ovary, the ovules should be borne on four distinct groups, each representing a part of half placenta. But in *S. grandiflorum* with four distinct placentae, the ovular orientation is in opposite direction on each half of the placenta (Figure 1 *l–k*), which is at variance with the usual orientation (Figure 1 *i*) as generally advocated[8]. Nevertheless, Puri's view[8] of half placenta can get support in *S. grandiflorum* only when its gynoecium is taken as tetracarpellary–a situation which is not commensurate with the existence of only two dorsals as observed against the four expected.

The existence of a residual concentric vascular bundle in the centre of the ovary in *S. grandiflorum* is another interesting feature, not found in other species of the genus. But this bundle certainly does not belong to the residual floral apex, as its morphological nature does not resemble the structure, i.e. endarch, conjoint and bicollateral. Nevertheless, its origin may be assumed from the congenital fusion of the four submarginal bundles of the two carpels forming one concentric bundle, which does not supply any organ but is consumed in the placenta itself.

From the foregoing discussion it is clear that floral anatomy of *S. grandiflorum* presents certain unique features which indicate its specialized nature among *Solanums*. The occurrence of perigynous condition, three traced sepal, sepal–petal–staminal tube, formation of false septum imitating tetracarpellary/tetralocular condition, a morphological tendency towards parietal placentation (with a unique type of placenta) and existence of a central carpellary residual bundle, all represent a sort of specialized condition for this species.

1. Lavania, S., Ph D thesis, Meerut University, Meerut, 1982.
2. Bhatt, G. P., Ph D thesis, Meerut University, Meerut, 1974.
3. Murray, M. A., *Bot. Gaz.*, 1945, **107**, 243–260.
4. Cutter, E. G., *Phytomorphology*, 1955, **5**, 274–286.
5. Carlquist, S, *Phytomorphology*, 1969, **19**, 332–366.
6. Cronquist, A., *An Integrated System of Classification of Flowering Plants*, Columbia Univ. Press, New York, 1987, pp. 1262.
7. Puri, V., *Bot. Rev.*, 1952, **18**, 603–651.
8. Puri, V., *J. Indian Bot. Soc.*, 1963, **42**, 189–198.
9. Raud, P. G., *Bull. Soc. Bot. Fr.*, 1963, **110**, 216–237.

ACKNOWLEDGEMENTS. I thank late Professor Y. S. Murty for his expert opinion on floral anatomical observations and Professor C. M. Govil for necessary guidance.

Received 12 April 1993; accepted 23 April 1993

An 18 mer sequence in a rat 1.3 kbp *Eco*RI repeat detects genetic polymorphism in humans

Lakshmi Ramachandra*, A. Radha Rama Devi and M. R. S. Rao*†**

*Department of Biochemistry, †Centre for Genetic Engineering and **Health Centre, Indian Institute of Science, Bangalore 560 012, India

DNA fingerprinting involves the typing of an individual's DNA content to produce somatically stable, individual-specific DNA fingerprints. This technique often uses hypervariable minisatellite (HVMS) sequences as the fingerprinting probe and has found extensive use in several disciplines. Recently, we sequenced a 1.3 kbp *Eco*RI repetitive DNA fragment, shown to harbour the meiotic DNA repair site(s) of rat pachytene spermatocytes. This 1.3 kbp clone contained four sequences sharing high homology to the various HVMS sequences reported in the literature. Here we show that one of the sequences can indeed detect polymorphism in human individuals and can be used for DNA fingerprinting.

HYPERVARIABLE minisatellite (HVMS) sequences are highly prevalent in eukaryotic genomes of a number of species including humans. Minisatellites consist of short G+C repeats present in tandem to form arrays. They display strand asymmetry, in that one strand has a high G content[1]. Though no overall sequence consensus has been noted, several families of minisatellites identified contain a consensus 'core' sequence of 10 to 15 bp (ref. 2). Minisatellite sequences display considerable polymorphism in terms of the number of repeats present in an array and also in the sequence composition of each individual repeat within the array. Taking advantage of the genetic polymorphism detected by these sequences at several loci in the genome, Jeffreys and coworkers[2,3] developed the principle of DNA fingerprinting. This technique initially utilized the core sequences of HVMS as probes to generate somatically stable, individual-specific DNA fingerprints. More recently, Ehtesham *et al.*[4] developed a novel probe for human DNA fingerprinting which contained chi-like sequences. DNA fingerprinting has found wide-spread application in several disciplines including forensics, paternity testing, ecological genetics, immigration laws and transplant screening to name a few. Over the years, this technique has undergone considerable refinement. Polymerase chain reaction (PCR) amplification of hypervariable loci, has considerably increased the sensitivity of DNA typing systems and has proved extremely useful when the DNA source is limiting or degraded[5].

The mechanism of generation of polymorphism has generated considerable debate over the last several years. Owing to the high homology of the 'core' sequence of the HVMS with that of the general recombination signal of *E. coli* (chi) it has often been postulated that these

sequences could be the eukaryotic initiators of recombination promoting among other things their own propagation[2]. Direct evidence for this hypothesis is, however, lacking. Debate on whether the propagation is due to germline or somatic events also exists. While similar minisatellite mutation rates in male and female germ cells suggest that hypervariability is a consequence of meiotic events[6], detection of new mutant minisatellite alleles, in early mouse development indicates that mutation events can also arise during mitosis[7].

Over the past several years, we have been studying DNA repair synthesis at the pachytene interval of meiosis in rat pachytene spermatocytes with an aim of understanding the significance of this event in the context of the events at this stage of meiosis mainly recombination.

Recently, we have analysed the meiotic DNA repair sites of rat pachytene spermatocytes and sequenced a member of the 1.3 kbp *Eco*RI repetitive DNA family (1.3 A), found to harbour the meiotic DNA repair sites[8]. The sequence contained (a) a $(CAGA)_6$ repeat, a $(CA)_{22}$ repeat, and (c) four sequences showing a high percentage of homology to the various HVMS sequences reported. Among these four sequences was an 18 mer sequence (5′ GGGAGGGAGTGAGGATTG 3′) sharing a 90% homology to a core sequence (GGNNGTGGGG) derived from a comparison of DNA sequences of several variable number tandem repeat loci described by Nakamura *et al.*[9], a 69% homology to the myoglobin core and the human consensus minisatellite core[2] and a 63% homology to the mouse MHC recombination hotspot[10] (Figure 1). In order to evaluate the significance of the HVMS sequences in the repair positive clone, we were interested in determining whether this sequence could detect genetic polymorphism in humans. Here we show that this sequence can indeed detect polymorphism in human individuals and could be used for DNA fingerprinting in the human population.

Genomic DNA was isolated from the peripheral blood of four related donors (consisting of a father, mother and a pair of identical twins) and four unrelated donors. Following digestion of the genomic DNA with *Hinf*I (which does not cut within the minisatellite sequences), the DNA samples were run on a 1% agarose gel and transferred to nylon membrane (gene screen plus du pont). The 18 mer oligonucleotide was end labelled with γ-^{32}P-ATP using T4 polynucleotide kinase. The nylon membrane was prehybridized for 30 min at 37°C in prehybridization solution (6 × SSC, 5 × Denhardt's solution, 0.05% sodium pyrophosphate, 100 μg ml^{-1} yeast tRNA and 0.5% sodium dodecyl sulphate). Hybridization was carried out for 30 h at 37°C in hybridization solution (6 × SSC, 5 × Denhardt's solution, 0.05% sodium pyrophosphate, 100 μg ml^{-1} yeast tRNA) containing 1×10^6 cpm ml^{-1} of the end labelled probe. Yeast tRNA has been used as the non-specific nucleic acid instead of salmon sperm DNA, to prevent any non-specific titering out of the probe. As is evident in Figure 2, the identical twins had identical fingerprints and most of the bands identified could be traced to one or the other parent. At the same time, the DNA samples from the four unrelated individuals showed hybridization patterns, each distinct from the other. Thus, it is clear that the 18 mer sequence from the rat *Eco*RI 1.3 kbp family can detect polymorphism in the human population. It may be pertinent to point out here that recently Mazzarella *et al.*[11] demonstrated that using PCR technique and syntenically equivalent tagged sites from human DNA, one can assemble corresponding genomic maps from other primates as well as rodents. Although the detection of polymorphism in humans by rat HVMS-like sequence is not that surprising, we would like to stress here that the availability of the oligonucleotide probe described in this study would be very valuable for DNA fingerprinting studies in Indian population.

```
Myoglobin core (2)          GGAGGTGGNCAGGRRG
                            ||||| || ||||
   Rat 1.3A                 GGAGGGAGTGAGGATT

Nakamura core (VNTR) (9)    GGNNGTGGGG
                            |||||||  ||
   Rat 1.3A                 GGGAGTGAGG

Human consensus minisatellite core (2)   GGAGGTGGGCAGGAXG
                                         |||||  |  ||||||
   Rat 1.3A                              GGAGGGAGTGAGGATT

Mouse MHC recombination hotspot (10)     GGAGGTAGGCAGGCAG
                                         |||||  ||  |||
   Rat 1.3A                              GGAGGGAGTGAGGATT
```

Figure 1. Comparison of the rat 1.3 A HVMS-like sequence with some of the reported HVMS, VNTR and recombination hotspot sequences.

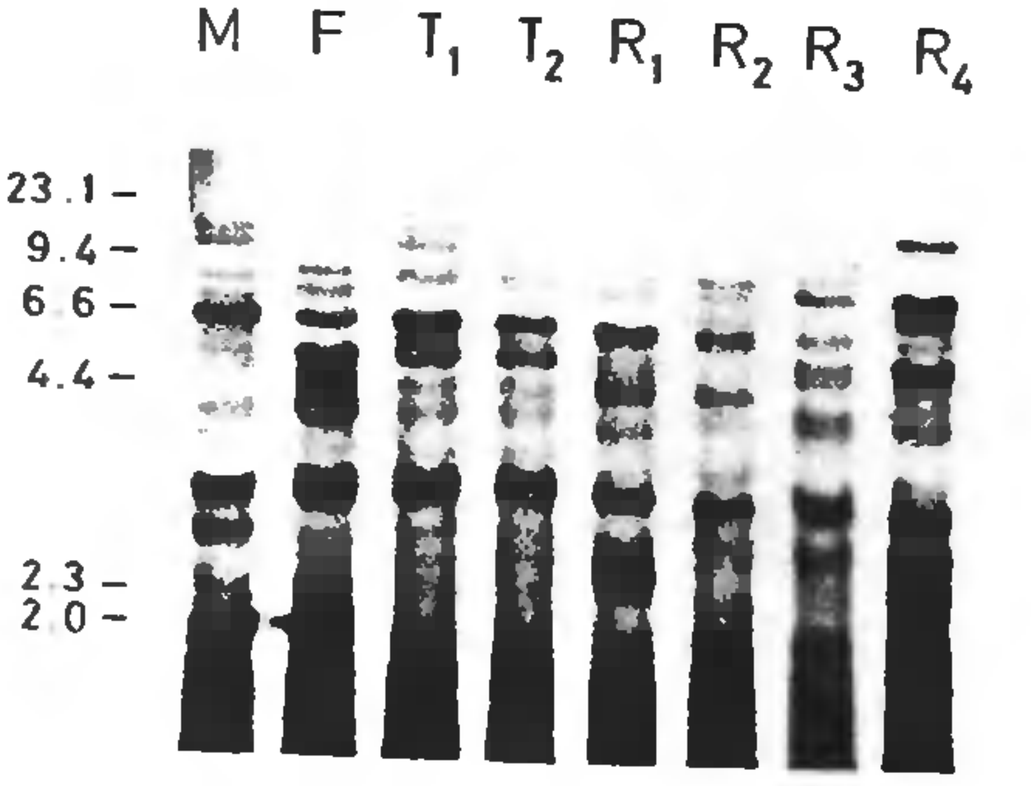

Figure 2. *Hinf*I digested human genomic DNA hybridized with 18 mer rat sequence. Genomic DNA samples were obtained from four related donors—mother (M), father (F) and a pair of identical twins (T_1, T_2) and four unrelated donors, R_1, R_2, R_3, R_4.

1. Jarman, A. P. and Wells, R. A., *Trends Genet.*, 1989, **5**, 367–371.
2. Jeffreys, A. J., Wilson, V. and Thein, S. L., *Nature*, 1985, **316**, 67–73.
3. Jeffreys, A. J., Wilson, V. and Thein, S. L., *Nature*, 1985, **316**, 76–79.
4. Ehtesham, N. Z., Das, A and Hasnain, S. E., *Gene*, 1992, **111**, 261–263
5. Jeffreys, A. J., Wilson, V., Neumann, R. and Keyte, J., *Nucleic Acids Res*, 1988, **16**, 10953–10971.
6. Jeffreys, A. J., Royle, N. J., Wilson, V. and Wong, Z., *Nature*, 1988, **332**, 278–281.
7. Kelly, R., Bulfield, G., Collick, A., Gibbs, M. and Jeffreys, A. J, *Genomics*, 1989, **5**, 844–856.
8. Ramachandra, L. and Rao, M.R. S., Manuscript submitted.
9. Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, M., Culver, M., Martin, C., Fujimoto, E., Hoff, M., Kumlin, E. and White, R., *Science*, 1987, **235**, 1616–1622.
10. Steinmetz, M., Stephan, D. and Fishcher Lindahl, K., *Cell*, 1986, **44**, 895–904.
11. Mazzerella, R., Manotanaro, V., Kekre, J., Reinbold, R., Ciccodicola, A., D'Urso, M. and Schlessinger, D., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 3681–3685.

ACKNOWLEDGEMENTS. This work was supported by a grant from the Department of Science and Technology, New Delhi, LR is a senior research fellow of the Council of Scientific and Industrial Research. The oligonucleotide probe was synthesized by the Oligonucleotide Core Facility at CGE, Indian Institute of Science, Bangalore.

Received 16 March 1993; revised accepted 7 May 1993.

Cryptocaryon irritans (Protozoa: Ciliata) infection among aquarium-held marine ornamental fish and its control

A. P. Lipton

Regional Centre of Central Marine Fisheries Research Institute, Mandapam 623 520, India

Ornamental fishes belonging to seven genera maintained in the marine aquarium in Mandapam were infected by the ciliate *Cryptocaryon irritans*, reported for the first time from India. In the affected fishes numerous whitish pustules were noticed on the body. The percentage mortality ranged from 4 to 100%. Bath treatment using 2 ppm chloramphenicol, followed by 5 ppm of copper sulphate after 6 h, controlled and eradicated the ciliates effectively. Hydrological and other possible environmental factors for the onset and spread of ciliate infection are discussed.

SEVERAL species of Protozoan ciliate parasites have been documented as causing considerable damage to marine fin fishes, particularly to those cultured or reared under controlled conditions[1,2]. Among the ciliates, *Cryptocaryon irritans*, which causes the disease cryptocaryonisis (white spot disease), has been reported by a few workers[3–8] and is considered as the marine counterpart of the 'ich' disease caused by *Ichthyopthirius multifilus* among the freshwater fishes[9]. Epizootics caused by Cryptocaryonisis have been described in marine aquarium fishes in Japan, Singapore and London[3, 10]. For example, in Japan, *C. irritans* affected 44 species of the 53 species stocked in marine aquaria[2]. Nigrelli and Ruggieri[11] have listed 27 species of marine fishes affected by Cryptocaryonisis in New York.

In the present investigation, eleven species of ornamental and other fishes belonging to seven genera were infected by *C. irritans*. The causative factors, the species affected and the possible control measures are discussed here.

During December 1988, heavy mortality was noticed among marine ornamental fishes. The fishes affected had numerous macroscopic glistening whitish pustules spread all over the body surface. Some were restless, exhibiting unusual swimming movements and respiratory distress. A few were lethargic and on the verge of collapse with excessive production of mucus, ultimately succumbing to death. Although the infected fish responded to external stimuli, their feeding intensity was poor. In some fishes, fins were infected, eroded and necrotic. The different species of fish affected by cryptocaryonisis and their mortality percentages are given in Table 1.

Except for the box fishes and cow fishes, all other species of fishes registered mortalities (Table 1). In these fishes and *Lethrinus* sp., the infection was in the initial stages. The temperature of the water in aquarium ranged from 28 to 29 °C; the dissolved oxygen ranged from 3.54 to 4.73 ml l^{-1} (average 3.84 ml l^{-1}) and the salinity was 27.25‰.

Observations of parasites from the moribund fishes under microscope indicated that their body was densely ciliated. The presence of characteristic ribbon-shaped nuclei together with buccal apparatus including three membranelles and one paroral membrane suggest that this organism is a ciliate belonging to Hymenostomatidae, which could be assigned as *Cryptocaryon irritans* species. Among the four stages, viz. trophonts, matured ones, tomonts and tomites, the trophonts stage is parasitic, which attach and live in the skin of fish. Although *C. irritans* has been recorded from marine aquarium fishes from other countries, there appears no report from India so far.

The infected live fishes were given external treatment by reducing the water level in each aquarium to 5 cm. As bacteria and fungi were also found on the eroded fins, chloramphenicol (CDH make) at 2 ppm was given for 3 min, followed by flushing with fresh seawater. After 6 h, copper sulphate at 5 ppm was given following the above procedure, the contents flushed by increasing the seawater level in each aquarium and the fresh seawater allowed to flow through each tank. After this pattern of