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Analysis of Cytomegalovirus Genomes with Restriction Endonucleases HinD III and EcoR-1

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Cleavage of genomes of eleven human, one simian, and one simian-related cytomegalovirus (CMV) isolate by the restriction endonucleases HinD III and EcoR-1 generated reproducible DNA fragments. The size range of CMV DNA fragments as estimated by contour length measurements in comparison with simian virus 40 form II DNA and by coelectrophoresis with EcoR-1 fragments of herpes simplex virus DNA varied between 15×10^6 and 0.5×10^6 daltons. Comparison of the cleavage products of each isolate in 1% agarose slab gels showed extensive comigration of fragments among the human CMV isolates. In the HinD III digests, three fragment bands comigrated among all human CMV isolates, and six fragments comigrated among most, but not all, human CMV isolates. In the EcoR-1 digests, nine fragment bands comigrated among all human CMV isolates, and five bands comigrated among most, but not all, human isolates. Each isolate had a distinctive electrophoretic profile with either HinD III or EcoR-1 digests. No two isolates had identical HinD III or EcoR-1 patterns although some isolates did share more general pattern similarities than others. No clear-cut subgrouping of isolates based on cleavage pattern characteristics could be discerned. Comparison of HinD III and EcoR-1 patterns showed that human isolates differ greatly from nonhuman CMV isolates. HinD III and EcoR-1 digests of each isolate contained both major and minor molar classes of DNA fragments that ranged from about 1 and multiples of 1 M down to about 0.25 M; however, the summed molecular weights for major molar fragments resulting from HinD III or EcoR-1 digests of several isolates closely approximated the molecular weight of 10^8 of the intact genome.

Cytomegalovirus (CMV) is a ubiquitous pathogen with a variety of clinical manifestations (18, 32). As with other herpes-group viruses, it is able to maintain latent infections with subsequent recurrence (6, 20; J. N. Dowling, A. Saslow, J. A. Armstrong, and M. Ho, Yale J. Biol. Med., in press; E. R. Alexander, personal communication; and our unpublished data), and it has recently been implicated as a possible oncogenic virus (1, 19). In contrast to the herpes simplex viruses, CMV requires a much longer infectious cycle, is more host cell-type specific, is more strongly cell associated, and has distinctive thermal and chemical lability characteristics in the extracellular state (2, 3, 25, 30, 34).

Several clinical isolates and tissue culture-adapted strains of CMV are available. Although a few immunological studies have been performed to ascertain the antigenic diversity among some of these isolates (4, 7, 17, 31-33; E. S. Huang, B. A. Kilpatrick, Y. T. Huang, and J. S. Pagano, Yale J. Biol. Med., in press), little is known about whether most isolates belong to the same or different CMV strains or whether a given strain is always isolated from similar clinical sources. Also unknown is whether individual CMV isolates are capable of exhibiting only specific or a variety of pathological characteristics. Only recently have genome homology studies been done. There is a high degree (80% DNA homology) of genetic relatedness among several human CMV isolates (Huang et al., in press) and no detectable homology between these human isolates and the simian and murine strains (15).

With the aim of comparing genetic and structural heterogeneity of the CMV genomes, we subjected the DNA of selected CMV isolates to analysis by restriction endonuclease digestion and coelectrophoresis. Since the degree of sensitivity of such a comparison is limited largely by the number of cleavage sites recognized by a given restriction enzyme, it is best to use an enzyme that will cleave the genome into size pieces sufficient to code individually for one or

a few proteins and still generate a distinctive electrophoretic pattern. With such digests, although comigration of fragments of DNA does not necessarily mean base sequence identity of these fragments, extensive comigration would indicate significant genetic or organizational similarity of the virus genomes. In this paper we report such a comparison based on analyses of CMV DNA with the restriction endonucleases EcoR-1 (9) and HinD III (29).

MATERIALS AND METHODS

Cells and virus isolates. The human fibroblastic cell strain WI-38 was cultivated as described previously (13). Eagle minimal essential medium contained 10% fetal calf serum and 100 μg each of penicillin and streptomycin per ml. Occasionally, kanamycin (100 μg/ml) was added.

Eleven human, one simian, and one simian-related (Colburn) CMV isolates were used in this study. The CMV isolates are listed in Table 1. All plaque purifications were done by terminal dilution.

Herpes simplex virus type I (strain KOS [HSV-KOS] [29]) was used as a marker and a control for digest conditions (see below).

Infection and labeling of viral DNA. WI-38 monolayers were infected with each isolate at a multiplicity of approximately 1 to 2 PFU/cell. After a 2-h adsorption period at 37 C, minimal essential medium containing 2% fetal calf serum was added, and the cells were incubated for 16 to 20 h at which time virus DNA synthesis begins (13). The medium was then replaced with low-phosphate minimal essential medium containing 2% dialyzed fetal calf serum and 10^{-5} M Na_2HPO_4, and 30 μCi of carrier free [^{32}P]orthophosphate per ml was added. The medium was changed every 4 days or as determined by the cytopathic effect.

TABLE 1. *CMV isolates analyzed with restriction endonucleases in this study*

CMV isolate	Origin	Source	Reference
AD-169	Adenoid tissue	W. Rowe	24, 33
Davis	Liver	T. Weller	33
C-87	Kidney	M. Benyesh-Melnick	3
TW-087	Cervix	E. S. Huang	4
Kerr	Urine	U.S. Naval Hospital, Chelsea, Mass.	33
Esp	Urine	Babies Hospital, New York	33
UW-1	Urine (CMV inclusion disease)	B. Wentworth	4
Town	Congenital infection	S. Plotkin	9
NC-496	Liver	Y. T. Huang N. C. Memorial Hospital, University of North Carolina	
RCH-234	Australian strain (CMV inclusion disease)	I. Jack	
Isolate-7	Denmark strain (CMV inclusion disease)	H. Anderson	
Colburn	Human brain	C. Alford	21
GR-2757	Monkey kidney	M. Benyesh-Melnick	7

Purification of virus and viral DNA. ^{32}P-labeled HSV-KOS DNA was purified as described (13) and was a gift from J. S. Shaw. All virus was harvested and purified exclusively from the extracellular media, and viral DNA was purified as described (13). Simian virus 40 DNA was purified from purified virions (14); form II DNA was separated from form I DNA by ethidium bromide-CsCl equilibrium centrifugation (22).

Restriction endonucleases. Restriction endonuclease EcoR-1 was purified from *Escherichia coli* strain Ry13 by the method of Greene et al. (9), and HinD III was purified from *Haemophilus influenzae* by the method of Smith and Wilcox (28).

Restriction enzyme digests. Purified ^{32}P-labeled viral DNA was dissolved in TBS (0.15 M NaCl–0.05 M Tris-hydrochloride, pH 7.4) and digested with either EcoR-1 or HinD III. EcoR-1 or HinD III digestion mixtures (20 μl), each containing 0.02 to 0.04 μg of viral DNA (specific activity, 6.62×10^5 counts/min per μg of DNA), 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, and 3 to 5 μl of enzyme, were incubated for 24 to 28 h at 37 C. Preparative digests contained 1 to 2 μg of ^{32}P-labeled DNA and 20 to 40 μl of enzyme. Digestion was stopped, and samples were prepared for layering on the electrophoresis gel by adding 1/10 volume of a tracking solution containing 0.1 M EDTA, 60% sucrose, and 0.5% bromophenol blue.

Slab gel electrophoresis. Vertical 0.7, 1, and 1.4% agarose (SeaKem) slab gels were used. Analytical gels were 1.5 by 150 by 250 mm; preparative gels were 3 mm thick. Agarose was dissolved in E buffer (40 mM Tris, 20 mM sodium acetate, and 1 mM EDTA), pH 7.2, and autoclaved. The E buffer also served as the electrode buffer. Gels were prerun 30 min to 1 h before loading samples.

The samples were electrophoresed at a constant voltage of 4 V/cm (about 45 mA) at room temperature with constant buffer recirculation until the bromophenol blue migrated to the bottom of the gel (8 to 8.5 h). The gels were then vacuum dried onto filter paper and exposed to X-ray film (Kodak RP/R2) for autoradiographs. Some preparative gels were not dehydrated in order to aid recovery of DNA; DNA fragment bands were localized by autoradiography at 4 C or by ethidium bromide staining. Optimum exposure time for analytical autoradiographs was about 1 week. Several autoradiographs were scanned with a Joyce Loebl microdensitometer (Tech/OP Instruments, Burlington, Mass.) for estimations of molar concentrations of DNA.

Electron microscopy and length measurements. After DNA fragment bands were localized in the preparative gel, individual bands or closely migrating size classes were cut out, and the DNA was recovered by electroelution. The DNA was ethanol precipitated at −20 C, centrifuged for 20 min at 10,000 rpm in a Sorvall HB-4 rotor, and redissolved

in TBS containing 5 mM EDTA. The formamide method for spreading DNA was used (5). A 50-μl spreading solution containing CMV DNA fragments, simian virus 40 form II DNA (as an internal length marker), 0.025 mg of cytochrome *c* per ml, 0.1 M Tris-hydrochloride, 10 mM Na_2 EDTA, and 40% formamide (pH 8.5) was spread onto a hypophase containing 10 mM Tris-hydrochloride, 1 mM Na_2 EDTA, and 10% formamide (pH 8.5). The film was allowed to stand 1 min, and samples were transferred to collodion-coated nickel grids (400 mesh). The gird samples were dehydrated and stained for 1 min with 10^{-5} M uranyl acetate in 90% ethanol, and the collodion films were stabilized against distortions from the electron beam by a thin carbon coating.

The grids were examined in an AEI EM 6-B electron microscope at 60 kV, and micrographs were taken at a calibrated magnification of ×6650. Micrographs were enlarged 27.5 times with a projector, and DNA molecules were traced onto paper and measured with a map measurer.

RESULTS

Restriction endonuclease digestion of CMV DNA. Preliminary screening of restriction enzymes showed that the endonucleases EcoR-1 and HinD III give satisfactory digestion products for comparison of a variety of CMV isolates. Either enzyme generates a characteristic and reproducible set of DNA fragments from each CMV isolate. The size range of fragments is such that the largest fragment from any given isolate represents no more than about a 15% segment of the genome, and the smallest fragment represents about 0.5% of the genome, assuming a molecular weight of 10^8 for the CMV genome (13).

Analysis of digestion products of CMV DNA by gel electrophoresis. Slab gels (conditions described above) were used for all analytical comparisons since previous screening experiments with several gel types had shown the 1% agarose slabs to provide optimum resolution for comparisons of CMV DNA fragments of the sizes generated with EcoR-1 and HinD III. Figure 1A and B show autoradiographs of analytical 1% agarose slab gels of ^{32}P-labeled DNA from several CMV isolates digested with HinD III and EcoR-1, respectively. Digestion patterns of the human CMV isolates showed several comigrating and several unique fragment bands. The Colburn isolate, obtained from human brain (21), and GR-2757, a simian isolate, showed little or no comigration with any human isolate. The Colburn isolate shared more comigration fragments with GR-2757 than with any human isolate. By DNA-DNA reassociation kinetics analysis, we have found that Colburn virus is more closely related to simian virus (Huang, Kilpatrick, Lakeman, and Alford, manuscript in preparation); Colburn is not considered as human CMV in this report. Although some human CMV isolates had very similar electrophoretic patterns, no two isolates had identical patterns. The number of comigrating fragments produced from EcoR-1 digestion was greater than the number from HinD III digestion for human CMV isolates.

Several interesting observations can be made from a comparison of these electrophoretic profiles. Some fragment bands were found in most or all cleavage products, and some virus isolates had generally very similar cleavage patterns.

(i) HinD III digests. HinD III digestion yielded several distinctive common comigrating DNA fragment bands (see Fig. 1A and 4). Specifically, (i) one DNA fragment band (3.0×10^6 daltons) comigrated among all CMV isolates, except in digests of the human isolates C-87 and Esp (marked with F, Fig. 4). (ii) A second band with a molecular weight of 5.2×10^6 comigrated with all CMV isolates, except the simian virus GR-2757 and the human isolates Esp and UW-1 (marked with B, Fig. 4). (iii) Three fragment bands (molecular weights, 6.0×10^6, 3.4×10^6, and 1.1×10^6) comigrated among all human CMV isolates (marked with A, E, and G, respectively, Fig. 4); however, one of these was seen as a closely migrating doublet in Town, AD-169, and UW-1 (marked with A, Fig. 4). (iv) Four separate bands comigrated with most, but not all, human isolates (C, D, H, and I, Fig. 4). Two of these bands (molecular weights, 0.64×10^6 and 1.0×10^6) comigrated with all human isolates, except Isolate-7 (marked with I, Fig. 4) and Town and UW-1 (marked with H, Fig. 4), respectively. Finally, two fragment bands (molecular weights, 4.0×10^6 and 3.6×10^6) comigrated with all human isolates, except Davis (marked with C, Fig. 4) and RCH-234, UW-1, and Town (marked with D, Fig. 4), respectively. Both of these latter two bands varied in intensity, and thus in molar amount, among these isolates (see below).

Simply stated, HinD III fragment bands A, B, C, D, E, F, G, H, I, and J were dominant, extensively comigrating bands among most or all of the human CMV isolates, with band F, found in both the simian isolate (GR-2757) and Colburn, and band B, found also in Colburn, being the only bands from either nonhuman isolate to significantly comigrate with the human isolates.

The similarities of the above and other comigrating fragment bands plus varying heterogeneity among digests resulted in several possi-

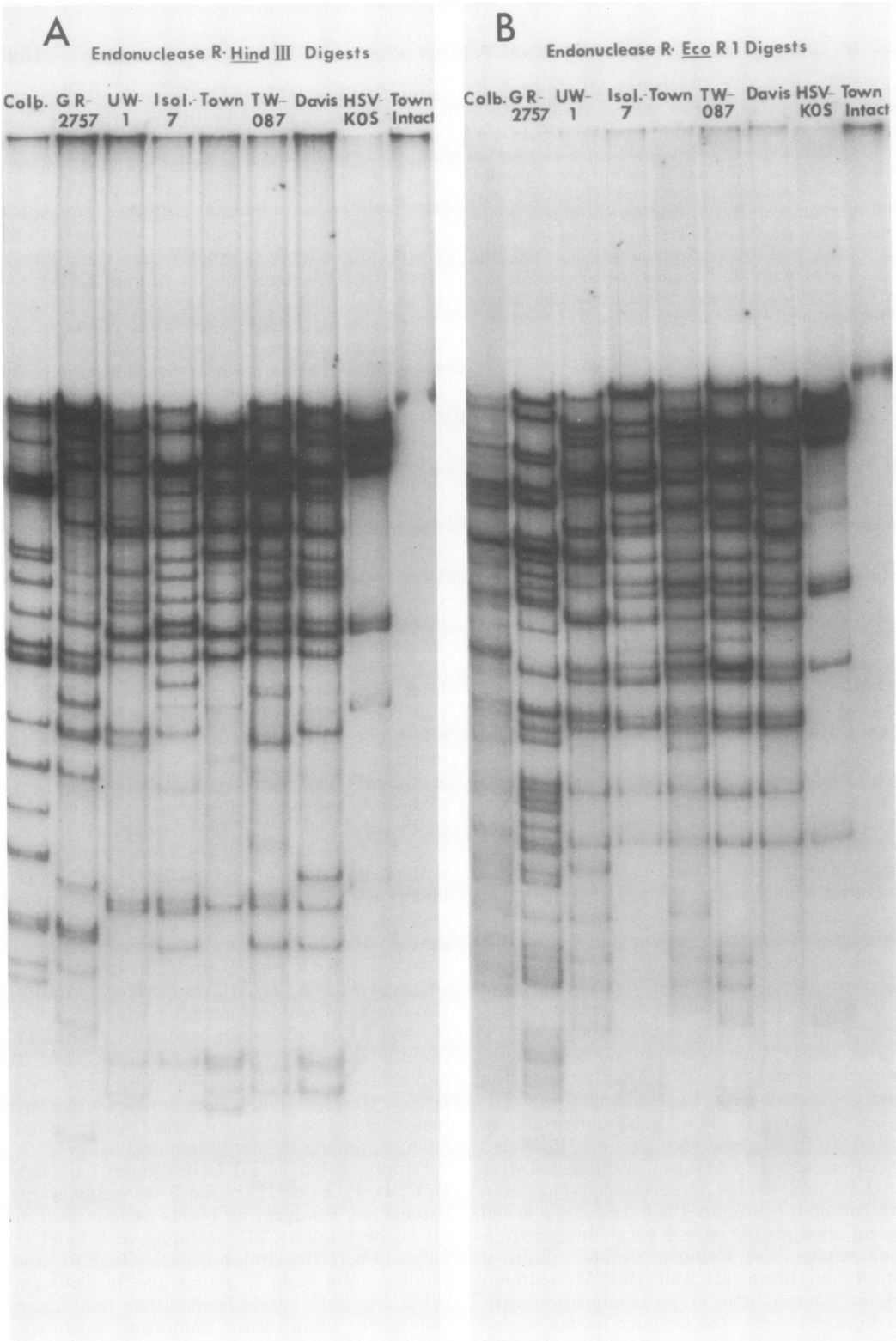

FIG. 1. *Coelectrophoresis comparison of DNA from CMV isolates by cleavage of the DNA with restriction endonucleases EcoR-1 and HinD III and separation of the fragments in 1% agarose gels. CMV DNA labeled with ^{32}P was purified, digested with EcoR-1 or HinD III, electrophoresed, and exposed to X-ray film for autoradiographs, as described in the text. KOS, a type 1 herpes simplex virus strain, was also used as a molecular weight marker and as a control for digestion conditions. HSV-KOS DNA in both (A) and (B) was digested with EcoR-1. (A) HinD III digests, (B) EcoR-1 digests.*

ble groupings of the human CMV isolates into similar patterns; however, two sets of patterns with varying degrees of similarity and heterogeneity continually emerged. These were Esp, C-87, AD-169, and NC-496 and Davis, TW-087, Kerr, and Isolate-7. Lesser similarities could be found between RCH-234, UW-1, and Town and members of both sets. Large portions of the patterns in each set comigrated. However, two through several major band differences existed among most of the isolates in these two sets. Two pairs of human CMV isolates (AD-169 and NC-496, Isolate-7 and Kerr) had very similar cleavage patterns. AD-169 and NC-496 had HinD III patterns differing by only two to three minor bands and two major bands, one of which was a comigrating doublet in AD-169. Isolate-7 and Kerr differed by the absence of three major and three minor bands between both patterns. The Colburn and GR-2757 patterns were largely different; however, several bands did comigrate between these two strains.

(ii) EcoR-1 digests. EcoR-1 digestion yielded several common comigrating fragments; however, no EcoR-1 fragment bands were found that comigrated among all CMV isolates (see Fig. 1B and 5). Specifically, (i) nine EcoR-1 fragment bands, ranging from 4.3×10^6 to 1.3×10^6 daltons, were found that comigrated among all of the human CMV isolates (A, B, D, E, F, G, H, J, K, respectively, Fig. 5). These were all major bands; however, one of these appeared in digests of AD-169, NC-496, and C-87 as a faint minor band very closely migrating under a higher-intensity major band (marked with A, Fig. 5). (ii) A group of three low-molecular-weight fragments (0.87×10^6 through 0.73×10^6) comigrated among all human isolates, except Davis and Isolate-7 (L, M, N, Fig. 5). (iii) Two additional comigrating fragment bands (3.6×10^6 and 1.8×10^6 daltons) were found that roughly divided the CMV isolates into two separate groups (Davis, Isolate-7, RCH-234, TW-087, Kerr, Esp, Town; Esp, Town, UW-1, AD-169, NC-496, C-87), with Town and Esp being the only isolates having both fragment bands (marked with C and I, respectively, Fig. 5). Several other fragment bands were found that comigrated among fewer human isolates.

Larger portions of the EcoR-1 patterns of each human CMV isolate comigrated identically with portions of several or all human CMV EcoR-1 patterns, and the difference between these isolates was less obvious than with the HinD III digests. Distinction among EcoR-1 digests of the human CMV isolates into separate groups was again unclear. However, specific pairs of human isolates did have more generally similar patterns (NC-496 and C-87; Davis and Isolate-7); the differences among these patterns existed mainly in the high-molecular-weight fragments (8.3×10^6 to 5.7×10^6). As with the HinD III digests, Colburn and GR-2757 shared few or no comigrating fragments with human CMV isolates. However, in contrast to the HinD III digest, the similarity of the DNA fragment patterns generated by EcoR-1 of Colburn and GR-2757 is not as obvious as patterns generated by HinD III of these two isolates. Again, no two patterns were identical.

Detemination of molar amounts and molecular weights of DNA fragments. The autoradiographs of all CMV DNA digests, using either enzyme, showed both high-intensity major bands and fainter minor bands. Quantitation of the molar amounts of DNA in each fragment band by calculations obtained through slicing and counting the radioactivity in the gel was not possible due to the close migration of many of the bands. Figure 2 shows a typical microdensitometer scan of a CMV (Town isolate) restriction digestion profile. By scanning an autoradiograph of electrophoretically sepa-

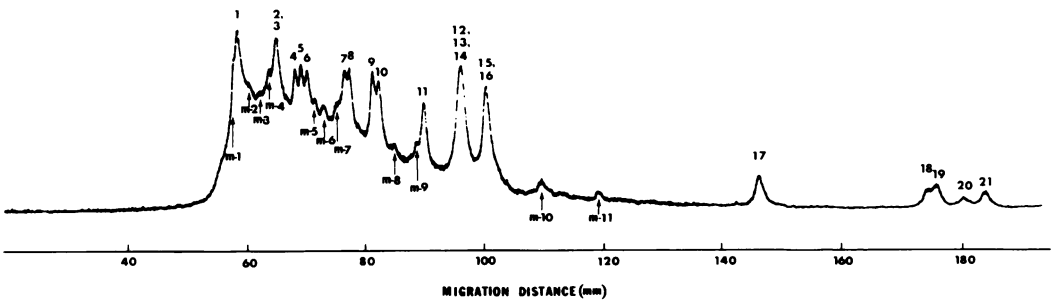

FIG. 2. *Microdensitometer scan of autoradiograph of electrophoretically separated CMV DNA fragments cleaved by HinD III. A Town DNA cleavage pattern (as shown in Fig. 1A) was scanned at a 1:5 scan-to-record ratio. Fragments are labeled by order of increasing mobility. Fragments found to be in "major" molar amounts are designated by numbers, and fragments in submolar "minor" amounts are designated by numbers prefixed by "m" (see Fig. 3).*

rated DNA fragments at a 1:5 scan-to-record ratio, it was possible to approximate the molar concentrations of the fragments in the profile. Major fragments were generated in amounts equivalent to about 1 copy (0.9 to 1.1) or multiples of 1 copy/genome, and minor fragments were generated in submolar amounts down to about 0.25 copies/genome (Fig. 3).

Town DNA fragments, generated by HinD III and electrophoretically separated, were measured by electron microscopy for contour lengths relative to simian virus 40 form II DNA (3.6×10^6 daltons) (12). As shown in Table 2, molecular-weight estimates of Town fragments from these contour length measurements agreed very well with estimates from coelectrophoresis of EcoR-1-digested HSV-KOS DNA fragments (27). Total molecular weights were determined from estimated molar amounts and electron microscope molecular weight measurements of Town DNA fragments. Table 3 lists the estimated total molecular weight of major DNA fragments generated by either HinD III or EcoR-1 from several of these CMV isolates. These total molecular weights ranged from 97 $\times 10^6$ to 105 $\times 10^6$ for the HinD III digest and from 95 $\times 10^6$ to 105 $\times 10^6$ for the EcoR-1 digests. These values agreed well with the estimated molecular weight of 10^8 of intact CMV DNA (13). Additionally, the total number of major fragments was large and consistent for all isolates in both the HinD III and EcoR-1 digests (Table 3).

The origin of the submolar bands is not clear. Since the ratio of noninfectious to infectious CMV particles is normally high (about 500:1 to 200:1) in a CMV seed preparation, the minor bands may be entirely or partly accounted for by defective genomes; this high ratio is common for the herpes-group viruses. Additionally, the peculiar lability of infectivity characteristic of CMV may be in some degree due to genome breaks, which could generate minor bands. However, it was clear that any population of defective genomes, if present, was not significantly fluctuating for the following reasons. (i) The same restriction fragment patterns (including background) were always obtained for any given isolate (several CMV DNA preparations were used, involving preparations from several virus passages; e.g., the Town isolate showed no pattern variations in passages 30 to 36). (ii) The restriction enzyme patterns were identical before and after plaque purification. These minor fragments were not due to partial digestion products. This was clear from the HSV-KOS digest control, other DNA digests in which known products were obtained (data not

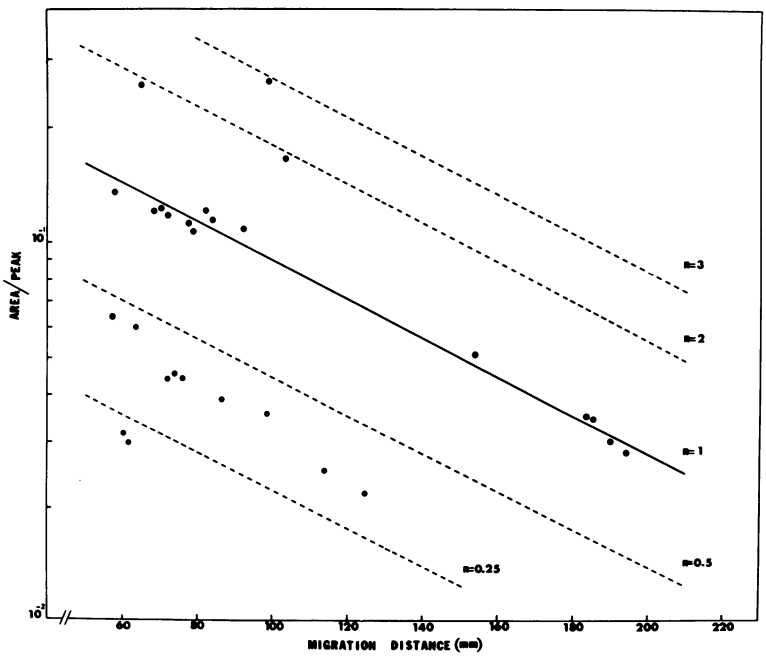

FIG. 3. *Estimation of molar amounts of fragments in HinD III cleavage pattern of Town DNA. The average area per peak in a microdensitometer profile (Fig. 2), scanned at a 1:5 scan-to-record ratio, was measured and plotted on a log scale versus the migration distance of the respective fragment. The solid line represents the assumed 1 M amounts. Dashed lines represent predicted supra- and submolar amounts.*

TABLE 2. *Molecular weight estimates for HinD III fragments of Town DNA by electron microscope measurements and by coelectrophoresis with EcoR-1 fragments of HSV-KOS*

Fragment[a]	Molecular weight[b] by electron microscopy ± standard deviation ($\times 10^6$)	Molecular weight by coelectrophoresis with HSV-KOS ($\times 10^6$)
m-1	13.25 ± 0.24	12.7
1	11.13 ± 0.18	11.5
m-2	9.98 ± 0.17	10.3
m-3		9.8
m-4	8.79 ± 0.40	9.0
2,3		8.3
4	7.29 ± 0.23	7.5
5		7.3
6		7.1
m-5	6.80[c]	6.8
m-6		6.3
m-7	5.97 ± 0.14	6.0
7		5.7
8		5.5
9	5.17 ± 0.22	4.9
10		4.7
m-8	4.55 ± 0.04	4.3
m-9	4.06 ± 0.22	3.9
11		3.7
12, 13, 14	3.39 ± 0.05	3.1
15, 16	2.95 ± 0.15	2.95
m-10	2.29 ± 0.19	2.35
m-11	1.87[c]	1.925
17	1.12 ± 0.16	1.125
18, 19, 20, 21	0.64 ± 0.13	

[a] Fragments are labeled as indicated in Fig. 2.

[b] Each molecular weight estimate by electron microscopy represents the corresponding single DNA band or closely migrating size class.

[c] Too few molecules were measured to accurately determine standard deviation for these two size classes.

TABLE 3. *Estimated total molecular weight[a] of major[b] DNA fragments in HinD III and EcoR-1 digests*

Virus	HinD III		EcoR-1	
	Total molecular weight of major fragments ($\times 10^6$)	No. of major fragments	Total molecular weight of major fragments ($\times 10^6$)	No. of major fragments
Esp	105	20		
C-87	100	21	100	29
NC-496	104	20	97	24
AD-169	100	20		
Davis	103	23	105	24
TW-087	105	24	95	25
Kerr	104	22		
Isolate-7	101	24	96	24
RCH-234	99	22	101	25
UW-1	104	21	102	28
Town	97	21	104	27
Colburn	104	20		

[a] Molecular weights of individual fragments were determined from plots of molecular weight by electron microscope measurements versus migration distance of coelectrophoresed Town DNA fragments generated by HinD III.

[b] Major fragments were determined as described in the legend to Fig. 3.

shown), and from experiments in which CMV DNA in a series of digestion reactions was subjected to restriction enzyme digestion over several hours with and without additional enzyme during the course of the digestion (data not shown). Digestion was complete within 5 h, and the limit digestion products were always identical to the digests shown here (see Fig. 1A and B). There was no obvious contamination of the HinD III and EcoR-1 digests by either HinD II or EcoR-1* activities, respectively (23). These unusual submolar amounts of DNA fragments are most likely due to either heterogeneity within CMV genomes or to a heterogeneous subset of DNA molecules in any preparation of CMV DNA.

In Fig. 4 major and minor bands from HinD III digests of Town DNA are labeled with the approximations obtained by microdensitometer scans and resultant area versus mobility plots of cleavage patterns. Major fragments are labeled by numbers, and minor fragments are labeled by numbers prefixed by "m." In both Fig. 4 and 5 the patterns are arranged according to the most common comigrating fragments.

DISCUSSION

HinD III or EcoR-1 digestion of several CMV isolates yields characteristic and reproducible products. Very similar electrophoretic patterns are obtained for a few isolates, and among several isolates large portions of the patterns comigrate; however, no two isolates have identical fragment migration patterns with either

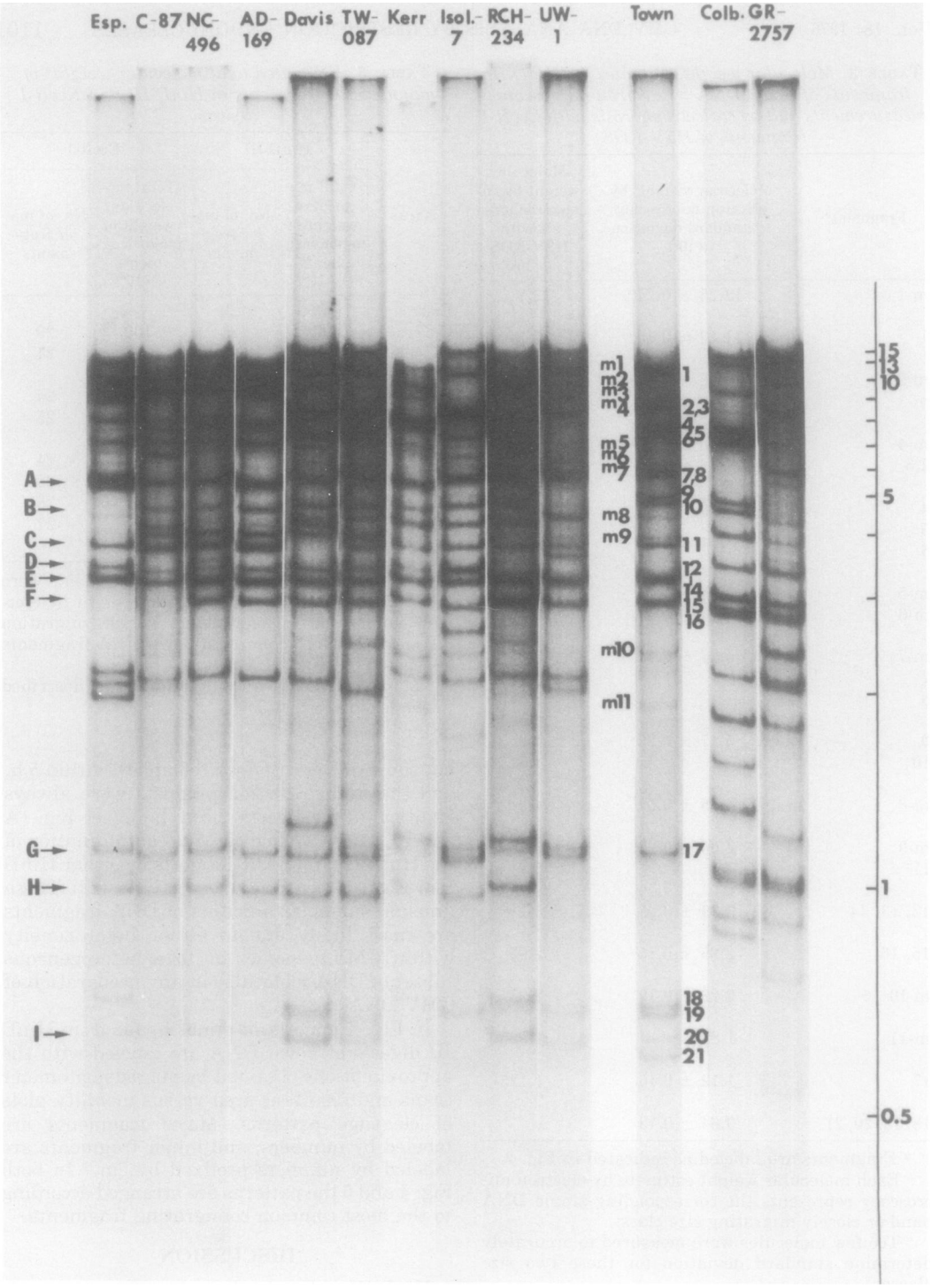

FIG. 4

FIG. 4 and 5. *Cleavage similarities within the HinD III and EcoR-1 digests of CMV DNA. The cleavage patterns were obtained as described in the text. Major molar fragments are designated by numbers, and minor molar fragments are designated by numbers prefixed by "m" (Fig. 4). Letters with arrows indicate specific co-migrating fragment types described in the text. The patterns within each figure are arranged according to general pattern similarities and according to the most common comigrating fragments. The molecular weight scale (×10⁶) is from electron microscope contour length measurements of HinD III cleaved Town DNA fragments measured relative to the contour length of simian virus 40 form II DNA. Figure 4, HinD III digests; Fig. 5, EcoR-1 digests.*

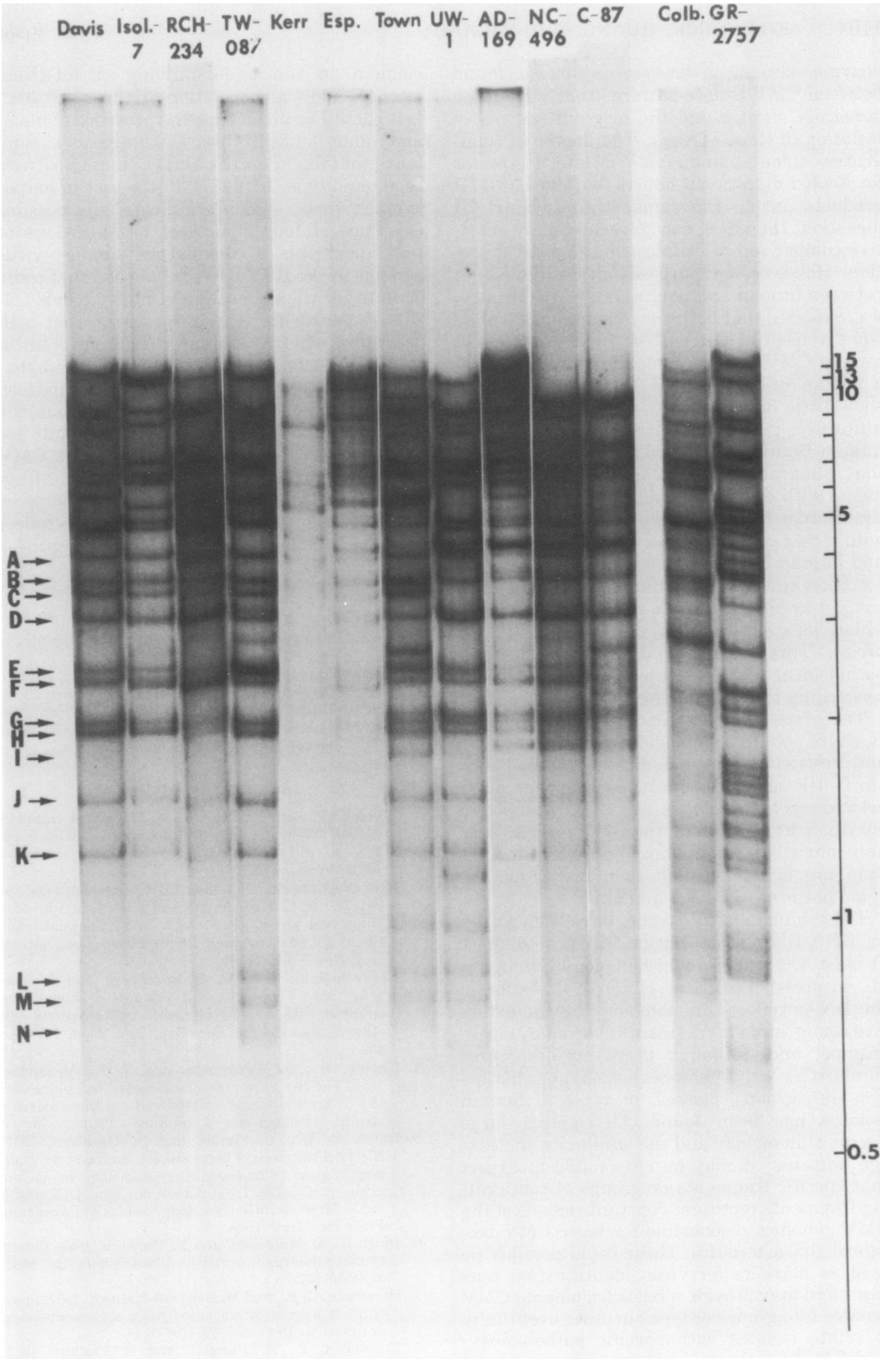
Davis
Isol.-7
RCH-234
TW-087
Kerr
Esp.
Town
UW-1
AD-169
NC-496
C-87
Colb.
GR-2757
A
B
C
D
E
F
G
H
I
J
K
L
M
N
15
13
10
5
1
0.5

FIG. 5

enzyme. Also, no obvious association was found between CMV isolate patterns that were more generally similar and the original sources of isolation of these viruses. The degree of comigration among human CMV isolates is greater for EcoR-1 digestion products than for HinD III products among the same strains. HinD III digestion, therefore, can provide a more sensitive comparison of isolates than EcoR-1 digestion. However, for purposes of distinguishing between human and nonhuman CMV isolates, it is expected that both enzyme products will be equally useful. HinD III or EcoR-1 digests of human CMV isolates compared with digests of a simian isolate (GR-2757) and with Colburn show little or no matching of fragments. Significantly, the Colburn isolate, obtained from human brain, has several HinD III fragments that comigrate with GR-2757 but few or no fragments with either enzyme that comigrate with the human CMV isolates. This finding along with recent comparisons of Colburn with simian and human CMV isolates by DNA-DNA reassociation kinetics and immunological cross-reactivity may be sufficient to consider the Colburn isolate as a simian CMV (21; Huang et al., in press). This finding could have interesting pathological and epidemiological implications concerning the simian CMVs.

The herpes simplex viruses, type I and type II, share about 48% nucleic acid homology (16), and restriction enzyme digests (EcoR-1 and HinD III) show distinctly different products between both serotypes (10, 27). Restriction enzyme patterns among the CMV isolates seen here obviously have many more similarities than are found between such restriction enzyme patterns of HSV I and II.

These human CMV strains have been shown by DNA-DNA reassociation kinetics to share at least 80% nucleic acid homology (Huang et al., in press). EcoR-1 or HinD III digestion of the DNAs reflect this homology by the extensive comigration of fragments cleaved by either enzyme, and, although there are important similarities and differences, no obvious distinction into groups, classes, or types of human isolates has been found. Given this large degree of homology and the similarity of cleavage patterns, it may be reasonable to expect that specific fragments or groups of comigrating fragments represent constant regions of the CMV genomes responsible for basic CMV biological characteristics. These could possibly be used as markers for virus identification purposes and may provide a basis for placing CMV isolates into groups or types in order eventually to relate isolates with specific pathobiology. Additionally, these may provide a useful element in the understanding of infection, latency, and transformation by these viruses.

It should be noted that the presence of major and minor bands in these CMV digests is similar to that found for the herpes simplex viruses type I and type II (10, 27). In the case of herpes simplex virus, evidence indicates the possible existence of four structural organizations or rearrangements of the herpes simplex virus genome in the HSV DNA population that could account for the generation of minor bands (11, 26). Experiments are in progress that will determine whether similar alterations occur in the CMV genome. Finally, it should be emphasized that, although each cleavage pattern contains minor bands, the findings that the total molecular weights of major fragments are the same or about the same as intact CMV DNA and that the total numbers of major fragments were both large and consistent support the view that these digests accurately reflect both the homology and heterology among these CMV isolates.

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