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Map Location of Homologous Regions Between Marek's Disease Virus and Herpesvirus of Turkey and the Absence of Detectable Homology in the Putative Tumor-Inducing Gene

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The DNA region having homology between Marek's disease virus and herpesvirus of turkey was assigned to the restriction map of Marek's disease virus by Southern blot hybridization. Under moderate conditions at the level of 15% mismatching, homology was found to be distributed throughout the Marek's disease virus genome. The long inverted-repeat regions (TR_L and IR_L), which are considered to play a significant role in tumorigenicity, did not show any homology to herpesvirus of turkey DNA.

Marek's disease (MD) is a lymphoproliferative malignant neoplasia of chickens caused by a herpesvirus (12). During the 1960s, the poultry industry encountered difficulties related to MD, leading to severe economic losses. Subsequent veterinary studies which characterized MD more fully revealed that this was the first known instance of a virus-induced, naturally occurring malignant disease which was successfully prevented by vaccination (1, 13). Thus, MD currently serves as a model for the development of vaccines for other herpesviruses which may have oncogenic potential.

Herpesvirus of turkey (HVT), although nononcogenic for chickens, is serologically related to MD virus (MDV) and is effectively used for vaccinating chickens against MD (13). However, nucleic acid hybridization studies have revealed only limited homology (at most 5%) between the DNAs of HVT and MDV (5, 9, 11). Because restriction maps are now available for MDV DNA (2), we are able to locate specific regions of the MDV genome which show sequence homology to HVT DNA. We report in this paper that the regions in the MDV genome homologous to HVT DNA are not restricted to a certain area of the MDV genome but rather appear to be widely distributed throughout the viral DNA. However, the long inverted-repeat regions, IR_L and TR_L, which appear to be related to the tumor-inducing function of MDV (4; K. Fukuchi, A. Tanaka, and M. Nonoyama, Proc. Natl. Acad. Sci. U.S.A., in press), do not show homology between MDV and HVT.

For the Southern blot hybridizations, a nick-translated HVT probe (average size, 150 to 200 base pairs, as determined by gel electrophoresis) was hybridized to cloned MDV *Bam*HI fragments on nitrocellulose filters (14, 18). HVT DNA was prepared as follows. Chicken embryo fibroblasts (11 days old) were infected with HVT strain FC126 in minimal essential medium with 2% fetal calf serum. When 80% of the cells showed a cytopathic effect, nucleocapsids were extracted by Nonidet P-40–Triton X-100 treatment, as described previously (19). Viral DNA was isolated by centrifugation through a 10 to 30% continuous glycerol gradient after sodium dodecyl sulfate-proteinase K digestion of nucleocapsids (2). Hybridization was carried out under three different levels of stringency. Hybridization under relaxed conditions was performed in 10 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.4]) at 66°C and yielded 5% homology, as determined by DNA-DNA reassociation kinetics (11). The guanidine-plus-cytosine content of both MDV and HVT DNAs is known to be 46% (10). The melting temperature at these relaxed conditions was calculated to be 103°C, and the percentage of base mismatching at 66°C was 27%, as determined by the following formulas (7): $T_m = 81.5 + 16.6(\log M) + 0.41(\%GC) - 0.72(\%F)$, and $\%mm = (T_m - T_h)/1.4$, where T_m is the melting temperature, M is the monovalent salt molarity, %GC is the percent guanidine-plus-cytosine content, %F is the percent formamide content, %mm is the percentage of base mismatching, and T_h is the hybridization temperature. Under moderate conditions, hybridization was performed in 6 × SSC with 50% formamide at 41°C and yielded a melting temperature of 63.6°C and a percentage of base mismatching at 41°C of 15%. Under stringent conditions, the hybridization temperature was increased to 50°C, and the percentage of base mismatching was 10%. The amount of MDV DNA used for the hybridizations was 1 μg for each fragment. *Bam*HI DNA cloned in bacterial plasmids pACYC184 and pBR322 and cosmid pHC79 was digested with *Bam*HI and electrophoresed with vectors through 0.6% agarose in Tris-acetate buffer (pH 8.0). Figure 1a shows ethidium bromide staining of digested, cloned MDV *Bam*HI fragments after electrophoresis.

Hybridizations were carried out with 10^8 cpm/μg per filter for 12 h at various temperatures. After hybridization, the filters were washed in the same buffer without the probe and at the same temperature as for the hybridizations for 1 h with shaking; they were then autoradiographed on Cronex X-ray film with intensifying screens at −80°C for 12 h. The HVT probe used was hybridized with all the HVT *Bam*HI fragments identified by ethidium bromide staining (data not shown).

The hybridization pattern under relaxed conditions is shown in Fig. 1b. Under relaxed conditions, <5% homology was reconfirmed by DNA-DNA reassociation kinetics (data not shown), as previously reported (11). *Bam*HI-A, -B, -C, -E, -F, $-I_3$, $-O_1$, and -Q hybridized strongly (Fig. 1b). The intensity of hybridization in this study was much stronger than previously reported results, and the number of hybridized bands detected in the hybridization experiments was

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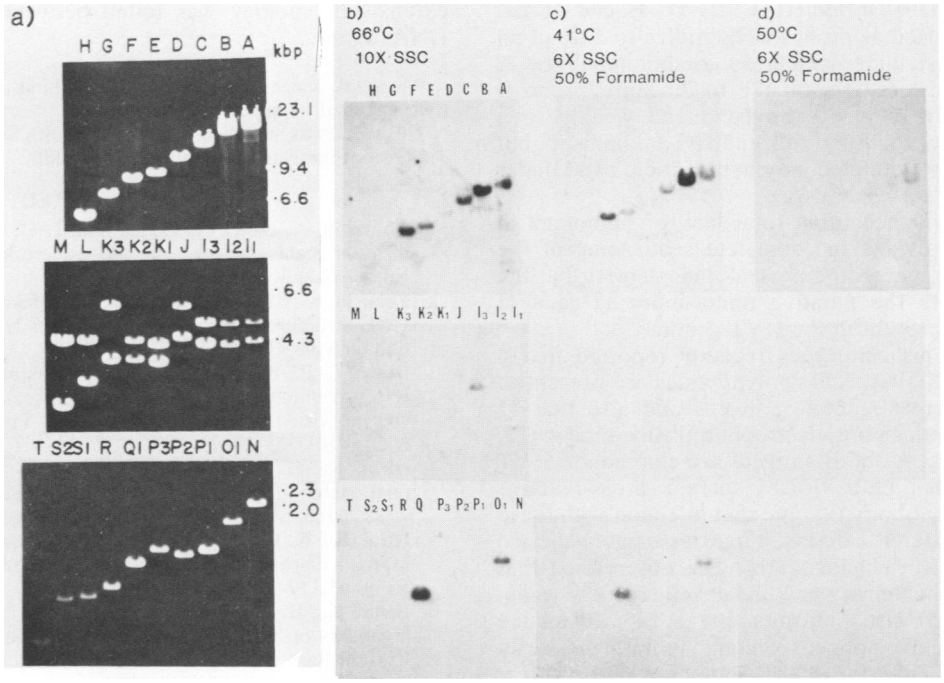

FIG. 1. Detection of homologous regions between HVT and MDV DNAs. (a) Ethidium bromide staining of MDV DNA *Bam*HI fragments. (b, c, and d) Hybridization patterns under three different conditions.

greater than in the previous study (11). This resulted from the use of 10 to 30 times more MDV DNA on the filters and the use of probes with 10 times more radioactivity. When the conditions were changed to moderate, the pattern of hybridization was identical to that under relaxed conditions (Fig. 1b and c). Under stringent conditions, nearly all of the bands that hybridized under moderate conditions became faint (Fig. 1d). Because we did not treat the hybridized filters with S1 nuclease, even partial hybridization of the probes could amplify the intensity of hybridization by the remaining parts of the probes, such as tails and loops. The homologous regions detected by this hybridization are summarized on the restriction map in Fig. 2. The homologous regions were scattered throughout the MDV genome, except for the long inverted-repeat regions *Bam*HI-I_2, -L, -D, and -H. It has previously been reported that genes in *Bam*HI-D and -H may play a significant role in oncogenesis; that is, *Bam*HI-D and -H are intact in pathogenic strains but are expanded in nonpathogenic strains by multiple repetitions of a small unit of 150 bp within the *Bgl*I-*Pst*I 1.5-kilobase-pair (kbp) fragment of *Bam*HI-D and -H (Fig. 2; Fukuchi et al., in press). Furthermore, the long inverted-repeat regions were transcribed in the nonproductive tumor cell line MKT-1, as determined by analysis of the cDNA of cytoplasmic mRNA (unpublished data). A reciprocal experiment confirmed the absence of detectable homology of MDV *Bam*HI-D and -H

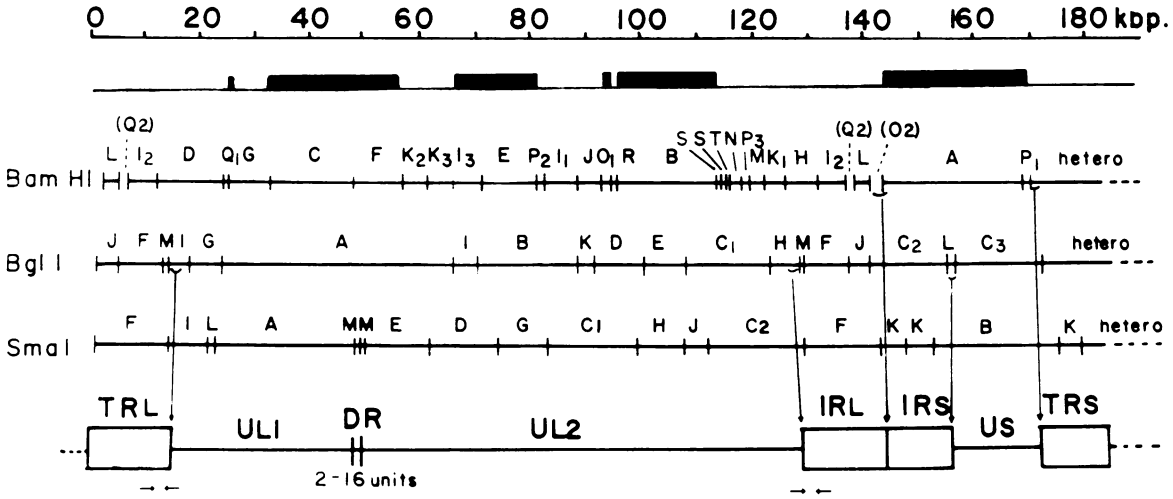

FIG. 2. Location of homologous regions between MDV and HVT DNAs on the restriction map of MDV strain GA DNA. Solid boxes indicate the homologous regions detected by Southern blot hybridization. → ← *Bgl*I-*Pst*I 1.5-kbp fragment.

to HVT DNA. ^{32}P-labeled *Bam*HI-A, -B, -D, -E, and -H (ca. 10^8 cpm/μg) were used as probes to hybridize to 1 μg of an HVT *Bam*HI digest under moderate conditions. Probe A hybridized to a smeared region (10 to 20 kbp), probe B hybridized strongly to a 4.0-kbp band and weakly to a 3.2-kbp band, and probe E hybridized to a 6.6-kbp band, but probes D and H did not detect any hybridizable band under these conditions (data not shown).

Although the evidence for a total lack of homology in *Bam*HI-D and -H awaits the complete sequencing of the *Bam*HI-D and -H regions, the present data suggest that the sequence related to the putative tumor-inducing gene of MDV may not be present in the HVT genome.

van Zaane and his colleagues recently reported that 4 major HVT- and MDV-specific polypeptides are present in infected cells as cross-reactive antigens and also that 11 other viral polypeptides are electrophoretically similar (20, 21). It is known that A and B antigens are common to HVT and MDV (22), that both viruses share a cross-reactive virus-neutralizing antibody (8), and that infection with these viruses generates Marek's disease tumor-associated surface antigens (MATSA) in chickens (16). The observation that HVT can protect chickens against the development of transplanted tumors (15) also indicates that HVT can induce common tumor-specific antigens which may not necessarily be identical to MATSA, for MATSA may not play a direct role in cell-mediated cytotoxicity (17).

The above immunological and antigenic relationships between HVT and MDV should reflect on the DNA homology of the two viruses. Several possibilities can be considered in explaining this discrepancy. (i) A homology of 5% in DNA sequences is equivalent to a polypeptide molecular weight of 400,000, which could account for the 4 observed major polypeptides and for the 11 polypeptides with structural similarities. (ii) Cross-reacting antigenic determinants are present in many polypeptides which are otherwise different in structure. (iii) More than 5% homology of DNA sequences is present in HVT and MDV DNAs under moderate-stringency conditions. As shown in previous papers (11) and in the present paper, precise homology between HVT and MDV DNAs is found only in <5% of the total viral genomes, as determined by reassociation kinetics, and yet Southern blot hybridization under low-stringency conditions detected homologies throughout the MDV genome. The following possibilities may be considered in explaining the nature of this hybridization. (i) MDV and HVT DNAs may code for the same or similar proteins which, however, do not hybridize to each other. This could occur if the third base of every codon of MDV and HVT DNAs diverged, giving rise to a 30% mismatch of sequences. Thus, without S1 nuclease digestion, these regions should be detected as hybridized fragments. However, the conditions used at the level of 15% mismatching still revealed significant hybridization throughout the genome. Thus, this possibility is not likely. (ii) A precise homology of 5%, which may be related to antigenic determinant sites, may be divided and distributed throughout the viral genome, and the intensity of hybridization may be amplified by the nonhybridized portions of the probes. (iii) Weak homologies, which can be removed by S1 nuclease digestion, may exist throughout the viral genome.

The nature of the homology remains to be detailed by DNA sequencing. It will also be important to determine which regions of homology contribute to the above-mentioned vaccinal immunity induced by viral infections. Gibbs et al. and Hirai et al. also recently reported similar findings, although with slightly different interpretations, i.e., that extensive homology was found between MDV and HVT DNAs (3, 6).

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