Chromosome mediated gene transfer of six DNA markers linked to the cystic fibrosis locus on human chromosome seven

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ABSTRACT

The DNA probes met and pJ3.11 are derived from loci on chromosome seven that are closely linked to, and probably flanking, the gene mutation causing cystic fibrosis (CF). We have shown that mitotic chromosomes from the cell line MNNG-HOS, which contains an activated met oncogene, can induce morphological transformation of mouse NIH-3T3 cells. Southern analysis of isolated transfectant cell lines with cloned dispersed repetitive human DNA sequences as probes demonstrated that several lines of transformed NIH 3T3 cells had stably incorporated large segments of chromosome seven DNA. Southern blot analysis also demonstrated the presence of met, pJ3.11 and several other single copy sequences that had been previously localised to chromosome 7 within the transgenomes. In this way a further four genetic markers were shown to be physically linked to met, and thus to CF. These probes may prove useful in confirming the order of loci around CF and in the prenatal diagnosis of this common autosomal recessive disease.

INTRODUCTION

Chromosomal localisation of DNA probes linked to the locus of the mutation causing cystic fibrosis (CF) has mapped this gene defect to 7q22-7q31.1 (1-5). The DNA markers pJ3.11 and met are linked to, and flanking CF at recombination fractions of approximately 0.01(6). Recombination fractions as small as this approach the limits possible for genetic linkage analysis of a recessive disorder. In order to study this region of the genome at the subcentimorgan level, and as part of a strategy to isolate the normal counterpart of the cystic fibrosis gene (CF^N), we have effected the chromosome mediated gene transfer (CMGT) of the met-pJ3.11 linkage group. DNA from the chemically transformed human osteosarcoma cell line MNNG-HOS has the ability to transform NIH 3T3 cells. The oncogene met responsible for this transformation was cloned and shown to be

unrelated to previously described transforming genes (7). In this paper we report that mitotic chromosomes derived from the MNNG-HOS cell line can confer the transformed phenotype on recipient NIH 3T3 cells. The close linkage of met and CF is a fortunate coincidence which provides a dominant selectable marker close to the locus of the most common autosomal recessive disease in the greater part of Europe and America (8).

Several groups have been able to demonstrate transfer of syntenic markers by CMGT using a variety of selection protocols, often relying upon metabolic selection with nucleotide analogues (9-12). Chromosome mediated gene transfer of regions of human chromosome 11p with H-ras-1 as the selectable marker has recently been described (13). Potential problems with this approach include possible rearrangement of transferred sequences, transfer of non-syntenic DNA and creation of transgenomes too small to contain sequences of interest neighbouring the selectable marker. We have attempted to assess the degree to which these phenomena have affected our transfectants using cloned repeat sequences, and single copy sequences regionally localised to human chromosome seven.

The size of the transgenomes may be estimated by quantitative hybridisation of labelled human DNA to dot-blots of transfectant DNA. Transgenome length can also be analysed by examination of the complexity of the pattern observed when transfectant DNA digested with restriction endonucleases is hybridised with members of the L1 (formerly Kpn1) repeat family. Studies indicate that there are approximately 10^4 - 10^5 members of this family dispersed throughout the haploid human genome, depending upon the particular probe used (14, 15). Genomic library screens with the L1 consensus sequences as probes indicate that these sequences are interspersed throughout the human genome (14, 15).

Differences in the sequences flanking L1 family members and divergence within the family produces a ladder of fragments (restriction fragment length variants (RFLVs)) when digested DNA is probed with the labelled consensus sequence. These RFLVs can serve as markers for a particular region of a

chromosome. In our studies several RFLVs that are present in a hybrid cell line whose only human component is chromosome seven can be demonstrated in the transfectants and used to assess both the extent and fidelity of transfer of chromosome seven DNA. This type of analysis has been used to study the transfer of regions of human chromosome eleven in CMGT experiments with H-ras-1 as the selectable marker (13).

DNA probes for several markers previously mapped to chromosome seven or isolated from a chromosome seven-specific library were analysed for their presence in the transfectants. Of particular interest were those cell lines containing met and pJ3.11, both closely linked to CF. The other markers were examined with the aim of localising further probes to the vicinity of CF. The presence or absence of markers in the hybrids allowed us to infer the physical order of the loci, and in conjunction with the available genetic data determine which transgenomes are likely to contain CF^N.

MATERIALS AND METHODS

Cells and Culture Conditions

MNNG-HOS is a derivative of a human osteosarcoma cell line HOS that has been transformed in vitro with N-methyl-N-nitro-N-nitroso guanidine (16). The cells were grown in Dulbeccos Modified Eagles Medium (DMEM) supplemented with 10% foetal calf serum (FCS) and grown under 10%CO₂ at 37°C.

Clone 21 (C121) is a mouse X human hybrid whose only human complement is chromosome seven (17). It was grown in RPMI 1640 supplemented with 10% FCS, glutamine and pencillin/gentamicin under 5% CO₂ at 37°C.

NIH 3T3 cells and the transfectants were grown in DMEM supplemented by 10% calf serum (CS), under 10%CO₂ at 37°C.

Chromosome Mediated Gene Transfer

The protocol used was essentially that of Weis and colleagues (18). MNNG-HOS cells were blocked in metaphase using colcemid at 0.1 ug/ml for 16-20 hours. The cells were then detached by shaking and washed in cold phosphate buffered saline. After collection by centrifugation the cells were resuspended in lysis buffer (10 mM HEPES, pH 7.1, 3mM CaCl₂)

and kept on ice for 25 minutes. Cells were lysed by passage through a gauge 21 needle (ten strokes of a syringe plunger). Chromosomes were collected by differential centrifugation and the purity of the chromosome preparation checked at each stage by Giesma staining and light microscopy. The final chromosome pellet was resuspended in cold transfection buffer (25 mM HEPES, pH 7.1, 134 mM NaCl, 5 mM KCl, 5 mM glucose, 0.7 mM Na_2HPO_4). The suspension was then brought to 125 mM CaCl_2 at room temperature and a precipitate allowed to form over 20-30 minutes. Chromosomes were then plated onto recipient cells at 20-50 chromosomes per cell, in 1ml aliquots per dish. Each transfection dish (100 mm) contained approximately 3×10^5 cells and DMEM supplemented with 10% CS (10ml). Four hours post-transfection the cells were given a four minute glycerol stock treatment (25 mM HEPES, pH 7.1, 134 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 15% glycerol (w/v)). The cells were then fed with DMEM supplemented with 10% CS and containing 2.5×10^{-7} M dexamethasone. Subsequently the medium was changed every three days and the cultures examined for the appearance of foci after 10-21 days. Positive control transfections using high molecular weight DNA from MNNG-HOS and negative controls using NIH 3T3 DNA were set up at the same time. The method for DMGT has been described previously (16). DNA was precipitated at 20ug/ml and applied to the cells in the same manner as given for the chromosomes.

Foci of transformed cells were subcloned into individual flasks and grown to mass culture. Aliquots of cells were injected subcutaneously into nude mice (5×10^5 cells per site) and solid tumour isolated after two to four weeks.

DNA Analysis

Cells were harvested with trypsin EDTA, collected by centrifugation and suspended in 10 mM Tris, 10 mM EDTA; SDS was added to 1%. The lysate was emulsified with phenol and the aqueous phase extracted with phenol:chloroform (1:1 v/v) and then with chloroform:isoamylalcohol (24:1 v/v). DNA then precipitated with sodium acetate and ethanol, spooled out and washed in 70% ethanol. The DNA was allowed to air dry and was then resuspended in 10 mM Tris, 1 mM EDTA. DNA concentrations

were measured using a Beckman DU-6 spectrophotometer operating between 320 and 220 nm at 15 nm/min.

Restriction endonucleases were purchased from Anglian Biotechnology Ltd., and used, in three fold excess, according to the manufacturers' recommendations. Agarose gel electrophoresis, Southern blotting (19) prehybridisation, hybridisation, posthybridisation wash and autoradiography conditions have all been described previously (20). Probes were labelled by the method of Feinberg and Vogelstein (21) after isolation of the relevant insert from low gelling temperature agarose gels.

For the quantitative dot-blot hybridisations 5 ug of hybrid DNA was loaded in duplicate onto a Schleicher and Schuell manifold and transferred to a Hybond^N membrane (Amersham International). 0.5-20 ng of human genomic DNA was added in duplicate to give hybridisation signals equivalent to that expected from transgenomes with a size range 0.12-24 Mb. Filters were probed with labelled total human DNA and signals quantified using a densitometer (Joyce-Loebl Chromoscan 3) and scintillation counting. Filters were washed to a stringency of 0.1 X SSC (1 X SSC is 0.15M sodium chloride, 0.015M sodium citrate, pH7.4). Filters were then stripped of signal by boiling in 0.1 X SSC for 30 minutes and hybridised with labelled mouse DNA. The quantitation of this signal gave a correction factor for the amount of DNA available for hybridisation at each dot.

Derivation of Probes

Met D, Met H (7) pJ3.11, pB79a, pJu48, pJu28, and pB78 (30) and 7C22 (5) have been described previously. PDGFR α was from J. Scott, CRC Harrow; TCR β was from M. Owen, University College, London; NJ3, a genomic clone of the COL1A2 gene was from F. Ramirez, Rutgers Medical School, New Jersey. Eight further probes were derived from a flow sorted chromosome seven library as described previously (5).

pBK11, pBK26 and pBK54 were obtained from J. Maio, Albert Einstein College of Medicine, New York. The α RI clone was cloned in this laboratory by Dr. S. Darling. Dr. S. Brown, St. Mary's Hospital, London, provided the mouse autosomal clone.

RESULTS

Chromosome Mediated Gene Transfer

Metaphase chromosomes from colcemid-treated MNNG-HOS cells were precipitated by calcium chloride and added to cultures of NIH 3T3 cells as described previously (18). The DNA mediated gene transfer (DMGT) of MNNG-HOS and NIH 3T3 cell DNA served as a positive and negative control respectively. Foci of transformed cells appeared on the positive control plates after 10-14 days culture. On average foci appeared 2-7 days later on the CMGT plates. No foci were seen on the negative controls. On one plate a large number of foci appeared and it was felt that the majority, if not all, had seeded from one parent: four foci were picked for analysis from this plate (E111, F111, G111 and H111). Six other independent foci were picked from different plates. The frequency of transformation was approximately 1 in 5×10^5 recipient cells for CMGT and 1 in 2×10^5 for DMGT.

Considerable differences in the phenotype of the transformed cell lines were observed. Line C11 was relatively slow growing, grew in an even monolayer and showed a high degree of anchorage dependence. V1a tended to grow as aggregations of cells and showed a high degree of anchorage independence. The other cell lines had characteristics that were intermediate between these two extremes.

L1 (Kpn1) RFLV Analysis

CMGT may induce rearrangements in the transferred sequences. Although there is no method of establishing the exact degree of rearrangement an estimate may be obtained using the L1 repeat probe. Figure 1 shows the hybridisation patterns obtained with the L1 probes when transfectant cell line DNA is digested with TaqI and PstI. A mouse-human cell hybrid, C121, whose only human component is chromosome seven contains a large number of fragments hybridising to L1. The high background due to superimposed fragments makes it very difficult to discern discrete RFLVs in the Pst I digest of C121, but from the Taq I digest (T, lane 7, Fig 1) it is apparent that most of the fragments present in line E111 are also present in C121. Some of the RFLVs present in E111 were not detected in C121. It is

Fig.1(A): RFLV pattern observed using the L1 probes in Taq I (T) and Pst I (P) digests of transfected DNA. M = mouse; 7 = clone 21; E = E111; D = D11; C = C11; B = B11; λ = Hind III digested lambda marker. X marks the position of a fragment present in C121, E111, C11 and B11, but not in D11. Y indicates a fragment present in line D11 only. Fig.1(B): Pst I RFLV pattern of lines VIa, Va, E111, F111 and D11.

possible that these result from rearrangements and/or the integration of DNA non-syntenic to met. It is also possible that novel fragments represent polymorphic variants between the two chromosomes seven, one from MNNG-HOS and the other from C121. Unfortunately, due to the background from the other chromosomes present in MNNG-HOS it is not possible to compare the RFLVs from the respective chromosomes seven. Although RFLV analysis can give an indication of the degree of rearrangement, it cannot determine the source.

TABLE 1
TRANSGENOME SIZE AND PRESENCE OF KNOWN CHROMOSOME
SEVEN MARKERS IN THE TRANSFECTANTS

		CELL LINE								
		E111	Va	C11	B11	D11	A1	V1a	Dd	Df
Transgenome size										
(Dot-blot) Mb.		13	10	5	6	5	6	3	3	4
Transgenome size										
(L1 RFLV's) Mb.		9.3	7.5	4.2	6	5.1	4.8	3	2	3
PROBE	CHROMOSOMAL LOCALISATION									
met	7q22-31.3	+	+	+	+	+	+	+	+	+
pJ3.11	7q22-7cen	+	+	+	+	-	-	-	-	-
pB79a	7q22-7cen	+	+	+	-	-	-	-	-	+
pJu48	7q22-7cen	+	+	-	-	-	-	-	-	-
pJu28	7q22-7cen	+	+	-	-	-	-	-	-	-
pB78	7q22-7pter	+	+	-	-	-	-	-	-	-
7C22	7q31.1-31.2	-	-	-	-	-	-	-	-	-
NJ3	7q21.3-22	-	-	-	-	-	-	-	-	-
TCRB	7q33-7q35	-	-	-	-	-	-	-	-	-
PDGFR	7q22-7pter	-	-	-	-	-	-	-	-	-
ARI	7cen	-	-	-	-	-	-	-	-	-

The correlation between transgenome size and presence (+) or absence (-) of chromosome seven markers is shown above (see Figure 3). Dd and Df are transfectants derived in DMGT experiments, while the other transfectants were produced by CMGT. Only those probes with an established sub-chromosomal localisation are shown. Eight other probes described in Materials and Methods were tested, but none were present within the transfectants.

Line D11 contains fragments present in other hybrids and C121, but also shows RFLVs shared with neither (an example is marked Y in Figure 1). Almost every band detected in line V1a is unique. Line Va also has several unique bands and as this hybrid contains several chromosome seven markers this may be indicative of rearrangement rather than uptake of non-syntenic DNA. Va and E111 contain the same six chromosome seven markers, but the RFLV patterns of these lines show almost no bands in common. Since the RFLV pattern in E111 is similar to a subset of C121 it is likely that Va is the cell line with a rearranged transgenome, which may be relevant to the more extreme transformed phenotype of Va. Line C11 shows a simple pattern with all RFLV's present in at least one other hybrid or C121. Since it is unlikely that uptake of non-syntenic DNA or

sequence rearrangement would produce identical RFLV's in independent foci we regard Cll as the most likely cell line to contain a transgenome faithfully transferred from MNNG-HOS.

E111, F111, G111 and H111 have identical RFLV's showing that these foci, which are all derived from the same transfection dish, represent the same transfer and integration event (E111 and F111 are shown in Figure 1B). The fact that the RFLV pattern in these lines showed no variation after multiple passages and growth in nude mice (data not shown) argues for the stability of the transgenome. The cell line consistently contained the six chromosome seven markers shown in table 1. Under the conditions used for our experiments no hybridisation of the L1 probe to mouse DNA was detected.

Size of the Transgenome

An estimate of the size of each transgenome was made using quantitative hybridisation of dot-blotted transfectant DNA. Labelled total human DNA was used as probe and the results are presented in table 1.

The human DNA content of each hybrid was also estimated from the complexity of the Pst I RFLV pattern produced on hybridisation of the L1 repeat probes pBK11 and pBK54. These probes were chosen as they do not contain internal Pst I sites and are less likely to give a significant overestimate of transgenome size. Sequence analysis of three cDNA and three genomic DNA clones complementary to the human L1 repeat did not reveal any internal Pst I sites (22). In order to maximise the chance that all L1 related fragments were counted we analysed autoradiographs obtained after short (16 hour) and long (56 hour) exposure times, and used electrophoresis runs of up to 50 hours at 45V. It was assumed that the distribution of the repeats was uniform over large distances with one sequence per 3×10^5 bp for the particular probes used (14). Results are shown in Table 1.

Hybridisation of metH to 5ug of Taq I digested transfectant DNA and 2.5ug human DNA is shown in Figure 2a. Results are consistent with one copy of the met sequence being present in these transgenomes. Hybridisation of pJ3.11 to dot blots of transfectant DNA (figure 2b) are also consistent with one sequence per transgenome.

Fig.2 Estimation of Transgenome Copy Number.

a) 5 μ g. of NIH 3T3 (M) and transfectant DNA and 2.5 μ g. of human DNA (Hu) were digested with Taq I and hybridised to oligolabelled metH. Lane 1 CII; lane 2 DII; lane 3 EII, lane 4 human DNA.

b) Left hand panel: 5 μ g dot blotted transfectant DNA and 2.5 μ g human DNA (H) probed with pJ3.11

Right hand panel: same filter stripped of signal and probed with a single copy, autosomal sequence from mouse to provide a control for the amount of DNA loaded.

Presence of Chromosome Seven Markers Within the Transgenomes

DNA from each cell line was digested with Pst I, blotted and hybridised with labelled single copy probes mapping to different regions of chromosome seven. The presence of pJ3.11 in several transfectants is shown in figure 3. A complete presentation of the results is given in table 1. All transfectants contain met, as expected. Four of seven contain the locus for pJ3.11 and three of these contain pB79a. Of these three transfectants two contain the loci of the DNA

Fig3. Presence of pJ3.11 in the Transgenomes.

Human (Hu), NIH 3T3 (M) and transfectant DNAs (B-D) were digested with Pst I, transferred to nylon filters and hybridised with labelled pJ3.11. This sequence is clearly present in lanes B (B11), C (C11), E (E11) and Va. Differences in signal intensity reflect differences in the quantity of DNA loaded in each lane.

probes pJu48, pJu28 and pB78. Thus, the two largest transgenomes contain the most chromosome seven markers.

DISCUSSION

Chromosomes from the MNNG-HOS cell line can transform NIH 3T3 cells following CMGT. The transfectants obtained using this technique contain transgenomes of between 3 and 13 Mb. Estimates of transgenome size using the L1 probe tended to be smaller than those obtained by quantitative hybridisation, although the relative sizes for different cell clones are similar and compatible with the number of chromosome seven markers seen in each hybrid. The differences in size estimation will be due to errors in our assumptions concerning the frequency and distribution of the L1 repeat, and to the technical problems of quantitative hybridisation. The human DNA content in our hybrids is within the range of that observed by other workers (13, 23).

Multiple copies of donor sequences have been observed in some studies (13, 24) but generally it seems that donor sequences are present, or at least expressed, at the single copy level (25-27). In our studies dosage analysis with single copy probes is consistent with the existence of one transgenome copy per transfectant.

Examination of the RFLV patterns has revealed differing degrees of rearrangement in the transferred sequences. We

expect that all transgenomes will suffer from rearrangement to some extent, but it is evident from the L1 RFLV analysis that lines C11, E111 and B11 have each integrated a large segment of intact chromosome seven DNA.

In order to assess the frequency of co-transfer of asyntenic markers Klobutcher and Ruddie screened hybrids selected for the transfer of the human TK gene (chromosome seventeen) for the presence of 20 isozyme markers whose genes were known not to be on chromosome seventeen (28). They found that 10% of lines expressed markers non-syntenic to TK. Since these 20 markers can only cover a small proportion of the genome we expect that the transfer of sequences non-syntenic to the selectable marker is considerably more common than this. Indeed, with the exception of C11, all transfectants contained L1 RFLV's unique to themselves.

The pair of linked loci pJ3.11 and met are present in four of seven transfectants. We have also demonstrated the presence of probes pB79a, pJu28, pJu48 and pB78 in some transgenomes. The frequency of CMGT of each marker, taken in conjunction with the physical localisations discussed below, suggests a tentative physical ordering of the probes as follows:

qter--pB79a--met--pJ3.11--pJu48/pJu28/pB78--cen

met is known to be activated by a segment of DNA from chromosome one inserted at the 5' end of the gene (29). If this is mediated by a simple chromosome one:seven translocation it is possible that CF^N may not be present in the transferred sequences. ie. met could be located on one of the translocations and CF^N on the reciprocal. However, as stated above, the most recent linkage data suggests that met and pJ3.11 flank CF and potentially four transfectants could contain CF^N.

The DNA sequence pB79a is found in one cell line created by DMGT (Df). This would imply close physical linkage (ie within 250kb) of pB79a and met, as it is very unlikely that pB79a and met were transferred independently. At first sight this may seem inconsistent with the observation that pB79a was not present in all CMGT derived transgenomes. However, a similar situation has been reported where cotransfer of GALK

and TK was observed in some DMGTs. Although cotransfer was more frequent following CMGT this did not occur in all cases (11). Since pB79a is not present in line B11 this suggests that pB79a and pJ3.11 flank met, although the orientation of this linkage group on the chromosome is uncertain. If this result is confirmed then the met activation event could have resulted from an interstitial insertion, rather than a simple translocation. pB79a shows close genetic linkage to CF, met and pJ3.11 (Wainwright and Estivill, personal communication).

Probes pJu28, pJu48 and pB78 have been shown to be physically linked to met, and presumably to CF. The former two probes have been previously mapped to 7q22-7cen and the latter probe to 7q22-7pter (30). Taking these localisations into account we have placed these loci centromeric to CF which maps to 7q22-7q32 (31). In support of this hypothesis it has been shown that whereas met, pJ3.11 and pB79a map within a deletion 7q22.2-7q31.3, pB78 does not (Wainwright, B.J., Scambler, P.J., and Williamson, K., manuscript submitted). When polymorphisms at these loci have been found a comparison of the physical and genetic maps can be made. No rearrangements of any single copy probe loci were detected on Southern analysis.

It is interesting that 7C22, known to be at 7q31.1-7q31.2 (Buckle, V.J., Scambler, P.J., and Wainwright, B.J., manuscript in press) is not present in these hybrids. 7C22 is linked to met at a recombination fraction of 0.045 (5) which could represent a physical length of over 5 Mb and hence the transgenome may be too small to include the 7C22 locus. Using L1 RFLV analysis the largest transfectant, E111, contains 9.3 Mb of human DNA, a significant proportion of which must extend centromerically from met and a minor proportion of which may not be from chromosome seven. Alternatively 7C22 may be further from met than the initial genetic studies suggest. The absence of COL1A2 and TCRB, probes loosely linked to met, is not surprising in view of the estimated transgenome sizes.

Porteus and colleagues reported an association of aberrant L1 RFLV patterns and extreme transformed phenotype (13). They suggested that co-transferred sequences had a modifying effect on H-ras-1 expression. Data from the transfectants we have

analysed are consistent with these findings with line V1a having an aberrant L1 pattern and most highly transformed phenotype. The met sequences showed no evidence of rearrangement in any cell line, in contradistinction to the novel H-ras-1 fragments seen in the experiments discussed above. However, the question of whether these rearrangements are the cause or effect of the transformation phenotype remains open.

The main objective of this work was to create a transfectant which contained the DNA markers known to be closely linked to and flanking CF. Such a transgenome should also be free of rearrangement and the co-integration of DNA non-syntenic to CF. Cl1 best fulfills these criteria; Ell1 contains a larger transgenome and, as the exact location of CF is not known, provides a margin for error. Accordingly cosmid libraries are to be constructed from both these lines. This should allow the cloning of probes even more closely linked to CF than those available at present. Once markers showing no recombinants with CF have been isolated overlapping cosmids from this area of the transgenome will be used to identify known or unknown coding regions that may be involved in the pathogenesis of cystic fibrosis.

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REFERENCES

1. Scambler, P.J., Wainwright, B.J., Farrall, M., Bell, J., Stanier, P., Lench, N., Bell, G., Kruyer, H., Ramirez, F. and Williamson, R. (1985) *Lancet*, **ii**, 1241-1242.
2. Tsui, L.-C., Buchwald, M., Barker, D., Bramen, J.C., Knowlton, K., Schumm, J.W., Eiberg, H., Mohr, J., Kennedy, D., Plavisc, N., Zsiga, M., Markiewicz, D., Akots, G., Brown, V., Helms, C., Gravius, T., Parker, C., Rediker, K. and Donis-Keller, H. (1985) *Science*, **230**, 1054-1057.
3. Wainwright, B.J., Scambler, P.J., Schmidtke, J., Watson, E.S., Law, H.-Y., Farrall, M., Cooke, H.J., Eiberg, H. and Williamson, R. (1985) *Nature*, **318**, 384-385.
4. White, R., Woodward, S., Leppert, M., O'Connell, P., Hoff, M., Herbst, J., Lalouel, J.-M., Dean, M. and Vande Woude, G. (1985) *Nature*, **318**, 382-384.
5. Scambler, P.J., Wainwright, B.J., Watson, E., Bates, G., Bell, G., Williamson, R. and Farrall, M. (1986) *Nucleic Acids Res.*, **14**, 1951-1956.
6. Beaudet, A., Bowcock, A., Buchwald, M., Cavalli-Sforza, L., Farrall, M., King, M.-C., Klinger, K., Lalouel, J.-M., Lathrop, G., Naylor, S., Ott, J., Tsui, L.-C., Wainwright, B., Watkins, P., White, R. and Williamson, R. *Am. J. Hum. Genet.* in press (scheduled for December)
7. Cooper, C.S., Park, M., Blair, D.G., Tainsky, M.A., Huebner, K., Croce, C.M. and Vande Woude, G.F. (1985) *Nature*, **311**, 29-33.
8. Raeburn, J.A. (1983) In Hodson, M.E., Norman, A.P. and Batten, J.C. (eds.), *Cystic Fibrosis*. Bailliere Tindall, London, pp. 1-12.
9. Willecke, K., Lange, R., Kruger, A. and Reber, T. (1976) *Proc. Acad. Sci. USA.*, **73**, 1274-1278.
10. Weis, J.H., Seidman, J.G., Housman, D.E. and Nelson, D.L. (1986) *Mol. Cell. Biol.*, **6**, 441-451.
11. de Jonge, A.J.R., de Smit, S., Kroos, M.A. and Renner, A.J.J. (1985) *Hum. Genetics*, **69**, 32-38.
12. Pritchard, C. and Goodfellow, P.N. (1986) *EMBO Jnl.*, **5**, 979-985.
13. Porteus, D.J., Morten, J.E.M., Cranston, G., Fletcher, J.M., Mitchell, A., van Heyningen, V., Fantes, J.A., Boyd, P.A. and Hastie, N.D. (1986) *Mol. Cell. Biol.*, **6**, 2223-2232.
14. Shafit-Zagardo, B., Maio, J.J. and Brown, F.L. (1982) *Nucleic Acids Res.*, **10**, 3175-3193.
15. Ru Hwu, H., Roberts, J.W., Davidson, E.H. and Britten, R.J. (1986) *Proc. Natl. Acad. Sci. USA.*, **83**, 3875-3879.
16. Cooper, C.S., Blair, D.G., Oskarsson, M.K., Tainsky, M.A., Eader, L.A. and Vande Woude, G.F. (1984) *Cancer Res.*, **44**, 1-10.
17. Croce, C.M. and Koprowski, H. (1974) *J. Exp. Med.*, **139**, 1350-1353.
18. Weis, J.H., Nelson, D.L., Przyborski, M.J., Chaplin, D.D., Mulligan, R.C., Housman, D.E. and Seidman, J.G. (1984) *Proc. Natl. Acad. Sci. USA.*, **81**, 4897-4883.
19. Southern, E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
20. Gilliam, T.C., Scambler, P., Robbins, T., Ingle, C., Williamson, R. and Davies, K.E. (1984) *Hum. Genetics*, **68**, 154-158.
21. Feinberg, A.P. and Vogelstein, B. (1984) *Analytical Biochemistry*, **137**, 266-267.

22. DiGiovanni, L., Hayes, S.R., Misra, R. and Jelinek, W.R. (1983) *Proc. Natl. Acad. Sci. USA.*, **80**, 6533-6537.
23. McBride, O.W. and Peterson, J.L. (1980) *Annu. Rev. Genet.*, **14**, 321-345.
24. Scangos, G.A., Huttner, K.M., Silverstein, S. and Ruddle, F.H. (1979) *Proc. Natl. Acad. Sci. USA.*, **76**, 3987-3990.
25. Miller, C.L., and Ruddle, F.H. (1978) *Proc. Natl. Acad. Sci. USA.*, **75**, 3346-3350.
26. Klobutcher, L.A., Miller, C.L. and Ruddle, F.H. (1980) *Proc. Natl. Acad. Sci. USA.*, **77**, 3610-3614.
27. Olsen, A.S., McBride, O.W. and Moore, D.E. (1981) *Mol. Cell. Biol.*, **1**, 439-448.
28. Klobutcher, L.A. and Ruddle, F.H. (1979) *Nature*, **280**, 657-660.
29. Park, M., Dean, M., Cooper, C.S., Schmidt, M., O'Brian, S.J., Blair, D. and Vande Woude, G.F. (1986) *Cell*, **45**, 895-904.
30. Bartels, I., Grzeschik, K-H., Cooper, D.N. and Schmidtke, J. (1986) *Am. J. Hum. Genet.*, **39**, 280-287.
31. Dean, M., Park, M., Le Beau, M.M., Robbins, T.S., Diaz, M.O., Rowley, J.D., Blair, D.G. and Vande Woude, G. (1985) *Nature*, **315**, 385.