*Proc. Natl. Acad. Sci. USA*
Vol. 82, pp. 732–736, February 1985
Biochemistry

# Molecular cloning and complete sequence determination of RNA genome of human rhinovirus type 14

**(recombinant DNA/translation frame/sequence comparisons/proteolytic cleavage sites)**

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*Communicated by Edward M. Scolnick, October 3, 1984*

ABSTRACT **The genomic RNA of human rhinovirus type 14 was cloned in *Escherichia coli* and the complete nucleotide sequence was determined. The RNA genome is 7212 nucleotides long. A single large open reading frame of 6536 nucleotides was identified, which starts at nucleotide 678 and ends 47 nucleotides from the 3′ end of the RNA genome. Comparisons of the specified proteins with those of other picornaviruses showed a striking homology (44–65%) between rhinovirus and poliovirus. The rhinovirus genomic RNA is rich in adenosine (32.1%) and strongly favors an adenosine or uridine in the third position of codons. The predicted map locations of all the rhinovirus structural and non-structural proteins and their proposed proteolytic cleavage sites are described.**

Rhinoviruses are the most important common cold viruses known. The name "rhinovirus" reflects the prominent nasal involvement seen in infections with these viruses. Human rhinoviruses (HRVs) are members of the picornavirus family, which also contains the entero- (polio, coxsackie), cardio- [encephalomyocarditis (EMC), Mengo], and aphthoviruses [foot-and-mouth disease virus (FMDV)] (1). As many as 115 HRV serotypes have been identified (2). Similar to other picornaviruses, the rhinoviruses contain a single-stranded RNA genome that serves as a monocistronic mRNA for the synthesis of all viral structural and non-structural proteins. Studies with poliovirus have indicated that a protein (VPg) is attached by a tyrosine-$O^4$-phosphodiester bond to the 5′ pUp of the RNA genome. In addition, all the picornaviruses contain a short poly(A) stretch at the 3′ end of their genomic RNA (3). A variety of experimental results have suggested that the genomic RNA of picornaviruses is translated into a single polypeptide from which functional viral proteins are derived by proteolysis.

The fact that the RNA genome of picornaviruses is infectious, illustrates the importance of knowing the sequence of the RNA genome. The complete nucleotide sequences of the genomic RNAs of polio, EMC, and FMD viruses recently have been determined (4–7). We now report the sequence of the RNA genome of a single HRV serotype.

## MATERIALS AND METHODS

**Virus Growth and Purification.** HRV type 14 (HRV-14) was obtained from the American Type Culture Collection and plaque-purified by standard techniques. The growth and purification of HRVs have been described recently (8).

**Genomic RNA Isolation.** Purified HRV-14 was digested with proteinase K (0.5 mg/ml) in 10 mM Tris Cl/1 mM EDTA/0.3% $NaDodSO_4$, pH 7.5, for 30 min at 37°C. Viral genomic RNA was separated from digested protein by oligo(dT)-cellulose chromatography (9). After precipitation with ethanol, the RNA was suspended in 0.3 ml of 10 mM Tris Cl/1 mM EDTA/0.5% $NaDodSO_4$, pH 7.5, and layered on a 12-ml preformed 15–30% (wt/wt) sucrose gradient in the same buffer but containing 0.1 M NaCl. RNA was sedimented at 23,000 rpm for 17 hr at 4°C in a Beckman SW40 rotor. Gradients were fractionated (0.4 ml) with an ISCO gradient fractionator and the $A_{260}$ of each fraction was measured. A major peak of viral RNA sedimenting at 35 S, relative to the position of marker 18S and 28S RNAs in a parallel gradient, was pooled and precipitated with ethanol.

**Construction of Double-Stranded cDNA.** The procedures used for making cDNA to the genomic RNA and converting the cDNA to oligo(dC)-tailed, double-stranded cDNA were as described by Maniatis *et al.* (10). To insert the oligo(dC)-tailed cDNA into pBR322, 25 ng of pBR322-oligo(dG) cloning vector was annealed with 13, 26, or 39 ng of oligo(dC)-tailed cDNA and used to transform calcium-treated *Escherichia coli* strain RRI (10). The transformants were selected on tetracycline-containing agar plates.

**Characterization of Selected Clones.** Colonies (354) were picked and each was used to inoculate 2 ml of L broth containing 12 mg of $Na_2HPO_4$, 6 mg of $KH_2PO_4$, 1 mg of NaCl, 2 mg of $NH_4Cl$, and 4 mg of glucose. The cultures were shaken at 37°C for 16 hr, then 1.5 ml of each culture was pelleted by centrifugation. Plasmid DNA was isolated by using the miniprep protocol of Holmes and Quigley (11). The resulting plasmid DNA preparations were each suspended in 50 μl of $H_2O$, 10 μl of each was digested with the restriction endonuclease *Pst* I, and the size of its cDNA insert was determined by electrophoresis in 1% agarose gel. Clones containing inserts of at least 1500 base pairs (bp) were mapped by colony-hybridization studies (12).

**Construction of Deletion Subclones.** Deletion clones were constructed as described by Hong (13). Briefly, 1 μg of isolated cDNA insert from either clone 7 or clone 186 was ligated to 2.8 μg of linearized, *Pst* I-digested pUC9 DNA (10). *E. coli* strain HB101 was transformed with each of the ligation mixtures and transformants were selected on agar plates containing ampicillin at 100 μg/ml. pUC9 plasmids containing clone-7 or clone-186 inserts were linearized with DNase I and digested with *Sal* I, and the resulting overhanging ends were filled in with DNA polymerase (10). After blunt-end ligation, the DNA was used to transform *E. coli* strain HB101 cells. Transformants were screened for size of inserts, and clones were selected that represented a distribution of sizes.

**Nucleic Acid Sequencing of cDNA Inserts.** Digestion of clones 7, 57, and 186 and their subsets of deletion clones with various commercially available restriction enzymes generated a set of overlapping DNA fragments. These fragments were $^{32}P$-labeled as described (10). Labeled DNA fragments were asymmetrically cut with a second restriction enzyme to generate fragments with only one radioactively

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Abbreviations: HRV, human rhinovirus; EMCV, encephalomyocarditis virus; FMDV, foot-and-mouth disease virus; PV-1, poliovirus type 1; bp, base pair(s).

labeled end. After isolation by electrophoresis in 5% acrylamide/bisacrylylcystamine gels (14), labeled DNA fragments were sequenced by using the chemical methods described by Maxam and Gilbert (15). Alternatively, synthetic deoxynucleotide primers and Sanger dideoxy sequencing techniques (16) were used to complete the determination of the nucleotide sequence. Both strands of cDNA were sequenced in most instances, and all sequence reactions were done at least twice. A continuous DNA sequence of 7227 nucleotides was obtained using the Intelligenetics software package. All $^{32}$P-labeled triphosphates were purchased from Amersham. DNA-modifying enzymes and restriction endonucleases were purchased from Boehringer Mannheim.

## RESULTS

**Cloning of Rhinovirus Double-Stranded cDNA.** Genomic RNA from plaque-purified HRV-14 was extracted and then purified by oligo(dT)-cellulose chromatography and sucrose gradient centrifugation. The 35S genomic RNA was used as a template for the synthesis of cDNA, using reverse transcriptase and an oligo(dT) primer. Following second-strand synthesis with DNA polymerase I, the cDNA was trimmed with nuclease S1 and tailed with oligo(dC) prior to insertion into the *Pst* I site of oligo(dG)-tailed pBR322. The newly constructed plasmids were then used to transform competent *E. coli* RRI cells. Each resulting cDNA clone was characterized for the size of its cDNA insert. Clones containing inserts of >1500 bp were analyzed by restriction endonuclease mapping and colony hybridization to determine their relationship to each other. Clones 7 (3230 bp), 57 (1910 bp), and 186 (3223 bp) were found to overlap and represent a 6784-nucleotide segment of the genomic RNA. Clone 186, which contained a 3′ poly(A) of 15 nucleotides hybridized to total cellular RNA from infected but not uninfected cells, and thus contained viral-encoded sequences (data not shown). The precise map location of each clone was determined from restriction digests and is shown in Fig. 1. Since clone 7 did not appear to extend far enough to represent the 5′-terminal region, a synthetic primer complementary to viral genome nucleotides 864–878 was used to generate a new set of cDNA clones as above. A new cDNA clone designated 198 was isolated, which overlapped cDNA clone 7 and extended 430 nucleotides toward the 5′ end of the genome

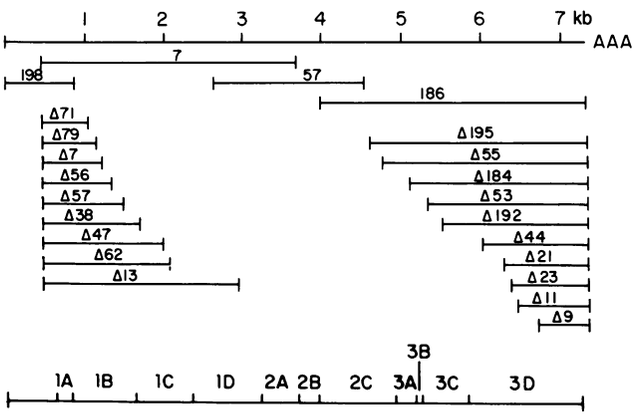


FIG. 1. Mapping of HRV-14 cDNA clones to the viral RNA genome. The topmost line represents the HRV-14 RNA genome; numbers above the line indicate sequence positions in kilobases (kb), and AAA, the poly(A) tail. The positions of cDNA clones 198, 7, 57, and 186 were determined by restriction endonuclease mapping. Deletion clones (designated by Δ) were constructed from cDNA clones 7 and 186 as described in *Materials and Methods*. The HRV-14 protein map is shown at the bottom and was determined by analogy to poliovirus. Protein nomenclature is that of Rueckert and Wimmer (17).

RNA. Sequence data (see below) indicated that clone 198 represents a sequence starting at genomic RNA nucleotide 13.

**Construction of Deletion Clones.** To aid in the rapid sequencing of cDNA clones 7 and 186, a subset of deletion clones representing various deletions from the 5′ and 3′ ends of clones 186 and 7, respectively, were constructed. Inserts of clones 7 and 186 were put into pUC9 plasmids that had been linearized with DNase I and cut with the restriction endonuclease *Sal* I. No *Sal* I or *Pst* I sites were found in the HRV-14 sequence. Following ligation and transformation of *E. coli*, clones were selected to give a set of clones that had inserts corresponding to various lengths of the original clones 7 and 186 (Fig. 1).

**Nucleotide Sequence of the Rhinovirus Genome.** The sequence of cDNA clone inserts were determined predominantly (>95%) by using the Maxam–Gilbert chemical sequencing method (15). The 5′ end of the RNA genome and regions lacking convenient restriction sites were sequenced by using dideoxy sequencing methods (16) in which synthetic primers were synthesized from sequenced regions and used to prime cDNA synthesis on the genomic RNA template. Deletion clones were labeled at the *Eco*RI or *Bam*HI sites of the pUC9 polylinker and sequenced by using the Maxam–Gilbert method. The first 6 nucleotides at the 5′ end were confirmed by sequential single-nucleotide extension of a $^{32}$P-labeled synthetic oligodeoxynucleotide representing genome nucleotides 7–27. The results of this experiment confirmed the 5′ sequence U-U-A-A-A-A for the HRV-14 RNA genome, since addition of only dTTP extended the primer by 4 nucleotides and addition of dATP and dTTP extended the primer by 6 nucleotides, whereas addition of dCTP and dGTP had no effect (data not shown). The first 10 nucleotides of poliovirus type 1 (PV-1) and HRV-14 are identical.

The Intelligenetics software package was used to merge sequence gel readings into a single DNA sequence containing 7227 nucleotides including the poly(A) tail (Fig. 2). Computer-generated translation of the DNA sequence in all three reading frames (Fig. 3) revealed a single long open reading frame 6537 nucleotides long. The open reading frame initiates at nucleotide 629, following four unused AUG codons in the same reading frame at nucleotide positions 59, 332, 395, and 419. In addition, there are nine AUG codons in the other two reading frames prior to the AUG at position 629.

Base composition of the HRV-14 genomic RNA, minus the poly(A), showed a high adenine content (32.1%), followed by uracil (27.3%), guanine (20.5%) and cytosine (20.1%). Codon frequency analysis supported the high adenine content by showing a strong preference for adenosine and uridine in the third position of codons. The triplet CGG does not occur at all, and GCG and UCG occur only two and three times, respectively. The predicted polyprotein contains only 1.1% tryptophan, 2.0% cysteine, 2.4% histidine, and 2.4% methionine, but it is rich in leucine (8.9%) and threonine (8.0%). The computer-generated lengths and molecular weights for all the viral proteins are listed in Table 1. This assignment of protein map positions was done solely by analogy to the PV-1 sequence. The postulated proteolytic cleavage sites (Table 2) must be considered tentative until protein sequences are obtained.

## DISCUSSION

HRVs are the leading cause of the common cold in humans (18). Although up to 115 antigenically distinct serotypes have been identified, little is known at the molecular level about any of the viruses. A knowledge of the RNA genome sequence is necessary for a full understanding of the functions of the encoded proteins involved in virus structure

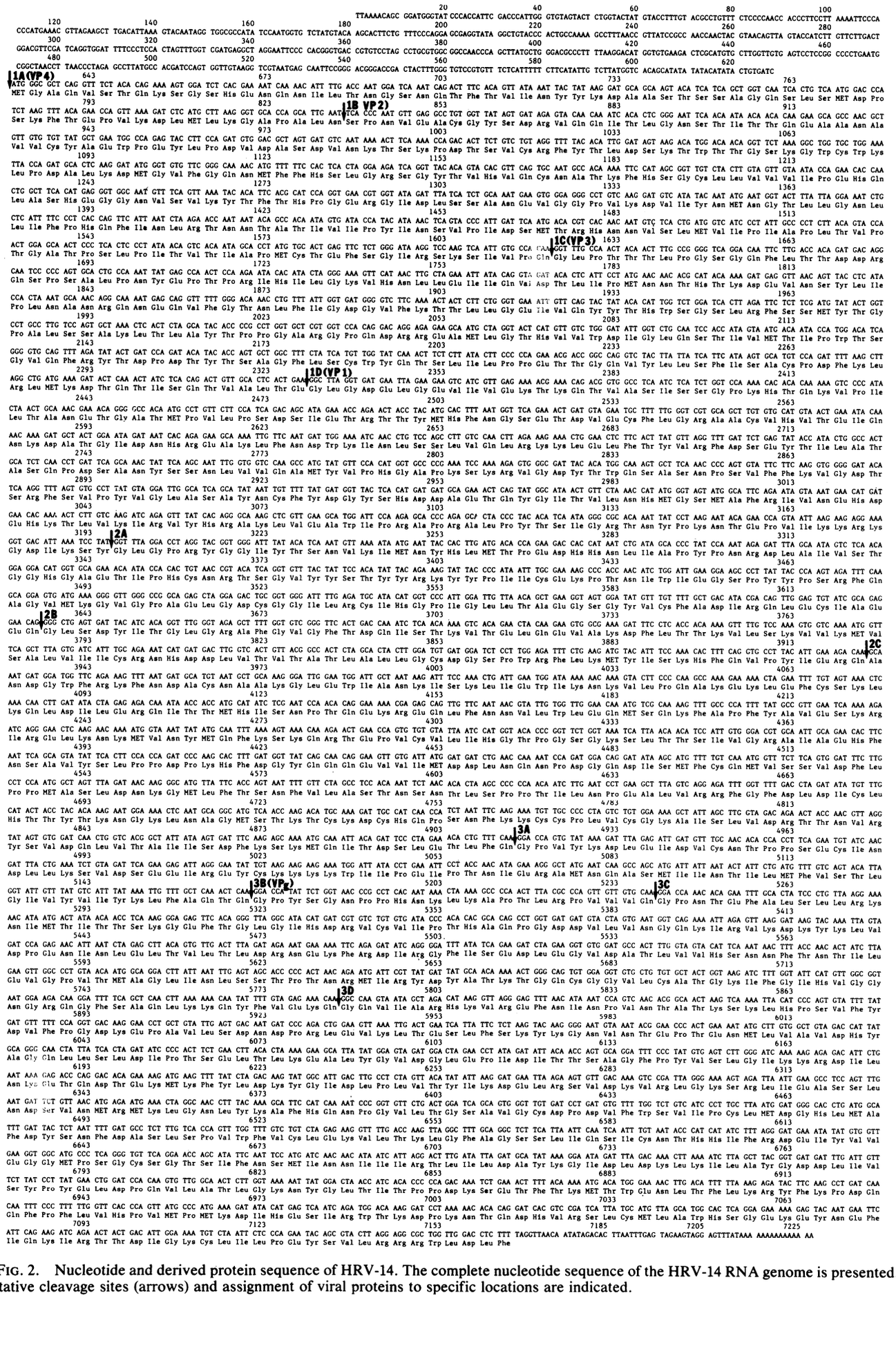


FIG. 2. Nucleotide and derived protein sequence of HRV-14. The complete nucleotide sequence of the HRV-14 RNA genome is presented. Putative cleavage sites (arrows) and assignment of viral proteins to specific locations are indicated.

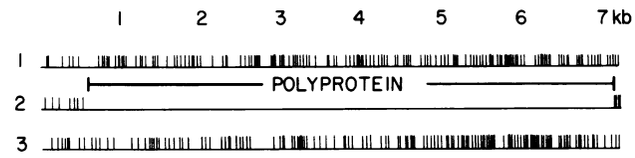


FIG. 3. Translation of HRV-14 genomic RNA. Computer translation of the sequence in Fig. 2 was performed in all three reading frames, numbered 1–3 on the left. The vertical lines designate the occurrence of stop codons. kb, Kilobases.

and replication. In addition, determination of the genome sequence allows definitive comparisons between the four families of picornaviruses to determine sequence conservation in viral proteins and evolutionary relationships between viruses.

We have cloned and sequenced the entire RNA genome of HRV-14. Four cDNA clones, designated 198 (866 bp, nucleotides 13–878), 7 (3231 bp, nucleotides 443–3673), 57 (1911 bp, nucleotides 2624–4534), and 186 (3224 bp, nucleotides 4004–7227), were isolated which overlapped and represented 99.8% of the entire genome sequence. Random deletion of the 3′ and 5′ ends of cDNA clones 7 and 186, respectively, yielded a subset of clones used to facilitate rapid sequencing of the cDNA. The map positions of all these clones are shown in Fig. 1.

Translation of the cDNA in all three reading frames shows a single large open reading frame 6637 nucleotides long with initiation 34 codons downstream at position 629. This would predict a 5′ 628-nucleotide nontranslated region, which contains four unused AUG codons upstream and in the same reading frame. The four unused AUG codons would initiate peptides with amino acid lengths of only 2, 8, 10, and 24. Analysis of the other two reading frames in the 5′ noncoding region shows nine additional initiation codons. Six of these would result in proteins <29 amino acids long. The remaining three (nucleotide locations 496, 526, and 595) fall within the first reading frame (Fig. 3) and could initiate an amino acid sequence of up to 77 amino acids, depending on which AUG was utilized as a start codon. Although these starts cannot be ruled out completely, they seem highly unlikely for the following reasons: First, they would result in relatively short peptides; second, they lack efficient flanking sequences for ribosome recognition as described by Kozak (19), which are present around the AUG at position 629; third, comparative studies among the three serotypes of poliovirus demonstrated that the AUG codons found in similar positions in the 5′ noncoding region are not conserved, in contrast to the strong homology found within the true coding sequence (20). In addition, comparison of the 1A (first capsid protein to be translated) sequences of HRV-14 and PV-1 shows that initiation at position 629 of HRV-14 gives a 1A sequence identical to that of PV-1 1A at positions 1–6 and at 13 of the first 17 amino acids.

Table 1. Size of predicted HRV-14 proteins

| Protein | Nucleotides | Number of amino acids | Predicted $M_r$ |
|---|---|---|---|
| Polyprotein | 629–7165 | 2178 | 242,593 |
| 1A (VP4) | 629–835 | 69 | 7,310 |
| 1B (VP2) | 836–1621 | 262 | 28,506 |
| 1C (VP3) | 1622–2329 | 236 | 26,198 |
| 1D (VP1) | 2330–3196 | 289 | 32,384 |
| 2A | 3197–3634 | 146 | 16,090 |
| 2B | 3635–3925 | 97 | 10,796 |
| 2C | 3926–4915 | 330 | 37,400 |
| 3A | 4916–5170 | 85 | 9,751 |
| 3B (VPg) | 5171–5239 | 23 | 2,470 |
| 3C | 5240–5785 | 182 | 19,999 |
| 3D | 5786–7165 | 460 | 52,217 |

Table 2. Predicted HRV-14 polyprotein cleavage sites

| Proteins | Cleavage site |
|---|---|
| 1A/1B | -Pro-Ala-Leu-Asn/Ser-Pro-Asn-Val- |
| 1B/1C | -Ile-Val-Pro-Gln/Gly-Leu-Gly-Asp- |
| 1C/1D | -Ala-Leu-Thr-Glu/Gly-Leu-Gly-Asp- |
| 1D/2A | -Ile-Lys-Ser-Tyr/Gly-Leu-Gly-Pro- |
| 2A/2B | -Ala-Glu-Glu-Gln/Gly-Leu-Ser-Asp- |
| 2B/2C | -Ile-Glu-Arg-Gln/Ala-Asn-Asp-Gly- |
| 2C/3A | -Thr-Leu-Phe-Gln/Gly-Pro-Val-Tyr- |
| 3A/3B | -Ala-Gln-Thr-Gln/Gly-Pro-Tyr-Ser- |
| 3B/3C | -Val-Val-Val-Gln/Gly-Pro-Asn-Thr- |
| 3C/3D | -Val-Glu-Lys-Gln/Gly-Gln-Val-Ile- |

Alignment of the HRV-14 5′ noncoding sequence to the first 600 bases of the PV-1 sequence shows an RNA homology in the range of 75%, depending on how the alignment is done. Interestingly, the major difference between them occurs just 5′ of the proposed initiation codon where HRV-14 is missing 114 bases that are present in the PV-1 5′ noncoding region. This is the precise location where each of the different poliovirus serotypes differs dramatically (20). The function of this region in picornaviruses remains unknown. In addition, computer programs predict a stem and loop structure at the 5′ end of the HRV-14 genome sequence that is very similar to that found in PV-1 (4).

Protein-to-protein comparisons between HRV-14, PV-1, EMCV, and FMDV sequences show that PV-1 and HRV-14 are closely related, whereas sequence homology to EMCV or FMDV is not apparent. The map positions of the viral proteins were determined solely by analogy to PV-1, since no amino acid sequence was determined directly from isolated proteins. However, based on the strong homology with PV-1 proteins and similarity in the predicted size of the HRV-14 viral proteins, the assignment of amino acid sequence to proteins appears to be correct. The general genomic organization is similar to those of other picornaviruses. Protein nomenclature used is the 4-3-4 system recently described by Rueckert and Wimmer (17). Comparisons of homologies for non-structural proteins between HRV-14 and PV-1 ranged from 44% in the protease gene 3C to 65% in the replicase gene 3D. Recent studies have postulated that the protease of picornaviruses is a cysteine protease with the cysteine at position 146 of the PV-1 3C within the active site (21). The protease gene of HRV-14 encodes the same conserved amino acid sequence (Gly-Gln-Cys-Gly-Gly-Val at residues 154–159) that is found in PV-1. A similar sequence (Gly-Trp-Cys-Gly-Ser-Ala) is found in EMCV protease (6).

Comparison of the four structural proteins gave interesting results. 1A (VP4), which is an internal structural protein, and 1B (VP2) show a 60% amino acid homology to 1A and 1B of PV-1. Although 1A shows conserved sequences throughout its length, 1B contains a very different region (amino acids 134–184), which corresponds to a PV-1 neutralization epitope (22) and is probably the same in HRV-14. Comparisons of 1D (VP1) and 1C (VP3), which contain most of the immunodominant neutralizing epitopes of PV-1, show only 47% and 44% homology, respectively. Comparisons of these

sequences have also allowed us to predict possible neutralizing sites. For example, the amino acid sequences 62–95 of 1C (VP3) and 91–95 of 1D (VP1) are unique to HRV-14 and map to known neutralizing sites in PV-1 (ref. 23; E. Emini, personal communication). No open reading frame large enough to code for the leader proteins found in EMCV and FMDV is found 5′ of the amino-terminal methionine codon of 1A (VP4) in HRV-14.

The viral-encoded protease of HRV-14, like those of other picornaviruses, is presumably crucial for proper proteolytic cleavage of the viral polyprotein and for effective replication of the virus. The predicted proteolytic cleavage sites are shown in Table 2. Like other picornaviruses, there is a predominance of Gln-Gly sites (6 of 10) in addition to a Glu-Gly, a Tyr-Gly, an Asn-Ser, and a Gln-Ala. Eight of the cleavage dipeptides are identical in both sequence and location to those in PV-1. The Glu-Gly cleavage site between 1C and 1D is not found in either PV-1 (4) or EMCV, but five are present in FMDV (7). Only one uncleaved Gln-Gly sequence exists in the HRV-14 sequence (within the protease region), but multiple uncleaved dipeptides identical to the other dipeptide cleavage sites are found. The Gln-Ala cleavage site, if utilized, is unique to HRV and not found in PV-1, EMCV, or FMDV. As in other picornaviruses, the flanking amino acid sequences around the cleavage sites do not fall into a specific pattern but do contain a proline either amino- or carboxyl-terminal to the cleaved amino acid pair in half the sites, which is similar to the situation in EMCV (6). This lack of uniformity of sequence surrounding the cleavage sites in all four picornaviruses clearly indicates involvement of secondary or tertiary structure in the precursor molecule.

The 3′ noncoding region is 47 nucleotides long in HRV-14. This compares to 72 in PV-1, 96 in FMDV, and 126 in EMCV (4–7). Both HRV-14 and FMDV have single stop codons, whereas PV-1 and EMCV have double stops. All four picornaviruses lack the polyadenylylation signal sequence A-A-U-A-A-A, which supports the finding that the poly(A) tail is transcribed from a minus-strand template.

Further sequence comparisons to other HRV serotypes will help to generate a genomic map of common and unique regions. This information will aid in mapping neutralization sites, active sites of the protease and the replicase, and viral receptor binding sites. Recent competition binding studies have shown clearly that HRV-14 competes with the vast majority of other HRV serotypes for attachment to HeLa cell receptors (8). Since this receptor is distinct from other picornaviruses, determination of this highly conserved region in HRV serotypes will be of major importance.

We thank Nancy Dunn and Peter Kniskern for synthesis of DNA primers and thank Ann Palmenberg (University of Wisconsin) for helpful discussions.

1. Cooper, P. D., Agol, V. I., Bachrach, H. L., Brown, F., Ghendon, Y., Gibbs, A. J., Gillespie, J. H., Lonberg-Holm, K., Mandel, B., Melnick, J. L., Mohanty, S. B., Povey, R. C., Rueckert, R. R., Schaffer, F. L. & Tyrrell, D. A. J. (1978) *Intervirology* **10,** 165–180.
2. Melnick, J. L. (1980) *Prog. Med. Virol.* **26,** 214–232.
3. Agol, V. I. (1980) *Prog. Med. Virol.* **26,** 119–157.
4. Kitamura, N., Semler, B. L., Rothberg, P. G., Larsen, G. R., Adler, C. J., Dorner, A. J., Emini, E. A., Hanecak, R., Lee, J. J., van der Werf, S., Anderson, C. W. & Wimmer, E. (1981) *Nature (London)* **291,** 547–553.
5. Racaniello, V. R. & Baltimore, D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4887–4891.
6. Palmenberg, A. C., Kirby, E. M., Janda, M. R., Drake, N. L., Duke, G. M., Potratz, K. F. & Collett, M. S. (1984) *Nucleic Acids Res.* **12,** 2969–2985.
7. Carroll, A. R., Rowlands, D. T. & Clarke, B. E. (1984) *Nucleic Acids Res.* **12,** 2461–2472.
8. Abraham, G. & Colonno, R. J. (1984) *J. Virol.* **51,** 340–345.
9. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
10. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
11. Holmes, D. S. & Quigley, M. (1981) *Anal. Biochem.* **114,** 193–197.
12. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
13. Hong, G. F. (1982) *J. Mol. Biol.* **158,** 539–549.
14. Hansen, J. N., Pheiffer, B. H. & Boehnert, J. A. (1980) *Anal. Biochem.* **105,** 192–201.
15. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
16. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
17. Rueckert, R. R. & Wimmer, E. (1984) *J. Virol.* **50,** 957–959.
18. Gwaltney, J. M. (1975) *Yale J. Biol. Med.* **48,** 17–45.
19. Kozak, M. (1981) *Nucleic Acids Res.* **9,** 5233–5252.
20. Toyoda, H., Kohara, M., Kataoka, Y., Suganuma, T., Omata, T., Imura, N. & Nomoto, A. (1984) *J. Mol. Biol.* **174,** 561–585.
21. Argos, P., Kamer, G., Nicklin, M. J. H. & Wimmer, E. (1984) *Nucleic Acids Res.* **12,** 7251–7267.
22. Emini, E. A., Jameson, B. A. & Wimmer, E. (1984) *J. Virol.* **52,** 719–721.
23. Emini, E. A., Jameson, B. A. & Wimmer, E. (1983) *Nature (London)* **304,** 699–703.