Volume 15 Number 23 1987 Nucleic Acids Research

Linking of the human immunoglobulin V_K and J_KC_K regions by chromosomal walking

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

Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie der Universität München, 8000 München 2, FRG

Received September 16, 1987; Revised and Accepted November 9, 1987

ABSTRACT

The linking of the human V_K and J_KC_K gene regions (abbreviations in ref. 1) by chromosomal walking is reported. Hybridization experiments with the DNA of a somatic cell hybrid containing the region between J_KC_K and the telomer show that none of the major V_K gene clusters is located downstream of C_K. The distance between the V_K and J_K genes was found to be 23 kb. The J_K proximal V_K gene is the B3 gene which is the only representative of subgroup IV in the genome. This gene and the neighbouring B2 gene (accompanying paper) are arranged in opposite orientation to J_KC_K and can therefore rearrange only by an inversion mechanism. This finding is used, together with previous data, to delineate the rearrangement processes in the Burkitt lymphoma derived cell line BL21 as comprising an inversion in the first and a deletion in the second step.

INTRODUCTION

The gene coding for the variable part of an immunoglobulin kappa light chain is assembled during B cell maturation by a somatic recombination process from V_K and J_K gene segments (reviews 2,3). During the last years a considerable number of V_K gene segments of man were cloned and grouped in large clusters (4-7). These clusters, however, could not be linked to the J_KC_K region on a physical map. Up to now in none of the mammalian immunoglobulin loci a linkage of the variable gene segments to the (D)-J-C regions could be established. Recently the single functional V gene segment of the chicken was linked to the J-C region and found to be arranged in the same transcriptional orientaton at a distance of only 1.7 kb (8). In the heavy chain locus of the horned shark V gene segments were found to be located at a distance of approximately 10 kb from the C gene segments (9). Another linkage of a V segment with the cognate J-C region was

described for the T cell receptor ß chain locus of the mouse where a single inverted V gene segment is located downstream of a C gene segment (10).

The orientation of the V_K gene segments to each other as well as to the J-C region is of importance for model considerations of the V_K-J_K joining mechanism. V-J rearrangements were first discussed in terms of a simple excision/deletion model (11,12) which requires the V and J genes to be oriented in the germline in the same transcriptional orientation. In later studies the finding of reciprocal recombination products of V_K and J_K flanks in lymphoid cells (13-17) led to the proposal of a sister chromatid exchange process (15,16) and to an inversion/deletion model for V_K-J_K recombination (17). The latter model requires some of the V_K gene segments to be inverted with respect to the transcriptional polarity of J_KC_K. With the exception of one region which contains inverted V_K pseudogenes (6) and of the B region (see below) all V_K gene segments of man were found to be in the same transcriptional orientation within the clusters (4-7). Based on the inversion/deletion model and on the finding of a duplication of a major part of the V_K locus Pech et al. (5) proposed that the duplicated parts of the locus are oriented inversely to one another. Thereby the genes of one cluster would rearrange by an inversion mechanism while the genes of the other one would lead to deletions upon rearrangement.

In order to clarify the situation we attempted to link one of the V_K gene segments containing regions with the J_KC_K region. The B region, containing the single V_KIV gene segment (18,19) and two other gene segments (20, accompanying paper), was isolated during our previous work on the cloning of V_K subgroup specific probes (18). Hybridization studies with lymphoid cell lines using probes from the V_KIV region had suggested (18) that this region may represent the J_K proximal V_K cluster. This notion was confirmed by pulsed field gel electrophoresis data (21) indicating that V_KIV and J_KC_K reside on a 220 kb SalI fragment, the distance being less than 150 kb. On the basis of these data it seemed feasible to link the two regions by chromosomal walking.

MATERIALS AND METHODS

Subclones, genomic library and hybridization conditions

Subclones for the chromosomal walking experiments were prepared in M13 phages (22). A genomic library was constructed from a size selected partial HindIII digest of DNA of the lymphoid cell line GM607 in the cosmid vector Lorist 2 (ref. 23) according to the protocol of ref. 24. The cell line GM607 which was obtained from the Human Mutant Cell Repository, Camden N.J., contains one germline K locus (K^o, K^+; ref. 25). A total of $6x10^5$ colonies were plated, transferred to GeneScreen Plus filters (New England Nuclear) and screened with self-ligated and nick-translated probes in a mixture of 4xSSC, 1xDenhardt's solution, 1% SDS, 0.1% pyrophosphate, 50 µg/ml sonicated salmon sperm DNA, 25 µg/ml sonicated E. coli DNA and 50 ng/ml ^{32}P-labelled probe at 68° C. Final washing was in 2xSSC, 1% SDS at 68° C (relaxed conditions). In one experiment the library filters were hybridized with 10 ng/ml labelled probe in 500 mM Na-phosphate, pH 7.2, 7% SDS, 1 mM EDTA, 100 µg/ml sonicated salmon sperm DNA, 50 µg/ml E. coli DNA and 0.1 µg/ml M13 DNA at 68° C. Final washing was in 40 mM Na-phosphate, pH 7.2, 1% SDS at 68° C.

Blot hybridizations were carried out by the SSC procedure used for the library filters except that the E. coli competitor DNA was omitted.

RESULTS

The J_K proximal V_KIV gene segment is inverted with respect to J_KC_K

To our surprise the first round of genomic walking from the 5' side of the V_KIV gene segment (clone mAF2/10; a in Fig. 1) led to a cosmid clone which bridges the gap between the B and J_KC_K regions. The two regions were also linked by walking steps from the J_KC_K side (clone m2132-2/2; b in Fig. 1). Restriction mapping of the clones obtained by chromosomal walking showed that the previously cloned B region (18) and the J_KC_K region (26) were separated only by 2 kb (Fig. 1).

Subsequent hybridization experiments of the cloned regions

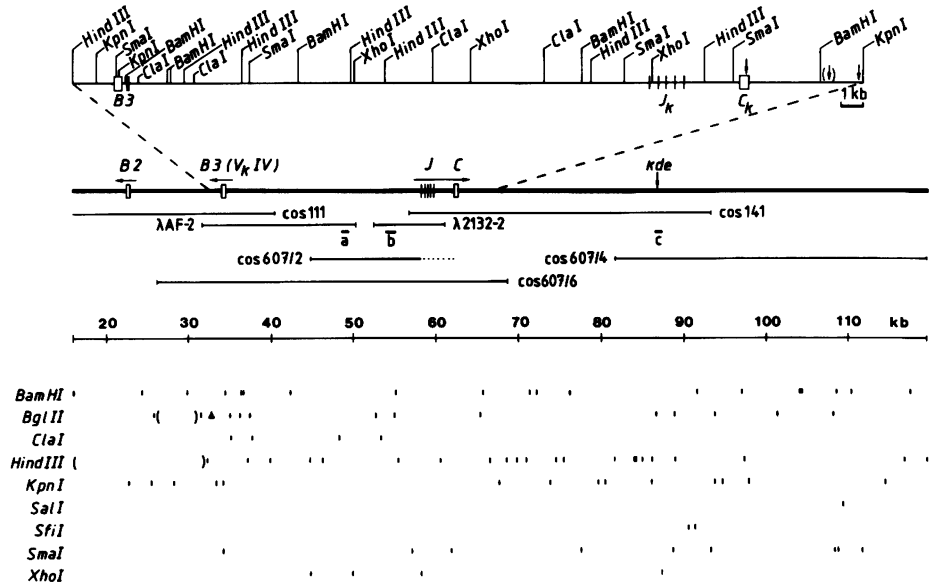

Figure 1. Restriction map of the B and J_KC_K regions. V_K and C_K gene segments are shown as open rectangles, J_K segments as vertical bars. The location of the kappa deleting element (26) is indicated. Transcriptional directions are symbolized by horizontal arrows. Only part of the B region is shown (see also ref. 18 and the accompanying paper, ref. 20). The clones cos 111 and AF-2 are described in ref. 18, clones cos 141 and λ2132-2 in ref. 26. Subclones used for the genomic walking experiments are designated a, b, and c; a is a 0.9 kb EcoRI fragment from λAF-2 cloned in M13mp10; b is a 1.75 kb PstI-BamHI fragment from λ2132-2 cloned in M13mp10; c is a 0.7 kb HindIII-KpnI fragment from cos 141 cloned in M13mp18. cos 607/2 contains in the dotted part an f fragment of the 5' J_KI flank and a 3' V_K flank (H.G.K. unpublished). A scale is given above the display of restriction enzyme cutting sites. Although the clones are derived from different individuals (cos 111 and cos 141 from placenta St., λAF-2 from placenta AF, λ2132-2 from the cell line GM2132, ref. 26, and the cos 607 clones from the cell line GM607, ref. 25) no restriction site polymorphisms for the nucleases shown were found in overlapping regions except for the BglII polymorphism in the B region indicated by a triangle (see text). The three vertical arrows in the C_K gene region indicate SacI sites for which a polymorphism had been previously detected (27; see text). BglII and HindIII sites were not determined in the regions indicated by brackets.

with V_K probes of the four subgroups under relaxed conditions showed that no further V_K gene segments are present between V_KIV and J_KC_K (not shown). Therefore the V_KIV gene segment is the J_K

proximal V_K segment, the distance being 23 kb (Fig. 1).

Interestingly, the V_KIV gene segment and its immediate neighbour, a V_K gene segment of subgroup III (ref. 20) are oriented inversely with respect to the transcriptional direction of J_KC_K. The J_K distal V_K gene segment B1, however, has the same transcriptional polarity as J_KC_K (20).

Due to the fact that the panel of rarely cutting nucleases is rather limited and that for instance no SalI site was found in the previously cloned regions (18,26 and Fig. 1) the pulsed field gel electrophoresis data did not allow to determine the relative orientation of the B and J_KC_K regions or to establish the exact distance between these regions. The finding of a SalI site and two closely spaced SfiI sites on the 3' side of C_K (Fig. 1) now allows to map the 220 kb SalI fragment which in pulsed field gel experiments contains the B and J_KC_K region (21). The data obtained from the present physical linkage are in full agreement with the pulsed field electrophoresis data of this region (21).

Recently a polymorphism of a SacI site downstream of C_K was reported (27). Two alleles of 3.7 kb and 5.0 kb, respectively, were detected using a C_K gene probe. We mapped the SacI sites in the vicinity of our cloned C_K segment (arrows in Fig. 1) and found that our clone harbours the 5.0 kb SacI fragment. Analogously a BglII polymorphism downstream of the B3 gene was detected (triangle in Fig. 1). 18 and 2 individuals were homozygous for the presence and absence of the BglII site, respectively; 3 individuals were heterozygous.

No V_K genes were detected downstream of C_K

Recently a V gene segment was found in an inverted position downstream of a C gene of the mouse T cell receptor ß chain (10). We had previously reported that no V_K gene segments are located within the 24 kb between C_K and the kappa deleting element (26). During the present chromosomal walking experiments the cloned region 3' of C_K was extended by another 33 kb (using the probe m141-3/2; c in Fig. 1). Again no V_K-like sequences were found using V_K gene probes of the four subgroups.

In order to clarify whether some V_K gene segments are located further downstream of C_K a somatic cell hybrid (28) was used which contains the $8q^+$ marker chromosome of the lymphoid

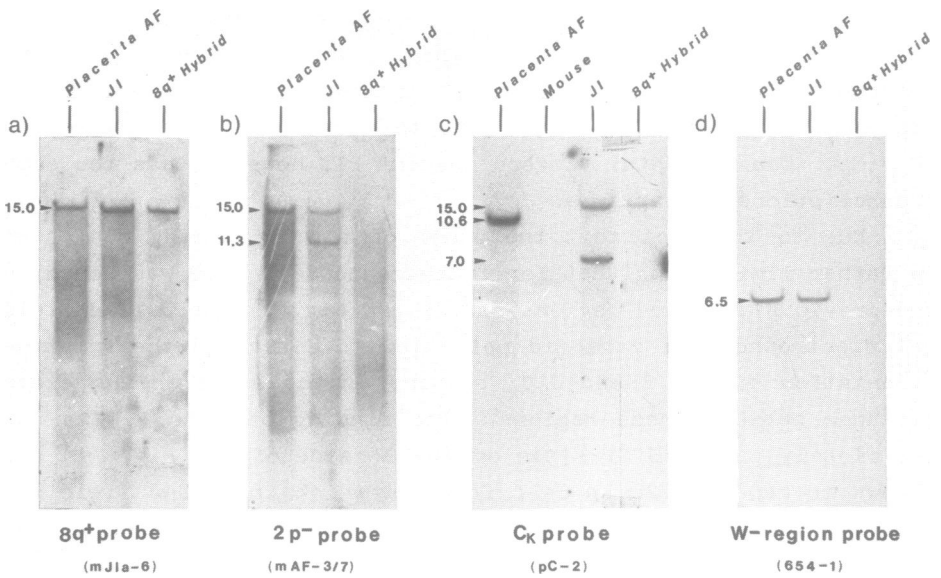

Figure 2. Blot hybridizations of DNA from a somatic cell hybrid containing an $8q^+$ marker chromosome and its parental cell lines. Southern blots were prepared from BglII (a,b,d) and BamHI (c) digests of DNAs from the cell line JI, mouse myeloma NP3, and a somatic cell hybrid derived from their fusion (clone 4-2L in ref. 28, containing the $8q^+$ marker chromosome). DNA from placenta AF was included for comparison. Hybridization was carried out with the probes indicated; the sizes of the fragments are given in kb. mJIa-6 is a probe derived from the chromosome 8 part of the $8q^+$ chromosome; mAF-3/7 is derived from the chromosome 8 part of the reciprocal $2p^-$ marker chromosome (29); pC-2 is a C_K containing subclone (26); m654-1 is an intergenic probe from the C_KW region of the V_K locus (6). DNA of the $8q^+$ hybrid did also not hybridize with probes from the A-O region (m659-2; ref. 7, W. Lorenz, unpublished) and the L region (m127-2; E. Huber, unpublished) of the K locus.

cell line JI. The $8q^+$ chromosome of JI which is the result of a reciprocal t(2;8) chromosomal translocation contains the region between J_KC_K and the telomer of chromosome 2 fused to chromosome 8 sequences (29). The hybrid cell line containing the $8q^+$ chromosome of JI was kindly provided by J. Erikson (28). The identity of the hybrid cell line was confirmed by hybridization with chromosome 8 (ref. 29) and J_KC_K probes (Fig. 2). Hybridization experiments with V_KI and V_KII gene probes did not allow clear conclusions because of crosshybridization with the mouse V_K genes of

the hybrid cells. Therefore unique intergenic probes of the cloned V_K gene regions W (6; Fig. 2d), L and A-O (4,5,7; data not shown) were hybridized to the $8q^+$ hybrid DNA. No signal was seen (e.g. Fig. 2d). We therefore conclude that none of the major V_K containing regions are located to the 3' side of C_K.

This finding is important because pulsed field gel electrophoresis experiments had shown (21) that one copy of the duplicated V_K gene regions is located on a 1.0 Mb NotI fragment and the other one on a 1.3 Mb NotI fragment, the latter one containing also the B and the J_KC_K regions. The lack of hybridization of the V_K cluster specific probes to the $8q^+$ chromosome places the 1.0 Mb fragment to the centromere side of C_K.

DISCUSSION

The finding of reciprocal flank recombination products (13,14) led to the formulation of new models for the mechanism of V_K-J_K recombination as occurring by sister chromatid exchange (15,16) or by an inversion/deletion process (17). The development of model systems for V_K-J_K recombination (30,31) and the finding of an inverted V gene segment in the mouse T cell receptor ß chain locus (10) had shown that inversions can indeed occur. Nevertheless the sister chromatid exchange model was hard to rule out on the basis of the known data. According to this model a V_K-J_K joint and its reciprocal flank recombination product (called f fragment) would segregate upon cell division. The finding of a V_K-J_K joint and its reciprocal f fragment in the same cell (32,33 and H.G.K. unpublished) is not consistent with a segregation mechanism and therefore supports an inversion model. Reciprocal recombination products had also been found earlier in a mouse myeloma (34).

Our finding of two inverted V_K gene segments in the germline fit the requirements of the inversion model (17). Furthermore, sister chromtid exchange involving V_K and J_K gene segments of opposite polarity would cause the loss of the part of the chromosome which contains the V_K-J_K joint. Rearranged V_KIV gene segments, however, were characterized by cloning and sequencing (18,19,33) and by hybridization studies of lymphoid cell lines (18). Thus all the data are in accordance with the inversion

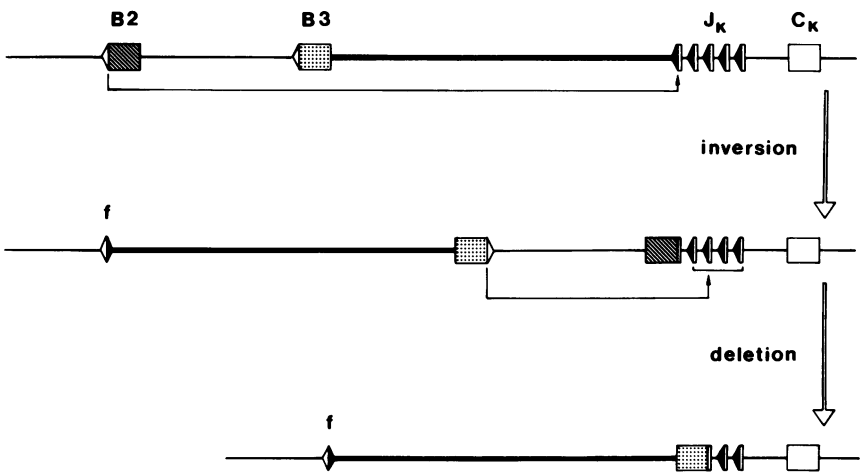

Figure 3. Consecutive DNA rearrangements in the cell line BL21 involving the B2 and B3 gene segments. The cell line contains a B3-J_K joint but it was not yet possible to demonstrate the existence of the KIV light chain in the cells. V_K, J_K, and the C_K gene segments are shown as rectangles. The 23 kb between B3 and J_K1 are depicted as a thick line. Recombination signal sequences (2) are shown as open and filled triangles and the f fragment as a rhombus. The partners of the recombination events are connected by arrows. The model rests on hybridization data obtained with B2, B3, and C_K gene probes and J_K1 and B2 flanking probes (18,35, 37). The J_K segment involved in the second rearrangement event could not be determined unambiguously; this is indicated by the bracket.

model of V_K-J_K recombination (17).

A simple inversion model would predict the V_KIV-J_K joint and its reciprocal f fragment to be present in the same cell line. However, in the three cell lines with a rearranged V_KIV gene investigated in our laboratory (cell lines BL21, BL41 and JI, ref. 18) the f fragments consist of J_K1 flanks joined to different V_K flanks (35). This confirms the earlier observations that the J_K1 containing f fragments are not reciprocal to the V_K-J_K joints of the respective cells (13-17,33,35,36).

It is clear from the structure of the B3-J_K1 region that the B3 gene and the J_K1 flank have to stay together or are deleted together. If one finds in a cell line a B3 gene rearranged to J_K2 to J_K5 and an f fragment containing a J_K1 flank two or more consecutive rearrangements should have taken place. A point in case is the cell line JI which contains a V_KIV-J_K4 joint and an f

fragment with a J_K1 flank and an unrelated V_K gene flank (18,35). Hybridization studies with somatic cell hybrids derived from JI cells (28,29) and in situ hybridizations showed that the f fragment and the V_KIV-J_K4 joint are located on the same chromosome in JI (S. Adolph and H. Hameister, unpublished). The first rearrangement in this cell line must have occurred by inversion leading to the formation of the observed f fragment. Such an inversion would bring the B3 and the B2 gene segments into the same transcriptional orientaton as J_KC_K. If the inverted B3 gene were then rearranged the B2 gene and other V_K genes would have been deleted. Since the B2 gene is clearly present on this chromosome of JI (37 and H.G.K. unpublished) a deletion step can be excluded and two further rounds of inversion have to be assumed, the last one leading to the productive V_KIV-J_K4 joint. The first and second inversion steps should have led to non-productive V_K-J_K joints. It was previously shown that consecutive rearrangements can indeed occur on the same chromosome (32,33).

The known structure of the B-J_KC_K region makes it also possible to reconstruct the sequence of rearrangements in the cell line BL21 (ref. 38). The structure found in this cell line and the likely sequence of events are illustrated in Fig. 3. The cell line has two rearranged kappa alleles, one of them being a B3-J_K joint (18). The B3 gene segment as well as the 3' flank of the B2 gene segment are deleted from the other chromosome (18,35, 37). The single f fragment found in this cell line is a joint of the B2 flank with the J_K1 flank (35). Accordingly the first rearrangement was a B2-J_K1 joint which resulted in the formation of the f fragment and brought the B3 gene segment into the same transcriptional polarity as J_KC_K (Fig. 3). A subsequent rearrangement of the V_KIV gene segment led to the deletion of the B2-J_K1 joint and created the situation found in BL21. In a similar way the rearranged structures can be explained which were found in the cell line BL41 (18,35) and in a cell line from a kappa chain deficient individual (20,33).

After the gross structure of the human K locus has been established (4-7,21 and the present paper) it should now be possible to delineate the course of rearrangement events in any human cell line.

ACKNOWLEDGMENT

We thank B. Bauriedel for expert assistance, P.H.R. Little for the cosmid vector Lorist 2 and J. Erikson for the somatic cell hybrids. F.-J.Z. is the holder of an A. Butenandt fellowship. The work was supported by Bundesministerium für Forschung und Technologie, Sonderforschungsbereich 304 and Fonds der Chemischen Industrie.

REFERENCES

1. Abbreviations: V,D,J,C, variable, diversity, joining and constant gene segments of the immunoglobulin genes; f fragments, recombination products of 3' VK flanks with 5' JK flanks; the designation of the clones is the following: m, λ, cos refer to M13, phage lambda and cosmid vectors; the cosmid clones from the cell line GM607 are designated cos607/ followed by the number of the clone.
2. Tonegawa, S. (1983) Nature 302, 575-581.
3. Honjo, T., and Habu, S. (1985) Ann. Rev. Biochem. 54, 803-830.
4. Pech, M., Jaenichen, H.-R., Pohlenz, H.-D., Neumaier, P.S., Klobeck, H.-G., and Zachau, H.G. (1984) J. Mol. Biol. 176, 189-204.
5. Pech, M., Smola, H., Pohlenz, H.-D., Straubinger, B., Gerl, R., and Zachau, H.G. (1985) J. Mol. Biol. 183, 291-299.
6. Pohlenz, H.-D., Straubinger, B., Thiebe, R., Pech, M., Zimmer, F.-J., and Zachau, H.G. (1986) J. Mol. Biol. 193, 241-253.
7. Straubinger, B., Huber, E., Lorenz, W., Osterholzer, E., Pargent, W., Pech, M., Pohlenz, H.-D., Zimmer, F.-J., and Zachau, H.G. (1987) J. Mol. Biol., in press.
8. Reynaud, C.A., Anquez, V., Dahan, A., and Weill, J.C. (1985) Cell 40, 283-291.
9. Hinds, K.R., and Litman, G.W. (1986) Nature 320, 546-548.
10. Malissen, M., McCoy, C., Blanc, D., Trucy, J., Devaux, C., Schmitt-Verhulst, A.-M., Fitch, F., Hood, L., and Malissen, B. (1986) Nature 319, 28-33.
11. Sakano, H., Hüppi, K., Heinrich, G., and Tonegawa, S. (1979) Nature 280, 288-294.
12. Seidman, J.G., Nau, M.M., Norman, B., Kwan, S.-P., Scharff, M., and Leder, P. (1980) Proc. Natl. Acad. Sci. USA 77, 6022-6026.
13. Steinmetz, M., Altenburger, W., and Zachau, H.G. (1980) Nucl. Acids. Res. 8, 1709-1720.
14. Selsing, E., and Storb, U. (1981) Nucl. Acids Res. 9, 5725-5735.
15. Van Ness, B.G., Coleclough, C., Perry, R.P., and Weigert, M. (1982) Proc. Natl. Acad. Sci. USA 79, 262-266.
16. Höchtl, J., Müller, C.R., and Zachau, H.G. (1982) Proc. Natl. Acad. Sci. USA 79, 1383-1387.
17. Lewis, S., Rosenberg, N., Alt, F., and Baltimore, D. (1982) Cell 30, 807-816.

18. Klobeck, H.-G., Bornkamm, G.W., Combriato, G., Mocikat, R., Pohlenz, H.-D., and Zachau, H.G. (1985) Nucl. Acids. Res. 13, 6515-6529.
19. Marsh, P., Mills, F., and Gould, H. (1985) Nucl. Acids Res. 13, 6530-6544.
20. Lorenz, W., Schäble, K.F., Thiebe, R., Stavnezer J., and Zachau H.G. (1987) submitted for publication.
21. Lorenz, W., Straubinger, B., and Zachau. H.G. (1987) Nucl. Acids Res., 15, 9667-9676.
22. Messing, J. (1983) in Methods in Enzymology, Vol 101, 20-78.
23. Gibson, T.J., Coulson, A.R., Sulston, J.E., and Little, P.F.R. (1987) Gene 53, 275-281.
24. Ish-Horowicz, D., and Burke, J.F. (1981) Nucl. Acids Res. 9, 2989-2998.
25. Klobeck, H.-G., Solomon, A., and Zachau, H.G. (1984) Nature 309, 73-76.
26. Klobeck, H.-G., and Zachau, H.G. (1986) Nucl. Acids Res. 14, 4591-4603.
27. Field, L.L., Tobias, R., and Bech-Hansen, T. (1987) Nucl. Acids Res. 15, 3942.
28. Erikson, J., Nishikura, K., Ar-Rushdi, A., Finan, J., Emanuel, B., Lenoir, G., Nowell, P.C., and Croce, C.R. (1983) Proc. Natl. Acad. Sci. USA 80, 7581-7585.
29. Klobeck, H.-G., Combriato, G., and Zachau, H.G. (1987) Nucl. Acids Res. 15, 4877-4888.
30. Lewis, S., Gifford, A., and Baltimore, D. (1984) Nature 308, 425-428.
31. Hesse, J.E., Lieber, M.R., Gellert, M., and Mizuuchi, K. (1987) Cell 49, 775-783.
32. Feddersen, R.M., and Van Ness, B.G. (1985) Proc. Natl. Acad. Sci. USA 82, 4793-4797.
33. Stavnezer, J., Kekish, O., Batter, D., Grenier, J., Balazs, I., Henderson, E., and Zegers, B.J.M. (1985) Nucl. Acids Res. 13, 3495-3514.
34. Höchtl, J., and Zachau, H.G. (1983) Nature 302, 260-263.35.
35. Deev, S.M., Combriato, G., Klobeck, H.-G., and Zachau, H.G. (1987) Nucl. Acids Res. 15. 1-14.
36. Selsing, E., Voss, J., and Storb, U. (1984) Nucl. Acids Res. 12, 4229-4246.
37. Mocikat, R., Klobeck, H.-G., and Zachau, H.G. (1987) Biol. Chem. Hoppe-Seyler, 368, 913-920.
38. Lenoir, G.M., Preud'homme, J.L., Bernheim, A., and Berger, R. (1982) Nature 298, 474-476.