A Sequence in M13 Phage Detects Hypervariable Minisatellites in Human and Animal DNA

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The term "DNA fingerprint" has been used to describe the extensive restriction fragment length polymorphism associated with hypervariable minisatellites present in the human genome. Until now, it was necessary to hybridize Southern blots to specific probes cloned from human genomic DNA in order to obtain individual-specific restriction patterns. The present study describes the surprising finding that the insert-free, wild-type M13 bacteriophage detects hypervariable minisatellites in human and in animal DNA, provided no competitor DNA is used during hybridization. The effective sequence in M13 was traced to two clusters of 15–base pair repeats within the protein III gene of the bacteriophage. This unexpected use of M13 renders the DNA fingerprinting technology more readily available to molecular biology laboratories.

EXTENSIVE RESTRICTION FRAGMENT length polymorphism (RFLP) associated with moderately repeated sequences has recently been described in the human genome (*1–4*). The hypervariable nature of these "minisatellites" has been exploited as a means of developing a powerful fingerprinting technique with applications in paternity testing, forensic medicine, and mapping of the human genome (*5, 6*). The probes used in such determinations were originally derived from segments of DNA discovered by chance within anonymous (*3*) or gene-associated pieces of genomic DNA (*1, 4, 7*). We report here that a sequence present in the protein III gene of the widely used M13 bacteriophage vector (*8*) allows detection of a distinct set of hypervariable minisatellites in human and animal DNA.

In the course of a systematic search of RFLPs associated with the thyroglobulin gene, we observed that a set of probes corresponding to DNA segments subcloned in the bacteriophage M13 gave different results when used in the classical hybridization mixture [Denhardt's medium plus herring sperm DNA (*9*)] or in a milk-based cocktail [Blotto medium (*10*)]. A complicated and highly polymorphic pattern was systematically observed with the enzyme Hae III in the latter condition. It soon became evident that the pattern was unrelated to the nature of the insert and that wild-type M13 bacteriophages gave the same results. Nine unrelated individuals displayed different patterns while monozygotic twins were indistinguishable (Fig. 1, A and B). Similar blots hybridized with an M13 probe in the presence of herring sperm DNA showed no polymorphism (Fig. 1C).

The logical explanation for this finding was that a segment in M13 hybridized to a hypervariable minisatellite that could be competed for by fish DNA. If this were true,

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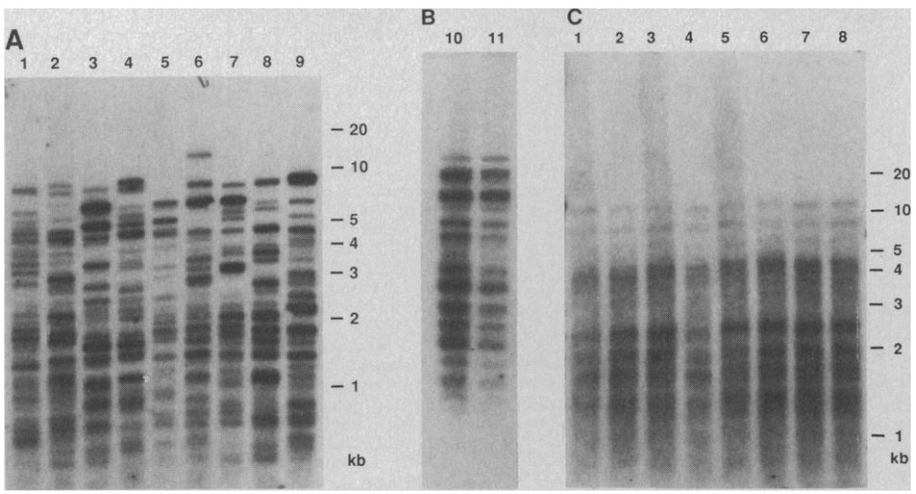

Fig. 1. Hypervariable polymorphic patterns detected in human DNA with wild-type M13 DNA as probe. Individuals 1 to 9 are unrelated Caucasians; 10 and 11 are monozygotic twins. Hae III–digested DNA (5 μg) from white blood cells was separated in a 1% agarose gel in 40 m*M* tris-acetate, 1 m*M* EDTA, *p*H 7.7, at a constant voltage of 9 V/cm for about 4 hours. The gels were soaked in 1.5*M* NaCl, 0.5*M* NaOH (2 × 30 minutes) followed by equilibration in 1*M* NH_4 acetate, 0.03*M* NaOH (2 × 30 minutes) and transfer overnight onto nitrocellulose filters (Schleicher & Schuell) in the same solution. Single-stranded M13 (200 ng) was labeled with ^{32}P-dATP to a specific activity of 0.5×10^{-9} to 1.0×10^{-9} cpm per microgram of DNA (*13*). After a 2-hour prehybridization, the filters were hybridized overnight at 42°C in the following solutions: (**A** and **B**) 40% formamide, 6× SSC (standard saline citrate), EDTA 5 m*M*, 0.25% dried skimmed milk; (**C**) same medium except that the powdered milk was replaced by 5× Denhardt's and denatured herring sperm DNA (100 μg/ml). The filters were all washed under identical conditions: twice for 15 minutes in 2× SSC, 0.1% SDS, room temperature; four times for 15 minutes in 2× SSC, 0.1% SDS, 65°C; and twice for 30 minutes in 1× SSC, 65°C. Filters were exposed to Kodak XAR-5 films at −70°C with an intensifying screen.

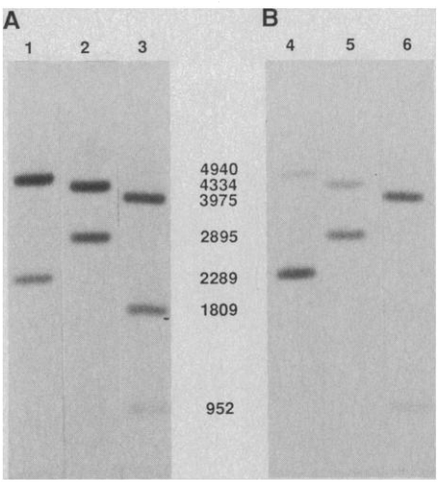

Fig. 2. Identification of M13 sequences hybridizing to herring sperm DNA. M13 RF DNA was digested with Xmn I (lanes 1 and 4), BstN I (lanes 2 and 5), and Cla I (lanes 3 and 6). Two identical blots were prepared and hybridized to a total M13 probe (**A**) or to M13 sequences selected by hybridization to herring sperm DNA (**B**). M13 RF DNA (200 ng) was digested with restriction endonucleases and identical blots were prepared as described (Fig. 1). (A) The filter was hybridized to a total ^{32}P-labeled M13 probe. (B) The filter was hybridized to labeled M13 sequences that had been selected by hybridization to herring sperm DNA. A 100-cm^2 nitrocellulose filter was saturated with herring sperm DNA by soaking it for 30 minutes into NH_4Ac 1*M*–NaOH 0.03*M* containing 2.5 mg/ml denatured herring sperm DNA and baked at 80°C for 2 hours. The filter was prehybridized for 2 hours in 40% formamide, 6× SSC, 5m*M* EDTA, 0.25% powdered milk at 42°C, and hybridized overnight to a total ^{32}P-labeled M13 DNA probe. After washing, the affinity-purified M13 sequences were eluted by boiling the filter for 10 minutes in 10 ml of 10 m*M* tris HCl *p*H 8, 1 m*M* EDTA. About 0.1% of the input radioactivity was recovered. Hybridization and washing of filters was as described in Fig. 1A.

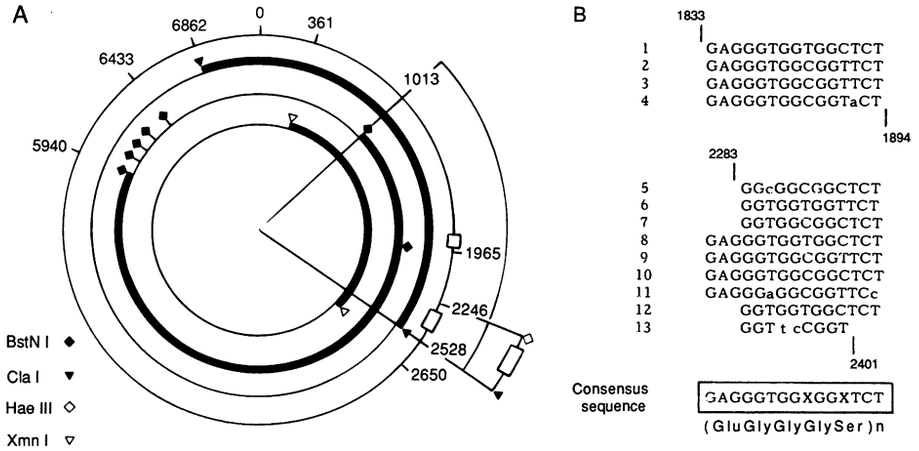

Fig. 3. (**A**) M13 restriction map. The thick lines show the restriction fragments that were preferentially recognized by the affinity-purified M13 sequences. The open boxes give the localization of the tandem repeats. (**B**) Alignment of tandem repeats present in the protein III gene of M13. The coordinates are as described in (*14*).

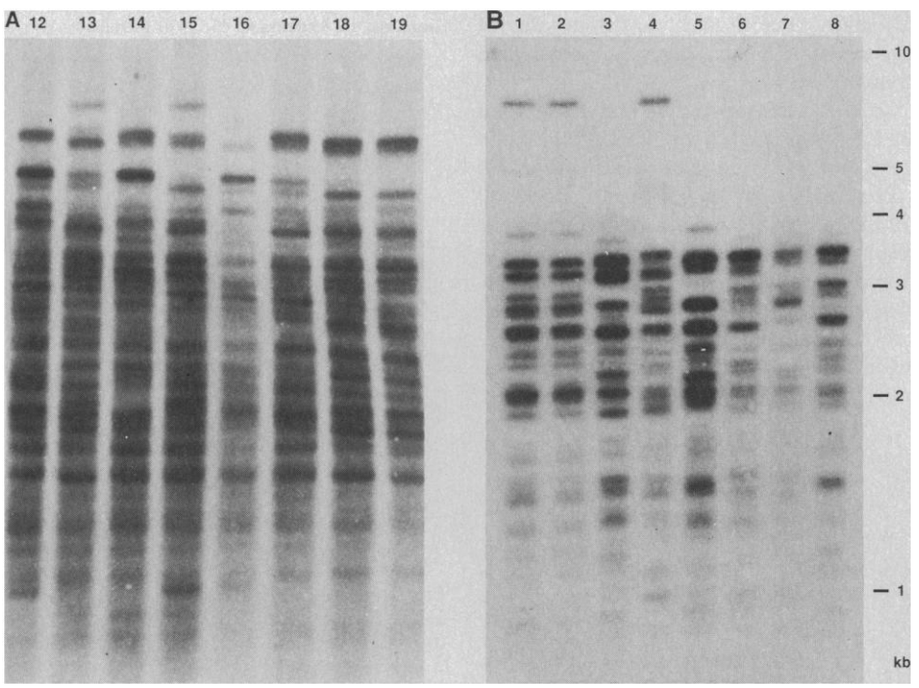

Fig. 4. Hypervariable polymorphic patterns detected in human (**A**) and bovine (**B**) DNA by hybridization to a tandem repeat present in M13 (*15*). (A) Individuals 12 to 17 are members of a Caucasian pedigree, 18 and 19 are monozygotic twins; (B) 1 and 2 are monozygotic twins obtained by embryo transfer of a surgically bisected bovine morula; bovines 3 to 8 are unrelated animals from the "Belgian White and Blue" breed. The 280-bp M13 Hae III–Cla I restriction fragment was obtained after agarose gel electrophoresis and electroelution. The fragment (100 ng) was labeled with ^{32}P to a specific activity of 0.5×10^{-9} to 1×10^{9} cpm per microgram of DNA (*13*). This probe was used in Southern blotting experiments as described in the legend to Fig. 1 with the milk-based cocktail for hybridization.

it should be possible to affinity-purify the bacteriophage sequence involved by a round of hybridization-selection to herring sperm DNA. A radioactively labeled M13 probe was purified in this way and hybridized to a Southern blot containing restriction fragments of the replicative form of M13 (Fig. 2B). Total, unpurified M13 was used as a control on a replicate blot (Fig. 2A). The results indicated that the affinity-purified probe was enriched in sequences corresponding to a region between coordinates 1013 and 2528 on the M13 map (Fig. 3A). Inspection of this region of the phage sequence revealed the presence of a 15-bp motif repeated in tandem at two places in the protein III gene and corresponding to $(Glu, Gly, Gly, Gly, Ser)_n$ (Fig. 3B). A 280-bp fragment essentially made of one of the repeat clusters was purified and used as a probe on a Southern blot of human DNA cleaved with Hae III. A typical hyperpolymorphic pattern was obtained (Fig. 4A). The pattern was clearly different from that obtained with Jeffreys' probe (*5, 6*) under similar conditions. Highly polymorphic patterns were also obtained with DNA of bovine (Fig. 4B), equine, murine, and canine origin (*11*), which extends the usefulness of the M13 to pedigree analyses in animal breeding.

Our results lead to the surprising conclusion that one of the most commonly used DNA vectors is able, by itself, to detect hypervariable minisatellites in a variety of mammals. The observation that fish DNA, which is frequently added in excess to hybridization mixtures, competes for the probe explains why this phenomenon had remained unnoticed. It also suggests that omission of carrier DNA during hybridization should help in finding additional probes of this type. Finally, the wide conservation of sequences showing cross-hybridization with the M13 repeat poses the question of their putative role or mechanism of maintenance during evolution. An interesting clue may be found in the recent discovery that another highly conserved minisatellite is associated with transposable elements (*12*).

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15. The sequence and procedure described in the present study are the subject of a pending patent.

16. The continuous support and critical interest of J. E. Dumont and R. Hanset are deeply acknowledged. We thank A. Deplano and M. Mairie for expert technical assistance and D. Leemans for the preparation of the manuscript. We are grateful to A. Massip for providing the bovine monozygotic twins. Supported by Actions Concertées (Ministère de la Politique Scientifique) and Association Recherche Biomédicale et Diagnostic a.s.b.l.

29 September 1986; accepted 12 December 1986