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Evidence for the Role of the P2 Protein of Human Rhinovirus in Its Host Range Change

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Human rhinovirus 39 (HRV39) is blocked in nonpermissive L cells at both adsorption and intracellular replication steps. We have selected a host range variant of HRV39 capable of bypassing the intracellular replication block and found it to have altered nonstructural P2 proteins. These alterations are similar to those reported earlier for host range variants of HRV2 (F. H. Yin and N. B. Lomax, J. Virol. 48:410–418, 1983). This observation suggests that the intracellular replication block for both HRV2 and HRV39 in mouse L cells is at the same step. We propose that the P2 protein is an essential viral component that cannot function in mouse L cells unless altered. This alteration occurs spontaneously in stocks of HRV39 during growth in permissive HeLa cells.

Human rhinoviruses (HRVs) possess a very limited host range and are normally capable of replicating only in cells of primate origin. Mouse L cells lack receptors for the major group of HRVs; thus, replication of the major group in L cells is blocked because the virus cannot adsorb (1). Adsorption blocks have been reported for other picornaviruses (12). Not surprisingly, host range variants of these viruses usually have altered structural proteins (8, 12). In contrast, the minor group of HRVs is able to adsorb to receptors on the nonpermissive mouse L cells (1). We have previously reported that HRV2 of the minor group not only can adsorb to L cells but also can uncoat and inhibit cellular synthesis. However, this virus fails to make viral RNA or viral progeny (13). Host range variants of HRV2 which can bypass this intracellular replication block have been isolated. We demonstrated that HRV2/L, a variant of HRV2 able to replicate productively in L cells, produces nonstructural P2 proteins with altered electrophoretic mobilities on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Our data also indicated that the alteration of the P2 proteins covaries with the host range and that P2 may be involved in viral RNA synthesis (13).

To further substantiate the covariation of P2 with host range, we attempted to select host range variants from a serologically and biologically distinct rhinovirus strain. We describe here HRV39, which belongs to the major group and cannot adsorb to L cells. We show that when this adsorption block is bypassed by RNA transfection, HRV39 is still unable to replicate in L cells. We have isolated a host range variant of HRV39 which is capable of productively transfecting L cells. This variant has P2 proteins with alterations similar to those found for HRV2/L. These observations strengthen the argument that bypass of the intracellular replication block in L cells is mediated by a step involving P2 proteins and that wild-type P2 proteins represent viral components that cannot function properly in L cells.

Both HRV2 (strain HGp) and HRV39 (strain 209) yield significant virus following one cycle of replication in HeLa cells; however, neither produces detectable virus following L-cell (strain LM) infection, as shown in Table 1. In contrast, the host range variant, HRV2/L, can replicate equally well in both HeLa and L cells, although producing somewhat lower yield per cell than the parent strain.

L cells have no receptors for HRV39. To determine whether other replication blocks also exist, viral RNA was used to transfect HeLa or L-cell monolayers. RNA was prepared from purified virions by protease digestion and oligo(dT)-cellulose chromatography as described by Callahan et al. (3). HeLa and L-cell monolayers were transfected with dilutions of RNA prepared in buffer containing 0.14 M LiCl, 1 mM $MgCl_2$, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and 1.2 mg of DEAE-dextran per ml (6). The cells were overlaid with growth medium and incubated at 34°C for 18 h, allowing for one cycle of virus replication, and virus yield was determined by plaque assay on HeLa cells. The relative infectivity of the viral RNA in HeLa and L cells is expressed as the log ratio of virus yield in the two hosts (Table 2). Viral RNAs of HRV2 and HRV2/L were used as controls. The viral RNA of HRV2 was able to transfect HeLa cells but not L cells (Table 2). Viral RNA of HRV2/L transfected HeLa and L cells equally well. Viral RNA of HRV39, although more productive in infecting HeLa cells, was capable of only limited infection in L cells, yielding approximately 25 PFU/120 ng of RNA.

Since there is no background of input virus from an infectious RNA assay, even low levels of virus recovered represent virus replication. This population recovered from the L-cell transfection included those variants capable of bypassing replication blocks. One such variant from HRV39-transfected L cells was plaque purified and propagated in HeLa cells and was referred to as HRV39/L. The parent strain was referred to simply as HRV39. Viral proteins of HRV39 and HRV39/L were compared by SDS-PAGE as described previously (13). Previous studies established that the host range variant of HRV2 has an altered nonstructural protein 2BC. While 2BC of HRV2 has an apparent molecular weight of 43,000, 2BC of HRV2/L has an apparent molecular weight of 45,000. Host range variant HRV39/L produced a protein with an apparent molecular weight of 45,000 (45K), while electrophoresis of proteins from the parent virus HRV39 showed a doublet band, one at an apparent molecular weight of 45,000 and one at an apparent molecular weight of 43,000 (Fig. 1).

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TABLE 1. Virus yield in HeLa and L cells[a]

Virus	One cycle yield (PFU/cell)	
	HeLa cells	L cells
HRV2	91	<0.03
HRV2/L	37	35
HRV39	76	<0.04

[a] HeLa and L-cell monolayers were infected with a multiplicity of infection of 5 for each virus indicated. Twenty-four hours after infection, the cells were subjected to two cycles of freezing and thawing; virus from the supernatant was titrated on HeLa cells by standard plaque assay.

Emini et al. have reported the antigenic conservation of the P2 region proteins between poliovirus, several echovirus and coxsackievirus strains, and rhinovirus types 2 and 14 (5). Therefore, we used antiserum against poliovirus P2 protein (kindly provided by R. Hanecak and E. Wimmer) to identify the P2 proteins of HRV39. The procedure of immunoprecipitation was that of Semler et al. (11). Antiserum against poliovirus 2BC specifically immunoprecipitated a doublet band at 43,000 and 45,000 molecular weight from HRV39-infected HeLa cell lysates, while the same antiserum immunoprecipitated a single band at 45,000 molecular weight from HRV39/L-infected HeLa cell lysates (Fig. 2). The smaller-molecular-weight band immunoprecipitated from both HRV39- and HRV39/L-infected lysates at an apparent molecular weight of 38,000 is presumably 2C, the cleavage product of 2BC. Antiserum prepared against purified HRV39 virion specifically precipitated viral structural proteins but did not precipitate proteins of 45,000, 43,000, or 38,000 molecular weight (data not shown). These results indicate that the 45K and 43K proteins of HRV39 and HRV39/L are the nonstructural protein 2BC.

Several possible explanations exist for the appearance of two bands at the 2BC position of HRV39. It is possible that the 43K protein is an additional cleavage product of the 45K 2BC protein which is present only in the parental HRV39 strain. Alternatively, the double band may indicate a mixed population of virions in the parent stock. We investigated the latter possibility by randomly selecting plaques of HRV39 and examining the P2 protein in these virus isolates. Ten plaques from HRV39 were plaque purified three times and propagated in HeLa cells; viral proteins from these isolates were labeled with [^{35}S]methionine and analyzed by one-dimensional SDS-PAGE. Each individual plaque isolate of HRV39 had only a single protein band which electrophoresed with a mobility corresponding to that of 2BC (Fig. 3). One-half of the plaque isolates had a protein with an apparent molecular weight of 43,000, while the other half had a protein with an apparent molecular weight of 45,000. Thus, the double 2BC band observed in the parent HRV39-infected cell lysates was due to a mixed population of virions. Also apparent in Fig. 3 is the variation in mobility of viral protein P1 in individual isolates (shown in lanes 6, 8, and 13), which had been identified by immunoprecipitation as the precursor polyprotein to the capsid proteins (unpublished results). This observed variation is independent of host range and will not be discussed here.

TABLE 2. Virus yield following HeLa and L-cell transfection

Viral RNA source	Total virus recovered[a] (PFU) after transfection in:		Relative infectivity[b]
	HeLa cells	L cells	
HRV2	8.0×10^5	0	>5.9
HRV2/L	7.7×10^2	1.7×10^2	0.7
HRV39	3.6×10^4	2.5×10^1	3.2
HRV39 isolate 1	4.1×10^5	0	>5.6
HRV39 isolate 2	2.5×10^5	5.0×10^2	2.7
HRV39 isolate 4	2.4×10^5	0	>5.4

[a] Total virus recovered represents 1 cycle yield following transfection of a HeLa or L-cell monolayer with 120 ng of viral RNA.

[b] Relative infectivity = log (total virus recovered after HeLa cell transfection/total virus recovered after L-cell transfection).

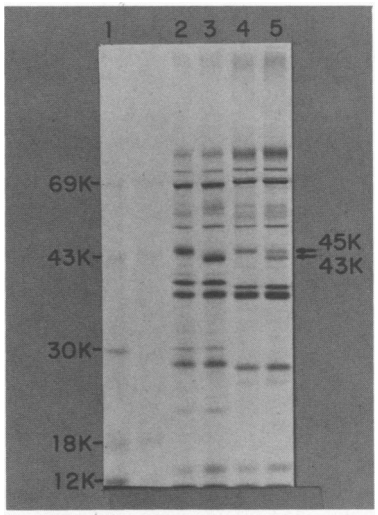

FIG. 1. Autoradiograph of viral proteins. HeLa cell monolayers were infected with the virus indicated and radiolabeled with [^{35}S]methionine. Lysates were subjected to electrophoresis on 10% polyacrylamide gels. Lanes: 1, molecular weight marker proteins; 2, HRV2/L proteins; 3, HRV2 proteins; 4, HRV39/L proteins; 5, HRV39 proteins.

To verify the correlation between the variations of the P2 protein with the host range in the HRV39 isolates, RNA

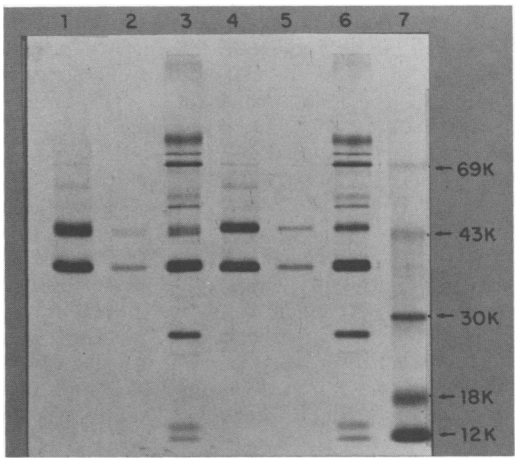

FIG. 2. Immunoprecipitation of viral proteins analyzed by SDS-PAGE. Radiolabeled infected cell lysates were prepared, incubated with polio 2BC antiserum, and immunoprecipitated with staphylococcus A cells as described previously (11). Lanes: 1 and 2, HRV39-infected cell lysate (30 and 6 μl of lysate, respectively); 4 and 5, HRV39/L-infected cell lysate (30 and 6 μl of lysate, respectively); 3 and 6, total cell lysate from HRV39- and HRV39/L-infected cells; 7, molecular weight markers.

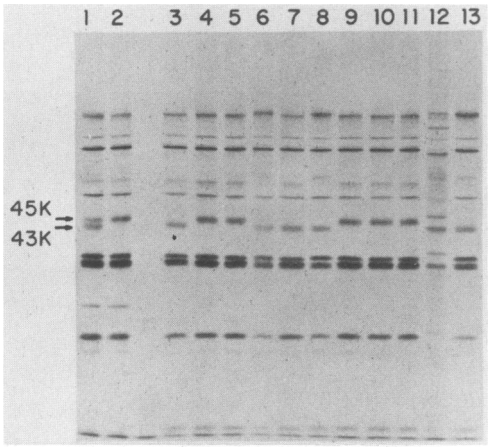

FIG. 3. Autoradiograph of viral proteins of HRV39 plaque isolates. Cells were infected with the viruses indicated and radiolabeled with [^{35}S]methionine, and lysates were electrophoresed on a 10% polyacrylamide gel. Lanes: 1, HRV39 proteins; 2, HRV39/L proteins; 3 to 11 and 13, proteins from HRV39 individual plaque isolates; 12, proteins from uninfected cells. Positions of the 43K and 45K proteins are indicated at left.

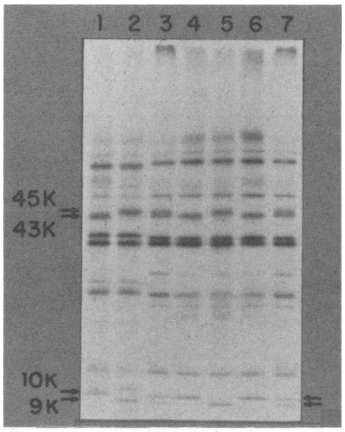

FIG. 4. Autoradiograph of viral proteins. ^{35}S-labeled infected cell lysates were analyzed on a 9 to 18% gradient polyacrylamide gel. Lanes: 1, HRV2 proteins; 2, HRV2/L proteins; 3 and 7, HRV39 proteins; 4, HRV39 (plaque isolate 1) proteins; 5, HRV39 (plaque isolate 2) proteins; 6, HRV39 (plaque isolate 4) proteins. Positions of the 9K, 10K, 43K, and 45K proteins are indicated at left.

from three representative HRV39 plaques was isolated and used to transfect both HeLa and L-cell monolayers. As indicated in Table 2, only RNA from HRV39 plaque isolate 2 (Fig. 3, lane 4; 2BC protein of 45,000 molecular weight) was able to transfect L cells; RNA from either HRV39 plaque isolate 1 (Fig. 3, lane 3; 2BC protein of 43,000 molecular weight) or HRV39 plaque isolate 4 (Fig. 3, lane 6; 2BC protein of 43,000 molecular weight, larger P1) was unable to transfect L cells. Thus, the 45K viral protein of HRV39 covaried with host range.

Our previous study with HRV2 indicated that the only other difference between HRV2 and HRV2/L which was detectable by SDS-PAGE was a low-molecular-weight protein. This protein, in HRV2-infected cell lysates, migrates as a 10K protein, while in HRV2/L-infected lysates it migrates as a 9K protein. This protein has subsequently been identified as 2B (unpublished results). A comparable alteration was observed in HRV39 and HRV39/L, in which the parent stock had a double band corresponding to apparent molecular weights of 10,000 and 9,000, while HRV39/L had a single band at an apparent molecular weight of 9,000 (Fig. 4).

Thus, both viral proteins 45K and 9K of HRV39 covary with host range. This observation is identical to what had been found for HRV2. Both host range variants were selected by passage through the nonpermissive L cells; no mutagen was used. The variants were found to preexist as a subpopulation in the parent stock. HRV2 and HRV39 are different not only serologically but also in their receptor-binding characteristics in mouse L cells. In view of the differences between these two viruses, our findings of parallel changes of the P2 proteins in their host range variants are not likely to have been fortuitous. We propose that the intracellular replication block of HRV2 and HRV39 is at the same step, and P2 proteins represent viral replication components that are cell specific; the 43K protein functions only in human cells, and the 45K protein functions in both mouse and human cells.

As noted previously, RNA from HRV2/L yielded approximately equal levels of progeny from L- or HeLa-cell transfection. However, transfection of L cells by the host range variant HRV39 plaque isolate 2 yielded fewer progeny than did a parallel HeLa cell transfection (Table 2), suggesting that in addition to the change of 2BC to the 45,000-molecular-weight form, other viral alterations may be required for more efficient replication of HRV in L cells.

The molecular basis for the alteration of the P2 proteins in the host range variants is under investigation. To date we have sequence information only for HRV2/L. Amino acid analysis of the N terminus of 2BC of HRV2/L showed no change of cleavage site at this junction. RNA genomic sequence predicted no change of amino acids in the 2A region, three changes in the 2B region, and two changes in the 2C region. Only one amino acid change, from lysine to glutamic acid in 2B, involves a change in the charge of the polypeptide (manuscript in preparation). In view of this finding, we cannot rule out the possibility that other proteins also have altered amino acids and that some of the changes could affect host range without visibly altering the electrophoretic mobilities of the viral protein. However, our data indicate that 2BC is an essential component in the change of host range, and the one-amino-acid change from lysine to glutamic acid in 2B could be sufficient to account for the altered electrophoretic mobility of 2BC.

The functions of these nonstructural proteins have not been clearly delineated; however, P2 proteins are thought to be involved in viral RNA replication for the following reasons. First, P2 proteins have always been found to be physically associated with the viral replication complex (2). Second, the locus for the RNA synthesis inhibitor, guanidine HCl, has been mapped for poliovirus and foot-and-mouth disease virus in the P2C region (9, 10). Third, we previously reported that the host range variant HRV2/L with altered P2 proteins has a decreased susceptibility to inhibitors of viral RNA synthesis but no change in susceptibility to inhibitors which affect other viral processes, such as adsorption or polyprotein cleavage (13); similar decreased susceptibility to RNA inhibitors has been found for HRV39/L (unpublished data). Lastly, recent reports identified a consensus sequence of a nucleotide-binding pocket in 2C of poliovirus, HRV2, and foot-and-mouth disease virus, indicating the possible role of P2 in carrying out a nucleoside triphosphate-dependent step in nucleic acid replication other than that of viral polymerase (4, 7). Therefore, we postulate that in L cells, both HRV2 and HRV39 are blocked at an RNA synthesis

step; the host range variants HRV2/L and HRV39/L can synthesize RNA in L cells because of a conformational modification of the P2 proteins which allows their interaction with formerly nonpermissive host factors.

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