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Physical map of the human immunoglobulin K locus and its implications for the mechanisms of V_K-J_K rearrangement

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ABSTRACT

Genomic regions containing numerous cloned V_K genes (abbreviations in ref. 2) were investigated by pulsed-field gel electrophoresis. 31 and 32 genes were linked within 1.0 and 1.3 Mb NotI fragments, respectively; the latter fragment includes also the J_KC_K gene segment. A 0.25 Mb NotI fragment comprises further 10 V_K genes. Since the transcriptional polarities of the V_K genes within the genomic regions are known the linking of the regions allows us now to answer unequivocally some longstanding questions concerning the mechanism of V_K-J_K rearrangement. The V_K genes of the 1.3 Mb NotI fragment except for the two J_K proximal ones (accompanying paper) are arranged in the same transcriptional polarity as J_KC_K and therefore must rearrange by a deletion mechanism. The V_K genes of the 1.0 Mb NotI fragment which has not yet been linked to V_KJ_K have identical polarity within the fragment. They should be arranged in opposite polarity to J_KC_K since reciprocal recombination products derived from them are known to exist; such recombination products must have been formed by inversion of oppositely oriented gene segments.

INTRODUCTION

The gene segments encoding the variable, joining and constant regions of the immunoglobulin light chains of the K type (V_K, J_K, C_K; ref. 2) have been extensively studied (3,4). Current objectives of such studies are to determine the size of the germline V_K gene repertoire and to contribute to the understanding of the mechanisms of V_K-J_K recombination (5). The investigation of human V_K genes started with the work of Bentley and Rabbitts (ref. 6 and earlier work). Cloning of 80 V_K genes in 1 Mb of DNA (7-9) and the application of pulsed-field gel electrophoresis described in the present paper allowed us now to delineate the structure of the human K locus and to clarify the transcriptional polarities of many V_K genes relative to the one of J_K-C_K. Knowing the relative polarities one can answer the longstanding question about

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the role of deletion and/or inversion in the mechanisms of the V_K-J_K rearrangement: certain specified V_K genes rearrange by a deletion and others by an inversion mechanism.

MATERIALS AND METHODS

The cell line PC-3 (ref. 10) was obtained from Flow Laboratories GmbH, Meckenheim, and low melting point agarose from GIBCO BRL GmbH, Eggenstein. Restriction nucleases were from Boehringer Mannheim GmbH and New England BioLabs GmbH, Schwalbach. The general techniques including the preparation of high molecular weight DNA, pulsed-field gel electrophoresis and the calibration of fragment sizes by multimers of lambda phage DNA and DNA of Saccharomyces cerevisiae chromosomes are described in refs. 11-13. Blotting and hybridization procedures followed ref. 14.

RESULTS

As illustrated in Fig. 1 most of the V_K genes were found in duplicated gene regions called Oa, Ob, Aa etc. (7-9). Just the B region which contains the only V_K gene of subgroup IV (15,16) occurs in one copy. Recently the B region was linked to J_K-C_K by chromosomal walking (17) and an O region was linked to an A region (9).

The method of pulsed-field gel electrophoresis and the availability of rarely cutting restriction nucleases have opened a new dimension of genome analysis (11-13). The application of the technique to the study of the human K locus was successful mainly because we had a large number of V_K gene probes and single copy walking probes available which permitted us to identify by blot hybridization (14) the fragments separated by PFG electrophoresis. The studies were carried out with DNA of the prostate carcinoma cell line PC-3 (ref. 10) because it seems to contain more unmethylated and therefore cleavable restriction sites than DNAs from some other sources (18). In several cleavage and hybridization experiments the K locus of PC-3 is indistinguishable from the one in the DNA of placenta St (18) which most of our work is done on. Some essential features of the evolving structure were confirmed in experiments with DNA from peripheral lymphocytes of several individuals.

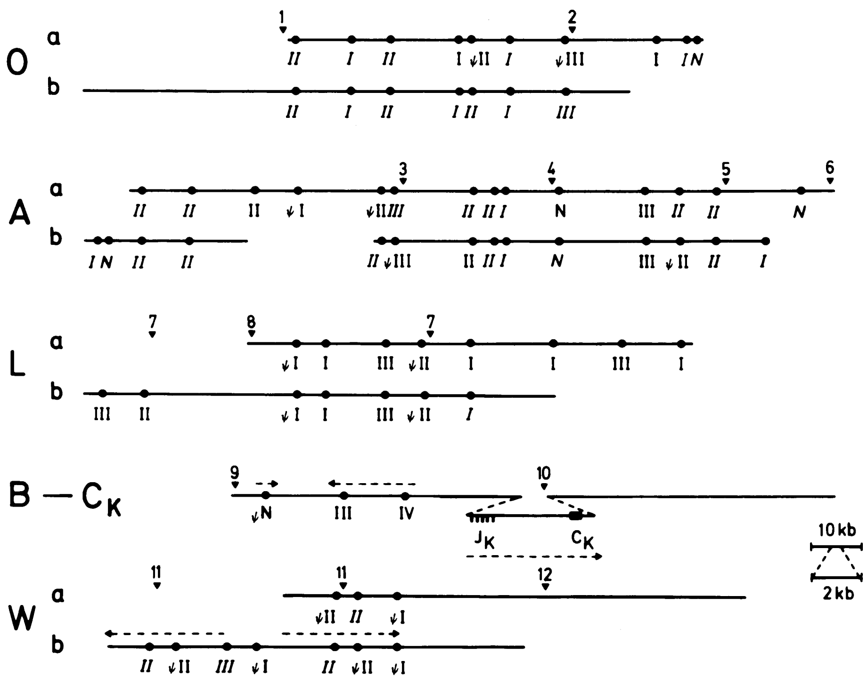

Fig. 1. Human V_K gene regions and the J_K-C_K gene region. The regions are drawn to scale. Genes are shown as dots. Subgroup assignment by hybridization is in italics, by sequence analysis in straight type. Regions: O, ref. 8 and W. Pargent, unpubl.; A, ref. 9; L, ref. 7 and E. Huber, unpubl.; B-J_K-C_K, refs. 15,17,23, 33. W, ref. 8 and F.-J. Zimmer, unpubl. Unpublished sequence data were contributed by C. Huber, E. Huber, H.-G. Klobeck, A. Meindl, K. Schäble, H. Smola, R. Thiebe. Not shown are a few unlinked clones whose V_K genes are located on chromosome 2 and the V_K gene regions which are transposed to other chromosomes (19). The numerals refer to the following hybridization probes: 1, 2, 12, m217-1, m143-1, m167-1, ref. 8; 3, 4, 6, m142-2, m163-1, m656-1, ref. 9; 5, m659-2, W. L., unpubl.; 7, m21-1, ref. 7; 8, m127-2, E. Huber, unpubl.; 9, m111-7, ref. 23; 10, pC-2, ref. 33; 11, m654-1, ref. 8. The transcriptional orientation of the gene region is from left to right; an inverted repeat in Wb and the opposite polarity of the B region genes are indicated by dashed arrows.

A prominent feature of the PFG blots are the 1.0 and 1.3 Mb NotI bands which appear with V_K gene probes (Fig. 2A and B) and with single copy probes of the O, A and L regions. The gene probes detect also smaller NotI fragments which in further experiments could be assigned to the W regions and to the V_K gene

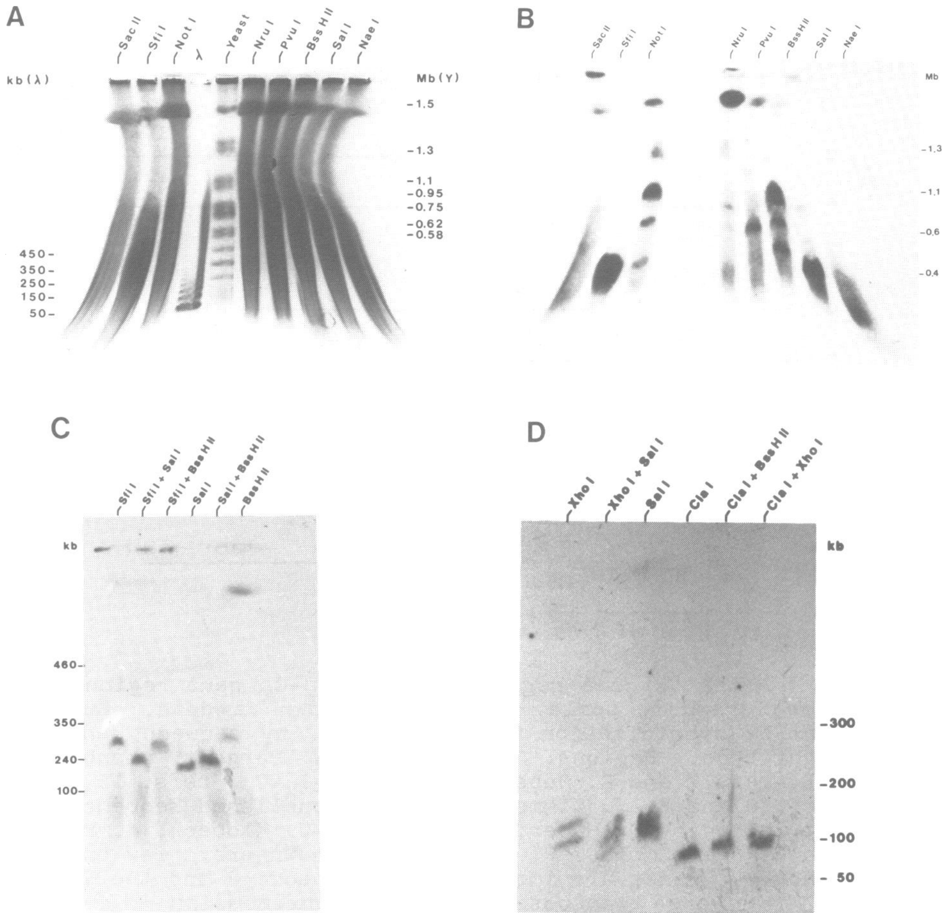

Fig. 2. Pulsed-field gel analysis of restriction nuclease digests of DNA from PC-3 cells (10). Three examples of PFG experiments are shown. In panels A-C and D the so-called OFAGE (12) and CHEF (13) techniques were used, respectively. Panels A,B: ethidium bromide stained gel and autoradiography of the corresponding blot hybridized with the V_{κ}II gene probe m607V-3 (ref. 34). The gel was run for 40 h at a 100-s pulse interval (6.5 V/cm). The sizes attributed to fragments in 0.6-1.3 Mb range hold only for a certain range of amounts of DNA applied per track and for the given electrophoresis conditions. Hybridization signals in the range above 1.5 Mb appear to be due to a compression zone. A very similar blot picture is seen with a V_{κ}I gene probe. Panels C and D: the gels were run for 37 and 35 h at 40- and 30-s pulse intervals, respectively (6 V/cm); blot hybridizations with probes 9 and 8 (Fig. 1), respectively.

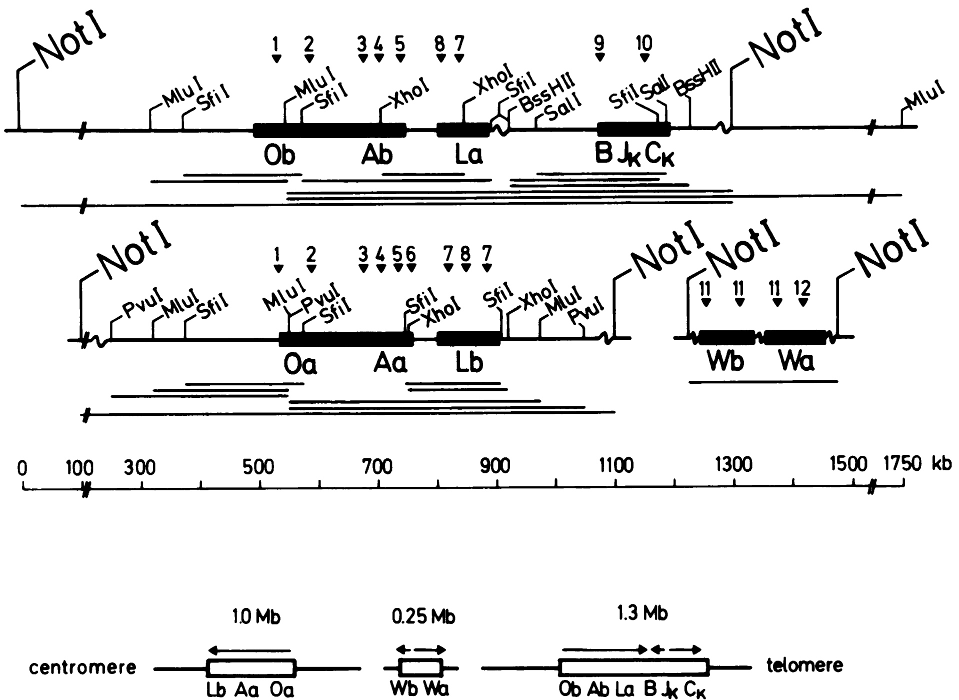

Fig. 3. Maps of three parts of the human K locus. The maps were constructed on the basis of numerous PFG blots most of which were hybridized consecutively with different probes in order to find out whether certain fragments are recognized by more than one probe. Only those cleavage sites, fragments and probes which are required for the construction of the maps are shown. The designation of the probes 1-12 is as in Fig. 1. Restriction sites in the cloned regions of the map were also identified in the respective cosmids. The interruptions in the cloned O-A regions (Fig. 1) are neglected in the drawings. The question whether the W regions are part of the K locus is discussed in the text; it cannot be decided yet whether both, Wa and Wb, are located on one 0.25 Mb NotI fragment or on two fragments of similar sizes; two NotI fragments of 0.4 and 0.6 Mb which contain Wb related sequences are not shown. The terminal regions of the maps without known restriction sites are shortened (-//-). Pairs of wavy lines (~~) mean that the stretches of DNA in between can be shifted to the left or to the right. The SfiI site(s) at the 3' side of La were mapped from upstream and downstream and it is uncertain whether there are one or two of them.

Bottom panel: a simplified scheme of the K locus emphasizing the transcriptional polarities (horizontal arrows; see text). The localization of the W region(s) in between the two large NotI fragments and the orientation of the W region(s) are hypothetical. For the opposite polarity within the Wb region see ref. 8.

regions which had been transposed to other chromosomes (19). When the NotI fragments were isolated on a micropreparative scale, re-cleaved with nucleases distinguishing between the a and b copies of the regions (7-9) and hybridized with the respective probes it turned out that Ob-Ab and La can be assigned to the 1.3 Mb NotI fragment, Oa-Aa and Lb to the 1.0 Mb one and Wa and Wb to a 0.25 Mb NotI fragment. B and C_K region specific probes hybridize only to the 1.3 Mb fragment. In Fig. 2C-D other cleavage patterns are shown which are crucial to the construction of the map of the K locus (Fig. 3).

The Leu2/T8 gene was reported to be closely linked to the K locus (20). Probes of the gene region were found to hybridize to other fragments than those hybridizing to the K locus probes, implying that the Leu2/T8 gene lies outside of the present map of the K locus.

DISCUSSION

A minimal estimate of the size of the K locus is 1.5 Mb. This figure is based on the assumptions that the 1.0 and 1.3 Mb NotI fragments are contiguous and that within these fragments the mapped regions are arranged in maximal proximity (Fig. 3). The figure does not include the W regions since it is still unclear whether they can be counted as parts of the K locus; they are located on chromosome 2, but at an unknown distance from the other regions and all six of the nine V_K genes of W which have been sequenced turned out to be pseudogenes (8; C. Huber, unpubl.). If the W regions are part of the K locus and if there are considerable distances between the mapped NotI fragments the actual size of the locus may be much larger than the minimal estimate. This does not mean that the number of V_K genes in the locus has to be much higher than 80 which is the number of genes we have isolated up to now. It is in fact suggested on the basis of a systematic comparison of blot hybridization patterns of digested genomic and cosmid clone DNAs that the final number may be somewhat but not much higher than 80 (A. Meindl, unpubl.).

On the basis of the PFG data some features of the mechanism of the V_K-J_K rearrangement can be established. All V_K genes of

the O, A and L regions are arranged within the regions in the same transcriptional polarities (7-9). The Ob-Ab and La regions were now linked by PFG to each other and to J_K-C_K and found to have overall identical polarity. Their V_K-J_K rearrangement therefore occurs by deletion of the stretch of DNA between the respective V_K and J_K gene segments. The assignment of polarities rests on the mapping especially of the MluI and XhoI sites in the 1.3 Mb NotI fragment. On the basis of the map it cannot be excluded that within the 1.3 Mb NotI fragment the Ob-Ab and La regions are located on the 3' side of C_K but this is highly unlikely since no signals were found in blot and in in situ hybridizations with probes from V_K gene regions on the part of chromosome 2 which in a t(2;8) translocation (21) extends from the J_K region to the telomer (17,22; S. Adolph and H. Hameister, unpubl.).

The distance between the J_K proximal V_KIV gene of the B region and J_K1 is 23 kb and the transcriptional orientation of this gene and the neighbouring V_KIII gene is opposite to the one of J_K-C_K (Fig. 1; refs. 17,23). The V_K-J_K rearrangement of these two J_K proximal V_K genes can therefore not occur by a deletion but should take place by an inversion mechanism. In accordance with such a mechanism a recombination product of the 3' flank of the V_KIII gene and the 5' flank of J_K1 was identified in lymphoid cell lines (24,25). Both, inversion (26) and sister chromatid exchange (27,28) mechanisms explain the fact that in some V_K-J_K rearrangements reciprocal recombination products of the V_K and J_K flanks, called f fragments, are found in addition to V_K-J_K joints (29). Also excision of the DNA between V_K and J_K, circularization and reinsertion into the genome can be invoked to account for the occurrence of f fragments but this is made unlikely by the localization of an f fragment on chromosome 2 by in situ hybridization (S. Adolph and H. Hameister, unpubl.). Sister chromatid exchange mechanisms are very unlikely since rearranged V_K genes and their cognate f fragments were found in the same cells (30; H.-G. Klobeck, unpubl.). Inversion is therefore the only mechanism which explains the joining of V_K and J_K gene segments of opposite polarity and is compatible with the present data, although complicated alternatives are hard to exclude. V_K-J_K inversions have also been shown to take place in model systems

(26,31) and an inversion was found in the T cell receptor ß locus (32).

The transcriptional polarity of the V_K genes of the Oa-Aa and Lb regions is uniform within the 1.0 Mb NotI fragment according to the results of the cloning (7-9) and the PFG work (Fig.3). Since no V_K genes were found on the 3' side of C_K (17) the 1.0 Mb NotI fragment should be located on the 5' side of the 1.3 Mb fragment. Furthermore, since we found f fragments with flanks of cloned V_K genes which are located on the 1.0 Mb NotI fragment (24; H.-G. Klobeck, unpubl.) we can turn the above argument around: f fragments are formed by an inversion mechanism and joining by inversion takes place only if V_K and J_K-C_K have opposite polarity. We therefore conclude that the V_K genes of the 1.0 Mb NotI fragments are arranged in opposite polarity to the O-A, L and J_K-C_K genes of the 1.3 Mb fragment. The opposite polarity of the duplicated V_K gene regions which was postulated before (7) is not yet proven by linking of the two NotI fragments but it is considered to be highly likely for mechanistic reasons.

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REFERENCES

1. Present address: Department of Plant Pathology, Cornell University, Ithaca, New York 14853, USA.
2. Abbreviations: V, J, C, variable, joining and constant gene segments of the immunoglobulin genes; for reasons of simplicity V_K gene segments are mostly called V_K genes; V_KI to V_KIV, variable gene segments of immunoglobulin light chains of the K type belonging to subgroups I to IV; V_KN, no subgroup assigned, since hybridization or sequence analysis do not conform to subgroups I to IV; , pseudogene (Fig. 1); f fragments, recombination products of 3' V_K flanks and 5' J_K flanks; PFG, pulsed-field gel electrophoresis; m127-2,

the second subclone prepared in M13 from the cosmid clone cos 127.
3. Tonegawa, S. (1983) Nature 302, 575-581.
4. Honjo, T. and Habu, S. (1985) Ann. Rev. Biochem. 54, 803-830.
5. Alt, F.W., Blackwell, T.K., DePinho, R.A., Reth, M.G. and Yancopoulos, G.D. (1986) Immunol. Reviews 89, 5-30.
6. Bentley, D.L. and Rabbitts, T.H. (1983) Cell 32, 181-189.
7. Pech, M., Smola, H., Pohlenz, H.-D., Straubinger, B., Gerl, R. and Zachau, H.G. (1985) J. Mol. Biol. 183, 291-299.
8. Pohlenz, H.-D., Straubinger, B., Thiebe, R., Pech, M., Zimmer, F.-J. and Zachau, H.G. (1987) J. Mol. Biol. 193, 241-253.
9. 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.
10. Kaighn, M.E., Narayan, K.S., Ohnuki, Y., Lechner, J.F. and Jones, L.W. (1979) Investigative Urology 17, 16-23.
11. Schwartz, D.C. and Cantor, C.R. (1984) Cell 37, 67-75.
12. Carle, G.F. and Olson, M.V. (1984) Nucl. Acids Res. 12, 5647-5664.
13. Chu, G., Vollrath, D. and Davis, R.W. (1986) Science 234, 1582-1585.
14. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
15. Klobeck, H.-G., Bornkamm, G.W., Combriato, G., Mocikat, R., Pohlenz, H.-D. and Zachau, H.G. (1985) Nucl. Acids Res. 13, 6515-6529.
16. Marsh, P., Mills, F. and Gould, H. (1985) Nucl. Acids Res. 13, 6531- 6544.
17. Klobeck, H.-G., Zimmer, F.-J., Combriato, G. and Zachau, H.G. (1987) accompanying paper.
18. Straubinger, B. (1987) Ph.D. Thesis, Fakultät für Chemie und Pharmazie, Universität München.
19. Lötscher, E., Grzeschik, K.-H., Bauer, H.G., Pohlenz, H.-D., Straubinger, B. and Zachau, H.G. (1986) Nature 320, 456-458.
20. Sukhatme, V.P., Vollmer, A.C., Erikson, J., Isobe, M., Croce, C. and Parnes, J.R. (1985) J. Exp. Med. 161, 429-434.
21. Erikson, J., Nishikura, K., Ar-Rushdi, A., Finan, J., Emanuel, B., Lenoir, G., Nowell, P.C. and Croce, C.R. (1983) Proc. Nat. Acad. Sci. USA 80, 7581-7585.
22. Klobeck, H.-G., Combriato, G. and Zachau, H.G. (1987) Nucl. Acids Res. 15, 4877-4888.
23. Lorenz, W., Schäble, K.F., Thiebe, R., Stavnezer, J. and Zachau, H.G. (1987) submitted for publication.
24. Deev, S.M., Combriato, G., Klobeck, H.-G. and Zachau, H.G. (1987) Nucl. Acids Res. 15, 1-14.
25. Stavnezer, J., Kekish, O., Batter, D., Grenier, J., Balazs, I., Henderson, E. and Zegers, B.J.M. (1985) Nucl. Acids Res. 13, 3495-3514.
26. Lewis, S., Gifford, A. and Baltimore, D. (1984) Nature 308, 425-428.
27. Van Ness, B.G., Coleclough, C., Perry, R.P. and Weigert, M. (1982) Proc. Nat. Acad. Sci. USA 79, 262-266.
28. Höchtl, J., Müller, C.R. and Zachau, H.G. (1982) Proc. Nat. Acad. Sci. USA 79, 1383-1387.
29. Steinmetz, M., Altenburger, W. and Zachau, H.G. (1980) Nucl. Acids Res. 8, 1709-1720.

30. Feddersen, R.M. and van Ness, B.G. (1985) Proc. Nat. Acad. Sci. USA 82, 4793-4797.
31. Hesse, J.E., Lieber, M.R., Gellert, M. and Mizuuchi, K. (1987) Cell 49, 775-783.
32. 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.
33. Klobeck, H.-G., Combriato, G. and Zachau, H.G. (1984) Nucl. Acids Res. 12, 6995-7006.
34. Klobeck, H.-G., Solomon, A. and Zachau, H.G. (1984) Nature 309, 73-76.