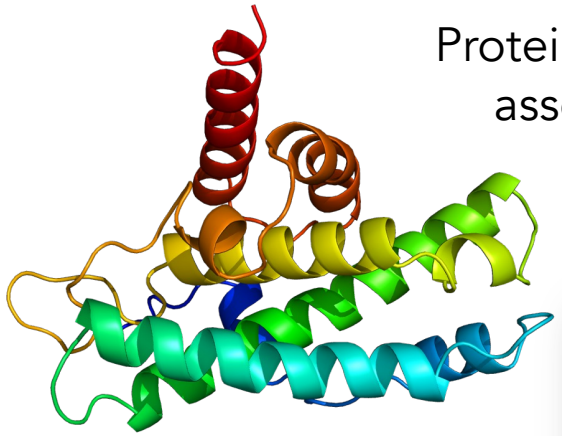
RB E IL CICLO CELLULARE

The Retinoblastoma susceptibility gene

Qual è la funzione di *Rb*?



Proteina nucleare di 128 aa, 105 KDa (pRb o RB)
assente o alterata in molte forme di tumori

Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product

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‡ Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA
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One of the cellular targets implicated in the process of transformation by the adenovirus E1A proteins is a 105K cellular protein. Previously, this protein had been shown to form stable protein/protein complexes with the E1A polypeptides but its identity was unknown. Here, we demonstrate that it is the product of the retinoblastoma gene. The interaction between E1A and the retinoblastoma gene product is the first demonstration of a physical link between an oncogene and an anti-oncogene.

Whyte et al., 1988

Funzioni *Rb*

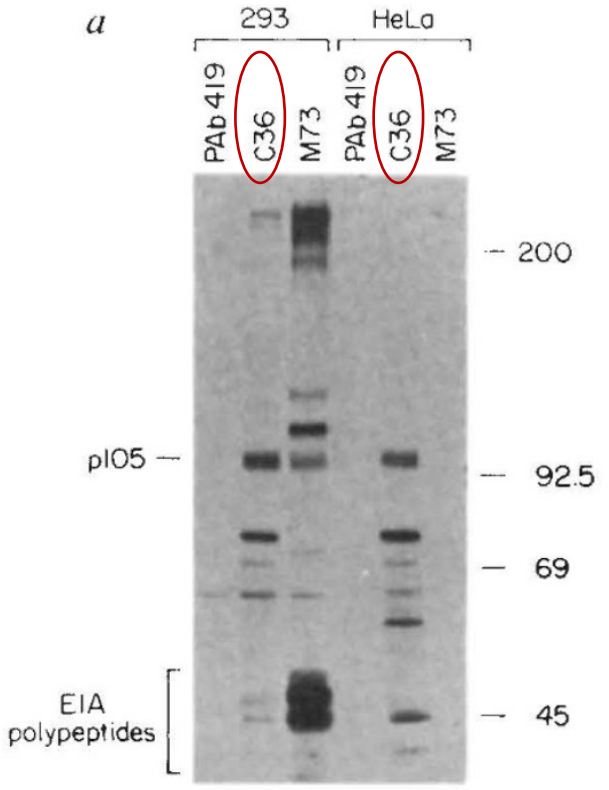
Oncogenes from some DNA viruses induce transformation and do not appear to have cellular homologues.

<u>Adenovirus oncogene E1A</u>:

- E1A can immortalize primary cells
- E1A cooperate with *ras* gene to transform cells in culture and these cells will induce tumours in animals.
- E1A oncoprotein interacts with several host polipteptides

C36 antibody that recognises a 105 kDa-E1A interaction protein present also in untransformed HeLa cells

Lee et al., 1987: Product or RB gene= 110KDa nuclear phosphoprotein



Prot 105 KDa è RB?

Whyte et al., 1988

Funzioni *Rb*

IP with c36 antibody of 3 cell lines with large deletions of *RB*

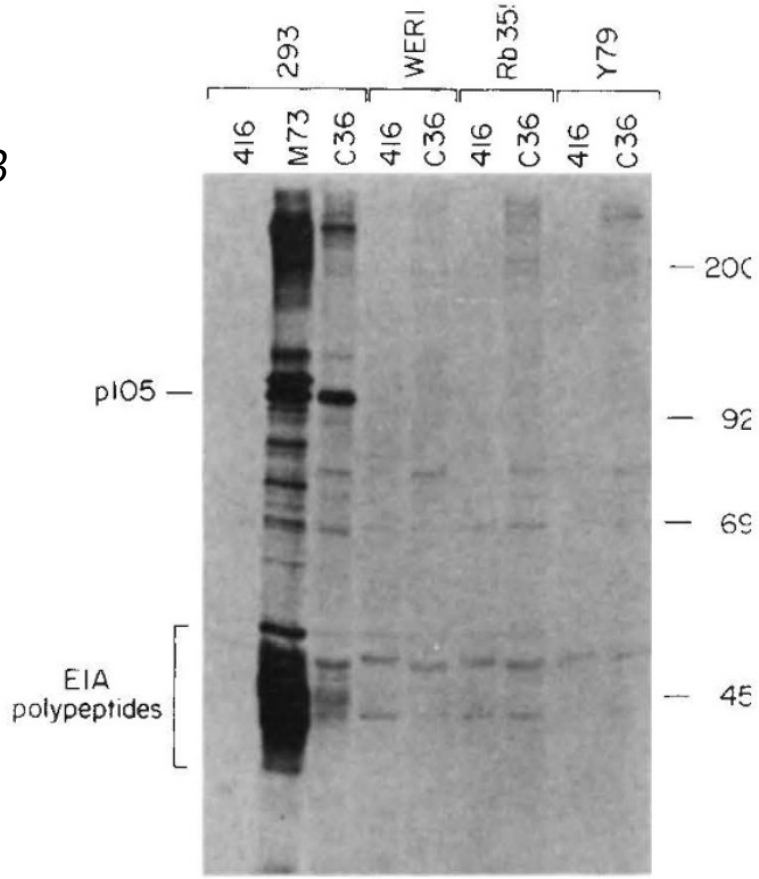
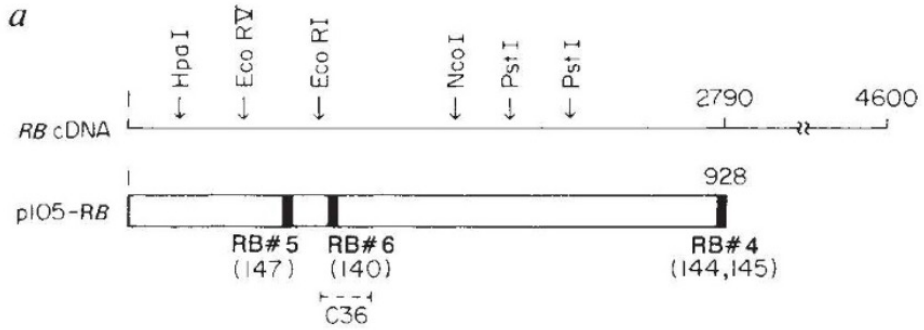


Fig. 2 Immunoprecipitations using the C36 monoclonal antibody from lysates of retinoblastoma cells. Cultures of 293, WERI-1, Y79, or RB355 cells were radiolabelled with [^{35}S]methionine, and lysates were precipitated with either C36, M73 or PAb416 monoclonal antibodies. PAb416 is a monoclonal antibody specific for SV40 large T antigen[32]. Immune complexes were collected on protein A-Sepharose beads and analysed on an 8% SDS-polyacrylamide gel by fluorography.

Whyte et al., 1988

Funzioni *Rb*

Preparation antibodies against pRB using a short fragment of the protein synthesized in vitro



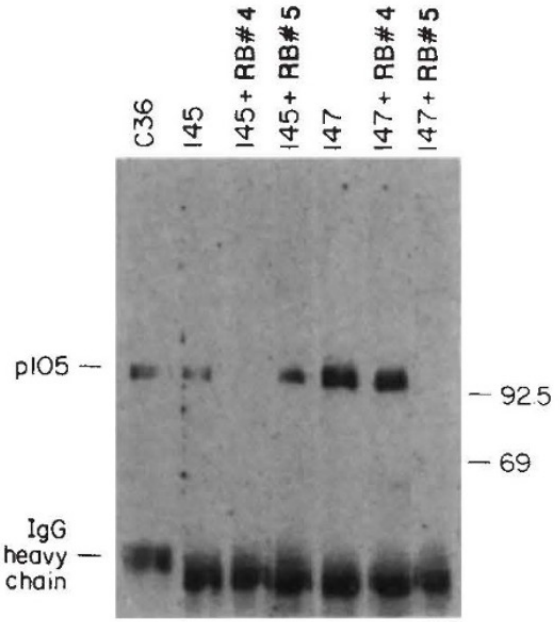
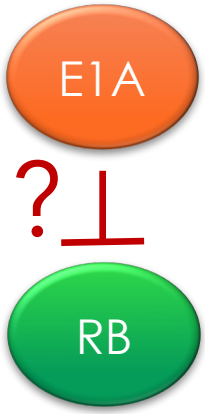


Fig. 5 Immunoblots of 105K E1A-associated protein with anti-RB antibodies. The E1A-associated 105K protein was immunoprecipitated from 293 cells using the M73 anti-E1A monoclonal antibody. The immunoprecipitated polypeptides were resolved by SDS-PAGE and then transferred to nitrocellulose membranes using standard immunoblotting techniques[52]. Strips were cut and reacted with C36, 145 (anti-RB#4), or 147 (anti-RB#5) antibodies. The binding of the anti-RB-peptide antibodies was performed with and without a saturating amount of peptide RB#4 or RB#5 and the addition of the appropriate peptide blocked the binding of these anti-peptide antibodies to 105K. After washing, the C36-reacted strips were probed with 10^6 c.p.m. of [^{125}I]labelled rabbit anti-mouse immunoglobulin (New England Nuclear) and the anti-RB-peptide-reacted strips were probed with 10^6 c.p.m. of [^{125}I]labelled goat anti-rabbit immunoglobulin antibodies (New England Nuclear). The location of the [^{125}I]labelled reagents was determined by autoradiography.

Whyte et al., 1988

Funzioni *Rb*

In vitro translation of RB
(different small peptides)
from *Rb* cDNA
Pepdides recognized both by
Anti-RB and C36 antibodies



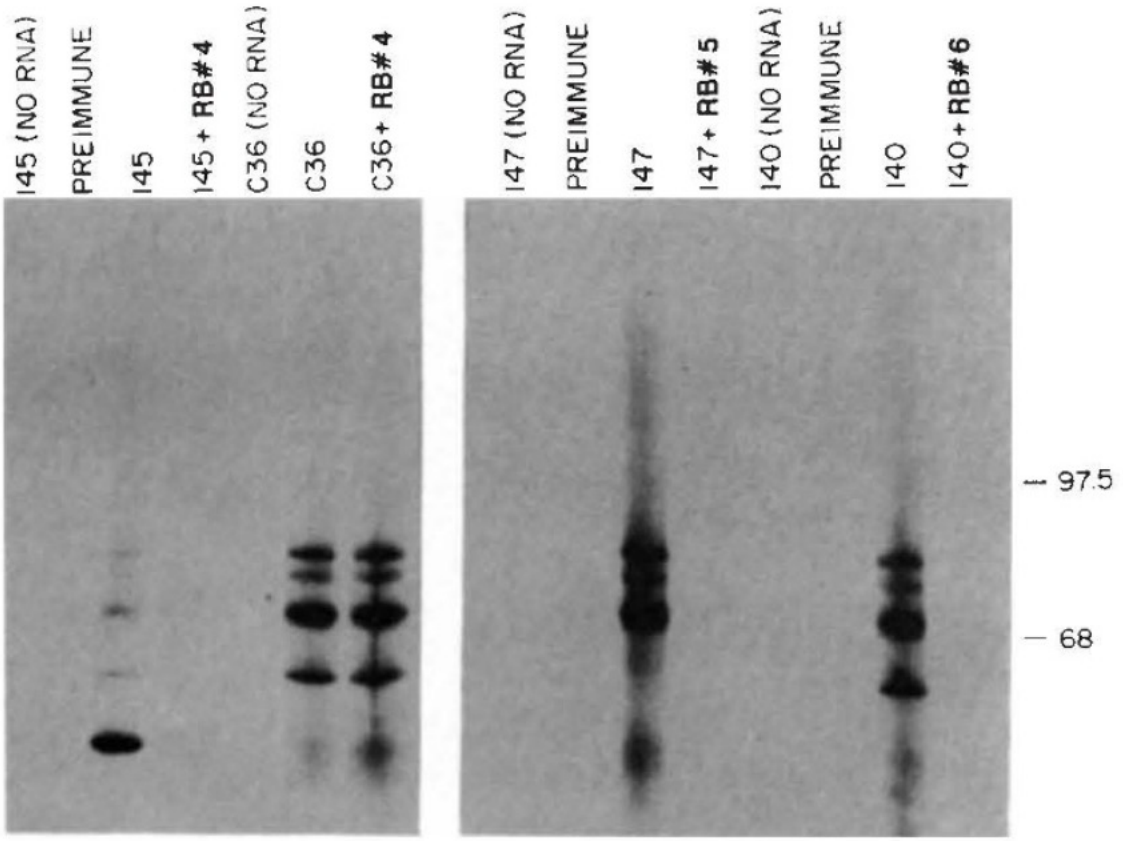
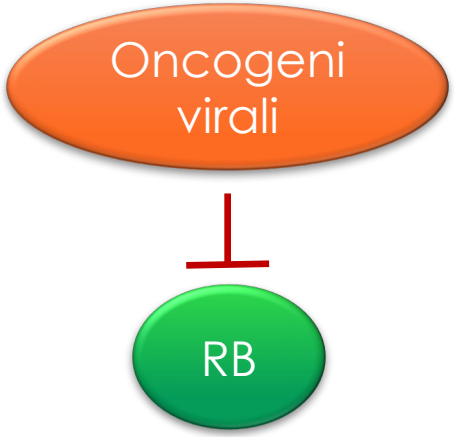


Fig. 6 Immunoprecipitation of polypeptides synthesized from the *in vitro* transcription/translation of *RB* cDNA. RB-related polypeptides synthesized *in vitro* were immunoprecipitated with C36, 145 (anti-RB#4), 147 (anti-RB#5) or 140 (anti-RB-#6) antibodies in the presence or absence of saturating amounts of peptide RB#4, RB#5, or RB#6. Preimmune rabbit sera were used in parallel immunoprecipitations as were rabbit reticulocyte lysates without addition of *RB* cRNA.

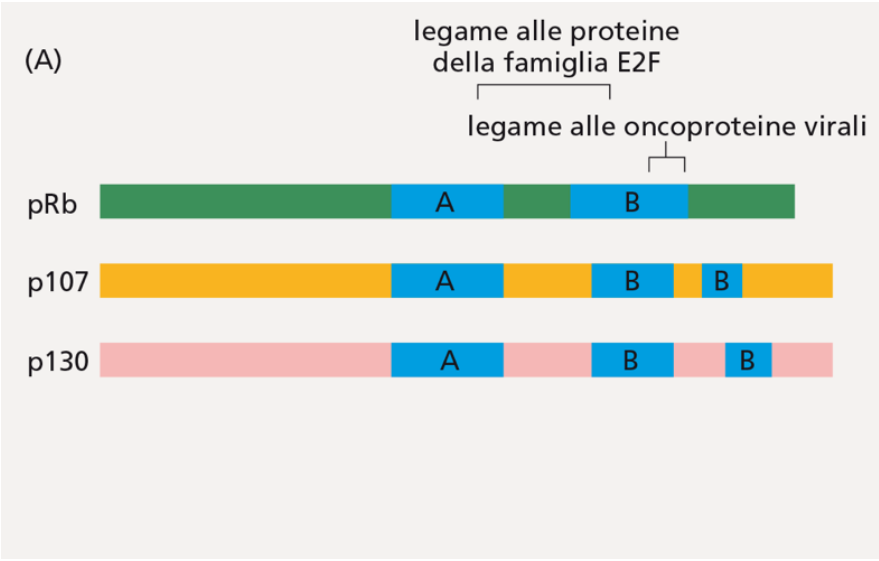
Whyte et al., 1988

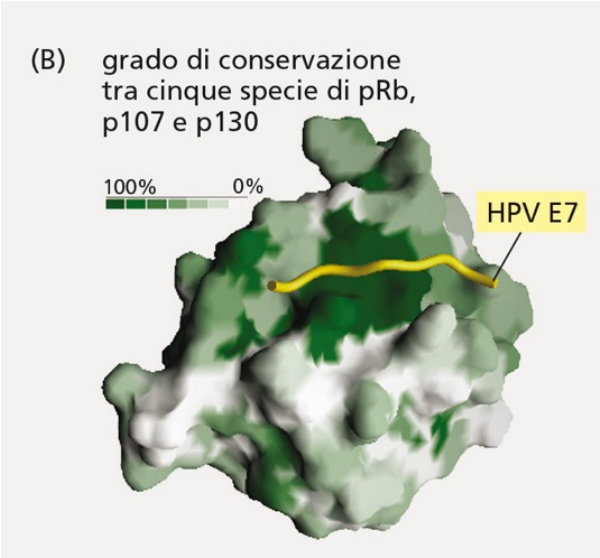
Funzioni *Rb*

Diverse oncoproteine virali legano RB → Inattivare RB serve per replicazione virale



Mutanti che non interagiscono hanno difetti di trasformazione → Interazione con RB è importante per trasformazione

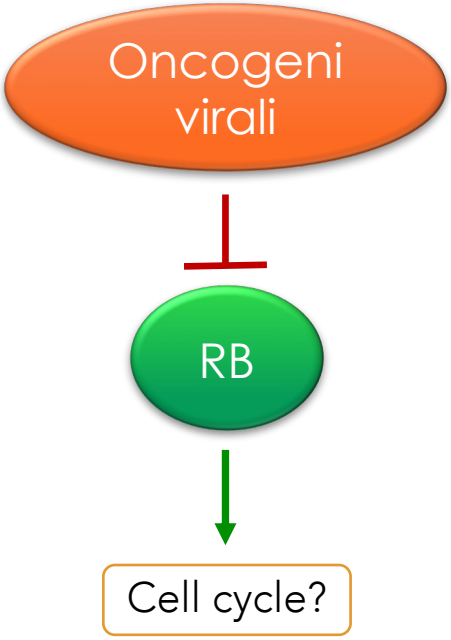




Weinberg, la Biologia del cancro

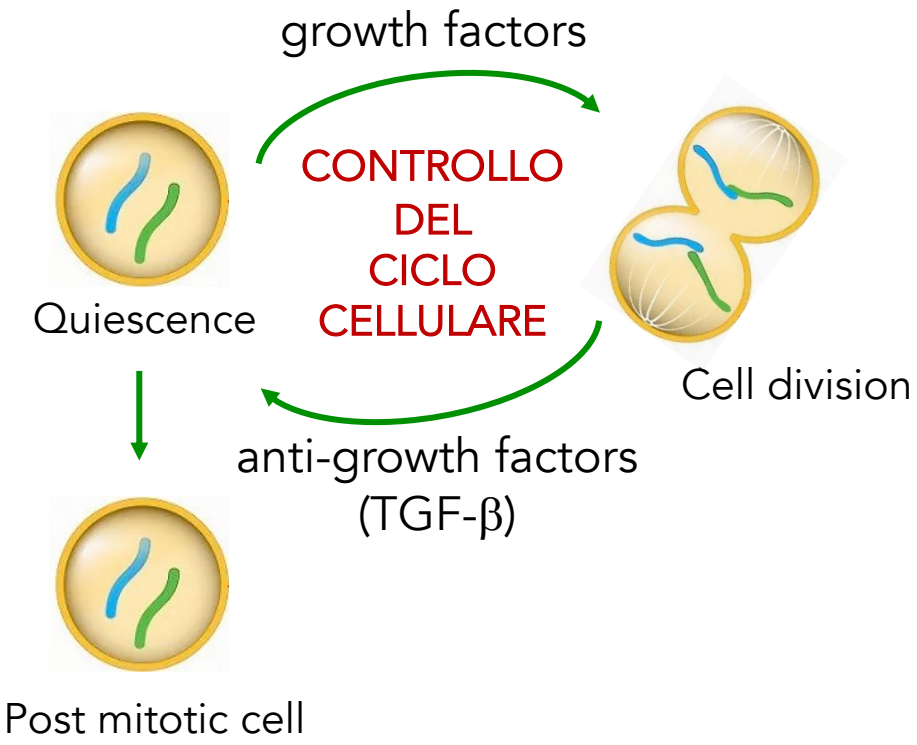
Funzioni *Rb*

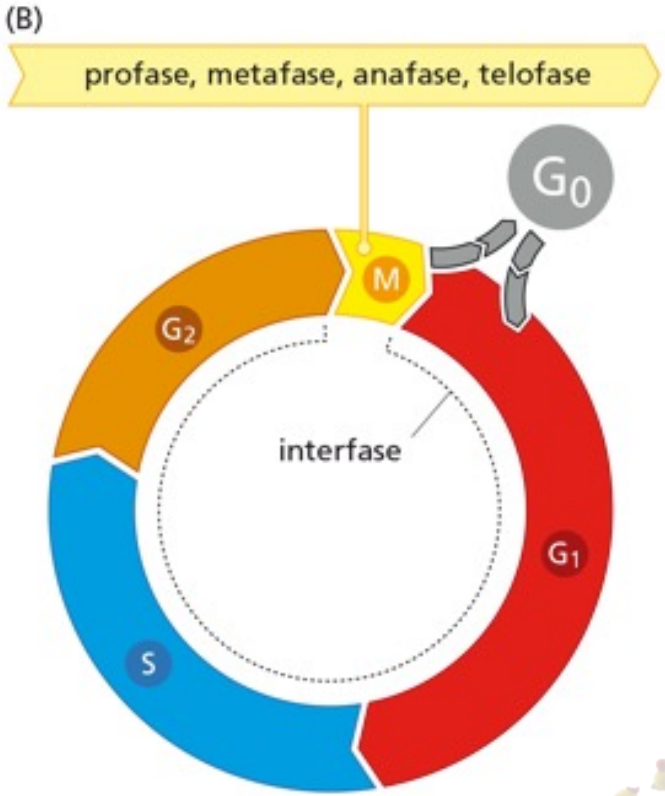
- Lee et al., 1987: RB è fosfoproteina
- Dulbecco et al., 1965 e seguenti: SV40 promuove ciclo cellulare
- Ludlow et al., 1989: SV40 T antigen lega RB non o poco fosforilato



DeCaprio et al., 1989

Controllo del ciclo cellulare

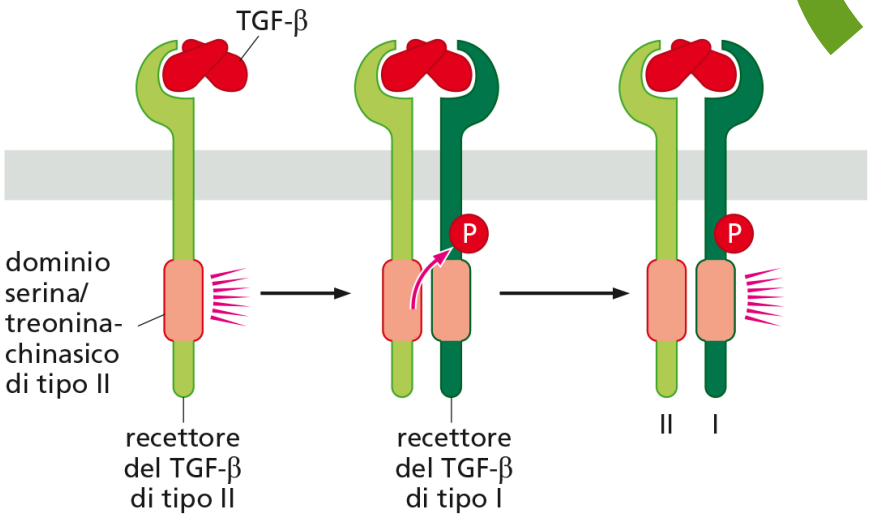


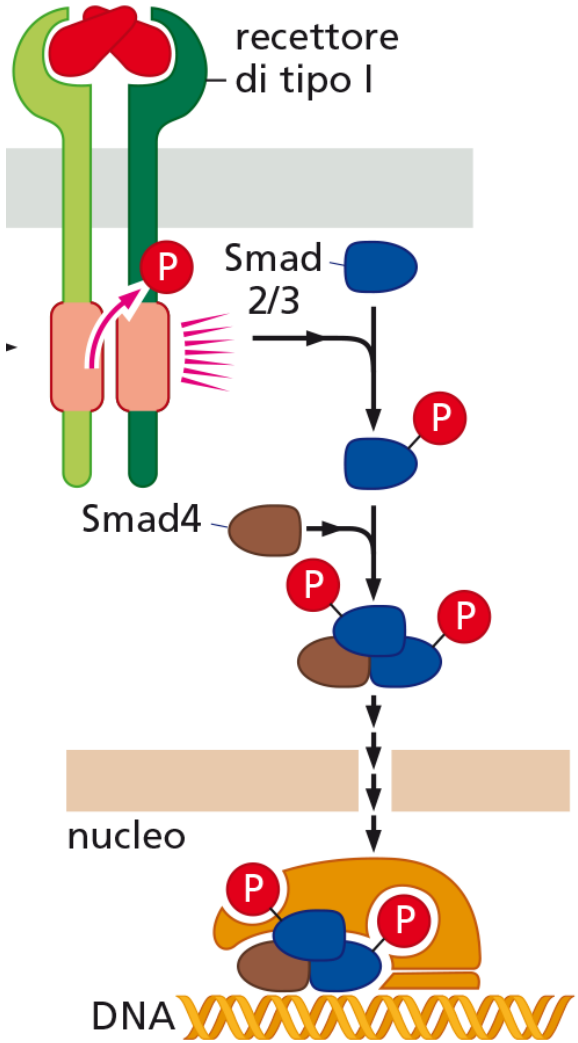


Weinberg, la Biologia del cancro

Via di TGF-β

- TGF-β (Fattore di crescita trasformante β)
- Recettore con attività Ser e Thr chinasica
- Attivazione simile a recettori TK

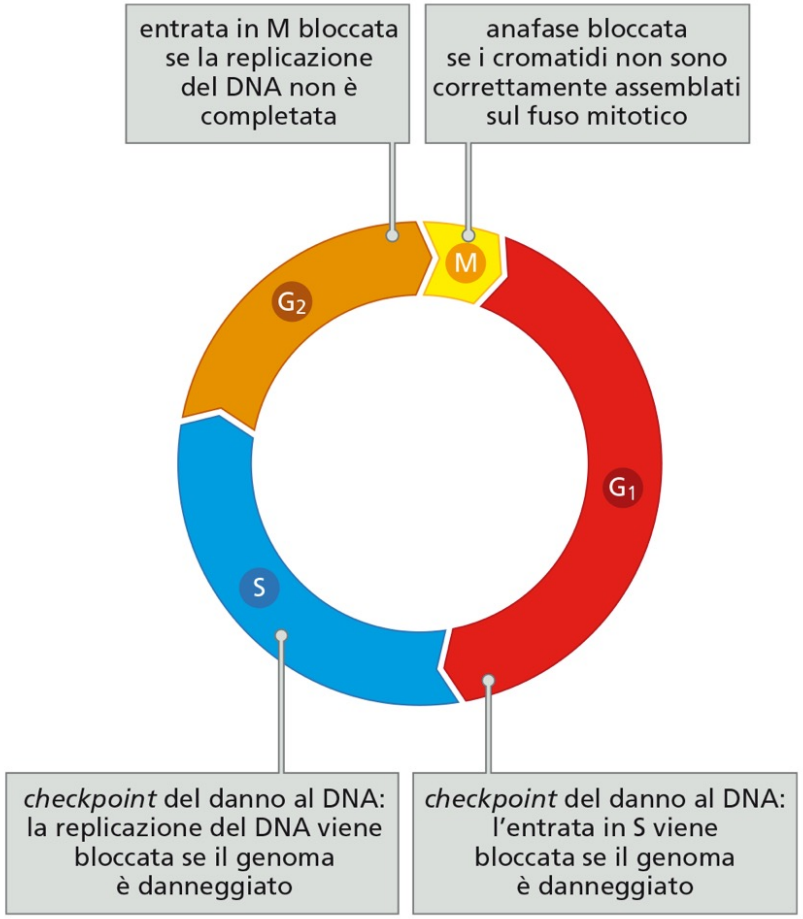


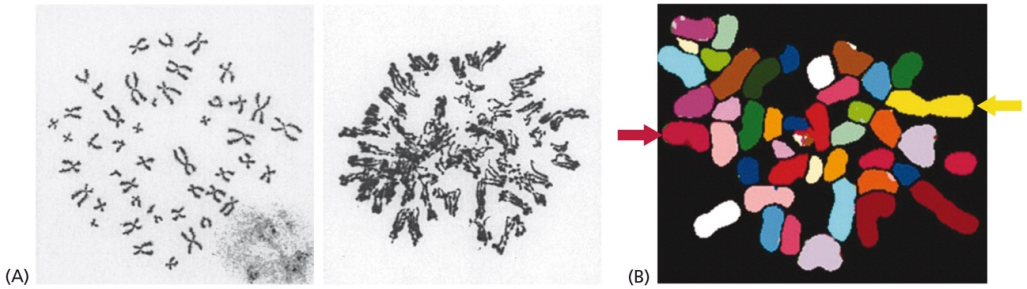


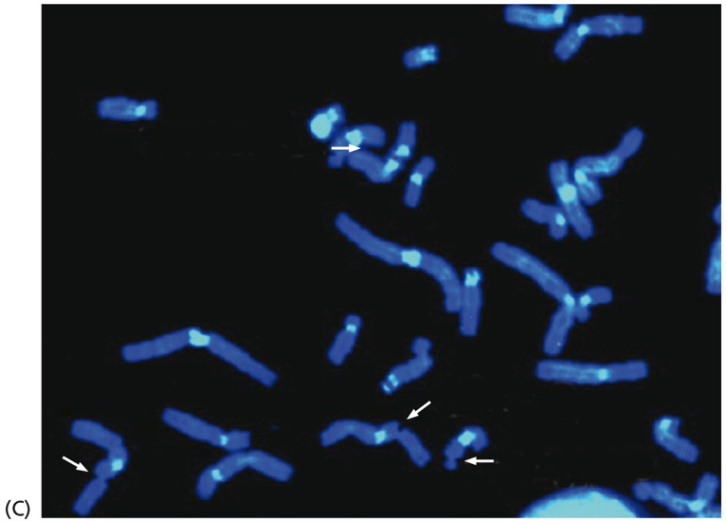
Weinberg, la Biologia del cancro

Controllo del ciclo cellulare

Il ciclo cellulare è unidirezionale e procede nella fase successiva solo se la precedente è terminata

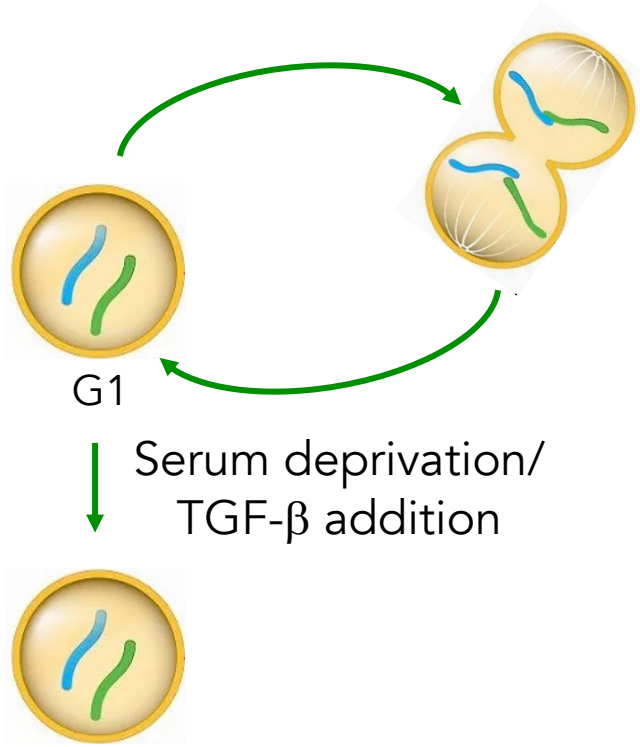


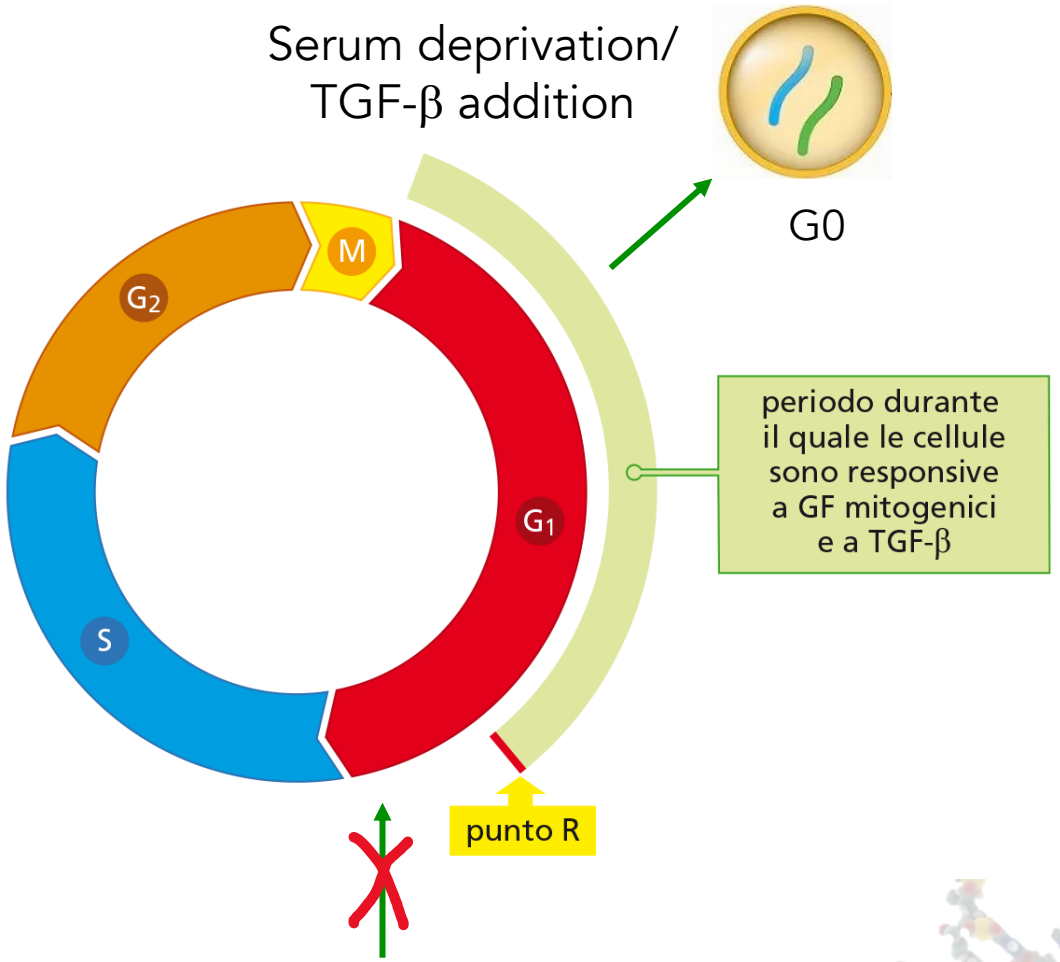




Weinberg, la Biologia del cancro

Controllo del ciclo cellulare





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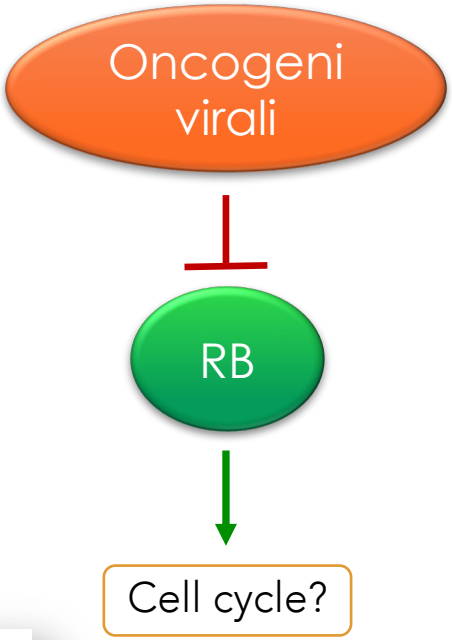


Cell, Vol. 58, 1085–1095, September 22, 1989, Copyright © 1989 by Cell Press

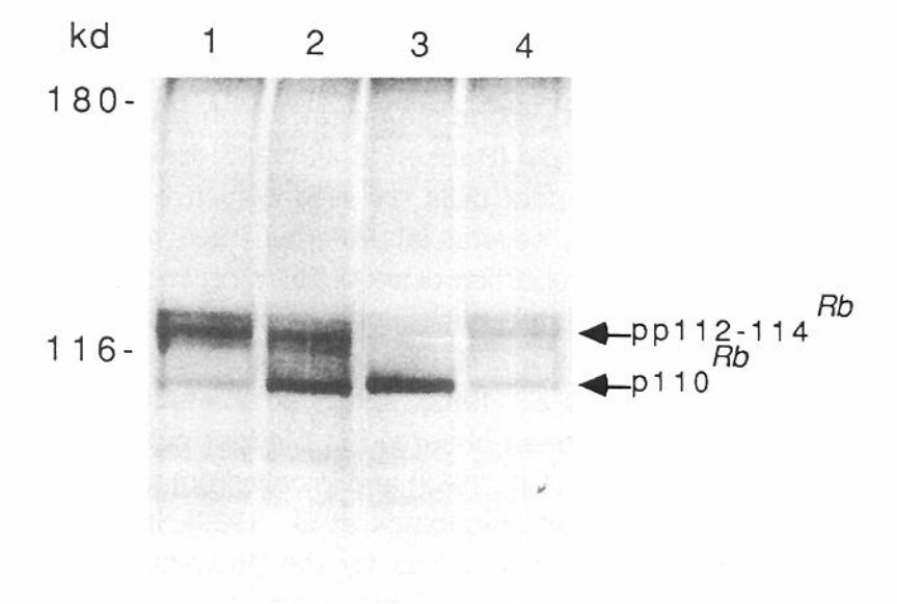
The Product of the Retinoblastoma Susceptibility Gene Has Properties of a Cell Cycle Regulatory Element

James A. DeCaprio,* John W. Ludlow,* Dennis Lyn
Yusuke Furukawa,* James Griffin,*
Helen Piwnica-Worms,† Chun-Ming Huang,‡
and David M. Livingston*

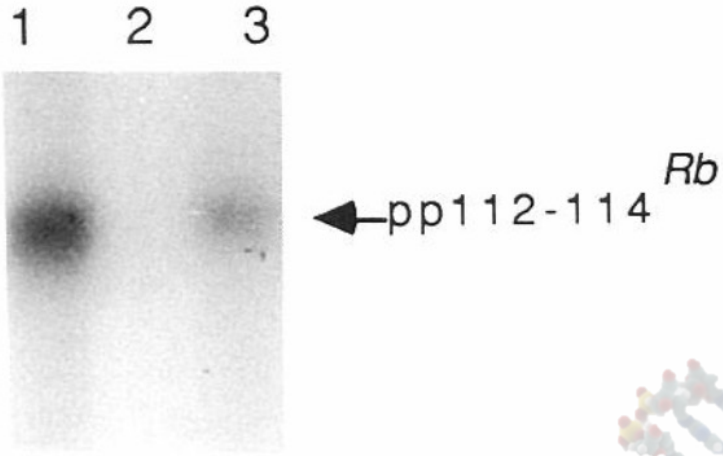
DeCaprio et al., 1989

Regolazione pRB nel ciclo

Western blot:
1. S phase-arrested cells (HU)
2. Factor depletion (36h) → + ECGF
3. Factor-depleted cells
4. Growing cells



Phosphorylation assay
1. Factor depletion (36h) → + ECGF
2. Factor-depleted cells
3. Growing cells



DeCaprio et al., 1989

Regolazione pRB nel ciclo

Fosforilazione pRB durante il ciclo avviene anche in vivo?

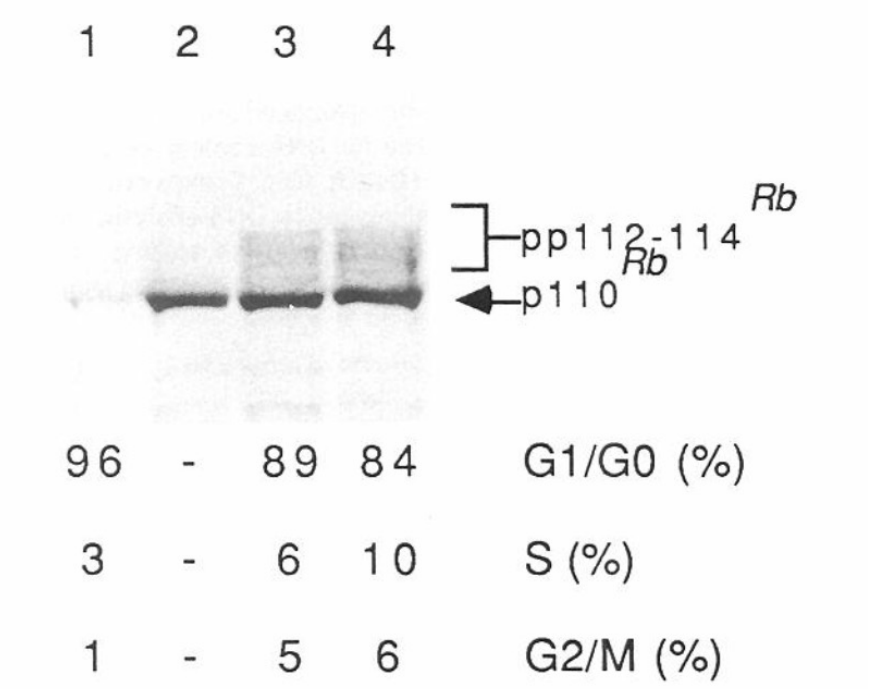


Figure 2. Western Blot for Rb in Resting and Activated T Lymphocytes

Lysates of an enriched population of T lymphocytes were prepared as described in Experimental Procedures. Aliquots of the culture to be analyzed were incubated in the presence of the two specific CD2 activating antibodies described in Experimental Procedures. Aliquots (10^6 cells) were removed before the addition of the antibodies (lane 1), and 24 (lane 2), 48 (lane 3), and 72 hr (lane 4) after addition for analysis of DNA content by cytofluorimetry and of Rb protein by Western blotting.

DeCaprio et al., 1989

Regolazione RB nel ciclo

Western blot in cultured elutriated HeLa cells

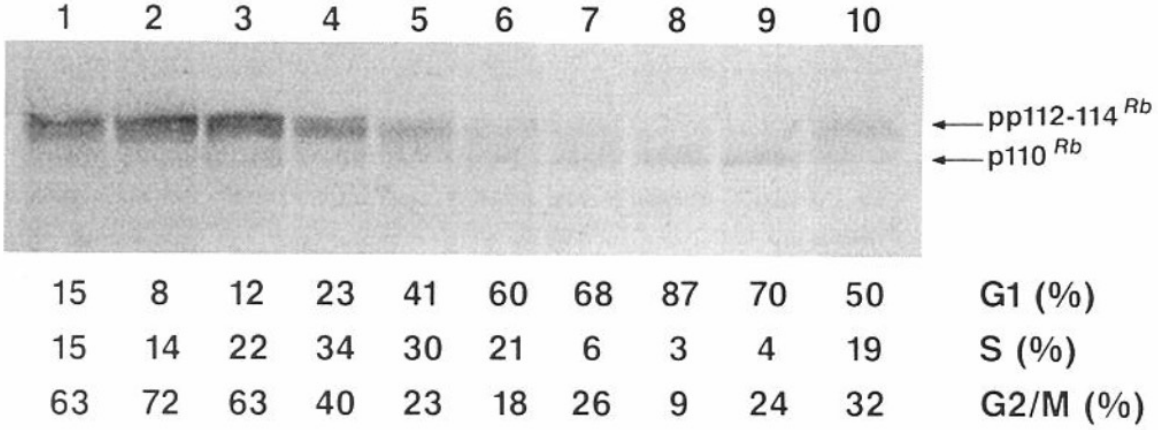
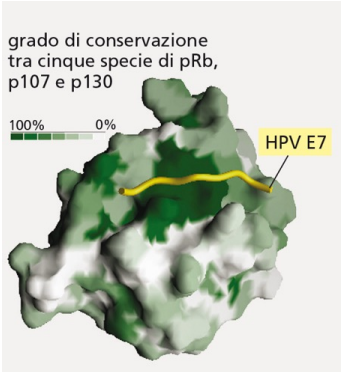


Figure 3. Western Blot for Rb in Elutriated HeLa cells

HeLa cells were elutriated and cytofluorimetrically analyzed for DNA content as descibed (Draetta and Beach, 1988; Draetta et al., 1988). Cells were aliquotted for DNA analysis, protein determination, and Western blotting. A non-elutriated population of HeLa cells is shown in lane 10.



Asn102-Glu114 of SV40 T antigen is the interaction domain with RB

Synthesis of a peptide containing wild- type Asn 102-Glu or the same sequence, but with a Lys for Glu substitution at position 107 (stable but not transforming)

DeCaprio et al., 1989

Regolazione RB nel ciclo

Synthesis of a peptide containing wild-type Asn 102-Glu or the same sequence, but with a Lys for Glu substitution at position 107 (stable but not transforming)

Saggio di competizione

IP con anti-SV40 ab (2, 4-8)
o con anti-pRB ab (1-3)

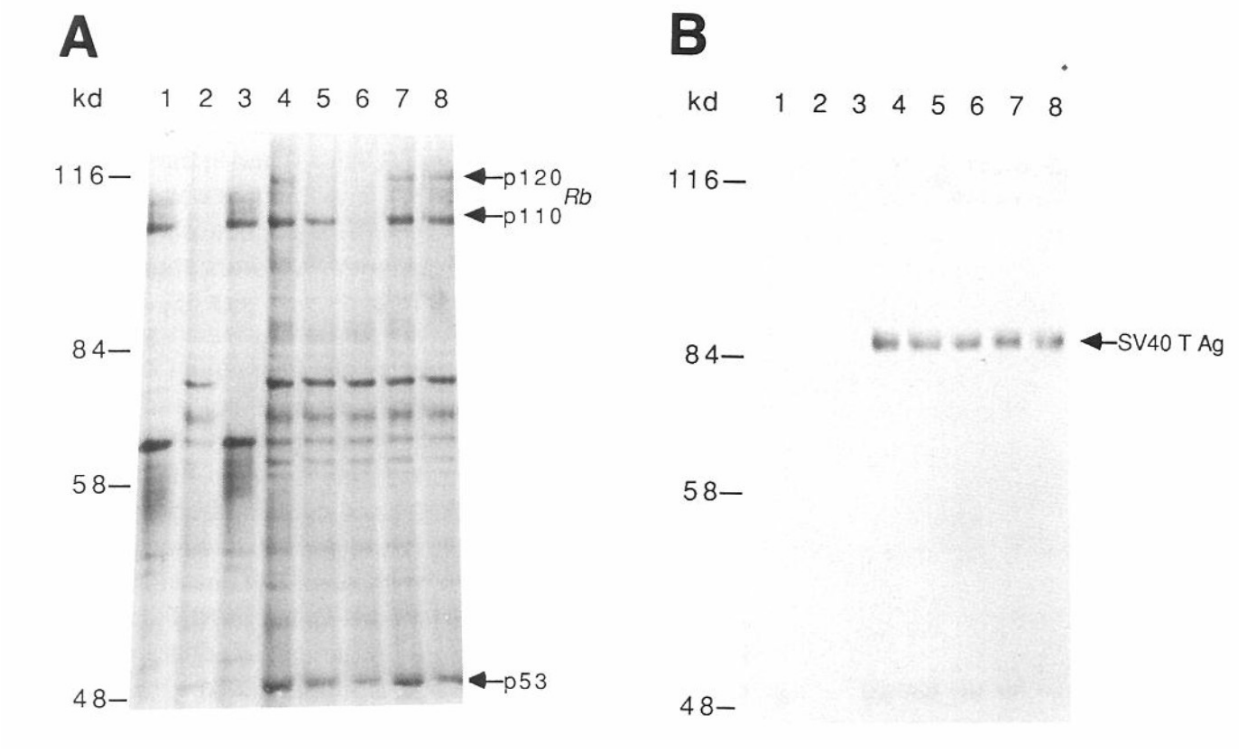


Figure 6. Peptide Mixing Experiment

CV-1P cells were labeled with [^{35}S]methionine (200 μCi/ml) for 3 hr, and lysed as described (DeCaprio et al., 1988). Aliquots of labeled lysate were mixed with wild-type peptide (NLFCSEEMPSSDDE) at 3 μM (lane 5) and 54 μM (lane 6) or mutant peptide (NLFCSKEMPSSDDE) at 3 μM (lane 7) and 54 μM (lane 8) for 60 min at 4°C. Then 100 μl of unlabeled SV80 cell extract (10 μg/μl) was added to each mixture (lanes 3–8) for an additional 60 min. Lysates were immunoprecipitated with an Rb monoclonal antibody (lanes 1 and 3), or with an SV40 T monoclonal antibody (lanes 2 and 4–8). Immunoprecipitates were eluted, and separated by electrophoresis through a 7.5% SDS–polyacrylamide gel. The gel was blotted onto nitrocellulose, and the paper immunostained for SV40 T (B). An autoradiogram was also obtained of this blot (A). The migration positions of p120 (Ewen et al., 1989), p110^{Rb} (DeCaprio et al., 1988; Ludlow et al., 1989), and p53 are indicated along the righthand border of (A). SV40 T is noted along the righthand border of (B). Molecular weight standards are indicated along the lefthand border of each panel.

DeCaprio et al., 1989

Regolazione RB nel ciclo

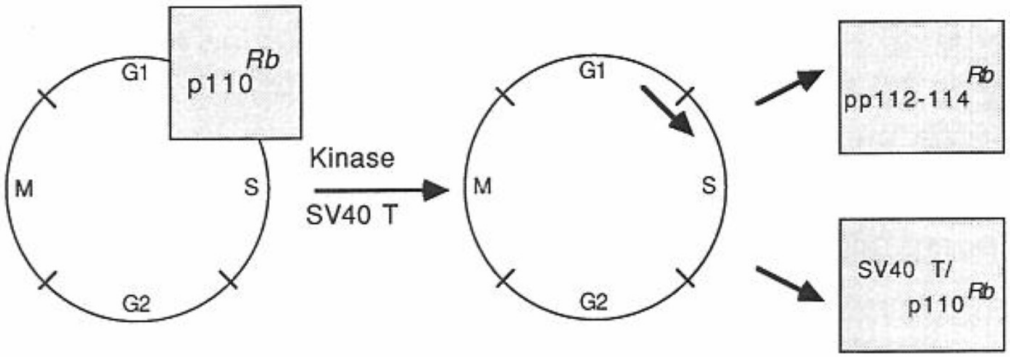


Figure 7. Model for the Growth Suppression Function of $p110^{Rb}$

It is proposed that $p110^{Rb}$ helps to provide a block to exit from G1 and, thereby, prevents the cell from progressing to S. The block can be removed by specific phosphorylation of $p110^{Rb}$ to yield $pp112–114^{Rb}$ or by binding to SV40 T. Once $p110^{Rb}$ is inactivated by phosphorylation or binding to SV40 T, the cell can advance to the DNA replication phase of the cycle.

Ipotesi: RB legata da oncoproteine virali è sequestrata e non può svolgere le sue funzioni

DeCaprio et al., 1989