

BIMM114: VIROLOGY

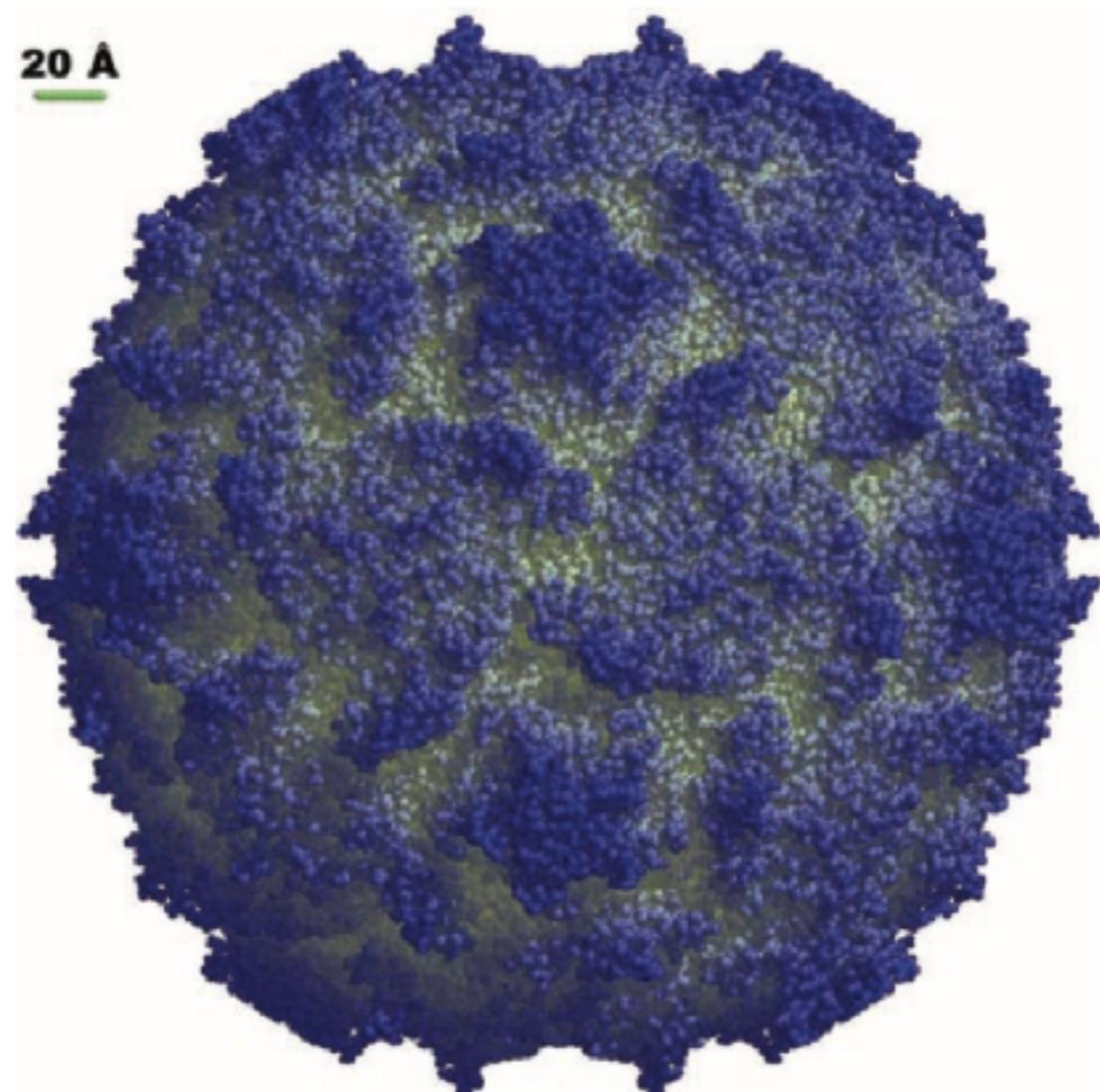


Figure 11.2 Picornavirus virion structure. Model of poliovirus virion determined by x-ray crystallography.

Matt Daugherty
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OUTLINE FOR TODAY'S LECTURE

Key concepts in this lecture:

Picornaviridae:

Diseases caused by members of the *Picornaviridae* family

Poliovirus, poliomyelitis and the polio vaccine

Viral life cycle: non-enveloped, +ssRNA, with some twists

Viral genome: VPg cap and an IRES structure

Polyprotein processing

Replication at membranes: efficiency from localization

Basic virology we've learned from studying these viruses

General concepts

R_0 - the basic reproductive number of a virus

Reverse genetics of viruses

Vaccines

Vaccines are safe and prevent death

Different types of vaccines and adjuvants

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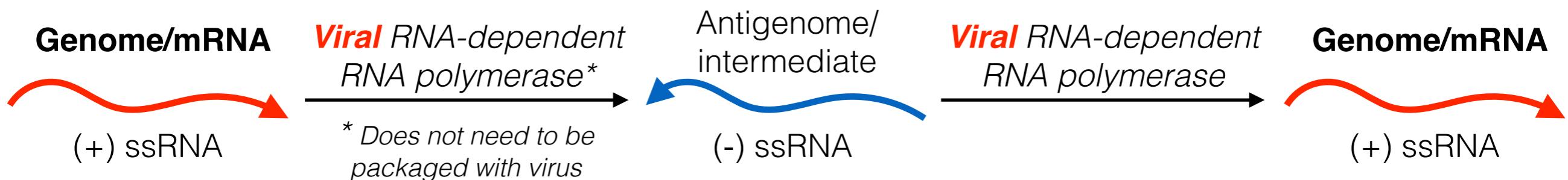
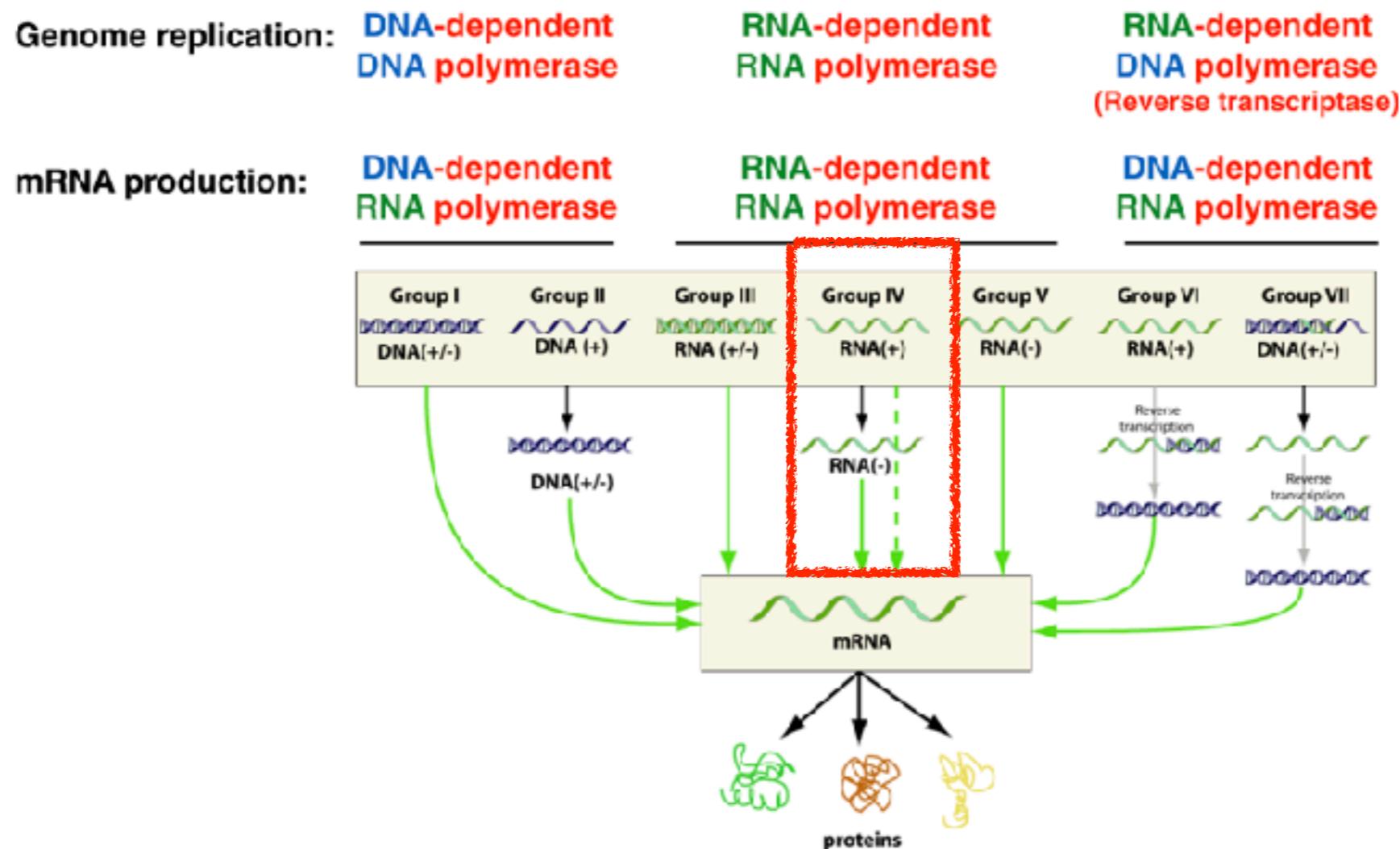
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FAMILY: *PICORNAVIRIDAE*

Pico-RNA-virus: Small RNA virus



FAMILY: *PICORNAVIRIDAE*

Pico-RNA-virus: Small RNA virus

Small non-enveloped viruses

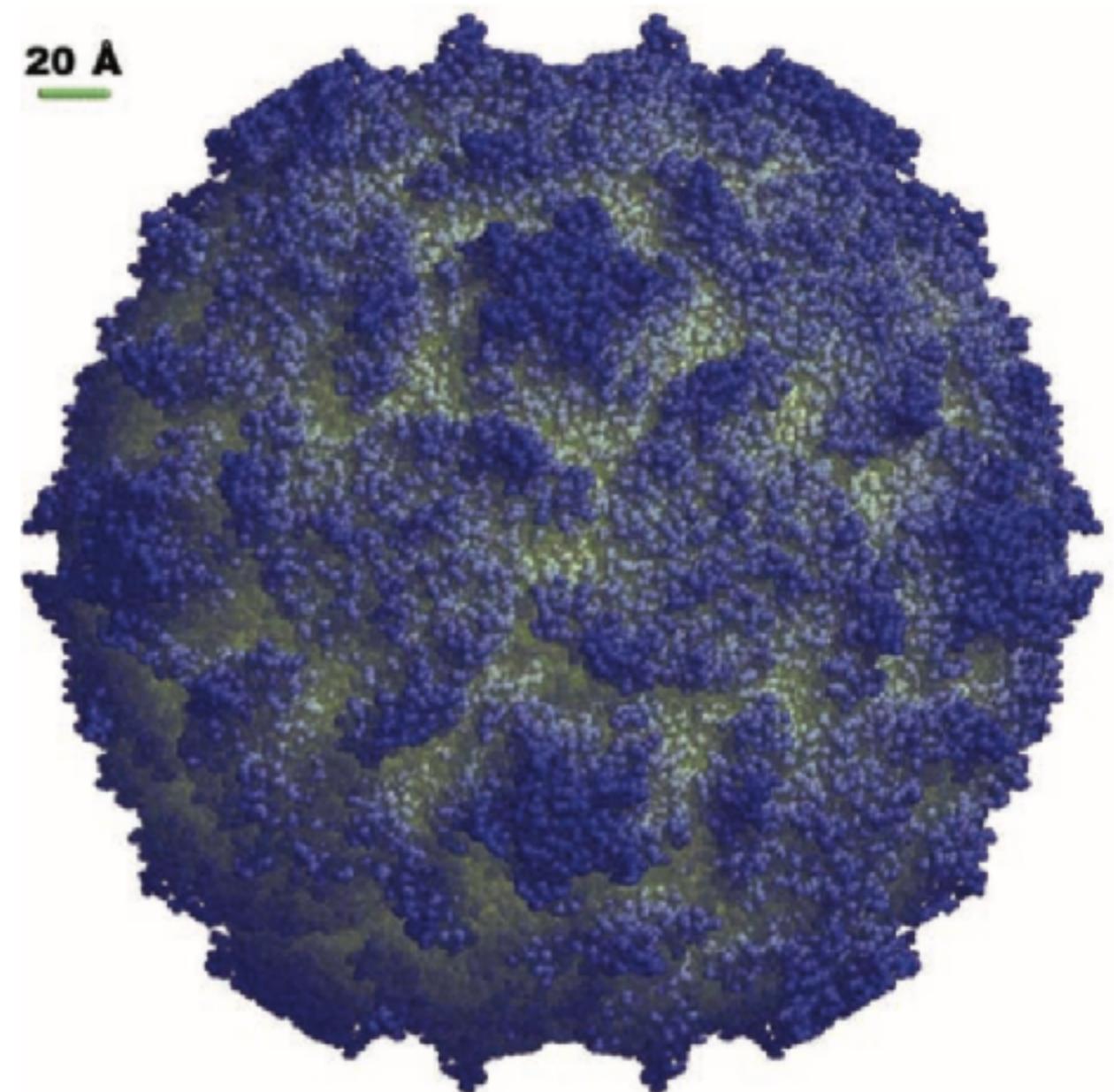


Figure 11.2 Picornavirus virion structure. Model of poliovirus virion determined by x-ray crystallography.

How do viruses get out of cells?

What does this mean for their environmental stability?

PATHOGENS IN THE *PICORNAVIRIDAE* FAMILY

Human pathogens

Poliovirus

During 1940's and 50's, paralyzed or killed >500,000 people per year.

Nearly eradicated by vaccine

Early model system for field of virology (from 1910 on)

Rhinoviruses ('the common cold' most often)

>50 million infections/year in US.

Hepatitis A virus

>100 million infections/year and ~100,000 deaths/year.

Coxsackieviruses

Hand-foot-and-mouth disease and some cardiac inflammatory diseases.

Other enteroviruses (infect gastrointestinal tract)

These are incorrectly called 'the stomach flu'

Non-human pathogens

Foot-and-mouth disease and several others (devastating for livestock)

Related viruses

Norovirus ('the cruise ship virus')

Formally a member of Picornaviridae, but much of the same biology

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POLIOVIRUS AND POLIOMYELITIS

Most infections are asymptomatic

~0.5% of poliovirus infections result in muscle weakening
Can weaken muscles for breathing

Still, most of those people fully recover

However, in some cases, people either die or
are permanently paralyzed/debilitated

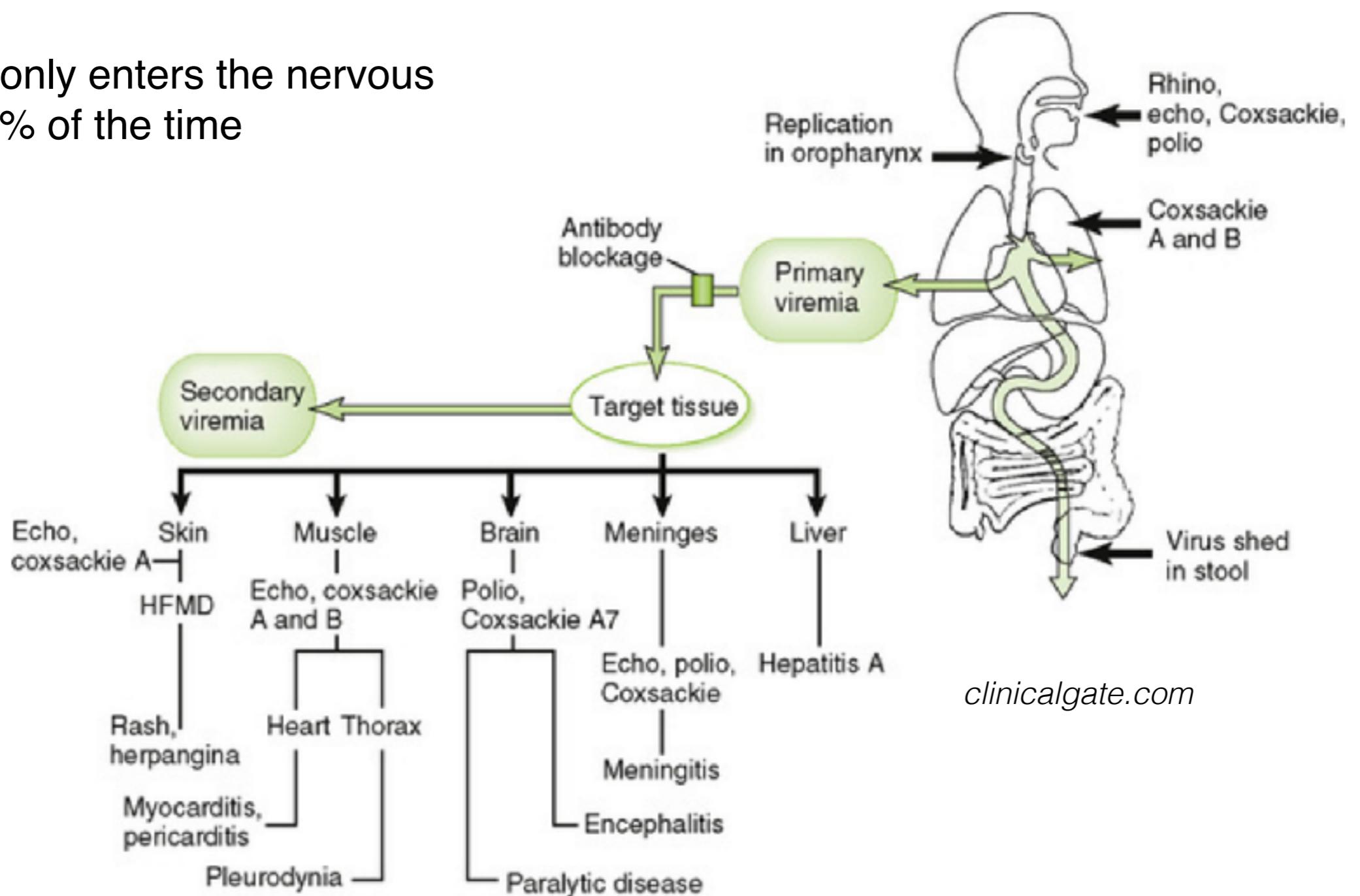
Though this is a very rare outcome, the fact that
many people got infected at some point in their
life made poliomyelitis a terrifying disease

This is one of the reasons we use chlorine in swimming pools



MOST PATHOGENESIS IS CAUSED BY 'OFF TARGET' REPLICATION OF VIRUS

Poliovirus only enters the nervous system ~1% of the time



POLIO VACCINES

Public fear of poliomyelitis was already high

In 1921, Franklin D. Roosevelt contracted poliomyelitis at age 39.
Caused life-long paralysis

Became a strong supporter of vaccine efforts

Two competing (and I really mean competing) vaccines:

1952: Salk vaccine

Jonas Salk

1914–1995



1961: Sabin vaccine

Albert Sabin

1906–1993



POLIO VACCINES

Salk vaccine (*Killed/inactivated*)

Induces antibodies, but not at intestinal route of infection

More costly and difficult to manufacture

Needs to be injected

No chance of reactivation

Sabin vaccine (*live attenuated*)

Natural route of infection, so protection there and can also spread to uninfected people

Less stable environmentally

Given orally

Small chance of reactivation

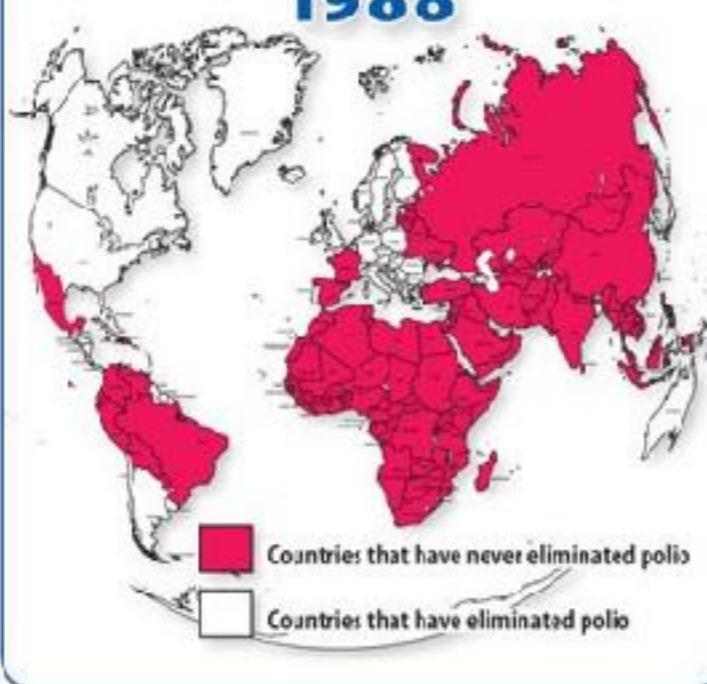
Table 35.6 Attenuated oral polioviruses: reversion to neurovirulence

Virus strain	Number of mutations	Number of amino acid changes	Number of mutations required for return to neurovirulence
Sabin strain 1	57	23	>10
Sabin strain 2	23	5	5–6
Sabin strain 3	~6	3	1–2

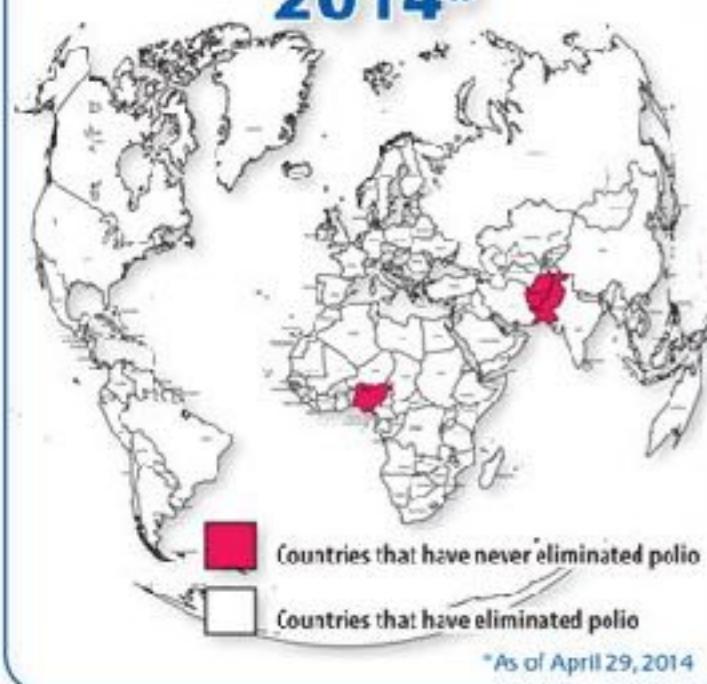
From Minor, P. D. (1992). The molecular biology of poliovaccines. Journal of General Virology 73, 3065–3077.

POLIOVIRUS IS NEARLY ERADICATED

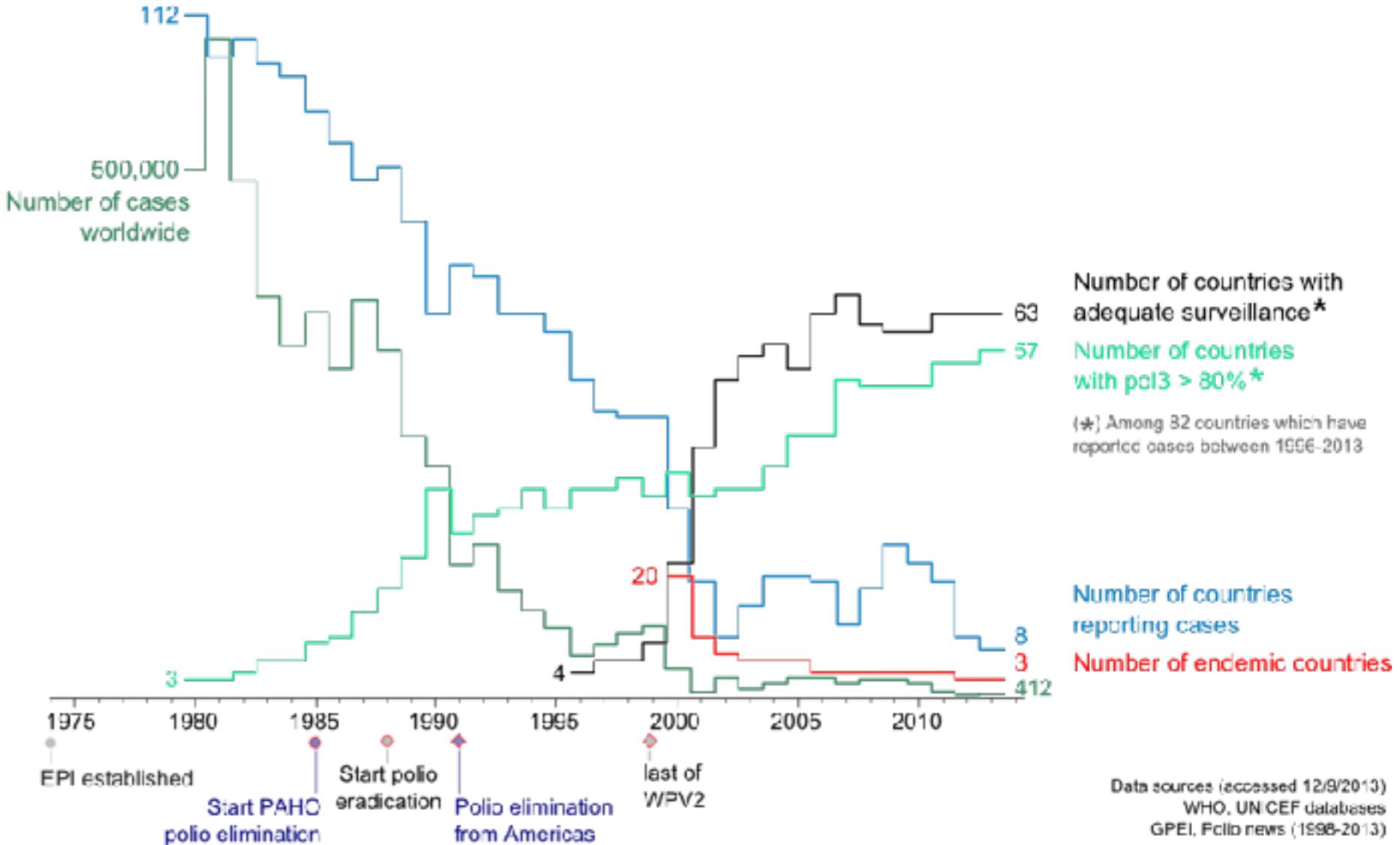
1988



2014*



Number of countries reporting cases



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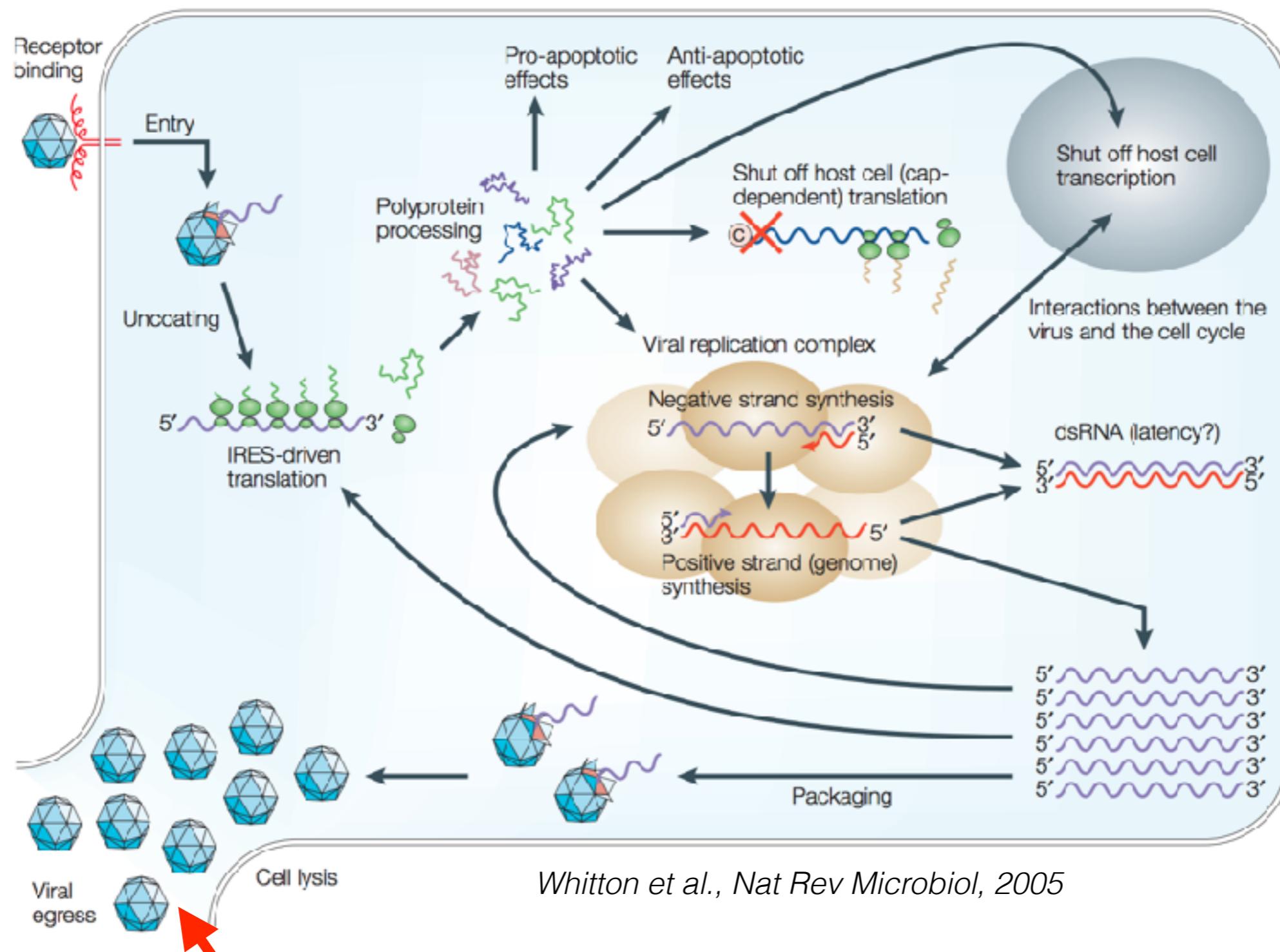
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Vaccines are safe and prevent death

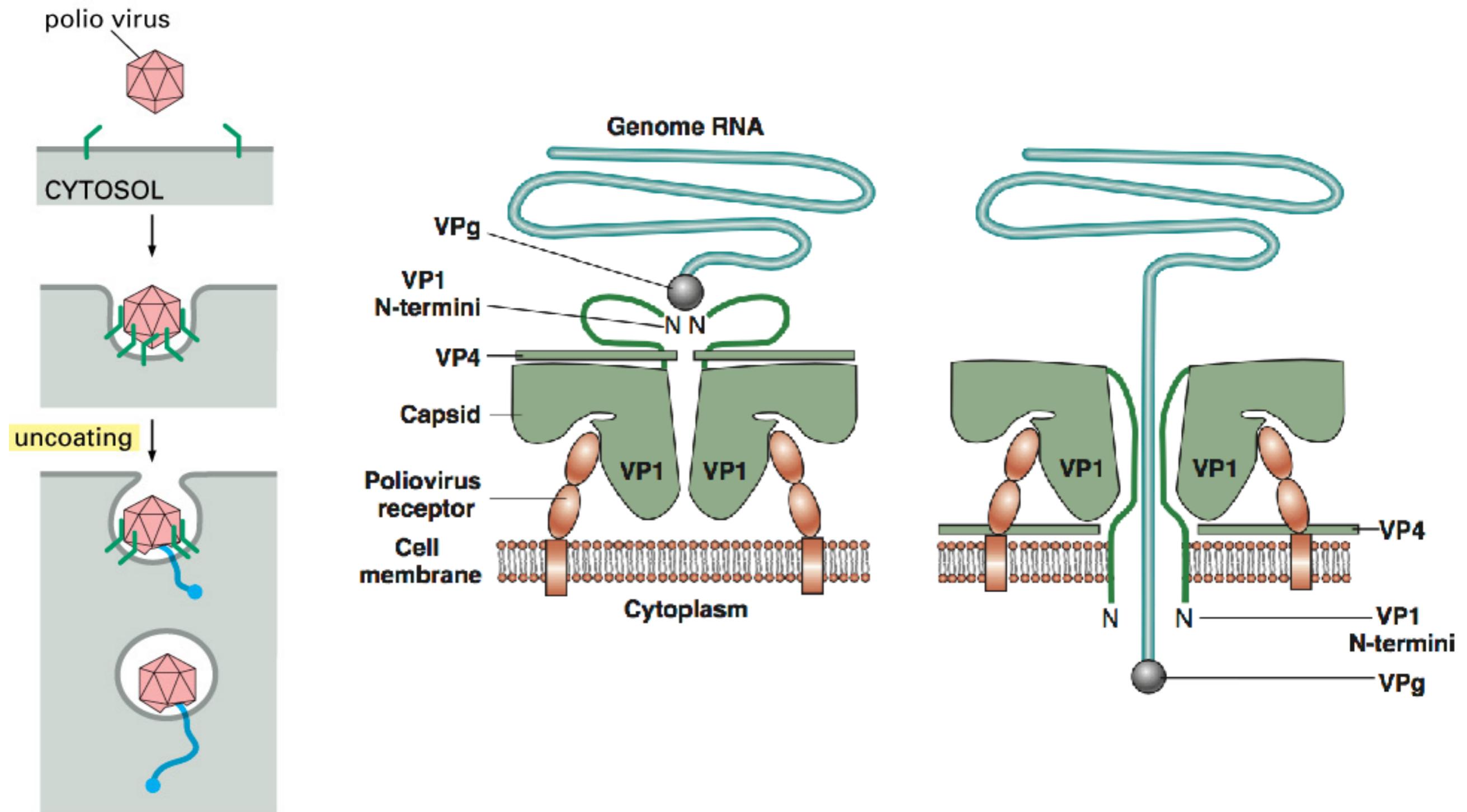
Different types of vaccines and adjuvants

PICORNAVIRUSES ARE NON-ENVELOPED LYtic VIRUSES

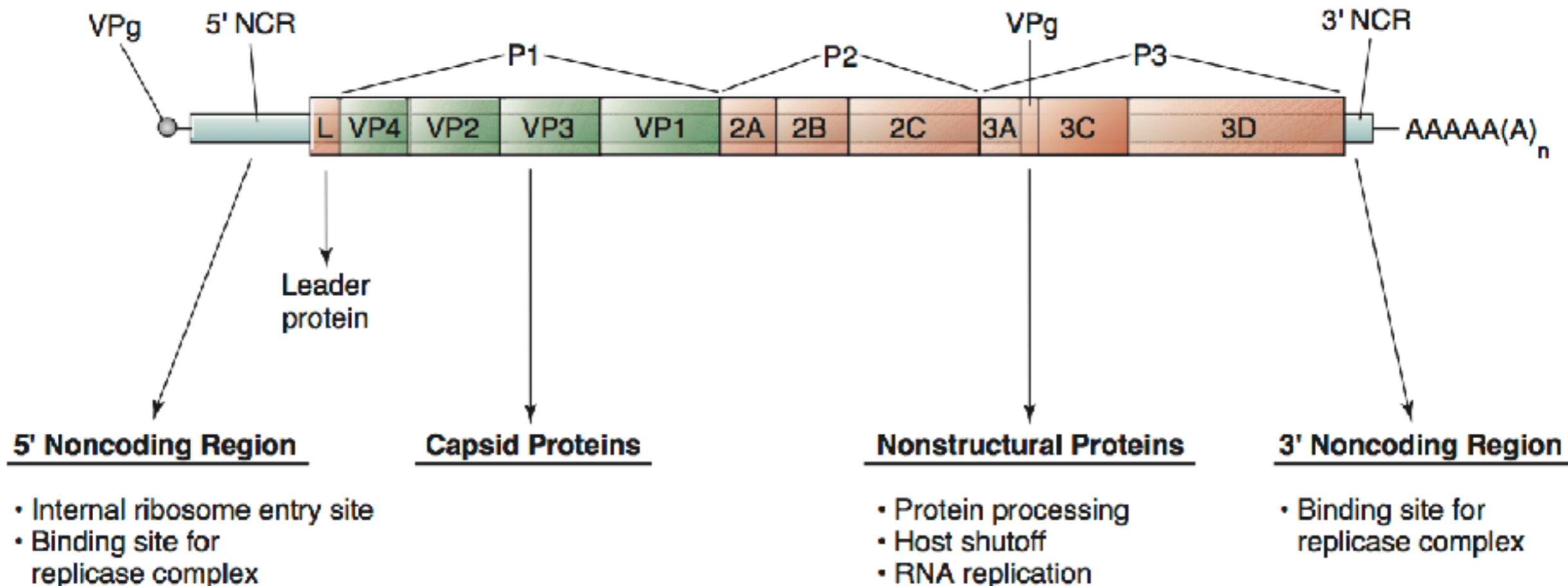


This can be $>10^5$ viruses released per infected cell!

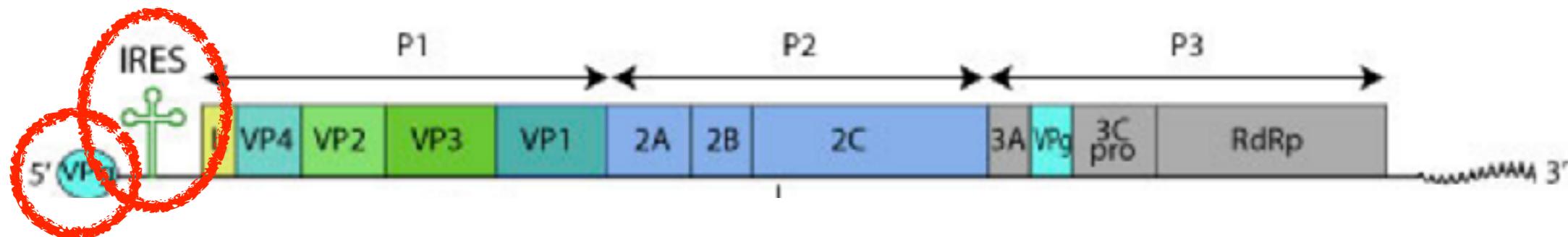
ATTACHMENT AND GENOME DELIVERY



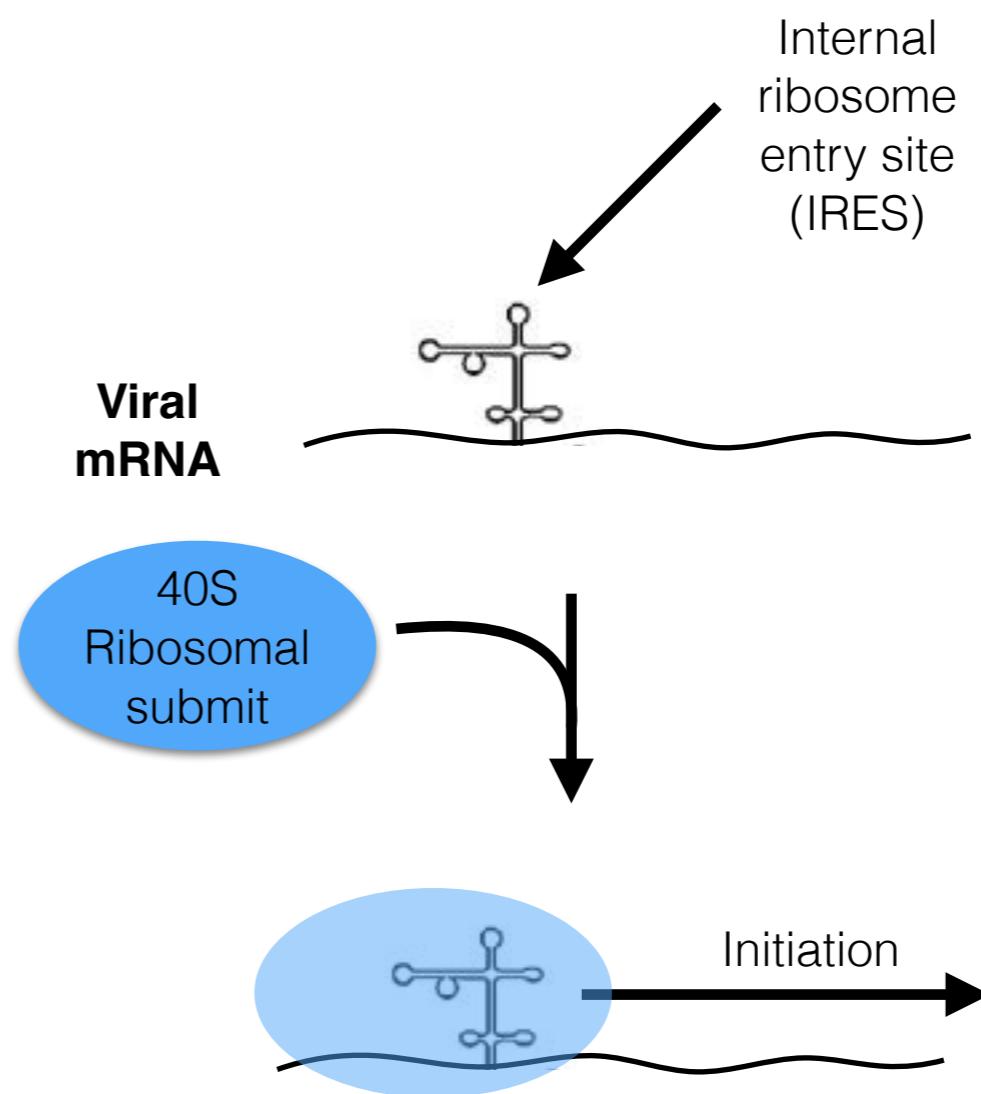
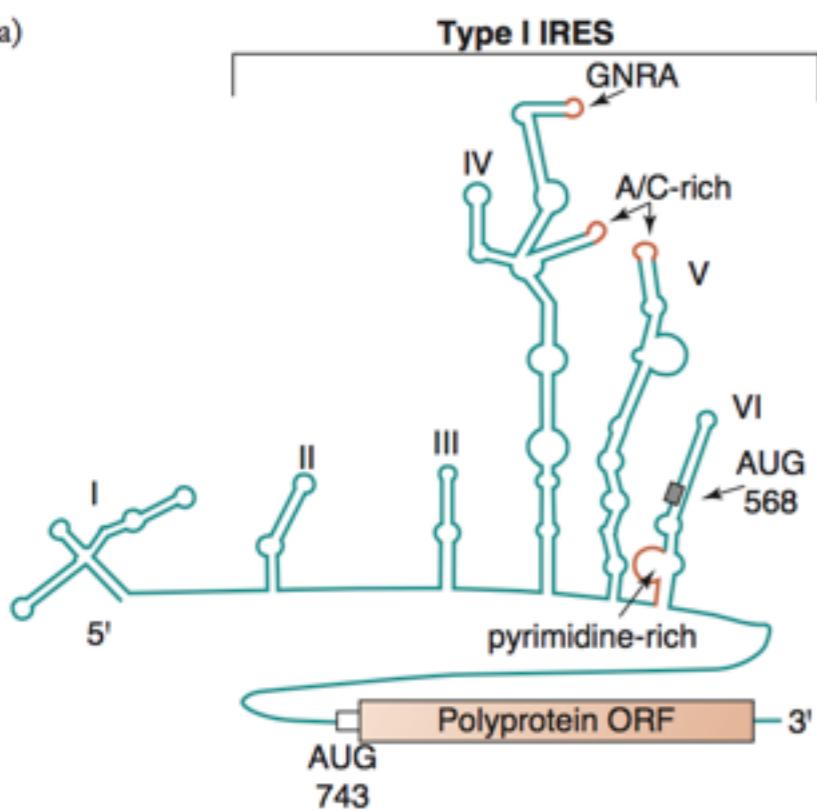
GENOME STRUCTURE: DOING A LOT WITH ONLY A LITTLE GENOME



IRES - INTERNAL RIBOSOME ENTRY SITE

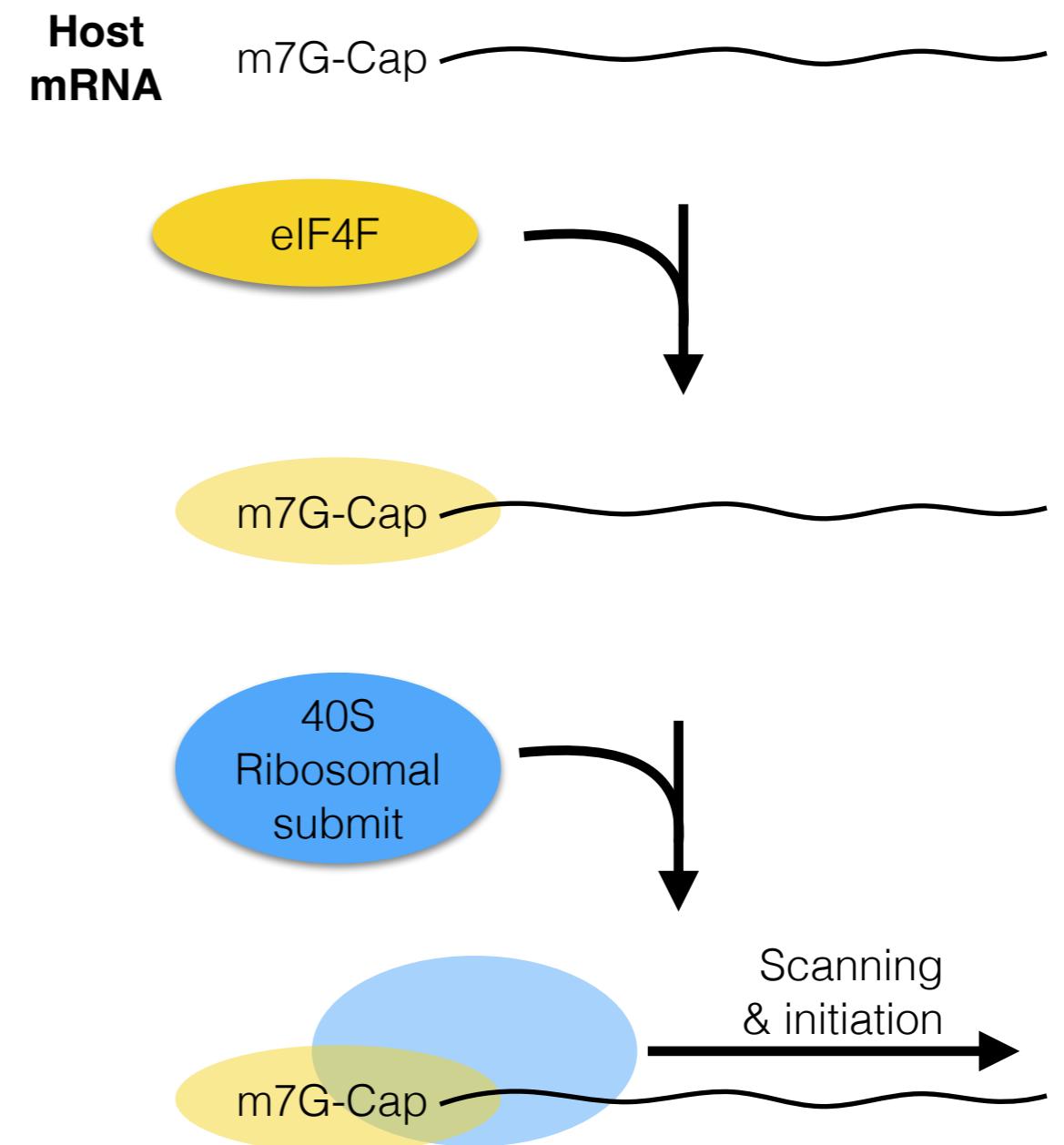
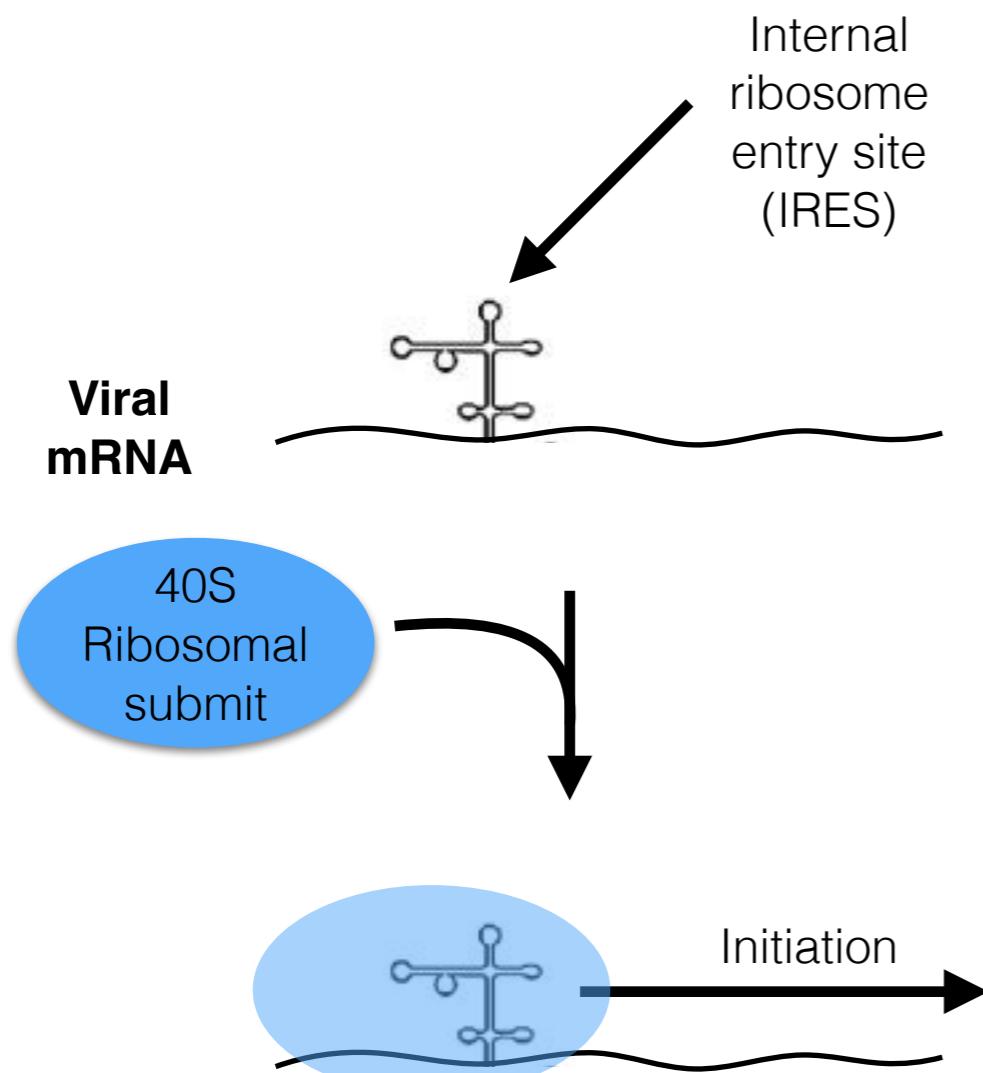


(a)



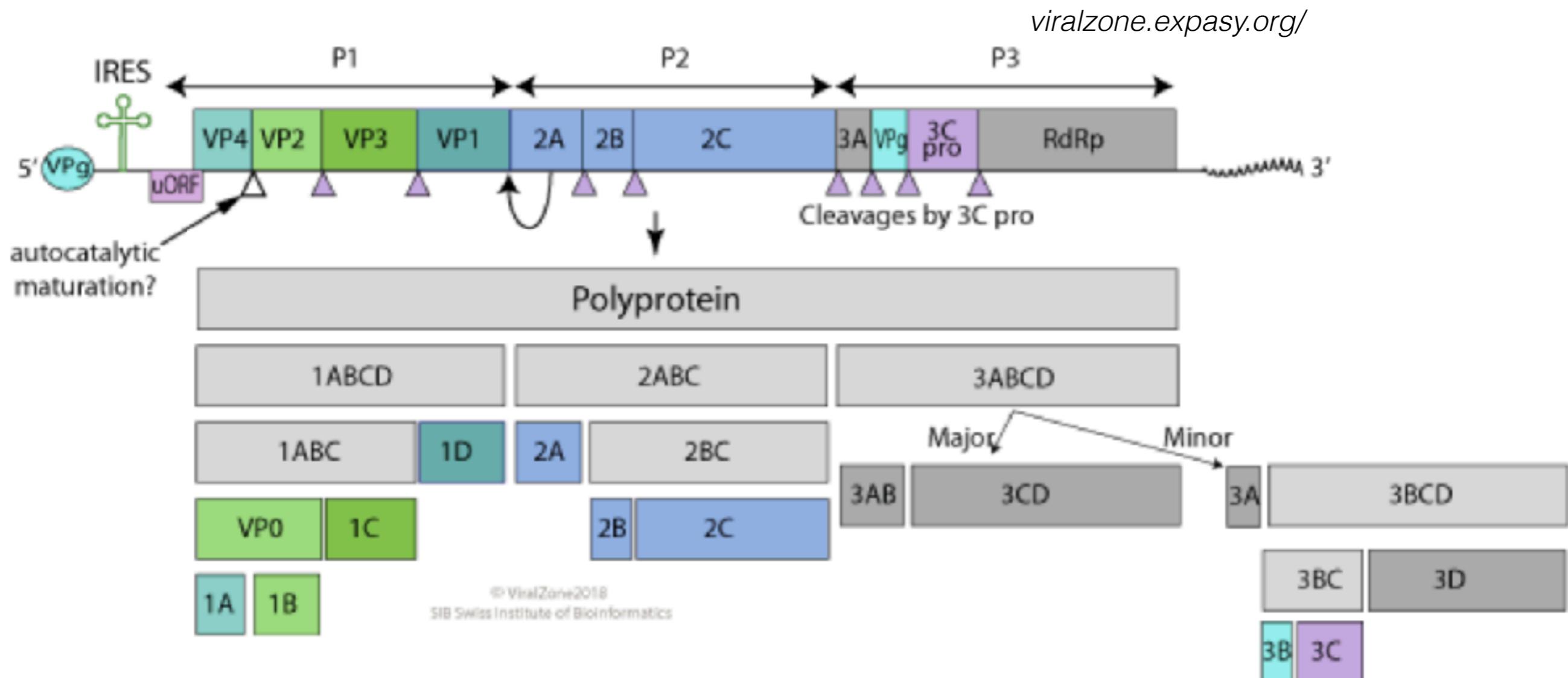
Also means that the 5' end doesn't have to have m7G cap.
Picornaviruses use a protein instead!

STRATEGIES TO DISRUPT HOST PROTEIN SYNTHESIS



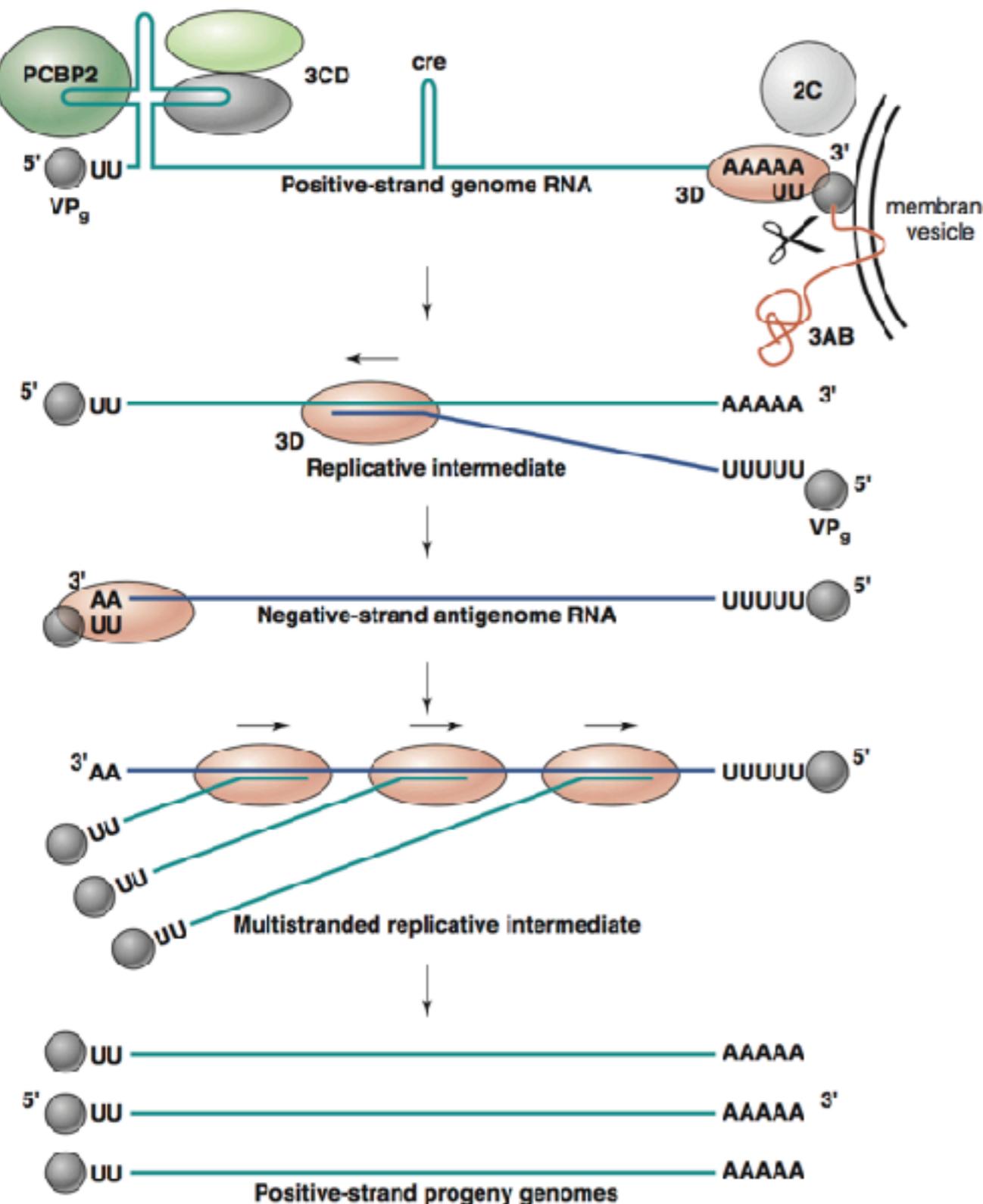
These viruses can cleave or degrade elf4F components to block host synthesis

VIRAL PROTEINS ARE ALL TRANSLATED AS A SINGLE POLYPROTEIN AND THEN CLEAVED



Viral proteases also serve to cleave host factors

REPLICATION HAPPENS AT MEMBRANES



This is a common theme in viruses

Allows the virus to localize proteins, production, etc.

Also may allow the virus to escape immune detection

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COOL STUFF WE'VE LEARNED FROM STUDYING MEMBERS OF THE *PICORNAVIRIDAE*

One of the best studied group of viruses

First animal virus discovered (foot-and-mouth disease virus)

First evidence of RNA-dependent RNA polymerase

Proteins are expressed as a polyprotein and cleaved by viral protease

IRES mediated cap-independent translation

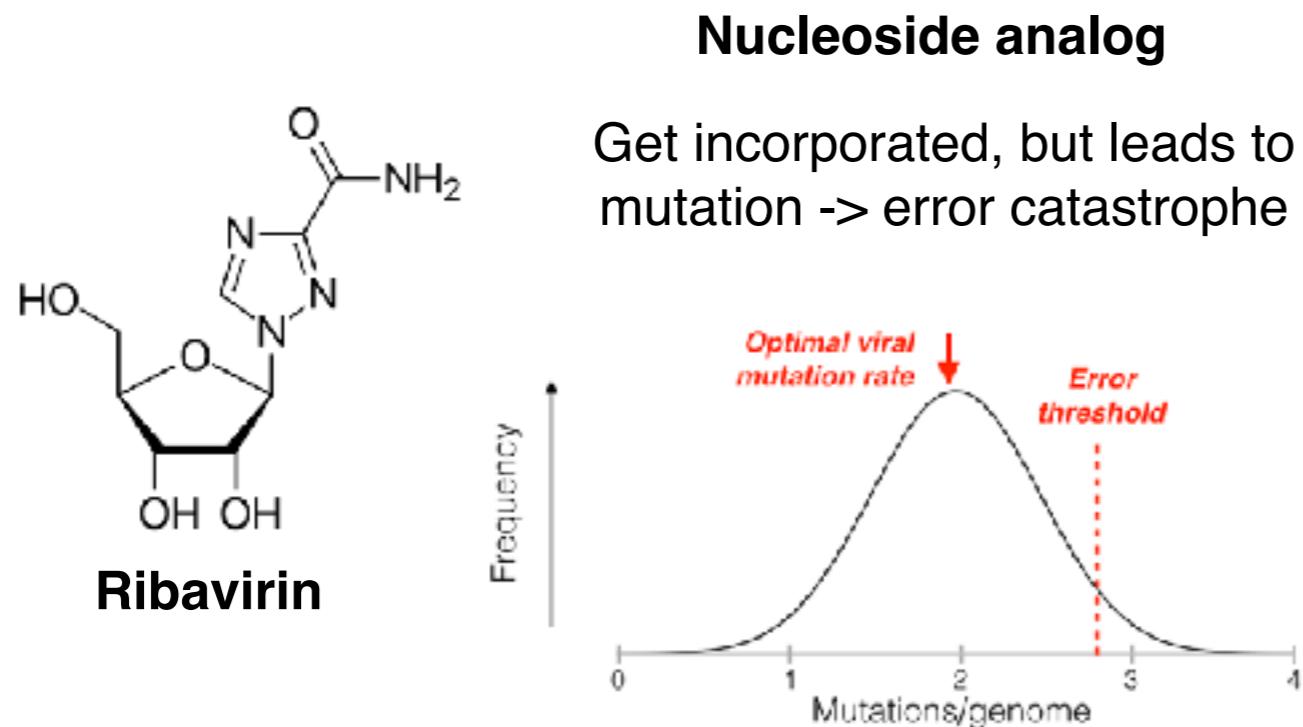
Vaccine research

Evolution of RNA viruses (e.g. error catastrophe, quasispecies, viral recombination)

COOL STUFF WE'VE LEARNED FROM STUDYING MEMBERS OF THE *PICORNAVIRIDAE*

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Mechanism of mutagenic drugs -> error catastrophe



Evolution of RNA viruses (e.g. quasispecies theory, viral recombination)

QUESTIONS?

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R_0 - THE BASIC REPRODUCTIVE NUMBER

One person is infected. How many more people will they infect in a naive group?

Called the “basic reproductive number” or R_0 (pronounced “R naught”)

$$R_0 = \text{Number of contacts in a given time} \times \text{Transmission probability per contact} \times \text{Duration of infection}$$

$R_0 > 1$ Epidemic continues

$R_0 < 1$ Epidemic dies out

R_0 - THE BASIC REPRODUCTIVE NUMBER

Estimates for some epidemic viruses:

Measles: 12-18

Polio: 5-7

Influenza: 1-3

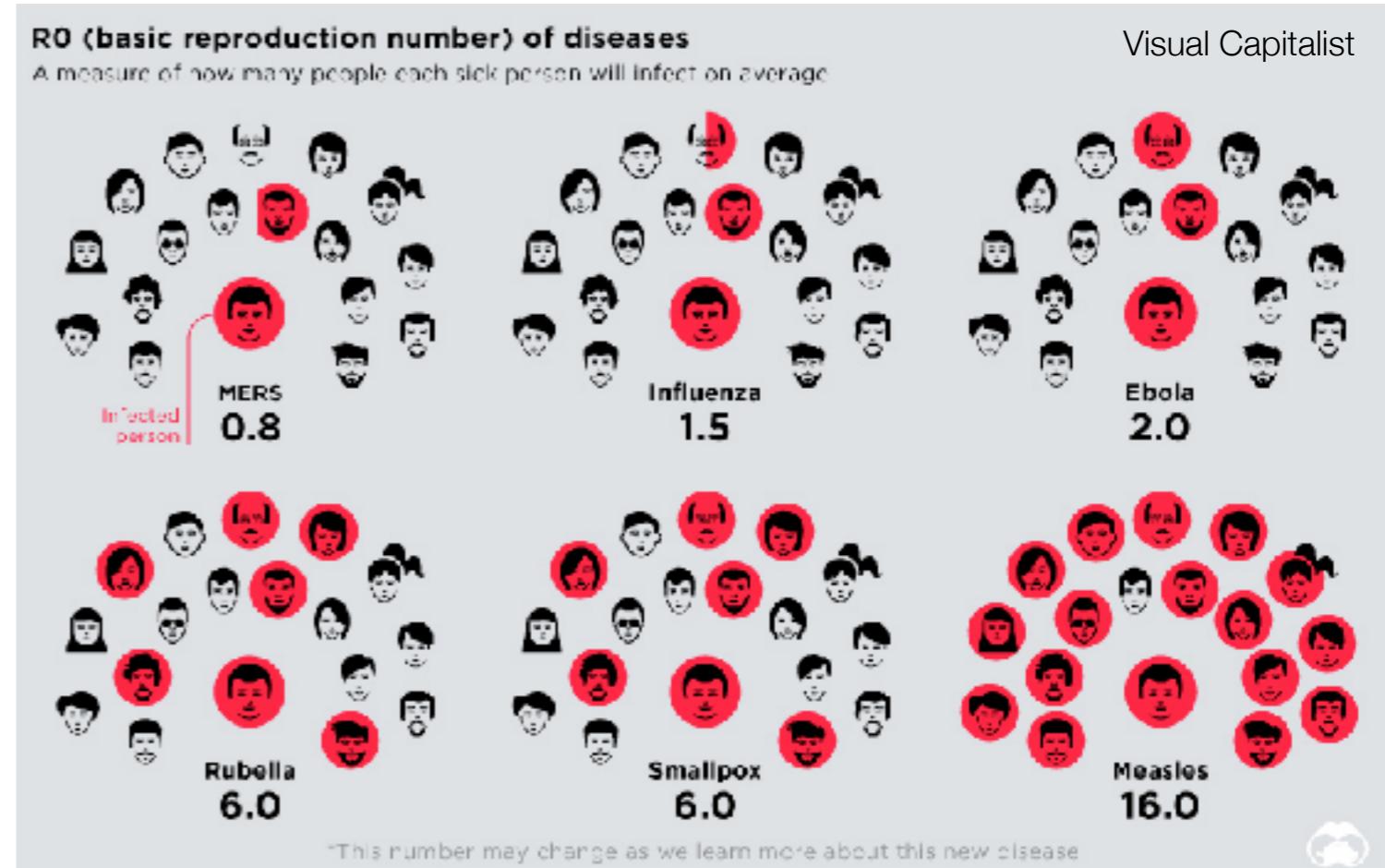
HIV: 2-5

Ebola (2013-2016): 1.5-2.5

MERS: <1

SARS-CoV-2 (original): 2-3

SARS-CoV-2 (variant): >5?



$$R_0 = \text{Number of contacts in a given time} \times \text{Transmission probability per contact} \times \text{Duration of infection}$$

What are two ways to reduce R_0 ?

SOCIAL DISTANCING, MASKING, ETC. REDUCE R_0

$$R_0 = \text{Number of contacts in a given time} \times \text{Transmission probability per contact} \times \text{Duration of infection}$$



Slide credit: Stephen Hedrick

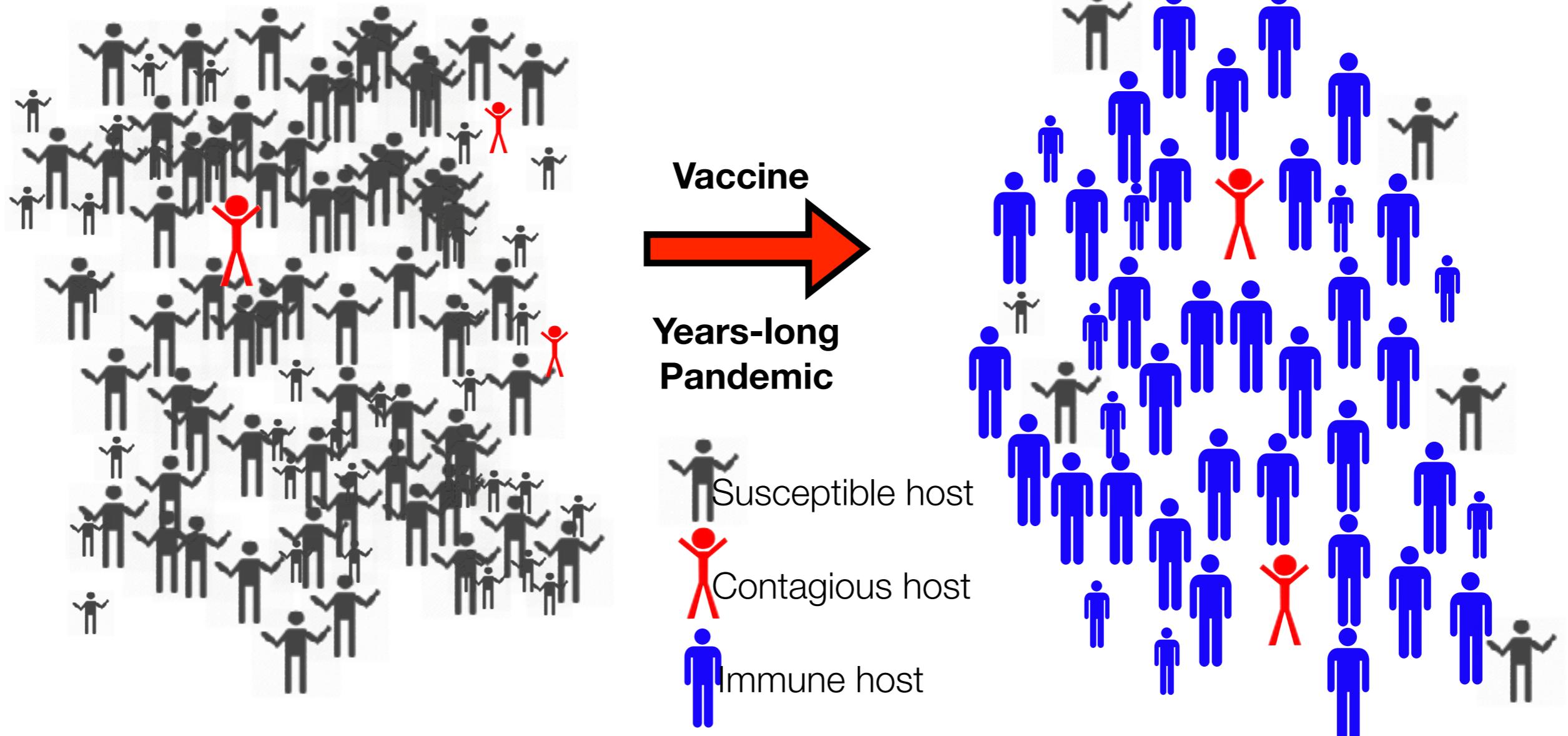
EFFECTIVE R CAN ALSO BE IMPACTED

$$R_0 = \text{Number of contacts in a given time} \times \text{Transmission probability per contact} \times \text{Duration of infection}$$

$$\text{Effective } R = R_0 \times \text{Fraction of susceptible population}$$

What is one way to reduce the effective R?

IMMUNE INDIVIDUALS REDUCE EFFECTIVE R



Slide credit: Stephen Hedrick

WHY DO VACCINES WORK?

This is the basis for herd immunity

$$\text{Effective } R = R_0 \times \text{Fraction of susceptible population}$$

Tells us how much of the population needs to be vaccinated

Let's assume measles virus has an R_0 of 10, what % of the population needs to be immune to prevent an outbreak?

Estimates for some epidemic viruses (making assumptions to make the math easier):

Measles: 10

Need >90% effective vaccination rate

Polio: 5

Need >80% effective vaccination rate

Influenza: 2

Need >50% effective vaccination rate

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REVERSE GENETICS IN RNA VIRUSES

We often want to test hypotheses about viruses by introducing mutations

Historically, this was done by randomly mutating the virus, looking for a phenotype and sequencing that virus

This is called “forward genetics”

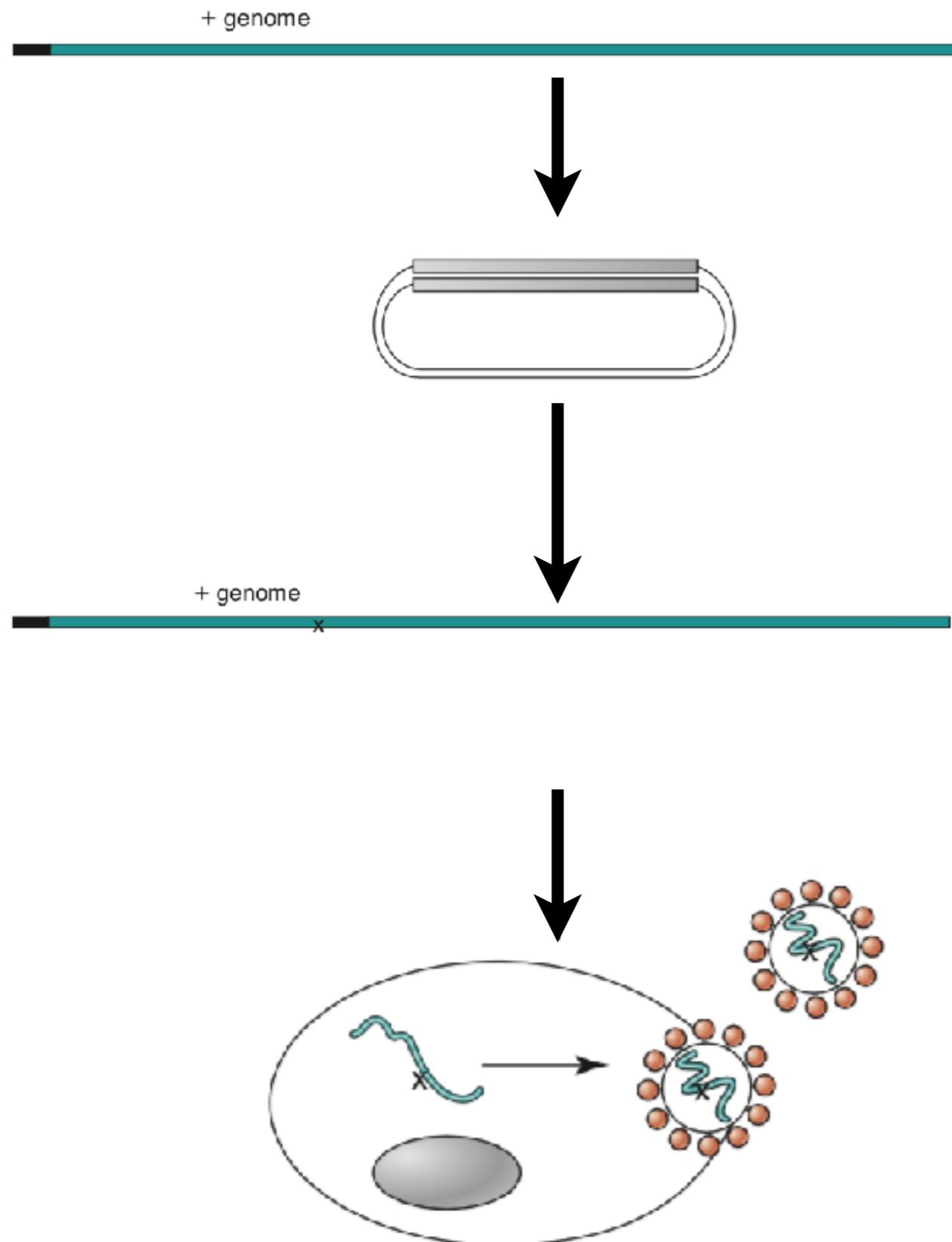
For many viruses, we can now go in and make individual mutations and test whether it produces a given phenotype

This is called “reverse genetics”

The ease of doing reverse genetics depends on the life cycle of the virus

Which Baltimore group of RNA viruses do you think will be easiest to do reverse genetics on?

REVERSE GENETICS IN RNA VIRUSES



Reverse transcribe viral genome and insert into vector

Can make mutations at this step via standard molecular biology techniques

Transcribe the RNA using a DNA-dependent RNA polymerase

Often use a phage polymerase (e.g. T7) because they are small, robust and have little regulatory requirements

Get mRNA into cells and recover virus

Either transfet or electroporate to get mRNA inside mammalian cell

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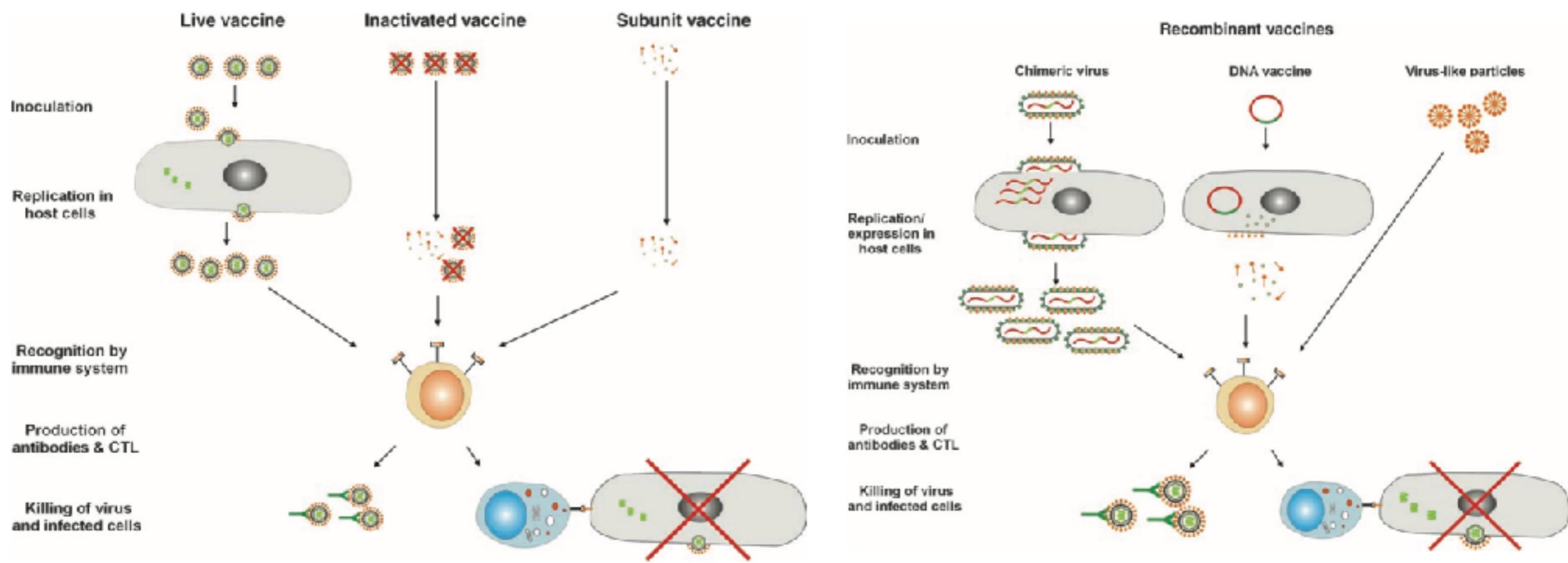
VACCINES HAVE BEEN EFFECTIVE AGAINST EMERGING AND ESTABLISHED VIRUSES

Pre-1700s	Chinese doctors use powdered smallpox scabs to “immunize” intranasally. Mediterranean-area doctors use directed leishmania-infected sandfly bites to induce long-term protection from reinfection.
1721	Lady Montagu brings concept of variolation (inoculation with pus from recovering smallpox victim) from Turkey to England.
1798	Jenner publishes <i>Variolae Vaccinæ</i> , the use of cowpox inoculation to protect against smallpox.
1885	Pasteur and collaborators introduce air-dried rabbit spinal cord as rabies vaccine.
1900	Walter Reed demonstrates that yellow fever is caused by a filterable virus.
1930–45	Introduction of vaccines for Japanese B encephalitis (1930), yellow fever (1935), and influenza (1936).
1946–75	Introduction of vaccines for polioviruses types 1–3 (Sabin attenuated strains and Salk inactivated virus); measles, mumps, and rubella viruses; tick-borne encephalitis virus; mouse brain, duck embryo, and tissue culture vaccines for rabies virus; inactivated influenza A and B viruses; and adenoviruses.
1975–present	Introduction of vaccines for hepatitis B virus, hepatitis A virus, varicella zoster virus (chickenpox), live, cold-adapted influenza virus, rotavirus, and human papillomavirus.

Also, antisera from infected people can be highly protective (Lassa, Ebola, etc)

Disease	Dates	Annual reported cases:		
		Pre-vaccination	Post-vaccination (1998)	Decrease (%)
Smallpox	1900–04	48,164	0	100
Poliomyelitis (paralysis)	1951–54	1,314	1*	100
Measles	1958–62	503,282	89	100
Mumps	1968	152,209	606	99.6
Rubella	1966–68	47,745	345	99.3
Congenital rubella syndrome	1958–62	823	5	99.4

HOW DO WE DEVELOP NEW VACCINES?



Live wild-type viruses	Vaccinia (cowpox) ^a	Whole inactivated viruses	Virus-like particles	Human papillomavirus
Live attenuated viruses	Adenovirus ^b Influenza A (cold adapted) Measles Mumps Polio (Sabin) Rotavirus (human-recombinant) Rubella (german measles) Varicella (chickenpox) Yellow fever	I Hepatitis A Influenza A Influenza B Polio (Salk) Rabies Tick-borne encephalitis	Chimeric virus	Rotavirus (human-bovine)

PROS AND CONS OF DIFFERENT TYPES

 Types	 Advantages	 Disadvantages	 Examples
Inactivated vaccines	Simple, quick to develop; high safety; low cost	Weaker immunity protection; short immunity period; antibody-dependent enhancement	Hepatitis A; Flu; Polio; Rabies
Recombinant protein vaccines	Safety; high efficiency; scale production	Protein antigen influenced by expression system selected	Hepatitis B
Attenuated influenza virus vaccines (Live-attenuated vaccines)	Prevent flu and COVID-19; low dose; easy application	Long development process	Measles, mumps, rubella (MMR combined vaccine); Rotavirus; Chickenpox; Yellow fever
Adenoviral vector vaccines	Safety; fewer adverse reactions	Pre-existing immunity	Ebola
Nucleic acid vaccines (RNA, DNA)	Simple production process; high security	No empirical basis	In clinical trials

Source: Xinhua News Agency, U.S. Department of Health & Human Services

CGTN

VACCINES ARE AN ACTIVE AREA OF RESEARCH

Live virus infection provides the best protection

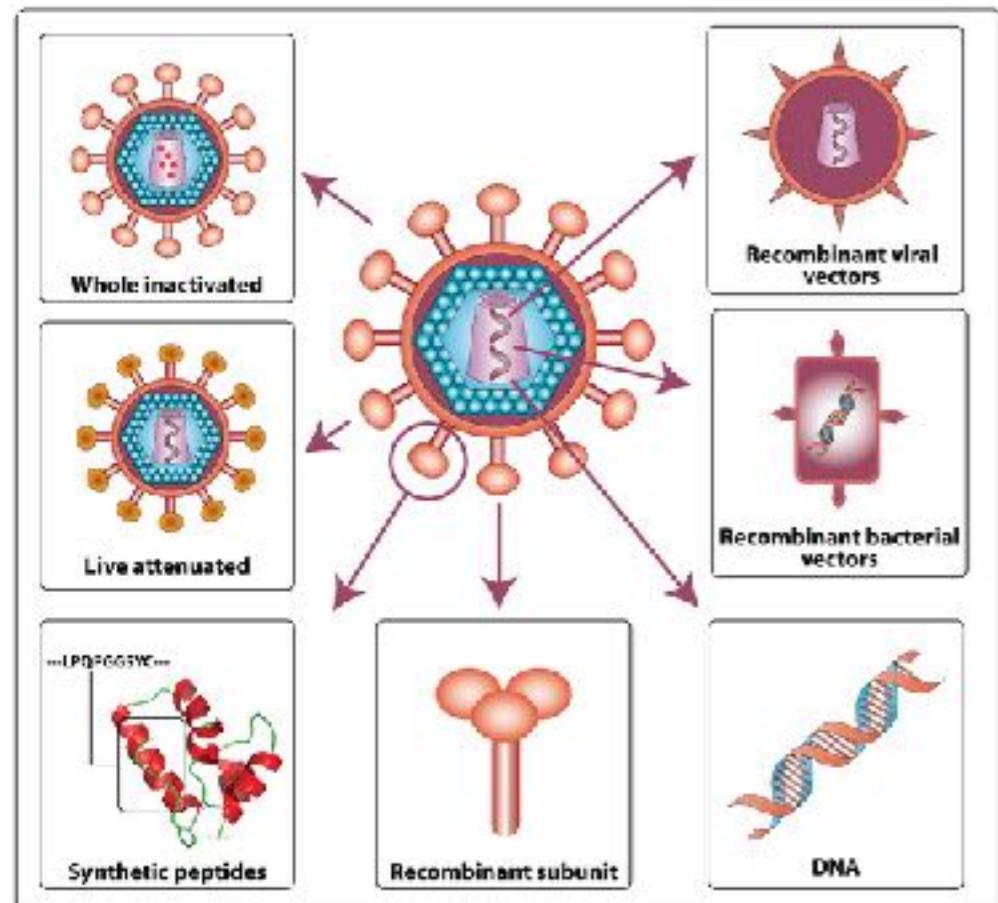
How to promote the same (cellular innate and adaptive) response with safer antigens?

Some viruses are resistant to common vaccine strategies

How to induce immunity to “hard to see” antigens?

A. Adjuvant systems	
Oil-in-water emulsions	Elicit strong humoral response without major toxicity.
Cholera toxin B subunit	Elicits strong humoral response without toxicity.
Toll-like receptor ligands	Elicit strong humoral and cellular immune responses.
• CpG motifs	Short, unmethylated DNA motifs that bind to TLR-9 and elicit potent, innate and cellular (Th-1-like) responses.
• Imiquimod/resiquimod	TLR-3 ligand that elicits strong humoral and cellular responses
• Detoxified lipopolysaccharide	TLR-4 ligand that mimics bacterial infection and stimulates strong humoral and cellular responses
Pulsed dendritic cells	Presentation of antigens to dendritic cells outside of the body and their reintroduction can lead to enhanced response to antigens.
Conjugates	A variety of molecules such as cytokines, monoclonal antibodies, and lectins can be conjugated to antigens to enhance immune reactions.
B. Delivery systems	
Liposomes	Antigens are trapped inside vesicles made from synthetic lipids, enhancing immune reactions. Stability and consistency have been major problems.
Immune stimulating complexes (ISCOM)	More complex liposomal preparations that retain the capacity to elicit strong immune responses, but are more stable.
Proteosomes	Auto-assembling vesicles containing bacterial outer membrane proteins from Neisseria that can trap antigens and promote strong humoral and cellular responses.
Plasmid DNA	An expression plasmid containing the gene that encodes a viral antigen is introduced into the body. Expression of this gene <i>in vivo</i> can lead to potent induction of both humoral and cellular responses.
Viral or bacterial vectors	Genes that encode viral antigens of interest can be inserted into non-pathogenic viral vectors (avian poxviruses, Semliki Forest virus) or bacterial vectors (attenuated <i>Salmonella typhi</i>) that retain some useful characteristics of the vector organisms (binding and entry, tissue tropism).
Virus-like particles	Viral proteins, alone or in combination, that can either spontaneously self assemble or be coaxed into assembling with lipids to form nanoparticles resembling viruses.

COVID-19 VACCINES



Gorry et al. - (2007). Retrovirology 4: 66

Whole inactivated: virus grown in eggs or in cell culture, then treated with chemicals, heat, or irradiation to “kill” it

Sinovac, others

Live attenuated: virus is passaged through experimental animals and cultured human cells, the viruses that grow the fastest eventually lose the properties that allow them to cause human disease

None?

Synthetic peptide or recombinant subunit: predicted proteins, or pieces of proteins are made in the lab and coupled to a benign protein that can provoke an immune response

Novavax, others

Recombinant viral vectors: a gene from the disease-causing virus is inserted into a virus that does not cause human disease

AstraZeneca, J&J

DNA or RNA: A gene from the virus is encapsulated into a lipid particle, and injected into a patient. The gene is taken up by cells, released, and translated into a viral protein

Moderna, Pfizer, others

Slide credit: Stephen Hedrick

CLASS 8 (THURSDAY 2/1)

Class 8 (Thursday 2/1)

First 30 minutes: going over R₀ calculations and any other questions

*Rest of class, we will discuss Vignuzzi et al. **Please read it before class.***

Some questions to focus on for the paper discussion

What is the main question the paper is trying to address?

What was known before this paper?

What is the hypothesis of the study?

What are the primary experimental approaches?

What are the main conclusions?

Are the conclusions supported by the data?

Are there any caveats about the paper?

What are the next steps?

Why am I having you read this paper?

Why is this paper important for the field of virology?

NEXT WEEK

Tuesday and Thursday will be guest lectures from Dr. Dustin Glasner, expert in Flaviviruses and viral pathogenesis

Tuesday: Flaviviridae and Togaviridae

Thursday: Viral pathogenesis

For Tuesday read the assigned reading in textbook:

Chapter 12 & 13

For Thursday, there are no pre-class readings