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Variable breakpoints in Burkitt lymphoma cells with chromosomal t(8;14) translocation separate *c-myc* and the IgH locus up to several hundred kb

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ABSTRACT

In about 80% of Burkitt's lymphoma cases, the tumour cell harbours a reciprocal chromosomal translocation which invariably transposes the coding exons 2 and 3 of c-myc from chromosome 8 to the immunoglobulin heavy chain locus on chromosome 14. Those t(8;14) translocations which disrupt chromosome 8 within or close to the c-myc gene are well documented. In this study we have focussed on t(8;14) translocations with the chromosomal breakpoint far upstream of c-myc. We analyzed the breakpoint position in 44 BL cell lines with t(8;14) translocations of different geographical origin and identified 9 cell lines with the breakpoint more than 14 kb upstream of c-myc. In these cell lines the positions of the translocation junctions on the derivative chromosomes $8q^-$ and $14q^+$ were mapped by pulsed field gel electrophoresis and multicolour fluorescence in situ hybridization. The breakpoints occur at distances between 55 and more than 340 kb upstream of c-myc with no preferential site on chromosome 8. On chromosome 14, however, the translocation breakpoints are clustered in a narrow region 5′ of the intron enhancer of the immunoglobulin heavy chain gene. In 7 of 9 cases, the enhancer is fused to the c-myc bearing sequences of chromosome 8. In two cases, the translocation has occurred in switch μ and downstream of Cμ, respectively. The impact of these results with respect to the hypothesis, that cis-regulatory sequences from the immunoglobulin heavy chain locus can deregulate c-myc expression in a manner sufficient for tumour formation, is discussed.

INTRODUCTION

Burkitt's lymphoma (BL) cells exhibit a reciprocal chromosomal translocation which fuses the proto-oncogene c-myc on chromosome 8 to one of the three immunoglobulin loci. The t(8;14) translocation, found in about 80% of BL, transposes the coding part of the c-myc gene onto the immunoglobulin heavy chain (IgH) locus. The 'variant' translocations t(2;8) and t(8;22) juxtapose c-myc to the κ and λ light chain genes, respectively (for a review see 1).

The t(8;14) translocations have been classified according to the position of the chromosomal breakpoints relative to the c-myc gene. Translocations with breakpoints in the first exon or intron of c-myc have been designated as class I, those with breakpoints immediately upstream of the gene as class II, and those with breakpoints distant as class III (2). Conclusive data regarding the location of breakpoints in cases with t(8;14) class III translocations are still missing.

On chromosome 14, the t(8;14) breakpoints are located between the variable and the constant part of the IgH locus, either 5′ of the intron enhancer (E_i) in a joining (J) or diversity (D) segment, or 3′ of the intron enhancer in the μ switch region (1–6). In the first case the intron enhancer remains on chromosome $14q^+$ linked to the c-myc gene in cis (Fig.2), while in the second case the intron enhancer is placed onto the reciprocal translocation chromosome $8q^-$.

E_i mediates B cell specific transcriptional control of the IgH locus (7–9). Recently, another enhancer at the very 3′ end of the IgH locus has been described in rats and mice (10, 11). An equivalent IgH downstream enhancer is likely to exist also in humans, but has not yet been identified. In the light chain loci of mouse and man analogous elements have been described: within the J_κ-C_κ intron (12) as well as downstream of the κ coding region (13, 14), and downstream in the λ light chain locus (15).

In the variant translocations t(2;8) and t(6;15) of BL and mouse plasmacytoma, respectively, the κ intron enhancer is invariably fused to the c-myc bearing chromosome. Since the distance between the enhancer and c-myc can be as large as 300 kb, these findings have led to the hypothesis, that Ig regulatory sequences might deregulate c-myc expression despite long distances between them (16, 17).

Functional evidence that IgH gene regulatory sequences can control c-myc expression in BL came from cell fusion experiments. In mouse fibroblast/BL hybrid cells, the IgH- as well as the c-myc gene are concomitantly downregulated

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('extinguished'). Thus in this context c-myc behaves like a B cell specific gene (18). Furthermore, transgenic mice harbouring a c-myc transgene under the control of an IgH intron enhancer develop B cell lymphomas, whereas no tumours arise in the absence of the intron enhancer (19).

In BL with t(8;14) class III translocation, little is known to date concerning the presence of the IgH regulatory sequences on $14q^+$ and their possible physical distance from c-myc. In this paper we present a detailed analysis of 14 out of 44 cell lines with this type of translocation. Using standard and pulsed field gel electrophoresis (PFGE) as well as fluorescence in situ hybridization we have localized the translocation breakpoints upstream of c-myc and within the IgH locus.

5'of BamHI	Bam to Eco	Eco to exonII	EBV pos/total	origin
6	4	7	17 / 17	eBL
1	1	14	8 / 16	sBL
2	-	9	11 / 11	NA BL
9	5	30	36 / 44	total

Figure 1. Variation of the sites of chromosomal translocation within the c-myc locus in Burkitt lymphomas of different geographic origin. BL is found with high incidence in Central Africa and New Guinea ('endemic BL', eBL) and with low incidence in the caucasian areas ('sporadic', sBL). The cases from North Africa (NA BL) are indicated seperatedly because an intermediate incidence has been described for this area. (20)

RESULTS

Identification of BL cell lines with chromosomal breakpoints far upstream of *c-myc*

To identify BL cells with the translocation breakpoint far upstream of c-myc, cell lines with t(8;14) translocations were analysed for the presence or absence of c-myc rearrangements by Southern analysis using a c-myc specific probe. Digestion with EcoRI and BamHI allowed to visualize rearrangements within a distance of 7 kb and 14 kb respectively 5′ of the first c-myc promoter. As shown in Fig.1, among 44 cell lines studied, 14 did not show a rearrangement within the EcoRI fragment. We conclude that these BL lines carry the breakpoint of the chromosomal translocation more than 7 kb upstream of c-myc. They were thus classified as cell lines with t(8;14) class III translocations and used for further analysis.

A comparison of the distribution of chromosomal breakpoint positions in tumours of different geographical origin, i.e. in areas with low, intermediate and high incidence of BL (20, 21), shows that the translocation sites far upstream of c-myc are predominantly found in cases from endemic areas, i.e. from Central Africa and New Guinea (Fig.1). These results confirm and extend the observations of Pellici et al. (22), Haluska et al. (4) and Shiramizu et al. (23) correlating the site of the translocation with the geographical origin of the tumour.

In 13 of 14 cell lines with t(8;14) class III translocation the chromosomal breakpoint is located within 340 kb upstream of *c-myc*

As is shown in Fig. 1 in 5 cell lines with t(8;14) class III translocation the chromosomal breakpoint was mapped between 7 kb (EcoRI) and 14 kb (BamH1) upstream of c-myc. Pulsed field gel electrophoresis (PFGE) was used to detect chromosomal breakpoints in the remaining 9 cell lines. The molecular structure of this type of translocation as well as the probes that were used in this study, are presented in Fig.2. Using the *5′myc* probe for hybridization, a region of 230 kb adjacent to the c-myc gene could be studied for rearrangements by PFGE analysis after digestion of the DNA with NotI plus SalI. Rearranged fragments were visualized in 5 cell lines (IARC/BL72, LY74, MAK-BL, MUK-BL, IARC/BL6; Fig.3), indicating that in these cell lines the chromosomal breakpoint is located within this region. The analysis of the remaining 4 cell lines by digestion with SfiI and hybridization with probe $p380_c$ detected rearrangements in IARC/BL9-12, IARC/BL74 and Namalwa but not in IARC/BL16 (Fig.4). In IARC/BL6 DNA, a rearrangement was observed in both experiments shown in Figs. 3 and 4 indicating that the breakpoint is located between the SfiI site at position 180 kb and the SalI site at position 230 kb upstream of c-myc.

In order to verify that the rearranged fragments represent indeed the t(8;14) translocations, the same filters were rehybridized with probes of the immunoglobulin heavy chain (IgH) gene (Fig.3). In 7 of the 9 cell lines at least one of the IgH probes comigrated with rearranged fragments that had also been visualized by the chromosome 8 probes (Fig.3, 5a), confirming that the chromosomal breakpoints are located within 340 kb upstream of c-myc. In Namalwa cells, probes C_μ and $5'E_i$ visualized fragments of different size than the rearranged band detected by probe $p380_c$ (Fig.5b) thereby not allowing to asses the chromosome 8 breakpoint. In IARC/BL16 cells no rearrangements were found with any of the chromosome 8 probes indicating that the chromosomal breakpoint in IARC/BL16 cells is located more than 340 kb upstream of c-myc. This was indeed confirmed as will be described below.

In 7 of the 9 cell lines the translocation juxtaposes the IgH intron enhancer and *c-myc* on chromosome $14q^+$

Chromosomal breakpoints in BL cell lines within the IgH locus have been described either within a rearranged VDJ segment or 3′ thereof, usually in the μ switch region ($S\mu$) (1−6). Only in the first situation the IgH intron enhancer is located on chromosome $14q^+$. In this case, the breakpoints are found within 2 kb including the VDJ region. In contrast the enhancer is placed on the reciprocal translocation chromosome $8q^-$ when the breakpoint occurrs within the switch region or further downstream.

To assess the chromosomal location of the intron enhancer, PFGE was carried out using the probes C_μ, $5'E_i$ and $3'E_i$ (see Fig.2). In 4 cases (IARC/BL72, LY74, MAK-BL and MUK-BL), the C_μ- as well as the $5'E_i$ probe were found on the same rearranged fragment that had also been visualized with chromosome 8 specific probes (Fig.3). In two cell lines (IARC/BL6 and IARC/BL9-12) only the $5'E_i$ probe comigrated with a rearranged fragment but not C_μ (for IARC/BL6 see Fig.3). This can be explained by switch recombination on the translocated chromosome. Therefore the chromosomal

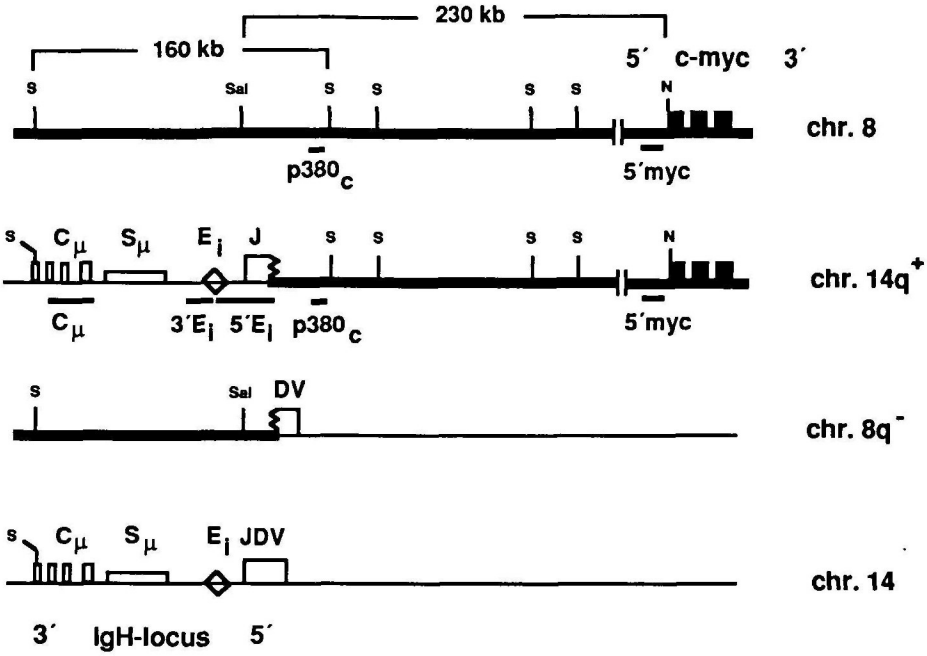


Figure 2. Structure of the breakpoint regions involved in t(8;14) class III translocation. c-myc on chromosome 8 (bolded) and the IgH-gene on chromosome 14 (thin-lined) are fused on chromosome $14q^+$ in 'head to head' orientation. Chromosomal breakpoints within 340 kb upstream of c-myc cause rearrangements within the 230 kb NotI/SalI fragment or the 160 kb SfiI fragment that can be visualized using probes *5′myc-* and $p380_c$. The map was established by Gemmill et al. (47) and Joos et al. (38). Note that the chromosome 8 and chromosome 14 specific sequences are scaled differently. Probes are indicated by bars. C_μ:, constant μ segment; S_μ: μ switch region; E_i: intron enhancer, J: joining region; D: diversity region; V: variable region. N: NotI, S: SfiI, Sal: SalI.

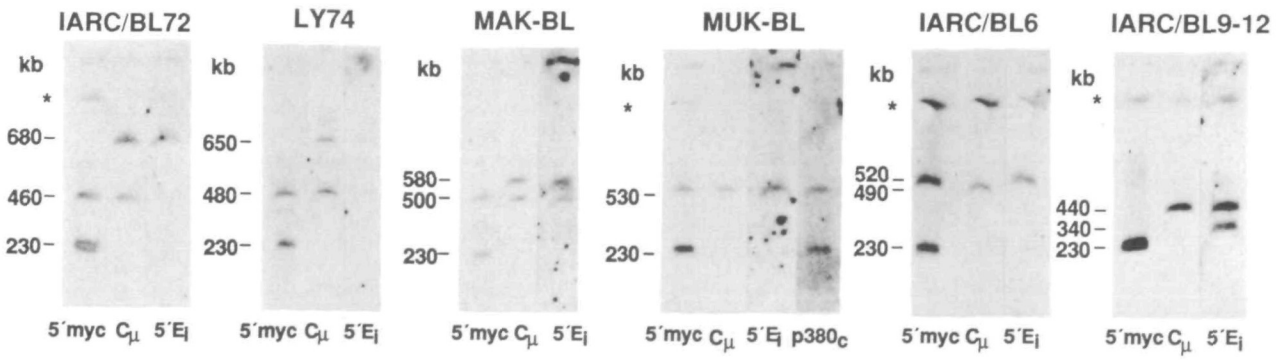


Figure 3. Comigration of rearranged restriction fragments in cell lines with t(8;14) class III translocation after digestion with NotI and SalI and consecutive hybridization with c-myc (*5′myc*) and IgH (C_μ, $5'E_i$) specific probes. The relative position of c-myc and the IgH locus and the origin of the hybridization probes is given in Fig. 2. In MUK-BL, IARC/BL6 and IARC/BL9/12 one C_μ allele has been deleted during switch recombination (see text). The asterix (*) denotes unspecific hybridization of DNA of large size not resolved under the conditions applied ('compression zone', 49).

breakpoints are located upstream of the IgH intron enhancer on chromosome $14q^+$ in these 6 cell lines.

A different picture emerged for IARC/BL74. As shown in Fig.5a, the probes C_μ and $p380_c$ detected the same rearranged fragment, whereas the $5'E_i$ and the $3'E_i$ probes visualized fragments of different size. The translocation breakpoint is thus located 3′ of the intron enhancer within the μ switch region.

In Namalwa and IARC/BL16 probes C_μ and $5'E_i$ visualized two fragments of identical size (for Namalwa see Fig.5b), not allowing to determine the breakpoints within the IgH gene. For further analysis of these cell lines in situ hybridization was applied (see below).

The results of PFGE mapping of both chromosomal breakpoints in all 9 cell lines are compiled and described in more detail in Fig.6.

Mapping of chromosomal breakpoints by using two colour fluorescence *in situ* hybridization

Fluorescence in situ hybridization allows a highly specific delineation of chromosomal target regions. High efficiency in target detection is achieved when probes larger than 5–10 kb are being used. Therefore we first isolated cosmid clones ('*cos-H4.1*','*cos-p380j9*' and '*cos-myc72*') by using the probes *H4.1*, *p380j9* and a c-myc probe representing the ClaI/EcoRI fragment

covering the third exon. The origin of the clones *cos-*$C_{\alpha 2}$ containing the $\alpha 2$ constant gene and λ-$C_{\mu\delta}$ spanning the IgH constant μ and δ region is described in Materials and Methods.

In order to pinpoint the chromosomal translocations, it was determined on which side of the breakpoints the probes occur. Multiple probe pairs were used for two colour fluorescence in situ hybridization on metaphase chromosomes as well as interphase nuclei. Combination of suitable probe pairs allowed to assess unambiguously the position of the breakpoints by either co-localization of chromosome 8 and 14 probes, or by separation of adjacent chromosome 8 probes (see schematic drawings in Fig.7). The results with the probe pairs critical for defining the chromosomal breakpoint are shown for IARC/BL16 in Fig. 7 A–F and for Namalwa in Fig. 7 G–L.

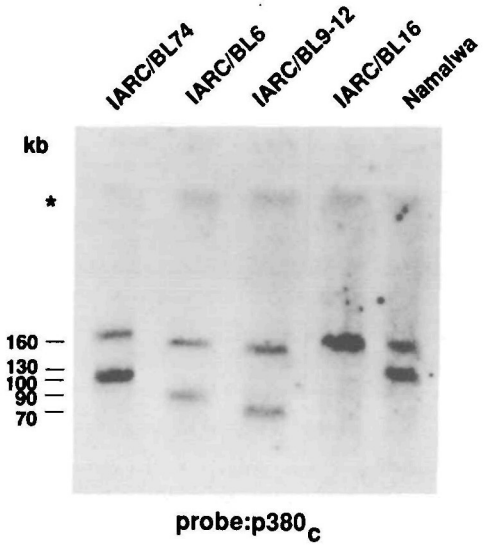


Figure 4. Rearranged fragments between 180 kb and 340 kb 5′ of c-myc in BL cell lines with t(8;14) class III translocation visualized by Southern blot analysis after digestion with SfiI and hybridization with probe *p380*$_c$ (see Fig.2). Note that in IARC/BL16 cells only the germline configuration is found. The compression zone is denoted by an asterix (see Fig.3).

After hybridization to metaphase chromosomes of IARC/BL16 cells, one c-myc and one λ-$C_{\mu\delta}$ specific signal was found on the normal chromosomes 8 and 14, respectively, whereas the second c-myc and λ-$C_{\mu\delta}$ signals colocalized on chromosome 14q$^+$ (Fig. 7A). This demonstrates that in IARC/BL16 cells the chromosome 14 breakpoint of the translocation is upstream of the λ-$C_{\mu\delta}$ fragment, presumably in the joining region (see schematic drawing in Fig. 7). Correspondingly in interphase nuclei again one c-myc and one λ-$C_{\mu\delta}$ signal colocalize (Fig. 7B, F). The same pattern was found when the *p380j9* cosmid and the phage λ-$C_{\mu\delta}$ were used for hybridization (not shown), indicating that the breakpoint is located proximal of *p380j9*. This is confirmed by colocalization of both c-myc and *p380j9* alleles (Fig.7C). On the other hand, when probes *H4.1* and *cos-myc72* or *H4.1* and *p380j9* were used, the signal from one allele colocalized as expected whereas the second *H4.1* signal was separated from *cos-myc72* or *p380j9* (Fig. 8D, E). This maps the breakpoint of the translocation between *H4.1* and *p380j9*. Since PFGE analysis revealed that the breakpoint is located more than 340 kb upstream of c-myc, it can now be narrowed down to a region of 160 kb between the SfiI site (at position 340 kb) and the NarI site (at position 500 kb) 5′ of c-myc.

In Namalwa cells the situation was more complex and thus more difficult to assess. Colocalization of the *cos-myc72* and λ-$C_{\mu\delta}$ probes was not observed (Fig.7H). In contrast, one c-myc

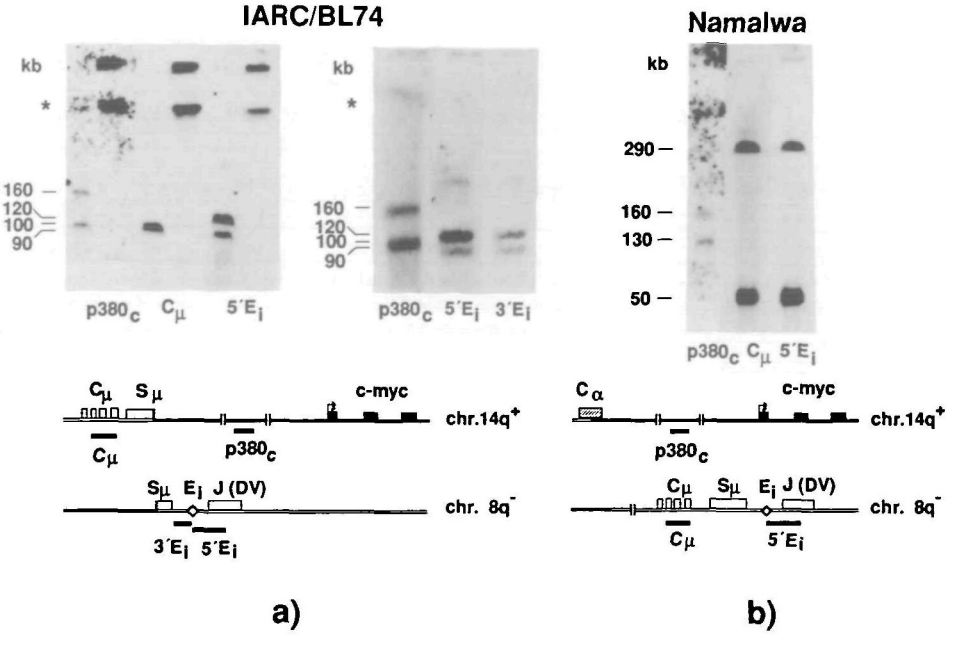


Figure 5. a) Identification of the chromosomal breakpoint within the switch region of C_μ (S_μ) in IARC/BL74 cells. Two PFGE Southern blots are shown. Only the C_μ probe comigrates with the rearranged fragment detected by probe *p380*$_c$ but not the probes located 5′ and 3′ of the intron enhancer (*5′E*$_i$ and *3′E*$_i$). The structure of chromosomes 14q$^+$ and 8q$^-$ is drawn schematically at the bottom part. b) Same analysis with Namalwa cells. The IgH probes C_μ and *5′E*$_i$ do not comigrate with the rearranged SfiI fragment of 130 kb detected by probe *p380*$_c$ (see Fig.2 for abbreviations).

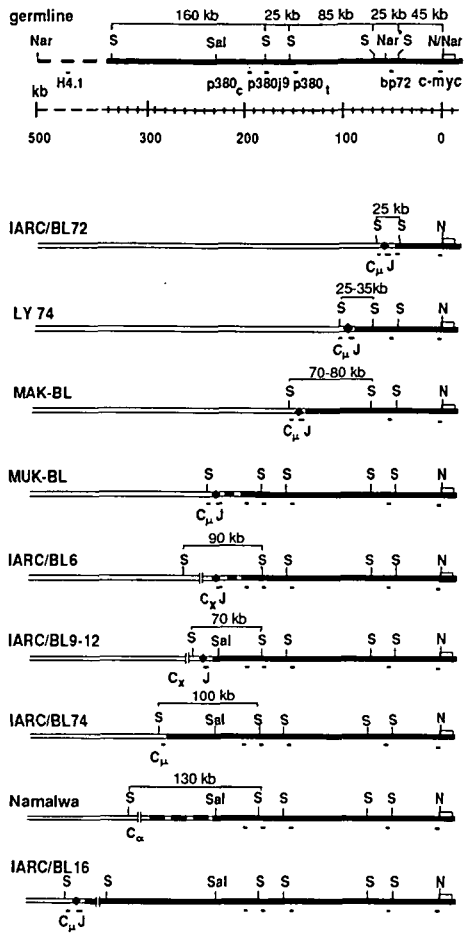


Figure 6. Structure of chromosome 14q+ in 9 cell lines with t(8;14) class III translocation. Bolded: chromosome 8 sequences, thin-lined: chromosome 14 sequences. The size of the rearranged SfiI fragments used to determine the position of chromosomal breakpoints upstream of c-myc more precisely are indicated. In **IARC/BL72** a probe representing the breakpoint of this cell line, termed *bp72*, is located on a 25 kb SfiI fragment, about 55 kb 5′ of c-myc. Sequence analysis revealed, that the breakpoint within the IgH gene occurred in the J6 segment of the joining region (38). In **LY74** and **MAK-BL** the distance between the breakpoint within the J-region of the IgH locus and a SfiI site immediatedly downstream of C_μ is about 10 kb. Therefore in LY74 the chromosomal breakpoint must be located 15 to 25 kb upstream of the SfiI site (at position 70 kb), i.e. between 85 and 95 kb upstream of c-myc. Correspondingly the chromosomal breakpoint in MAK-BL cells was mapped between 120 and 130 kb upstream of the gene. In **MUK-BL** and **IARC/BL6** an identical fragment pattern was observed after digestion with NotI/SalI and hybridization with either the c-myc or the *p380c* probe (for MUK-BL see Fig.3). Thus the chromosomal breakpoint must be located between probe *p380c* and the SalI site, i.e. between 200 and 230 kb 5′ of c-myc. Since the intron enhancer is present on 14q+, the breakpoint within the IgH locus is located presumably within 2 kb upstream of the intron enhancer E_i (see text). In **IARC/BL9-12** a rearranged SfiI fragment of 70 kb was visualized with probe *p380c* (Fig.4) but no rearrangement was found after NotI/SalI digestion, i.e. within 230 kb upstream of c-myc, using *5′myc* as probe (Fig.3). Consequently, the breakpoint is located within 20 kb proximal to the SalI site. The breakpoint in the IgH locus maps again within 2 kb upstream of E_i (see text). In **IARC/BL74** the distance between the chromosomal breakpoint and the SfiI site downstream of C_μ is about 5 kb. The remaining part of the 100 kb SfiI fragment, detected by probe *p380c* is located proximal to the SfiI site 180 kb 5′ of c-myc. Thus in IARC/BL74 the chromosomal breakpoint maps about 275 kb upstream of the c-myc gene. Assignement of the chromosome 14 breakpoint to the IgH switch region was described in the text. The breakpoint position in **Namalwa** and **IARC/BL16** cells was concluded from two colour fluorescence in situ hybridization (see text). N: NotI, Nar: NarI, Sal: SalI, S: SfiI, C_α: α constant gene segment, C_x: undetermined constant gene segment of the IgH locus.

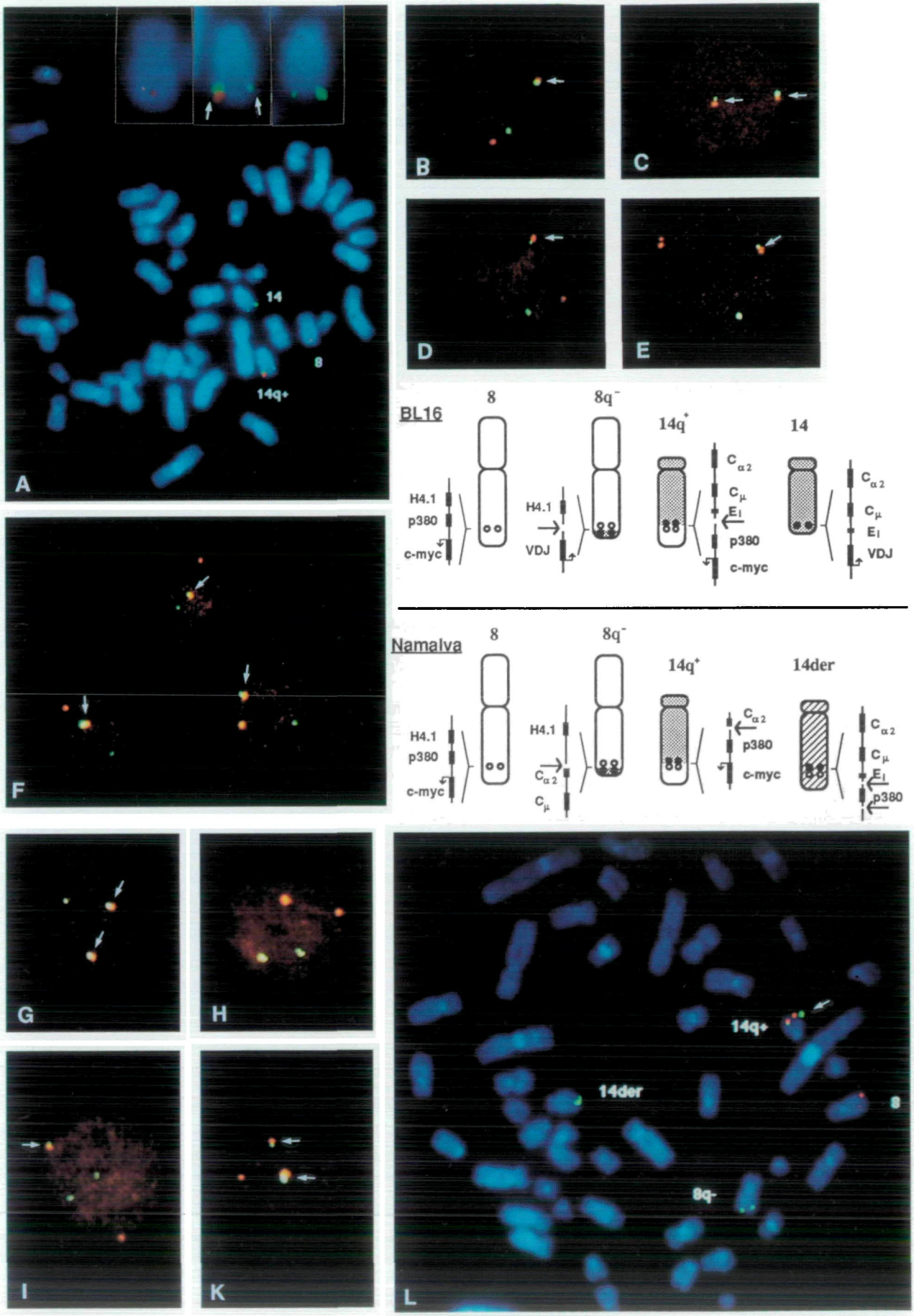
allele colocalized with the constant α2 probe (Fig.7I and L). This indicates that in Namalwa cells another immunoglobulin heavy chain isotype gene region was involved in the translocation event. On chromosome 8 the breakpoint maps between *p380j9* and *H4.1*, consistent with the data obtained by PFGE analysis. The breakpoint can thus be assigned to a region between 230 kb and 340 kb upstream of c-myc. Two probes, *cos-*$C_{\alpha 2}$ and *cos-p380j9*, generated three hybridization signals (Fig. 7.G, K). The third signal of *cos-*$C_{\alpha 2}$ can best be explained by assuming that this cosmid spans the breakpoint of the translocation and thus visualized additionally the reciprocal product of the translocation on chromosome $8q^-$. This explains also the colocalization of $C_{\alpha 2}$ and *H4.1* signals on chromosome $8q^-$ (not shown). In situ hybridization showed, that the third *cos-p380j9* signal is localized on one of the marker chromosomes known to exist in Namalwa cells (24, 25). It is likely composed of chromosome 14 and chromosome 8 specific sequences as well as further material of unknown origin (14der in Fig 7 L).

DISCUSSION

In this study, we have mapped the position of breakpoints of t(8;14) class III translocations on chromosome 8 as well as on chromosome 14 in BL cell lines. Of 44 cell lines studied, 9 had the breakpoint more than 14 kb 5′ of c-myc. The breakpoints of these lines were analyzed in more detail. In two cases, where PFGE failed to provide conclusive data, two colour fluorescence in situ hybridization allowed to asses the chromosomal breakpoints. On chromosome 8 the breakpoints were found to be scattered within a region of more than 340 kb upstream of c-myc. The breakpoints are not clustered in the region 180 kb upstream of c-myc which was previously described as the breakpoint aerea of 380, Daudi, P3HR1 and EW36 BL cells (4, 26). The distribution of breakpoints upstream of c-myc mirrors the distribution of breakpoints in the variant translocations downstream of the gene. In both cases they are scattered over a distance of slightly more than 300 kb with limited clustering of breakpoints at 140 and 180 kb distant from c-myc (27 and this study).

Downstream of c-myc a transcription unit called PVT has been shown to be affected by the majority of the variant t(2;8) and t(8;22) translocations (28, 29). However there is no evidence for a role of PVT in BL pathogenesis (30). The homogeneous distribution of t(8;14) class III breakpoints over a large aerea also argues against a gene that would play a role in BL tumorigenesis in addition to c-myc.

It is generally believed, that regulatory elements in the IgH locus control c-myc in cis in BL cells. In order to identify the putative regulatory elements, it is essential to know the relative position and the distances between c-myc and the immunoglobulin genes resulting from the translocation events. In 7 of 9 cases the breakpoint of the translocation with regard to the IgH locus is in the VDJ region. In these cases the distance between c-myc and the intron enhancer is given by the distance between the breakpoint on chromosome 8 and c-myc. The distance varies between 55 kb in IARC/BL72 and more than 340 kb in IARC/BL16 cells. Two cases, Namalwa and IARC/BL74, were exceptional: In Namalwa cells, the translocation occured even in another heavy chain isotype gene ($C_{\alpha 2}$). In rats and mice but not yet in humans a 'downstream enhancer' has been identified 3′ of the constant α gene (10, 11). Given the high degree of functional conservation between the murine and human


A
14
8
14q+
B
C
D
E
BL16
8
8q⁻
14q⁺
14
H4.1
p380
c-myc
VDJ
Cα2
Cμ
Eι
F
Namalva
14der
G
H
I
K
L
14q+
14der
8
8q-

immunoglobulin gene loci, there is a high likelihood, that the human IgH locus also bears a downstream enhancer element. In Namalwa cells the translocated part of chromosome 8 would thus be close to this putative element.

In IARC/BL74 cells, a sporadic BL with the breakpoint about 280 kb upstream of c-myc, the translocation within the IgH locus has targeted the μ switch site rather than the joining region. According to these data and the physical map of the human IgH locus (31) the distance between c-myc and the putative downstream enhancer would be in this case in the range of 600 kb. These distances are very large and it might be difficult to explain the action of cis elements over such big regions. The cis-activating function of enhancers could be dependent on the linear distance between the enhancer and the target promoter on the DNA or, more generally, on their spatial distance in chromatin. We are presently trying to determine the distance between the Ig enhancers and c-myc in BL t(8;14) class III translocations in interphase chromatin by multicolour fluorescence in situ hybridization combined with 2-D and 3-D analysis.

Interestingly we observed a strong prevalence of class III translocations in endemic vs. sporadic BL with t(8;14) translocations. This is in agreement with Pellici et al. (22) and Haluska et al. (4). Of 17 endemic BL cell lines with t(8;14) translocation, 10 were class III. In contrast, only 2 of 16 sporadic BL had the breakpoints more than 7 kb upstream of c-myc. It is tempting to speculate that in endemic BL a less stringent deregulation of c-myc, reflected by a greater variability of the breakpoint positions, would suffice for tumour induction or development, due to the presence of a cooperating pathogen. Epstein–Barr Virus (EBV) which immortalizes B-cells in vitro, is a good candidate for such a cofactor, since all endemic BL carry the EBV genome. In contrast, we found only 50% (8/16) EBV positive cases among sporadic BLs.

MATERIALS AND METHODS

Tumour cell lines

All cell lines were grown as stationary suspension cultures at 37°C in an atmosphere of 5% CO_2 and 95% air in RPMI1640 supplemented with 10% fetal calf serum, 300 μg/ml glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin and 1 μg/ml amphotericin B. The cell lines used contain t(8;14) translocations as shown by cytogenetic analysis, and were derived as follows: IARC307, IARC/BL6, IARC/BL9-12, IARC/BL16, IARC/BL18, IARC/BL72, LY74 and IARC/BL74 established at the International Agency of Research on Cancer at Lyon (32, 33); MUK-BL and MAK-BL from A.Rickinson (34); P3HR1 from Y.Hinuma (35); Daudi and Namalwa from G.Klein (36, 37).

Cloned probes

Chromosome 8: For detection of rearrangements within c-myc, a 1.3 kb EcoRI/ClaI fragment representing the third exon was used. For PFGE analysis a HindIII/ClaI fragment (*'5'myc'*), located immediatedly 5' of c-myc promoters P1/P2 was used. *bp72* represents the chromosomal breakpoint of the BL cell line IARC/BL72 (38). p380j9 0.8Ss (*'p380j9'*) is close to the t(8;14) translocation breakpoints of the cell lines 380, EW36, Daudi and P3HR-1 (4, 26). pEW36-9;H2,0 (*'$p380_t$'*) is a 2 kb HindIII fragment, which was obtained by chromosomal walking about 25 kb in telomeric direction from *p380j9*. pEW36-7D;SsB0.6 (*'$p380_c$'*) is a 0.6 SstI/BamHI fragment located about 20 kb in centromeric direction from *p380j9*, also obtained by chromosomal walking (38). *H4.1* represents a HPV18 integration site in HeLa cells (39). The 1.3 kb EcoRI/ClaI myc probe, the *p380j9* probe and the *H4.1* probe were used to isolate three cosmids (*'cos-myc72'*, *'cos-p380j9'* and *'cos-H4.1'*) from a cosmid library of IARC/BL72 (38).

Chromosome 14: Probe C_μ is a 1.2 kb EcoRI fragment, containing the first and second C_μ-exon (40). The enhancer probes were obtained by subcloning a genomic 3.1 kb EcoRI/HindIII fragment (41) which overlaps the IgH intron enhancer and part of the joining gene segment resulting in a 2,2 kb EcoRI/BglII fragment (*5'E_i*) and a 0,9 kb BglII/HindIII fragment (*3'E_i*). Probes for in situ hybridization: phage λ-$C_{\mu\delta}$ isolated from a EMBL3 library of IARC/BL18 DNA and spanning the IgH constant μ and δ region; cosmid *cos-*$C_{\alpha 2}$ containing the $C_{\alpha 2}$ gene segment isolated from a cosmid library of human placenta DNA: a 780 bp hybridization probe was generated by PCR using C_α specific primers according to the sequence described (42) (5'ATCCAGAATTCAGAAGCGAA-CCTCACGTG 3' and 5' ATGGTGAATTCTGTGTGAAGGCCAGCGGC 3'). Isolated α1- and α2-cosmids were discriminated by hybridization with a $C_{\alpha 2}$ specific oligonucleotide (5' GTACAGCCTGTCCTCCTGGGGACA-TGGCAT 3').

Large-scale mapping using pulsed field gel electrophoresis (PFGE)

For PFGE analysis the protocol described by Smith et al. (43) was followed using the CHEF-II system (Biorad). PFGE-gels were blotted to nylon filters which were hybridized according to Maniatis et al. (44) using DNA probes labelled by the random priming method (45).

Fluorescence *in situ* hybridization

In situ hybridization to acetic acid fixed mitotic chromosomes and interphase nuclei was according to Lichter et al. (46). For two colour hybridizations, probes were labelled with biotin or digoxigenin and detected via FITC and rhodamine. Chromosomes were counterstained with DAPI. For documentation digitized images of FITC, rhodamine or DAPI fluorescence were generated using a confocal laser scanning microscope (LSM 10, Zeiss, Oberkochen) as described by Lichter et al. (46; Fig. 7 B–K) or by a cooled CCD camera (Photometrix, Tucson, AZ) as described by Boyle et al.(47; Fig. 7A, L) and overlayed electronically.

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ABBREVIATIONS

BL: Burkitt's lymphoma; IgH-gene: immunoglobulin heavy chain gene; PFGE: pulsed field gel electrophoresis; FITC: Fluoresceine-iso-thiocyanate; DAPI: 4'6'-Diamidine-2-phenylindole-dihydrochloride.

REFERENCES

1. Bornkamm,G.W., Polack,A. and Eick,D. (1988) In Klein,G.(ed.), Cellular Oncogene Activation, Dekker,M.,Inc., New York, Basel, pp.223–273.
2. Cory,S. (1986) *Adv. Cancer Res.*, **47**, 189–234.
3. Neri,A., Barriga,F., Knowles,D.M., Magrath,I.T. and Dalla-Favera,R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 2748–2752.
4. Haluska,F.G., Finger,S., Tsujimoto,Y. and Croce,C.M. (1986) *Nature*, **324**, 158–161.
5. Haluska,F. G., Tsujimoto,Y. and Croce,C.M. (1987) *Proc. Natl. Acad. Sci. USA*, **85**, 6835–6839.
6. Buluwela,L., Albertson,D.G., Sherrington,P., Rabbitts,P.H., Spurr,N. and Rabbitts,T.H. (1988) *EMBO J.*, **7**, 2003–2010.

Figure 7. Two colour in situ hybridization of interphase nuclei and metaphase spreads in the cell lines IARC/BL16 (A–F) and Namalwa (G–L). Pairs of biotin and digoxigenin labelled probes were hybridized as described in Material and Methods. The following combinations of probes are shown: A, B, F: *cos-myc72* (red)/λ-$C_{\mu\delta}$ (green); C: *cos-myc72* (green)/*cos-p380j9* (red); D: *cos-myc72* (red)/*cos-H4.1* (green); E: *cos-p380j9* (red)/*cos-H4.1* (green); G: *cos-p380j9* (red)/*cos-*$C_{\alpha 2}$ (green); H: *cos-myc72* (red)/λ-$C_{\mu\delta}$ (green); I, L: *cos-myc72* (red)/*cos-*$C_{\alpha 2}$ (green); K: *cos-myc72* (green)/*cos-p380j9* (red). Schematic drawings show the structure of the translocated chromosomes in both cell lines. In panel A, the chromosomes showing hybridization signals are also shown in the insert.

7. Queen,C. and Baltimore,D. (1983) *Cell*, **33**, 741–748.
8. Gillies,S.D., Morrison,S.L., Oi,V.T. and Tonegawa,S. (1983) *Cell*, **33**, 717–728.
9. Banerij,J., Ohlson,L. and Schaffner,W. (1983) *Cell*, **33**, 729–740.
10. Pettersson,S., Cook,G.P., Brüggemann,M., Gareth,T.W. and Neuberger,M.S. (1990) *Nature*, **344**, 165–168.
11. Lieberson,R., Giannini,S.L., Birshtein,B.K. and Eckhard,L.A. (1991) *Nucleic Acids Res.*, **19**, 933–937.
12. Potter,M., Weir,L. and Leder, P. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7161–7165.
13. Meyer,K.B. and Neuberger,M.S. (1989) *EMBO J.*, **8**, 1959–1964.
14. Müller,B., Stappert,H. and Reth,M. (1990) *Eur. J. Immunol.*, **20**, 1409–1411.
15. Hagman,J., Rudin,C.M., Haarsch,D., Chaplin,D. and Storb,U. (1990) *Genes Dev.*, **4**, 978–992.
16. Nishikura,K., ar-Rushdi,A., Erikson,J., deJesus,E., Dugan,D. and Croce,C. (1984) *Science*, **224**, 399–402.
17. Croce,C.M., Erikson,J., Huebner,K. and Nishikura,K. (1985) *Science*, **227**, 1235–1238.
18. Nishikura,K., ar-Rushdi,A., Erikson,J., Watt,R., Rovers,G. and Croce, C.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 4822–4826.
19. Adams,J.M., Harris,A.W., Pinkert,C.A., Corcoran,L.M., Alexander,W.S., Cory,S., Palmiter,R.D. and Brinster, R.L. (1985) *Nature*, **318**, 533–538.
20. Ladjadj,Y., Philip,T., Lenoir,G.M., Tazerout,F.Z., Bendisari,K., Boukheloua,R., Biron,P., Brunat-Mentigny,M. and Aboulola,M. (1984) *Br. J. Cancer*, **49**, 503–512.
21. Parkin,D.M., Sohier,R. and O'Conor,G.T. (1985) In Lenoir,G.M., O'Conor,G.T. and Olweny,C.L.M. (eds.), Burkitt's Lymphoma. IARC scientific publications (No. 60), International agency for research on cancer, Lyon, pp.155–164.
22. Pelicci,P.G., Knowles,D.M., Magrath,I. and Dalla-Favera,R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 2984–2988.
23. Shiramizu,B., Barriga,F., Neequaye,J., Jafri,A., Dalla-Favera,R., Neri,A., Guttierez,M., Levine,P. and Magrath,D. (1991) *Blood*, **77**, 1516–1526.
24. Wurm,F., Polastri,G., Hilfenhaus,J., Harth,H. and Zankl,H. (1985) *Develop. Biol. Standard*, **60**, 393–403.
25. Ruppersberger,P. Arnold,M., Zankl,H. and Scherthan,H (1991) *Genes, Chromosomes and Cancer*, **3**, 394–399.
26. Haluska,F.G., Tsujimoto,Y. and Croce,C.M. (1988) *Nucleic Acids. Res.*, **5**, 2077–2085.
27. Henglein,B., Synovzik,H., Groitl,P., Bornkamm,G.W., Hartl,P. and Lipp,M. (1989) *Mol. Cell. Biol.*, **9**, 2105–2113.
28. Shtivelman,E. and Bishop,J.M. (1989) *Mol. Cell. Biol.*, **9**, 1148–1154.
29. Shtivelman,E., Henglein,B., Groitl,P., Lipp,M. and Bishop,J.M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 3257–3260.
30. Shtivelman,E. and Bishop,J.M. (1990) *Mol. Cell. Biol.*, **10**, 1835–1839.
31. Hofker,H.M., Walter,W.A. and Cox, D.W. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 5567–5571.
32. Lenoir,G.M., Vuillaume,M. and Bonnardel,C. (1985) In Lenoir,G.M., O'Conor,G.T. and Olweny,C.L.M. (eds.), Burkitt's Lymphoma. IARC scientific publications (No. 60), International agency for research on cancer, Lyon, pp.309–318.
33. Bernheim,A., Berger,R. and Lenoir,G.M. (1983) *Cancer Genet. Cytogenet.*, **8**, 223–229.
34. Rooney,C.M., Gregory,C.D., Rowe,M., Finerty,S., Edwards,C., Rupani,H. and Rickinson,A.B. (1986) *J. Natl. Cancer Inst.*, **77**, 681–687.
35. Hinuma,G. and Grace,J.T.(1967) *Proc. Soc. Exp. Biol. Med.*, **124**, 107–111.
36. Klein,E, Klein,G. Nadkarni,J.S., Nadkarni,J.J., Wigzell,H. and Cliffort,P. (1968) *Cancer Res.*, **28**, 1300–1310.
37. Klein,G., Dombos,L. and Gothoskor,B. (1972) *Int. J. Cancer*, **10**, 44–57.
38. Joos et al. (1992) *Cancer Res.*, In Press.
39. Dürst,M., Croce,C.M., Gissmann, L., Schwarz,E. and Hübner,K. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1070–1074.
40. Forster,A., Hobart,M., Hengartner,H. and Rabbitts,T.H. (1980) *Nature*, **286**, 897–899.
41. Lawn,R.M., Fritsch,E.F., Parker,R.C., Blake,G. and Maniatis, T. (1978) *Cell*, **15**, 1157–1174.
42. Flanagan,J.G., Lefranc,M.P. and Rabbitts,T.H. (1984) *Cell*, **36**, 681–688.
43. Smith,C.L., Klco,S.R., Cantor,C.R. (1988) In Davies,K.(ed.), Genome Analysis: A Practical Approach., IRL Press, McLean, VA., pp.41–72.
44. Maniatis,T., Fritsch,E.F. and Sambrook,J. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor.
45. Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **132**, 6–13.
46. Lichter,P., Tang,C.T., Call,K., Hermanson,G., Evans,G.A., Housman,D. and Ward,D.C. (1990) *Science*, **247**, 64–69.
47. Boyle,A.L., Feltquite,D.M., Dracopoli, N.C., Housman, D.E. and Ward,D,C. (1992) *Genomics*, **12**, 106–115.
48. Gemmill,R.M., Coyle-Morris,J., Ware-Uribe,L., Pearson,N., Hecht,F., Brown,RS., Li,F.P. and Drabkin,H.A. (1989) *Genomics*, **4**, 28–35.
49. Mathew,M.K., Smith,C.L. and Cantor,C.R. (1988) *Biochemistry*, **27**, 9210–9216.