# Genetic analysis of picornaviruses

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During the past year, genetic studies of picornaviruses, vastly facilitated by the application of infectious picornaviral cDNAs and RNAs, have contributed to our understanding of the function of individual picornavirus polypeptides and to the genetic processes that operate in these small RNA viruses. Especially notable were the demonstrations that the RNA-dependent RNA polymerase may have a function in RNA synthesis as an uncleaved precursor polypeptide, and that a mutation in the polymerase can be complemented in *trans*, in contrast to data obtained from previously studied polymerase mutants. A new *in vitro* system, in which positive-strand sythesis, negative-strand synthesis and RNA packaging were all observed, will facilitate further studies into the mechanism of these processes.

## Introduction

Cloned picornavirus cDNAs that are either themselves infectious [1–4] or can serve as templates for infectious RNAs [4–9] have conferred the ability to make and study defined mutations to the field of picornaviral genetics. The study of genetics in the system can be divided into two subdisciplines. The tools and logic of the genetic system may be used to investigate the biochemistry of individual proteins or RNAs and their interactions with other gene products. Alternatively, the primary focus of a study may be the processes of transmission genetics: genome replication, complementation and recombination. This review highlights some of the genetic processes that occur in picornaviruses, and the functions of individual viral polypeptides are discussed. Models demonstrating the dependence of RNA synthesis on translation of template RNA are presented.

For a parasite like a virus, genetic approaches can also be used to study the interaction of the viral genome with its host organism. Because of space constraints, the effects of the picornaviral infections on host cells and the exploitation of those host cells by picornaviruses will not be discussed in this review. The reader is referred to several recent, interesting reports on these subjects [10•,11••,12• –14•,15••].

## New information on RNA structure and function

The genomic structure of picornaviral RNAs is schematically shown in Figure 1. The 5' non-coding regions of picornaviral RNAs vary from 600–1200 nucleotides in length, and must perform at least two functions. At the 5' end of the viral mRNAs, sequences from 140–630, 403–811 and 369–805 in poliovirus [16], encephalomyocarditis virus [17] and foot-and-mouth disease virus [18,19], respectively, confer the ability for picornaviral RNAs to be translated in cells by the unusual mechanism of internal ribosome binding. RNA sequences and factors mediating this process are under intense scrutiny; the state of this research has been recently reviewed [20•]. Cap-independent translation mediated by internal binding of ribosomes to specific sequences in 5' non-coding sequences was thought, until this year, to be limited to the picornaviruses. Macejack and Sarnow [21••], however, report that a cellular RNA, encoding the immunoglobulin heavy-chain binding protein, can be translated by internal ribosome binding as well. Thus, picornaviruses did not 'invent' a new mechanism of translation, but hijacked one used by cellular messages [21••]. Sequences in the 5' non-coding region outside the internal ribosome binding region are also important for translation initiation in poliovirus. A small-plaque mutant whose genome harbors a small insertion at nucleotide 21 clearly displays a primary defect in translation [22].

It would seem reasonable that sequences complementary to the 5' end of the positive strands, and constituting the 3' end of the negative strands, should also be involved in the initiation of positive-strand synthesis. Andino *et al.* [23••] investigated both the RNA structure formed by the 3'-terminal 90 nucleotides of the poliovirus negative strand, and the similar RNA structure formed by the complementary 5'-terminal nucleotides of the positive strand. Chemical and enzymatic probing of the positivestrand RNA structure, as well as site-directed mutagenesis, confirmed the presence and functional importance

of the RNA secondary structure predicted for this region [24–26]. Clever mutagenesis, creating G–U base pairs on one strand and C–A mismatches on its complement, was used to test whether the RNA structure was functionally required in its positive- or negative-strand version. Quite surprisingly, the intricate clover-leaf structure seems to be needed, not at the 3' end of the negative strand from which RNA synthesis must initiate, but at the 5' end of the positive strand! The phenotypes of mutants showed that the requisite function is in RNA synthesis, specifically in positive-strand synthesis (although this latter suggestion comes only from measuring the steady-state ratio of positive to negative strands in non-conditional mutants). Taken together, these findings suggest that the structure of the extreme 5' end of the poliovirus positive strand may be important for its own synthesis, either as a nascent RNA or as the template of the template of nascent positive-strand RNAs [23••].

## New information on poliovirus proteins

### RNA-dependent RNA polymerase and other RNA-replication proteins

What proteins interact with 3D, the viral polymerase, during picornaviral RNA synthesis? Andino et al. [23••] have identified at least one complex that appears to be required for poliovirus RNA synthesis. Using non-denaturing gel electrophoresis, a complex formed in vitro between the RNA and either a host protein or proteins

from both uninfected and poliovirus-infected HeLa cells has been identified [23••]. Using extracts from infected cells, a new complex was formed, and was found to contain 3CD, the uncleaved 'precursor' of both the 3D polymerase and the 3C protease. A role for 3C coding sequences in RNA synthesis had been anticipated by earlier work by Andino et al. [27], who showed that certain phenotypic revertants of mutations in the 5' non-coding region clover-leaf RNA structure mapped to 3C. The recent work showed that mutations in the RNA clover-leaf or in the coding region of 3C resulted in the same in vitro phenotype (the loss of formation of the large complex), and similar in vivo defects in RNA synthesis [23••]. Therefore, there is abundant structural and genetic evidence demonstrating the existence of a functional complex between the 5'-most RNA sequences of the poliovirus positive strand and 3CD. Whether the host protein is still present in this larger complex has not yet been shown, but the idea remains tantalizing.

The function of this complex in RNA replication remains to be established. Models to explain the importance of an RNA–protein complex at the 5' end of the positive strand in positive-strand synthesis include possible roles in RNA stability, in separating the nascent positive strands from their templates, and in factor recruitment, that is, keeping proteins required for RNA synthesis close by for synthesis of the positive strand's 'grandchildren' [23••].

Is there any difference between the protein requirements for positive and negative strand synthesis? Recently Gia-
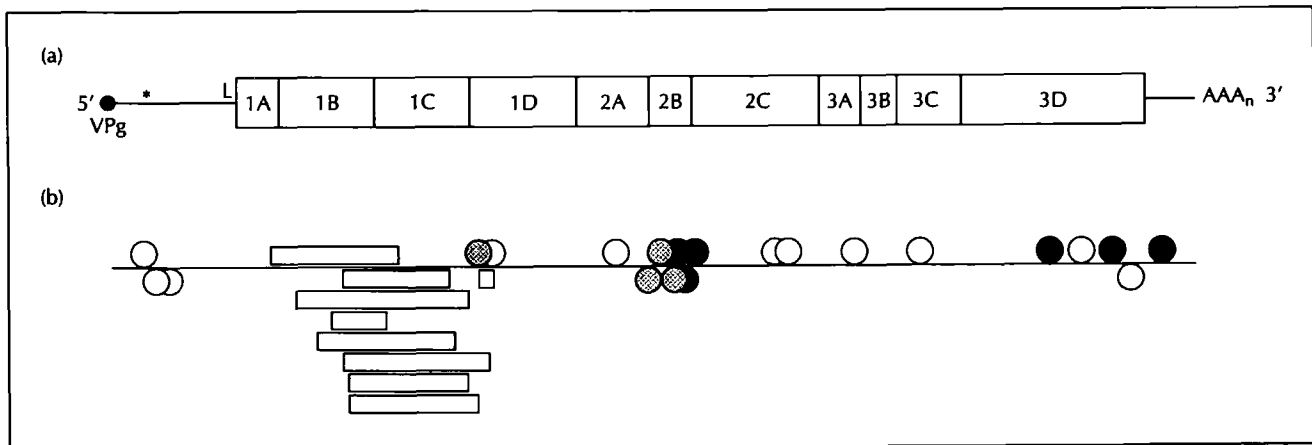


**Fig. 1.** (a) Genetic map of picornaviruses, approximately to scale. The sizes of picornaviral genomes range from 7209–8450 nucleotides [50]. The single open reading frame is shown by the open box. The 5' and 3' non-coding regions of RNA are shown as lines and the individual viral proteins, at their terminal processing stages, are designated within the box. Proteins 1A, 1B, 1C and 1D are the viral capsid proteins VP4, VP2, VP3 and VP1, respectively; 2A and 3C are viral proteases; 2B, 2C and 3A are polypeptides which, when mutant, affect viral RNA synthesis; 3B is VPg, the 22 amino-acid peptide covalently linked to the 5' end of both the positive- and the negative-RNA strands; and 3D is the RNA-dependent RNA polymerase. Each of these proteins may play more than one role, function in the context of a larger precursor, or both. Furthermore, RNA structures within the coding regions, as well as in the 5' and 3' non-coding regions, may fulfill a function. Important differences among the picornaviruses exist. For example, the location of the poly (C) region present in cardioviruses (for example, encephalomyocarditis virus) and apthoviruses (for example, foot-and-mouth disease virus) is marked with an asterisk; the small 'leader' polypeptide present at the beginning of the single open reading frame in cardioviruses, apthoviruses and Theiler's viruses is marked by an L; the coding region for VPg is tandemly repeated 3 times in foot-and-mouth disease virus (reviewed in [50]). (b) 'Complementation' map of poliovirus. Circles denote mutations of only a few amino acids or nucleotides; rectangles denote larger deletions and suggest their relative sizes [39]. Open circles; recessive mutations, those which can be complemented by other mutants or rescued by wild-type virus [38,43,44]. Black circles; cis-dominant mutations, which cannot be complemented by other mutants or rescued by wild-type virus [38,43,44,51]. Grey circles; trans-dominant mutations, which can inhibit the growth of coinfecting wild-type or mutant virus [38,43,45•]. The recessive alleles in the 5' non-coding region are 'complemented' by coinfecting viruses because of the increased inhibition of host protein synthesis during coinfection [51].

chetti and Semler [28] have reported another interesting example of a picornavirus mutant in a protein-coding region with a strand-specific defect in RNA synthesis. A mutant derived from a poliovirus cDNA bearing a mutation in the 3A coding region displayed defects in translation [29] and RNA replication [28]. The results of further *in vivo* and *in vitro* experiments are consistent with the idea that the primary defect of this mutant is in positive-strand synthesis. Unfortunately, the dot-blot analysis used in these studies for the quantitative determination of negative strands in the presence of excess positive strands can be quite misleading [30•]. Notwithstanding, this result suggests that the role of 3A, whose only suspected function is in the VPg precursor 3AB, may be more important in positive- than in negative-strand synthesis. That the two strands could be primed differently is quite conciliatory, given the controversy over the mechanism of RNA priming, which is discussed below.

Astonishingly, a cell-free extract in which poliovirus negative-strand synthesis, positive-strand synthesis and RNA packaging can all be accomplished has recently been developed by Molla *et al* [31••]. In this system, a translation-competent extract prepared from uninfected HeLa cells was programmed with positive-strand poliovirus RNA. Following incubation *in vitro* for 15 h, infectious poliovirions could be recovered. That the authors did not merely observe packaging of the RNA molecules that were added to the extract was argued by the presence of negative-strand RNA following, but not preceding, incubation. That *de novo* RNA synthesis occurred in these extracts was shown by the dependence of infectious virion production on the presence of all four dNTPs, and by the inhibition of virion production by guanidine hydrochloride, an inhibitor of picornavirus RNA synthesis *in vivo*. When the *in vitro* extracts were programmed with positive-strand RNA from a guanidine-resistant mutant, however, the synthesis of infectious virions was not inhibited by guanidine hydrochloride. This *in vitro* system will provide a wealth of experimental information about the protein and RNA requirements for the initiation of positive-strand synthesis, negative-strand synthesis, RNA packaging and virion morphogenesis, as all these processes presumably occur *in vitro*, thus accounting for the production of infectious virus [31••].

**Capsid structure and assembly**

The mechanism of picornavirus assembly and of viral RNA packaging can be investigated by mutating the capsid proteins and identifying the types of presumed assembly intermediates that accumulate in cells infected with the mutant viruses. Marc *et al* [32] have pioneered the study of 'dead' mutants by investigating the assembly pathway in cells transfected with mutant poliovirus RNA that does not give rise to viable virus. Using this technique, the role of myristoylation of capsid proteins 1A and 1AB has been explored. In wild-type poliovirions, the amino-terminal glycine of 1A and 1AB is myristoylated co-translationally [33]. Upon changing the terminal glycine to an alanine residue, myristoylation was found to be totally abolished [32]. In this mutant, assembly about the fivefold axis (of pentameric intermediates) was found to be quite slow, and the few intact virion-like structures

formed were not infectious. In mutants in which myristoylation is only 40–60% inhibited, Moscufo *et al* [37] showed that myristoylated proteins were selected with a twofold preference in the assembly of the pentameric intermediates, and a 20-fold preference in intact, virion-like structures. Therefore, myristoylation is thought to be important at several stages of viral assembly [32,37]. Interestingly, these *in vivo* studies demonstrate little effect of myristoylation on RNA replication or even on proteolytic processing, although processing effects have previously been observed in *in vitro* experiments.

## Genetic processes

### RNA replication

The mechanism of priming picornavirus RNA synthesis remains a subject of controversy. One school of thought contends that a host factor, terminal uridylyl transferase (TUTase), adds non-templated U's to the 3' ends of the would-be template RNAs. This could create a small hairpin for priming of the new strand. The negative strands of picornaviruses begin with poly (U), owing to its synthesis from the poly (A) at the 3' ends of the positive strands (Fig. 1). In addition, the initial two nucleotides of all picornaviral positive strands are U's. The second school of thought draws support from a series of experiments, including the observation of free, di-uridylylated VPg (a 22 amino-acid peptide) in infected cells. This structure, VPg–pU–pU, could be used as the primer for both positive- and negative-strand synthesis (reviewed in [35]).

In accord with a previous, similar report for coxsackie B3 viral RNA [37], Harmon *et al* [36•] reported that the initial two nucleotides of poliovirus RNA were not required for infectivity of RNA transcripts made *in vitro*. Interestingly, the recovered viral RNA contained the two U's reinstated at the 5' end of all of the progeny positive strands. With the two A's missing from the 3' end of the first negative strands, it is difficult to imagine how TUTase action could create the hairpin to facilitate the priming described in the first model, discussed above. Of course, the polymerase may not require the two A–U base pairs to recognize the template–primer loop created by TUTase. In contrast, priming by VPg–pU–pU could quite easily restore these non-templated nucleotides, and this evidence can be interpreted as support for the VPg–pU–pU priming model [36•,37].

Without quantitation of the specific infectivity of mutant RNAs missing the first two uridine residues, it remains possible that the mutant negative strands could have been polyadenylated in the cell, regenerating either templated, hairpin-primed, or VPg–pU–pU-primed synthesis of the missing nucleotides. Several instances of such low-frequency correction of laboratory-induced defects in the cell can be found in picornavirology, including both the elongation of short poly (A) tails and poly (C) tracts [9] and the removal of extra nucleotides from the 5' ends [3]. Clearly, further experiments, preferably in a truly representative *in vitro* RNA replication system [31••], are needed to understand the mechanism of initiation of RNA replication in picornaviruses.

## Complementation

Several lines of evidence have pointed to an unusual relationship between translation and RNA replication in picornaviruses [38]. Most strikingly, deletion mutants and defective interfering (DI) genomes of picornaviral RNAs can be replicated in the presence or absence of helper virus only if the 3′ end of the defective genome remains in frame with the initiation codon at the beginning of the polyprotein. This phenomenon was first noticed during the sequencing of poliovirus DI genomes, when numerous isolates were found to contain deletions of different sizes, all in the region of the genome encoding the viral capsids and all retaining the downstream coding regions in frame [39]. This has since been confirmed using deletion variants synthesized *in vitro* from engineered infectious cDNAs [40,41,42•]; only in-frame deletions gave rise to replicatable RNA. These data can be explained most simply by either a requirement for certain poliovirus pro-

teins in *cis*, or a requirement for the translation of the entire poliovirus genome for its successful replication.

Several poliovirus mutants whose mutations map to coding regions for proteins involved in RNA replication have been shown to be *cis*-dominant in plaque assays. One mutation in 3A, one in 3D, and four in 2B [43,44,45•] demonstrated an inability to be rescued or complemented during coinfections with either wild-type or mutant viruses. It is possible that RNA structures in these regions are so critical and sensitive to disruption that all these mutations are, in fact, *cis*-dominant RNA-structure mutants. Alternatively, 2B, 3A, and 3D may be proteins that act in *cis*, on the genomes from which they are translated. Interestingly, Charini *et al.* [46••] have recently reported that a small-plaque mutant resulting from a single amino-acid insertion in the 3D (polymerase) coding region was clearly recessive during coinfection with wild-
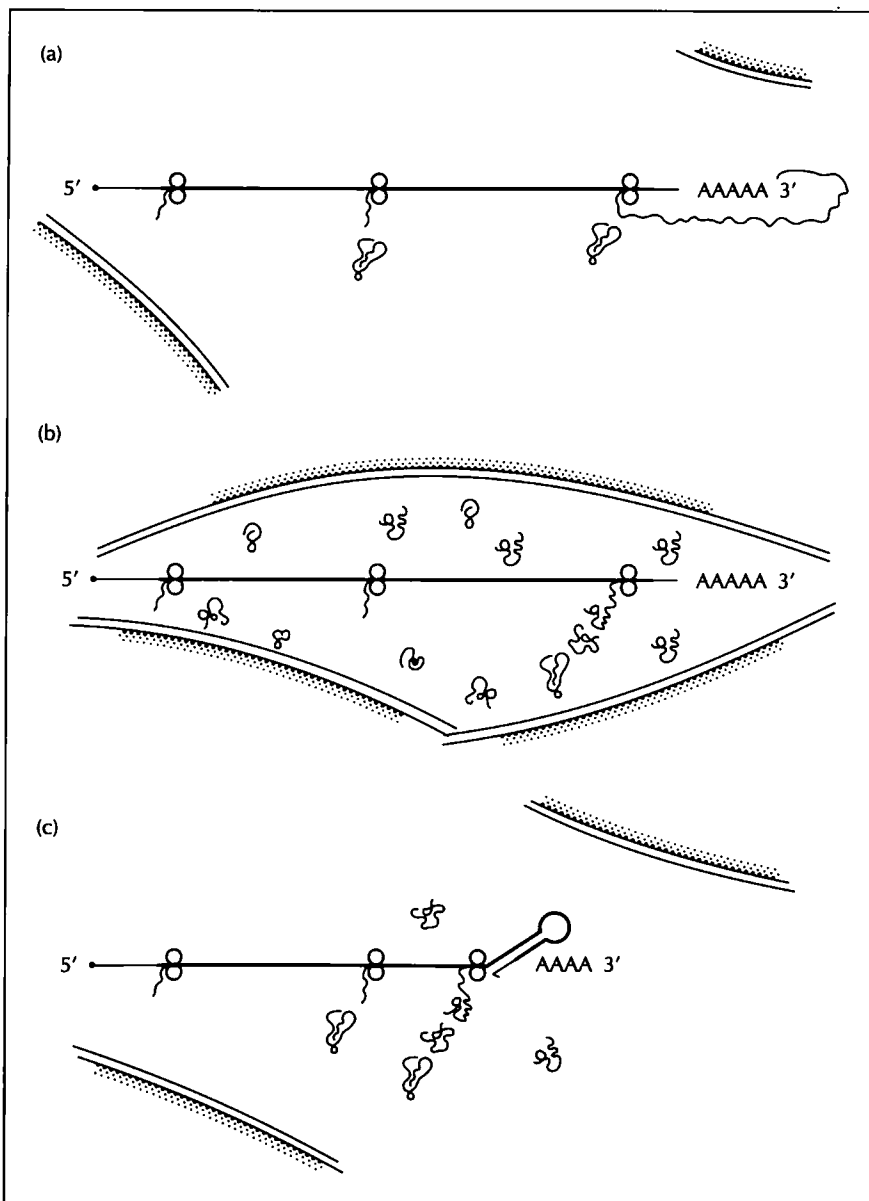


Fig. 2. Models for the replication of picornaviral RNA and its dependence on the presence of an in-frame coding region. These models do not address the mechanism of priming of negative- or positive-strand synthesis, but simply the relationship of RNA synthesis to translation. For the sake of simplicity, only the initiation of negative-strand synthesis is depicted. Translation of poliovirus proteins is known to be associated with membranes [50], which are schematically represented. (a) Nascent replication proteins are required, in *cis*, for the initiation of negative- or positive-strand synthesis. Only newly synthesized proteins are in the proper conformation for the initiation of RNA replication. (b) Replication proteins are sequestered in the cell near the RNA from which they were translated, along with the descendants of that RNA. Most proteins translated from other genomes have no access to the genome shown; however, any proteins able to penetrate the compartment could work in *trans*. (c) An RNA structure at the 3′ end of the genome prevents the positive strand from acting as a template for negative-strand synthesis until the RNA structure is denatured by the translational apparatus. Thus, the act of translation itself, as well as the translation products, are required for the initiation of negative-strand synthesis.

type virus. Carefully planned controls showed that the observed rescue of this mutant virus by the coinfecting wild-type virus was not the result of genetic recombination. Furthermore, three alleles of 2B that could not be complemented by other mutant viruses have been demonstrated by Johnson and Sarnow [45•] to show *trans*-dominance, that is, to exert some dosage-dependent interference with the growth of coinfecting wild-type virus. Thus, at least some function or functions of 3D, and possibly of 2B, can be contributed in *trans*. A 'complementation map' of the poliovirus genome is shown in Figure 1b.

Three models attempting to reconcile these somewhat contradictory findings are presented (Fig. 2) [38–41,42•,44,45•,46••]. Model A suggests that replication proteins must be newly synthesized to associate with the template or to initiate RNA replication. These nascent proteins would transiently occupy a conformation in which they could initiate negative-strand synthesis from the RNA strand from which they were translated. To explain the recessive 3D and the *trans*-dominant 2B mutants discussed above, one must then invoke other, *trans*-acting functions for 2B and 3D that are defective in those particular mutants. Model B suggests that the apparent *cis* action of certain poliovirus proteins could result from compartmentalization inside the cell. Then, the local concentration of proteins translated from a particular RNA or its siblings would be quite high relative to that of proteins translated from other, coinfecting RNAs. No replication of out-of-frame DI particle genomes would occur due to the absence of the local high concentration of proteins required for RNA replication. Again, certain functions of 2B and 3D, which operate outside the compartments involved in RNA replication, could require these proteins only at lower concentrations. In model C, an RNA structure in the positive strand is hypothesized to be inhibitory to the initiation of negative-strand synthesis. Until this structure is removed by the act of translation, no RNA replication can occur. In this model, all replication proteins can in principle act in *trans*, and the *cis*-dominant mutations in the coding regions for 2B, 3A and 3D are in fact mutant in their RNA structures. Perhaps within the year this quandary will be resolved?

## Recombination

Recent and not-so-recent advances in our understanding of RNA recombination between picornaviral genomes have been reviewed in the past year [47•]. During 1991, the most exciting discovery in RNA recombination came from another field of virology. It was thought that alphaviruses, also positive-strand viruses, did not undergo genetic recombination until, in 1988, sequence analysis revealed that western encephalitis virus apparently arose by genetic recombination [48]. In 1991, Weiss and Schlesinger [50••] reported that, under conditions of stringent selection for viability, recombinant Sindbis virus RNA could be observed in the laboratory. Thus far, the list of RNA viruses known to undergo genetic recombination contains only positive-strand eukaryotic RNA viruses, including picornaviruses, coronaviruses, alphaviruses and bromoviruses. This situation is certain to develop during the year.

## Conclusions

Mutations in the viral coding regions, and especially in RNA structures in the 5' non-coding region, have provided new insights into the mechanisms of viral translation, RNA synthesis and assembly. In addition, several new findings in the areas of complementation and RNA replication have supplied surprising twists.

The recently-developed *in vitro* system for poliovirus RNA synthesis and packaging will provide an invaluable tool to test the functions of viral RNA sequences and viral and cellular proteins in picornaviral replication. Furthermore, unsolved quandaries such as the mechanism or mechanisms of priming RNA synthesis, and the relationship between the initiation of viral RNA synthesis and translation may yield to an assault that includes both genetic analysis and *in vitro* biochemistry that faithfully mimics viral replication in an infected cell.

## Acknowledgements

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
•    of special interest
••    of outstanding interest

1.    RACANIELLO VR, BALTIMORE D: Cloned Poliovirus Complementary DNA is Infectious in Mammalian Cells. *Science* 1981, 214:916–919.

2.    KANDOLF R, HOFSCHNEIDER PH: Molecular Cloning of the Genome of the Cardiotropic Coxsackie B3 Virus: Full-length Reverse-transcribed Recombinant cDNA Generates Infectious Virus Mammalian Cells. *Proc Natl Acad Sci USA* 1985, 82:4818–4822.

3.    OMATO T, KOHARA M, SAKAI Y, KAMEDA A, IMURA N, NOMOTO A: Cloned Infectious Complementary DNA of the Poliovirus Sabin 1 Genome: Biochemical and Biological Properties of the Recovered Virus. *Gene* 1984, 32:1–10.

4.    COHEN JI, TICEHURST JR, FEINSTONE SM, ROSENBLUM B, PURCELL RH: Hepatitis A Virus cDNA and its RNA Transcripts are Infectious in Cell Culture. *J Virology* 1987, 61:3035–3039.

5.    MIZUTANI S, COLONNO RJ: *In Vitro* Synthesis of an Infectious RNA from cDNA Clones of Human Rhinovirus Type 14. *J Virology* 1985, 56:628–632.

6.    VAN DER WERF S, BRADLEY J, WIMMER E, STUDIER FW, DUNN JJ: Synthesis of Infectious Poliovirus RNA by Purified T7 RNA Polymerase. *Proc Natl Acad Sci USA* 1986, 83:2330–2334.

7.    DEUCHLER M, SKERN T, BLAAS D, BERGER B, SOMMERGRUGER W, KUECHLER E: Human Rhinovirus Serotype 2: *In Vitro* Synthesis of an Infectious RNA. *Virology* 1989, 168:159–161.

8.    TANGY F, MCALLISTER A, BRACHIC M: Molecular Cloning of the Complete Genome of Strain GDVII of Theiler's Virus

and Production of Infectious Transcripts. *J Virology* 1989, 63:1101–1106.

9. ZIBERT A, MAASS G, STREBEL K, FALK MM, BECK E: Infectious Foot-and-Mouth Disease Virus Derived from a Cloned Full-length DNA. *J Virology* 1990, 64:2467–2473.

10. CLARK ME, DASGUPTA A: A Transcriptionally Active Form of
• TFIIIC is Modified in Poliovirus-infected HeLa Cells. *Mol Cell Biol* 1990, 10:5106–5113.
Physical alterations in complexes between transcription factor TFIIIC and DNA in poliovirus-infected cells were documented and found to correlate with the inhibition of polymerase III-mediated transcription in infected cells. The possibility that either dephosphorylation or limited proteolysis of TFIIIC are responsible for transcription inhibition are discussed. This paper thus adds to the short list of cellular proteins that are altered in picornavirus-infected cells.

11. REN R, CONSTANTINI F, GORGACZ EF, LEE JJ, RACANIELLO VR:
•• Transgenic Mice Expressing a Human Poliovirus Receptor: a New Model of Poliomyelitis. *Cell* 1990, 63:353–362.
The introduction of a cloned poliovirus receptor into transgenic mice not only rendered them infectable by Mahoney type 1 poliovirus, but allowed them, when infected, to develop a pattern of neurovirulent disease quite similar to human poliomyelitis. The transgenic mice provide both a useful model for pathogenesis and an excellent *in vivo* system to study the molecular requirements for productive cell entry to poliovirus.

12. DMITRIEVA TM, NORKINA KB, AGOL VI: Encephalomyocarditis
• Virus RNA Polymerase Preparations, with and without RNA Helicase Activity. *J Virology* 1991, 65:2714–2717.
Preparations of encephalomyocarditis virus RNA-dependent RNA polymerase were shown to perform strand-displacement synthesis by removing long stretches of annealed, complementary RNA from RNA templates during synthesis. Upon purification of the polymerase, the strand-displacement, but not the RNA replication, activity was lost. Whether the 'accessory factor' is a single-stranded RNA-binding protein, a processivity factor for polymerase, or an RNA helicase as suggested by the authors, the ability for RNA-dependent RNA polymerase to displace strands during RNA synthesis is of great interest.

13. MORRISSEY LM, KIRKEGAARD K: Regulation of a Double-
• stranded RNA Modification Activity in Human Cells. *Mol Cell Biol* 1991, 11:3719–3725.
The RNA modification activity that converts adenosine residues to inosine in double-stranded RNA was found to be inhibited in cells infected with synthetic or natural double-stranded RNAs. Thus, the activity is probably not antiviral, as has been suggested by others.

14. SELINKA HC, ZIBERT A, WIMMER E: Poliovirus can Enter and
• Infect Mammalian Cells by Way of an Intercellular Adhesion Molecule 1 Pathway. *Proc Natl Acad Sci USA* 1991, 88:3598–3602.
When the extreme terminal immunoglobulin-like domain of the poliovirus receptor, fused directly with the transmembrane and cytoplasmic domains, was expressed on the surface of mouse cells, it was found to be sufficient to allow poliovirus infection. Furthermore, the expression of this domain attached to a truncated version of ICAM-1, another intracellular adhesion molecule that is the receptor for rhinovirus, conferred susceptibility to poliovirus. Whatever the mechanism of cell entry by picornaviruses, the membrane and cytoplasmic portions of their cellular receptors are interchangeable.

15. YEATES TO, JACOBSON DH, MARTIN A, WYCHOWSKI C, GIRARD
•• M, FILMAN DJ, HOGLE JH: Three-dimensional Structure of a Mouse-adapted Type 2/Type 1 Poliovirus Chimera. *EMBO J* 1991, 10:2331–2341.
A chimeric poliovirion, composed of poliovirus Mahoney type 1 except for the external 'BC' loop, which is derived from the mouse-adapted Lansing type 2 poliovirus, was found to differ in structure from Mahoney type 1 exclusively in the region of the loop. Thus, the ability to utilize a different cellular receptor can be conferred by external loop sequences alone. This finding argues against the hypothesis that poliovirus binds functionally to its cellular receptor only via the viral 'canyon', a recession around the fivefold axis of the virion.

16. PELLETIER J, SONENBERG N: Internal Binding of Ribosomes to the 5' Noncoding Region of a Eukaryotic mRNA: Translation of Poliovirus. *Nature* 1988, 334:320–325.

17. JANG SK, KRÄUSSLICH HG, HICKLIN MJH, DUKE GM, PALMENBERG AC, WIMMER E: A Segment of the 5' Nontranslated Region of Encephalomyocarditis Virus RNA Directs Internal Entry of Ribosomes during *In Vitro* Translation. *J Virology* 1988, 62:2636–2643.

18. BELSHAM GJ, BRANGWYN JK: A Region of the 5' Noncoding Region of Foot-and-Mouth Disease Virus RNA Directs Efficient Internal Initiation of Protein Synthesis within Cells: Involvement with the Role of L Protease in Translational Control. *J Virology* 1990, 64:5389–5395.

19. KUHN R, LUZ N, BECK E: Functional Analysis of the Internal Translation Site of Foot-and-Mouth Disease Virus. *J Virology* 1990, 64:4625–4631.

20. JACKSON RJ, HOWELL MT, KAMINISKI A: The Novel Mecha-
• nism of Initiation of Picornavirus RNA Translation. *Trends Biochem Sci* 1990, 15:477–483.
A current, critical review of the mechanism of translation of picornaviral RNAs. The RNA structures and sequences of the various internal ribosomal entry site sequences are compared, and the known effects of mutagenizing these sequences discussed.

21. MACEJAK DG, SARNOW P: Internal Initiation of Translation
•• Mediated by the 5' Leader of a Cellular mRNA. *Nature* 1991, 353:90–94.
Sequences from the 5' non-translated region of mRNA encoding the human immunoglobulin heavy chain-binding protein were found to confer internal ribosome binding when placed upstream of the second cistron in dicistronic hybrid RNAs. This is the first example of a cellular RNA translated by a mechanism previously thought to be the exclusive domain of picornaviruses.

22. SIMOES EAF, SARNOW P: An RNA Hairpin at the Extreme 5' End of the Poliovirus Genome Modulates Viral Translation in Human Cells. *J Virology* 1991, 65:913–921.

23. ANDINO RM, RIECKHOF GE, BALTIMORE D: A Functional Ribonu-
•• cleoprotein Complex Forms around the 5' End of Poliovirus RNA. *Cell* 1990, 63:369–380.
This paper reports the mutagenesis, chemical probing and protein-binding properties of an RNA secondary structure at the 5' end of the poliovirus positive strand. Studies of mutant viruses and RNAs suggest that the structure and the protein complexes it forms are important for positive strand synthesis, a surprising result.

24. RIVERA R, WELCH JD, MAIZEL JV: Comparative Sequence Analysis of the 5' Noncoding Region of the Enteroviruses and Rhinoviruses. *Virology* 1988, 165:42–50.

25. SKINNER MA, RACANIELLO VR, DUNN G, COOPER J, MINOR PD, ALMOND J: New Model for the Secondary Structure of the 5' Non-coding RNA of Poliovirus is Supported by Biochemical and Genetic Data that also Show that RNA Secondary Structure is Important in Neurovirulence. *J Mol Biol* 1989, 207:379–392.

26. LEE SY, ZUKER M: Common Structures of the 5' Non-coding RNA in Enteroviruses and Rhinoviruses: Thermodynamical Stability and Statistical Significance. *J Mol Biol* 1990, 216:729–741.

27. ANDINO R, RIECKHOF GE, TRONO D, BALTIMORE D: Substitutions in the Protease (3C^pro) Gene of Poliovirus Can Suppress a Mutation of the 5' Noncoding Region. *J Virology* 1990, 64:607–612.

28. GIACHETTI C, SEMLER BL: Role of a Viral Membrane Polypeptide in Strand-specific Initiation of Poliovirus RNA Synthesis. *J Virology* 1991, 65:2647–2654.

29. GIACHETTIC C, SEMLER BL: Molecular Genetic Analysis of Poliovirus RNA Replication by Mutagenesis of a VPg Precursor Polypeptide. In *New Aspects of Positive-strand Viruses* edited by Brinton MA, Heinz FX [book]. Washington DC, American Society for Microbiology 1990, pp 83–93.

30. NOVAK JE, KIRKEGAARD K: Improved Method for Detecting
• Poliovirus Negative Strands Used to Demonstrate Specificity of Positive-strand Encapsidation and the Ratio of Pos-

itive to Negative Strands in Infected Cells. *J Virology* 1991, 65:3384–3387.

A solution hybridization approach for the quantitative determination of negative strands as well as positive viral RNA strands in poliovirus-infected cells is described, and the types of artifacts that can be generated by the use of filter hybridization are documented. The specificity of packaging positive strands, as compared to negative strands, was demonstrated to be greater than 600-fold.

31. MOLLA A, PAUL AV, WIMMER E: **Cell-free, De Novo Synthesis**
•• **of Poliovirus.** *Science* 1991, 253:1647–1651.

An *in vitro* system containing HeLa cell extract and positive-strand poliovirus RNA was shown to direct the synthesis of intact, infectious virions. A variety of control experiments strongly suggest that the production of virions involved negative-strand synthesis, positive-strand synthesis and virion assembly, all in the same test tube, making this paper a milestone in the fields of viral RNA synthesis and morphogenesis.

32. MARC D, GIRARD M, VAN DER WERF S: **A Gly¹ to Ala Substitution in Poliovirus Capsid Protein VP0 Blocks its Myristoylation and Prevents Viral Assembly.** *J Gen Virology* 1991, 72:1151–1157.

33. CHOW M, NEWMAN JFE, FILMAN DJ, HOGLE JM, ROWLANDS DJ, BROWN F: **Myristoylation of Picornavirus Capsid Protein VP4 and its Structural Significance.** *Nature* 1987, 327:482–486.

34. MOSCUFO N, SIMONS J, CHOW M: **Myristoylation is Important at Multiple Stages in Poliovirus Assembly.** *J Virology* 1991, 65:2372–2380.

35. SEMLER BL, KUHN RJ, WIMMER E: **Replication of the Poliovirus Genome.** In *RNA Genetics, Vol 1* edited by Domingo E, Holland JJ, Ahiquist PA [book]. Boca Raton, Florida: CRC Press, 1988, pp 23–48.

36. HARMON SA, RICHARDS OC, SUMMERS DF, EHRENFELD E: **The**
• **5′ Terminal Nucleotides of Hepatitis A Virus RNA, but not Poliovirus RNA, are Required for Infectivity.** *J Virology* 1991, 65:2757–2760.

As was originally shown for coxsackievirus B3 [37], the most 5′ terminal nucleotides, even if they were not present in a transfected RNA, were found to be regenerated during the ensuing infection. This is interpreted as support for the non-templated synthesis of the initial two nucleotides of picornaviruses. That these nucleotides were required for the infectivity of hepatitis A viral RNA is thought to result from their additional involvement in a crucial RNA structure in this virus.

37. KLUMP WM, BERGMANN I, MULLER BC, AMEIS D, KANDOLF R: **Complete Nucleotide Sequence of Infectious Coxsackievirus B3 cDNA: Two Initial 5′ Uridine Residues are Regained During Plus-strand RNA Synthesis.** *J Virology* 1990, 64:1473–1583.

38. SARNOW P, JACOBSON SJ, NAJITA L: **Poliovirus Genetics.** *Curr Top Microbiol Immunol* 1990, 161:155–188.

39. KUGE S, SAITO I, NOMOTO A: **Primary Structure of Poliovirus Defective-interfering Particle Genome and Possible Generation Mechanisms of the Particles.** *J Mol Biol* 1986, 192:473–487.

40. HAGINO-YAMAGISHI K, NOMOTO A: **In Vitro Construction of Poliovirus Defective Interfering Particles.** *J Virology* 1989, 63:5386–5392.

41. KAPLAN G, RACANIELLO VR: **Construction and Characterization of Poliovirus Subgenomic Replicons.** *J Virology* 1988, 62:1687–1696.

42. CHOI WS, PAL-GHOSH R, MORROW CD: **Expression of Human**
• **Immunodeficiency Virus Type 1 (HIV-1) gag, pol, and env Proteins from Chimeric HIV-1-Poliovirus Minireplicons.** *J Virology* 1991, 65:2875–2883.

Coding regions from HIV-1 proteins were inserted into poliovirus 'minireplicons', RNA genomes transcribed *in vitro* bearing deletions in the capsid-encoding region of the genome. The transfected RNAs were replicated in the absence of helper virus, and HIV antigens were successfully expressed in the transfected cells. Although the progeny genomes were not packaged, the idea of using poliovirus vaccines expressing foreign antigens intracellularly, with the attendant possibilities for the induction of mucosal immunity, is exciting.

43. BERNSTEIN HD, SARNOW P, BALTIMORE D: **Genetic Complementation among Poliovirus Genomes Derived from an Infectious cDNA Clone.** *J Virology* 1986, 60:1040–1049.

44. LI JP, BALTIMORE: **Isolation of Poliovirus 2C Mutants Defective in Viral RNA Synthesis.** *J Virology* 1988, 62:4016–4021.

45. JOHNSON KL, SARNOW P: **Three Poliovirus 2B Mutants Ex-**
• **hibit Noncomplementable Defects in Viral RNA Amplification and Display Dosage-dependent Dominance over Wild-type Poliovirus.** *J Virology* 1991, 65:4341–4349.

In this work, a lack of complementation was shown with two new alleles of 2B displaying primary defects in RNA synthesis. Three 2B mutants were, however, shown to interfere with wild-type infections, thereby supporting the argument that 2B can function in *trans* to interfere with wild-type virus.

46. CHARINI WA, BURNS CC, EHRENFELD E, SEMLER BL: **Trans Res-**
•• **cue of a Mutant Poliovirus RNA Polymerase Function.** *J Virology* 1991, 65:2655–2665.

A temperature-sensitive poliovirus mutant whose defect maps to the RNA-dependent RNA polymerase coding region was shown to be complementable for RNA replication during coinfection with a wild-type virus. This observation is discussed in the context of various models of *cis*-action of poliovirus replication proteins.

47. JARVIS TC, KIRKEGAARD K: **The Polymerase in its Labyrinth:**
• **Mechanisms and Implications of RNA Recombination.** *Trends Genet* 1991, 7:186–191.

The incidence and mechanism of RNA recombination in viruses is reviewed. Evidence for copy-choice replication and recombination in DNA-based replication systems is discussed, and processive and distributive models for copy-choice recombination are compared.

48. HAHN CS, LUSTIG S, STRAUSS EG, STRAUSS JH: **Western Equine Encephalitis Virus is a Recombinant Virus.** *Proc Natl Acad Sci USA* 1988, 85:5997–6001.

49. WEISS BG, SCHLESINGER S: **Recombination Between Sindbis**
•• **Virus RNAs.** *J Virology* 1991, 65:4017–4025.

Deleted or mutated Sindbis viral RNAs, generated *in vitro*, were transfected into cells. At a readily detectable frequency, infectious virus was generated, apparently by RNA recombination. This is the first report of RNA recombination among alphaviruses in a laboratory situation, and lends support to the idea that recombination among viral RNAs may be the rule rather than the exception.

50. RUECKERT RR: **Picornaviridae and their Replication.** In *Virology* edited by Fields BN, Knipe DM [book]. New York: Raven Press, 1990, pp 507–548.

51. TRONO D, ANDINO R, BALTIMORE D: **An RNA Sequence of Hundreds of Nucleotides at the 5′ End of Poliovirus RNA is Involved in Allowing Viral Protein Synthesis.** *J Virology* 1988, 62:2291–229.

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