Abstract. The complete nucleotide sequence of genome segment 4 from the human group C rotavirus (Bristol strain) was determined. Comparison of the nucleotide sequences of the genome termini with the consensus 5' and 3' terminal non-coding sequences of the human group C rotavirus genome revealed characteristic 5' and 3' sequence motifs. Human group C rotavirus genome segment 4 is 2,166bp long and encodes a single open reading frame of 2,082 nucleotides (693 amino acids) starting at nucleotide 55 and terminating at nucleotide 2,136 giving a 3' untranslated region of 30 nucleotides. Alignment with the porcine group C VP3 equivalent gene showed the human gene is one amino acid longer, and that the proteins have 84.1% amino acid sequence identity. A conserved potential nucleotide binding motif shared with the porcine VP3 sequence was identified. Analogy with the group A rotaviruses suggested that the genome segment 4 encodes the group C rotavirus guanylyltransferase.

Key Words: Rotavirus, dsRNA, VP3 gene

Introduction

Rotaviruses are the most important cause of acute gastroenteritis in infants, young children and animals (1). The infectious virions are morphologically identical, 70nm in diameter and have a protein shell composed of three distinct layers (2). Each particle contains a genome comprising 11 segments of double stranded RNA. The complete nucleotide sequence of the simian rotavirus SA11 is 18,555bp with the genome segments ranging in size from 667bp for segment 11 to 3,302bp for segment 1 (3,4).

Despite overall similarities in morphology and genome structure rotaviruses have been subdivided into seven separate serogroups labelled from A to G (5). However, rotaviruses in only groups A, B, and C have been documented as human pathogens.

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence database and have been assigned the accession number X96697.

Acute nonbacterial gastroenteritis in children is caused mainly by group A rotaviruses. The group A viruses are not a significant cause of disease in adults, however major outbreaks of adult diarrhoea in China have been associated with human group B rotaviruses (6). In contrast, Group C rotaviruses cause sporadic infections worldwide involving both adults and children (5).

We used a sequence-independent single primer amplification technique to allow the molecular cloning of the Bristol strain of human group C rotavirus (7). This method is designed to produce full length cDNA for each segment of the genome which can be amplified together in a single reaction. The method works by adding a single primer to the 3' termini of the double stranded RNA allowing cDNA synthesis to occur simultaneously for both strands using a complementary primer. Thus the technique defines the 5' and 3' termini of each genome segment in full length cDNA copies. The purpose of this work was to identify and characterise cDNA representing the core protein VP3. We present the
complete nucleotide sequence and the gene coding assignment of human group C rotavirus VP3.

Materials and Methods

Human Group C Rotavirus dsRNA

The human group C rotavirus isolate (Bristol strain) has been described previously (8). This isolate was confirmed as a group C rotavirus by the characteristic 'electropherotype' on SDS-PAGE. Human group C rotavirus dsRNA was extracted and purified from clinical specimens using RNAzol B™ and the Geneclean II™ method as previously described (9).

Screening for VP3 Recombinants

A cDNA library of the Bristol human group C rotavirus was constructed by single primer amplification as described previously (7). Recombinants from the library were sequenced and compared to known rotaviral sequences in the EMBL nucleotide sequence database. Recombinants which matched rotavirus VP3 sequences were selected for further analysis.

Gene Coding Assignment

A probe for the gene coding assignment was prepared using the PCR to amplify a full-length VP3 insert. Probe DNA (100ng) consisting of this PCR product was labelled with α [32P] dATP using the Prime-a-gene kit (Promega). Electrophoretic profiles of human (Bristol) group C rotavirus dsRNA were electroblotted onto Hybond™ N membranes and hybridisation was performed as described (9).

Sequence Analysis

Recombinant M13 templates, prepared using standard techniques were sequenced with universal primer using the Taq dyedeoxy™ terminator sequencing kit (Applied Biosystems) and analysed on an Applied Biosystems model 373A sequencer according to the manufacturers' instructions. A series of internal custom primers were used to extend sequences obtained using universal primer.

Results and Discussion

Gene Coding Assignment

Northern blot analysis of group C rotavirus dsRNA with a probe representing the full-length VP3 insert indicates that this gene hybridises to either genome segment 3 or 4 (Fig. 1). In human

![Fig. 1. Northern blot showing the genome coding assignment for the human group C rotavirus VP3 gene. The relative migration positions of all eleven dsRNA segments, following SDS-PAGE and transfer to a Hybond N membrane (7) are shown diagrammatically in lane 1. A similar strip of genomic dsRNA probed with a full-length [32P] labelled PCR generated probe to the VP3 gene is shown in lane 2.](image-url)