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Sequence Relationships Between the Genome Segments of Human and Animal Rotavirus Strains

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The sequence relationships of a range of cultivable and noncultivable human and animal rotaviruses were investigated by hybridization of rotavirus cDNA probes to genomic RNAs immobilized on diazobenzyloxymethyl paper. Under conditions of low stringency (34% base mismatch tolerated) most genome segments exhibited partial homology except for genes 4 and 5. In contrast, under more stringent conditions of hybridization in which no more than 8% base mismatch was tolerated, few segments exhibited homology. Generally the human and animal rotaviruses were found to possess distinct nucleic acid sequences that exhibit only a low order of sequence relatedness. These results are consistent with the notion that both cumulative changes in nucleic acid sequences and the interchange of segments may be involved in the evolution of distinct rotavirus strains.

The rotaviruses not only are important etiological agents of diarrheal disease in humans, but also occur widely among animal populations. Rotaviruses have been recorded in bovine (18), equine (10), porcine (22, 29), and canine (7) populations, and virus has been isolated from a number of other animal species (12, 25).

In the accompanying paper (24) we describe a technique developed to permit investigation of the sequence relationships between different rotavirus RNAs. The procedure is based on the hybridization of cDNA probes specific for the RNA of a particular rotavirus to the viral genomic RNA, which has first been resolved by polyacrylamide gel electrophoresis and then immobilized by blotting onto diazobenzyloxymethyl (DBM) paper. Varying degrees of sequence relationship were detected among the human strains studied by this method. In some instances evidence was obtained for the absence of sequence relationship between a single human rotavirus gene and cDNA probes copied from genes of similar viruses isolated at a comparable time. Other human isolates showed evidence of major sequence differences in most of the RNA segments, implying that the nucleic acids of these strains have a distinct origin.

The Reoviridae in general (4) and the rotaviruses in particular (14) are known to interchange segments at a high frequency under suitable conditions in culture. An attractive explanation for the evolution of distinct strains might therefore be based on the interchange of segments between the various animal species which act as reservoirs of infection. Interchange of genes in this fashion has been suggested as a possible mechanism for the evolution of new strains of orbivirus (11, 27). Furthermore, reassortment of genes is a likely mechanism for the development of new influenza A virus strains (21): the H3 subtypes, as compared to preexisting subtypes, exhibit entirely different nucleic acid sequences for their hemagglutinin genes (3). These genes are thought to have originated from the avian reservoir (17, 21).

This communication reports the results of a series of cross-hybridization analyses in which the extent of sequence relationships between a series of cultivable and wild-type rotavirus strains was investigated. The aim of the work has been to search for evidence of sequence identity or relatedness between rotavirus genes of different origin. The results obtained imply that interchange of genomic segments may have occurred during the evolution of these viruses and that the genomes examined have been subjected to major sequence divergence during the evolution of this group of viruses.

MATERIALS AND METHODS

Virus and RNA. Cultivable rotaviruses were propagated as described elsewhere (24), and the genomic RNA was extracted. Wild strains of rotavirus were purified from fecal material by the method of Croxson and Bellamy (5, 6), and the RNA was extracted by detergent lysis. The method of Rodger and Holmes (23) has been adopted to describe the source and year of isolation of these strains.

cDNA probes and transfer of RNA to DBM paper. For synthesis of cDNA probes and transfer of RNA to DBM paper the method of Street et al. (24) was used.

For the synthesis of the canine rotavirus probe, the double-stranded RNA was first purified by isopycnic sedimentation on cesium sulfate-ethidium bromide gradients (24).

Hybridization. For hybridization of cDNA probes to immobilized RNA essentially the method of Street et al. (24) was followed. A mixed cDNA probe specific for the RNA of a particular rotavirus strain was hybridized to other rotavirus RNAs which had been immobilized on DBM paper. This method permits the fraction of a genome segment exhibiting homology to be estimated by comparison with the radioautograph of the corresponding homologous hybridization. The sequence relatedness of that portion of the genome which exhibits homology can then be determined by carrying out hybridization at different stringencies after adjustment of the formamide concentration (1). Hybridization at 52°C in the absence of formamide detects sequences which possess up to 34% base mismatch, whereas hybridization in the presence of 50% formamide detects sequences having only 8% base mismatch or less (24).

RESULTS

Diversity of gel electrophoretic profiles of rotavirus RNAs. Figure 1 presents a typical result of an electrophoretic analysis in which a series of animal and human rotavirus RNAs were resolved on the same polyacrylamide gel. A number of differences could be detected when the migration rates of the individual segments were compared. This result confirms the observations of previous investigators (5, 9, 13, 15, 23, 26) that the "electropherotype" of the viral RNA is a distinctive feature of rotavirus isolates. However, certain isolates (Nebraska calf diarrhea virus [NCDV], lane 1; Northern Ireland bovine rotavirus [NI], lane 2) were not distinguished from each other by this procedure, whereas some of the other bovine isolates (for example BO/DUN/25/79 and BO/DUN/70/79) were distinguishable on the basis of the mobility of segments 7, 8, and 9. Other genes (for example, gene 5) showed considerable variation in electrophetic mobility.

Sequence relationships of selected rotaviruses with SA11 virus cDNA probes. The series of rotavirus RNAs shown in Fig. 1 and other comparable series from separate acrylamide gels were transferred to DBM paper by transverse electrophoresis (24). The immobilized viral RNAs were then probed with cDNA sequences specific for all 11 simian 11 (SA11) virus genes.

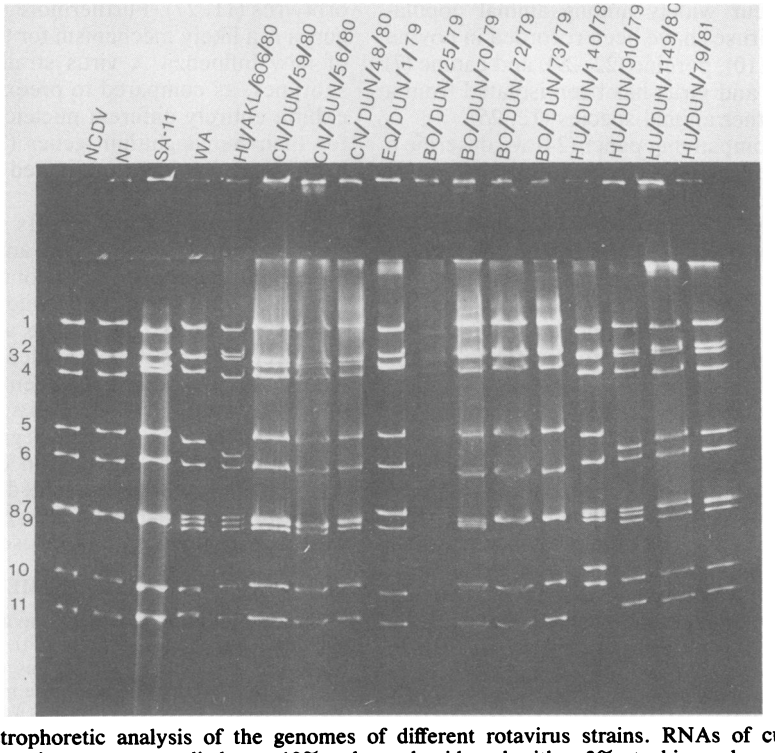

FIG. 1. Electrophoretic analysis of the genomes of different rotavirus strains. RNAs of cultivable and noncultivable rotaviruses were applied to a 10% polyacrylamide gel with a 3% stacking gel prepared by the method of Laemmli (16). Electrophoresis was for 9 h at 150 V/cm. The gel was then stained in 10 μg of ethidium bromide per ml and photographed under UV light. NCDV, Nebraska calf diarrheal virus; NI, Northern Ireland bovine rotavirus; Wa, human cultivable rotavirus type 2 isolate (30); SA11, simian 11 virus. Strain description of wild isolates is by the convention of Rodger and Holmes (23): BO, bovine; CN, canine; EQ, equine; HU, human.

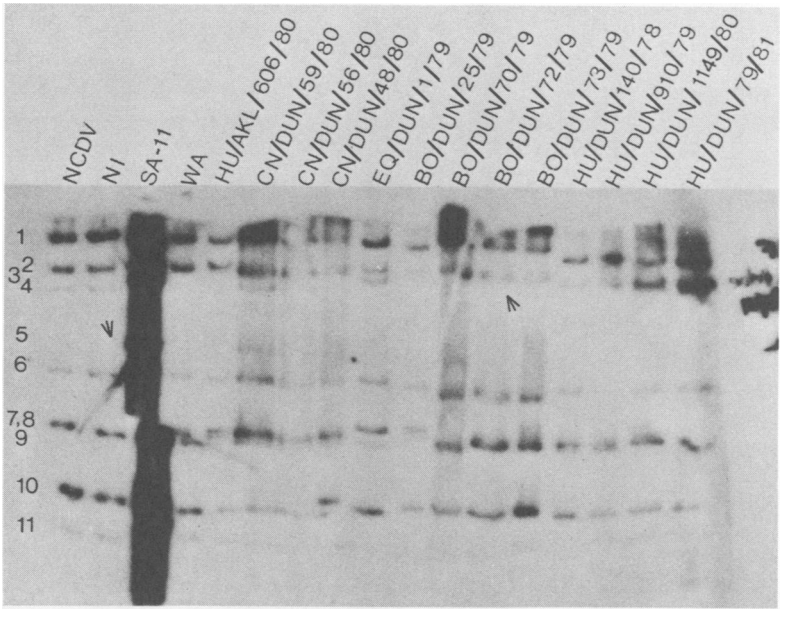

FIG. 2. Northern blot sequence analysis of rotavirus genomic RNAs, utilizing [^{32}P]cDNA mixed probes specific for the genome of SA11 virus. For details of the hybridization procedure see references 1 and 24. Arrows indicate examples of isolates in which genes 4 and 5 show no detectable homology with the SA11 probe. Hybridization was at 52°C in the absence of formamide (34% base mismatch tolerated).

Figure 2 presents the results obtained when the hybridization was carried out under low stringency in the absence of formamide. Hybridization occurred between the SA11 probe and many of the rotavirus genes of the strains examined. Two exceptions are obvious: genes 4 and 5 showed little evidence for homology with the SA11 genome under these conditions of hybridization, which should detect sequences that on average have base mismatches as great as 34% (examples of missing bands are arrowed in Fig. 2). It may be concluded that cDNA probes specific for genes 4 and 5 of SA11 show little or no relationship with genes 4 and 5 of the other strains examined. This was confirmed by hybridization at a lower temperature (37°C), which also failed to detect homology (data not shown). Overall the homology detected was generally low as compared to the homologous SA11/SA11 hybridization (channel 3, Fig. 2).

Sequence relationships with bovine and canine isolates. Figures 3 and 4 present the results of a series of hybridizations similar to those presented in Fig. 2. Probes specific for NCDV genes and those of the wild-type canine virus strain CN/DUN/59/80 were prepared and applied to the DBM-immobilized genome RNAs at both high and low stringency. For the NCDV probes at low stringency (Fig. 3a), gene 5 again exhibited little evidence for homology with the equivalent gene of human, canine, equine, or simian origin. Homology was evident, however, between the cDNA probe and gene 5 of other bovine isolates. It is also notable that the NCDV probe showed some homology with SA11 gene 4 (confirming the relationship already established in Fig. 2), but none with the human or the wild-type bovine isolates examined (arrow, Fig. 3a).

When the stringency of hybridization conditions was increased, few nonbovine genes showed homology with the NCDV probe. A notable exception from the series shown in Fig. 3b were genes 6 through 11 of the SA11 virus, which demonstrated strong homology. This implies that NCDV exhibits considerable sequence relatedness to these genes of this simian strain, but not to genes 1 through 5. This relationship was less evident but detectable in the reciprocal high-stringency hybridization shown earlier (Fig. 2), in which an SA11 probe was hybridized to the immobilized NCDV genome.

Figure 3 also demonstrates that the Northern Ireland bovine rotavirus (NI) and NCDV, despite their diverse geographical origin, exhibited very close sequence relationships.

A similar cross-hybridization analysis was carried out on the same series of isolates by using a cDNA probe copied from the RNA of the wild-type canine strain CN/DUN/59/80 (Fig. 4a). Of the various gene 5 bands, only the equine gene showed homology with the corresponding canine probe. Gene 4 of NCDV, Northern Ire-

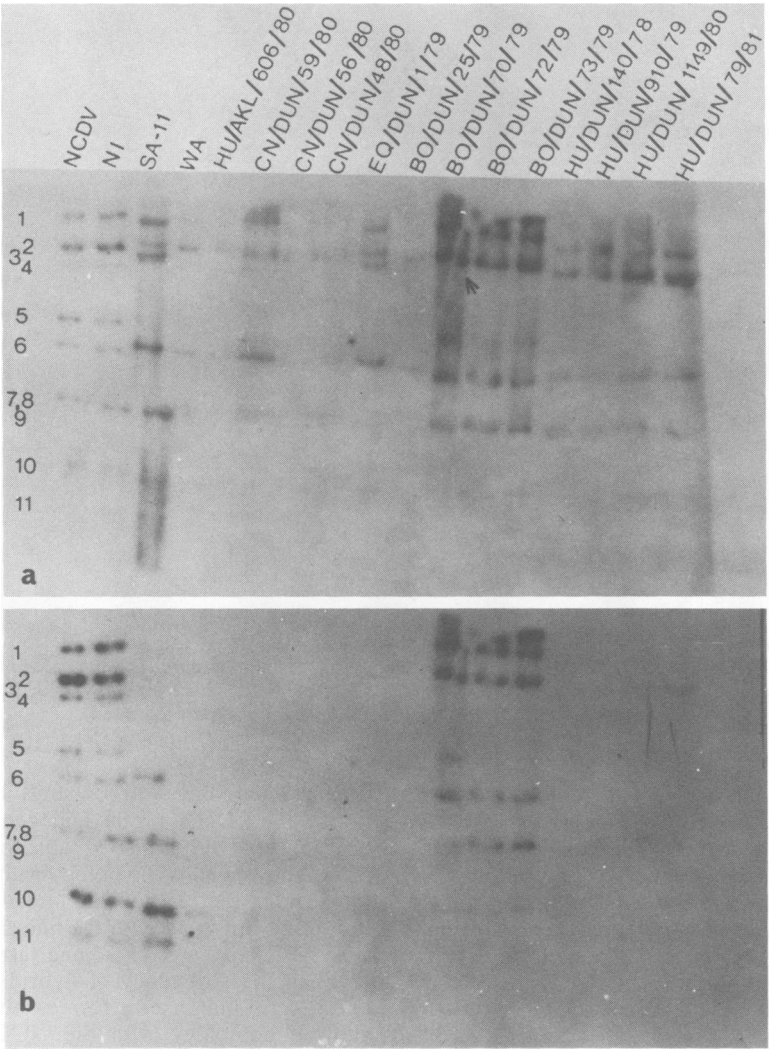

FIG. 3. Northern blot sequence analysis of rotavirus genomic RNAs, utilizing [^{32}P]cDNA mixed probes specific for NCDV. For details of the hybridization see references 1 and 24. Arrow in (a) indicates evidence for the absence of homology between gene 4 of NCDV and the equivalent gene of the four wild-type bovine strains examined. (a) Low-stringency hybridization (0% formamide, 52°C); (b) high-stringency hybridization (50% formamide, 52°C).

land bovine rotavirus, and EQ/DUN/1/79 exhibited homology with the probe, but gene 4 of the human cultivable strain did not (arrow, Fig. 4a). At high stringency (Fig. 4b) few sequences of near identity were revealed, confirming that the CN/DUN/59/80 isolate shares few regions of sequence identity with the other strains examined.

Sequence relationships between human and bovine rotaviruses. In Fig. 3b there is some evidence that small regions of the NCDV genome share sequences of near identity with those of certain human isolates (see channels 14 through 17 of Fig 3). To determine whether the bovine rotavirus genome exhibits homology with a wider selection of human rotavirus genomes, an NCDV probe was hybridized to a series of human isolates (24).

Figure 5 reveals that certain human isolates indeed contain minor sequences closely related to those of the bovine virus. This was particularly evident for segments 2 or 3 of the "short" 1978 isolates. It is also evident from Fig. 5b that segment 11 of the short human genomes and segment 10 of the "long" genomes also possess small regions of closely related sequence.

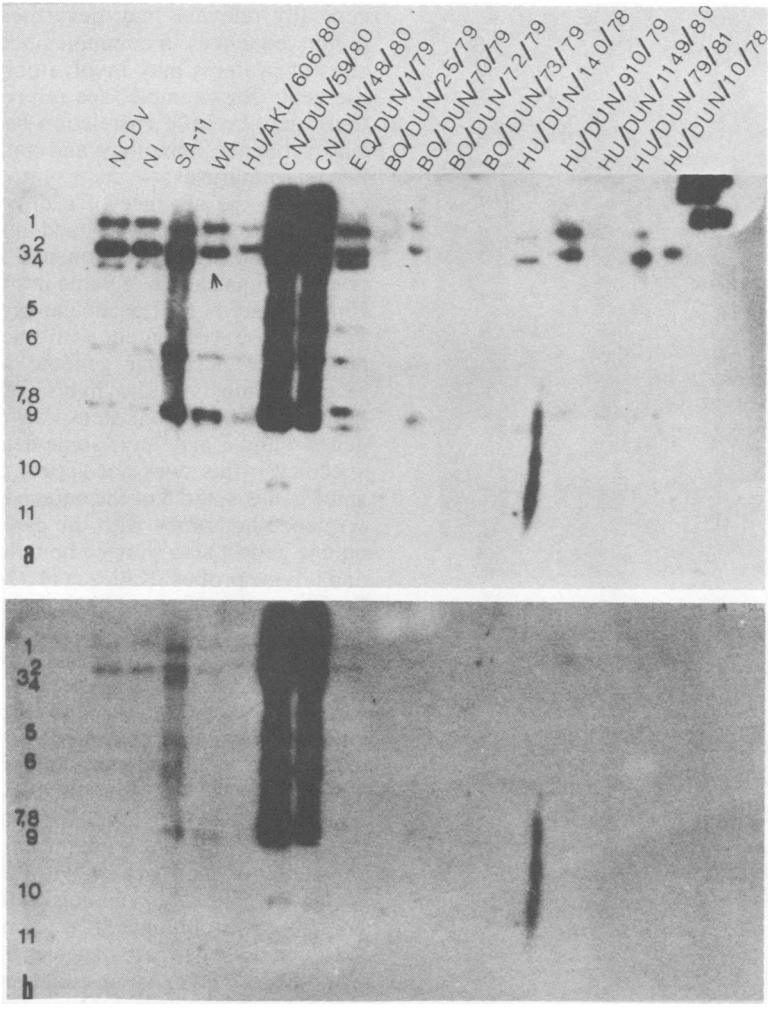

FIG. 4. Sequence analysis using a [^{32}P]cDNA mixed probe specific for the wild-type canine isolate CN/DUN/59/80. For details of hybridization see references 1 and 24. Arrow in (a) indicates the absence of homology between the probe and gene 4 of the human rotavirus strain Wa. (a) Low-stringency hybridization (0% formamide, 52°C); (b) high-stringency hybridization (50% formamide, 52°C).

DISCUSSION

By analogy with influenza viruses (17), both antigenic "shift" and "drift" might be anticipated to occur among the rotaviruses in view of their known ability to interchange genome segments during mixed infections of cultured cells (14). However, in an investigation of sequence relationships between a relatively small number of field isolates, it is unlikely that definitive evidence for interchange of genome segments would be found. Segment interchange, nevertheless, is an attractive hypothesis to explain the results obtained here, since it would account for the lack of detectable homology observed for genes 4 and 5 in strains which at the same time demonstrated homology in most other segments. Antigenic drift could account for the differences between those isolates which exhibit some base sequence homology but which are shown to be quite distinct when hybridizations are carried out under more stringent conditions.

The serological relationships existing between various rotavirus strains have yet to be well characterized because of difficulties encountered with the serological typing of this group of viruses (2, 32). Furthermore, nucleic acid homology need not necessarily lead to antigenic similarity and vice versa. The rotaviruses possess a segmented genome, and thus only a few segments are likely to code for important antigenic proteins. Those segments which are anti-

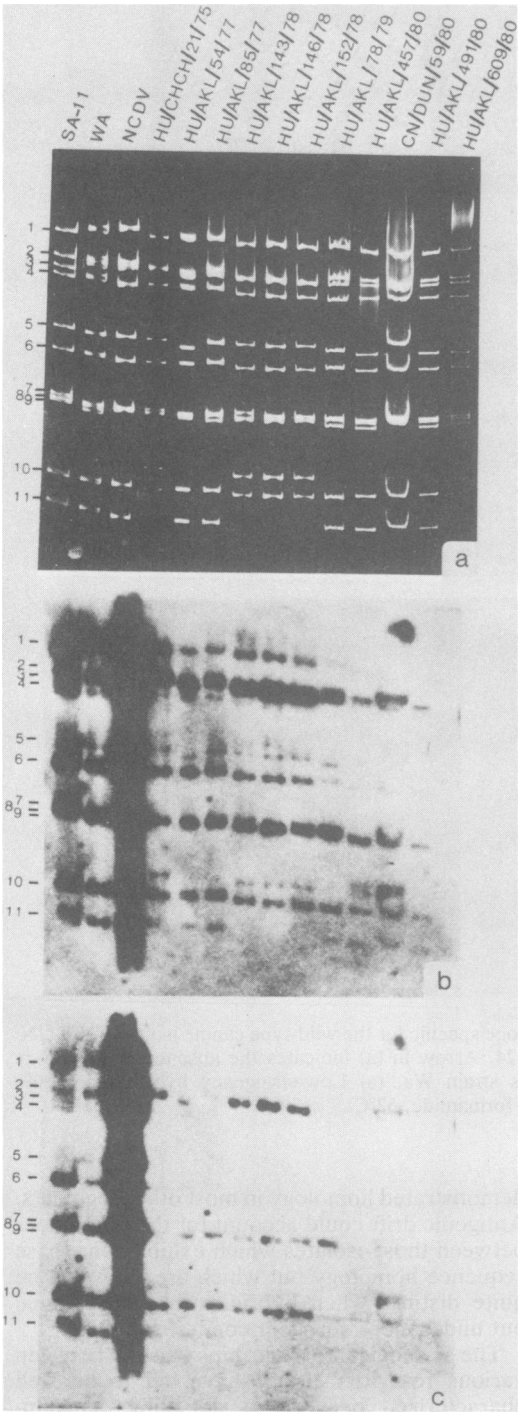

FIG. 5. Sequence relationships between human and bovine rotaviruses. (a) Ethidium bromide-stained gel showing electrophoretic separation of the genomes of a series of human rotavirus isolates collected between 1975 and 1980. Short electropherotypes (channels 4, 7, 8, and 9) are those in which the mobilities of the smallest segments differ as a result of a major change in the mobility of segment 11 (see reference 8). (b) The gel shown in (a) was blotted onto DBM paper and hybridized with a [^{32}P]cDNA mixed probe specific for the cultivable bovine strain NCDV. Low-stringency hybridization (10% formamide, 52°C). (c) High-stringency hybridization (50% formamide, 52°C) corresponding to (b) above.

genically relevant may nevertheless hold only minor sequences in common since the antigenic sites of proteins may involve only a portion of the gene (for example, see reference 28). Thus there may be little correlation between nucleic acid sequence homology and antigenic properties of rotaviruses.

Despite the absence of a direct relationship between serological type and nucleic acid sequence, the study of sequence homology between strains can be of value in two other ways. First, it may permit particular properties of the virus to be correlated with the presence or absence of particular genome segments. For example, most of the hybridization evidence presented here is consistent with the notion that genes 4 and 5 may have some degree of species specificity: this does not appear to be absolute since genes 4 and 5 of the equine isolate showed sequence homology with the canine probe, and equine gene 4 also showed homology with SA11 and bovine probes. Kalica et al. (14) investigated the properties of recombinants formed between bovine and human viruses and also concluded that genes 4 and 5 may be involved in the ability of the virus to grow in particular cell types.

Second, a knowledge of the sequence relationships between different rotaviruses might permit assessment of the relative importance of shift and drift in the evolution of these viruses. Mechanisms that might be proposed to account for the evolution of the rotaviruses need to account for the complex and generally low order of sequence relationship demonstrated here. The common morphology (12), common antigens (31), and ability to cross species barriers (19, 20) all argue in favor of a common evolutionary origin for these viruses. A combination of segment interchange and progressive evolutionary divergence would thus account well for the unexpected low order of sequence relationship that we have found between human, simian, bovine, canine, and equine rotaviruses.

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