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Analysis by RNA-RNA Hybridization Assay of Intertypic Rotaviruses Suggests that Gene Reassortment Occurs In Vivo

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Antigenic characterization of human and animal rotaviruses by the plaque reduction neutralization assay has shown the existence of naturally occurring intertypes. Antiserum to M37, a rotavirus strain isolated from an asymptomatic neonate, neutralizes both Wa and ST3 strains, which are classified as serotype 1 and serotype 4 human rotaviruses, respectively. Likewise, antiserum to SB-1A, a porcine rotavirus, neutralizes rotavirus strains belonging to serotype 4 or 5. Plaque reduction neutralization assay of reassortant rotaviruses produced in vitro from these intertypes indicates that these viruses share one antigenically related outer capsid protein, VP3, with one serotype and another antigenically related outer capsid protein, VP7, with the other serotype. Thus, M37 is related to ST3 on the basis of its fourth-gene product, VP3, and to Wa on the basis of its ninth-gene product, VP7, whereas SB-1A is related to Gottfried (serotype 4 porcine rotavirus) via VP7 and to OSU (serotype 5 porcine rotavirus) via VP3. RNA-RNA hybridization studies revealed a high degree of homology between the VP3 or VP7 gene segments responsible for shared serotype specificity. Thus, the fourth gene segments of M37 and ST3 were highly homologous, while M37 and Wa had homology between their ninth gene segments. SB-1A and Gottfried were homologous not only with respect to the ninth gene but had complete homology in all other genes except the fourth gene. The fourth gene of SB-1A was highly homologous with the fourth gene of OSU. These observations suggested that SB-1A was a naturally occurring reassortant between Gottfried-like and OSU-like porcine rotavirus strains. Our observations also suggested that intertypes may result from genetic reassortment in nature.

Rotaviruses are an important cause of infantile diarrhea in a wide variety of mammalian species, including humans. The need for a safe and effective vaccine has been widely recognized and has stimulated considerable research into the antigenic and genetic diversity of rotaviruses (1, 6, 13).

The genome of rotaviruses consists of 11 segments of double-stranded (ds) RNA which reassort with high efficiency during coinfection of cells in tissue culture. Analysis of these reassortant rotaviruses has been helpful in establishing gene-product relationships. For example, the fourth gene codes for VP3, an outer capsid protein which is the site for trypsin activation of infectivity, restriction of growth of human rotaviruses in tissue culture, and hemagglutination activity of certain rotaviruses (5, 11). The sixth gene codes for VP6, an inner capsid protein which bears common and subgroup antigens (5, 12). The eighth or ninth gene, depending on the virus strain, codes for VP7, an outer capsid protein which represents the major neutralization antigen (4a, 5, 7, 8, 11, 12, 15, 16). Study of various human and animal rotaviruses by plaque reduction neutralization assay has revealed at least seven different serotypes (9, 10, 17). Moreover, certain strains appear to be intertypes which bridge two different serotypes. For example, M37, a human rotavirus strain that causes asymptomatic infection in neonates, shares neutralization specificity with serotype 1 and serotype 4 viruses: antiserum to serotype 1 neutralizes M37, whereas antiserum to M37 neutralizes both serotype 1 and serotype 4 human rotavirus strains (9). In addition, SB-1A, a porcine rotavirus, shares neutralization specificity, reciprocally, with both serotype 4 and serotype 5 rotaviruses (10). Recent studies performed with reassortant rotaviruses have shown that the VP3 and VP7 of the M37 human rotavirus are responsible for its shared neutralization specificities with serotype 4 and serotype 1 rotaviruses, respectively, and that these outer capsid proteins of the SB-1A porcine rotavirus are also responsible for its shared neutralization specificities with OSU (serotype 5) and Gottfried (serotype 4) porcine rotaviruses, respectively (7, 8). The shared neutralization specificity between SB-1A and Gottfried rotaviruses was shown to be mediated through VP7 in an indirect manner. The reassortant rotavirus SB-1A × DS-1 (18-1), which derives all of its genes except gene 9 from SB-1A and contains the VP7 gene (gene 8) of DS-1, was not neutralized by hyperimmune serum to the Gottfried strain (7). Likewise, the Gottfried strain was not neutralized by hyperimmune serum to this reassortant rotavirus (7). Thus, substitution of the VP7 protein of DS-1 for the corresponding protein of SB-1A abrogated the shared neutralization specificity between SB-1A and Gottfried strains of porcine rotavirus.

In this study we used the RNA-RNA hybridization technique performed under stringent conditions to examine the genetic relationships among the intertypic rotaviruses and their bridging serotypes, especially with respect to the genes coding for VP3 and VP7.

MATERIALS AND METHODS

Viruses. The following naturally occurring, tissue culture-adapted rotaviruses were used: human rotaviruses Wa (serotype 1), DS-1 (serotype 2), M37 (serotype 1 and serotype 4), and ST3 (serotype 4); porcine rotaviruses Gottfried (serotype 4), SB-1A (serotype 4 and serotype 5), and OSU (serotype 5); and bovine rotavirus UK (serotype 6). The following reassortant rotaviruses derived from earlier in vitro experiments were also used: Wa × UK (18-1) and ST3 × UK (52-1-1), both of which derive 10 gene segments from UK and only 1 gene segment, the ninth,

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TABLE 1. Relationships among porcine rotaviruses studied by neutralization and RNA-RNA hybridization[a]

Porcine rotavirus	Gottfried (serotype 4)				OSU (serotype 5)			
	Neutralization	Hybridization with gene			Neutralization	Hybridization with gene		
		VP3	VP7	All others		VP3	VP7	All others
SB-1A (serotype 4 and 5)	High titer, reciprocal	0[b,c]	+[b]	9 hybrids similar to genomic dsRNAs[b]	High titer, reciprocal	+[b,c]	0[b,d]	6 hybrids; parental origin not assignable[b]
Gottfried (serotype 4)					No neutralization	0[b,e]	0[b,f]	6 hybrids; parental origin not assignable[b]

[a] The conditions for the hybridization reactions are noted individually. Genes of human DS-1 rotavirus did not produce hybrids with labeled ssRNA transcripts of porcine rotaviruses and vice versa.
[b] Full complement of dsRNA genes and labeled full ssRNA transcripts.
[c] Full complement of dsRNA genes of DS-1 × SB-1A reassortant with only fourth gene derived from SB-1A and labeled full ssRNA transcripts of Gottfried or OSU.
[d] Full complement of dsRNA genes of DS-1 × SB-1A reassortant with all but the ninth gene derived from SB-1A and labeled full ssRNA transcripts of OSU compared with pattern of SB-1A dsRNAs and full labeled ssRNA transcripts of OSU.
[e] Isolated fourth dsRNA gene of OSU and labeled full ssRNA transcripts of Gottfried.
[f] Isolated ninth dsRNA gene of Gottfried and full labeled ssRNA transcripts of OSU.

which codes for VP7, from their human rotavirus parent (12, 16); SB-1A × DS-1 (17-1), which derives 10 gene segments from DS-1 and only 1 segment, the fourth, which codes for VP3, from its animal rotavirus parent; and SB-1A × DS-1 (18-1), which derives 10 gene segments from SB-1A and only 1 gene segment, the eighth, which codes for VP7, from its human rotavirus parent (7).

Preparation of RNA. Genomic dsRNAs were extracted from lysates of infected Ma104 cells with phenol-chloroform as described previously (15). In certain instances, individual gene segments were isolated by separating the 11 genomic RNAs by polyacrylamide gel electrophoresis (10% preparatory gels), staining the RNA segments with ethidium bromide, and cutting the desired segments out of the gel. Samples of gel containing the individual gene segments were then homogenized by passing the material through an 18-gauge needle, followed by incubation in an equal volume of acrylamide extraction buffer (500 mM ammonium acetate, 1 mM EDTA [pH 8.0]) for 12 h at 37°C. The resulting mixture was then briefly centrifuged to remove the gel fragments, and the liquid phase was precipitated with ethanol at −20°C overnight. ^{32}P-labeled single-stranded (ss) RNAs were transcribed from viral cores of selected rotaviruses by the method described by Flores et al. (2), except that newly synthesized transcripts were precipitated with 2 M lithium chloride. Labeled transcripts were stored at −70°C until used.

Hybridization. To assess genetic relatedness, denatured dsRNAs (either an individual gene segment or the complete genome) of a virus were hybridized to [^{32}P]ssRNA probes prepared from other strains. The dsRNAs were diluted in 1 mM EDTA, heated at 100°C for 2 min, and then quenched on ice for 2 min. Approximately 10,000 cpm of the [^{32}P]RNA probes in hybridization buffer (100 mM NaCl, 50 mM Tris [pH 8], 0.1% sodium dodecyl sulfate) was added to the denatured dsRNAs, and hybridization was allowed to take place at 65°C for 14 h. Hybridized RNAs were precipitated with 2 or 3 volumes of ethanol, and after being held overnight at −20°C, the RNAs were pelleted (12,000 × *g*), taken up in 20 μl of sample buffer, and electrophoresed in a 10% polyacrylamide gel at 20 mA for 14 h. After being stained with ethidium bromide and examined under UV light, the gels were dried and autoradiographed on Kodak X-Omat AR film for about 24 to 48 h. The formation of hybrid segments under the stringent conditions used in this study indicates a relatively high degree of homology. This is indicated by the failure, under similar conditions, of hybrid VP7 gene segments to form during pairwise comparisons among rotavirus strains Wa, UK, SA-11, and Nebraska calf diarrhea virus despite 75% sequence homology among the VP7 glycoprotein genes of these strains (4). However, the level of homology above 75% that is required for hybrid formation has not been established.

RESULTS

Cross-hybridization analysis of porcine rotavirus strains (serotype 4 or 5). SB-1A is a porcine rotavirus strain that shares reciprocal neutralization specificity with both serotype 4 and serotype 5 rotaviruses and has thus been classified as an intertype exhibiting both serotypes (10). When the denatured genomic RNAs of SB-1A were hybridized to the ^{32}P-labeled probe of ssRNA transcripts prepared from the porcine Gottfried strain of rotavirus (serotype 4), 10 hybrid gene segments were produced (Table 1). The hybridization pattern was identical to the homologous Gottfried-Gottfried hybridization except for the absence of a hybrid corresponding to the fourth gene segment (Fig. 1). In addition, at least seven major hybrid segments were formed following hybridization of SB-1A genomic RNAs to the ^{32}P-labeled probe of the porcine OSU strain of rotavirus (serotype 5) (Table 1). The genes which were involved in forming these hybrids could not be determined with certainty. However, hybrids were observed that exhibited a migration pattern similar, if not identical, to those of the 1st, 2nd or 3rd, 4th, 8th, 10th, and 11th genes of the homologous OSU-OSU hybridization. Of interest, the OSU-Gottfried hybridization pattern was identical to the SB-1A-OSU hybridization pattern except that the former lacked a hybrid segment with a migration pattern similar to that of the fourth gene of the homologous OSU-OSU hybridization (Fig. 1). These observations suggested that the SB-1A neutralization specificities that were shared with serotype 4 and serotype 5 rotaviruses were mediated by VP7 and VP3, respectively.

To confirm this interpretation, additional studies were performed with individual gene segments of these viruses and with reassortant viruses. The reassortant rotavirus SB-1A × DS-1 (17-1) contained the fourth gene of SB-1A, but its remaining genes were derived from DS-1. Since genomic dsRNAs of DS-1 failed to form hybrids with SB-1A, OSU, or Gottfried under the conditions used in this study, any hybrids resulting from the hybridization between reassortant 17-1 and the probes derived from the porcine strains would indicate homology between the fourth gene of SB-1A

and the probe. With this approach, the absence of hybrid formation between the fourth gene of SB-1A (as present in the 17-1 reassortant) and Gottfried and the existence of significant homology between the fourth gene of SB-1A (17-1) and OSU were confirmed (Fig. 2, Table 1). The electrophoretic mobility of the hybrid formed between the fourth gene of SB-1A and the OSU probe was similar but not identical to the homologous OSU-OSU fourth-gene hybrid (Fig. 2). There was also no hybrid formation between the fourth gene of SB-1A (17-1) and the probe derived from ST3, a serotype 4 human rotavirus. Homology was not demonstrated between the fourth gene of OSU, which had been isolated from the complete genomic dsRNAs of OSU, and probes derived from Gottfried or ST3 (Table 1), nor did any hybrid segments form between the isolated fourth gene of Gottfried and an ST3 probe, indicating that the shared neutralization specificity of these two serotype 4 viruses was probably mediated by VP7.

The genetic relationships among the ninth gene segments of the porcine rotaviruses was also examined. The ninth gene of OSU codes for VP7 (4a); the ninth gene of Gottfried and SB-1A also codes for VP7, although this has been shown in an indirect manner. Reassortants between DS-1 and Gottfried or SB-1A which derive all their genes except the ninth gene from their animal rotavirus parent lose their animal rotavirus VP7 neutralization specificity and gain the DS-1 VP7 neutralization specificity (7; Hoshino et al., unpublished studies). It was difficult to achieve the degree of separation among the seventh, eighth, and ninth genes necessary to completely isolate the ninth gene segment. Hybridization of full genomic dsRNAs of SB-1A to the Gottfried probe had established complete homology between all genes except gene four. On the other hand, the isolated ninth gene of Gottfried failed to form a hybrid with the OSU probe (Table 1). The ninth gene was not successfully isolated from either SB-1A or OSU. Thus, it was necessary to demonstrate the relationship between the ninth genes of these viruses in an indirect fashion.

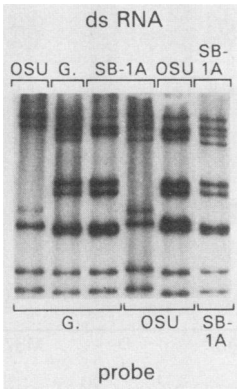

FIG. 1. Hybridization of genomic dsRNAs of porcine rotaviruses OSU, Gottfried (G.), and SB-1A to ^{32}P-labeled full ssRNA transcripts of Gottfried, OSU, or SB-1A. The hybridization pattern of the dsRNAs of SB-1A to the Gottfried probe (lane 3) was indistinguishable from the homologous Gottfried-Gottfried (lane 2) and SB-1A-SB-1A (lane 6) hybridization patterns except for the absence of a hybrid band corresponding to the fourth gene of the homologous reactions. In contrast, the hybridization pattern of dsRNAs of SB-1A to the OSU probe (lane 4) differed significantly from the homologous OSU-OSU pattern (lane 5). However, there was a hybrid band with a migration distance very similar to that of the fourth gene of the homologous reaction. A hybrid band corresponding to the fourth gene was absent in the OSU-Gottfried hybridization (lane 1).

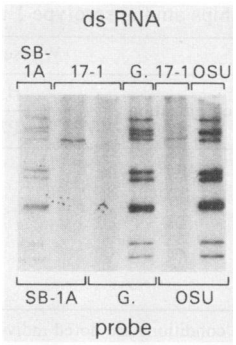

FIG. 2. Hybridization of genomic dsRNAs of reassortant SB-1A × DS-1 (17-1) (which derives only the fourth gene coding for VP3 from its animal rotavirus parent SB-1A) to ^{32}P-labeled full ssRNA transcripts of SB-1A, Gottfried (G.), or OSU. Since genomic dsRNAs of human rotavirus DS-1 failed to form hybrids with SB-1A, Gottfried, or OSU probes, any hybrid formation between dsRNAs of 17-1 and these porcine rotavirus probes can be attributed to homology between the fourth genes of these porcine rotaviruses. The homologous hybridization pattern between the fourth gene of the reassortant 17-1 and the SB-1A probe is shown in lane 2. Homology was not demonstrated between the fourth genes of SB-1A and Gottfried (lane 3) but significant homology was present between the fourth genes of SB-1A and OSU (lane 5).

The genomic dsRNAs of (i) reassortant rotavirus SB-1A × DS-1 (18-1), which derives all its genes except the ninth gene from SB-1A, and (ii) SB-1A were hybridized to OSU probes. The reassortant 18-1-OSU and SB-1A-OSU hybrid segments were identical, suggesting that the ninth gene of SB-1A failed to form a hybrid with OSU (Fig. 3, Table 1).

Cross-hybridization among human rotavirus strains Wa (serotype 1), M37 (serotype 1 and 4), and ST3 (serotype 4). The isolated fourth gene of Wa failed to form a hybrid with the M37 probe. However, significant homology between the ninth gene of Wa present in a single gene substitution reassortant prepared with UK virus and the M37 probe was demonstrated (Table 2). Genomic dsRNAs of reassortant rotavirus Wa × UK (18-1) were hybridized to the M37 probe (Fig. 4). This reassortant derives all its genes from the UK parent except the ninth gene, which it derives from Wa. Since genomic dsRNAs of UK did not form hybrids with M37, the hybrid observed between the reassortant and M37 was interpreted as indicating homology between the ninth gene of Wa and the M37 probe. In a similar fashion, the fourth and ninth genes of another serotype 1 human rotavirus, D strain, were hybridized with the M37 probe, again demonstrating significant homology between the ninth genes, but hybrid formation was not detected between the fourth genes. The fourth gene of ST3 was highly homologous to the M37 probe, but hybrids were not demonstrated between the genomic dsRNAs of reassortant rotavirus ST3 × UK (52-1-1), which contained the ninth gene of ST3 and the remaining 10 genes from UK, and the M37 probe (Fig. 4, Table 2). The isolated fourth gene of Wa and the ninth gene of Wa in the Wa × UK (18-1) reassortant did not hybridize with the ST3 probe (Table 2).

DISCUSSION

In a recent study it was shown that the naturally occurring intertype M37, a human rotavirus strain that causes asymp-

TABLE 2. Relationships among serotype 1 and serotype 4 human rotaviruses studied by neutralization and RNA-RNA hybridization[a]

Human rotavirus	Wa (serotype 1)				ST3 (serotype 4)			
	Neutralization	Hybridization with gene			Neutralization	Hybridization with gene		
		VP3	VP7	All others		VP3	VP7	All others
M37 (serotype 1 and 4)	High titer, reciprocal	0[b]	+[c]	6 hybrids; parental origin not assignable[d]	M37 antiserum neutralized ST3 to high titer but reciprocal titer low	+[e]	0[f,g]	6 hybrids; parental origin not assignable[d]
Wa (serotype 1)					No neutralization	0[b]	0[c,f]	7 hybrids; parental origin not assignable[h]

[a] Hybridization reaction conditions are noted individually. Genes of bovine UK rotavirus did not produce hybrids with labeled ssRNA transcripts of human rotaviruses and vice versa.
[b] Isolated fourth dsRNA gene of Wa and labeled full ssRNA transcripts of M37 or ST3; also isolated fourth dsRNA gene of M37 and labeled full ssRNA transcripts of Wa.
[c] Full complement of dsRNA genes of UK × Wa reassortant with only ninth gene derived from Wa and labeled full ssRNA transcripts of M37 or ST3.
[d] Full complement of dsRNA genes of Wa or ST3 and labeled full ssRNA transcripts of M37.
[e] Isolated fourth dsRNA gene of ST3 and full ssRNA transcripts of M37; also isolated fourth dsRNA gene of M37 and full ssRNA transcripts of ST3.
[f] Full complement of dsRNA genes of UK × ST3 reassortant with only ninth gene derived from ST3 and labeled full ssRNA transcripts of M37 or Wa.
[g] Full complement of dsRNA genes of M37 and labeled full ssRNA transcripts of UK × ST3 reassortant with only ninth gene derived from ST3.
[h] Full complement of dsRNA genes of Wa and labeled full ssRNA transcripts of ST3.

tomatic neonatal infection, shares neutralization specificities with a serotype 1 human rotavirus (Wa) as well as a serotype 4 human rotavirus (ST3). These relationships were ascribed to the antigenically related outer capsid proteins VP7 and VP3, respectively (8). A similar study of the porcine rotavirus intertype SB-1A demonstrated that it shared neutralization specificities with a serotype 4 porcine rotavirus (Gottfried) as well as a serotype 5 porcine rotavirus (OSU); these relationships were also ascribed to the related outer capsid proteins VP7 and VP3, respectively (7).

The aim of this study was to examine the genetic relatedness of the fourth or ninth genes (which code for VP3 and VP7, respectively) of these naturally occurring intertypes

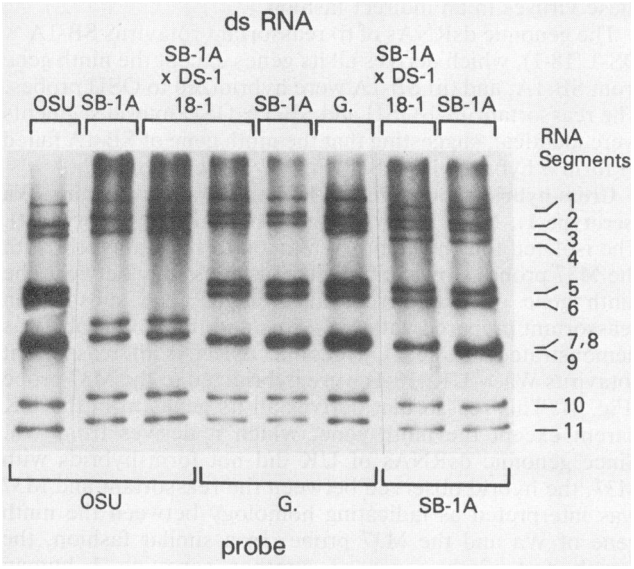

FIG. 3. Hybridization of genomic dsRNAs of reassortant SB-1A × DS-1 (18-1) (which derives all its genes except the ninth gene from its animal rotavirus parent SB-1A and contains the eighth gene, coding for VP7, from its human rotavirus parent DS-1) and of porcine rotaviruses OSU, SB-1A, and Gottfried (G.) to ^{32}P-labeled full ssRNA transcripts of OSU, Gottfried, and SB-1A. The hybridization pattern of the dsRNAs of SB-1A to the OSU probe (lane 2) was indistinguishable from that of the dsRNAs of SB-1A × DS-1 (18-1) to the OSU probe (lane 3). This finding suggests that the ninth gene of SB-1A failed to form a hybrid with OSU, since the reassortant lacked the ninth gene of SB-1A yet formed a pattern identical to that of SB-1A when hybridized to the OSU probe. In contrast, the hybridization pattern of the dsRNAs of the reassortant to the Gottfried or SB-1A probe (lane 4 or 7) differs from the pattern of the dsRNAs of SB-1A to the Gottfried or SB-1A probe (lanes 5 and 8, respectively) in that a hybrid band corresponding to the ninth gene segment was absent in the hybridizations with the reassortant dsRNAs.

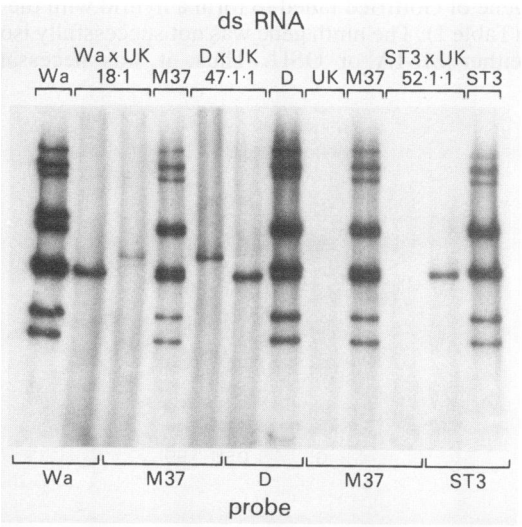

FIG. 4. Hybridization of genomic dsRNAs of single human rotavirus gene substitution reassortants Wa × UK (18-1), D × UK (47-1-1), and ST3 × UK (52-1-1) (which derive only the ninth gene coding for VP7 from their respective human rotavirus parents) to ^{32}P-labeled full ssRNA transcripts of M37. Since genomic dsRNAs of UK bovine rotavirus failed to form hybrids with the human rotavirus M37 probe (lane 8), any hybrid formation between the dsRNAs of 18-1, 47-1-1, or 52-1-1 and the M37 probe can be attributed to homology between the ninth genes of these human rotaviruses. Thus, the single hybrid bands present in the 18-1-M37 and 47-1-1-M37 reactions (lanes 3 and 5, respectively) demonstrated homology between the ninth genes of human rotaviruses Wa or D and M37, respectively. Homology was not demonstrated between the ninth genes of ST3 and M37 (lane 10).

with the corresponding genes of their bridging serotypes. Hybridization studies demonstrated homology between the fourth but not the ninth genes of M37 and ST3 and between the ninth but not the fourth genes of M37 and Wa. Homology was not observed between the fourth or ninth genes of Wa and ST3. Thus, the antigenically similar VP3s of M37 and ST3 and VP7s of M37 and Wa represent products of genes which have a high degree of homology.

Similar observations were made with porcine rotavirus intertypes; homology was demonstrated between the fourth but not the ninth genes of SB-1A and OSU and between the ninth but not the fourth genes of SB-1A and Gottfried. Homology was not demonstrated between the fourth or ninth genes of Gottfried and OSU. Thus, the antigenically similar VP3s of SB-1A and OSU are coded for by genes which exhibit significant homology, as are the antigenically similar VP7s of SB-1A and Gottfried. It is interesting that some degree of homology could also be demonstrated between the ninth genes of ST3 and Gottfried or SB-1A, suggesting that these porcine and human rotaviruses share serotype 4 specificity on the basis of antigenically related VP7s.

Our hybridization data also suggested that some intertypes may represent natural reassortants. The observations relating to SB-1A were particularly persuasive, as SB-1A and Gottfried were totally homologous in all gene segments except the fourth gene, and this segment of SB-1A was highly homologous with the corresponding gene of OSU.

Hybridization also indicated that a high degree of homology between the fourth or ninth genes of these intertypic rotaviruses (M37 and SB-1A) and their bridging serotypes was associated with an antigenically related VP3 or VP7, respectively. Conversely, the absence of hybrid segment formation under stringent conditions correlated with the absence of shared neutralization specificities. Other studies have examined the genetic relatedness among the fourth or ninth genes of human rotaviruses that cause symptomatic or silent infection and that have the neutralization specificity of serotype 1, 2, 3, or 4. The results have shown that a high level of homology between the corresponding ninth genes of two viruses is regularly associated with shared neutralization specificity attributable to VP7 (K. Midthun, J. Flores, A. Z. Kapikian, and R. M. Chanock, submitted for publication). However, this is not the case for the fourth gene, because in some instances hybrid formation is not associated with shared neutralization specificity (3). It should also be pointed out that failure to demonstrate homology under these stringent conditions of hybridization does not exclude serotypic relatedness. For example, rhesus rotavirus, a simian rotavirus, does not form hybrids with strain P, a human rotavirus, although both belong to serotype 3 and have a shared neutralization specificity attributable to VP7 (14, 15). The porcine strains OSU (serotype 4) and SB-1A (serotype 4 and serotype 5) share a one-way cross-reactivity with the canine strain CU-1 (serotype 3) on the basis of an antigenically related VP3, and CU-1 demonstrates a one-way cross-reactivity with the porcine strain Gottfried (serotype 4), probably on the basis of an antigenically related VP7 (7). However, genetic homology could not be demonstrated between CU-1 and the three porcine strains. Among rotaviruses derived from the same species, however, failure to form hybrids between the fourth or ninth gene appears to indicate absence of shared neutralization specificity related to VP3 or VP7, respectively. This relationship holds true for the human rotaviruses (Wa, M37, and ST3) and porcine rotaviruses (Gottfried, SB-1A, and OSU) examined in this study as well as additional human rotaviruses currently under study.

It appears that some intertypes may be the product of in vivo reassortment and that the antigenic similarity between VP3 or VP7 of the intertypic rotaviruses and the corresponding protein of their bridging serotypes is associated with a high degree of homology between the genes coding for the related proteins (Tables 1 and 2).

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