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M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms

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Hypervariable polymorphic patterns were detected with M13 phage DNA as a probe in genomic DNA of organisms belonging to different taxonomic groups including animals (vertebrates and invertebrates), plants and microorganisms. Individual-specific restriction pattern analysis (DNA fingerprinting) with this probe proved to be useful for individual identification, analysis of somatic stability and paternity testing in man. The nuclear type of inheritance indicates that the hypervariable DNA regions in question are located in the chromosomes, not in the mitochondrial DNA. The data obtained also demonstrate a potential range of M13 DNA applications as a probe for DNA fingerprinting of animals, plants and microorganisms, particularly for the determination of inbred lines, identification of bacterial strains and establishing stock, variety and strain distinctions.

M13 phage DNA; DNA fingerprinting; Restriction fragment length polymorphism

1. INTRODUCTION

Several regions of the human genome are highly variable in populations because the number of repeats in these regions of a short 'minisatellite' sequence varies at high frequency [1]. Jeffreys et al. [2–4], using probes consisting of tandem repeats of the 'core' sequences, have detected many hypervariable minisatellites simultaneously, to produce an individual-specific DNA fingerprint with applications in forensic and parentage studies. Recently it has been shown that wild-type M13 bacteriophage DNA [5] or its recombinants [6] under appropriate hybridization conditions could detect hypervariable minisatellites in a variety of mammalian DNAs. According to Vassart et al. [5], the effective sequence in M13 was traced to two clusters of 15-bp repeats within the protein III gene of the bacteriophage. We show here that DNA fingerprinting with M13 phage DNA as a probe and its general usage for study of biological relationships can be applied to organisms of different taxonomic groups including animals (vertebrates and invertebrates), plants and microorganisms.

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2. MATERIALS AND METHODS

Samples of high molecular mass DNA from blood and tissues were prepared as described [7–9]. These DNAs were digested with appropriate restriction enzyme, electrophoresed through a 20-cm long 1% agarose gel and transferred by blotting to a Schleicher and Schuell nitrocellulose membrane filter (BA85). Single-stranded M13 DNA was labeled to a specific activity of 1.0×10^9 cpm/μg of DNA using a hybridization [10], sequencing or random [11] primers. The hybridization solution contained 4 × SSC, 0.1% SDS, 10 × Denhardt's solution. Incubation went on for 16–20 h at 65°C. After the hybridization, the filters were washed in 1 × SSC, 0.1% SDS at 65°C, and exposed to X-ray film.

3. RESULTS

The results, presented in fig.1, illustrate the blot-hybridization analysis with radioactively labeled M13 DNA of human DNA, fragmented by the *Bsp*RI restriction endonuclease, which yields a

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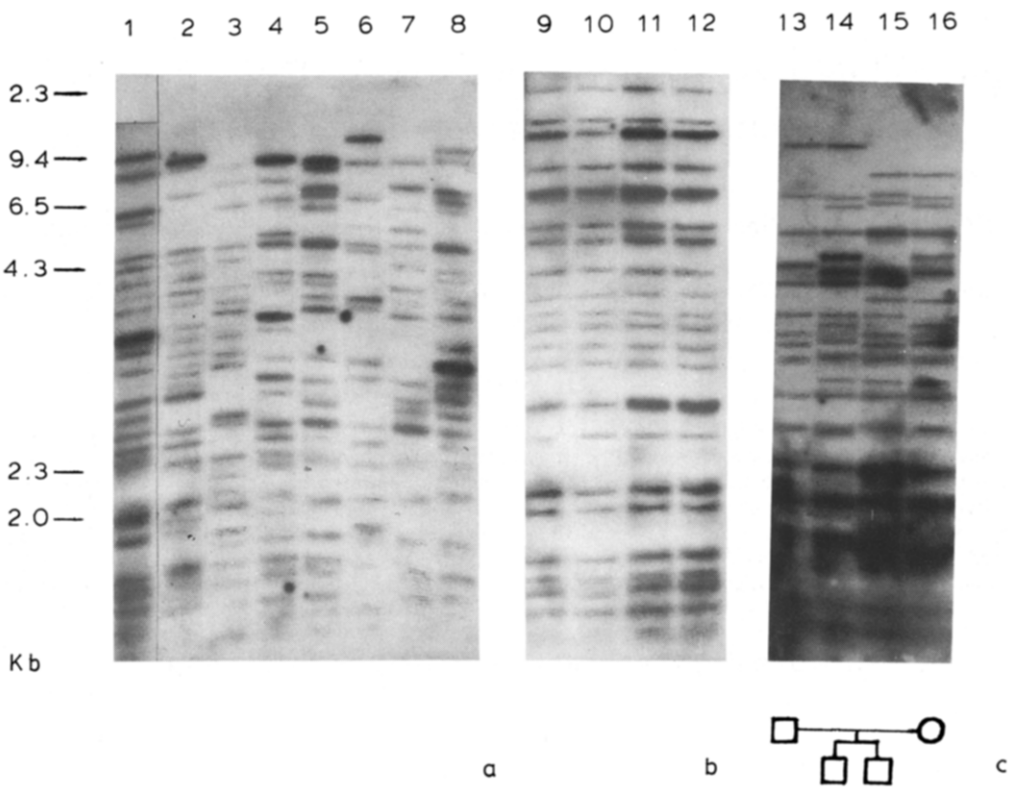

Fig.1. Individual polymorphism (a), somatic stability (b), and paternity testing (c) in man. Lanes 1–8, blood DNAs of unrelated individuals digested with *Bsp*RI; lanes 9–12, heart, liver, stomach and thyroid DNAs of one individual, digested with *Bsp*RI; lanes 13–16, blood DNAs of the father, two sons, and mother digested with *Bsp*RI + *Alu*I. Southern blots of digests of 10 μg samples of DNA were hybridized with ^{32}P-labeled M13 phage DNA.

wide spectrum of hybridizable bands. Meanwhile, as we have shown [6], other restriction endonucleases can also be used for this kind of analysis. In general, the variability of DNA fingerprints for unrelated individuals (fig.1a) was quite similar to that described by Vassart et al. [5]. The somatic stability of hypervariable patterns was studied for heart, liver, stomach, thyroid gland (fig.1b), brain, adrenal, gall-bladder and testis DNAs isolated from the same individual. The DNA fingerprints in all cases were identical and depended only on the restriction enzymes involved. The parentage analysis (fig.1c) revealed that the father's and mother's DNA fingerprints were different as they should be for two unrelated individuals, two brothers had a far larger number of coinciding hypervariable fragments, the variants of hypervariable regions were inherited by the children from their parents (approx. half of them were inherited from the father). The nuclear type of inheritance indicates that the hypervariable DNA regions in question are located in the chromosomes, not in mitochondrial DNA.

As has been reported earlier [5,6] the hypervariable sequences hybridizing to M13 phage DNA can be found in the genomes of some mammals. We have applied this DNA fingerprinting technology to study genomes of organisms belonging to different taxonomic groups. It was found (fig.2) that all the DNAs studied (of animal, plant and bacterial origin) exhibit complex hybridization patterns with size distribution of hybridizable DNA fragments from 0.5 to 10 kb, indicating the presence of multiple regions of homology with the hybridization probe. Significantly, the number of characteristic bands in bacterial DNA is less than in higher organisms.

Our subsequent experiments were designed to

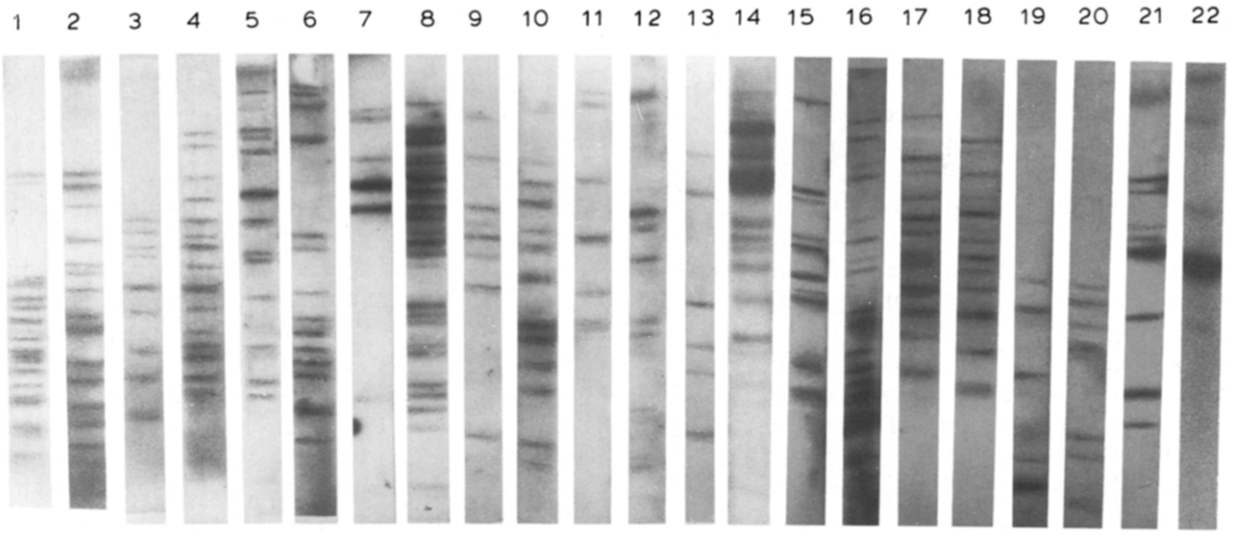

Fig.2. DNA fingerprints of animal, plant and bacterial genomes. ^{32}P-labeled M13 DNA or recombinant M13/JIN600 DNA [6] was hybridized with Southern blots of restriction endonuclease digests of DNA from mouse (1), cow (2), pig (3), rabbit (4), chicken (5), sheep (6), loach (7), *Drosophila melanogaster* (8), *Bombyx mori* (9), *Blatta orientalis* (10), *Chrysomela gamellata* (11), *Anodonta celensis* (12), *Lumbricus terrestris* (13), *Echinococcus granulosus* (14), cotton (15), barley (16), soybean (17), orange (18), *Poncirus trifoliatae* (19), yeast (20), *Vibrio cholerae* (21), *Escherichia coli* (22). 1–5 μg samples of DNA were digested with *Bsp*RI (1–7,9,15,19), *Eco*RI + *Hin*dIII (8), *Bsp*RI + *Alu*I (10–13,17), *Hin*dIII (14,18,20–22), *Hin*dIII + *Bsp*RI (16). K_b values are not indicated: the results of several independent experiments shown are not graphically equalized.

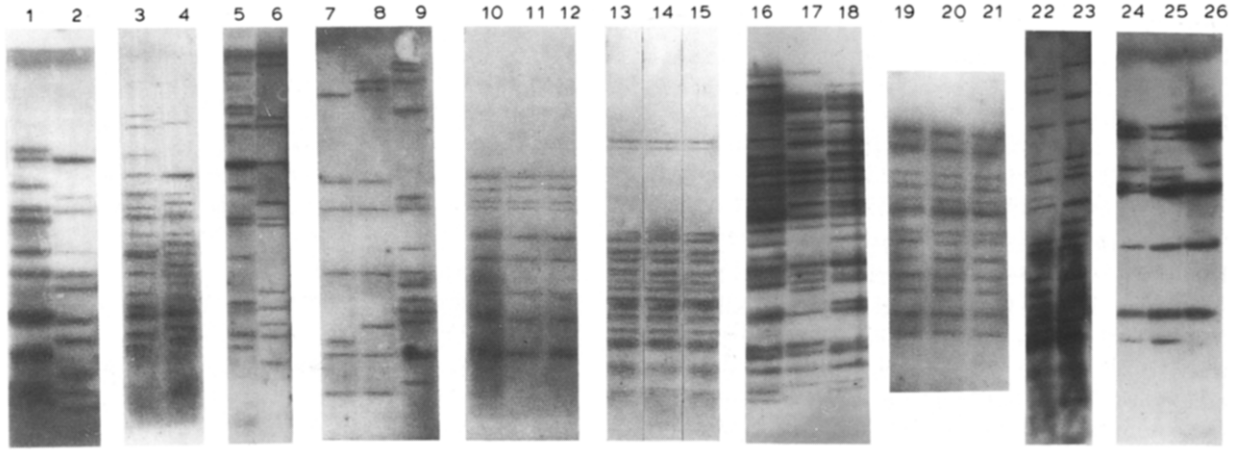

Fig.3. Hypervariable polymorphic patterns of eukaryotic and prokaryotic DNAs. ^{32}P-labeled M13 DNA or recombinant M13/JIN 600 DNA [6] was hybridized with the DNAs of two unrelated oxen (1,2), two unrelated rabbits (3,4), two unrelated chickens (5,6), two related and one unrelated lamb (7–9), three young sibling pigs (10–12), three mice of the BALB/c line (13–15), three stocks of *D. melanogaster* (Canton, 19 w, Domodedodvskaya) (16–18), three individuals of Oregon RC of *D. melanogaster* (inbred stock from Dr E. Ananiev) (19–21), two varieties (waxy, MB-1) of barley (22,23), three collection strains (1520, RV-79, 1620) of *V. cholerae* (24–26). DNAs were digested with *Bsp*RI (in experiments 1–15), *Eco*RI + *Hin*dIII (in experiments 16–21), *Bsp*RI + *Hin*dIII (in experiments 22,23), *Hin*dIII (in experiments 24–26).

establish the potential range of M13 DNA applications as a probe for the DNA fingerprinting of animals, plants and microorganisms, specifically for the determination of species and inbred lines, stock, variety and strain distinctions (fig.3). As in the case of human DNA, the patterns of unrelated animals reveal a marked individual polymorphism (lanes 1–9). Meanwhile two related lambs show more similar hybridization patterns (lanes 7,8) and the patterns are almost identical for three young sibling pigs (lanes 10–12) and for three mice of the same line (lanes 13–15). Three examined stocks of one *Drosophila* species (*D. melanogaster*) exhibit its own distinctive pattern of hybridization (lanes 16–18) while various individuals of the same stock are indistinguishable (e.g. see lanes 19–21). DNA fingerprinting is of special interest for plant genetics. The data in fig.3 (lanes 22,23) show that hypervariable polymorphic patterns can be obtained in the same way for barley, demonstrating the possibilities for characterizing inter-variety distinctions and identifying varieties. Modern microbiology and practical bacteriology are known to encounter considerable difficulties in systematizing microorganisms, identifying strains and determining the purity of bacterial cultures. Fig.3 (lanes 24–26) shows the results of DNA fingerprinting for three collection strains of *Vibrio cholerae*. The distinctive hybridization patterns of the three strains suggest new opportunities for the identification of bacteria, specifically bacterial pathogens.

4. DISCUSSION

The results presented in this paper make it possible to anticipate that M13 phage DNA will be of general use in DNA fingerprinting of animals, plants and microorganisms, in particular for the analysis of sort, determination of species, classification and registration of inbred lines and strains, as well as for establishing stock, variety and strain distinctions. Our data also indicate that M13 DNA as well as Jeffreys' minisatellite probe [2] can be used for individual identification, analysis of somatic stability and paternity testing in man. Besides the applications shown in this paper, it may hold some promise for population genetics, establishing evolutionary kinship and analysis of genome instability.

The data obtained indicate that the DNA fingerprint pattern for some hypervariable fragments is largely independent of the restriction enzyme (*Bsp*RI, *Alu*I, or a mixture of both *Bsp*RI and *Alu*I, data not shown). So, we can suggest these fragments to be multiple-repeated tandem-organized sequences of some elementary unit devoid of cleavage sites for the restriction endonucleases used.

We should say here that the cause of the universal occurrence of these sequences, hybridizing to M13 phage, in a wide range of living organisms – eukaryotes, prokaryotes and viruses – remains to be elucidated. Vassart et al. [5] have previously shown that the 15-bp motif repeated in tandem at two places in the M13 phage DNA was responsible for the hyperpolymorphic hybridization patterns in human DNA. Significantly, our hybridization pictures obtained with invertebrate, plant and bacterial DNAs completely disappeared in the presence of human or fish DNA used as nonspecific carrier. It could mean that the similar sets of hypervariable sequences were detected by M13 DNA in all these genomes. However, we realize the necessity of cloning and sequencing of hybridizable elements from these organisms for the direct proof of this idea. This is now being investigated.

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