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Molecular Epidemiology of DNA Viruses: Applications of Restriction Endonuclease Cleavage Site Analysis

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Restriction endonucleases which cleave DNA at specific nucleotide sequences can be used to produce a set of DNA fragments of a viral genome which, when separated by gel electrophoresis, gives a characteristic "fingerprint" for that virus genome. This simple technique has been used to identify and classify DNA viruses of the herpes, adeno, and papova virus groups. Small variants within a given type (e.g., herpes simplex type I) are genetically stable and permit study and identification of individual strains of viruses. Such analyses have recently been applied to study the epidemiology of some DNA virus outbreaks. Restriction endonuclease fingerprinting provides a useful addition to methods for virus identification and classification.

A fundamental assumption which underlies all methods of virus diagnosis and classification is that the biological and biochemical characteristics of a given virus are determined by its genetic composition, and that this genetic plan is an essentially invariant and unique property of the virus. Immunological classifications rely on antibodies to distinguish proteins of different conformations. Such differing conformations reflect an underlying difference in the nucleotide sequence in the viral gene encoding the protein in question. Ultimately, then, virus identification and classification devolves to knowledge of the relationships between the genetic constitutions (i.e., nucleotide sequences) of various viruses.

Until relatively recently only indirect methods for comparison of nucleotide sequence relationships were available. Immunological cross-reactivity, heat stability, and electrophoretic mobility are all properties which depend on the amino acid composition and arrangement in virus proteins, and, in turn, reflect nucleotide sequences. Now, however, relatively simple methods are available which allow direct analysis and comparison of the genomes oi viruses. For a very few small bacterial viruses the total nucleotide sequence of the entire genome is known [1]. This is true also for the papovavirus SV-40 [2,3]. Methods for total nucleotide sequence determinations are being developed rapidly, but complete sequence analysis is still a major undertaking. More limited but direct information about nucleotide sequences can be obtained by analysis of the size distributions and composition of polynucleotide fragments produced by nucleotide-sequence-specific cleavage of virus genomes. It is this latter approach, applied to DNA-containing viruses, which will be discussed in this work.

Two classes of nucleases have been employed as sequence-specific cleavage

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reagents. Ribonuclease, which cleaves RNA chains after a specific nucleotide (e.g., RNase Tl cleaves after every guanylate (G) residue), produces a set of fragments, the sizes of which are determined by the position of the specific residue in the viral genome. Such sets of fragments can be resolved by 2-dimensional chromatographic separations to yield a "fingerprint." Comparison of these fingerprints for two RNA genome viruses can show if the two viruses have the same or different distribution of G residues in their genomes. This method has been invaluable in the study of oncornaviruses and the relationships between multiple endogenous retroviruses from the same animal [4,5].

For analysis of DNA-containing viruses a class of nucleases called bacterial restriction endonucleases has been employed. This terminology reflects the known biological function of some of these nucleases. They recognize and cleave at specific nucleotide sequences present in foreign DNA, but absent in the DNA of the cell which produces the enzyme; thus entry of foreign DNA is "restricted." In the present context, it is sufficient to note two features of these nucleases. First, they come from a variety of organisms and exhibit a number of DNA-sequence specificities. This means that many different DNA sequences can be identified. Second, these enzymes, for the most part, are easy to prepare and store [6], and many are commercially available.

The general idea behind the use of restriction endonuclease for virus identification is illustrated in Fig. 1. Viral DNA is isolated by a suitable method, incubated with a specific endonuclease until all DNA sequences which are susceptible to the nuclease have been cleaved. The set of fragments are then resolved on the basis of size by gel electrophoresis. Usually agarose gel is used in this electrophoresis. The large fragments are retarded most by the sieving effect of the gel so an inverse relationship between size and migration is observed. The position of the DNA fragments can be determined by radioautography on X-ray film if the viral DNA is labeled, or the DNA can be visualized and photographed directly by the fluorescence of a dye, ethidium bromide, which binds to the DNA when included in the agarose gel. Routine work requires less than 1 ug DNA per sample for fluorescent detection and 5000 counts per minute radioactive label for autoradiographic detection. One microgram of DNA is obtained from about 5×10^9 particles of herpesvirus and 1.5×10^{11} particles of papovavirus. Since the particle to plaque-forming-unit ratio is high for most animal virus stocks, considerably less biological activity is needed.

The first application of restriction endonuclease cleavage site analysis to comparative virology was by Mulder and his colleagues [7] who studied the *Eco*RI cleavage patterns of several adenoviruses (serotypes 2,3,5,7,12) and an Ad-SV-40 hybrid virus (Ad2+ND1). They showed that each serotype was distinct and found that two independent members of the Ad7 serotype gave related but distinguishable patterns.

We [8,9] applied this same approach to herpesvirus DNA analysis and were able to classify herpes simplex virus isolates into two groups on the basis of endonuclease cleavage patterns, and that these groups were the same as that obtained by biological characterization (i.e., HSV-1 and HSV-2) (Fig. 2). Furthermore, however, we noted intratypic variation; that is, various isolates of HSV-1 showed strain-specific differences which could be used to further subclassify these HSV-1 isolates. The power and clarity of this approach was immediately apparent and the use of restriction endonucleases for studies in "molecular epidemiology" [10] has progressed rapidly in the past five years. Lonsdale [11] has described technical modifications which allow rapid, accurate diagnosis of HSV-1 and HSV-2 on a large scale; fifty or more isolates can be analyzed by one worker in 4–5 days. Buchman et al. [12] employed the

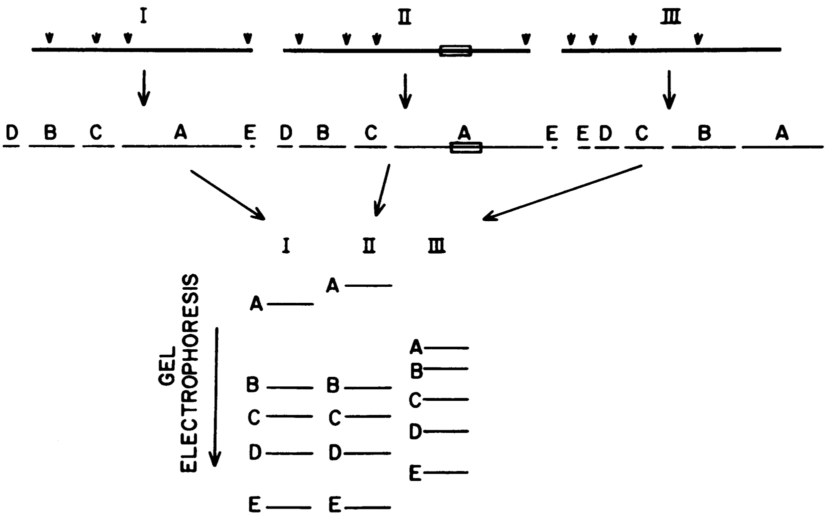

FIG. 1. Illustration of principles of restriction endonuclease cleavage site analysis. The linear DNA (double-stranded) genomes of three hypothetical viruses to be compared are indicated I,II,III. Suppose a specific nucleotide sequence, e.g., GAATTC, the cleavage site for nuclease EcoRI, occurs at four sites in each genome as indicated by arrows. Genome I and II are identical except for a substantial DNA insertion mutation in genome II. Genome III has none of the sequences in question located in positions analogous to genomes I or II. Cleavage of these DNAs at the sites marked by arrows results in five fragments (A–E) in each case. If these DNA fragments are separated according to size in adjacent tracks in a gel electrophoresis experiment the result will be as diagrammed: fragments B,C,D, and E of samples I and II will co-migrate and fragment A from each virus will differ. The fragments from genome III will co-migrate with none of those from genomes I and II. (Occasionally co-migration of non-identical fragments of similar size is noted. This sort of ambiguity is resolved by analysis with an endonuclease with a different specificity.) It should be noted that knowledge of the cleavage site maps at the top are not essential to be able to deduce the fact that genomes I and II are related to each other, but not to genome III.

intratypic variation noted previously [8,13] to follow the course of a nosocomial outbreak of HSV-1.

The study of other herpesviruses has been aided by restriction endonuclease analysis. The Epstein-Barr Virus is thought to be a causative agent of several quite distinct clinical entities: infectious mononucleosis (IM), Burkitt's lymphoma (BL), and nasopharyngeal carcinoma (NPC). Comparison of virus strains from patients with each of these conditions might be expected to answer the question of the existence of subtypes which are responsible for the differing clinical diseases. Initial comparison of BL-derived EBV DNA with IM-derived EBV DNA showed only very minor differences [14]. Later, more extensive studies confirmed the initial conclusion that major, disease-specific subgroups of EBV isolates do not exist [15].

Another clinical problem to be attacked with restriction endonuclease cleavage

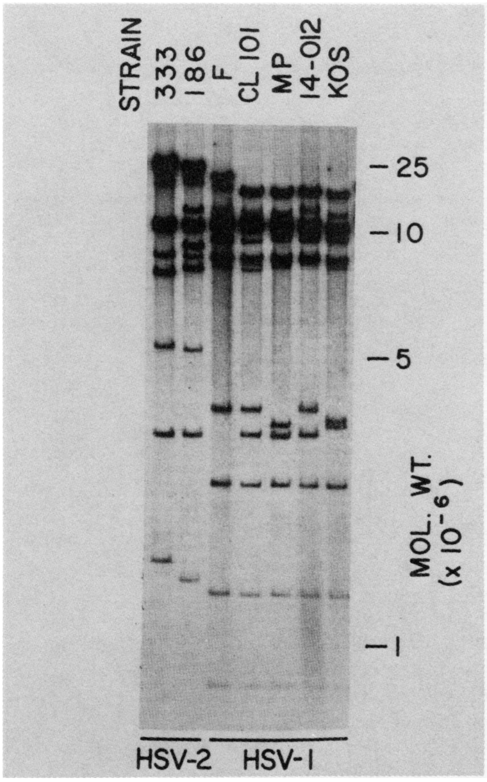

FIG. 2. Autoradiograph of *Eco*RI digestion products of ^{32}P labeled DNA isolated from five HSV-1 and two HSV-2 strains. The origin of the electrophoresis was at the top and the gel was 0.5 percent agarose. The molecular weights of the fragments were estimated by comparison to known size standards run in adjacent tracks (not shown). The molecular weight of uncleaved HSV DNA isolated from virions is about 100×10^6 daltons.

analysis is the relationship of varicella virus (chicken pox) to herpes zoster virus (shingles). Immunological studies suggested that the same virus (VZV) caused both diseases. This conclusion was more firmly substantiated by direct analysis of DNA from varicella virus and zoster virus [16]. Interestingly, the minor, intratypic strain variations noted in HSV were not seen in VSV; all five isolates, even from geographically distant sources, were identical [16,17].

Human cytomegalovirus (HCMV) has been analyzed for strain variations and although strain-specific differences were noted, no subgrouping of strains was possible [18,19]. On the basis of restriction endonuclease cleavage patterns, however, Kilpatrick et al. [19] were able to identify the Colburn strain of CMV as a simian CMV rather than a human CMV, even though the Colburn strain was isolated from human brain tissue.

In the papovavirus group of DNA viruses, SV-40 and polyomavirus have been extensively characterized by cleavage site analysis, and more recently the human papovavirus JCV and BKV have come under extensive study [20]. This technology provided rapid and definitive proof that SV-40, JCV, and BKV were distinct viruses.

These examples demonstrate the application of very recent progress in molecular biology to problems of considerable biological and clinical interest. The methods for analysis of DNA virus genomes by restriction endonuclease cleavage patterns are sufficiently simple and reproducible to be of use in a variety of areas: virus identification and classification, epidemiological studies, and virus-host interactions.

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