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N segment insertion and region-directed somatic hypermutation in a Kappa gene of a t(2;8) chromosomal translocation

H.-Gustav Klobeck, Gabriele Combriato and Hans G.Zachau

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, FRG

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ABSTRACT

A detailed molecular analysis of both reciprocal recombination products of the variant t(2;8) chromosomal translocation of the Burkitt lymphoma derived cell line JI and their germline counterparts was carried out. The breakpoint on chromosome 8 is localized 28 kb to the 3' side of the c-myc protooncogene, the breakpoint on chromosome 2 was found to be within an aberrrantly rearranged V_K gene (abbreviations ref.1). Novel features of the immunoglobulin moiety involved in this process include (a) insertion of extra nucleotides in the V-J junction which have the characteristics of a N segment as it has been found up to now only in heavy chain and T cell receptor genes; (b) the occurrence of somatic mutations in $8q^+$ and not in $2p^-$. These data allow a reconstruction of the course of events in the cell line JI; (c) remarkable sequence regularities at the chromosomal breakpoints consisting of symmetrically placed dinucleotides and elements related to the hepta- and nonanucleotide recombinase recognition sequences are discussed in the context of the translocation mechanism.

INTRODUCTION

Reciprocal chromosomal translocations involving the immunoglobulin gene loci on chromosomes 2 (Kappa light chain genes), 14 (heavy chain genes) and 22 (lambda light chain genes) and the c-myc oncogene on chromosome 8 are characteristic for the B-cell malignancy Burkitt's lymphoma (reviews e.g. 2-4). It is generally accepted that the translocations which lead to a apposition of immunoglobulin genes and c-myc result in a deregulation of the c-myc oncogene (reviewed in ref. 5), thereby promoting the tumour phenotype. In the t(8;14) translocations which constitute the majority of the Burkitt lymphomas the c-myc oncogene is moved to chromosome 14, while in the variant translocations t(2;8) and t(8;22) the protooncogene stays on chromosome 8. The translocation breakpoints on chromosome 8 are not clustered but dispersed over a region of more than 100 kb surrounding the c-myc

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oncogene (reviewed in ref. 3). The breakpoints on the chromosomes containing immunoglobulin genes however show a clear preference for heavy chain switch regions and the J regions of light chain genes, which can be interpreted to reflect an involvement of the immunoglobulin gene recombinase(s) in the translocation process.

The Burkitt lymphoma derived cell line JI (K^+,K^-) contains the variant t(2;8) chromosomal translocation (6) found in a few percent of Burkitt lymphomas (2-5). DNA hybridization studies of somatic cell hybrids (prepared from the human cell line JI and rodent cells) using a V_K and a c-myc probe had indicated that the translocation breakpoint on chromosome 2 resides within the V_K locus (7). Recently the breakpoint on chromosome 8 could be localized within a region 25-32 kb 3' of c-myc (8).

We have previously reported structural data of the productively rearranged Kappa light chain gene (9) and a reciprocal recombination product (10) of this cell line. We now extended our studies of the cell line by cloning the aberrantly rearranged Kappa gene, which unexpectedly was found to contain the chromosomal breakpoint. The molecular analysis of the reciprocal translocation products revealed a number of novel features of the immunoglobulin partner of the translocation process.

MATERIALS AND METHODS

The cell line JI was originally established from Burkitt lymphoma tissue derived from a german case of the B-cell malignancy (6).

High molecular weight DNA used for the construction of the genomic libraries was isolated from cultured cells as described in ref. 11, except that the liquid nitrogen step was omitted.

Partial genomic libraries from size selected DNA were constructed in the λ phage EMBL 3 (ref.12) as described (13). DNA fragments were subcloned in M13 phages (14) and sequenced by the dideoxy chain termination method (15).

RESULTS AND DISCUSSION

Analysis of the reciprocal translocation products and their germline counterparts

The course of our analysis of the t(2;8) chromosomal translocation products of the cell line JI is shown in Fig.1. The

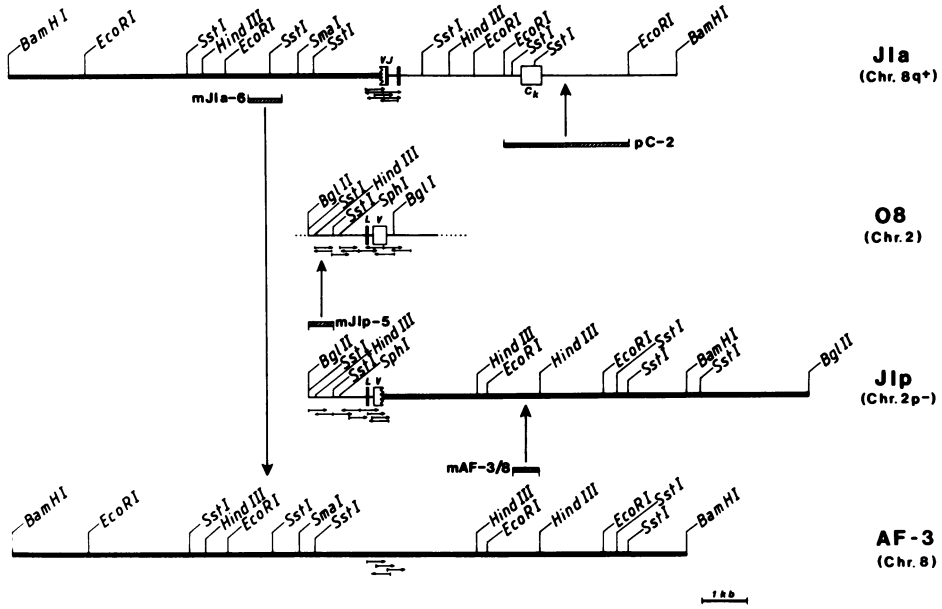

Figure 1. Course of analysis of the t(2;8) chromosomal translocation in JI cells. The C_K, J_K, and V_K gene segments are shown as open rectangles, chromosome 8 sequences as thick lines. The chromosomal origin of the fragments is indicated in brackets. O8 is a V_KI gene segment of the clone cos 146 (ref.17). Subclones used for the screening of the partial genomic libraries of JI DNA and placenta AF DNA are shown as hatched bars. The order of the cloning steps is indicated by vertical arrows and the extent and direction of DNA sequencing by horizontal arrows.

initial screening of a size selected genomic library of JI (13-17 kb Bam HI fragments) was done with the C_K probe pC-2 (ref.13) and led to the isolation of the aberrantly rearranged kappa allele JIa (the functional allele was already described in ref. 9). Hybridization data indicated that JIa contains a rearranged V_K gene which consists only of the 3' part of a V_K gene segment joined to the J_K4 segment, thus representing a truncated V_K gene. A subclone (mJIa-6 in Fig.1) from the 5' flank of the crippled V_K gene was prepared and hybridized with DNA of a panel of human-rodent somatic cell hybrids (previously used in ref.16). This experiment clearly showed that mJIa-6 is derived from chromosome 8 (data not shown).

For the following two cloning steps non-repetitive sequences

were selected as subclones by blot hybridization with nick-translated placenta DNA; the subclones were then used for the size selection and the cloning of the desired fragments from genomic libraries.

The germline chromosome 8 clone AF-3 was isolated by screening a size selected genomic library (13-17 kb Bam HI fragments of placenta AF) with the probe mJIa-6. Fragment AF-3 contains no sequences homologous to V_K genes but is particularly abundant with repetitive sequences. The subclone mAF-3/8 then served to clone from JI DNA (9-13 kb Bgl II fragments) the reciprocal translocation product to JIa which is called JIp.

The final cloning step was carried out by hybridization of blots of cosmid clones containing V_K gene segments (e.g. ref.17) with the subclone mJIp-5 from the chromosome 2 moiety of JIp. This led to the identification of the parent chromosome 2 sequence including the V_KI gene 08. Identification rests in each case on restriction mapping, hybridization with rodent-human cell hybrids and extensive sequence data.

Localization of the chromosomal breakpoint

Previous work has indicated that the t(2;8) chromosomal translocation in JI cells had occurred by breakage of chromosome 8 approximately 25-32 kb downstream of the c-myc gene (8) and of chromosome 2 within the cluster of V_K gene segments (7). The breakpoint on chromosome 8 can now be localized 28 kb downstream of c-myc by a combination of the maps of ref.8 and of Fig.1 of the present paper. Recent chromosomal in situ hybridization studies led to the proposal that the breakpoint on chromosome 2 is located between the V_K and J_K clusters (18,19). According to our data this breakpoint lies exactly within the rearranged V_KI gene 08.

It was recently shown that the human Leu-2/T8 gene segregates with the $8q^+$ chromosome of JI cells in somatic cell hybrids (20). This led to the conclusion that Leu-2/T8 maps either to the 3' side of C_K or between the V_K gene segments and C_K. Our localization of the breakpoint on chromosome 2 now places the Leu-2/T8 gene clearly on the 3' side of C_K.

A N segment in a rearranged V_K gene

The 5' half of the non-productively rearranged V_KI gene was

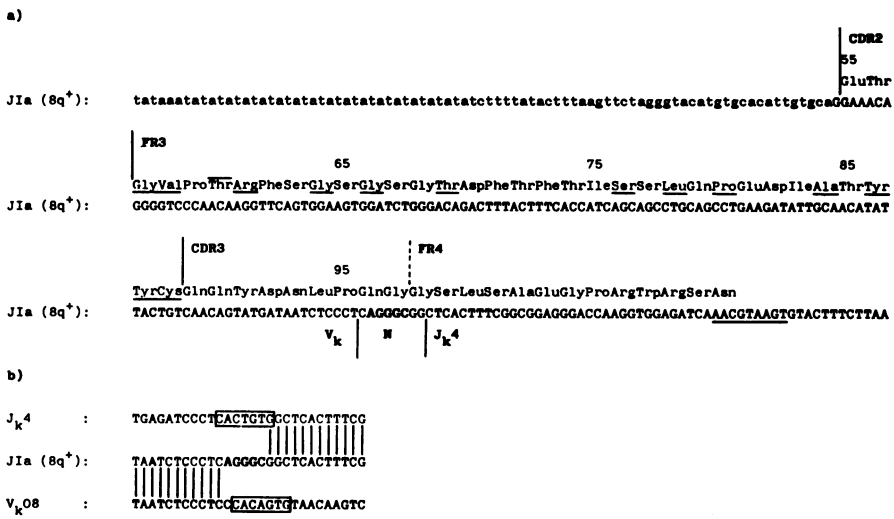

Figure 2. Nucleotide and deduced amino acid sequences of the aberrantly rearranged allele of JI (a) and comparison of the sequence around the V-J junction with the germline J_K4 and V_K 08 sequences (b). Chromosome 2 and 8 derived nucleotides are in capital and small letters, respectively, a putative N segment in bold face capital letters. FR, CDR: framework and complementarity determining regions, respectively. The sequences were obtained using the strategies shown in Fig.1. Only the relevant parts of the sequences are shown; the entire sequences are being transmitted to the EMBL nucleic acid data library. (a) The region homologous to a V_K gene was determined by comparison with known V_K sequences and the germline counterpart 08. Invariant amino acid residues (25) characteristic for the V_K subgroup I are underlined, the single altered amino acid residue (pos. 60, line above the symbol) is attributed to a somatic mutation (Fig.3a). Numbering is according to ref. 25. The donor splice site of the J_K4 gene segment is underlined. The formal translation of the $8q^+$ sequence is not continued into the chromosome 8 derived moiety although the open reading frame continues throughout the stretch of simple sequence DNA. (b) The heptanucleotide recognition sequences (5) are boxed. The J_K4 sequence is taken from ref. 41. The germline gene segment 08 is a potentially functional gene segment; its full sequence will be published elsewhere.

sequenced on fragment JIp (Chr. $2p^-$), its 3' half on fragment JIa (Chr. $8q^+$), and the corresponding germline V_KI gene segment from cos 146 as shown in Fig.1. It is seen from the chromosome $8q^+$ derived sequence (Fig.2a) that eight nucleotides are interspersed between codon 95 which is normally the last codon of a V_KI gene segment and the first codon of J_K4. This results in a frameshift within J_K4. At least the five nucleotides AGGGC do not

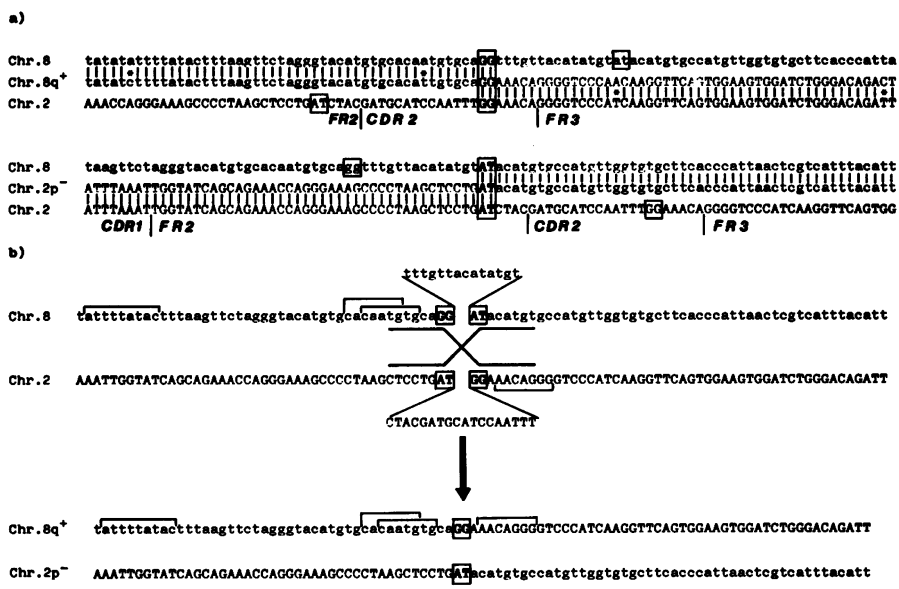

Figure 3. Alignment of the nucleotide sequences around the translocation breakpoints and a model for the t(2;8) chromosomal translocation of JI. Capital and small letters and abbreviations are as in Fig.2. Only parts of the sequences (Figs.1 and 2) are shown. The dinucleotides marking the breakpoints are boxed. (a) Nucleotide differences between chromosome $8q^+$ and chromosome 2 and 8 sequences are marked by asterisks. (b) Sequences related to the immunoglobulin recognition sites (23) are indicated by brackets.

originate from the germline V_KI sequence 08 or from the J_K4 sequence (Fig.2b). Such an inserted piece of DNA can arise either from a germline encoded sequence like the D elements of heavy chain genes or from a template independent addition of nucleotides by terminal deoxynucleotidyl transferase yielding a N segment (21-24).

Neither a D nor a N segment has been found yet in rearranged Kappa light chain genes (22,23) or proteins (25). If D elements existed in the germline of the Kappa locus one would expect to have found them in one of the numerous sequenced V_K genes or proteins. The transferase, on the other hand, is known to occur in B cells although at an early stage of development when the heavy chain genes rearrange (21-23). If this enzyme has been involved in the formation of a N_K segment one would then have to

assume that the V_K-N-J_K formation had occurred early in the ontogeny of the JI cells or, less likely, that it had been caused by a late appearance of the terminal transferase. It cannot be ruled out that a different enzyme was involved but the high GC content of the insert is in accord with the known GC preference of the transferase. On the basis of these arguments we consider it very likely that the extra nucleotides in JIa represent a N segment. It should be mentioned that the formation of a N-like segment in a Kappa gene was also observed when a Kappa gene construct was introduced into pre-B cells (26).

The t(2;8) chromosomal translocation precedes the productive Kappa gene rearrangement.

An interesting feature of the rearranged and translocated sequence of chromosome $8q^+$ is the presence of nucleotide sequence differences as compared to the respective parts of the parent chromosomes 2 and 8 (marked by asterisks in Fig.3a). In contrast no such differences were found between the $2p^-$ sequence and its germline counterparts (1.6 kb and 0.2 kb of sequence determined on the chromosome 2 and 8 derived parts, repectively; data not shown). The clustering of four sequence differences within 90 bp (Fig.3a) and the absence of base changes in the adjacent 300 bp (not shown) indicate that the differences result from a hypermutation process (review 23). This process apparently affected both the truncated V_KJ_K gene and the adjacent chromosome 8 derived part of chromosome 8.

In JI the productively rearranged V_KIV gene was also affected by the somatic mutation process (9). It is interesting to note that the mutations seem to be restricted to a certain region around a V_KJ_K junction and that the hypermutation process is not directed by the major part of a V_K gene segment or its 5' flanking region. Furthermore this mechanism does not discriminate between functional and non- functional rearranged Kappa genes (27), and does not affect reciprocal recombination products like f fragments (10). A simple correlation between transcriptional activity and the hypermutation mechanism is also not very likely since the aberrantly rearranged Kappa allele in JI (chromosome $8q^+$) is not transcribed at a rate similar to the productively rearranged allele (7; data confirmed in our laboratory). It can

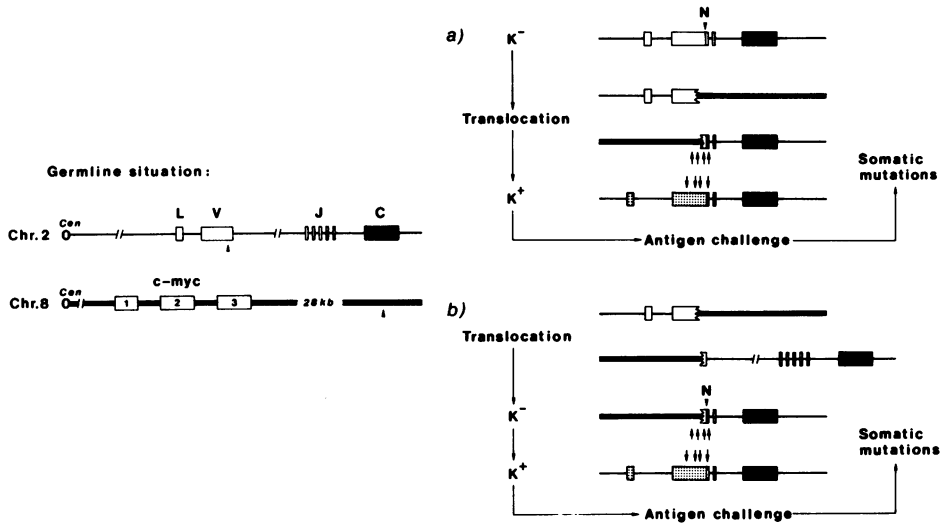

Figure 4. Two alternatives for the sequence of DNA rearrangements in JI cells. The germline situation of chromosomes 2 and 8 is shown for comparison. Drawings are not to scale. Exons are shown as rectangles, the C_K exon is shaded, c-myc exons are numbered . Chromosome 8 is depicted by a thick line; Cen, centromer region of the chromosomes. The breakpoints are marked by an arrowhead. (a) The aberrant Kappa gene rearrangement (K^-) precedes the chromosomal translocation. Insertion of the N segment (N) is marked by an arrow. The productively rearranged V_KIV gene (K^+)is dotted, somatic mutations are marked by arrows.
(b) The chromosomal translocation precedes the aberrant kappa gene rearrangement. Additional V_K gene segments are present in the germline between the V_K gene segment which is involved in the translocation and the J_K region (not shown).

therefore be concluded that the somatic hypermutation machinery is likely to be guided by sequences of the J_K-C_K region.

Since it is accepted that the hypermutation process takes place only after contact of lymphoid cell clones with antigen (28,29), the functional rearrangement (V_KIV-J_K4, ref. 9) must have occurred after the t(2;8) chromosomal translocation. There are two alternatives for the sequence of events in the JI cell line as outlined in Fig.4. It cannot be decided clearly whether the chromosomal translocation took place before or after the aberrant Kappa gene rearrangement occurred. However, we feel that an early V_K-J_K rearrangement including the addition of a N segment and subsequent chromosomal translocation involving a

strongly transcribed rearranged V_K gene (Fig.4a) is a more plausible sequence of events than a translocation involving a germline V_K gene segment and subsequent (V_K)-J_K rearrangement of the same gene segment (Fig.4b).

Structural features possibly involved in the translocation process.

No common translocation mechanism has emerged yet in Burkitt lymphomas (2-4). Homologous recombinations are clearly not responsible for the t(2;8) chromosomal translocations and only in a minority of lymphomas switch recombination enzymes might have been involved. Recently in a number of chromosomal translocations sequences resembling the hepta- and nonanucleotide recognition sequences involved in the rearrangement of the immunoglobulin genes were observed near the breakpoints (30-33). This led the authors to postulate that immunoglobulin gene recombinase(s) are involved also in those translocation processes.

The JI translocation may also fall into this group since recognition site related sequences are found at the breakpoints on chromosome 8 and, though less well conserved, on chromosome 2 (Fig.3b). While the hepta- and nonanucleotides on chromosome 8 fit the consensus sequences well, only five nucleotides of the consensus heptanucleotide and no nonanucleotide box are found on chromosome 2. The absence of nonanucleotide sequences was observed also in other specific recombinations (e.g. 34-37).

Although these findings indicate an involvement of the immunoglobulin gene recombinase(s) in the translocation process there are at least two features which do not fully parallel V-J recombination events. In all known heptanucleotide recognition sequences the first three nucleotides (CAC) are strongly conserved which is not the case in the chromosome 2 heptanucleotide (AAC). Furthermore, relatively large regions of chromosome 2 (20 bp) and chromosome 8 (16 bp) were deleted upon rearrangement, which is unusual for the immunoglobulin gene recombinase(s). In addition in most variant translocations in B cell malignancies of mouse and man no such recombination signal sequences can be found (e.g. refs. 38,39). It may be appropriate to consider these chromosomal translocations as products of recombinase mediated reactions and/or of illegitimate recombination processes.

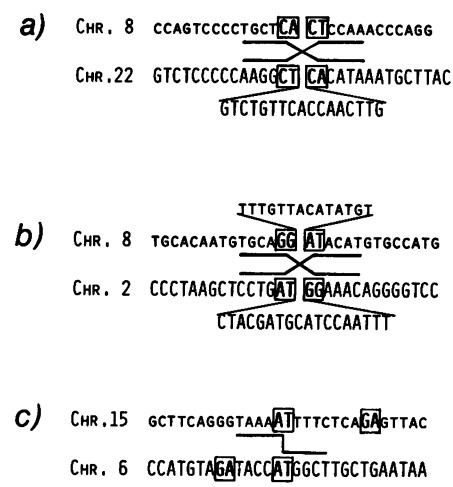

Figure 5. Symmetrically placed dinucleotides at the chromosomal breakpoints of variant translocations in B-cell lymphomas. The dinucleotides are boxed and the recombination sites are indicated. The sequences are from (a) a (8;22) chromosomal translocation (38), (b) the t(2;8) chromosomal translocation of JI (this paper) and (c) a t(6;15) chromosomal translocation of a mouse plasmacytoma (39). Only one of the reciprocal fragments was sequenced, putative symmetrically placed dinucleotides which might have been involved in the process are indicated.

In this context it is interesting to note that the reciprocal recombination between chromosomes 2 and 8 in JI occurred exactly in between a pair of inversely oriented dinucleotides and led to the deletion of the regions between the dinucleotides (Fig.3b). We have no clues as to the underlying mechanistic process(es) but it is noteworthy that inversely oriented dinucleotides, although different ones, are observed in analogous positions in other variant translocations (refs. 38,39; Fig.5).

Concluding remarks

The question how the translocation of immunoglobulin loci to the vicinity of the c-myc oncogene promotes the tumour phenotype remains open (reviews 2-5). Like others (40) we have not been able to detect a transcript from the region downstream of c-myc which is involved in the translocation (data not shown). A detailed study of the region between the c-myc gene and the translocation breakpoint with respect to chromatin structure and possibly also a search for encoded genes may lead to a better

understanding of the deregulation of c-myc transcription by chromosomal translocations.

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REFERENCES

1. Abbreviations: V_K, J_K, C_K, variable, joining, and constant segments of immunoglobulin kappa light chain genes; D, diversity gene segment of immunoglobulin heavy chain genes;
2. Klein, G. and Klein, E. (1985) Immunol. today 5, 208-215.
3. Cory, S. (1986) Advances in Cancer Res. 47, 189-234.
4. Lenoir, G.M. and Bornkamm, G.W. (1987) Advances in viral oncol. 7, 173-206.
5. Rabbitts, T.H. (1985) Trends in Genetics 1, 327-331.
6. Bornkamm, G.W., Kaduk, B., Kachel, G., Schneider, U., Fresen, K.O., Schwanitz, G., and Hermanek, P. (1980) Blut 40, 167-177.
7. Erikson, J., Nishikura, K., ar-Rushdi, A., Finan, J., Emanuel, B., Lenoir, G., Nowell, G. and Croce, C.M. (1983) Proc. natn. Acad. Sci. U.S.A. 80, 7581-7585.
8. Sun, L.K., Showe, L.C. and Croce, C.M. (1986) Nucl. Acids. Res. 14, 4037-4050.
9. Klobeck, H.-G., Bornkamm, G.W., Combriato, G., Mocikat, R., Pohlenz, H.D. and Zachau, H.G. (1985) Nucl. Acids Res. 13, 6515-6529.
10. Deev, S.M., Combriato, G., Klobeck, H.-G. and Zachau, H.G. (1987) Nucl. Acids Res. 15, 1-14.
11. Blin, N. and Stafford, D.W. (1976) Nucl. Acids Res. 3, 2303-2308.
12. Frischauf, A.M., Lehrach, H., Poustka, A. and Murray, N. (1983) J. Mol. Biol. 170, 827-842.
13. Klobeck, H.-G., Combriato, G. and Zachau, H.G. (1984) Nucl. Acids Res. 12, 6995-7006.
14. Norrander, J., Kempe, T. and Messing, J. (1983) Gene 26, 101- 106.
15. Sanger, F., Coulson, A., Barrell, B., Smith, A. and Roe, B. (1980) J. Mol. Biol. 143, 161-178.
16. Lötscher, E., Grzeschik, K.-H., Bauer, H.G., Pohlenz, H.D., Straubinger, B. and Zachau, H.G. (1986) Nature 320, 456-458.
17. Pohlenz, H.D., Straubinger, B., Thiebe, R., Pech, M., Zimmer, F.J. and Zachau, H.G. (1987) J. Mol. Biol. 193, 241-253.
18. Malcolm, S., Davis, M. and Rabbitts, T.H. (1985) Cytogenet. cell. Genet. 39, 168-172.
19. Hameister, H. and Adolph, S. (1986) Hum. Genet. 73, 73-76.
20. Sukhatme, V.P., Vollmer, A.C., Erikson, J., Isobe, M.,

Croce, C.M. and Parnes, J.R. (1985) J. Exp. Med. 161, 429-434.
21. Desiderio, S.V., Yancopoulos, G.D., Paskind, M., Thomas, E., Boss, M.A., Landau, N., Alt, F.W. and Baltimore, D. (1984) Nature 311, 752-755.
22. Yancopoulos, G.D. and Alt, F.W. (1986) Ann. Rev. Immunol. 4, 339-368.
23. Tonegawa, S. (1983) Nature 302, 575-581.
24. Kronenberg, M., Siu, G., Hood, L.E. and Shastri, N. (1986) Ann. Rev. Immunol. 4, 529-591.
25. Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M. and Perry, H., Sequences of Proteins of Immunological Interest (National Instutute of Health, Bethesda 1983).
26. Lewis, S., Gifford, A. and Baltimore, D. (1985) Science 228, 677-685.
27. Pech, M., Höchtl, J., Schnell, H. and Zachau, H.G. (1981) Nature 291, 668-670.
28. Berek, C., Griffiths, G.M. and Milstein, C. (1985) Nature 316, 412-418.
29. Manser, T. and Gefter, M.L. (1986) Eur. J. Immunol. 16, 1439-1444.
30. Tsujimoto, Y., Jaffe, E., Cossman, J., Gorham, J., Nowell, P.C. and Croce, C.M. (1985) Nature 315, 340-343.
31. Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E. and Croce, C.M. (1985) Science 229, 1390-1393.
32. Haluska, F.G., Finver, S., Tsujimoto, Y. and Croce, C.M. (1986) Nature 324, 158-161.
33. Lipp, M. and Hartl, P. (1986) Curr. Topics Microbiol. Immunol. 132, 162-168.
34. Höchtl, J. and Zachau, H.G. (1983) Nature 302, 260-263.
35. Durdik, J., Moore, N.W. and Selsing, E. (1984) Nature 307, 749-752.
36. Reth, M., Gehrmann, P., Petrac, E. and Wiese, P. (1986) Nature 322, 840-842.
37. Kleinfeld, R., Hardy, R.R., Tarlinton, D., Dangl, J., Herzenberg, K.A. and Weigert, M. (1986) Nature 322, 843-846.
38. Hollis, G.F., Mitchell, F.K., Battey, J., Potter, H., Taub, R., Lenoir, G.M. and Leder, P. (1984) Nature 307, 752-755.
39. Webb, E., Adams, J.M. and Cory, S. (1984) Nature 312, 777-779.
40. Graham, M. and Adams, J.M. (1986) EMBO J. 5, 2845-2851.
41. Hieter, P.A., Maizel,J.V. and Leder, P. (1982) J. Biol. Chem. 257, 1516-1522.