DNase I-defined chromatin configuration of the human CD3 gene cluster

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ABSTRACT

The three CD3 genes on human chromosome 11q23 encode proteins (γ , δ and ϵ) which form part of the antigen receptor on T lymphocytes. All three genes are clustered within 50 kb and are activated approximately contemporaneously during the early stages of T cell ontogeny. In order to pinpoint potential regulatory sequences important for locus activation and tissue-specific gene expression, the chromatin structure of almost 90 kb of this region has been probed in five cell lines using the endonuclease pancreatic DNase I. A set of DNase I hypersensitive (HS) sites has been defined in T cell chromatin, five of which were strong and not found in non-T cells, with the exception of the erythroleukaemia cell line K562, in which three sites were weakly expressed, correlating with a low level of δ mRNA. The subset of five HS sites map close to the CD3 genes and lie in regions which contain elements of defined function: the γ promoter; the δ promoter and its 3' enhancer; and the ϵ promoter and its 3' enhancer. Since no further major T cell-restricted HS sites lie within the 90kb of the CD3 locus analysed, these five regions may contain all the sequences important for CD3 gene expression.

INTRODUCTION

CD3 is a complex of three cell surface proteins (γ , δ and ϵ) which forms part of the antigen receptor on T cells (reviewed in ref. 1). These proteins are thought to be involved in the generation of an internal mitogenic signal after the T cell has encountered antigen (2). cDNA cloning has demonstrated that the CD3 genes are homologous and are member of the immunoglobulin (Ig) superfamily (3–5). Moreover, the three genes are clustered and closely linked in human (6, 7), mouse (8) and sheep (W.Hein and A.T., unpublished data).

While the cluster of CD3 genes probably arose during evolution by two gene duplication events, an intriguing question is whether their continued close proximity indicates the use of common regulatory sequences. Two points of particular interest with regard to the organisation and expression of the CD3 genes are the following: First, the CD3- γ and - δ genes are divergently transcribed, their cap sites being separated by only 1.6 kilobases (kb) (9, 10). Such a back-to-back arrangement of gene pairs is often exploited in lower eukaryotes (e.g. 11, 12), where a regulatory element situated between the two promoters is used

by both genes. The implication, therefore, is that a shared element or elements might be found between the CD3- γ and - δ genes; Secondly, all three CD3 genes are activated at approximately the same stage of development of the T cell lineage (reviewed in ref. 13). The clustering of CD3 genes may facilitate their similar patterns of expression during ontogeny, perhaps by an alteration of the chromatin structure of the whole locus. In the β -globin (14) and CD2 (15) loci, candidate elements which may effect such local changes in chromatin configuration have been described. An attractive hypothesis, therefore, is that an analogous element, termed a dominant control region (DCR), may also be situated close to the CD3 gene cluster.

Elements important for gene expression, such as promoters, enhancers and DCRs, are often associated with DNase I hypersensitive (HS) sites in intact nuclei (14, 16). HS sites are regions of the chromosome which are relatively free of nucleosomes and are therefore more susceptible to endonuclease (e.g. DNase I) digestion than surrounding chromatin. The altered chromatin structure at HS sites is thought to allow access to underlying DNA sequences of *trans*-acting factors important for gene expression or chromosome function (16). Hence, mapping HS sites offers a relatively rapid means of identifying possible regulatory sequences. In this paper experiments are described which examine almost 90 kb of DNA containing the human CD3 genes for HS sites in several different cell types.

MATERIALS AND METHODS

Cosmid clones

Screening of a partial HindIII digest cosmid library and isolation of cosmids c γ 3, c δ 11A and c ϵ 1 have been described (6). Clone c δ 12A was isolated from the same library using a partial CD3- δ cDNA clone (17). For cloning c π 1, a 1.0 kb EcoRI fragment (termed π 1), mapping approximately 16 kb 5' of CD3 ϵ (Fig. 1), was isolated from cosmid c ϵ 1 and used to screen the library after prehybridisation with sonicated total human DNA to remove repetitive sequences (18).

Cells

HSB2 and SB are T and B cell lines, respectively, derived from a child with acute lymphoblastic leukaemia and obtained from ATCC (Rockville, MD). JM is a T cell line established by Schneider et al. (19). K562 (20) and COLO320 (21) are cell lines derived from an erythroleukaemia and a colon carcinoma, respectively. All cells were maintained in RPMI 1640 medium supplemented with 10% foetal calf serum (heat inactivated).

DNase I hypersensitivity assay

The method is based on that of Yagi et al. (22). All procedures were carried out on ice in Eppendorf tubes. Cells (2×10^7) were recovered and washed twice in phosphate-buffered saline, washed once in RSB (10mM TrisHCl pH7.5, 10mM NaCl, 5mM $MgCl_2$, 0.1mM PMSF), then resuspended in 1ml RSB plus 0.5% NP40. After 5min on ice, nuclei were recovered by centrifugation, washed three times in RSB and once in buffer A (100mM NaCl, 50mM TrisHCl pH8.0, 3mM $MgCl_2$) plus 1mM EGTA, 1mM PMSF. Finally, nuclei were taken up in 600 μ l buffer A plus 1mM $CaCl_2$, 1mM PMSF. Two 100 μ l portions were taken as controls: one (denoted 'N' in Figs. 2 and 3) was added immediately to 2 $\times$ TNE/SDS (100mM TrisHCl pH8.0, 100mM NaCl, 10mM EDTA, 1% SDS) plus 100 μ g/ml proteinase K (Boehringer), while the second ('O' in Figs. 2 and 3) was incubated for 10min at 37°C without DNase I before also being added to 2 $\times$ TNE/SDS/proteinase K. To the remaining 400 μ l of nuclei, 3u DNase I (RQ1; Pharmacia) was added and equal aliquots incubated at 37°C for 1, 2, 5 or 10min before stopping the reaction with 2 $\times$ TNE/SDS/proteinase K. All aliquots were then allowed to undergo proteinase K digestion overnight at 37°C after which DNA was recovered by phenol/chloroform extraction and ethanol precipitation. Restriction enzyme digests and agarose gel fractionation followed standard procedures (23). Gels were blotted overnight onto Zetaprobe (Bio-Rad) nylon membranes in 0.4M NaOH after brief (10min) depurination with 0.25M HCl, and 30min incubation in 0.5M NaOH, 1.5M NaCl.

RNA gels

Approximately 2 μ g polyA⁺-enriched RNA from cell lines were separated in formaldehyde/agarose gels according to standard procedures (23). Gels were soaked in water for 30min before blotting 4–5hr in 50mM NaOH onto Zetaprobe membranes. Filters were rinsed in 2 $\times$ SSC (1 $\times$ SSC is 150mM NaCl, 15mM sodium citrate, pH7.0) before hybridisation.

Filter hybridisations

Both DNA and RNA filters were prehybridised (approximately 1hr; 65°C) in 3 $\times$ SSPE (1 $\times$ SSPE is 180mM NaCl, 10mM NaH_2PO_4 , 1mM EDTA, pH7.4), 1% dried skimmed milk (Carnation), 0.5% SDS, 500 μ g/ml salmon sperm DNA and hybridised (overnight; 65°C) in prehybridisation solution plus 10% dextran sulphate (Pharmacia) and ³²P-labelled probes. Washes were to 0.2 $\times$ SSC, 0.1% SDS, 65°C for DNA hybridisations, and to 3 $\times$ SSC, 0.1% SDS, 65°C for RNA filters. DNA filters were reprobbed after stripping with a boiling solution of 0.1 $\times$ SSC, 0.1% SDS and autoradiography to check for removal of signal. RNA filters were stripped in 0.1 $\times$ SSC, 0.1% SDS, 70°C, 30min, before reprobbed.

Probes

All but three of the ten probes were genomic fragments, subcloned from lambda or cosmid clones of the CD3 locus. The map positions of these fragments are shown in Fig. 1. *Probe 1*: 0.9 kb EcoRI; 2: 1.4 kb HindIII-EcoRI; 3: 0.4 kb BamHI-SmaI; 4: 0.5 kb EcoRI-XhoI, derived from CD3- γ cDNA pJ6T3 γ -2 (3); 5: 1.1 kb BamHI-SalI; 6: 0.3 kb BamHI, derived from CD3- δ cDNA clone pJM3A10 (17); 7: 0.6 kb SalI-HindIII; 8: 0.7 kb EcoRI-HindIII, derived from CD3- ϵ cDNA clone pJM4C11 (9); 9: 0.9 kb HindIII-BamHI; 10: 1.4 kb SphI. For RNA filter hybridisations, probes 1, 3, 4 (γ), 6 (δ) and 8 (ϵ) were used, together with a rat glyceraldehyde phosphate dehydrogenase (GAPDH) probe (24) as control. Labelling of probes was by random oligonucleotide directed priming (25).

RESULTS

Overlapping cosmid clones containing the CD3 cluster were obtained previously (6). Two further clones, c δ 12A and c π 1, are described here which extend the cloned region to 94 kb (see Materials and Methods; Fig. 1). The CD3 cosmids have been subcloned as EcoRI fragments in plasmid vectors. Seven single

Figure 1. The human CD3 gene cluster and its associated DNase I hypersensitive sites. The cloned region of chromosome 11q23 containing the three CD3 genes is shown, with arrows denoting transcriptional orientation and black boxes signifying exons. The exon structure of the hatched region at the end of the γ gene, consisting of 3'-untranslated sequences (3), has not been determined. DNase I hypersensitive sites in T cells are lettered and indicated by arrows, with shorter arrows implying a weaker site. A 'weaker' site is empirically defined as appearing after only 5' of DNase I digestion, in contrast to 'normal' sites which were apparent after 2'. Probes, as detailed in Materials and Methods, are numbered open boxes. Restriction sites flanked by parentheses are not cut in noncloned DNA, probably due to methylation.

copy probes were isolated from these subclones which, together with three cDNA clones, allowed analysis of almost 90 kb of the locus for the presence of HS sites (Fig. 1).

DNase I hypersensitive sites associated with the CD3- γ/δ gene pair

Nuclei were prepared from five human cell lines: JM and HSB2 (T cell), SB (B cell), K562 (erythroleukaemia) and COLO320 (colorectal carcinoma). They were then subjected to a time course of DNase I digestion followed by DNA extraction and Southern blot analysis using several different restriction enzymes. Six probes were used to detect HS sites in the vicinity of the CD3- γ and - δ genes (Fig. 2). Probes 1 and 2, in combination with HindIII, EcoRI or BamHI digests, showed no DNase I hypersensitivity in the region far 3' of the CD3- γ gene (e.g. Fig. 2a). However, probes 3–6 revealed HS sites in closer proximity to the γ/δ gene pair. Six sites (A–F) were open in the T cell

lines examined (Fig. 1). Fig. 2b shows site A to be present also in COLO320 and K562: probe 3 hybridises to a 6.2 kb EcoRI fragment which is cut by DNase I to a smaller 4.4 kb band. This, therefore, positions the HS site with respect to the EcoRI sites producing the 6.2 kb fragment (Fig. 1). HindIII and BamHI digests (not shown) confirmed the map position. Since site A is also expressed in the B cell line SB (not shown; Table 1), it may be constitutive, i.e. generally expressed in different cell types.

Other HS sites have a more restricted expression, being found in the T cell lines only (sites C, H and I) or additionally in one other cell line (sites B, D and E). Site B (Fig. 2c) seems to map within, or very close to, exon 7 of the CD3- γ gene and is found in COLO320 also. Site C is located close to the 5' end of the γ gene and probably defines the promoter. Thus, in Fig. 2d, a 4.2 kb BglII-HindIII fragment hybridising to probe 5 is reduced by DNase I to 3.0 kb in the T cell line HSB2, but not in the B cell, SB. Since BglII cuts approximately 350 bp upstream of

Figure 2. Definition of DNase I hypersensitive sites around the CD3- γ/δ gene pair. A time course of DNase I digestion for the different cell lines is shown (from the left for each cell the order is N, O, 1', 2', 5', 10'; detailed in Materials and Methods). The alphanumeric at the bottom left corner of each panel indicates the combination of probe and restriction endonuclease used (so, '1H' means probe 1, HindIII digest). Abbreviations of enzyme names: H, HindIII; R, EcoRI; B, BglII; Bm, BamHI. Numbers to the right of each panel are the sizes in kilobases of the corresponding band detected by Southern hybridisation.

Table 1 Summary of HS sites in five human cell lines

HS site	cell line					probable associated functional elements
	JM	HSB2	SB	K562	COLO320	
A	+	+	+	~	+	?
B	+	+	-	-	+	?
C	+	+	-	-	-	γ promoter
D	+	+	-	~	-	δ promoter
E	+	+	-	~	-	δ enhancer
F	ND	+	-	ND	ND	?
G	+	+	-	~	-	ϵ enhancer
H	+	+	-	-	-	ϵ promoter
I	+	+	-	-	-	?

+ = present; - = not present; ~ = weakly present; ND = not determined

Figure 3. Definition of DNase I hypersensitive sites around the CD3- ϵ gene. For explanation of symbols, see legend to Fig. 2.

the CD3- δ transcription initiation region (26), and HindIII cuts in the first intron of the γ gene, site C can be positioned close to the γ promoter.

Fig. 2e, a BamHI digest hybridised with probe 5, shows a 3.4 kb fragment which is reduced to 3.1 kb in JM, due to DNase I cutting at site D, close to the start of CD3- δ gene transcription. The 1.6 kb fragment, also seen in Fig. 2e, is the result of further digestion at HS site C. Site E, 0.3–0.4 kb 3' of the CD3- δ gene is demonstrated by the 8.3 kb DNase I-cut subfragment of a 13.2 kb HindIII band detected by probe 5 (Fig. 2f). Further reduction of this fragment, to 4.3 kb and 2.9 kb bands, is due to cutting at sites D and C, respectively. Interestingly, Fig. 2f also shows that sites D and E, flanking the CD3- δ gene, are weakly present in K562, although site C is not found in this cell line. None of the latter three sites is found in COLO320. Fig. 2g–h confirm the location of Site E 3' of the CD3- δ gene. In mouse there is an equivalent HS site 3' of the δ gene in a region which has been shown to have T cell-specific enhancer activity (27). A 3' enhancer has also recently been demonstrated for the human δ gene (C. Terhorst, personal communication), which probably coincides with HS site E. Fig. 2h shows further that a 6.2 kb BglII-HindIII band is initially reduced to 5.0 kb in the T, but not in the B, cell, implicating a weak HS site (F) further 3' of the CD3- δ gene.

DNase I hypersensitive sites associated with the CD3- ϵ gene

HS sites flanking the CD3- ϵ gene were demonstrated using probes 8–10 after probe 7 failed to detect any sites in the δ - ϵ intergenic

region (not shown). In Fig. 3a probe 8 hybridises to two HindIII bands of 8.4 kb and 2.8 kb. In the T cell line JM, the larger band is reduced to 2.0 kb by DNase I digestion, which positions site G approximately 0.8 kb downstream of the last exon of the CD3- ϵ gene. This HS site probably coincides with that recently reported by Clevers et al. (28), which is associated with an enhancer operational in T cells. A number of subbands, also seen in the JM tracks of Fig. 3a, may define further minor HS sites close to site G. Site G appears weakly in K562 also and therefore is not absolutely T cell-specific.

Probes 9 and 10, when hybridised to EcoRI-digested DNA, highlight a 19.4 kb band, which in JM is reduced by DNase I to a band of 7.8 kb and again subsequently to 6.6 kb (Fig. 3b, c). Further analysis (Fig. 3d) showed that this pattern was consistent with the expression of two T cell-specific HS sites, H and I, mapping to the CD3- ϵ gene promoter and approximately 7.0 kb upstream of the gene, respectively.

Expression of CD3 genes

The CD3 genes are expressed in thymocytes and mature T cells, but generally not in other lineages. An exception is the natural killer (NK) cell, where CD3- ϵ , but not γ or δ mRNA, is detected at low levels (29, 30). Since some of the HS sites defined in this report are found also in non-T cells (Table 1), it was of interest to test for CD3 mRNA in the different cell lines examined.

Northern analysis of polyA⁺-enriched RNA from JM, SB, K562 and COLO320 showed expression of the three CD3 genes

Figure 4. Expression of CD3 genes in cell lines used for HS studies. Northern analysis of polyA⁺-enriched RNA of JM, SB, K562 and COLO320 probed for (a) CD3- γ (probe 4); (b) CD3- δ (probe 6); (c) CD3- ϵ (probe 8); or (d) GAPDH as control. Filters were stripped and rehybridised, or identical gels used, for the different probes.

in the T cell, as expected (Fig. 4). The non-T cells were negative for the presence of CD3 mRNA, with the exception of K562, which showed a low level of δ mRNA (Fig. 4b). This correlates with the weak presence in the erythroleukaemia cell line of HS sites D and E, which flank the CD3- δ gene and suggests at least partial control of δ gene expression by elements at these sites. Clearly, however, the CD3- ϵ gene is not expressed in K562 (Fig. 4c), despite HS site G being partially accessible to DNase I in this cell line (Fig. 3).

HS site A, present in all five cell lines tested and therefore not T cell-specific, could indicate the presence of a new generally expressed gene 3' of the CD3- γ gene. Northern analysis with probes 1 and 3 (not shown) did not reveal any transcript from this region, however.

DISCUSSION

The organisation of genes into clusters often has implications for the evolution and expression of the genes involved. So, for example, clustering of the Ig heavy chain constant region genes reflects their evolution from a common ancestral gene, but is also necessary for isotype switching by DNA rearrangement or alternative pre-mRNA splicing mechanisms (reviewed in ref. 31). Gene clustering may also facilitate coordinate regulation, a question particularly relevant to the CD3 locus, where all three CD3 genes are activated approximately contemporaneously during T cell development (13). As an initial step in the analysis of the regulation of genes of the CD3 cluster, we have looked

for associated DNase I hypersensitive (HS) sites, nucleosome-free regions of chromatin which are frequently correlated with the presence of regulatory sequences (16).

Nine HS sites have been defined in the CD3 locus, which are all open in the T cells examined (Table 1). Three of these, sites C, D and H, map to the promoter regions of the γ , δ and ϵ genes, respectively. The CD3 gene promoters have been shown to be weak when acting without an enhancer and to exhibit no tissue specificity in transient expression assay systems (27, 28, 32). This latter characteristic is worth comment since hypersensitivity at these promoter sites is clearly tissue restricted. Thus, while the promoters themselves are not tissue specific, efficient access of the transcription machinery may be. Two further HS sites (G and E) can be assigned to regions of known function: HS site G, immediately downstream of the CD3- ϵ gene, has been previously defined (28) and lies in a region containing an enhancer functional in T cells but not in HeLa or B cells. Site E, situated 3' of the CD3- δ gene, probably also coincides with an enhancer location (C. Terhorst, personal communication), as has been demonstrated for the mouse gene (27). The remaining four HS sites (A, B, F and I) are not associated with currently recognised functional sequences and may therefore give clues to the location of new regulatory elements. However, since site A is constitutive and the other three sites are relatively weak, it is perhaps unlikely that they define sequences of major importance for tissue-specific expression.

It was expected that, since expression of the CD3 genes is largely restricted to the T cell lineage, the presence of HS sites would also be tissue specific. Surprisingly, only three sites were uniquely present in T cells and not in the three other cell types tested: C (γ promoter); H (ϵ promoter); and I (upstream of the ϵ gene). Other HS sites were also found in at least one non-T cell (Table 1). Perhaps significantly, a low level of CD3- δ mRNA in the erythroleukaemia cell line K562 was correlated with weak accessibility of HS sites D and E flanking this gene. The presence of HS site A in all cell lines, and of B in COLO320, both associated with the CD3- γ gene, did not, however, correlate with γ expression. Indeed, expression of the CD3- γ gene has not, so far, been observed outside the T cell lineage, whereas the δ and ϵ genes display a less absolute expression pattern (29, 30; this paper).

Since no strong, T-cell specific HS sites are apparent in the 20kb or so of DNA flanking the CD3- γ and - ϵ genes, it is possible that all the significant regulatory regions pertaining to CD3 gene expression have now been identified and are situated close to the genes themselves, although the presence of additional, more distant elements cannot be excluded. That known functional elements locate in the DNA adjacent to the CD3 genes does not preclude the presence of others in their vicinity. Thus, in the DCR of the β -globin locus, an enhancer is situated close to, or may overlap with, sequences responsible for high-level, copy-dependent, position-independent gene expression in transgenic mice (33–35). It will now therefore be important to assess whether the elements defined by the HS sites described here are sufficient for expression of the CD3 genes. The most stringent test for correct expression is a developmental, i.e. transgenic, assay. It is clear that the human CD3- ϵ gene, when introduced into transgenic mice, is expressed in T cells and not in other tissues tested (28, 36). However, in both the above reports, mice carrying high copy numbers of the transgene were used. Such mice can show aberrant levels of gene expression, and therefore it will be necessary to examine low copy mice for regulation studies. Preliminary results using transgenic mice bearing low

transgene copy numbers suggest that appropriate expression is seen with the CD3- ϵ gene, but not with the CD3- γ/δ gene pair (C. Olsson, A. de la Hera, U. Müller and A.T., in preparation).

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REFERENCES

- Clevers, H., Alarcon, B., Wileman, T. and Terhorst, C. (1988) *Annu. Rev. Immunol.*, **6**, 629–662.
- van Wauwe, J.P., De Mey, J.R. and Goossens, J.G. (1980) *J. Immunol.*, **124**, 2708–2713.
- Krissansen, G.W., Owen, M.J., Verbi, W. and Crumpton, M.J. (1986) *EMBO J.*, **5**, 1799–1808.
- Gold, D.P., Clevers, H., Alarcon, B., Dunlap, S., Novotny, J., Williams, A.F. and Terhorst, C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 7649–7653.
- Williams, A.F. (1987) *Immunol. Today*, **8**, 298–303.
- Tunnacliffe, A., Olsson, C., Buluwela, L. and Rabbitts, T.H. (1988) *Eur. J. Immunol.*, **18**, 1639–1942.
- Evans, G.A., Lewis, K.A. and Lawless, G.M. (1988) *Immunogenetics*, **28**, 365–373.
- Letourneur, F., Mattei, M.-G. and Malissen, B. (1989) *Immunogenetics*, **29**, 265–268.
- Tunnacliffe, A., Buluwela, L. and Rabbitts, T.H. (1987) *EMBO J.*, **6**, 2953–2957.
- Saito, H., Koyama, T., Georgopoulos, K., Clevers, H., Haser, W.G., LeBien, T., Tonegawa, S. and Terhorst, C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 9131–9134.
- Osley, M.A., Gould, J., Kim, S., Kane, M. and Hereford, L. (1986) *Cell*, **45**, 537–544.
- Baum, J.A., Geever, R. and Giles, N.H. (1987) *Mol. Cell. Biol.*, **7**, 1256–1266.
- Tunnacliffe, A. (1990) *Expl. Clin. Immunogenet.*, **7**, 43–52.
- Grosveld, F., Blom van Assendelft, G., Greaves, D.R. and Kollias, G. (1987) *Cell*, **51**, 975–985.
- Greaves, D.R., Wilson, F.D., Lang, G., and Kioussis, D. (1989) *Cell*, **56**, 979–986.
- Gross, D.S. and Garrard, W.T. (1988) *Annu. Rev. Immunol.*, **57**, 159–197.
- Rabbitts, T.H., Lefranc, M.P., Stinson, M.A., Sims, J.E., Schroder, J., Steinmetz, M., Spurr, N.L., Solomon, E. and Goodfellow, P.N. (1985) *EMBO J.*, **4**, 1461–1465.
- Sealey, P.G., Whittaker, P.A. and Southern, E.M. (1985) *Nucleic Acids Res.*, **13**, 1905–1922.
- Schneider, U., Schwenk, H.U. and Bornkamm, G. (1977) *Int. J. Cancer*, **19**, 621–626.
- Luzzio, C.B. and Luzzio, B.B. (1975) *Blood*, **45**, 321–334.
- Alitalo, K., Schwab, M., Lin, C.C., Varmus, H.E. and Bishop, J.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1707–1711.
- Yagi, M., Gelinas, R., Elder, J.T., Peretz, M., Papayannopoulou, T., Stamatoyannopoulos, G. and Groudine, M. (1986) *Mol. Cell. Biol.*, **6**, 1108–1116.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor.
- Fort, P., Marty, L., Piechaczyk, M., El Sabrouy, S., Dani, C., Jeanteur, P. and Blanchard, J.M. (1985) *Nucleic Acids Res.*, **13**, 1431–1442.
- Feinberg, A.P. and Vogelstein, B. (1983) *Anal. Biochem.*, **132**, 6–13.
- Tunnacliffe, A., Sims, J.E. and Rabbitts, T.H. (1986) *EMBO J.*, **5**, 1245–1252.
- Georgopoulos, K., van den Elsen, P., Bier, E., Maxam, A. and Terhorst, C. (1988) *EMBO J.*, **7**, 2401–2407.
- Clevers, H., Lonberg, N., Dunlap, S., Lacy, E. and Terhorst, C. (1989) *EMBO J.*, **8**, 2527–2535.
- Biassoni, R., Ferrini, S., Prigione, I., Moretta, A. and Long, E.O. (1988) *J. Immunol.*, **140**, 1685–1689.
- Anderson, P., Caligiuri, M., Ritz, J. and Schlossman, S.F. (1989) *Nature*, **341**, 159–162.
- Honjo, T., Alt, F.W. and Rabbitts, T.H. (eds.) (1989) *Immunoglobulin Genes*. Academic Press, London.
- Clevers, H.C., Dunlap, S., Wileman, T.E. and Terhorst, C. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8156–8160.
- Tuan, D.Y.H., Solomon, W.B., London, I.M. and Lee, D.P. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 2554–2558.
- Blom van Assendelft, G., Hanscombe, O., Grosveld, F. and Greaves, D.R. (1989) *Cell*, **56**, 969–977.
- Forrester, W.C., Novak, U., Gelinas, R. and Groudine, M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 5439–5443.
- Tunnacliffe, A., Olsson, C. and de la Hera, A. (1989) *Int. Immunol.*, **1**, 546–550.