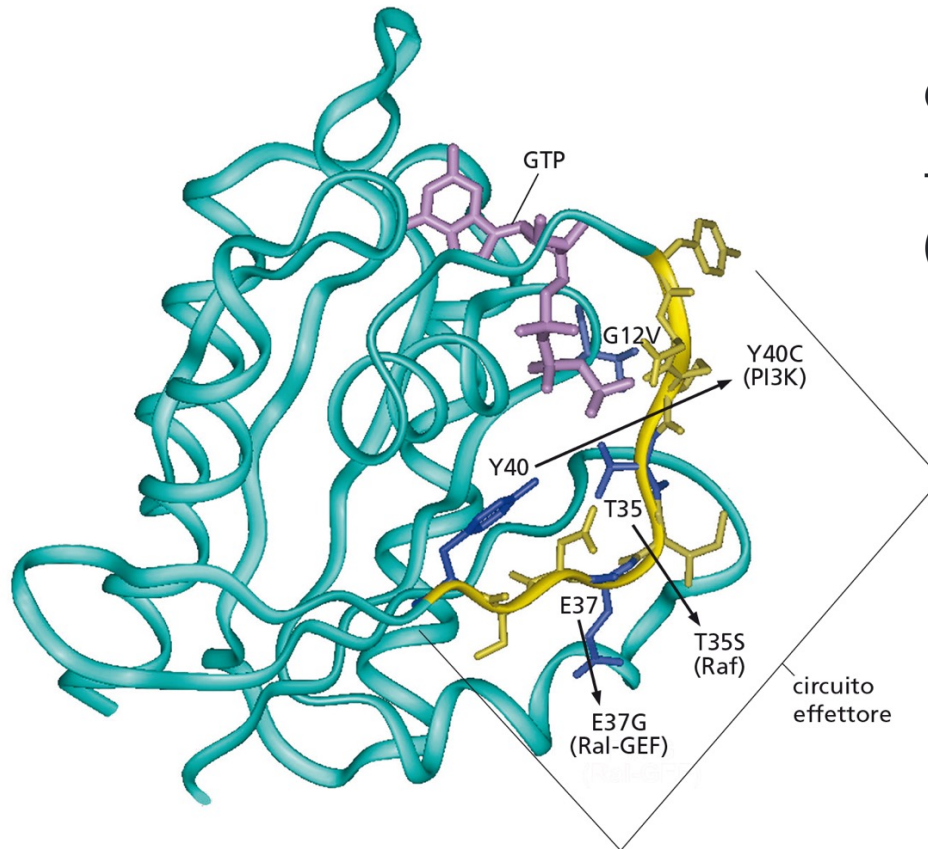


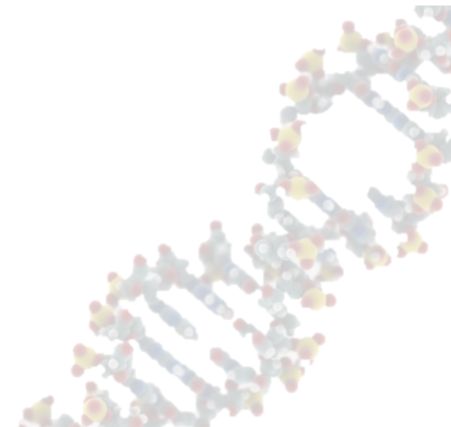
LE VIE ATTIVATE DA RAS



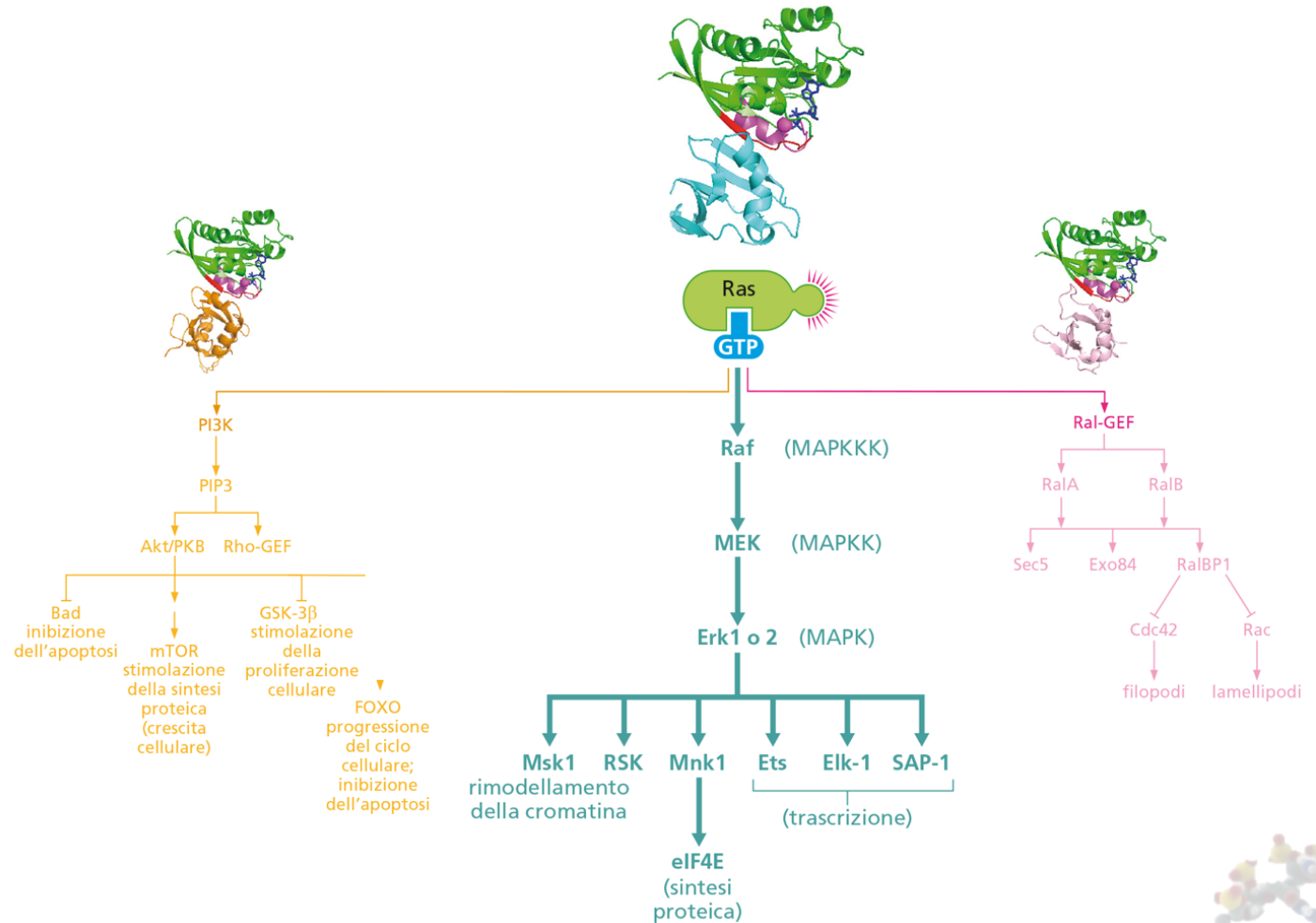
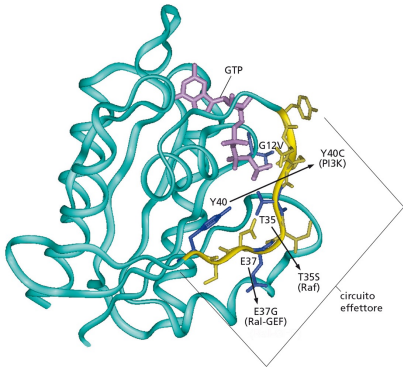
RAS



Circuito effettore di Ras
- Interagisce con diverse proteine
(almeno 3): EFFETTORI di Ras

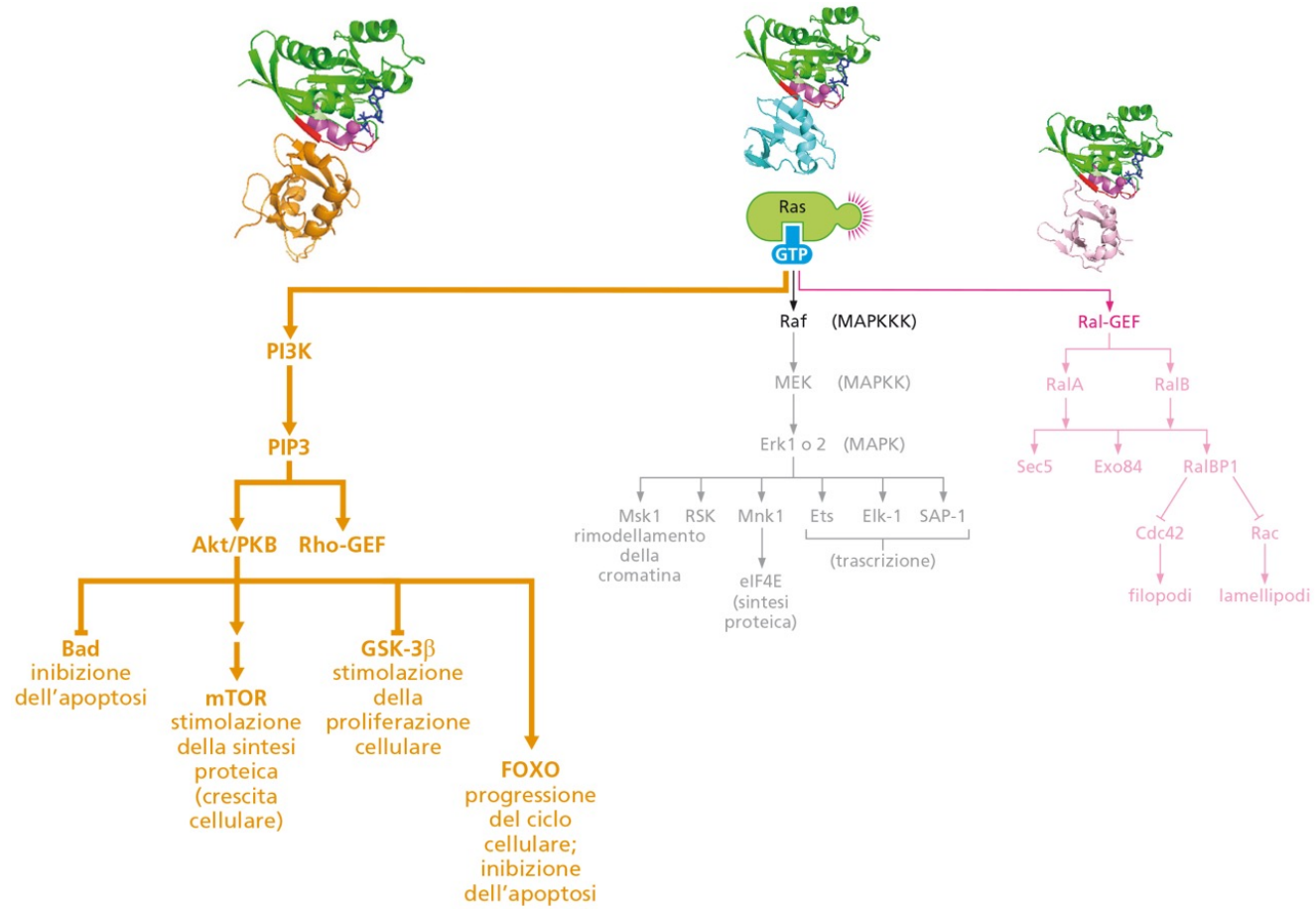
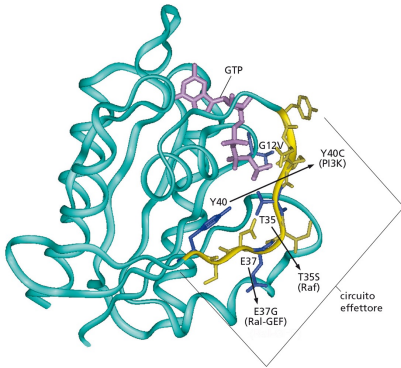


Ras e la via delle MAPK



Attiva geni precoci Jun e Fos che insieme formano fattore trascrizionale AP-1
 Attivazione questa via causa indipendenza dall'ancoraggio e perdita di inibizione da contatto. Molto attiva nei tumori

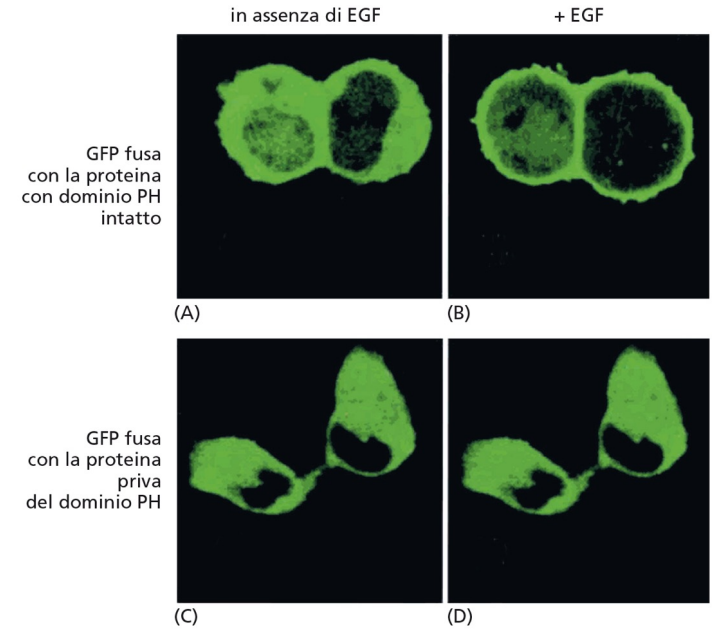
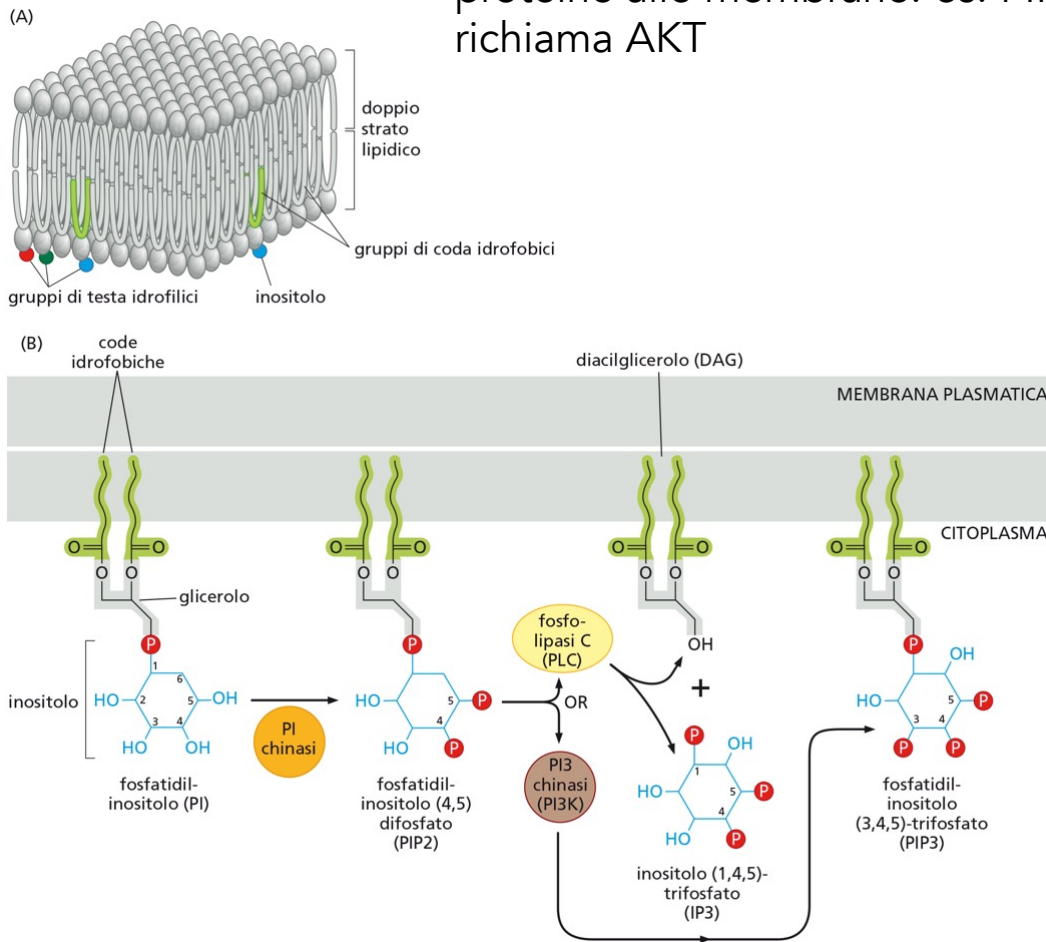
Ras e la via delle PI3K



Inositolo ha ruolo centrale nella regolazione delle membrane oltre a quello strutturale: se fosforilato si stacca dalle membrane e può funzionare da secondo messaggero. Attivazione questa via sopprime apoptosi

Ras e la via delle PI3K

A seconda dello stato di fosforilazione, l'inositolo fosforilato può funzionare da secondo messaggero o richiamare altre proteine alle membrane: es. PIP3 richiama AKT



AKT lega PIP3 attraverso dominio PH e controlla la crescita cellulare, l'apoptosi

Ras e la via delle PI3K

AKT controlla la crescita e la proliferazione cellulare, l'apoptosi

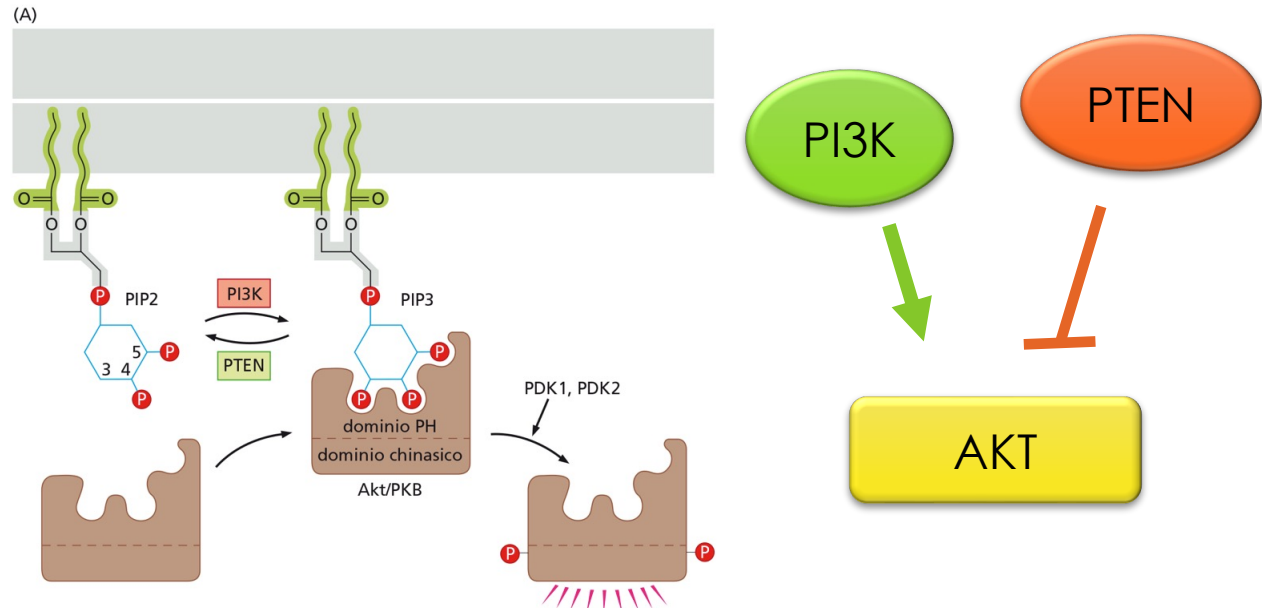
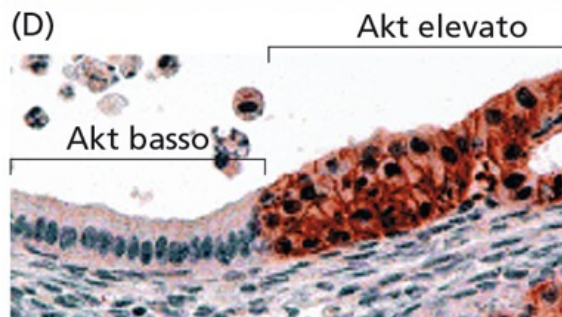
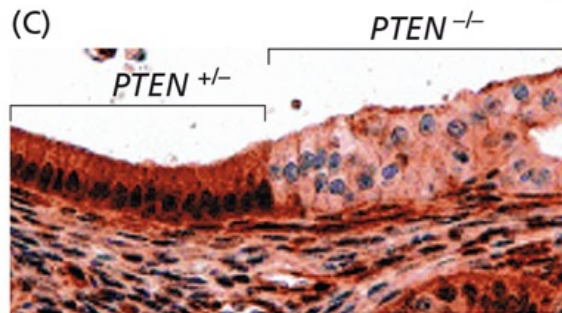
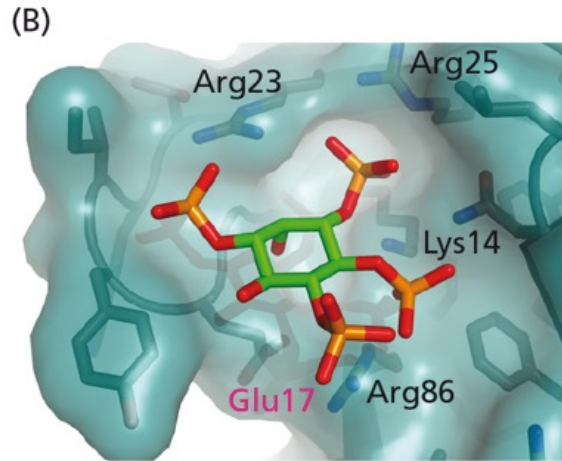
TABELLA 6.3

Effetti di Akt/PKB sulla sopravvivenza, la proliferazione e la crescita cellulare

Effetto biologico	Substrato di Akt/PKB	Descrizione	Conseguenza funzionale
Antiapoptotico			
	Bad (proapoptotico)	Antagonista di Bcl-X; come Bad, appartiene alla famiglia di proteine Bcl-2 che controllano i pori della membrana mitocondriale (Sezione 9.13).	inibizione
	caspiasi-9 (proapoptotico)	Componente della cascata di proteasi che regolano il programma dell'apoptosi (Sezione 9.13).	inibizione
	I κ B chinasi, abbreviata IKK (antiapoptotica)	Attivata dalla fosforilazione di Akt/PKB (Sezione 6.12)	attivazione
	Fattore di trascrizione (TF) FOXO1, in precedenza chiamato FKHR TF (proapoptotico)	La fosforilazione impedisce la sua traslocazione nucleare e l'attivazione di geni proapoptotici.	inibizione
	Mdm2 (antiapoptotico)	Attivato tramite fosforilazione da parte di Akt/PKB; innesca la distruzione di p53 (Sezione 9.7).	attivazione
Proliferativo			
	GSK-3 β (antiproliferativo)	Fosforila β -catenina, ciclina D1 e Myc (Sezioni 7.11, 8.3, 8.9), causando la loro degradazione; inattivato tramite fosforilazione da parte di Akt/PKB.	inibizione
	FOXO4, in precedenza chiamato AFX (antiproliferativo)	Induce l'espressione dell'inibitore di CDK p27 ^{Kip1} (Sezione 8.4) e di alcuni geni proapoptotici; viene esportato dal nucleo a seguito della fosforilazione da parte di Akt/PKB.	inibizione
	p21 ^{Cip1} (antiproliferativo)	Inibitore di CDK, come p27 ^{Kip1} (Sezione 8.4). Lascia il nucleo a seguito della fosforilazione da parte di Akt/PKB; nel citoplasma, la forma fosforilata di p21 ^{Cip1} inibisce le caspasi, acquisendo pertanto funzioni antiapoptotiche (Sezione 9.13).	inibizione
Crescita			
	Tsc2 (anticrescita)	La sua fosforilazione da parte di Akt/PKB fa sì che il complesso Tsc1/Tsc2 si dissoci, permettendo l'attivazione di mTOR, che quindi aumenta la sintesi proteica (Sezione 16.15).	inibizione

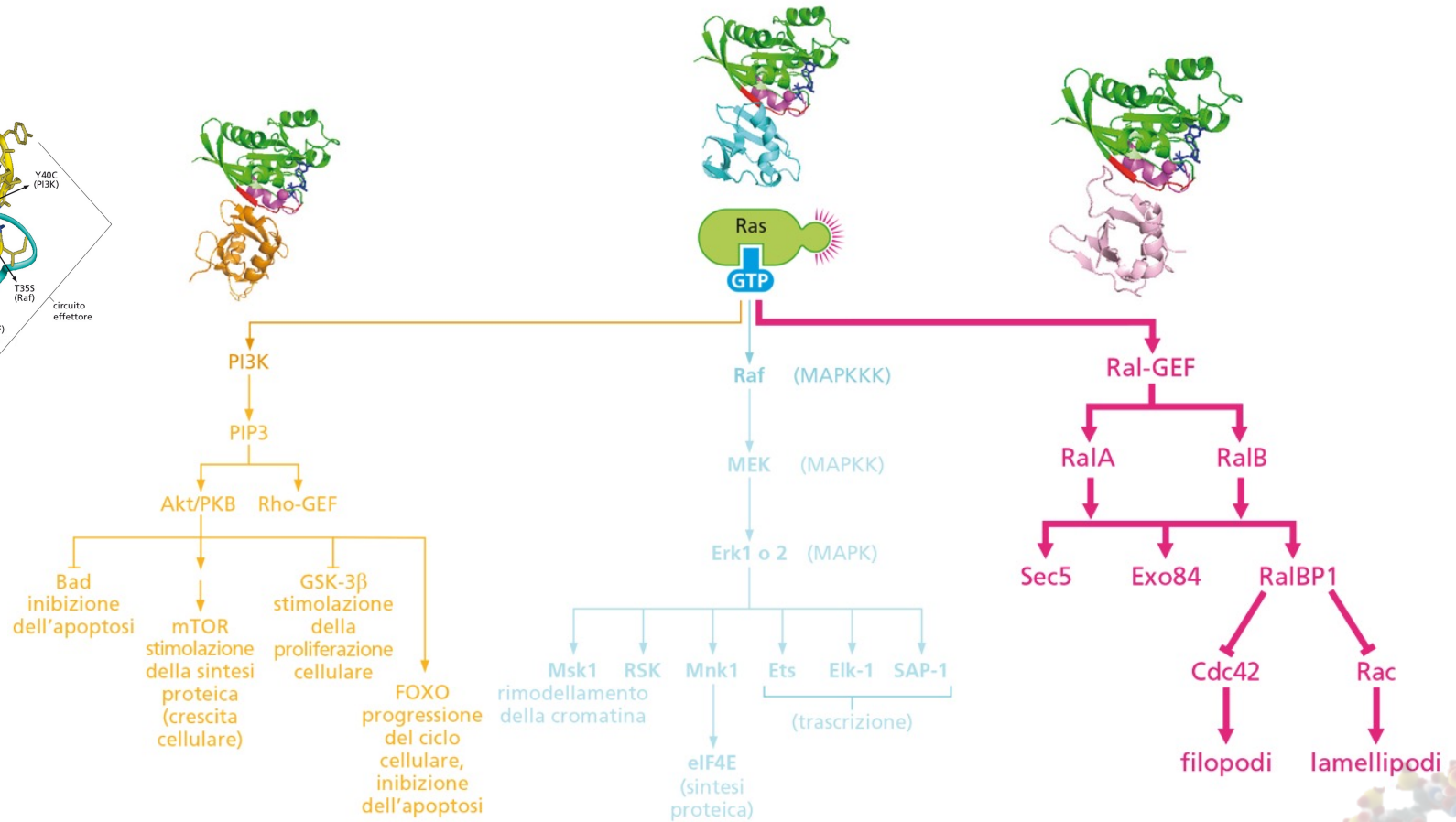
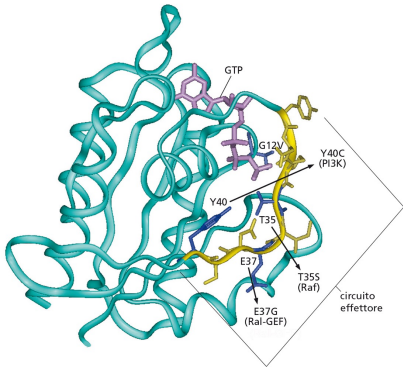
Ras e la via delle PI3K

AKT controlla la crescita e la proliferazione cellulare, l'apoptosi



Attività di AKT è molto regolata ed è deregolata in cellule tumorali

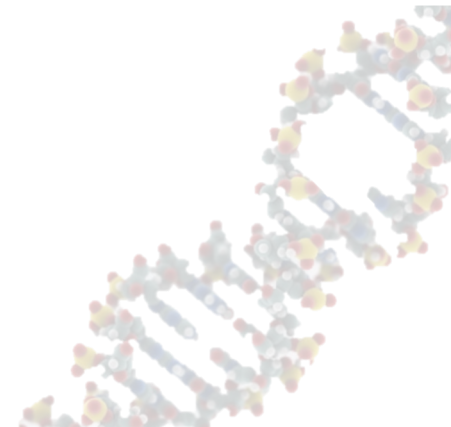
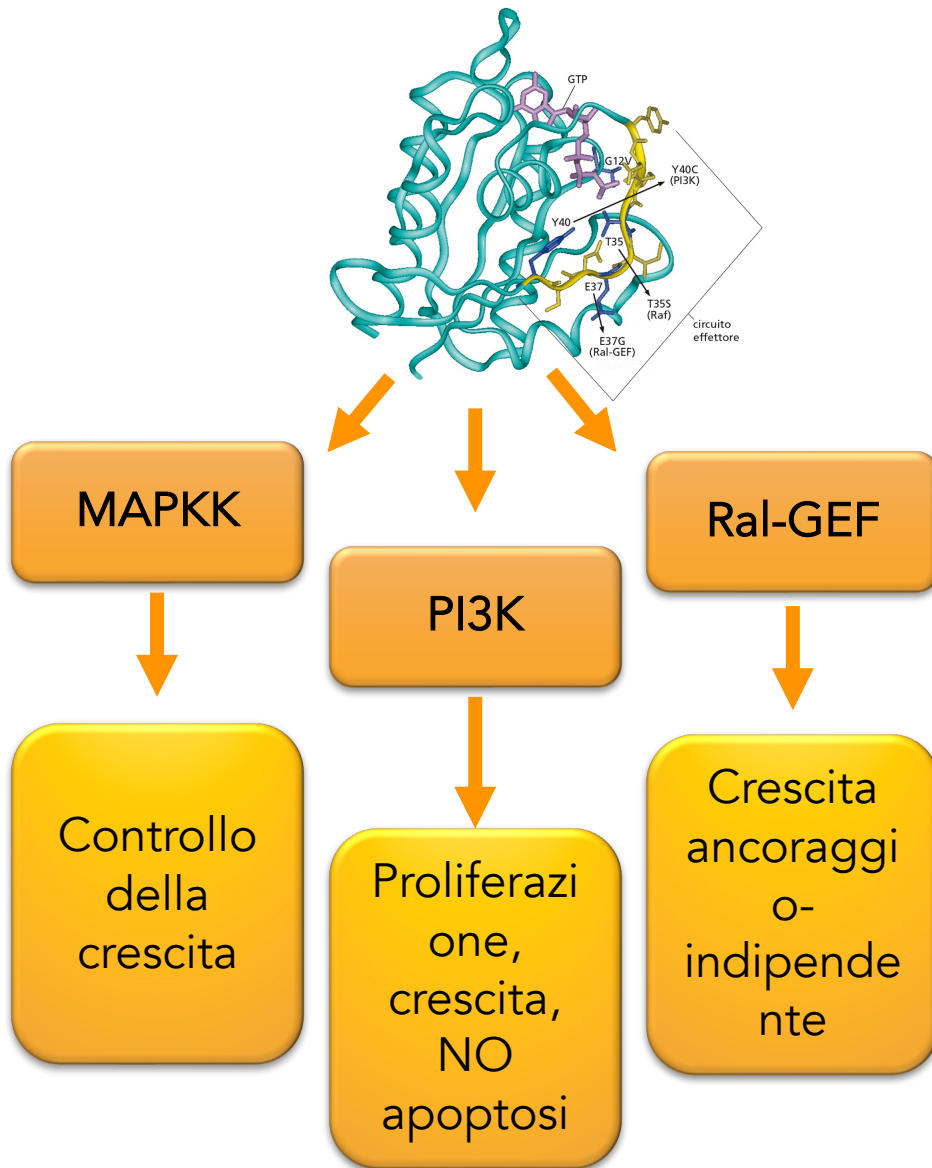
Ras e la via di Ral



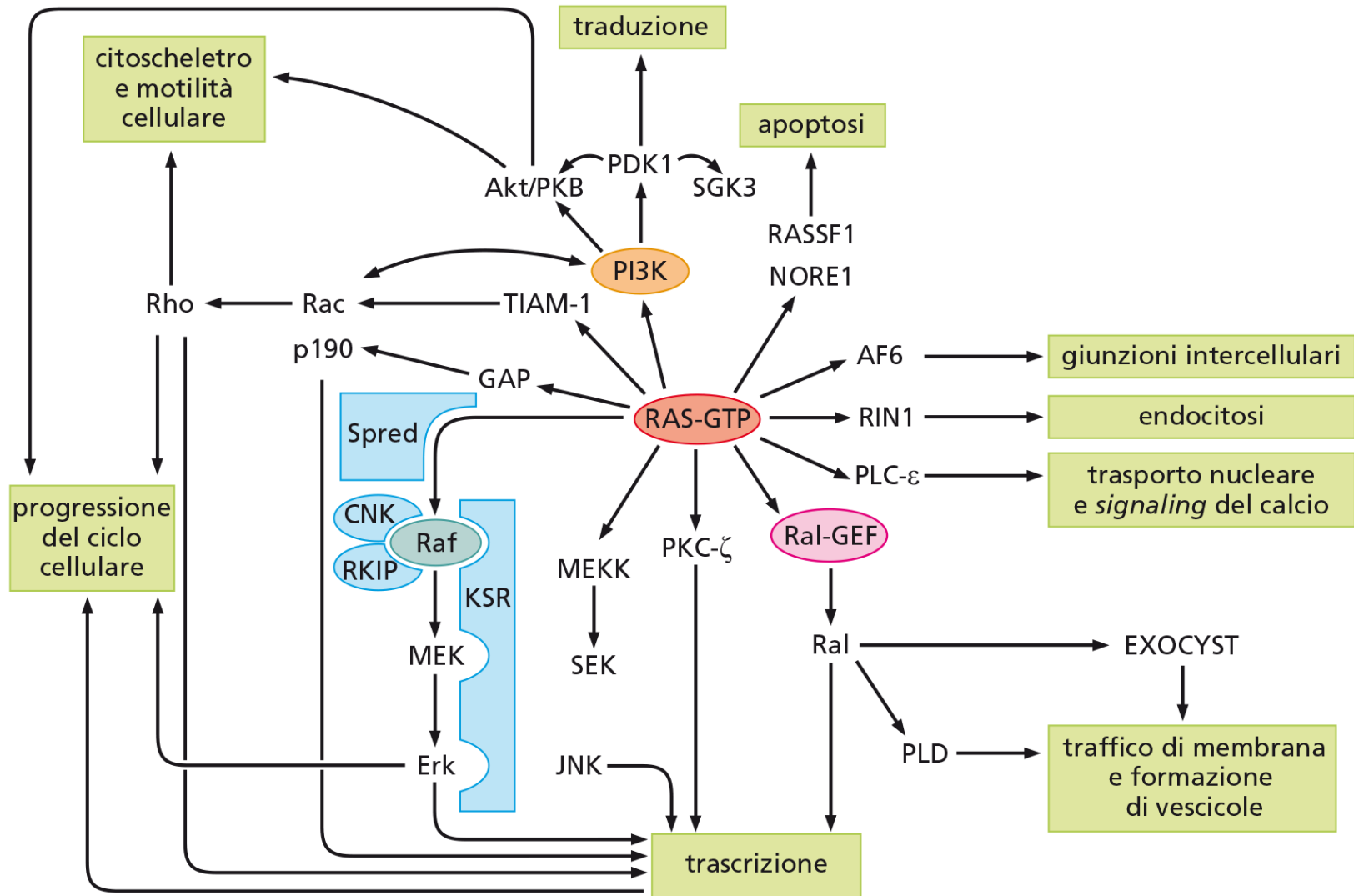
Ral è G protein come Ras
Regola crescita ancoraggio-indipendente

Effetto pleiotropico di Ras

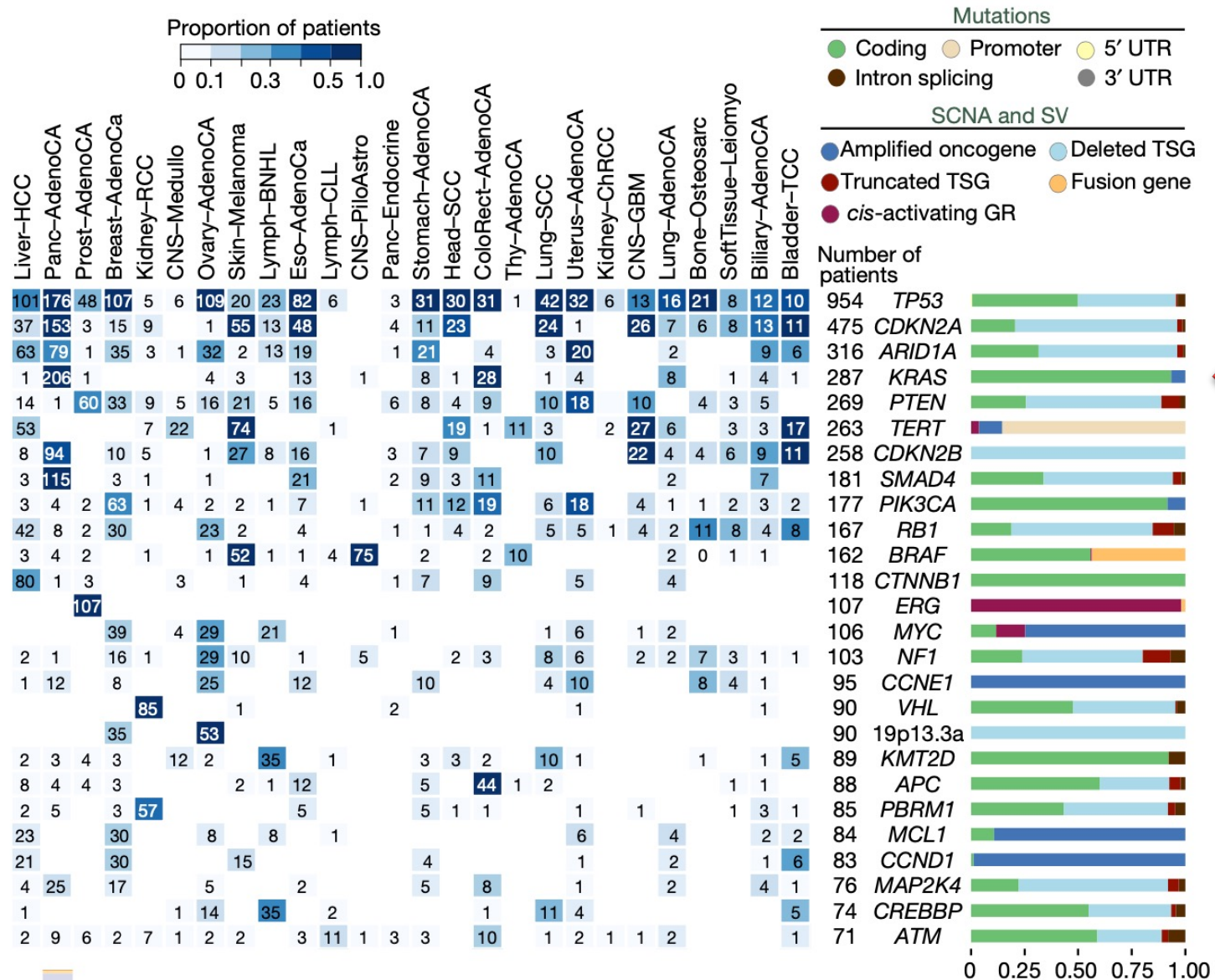
Molti oncogeni anche nelle via e valle di Ras, ma con potenziale oncogenico < a quello di Ras



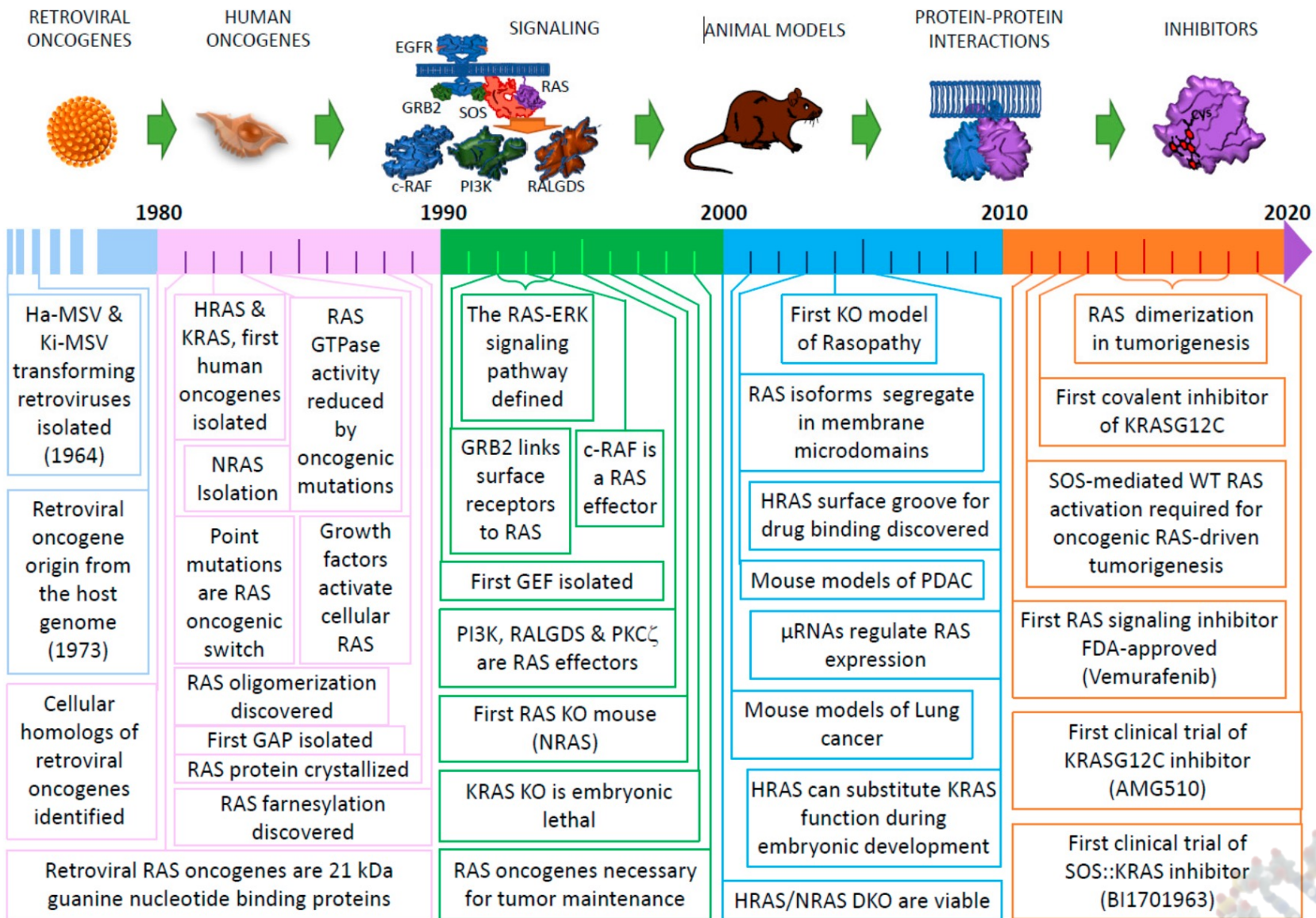
Le vie effettrici di Ras



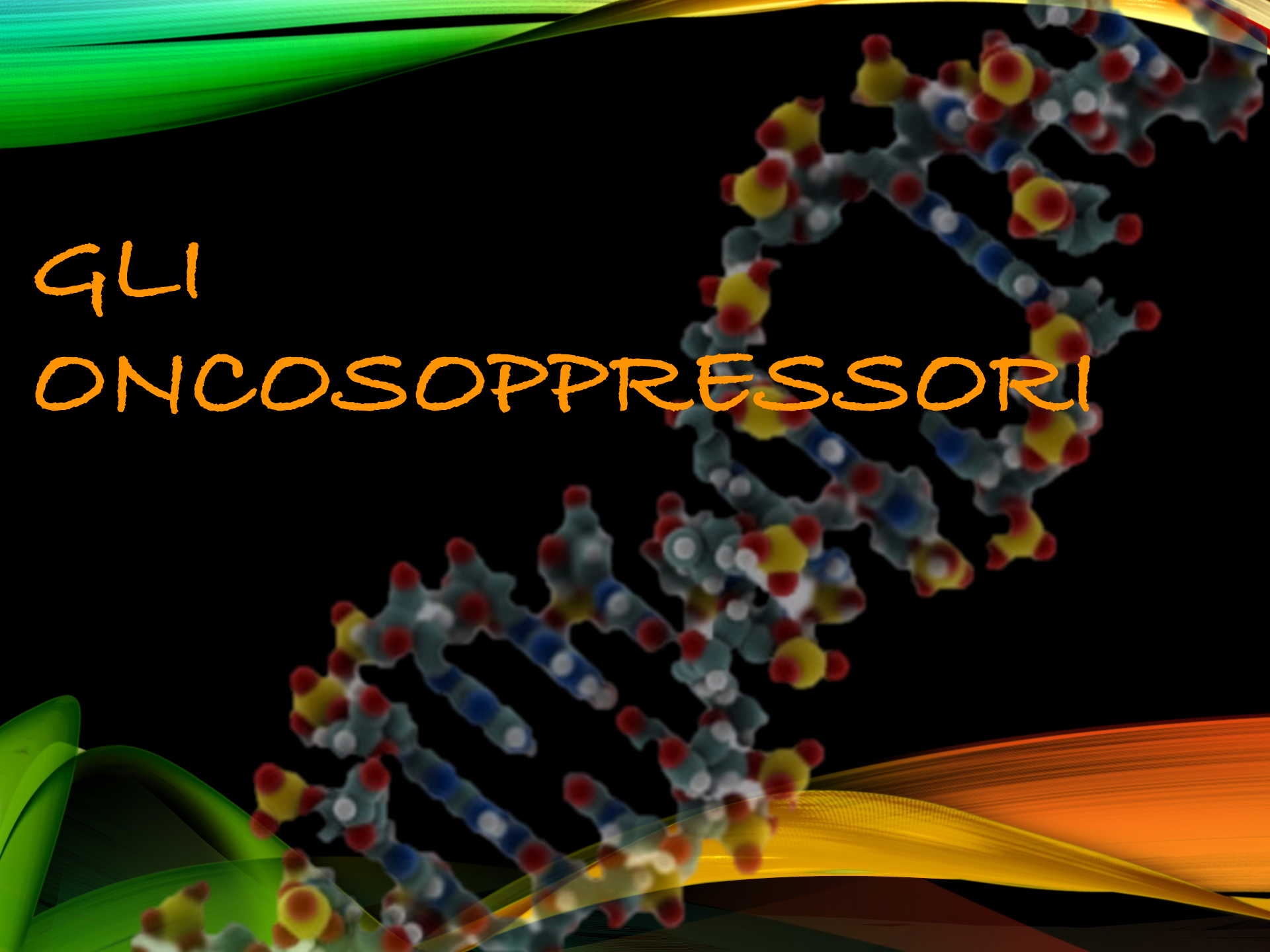
Ras nel XXI secolo



Ras nel XXI secolo



GLI ONCOSOPPRESSORI



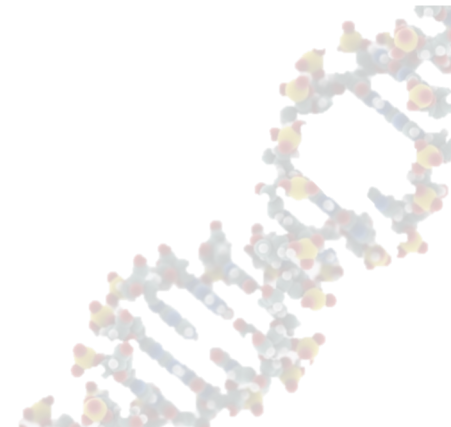
Tumor suppressor genes

Studi su virus oncogeni → Oncogeni hanno effetto dominante per trasformazione cellulare

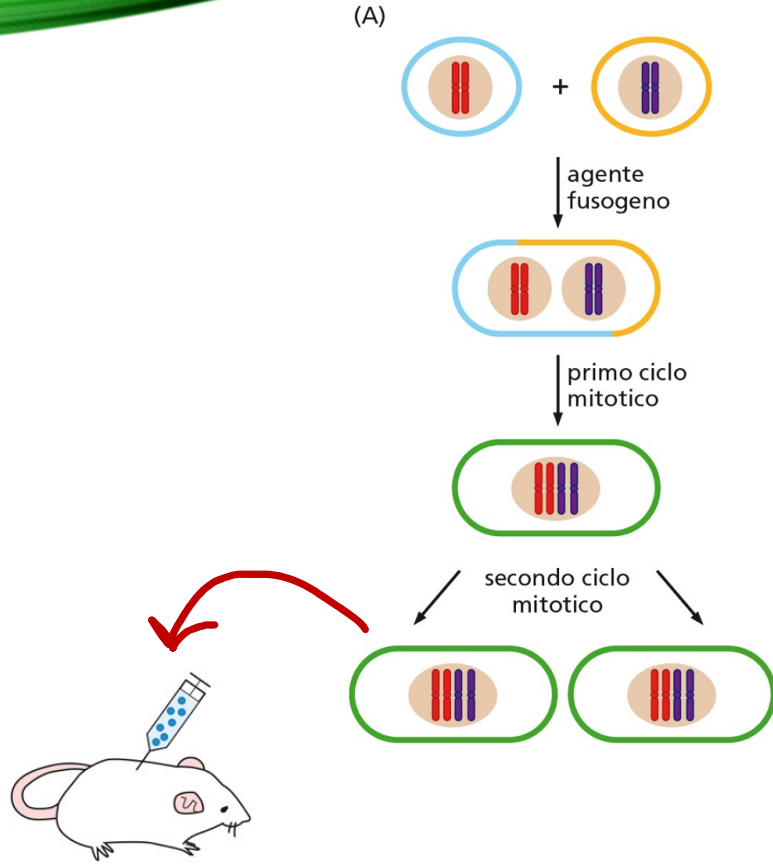
MA

Il cancro non ha origine virale....

Manca qualcosa nella genetica del cancro!

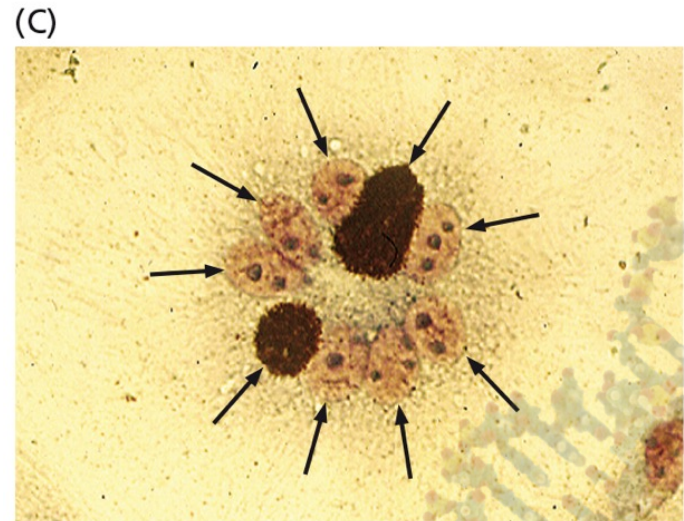
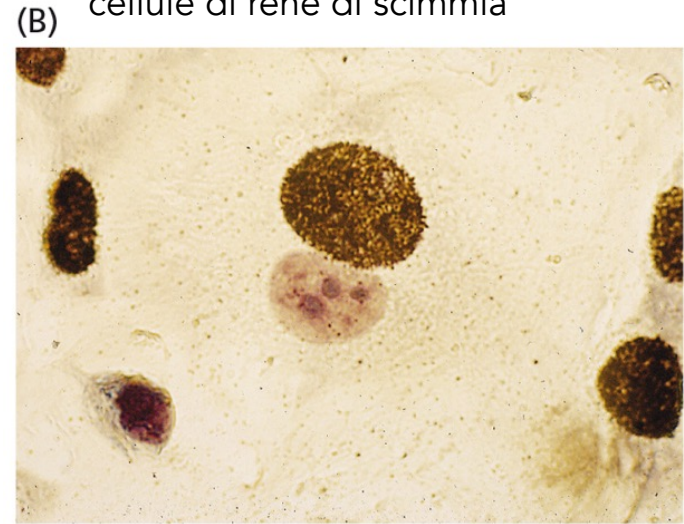


Dominanza/recessività del fenotipo tumorale

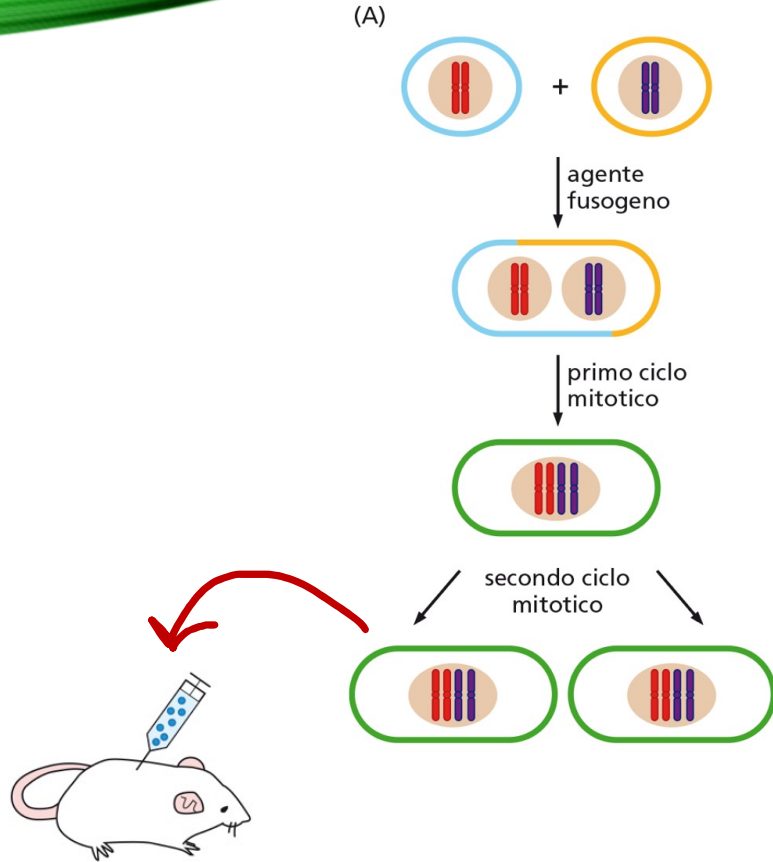


Il fenotipo tumorale trasformante è dominante?

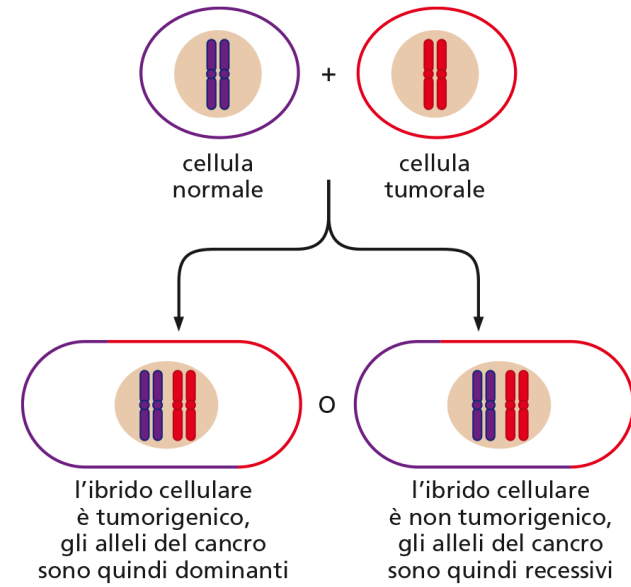
NIH3T3 di topo radiomarcate + cellule di rene di scimmia



Dominanza/recessività del fenotipo tumorale



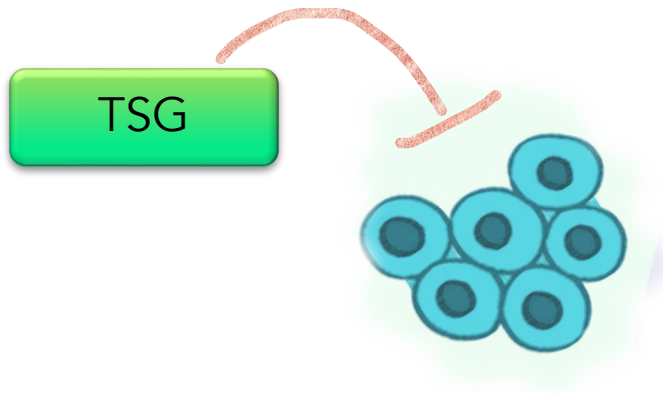
Il fenotipo tumorale trasformante è dominante?



Fenotipo maligno recessivo in maggior parte esperimenti che non coinvolgono virus

Tumor suppressor genes

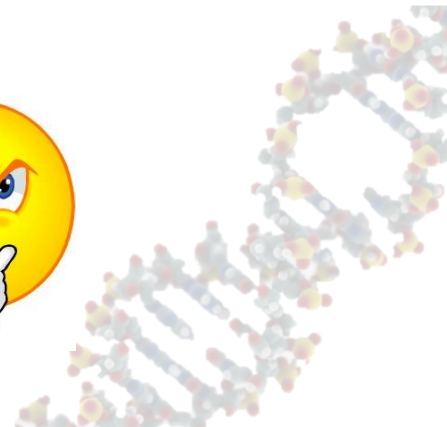
Ipotesi:



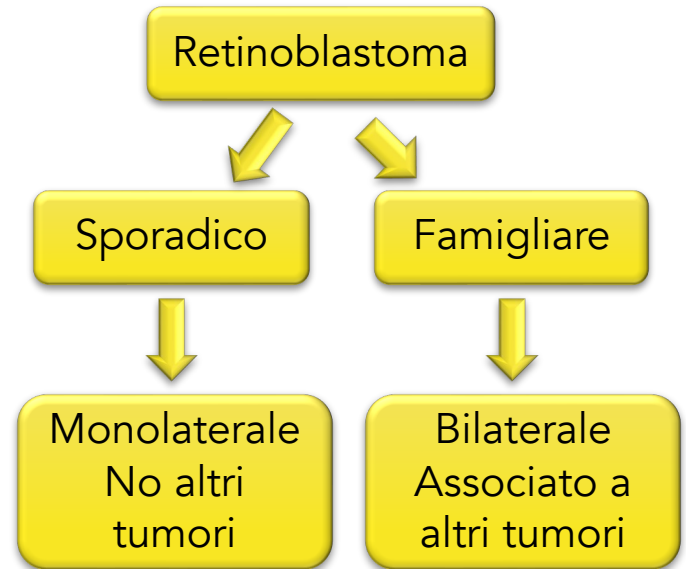
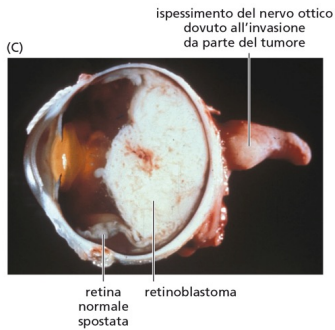
Perdita di funzione causa tumore:

-più facile che mutazione causi perdita di funzione rispetto a guadagno ✓

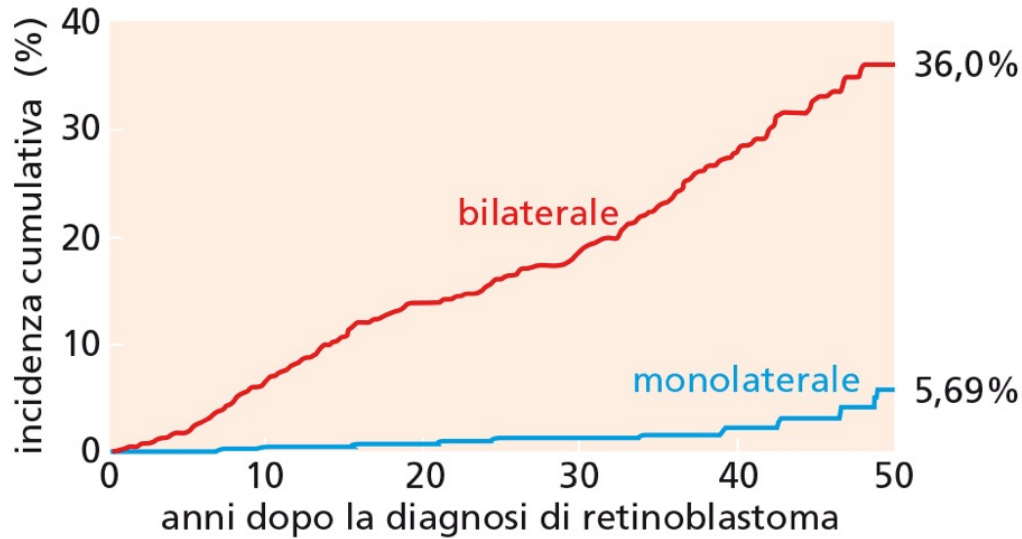
-MA... cellule di mammifero sono diploidi!
2 eventi mutazionali per avere omozigote
Freq.: $10^{-6} * 10^{-6} = 10^{-12} \rightarrow$ prob troppo bassa!



Il retinoblastoma



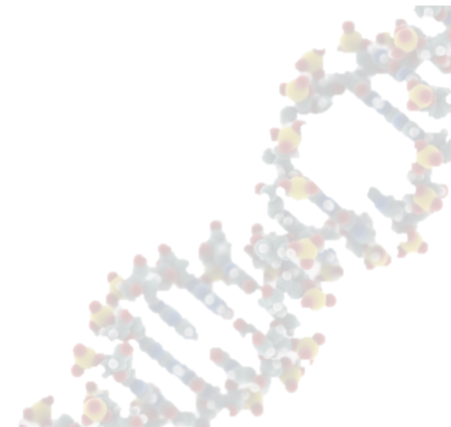
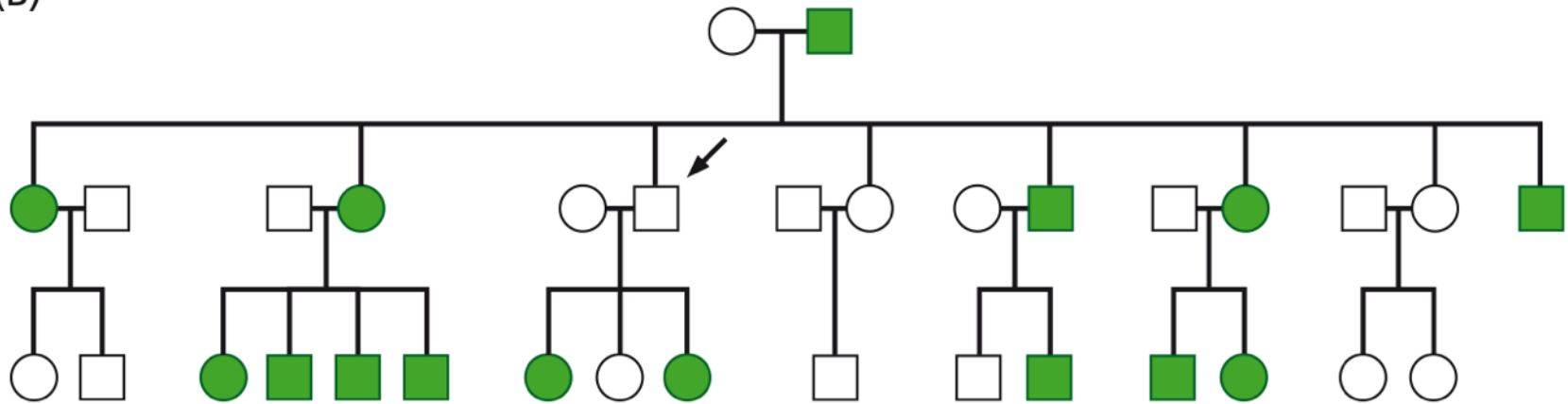
(A) tumori non retinici in pazienti con retinoblastoma



Il retinoblastoma

Eredità mendeliana nei casi familiari

(B)



II retinoblastoma

Proc. Nat. Acad. Sci. USA
Vol. 68, No. 4, pp. 820-823, April 1971

Mutation and Cancer: Statistical Study of Retinoblastoma

ALFRED G. KNUDSON, JR.

Graduate School of Biomedical Sciences and M. D. Anderson Hospital and Tumor Institute,
The University of Texas at Houston, Houston, Texas 77025

Communicated by James V. Neel, February 8, 1971

ABSTRACT Based upon observations on 48 cases of retinoblastoma and published reports, the hypothesis is developed that retinoblastoma is a cancer caused by two mutational events. In the dominantly inherited form, one mutation is inherited via the germinal cells and the second occurs in somatic cells. In the nonhereditary form, both mutations occur in somatic cells.

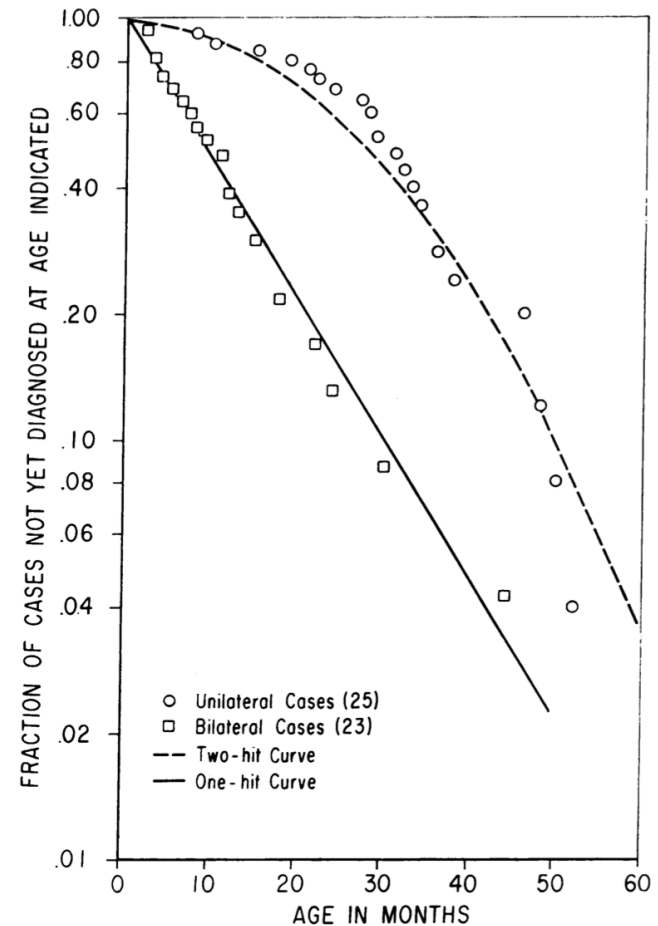
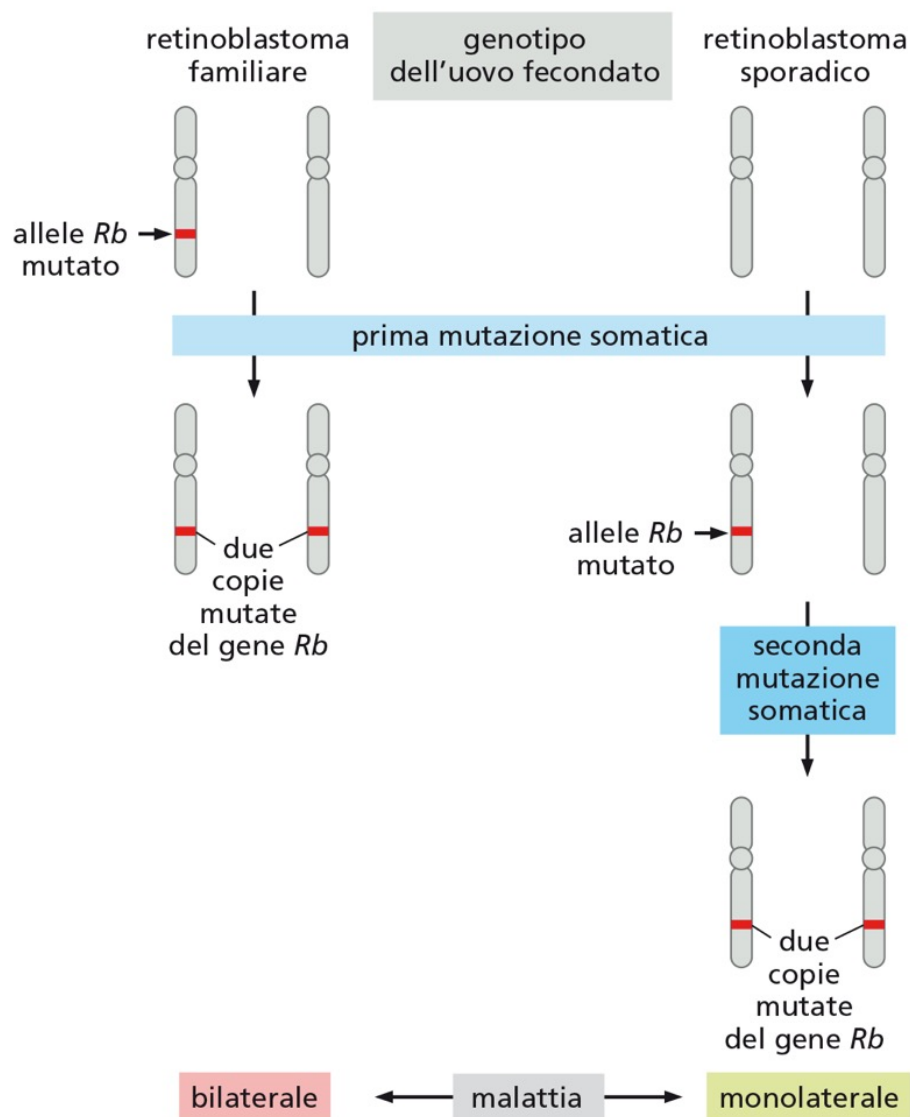
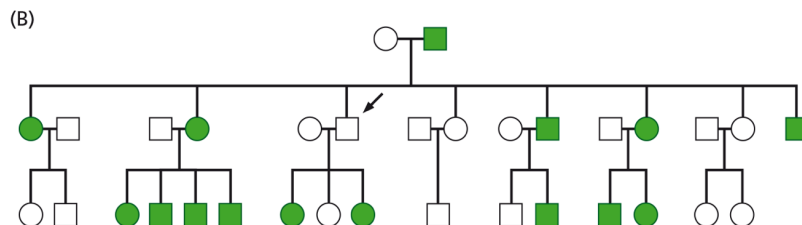


FIG. 1. Semilogarithmic plot of fraction of cases of retinoblastoma not yet diagnosed (S) vs. age in months (t). The one-hit curve was calculated from $\log S = -t/30$, the two-hit curve from $\log S = -4 \times 10^{-5} t^2$.

Il retinoblastoma



Mutazioni *Rb* sono dominanti sull'organismo ma recessive a livello cellulare

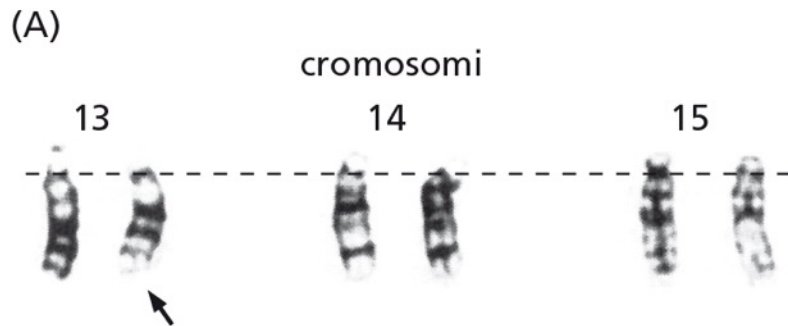


Come si forma Retinoblastoma sporadico? Probabilità di 2 eventi di mutazione indipendenti è troppo bassa rispetto ai casi osservati!

Idea: seconda mutazione non è casuale

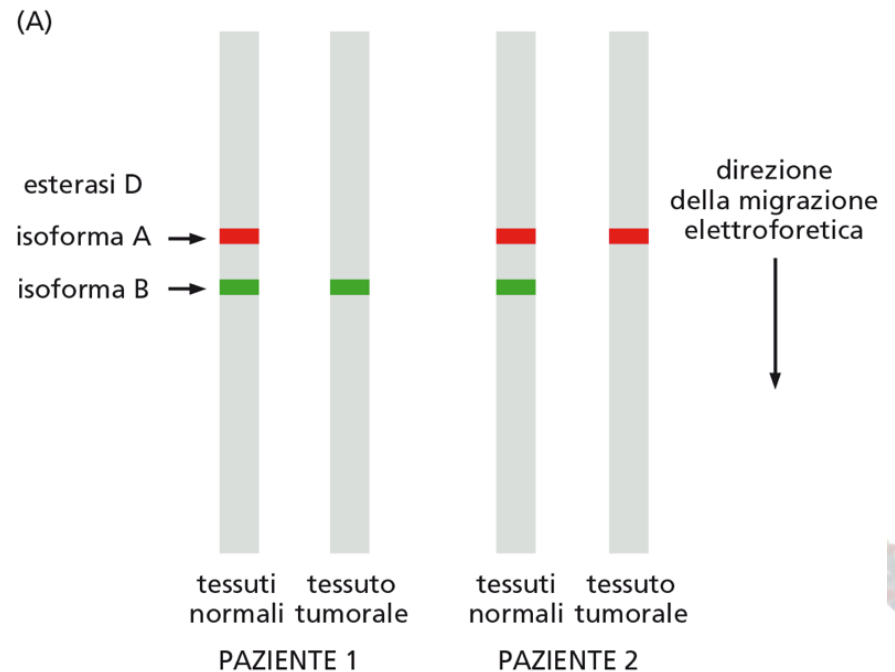
Identificazione gene *Rb*

1976: analisi citologiche su cromosomi metafasici di cellule normali e tumorali di un bambino di 6 anni con Retinoblastoma



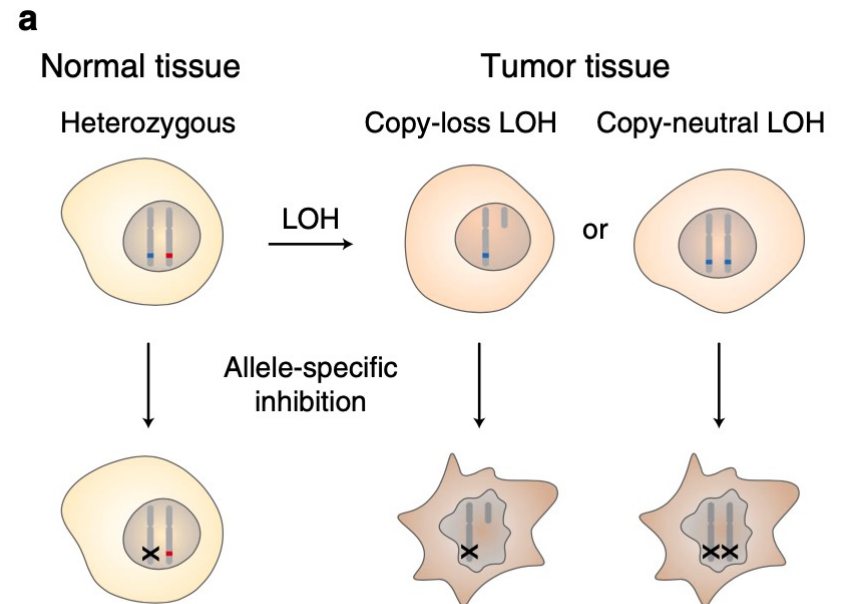
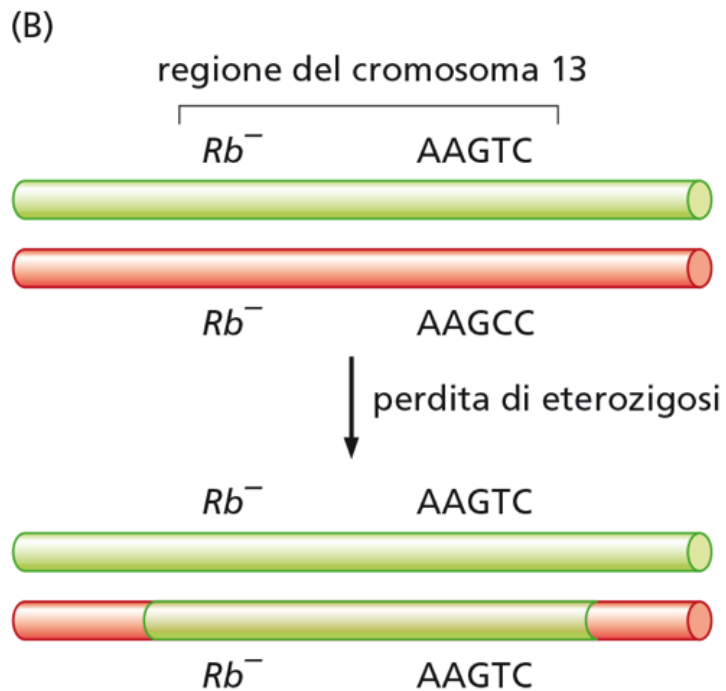
Delezione su braccio lungo del cromosoma 13
13q14

Gene per esterasi D nella stessa regione: 2 alleli danno prodotti di dimensioni diverse



Perdita di eterozigosità

Come si forma Retinoblastoma sporadico?



Mutazioni nel gene *Rb*

Clonato un frammento umano da una library che riconosce delezioni 13q14 in diversi retinoblastomi

Frammenti da questo usati per clonare gene

A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma

Stephen H. Friend^{*†}, Rene Bernards^{*}, Snezna Rogelj^{*}, Robert A. Weinberg^{*‡}, Joyce M. Rapaport[§], Daniel M. Albert[§] & Thaddeus P. Dryja[§]

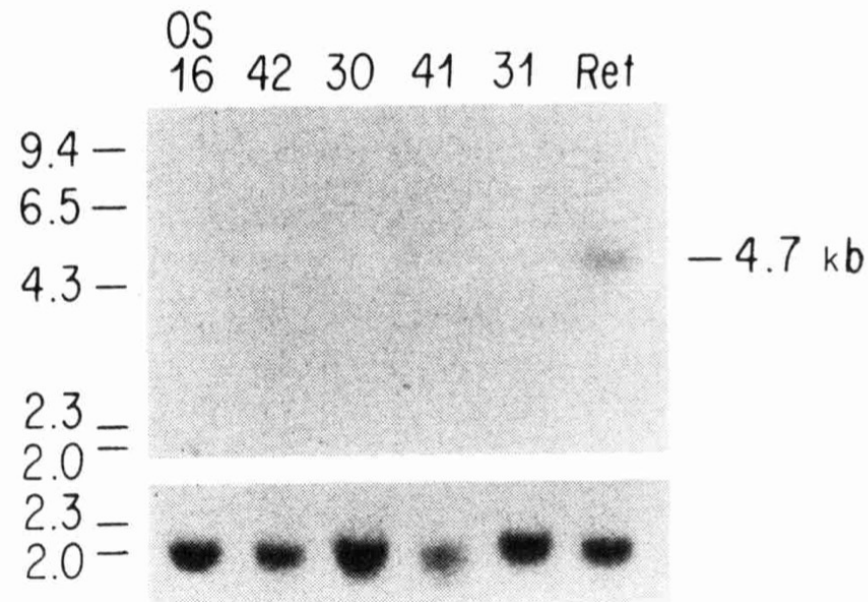


Fig. 2 Northern blot analysis of RNA isolated from retinal and osteosarcoma cells. RNA (20 μ g) was loaded from osteosarcoma tumour No. 16; four retinoblastomas: Nos 42, 30 (Y-79, ref. 30), 41 (WERI-1, ref. 31) and 31; and the adenovirus-12-transformed retinal cells (ref. 28). *a*, Samples probed with p4.7R insert. The transcript detected in the retinal cells is 4.7 kb in size. Molecular mass markers indicated on the left. *b*, After washing the filter, it was rehybridized with a probe derived from the rat tubulin locus³².

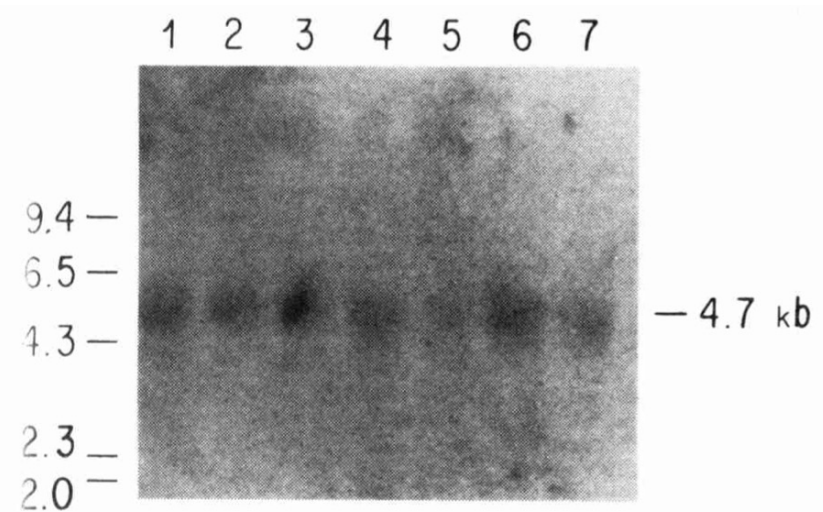


Fig. 3 Northern blot analysis of RNA isolated from various tumour cell lines. RNA was prepared and loaded as in Fig. 2. Lane 1, LX-1 (small-cell carcinoma of the lung); lane 2, SKNSH (neuroblastoma); lane 3, CAKI-1 (renal clear cell carcinoma); lane 4, MEL-5 (melanoma); lane 5, SKNMC (neuroblastoma); lane 6, SKRC-2 (renal carcinoma); lane 7, ACHN (renal adenocarcinoma). ³²P-labelled p4.7R insert was used as a probe. Positions of molecular mass markers are indicated on the left.

Mutazioni nel gene *Rb*

A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma

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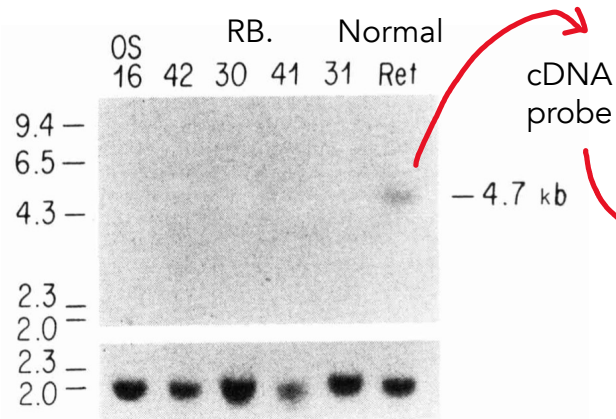


Fig. 2 Northern blot analysis of RNA isolated from retinal and osteosarcoma cells. RNA (20 µg) was loaded from osteosarcoma tumour No. 16; four retinoblastomas: Nos 42, 30 (Y-79, ref. 30), 41 (WERI-1, ref. 31) and 31; and the adenovirus-12-transformed retinal cells (ref. 28). *a*, Samples probed with p4.7R insert. The transcript detected in the retinal cells is 4.7 kb in size. Molecular mass markers indicated on the left. *b*, After washing the filter, it was rehybridized with a probe derived from the rat tubulin locus³².

Screening su
campioni di DNA
tumorale da 40
retinoblastomi, 8
osteosarcomi e 2
tumori
indifferenziati

3 diversi pattern
aberranti

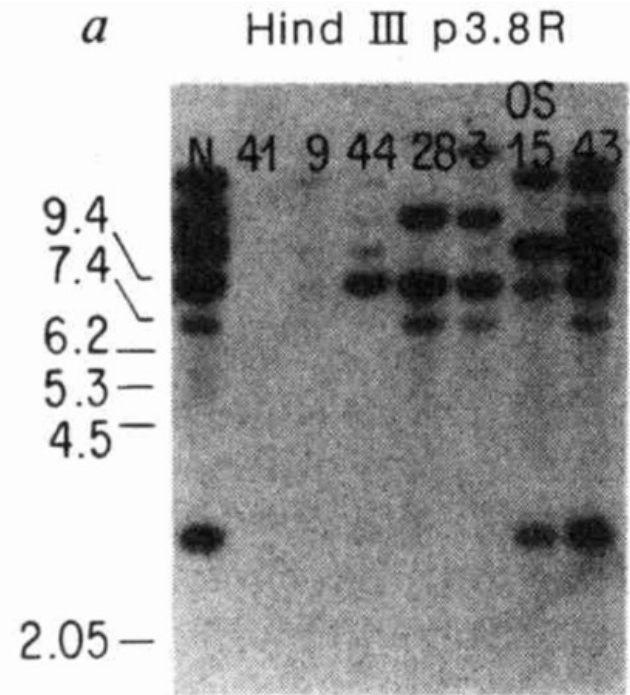


Fig. 4 Southern blot analysis of retinoblastoma, osteosarcoma and normal DNAs probed with subclones of p4.7R (Fig. 1). The first lane (N) contains lymphocyte DNA from a normal, healthy human. The adjacent lanes have DNA from the following tumours: retinoblastoma No. 41 (WERI-1, ref. 31); retinoblastoma No. 9; retinoblastoma No. 44; retinoblastoma No. 28; retinoblastoma No. 3; osteosarcoma OS-15 (ref. 24); and retinoblastoma No. 43. DNA (4 µg per lane) was digested with *Hind*III. *a*, Probed with a 3.8-kb *Eco*RI/*Eco*RI subcloned fragment of p4.7R (see Fig. 1). *b*, Probed

Mutazioni nel gene *Rb*

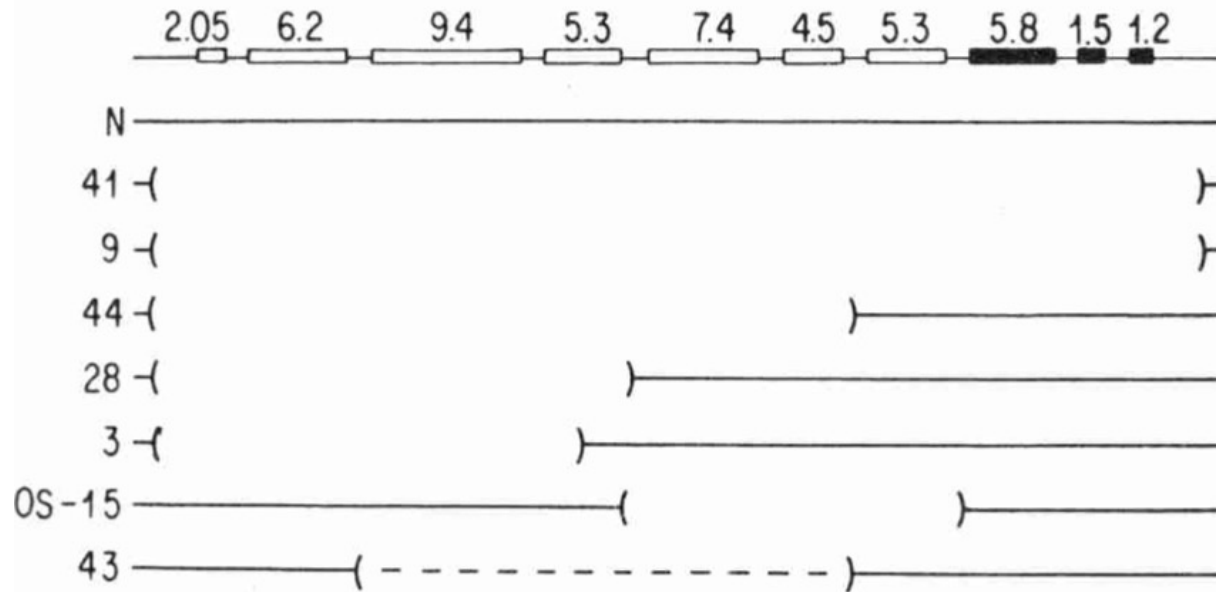


Fig. 5 Schematic representation of deletions in the genomic DNA from six retinoblastomas and an osteosarcoma. The genomic *Hind*III fragments detected by the 3.8- and 0.9-kb *Eco*RI fragments, denoted by open and closed bars, respectively (see Fig. 4), are shown at the top according to their relative positions in the genome and labelled with sizes in kilobases. Normal and tumour DNAs, numbered as in Fig. 4, have solid lines where both copies of a given genomic fragment are present. Dotted line between brackets, heterozygous deletion; open brackets, homozygous deletion.

Mutazioni nel gene *Rb*

Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6017-6021, August 1988
Genetics

Molecular mechanism of retinoblastoma gene inactivation in retinoblastoma cell line Y79

(nonhomologous recombination/mutation/cancer genetics)

EVA Y.-H. P. LEE*, ROBERT BOOKSTEIN*, LIH-JUAN YOUNG*, CHI-JEN LIN†, MICHAEL G. ROSENFELD†, AND WEN-HWA LEE*

*Department of Pathology, M-012, Center for Molecular Genetics, and †Department of Medicine, University of California, San Diego, La Jolla, CA 92093

Y79: linea cellulare di retinoblastoma da paziente con RB familiare

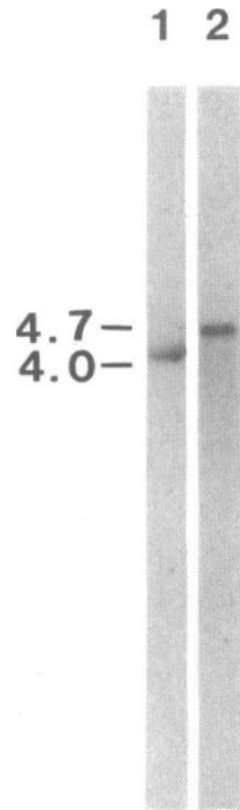


FIG. 1. RNA blotting analysis of retinoblastoma cell line Y79 and normal fetal retina. Five micrograms of polyadenylated RNA prepared from Y79 cells (lane 1) and fetal retina (lane 2) were separated by electrophoresis in 1% formaldehyde/agarose gels and transferred to nitrocellulose filters with $20 \times$ SSC ($1 \times$ SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7). Filters were hybridized with 32 P-labeled RB4.5. The transcript sizes (in kb) are given at left.

cDNA → Restriction analysis

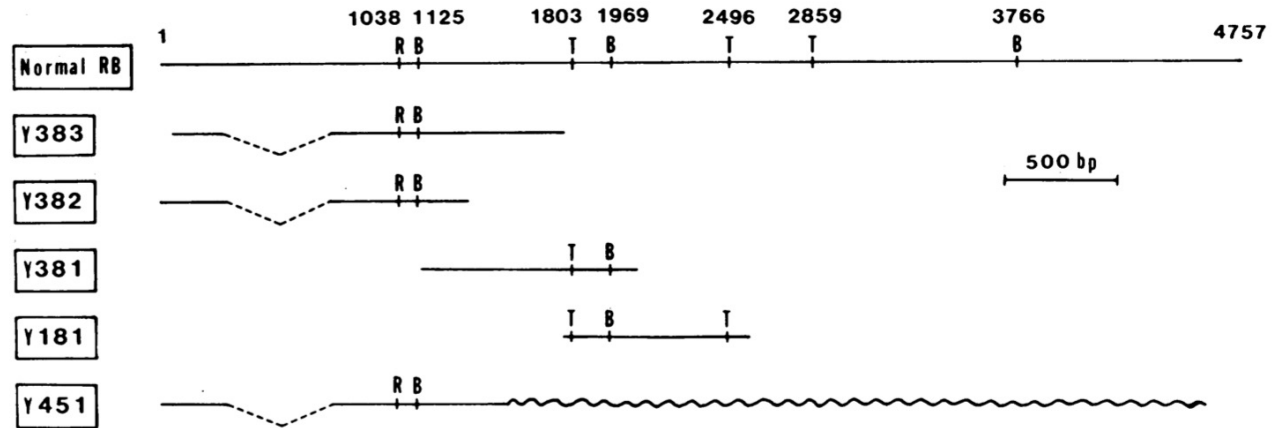


FIG. 2. Comparison of restriction maps of normal (RB4.7) and Y79 RB cDNA clones. Dashed lines indicate regions missing from Y79 RB cDNAs. Wavy lines represent regions not hybridizing with normal RB cDNA. B, *Bgl* II; R, *Eco*RI; T, *Taq* I.

Mutazioni nel gene *Rb*

Delezione introni 2-6 → Effetto sul prodotto?

IP

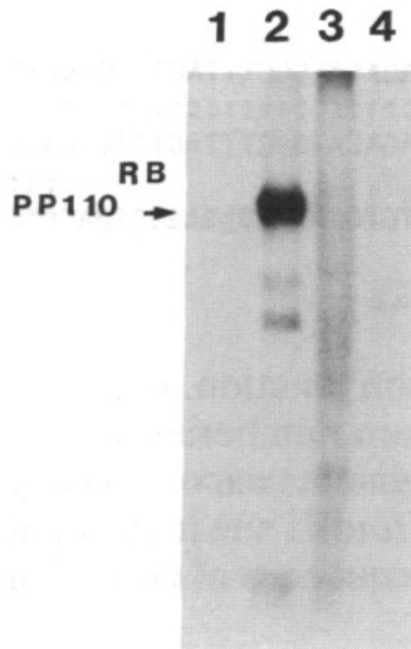


FIG. 6. Immunoprecipitation of RB protein. LAN-1 (lanes 1 and 2) and Y79 (lanes 3 and 4) cells were labeled with [³²P]phosphoric acid for 4 hr. Cell lysates were immunoprecipitated with either preimmune serum (lanes 1 and 3) or an affinity-purified polyclonal antibody (lanes 2 and 4) that recognizes the RB protein (14). Immunoprecipitates were separated in 7.5% NaDodSO₄/polyacrylamide gels that were autoradiographed overnight.