The Evolution of the Cell State Splitter: Motility to Embryogenesis

Presented in the International Embryo Physics Course

http://www.embryophysics.org

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By

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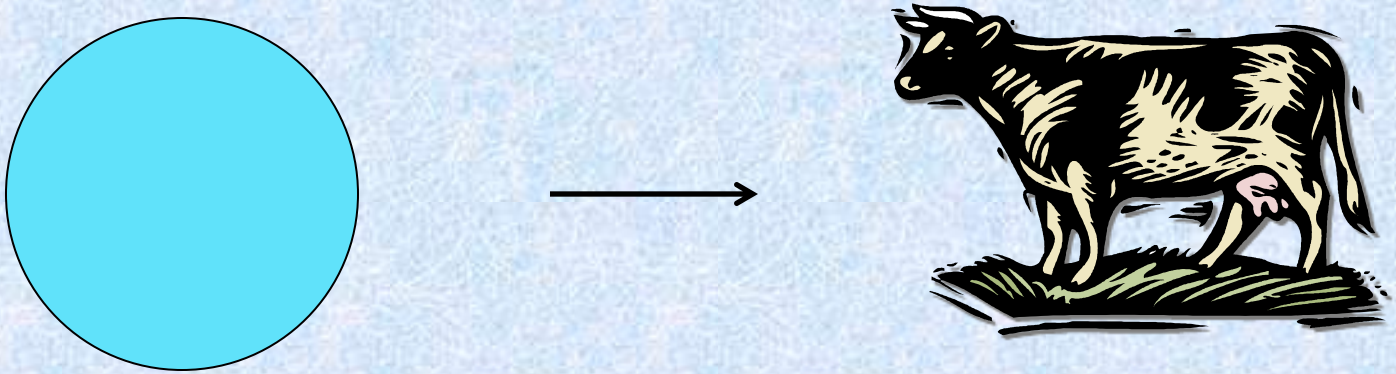
Embryogenesis: The Fundamental Problem

Given a spherical cow….



Fundamental Problem

- Go from one cell to many cells with many different functions.
- Get the different cells into
  - The right place
  - The right time
  - The right numbers

Normal mammalian development

1. Fertilized egg goes through multiple cell divisions to form a loose ball.
2. Cells on the outside of the ball form the trophoblast (future placenta, membranes)
3. Inner cells form inner cell mass which will be the embryo proper.
4. Embryo forms three basic cell layers
    1. Endoderm
    2. Mesoderm
    3. Ectoderm

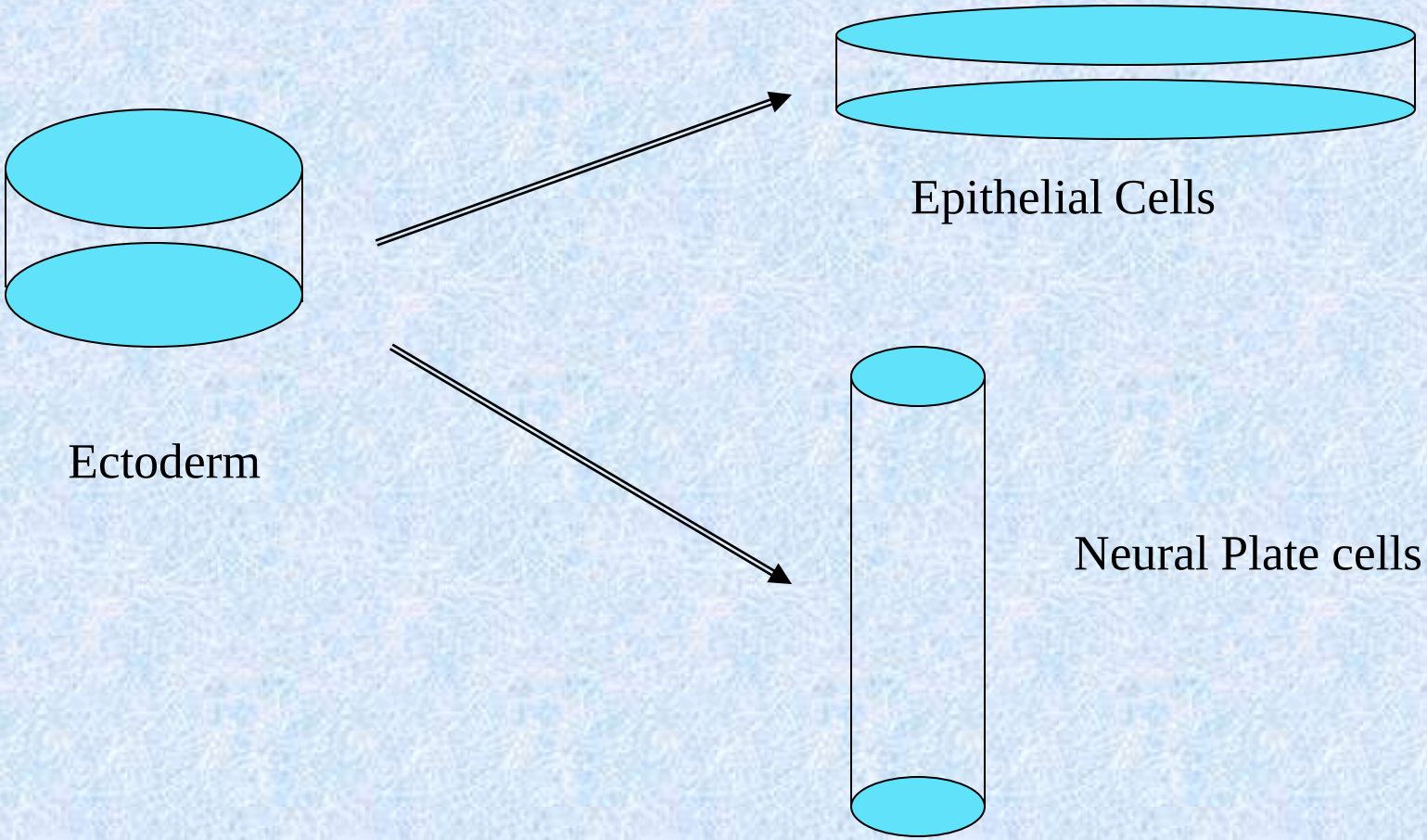
Gastrulation

1. Endoderm and mesoderm is tucked inside
2. Ectoderm stretches to cover the entire outside.

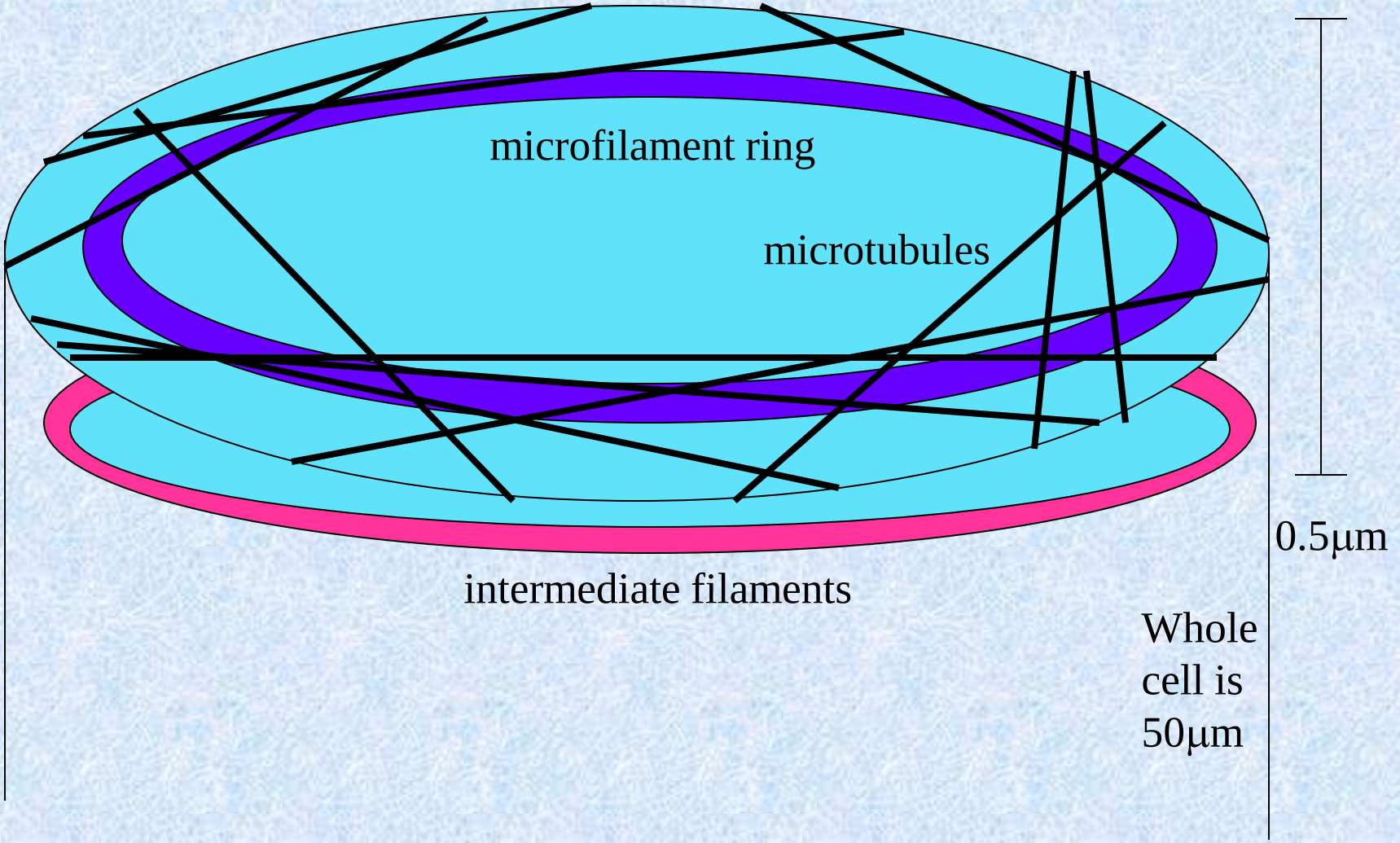
Neurulation

1. Ectoderm – forms all cells of the skin, neural tissue, brain, pigment cells, sweat glands
2. One half of ectoderm becomes neural tissue
3. One half becomes epithelial tissue
4. Neural ectoderm forms flat plate
5. Flat plate of neural cell sinks in the middle and the edges rise and join to form a tube.
6. Tube becomes fold to form brain, spinal cord

Ectoderm Differentiation

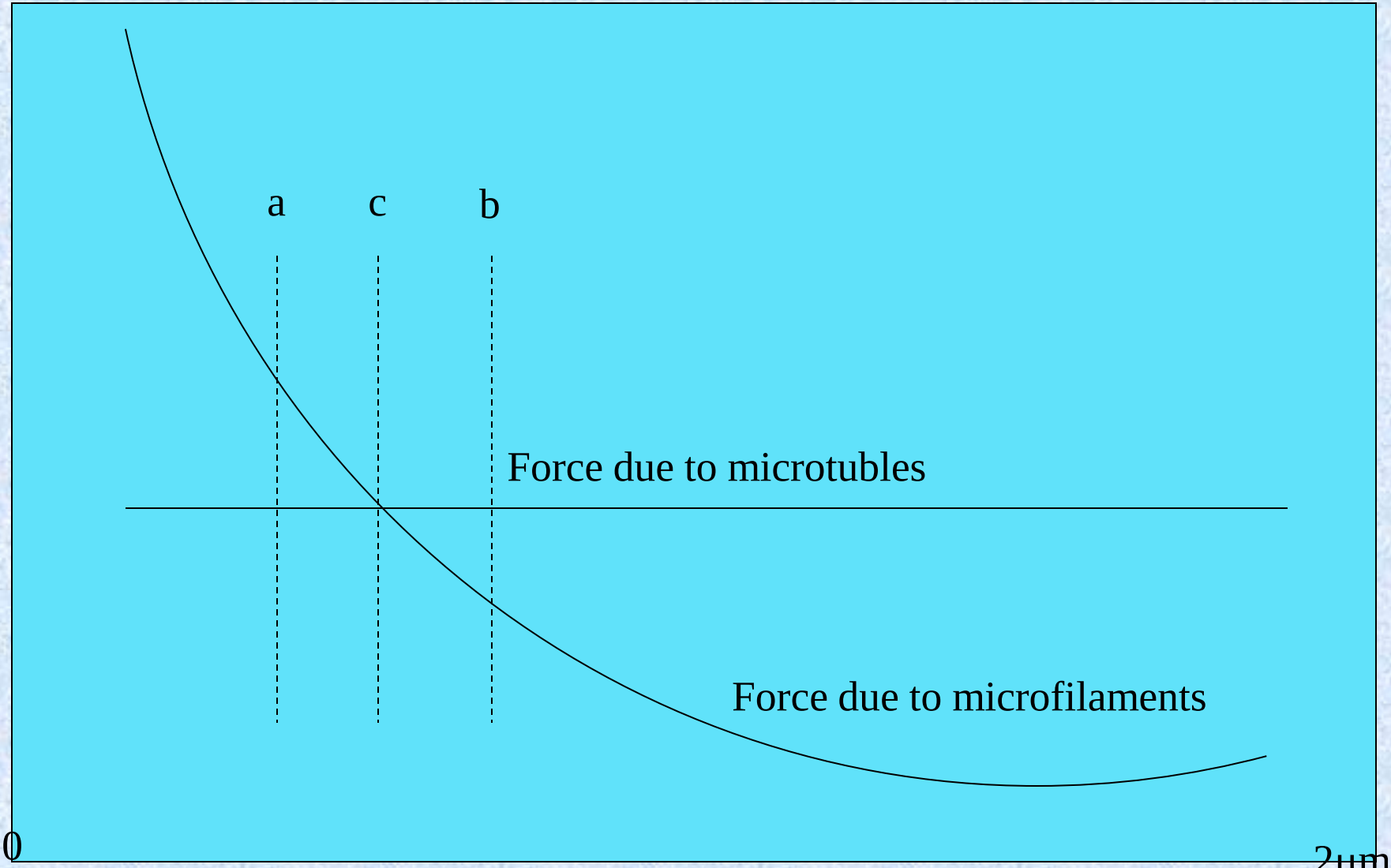


Apical end of ectoderm cell



The Cell State Splitter Model

- Cell state splitter is an organelle
  - Microtubules
  - Intermediate filaments
  - Actin filaments
- Microtubules exert steady force by polymerization
- Actin filaments in a ring exert a force that is proportional with the diameter (thick ring large force, thin ring less force)
- Intermediate filaments are elastic (resist change)


a
c
b
Force due to microtubles
Force due to microfilaments
0
2μm
Cell Diameter

Force balance

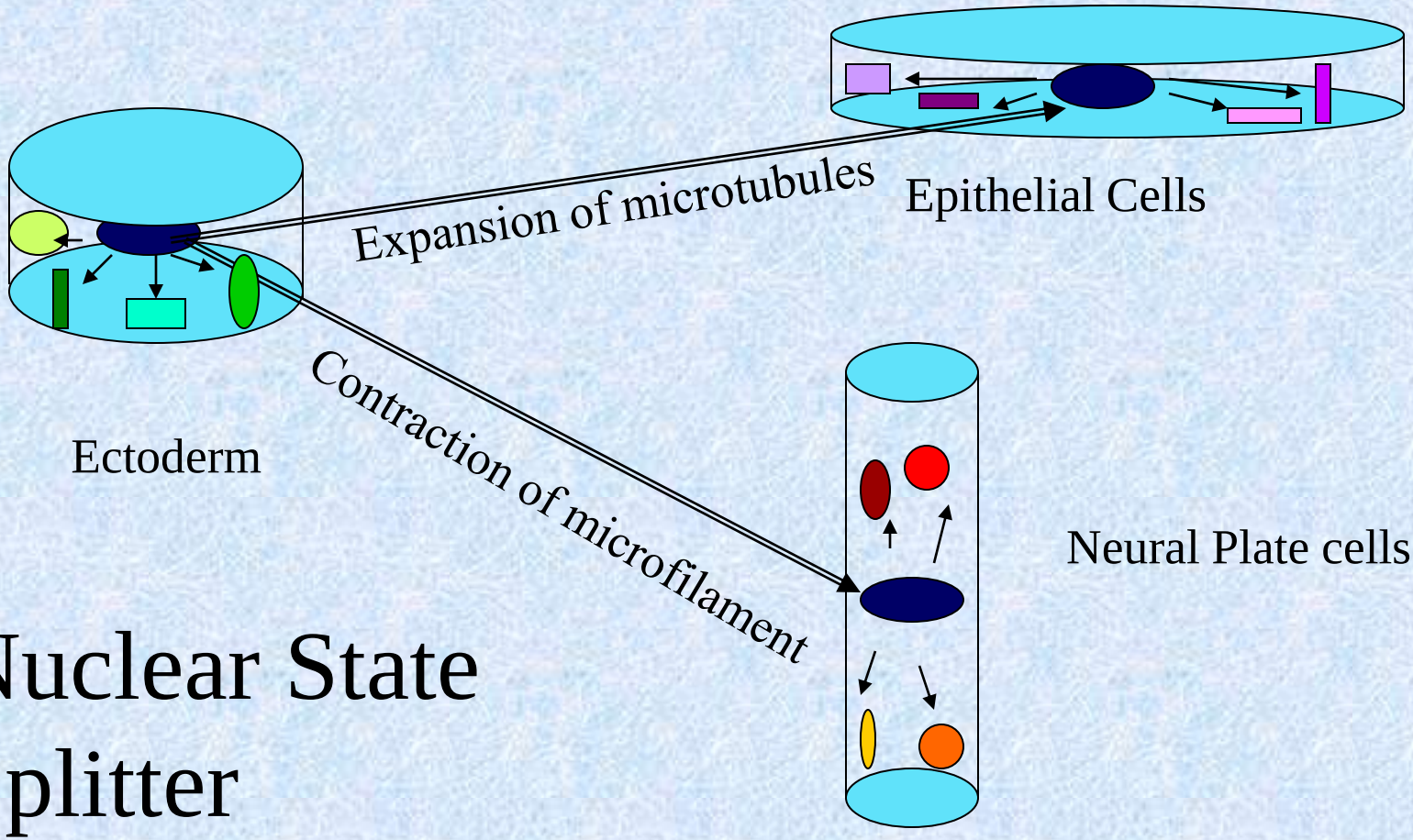
a) $F_{microfilament} > -F_{microtubles}$ = contraction

b) $F_{microfilament} < -F_{microtubles}$ = expansion

c) $F_{microfilament} = -F_{microtubles}$

Bistable organelle – resolve to one of possible two states

Ectoderm Differentiation



Nuclear State Splitter

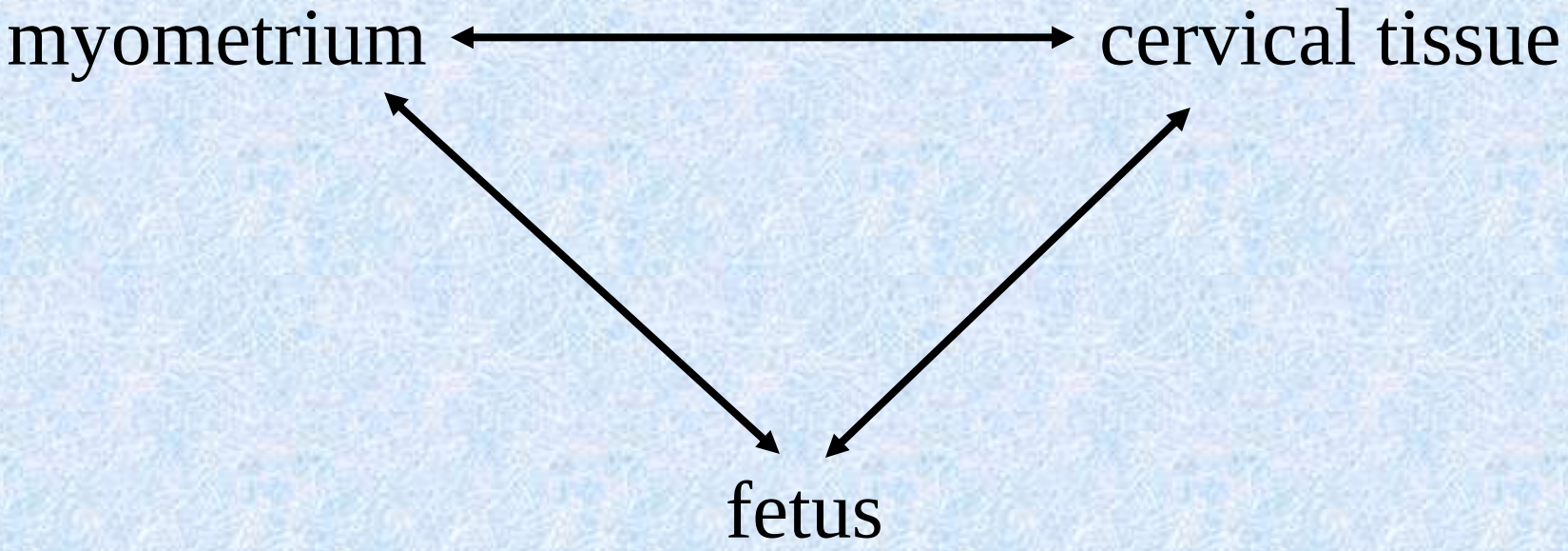
A bit of embryological jargon

- **Induction** = signal from cell to cell and from exterior of cell into nucleus that it is time to change into a new cell.
- (Determination)
- **Differentiation** = process of changing from one cell type to another.
- (Begins with changes in gene expression.)
- Cell state splitter = induction
- Nuclear state splitter = differentiation

Advantages to our model

- Requires physics – mechanical forces
- Mechanical forces trigger either an expansion or a contraction in a localized area.
- Genome responds to mechanical signal.
- Genome makes next cell state splitter and waits for next signal.
- Cell need not "know" anything going on around it or what any other cell is doing.
  - No reacting, reading, assessing, mediating, influencing, communicating, controlling, knowing required by any cell.

Initiation of labor



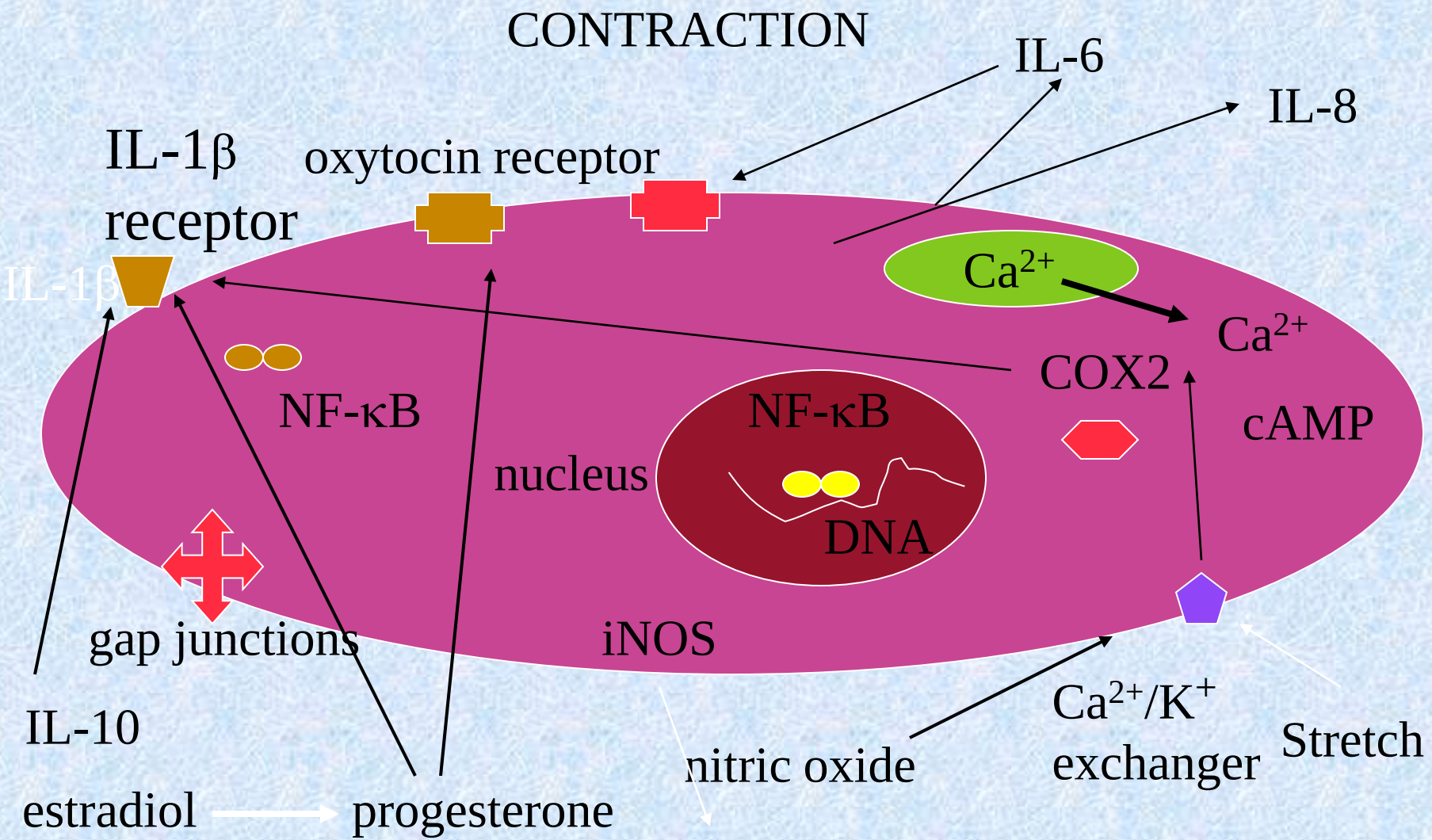
Initiation includes both mechanical (especially shear stress) of myometrium and chorioamniotic interface, hormonal signals, signals from the immune system via release of specific cytokine.

Myometrium

A signal, likely mechanical, perhaps chemical, perhaps both (IL-1β or TNF-α), occurs due to changes in the cervix. The result is an entire set of new proteins is expressed as labor begins.

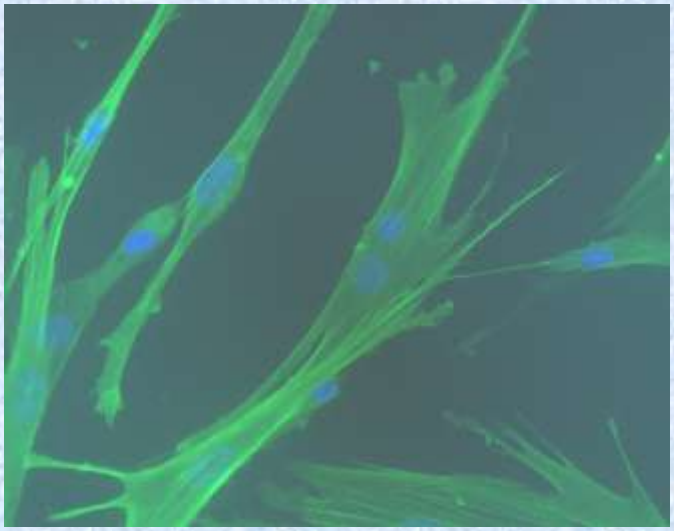
1) Ion channels (regulate membrane potential)
2) Agonist receptors to compounds that control the strength of labor contractions
3) GAP junctions to allow coordinated cell-cell coupling of the uterus.
4) cAMP down regulated

UtSM Cell Activation

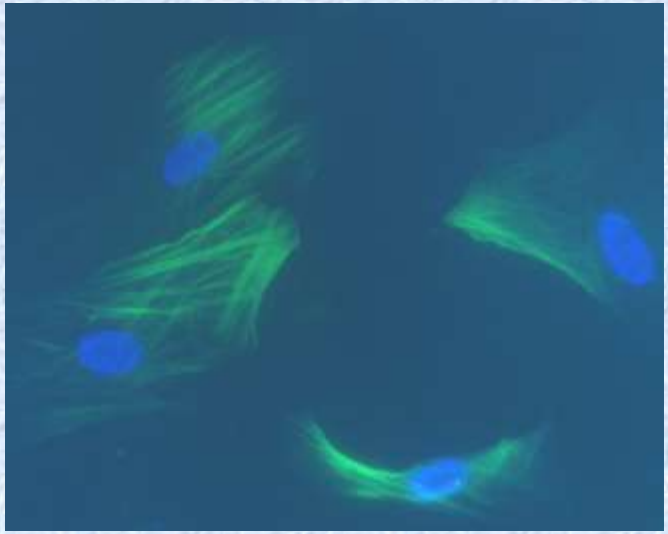


Fully Activated UtSM Cell.

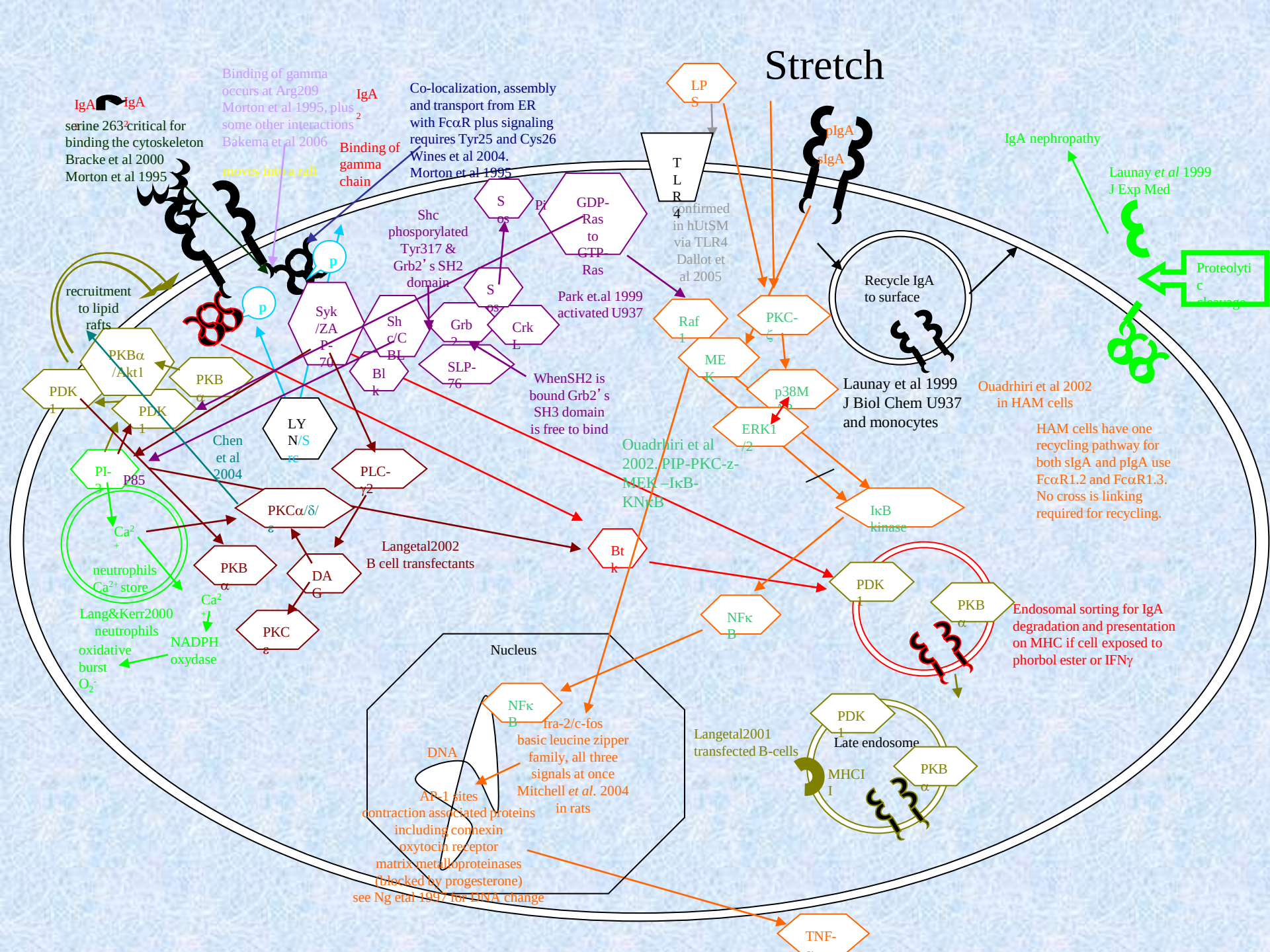
UtSM-CS04 cells (P3) prepared by the explant method stain for markers of smooth muscle cells: α-smooth muscle actin and calponin.

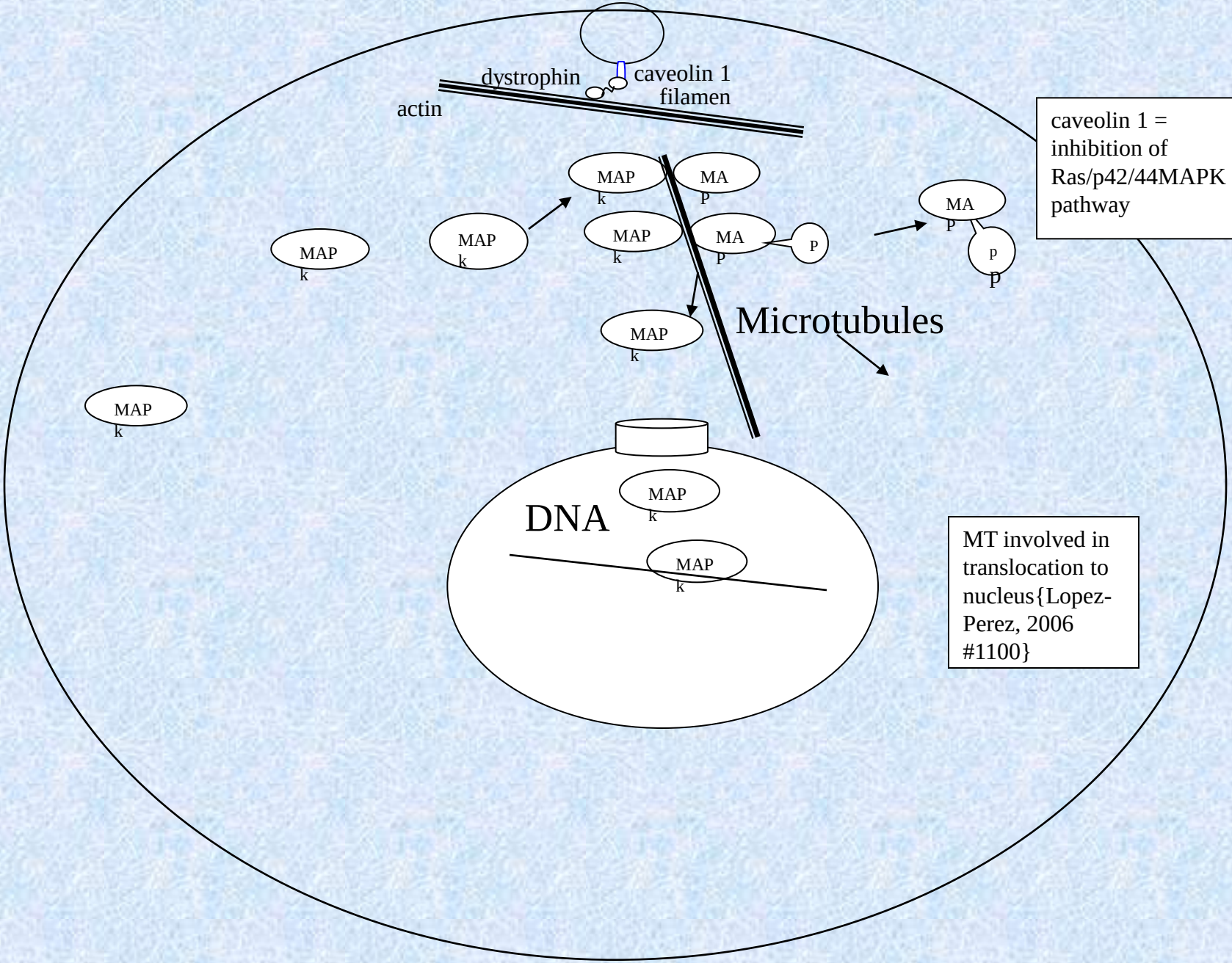


α-smooth muscle actin

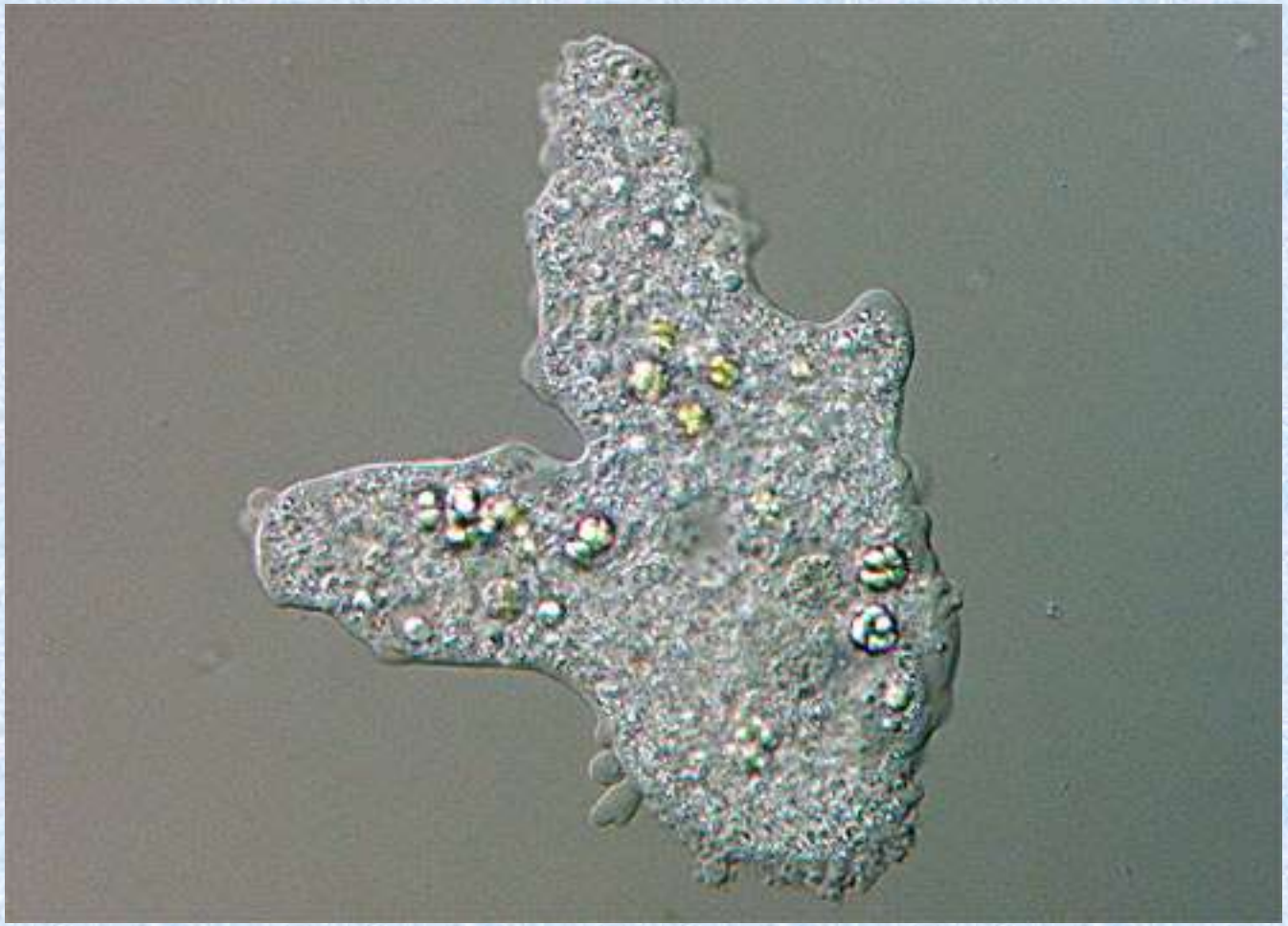


calponin


Stretch
IgA
IgA
serine 263 critical for binding the cytoskeleton
Bracke et al 2000
Morton et al 1995
Binding of gamma occurs at Arg209
Morton et al 1995, plus some other interactions
Bakema et al 2006
moves into a raft
IgA
2
Binding of gamma chain
Co-localization, assembly and transport from ER with FcαR plus signaling requires Tyr25 and Cys26
Wines et al 2004.
Morton et al 1995
LPS
TLR4
confirmed in hUtSM via TLR4
Dallot et al 2005
pIgA
sIgA
IgA nephropathy
Launay et al 1999
J Exp Med
Proteolytic cleavage
recruitment to lipid rafts
P
P
Syk /ZAP-70
Shc/CBL
Grb2
Crk L
Sos
Sos
Pi
GDP-Ras to GTP-Ras
Shc phosporylated Tyr317 & Grb2's SH2 domain
Park et.al 1999 activated U937
SLP-76
Blk
WhenSH2 is bound Grb2's SH3 domain is free to bind
Recycle IgA to surface
Raf 1
PKC-ζ
MEK
p38MAPK
ERK1/2
Launay et al 1999
J Biol Chem U937
and monocytes
Ouadrhiri et al 2002
in HAM cells
HAM cells have one recycling pathway for both sIgA and pIgA use FcαR1.2 and FcαR1.3. No cross is linking required for recycling.
PKBα/Akt1
PKBα
PDK1
PDK1
LYN/Src
Chen et al 2004
PI-3
P85
PLC-γ2
PKCα/δ/ε
Ca2+
neutrophils Ca2+ store
Lang&Kerr2000 neutrophils oxidative burst O2-
Ca2+
NADPH oxydase
PKBα
DAG
PKCε
Langetal2002
B cell transfectants
Btk
Ouadrhiri et al 2002. PIP-PKC-z-MEK –IκB-KNκB
IκB kinase
NFκB
PDK1
PKBα
Endosomal sorting for IgA degradation and presentation on MHC if cell exposed to phorbol ester or IFNγ
Nucleus
NFκB
fra-2/c-fos basic leucine zipper family, all three signals at once Mitchell et al. 2004 in rats
DNA
AP-1 sites
contraction associated proteins including connexin
oxytocin receptor
matrix metalloproteinases
(blocked by progesterone)
see Ng etal 1997 for DNA change
Langetal2001 transfected B-cells
PDK1
Late endosome
MHCI
PKBα
TNF-

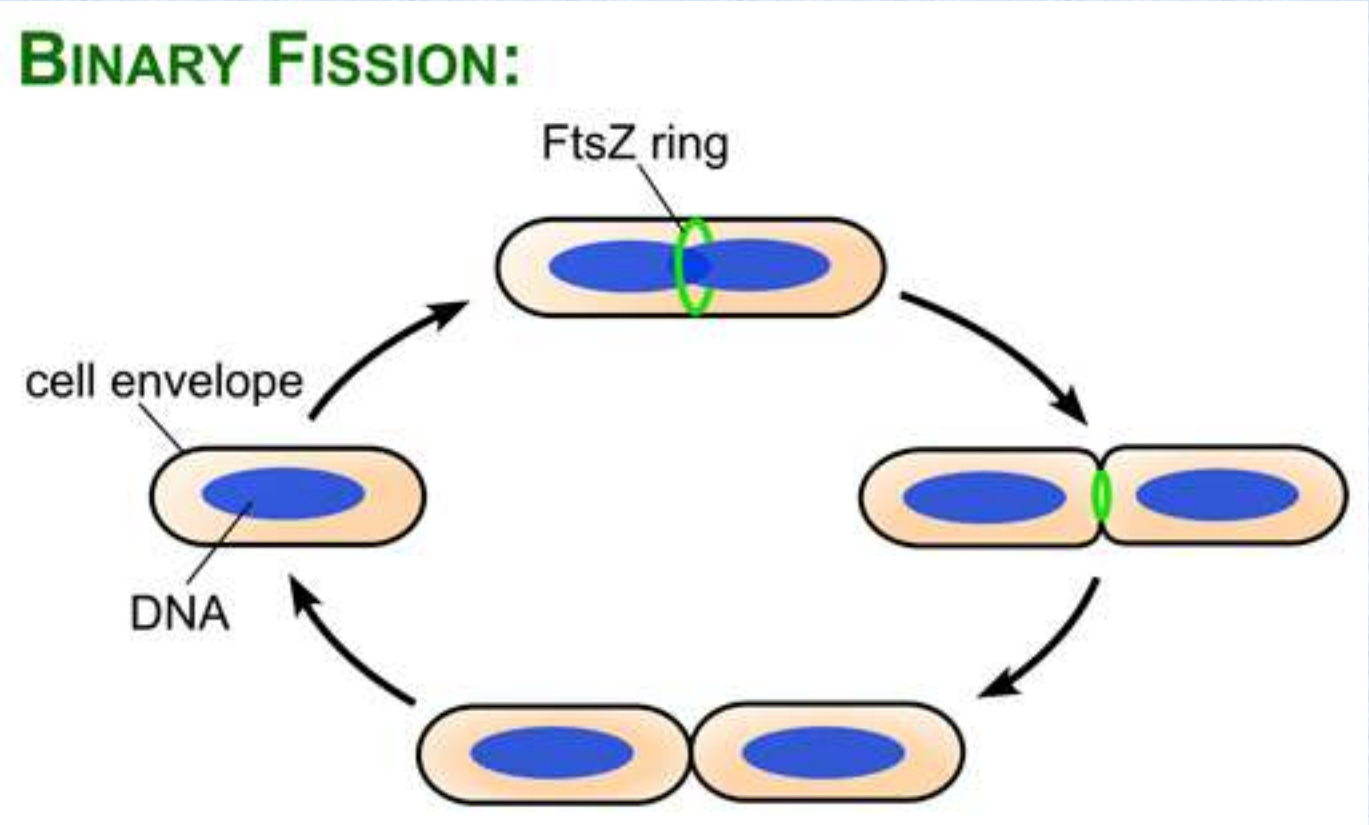

dystrophin
caveolin 1
filamen
actin
MAP
k
MA
p
MAP
k
MAP
k
MA
p
p
MA
p
p
p
MAP
k
Microtubules
MAP
k
MAP
k
DNA
MAP
k
MAP
k
caveolin 1 = inhibition of Ras/p42/44MAPK pathway
MT involved in translocation to nucleus{Lopez-Perez, 2006 #1100}

Where Did This System First Evolve From?



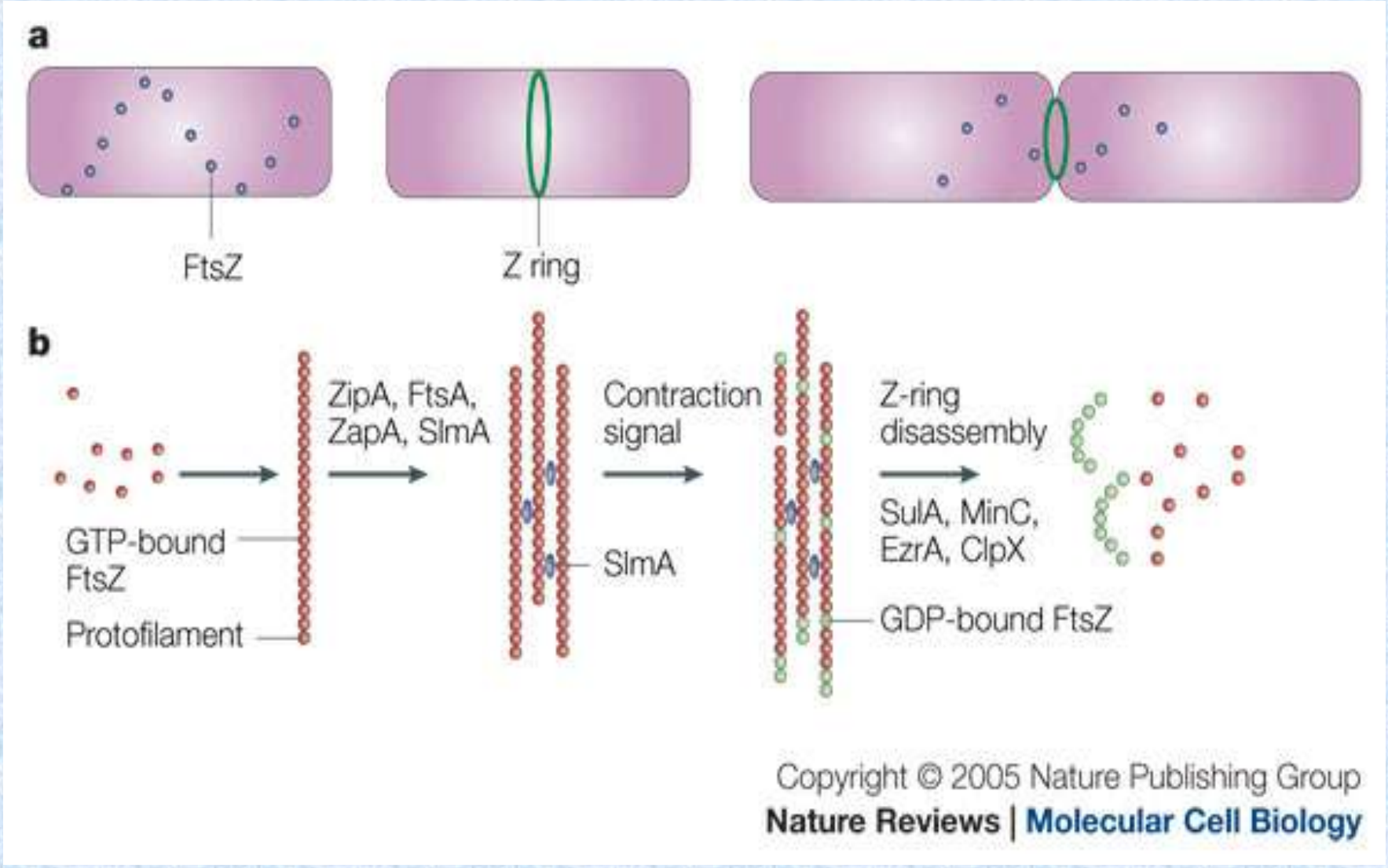
http://www.microscopyu.com/staticgallery/dxm1200/images/amoeba.jpg

Where Did Microtubules First Evolve From?

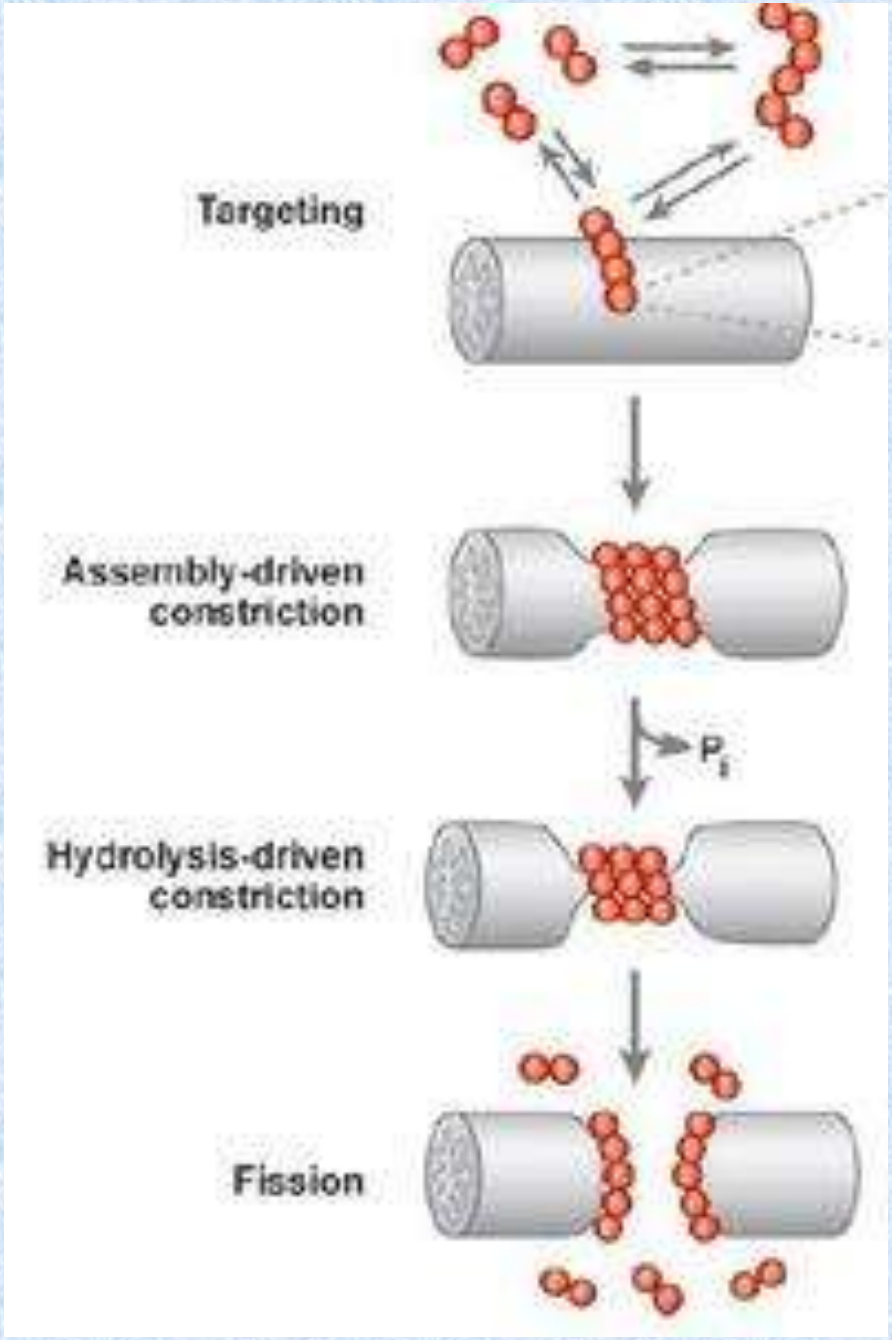


http://www.micro.cornell.edu/cals/micro/research/labs/angert-lab/images/binary_fission.jpg

Divide Now? Yes or No.



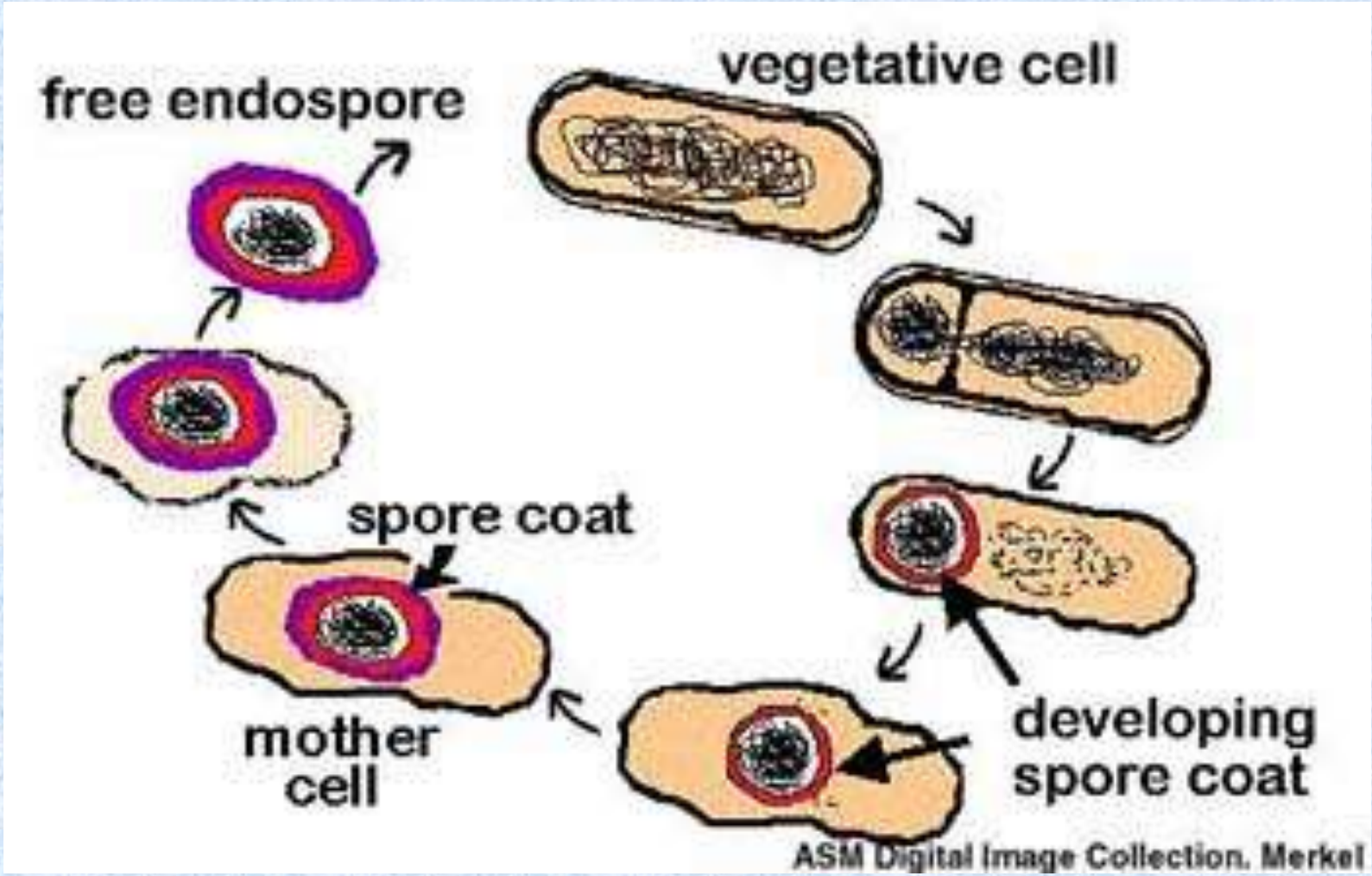
http://www.micro.cornell.edu/cals/micro/research/labs/angert-lab/images/binary_fission.jpg



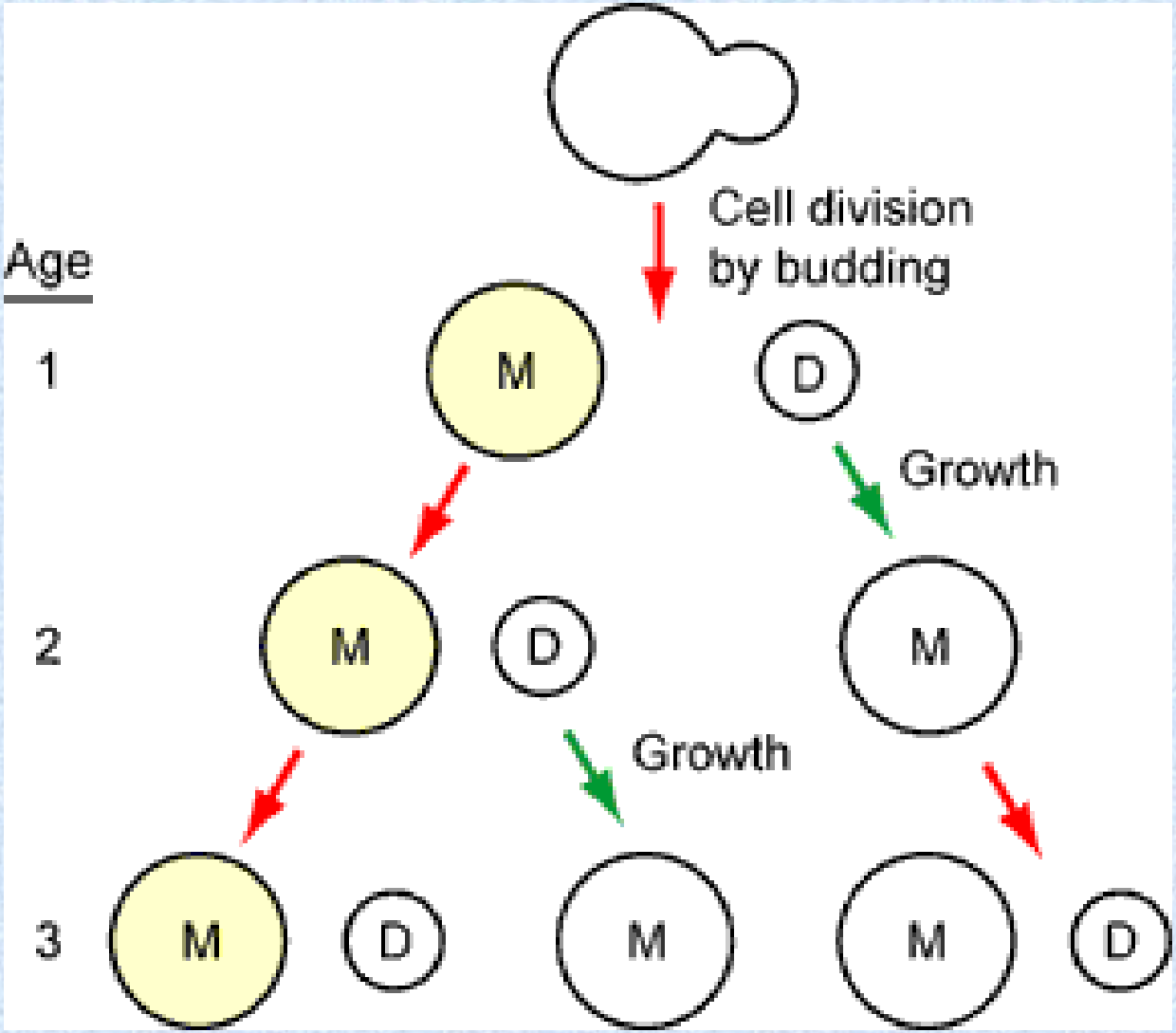
Divide Now?
Yes or No.

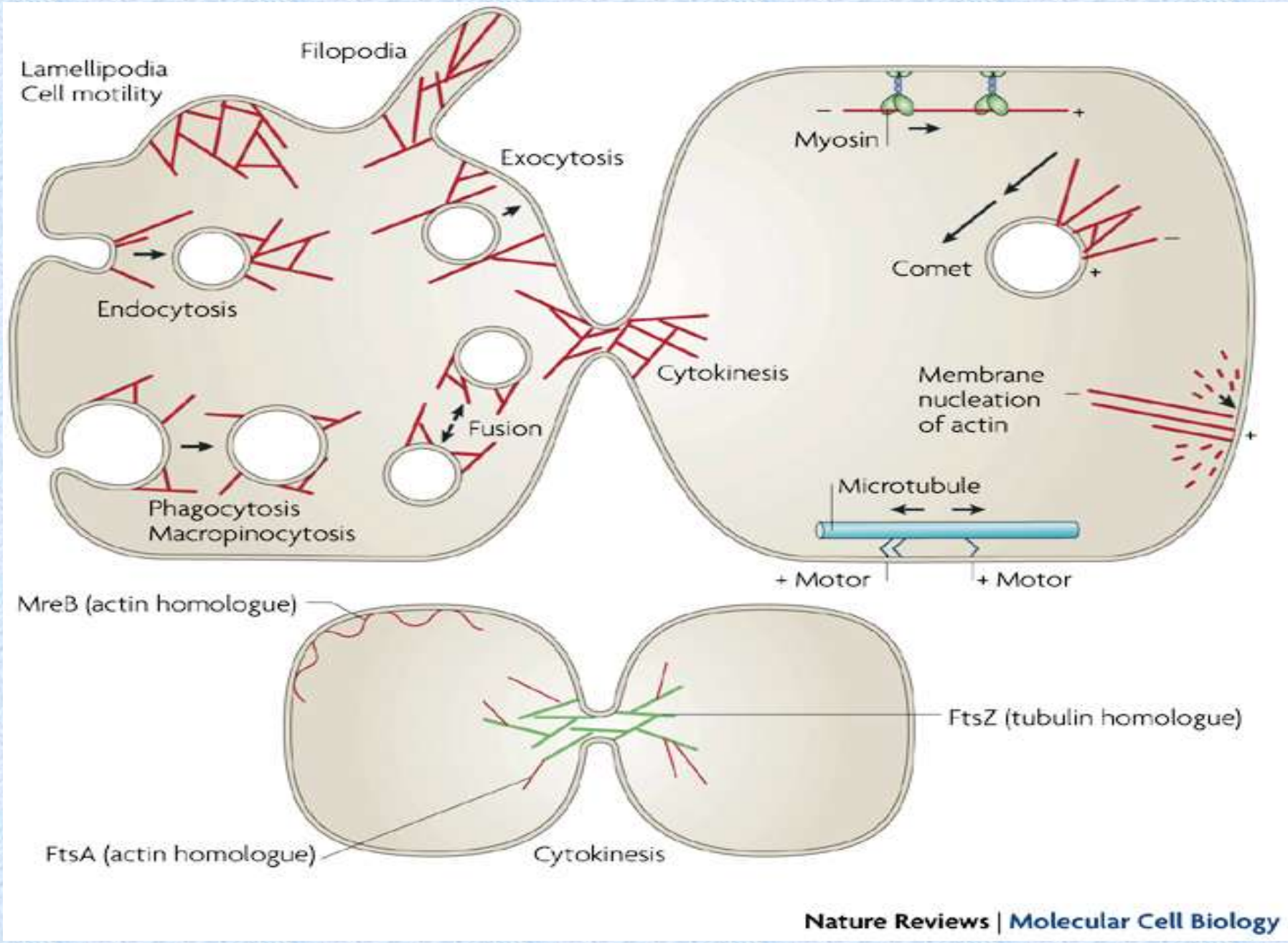
FtsZ = early tubulin

Divide and make a spore instead? Yes or No.



Divide and make two cells types





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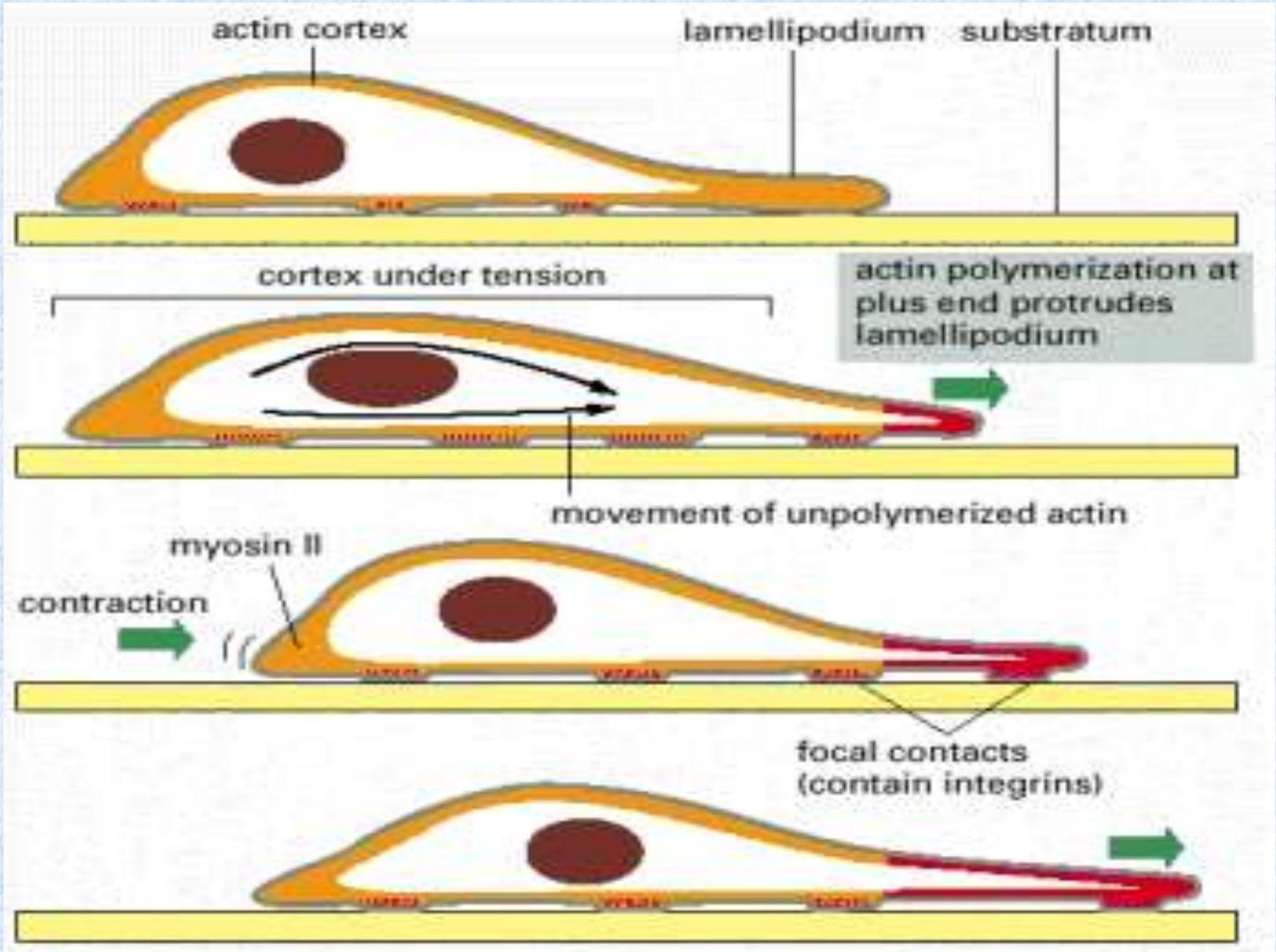
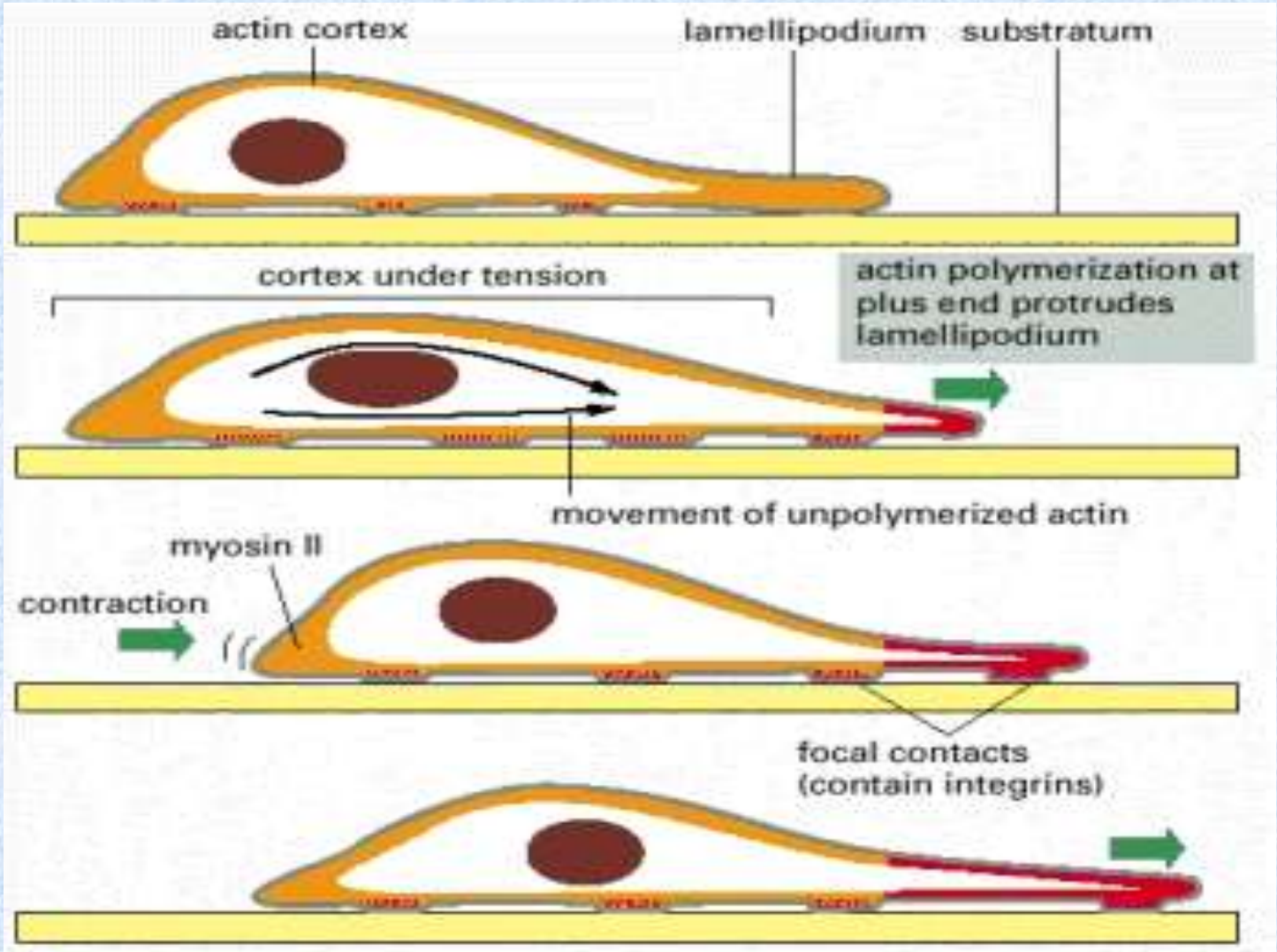
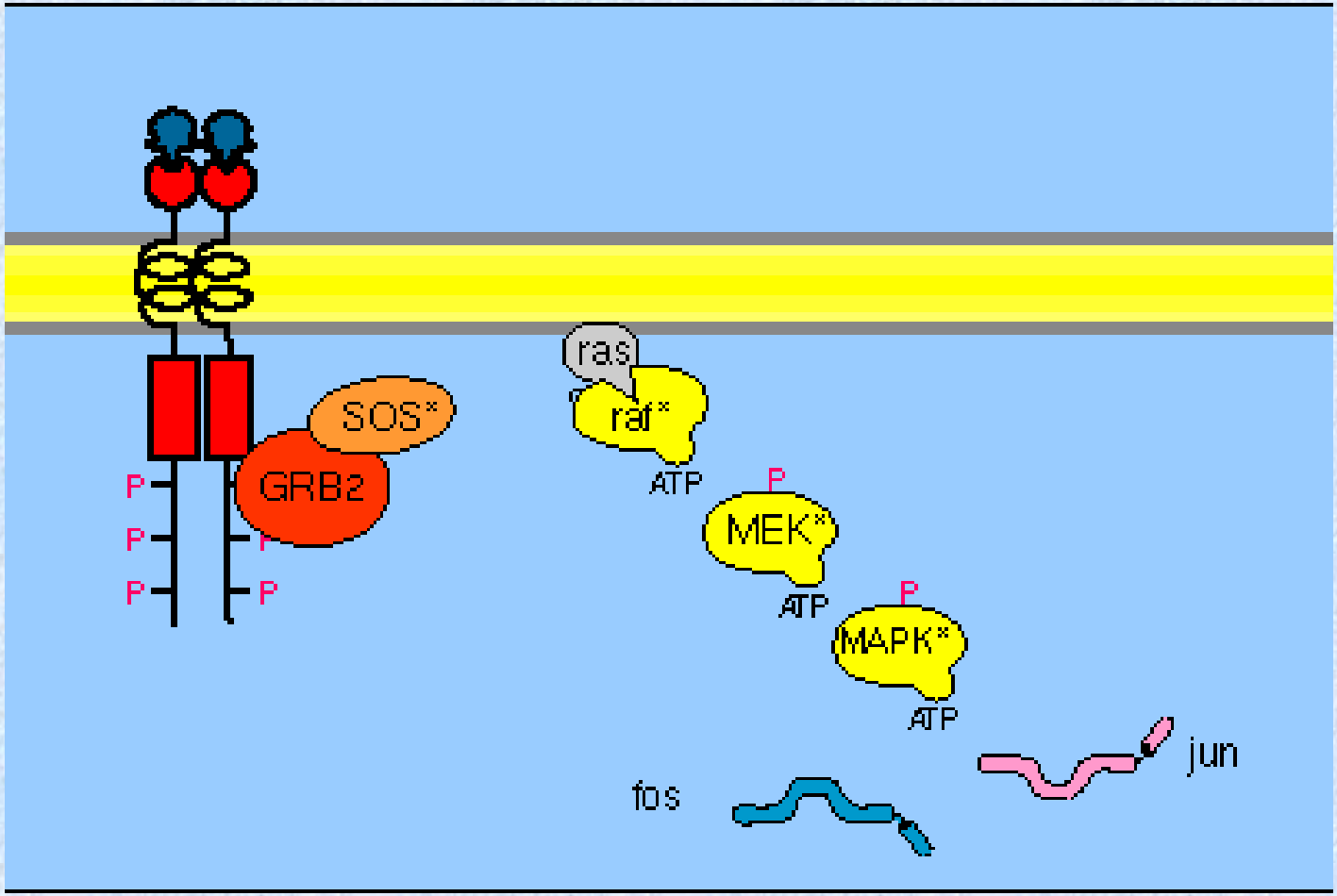
Where Did Actin Come From?

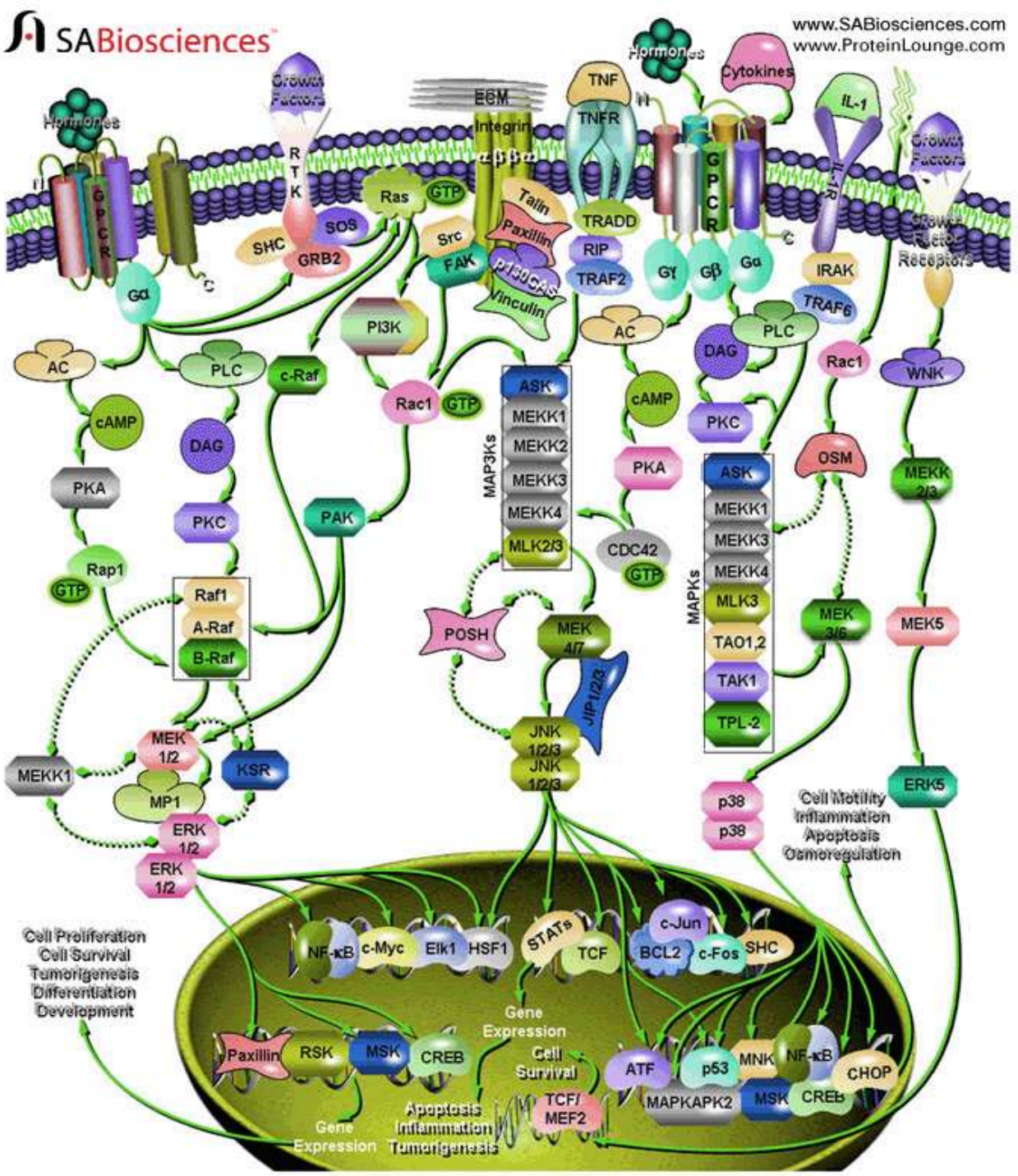


Move towards, Move Away?



Actin Contraction or Microtubule Elongation?

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www.ProteinLounge.com
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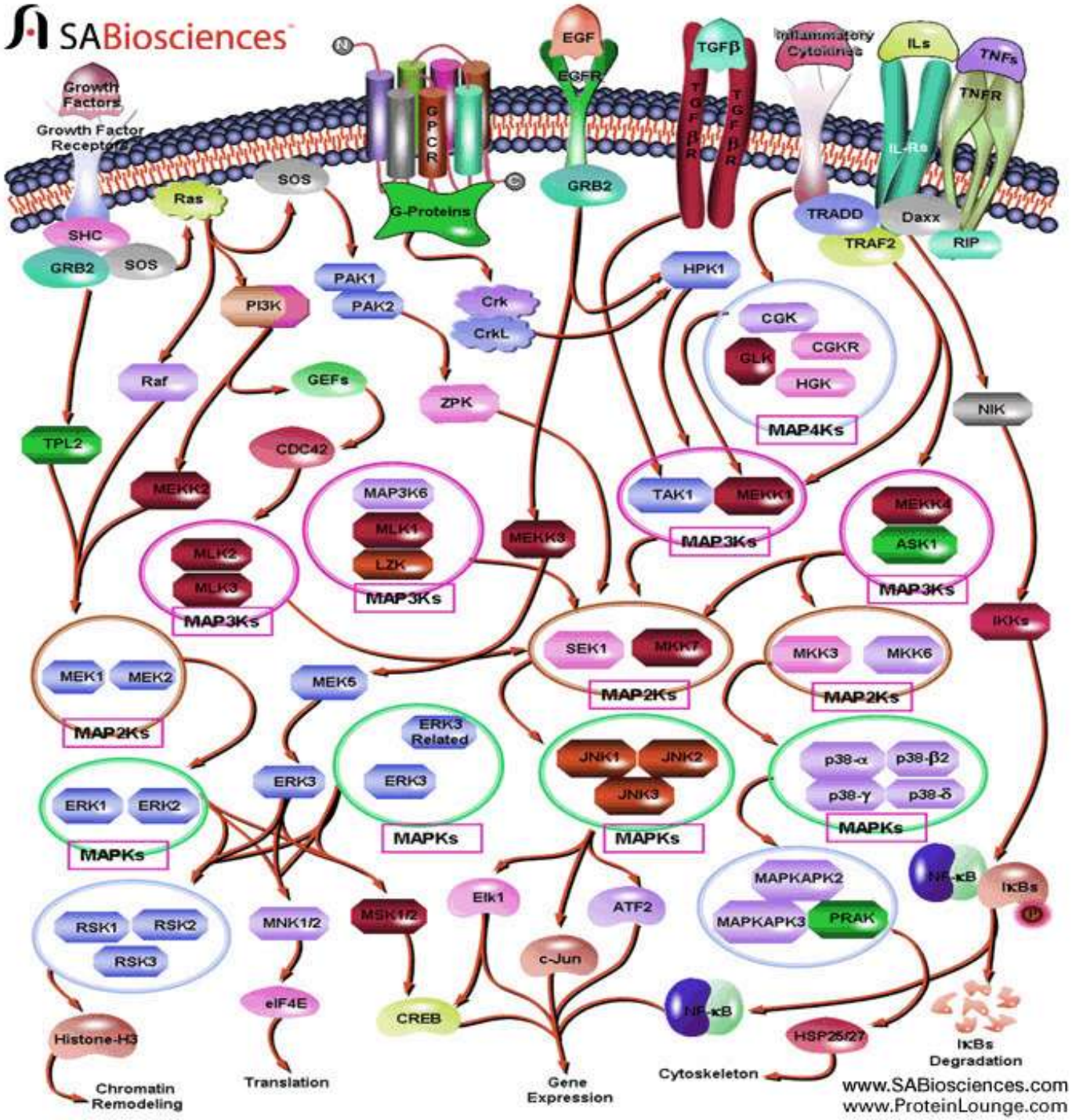
Growth Factors
ECM
Integrin
αβββ
TNF
TNFR
Cytokines
IL-1
IL-1R
Growth Factor Receptors
RTK
GPCR
Ras
GTP
SOS
SHC
GRB2
Src
FAK
p130CAS
Talin
Paxillin
Vinculin
TRADD
RIP
TRAF2
Gγ
Gβ
Gα
IRAK
TRAF6
PLC
DAG
AC
cAMP
PKA
PKC
c-Raf
PI3K
PAK
Rac1
GTP
Rap1
Raf1
A-Raf
B-Raf
MEK 1/2
MEKK1
KSR
MP1
ERK 1/2
MAP3Ks
ASK
MEKK1
MEKK2
MEKK3
MEKK4
MLK2/3
POSH
MEK 4/7
JIP1/2/3
JNK 1/2/3
CDC42
MAPKs
MLK3
TAO1,2
TAK1
TPL-2
MEK 3/6
p38
OSM
MEKK 2/3
MEK5
ERK5
WNK
Cell Motility
Inflammation
Apoptosis
Osmoregulation
Cell Proliferation
Cell Survival
Tumorigenesis
Differentiation
Development
NF-κB
c-Myc
Elk1
HSF1
STATs
TCF
c-Jun
BCL2
c-Fos
SHC
Gene Expression
Paxillin
RSK
MSK
CREB
Cell Survival
ATF
p53
MNK
NF-κB
CHOP
MAPKAPK2
MSK
CREB
TCF/ MEF2
Apoptosis
Inflammation
Tumorigenesis
Gene Expression


SABiosciences
Growth Factors
Growth Factor Receptors
GPCR
G-Proteins
EGF
EGFR
GRB2
TGFβ
TGFβR
TGFβR
Inflammatory Cytokines
ILs
TNFs
TNFR
IL-1R
TRADD
Daxx
TRAF2
RIP
SOS
Ras
SHC
GRB2
SOS
PAK1
PAK2
PI3K
Crk
CrkL
HPK1
CGK
CGKR
GLK
HGK
MAP4Ks
NIK
Raf
GEFs
ZPK
TPL2
CDC42
MEKK2
MAP3K6
MLK1
LZK
MAP3Ks
MLK2
MLK3
MAP3Ks
MEKK3
TAK1
MEKK1
MAP3Ks
MEKK4
ASK1
MAP3Ks
IKKs
MEK1
MEK2
MAP2Ks
MEK5
SEK1
MKK7
MAP2Ks
MKK3
MKK6
MAP2Ks
ERK3 Related
ERK3
MAPKs
ERK3
ERK1
ERK2
MAPKs
JNK1
JNK2
JNK3
MAPKs
p38-α
p38-β2
p38-γ
p38-δ
MAPKs
NF-κB
IκBs
P
MAPKAPK2
MAPKAPK3
PRAK
RSK1
RSK2
RSK3
MNK1/2
MSK1/2
Elk1
ATF2
c-Jun
eIF4E
CREB
NF-κB
HSP25/27
IκBs Degradation
Histone-H3
Chromatin Remodeling
Translation
Gene Expression
Cytoskeleton
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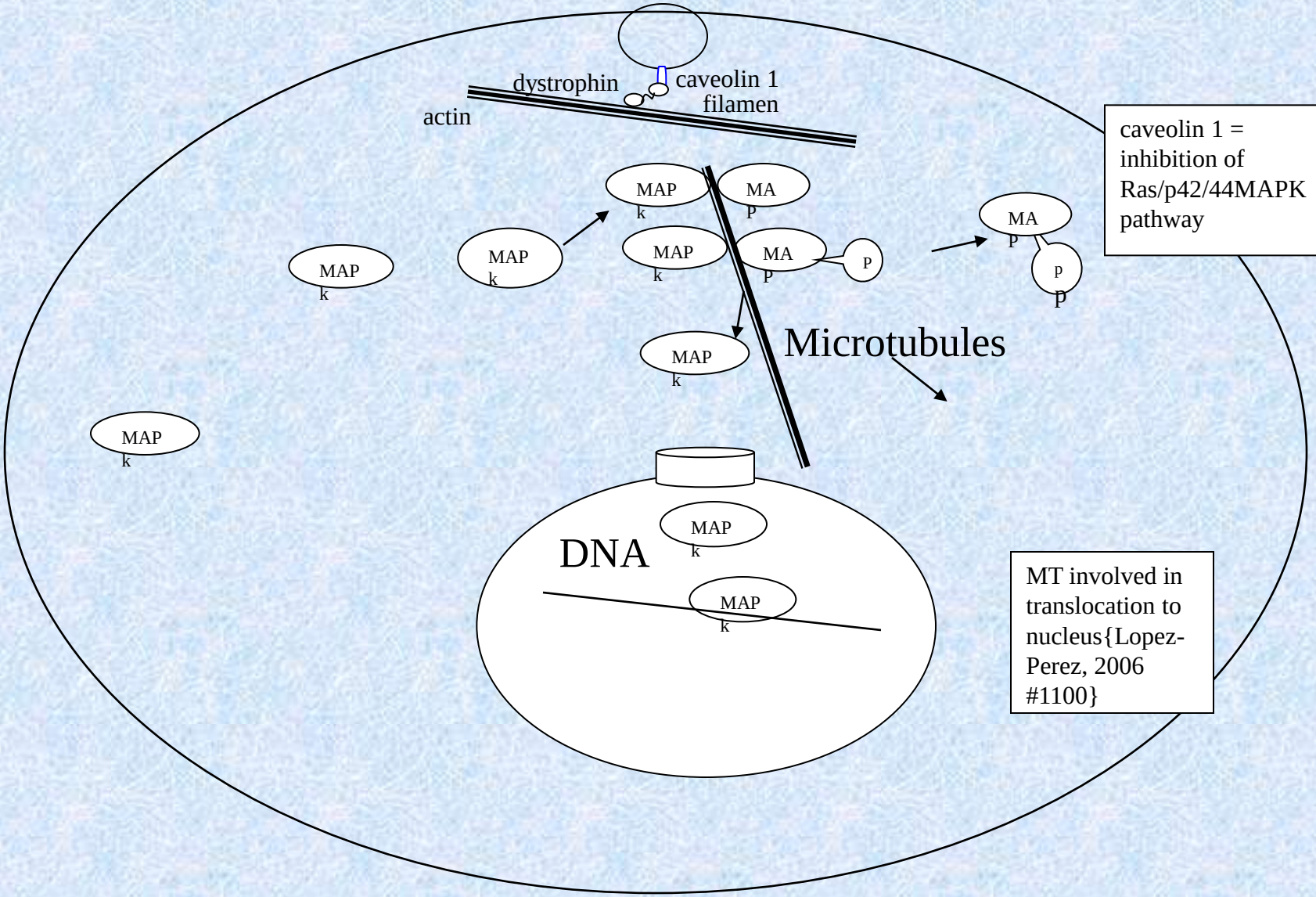
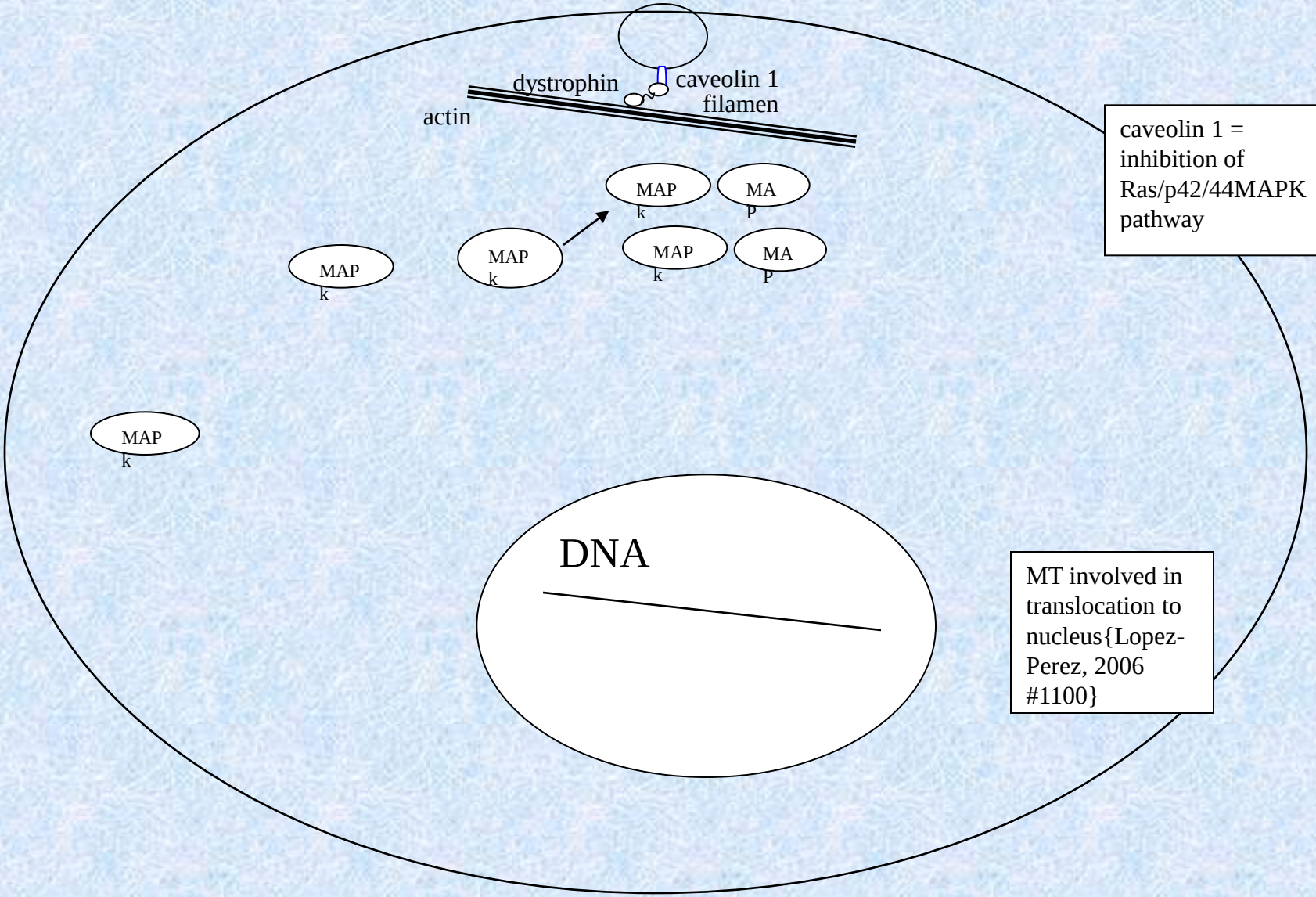


Microtubules present - Signal Sent



Microtubules absent - No Signal

All signal transduction systems, evolved from “move to, move away from” “divide, don’t divide”.

- For the complex multicellular organism, the environment to which the cell responds now includes the environment created by all the cells surrounding an individual cell and by that cell itself.
- The original and simple move away, move towards system, with cross talk, dependant on external stimulus has replaced the external stimuli with self-created and internalized stimuli.
- External stimuli cannot be controlled and regulated but self created internalized stimuli can be.

Conclusion

- Signal transduction = nuclear state splitting
- Cytoskeleton is a mechanically sensitive structure responding to both internal and external signals.
- All signal transduction (changes in gene expression) require aggregates of proteins moving on and/or off the cytoskeleton.
- All these systems evolved from simple components required for cell division and motility in the early single protocelluar organism.
- Current understanding of biology which does not include the physical effects of cytoskeleton are inadequate

Take home message

- Follow the cytoskeleton, see what it is dong and everything else follows from that.