EXISTENCE OF BOTH KAPPA AND LAMBDA LIGHT CHAIN MESSENGER RNA SEQUENCES IN MOUSE MYELOMA, MOPC-104E, KNOWN AS A LAMBDA CHAIN PRODUCER

Masao Ono and Masaya Kawakami

Molecular Biology Laboratory, School of Medicine, Kitasato University, Sagamihara, Japan

Tohru Kataoka and Tasuku Honjo

Department of Physiological Chemistry and Nutrition, Faculty of Medicine, University of Tokyo, Tokyo, Japan

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SUMMARY: A mouse myeloma, MOPC-104E, which is known to synthesize and secrete λ type light chain protein as a constituent of immunoglobulin M, was shown to contain mRNA sequences coding for κ as well as λ type light chain protein. Light chain mRNA sequences were quantitated by nucleic acid hybridization reaction using radioactive DNA complementary to light chain mRNAs which had been purified from other myelomas. The amount of κ type light chain mRNA present in MOPC-104E is almost equivalent to that of λ type light chain mRNA. κ chain mRNA was not separated from λ chain mRNA either by centrifugation in sucrose density gradient or by polyacrylamide gel electrophoresis in formamide.

INTRODUCTION: It seems to be generally taken for granted that immunoglobulin mRNA derived from a mouse myeloma encodes the immunoglobulin protein secreted by the myeloma. In fact, this had been proved in most of the myelomas (1-6), although some mRNA had not been well characterized (7,8). MOPC-104E myeloma is known to produce λ type light (L) chain and to secrete it as a constituent of IgM (9). Upon purification of L chain mRNA from MOPC-104E we have found that the myeloma contains κ type L chain mRNA as much as λ type L chain mRNA. The results give warning against using MOPC-104E mRNA or other not-well-characterized myeloma mRNA as probe for analysis of immunoglobulin genes or mRNAs.

MATERIALS AND METHODS

Mouse myeloma cell lines were kindly supplied by Dr. M. Potter, NIH. MOPC-104E (Kyoto) is a cultured cell line donated by Dr. Y. Namba of Kyoto University (10). MOPC-104E (Osaka) was provided by Dr. T. Kishimoto of Osaka University. Purification of L_κ chain mRNAs from MOPC-41 and MOPC-31C myelomas

Abbreviations: cDNA, complementary DNA; Crt value, product of concentration of nucleotide sequences of RNA and time of incubation (mole of nucleotides x sec/liter); $Crt_{1/2}$, Crt value at 50% hybridization; L_κ and L_λ chains, kappa and lambda light chains of immunoglobulin protein

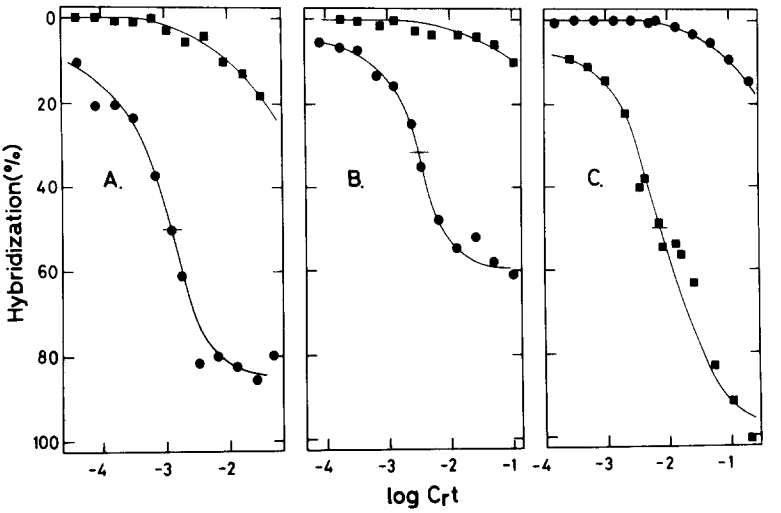

Fig. 1. Cross-hybridization of L_κ and L_λ cDNAs with myeloma RNAs. Total reaction mixture (150µl) contained 12,400cpm (4.1ng) of MOPC-31C L_κ cDNA or 9,650cpm (0.97ng) of RPC-20 L_λ cDNA. Fifteen µl aliquots were removed at appropriate intervals. The RNA was present in at least 15-fold excess over cDNA. A. MOPC-31C immunoprecipitated RNA. B. MOPC-41 RNA. C. RPC-20 RNA. Hybridization with L_κ cDNA (●); L_λ cDNA (■). Endogenous S_1 nuclease resistance (2.6% for L_κ cDNA and 2.0% for L_λ cDNA) was subtracted from all values.

and preparation of L_κ chain cDNA from respective mRNA have been described (1, 11). L_λ chain cDNA was prepared from L_λ chain mRNA purified from RPC-20 myeloma (5,6). Unless otherwise specified, mRNA preparations employed for hybridization were partially purified by two successive chromatographies on an oligo (dT)-cellulose column as described before (1). Total cytoplasmic RNA was obtained according to Kirby's method (13). Hybridization reactions were carried out in 0.6M NaCl at 75°C and assayed by S_1 nuclease digestion as described (1). Other sources of reagents have been described (1,5,6,11,12).

RESULTS

Characterization of hybridization probe

L_κ chain cDNA prepared from MOPC-41 mRNA has been shown to hybridize specifically to L_κ chain mRNA (1,6). Reciprocally, L_λ chain cDNA prepared from RPC-20 mRNA has been demonstrated to be specific for L_λ chain mRNA (1,6). In these myelomas the chain specificity of L chain mRNA present coincides with that of L chain protein secreted by the myeloma. In order to confirm these results we have examined specificity of L_κ chain cDNA prepared from MOPC-31C mRNA (11). As shown in Fig. 1, MOPC-31C cDNA hybridized with L_κ chain mRNAs derived from

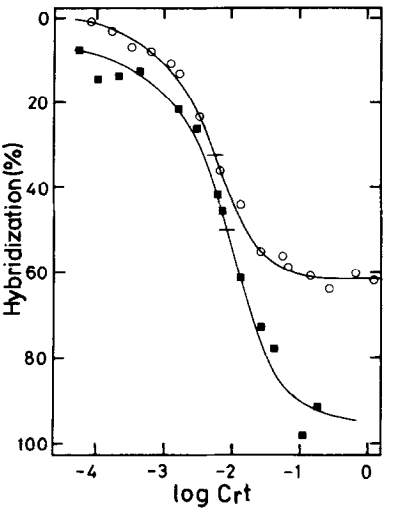

Fig. 2. Hybridization of MOPC-104E (Kyoto) RNA with L_κ and L_λ cDNA. Hybridization was carried out as described in Fig. 1. Hybridization with L_κ cDNA (O); L_λ cDNA (■).

MOPC-31C and MOPC-41 myelomas. The $Crt_{1/2}$ values (1.2×10^{-3} for MOPC-31C mRNA and 3×10^{-3} for MOPC-41 mRNA) of each hybridization are comparable to that reported for respective mRNA (1,11). The lower extent of hybridization to the heterologous mRNA reflects the proportion of the cDNA complementary to the constant region sequence. In contrast, MOPC-31C cDNA hardly hybridized with L_λ chain mRNA derived from RPC-20 myeloma at Crt values where L_λ chain (RPC-20) cDNA hybridized to completion (Fig. 1C). A slight hybridization observed at high Crt values may reflect a contaminant L_κ chain mRNA sequence present at less than 1/100th concentration of L_λ chain mRNA in RPC-20 myeloma (1). If the slight hybridization is due to a contaminant L_λ cDNA present in MOPC-31C L_κ cDNA, the $Crt_{1/2}$ values for hybridization of RPC-20 mRNA with L_κ (MOPC-31C) and L_λ (RPC-20) cDNAs should be identical.

L_λ chain cDNA prepared from RPC-20 mRNA showed little hybridization with L_κ chain mRNAs at Crt values comparable to hybridization with L_κ chain cDNA (Fig. 1A, B), whereas the L_λ chain cDNA hybridized with homologous mRNA at low $Crt_{1/2}$ value (8×10^{-3})(Fig. 1C). These results clearly indicate that

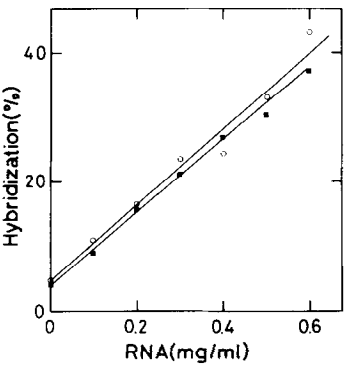

Fig. 3. Hybridization of MOPC-104E (NIH) cytoplasmic RNA with L_κ and L_λ cDNA. Increasing amounts of MOPC-104E cytoplasmic RNA were hybridized with MOPC-41 L_κ cDNA (2,200cpm) and RPC-20 L_λ cDNA (2,400cpm) in 200µl reaction mixture for 36 min. Hybridization with L_κ cDNA (O); L_λ cDNA (■).

MOPC-31C L_κ chain cDNA and RPC-20 L_λ cDNA are specific for the respective chain type.

Presence of both κ and λ chain mRNA sequences in MOPC-104E myeloma

The concentrations of L_κ and L_λ chain mRNA sequences in MOPC-104E (Kyoto) myeloma were quantitated by hybridization with respective chain-type specific cDNA (Fig. 2). There is, as expected, extensive hybridization between RPC-20 cDNA and mRNA derived from a L_λ chain producing tumor, MOPC-104E (Kyoto). A $Crt_{1/2}$ value of 9×10^{-3} is comparable to RPC-20 mRNA (Fig. 1C). Unexpectedly, hybridization between MOPC-104E mRNA and MOPC-31C cDNA attained 60% hybridization with a $Crt_{1/2}$ value of 7×10^{-3}. Since the $Crt_{1/2}$ values are quite similar to each other, the amounts of L_κ and L_λ chain mRNA sequence in MOPC-104E mRNA are almost equivalent. The lower extent of hybridization indicates that the L_κ chain sequence present in MOPC-104E myeloma has a variable region sequence different from MOPC-31C L_κ chain. Similarly, almost equivalent amounts of L_κ and L_λ chain mRNA sequences were present in total cytoplasmic RNA prepared from MOPC-104E myeloma (NIH) which had been maintained independently from MOPC-104E (Kyoto)(Fig. 3). Polysomal RNA prepared from MOPC-104E (Osaka) also contained L_κ chain mRNA slightly more than L_λ chain mRNA (unpublished observation).

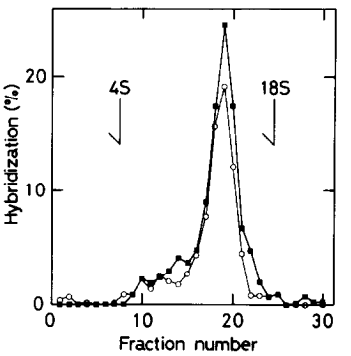

Fig. 4. Sucrose gradient centrifugation of MOPC-104E (NIH) RNA. After heat quenching, cytoplasmic RNA of MOPC-104E tumor (1.75mg) was layered on a 5-22% (w/v) sucrose gradient and centrifuged at 37,000rpm for 15h at 15°C in a Hitachi RPS 40T rotor (1,11). Fifteen µl of each fraction was hybridized with either MOPC-41 L_κ cDNA (1,270cpm) or RPC-20 L_λ cDNA (773cpm) in a 200µl reaction mixture for 30 min. Locations of 18S ribosomal RNA and tRNA (4S) markers were indicated in the figure. Hybridization with L_κ cDNA (O); L_λ cDNA (■). Endogenous S_1 nuclease resistance was subtracted from all values.

Characterization of light chain mRNA sequences present in MOPC-104E

The molecular size of L_κ and L_λ chain mRNA present in the MOPC-104E (NIH) cytoplasmic RNA fraction was analyzed by sedimentation in sucrose density gradient under conditions which abolish nonspecific aggregation of RNA (1)(Fig. 4). Both L_κ and L_λ chain mRNA sedimented together with a single peak of 12.5S which was known as the size of immunoglobulin light chain mRNA (1-8,11). The MOPC-104E L chain mRNA preparation purified by the method successfully employed for purification of MOPC-41 and RPC-20 mRNAs (1,5) migrated as a single band upon polyacrylamide gel electrophoresis in formamide, although this preparation contained equivalent amount of both L_κ and L_λ chain mRNA sequences. Both L_κ and L_λ chain mRNAs present in MOPC-104E contain poly(A) sequences because they were adsorbed to oligo(dT)-cellulose.

DISCUSSION

Available evidence indicates that three lines of MOPC-104E myeloma, which had been maintained in different laboratories, contain L_κ chain mRNA in a quantity almost equivalent to that of L_λ chain mRNA. Schechter (4) reported that the

λ type mRNA preparation purified from MOPC-104E myeloma contained about 3% of κ type mRNA. The discrepancy is probably attributable to their purification method which includes immunoprecipitation of polysomes using antibodies against L_λ chain protein. Myeloma tumors producing two classes of antibody have been observed (14). Investigators from several laboratories including our own (1,2,4) reported that light chain mRNA preparations from some myelomas contain a tiny amount of light chain mRNA sequences of the heterologous type. In these cases the major sequence is usually orders of magnitude more than the contaminant sequence, which could arise from circulating lymphocytes present in tumor tissues or from leaking transcription of the repressed gene in the myeloma cells.

Following questions should arise: 1) whether an individual MOPC-104E myeloma cell produces both L_κ and L_λ chain mRNA sequences; 2) whether the L_κ chain mRNA sequence is translated *in vivo* and the product is secreted from the cells. Hausman and Bosma (15) reported that they were unable to detect any L_κ chain protein in IgM secreted by MOPC-104E. However, a variant of MOPC-104E which was obtained by the alternate passage between *in vitro* culture and subcutaneous propagation in mice, produced IgM containing L_κ instead of L_λ chains and an altered μ type H chain. It is interesting to know whether the variant contains L_λ chain mRNA or not. At present we have no definitive answer to any of the above questions. We are trying to clone the MOPC-104E cell line and to study immunological properties of protein secreted by the myeloma, which will answer these questions.

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