Signaling Hierarchy

Mammary Epithelial Cells

Mammary Epithelial
Signaling Hierarchy

- **Stages operational during pregnancy (just before the onset of lactation to completion of lactation)**
- **Epithelial cells - ECM are the tissue level players**
- **Construction and destruction of steps with various ‘go’ checkpoints to the next step**

Signaling Hierarchy

- **Flow of information between cells and tissues are integrated into a signaling hierarchy that is :**

  a) constructed and then b) dismantled in a cyclical manner
- **First tier of hierarchy involves mechanical signals : cell rounding that trigger lactoferrin gene expression**
- **Rounded cells deposit ECM and initiate a laminin mediated hierarchy leading to biochemical signal transduction and activation of a wide range of genes**

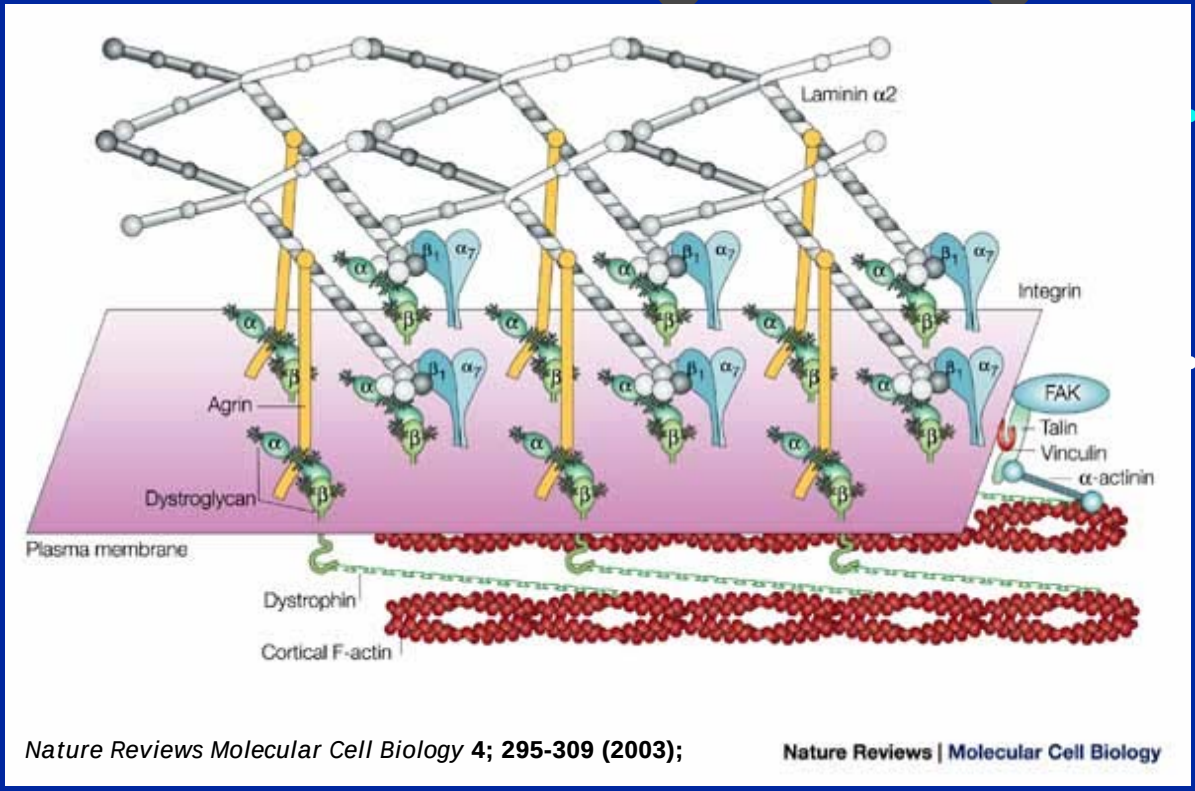
Signaling Hierarchy

- The third tier of hierarchy signaling relies on the ECM morphogenesis, wherein presence of ECM directs cell polarity, formation of central lumen and expression of WAP.
- WAP is expressed late in pregnancy and just before the onset of lactation.
- Dismantling of this hierarchy begins at weaning is mediated by ECM-degrading enzymes, which act in a development stage manner to induce programmed cell death.

Architecture

- Composition of ECM is important: e.g. myoblast proliferate (Fn) or form tubles (In)
- Decreased adhesion to rigid substratum: *mechanical in nature*
- Increased cell rounding
- Reorganization of cytoskeleton (markers)

Laminin Signaling

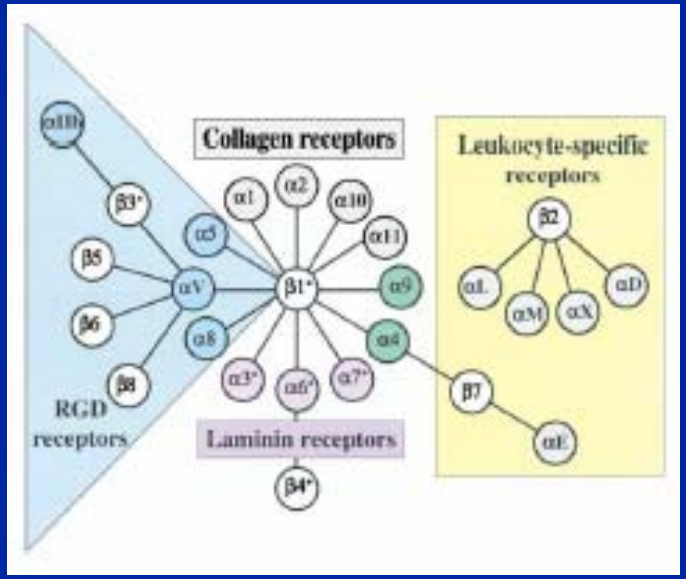


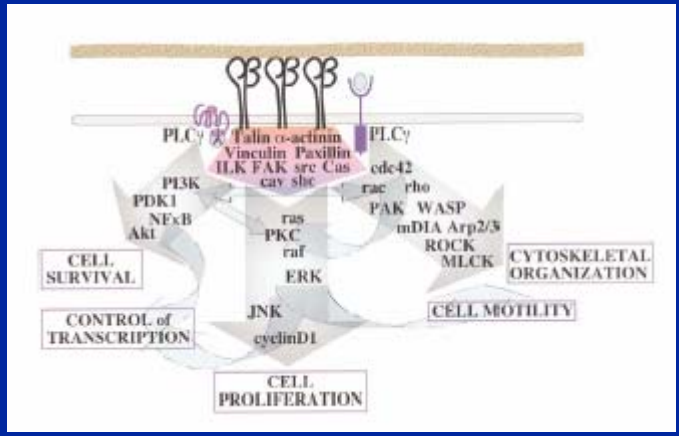
Nature Reviews Molecular Cell Biology **4; 295-309 (2003);**

Nature Reviews | Molecular Cell Biology

- **Integrin link very important signaling: inside-out and outside-in**
- **Laminin-specific integrin clustering and activation - laminin based cytoskeleton reorganization**

Integrin Family & Signaling





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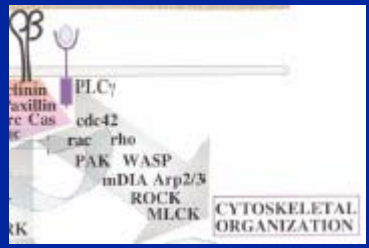
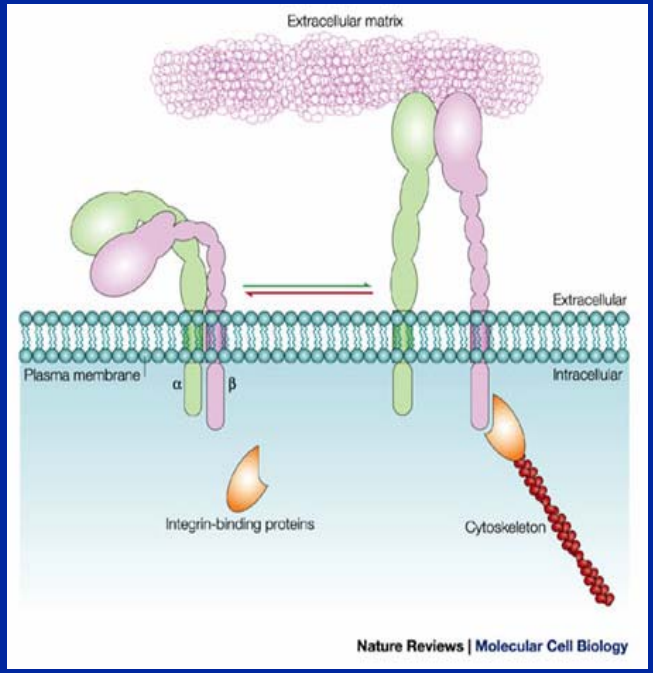
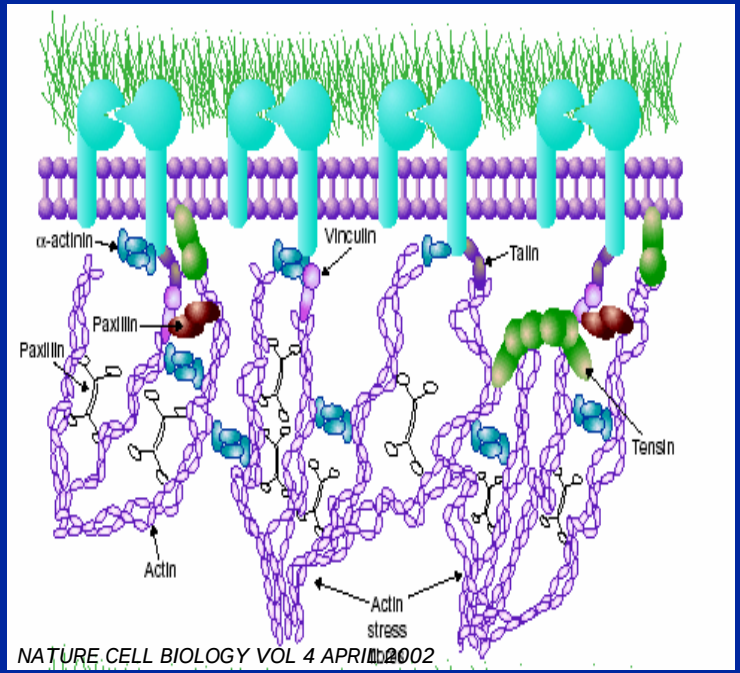
Integrin Signaling

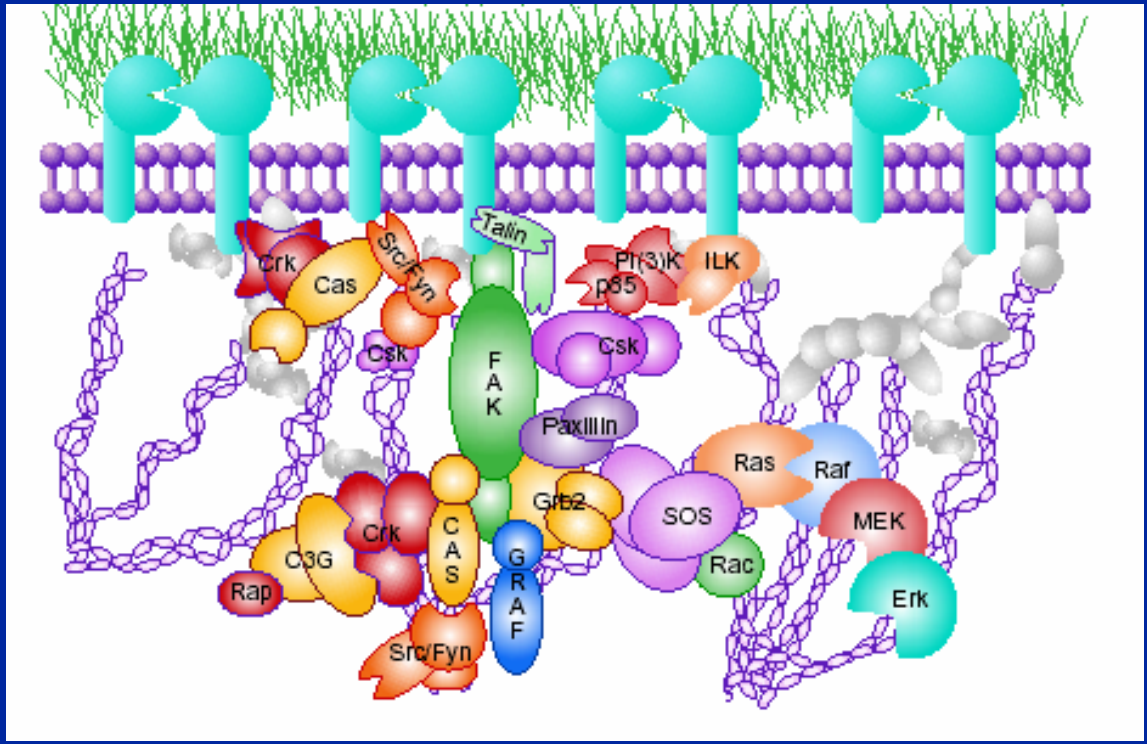






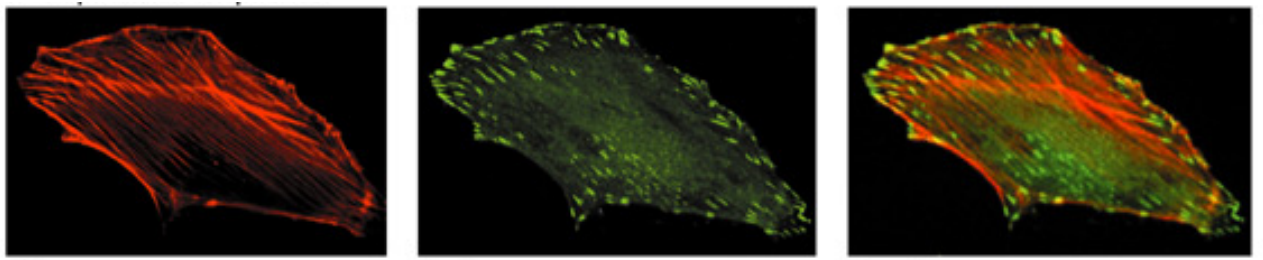
NATURE CELL BIOLOGY VOL 4 APRIL 2002

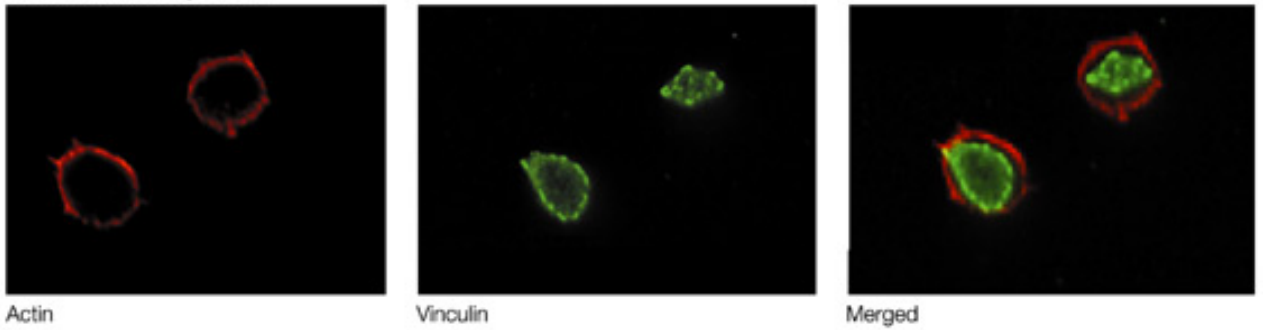
Cytoskeleton & Integrin signaling



NATURE CELL BIOLOGY VOL 4 APRIL 2002

Actin/Vinculin Complex

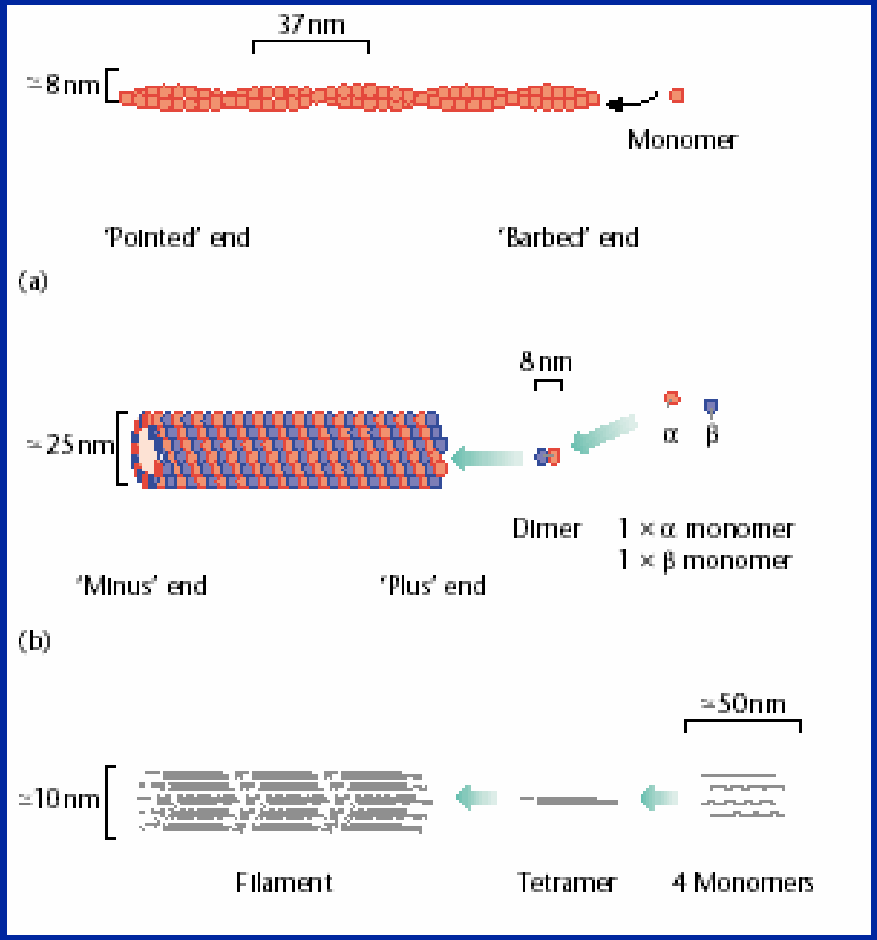




Nature Reviews | Molecular Cell Biology

Cytoskeleton

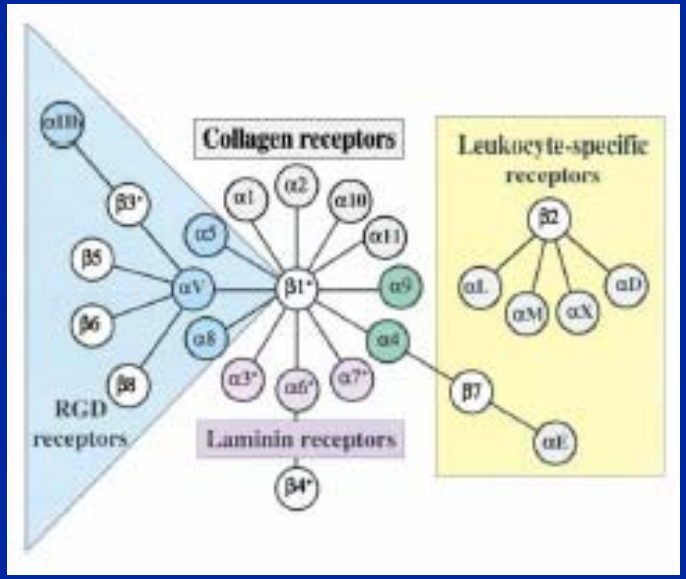
- **Microfilaments [actin monomers]**
- **Microtubules [α and β – tubulin]**
- **Intermediate filaments [various different types of monomers**

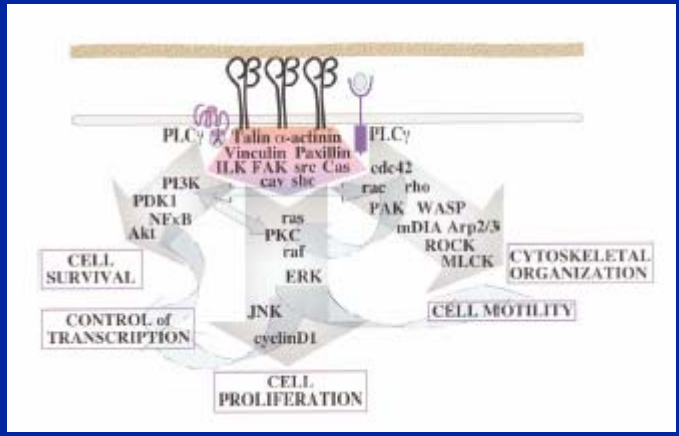


Cell-ECM contact

- **Cell shape is a set point for proliferation *versus* differentiation**
- **Integrin signaling - Cross talk to make sure that differentiation signaling is different from proliferation**
- **Cessation of proliferation – exit cell cycle**
- **Decreased AP1 transcription factor activity**

Integrin Family & Signaling





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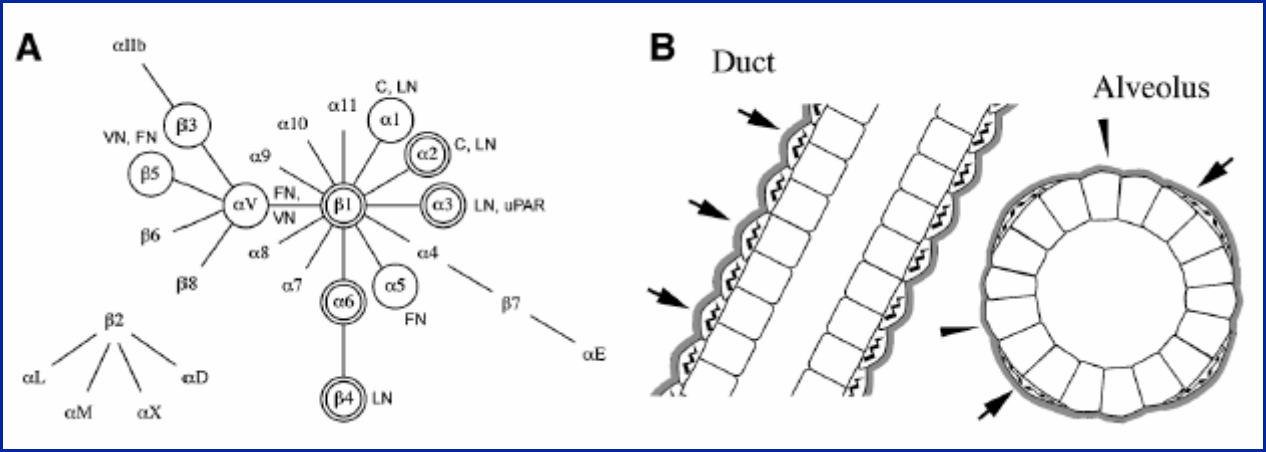
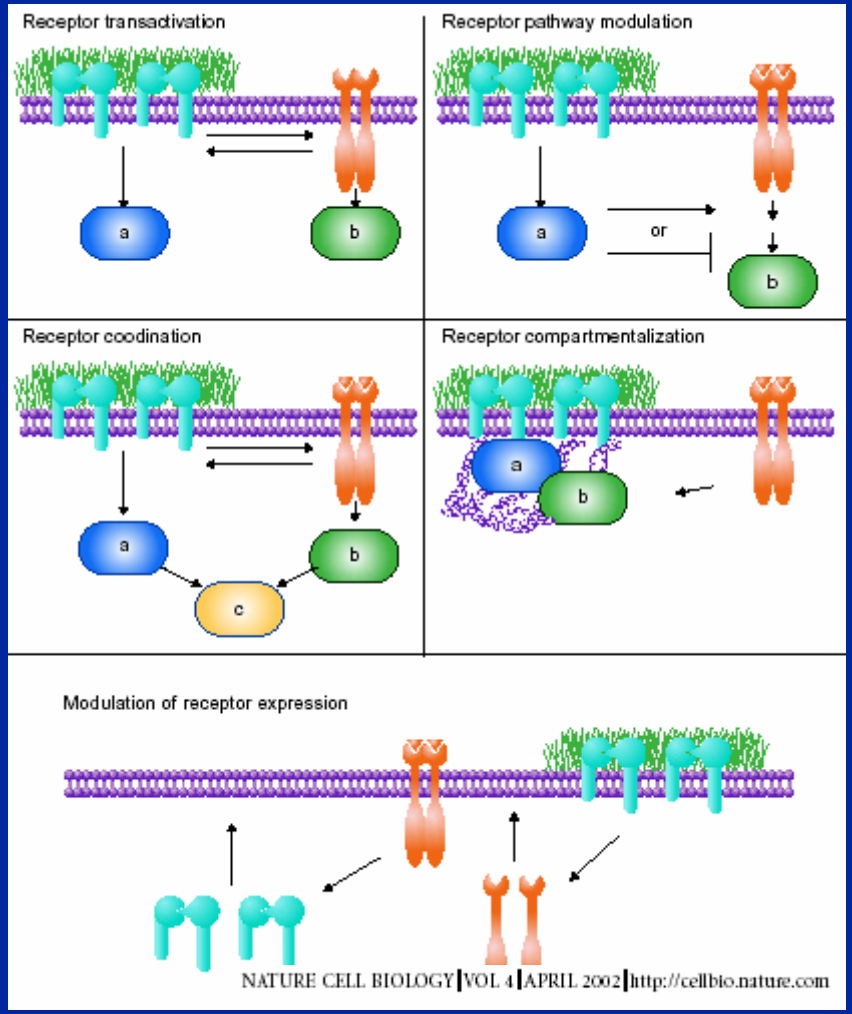
Integrins

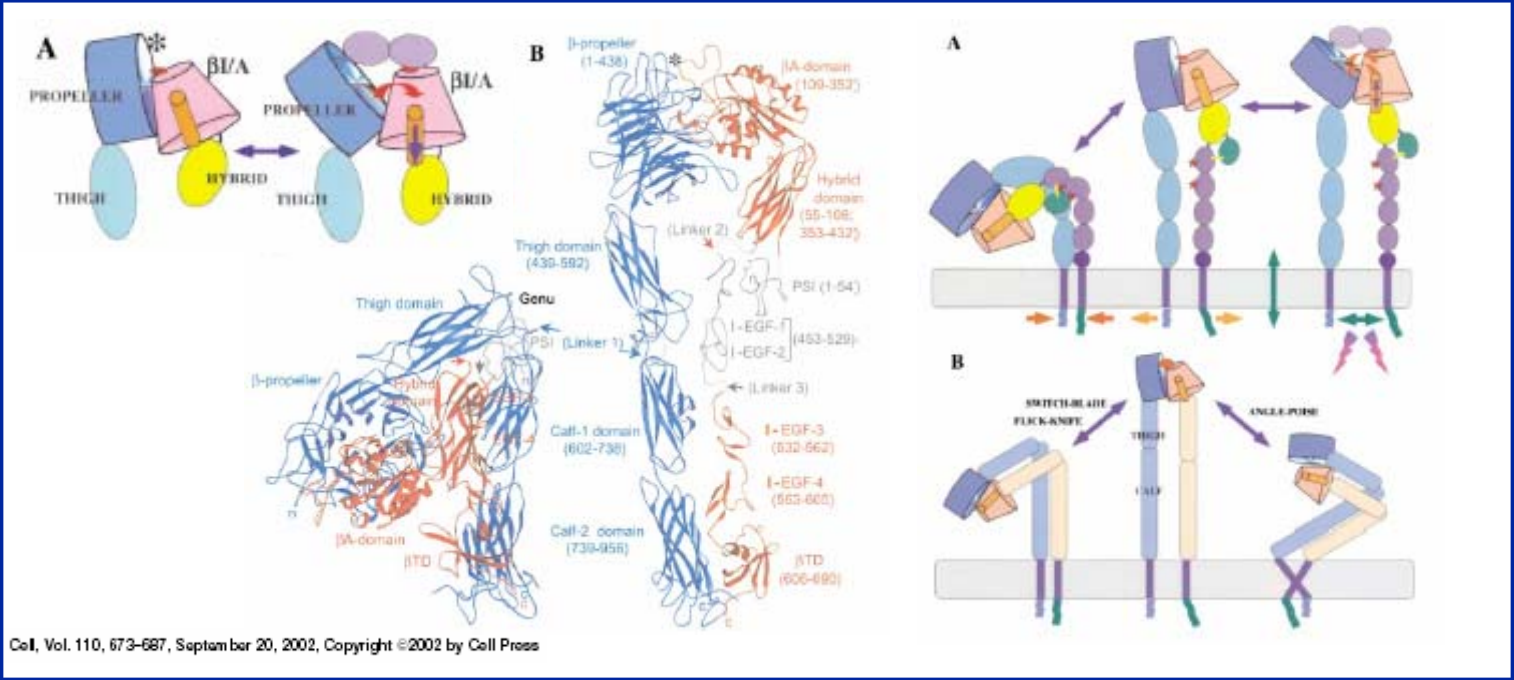
Mammary Gland Development



Integrin Signaling Diversity

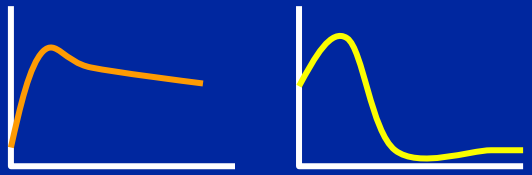


Integrin Signaling I

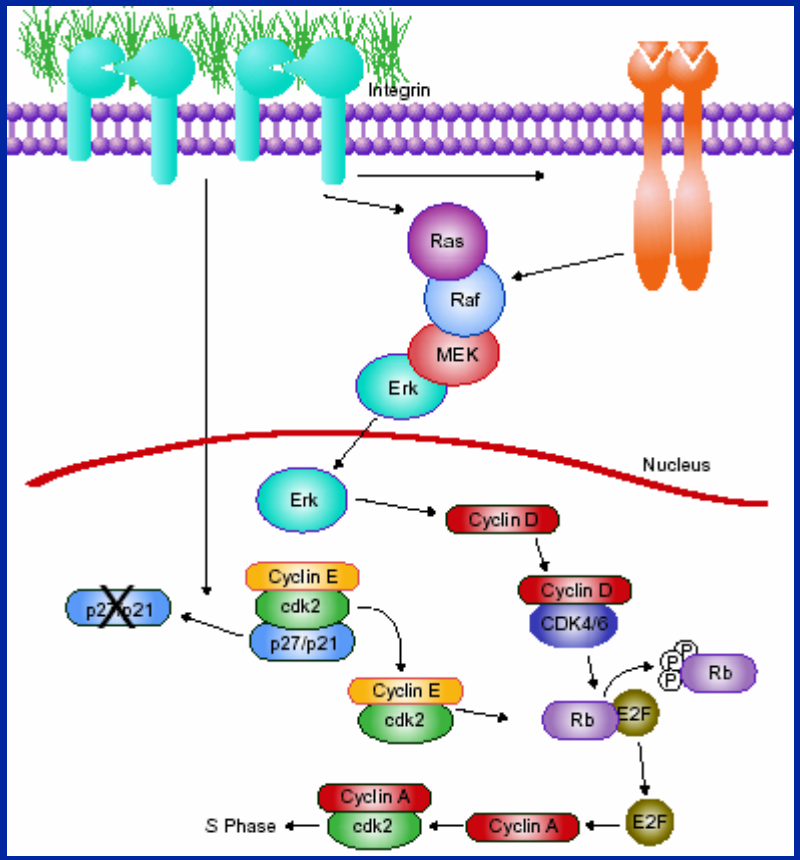


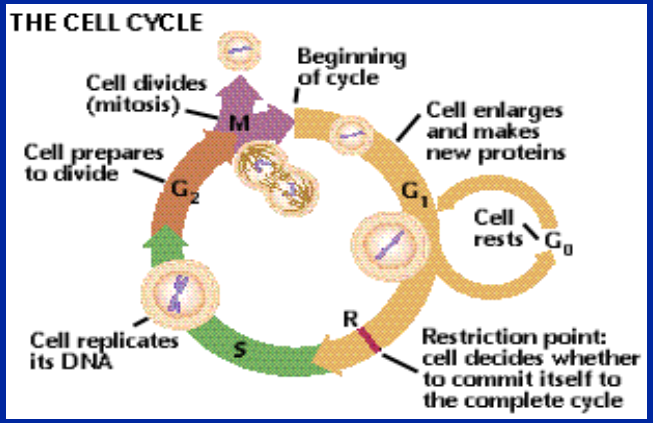
Integrin Signaling II

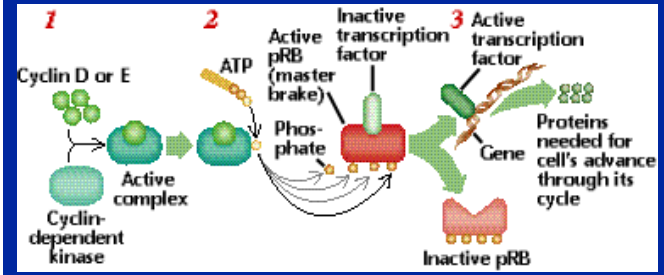
- Cross talk to make sure that differentiation signaling is different from proliferation
- Modulation of insulin signal transduction pathway MAP kinase pathway
- coupled with growth factor signaling: kinetic activation of transcription factors is modulated



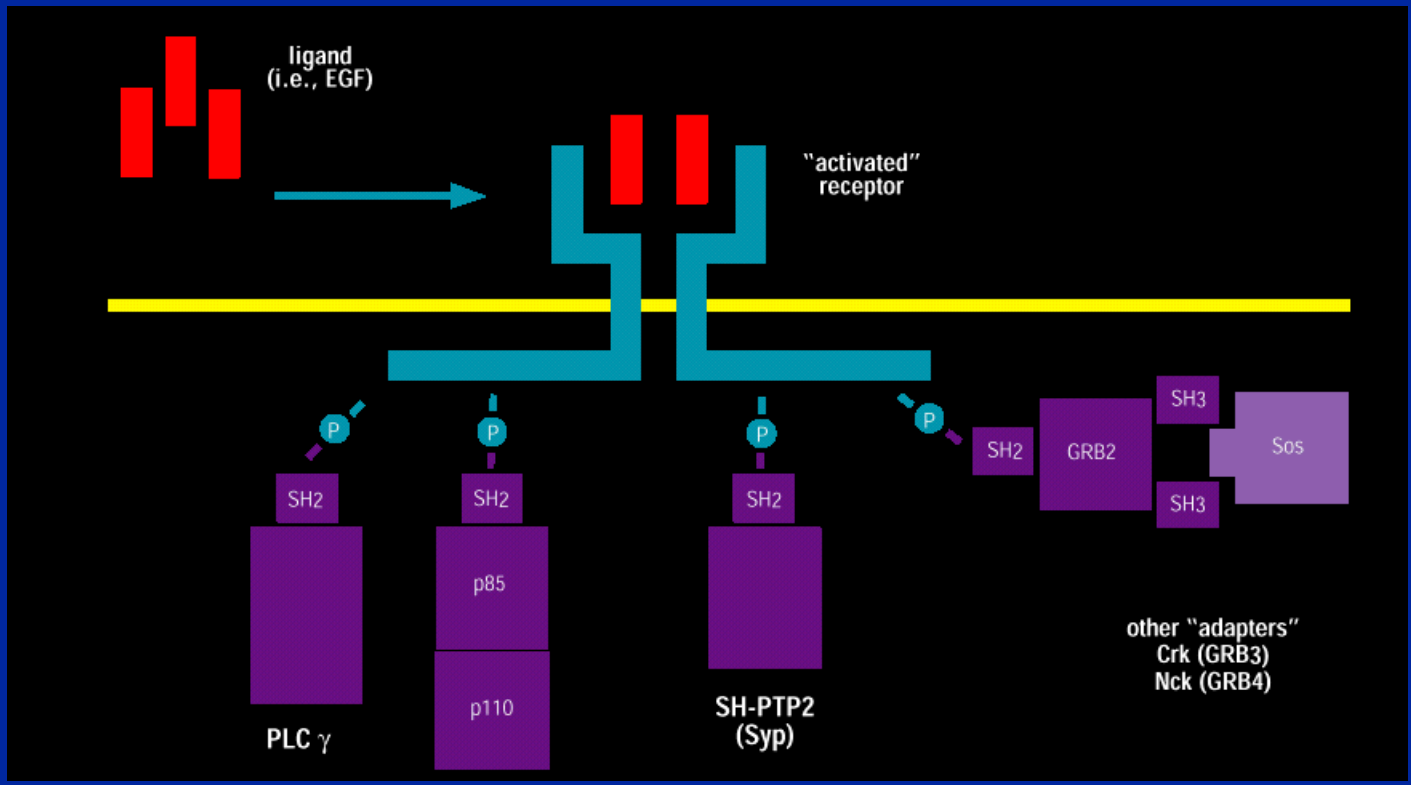
Integrin Coupled with growth factor Signaling: Cell Cycle *in* or *out*



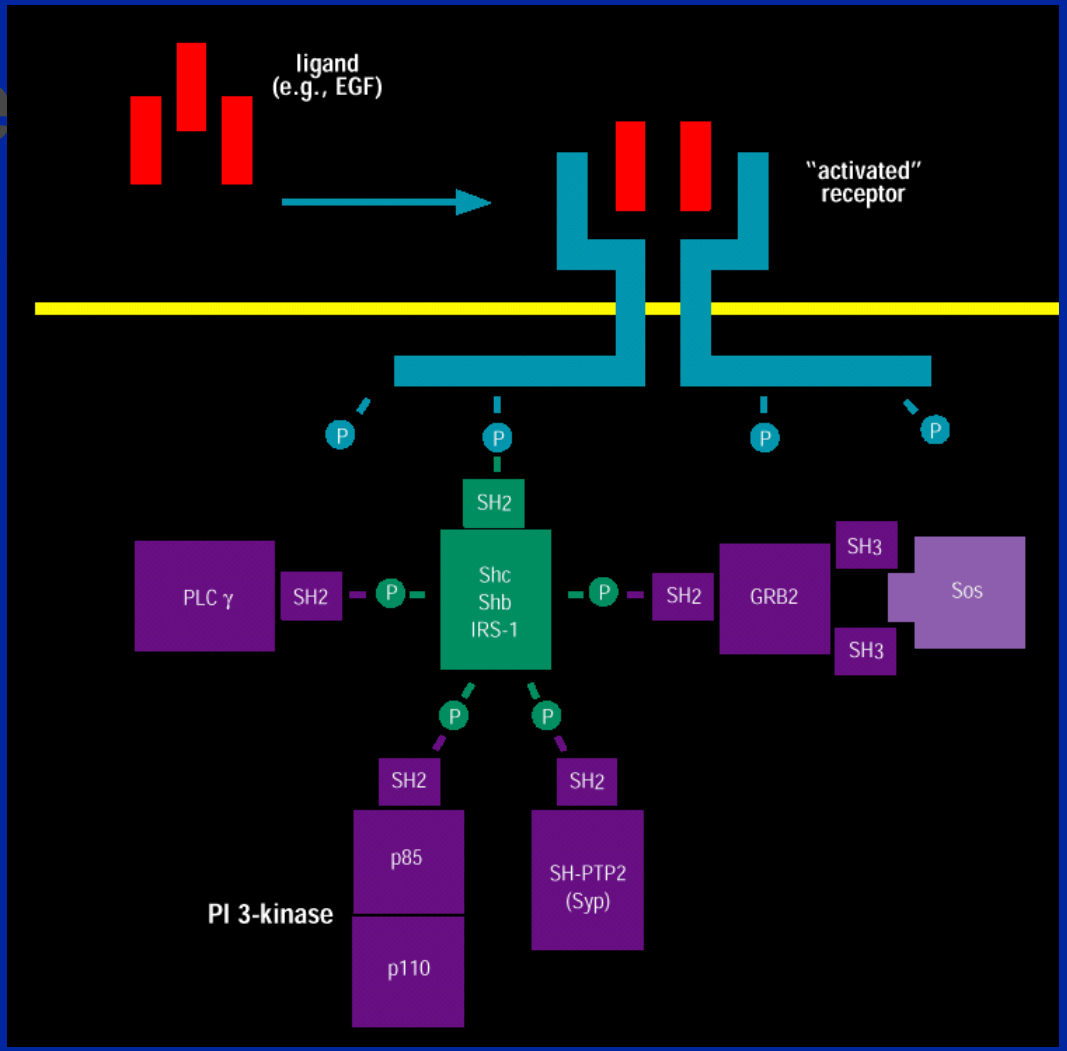




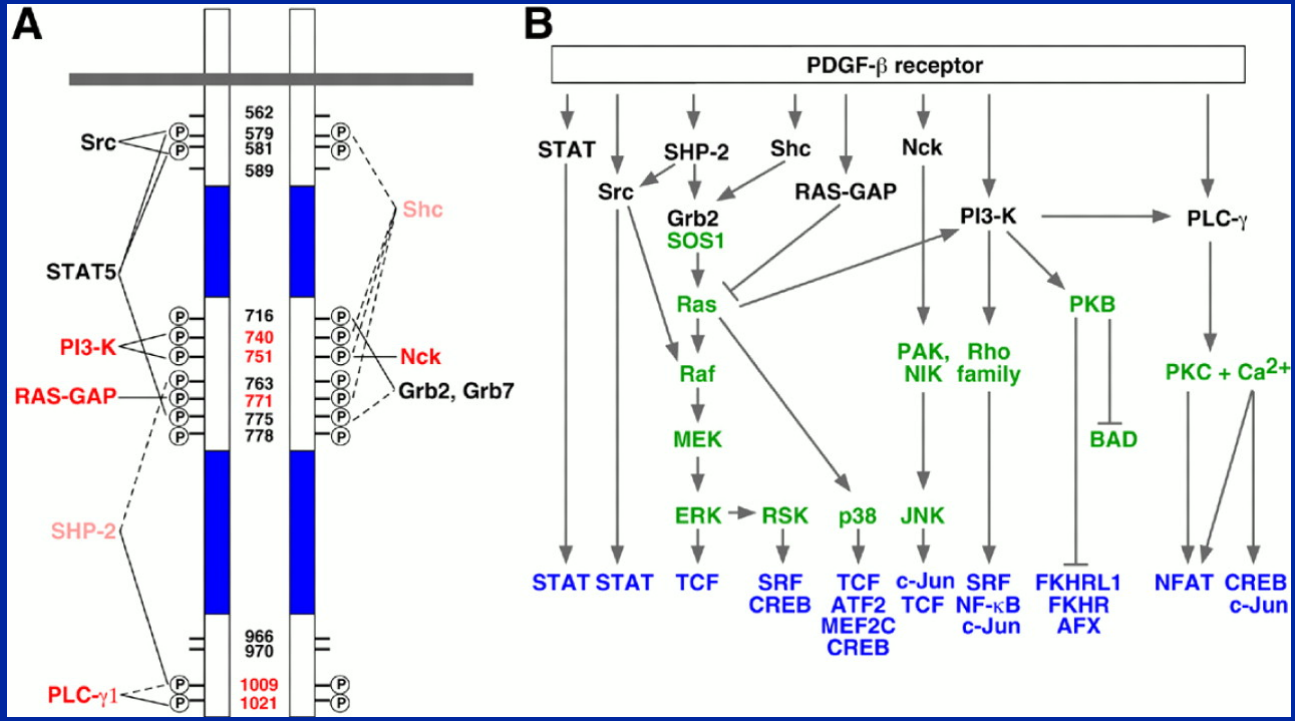
Growth Factor Signaling



Substrate Mediated Signaling

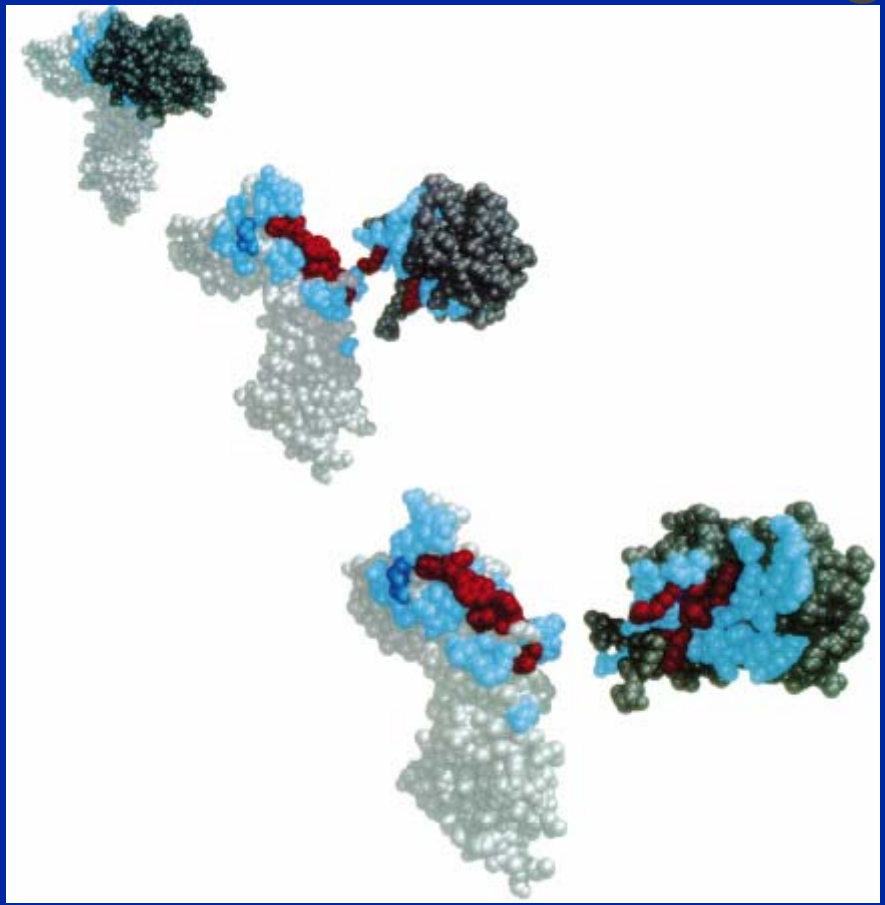


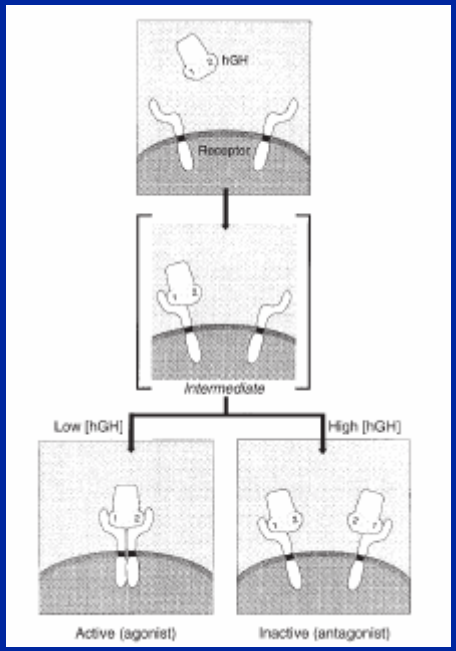
Growth Factor Signaling



Hormone Binding

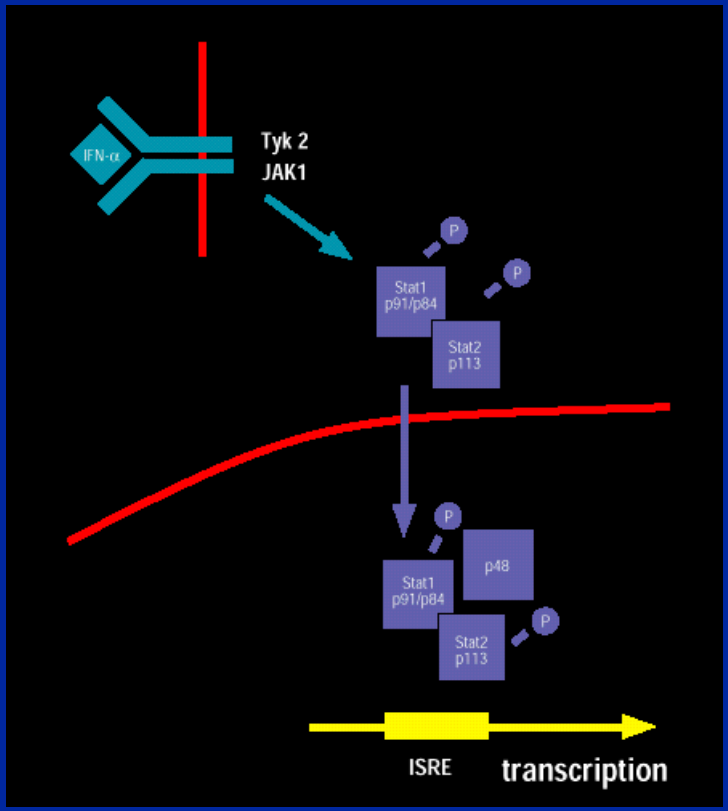
Hormone Binding



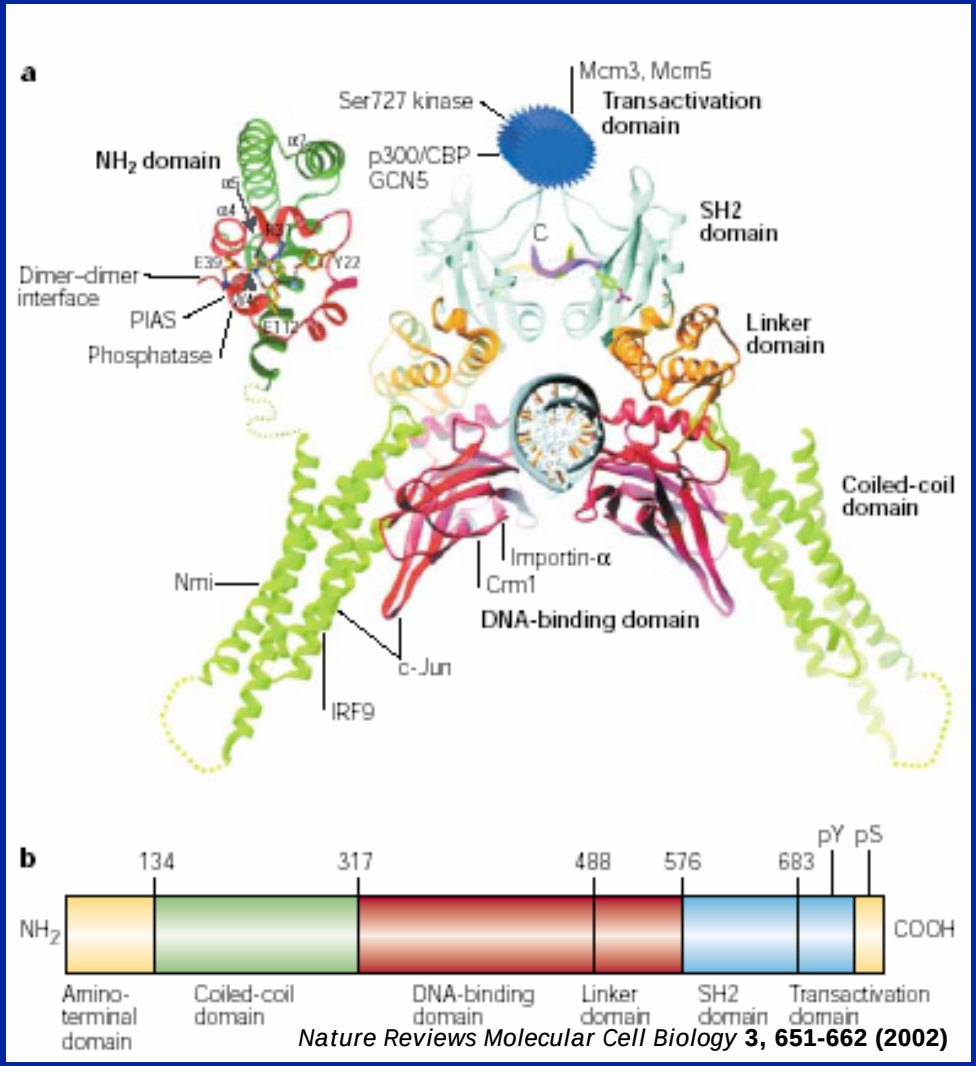


Differentiation Specific Effects

- STAT-5 interacts with activated prolactin receptor: gets P, and becomes a transcription factor
- BCE-1 contains STAT-5 binding sites
- beta-casein expression is on



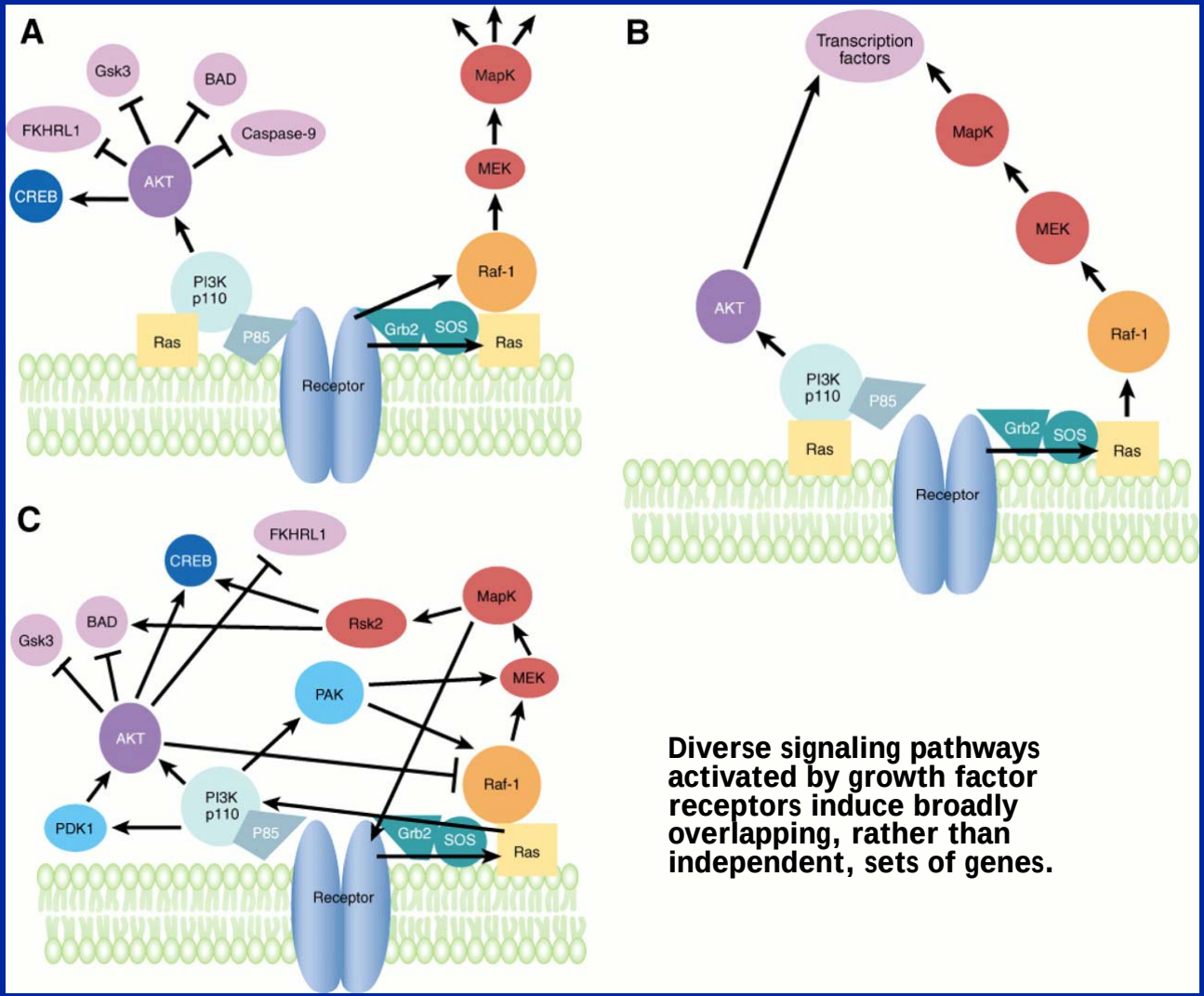
STATs



Nature Reviews Molecular Cell Biology **3, 651-662 (2002)**

Laminin Signaling

- **Differentiation specific elements are activated** *(BEC-1 which contains C/EBP binding: ECM responsive elements)*
- **Right ECM for the proper loading of the transcription factors: appropriate histone organization**
- **BEC-1 leads to prolactin based activation of STAT-5 leading to beta-casein expression**

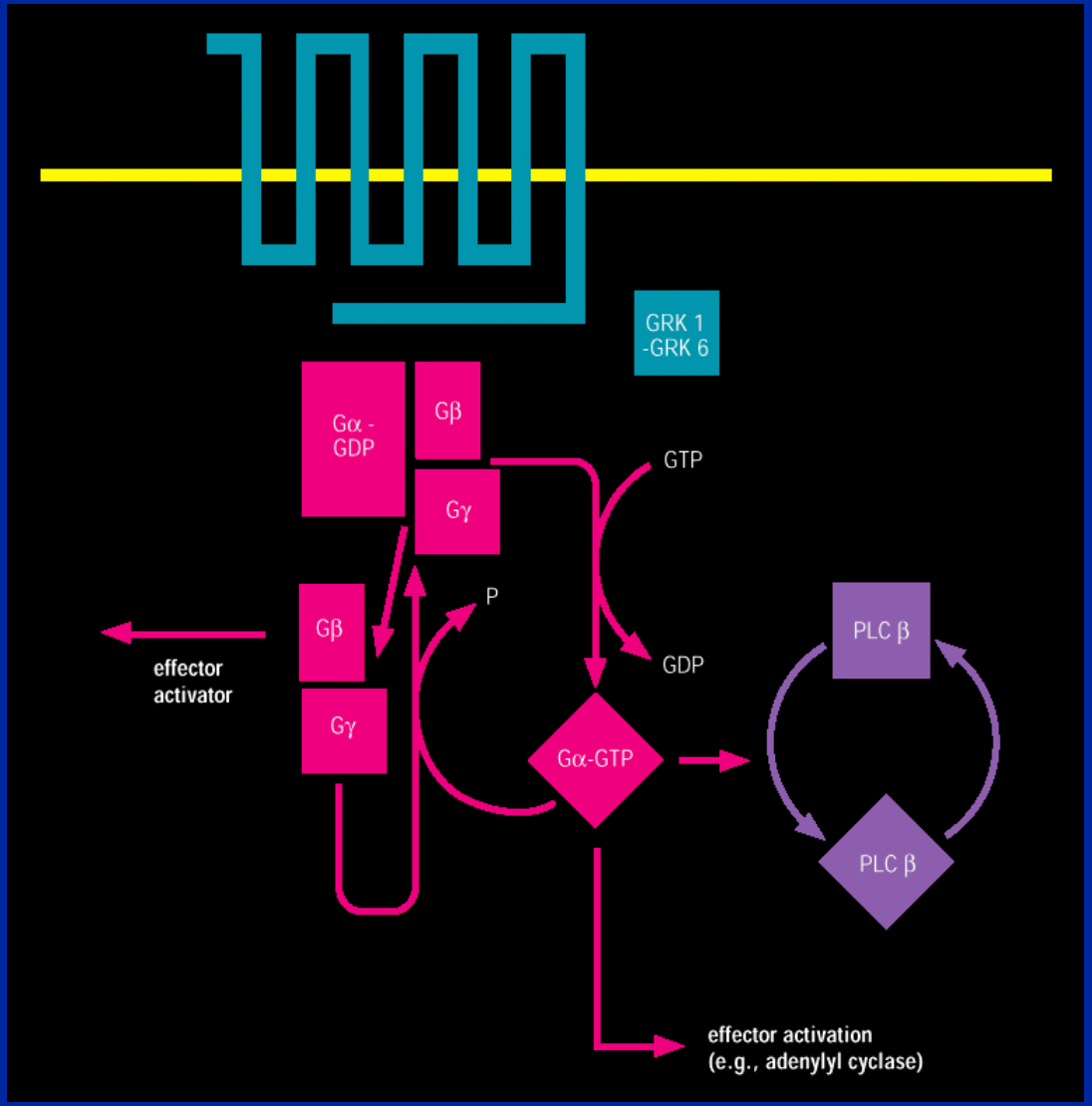


Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes.

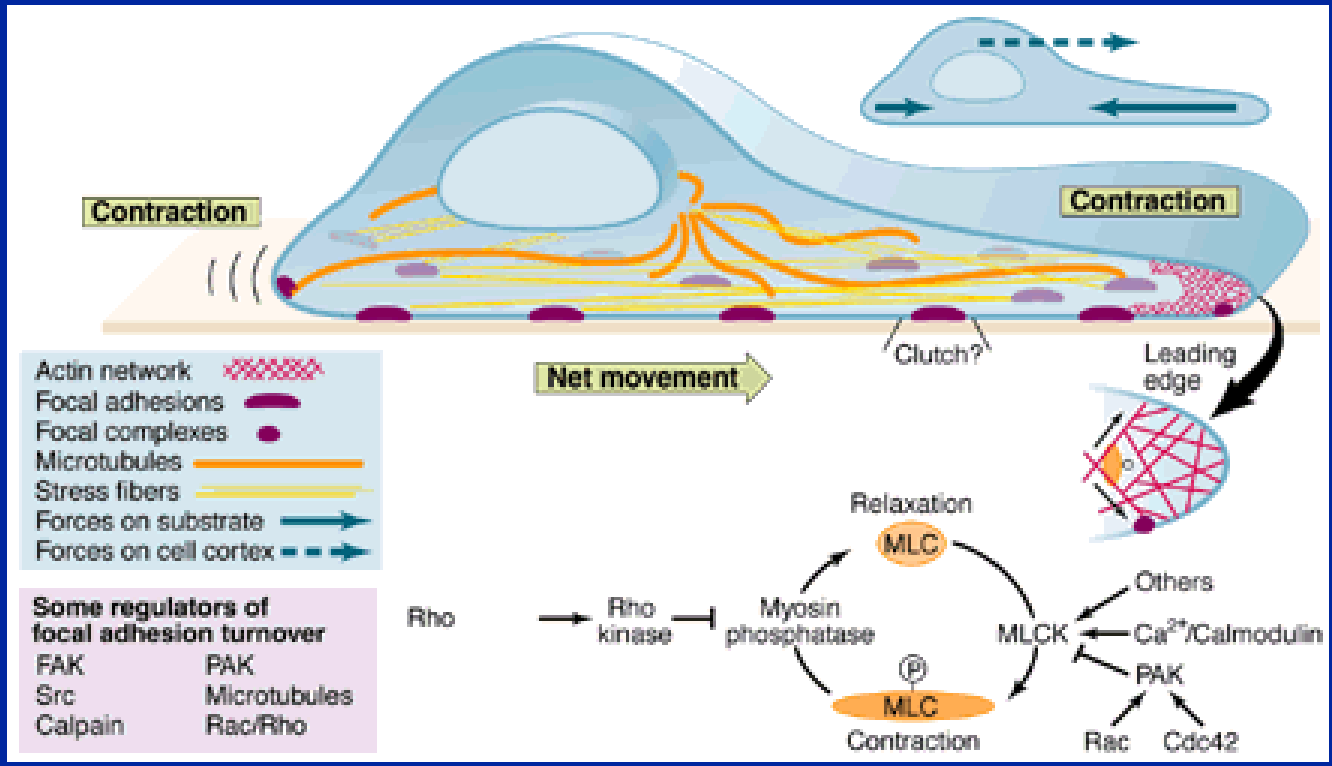
Ready for Third tier

- **Reciprocal cell-mediated changes in ECM composition**
- *rigid substratum results in flattening, de-differentiation and beta-casein production off*
- **The next signal is for the cells to migrate and this requires a change in the FAK based signaling as well as ECM-integrin-cell interactions**
- **Cell spreading on ECM - actin stress fibers**

G-protein coupled receptor activation

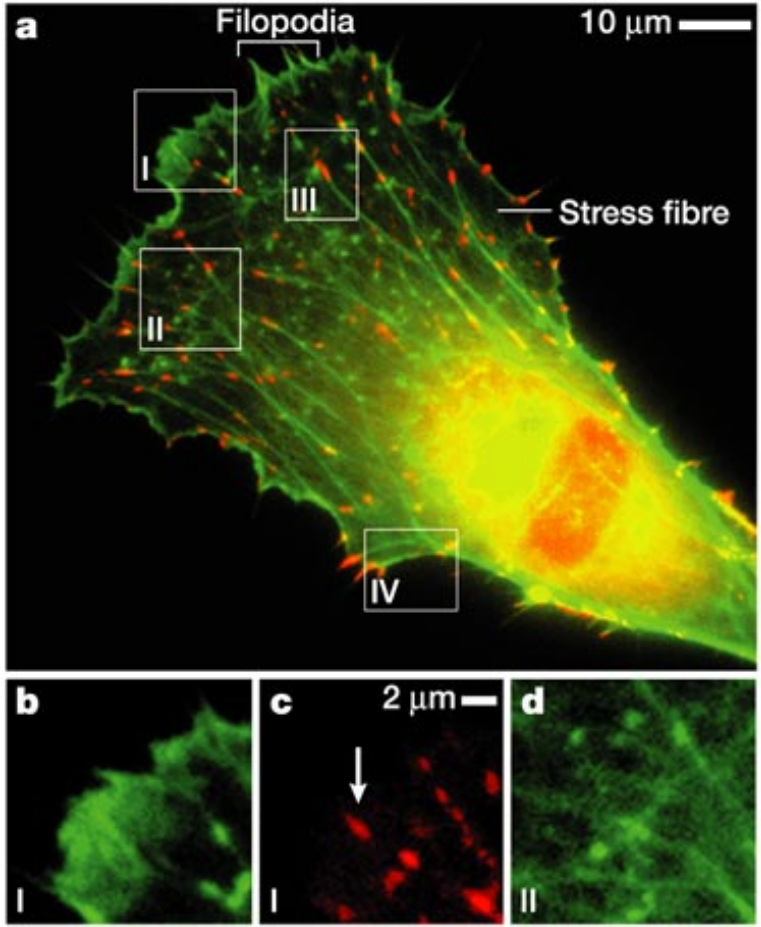


Migration


Contraction
Contraction
Clutch?
Net movement
Leading edge
Actin network
Focal adhesions
Focal complexes
Microtubules
Stress fibers
Forces on substrate
Forces on cell cortex
Some regulators of focal adhesion turnover
FAK
Src
Calpain
PAK
Microtubules
Rac/Rho
Rho
Rho kinase
Myosin phosphatase
Relaxation
MLC
MLCK
Others
Ca2+/Calmodulin
PAK
Rac
Cdc42
P
MLC
Contraction

Migrating cells

- Entire complex
- FA and migration
- Migration



Nature Reviews Molecular Cell Biology 3, 957-964 (2002)

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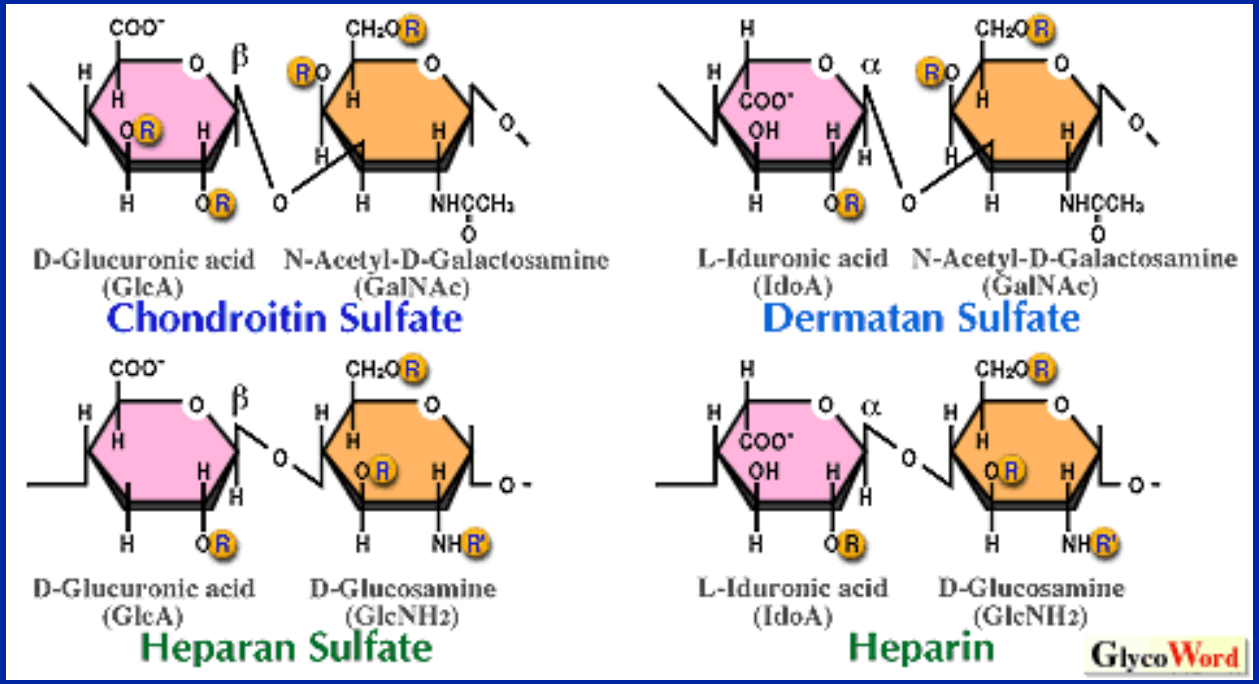
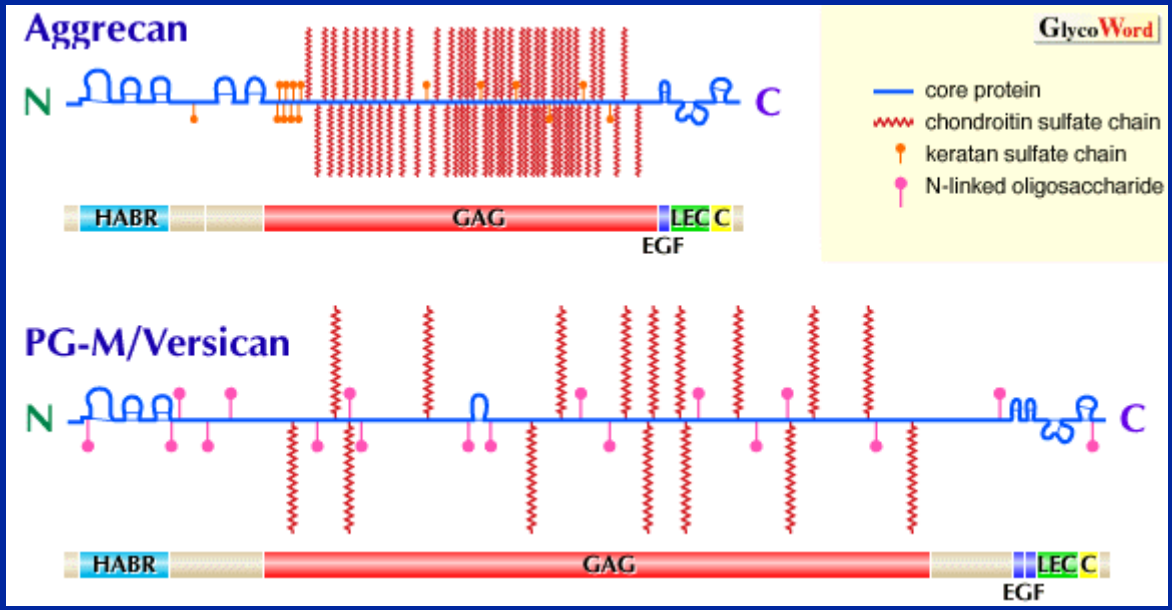
Migration, proliferation & differentiation

- Growth factors in the ECM become key for sending proliferative signals: FGF
- Polarization of the cells leads to self-assembly and the formation of alveoli like structures: morphogenesis (HGF)
- Production and deposition of new ECM
- Down regulation of TGF-β

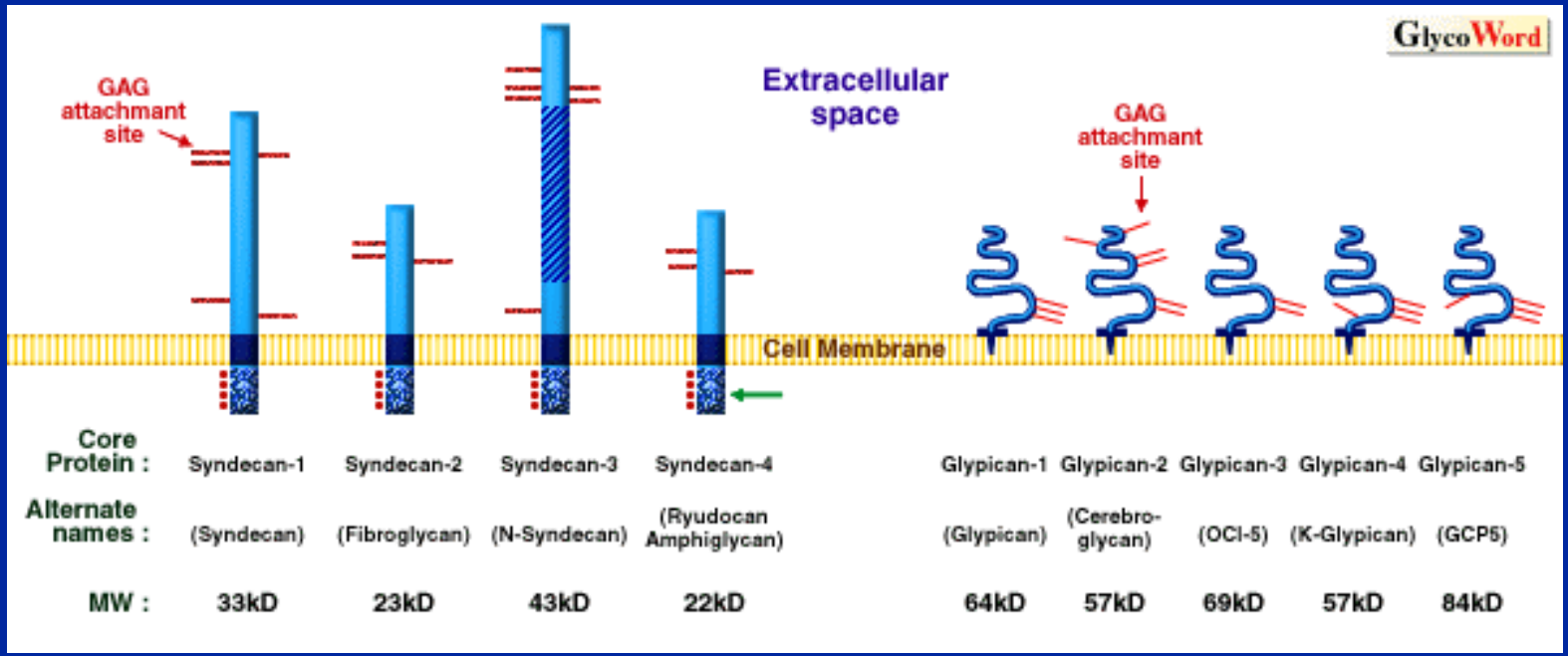
Glycosaminoglycans



Core: ECM CS

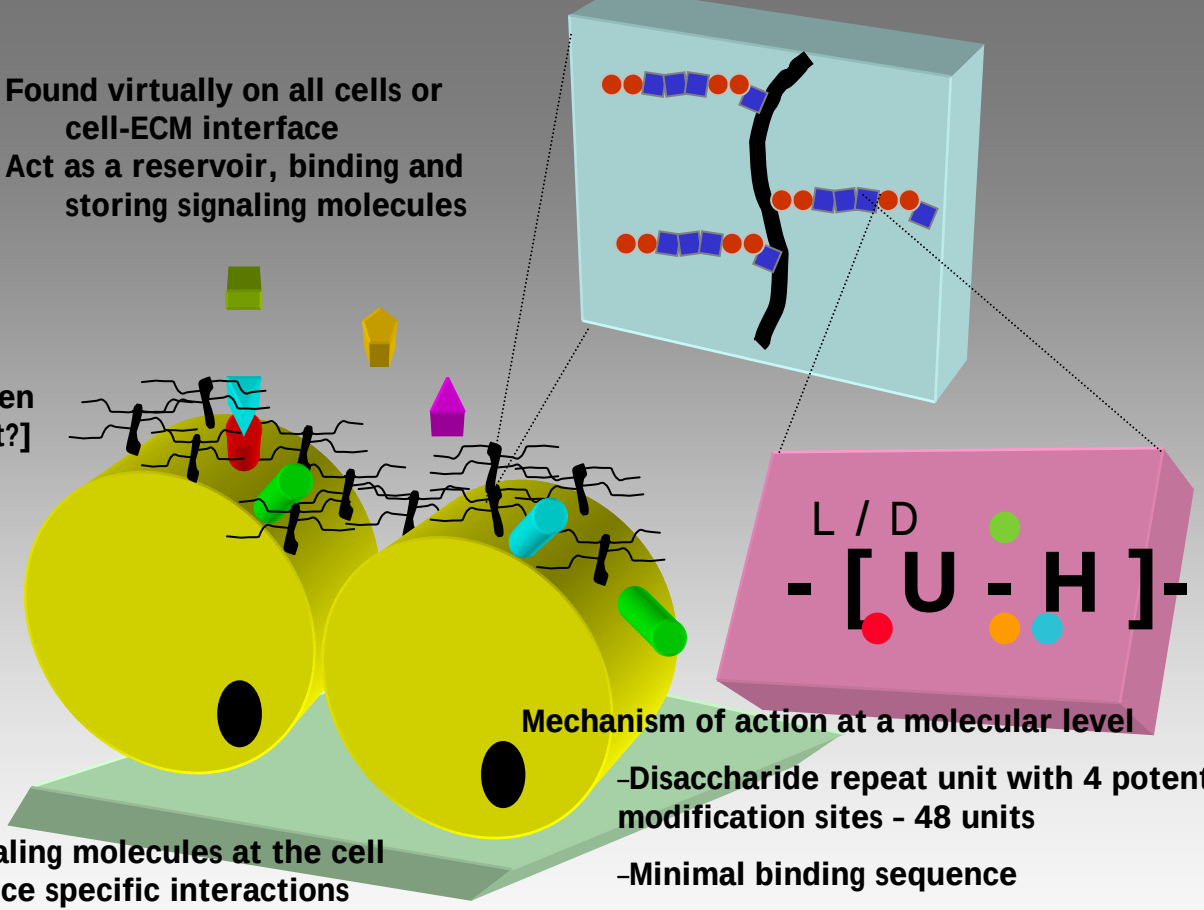

Aggrecan
N
C
HABR
GAG
LEC
C
EGF
GlycoWord
core protein
chondroitin sulfate chain
keratan sulfate chain
N-linked oligosaccharide
PG-M/Versican
N
C
HABR
GAG
LEC
C
EGF

Core: Cell Surface HSGAG



Found virtually on all cells or cell-ECM interface
Act as a reservoir, binding and storing signaling molecules

GAGs influence diffusion of signaling molecules - morphogen movements [gradient?]



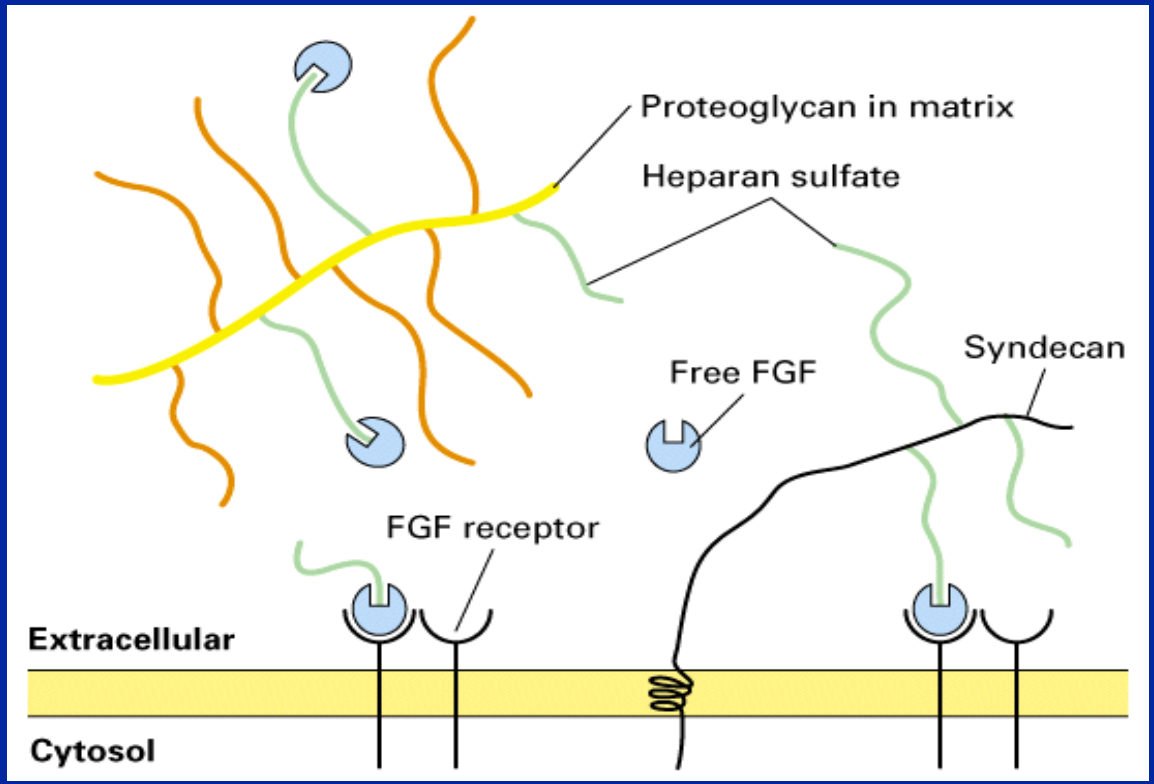
Mechanism of action at a molecular level

- Disaccharide repeat unit with 4 potential modification sites - 48 units
- Minimal binding sequence
- I conformation modulate helix [kink] to make maximum contact with protein surface
- Specificity may arise from spacing of binding sites

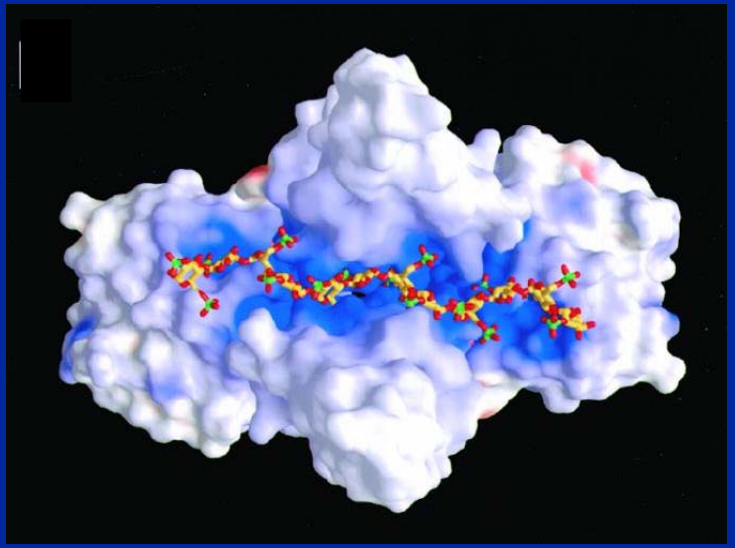
Modulating of signaling molecules at the cell surface via sequence specific interactions

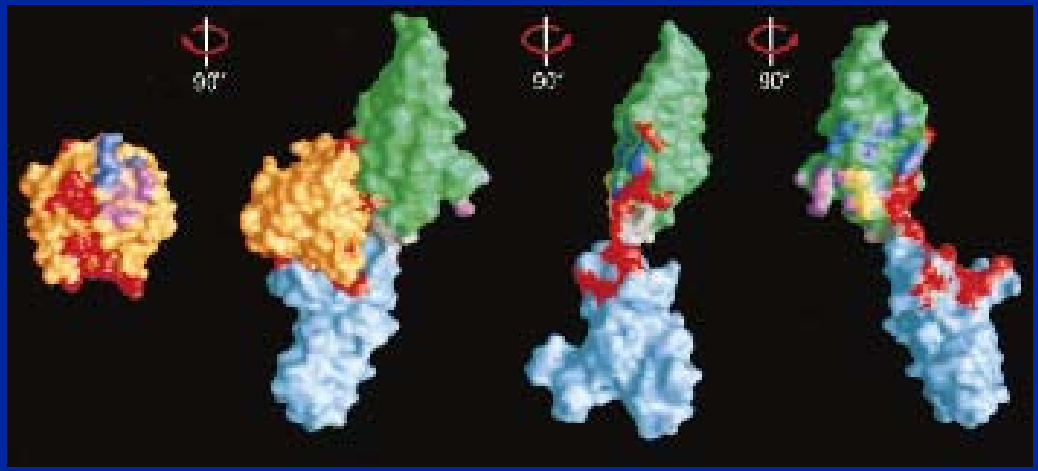
- Platform for ligand-receptor interactions
- Specificity for ligand-receptor complex [FGF-FGFRs]

ECM and Growth Factor

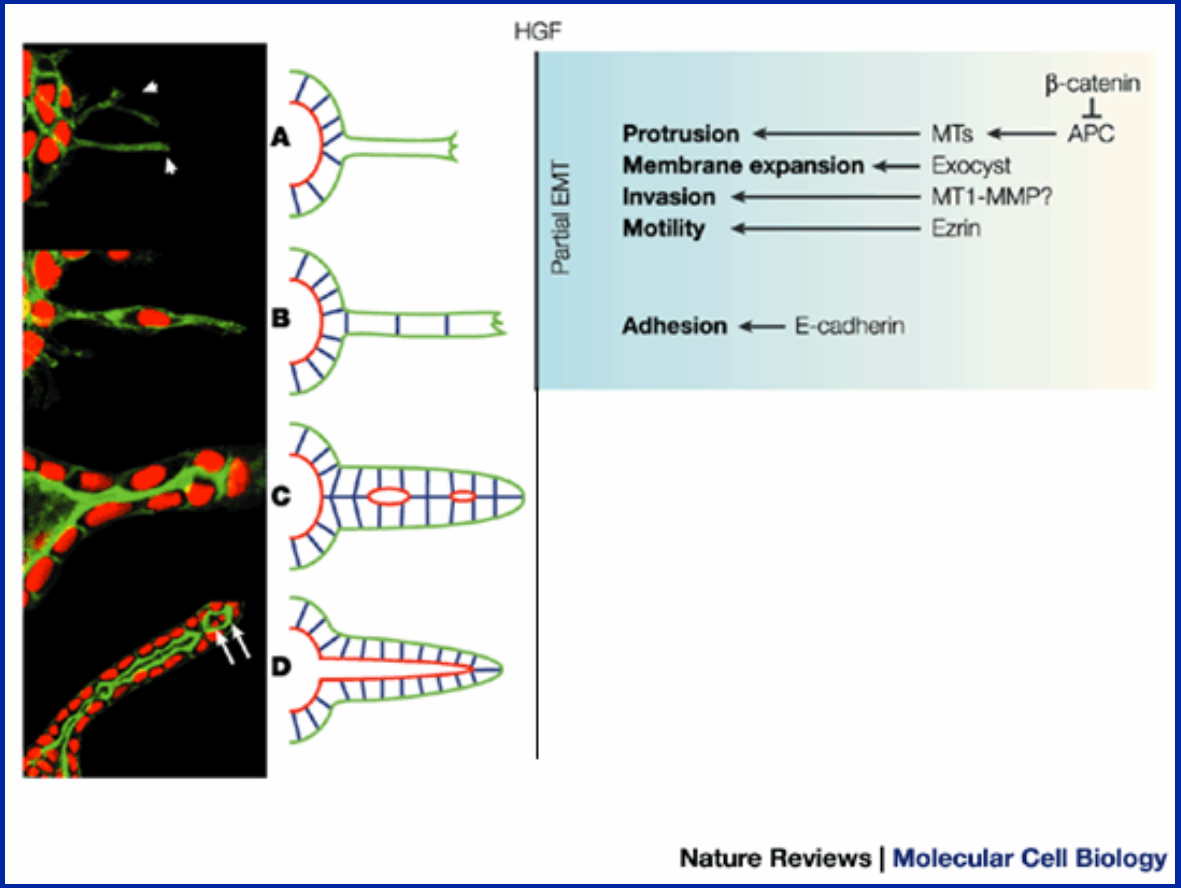


FGF-FGFR complex

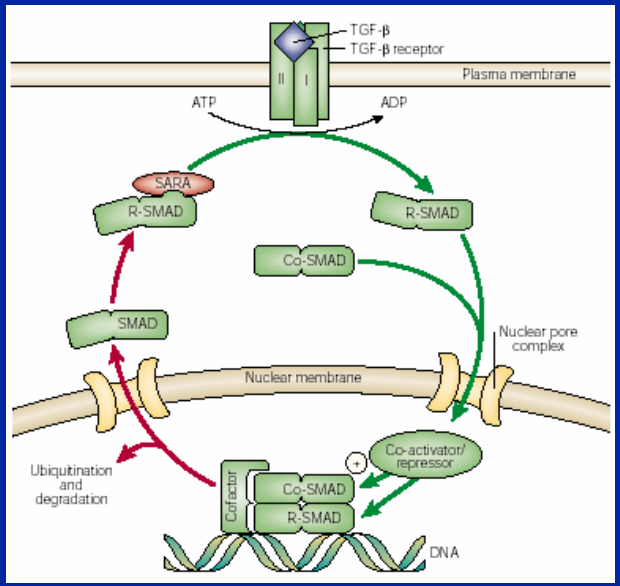


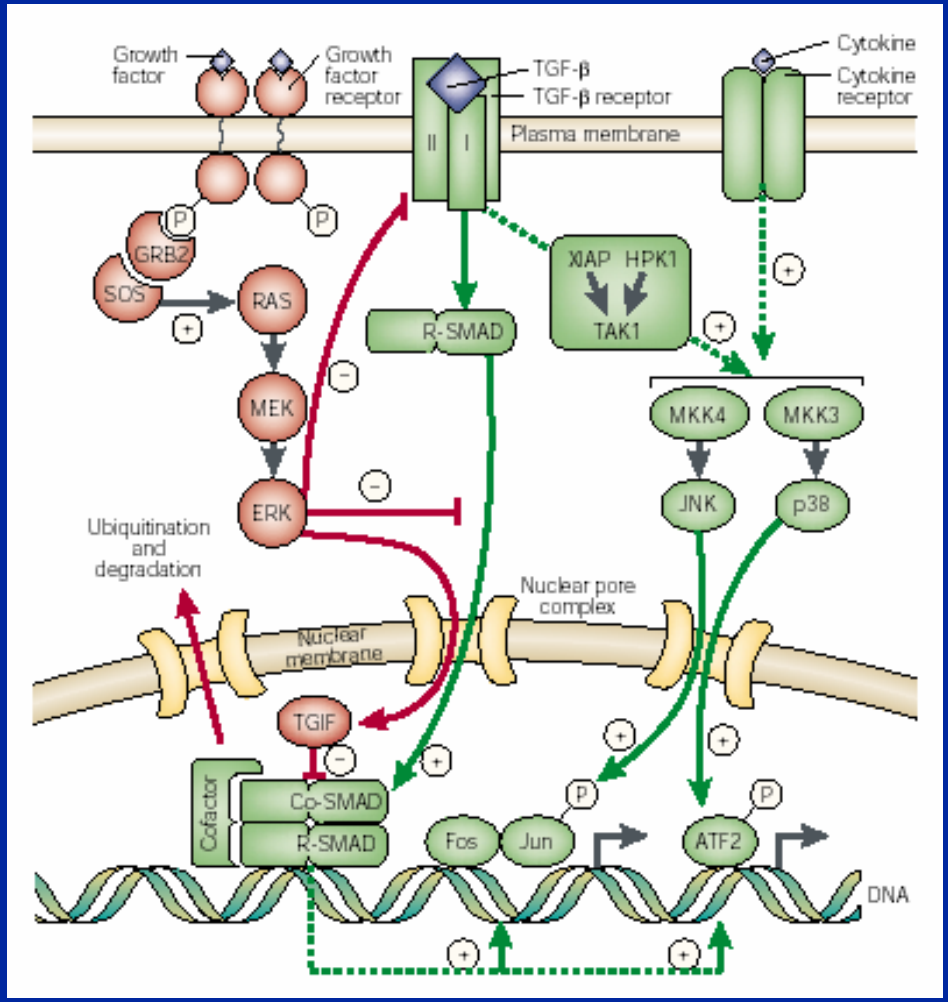


Tube formation



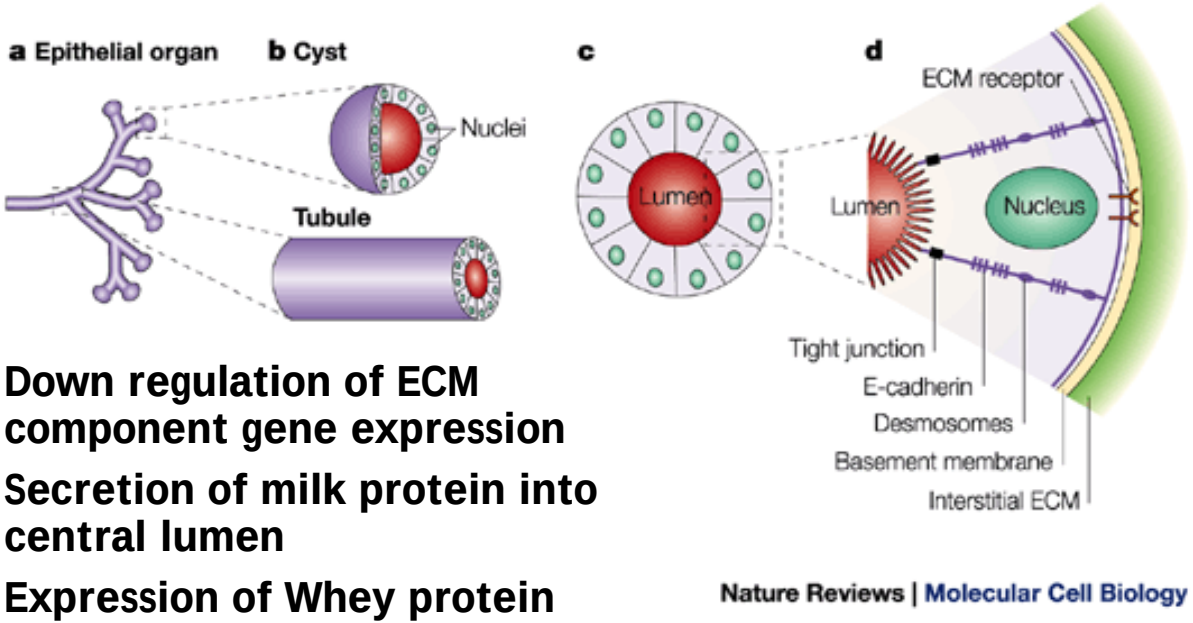
TGF-beta





Nature Reviews Molecular Cell Biology **1, 169-178 (2000**

Morphogenesis

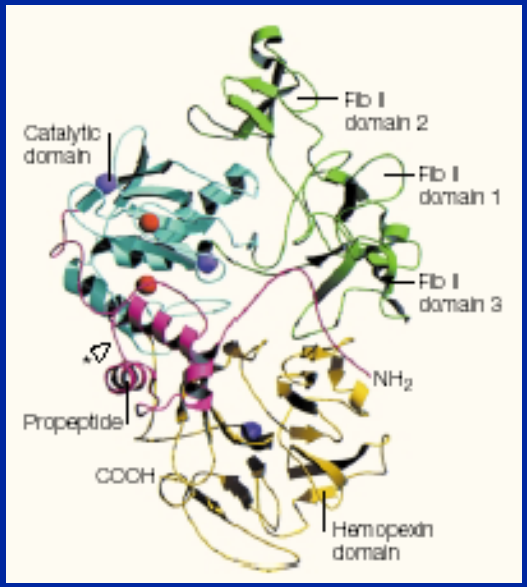


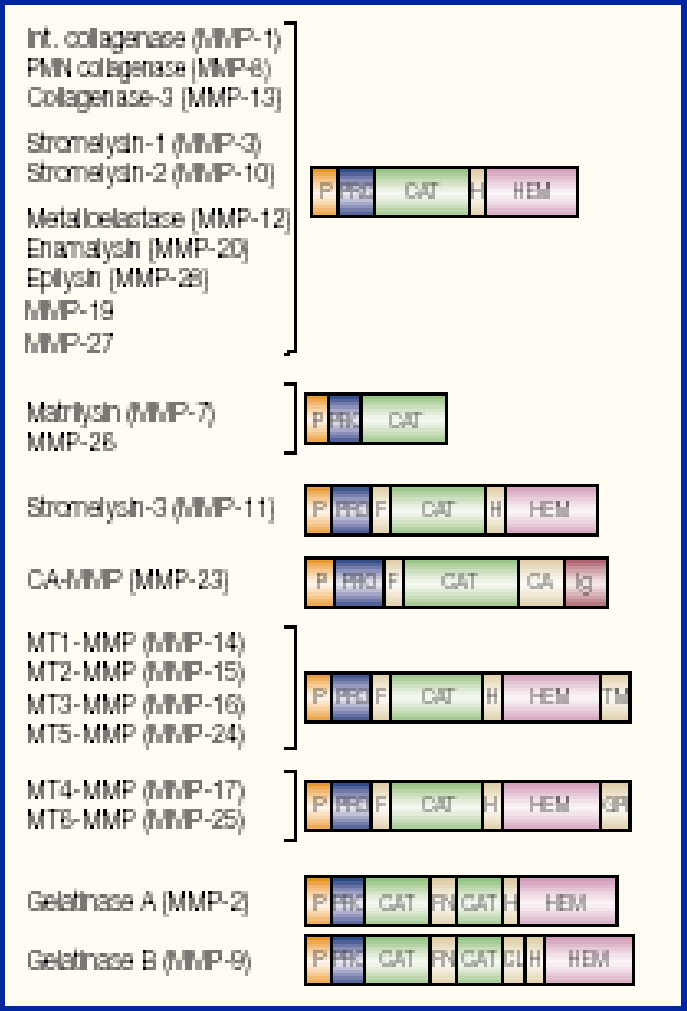
- **Down regulation of ECM component gene expression**
- **Secretion of milk protein into central lumen**
- **Expression of Whey protein**

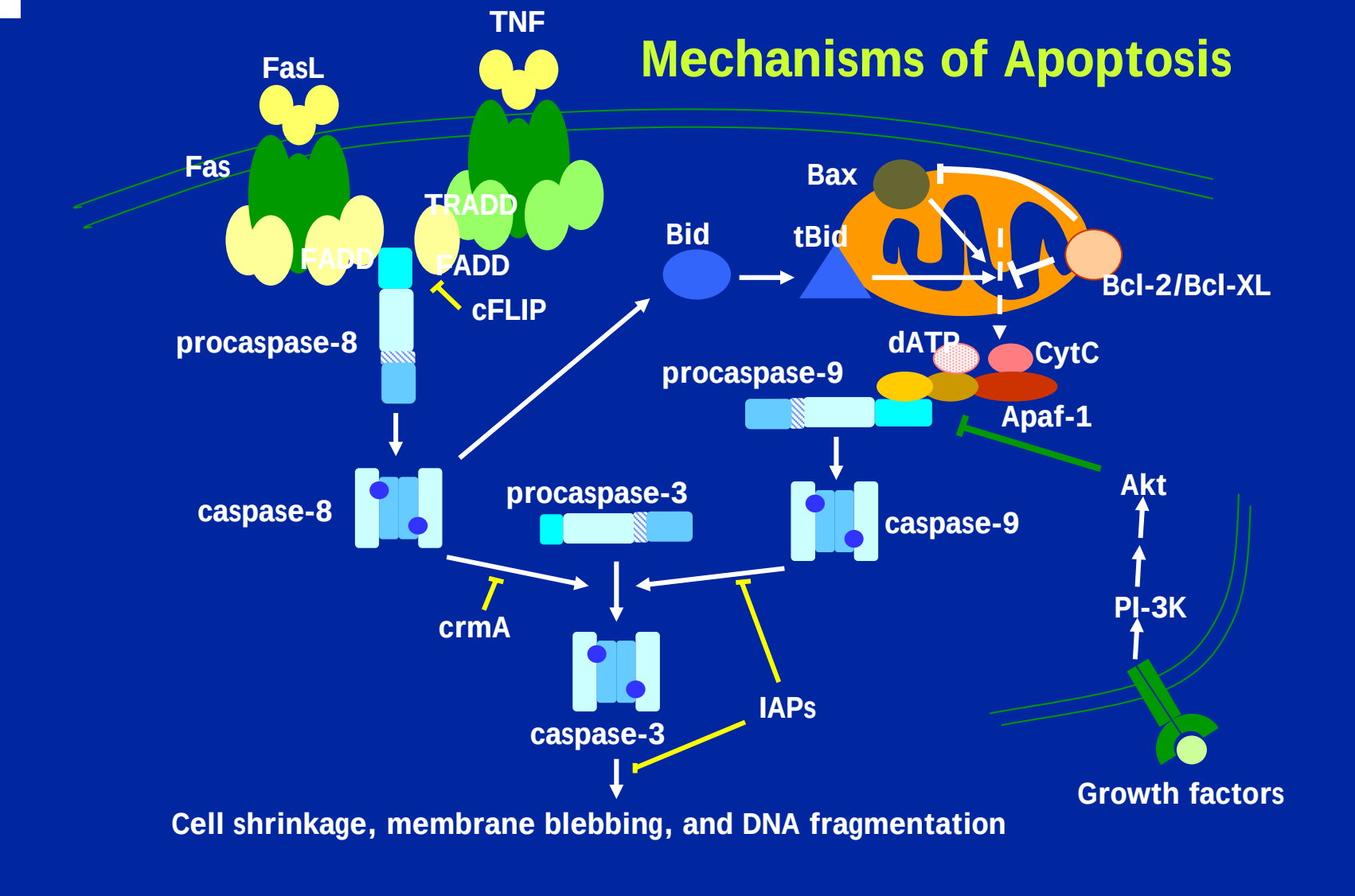
Destruction & Involution

- Inhibition of milk protein expression
- Increased Matrix Metalloprotease production
- Decreased production of MM inhibitors
- Basement membrane destruction and Enactin fragmentation and increase tenascin production
- Loss of cell function
- ICE dependent apotosis

MMPs


Fib II
domain 2
Catalytic
domain
Fib II
domain 1
Fib II
domain 3
NH_2
Propeptide
COOH
Hemopexin
domain


Int. collagenase (MMP-1)
PMN collagenase (MMP-8)
Collagenase-3 (MMP-13)
Stromelysin-1 (MMP-3)
Stromelysin-2 (MMP-10)
Metalloelastase (MMP-12)
Enamelysin (MMP-20)
Epilysin (MMP-28)
MMP-19
MMP-27
Matrilysin (MMP-7)
MMP-26
Stromelysin-3 (MMP-11)
CA-MMP (MMP-23)
MT1-MMP (MMP-14)
MT2-MMP (MMP-15)
MT3-MMP (MMP-16)
MT5-MMP (MMP-24)
MT4-MMP (MMP-17)
MT6-MMP (MMP-25)
Gelatinase A (MMP-2)
Gelatinase B (MMP-9)
P
CAT
H
HEM
F
CA
Ig
TM
GPI


Mechanisms of Apoptosis
TNF
FasL
Fas
TRADD
FADD
FADD
cFLIP
procaspase-8
caspase-8
Bid
tBid
Bax
Bcl-2/Bcl-XL
dATP
CytC
Apaf-1
procaspase-9
caspase-9
procaspase-3
crmA
caspase-3
IAPs
Akt
PI-3K
Growth factors
Cell shrinkage, membrane blebbing, and DNA fragmentation

Key Points: I

- **Flow of information between cells and tissues are integrated into a signaling hierarchy that is :**

  a) constructed and then b) dismantled in a cyclical manner
- **First tier of hierarchy involves mechanical signals : cell rounding that trigger lactoferrin gene expression**
- **Second tier: Rounded cells deposit ECM and initiate a laminin mediated hierarchy leading to biochemical signal transduction and activation of a wide range of genes**

Key Points: II

- **The third tier of hierarchy signaling relies on the ECM morphogenesis, wherein presence of ECM directs cell polarity, formation of central lumen and expression of WAP.**

  WAP is expressed late in pregnancy and just before the onset of lactation.

- **Fourth tier: Dismantling of this hierarchy begins at weaning is mediated by ECM-degrading enzymes, which act in a development stage manner to induce programmed cell death.**

Summary

- Signaling hierarchy emerges as a universal integrator of function for a given physiology
- Fundamental cellular processes modulated by biochemical signals- cycles of growth, differentiation, morphogenesis and apoptosis
- Molecular (biochemical, mechanical, physical interactions) - cellular - tissue - organ system