



F C Ciências
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Cell invasion and metastasis: Role of Rho GTPases

Paulo Matos

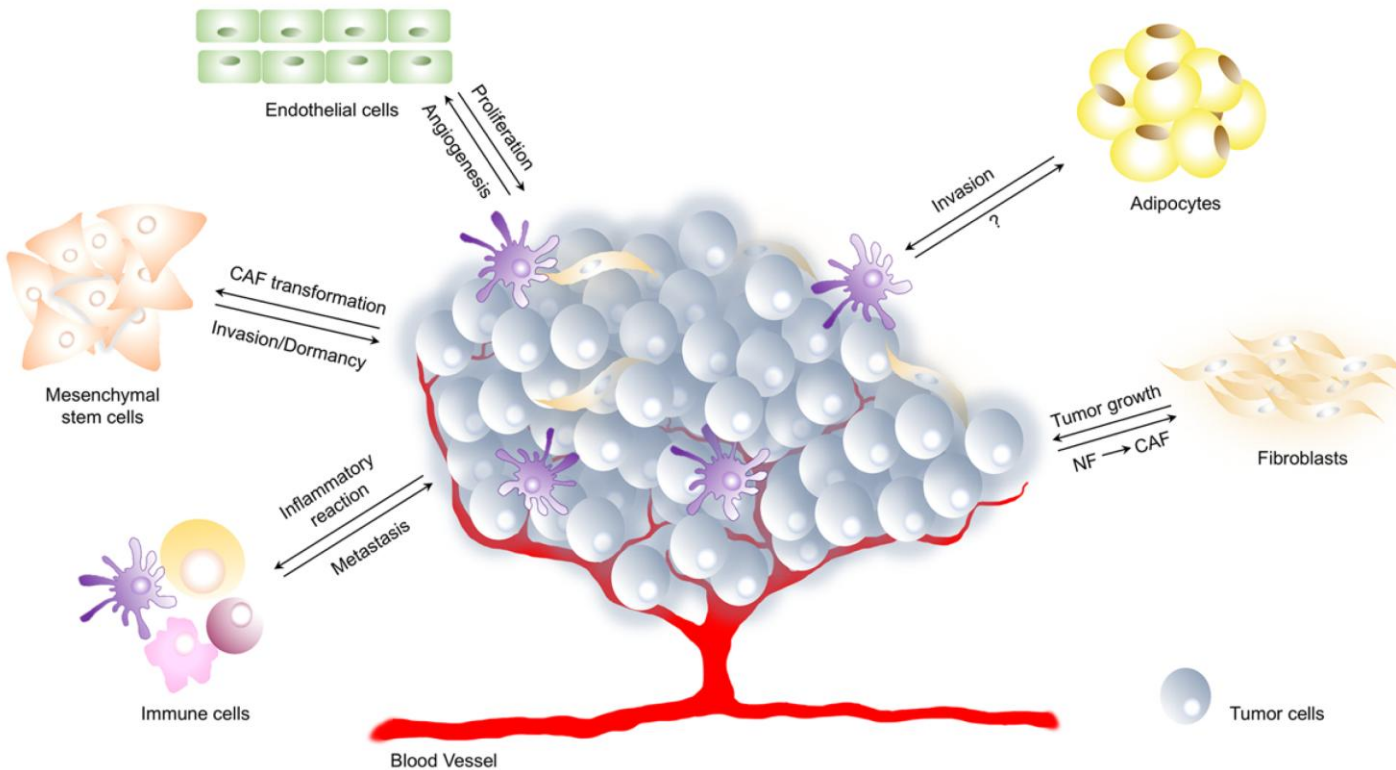
2024-2025



Intercellular signaling...

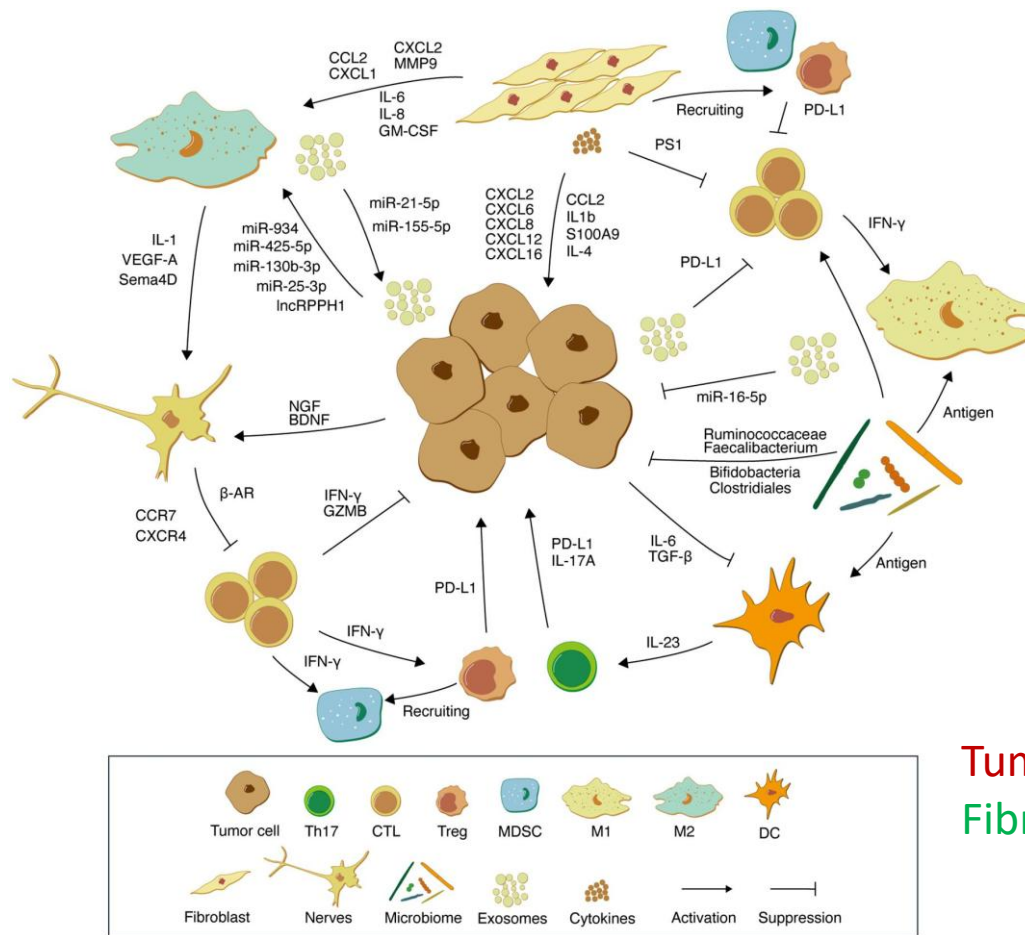
...in the tumor microenvironment

The interaction of tumor cells with their microenvironment is known to modulate the genetic and epigenetic events that determine the development of cancer...



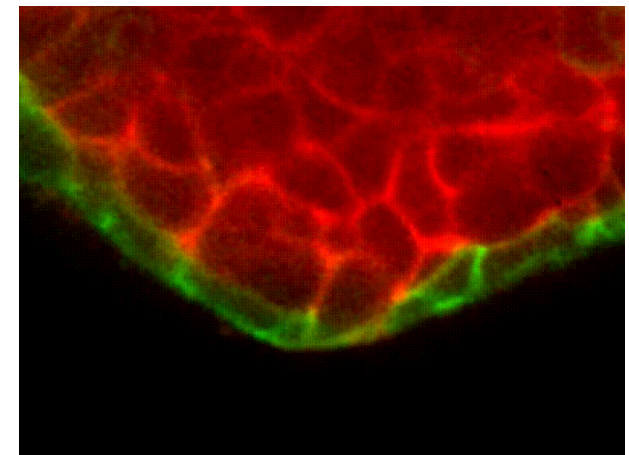
Intercellular signaling...

...is hijacked by tumor cells!

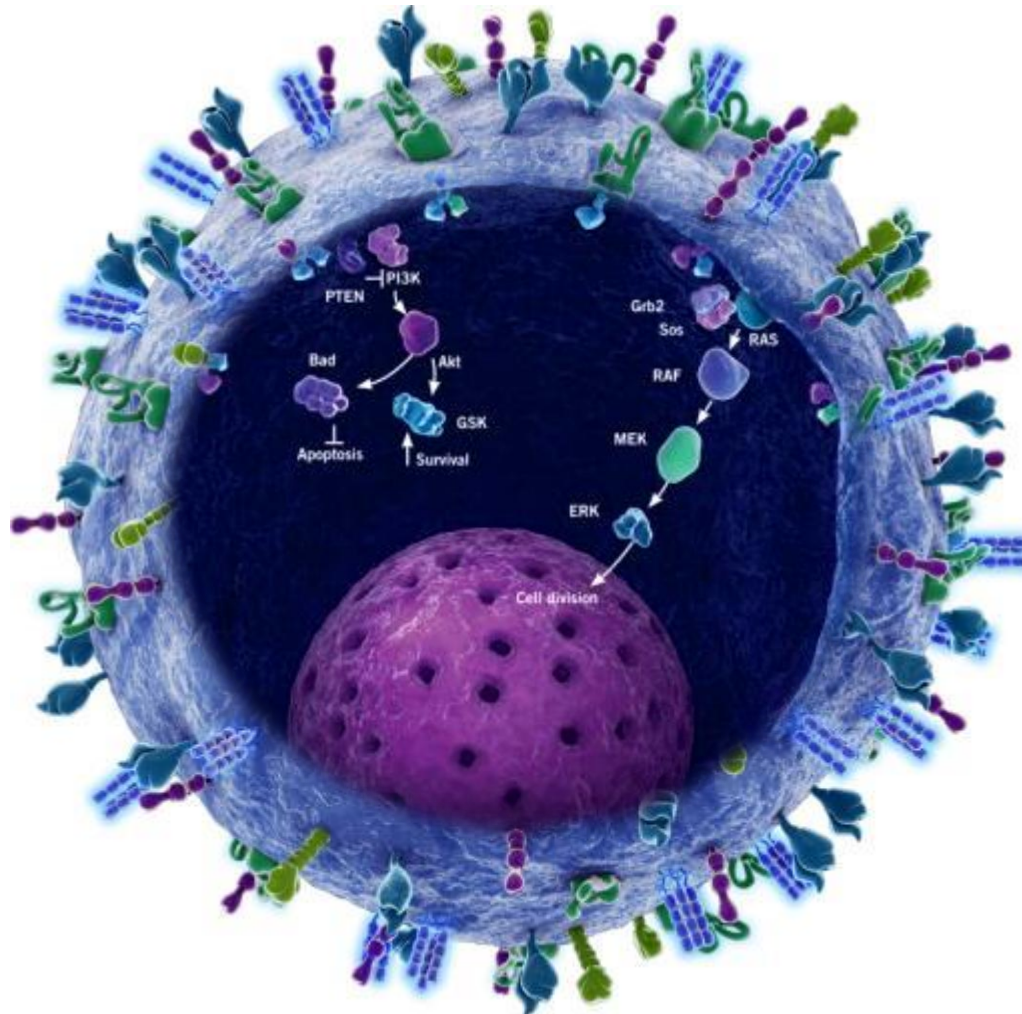


Tumor cells hijack the signaling cues (cytokines, chemokines, growth factors, etc.) that stromal cells use to communicate and use them to boost their own growth, survival and **invasion...**

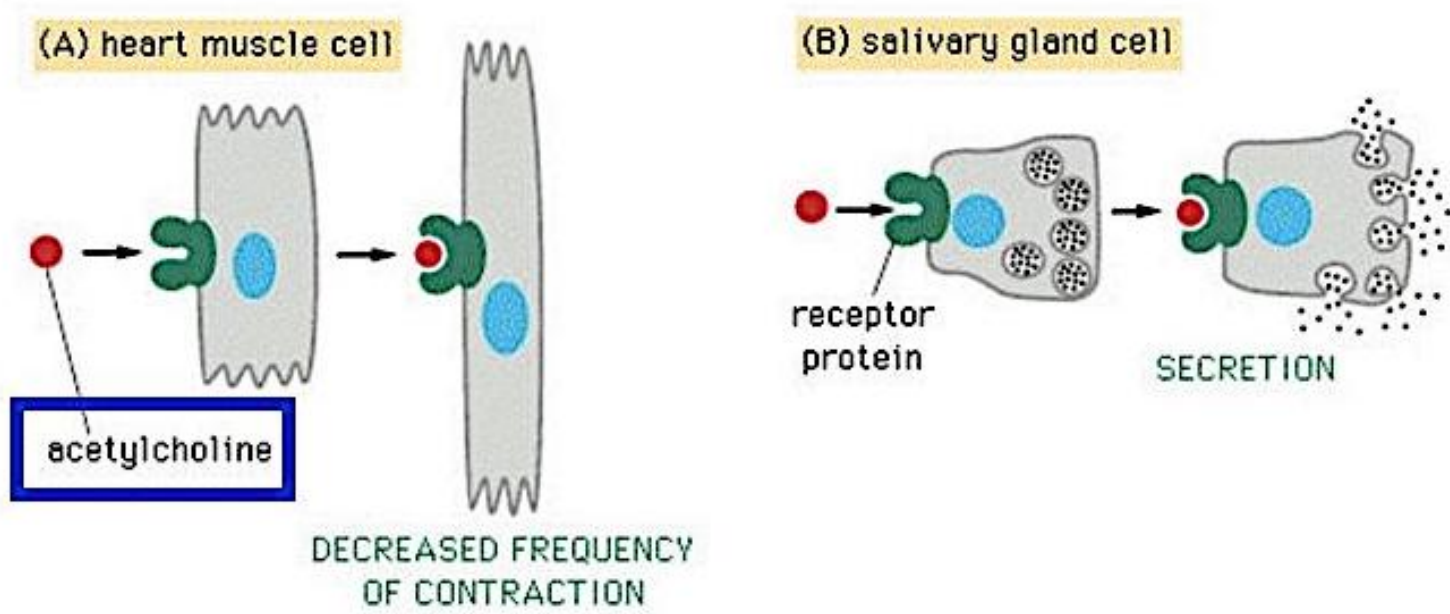
Tumor cells
Fibroblasts



Dysregulated Intracellular signaling...



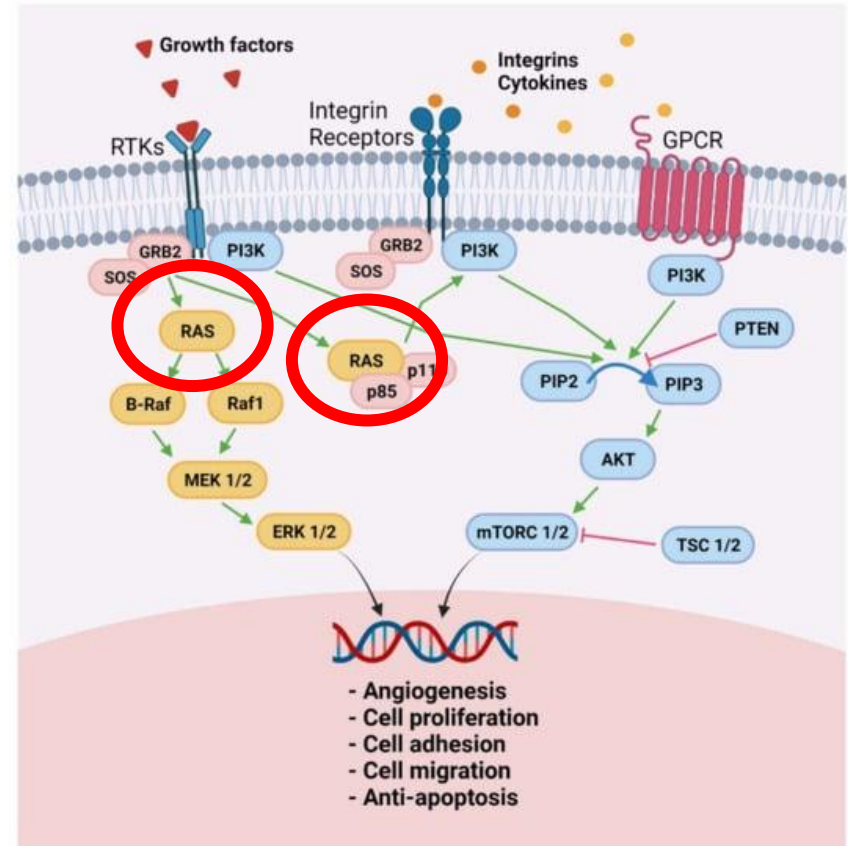
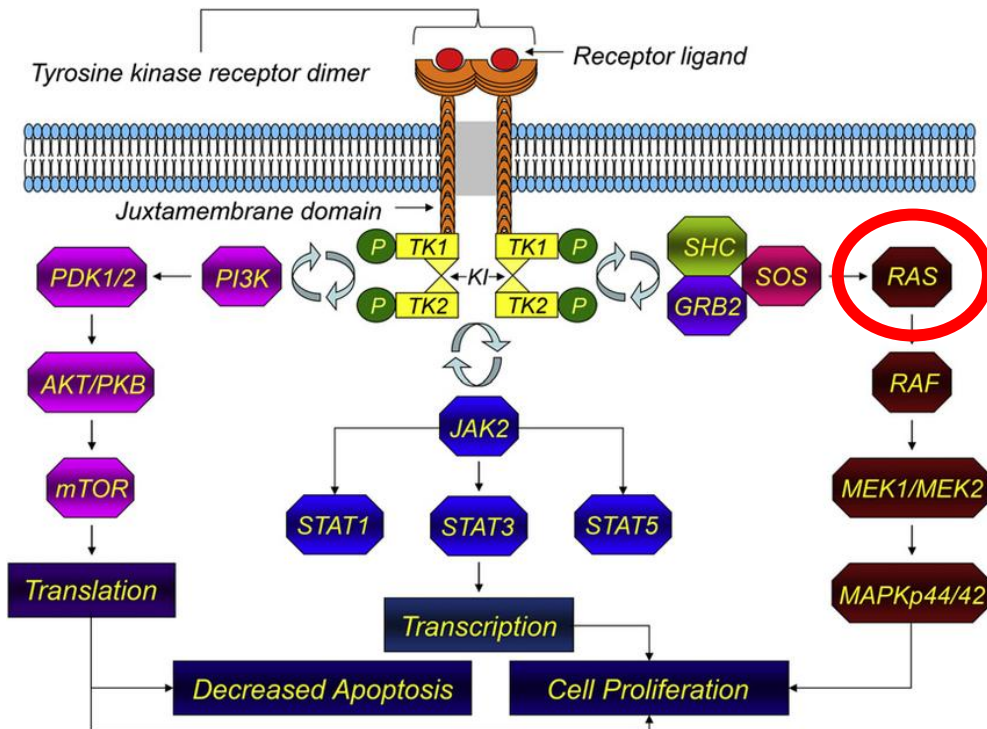
Same ligand, same receptor, different cell type...different response?



Different intracellular signaling machinery!

Tumors adapt by making changes in the intracellular signaling machinery...

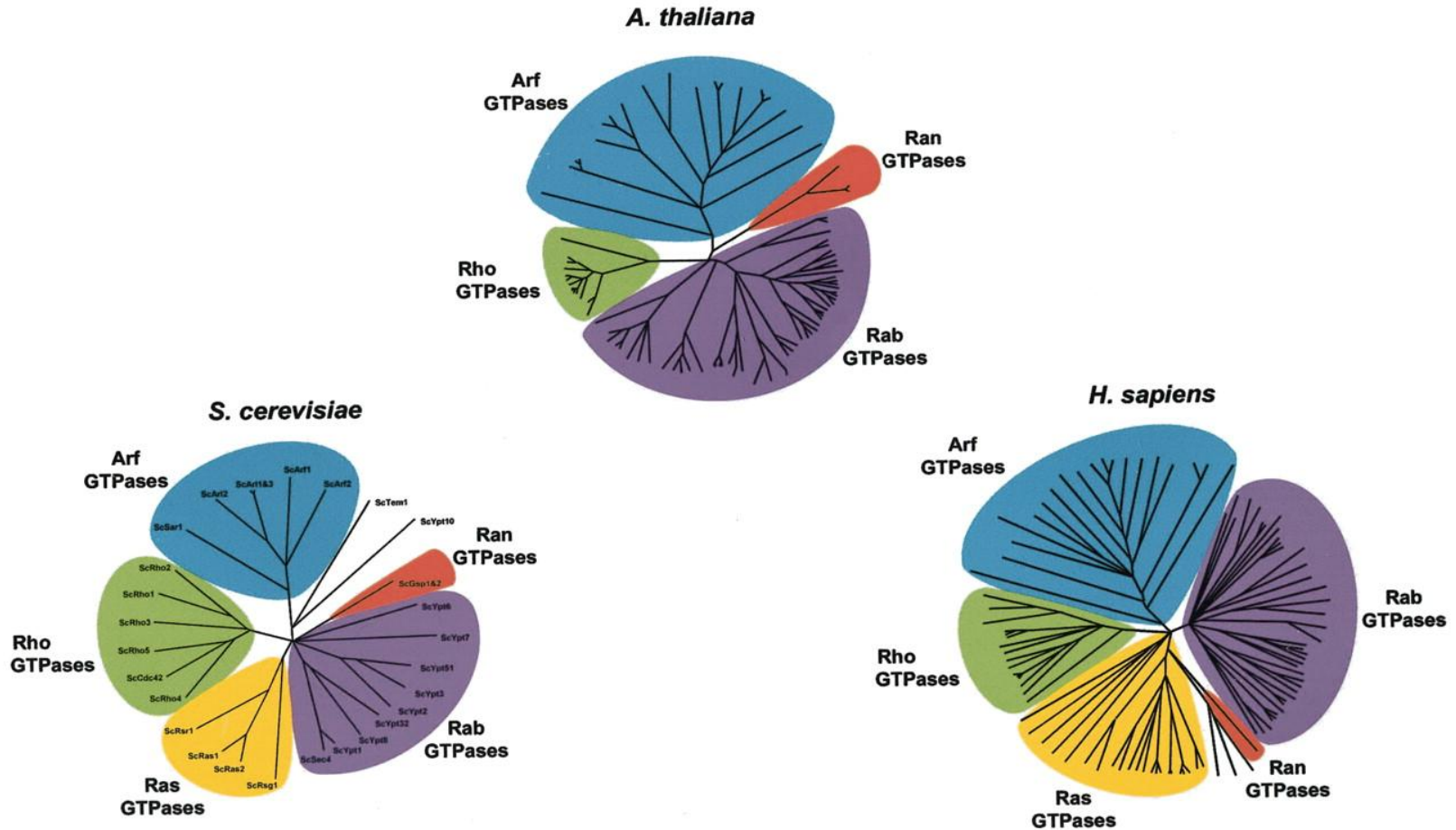
Same receptor, multiple pathways...



Different receptors, shared pathways...

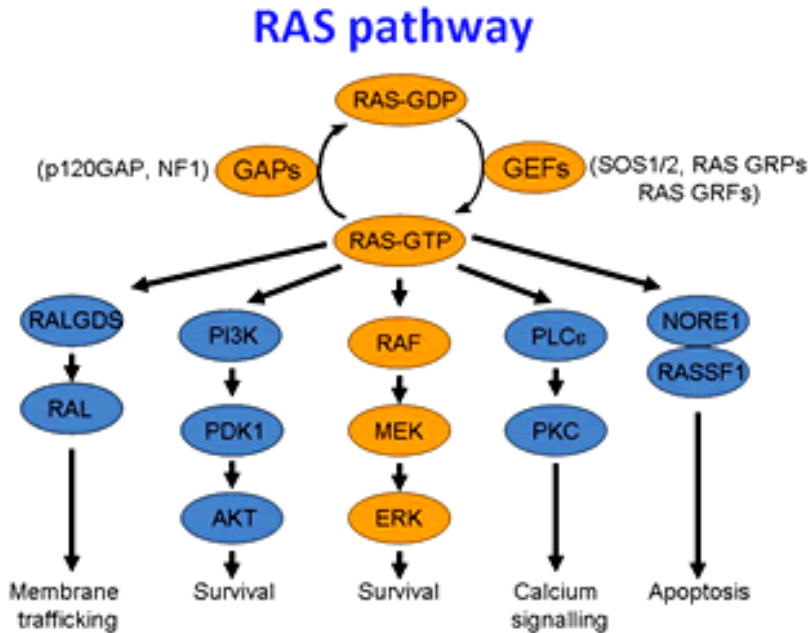
Changes in the abundance, activity, subcellular localization, etc., of signaling proteins...

Small GTPases



Monomeric GTP-binding proteins with a molecular mass of 20-40 kDa.

The prototype...Ras GTPase



- Ras (Rat sarcoma) was first identified as the transforming principle of the Harvey and Kirsten strains of rat sarcoma viruses.

Harvey, JJ (1964) *Nature*.

Kirsten, W.H., Mayer, L.A. (1967) *J.Natl.Cancer Inst.*

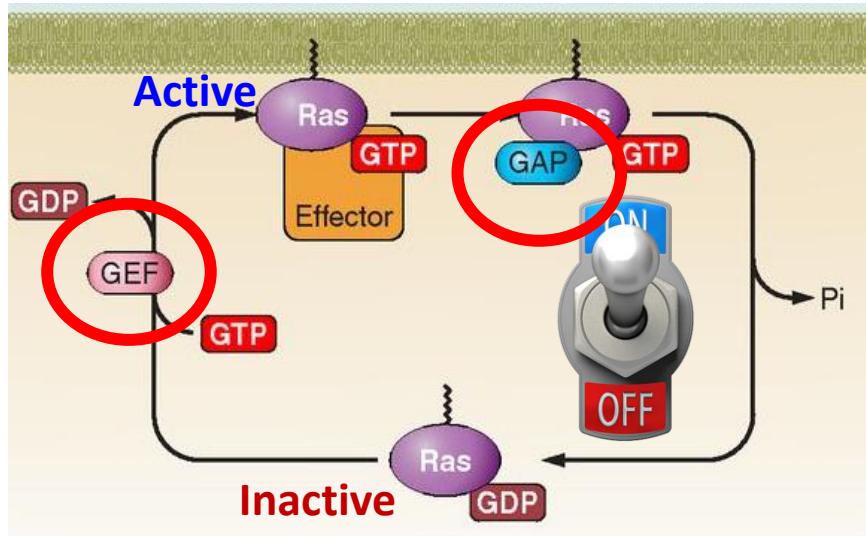
- Endogenous cellular *Ras* genes were subsequently identified in human cells.

- There are three *Ras* genes in humans:

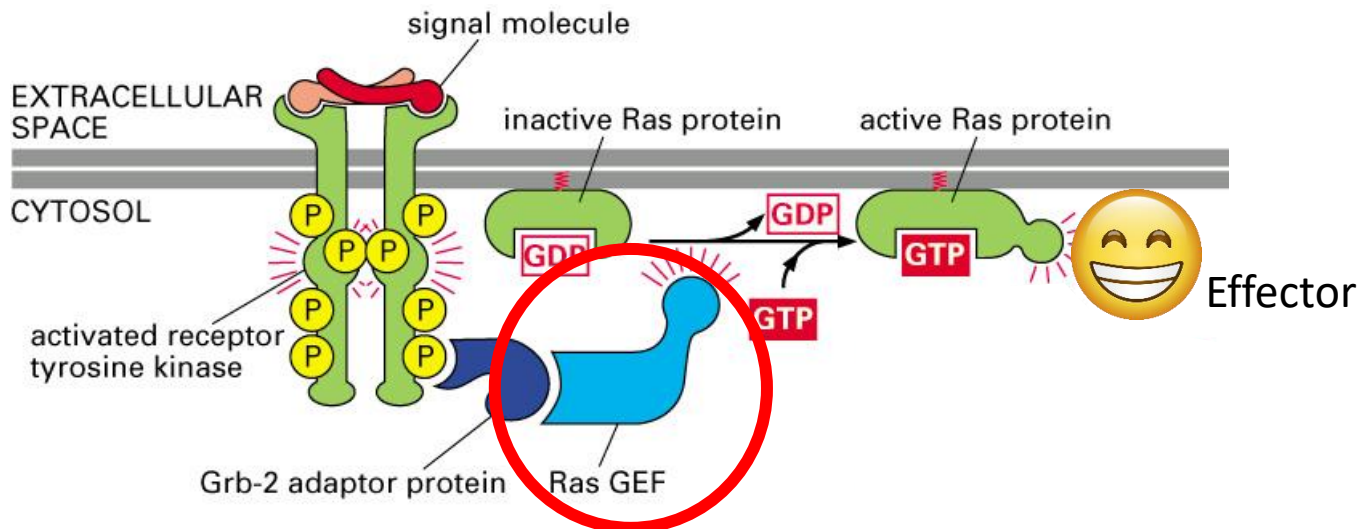
- ✓ **HRAS**
- ✓ **KRAS**
- ✓ **NRAS**

**Mutated in
>40% of cancers**

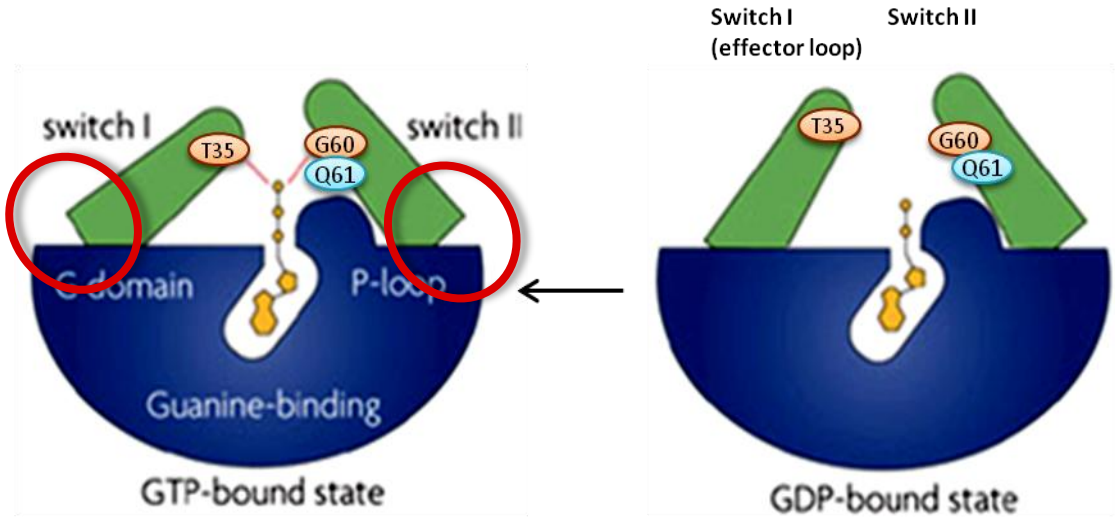
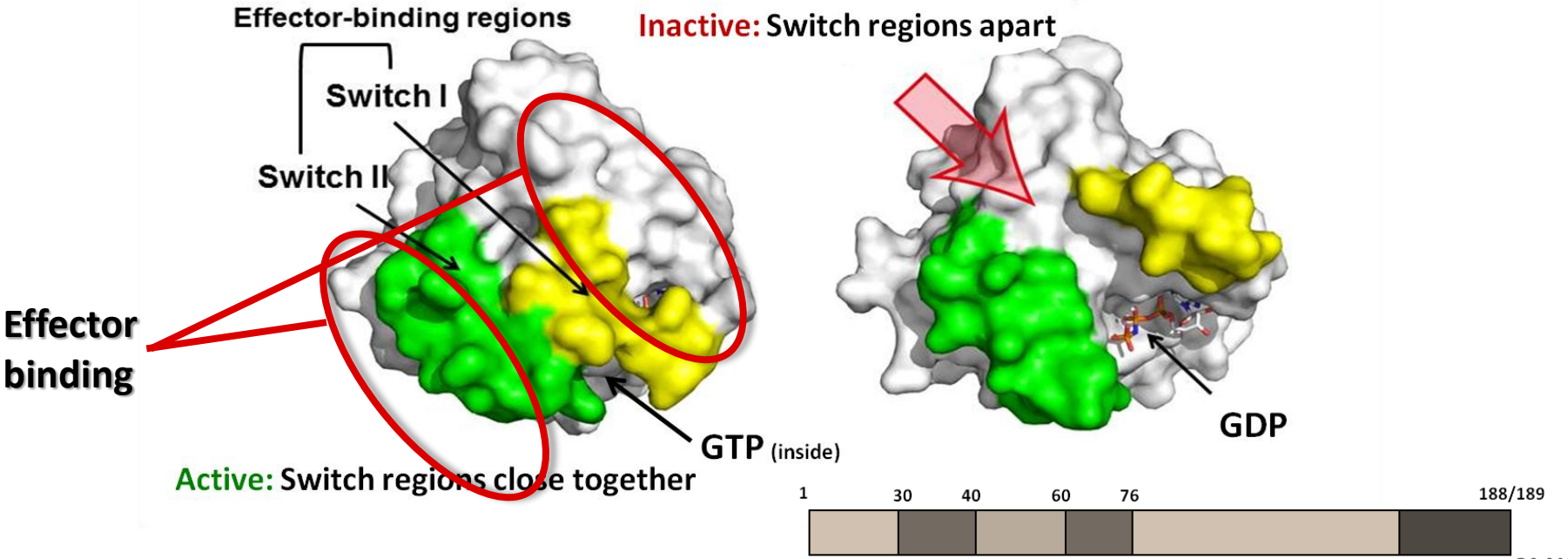
Small GTPases: The molecular switches



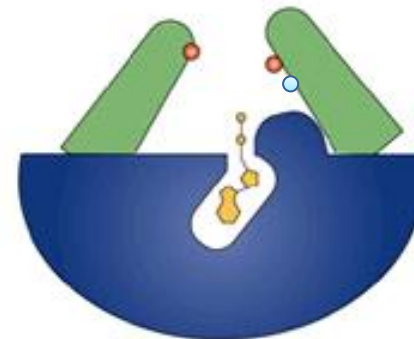
By cycling between an **inactive** (GDP-bound) and an **active** (GTP-bound) state, small GTPases act as **molecular switches** that turn on and off signal transduction **downstream** of cell surface receptors.



Ras structural switch

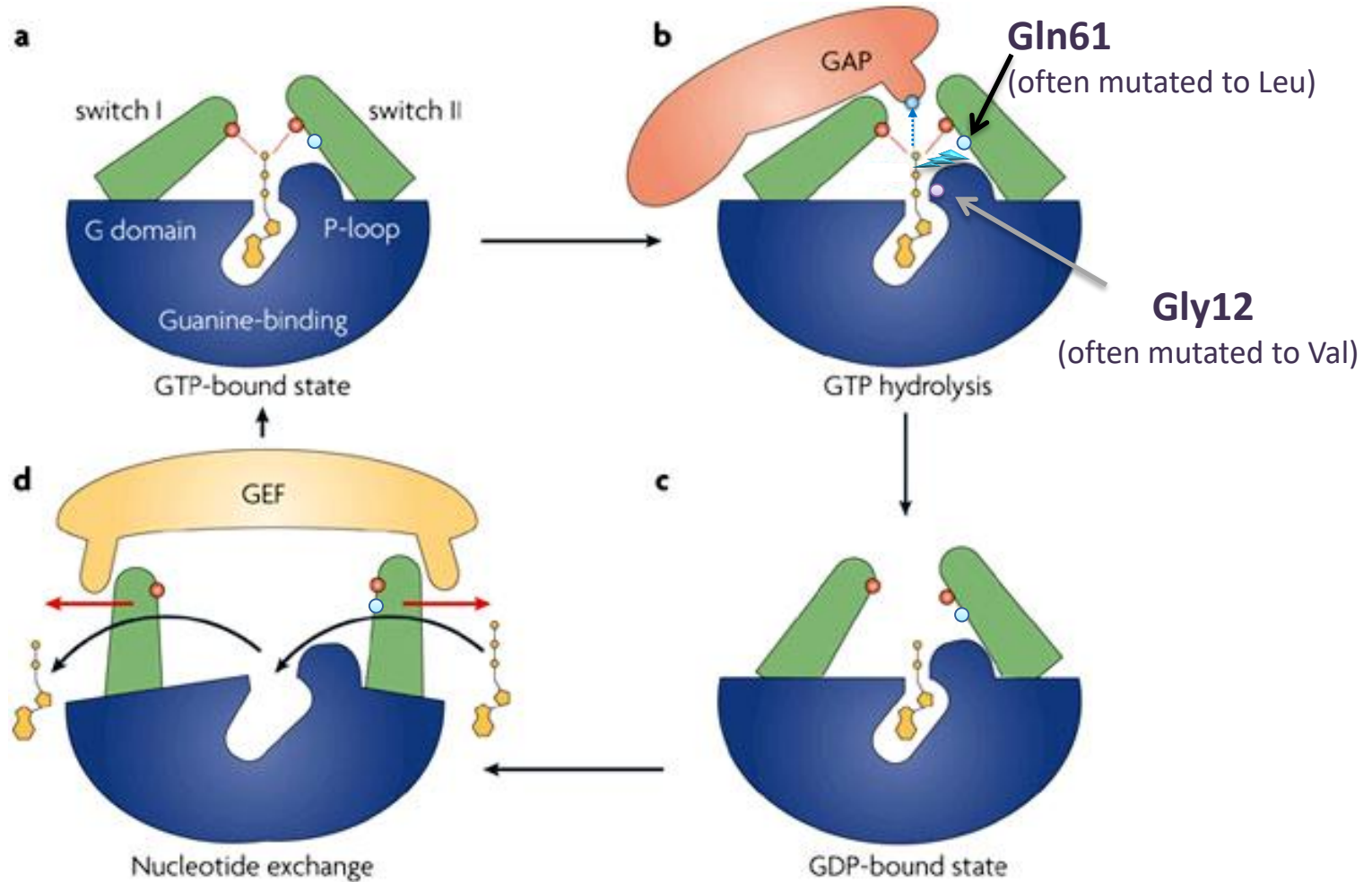


GEFs & GAPs: how they work?



GDP-bound state

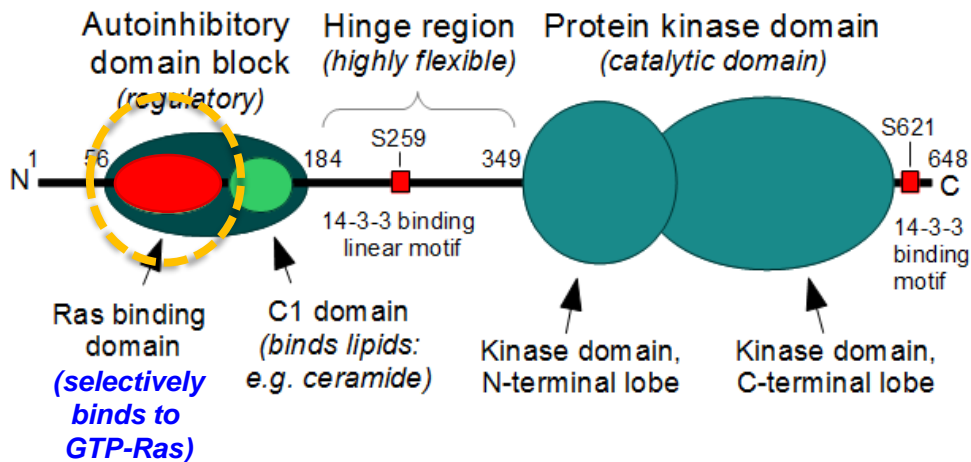
GEFs & GAPs: how they work?



Assessing the level of Ras activation

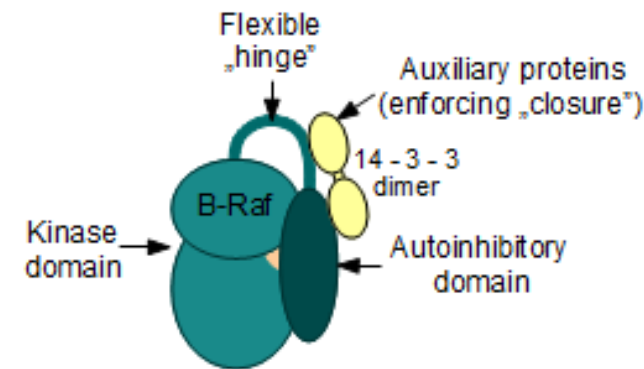
The RBD pulldown assay

Schematic architecture of the human c-Raf kinase

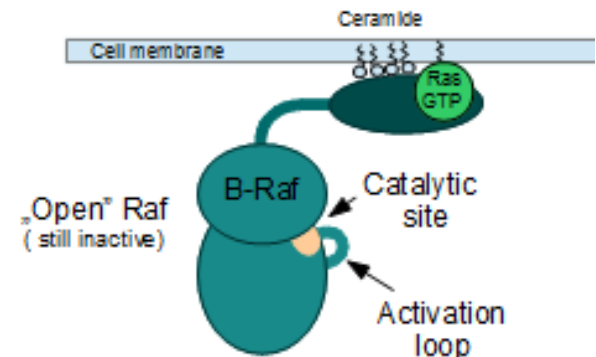


Upon the selective binding of GTP-Ras to Raf RBD, the intramolecular interaction of the inhibitory domain and the kinase domain is relieved, rendering the enzyme active.

Inactive state

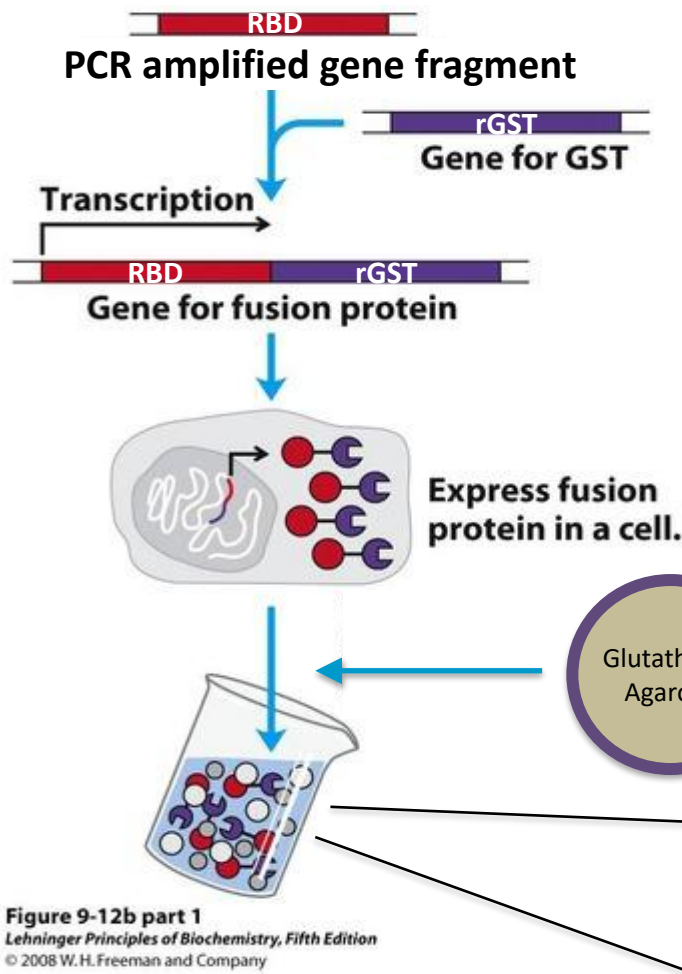


Relief of autoinhibition

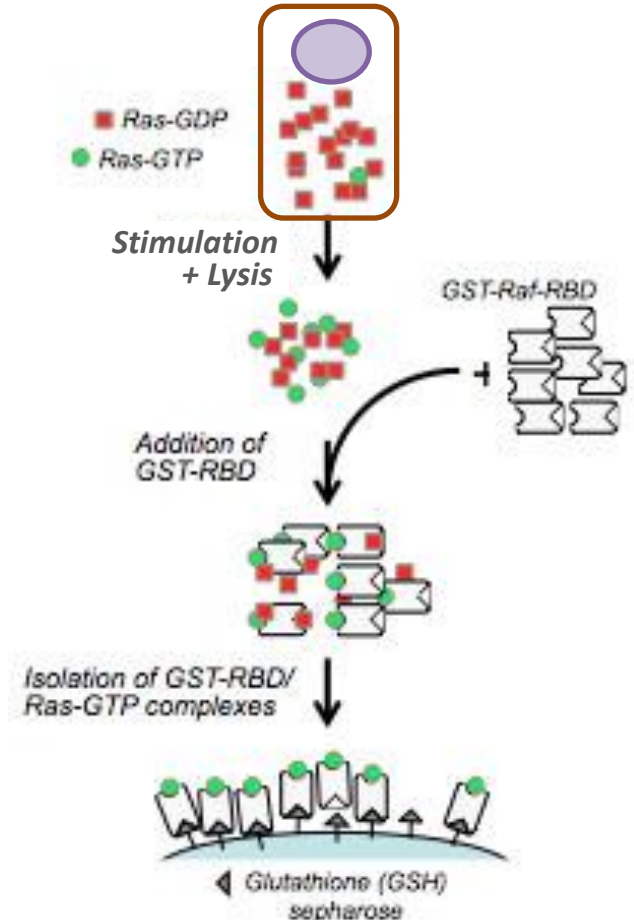


Assessing the level of Ras activation

The RBD pulldown assay

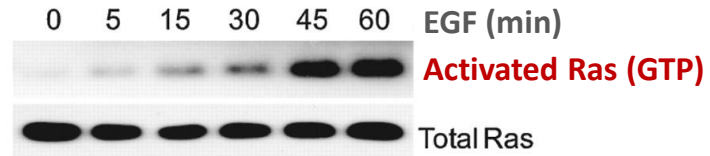
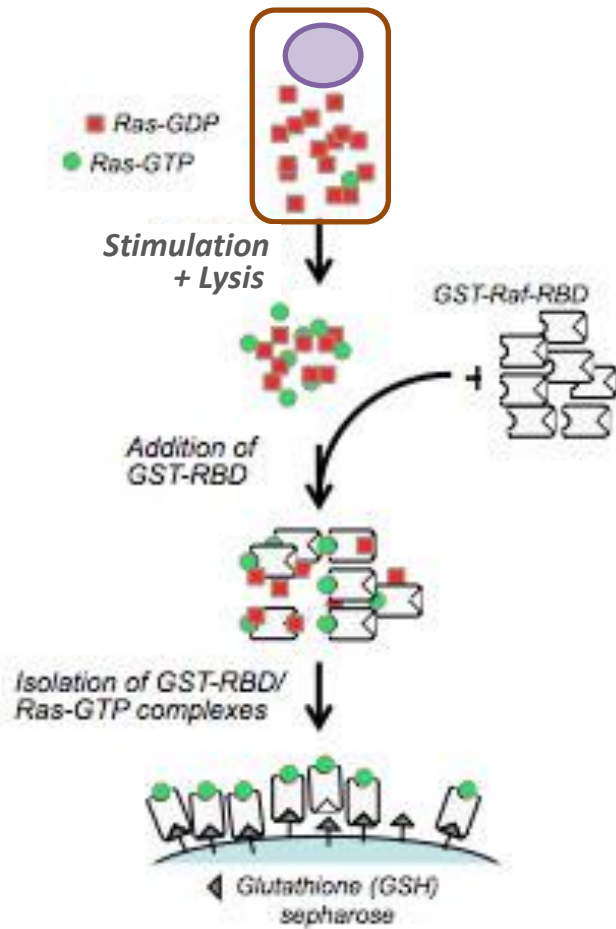


Glutathione-S-transferase (GST) is a small enzyme (purple) that binds glutathione

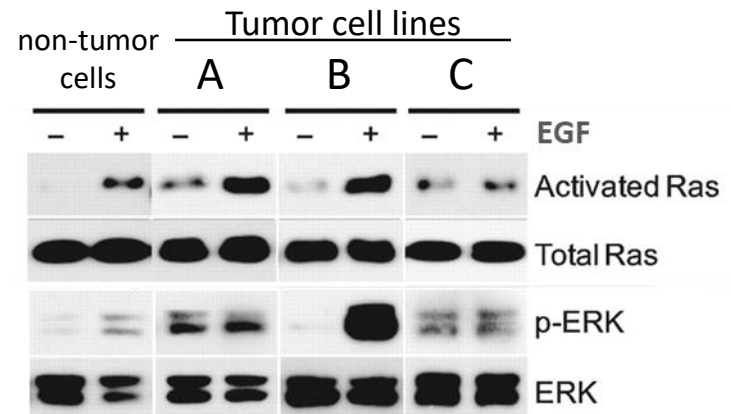
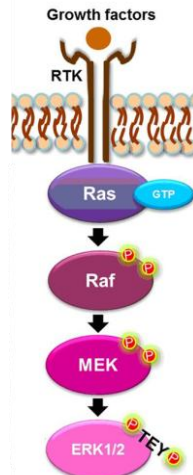


Assessing the level of Ras activation

The RBD pulldown assay



Which tumor cell line has a RAS activating mutation?



Ras superfamily...

More than 150 members

Rab+Ran
71 members

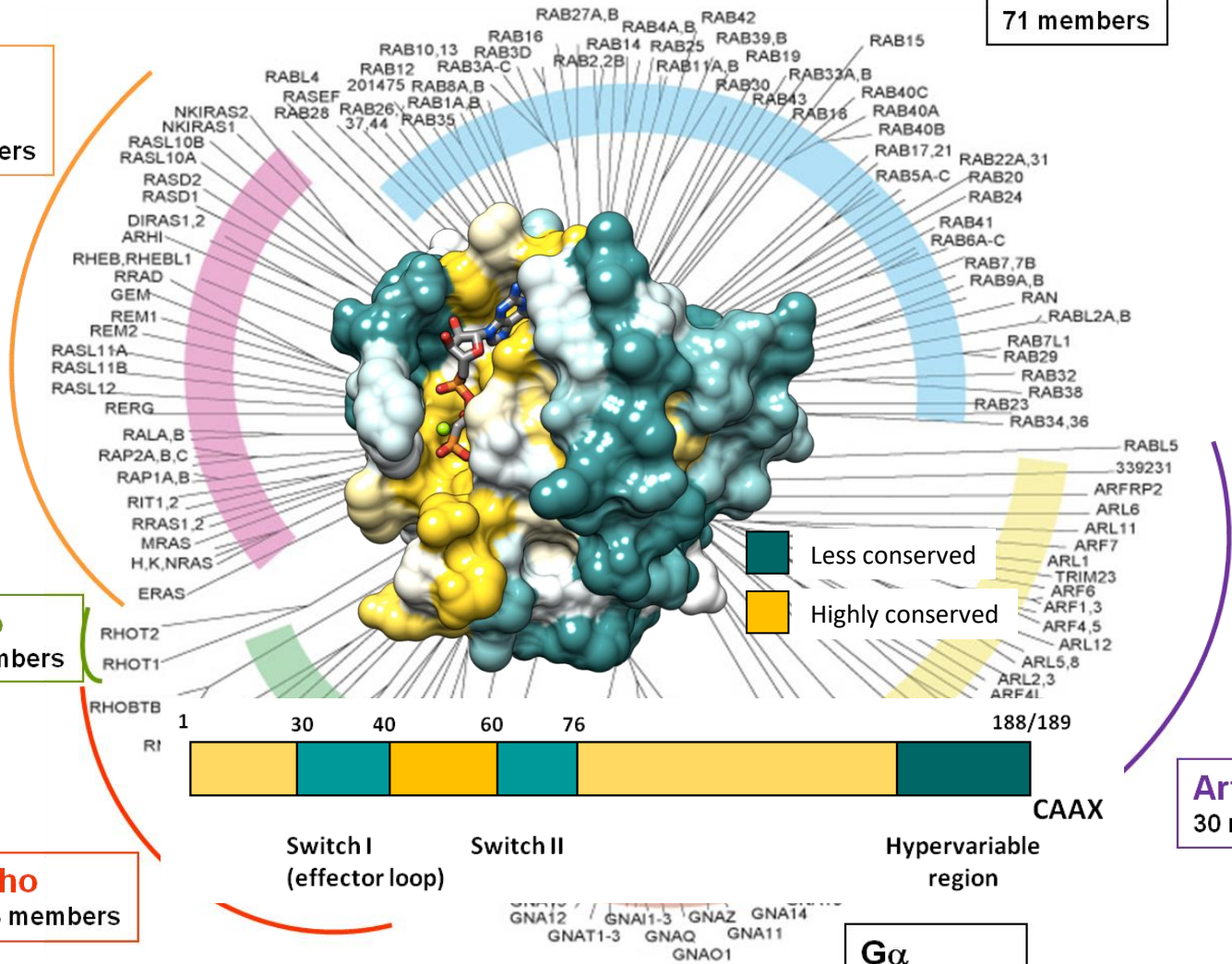
Ras+RGK
35 members

Miro
2 members

Rho
23 members

Gα
16 members

Arf
30 members



Colicelli, Science STKE., 2004

Ras superfamily...

Ras subfamily

Biological function

Ras

Cell growth

Arf

Vesicular transport

Rab

Membrane trafficking

Miro

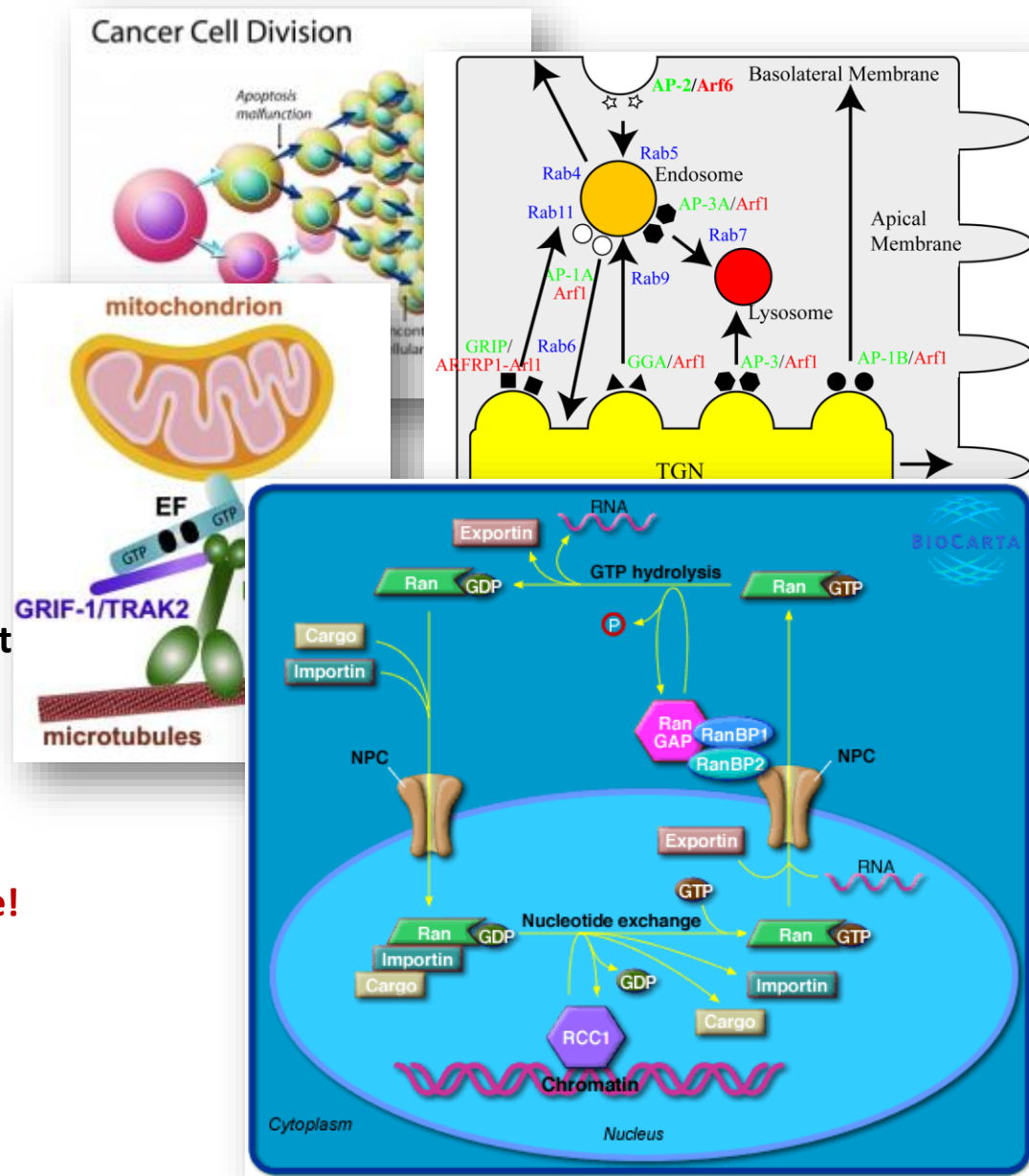
Mitochondria transport

Ran

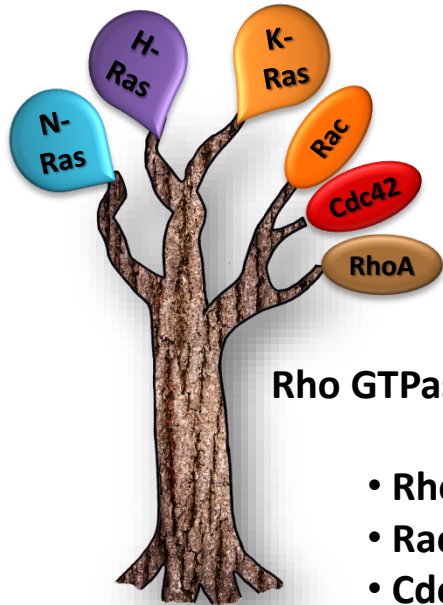
Nuclear transport

Rho

cytoskeletal dynamics,
cell motility...and more!



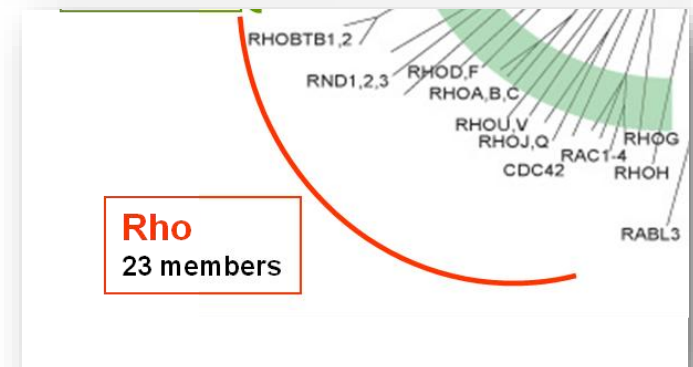
The Rho family of small GTPases...



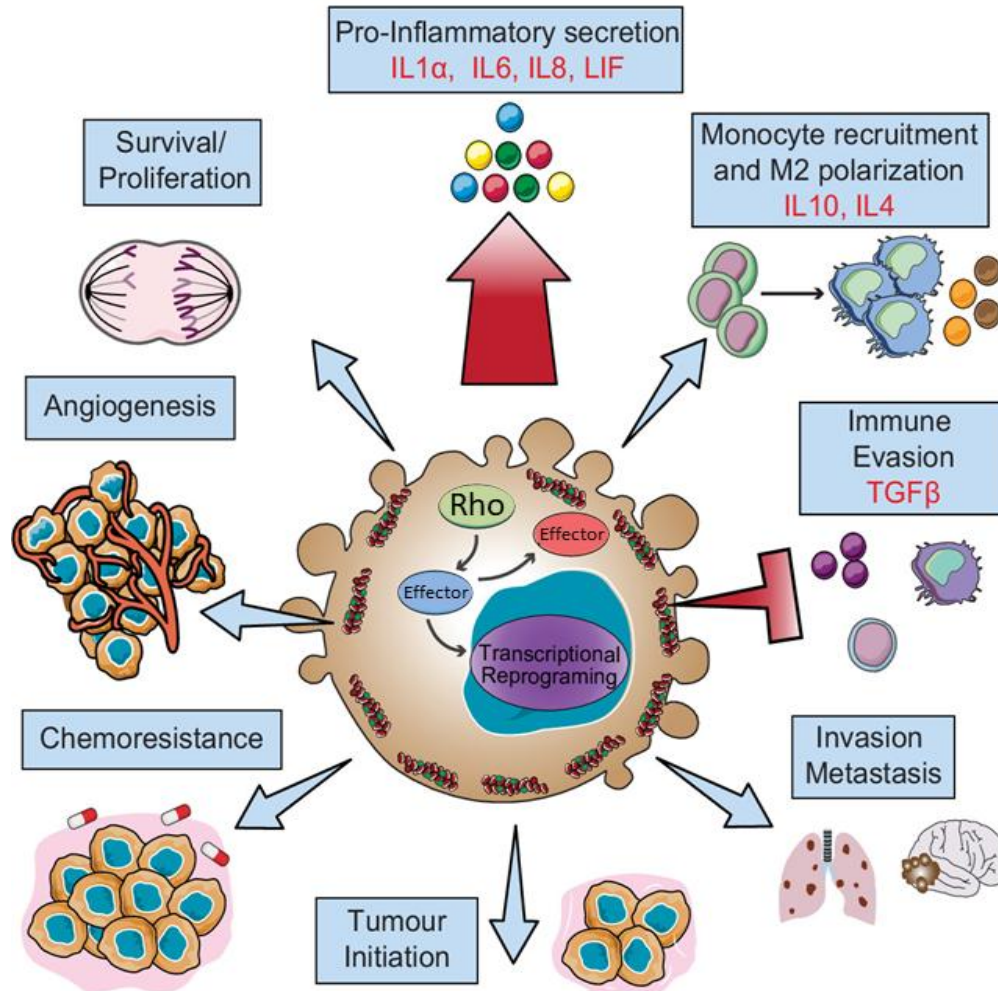
Rho GTPase family **canonical members**:

- RhoA
- Rac1
- Cdc42

...These are the most well-characterized ...

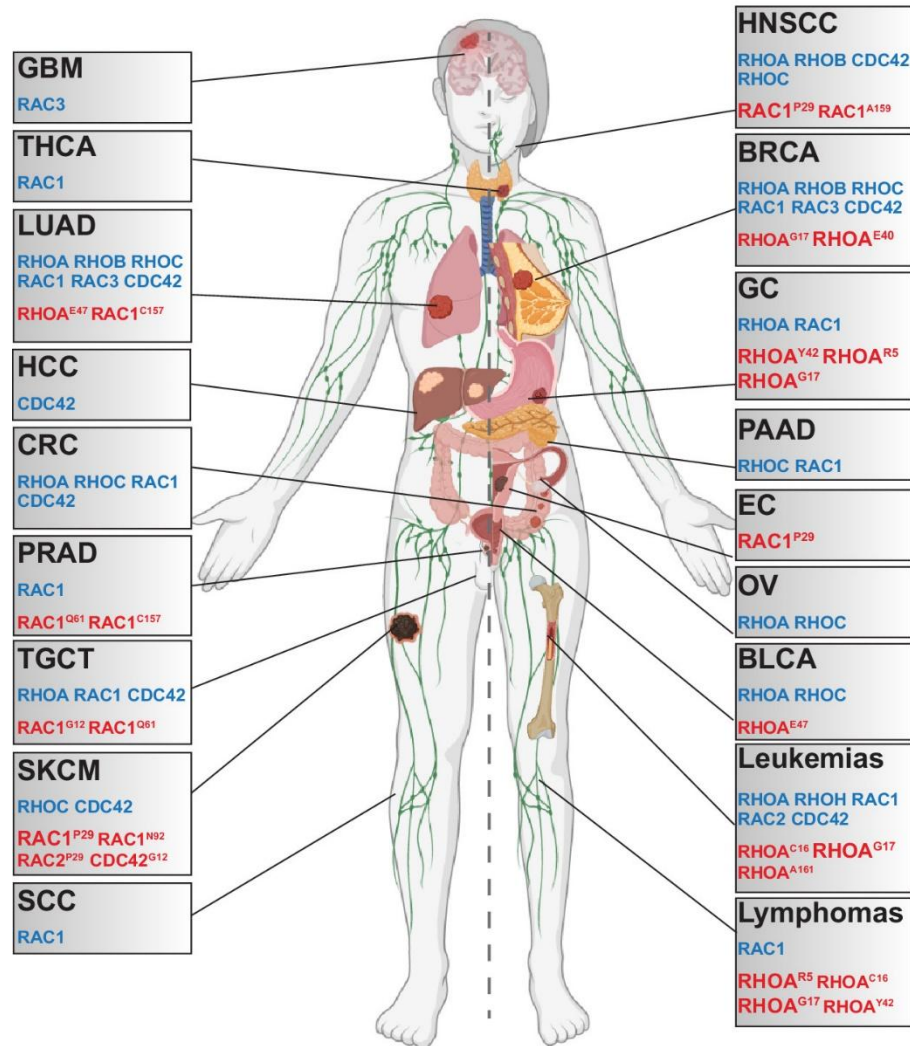


Dysregulated Rho GTPase signaling in cancer

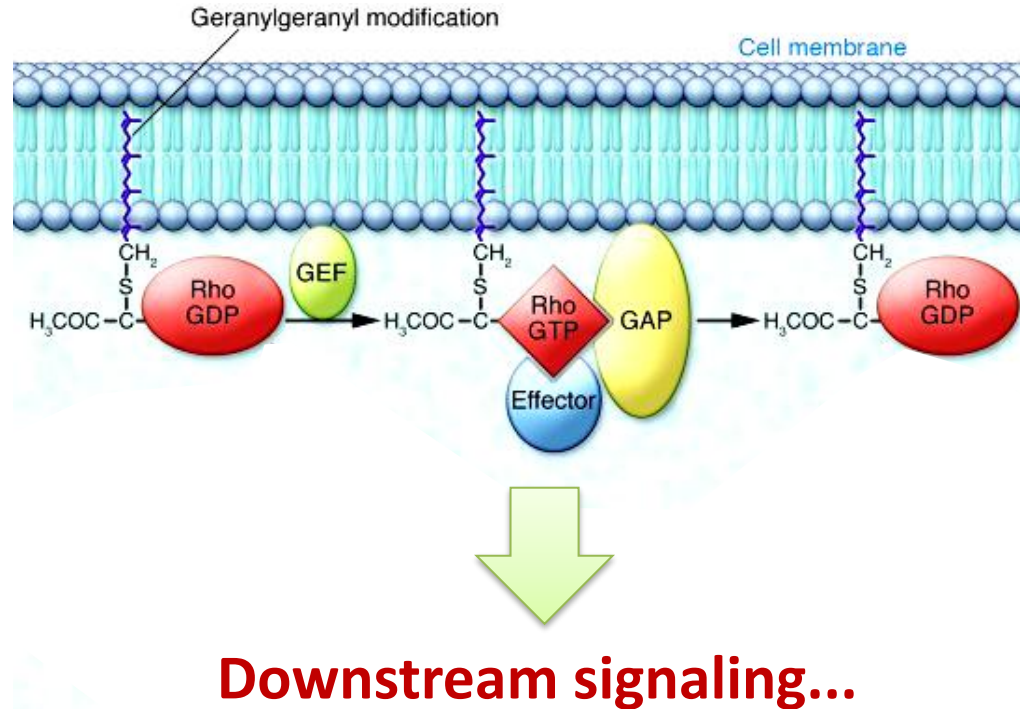


Dysregulated Rho GTPase signaling in cancer

Rho GTPase **mutations** / **altered expression** in cancer



Regulation of Rho GTPases



Mutations and dysregulated expression of RhoGEFs in cancer

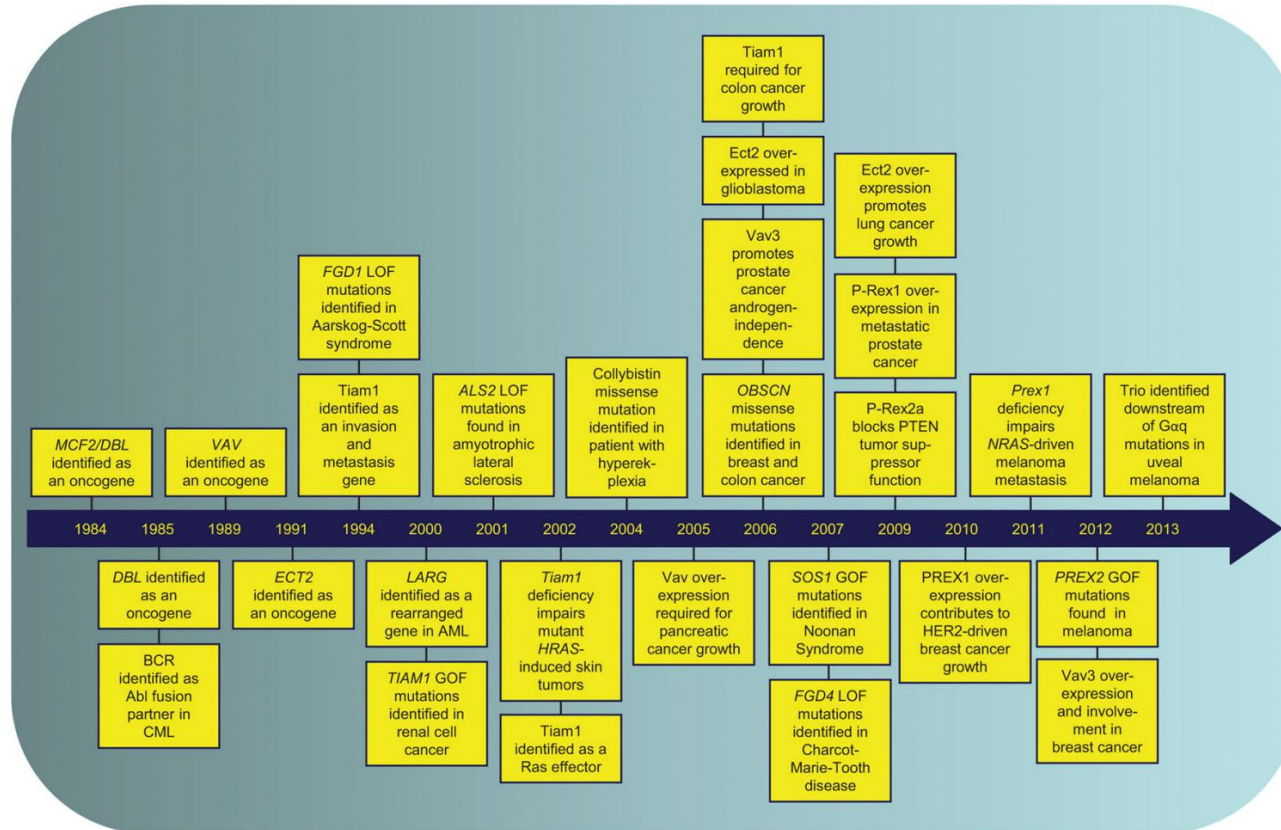


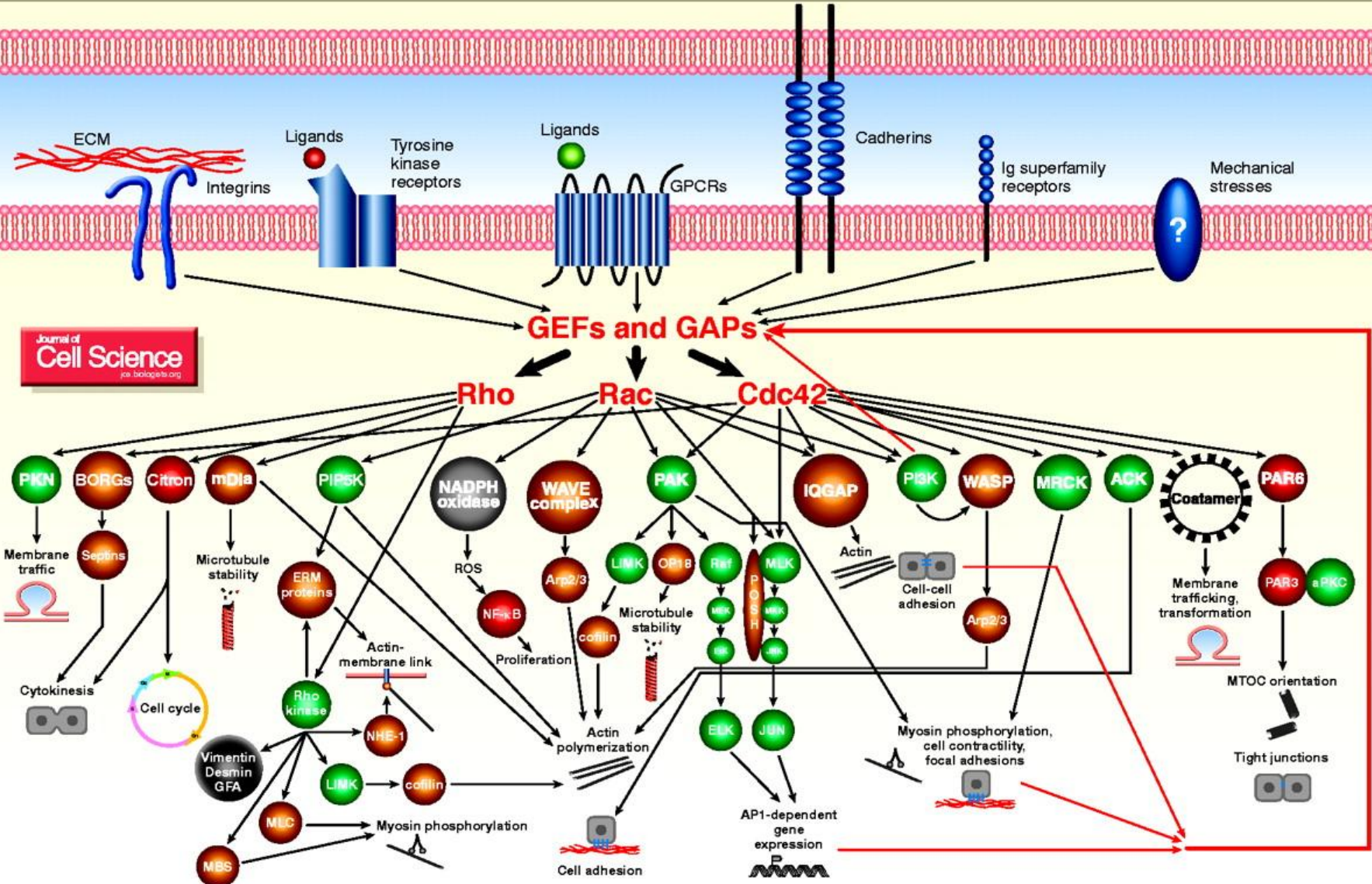
Figure 2. Key discoveries linking Dbl RhoGEFs to human disease

We highlight representative discoveries in the study of RhoGEFs that link their aberrant function to human disease. Gain-of-function, GOF; loss-of-function, LOF.

Adapted from:

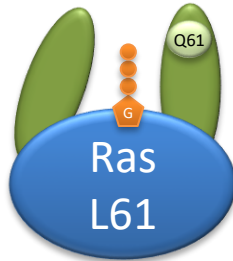
Rho Signalling at a Glance

Martin Schwartz

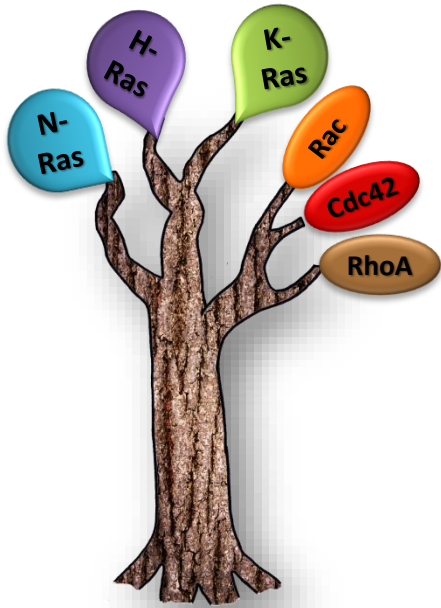


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jcs.biologists.org

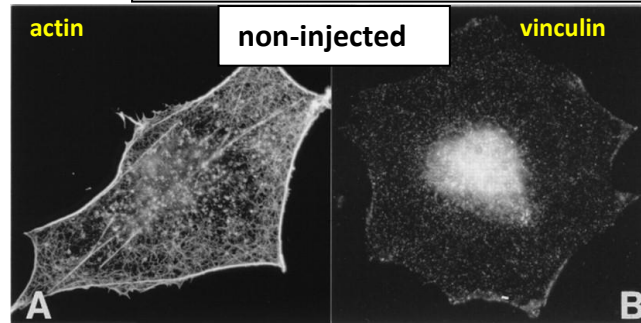
Rho GTPases & actin cytoskeleton



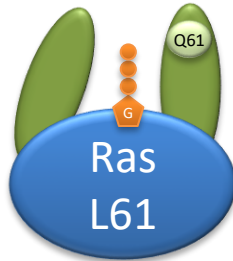
- GTPase activity-deficient mutant (Gln to Leu at codon 61)



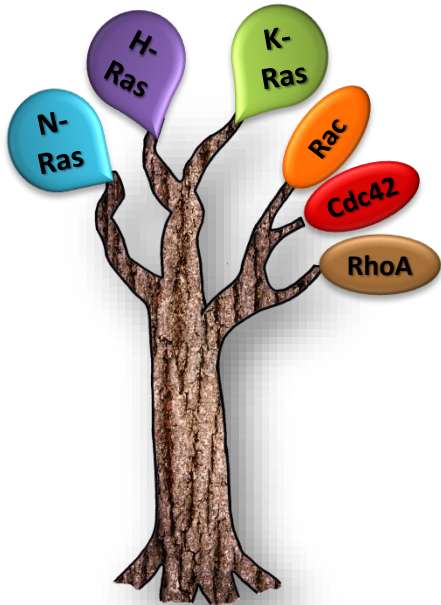
Microinjection of constitutively active Rho GTPases into Swiss 3T3 fibroblasts induce specific responses in the organisation of the actin filament system



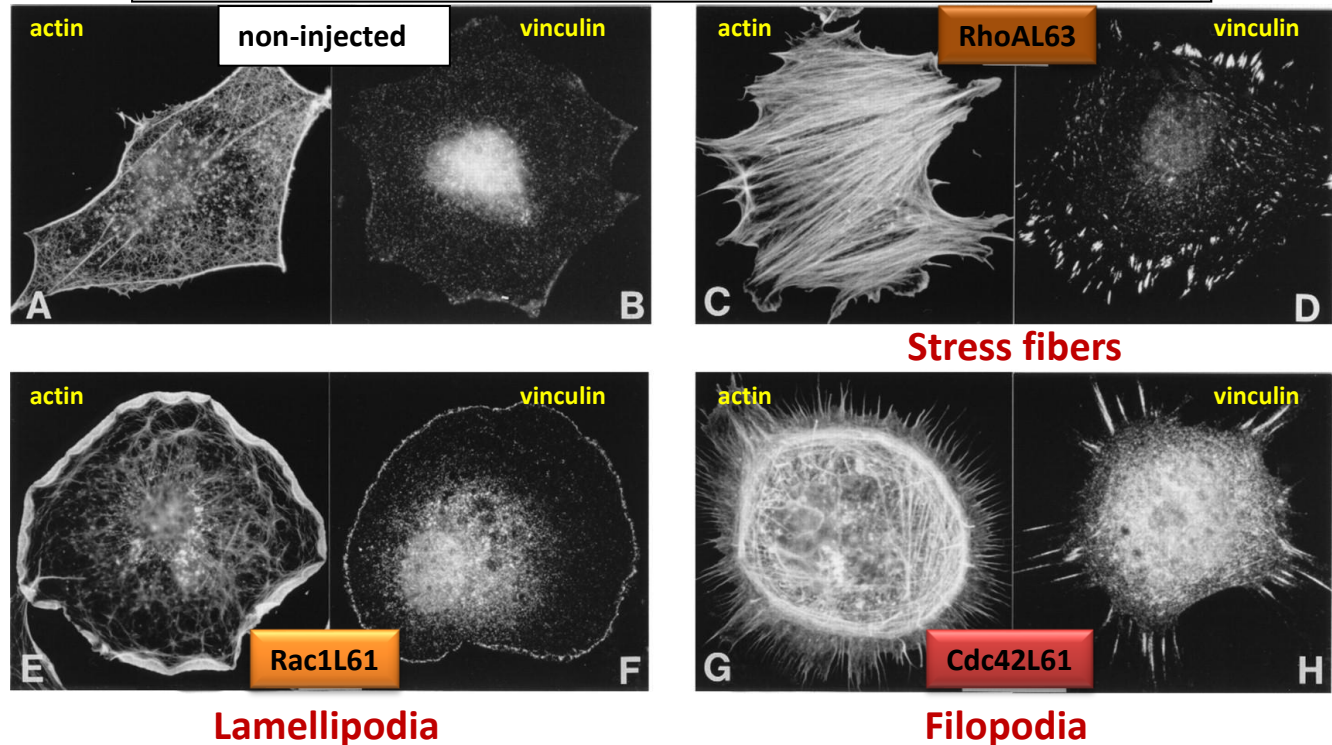
Rho GTPases & actin cytoskeleton



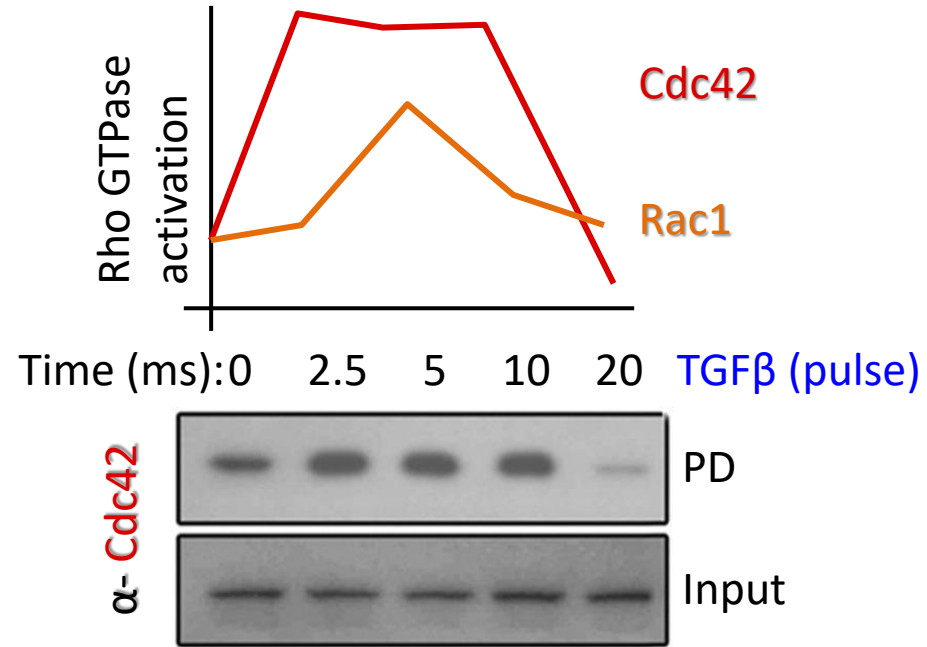
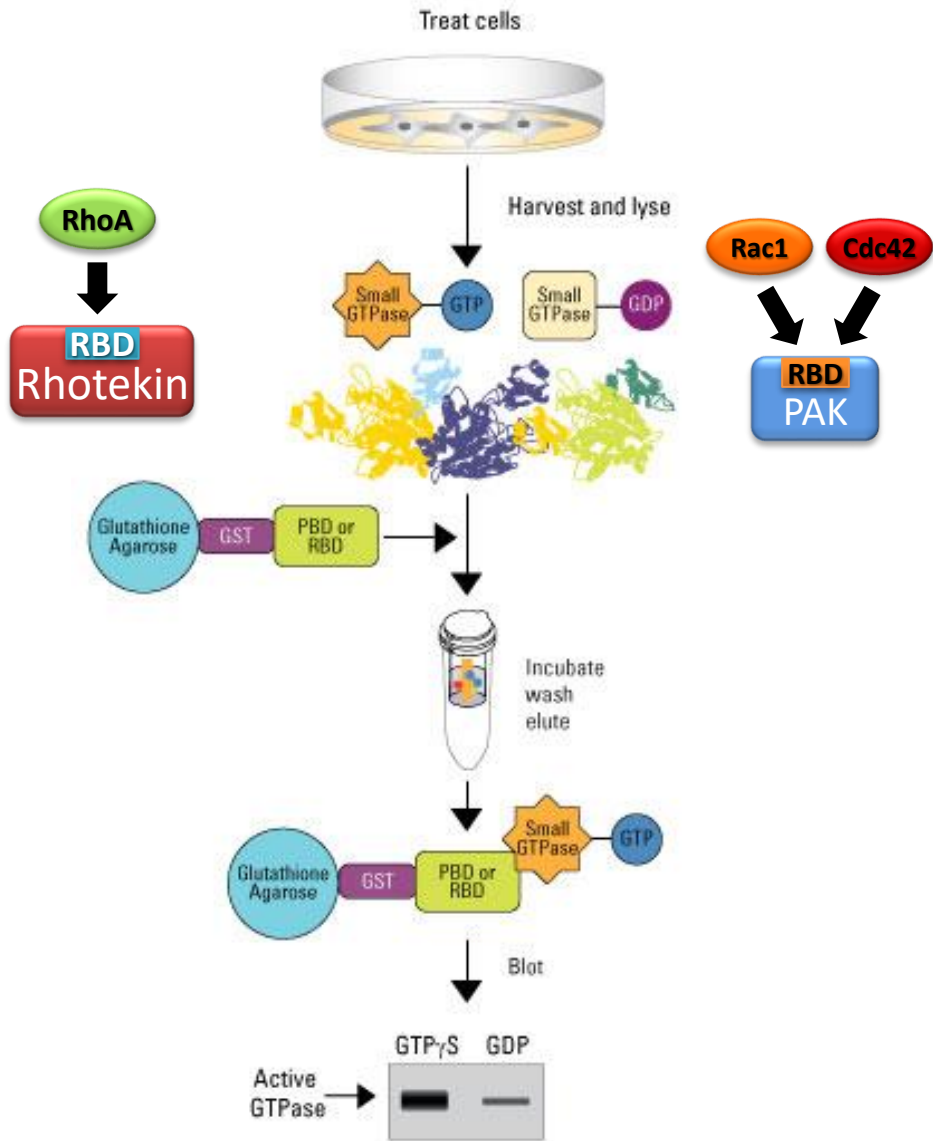
- GTPase activity-deficient mutant (Gln to Leu at codon 61)



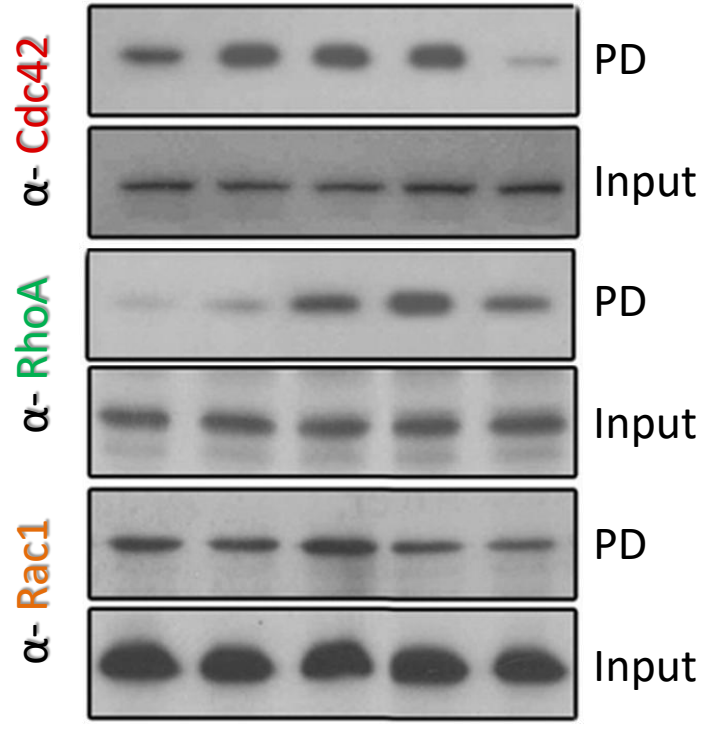
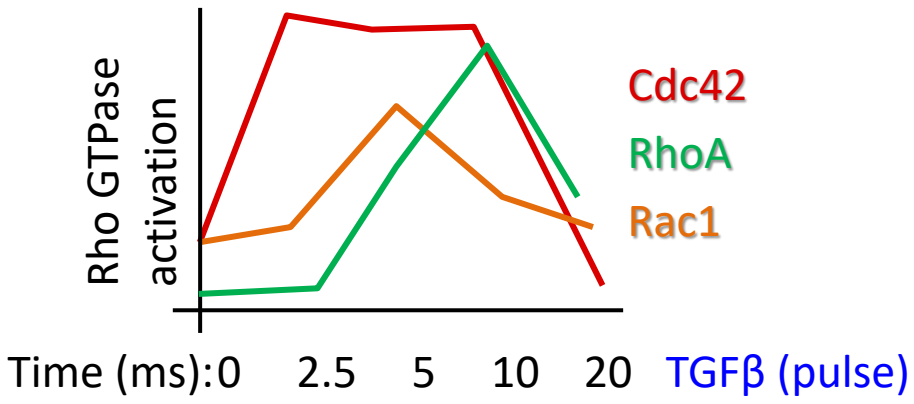
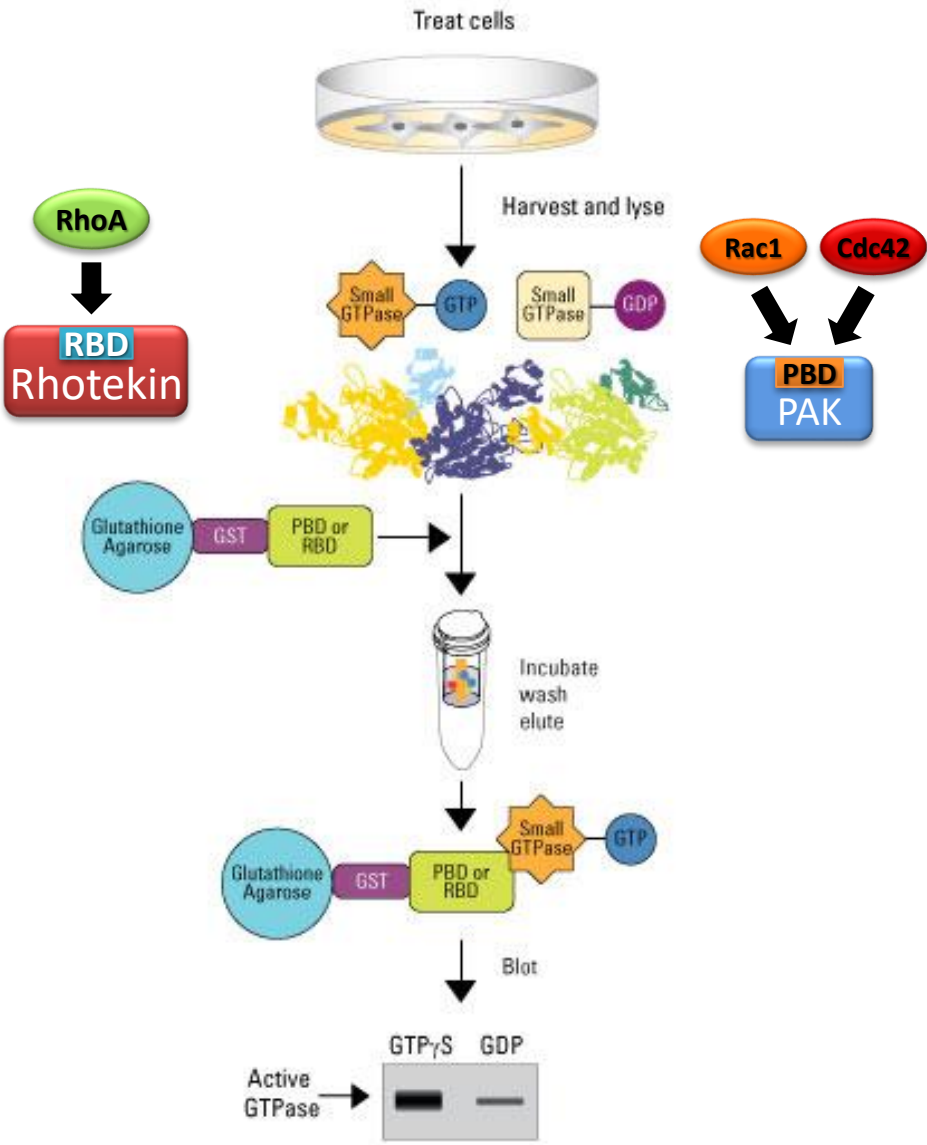
Microinjection of constitutively active Rho GTPases into Swiss 3T3 fibroblasts induce specific responses in the organisation of the actin filament system



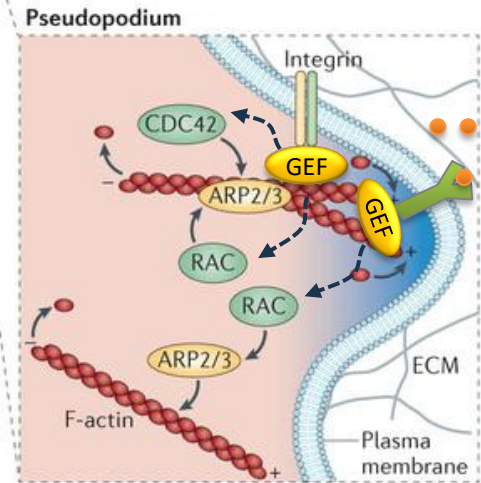
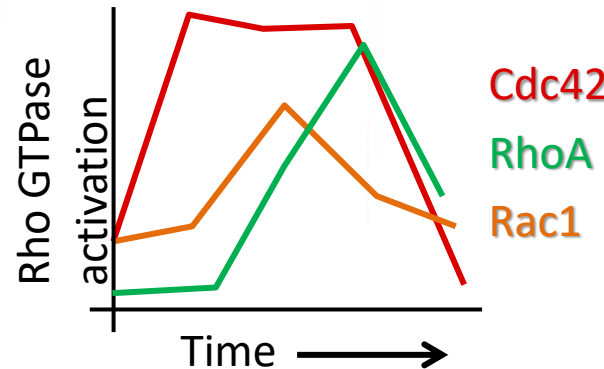
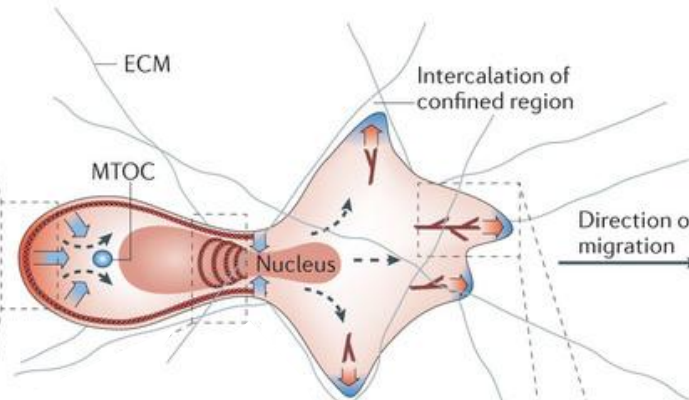
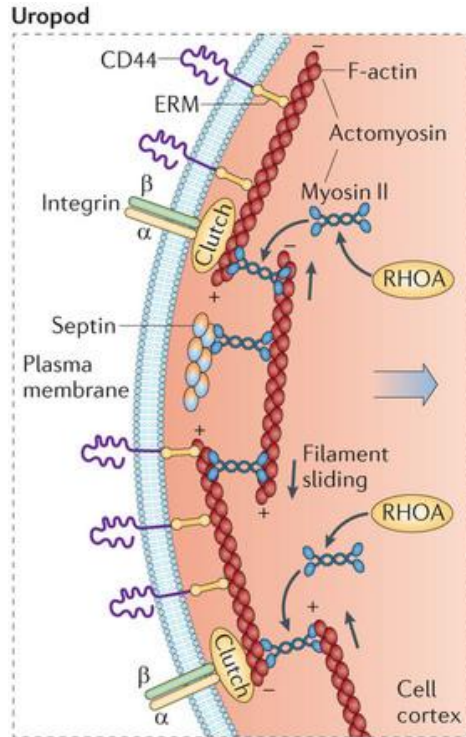
Assessing Rho GTPase activation level



Assessing Rho GTPase activation level



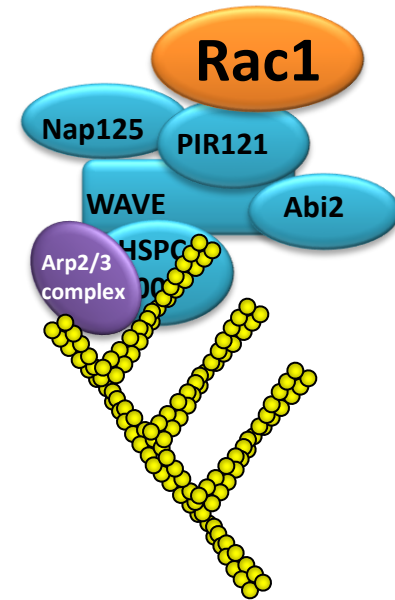
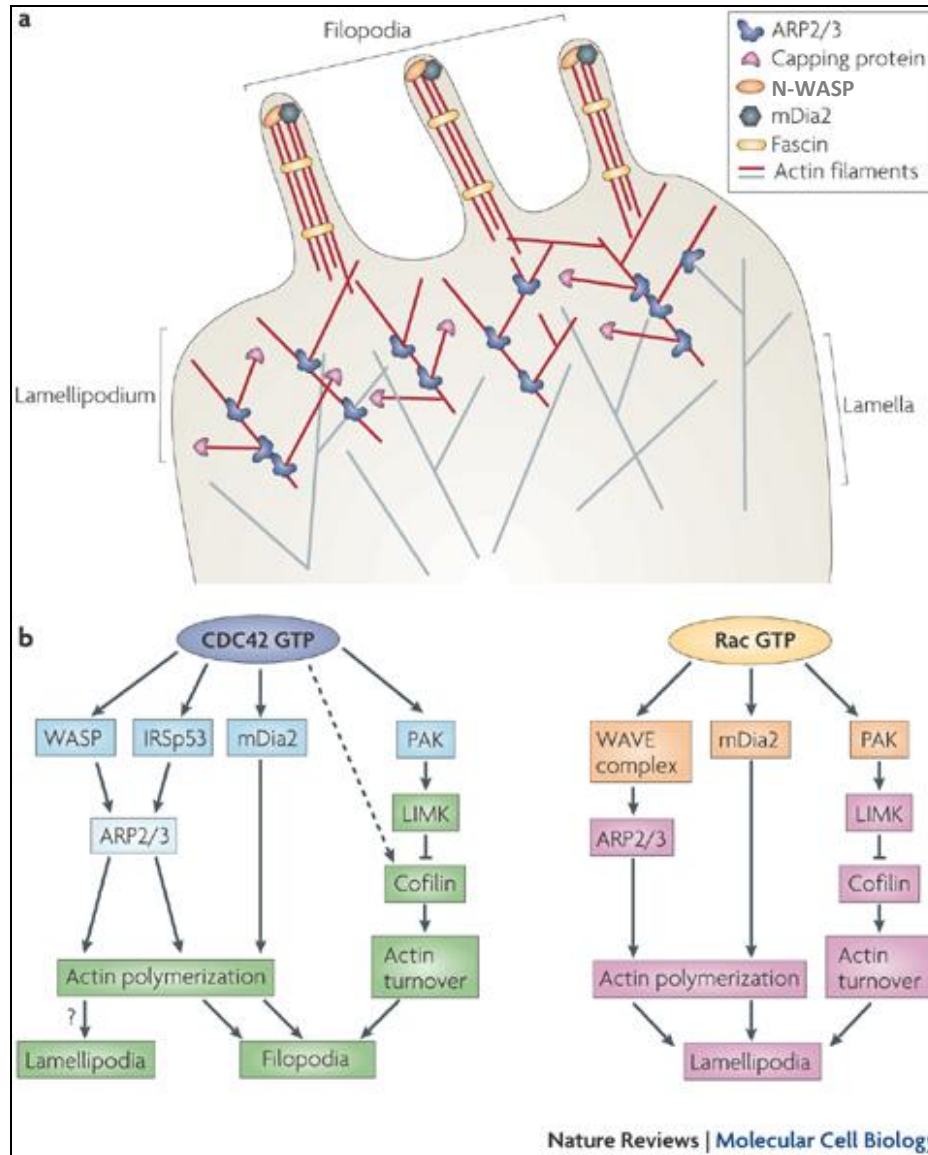
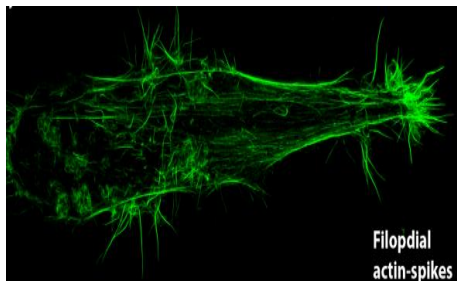
Rho GTPases, ERMs & Receptors



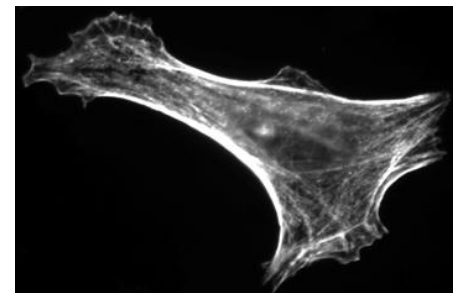
Signalling downstream of Cdc42 and Rac1



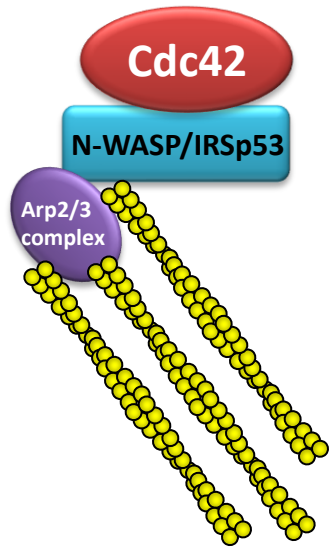
Activation of actin polymerization by N-WASP/IRSp53



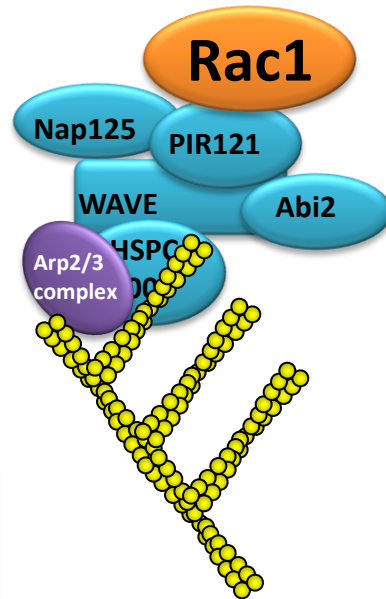
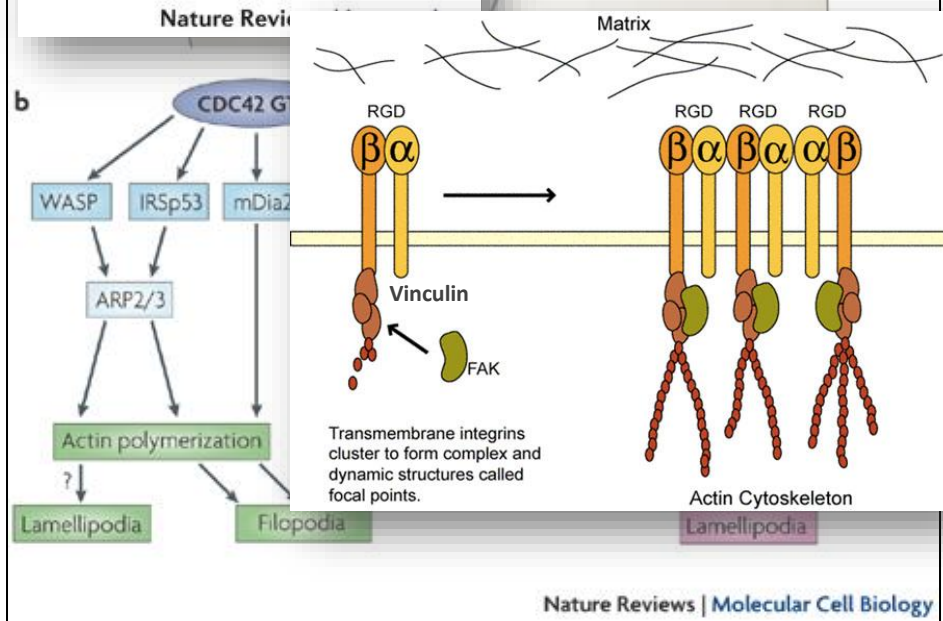
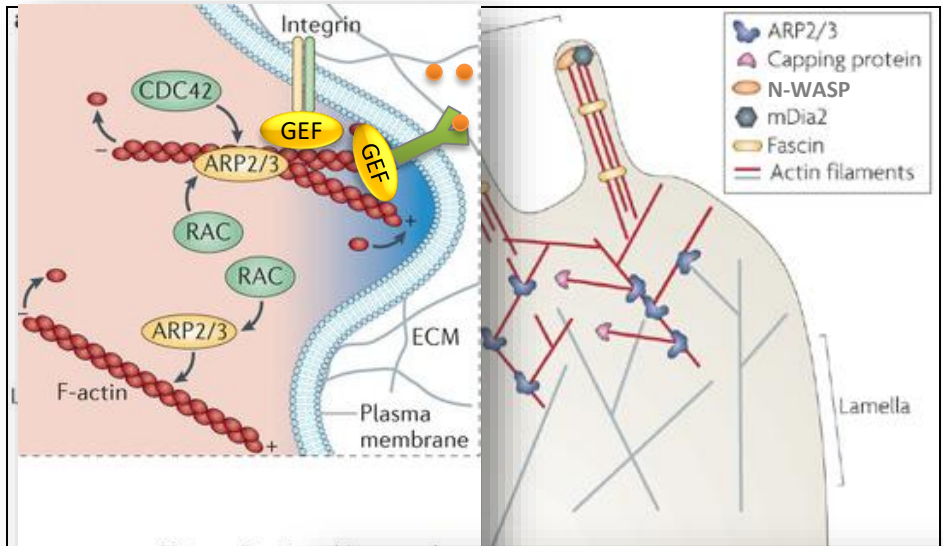
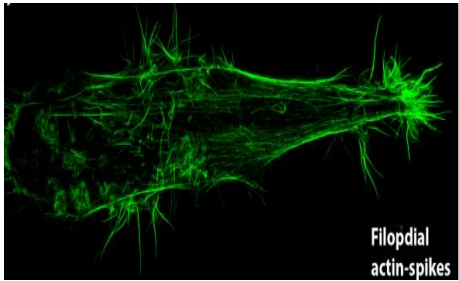
Activation of actin polymerization by WAVE complex



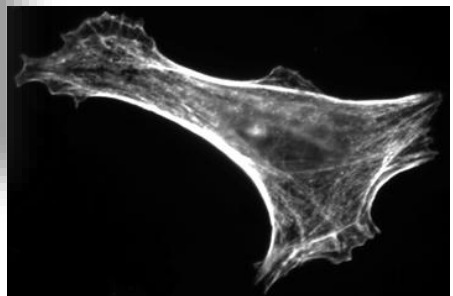
Signalling downstream of Cdc42 and Rac1



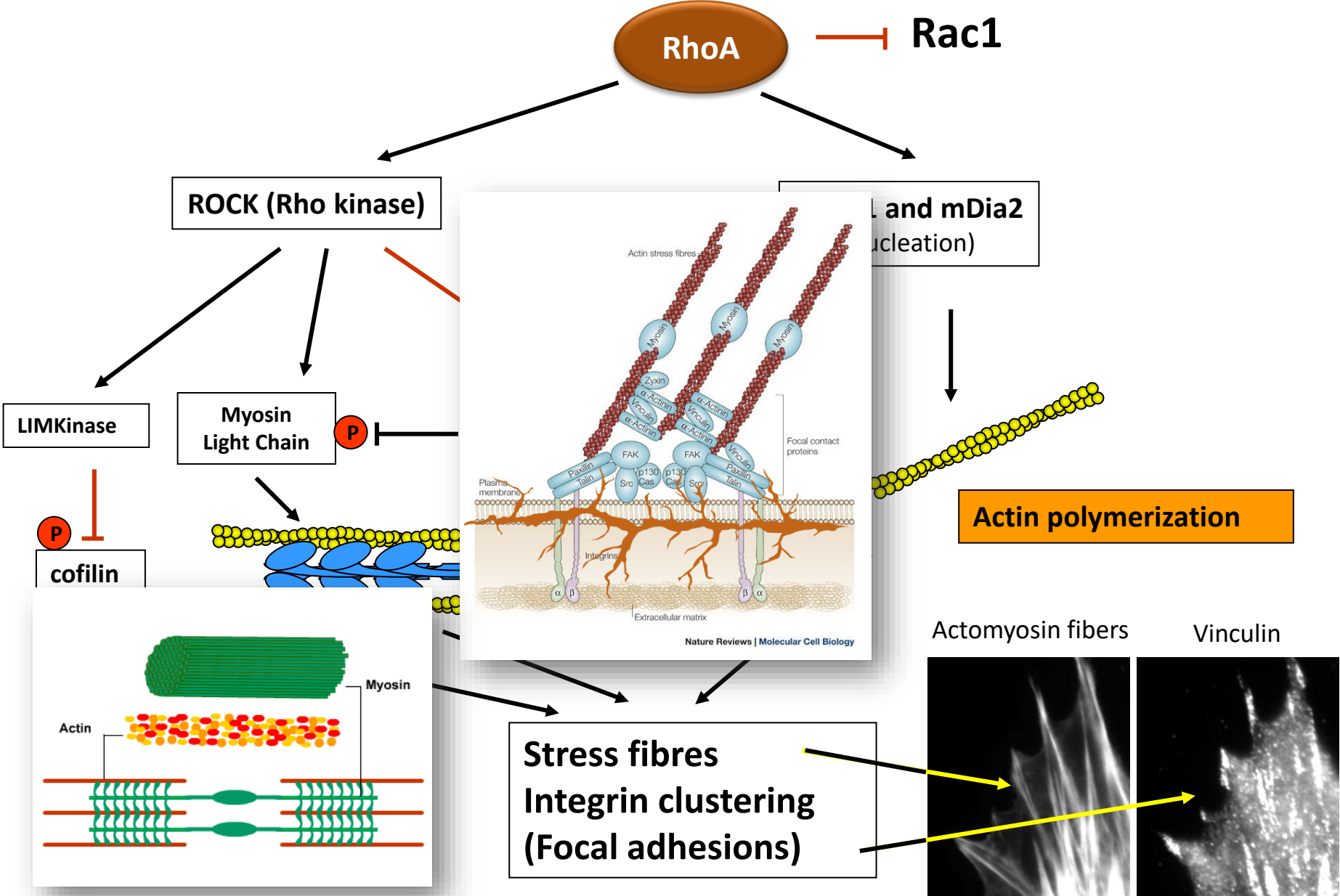
Activation of actin polymerization by N-WASP/IRSp53



Activation of actin polymerization by WAVE complex



Signalling downstream of RhoA

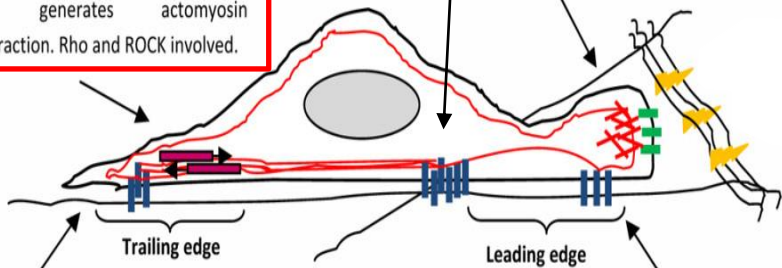


Rho GTPases & cell migration

3. Focal adhesion maturation: adhesion complex clustering and actin fiber anchoring. Rac involved.

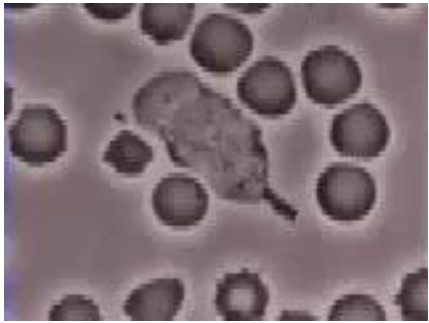
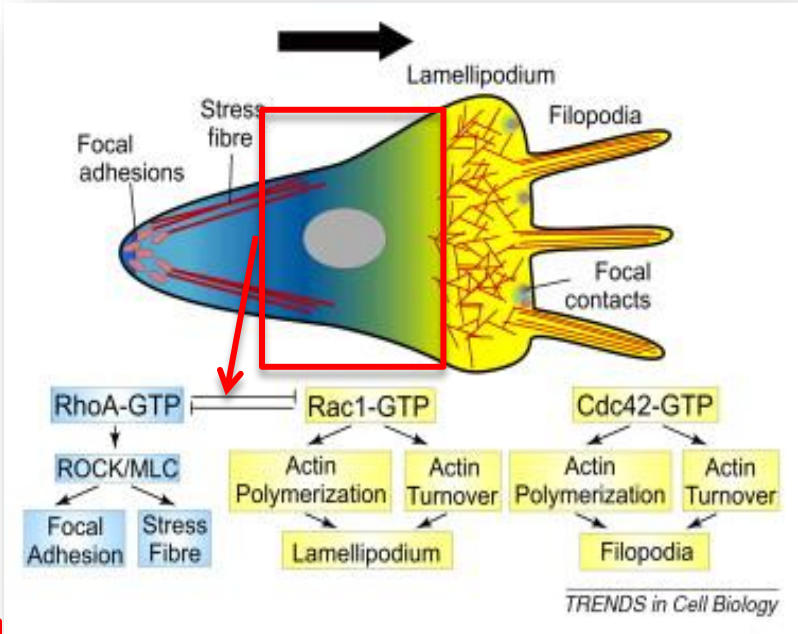
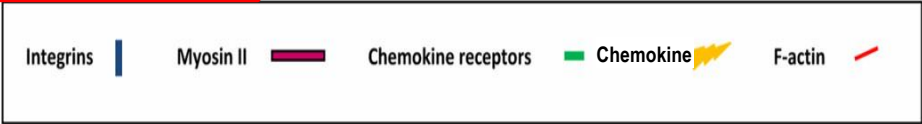
4. Cell contraction by actomyosin: active myosin II binds to actin filaments (then termed actomyosin) and generates actomyosin contraction. Rho and ROCK involved.

1. Protrusion of the leading edge: actin polymerization at the leading edge of cell. Rac, Cdc42 and Arp2/3 involved.



5. Detachment of the trailing edge: contractile force generated by actomyosin structures (RhoA involved) and ECM adhesion destabilization (Rac inhibition by RhoA) enables trailing edge detachment and translational movement.

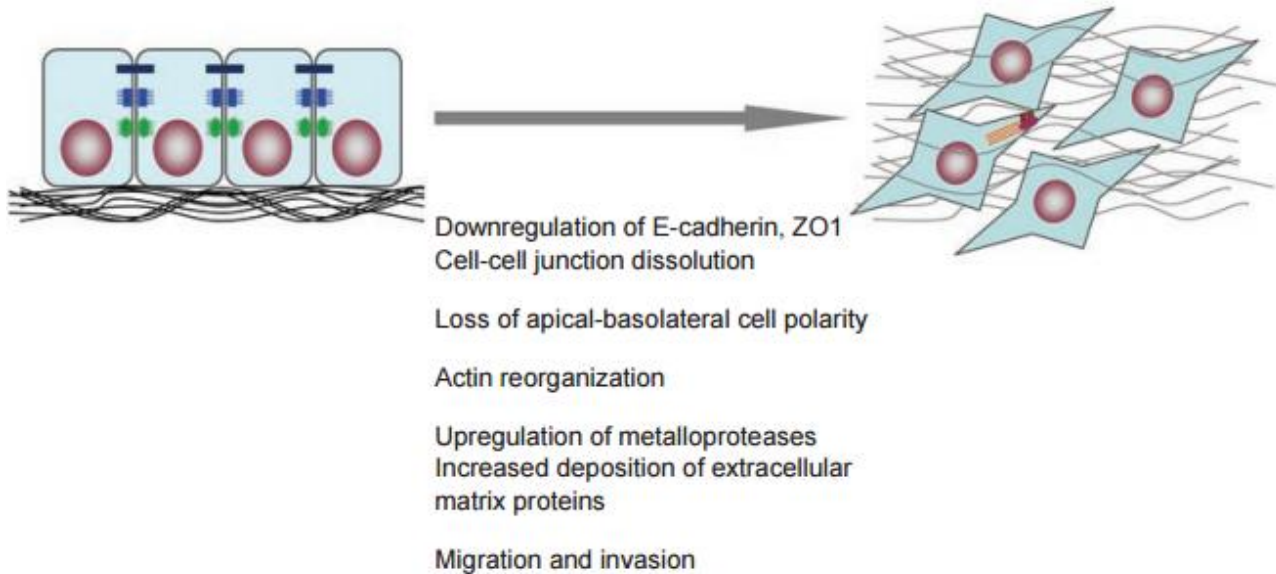
2. Cell-matrix interaction and formation of focal contacts: integrins come into contact with ECM ligands and cluster in the cell membrane. Rac involved.



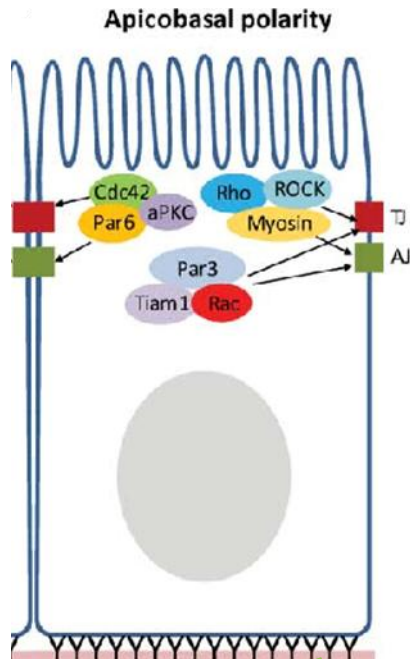
Mesenchymal Cell migration...

Rho GTPases in Epithelial-Mesenchymal Transition (EMT)

Atered Rho GTPase Signaling & localization

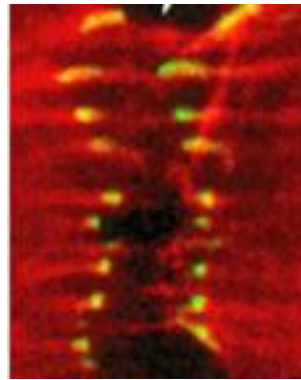


Rho GTPases in epithelial polarity

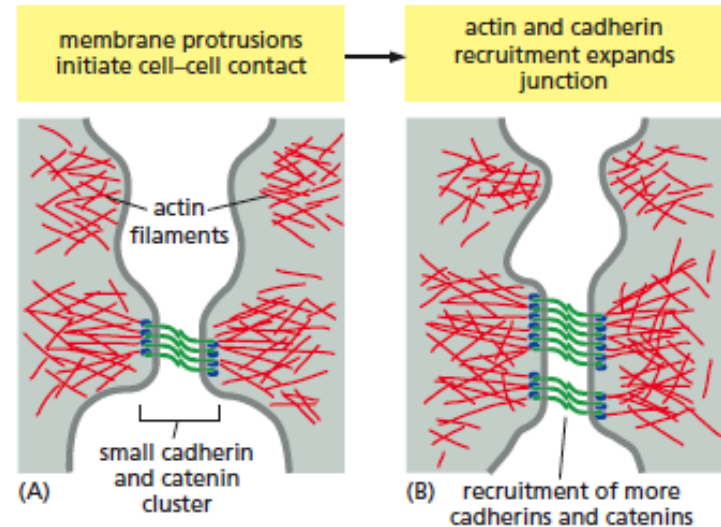


Epithelial Stimuli

RAC & Cdc42

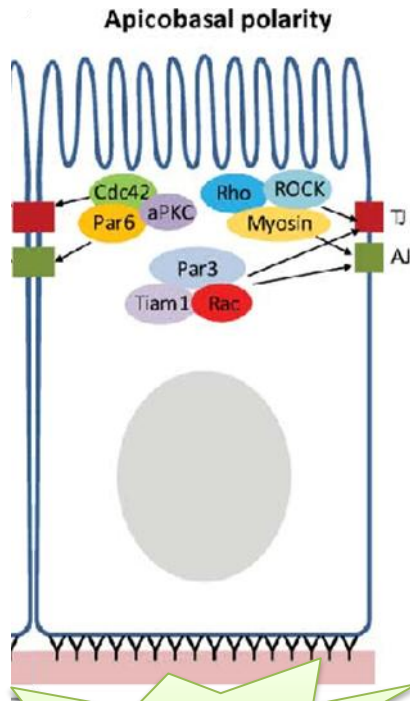


Actin
E-cadherin



Together with **PAR proteins**, RAC and CDC42 promote cell-cell adhesion through the formation and maturation of adherens (AJ) and tight (TJ) junctions...

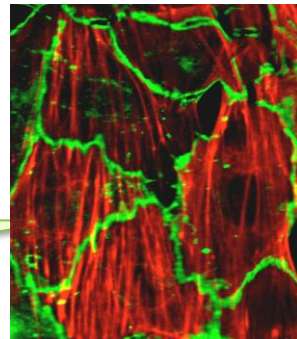
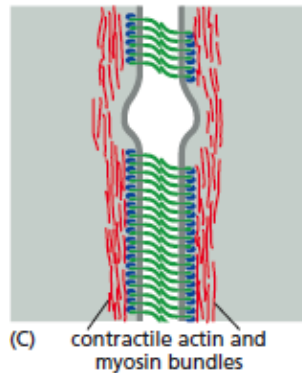
Rho GTPases in epithelial polarity



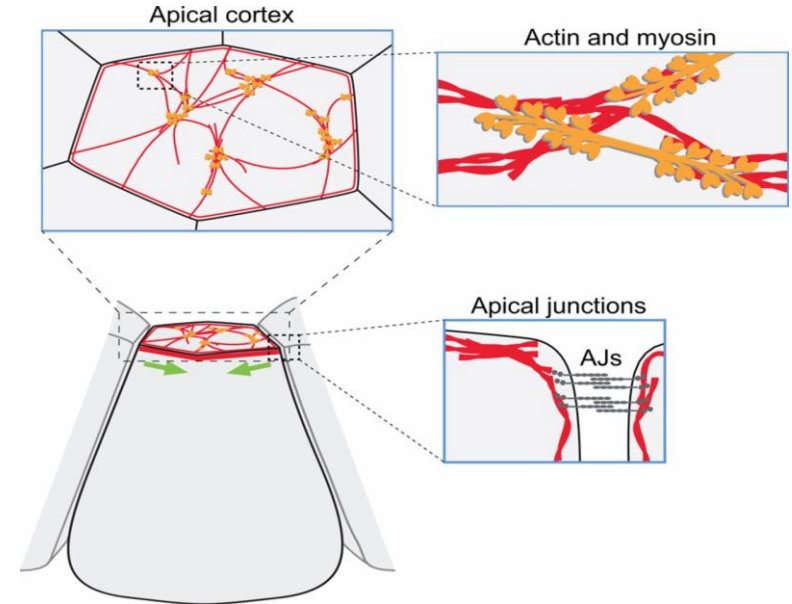
Epithelial Stimuli

RhoA

actin remodeling and myosin recruitment expands the adherens junction



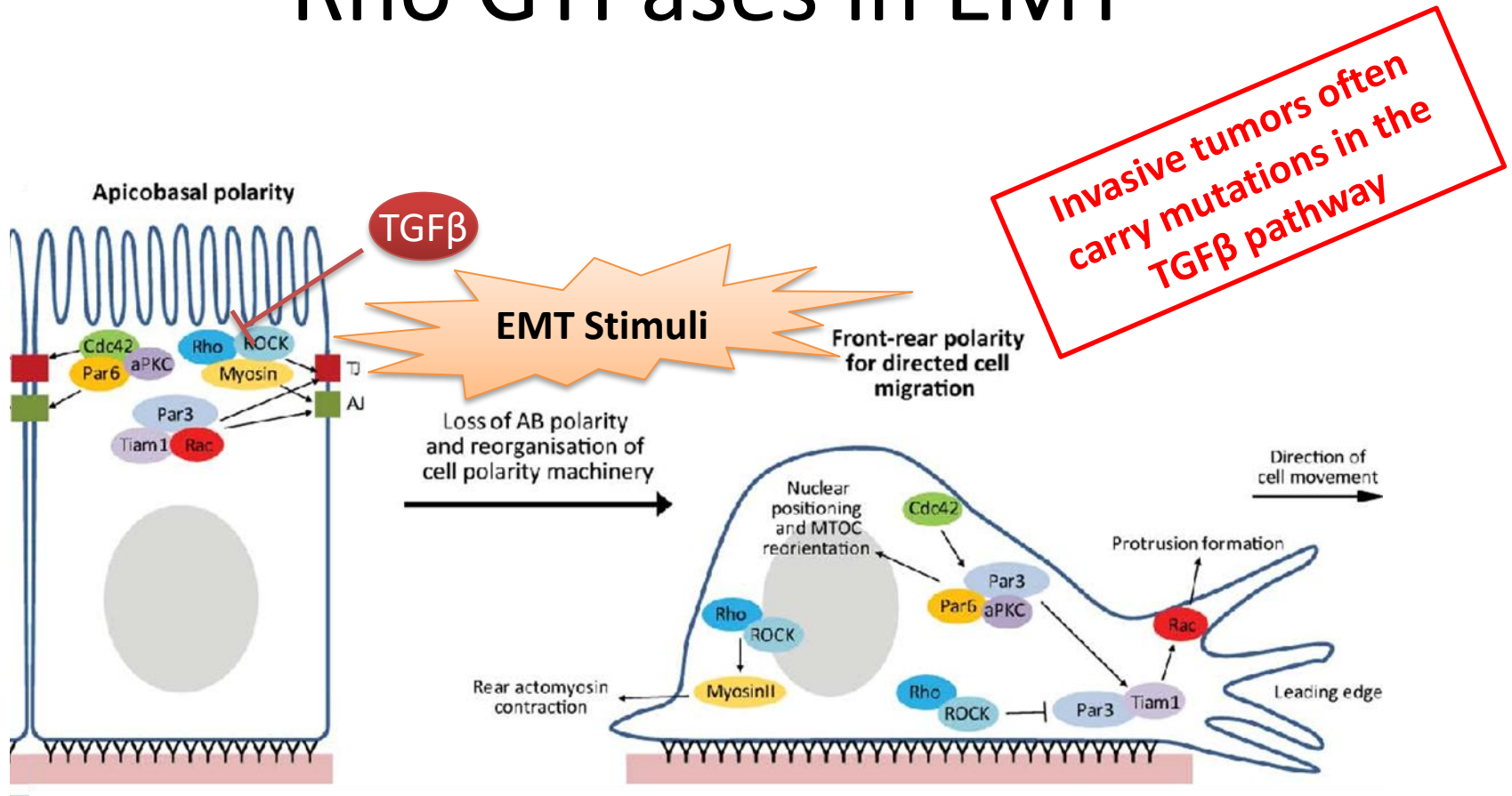
Actin
E-cadherin



Apical actomyosin belt:

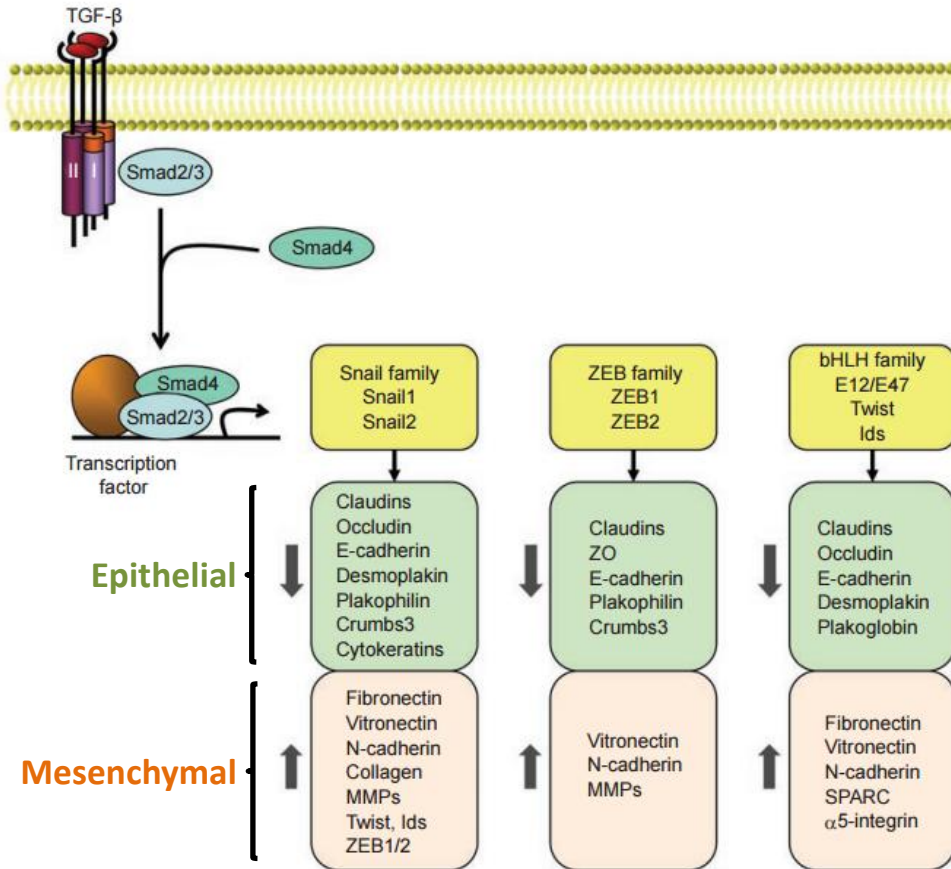
- ✓ Pulls cells together;
- ✓ Promotes barrier function;
- ✓ Stabilizes **Tight Junctions**;
- ✓ Sustains polarity

Rho GTPases in EMT



TGFβ signaling has been shown to play an important role in EMT by **inhibiting RhoA** and triggering actomyosin ring breakdown and promoting the **loss of apicobasal polarity**.

EMT transcriptional reprogramming by TGFβ

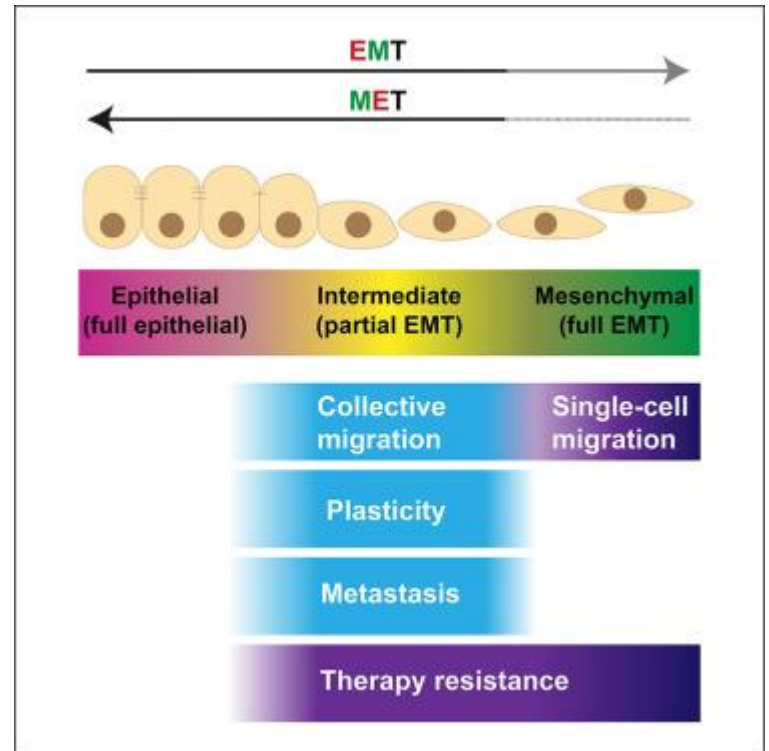
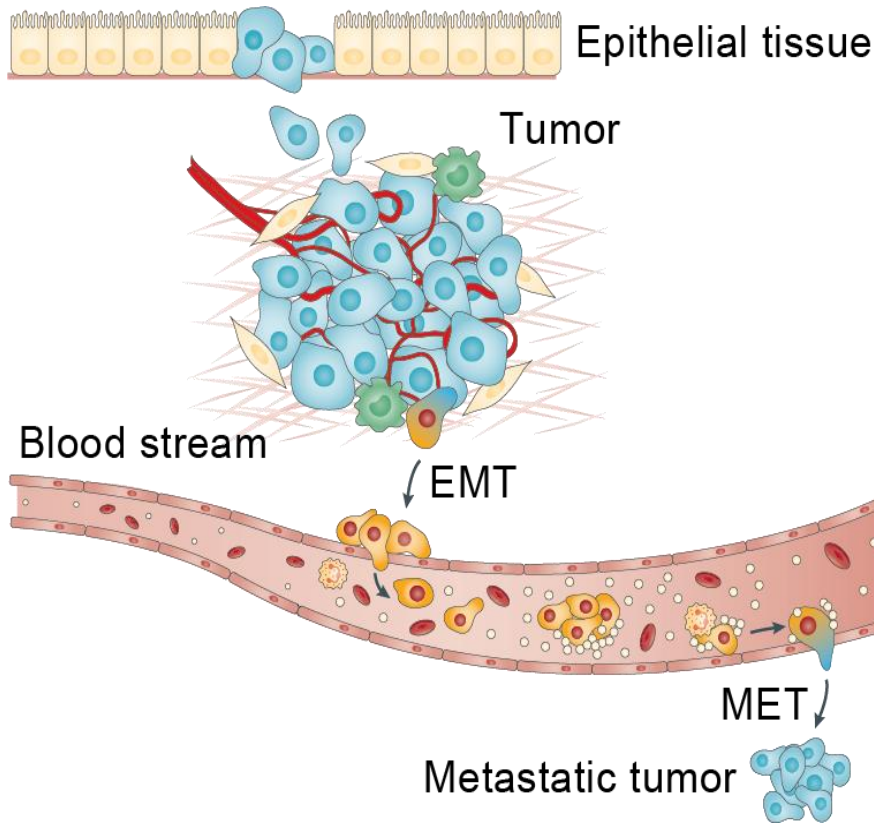


✓ TGFβ also induces **transcriptional reprogramming**, **repressing epithelial** genes and promoting the expression of **mesenchymal proteins**;

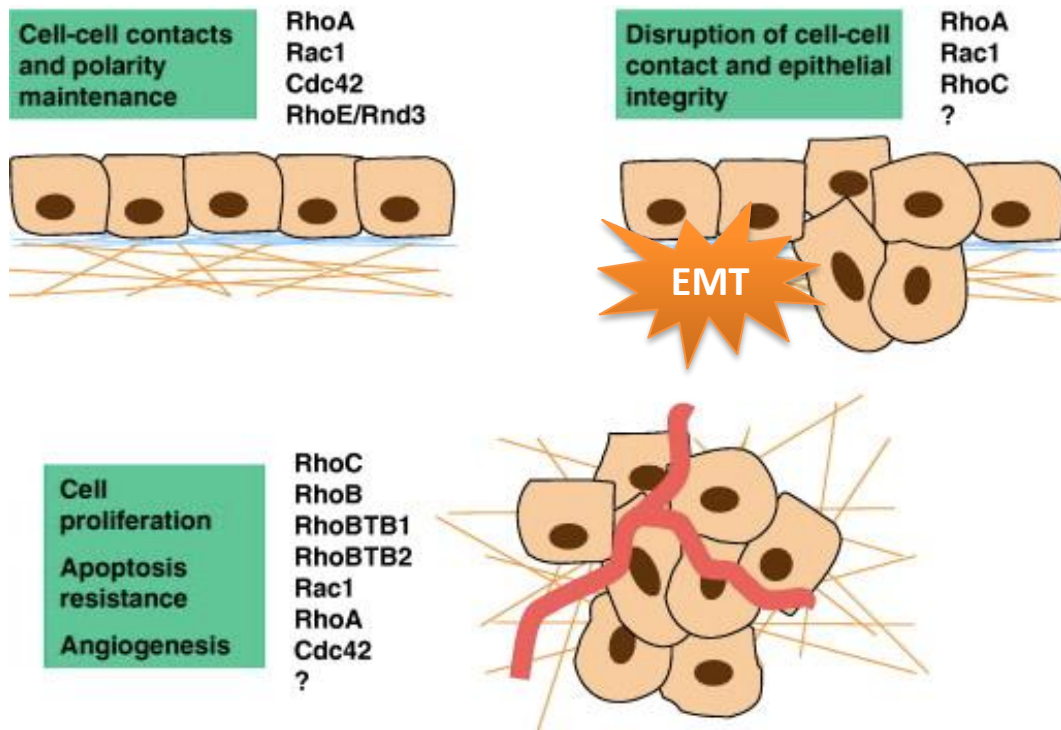
✓ **Changes in expression of polarization protein** also alters the localization and activity of **RAC & CDC42**, promoting **loss of lateral cell-cell adhesions** and altering integrin-mediated **cell-matrix adhesion** .

✓ This progresses the **loss of apical-basal polarity** and promotes **EMT!**

EMT – MET plasticity in metastasis



Rho GTPases, EMT & metastasis



- ✓ Several RhoGEFs have been implicated in invasion & metastasis (e.g., The Rac1 GEF Tiam1 - *T-cell lymphoma invasion and metastasis*)
- ✓ Dysregulated activity of several GTPases has been associated with EMT and invasive behavior.
- ✓ **Rac1, RhoA** activity have been **directly implicated in the invasive process**

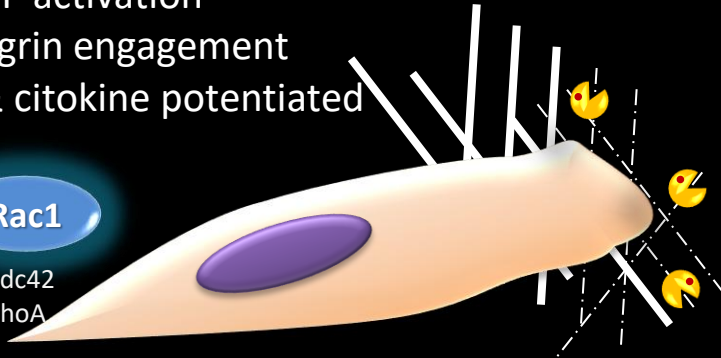
Invasive phenotype...

MESENCHYMAL MOTILITY

- ✓ Dense/Stiff ECM
- ✓ MMP activation
- ✓ Integrin engagement
- ✓ GF & cytokine potentiated
- ✓ ...

Rac1

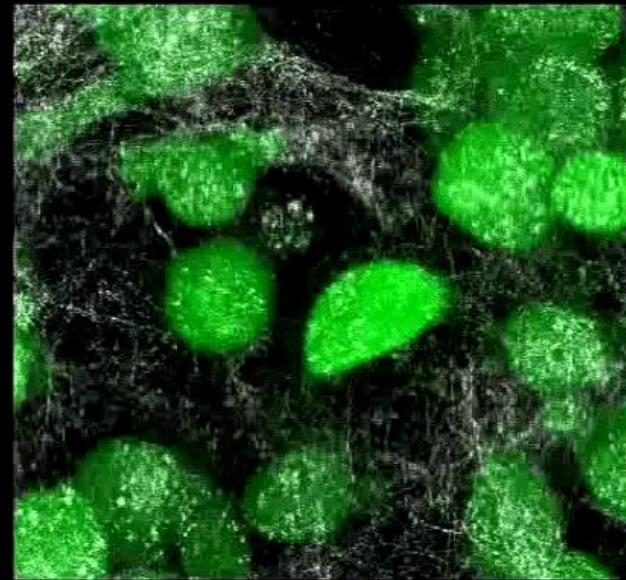
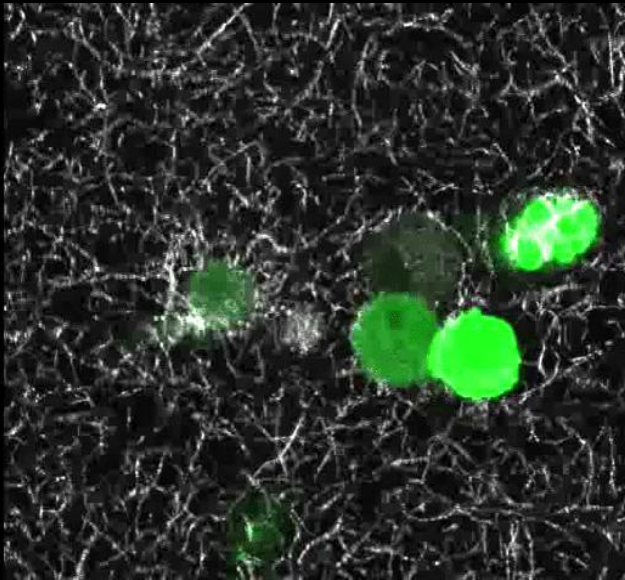
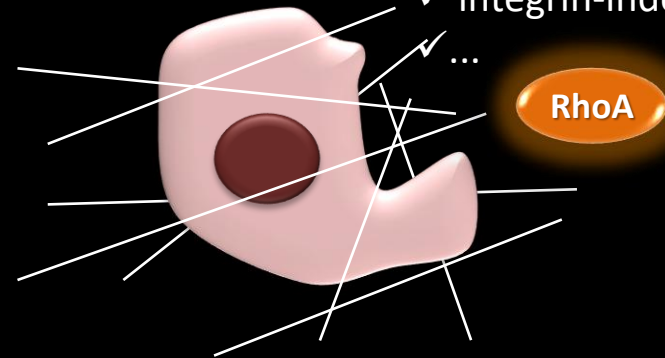
- Cdc42
- RhoA



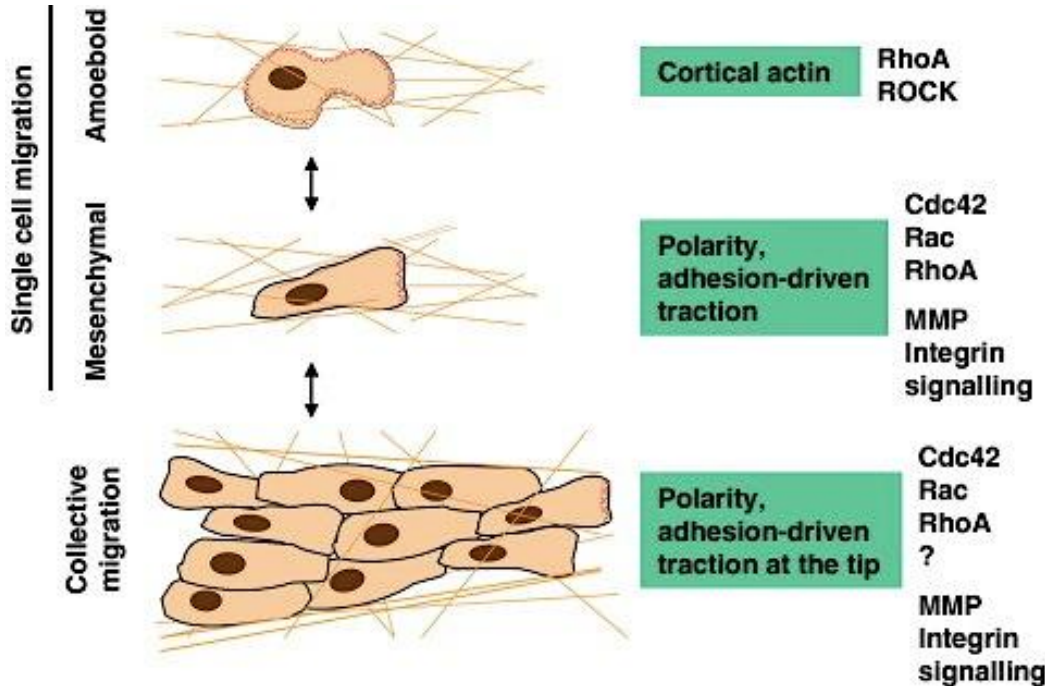
AMOEBOID MOTILITY

- ✓ Loose/Soft ECM
- ✓ MMP-independent
- ✓ integrin-independent

RhoA

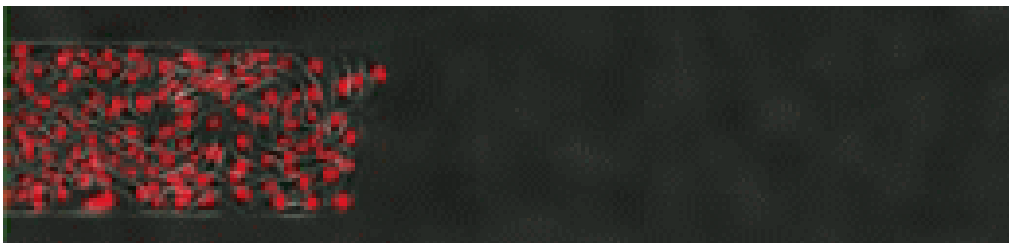


Rho GTPases & cancer metastasis



Collective Migration:

- ✓ In collective cell invasion **cells maintain cell-cell adhesions**.
- ✓ A leading cell at the **tip generates migratory traction** while the rest of the cells in the group are mostly dragged passively.
- ✓ **Degradation of the ECM** by matrix metalloproteases (MMPs) is **essential** for this kind of collective migration.
- ✓ Leading cells can **convert from mesenchymal to amoeboid** migration, depending on the extracellular conditions, and **from collective to single-cell**, to transverse into blood and lymph vessels.



Collective migration of MDCK epithelial cells on a fibronectin strip.

Further reading

- Rho GTPase signaling in cancer progression and dissemination
<https://doi.org/10.1152/physrev.00045.2020>
- Dysregulation of Rho GTPases in Human Cancers
<https://doi.org/10.3390/cancers12051179>
- Small GTPases orchestrate cell-cell communication during collective cell movement
<https://doi.org/10.1080/21541248.2017.1366965>
- Guanine nucleotide exchange factors for Rho GTPases (RhoGEFs) as oncogenic effectors and strategic therapeutic targets in metastatic cancer
<https://doi.org/10.1016/j.cellsig.2023.110749>

