COMPARATIVE HYBRIDIZATION STUDIES WITH AN IMMUNOGLOBULIN LIGHT CHAIN mRNA FRACTION AND NON-IMMUNOGLOBULIN mRNA OF MOUSE

T.H. RABBITTS* and J.O. BISHOP

Department of Genetics, University of Edinburgh, West Main Road, Edinburgh EH9 3JN, Scotland

and

C. MILSTEIN and G.G. BROWNLEE

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

Received 17 December 1973

1. Introduction

The messenger RNA (mRNA) for the mouse immunoglobulin light chain has been partially purified from several different mouse myelomas [1–5]. A mRNA fraction prepared from the tumour line MOPC 21 has been shown to consist of between 25% and 50% pure L-chain mRNA [6]. Such a mRNA preparation is of potential value, in DNA–RNA hybridization experiments using the DNA excess technique [7,8] for assessing the number of genes for both the variable (V) and the constant (C) region of the immunoglobulin molecule. Such experiments should help to define the extent of antibody diversity encoded as stable information in the genome and the corresponding importance of diversification.

We report here that the hybridization pattern of a 13 S mRNA fraction, which contains L-chain mRNA (the LE fraction), consists of components which hybridize with repetitive and with non-repetitive elements in the DNA. A similar biphasic pattern is also shown to occur with a mRNA fraction prepared from mouse fibroblasts (which do not contain immunoglobulin mRNA) and a mRNA fraction from the non-membrane bound polysomes of myeloma cells. A biphasic pattern has previously been reported using total mRNA from rat myoblasts [9]. Competition experiments indicate that the sequences involved in the hybridization of the LE fraction and the fibroblast mRNA are not identical (in either transition). Hence it seems that the biphasic hybridization profile is a general property of the mRNAs studied.

* Please address correspondence to T.H. Rabbitts at the Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

2. Methods

^{32}P-labelled mRNA and 18 S rRNA were prepared from P3K cells (a cell line derived from MOPC 21 myeloma cells [10]) as described in ref. [6]. The degree of purity of the mRNA was assayed by fingerprint techniques [6]. The fraction which is rich in L-chain mRNA will be named the LE fraction.

Secondary cultures of fibroblasts were prepared from Balb/c mouse embryos. mRNA was ^{3}H-labelled by incubating cells for 30 min at 37°C in the presence of 0.04 μg/ml actinomycin D (Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.) followed by 3 hr with 100 μCi/ml [^{3}H]uridine (The Radiochemical Centre, Amersham, Bucks., U.K., 20 Ci/mmol). mRNA was prepared from total polysomes by proteinase K treatment and oligo dT chromatography [3]. The mRNAs were sedimented in 5–20% sucrose density gradients containing 0.1 M NaCl, 0.01 M Tris–HCl, pH 7.5 and 0.5% sodium dodecyl sulphate (20 hr, 24 000 rpm, 25°C, SW. 27 Spinco Model L2)

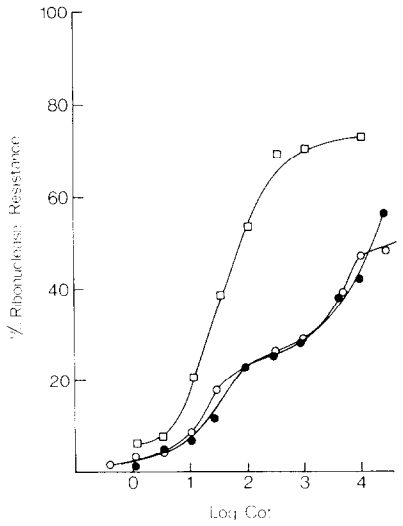

Fig. 1. Hybridization of ^{32}P-RNA to nuclear DNA in DNA excess. 5 mg samples of DNA were mixed with either 10^{-2} μg ^{32}P-LE mRNA fraction in the presence of 30 μg unlabelled 28 S rRNA and 30 μg unlabelled 18 S rRNA or with 10^{-2} μg ^{32}P-18 S rRNA alone. The individual mixes were boiled for 5 min in 0.1 × SSC and than annealed at 70°C in 2 × SSC. Samples, taken at various times, were assayed for resistance to digestion by pancreatic ribonuclease: intrinsic ribonuclease resistances are subtracted from each point. C_0 is initial DNA concentration (mol nucleotide litre^{-1}); t is time in seconds; (□——□——□) 18 S rRNA plus myeloma DNA; (○——○——○) LE mRNA plus myeloma DNA; (●——●——●) LE mRNA plus liver DNA.

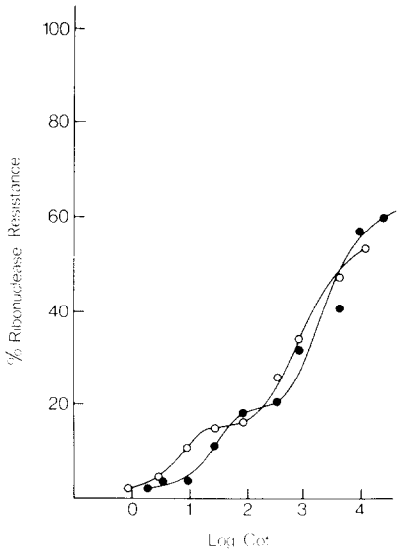

Fig. 2. Hybridization of fibroblast mRNA and myeloma-free polysomal mRNA to myeloma DNA in DNA excess. 5 mg myeloma DNA plus 10 000 cpm 13 S ^{3}H-mRNA from fibroblasts or 10^{-2} μg 13 S ^{32}P-mRNA from myeloma-free polysomes were annealed as in fig. 1. (○——○——○) ^{3}H-mRNA of fibroblasts; (●——●——●) ^{32}P-mRNA of myeloma free polysomes.

and the 13 S mRNA fraction was precipitated by the addition of two volumes of ethanol. Unlabelled fibroblast total polysomal mRNA was prepared from roller cultures.

Nuclear DNA was prepared from MOPC 21 myeloma solid tumours and Balb/c liver essentially as described [11] and DNA excess hybridization was carried out in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate) at 70°C [7,8].

3. Results

The hybridization profiles of ^{32}P-18 S rRNA and ^{32}P-LE mRNA are shown in fig. 1. The 18 S rRNA profile has a single transition of hybridization with $Cot^{h}_{1/2}$ value (the Cot value corresponding to the half point of a transition [12]) of about 30. This value indicates a reiteration frequency of about 170 copies per haploid genome (Bishop et al. [13]). The hybridization of the LE mRNA fraction to either myeloma or liver DNA, however, shows distinct transitions at $Cot^{h}_{1/2}$ = 20 and at $Cot^{h}_{1/2}$ = 3200. These transitions correspond to genes reiterated about 250 times for the first transition and 1 to 2 for the second transition. The fast transition ($Cot^{h}_{1/2}$ = 20) is very close to that obtained with 18 S rRNA but it is not attributable to contaminating ^{32}P-rRNA fragments since no contamination with rRNA could be detected in the fingerprint assay. In addition, the hybridization experiments were conducted in the presence of sufficient unlabelled rRNA to dilute out completely any possible ^{32}P-rRNA hybridization [14]. Similar hybridization experiments employing kidney DNA demonstrated an identical biphasic profile with L-chain mRNA.

Fig. 2 shows the hybridization pattern of 13 S mRNA fractions isolated from mouse fibroblasts and from free (i.e. not membrane-bound) polysomes of

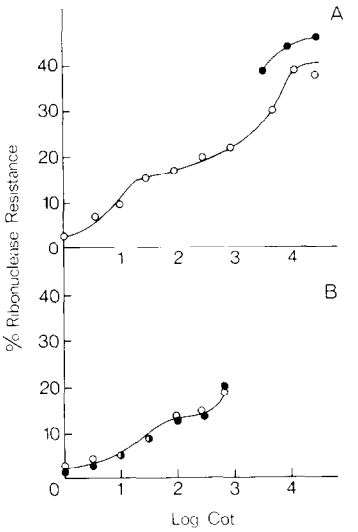

Fig. 3. Competition hybridization of ^{32}P-LE mRNA fraction vs unlabelled fibroblast mRNA. (A) High Cot transition competition. 5 mg myeloma DNA were annealed (as described for fig. 1) with 10^{-2} μg ^{32}P-LE mRNA in the presence of unlabelled fibroblast total mRNA (DNA/competitor fibroblast mRNA = 384). A control tube (3 mg DNA plus 6×10^{-3} μg ^{32}P-LE mRNA minus competitor RNA) was annealed separately to high Cot. (●——●——●) Control hybridization (no competitor); (○——○——○) hybridization in the presence of competitor. (B) Low Cot transition competition. 0.1 mg samples of DNA were annealed (as described for fig. 1) with 4×10^{-3} μg ^{32}P-LE mRNA in the presence or absence of unlabelled fibroblast total mRNA to a final Cot 600. (DNA/competitor fibroblast RNA = 1). (●——●——●) Control hybridization (no competitor); (○——○——○) hybridization in the presence of competitor.

P3K cells. Both curves show a biphasic profile very similar to that observed with the LE mRNA fraction. Competition hybridization experiments were carried out to determine whether the DNA sequences involved in the hybridization of 13 S mRNA from fibroblasts and the LE mRNA fraction from myelomas were the same. The hybridization pattern of the LE mRNA fraction was found to be largely unaffected by the presence of unlabelled mRNA prepared from fibroblasts (fig. 3). Some competition does, however, appear to occur under conditions where only material hybridizing to limited DNA copies will be competed (fig. 3A). Control homologous competition experiments using labelled and unlabelled fibroblast mRNA showed that the high and low DNA: RNA ratios used were sufficient to abolish completely the homologous high and low Cot transitions respectively.

4. Discussion

The results described in the present paper show that a biphasic hybridization curve is obtained when the myeloma mRNA fraction, which is rich in L-chain mRNA (LE fraction), is hybridized to nuclear DNA. Before any conclusions can be drawn from these results, however, two points must be carefully considered. Firstly, the LE fraction is not completely pure L-chain mRNA [6] and so the contribution of contaminating RNA species will be important. Secondly, repetitive and non-repetitive elements are also present in mRNA fractions prepared from myeloma free polysomes (which synthesize a negligible amount of L-chain [15] and from fibroblast polysomes (which synthesize no immunoglobulin). Two phase transitions have also been described in mRNA from rat myoblasts [9]. Furthermore such hybridization characteristics occur with rat nuclear RNA [7] and it appears that mRNA from *Xenopus* embryos contain sequences corresponding to repetitive and non-repetitive DNA sites [16,17] although the repetitive component in *Xenopus* seems to represent a smaller proportion of the total mRNA. The biphasic hybridization curve is, therefore, not restricted to immunoglobulin mRNA. The inability of fibroblast mRNA to compete with the repetitive sequences present in the LE mRNA fraction indicate, however, that these sequences are either absent or they represent a very low proportion of the fibroblast mRNA. The problem is further complicated when consideration is given to the experimental conditions. It is known that departure from true DNA excess hybridization conditions with a single mRNA species results in progressive reduction in the amount of hybridization which occurs with that particular species [14]. The DNA: RNA ratio employed in the present experiments was, in fact, insufficient to achieve vast DNA excess for a single mRNA species, so that the high Cot transition shown in fig. 1 is undoubtedly due to a heterogeneity of mRNA species. The bulk of the RNA species involved in the high Cot transition appears to be myeloma-specific, however, in so far as they are not

competed out by fibroblast mRNA. The L-chain specific mRNA consists of three major portions of approximately equal size: the V-region, the C-region and the untranslated bases [6]. The contribution of these L-chain mRNA sequences to the observed high and low Cot transitions is not yet clear.

Acknowledgements

We thank Mr G. Smith for technical assistance and S.R.C. for financial support.

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