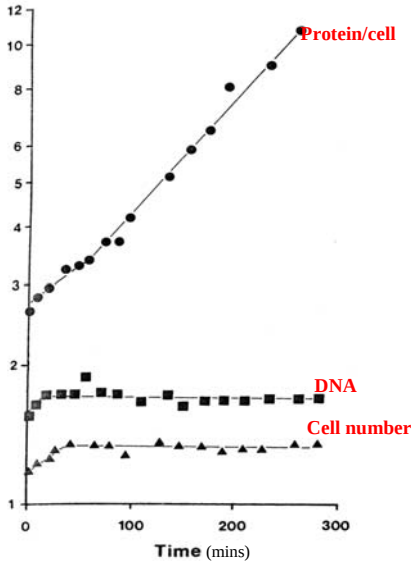
Conclusions from the constant IGF-1 + variable GGF experiments

1. Schwann cell size at division depends on the rates of cell growth and cell-cycle progression

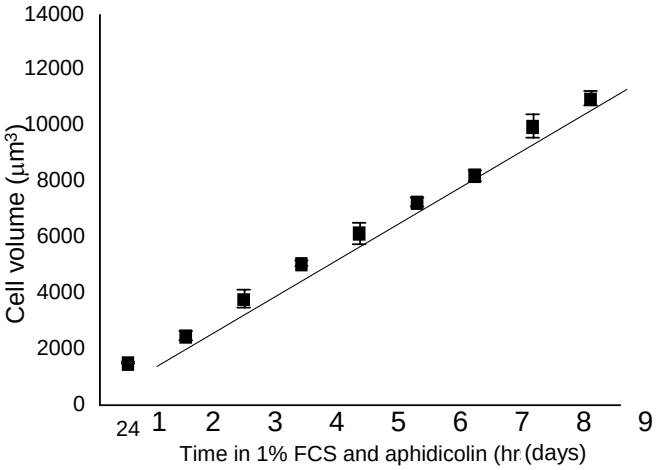
2. These rates depend on the concentrations of extracellular signals that stimulate cell growth, cycle progression, or both

The findings do not exclude that Schwann cells have a **cell-size checkpoint**

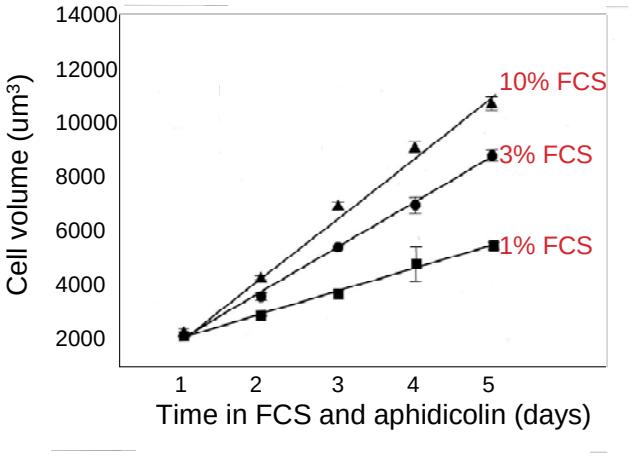
With mutant fission yeast that are blocked in S phase, large cells grow faster than small cells (Nurse and Nasmyth '72)



With rat Schwann cells that are arrested with aphidicolin, large cells grow at the same rate as small cells



Schwann cell growth rate increases with increasing concentration of FCS

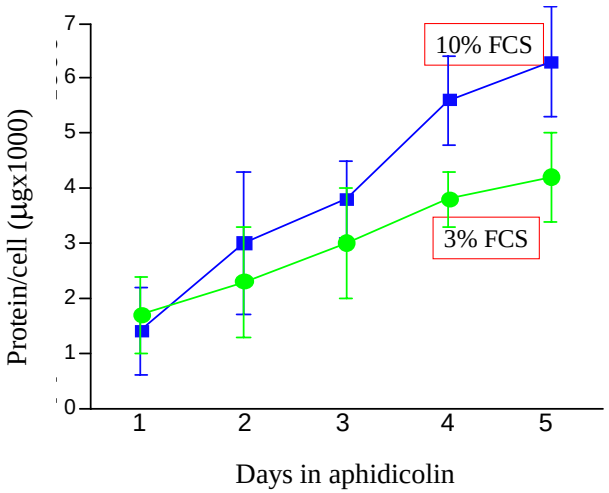


Why do Schwann cells and yeast cells behave differently?

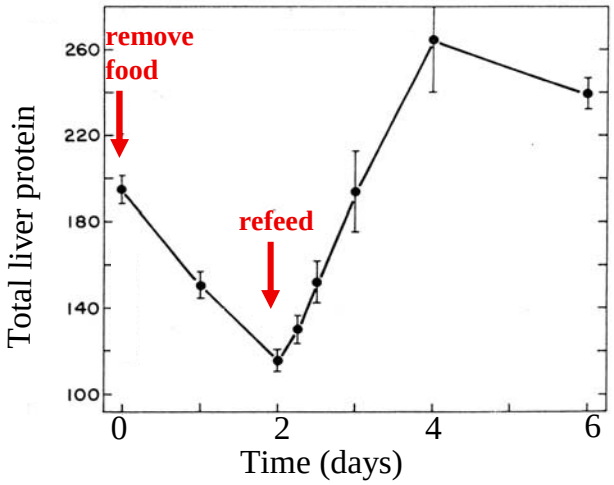
1. Assays?

2. Aphidicolin?

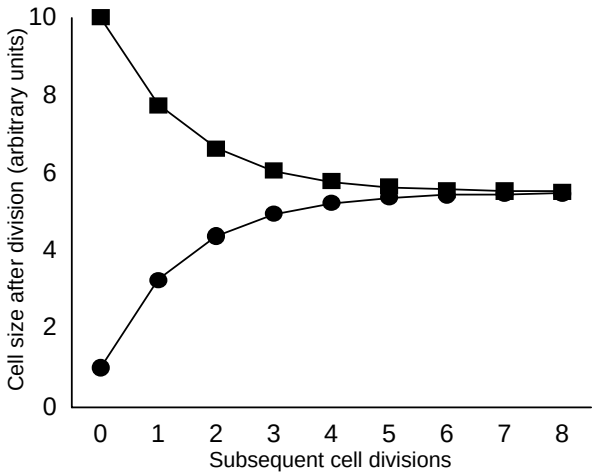
Increase in protein per cell in aphidicolin-treated Schwann cells in 3% or 10% FCS

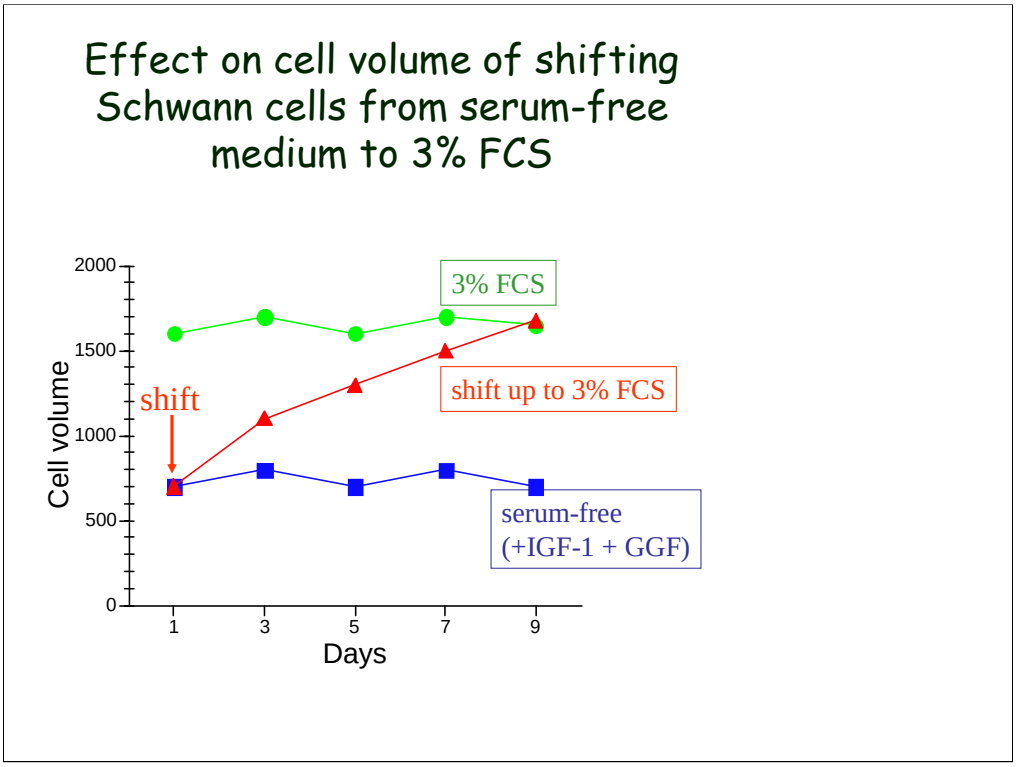


Regrowth of mouse liver after starvation (Hutson and Mortimore, 1982)



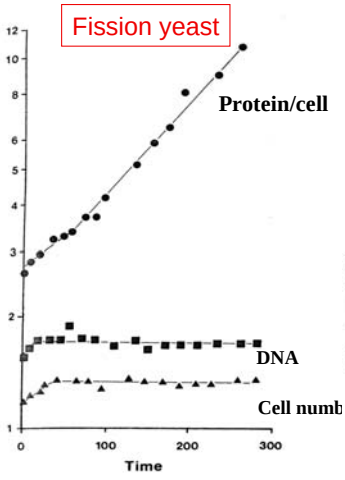
If big cells and small cells grow and proliferate at the same rate, they don't need a cell-size checkpoint to maintain their size (Robert Brooks '81)



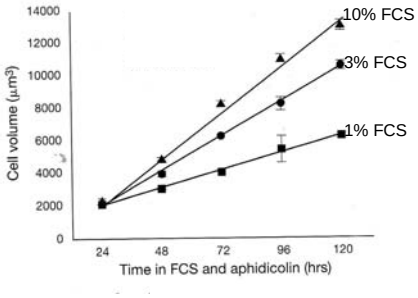

Effect on cell volume of shifting Schwann cells from serum-free medium to 3% FCS
3% FCS
shift up to 3% FCS
shift
serum-free
(+IGF-1 + GGF)
Cell volume
2000
1500
1000
500
0
1
3
5
7
9
Days

Why is cell growth control different in yeasts and Schwann cells?

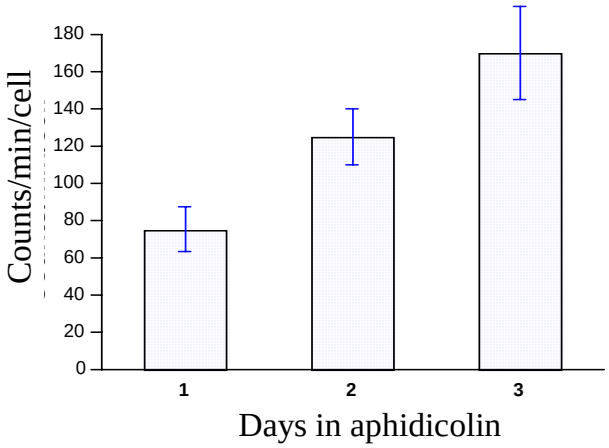
Fission yeast



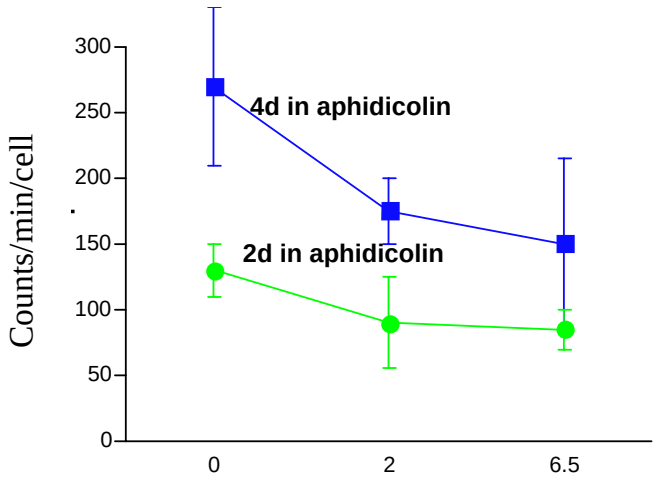
Schwann cells



Protein synthesis is greater in big Schwann cells than in little Schwann cells (assessed by a 2h pulse of ^{35}S-methionine)

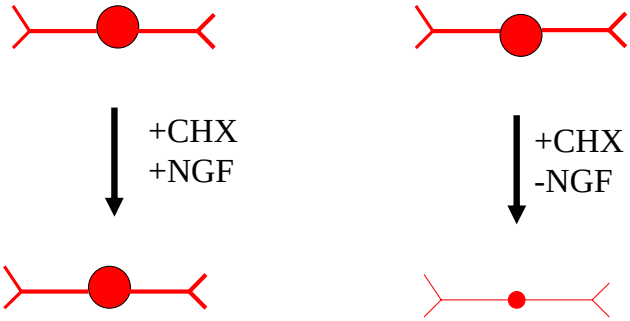


Protein degradation is greater in big Schwann cells than in small Schwann cells

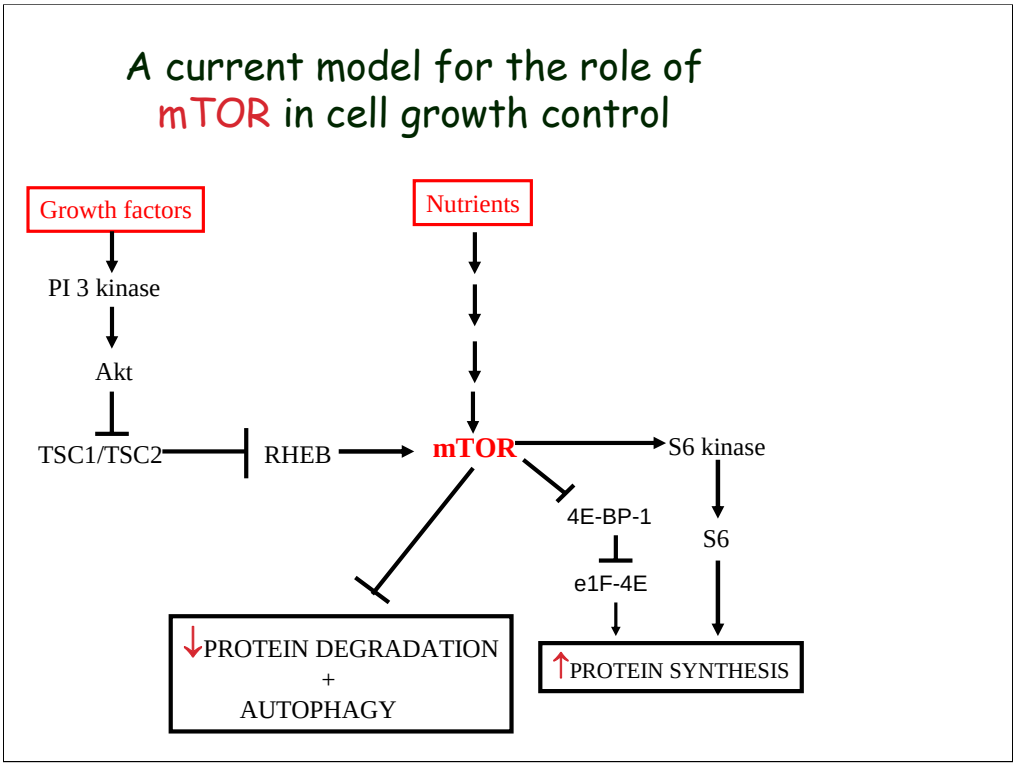


Hrs after 2 hr pulse with ^{35}S-methionine

NGF is required for sympathetic neurons to shut off protein degradation when protein synthesis is blocked with cycloheximide (CHX)



Franklin and Johnson'98


A current model for the role of mTOR in cell growth control
Growth factors
Nutrients
PI 3 kinase
Akt
TSC1/TSC2
RHEB
mTOR
S6 kinase
4E-BP-1
S6
e1F-4E
↓PROTEIN DEGRADATION + AUTOPHAGY
↑PROTEIN SYNTHESIS