6. This approach was undertaken with some trepidation because numerous reports [N. A. de Vries, *Am. J. Phys. Med.* **47**, 10 (1968); S. Herring, *Am. Zool.* **17**, 954 (1977); J. C. Hickey, J. B. Woelfel, L. Rinear, *J. Prosthet. Dent.* **7**, 273 (1957); V. T. Inman, N. J. Ralston, J. B. De, C. M. Saunders, B. Feinstein, E. W. Wright, Jr., *Electroencephalogr. Clin. Neurophysiol.* **4**, 187 (1952); B. Jonsson and S. Reichman, *Acta Morphol. Neerl.-Scand.* **7**, 73 (1968); P. V. Komi and E. R. Buskirk, *Electromyography* **10**, 357 (1970); R. S. Person and U. M. Kushnarev, *Biophysics* **8**, 302 (1963); H. J. Ralston, *Am. J. Orthodont.* **47**, 521 (1961); E. N. Zuniga, X. T. Truong, D. G. Simons, *Arch. Phys. Med. Rehabil.* **51**, 264 (1970)] suggest that EMG activity compared among different electrodes (of very similar configuration), from different portions of a single muscle, and from placements in the same muscle of multiple individuals show sufficient differences to raise questions about the validity of the results. The present system appears to overcome some of these difficulties.
7. Autografts are referred to as stabilized when the fiber areas in the core of the muscle are similar to those of peripheral fibers. In cats, this stage is attained after approximately 280 days after transplantation [see J. A. Faulkner *et al.* (*2*)].
8. Electromyograms were recorded from the EDL autografts and adjacent ATB for periods ranging from 350 to 377 days after transplantation. The EMG activities were recorded 1 to 2 days before the animals were killed to permit analysis of the autografts for morphological and physiological characteristics. For transplant techniques, histological and histochemical profiles, and contractile properties of transplanted EDL muscles in cats, see J. A. Faulkner *et al.* (*3*); L. C. Maxwell *et al.* (*4*); S. A. Mufti *et al.* (*5*).
9. All electrodes were formed of 0.076-mm Teflon-insulated stainless steel wires (Medwire) spirally twisted about one another for approximately 2 to 3 cm. The free ends were bent backward into hooks after the insulation had been removed from the distal 2 mm of each wire [see G. C. Gorniak, *J. Morphol.* **157**, 427 (1977)]. The length of the bare ends was 2.16 ± 0.166 mm and the resistance, 42.6 ± 1.01 kohm (mean ± standard deviation, $N = 40$). Electrodes were inserted surgically into the muscles under Vetalar and local anesthesia with leads running subdermally to the hip and from there to a four-channel telemetry transmitter (Bio-sentry 7140). Outputs from the receivers were passed through Tektronix FM 122 preamplifers and Honeywell 117 DC Accudata amplifiers and stored on a Honeywell 5600 medium bandpass 1-inch tape recorder. Signals from four channels at a time were later analyzed on a modified Hewlett-Packard 2100A minicomputer, the number of spikes per unit time being counted as well as the mean amplitude (40 mv/unit) of spikes during each interval. A separate control permitted subtraction of noise from the zone scanned. The tape was marked electronically, permitting parallel analysis of additional records, and the digitized EMG data were stored on tape cassettes for further statistical analyses. The EMG activities were scanned in serial time sections, normally set at 200-msec intervals.
10. Control EDL muscles show a mean number of spikes of 15.92 ± 0.758 ($N = 82$) and a mean amplitude of 24.15 ± 1.67 ($N = 82$). For control ATB, the mean number of spikes is 15.37 ± 0.826 ($N = 82$) and the mean amplitude is 23.54 ± 1.85 ($N = 82$); both mean number of spikes and mean amplitude are not significantly different from those of control EDL muscle at a significance level of $P = .10$. However, three of the adjacent ATB muscles show significantly different ($P < .05$) mean numbers of spikes from those of control ATB muscles, ranging from 19.23 ± 1.36 ($N = 82$) to 20.16 ± 2.27 ($N = 38$). The ATB muscles were studied to determine whether transplantation procedures affect the EMG activity pattern of nontransplanted muscles adjacent to autografts, perhaps reflecting compensatory action of the synergistic ATB muscle for the loss of EDL function.
11. In those autografts with a significantly greater number of spikes and lower amplitude ($P < .01$), the mean number of spikes ranged from 19.37 ± 1.25 ($N = 53$) to 26.77 ± 0.73 ($N = 115$) and the mean amplitude ranged from 11.84 ± 0.73 ($N = 115$) to 14.75 ± 0.99 ($N = 53$). In autografts showing a significantly lower number of spikes and greater amplitude ($P < .01$), the mean number of spikes ranged from 11.72 ± 1.58 ($N = 37$) to 12.20 ± 0.98 ($N = 91$) and the mean amplitude ranged from 31.87 ± 1.16 ($N = 81$) to 38.97 ± 2.48 ($N = 37$).
12. Correlation coefficients (r) for two variables were calculated for all the EDL autografts studied. Correlation coefficients below 0.63 are not considered significant at a level of .01 [K. Diem and C. Lentner, *Scientific Tables* (Geigy, Basel, 1970)]. Fibers classified as oxidative showed distinct succinate dehydrogenase activity as well as subsarcolemmal aggregates of diformozon, especially near capillaries [L. C. Maxwell *et al.* (*4*)]. Time denotes the number of days after transplantation.
13. F. Buchthal and D. Rosenfalck, in *Muscular Dystrophy in Man and Animals*, G. H. Bourne and M. N. Golarz, Eds. (Hafner, New York, 1963), p. 194; J. E. Desmedt and S. Borenstein, in *Explanatory Concepts in Muscular Dystrophy*, A. T. Milhorat, Ed. (Excerpta Medica, Amsterdam, 1974), vol. 2, p. 555; J. Kopec, I. Nausmanova-Petrusewicz, M. Rawski, M. Wolynski, in *New Developments in Electromyography and Clinical Neurophysiology*, J. E. Desmedt, Ed. (Karger, Basel, 1973), vol. 1, p. 477; A. J. McComas and S. J. Mossewy, in *Research in Muscular Dystrophy*, Muscular Dystrophy Group, Eds. (Pitman Medical, New York, 1965), p. 317; A. J. McComas, R. E. P. Sica, M. J. Cambell, in *New Developments in Electromyography and Clinical Neurophysiology*, J. E. Desmedt, Ed. (Karger, Basel, 1973), vol. 1, p. 55; E. Ståberg and J. Ekstedt, in *ibid.*, p. 113.
14. H. P. Ludin, in *New Developments in Electromyography and Clinical Neurophysiology*, J. E. Desmedt, Ed. (Karger, Basel, 1973), vol. 1, p. 400.
15. C. H. Håkansson, *Acta Physiol. Scand.* **37**, 13 (1956); Ludin (*14*); J. R. Warmolts and W. K. Engel, in *New Developments in Electromyography and Clinical Neurophysiology*, J. E. Desmedt, Ed. (Karger, Basel, 1973), vol. 1, p. 35.
16. We thank J. Beach and T. Harkaway for aid in developing the computer hardware and programs, L. C. Maxwell and T. P. White for their assistance with the histochemical and biochemical assays, and B. D. Clark, L. C. Maxwell, and T. C. Scanlon for their comments on the manuscript. This study was supported by NIH 5 F32 Am05368, NSF DEB 77-02605, and NIH 05R1 Am18727.

3 October 1978; revised 26 December 1978

Nuclear Transcripts of Mouse Heavy Chain Immunoglobulin Genes Contain Only the Expressed Class of C-Region Sequences

Abstract. *In plasmacytoma cells producing IgG, IgA, or IgM immunoglobulin heavy chains, the large precursors of the heavy chain messenger RNA's contain nucleotide sequences that specify only the expressed class of constant region. This indicates that the switch from one class of heavy chain to another during B cell ontogeny does not occur by altered processing of a complex gene transcript.*

During the course of their ontogeny B lymphocytes produce heavy (H) chain immunoglobulins in which variable region (V_H) sequences of a characteristic idiotype can be associated with a variety of constant region (C_H) sequences (*1*). Thus, the commitment to produce the M, G, or A class of immunoglobulins is presumed to reflect a switch between V_HC_μ and V_HC_γ or V_HC_α (*2*) expression. Two types of molecular models have been proposed to account for this switch: one involving structural rearrangements of the V_H and C_H genes (*3*) and the other, variable processing of a complex messenger RNA (mRNA) precursor containing information for all of the C_H sequences (*4*). Some doubt has been cast on the RNA processing model by the observation that a hybridoma line formed by fusing $V_{H_1}C_\mu$- and $V_{H_2}C_\gamma$-producing cells does not produce a $V_{H_2}C_\mu$ hybrid immunoglobulin (*5*). However, this negative evidence is only inferential because it presumes capabilities of processing enzymes about which essentially nothing is known.

We have made a more direct test of the processing model by examining the nuclear transcripts in cells producing immunoglobulins of one particular class for the presence of sequences specifying other classes. This test is based on our ability to detect the nuclear precursors of H-chain mRNA's by their specific hybridization to cloned complementary DNA (cDNA) sequences (*6*). We have observed that the precursors of γ-, α-, and μ-chain mRNA's contain only the sequences of that particular class, a result that is inconsistent with the RNA processing model.

The myeloma cell line MPC-11, which produces γ_{2b} chains, and plasmacytoma tumors J558 and PC3741, which produce α and μ heavy chains, were used as the source of nuclear RNA and cytoplasmic mRNA (*7, 8*). Nuclear RNA's were extracted by the hot phenol method of Scherrer (*9*) from MPC-11 nuclei purified with detergent (*6*) and from tumor nuclei purified with citric acid (*10*). Cytoplasmic mRNA's were extracted from membrane-bound polyribosomes and further purified as indicated (*8*). Polyadenylate [poly(A)]-containing nuclear RNA was isolated by chromatography on oligodeoxythymidylate cellulose and size-fractionated on methylmercury hydroxide–agarose gels (*6*). The RNA molecules containing sequences coding for this H-chain constant region were identified by the RNA transfer technique of Alwine *et al.* (*11*) with the use of nick-translated cloned cDNA probes of ≥ 25 count/min per picogram (*8*).

The construction and characterization of the chimeric plasmid $p\gamma_{2b}(11)^7$ containing a 1-kilobase-pair (kbp) segment of γ_{2b} C-region nucleotide sequences derived from the H-mRNA of MPC-11 cells has been described (*6*). The plasmids

0036-8075/79/0608-1087$00.50/0

containing μ and α nucleotide sequences, pμ(3741)[9] and pα(558)[13], were made and characterized by similar procedures, starting with H-mRNA's from PC3741 and J558 cells. Their authenticity was verified by the hybrid-arrest translation assay (*12*), and the size of the immunoglobulin nucleotide sequence inserts was estimated by restriction analysis (*6*) to be about 0.9 kbp. Since these inserts are approximately half the length of the H-mRNA's, they presumably contain a substantial proportion of the C-region sequences.

In MPC-11 cells the nuclear precursors of a γ_{2b} class H-mRNA have been shown to consist of an 11-kilobase (kb) component, which appears to be the initial transcript of the H-chain gene, a 3.7-kb intermediate component, and a 1.8-kb component the size of mature H-mRNA (*6*). These components are detected as discrete bands on a diffuse background of partially degraded RNA when the nuclear RNA is fractionated on denaturing gels and hybridized with a labeled γ_{2b} probe (Fig. 1A). When the same nuclear RNA was incubated with the α or μ probes, no hybridization was detected (Fig. 1, B and C). That the probes could have detected α- and μ-mRNA sequences if they were present in the γ_{2b} precursors is demonstrated by their ability to hybridize with their corresponding cytoplasmic mRNA's (Fig. 1, D to F). These data indicate that the nuclear precursors of the γ_{2b}-mRNA, which contain sequences coding for the γ_{2b} C-region, do not contain sequences coding for the α or μ C-region.

The same type of analysis was carried out with nuclear RNA's from plasmacytoma cells expressing C_α and C_μ genes (Fig. 2). For these experiments we used poly(A)-containing nuclear RNA, which is significantly enriched in H-mRNA sequences over the total nuclear RNA (*6*), and hence affords a higher level of detectability. The poly(A)-containing nuclear RNA from the α-chain-producing tumor, J558, contains components of 8.2, 2.1, and 1.65 kb that hybridize with the α-sequence-containing probe (Fig. 2A). In addition, there is a background of polydisperse components < 8.2 kb, which probably represents breakdown products of the 8.2-kb and 1.65-kb components. The 1.65-kb component corresponds in size to the cytoplasmic H-mRNA from these cells (Fig. 1E). This same nuclear RNA preparation did not exhibit any detectable hybridization with the μ and γ_{2b} probes (Fig. 2, B and C), indicating that the nuclear transcripts of α-mRNA contain only C_α coding sequences. The poly(A)-containing nuclear RNA from the μ-chain-producing tumor, PC3741, contains two discrete components that hybridize to the μ probe (Fig. 2D): a large component of approximately 10.5 kb, and a 2.1-kb component corresponding in size to the mature μ-mRNA (Fig. 1F). A diffuse background of putative degradation products < 10.5 kb is also observed. There is no detectable hybridization of this nuclear RNA with either the γ_{2b} or the α probe (Fig. 2, E and F).

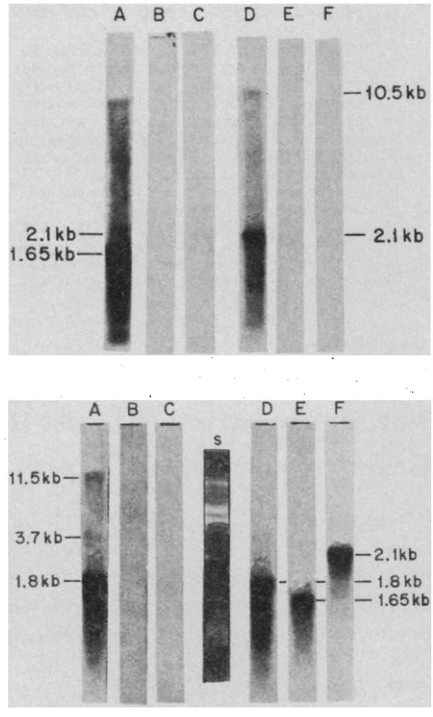

Fig. 1 (top). Analysis of nuclear RNA sequences coding for the H-chain constant region in MPC-11 cells. Nuclear RNA (30 μg per track) was subjected to electrophoresis on methylmercury hydroxide-agarose gels, transferred to paper, and hybridized with ^{32}P-labeled plasmids (~ 50 count/min per picogram) containing γ_{2b} sequences (A), α sequences (B), and μ sequences (C). The ^{32}P-labeled plasmids were also hybridized, respectively, with γ_{2b}-mRNA from MPC-11 cells (D), α-mRNA from J558 cells (E), and μ-mRNA from PC3741 cells (F). Track D contained 5 μg of total cytoplasmic RNA; tracks E and F contained 20 ng of H-mRNA purified by oligodeoxythymidylate-cellulose chromatography and sedimentation through one sucrose gradient. The autoradiographs were intentionally overexposed (6 days with an intensifier screen) in order to maximize detection of the less abundant, large nuclear transcripts. The center track shows a pattern (ethidium bromide-stained) of MPC-11 nuclear RNA. Prominent bands of precursor ribosomal RNA and ribosomal RNA (45*S*, 32*S*, 28*S*, 18*S*) are visible over a diffuse background of heterogeneous nuclear RNA. The magnification and electrophoresis conditions for this track were different from those used in tracks A to C. Fig. 2 (bottom). Analysis of nuclear RNA sequences coding for H-chain constant regions produced by plasmacytoma cells J558 (A to C) and PC3741 (D to F). Poly(A)-containing nuclear RNA (9 μg per track for J558 and 5 μg per track for PC3741) was analyzed as described (Fig. 1) by hybridization with ^{32}P-labeled (~ 30 count/min per picogram) α sequences (A and E), γ_{2b} sequences (B and F), and μ sequences (C and D). The autoradiographic exposure (with intensifier screen) was 16 hours (A and D) and 4 days (B, C, E, and F).

These results argue against the existence of multiple C-region sequences in the nuclear precursors of either γ_{2b}-, α-, or μ-mRNA's, and thus provide direct evidence against the RNA processing model as an explanation for the ontogenetic switch in expression of C_H genes. A particular mRNA precursor appears to contain only that C-region sequence characteristic of its mature mRNA product. Thus, the more plausible models would seem to be those that assume some type of DNA rearrangement during the course of B lymphocyte differentiation. Such gene rearrangements are already known to occur for V and C light chain genes (*13*).

K. B. MARCU,* U. SCHIBLER†
R. P. PERRY
Institute for Cancer Research,
Fox Chase Center,
Philadelphia, Pennsylvania 19111

References and Notes

1. A. R. Lawton and M. D. Cooper, *Contemporary Topics in Immunobiology* (Plenum, New York, 1974), vol. 3, p. 193.
2. The genetic elements coding for the C_H sequences of the M, G, and A classes are designated C_μ, C_γ, and C_α, respectively; those coding for different V_H sequences are designated V_{H_1}, V_{H_2}, V_{H_3}, and so forth.
3. W. J. Dreyer, W. R. Gray, L. Hood, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 353 (1967); J. A. Gally and G. M. Edelman, *Nature (London)* **227**, 341 (1970); T. Honjo and T. Kataoka, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2140 (1978).
4. S. Tonegawa, A. M. Maxam, R. Tizard, O. Bernard, W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1485 (1978); T. H. Rabbitts, *Nature (London)* **275**, 291 (1978).
5. M. J. Shulman and G. Kohler, *Nature (London)* **274**, 917 (1978).
6. U. Schibler, K. B. Marcu, R. P. Perry, *Cell* **15**, 1495 (1978).
7. K. B. Marcu, U. Schibler, R. P. Perry, *J. Mol. Biol.* **120**, 381 (1978).
8. K. B. Marcu, O. Valbuena, R. Perry, *Biochemistry* **17**, 1723 (1978).
9. K. Scherrer, *Fundamental Techniques in Virology* (Academic Press, New York, 1969), pp. 413–432.
10. U. Schibler, T. Wyler, O. Hagenbuchle, *J. Mol. Biol.* **94**, 503 (1976).
11. J. C. Alwine, D. J. Kemp, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5350 (1977).
12. B. M. Paterson, B. E. Roberts, E. L. Kuff, *ibid.*, p. 4370.
13. S. Tonegawa, C. Brack, N. Hozumi, G. Matthyssens, R. Schuller, *Immumol. Rev.* **36**, 73 (1977); T. H. Rabbitts and H. Forster, *Cell* **13**, 319 (1978).
14. We thank Dr. Martin Weigert for providing the plasmacytoma tumors and Drs. Oliver Smithies and Fred Blattner at the University of Wisconsin-Madison for the use of their P3 facility. Supported by a grant from the National Science Foundation, grants from the National Institutes of Health, and an appropriation from the Commonwealth of Pennsylvania.
* Present address: Department of Biology, State University of New York at Stony Brook.
† Present address: Institut Suisse de Recherches Experimentales, Lausanne, Switzerland.

7 December 1978; revised 12 February 1979