Viral Vectors In The Research Laboratory: Just How Safe Are They?

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Learning Objectives

- Recognize hazards associated with viral vectors in research and animal testing laboratories.
- Interpret viral vector modifications pertinent to risk assessment.
- Understand the difference between gene delivery vectors and viral research vectors.

Outline

- Introduction to Viral Vectors
- Lentiviral Vectors (+RNA virus)
- Adenovirus Vectors (DNA virus)
- Novel (-)RNA virus vectors
- Conclusions

Increased Use of Viral Vectors in Research

- Difficulties in DNA delivery to mammalian cells
  - <50% with traditional transfection methods
  - Up to ~90% with viral vectors
- Increased knowledge about viral systems
- Commercialization has made viral vectors more accessible
- Many new genes identified and cloned (transgenes)
- Gene therapy

What is a Viral Vector?

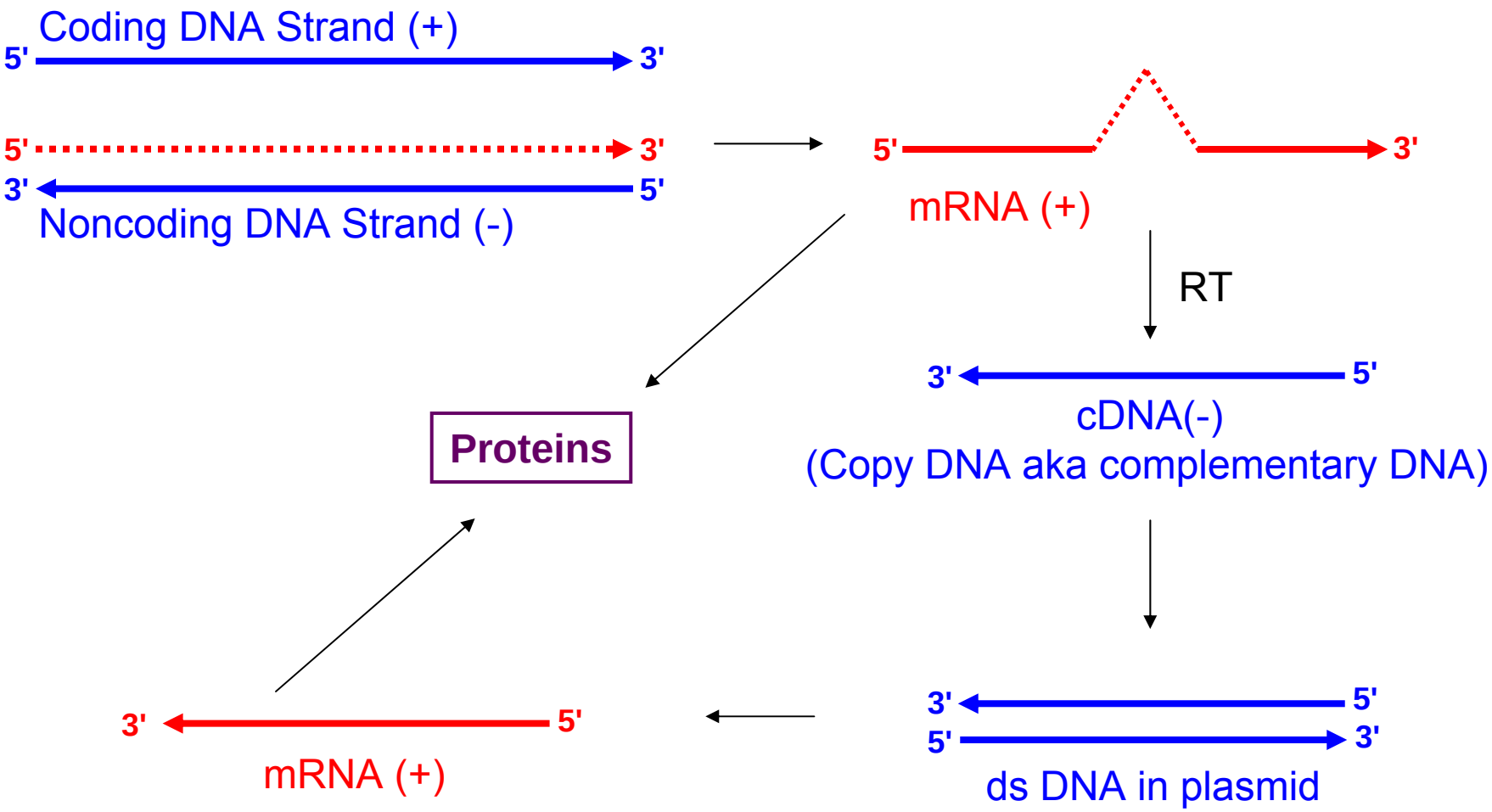
- Viral Vector: A viral genome with deletions in some or all essential genes and possibly insertion of a transgene

- Plasmid: Small (~2-20 kbp) circular DNA molecules that replicates in bacterial cells independently of the host cell chromosome

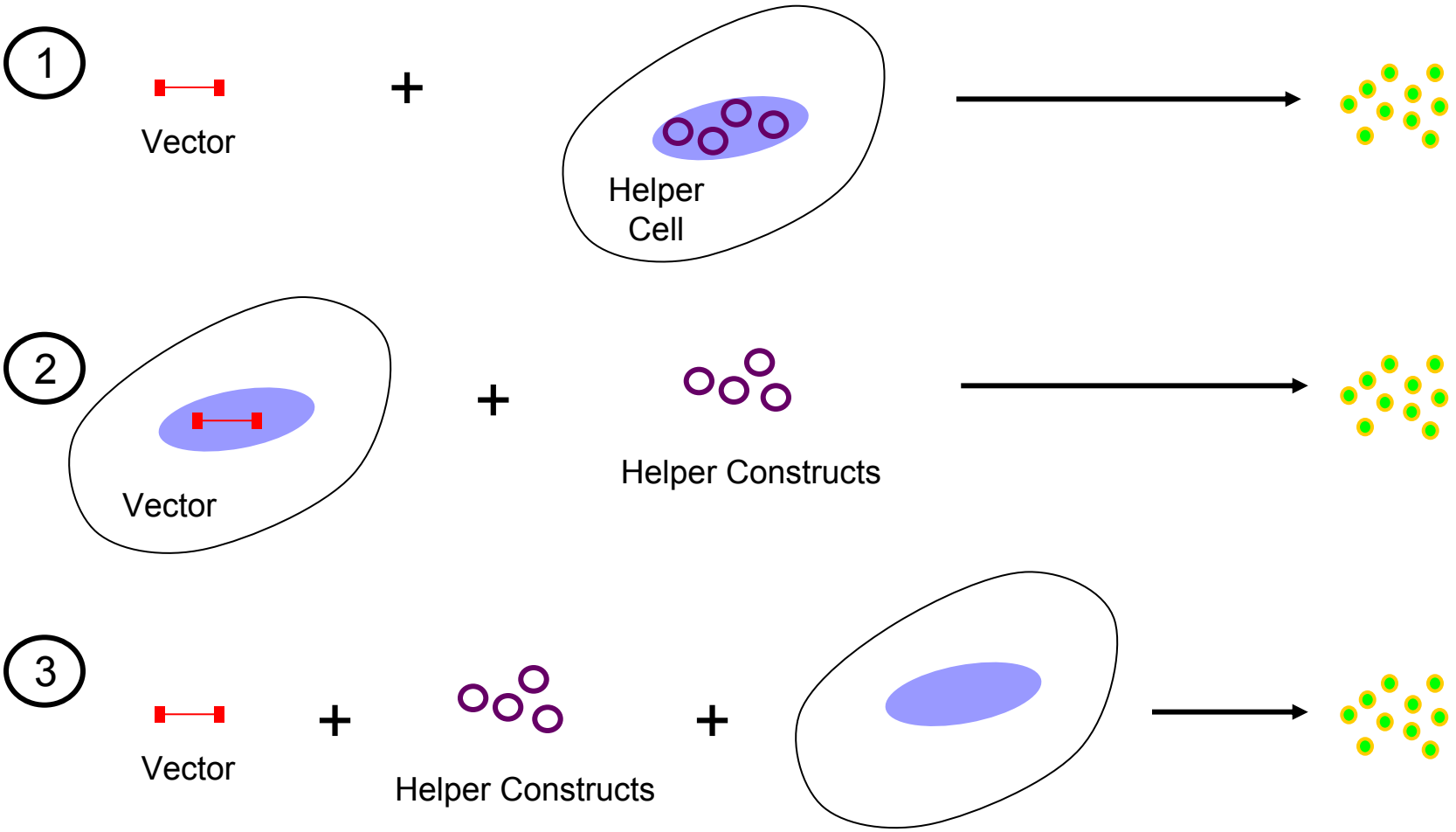
Molecular Biology Essentials

- Flow of genetic information
- Nucleic acid polarity
- Understanding cDNA
- *cis* and *trans*-acting sequences

Genetic flow & nucleic acid polarity



Viral Vector Design and Production



Note: These viruses are replication-defective but still *infectious*.

The Marketing of Lentiviral Vectors

ViraPower™ Lentiviral Expression Systems (Invitrogen)

Features of Lentiviral Vectors

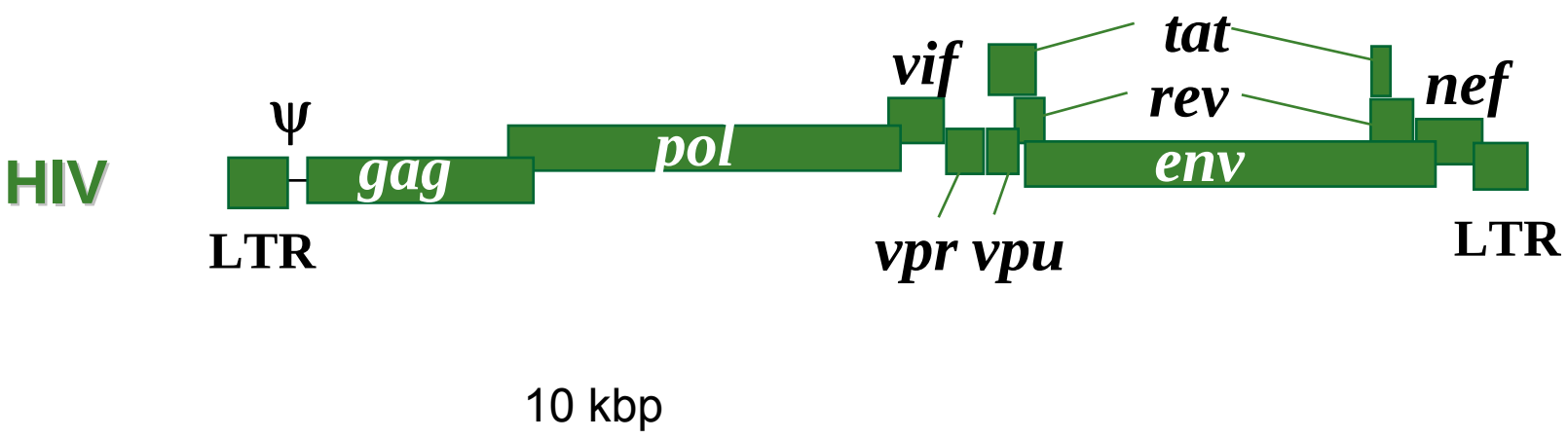
- Naturally integrate into chromosome
  - Long term persistence
- Infect resting as well as dividing cells
- Do not harm target cells as they enter
- Up to ~8 kb of foreign gene sequences can be packaged
- Relatively convenient packaging systems
- Can be manufactured in large quantities

Lentivirus is a genus, *not* a species

- Family: *Retroviridae*
  - Genus: *Lentivirus*
    - *Human immunodeficiency virus 1* (human)
    - *Human immunodeficiency virus 2* (human)
    - *Simian immunodeficiency virus* (monkey)
    - *Bovine immunodeficiency virus* (cow)
    - *Feline immunodeficiency virus* (cat)
    - *Caprine arthritis encephalitis virus* (goat)
    - *Visna/maedi virus* (sheep)

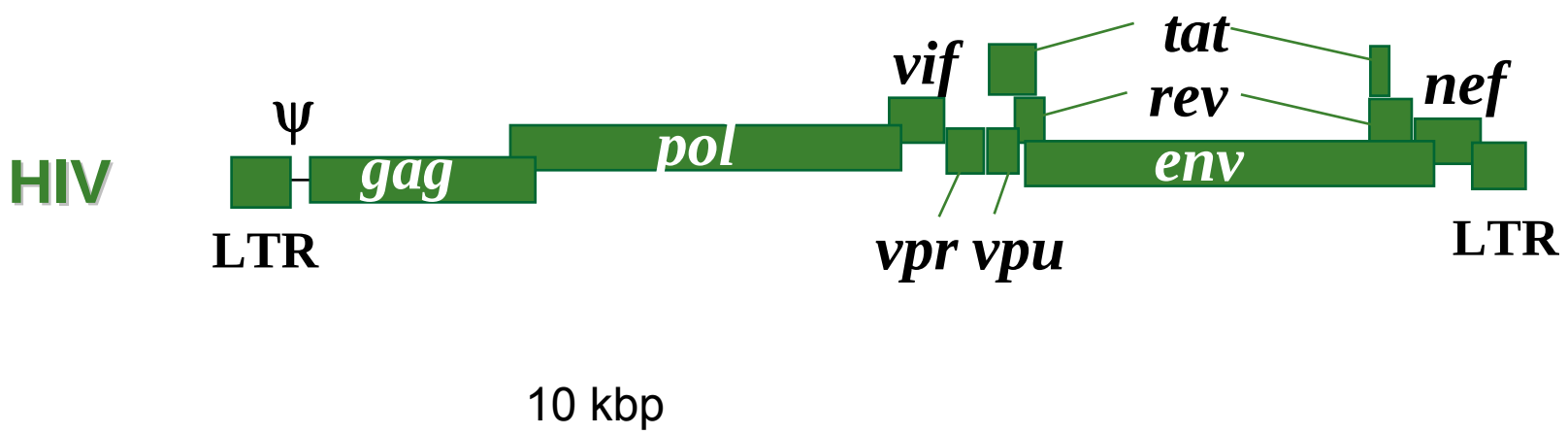
Generations of HIV vectors

- First Generation Vectors
  - Ψ minus helper constructs and split genome (*gag/pol* & *env*); three plasmids with pseudotyping
- Second Generation Vectors
  - Deletion of HIV accessory genes *vpr*, *vif*, *vpu* and *nef*



Generations of HIV vectors

- Third Generation Vectors
  - Four plasmids, *tat* eliminated, *rev* supplied *in trans*
- Fourth Generation Vectors
  - Self Inactivating Vectors (SIN); 3 of 9 HIV genes left



HIV vector hazards

- Integration
- Transgene
- High mutation rate
- High transduction efficiencies
- Amphotropic envelope (broad tropism)
- Recombination
- Seroconversion

The marketing of Adenovirus vectors

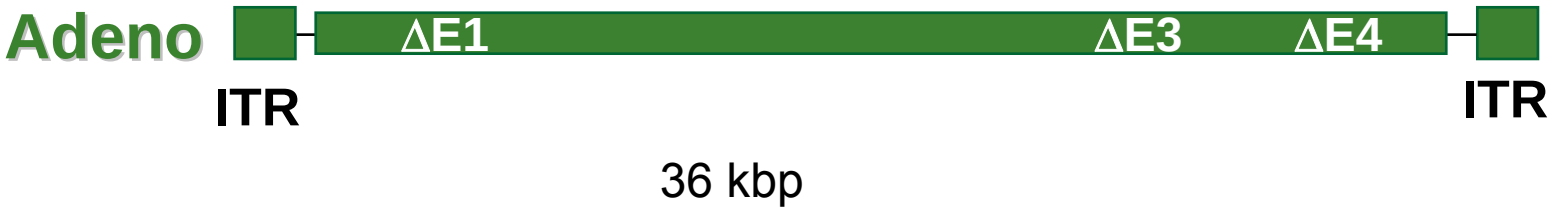
AdEasy™ Adenoviral Vector System (Stratagene)

Features of Adenovirus Vectors

- Relatively Safe (millions of recruits vaccinated)
- Ad2 and Ad5 do not cause cancer
- Rarely integrate into the chromosome
- Transient expression only
- Infects many cell types and resting cells
- Aerosol delivery
- Can accept large foreign DNA inserts
- Stable and amenable to purification (10^{11})
- Generate strong immune response

Generations of Adenovirus vectors

- First Generation Vectors
  - Two early genes deleted (E1 or E1/E3)
- Second Generation Vectors
  - Three early genes deleted (E1, E3, E4)
  - Commercialized as AdEasy (He *et al*., 1998)
- Third Generation Vectors
  - "Gutless vectors"
  - All viral genes deleted; only essential *cis*-acting sequences retained



Adenovirus vector hazards

- Recombination with wild type strains
- Recombination during virus production
- Contamination with helper virus (gutless)
- Transgene
- Immune response
- Difficulties in detecting exposure (prevalence)
- Altered tropism – capsid and fiber proteins

(-) RNA Viruses

- Nonsegmented
  - *Filoviridae* (Ebola)
  - *Rhabdoviridae* (Rabies)
  - *Paramyxoviridae* (Hendra & Nipah)
- Segmented
  - *Orthomyxoviridae* (Flu)
  - *Bunyaviridae* (Hanta)
  - *Arenaviridae* (Lassa)

Minus-Strand RNA Virus Vectors

Generation of biologically contained Ebola viruses

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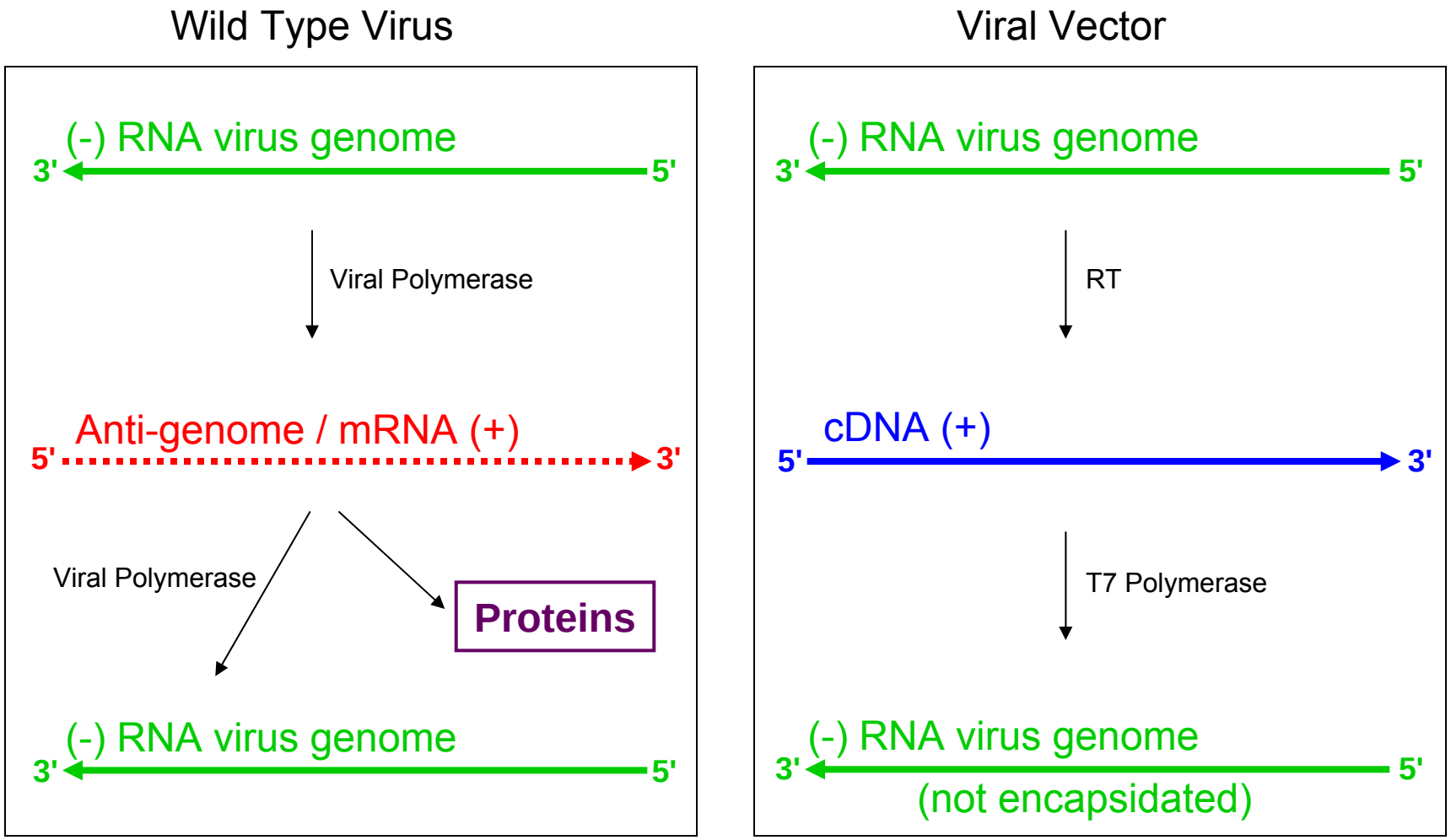
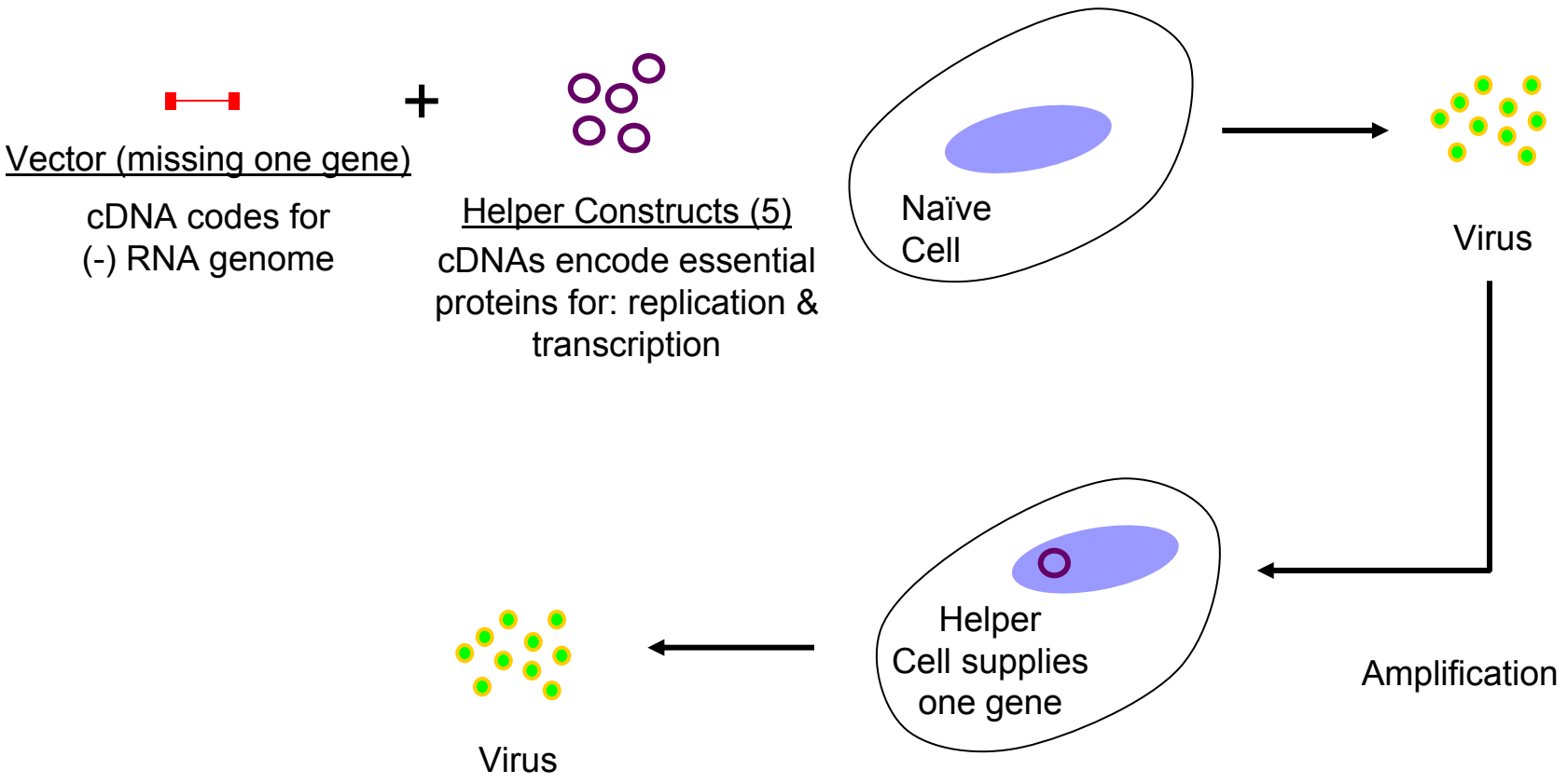
Edited by Peter Palese, Mount Sinai School of Medicine, New York, NY, and approved December 12, 2007 (received for review August 25, 2007)

PNAS **January 29, 2008** vol. 105 no. 4 **1129–1133**

Nucleic Acid Polarity for (–)RNA Viruses



Viral Vector Design and Production for the Ebola Virus Vector



(-) RNA Vector Hazards

- Includes RG3 and RG4 agents
- Little known about recombination for (-) RNA viruses
- Little known about the replication cycles for these viruses
- Such unknowns create difficulties with regard to accurate risk assessments

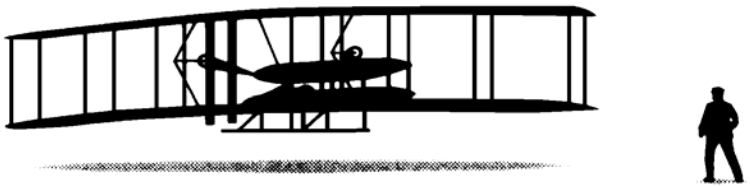
Animal Studies

- Push to move animals to lower containment levels
- Excretions and secretions
- Issues of mixed waste (bio and chemical)
- Proper equipment for containment
- Recombination with endogenous sequences or wild type species

Conclusions

- Viral vectors can be handled safely
- Realize that modern vector systems are becoming increasingly complex
- Understanding all hazards → accurate risk assessments
- Know the purpose and potential uses of the vector with respect to its design
- Pay attention to the transgene, especially when function not fully understood
- Be cautious in prematurely lowering containment levels for novel vector types

Questions?



“The difficulty lies, not in the new ideas, but in escaping from the old ones, which ramify, for those brought up as most of us have been, into every corner of our minds.”

John Maynard Keynes (1936)