with iron carbonyl particles and subsequent removal of iron-loaded monocytes by a magnet. This mononuclear fraction was incubated in ice with AET (2-aminoethylisothioronium bromide hydrobromide)-treated sheep red blood cells (SRBC) to allow for rosette formation. Non-erythrocyte (E) rosette-forming cells were recovered after application of the whole SRBC mononuclear fraction to a lymphocyte-separating medium gradient. This nonadherent non-E rosetting fraction was constituted of at least 50% B cells, less than 1% monocytes, and variable amounts of lymphocytes with the natural killer (NK) phenotype, as assessed with mouse monoclonal antibodies OKB7, B77.1, and B73.1, respectively. We refer to this non-E rosetting B-enriched lymphocyte fraction as B cells.

7. Human Tg was isolated from normal thyroid tissue obtained at autopsy [S. H. Roman, F. Korn, T. F. Davies, *Clin. Chem.* **30**, 246 (1984)]. Tissue was homogenized in phosphate-buffered saline (PBS) (*p*H 7.2) and centrifuged at 100,000*g*, and the supernatant was applied to a Sephadex G-200 (Pharmacia) column. The Tg was purified to homogeneity by application of the first eluted peak to a Sepharose 6B (Pharmacia) column. Tg was stored in aliquots at −70°C. Semipurified tetanus toxoid (TT) was obtained from the Commonwealth of Massachusetts (Department of Health, Boston, MA) and fractionated to homogeneity by gel filtration on a Sephadex (Pharmacia) G-150 column. Both Tg and TT were labeled with *n*-hydroxysuccinimidobiotin (Sigma) in 0.1*M* carbonate buffer, *p*H 8.5, at a protein-to-biotin ratio of 4:1 followed by exhaustive dialysis against PBS.
8. Approximately 2×10^7 B cells (5×10^6 per milliliter) were incubated for 2 hours in ice-chilled sterile Hanks balanced salt solution without Ca^{2+} and Mg^{2+}, without phenol red, and with 1% bovine serum albumin (BSA-HBSS) containing biotinylated Tg ($4.50 \times 10^{-9}M$). The cells were washed with cold BSA-HBSS and then allowed to react with FITC-avidin ($1.56 \times 10^{-7}M$) in cold BSA-HBSS for 1 hour. A smaller sample of B cells (10^6) was simultaneously incubated with BSA-HBSS devoid of biotinylated Tg and allowed to react with FITC-avidin under similar conditions. After further washing with cold BSA-HBSS, cells from both samples were resuspended at a density of 10^6 cells per milliliter in the same medium and at different times applied to a Becton and Dickinson 440 FACS with an Argon 466 laser.
9. EBV used to infect B lymphocytes was obtained from culture fluids of B95-8 marmoset lymphoma cells incubated at 37°C in the presence of $1.62 \times 10^{-8}M$ 4-phorbol 12β-myristate 13α-acetate (TPA, Sigma). This virus preparation had a titer of 5×10^5 transforming units per milliliter, one unit being the minimum amount of virus-transforming 10^4 purified human B cells.
10. Limiting dilution experiments were performed to assess the degree of enrichment after cell sorting. The ratio of B cells producing IgM reactive with Tg to total B cells producing IgM was: unsorted cells, 1:100; positively sorted cells, 1:3.5; and negatively sorted cells, 1:10,000. The ratio of B cells producing IgM reactive with TT to total B cells producing IgM was: unsorted cells, 1:80; positively sorted cells, 1:2.5, negatively sorted cells, 1:50,000.
11. P. Casali, M. Nakamura, A. L. Notkins, in preparation.
12. D. Kozbor, M. Steinitz, G. Klein, S. Koskimies, O. Mäkelä, *Scand. J. Immunol.* **10**, 187 (1979).
13. D. Kozbor and J. C. Roder, *J. Immunol.* **124**, 1275 (1981).
14. F. Taub, J. Satoh, C. Garzelli, K. Essani, A. L. Notkins, in (*5*), pp. 263–275.
15. C. Garzelli *et al.*, *J. Virol.* **52**, 722 (1984).
16. M. Nakamura, T. F. Davies, A. L. Notkins, P. Casali, in preparation; M. Nakamura, F. Ginsberg-Fellner, A. L. Notkins, P. Casali, in preparation. In addition, by limiting dilution analysis, recent experiments showed that B cells making IgG and IgA antibodies to Tg or insulin, albeit at low frequency, are present in the circulation of healthy subjects and are transformable by EBV (*11*).
17. A. L. Notkins and B. S. Prabhakar, *Ann. N.Y. Acad. Sci.* **475**, 123 (1986).

18. We thank B. Perussia and G. Trinchieri for their steady supply of mouse monoclonal antibodies to human lymphocyte subpopulations. We also thank S. A. Barbieri and J. Wheeler for their expert technical help and E. Mange for typing the manuscript.

23 May 1986; accepted 25 August 1986

Synchronized Rearrangement of T-Cell γ and β Chain Genes in Fetal Thymocyte Development

WILLI BORN, GARY RATHBUN, PHILIP TUCKER, PHILIPPA MARRACK, JOHN KAPPLER

Kinetics of mouse T-cell γ gene rearrangements in ontogeny were determined as an approach to understanding the possible role of these genes in the development of fetal thymocytes. Two of these genes (Cγ1 and Cγ2) rearranged rapidly during days 14 to 17 of the gestational period in BALB/c mice. Moreover, these rearrangements seemed to be tightly synchronized with rearrangements of T-cell receptor β chain genes in the same cells. It is suggested that the early transcriptional activity of γ genes, which precedes that of β chain genes, may not reflect the functional activation of these genes. Nevertheless, productive and therefore potentially functional γ gene rearrangements precede surface expression of T-cell receptors in the thymus by 2 to 3 days, which is compatible with a role for γ gene products in thymocyte development prior to antigen-specific stages.

WHEN ATTEMPTS WERE BEING made to clone the genes of the α and β chains of T-cell receptors for antigen, a third, structurally similar group of genes was found and designated γ (*1, 2*). The organization of these γ genes in variable (V), joining (J), and constant (C) segments placed them within the immunoglobulin-like superfamily of cell-surface receptor genes. Beyond this, T-cell specific rearrangement and expression and high concentrations of γ messenger RNA (mRNA) in fetal thymus populations at days 14 and 15 suggested a role for these genes early in T-cell development (*1, 3–5*). At least in humans, the protein chains encoded by γ genes have now been found (*6, 7*), but their possible functions have remained uncertain.

To help clarify this issue we have analyzed the developmental period in which γ genes rearrange to form the complete VJC-structures that are necessary for function. This study was carried out with a large panel of mouse fetal thymus hybridomas made by fusing thymocytes from fetal mice of different ages to the AKR thymoma, BW5147. These hybridomas appear to be representative of the thymocyte populations from which they were prepared because, for example, the occurrence of β gene rearrangements in the hybridomas correlates well with the kinetics of these events in unfractionated thymocytes (*8, 9*). The studies we report here allowed us to establish the point in fetal life at which γ protein might begin to function and to correlate this time with other events in T-cell differentiation, in particular β chain gene rearrangements (*10*) and intrathymic expression of the α/β heterodimeric receptor for antigen (*11*).

There are at least three highly homologous γ constant regions in the mouse genome (*12*), located on chromosome 13 (*13*). A fourth Cγ gene with little homology to the others has recently been found (*14*). We have analyzed rearrangements associated with the two functional genes of the three Cγ genes that were first described by Hayday *et al.* (*12*). These Cγ segments have been found on three restriction fragments after digestion of germline DNA with the restriction enzyme Eco RI, and have been named correspondingly Cγ 10.5, Cγ 13.4 and Cγ 7.5. In the meantime, a more simple nomenclature has been used by several groups, in which the four known γ constant regions have been assigned arbitrary numbers. In this report we have followed the terminology used recently by Iwamoto *et al.* (*14*): Cγ 10.5 = Cγ1, Cγ 3.4 = Cγ2, Cγ 7.5 = Cγ3, a nonfunctional gene, and the new γ constant region = Cγ4.

Consistent with previous studies we found that Cγ1 and Cγ2 were frequently rearranged in our hybridomas. However, somewhat surprisingly in the view of previous examinations of γ mRNA levels in early fetal thymocytes, we found rearrangements involving Cγ1 and Cγ2 to be tightly synchronized with T-cell receptor β chain gene

W. Born, P. Marrack, J. Kappler, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.
G. Rathbun and P. Tucker, University of Texas Health Sciences Center at Dallas, Dallas, TX 75235.

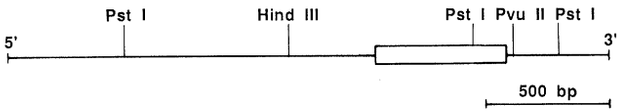

Fig. 1. Restriction map of cDNA clone γ 7.1. The cDNA clone γ 7.1 was derived from an adult normal mouse cDNA library. This library was constructed with mRNA from mouse strain C57BL/ka cloned into the Eco RI site of phage vector λgt10 by standard techniques (*25*). The library was screened with an oligonucleotide probe complementary to the first 48 nucleotides of the published constant portion of mouse γ cDNA clone pHDS4/203 (*1*). The screening of approximately 1.2×10^6 plaques revealed seven clones hybridizing to the oligomer, two of which (γ2 and γ7) were subcloned into the plasmid vector pEMBL9 (*15*) and further characterized by restriction mapping (the sites of restriction enzymes Pst I, Hind III, and Pvu II are indicated) and sequencing. A map of the entire subclone γ 7.1 is shown.

rearrangements, both in ontogeny and at the level of clonal development. The γ rearrangements observed seemed to be direct results of V to J joining, suggesting that diversity (D) γ segments do not exist for the two rearranging genes examined. The kinetics of Cγ rearrangements are compatible with a role for their products in thymocyte development shortly before expression of T-cell receptors for antigen.

To obtain probes to the mouse Cγ region and follow γ gene rearrangements, we isolated a Cγ gene complementary DNA (cDNA) clone, and subcloned it into the plasmid vector pEMBL9 (*15*). The exon-intron structure of a subclone, pγ 7.1, was determined by restriction mapping and nucleotide sequencing (*16*) (see Fig. 1). pγ 7.1 has properties of a processed germline transcript. The correctly spliced constant portion is flanked by a 5′ germline sequence, and a normal 3′ untranslated portion. Southern analysis of Eco RI–digested BALB/c liver DNA with the entire insert of pγ 7.1 as a probe revealed strong hybridization only to the three Cγ gene fragments found by Hayday *et al.* (*12*), but not to the fourth γ constant region or to V genes or J segments alone. Absence of the known V and J segments in this clone has been confirmed by sequencing (*16*).

The insert of pγ 7.1 was used as a probe to screen Southern blots of genomic DNA derived from a panel of fetal liver, fetal thymus, and adult thymus hybridomas (Fig. 2). As noted previously (*17*), fragments of Eco RI–digested germline DNA, hybridizing with pγ 7.1, were of different sizes in AKR and BALB/c mice. Blots of DNA from BW5147 and comparison with the AKR germline pattern suggested that this cell line has a rearrangement associated with Cγ1 (reflected in a 10.5-kb to 22-kb change in band size), and contains germline Cγ2 (at 16.0 kb) and Cγ3 (at 6.6 kb) genes but no germline Cγ1. The fact that blots of BW5147-derived DNA always resulted in fainter hybridization patterns for all three bands than blots of equal amounts of AKR or BALB/c germline DNA suggested that the BW5147 genes are represented in the cell as one allele each, in contrast to the diploid representation in normal cells.

Rearrangements involving Cγ1 and Cγ2 are relatively limited, a fact that facilitated analysis of the DNA's of our hybridomas. It has been shown for some mouse strains, for example, BALB/c, that rearrangements of a Vγ to Cγ1 changes the 10.5-kb Eco RI fragment to 16 kb, and rearrangements of the different Vγ's to the Cγ2 gene usually involve increasing the Eco RI fragment size from 13.4 kb to 22 kb, or similar sizes (*12*). Some of the normal T-cell–derived rearranged Cγ fragments therefore corresponded in size to fragments already present in BW5147. The presence or absence of normal T-cell–derived rearranged DNA in such a band found in hybridoma DNA could easily be identified, however, by a diminution or absence of the normal cell-derived germline band, accompanied by a correspondingly more intense hybridization pattern in one of the rearranged bands (Fig. 2). Hybridoma 7DTE-1 did not contain any rearrangements. 8DTE-8 contained a rearrangement involving Cγ2 in one allele only, since the 13.4-kb band was comparatively diminished in intensity, but was not absent, whereas hybridization in the 22-kb band was more intense when the BW5147-derived 16-kb band was used as an internal reference. Similarly, 8DTE-3 contained a rearrangement in one allele involving Cγ1, 7DTE-6 contained rearrangements involving Cγ1 and Cγ2, each in one allele only. 8DTE-5 contained a rearrangement involving Cγ2. Comparison with the BW5147-derived bands suggested that in this hybridoma only one γ allele from the normal cell parent was present. The hybridization pattern of 8DTE-1 suggested that this hybridoma contained two normal parent-derived γ alleles, and that in this case both Cγ1 genes had undergone rearrangement.

Rearrangements of Cγ3 were not seen, because Eco RI separates J segment–containing from C segment–containing fragments in this gene (*12*). It is unlikely, however, that this gene contributes to the expression of functional γ gene products, since it contains a 15-bp insertion in the second exon of the constant region that renders this exon defective (*12*).

Hybridomas 8DTE-2 and 8DTE-6 both contained rearrangements involving Cγ2, yet these rearrangements differed somewhat from the commonly seen pattern in that the rearranged bands fell between the 22-kb and the 16-kb bands. Patterns like these occurred in 9 of 55 rearrangements involving Cγ2. Similar heterogeneity was not found in the 65 rearrangements involving Cγ1 we analyzed. Therefore, this observation might indicate that Cγ2 can undergo several different rearrangements, for example, because of the existence of multiple V genes or J segments connected with this particular gene. In this context it should be noted that three new rearranged Vγ genes have recently been found in murine fetal thymocytes (*18*). In a total of 144 hybridomas analyzed we never found deletions of Cγ gene–containing germline bands without corresponding rearrangements. This suggested that each of the three γ genes analyzed functions as an independent unit, using its own V and J genes, without spatial overlaps with the other γ genes studied. In this, the mouse and human γ loci may be different, since in the case of human γ genes deletions of a proximal C segment as a consequence of rearrangements

Table 1. Rearrangements of γ genes in mouse ontogeny. Hybridomas that had lost both γ alleles were excluded from the analysis.

Time of gestation	Number of hybridomas analyzed	Total number of hybrids with			Number of γ rearrangements per total number of hybrids with γ rearrangements
		Rearrangements of γ (%)	Rearrangements involving Cγ2 (%)	Rearrangements involving Cγ1 (%)	
		Fetal tissue			
Day 14 liver	24	None	None	None	None
Day 14 thymus	20	3 (15)	2 (10)	1 (5)	1.0
Day 15 thymus	23	10 (43)	8 (34)	4 (17)	1.2
Day 16 thymus	26	18 (69)	8 (31)	16 (62)	1.4
Day 17 thymus	19	15 (79)	10 (67)	11 (73)	2.1
		Adult tissue			
Week 10 thymus	23	20 (87)	13 (57)	17 (74)	1.7

to another, more distal C segment have been frequently observed (*19, 20*).

As in the case of β genes, we found no rearrangements of γ genes in any of the fetal liver hybrids (Table 1). This places an upper limit on the percentage of fetal liver cells that could contain such genes and suggests that γ genes may not rearrange until precursor cells reach the thymus. This observation also indicates that BW5147 by itself does not induce rearrangement at this locus in the genes of normal cells to which it is fused.

In fetal thymus hybrids, only a few γ gene rearrangements were seen at day 14 and these always involved only one allele (Fig. 3 and Table 1). This finding was unexpected because of the previously reported transcriptional activity of these genes, which occurs at high levels at this time (*4, 5*). After day 14 there was a rapid increase in the fraction of hybrids with γ gene rearrangements. At day 17 or in adult thymus hybridomas, γ gene rearrangements usually had occurred in more than one gene or in both alleles of one gene. In these most mature samples up to four γ gene rearrangements per cell were observed. Therefore, γ genes seem to rearrange in the same way as Ig or β genes, in an ordered progression (*10, 21*) during cell differentiation.

Table 2. Coincidence of γ and β gene rearrangements.

Time of gestation	Hybridomas without β rearrangements*		Hybridomas with β rearrangements	
	With γ rearrangements	Without γ rearrangements	With γ rearrangements	Without γ rearrangements
Day 14	1	15	1	2
Day 15	0	11	9	2
Day 16	1	3	18	3
Day 17	0	1	11	3
Total	2	30	39	10

*Hybridomas that had lost all γ or β alleles were excluded; adult thymus hybridomas all contained rearranged β complexes and are therefore not shown here.

Rearrangements involving Cγ2 were predominant at days 14 and 15, whereas later on rearrangements involving Cγ1 were seen more frequently (Table 1). Thus it seems possible that these two genes tend to rearrange sequentially in thymocyte differentiation.

We compared the kinetics of β and γ gene complex rearrangements in mouse ontogeny as represented by our collection of hybridomas. As shown in Fig. 3, lines 1 and 2, rearrangements involving these genes occurred with almost identical kinetics. Because the construction of a functional β chain gene involves both D-to-J and V-to-DJ movements, however, these identical kinetics do not mean that functional β and γ proteins appear at the same time in thymus ontogeny. A more useful comparison is that of γ gene with β gene rearrangements, which are more complex than those of D-to-J. These non-D-to-J rearrangements (*10*) include VDJ joinings. These are indicated by line 3 of Fig. 3, and clearly lag about 2 days behind those of γ.

Since non-D-to-J β rearrangements included all configurations that were classified as "unidentified but involving either Cβ1 or Cβ2" (*10*), and therefore included functional VDJ as well as aberrant rearrangements, the estimate of functional β genes is probably too high. With regard to γ gene rearrangements our analysis cannot reveal the apparently frequent mistakes in the joining process in this locus or in the splicing of γ gene mRNA (*22*). With these caveats, our kinetic data still allow for a functional role of γ genes in thymocyte development about 2 days before T-cell receptors are expressed for the first time. Thus, although for different reasons, we agree with previous conclusions that γ genes may have a physiological function before or early during T-cell receptor expression (*4, 5*).

We also analyzed individual hybridomas for the coincidence of γ and β gene rearrangements (Table 2). Among 81 hybridomas studied only two had rearranged γ complexes in the presence of unrearranged β complexes in both alleles, whereas 39 had rearranged γ complexes in the presence of various β gene rearrangements. Thus γ gene rearrangements are tightly linked to β gene rearrangements in individual thymocyte development. The nature of this linkage remains unclear, but it may be based on common usage of recombinases (*23*) and synchronized activation of the DNA substrate. Finally, the fact that about 20% of the hybridomas with β gene rearrangements did not have detectable γ gene rearrangements might indicate two alternative path-

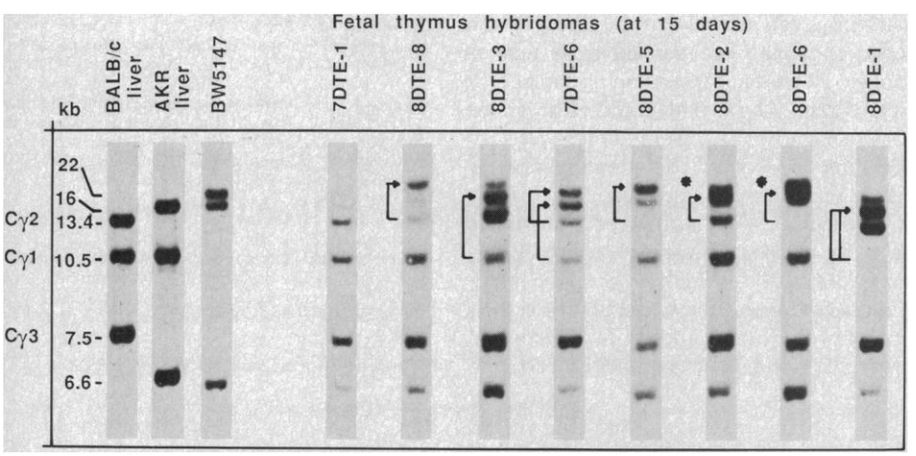

Fig. 2. Examples of γ chain gene rearrangements. Genomic DNA's of germline controls from liver of BALB/c and AKR mice, of the fusion line BW5147, and of thymus hybridomas generated with BALB/c fetal thymocytes at day 15 of gestation were digested with the restriction enzyme Eco RI and analyzed by electrophoresis in 0.7% agarose gels, Southern blotting, and densitometry. Sizes of rearranged restriction fragments in kilobases were estimated by comparison with a Hind III digest of λ phage DNA and known γ chain germline bands (*12*). As a probe, the entire insert of pγ 7.1 was used. All examples are derived from the same gel. To identify overlapping bands, we scanned the autoradiograms with an ELISA reader (Biotek Instruments, models EL307) at 550 nm, after partly covering the lens (slit approximately 500 μm wide). The linear range of absorbance was determined by exposing the film to serial dilutions of radioactive label, and hybridization bands were recorded as peaks of absorbance. Densitometry was used to identify rearrangements in one allele in the presence of another unrearranged allele. Since rearrangements involving Cγ3 cannot be seen in Eco RI digests, the 7.5-kb Cγ3 germline band could be used in most cases as a reference: the relative hybridization in this band compared to the three BW-derived bands was used to estimate the number of normal cell-derived γ alleles (2, 1, or 0), and comparison of the 7.5-kb germline band with the other two germline bands (10.5 and 13.4 kb) was used to detect rearrangements involving Cγ1 or Cγ2. In most cases, diminished or absent hybridization in either germline band was associated with a stronger hybridization pattern in the predicted size range (22 or 16 kb). These rearrangements overlap with the larger two of the BW-derived bands, but comparison with the third BW-derived band (6.6 kb) allows their identification. In some cases, however, diminished or absent hybridization in the 13.4-kb germline bands was associated with a new band at approximately 19 kb, between the 22- and 16-kb bands. Lack of resolution in this part of the gels did not allow a precise size determination.

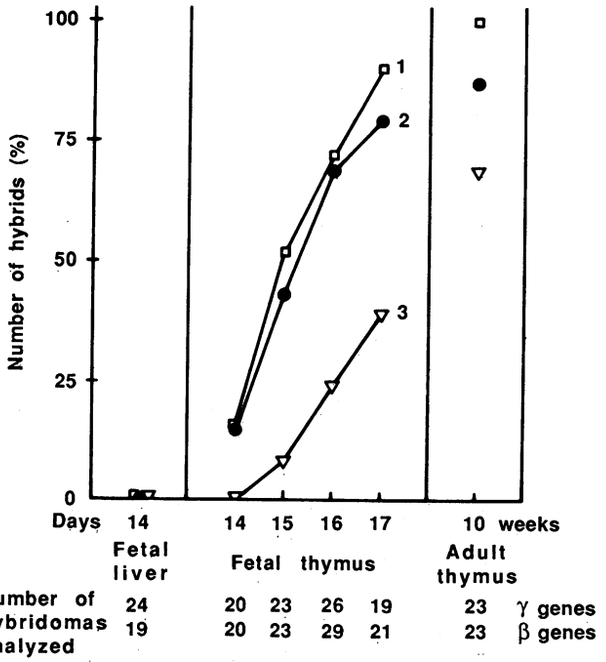

Fig. 3. Kinetics of γ and β complex rearrangements in mouse ontogeny. Rearrangements of β complex in fetal thymus and other hybridomas were analyzed as described (*10*). Briefly, DNA from individual hybridomas was digested with Hind III, Hpa I, or Pvu II and analyzed by Southern blotting. Filters were hybridized with a cDNA probe complementary to Jβ26 and Cβ2. The rearrangements that had occurred in each hybridoma were deduced from the band patterns so obtained, and grouped into partial D-to-J joinings and other rearrangements involving Cβ1 and Cβ2. The fractions of hybridomas (%) with γ and β gene rearrangements are plotted against ontogenetic time. The curves represent total β gene rearrangements (curve 1), total γ gene rearrangements (curve 2), and potentially functional β gene rearrangements only (all rearrangements except D-to-J joinings) (curve 3). Also indicated are the numbers of hybridomas that were analyzed for β and γ gene rearrangements at each time point.

ways of differentiation of thymocytes or that β gene rearrangement can precede γ gene rearrangement.

This leaves us with a discrepancy between the previously reported, high transcriptional activity of γ genes early in development (days 14 and 15) and the comparatively slow kinetics of gene rearrangements. One is tempted to speculate that other, by our probe undetected, genes are involved, a possibility that would explain the observation by Haars *et al.* (*9*) of an early rearrangement in fetal liver and thymus. Alternatively, early transcripts may reflect rearrangements at the nonfunctional Cγ locus that would not be detected in our analyses, or they may indicate that a few early cells make large quantities of γ message or that many of the early transcripts are derived from unrearranged genes that make them physiologically irrelevant. Our own data and observations of other groups (*22, 24*) indicate that such germline transcripts exist, exemplified by clone γ 7.1 in this report. Also, other sterile transcripts have been observed that initiate 5′ to Jγ and contain correctly spliced Jγ Cγ junctions (*24*). Whether germline transcripts comprise a major portion of early γ mRNA, or whether a few loci are activated early to give high transcriptional activity, the phenomenon of an approximately 50-fold decrease in γ transcript levels (*4*) in the presence of increasing numbers of rearranged genes has no precedent in immunoglobulin or T-cell receptor α and β genes and may be connected with a unique role of γ genes.

REFERENCES AND NOTES

1. H. Saito *et al.*, *Nature* (*London*) **309**, 757 (1984).
2. H. Saito *et al.*, *ibid.* **312**, 36 (1984).
3. D. M. Kranz *et al.*, *ibid.* **313**, 752 (1985).
4. D. H. Raulet, R. D. Gasman, H. Saito, S. Tonegawa, *ibid.* **314**, 103 (1985).
5. H. R. Snodgrass, Z. Dembić, M. Steinmetz, H. von Boehmer, *ibid.* **315**, 232 (1985).
6. M. B. Brenner *et al.*, *ibid.* **322**, 145 (1986).
7. I. Bank *et al.*, *ibid.*, p. 179.
8. H. R. Snodgrass, P. Kisielow, M. Kiefer, M. Steinmetz, H. von Boehmer, *ibid.* **313**, 592 (1985).
9. R. Haars *et al.*, *J. Exp. Med.* **164**, 1 (1986).
10. W. Born, J. Yagüe, E. Palmer, J. Kappler, P. Marrack, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2925 (1985).
11. N. Roehm *et al.*, *Cell* **38**, 577 (1984).
12. A. C. Hayday *et al.*, *ibid.* **40**, 259 (1985).
13. D. Kranz *et al.*, *Science* **227**, 941 (1985).
14. A. Iwamoto *et al.*, *J. Exp. Med.* **163**, 1203 (1986).
15. J. Messing, *Methods Enzymol.* **101**, 20 (1983).
16. G. Rathbun *et al.*, in preparation.
17. J. S. Heilig *et al.*, *Nature* (*London*) **317**, 68 (1985).
18. R. D. Garman, P. J. Doherty, D. H. Raulet, *Cell* **45**, 733 (1986).
19. M. P. Lefranc, A. Forster, T. H. Rabbitts, *ibid.* **319**, 420 (1986).
20. T. Quertermous *et al.*, *Science* **231**, 252 (1986).
21. F. W. Alt *et al.*, *EMBO J.* **3**, 1209 (1984).
22. T. Mak, personal communciation.
23. G. D. Yancopoulos, T. K. Blackwell, H. Suh, L. Hood, F. W. Alt, *Cell* **44**, 251 (1986).
24. G. K. Sim, personal communication.
25. T. Maniatis, E. F. Fritsch, J. Sambrook, Eds., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982). The C57BL/ka adult thymus cDNA library was constructed by H. Gershenfeld. It has been plated, grown, and analyzed as described in: R. Davis *et al.*, *Cold Spring Harbor Advanced Bacterial Genetics: A Manual for Genetic Engineering* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1980).

26. We thank H. Gershenfeld and I. Weissman for the thymus cDNA library, E. Palmer for advice and support, J. White for technical assistance, and D. Thompson for secretarial help. Supported by USPHS grants AI 18785, AI17134, and AI 18016 and by American Cancer Society research grant IM-49.

5 May 1986; accepted 18 September 1986