

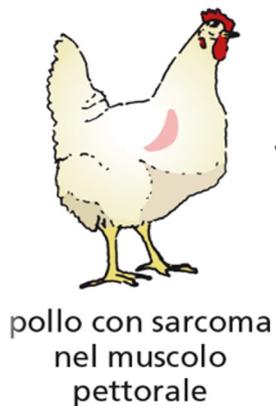
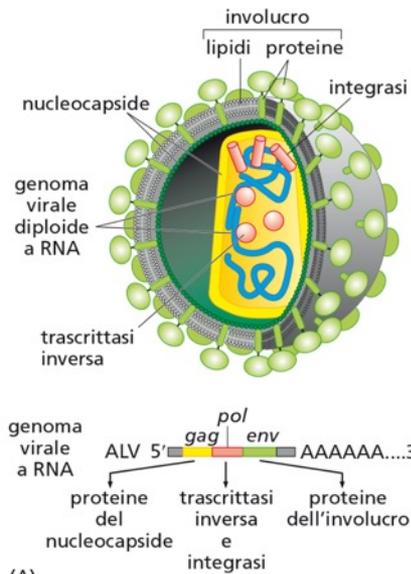
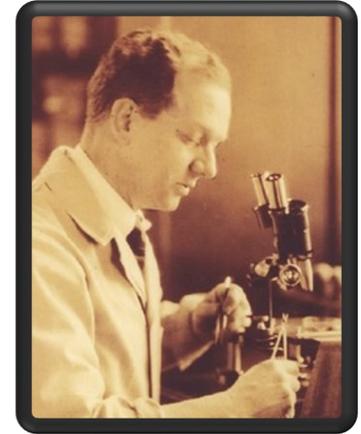
GLI ONCOGENI VIRALI



Rous Sarcoma Virus (RSV)

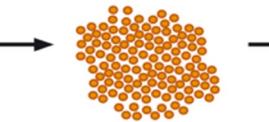
Scoperta e studio dei virus oncogeni conferma che il cancro è una malattia genetica

Peyton Rous scopre il virus del sarcoma di pollo o RSV (1910)



pollo con sarcoma nel muscolo pettorale

rimozione del sarcoma e sua triturazione in piccoli pezzetti



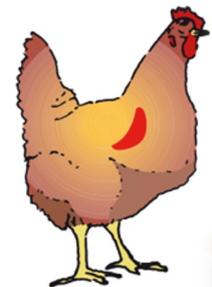
omogenizzazione del tumore con sabbia



raccolta del filtrato ottenuto passando l'omogenato attraverso il filtro a pori sottili



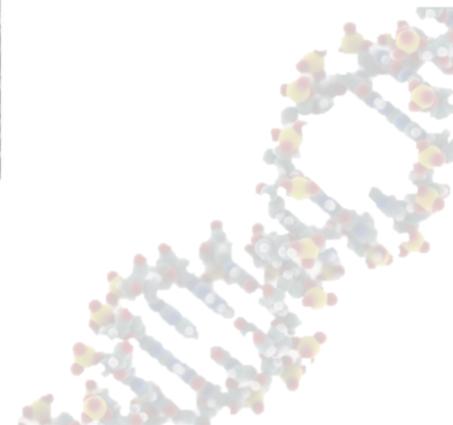
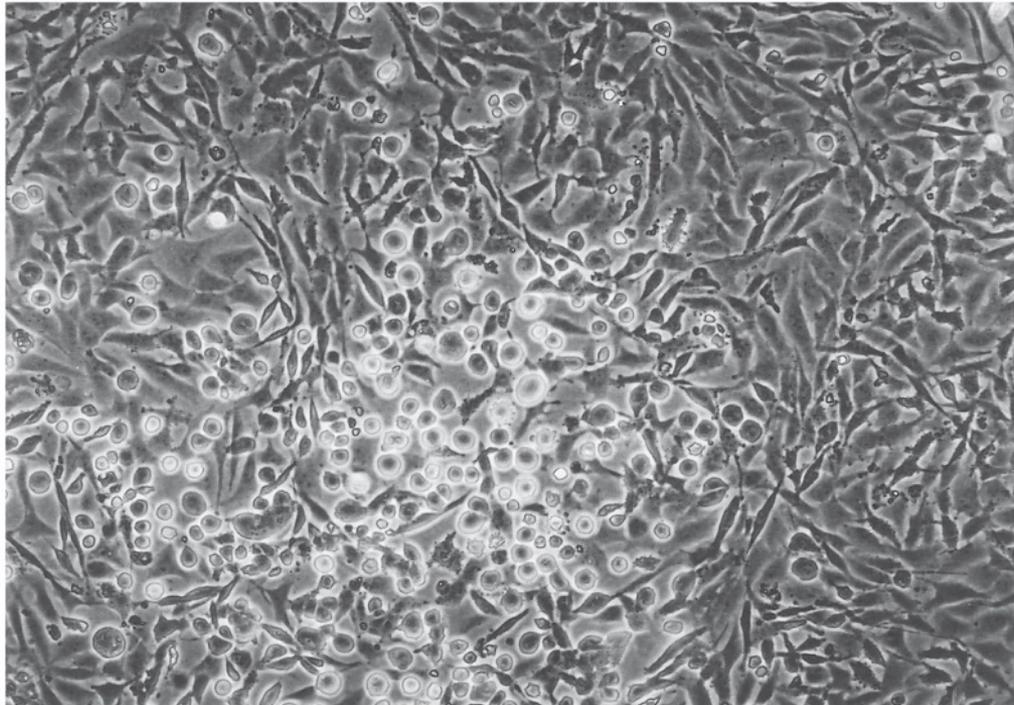
iniezione del filtrato in un giovane pollo



comparsa del sarcoma nel pollo inoculato

RSV trasforma cellule *in vitro*

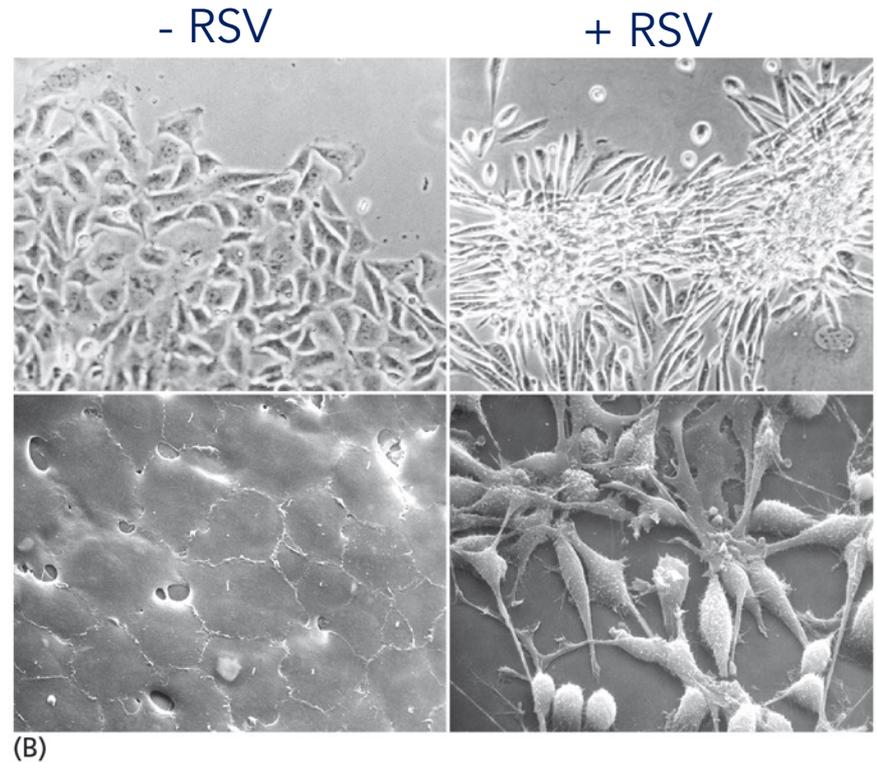
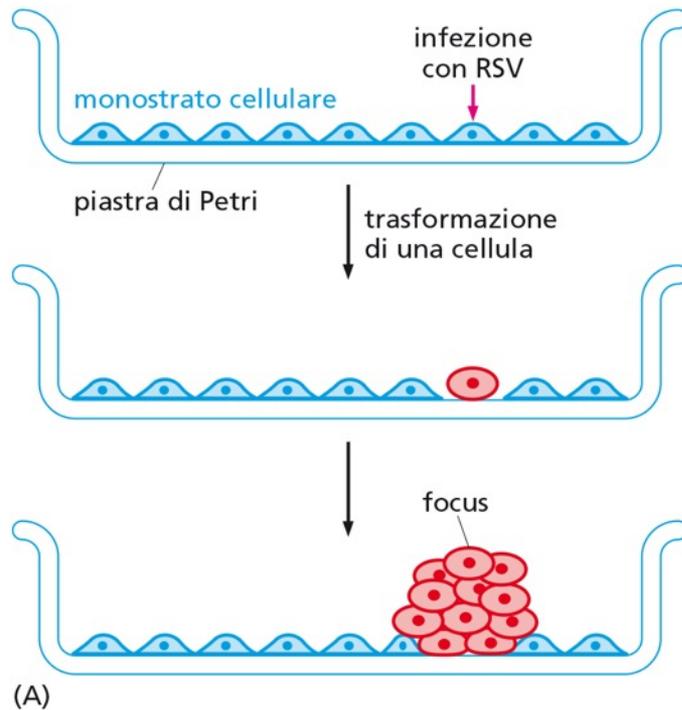
Harry Rubin e Howard Temin, 1959: preparazioni di RSV inoculate in fibroblasti di pollo coltivate in piastre Petri crescono indefinitamente (foci)



RSV trasforma cellule *in vitro*

Cellule normali in piastra Petri formano un monostrato.

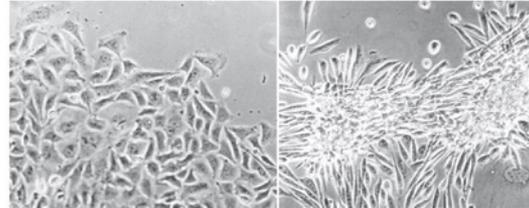
Infezione con RSV fa formare foci di cellule derivate da una cellula infettata (cloni)



La presenza continua di RSV è necessaria per mantenere il fenotipo trasformato?

RSV serve per mantenere il fenotipo trasformato

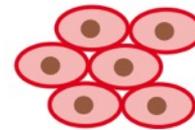
Mutante ts di RSV:
trasforma cellule di pollo a
37°C ma non a 41°C →
cellule infettate hanno
fenotipo ts



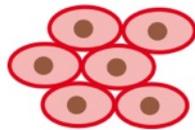
infezione
con un mutante RSV



normale
morfologia



morfologia
trasformata



temperatura non permissiva

temperatura
41 °C
37 °C

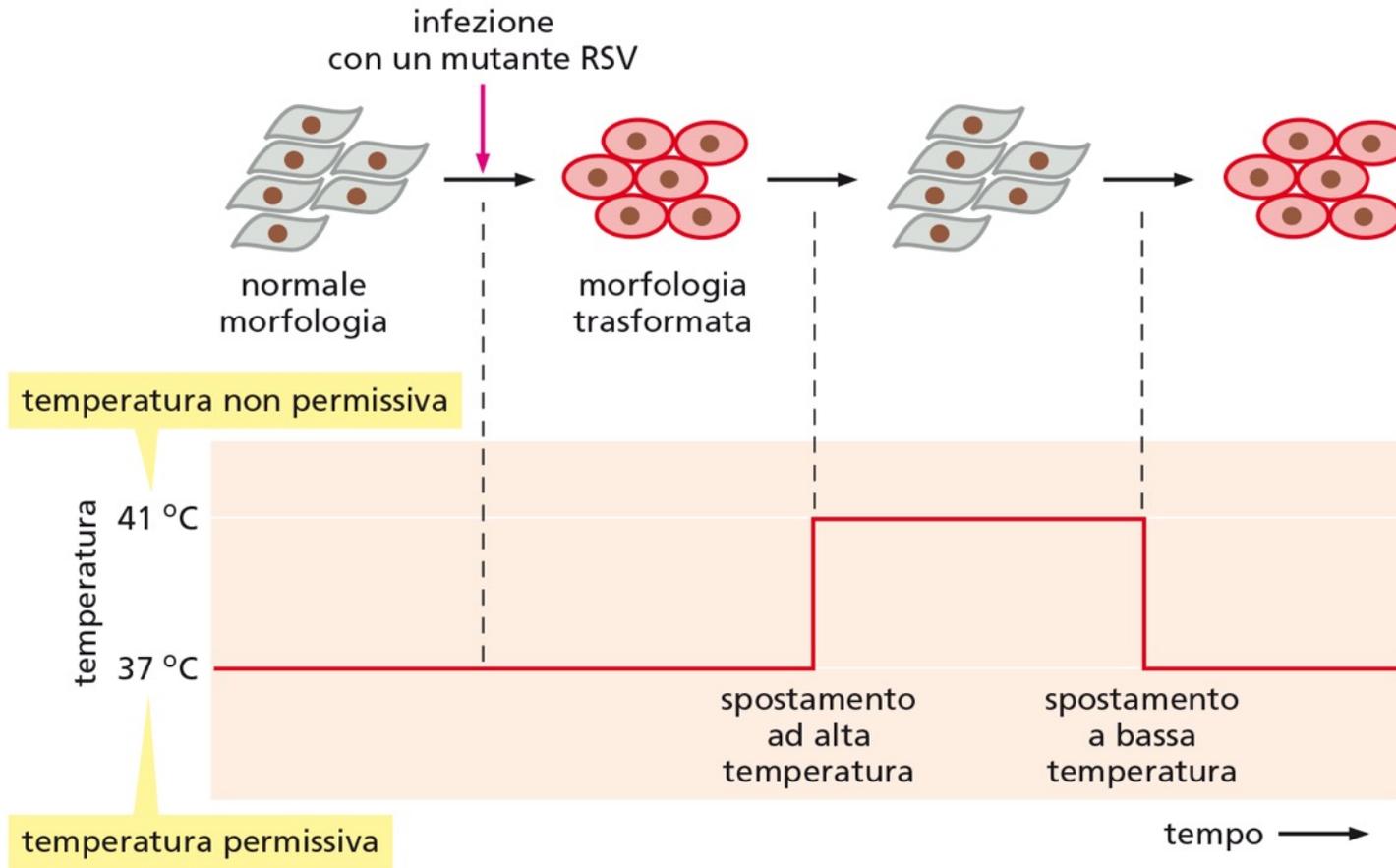
temperatura permissiva

spostamento
ad alta
temperatura

spostamento
a bassa
temperatura

tempo →

RSV serve per mantenere il fenotipo trasformato



GENE TRASFORMANTE VIRALE SERVE PER INIZIARE E MANTENERE FENOTIPO TRASFORMATO

Retrovirus oncogeni

A History of Cancer Research: Retroviral Oncogenes

Joseph Lipsick

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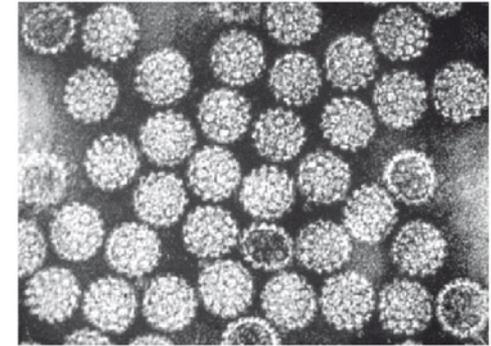
Correspondence: lipsick@stanford.edu



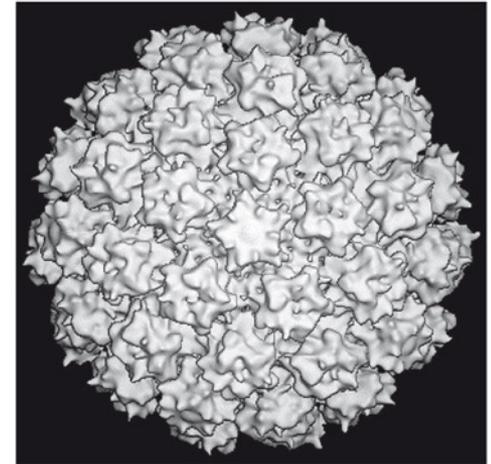
Figure 9. Cover of the abstract book for the meeting on RNA Tumor Viruses at Cold Spring Harbor Laboratory on May 25–29, 1983. Some (but not all) of the discoverers of various retroviral oncogenes are depicted as the animals from which the viruses were isolated. (Illustration © Jamie Simon.)

Virus a DNA e cancro

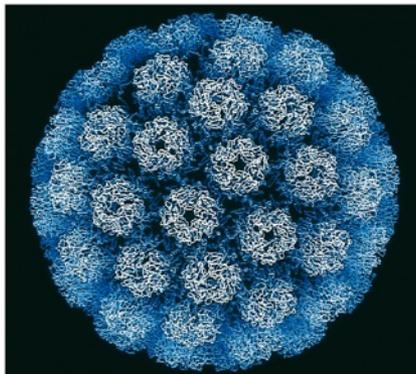
- Virus del papilloma di Shope causa papilloma della cute
- Più di 100 virus del papilloma (HPV, *human papilloma virus*)
- SV40: virus della scimmia che produce numerosi vacuoli nelle cellule infettate prima di lisarle.
- SV40 non si replica in cellule di roditori (non permissive), ma occasionalmente causa tumori.
- Adenovirus umani non si replicano in cellule di roditori, ma occasionalmente causa tumori.
- Alcuni virus erpetici causano linfomi



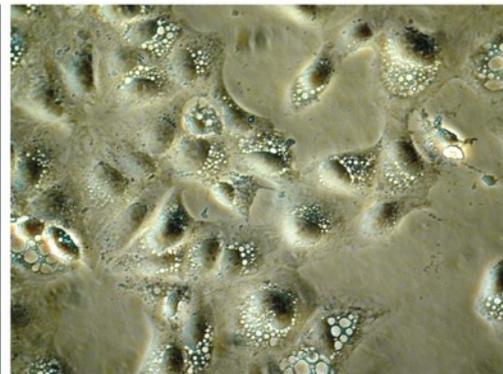
(A)



(B)



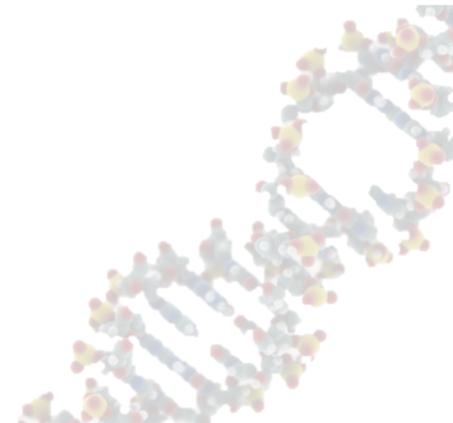
(A)



(B)

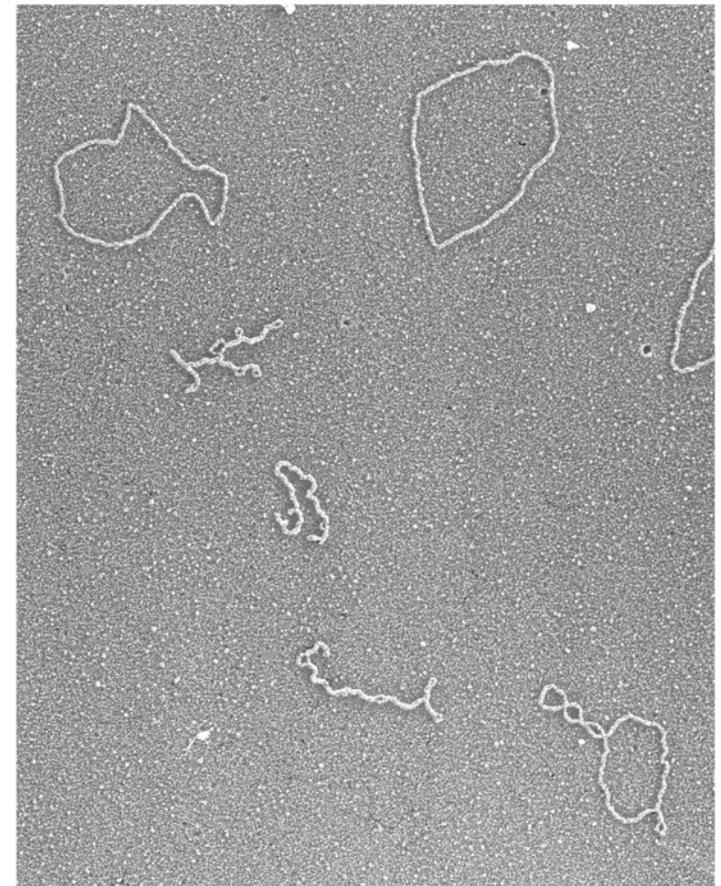
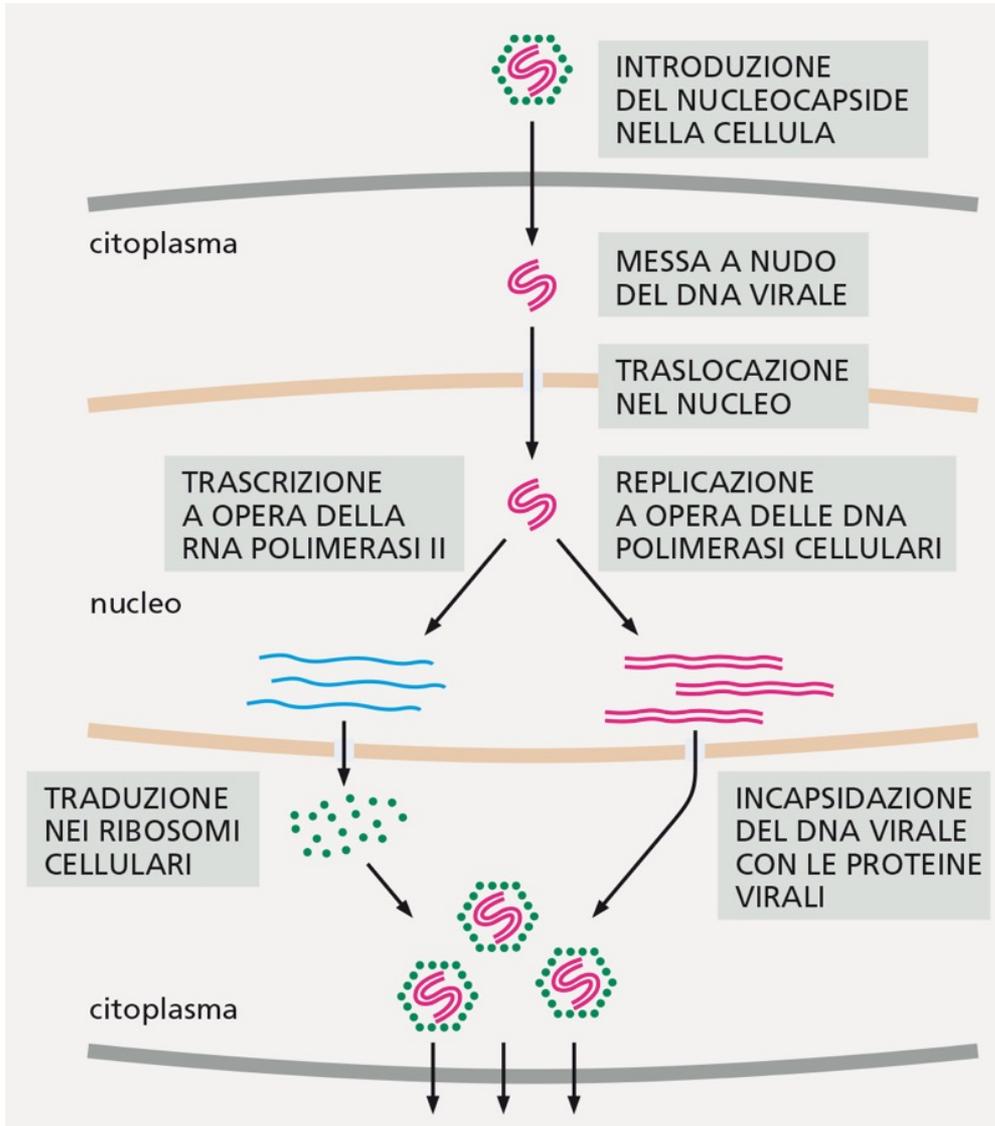
Virus e cancro

- Virus hanno genoma piccolo
- Ricerca dei geni che causano trasformazione

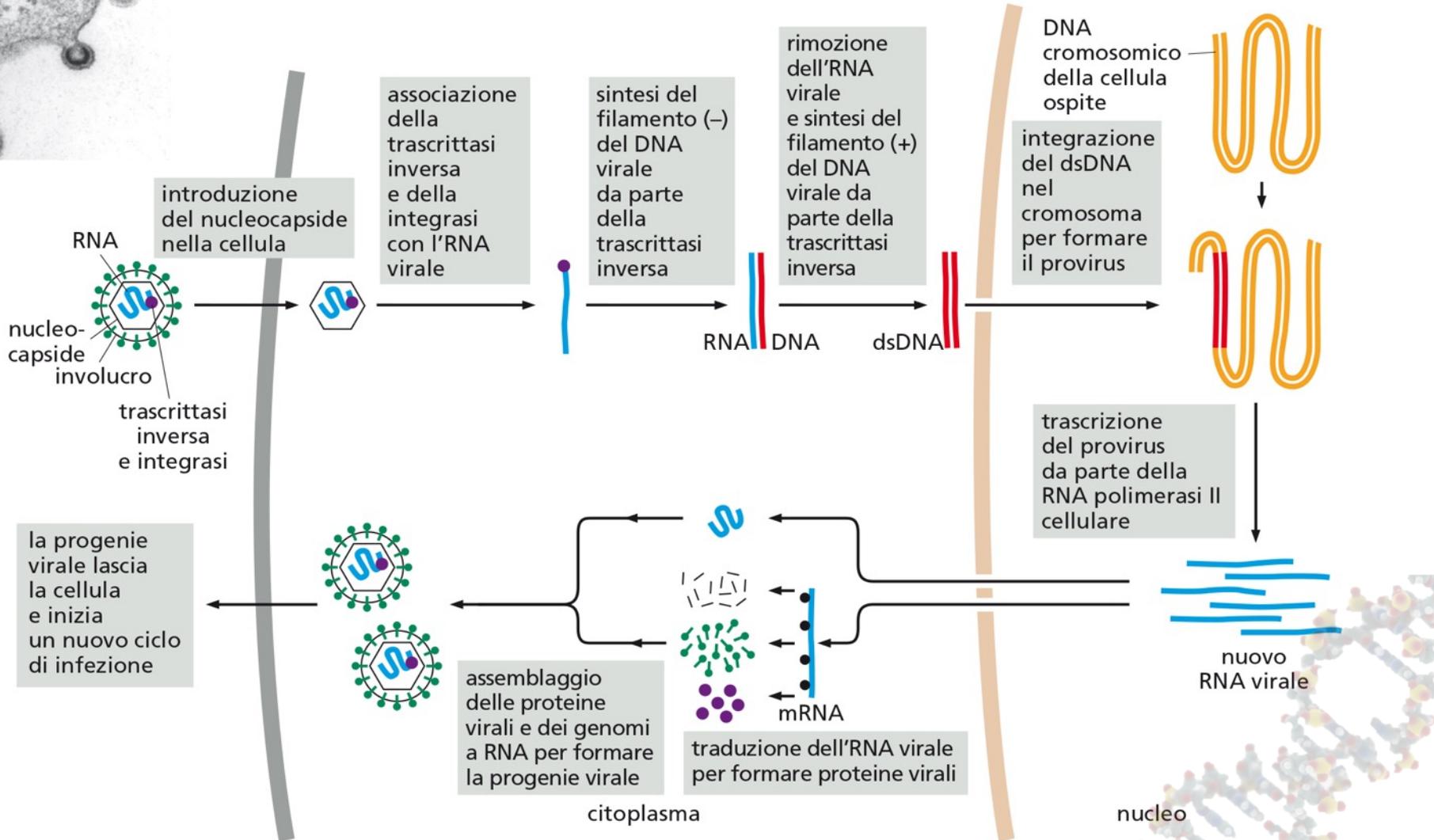
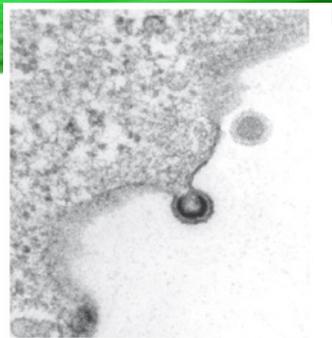


Virus a DNA

Genoma a DNA circolare piccolo:
pochi geni



Virus a RNA



Virus oncogeni

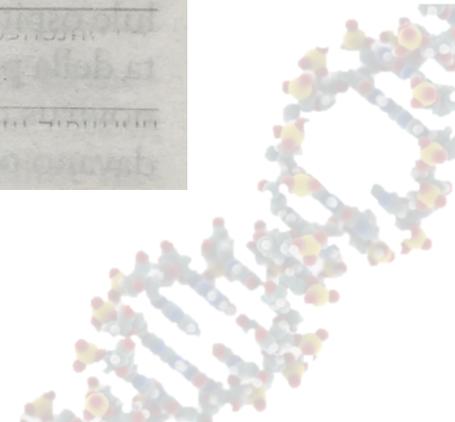
TABELLA 3.1

Genomi di alcuni virus oncogeni

	Famiglia	Dimensione del genoma (kb)
Virus a DNA		
Virus dell'epatite B (HBV)	<i>Hepadnaviridae</i>	3
SV40/polioma	<i>Polyomaviridae</i> *	5
Papilloma virus umano 16 (HPV)	<i>Papillomaviridae</i> *	8
Adenovirus umano 5	<i>Adenoviridae</i>	35
Virus erpetico umano tipo 8 (HHV-8; KSHV)	<i>Herpesviridae</i>	165
Virus del fibroma di Shope	<i>Poxviridae</i>	160
Virus a RNA		
Virus del sarcoma di Rous (RSV)	<i>Retroviridae</i>	9
Virus umano della leucemia a cellule T (HTLV-1)	<i>Retroviridae</i>	9

Adattata da G.M. Cooper, *Oncogenes*, 2ª edizione, Boston: Jones e Bartlett Publishers, 1995.

* Inclusi inizialmente nella famiglia dei papova virus (N.d.T.).



Virus oncogeni e trasformazione

Transformation assay or focus formation assay:

-in vitro

-immortalized cells

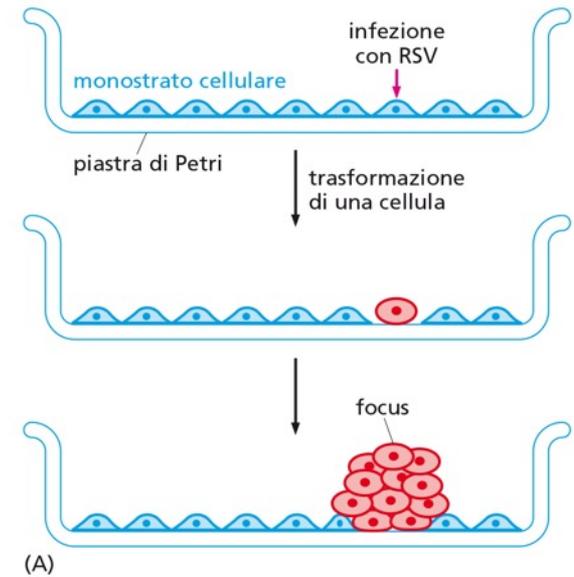


TABELLA 3.2

Proprietà delle cellule trasformate

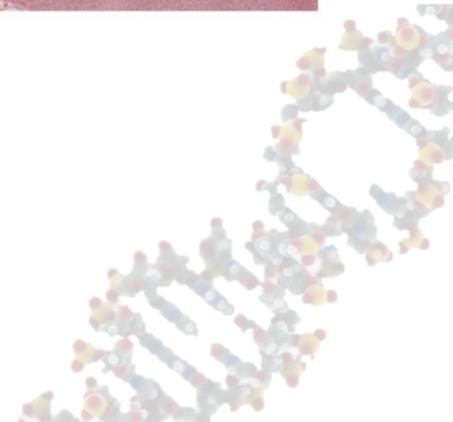
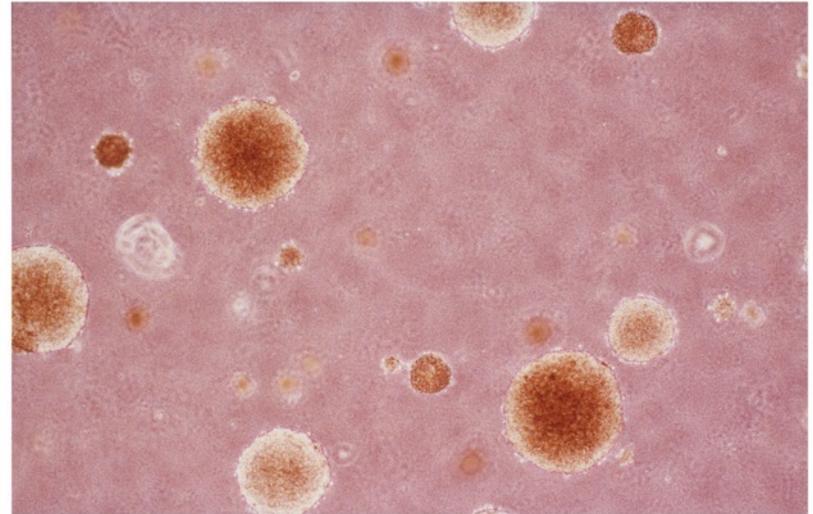
- Alterata morfologia (forma rotondeggiante, rifrangente al microscopio a contrasto di fase)
- Mancanza di inibizione da contatto (capacità di crescere una sull'altra)
- Capacità di crescita senza adesione al substrato solido (indipendenza dall'ancoraggio)
- Capacità di proliferare indefinitamente (immortalizzazione)
- Ridotta richiesta di fattori di crescita
- Alta densità di saturazione (capacità di accumulare un grande numero di cellule nelle piastre di coltura)
- Incapacità di bloccare la proliferazione in risposta alla mancanza di siero
- Aumentato trasporto di glucosio
- Tumorigenicità

Adattata da S.J. Flint, L.W. Enquist, R.M. Krug et al., *Principles of Virology*, Washington, DC: ASM Press, 2000.

Virus oncogeni e trasformazione

Soft agar colony formation assay:

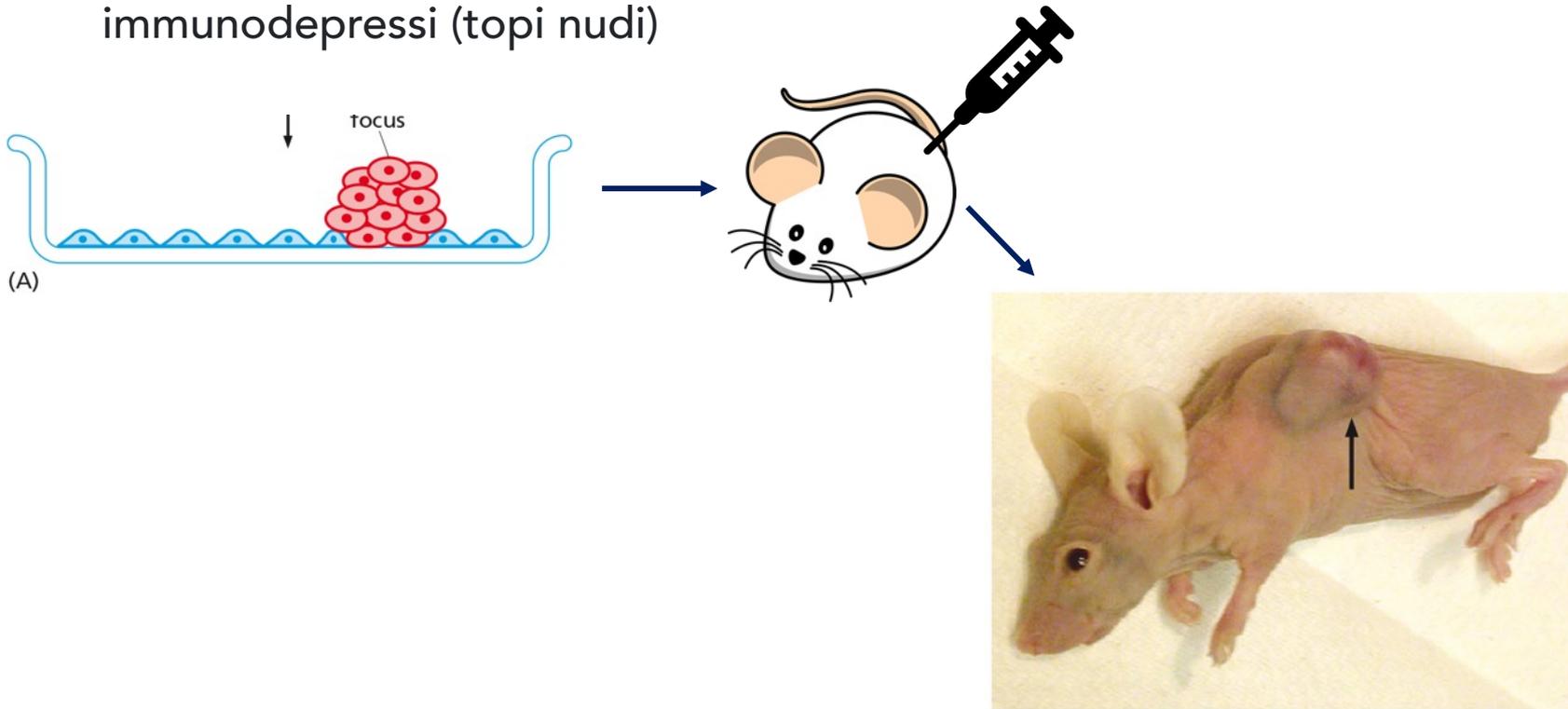
anchorage-independent growth is the ability of transformed cells to grow independently of a solid surface



Virus oncogeni e trasformazione

Saggio di tumorigenicità in vivo:

- Cellule trasformate in vitro
- Inoculo sottocute in topi dello stesso ceppo (singenico) oppure in topi immunodepressi (topi nudi)

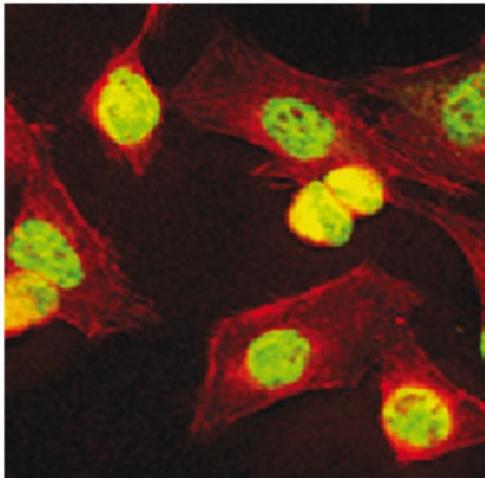


Virus oncogeni e trasformazione

EXP con mutanti ts di RSV → genoma virale mantenuto in cellule trasformate

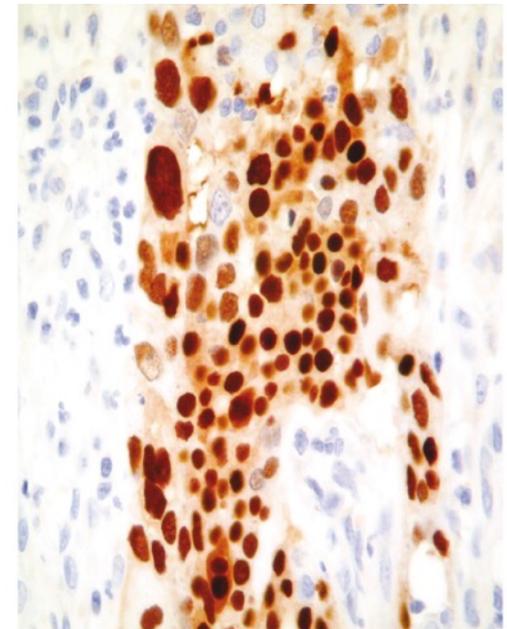
Anche trasformazione da parte di SV40 o virus del poliovirus sembrava richiedere presenza continua del genoma virale.

Sieri da topi infettati con SV40 molto reattivi contro un antigene tumorale (large T antigen)



IF con anti-LT. Verde

COME?



(B) IHC con anti-LT. Marrone

I genomi dei virus oncogeni sono integrati nelle cellule trasformate

THE INTEGRATED STATE OF VIRAL DNA IN SV40-TRANSFORMED CELLS*

By J. SAMBROOK, H. WESTPHAL, † P. R. SRINIVASAN, ‡ AND R. DULBECCO

SALK INSTITUTE FOR BIOLOGICAL STUDIES, SAN DIEGO, CALIFORNIA

Communicated June 12, 1968

Viral form II behaves as linear DNA in these gradients and remains with the cell DNA

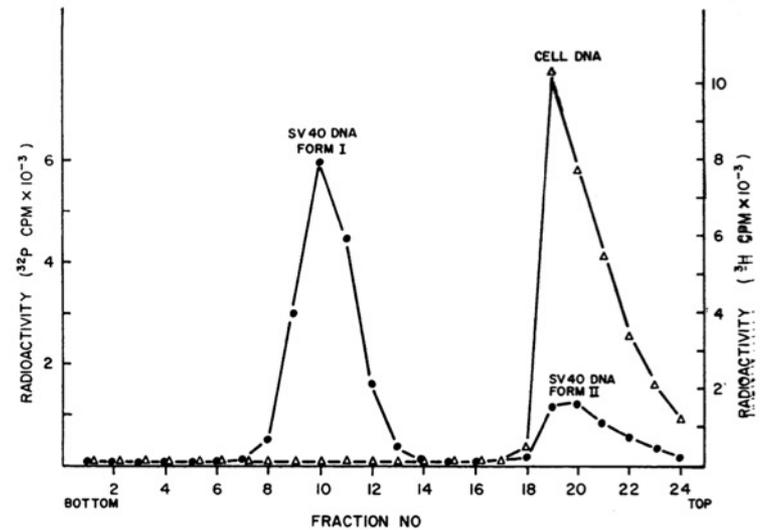


FIG. 1.—Equilibrium centrifugation of a mixture of 0.2 μg purified SV40 P^{32} -DNA (I and II) and 100 μg cell H^3 -DNA in 5.00 ml CsCl , density 1.57 gm/ml, 100 $\mu\text{g}/\text{ml}$ ethidium bromide for 36 hr at 43 krpm, 20°C in a Spinco 50 angle head. Fractions were collected and the DNA was precipitated and counted as described.

Discussion.—In these experiments, DNA's isolated by a variety of methods from SV3T3 and Py3T3 cells have been hybridized with RNA made *in vitro* with SV40 DNA as template. In every case, it was possible to isolate a DNA fraction from the SV3T3 cells that hybridized to a greater extent than the comparable DNA fraction from the control cell line, Py3T3. This increased hybridization is equivalent to 20 SV40 genomes per cell.

I genomi dei virus oncogeni sono integrati nelle cellule trasformate

TABLE 1. *Hybridization of DNA from transformed cells after centrifugation in CsCl/ethidium bromide gradients.*

Cell type	Fraction from Gradient (cpm)	
	V	C
SV3T3	69 ± 5	358 ± 2
Py3T3	64 ± 7	129 ± 12
Py3T3 + 20 SV40 genomes per cell	385 ± 11	198 ± 5

H^3 -RNA complementary to SV40 (form I) DNA was hybridized with the indicated fractions. The amount of DNA remaining on the filters after hybridization was determined and the figures in column C are the hybrid cpm normalized to 50 μ g DNA. The figures in column V are uncorrected hybrid cpm. The H^3 -cRNA input per filter was 1.2×10^5 cpm in this experiment.

TABLE 3. *Hybridization of DNA extracted from chromosomes and nuclei.*

Source of DNA	Cpm hybridized
SV3T3 chromosomes	680 ± 63
SV3T3 nuclei*	690 ± 87
Py3T3 nuclei	290 ± 32
SV3T3 nuclei	740 ± 47

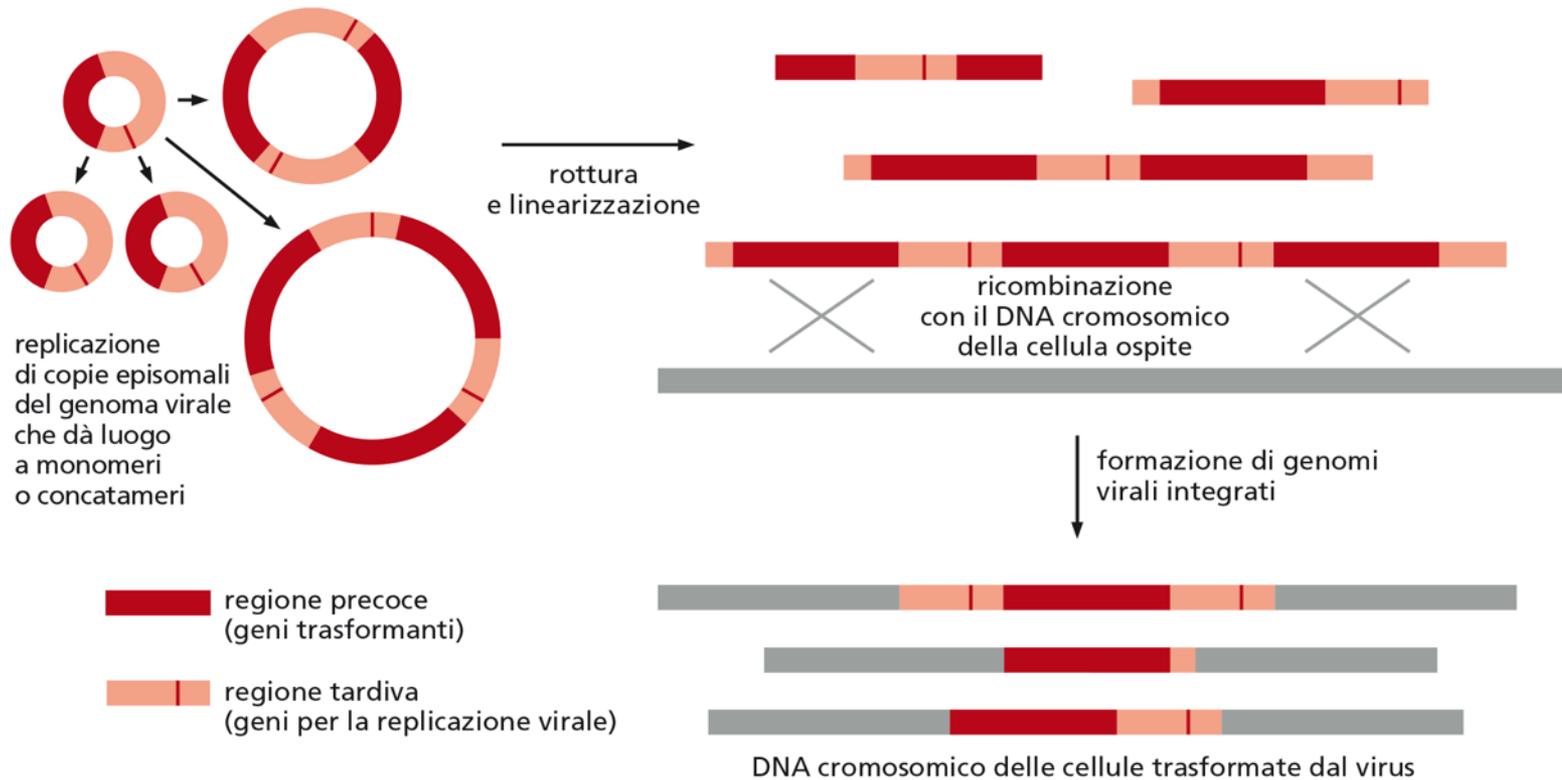
H^3 -RNA complementary to SV40 (form I) DNA was hybridized with the indicated DNA. The amount of DNA remaining on the filters after hybridization was determined and the hybrid cpm have been normalized to 50 μ g DNA. The H^3 -cRNA input in this experiment was 2.1×10^5 cpm/filter.

* DNA extracted from nuclei of SV3T3 cells treated with colcemid.

Thus the results presented here provide evidence that the viral DNA molecules in SV3T3 cells are integrated with cell DNA by alkali-stable covalent linkages.

Integrazione genoma SV40

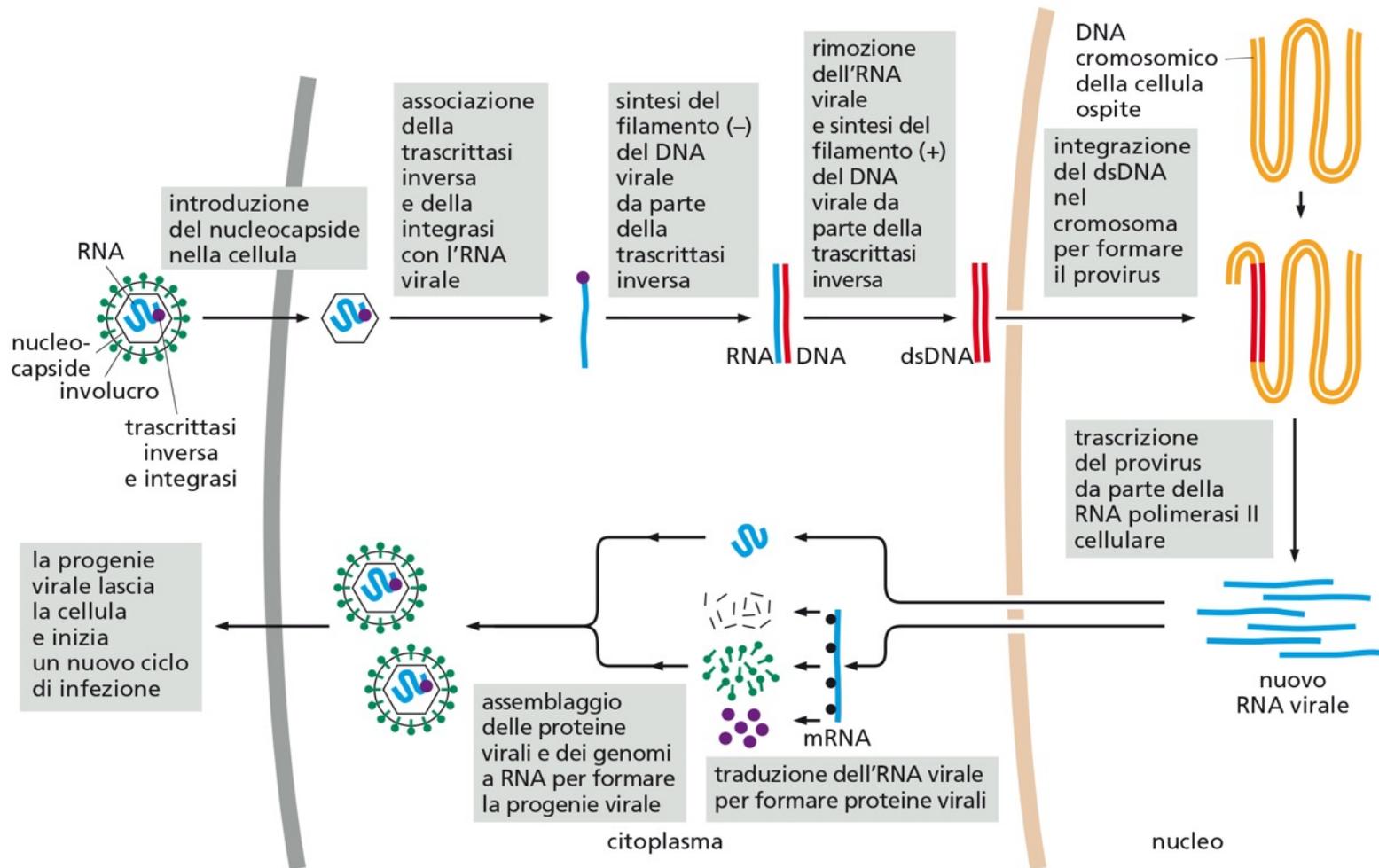
DNA virale integrato in cromosomi si trasmette alle cellule figlie → **COME SI INTEGRA IL GENOMA VIRALE?**



Meccanismo comune a molti virus oncogeni a DNA: >99,7% dei carcinomi della cervice uterina hanno frammento di HPV integrato nelle cellule

Weinberg, La Biologia del cancro.

Integrazione genoma RSV



Integrazione è passaggio essenziale nel ciclo replicativo dei retrovirus, mentre integrazione di genomi (o frammenti) di virus a DNA è evento raro ($\ll 1/1000$ infezioni)

Il genoma di RSV e il gene *src*

2 classi di virus RSV mutanti:

- classe 1: si replicano ma non trasformano
- classe 2: trasformano ma non si replicano e non formano particelle virali

Geni richiesti per trasformazione sono diversi da quelli per replicazione

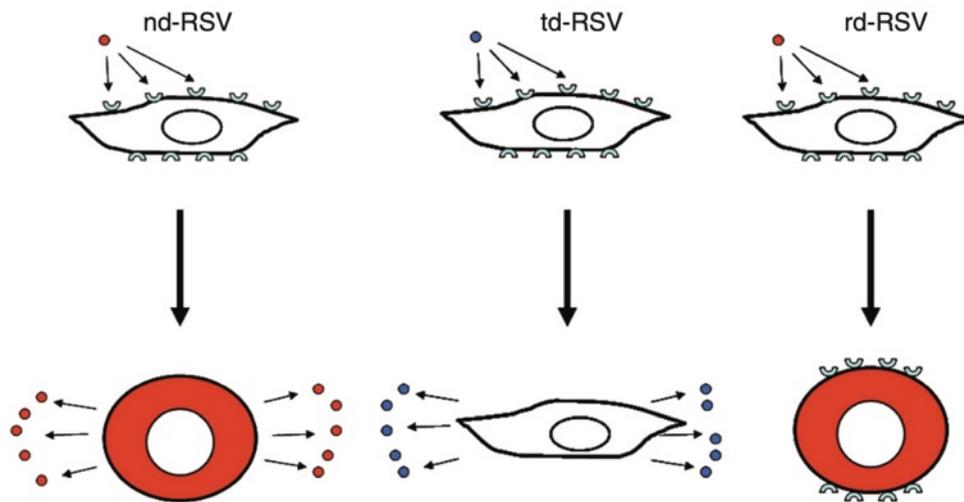


Figure 1. The biology of nondefective (nd), transformation-defective (td), and replication-defective (rd) forms of Rous sarcoma virus (RSV). Note that avian leukosis virus (ALV), Rous-associated virus (RAV), and replication-inhibiting factor (RIF) all behave like the td-RSV mutant shown here. Round, red cells are morphologically transformed. Cell surface receptors available for viral infection are indicated by light blue semicircles. These receptors are no longer available following infection with nd-RSV or td-RSV (interference). The Bryan strain of RSV contains a mixture of td-RSV and rd-RSV genomes within virions containing envelope proteins encoded by td-RSV. (Modified from Martin GS. 2004. *Oncogene* **23**: 7910–7917, with permission, from Springer Nature.)

Il genoma di RSV e il gene *src*

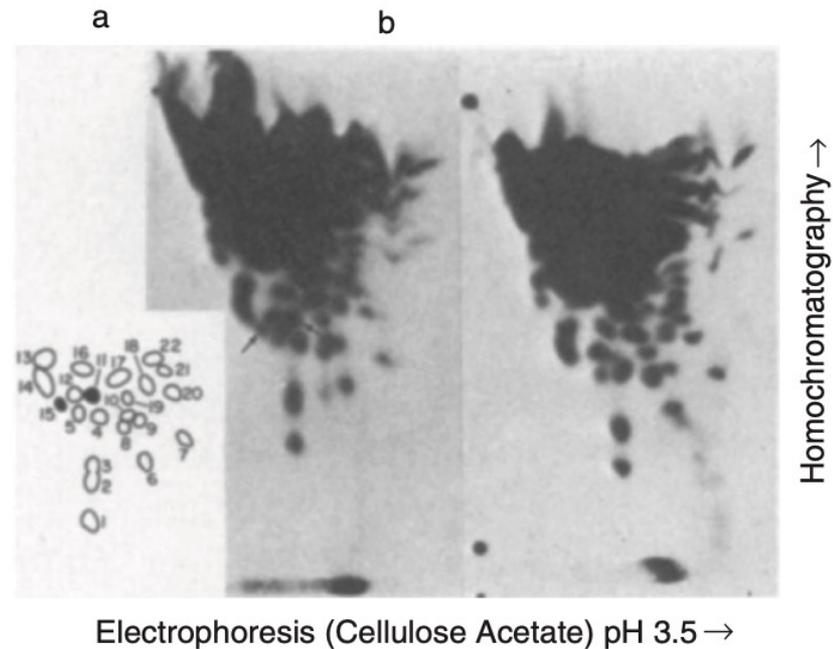
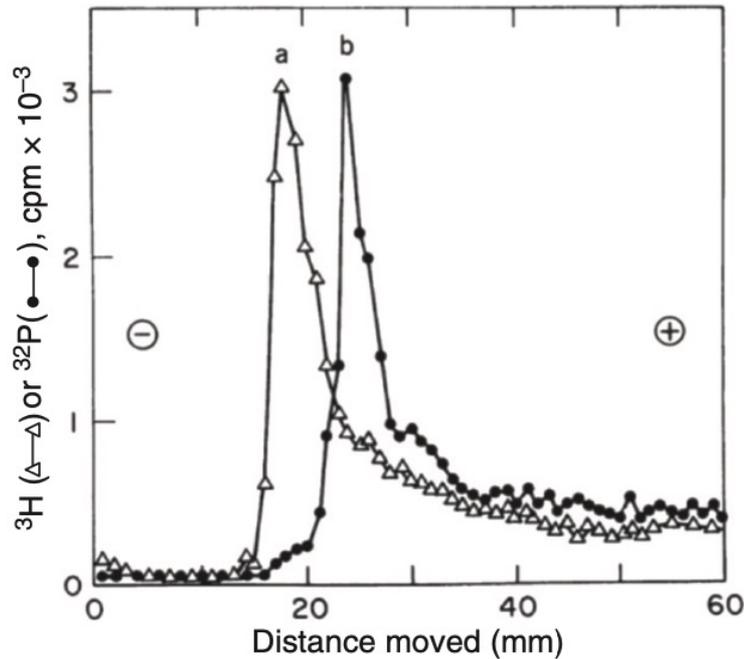


Figure 3. Comparative analyses of genomic RNA from an isogenic pair of Prague C Rous sarcoma viruses (RSVs), the parental nondefective (nd) strain (a) and a mutant transformation-defective (td) strain (b). (Left) Co-electrophoresis of nd-RSV labeled with [^3H]-uridine and td-RSV labeled with [^{32}P]-phosphate. (Right) Two-dimensional “fingerprinting” of [^{32}P]-phosphate-labeled RNA fragments created by digestion with T1 RNase. The shaded dots and the arrows indicate oligonucleotide spots unique to the nd virus. (Reprinted from Lai MC, et al. 1973. *Proc Natl Acad Sci* **70**: 2266–2270, with permission.)

Il genoma di RSV e il gene *src*

Confronto genoma RSV con quello di virus simili non trasformanti (ALV, *avian leukosis virus*) → piccola sequenza in più nel genoma di RSV → 1 gene? **src**



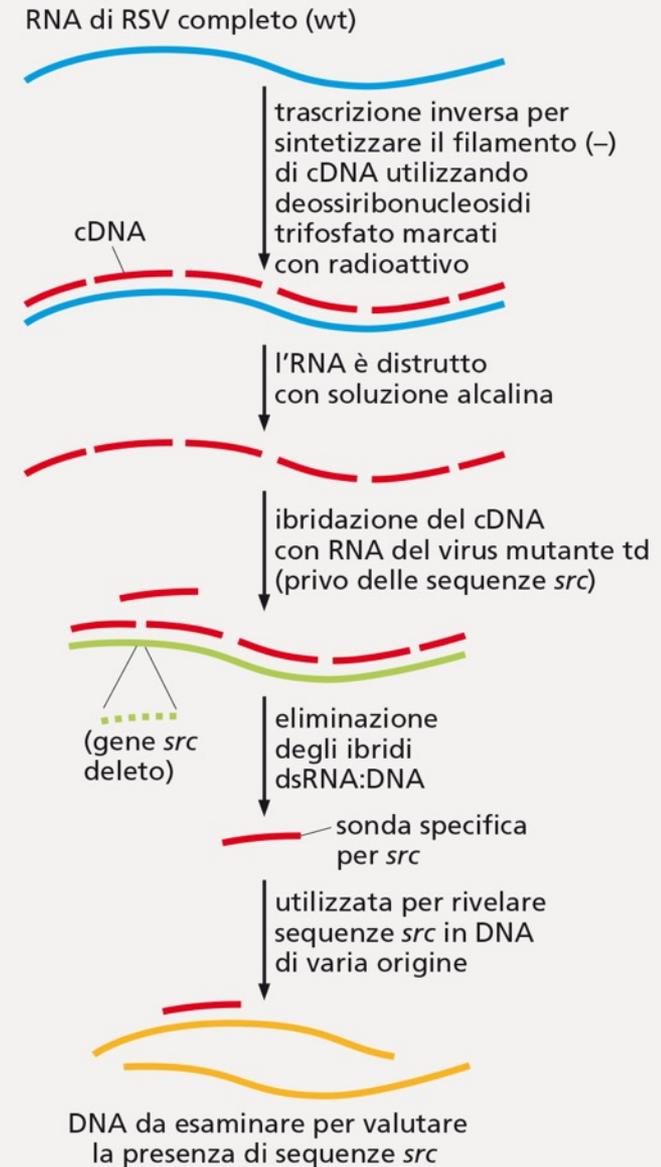
Il gene *src* è presente anche in cellule non infettate

Nature Vol. 260 March 11 1976

D. Stehelin, H.E. Varmus, J.M. Bishop

DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA

Costruzione di una sonda per il gene *src*



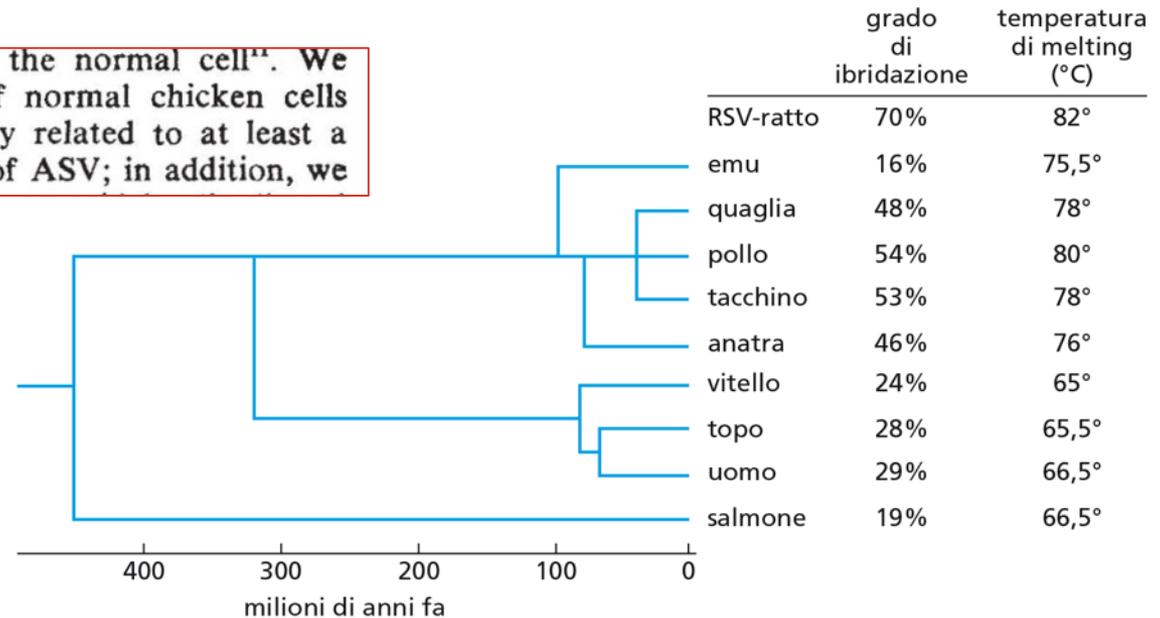
Il gene *src* è presente anche in cellule non infettate

Table 1 Homology between cDNA_{src} and normal DNAs

Assay	Hybridisation conditions		Extent of reaction between cDNA _{src} and DNA from						
	[Na ⁺]	Temperature	Chicken	Quail	Turkey	Duck	Emu	Mouse	Calf
S1	0.9 M	68°	52%	46%	48%	45%	24%	<2%	<2%
HAP	0.9 M	68°					36%		<5%
HAP	1.5 M	59°					54%		

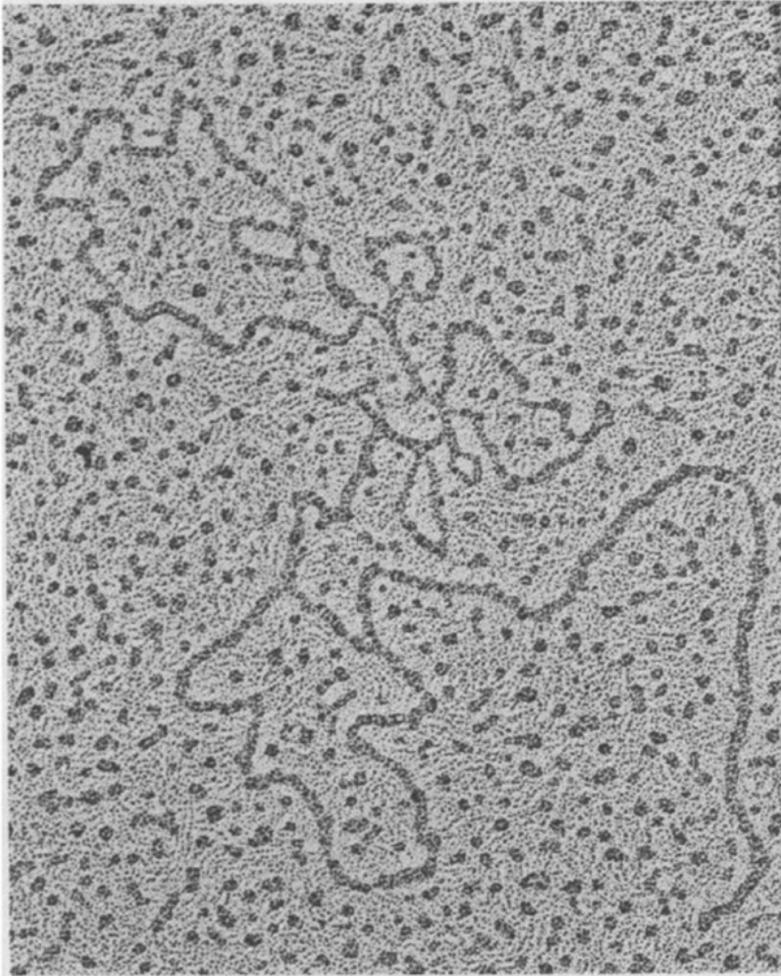
DNA was extracted from 10-11-d-old embryos of chickens, ducks and quails, 3-d-old mice (strain RIII), livers of adult turkeys, liver and heart of a 22-d-old emu, and calf thymus. Reaction mixtures containing denatured DNA (8 mg ml⁻¹) and ³H-cDNA_{src} (0.32 ng ml⁻¹, 7,000 c.p.m. ml⁻¹) in a final volume of 0.3 ml were incubated at either 59 or 68 °C for 48 h. Samples incubated at 59 °C were in 1.5 M NaCl (final C_{0t} = 40,000), those incubated at 68 °C were in 0.9 M NaCl (final C_{0t} = 32,000); all reactions also contained 0.001 M EDTA-0.02 M Tris-HCl, pH 7.4. Duplex formation was measured by either hydrolysis with S1 nuclease²⁸ (in 0.3 M NaCl at 50 °C) or fractionation on hydroxyapatite (HAP) (samples adsorbed in 0.14 M sodium phosphate at 50 °C).

part of the genetic complement of the normal cell¹¹. We demonstrate here that the DNA of normal chicken cells contains nucleotide sequences closely related to at least a portion of the transforming gene(s) of ASV; in addition, we



Il gene *src* è presente anche in cellule non infettate

A



B

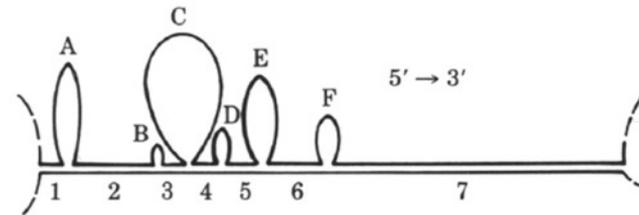
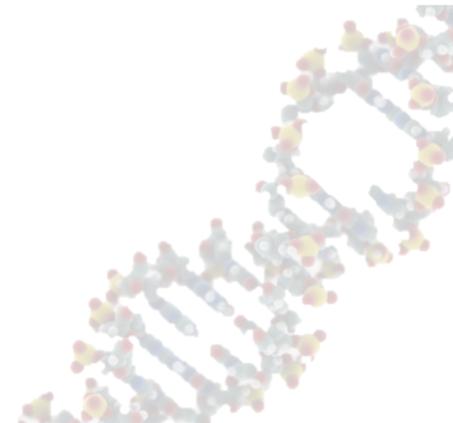
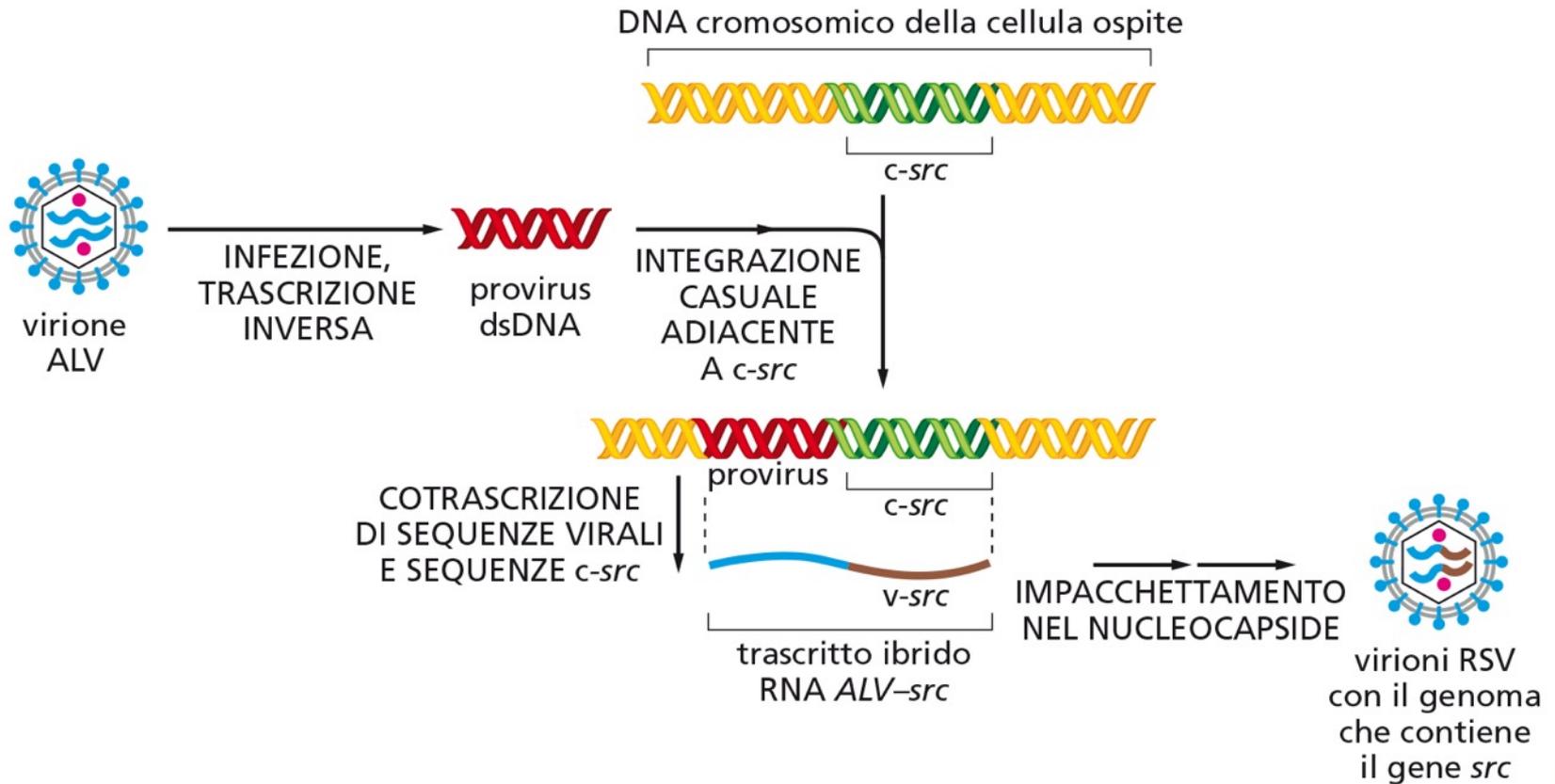


Figure 8. (A) Electron micrograph and (B) schematic diagram of a heteroduplex between molecular clones of *c-src* and *v-src*. The loops represent introns present in *c-src* but not *v-src*. (Reprinted from Parker RC, et al. 1981. *Proc Natl Acad Sci* **78**: 5842–5846, with permission.)



V-src e c-src



ONCOGENE: gene strettamente correlato a gene normale (proto-oncogene) ma con funzioni diverse, capace di trasformazione neoplastica.

PROTO-ONCOGENE: gene cellulare che può trasformarsi in potente oncogene

Proto-oncogeni e oncogeni virali

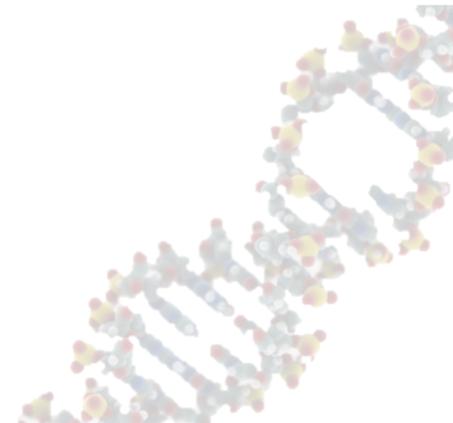
- Virus della mielocitomatosi MC29: oncogene *v-myc* deriva da proto-oncogene *c-myc*.
- Virus ratto-topo che causa sarcoma di Harvey e di Kirsten: oncogeni *H-ras* e *K-ras*

Retrovirus rapidamente trasformanti e oncogeni acquisiti ^a				
Nome del virus	Oncogene virale	Specie	Malattia principale	Natura dell'oncoproteina
Sarcoma di Rous	<i>src</i>	pollo	sarcoma	TK non recettoriale
Sarcoma Y73/Esh	<i>yes</i>	pollo	sarcoma	TK non recettoriale
Sarcoma di Fujinami	<i>fps^b</i>	pollo	sarcoma	TK non recettoriale
UR2	<i>ros</i>	pollo	sarcoma	RTK; ligando sconosciuto
Mielocitomatosi 29	<i>myc</i>	pollo	leucemia mieloide ^c	fattore trascrizionale
Virus 2 di Mill Hill	<i>mil^d</i>	pollo	leucemia mieloide	<i>ser/tr</i> chinasi
Mieloblastosi aviaria E26	<i>myb</i>	pollo	leucemia mieloide	fattore trascrizionale
Mieloblastosi aviaria E26	<i>ets</i>	pollo	leucemia mieloide	fattore trascrizionale
Eritroblastosi aviaria ES4	<i>erbA</i>	pollo	eritroleucemia	recettore ormone tiroideo
Eritroblastosi aviaria ES4	<i>erbB</i>	pollo	eritroleucemia	EGF RTK
Sarcoma murino 3611	<i>raf^e</i>	topo	sarcoma	<i>ser/tr</i> chinasi
SKV 770	<i>shi</i>	pollo	endotelioma (?)	fattore trascrizionale
Reticoloendoteliosi	<i>rel</i>	tacchino	linfoma delle cellule B immature	fattore trascrizionale
Leucemia murina di Abelson	<i>abl</i>	topo	linfoma delle cellule pre-B	TK non recettoriale
Leucemia murina di Moloney	<i>mos</i>	topo	sarcoma, eritroleucemia	<i>ser/tr</i> chinasi
Sarcoma murino di Harvey	<i>H-ras</i>	ratto, topo	sarcoma	proteina G
Sarcoma murino di Kirsten	<i>K-ras</i>	topo	sarcoma	proteina G
Sarcoma murino FBJ	<i>fos</i>	topo	osteosarcoma	fattore trascrizionale
Sarcoma felino di Snyder-Theilen	<i>fes^f</i>	gatto	sarcoma	TK non recettoriale
Sarcoma felino di McDonough	<i>fms</i>	gatto	sarcoma	CSF-1 RTK
Sarcoma felino di Gardner-Rasheed	<i>fgr</i>	gatto	sarcoma	TK non recettoriale
Sarcoma felino di Hardy-Zuckerman	<i>kit</i>	gatto	sarcoma	fattore di crescita RTK
Sarcoma della scimmia	<i>sis</i>	scimmie lagotrici	sarcoma	PDGF
AKT8	<i>akt</i>	topo	linfoma	<i>ser/tr</i> chinasi
Virus aviario S13	<i>sea</i>	pollo	leucemia eritroblastica ^g	RTK; ligando sconosciuto
Leucemia mieloproliferativa	<i>mpl</i>	topo	mieloproliferazione	recettore TPO
Pollame regionale Lab v. 30	<i>eyk</i>	pollo	sarcoma	RTK; ligando sconosciuto
Virus del sarcoma aviario CT10	<i>crk</i>	pollo	sarcoma	adattatori SH2/SH3
Virus del sarcoma aviario 17	<i>jun</i>	pollo	sarcoma	fattore trascrizionale
Virus del sarcoma aviario 31	<i>qin</i>	pollo	sarcoma	fattore trascrizionale ^h
Virus del sarcoma AS42	<i>mof</i>	pollo	sarcoma	fattore trascrizionale
Virus Cas NS-1	<i>cbl</i>	topo	linfoma	fattore di ubiquitinazione SH2-dipendente

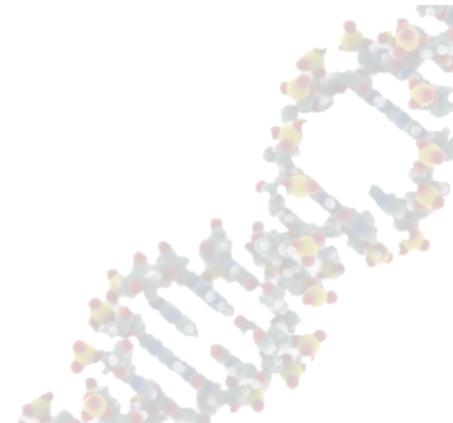
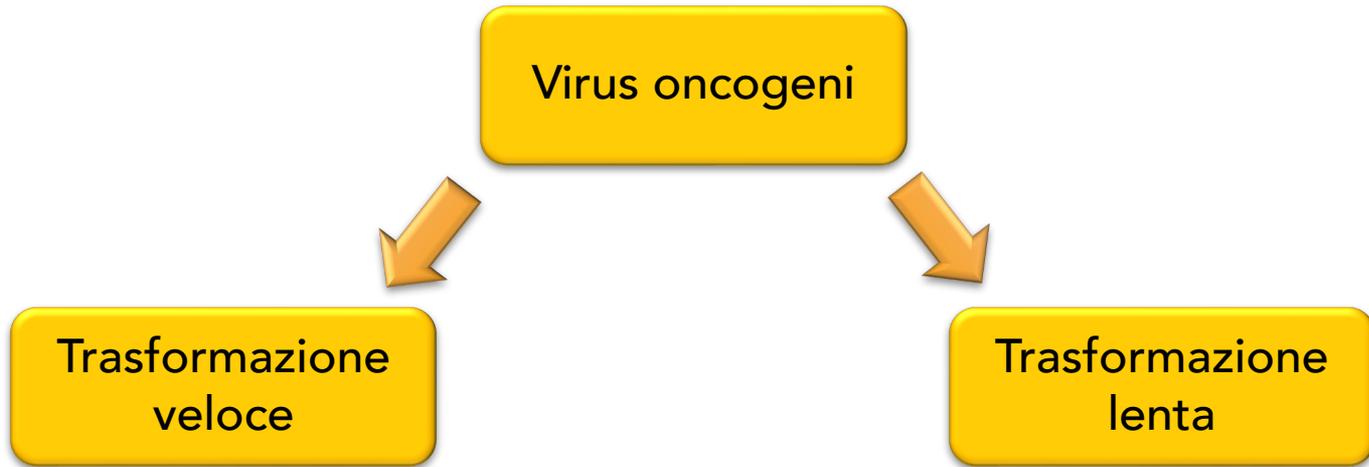
Nomenclatura

- Oncogeni non umani: *src* o *myc*
- Proto-oncogeni non umani: *c-src* o *c-myc*
- Geni correlati: *N-myc*, *L-myc*
- Prodotto genico di oncogeni non umani: *Src* o *Myc*

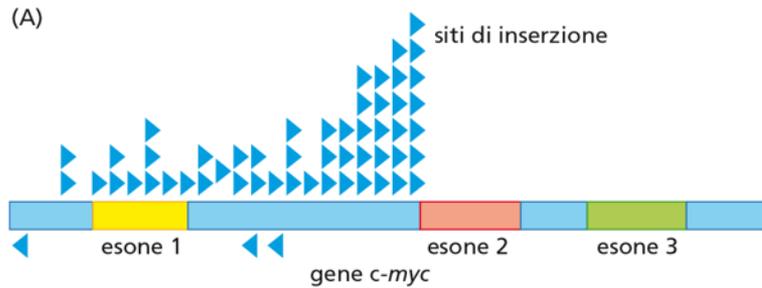
- Geni umani: *MYC*
- Prodotti genici umani: *MYC*



Virus oncogeni

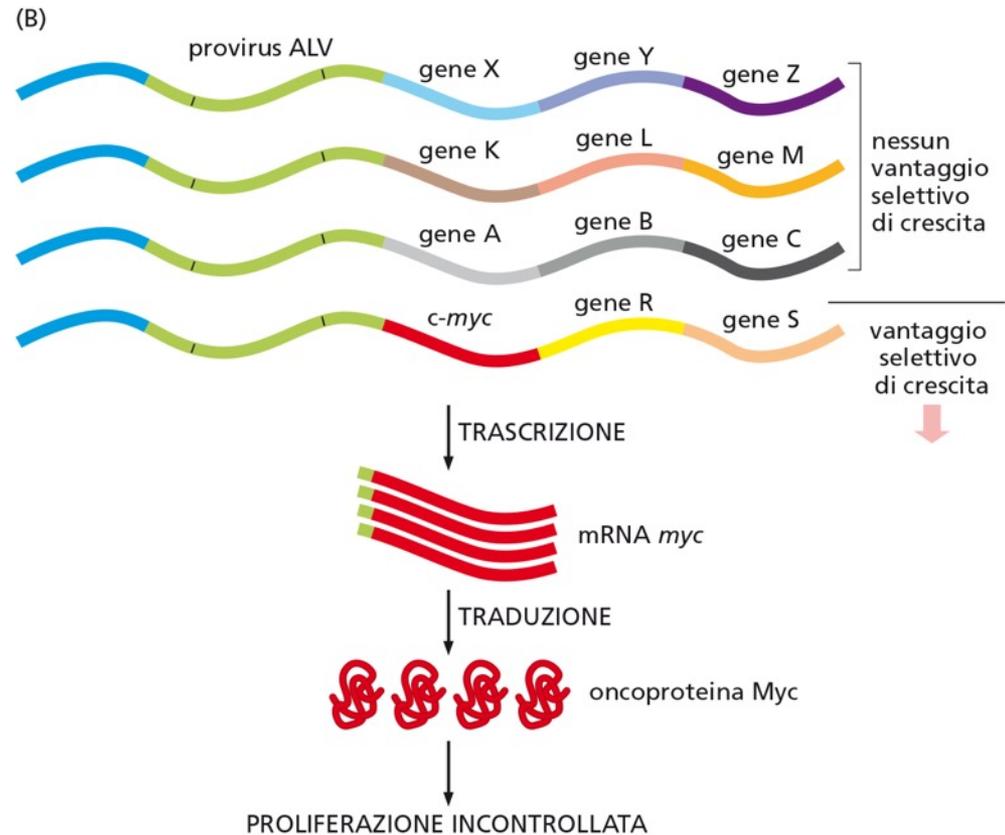


Mutagenesi inserzionale



In cellule leucemiche provirus ALV integrato in sito adiacente a *c-myc*

Trascrizione *c-myc* influenzata da promotore virale



Virus oncogeni

