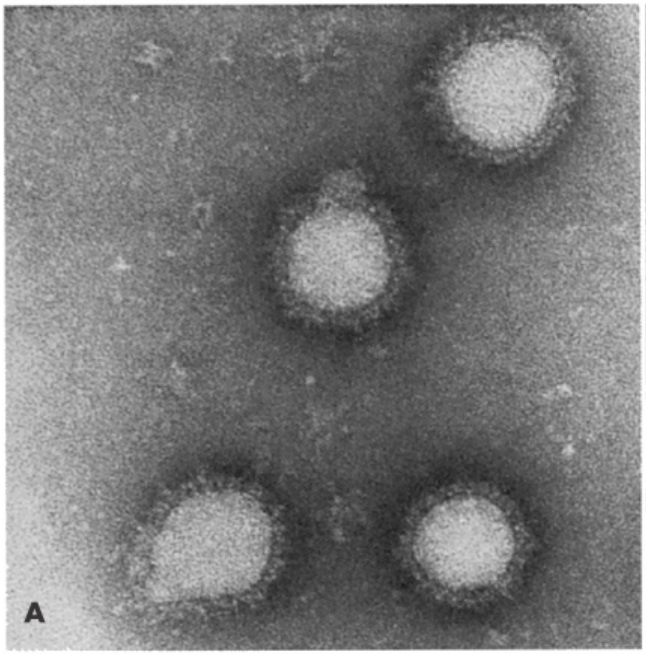
VIROLOGY

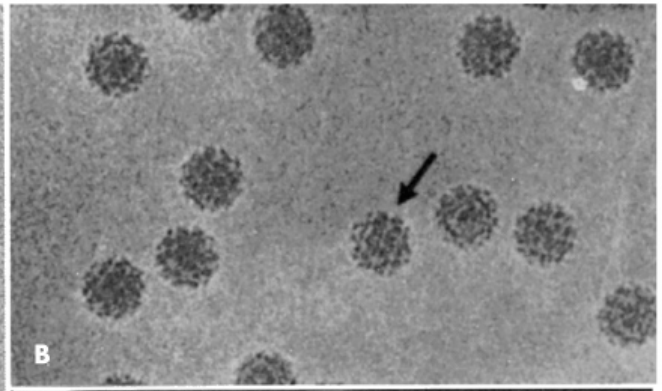
Engineering Viral Genomes: Alphavirus Vectors

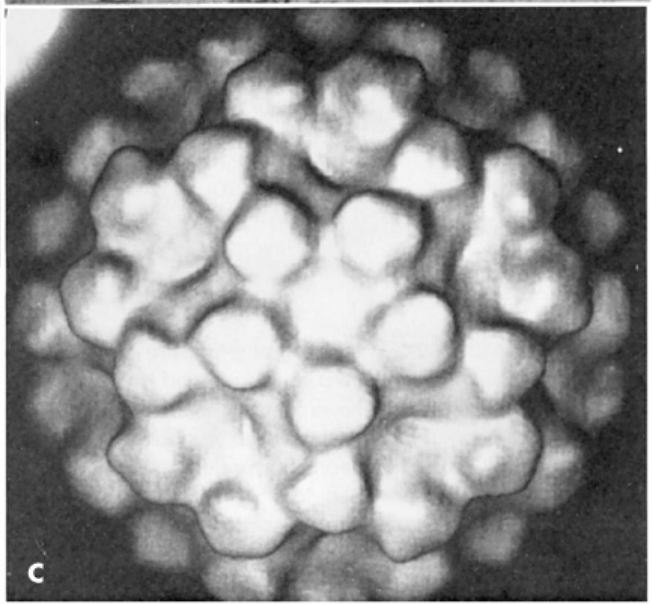
Viral vectors

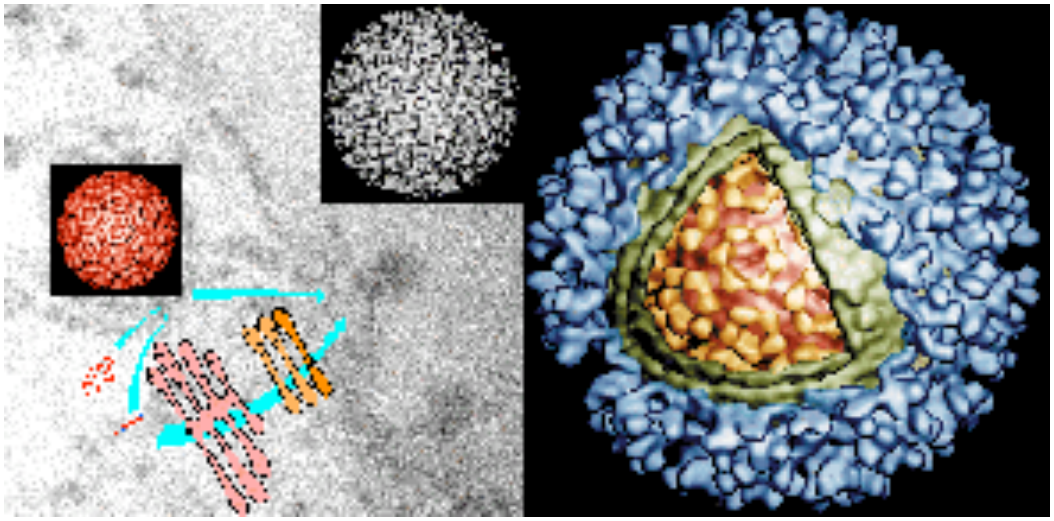
Virus	Insert size	Integration	Duration of expression	Advantages	Potential disadvantages
Adeno-associated virus	~4.5–9 (?) kb	Low efficiency	Long	Nonpathogenic, episomal, infects nondividing cells	Immunogenic, toxicity, small packaging limit
Adenovirus	2–38 kb	No	Short	Efficient gene delivery, infects nondividing cells	Transient, immunogenic
Alphavirus	~5 kb	No	Short	Broad host range, high level expression	Virulence
Epstein-Barr virus	~120 kb	No; episomal	Long	High capacity, episomal, long-term expression	
Gammaretrovirus	1–7.5 kb	Yes	Shorter than formerly	Stable integration	May rearrange genome, insertional mutagenesis require cell division
Herpes simplex virus	~30 kb	No	Long in central nervous system, short elsewhere	Infects nondividing cells; neurotropic, large capacity	Virulence, persistence in neurons, immunogenic
Lentivirus	7–18 kb	Yes	Long	Stable integration; infects nondividing and terminally differentiated mammalian cells	Insertional mutagenesis
Poliovirus	~300 bp for helper-free virus; ~3 kb for defective virus	No	Short	Excellent mucosal immunity	Limited capacity; reversio to neurovirulence
Rhabdovirus	Unknown	No	Short	High-level expression, rapid cell killing	Virulence, highly cytopathic
Vaccinia virus	At least ~25 kb, probably ~75–100 kb	No	Short	Wide host range, ease of isolation, large capacity, high-level expression	Transient, immunogenic

Structure of Alphaviruses



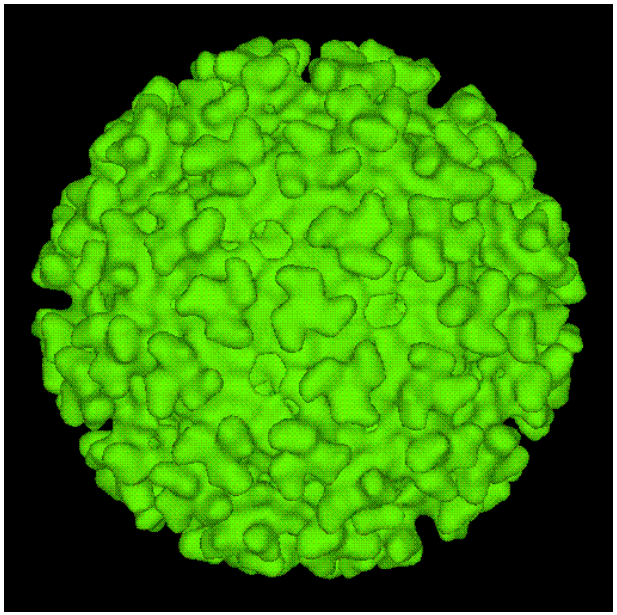


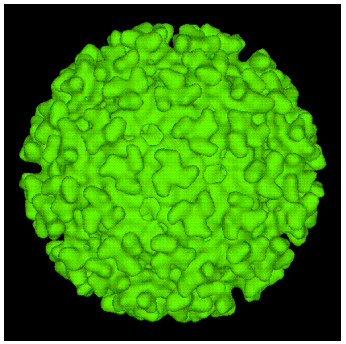




Medically important Alphaviruses

Virus	Vettore	Ospite	Distribuzione	Malattia
Alphavirus				
Sindbis*	*Aedes* ed altre zanzare	Uccelli	Africa, Australia, India	Clinicamente non evidente
Semliki Forest*	*Aedes* ed altre zanzare	Uccelli	Africa orientale ed occidentale	Clinicamente non evidente
Encefalite equina venezuelana	*Aedes*, *Culex*	Roditori, cavalli	Nord e Sud America, America centrale	Sistemica lieve; encefalite grave
Encefalite equina dell'est	*Aedes*, *Culiseta*	Uccelli	Nord e Sud America, Caraibi	Sistemica lieve; encefalite
Encefalite equina dell'ovest	*Culex*, *Culiseta*	Uccelli	Nord e Sud America	Sistemica lieve; encefalite
Chikungunya	*Aedes*	Uomo, scimmia	Africa, Asia	Febbre, artralgia, artrite

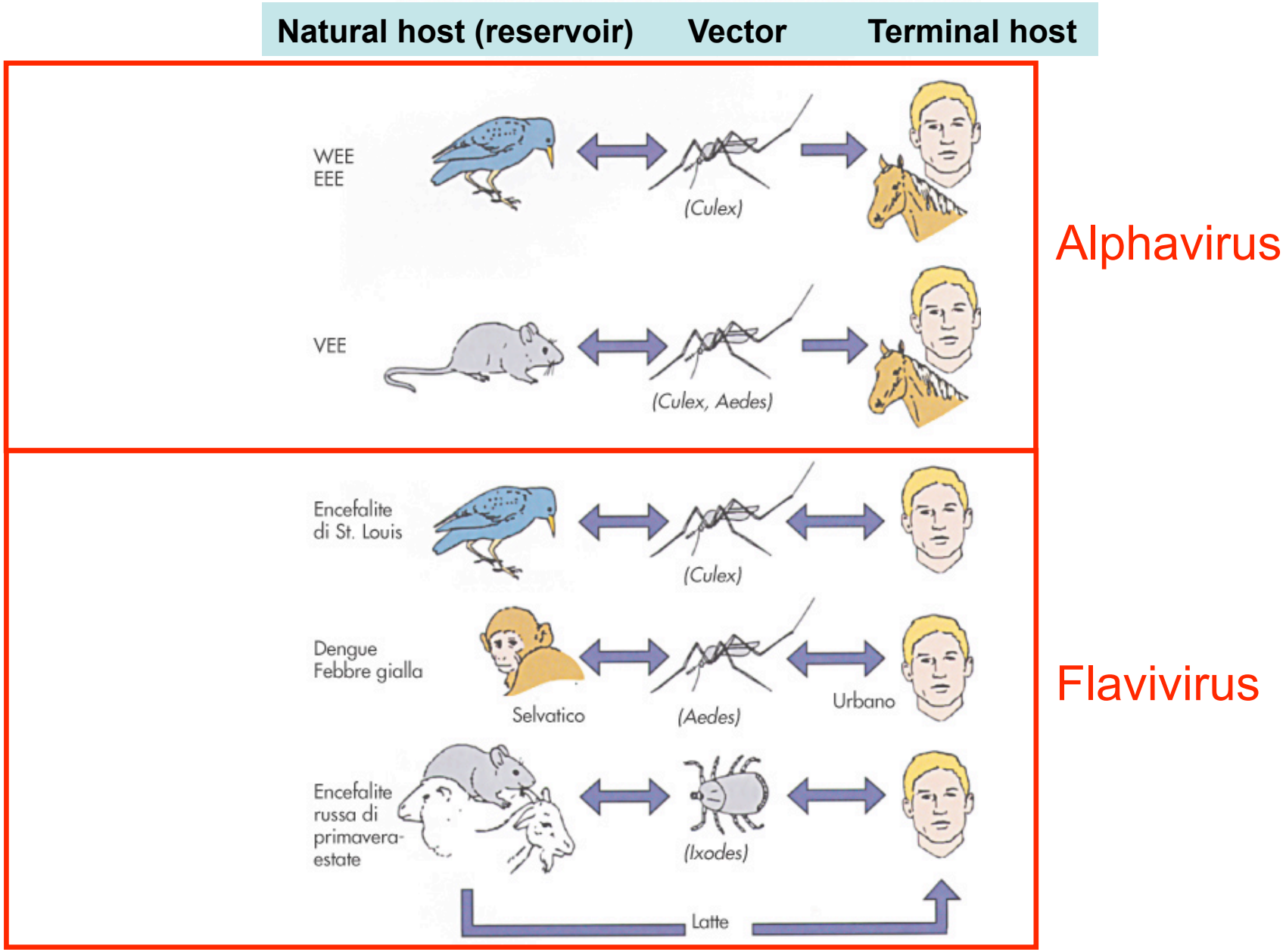




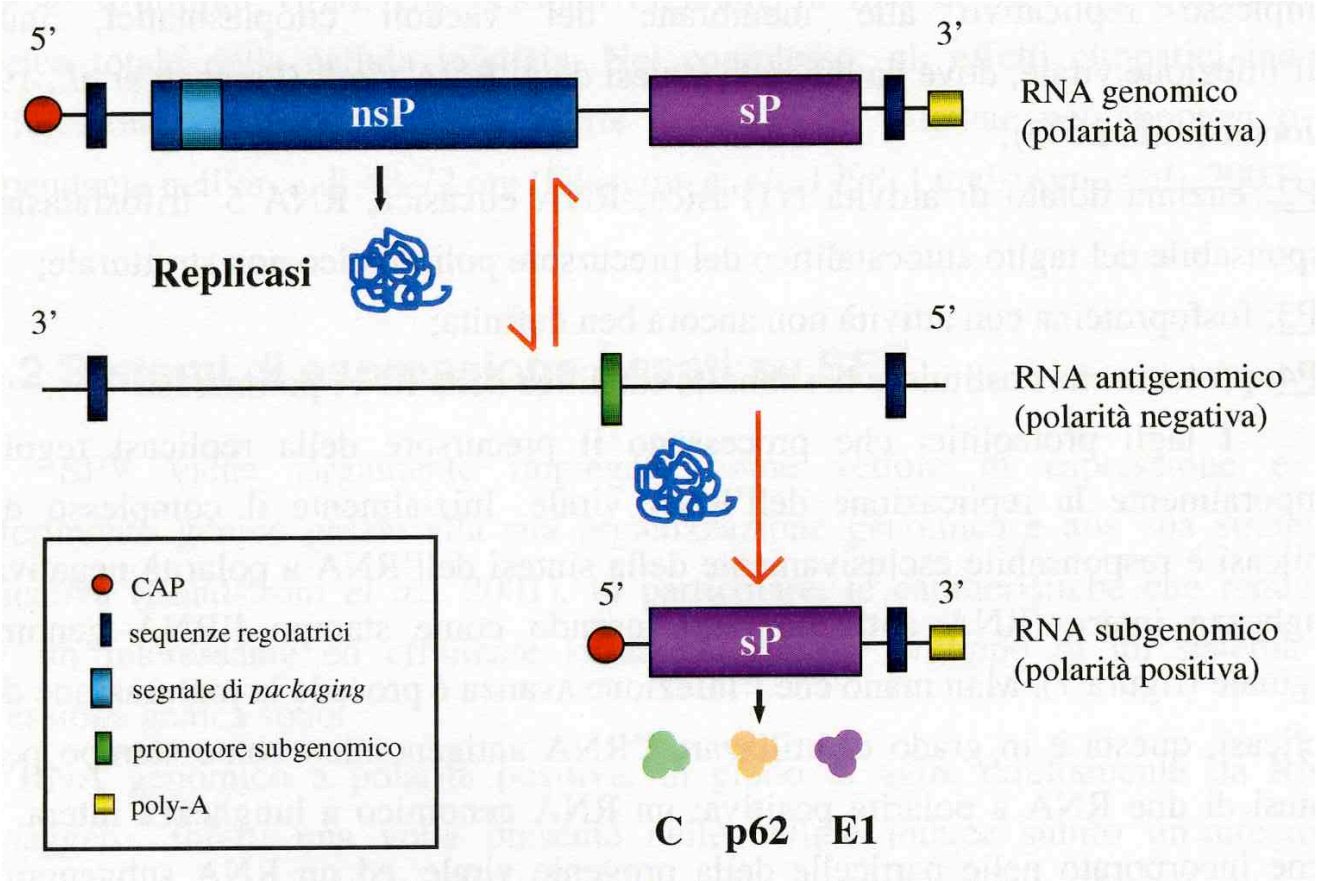
Medically important Alphaviruses

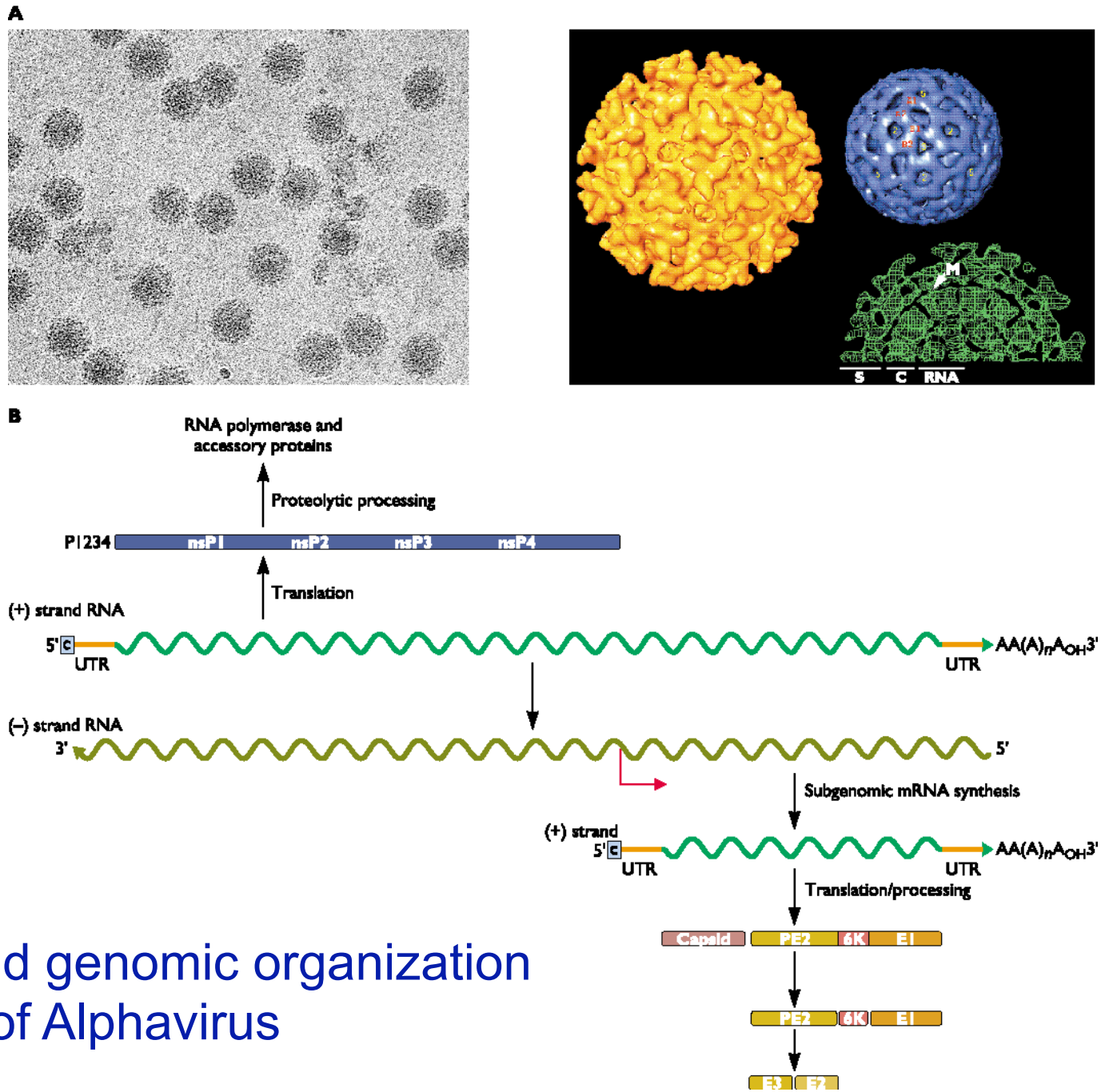
Virus	**Human Disease**	**Vertebrate Reservoir**	**Distribution**
Sindbis virus	Rash, arthritis	Birds	Europe, Africa, Australia
Semliki forest virus	Rash	Birds	Africa
O'nyong'nyong virus	Rash	Primates	Africa
Chikungunya virus	Rash	Primates, humans	Africa, India, SE Asia
Mayaro virus	Rash	Primates, humans	South America
Ross River virus	Rash	Mammals, humans	Australia, South Pacific
Barmah Forest virus	Fever, malaise, rash, joint pain, muscle tenderness	Humans	Australia
Eastern equine encephalitis virus	Encephalitis	Birds	Americas
Western equine encephalitis virus	Encephalitis	Birds, mammals	North America

Alphaviruses and Flaviruses as **Ar**thopod-**bo**rne (Arbo)virus



Alphavirus: **Semliki Forest Virus** (SFV) genome transcription and replication





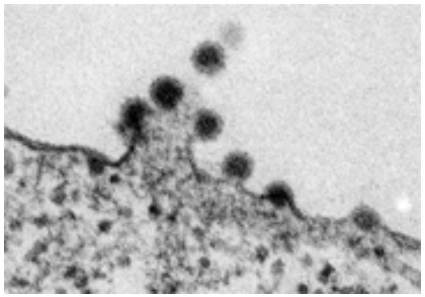
Structure and genomic organization of Alphavirus

Alphavirus replication

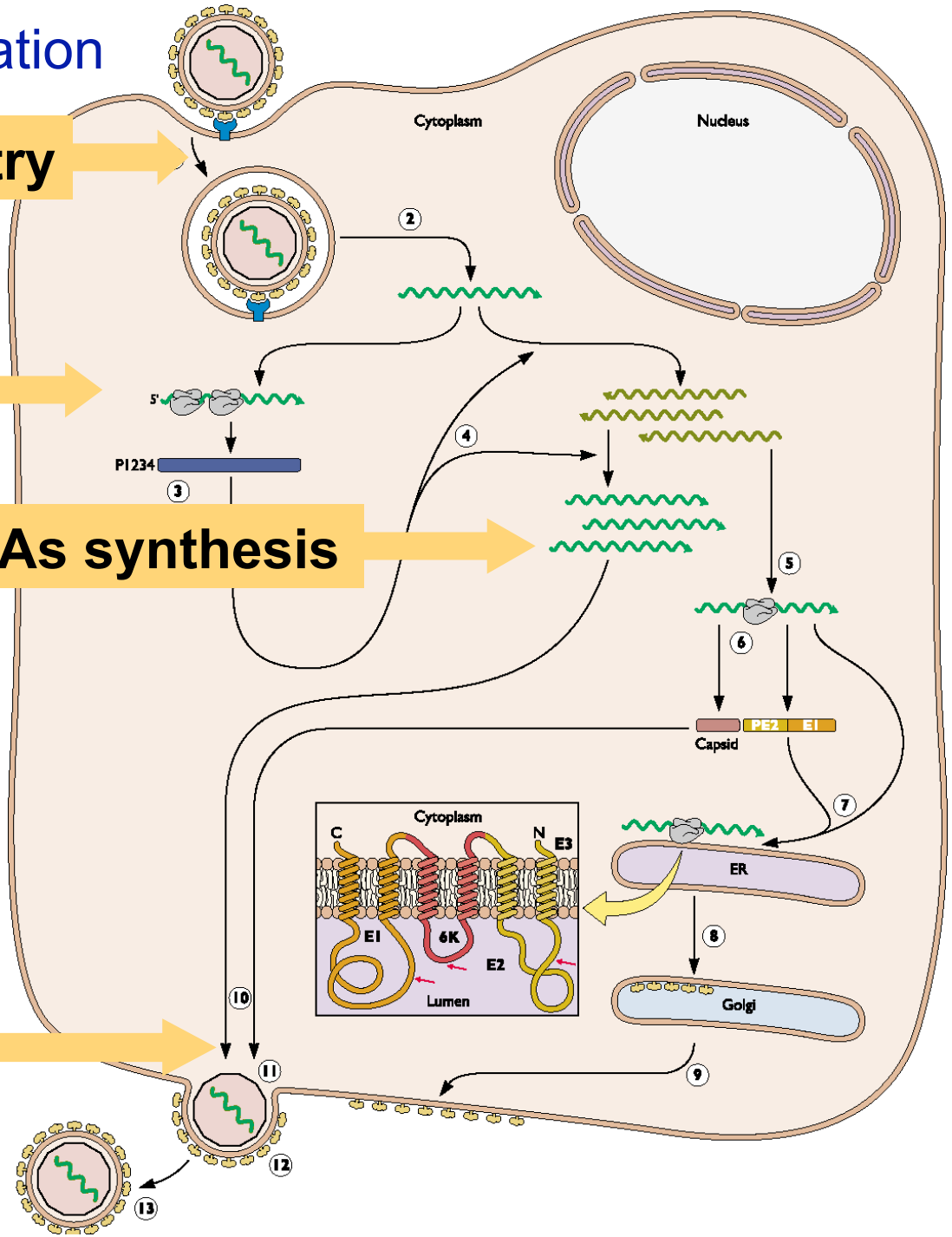
Adsorption & entry

nsP translation

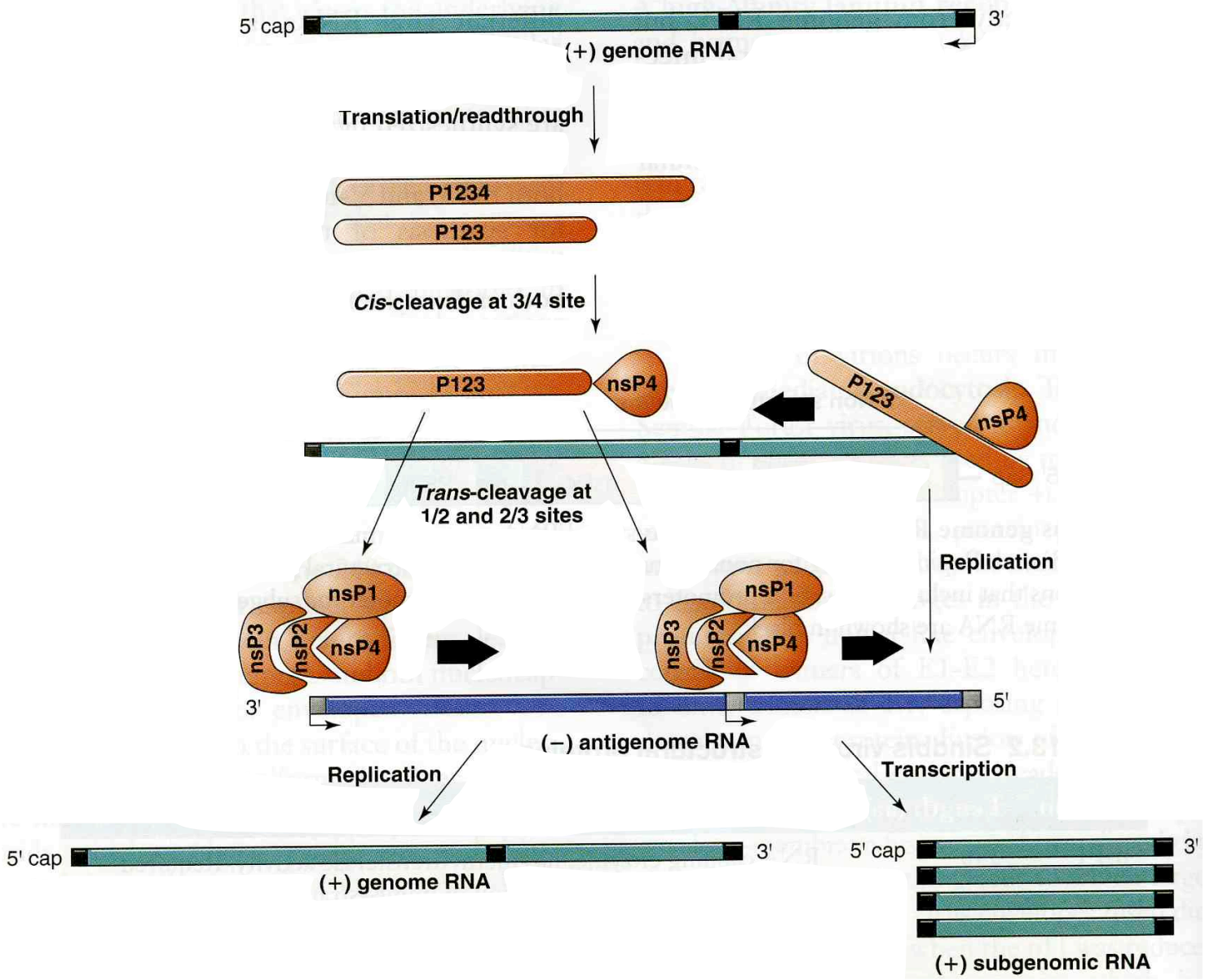
(+) RNAs synthesis



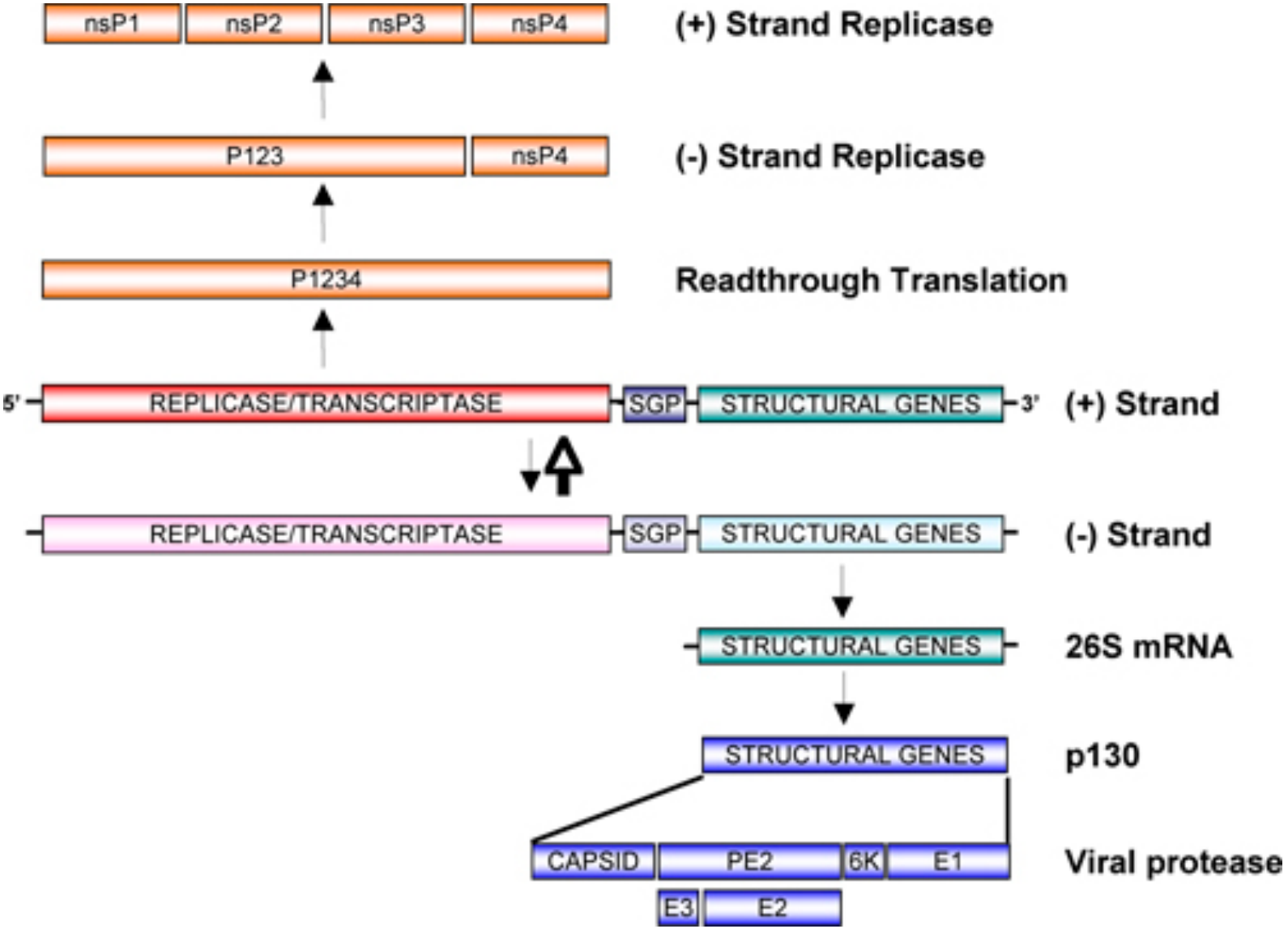
Assembly



Alphavirus: model for the temporal regulation of minus- and plus-strand RNA synthesis

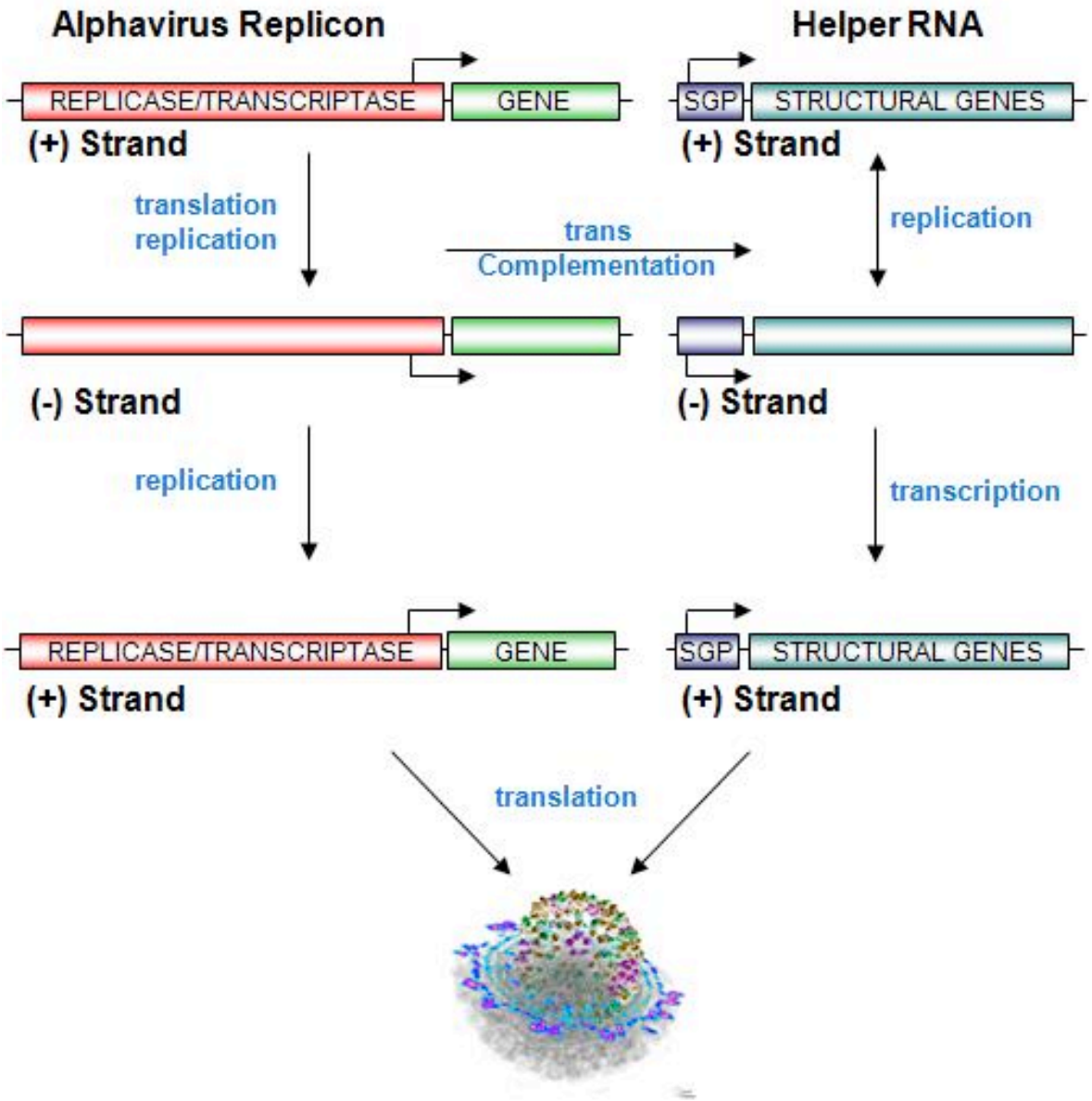


Alphavirus genome transcription and replication



Favorable Features of Alphavirus Vectors

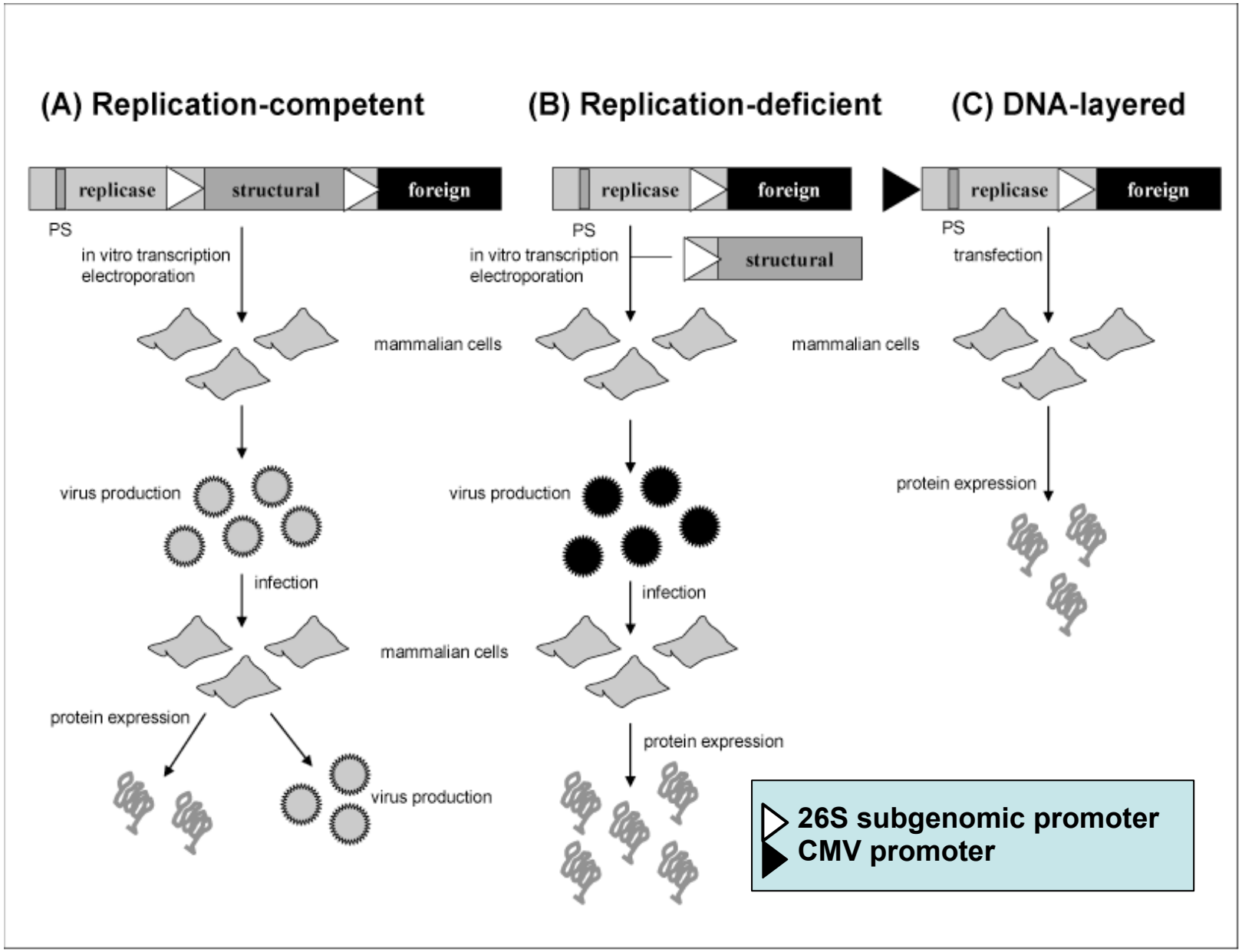
- Ability to infect a broad range of cell types
- High rate of RNA synthesis
- High rate of subgenomic RNA translation
- High transient expression levels (up to 50% of total cell protein)
- Cytoplasmic replication
- Rapid production of high titer (10^9-10^{10}pfu/ml)
- Induction of apoptosis (cancer therapy)



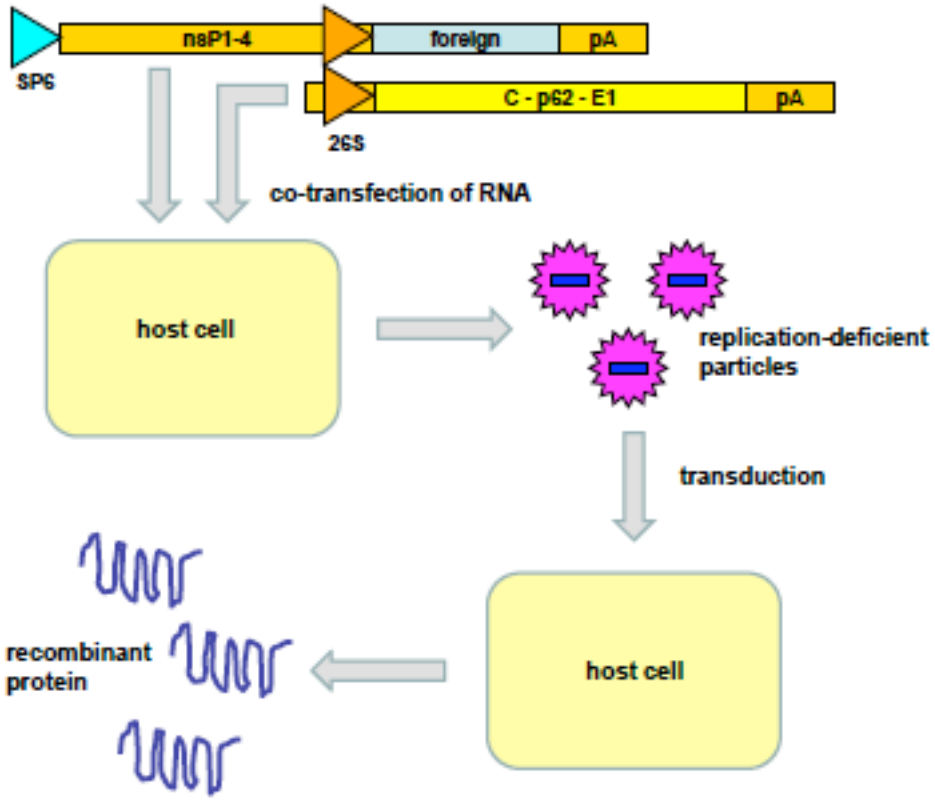
RECOMBINANT ALPHAVIRAL VECTORS

In order to create a viral vector from alphaviruses, the structural genes are replaced with the therapeutic transgene. As a consequence, during vector production the structural genes have to be provided in trans by co-transfection with a helper plasmid.

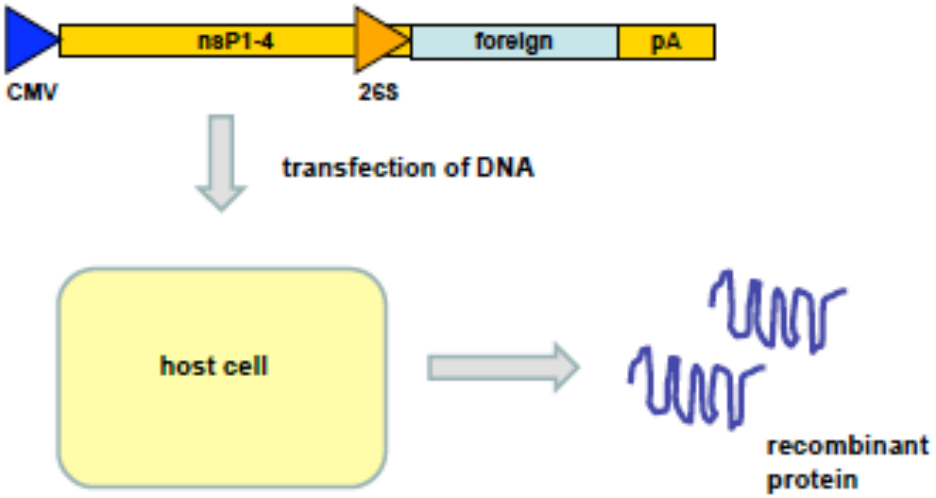
Schematic presentation of Alphavirus expression systems



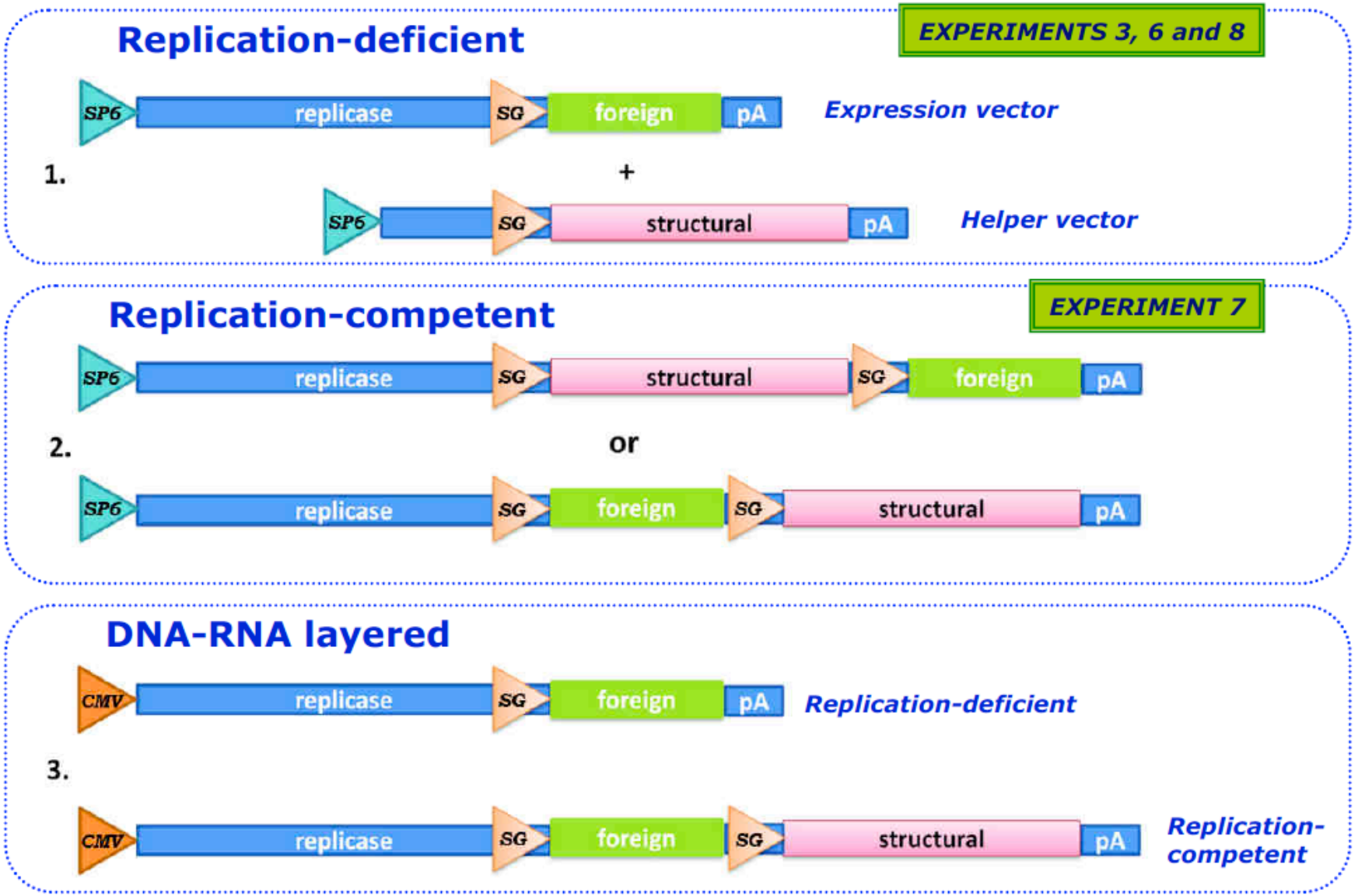
Alphavirus vector systems: Replication-deficient RNA vectors



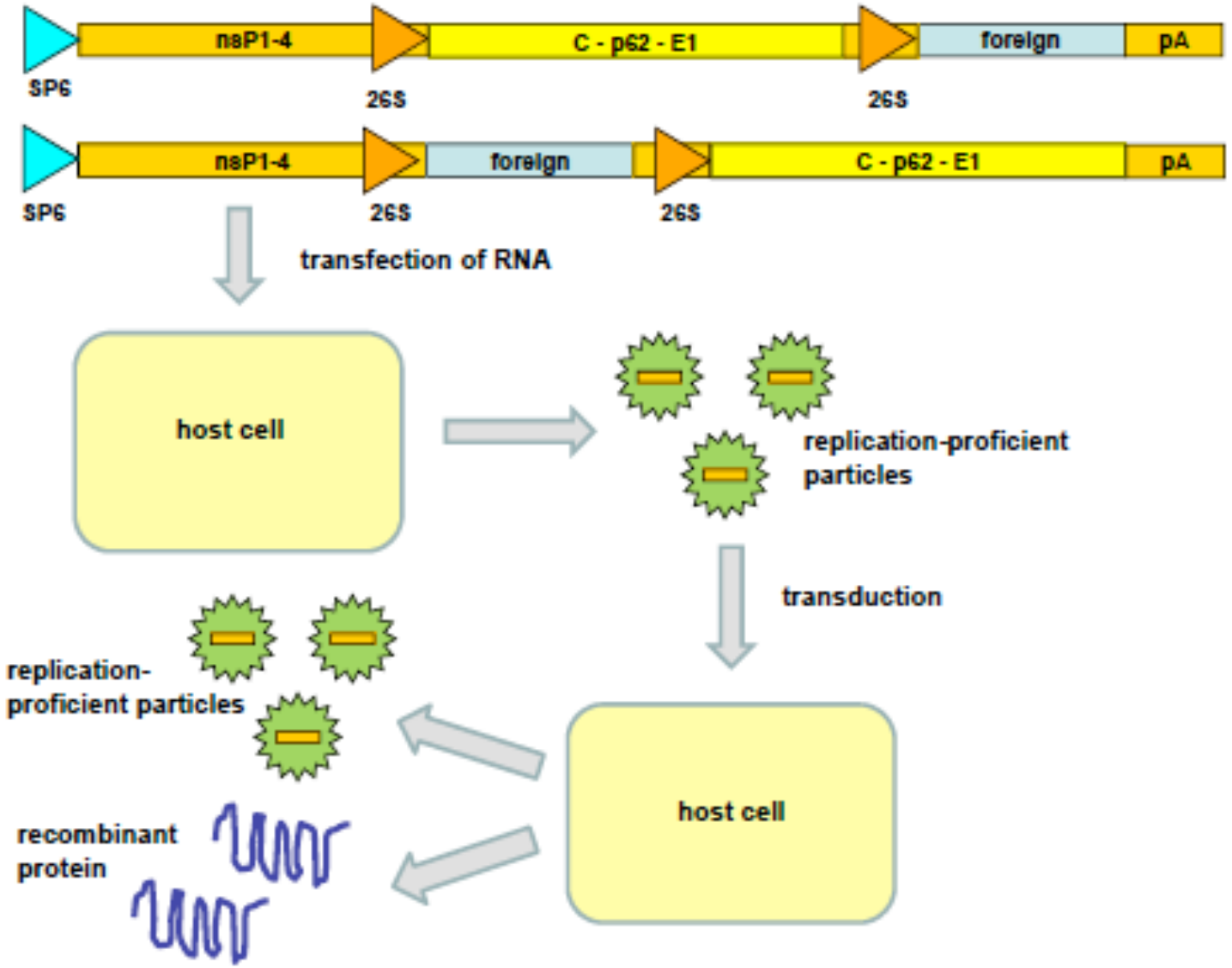
Alphavirus vector systems: Layered DNA/RNA vectors



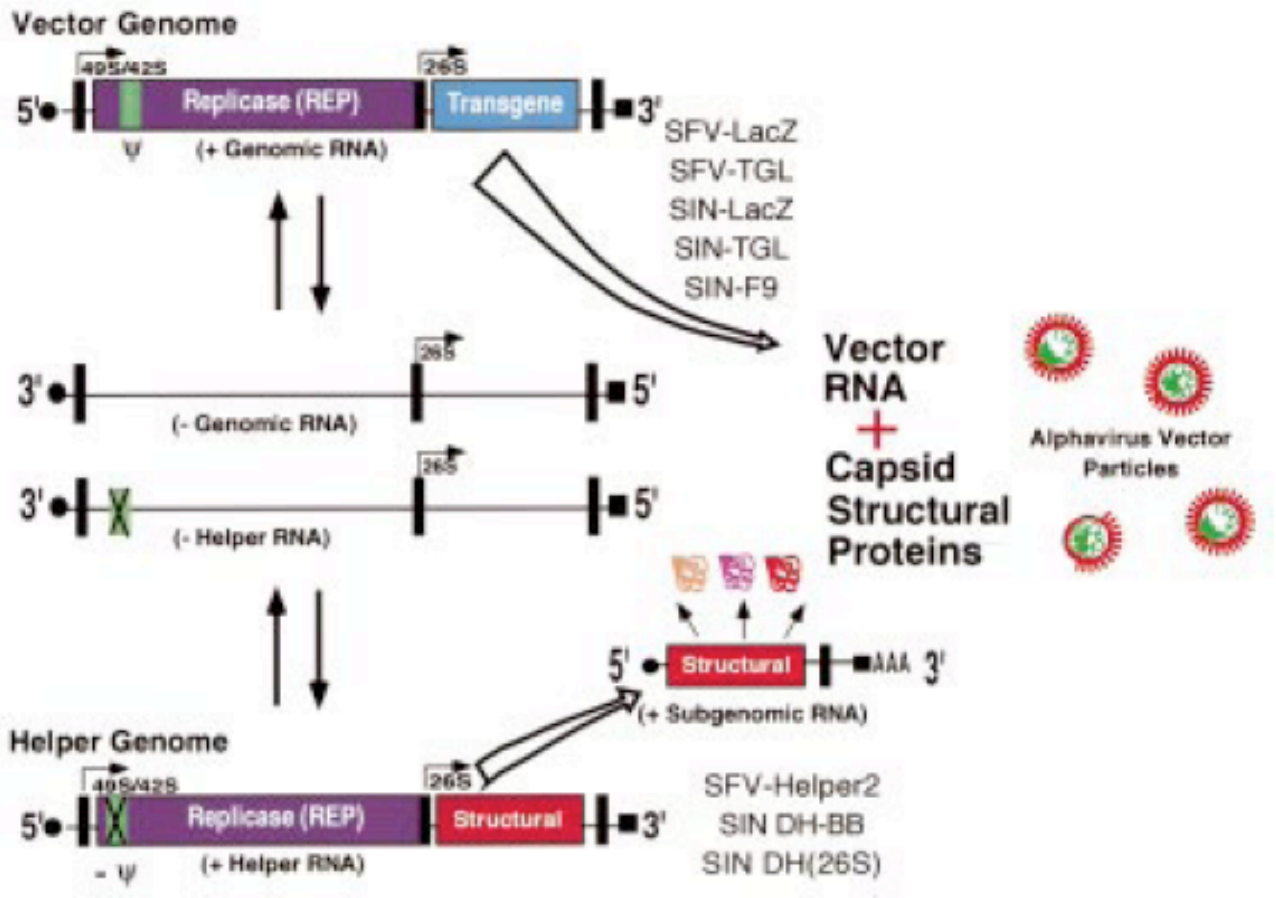
Schematic presentation of Alphavirus expression systems



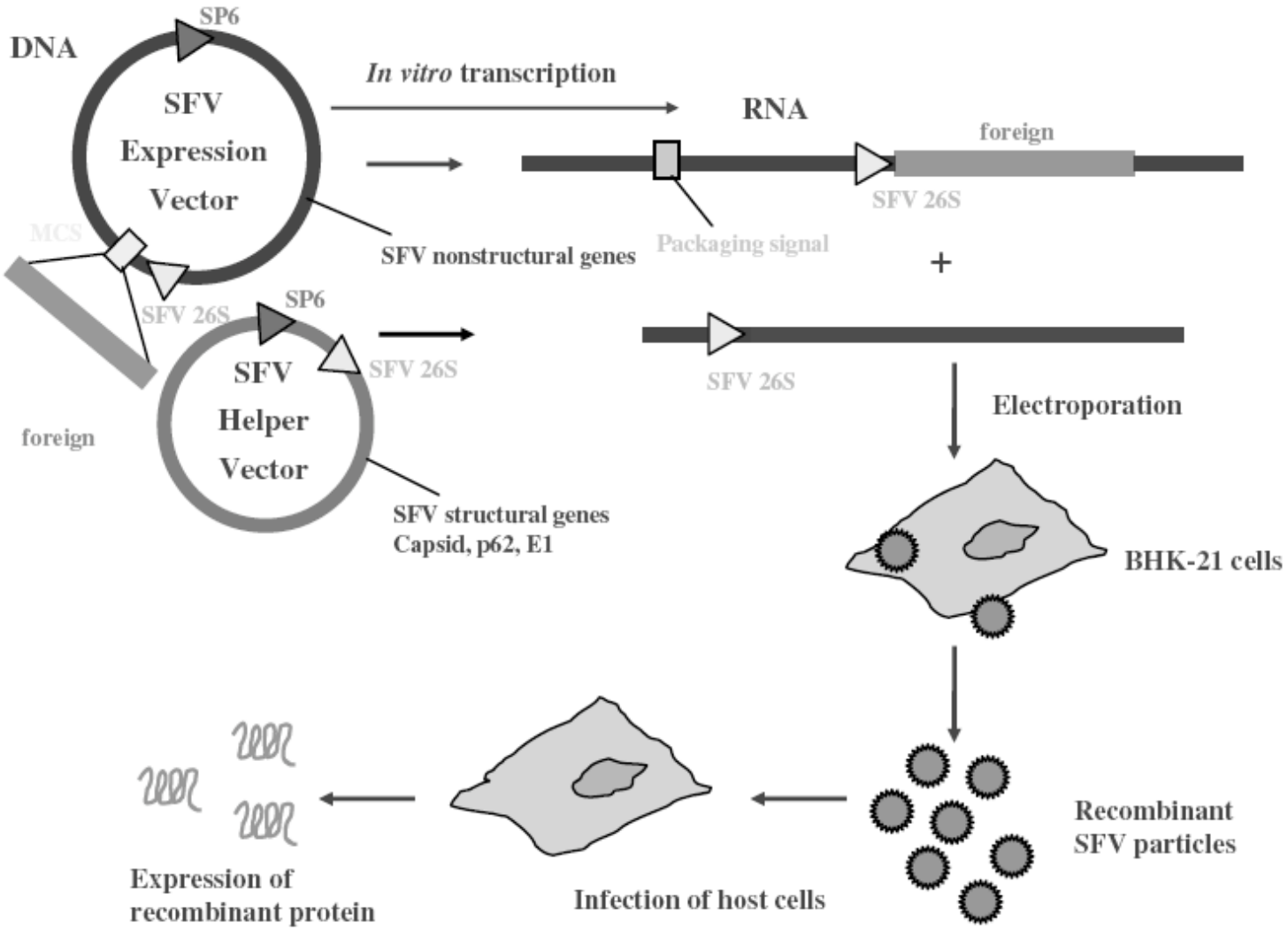
Alphavirus vector systems: Replication-proficient



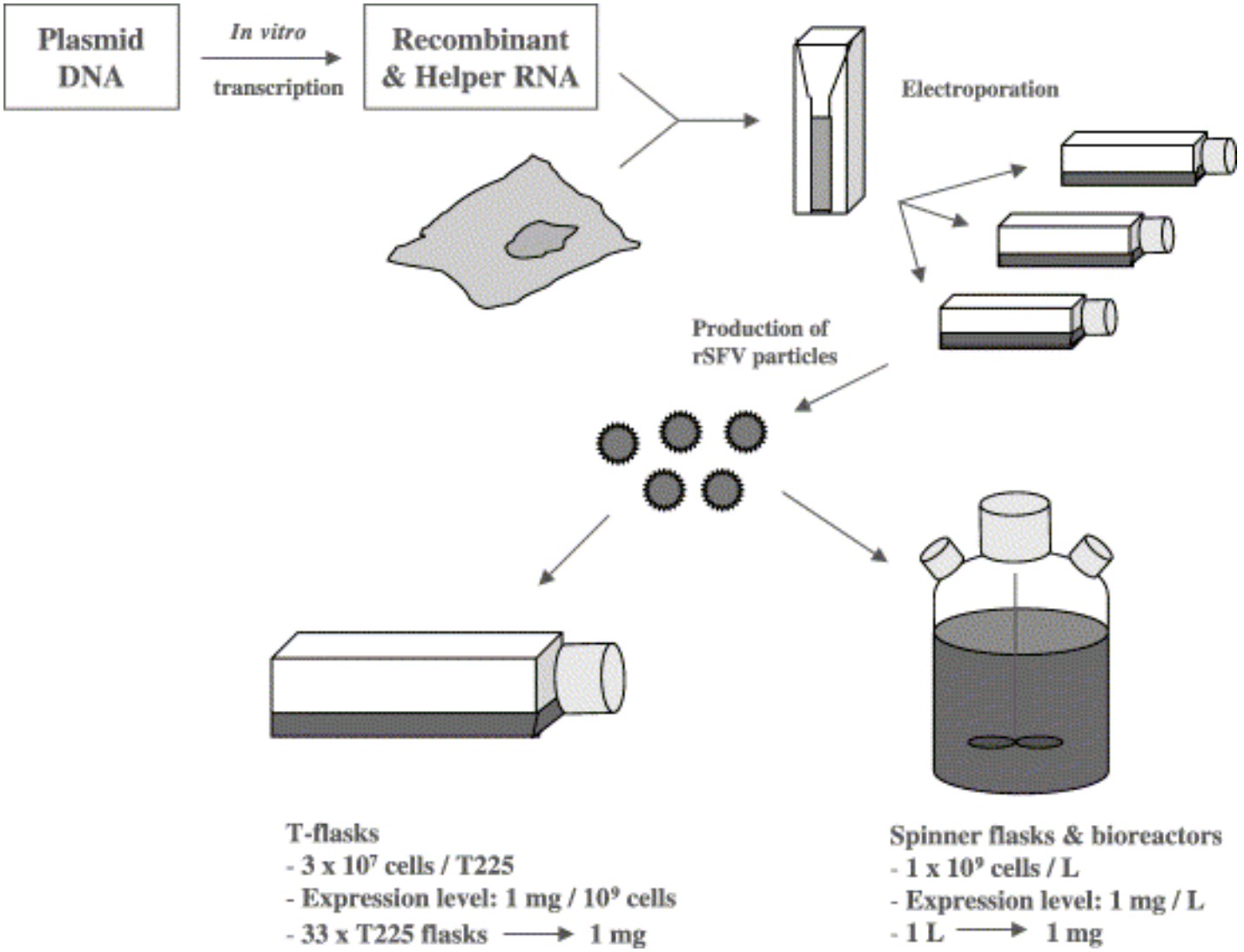
1st generation replication-defective alphavirus vector system



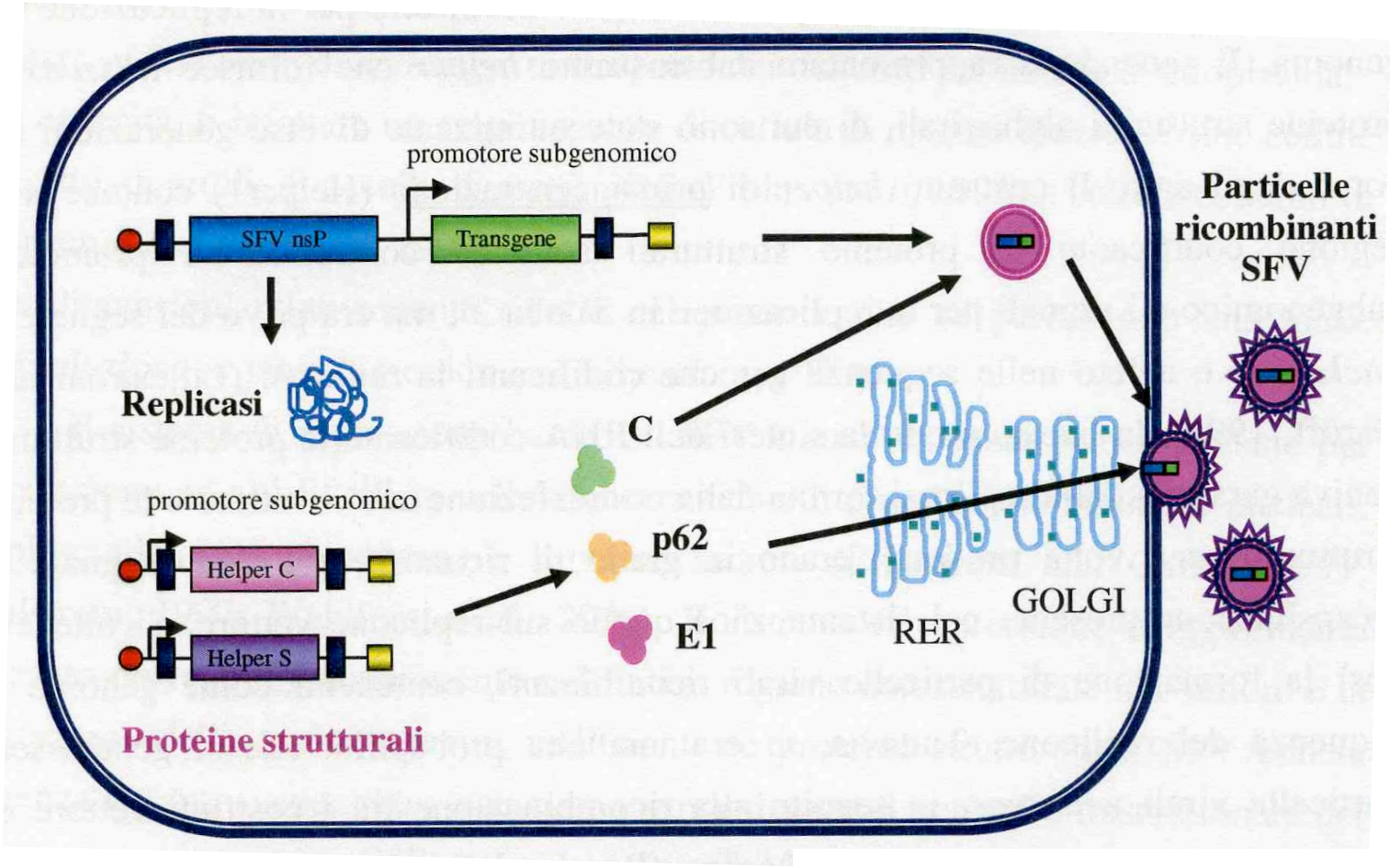
1st generation of replication-deficient SFV particles



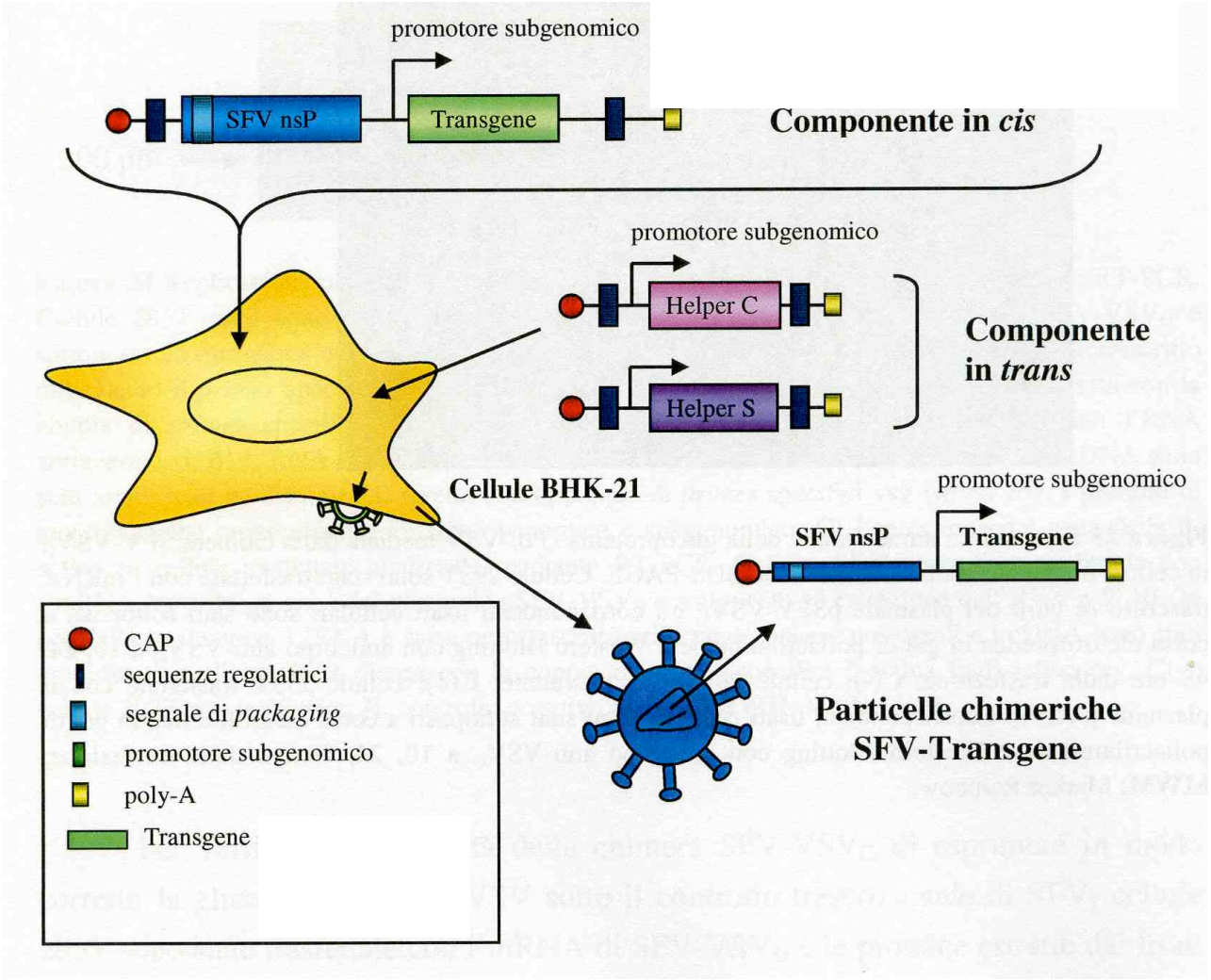
Large scale production of recombinant 1st generation SFV particles



3rd generation replication-defective alphavirus vector system

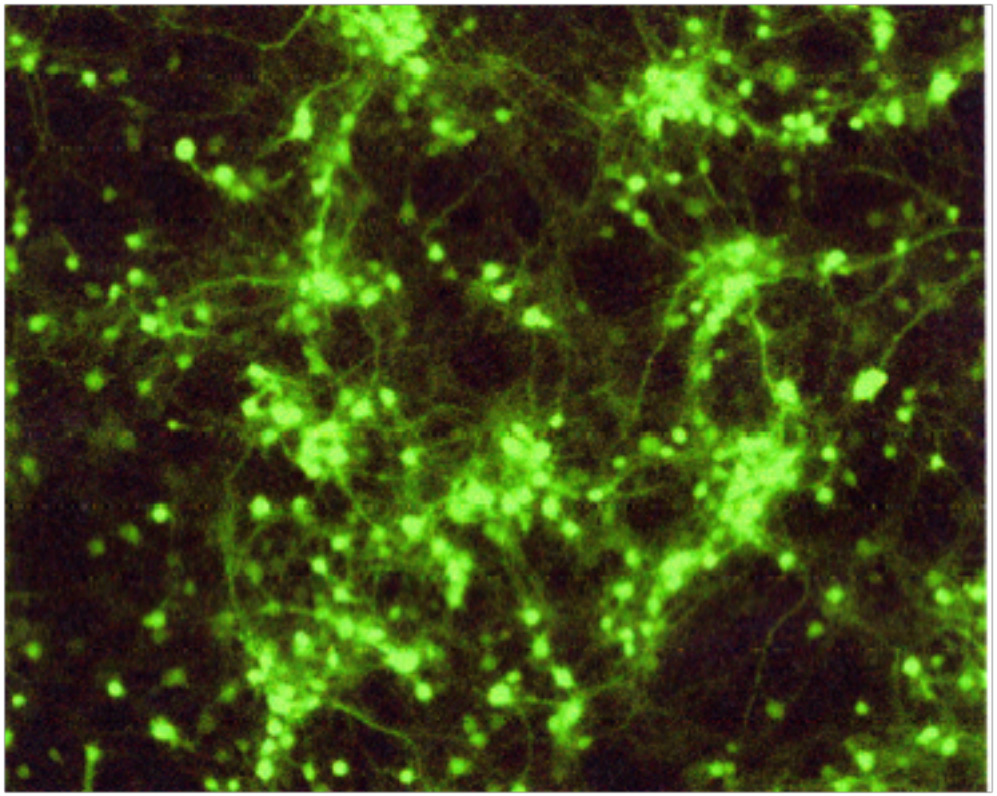


3rd generation of replication-deficient SFV particles



Aphavirus vectors applications

Virus	*Application*	*Objectives*
SFV	*Gene expression*	
	Cell lines	Protein characterization
	Large-scale production	Drug screening, structural biology
	Primary neurons	Localization, electrophysiology
	Hippocampal slices	Localization, electrophysiology
	In vivo, rat brain	Localization, duration
	Gene therapy	
	Cancer vaccines	Tumor protection, tumor regression
	Tumor cell lines	Transduction, cell killing
	Intratumoral injection	Tumor regression
	Liposome-encapsulation	Tumor-targeted gene delivery, therapy
SIN	*Gene expression*	
	Cell lines	Expression of toxic proteins
	Primary neurons	Expression, localization
	Hippocampal slices	Localization
	Mouse brain	Localization, duration
	Gene therapy	
	Cancer vaccines	Tumor protection, tumor regression
	Envelope modifications	Tumor targeting
	Systemic delivery	Tumor targeting
VEE	*Gene therapy*	
	Cancer vaccines	Tumor protection, tumor regression



Expression of GFP in primary rat hippocampal neurons. Primary hippocampal neurons were infected with SFV-GFP at a multiplicity of infection (MOI) of 10 and visualized by fluorescence microscopy at 2 days post-infection.

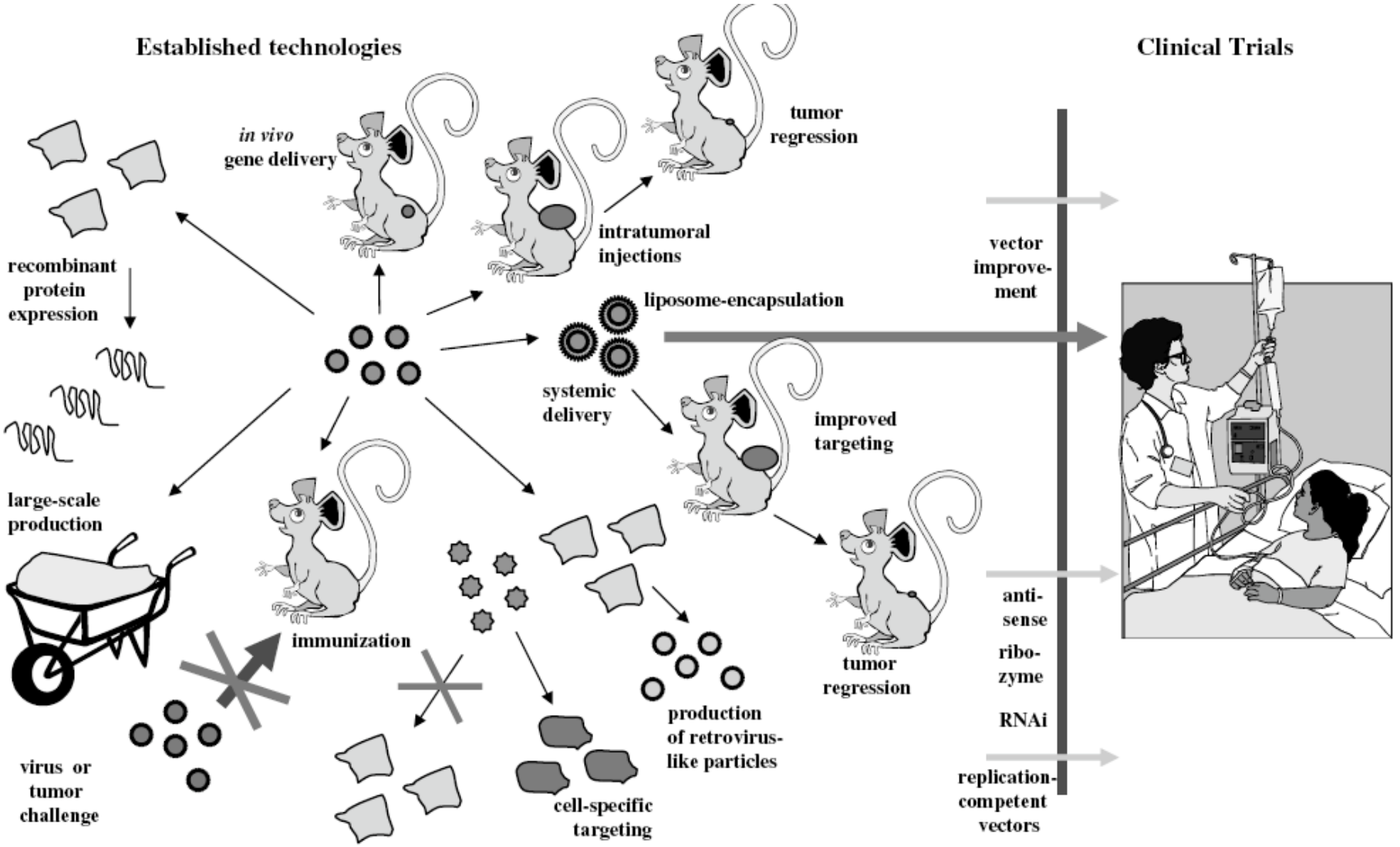
Alphavirus vectors Advantages

- Easy to manipulate on the cDNA level
- Fast and hightrangene expression level
- Broad host cell range
- Alphavirus have a natural potential to kill cancer cells – anticancer agents
- Alphavirus are potent activators of innate immunity – good for vaccine development

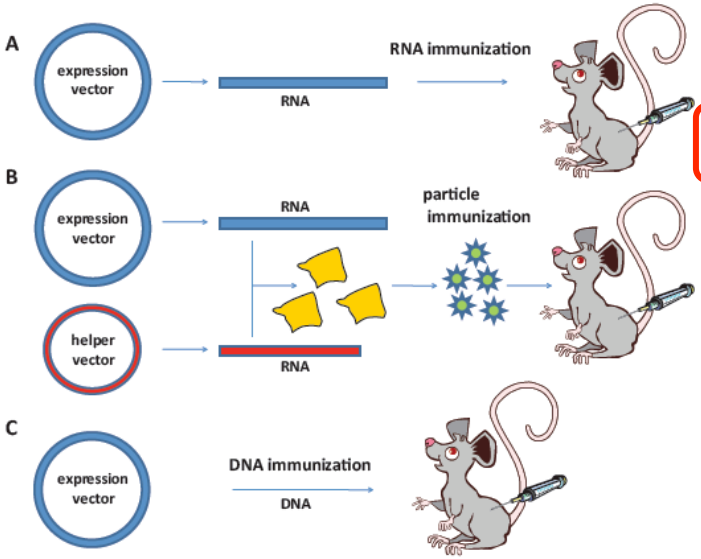
Alphavirus vectors Disadvantages

- Inability to regulate gene expression
- Possible biological hazard (SFV –BSL2; CHIKV – BSL3; VEEV - BSL4)
- High citotoxicity in infected cells
- Relatively short-term transgene expression, which lasts *in vivo* for 5-7 days
- Induction of apoptosis

Aphavirus vectors applications



Aphavirus vectors in vaccine development



Target	Gene	Vector/Delivery	Immunization	Response	Reference
Brain tumor	IL-12	SFV / Particles	Mouse	Mouse	[93]
Cervical cancer	HPVE6-E7	SFV / Particles	Mouse	Tumor protection	94]
Glioma	B16, 203	SFV / Particles	Mouse	Tumor protection	[95]
Melanoma	MDA/trp-2	VEE / Particles	Mouse	Therapeutic effect	[42]
Tumor	β-galactosidase	SFV / RNA	Mouse	Tumor protection	[38]
Tumor	HPVE7	VEE / Particles	Mouse	Tumor protection	[40]
Tumor	HPVE6E7+IL12	SFV / Particles	Mouse	Anti-tumor activity	[41]
Tumor	HPVE7-VP22	SIN / Particles	Mouse	CD8+ T-cell response	[96]
Tumor	IL-12	SFV / Particles	Mouse	Tumor protection	[97]
Tumor antigen	MHC class II	SFV / Particles-DNA	Mouse	Immunogenicity	[98]
Tumor antigen	P185	SFV / Particles	Mouse	CTL, tumor protection	[39]
Tumor antigen	trp-1	SIN / DNA	Mouse	Antitumor activity	[99]

CTL: Cytotoxic T-lymphocyte activity; HPV: Human papillomavirus; IL: interleukin; MDA: Melanoma differentiation antigen; MHC: Major histocompatibility complex; SFV: Semliki Forest virus; SIN: Sindbis virus; trp: tyrosine-related protein; VEE: Venezuelan equine encephalitis virus

Table 3: Vaccine development for cancer targets.

Virus	Target	Vector/Delivery	Immunization	Response	Reference
NS3 (p80)	SFV / DNA	Mouse	CTL	CMI	[63]
CSFV	E2	SFV / DNA	Swine	CSFV protection	[24]
EAV	G, L, M	VEE / Particles	Mouse	Neutralizing Abs	[64]
Ebola	NP	VEE / Particles	Mouse	Ebola protection	[18]
	NP, GP	VEE / Particles	Guinea pig	Ebola protection	[65]
	VP24, 30, 35, 30	VEE / Particles	Mouse	Ebola protection	[66]
Hepatitis B	cAg	SIN / DNA	Mouse	Specific Abs	[67]
	sAg	SIN / DNA	Mouse	Specific Abs	[67]
Hepatitis C	cAg	SFV / Particles, DNA	Mouse	CTL	[68]
	NS3	SFV / Particles	Mouse	Cellular	[69]
HeV	Glycoprotein	VEE / Particles	Mouse	Neutralizing Abs	[25]
HIV-1	env	SFV / Particles	Mouse	Humoral	[15]
	gp41	SFV / Particles	Mouse	Monoclonal Abs	[16]
	MA/CA	VEE / Particles	Mouse	Humoral, CTL	[17]
HPV	16E7	SFV / DNA	Mouse	CTL	[70]
	16E7-VP22	SIN / Particles	Mouse	CD8+ T cell response	[71]
	E7-hsp70	SIN / Particles	Mouse	CTL	[72]
HSV-1	gpB	SIN / Particles	Mouse	HSV protection	[73]
	gpB	SIN / DNA	Mouse	CTL, protection	[74]
IBDV	VP2	SFV / Particles, DNA	Chicken	Specific Abs	[75]
Influenza	HA	SFV / Particles	Mouse	Systemic response	[13]
	HA	SFV / DNA	Mouse	Humoral, cellular	[76]
	HA	VEE / Particles	Chicken	Influenza protection	[14]
	HA	VEE / Particles	Swine	Influenza protection	[20]
	HA	VEE / Particles	Swine	Specific Abs	[21]
	NP	SFV / Particles, RNA	Mouse	Humoral, CTL	[77]
	NP	SFV / Particles, RNA	Mouse	CTL	[78]
JEV	prM-E, NS1-2A	SIN / Particles	Mouse	JEV Abs	[79]
Lassa	N	VEE / Particles	Mouse	Immune response	[19]
LIV	prME	SFV / Particles	Mouse	LIV protection	[80]
	prME, NS1	SFV / Particles	Sheep	LIV protection	[81]
MBGV	GP, NP, VP35	VEE / Particles	Guinea pig	MBGV protection	[82]
	GP, NP	VEE / Particles	Macaques	MBGV protection	[83]
Measles	HA, FUd	SIN / DNA	Mouse	Measles protection	[29]
	HA, FUd	SIN-VEE / Particles	Macaques	Measles protection	[30]
MVE	prME, E	SFV / Particles	Mouse	Neutralizing Abs	[84]
NiV	Glycoproteins	VEE / Particles	Mouse	Neutralizing Abs	[25]
NLV	VLP	VEE / Particles	Mouse	Immune response	[85]
Rabies	G	SIN / DNA	Mouse	Rabies protection	[32]
RSV	F, G	SFV / DNA, RNA	Mouse	RSV protection	[86]
	F, G	SFV / Particles	Mouse	RSV protection	[87]
SARS-CoV	Glycoprotein	VEE / Particles	Mouse	SARS-CoV protection	[23]
SEOV	M, S	SIN /Particles, DNA	Hamster	SEOV protection	[88]
SHIV	env	SFV / Particles	Macaques	T cell prolif. Response	[89]
Vaccinia	A33R, B5R	VEE / Particles	Mouse	Vaccinia protection	[31]

Abs: Antibodies; BVDV: Bovine viral diarrhea virus; CMI: Cell-mediated immune response; CSFV: Classical swine fever virus; CTL: Cytotoxic T-lymphocyte activity; EAV: Equine arteritis virus; HBV: Hepatitis B virus; HBC: Hepatitis C virus; HeV: Hendra virus; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; HSV: Herpes simplex virus; IBDV: Infectious bursal disease virus; JEV: Japanese encephalitis virus; LIV: Loupin ill virus; MBGV: Marburg virus; MVE: Murray Valley encephalitis virus; NiV: Nipah virus; NLV: Norwalk-like virus; RSV: Respiratory syncytial virus; SARS-CoV: Severe acute respiratory syndrome corona virus; SEOV: Seoul virus; SFV: Semliki Forest virus; SHIV: Simian-human immunodeficiency virus; SIN: Sindbis virus; VEE: Venezuelan equine encephalitis virus

Table 1: Vaccine development for viral targets.