J CHAIN IS ENCODED BY A SINGLE GENE UNLINKED TO OTHER IMMUNOGLOBULIN STRUCTURAL GENES*

By MAYUMI YAGI,‡ PETER D'EUSTACHIO,§ FRANK H. RUDDLE, and MARIAN ELLIOTT KOSHLAND‖

From the Department of Microbiology and Immunology, University of California, Berkeley, California 94720, and the Department of Biology, Yale University, New Haven, Connecticut 06520

Considerable progress has been made in understanding the function and biosynthesis of the immunoglobulin J chain. Functional studies have shown that J chain is required for the assembly of the polymeric immunoglobulins; it initiates polymerization by forming a disulfide bridge between two monomer subunits and is incorporated into the secreted product in a ratio of one J chain per polymer (1, 2). Biosynthetic studies have established that J chain is produced as a consequence of antigen or mitogen stimulation. Unstimulated populations of splenic lymphocytes contain little or no J chain, but they respond to mitogen stimulation with a rapid and dramatic increase in J chain levels (3–5). Moreover, analyses of lymphoid cell lines have indicated that J chain synthesis is activated at the level of transcription. Lymphomas with the properties of virgin B cells lack detectable J chain-specific RNA, whereas Ig-secreting plasmacytomas contain both precursor and mature J chain RNA (6, 7).

In contrast, little or no progress has been made in understanding the genetics of the J chain. Investigations using classical genetic methods have been hampered by the absence of detectable polymorphic markers or mutant forms of the J protein. However, recent advances in recombinant DNA technology and somatic cell hybridization have provided the means to overcome these difficulties. We have applied such techniques to determine the chromosomal location and copy number of the murine J chain gene. The gene was mapped by screening the DNA from different mouse × hamster cell hybrids (8) for the presence of J chain-specific sequences. The number of gene copies was assessed by digesting lymphoid DNA with *Eco* RI endonuclease and comparing the ability of the digest and a J chain cDNA-containing plasmid to hybridize with a J chain probe.

Materials and Methods

Somatic cell hybrids formed between the Chinese hamster cell line E36 and various primary mouse cells or established mouse cell lines were grown, karyotyped, and tested for isoenzyme markers as described previously (8). The murine B lymphoma, WEHI 231, was obtained from Dr. W. Raschke, Salk Institute, La Jolla, CA. The cells were maintained in Dulbecco's modified

* Supported by grants GM-09966 (to Dr. Ruddle) and AI-07079 (to Dr. Koshland) from the National Institutes of Health, and training grant CA-09179 (to Dr Yagi).

‡ Present address: Tumor Biology, Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104.

§ Special Fellow of the Leukemia Society of America.

‖ To whow correspondence should be addressed at the Department of Microbiology and Immunology, University of California, Berkeley, CA 94720.

Eagle's medium containing 4,500 mg glucose/liter medium, and supplemented with 10% fetal calf serum and 5×10^{-5} M 2-mercaptoethanol. Karyotype analysis was performed according to the method of D'Eustachio et al. (8). High-molecular weight DNA was isolated from the somatic cell hybrids by the method developed for the chromosomal location of the murine light chains (8). DNA was prepared from the WEHI 231 cells by a modification (9) of the method of Gross-Bellard et al. (10).

For the mapping of the J chain gene, DNA from the somatic cell hybrids was digested with *Eco* RI endonuclease, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose (11). The J chain cDNA insert from a pBR322 plasmid (6) was labeled with $\alpha[^{32}P]$ deoxycytidine triphosphate and $\alpha[^{32}P]$deoxythymidine triphosphate to a specific activity of 1×10^8–3×10^8 cpm/μg by nick translation (12). In addition to the J chain cDNA insert, ^{32}P-labeled pBR322 plasmid containing the α-fetoprotein sequence "B" genomic DNA (13) was used as a probe. Hybridization of the probes to nitrocellulose was performed essentially as described by Wahl et al. (14). With the α-fetoprotein probe 40% (vol/vol) formamide was omitted from the presoak and hybridization buffers. With both probes excess unhybridized label was removed by washing the filters sequentially at 65°C in buffers containing 0.1% (wt/vol) sodium dodecyl sulfate and 1.0 × sodium saline citrate (SSC),[1] 0.5 × SSC, and 0.3 × SSC (1.0 × SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7.4). The filter probed with α-fetoprotein plasmid was further washed in 0.1 × SSC at 65°C. Autoradiography was performed at −70°C using Kodak X-OMat R film (Eastman Kodak Co., Rochester, NY) and Dupont Cronex "Lighting-Plus" intensifying screens (Dupont Instruments, Wilmington, DE).

For quantitation of the number of J chain genes, the pBR322 plasmid containing an 1,100 bp J chain cDNA insert (6) served as the internal standard. A stock solution was prepared by adjusting the plasmid DNA to a concentration of 1 mg/ml as judged by its absorption at 260 nm and an $\epsilon_{1\%}$ of 200. The stock solution was diluted $1{:}10^5$, 5 μl added to the first tube, and twofold serial dilutions were made. Aliquots containing 10 μg of WEHI 231 DNA were added to each tube, and the mixtures were digested with *Eco* RI endonuclease and analyzed by the Southern blotting technique as described above. The resulting autoradiographs were scanned by use of a "Quick-Scan" densitometer (Helena Laboratories, Beaumont, TX). The scans were photocopied and the peaks drawn in, cut out, and weighed on a Mettler balance.

Results

Chromosomal Location of the J Chain Gene. The mouse J chain gene was mapped using a series of mouse x hamster hybrids known to have differentially segregated mouse chromosomes (8). DNA from the somatic hybrids and two parental lines was digested with *Eco* RI endonuclease and analyzed for the presence of J chain sequences by the Southern blotting technique. The autoradiograph (Fig. 1 A) showed a single J chain-specific fragment of 6.4 kilo-base pair (kb) in the DNA from the mouse fibroblast line A9 (lane 1). However, no fragments were detected in the DNA from the hamster parental line E36 (lane 2). This result was not surprising because the probe used contained primarily the 3′ untranslated region of the J chain message and such sequences are believed to diverge in evolution at a faster rate than the coding sequences. Similar data were obtained in analyses of the DNA of other species; the mouse J chain cDNA probe was found to hybridize slightly with *Eco* RI-digested DNA from the closely related rat, but not with comparable preparations from the more distantly related rabbit and human species.

A J chain-specific fragment of 6.4 kb was identified in two of the somatic hybrid DNA preparations, BEM 1-4 and MACH 7A13-3B3 (lanes 4 and 5, Fig. 1 A). Even after prolonged exposure, no bands were observed in any of the other hybrid lines. By comparing these restriction patterns with the content of mouse chromosomes in hybrid cells (Table I), the J chain gene could be assigned to chromosome 5. Only this

[1] *Abbreviations used in this paper:* bp, base pair; kb, kilo-base pair; SSC, sodium saline citrate.

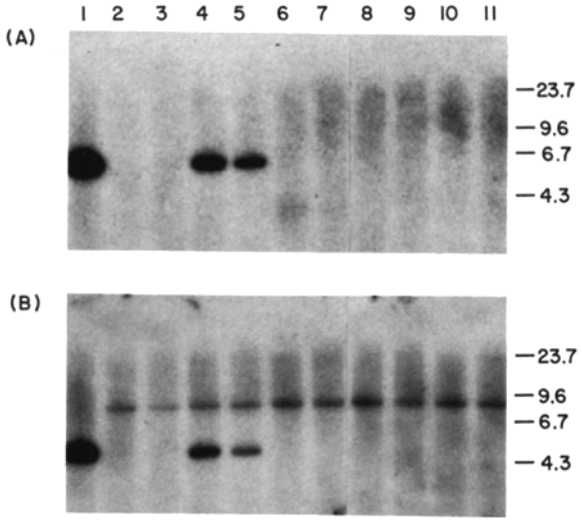

FIG. 1. J chain (A) and α-fetoprotein (B) gene fragments. 25-μg samples of DNA from mouse (A9), Chinese hamster (E36), and hybrid cell lines were digested with *Eco* RI restriction endonuclease, fractionated by electrophoresis on a 1% agarose slab gel made in 0.16 M Tris, 0.08 M sodium acetate, 0.08 M NaCl, 0.005 M EDTA, pH 8, transferred to nitrocellulose (7), and hybridized with a ^{32}P-labeled J chain cDNA probe (A). Following autoradiography, the radiolabeled probe was removed by incubating the filter for 20 min in 250 ml 0.1 M NaOH − 2 × SSC at room temperature with gentle shaking, and rinsing it with two 250 ml portions of 2 × SSC. The filter was then hybridized with a ^{32}P-labeled α-fetoprotein probe (B). Lane 1, A9 DNA; 2, E36 DNA; 3, BEM 1-6; 4, BEM 1-4; 5, MACH 7A13-3B3; 6, MACH 4A63; 7, MACH 4A64 A-1; 8, MAE 28A; 9, MAE 32; 10 ECm4c; 11, R44.1.

TABLE I

Hybrid Cell Lines Tested for J Chain and α-Fetoprotein Genes

Hybrid	Mouse chromosomes present*	Reaction with probes	
		J	α-feto-protein
BEM 1-6	1, 2, 3, 4, 5, 6, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, X	−	−
BEM 1-4	1, 2, 3, *5*, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19, X	+	+
MACH 7A13-3B3	2, *5*, 7, 9, 12, 13, 14, 15, 17, 18, 19	+	+
MACH 4A63	2, 7, 12, 13, 15, 16, 17, 18, 19	−	−
MACH 4A64 A-1	1, 2, 7, 12, 15, 17, 19	−	−
MAE 28A	12, X	−	−
MAE 32	16, X	−	−
ECm4c	14, 15	−	−
R44.1	17	−	−

* Detailed analyses of these cell lines have been published previously (6). All cell lines contained a full set of Chinese hamster (E36) chromosomes. Mouse chromosomes detected at a frequency ≥0.20 per cell were scored as present. This frequency corresponds to the approximate limit of detection at a single-copy mouse gene sequence against a diploid or tetraploid Chinese hamster background under our assay conditions (6).

chromosome was shared by the BEM 1-4 and MACH 7A13-3B3 lines and missing from all the remaining lines. To confirm the location of the J chain gene, the J chain probe was stripped from the filter (15), and the filter was rehybridized with a probe to another gene sequence mapped to chromosome 5, α-fetoprotein (16). Nick-trans-

lated plasmid carrying a 4.75 kb *Eco* RI fragment (B) of the mouse α-fetoprotein gene (13) hybridized strongly with the corresponding fragment in the DNA from mouse fibroblast cells (lane 1, Fig. 1 B) and the same two hybrid lines as the J chain probe (lanes 4 and 5, Fig. 1 B). In addition, the α-fetoprotein probe recognized an 8 kb fragment arising from the DNA of the hamster parent.

Number of J Chain Genes in the Mouse Genome. As a first approach to determining the number of J chain genes, the structure of J chain sequences in the mouse genome was analyzed by restriction mapping. The patterns obtained indicated that both the coding and noncoding regions were homogeneous with respect to endonuclease cleavage sites. For example, when the 1,100-base pair (bp) J chain cDNA insert was used as a probe, only a single band of hybridization was observed. Digestion with *Eco* RI enzyme yielded the J chain-specific fragment of 6.4 kb shown in Fig. 1 A; digestion with *Bam* HI, *Msp* I, and *Hin* dIII gave single fragments of 6.2, 7.5, and 8.0 kb, respectively. A similar homogeneity in fragment size was seen with other cDNA probes that hybridized with fragments containing 5′ flanking sequences. These findings suggested that the J chain gene is present in low copy number.

To obtain more definitive evidence, the amount of the *Eco* RI fragment hybridizing to the J chain cDNA probe was determined by comparison to a known standard. The WEHI 231 lymphoma was chosen as the source of the DNA because karyotype analysis showed that the cells have a near-diploid chromosomal content (Fig. 2). In 33 individual cells examined, the mean number of chromosomes was 41.5 with a standard error of 0.78, and chromosome 5 was present in 1.77 copies per cell with a standard deviation of 0.08. The pBR322 plasmid containing 1,100 bp of J chain cDNA was chosen as the standard. This plasmid has the advantage that the cDNA was inserted in the *Pst* I site, and thus the plasmid could be cleaved at its single *Eco* RI site to yield

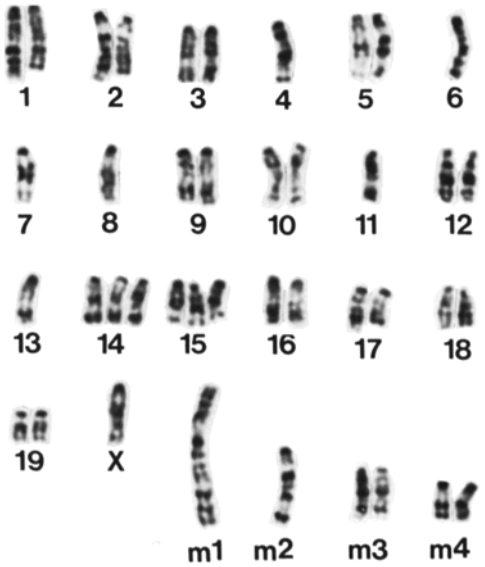

FIG. 2. Karyotype of a WEHI 231 cells. The cell line is near diploid, most cells having between 40 and 42 chromosomes and at least one normal copy of each chromosome. Four marker chromosomes (m1–m4) were found in most of the cells examined, and one copy each of chromosomes 1, 2, and 17 (shown here as the right hand member of the pair) had suffered a small terminal deletion.

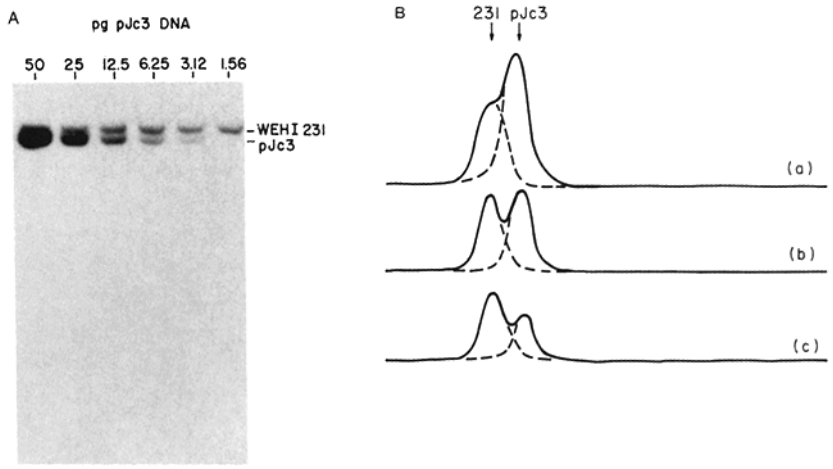

FIG. 3. Estimation of J chain gene number (A). Mixtures of a constant amount (10 μg) of genomic DNA from the mouse cell line WEHI 231 and variable amounts of plasmid pJc3 DNA were digested with *Eco* RI enzyme, electrophoresed in agarose, blotted onto nitrocellulose, and hybridized with ^{32}P-labeled pJc3 probe, as described in Materials and Methods. (B) Densitometric tracings of tracks containing 25(a), 12.5(b), and 6.25(c) pg of plasmid DNA. To determine the relative areas under the peaks, the line defining each peak was extrapolated back to the baseline (----), and the peak area was cut out of the tracing and weighed (Table II).

a fragment of 5.5 k. The slightly smaller size of the plasmid fragment allowed it to be separated from the 6.4 kb *Eco* RI genomic fragment by gel electrophoresis. At the same time, the plasmid and genomic fragments were similar enough in size that their transfer efficiencies to nitrocellulose could be assumed to be equal. For the measurements, varying amounts of plasmid DNA, 1.6–50 pg, were added to a constant amount, 10 μg, of WEHI 231. The amounts of plasmid standard used were based on the following calculation: if the haploid mouse genome is 3.5×10^{-12} g, then 10 μg of DNA should contain $(10 \times 10^{-6}\ \text{g})/(3.5 \times 10^{-12}\ \text{g/copy}) = 2.86 \times 10^{6}$ copies of a given unique sequence. 2.86×10^{6} copies of a 5.5 kb plasmid correspond to $(2.86 \times 10^{6}\ \text{copies} \times 5.5 \times 10^{3}\ \text{base pairs} \times 650\ \text{daltons/bp})/(6.02 \times 10^{23}\ \text{daltons/g}) = 16.97 \times 10^{-12}$ g, i.e. 17 pg.

The mixtures were then digested with *Eco* RI, electrophoresed, blotted, and hybridized with the ^{32}P-labeled 1,100 bp J chain cDNA insert. The resulting autoradiograph (Fig. 3A) was scanned with a densitometer and the relative extent of hybridization was determined by cutting out and weighing each peak. The densitometer scans for the lanes containing 25, 12.5, and 6.25 pg of plasmid DNA are shown in Fig. 3B, and the corresponding measurements of the areas under the peaks are given in Table II. The extent of hybridization of the genomic fragment ranged from 0.68 to 1.81 of that obtained with the plasmid standards (column 5, Table II). It was calculated from these data that the amount of genomic fragment represented on the average 0.8 of the theoretically expected value for one copy (column 6, Table II) and 0.9 of the experimentally determined value of 0.89 copies (1.77/2). These results indicated that, as precisely as can be determined by this method, there is only one J chain gene per haploid mouse genome.

TABLE II

Quantitation of J Chain-specific Hybridization

cDNA added	Peak size*		Genomic DNA/cDNA		J chain gene copies
	cDNA	Genomic DNA	Expected‡	Observed	
pg		*mg*			
25	46.30	32.20	0.68	0.68	1.0
12.5	26.00	26.45	1.36	1.02	0.75
6.25	12.50	22.60	2.72	1.81	0.67

* The densitometer scans were photocopied. The peaks were drawn in, cut out, and weighed on a Mettler balance.

‡ The expected ratio was that calculated for one copy per haploid genome.

Discussion

The genetic analyses presented in this paper indicate that the single J gene found in the haploid mouse genome is located on chromosome 5, unlinked to other immunoglobulin structural genes. These results contribute to the understanding of J chain gene expression during B cell differentiation. The J protein is required at two widely separated stages of differentiation, first for the assembly and secretion of pentamer IgM that follows a primary antigenic challenge in all B cells, and later for the production of polymer IgA in that subpopulation of cells which have undergone one or more heavy chain class switches and are synthesizing alpha chains (1). The finding that the J chain gene exists in only one copy per haploid genome means that the same gene product is used for the polymerization of both IgM and IgA. Moreover, the existence of a single gene may explain the synthesis of J chain in cells that secrete monomer Ig and thus represent intermediates in the heavy chain switch process. Although J chain is apparently not necessary for the production of IgG and IgE, no cell line actively secreting these Ig classes has been found to lack J chain (17–21). Its continued expression could be a mechanism to ensure that a J chain product is available in the event of a switch to IgA production. The data do not, however, eliminate the possibility that J chain may also play some other role in antigen-induced B cell differentiation.

The genetic analyses of the J chain also contribute to an understanding of the signaling of B cell differentiation The encounter of a virgin B cell with antigen is known to have several coordinated effects: activation of J chain synthesis (3, 4), a shift in μ chain synthesis from the membrane to the secreted form (22, 23), and an increase in the rate of μ chain, and light chain synthesis that parallels that of the J chain (5). The location of the J chain gene on chromosome 5 indicates that these differentiative changes are mediated by control mechanisms operating on three different loci on three different chromosomes. To generate such multiple effects, the interaction of antigen or mitogen at the B cell surface must induce either a series of separate signals or a single signal that has a cascade action on loci at other chromosomes. Which of these two signaling mechanisms is responsible for the differentiative changes observed and how the changes are coordinated present a challenging problem in eukaryotic gene expression.

Summary

Immunoglobulin J chain mediates the polymerization of both IgM and IgA immunoglobulins. Its synthesis is closely regulated in B lymphocytes, apparently at the level of RNA transcription. To define the genetic bases of this regulation, we have determined the location and number of J chain genes in the mouse. Analysis of DNA from a group of somatic cell hybrids containing various mouse chromosomes on a constant background of Chinese hamster chromosomes indicated that this gene is located on mouse chromosome 5, unlinked to immunoglobulin heavy and light chain structural genes. Restriction mapping experiments further suggested the existence of a single J chain gene per haploid genome. This result was confirmed by quantitative analyses of band intensities yielded by Southern blots of mouse genomic DNA and J gene-containing plasmid DNA.

We are grateful to Dr. Robert Tijan for his valuable suggestions on determining gene copy number, and would like to thank Dr. Shirley Tilghmann for the gift of the mouse α-fetoprotein probe.

Received for publication 9 June 1981 and in revised form 2 November 1981.

References

1. Koshland, M. E. 1975. Structure and function of the J chain. *Adv. Immunol.* **10:**41.
2. Chapuis, R. M., and M. E. Koshland. 1974. Mechanism of IgM polymerization. *Proc. Natl. Acad. Sci. U. S. A.* **71:**657.
3. Mestecky, J., R. J. Winchester, T. Hoffman, and H. G. Kunkel. 1977. Parallel synthesis of immunoglobulins and J chain in pokeweed mitogen-stimulated normal cells and in lymphoblastoid cell lines. *J. Exp. Med.* **145:**760.
4. Mather, E. L., and M. E. Koshland. 1977. The role of J chain in B cell activation. *In* Immune System: Genetics and Regulation. E. E. Sercarz, L. A. Herzenberg, and C. F. Fox, editors. Academic Press Inc., New York. 727–733.
5. Roth, R. A., E. L. Mather, and M. E. Koshland. 1979. Intracellular events in the differentiation of B lymphocytes to pentamer IgM synthesis. *In* Cells of Immunoglobulin Synthesis. H. Vogel and B. Pernis, editors. Academic Press Inc., New York. 141–151.
6. Mather, E. L., F. W. Alt, A. L. M. Bothwell, D. Baltimore, and M. E. Koshland. 1981. Expression of J chain RNA in cell lines representing different stages of B lymphocyte differentiation. *Cell.* **23:**369.
7. McCune, J. M., S. M. Fu, and H. G. Kunkel. 1981. J chain biosynthesis in pre-B and other possible precursor B cells. *J. Exp. Med.* **154:**138.
8. D'Eustachio, P., A. L. M. Bothwell, T. K. Takaro, D. Baltimore, and F. H. Ruddle. 1981. Chromosomal location of structural genes encoding murine immunoglobulin λ light chains. *J. Exp. Med.* **153:**793.
9. Yagi, M., and M. E. Koshland. 1981. Expression of the J chain gene during B cell differentiation is inversely correlated with DNA methylation. *Proc. Nat. Acad. Sci. U. S. A.* **78:**4907.
10. Gross-Bellard, M., P. Oudet, and P. Chambon. 1973. Isolation of high-molecular-weight DNA from mammalian cells. *Eur. J. Biochem.* **36:**32.
11. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98:**503.
12. Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113:**237.

13. Kioussis, D., F. Eiferman, P. van de Rijn, M. B. Gorin, R. S. Ingram, and S. M. Tilghman. 1981. The evolution of α-fetoprotein and albumin. II. The structures of the α-fetoprotein and albumin genes in the mouse. *J. Biol. Chem.* **256:**1960.
14. Wahl, G. M., M. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. U. S. A.* **76:**3683.
15. McGrogan, M., D. J. Spector, C. J. Goldenberg, D. Halbert, and H. J. Raskas. 1979. Purification of specific adenovirus 2 RNAs by preparative hybridization and selective thermal elution. *Nucl. Acids. Res.* **6:**593.
16. D'Eustachio, P., R. S. Ingram, and S. M. Tilghman. 1981. Murine α-fetoprotein and albumin: two evolutionarily linked proteins encoded on the same mouse chromosome. *Somat. Cell Genet.* **7:**289.
17. Kaji, H., and R. M. E. Parkhouse. 1974. Intracellular J chain in mouse plasmacytomas secreting IgA, IgM and IgG. *Nature (Lond.).* **249:**45.
18. Kaji, H., and R. M. E. Parkhouse. 1975. Control of J chain biosynthesis in relation to heavy and light chain synthesis, polymerization and secretion. *J. Immunol.* **114:**1218.
19. Mosmann, T., and R. Baumal. 1975. Synthesis but not secretion of J chain by variant mouse myeloma cells which lose α-chain-synthesizing ability. *J. Immunol.* **115:**955.
20. Mestecky, J., J.-L. Preud'homme, S. S. Crago, E. Mihaesco, J. T. Prchal, and A. J. Okos. 1980. Presence of J chain in human lymphoid cells. *Clin. Exp. Immunol.* **39:**371.
21. McHugh, Y., M. Yagi, and M. E. Koshland. 1980. The use of J and μ chain analyses to assign lymphoid cell lines to various stages in B cell differentiation. *In* B Lymphocytes in the Immune Response: Functional, Developmental, and Interactive Properties. N. Klinman, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier/North-Holland, New York. 474.
22. Alt, F. W., A. L. M. Bothwell, M. Knapp, E. Siden, E. Mather, M. Koshland, and D. Baltimore. 1980. Synthesis of secreted and membrane-bound immunoglobulin μ heavy chains is directed by mRNAs that differ at their 3′ ends. *Cell.* **20:**293.
23. Rogers, J., P. Early, C. Carter, K. Calame, M. Bond, L. Hood, and R. Wall. 1980. Two mRNAs with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin μ chain. *Cell.* **20:**303.