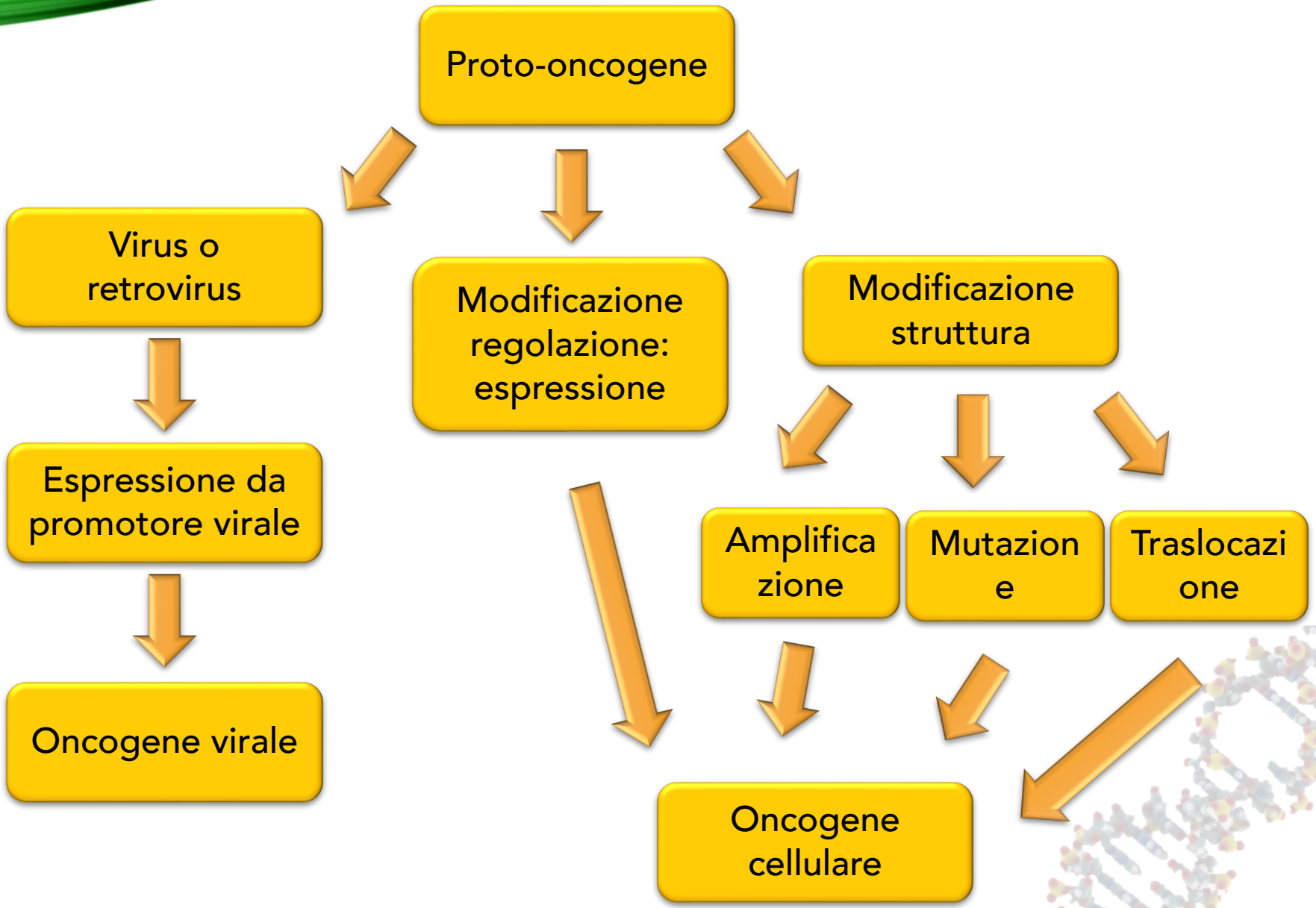
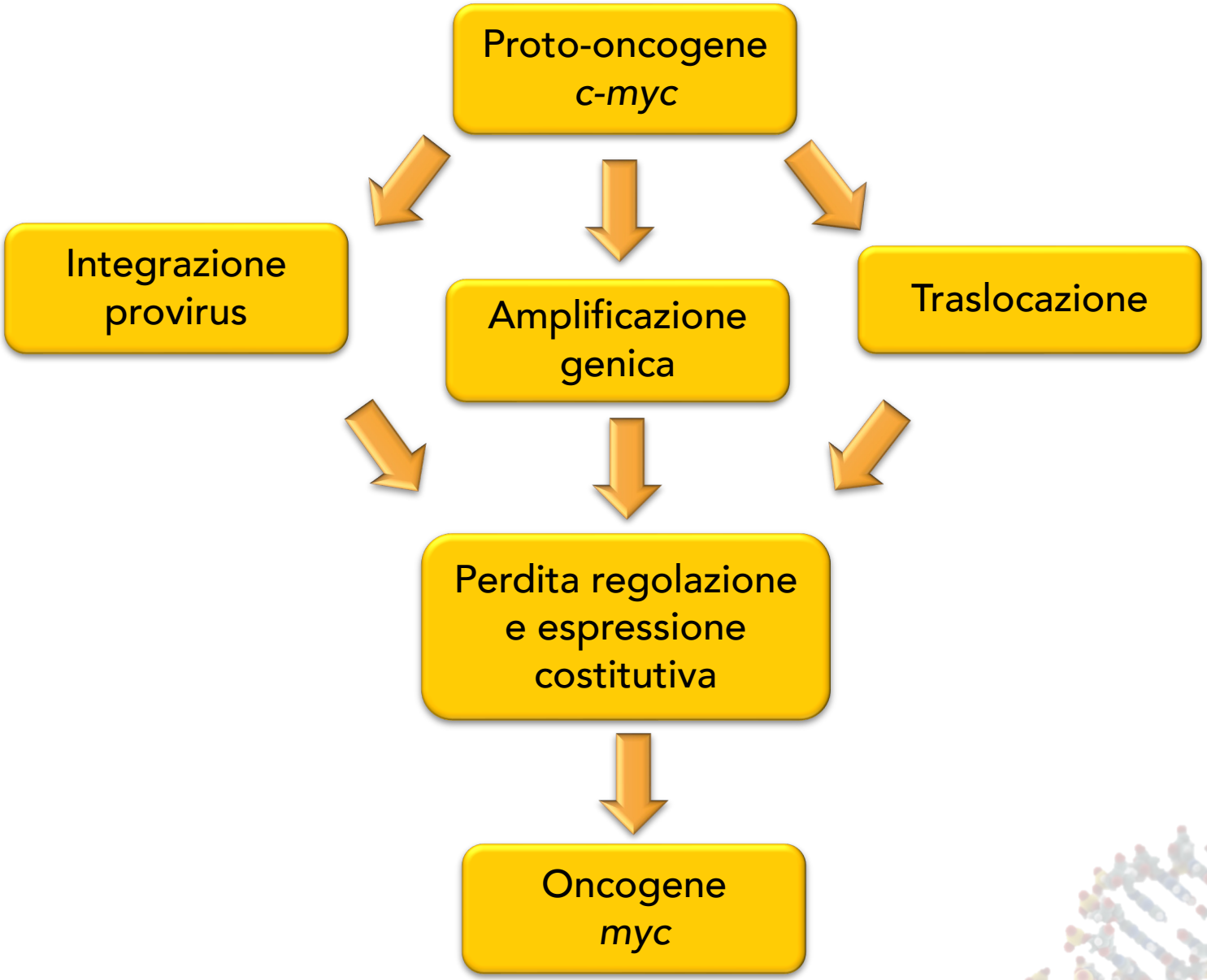
ONCOGENI E LORO ATTIVAZIONE

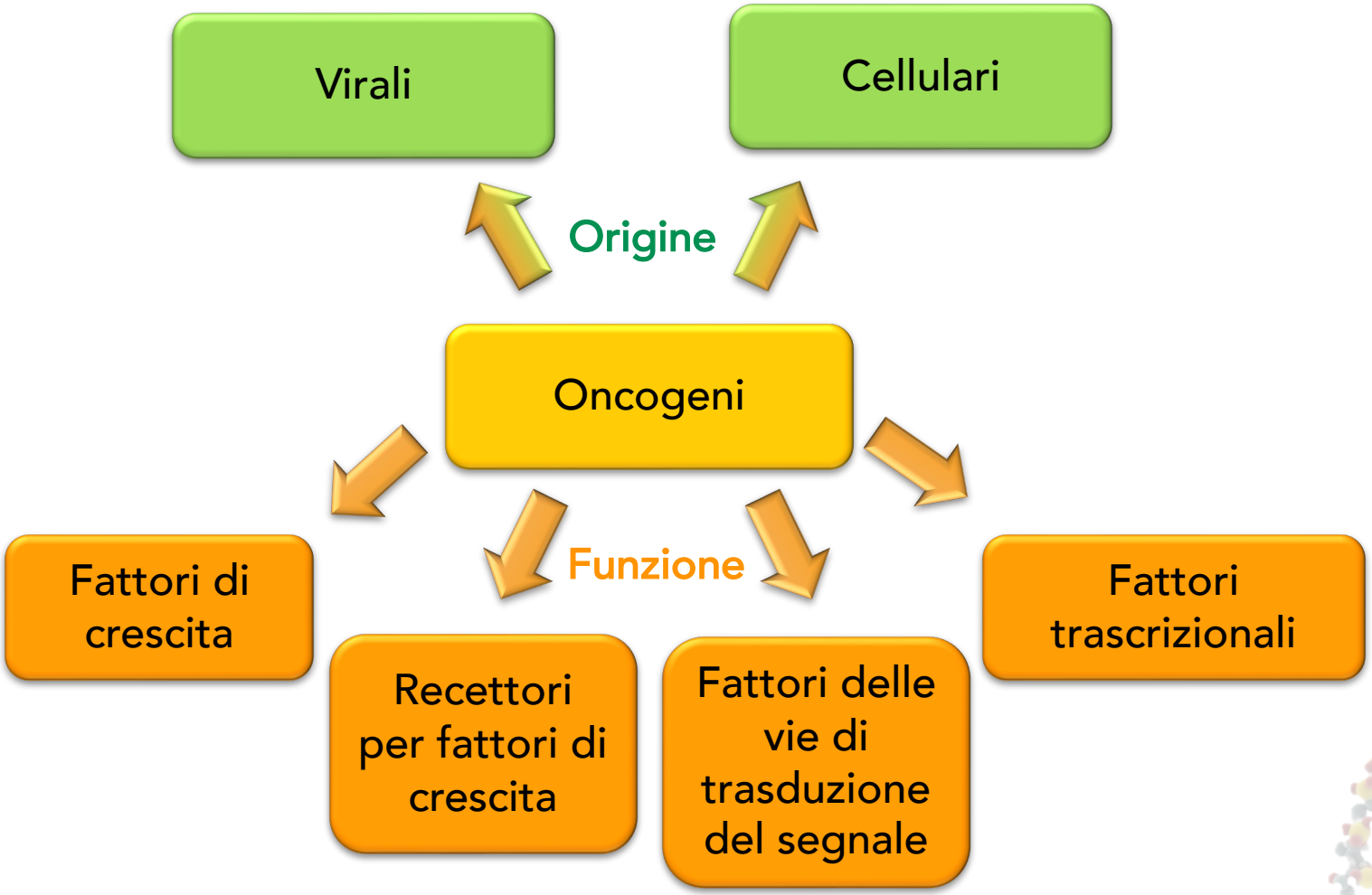
Attivazione oncogeni



Attivazione c-*myc*

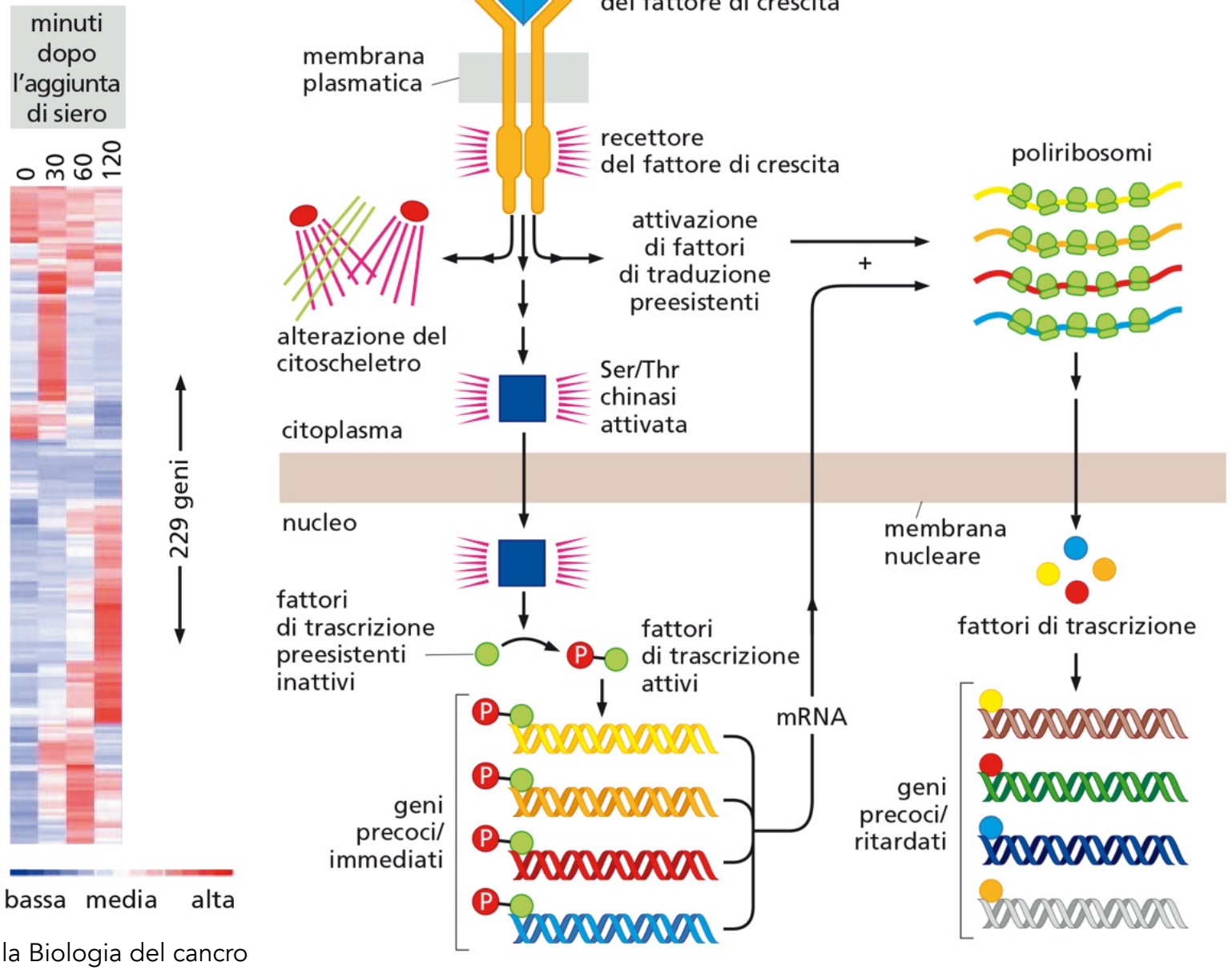


Oncogeni



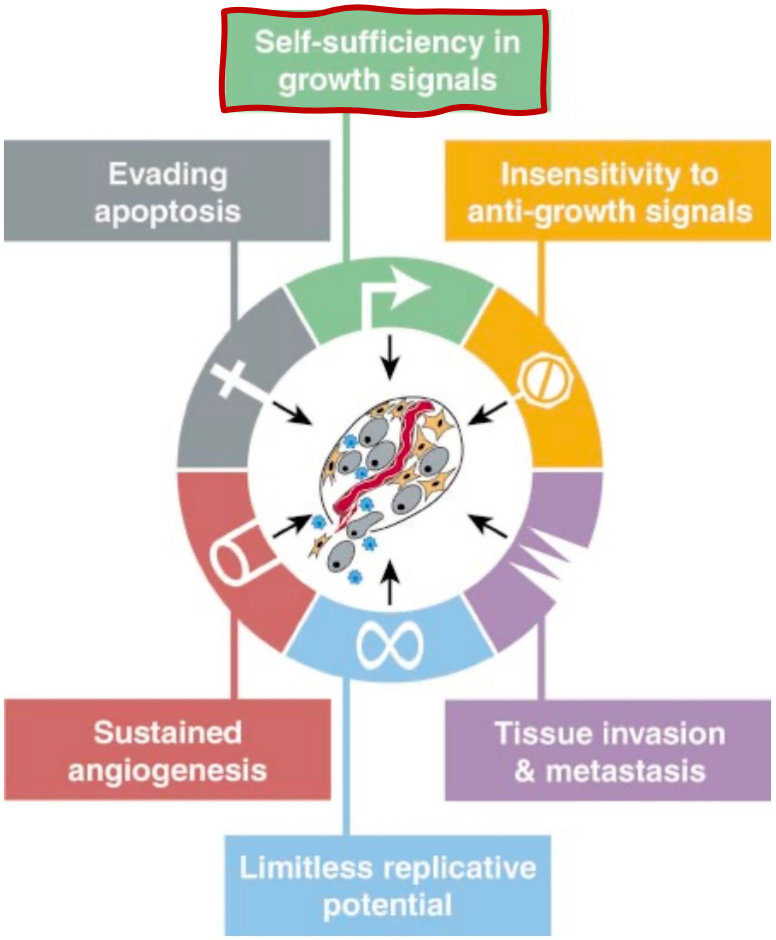
Weinberg, La Biologia del cancro.

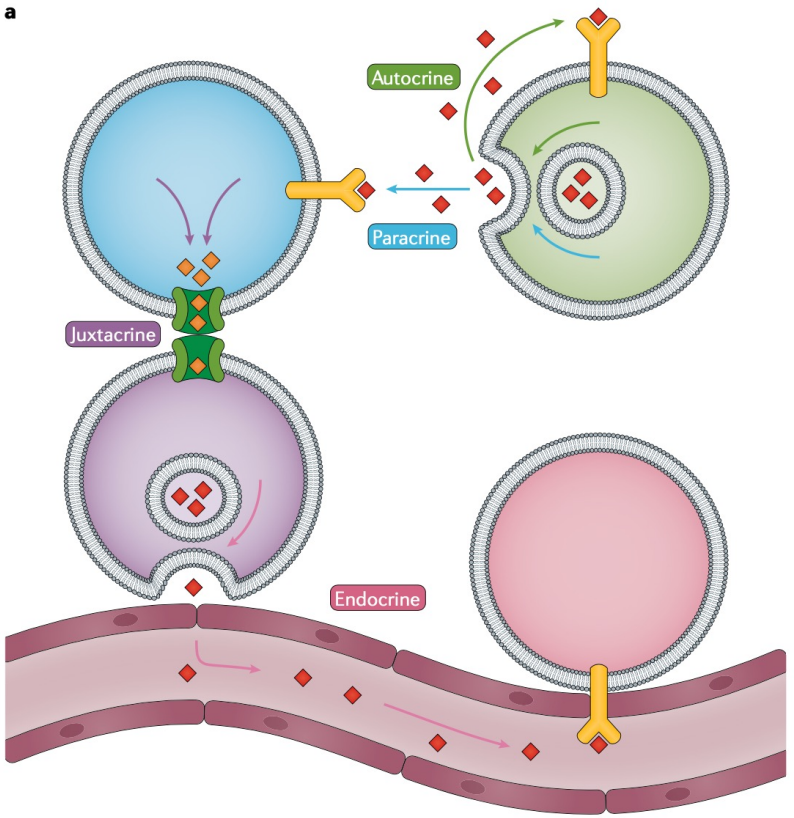
Vie di trasduzione del segnale



Weinberg, la Biologia del cancro

The hallmarks of cancer

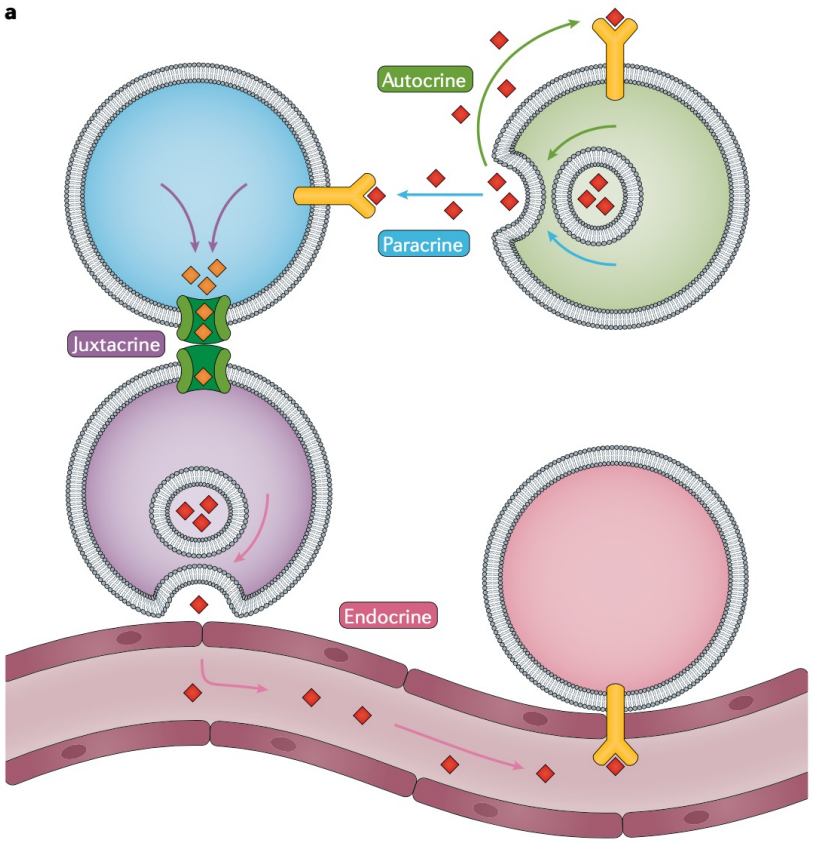




Cell–cell interactions orchestrate organismal development, homeostasis and single-cell functions. Signalling through peptides

Hanahan & Weinberg, 2000; Armingol et al., 2021

Cell-cell signalling



Armingol et al., 2021

<u>Types of cell–cell interactions and communication.</u>

Autocrine signalling: intracellular communication whereby cells secrete ligands that are used to induce a cellular response through cognate receptors for those molecules expressed on the same cell.

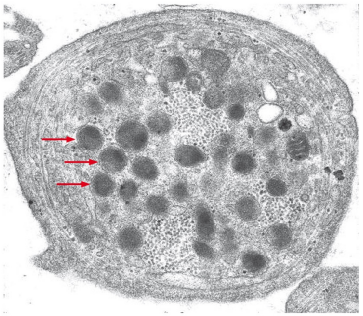
Paracrine cell–cell communication does not require cell–cell contact, rather depending on the diffusion of signalling molecules from one cell to another after secretion.

Juxtacrine, that is, contact-dependent, cell–cell communication relies on gap junctions or other structures such as membrane nanotubes to pass signalling molecules directly between cells, without secretion into the extracellular space.

Endocrine cell–cell communication represents intercellular communication whereby signalling molecules are secreted and travel long distances through extracellular fluids such as the blood plasma; typical mediators of this communication are hormones.

Fattori di crescita

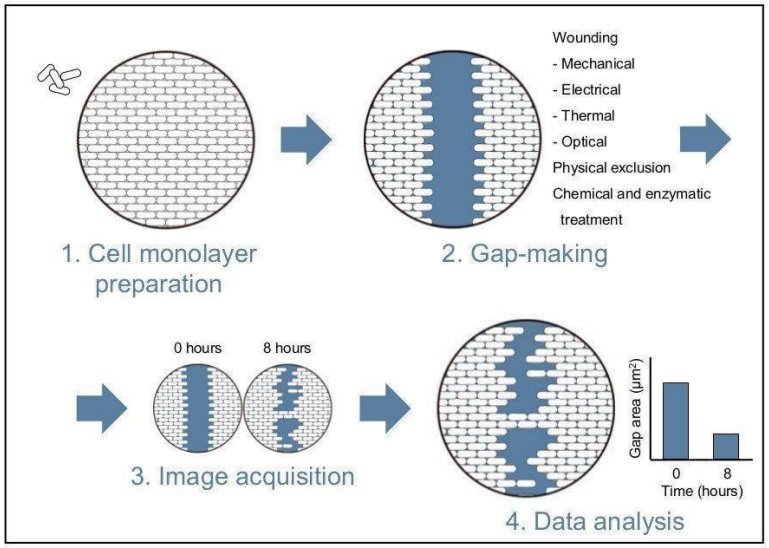
Cellule coltivate in capsule Petri non si dividono se non è aggiunto siero



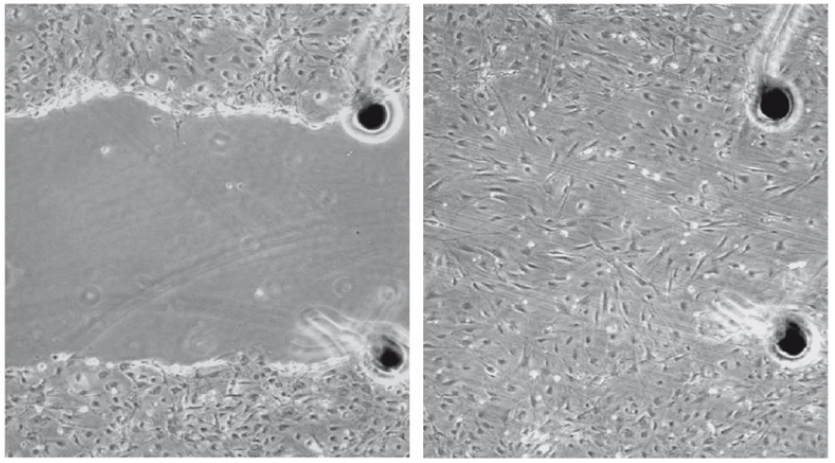
Platelet-derived growth factor (PDGF):
dimeric glycoprotein composed of two A subunits (PDGF-AA), two B subunits (PDGF-BB), or one of each (PDGF-AB).

Fibroblasti in coltura con aggiunta PDGF-BB

Wound healing assay



PDGF-R+

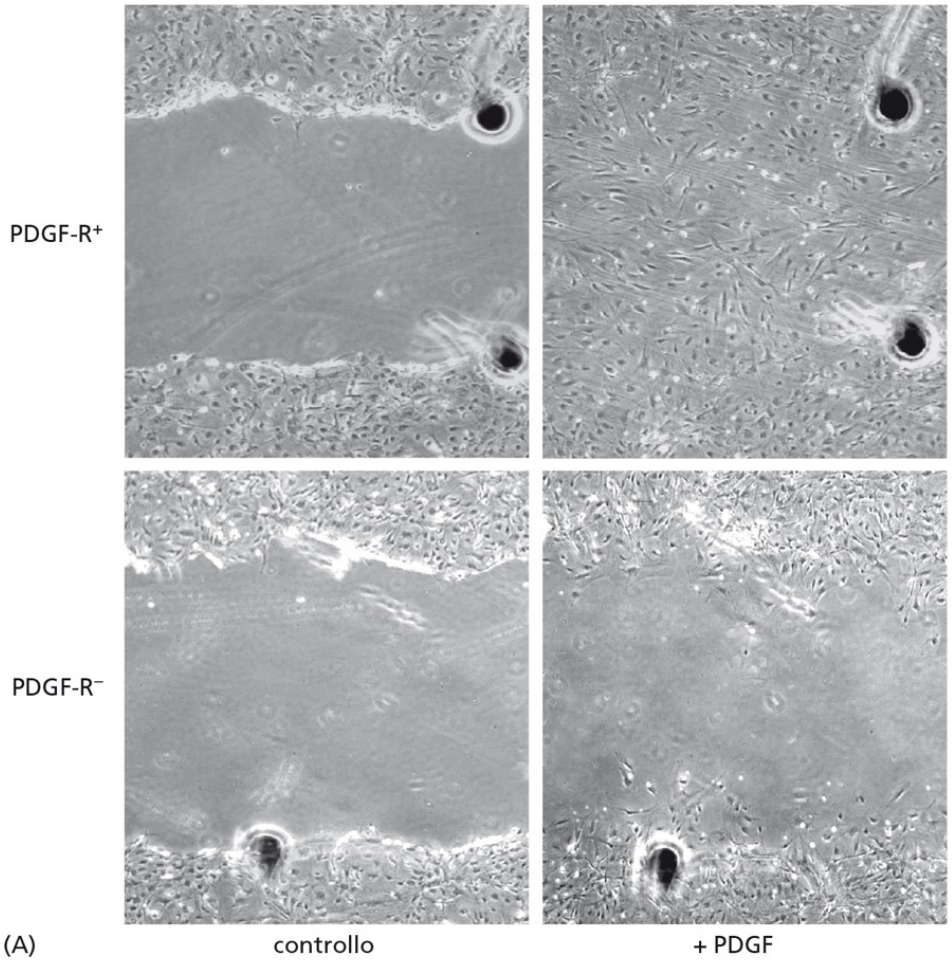


PDGF è potente stimolatore della proliferazione cellulare dei fibroblasti: MITOGENO

Weinberg, la Biologia del cancro

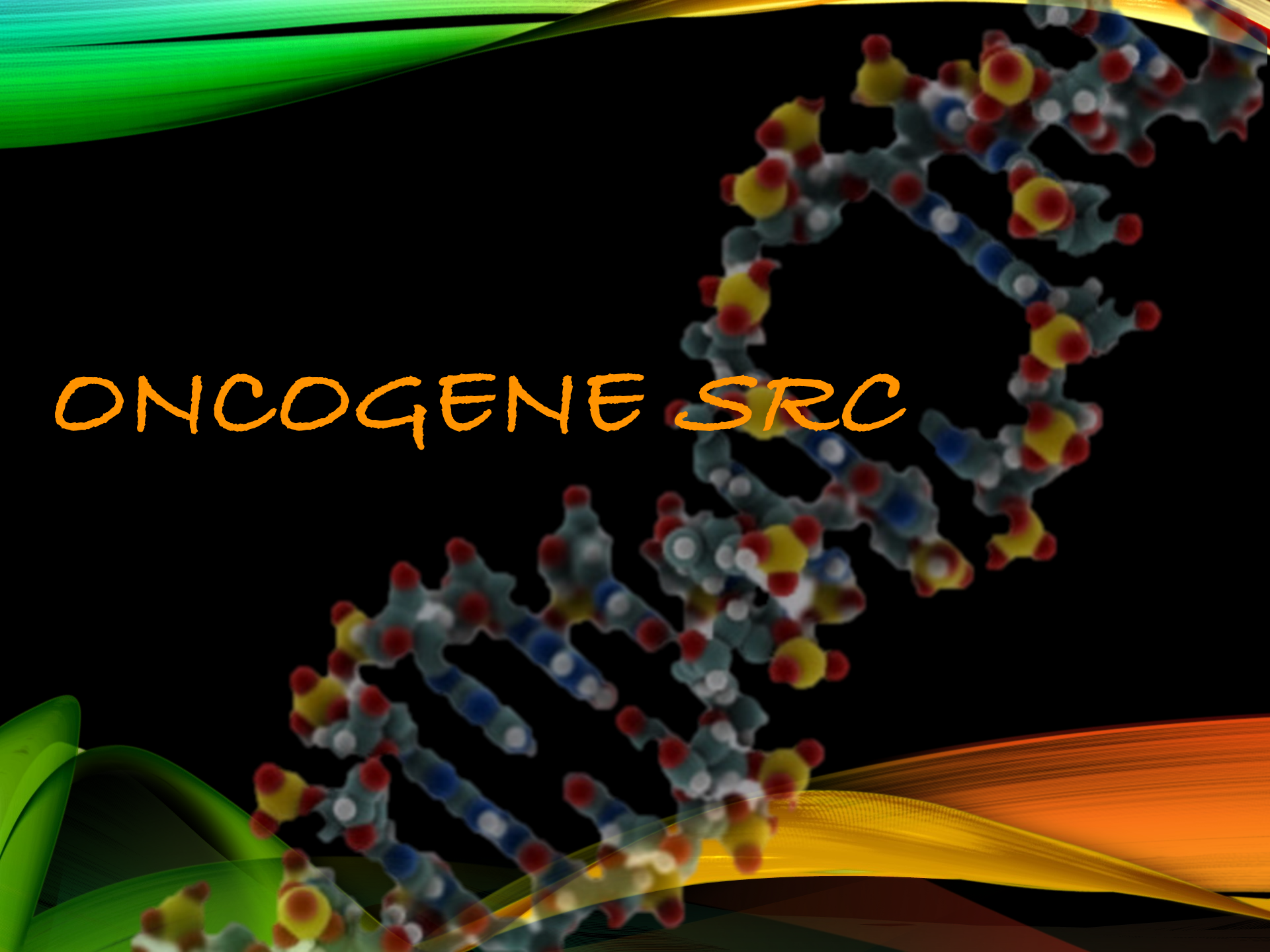
Fattori di crescita e recettori

Il segnale portato dai GF viene recepito da appositi recettori



Weinberg, la Biologia del cancro

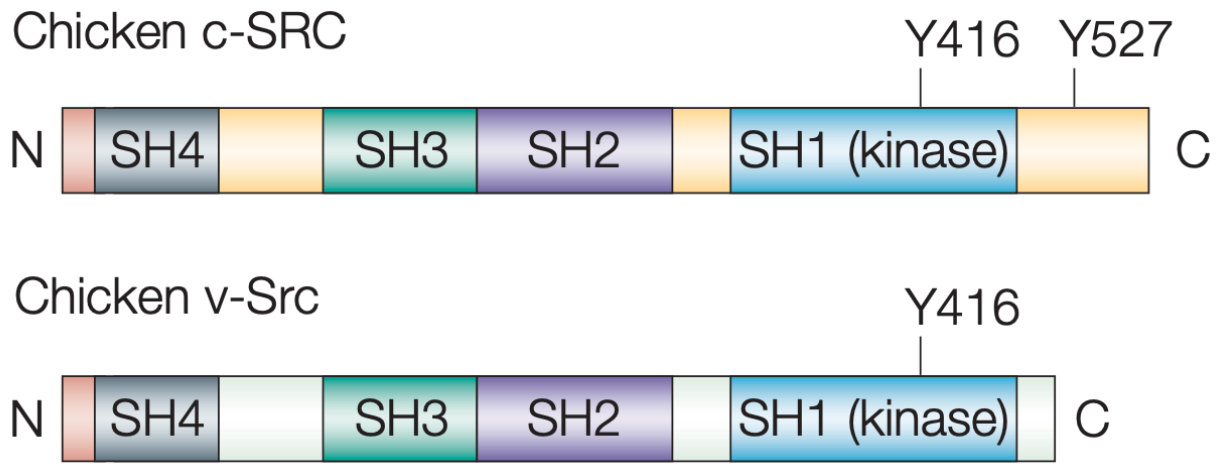
ONCOGENE SRC



Identification of Src gene

- 1900: Agente filtrante del sarcoma di Rous
- 1970: identificati mutanti ts RSV che dimostravano che virus serviva per mantenere lo stato tarsformato
- 1980s: identificato e sequenziato gene *SRC* di pollo
- *v-src* manca di una porzione C-terminale e contiene numerose mutazioni puntiformi
- *c-src* trasforma meno di *v-src*

533 aa, 60 kDa



Yeatman, 2004

Identification of Src protein

Purchio et al., 1978:

Immunoprecipitate of Src protein.

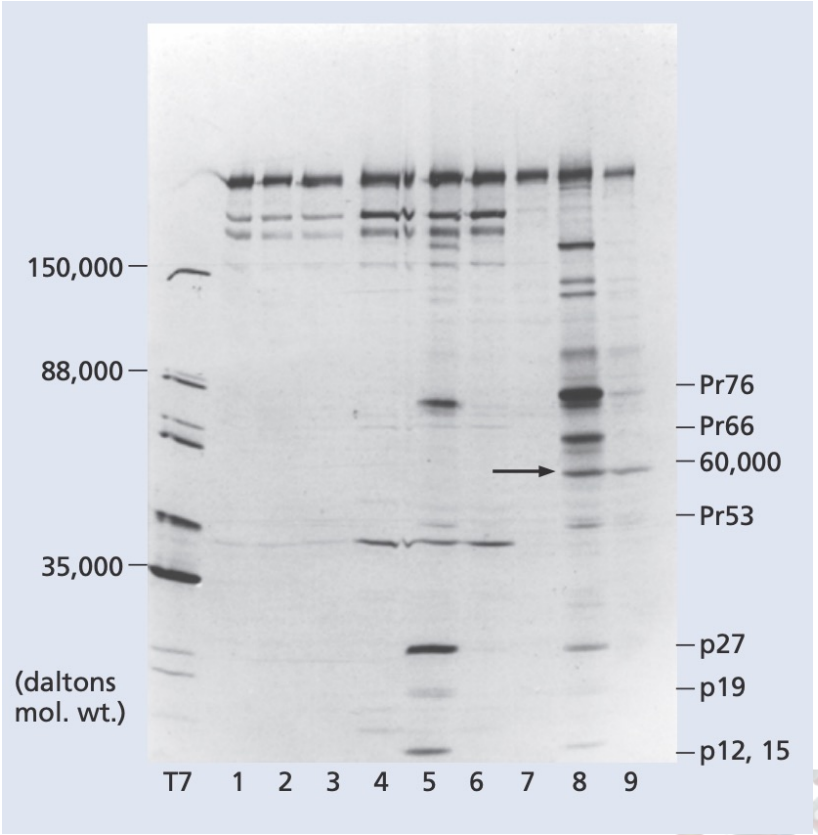
T7: Molecular weight markers

1–3: lysate of normal, uninfected cells

4–6: lysate of cells infected with an RSV mutant with a deleted src gene

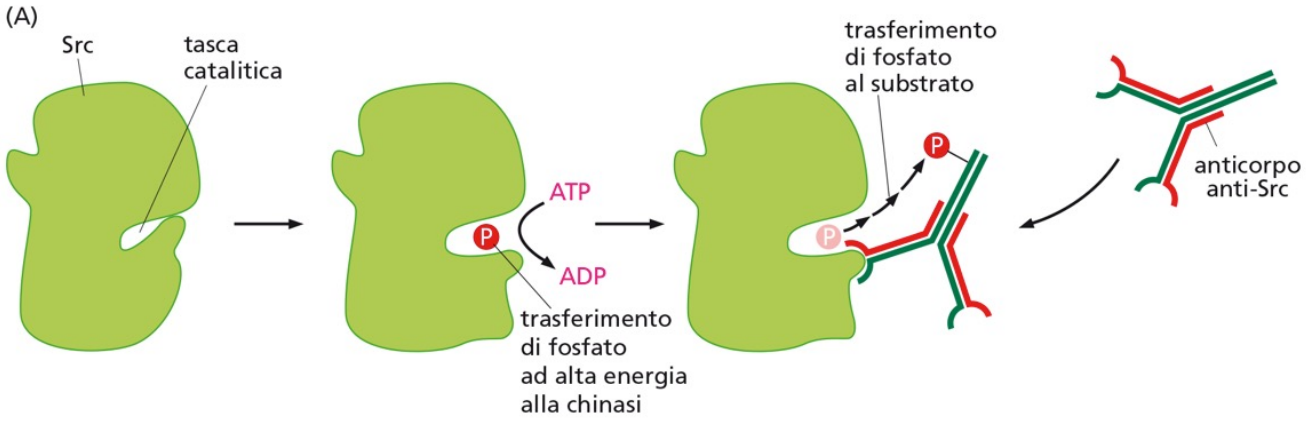
7–9: lysate of cells infected with wild-type RSV (7: normal rabbit serum; 8: tumor-bearing rabbit serum; 9: same as channel 8 except pre-incubated with lysate of RSV particles.

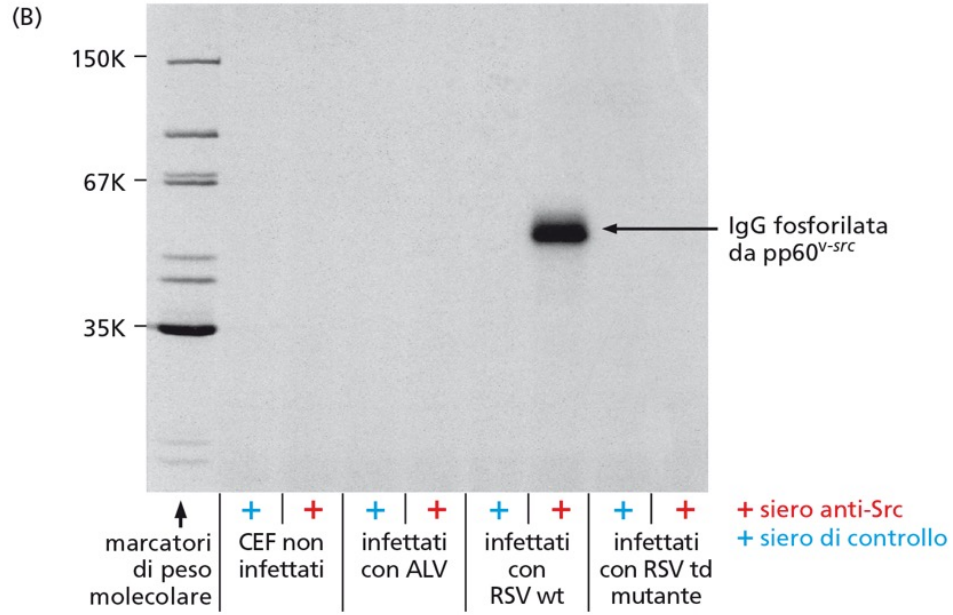
Qual è la funzione di SRC?



Purchio et al., 1978; Aleshin & Finn, 2010; Weinberg, la Biologia del cancro

Clonaggio e caratterizzazione Src



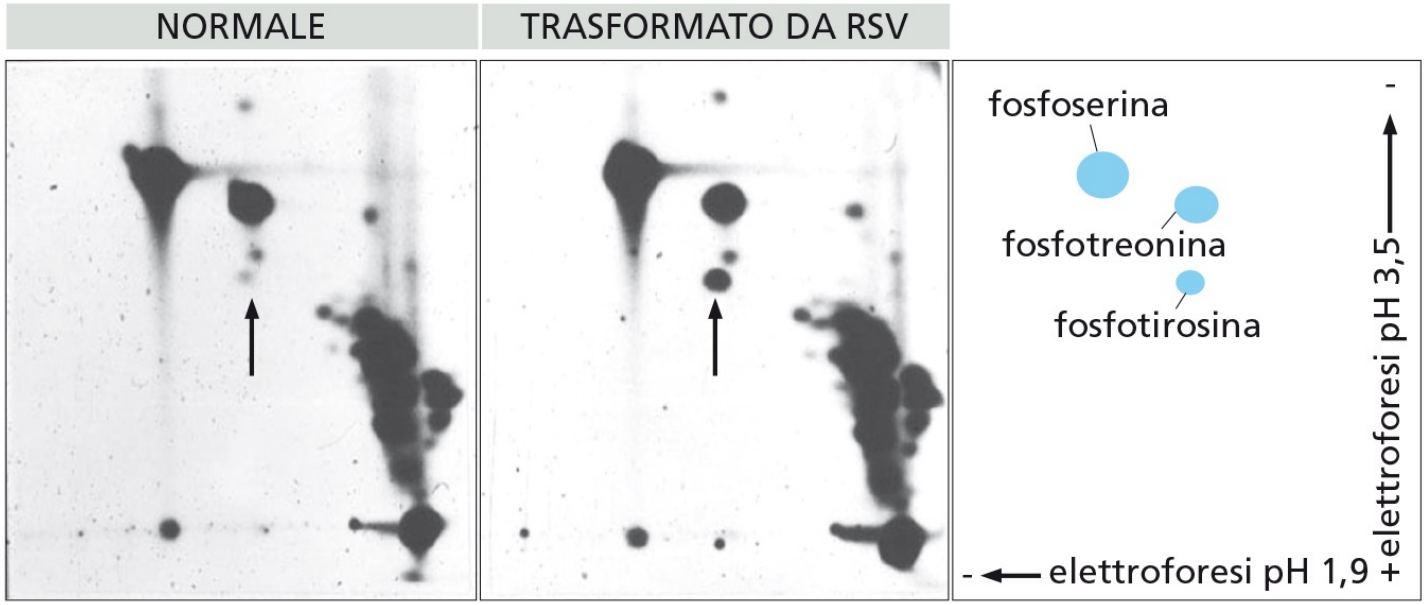


- Src è una protein chinasi
- Src è una fosfoproteina

Weinberg, la Biologia del cancro

Caratterizzazione Src

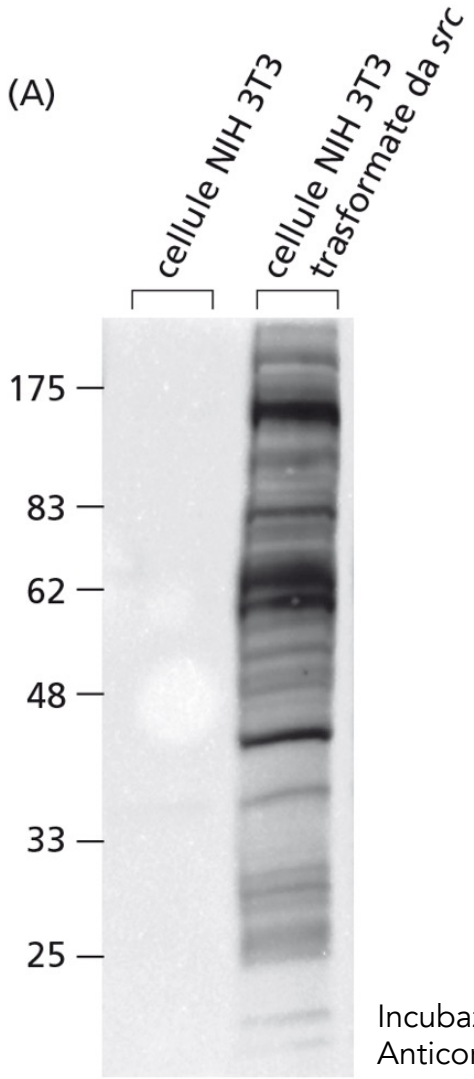
Elettroforesi bidimensionale per rilevare residui forsforilati



- Fosforilazione Tyr indotte da v-Src
- Varianti mutate di Src che non fosforilano non trasformano → Src funziona da oncogene attraverso la sua attività chinasica

Weinberg, la Biologia del cancro

Caratterizzazione Src



Incubazione con
Anticorpi anti fosfo-Tyr

- Src fosforila residui di tirosina
- Noti 50 diversi substrati di Src
- Effetto pleiotropico
- Fosforilazione Tyr specfico per Src: phspho-Tyr è specifico per vie di segnalazione mitogeniche in mammifero

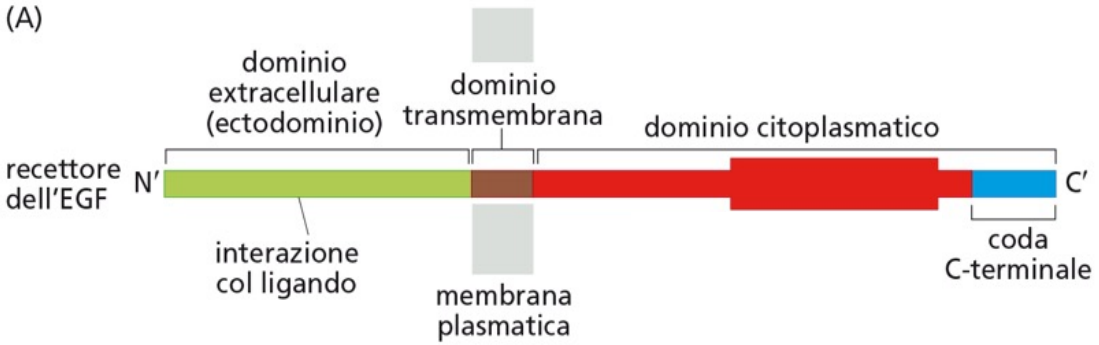
Weinberg, la Biologia del cancro

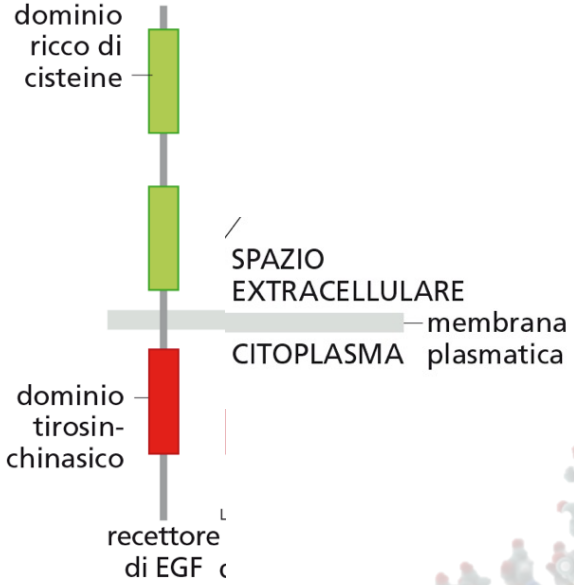
Recettore EGF

Studi oncogene Src

Studi trasduzione del segnale

Funzione Src



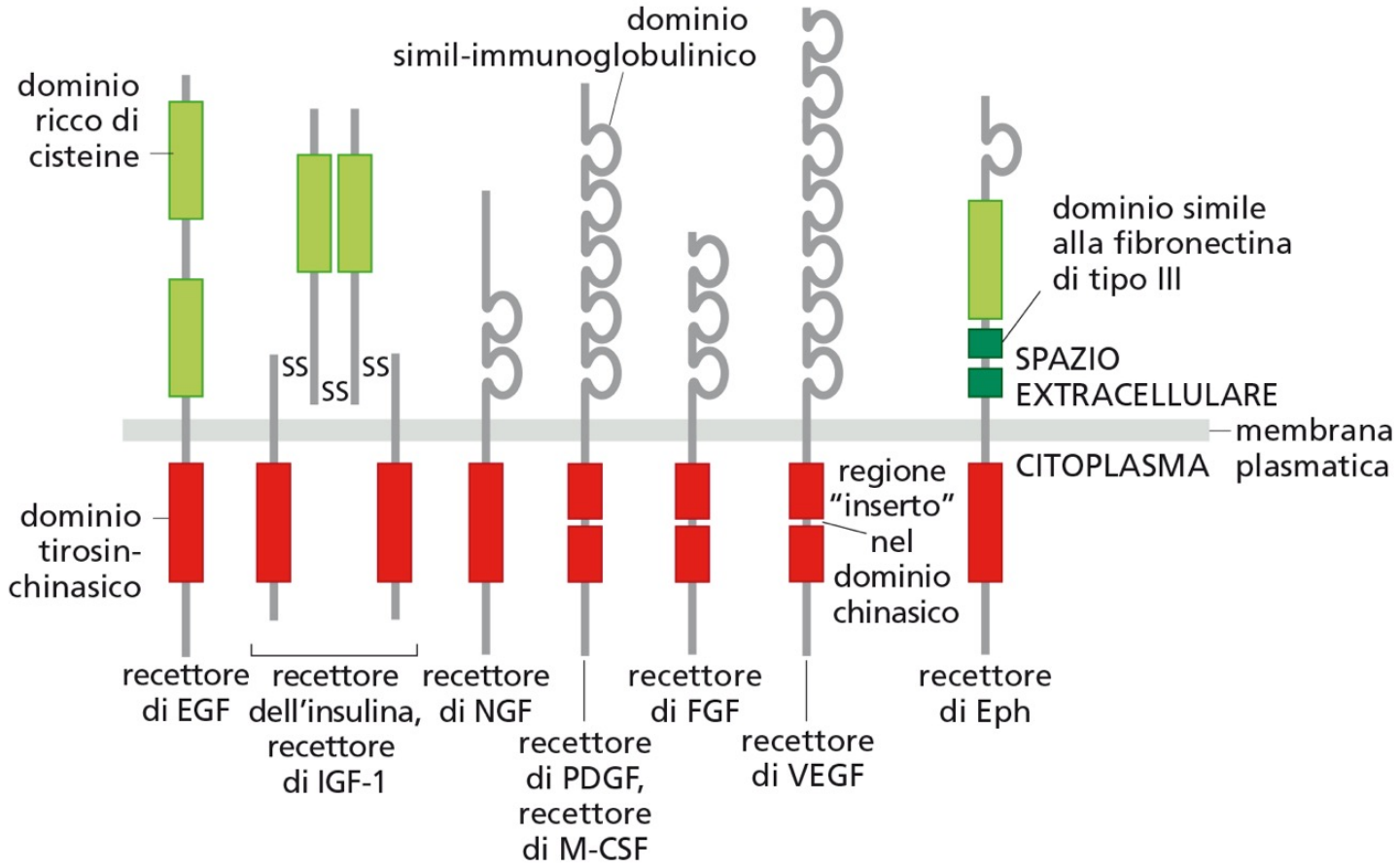


- Src fosforila residui di tirosina

Weinberg, la Biologia del cancro

I recettori tirosin chinasici

Famiglia di recettori, ciascuno specifico per 1 o una famiglia di GF



Nell'uomo ci sono almeno 59 geni che codificano per recettori TK

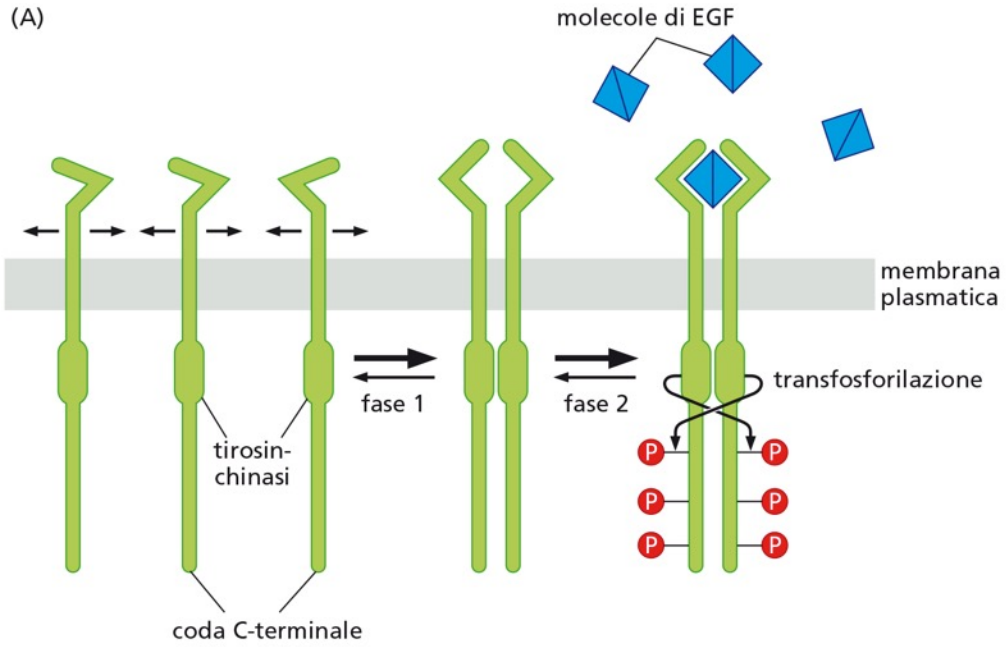
Weinberg, la Biologia del cancro

Modello di attivazione dei recettori TK

Dimerizzazione (omo- o etero-) e fosforilazione in trans:

-libera tasca catalitica da ansa di attivazione

-fosforilazione di tanti residui sulla porzione citosolica



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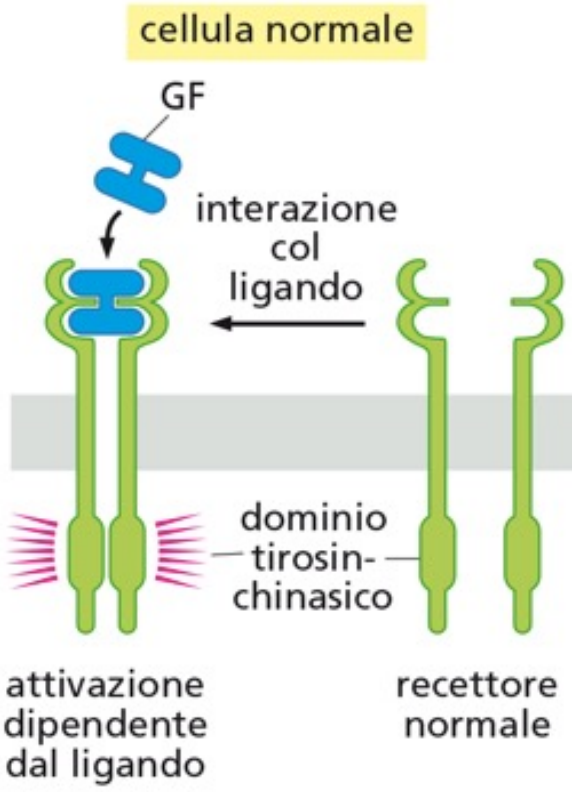
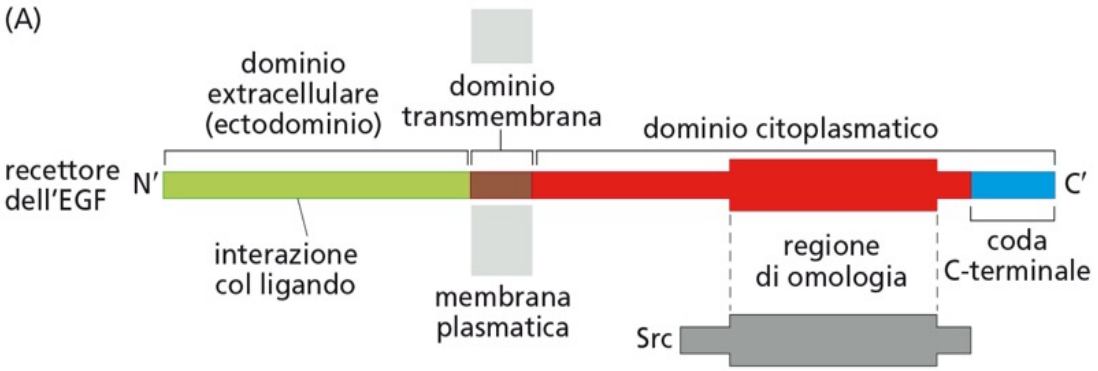
Recettore EGF



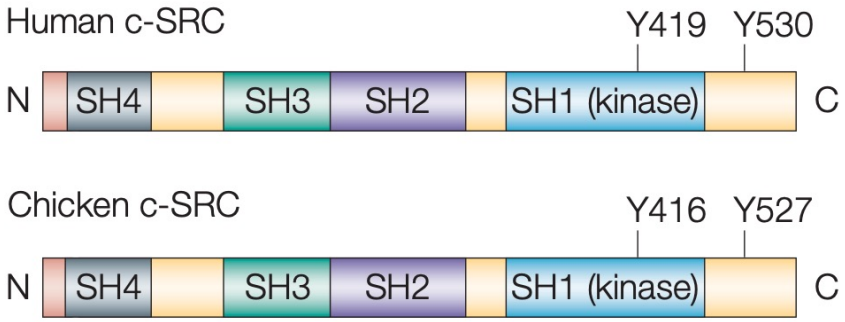


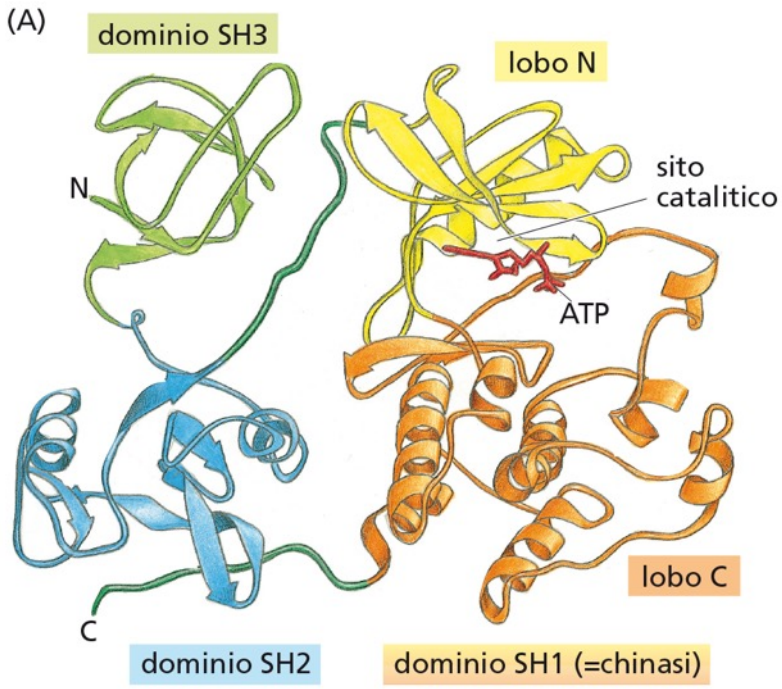
- Omologia di sequenza tra Src e la porzione citoplasmatica del recettore per EGF

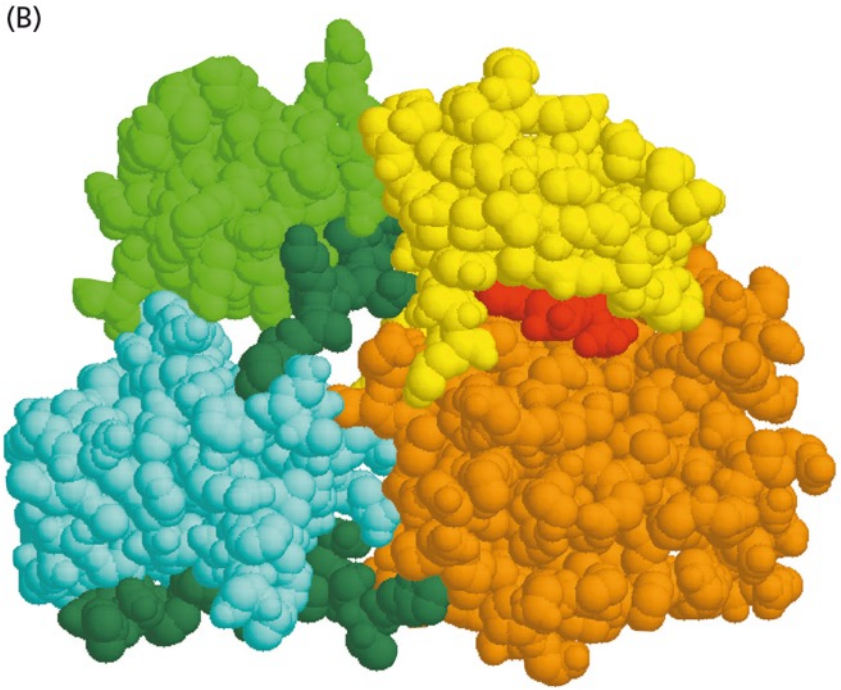
Weinberg, la Biologia del cancro

Struttura di Src

Struttura a domini

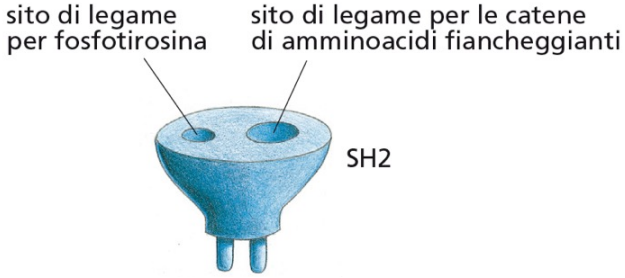


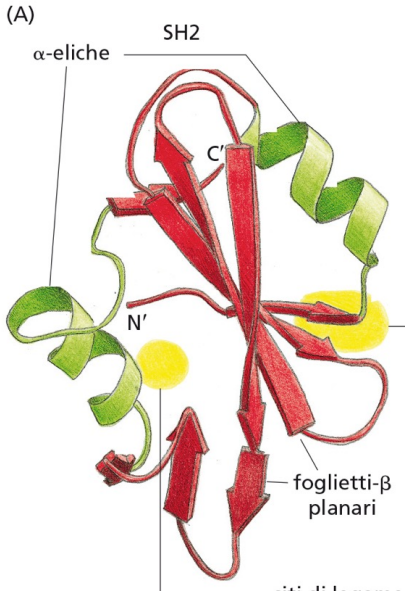


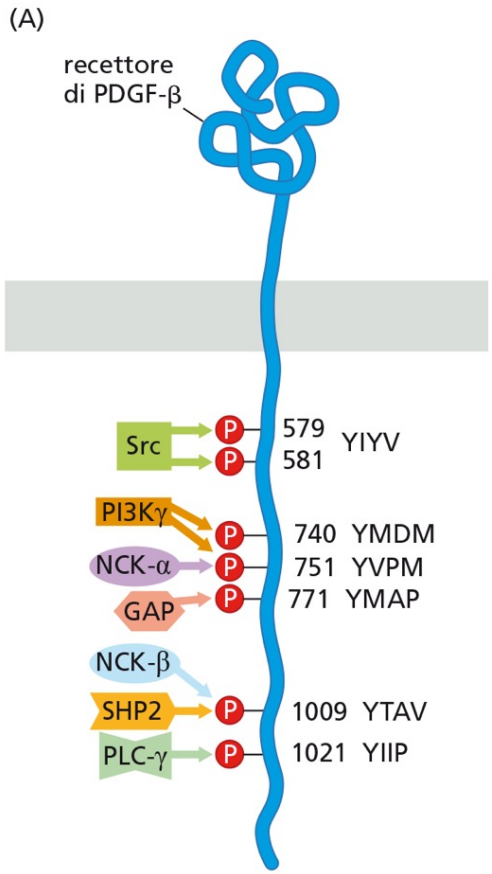


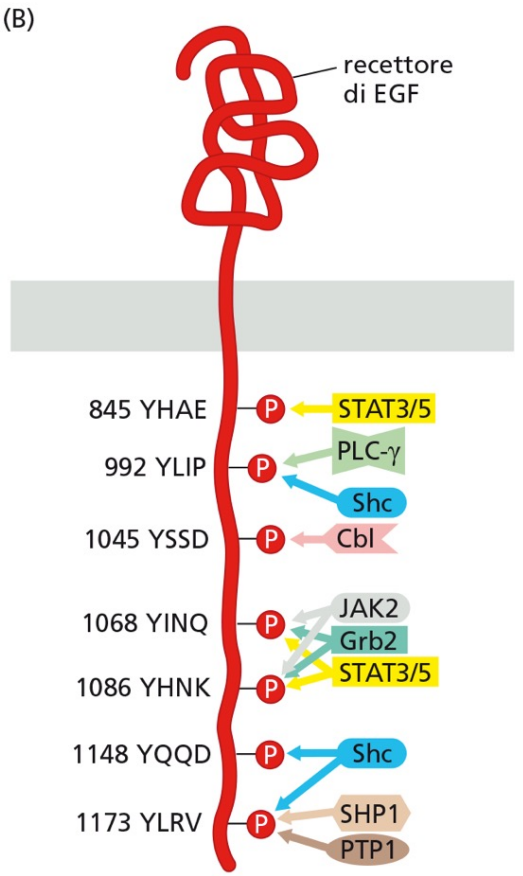
Weinberg, la Biologia del cancro

Dominio SH2









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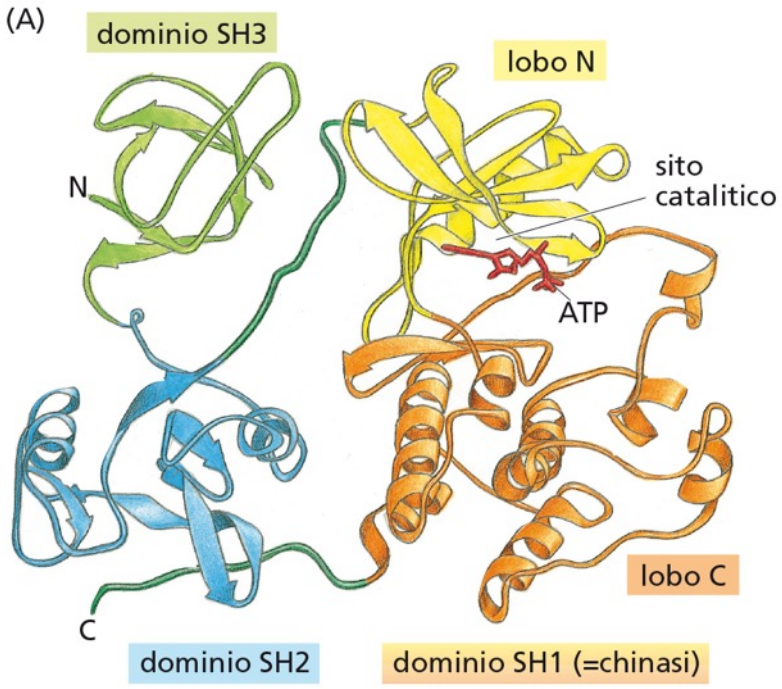
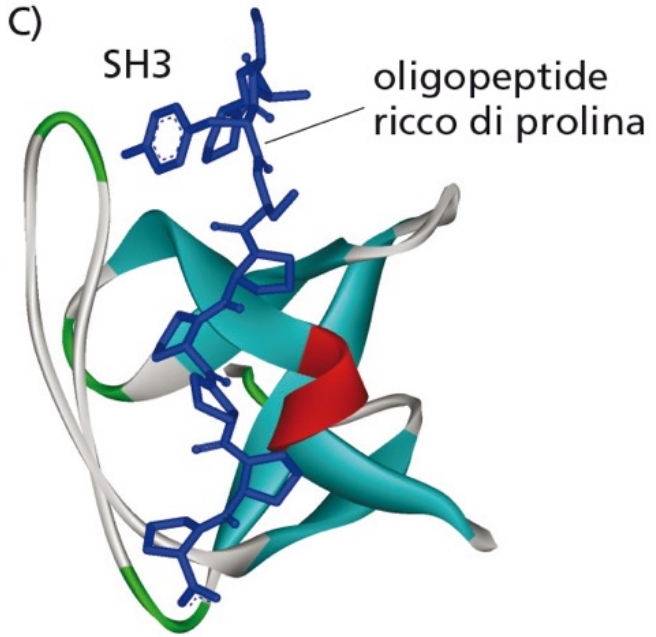
Dominio SH3

In genere legano domini ricchi di Prolina ma non sempre

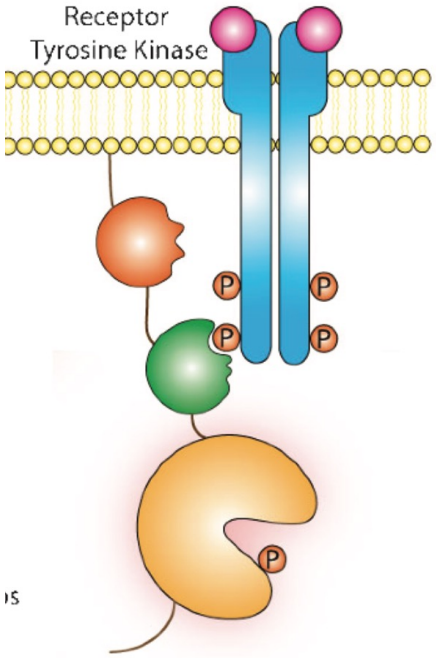
Nell'uomo ci sono 253 domini SH3

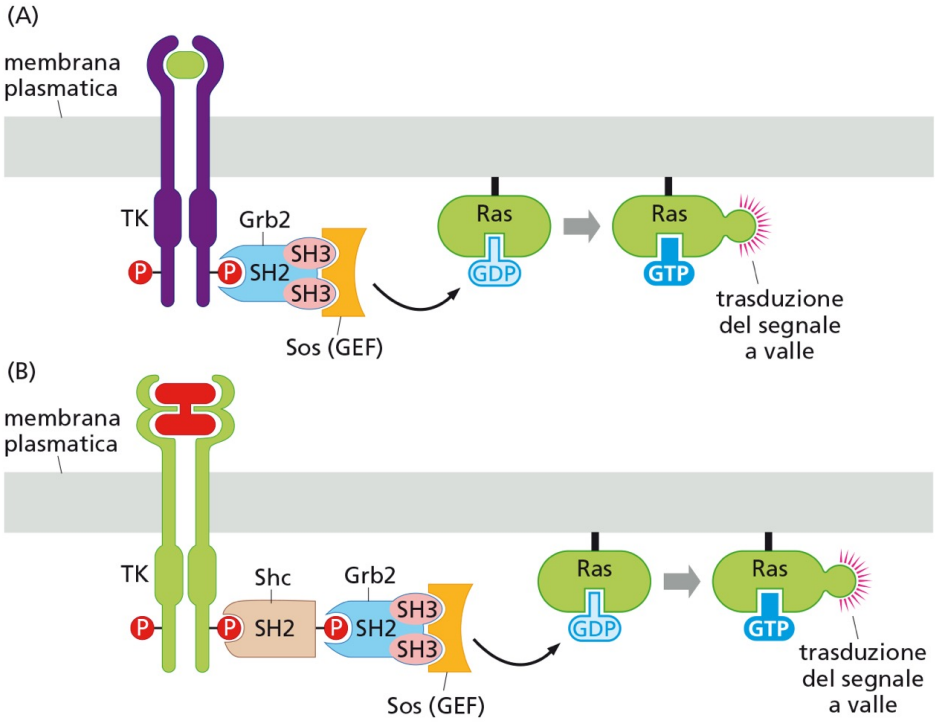


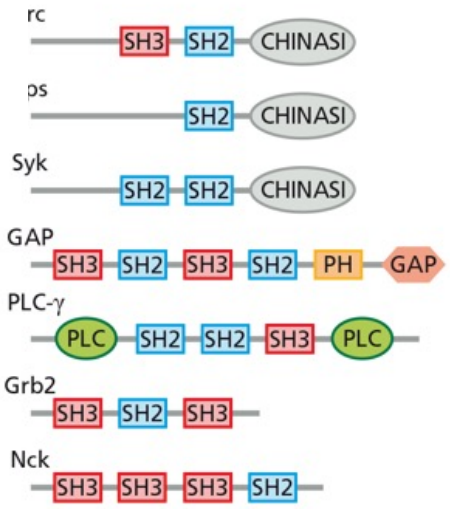


Weinberg, la Biologia del cancro

Proteine con domini SH2 e SH3 e attivazione di Ras



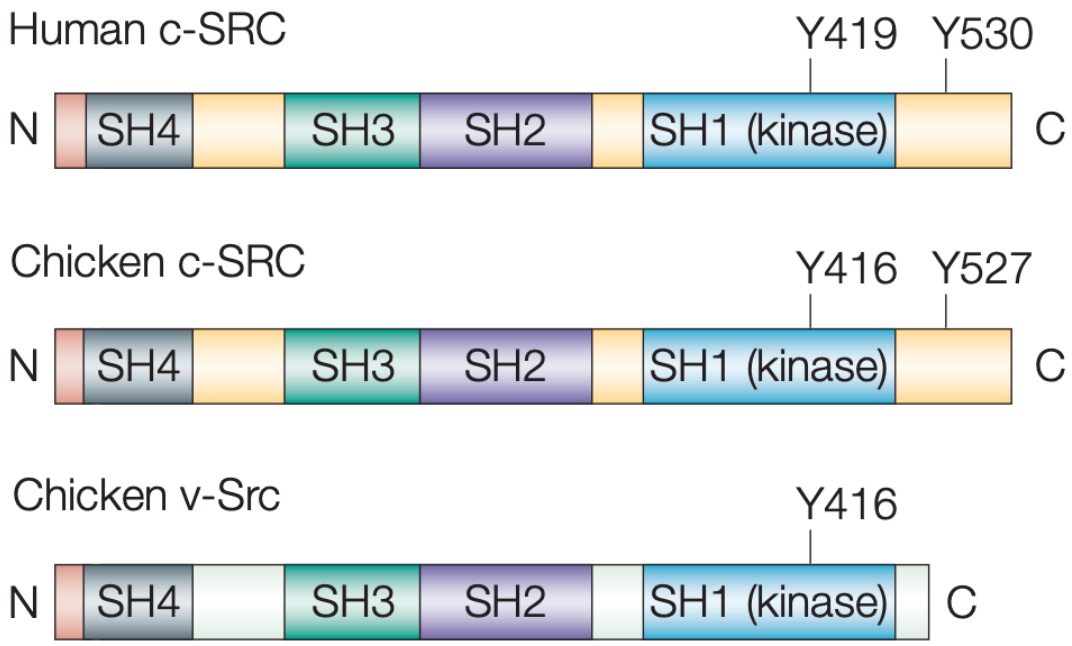




Weinberg, la Biologia del cancro

Clonaggio e caratterizzazione Src

- *v-src* con mitazioni in dominio SH1 catalitico non trasforma
- *c-src* anche se sovraespresso non trasforma

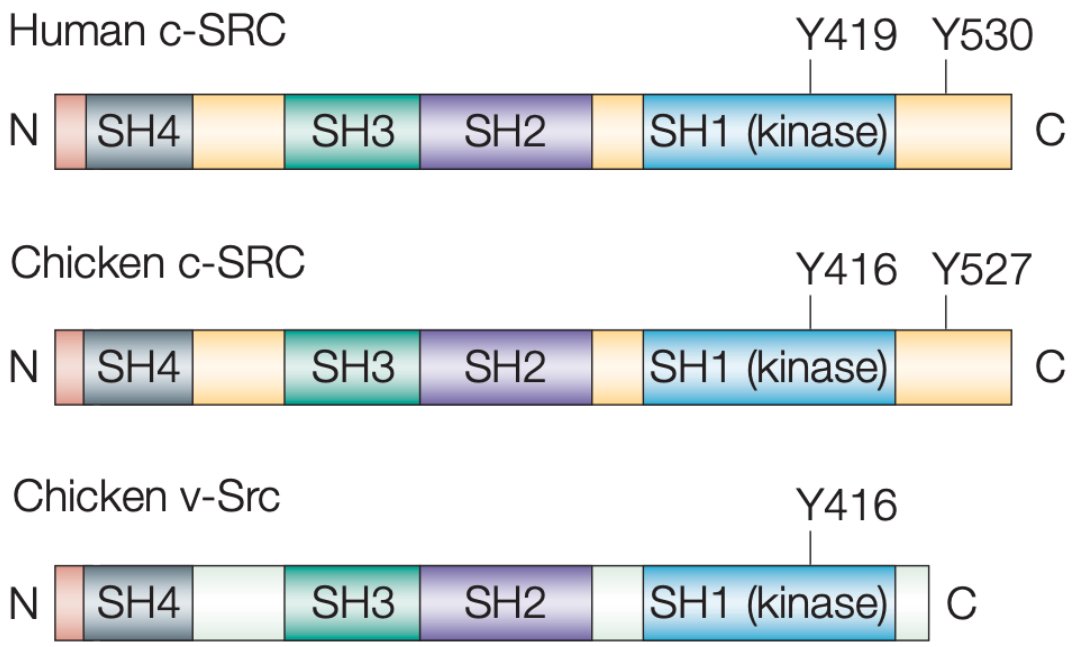


Yeatman, 2004; Aleshin & Finn, 2010

Clonaggio e caratterizzazione Src

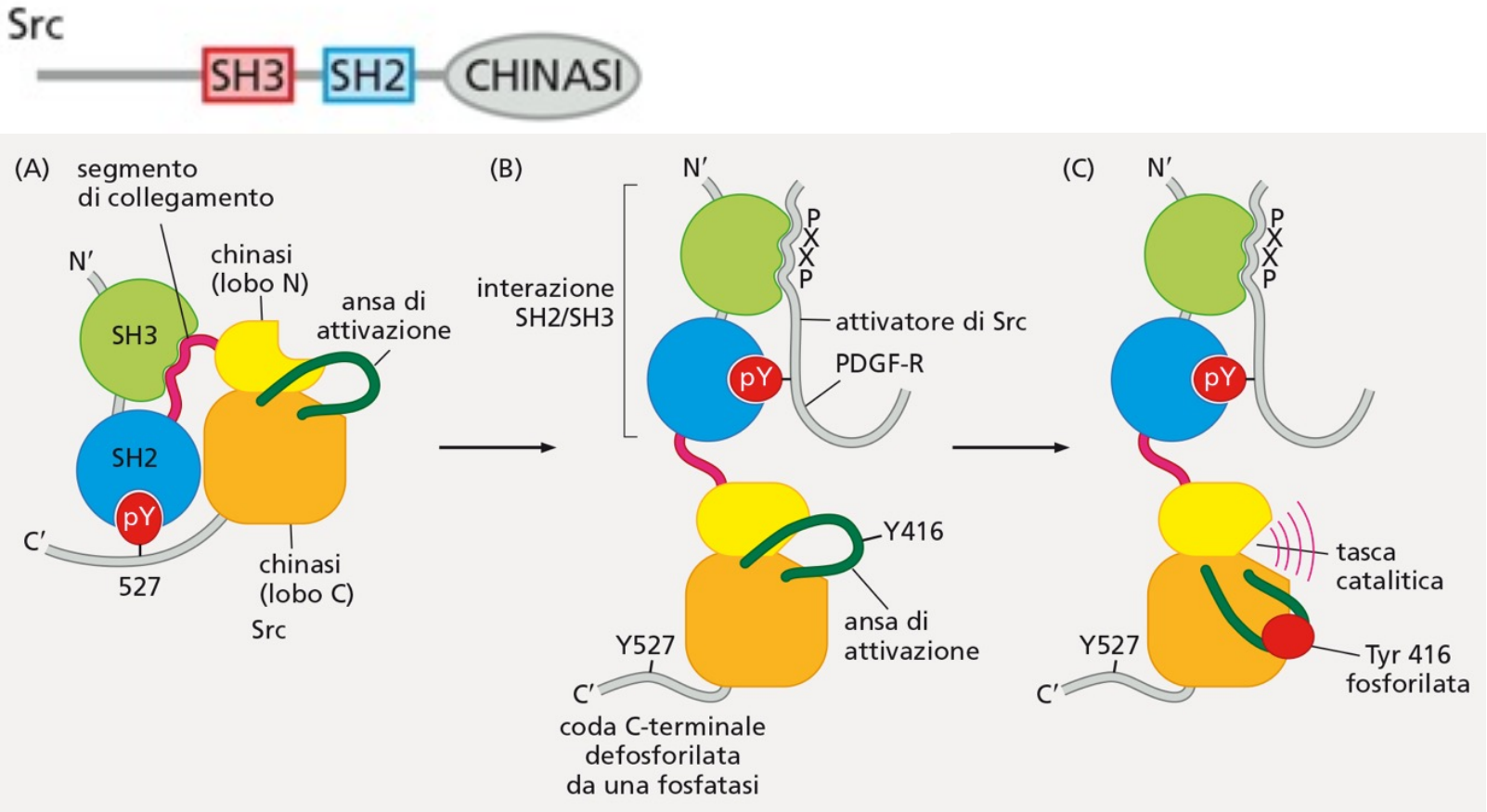
533 aa, 60 kDa

four SRC homology (SH) domains and a C-terminal segment containing a negative regulatory tyrosine residue (Tyr530)



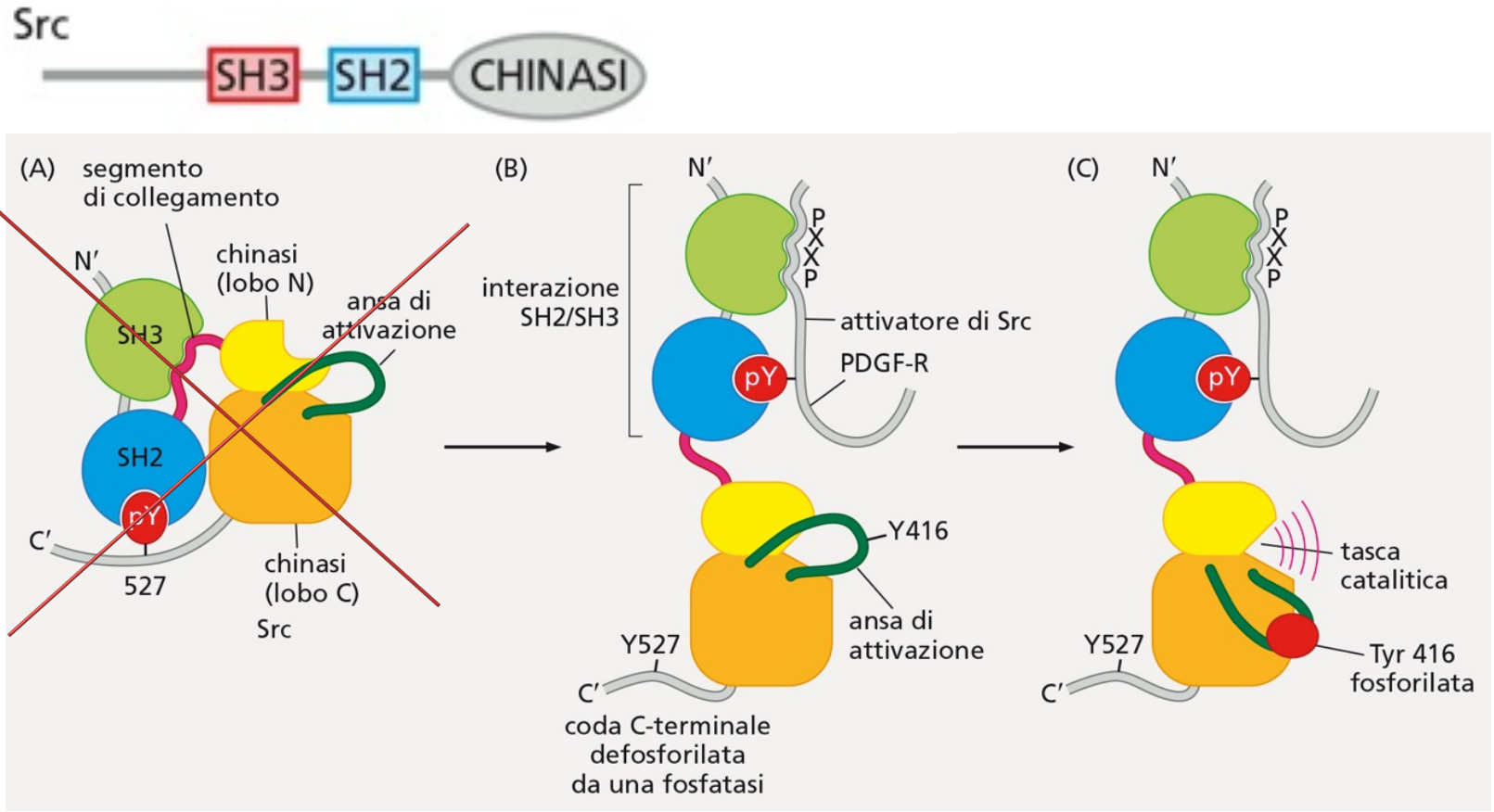
Yeatman, 2004; Aleshin & Finn, 2010

Regolazione di Src



Weinberg, la Biologia del cancro

Src e la sua attivazione a oncoproteina



Weinberg, la Biologia del cancro

Src e la sua attivazione a oncoproteina

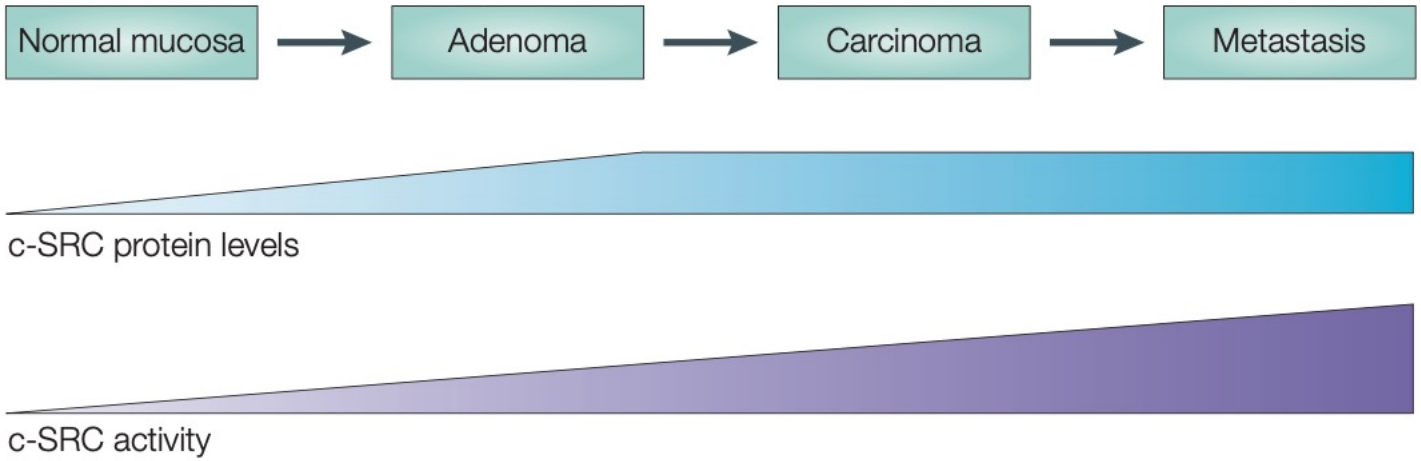
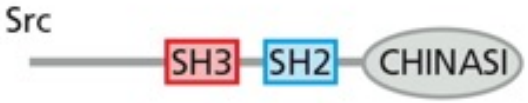


Figure 4 | **Levels of c-SRC expression and activity in colorectal cancer progression.** Sporadic colon cancer is frequently the result of the sequential accumulation of numerous genetic alterations, with neoplastic progression from normal mucosa, to formation of polyps (adenomas), to invasive cancer (carcinoma), which then metastasizes. Both c-SRC overexpression and c-SRC activation, with increased specific activity, are prominent features of the adenoma–carcinoma sequence. Both c-SRC overexpression and activation seem to be early features of adenoma formation, whereas continuing increases in c-SRC activity are seen with progression to cancer and later metastatic stages.

Aleshin & Finn, 2010

Src e la sua attivazione a oncoproteina



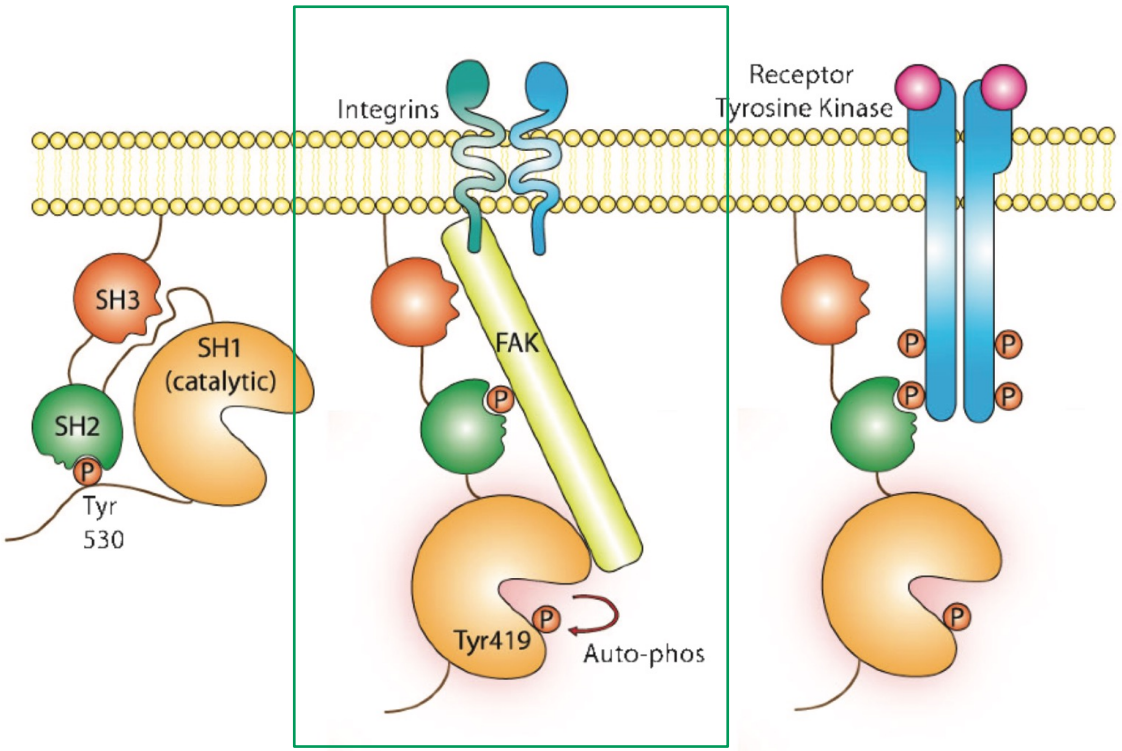
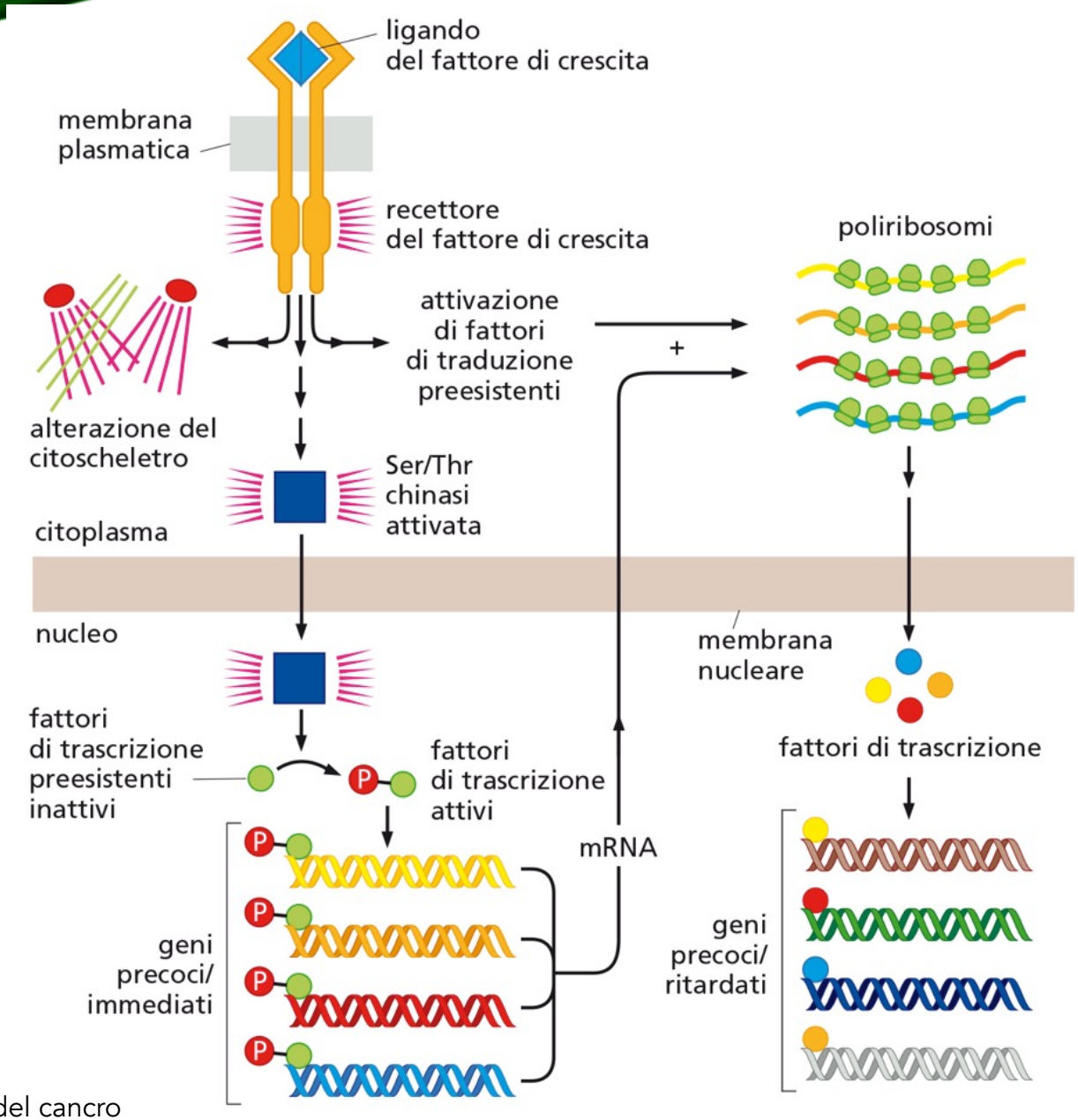


Figure 1. Methods of SRC activation and inactivation. Phosphorylation of Tyr530 at the C-terminus locks the protein in a closed, inactive conformation stabilized through interactions between the SH3 and kinase domains. Dephosphorylation of Tyr530 and autophosphorylation of Tyr419 within the catalytic domain allow SRC to assume an open, active conformation. SRC activity is also regulated by receptor tyrosine kinases and direct binding of FAK to the SH2 domain.

Aleshin & Finn, 2010

Vie di trasduzione del segnale

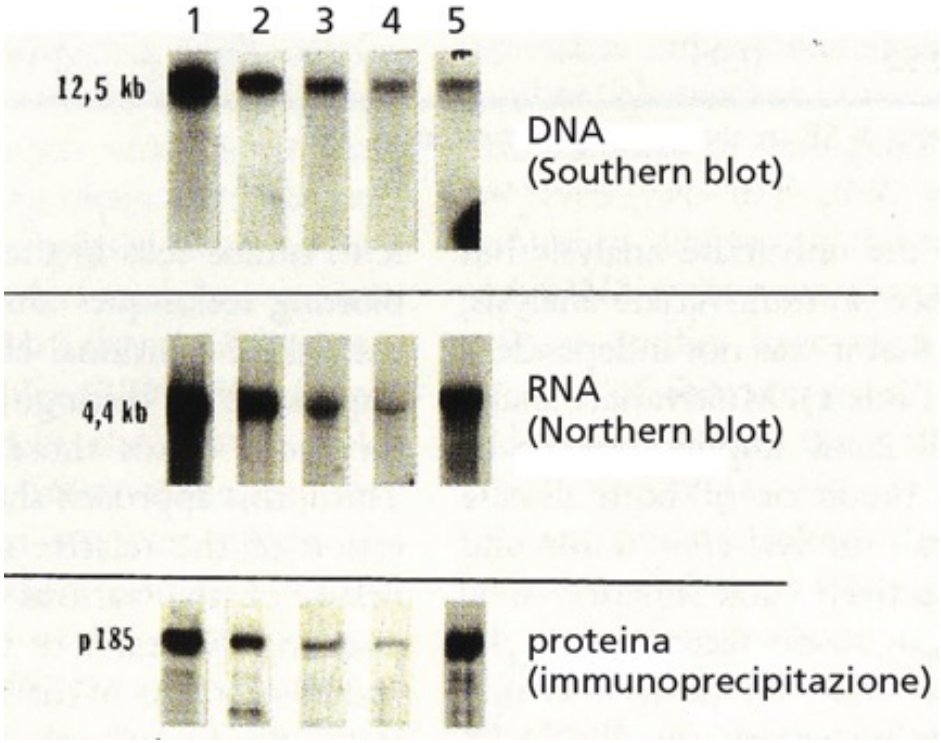


Weinberg, la Biologia del cancro

ONCOGENE HER2

Aumento dell'espressione genica di *erbB2/neu/HER2*

Oncogeni associati a retrovirus presenti in numero di copie aumentato in tumori umani: oltre a *myc*, *erbB* (eritroblastosi aviaria, amplificato in tumori gastrici, mammari e cerebrali).
HER2: appartiene a famiglia *erbB* ed è amplificato in molti tumori mammari umani

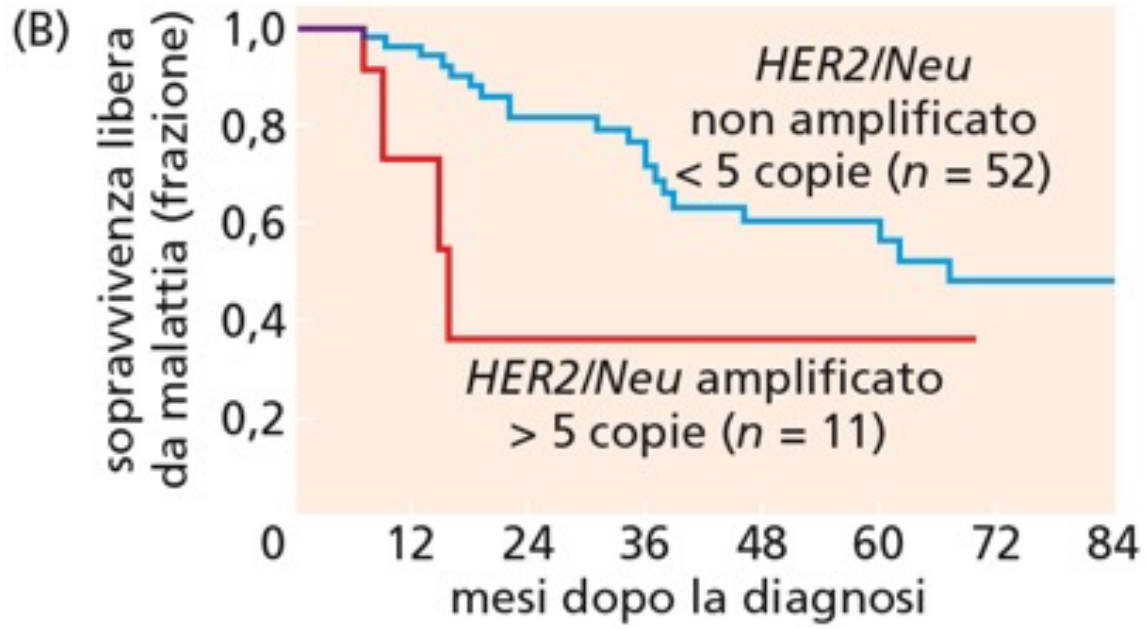


Amplificazione *HER2* in 30% dei casi di carcinoma mammario umano

Analisi di 5 tumori mammari indicano diverse vie di iperattivazione di HER2

Weinberg, la Biologia del cancro

Aumento dell'espressione genica di *erbB2/neu/HER2*

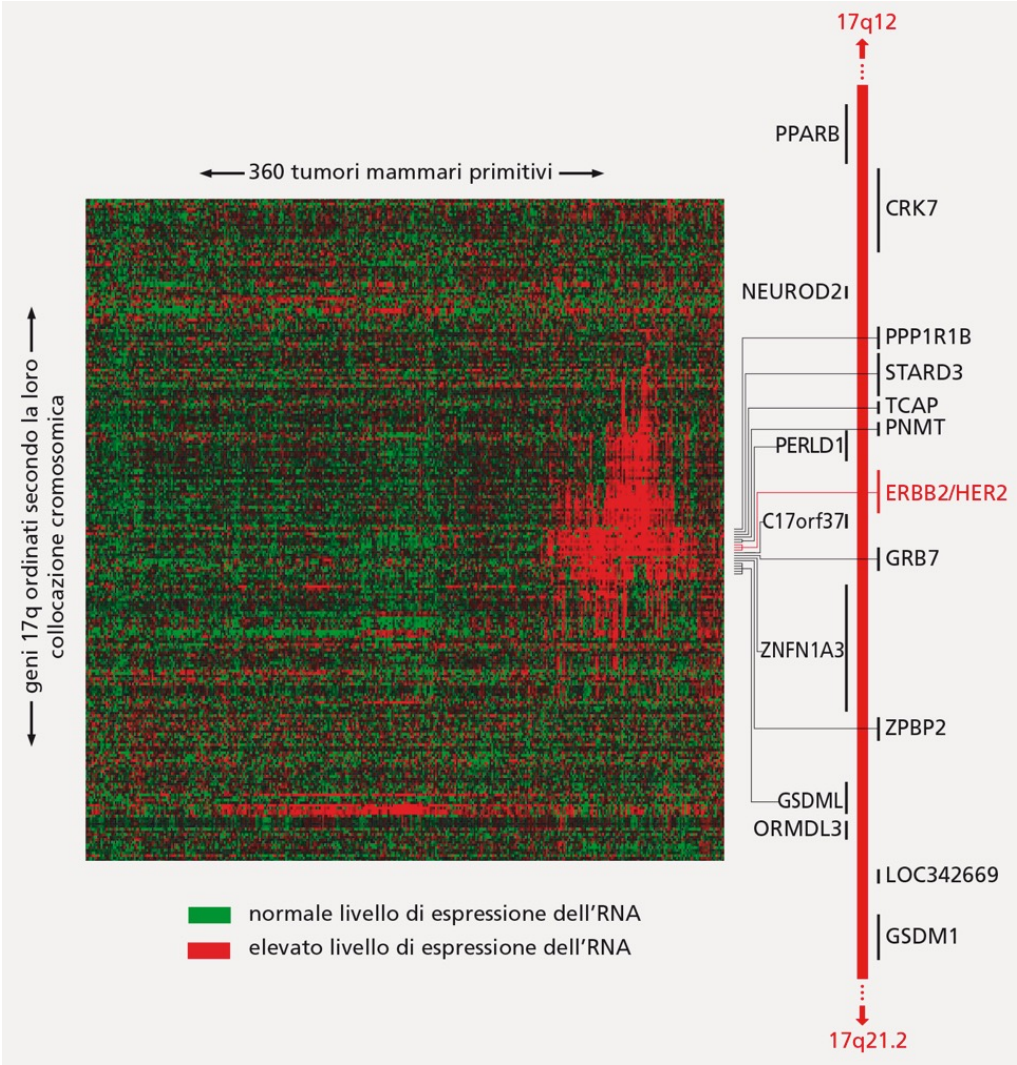


Amplificazione *HER2* nel carcinoma mammario umano associato a cattiva prognosi

Amplificazione HER2 induce proliferazione ma protegge anche da apoptosi

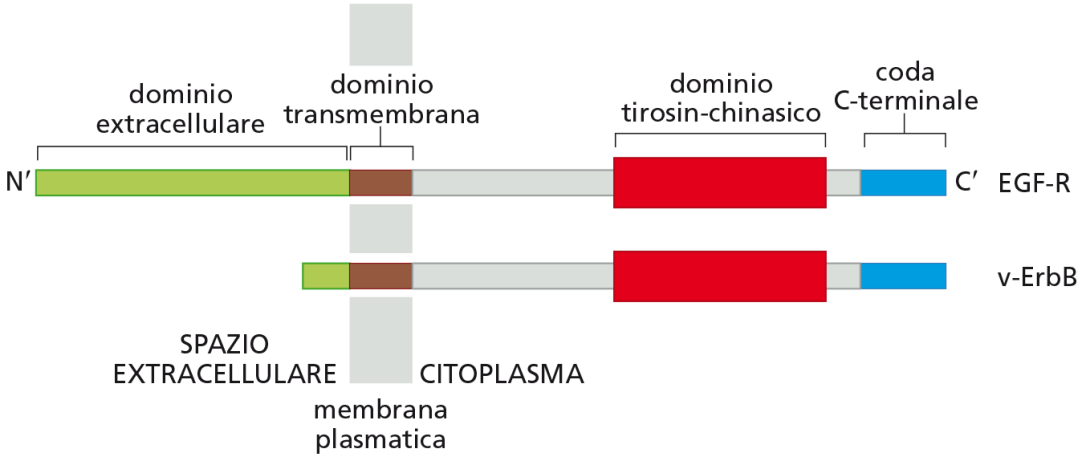
Weinberg, la Biologia del cancro

Aumento dell'espressione genica di *erbB2/neu/HER2*



Oncogene *erbB*

- Oncogene virale *erbB* identificato nel virus dell'eritroblastosi aviaria (AEV)
- 1984: sequenza *erbB* simile a recettore EGF
- HER2 is the product of the *erbB2* gene (chromosome 17), cloned in 1983 (Sato et al., 1983).

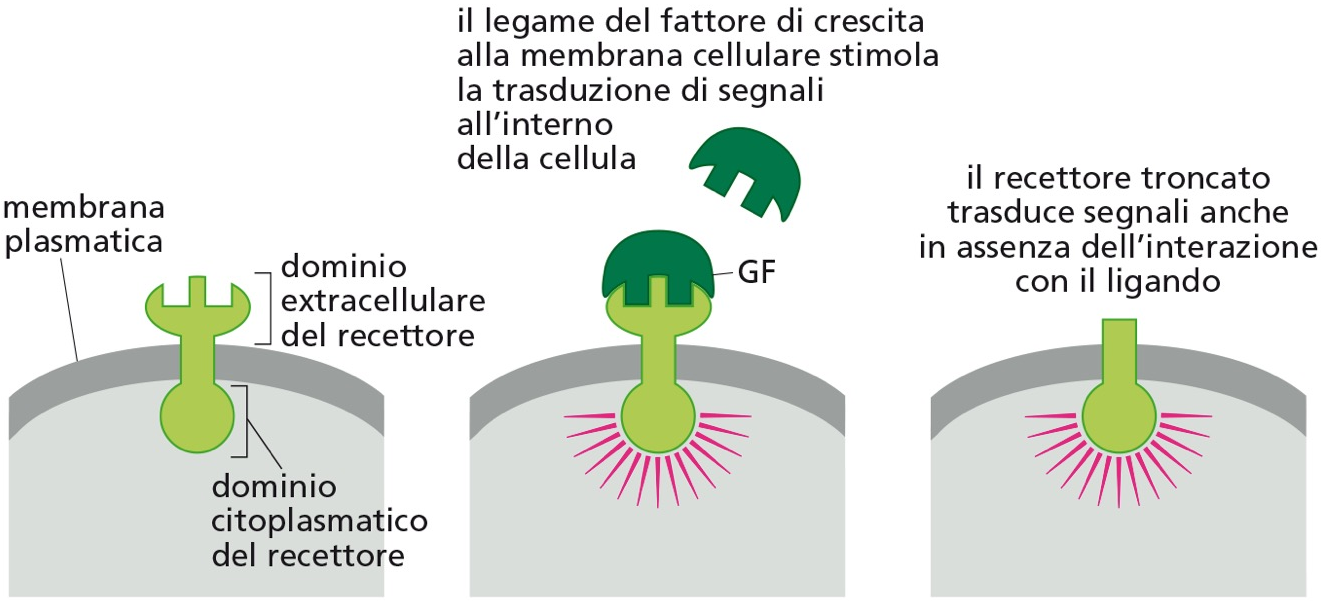


- In *erbB* manca sequenza che interagisce con EGF
- Stimolazione costitutiva della via di trasduzione del segnale indipendente da EGF

Weinberg, la Biologia del cancro

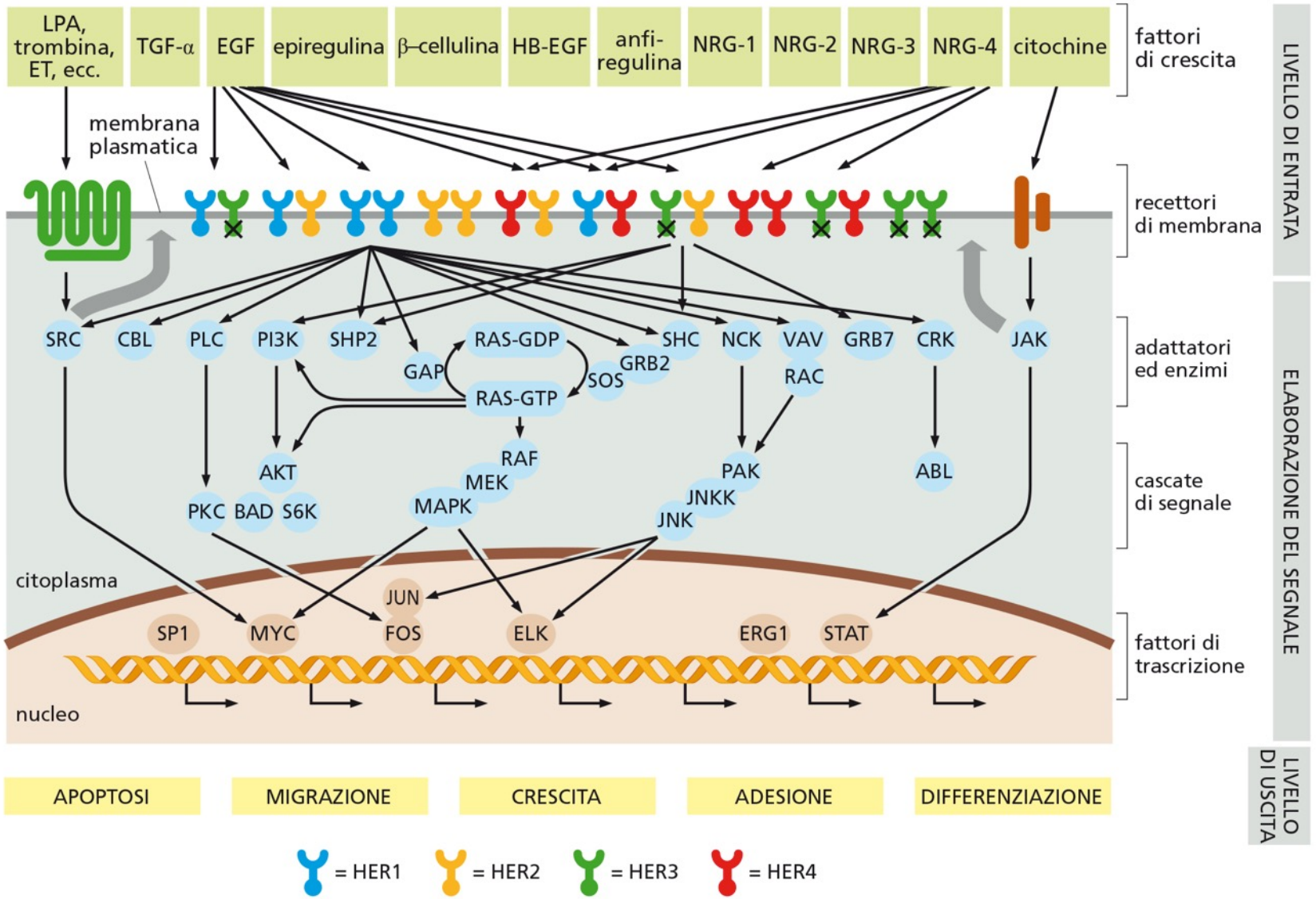
Mutazioni che producono proteine tronche

- Caratteristiche di molti oncogeni per recettori: perdita di regolazione da ligando



- Delezione regioni regolative: es. domini inibitori o riconosciuti da inibitori

Recettori per EGF: HER



Weinberg, la Biologia del cancro

erbB and HER2

- HER2 activating mutations act as oncogenic drivers in various cancer types in humans
- causative for cancer initiation and progression in multiple tumor types

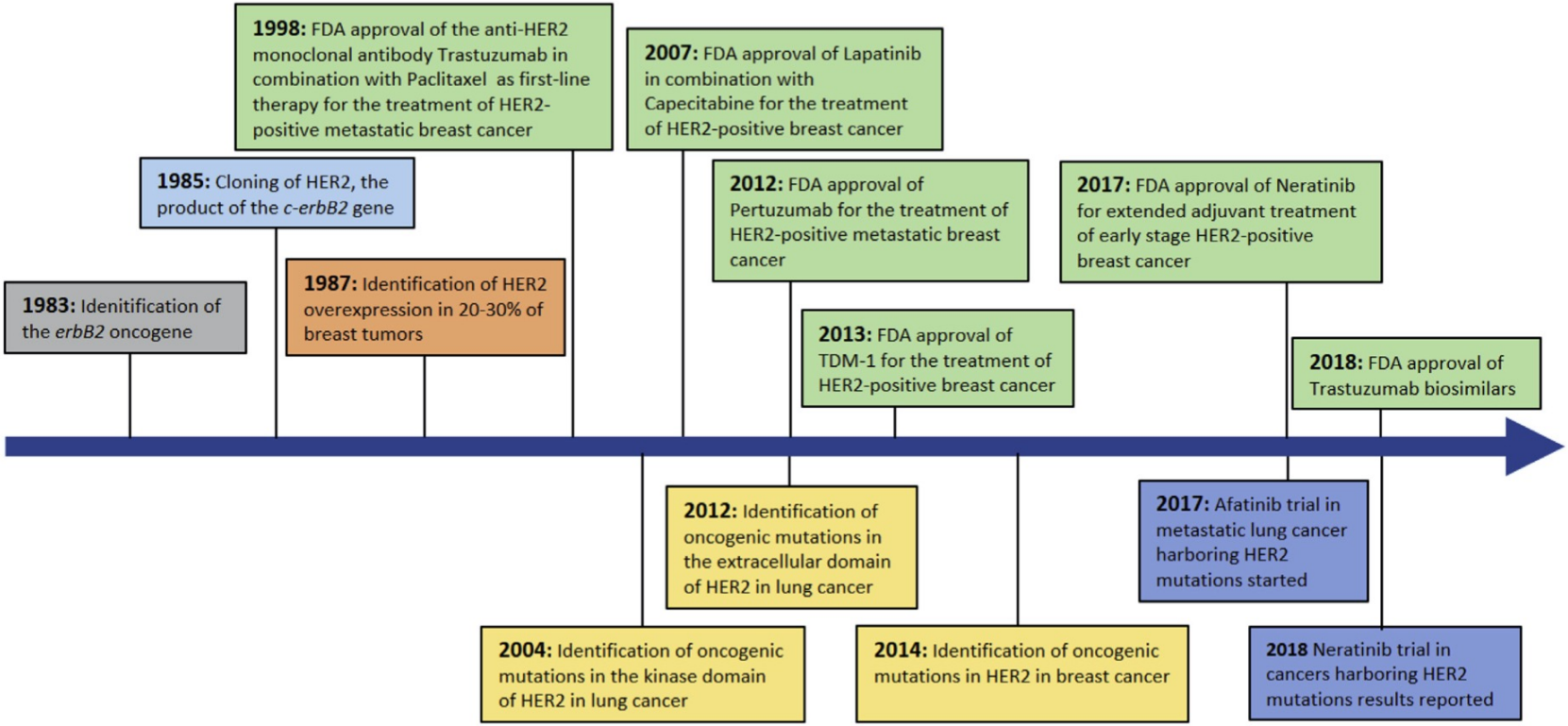


Fig. 1. Timeline of key advances relating to the biology and therapeutic targeting of HER2 signaling. Milestone discoveries that are relevant to HER2 amplified/overexpressing (boxes above the timeline arrow) and HER2 mutant tumors (boxes below the timeline arrow). Key events relating to the following fields of study are color coded as follows: FDA approval of anti-HER2 drugs (green); identification of HER2 mutation in cancers (yellow); clinical trial enrolling HER2 mutated cancers (blue).

Cocco et al., 2019