

In a future paper, we will examine strategies to search for the stringiness of an anisotropy pattern in more detail by running Monte Carlo experiments on our anisotropy map. We will also use Monte Carlo experiments to determine the proper limits on $G\mu/c^2$ from small-angular-scale experiments. Finally, we will also attempt a more quantitative estimate of other anisotropies

predicted by cosmic string models, which might interfere with the stringy nature of the anisotropies discussed here.

This work was supported in part by the NSF (at Berkeley and Chicago), the DOE (at Fermilab and IGPP) and by NASA (at Fermilab).

Received 20 June; accepted 10 August 1988.

1. Brandenberger, R. *Int. J. mod. Phys. A* **2**, 77-131 (1987).
2. Vilenkin, A. *Phys. Rev. D* **24**, 2082-2089 (1981).
3. Kaiser, N. & Stebbins, A. *Nature* **310**, 391-393 (1984).
4. Gott, R. *Astrophys. J.* **288**, 422-427 (1985).
5. Bennett, D. P. & Bouchet, F. R. *Phys. Rev. Lett.* **60**, 257-260 (1988).
6. Bouchet, F. R. *8th Moriond Astrophysics Meeting: Dark Matter* (ed. Frontieres, initial?) (Reidel, Dordrecht) (in the press).
7. Albrecht, A. & Turok, N. *Phys. Rev. Lett.* **54**, 1868-1871 (1985).
8. Stebbins, A. *Astrophys. J.* **327**, 584-613 (1988).
9. Brandenberger, R. & Turok, N. *Phys. Rev. D* **33**, 2182-2195 (1986).
10. Traschen, J., Turok, N. & Brandenberger, R. *Phys. Rev. D* **34**, 919-930 (1986).
11. Melchiorri, F., Melchiorri, B., Ceccarelli, C. & Pietranera, L. *Astrophys. J.* **250**, L1 (1981).
12. Readhead, A. & Sargeant, W. in *IAU Symposium 130: Large Scale Structure of the Universe* (ed. Audouze, J.) (Reidel, Dordrecht, 1988).
13. Hogan, C. J. & Rees, M. J. *Nature* **311**, 109-114 (1984).
14. Davis, R. L. *Phys. Lett.* **161B**, 285-288 (1985).
15. Bennett, D. P. *Phys. Rev. D* **34**, 3592-3607 (1986).
16. Silk, J. & Vilenkin, A. *Phys. Rev. Lett.* **53**, 1700-1703 (1984).
17. Turok, N. & Brandenberger, R. H. *Phys. Rev. D* **33**, 2175-2181 (1986).
18. Brandenberger, R., Kaiser, N., Schramm, D. & Turok, N. *Phys. Rev. Lett.* **59**, 2371-2374 (1987).
19. Bertschinger, E. & Watts, P. N. *Astrophys. J.* **328**, 23-33 (1988).

Amplification and analysis of DNA sequences in single human sperm and diploid cells

Honghua Li*, Ulf B. Gyllenstein†, Xiangfeng Cui*, Randall K. Saiki†, Henry A. Erlich† & Norman Arnheim*

* Molecular Biology Section, Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-0371, USA

† Department of Human Genetics, Cetus Corporation, 1400 53rd Street, Emeryville California 94608, USA

The use of the polymerase chain reaction for analysing DNA sequences in individual diploid cells and human sperm shows that two genetic loci can be co-amplified from a single sperm, which may allow the analysis of previously inaccessible genetic phenomena.

THE construction of genetic maps in higher organisms requires analysis of the progeny of selected matings or calculation of linkage relationships by pedigree analysis. In humans only the latter is possible. Using restriction fragment length polymorphisms (RFLPs), there has been significant progress towards the construction of a human linkage map (for review see ref. 1). To locate genes with known phenotypic effects relative to RFLP markers there has been a concerted effort to establish a panel of genetic markers at about 10 cM (1 cM = 1% recombination) intervals so that no gene will be further than 5 cM away from an RFLP (ref. 2). Pedigree analysis is thought to be able to measure genetic distances of about 1 cM encompassing about 1,000 kilobases (kb) of DNA with statistical reliability. The analysis of smaller distances requires such a large number of individuals from informative families that it is impractical. If the genotype of large numbers of individual sperm could be determined, the measurement of genetic recombination over shorter physical distances could be accomplished at a resolution far greater than that currently possible and without family studies. We have therefore attempted to define the conditions required to analyse DNA sequences in single cells using the polymerase chain reaction (PCR, refs 3 and 4). We studied single diploid cells and then single sperm from an individual heterozygous at two genetic loci found on non-homologous chromosomes. For each locus we determined which of the two alleles were present in any one sperm and analysed the independent assortment of the alleles at a single locus and the independent segregation of genes on non-homologous chromosomes.

Analysis in single diploid cells

We studied the human β -globin gene locus in individual diploid cells from two tissue culture cell lines. One was derived from a

homozygous individual for the sickle-cell mutation at codon six (β^S); the other was homozygous for the normal β^A allele. PCR primers that amplify a β -globin fragment containing codon six and allele-specific oligonucleotide probes (ASO) which can distinguish between these two alleles have already been described^{3,5}. We co-cultivated the cells homozygous for β^A and cells homozygous for β^S in the same tissue culture flask for several days. Individual cells from this mixture were drawn into a thin plastic pipette during observation under a phase-contrast microscope. Each individual cell was delivered into a PCR tube containing a lysis solution and, after incubation, PCR buffer containing deoxyribonucleotides, *Taq* DNA polymerase⁶ and a set of PCR primers that amplify the informative region of the globin gene was added. After DNA denaturation 50 cycles of amplification were performed⁶. Aliquots from each sample of amplified product were hybridized separately with the β^A and β^S probes after fixation to nylon membranes^{5,7}. The results of this co-cultivation experiment are shown in Fig. 1. Out of the 37 cells analysed, 84% hybridized with only one of the two allele-specific probes; 19 with the β^A probe and 12 with the β^S probe. None of the 12 control tubes, which received water instead of a cell, was positive, indicating that DNA contamination was insignificant. No sample hybridized with both probes, indicating that a single cell only was introduced into each tube and that DNA from lysed cells present in the co-cultivation mixture did not adhere to individual cells.

The amount of amplified β -globin gene product produced by PCR of a single cell was determined by comparing the intensity of the hybridization signal obtained with that from known amounts of plasmid DNA carrying the globin gene spotted on the same filter (Fig. 1). We estimate that, starting with a diploid amount of globin DNA (3.3×10^{-9} fmol), between 5 and 500 fmol of PCR product was produced in 50 cycles. This is equivalent to an average amplification ratio of 7.6×10^{10} with

† To whom correspondence should be addressed.

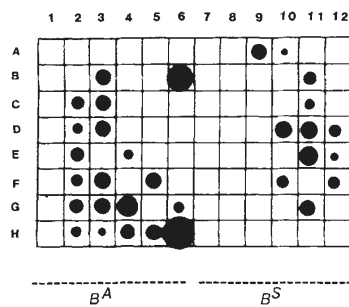


Fig. 1 PCR analysis of the β -globin gene in individual tissue culture cells. Each of two aliquots from the PCR products of a single cell were placed in the same row of the dot blot apparatus separated by six columns. Half of the filter was hybridized to the β^A ASO and the other to the β^S ASO. 1A-1H (7A-7H) and 2A, 2B (8A, 8B) were water blanks. 6H and 12H are aliquots of PCR product of purified β^A DNA. One, 3, 10 and 30 fmol of β^S gene containing plasmid were dotted as hybridization standards at positions a, b, c and d respectively. The remaining samples were individual tissue culture cells. After washing three times single cells were selected from a cell suspension ($1-3 \times 10^6 \text{ ml}^{-1}$) with a Zeiss phase-contrast microscope at $\times 100$ using a plastic needle with a 0.1-mm-diameter opening made by pulling a flamed disposable 1-ml-graduated plastic pipet. Each single cell sample was delivered into a 0.5 ml plastic microfuge tube containing 10 μl autoclaved distilled water. The cells were lysed using a slight modification of a published sperm lysis procedure¹⁶. The sample was adjusted to a final volume of 20 μl containing $1 \times$ PCR reaction buffer (ref. 6; 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl_2 , 0.1 mg ml^{-1} gelatin), 0.05 mg ml^{-1} proteinase K, 20 mM DTT, and 1.7 μM SDS. After one hour at 37 $^\circ\text{C}$, samples were heated to 85 $^\circ\text{C}$, and suspended in 100 μl PCR reaction mix containing $1 \times$ PCR buffer, 1 μM each oligonucleotide PCR primer, 187.5 μM each of dATP, dCTP, dGTP and dTTP, 100 ng *E. coli* DNA, 2 units of *Thermus aquaticus* thermostable DNA polymerase (Perkin Elmer-Cetus Instruments), and 60 μl mineral oil to prevent evaporation. The PCR reactions were carried out on a DNA Thermal Cycler (Perkin Elmer-Cetus Instruments). After heating at 95 $^\circ\text{C}$ for 10 min to ensure DNA denaturation, each cycle of PCR consisted of incubation at 95 $^\circ\text{C}$ for 15 s, 15 s incubation at 54 $^\circ\text{C}$ and a 1 min incubation at 72 $^\circ\text{C}$. After fifty cycles of PCR, dot blot analysis of 20 μl samples of the PCR reaction were carried out using β^S - and β^A -allele specific probes⁵.

an average efficiency per cycle of 65%. The precise extent of amplification may have been slightly lower if these cell lines contained more than two copies of chromosome 11. Considering the extent of amplification, elimination of all sources of possible contamination is critical to the success of these experiments.

Analysis in single human sperm

The genotype of single sperm derived from an individual heterozygous at the gene coding for the low density lipoprotein receptor (the *LDLr* gene), which has been localized to chromosome 19 (ref. 8), was analysed next. We adapted the detection of an *LDLr* polymorphism⁹ to PCR and ASO analysis using DNA sequence information (D. Russell, unpublished data), with the PCR primers and probes shown in the Fig. 2 legend. The size of the expected PCR product was 254 base pairs (bp).

Sperm were purified from a semen sample by centrifugation through a sucrose step gradient¹⁰ and stored for 8 months at -20°C . Individual sperm were drawn into a fine plastic needle under microscopic observation and delivered to a tube for lysis and amplification. In a series of experiments we analysed the *LDLr* genotypes in 80 individual sperm; typical results from one such experiment are shown in Fig. 2. Altogether 55% of

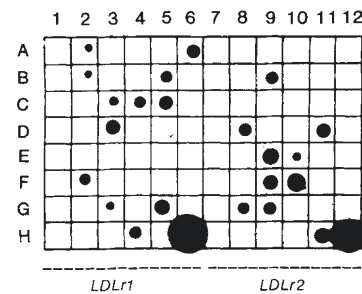


Fig. 2 PCR analysis of the LDL receptor locus of individual human sperm. 1A-1H (7A-7H) are water blanks. 6H and 12H are aliquots of amplified DNA from an *LDLr1/LDLr2* heterozygote. The remaining samples are from individual sperm. The organization of the samples on the dot blot is as in Fig. 1. Sperm were purified from semen by a modification of a published procedure¹⁰: 0.5 ml semen was mixed with 3 ml of 40% sucrose. The mixture was applied to the top of a sucrose step gradient made by adding 3.5 ml 90%, 70% and 50% (w/v) sucrose successively to a 15-ml graduated plastic tube (Falcon 2095). The sample was spun at 14,500g for two hours at room temperature. 0.5 ml of the interface between 70% and 90% sucrose was collected and applied to an identical sucrose gradient. This was repeated twice more. Single sperm were isolated in the same way as individual diploid cells using a sperm suspension at a concentration of $1 \times 10^5 \text{ sperm ml}^{-1}$. The sequence of the two primers used for PCR of the *LDLr* locus were 5'-AGTGCCACCGCCTCACAGG3' and 5'-CCTCTCACACCAGTTCACCT3'. The ASO for the *LDLr1* allele had the sequence 5'-AGGATATGGTCTCTTCCA3' whereas the *LDLr2* ASO had the sequence 5'-TGGAAGAGAACCATATCCT3'. The PCR and dot blot analysis were performed as in Fig. 1 except that the final washes of the filters hybridized with the *LDLr* probes (and *HLA DQA* probes, see Fig. 3) were at 56 $^\circ\text{C}$.

the sperm gave a hybridization signal. Twenty-two carried one allele; 21 the other. Only one sample was positive with both probes. Sixteen additional control tubes which received all of the reagents but no sperm did not give any hybridization signal. The distribution of the two amplified alleles obeyed Mendel's law of independent segregation, indicating that the PCR reactions were initiated with a single meiotic product and that no contaminating diploid DNA sequences were present.

Independent assortment of chromosomes

We attempted to amplify simultaneously DNA sequences at two different loci on non-homologous chromosomes in a single sperm. Our sperm donor was heterozygous at the *HLA DQ- α* locus (*DQA*) on chromosome six, as well as at the *LDLr* gene. Primers for amplification of the first gene and probes to distinguish between allelic variants have been described previously^{5,11}. The predicted size of the PCR product was 242 bp. Initial experiments in which primers for both loci were present throughout the entire amplification experiment were unsuccessful, so we performed only the first 20 amplification cycles in the presence of both primer pairs. After this primary amplification, 1/50 of the reaction mixture was placed in a tube and diluted with a PCR solution containing the *HLA* primers only and another aliquot was diluted with a PCR mix containing the *LDLr* primers only. After an additional 45 cycles of amplification, part of each secondary reaction was hybridized to either of the two ASOs for that locus. A total of 150 individual sperm were analysed, in a series of such experiments (Table 1; representative data are shown in Fig. 3). Twenty-seven samples did not exhibit amplification of either locus, but we did detect hybridization signals in 123 samples (82%). In nine of these samples, two alleles from at least one of the two loci were

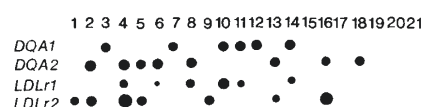


Fig. 3 PCR analysis of individual sperm simultaneously amplified with *HLA DQA* and *LDLr* primers and analysed with allele-specific probes for the *DQA* alleles 1 and 2 (*DQA1*, *DQA2*) and *LDLr* alleles 1 and 2 (*LDLr1*, *LDLr2*). Water blanks are in positions 19–21. Examples of sperm where one allele at each locus is amplified are shown in positions 2, 5, 6, 8, 10, 11, 13, 14 and 16. The remaining samples can be divided into those that amplified more than one allele at a locus (position 4), only one allele at one locus (positions 1, 3, 7, 9, 12, 17, 18) or neither allele (position 15). During the first 20 cycles both loci were amplified simultaneously with both sets of primers present at 1 μ M. A 2 μ l aliquot from each reaction was then added to each of two tubes with 100 μ l fresh PCR reaction buffer containing only one of the primer pairs. Forty-five additional PCR cycles were then performed.

detected and these samples were excluded from further analysis. These samples may have contained two sperm of different genotypes. On the other hand these nine samples might have resulted from non-disjunction events. The analysis of only two loci cannot distinguish between these possibilities.

Among the remaining 114 sperm, 96 could be typed at the *LDLr* locus with 45 having *LDLr1* and 51 having *LDLr2*. The two alleles segregate in the expected 1:1 ratio (Chi square = 0.375, $0.75 > P > 0.5$, 1 degree of freedom). Eighty-eight could be typed at the *DQA* locus: 53 had the *DQA1* allele, 35 the *DQA2*. The segregation of the *DQA* alleles with the expected 1:1 ratio is at the borderline of statistical significance (Chi square = 3.68, $0.1 > P > 0.05$, 1 degree of freedom). This could represent a statistical fluctuation, unequal amplification of the two *DQA* alleles resulting from base mismatches between the PCR primers and the *DQA* DNA sequence of our particular donor due to the extensive polymorphism of this locus, or some unusual genetic phenomenon such as segregation distortion.

Seventy of the 114 sperm (61%) that gave hybridization signals could be typed at both loci. The independent segregation of chromosome six and chromosome 19 should result in the equally frequent occurrence of the four possible gametes: *DQA1*, *LDLr1*; *DQA1*, *LDLr2*; *DQA2*, *LDLr1*; *DQA2*, *LDLr2*. We actually observed 21, 18, 14 and 17 of each type respectively; the difference is not statistically significant (Chi square = 1.43, $0.75 > P > 0.5$, 3 degrees of freedom). These results show that we can reliably and with reasonable efficiency simultaneously determine the genotype of individual sperm at two distinct genetic loci.

Among the 114 samples that gave a hybridization signal, 18 showed one of the two *DQA* alleles but an *LDLr* product could not be detected. Twenty-six sperm showed one of the two *LDLr* alleles but no amplification of the *HLA* locus. These sperm could be nullisomic for one of the two chromosomes we studied due to a non-disjunction event. There may also have been a failure to amplify one of the two loci. Because we only looked at a single locus on each chromosome we cannot distinguish between these possibilities.

The relative frequency of successful amplification of the *LDLr* and *DQA* loci in single sperm is approximately the same. Among 141 samples, 88 amplified *DQA* and 96 amplified *LDLr*. We are unable to calculate accurately the absolute frequency of success because we cannot be sure that every one of the 141 samples did in fact contain a sperm. As a result, we cannot use our data to calculate accurately the expected frequency of samples with a single allele amplified at both loci, at only one locus or at neither locus assuming that amplification at each locus is an independent event. Amplification of both loci may not be independent events if sperm lysis is not uniform from sample to sample. Thus the assessability of one of the target DNA

molecules to the PCR reagents might be positively correlated with the accessibility of the other. Improving the absolute rate of successful amplification of single sperm may depend upon improving lysis procedures, thereby enhancing target DNA accessibility.

Discussion

The analysis of the genotype of single sperm at the DNA level is a unique tool for the study of problems in human genetics considered intractable using current approaches. To date there have been no practical methods for accurate measurement of genetic distances of less than 1 cM. A significant advantage of the approach described here is that a large number of meiotic products can be examined from a single individual allowing determination of the recombination frequency between genetic markers which are physically very close. In conjunction with gel electrophoresis procedures for very large DNA fragments and chromosome-walking data it should be possible to measure the frequency of recombination between genetic markers whose physical distance apart is known precisely. This would allow the analysis of recombination frequency as a function of physical distance and a test of the conventionally accepted value of one per cent recombination per million base pairs¹² for specific chromosomal regions. Because it should be possible to obtain statistically significant data on recombination frequencies from a single individual, it should also be possible to determine for the first time whether different individuals have the same or different rates of recombination for the same interval.

The ability to measure recombination over short physical distances will be especially useful in the study of recombination hot spots. The effect that a hot spot can have on recombination between flanking markers depends upon how far away the flanking marker loci are from the ends of the hot spot. Thus a 1-kb DNA segment with a recombination potential which is 10-fold greater than normal DNA will have very little effect on recombination measured between markers that are 500 kb to either side of the hot spot. Pedigree analysis cannot measure recombination over the short intervals typical of many of the hot spot regions that have been deduced from population genetics data^{13–15}, given the number of informative families required and the effort involved in obtaining the data. With PCR, we can envisage typing as many as 500 meiotic products in a week. Decreasing the number of samples containing two sperm and increasing the efficiency of amplification of both loci simultaneously will be required for the highest resolution

Table 1 Amplification of sequences at two different loci

Total number of sperm examined	150
No signal	27
<i>DQA1</i> , <i>LDLr1</i>	21
<i>DQA1</i> , <i>LDLr2</i>	18
<i>DQA2</i> , <i>LDLr1</i>	14
<i>DQA2</i> , <i>LDLr2</i>	17
<i>DQA1</i>	14
<i>DQA2</i>	4
<i>LDLr1</i>	10
<i>LDLr2</i>	16
<i>DQA1</i> , <i>LDLr1</i> , <i>LDLr2</i>	2
<i>DQA2</i> , <i>LDLr1</i> , <i>LDLr2</i>	1
<i>DQA1</i> , <i>DQA2</i>	1
<i>LDLr1</i> , <i>LDLr2</i>	2
<i>DQA1</i> , <i>DQA2</i> , <i>LDLr1</i> , <i>LDLr2</i>	3
Controls	32
No signal	29
<i>LDLr1</i>	2
<i>LDLr2</i>	1

studies. In linkage experiments a fraction of the samples that contain two sperm and which have amplified only one of the two alleles at each locus will generate 'false recombinants'. We expect that the frequency of false recombinants can be minimized by careful attention to sperm isolation and the enhancement of sperm lysis and amplification efficiency. If very large numbers of sperm could be analysed with great reliability, some mutational events which cannot be analysed by conventional methods might eventually be studied.

Our ability to haplotype sperm at the DNA level will provide a fundamentally new approach to studying human recombination and may also be useful in determining the physical order of DNA polymorphisms which are so tightly linked that they cannot be resolved by additional family analysis. This may be especially significant in the case of random RFLPs tightly linked to disease-causing loci. The genetic distances between the random RFLPs could be accurately determined and the RFLPs ordered with respect to one another by three point crosses, provided that simultaneous amplification of 3 marker loci could be made efficient enough for single sperm experiments. Such fine structure maps might be of great value in attempts to locate the disease-causing locus itself. Of course because sperm do not exhibit disease phenotypes, an unknown disease locus cannot be directly mapped relative to polymorphisms in this way.

The analysis of single sperm in species that cannot be exten-

sively bred or have exceptionally long generation times may be the only practical way of making genetic maps for these species.

One immediate practical application of the analysis of individual sperm is in the area of forensic medicine. *HLA* typing for paternity determinations or identification of criminals is often hampered by the inability to determine the haplotype of the suspected individuals because this would require the analysis of close relatives. *HLA* analysis of individual sperm from a suspect would allow the linkage phase of the *HLA* markers to be unambiguously determined and thus increase the probability of inclusion or exclusion.

Finally, the ability to study DNA sequences in individual diploid cells will make it possible to study cell-to-cell variation in developmental processes involving DNA rearrangements or other genetic alterations. It is also likely that analysis of messenger RNAs in single cells would be possible if efficient reverse transcription could be carried out before PCR was initiated. Prenatal diagnosis on a single cell derived from a pre-implantation embryo resulting from *in vitro* fertilization is also conceivable.

We thank L. Goda, D. Spasic and C. Levenson for synthesis of the DQA primers and probes. U.B.G. was supported by a Knut and Alice Wallenberg Foundation Fellowship. This work was supported in part by a grant from the National Institute of General Medical Sciences (N.A.).

Received 11 May; accepted 15 June 1988.

1. Donis-Keller, H. *et al.* *Cell* **51**, 319-337 (1987).
2. Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. *Am. J. hum. Genet.* **32**, 314-331 (1980).
3. Saiki, R. K. *et al.* *Science* **230**, 1350-1354 (1985).
4. Mullis, K. B. & Faloona, F. A. *Meth. Enzym.* **155**, 335-350 (1987).
5. Saiki, R. K., Bugwan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. *Nature* **324**, 163-166 (1986).
6. Saiki, R. K. *et al.* *Science* **239**, 487-491 (1988).
7. Conner, B. J. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 278-282 (1983).
8. Lindgren, V., Lusky, K. L., Russel, D. W. & Francke, U. *Proc. natn. Acad. Sci. U.S.A.* **82**, 8567-8571 (1985).

9. Hobbs, H. H., Esser, V. & Russell, D. W. *Nucleic Acids Res.* **15**, 379 (1987).
10. Puwaravutpanich, T. & Panyim, S. *Expl. Cell Res.* **90**, 153-158 (1975).
11. Scharf, S. J., Horn, G. T. & Erlich, H. A. *Science* **233**, 1076-1078 (1986).
12. Renwick, J. H. *Br. med. Bull.* **25**, 65-73 (1969).
13. Chakravarti, A. *et al.* *Am. J. hum. Genet.* **36**, 1239-1258 (1984).
14. Chakravarti, A., Elbein, S. C. & Permutt, M. A. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1045-1049 (1986).
15. Migone, N., de Lange, G., Piazza, A. & Cavalli-Sforza, L. L. *Am. J. hum. Genet.* **37**, 1146-1163 (1985).
16. Gill, P., Jeffreys, A. J. & Werrett, D. J. *Biotechnology* **3**, 1008-1012 (1985).

LETTERS TO NATURE

No cometsimals in the inner Solar System

D. T. Hall & D. E. Shemansky

Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona, USA

Ultraviolet measurements made by Voyager 2, apparently showing a rapid decrease in hydrogen Lyman- α emission with distance from the Sun, were taken by Donahue *et al.*¹ as evidence for a source of atomic hydrogen in the very local interstellar medium (VLISM). The suggested source¹ is a class of small comets, at solar distances of ~ 1 AU, that produce atomic hydrogen as their icy mantle is evaporated. This claim has been adduced as evidence for a theory² that the Earth is subject to a large influx of cometary material, significantly affecting atmospheric evolution. Here we analyse again the Voyager 2 data, and show that no source of hydrogen in the VLISM is required other than the inflow of neutral atoms; the original analysis was apparently flawed by an erroneous transcription of tabulated data (T. M. Donahue, personal communication).

Although Donahue *et al.*¹ claimed to find a cometary source of hydrogen, the estimated flux was $\sim$ seven orders of magnitude smaller than required by the theory of Frank *et al.*². But by postulating a different and more numerous kind of comet, Frank

et al. have claimed³ that the Voyager data, the most important direct evidence for small comets and the crucial data in obtaining limits on sources of volatile compounds such as H₂O, can be made to agree with their theory.

The observations in question are obtained with Voyager 2 shortly after launch¹. Figure 1 shows the observing geometry looking down on the north pole of the Solar System. A number of observations, in a direction approximately downstream with respect to the inflowing VLISM neutral gas, were obtained with the spacecraft located near 1 AU. Further measurements were obtained with the spacecraft located at $r_0 \sim 1.3$ AU and beyond, with all observations approximately downstream as shown in Fig. 1. The analysis of these data by Donahue *et al.*¹ indicated that the measurements near 1 AU in a direction almost tangential to the Earth's orbit demonstrated intensities in excess of a normal VLISM model. The excess near 1 AU was attributed to the influx of cometsimals. We argue that the stronger signal obtained near 1 AU is simply a consequence of observing geometry.

The search for a local source of hydrogen in the Voyager data was limited by Donahue *et al.*¹ to observations in the VLISM downstream direction (Fig. 1). The observations obtained with the spacecraft positioned near 1 AU were necessarily at a high angle ($\beta \approx 90^\circ$; see Fig. 1) to the antisolar direction because the spacecraft was positioned in the vicinity of 0° right ascension (α) (Fig. 1). Later observations with the spacecraft positioned near 2 AU were obtained with a viewing direction more closely aligned with the antisolar direction. A non-negligible fraction