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DETECTION OF SMALL AMOUNTS OF HUMAN DNA IN HUMAN–RODENT HYBRIDS

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SUMMARY

A method of measuring semi-quantitatively small amounts of human DNA in irradiated human × mouse and irradiated human × Chinese-hamster somatic cell hybrids is described. One method uses molecular hybridization of cell DNA bound to nitrocellulose filters with a cRNA probe to C_0t 0–1 human DNA. Alternatively hybrid cell DNA is reassociated in solution with a C_0t 0–1 fraction of nick-translated human DNA. Formamide buffers give specificity to the reaction. The detection limit of the filter method is 0·2–0·5 % equivalents and reassociation kinetics 0·005–0·01 % equivalents of a human genome.

Experiments with cell hybrids suggest that a fragment of repetitive DNA may be retained along with the selected genes after a cell fusion. In one case however, of a hybrid cell in which malignancy is suppressed, highly repetitious sequences were not found.

INTRODUCTION

When a cell with a genome fragmented by irradiation is fused with a normal unirradiated cell, the resulting hybrid undergoes extensive loss of the genetic material of the irradiated parent, and only a small chromosomal fragment may be retained: this is the basis of a method of gene mapping (Goss & Harris, 1975, 1977). Cell hybrids made by chromosome-mediated gene transfer have a similar end result (McBride & Ozer, 1973; Miller & Ruddle, 1978). In both of these cases, the usual way of determining the total contribution of chromosomal material of each parent to the hybrid, by karyotyping, may be applicable either with difficulty or not at all.

Molecular hybridization is a method commonly used for detecting and quantitating viral DNA and other specific sequences (e.g. Y-chromosome-specific DNA; Kunkel *et al.* 1977) in cells. The method has been used occasionally to measure total foreign DNA (as opposed to specific sequences) in cell hybrids (Coon, Horak & Dawid, 1973; Jones, 1977), although the sensitivity was not very high, 2–5 % equivalent genomes of foreign DNA. It was thought worthwhile to see how far hybridization methods could be useful as a measure of the heterologous DNA in animal cell hybrids. The results show that filter hybridization will detect the equivalent of 0·5 % of a human genome, and reassociation kinetics the equivalent of 0·005–0·01 %, in a rodent × human cell hybrid. The reassociation method also gives information about sequences. Application of the method to several cell hybrids containing human DNA fragments is described.

METHODS

Cells

A9, an HPRT$^-$ derivative of the mouse L cell, was normal laboratory stock.

A9–Daudi clones. Various clones derived from a Sendai virus fusion of A9 with Daudi, a Burkitt lymphoma-derived B-type lymphocytic line have been described (Allderdice *et al.* 1973). Clones were from the laboratory stock and were karyotyped co-incidentally with these experiments (see Results).

Wg3-h is a Chinese-hamster DON line derivative lacking HPRT.

$R_4$93 is a hybrid cell, Wg3-h × irradiated human lymphocyte, made by the Sendai virus method. The lymphocyte had received 40 J kg^{-1} γ-rays before cell fusion.

$R_4$93(6TG) was $R_4$93, back selected in 6 μg/ml 6-thioguanine.

$R_6$2 is similar to $R_4$93 except that the lymphocyte had received 60 J kg^{-1} γ-rays before fusion. Chinese-hamster × lymphocyte fusions were originally made by Dr Stephen Goss.

PG19 is a HPRT$^-$ cell line derived from a spontaneous melanoma in a C57 Black mouse (Jonasson, Povey & Harris, 1977).

B_2 and D_1 were clones from a PG19 × MRC-5 (human fibroblast) fusion in which the MRC-5 had received 200 J kg^{-1} γ-rays before fusion. These fusions were originally made by Professor H. Harris.

Cl19S16TG is a PG19 × human lymphocyte hybrid cell, described by Jonasson & Harris (1977).

Cell lines (except Raji) were grown in monolayer culture in MEM. Hybrid cells were grown similarly but with the addition of HAT.

Raji cells were grown in suspension culture in DMEM with 20% foetal calf serum.

Preparation of DNA

Cell lines or hybrid cells were grown in roller bottles at 37 °C, trypsinized from the surface, washed twice with ice-cold PBS (20 ml per ml packed cell volume) and resuspended in the same volume of PBS. 1 vol. of sarkosyl-EDTA-Tris (3·5% sarkosyl + 0·075 M Tris pH 7·5, 0·025 M EDTA) was added and the mixture stood overnight at room temperature. It was then heated at 65 °C for 10 min followed by incubation with 20 μg/ml heated RNase for 30 min at 37 °C, and then pronase (1–2 mg/ml, 3 h at 37 °C). CsCl was added to give a density of 1·700 g cm^{-3} and the solution centrifuged to equilibrium at 20 °C. DNA-containing fractions were dialysed against 1 × SSC (1 × SSC is 0·15 M NaCl + 0·015 M trisodium citrate pH 7·2) and stored at —70 °C. DNA for reassociation was further treated by degradation in 0·3 M NaOH at 100 °C for 15–20 min (Sharp, Pettersson & Sambrook, 1974) followed by dialysis. DNA concentrations were measured by the diphenylamine method of Burton (1956).

Preparation of cRNA

Sheared human placental DNA (Calbiochem) was reassociated to C_0t 1 in 0·12 M phosphate buffer at 60 °C and isolated on hydroxylapatite (HAP). C_0t 0·5–10 DNA was prepared in the same way. 5 μg reassociated DNA were transcribed with *E. coli* RNA polymerase (Boehringer Corp. Ltd) in 40 mM Tris-HCl, pH 7·9, 0·15 M KCl, 10 mM $MgCl_2$, 0·1 mM EDTA, 0·1 mM 2-mercaptoethanol and 0·15 mM GTP. 100 μCi of tritiated ATP, CTP, UTP were added, incubated at 37 °C for 3 h and the product isolated on Sephadex G50. It was freeze-dried and stored at —70 °C.

Hybridization on filters

DNA (20 μg per filter) in 0·1 × SSC was denatured by adding 1 vol. 0·5 M NaOH and standing at about 30 °C for 15–20 min. It was neutralized by adding 2 vol. ice-cold neutralization mixture (12 × SSC : 1 M Tris-HCl pH 7·1 : distilled water : conc. HCl, 100 : 60 : 140 : 3). The neutralized DNA was applied to the filter under slight vacuum and the filter washed with 10 ml 6 × SSC: it was then removed from the filtration apparatus, air-dried for 3 h and baked

at 80 °C *in vacuo* for 18 h. Filters were prepared in triplicate. Filters for hybridization were preincubated for 1 h at 37 °C in 50 % formamide + 6 × SSC, the preincubation buffer removed and cRNA (90000 cpm) in the same buffer was added. Hybridization was at 50 °C for 48 h, after which the filters were washed (twice each time) with 50 % formamide – 6 × SSC, 6 × SSC, 2 × SSC, and then incubated with RNase (25 μg/ml final concentration) in 2 × SSC for 60 min at room temperature. Filters were washed in a large volume of 12 × SSC. Filters were then heated at 70 °C for 45 min in 1·0 M perchloric acid to hydrolyse the nucleic acid, the O.D.$_{260}$ of the hydrolysate measured, and counted in a scintillation counter. Counts were corrected to a known DNA concentration from the O.D.$_{260}$.

Nick translation of DNA

Probes of lower repetitiveness (C_0t 1–10) were nick translated after isolation of the fraction by 2–3 repeated reassociations. Probes of higher repetitiveness (C_0t 0–1) were usually made by nick translation of total DNA and isolation of the probe by reassociation to the appropriate C_0t.

Nick translation followed the method of Rigby, Dieckmann, Rhodes & Berg (1977) or Maniatis, Jeffrey & Kleid (1975). 5 μg DNA were nick translated using DNA polymerase I (Boehringer Corp. Grade I or Miles Lab. Ltd): the DNA did not need to be nicked before the reaction. The product of the nick translation was isolated on HAP at room temperature and either used (C_0t 1–10) or further processed by reassociation (C_0t 0–1). The final specific activity was $> 10^7$ cpm/μg DNA.

Buffers

1 × SSC is 0·15 M NaCl + 0·015 M trisodium citrate: phosphate buffer (PB) is an equimolar mixture of Na_2HPO_4 and NaH_2PO_4, pH 6·8: 40 % FSSPE buffer is 40 % (v/v) formamide + 1 M NaCl + 1 % SDS + 0·12 M PB + 0·1 mM EDTA.

RESULTS

It was clear at the outset that since no single DNA sequence was known to be widely distributed in the genome, the hybridization probe would be heterogeneous and contain a spectrum of sequences. Since much evidence suggests that some repetitious sequences at least are interspersed with unique sequence DNA in a wide variety of eukaryotes, both simple (Kram, Botchan & Hearst, 1972; Davidson, Hough, Amenson & Britten, 1973; Graham, Neufeld, Davidson & Britten, 1974; Davidson, Hough, Klein & Britten, 1975; Manning, Schmid & Davidson, 1975) and mammalian (Pearson, Wu & Bonner, 1978; Saunders *et al.* 1972; Marx, Allen & Hearst, 1976; Deininger & Schmid, 1976), these sequences seemed most appropriate as the basis of the probe.

Therefore, the properties of a cRNA probe prepared by transcribing isolated C_0t 0–1 human placental DNA with *E. coli* RNA polymerase and ^{3}H-nucleotide triphosphates (see Methods) were investigated. Human placental DNA was used as the origin of the probe rather than cell DNA to avoid any special relationship between the probe and the DNA to be analysed.

The relationship between the amount of human probe hybridized and the amount of human DNA in a cell hybrid was tested in the following way. DNA was isolated from several mouse (A9, HPRT$^-$, fibroblast) × human (Daudi, Burkitt lymphoma derived B-lymphocyte) clones of known karyotype. The karyotype analysis (by banding) was kindly provided by Mr M. Burtenshaw and Dr E. P. Evans. 20 μg

A9-Daudi DNA and human DNA (separately) were denatured, bound (in triplicate) to 13-mm nitrocellulose filters and hybridized in 6 × SSC – 50% formamide at 50 °C for 48 h, with cRNA to C_0t 1 human DNA (9×10^4 cpm). Time-course experiments (not shown) indicated that there was no additional hybridization after 48 h. The amount of human DNA in the hybrid cells was calculated from the karyotype and published measurements (Bosman, van der Ploeg, van Duijn & Schaberg, 1977) of the DNA content of each human chromosome. These values were compared with the DNA content derived from hybridization using human DNA standards. Previous experiments (unpublished) had shown that under the conditions of the hybridization there was little cross-hybridization between cell DNA and Epstein–Barr virus DNA (which the hybrid cells contain), and the hybridization was not related to the viral DNA content. The EBV DNA content of the A9–Daudi clones did not parallel their human DNA content.

Table 1. *Human DNA content of human–mouse hybrid clones measured by hybridization, compared with that derived from karyotype and photometric data*

Cell DNA	Hybridization per 8 μg DNA, cpm	% human equivalent DNA (hybridization)	Most frequent* chromosomes	% human equivalent DNA (cytology)*
Human placental	3774	100	—	—
Mouse A9	74	—	—	—
A9/Daudi				
Cl10	794	19·1	13, 7, 3, 10, 4, 15, 14	17·0
P3	636	14·8	21, 22, 5, 3, 1 and small number of most others	13·7
Cl9	502	11·3	15, 9, 5, 22	8·0
P2	467	10·4	13, 21, 18, 7, 4	12·2
Cl6	280‡	6·3	13, 10, 3	5·6

* 25 cells karyotyped and a complete set of diploid human chromosomes totals 50 copies of each chromosome. Only chromosomes with > 10 copies per 25 cells (i.e. 20 % full complement) recorded, in decreasing order of frequency.

† Calculated from karyotype and photometric data (Bosman *et al.* 1977).

‡ Different experiment.

The results are shown in Table 1. About 4·2% of the added counts hybridized to total human DNA. There are 2 likely reasons for this: (1) the very stringent hybridization conditions; and (2) the heterogeneous nature of the probe, which may contain up to 50% unique and non-hybridizing sequences.

Table 1 shows that there is quite good agreement between the DNA content derived from cRNA hybridization and from karyotyping. This table also shows that at the whole chromosome level, the sequences measured are not chromosome specific. Thus Cl6, whose human DNA content was derived almost entirely from human chromosomes 13, 10 and 3, was as accurately estimated as P_3 whose DNA was derived from chromosomes 21, 22, 5, 3 and 1 and very small numbers of most of the others.

This suggests that satellite DNAs are not the major components being hybridized since they tend to be associated with specific chromosomes (Gosden *et al.* 1975). There are undoubtedly other fast-reassociating sequences with similar properties but different base sequences in the probe.

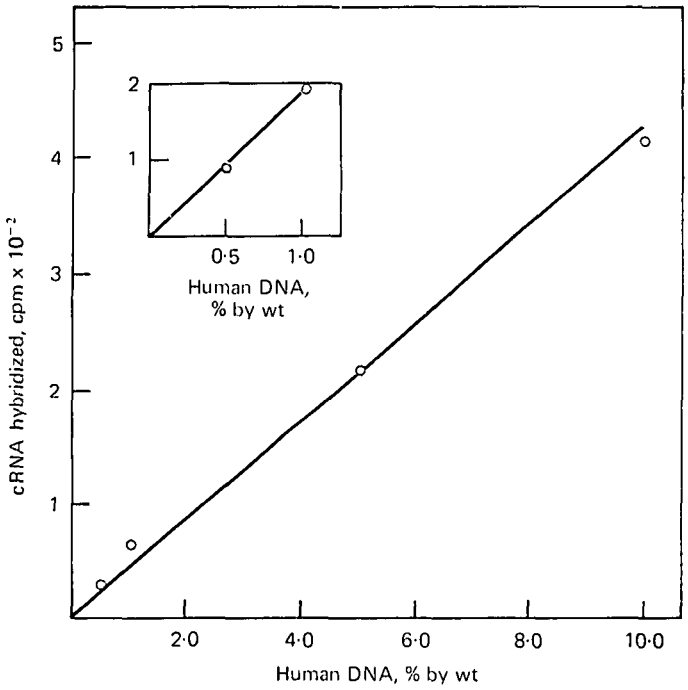

Fig. 1. Filter hybridization of cRNA to C_0t 1 human DNA with human–mouse and human–hamster DNA mixtures. Ordinate: cpm cRNA hybridized/8 μg DNA. Abscissa: % by weight human DNA in human–mouse DNA mixture; 9×10^4 cpm cRNA added. Inset: same but lower % human DNA in human–Chinese-hamster DNA mixture; $2{\cdot}5 \times 10^5$ cpm cRNA added.

The method was extended to lower amounts of human DNA by examining the hybridization of cRNA with mouse or Chinese hamster DNA containing various percentages by weight of human DNA. 20-μg DNA mixtures were bound (in triplicate) to nitrocellulose filters and hybridized under the same conditions as before.

Fig. 1 shows the relationship between the percentage of human DNA (by weight) and hybridized counts in human–mouse or human–Chinese hamster DNA mixtures. A linear relation clearly extends down to about 99·5% mouse DNA+0·5% human DNA mixtures. Chinese-hamster–human DNA mixtures gave similar results (inset). The sensitivity of the method can be increased further by increasing the concentration of probe, although the cross-hybridization with mouse and hamster DNA increases also; 0·2% of human DNA is probably the least that this method will detect.

The filter hybridization method has been applied to several somatic cell hybrids. The cells were grown as described in Methods and DNA isolated, denatured and

bound to nitrocellulose filters. Calibration mixtures of known amounts of human and Chinese-hamster or human and mouse DNA were bound to filters at the same time as the hybrid cell DNA. Filters were hybridized with 9×10^4 cpm cRNA in $6 \times$ SSC $+ 50\%$ formamide at 50 °C for 48 h, and hybridized counts measured. Human DNA content was determined from the calibration. The results are given in Table 2.

Table 2. *Human DNA content of Chinese-hamster × human and mouse × human hybrid cells measured by filter hybridization*

Hybrid cell	Description of hybrid cell	cRNA probe	Equivalents human DNA: Cytology, %	Equivalents human DNA: Hybridization, %
Cl2D	Chinese-hamster lung fibroblast (Wg3-h) × normal human lymphocyte 1 X-chromosome per cell	C_0t 1	2·6	2
R_493	Chinese-hamster (Wg3-h) × irradiated (40 J kg^{-1}) human lymphocyte. Small chromosome fragment ? human. HPRT+G6PD+ SAX+	C_0t 1	Visible fragment	1·9
R_493(TG)	R_493 back-selected in HAT HPRT−	C_0t 1	0	0
R_62	Chinese-hamster (Wg3-h) × irradiated (60 J kg^{-1}) human lymphocyte. No additional visible fragments. HPRT+G6PD−	C_0t 1	—	0·6
B_2	Mouse melanoma (PG–19) × irradiated (200 J kg^{-1}) human diploid fibroblast	C_0t 1	0·02	0·6
D_1	Mouse melanoma (PG–19) × irradiated (200 J kg^{-1}) human diploid fibroblasts	C_0t 1	5	4
Cl2D	As above	C_0t 0·5–10	2·6	2·8
R_62	As above	C_0t 0·5–10	—	0·65

Chinese-hamster × human hybrid cells were originally isolated by Dr Stephen Goss. Mouse melanoma (PG19) × human hybrid cells were isolated by Prof. H. Harris.

These results show that whenever a large amount of human DNA was measured, e.g. Cl2D with 1 human X-chromosome (2·5% of a human genome) or D1 with 5–6% human, there was reasonably good agreement between the hybridization value and cytological expected value. However, cells with a human chromosome fragment either visible ($R_4$93) or not visible ($R_6$2, B_2) gave hybridizable DNA values greater than expected (see Discussion).

A cRNA probe to human DNA of lower repetitiveness (C_0t 0·5–10·0) has also been tested with appropriate calibrations, and human DNA in Cl2D and $R_6$2 investigated. The method used was identical with that used before. Values of 2·8 and 0·65% were obtained (Table 2), in good agreement with the values obtained with the C_0t 1 probe (2 and 0·75%, respectively).

Although the human DNA sequences at the concentration used here are not

saturated by the probe, the linearity of the calibration suggests that saturation is not essential provided known mixtures are hybridized at the same time as the unknowns.

Reassociation kinetics is a more sensitive and informative method of examining DNA sequences, and the potential of this method was therefore tested. The principles of reassociation kinetics are well known (Wetmur & Davidson, 1968; Britten & Kohne, 1968), although there are problems of interpretation when heterogeneous probes are used (Sharp *et al.* 1974), as is the case in the present experiments.

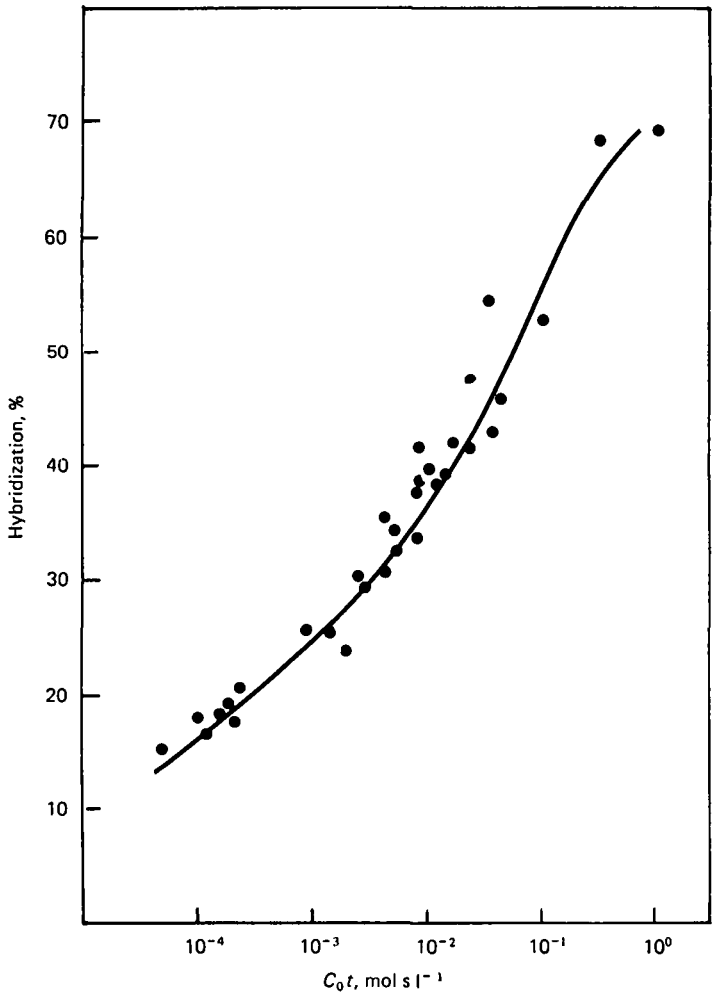

Fig. 2. Reassociation of C_0t 1 DNA probe from uniformly labelled Raji cell DNA (—) and from nick-translated human placental DNA (●) in 40 % FSSPE buffer at 50 °C. Ordinate: % total cpm in d.s. DNA (hybridization). Abscissa: C_0t, mol s l^{-1}.

^{3}H-human DNA probes were prepared by nick translation with DNA polymerase I in the presence of ^{3}H-deoxynucleotide triphosphates as described in Methods.

An essential to using the probe was to establish that all sequences in the nick-translated DNA were equally labelled. This was done by comparing the

nick-translated probe reassociation with that of uniformly labelled C_0t 0–1 DNA. The experiment was conducted as follows. Uniformly labelled C_0t 1 DNA was isolated from Raji cell DNA after the cells had been grown from low density in medium containing 60 μCi [^{3}H]thymidine/ml. Human DNA was added to give an appropriate reassociation rate, in a final volume of 50 μl of 40% FSSPE buffer. Similarly 8000 cpm nick-translated C_0t 1 DNA and unlabelled human DNA were mixed in a final volume

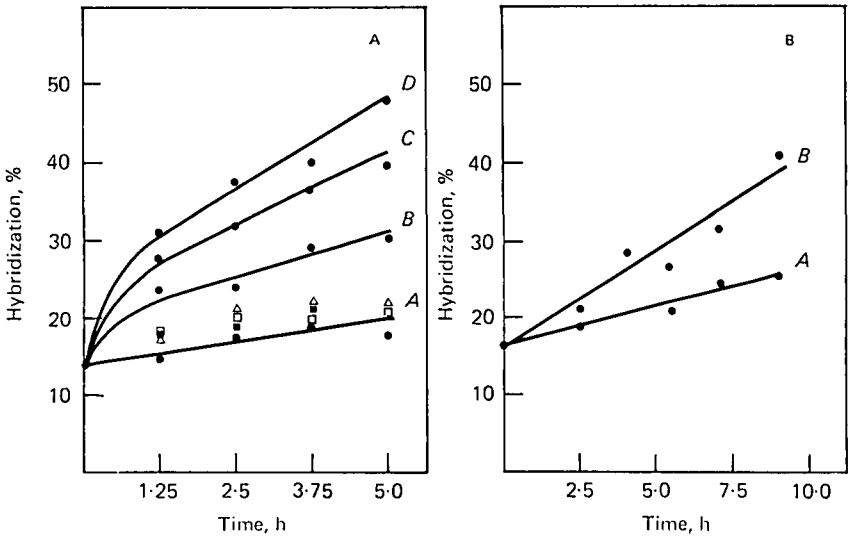

Fig. 3. A. Reassociation of nick-translated C_0t 1 DNA probe with human DNA (●) or mouse DNA (■, △, □) in 40% FSSPE at 50 °C. *A*, probe alone; *B*, *C*, *D*, with added human DNA (0·2, 0·6 and 1·0 μg DNA/ml, respectively). ■, with 1500 μg DNA/ml; △, with 3000 μg DNA/ml; □, with 4500 μg DNA/ml. Ordinate: % cpm in d.s. DNA (hybridization). Abscissa: time, h. B. Reassociation of nick-translated C_0t 10^{-3} – 1 human DNA probe with total human DNA. *A*, probe alone; *B*, probe with 0·3 μg human DNA/ml. Ordinate: % cpm in d.s. DNA (hybridization). Abscissa: time, h.

of 50 μl in 40% FSSPE buffer. Both samples were overlaid with 40 μl paraffin oil and stoppered. The DNA was denatured by heating to 105 °C for 7 min and the reassociation started by immersing the tubes in an accurately controlled waterbath at 50 ± 0·05 °C. Samples (12-μl) were removed at intervals into 1·0 ml 0·12 M phosphate buffer + 0·04% SDS at 4 °C and stored at 4 °C, until all the samples had been collected. Samples were fractionated at 60 °C in a 0·9-ml column of hydroxylapatite (HAP) equilibrated with 0·12 M phosphate buffer. Single-stranded DNA was washed through the column with 8·0 ml 0·12 M PB at 60 °C and double-stranded DNA eluted with 8·0 ml of 0·4 M PB. The reassociation curve is shown in Fig. 2, from which it is clear that the line (Raji C_0t 1 DNA reassociation) is well represented by the points (nick translation C_0t 1 DNA), and therefore the nick-translated probe can be considered uniformly labelled. The reassociation became much slower after about 60% hybridization, as expected of a probe which contains about 30–40% single-stranded material (Marx *et al.* 1976).

The reassociation rate in 50% FSSPE buffer at 50 °C was similar to that in 0·12 M phosphate buffer at 60 °C. $C_0t_{0·5}$ for C_0t 1 DNA reassociation in formamide buffer was (Fig. 2) $2–3 \times 10^{-2}$ mol s l^{-1}, compared with the value of 2×10^{-2} in 0·12 M phosphate buffer obtained by Marx *et al.* (1976). The accelerating effect of high monovalent ion concentration (Britten, Graham & Neufeld, 1974), 1·18 M in this buffer, is counterbalanced by the formamide concentration and lower reassociation temperature (McConaughy, Laird & McCarthy, 1969).

Fig. 3A shows the reassociation of nick-translated, C_0t 1 DNA in the presence of various concentrations of human total DNA (0·2, 0·6, 1·0 μg/ml final conc.), and also 1500, 3000, and 4500 μg/ml PG19 mouse DNA (see legend to figure). The reassociation with human DNA is clearly biphasic, with a rapid early phase followed by a slower phase: self-reassociation of the probe alone followed a linear path. Fig. 3A shows that the rate of reassociation of the nick-translated probe varied with the added human DNA concentration. The sequences reassociated in the fast phase (at 1 μg/ml) correspond with a C_0t of about 2×10^{-2}, in the range of 'intermediate' repetitive sequences: the apparently linear portion of the curve represents reassociation of less-repetitive DNAs. If the slope of the linear part of the reaction was plotted against DNA concentration then a reasonably straight line resulted. In contrast, mouse DNA, even at a 15–22000 times higher concentration did not show a progressive interaction with the probe DNA. There may be an early phase of increased reassociation, but after 3·75–5 h the rate was not significantly different from the probe alone. It was concluded therefore that up to 4500 μg mouse DNA/ml did not interact significantly with human probe DNA in the hybridization buffer.

If the faster reassociating sequences were removed from either the unlabelled human DNA sample or the probe, then the initial phase of the hybridization did not take place. This is shown in Fig. 3B where sequences reassociating at $C_0t < 10^{-3}$ were removed from the probe, by an appropriate reassociation and HAP separation. The probe was then reassociated with 0·3 μg/ml final concentration of total human DNA in 40% FSSPE buffer at 50 °C, and the hybridization plotted as a function of time. The slope of this reassociation was in good agreement with the expected slope derived from the linear phase of total DNA reassociation with total probe (Fig. 3A). Probe self-reassociation followed the usual linear time course.

Experiments have also been made with a probe to C_0t 1–10 human DNA, i.e. of rather low repetitiveness, but although these probes can be used the long reassociation times to achieve a reasonable hybridization make them inconvenient to use. They are also contaminated with presumptive unique sequence DNAs in spite of multiple purification steps to remove them. Another disadvantage is that their relatively low degree of repetition in the genome could mean a loss of sensitivity in the detection of small fragments of DNA.

The reassociation method has been applied to 2 clones, R_62, found to contain 0·6–0·7% equivalent of a human genome by the filter method (Table 2) and Cl19S16-TG, a PG19 × human lymphocyte hybrid (see Discussion). Fig. 4A shows the time-course of reassociation of 1 mg/ml R_62 DNA with nick-translated C_0t 0–1 probe. There was a fast reassociation complete in about 1·5–2 h, followed by a rate not

significantly different from the probe alone rate. It is clear by comparison with Fig. 3A that the slower reassociating sequences are in much lower concentration than expected from the extent of the fast phase. Based on the extent of reassociation of the fast component, DNA in this clone is approximately 0·2% equivalents of a human genome, lower than the value found by filter hybridization.

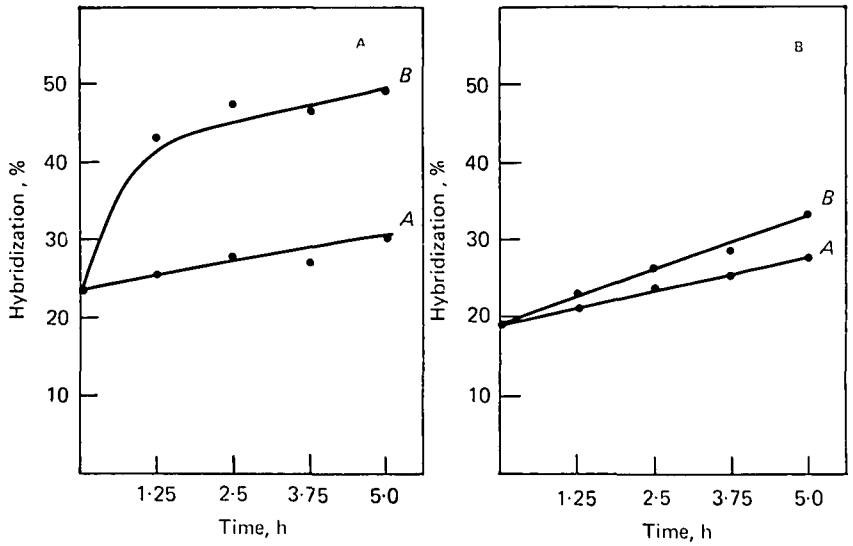

Fig. 4. A. Reassociation of nick-translated C_0t 1 human DNA probe with $R_0$2 DNA. *A*, probe alone; *B*, probe + 1000 μg/ml $R_0$2 DNA. Ordinate: % cpm in d.s. DNA (hybridization). Abscissa: time, h. B. Reassociation of nick-translated C_0t 1 human DNA probe with Cl19S16TG DNA. *A*, probe alone; *B*, probe + 3000 μg Cl19S16TG DNA/ml. Ordinate: % cpm in d.s. DNA (hybridization). Abscissa: time, h.

In contrast, Cl19S16TG does not show a fast-reassociating phase at all (Fig. 4B) and clearly this hybrid contains no very repetitive human sequences. However, the progressive reassociation rate shows that less-repetitive sequences are present, and comparison with Fig. 3A suggests a value of 0·005–0·01% equivalents of human DNA in this hybrid.

DISCUSSION

These experiments show that molecular hybridization methods can be used under suitable conditions to measure semi-quantitatively the amount of human DNA in human somatic cell hybrids. The reassociation method is more informative about the types of human sequences in the hybrid and is more sensitive but the filter method is simpler to use. The lower limit of the method is around 0·005–0·01% equivalents of a human genome ('equivalents' has the same meaning as 'genome equivalents' in tumour virus assays). The good agreement (Table 1) between the DNA content of A9 × Daudi hybrids determined from the karyotype and by molecular hybridization on filters confirms that the hybridization is not chromosome-specific. Although

human satellite DNAs are most probably involved in the hybridization (with C_0t 0–1 probes), they are only a fraction of the sequences in the probe (Marx *et al.* 1976), and reassociation and filter methods can be used when these sequences are absent from the system. The reassociation method may give lower values for the DNA content than the filter method (see later), although the evidence is not yet conclusive.

A surprising feature of experiments with cell hybrids made from one irradiated human parent, is the relatively large amount of human DNA retained along with the selected gene, HPRT; e.g. in $R_4$93, $R_6$2, B_2 (Table 2). HPRT (a trimer of mol. wt. 75–85000 (Fenwick *et al.* 1977)) is probably encompassed by less than 5×10^3 b.p., about 3×10^{-3}% of the X-chromosome. Hybrid cell $R_6$2 which had only HPRT activity (PGK$^-$, αGAL$^-$ or G6PD$^-$ (S. Goss, personal communication)) however, contained DNA equivalent to 0·6% equivalent of a human genome, about 25% of that in the X-chromosome (Table 2). The value from reassociation kinetics was 0·2%, about 8% of that in the X. This DNA reassociated in the fast-intermediate class ($C_0t = 2 \times 10^{-2}$). No slower sequences were detected (cf. Fig. 4A and Fig. 3A, curve *C*). $R_4$93, a similar hybrid but containing a larger human fragment and 1·9% equivalents of a human genome (around 75% of an X) had HPRT and G6PD activities (S. Goss, personal communcation). These results suggest that HPRT is either normally linked to a very large piece of repetitive DNA, or that recombination can occur between a small HPRT-containing fragment and a large repetitive DNA segment. In either case, the association is close since back selection of $R_4$93 with 6-thioguanine caused loss of the HPRT and repetitive sequences together. The human repetitive sequences are clearly not spread around the Chinese-hamster genome. More needs to be known about DNA sequence distribution along the human X-chromosome for this to be resolved.

Cl19S16TG presents a very different picture. This clone, PG19 mouse melanoma × human lymphocyte (Jonasson & Harris, 1977), contained no DNA detectable by filter hybridization. It also contained no human chromosomal material identifiable by cytology, but suppression of malignancy (Harris *et al.* 1969; Wiener, Klein & Harris, 1974; Jonasson *et al.* 1977) was very strong (Jonasson & Harris, 1977), coupled with changes in cell-surface glycoproteins (Bramwell & Harris, 1978). Re-check of the malignancy suppression of this clone showed that it had not changed since Jonasson & Harris (1977). Injection of 5×10^5 cells into nude mice produced 0/10 tumours in 3 months.

The nature of the association between the human fragments and the mouse or Chinese-hamster parental genome in any of the clones examined is unknown (except $R_4$93 which had a small chromosomal fragment of possibly human origin). Among the possibilities are: (*a*) free existence of the fragment, synchronized to the parental cell cycle ($R_4$93 ?); (*b*) translocation of the fragment to a terminal position on a parental chromosome; (*c*) non-integrative association; and (*d*) integrative association.

The relation between EBV and human DNA in lymphoblastoid cell lines typifies (*c*) and (*d*) (Adams, Lindahl & Klein, 1973; Kaschka-Dierich *et al.* 1976; Adams & Lindahl, 1975; Lindahl *et al.* 1976).

Whatever the association, it is interesting that in Cl19S16TG, cells with

malignancy highly suppressed can contain only a small amount (around 3×10^5 b.p.) human DNA.

Probes to human DNA could have other uses. One use currently under investigation is to identify human sequences in recombinant plasmids by hybridization (Grunstein & Hogness, 1975). Cell hybrids such as those used in the present experiments may provide convenient starting material for the isolation of some human DNA sequences.

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