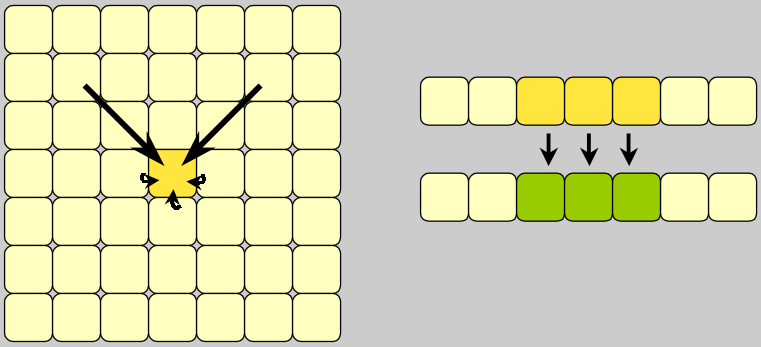
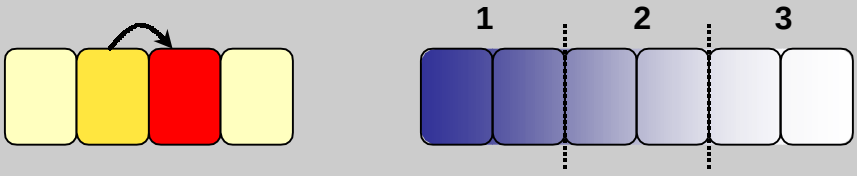
Development in multicellular organisms is controlled by intercellular signalling



Inductive vs. Morphogen Signals

Working hypothesis: these are regulated with different logic



Concentration independent

Threshold

Binary

Concentration dependent

Direct relationship between signal intensity and outcome

Both can produce 'complex' patterns

Signalling components

Signalling logic

The initiation of signalling is regulated by ligand availability

?

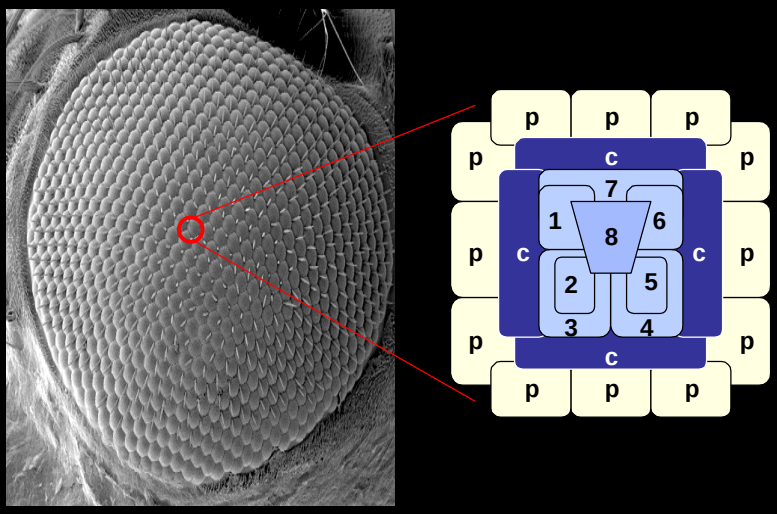
Freeman and Gurdon 2002

Few pathways control development

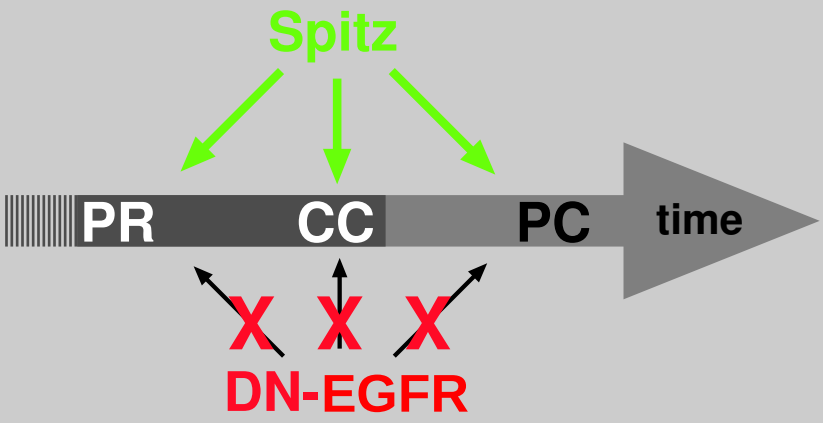
- Receptor tyrosine kinases
- Notch
- Hedgehog
- Wnt
- TGFb
- G-protein coupled receptors
- PI-3 kinase/phosphoinositide

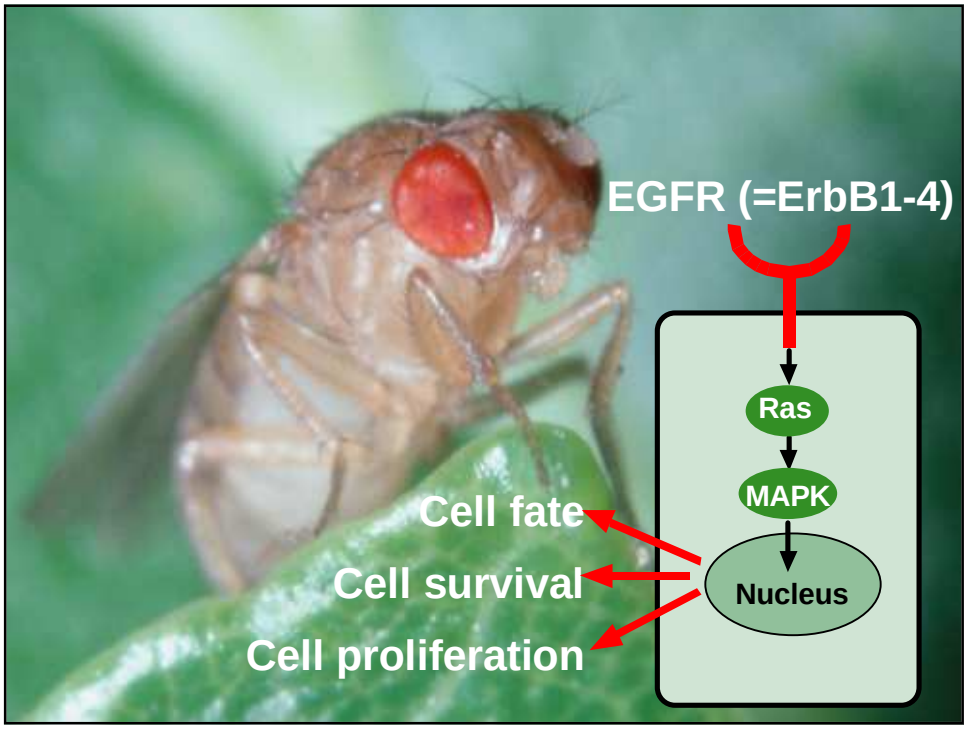
A cell's response to a signal is determined by its developmental context, not by the nature of the signal

Multiple cell types in the developing ommatidium are determined by EGFR



EGFR triggers each cell type





Signal regulation is paramount

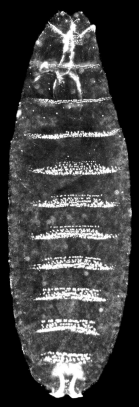
Precise

Robust/stable

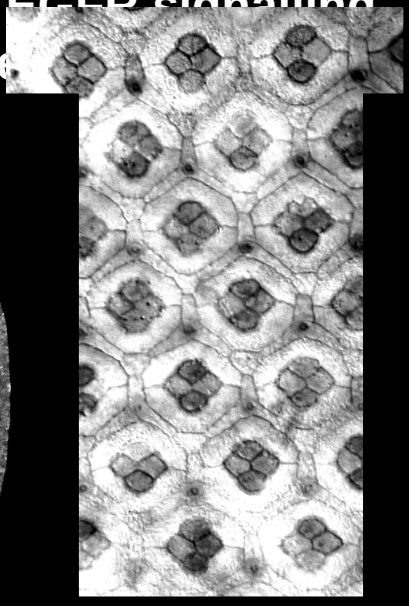
Versatile

Integrated control of EGFR signalling regulates developme

Egg

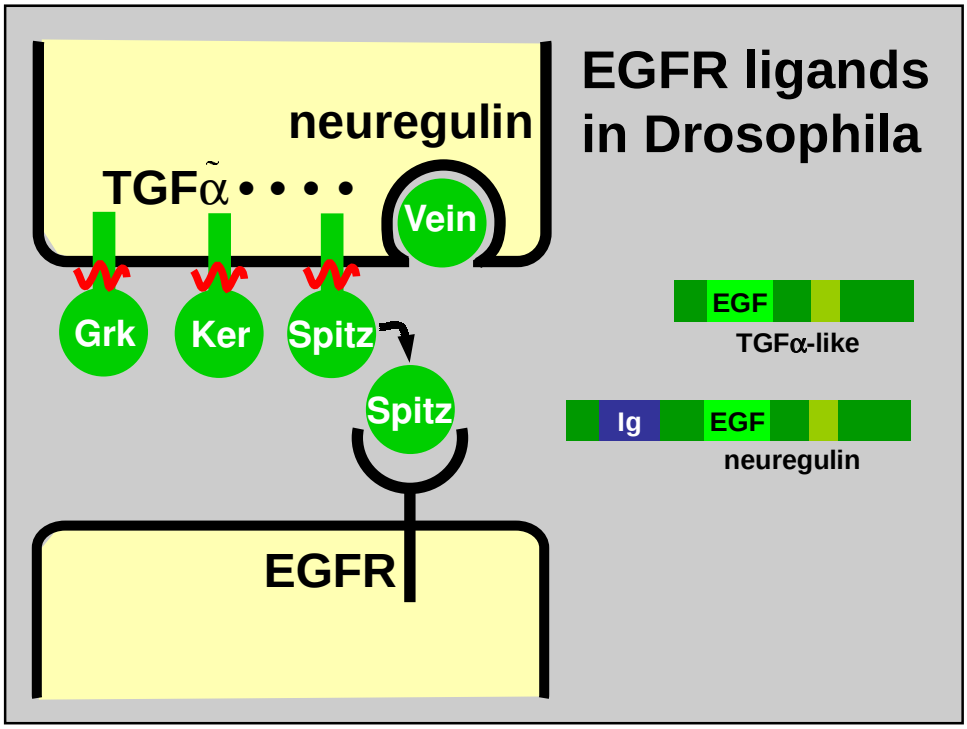


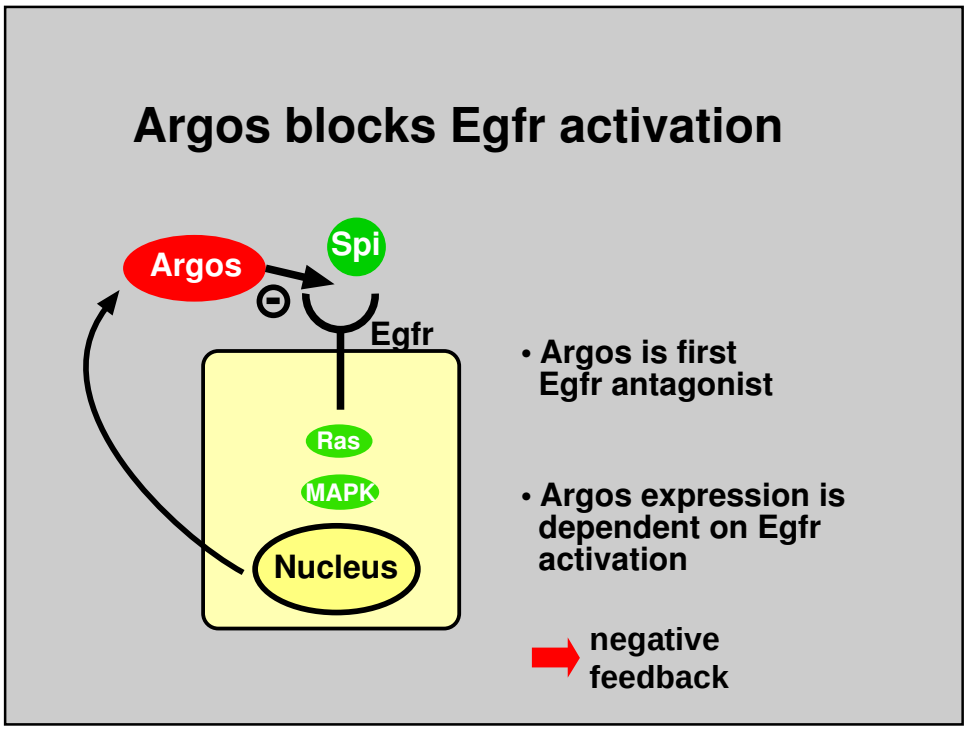
Embryo



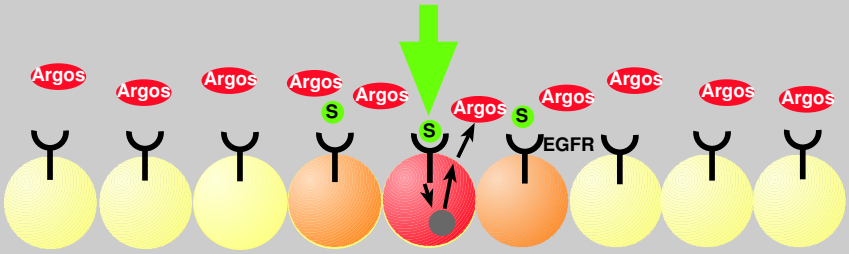
Eye

Genetics provides unbiased method for identifying physiologically-significant components/regulators


EGFR ligands in Drosophila
neuregulin
TGFα
Vein
Grk
Ker
Spitz
Spitz
EGFR
EGF
TGFα-like
Ig
EGF
neuregulin


Argos blocks Egfr activation
Argos
Spi
Egfr
Ras
MAPK
Nucleus
• Argos is first Egfr antagonist
• Argos expression is dependent on Egfr activation
negative feedback

Remote (or local) inhibition by Argos



Short range activator, long range inhibitor

Properties:

Range of inhibition could be determined by duration of a cell' s susceptibility to Argos

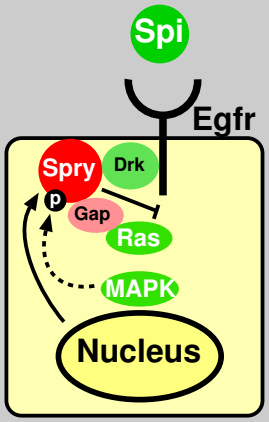
Can potentially stabilise a gradient of activity, or convert graded signal to binary output

NB

Our models of signalling logic are based on experiment and intuition

They are not mathematically tested and may be wrong

Sprouty: an intracellular inhibitor of Ras signalling



- Potent inhibitor of Ras activation
- MAP kinase target
- Binds Gap1 and Drk
- Tightly bound to inner face of plasma membrane
- Acts in negative feedback control of signalling
- Mammalian homologues

Novel feedback inhibitors of EGFR

Argos

Extracellular
Diffusible
Egfr-specific

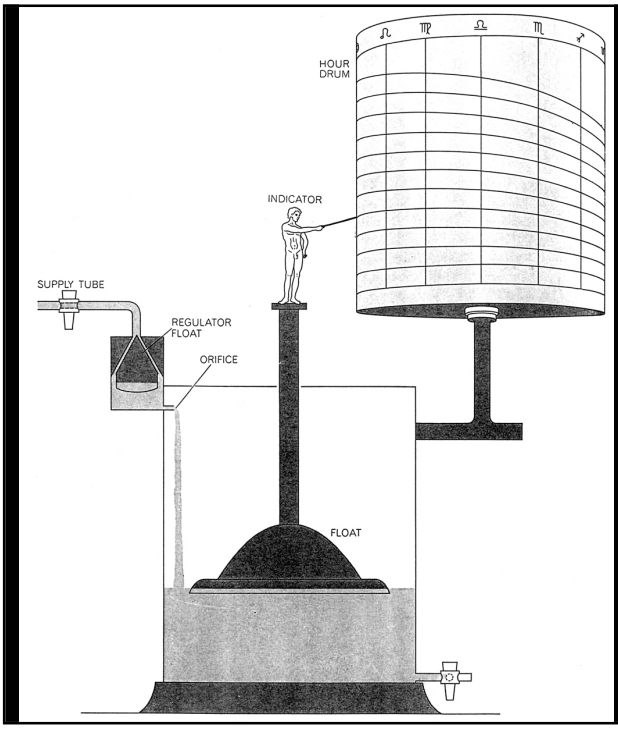
Kekkon-1

Ghiglione et al. 1999

Membrane-spanning
Egfr-specific
Binds Egfr

Sprouty

Intracellular
Blocks all RTKs
Binds membrane
Binds Drk (Grb2)
Binds Gap1



Negative feedback in 3rd century BC

Waterclock by Ktesibios of Alexandria

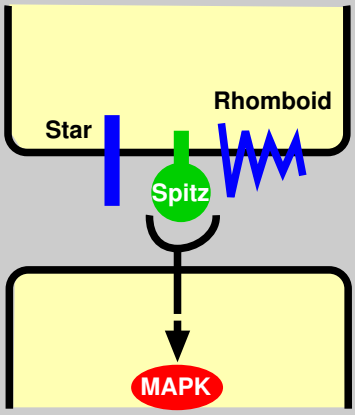
Negative feedback

Stabilises

Limits

Patterns

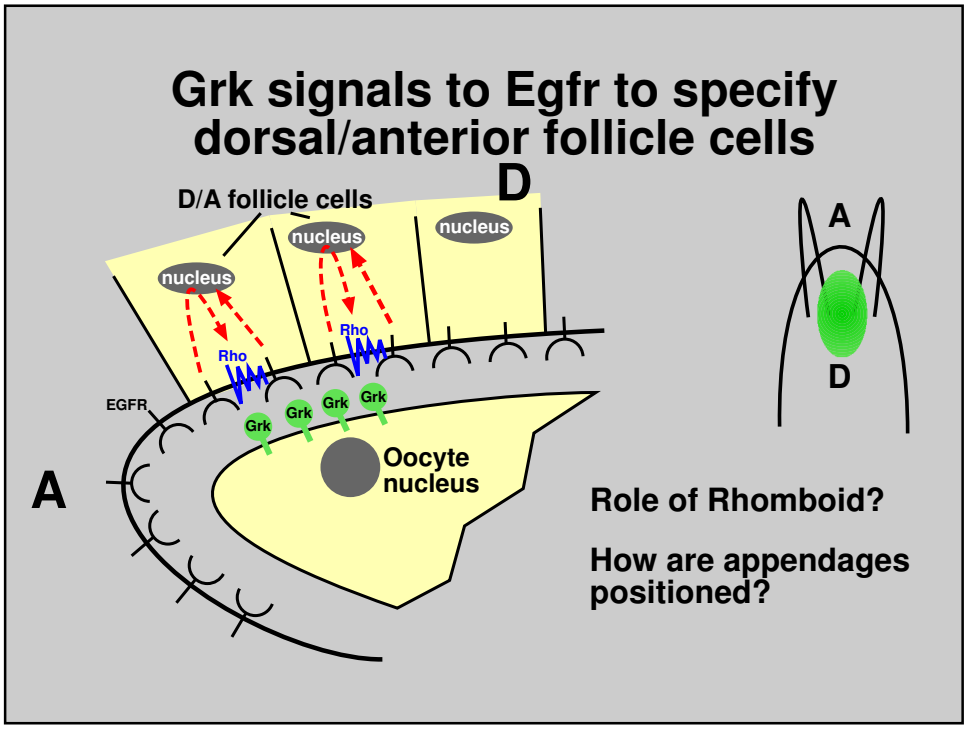
Rhomboid is a key activator of EGFR signalling

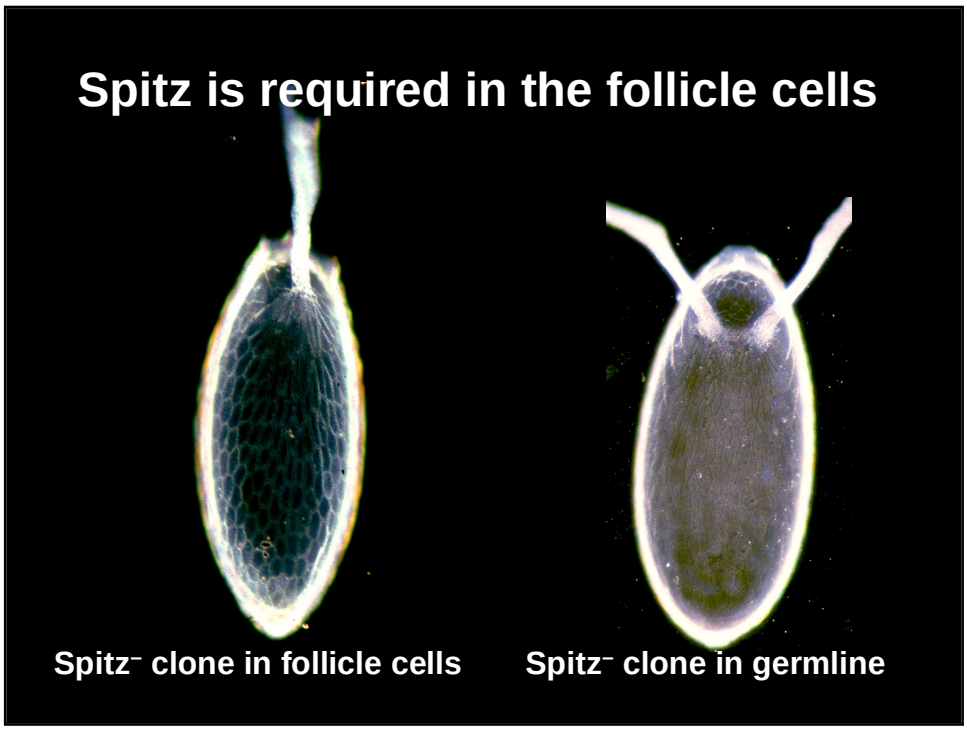


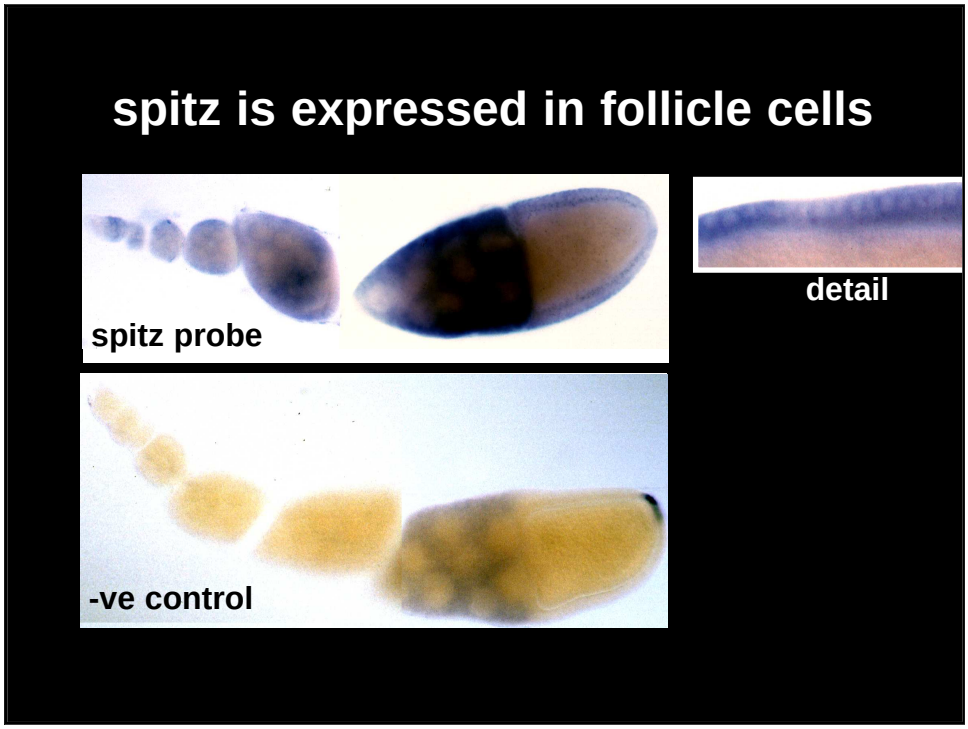
Necessary

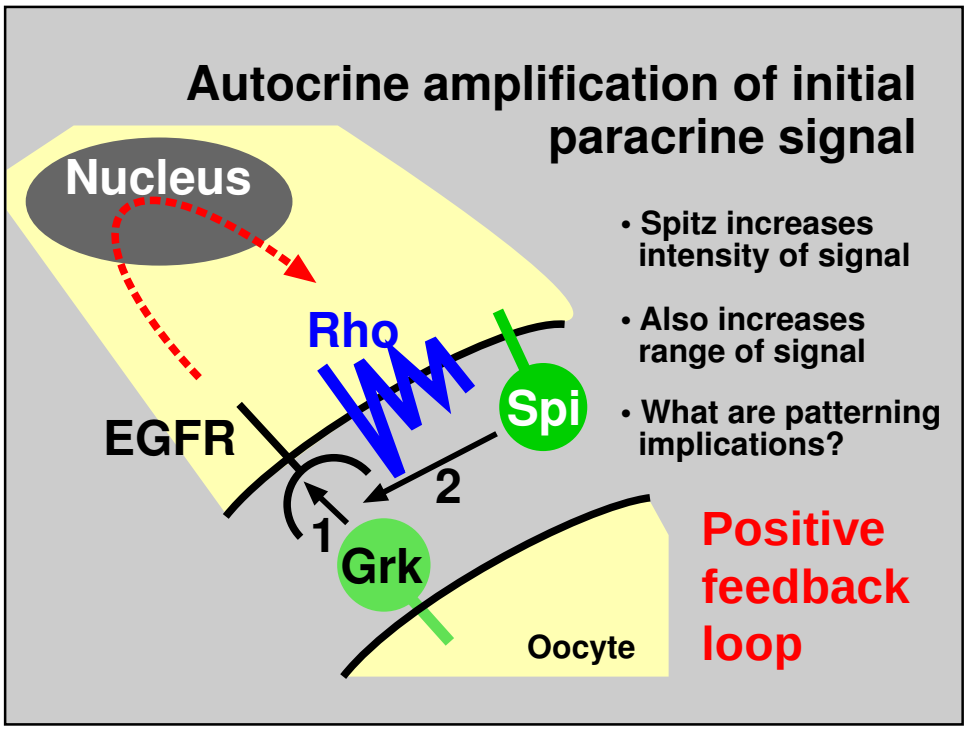
Sufficient

Expression prefigures activation

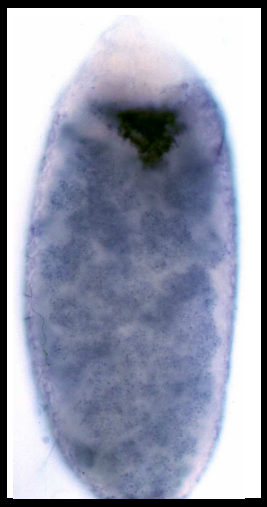

Grk signals to Egfr to specify dorsal/anterior follicle cells
D
D/A follicle cells
nucleus
nucleus
nucleus
Rho
Rho
EGFR
Grk
Grk
Grk
Grk
Oocyte nucleus
A
A
D
Role of Rhomboid?
How are appendages positioned?


Spitz is required in the follicle cells
Spitz⁻ clone in follicle cells
Spitz⁻ clone in germline


spitz is expressed in follicle cells
spitz probe
detail
-ve control

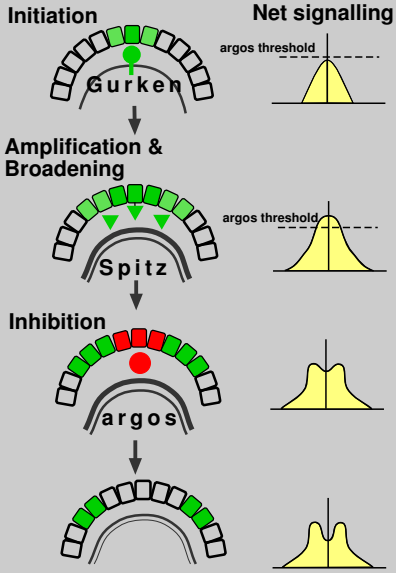

Autocrine amplification of initial paracrine signal
Nucleus
Rho
Spi
EGFR
2
1
Grk
Oocyte
• Spitz increases intensity of signal
• Also increases range of signal
• What are patterning implications?
Positive feedback loop

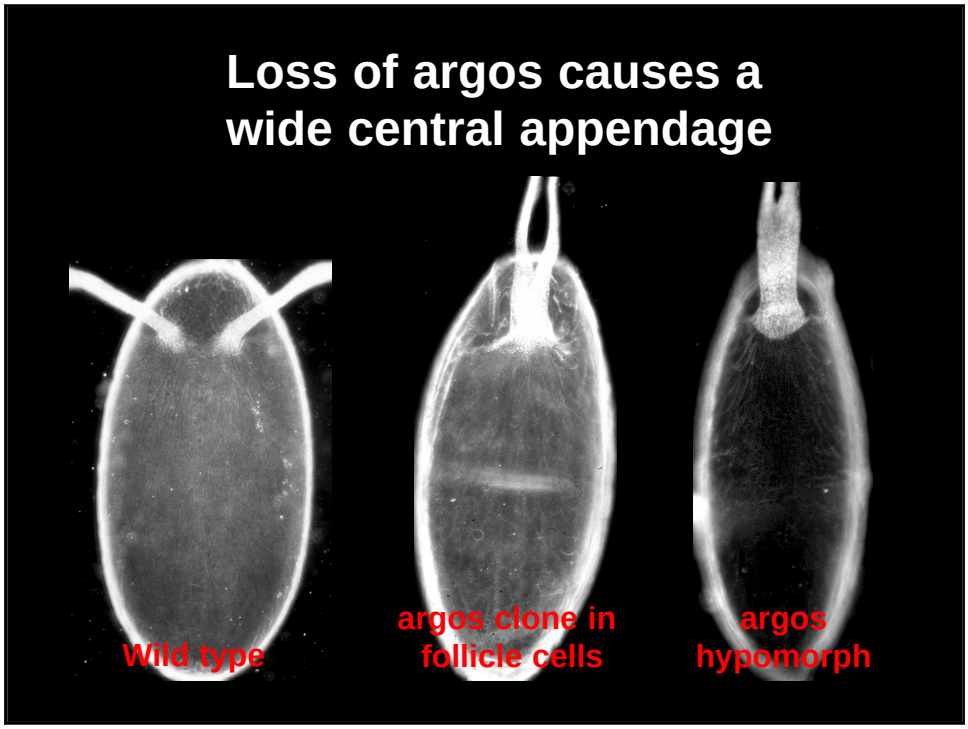
argos is expressed in dorsal anterior of oocyte

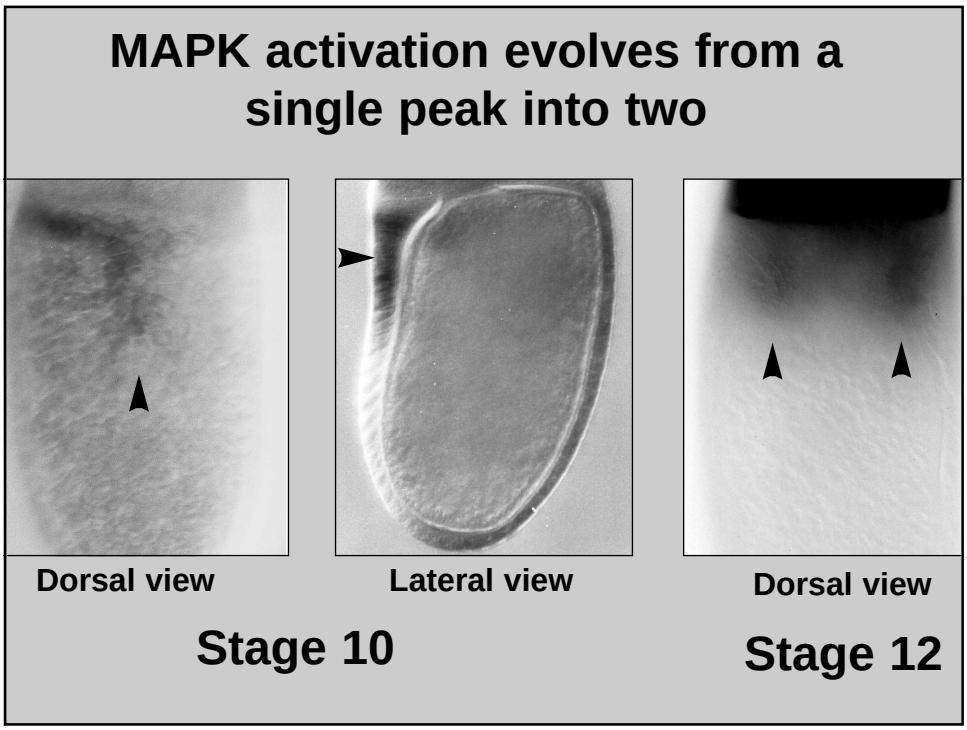


Stage 12 oocyte

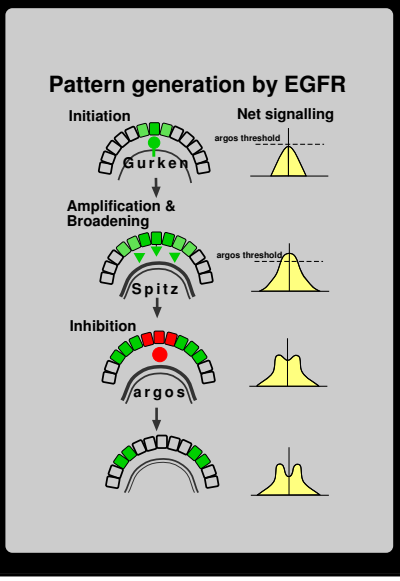
Pattern generation by EGFR



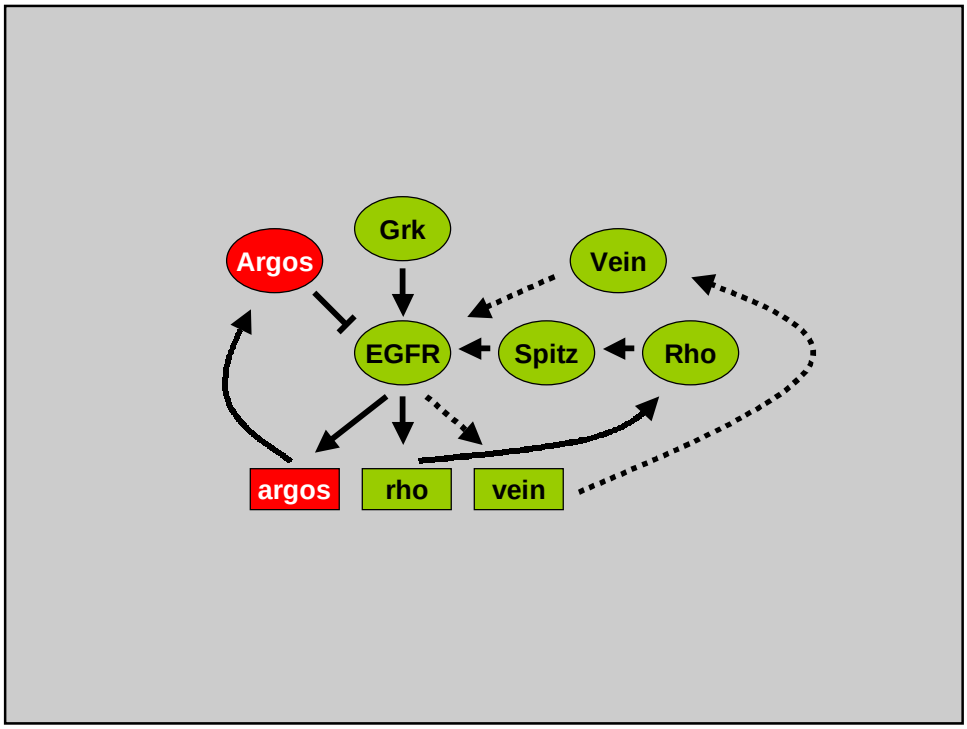

Loss of argos causes a
wide central appendage
Wild type
argos clone in
follicle cells
argos
hypomorph


MAPK activation evolves from a
single peak into two
Dorsal view
Lateral view
Dorsal view
Stage 10
Stage 12

Interlocking negative and positive feedback loops pattern the egg

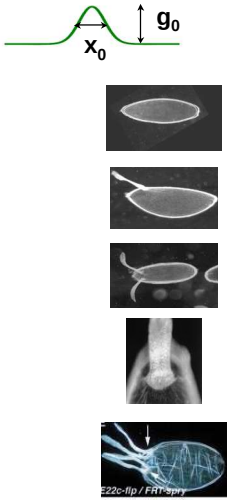


An automatic pattern-generating circuit

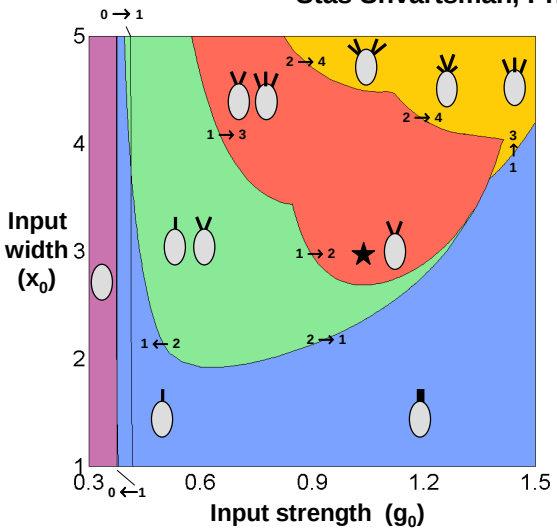


Possible Phenotypes:

effect of input parameters



Stas Shvartsman, Princeton



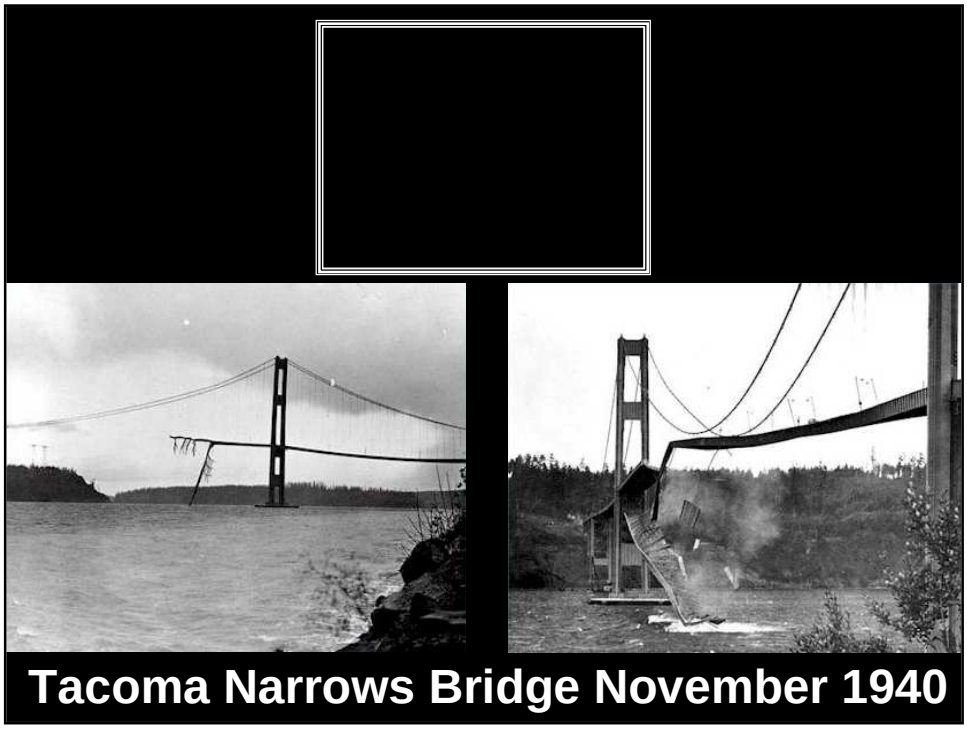
Positive feedback

Produces binary response

Amplifies

Can lead to dangerous instability

(don' t forget transcriptional autoregulation)



ErbB receptors in disease

Cancer
brain
head/neck
oesophagus
mouth
breast
lung
stomach
pancreas
kidney
colon
prostate
cervix
ovary

Other
psoriasis
atherosclerosis

Therapeutic opportunities
wound healing
nerve injuries

Significant role for positive feedback

Lewis, you mentioned earlier that the types of forces that cells generate are very few, and that small changes in any of them can have profound influences on the end result. One would think, then, that there should be lots of feedback mechanisms, to ensure reproducibility...

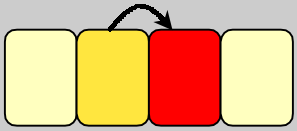
L.W.: Very little feedback at all -one of the most remarkable features about development is the virtual total absence of feedback.

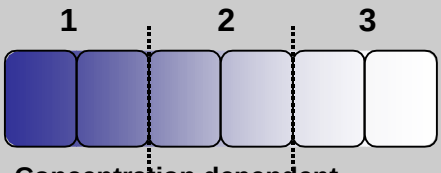
A.G.B.: but there is feedback! Development has lots of regulation, and that is a form of feedback. Most of developmental operation involve counteracting forces, they are done by antagonisms. The way the HLH products work is by titrating each other!

L.W.: Titration is not feedback: a threshold is not a thermostat. Negative feedback has a well-defined classical meaning: you actually have to measure something, and then if you have too much you make less, and if you have too little you make more. There is no feedback in development, nor even in the regulation of developmental genes: if you put extra copies of bicoid, you make more bicoid proteins

Lewis Wolpert and Antonio Garcia-Bellido interviewed by Alain Ghysen Int J Dev Biol 1998

Inductive vs. Morphogen Signals
Part 2





Concentration independent

Threshold

Binary

Concentration dependent

Direct relationship between signal intensity and outcome

Feedback common (adds robustness and makes response digital)

Much less feedback(?) (uncouples input and output signal strength)

Enzyme signal cascades (amplification of input signal)

Signal cascades more dependent on protein-protein interactions

NB
Both morphogens and inductive signals can be used to elaborate complex patterns

Canalization

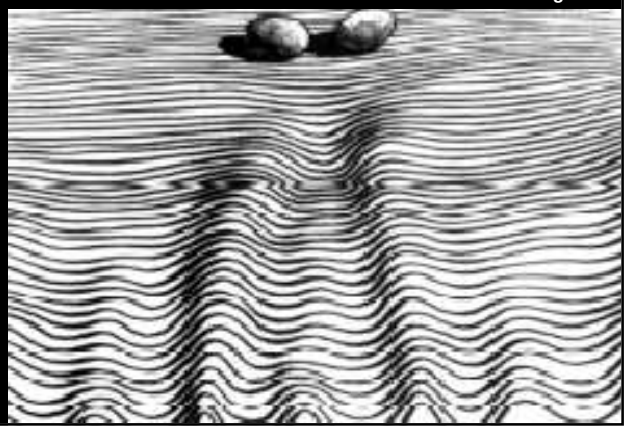
Robustness against perturbation

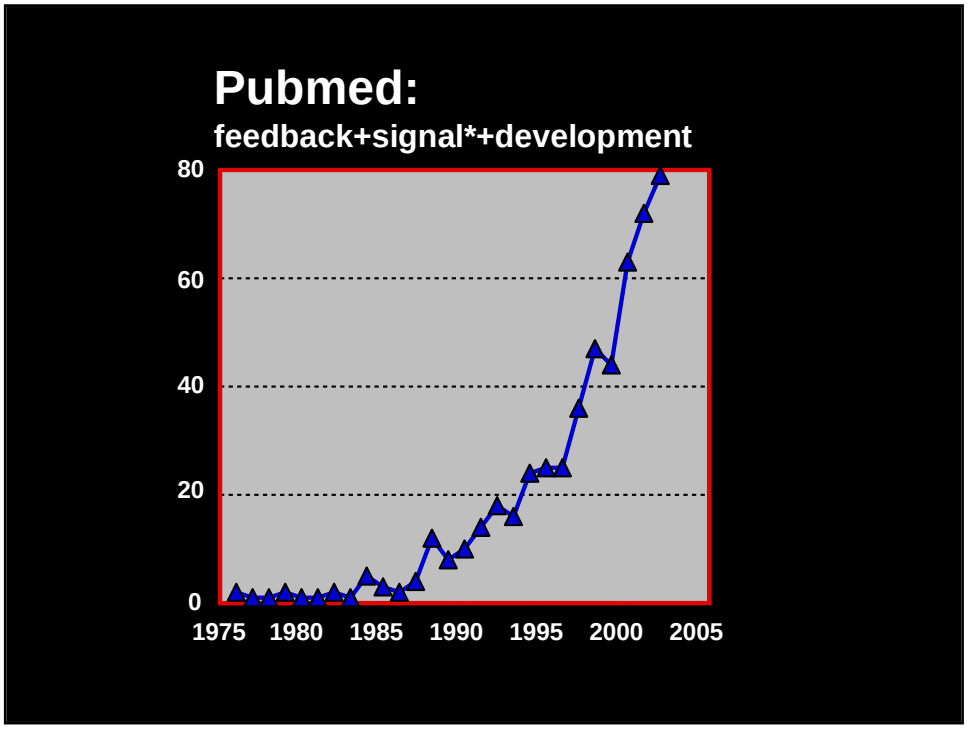
Negative feedback

All or nothing response: no half-way points

Positive feedback


Conrad Waddington

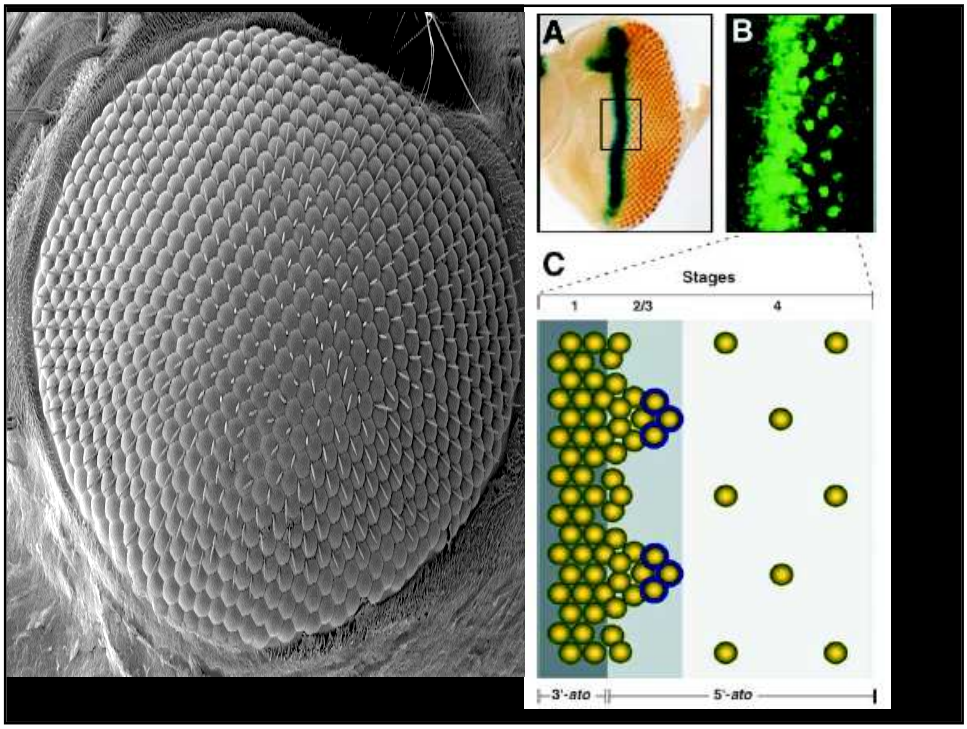


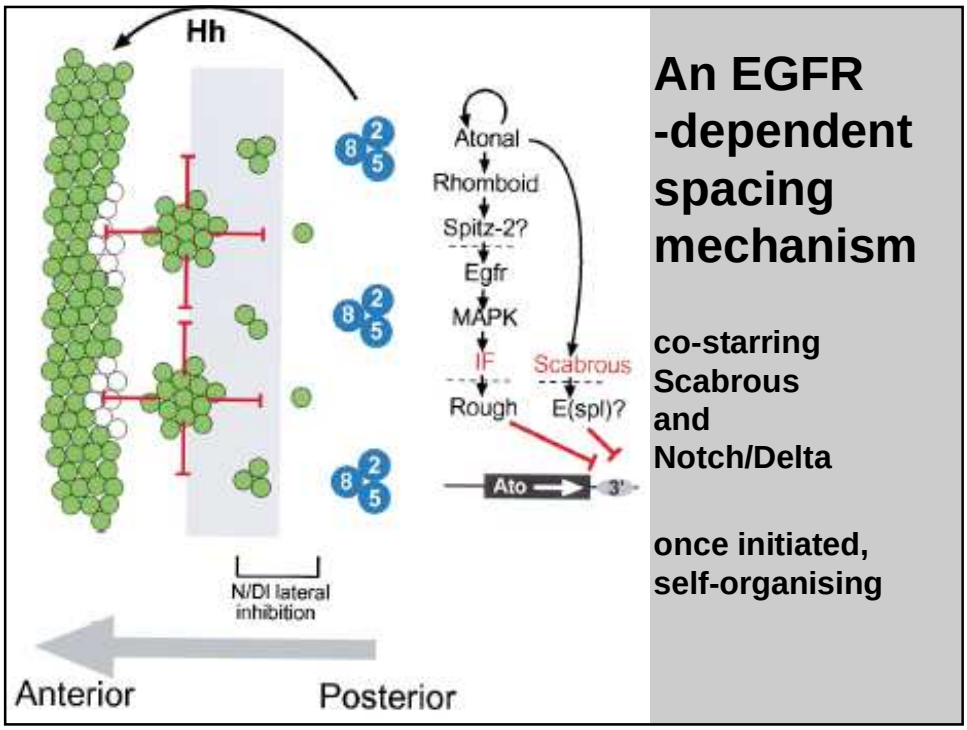


Patterning the fly eye by EGFR

Multiple roles

How is the ' crystalline' array formed?





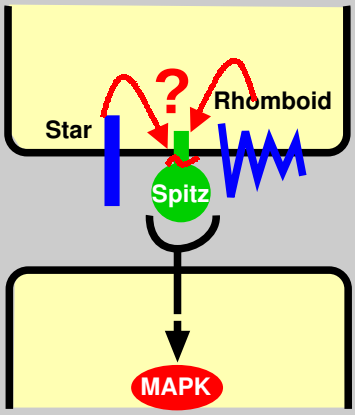
An EGFR -dependent spacing mechanism

co-starring Scabrous and Notch/Delta

once initiated, self-organising

A new molecular mechanism for intercellular signalling

Rhomboid and Star are principal activators of Egfr signalling



Necessary

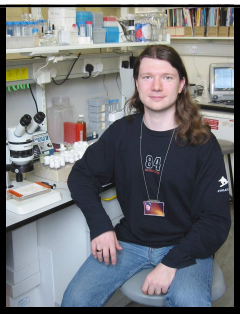
Sufficient

Expression prefigures activation

A conserved family of Rhomboids

ElegansE1344687
ElegansQ19821
Drosophila2
Drosophila1
Drosophila3
Human
Rat
Cerevisiae246c
Pombe2
HaemophilusGLPG
EcoliGLPG
ArabidopsisO81073
ArabidopsisO82765
Sugarcane
BsubtilisGLPG
BsubtilisYDCA
Mycobacteriumtub2
Pyrococcus
Providencia
Acinetobacter
Cerevisiae101w
Pombe1
TreponemaGLPG
Archaeoglobus
Dictyrho
Streptomyces
Aquifex
Synechocystis
Mycobacyeriumtub1
Mycobcteriumleprae

Jeff Lee

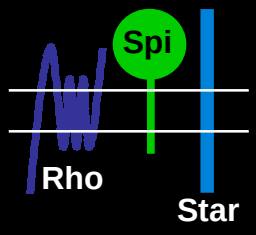


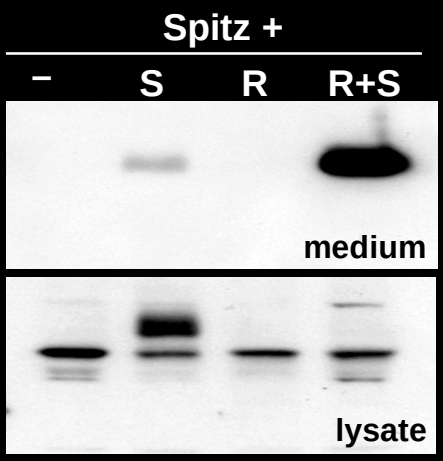
Sin Urban

Angus McQuibban

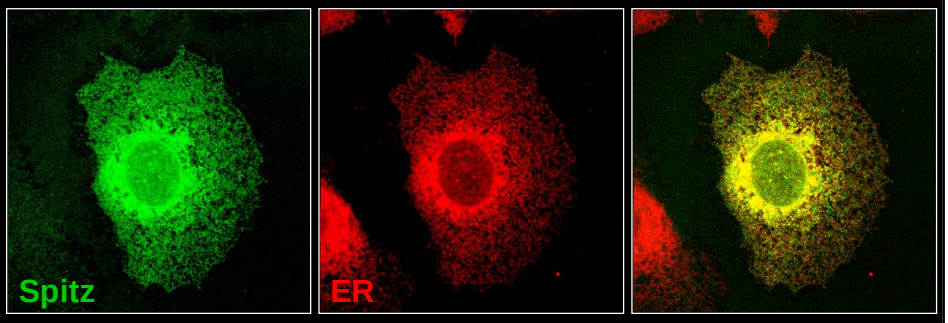
Olli Lohi

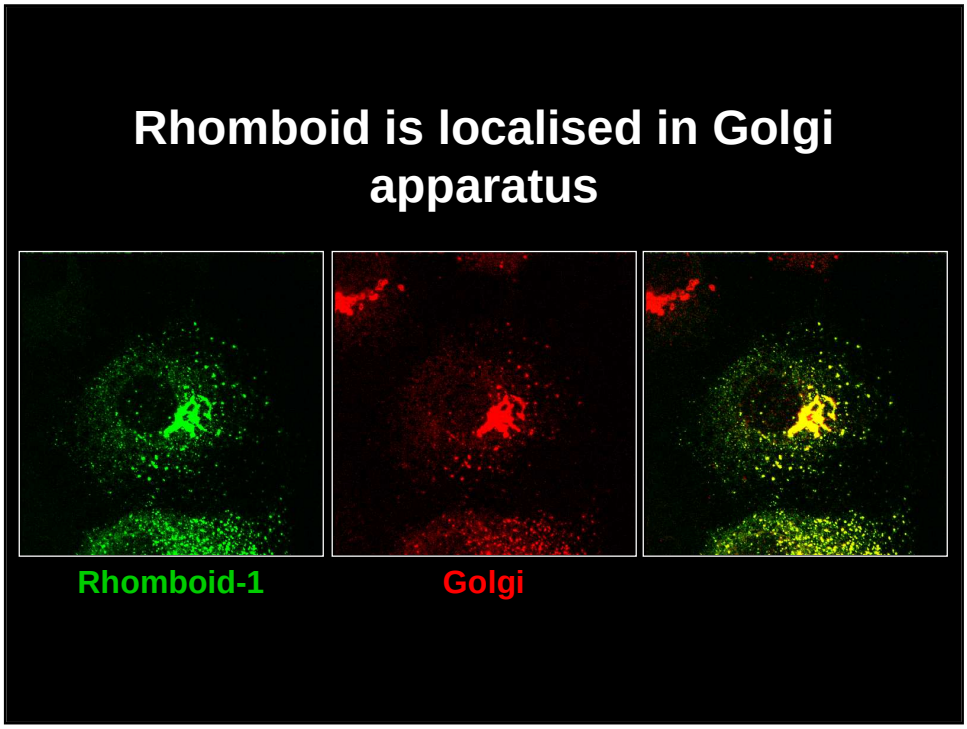
Rhomboid and Star-dependent Spitz cleavage in Cos cells

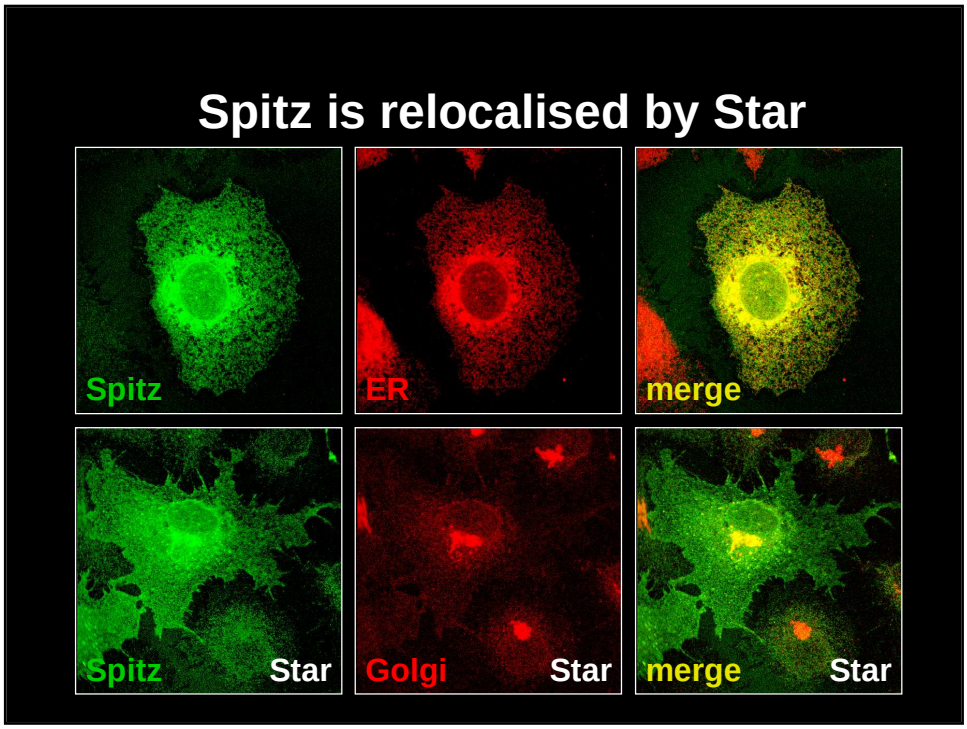


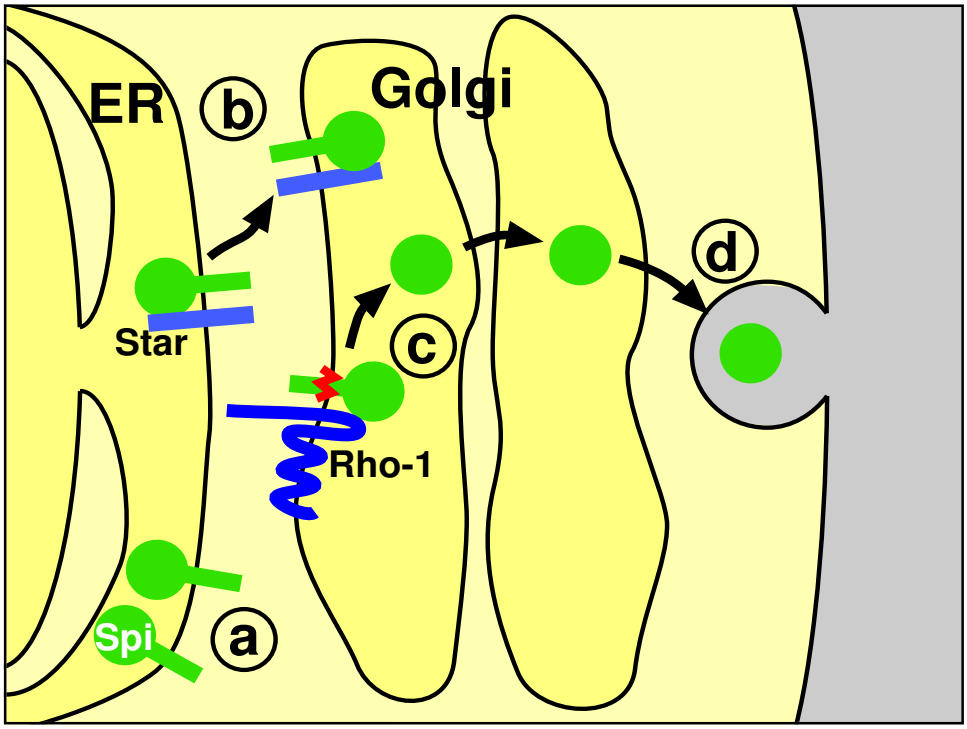


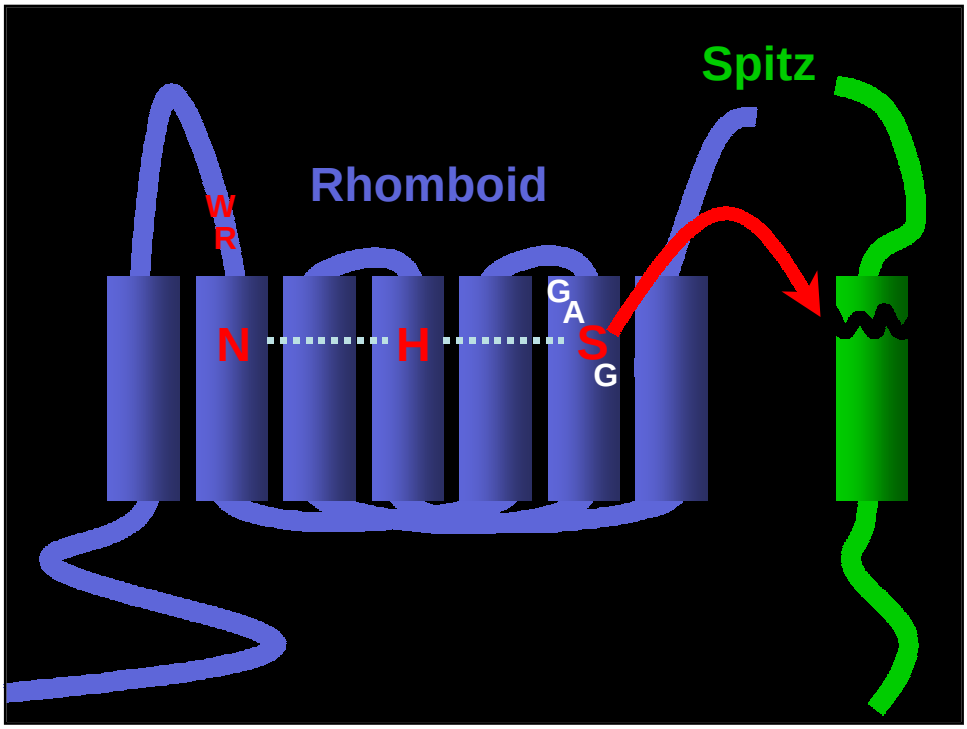
Spitz is localised in ER

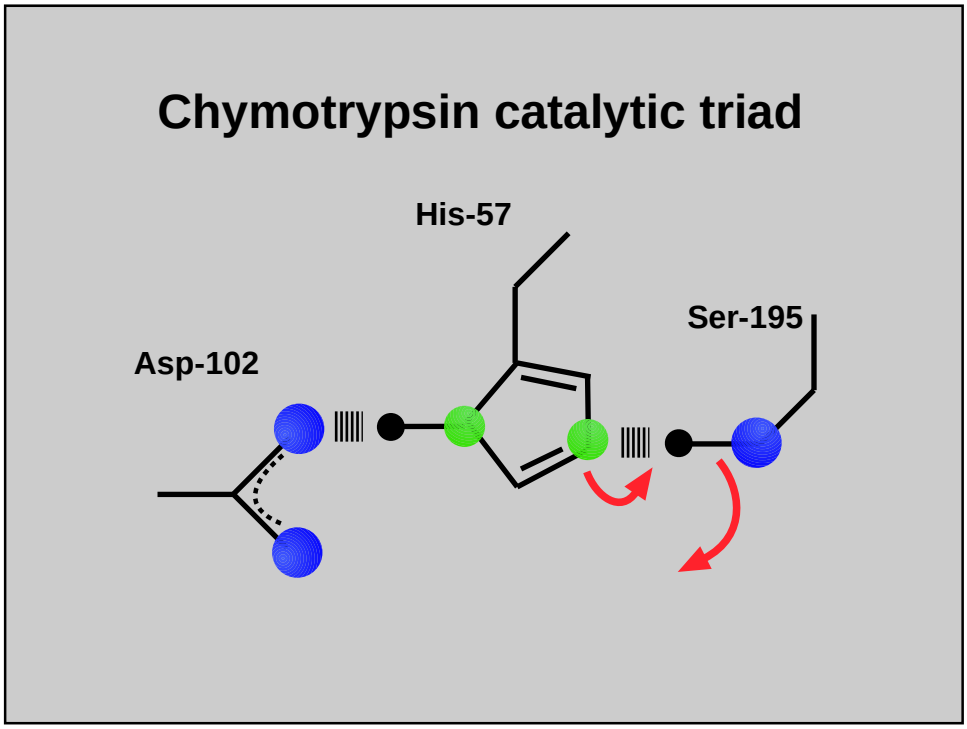


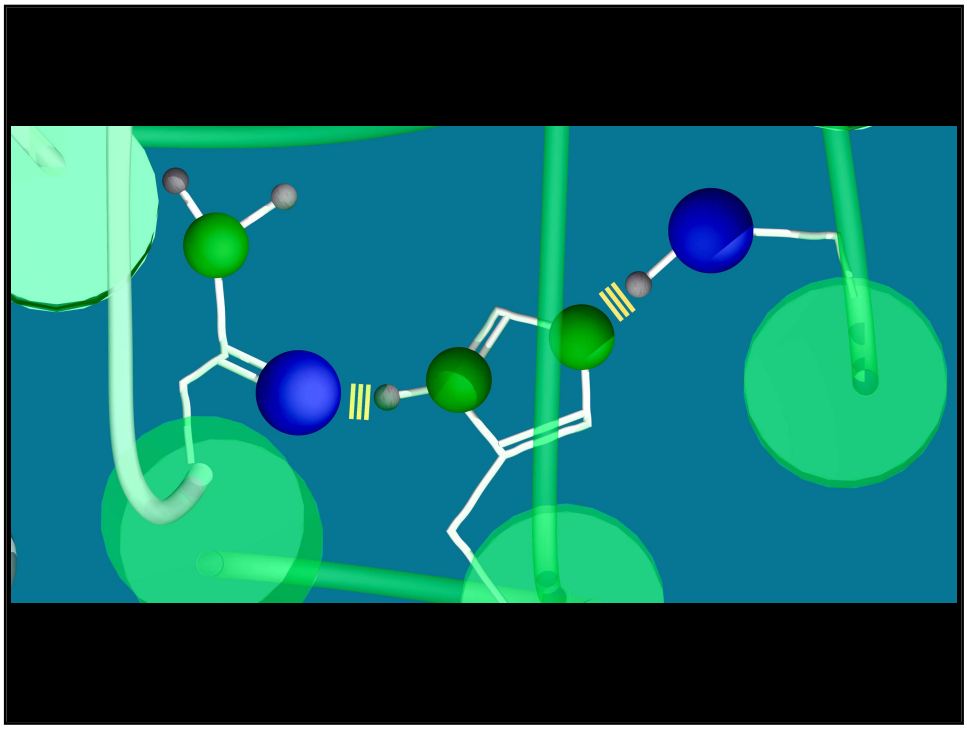

Rhomboid is localised in Golgi apparatus
Rhomboid-1
Golgi


Spitz is relocalised by Star
Spitz
ER
merge
Spitz
Star
Golgi
Star
merge
Star


ER
b
Golgi
Star
c
d
Rho-1
Spi
a


Spitz
Rhomboid
W
R
N
H
G
A
S
G


Chymotrypsin catalytic triad
His-57
Ser-195
Asp-102



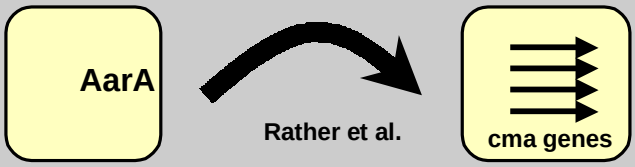
Regulated Intramembrane Proteolysis…

Site 2 protease	Polytopic membrane proteins
Presenilin	Metallo- and aspartyl proteases
	Release cytoplasmic domains
	of SREBP, Notch, APP etc
Rhomboid	Polytopic membrane protein
	Serine protease
	Releases luminal domains
	eg growth factors

…a widespread signalling mechanism

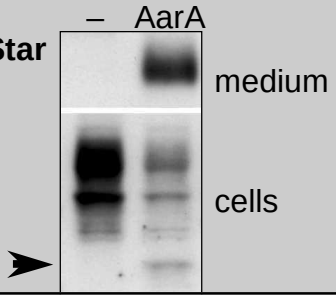
Bacterial rhomboids

Providencia stuartii

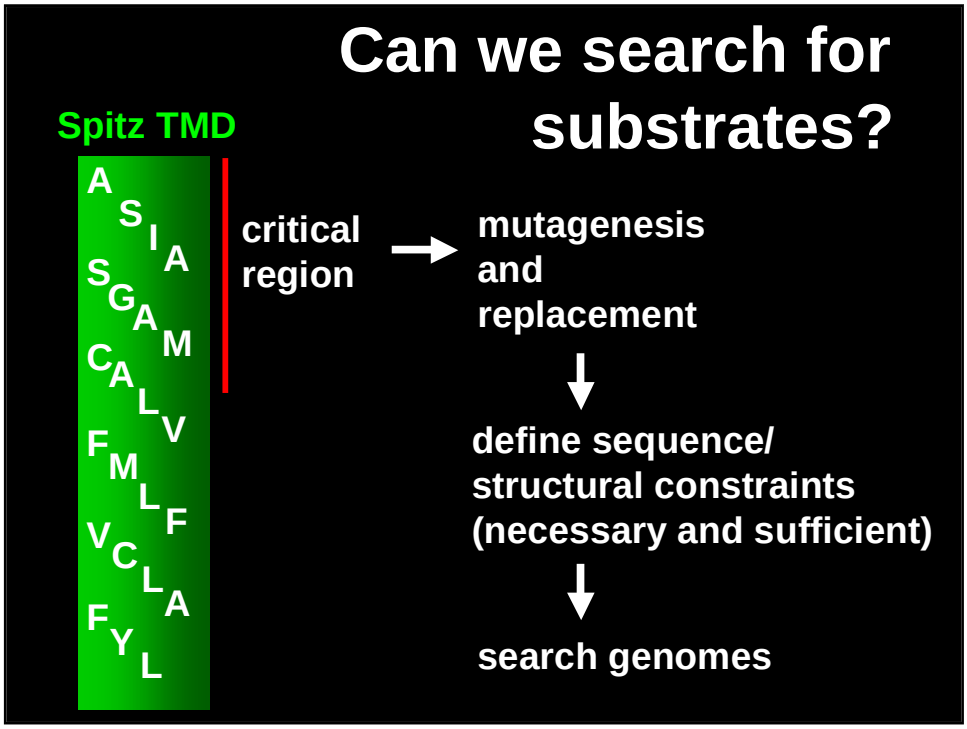


AarA = Rhomboid Gallio & Kylsten

Spitz + Star



Providencia Rhomboid cleaves Drosophila substrate

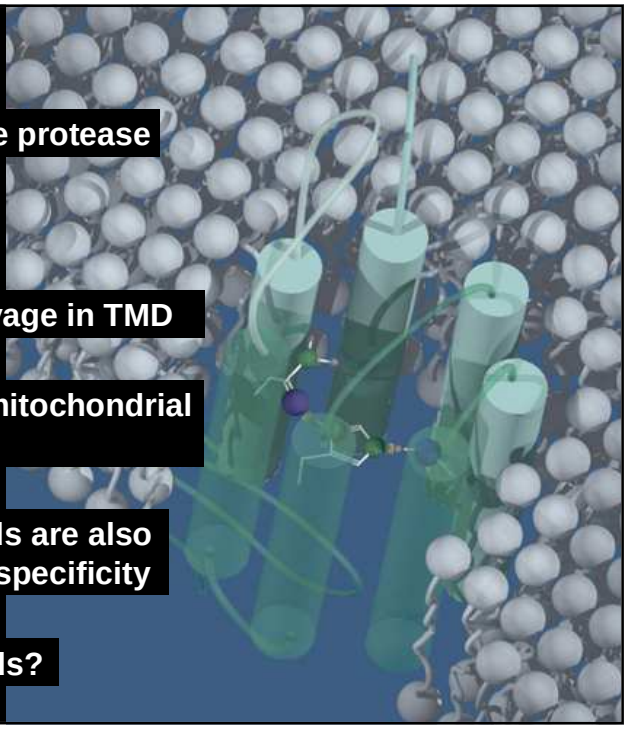


Mouse genome

20-30 candidates

Testable number!

Rhomboid



intramembrane serine protease

high specificity

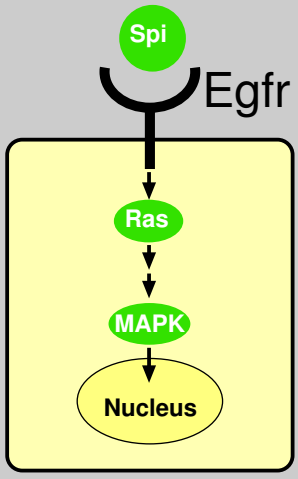
recognition and cleavage in TMD

rhomboids regulate mitochondrial membrane dynamics

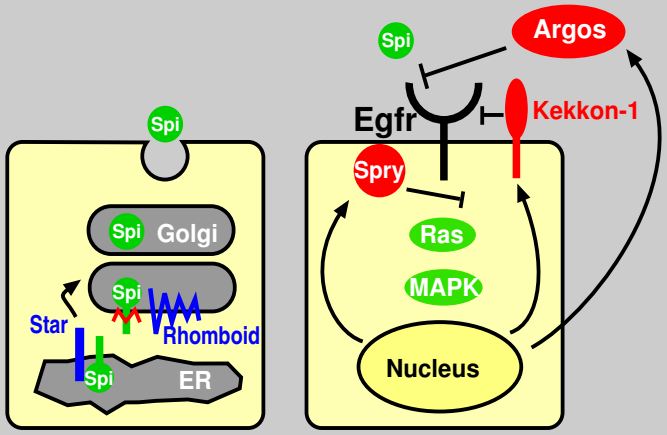
prokaryotic rhomboids are also proteases and share specificity

mammalian rhomboids?

Drosophila EGF receptor



Regulators of Egfr signalling in flies



Regulated intercellular signalling controls development

Feedback (positive and negative) is a key regulatory principle of developmental signalling

Inductive signalling is not a poor relation of morphogen signalling: both can elaborate complex patterns