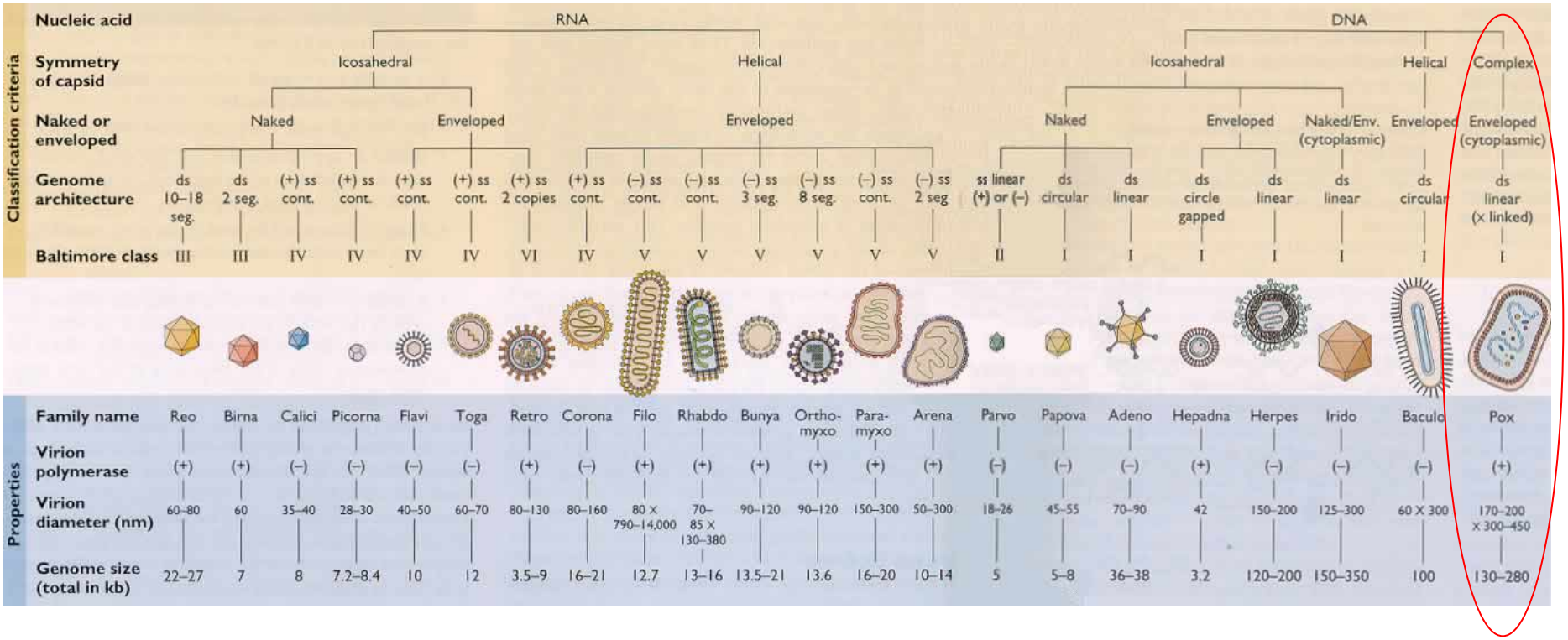
Molecular Virology

Vaccinia Virus

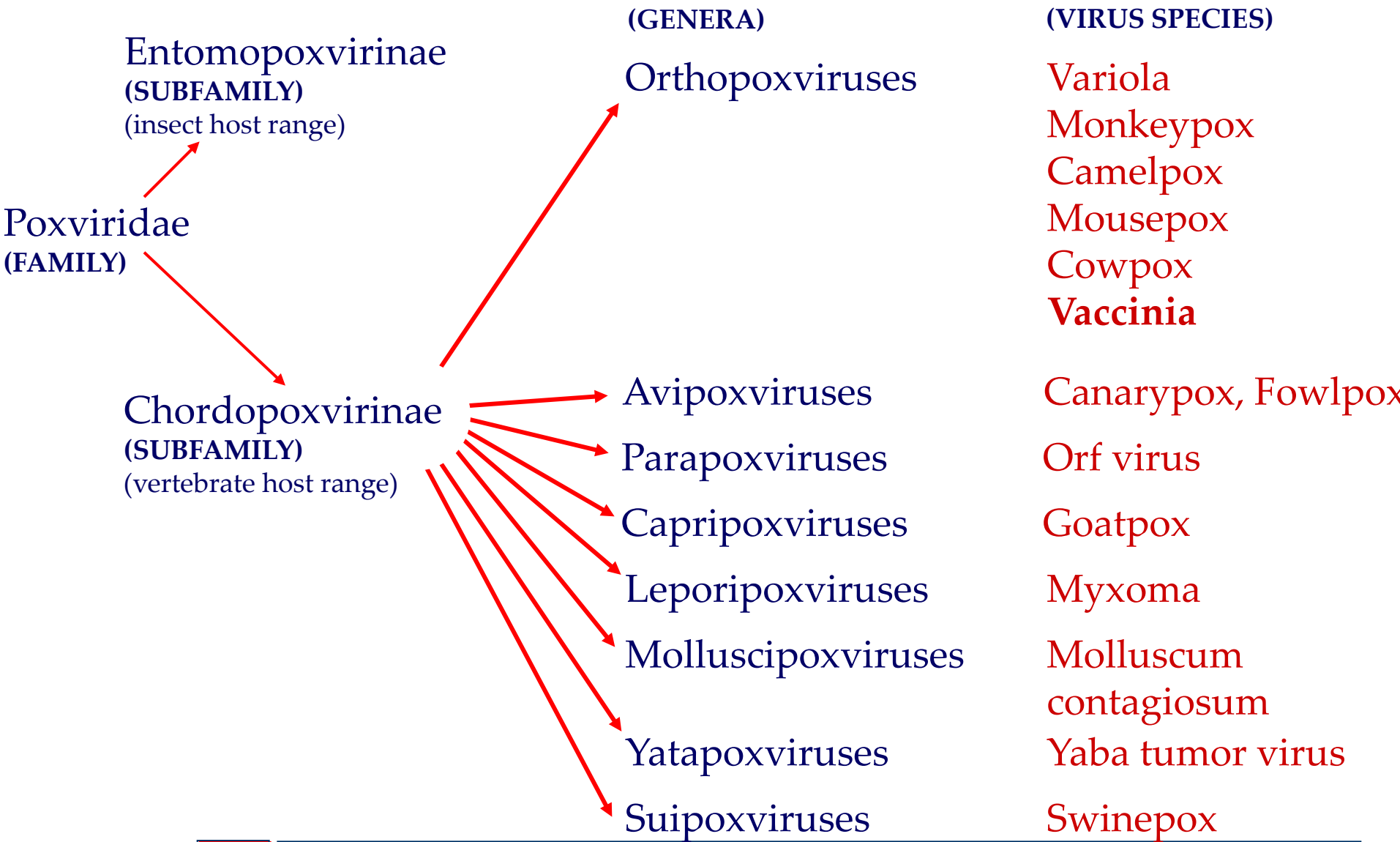
Barbara Schnierle
Department of Virology

Paul-Ehrlich-Institut

The hierarchical virus classification system



Familiy Poxviridae



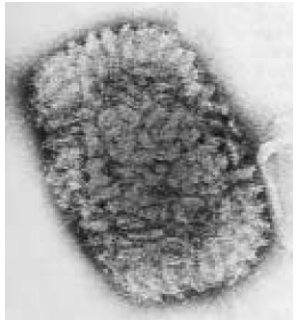
Origin of the different virus species

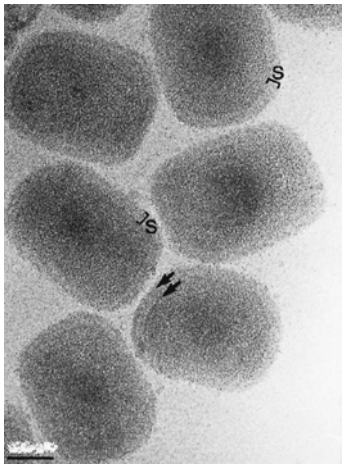
Unterfamilie	Gattung	Art	Ursprünglicher, natürlicher Wirt
Chordopoxvirinae	Orthopoxvirus	Variolavirus Vacciniavirus Kuhpockenvirus Kamelpockenvirus Ectromeliavirus Affenpockenvirus Waschbärpockenvirus Stinktierpockenvirus	Mensch ? Nager Kamel Nager Eichhörnchen Waschbär Stinktier
	Parapoxvirus	Pseudokuhpockenvirus	Rind
	Avipoxvirus	viele Arten	Vögel
	Capripoxvirus	Schafpockenvirus Ziegenpockenvirus	Schaf Ziege
	Leporipoxvirus	Myxomvirus Fibromatosevirus	Kaninchen Hase, Kaninchen, Eichhörnchen
	Suipoxvirus	Schweinepockenvirus	Schwein
	Molluscipoxvirus	Molluscum-contagiosum-Virus	Mensch
	Yatapoxvirus	Tanapockenvirus Yabapockenvirus	Nager? Affen?
Entomopoxvirinae	Entomopoxvirus A	Pockenviren der Käfer, Schmetterlinge und Fliegen	
	Entomopoxvirus B		
	Entomopoxvirus C		

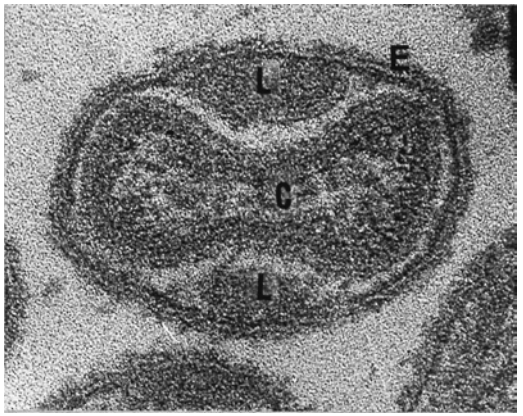
Poxviruses

- large viruses with linear double stranded DNA genome
  - 130 000 - 370 000 bp

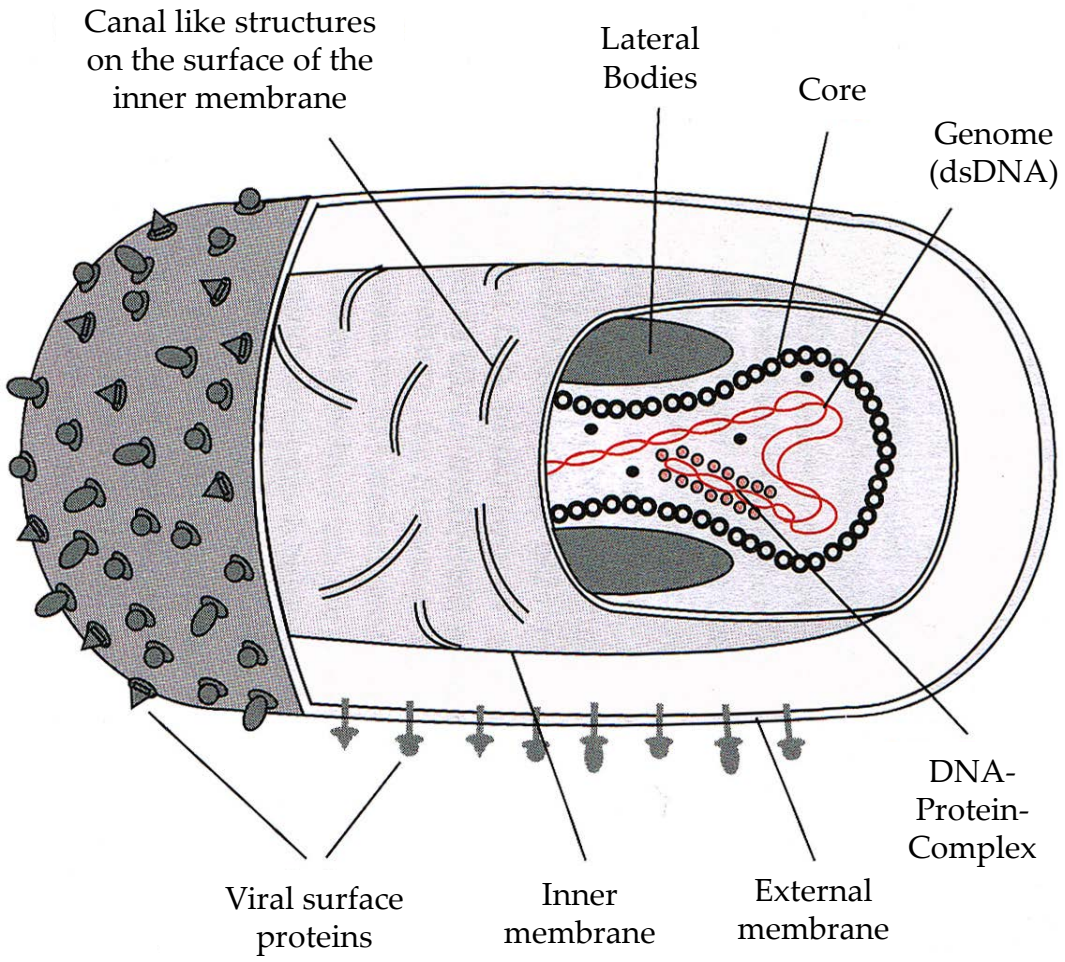
- complex architecture
  - enveloped
  - oval or brick-shaped (300 x 240 x 150 nm in size)
  - replicate entirely in the cytoplasm of infected cell
  - encode 150 – 200 viral proteins; 1/3 non-essential



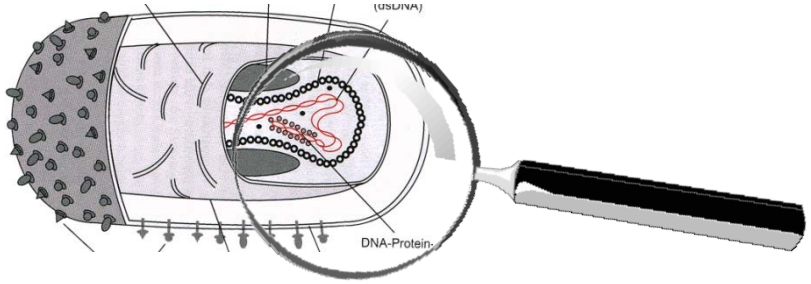


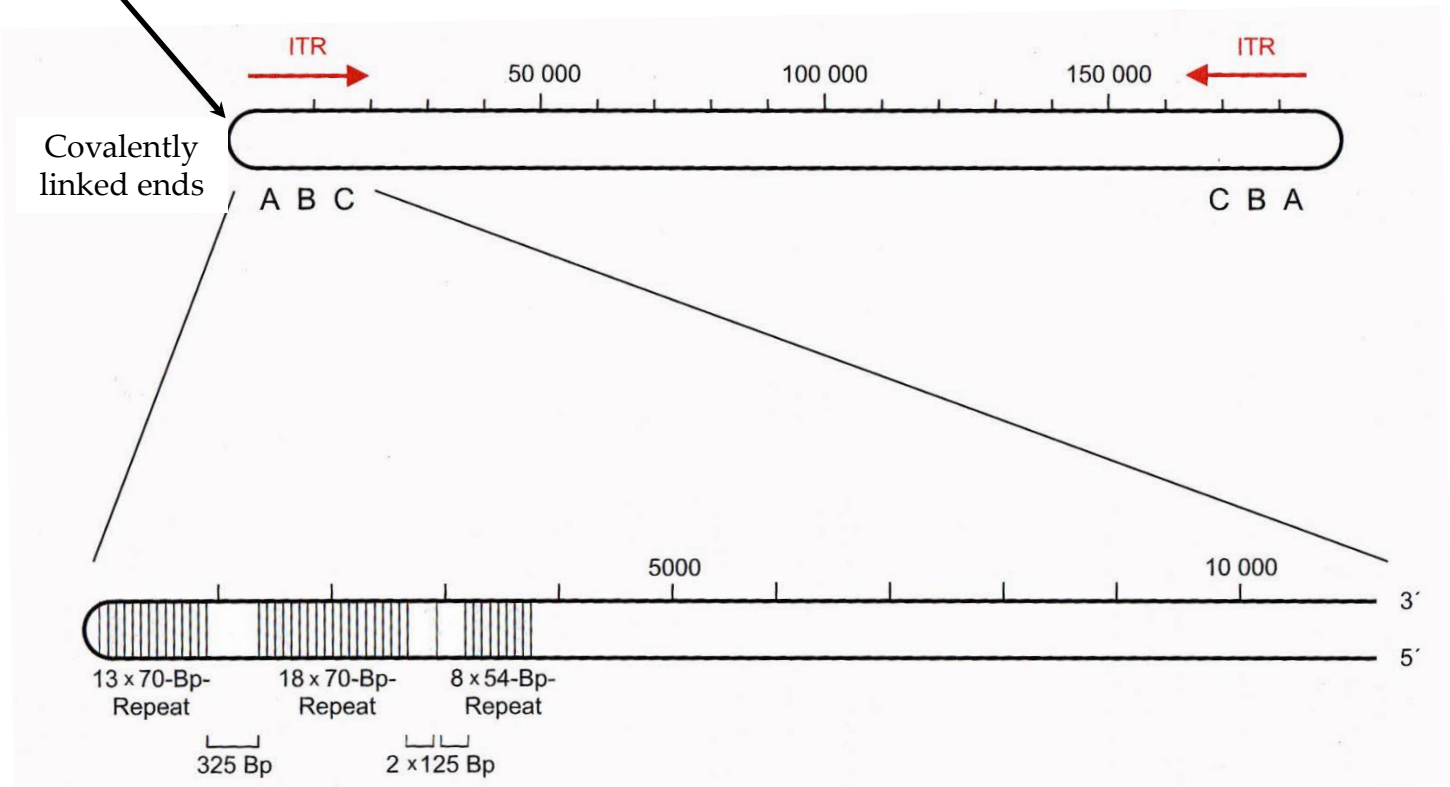


Poxvirus particle

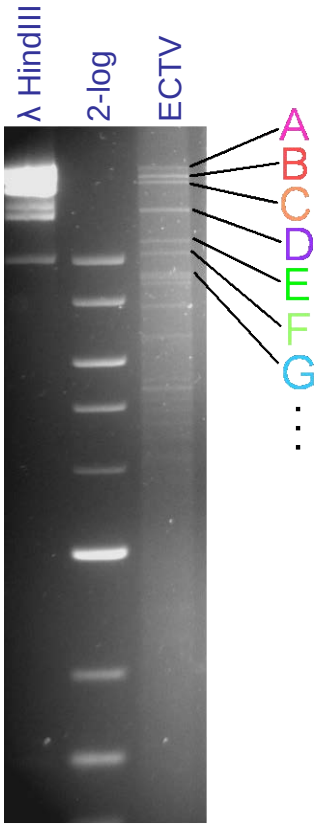


Vaccinia virus genome





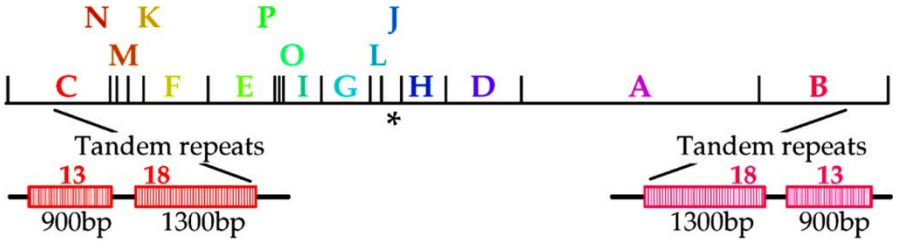
Viral genome

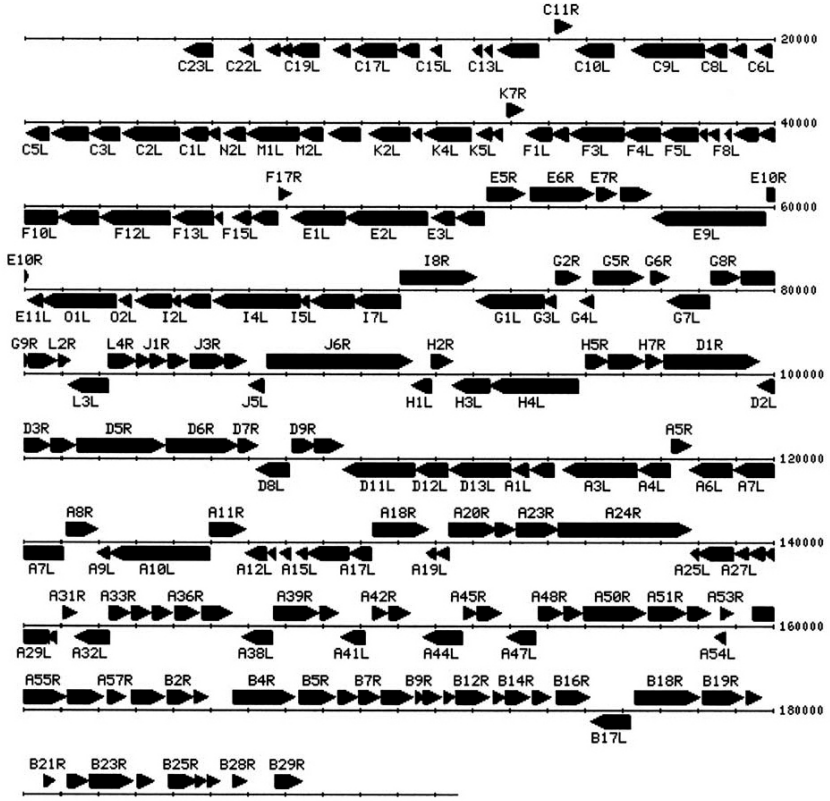


HindIII restriction analysis of ECTV by Dr. Meike Gratz

Naming for VACV genes:

HindIII restriction endonuclease DNA fragment letter + OFR number + direction of the ORF

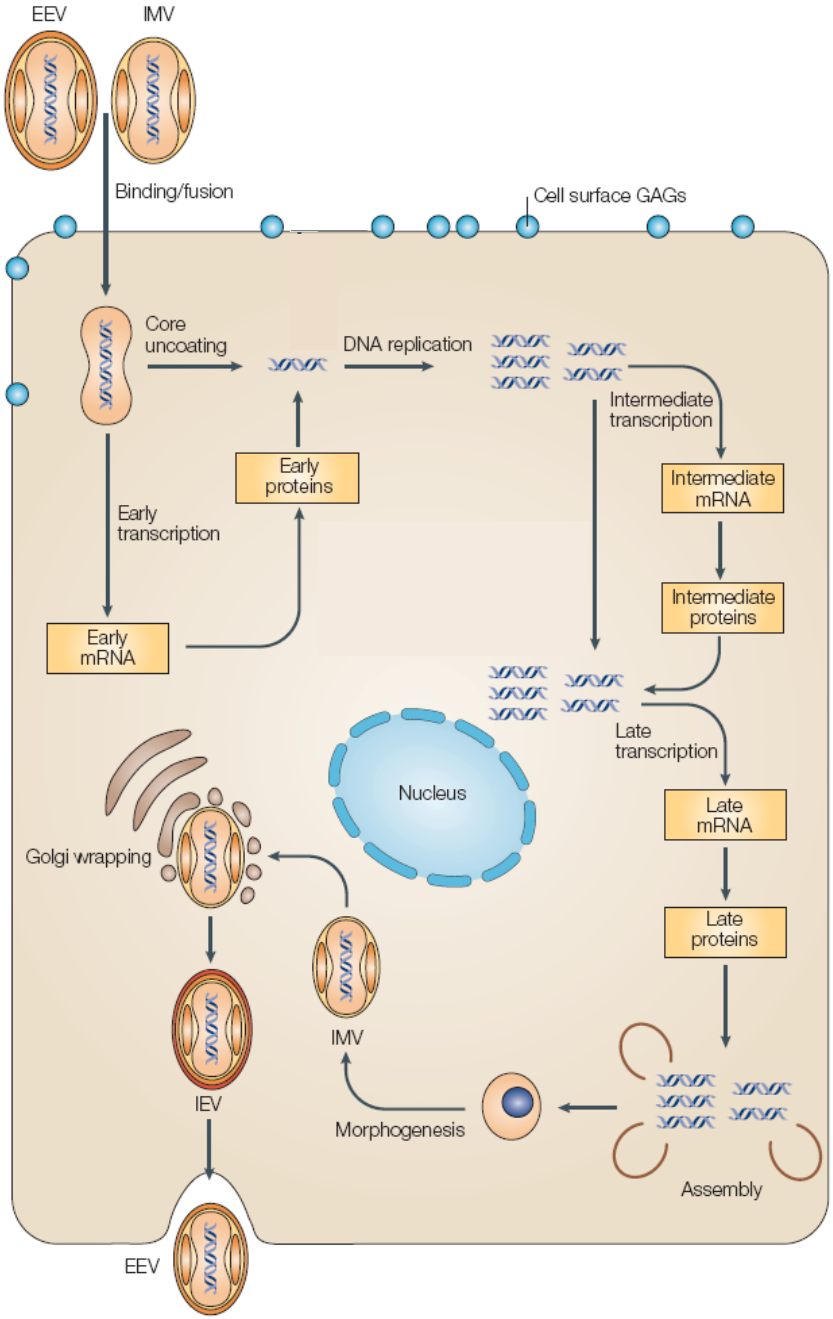




~ 150 – 200 viral proteins

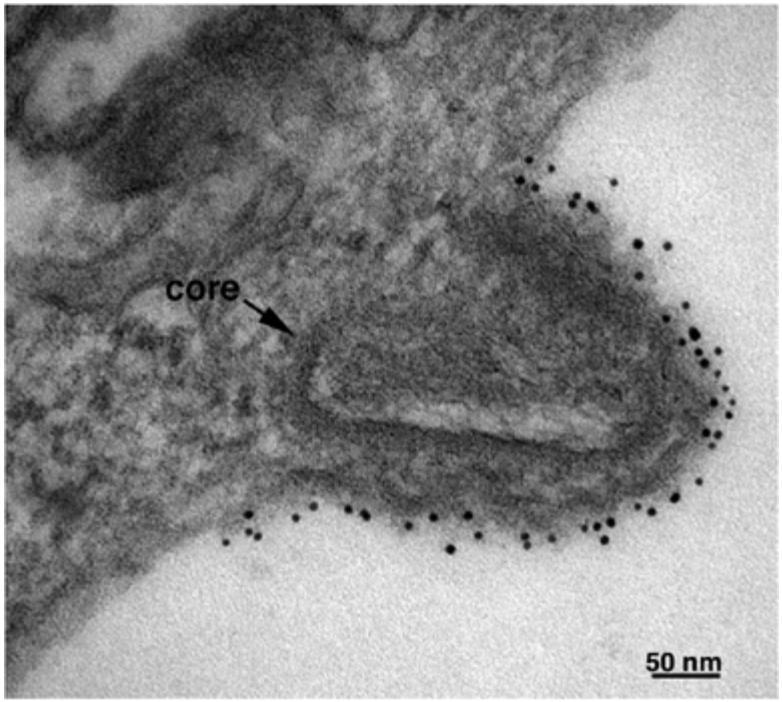
Poxvirus life cycle

- entry
- cascade-like gene expression
  - early
    ->DNA replication
  - intermediate
  - late
- assembly & morphogenesis
- egress



Entry

- receptor is unknown
- putative entry complex of eight VACV transmembrane proteins has been recently identified
- two mechanism proposed
  => pH-dependent fusion with the plasma membrane
  => actin-mediated internalization mechanism
- both pathways may be used depending on the virus strain and cell type
- viral particles contain proteins that facilitate initial attachment to cells by binding to glycosaminoglycans
  (chondroitin sulfat + heparan sulfate)



Immunoelectron microscopy showing VACV MV fusing with the plasma membrane at neutral pH.

Cytoplasmic poxvirus replication

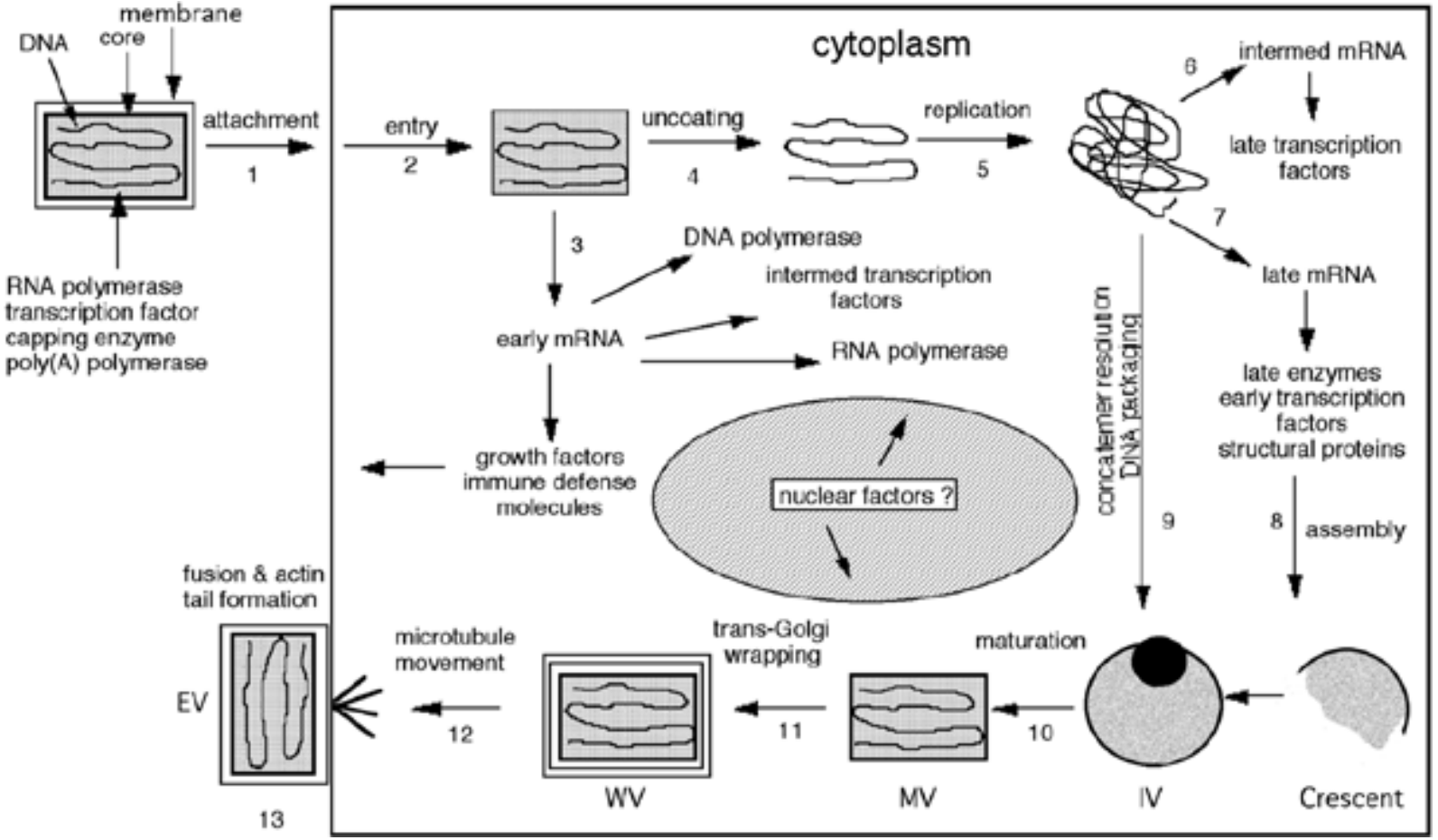
- Poxviruses replicate entirely in the cytoplasm – cannot use cellular enzymes for replication

- code for enzymes responsible for:
  - => DNA replication
  - => transcription
  - => RNA modification
  - => nucleic acid metabolism

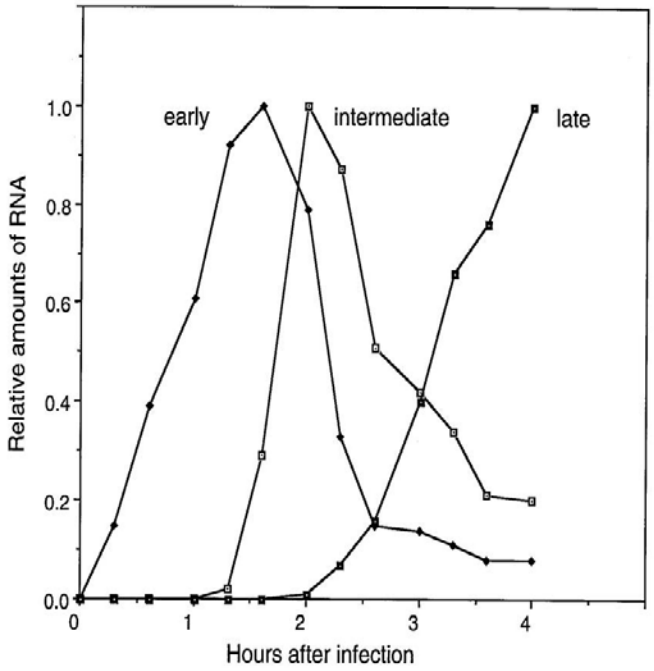
- for example:
  - => DNA-dependent RNA-polymerase
  - => Poly(A) polymerase
  - => Capping enzyme complex
  - => DNA-Topoisomerase

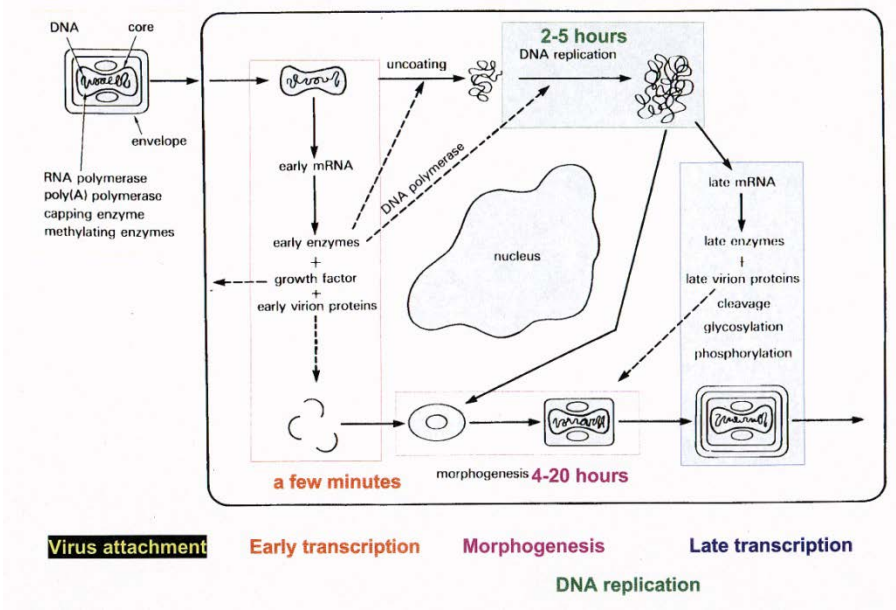
- enzymes needed for the early procedures (early transcription, RNA modification, DNA replication) are packed within the virus capsid

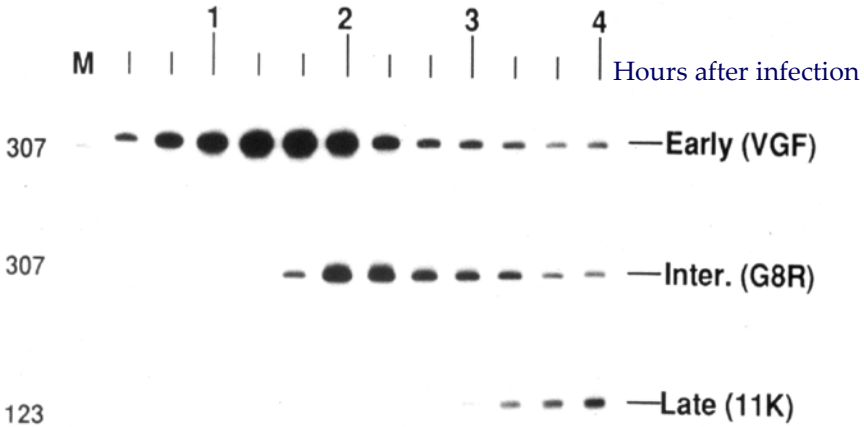
Poxvirus replication



Temporal synthesis of VV mRNAs



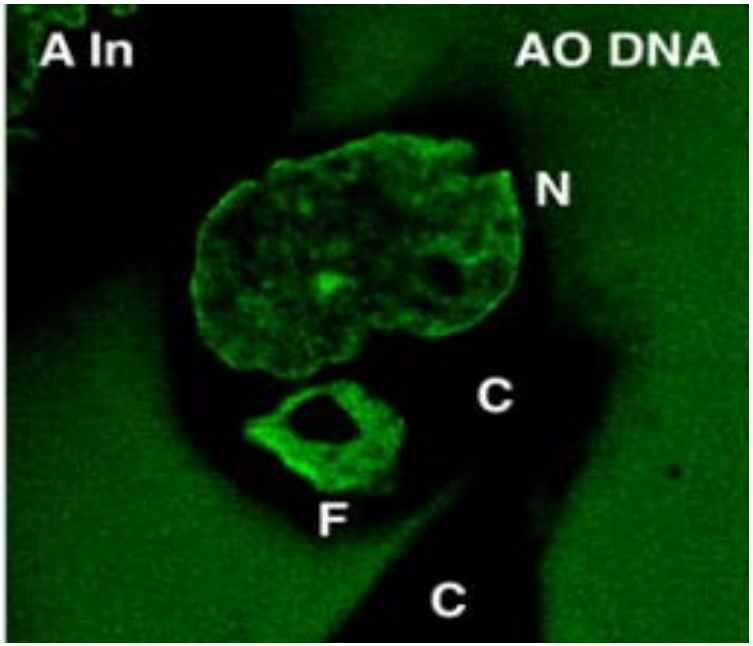




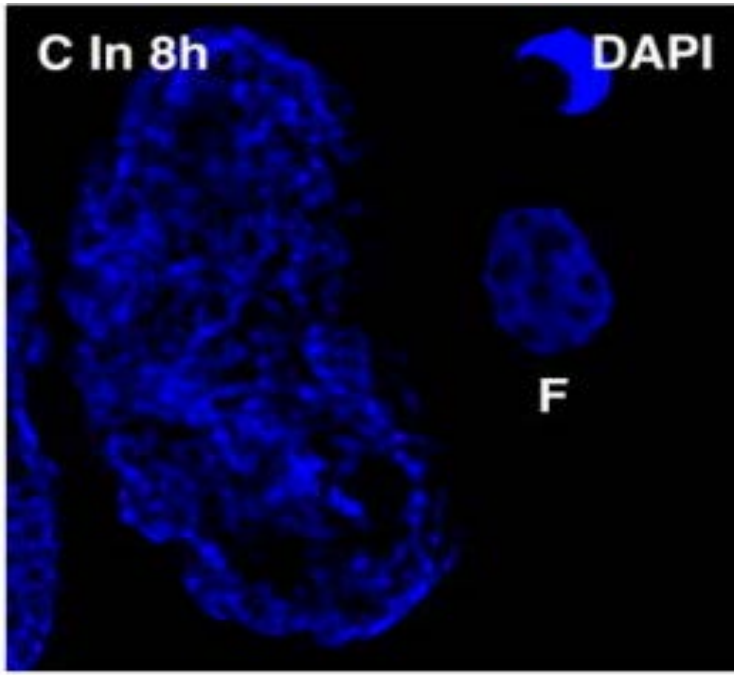
Viral factories

Vaccinia virus infected cells …

…stained with acridine orange

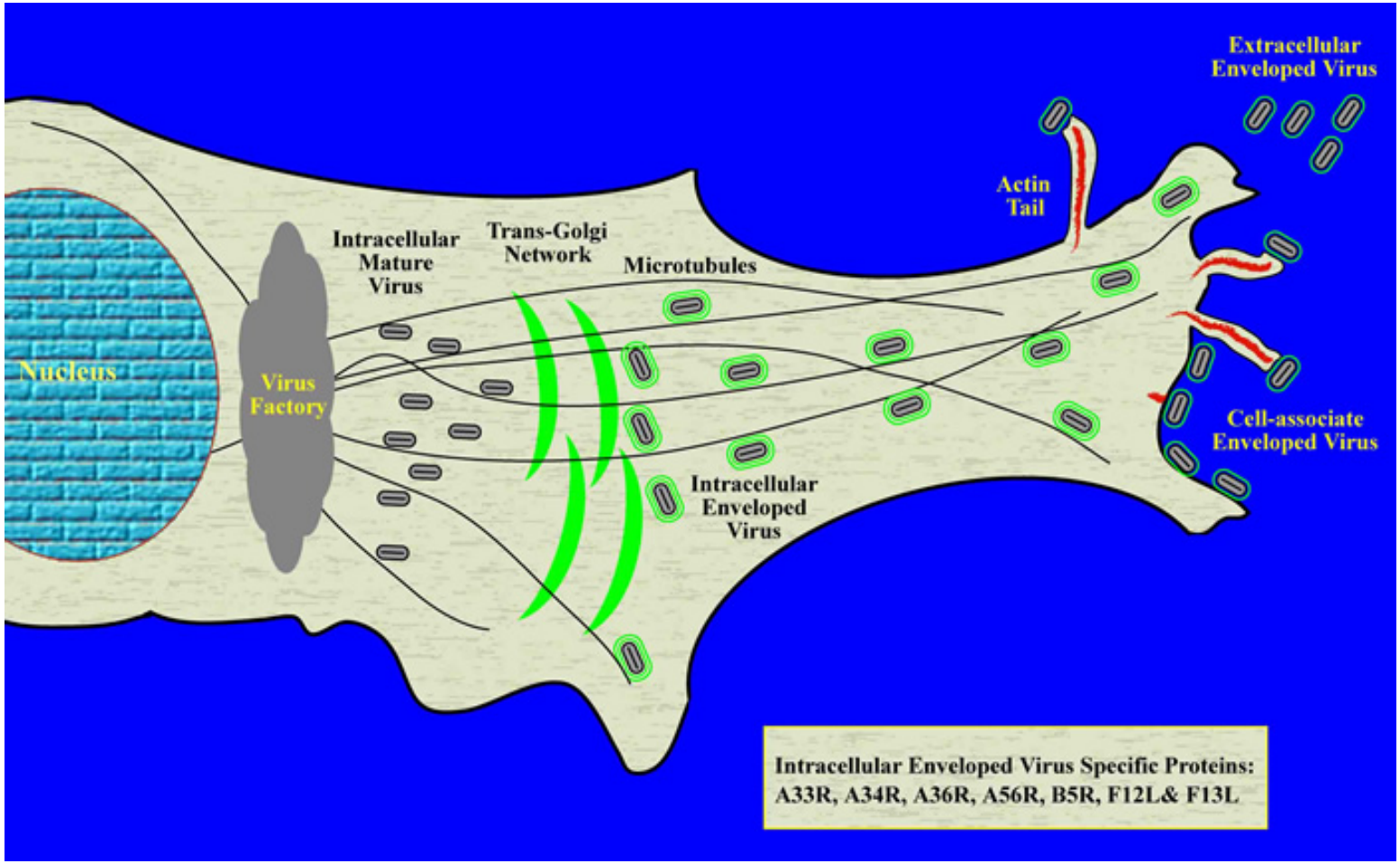


…stained with DAPI

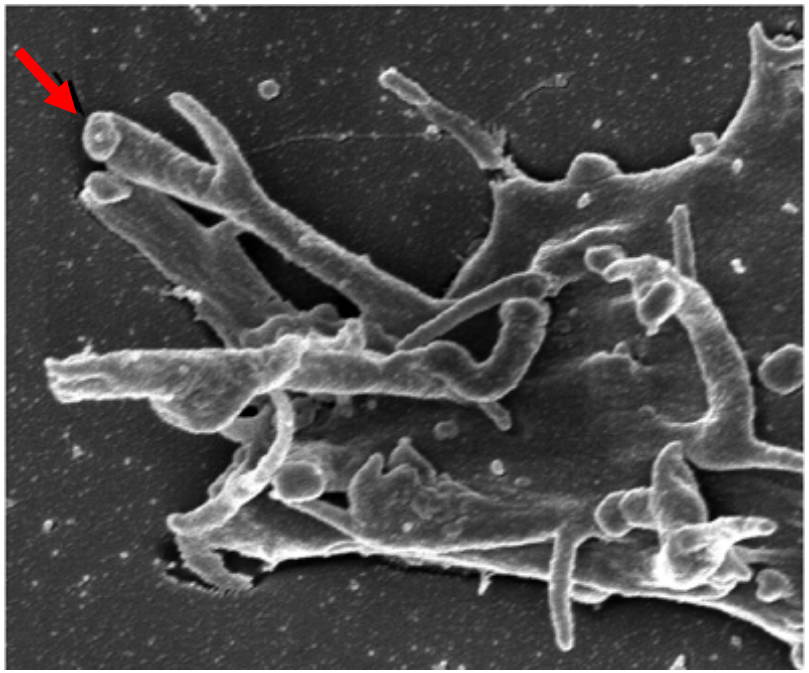


c = cytoplasm n = nucleus f = viral factory

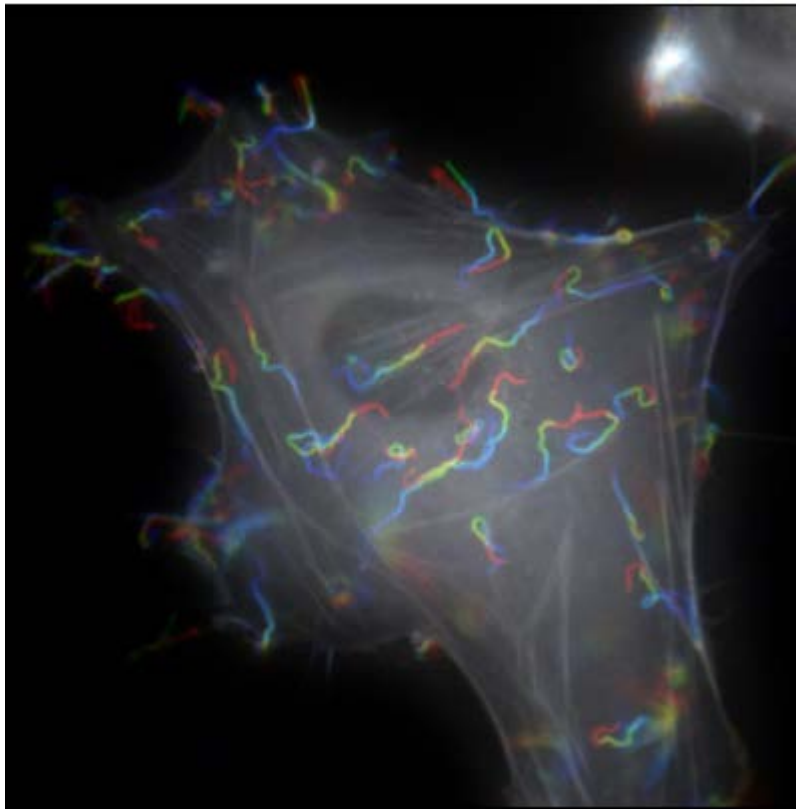
Vaccinia virus motality



Poxvirus infected cell

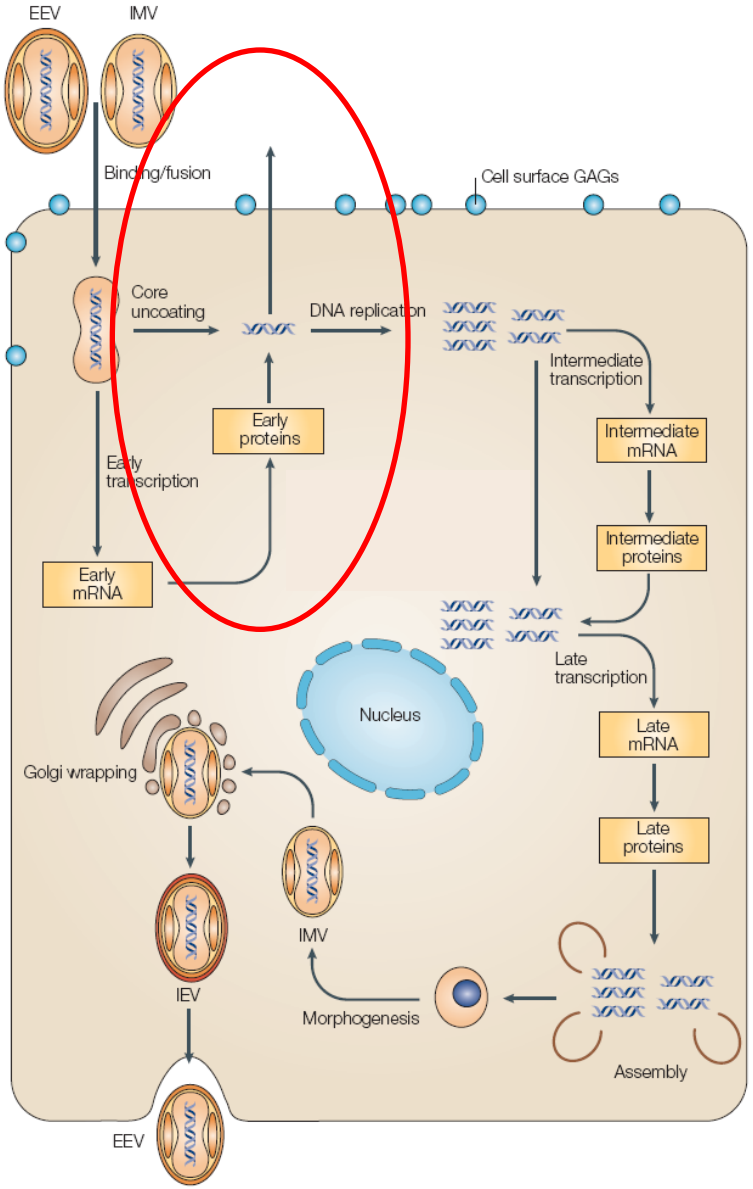


Scanning electron microscopy of cell infected with VACV. Arrow points to an EV at the tip of one of many virus-induced actin-containing microvilli.



Fluorescence image of the paths taken by vaccinia induced actin tails over a 5 minute period. By Michael Way

Poxviruses and immune evasion



Immune evasion

Extracellular

cytoplasmic

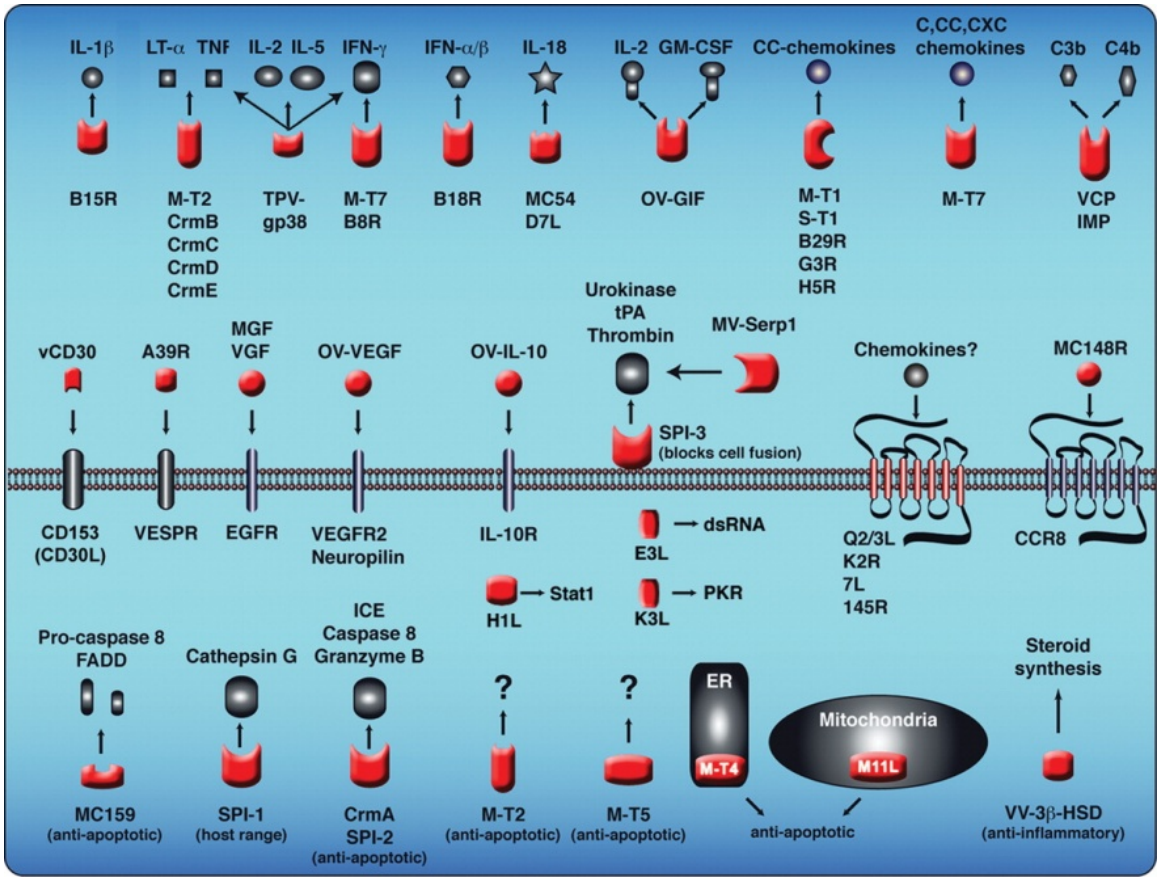


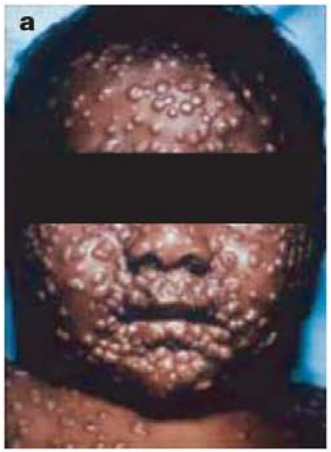
Table 1
Poxvirus immune modulators

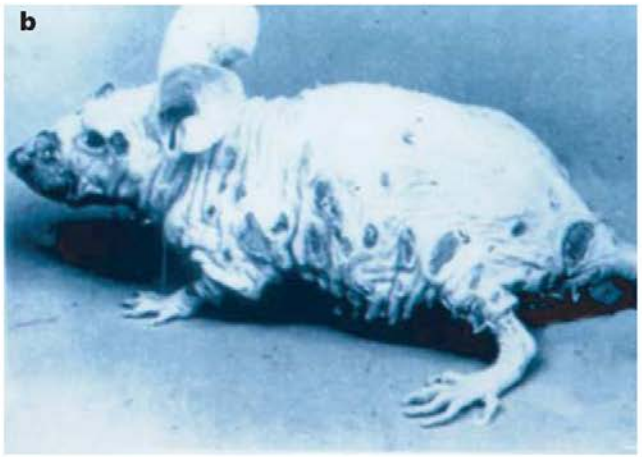
Function or class	Genes	Primary references
TNF receptor	MYX T2, SFV T2	Upton et al. (1987), Smith et al. (1991a)
	VV crmC	Howard et al. (1991)
	CPV crmB, C, D, E	Hu et al. (1994), Smith et al. (1996), Loparev et al. (1998), Saraiva and Alcami (200
	ECT crmD	Loparev et al. (1998)
	VAR crmB	Shchelkunov et al. (1993)
IFN-γ receptor	MYX T7	Upton et al. (1992)
	VV B8R, CPV, ECT	Alcami and Smith (1995), Mossman et al. (1995a)
IFN-α/β receptor	VV B18R	Symons et al. (1995), Colamonici et al. (1995)
IL-1β receptor	VV B15R	Spriggs et al. (1992), Alcami and Smith (1992)
IL-18 BP	MCV 54L	Xiang and Moss (1999b)
	VV, CPV, ECT D7L	Smith et al. (2000), Born et al. (2000)
P1 Asp serpins	crmA/SPI-2	Pickup et al. (1986), Ray et al. (1992)
	MYX SERP2	Petit et al. (1996)
P1 Arg serpins	MYX SERP1	Upton et al. (1990)
	SPI-3	Law and Smith (1992), Turner and Moyer (1995)
Toll/IL-1 receptor	VV A52R, A46R	Smith et al. (1991b), Bowie et al. (2000)
Chemokine	MC148	Senkevich et al. (1997), Krathwohl et al. (1997), Damon et al. (1998)
Chemokine binding	MYX T7	Lalani et al. (1997)
	MYX T1	Graham et al. (1997), Smith et al. (1997)
	RPV, CPV P35 (vCCI)	Smith et al. (1997), Graham et al. (1997)
Complement control	VV VCP, CPV IMP	Kotwal and Moss (1988), Kotwal et al. (1989)
Semaphorin	VV A39R	Comeau et al. (1998)

Orthopoxviruses

Systemic infections

variola virus	⇨	human
ectromelia virus	⇨	mouse
camelpox virus	⇨	camel





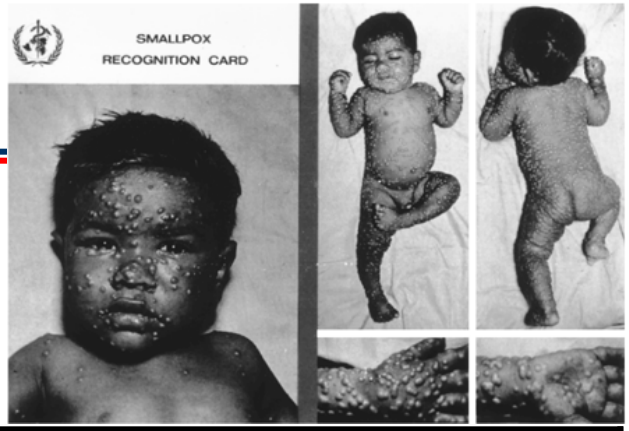
Variola virus

- eldest known communicable disease
- Historians speculate: that it must have emerged sometime after the first agricultural settlements, about 10,000 BC.
- earliest evidence: skin lesions of Egyptian mummies (Ramses V) from the 18th and 20th dynasties (1570-1085 BC)
- written descriptions of the disease did not appear until the 4th century AD in China and the 10th century in southwest Asia
- disease killed estimated 400,000 Europeans each year during the 18th century & was responsible for one third of blindness
- fatality: 10-40% (children over 80%)
- today: eradicated => benefit from vaccination with **Vaccinia virus**

Systemic disease pathogenesis of poxviruses

Entry	Skin (via contact) or respiratory tract
Site of primary replication	Skin entry: virus replicates in the Malphigian layer of the epidermis + dermal infection (fibroblasts + histiocytes) => responsible for subsequent movement of virus to the lymphatics Respiratory tract entry: primary infection in the mucosal surfaces of the nasopharyngeal tract, then moving to regional lymphatics
Virus spread	Primary viremia (first week): Virus moves from the regional lymphatics to the bloddstream to cause primary viremia, then multiplies in the spleen, liver, bone marrow and othe reticuloendothelial organs. Second viremia (second week): Virus enters leucocytes in small blood vessels in dermis

Smallpox disease



Incubation Period (Duration: 7 to 17 days) ***Not contagious***	**Exposure to the virus** is followed by an incubation period during which people do not have any symptoms and may feel fine.
Initial Symptoms *(Prodrome)* (Duration: 2 to 4 days) ***Sometimes contagious***	The **first symptoms** of smallpox include fever, malaise, head and body aches, and sometimes vomiting.
Early Rash (Duration: about 4 days) ***Most contagious***	A **rash emerges** first as small red spots on the tongue and in the mouth. These spots develop into sores that break open and spread large amounts of the virus into the mouth and throat.
Pustular Rash (Duration: about 5 days) ***Contagious***	The bumps become **pustules**—sharply raised, usually round
Pustules and Scabs (Duration: about 5 days) ***Contagious***	The pustules begin to form a crust and then **scab**. By the end of the second week after the rash appears, most of the sores have scabbed over.
Resolving Scabs (Duration: about 6 days) ***Contagious***	The scabs begin to fall off, leaving marks on the skin that eventually become pitted **scars**. Most scabs will have fallen off three weeks after the rash appears.
Scabs resolved ***Not contagious***	Scabs have fallen off. Person is no longer contagious

CDC – Homepage (adapted)

First protection - Variolation in China

China in the sixth century:
Chinese physicians ground dried scabs from smallpox victims along with musk and applied the mixture to the noses of healthy people.
The goal was to cause a mild infection of smallpox and stimulate an immune response that would give the person immunity from the natural infection.
This process was called *variolation*.

Fatality rate: 1:10

This method was utilized as late as to the 1960s and 1970s in some parts of Ethiopia, western Africa, Afghanistan, and Pakistan.

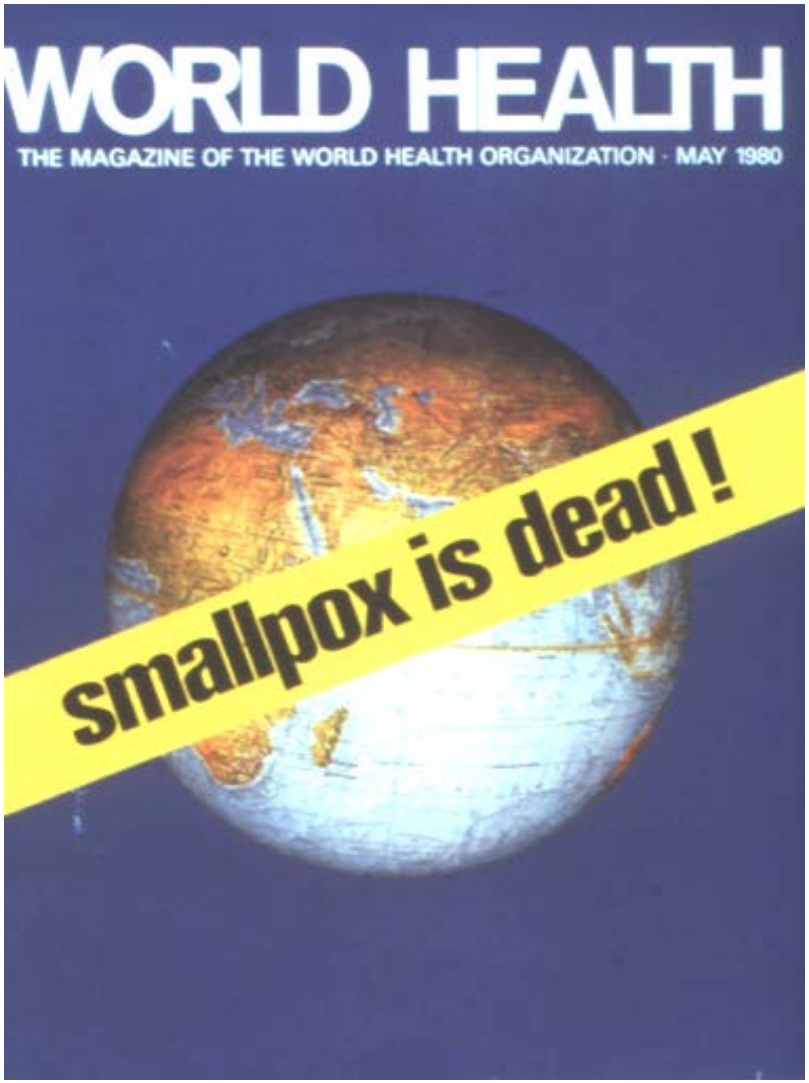
Edward Jenner 1789

Bovine analogue of poxvirus = Vaccinia virus

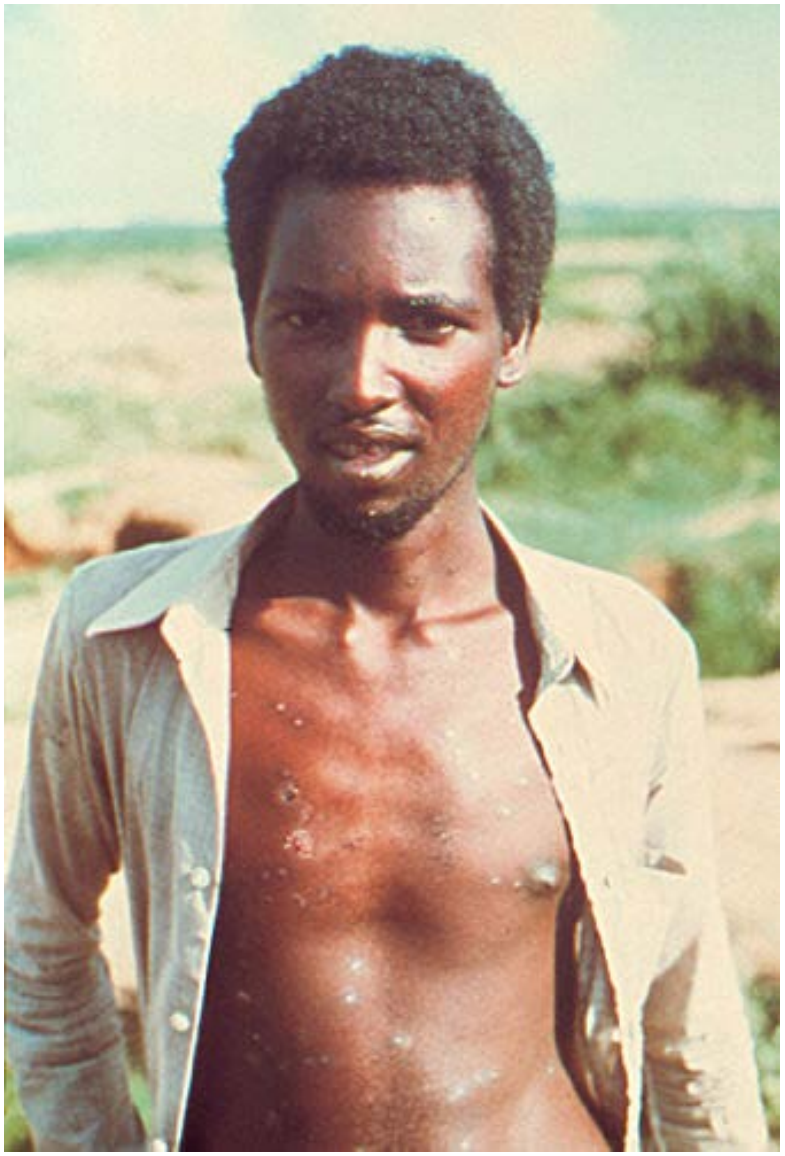
Long lasting protecting immunity against poxviral infections

"**Vaccination**"
(vacca = latin word for „cow")

1980 – WHO declared:



Last known poxvirus infected human 1977



23-year old

Ali Maow Maalin,
Merka, Somalia

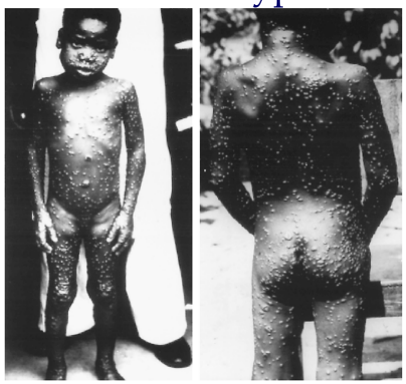
Eradicated poxviruses?

Risk of bioterroristic use

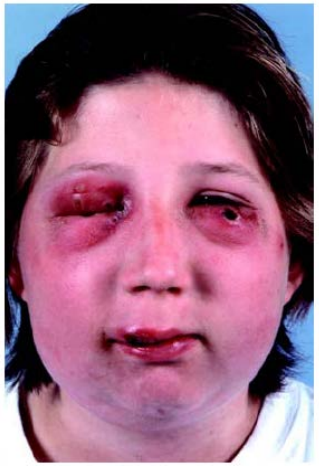
Variola major (smallpox) and other related poxviruses:
NIAID (national institute of allergy and infectious disease) **category A pathogen**

Zoonotic infections

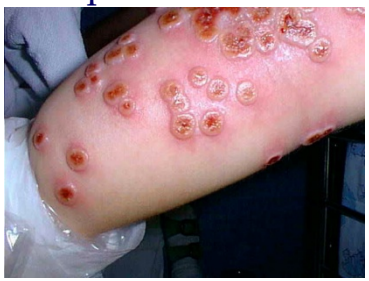
Human monkeypox



Cowpox lesions



Cowpox lesions

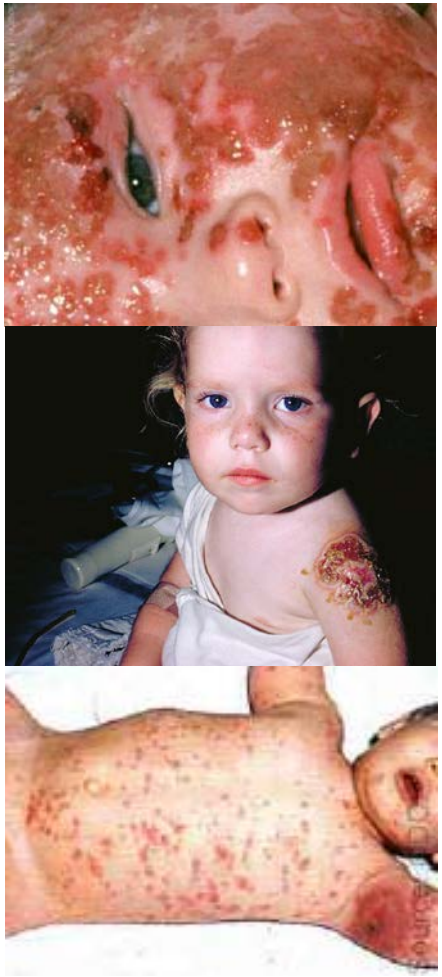


Severe side reactions to licensed vaccines

Severe side reactions to licensed vaccines

- serious reactions
  ~ 1,000 people for every 1 million people

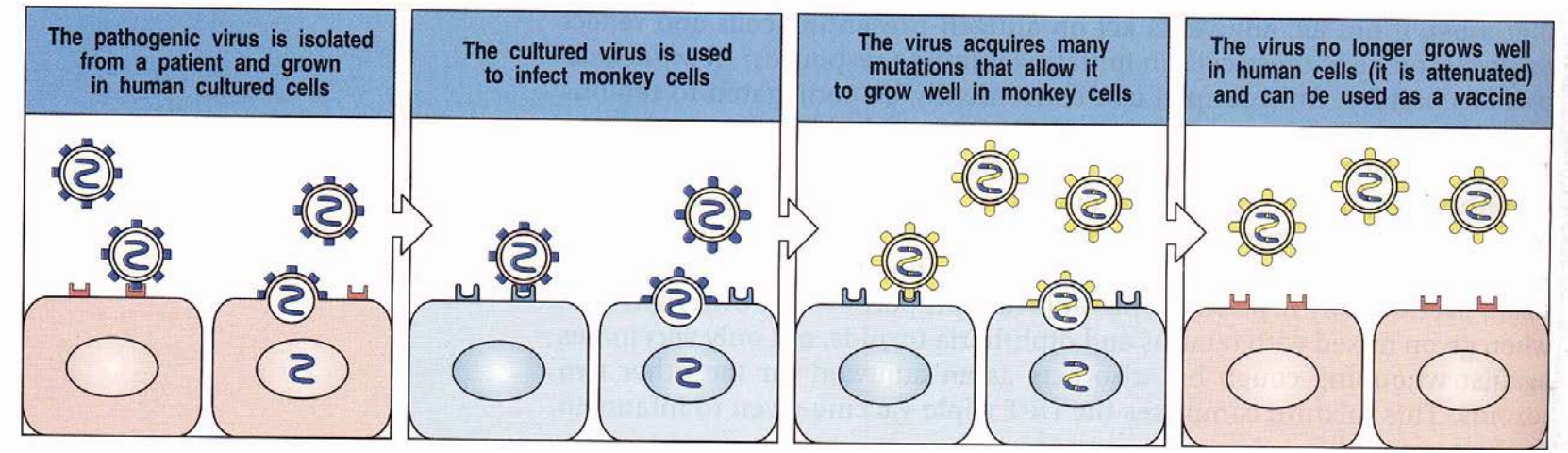
- life-threatening reactions
  ~ 14 to 52 people per 1 million people
  → eczema vaccinatum
  progressive vaccinia (v.necrosum)
  generalized vaccinia
  postvaccinal encephalitis



Live vaccines

- Live attenuated organisms:
  Virulence has been artificially reduced

  Vaccinia MVA, NYVAC

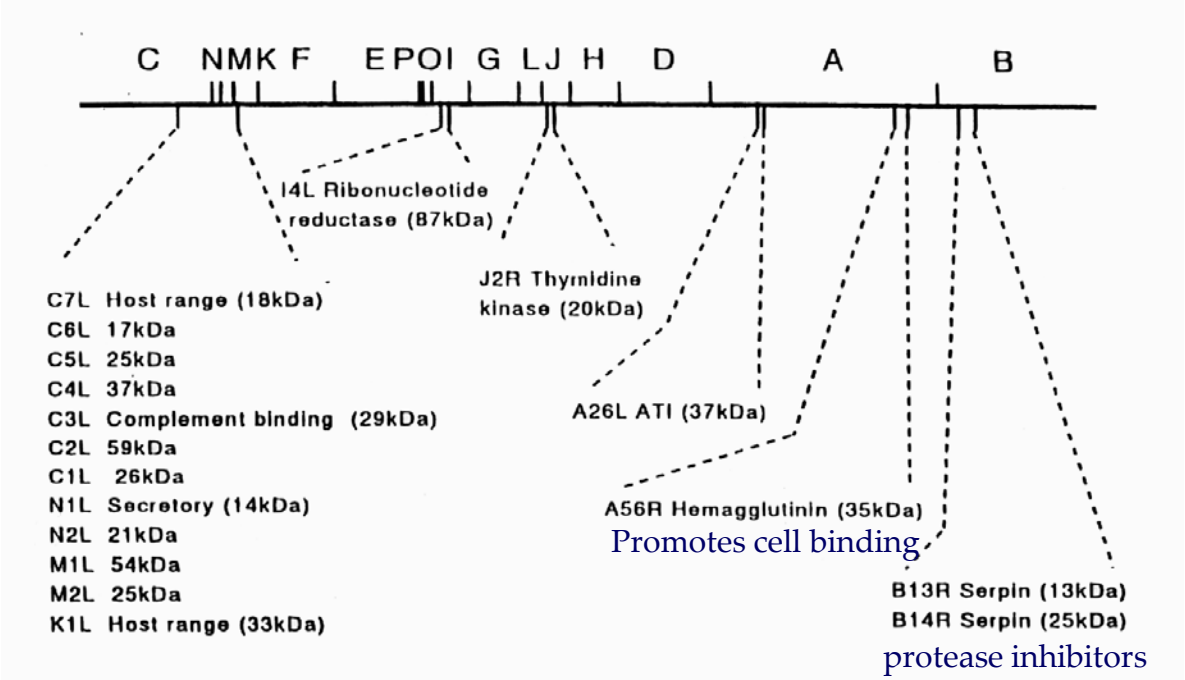


Selection of mutants that replicate poorly in human cells

Vaccinia virus NYVAC

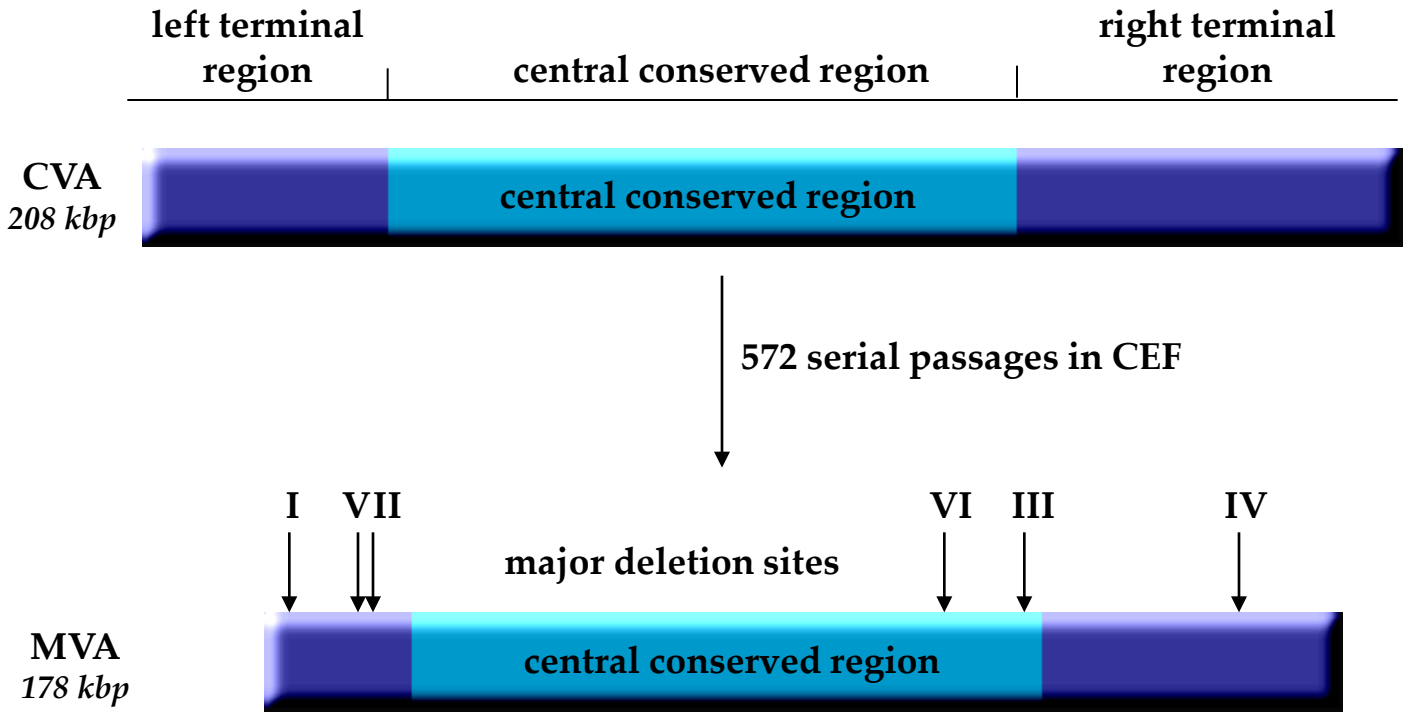
Virulence has been reduced by deletion of virulence genes by recombinant DNA technology.

Deletion of 18 ORFs



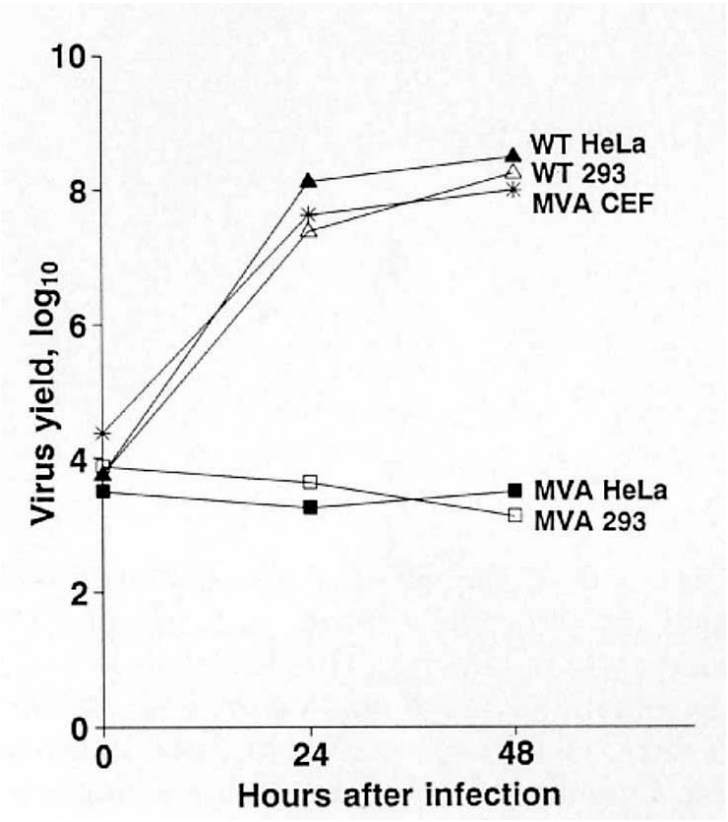
Modified vaccinia virus Ankara (MVA)

- derived by serial passage in chicken embryo fibroblasts
- substantial loss of genetic information (~ 15%)

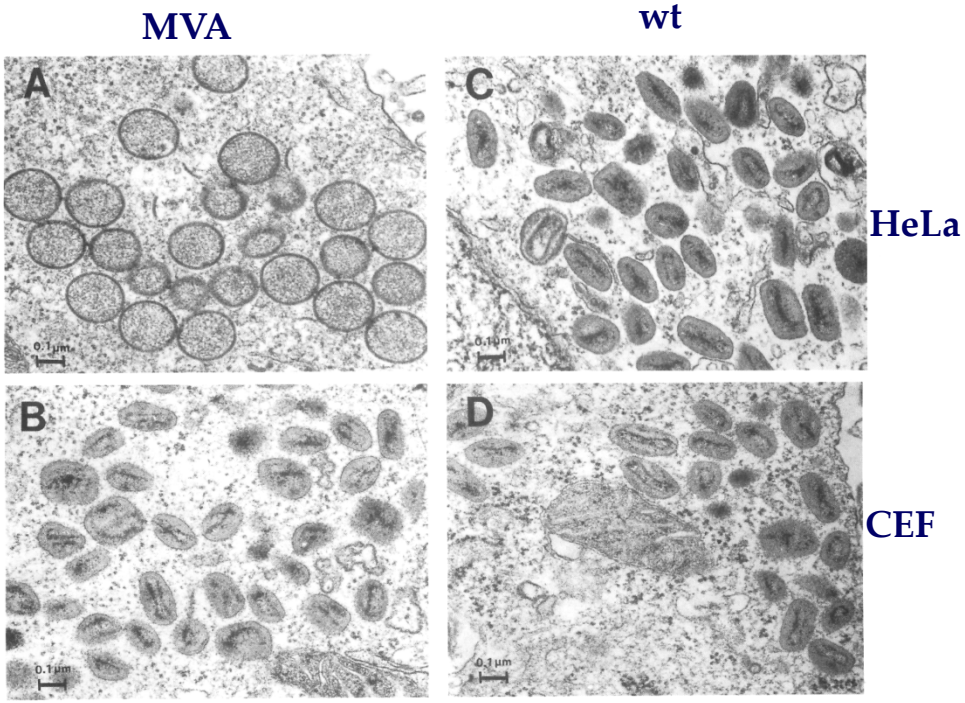


Phenotype of attenuated MVA …

… after 500 passages on chicken embryo fibroblasts

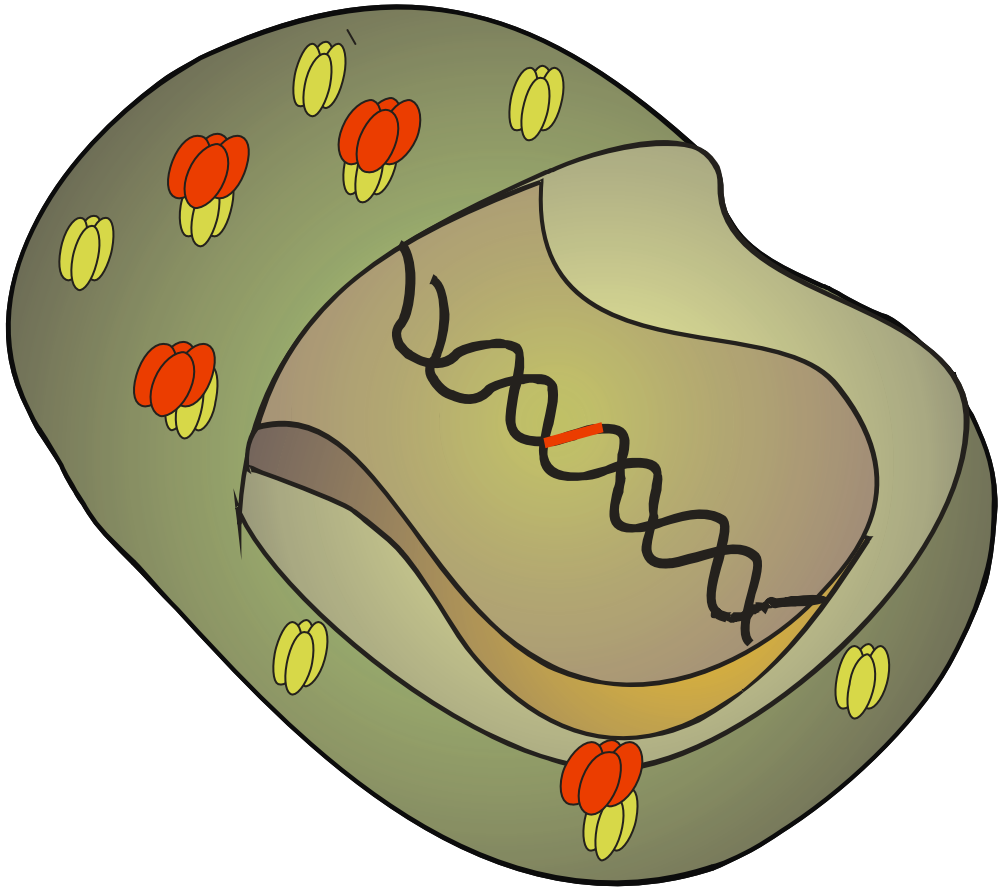


no replication in human cells



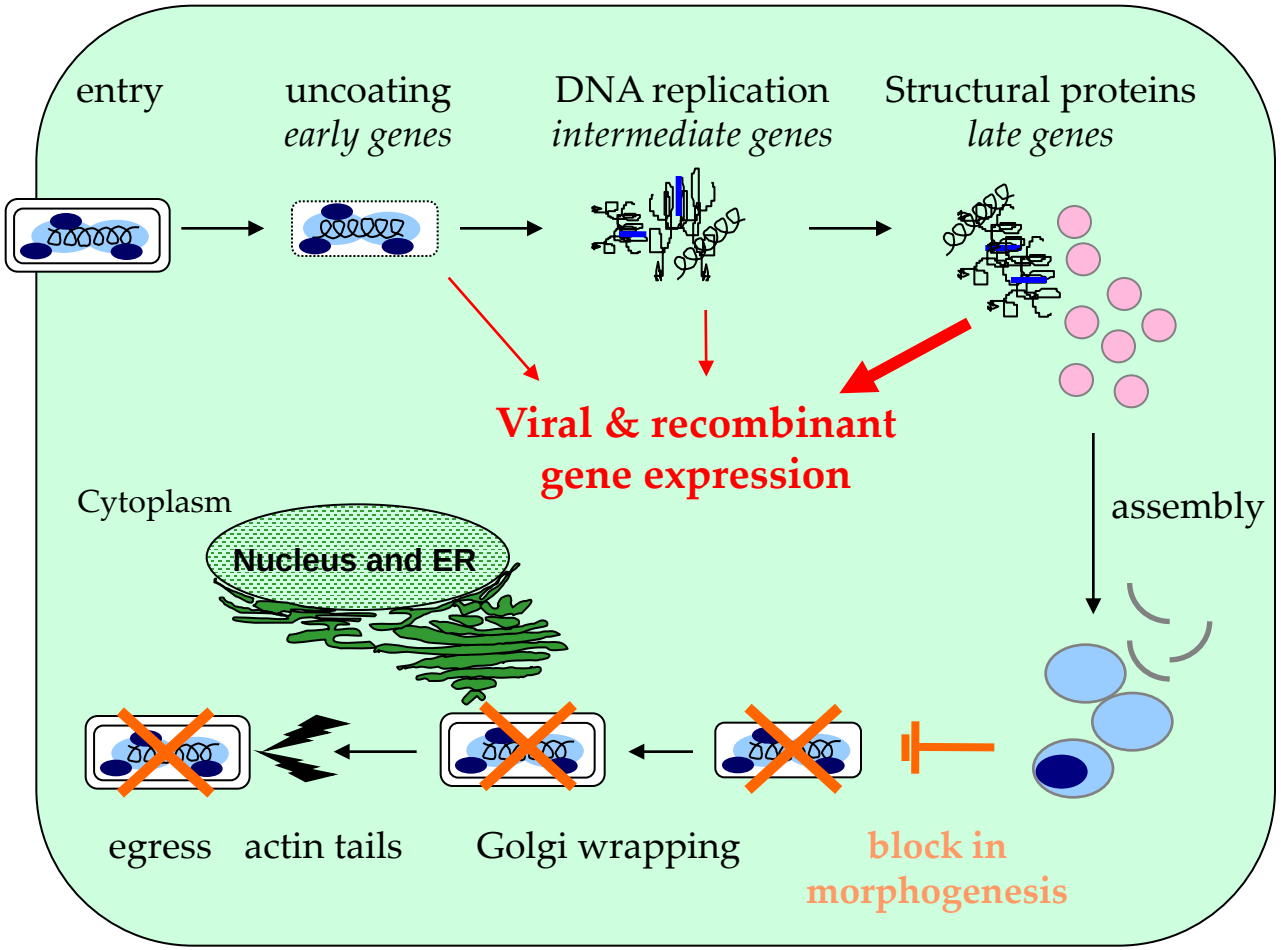
defect in viral morphogenesis, but not in protein synthesis

Vaccinia virus as vectors



Slide by Dr. Ulrike Mettler

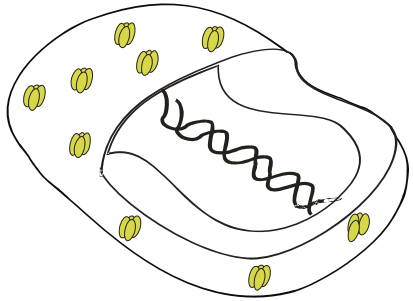
MVA for gene expression and vaccination



Expression system vaccinia virus

Expression plasmid

Vaccinia virus-T7 Polymerase



Transfection

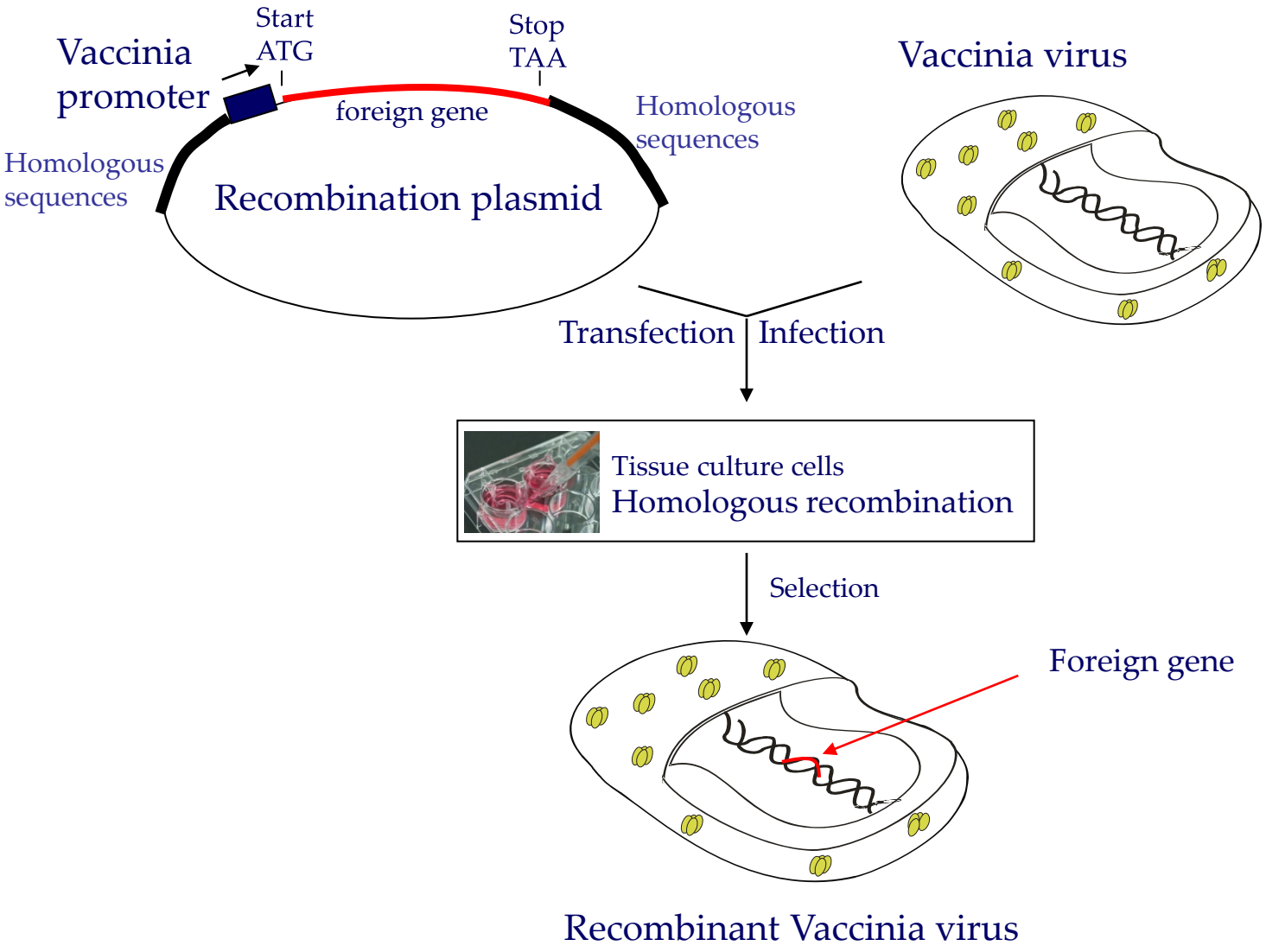
Infection

Tissue culture cells

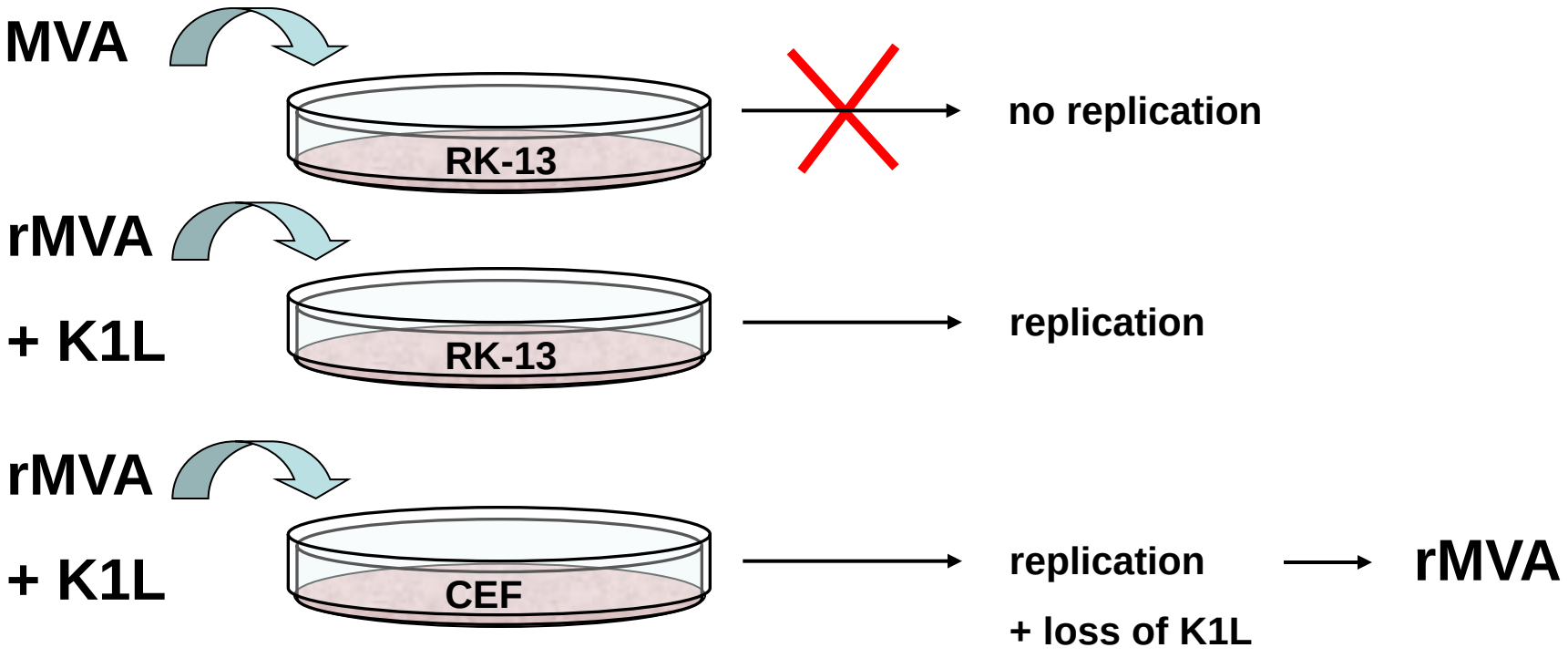
Purification

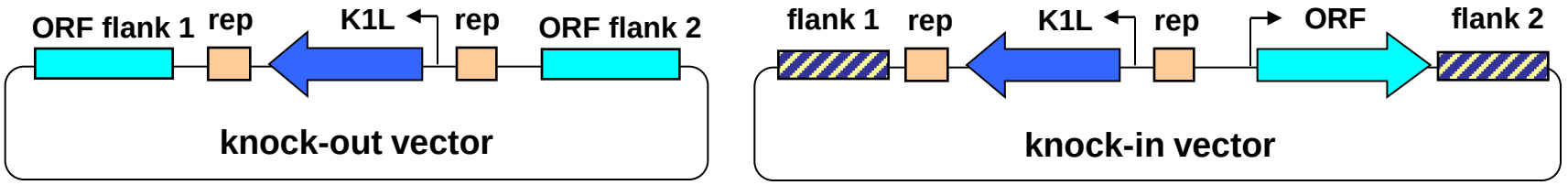
recombinant **protein X**
(up to 10% of total cell protein amount)

Production of recombinant vaccinia viruses



Recombinant MVAs – K1L selection





Slide by Dr. Karin Sperling

Clinical evaluation of MVA vector vaccines

Target disease	Clinical trial
Smallpox	Phase III planned 2010, prophylaxis sold to government as vaccine under development
AIDS	Phase I/II, therapeutic
Influenza	Phase I, prophylaxis
Tuberculosis	Phase I, prophylaxis
Malaria	Phase I, prophylaxis
Measles	Phase I/II, prophylaxis
Cervical cancer	Phase I/II, immunotherapy
Breast cancer	Phase I/II, immunotherapy
Hepatitis C	Phase II, therapeutic
Melanoma	Phase I/II, immunotherapy
Colorectal cancer	Phase III, immunotherapy
Anthrax	Phase I planned 2010