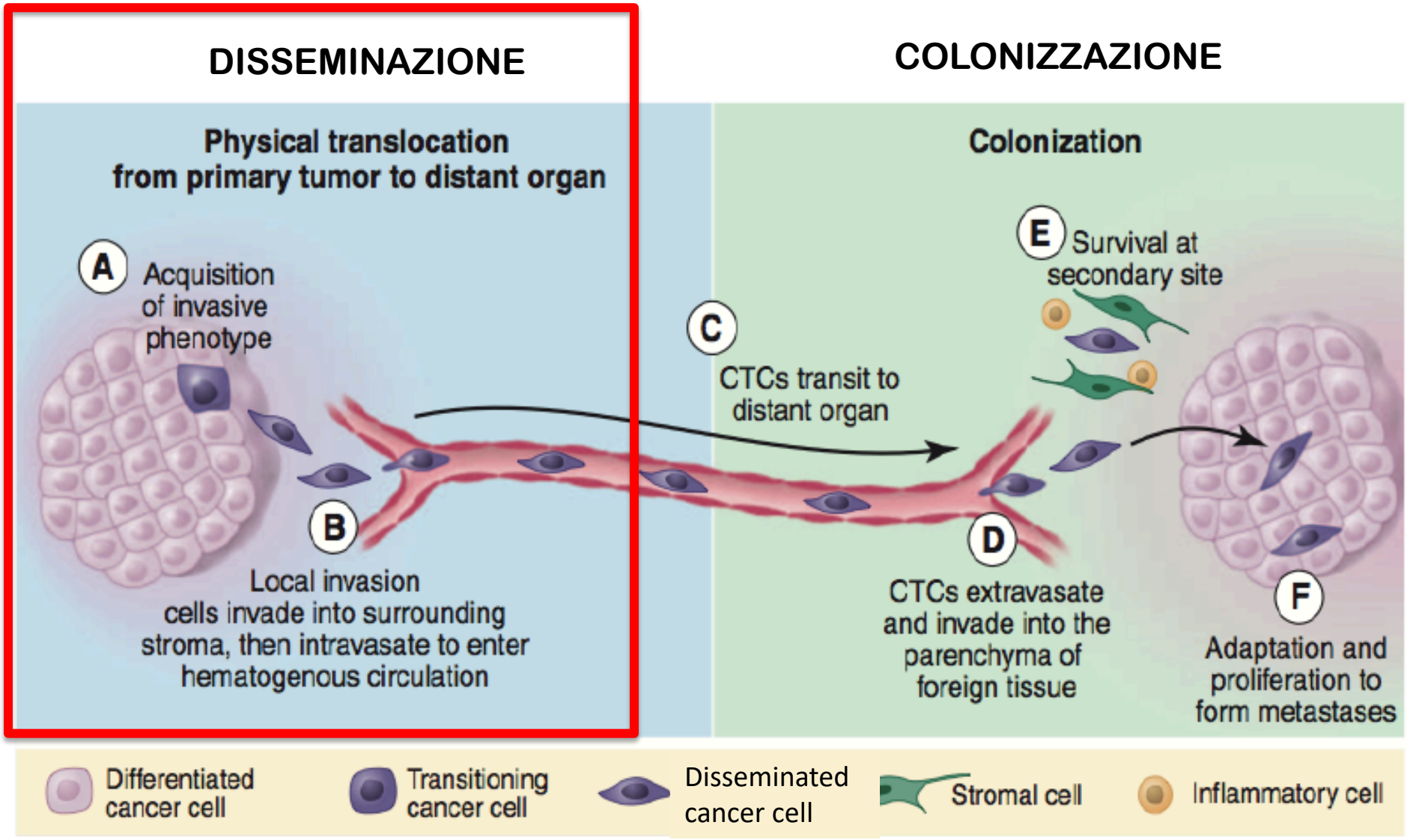
Corso di Biologia Cellulare del Cancro AA 2019-2020

La transizione epitelio-mesenchimale

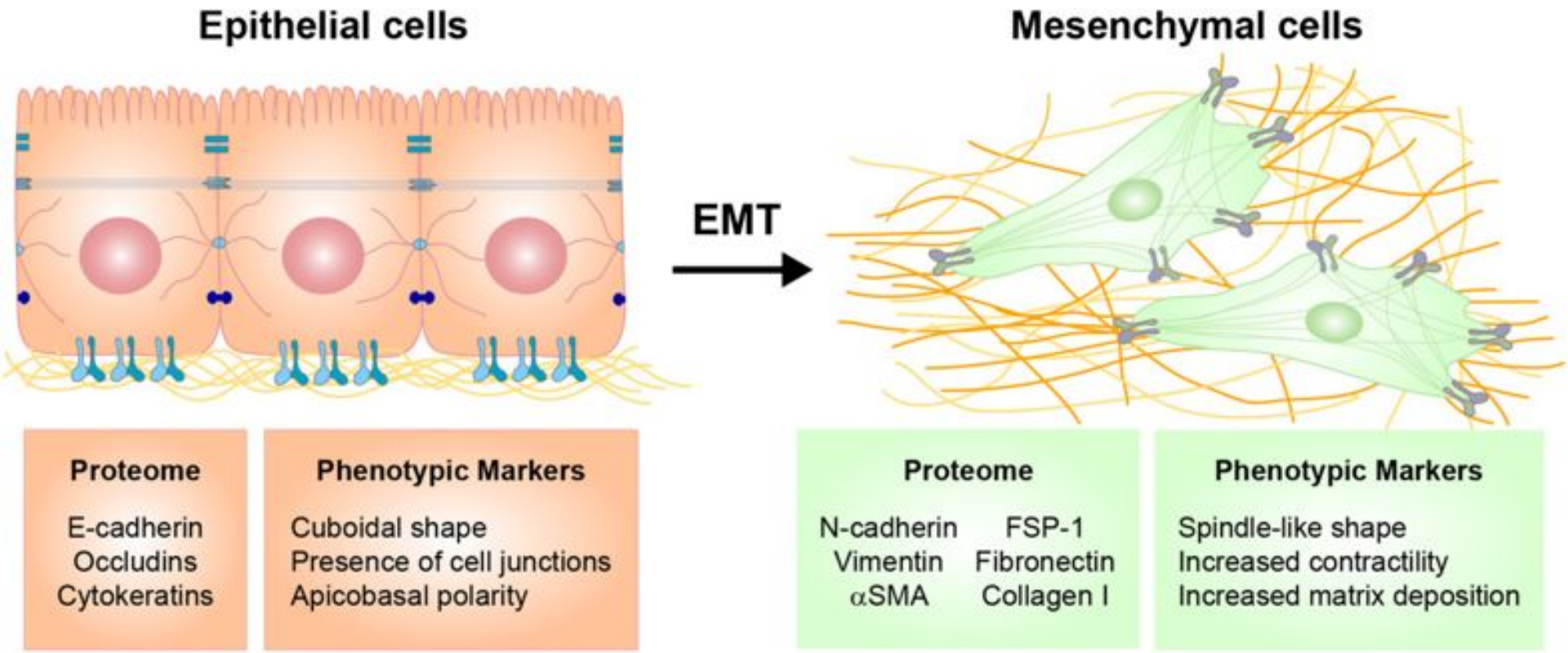
La cascata invasione-metastasi



EMT

MET

La TRANSIZIONE EPITELIO-MESENCHIMALE



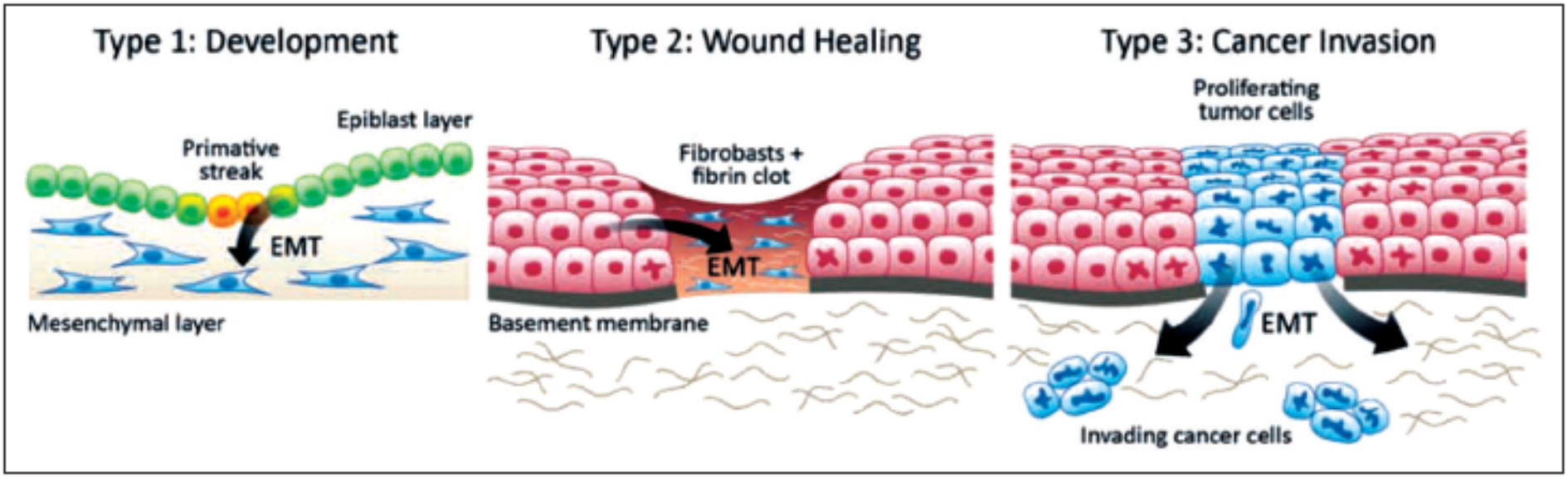
Ruoli fisiologici e patologici della EMT

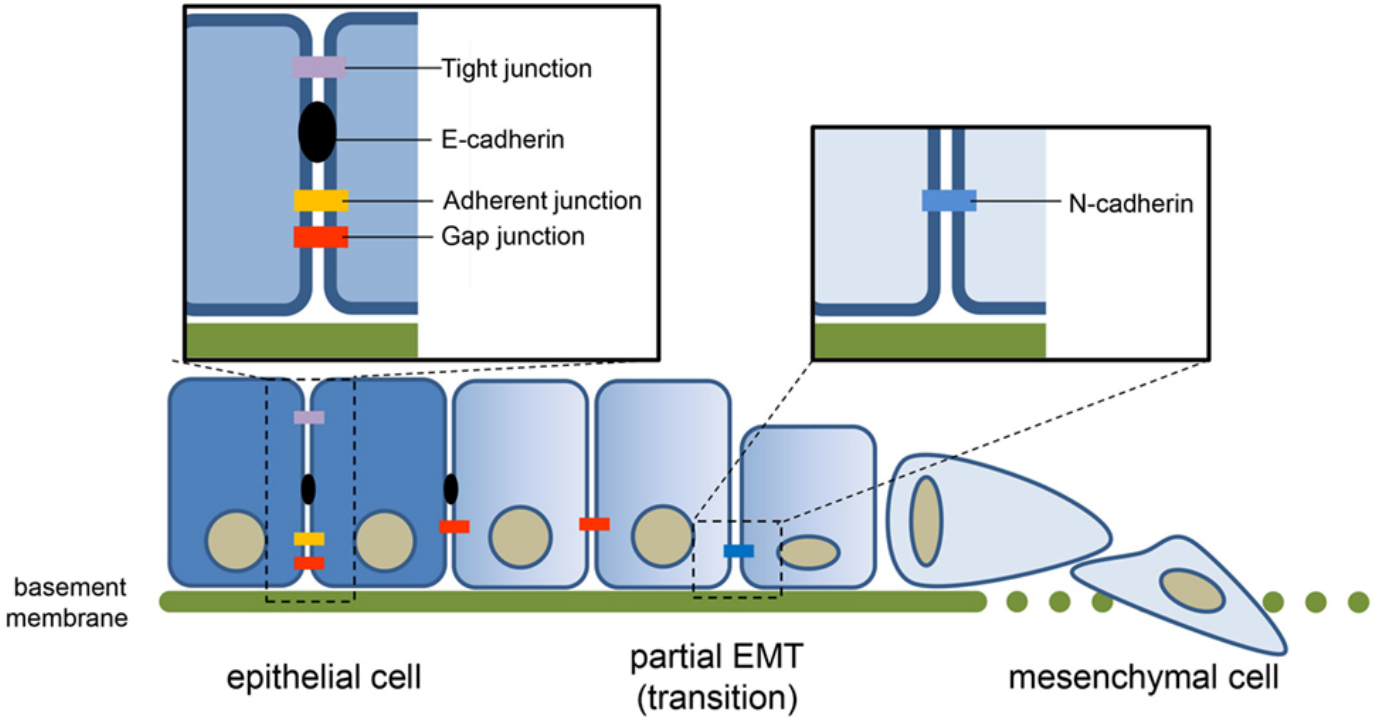
Embriogenesi

Riparazione delle ferite
(Wound healing)

Fibrosi

Cancro





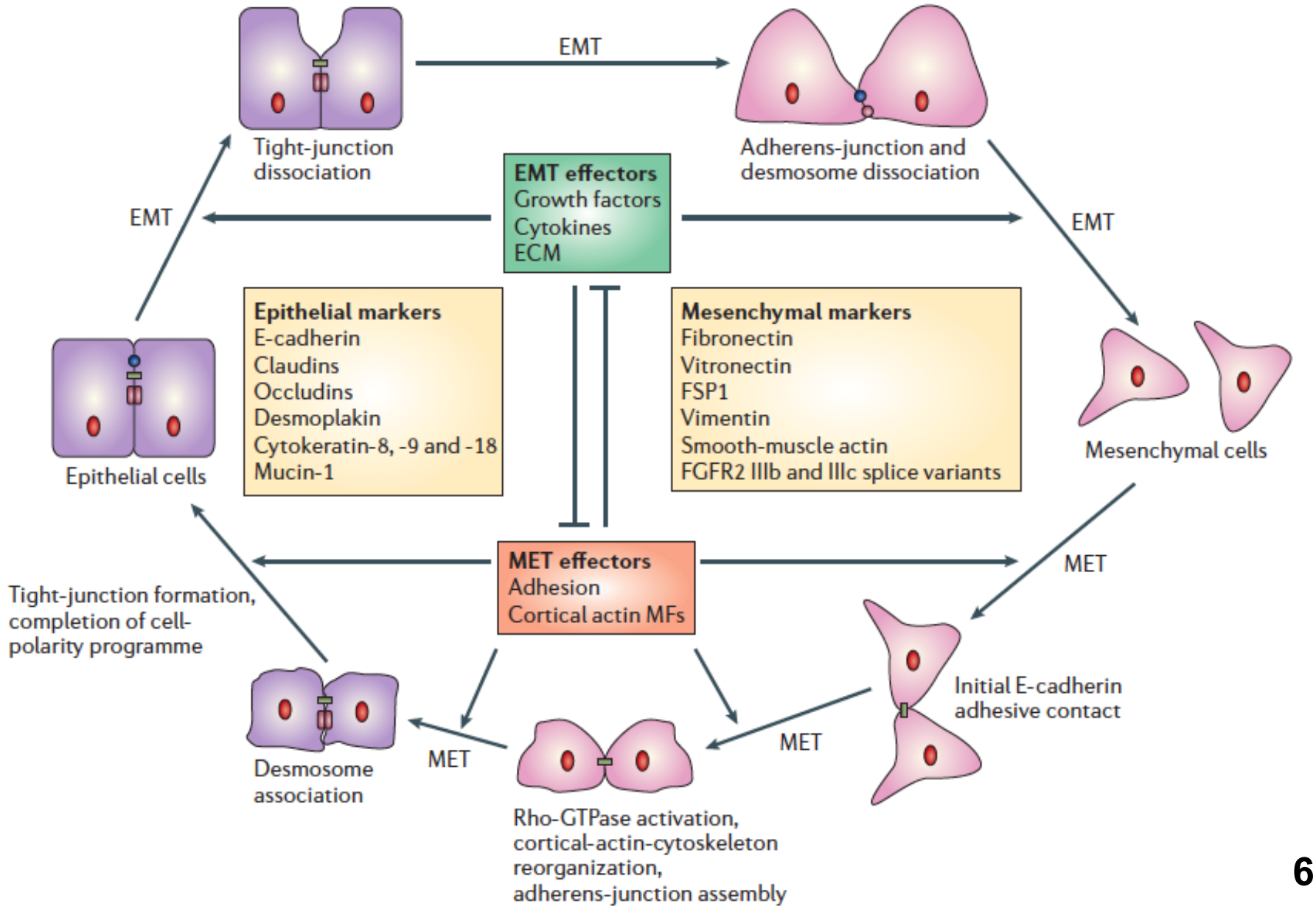
EMT inducers

epithelial to mesenchymal transition

Phenotype

Loss of differentiation Junctions dissociation Loss of apical-basal polarity Loss of epithelial markers (e.g. E-cadherin, claudins, cytokeratin) Increase of transcription factors (e.g. Snail, Slug, ZEB, Twist)	Cytoskeleton reorganization Migration Secretion of MMPs Basemenent membrane degradation Invasion Acquisition of mesenchymal markers (N-cadherin, vimentin, α-SMA) Increase of transcription factors (e.g. Slug)

SWITCH FENOTIPICO EMT/MET



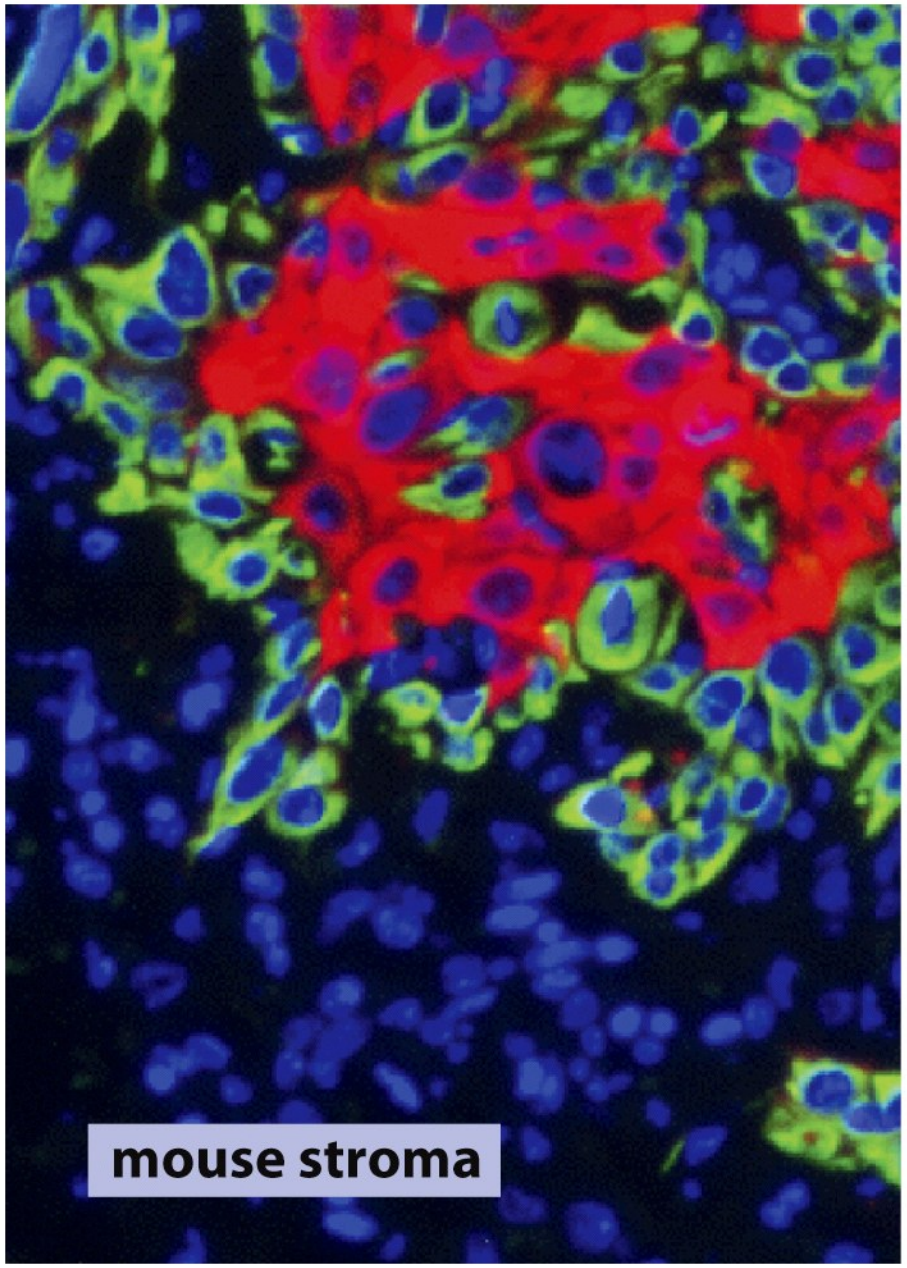


Figure 14.19c *The Biology of Cancer* (© Garland Science 2007)

Lo SWITCH delle caderine facilita l'invasione

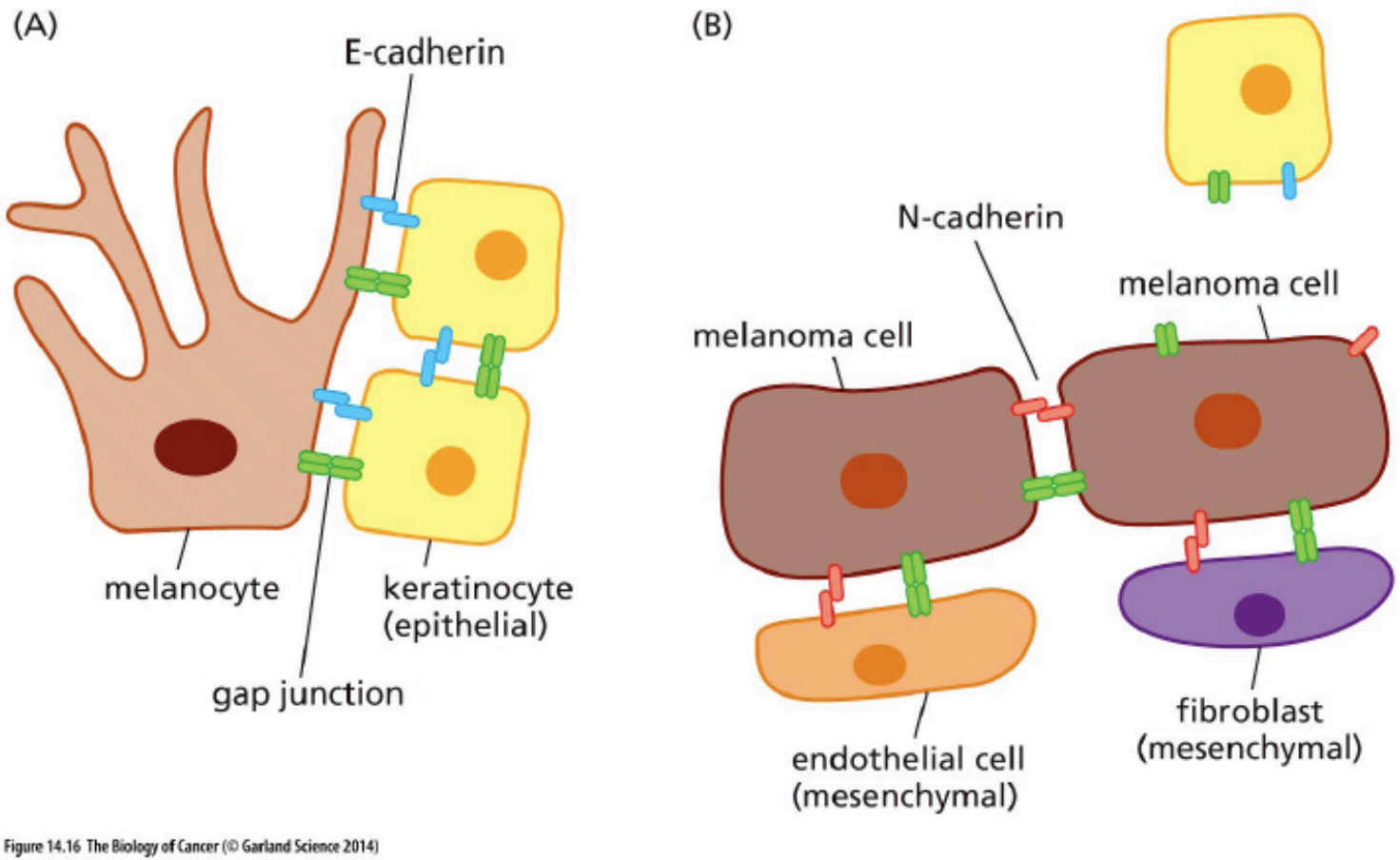
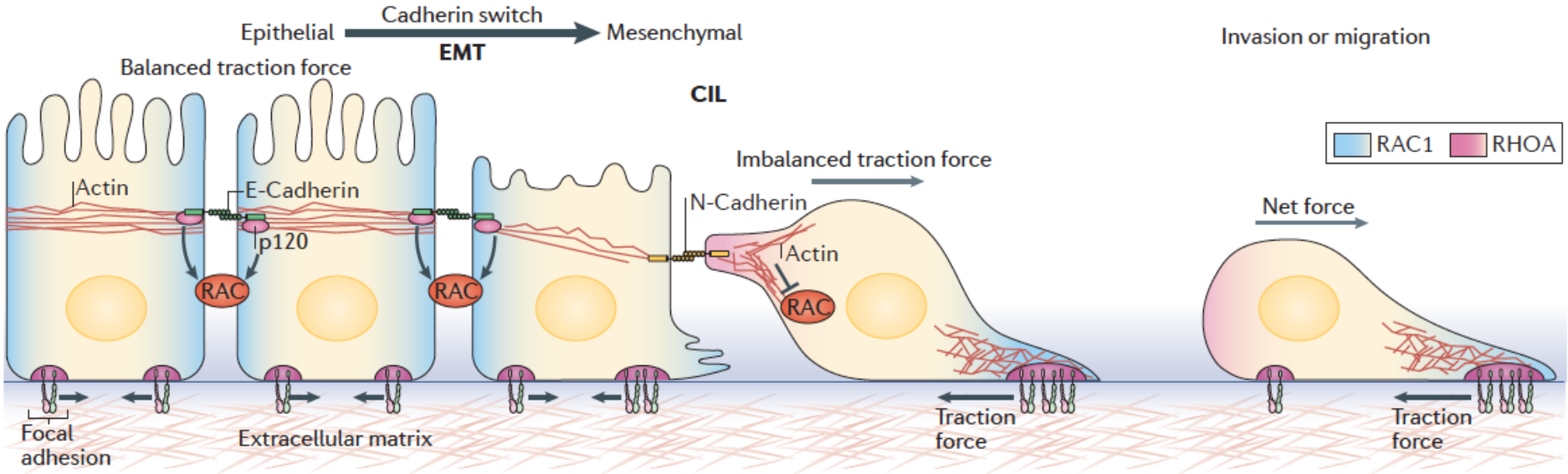


Figure 14.16 The Biology of Cancer (© Garland Science 2014)

When melanocytes become transformed in melanoma cells they shift from **E-Cadherin to N- Cadherin** thus extricating from keratinocytes and making more interactions via N-cadherin to stromal cells facilitating cell migration and invasion.

Lo switch delle caderine facilita la perdita di polarità e rimuove l'inibizione della migrazione

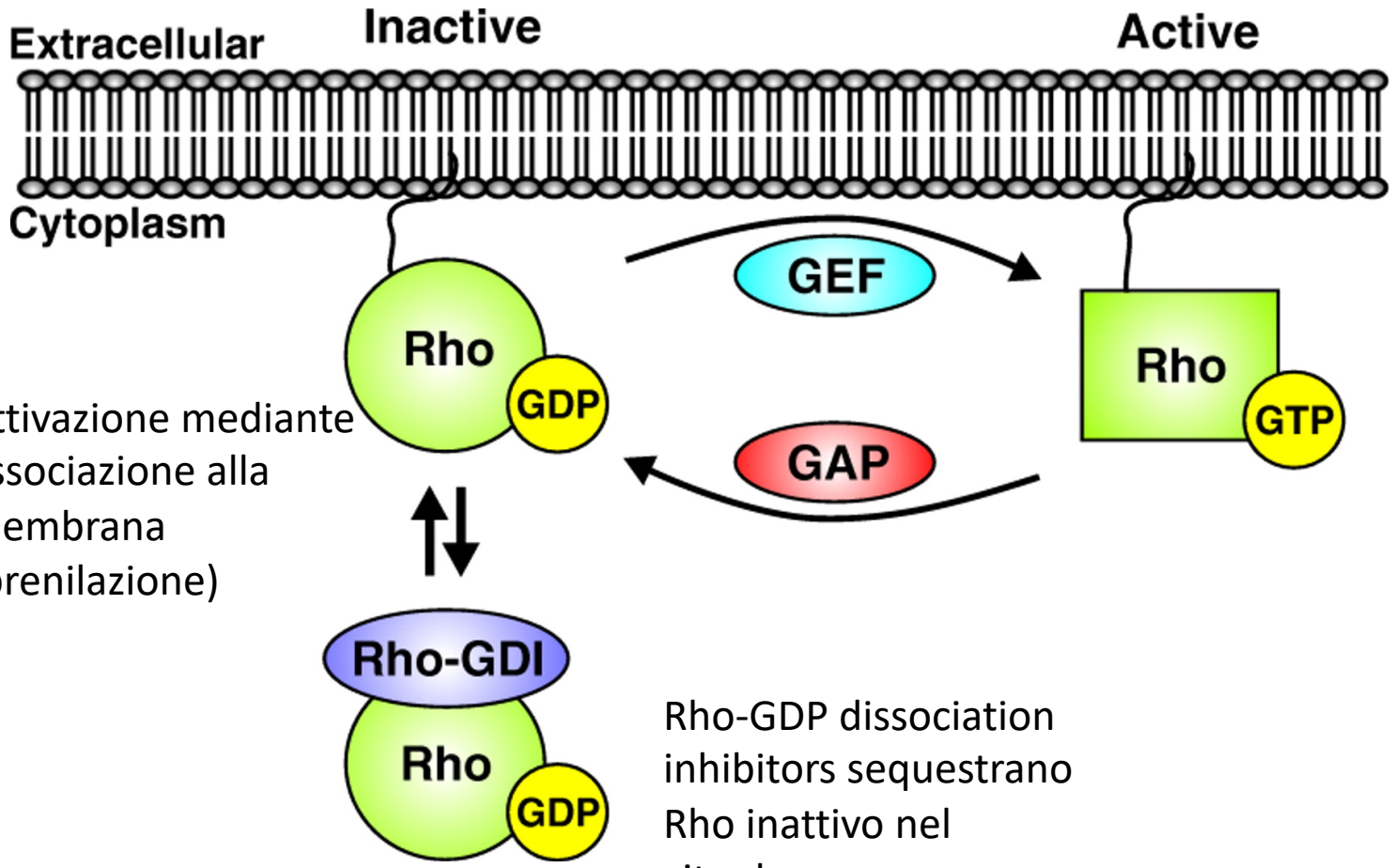


E-Cadherin suppresses EMT by signalling to other adhesion components, such as p120 catenin, which polarizes the small GTPase **RAC1 towards cell–cell junctions.**

N-cadherin expression promotes polarization of **RAC1 activity towards the leading edge** of cells to generate asymmetric traction stress.

Stramer et al., Nature Revies on Cancer 2016

IL CICLO DI ATTIVAZIONE DELLE GTPasi della famiglia di RHO

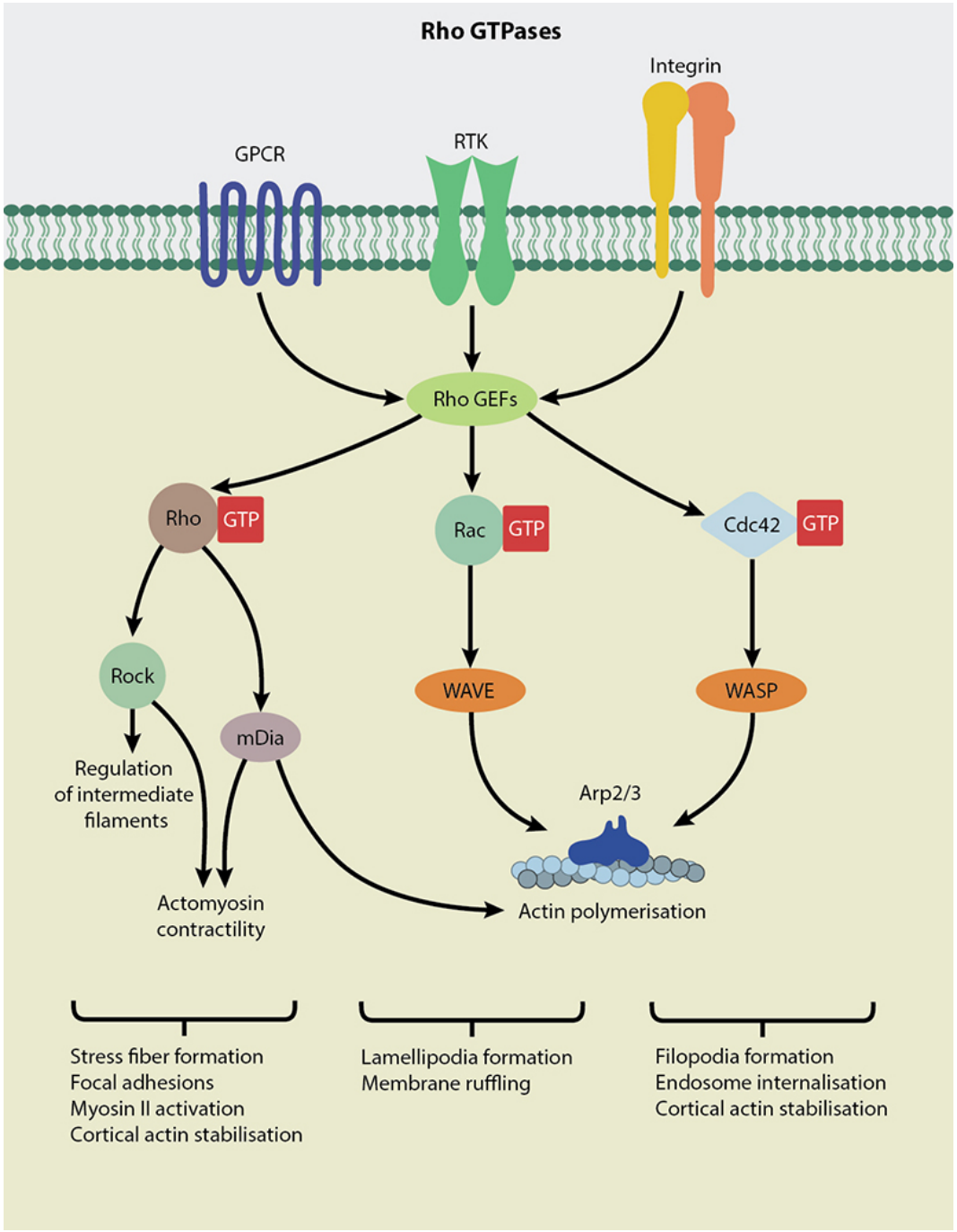


Attivazione mediante associazione alla membrana (prenilazione)

Rho-GDP dissociation inhibitors sequestrano Rho inattivo nel citoplasma

Stephan Huveneers, and Erik H. J. Danen J Cell Sci 2009;122:1059-1069

Journal of Cell Science


Rho GTPases
Integrin
GPCR
RTK
Rho GEFs
Rho
GTP
Rac
GTP
Cdc42
GTP
Rock
mDia
WAVE
WASP
Regulation of intermediate filaments
Actomyosin contractility
Arp2/3
Actin polymerisation
Stress fiber formation
Focal adhesions
Myosin II activation
Cortical actin stabilisation
Lamellipodia formation
Membrane ruffling
Filopodia formation
Endosome internalisation
Cortical actin stabilisation

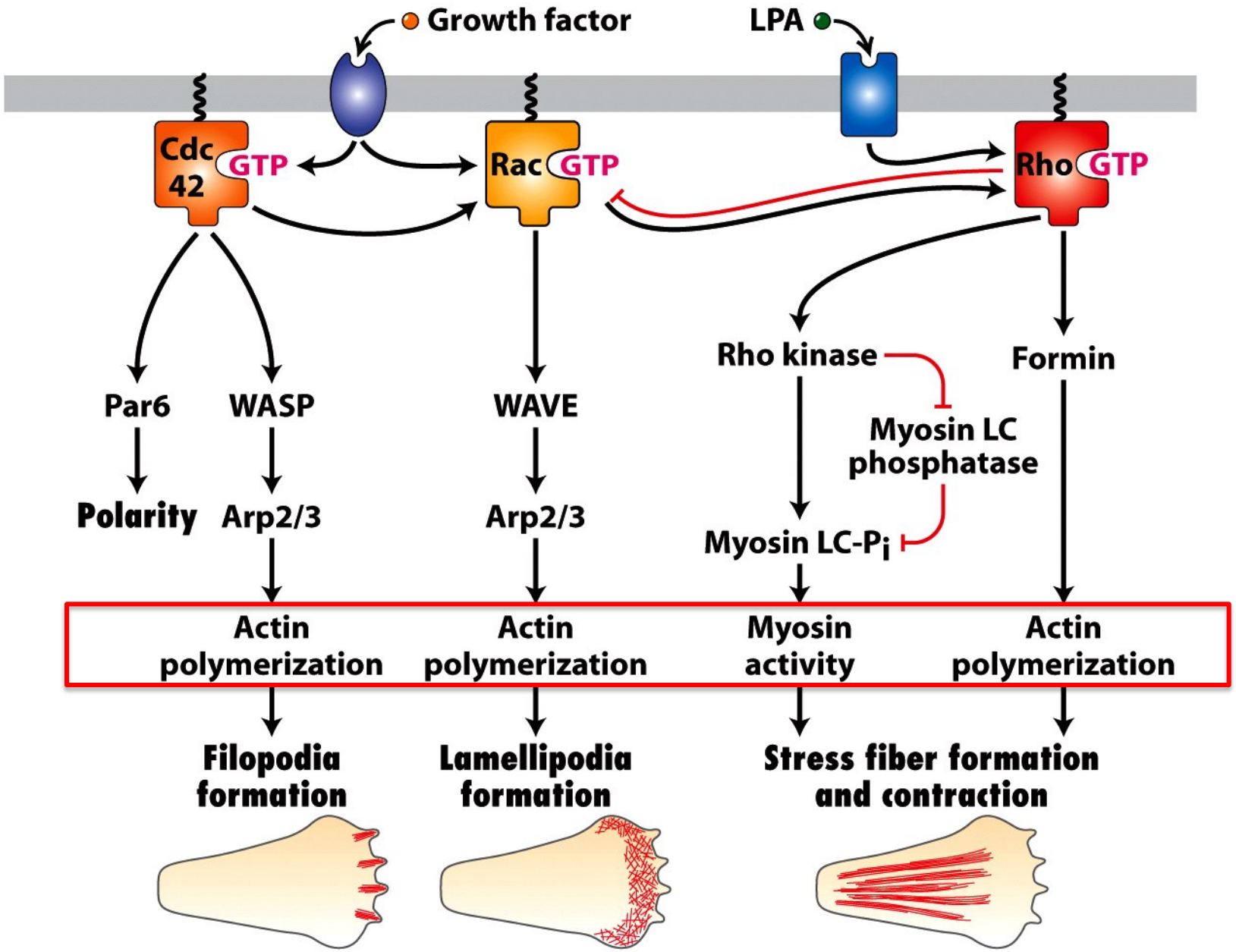
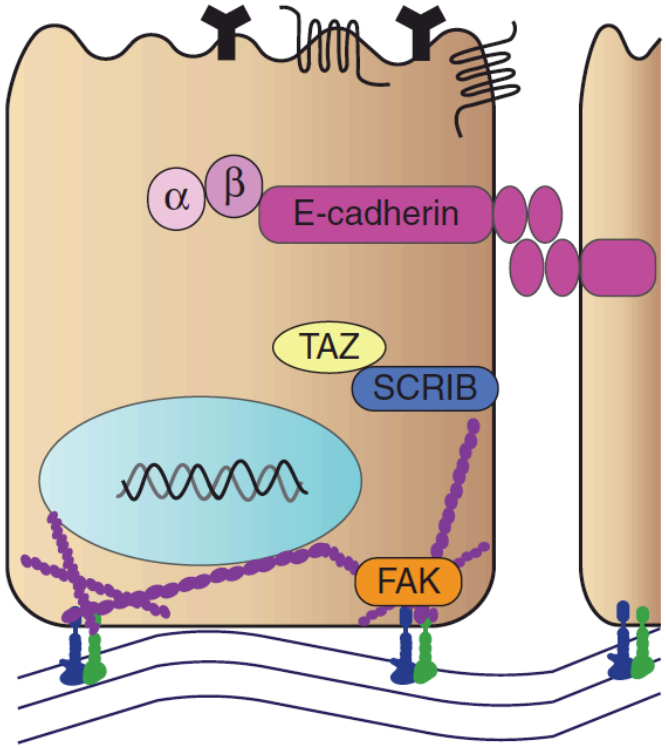


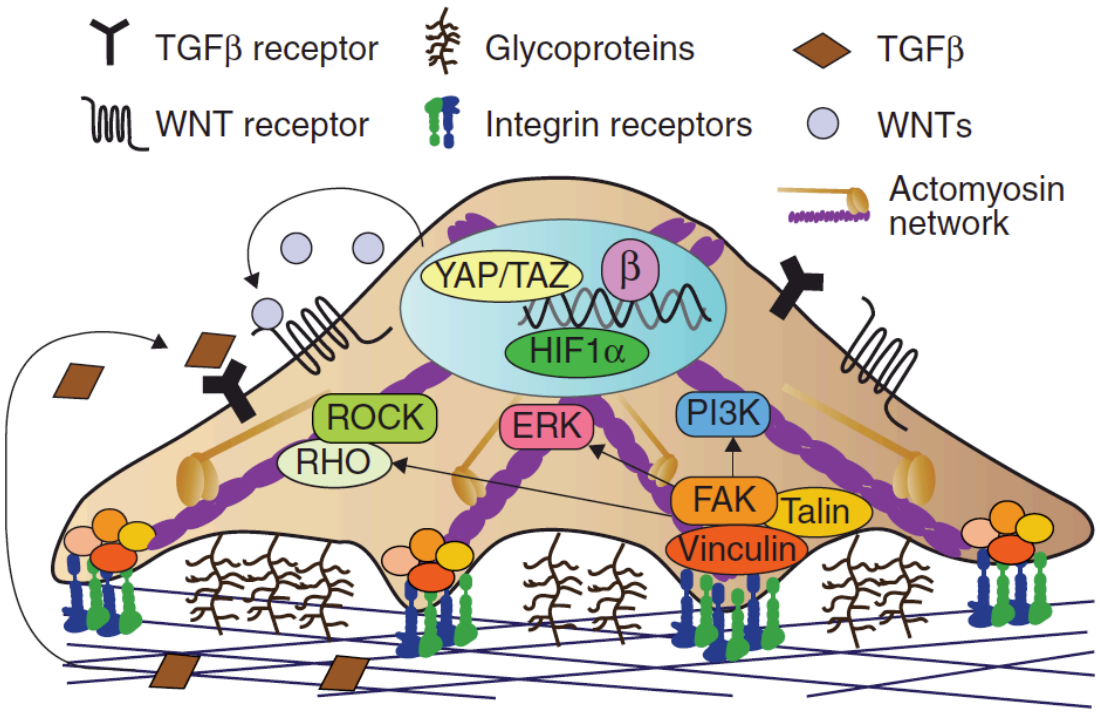
Figure 17-42
Molecular Cell Biology, Sixth Edition

invadopodia

Il signalling delle integrine attiva Rho

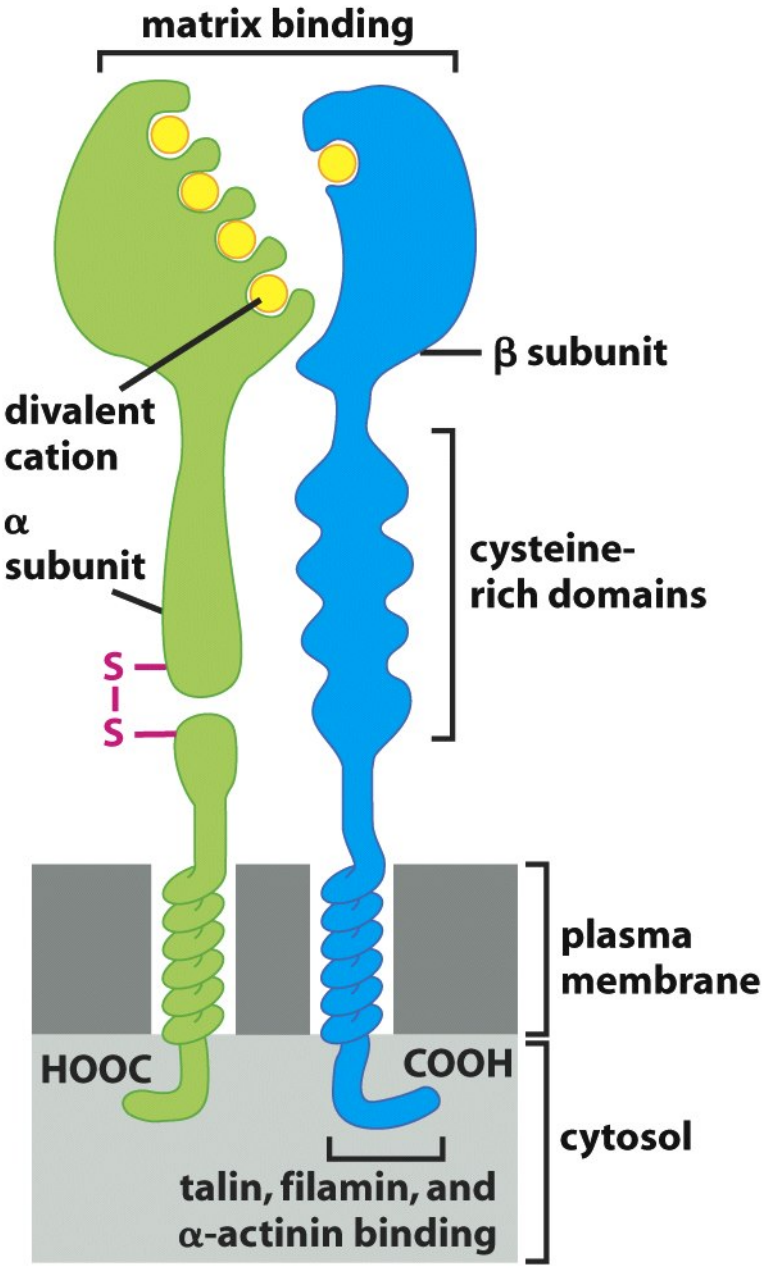


Low tension

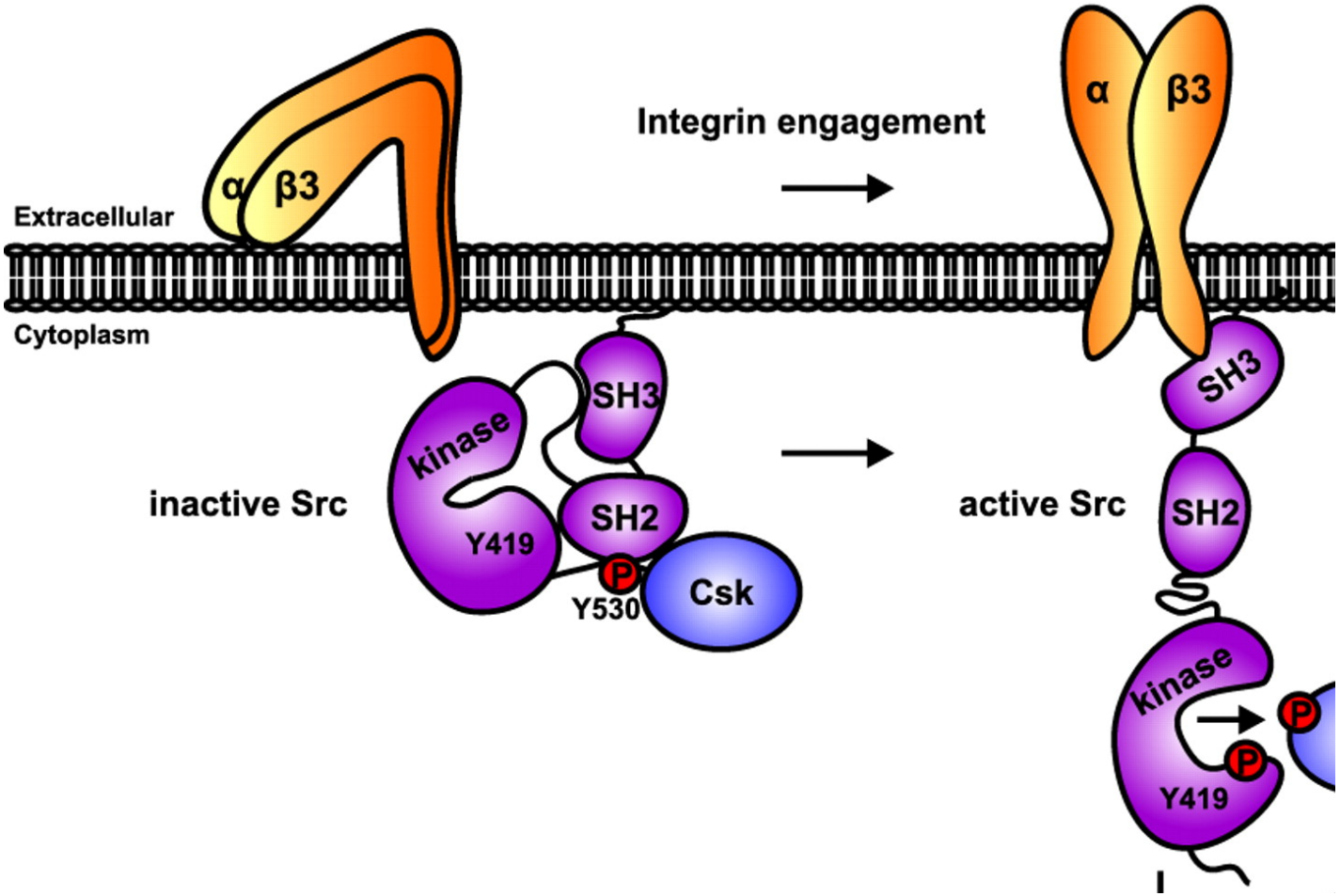


High tension

Le integrine mediano le interazioni cellula-ECM

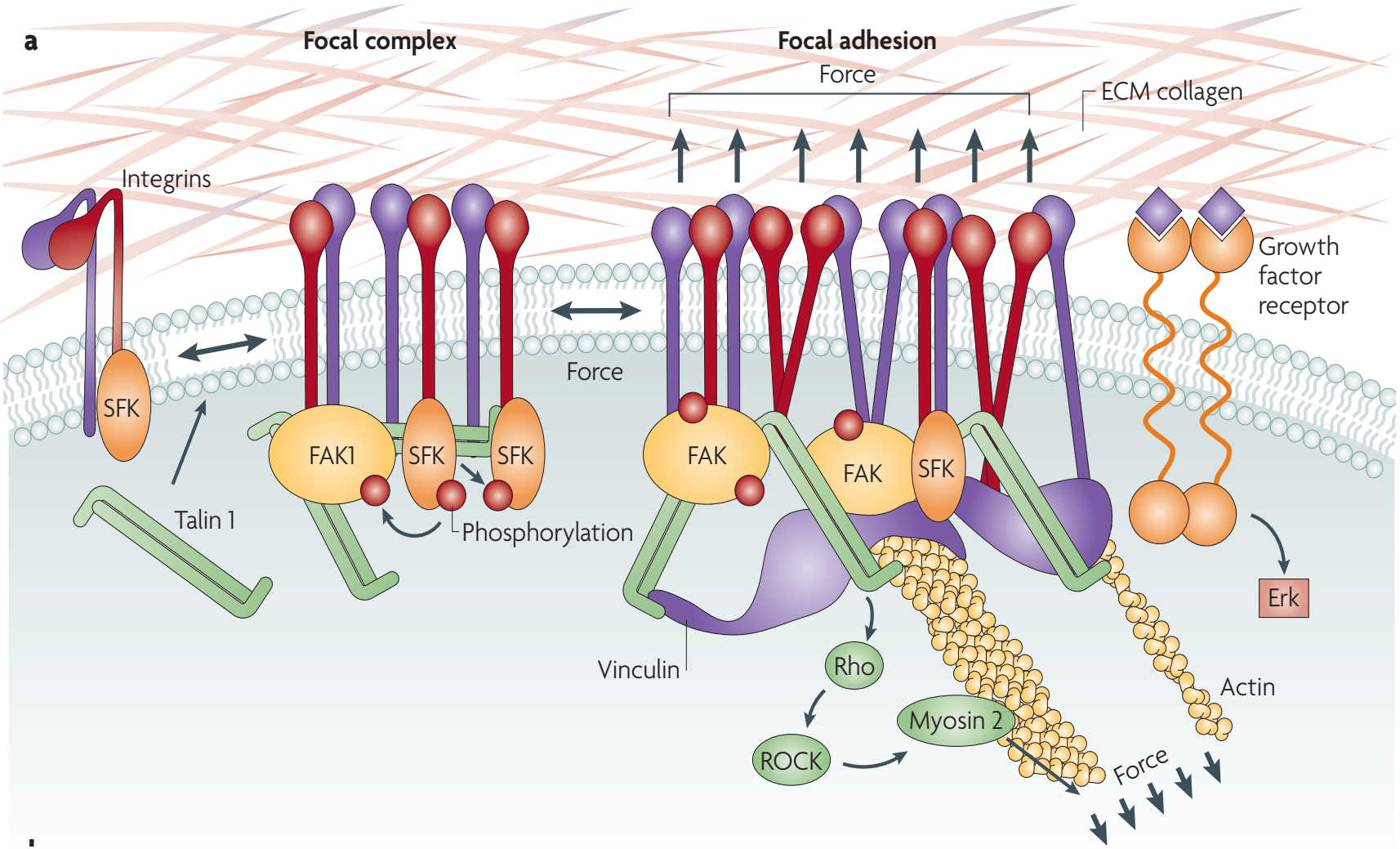


II Attivazione della chinasi Src



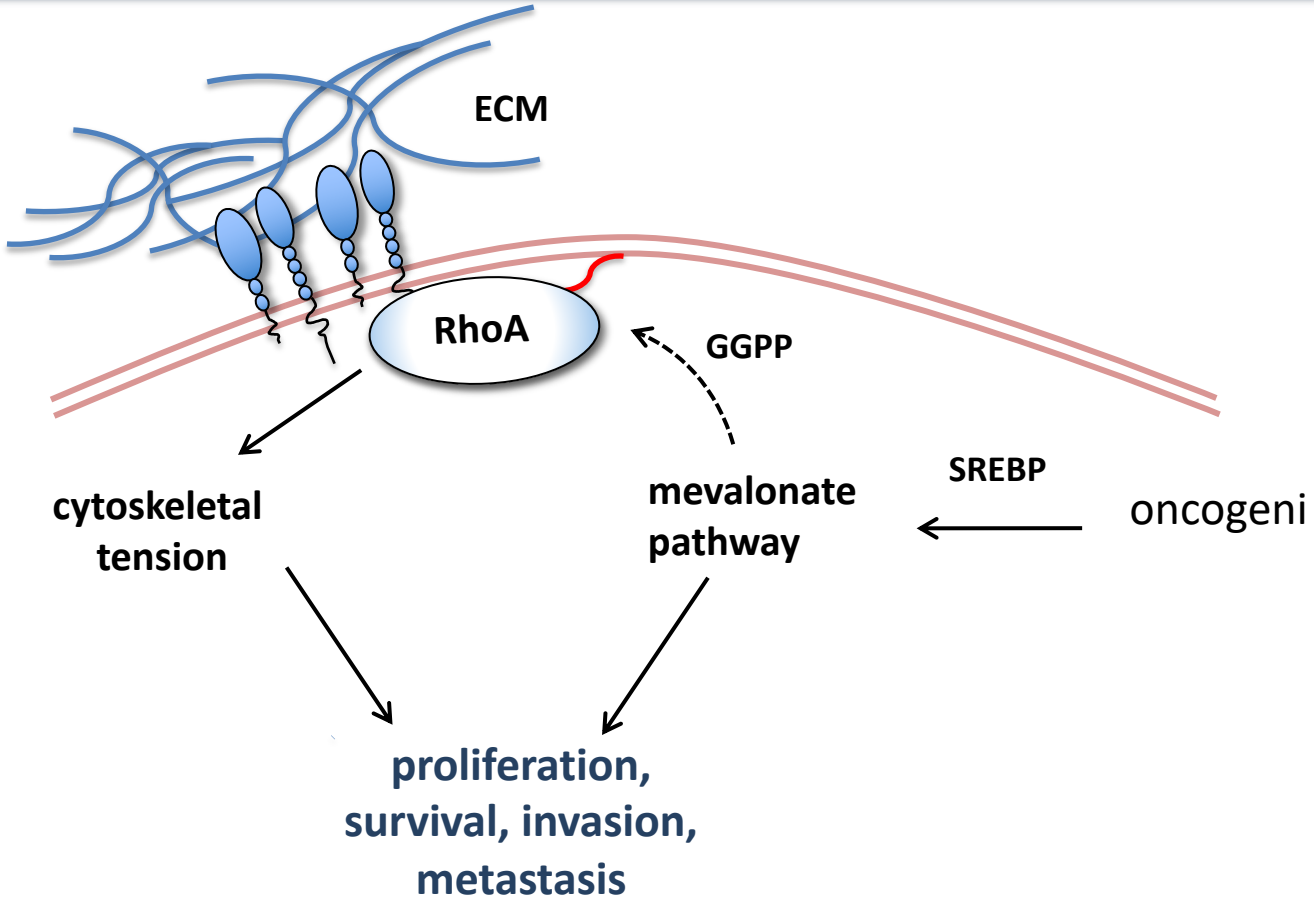
Stephan Huveneers, and Erik H. J. Danen J Cell Sci 2009;122:1059-1069

Adesioni focali: attivazione del signaling delle integrine.

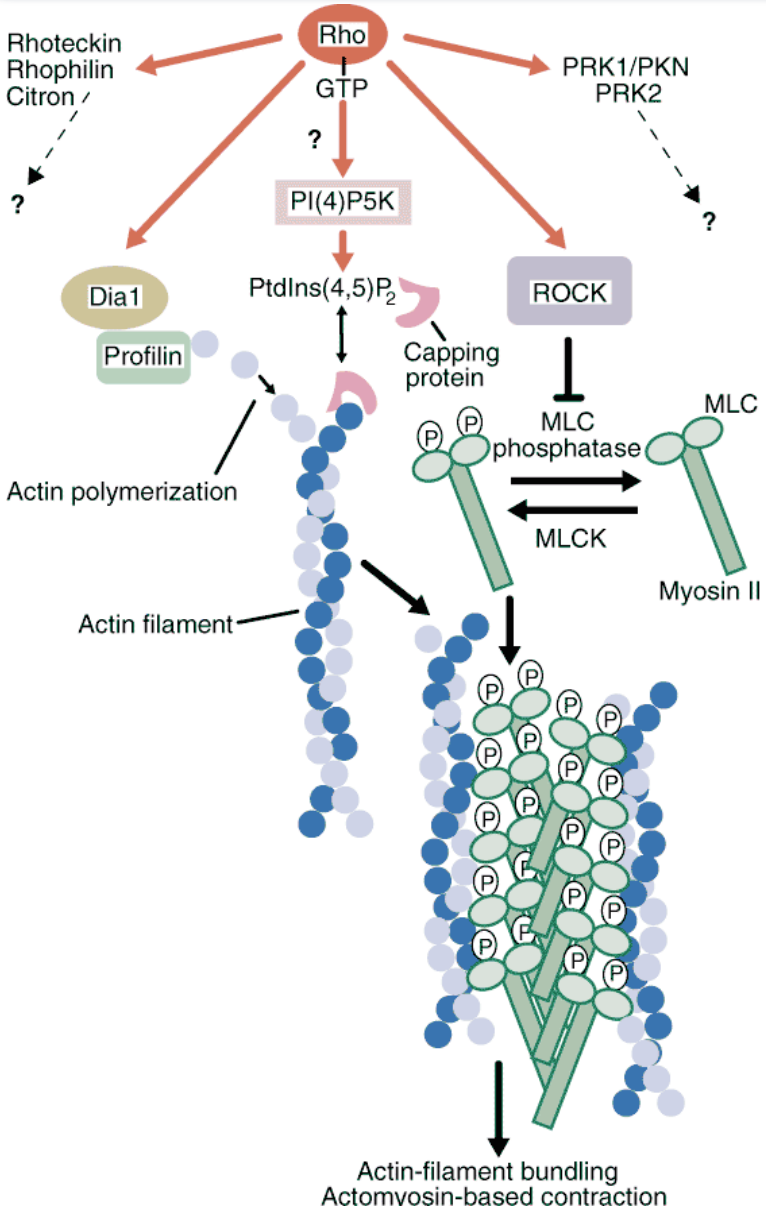


Impatto su: sopravvivenza, proliferazione e progressione tumorale

La via del mevalonato potenzia le attività tumorigeniche di RhoA favorendo la sua localizzazione alle adesioni focali via GGPP



Le GTPasi Rho e la dinamica del citoscheletro actomiosinico



La GTPasi Rho induce polimerizzazione della F actina e contrattilità actomiosinica

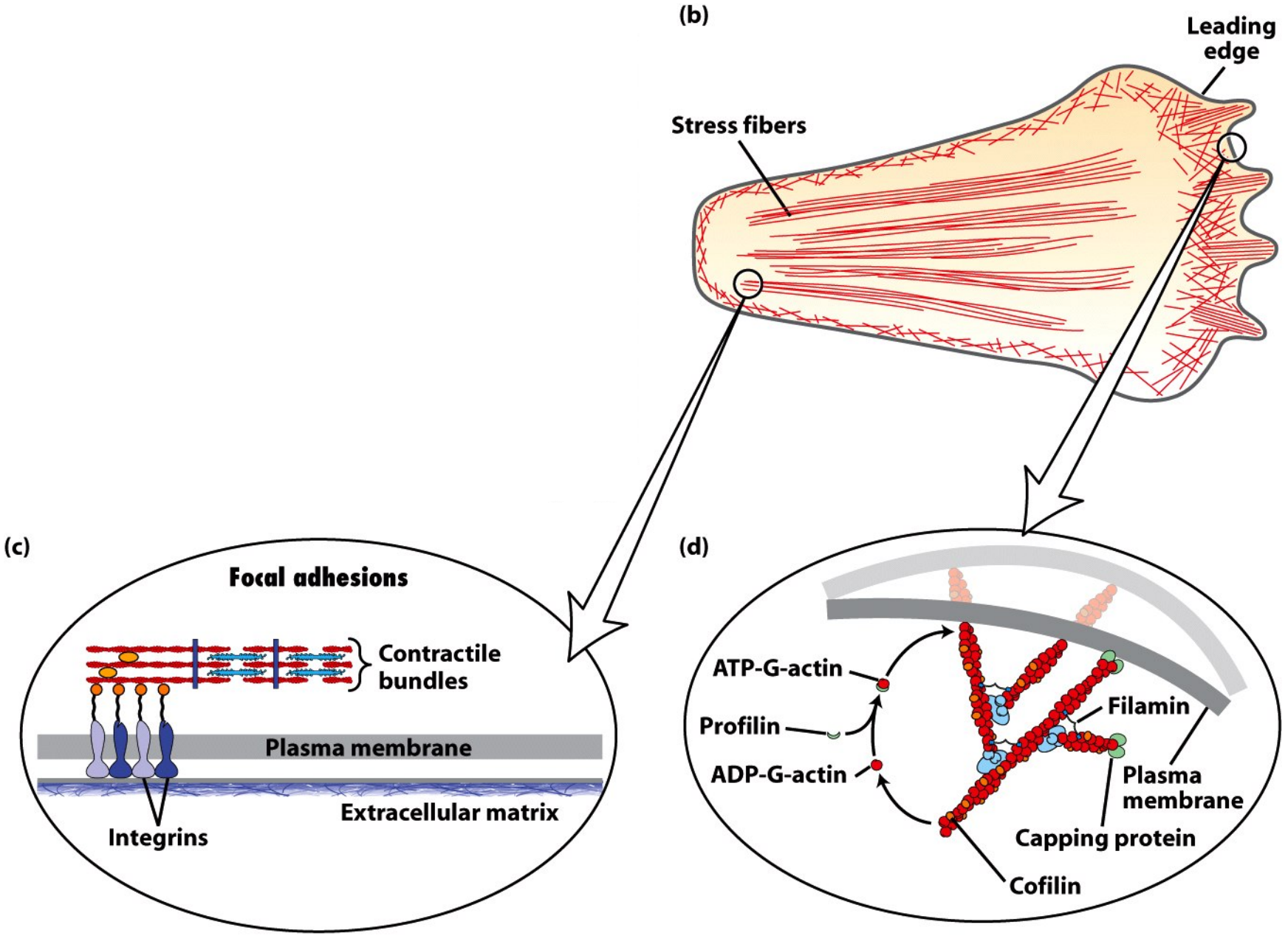
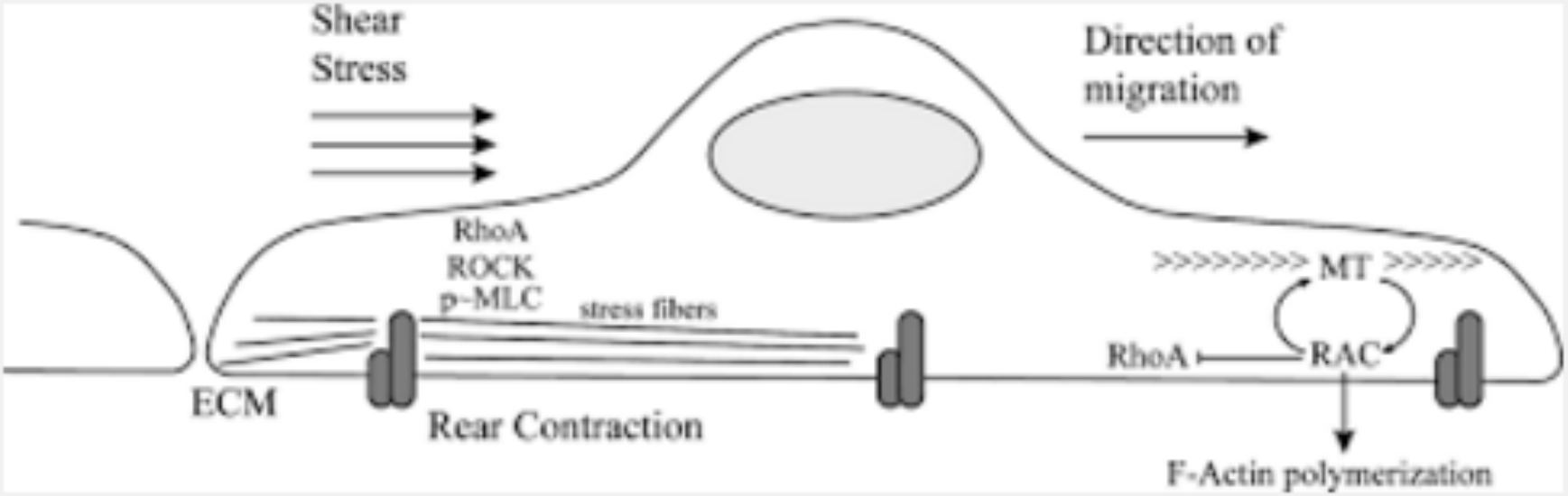


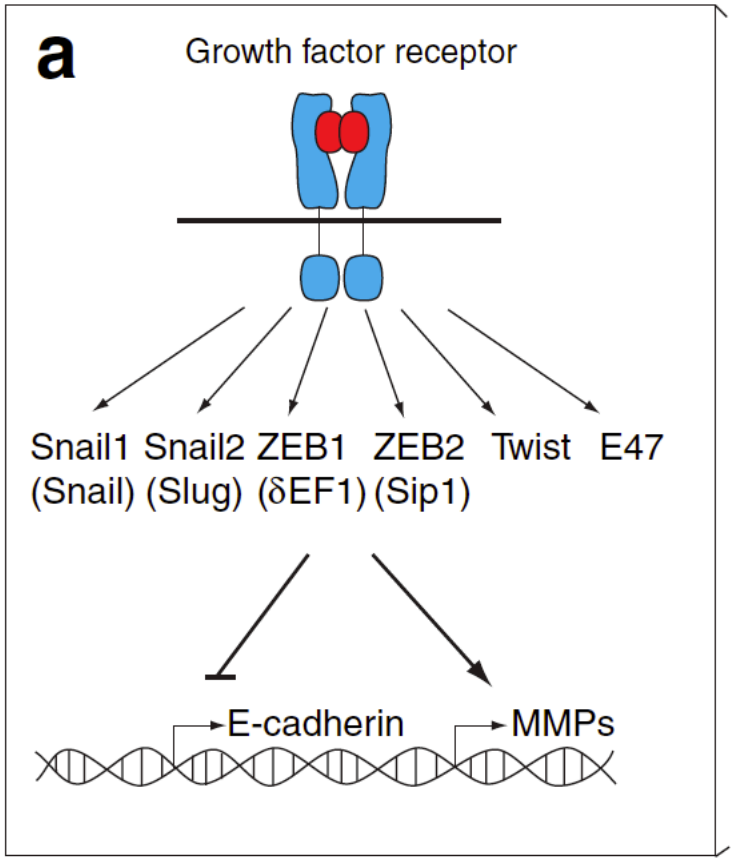
Figure 17-39
Molecular Cell Biology, Sixth Edition

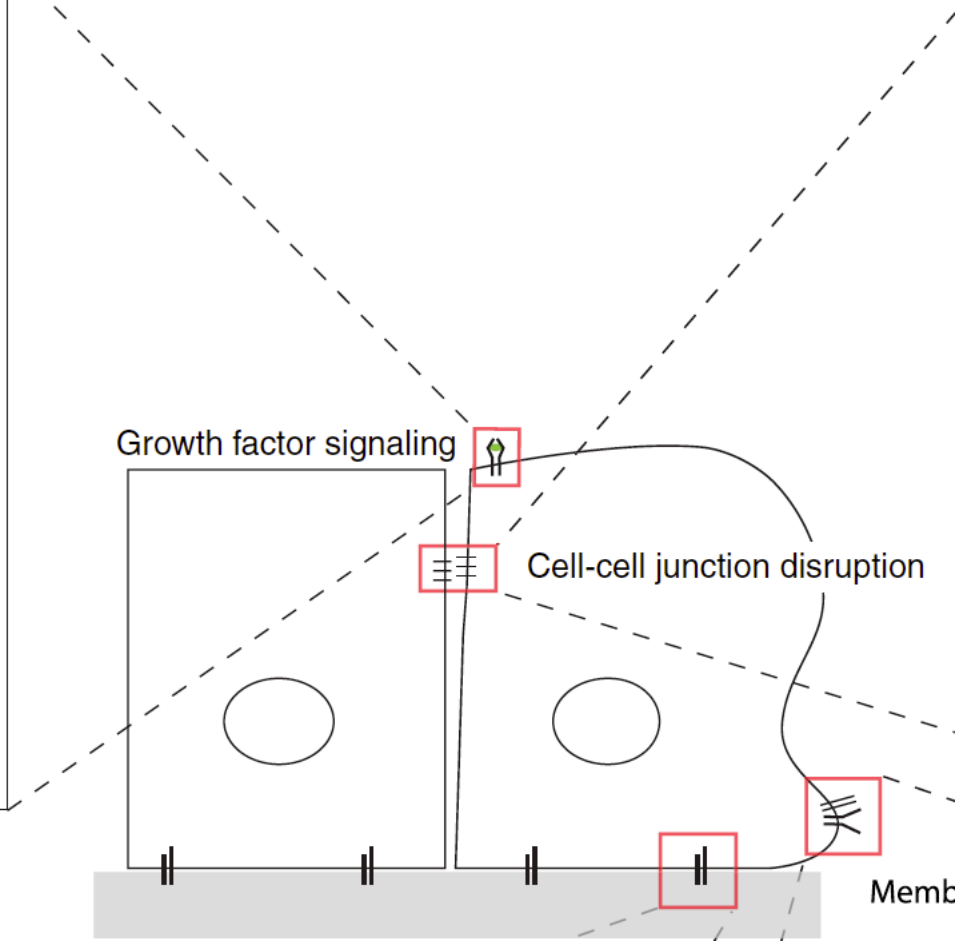


Le GTPasi Rho, Rac e cdc42 e controllano la organizzazione dell'actina e delle adesioni focali.

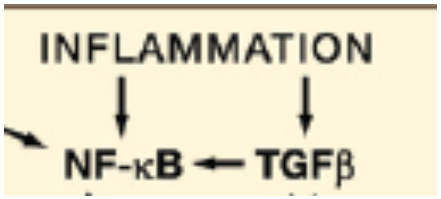
La migrazione dipende da **attivazione localizzata di qs proteine in piccoli, limitati domini di membrana**.

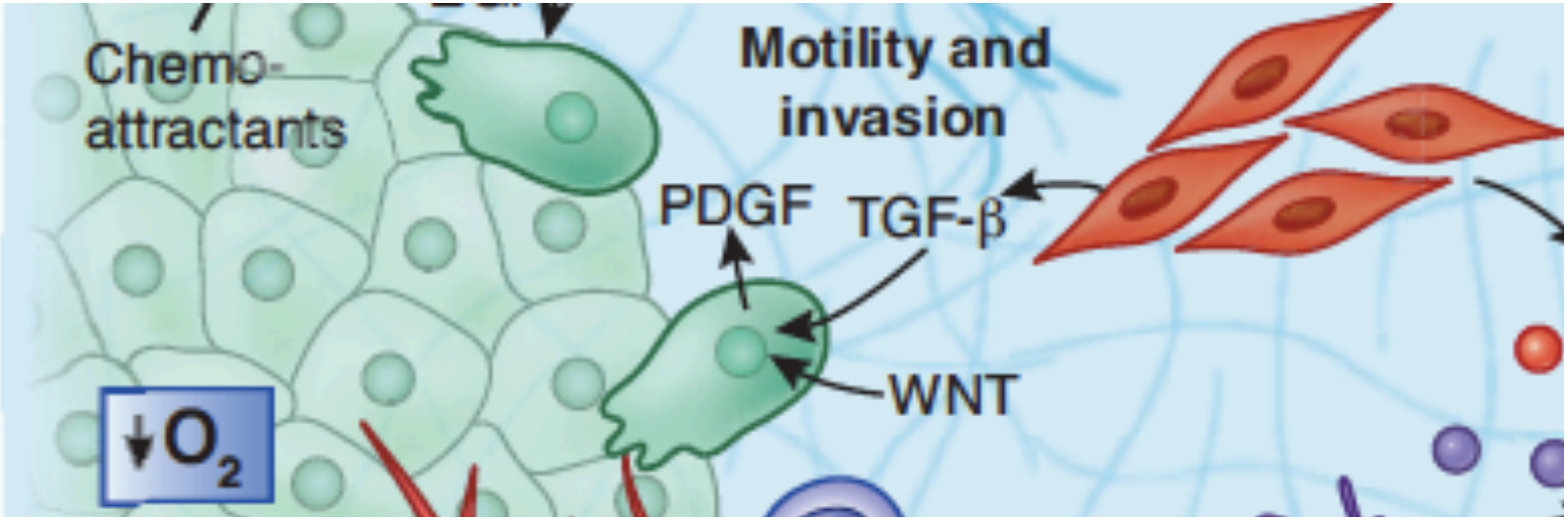
Gli effettori del programma genico della EMT: TWIST SNAIL e ZEB:

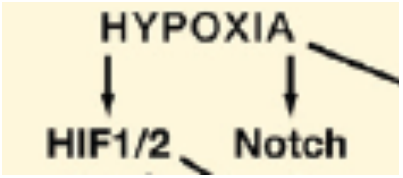




ORIGINE DEI SEGNALI DI EMT







Stroma(CAFs)
TFGβ e WNT

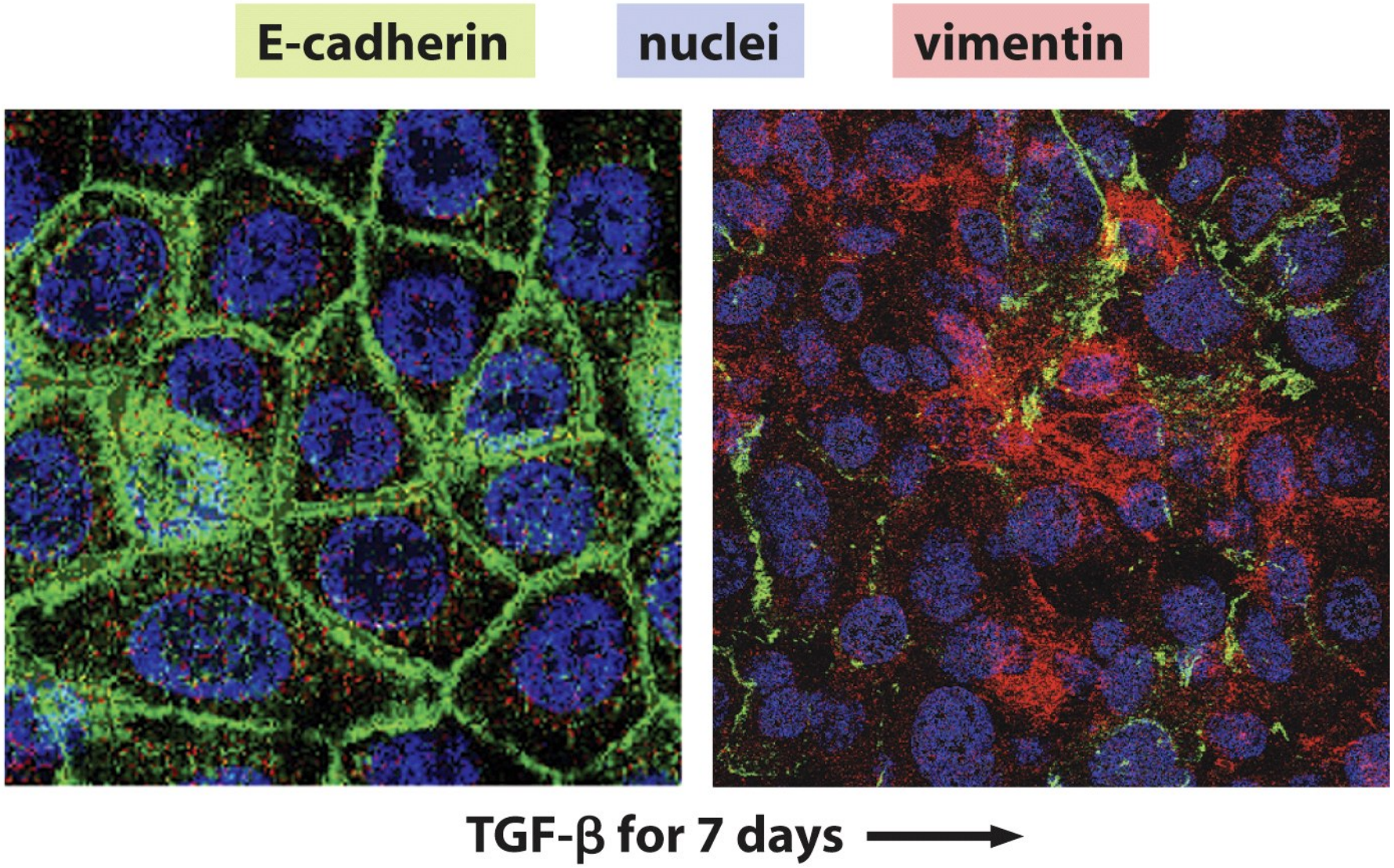
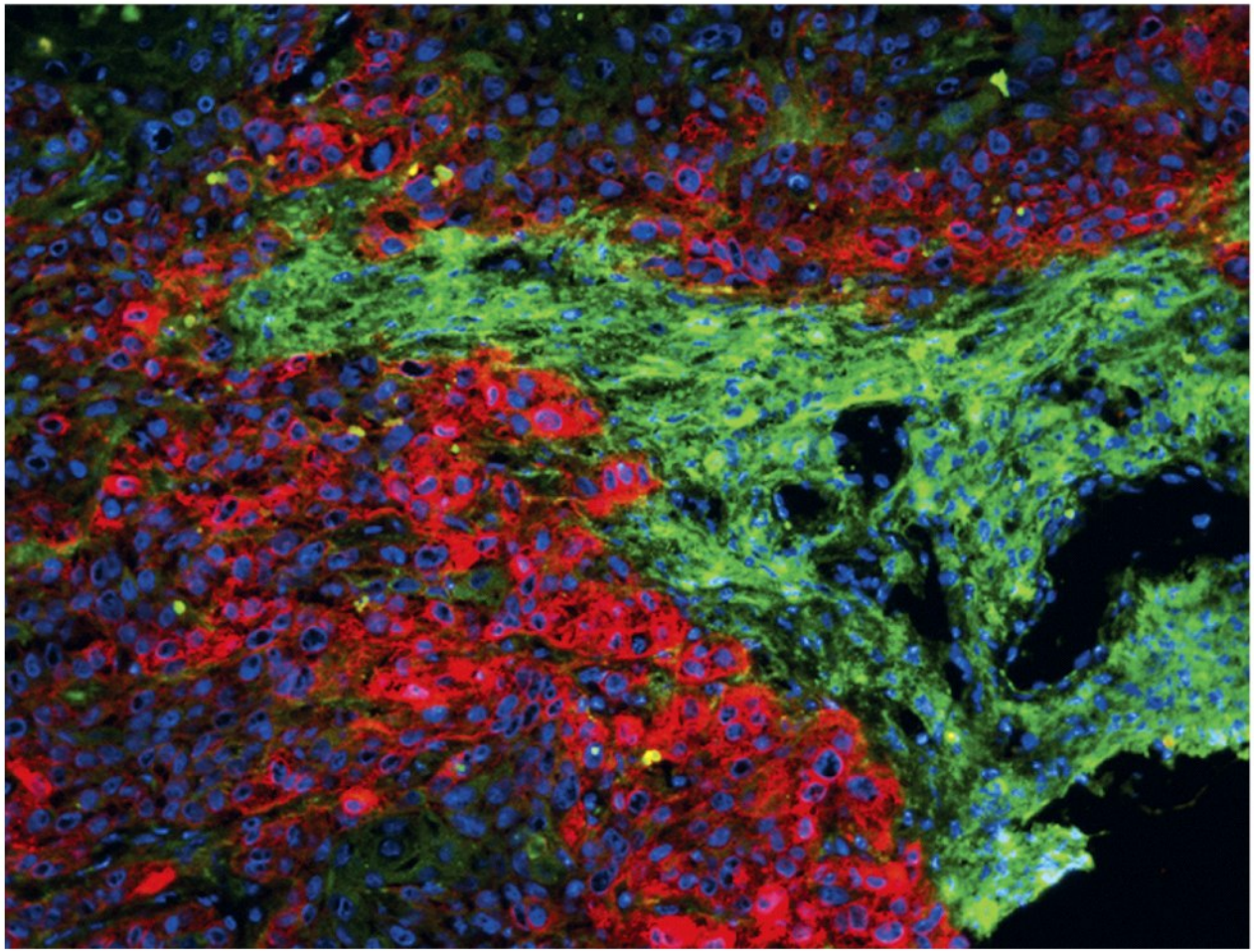


Figure 14.20a *The Biology of Cancer* (© Garland Science 2007)

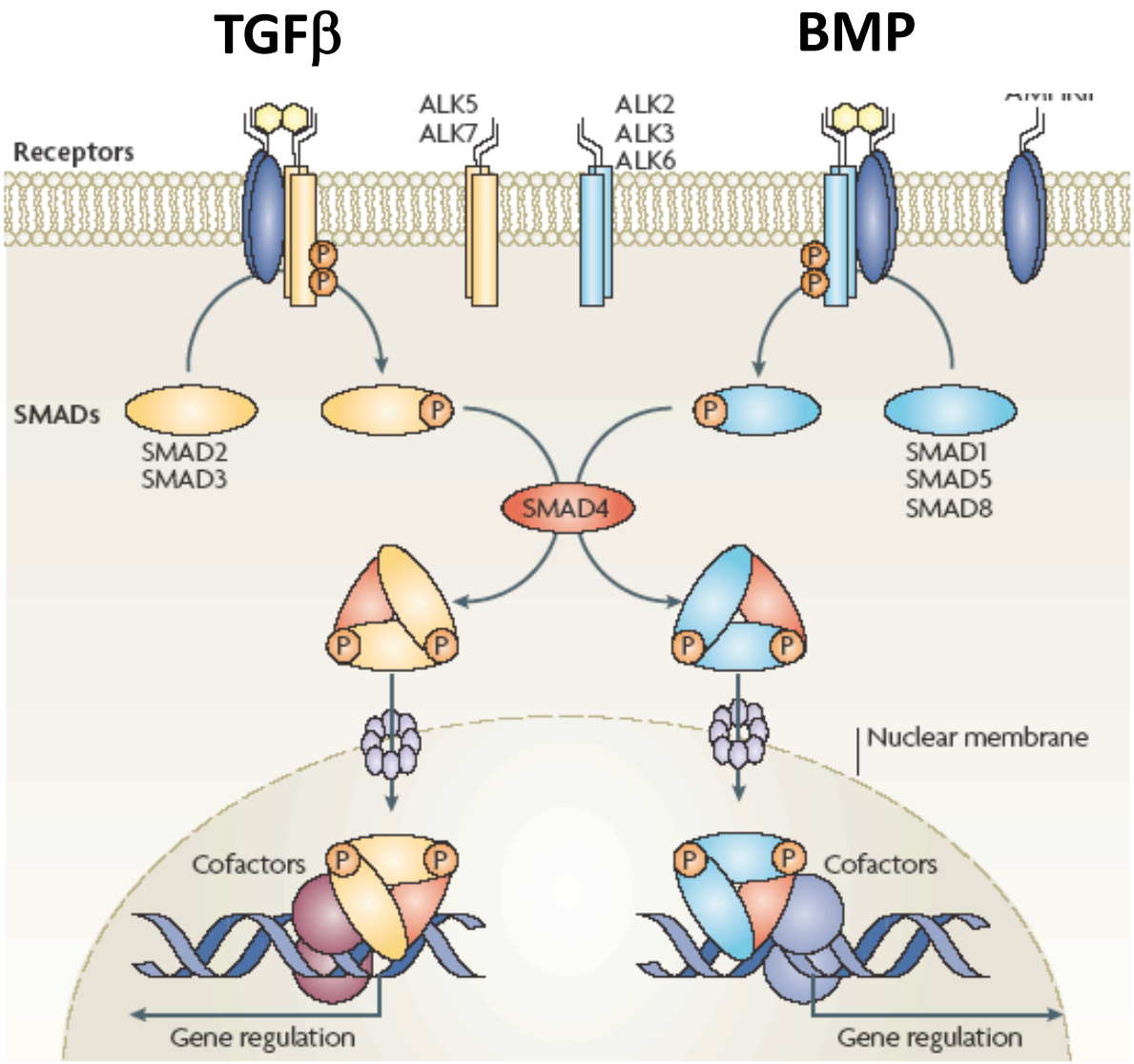
TGF-β (stromal cells)



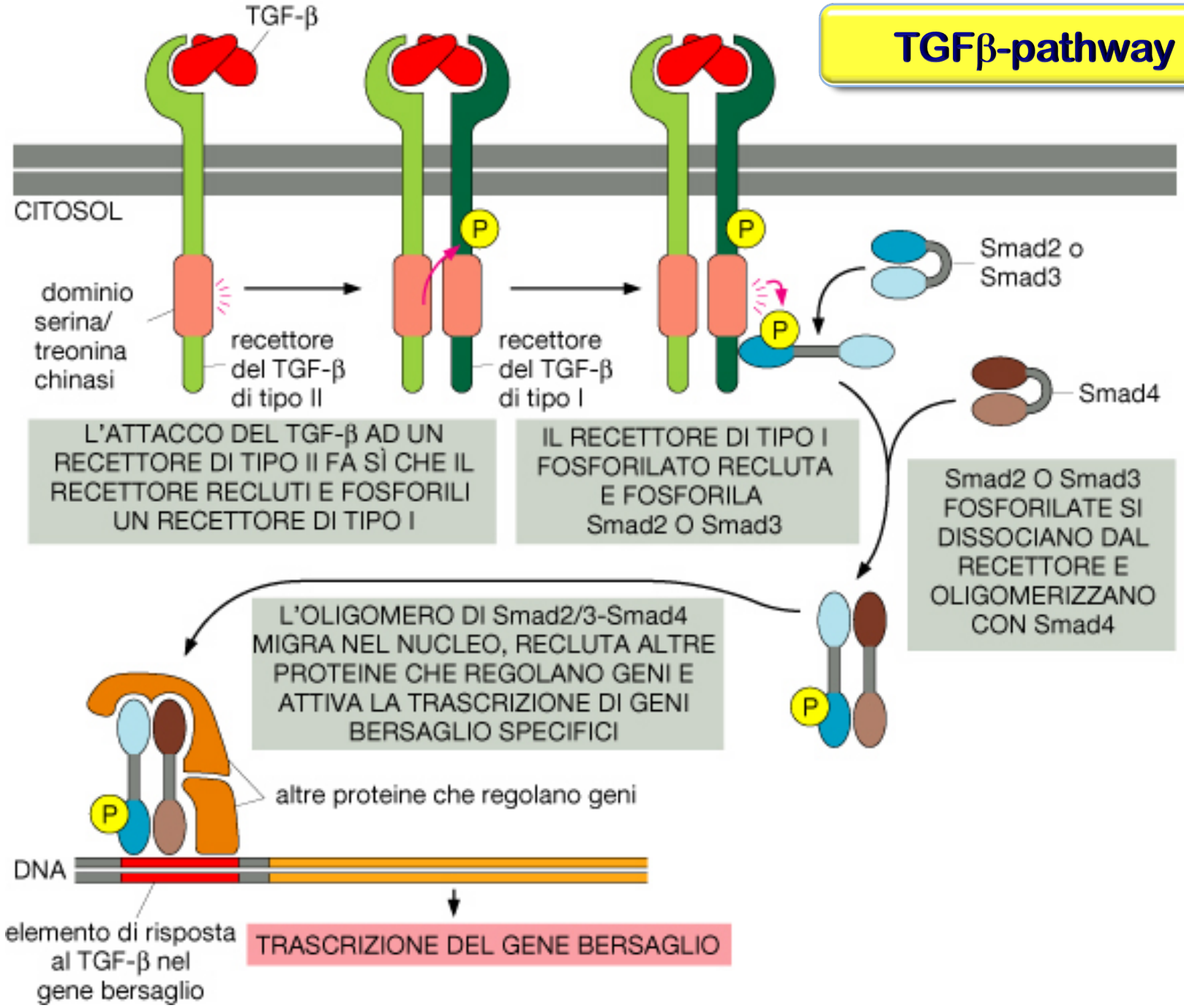
$\alpha_v\beta_6$ integrin (epithelial cells)

Figure 14.20e *The Biology of Cancer* (© Garland Science 2007)

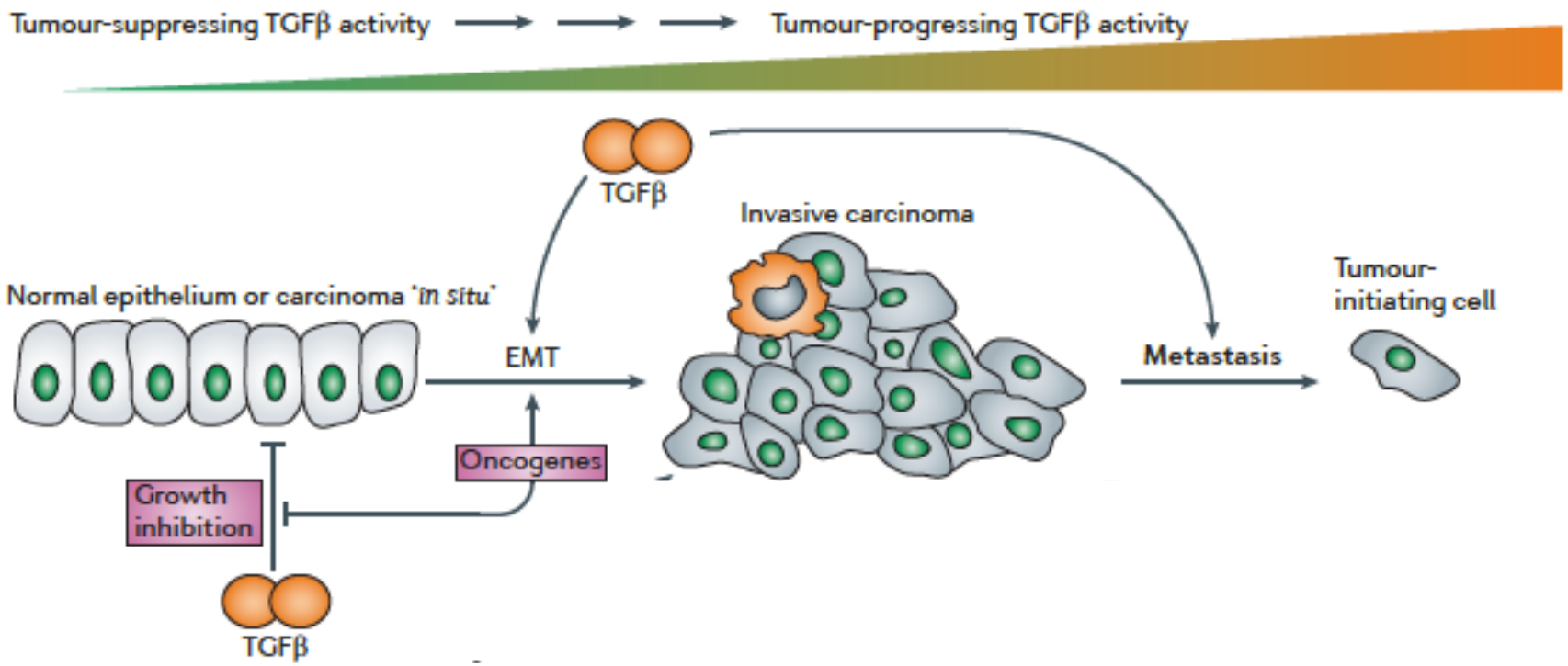
La pathway di TGFβ



TGFβ-pathway

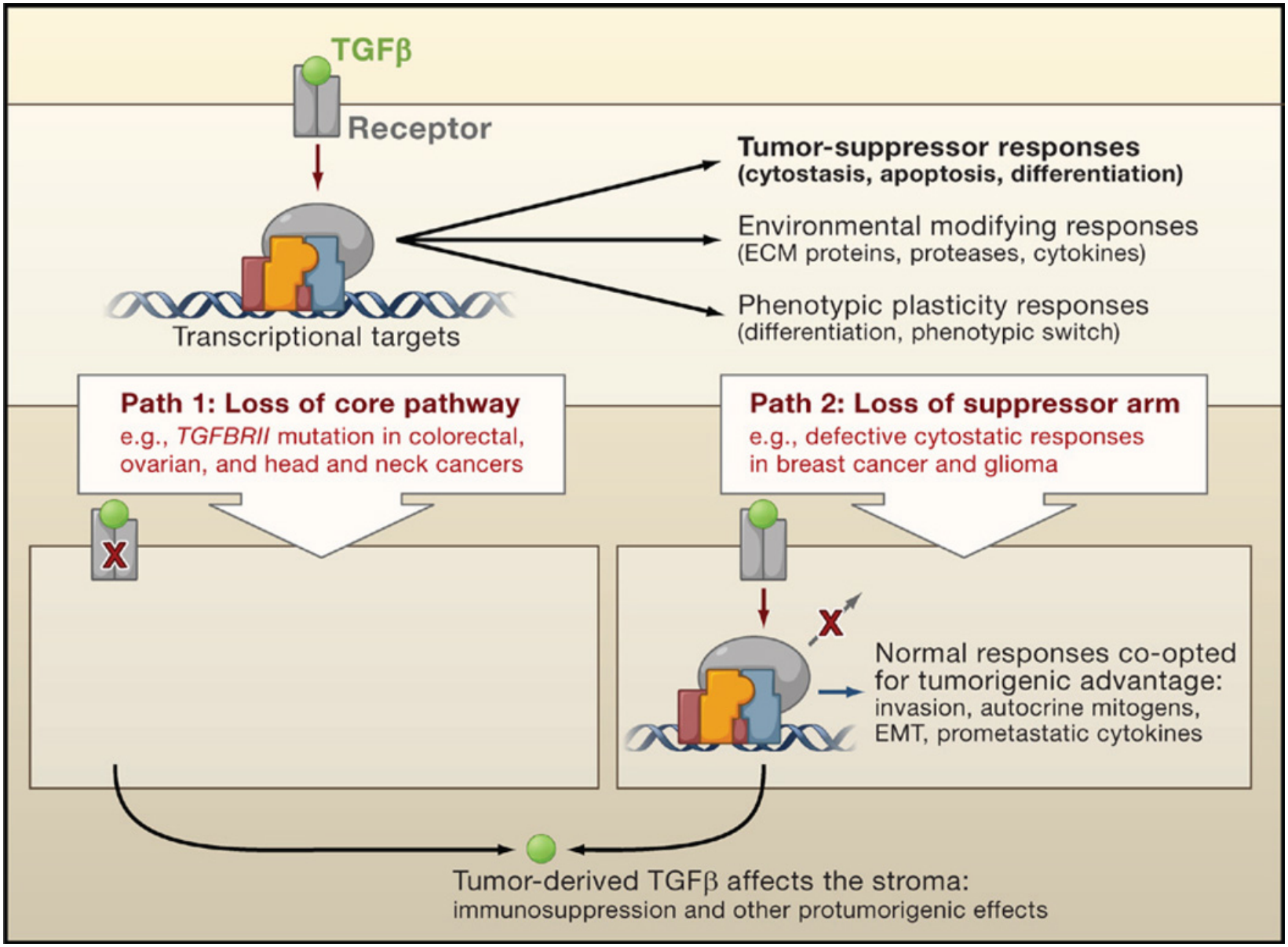


Il doppio ruolo della pathway di TGFβ nel cancro

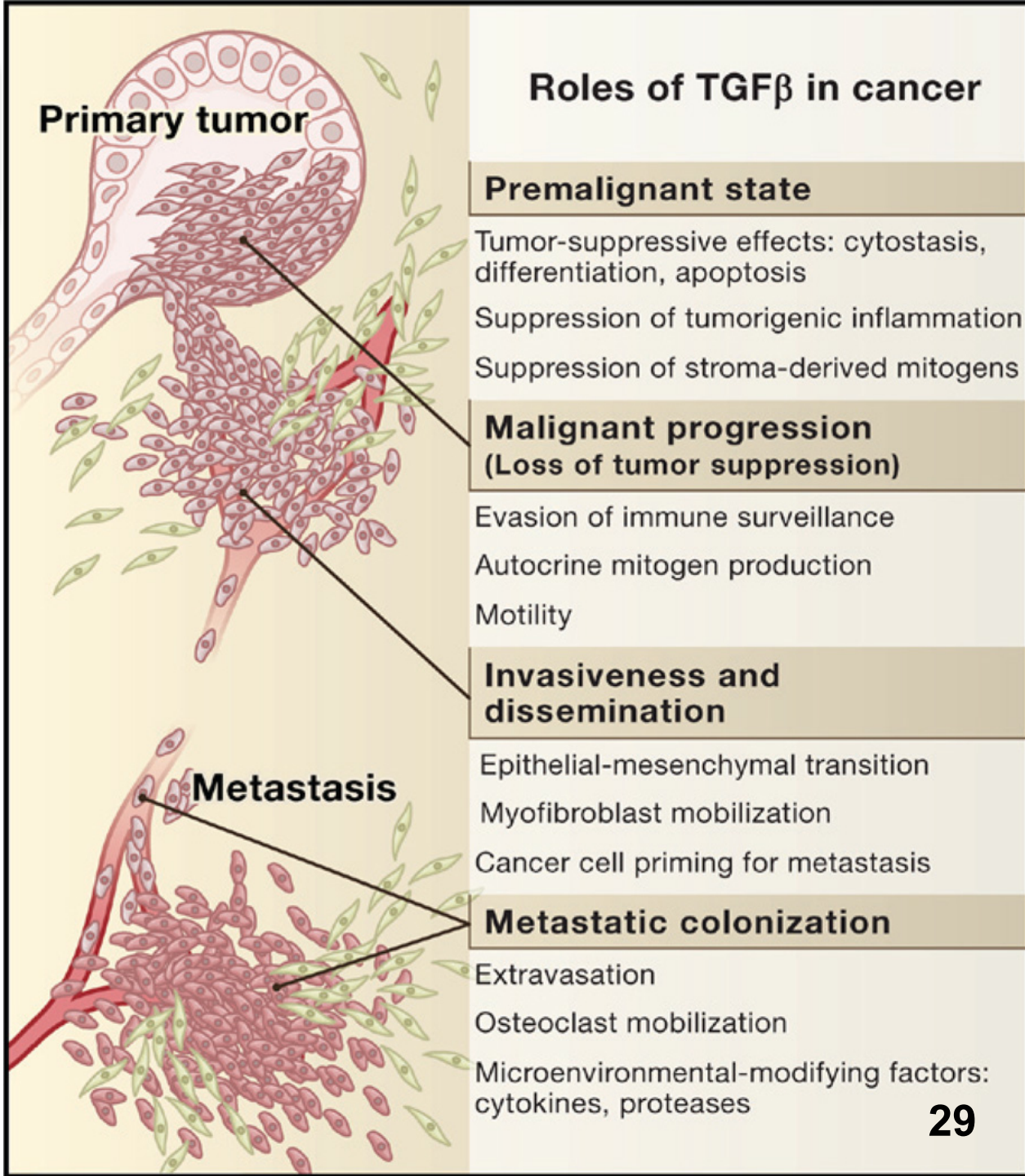


Akhurst & Hata, Nat Rev Drug Disc 2012

Alterazioni della pathway di TGFβ nel cancro

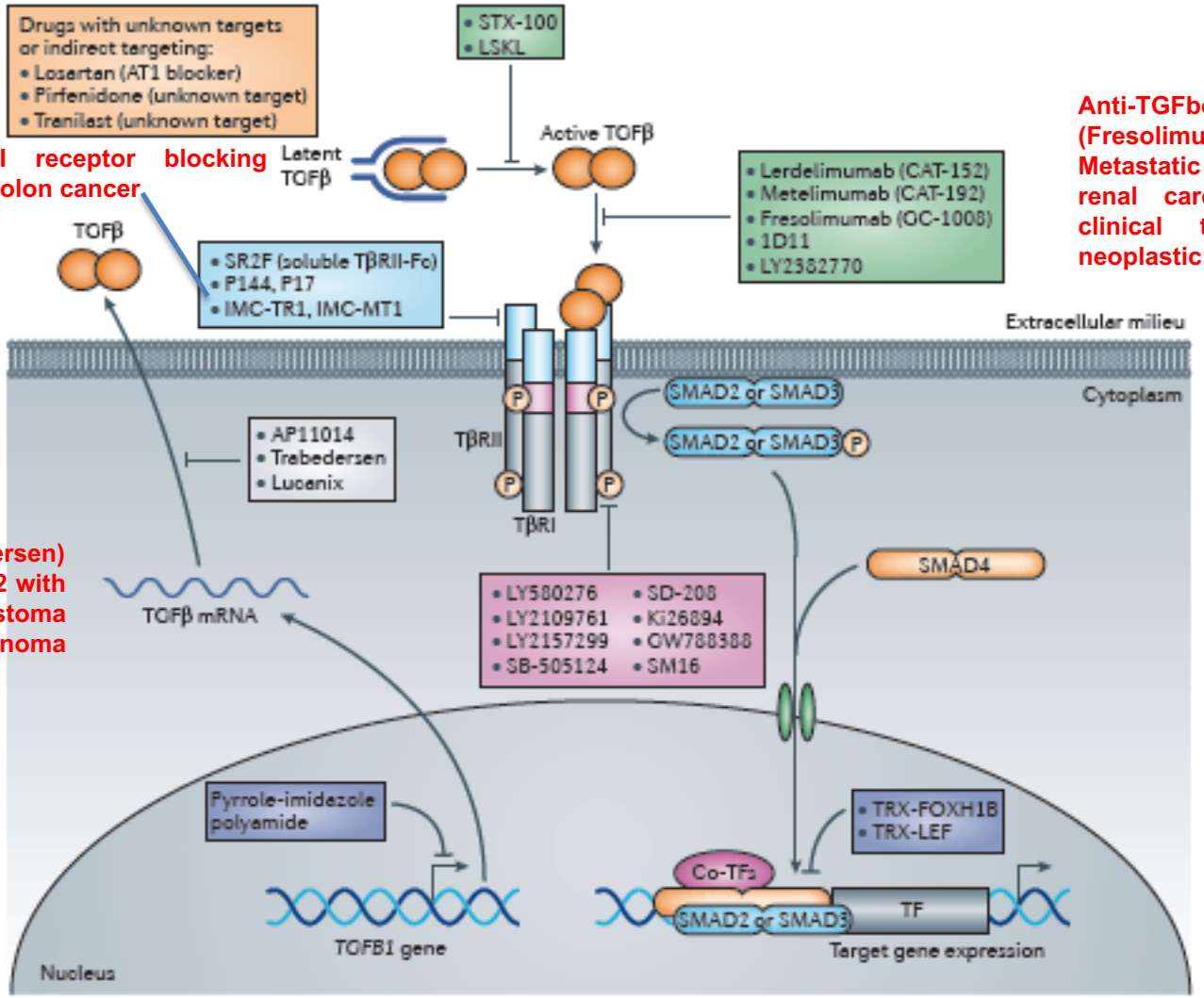


Il doppio ruolo della pathway di TGFβ nel cancro



J Massague, Cell 2008

Strategie farmacologiche per inibire la TGF-β pathway nel cancro e nelle malattie fibrotiche

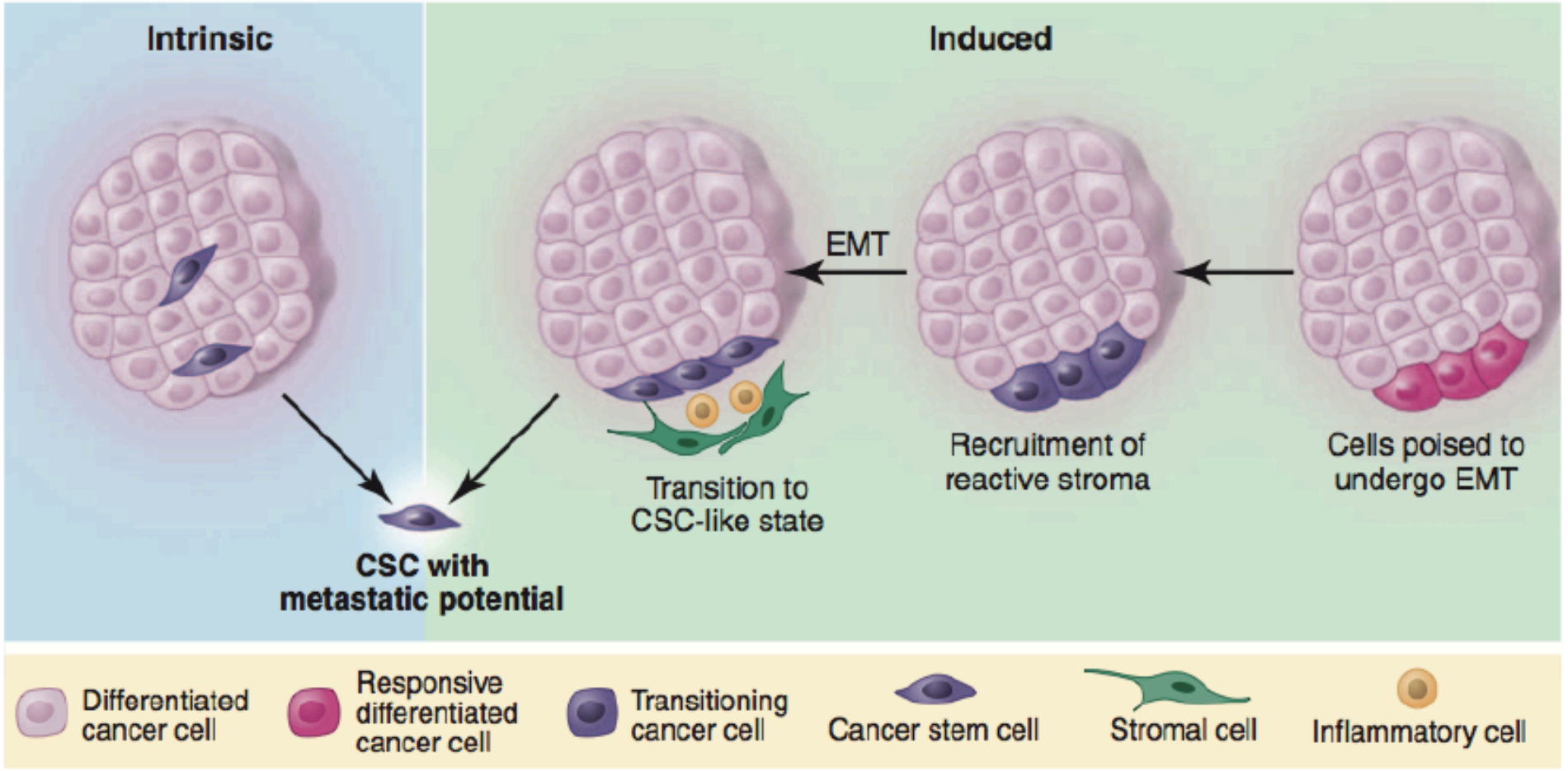


e.g. (IMC-TR-1) TGFβII receptor blocking antibody for breast and colon cancer

Anti-TGFbeta1 antibody e.g. (Fresolimumab) Metastatic melanoma and renal carcinoma (phase I clinical trial) and non-neoplastic applications

e.g. (AP12009 = Trabedersen) Targeting excess TGFβ2 with ASO-RNAi in glioblastoma and pancreatic carcinoma (phase III clinical trial)

Il programma di EMT conferisce caratteristiche STAMINALI generando TUMOR INITIATING CELLS (CSCs) essenziali per la metastasi e chemioresistenti

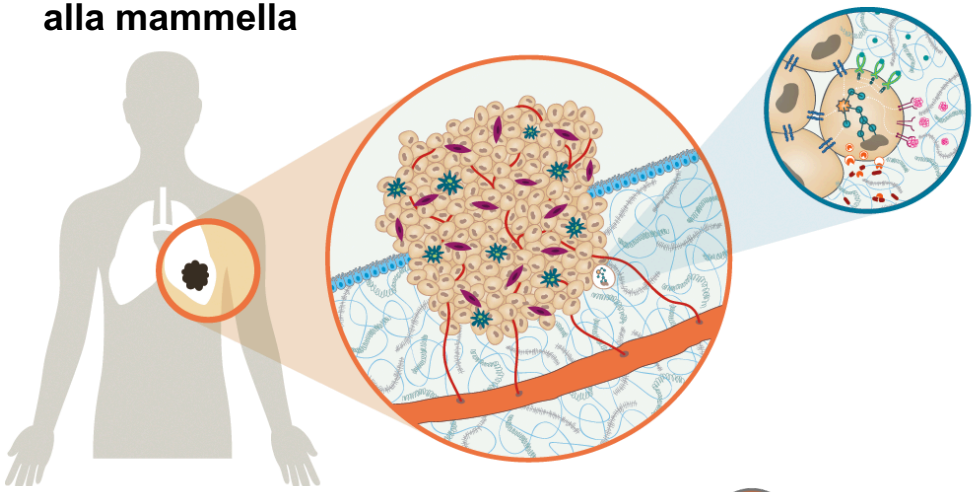


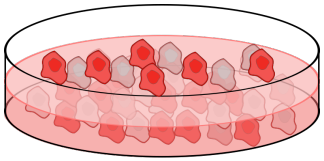
Il riposizionamento dei farmaci

Farmaci approvati da EMA/FDA e commercializzati per qualsiasi malattia

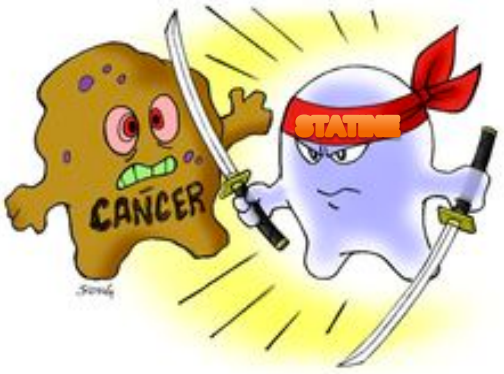
Testare in vitro l'efficacia antitumorale dei farmaci

Paziente con tumore alla mammella

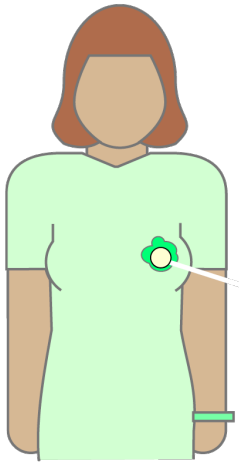




Es. Identificazione delle statine (anticolesterolemici) come potenziali farmaci antitumorali



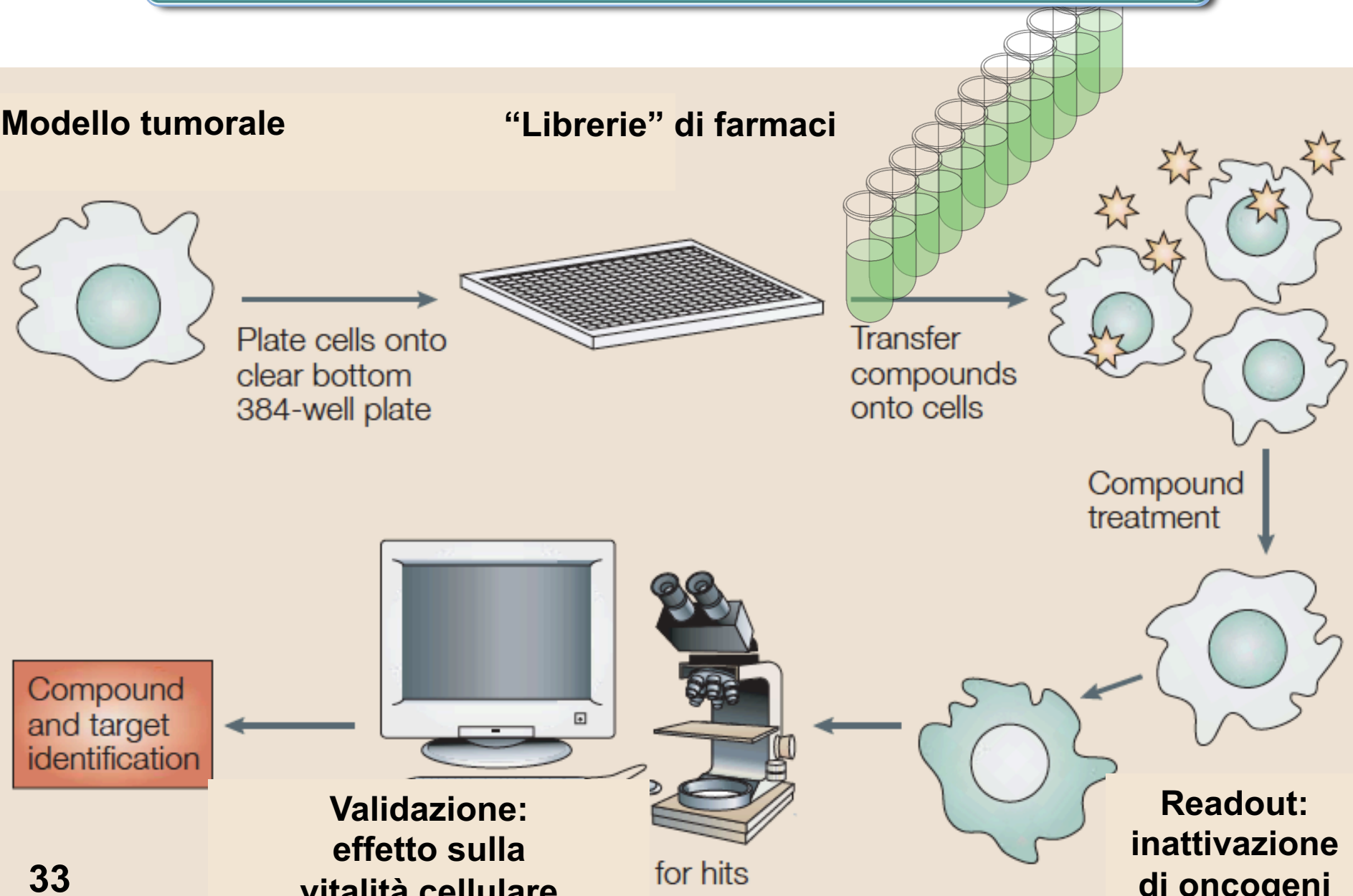
Trial clinico per l'efficacia delle statine in pazienti con tumore al seno in combinazione con le terapie standard



Screening per il riposizionamento di farmaci

Modello tumorale

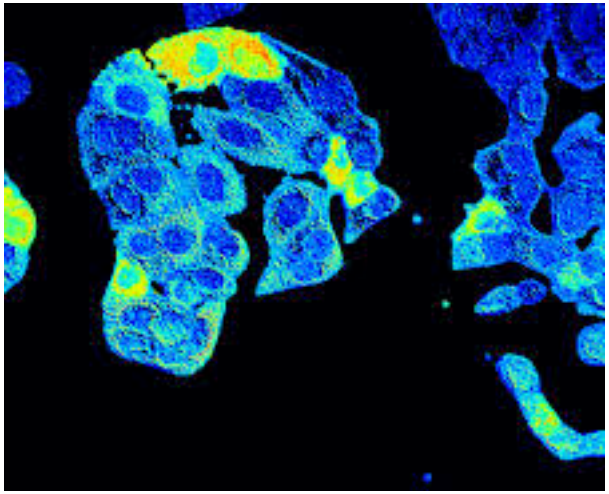
“Librerie” di farmaci



Validazione: effetto sulla vitalità cellulare

Readout: inattivazione di oncogeni

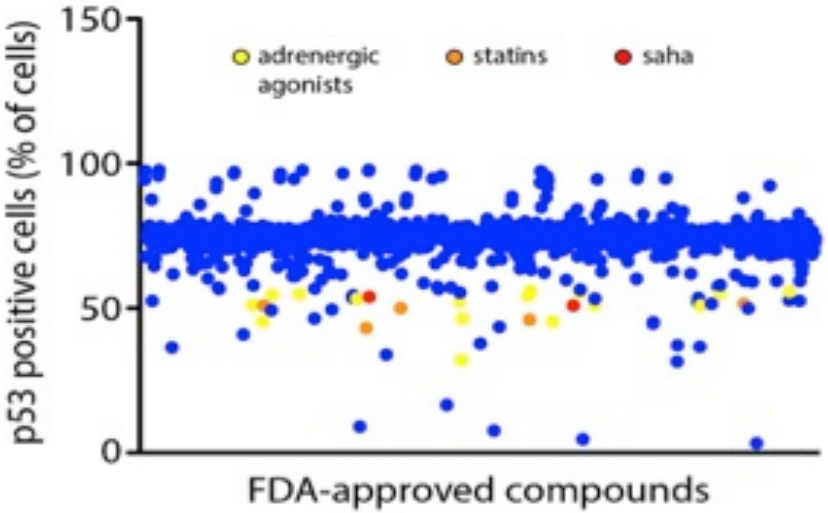
High content screening systems

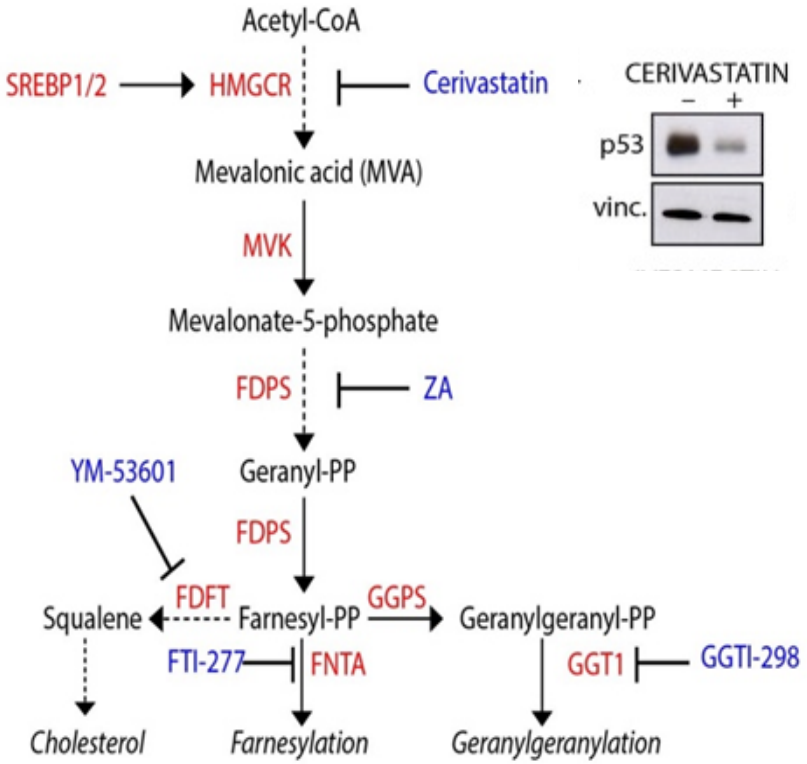


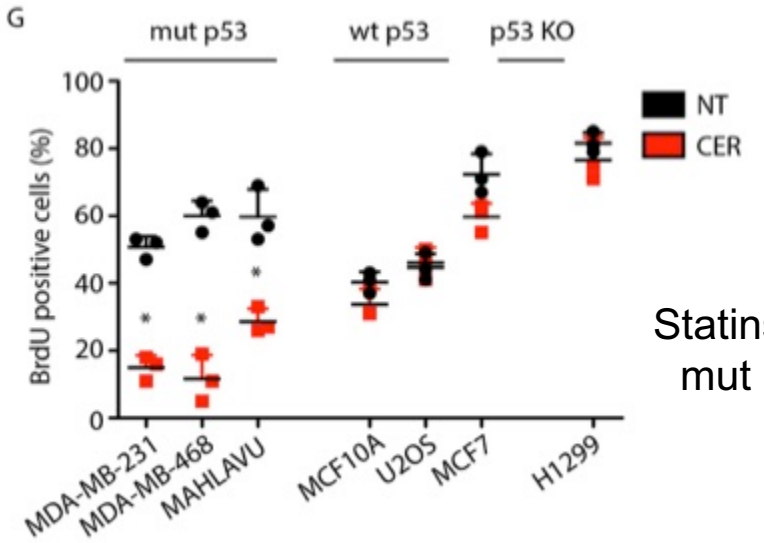
Next generation confocal high content screening system, designed to reliably discriminate phenotypes of complex cellular models, such as primary cells and 3D microtissue, **integrated with automated microplate loader and liquid handling robot station** for automated transfection of cells in 96- and 384-well microplates and assay preparation

Identification of molecules affecting mutant p53 protein levels by high content high-throughput screening

A



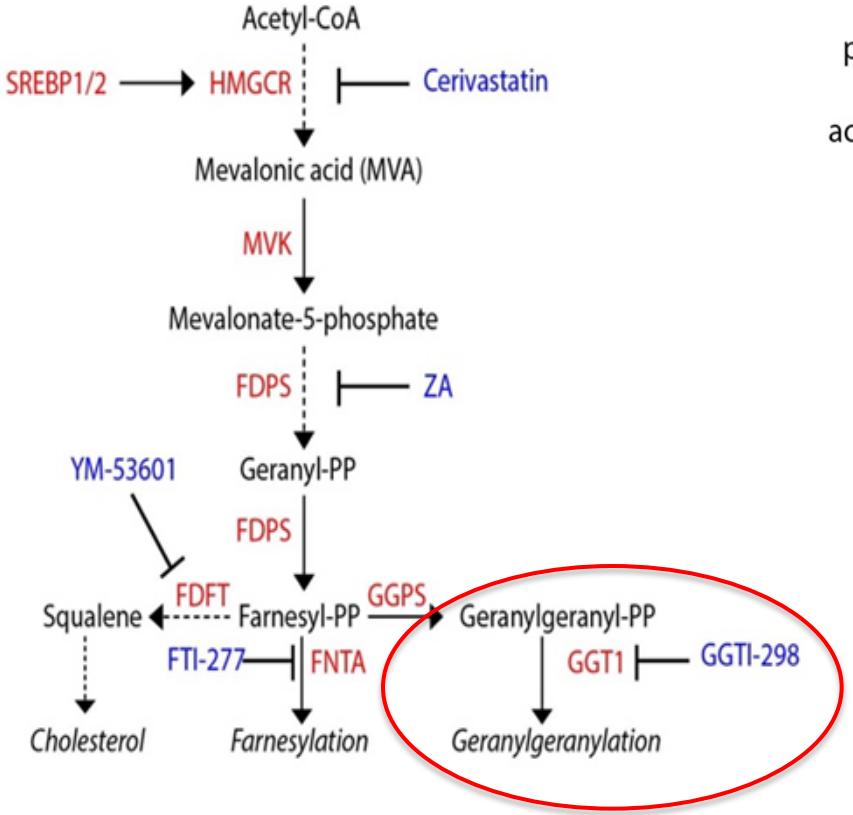


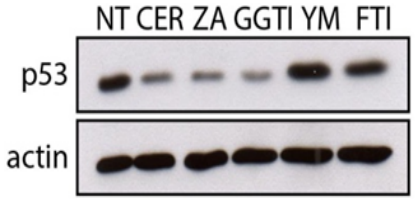


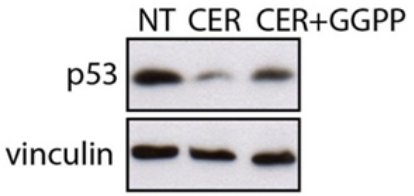
Statins inhibit proliferation of mut p53 expressing cells

Ingallina et al, *Nat Cell Biol* 2018

Geranyl-geranylation is required for mutp53 stabilization

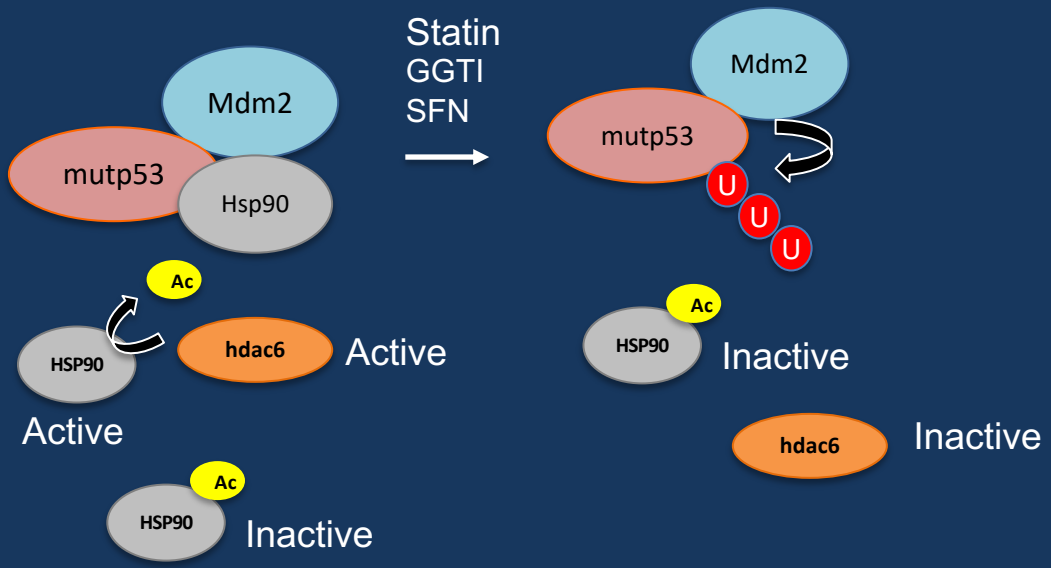






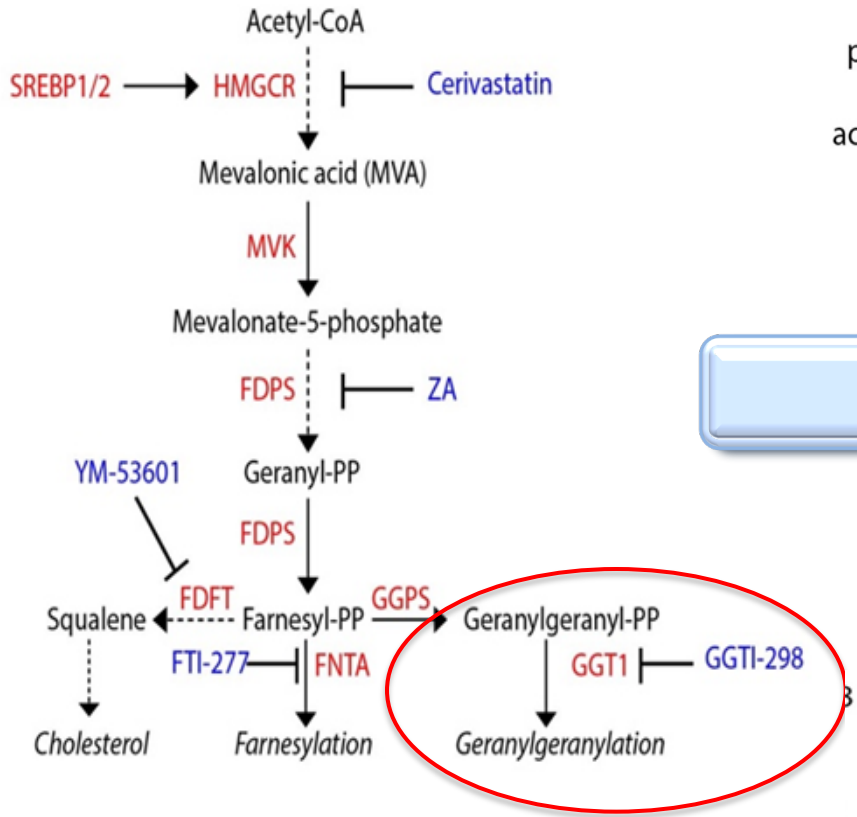
Key findings

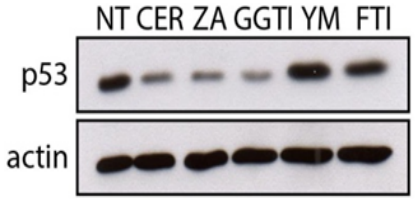
- ✓ Statins and other MVA pathway inhibitors are potent mutant p53 inhibitors.
- ✓ Statins cause mutant p53 degradation through MDM2 by inactivating Hsp90 and HDAC6

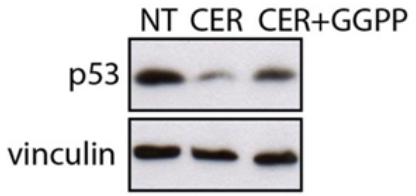


- ✓ The MVA pathway sustains mutant p53 accumulation in cancer cells.
- ✓ Loss of Geranyl-Geranyl-Phyrophosphate induces mutant p53 degradation.

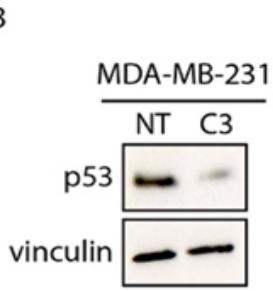
Geranyl-geranylation is required for mutp53 stabilization

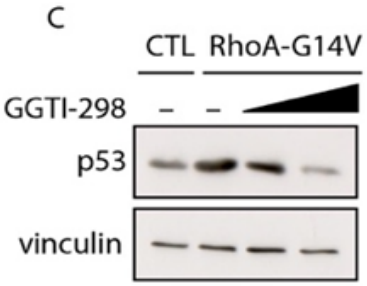






And the substrate is… RhoA





L'attivazione di RhoA induce la stabilizzazione di mutp53