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VIRUS 00523

Detailed structural analysis of a genome rearrangement in bovine rotavirus

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(Accepted 21 June 1989)

Summary

A genome rearrangement involving RNA segment 11 of a bovine rotavirus has been analysed by molecular cloning and sequencing. This revealed that the rearranged genome segment was generated by a head to tail concatemerisation of two almost full length copies of segment 11. The upstream copy of the gene has lost its 3' end and the downstream copy its 5' end. The truncation of the upstream copy of the gene occurs within the termination codon for VP11 converting it from a UAG to a UGA, the rearranged gene is therefore still able to encode a normal VP11. The possible mechanisms by which this rearrangement may have been generated are discussed.

Rotavirus; Genome rearrangement; Epidemiological variation

Introduction

Rotaviruses are major medical and veterinary pathogens. Epidemiological variation within these viruses and the mechanisms that are involved in generating it are consequently of considerable interest to strategies aimed at effective virus vaccine development. Five distinct groups of rotavirus (A-E) have been defined using both serological and nucleic acid based criteria (Pedley et al., 1983, 1986). There is no serological cross-reaction between viruses from different groups and the available nucleic acid data would suggest that each group represents an isolated gene pool with no exchange of genes across the group boundaries (McCrae, 1987). Currently numbers of isolations of group A rotaviruses greatly exceed those from any of the

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other groups and molecular studies have focused on isolates from this group. Extensive genome profile analysis of group A rotaviruses has revealed that whilst variation in the electrophoretic mobility of individual RNA segments is observed, in general the overall 4,2,3,2 pattern of segment migration is highly conserved. Group A rotaviruses exhibiting major perturbations in their genome profiles have however been reported, the earliest being the 'short-profile' isolates of human rotavirus (Espejo et al., 1980). Gene coding assignments have shown these were due to a major shift in the size of gene 11 (Dyall-Smith and Holmes, 1981). Subsequently 'super-short' profile isolates of human rotavirus were reported (Hasegawa et al., 1984; Albert, 1985). More recently there have been reports of group A viruses being isolated from humans (Besselaar et al., 1986), calves (Pocock, 1987), pigs (Bellinzoni et al., 1987) and rabbits (Thouless et al., 1986), in which the segment 11 was replaced by an RNA segment migrating between segments 6 and 7. Rearrangements of other genome segments have also been reported for human (Pedley et al., 1984), bovine (Hundley et al., 1985) and rabbit rotaviruses (Thouless et al., 1986). Pocock (1987) analysed the proteins encoded by transcripts coming from the new RNA segment in one of the calf isolates exhibiting a rearranged gene 11. This showed that despite being approximately twice the length of the normal gene 11, transcripts from the new RNA encoded the normal protein product of gene 11, namely vp11 (McCrae and McCorquodale, 1982a). We have undertaken a detailed structural analysis of the new gene (gene x) from one of these calf isolates, using c-DNA cloning and sequencing, to ascertain the exact nature of the rearrangements involved in its generation.

Materials and Methods

Viruses and cell culture

The bovine virus strain (C7/183, see Pocock, 1987) showing an altered genome profile used in this study was kindly provided by Dr. D. Pocock. Virus was propagated in and purified from the African Green Monkey kidney cell line BSC-1 as previously described (McCrae and Faulkner-Valle, 1981).

Extraction and analysis of viral RNA

Genomic double-stranded (ds) RNA was extracted from purified virions as previously described (McCrae and McCorquodale, 1982a). Fractionation of the dsRNA on polyacrylamide gels and hybridisation studies following transfer of the fractionated segments to DPT paper were carried out as previously described (Pedley et al., 1984).

Isolation of full length c-DNA clones of segment X

The protocol used to obtain full length c-DNA of segment X broadly followed that of McCrae and McCorquodale (1982b), developed for c-DNA cloning of

genomic RNA segments from the UKtc strain of bovine rotaviruses. Briefly this involved addition of Poly A tails to unfractionated genomic RNA and reverse transcription of denatured polyadenylated RNA segments using oligo-dT as a primer to produce ds c-DNA in a single step reaction. Followed by fractionation of the c-DNA on denaturing alkaline agarose gels and excision of the c-DNA band corresponding to full length segment X. The addition of homopolymer C tails to the c-DNA using terminal transferase allowed its cloning into the vector pAT 153, which had been cut at the *Pst*I site and tailed with G residues. The vector-c-DNA mixture was then transformed into competent *E. coli* (strain MC1061) and plated onto tetracycline-containing plates. Colonies were then screened on ampicillin containing plates and those colonies showing an Amp^r phenotype were screened by the Grunstein and Hogness (1975) colony lift hybridisation procedure, using a segment 11 specific probe. The plasmids present in colonics giving a positive

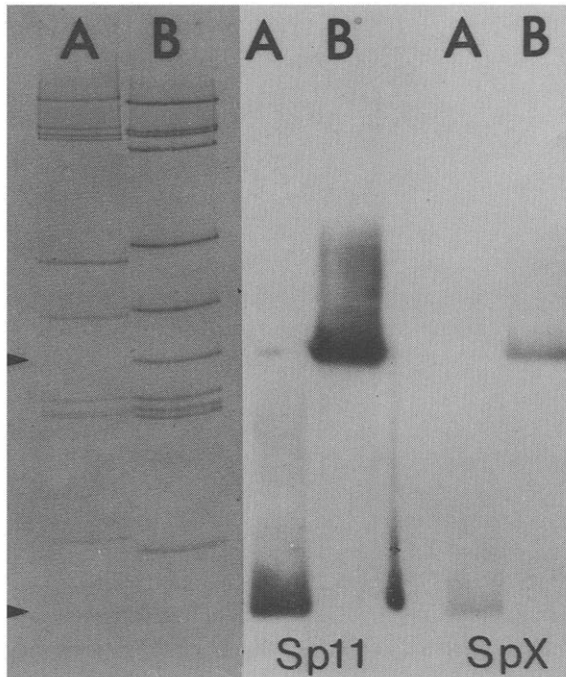


Fig. 1. Comparative genome profile and Northern blot analysis of the UKtc strain of bovine rotavirus and C7/183. The extraction of genomic RNA, its fractionation on polyacrylamide gels, its transfer to and northern blot analysis on DPT paper were all carried out as described in Materials and Methods. In all three panels track A is that given by the UKtc strain and track B that of C7/183. The left panel of two tracks shows a stained gel of the genome profiles of the two viruses with arrows used to indicate the migration positions of normal RNA segment 11 and the rearranged RNA segment X. The centre panel shows a Northern transfer of tracks such as those in the left panel probed with a c-DNA probe derived from RNA segment 11 of the UKtc strain of virus. The right panel of two tracks shows a Northern blot probed with a full length c-DNA clone of RNA segment X of C7/183.

hybridisation signal were then screened using the Birnboim and Doly (1979) mini plasmid prep method to identify the clones carrying a full length copy of segment X.

DNA sequence analysis

This was carried out using the Sanger di-deoxy method (Sanger et al., 1980) following sub-cloning of pieces of gene X c-DNA into m13 sequencing vectors.

Results

When the genome of C7/183 was compared on 6% polyacrylamide gels with that of the UKtc strain of bovine rotavirus, the absence of a band migrating in the position of RNA segment 11 and the presence of an additional band (segment X) migrating between RNA segments 6 and 7 originally reported by Pocock (1986), was confirmed (Fig. 1). When the fractionated RNA segments of these two viruses were transferred to DPT paper and hybridized with a c-DNA probe derived from segment 11 of UKtc, as expected the positive control gave an appropriate hybridisa-

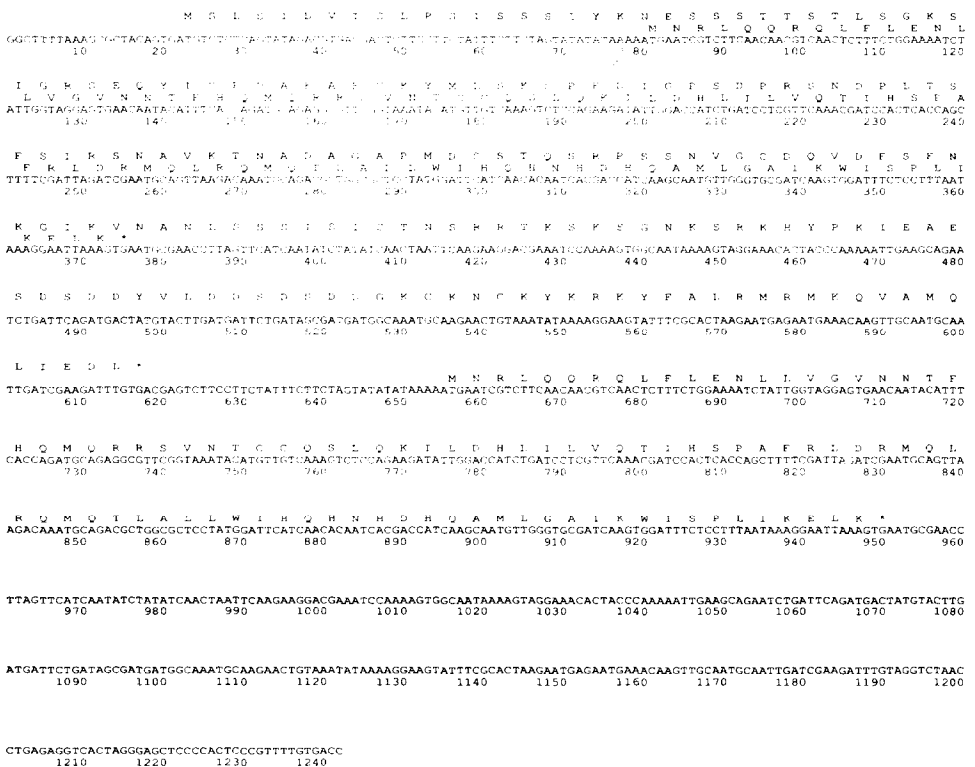


Fig. 2. Complete nucleotide and protein sequences of RNA segment X of C7/183.

M S L S I D V T S L P S I S S S I Y
 GCCTTTAAAGCGCTACAGTGATGCTCTCAGTATAGACGTGACGAGTCTTCTCTTATTCTCTAGTATATAT⁷⁵

 GCCTTTAAAGCGCTAAAGTCATGATCTCAGTATTGACGTGACGAGTCTTCTCTTATTCTCTAGTATATAT⁷⁵
 M V L S I D V T S L P S I S S S I Y

M N R L Q Q R Q L F L E N L L V G V N N I F H Q
 K N E S S S T T S T L S G K S I G R S E Q Y I S P
 AAAAAATGAATCGCTTCTCAACAACGCTCAACTCTTTCTGAAAAATCTATTGGTAGGAGTGAACAATACATTTCCACCA¹⁵⁰

 AAAAAATGAATCATCTTCAACAGCGCTCAACTCTTTCTGAAAAATCTATTGGTAGGAGTGAACAATATGTTTCACCA¹⁵⁰
 K N E S S S T A S T L S G K S I G R S E Q Y V S P
 M N H L Q Q R Q L F L E N L L V G V N N M F H Q

M Q R R S V N T C C Q S L Q K I L D H L I L V Q T
 D A E A E G K Y M L S K S P E D I G P S D P R S N
 GATGCAGAGCGCTTCGGTAAATACATGTTGTCAAAGTCTCCAGAAGATATTGGACCATCTGATCCTCGTTCAAAAC²²⁵

 GATGCAGAAGCTCCAGTAATACATGCTGTGCAAGTCTCCAGAAGATATTGGACCATCTGATTCGCTTCAAAAC²²⁵
 D A E A S S V N T L S K S P E D I G P S D S A S N
 M Q K R P V N T C C R S L Q K I L D H L I L L Q T

I H S P A F R L D R M Q L R Q M Q T L A L L W I H
 D P L T S F S I R S N A V K T N A D A G A P M D S
 GATCCACTCACCAGCTTTTCGATTAGATCGAATGCAGTTAAGACAAATGCAGACGCTGGCGCTCTATGGATTCA³⁰⁰

 GATCCACTCACCAGCTTTTCGATTAGATCAAAATGCAGTTAAGACAAATGCAGACGCTGGCGGTCTATGGATTCA³⁰⁰
 D P L T S F S I R S N A V K T N A D A G V S M D S
 I H S P A F R L D Q M Q L R Q M Q T L A C L W I H

Q H N H D H Q A M L G A I K W I S P L I K E L K *
 S T Q S R P S S N V G C D Q V D F S F N K G I K V
 TCAACACAATCAGCACCATCAAGCAATGTTGGTGGCATCAAGTGGATTCTCCTTTAATAAAGCAATTAAGTG³⁷⁵

 TCAGTACAATCAGCACCATCAACTAACCTTGGTGGCATCAAGTGGATTCTCCTTTAATAAAGCAATTAAGTG³⁷⁵
 S V Q S R P S S N V G C D Q V D F S F N K G I K V
 Q Y N H D H Q V T L G A I K W I S P L I K E L K *

N A N L S S S I S I S T N S R R T K S K S G N K S
 AATGCCAACTTAGTTCATCAATATCTATCAACTAATTCAGAAGGACGCAAAATCCAAAAGTGGCAATAAAAGT⁴⁵⁰

 AATGCCAACTTAGTTCATCTATATCAGTGTCAACAAATTCAGAAGGAGGAGTCCAAAATGACCATAAAGT⁴⁵⁰
 N A N L D S S I S V S T N S R K E K S K N D H K S

R K H Y P K I E A E S D S D D Y V L D D S D S D D
 AGGAAACACTACCCAAAATGAAGCAGAATCTGATTGATGACTATGCTACTTGATGATTCTGATAGCGATGAT⁵²⁵

 AGGAAACACTACCTAGAATTGAAGCAGAATCCGACTCAGATGATTACGCTACTTGACGATTGACAGCAGTATGAT⁵²⁵
 R K H Y P R I E A E S D S D D Y V L D D S D S D D

G K C K N C K Y K R K Y F A L R M R M K Q V A M Q
 GGCAATGCAAGAACTGAAATATAAAGGAAGTATTTCCGACTAAGAATGAGAATGAAACAAGTTCGAATGCAA⁶⁰⁰

 GGCAATGTAAGAACTGAAATATAAAGGAAGTATTTCCGACTGAGAATGAGAATGAGCAAGTTCGCATGCAA⁶⁰⁰
 G K C K N C K Y K R K Y F A L R M R M K Q V A M Q

L I E D L *
 TTGATCGAAGATTGTGA⁶¹⁷ 1193GTCTAACCTGAGAGGCTCACTAGGAGCTCCCACTCCCGTTTTGTGACC¹²⁴²

 TTGATCGAAGATTGTGAG⁶¹⁷ 618GTCTGACCTGAGAGGCTCACTAGGAGCTCCCACTCCCGTTTTGTGACC⁶⁶⁷
 L I E D L *

Fig. 3. Comparison of the RNA and Protein sequences of the unique sequences of RNA segment X (top lines) and the corresponding sequences from the UKtc bovine rotavirus RNA segment 11. Positions of sequence identity are denoted by asterixes. The protein sequence of both VP11 and that derived from translating the shorter open reading frame are both shown. Differences in amino acid sequence are underlined for the segment X-derived translation product.

tion signal (Fig. 1). This probe also hybridized to segment X of C7/183, indicating that in this virus only this RNA segment contained sequences derived from the normal segment 11 RNA (Fig. 1). When the converse hybridisation analysis was carried out using a full length c-DNA probe obtained by cloning segment X of C7/183 (see later), it hybridized to its homologous RNA as expected (Fig. 1). When hybridising to the UKtc profile this probe only gave a signal with segment 11 (Fig. 1). The failure of the full length c-DNA probe of segment X to give a hybridisation signal with any rotavirus RNA segment other than itself or segment 11, indicated that it only contains sequences from segment 11 and is not composed of a mosaic of sequences derived from a number of rotavirus RNAs. This result did not exclude the possibility that the extra sequences present in segment X compared to the normal segment 11 which it replaced, were not derived from something completely unconnected to rotaviruses.

To obtain a definitive picture of the precise structural rearrangement involved in generating segment X, full length c-DNA clones of it were isolated using a cloning strategy developed for cloning dsRNA molecules. Sequencing of a full length clone showed (Fig. 2) that segment X is 1242 base pairs long and is made up of a head to tail partial duplication of the normal segment 11. The sequence of gene 11 from the UKtc strain has been determined (Ward et al., 1985) and therefore the sequence of segment X can be compared with it. If the duplication is disregarded and the two sequences compared (Fig. 3), then at the nucleotide level there is 92% sequence conservation. This translates at the protein level to there being 17 amino acid changes (91% conservation) in VP11 and 11 changes (88% conservation) in the putative protein encoded by the shorter open reading frame in the gene (Fig. 3). The only clustering of changes that is evident is a 135 nucleotide stretch extending from

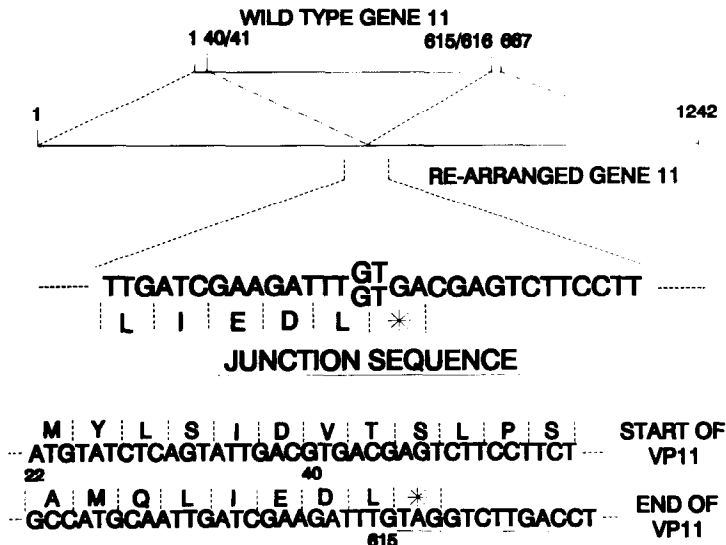


Fig. 4. Map of the structural organisation of RNA segment X and detailed sequence at the fusion point.

nucleotides 385 to 520 which contains 25 of the 63 nucleotide changes (40%) and 8 (47%) of the amino acid changes. The other comparative points of note are the absence of any sequence change in the terminally conserved 8–10 nucleotides (McCrae and McCorquodale, 1983) and the presence of only 4 nucleotide changes in the terminal 50 nucleotides at each end of the gene. This agrees with the RNA segment specific terminal conservation previously proposed from RNA fingerprinting studies (Clarke and McCrae, 1983) (Fig. 3). Detailed examination of the sequences at the point of fusion between the two copies of segment 11 (Fig. 4) showed that the upstream copy, which began at nucleotide 1 of the normal gene sequence, stops at base 615 or 616 (because of the sequence at the fusion point the precise base at which fusion occurred cannot be defined). It is then fused to nucleotide 40 or 41 of the downstream copy, which proceeds through to give the normal 3' end of segment 11 with no sequence differences between upstream and downstream copies of the repeated region. The effect of the fusion is to leave the upstream copy of segment 11 with exactly the same length of open reading frame as in a normal version of the gene. The fusion does, however, change the termination codon for VP11 from its normal UAG to a UGA (Fig. 4).

Discussion

There have now been a variety of reports of group A rotaviruses exhibiting unusual genome profiles. In the case of the original observations made in human virus isolates from immunodeficient children (Pedley et al., 1984) and some of the bovine isolates generated by high multiplicity passage in tissue culture (Hundley et al., 1985), this has been shown to involve covalent concatemerisation of rotavirus segments. The isolate studied here was interesting in being the first natural isolate that has been adapted to routine growth in tissue culture and therefore could be analysed in detail. The original work of Pocock (1987) on the isolate showed that despite the absence of a normal genome segment 11, the virus was not defective. Also when transcripts of segment X were translated in an *in vitro* translation system they were found to encode a protein that co-migrated with VP11 (Pocock, 1987), the only protein product identified to date as being coded for by RNA segment 11 (McCrae and McCorquodale, 1982a). The sequence analysis carried out on segment X in this study has provided an explanation for these observations. Thus the sequence rearrangement that has occurred has no effect on the protein coding potential of the 5' copy of segment 11, the fusion having taken place within the normal termination codon for VP11, thereby changing it from UAG to UGA. The 3' copy of segment 11 in the rearrangement begins at nucleotide 40/41 of the normal gene sequence. This is downstream of the initiation codon for VP11 (nucleotides 22–24) and therefore this copy of the coding sequence cannot give rise to protein.

Sequence analysis has now been carried out on RNA segment 11 from several different rotaviruses. This has revealed that in addition to the long open reading frame encoding VP11, there is a second conserved open reading frame extending from nucleotide 80 to 373 (Fig. 2). This frame is shifted +1 with respect to that for

VP11. To date the putative protein product that it encodes has not been identified. However, if it is found that RNA segment 11 is indeed bi-cistronic, then it is interesting to note that as a result of the rearrangement generating segment X, it has become diploid for the protein potentially encoded by this second reading frame (Fig. 2).

Considering the mechanism by which the genome rearrangement studied here was generated, the first important observation is that segment X only contains viral sequence information. Therefore it did not involve recombination between segment 11 sequences and a cellular transcript. The two mechanisms that can be most easily envisaged as generating a concatemeric genome rearrangement such as that seen in this study both involve aberrant transcription events. In the 'transcriptional slippage' model, the RNA polymerase of the virus would have transcribed normally until reaching nucleotides 615/616 of segment 11, then slipped backwards along its template to nucleotides 40/41 and begun transcribing again proceeding to the normal 3' end of the RNA. The alternative 'copy choice' model, for which there is evidence from other virus systems (Bujarski et al., 1986; Makino et al., 1986; Kirkegaard and Baltimore, 1986), involves the viral transcriptase detaching from its template strand and 'jumping' to another template strand before beginning to transcribe again. This second mechanism is less attractive here, since it would have to explain why the polymerase did not 'jump' to another RNA segment and why its transcription of the downstream copy of the gene began some way into the gene and not at the end. Finally it would demand that the transcriptase did not release the first transcript during the jumping process.

One of the most interesting aspects of the concatemerisation of RNA segments observed in rotaviruses is that it provides a possible alternative to genome segment re-assortment for these viruses to achieve rapid change in their antigenicity. In demonstrating that this alternative is a potential reality, the sequence analysis of segment X has proved somewhat disappointing since the rearrangement has not resulted in any change in coding potential. However, this is the first example of concatemerisation of RNA that has been analysed in sufficient detail to allow its protein coding implications to be defined. There has already been one report in which concatemerisation of a bovine rotavirus RNA has been associated with a change in the proteins synthesized by the viral genome (Hundley et al., 1985). It will be interesting to see, when this and other examples are analysed in detail, whether this potentially completely new method of rapidly generating antigenic change in rotaviruses is in fact used.

Acknowledgements

This work was supported by grants from the MRC and AFRC. M.A.M. is a Lister Institute Fellow.

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(Received 22 May 1989; revision received 20 June 1989)