Volume 11 Number 19 1983 Nucleic Acids Research

The structure of the mouse immunoglobulin in γ_3 membrane gene segment

Michael Komaromy[1], Linda Clayton[1,2], John Rogers [1,3], Stella Robertson[4,6], Jack Kettman[4] and Randolph Wall[1,5]

[1]Molecular Biology Institute, University of California, Los Angeles, CA 90024, [4]Dept. Microbiology, University of Texas Health Sciences Center, Southwestern Medical School, Dallas, TX 75235, and [5]Dept. Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024, USA

Received 20 June 1983; Revised and Accepted 6 September 1983

ABSTRACT

The genomic region containing the mouse immunoglobulin $\gamma 3$ heavy chain membrane (M) exons has been located and sequenced. The exon structure is highly similar to that of the other mouse γ chains, with strong sequence conservation in the coding regions and the intron 5' to the M1 exon. The intron between M1 and M2 shows moderate sequence homology but very strong conservation of size. RNA blots suggest that γ_3 membrane exon usage is similar to that seen in other immunoglobulin membrane heavy chain mRNAs. The transmembrane region contains the invariant residues which have been noted in all other heavy chain sequences and which were previously proposed to be interactive in a two-chain model for insertion through the lymphocyte membrane. Conserved residues with similar spacing have been seen in class II histocompatibility antigens, which are also two-chain transmembrane molecules, but not in class I antigens, which span cell membranes with a single chain.

INTRODUCTION

Immunoglobulin chains are composed of structurally defined domains which correspond to the biological or functional properties of the molecules. Variable region domains are involved in antigen recognition and binding. Constant region domains define the different classes of immunoglobulins (IgM, IgG, etc.) and carry out various effector functions such as complement fixation, transplacental mobility and release of histamine from mast cells. In active immunoglobulin genes, the complete variable region (generated by DNA rearrangements) as well as the constant region in light chains and the individual constant region domains in heavy chains are encoded in DNA as distinct structural gene segments (exons) which are separated from each other by noncoding intervening sequences (introns). We discovered another class of exons, the M or membrane exons, which include a hydrophobic peptide sequence likely to anchor the membrane-bound form of IgM on the surface of B lymphocytes (1,2). In the μ heavy chain gene, the coding sequences for the secreted C-terminal region are contiguous with the C_H4 domain, while the

© IRL Press Limited, Oxford, England.

membrane C-terminal coding sequences (the M exons) are located approximately 2 kilobases (kb) to the 3'-side of the C_H4 domain (2). It has been proposed that the expression of membrane or secreted IgM is regulated by RNA processing events which generate μ mRNAs with alternative 3'-sequences coding for either membrane (μ_m) mRNA or secreted (μ_s) mRNA C-termini (1-4). In the nuclear processing pathway for μ_m mRNA, the M exons are spliced into the sequence GGT-AAA (coding for Gly-Lys) which closely resembles an upstream consensus splice site (5) at the end of the C_H4 domain. The μ_s C-terminal sequences are replaced by the μ_m exons in this process. We have proposed that the developmentally regulated processing of μ_m or μ_s mRNA occurs through the choice of alternative poly(A) addition sites in the μ transcription unit (1,2). This proposal is based on general findings that poly(A) addition precedes splicing in eukaryotic mRNA processing (6-10).

Because all immunoglobulin heavy chains now sequenced at either the DNA or protein level have GGT-AAA and Gly-Lys at the end of their final domain (11-14), we previously predicted that all heavy chains might use the RNA processing mechanism just described to generate membrane or secreted forms (1-5). Recently, we determined that the γ_1 and γ_{2b} subclasses of mouse heavy chains have membrane C-terminal gene segments located to the 3'-side of the different C_H3 domains (16). This has also been shown for the γ_{2a} (17) and δ (18-21) heavy chain genes.

Here we describe hybridoma cell lines which produce both secreted and membrane-bound forms of IgG_3. These cells contain two species of γ_3 mRNA encoding membrane and secreted heavy chains. By analogy to the μ, δ, γ_1, γ_{2a} and γ_{2b} genes, the γ_3 membrane exons are located 2 kb to the 3'-side of C_H3 domain. The nucleotide sequence of the γ_3 M_1-exon contains a hydrophobic core whose sequence is highly conserved in relation to other transmembrane sequences of heavy chain M-exons. These findings are presented in a model for insertion of immunoglobulin heavy chains in lymphocyte membranes.

METHODS

The cell lines used have been described (22). The mouse γ_3 gene clone (pγ3C6) and cDNA clone (p606γ3-1) were kindly provided by M. Davis and S. Kim. Nucleotide sequencing was done as described (23), using the M13 system. All enzymes were purchased from New England Bio Labs, Boehringer-Mannheim and P-L Biochemicals.

RESULTS

1. Hybridoma lines making membrane and secreted γ_3 chains

The parental hybridoma line B1, which secretes both IgM and IgG anti-arsonate antibodies is a spontaneous double-producing line from the fusion product of the MPC-11 x 45 6TG plasmacytoma line (received from M.D. Scharff, Albert Einstein, Bronx, New York) and immune A/J spleen cells which had been stimulated *in vitro* by dextran sulfate (DxS) and lipopolysaccharide (LPS) as described by Robertson *et al*. (22). Briefly, A/J mice were immunized intra-peritoneally (IP) with p-azophenylarsonate coupled to keyhole limpet hemocyanin (Ar-KLH) in adjuvant, rested, and injected (IP) with Ar-KLH in saline. Three days later the spleen was removed, teased into a single cell suspension, and cultured at low density (2×10^5 cells/ml) in the presence of 30 µg/ml DxS and 10 µg/ml *S. typhosa* LPS. The *in vitro* expanded spleen cells were then fused with the MPC-11 plasmacytoma line at a ratio of 10:1 using 35% polyethylene glycol (PEG1000) according to the method of Galfre *et al*. (24). After fusion the cells were plated at limiting dilutions at 4×10^2 to 4×10^5 cells/microtiter well and grown in the presence of Littlefield's HAT selection media (25). The hybrid clone supernatants were then scored for anti-Ar antibodies in a solid phase radioimmunoassay (RIA) and were typed for subclass by gel diffusion. Hybrid lines were carried *in vitro* and as ascitic tumors in CAF_1 mice.

The A/J mice used for the particular fusion from which the B1 line was derived had been hyperimmunized with Ar-KLH: 500 µg Ar-KLH in complete Freund's adjuvant IP followed by three injections of 250 µg Ar-KLH in incomplete adjuvant and one of Ar-KLH in saline at intervals of two weeks. After resting for eight weeks the animals were given Ar-KLH (250 µg) in saline. Three days later three animals were sacrificed and their spleen cells were pooled for the in vitro expansion and hybridization procedure as described above. When the cells from the day 4 mitogen stimulated cultures were fused with MPC-11 cells and plated at 2×10^5 cells/well, approximately 12% of the wells contained hybrids producing anti-Ar-antibodies. One well, E3, which was producing both IgM and IgG2b, was subcloned twice (F11-H8) and injected into a CAF_1 mouse to produce ascites. The cells were recovered after one passage, cultured and recloned, producing the B1 line which secreted predominantly IgM and little IgG2b. After this line was carried in mice for approximately 3 months (5 passages) the hybrid cells were harvested from ascitic fluid and cultured. The line recovered was now secreting predominantly IgM and IgG3 with a trace of IgG2b and thus represented a spontaneous double-pro-

ducing line. All studies concerning antibody structure, cell surface properties, karyotype, and mRNA have been performed on this B1 double-producer and its subclones.

2. Characteristics of Cells Making Membrane and Secreted γ_3 Chains

The B1 hybridoma cell line and its double-producing subclones, B1C and B1G9 have between 64 and 72 chromosomes. Based on Giemsa banding and secondary constriction karyotypic studies, the B1 line has 2 A/J 12 chromosomes and one Balb/c 12 chromosome. In addition, both the B1C and B1G9 lines appear to have duplicated one A/J chromosome 12 and B1C has lost the Balb/c chromosome 12 (22). The lines secrete varying amounts of IgM and IgG3 antibodies with the B1 line secreting higher amounts of IgM and B1G9 secreting mainly IgG3. All three lines secrete IgG2b, which was the isotype secreted by the parent line. By cell sorter analysis, all three lines bear membrane IgG3 and the B1 line membrane IgM.

3. Cells Making Membrane and Secreted IgG_3 Contain Two Species of γ_3 Heavy Chain mRNA

Cytoplasmic poly(A)-containing mRNA was isolated from two subclones of the IgM B1 hybridoma and analyzed by RNA blots. The γ_3 mRNAs were initially identified by hybridization to a cloned cDNA probe identified by nucleotide sequencing (J. Rogers and L. Clayton, unpublished results) or to a γ_3 genomic DNA probe identified by its hybridization to the cloned γ_3 cDNA probe and nucleotide sequencing. These hybridoma cell lines were originally constructed by fusion with a γ_{2b}-producing MPC 11 cell line; however, the presence of γ_{2b} mRNA in these cells does not complicate the results as the hybridization conditions employed effectively discriminate between the mRNAs for these two subclasses of γ heavy chains.

The IgM B1C subclone produces 3.2 kb and 1.7 kb γ_3 mRNA species as well as a 2.1 kb μ mRNA (Fig. 1). The 2.1 kb μ mRNA codes for secreted μ_s chains. The 2.35 kb μ mRNA which codes for membrane μ_m chains is not detectable in IgM B1C. Thus, these cells make both membrane and secreted IgG_3 but only secreted IgM. The IgM B1G9 subcloned cells contain the 3.2 kb and 1.7 kb γ_3 mRNAs but lack any μ mRNA.

4. Structure of the γ_3 M-Region

By analogy to the finding for μ, δ , γ_3, γ_{2a} and γ_{2b}, we presumed that the 3.2 and 1.7 kb γ_3 mRNA species contain alternative COOH terminal coding sequences. The origin of such sequences in the 3.2 kb γ_3 mRNA species was examined by hybridization to isolated restriction fragments from the γ_3 genomic clone (Fig. 2). The pγ3C6 clone contains a 6.6 kb BamH I insert.

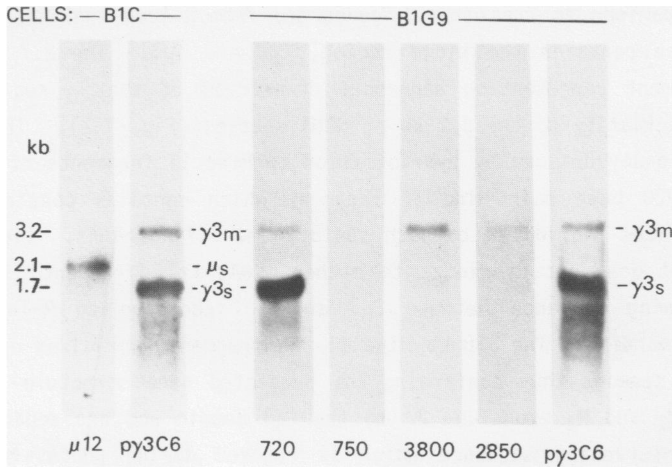

Figure 1. RNA blot analysis of mRNA from the B1C and B1G9 cell lines. See Figure 2 and text for explanation of probes. RNA sizes were based on cellular RNA standard sizes of 1.84 kb (18S), 5.00 kb (28S), 6.35 kb (32S), 11.8 kb (45S), and E. coli ribosomal RNA sizes of 1541 nucleotides (16S) and 2904 nucleotides (23S).

Comparison of restriction maps and nucleotide sequences from p606γ3-1 and pγ3C6 indicates that this insert contains sequences 3' to the BamH I site located at amino acid 164 in the middle of the γ_3 C_H1 domain (J. Rogers and L. Clayton, unpublished results; 26,27). The pγ3C6 DNA was cut by BamH I and Xba I and two fragments isolated on an agarose gel. The 3.9 kb BamH I-Xba I fragment contains the distal portion of the γ_3 C_H1 domain and regions 3' to

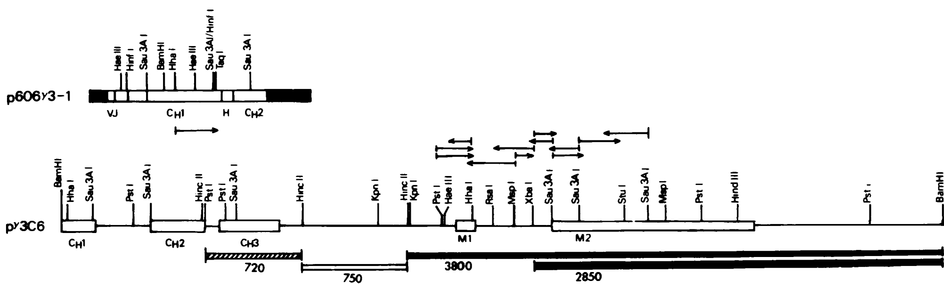

Figure 2. Restriction enzyme maps of p606γ3-1 and pγ3C6. Arrows indicate the direction and extent of nucleotide sequence analysis. Bars indicate probes used in Figure 1. The hatched bar hybridized to 1.85 kb γ_{3s} RNA, the solid bars hybridized to 3.2 kb γ_{3m} RNA, and the open bar did not hybridize with either cytoplasmic γ_{3m} RNA species.

it. In comparison to the other γ genes and from R-loop analysis (26) this fragment also contains the hinge region, C_H2 and C_H3. The 2.7 kb Xba I-Bam HI fragment representing sequences downstream of the γ_3 region domain hybridizes primarily to the 3.2 kb γ_3 mRNA species (Fig. 1,2). The M exons were more closely defined by hybridization to Hinc II fragments of the pγ3C6 DNA. The 720 base pair Hinc II fragment which contains constant region domain sequences hybridizes to both the 3.2 and 1.7 kb mRNAs. The 750 base pair fragment does not hybridize to either mRNA; this fragment lies within the intervening sequence between C_H3 and M_1 according to R-looping and restriction mapping. The 3.8 kb Hinc II DNA fragment hybridizes only to the 3.2 kb mRNA species thus confirming the predicted gene structure for the M exons; the M_1 and M_2 exons lie 3' to the C_H3 domain and are separated from it by an intervening sequence which is removed during processing of the primary transcript. In addition, RNA dot hybridization experiments show that the $M_1 \rightarrow M_2$ intron is removed during γ_3 mRNA biogenesis (data not shown).

The sequence of the γ_3 M_1 exon was determined according to the strategy shown in Figure 2. The nucleotide sequence and deduced amino acid sequence is shown in Figure 3.

DISCUSSION

The nucleotide sequences of the γ_3 M_1 and M_2 exons are highly conserved in comparison to other γ heavy chains (28). As has been shown for μ (1,2), γ_1 and γ_{2b} (16,28) and γ_{2a} (28), the γ_3 M_1 contains a highly acidic polypeptide sequence followed by a hydrophobic core. The γ_3 protein sequence differs from γ_1 in 4 amino acids out of the first 12 and from γ_{2b} in five positions. The hydrophobic core sequences of γ_3 and γ_{2b} are identical; this sequence differs from γ_1 in one position.

The patchwork pattern of nucleotide sequence homology observed for γ_1 and γ_{2b} (16) is also obvious in γ_3. The 33 bases preceding the 5' splice site are highly conserved. There are four differences between γ_1 and γ_3 (88% homology) and one difference between γ_{2b} and γ_3 (97% homology). This is followed by a region of higher divergence within the acidic portion of the protein sequence. Here there are 6 out of 38 differences (84% homology) between γ_1 and γ_3 and 8 out of 38 (76% homology) differences between γ_{2b} and γ_3. However, as previously mentioned, the overall charge of the protein sequence in this region is maintained. The following 93 nucleotides leading to the splice site at the 3' end of M_1 show only two differences between γ_3 and the γ_1 and γ_{2b} sequences (98% homology). However, 3' to the splice site the

100
TGCAGCTTTCTCCTGGGCCTCCATGCAGCCTCCTGCCACACAGGGAATGGCCCTAGCTCTACCTTGTTGGGACAAACACTGACTTTCCTCTCTGTTCAGA

GluLeuGluLeuAsnGlyThrCysAlaGluAlaGlnAspGlyGlnLeuAspGlyLeuTrpThrThrIleThrIlePheIleSerLeuPheLeuLeuSerVal
GCTGGAACTGAATGGGACCTGTGCTGAGGCCCAGGATGGGGAGCTGGACGGGCTCTGGACGACCATCACCATCTTCATCAGCCTCTTCCTGCTCAGCGTG
M1

300
CysTyrSerAlaSerValThrLeuPheLys
TGCTACAGCGCCTCTGTCACCCTGTTCAAGGTCATCCCTATTTCCTCCATCCATCCTCACAATATCTACACTATACTGCTATCCCTACCTGTCCCATACT

400
GTCTCAATGTCTCCCATGTCATCATATGTCATCTTCCACATAGTCATCCACTGTACTTACACCACCCTCTCCCTGTCCCTCTGTCTATACTGTGCCCACA

500
TTGTCCATGCTGTCCCTNANTTTGTCTTGACACTCCCGCAAGTTGTCACCCACCCTATCCATGCACCGTCTCCAATATGATCTGCACACTACGTTCCTAA

600
TTGCCGGTCCCCACTCTATTCACACCTGGTCCTCACCAAATCCCTACACTGTCCCTACAATGCCCCTTGCTATAGCTACACACCGTCTCAAAACTCTACT

700
CACACCTTGCCTTACAATGTCACTGTCTTATCCTCACTCTAGATATATGACATCCCAAGATTGGGAGCTGCCCTGGCTTAGTGTATAATGAGGCAGGGAT

ValLysTrpIlePheSerSerValValGlnValLysGlnThrAla
GACCTGTGAAGGGACTCAGGTCAGGCACATAACATCTCACTTTTGCATCTCACAGGTGAAGTGGATCTTCTCCTCAGTGGTGCAGGTGAAGCAGACGGCC
M2

900
IlePheAspTyrArgAsnMetIleGlyGlnGlyAlaStop
ATCCCTGACTACAGGAACATGATTGGACAAGGTGCCTAGCCTGTCTCTTCAGAAGGTGCCAGAGCTTGCTAGCCCTCTAGGACCAAGTCCATGCTGCTGA

1000
GCTGCCAGACACACGGGTTACACTGCCTTCTCATCTCANCATCCTTTGATCTTATGGCTCTGACTCTGCTACCCAGGTCCTTCCACATTTGGAGCAGCAT

1100
GTGGNAAAGCAGGTTTGCCCAGACCATAGCCAACAGGACCCAAGCACCACTACCACAGCCAAAACAGTGGATAGGGTCAAAACTGCTGCTGTGTTTCTTC

1200
TACATGGTGTCTAGCCCTCAGCCAGGACCTCTCCCAGATGGCTGCATCACCTGCACTCCATTTAGATGCAGATGAGCCTTAGAGCCAGCCCTGTCTATAC

1300
AGTCCTCTGAAACTGCTCTCTGAGAGAAGGAATTTCCATGTGTCTCTAAGGCTGAGAAGGCCTCAAAGGGAACTCTTTGGGAAGGACTGCCAAAGGTGAC

1400
TCTAATTGGCCTTGCACCCAACCAGTGCCCTGCCGTGTTTTGTCACTTCTTGGAATGTAAGATGGGGCATCGTTGTGAAGACCTCTTTCCTGCCATCCAT

GCAACATGATGATGTTTGATGATC

Figure 3. Nucleotide sequence of the γ_3 M exon region. M1 and M2 are indicated, with their respective amino acid sequences written in italics.

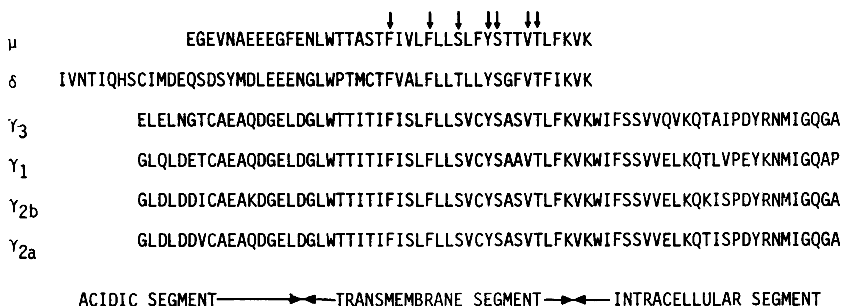

Figure 4. Comparison of amino acid sequences of M exons from mouse immunoglobulin heavy chain genes.

sequences diverge completely. Thus the sequence of the γ_3 M_1 exon and regions immediately flanking it show a pattern of homology identical to that noted for γ_1 and γ_{2b} (16).

We previously proposed (16) that the conservation of sequence observed in the M exons of γ immunoglobulins is necessary for the formation of a dimer within the membrane. Possible models of such a dimer include one in which the α-helices cross and interact at 7 "contact" residues. It is noteworthy that these contact residues are identical and conserved in their placement in all mouse immunoglobulin transmembrane segments (with the exception of one S > T in the sequence) (Fig. 4). The homology of the M1 exons in all known mouse immunoglobulins is much better than that seen between the constant region domains of different immunoglobulins (28).

With regard to the two-chain model, we have noted the presence of conserved residues in other molecules which are structurally related to immunoglobulins and which are anchored in the cell membrane by two chain complexes. These are class II HLA or H-2 molecules which are involved in lymphoid cell interactions. For example, the HLA-DR_β and HLA-DR_α (29-32) transmembrane regions contain six conserved residues with spacing like the heavy chain transmembrane contact sites. The Aα, Aβ, and Eα chains show similar constraints (33,34; E. Choi and J. Seidman, personal communication). In contrast, none of the sequenced class I H-2 or HLA chains which are anchored in membranes by single chains contain conserved residues at these sites (35-43, reviewed in 44).

The high degree of conservation (85%, 4 differences out of 27 amino acids) seen in the M2 exons is not as easy to explain. The function of the protein encoded by the M2 exon, the intracellular segment, is not known, so discussion of selective pressure is purely speculative. Alternatively,

conservations may be due to limited genetic exchange or gene conversion, as proposed for the γ_{2a} gene (45). It has been observed that exons and introns in closely related genes can evolve at different rates, and arguments have been made for crossover events involving separate sequence domains (46). Such events, in this case, would have to be postulated to occur almost entirely within the exons, since the intron between M1 and M2, while exhibiting conservation of size, shows limited sequence homology between γ_3 and γ_{2a} or γ_{2b}. Curiously, the much larger CH3 → M1 intron exhibits strong homology (about 90%) between γ_1, γ_{2a}, γ_{2b} and the sequenced region of γ_3. This is comparable to the values seen for the other introns in the γ genes (47,48).

The factors controlling the production of membrane vs. secreted immunoglobulins are not understood. It is clear that one of the first steps in the B cell lineage is the switch from μ_m to μ_s. As known for other hybridoma lines fused with myeloma cell parents, the B1C subclone used here produces μ_s, γ_m, γ_s but no μ_m. This indicates that the expression of μ and γ M exons is under different control. It is possible that the sequences in the M2 exon have a role in this differentiation. The M2 coding region for μ contains only 2 amino acids, as opposed to the 27 amino acids in the M2 γ family. In addition, the 3' untranslated regions in the γ M2 exons range from 1.3-1.8 kbp, while the corresponding μ region is only 267 bp. Experiments are in progress to determine if control of antigen reactivity of lymphocytes resides in these sequences.

The question of the mechanism of the co-expression of μ and γ_3 remains. The cells contain three copies of chromosome 12 (22). This raises the possibility that transcription of the μ and γ genes occurs on rearranged heavy chain genes on different chromosomes. A second possibility is that there has been a duplication of the V region, so that both the μ and γ_3 genes have a rearranged V region. This would, as would the first possibility, result in separate transcription units for the two heavy chain genes. Support for separate transcription units comes from analysis of subclones of B1 which have lost either μ or γ_3 expression during cell passage. A third possible mechanism would involve a large complex transcription unit containing a rearranged V region and spanning the μ and γ_3 gene segments. The μ_s, γ_m and γ_s would be generated by differential RNA splicing. This latter possibility has been suggested for the co-expression of IgM and IgE (49). Resolution of these possible mechanisms will be necessary to establish the biological relevance of these cells co-expressing IgM and IgG to B cell development.

ACKNOWLEDGEMENTS

We would like to thank Drs. Mark Davis and Stuart Kim for the clones pγ3C6 and p606γ_{3-1}. We thank Dr. Donald Capra for cells and stimulating discussion. Carla Carter and Tatjana Loh provided excellent technical support, and Jane Kobayashi and Mayo Yerington were invaluable during preparation of the manuscript. This work was supported by NIH grants AI 13410 and CA 12800, and NSF grant PCM 7924876.

[2]Present address: Department of Medicine, Stanford University, Palo Alto, CA 94305, USA

[6]Present address: Alcon Laboratories, 6201 S. Freeway, Fort Worth, TX 76134, USA

[3]Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

REFERENCES

1. Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L. and Wall, R. (1980) Cell 20, 303-312.
2. Early, P., Rogers, J., Davis, M., Calame, K., Bond, M., Hood, L. and Wall, R. (1980) Cell 20, 313-319.
3. Williams, P.B., Kubo, R.T. and Grey, H.M. (1978) J. Immunol. 121, 2435-2439.
4. Alt, F.W., Bothwell, A.L.M., Knapp, M, Siden, E., Mather, E., Koshland, M. and Baltimore, D. (1980) Cell 20, 293-301.
5. Rogers, J. and Wall, R. (1980) Proc. Nat. Acad. Sci. USA 77,1877-1879.
6. Nevins, J.R. and Darnell, J.E., Jr. (1978) Cell 15, 1477-1493.
7. Lai, C.-J., Dhar, R. and Khoury, G. (1978) Cell 14, 971-982.
8. Schibler, U., Marcu, K.B. and Perry, R.P. (1978) Cell 15,1495-1509.
9. Gilmore-Hebert, M. and Wall, R. (1979) J. Mol. Biol. 135, 879-891.
10. Darnell, J.E., Jr. (1982) Nature 297, 365-371.
11. Honjo, T., Obata, M., Yamawaki-Kataoka, Y., Kataoka, T., Kawakami, T., Takahashi, N. and Mano, Y. (1979) Cell 18, 559-568.
12. Tucker, P.W., Marcu, K., Newell, N., Richards, J. and Blattner, F.R. (1979) Science 206, 1303-1306.
13. Yamawaki-Kataoka, Y., Kataoka, T., Takahashi, N., Obata, M. and Honjo, T. (1980) Nature 283, 786-789.
14. Tucker, P.W., Slighton, J.L., and Blattner, F.R. (1981) Proc. Acad. Nat. Sci. USA 78, 7684-7688.
15. Wall, R., Choi, E., Carter, C., Kuehl, M. and Rogers, J. (1980) Spring Cold Harbor Symp. Quant. Biol. 45, 879-885.
16. Rogers, J., Choi, E., Souza, L., Carter, C., Word, C., Kuehl, M., Eisenberg, D. and Wall, R. (1981) Cell 26, 19-27.
17. Tyler, B.M., Cowman, A.F., Adams, J.M. and Harris, A.W. (1981) Nature 293, 406-408.
18. Tucker, P.W., Liu, C.P., Mushinski, J.F. and Blattner, F.R. (1980) Science 209, 1353-1360.
19. Moore, K.W., Rogers, J., Hunkapillar, T., Early, P., Nottenberg, C., Weissman, I., Bazin, H., Wall, R. and Hood, L. (1981) Proc. Nat. Acad. Sci. USA 78, 1800-1804.
20. Mushinski, J.F., Blattner, F.R., Owens, J.D., Finkelman, F.D., Kessler, S.W., Fitzmaurice, L., Potter, M. and Tucker, P.W. (1980) Proc. Nat. Acad.Sci. USA 77, 7405-7409.
21. Maki, R., Roeder, W., Traunecker, A., Sidman, C., Wabl, M., Raschke, W.

and Tonegawa, S. (1981) Cell 24, 353-365.
22. Robertson, S.M., Clayton, L., Dev, V.G., Wall, R., Capra, J.D. and Kettman, J.R. (1982) Fed. Proc. 41, 2502-2506.
23. Heidecker, G., Messing, J. and Gronenborn, B. (1980) Gene 10,69-73.
24. Galfre, G., Howe, S.C. and Milstein, C. (1977) Nature 266, 550-552.
25. Littlefield, J.W. (1964) Science 145, 709-710.
26. Tyler, B.M., Cowman, A.F., Gerondakis, S.D., Adams, J.M. and Bernard, O. (1982) Proc. Nat. Acad. Sci. USA 79. 2008-2012.
27. Adams, J.M., Gough, N.M., Webb, E.A., Tyler, B.M., Jackson, J. and Cory, S. (1980) Biochem. 19, 2711-2719.
28. Yamawaki-Kataoka, Y., Nakai, S., Miyata, T. and Honjo, T. (1982) Proc. Nat. Acad. Sci. USA 79, 2623-2627.
29. Korman, A.J., Auffray, C., Schamboeck, A. and Strominger, J.L. (1982) Proc. Nat. Acad. Sci. USA 79, 6013-6017.
30. Lee, J.S., Trowsdale, J., Travers, P.J., Carey, J., Grosveld, F., Jenkins, J. and Bodmer, W.F. (1982) Nature 299, 750-752.
31. Larhammar, D., Schenning, L., Gustafsson, K., Wiman, K., Claesson, L., Rask, L. and Peterson, P. (1982) Proc. Nat. Acad. Sci. USA 79, 3687-3691.
32. Bregere, F., Abastado, J.P., Krist, S., Rask, L., Lalanne, J.L., Garoff, H., Cami, B., Wiman, K., Larhammar, D., Peterson, P.A., Gachelin, G., Kourilsky, P. and Dobberstein, B. (1982) Nature 292, 78-81.
33. Benoist, C.O., Mathis, D.J., Kanter, M.R., Williams, V.E., II and McDevitt, H.O. (1983) Proc. Nat. Acad. Sci. USA 80, 534-538.
34. McNicholas, J., Steinmetz, M., Hunkapillar, T., Jones, P. and Hood, L. (1982) Science 218, 1229-1232.
35. Coligan, J.E., Kindt, T.J., Uehara, H., Martinko, J. and Nathenson, S.G. (1981) Nature 291, 35-39.
36. Krist, S., Bregegere, F., Rask, L., Cami, B., Garoff, H., Daniel, F., Wiman, K., Larhammar, D., Abastado, J.P., Gachelin, G., Peterson, P.A., Dobberstein, B. and Kourilsky, P. (1981) Proc. Nat. Acad. Sci. USA 78, 2772-2776.
37. Auffray, C. Korman, A.J., Roux-Dosseto, M., Bono, R. and Strominger, J. (1982) Proc. Nat. Acad. Sci. USA 79, 6337-6341.
38. Evans, G.A., Margulies, D.H., Camerini-Otero, R.D., Ozato, K. and Seidman, J.G. (1982) Proc. Nat. Acad. Sci. USA 79, 1994-1998.
39. Malissen, M., Malissen, B. and Jordan, B.R. (1982) Proc. Nat. Acad. Sci. USA 79, 893-897.
40. Moore, K.W., Sher, B.T., Sun, Y.H., Eakle, K. and Hood, L. (1982) Science 215, 679-682.
41. Reyes, A.A., Schöld, M., Itakura, K. and Wallace, R.B. (1982) Proc. Nat. Acad. Sci. USA 79, 3270-3274.
42. Krist, S., Roberts, L. and Dobberstein, B. (1983) The EMBO Journal 2, 245-254.
43. Lalanne, J.-L., Delarbre, C., Gachelin, G. and Kourilsky, P. (1983) Nucleic Acids Res. 11, 1567-1577.
44. Hood, L., Steinmetz, M. and Malissen, B. (1983) Ann. Rev. Imm. 1, 529-568.
45. Ollo, R. and Rougeon, F. (1983) Cell 32, 515-523.
46. Miyata, T., Yasunaga, T., Yamawaki-Kataoka, Y., Obata, M. and Honjo, T. (1980) Proc. Nat. Acad. Sci. USA 77, 2143-2147.
47. Ollo, R., Auffray, C., Morchamps, C. and Rougeon, F. (1981) Proc. Nat. Acad. Sci. USA 78, 2442-2446.
48. Yanawaki-Kataoka, YU., Miyata, T. and Honjo, T. (1981) Nucleic Acids Res. 9, 1365-1381
49. Yaoita, Y., Kumagai, Y., Okumura, K. and Honjo, T. (1982) Nature 297, 697-699.