

VIROLOGIA 2005/2006

APRESENTAÇÃO 4

Maria Filomena Caeiro

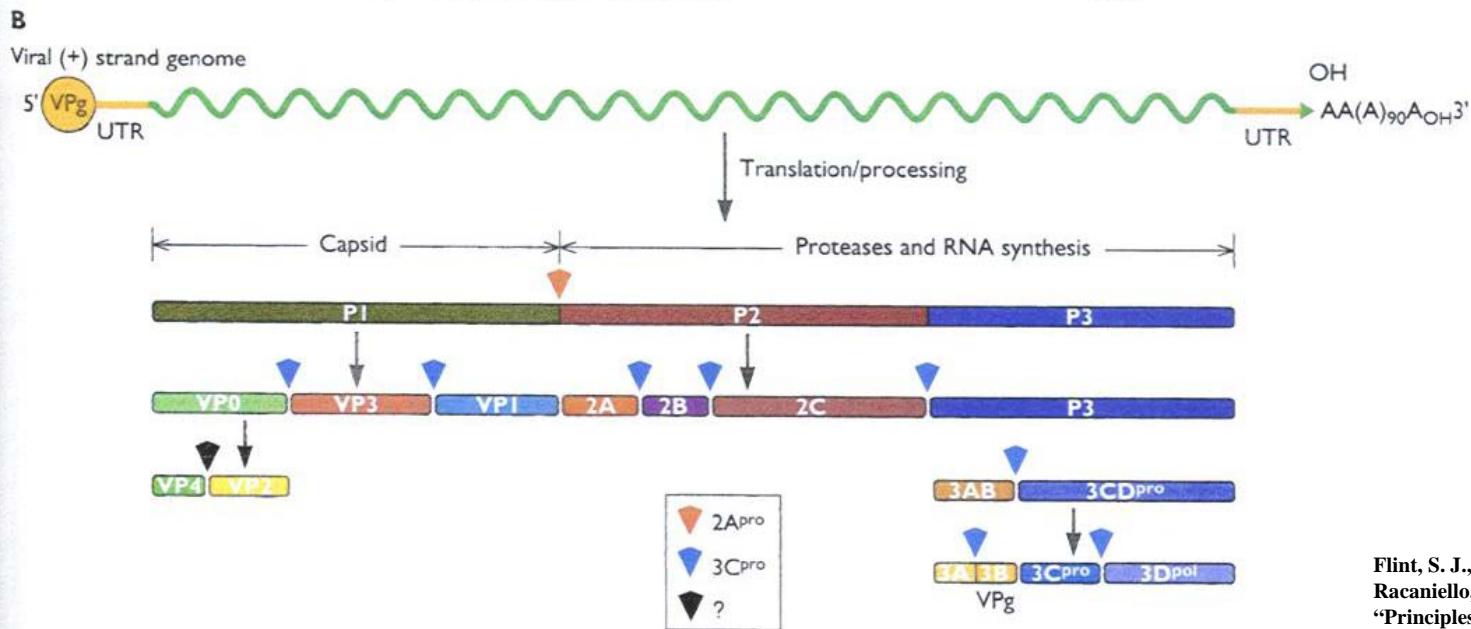
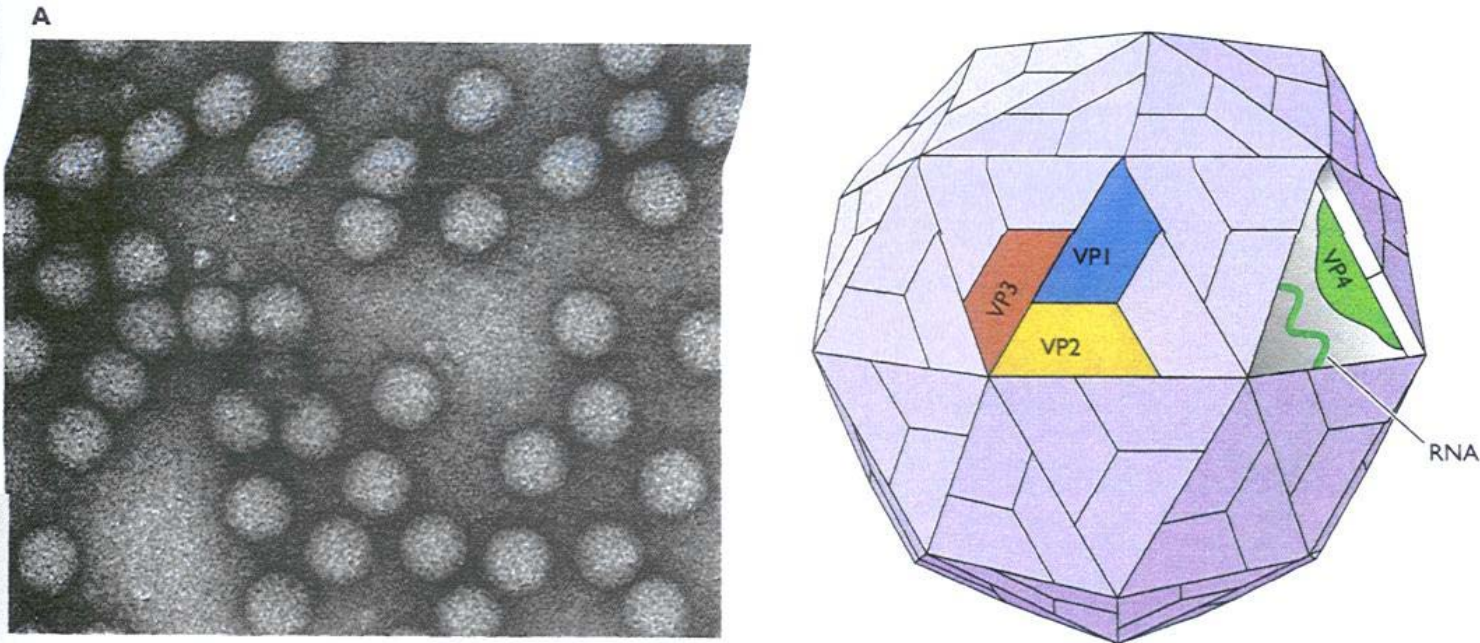
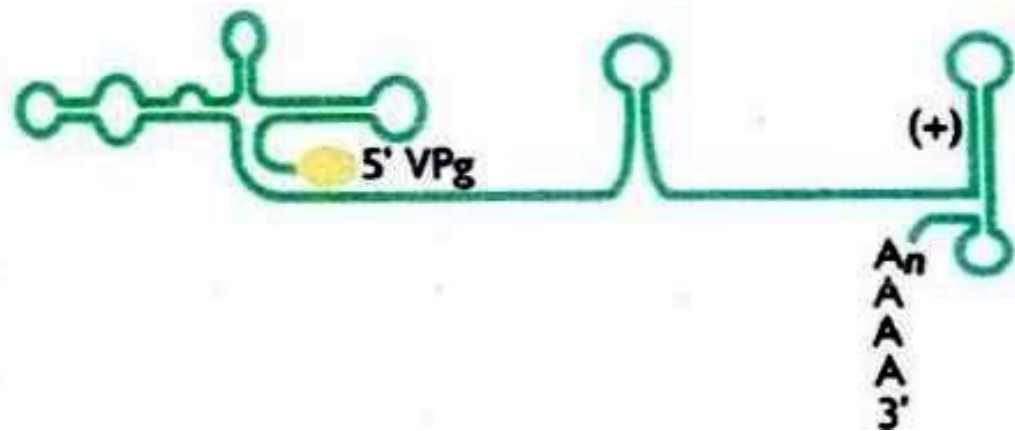


Figure 13 Structure and genomic organization

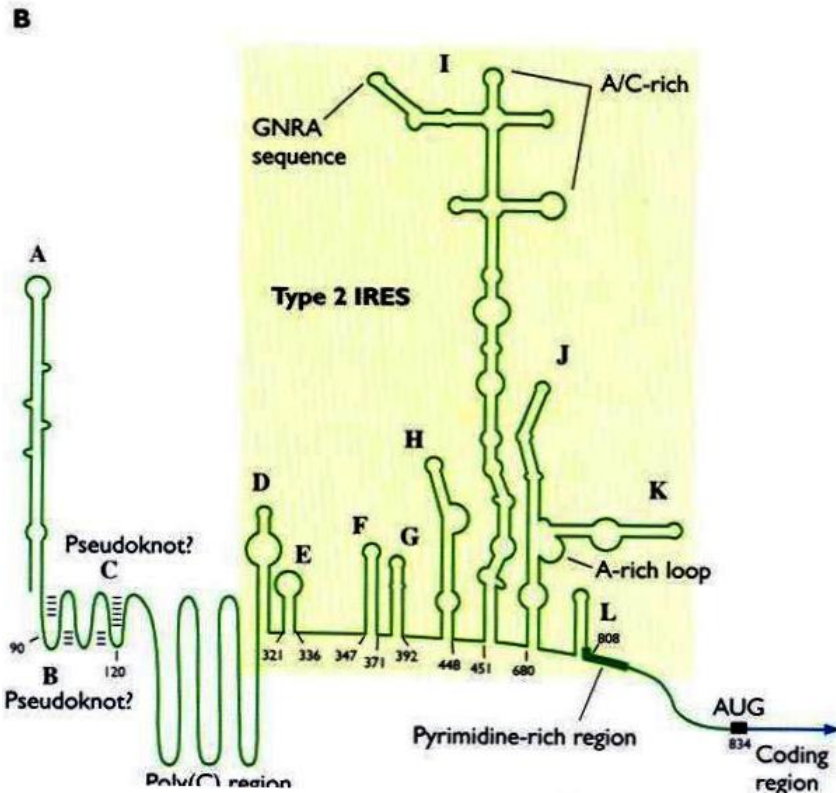
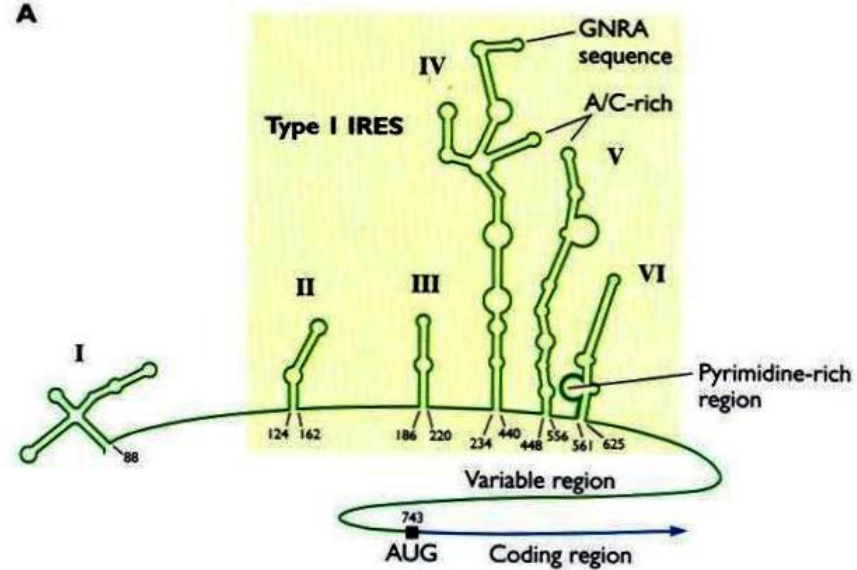
Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

Poliovirus

- 5'-linked VPg primer
- 5' cloverleaf
- 3' pseudoknot
- *cis*-acting replication element in coding region



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

Key experiment: discovery of the IRES

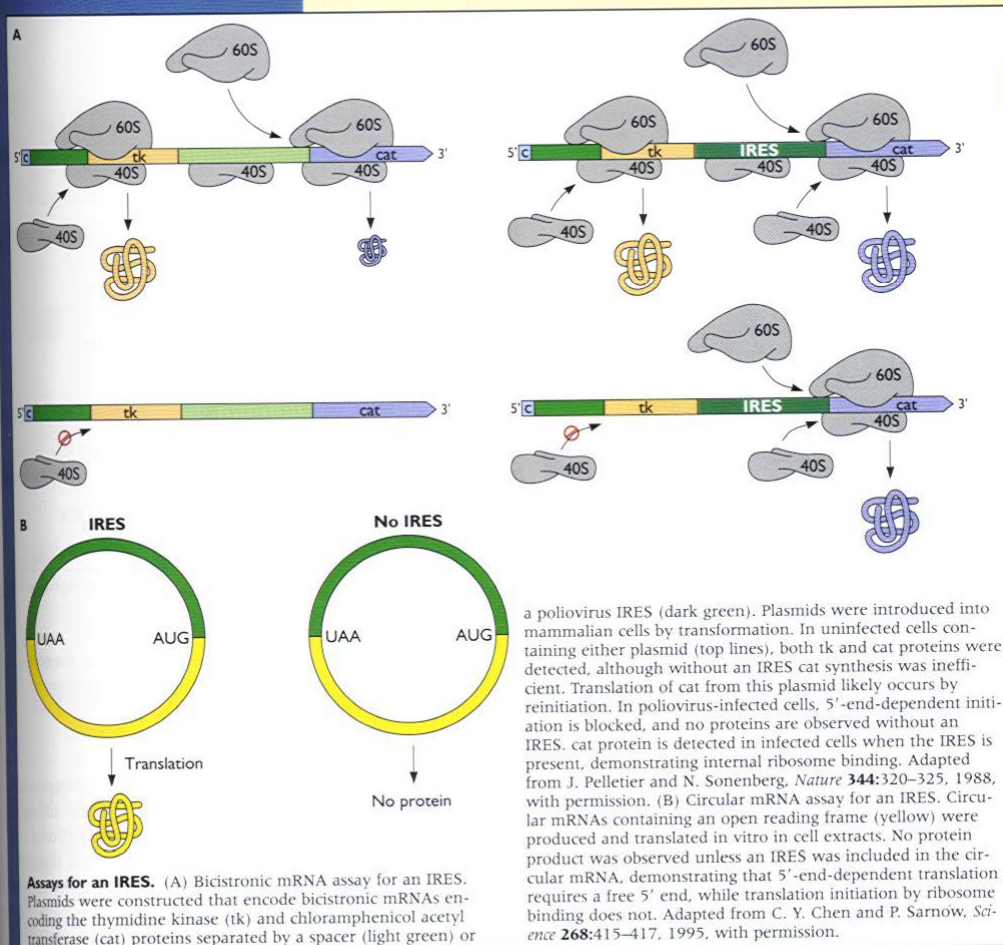
The hypothesis that poliovirus mRNA is translated by internal ribosome binding, however, should not require a free 5' end. To test this hypothesis, Peter Sarnow created circular mRNAs with and without an IRES. The circular mRNA was translated only if an IRES was present (panel B). This experiment formally proved that translation initiation directed by an IRES occurs by internal binding of ribosomes and does not require a free 5' end.

It had long been known that circular mRNAs cannot be translated by 5'-end-

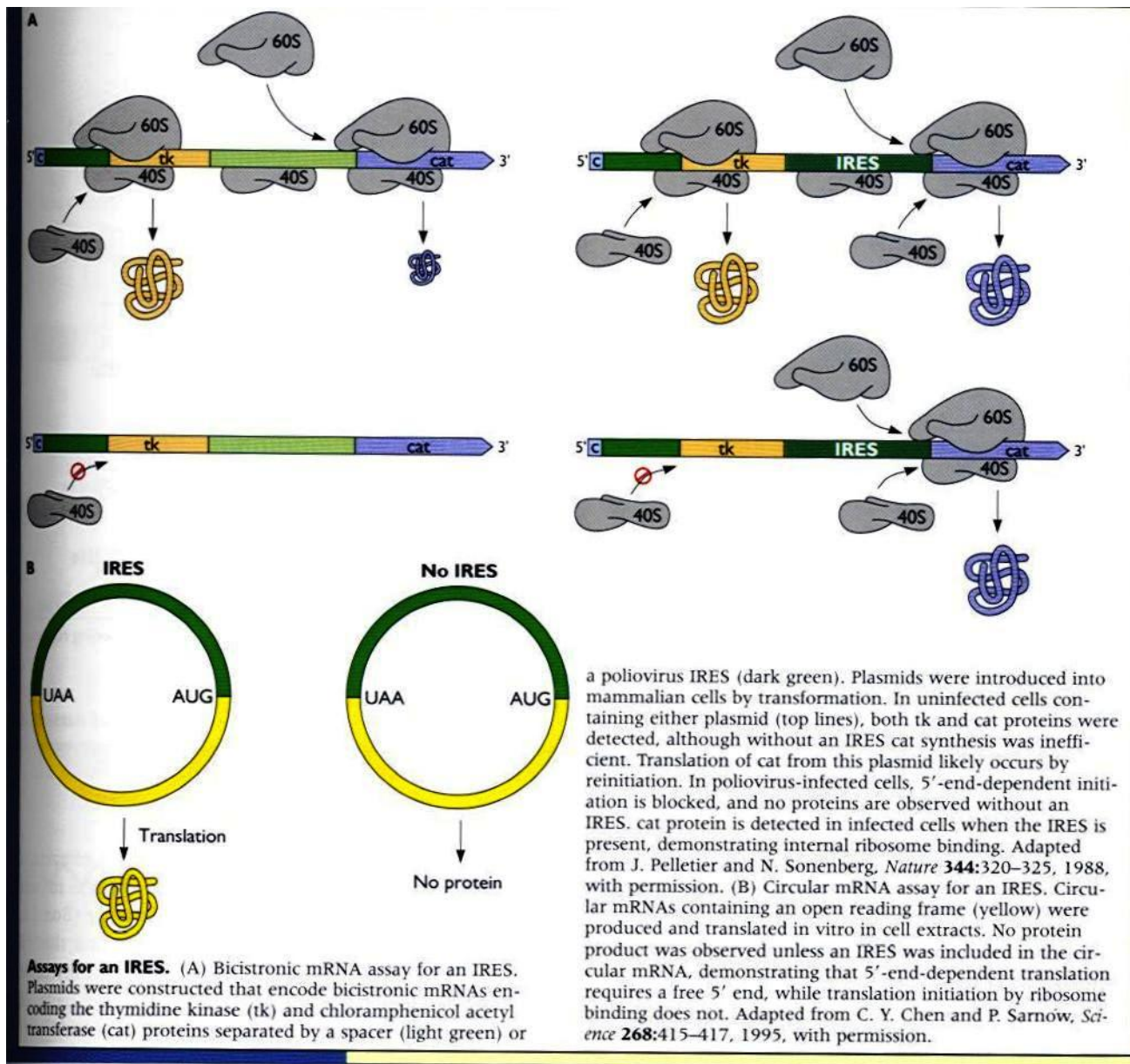
dependent initiation. Translation by internal ribosome binding, however, should not require a free 5' end. To test this hypothesis, Peter Sarnow created circular mRNAs with and without an IRES. The circular mRNA was translated only if an IRES was present (panel B). This experiment formally proved that translation initiation directed by an IRES occurs by internal binding of ribosomes and does not require a free 5' end.

Chen, C. Y., and P. Sarnow. 1995. Initiation of protein synthesis by the eukaryotic translational apparatus on circular RNAs. *Science* 268:415-417.

Pelletier, J., and N. Sonenberg. 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature (London)* 334:320-325.



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

Use of the IRES in cloning and expression vectors

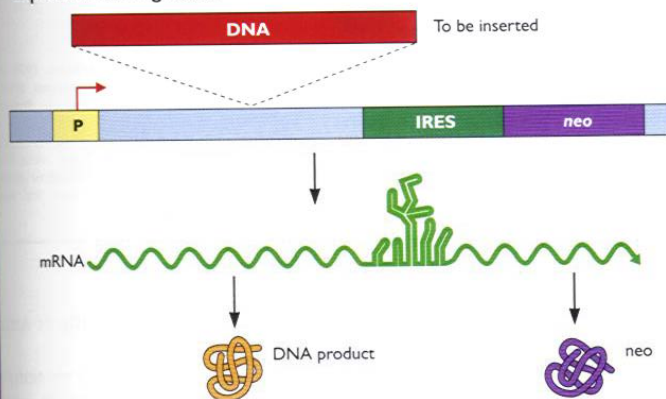
The IRES has been used widely in the expression and cloning of foreign genes in eukaryotes. One strategy for the expression of genes in mammalian cells is to produce mRNAs in the cytoplasm by a bacterial DNA-dependent RNA polymerase, such as T7 RNA polymerase. Such mRNAs are poorly translated because they are not capped; inclusion of an IRES in the 5' untranslated region allows them to be translated efficiently.

Another application of the IRES is in the functional cloning of new genes (figure panel A). A DNA library is made by using a cloning vector that produces a bicistronic mRNA encoding both the desired gene and a selectable marker. This library is introduced into cells which are then screened for expression of the desired protein. The use of a selectable

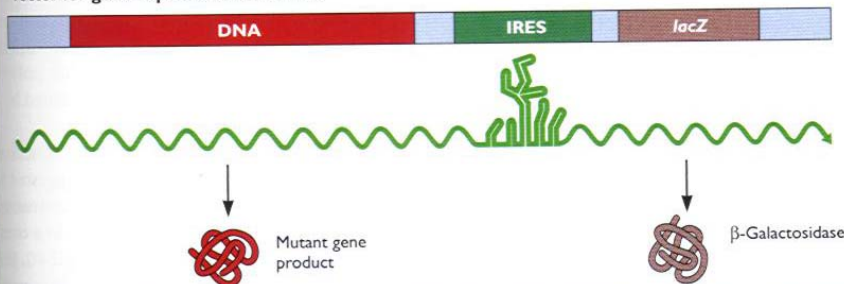
marker on the same mRNA increases the efficiency of screening because most transformants also express the encoded gene.

IRESs have also been used in the isolation of mutant mice by homologous recombination in embryonic stem cells. Bicistronic vectors have been designed to produce mRNA encoding the altered protein and the *lacZ* gene, separated by an IRES (panel B). Cells in which this DNA is integrated into the wild-type gene are selected; these cells express a mutant form of the protein and the β -galactosidase enzyme. The latter can be readily assayed in tissue sections by staining with a chromogenic substrate, and since it is encoded on the same mRNA as the targeted gene product, it serves as a marker for mRNA expression.

A Expression cloning vector

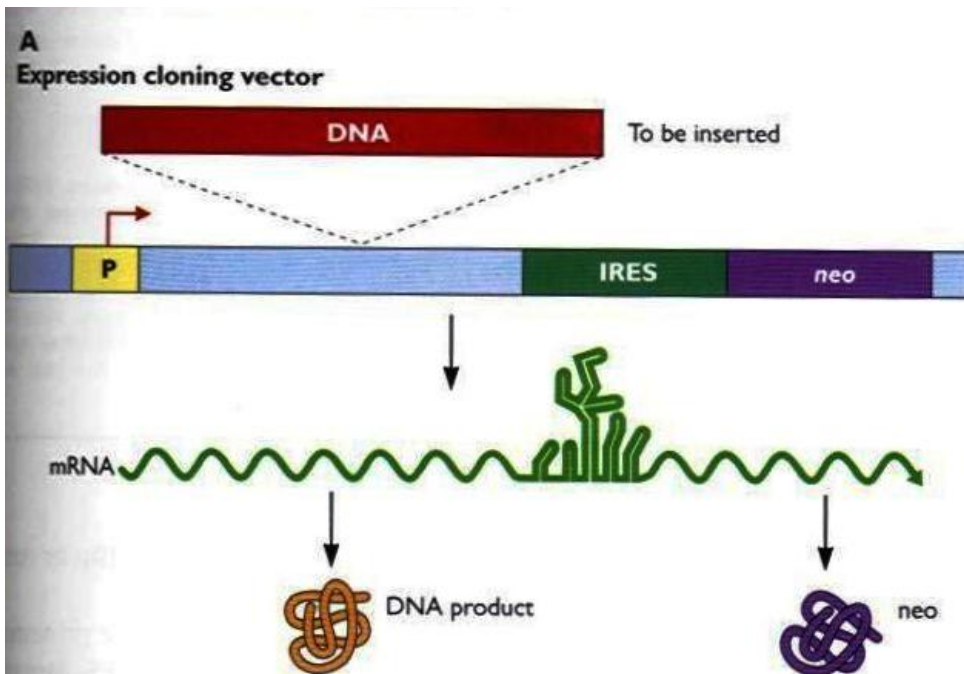


B Vector for gene replacement in mice



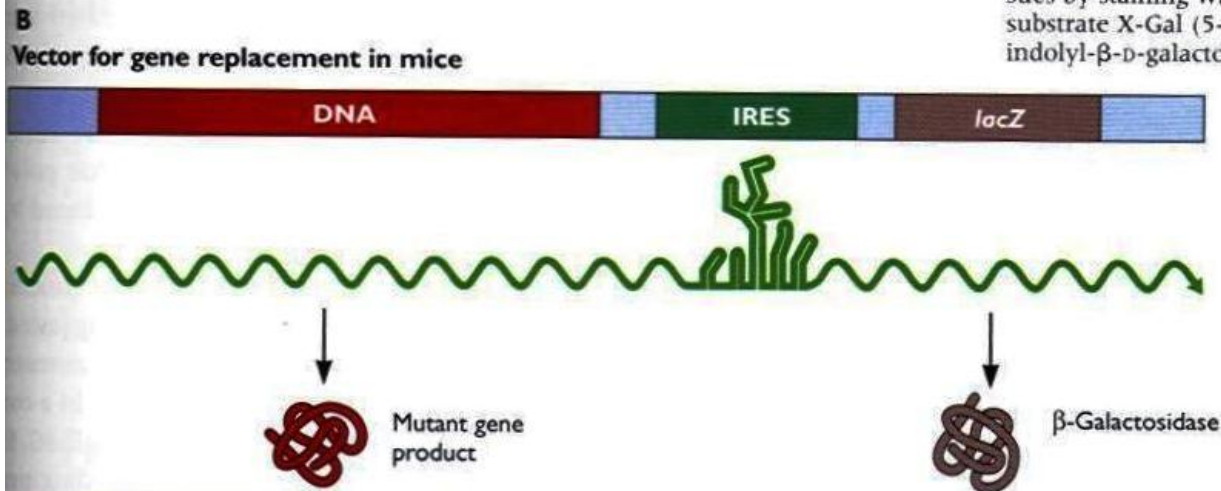
Uses of the IRES in molecular biology.

(A) Design of plasmids for expression cloning. The expression library is created by inserting DNA into a site on the plasmid downstream of a promoter. The foreign DNA is followed by an IRES linked to a selectable marker such as neomycin resistance. The mRNA produced from this plasmid DNA, after introduction into mammalian cells, encodes the cloned DNA product and the protein conferring neomycin resistance. (B) Vector for gene replacement in mice. In this example, the goal is to replace the gene with a mutant version. The targeting plasmid consists of mutant DNA followed by an IRES and the *lacZ* gene. The flanking light blue bars represent sequences from the mouse gene that will mediate homologous recombination. After replacement of the endogenous gene with this synthetic version, mRNA will be produced that encodes the mutant gene product as well as the β -galactosidase protein. The latter can be detected in tissues by staining with the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside).



Uses of the IRES in molecular biology.

(A) Design of plasmids for expression cloning. The expression library is created by inserting DNA into a site on the plasmid downstream of a promoter. The foreign DNA is followed by an IRES linked to a selectable marker such as neomycin resistance. The mRNA produced from this plasmid DNA, after introduction into mammalian cells, encodes the cloned DNA product and the protein conferring neomycin resistance. (B) Vector for gene replacement in mice. In this example, the goal is to replace the gene with a mutant version. The targeting plasmid consists of mutant DNA followed by an IRES and the *lacZ* gene. The flanking light blue bars represent sequences from the mouse gene that will mediate homologous recombination. After replacement of the endogenous gene with this synthetic version, mRNA will be produced that encodes the mutant gene product as well as the β -galactosidase protein. The latter can be detected in tissues by staining with the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside).



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

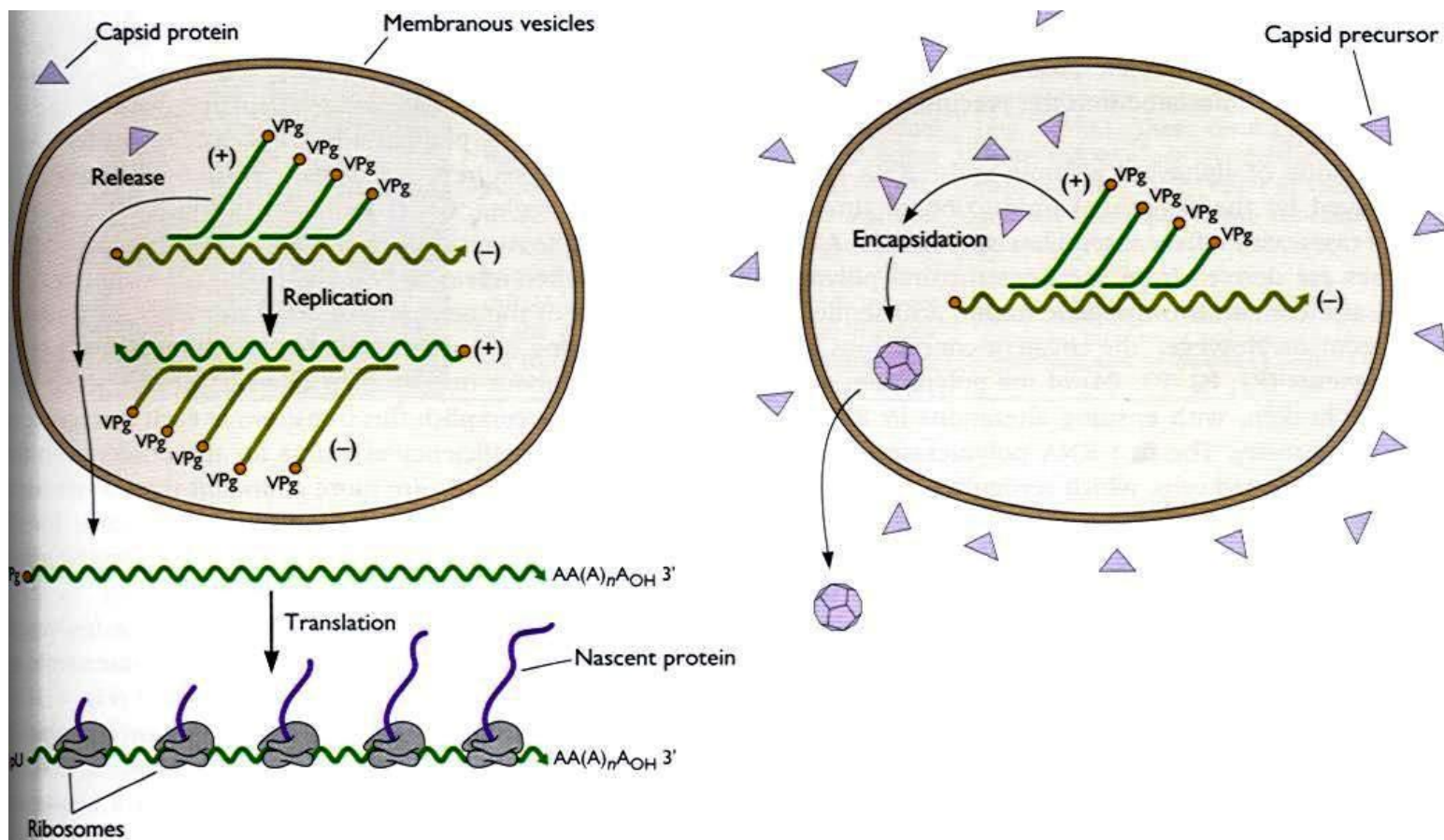
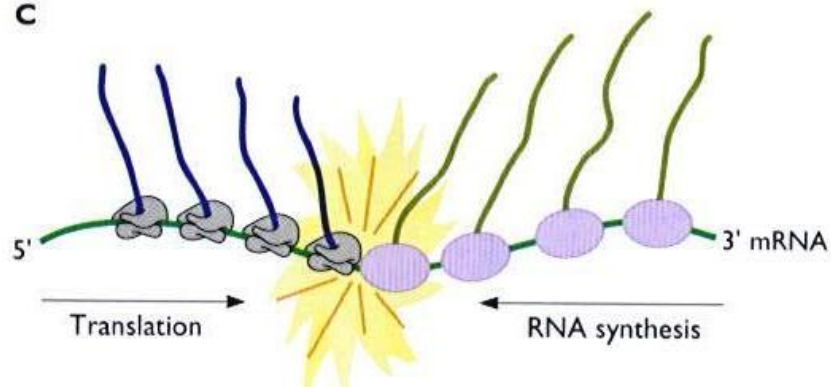
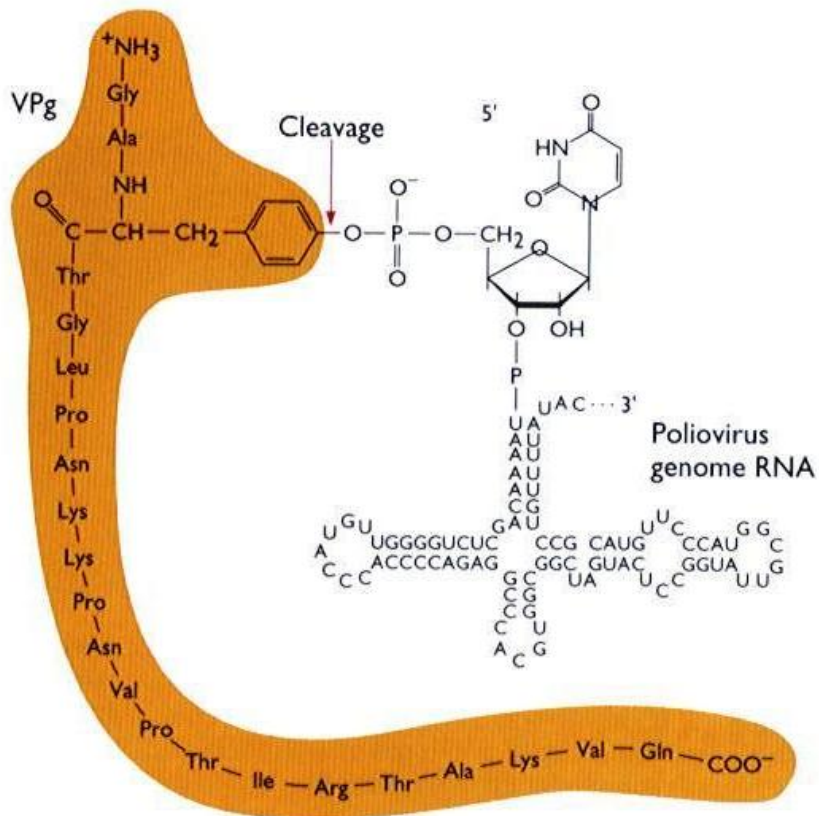


Figure 6.15 Hypothetical model of how polioviral (+) strand RNAs play different roles in infected cells. (+) strand RNA synthesis occurs on membranous vesicles. (A) When the concentration of capsid proteins is low, encapsidation of genomic RNA does not occur. Some of the newly synthesized genomic RNAs are retained on the vesicles and participate in further genomic RNA replication. The genomic RNAs released from the vesicles can serve as viral mRNAs. These mRNAs, when found on polyribosomes, lack the 5'-terminal VPg protein. (B) When the concentration of capsid proteins is high, encapsidation of genomic RNA is favored, and RNAs do not enter the replication/translation pathways.

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2000). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

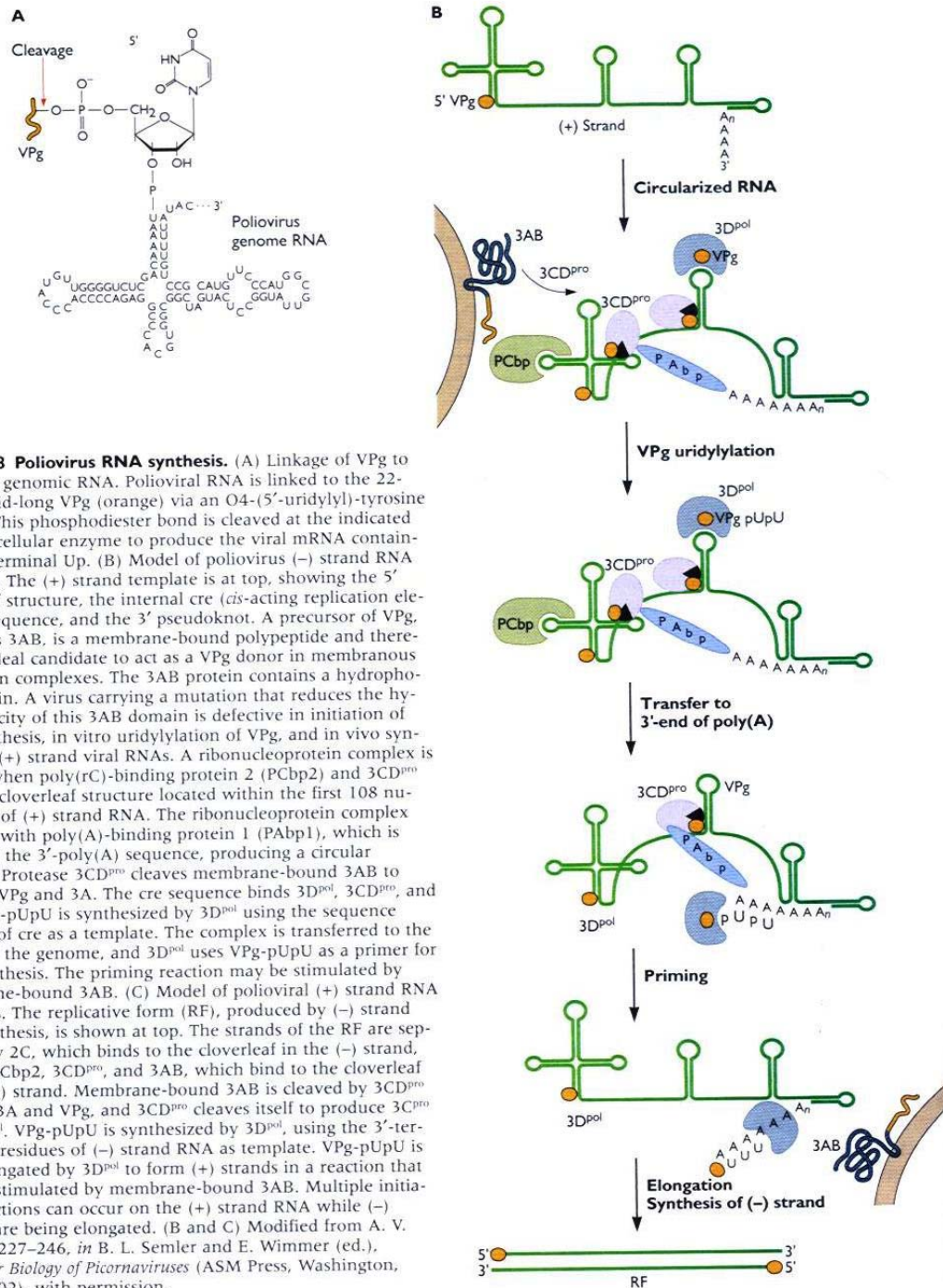
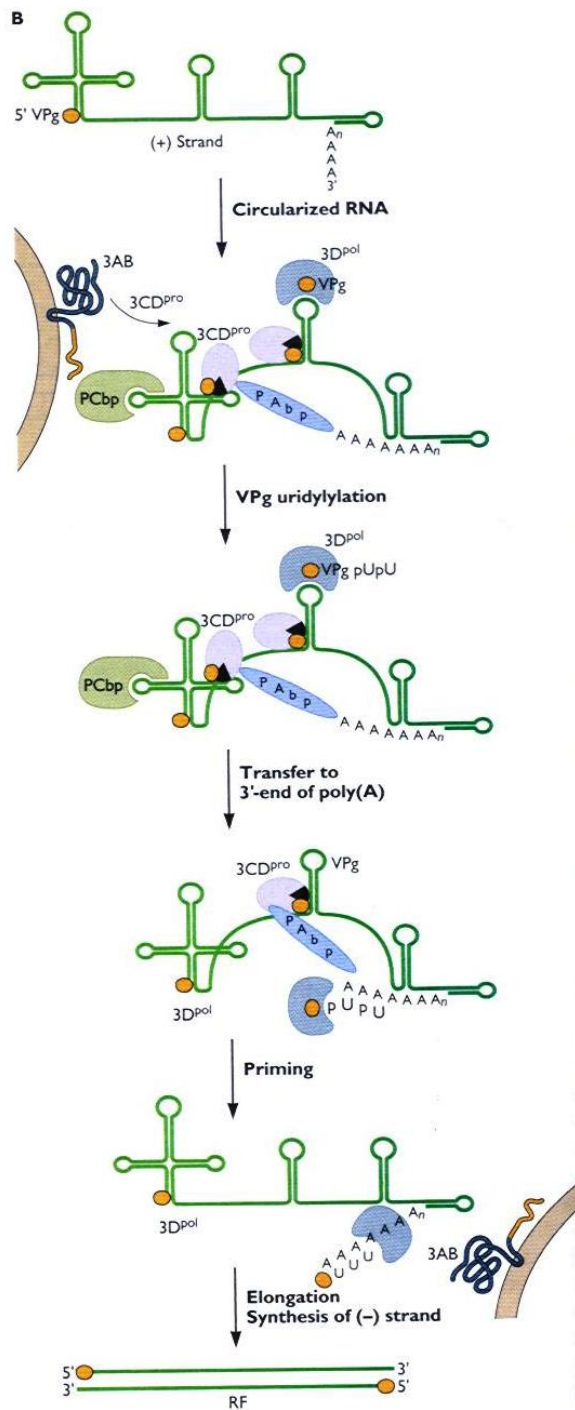


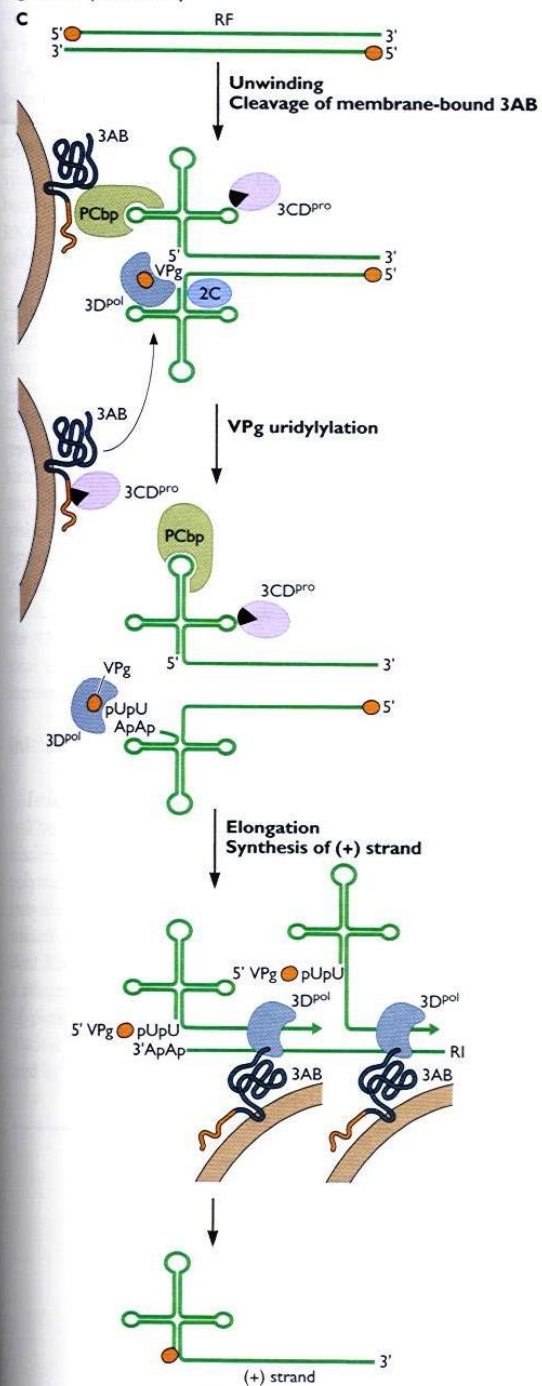
Figure 6.8 Poliovirus RNA synthesis. (A) Linkage of VPg to poliovirus genomic RNA. Poliovirus RNA is linked to the 22-amino-acid-long VPg (orange) via an O4-(5'-uridylyl)-tyrosine linkage. This phosphodiester bond is cleaved at the indicated site by a cellular enzyme to produce the viral mRNA containing a 5'-terminal Up. (B) Model of poliovirus (-) strand RNA synthesis. The (+) strand template is at top, showing the 5' cloverleaf structure, the internal *cre* (*cis*-acting replication elements) sequence, and the 3' pseudoknot. A precursor of VPg, known as 3AB, is a membrane-bound polypeptide and therefore an ideal candidate to act as a VPg donor in membranous replication complexes. The 3AB protein contains a hydrophobic domain. A virus carrying a mutation that reduces the hydrophobicity of this 3AB domain is defective in initiation of RNA synthesis, *in vitro* uridylation of VPg, and *in vivo* synthesis of (+) strand viral RNAs. A ribonucleoprotein complex is formed when poly(rC)-binding protein 2 (PCbp2) and 3CD^{pro} bind the cloverleaf structure located within the first 108 nucleotides of (+) strand RNA. The ribonucleoprotein complex interacts with poly(A)-binding protein 1 (PABp1), which is bound to the 3'-poly(A) sequence, producing a circular genome. Protease 3CD^{pro} cleaves membrane-bound 3AB to produce VPg and 3A. The *cre* sequence binds 3D^{pol}, 3CD^{pro}, and VPg. VPg-pUpU is synthesized by 3D^{pol} using the sequence AAACA of *cre* as a template. The complex is transferred to the 3' end of the genome, and 3D^{pol} uses VPg-pUpU as a primer for RNA synthesis. The priming reaction may be stimulated by membrane-bound 3AB. (C) Model of poliovirus (+) strand RNA synthesis. The replicative form (RF), produced by (-) strand RNA synthesis, is shown at top. The strands of the RF are separated by 2C, which binds to the cloverleaf in the (-) strand, and by PCbp2, 3CD^{pro}, and 3AB, which bind to the cloverleaf in the (+) strand. Membrane-bound 3AB is cleaved by 3CD^{pro} to form 3A and VPg, and 3CD^{pro} cleaves itself to produce 3C^{pro} and 3D^{pol}. VPg-pUpU is synthesized by 3D^{pol}, using the 3'-terminal A residues of (-) strand RNA as template. VPg-pUpU is then elongated by 3D^{pol} to form (+) strands in a reaction that may be stimulated by membrane-bound 3AB. Multiple initiation reactions can occur on the (+) strand RNA while (-) strands are being elongated. (B and C) Modified from A. V. Paul, p. 227–246, in B. L. Semler and E. Wimmer (ed.), *Molecular Biology of Picornaviruses* (ASM Press, Washington, D.C., 2002), with permission.

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

Figure 6.8 (continued)



Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. and Skalka, A. M. (2004). "Principles of Virology. Molecular Biology, Pathogenesis, and Control". 2nd edition. ASM Press.

Caliciviruses

Virus	Disease
Norovirus <ul style="list-style-type: none">• Desert Shield virus• Lordsdale virus• Mexico virus• Norwalk virus• Hawaii virus• Snow Mountain virus• Southampton virus	Gastroenteritis
Sapovirus <ul style="list-style-type: none">• Sapporo virus	

Epidemiology	
Transmission <ul style="list-style-type: none">• Fecal-oral route from contaminated water and food	Distribution of virus <ul style="list-style-type: none">• Ubiquitous• No seasonal incidence
At risk or risk factors <ul style="list-style-type: none">• Children in day care centers• Schools, resorts, hospitals, nursing homes, restaurants, cruise ships (due to infected food handlers)	Vaccines or antiviral drugs <ul style="list-style-type: none">• None

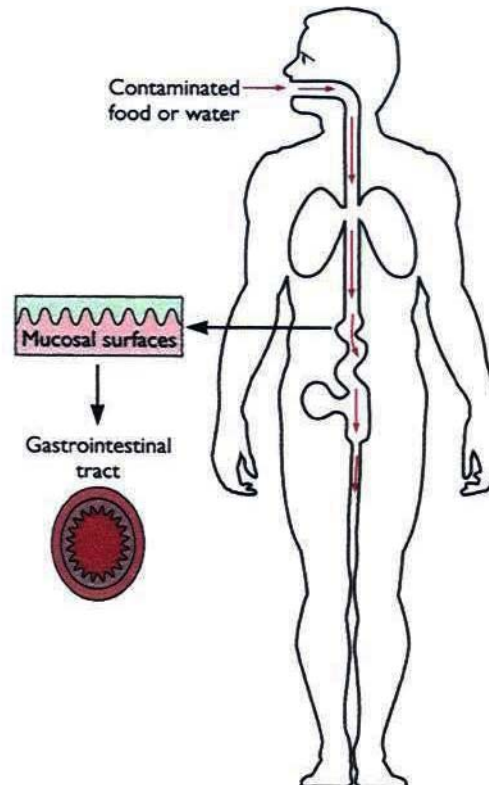
Disease mechanisms

Viruses are resistant to detergents, drying, acid

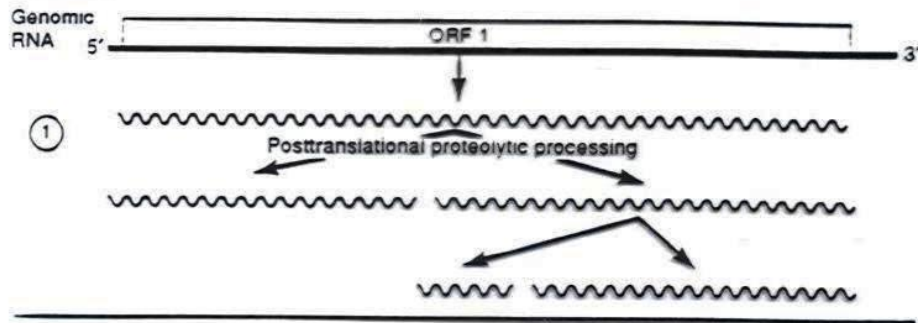
Transmitted by fecal-oral route (contaminated water and food)

Viruses infect intestinal brush border, preventing proper absorption of water and nutrients

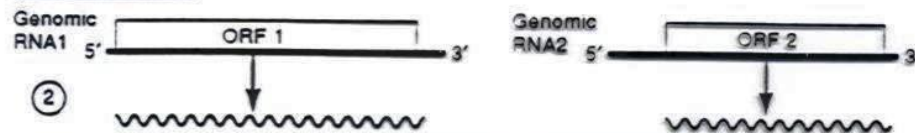
Viruses cause diarrhea, vomiting, abdominal cramps, nausea, headache, malaise, fever



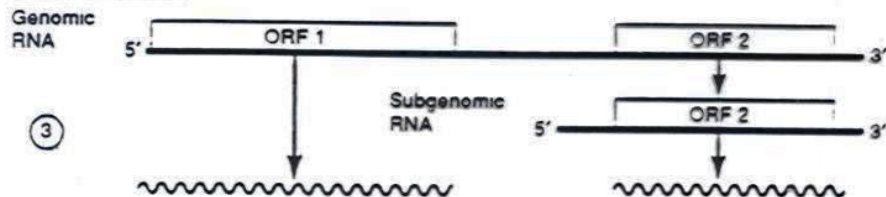
Polyprotein



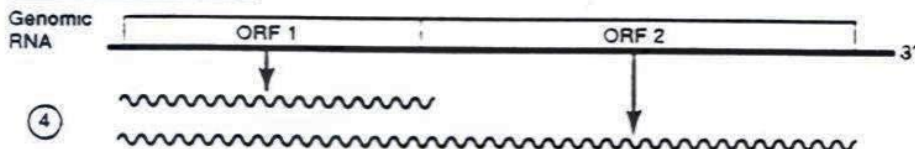
Multipartite genome



Subgenomic RNAs



Translational readthrough



Translational frameshift

The ribosome bypasses a stop codon in Frame 0 by switching back one nucleotide to Frame -1 at a UUUAG sequence before continuing to read triplets in Frame -1 to give a fusion or transframe protein.

