

## Signaling Hierarchy

*Mammary Epithelial Cells*

*Part I & II*

## Mammary Epithelial Cell

*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

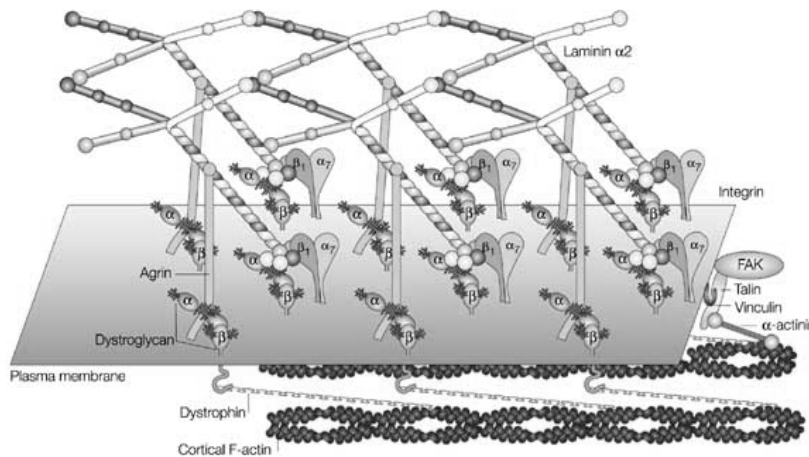
## Signaling Hierarchy

- 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 tubes (ln)
- Decreased adhesion to rigid substratum: *mechanical in nature*
- Increased cell rounding
- Reorganization of cytoskeleton (markers)

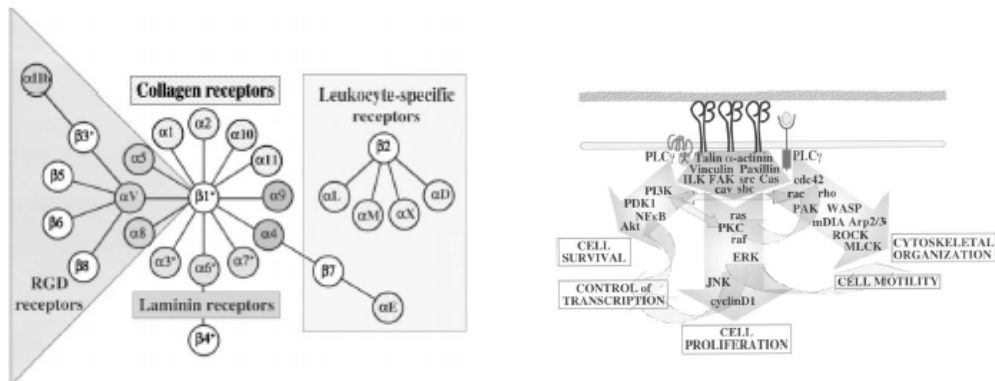
## Laminin Signaling



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

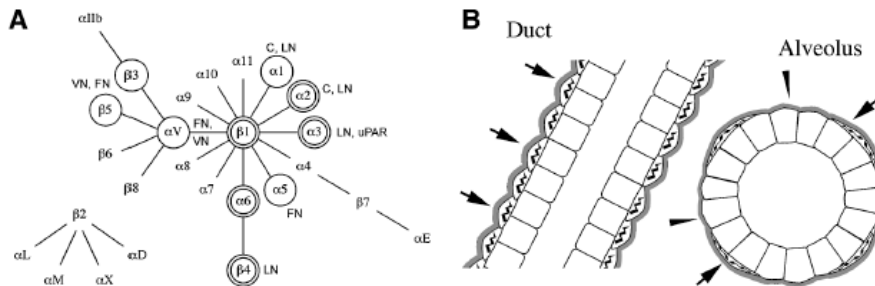
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# Integrin Family & Signaling

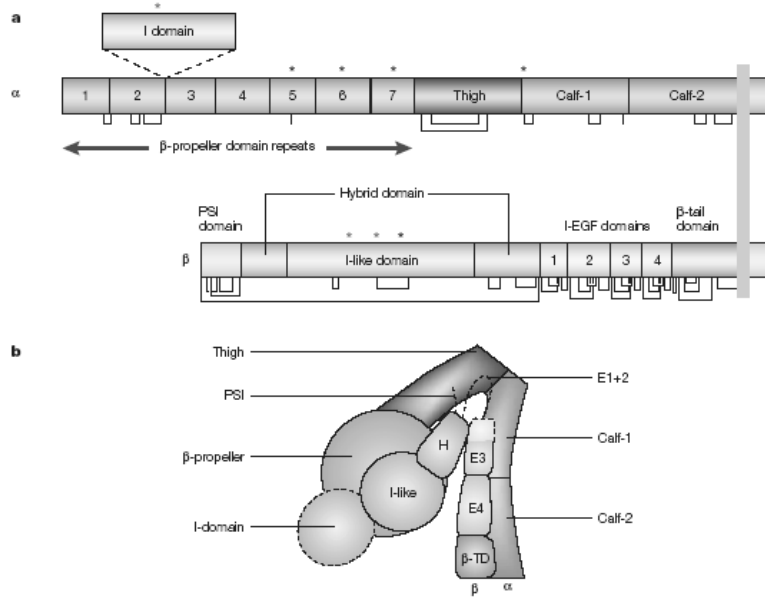


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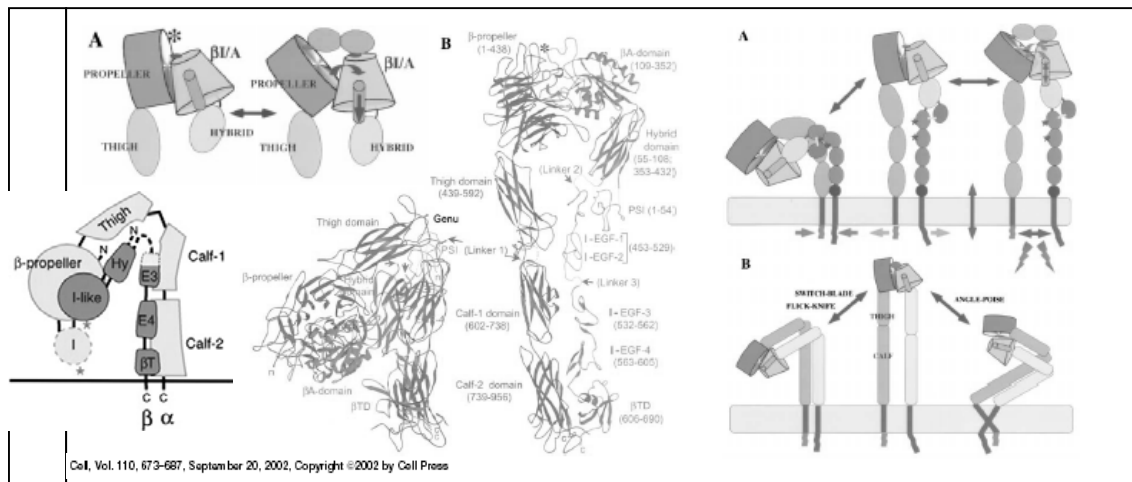
## Integrins *Mammary Gland Development*



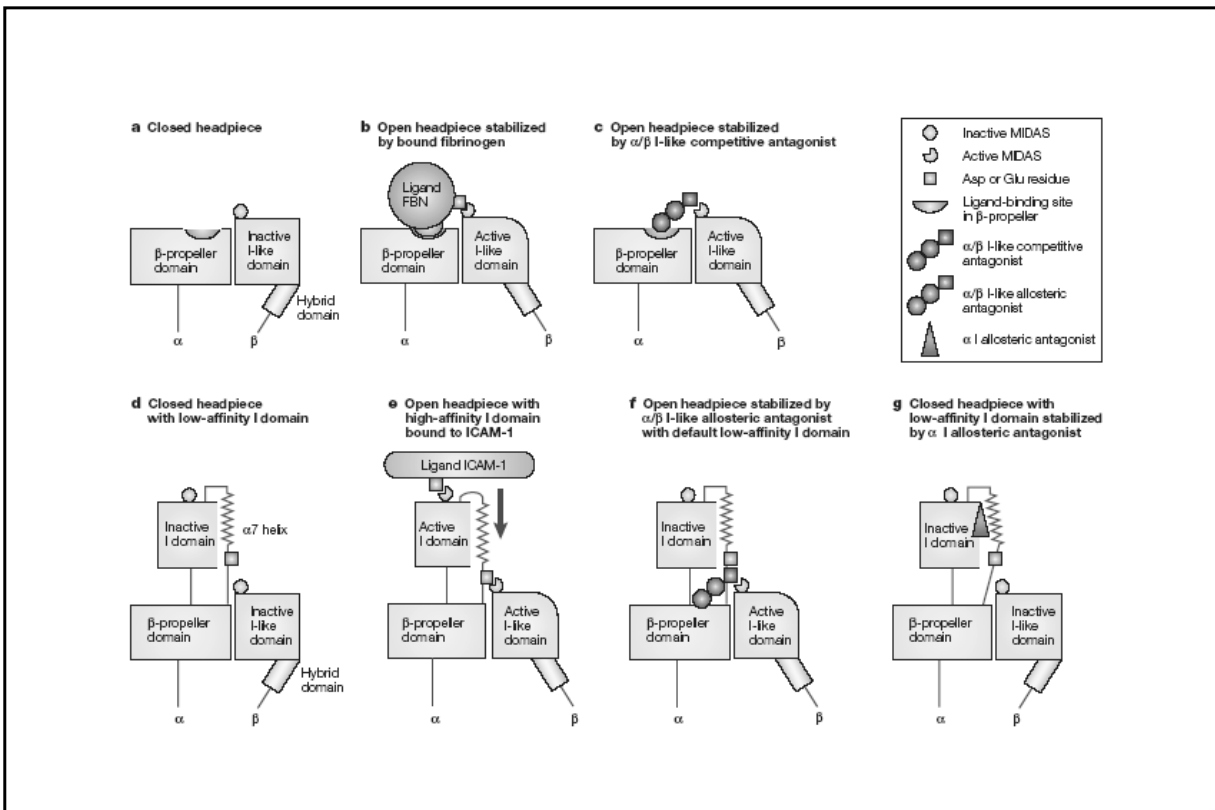
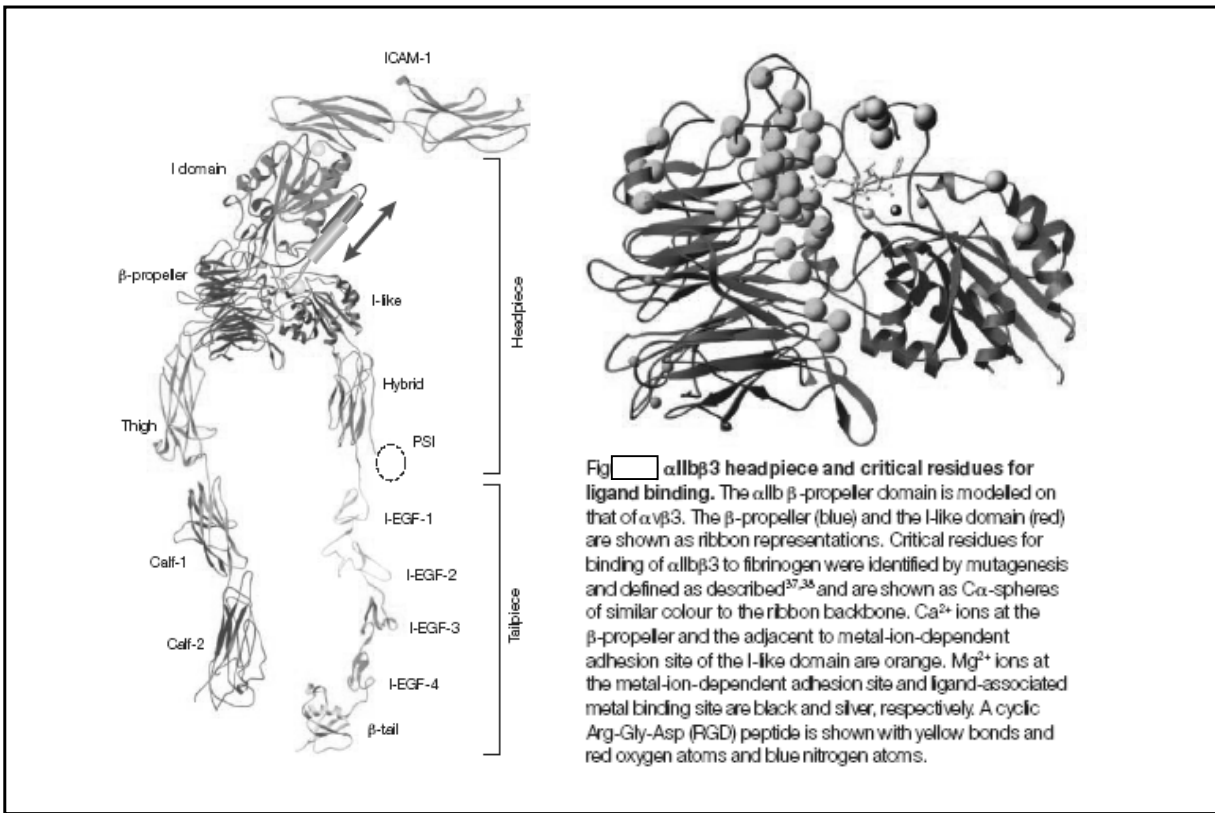
## Integrin chains



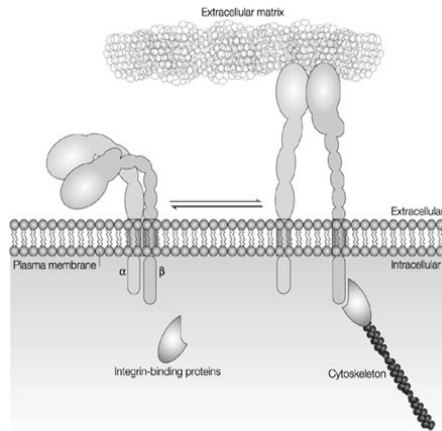
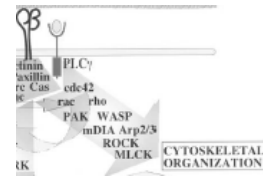
## Integrin Signaling I



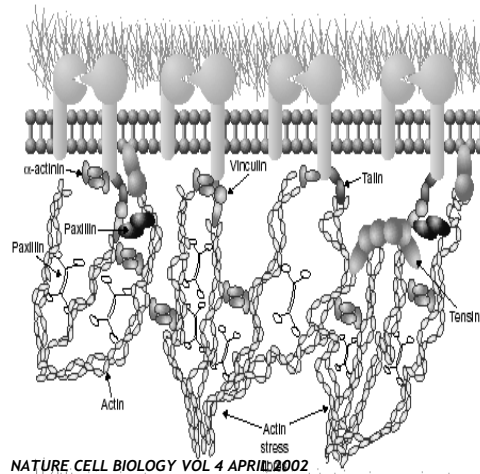
# BE 440. Analysis of Biological Networks



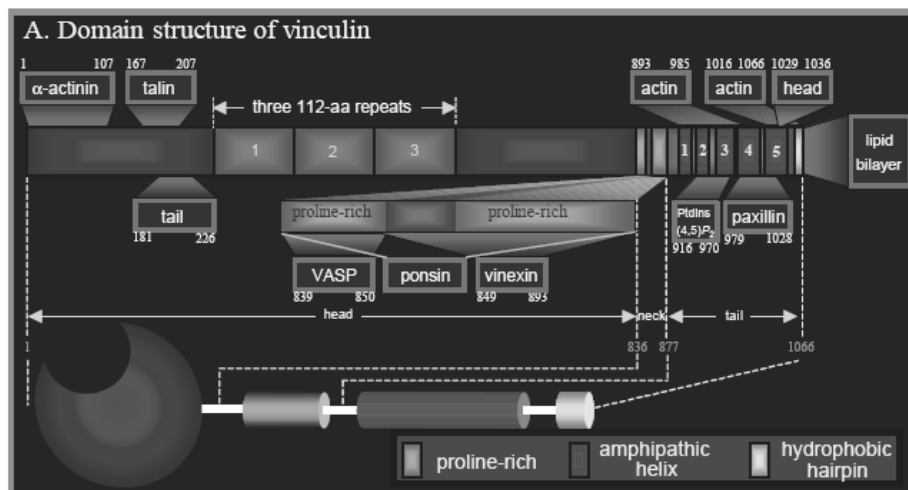
## Integrin Signaling



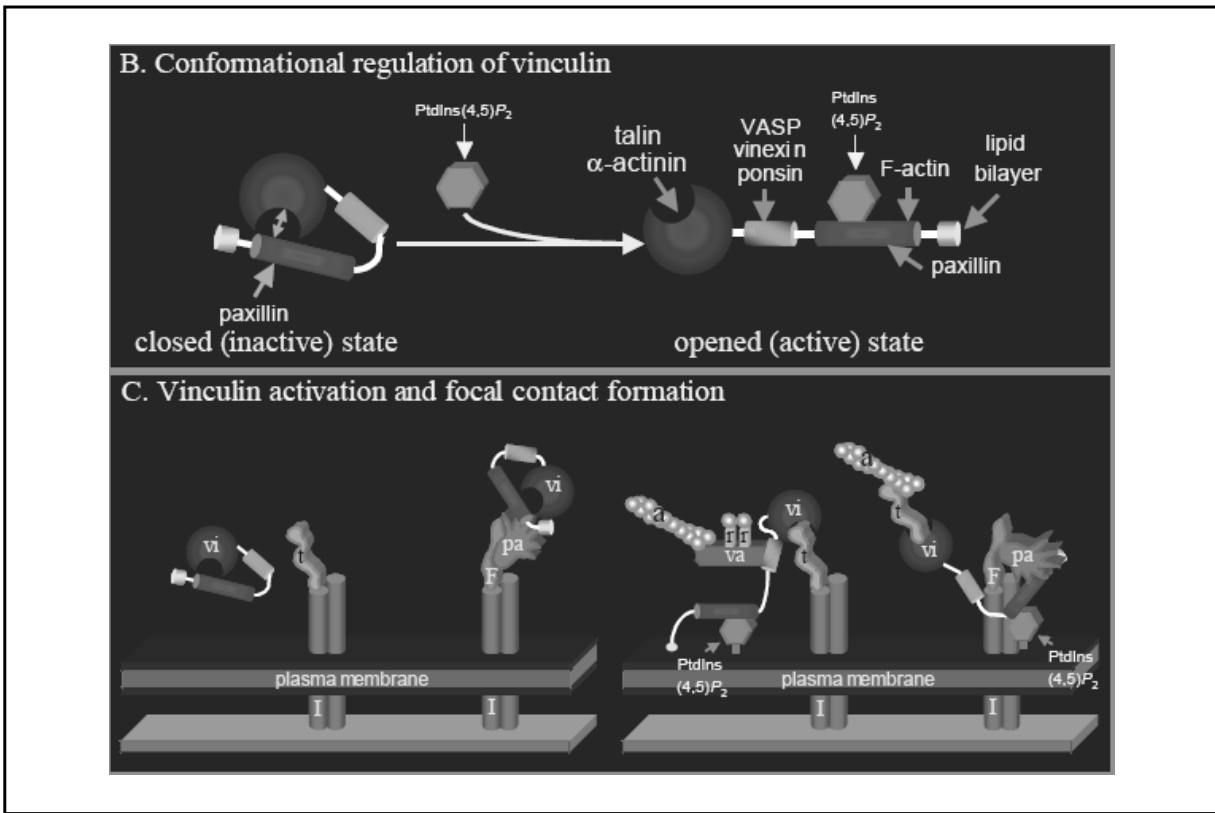
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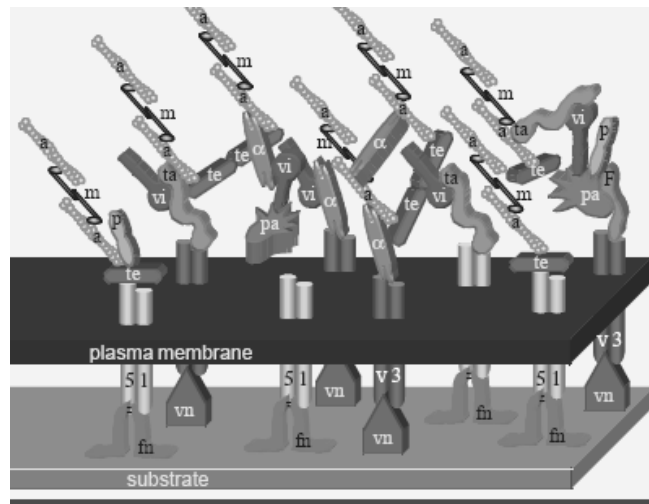
## Vinculin



# BE 440. Analysis of Biological Networks

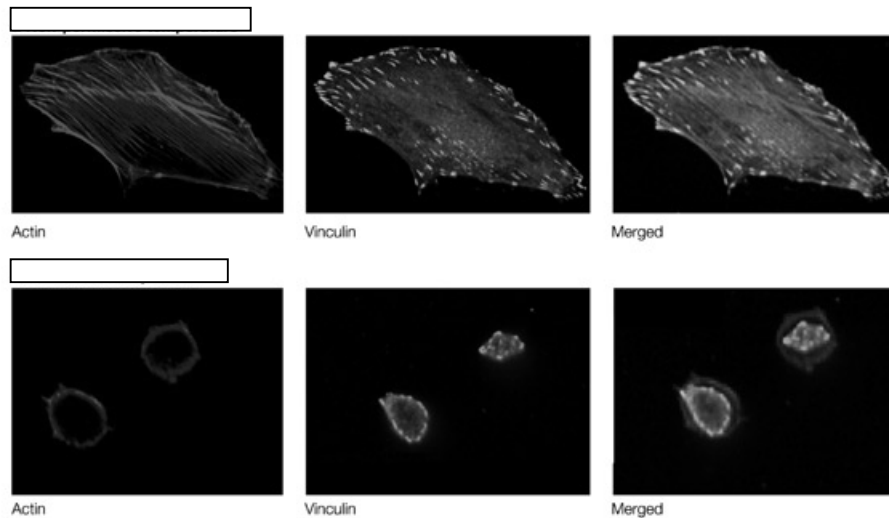


## Adhesion Complex





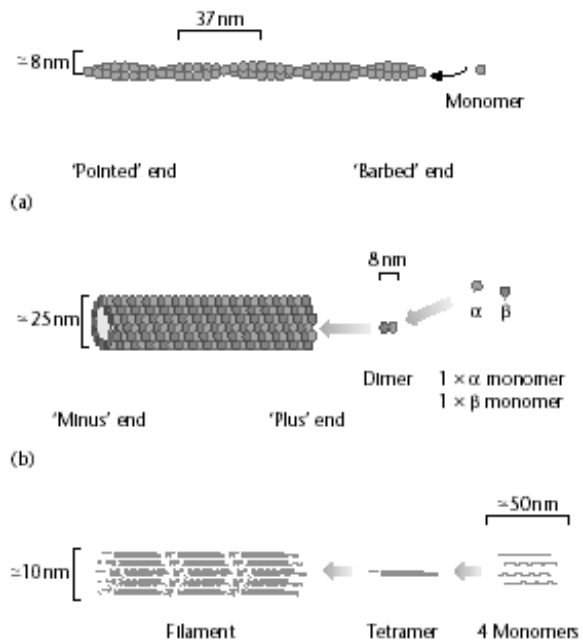
## Actin/Vinculin Complex



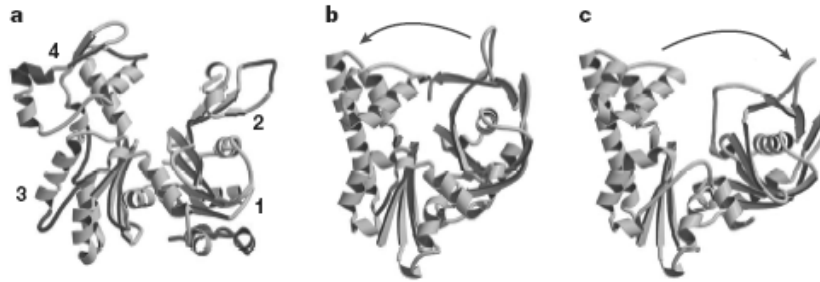
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## Cytoskeleton

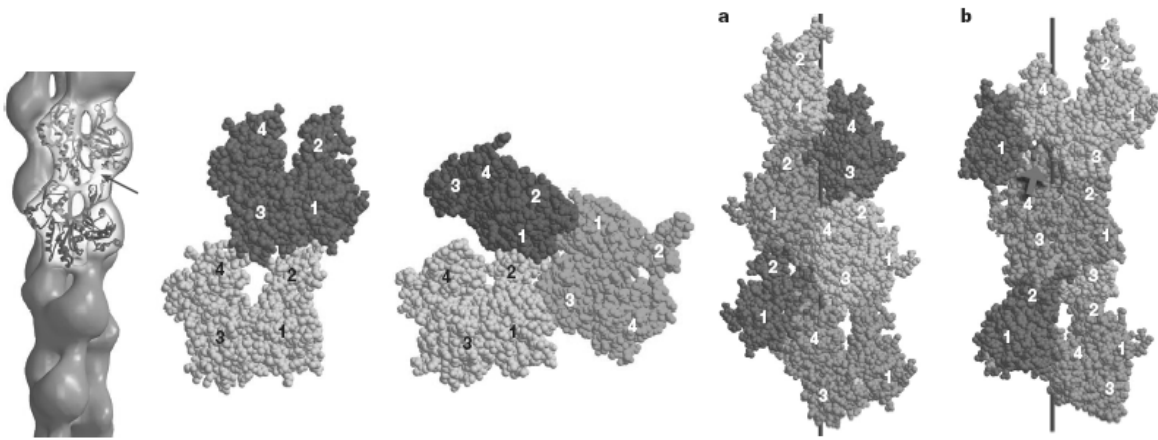
- Microfilaments [actin monomers]
- Microtubules [ $\alpha$  and  $\beta$  - tubulin]
- Intermediate filaments [various different types of monomers]



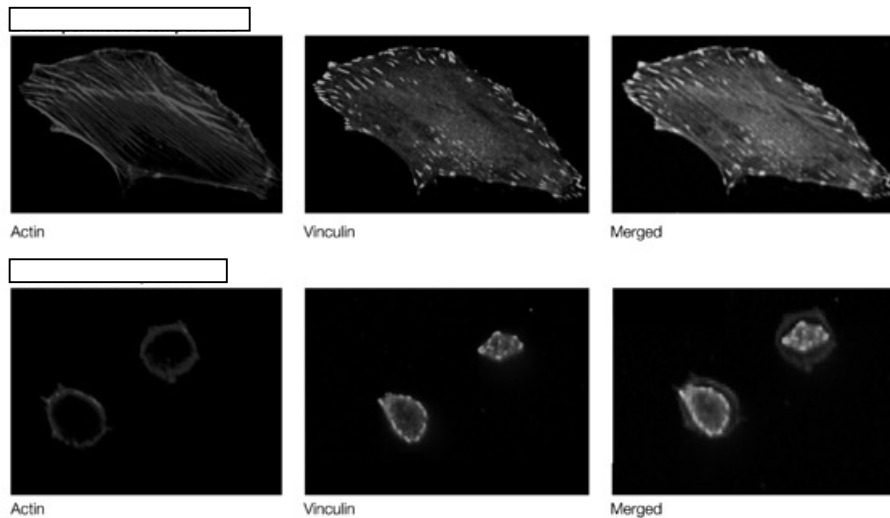
## Properties of the actin subunit



## Actin Polymerization

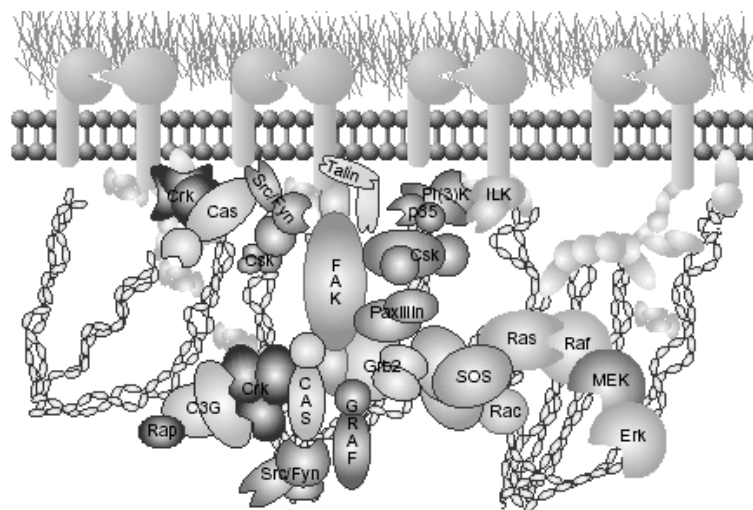


## Actin/Vinculin Complex



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## Cytoskeleton & Integrin signaling

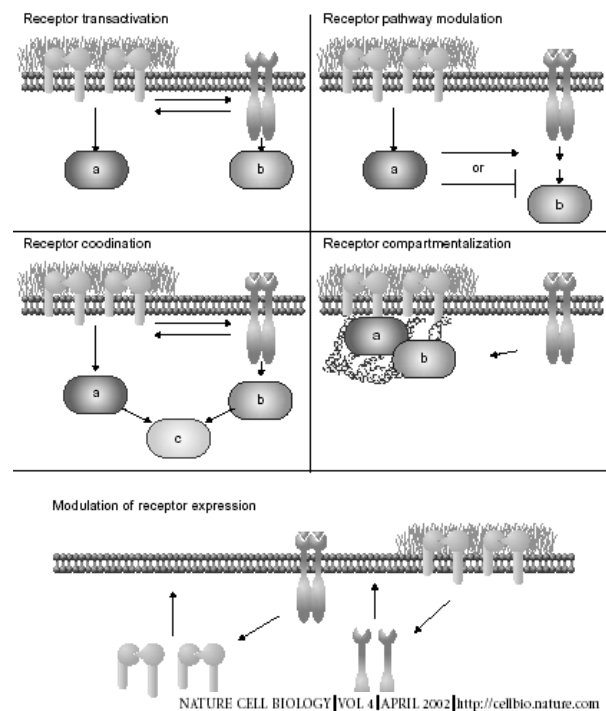


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## Cell-ECM contact

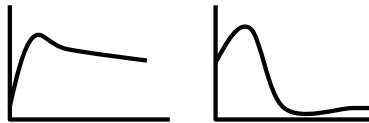
- Cell shape is a set point for proliferation *versus* differentiation
- Integrin signaling - Cross talk to make sure that differentiation signaling is different from proliferation
- Cessation of proliferation - exit cell cycle
- Decreased AP1 transcription factor activity

## Integrin Signaling Diversity

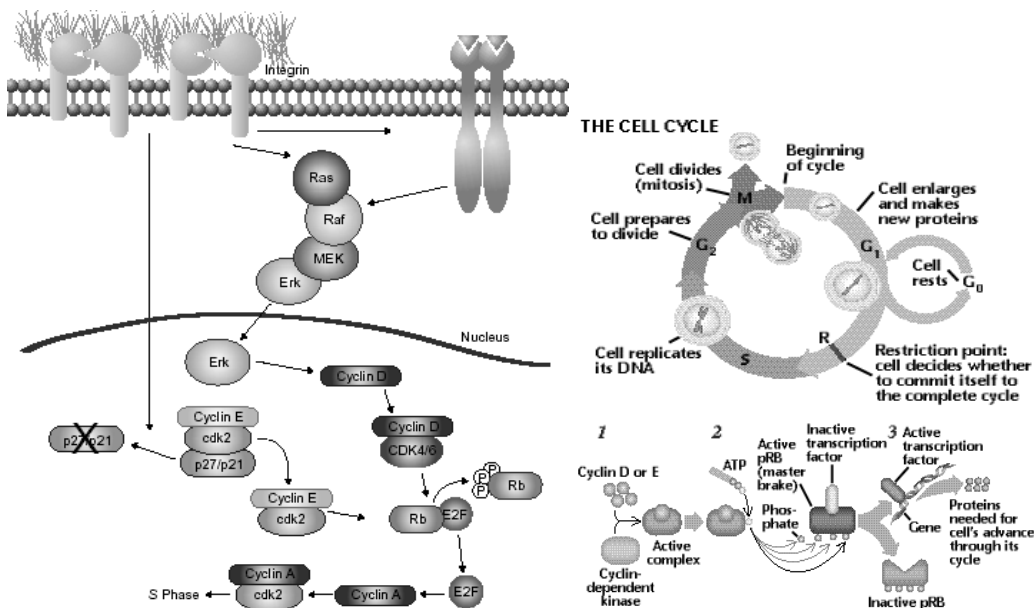


## Integrin Signaling II

- 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



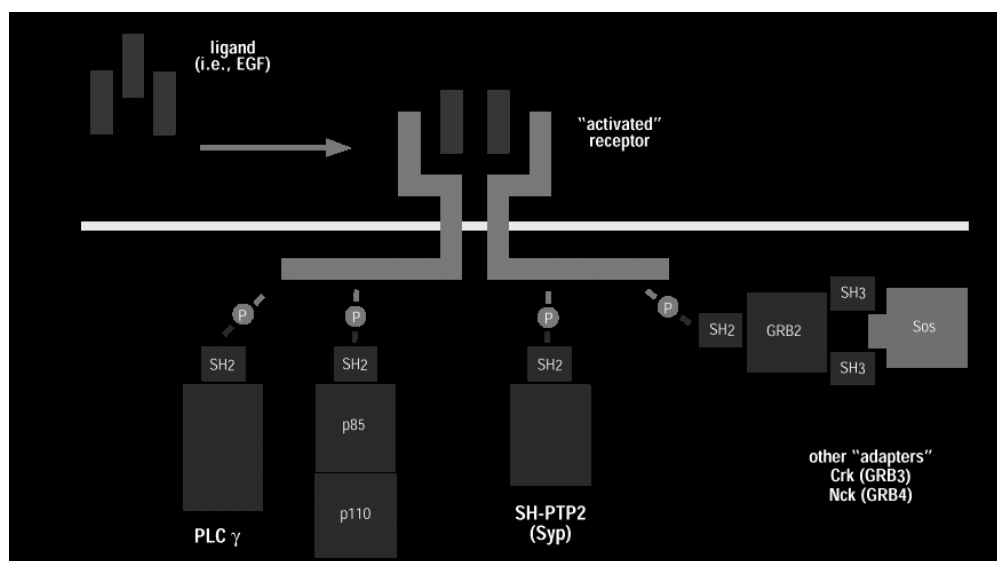
## Integrin Coupled with growth factor Signaling: Cell Cycle *in* or *out*



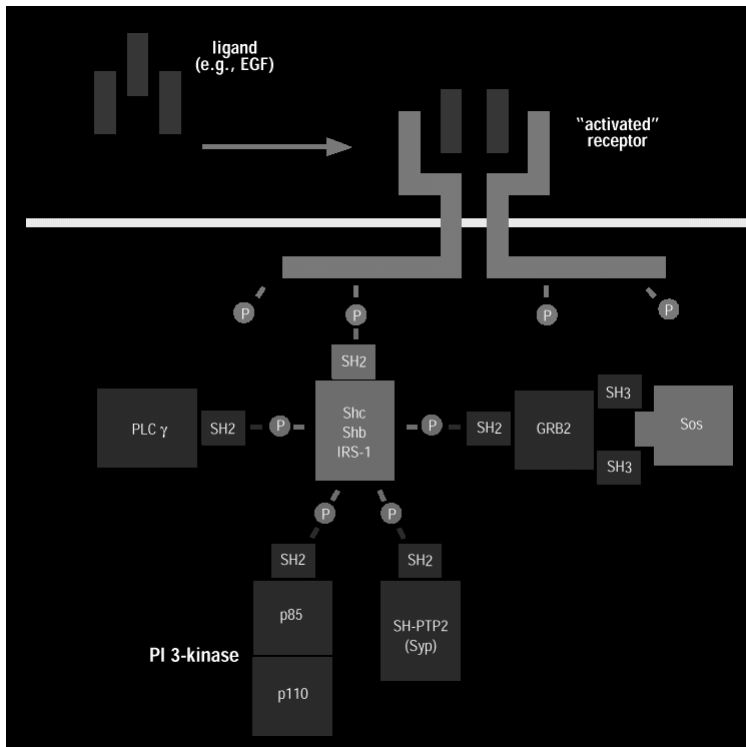
## Receptor Mediated Signaling

	Horizontal receptors	Vertical receptors
Typical ligand	Protein	Small molecule
Signaling induced by	Change in oligomeric state	Structural change in pre-associated receptor; no change in quaternary state
Response time	Slow	Fast
Typical cellular response	Change in transcription, translation, replication or apoptosis	Reversible metabolic change
Evolutionary origin	Eukaryotes (recent)	All species (old)
Example	Cytokine receptors	G protein-coupled, ligand-gated ion channels

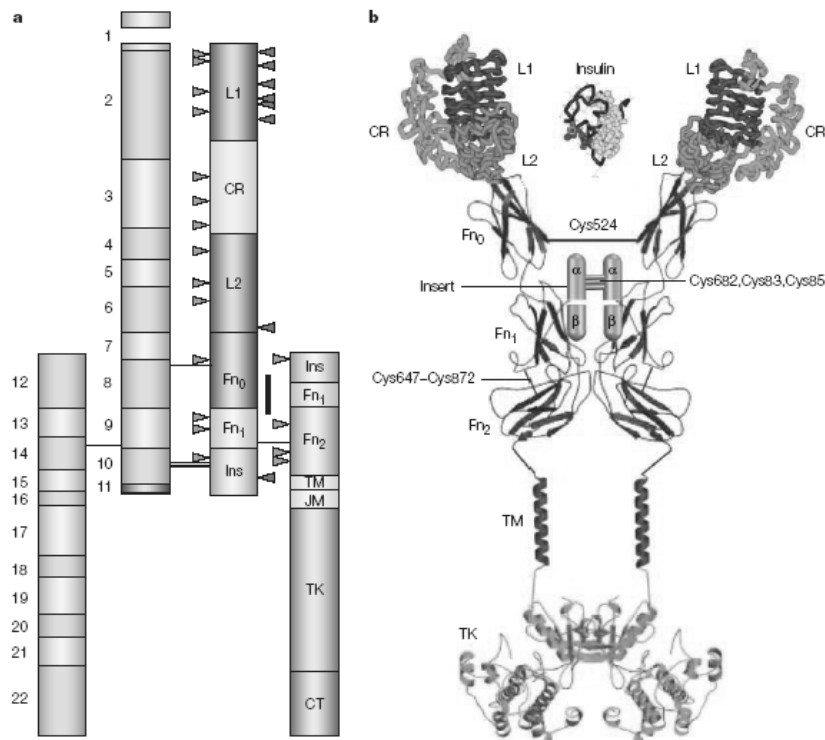
## Growth Factor Signaling



## Substrate Mediated Signaling

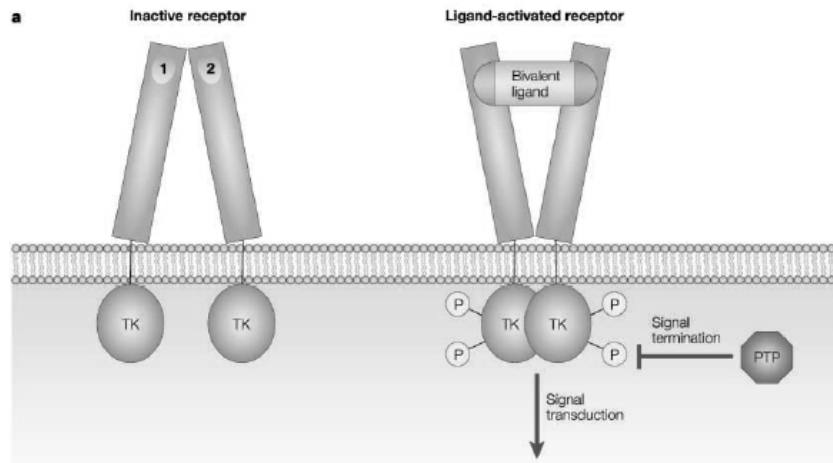


## IGF signaling

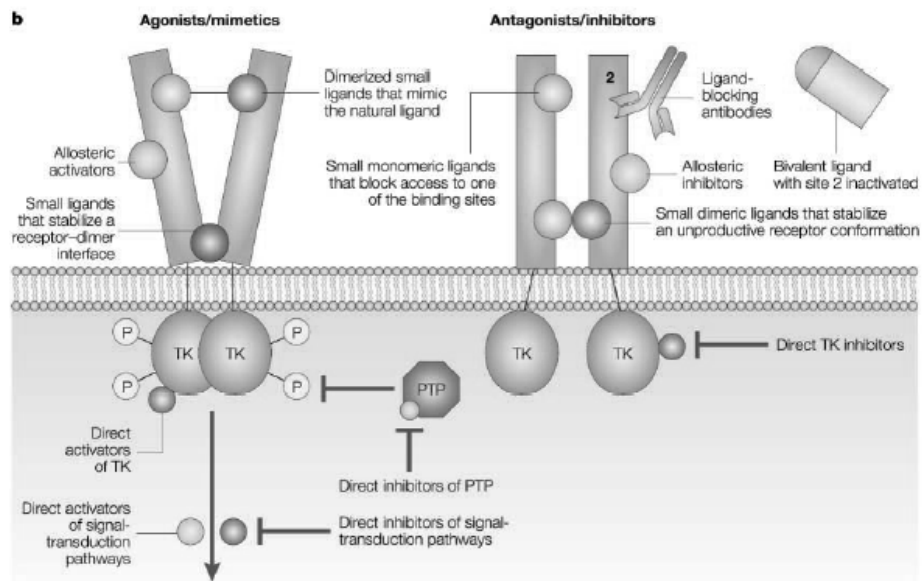


# BE 440. Analysis of Biological Networks

## IGF

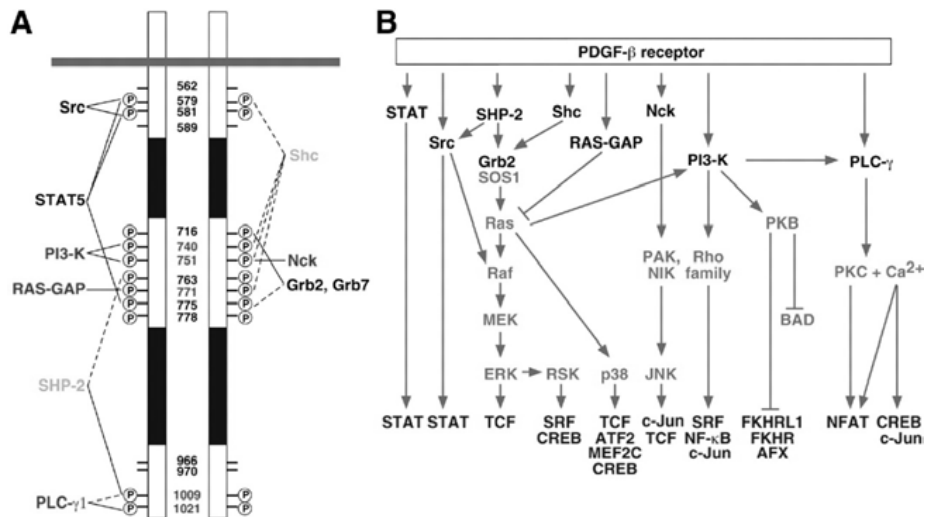


## IGF

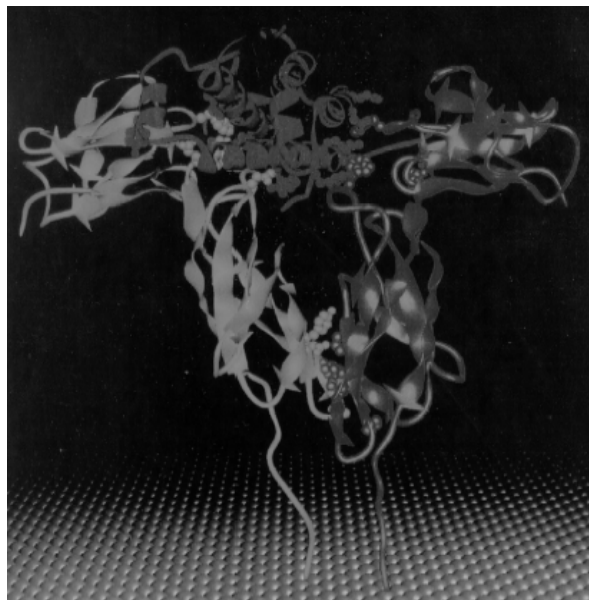




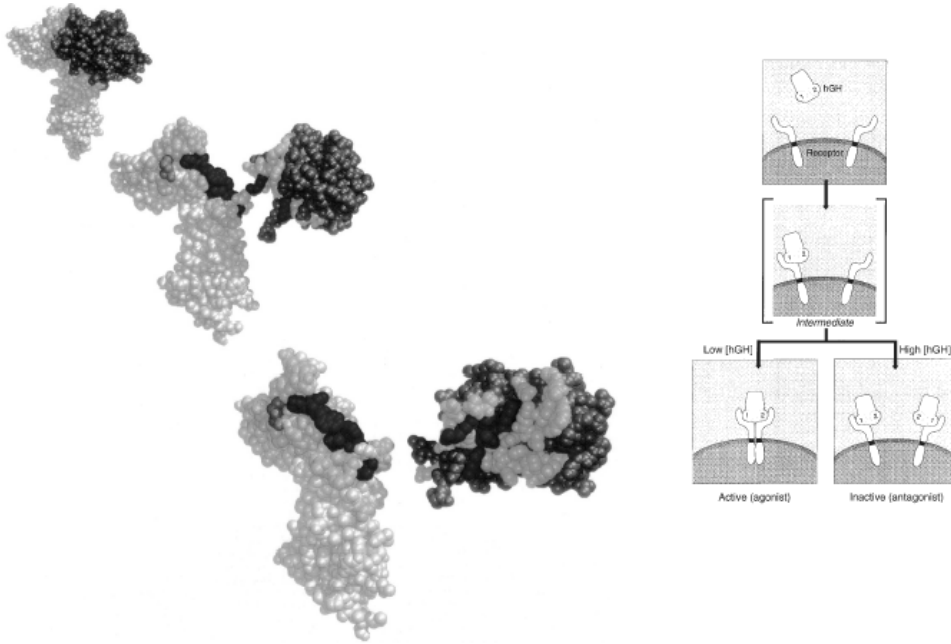
## Growth Factor Signaling



## Hormone Binding

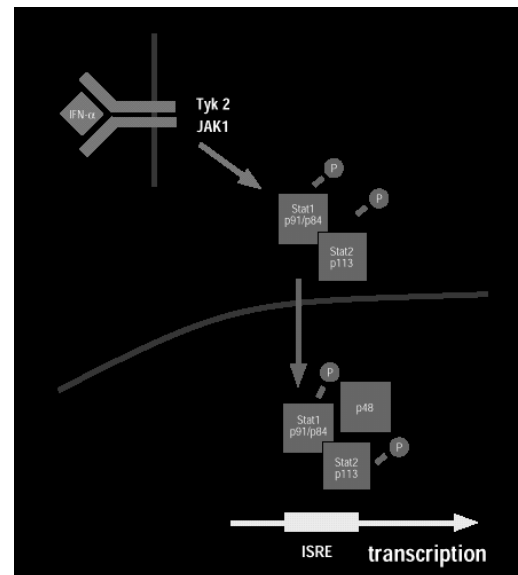


## Hormone Binding

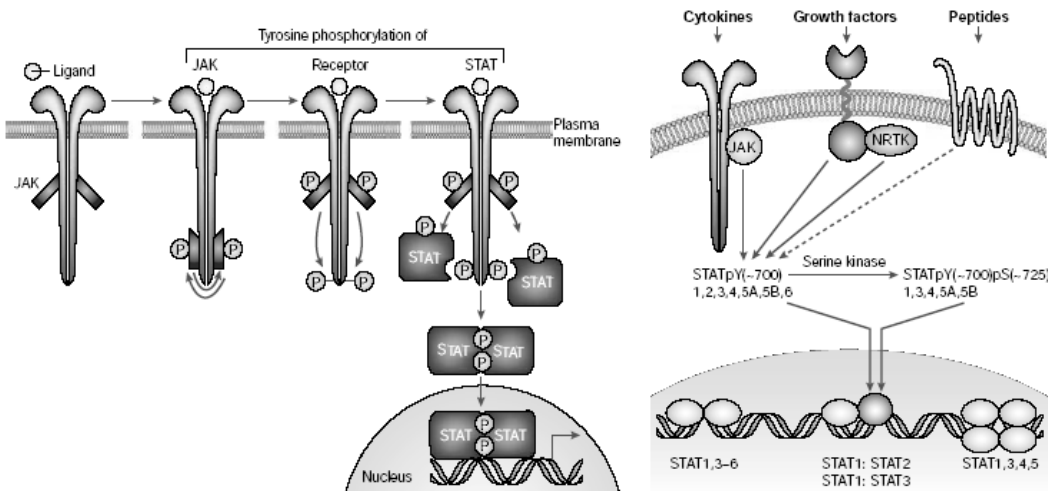


## Differentiation Specific Effects

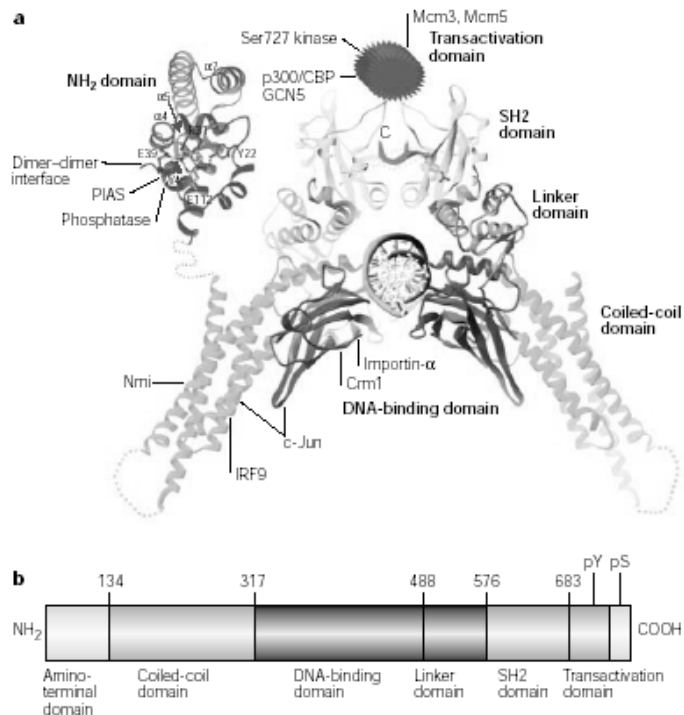
- STAT-5 interacts with activated prolactin receptor: gets P, and becomes a transcription factor
- BCE-1 contains STAT-5 binding sites
- beta-casein expression is on



## STAT pathway

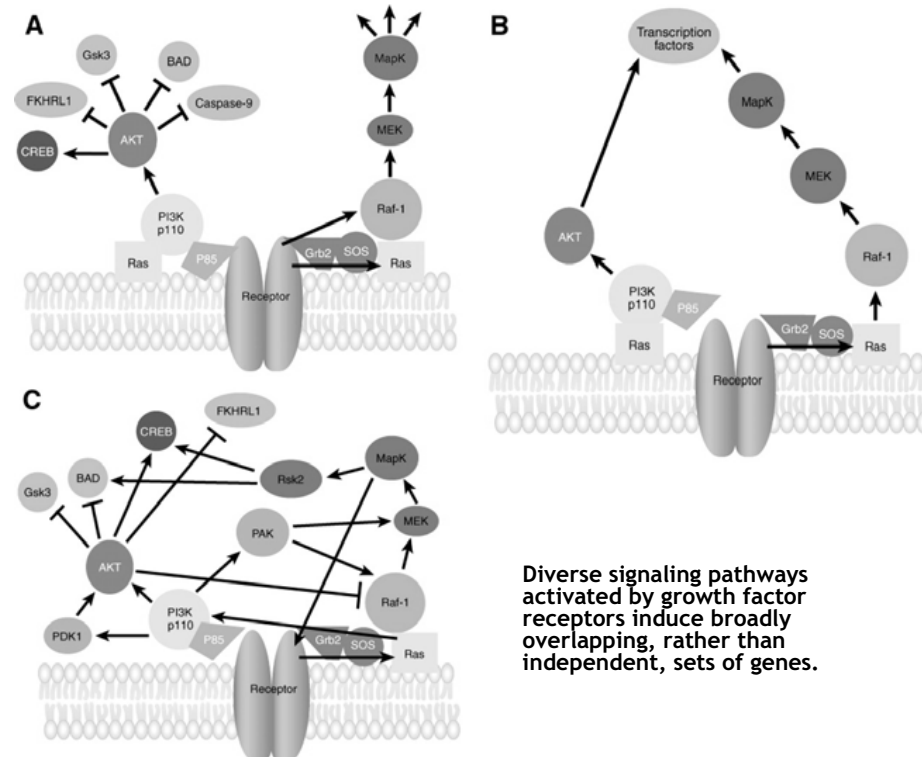


## STATs



## 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



# BE 440. Analysis of Biological Networks

## Micro array Prolactin putative target genes

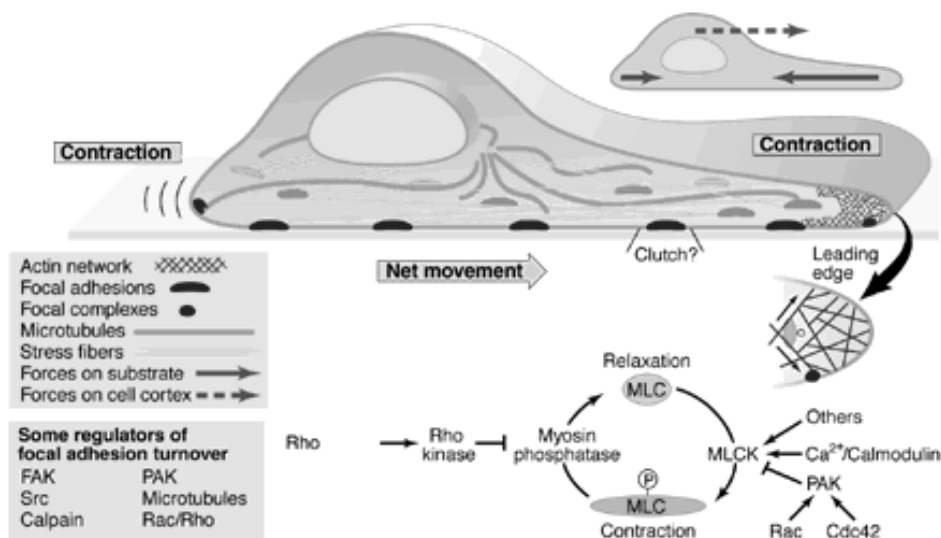
Table 1. Putative Prolactin Target Genes

Genes Related to Milk Secretion	
X09037	<i>M. musculus</i> mRNA for WDM1 protein
W18308	Mouse ferritin heavy chain gene
x04673	Adipin
X61431	ACYL-COA-BINDING PROTEIN
W44201	Sim. to PROTEIN TRANSPORT PROTEIN SEC23
Y00516	Mouse mRNA for aldolase A:FRUCTOSE-BISPHOSPHATE ALDOLASE A
M32599	Mouse glyceraldehyde-3-phosphate dehydrogenase mRNA
A001776	Sim. to GLUCOSE-6-PHOSPHATE ISOMERASE (PHOSPHOGLUCOSE ISOMERASE)
X05277	Mouse hexokinase mRNA
x04400	Casein beta
L09104	<i>M. musculus</i> glucose phosphate isomerase mRNA, 3' end
M21285	Mouse stearyl-CoA desaturase gene
AA117004	Sim. to ER LUMEN PROTEIN RETAINING RECEPTOR (KDEL RECEPTOR) (P23)
X02520	Lactate dehydrogenase 1, A chain
W09599	Sim. to FATTY ACID-BINDING PROTEIN
Calcium Metabolism	
X07991	Calcitonin
M27844	Parvalbumin
W20937	Sim. to CALCIUM-TRANSPORTING ATPASE SARCOPLASMIC RETICULUM TYPE
Cytoskeleton	
x51438	Vimentin
AA158865	Sim. to ACTIN 1 (FRAGMENT)
X13297	ACTIN, AORTIC SMOOTH MUSCLE
J04953	Mouse gelsolin gene
X14425	Mouse mRNA for profilin:PROFILIN I
U20365	<i>M. musculus</i> smooth muscle gamma actin mRNA
Growth Factors	
AA002605	Mouse insulin-like growth factor II (IGF-II) gene, 5' flank
Extracellular Matrix	
X04017	Mouse mRNA for cysteine-rich glycoprotein SPARC
x14104	Nidogen
x17099	Mouse COL1A2 mRNA for pro-alpha-2(I) collagen
x55582	Procollagen, type VI, alpha 2
U08920	<i>M. musculus</i> alpha 1 type I collagen gene, partial cds and 3' flanking region
ET61037	Lectin, galactose binding, soluble 1
x72862	<i>M. musculus</i> gene for beta-3-adrenergic receptor:Adrenergic receptor, beta 3
X73523	Mouse mRNA for matrix Gta protein (MGF)
W75372	Procollagen, type IX, alpha 2
Signal Transduction	
x15258	Sim. to INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 4 PRECURSOR (IGFBP-4)
W55899	Sim. to GUANINE NUCLEOTIDE-BINDING PROTEIN G(i)(G13)(G12) BETA SUBUNIT 2
D10024	Mouse mRNA for protein-tyrosine kinase substrate p36 (calpactin I heavy chain)
X55251	<i>M. musculus</i> mRNA for E-selectin ligand-1
L29108	<i>M. musculus</i> mRNA for GTP-binding protein
X05788	<i>M. musculus</i> mRNA for DCC tumour suppressor
L09992	Cathepsin D
M16258	RAB1, member RAS oncogene family
Heat Shock	
AA162643	Sim. to HEAT SHOCK COGNATE 71 KD PROTEIN
U73744	<i>M. musculus</i> heat shock 70 protein (Hsc70) gene
AA105022	Sim. to HEAT SHOCK PROTEIN HSP 90-BETA (HSP 84) (HSP 90)
Miscellaneous	
W41617	<i>M. musculus</i> cytochrome c oxidase subunit VIII precursor (Cox81) mRNA
Z33398	<i>M. musculus</i> RPS3a gene
M76191	Mouse elongation factor 2 (eF2) mRNA, 3' and
AA154007	Sim. to POL POLYPROTEIN REVERSE TRANSCRIPTASE
X05251	Mouse mRNA with homology to yeast L01 ribosomal protein gene
x54691	Cytochrome C oxidase, subunit IV
W99176	Sim. to THIOL-SPECIFIC ANTIOXIDANT PROTEIN (PRP)
Z59159	<i>M. musculus</i> mRNA for Sirt1
M24283	Mouse testosterone 16-alpha-hydroxylase (CB) gene
AA159107	Mouse COX11 mRNA for cytochrome c oxidase VIc (EC 1.9.3.1)
D00466	Mouse apolipoprotein E mRNA
x82067	<i>M. musculus</i> thioredoxin-dependent peroxide reductase (tpx) mRNA

## 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

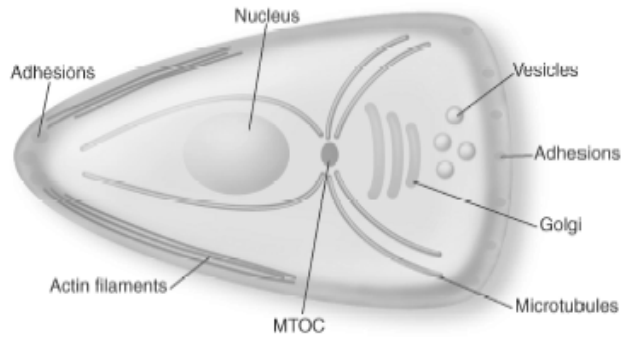
## Migration



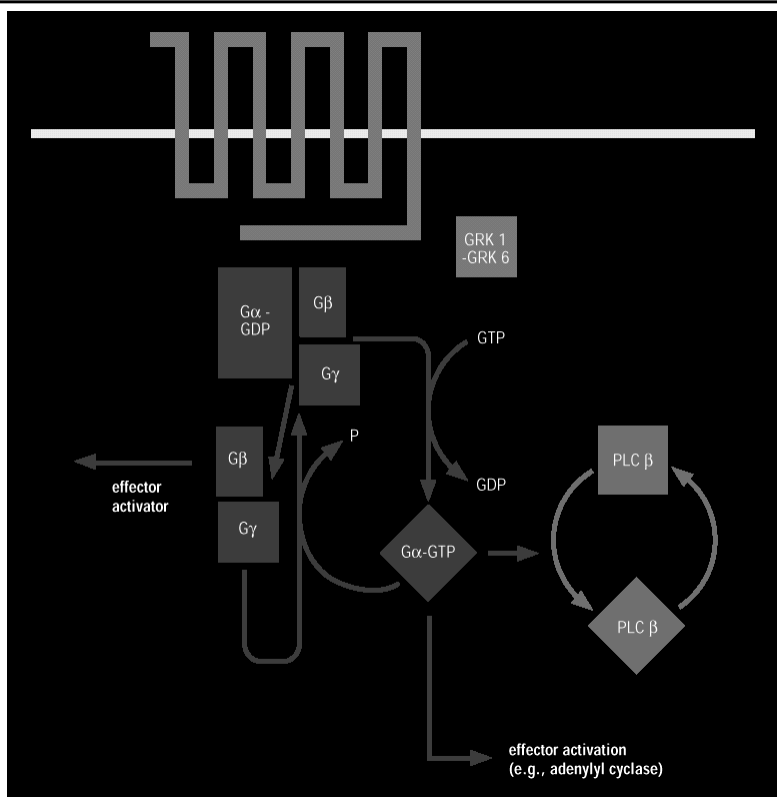
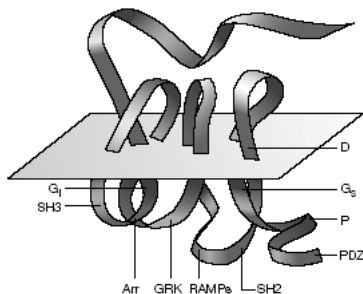
## Steps in migration *polarization*

### A. Cell Polarization

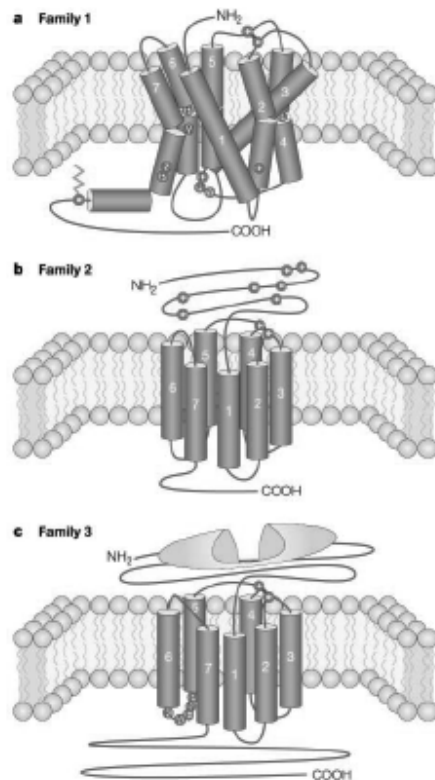
Regulators of Polarity	
Side/Rear	Front
PTEN	Activated Cdc42 & Rac
Myosin II	Cdc42/PARs/aPKC
	PIP <sub>3</sub>
	Activated integrin
	MTOC/Golgi
	Microtubules



## G-protein coupled receptor activation

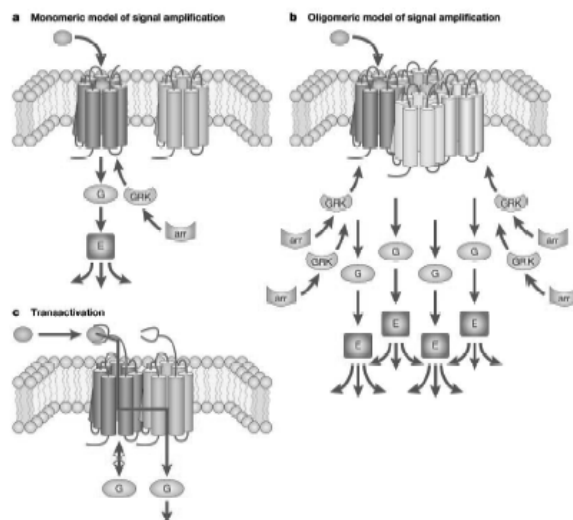


## GPCRs



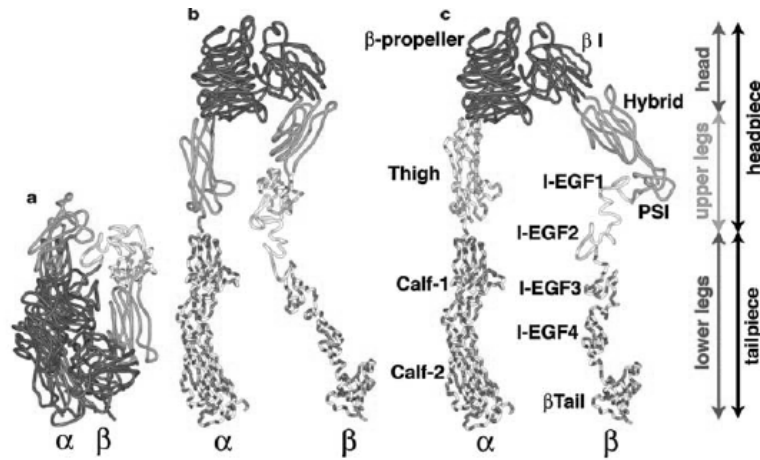
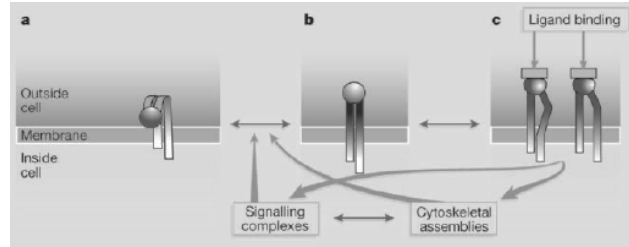
Receptor	Receptor
<b>Family 1 receptors</b>	5-HT <sub>1a</sub> -5-HT <sub>1c</sub> serotonin
A <sub>1</sub> adenosine	A <sub>1</sub> adenosine-D <sub>2</sub> dopamine
β <sub>2</sub> -adrenoceptor	A <sub>2</sub> adenosine-mGlu <sub>1</sub>
AT <sub>1</sub> angiotensin II	A <sub>2b</sub> adenosine-P2U <sub>1</sub> putnergic
B <sub>2</sub> bradykinin	A <sub>2c</sub> adenosine-D <sub>3</sub> dopamine
CCR2 chemokine	AT <sub>1</sub> -AT <sub>2</sub> angiotensin
CCR5 chemokine	AT <sub>1</sub> angiotensin-B <sub>2</sub> bradykinin
CXCR4 chemokine	CCR2-CCR5 chemokine
D <sub>1</sub> dopamine	D <sub>2</sub> -D <sub>4</sub> dopamine
D <sub>2</sub> dopamine	GABA <sub>A(α5)</sub> -GABA <sub>A(α2/3)</sub>
D <sub>3</sub> dopamine	M <sub>1</sub> -M <sub>4</sub> muscarinic acetylcholine
H <sub>1</sub> histamine	MT <sub>1</sub> -MT <sub>2</sub> melatonin
H <sub>2</sub> histamine	SSTR <sub>1-5</sub> -SSTR <sub>6</sub> somatostatin
Luteinizing hormone/hCG	SSTR <sub>1a</sub> somatostatin-μ-opioid
MT <sub>1</sub> melatonin	SSTR <sub>1b</sub> -SSTR <sub>6</sub> somatostatin
MT <sub>2</sub> melatonin	SSTR <sub>6</sub> somatostatin-D <sub>2</sub> dopamine
M <sub>1</sub> muscarinic acetylcholine	T1R1-T1R3 amino-acid taste
M <sub>2</sub> muscarinic acetylcholine	T1R2-T1R3 amino-acid taste
μ-opioid	μ- and κ-opioid
δ-opioid	μ- and δ-opioid
κ-opioid	δ-opioid-β <sub>1</sub> adrenoceptor
5-HT <sub>2</sub> serotonin	κ-opioid-β <sub>2</sub> adrenoceptor
5-HT <sub>2a</sub> serotonin	
SSTR <sub>1a</sub> somatostatin	
SSTR <sub>1b</sub> somatostatin	
SSTR <sub>2</sub> somatostatin	
SSTR <sub>3</sub> somatostatin	
SSTR <sub>4</sub> somatostatin	
SSTR <sub>5</sub> somatostatin	
Thyrotropin	
V <sub>1</sub> vasopressin	
<b>Family 2 receptors</b>	
IgG hepta	
Gonadotropin-releasing hormone	
<b>Family 3 receptors</b>	
Metabotropic mGlu <sub>1</sub>	
Metabotropic mGlu <sub>2</sub>	
Ca <sup>2+</sup> -sensing	
GABA <sub>A(α10)</sub>	
GABA <sub>A(α20)</sub>	
<b>Family 4 receptor</b>	
Yeast α-factor receptor	

## GPCR -oligomerization





## Integrins



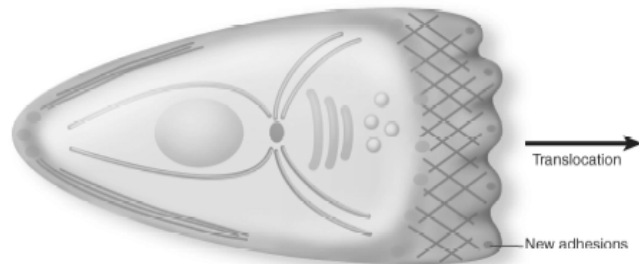
## Steps in migration

### *Protrusion and Adhesion Formation*

#### B. Protrusion and Adhesion Formation

##### Actin Polymerization

Nucleation	Polymerization/Organization
Arp2/3 complex	Profilin
WAVE/WASP	ENA/VASP
Rac/Cdc42	ADP/Cofilin
	Capping proteins
	Cross linkers



##### Adhesion Formation

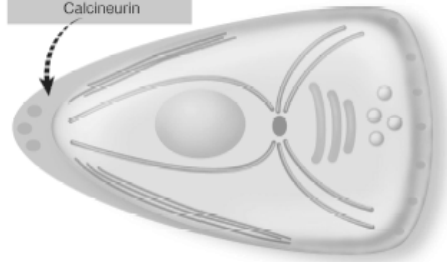
Integrin Activation	Integrin Aggregation
Talin	Rac/Cdc42
PKC	
Rap1	
PI3K	

## Steps in migration

### *Rear Retraction*

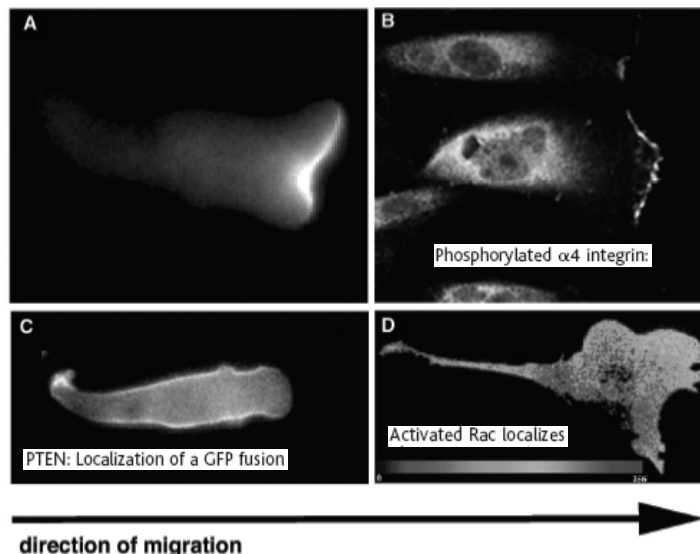
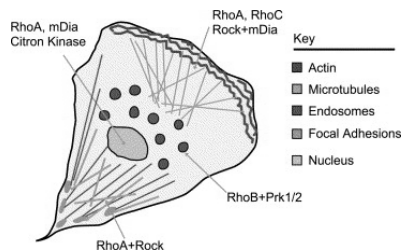
#### C. Rear Retraction

Rear Retraction  
 Adhesion Disassembly and Retraction  
 FAK/Src/ERK  
 Myosin II  
 Microtubules  
 Rho  
 Ca<sup>2+</sup>  
 Calpain  
 Calcineurin



## Steps in migration

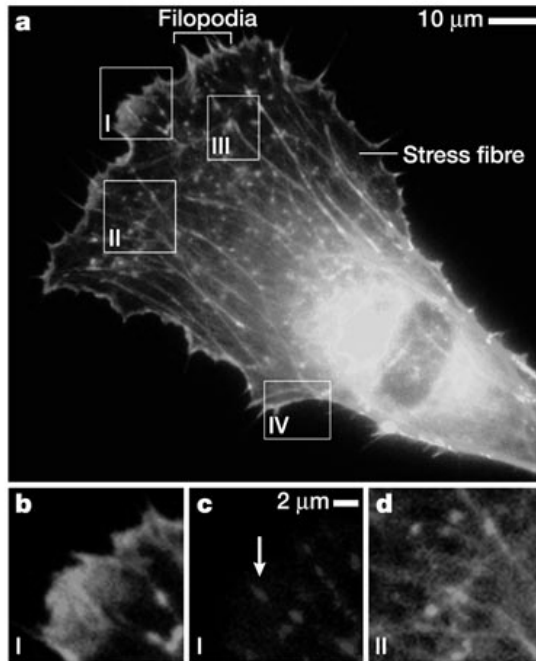
PIP<sub>3</sub>: Leading-edge localization of a green fluorescent protein (GFP)



# BE 440. Analysis of Biological Networks

## Migrating cells

- Entire complex
- FA and migration
- Migration



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

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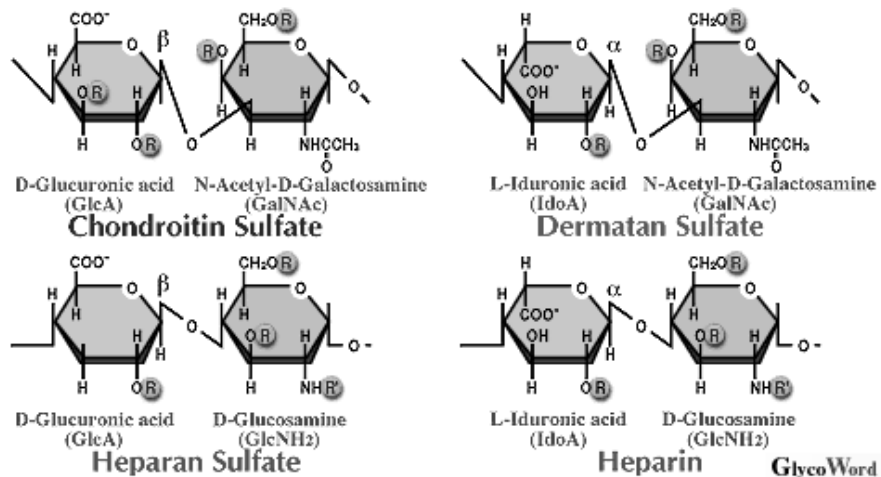
## alpha5 integrin clustering, diffusion and interactions in the cell

- Image Correlation Microscopy (ICM) allows the detection of submicroscopic alpha5 integrin clusters with 3-4 proteins.
- Two color ICM shows that alpha5 integrin and alpha-actinin localize and move together even in regions of the cell with no discernable adhesions.
- A temporal ICM analysis reveals heterogeneity in both alpha5 integrin and alpha-actinin dynamics across the cell with the proteins being more dynamic in regions of the cell that are ruffling and protruding.
- When adhesions disassemble three proteins have very different fates:
  - alpha5 integrin diffuses away slowly,
  - alpha-actinin moves away with a directed motion and
  - paxillin diffuses away rapidly into the cytosol.

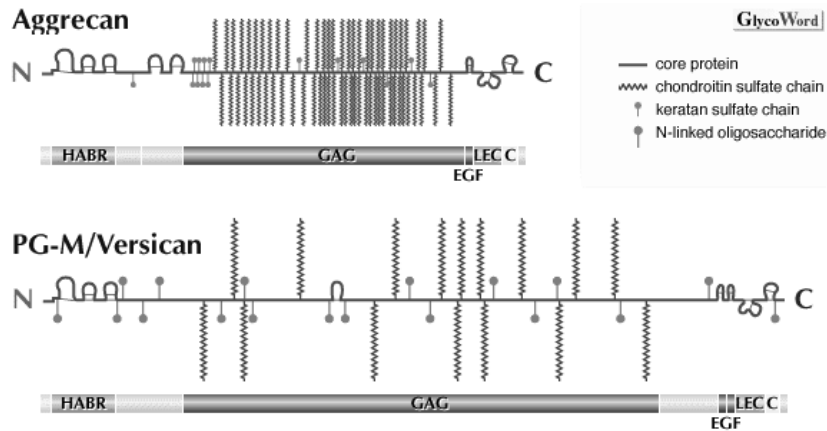
## Migration, proliferation & differentiation

- 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- $\beta$

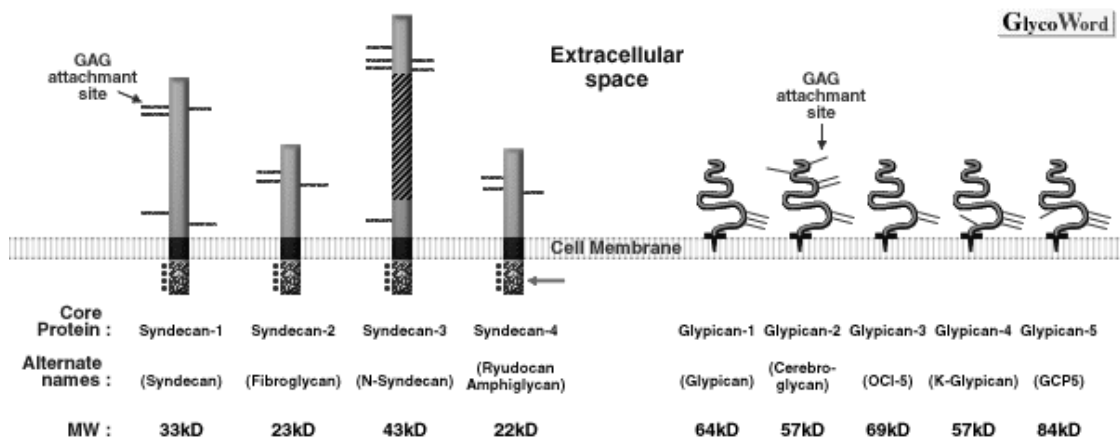
## Glycosaminoglycans



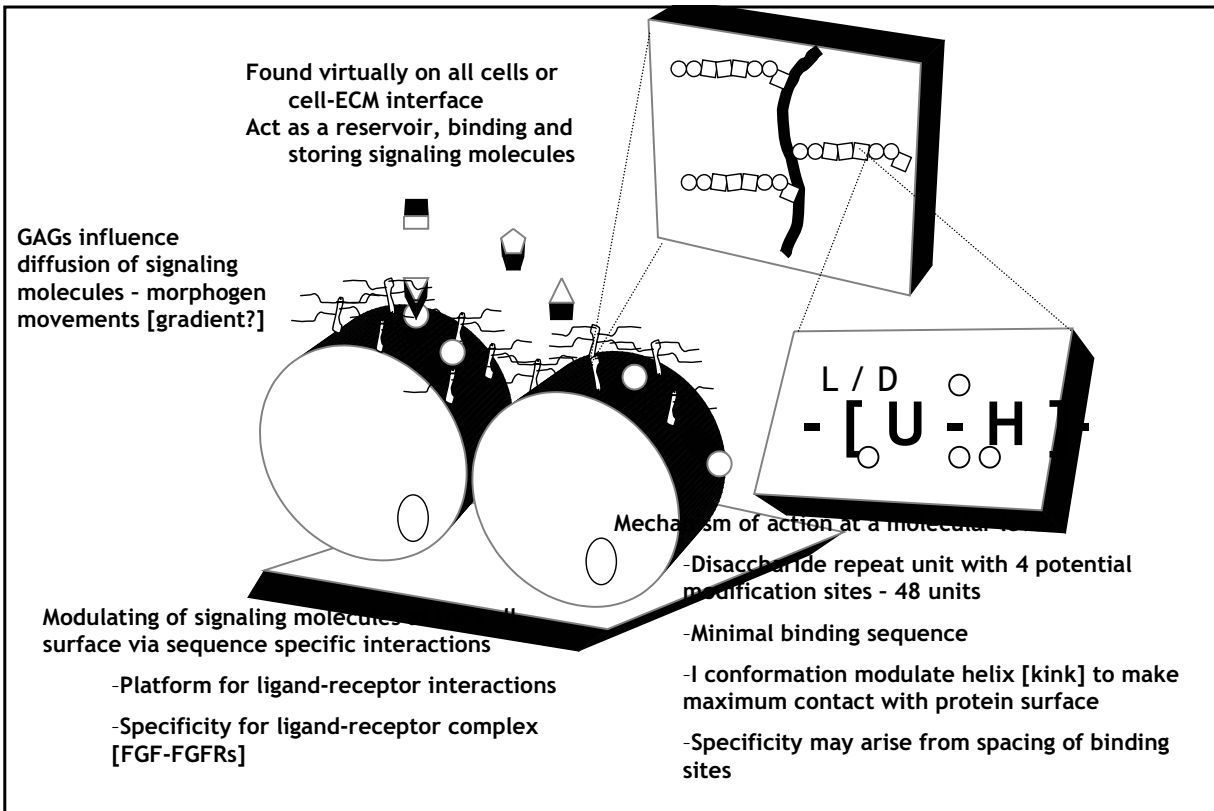
## Core: ECM CS



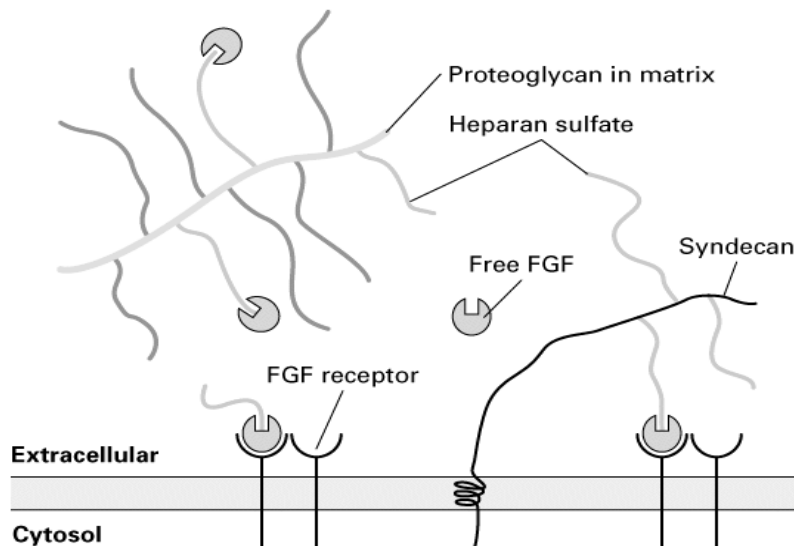
## Core: Cell Surface HSGAG



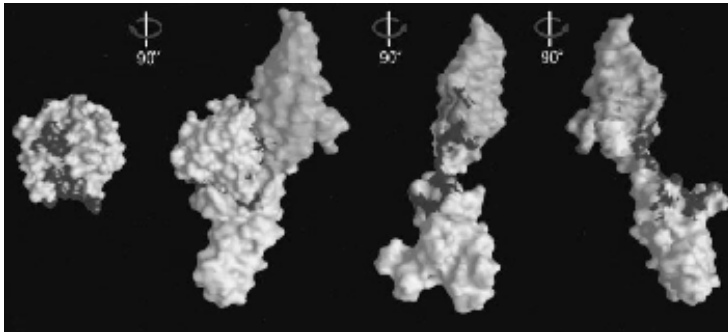
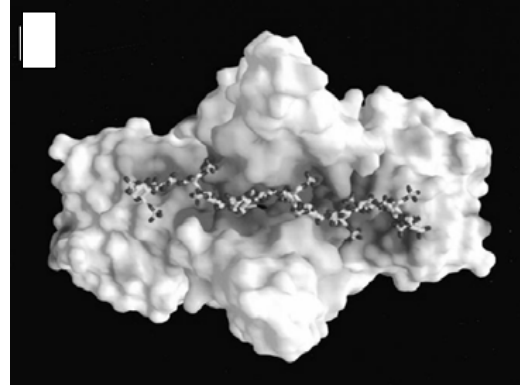
# BE 440. Analysis of Biological Networks



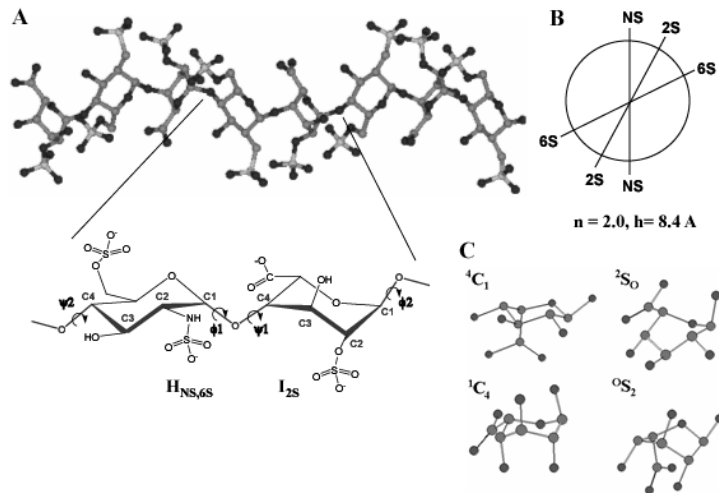
## ECM and Growth Factor



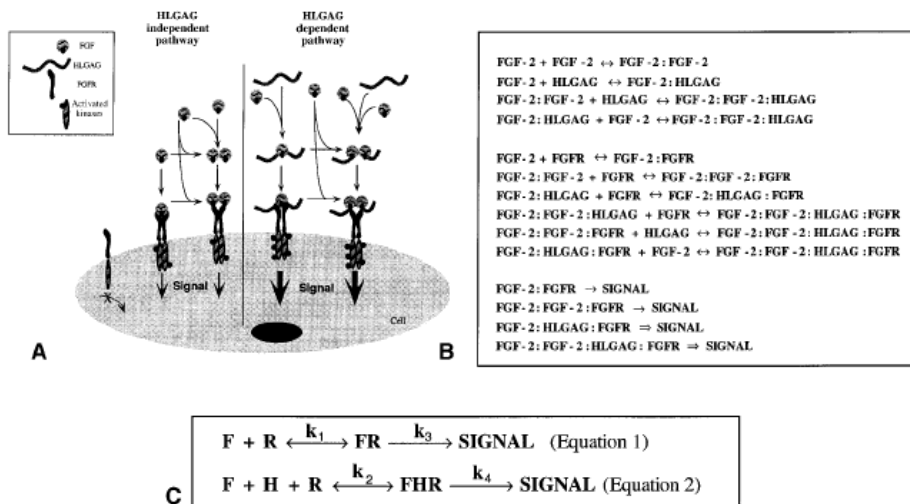
## FGF-FGFR complex



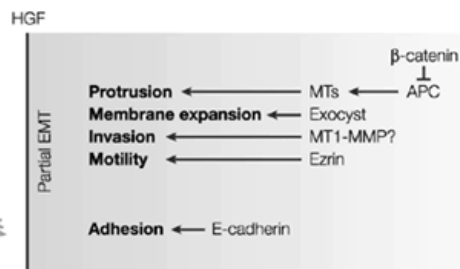
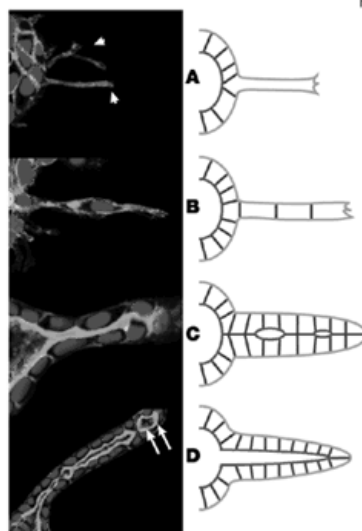
## Conformation of H



## FGF-FGFR-H mediated signaling



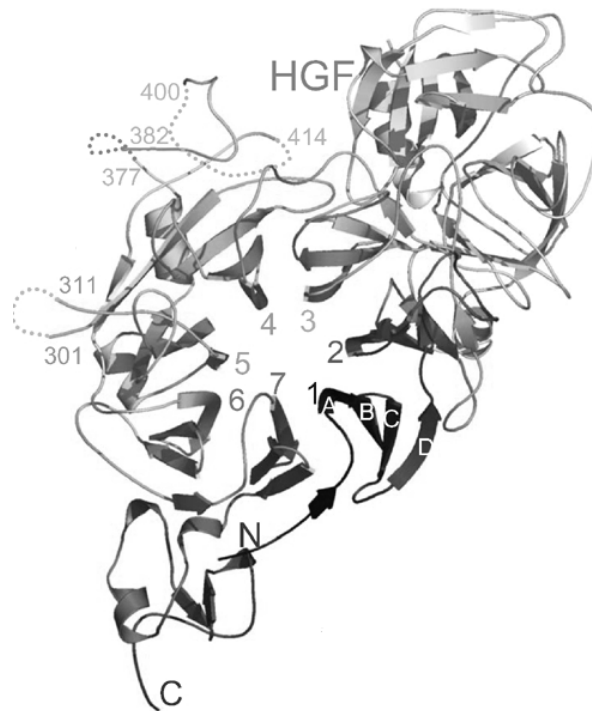
## Tube formation



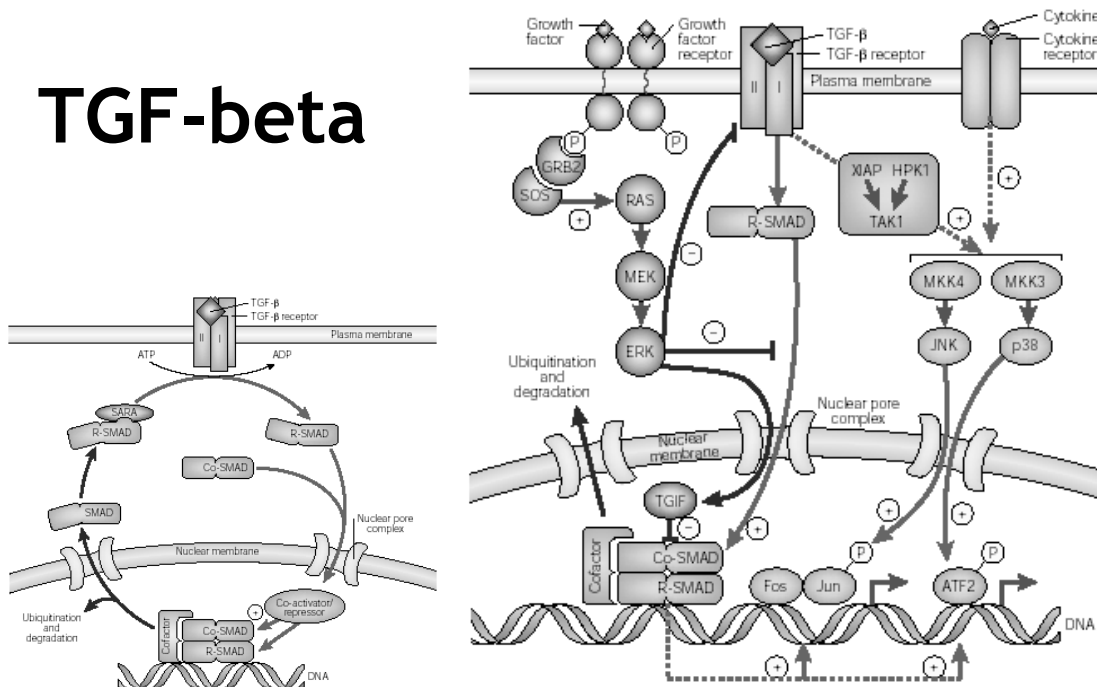


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## HGF

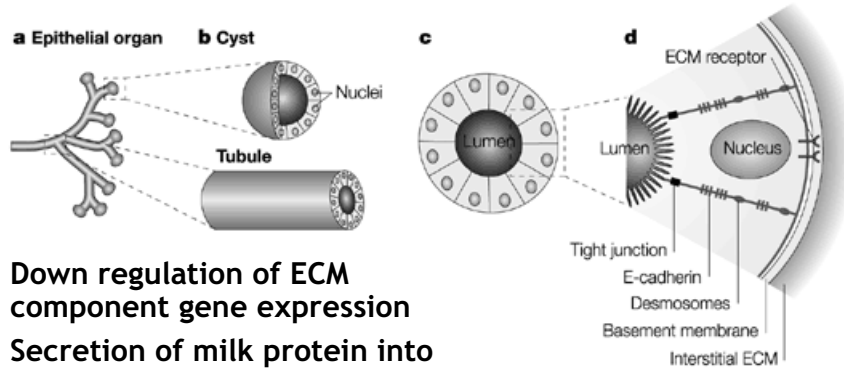


## TGF-beta



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

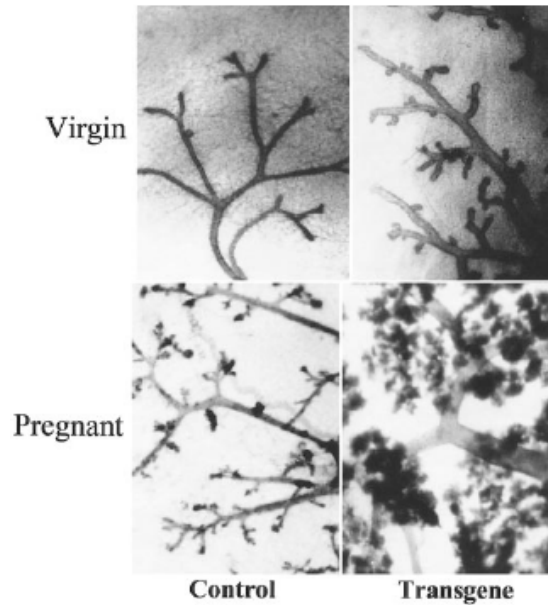
## Morphogenesis



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

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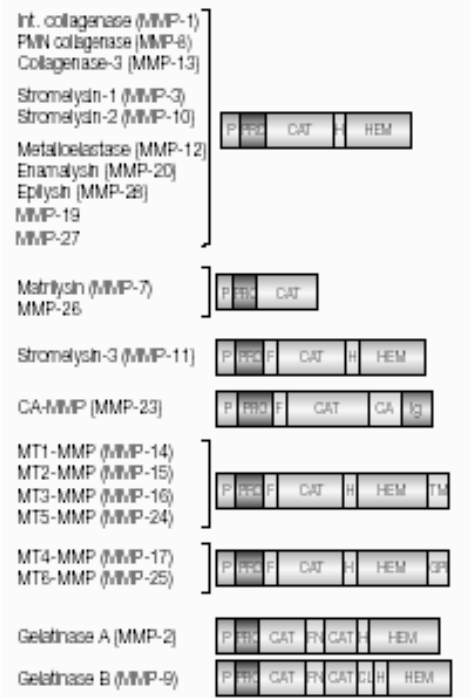
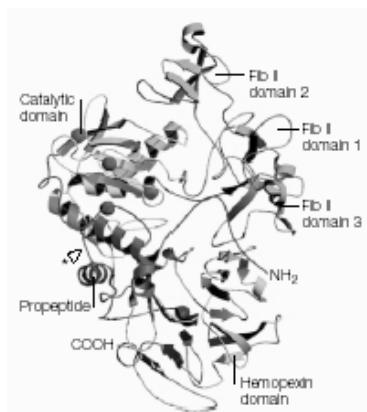
## Heparanase over expression



## Destruction & Involution

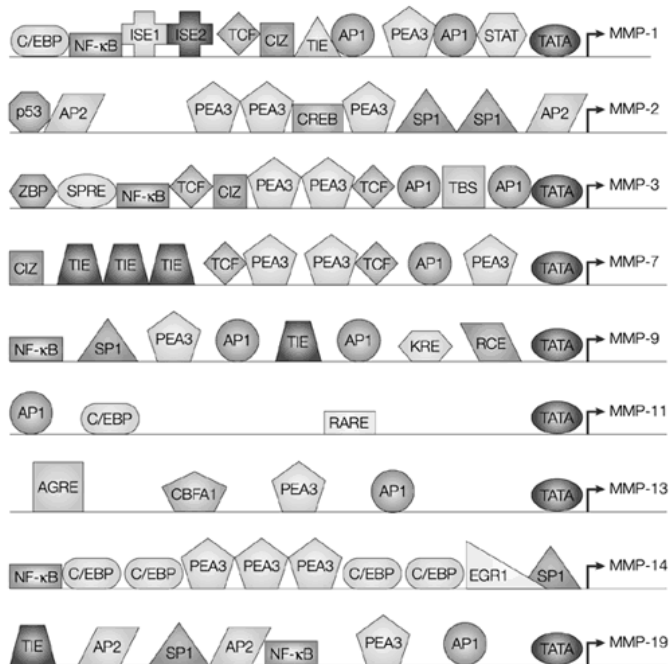
- Inhibition of milk protein expression
- Increased Matrix Metalloprotease production
- Decreased production of MM inhibitors
- Basement membrane destruction and Enactin fragmentation and increase tenascin production
- Loss of cell function
- ICE dependent apoptosis

## MMPs



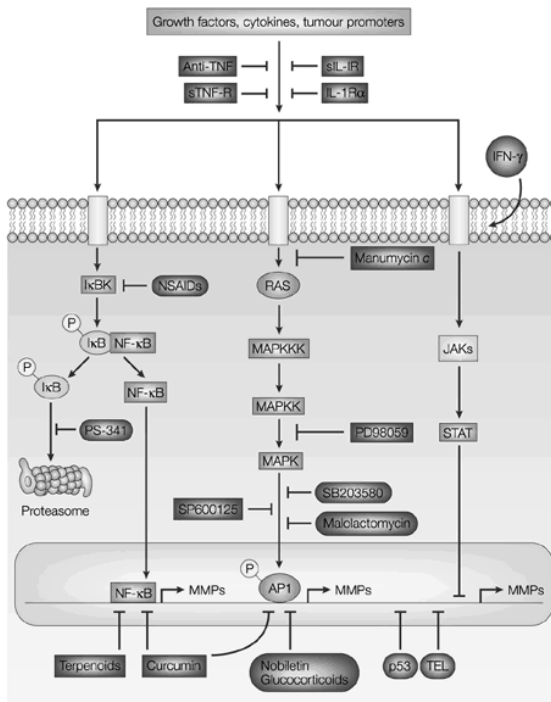
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## MMP gene regulation



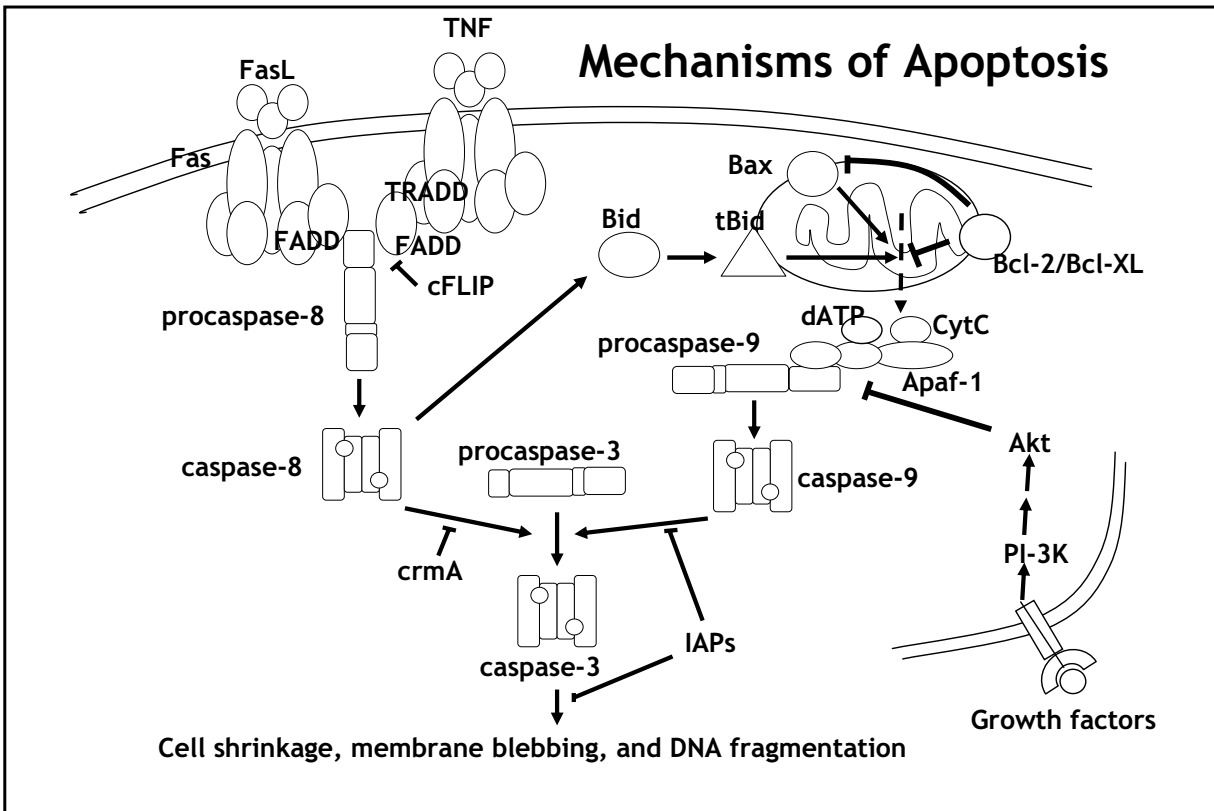
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## MMP regulation



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# BE 440. Analysis of Biological Networks



## 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