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Mutual Homology of Mouse Immunoglobulin γ-Chain Gene Sequences†

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ABSTRACT: We have assessed the relative homology of mouse immunoglobulin heavy-chain gene sequences using complementary DNAs (cDNAs) synthesized against γ-chain mRNAs (γ1, γ2a, γ2b, and γ3) purified from mouse myelomas. cDNAs complementary to the γ-chain mRNAs did not cross-hybridize with the μ- and α-chain mRNAs, whereas they cross-hybridized to significant extents (22–66%) with the γ-chain mRNAs of other subclasses. The heterologous hybrids formed, however, melt at 5–13 °C lower temperatures as compared to the homologous hybrids, indicating that significant portions of the heterologous hybrids are mismatched. The rates of the cross-hybridization reactions are 2- to 17-fold slower than those of the homologous hybridization reactions. Therefore, the γ-chain gene sequences of four subclasses share a part of homology with each other, but they are different enough to be measured separately. Cross-hybridization analyses indicate that the γ2a and γ2b genes are the most closely related, while the γ1 and γ3 genes are the least related among the γ subclass genes.

Immunoglobulin heavy chains are encoded by a family of V[1] region genes and a set of C region genes (reviewed by Eichmann, 1975). In order to study the organization of immunoglobulin heavy-chain genes, it is essential to isolate the specific probe for each heavy chain gene. Recently we have succeeded in purifying mRNAs encoding mouse immunoglobulin heavy chains (Ono et al., 1977). Using purified mRNAs we have synthesized and purified complementary DNAs (cDNAs) corresponding to the heavy chains of all four γ subclasses, namely, γ1, γ2a, γ2b, and γ3 chains.

In this paper we will report the mutual homology among four γ-chain gene sequences. The extent and rate of hybridization and the thermal stability of hybrids formed showed that although these gene sequences share partial homology with each other, each cDNA is specific to its own subclass.

Materials and Methods

Materials. Mouse myeloma tumors were kindly supplied by Dr. M. Potter of National Institutes of Health, except that MC 101 was provided from Dr. Migita of Kanazawa University. Tumors were maintained as described (Swan et al., 1972). ^{3}H-Labeled dCTP (24 Ci/mmol) was obtained from the Radiochemical Center, Amersham, England, and ^{3}H-labeled dATP and dGTP (12 Ci/mmol) were purchased from New England Nuclear. Hydroxylapatite of DNA grade was obtained from Bio-Rad Lab.

Preparation and Purification of mRNAs and cDNAs. Preparation and purification of heavy-chain mRNAs derived from mouse myelomas MOPC 31C (γ1), HOPC 1 (γ2a), MPC 11 (γ2b), and J 606 (γ3) were done as described in the previous report (Ono et al., 1977). Purities of γ1, γ2a, γ2b, and γ3 mRNAs were 97, 63, 80, and 100%, respectively, as assayed by hybridization kinetic analysis. Detailed characterization of mRNAs will be described elsewhere. [^{3}H]cDNAs complementary to the mRNAs were synthesized using [^{3}H]dCTP, [^{3}H]dATP, and [^{3}H]dGTP by avian myeloblastosis virus reverse transcriptase and purified as described (Honjo et al., 1974). The specific radioactivity of [^{3}H]cDNA was 1.6 × 10^7 cpm/μg. Although each cDNA showed a single transition in hybridization kinetic analysis to corresponding mRNAs (Ono et al., 1977), γ2a and γ2b cDNAs showed, in our preliminary experiments, the presence of some quantity of contaminants in their preparations. As the contaminants seem to come from minor mRNA species present in common in tumor cells, they were removed by hybridizing γ2a and γ2b

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[1] Abbreviations used: V and C regions, variable and constant regions; cDNA, synthetic DNA complementary to mRNA; Cot (Crt) values, product of concentration of nucleotide sequences of DNA (RNA) and time of incubation (mol of nucleotides × s/L).

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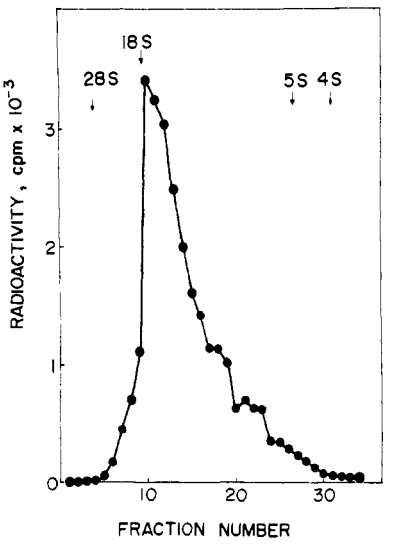

FIGURE 1: Electrophoretic pattern of MPC 11 cDNA. Purified MPC 11 [^{3}H]cDNA was run on polyacrylamide gel electrophoresis in formamide. The reference position of 28S, 18S, 5S, and 4S rRNA from mouse myeloma is also indicated.

cDNA with a crude mRNA preparation derived from a tumor (MOPC 511) producing α chain. After hybridization to a Crt value of 40, the reaction mixture was loaded on a hydroxylapatite column and cDNA remaining as single stranded was eluted with 0.14 M phosphate buffer. By this purification, about 65% of the original cDNA was recovered.

Determination of cDNA Length. [^{3}H]cDNAs were electrophoresed at 130 V for 3 h at room temperature in 4% polyacrylamide gels made up in 99% formamide (Staynov et al., 1972). After electrophoresis the gel was cut into 2-mm thick slices. Each slice was solubilized in 20% H_2O_2 and 20% perchloric acid at 70 °C for 2 h (Mahin & Lofberg, 1966), and then radioactivity was measured by adding 10 mL of Aquasol-2 (New England Nuclear).

cDNA–RNA Hybridization. Hybridization reactions were carried out as described (Honjo et al., 1974). Hybridization was assayed with S1 nuclease digestion as described (Honjo et al., 1976). RNAs were partially purified from various myeloma tumors up to the dT1 stage (Honjo et al., 1974), which comprise about 4% pure mRNA. Briefly, the homogenate of myeloma tumors was centrifuged to remove nuclei and the total cytoplasmic RNA was isolated by phenol—methacresol extraction (Kirby, 1968). mRNA was purified by oligo(dT)-cellulose column chromatography.

Thermal Denaturation of Hybrids. Hybridization was carried out in 0.6 M NaCl–0.2 mM EDTA–20 mM Tris-HCl, pH 7.2, at 75 °C. When a Crt value of 0.5 was attained, the hybridization mixture was immediately chilled and diluted to 0.25 M NaCl. The thermal stability of cDNA–RNA hybrids was determined by measurement of the S1 nuclease resistance upon heating (Honjo et al., 1976).

Results

Characterization of cDNAs. Complementary DNAs were synthesized against γ-chain mRNAs of four subclasses which were highly purified from MOPC 31C (IgG 1), HOPC 1 (IgG 2a), MPC 11 (IgG 2b), and J 606 (IgG 3) myelomas (Ono et al., 1977). The sizes of the [^{3}H]cDNAs were determined by polyacrylamide gel electrophoresis in 99% formamide. A typical electrophoresis pattern is shown in Figure 1. The size distribution patterns were rather heterogeneous with an average chain length of about 1500 nucleotides, having a peak length of 1500, 1900, 1900, and 1600 nucleotides for MOPC

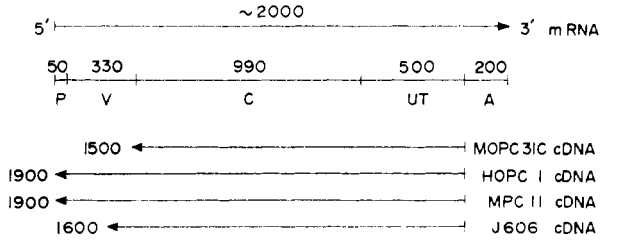

FIGURE 2: Diagramatic representation of γ-class mRNA and cDNAs. P, precursor region sequence determined from the amino acid sequence of the MOPC 315 precursor (Jilka & Pestka, 1977); V, variable region sequence; C, constant region sequence; UT, untranslated sequence; A, poly(A) sequence. Numbers in bases.

31C (γ1), HOPC 1 (γ2a), MPC 11 (γ2b), and J 606 (γ3) cDNA, respectively. γ-Chain mRNAs are 2000 nucleotides long (Ono et al., 1977), of which the V region and C region sequences occupy 330 and 990 nucleotides, respectively. Nucleotide sequence analysis of MOPC 31C cDNA inserted in a plasmid indicates that the untranslated sequence of about 500 nucleotides is present at the 3′ end of mRNA (Honjo and Leder, unpublished observation). Assuming that the mRNAs contain poly(adenylic acid) of 200 nucleotides, these cDNAs include almost the entire C region sequence but do not necessarily include the V region sequence (Figure 2).

Cross-Hybridization within a Subclass. To determine whether these cDNAs extend into the V region sequence or not, the cDNAs were hybridized with partially purified (dT1 stage) mRNAs derived from myelomas producing other γ chains of the same subclass, namely, heavy-chain proteins with the same C region sequence and a different V region sequence.

As shown in Figure 3, MOPC 31C (γ1) cDNA hybridized with MC 101 (γ1) mRNA to the extent similar to that with MOPC 31C mRNA. However, HOPC 1 (γ2a), MPC 11 (γ2b), and J 606 (γ3) cDNAs hybridized to UPC 10 (γ2a), MOPC 141 (γ2b), and FLOPC 21 (γ3) mRNAs, respectively, to less extents than those to the respective homologous mRNAs; the final extents of hybridization obtained for the heterologous mRNAs were about 85 (γ2a) and 90% (γ2b and γ3) of those obtained for the homologous mRNAs. HOPC 1, MPC 11, and J 606 cDNAs seem to be long enough to extend into the V region sequence, while MOPC 31C cDNA covers only the constant region sequence.

The $Crt_{1/2}$ values of these cross-hybridizations are comparable to those of the homologous hybridizations, which indicates the presence of different but roughly similar quantities of mRNA among different tumors. The thermal denaturation profiles of hybrids formed between cDNAs and corresponding partially purified mRNAs were sharp and undistinguishable from those obtained for hybrids with highly purified mRNAs (data not shown).

Cross-Hybridization between γ-Chain Genes of Different Subclasses. The γ-chain cDNAs derived from MOPC 31C (γ1), HOPC 1 (γ2a), MPC 11 (γ2b), and J 606 (γ3) mRNAs were examined for their cross-hybridizability to other mRNA sequences. [^{3}H]cDNA probes were hybridized to large excess of mRNAs from various myelomas and assayed for their extents of cross-hybridization and thermal stability of hybrids formed. All possible sets of cross-hybridizations were examined as shown in Figures 4 and 5. It is clear that, although each cDNA probe reacted extensively (89–94%) with mRNA of the identical subclass, it hybridized poorly with mRNAs of other subclasses and classes under the conditions used. Between the γ-chain subclasses, only small extents (20–39%) of cross-hybridization were observed except in the case of the γ2a–γ2b cross-hybridization where the extent of cross-hy-

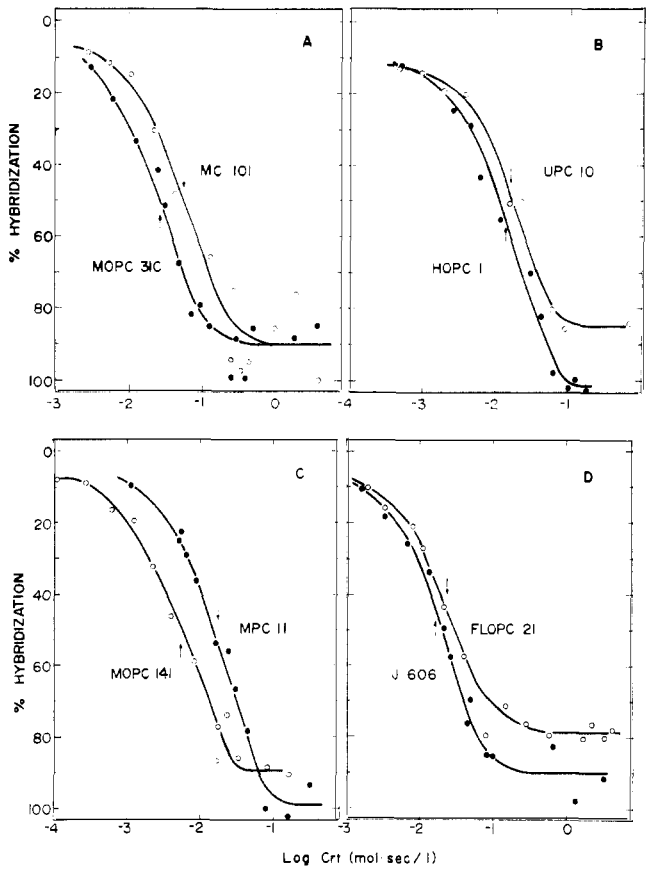

FIGURE 3: Cross-hybridization of γ1, γ2a, γ2b, and γ3 cDNAs with mRNAs of the corresponding subclasses. Hybridization reactions were carried out as described under Materials and Methods. A, MOPC 31C cDNA (γ1) with γ1 mRNAs from MOPC 31C and MC 101 myelomas. B, HOPC 1 cDNA (γ2a) with γ2a mRNAs from HOPC 1 and UPC 10 myelomas. C, MPC 11 cDNA (γ2b) with γ2b mRNAs from MPC 11 and MOPC 141 myelomas. D, J 606 cDNA (γ3) with γ3 mRNAs from J 606 and FLOPC 21 myelomas. Arrows indicate $Crt_{1/2}$ points. $Crt_{1/2}$ values for mRNAs are 2.7×10^{-2} (MOPC 31C), 5.6×10^{-2} (MC 101), 1.4×10^{-2} (HOPC 1), 1.6×10^{-2} (UPC 10), 1.8×10^{-2} (MPC 11), 5.5×10^{-3} (MOPC 141), 1.65×10^{-2} (J 606), and 2.4×10^{-2} (FLOPC 21).

bridization reached as high as 60% of the input cDNA. The possibility that the small extents of cross-hybridization are due to contaminants present in the cDNA probes was excluded by the experiments in which the γ1 gene cloned in a phage did hybridize to similar extents with all the other γ-chain mRNAs (Honjo, unpublished data). α- and μ-chain mRNAs did not hybridize to the γ-chain probes at any significant degree even at a Crt value of 10 or 100. Neither did liver mRNA hybridize with any of the γ-class probes. The results indicate that the γ-chain cDNAs used are not contaminated with other sequences present in myelomas or liver.

Since a $Crt_{1/2}$ value is inversely proportional to a rate of hybridization reaction, relative rates of cross-hybridization of a single mRNA to different cDNAs were calculated from $Crt_{1/2}$ values obtained in Figures 4 and 5. Table I shows that the heterologous hybridization reactions are 2- to 17-fold slower than the homologous reactions. It seems that the smaller the extent of cross-hybridization, the slower the reaction rate. For example, J 606 mRNA hybridized with MOPC 31C cDNA to 26% with a 17-fold slower reaction rate as compared to that with J 606 cDNA. On the other hand, HOPC 1 mRNA hybridized with MPC 11 cDNA to 60%, with a twofold slower rate than that with HOPC 1 cDNA. Retardation in a hybridization rate has been reported for imperfectly matched DNA–DNA or mRNA–cDNA hy-

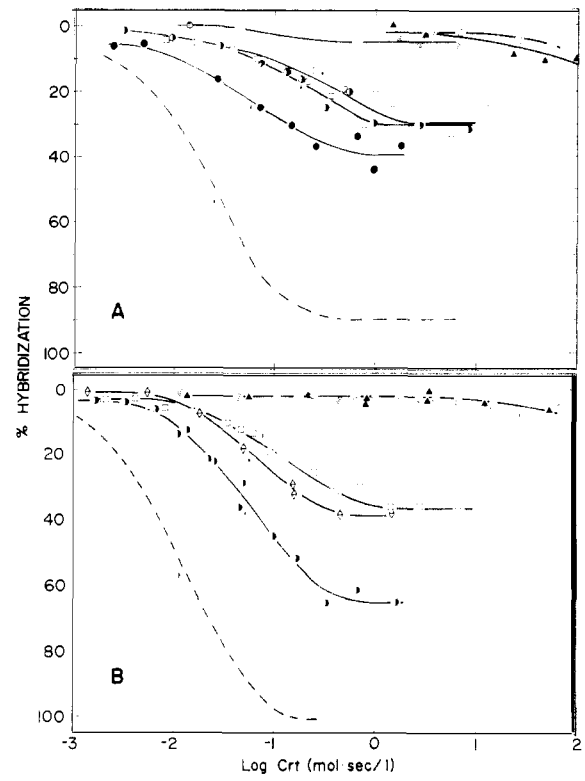

FIGURE 4: Cross-hybridization of γ1 and γ2a cDNAs with mRNAs of other subclasses. Hybridization reactions were carried out as described under Materials and Methods. Arrows indicate $Crt_{1/2}$ points. $Crt_{1/2}$ values are shown in Table I. Hybridization of each cDNA with homologous mRNA is also shown on these figures in dotted line. A, MOPC 31C cDNA (γ1). B, HOPC 1 cDNA (γ2a). ◈, MOPC 31C mRNA (γ1); ●, HOPC 1 mRNA (γ2a); ◑, MPC 11 mRNA (γ2b); □, J 606 mRNA (γ3); ▲, MOPC 104E mRNA (μ); ⊙, MOPC 511 mRNA (α); ○, liver mRNA.

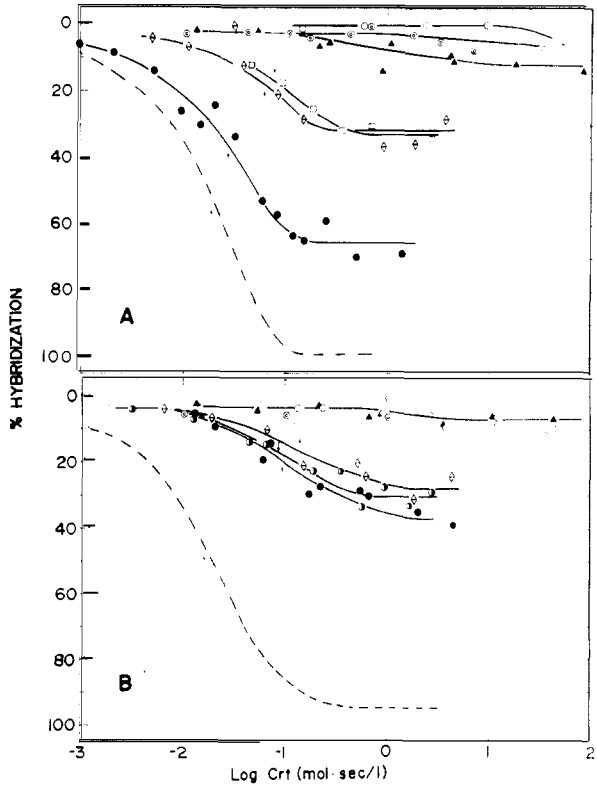

FIGURE 5: Cross-hybridization of γ2b and γ3 cDNAs with mRNAs of other subclasses. Condition of hybridization and explanations of symbols are described in the legend of Figure 4. A, MPC 11 cDNA (γ2b); B, J 606 cDNA (γ3).

Table I: Summary of Cross-Hybridization

mRNA		cDNA MOPC 31C	HOPC 1	MPC 11	J 606
MOPC 31C	$Crt_{1/2} \times 10^2$	2.7	5.9	6.1	14
	rate[d]	1	0.46	0.44	0.19
	extent[b] (%)	100	41	29	22
	ΔT_m[c]		10.4	10.1	12.9
HOPC 1	$Crt_{1/2} \times 10^2$	8.9	1.4	2.8	9.1
	rate[d]	0.16	1	0.5	0.15
	extent[b] (%)	43	100	63	37
	ΔT_m[c]	11.5		5.2	6.4
MPC 11	$Crt_{1/2} \times 10^2$	19	5.4	1.8	13
	rate[d]	0.10	0.33	1	0.14
	extent[b] (%)	32	66	100	30
	ΔT_m[c]	11.6	5.7		8.9
J 606	$Crt_{1/2} \times 10^2$	30	7.9	8.4	1.65
	rate[d]	0.06	0.21	0.20	1
	extent[b] (%)	29	37	31	100
	ΔT_m[c]	13.0	6.4	9.7	

[a] The relative rate of hybridization of a single mRNA to different cDNAs is shown by taking the rate of the homologous hybridization as 1. [b] The relative extent of hybridization of a single mRNA to different cDNAs is normalized by taking that of the homologous reaction as 100%; the actual final extents for the homologous reactions varied from 89 to 94%. [c] Difference of T_m values of the heterologous hybrids formed between a single cDNA and different mRNAs from that of the homologous hybrids. T_m values for the homologous hybrids of MOPC 31C, HOPC 1, MPC 11, and J 606 cDNAs are 93, 90, 92, and 94 °C, respectively.

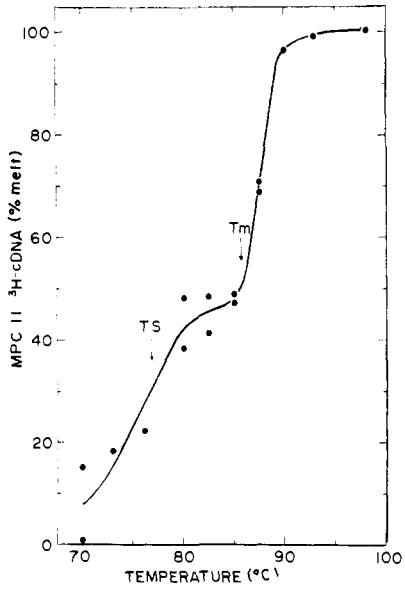

FIGURE 6: Thermal stability profile of hybrid formed between MPC 11 cDNA (γ2b) and HOPC 1 mRNA (γ2a). MPC 11 cDNA was hybridized with HOPC 1 mRNA to a Crt value of 0.5 as described under Materials and Methods. The final extent of hybridization was 65%. The temperature at which a half of the hybrids melt (T_m) was 86 °C, and the temperature at which a half of total hybridizable cDNA (see text) remained as hybrid form (TS) was 78 °C. Arrows indicate positions of T_m and TS.

bridization (Kohne et al., 1972; Leder et al., 1973).

The thermal stability of hybrids formed was measured by resistance to S1 nuclease digestion (Figure 6). Differences in T_m values between the homologous and heterologous hybrids and final extents of the cross-hybridization are also summarized in Table I. The heterologous hybrids formed melt at 5–13 °C lower temperatures as compared to the homologous hybrids, indicating that significant portions of the heterologous hybrids are not congruently base-paired. A ΔT_m value and extent of hybridization for a given combination of mRNA and cDNA were, as expected, in good agreement with those for the reciprocal combination of cDNA and mRNA.

Table II: Relative Homologies between γ-Chain Gene Sequences

gene sequences	ΔTS[a]	% divergence[b]
γ1–γ2a	25	38
γ1–γ2b	30	45
γ1–γ3	42	63
γ2a–γ2b	13	20
γ2a–γ3	25	38
γ2b–γ3	30	45

[a] Difference of thermal stability for total hybridizable cDNA used between the cross-hybrid and the homologous hybrid. [b] Calculated by ΔTS × (1.5% nucleotide pairs substituted/1 °C lowering of TS) according to Kohne et al. (1972).

From the extent of hybridization and the thermal stability of hybrids, it is possible to estimate the evolutional proximity among the γ genes. The temperature (T_m) at which a half of the hybrids melt is used often as a measure of divergence in nucleotide sequences. However, a T_m value of hybrid depends on the temperature at which hybrids are formed. Thus we used the temperature (TS) at which 50% of the total hybridizable cDNA remains in a hybrid form (Kohne et al., 1972) to estimate the divergence of nucleotide sequences among γ-chain genes. The total hybridizable cDNA represents the fraction of C region sequence which varied from 85 (γ2a) to 100% (γ1) of cDNAs (Figure 3). Table II summarizes ΔTS values of cross-hybrids and percentages of nucleotide substitution calculated. It is evident from this table that γ2a and γ2b gene sequences are most closely related, showing 80% homology in nucleotide sequences. On the other hand, 63% of nucleotide sequences seems to be substituted between γ1 and γ3 chain genes, indicating that the γ1 and γ3 genes are most distantly related among the γ gene subclasses. A close relationship between γ2a and γ2b chain is also reported based on serological studies and tryptic peptide maps of the Fc fragment of these proteins (Potter, 1972).

Discussion

We have shown that the γ-chain genes are different from each other although they share partial homology in nucleotide sequence. The divergence in nucleotide sequence is manifested as the low extent of cross-hybridization, reduced thermal stability of heterologous hybrids, and retardation in a rate of cross-hybridization (Figures 4 and 5 and Table I). These properties enable us to distinguish the γ-chain gene sequences from one another distinctively. Class specificity of the γ-chain cDNAs is certified by the fact that none of them cross-hybridized with the μ- and α-chain mRNA.

The specificity of the γ-chain cDNAs offers a great virtue to quantitate the γ gene sequences represented in mouse genome. A relatively small extent and a decreased rate of cross-hybridization indicate that, in the case of Cot analysis, the formation of homologous hybrids is predominant and completes almost before the formation of heterologous hybrids. Each γ-chain cDNA has little chance to form heterologous hybrids since reassociation of the γ-chain gene DNA proceeds faster, leaving little heterologous γ gene sequences available for hybridization with the cDNA. Taking all these into consideration, we assume that the contribution of cross-hybridization to the Cot curve would be negligible. This assumption has been verified by sharp melting profiles of cDNA–DNA hybrids and monophasic unique kinetic curves (Honjo & Kataoka, 1978). Although the γ1, γ2a, γ2b, and γ3 genes are shown to present one copy each per haploid genome in mouse normal tissues, the allelic deletion of these

genes was reported in mouse myelomas (Honjo & Kataoka, 1978).

Since the γ-chain gene sequences did not show any homology with other class (α and μ) gene sequences, early divergence of classes of immunoglobulins must have occurred. This observation agrees with phylogenetic evidence that lower vertebrates such as sharks appear to have only IgM and amphibians have IgM and IgG, although all these classes in addition to IgA commonly exist in mammalians (Fudenberg et al., 1972). The emergence of subclasses is probably a result of recent evolution because the members of the γ-chain gene share considerable homology with each other. Pink et al. (1971) have concluded that subclasses in several species have evolved after differentiation of species. The amino acid sequence studies on γ1 chain (Milstein et al., 1974) and γ2a chain (Fougereau et al., 1976), which are the only two cases that the complete amino acid sequences of mouse immunoglobulin heavy chains are determined, showed that 62% of the C region sequences of two chains is homologous. Our estimation that nucleotide sequence homology between the γ1 and γ2a genes is about 62% (Table II) is in agreement with the amino acid sequence data.

Acknowledgments

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Cell-Free Translation of Mammalian Myosin Heavy-Chain Messenger Ribonucleic Acid from Growing and Fused-L_6E_9 Myoblasts†

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ABSTRACT: An mRNA-dependent reticulocyte cell-free protein synthesizing system very efficient in the translation of myosin heavy-chain mRNA from a rat myogenic cell line is described. This system exhibits a high degree of fidelity with regard to the spectrum and relative proportion of the different proteins synthesized from a sample of cytoplasmic RNA as compared to the proteins synthesized in vivo by the cells from which the RNA is prepared. The main feature of this system is the use of a K^+ and Cl^- concentration similar to those of the reticulocyte cytoplasm. Using this system, myosin heavy chain, identified by low-salt precipitation, electrophoretic mobility, and partial peptide analysis, represents 17% of the total protein synthesis when cytoplasmic RNA from well-fused L_6E_9 cells is used. Furthermore, when RNA preparations from growing myoblasts, that when analyzed in other cell-free translational systems seem not to contain any myosin heavy-chain mRNA, are tested in the system reported here, they are proven to contain high amounts of translatable myosin heavy-chain mRNA.

One of the most efficient eukaryotic systems for studying protein synthesis in vitro is the exogeneous mRNA-dependent unfractionated reticulocyte lysate (Hunt & Jackson, 1974; Pelham & Jackson, 1976). In vitro protein synthesizing systems provide a biological assay for the presence of functional mRNAs for specific proteins. By employing identical RNA concentrations from cultures at different stages of differentiation, it is possible to obtain an estimate of the amount of translatable mRNA for a specific protein present at one stage of differentiation relative to another. Such experiments can be coupled with in vivo studies on the accumulation of specific proteins during the course of differentiation to determine whether the controls on the synthesis of these proteins occur at a translational or a pretranslational level.

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